

Computer vision profiling of neurite outgrowth dynamics reveals spatiotemporal modularity of Rho GTPase signaling

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Rho guanosine triphosphatases (GTPases) control the cytoskeletal dynamics that power neurite outgrowth. This process consists of dynamic neurite initiation, elongation, retraction, and branching cycles that are likely to be regulated by specific spatiotemporal signaling networks, which cannot be resolved with static, steady-state assays. We present NeuriteTracker, a computer-vision approach to automatically segment and track neuronal morphodynamics in time-lapse datasets. Feature extraction then quantifies dynamic neurite outgrowth phenotypes. We identify a set of stereotypic neurite outgrowth morphodynamic behaviors in a cultured neuronal cell system. Systematic RNA interference perturbation of a Rho GTPase interactome consisting of 219 proteins reveals a limited set of morphodynamic phenotypes. As proof of concept, we show that loss of function of two distinct RhoA-specific GTPase-activating proteins (GAPs) leads to opposite neurite outgrowth phenotypes. Imaging of RhoA activation dynamics indicates that both GAPs regulate different spatiotemporal Rho GTPase pools, with distinct functions. Our results provide a starting point to dissect spatiotemporal Rho GTPase signaling networks that regulate neurite outgrowth.

Introduction

Neurite outgrowth is a prerequisite step to produce the axons and dendrites that wire the adult brain. Neuronal cell migration is crucial for brain morphogenesis. In cell culture, neurite outgrowth is highly dynamic and involves a series of dynamic morphogenetic processes (MPs) such as neurite initiation, elongation, branching, growth cone motility, and collapse (da Silva and Dotti, 2002). Cultured neuronal cells are also highly motile, which might reflect the process of neuronal migration

(Nadarajah and Parnavelas, 2002). Specific spatiotemporal signaling networks control the cytoskeletal, trafficking, and adhesion dynamics required for each MP to occur. Because neurite outgrowth and cell migration use overlapping cellular machineries, it is conceivable that some molecular components might regulate both processes. These MPs fluctuate on length and time scales of tens of microns and minutes to hours, which have been missed in steady-state neurite outgrowth measurements, most often at a late differentiation stage. Identification of MP-specific signaling networks thus requires the quantification their morphodynamics.

Rho GTPases are key regulators of the cytoskeletal dynamics that regulate neuronal cell morphogenesis (da Silva and Dotti, 2002). Their activity is tightly controlled in time and space by guanine nucleotide-exchange factors (GEFs; Rossman et al., 2005) and GTPase-activating proteins (GAPs; Moon and Zheng, 2003) that activate and deactivate GTPases, respectively. This regulation, as well as coupling of Rho GTPases to specific downstream effectors, dictates their cytoskeletal output at any given subcellular localization (Pertz, 2010). Current models

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Abbreviations used in this paper: CV, computer vision; FRET, Förster resonance energy transfer; GAP, GTPase-activating protein; GEF, guanine nucleotide-exchange factor; HDS, hierarchical data structure; HS, high stringency; KD, knock-down; LS, low stringency; MDS, morphodynamic signature; MLCK, myosin light chain kinase; MP, morphogenetic process; MRCK, myosin-related dystrophy kinase myosin light chain kinase; pMLC, myosin light chain phosphorylation.

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state that Rac1 and Cdc42 regulate neurite outgrowth, whereas RhoA controls neurite collapse (da Silva and Dotti, 2002). However, multiple GEFs, GAPs, and effectors are ubiquitously expressed by cells and outnumber their cognate Rho GTPases (Moon and Zheng, 2003; Rossman et al., 2005). This raises the question of the significance of this signaling complexity.

We present NeuriteTracker, a computer vision (CV) platform to track neuronal morphodynamics from high-content time-lapse imaging datasets. Automatic extraction of a large set of morphological and morphodynamic features, coupled with adequate statistical analysis, can then quantify the dynamics of neuronal morphogenesis. Our pipeline identifies distinct, stereotyped morphodynamic phases during neuronal cell morphogenesis and quantifies a set of morphodynamic phenotypes in a siRNA screen targeting a candidate Rho GTPase interactome. This provides insight into the spatiotemporal Rho GTPase signaling networks regulating distinct MPs. As proof of concept for our screen, we show that two RhoA-specific GAPs regulate two distinct spatiotemporal RhoA signaling networks controlling different cytoskeletal outputs. Our data provide an initial resource to study the complex spatiotemporal Rho GTPase signaling networks that regulate neuronal cell morphogenesis.

Results

High-content live-cell imaging pipeline

To study neuronal dynamics, we used neuronal-like mouse N1E-115 neuroblastoma cells. To visualize cell morphology, we used a bicistronic vector that expresses Lifeact-GFP, a fusion of GFP with the F-actin binding peptide Lifeact (Riedl et al., 2008), and a nuclear localization NLS-mCherry fusion, which labels the nucleus for cell detection (Fig. 1 a). This construct can be expressed at a high level without affecting neurite outgrowth (Fig. S1, a and b) and provides homogeneous high contrast on neurites and somata for imaging with air objectives (Fig. 1 b and Video 1). An additional benefit of our reporter is that it labels F-actin-containing branches not apparent using the classic microtubule staining used in neurite outgrowth assays. To perturb different signaling molecules, we cotransfected our reporter plasmid with siRNAs in nondifferentiated cells. These cells were subsequently differentiated by serum starvation, replated on laminin-coated coverslips, and allowed to extend neurites for 19.6 h (Fig. S1 c). As proof of concept, we evaluated knockdown (KD) of previously characterized proteins. *MKK7* KD leads to highly unstable, short neurites owing to loss of microtubule bundling in the neurite shaft (Feltrin et al., 2012). *RhoA* KD leads to increased neurite outgrowth (Hirose et al., 1998) through loss of growth cone collapse controlled by RhoA (Fritz et al., 2013). *SrGAP2* KD leads to stabilization of the growth cone and soma lamellipodial veils, increasing neurite outgrowth and branching, as well as cell spreading and migration (Pertz et al., 2008). Our protocol enabled efficient KD of these proteins, which recapitulated the expected phenotypes (Fig. S1, d–f). We also identified a nontargeting siRNA that did not affect neurite outgrowth (Fig. S1 g). To perform high-content live-cell imaging, we optimized our microscope setup for fast, two-color imaging of multiple wells. Time-lapse analysis started 3 h postplating, a time point at which initial neurites already protruded, rendering cells resistant to phototoxicity. An automated image analysis routine was then used to identify 10 fields of view per well containing an adequate number of cells

exhibiting a specific range in fluorescence intensities and cell areas (Fig. S1, h–j). We then time-lapsed neurite outgrowth dynamics in 10 fields of view per well across a 24-well plate with 12-min time resolution for a total of 19.6 h.

NeuriteTracker pipeline to quantify neuronal dynamics

To analyze the time-lapse datasets, we engineered NeuriteTracker, an automated CV pipeline to segment and track soma and neurite morphodynamics described in Materials and methods (Fig. 1, b and c; and Video 2). First, mCherry-NLS-labeled nuclei and their associated somata were identified using maximally stable extremal regions and a fast marching region growing step (Fig. 1 c, steps 1 and 2). Second, a graph-association tracking method was applied over the time-lapse data to provide a unique label for each tracked cell over time (Fig. 1 c, steps 3 and 4). Neurites were extracted for each cell by applying a calibrated Hessian-based filter, computing a geodesic distance combining intensity and geometric distance, and applying a threshold (Fig. 1 c, steps 5–7). Candidate terminals were identified, and neurite filaments were detected by backtracing and reconstruction with a minimal spanning tree (Fig. 1 c, step 8). Again applying the graph association to the neurites yielded automatic segmentation and tracking of individual cell nuclei, soma, and neurites (Fig. 2 a and Video 2). Our pipeline provided robust results across a wide variety of fluorescence intensities that result from transient transfection and was evaluated against human annotated ground-truth datasets (Fig. S2 and Videos 3 and 4).

To describe the segmented cells, we defined a series of parameters that describe nucleus, soma, and neurite morphology, the neurite being modeled as a tree arborescence (Figs. 2 b and S3; also see Materials and methods, Definition of nuclei and soma parameters and Definition of neurite parameters). These data were then organized in a hierarchical data structure (HDS) that models the neurite, as well as the nucleus and soma geometries, at a given time point (Fig. 2 c). A graph representation of this HDS can then be rendered (Fig. 1 c, step 9). Finally, we used these parameters and the HDS to extract an as-comprehensive-as-possible set of features that describe nuclei, soma, and neurite morphology and morphodynamics. For each cell, 28 “static” features that provide measurements relevant to cell morphology at one specific time point were extracted from the HDS and stored in a feature vector as described in Materials and methods (Definition of static features). The dynamic evolution of some of these features is shown in Fig. 2 d. Furthermore, we computed five layers of dynamic features, which provide summaries of the temporal evolution of static features on a per-cell basis (described in Materials and methods, Definition of dynamic features). These include the mean value of static features through time (1), the time a feature expands/contracts (2/3), the number of inflection points during feature evolution (4), and the frequency of the expansion of a feature (5). This provides a comprehensive description of neurite and soma morphodynamics. Importantly, these different layers of dynamic features are to some extent redundant, and a selection of a specific set of features can be required to avoid redundancy during their analysis.

We also used our CV pipeline to track and segment neuronal morphodynamics of Lifeact-GFP/NLS-mCherry-transfected Pheochromocytoma PC-12 cells plated on collagen and stimulated with nerve growth factor. The raw images, their superimposed HDS graph representations, and the temporal

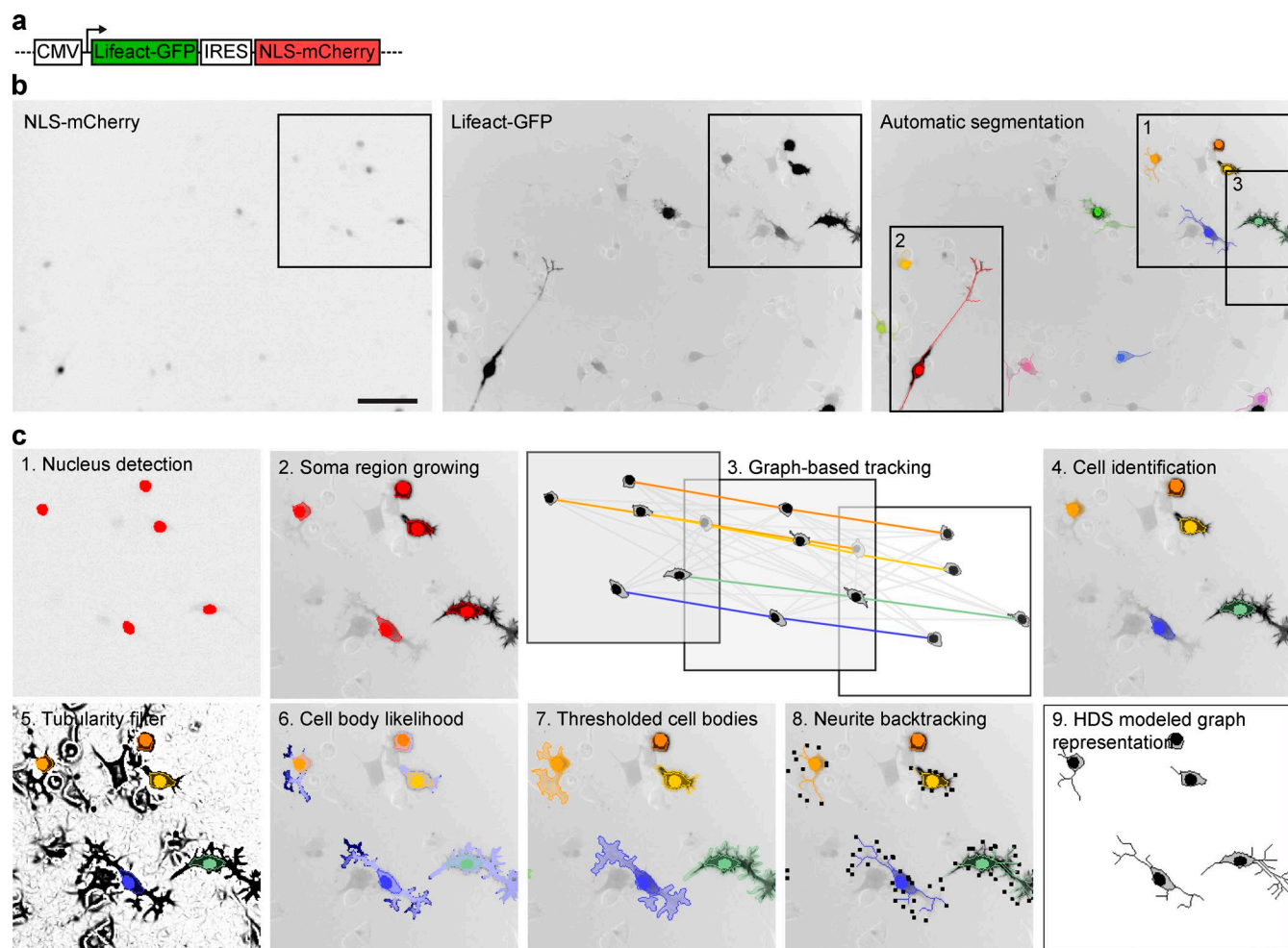


Figure 1. Tracking and segmentation of neuronal morphodynamics. (a) Schematics of Lifeact-GFP/NLS-mCherry expression vector. (b) Raw NLS-mCherry channel (left), raw Lifeact-GFP channel (middle), and Lifeact-GFP channel (right) with superimposed nucleus, soma, and neurite segmentations color-coded according to cell identity. Bar, 100 μ m. Fluorescent images are shown in inverted black-and-white contrast. (c) Different segmentation steps highlighted in magnification from inset 1 from panel b.

evolution of some extracted features are shown in Fig. S4 (a–c) and indicate that our CV pipeline is sufficiently versatile to be applied to different neuronal-like cell types.

We provide access to the MATLAB code of NeuriteTracker through the Github platform at <https://github.com/sgbasel/neuritetracker>. Analysis of different neuronal morphologies requires the tuning of a small set of parameters, which can be conveniently performed using a graphical user interface. Ultimately, NeuriteTracker provides both the extracted features in the HDS format in a CSV file, as well as a graph representation of the HDS. A test time-lapse dataset and manual are also provided.

Functional analysis of a Rho GTPase signaling network

As a proof of concept to validate NeuriteTracker, we performed an siRNA screen targeting a potential Rho GTPase interactome. We used a bioinformatics approach to mine a proteomics dataset of purified neurite and soma fractions of N1E-115 cells (Pertz et al., 2008) to identify GEFs, GAPs, effectors, and their interacting proteins that potentially interact with a panel of Rho GTPases (Fig. 3 a; described in Materials and methods). This revealed a complex interactome consisting of 219,

mostly neurite-enriched, proteins. Gene ontology analysis revealed a large coverage of cellular processes relevant to adhesion and cytoskeletal dynamics and axonal guidance (Fig. 3 b). We used three distinct siRNAs per gene, leading to a total of 657 perturbations (siRNA sequences are described in Table S1). These were distributed among 32 experiments, with each 24-well plate containing one negative, nontargeting and one positive, *MKK7*-targeting siRNA control. For each of these 721 wells (657 perturbations + 64 controls), we acquired 10 fields of view, therefore accounting for a total of 7,210 time-lapse movies. We then developed a statistical analysis pipeline to analyze this dataset.

Morphodynamic signature extraction

To quantify the effect of perturbations on neuronal dynamics, we considered 28 static features measured on the whole-cell population at all the different time points of a time-lapse dataset. This provides the distribution of feature values throughout the whole lifetime of the cell population (Fig. 4 a, population-level features). We also considered the “frequency expansion” feature set that consists of 18 features that summarize the dynamics of single cells throughout the movie (Fig. 4 a, dynamic cell-level features).

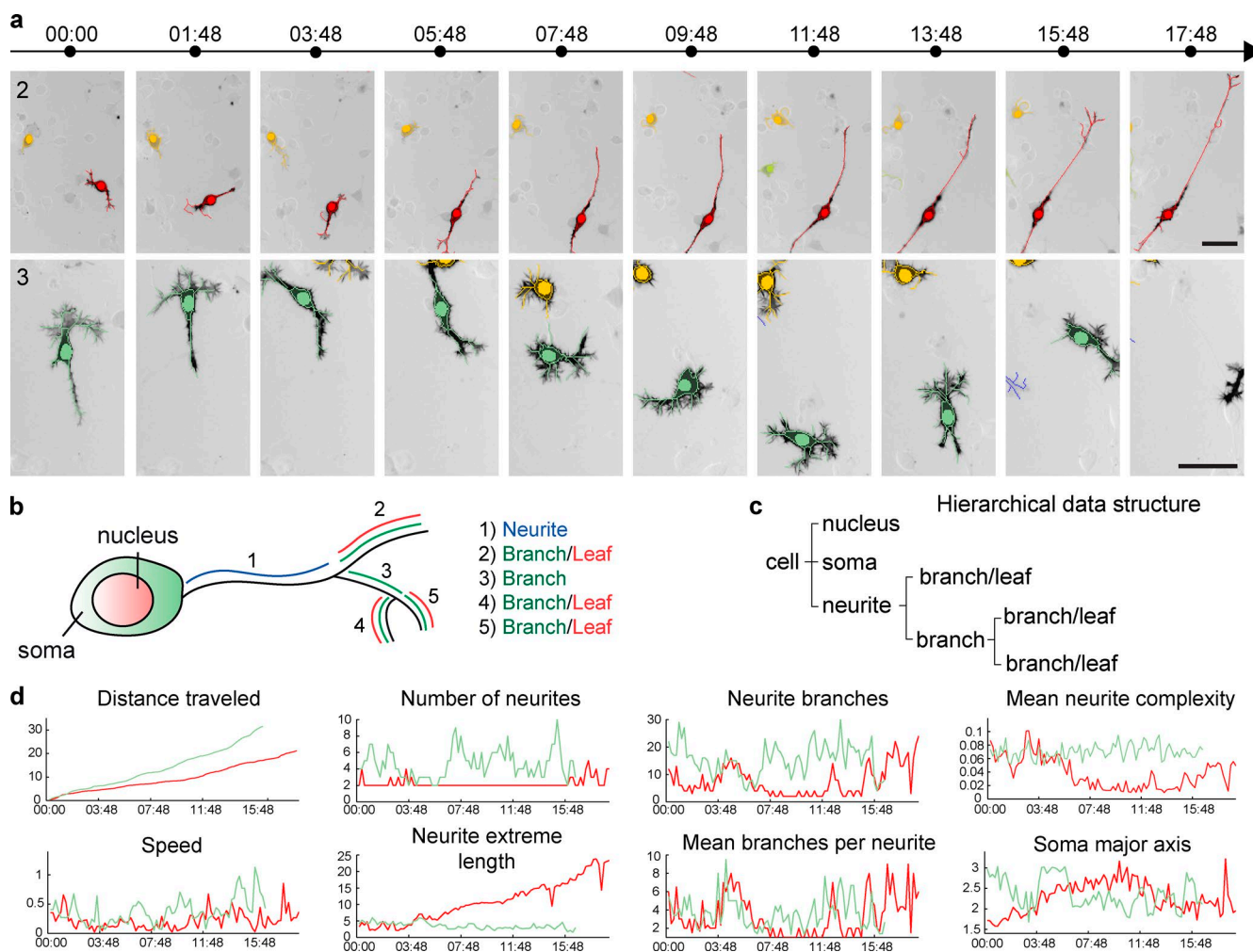


Figure 2. Feature extraction from time-lapse datasets. (a) Example of segmentation of two cells from a time-lapse dataset (from insets 2 and 3 in Fig. 1 b). Nucleus, soma, and neurite segmentations are color-coded for cell identity. Time, h:min. Bar, 50 μ m. (b) Schematics describing neuronal cell morphology, and how different neuronal parameters are extracted for definition of neuronal arborescence. (c) Example of the HDS that models the schematic cell in b. (d) Temporal evolution of a set of features for the two cells shown in panel a.

For each feature, we used a z -test to compare feature value distributions of control and siRNA-transfected cells (Fig. 4 b). The z -score polarity indicates if a specific feature value augments or decreases when control and KD cells are compared. All the details relevant to our data analysis pipeline are explained in Materials and methods. To mitigate any potential off-target effects, we computed mean z -scores from the three siRNAs, according to the rule that if at least two of three siRNAs produce the same phenotype for a given feature (Echeverri et al., 2006), then this phenotype is on target (Fig. 4 c). This approach provides a mean z -score vector that indicates penetrance and polarity for each feature in response to a siRNA perturbation (Lefort et al., 2015). We refer to this z -score vector as the morphodynamic signature (MDS) of the target gene.

We then reduced the feature space by two successive steps. First, we evaluated the feature robustness at different stringency levels by comparing the MDS of the *MKK7*-KD positive control phenotype across the 32 independent performed experiments. Specifically, we selected features that exhibited a statistically relevant z -score with identical polarity in at least 65% (low stringency [LS]) or 90% (high stringency [HS]) of the 32 *MKK7* KD control experiments. Second, we selected features that have

high information content; we retained features that exhibit a statistically significant z -score in at least 40% of the 219 genes across the siRNA screen. This especially affected “dynamic” features, because those require integration of a large number of measurements over time, leading to a lower measurement sample size than static features. This reduced the feature space to 32%/78% (LS feature set) and 75%/95% (HS feature set) static/dynamic features, providing robust feature sets that are highly informative about neuronal morphology and dynamics.

We evaluated the validity of our pipeline by extracting MDSs associated with our previously characterized *SrGAP2*, *MKK7*, and *RhoA* KD phenotypes (Figs. 4 d and S1 e). This revealed that our approach successfully captures morphological and morphodynamic phenotypes (Fig. 4, e and f). *SrGAP2* KD led to increased neurite number, length, and branching, as well as increased soma migration, which further correlate with increased cell and nucleus eccentricity. As expected, *MKK7* KD led to reduced frequency of neurite extension events, short neurites, and low soma motility. *RhoA* KD led to reduced collapse frequency, producing longer neurites, with reduced branching. Some of these features can be easily assessed by visual inspection of static images (neurite length/branching)

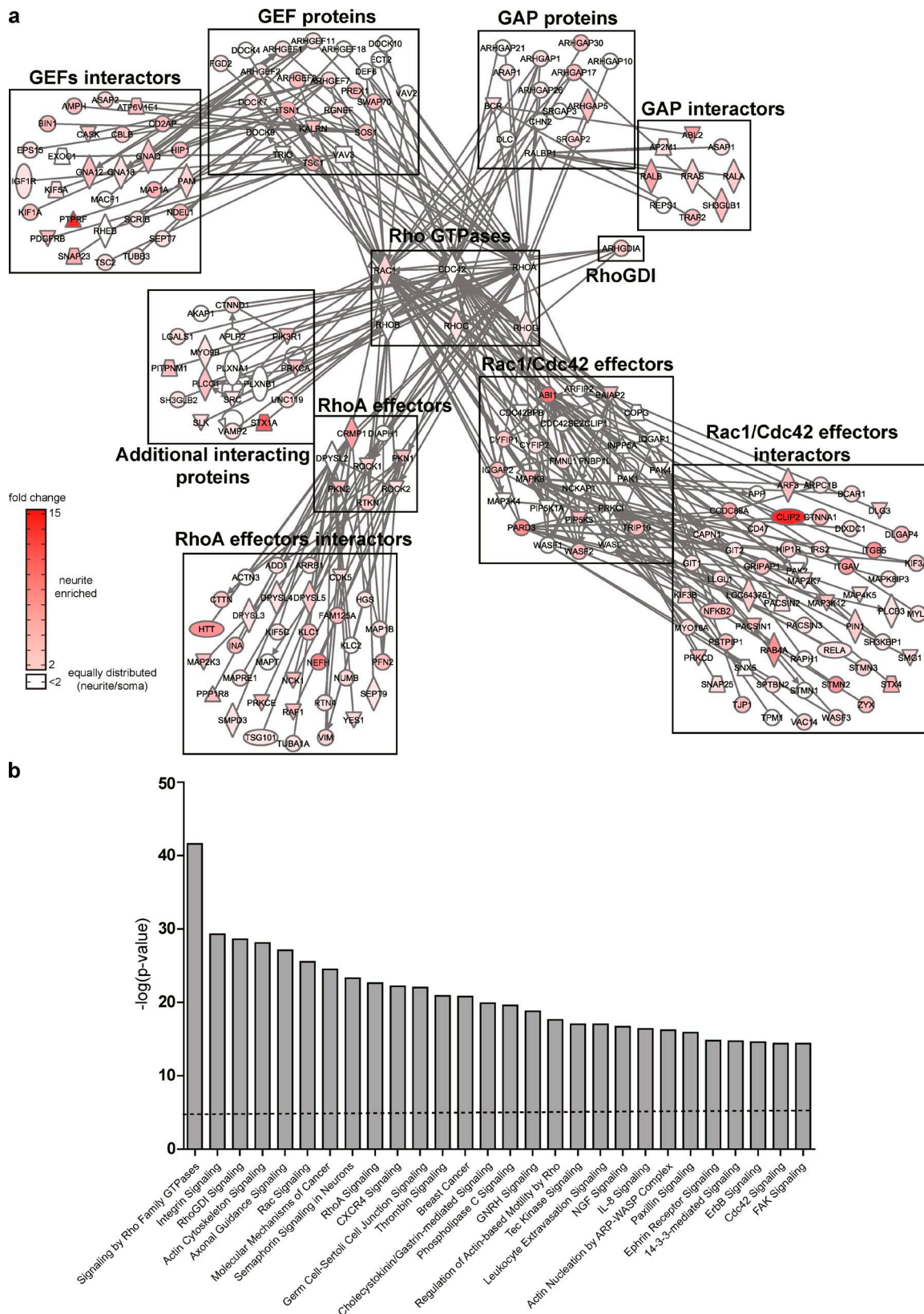


Figure 3. **Identification of a candidate Rho GTPase signaling network.** (a) Rho GTPase protein interaction network as determined by the strategy described in Materials and methods. Different families of proteins known to interact with six expressed Rho GTPases are shown. Proteins are named by their official gene symbol according to Entrez Gene. Relative neurite protein enrichment is color-coded according to the color scale bar at the bottom of the figure. Plain lines represent direct protein-protein interaction. Icons are color-coded according to the color scale bar. (b) Gene Ontology analysis of the most significant canonical pathways present in the protein network shown in panel a. Dotted line represents significance threshold as measured by Fisher's exact test ($P < 0.05$).

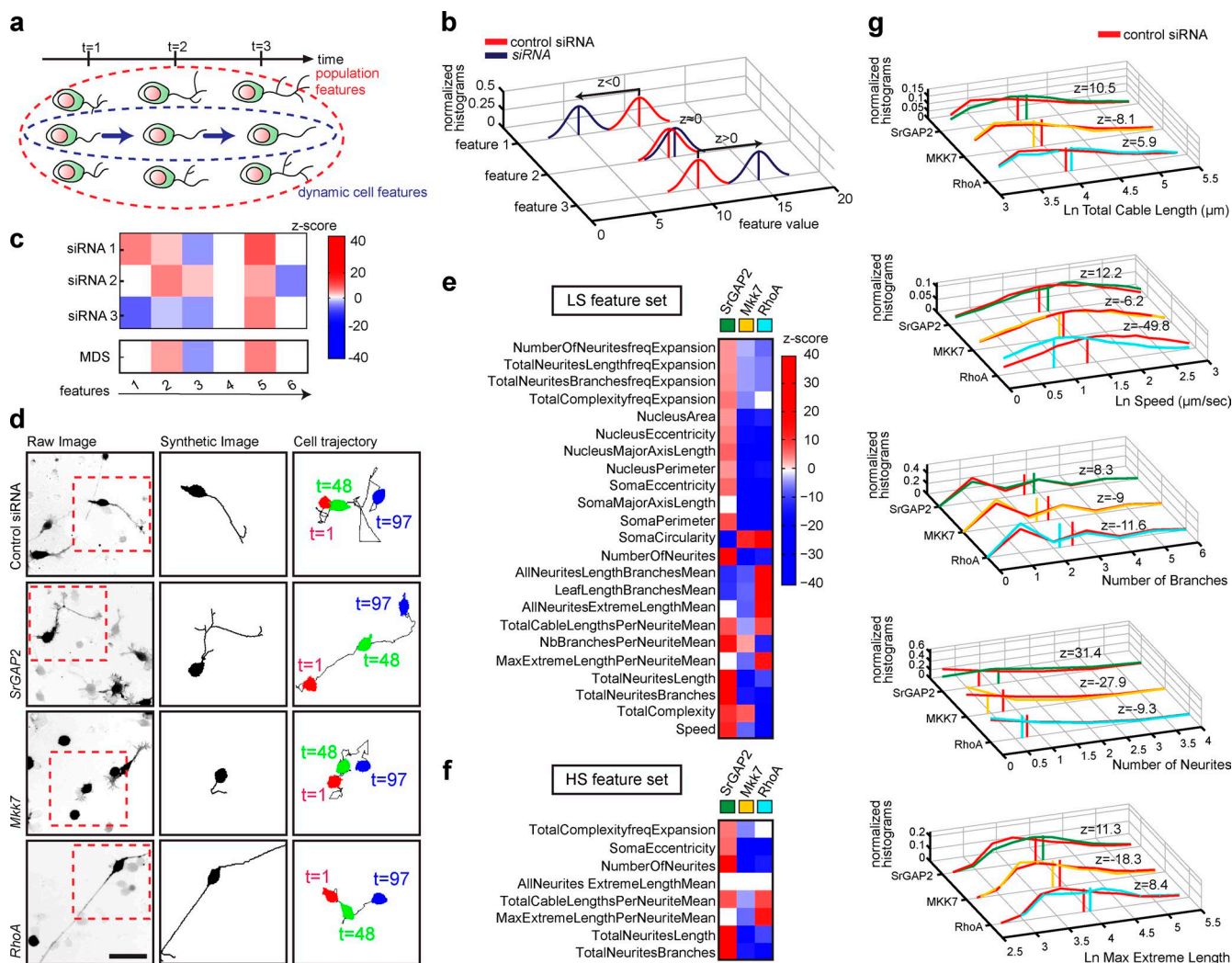


Figure 4. Morphodynamic signature extraction. (a) Schematics of two distinct feature analysis levels. Static population-level and dynamic cell-level features are considered. (b) Feature extraction procedure. z-score vector defines statistical significance, penetrance, and polarity of measured feature distributions of control compared with siRNA-treated cells. (c) Deconvolution of RNAi OFF-target effects. For each given feature: (1) if the three siRNA perturbations lead to a z-score with identical polarity, the mean z-score is computed; (2) if two of three siRNA perturbations lead to a z-score with identical polarity, the mean z-score for the two features with the same polarity is computed; (3) if the three siRNA perturbations yield three different z-score polarities, then the z score is set to 0. (d) Raw image (left, inverted black-and-white contrast), HDS graph representation (middle), and cell migration tracks (right) for SrGAP2, MKK7, and RhoA siRNA perturbations are shown. Bar, 50 μ m. (e and f) Gene-associated MDSs represented by a color map. (e) LS feature set; (f) HS feature set. z-score scale is also shown. (g) Normalized histograms of representative feature distribution profiles. The data were normalized dividing each histogram value by the histogram area. Vertical bars represent the mean. The z-score associated with each feature is also shown.

or time-lapse datasets (soma speed). Others, such as soma circularity, are more subtle and can be detected only by our CV approach. Plotting feature distribution in the different perturbed states indicates the cellular heterogeneity associated with our cell system (Fig. 4 g).

siRNA screen analysis

A set of quality-control metrics showed that a similar number of cells per field of view and NLS-mCherry fluorescence intensities occurred in the different experimental plates (Fig. 5, a and b). Quantification of off-target effects across the features, throughout the screen, revealed that 43% of the measurements led to identical z-score polarities for all three siRNAs; 52% of the measurements had two of three siRNAs yielding identical z-score polarities, whereas in only 5% of the cases, one siRNA led to a statistically nonsignificant z-score and the two other yielded two distinct polarities. Further, this low level of

off-target effect was not biased to a specific set of experimental plates (Fig. 5 c). Finally, the positive MKK7-KD control associated with each plate yielded a stable MDS across the different experimental plates (Fig. 5 d), indicating the stability of our pipeline. Each siRNA perturbation led to a quantifiable MDS with a mean z-score of 1.2 ± 1.9 for the least penetrant and 40.9 ± 21.6 for the most penetrant phenotype. Even in the least penetrant phenotypes, a subset of features displayed statistically relevant z-scores.

To identify sets of gene perturbations that lead to common MDSs, we binarized z-score vectors to give equal weight to all features and performed bottom-up, hierarchical clustering. Similar clustering results were observed whether z-score matrices were binarized or not (e.g., the z-score value was taken into account). Iterative evaluation of different dendrogram thresholds was performed by visual inspection of the quality of the clusters (Fig. 5, e–h), using HDS representations to evaluate

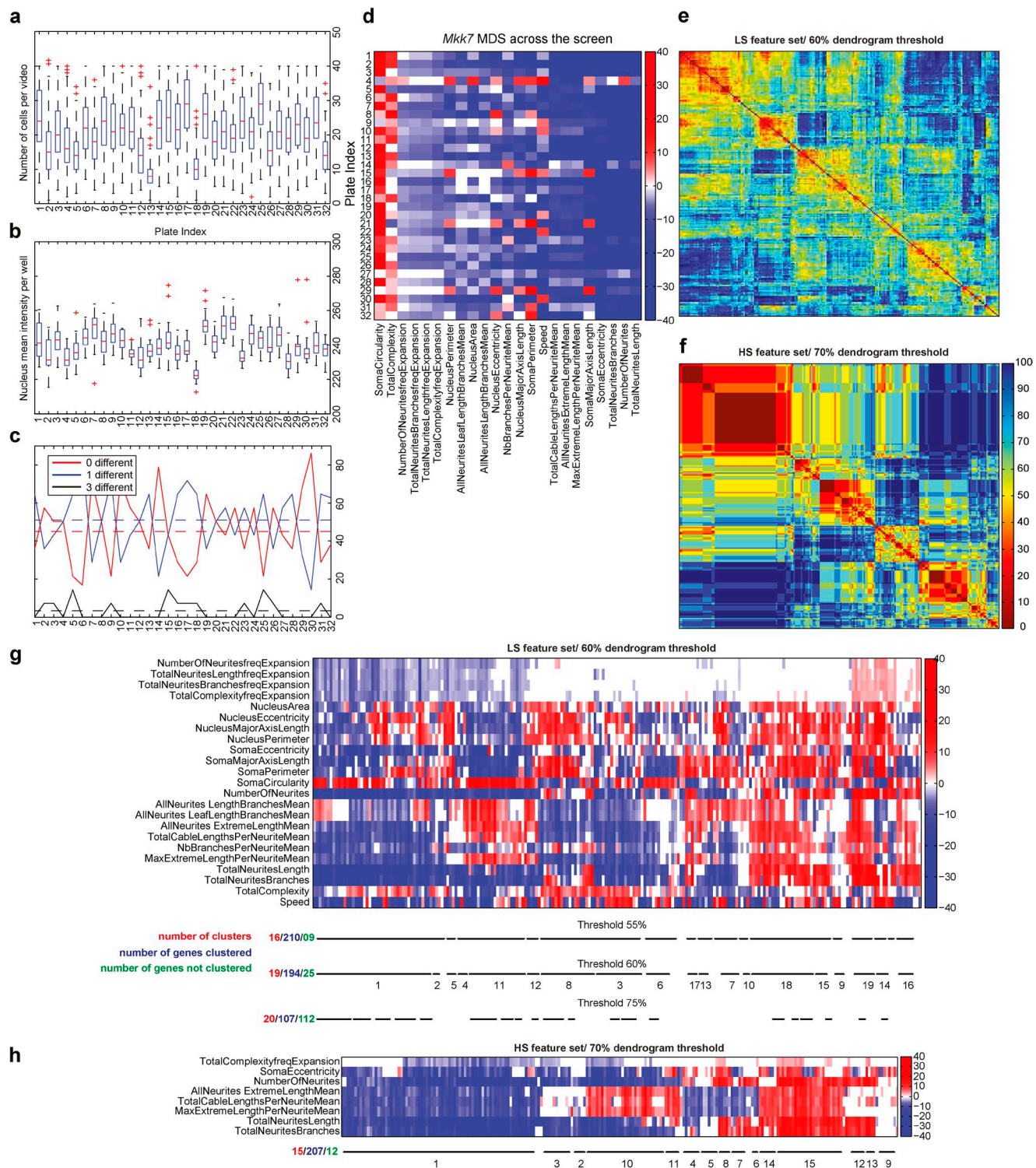


Figure 5. siRNA screen quality controls and MDS hierarchical clustering. (a) Boxplots quantification of number of cells per field of view across 32 independent experiments. Median (red line), interquartile range (box), and domain (dashed line) are represented. The outliers are plotted individually. (b) Boxplot quantification of NLS-mCherry mean fluorescence intensity across 32 independent experiments. Median (red line), interquartile range (box), and domain (dashed line) are represented. The outliers are plotted individually. (c) Per-plate off-target effect analysis across 32 independent experiments. The analysis takes in consideration the off-target effect of the LS feature set. Black lines indicate the three siRNAs generate different phenotypes. Blue line indicates that two siRNAs generate the same phenotype, but the phenotype of the third siRNA is different. Red lines indicate that the three siRNAs generate the same phenotype. For each plate, the proportion of each case is represented by continuous lines. The dashed line represents the mean value of the case across the whole screen. (d) MDS of MKK7-KD cells across 32 independent experiments. z-score vector maps are shown for each experimental plate. Right bar indicates z-score color code. (e and f) L₀ distances comparing all the MDS to each other for low- (e) and high-stringency (f) feature sets. L₀ distances were normalized to range between 0 and 100 (percentage). L₀ distances are color-coded so that warm colors indicate low distances and cold colors indicate high distances. (g) MDSs hierarchical clustering using the LS feature set. MDS z-scores are color-coded according to the color scale. Identified clusters for different dendrogram thresholds are shown by black lines. The number of clusters, genes clustered, and nonclustered genes are also indicated. (h) MDSs hierarchical clustering using the HS feature set with a dendrogram threshold of 70%.

the similarity of penetrant phenotypes for different gene perturbations within a cluster. The rationale to identify cells with penetrant phenotypes is mentioned in the next paragraph. For the LS feature set, a good compromise was obtained with a 60% dendrogram threshold, meaning that 60% of the features of the MDSs within a cluster have the same polarity. This led to the identification of 19 phenoclusters that comprise 194 of 219 genes (Fig. 5, e and g). Using the HS feature set led to the identification of 15 clusters that comprise 207 of the 219 genes with a 70% dendrogram threshold (Fig. 5, f and h). Any of the nonclustered genes also led to phenotypes, but these have to be inspected on a case-by-case basis.

For intuitive display, we manually sorted phenoclusters according to the overall polarity of their MDSs (Figs. 6 and S4 d). We then described the phenoclusters at different complexity levels and focused first on static, then on dynamic phenotypes. Phenotypes that can be explained by static features led us to group the different clusters in five large phenotypic categories: short neurites (group 1: C1–C7), spread soma multiple and/or branched short neurites (group 2: C8), normal neurites with soma phenotypes (group 3: C9–C10), long/unbranched neurites (group 4: C11–C13), and long/branched neurites (group 5: C14–C19). To provide a visual representation of these phenotypic categories at the static level, we used a mathematical model that selects specific cells with a highly penetrant phenotype according to three visually intuitive features (number of neurites, number of branches, and maximum extreme length per neurite mean). These are then represented using HDS-modeled graph representations, providing a representative example of the phenotype (Fig. 6, Fig. S4 d, and Video 5). Within these static-level phenotype groups, the different subclusters are characterized by additional static features that are mostly relevant to nucleus and soma morphology and that are less visually evident. Dynamic features such as cell migration (speed) and features relevant to neurite outgrowth dynamics (total neurite length frequency expansion, etc.) then discriminate further clusters. Representative dynamic phenotypes were selected using our mathematical model using the same three features set but additionally including the total neurite length frequency expansion feature (Videos 6 and 7). Phenoclusters computed using LS and HS feature sets were similar, except for some sensitive phenotypes observed within cluster 8 (LS feature set; Figs. 6 and S4 d). The lower amount of clusters when the HS feature set is used can mostly be explained by the loss of the cell migration feature. Schematics and statistics of the whole workflow of our pipeline are described in Fig. S5. A web application based on the openBIS data management platform (Bauch et al., 2011) illustrates the static and dynamic phenotypes associated to each siRNA perturbation using the LS feature set (<https://mat-openbis.dbm.unibas.ch:8443/openbis/webapp/sinergia/>). This platform provides access to the z-score vector associated with each siRNA perturbation, as well as to the mean MDS. 10 representative images, as well as 10 representative time-lapse datasets of penetrant phenotypes (selected using the approach described at the beginning of this paragraph), and their matched experimental controls are also provided.

Characterization of stage-specific neuronal dynamics

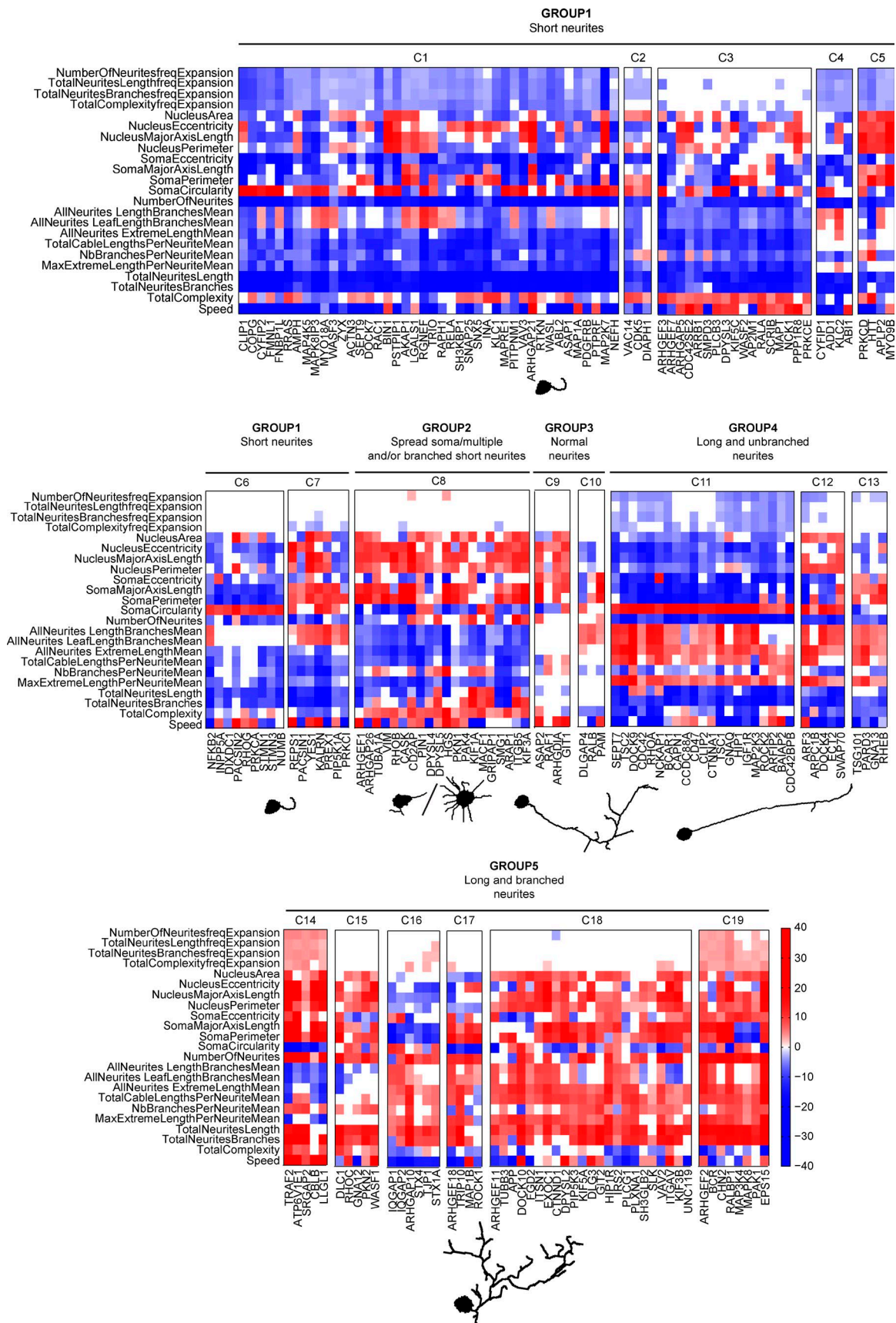
Neuronal differentiation involves multiple temporal episodes with distinct morphodynamic behaviors that have until now been described only qualitatively (da Silva and Dotti, 2002).

To get quantitative insight into neuronal morphodynamics associated with different temporal episodes, we took advantage of the large control and *MKK7*-KD dataset from our siRNA screen dataset. We studied MDSs associated with sequential 1-h episodes from our 20-h time-lapse datasets in control cells. For scale-free representation, we plotted the temporal evolution of the coefficient of variation of a panel of features. Because only a fraction of the cell population extends robust neurites, we considered the whole population but also gated on the 10% of cells with the most robust neurite outgrowth (“all neurites extreme length mean feature”). At the whole-cell population level (Fig. 7 a), robust initiation of multiple short and branched neurites is observed at the onset of differentiation and then decreases during a first phase that lasts 7 h. This period is also associated with a low soma motility state. After 7 h, a small cell population extends a lower number of long unbranched neurites (Fig. 7 c), and soma motility steadily increases during the remaining period studied. We used a mathematical model to identify cells that represent the mean static phenotypes relevant to select temporal bins of the 10% cells with longest neurites (Fig. 7 c). These results quantitatively define two distinct phases with different morphodynamic behaviors during the neurite outgrowth process.

We then applied the same approach to study the short, unstable neurites *MKK7*-KD phenotype (Fig. 7, b and d). Although short neurites were observed in phase 2, temporal analysis surprisingly revealed a burst of neurite outgrowth in phase 1 compared with control cells, which previously had eluded our visual inspection of time-lapse movies. Other parameters such as neurite and branch numbers, as well as cell migration speed, were not affected. These results suggest that *MKK7* regulates two distinct functions, one occurring at an early and the other at a late phase during neurite outgrowth. Feature distribution plots again represent the heterogeneous, but statistically significant, behavior of cell populations (Fig. 7 e).

Spatiotemporal regulation of RhoA activation by two RhoA-specific different GAPs

As a proof of concept for the validity of our siRNA screen, we sought to explore some relevant neurite outgrowth phenotypes with respect to spatiotemporal Rho GTPase activation dynamics. We focused on an apparently paradoxical result of our screen that is centered around RhoA and two RhoA-specific GAPs: *ARHGAP5* (p190RhoGAP; Ridley et al., 1993; Arthur and Burridge, 2001) and *DLC1* (deleted in liver cancer; Kim et al., 2009; Braun and Olayioye, 2015). Consistently with the classic model (Hirose et al., 1998), we observed that *RhoA* KD led to longer neurites, which results in decreased neurite collapse events (Fig. 6, cluster 11). Furthermore, as expected, *ARHGAP5* KD, which is expected to augment RhoA activity, led to short neurites owing to increased neurite collapse (Fig. 6, cluster 3). In marked contrast, *DLC1* KD, which is also expected to augment RhoA activity, surprisingly led to an increase in neurite outgrowth (Fig. 6, cluster 15). As we had previously shown (Fritz et al., 2013), two distinct spatiotemporal RhoA activation pools, most likely involving different signaling complexes, are associated with growth cone advance and collapse in N1E-115 cells. We therefore hypothesized that each GAP regulates one of the specific RhoA signaling complexes. During growth cone advance, RhoA activity localizes to the tip of filopodial F-actin bundles (Fig. 8, a and c; and Video 8). In contrast, RhoA is



activated in retraction fibers at the tip of spontaneously collapsing growth cones (Fig. 8, b and d; and Video 8). Importantly, RhoA activity correlates only with its downstream cytoskeletal output, myosin light chain phosphorylation (pMLC) during growth cone collapse (Fig. 8 e), suggesting that this specific process is regulated by Rho kinase. In contrast, low pMLC signal was observed during growth cone advance, suggesting that RhoA regulates a distinct effector pathway involved in filopodium formation.

To explore the possibility that DLC1 and ARHGAP5 regulate two distinct spatiotemporal pools of RhoA activity, we performed a series of experiments in which we knocked down *DLC1*, *ARHGAP5*, or both. For these experiments, we selected the single siRNAs of which the feature vector most resembled the mean MDS. Quantitative PCR indicated potent reduction of *DLC1* and *ARHGAP5* messages in both single and double KD (Fig. 9 a). Quantification of steady-state neurite outgrowth levels recapitulated the results observed using our CV approach: *DLC1* KD led to increased whereas *ARHGAP5* KD led to reduced neurite outgrowth (Fig. 9, b and c). Combined *DLC1/ARHGAP5* KD led to reduced neurite outgrowth, indicating that, at this specific length scale, *ARHGAP5* loss of function is dominant over *DLC1* loss of function. Because RhoA spatiotemporal signaling patterns are evident only when the growth cone is examined (see Fig. 8 and Fritz et al., 2013), we zoomed in on the cytoskeletal and spatiotemporal RhoA activation phenotypes at this specific length scale (Fig. 9, d–f). *DLC1* KD cells exhibited longer filopodia that extended out of the growth cone veil (Fig. 9 d). Rather than being focused at the tip of filopodial F-actin bundles, RhoA was activated throughout the filopodium (Fig. 9, e and f). This suggests that *DLC1* functions by locally deactivating RhoA to shape its focused activity pattern at the tip of F-actin bundles. In *DLC1* KD cells, the aberrant RhoA activation pattern then leads to longer and more stable filopodia, which favor steady growth cone motility (Video 9). Ultimately, this can explain the increased neurite outgrowth phenotype observed at the length scale of a whole cell. *ARHGAP5* KD cells did not display a specific filopodium phenotype as with *DLC1* KD (Fig. 9 d). Instead, rather than being confined to filopodia, aberrant RhoA activity was potently upregulated not only throughout filopodia structures but also within the growth cone (Fig. 9, e and f). Furthermore, *ARHGAP5* KD growth cone filopodia exhibited “frozen” morphodynamics, suggestive of excessive actomyosin contractility (Video 9). This aberrant cytoskeletal state then most likely is highly permissive for the increased growth cone collapse frequency observed at larger time scales. Double KD of *ARHGAP5* and *DLC1* revealed a hybrid phenotype (Fig. 9, d–f). Long filopodia, with activated RhoA throughout the filopodium were observed as for the *DLC1* KD phenotype. Additional RhoA activity throughout the growth cone body was observed as for the *ARHGAP5* phenotype. The latter pool of aberrant RhoA activity might then lead to excessive contractility that favors growth cone collapse, ultimately leading to short neurites in double-KD cells (Fig. 9, b and c). These results strongly suggest that *DLC1* and *ARHGAP5* respectively regulate filopodia formation and growth cone advance, versus actomyosin contractility and growth cone collapse.

Discussion

We present NeuriteTracker, a robust CV pipeline to track morphodynamics of neurons transfected with a genetically encoded, nonperturbing, fluorescent morphology reporter that can be time-lapsed for hours using air objectives in different neuronal-like cell types (Fig. S4, a–c). Automatic identification of multiparametric MDSs provides the throughput to analyze large time-lapse datasets and detects complex dynamic phenotypes that are difficult to discern by human visual inspection. The benefit of using dynamic features is demonstrated by the finding that they stratify phenotypes in additional clusters when only static features are considered during the analysis of our siRNA screen. Transiently occurring phenotypes can also be identified when specific temporal episodes are taken into account (Fig. 7, c and d), at the expense, however, that larger datasets are required to obtain adequate statistical power. Our approach identified two discrete neuronal differentiation episodes with specific morphodynamic states in an unbiased fashion (Fig. 7, a and b). Furthermore, we analyzed a Rho GTPase interactome that provides substantial coverage of the cytoskeleton regulating machinery controlling neurite outgrowth, with all candidates yielding a quantifiable phenotype. Our unbiased approach that uses unsupervised clustering of the MDSs uncovers a relatively small phenotypic space that is already visible using a small feature set (LS feature set, 19 clusters; HS feature set, 15 clusters). The benefit of using a larger feature set is then simply that it provides more information about the phenotypes. Our results are consistent with the recent finding of a similarly small phenotypic space in a perturbation screen targeting cell morphology in *Drosophila melanogaster* hemocytes (Yin et al., 2013). Importantly, cell migration was also impacted by many gene perturbations, revealing that the cytoskeletal regulation machinery regulating neurite outgrowth and cell migration is to a large extent shared.

Current models of Rho GTPase function in neurite outgrowth posit that Rac1/Cdc42 regulate neurite extension, whereas RhoA controls neurite retraction (Hirose et al., 1998; da Silva and Dotti, 2002; Govek et al., 2005). However, such simple models are not consistent with the apparent complexity of Rho GTPase signaling in which GEFs (Rossman et al., 2005), GAPs (Moon and Zheng, 2003), and effectors (Pertz, 2010) outnumber their cognate Rho GTPases. The rationale behind our screen was to explore the significance of the large potential Rho GTPase interactome we had previously unveiled using a proteomics approach (Pertz et al., 2008; Fig. 3). Indeed, our screen revealed that the different proteins of this potential Rho GTPase interactome are all important for the neurite outgrowth process. Thus, our results suggest a complex scenario in which multiple spatiotemporal Rho GTPase signaling networks, most likely consisting of GEFs, GAPs, GTPases, effectors, and their associated proteins, fine-tune different MPs.

Our proof-of-concept study in which we perturbed two RhoA-specific GAPs, which surprisingly led to two distinct global neurite outgrowth phenotypes, illustrates how this spatiotemporal signaling complexity will have to be tackled in the

Figure 6. Hierarchical clustering identifies MDS phenoclusters. Hierarchical clustering of MDSs using the LS feature set. Phenotypic clusters were manually sorted by the overall polarity of their MDSs. Representative images of major phenotype classes comprising multiple clusters were identified by a mathematical model described in Materials and methods section Identification of phenocluster representative cells. These representative images do not take into account any dynamic feature.

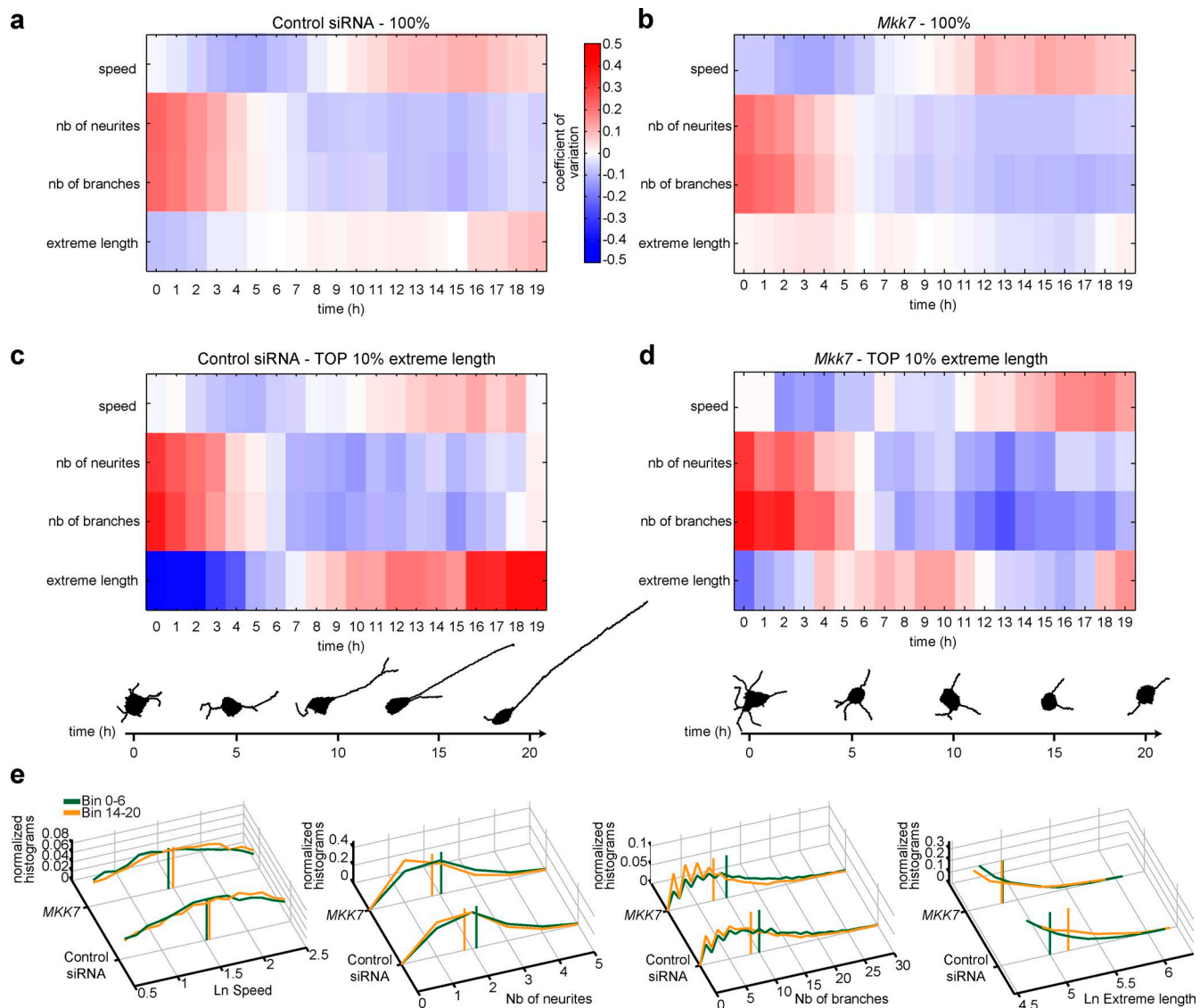


Figure 7. Temporal analysis of neuronal dynamics in control and MKK7-KD cells. (a–d) Control and MKK7-KD time-lapse sequences from multiple experimental plates were divided in 19 1-h bins. Coefficient of variation of selected features for each temporal bin for control (a and c) and MKK7 KD (b and d) cells. Measurements for the whole-cell population (a and b) and the 10% of cells with the highest neurite extreme length (c and d) are shown. Representative HDS graph representations of cells from the tenth percentile at the 1-, 5-, 10-, 15-, and 20-h time points have been identified using a mathematical model (Materials and methods; c and d). (e) Feature distributions at the two different time point intervals (1–6 h and 14–20 h). Vertical bars display distribution means.

future. Our data suggest that the two RhoA-specific GAPs regulate two distinct spatiotemporal RhoA signaling complexes, one involved in regulation of filopodium formation during growth cone advance and the other involved in the generation of contractility necessary for growth cone collapse. Our data suggest that ARHGAP5 is involved in the regulation of growth cone collapse because its perturbation leads to aberrant RhoA activity not only within unprotrusive filopodia but also within the body of the growth cone, which further correlates with frozen growth cone morphodynamics and ultimately with short neurites. This conceptually fits well with the function of RhoA in growth cone collapse, as exemplified by decreased instance of neurite retraction leading to longer neurites in the *RhoA* KD phenotype. Given the different global neurite outgrowth phenotype induced by *DLC1* KD, we propose that *DLC1* regulates a RhoA-dependent function distinct from that of ARHGAP5.

Our data strongly suggest that *DLC1* is involved in the fine spatial regulation of the focused RhoA activation pool at the tip of F-actin bundles, which most likely involves reaction–diffusion systems as previously documented for RhoA (Bement and von Dassow, 2014). This specific spatiotemporal RhoA activity pool might serve to locally position the formin mDia1 to polymerize F-actin to enable filopodia extension (Goh et al., 2012). *DLC1* loss of function might then compromise RhoA deactivation, leading to widening of the RhoA activation domain. This in turn might lead to aberrant formin regulation and longer and more stable filopodia that favor growth cone advance, ultimately leading to increased neurite outgrowth. Our *ARHGAP5/DLC1* double-KD data clearly show a hybrid phenotype with characteristic features from both single KDs, strongly suggesting that both GAPs specifically regulate the two distinct cytoskeletal processes of growth cone advance and collapse.

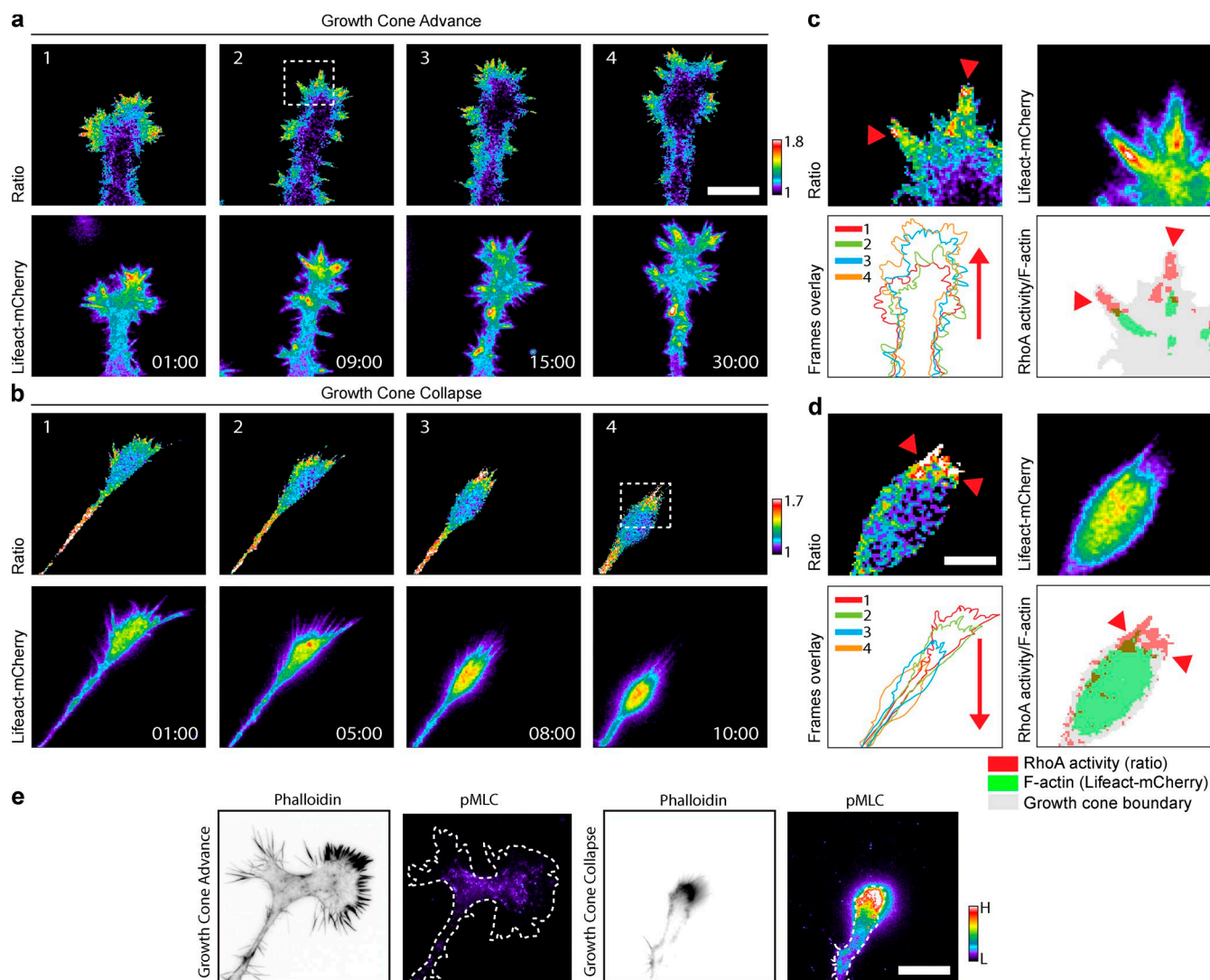


Figure 8. Dynamics of RhoA activation in advancing and collapsing growth cones. (a and b) Time series showing RhoA activation in advancing and collapsing growth cones from differentiated N1E-115 cells cotransfected with the RhoA2G FRET probe and plenti-Lifeact-mCherry. (top) Ratio (RhoA activation) images. (bottom) Lifeact-mCherry (F-actin) images. Time scale, min:s. Bar, 10 μ m. (c and d, top left and right) High-magnification images of advancing and collapsing growth cones outlined in the top rows of panels a and b. Red arrowheads point to the RhoA activity zone. (bottom left) Overlay of the time series frames showed in the top rows of panels a and b. Red arrows indicate the advance or collapse direction. (bottom right) Overlay images of the top-left and right panels of c and d highlight the RhoA activity zone (red) over the filopodia F-actin bundles (green). Red arrowheads point to the RhoA activity zone. Bar, 2 μ m. (e) High-resolution images of advancing and collapsing growth cones from differentiated N1E-115 cells stained for phalloidin and pMLC are shown. Bar, 10 μ m.

These observations have important consequences for the interpretation of our global neurite outgrowth phenotypes. Many of the neurite outgrowth phenotypes observed in our siRNA screen might emerge from aberrant spatiotemporal Rho GTPase signaling patterns occurring at the micrometer-length scale at which the growth cone functions, which might then propagate at the whole-cell scale. The information-rich, multi-parametric phenotypic signatures extracted by our CV pipeline therefore do not allow to unambiguously decipher the spatio-temporal complexity of Rho GTPase signaling. Deconvolving this molecular complexity of Rho GTPase signaling will therefore require evaluation of molecular perturbations at multiple time and length scales.

Finally, we nonexhaustively discuss some potential Rho GTPase signaling networks suggested by our screen (Fig. 10). The apparently conflictual phenotypes caused by DLC1 and

ARHGAP5 KD, which can be understood from the experiments performed in Fig. 9, are illustrated in Fig. 10 a. Our approach detects a continuum of similar phenotypes for KD of members of a well-known Rac1 effector pathway, consisting of the effectors Wave2 (WASF2) and N-WASP (WASL), as well as additional interactors known to regulate the Arp2/3 complex (Machesky et al., 1999; Banzai et al., 2000; Bradley and Koleske, 2009; Tahirovic et al., 2010), of which loss of function leads to short neurites (Fig. 10 b). Surprisingly, the *Rac1* “short neurites” KD phenotype (group 1: clusters 1 and 3) is also phenocopied by multiple Rac1-specific GEFs: KD of GEFs such as ARHGEF7 (ten Klooster et al., 2006; β -PIX), DOCK7 (Pineiro and Gertler, 2006), or Trio (Fig. 10 c; Bellanger et al., 2000). This suggests the existence of multiple complex spatiotemporal Rac1 pathways that regulate neurite protrusion. Consistently with the classic model in which RhoA regulates myosin contractility to enable

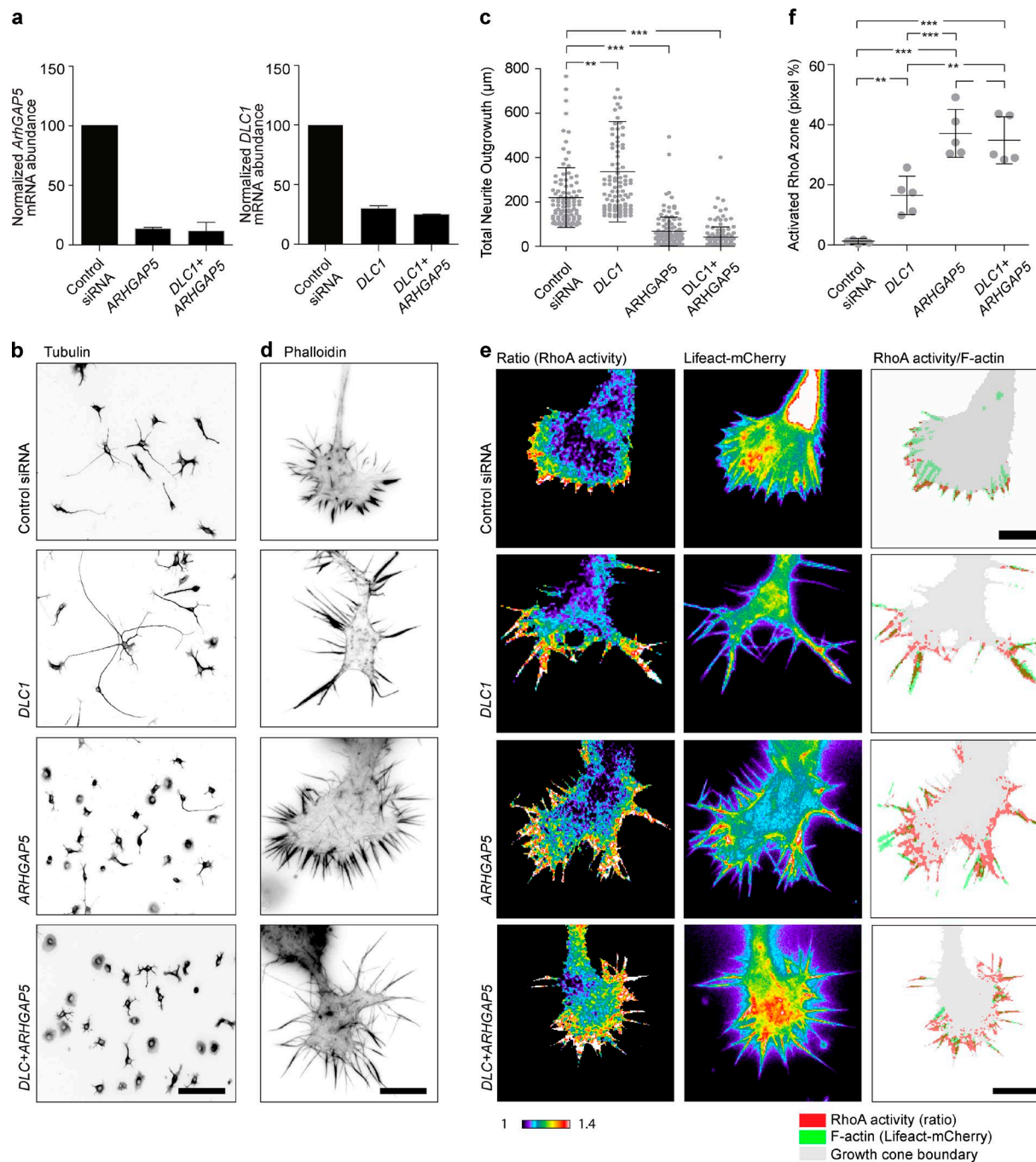


Figure 9. Characterization of *DLC1*, *ARHGAP5*, and double-KD phenotypes. (a) Quantitative RT-PCR of *DLC1*, *ARHGAP5*, and double *DLC1* and *ARHGAP5* KD efficiencies in N1E-115 cells. Mean \pm standard deviations from four replicates are shown. (b and c) Quantification of global neurite outgrowth phenotypes of the three perturbations. (b) Representative images of α -tubulin-stained N1E-115 KD cells. Inverted black-and-white contrast. (c) Quantification of total neurite outgrowth. Images were segmented and quantified using the Metamorph neurite outgrowth plugin. The single siRNAs that produced the z-score vector with lowest distance to the mean MDS were used for KD. Population mean \pm standard deviations are shown for the whole-cell population ($n = 150$ cells). One-way ANOVA with Bonferroni's multiple comparison test was used. ***, $P < 0.0001$. (d) High-resolution growth cone images of phalloidin-stained, control, and KD growth cones. All experiments were performed and stained simultaneously and acquired with identical acquisition settings. (e) Spatiotemporal RhoA activation patterns in response to the three perturbations. Ratio (RhoA activation), Lifeact-mCherry (F-actin), and overlay representative images of both signals are shown. Cells were cotransfected with the RhoA2G FRET probe, a Lifeact-mCherry construct, and the indicated siRNA. Ratio and F-actin images are color-coded for signal intensity. Ratio images have been scaled identically across experiments. Overlay images highlight the RhoA activity zone (red) over the filopodia F-actin bundles (green). (f) Quantification of RhoA activation in the three perturbed states. The RhoA activity zone was calculated as the pixel percentage of the growth cone RhoA activity area over the growth cone p-domain area. Population mean \pm standard deviation are shown for $n = 5$ cells/sample. One-way ANOVA with Bonferroni's multiple comparison test was used. ***, $P < 0.0001$. Bars: (b) 100 μ m; (d) 10 μ m; (e) 10 μ m.

collapse (Hirose et al., 1998), *ROCK2* KD, a RhoA-effector protein regulating myosin light chain kinase (MLCK; Amano et al., 1996; Matsui et al., 1996; Borisoff et al., 2003; Govek et al.,

2005), phenocopies RhoA KD. This is also true for Ect2 KD, a GEF that can activate RhoA (Fig. 10 d; Schmidt and Hall, 2002; Tsuji et al., 2011), suggesting that Ect2, RhoA, and ROCK2

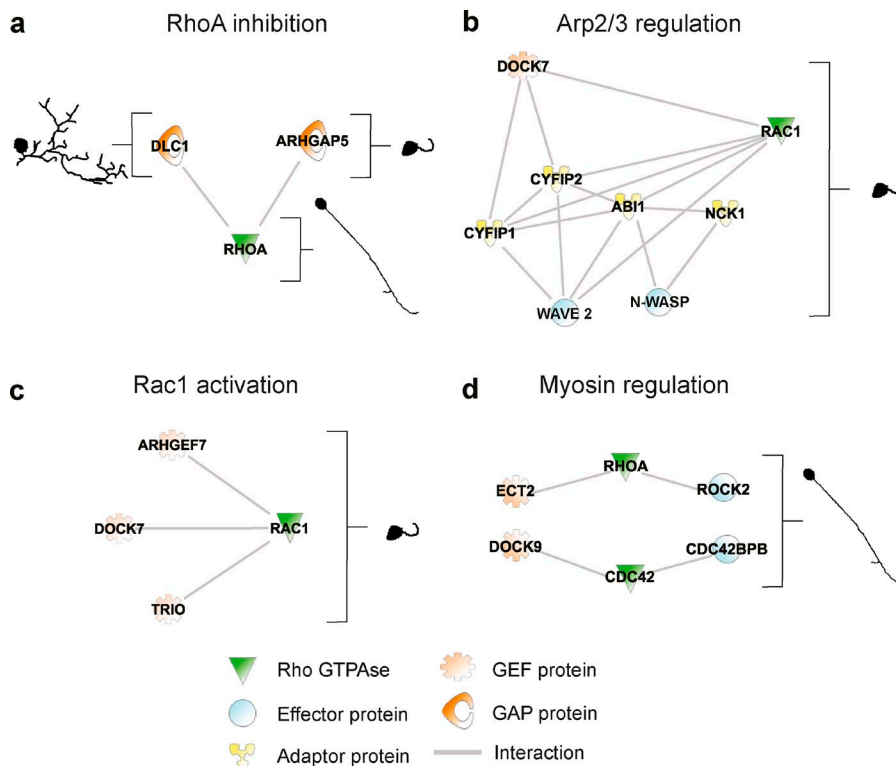


Figure 10. Selected potential Rho GTPase functional networks identified in the RNAi screen. Potential Rho GTPase signaling networks were identified using Ingenuity Pathway Analysis based on high-confidence protein–protein interactions or literature-based knowledge. Representative static phenotypes corresponding to the RNAi perturbations are shown. Each solid line represents a direct protein–protein interaction. A legend identifies the functions of the proteins in the network. (a) Multiple functions for distinct RhoA-specific GAPs. (b) Rac1-dependent effector pathway controlling the Arp2/3 complex. KD of these proteins all lead to a “short neurites” phenotype (group 1: clusters 1, 3, and 4). (c) Potential regulation of Rac1 by multiple Rac1-specific GEFs. (d) Potential Rho GTPase signaling networks regulating myosin light chain. All phenotypes correspond to a “long and unbranched neurites” phenotype (group 4 clusters 11 or 12).

might function together to promote neurite collapse. However, this is not phenocopied by KD of other RhoA-specific GEFs (*ARHGEF3*: C3; *TRIO*: C1; *ARHGEF11* and *ARHGEF18*: C18; and *ARHGEF2*: C19), suggesting additional mechanisms of spatiotemporal RhoA activation. Another novel finding was that KD of *Cdc42*, *DOCK9* (a *Cdc42*-specific GEF; Lin et al., 2006) and *Cdc42BPB* (a *Cdc42* effector also called myosin-related dystrophin kinase [MRCK]; Leung et al., 1998; Chen et al., 1999) phenocopy *RhoA* KD (Fig. 10 d). Because MRCK also regulates MLCK and contractility, a *DOCK9*, *Cdc42*, and MRCK signaling network might therefore also regulate growth cone collapse. It has been shown that the simultaneous phosphorylation of MLC, induced by RhoA and *Cdc42* via ROCK and MRCK, is crucial to promote actomyosin contractility and guarantee proper migration (Wilkinson et al., 2005). However, this cooperative mechanism between these two GTPases has never been shown in neuronal cells. These results suggest that RhoA and *Cdc42* may cooperate to regulate actomyosin contractility to control growth cone collapse and neurite retraction. Our CV approach provides a first estimate of the complexity of a spatiotemporal Rho GTPase signaling network during neuronal morphodynamics. This provides an initial resource that will allow researchers to gate on relevant morphodynamics processes to dissect these complex spatiotemporal networks at multiple time and length scales.

Materials and methods

Overview of wetlab experiments

DNA constructions. For simultaneous expression of Lifeact-GFP and NLS-mCherry, we created a bicistronic expression vector consisting of Lifeact-GFP, an IRES sequence (derived from a pIRES vector; Clontech), and NLS-mCherry (Fig. 1 a). This construct was built using InFusion technology (Clontech) and was cloned in a pcDNA3.1(+) eukaryotic expression vector (Invitrogen).

Accession numbers. The accession number for the pcDNA Lifeact-GFP/NLS-mCherry construct is Addgene 69058.

Cell culture and transfection. N1E-115 neuroblastoma cells (American Tissue Culture Collection) were cultured in DMEM supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin. For differentiation, N1E-115 cells were starved for 24 h in serum-free Neurobasal medium (Invitrogen) supplemented with 1% L-glutamine and 1% penicillin/streptomycin. For the double siRNA-mediated KD and plasmid transfection, cells were transfected as previously described (Chong et al., 2006), using 400 ng of the plasmid pcDNA-Lifeact-GFP-IRES-NLS-mCherry and 20 pmol Stealth Select siRNAs (Invitrogen). 1 μ l of Transfectin (Bio-Rad) was used as transfection reagent. 48 h posttransfection, cells were starved in Neurobasal medium. 72 h posttransfection, cells were detached with Puck's saline and replated on a glass-bottom 24-multiwell plate (MatTek) coated with 10 μ g/ml laminin (Millipore). Approximately 3 h after seeding, cells were immediately used for time-lapse imaging. For transfection of both siRNAs and plasmids, cells were transfected as previously described (Feltrin et al., 2012). PC12 Neuroscreen-1 subclone cells (Chen et al., 2012) were cultured in DMEM with 1% L-glutamine, supplemented with 10% horse serum, 5% FBS, and 1% penicillin/streptomycin. Cells were culture on 50 μ g/ml collagen solution from bovine skin (Sigma-Aldrich). For differentiation, PC12 Neuroscreen-1 cells were starved for 24 h in DMEM with 1% L-glutamine supplemented with 1% glucose, 1% penicillin/streptomycin, and 1% horse serum; 50 ng/ml NGF was added to the medium to induce differentiation. For PC12 transfection, cells were transfected as previously described (Chong et al., 2006), using 400 ng of the plasmid pcDNA-Lifeact-GFP-IRES-NLS-mCherry. 0.8 μ l of Transfectin (Bio-Rad) was used as transfection reagent. 24 h posttransfection, cells were starved in DMEM medium. 72 h posttransfection, cells were gently detached using a cell scraper and replated on a glass-bottom 24-multiwell plate (MatTek) coated with 50 μ g/ml collagen solution from bovine skin (Sigma-Aldrich).

Immunofluorescence. N1E-115 cells were washed in PBS, fixed in PBS containing 4% PFA (Sigma-Aldrich) for 10 min, and permeabilized

in PBS containing 1% Triton X-100 for 2 min. Coverslips were washed in PBS for 20 min and blocked in 2% BSA and 0.1% Triton X-100 in PBS for 15 min. Cells were stained with primary antibodies for 1 h (Alpha Tubulin Clone DM1A; Sigma-Aldrich), phospho-myosin light chain 2 (Cell Signaling Technology), and then with secondary antibodies for 30 min (Alexa Fluor 555-labeled phalloidin, Alexa Fluor 546 secondary antibody, and DAPI for 30 min; Invitrogen).

Microscopy and image acquisition. All experiments were imaged using an inverted Eclipse Timicroscope (Nikon) controlled by Metamorph software (Universal Imaging). Laser-based autofocus, a CFI Plan Apo Lambda 10 \times (NA 0.45; Nikon) objective, and a Cool-Snap HQ2 camera (Roper Scientific) were used throughout. For live-cell imaging experiments, rapid switching between the GFP and mCherry channels was performed using two independent LED lamps with excitation at 470 and 585 nm (Cool LED) with a multiband-pass EGFP/mCherry filter cube (Chroma Technology Corporation). All experiments were performed in serum-free Neurobasal medium (Invitrogen) supplemented with 1% L-glutamine and 1% penicillin/streptomycin. For measurements of neurite outgrowth at steady state, differentiated, siRNA-transfected cells were replated on 18-mm glass coverslips coated with 10 μ g/ml laminin. 24 h after plating, the cells were fixed and stained with DAPI and an antitubulin antibody. DAPI and tubulin images were acquired in a 5 \times 5 matrix of fields of view. Image analysis was performed using the Metamorph neurite outgrowth plugin (Universal Imaging). Selected fields of view were then acquired using a CFI Plan Apo Lambda 10 \times (NA 0.45) objective (Nikon).

For time-lapse imaging of neurite outgrowth dynamics in cells expressing Lifeact-GFP and NLS-mCherry, 25,000 transfected N1E-115 cells were replated on a glass-bottom, 24-well plate (MatTek) coated with 10 μ g/ml laminin. 4 h after plating, 10 selected fields of view per well were imaged in Neurobasal medium (Invitrogen) in a heated closed chamber using a CFI Plan Apo Lambda 10 \times (NA 0.45) objective (Nikon). Selection of fields of view was performed using Metamorph software. For that purpose, the GFP channel was acquired in a 7 \times 7 matrix of fields of view for each of the 24 wells. A macro was then used postacquisition to select 10 fields of view containing an adequate number of cells exhibiting a specific range in fluorescence intensities and cell areas. This ensured that each field of view displayed a similar number of cells and fluorescence intensities and excluded fields of view with cellular debris. The selected fields of view were automatically translated in a stage position list, which was used for the time-lapse experiment. This resulted in acquisition of 240 fields of view in GFP and mCherry channels throughout the 24-well plate every 12 min for a total of 19.6 h.

For imaging of F-actin and/or RhoA activation dynamics, N1E-115 cells were imaged using a Plan Apo VC 60 \times (NA 1.4) objective (Nikon) in Neurobasal medium (Invitrogen). Förster resonance energy transfer (FRET) imaging was performed as described before (Fritz et al., 2013). Ratio imaging analysis was performed with Biosensor Processing Software 2.1 (<http://lccb.hms.harvard.edu/software.html>). Images were thresholded on each channel; shade, background, and photobleach corrected; masked; and registered before ratios were calculated. Ratio images are color-coded so that warm and cold colors represent high and low biosensor activity, respectively.

For time-lapse imaging of neurite outgrowth dynamics in PC-12 Neuroscreen-1 cells, 80,000 transfected Neuroscreen-1 cells were replated on a glass-bottom, 24-well plate (MatTek) coated with 50 μ g/ml collagen solution from bovine skin (Sigma-Aldrich). 30 min after seeding, 50 ng/ml NGF was added to the medium to induce differentiation. After 6 h, selected fields of view per well were imaged in DMEM medium (Invitrogen) plus NGF in a heated closed chamber using a CFI Plan Apo Lambda 10 \times (NA 0.45) objective (Nikon).

Identification of a Rho GTPase signaling network. To identify a Rho GTPase signaling network, we took advantage of our recent proteomic analysis of purified neurite and soma fractions of N1E-115 cells (Pertz et al., 2008). This dataset provides spatial information about the subcellular localization (neurite/soma) for 4,855 proteins through the measurement of relative protein enrichment in purified neurite and soma fractions. Using Ingenuity Pathways Analysis (IPA) software, we first identified Rho GTPases expressed in our cell system (Rac1, Cdc42, RhoA, RhoB, RhoC, and RhoG). We then used these as “bait” to identify proteins that are documented to interact directly with these Rho GTPases by interrogating the Ingenuity Pathways Knowledge Base, which is a systemwide database of biological pathways created from multiple relationships of proteins, genes, and diseases. Only considering proteins identified in our proteomics screen to be neurite-enriched or equally distributed in neurite and soma fractions, we then manually sorted these proteins in GEFs, GAPs, RhoA effectors, Rac1 and Cdc42 effectors, and additional interacting proteins. In a second round, we then used these different protein groups as baits to identify additional interactors that might work upstream of GEFs and GAPs or downstream of effectors. This yielded a Rho GTPase signaling network of 219 proteins (Fig. 3 a), in which most proteins are significantly enriched in the neurite. We used Ingenuity analysis pathway software to perform a Gene Ontology analysis of the protein ensemble in this signaling network (Fig. 3 b). As expected, we observed enrichment of Rho GTPase, adhesion, cytoskeleton, and axonal Gene Ontology terms. Our approach reveals the existence of a neurite-enriched, Rho GTPase signaling network of unanticipated complexity.

Overview of CV analysis

We developed a CV pipeline to segment and track somata and neurites in time-lapse datasets. It extracts and stores representative models for each cell over time. The pipeline is summarized in Fig. 1 c. From these models, we extract features that are used to characterize the morphodynamic phenotype of the cell. The resulting data are stored in an HDS representing the cell (Fig. 2 b). For a cell detected at a specific time point, the top of the hierarchy represents the detected cell, with subcellular components nucleus, soma, and one or more neurite nodes (Fig. S3). The neurite is the trunk attached to the soma (Fig. 2 b, blue segment), and this can be followed by a tree structure of branches, which are any intersection points between neurite elements (Fig. 2 b, green segments), and leaf nodes, which are branches situated at the extremity of the neurite arborescence (Fig. 2 b, red segments). The CV algorithm first segments the nucleus and soma of each cell. Detected cells in each frame of the movie are linked using a graph-based tracking algorithm to identify individual cells over time. Next, neurites arbors are extracted for each detection by segmenting the full cell body and reconstructing tree structures corresponding to the neurites. Finally, detected neurites are tracked over time using a similar method as for the somata, and the tracked cell is stored in the HDS data structure. Validation of soma and neurite segmentation and tracking against human annotated ground truth is also shown.

Nucleus segmentation. We extracted a set of nucleus detections $\{d^1, \dots, d^k\}$ from the NLS-mCherry channel of every image (Fig. 1 c) using the MSER (Maximally Stable Extremal Regions) feature detector (Matas et al., 2004) from the VLFeat library followed by a morphological filling operation. The MSER detector is a robust method to find regions that are stable over a wide range of thresholds while remaining insensitive to contrast change. The minimum and maximum size parameters were fixed at 70 and 170; all other parameters used default settings.

Soma region growing. Using the segmented nuclei as seed regions, somata segmentation is accomplished using a region growing

and competition algorithm on the Lifeact-GFP channel (Fig. 1 c). For every pixel in the image, we compute the geodesic distance to each nucleus, combining the local intensity differences and the Euclidean distance. For each detected nucleus d^k , we defined a potential:

$$P_k(x) = \frac{1}{A \exp\left(\frac{(I(x) - \mu_k)^2}{2\beta^2 \sigma_k^2}\right) + 1},$$

where μ_k and σ_k are the mean and standard deviation of the intensities of the pixels describing d^k , respectively, and β is a multiplicative factor of σ_k representing a tolerance of variation between the local soma intensity and the local background. The geodesic distance U_k associated with nucleus d^k is defined such that it increases with geometric or intensity distance from the nucleus, as the solution of the Eikonal equation $\|\nabla U_k\| = P_k$ such that $U_k(d^k) = 0$. A distance map to all detected nuclei is given by $U(x) = \min_{k=1, \dots, K} U_{k(x)}$, which we approximate by computing the solution of $\|\nabla U\| = P$ such that $U(d^k) = 0$; for all k where

$$P(x) = \frac{1}{A \exp\left(\frac{(I(x) - \mu_k)^2}{2\beta^2 \sigma_k^2}\right) + 1}$$

and the index of the closest region k is decided during the propagation of a variant of the Fast Marching algorithm. The main advantage of this approach over the original (Benmansour and Cohen, 2009) is that the potential values are decided during the propagation. The somata segmentations s^k are obtained by thresholding $s^k = R^k \cap \{U < T_g\} \cap \{D < T_e\}$, where $\{R^k\}$ is the Voronoi tessellation associated with U the set of nuclei $\{d^k\}$, the Euclidean distance to the set of nuclei is denoted D , and the geodesic and Euclidean thresholds are denoted T_g and T_e , respectively. For all our experiments, we set parameter values $A = 1e7$, $\beta = 1.5$, $T_g = 2e - 6$, and $T_e = 7$.

Detection filtering. Cell detections $c_t^i = (d_t^i, s_t^i)$ are filtered before tracking is performed to retain the most reliable detections. Detections too close to the image boundary are ignored (distance from nucleus centroid < 10 pixels). The minimum tolerated nuclei circularity is 0.2, and the maximum is 0.85.

Cell tracking and identification. For each time-lapse sequence, an algorithm searches through the full set of cell detections $c_t^i = (d_t^i, s_t^i)$ and iteratively associates the most similar cells to track them over time (Fig. 1 c). We construct a graph $G = (C, E)$, where each node $c_t^i \in C$ corresponds to a detection. For each cell c_t^i in time step t , edges $e \in E$ are formed between c_t^i and all past and future detections within a time window W . A weight w_e measuring the similarity between each pair of cell detections is assigned to each edge. The weight takes into account spatial distance, temporal distance, and differences in shape between the two cells. The weight is given by $w_e = \alpha \|c_{t_1}^i - c_{t_2}^j\| + \beta |t_1 - t_2| + \gamma f(v_{t_1}^k, v_{t_2}^l)$, where $e^{k,l}$ connects $c_{t_1}^i$ and $c_{t_2}^j$, and v^k is a shape feature vector containing c_t^k 's area, perimeter, mean intensity, and major and minor axis lengths of a fitted ellipse. f evaluates differences between a feature a extracted from c_t^k and c_t^l as

$$f(a^k, a^l) = \left| \frac{a^k - a^l}{a^k + a^l} \right|.$$

According to this formulation, the tracking solution corresponds to the set of edges $E' \subset E$ that minimizes the cost $\sum_{e \in E'} w_e$. To perform the minimization, we use a graph association tracking algorithm that iteratively selects an edge with minimum cost and adds it to the tracking set until a cost threshold is reached (see below). The output of this algorithm is a set of lists of cell detections corresponding to the instantaneous location of each neuron over time. Using these lists, we assign identities to the detections belonging to each neuron.

Neurite segmentation and association. Neurites are detected for each neuron using a three-step process. First, the image is partitioned into regions, one assigned to each tracked cell. The probability of a pixel u being assigned a label $J_i(u)$ corresponding to neuron i is deduced using Bayes' rule:

$$P(J_i(u) = i | C_t, I_t) = \frac{P(C_t, I_t | J_i(u) = i)}{\sum_{\eta=1}^m P(C_t, I_t | J_i(u) = \eta)},$$

where I_t is the image at time t , $C_t = \{c_t^1 \dots c_t^m\}$ is the set of cells present in it, and $P(J_i(u))$ is a uniform distribution. The numerator is modeled as the probability of the path L that connects maximally the pixel u to the cell

$$c_t^i, P(C_t, I_t | J_i(u) = i) = \max_{L: u \rightarrow c_t^i} \prod_{l_r \in L} P(I_t(r) | l_r),$$

where l_r are indicator variables for the locations forming the path L . Optimal maxima can be found by minimizing the negative likelihood using geodesic shortest path, which produces a partitioning of the image into connected components assigned to each cell. Next, a tubularity measurement is computed for each pixel (Sethian et al., 1999) to identify likely neurites. Cells that were detected but not tracked have a suppressed tubularity value to avoid false positives on the cell boundary. A sigmoid function is fitted to the tubularity score using maximum likelihood to obtain a potential P_r . A front propagation Fast Marching algorithm is simultaneously launched from all cells (Benmansour and Cohen, 2009), which solves the Eikonal equation $\|\nabla U_i\| = P_r$ and $\|\nabla L_i\| = 1$. This yields a geodesic distance map U_i which serves as the cell body likelihood for each neuron (Fig. 1 c), the associated tessellation V_r , and the map of the Euclidean lengths L of the geodesics. The geodesic distance map is thresholded with a soft threshold T_s , yielding cell bodies for each neuron (Fig. 1 c). Candidate terminal points for the neurites are identified as the local maxima of L_i in each thresholded region. Candidate neurite tracings are found by back-propagating the geodesic distance U_i from the local maxima. Only those points for which $U_i > T_h$ is above a hard threshold T_h are retained. The idea is that even if the candidate endpoints detected in the previous step are not necessarily all correct, they will quickly converge to interesting elongated structures during the back propagation. Finally, a graph representing a each full neurite tree is constructed as a Minimum Spanning Tree of all back-propagated points, starting from a root touching the soma (Fig. 1 c). Neurites less than 30 pixels in length are removed.

Neurite tracking. Neurites are tracked using the same graph-association algorithm used to track cells. Instead of nucleus centroids, the positions of the centroids of the neurite trees are tracked. Edges connect neurites that emanate from the same cell. The weight w_e of an edge connecting two neurites N_i^j and N_i^l is assigned according to spatial distance and a shape measure:

$$w_e = w_{TCL} (TotalCableLength(N_i^j); TotalCableLength(N_i^l)) + w_{Centroid} \|Centroid(N_i^j) - Centroid(N_i^l)\| + w_{SomaContact} \|SomaContract(N_i^j) - SomaContract(N_i^l)\|,$$

where w_{TCL} , $w_{Centroid}$, and $w_{SomaContract}$ are the weights, $TotalCableLength(N)$ is the total cable length of a neurite N , $Centroid(N)$ is its centroid, $SomaContract(N)$ is its contact point with the soma, and

$$f(a^k, a^l) = \left| \frac{a^k - a^l}{a^k + a^l} \right|.$$

Unlike the weights used for cell tracking, the neurite weights ignore temporal distance because each neurite is already associated to a tracked cell body.

Graph association tracking. We construct a graph $G = (C, E)$ where each node $c_i^k \in C$ corresponds to a detection. For each cell c_i^k in time step t , edges $e \in E$ are formed between c_i^k and all past and future detections within a time window W . A weight w_e is assigned to each edge according to spatial and temporal distances, and a shape measure $w_e = \alpha \|c_i^k - c_j^l\| + \beta |t_1 - t_2| + \gamma f(v_i^k, v_j^l)$ where $e^{k,l}$ connects c_i^k and c_j^l , and v^k is a shape feature vector containing c_i^k 's area, perimeter, mean intensity, and major and minor axis lengths of a fitted ellipse. f evaluates differences between a feature a extracted from c_i^k and c_j^l as

$$f(a^k, a^l) = \left| \frac{a^k}{a^k + a^l} - \frac{a^l}{a^k + a^l} \right|.$$

To minimize this cost function, we adopt a greedy selection algorithm that iteratively selects an edge with minimum cost \hat{w}_e and adds it to the set E' , removing future and past connections from the detections $e^{k,l}$ connects. The algorithm iterates until the minimum cost \hat{w}_e is greater than a threshold T . The track for neuron i is extracted from E' by traversing the graph (C, E') and appending linked nucleus detections to X^i .

Algorithm 1. Greedy tracking association.

Start with an empty set E' .

- (1) Repeat. Find edge $\hat{e}^{k,l}$ with minimum cost \hat{w}_e . Add $\hat{e}^{k,l}$ to E' , linking detections d_{i1}^k and d_{i2}^l . Remove $\hat{e}^{k,l}$ from E .
- (2) If $t_1 < t_2$, then: remove edges between d_{i1}^k and future detections (where $t > t_1$) from E . Remove edges between d_{i2}^l and past detections (where $t < t_2$) from E .
- (3) Else, remove edges between d_{i1}^k and past detections (where $t < t_1$) from E . Remove edges between d_{i2}^l and future detections (where $t > t_2$) from E .
- (4) End if, until $\hat{w}_e > T$.

Soma segmentation validation. The ability of our method to segment nuclei and somata from static images was evaluated using a set of three hand-annotated movies selected randomly from the screen. The data were annotated according to the following protocol. First, annotators should label only cells that appear in the video for a sufficient amount of time (five frames). Second, they should label only cells with good fluorescent reporter expression. Finally, annotators should carefully trace the nucleus and soma of cells that fit this criterion. We then used our software to automatically segment the same cells that were annotated by experts. For the quality control analysis, the annotation and automatic segmentation areas in each frame are divided into regions and assigned a label from the following list: (a) OK: the nucleus/soma is correctly segmented; (b) FP (false positive): area of nucleus/soma segmentation that does not exist in the annotation; (c) FN (false negative): area of the nucleus/soma annotation that is not recovered by the segmentation.

The results of the validation can be found in Fig. S2 and Video 3. In total, 790 annotations outlining the nucleus and somata were collected from the movies and evaluated.

The automatic nucleus segmentation correctly recovered 94.5% of the area annotated as nuclei by the experts. 35.2% of the area of the segmentation fell outside of the annotation and was considered to be false positive. 5.5% of the area of the annotation was not recovered by the segmentation and was considered false negative. In general, the nucleus segmentation was very accurate but tuned to be slightly larger than the annotation, which explains the difference between the false-positive and false-negative rates.

The automatic soma segmentation correctly recovered 81.1% of the area annotated as soma by the experts. 19.1% of the segmentation area fell outside of the annotation, and was considered to be false positive. 18.9% of the area of the annotation was not recovered by the segmentation and was considered false negative.

Cell-tracking validation. To evaluate the ability of our method to track cells over time, we annotated 20 videos chosen randomly from the screen data. The data were annotated according to the following protocol. Any cell that our software attempted to track for more than five frames was labeled and added to the ground truth. Labeling a cell consisted of placing a bounding box around the soma for every frame in which the cell is present in the video. We compared these annotations to the soma segmentations recovered by our method using a standard tracking evaluation procedure (Smith et al., 2008). In each frame, every cell is assigned one or more states from the following list: (a) OK: the cell is correctly tracked; (b) FP (false positive): a cell was detected that does not exist in the annotation; FN (false negative): a cell appearing in the annotation was not detected; (c) MT (multiple tracker): the area of cell in the annotation was shared among multiple tracked cells; MO (multiple objects): the area of a tracked cell covered more than one annotated cells; (d) FI (false identification): an error was made in maintaining the identity of a cell over time (e.g., a detected cell label switched from one annotated cell to another).

A correct detection occurs if a labeled detection sufficiently overlaps the correct corresponding annotation. To establish correspondences between detections and annotations, we use the coverage test which checks the $F1$ -measure of the overlap between the area of the detection and the annotation:

$$F1 = \frac{2 * \frac{|d \cap a|}{d} * \frac{|d \cap a|}{a}}{\frac{|d \cap a|}{d} + \frac{|d \cap a|}{a}},$$

where $|d \cap a|$ is the intersection of the detection d with the annotation a , and $|\cap|$ is the cardinality operation. If the overlap is greater than a threshold $F1 > 0.33$, an association is made between the detection and the annotation.

The results of the validation can be found in Fig. S2 and Video 4. In total, 58,064 total cell detections were evaluated. The automatic tracking was correct in more than 90% of the cases. The most common errors were false detections (6.8%), followed by false identifications (2.6%). The remaining errors all occurred with <1% frequency.

Neurite validation. To evaluate the ability of our method to recover neurites, we compared automatic neurite detections against 128 hand-annotated cells selected randomly from the screen. Each annotation was performed using the TrackEM2 plugin for ImageJ (Schneider et al., 2012). The protocol for the evaluation is a slightly modified version of the TREES protocol (Cuntz et al., 2010), where a hard decision is made as to whether a pixel belonging to a neurite is correctly detected instead of a soft matching score. Each detected neurite pixel must fall within a specified distance ($\sigma = 4.5$ pixels) of an annotated neurite pixel to be counted as correct. If it is outside of this range, it is considered a false detection. If no neurite detection falls within the specified distance of an annotation, the annotation is considered a missed detection. The results of our evaluation show that the neurite missed detection rate is just below 30% (Fig. S2 m). The false detection rate is ~27%. The disagreement between the automatic method and the annotator might seem high, but it is in line with the disagreement between annotators (Fig. S2 n).

Overview of the statistical analysis

Features are extracted from the HDSs representing tracked cells and stored in a matrix format. Next, a feature profile is created for each siRNA, and the profiles for siRNAs corresponding to the same gene are combined to mitigate off-target effects and build a phenotypic signature for that gene. A robust subset of the complete set of collected features is selected and used for phenotypic clustering. A mathematical model

allows automatic selection of representative cells as prototypical examples of each phenocluster. Finally, we perform a temporal analysis comparing the evolution of various features characterizing cell behavior over time.

Definition of features describing soma and neurite morphodynamics. To generate a list of features that describe cell morphology and morphodynamics, we first defined a series of parameters that describe cell components, such as the nucleus, soma and neurite, at a given time point (see the following paragraphs). In a second step, these parameters are then used to compute static and dynamic features.

Definition of nuclei and soma parameters. Area: area of a nucleus or a soma. Perimeter: perimeter of a nucleus or a soma. Major axis length and minor axis length: an ellipse is fitted through the nucleus and the soma. Major axis A and a minor axis B of the ellipse are measured. Orientation: orientation of the major axis length A of the fitted ellipse (radians). Eccentricity: eccentricity of the fitted ellipse: $\sqrt{(a^2 - b^2)}/a$. Circularity: distance measured between a pure circle and the real shape of the nucleus or the soma.

Definition of neurite parameters. We modeled the neurite arborescence using different elements. A neurite is considered as the trunk of a tree. We define a tree root as the attachment point between the neurite and the soma, a branch point as any intersection point between neurite elements, and the extreme point as the extremities of the arborescence. The root is the neurite segment between the tree root and the first branch point, a branch is any elementary part of the tree, and a leaf is a branch situated at the extremity of the neurite arborescence. Using this notation, we extracted a series of parameters that describe the different components of a neurite, and that are shown below. A schematic representation of these parameters is shown in Fig. S3.

Extreme length: distances between the tree root and the extreme points. Total cable length: sum of the lengths of the root and the branches. Complexity: number of branches in the tree divided by the total cable length of the tree. Number of neurites/branches: number of roots or branches belonging to a cell. Branches length: total length of the branches of a cell. Leaf branches length: total length of the leaves of a cell.

Definition of static features. Using the nuclei, soma, and neurite parameters, we created a set of static features, which are described below. Nucleus area: nucleus area of the cell per frame, in pixels. Nucleus eccentricity: nucleus eccentricity of the cell per frame. Nucleus major axis length: nucleus major axis length of the cell per frame. Nucleus minor axis length: nucleus minor axis length of the cell per frame. Nucleus orientation: nucleus orientation of the cell per frame. Nucleus perimeter: nucleus perimeter of the cell per frame. Nucleus circularity: nucleus circularity of the cell per frame. Soma area: soma area of the cell per frame. Soma eccentricity: soma eccentricity of the cell per frame. Soma major axis length: soma major axis length of the cell per frame. Soma minor axis length: soma minor axis length of the cell per frame. Soma orientation: soma orientation of the cell per frame. Soma perimeter: soma perimeter of the cell per frame. Soma circularity: soma circularity of the cell per frame. Number of neurites: number of roots of the cell per frame. All neurites length branches mean: mean branch length of the cell per frame. All neurites leaf length branches mean: mean leaf length of the cell per frame. All neurites extreme length mean: mean extreme length of the cell per frame. Total cable lengths per neurite mean: mean neurite total cable length of the cell per frame. Complexity per neurite mean: mean neurite complexity of the cell per frame. Number of branches per neurite mean: mean number of branches per neurite of the cell per frame. Max extreme length per neurite mean: mean maximum extreme length per neurite of the cell per frame. Total neurites length: sum of the total cable length of each neurite of the cell per frame. Total neurites branches: sum of the num-

ber of branches of each neurite of the cell per frame. Total complexity: “total neurites branches” divided by “total neurites length” per frame. Distance traveled: distance traveled by the soma of the cell between two consecutive frames. Speed: instantaneous speed of the cell between two consecutive frames. Acceleration: instantaneous acceleration of the cell between two consecutive frames.

Definition of dynamic features. For the dynamic cell features, starting from the initial set of static features, we created five layers of dynamic cell features such as mean value of the feature through time, time a feature expands/contracts, number of inflection points in the feature evolution, and frequency of expansion (freq expansion) of a feature. Please note that to avoid redundancy, only a subset of dynamic features (freq expansion) was taken in account during the analysis of our siRNA screen.

Next, we provide the description of the four layers of dynamic cell features. Note that in the example we only listed the freq expansion features; however, the same set of features is also valid for the other four layers.

Let $F(t)$ be the feature value at time t and let $\Delta F(t) = F(t) - F(t - 1)$ be the feature difference between the two consecutive frames $t - 1$ and t .

Mean: The mean value of the feature through the entire movie. This gives rise to the following features. Time expanding: the number of times where $\Delta F(t)$ is positive, which is the number of times where the feature changes positively. Time contracting: number of times where $\Delta F(t)$ is negative which is the number of times where the feature changes negatively. Number inflection points: number of times where $\Delta F(t)$ switches from a positive value to a negative value and from a negative value to a positive value. Freq expansion: number of times where $\Delta F(t)$ is positive divided by the number of frames where the cell appears. This is the number of times per second where the feature changes positively.

This gives rise to the following features. Nucleus area freq expansion: number of times per second where the nucleus area of the cell changes positively. Nucleus eccentricity freq expansion: number of times per second where the nucleus eccentricity of the cell changes positively. Nucleus major axis length freq expansion: number of times per second where the nucleus major axis length neurites of the cell changes positively. Nucleus minor axis length freq expansion: number of times per second where the nucleus minor axis length neurites of the cell changes positively. Nucleus orientation freq expansion: number of times per second where the nucleus orientation neurites of the cell changes positively. Nucleus perimeter freq expansion: number of times per second where the nucleus perimeter of the cell changes positively. Nucleus Circularity Freq Expansion: Number of times per second where the nucleus circularity of the cell changes positively. Soma area freq expansion: number of times per second where the soma area of the cell changes positively. Soma eccentricity freq expansion: number of times per second where the soma eccentricity of the cell changes positively. Soma major axis length freq expansion: number of times per second where the soma major axis length of the cell changes positively. Soma minor axis length freq expansion: number of times per second where the soma minor axis length of the cell changes positively. Soma orientation freq expansion: number of times per second where the soma orientation of the cell changes positively. Soma perimeter freq expansion: number of times per second where the soma perimeter of the cell changes positively. Soma circularity freq expansion: number of times per second where the soma circularity of the cell changes positively. Number of neurites freq expansion: number of times per second where the number of neurites of the cell changes positively. Total neurites length freq expansion: number of times per second where the total neurites length of the cell changes positively. Total neurites branches

freq expansion: number of times per second where the total neurites branches of the cell changes positively. Total complexity freq expansion: number of times per second where the total complexity of the cell changes positively.

Data formatting. Data extracted by the segmentation and tracking steps are stored in a nonvectorial form, which must be converted into a matrix for statistical analysis. The analysis is performed on two groups of features: “population” features, which provide instantaneous characterizations of a cell or groups of cells; and “dynamic cell” features, which characterize a particular cell over time (Fig. 4 a). The population features account for several static shape measurements. The population tracking data structure takes the form $\text{movie}(m).\text{cell}(c).\text{time}(t).\text{feature}(f)$, where $m \in \{1, \dots, M\}$ is the movie index, $c \in \{1, \dots, C_m\}$ is the cell index, $t \in \{1, \dots, T\}$ is the time index, and $f \in \{1, \dots, F\}$ is the feature index. For analysis, it is aggregated and converted to a matrix with $T \sum_{m=1}^M \sum_{c=1}^{C_m}$ rows and F columns; i.e., one row per movie/cell/frame and one column per feature. The dynamic cell features capture both shape and c : $\text{feature}(f)$ is aggregated and organized into a matrix with $T \sum_{m=1}^M \sum_{c=1}^{C_m}$ rows and F columns; i.e., one row per movie/cell and one column per feature.

Single siRNA profiles. To characterize the phenotype associated with a perturbation, we create a profile for each siRNA using measurements extracted from the movies. Each siRNA profile measures whether the siRNA perturbation increased or decreased various measurements with respect to the control (Fig. 4 b). A siRNA profile $r = \{r_1, \dots, r_F\}$ is a vector of same dimension as the feature vector described above, where $r_f \in \mathbb{R}$ and F denotes the number of features. It is constructed by performing a z -test with 5% significance, comparing for each feature the perturbation population against the control population. If the statistical test indicates that a significant difference exists, the siRNA profile entry for that feature takes the value of the z -score. If the difference is insignificant, the profile takes a value of zero. More specifically, for a feature f the z -score is given by $z_f \in \mathbb{R}$ and $h_f \in \{0, 1\}$ is the result of the statistical test. If the mean of the two distributions are significantly different the result is $h_f = 1$, otherwise $h_f = 0$. The siRNA profile is given by $\forall f = 1, \dots, F, r_f = h_f z_f$, where r_f denotes a signed distance measure between the distribution of the control and the perturbed feature. If $r_f > 0$, the polarity is positive and the mean feature value is higher for the siRNA than for the control. If $r_f < 0$, the polarity is negative and the mean feature value is lower for the siRNA than for the control. If $r_f = 0$, the polarity is null and the mean feature value is the same for the siRNA and the control.

Morphodynamic signatures. To mitigate siRNA off-target effects, we create a gene-specific MDS by “averaging” the profiles of the three siRNAs targeting that gene according to the rules described below (Fig. 4 c). As previously proposed (Echeverri et al., 2006), if at least two of three siRNAs produce the same phenotype, this phenotype can be considered on target. We applied this rule to each independent feature, if the feature had the same polarity for two of three siRNAs (positive, negative, or null), it was considered on target. If all three siRNA polarities agree, the MDS score is the mean of the z -scores. If two of three siRNA polarities agree, the MDS is the mean z value of the two siRNAs profiles with the same polarity. In all other cases, we

set the z MDS score to zero. Formally, let $r^i = \{r_1^i, \dots, r_F^i\}$ be a siRNA profile, where F denotes the number of features, and i indexes the three siRNAs $i \in \{1, 2, 3\}$. Let $\epsilon \in \{-1, +1\}$ be a binary value and $N(\epsilon) = |\{i \text{ s.t. } \epsilon r^i > 0\}|$ be the number of siRNA perturbations resulting in a positive polarity ($\epsilon = 1$) or negative polarity ($\epsilon = -1$). The MDS $g = \{g_1, \dots, g_F\}$ is given by the mean siRNA profile of those that have the same polarity:

$$\forall f = 1, \dots, F, g_f = \begin{cases} \frac{1}{N(\epsilon^*)} \sum_{i \text{ s.t. } \epsilon^i r^i > 0} r_f^i & \text{if } N(\epsilon^*) > 1 \\ 0 & \text{otherwise} \end{cases},$$

where

$$\epsilon^* = \arg \max_{\epsilon} N(\epsilon).$$

For instance, for a given feature, if two siRNAs yield significantly positive z -scores and if the third siRNA molecule yields a negative z -score, then the feature f value of the gene profile is the mean z -score of the two first RNA molecules. In Table 1, we provide an example of how the MDS is computed from three siRNA feature profiles.

To determine whether a feature is robust, we checked whether the z -score of a feature for the positive MKK7 KN control is consistent across at least 65% (LS features) or 90% (HS features) for the 32 experimental plates (compared with the negative, nontargeting control). Formally, let F be the full set of collected features. We reduce this to a subset $\hat{F} = \{\hat{f}_1, \dots, \hat{f}_{\hat{P}}\} \subset F$ such that $f \in \hat{F}$ if, and only if:

$$\max \{ |\{p \text{ s.t. } r_f^p > 0\}|, |\{p \text{ s.t. } r_f^p < 0\}|, |\{p \text{ s.t. } r_f^p = 0\}| \} > PS,$$

where P is the number of plates, r_f^p is the siRNA profile of the MKK7 control in the plate p , and $S \in [0, 1]$ is a threshold value. By setting $S = 0.65$ (LS) and 0.9 (HS), we reduce the size of the feature space to 23 (LS) and 8 (HS) features.

Phenocluster identification. We used bottom-up hierarchical clustering (Bakal et al., 2007; Yin et al., 2013) to identify sets of genes that share common MDSs. To accomplish this, we first defined a distance metric $D(i, j)$ allowing us to compare pairs of MDSs g^i and g^j . We used a straightforward method based on the MDS polarity. The proposed distance depends on the signed distance between the gene profiles: $D(i, j) = D(\text{sign}(g^i), \text{sign}(g^j))$, where $\text{sign}(g^i) = \{\text{sign}(g_1^i), \dots, \text{sign}(g_F^i)\}$, $\text{sign}(g_j^i) = 0$ if $g_j^i = 0$, $\text{sign}(g_j^i) = 1$ if $g_j^i > 0$, and $\text{sign}(g_j^i) = -1$ if $g_j^i < 0$. In other words, two genes have a similar phenotype if their features have the same polarity. This can be computed by using the L_0 distance:

$$D(i, j) = \frac{1}{F} |\{f \text{ s.t. } \text{sign}(g_f^i) \neq \text{sign}(g_f^j)\}| = \frac{1}{F} \|\text{sign}(g_f^i) - \text{sign}(g_f^j)\|_0,$$

which gives a percentage of features that are matching between two genes. Evaluation of the distance between two clusters was performed by computing the L_0 distance between the two mean points of the clusters. We set the minimum number of genes per cluster to three, with the consequence that if a cluster is composed of fewer than three genes, we consider these genes as unclustered. The number of clusters also depends on the dendrogram

Table 1. An example of how the MDS is computed from three siRNA feature profiles

Profiles	1	2	3	4	5	6	7	8	9	10
siRNA1	0	0	0	0	0	-15	10	0	2	-12
siRNA2	0	0	0	5	8	8	8	-6	-11	-10
siRNA3	0	10	-5	-8	10	4	6	-4	-15	-8
MDS	0	0	0	0	9	6	8	-5	-13	-10

threshold, which gives the minimum percentage of matching features in each cluster. This percentage is directly connected to the L_0 distance. If this threshold is too high, there are few clusters; if the threshold is too low, the clusters are too noisy. We came to a compromise by iteratively testing different threshold values, visually evaluating the quality of both the clusters and the identified phenotypes by inspection of HDS graph representations of cells identified using a mathematical model to select penetrant phenotypes. The mathematical model is described below. We then chose the 60% (LS feature set) and 70% (HS feature set) threshold, implying that 60% or 70% of the features match in a given cluster.

Identification of phenocluster representative cells. For each phenocluster, we identified representative cells with a highly penetrant phenotype. We then provide static or time-lapse datasets using graph representations of the HDS extracted for these cells (Video 5). For that purpose, we first identified the single siRNA profile that has the highest similarity with the mean MDS. We then selected the single siRNA profile with the highest number of on-target features. We then selected cells with a highly penetrant phenotype. More specifically, for a given gene i , we looked for a cell $x_h^i \in \{x_1^i, \dots, x_N^i\}$, where $x_n^i \in \{x_{n,1}^i, \dots, x_{n,F}^i\}$, such that this cell has features strongly consistent with the centroid \bar{x} . The optimization formulation is:

$$\hat{n} = \arg \max_n \sum_{f=1}^F \frac{\text{sign}(g_f^i) x_{f,n}^i - x_f}{\sigma_f},$$

where σ_f is the standard deviation of the feature f (computed from all the data), x_f is the centroid value of the feature f for the control, $\text{sign}[g_f^i] = 1$ if the gene profile g_f^i is positive, and $\text{sign}[g_f^i] = -1$ otherwise. For this analysis, we focused on a subset of features that are easily visually interpretable. Specifically, we used the following features: number of neurites, total cable length per neurite mean, and number of branches.

Temporal analysis of neurite outgrowth dynamics. We used our large amount of time-lapse datasets of control and MKK7-KD cells (320 movies each acquired in 32 independent experiments) to analyze the morphodynamic behavior associated with specific temporal episodes during the neurite outgrowth process. For this, we divided each time-lapse series (of ~ 20 h) into 20 temporal bins and computed the mean of the feature value in each bin. To compare the temporal evolution of a feature, we normalized the feature value by its variance. Let $A = \{x_1, \dots, x_N\}$, where $x_n \in \mathbb{R}$, be the values of a feature for all the cells of a control. We denote by μ and σ^2 the mean and variance of A , respectively. Let $A^b = \{x_1^b, \dots, x_N^b\}$, where $x_n^b \in \mathbb{R}$ is a subset $A^b \subset A$ such that x_n^b is restricted in the temporal bin b . Similarly, we denote by μ_b and σ_b^2 the mean and variance of A^b , respectively. We normalized the data as follows:

$$x_n^b \leftarrow \frac{x_n^b - \mu_b}{\sigma_b}.$$

Additionally, we illustrated this temporal analysis by selecting representative cells in Fig. 5 (A and B). Each cell is automatically selected from a model. Let $X = \{X_1, \dots, X_F\}$ be a model for a targeted cell, where $X_f \in \mathbb{R}$ is chosen regarding the variation of μ_b in the analysis of Fig. 5 (A and B). The method consists of selecting the cell $x_h \in \{x_1, \dots, x_N\}$, where $x_n \in \mathbb{R}^F$, such that $\hat{n} = \arg \min_n \|x_n - X\|^2$. To filter the cells without neurite, we gated on the 10% cells with longest neurites.

Online supplemental material

Fig. S1 shows high-content live-cell imaging platform and RNAi pipeline. Fig. S2 shows an evaluation of the automatic soma and

neurite segmentation and soma tracking. Fig. S3 shows the definition of parameters for feature extraction. Fig. S4 shows PC-12 cell neuronal differentiation tracking and segmentation and hierarchical clustering of MDSs using the HS feature sets. Fig. S5 shows a summary of workflow and statistics of the RNAi screen. Video 1 is raw movies of Lifeact-GFP/NLS-mCherry channels in N1E-115 cells. Video 2 shows dynamic visualization of different steps in the computer vision pipeline. Video 3 shows nuclei and soma segmentation validation. Video 4 shows soma detection and tracking validation. Video 5 shows HDS-modeled graph representations of five global phenotypical classes. Video 6 shows HDS-modeled graph representations of MDSs of dynamic short neurite phenotypes in the LS map. Video 7 shows HDS-modeled graph representations of MDSs of dynamic long and branched neurite phenotypes in the LS map. Video 8 shows RhoA activation dynamics in advancing and collapsing growth cone in N1E-115 cells. Video 9 is high-resolution time-lapse imaging of *DLC1* and *ARHGAP5* KD growth cones in N1E-115 cells. Table S1 lists a description of all siRNAs in the RNAi library. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.201506018/DC1>.

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Author contributions: O. Pertz and K. Smith initially conceived the study; L. Fusco performed all experiments; K. Smith, F. Benmansour, G. Gonzalez, and P. Fua led the image segmentation effort; R. Lefort and F. Fleuret led the statistical analysis effort; C. Barillari and B. Rinn designed the OpenBis-based image database; O. Pertz and L. Fusco designed and interpreted the FRET validation experiments. O. Pertz, K. Smith, L. Fusco, and R. Lefort wrote the paper.

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Table S1. All siRNAs in the RNAi library

Sample lot	siRNA name	Sense sequence	Antisense sequence
154653A01	Arhgef1/MSS205912	GGAGAUUCUGCACCAUGUCAAUCAA	UUGAUUGACAUGGUGCAGAAUCUCC
154653A03	Arhgef1/MSS205911	GCGAGUGACCAAAGACAAAGCUAUA	UAUAGCUUUGUCUUUGGUCACUCGC
154653A05	Arhgef1/MSS205910	CCCUGUUCUCGAUCGCUUGAUGAA	UUCAUCAAGCGAUCGAGGAACAGGG
154653A07	Kalrn/MSS285208	CGGAACGCCGAGGUGUUCUCAAAU	AUUUGAGGAACACCUCGGCGUUCGG
154653A09	Kalrn/MSS285209	GAAACAGGAGAAUAUUACCUCUCAA	UUGAGAGGUAUAUUUCUCCUGUUUC
154653A11	Kalrn/MSS285210	GAUGGCUUCUCAGGGCGCAACUGCA	UGCAGUUGCGCCCUGAGAAGCCAUC
154653B01	Arhgef11/MSS277794	GGACCUGCUGGAGAGUGACAGUUCA	UGAACUGUCACUCUCCAGCAGGUCC
154653B03	Arhgef11/MSS277793	CACAUCCAGCUUACUUGGUAGUAUU	AAUACUACCAAGUAAGCUGGAUGUG
154653B05	Arhgef11/MSS210532	GCAAGGAGAGUCGAUUCAGCUCUU	AAGAGCUGGAAUCGACUCUCCUUGC
154653B07	Arhgef18/MSS272159	CACUAAAGAUCAUGCUGAAAGUAUA	UAUACUUUCAGCAUGAUCUUUAGUG
154653B09	Arhgef18/MSS200224	GCAGAUUCUCAGAUCCGCCAUGAGU	ACUCAUGGCCGAUCUGAGAAUCUGC
154653B11	Arhgef18/MSS200223	CCACUGUCCUGGAAUUAGAGCUGGU	ACCAGCUCUAAUUCAGGACAGUGG
154653C01	BC067047/MSS219653	GCCAUGAAGACUGUCUGCUCCAACA	UGUUGGAGCAGACAGUCUUAUGGC
154653C03	BC067047/MSS219654	CCAUGUCCACUACGAGUUCUCAA	UUUUGGAACUCGUAGUGGGACAUGG
154653C05	BC067047/MSS282431	CCUUCUCCUUUAAGCAGCUGGAUGA	UCAUCCAGCUGCUUAAAGGAGAAGG
154653C07	Rgnef/MSS201356	GGUAACCGACUUGGCAGUCUCUGAU	AUCAGAGACUGCCAAGUCGGUUACC
154653C09	Rgnef/MSS201357	GAGAUGUACGAAUCCACACCAAUU	AAUUGGUGUGGAUUUCGUACAUCUC
154653C11	Rgnef/MSS201358	CCAACAGAACUGAAGAUAGACAUUU	AAAUGUCUAUCUUCAGUUCUGUUGG
154653D01	Arhgef2/MSS205909	GGAUUCCAGCCAGAAGGAUCGGAU	AUUCGGAUCCUUCUGGCUGGAAUCC
154653D03	Arhgef2/MSS205908	ACCCGGAACUUUGUCAUCCAUCGUU	AACGAUGGAUGACAAAGUCCGGGU
154653D05	Arhgef2/MSS205907	CACUGCUUUGCAGUCUGUCUCCCUU	AAGGGAGACAGACUGCAAAGCAGUG
154653D07	Arhgef3/MSS230875	GGCUCAACUGUAUCCGUCAGGCAAA	UUUGCCUGACGGAUACAGUUGAGCC
154653D09	Arhgef3/MSS230874	CCAGCAGCACCUGGAAGAAGCUAUA	UAUAGCUUCUCCAGGUGCUGCUGG
154653D11	Arhgef3/MSS230873	GAGAAAUCCUGAGGCAUACACCAA	UUUGGUGUAUGCCUCAGGAUUUCUC
154653E01	Sos1/MSS209264	GAAAGGAUAUAUUACGCCUGUGCA	UGCACAGGCUGAAUAUAUCCUUUC
154653E03	Sos1/MSS209266	GAGUUCAUAAUGGAAGGAACUCUUA	UAAGAGUCCUUCUUAUGAACUC
154653E05	Sos1/MSS277177	CCUCCCACAGUUGAGUGGCACAUAA	UUUUGGCCACUCAACUGUGGGAGG
154653E07	Swap70/MSS277502	CAAUCAGGGCUACAUGCCAUUUUA	UAAUAUAGGCAUGUAGCCCUGAUUG
154653E09	Swap70/MSS277503	CAGAGGACAAGUAUCCACUAAUUAU	AUAAUUAGUGGAUACUUGUCCUCUG
154653E11	Swap70/MSS277504	CAGAAGAGAUUGAAUACCUGCUUAA	UUUAGCAGGAUUAUACUUCUUCUG
154653F01	Arhgef7/MSS285069	GAGUUCAGCAAACACUUCAUUUUA	UAAUAUAGAAGUGUUUGCUGAACUC
154653F03	Arhgef7/MSS225490	CCAACUGGUAGUACGAGCCAAGUUU	AAACUUGGCUCGUACUACCAGUUGG
154653F05	Arhgef7/MSS225488	GAGGACCUAGGAGAGUUCUUGGAAA	UUUCCAUGAACUCUCCUAGGUCCUC
154653F07	Def6/MSS215619	AGGAGCUGCUCAAGUCUUAUCUGGUA	UACCAGAUAGACUUGAGCAGCUCCU
154653F09	Def6/MSS280278	GCUUUCGUCUUAUGGUGCCUCUUA	UGAAGAGGCACCAUAGACGGAAGC
154653F11	Def6/MSS215620	UGCAUCCGAUCGAGCCAGGAGAUAA	UUUUCUCCUGGCUCGAUCGGAUGCA
154653G01	Trio/MSS278694	CAGCGUGCCCAAGCGAGCCAAUGAU	AUCAUUGGCUCGCUUGGGCACGCUG
154653G03	Trio/MSS278695	CCUCAGGAGGCUCAUUUCCUUAUUUA	UAAUAAGGAAAUGAGCCUCCUGAGG
154653G05	Trio/MSS278696	GAGAAAGCUGUAGAAGUCAUGUGCA	UGCACAUGACUUCUACAGCUUUCUC
154653G07	Tsc1/MSS227144	GGAGGAACACAAUGCAGCAAUGAAA	UUUCAUUGCUGCAUUGUGUCCUCC
154653G09	Tsc1/MSS227145	UGGAACUGGAGAUCUAGACUUGUA	UACAAGUCUAGGAUCUCCAGUCCA

154653G11	Tsc1/MSS227146	GACACCAUGGUGACCCAACUGCAUA	UAUGCAGUUGGGUCACCAUGGUGUC
154653H01	Dock10/MSS210019	CCAGGAAGAACUGAGGUCCCAUUAU	AUAAUGGGACCUCAGUUCUCCUGG
154653H03	Dock10/MSS210018	GCACAGGUCUCAGACUUUACCUAUA	UAUAGGUAAAAGUCUGAGACCUGUC
154653H05	Dock10/MSS210017	CCAGAGGUGUCAGACUUCUUCAGCA	UGCUGAAGAAGUCUGACACCUCUGG
154653H07	Vav2/MSS238640	GACAAAUGGGCGGAUCGGUUGGUUU	AAACCAACCGAUCCGCCCAUUUGUC
154653H09	Vav2/MSS238641	CCUCAAGUCUGCCAUGACAAAUUU	AAAUUUGUCAUGGCAGACUUUGAGG
154653H11	Vav2/MSS238642	GGUACAACUUUGCUGCUCGAGACAU	AUGUCUCGAGCAGCAAAGUUGUACC
154654A01	Dock4/MSS280225	CAGAUAGACAUUAUCAGCAACUUCU	AGAAGUUGCUGAUAAUGUCUAUCUG
154654A03	Dock4/MSS280226	GAGCAAAGAAGGAGUGGCAACUUUA	UAAAGUUGCCACUCCUUCUUUGCUC
154654A05	Dock4/MSS280227	UAUCACCGAGAUGACAUAACUUUA	UAAAGUUGAUGUCAUCUCGGUGAUA
154654A07	Dock7/MSS228219	CCACGGUACUCUGACCCUCAGAUAA	UUAUCUGAGGGUCAGAGUACCGUGG
154654A09	Dock7/MSS289303	CCGUAGUCCGGUUUCUUCUUCUUCU	AGAAGAUGAAGAAACCGGACUACGG
154654A11	Dock7/MSS289304	CGUCAAUUAUAAAUCGACUUCACAA	UUGUGAAGUCGAUUUAUAAUUGACG
154654B01	Vav3/MSS226475	GGUGGGCUGGUUCCAUCACAUUAU	AUAUGUGGAUGGAAACCAGCCCACC
154654B03	Vav3/MSS226477	GGGAGCUCUCCCUAUUGAAAGGAGA	UCUCCUUCAAUAGGGAGAGCUCCC
154654B05	Vav3/MSS285951	GGAAAGAUUGCAGGCAGAAACUGAA	UUCAGUUUCUGCCUGCAAUCUUUCC
154654B07	Centd2/MSS229844	GGCCUGUCAAGAGCCUCAAGUCUA	UAGACUUUGAGGCUCUUGACAGGCC
154654B09	Centd2/MSS229845	UCCACGAUCGCUACUUAUUCUCAA	UUGAGAAUGAAGUAGCGAUCGUGGA
154654B11	Centd2/MSS290899	AGGUGGAGACUGAACAGCAUGUUA	UUAACAUGCUGUUCAGUCUCCACCU
154654C01	Dock9/MSS200569	GCAUGCUCAGAACCUGCUGAUAAA	UUUAUCAGCAGGUUCUUGAGCAUGC
154654C03	Dock9/MSS272388	GAUCUCCAGCUUGACUACUCCUUA	UUAAGGAGUAGUCAAGCUGGAGAUC
154654C05	Dock9/MSS272389	UGGAUAUGAUCAAACUGCAGCUCAA	UUGAGCUGCAGUUUGAUCAUAUCCA
154654C07	Ect2/MSS203768	CCUUCCAUCGAACCUCAAAGAAA	UUUCUUUGAAGGUUCGAAUGGAAGG
154654C09	Ect2/MSS203769	GGGAUGAAAGGUGCACUCACCUUAU	AUAAGGUGAGUGCACCUUUAUCCC
154654C11	Ect2/MSS274062	CAGAUUAUCUUUGAUGUGCAUAUGAA	UUCAUAUGCACAUCAAAGAUUUCUG
154654D01	Arhgap1/MSS238961	GCUAAAGAUGCUGCCAUCACCCUCA	UGAGGGUGAUGGCAGCAUCUUUAGC
154654D03	Arhgap1/MSS238962	CCAACACUAACUUGGCUGUAGUCUU	AAGACUACAGCCAAGUUAGUGUUGG
154654D05	Arhgap1/MSS238963	UGACACAGCAGGUUCUCCAAACUCU	AGAGUUUGGAGAACCUGCUGUGUCA
154654D07	Arhgap10/MSS234252	GCGAGGGCUGAUUCCACAGAAUUAU	AUAAUUCUGUGGAAUCAGCCCUCGC
154654D09	Arhgap10/MSS234253	ACAUUCAGAAUACACGGAAUCGAUU	AAUCGAUUCGUGUAUUCUGAAUGU
154654D11	Arhgap10/MSS294204	CAAGUCACUCGAAGCAGAAUCUAAU	AUUAGAUUCUGCUUCGAGUGACUUG
154654E01	Fgd2/MSS218495	CCUGCUACACAUUUCUCACUGGAAA	UUUCCAGUGAGAAAUGUGUAGCAGG
154654E03	Fgd2/MSS218496	GACCCAAUGGAACGCUACCUGGUUU	AAACCAGGUAGCGUCCAUUGGGUC
154654E05	Fgd2/MSS218497	CCCUACUGGGCUAUCAGGUGAUUUC	GAAAUACCCUGAUAGCCCAGUAGGG
154654E07	Itsn1/MSS275155	CAGCUGGCUUCAUAUGGAAUCUUU	AAAGAUUCCAUAUUGAAGCCAGCUG
154654E09	Itsn1/MSS275156	CAGAAGAAUUUAUCCUAGCUAUGCA	UGCAUAGCUAGGAUAAAUUCUUCUG
154654E11	Itsn1/MSS275157	CAGGCAAGAACUAUUCUCAUGCAAU	AUUGCAUGAGAAUAGUUCUUGCCUG
154654F01	Arhgap17/MSS230246	CCUGGAGAGGUAGAAUUCUAUGUAU	AUACAUUGAAUUCUACCUCUCCAGG
154654F03	Arhgap17/MSS230247	ACUGUGCGUUCAAUGUGCCACCAUU	AAUGGUGGCACAUUGAACGCACAGU
154654F05	Arhgap17/MSS230248	CAACUUGCAGCAGACAUGUACAACU	AGUUGUACAUGUCUGCUGCAAGUUG
154654F07	Arhgap21/MSS230777	CCAUCCCAAGGAAUGGAGAUUAAA	UUUAUAUCUCCAUCCUUGGGAUGG
154654F09	Arhgap21/MSS230778	CCACCACAGUCACCUGACCAAAUAA	UUUUUUGGUCAGGUGACUGUGGUGG

154654F11	Arhgap21/MSS230779	ACGAUGCACGCAGAGAGGUCCAUAU	AUAUGGACCUCUCUGCGUGCAUCGU
154654G01	Arhgap26/MSS230710	ACAAAUUAUCAAGGAGCUCAUCAA	UUGAUGAGCUCCUUGAUGAAUUUGU
154654G03	Arhgap26/MSS291522	CAGGAGCAGAGGAACAGCAUCAUCA	UGAUGAUGCUGUUCCUCUGCUCCUG
154654G05	Arhgap26/MSS291523	CGGGCACCGUCUUCGAUAAUGUUCA	UGAACAUUAUCGAAGACGGUGCCCG
154654G07	Rhog/MSS225960	CCUCCUAUGAGAACGUGAGGCACAA	UUGUGCCUCACGUUCUCAUAGGAGG
154654G09	Rhog/MSS225961	ACCCUCAGACCAACGUCUUCGUCAU	AUGACGAAGACGUUGGUCUGAGGGU
154654G11	Rhog/MSS285643	CCUGAGAGCCCAGCCUGAUACCCUA	UAGGGUAUCAGGCUGGGCUCUCAGG
154654H01	Arhgap30/MSS213146	CCACAGAUECCGACCCUACCAUACUA	UAGUAUGGUAGGGUCGGAUCUGUGG
154654H03	Arhgap30/MSS213147	GGAGGUUCGAGUGCAGUCCAUAUGUU	AACAAUGGACUGCACUCGAACCUCC
154654H05	Arhgap30/MSS213148	CCUGUCUCUGGAUGAUACUCAGUAU	AUACUGAGUAUCAUCCAGAGACAGG
154654H07	Arhgap5/MSS202178	UCGAGAGUUUCUGUCUACCACUAAA	UUUAGUGGUAGACAGAAACUCUCGA
154654H09	Arhgap5/MSS273270	GCGAUCCAUUUAGUGUGGAUCUCAU	AUGAGAUCACACUAAAUGGAUCGC
154654H11	Arhgap5/MSS273271	GCCGGAUCCUUUGAUUCCAUAUUCA	UGAAUAUGGAAUCAAGGAUCCGGC
154656A01	Arhgdia/MSS208259	GAAGGAAGGUGUGGAGUACCGGAUA	UAUCCGGUACUCCACACCUUCCUUC
154656A03	Arhgdia/MSS208260	GGAGAGCUUCAAGAAACAGUCGUUU	AAACGACUGUUUCUUGAAGCUCUCC
154656A05	Arhgdia/MSS208261	GACUGACUACAUGGUCGGGAGCUAU	AUAGCUCCCGACCAUGUAGUCAGUC
154656A07	Crmp1/MSS203237	CCCGGUGGCAUUGAUGUCAACACUU	AAGUGUUGACAUCAAUGCCACCGGG
154656A09	Crmp1/MSS203238	CCAGGAAGAAAGGCCCUUUGUCUU	AAGACAAGAGGGCCUUCUUCUGG
154656A11	Crmp1/MSS203239	ACUGUCCCUACAGUACUGCUCAGAA	UUCUGAGCAGUACUGUAGGGACAGU
154656B01	Bcr/MSS201314	GGACUGUCAUCGACAUGAAUGGAU	AUUCCAUAUCUGUCGAUGACAGUCC
154656B03	Bcr/MSS201315	CCCAGACCCUGCGGAUACUAUGUUA	UAACAUAGUAUCCGCAGGGUCUGGG
154656B05	Bcr/MSS272842	CCAGGCACUGAAGGCAGCCUUUGAU	AUCAAAAGGCUGCCUUCAGUGCCUGG
154656B07	Chn2/MSS245507	CCCUGAAACUGUACUUCGAGACUU	AAGUCUCGGAAGUACAGUUUCAGGG
154656B09	Chn2/MSS245508	CCAAUGACUGCCAGCCAGAUCUCAA	UUGAGAUCUGGCUGGCAGUCAUUGG
154656B11	Chn2/MSS245509	GCGCUACCUGAUGAUCCACCUGAAA	UUUCAGGUGGAUCAUCAGGUAGCGC
154656C01	Diap1/MSS203555	UGCCACUGACGAGAAGGACAAGUUU	AAACUUGUCCUUCUCGUCAGUGGCA
154656C03	Diap1/MSS203556	GCCAUCCUCUUAAGCUACAGUUCA	UGAACUGUAGCUUGAAGAGGAUGGC
154656C05	Diap1/MSS203557	ACCUGCAGAAGAGCUUAGAUCAGAU	AUCUGAUCUAAGCUCUUCUGCAGGU
154656C07	Dpysl2/MSS203240	CCAACCACUCCAGACUUUCUCAACU	AGUUGAGAAAGUCUGGAGUGGUUGG
154656C09	Dpysl2/MSS203241	CCAGCUGACGGAUUCCCAGAUCAUAU	AUAGAUCUGGGAAUCCGUCAGCUGG
154656C11	Dpysl2/MSS203242	GACUCCAGCCUCAUCAGCUAAGACA	UGUCUUAGCUGAUGAGGCUGGAGUC
154656D01	Dlc1/MSS224945	GCUUAUGAUGUGGCAGACAUGUUA	UUAACAUGUCUGCCACAUCAUAAGC
154656D03	Dlc1/MSS224946	GCAGCCGCCUGAGCAUCUAUGAUAA	UUAUCAUAGAUGCUCAGGCGGCUGC
154656D05	Dlc1/MSS224947	GGUUGAUCAUCAGUGCUCCCAUAUCU	AGAAUGGGAGCACUGAUGAUCAACC
154656D07	Ralbp1/MSS208646	UGCAGAUCAAGCAUCCGGGUCCUGUA	UACAGGACCCGAUUGCUGAUCUGCA
154656D09	Ralbp1/MSS208647	GGGCGGGGAUAAAGGACUUAUCUAAA	UUUAGAUAAGUCCUUUAUCCCGCCC
154656D11	Ralbp1/MSS208648	GAGAUCAAGGAGACAGGAGUUCUUU	AAAGGAACUCCUGUCUCCUGAUCUC
155026A03	Pkn1/MSS241556	CCUAUCUGCUCAUCCACCAACCUGA	UCAGGUUGGUGGAUGAGCAGAUAGG
155026A04	Pkn1/MSS282993	CAGUCCCAAUGCAUCUCCAGGUGCU	AGCACCUGGAGAUGCAUUGGGACUG
154656E05	Pkn1/MSS282994	GAGCUGCCUUCAGAGACCCAGGAGA	UCUCCUGGGUCUCUGAAGGCAGCUC
154656E07	Pkn2/MSS201191	GGAUAAUUGUUGCUAGAUACAGAA	UUCUGUAUCUAGCAACAAGUUAUCC
154656E09	Pkn2/MSS201193	CACUAGAGCAAAGGUUAAAUGAGCU	AGCUCAUUUAACCUUUGCUCUAGUG

154656E11	Pkn2/MSS272752	CCGUCCAGACCAAUGAGCUGGCUUU	AAAGCCAGCUCAUUGGUCUGGACGG
154656F01	Srgap2/MSS204395	ACAUGAAGGUCUGGAUGCUAUUGAA	UUCAAUAGCAUCCAGACCUUCAUGU
154656F03	Srgap2/MSS204396	UCACAAGUAGAAGUGAACGACAUAA	UUAUGUCGUUCACUUCUACUUGUGA
154656F05	Srgap2/MSS274401	GAAGCACCAAGGACCAGCAAUUUAA	UUAAAUUGCUGGUCCUUGGUGCUUC
154656F07	Rock1/MSS208676	GCUGCUCAGCAAAUUUGAAUUGAUU	AAUCAUUUCAAUUUGCUGAGCAGC
154656F09	Rock1/MSS208677	GCACGCCUAACUGACAAGCACCAAU	AUUGGUGCUUGUCAGUUAGGCGUGC
154656F11	Rock1/MSS276868	CAGAAAUGCAGAACUUCAAACUJAA	UUAAGUUUGAAGUUCUGCAUUUCUG
154656G01	Srgap3/MSS281821	CCGUGAACUGUUGGUUUCUGGUACU	AGUACCAGAUACCAACAGUUCACGG
154656G03	Srgap3/MSS281822	CACGAUGUCUCUGAUUCUCAUGAUU	AAUCGAUGAGAUACAGAGACAUCGUG
154656G05	Srgap3/MSS281823	GAGAGCCAACCAGCAGGAAACAGAA	UUCUGUUUCCUGCUGGUUGGCUCUC
154656G07	Cdc42/MSS247082	CCUUUCUUGCUUGUUGGGACCCAAA	UUUGGGUCCCAACAAGCAAGAAAGG
154656G09	Cdc42/MSS247084	GGUGCCUGAGAUAAUCUACCACUGU	ACAGUGGUGAGUUUUCUCAGGCACC
154656G11	Cdc42/MSS273586	CAAGAACAAACAGAAGCCUAAUJACU	AGUAAUAGGCUUCUGUUUGUUCUUG
154656H01	Rock2/MSS208679	CCGGACCCAUGGAUCAGAGAUAAUU	AAUUAUCUCUGAUCCAUGGGUCCGG
154656H03	Rock2/MSS208680	CCUCGUCAACCUUAUGAGUAAUUAU	AUAAUUACUCAUAAGGUUGACGAGG
154656H05	Rock2/MSS208681	GCAGGAAACUCAGAAGCGUUGUCUU	AAGACAACGCUUCUGAGUUUCCUGC
154656H07	Rtkn/MSS208739	CCACUGUUCACUAUCGUCAUCAACA	UGUUGAUGACGAUAGUGAACAGUGG
154656H09	Rtkn/MSS208740	CCCAAUCCAGAGGUUUCUACAGCA	UGCUGUAGAAACCUCUGGAUUUGGG
154656H11	Rtkn/MSS208741	GCGCUUUGGCAGCUCUUCUUUGACA	UGUCAAGAAGAGCUGCCAAAGCGC
154657A01	Rac1/MSS237707	CCGCAGACAGACGUGUUCUUAAUUU	AAAUUAAGAACACGUCUGUCUGCGG
154657A03	Rac1/MSS237708	GGCGAAAGAGAUCCGUGCUGUCAA	UUUGACAGCACCGAUCUCUUUCGCC
154657A05	Rac1/MSS237709	GCCUGCUCAUACAGUUACACGACCAA	UUGGUCGUGUAACUGAUGAGCAGGC
154657A07	RhoA/NM_016802.4_stealth_3	CAUGCUCACGCGUCUGCCAUGAUU	AAUCAUGGCAGACGCGUGGAGCAUG
154657A09	RhoA/NM_016802.4_stealth_46	CAGGGCGUGGAUGCGUUCUUGAGCA	UGCUCAAGAACGCAUCCACGCCUCUG
154657A11	RhoA/NM_016802.4_stealth_652	GACACCGAUGUUUAUUAUGAUGUGU	AACACAUCAAUAUAACAUCGGUGUC
154657B01	Abi1/MSS272917	CAAAGCAUAUACAACUCAAUUCUA	UAGAGAUUGAGUUGUAUAUGCUUUG
154657B03	Abi1/MSS272918	CAUUGGCCAACAAUGUGCUCCAGCU	AGCUGGAGCACAUUGUUGGCCAAUG
154657B05	Abi1/MSS272919	UGGAUGAUGUGGGCCAUGGAGUUAA	UUAACUCCAUGGCCCACAUCAUCCA
154657B07	Arfp2/MSS233820	CCAGAAACAGCUGGAGCAGACUCUU	AAGAGUCUGCUCCAGCUGUUUCUGG
154657B09	Arfp2/MSS233821	UGAUGCACAAGCAGCUGCUGCUCUU	AAGAGCAGCAGCUGCUUGUGCAUCA
154657B11	Arfp2/MSS293819	CCCGGACUGUGGAUCUGGAGCUAGA	UCUAGCUCCAGAUCCACAGUCCGGG
154657C01	Rhob/MSS247025	GCAUCAACUGCUGCAAGGUGCUAUG	CAUAGCACCUUGCAGCAGUUGAUGC
154657C03	Rhob/MSS247026	ACACCGACGUCAUCCUUAUGUGCUU	AAGCACAUAAAGGAUGACGUCGGUGU
154657C05	Rhob/MSS247027	GCAUCCAAGCCUAUGACUACCUCGA	UCGAGGUAGUCAUAGGCUUGGAUGC
154657C07	Rhoc/MSS235982	GAGAGCUGGCCAAGAUGAAACAGGA	UCCUGUUUCAUCUUGGCCAGCUCUC
154657C09	Rhoc/MSS235984	UGAGAACUAUAUAGCCGACAUCGAA	UUCGAUGUCGGCUAUAUAGUUCUCA
154657C11	Rhoc/MSS273269	GCGGGCCUACAGGUCCGGAAGAAUA	UAUUCUUCGCGACCUGUAGGCCCGC
154657D01	Baiap2/MSS235629	GAUGCAACAGAUGGCCAAUAGCAAU	AUUGCUAUUUGCCAUCUGUUGCAUC
154657D03	Baiap2/MSS235630	GGCCAAGAACUCUGCUGCUUACCAU	AUGGUAAGCAGCAGAGUUCUUGGCC
154657D05	Baiap2/MSS272620	CAGUGCCACCUGAGCUGGCACCAUU	AAUGGUGCCAGCUCAGGUGGCACUG
154657D07	Cdc42bpb/MSS211616	GGGCAGAAUGGAAUAGAAGACUUUA	UAAAGUCUUCUAUCCAUCUGCCC
154657D09	Cdc42bpb/MSS211618	GCACAAGCAAUUGGUUGAGGCUUCA	UGAAGCCUCAACCAAUUGCUUGUGC

154657D11	Cdc42bpb/MSS278290	CAAACUGAAGGAAUCGGAAGCGAAA	UUUCGCUUCCGAUUCUUCAGUUUG
154657E01	Cdc42SE2/NM_178626.3_stealth_559	UGAGUGAAUUCUGGUUGUGUUUCAA	UUGAAACACAACCAGAAUUCACUCA
154657E03	Cdc42SE2/NM_178626.3_stealth_564	GAAUUCUGGUUGUGUUUCAACUGCU	AGCAGUUGAAACACAACCAGAAUUC
154657E05	Cdc42SE2/NM_178626.3_stealth_570	UGGUUGUGUUUCAACUGCUGUAUUG	CAAUACAGCAGUUGAAACACAACCA
154657E07	Pak4/MSS245585	GCAAAGACGUGAACUGCUCUCAAU	AUUGAAGAGCAGUUCACGUCUUUGC
154657E09	Pak4/MSS245586	ACCGUGACAUCAAGAGUGACUCUAU	AUAGAGUCACUCUUGAUGUCACGGU
154657E11	Pak4/MSS291252	AACUAGCAGCUGGCAGGCCUUUAA	UUAAAGGGCCUGCCAGCUGCUAGUU
154657F01	Clip1/MSS226157	CCGAAGAAGGCUAGAGUCCAGUAAA	UUUACUGGACUCUAGCCUUCUUCGG
154657F03	Clip1/MSS226158	GGAGCUGAAGGUGUCCUUUAGCAAA	UUUGCUAAAAGGACACCUUCAGCUCC
154657F05	Clip1/MSS226159	GGCCAAGCUGAUGAAGAUCAUUAAA	UUUAAUGAUCUUCUUCAGCUUGGCC
154657F07	Copg/MSS249938	GGGUCUUUAACGAAACUCCCAUCAA	UUGAUGGGAGUUUCGUUAAAGACCC
154657F09	Copg/MSS249939	ACCACGGAAGCAACUGAGGCUUUCU	AGAAAGCCUCAGUUGCUUCCGUGGU
154657F11	Copg/MSS249940	UCCUGCAUCGCUGAGGAUGUCAUCA	UGAUGACAUCUCAGCGAUGCAGGA
154657G01	Pard3/MSS234950	CGACAGCUGGCUUUCUCAAGCAGAA	UUCUGCUUGAGAAAGCCAGCUGUCG
154657G03	Pard3/MSS294614	GACCCAGCUUUAACUGGCCUUUCCA	UGGAAAGGCCAGUUAAGCUGGGUC
154657G05	Pard3/MSS294615	CGCAGGAUCUCACACUCCUCUACA	UGUAGAGGGAGUGUGAGAUCUGCG
154657G07	Pip5k1a/MSS237569	GGGCUUCUCAGAAAAGACGAGAGAA	UUCUCUCGUUCUUUCUGAGAAGCCC
154657G09	Pip5k1a/MSS237570	GGCGCUCUAUUCCACAGCUAUGGAA	UUCCAUAGCUGUGGAAUAGAGCGCC
154657G11	Pip5k1a/MSS237571	CCUCAGACUCCACCUUUGGAGGAAA	UUUCCUCCAAAGGUGGAGUCUGAGG
154657H01	Cyfp1/MSS208988	CCAACCGGUUUGUUCGGACAGUAUU	AAUACUGUCCGAACAAACCGGUUGG
154657H03	Cyfp1/MSS208989	GGCAAUUGGACGGUUUGAAAGUGAA	UUCACUUCAAACCGUCCA AUUGCC
154657H05	Cyfp1/MSS277058	CAGGAUGUACUUGACACCCAGCGAA	UUCGCUGGGUGUCAAGUACAUCUG
154657H07	Cyfp2/MSS233795	CCAUCCUGUUCUGCCUCCUCAUAGA	UCUAUGAGGAGGCAGAACAGGAUGG
154657H09	Cyfp2/MSS233796	GCCAUGUUCGGAGAAGCCAUCAUA	UAUGAUUGGCUUCUCGGAACAUGGC
154657H11	Cyfp2/MSS233797	ACAACAUCUGUGAGCAGAUGGUUCA	UGAACCAUCUGCUCACAGAUGUUGU
154658A01	Pip5k3/MSS207627	GCCUUCAUCAAAAGAGUCCUUAUUUA	UAAUAAGGACUCUUUGAUGAAGGC
154658A03	Pip5k3/MSS207628	UGGCAGUGUUCAGGCCAAGUCUAUU	AAUAGACUUGGCCUGAACACUGCCA
154658A05	Pip5k3/MSS276330	CCGAAGAUGAUAGAUUCAUUCUGAA	UUCAGAAUGAAUCUAUCAUCUUCGG
154658A07	Prkci/MSS207677	GGACAAUGUACUGCUAGACUCUGAA	UUCAGAGUCUAGCAGUACA UUGUCC
154658A09	Prkci/MSS207678	GCAUCCCGGUUCUCUGUCUGUAAA	UUUACAGACAGAGAACGCGGGAUGC
154658A11	Prkci/MSS207679	CCAAAGUACUGUUGGUUCGGUUAUU	UUUAACCGAACCAACAGUACUUGG
154658B01	Fmnl1/MSS226608	UCCGACUUCAGAGUCUGGAUGCGUU	AACGCAUCCAGACUCUGAAGUCGGA
154658B03	Fmnl1/MSS226609	GGAAACAGACGUUGUUGCACUACUU	AAGUAGUGCAACAACGUCUGUUUCC
154658B05	Fmnl1/MSS286071	CAGAGACUUCGGAGCGCAUGAACA	UGUUCAUGCGCUCGGAGUCUCUG
154658B07	Fnbp1l/MSS210755	GGUAAUUCUCAGAUUUACAAGCAA	UUGCUUGUAAAUCUGAGGAAUJACC
154658B09	Fnbp1l/MSS210756	UGAGGCAUGGCUCUCUGAAGUUGAA	UUCAACUUCAGAGAGCCAUGCCUCA
154658B11	Fnbp1l/MSS210757	GCAGUAAAGGUGCAGUAACUUAUUAU	AUAUAAGUUAACUGCACCUUUACUGC
154658C01	Trip10/MSS246881	GCUGAGCGCCUAGAUCAGGACAUUA	UAAUGUCCUGAUCUAGGCGCUCAGC
154658C03	Trip10/MSS246882	ACGACUCACAGGUCCUAUCGAAUU	AAUUCGAUGAGGACCUGUGAGUCGU
154658C05	Trip10/MSS246883	UGGAGCAGGCUUAUGCUAAGCAACU	AGUUGCUUAGCAUAAGCCUGCUCCA
154658C07	Wasf1/MSS234749	CCCUUACAGGGAUGAUGGUAAGGAA	UUCUUAACCAUCAUCCUGUAAGGG
154658C09	Wasf1/MSS234750	ACCCUGCCUGUAAUCAGUGACGCAA	UUGCGUCACUGAUUACAGGCAGGGU

154658C11	Wasf1/MSS234751	CCCUGGCAAAUUAUUUAGACAACU	AGUUGUCUAAUUAUUUUGCCAGGG
154658D01	Inpp5a/MSS238163	UCGACUACUUAACCAGGAUGUCUU	AAGACAUCCUGGUUGAAGUAGUCGA
154658D03	Inpp5a/MSS238164	CCUCCAUGUCCCAUGUGGACAAAUU	AAUUUGUCCCAUUGGGACAUUGGAGG
154658D05	Inpp5a/MSS238165	UCCAACUUAGUGGCCUGGGAGACAA	UUGUCUCCCAGGCCACUAAGUUGGA
154658D07	Wasf2/MSS216650	CCUAGCGAUACAAGCGAGCUGGAU	AUUCAGCUCGCUUGUAUCGCUAGG
154658D09	Wasf2/MSS216651	CCUCUUAACAAUCUCAGUCCUUA	UGUAAGGACUGAGAUUGUUAAGAGG
154658D11	Wasf2/MSS216652	GCAGGACACCAAGGAUUAUCAUGAAA	UUUCAUGAUAUCCUUGGUGUCCUGC
154658E01	Iqgap1/MSS219911	GACCCUGUGGAUUAUUACAAGUCUU	AAGACUUGUAAAUAUCCACAGGGUC
154658E03	Iqgap1/MSS219912	CCAACAAGAUGUUUCUGGGCGAUAA	UUUUCGCCAGAAACAUUCUUGUUGG
154658E05	Iqgap1/MSS219913	UGAACAAAGCCUUGUCUCAUGAAGAA	UUCUUCAUGAGACAAGGCUUGUUCA
154658E07	Iqgap2/MSS285190	CAGCAAACCCUGAACGCACUUAUA	UAUUAAGUGCGUUCAGGGUUUGCUG
155026B07	Iqgap2/MSS285191	GAUGACCUUCAAACCAACAGUUUA	UAAACUGGUUGGUUUGAAGGUCAUC
154658E11	Iqgap2/MSS285192	CAUAGCUAUUAUGAGGCUAUUGAA	UUCAAUAGCCUCAUUAUAGCUAUG
154658F01	Wasl/MSS292313	GCAGUGGUGUCGCUUGUCUGGUUAA	UUAACCAGACAAGCGACACCACUGC
154658F03	Wasl/MSS292314	GAACAAGAGCUAUACAUAACUUG	CAAAGUUAUUGUAUAGCUCUUGUUC
154658F05	Wasl/MSS292315	GAGAUACUUGUCAAGUAGCUCUUA	UUAAGAGCUACUUGACAAGUAUCUC
154658F07	Abl2/MSS201506	CCACUGAGAGUGACCCUAAUCUCUU	AAGAGAUUAGGGUCACUCUCAGUGG
154658F09	Abl2/MSS201507	CCGACUUUGGUUUAAGUAGACUGAU	AUCAGUCUACUUAACCAAAGUCGG
154658F11	Abl2/MSS272920	UACGUGACAGCUGAGAGCCGCUUUA	UAAAGCGGCUCUCAGCUGUCACGUA
154658G01	Map3k4/MSS218531	CCUCCUUCCUCAGAGCAGAAGUGUA	UACACUUCUGCUCUGAGGAAGGAGG
154658G03	Map3k4/MSS218532	GCUCGAGUCAGAGGAAGAUUCCAUA	UAUGGAAUCUCCUCUGACUCGAGC
154658G05	Map3k4/MSS218533	GCCAGCAGGCAUUGUUUGACUUCUA	UAGAAGUCAAAACAAUGCCUGCUGGC
154658G07	Mapk8/MSS218561	GCGCCUACCGAGAACUAGUUCUUAU	AUAAGAACUAGUUCUCGGUAGGCGC
154658G09	Mapk8/MSS218563	CCUCUUUCCAGGAAGGGACUAUAUU	AAUAUAGUCCCUUCCUGGAAAGAGG
154658G11	Mapk8/MSS281895	CAAGCACCUUCACUCUGCUGGAUUU	AAUUCAGCAGAGUGAAGGUGCUUG
154658H01	Ap2m1/MSS202076	GACGGAGAGUUUGAACUCAUGAGAU	AUCUCAUGAGUUCAAACUCUCCGUC
154658H03	Ap2m1/MSS273197	CAAGCAAGAGUGGUAAGCAGUCGAU	AUCGACUGCUUACCACUCUUGCUUG
154658H05	Ap2m1/MSS273198	GCGACUCAGCAAGUUUGACUCUGAG	CUCAGAGUCAACUUGCUGAGUCGC
154658H07	Ddef1/MSS273948	GACUACAACUCGCCCACCACGUCCA	UGGACGUGGUGGGCGAGUUUGUAGUC
154658H09	Ddef1/MSS273949	ACGCGGCUGCACAAUCGAGGAACA	UGUCCUGCAGUUGUGCAGCCGCGU
154658H11	Ddef1/MSS273950	UCACGCUGCUGGAGGAGGCUCUAGA	UCUAGAGCCUCCUCCAGCAGCGUGA
154659A01	Nckap1/MSS284790	CAGUGUACUCUUAGUGACCAGUUUA	AUAACUGGUCACUAAGAGUACACUG
154659A03	Nckap1/MSS284791	GGCGUUGUAUUACUUUAUUAUUGUA	UACAAAUGUAAAGUAAUACAACGCC
154659A05	Nckap1/MSS284792	GCAAUAGAAGGACACUGCAACAAUA	UAUUGUUGCAGUGUCCUUCUAUUGC
154659A07	Pak1/MSS237494	GGAAUGGAUGGCUCUGUCAAGUUA	UUAACUUGACAGAGCCAUCCAUCC
154659A09	Pak1/MSS237495	UGUGCCGAGAGUGUCUACAAGCUUU	AAAGCUUGUAGACACUCUCGGCACA
154659A11	Pak1/MSS237496	GGGCAUUUAUGGCAAUUGAAUUGAUU	AAUCAUUUCAAUUGCCAUAUUGCCC
154659B01	Rala/MSS225866	GGUAACAAGUCAGAUCUAGAAGAU	UAUCUUCUAGAUCUGACUUGUUAAC
154659B03	Rala/MSS225868	ACAGAGCUGACCAGUGGAACGUUA	UUAACGUUCCACUGGUCAGCUCUGU
154659B05	Rala/MSS285599	GCAGGAGGACUAUGCUGCAAUUAAGA	UCUAAUUGCAGCAUAGUCCUCCUGC
154659B07	Ralb/MSS287585	CCCACCAAAGCUGACAGUUACAGAA	UUCUGUAACUGUCAGCUUUGGUGGG
154659B09	Ralb/MSS287586	CAUCACAGAGCACGAGUCUUUCACA	UGUGAAAGACUCGUGCUCUGUGAUG

154659B11	Ralb/MSS287587	CAGCCGAGUUCAGGGAACAGAUUCU	AGAAUCUGUUCCCUGAACUCGGCUG
154659C01	Reps1/MSS208601	GGAAAUACCGUAGAUGGGUACAGUA	UACUGUACCCAUCUACGGUAUUUCC
154659C03	Reps1/MSS208602	CCAGUGGAUGCAGAUGGUCUAAUAA	UUUUUAGACCAUCUGCAUCCACUGG
154659C05	Reps1/MSS208603	CCAUUCAGCCUGACCUAAACGGAUU	AAUCCGUUUAGGUCAGGCUGAAUGG
154659C07	Gna13/MSS204752	CCAGCUGGGUGAGUCUGUAAAGUAU	AUACUUUACAGACUCACCCAGCUGG
154659C09	Gna13/MSS204753	GGCAUCCAUGAGUACGACUUUGAAA	UUUCAAGUCGUACUCAUGGAUGCC
154659C11	Gna13/MSS274579	UGGAGACAAGUUGAUUGGCAUUUGAU	AUCAAAUGCCAUCAACUUGUCUCCA
154659D01	Rras/MSS276892	CCUCCGGUCUGAAGCCUCCUCUUUC	GAAAGAGGAGGCUUCAGACCGGAGG
154659D03	Rras/MSS276893	GACUUACUUUGAGGCCUCAGCCAAA	UUUGGCUGAGGCCUCAAGUAAGUC
154659D05	Rras/MSS276894	GCGCUGUCCGGAAAUACCAGGAACA	UGUUCCUGGUUUUCCGGACAGCGC
154659D07	Sh3glb1/MSS225755	GCUAAUUGGGAGAGAGAGGAAAUCAA	UUGAUUUCCUCUCUCUCCCAUJAGC
154659D09	Sh3glb1/MSS225757	ACCAGUGCCAUAUGCUUUGUCAAAU	AUUUGACAAAGCAUAUGGCACUGGU
155026B10	Sh3glb1/MSS285450	CAAUUACCUACUUAGAACUUCUCAA	UUGAGAAGUUCUAAGUAGGUAAUUG
154659E01	Gnaq/MSS204770	CCCUUCCUAUCUGCCUACACAACAA	UUGUUGUGUAGGCAGAUAGGAAGGG
154659E03	Gnaq/MSS204772	CCCUUUGACUUACAAAGUGUCAUUU	AAAUGACACUUUGUAAGUCAAGGG
154659E05	Gnaq/MSS274590	CGCUUAGCGAAUAUGAUCAAGUUCU	AGAAUUGAUCAUAUUCGCUAAGCG
154659E07	Hip1/MSS238296	ACUACACUGUCAAGCUGCUCUUCAA	UUGAAGAGCAGCUUGACAGUGUAGU
154659E09	Hip1/MSS238297	UGGCUGCAUCCAAGGUGAAAGCGAA	UUCGCUUUCACCUUGGAUGCAGCCA
154659E11	Hip1/MSS277974	CAGAAGAUUUAGUGAGCUUCUGCA	UGCAGAAGCUCACUAAUAUCUUCUG
154659F01	Traf2/MSS212078	CCCUGAGUAACAAGGUGCAACAGCU	AGCUGUUGCACCUUGUUACUCAGGG
154659F03	Traf2/MSS212079	GGACCAUGUUAGAGCAUGCAGCAAA	UUUGCUGCAUGCUCUAACAUGGUCC
154659F05	Traf2/MSS212080	GGAAGAUUCUCUGACUUCACCAGAAA	UUUCUGGUGAAGUCAGAGAUCUUC
154659F07	Amph/MSS211646	GGAAGCACGAGAGCUUGCAAUCAGU	ACUGAUUUGCAAGCUCUGCUUUC
154659F09	Amph/MSS211647	GCGCAGCAGGAAGCUUGUGGAUUUAU	AUAAUCCACAAGCUUCCUGCUGCGC
154659F11	Amph/MSS211648	GGAUAAUUUAGCAGCAAUCAAAGGUA	UACCUUUUGAUUUGCUGCUAAAAUUC
154659G01	Igf1r/MSS205378	ACCACGGUAGCUGACACCUACAUA	UAUUGUAGGUGUCAGCUACCGUGGU
154659G03	Igf1r/MSS275035	UCCUGAAGGGCAAUCUGCUUAUUAA	UUAAUAAGCAGAUUGCCCUUCAGGA
154659G05	Igf1r/MSS275036	CGGAAAGAUGUACUUUGCUUUCAAU	AUUGAAAGCAAAGUACAUCUUUCCG
154659G07	Kif1a/MSS205763	CCGAGACUCCGUUUUGACAUGGCUU	AAGCCAUGUCAAUACGGAGUCUCGG
154659G09	Kif1a/MSS205764	GAGCUGGAAGCCAAUGGCGAUUACA	UGUAAUCGCCAUUGGCUUCCAGCUC
154659G11	Kif1a/MSS205765	GAGGUGACCAAGUCCUUCAUUCGAAU	AUUCGAUGAAGGACUUGGUCACCUC
154659H01	Ddef2/MSS277636	GAACAGAGAGGAACGGGAACCUCUA	UAGAGGUUCCCGUUCUCUCUGUUC
154659H03	Ddef2/MSS277637	GAGAAGAAGUGUUUCGACCUCUAU	AUAUGAGGUCGAAACACUUCUUCUC
154659H05	Ddef2/MSS277638	CAGACUUAGGAAGCGCGUUUCUGAA	UUCAGAAACGCGCUUCCUAAGUCUG
154659H07	Atp6v1e1/MSS202278	UAAGCACAUAGAUGGCUUUCAUUGAA	UUCAAUGAAAGCCAUAUGUGCUUA
154659H09	Atp6v1e1/MSS202279	GAAAUAGCUGGUGGCGUUGAGAUCU	AGAUCUCAACGCCACCAGCUAUUUC
154659H11	Atp6v1e1/MSS273322	AGCAGAGACUCAGUAAGGUGGUAAA	UUUACCACCUUACUGAGUCUCUGCU
154660A01	Kif5a/MSS205791	CCUCCGGGAUGAGAUUAAUGAGAAA	UUUCUCAUUAUUCUCAUCCCGGAGG
154660A03	Kif5a/MSS205792	ACAACCUUCGCAAGCUGUUCGUUCA	UGAACGAACAGCUUGCGAAGGUUGU
154660A05	Kif5a/MSS275221	GAUGAGAACCUUGAAUUCACAUUA	UAAUGUGGAAUUCAGGUUCUCAUC
154660A07	Macf1/MSS201569	GGAGAACCAACUUUCUGCAUCUAAA	UUUAGAUGCAGAAAGUUGGUUCUCC
154660A09	Macf1/MSS272959	CAGGCUUUAGUUGACUGGUUGUACA	UGUACAACCAGUCAACUAAAGCCUG

154660A11	Macf1/MSS272960	GAGUUCCAGGAAAGCUUCAAGAAUA	UAUUCUUGAAGCUUUCUGGAACUC
154660B01	Bin1/MSS220005	GAUGACGCAUUUGUCCCUGAGAUC	UGAUCUCAGGGACAAAUGCGUCAUC
154660B03	Bin1/MSS220006	CCUGGCAGGGAUGAAGCAAACAAGA	UCUUGUUUGCUUCAUCCCUGCCAGG
154660B05	Bin1/MSS282617	UCGGACCUAUCUGGCUUCUGUUAAA	UUUAACAGAAGCCAGAUAGGUCCGA
154660B07	Mtap1a/MSS206790	CCUCCACUCUGACACUCCAACGUUU	AAACGUUGGAGUGUCAGAGUGGAGG
154660B09	Mtap1a/MSS206791	CCACUCUGGAGAUCAUGAAGCUAAU	AUUAGCUUCAUGAUCUCCAGAGUGG
154660B11	Mtap1a/MSS275827	CAGAACACAGAAGCAAGGUUGAUAA	UUAUCAACCUUGCUUCUGUGUUCUG
154660C01	Cask/MSS202616	GGCCUUACUUCAGACUCAUGAUGUA	UACAUCAUGAGUCUGAAGUAAGGCC
154660C03	Cask/MSS202617	CCUUCAGUGUUGUACGGCGAUGUAU	AUACAUCGCCGUACAACACUGAAGG
154660C05	Cask/MSS202618	GGAGCGGGAU CGUUAUGCCUACAAA	UUUGUAGGCAUAACGAUCCCGCUCC
154660C07	Cblb/MSS209651	GGUCUUGUGAUAAUGGUCAUUGUAU	AUACAAUGACCAUUAUCACAAGACC
154660C09	Cblb/MSS209652	GGAUUCUCACAAAGCUGUCCCUAAU	AUAAGGGACAGCUUUGUGAGAUUCC
154660C11	Cblb/MSS277402	CGAAGCGAGCGAUCCGGCUCUUUAA	UUAAAGAGCCGGAUCGCGCUCUUCG
154660D01	Ndel1/MSS234683	ACUCUGGACUGUGAGAAGAUGGAUU	AAUCCAUCUUCUCACAGUCCAGAGU
154660D03	Ndel1/MSS234685	GAACGAAUUGCAUUCUUGAGAAAGUG	CACUUUCUAAAGAAUGCAUUUCGUUC
154660D05	Ndel1/MSS294501	CAACAGGAAGUGACCCGCAAGUCUG	CAGACUUGCGGGUCACUCCUGUUG
154660D07	Pam/MSS207392	CCAGUGUUUCAAGACUGACACCAA	UUUGGUGUCAGUCUUGAAACACUGG
154660D09	Pam/MSS207393	GAGUACUGGGAAGACUCCGAGGAAA	UUUCCUCGGAGUCUUCCAGUACUC
154660D11	Pam/MSS207394	CCAGCAUGGACACUGUCCACCAUAU	AUAUGGUGGACAGUGUCCAUGCUGG
154660E01	Cd2ap/MSS273555	CAGAGAAGAAAGCUUUCUUCUAAA	UUUAGAGGGAAAGCUUUCUUCUCUG
154660E03	Cd2ap/MSS273556	GAUGCCUUUGUAGCCAGGAACUCAA	UUGAGUUCUGGCUACAAAGGCAUC
154660E05	Cd2ap/MSS273557	CCAAGGAUGACAAUUUGCCCAUCAA	UUGAUGGGCAAUUGUCAUCCUUGG
154660E07	Eps15/MSS203951	CCCUUAAACUGAAUGAUCCAUUUCA	UGAAAUGGAUCAUUCAGUUUAAGGG
154660E09	Eps15/MSS203952	CCCAGGCAUUGAUAGUCCCAAAGAA	UUCUUUGGGACUAUCAUUGCCUGGG
154660E11	Eps15/MSS203953	AGAUGAAGUUCAAAGGGAGAGUAUU	AAUACUCUCCCUUUGAACUUAUCU
154660F01	Pdgfrb/MSS207503	GAGAGUGACGGUGGCUACAUGGAUA	UAUCCAUGUAGCCACCGUCACUCUC
154660F03	Pdgfrb/MSS207504	GAGCAAGGAUGAAUCUAUAGAUUAC	GUAAUCUAUAGAUUCAUCCUUGCUC
154660F05	Pdgfrb/MSS207505	GCCUCUACACCACUUUGAGUGAUGU	ACAUCACUCAAGUGGUGUAGAGGC
154660F07	Ptprf/MSS237674	GCCAACCAUCAUCCAGGAUGUCAU	AUGACAUCCUGGAUGAUGGGUUGGC
154660F09	Ptprf/MSS237675	CGCCCAUGCCGU AUGUGAAAUGGAU	AUCCAUUUCACAUACGGCAUGGGCG
154660F11	Ptprf/MSS237676	GCACUGAGACCUAUGGCCUCAUUA	UGAAUGAGGCCAUAGGUCUCAGUGC
154660G01	Exoc1/MSS229985	GAAGACCUGGAUGGAGGCUCGUUAU	AUAACGAGCCUCCAUCCAGGUCUUC
154660G03	Exoc1/MSS229986	GGAGGUGGACCAGAUUGAACUGAAA	UUUCAGUUCAAUCUGGUCCACCUC
154660G05	Exoc1/MSS229987	GGUCGACGCCAAAGAUGCCAUCAAA	UUUGAUGGCAUCUUUGGCGUCGACC
154660G07	Gna12/MSS204749	CCAUCGUCAACAACAAGCUCUUCUU	AAGAAGAGCUUGUUGUUGACGAUGG
154660G09	Gna12/MSS204750	GGGAUCGUGGAACAUGACUUCGUU	AACGAAGUCAUGUCCACGAUUC
154660G11	Gna12/MSS204751	GGAGAAGGUGAAGUCUGUGAGCAUU	AAUGCUCACAGACUUCACCUUCUCC
154660H01	Rheb/NM_053075.3_stealth_184	CCUCAUUGACAAUUCAGUUUGUUGA	UCAACAAACUGAAUUGUCAUAGAGG
154660H03	Rheb/NM_053075.3_stealth_478	GAAAUAGAAGGACCUGCAUAUGGA	UCCAUAUGCAGGUCCUUCUUAUUUC
154660H05	Rheb/NM_053075.3_stealth_1013	UACUAUUAACUAGGCUUCAUAUAU	AUAUAUUGAAGCCUAGUUAUAUGUA
154660H07	Scrib/MSS200606	GACCCUACAGAUGAGGGCAUCUUUA	UAAAGAUGCCUCAUCUGUAGGGUC
154660H09	Scrib/MSS200607	CCAGCCAUGUGAAGCAGGCGUAUA	UAUACGCCUGCUUCACAUUGGCUGG

154660H11	Scrib/MSS272416	CAAGGAGAAAGAGUUGGCCAGUCA	UUGACUGGCCAACUCUUUCUCCUUG
154661A01	Sept7/MSS214988	GCACAGCACAAAGAAUUGAGGAAA	UUUCCUCUAAUUCUUUGUGCUGUGC
154661A03	Sept7/MSS214989	GAGUGAACAGACGUCAGAUGCCUGA	UCAGGCAUCUGACGUCUGUUCACUC
154661A05	Sept7/MSS214990	GCCAACCUCCCAAUAAGUGUACA	UGUACACUUGAUUUUGGGAGGUUGGC
154661A07	Dlg3/MSS284974	CAGGACAGGGAUGAUUGAGUCUAAU	AUUAGACUCAAUCAUCCCUGUCCUG
154661A09	Dlg3/MSS284975	GAGACUGCAGCAAGCACAAUUAU	AUAAAGUUGUGCUUGCUGCAGUCUC
154661A11	Dlg3/MSS284976	CAGCCUAUCGGUGAAUGGCAGUGAU	AUCACUGCCAUCACCGAUAGGCUG
154661B01	Snap23/MSS209234	GGCAGCAUCCUAGGCAACCUAAAGA	UCUUUAGGUUGCCUAGGAUGCUGCC
154661B03	Snap23/MSS209236	GACAACUCACCUAGCAAUGUAGUAU	AUACUACAUUGCUAGGUGAGUUGUC
154661B05	Snap23/MSS277154	CAGUGGUGGAUACAUAUAAACGUUA	UAUACGUUUAUGUAUCCACCACUG
154661B07	Tsc2/MSS212132	CCGCUGGACUACAAGUGCAACCUAU	AUAGGUUGCACUUGUAGUCCAGCGG
154661B09	Tsc2/MSS212134	CCCUCCAUGAAGACCUCCAUGAAA	UUUCAUGGAGGUCUUAUUGGAGGG
154661B11	Tsc2/MSS278551	CAGAGACCUGAAUCCUCUCUCUUA	UUAAGAGAGAGGAUUCAGGUCUCUG
154661C01	Dlgap4/MSS279341	CAGCUGUCCAUGAGGACAUCAGCA	UGCUGAUGUCCUCAUUGGACAGCUG
154661C03	Dlgap4/MSS279342	CAGCCAAAUCCAAGGCGGCAGUGAG	CUCACUGCCGCCUUGGAUUUGGCUG
155023G01	Dlgap4/MSS279343	CAAGCAGCGUCAGGAGGCCAGAAAG	CUUUCUGGCCUCCUGACGCUGCUUG
154661C07	Git1/MSS211304	GCACCAUGAACUGGCAGAAAGGCUA	UAGCCUUUCUGCCAGUUAUGGUGC
154661C09	Git1/MSS211305	CCAGCACCGAAGACGUCAUCCUAAA	UUUAGGAUGACGUCUUCGGUGCUGG
154661C11	Git1/MSS278162	CCAAAGACCUCAGCAAGCAACUGCA	UGCAGUUGCUUGCUGAGGUCUUUGG
154661D01	Tubb3/NM_023279.2_stealth_752	CCACCUCUUUGAUUCCUUGGUCA	UGACCAGGGAAUCGAAGGGAGGUGG
154661D03	Tubb3/NM_023279.2_stealth_1508	CCAGCUCAUUAAGGCUCCCAGGUUA	UAACCUGGGAGCCCUAUAGAGCUGG
154661D05	Tubb3/NM_023279.2_stealth_1605	CCUCUGUAUUUAUGUUGCUUAUUCG	CGAAUAAGCAACAUAUAAUACAGAGG
154661D07	App/MSS202133	UCAGGAUUUGAAGUCCGCCAUCAA	UUUGAUGGCGGACUUCAAAUCUGA
154661D09	App/MSS202134	GACCAGGUUCUGGGCUGACAAACAU	AUGUUUGUCAGCCCAGAACCUGGUC
154661D11	App/MSS202135	GCGGAUGGAUGUUUGUGAGACCCAU	AUGGGUCUCACAAACAUCAUCCGC
154661E01	Git2/MSS218579	GGGCAGACAGAAAUUAGCUCGUAUU	AAACCGAGCUAAUUCUGUCUGCCC
154661E03	Git2/MSS218580	CAUGCUCUGGCAAGGUGAUGGCUUA	UAAGCCAUCACCUUGCCAGAGCAUG
154661E05	Git2/MSS218581	UCAUGAGUGGGAGGCGGAAAGCUAA	UUAGCUUUCGCCUCCCACUCAUGA
154661E07	Gripap1/MSS225731	UCAGCAACCUGAAGCAGGAGGUAAA	UUUACCUCCUGCUUCAGGUUGCUGA
154661E09	Gripap1/MSS225732	UCGAGGAGCUGGUUCUGUCUGAGAU	AUCUCAGACAGAACCAGCUCCUCGA
154661E11	Gripap1/MSS225733	GGCAUCUGAGUUAACAGCAUCGUCAA	UUGACGAUGCUGUAACUCAGAUGCC
154661F01	Arf3/MSS235970	GCAAGAAGGAGAUGCGCAUCCUGAU	AUCAGGAUGCGCAUCUCCUUCUUGC
154661F03	Arf3/MSS235971	ACAGCAAUGAUCGGGAGCGAGUGAA	UUCACUCGCUCCCGAUCAUUGCUGU
154661F05	Arf3/MSS235972	CCAGAACACCCAAGGCUUGAUUUU	AAAUUAUAAGCCUUGGGUGUUCUGG
154661F07	Arpc1b/NM_023142.2_stealth_185	CCAACAACCAUGAGGUGCACAUCUA	UAGAUGUGCACCUCAUGGUUGUUGG
154661F09	Arpc1b/NM_023142.2_stealth_887	CGGGCCACGACUGCUUCCCGGUGCU	AGCACCGGGAAGCAGUCGUGGCCCG
154661F11	Arpc1b/NM_023142.2_stealth_1125	CAAGUGCUCACAGUUCUGCACUACA	UGUAGUGCAGAACUGUGAGCACUUG
154661G01	Hip1r/MSS219893	UCAUCCUGGGCAGCAUCAUGAGAA	UUCUCAUGAUGCGUGCCCAGGAUGA
154661G03	Hip1r/MSS219894	CCACUACACAGUGAAGCUCAUGUUU	AAACAUGAGCUUCACUGUGUAGUGG
154661G05	Hip1r/MSS219895	GACCAGUAUGGACACCUGGUGAAUA	UAUUCACCAGGUGUCCAUAUCUGGUC
154661G07	Irs2/MSS222825	GGCGAACUCUAUGGGUACAUGAGCA	UGCUCAUGUACCCAUAAGAUUCGCC
154661G09	Irs2/MSS222826	GGUUUCCAGAACGGCCUCAACUAUA	UAUAGUUGAGGCCGUUCUGGAAACC

154661G11	Irs2/MSS284072	CAUCGGGCUUGAAGCGGCUAAGUCU	AGACUUAGCCGCUUCAAGCCCGAUG
154661H01	Bcar1/MSS273814	GACCGCACCUUGCAUGCCAAGCUUA	UAAGCUUGGCAUGCAAGGUGCGGUC
154661H03	Bcar1/MSS273815	ACAGAUGCAGUGGACGCCUUCUUA	UGAAGAAGGCGUCCACUGCAUCUGU
154661H05	Bcar1/MSS273816	CCUCAAGAUUCUGGUUGGCAUGUAU	AUACAUGCCAACCAGAAUCUUGAGG
154661H07	Itgav/MSS205547	GGUGUGGAUCGAGCUGUCUUAUACA	UGUAUAAGACAGCUCGAUCCACACC
154661H09	Itgav/MSS205548	CAGACCCGUUGUCACUGUAAAUGCU	AGCAUUUACAGUGACAACGGGUCUG
154661H11	Itgav/MSS205549	CCAAGUGGCAGAAAUCAUAUCUAAA	UUUAGAUUGAUUUUCUGCCACUUGG
154662A01	Capn1/MSS236087	ACCAAACUGGAAAUCUGCAACCUUA	UAAGGUUGCAGAUUUCCAGUUUGGU
154662A03	Capn1/MSS236088	CCCUGAUGAGAAAGUUCUCUCUGAA	UUCAGAGAGAACUUUCUCAUCAGGG
154662A05	Capn1/MSS236089	CCGAUAUCCGUGAUUUAGAGGCUAU	AUAGCCUCUAAAUCACGGAUAUCGG
154662A07	Ccdc88a/MSS201023	CCCAGGAAGUGAAGUUGUUAUCUCUA	UAGAGUAACAACUUCACUUCCUGGG
154662A09	Ccdc88a/MSS201024	ACACCAAGCACUCAAGGAAAGAUAA	UUUUCUUUCCUUGAGUGCUUGGUGU
154662A11	Ccdc88a/MSS201025	GGCCAGGUCAGUAAGUGGAAAGACU	AGUCUUUCCACUUACUGACCUGGCC
154662B01	Itgb5/MSS205565	GGACUAUCCAUCGCUUGCCUUGCUU	AAGCAAGGCAAGCGAUGGAUAGUCC
154662B03	Itgb5/MSS205566	GCCUACAAUUGCACAU GCGGCU GUA	UACAGCCGCAUGUGCAAUUGUAGGC
154662B05	Itgb5/MSS275142	CAGGAGGCUUGGCUUUGCUUCUACA	UGUAGAAGCAAAGCACAGCCUCCUG
154662B07	Kif3a/MSS205781	CAGGACUCCUUAAGAGGGAACUCAA	UUGAGUUCCCUCUUAAGGAGUCCUG
154662B09	Kif3a/MSS205782	GGGUAAGAAGCAGACCCAGAGGUUA	UAACCUCUGGGUCUGAUCUUUACCC
154662B11	Kif3a/MSS205783	UCGGUGCGACUAAUAUGAACGAGCA	UGCUCGUUCAUUAUAGUCGCACCGA
154662C01	Cd47/MSS205575	GAGAAAGGAGGUUGCAAUUAGUUU	AAACUAAUUUGCAACCUCUUUCUC
154662C03	Cd47/MSS205576	GAUCAUAGCUCUAGCAGAACUACUU	AAGUAGUUCUGCUAGAGCUAUGAUC
154662C05	Cd47/MSS275144	CGCGAUGCCAUGGUGGGAAACUACA	UGUAGUUUCCCACCAUGGCAUCGCG
154662C07	Clip2/MSS219153	GAAGACCGGUGUGGUGCGAU AUGUU	AACAUUUCGCACCACACCGGUCUUC
154662C09	Clip2/MSS282161	CGGGUGGAAGAAAGAUCCAUCACCA	UGGUGAUGGAUUUCUUCUCCACCCG
154662C11	Clip2/MSS282162	UGCCCGGCUCUACAGGGCAUCUUA	UGAAGAUGCCUGUAGAGCCGGGCA
154662D01	Kif3b/MSS275218	CAGCAGGAGGUAGACAUCAAGACCA	UGGUCUUGAUGUCUACCUCUGCUG
154662D03	Kif3b/MSS275219	AGCUAAAGCUCAAGCAUCUUAUUAU	AUAUAAGAUGCUUAGCUUUUAGCU
154662D05	Kif3b/MSS275220	CAGCUCACGGUCGCAUGCAAUCUUU	AAAGAUUGCAUGCGACCGUGAGCUG
154662D07	Llg1/MSS237065	GCCACACAGUCUGUGGACAACGUUU	AAACGUUGUCCACAGACUGUGUGGC
154662D09	Llg1/MSS237066	GCGAGAAGCUUAAGCAGGAGCUCUU	AAGAGCUCUGCUUAAGCUUCUCGC
154662D11	Llg1/MSS275350	CAGAAGGUUGCGCUUUGCAAGUACA	UGUACUUGCAAAGCGCAACCUUCUG
154662E01	Ctnna1/MSS202658	UCCUGGAGACCA AUGUCCCUUAUU	AAUAGAGGGACAUUGGUCUCCAGGA
154662E03	Ctnna1/MSS202659	GCUGAAAGUUGUGGAAGAUGGUUAU	UAUACCAUCUCCACAACUUUCAGC
154662E05	Ctnna1/MSS273499	CAAUAGUAAAGGGCCGUCUAAUAAA	UUUAUUAGACGGCCCUUACUAUUG
154662E07	Dixdc1/MSS221126	GCACCUGCACUAAGGUGCUCUAUUU	AAAUAGAGCACCUUAGUGCAGGUGC
154662E09	Dixdc1/MSS221127	UCGGAUGCAUCAGACGUCAGCGAAA	UUUCGCUGACGUCUGAUGCAUCCGA
154662E11	Dixdc1/MSS221128	GCGGGCUCUUGUGCAGCAAUAUGAA	UUCAUAUUGCUGCACAAGAGCCCGC
154662F01	Map2k7/MSS218514	UGAUGGACCUGGAUGUAGUACUCAA	UUGAGUACUACAUCAGGUCCAUCA
154662F03	Map2k7/MSS218515	CCAGUCAUUUGUCAAAAGACUGCCUU	AAGGCAGUCUUUGACAAAUGACUGG
154662F05	Map2k7/MSS281882	CCAGCGUUAUCAGGCAGAAUCAAU	AUUGAUUUCUGCCUGAUAAACGCUGG
154662F07	Map3k12/MSS218522	GACCUGAAGUCACCCAACAUGC UAA	UUAGCAUGUUGGGUGACUUCAGGUC
154662F09	Map3k12/MSS218523	GACUCAGACUGUGACAGCACUGAAU	AUUCAGUGCUGUCACAGUCUGAGUC

154662F11	Map3k12/MSS281884	CAACACUGAUGAGCGACCAGAUGAA	UUCAUCUGGUCGCUCAUCAGUGUUG
154662G01	Rab4a/MSS208367	GGCUUUCAUGCAGUGUGCAAGGAAA	UUUCCUUGCACACUGCAUGAAAGCC
154662G03	Rab4a/MSS208368	GGAAAUGCGGGAACUGGCAAAUCUU	AAGAUUUGCCAGUUCCCGCAUUUCC
154662G05	Rab4a/MSS276696	GGAGUGGAAUUUUGGCUCAAAGAUAA	UUUUCUUUGAGCCAAAUUCCACUCC
154662G07	Map4k5/MSS223262	GGCUGCCACAAAUGCUGCAUAGUUA	UAACUAUGCAGCAUUUGUGGCAGCC
154662G09	Map4k5/MSS223263	CCCACGCAAUCAUUCGUCAUACCAU	AUGGUAUGACGAAUGAUUGCGUGGG
154662G11	Map4k5/MSS223264	GGAAAUGUACGUGGCUCUACGUCAU	AUGACGUAGAGCCACGUACAUUUCC
154662H01	Mapk8ip3/MSS220022	CAUCCGCUGCAUGGCUGUUGUGAAU	AUUCACAACAGCCAUGCAGCGGAUG
154662H03	Mapk8ip3/MSS220023	GCCACAUGAAGAGGACUCUAGCAAU	AUUGCUAGAGUCCUCUUAUGUGGC
154662H05	Mapk8ip3/MSS220024	AGGAAAUGUGCUGGCCACUCUCAAU	AUUGAGAGUGGCCAGCACAUUCCCU
154662H07	Raph1/MSS293907	CAAAUCUCACUGUGGCUACAGUUUA	UAAACUGUAGCCACAGUGAGAUUUG
154662H09	Raph1/MSS293908	CCACUCUAUUCAGUCUGACAGCGGA	UCCGCUGUCAGACUGAUUAGAGUGG
154662H11	Raph1/MSS293909	CAGCAUGGAUUCUCUGGAUUAUUGAU	AUCAAUAUCCAGAGAAUCCAUGCUG
154663A01	Rela/MSS208587	GCAGUAUCCAUAGCUUCCAGAACCU	AGGUUCUGGAAGCUAUGGAUACUGC
154663A03	Rela/MSS208588	GCAGAAAGAAGACAUUGAGGUGUAU	AUACACCUCAAUGUCUUCUUCUGC
154663A05	Rela/MSS276820	CCGAGCUCAGAUCUGCCGAGUAAA	UUUACUCGGCAGAUUCUUGAGCUCGG
154663A07	Myo18a/MSS221405	CCUUCUGACGAUGGCAGCUUGAAGA	UCUUCAAGCUGCCAUCGUCAGAAGG
154663A09	Myo18a/MSS221406	UCGAGUCCUGAGCUGGUGACUAAA	UUUAGUCACCAGCUCAGGGACUCGA
154663A11	Myo18a/MSS221407	CAGCUGUAAAGGCACGGAAAGCGAU	AUCGCUUUCGUGCCUUUACAGCUG
154663B01	Sh3kbp1/MSS226688	GGAGAUUAUGUGACUCUCAUAUA	UAUUGAUGAGAGUCACUAUAUCUCC
154663B03	Sh3kbp1/MSS226689	ACUGGAUUUACAGAAGCCUUCAGUU	AACUGAAGGCUUCUGUAAAUCCAGU
154663B05	Sh3kbp1/MSS226690	GGUUGCAGAUUGGAAGUGAACGACAU	AUGUCGUUCACUCCAUCUGCAACC
154663B07	Smg1/MSS279870	GAGGACUGUCUUAUCCCACUCUUUA	UAAAGAGUGGGAUAAAGACAGUCCUC
154663B09	Smg1/MSS279871	CGGAAAGUUGUGGACAGAAUCUUCA	UGAAGAUUCUGUCCACAACUUUCCG
154663B11	Smg1/MSS279872	CCUCAUAUUUAUUCUGCAAUAGUA	UACUAUUGCAGGAUAUAAUAUGAGG
154663C01	Nfkb2/MSS207035	CCAAGGACAUGACUGCUCAAUUUUA	UUAAAUUGAGCAGUCAUGUCCUUGG
154663C03	Nfkb2/MSS207036	CCCAAUAUUGCACAUGCCUGAUUU	AAAUCAGGCAUGUGCAAUAUUUUGGG
154663C05	Nfkb2/MSS207037	CCAACUCUUACUCGCCUCCUUCUAA	UUAGAAGGAGGCGAGUAAGAGUUGG
154663C07	Pacsin1/MSS239516	CCAUGACCCGGGAGAUGAACAGUAA	UUACUGUUCAUCUCCCGGGUCAUGG
154663C09	Pacsin1/MSS239517	GCAGCUAUGACCGAGGCCAAACAUA	UAUGUUUGGCCUCGGUCAUAGCUGC
154663C11	Pacsin1/MSS239518	AGGAGCAGGAUGAGCUCAGCUUCA	UUGAAGCUGAGCUCAUCCUGCUCCU
154663D01	Snap25/MSS237950	GCAAGGCGAACAACUGGAACGCAUU	AAUGCGUUCAGUUGUUCGCCUUGC
154663D03	Snap25/MSS237952	GGGCAAUAUUCAGGAUGGAGUAGUG	CACUACUCCAUCCUGAUUAUUGCCC
154663D05	Snap25/MSS277146	GCCAUCAGUGGUGGCUUCAUCCGCA	UGC GGAUGAAGCCACCACUGAUGGC
154663D07	Snx5/MSS229554	GGAAUAUGAUCAGGAUCUAAGUGUU	AACACUUAGAUCUGAUCAUAUUC
154663D09	Snx5/MSS229555	UCAACGGAAGAGAGUGGCAGCAUU	AAUGCUGCCACUCUCUCCGUUUGA
154663D11	Snx5/MSS290594	CCCAUCGCUUCAGAUUCGACAUACCU	AGGUAUGUCGAUCUGAAGCGAUGGG
154663E01	Pacsin2/MSS215933	GCUGGUAGAGAAGGGACCACAGUAU	AUACUGUGGUCCCUUCUCUACCAGC
154663E03	Pacsin2/MSS215934	ACAGGGUUGGUGCAAGGGACGUUUA	UAAACGUCCCUUGCACCAACCCUGU
154663E05	Pacsin2/MSS215935	GAGCAUCAAAAGCAGCAGAUUCGGUA	UACCGCAUCUGCUGCUUUGAUGCUC
154663E07	Spnb3/MSS277255	CCCAAUGUCAAUUGGCACAACUUUA	UAAAGUUGUGCAUUGACAUUGGG
154663E09	Spnb3/MSS277256	CCCACUGACUUUGACAGCUUGGAGA	UCUCCAAGCUGUCAAGUCAGUGGG

154663E11	Spnb3/MSS277257	CAGUCAAGAUUAUGAUUGACCAUGAA	UUCAUGGUCAAUCAUAUCUUGACUG
154663F01	Pacsin3/MSS294410	CCAGUAGCGACAAGUCCAUGAACU	AGUUCAUGGAACUUGUCGCUACUGG
154663F03	Pacsin3/MSS294411	CCGUAAAAGCUCAGAAGCCCUGGCUA	UAGCCAGGGCUUCUGAGCUUUACGG
154663F05	Pacsin3/MSS294412	GAGGCUGAAGGAGGUUGAGGCUUCU	AGAAGCCUCAACCUCCUUCAGCCUC
154663F07	Pak2/MSS212562	GCAGUGCUGGAUGUCUUGAAGUUCU	AGAACUUAAGACAUCCAGCACUGC
154663F09	Pak2/MSS212563	CCAGUUCGGAUGAGCAGUACCAUUU	AAUUGGUACUGCUCAUCCGAACUGG
154663F11	Pak2/MSS278828	CAGCCAAUCACAGUUUGAAACCUUU	AAAGGUUUCAAACUGUGAUUUGCUG
154663G01	Stmn1/NM_019641.4_stealth_2	CAGCCAAUGCGGAGCGCCUGGUCUG	CAGACCAGGCGCUCCGCAUUGGCUG
154663G03	Stmn1/NM_019641.4_stealth_10	GCGGAGCGCCUGGUCUGGGUACGU	ACGUGACCCAGACCAGGCGCUCCGC
154663G05	Stmn1/NM_019641.4_stealth_705	GACAGAUAGUCUAGAAGCCGAUGUA	UACAUCGGCUUCUAGACUAUCUGUC
154663G07	Stmn2/MSS237848	GCAGGAACAAGGAACUGCAGGUUGA	UCAACCUGCAGUCCUUGUCCUGC
154663G09	Stmn2/MSS237849	UCCACGAACUCUAGCUUCUCCAAAG	CUUUGGAGAAGCUAGAGUUCGUGGA
154663G11	Stmn2/MSS237850	CCUGUCUCUGGAGGAGAUUCAGAAA	UUUCUGAAUCUCCUCCAGAGACAGG
154663H01	Pin1/NM_023371.3_stealth_1262	CCUCAGCACACAGACAGACUGCUUA	UAAGCAGUCUGUCUGUGUGCUGAGG
154663H03	Pin1/NM_023371.3_stealth_1302	CACCCAGCAUCUGCCACCCACUUCU	AGAAGUGGGUGGCAGAUUGCUGGGUG
154663H05	Pin1/NM_023371.3_stealth_1365	UCUCCAGGUGCUGUUUCCAUGGUAA	UUACCAUGGAAACAGCACCUGGAGA
154663H07	Plcb3/MSS207740	CGGCGCGGGAGUAAGUUCAUAAA	AUUUGAUGAACUACUCCCGCGCCG
154663H09	Plcb3/MSS207741	GGAUACCACUAUGUCUGCCUGCGAA	UUCGCAGGCAGACAUAGUGGUAUCC
154663H11	Plcb3/MSS276375	CAGGACUAUGCGGAGGCCUUGAUUA	UAAUCAAGGCCUCCGCAUAGUCCUG
154664A01	Stmn3/NM_009133.3_stealth_343	UCGCUCAUUUUGCUCUUCUACU	AGUAGAAGCAGGAGCAAAUGAGCGA
154664A03	Stmn3/NM_009133.3_stealth_440	GCCAGAGCUUCGAGGUCAUCCUCAA	UUGAGGAUGACCUCGAAGCUCUGGC
154664A05	Stmn3/NM_009133.3_stealth_807	GCAGCGGGAGGAAAUGUCUGGCUAA	UUAGCCAGACAUUCCUCCCGCUGC
154664A07	Stx4a/MSS238052	GGGAGAUGAUCAACCGCAUCGAGAA	UUCUCGAUGCGGUUGAUCAUCUCCC
154664A09	Stx4a/MSS238053	GCAGUAUCCGAGAGCUCCAUGAGAU	AUCUCAUGGAGCUCUCGGAUACUGC
154664A11	Stx4a/MSS238054	GCAGAACCUGCGAGAGGAGAUCAAA	UUUGAUCUCCUCUCGCAGGUUCUGC
154664B01	Prkcd/MSS207669	CCCUUCCUGCGCAUCUCCUUAUUA	AAUUGAAGGAGAUUGCAGGAAGGG
154664B03	Prkcd/MSS207670	GGACACAACAGAGUCUGUCGGAUA	UAUUCCGACAGACUCUGUUGUGUCC
154664B05	Prkcd/MSS276343	CAUGAACCGUCGUGGAGCAUUA	UUUAAUGGCUCCACGACGGUUAUG
154664B07	Pstpip1/MSS276532	GCAAUGCCCUGUGGGUGCAGUUA	UUACAGUGCACCCACAGGGCAUUGC
154664B09	Pstpip1/MSS276533	GAGAGAGACAGAAAGAGCAGCGGAA	UUCCGCUGCUCUUUCUGUCUCUCUC
154664B11	Pstpip1/MSS276534	GAGGCAGAAAGAGUGUACAGGCAA	UUUGCCUGUACACUCUUUCUGCCUC
154664C01	Tjp1/MSS211847	GCACGCAUCACAGCCUGGUUGUUUA	UAAACAACCAGGCUGUGAUGCGUGC
154664C03	Tjp1/MSS211848	GCAAAGAGAUGAGCGGGCUACCUUA	UAAGGUAGCCCGCUCAUCUCUUUGC
154664C05	Tjp1/MSS211849	GGGCCUACACUGAUCAAGAACUAGA	UCUAGUUCUUGAUCAGUGUAGGCC
154664C07	Tpm1/MSS247946	GAGAGAGGCAUGAAAGUCAUUGAAA	UUUCAUGACUUUCAUGCCUCUCUC
154664C09	Tpm1/MSS247947	GGCUGAGUUUGCAGAGAGAUCAUA	UACUGAUCUCUCUGCAAACUCAGCC
154664C11	Tpm1/MSS278512	UGGAUCAGACUUUACUGGAGCUAAA	UUUAGCUCCAGUAAAGUCUGAUCCA
154664D01	Vac14/MSS214907	GGAUCUGGAAGUGUUGGCAGAAA	AAUUUCUGCCAACACUCCAGAUCC
154664D03	Vac14/MSS214908	CACUCCCACCAUGAACUCCUACUUU	AAAGUAGGAGUUAUGGUGGGAGUG
154664D05	Vac14/MSS214909	UCUUCACAGCUGAGGAACCAGCUCAA	UUGAGCUGGUUCCUCAGCUGGAAGA
154664D07	NM_146100.4_stealth_89	CCGCCUCCUCCUACCGCAAGGUGUU	AACACCUUGCGGUAGGAGGAGGCGG
154664D09	NM_146100.4_stealth_1646	CCGCUAUAGAAUGUCUGCAAGGCUU	AAGCCUUGCAGACAUUCUAUAGCGG

154664D11	NM_146100.4_stealth_2138	CGUAUGCCCUUUAGCUAGCUCUUCU	AGAAGAGCUAGCUAAAGGGCAUACG
154664E01	Wasf3/MSS217310	UCCCAACCCUGUUGCUGACAUUUUAU	AUAAAUGUCAGCAACAGGGUUGGGA
154664E03	Wasf3/MSS217311	GGCCACCAUCUUGUCUAGACGCAUU	AAUGCGUCUAGACAAGAUGGUGGCC
154664E05	Wasf3/MSS217312	GCCAGGAGUGGAAUAUGAUGGCGUA	UACGCCAUCAUUUUCCACUCCUGGC
154664E07	Zyx/MSS238955	CCCACCAGUUGCCACUCCAUUUUGUU	AACAAAUGGAGUGGCAACUGGUGGG
154664E09	Zyx/MSS238956	GGCUCCUAAAUUUACUCCCGUGGUU	AACCACGGGAGUAAAUUUAGGAGCC
154664E11	Zyx/MSS238957	CAGAUGACAACGGCUGUUUCCUCU	AGAGGGAAACAGCCGUUGUCAUCUG
154664F01	Kif5c/MSS275222	CCGGGACAGCAAGAUGACUCGGAUU	AAUCCGAGUCAUCUUGCUGUCCCGG
154664F03	Kif5c/MSS275223	CAAGGCCUUGGAGAGUGCGCUGAAA	UUUCAGCGCACUCUCCAAGGCCUUG
154664F05	Kif5c/MSS275224	GGAAGUUACAUGAUCCUCAGCUUAU	AUAAGCUGAGGAUCAUGUAACUUC
154664F07	Klc1/MSS205817	GAGCACAACUCCAUCUGCAGAGUU	AACUCUGCAGGAUGGAGUUGUGCUC
154664F09	Klc1/MSS205818	UCACGCAGGAUGAGAUAUCUCUAA	UUAGAGAUGAUCUCAUCCUGCGUGA
154664F11	Klc1/MSS205819	GCCGUUGGAUGAUCUCUUCCCAAAU	AUUUGGGAAGAGAUAUCCAACGGC
154664G01	Actn3/MSS201671	CCAAUGUCAAUAAAGCCCUAGACUU	AAGUCUAGGGCUUUUUGACAUUGG
154664G03	Actn3/MSS201672	CCUCUAUGGAGAGAGUGACCUCUGA	UCAGAGGUCACUCUCUCCAUAAGAGG
154664G05	Actn3/MSS201673	GCUCUGGACUACGUGGCUUUCUCUA	UAGAGAAAGCCACGUAGUCCAGAGC
154664G07	Add1/MSS201758	GGGAGCAGGAAUUUGAAGCUCUUUAU	AUAAGAGCUUCAAUUCCUGCUCCC
154664G09	Add1/MSS201759	GAAGAAGAGGGUGUCUAUGAUUCUA	UAGAAUCAUAGACACCCUCUUCUUC
154664G11	Add1/MSS201760	GCCAAGUGUAUUGUGCACAUCACACA	UGUGGAUGUGCACAUAACACUUGGC
154664H01	Klc2/MSS236981	CGACCCUCAACAUCUGGCUGUUCU	AGAACAGCCAGAUUGUUGAGGGUCG
154664H03	Klc2/MSS236982	GAGGAGGUGGAAUACUACUACCGGA	UCCGGUAGUAGUAUUCCACCUCUC
154664H05	Klc2/MSS236983	GAGACCCUGUACAAGGAGAUCCUUA	UAAGGAUCUCCUUGUACAGGGUCUC
154664H07	NM_008634.2_stealth_203	CACCGCUUCCUAGACAGCAAGUUCU	AGAACUUGCUGUCUAGGAAGCGGUG
154664H09	NM_008634.2_stealth_7616	CAGUUUUUACCUGCCAGAUGCUAUA	UAUAGCAUCUGGCAGGUAAUAACUG
154664H11	NM_008634.2_stealth_8228	CACACGGCAUAAUGUAGCAAGUGUA	UACACUUGCUCACUUAUGCCGUGUG
154665A01	Arrb1/MSS201215	GGAAAGCUCACUGUCUACCUGGGAA	UUCCCAGGUAGACAGUGAGCUUUC
154665A03	Arrb1/MSS201216	CCUUCACCUUUGAGAUCCCGCCAAA	UUUGGCGGGAUCUCAAGGUGAAGG
154665A05	Arrb1/MSS272777	GACACCAAUGAUGACGACAUUGUAU	AUACAAUGUCGUCAUCAUUGGUGUC
154665A07	Cdk5/MSS247085	GGAGGGACCUGUUGCAGAACCUAUU	AAUAGGUUCUGCAACAGGUCCCUC
154665A09	Cdk5/MSS247086	GGGAGAUCUGUCUACUCAAGAACU	AGUUCUUUGAGUAGACAGAUCCCC
154665A11	Cdk5/MSS247087	CCAUGACCAAGCUGCCAGACUAUAA	UUUAUGUCUGGCAGCUUGGUCAUGG
154665B01	Map2k3/MSS240938	GGAUCAACCCUGAACUGAAUCAGAA	UUCUGAUUCAGUUCAGGGUUGAUCC
154665B03	Map2k3/MSS240939	ACCUUCAUCACUAUCGGAGACAGAA	UUCUGUCUCCGAUAGUGAUGAAGGU
154665B05	Map2k3/MSS281881	UGGAGCUGAUGGAACACCCAUUCUU	AAGAAUGGGUGUCCAUCAGCUCCA
154665B07	Mapre1/MSS274053	CCGAAGAAACCUCUCGGCUCCAGUA	UACUGGAGCCGAGAGGUUUCUUCGG
154665B09	Mapre1/MSS274054	CAUUGCAACACAGAGGACUACUGCA	UGCAGUAGUCCUCUGUGUUGCAAUG
154665B11	Mapre1/MSS274055	CAGCAGGUCAAAGUACUGAAGCUUA	UAAGCUUCAGUACUUUGACCUGCUG
154665C01	Cttn/MSS247133	GGAGAAGCAUGAGUCUCAGAAAGAU	AUCUUUCUGAGACUCAUGCUUCUCC
154665C03	Cttn/MSS247134	GGUGGAUCAGUCUGCUGUAGGCUUU	AAAGCCUACAGCAGACUGAUCCACC
154665C05	Cttn/MSS247135	GGCCAUGAGUACCAGUCGAAGCUUU	AAAGCUUCGACUGGUACUCAUGGCC
154665C07	Mapt/MSS275836	CAGUCGAAGAUUGGCUCCUUGGAUA	UAUCCAAGGAGCCAAUCUUCGACUG
154665C09	Mapt/MSS275837	CAGGAGGUGGCAAGGUGCAGAUAAU	AUUUUCUGCACCUUGCCACCUCUG

154665C11	Mapt/MSS275838	CAGGAGGUGGCCAGGUGGAAGUAAA	UUUACUUCCACCUGGCCACCUCCUG
154665D01	Dpysl3/MSS238620	AGGGAUUGACCACAGUGGACGAUUU	AAUUCGUCCACUGUGGUGAUUCCCU
154665D03	Dpysl3/MSS278607	CAGCUCUCUGGAAGUGCCCACUGUA	UACAGUGGGCACUUCCAGAGAGCUG
154665D05	Dpysl3/MSS278608	CCUCUGGUGGUUAUCUGCCAGGGCA	UGCCCUGGCAGAUAAACCACCAGAGG
154665D07	Dpysl4/MSS218676	GCCAGUGUACUGACGGUCAGAUUA	UAUAUCUGACCGUCAGUACACUGGC
154665D09	Dpysl4/MSS218677	CGUGCAUCCUGUUCAGGCAAGAUCU	AGAUCUUGCCUGAACAGGAUGCACG
154665D11	Dpysl4/MSS281959	CCAAGAGCCAUAAACCUGAAUGUAGA	UCUACAUUCAGGUUAUGGCUCUUGG
154665E01	Nck1/MSS206957	GAGAGAGGAUGAGUUGUCAUUGAUA	UAUCAAUGACAACUCAUCCUCUCUC
154665E03	Nck1/MSS206958	UCGUGACAGUGAAUCUUCGCCAAAU	AUUUGGCCGAAGAUUCACUGUCACGA
154665E05	Nck1/MSS275938	UGGCGAGUUCGAAAUUCCAUGAAUA	UAUUAUGGAAUUUCGAACUCGCCA
154665E07	NM_010904.3_stealth_17	CGGACCGGGGCCACCGCGGAUAUAAA	UUUAUAUCCGCGGUGGCCCGGUCCG
154665E09	NM_010904.3_stealth_1032	CAGCCAAAGUGAACACAGAUGCUAU	AUAGCAUCUGUGUUCACUUUGGCUG
154665E11	NM_010904.3_stealth_3945	CCACUUUCAAUUGCUUCCAUGCAAU	AUUGCAUGGAAGCAAUUGAAAGUGG
154665F01	Dpysl5/MSS227180	GAAUCGAGAUCAGCCAUCCAGAGGA	UCCUCUGGAUGGCUGAUCUCGAUUC
154665F03	Dpysl5/MSS227181	GGCAAGGUGGUGAACGAUGACUGUA	UACAGUCAUCGUUACCACCUUGCC
154665F05	Dpysl5/MSS227182	AGGCAGGUCGAGCGGUUUUUGGUAA	UUACCAAUACC GCUCGACCUGCCU
154665F07	1110012M11Rik/MSS246023	ACGUGAAGCUGAGUGGGAAGACUAA	UUAGUCUUCCACUCAGCUUACAGU
154665F09	1110012M11Rik/MSS246025	UGGACAUGCAGAUUCGUGAUGGACAA	UUGUCCAUCACGAUCUGCAUGUCCA
154665F11	1110012M11Rik/MSS292510	CAGACAUUGAGAAGGAGUAUAACUA	UAGUUAUACUCCUUCUCAUGUCUG
154665G01	Numb/MSS207221	ACUGGAAAGUGUAGCUUCCAGUUA	UAACUGGGAAGCUACACUUUCCAGU
154665G03	Numb/MSS207222	GGCGAUGGAUCUGUCAUUGUUUCAU	AUGAAACAAUGACAGAUCCAUCGCC
154665G05	Numb/MSS207223	GGCCACGUAGAAGUUGAUGAGUCAA	UUGACUCAUCAACUUCUACGUGGCC
154665G07	Pfn2/MSS207567	GACUGCACAAUGGACAUCGGGACAA	UUGUCCGGAUGUCCAUUGUGCAGUC
154665G09	Pfn2/MSS207568	UCCAGAGCAUCACGCCAGUAGAAAU	AUUUCUACUGGCGUGAUGCUCUGGA
154665G11	Pfn2/MSS276301	CCGGGAGAGUCUUGGUCUUUGUAAU	AUUACAAAGACCAAGACUCUCCGG
154665H01	Hgs/MSS205115	UGGAGGUGCAGAGACAGCUAGCUAU	AUAGCUAGCUGUCUCUGCACCUGCA
154665H03	Hgs/MSS205116	GGAGUCAGACUGGGAGUCCAUUCUA	UAGAAUGGACUCCAGUCUGACUCC
154665H05	Hgs/MSS205117	CCUGCUAUGAGCAGCUGAACAAGAA	UUCUUGUUCAGCUGCUCAUAGCAGG
154665H07	Htt/MSS205084	GGUUCGACAUGUUGCUGCAACAUCA	UGAUGUUGCAGCAACAUGUCGAACC
154665H09	Htt/MSS274794	GAUGGCUGUGAAGGCAGCUUCUAUA	UAUAGAAGCUGCCUUCACAGCCAUC
154665H11	Htt/MSS274795	CAUCAAAAGCUUUGAUGGAUUCUAAU	AUUAGAAUCCAUCAAGCUUUGAUG
154666A01	Ppp1r8/MSS200043	GGAACCUCACAAGCCUCAACAGAUU	AAUCUGUUGAGGCUUGUGAGGUUCC
154666A03	Ppp1r8/MSS200044	UCAGACAUUGCCAUCGGCUGUGAAA	UUUCACAGCCGAUGGCAAUGUCUGA
154666A05	Ppp1r8/MSS272038	CGAGGGCAUACACUCUGCGAGAGAA	UUCUCUCGCAGAGUGUAUGCCCUCG
154666A07	Prkce/MSS207671	GGACGUUAUCCUACAAGACGAUGAU	AUCAUCGUCUUGUAGGAUAACGUCC
154666A09	Prkce/MSS207672	GCGGGAAGAGCCAAUACUUACACUU	AAGUGUAAGUAUUGGCUCUUCGCCG
154666A11	Prkce/MSS207673	GGACGACUUGUUCGAAUCCAUCUUU	AAGGAUGGAUUCGAACAAGUCGUCC
154666B01	Raf1/MSS235712	GGAGAUGUUGCAGUAAAGAUCUAA	UUAGGAUCUUUACUGCAACAUCUCC
154666B03	Raf1/MSS235713	CCCACAUCAACAACCGAGACCAGAU	AUCUGGUCUCGGUUGUUGAUGUGGG
154666B05	Raf1/MSS235714	GGUGUGAAGGCAGCAGUCUCUACAA	UUGUAGAGACUGCUGCCUUCACACC
154666B07	Pitpnm1/MSS207644	GGGAAACAAGCGGAUCGACUAUUCA	UGAAUAGUCGAUCCGCUUGUUUCCC
154666B09	Pitpnm1/MSS207645	UCAUCCUCCCGUUCUCCUACUCAU	AUGAGUAGGAGGAACGGGAGGAUGA

154666B11	Pitpnm1/MSS207646	GGAUAGAACUGAGCAUGGCUGACAU	AUGUCAGCCAUGCUCAGUUCUAUCC
154666C01	Rtn4/MSS229196	GGAGUGGUGUUUGGUGCCAGCUUAAU	AUAAGCUGGCACCAAACACCACUCC
154666C03	Rtn4/MSS229197	GGCGUCUCUUCUUAGUUGAUGAUUU	AAAUCAUCAACUAAGAAGAGACGCC
154666C05	Rtn4/MSS229198	GGGUGUGAUCCAAGCUAUCCAGAAA	UUUCUGGAUAGCUUGGAUCACACCC
154666C07	Sept9/MSS244103	CCAGCAACAUCCACUUCGAAGCCUA	UAGGCUUCGAAGUGGAUGUUGCUGG
154666C09	Sept9/MSS244104	CCCAAGACGAUCGAAAUCAAGUCGA	UCGACUUGAUUUUCGAUCGUCUUGGG
154666C11	Sept9/MSS244105	UGCUGUCCAACGGCAUUGACGUGUA	UACACGUCAAUGCCGUUGGACAGCA
154666D01	Plcg1/MSS207749	GAGCUCAGGCUGAACAUUAGCUGAU	AUCAGCAUUAUGUUCAGCCUGAGCUC
154666D03	Plcg1/MSS207750	CCUACCUCUGUGAUGUACUCCGAGA	UCUCGGAGUACAUCACAGAGGUAGG
154666D05	Plcg1/MSS207751	GGAUCGUAACCGAGAGGAUCGUUAU	UAUACGAUCCUCUCGGUUACGAUCC
154666D07	Plxna1/MSS207786	GGAUUGGCAACUUUGUCAUUGACAA	UUGUCAAUAGACAAAGUUGCCAUUCC
154666D09	Plxna1/MSS207787	UGGAGGUACCUGGAUUGGCAUCGAA	UUCGAUGCCAAUCCAGGUACCUCCA
154666D11	Plxna1/MSS276393	GACAGGGAAGAAGUUUGCAUCUGUG	CACAGAUGCAAACUUCUUCUCCUGUC
154666E01	Smpd3/MSS226812	CCUCAGAUCAAGAUCUACAUCGAUU	AAUCGAUGUAGAUCUUGAUCUGAGG
154666E03	Smpd3/MSS226813	CCCUUUGCCUUUCUCGGGUUCAUCU	AGAUGAACCCGAGAAAGGCCAAAGGG
155212E02	Smpd3/MSS286183	GGACCGGCUGUUGGCCUCUUUCAUA	UAUGAAAGAGGCCAACAGCCGGUCC
154666E07	Tsg101/MSS238580	CCAGGCAGAGCUUAAUGCCUUGAAA	UUUCAAGGCAUUAAGCUCUGCCUGG
154666E09	Tsg101/MSS238581	GAUUCUAAAUCUGUAUGCAGAGGAA	UUCCUCUGCAUACAGAUUUUAGAAUC
154666E11	Tsg101/MSS278552	CCCAGUGCGUUAUCGAGGUAAUAUA	UAUAUUACCUCGAUAACGCACUGGG
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155212E04	Plxnb1/MSS215125	GGGACUUAAGAAAGUGCAGAUCCA	UGGAUCUGCACUUUCUUAUAGUCCC
154666F05	Plxnb1/MSS280068	CCGACUCUUGCACCUUGGAUGUUGA	UCAACAUCCAAGGUGCAAGAGUCGG
154666F07	Prkca/MSS207659	UCCAAAUGGGCUUUCGGAUCCUUAU	AUAAGGAUCCGAAAGGCCAUUUGGA
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154666G03	NM_011653.2_stealth_725	ACCUAAACAGGUUGAUAGGUCAAU	AUUUGACCUAUCAACCUGUUUAGGU
154666G05	NM_011653.2_stealth_726	CCUAAACAGGUUGAUAGGUCAAUU	AAUUUGACCUAUCAACCUGUUUAGG
154666G07	Vim/MSS238655	GCCAUCAACACUGAGUUCAAGAACA	UGUUCUUGAACUCAGUGUUGAUGGC
154666G09	Vim/MSS238656	GGUGAUCAAUGAGACUUCUCAGCAU	AUGCUGAGAAGUCUCAUUGAUCACC
154666G11	Vim/MSS278712	CACCUGUGAAGUGGAUGCCCUUAAA	UUUAAGGGCAUCCACUUCACAGGUG
154666H01	Sh3glb2/MSS279238	CGGAGCAGCAGAGCGGGAUUUCAUU	AAUGAAAUCCCGCUCUGCUGCUCCG
154666H03	Sh3glb2/MSS279239	CCAAAGCCACGACGGUGCCUGACUU	AAGUCAGGCACCGUCGUGGCCUUUGG
154666H05	Sh3glb2/MSS279240	ACUAGACCUCGUAAUUACAUUCUAU	AUAGAAUGUAAUUACGAGGUCUAGU
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154667A01	Yes1/MSS212966	CCGAGAUCUCCGAGCUGCUAAUAUU	AAUAUUAGCAGCUCGGAGAUCUCGG
154667A03	Yes1/MSS212968	CCUUUAAGAAGGGUGAACGAUUUCA	UGAAAUCGUUCACCCUUCUAAAAGG
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154667A07	Src/MSS238007	GCCCGGCUCAUAGAAGACAACGAUU	AUUCGUUGUCUUCUAUGAGCCGGGC
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154667B03	Akap1/MSS201873	CCAACCCUAAGAAGGUUGACCUUUAU	AUAAGGUCAACCUUCUUAGGGUUGG
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154667D03	Ctnnd1/MSS202668	GAAGCAGUGUGGACCUGCAUCGUUU	AAACGAUGCAGGUCCACACUGCUUC
154667D05	Ctnnd1/MSS202669	CCCAGGAUCACAACCACCUUCUGUA	UACAGAAGGUGGUUGUGAUCCUGGG
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154667D11	Lgals1/MSS275332	UGGUCGCCAGCAACCUGAAUCUCAA	UUGAGAUUCAGGUUGCUGGCGACCA
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154667E05	Vamp2/MSS278660	UCAUCCUCAUCAUCAUCGUUUUA	UAAACGAUGAUGAUGAUGAGGAUGA
154667E07	Myo9b/MSS237347	CCUCCUUCUGAAGAGUAAAGGUUAU	AUACCUUUACUCUUCAGGAAGGAGG
154667E09	Myo9b/MSS237348	UGACUGAAGCCAACCUUGCUCAGAA	UUCUGCAGCAGGUUGGCUUCAGUCA
154667E11	Myo9b/MSS237349	ACAGGCAGAACUACCAGAUUUGGAAA	UUUCCAAUCUGGUAGUUCUGCCUGU
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154667F03	Pik3r1/MSS276325	CCCACUACUGUAGCCAACAACAGCA	UGCUGUUGUUGGCUACAGUAGUGGG
154667F05	Pik3r1/MSS276326	GAGACAUCUCAAGGGAAGAAGUGAA	UUCACUUCUUCCCUUGAGAUGUCUC
154667F07	Actin_(Mouse)	ACGUUGACAUCCGUAAAGACCUCUA	UAGAGGUCUUUACGGAUGUCAACGU
154667F09	Srgap1/MSS202046	GAUCAUAGCCGAGUAUGAAAGUCAA	UUGACUUUCAUACUCGGCUAUGAUC
154667F11	Srgap1/MSS202047	GACUGACCGCCAUUCUGAUAGCUAU	AUAGCUAUCAGAAUGGCGGUCAGUC
154667G01	Srgap1/MSS202048	ACCAACGCCUCCGUGUUCAAGUAUU	AAUACUUGAACACGGAGGCGUUGGU
154667G03	Actin_(Mouse).1	ACGUUGACAUCCGUAAAGACCUCUA	UAGAGGUCUUUACGGAUGUCAACGU
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154667H05	Srgap3/MSS281821	CCGUGAACUGUUGGUUUCUGGUACU	AGUACCAGAUACCAACAGUUCACGG
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154667H11	Clasp1/MSS293730	CGGCACAGUGUUGCCAAGUCUAAUA	UAUUAGACUUGGCAACACUGUGCCG
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154668D06	Clasp2/MSS233612	CCCAAACGCCAAGCUUGAAGGUAAA	UUUACCUUCAAGCUUGGCGUUUGGG
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154668D10	Clasp2/MSS233614	GGGUCUAAUACAGGGUUUAUGAUAAU	AUUUAUCAUAAACCCUGUAUUAGACCC
154668D12	Robo1/MSS208673	CAGCCAUGGUGAAUCGGAGUGGUUA	UAACCACUCCGAUUCACCAUGGCUG
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154668H10	NM_001167745.1_stealth_678	GAGAUACUUGUCAAGUAGCUCUUA	UUAAGAGCUACUUGACAAGUAUCUC
154668H12	Actin_(Mouse).2	ACGUUGACAUCCGUAAAGACCUCUA	UAGAGGUCUUUACGGAUGUCAACGU
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154669A04	Slit1/MSS209184	CCAGGGCCAUGUCCGUGUUAGUUUAU	AUAACUAACACGGACAUGGCCUUGG
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154669C06	Sdc4/MSS238087	GGAACUGGAAGAGAAUGAGGUCAUU	AAUGACCUCAUUCUCUUCCAGUUCC
154669C08	Abl1/MSS235748	ACCACUUGGUGAAGGUGGCUGAUUU	AAAUCAGCCACCUUCACCAAGUGGU
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154669D06	Cables1/MSS226996	GGCCAUGAGCCUGAAGGAGAUCAUU	AAUGAUCUCCUUCAGGCUCAUGGCC
154669D08	Cables2/MSS217424	UCCCUCUAUAUCAAACUGACAUUGAG	CUCAAUGUCAGUUUGAUUAUGAGGGA
154669D10	Cables2/MSS217426	GCGUGUACUCAUCUUUGCUUCAUAC	GUAUGAAGCAAAGAUGAGUACACGC
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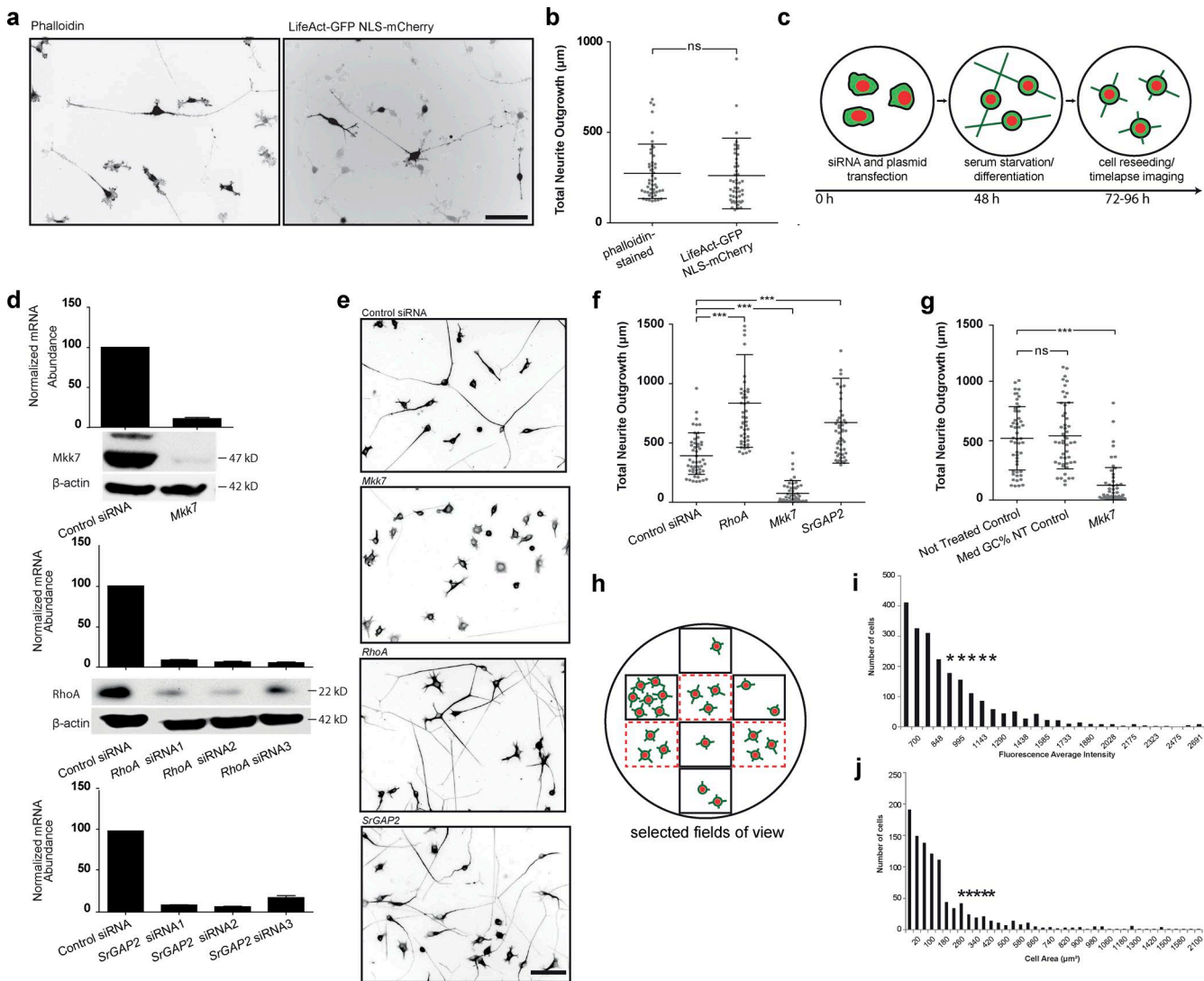


Figure S1. High-content live-cell imaging platform and RNAi pipeline. (a) Neurite outgrowth representative images of untransfected, phalloidin-stained versus LifeAct-GFP/NLS-mCherry transfected, unstained cells. Inverted black-and-white contrast is shown. Bar, 100 μm . (b) Total neurite outgrowth quantification of a. Images were segmented and quantified using the Metamorph neurite outgrowth plugin using phalloidin stainings (nontransfected cells) or the LifeAct-GFP signal (reporter-transfected cells). Population mean \pm standard deviations are shown for 20% cells with highest total neurite outgrowth ($n = 50$ cells). t test was used. Ns, nonsignificant. (c) Schematic representation of transfection and cell differentiation during the siRNA screen. In brief, cells are seeded on day 1, transfected with siRNAs or reporter plasmids on day 2, differentiated by serum starvation on day 3, and replated on laminin-coated coverslips on day 4 for 20 h (time-lapse experiments) or 24 h (steady-state neurite outgrowth measurements). (d) Western blot and quantitative PCR quantification of *Mkk7*, *RhoA*, and *SrGAP2* KD efficiencies. Mean \pm standard deviations from at least three experiments are shown for quantitative PCR data. (e) Representative images of α -tubulin-stained *Mkk7*, *RhoA*, and *SrGAP2* KD cells. Bar, 100 μm . (f) Total neurite outgrowth quantification of *Mkk7*, *RhoA*, and *SrGAP2* KD experiments. Images were segmented and quantified using the Metamorph neurite outgrowth plugin. (g) Total neurite outgrowth quantifications of nontransfected cells, cells transfected with a nontargeting control siRNA with medium GC content, or *Mkk7* siRNA. (f and g) Population mean \pm standard deviation is shown for 20% cells with longest total neurite outgrowth are shown ($n = 50$ cells). One-way ANOVA with Bonferroni's multiple comparison test was used. ***, $P < 0.0001$. (h) Schematics of field-of-view selection with appropriate cell numbers and fluorescence intensities. 3 h after replating of reporter- or siRNA-transfected cells, a Metamorph macro was used to comprehensively scan multiple fields of view of each well of the 24-well plate. On each field of view, cell somata were then automatically segmented, and their area and mean fluorescence intensity were computed. 10 fields of view with appropriate cell number and fluorescence intensities were then selected (represented by a dashed red outline). (i and j) Fluorescence intensity (i) and cell area (j) criteria used to select appropriate fields of view. Occurrence plots of fluorescence intensity (i) and cell area (j) values are shown. Fields of view containing cells with values denoted by multiple asterisks were selected.

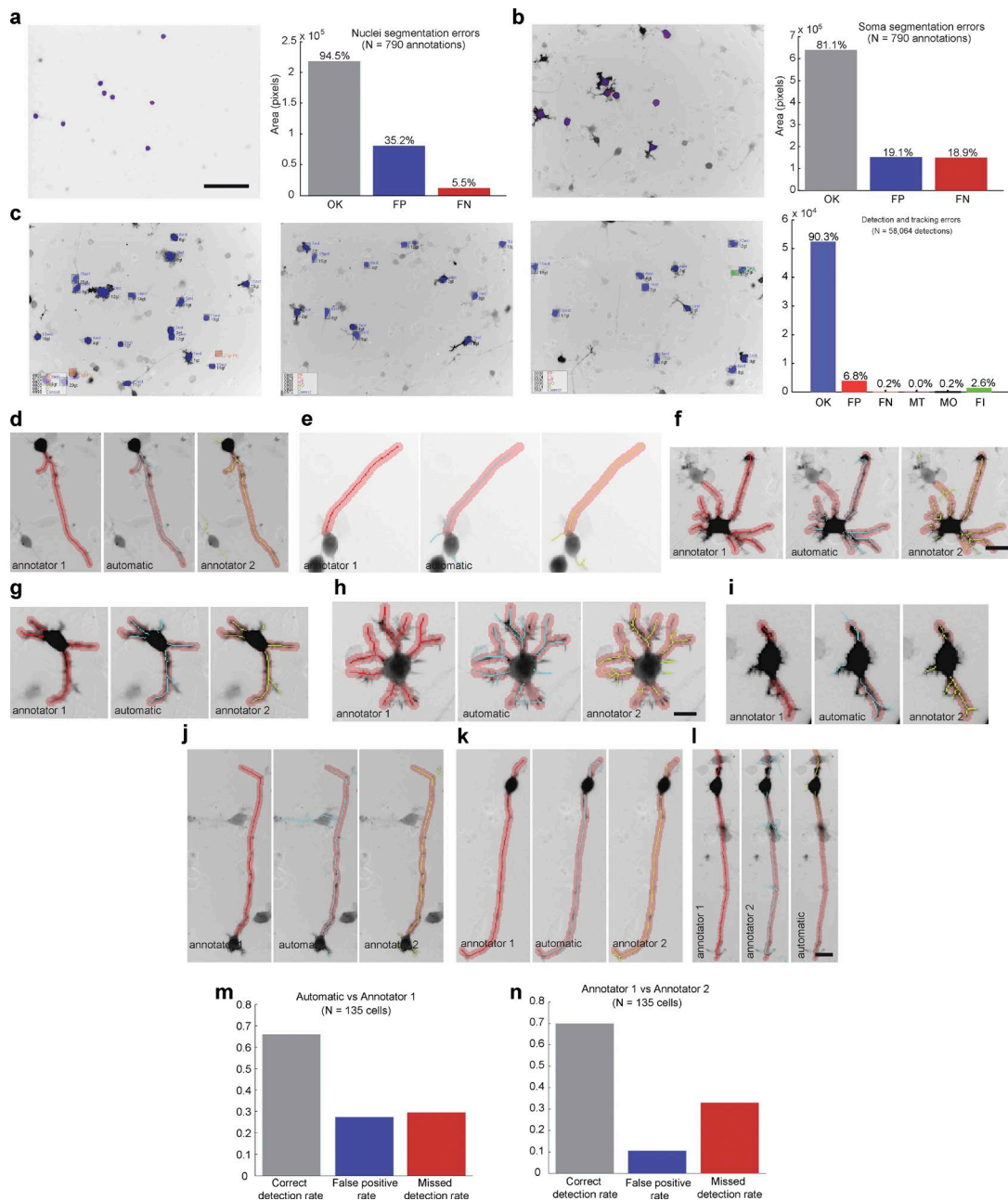


Figure S2. Evaluation of the automatic soma and neurite segmentation and soma tracking. (a) Nucleus segmentation evaluation. The ability of our method to segment nuclei was evaluated using a set of three hand-annotated movies from the screen. A total of 790 cells were annotated and then compared with the corresponding automatic segmentation. The annotation is shown as a red area in the left panel, and the automatic segmentation is depicted by the blue area. The overlap between the two areas is considered correct (the gray bar in the right panel). False positives occur in areas where the detection is present but without an annotation (blue bar in the right panel). False negatives occur where the annotation is not covered by a detection (red in the right panel). (b) Soma segmentation evaluation. The ability of our method to segment somata was evaluated using a set of three hand-annotated movies from the screen. A total of 790 cells were annotated and then compared with the corresponding automatic segmentation. The annotation is shown as a red area in the left panel, and the automatic segmentation is depicted by the blue area. The overlap between the two areas is considered correct (the gray bar in the right panel). False positives occur in areas where the detection is present but without an annotation (blue bar in the right panel). False negatives occur where the annotation is not covered by a detection (red in the right panel). (c) Tracking validation. The ability of our method to detect and identify cells over time was evaluated using a set of 20 hand-annotated movies from the screen. A total of 58,064 detections were annotated (gray bounding box surrounding the soma) if the cell was tracked for more than five frames. Automatic detections are color-coded according to the evaluation result: blue indicates correctly detected; red, false positive; orange, false negative; brown, the tracker covered multiple annotations; magenta, the annotation was covered by multiple trackers; and green, an error occurred in maintaining the identity of the annotation. In the right panel, the results show that 90.3% of annotated cells were correctly tracked. Videos of the 20 evaluation sequences appear in Video 4. Bar, 100 μ m (a–c). (d–l) Segmentations for nine example cells. Raw images are shown in inverted black/white contrast. In each panel, the first image shows the neurites annotated by expert 1 in red. The shaded region denotes the area where a matching annotation is considered valid (tolerance 4.5 pixels = 9 μ m). In the second image, the annotations recovered using our automatic algorithm are shown in cyan. The third image shows the neurite annotation from a second expert. Bars: (d–f) 30 μ m; (g–i) 20 μ m; (j–l) 50 μ m. (m) Bar graph demonstrating the ability of the algorithm to recover segmentations provided by annotator 1. (n) Bar graph showing the disagreement between annotator 1 and annotator 2 on the same set of cells. The level of disagreement between the two annotators is similar to that between the automatic method and annotator 1.

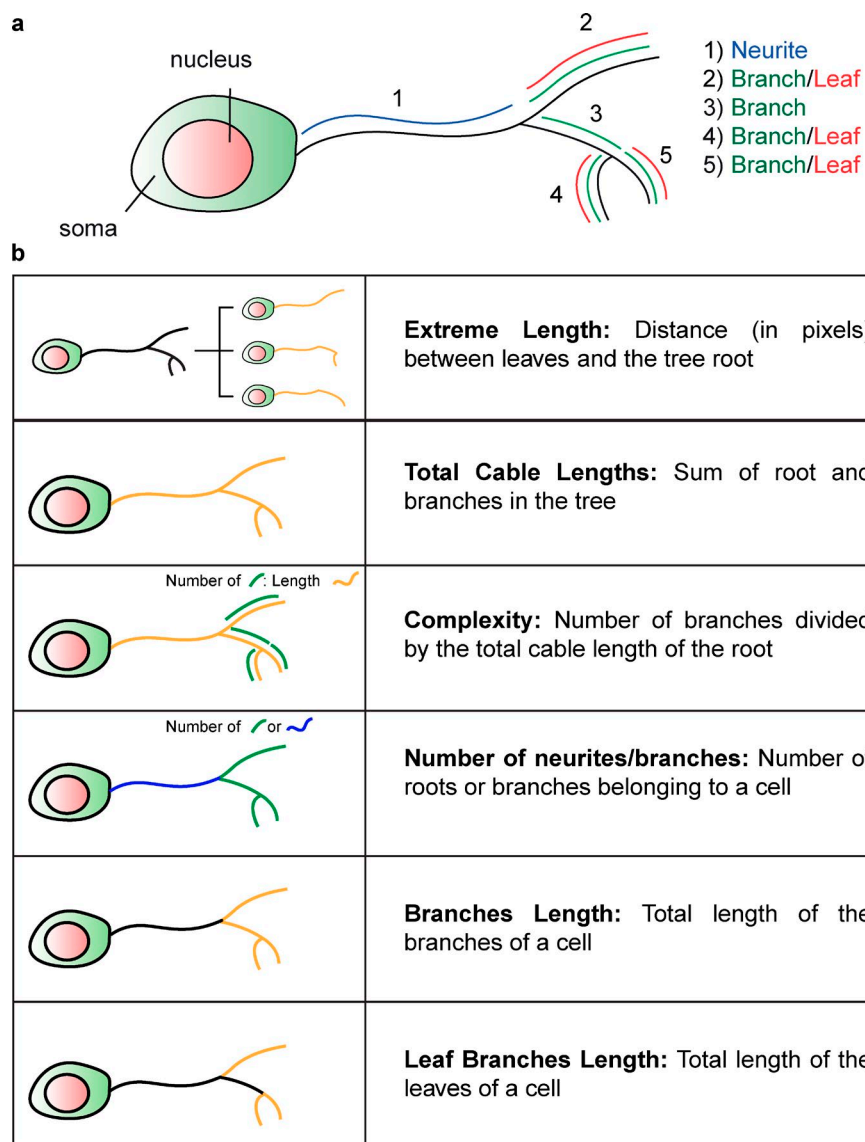


Figure S3. **Definition of parameters for feature extraction.** (a) Scheme representing different nucleus, soma and neurite components, as already shown in Fig. 2 b. (b) Explanatory schematics of different neurite-related parameters subsequently used to compute morphological and morphodynamic features.

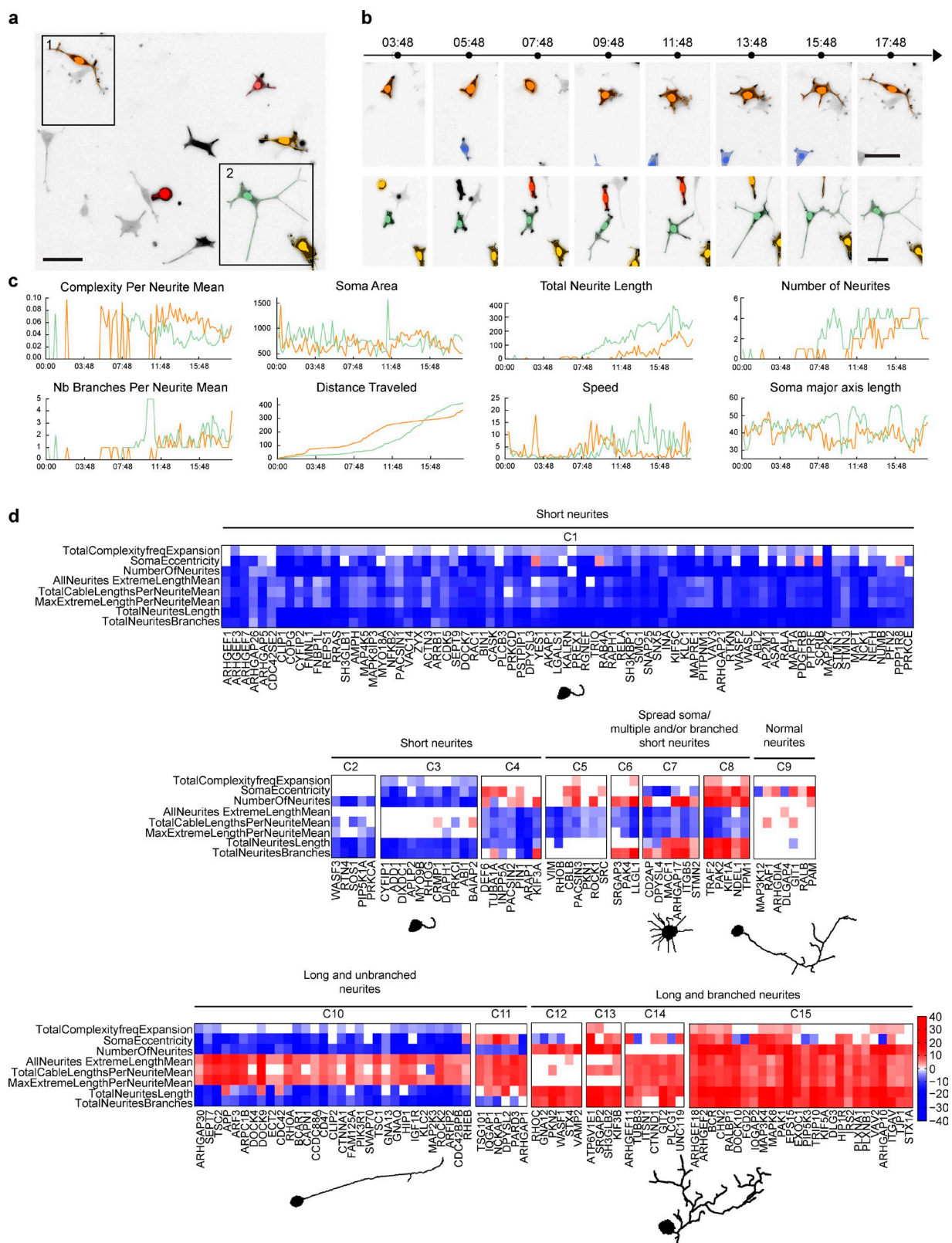


Figure S4. **PC-12 cell neuronal differentiation tracking and segmentation and hierarchical clustering of MDSs using the HS feature sets.** (a) Schematics of PC-12 Lifeact-GFP channel with superimposed nucleus, soma, and neurite segmentations color-coded according to cell identity. Bar, 100 μ m. (b) Example of segmentation of two cells (from insets 1 and 2, shown in panel a). Nucleus, soma, and neurite segmentations are color-coded for cell identity. Time, h:min. Bar, 50 μ m. (c) Temporal evolution of a set of features for the two cells shown in panel b. (d) Hierarchical clustering of MDSs using the HS feature set. Phenotypic clusters and representative images were extracted as described in Fig. 6.

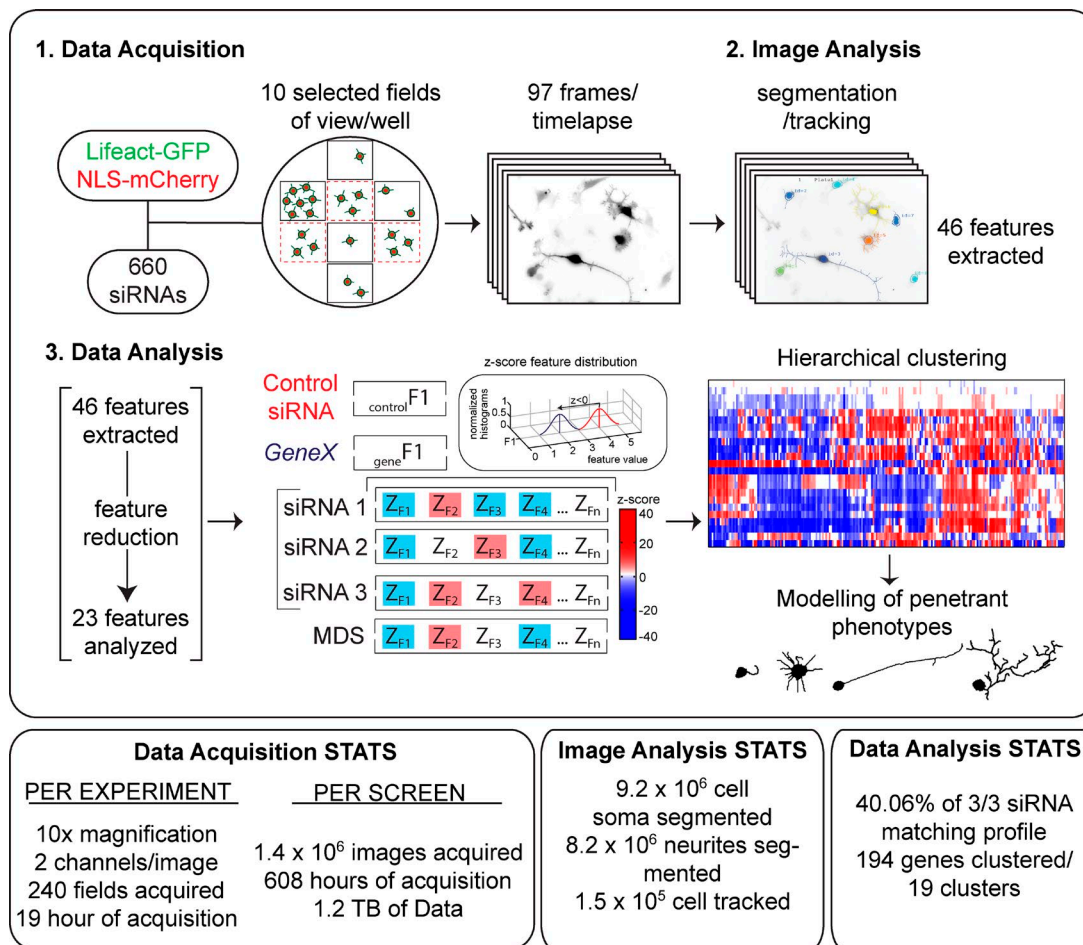
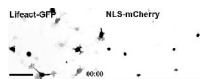
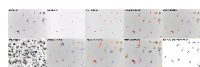


Figure S5. Summary of workflow and statistics of the RNAi screen.



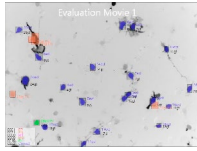
Video 1. **Raw movies of Lifeact-GFP/NLS-mCherry channels in N1E-115 cells.** Time-lapse video of N1E-115 cells cotransfected with control siRNA and Lifeact-GFP/NLS-mCherry is shown in inverted black-and-white contrast. Left: Lifeact-GFP channel; right: NLS-mCherry channel. Time scale is in h:min. Bar, 100 μ m.



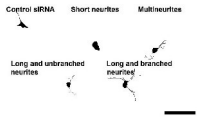
Video 2. **Dynamic visualization of different steps of the computer vision pipeline.** Different steps of the computer vision pipeline. From top left to bottom right: Lifeact-GFP channel, NLS-mCherry channel, detected nuclei marked in red, soma segmentation by region growing, cell identification over time using graph-based tracking, neurite-like structures detected using Hessian-based filter, calibrated likelihood of cell bodies, cell body segmentations after thresholding, backtracing neurite structures from candidate terminals, and final HDS-model graph representation. Bar, 100 μ m.



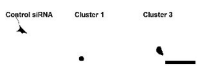
Video 3. **Nuclei and somata segmentation evaluation.** The ability of our method to segment nuclei and somata was evaluated using a set of three hand-annotated movies from the screen. A total of 790 cells were annotated and then compared with the corresponding automatic segmentation. The annotation is shown as a red area, and the automatic segmentation is depicted by the blue area. Quantifications of correct detections, false positives, and false negatives are shown in Fig. S2.



Video 4. **Soma detection and tracking validation.** The ability of our method to detect and identify cells over time was evaluated using a series of 20 hand-annotated movies from the screen. A total of 58,064 hand annotations appear as gray boxes surrounding the soma. Results of the automatic detection and tracking are color coded according to the result: blue, correctly detected; red, false positive; orange, false negative; brown, the tracker covered multiple annotations; magenta, the annotation was covered by multiple trackers; green, an error occurred in maintaining the identity of the annotation.



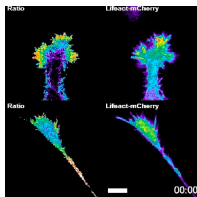
Video 5. **HDS-modeled graph representations of five global phenotypical classes.** HDS-modeled graph representations time-lapse video of the five phenotypical classes extracted from Fig. 6 is shown in inverted black-and-white contrast. From top left to bottom right: control siRNA, short neurite phenotype, spread soma/multiple and/or branched short neurites phenotype, long and unbranched neurite phenotype, and long and branched neurite phenotype. Bar, 100 μ m.



Video 6. **HDS-modeled graph representations of MDSs of dynamic short neurite phenotypes in the LS map.** HDS-modeled graph representations time-lapse video of the short neurite phenotypes extracted from Fig. 6 is shown in contrast. Left: control siRNA; middle: short neurite with low neurite dynamic phenotype (cluster 1); left: short neurite with normal neurite dynamic phenotype (cluster 3). Bar, 100 μ m.



Video 7. **HDS-modeled graph representations of MDSs of dynamic long and branched neurite phenotypes in the LS map.** HDS-modeled graph representations time-lapse video of the dynamic long and branched neurite phenotypes extracted from Fig. 6 is shown in inverted black-and-white contrast. Left: control siRNA; middle: long and branched neurite with normal neurite dynamic phenotype (cluster 18); left: long and branched neurite with high neurite dynamic phenotype (cluster 19). Bar, 100 μ m.



Video 8. **RhoA activation dynamics in advancing and collapsing growth cone in N1E-115 cells.** RhoA activation (ratio images) and Lifeact-mCherry (F-actin) were acquired by time-lapse epifluorescence microscopy using a Nikon Eclipse Ti microscope at one frame every 1 min. Movie plays at 5 fps. Bar, 10 μ m. Time scale, min:s. Movie corresponds to Fig. 8 (a and b).



Video 9. **High-resolution time-lapse imaging of Dlc1 and p190RhoGAP KD growth cones in N1E-115 cells.** Lifeact-mCherry (F-actin) was acquired by time-lapse epifluorescence microscopy using a Nikon Eclipse Ti microscope at one frame every 5 s. Movie plays at 2 fps. Bar, 5 μ m. Time scale, min:s.

Provided online is Table S1, which is a description of all siRNAs in the RNAi library.