Supplemental Data

Supplementary Figure 1. Proteins identified in the screen localising to neurite tips after NGF stimulation. PC12 cells were transfected with respective GFP-ORFs and incubated for 12 hours at 37° C. Thereafter cells were stimulated with NGF for 24 hours, fixed and stained with ConA-Alexa647 to highlight the outline of the cells. Each panel consists of two images – the left one shows the localisation of the GFP-tagged protein and the right one shows the merge between ConA-Alexa647 depicted in red and GFP-ORF in green. In the case of Sorbin and ?-BAR, a DIC image is shown instead of the merged fluorescence image. Above each panel the protein name and SwissPROT ID are shown. Arrows point to the protein accumulation in the neurite tip. Scale bar = 10 μ m.



Supplementary Figure 2. Statistical analysis of proteins affecting neurite outgrowth when over-expressed in PC12 cells. Cells were assayed for neurite outgrowth as described in Materials and Methods. For every protein tested the average neurite length and average number of neurites per cell were calculated for both transfected cells and surrounding non-transfected cells. The left column shows statistical analysis of GFP (A) and control proteins (C,E) known to have an effect on neurite outgrowth upon over-expression, the right column (B, D, F) shows statistical analysis of identified candidate proteins which have similar effects to their corresponding controls. Each panel consists of four graphs; the upper two graphs show the distribution of neurite length in non-transfected and transfected cells with average length ± S.D. indicated in the corner, while the two lower graphs show the distribution of neurite number per cell in non-transfected and transfected cells with average number of neurites per cell \pm S.D. indicated in the corner. A minimum of 60 cells were counted. Statistical significance was tested with the student's t-test, p<0.0001. Statistical analysis of other proteins tested in the screen can be found on the web site http://neurite.embl.de.



Supplementary Figure 3. Proteins that affected neurite outgrowth when overexpressed. PC12 cells were transfected with respective GFP-ORFs and incubated for 12 hours at 37°C. Thereafter cells were stimulated with NGF for 24 hours, fixed and stained with ConA-Alexa647 to highlight the outline of the cells. Each panel consists of an image showing an over-expression phenotype of the respective GFP-ORF (an image is the merge between ConA-Alexa647 depicted in red and GFP-ORF in green). At the side, protein name, SwissPROT ID and sub-cellular localisation are specified. Scale bars = 10μ m. (A) – Proteins that affect the number of neurites per cell when over-expressed (SNAP25 phenotype) (B) – Proteins that affect the number of neurites per cell when over-expressed (Cdc42 phenotype) (C) – Proteins that affect the neurite length when over-expressed (RhoA phenotype).

A	coiled-coil domain containing 8	10 5° C	frizzled homolog 7, FZD7
	Q9H0W5		Q96B74
	plasma membrane, neurite tips		plasma membrane
	RP/EB family member 3, EB3		
2KL	Q9UPY8		
	cytoskeleton - microtubules		

в		Hypothetical protein		muscarinic receptor 2, CHRM2	
		Q9H0H6		Q4VBK6	
	<u> </u>	plasma membrane		plasma membrane	
	Same Bar	Hypothetical protein		Transmembrane protein 10, TM10	
		Q69YW2	× *	Q96PE5	
	and the second	plasma mebrane, golgi	and the second s	plasma membrane, golgi	





SACM1L
Q9NTJ5
Golgi

D		TM4SF10		Hypothetical protein, ZNRF1	
		Q9BQJ4		Q9H083	
	- I	plasma membrane)))) 	plasma mebrane, Golgi, cytoplasm	
		Hypothetical protein	i je	Sorbin, SH3P12	
		Q9BQI0		Q9UFT2	
		cytoplasm, Golgi, cytoskeleton	Sur Andrew	cytoskeleton, neurite tips	
		Hypothetical protein		Hypothetical protein	
	Q9Y4P9	Q9Y4P9			
	cytoskeleton	cytoskeleton			
	·	CRIB-containing BORG2 protein	E S	Kinesin family member 2C, KIF2C	
	00	Q9UKI2		Q99661	
	_	cytoplasm, cytoskeleton		cytoplasm	
	4	Synaptopodin-2, SYP-2		RP/EB family member 2, EB2	
	Q9UMS6		Q15555		
		cytoskeleton		cytoplasm, cytoskeleton	
		Kinesin-associated protein 3, KAP3			
		Q5VXW0			

cytoplasm

Supplementary figure 4. Changes in gene expression 24 hours after NGF treatment measured by real-time quantitative RT-PCR. Relative gene expression levels of the identified proteins and controls were examined using real-time quantitative reverse-transcription PCR (real-time qRT-PCR). RNA was isolated from PC12 cells before and 24h after NGF stimulation and expression changes were determined as described in Materials and Methods. Bars are representing relative expression level changes after 24 hours of NGF stimulation \pm S.D.



Supplementary Figure 5. Effect of Gsk3ß inhibition on neurite number. Cells were stimulated with NGF (100 ng/ml) and with increasing concentrations of CHIR99021. Cells were left to differentiate for 24 hours. They were fixed and stained with ConA-Alexa647 to highlight the outline of the cells. (A) – PC12 cells after 24 hours of NGF stimulation (B) – PC12 cells after NGF stimulation in the presence of 8μ M CHIR99021 stimulation. Scale bars = 10μ m. (C) – Graphs show the changes in the percentages of cells with 1,2,3 and 4 or more neurites in response to increasing CHIR99021 concentrations. 20 images with 20x objective were taken and the number of neurites was scored on a minimum of 250 cells. Each experiment was repeated twice.



Supplementary Video 1. O14782 (Kif3C) dynamics during neurite outgrowth. PC12 cells were analysed by time-lapse microscopy as described in Materials and Methods. They were treated with NGF (100 ng/ml), mounted on the microscope and a transmission (DIC) and fluorescent image was acquired every three minutes for 8 hours. Note O14782 accumulation at the discrete points at the plasma membrane even before any degree of morphological differentiation is visible. Time is indicated in the upper left corner. Scale bar = $20 \,\mu$ m.

Supplementary Video 2. Q8TBG9 (synaptoporin) dynamics during neurite outgrowth. PC12 cells were analysed by time-lapse microscopy as described in Materials and Methods. They were treated with NGF (100 ng/ml), mounted on the microscope and a transmission (DIC) and fluorescent image was acquired every three minutes for 10 hours. Note that Q8TBG9 accumulates at the neurite tip after the neurite formed. Time is indicated in the upper left corner. Scale bar = $20 \,\mu m$.

Supplementary Video 3. Q9UKI2 (Borg2) dynamics during neurite outgrowth. PC12 cells were analysed by time-lapse microscopy as described in Materials and Methods. They were treated with NGF (100 ng/ml), mounted on the microscope and a transmission (DIC) and fluorescent image was acquired every three minutes for 13 hours. Note the appearance of small patches of Borg2 accumulation on the plasma membrane. Time is indicated in the upper left corner. Scale bar = $20 \,\mu m$.

Supplementary Video 4. Q9BQI0 (hypothetical protein) dynamics during neurite outgrowth. PC12 cells were analysed by time-lapse microscopy as described in Materials and Methods. They were treated with NGF (100 ng/ml), mounted on the microscope and a transmission (DIC) and fluorescent image was acquired every three minutes for 11 hours. Time is indicated in the upper left corner. Scale bar = $20 \,\mu$ m.

Supplementary Table 1. Localisation of selected proteins in Vero and PC12 cells. Proteins are sorted according to their localisation in Vero cells.

Plasma membrane

Protein Name	Swissprot ID	Accession Number / clone ID	localisation in Vero cells	localisation in PC12 cells
Actin-like protein 6A	O96019	AL136608 / DKFZp564J2222	plasma membrane	plasma membrane
Hypothetical protein	Q9H097	AL136887 / DKFZp434K1172	plasma membrane	plasma membrane
Hypothetical protein	Q8N3J6	AL834270 / DKFZp761G128	plasma membrane	plasma membrane
Synaptotagmin-3,SYT3 (control)	Q9BQG1	AL136594 / DKFZp7610132	plasma membarne + endosomes, lysosomes	vesicles
MARCKS-like 1	P49006	AL713653 / DKFZp547C2413	plasma membrane	unknown
Junctional adhesion molecule 1, JAM1	Q9Y624	AL136649 / DKFZp564H1562	plasma membrane + junctional adhesions	plasma membrane
htes3_73k11		DKFZp434K1173	plasma membrane + mitochondria	no NGF - nucleus + cytoplasm, with NGF - vesicles
Hypothetical protein	Q8N3L4	AL834247 / DKFZp451E012	plasma membrane + cytoplasm	vesicles + cytoplasm
Hypothetical protein	Q8N3S9	AL831962 / DKFZp451D112	plasma membrane + actin	plasma membrane + golgi + neurite tips
coiled-coil domain containing 8, CCDC8	Q9H0W5	AL136609 / DKFZp564K0322	plasma membrane	plasma membrane + neurite tips
Hypothetical protein	Q9H0H6	AL136793 / DKFZp434A171	plasma membrane	plasma membrane
frizzled homolog 7, FZD7	Q96B74	BC015915 / IMAGE:4549389	plasma membrane + ER	plasma membrane + Golgi
cholinergic receptor, muscarinic 2, CHRM2, isoform a	Q4VBK6	AL832585 / DKFZp451H2216	plasma membrane + ER + Golgi	plasma membrane

Hypothetical protein	Q69YW2	AL137507 / DKFZp761P211	plasma membrane + ER + Golgi	plasma membrane + Golgi
TBC1 domain family member 3, Rab GTPase- activating protein	Q8IZP1	AL136860 / DKFZp434P2235	plasma membrane	plasma membrane
Transmembrane 4 superfamily member 10, TM4SF10	Q9BQJ4	AL136550 / DKFZp761J17121	plasma membrane	plasma membrane + vesicles
Annexin A4 (control)	P09525		not tested	plasma membrane + cytoplasm
Calcium/calmodulin- dependent protein kinase type II beta (control)	Q13554		not tested	plasma membrane + cytoplasm

Plasma membrane and Golgi

Protein Name	Swissprot ID	Accession Number / clone ID	localisation in Vero cells	localisation in PC12 cells
Guanine nucleotide binding protein, alpha inhibiting activity polypeptide1	Q9UGA4	AL049933 / DKFZp564K1216	plasma membrane + Golgi	plasma membrane + Golgi
Integral membrane protein 2C, Transmembrane protein BRI3	Q9NQX7	AL136603 / DKFZp564L1216	plasma membrane + Golgi + ER	Golgi
Hypothetical protein	Q9BQG9	AL136580 / DKFZp761H2024	plasma membrane + Golgi	plasma membrane + Golgi
GPR85, Super conserved receptor expressed in brain 2	Q9NPD1	AL161959 / DKFZp761L08121	plasma membrane + Golgi + ER	plasma membrane + Golgi + vesicles
Protein kinase STYK1	Q9NSH1	AL353940 / DKFZp761P1010	plasma membrane + Golgi	plasma membrane + Golgi + vesicles
prominin 1	Q96EN6	BC012089 / IMAGE:4644690	plasma membrane + Golgi	plasma membrane + Golgi + vesicles
Complement component C1q receptor, CD93 antigen	Q9NPY3	BC028075 / IMAGE:5216061	plasma membrane + Golgi + ER	plasma membrane + Golgi
Protein C20orf98	Q9GZP1	AL136915 / DKFZp586F1919	plasma membrane + Golgi + endosomes, lysosomes	Golgi + vesicles
Sodium channel beta-3 subunit, CIB3	Q9NY72	AL136589 / DKFZp761F182	plasma membrane + Golgi + ER	Golgi

Hypothetical protein PRO0971, Gamma-BAR splice variant	Q96GG6	BC009485 / IMAGE:3623656	plasma membrane + Golgi	Golgi + neurite tips
Synaptoporin (control)	Q8TBG9	AL834457 / DKFZp547J226	plasma membrane + Golgi + ER	plasma membrane + Golgi + neurite tips
Transmembrane protein 10, TM10	Q96PE5	AL834229 / DKFZp761K0421	plasma membrane + Golgi	plasma membrane + Golgi
Gamma-BAR, AP-1 interacting protein	Q9H0V0	AL136628 / DKFZp564C182	plasma membrane + Golgi	Golgi + neurite tips
Hypothetical protein, zinc and ring finger 1, ZNRF1	Q9H083	AL136903 / DKFZp434E229	plasma membrane + Golgi	plasma membrane + Golgi
Synaptosomal-associated protein 25 (SNAP-25) (control)	P60880		not tested	plasma membrane + Golgi

Golgi

Protein Name	Swissprot ID	Accession Number / clone ID	localisation in Vero cells	localisation in PC12 cells
Hypothetical protein	Q9BQG3	AL136588 / DKFZp761D112	Golgi + ER	Golgi
Hypothetical protein	Q9H0W4	AL136610 / DKFZp564K0822	Golgi + ER	cytoplasm
Hypothetical protein	Q9UFC6	AL133049 / DKFZp434E1315	Golgi	Golgi
Hypothetical protein	Q9H076	AL136916 / DKFZp586G1919	Golgi	Golgi + vesicles
NDRG2 protein	Q9UN36	AL136574 / DKFZp761J1923	Golgi	cytoplasm
NDRG4 protein (Brain development-related molecule 1)	Q9ULP0	AL136584 / DKFZp761P2324	Golgi	cytoplasm
Hypothetical protein	Q9H0B8	AL136861 / DKFZp434B044	Golgi + ER	cytoplasm
Secretory carrier-associated membrane protein 5	Q8N3M4	AL834226 / DKFZp761D1321	Golgi + ER	Golgi + vesicles
Hypothetical protein	Q9H0V1	AL136626 / DKFZp564C012	Golgi + ER	Golgi + vesicles

Hypothetical protein	Q8N3G9	AL834349 / DKFZp761H2017	Golgi + ER + endosomes, lysosomes	vesicles
Synaptotagmin 13	Q9P2C0	AL512743 / DKFZp547F213	Golgi	Golgi + vesicles
ITBA1 protein (CXorf12 protein)	Q14656	BC008203 / IMAGE:3868838	Golgi + ER	Golgi + vesicles
Hypothetical protein	Q6UWH4	AL832589 / DKFZp451L1216	Golgi	Golgi
Proprotein convertase subtilisin/kexin type 5, preproprotein	Q96EP4	AL834522 / DKFZp667I0310	Golgi + ER	Golgi
Deoxyribonuclease gamma precursor, Deoxyribonuclease I- like 3	Q13609	BC015831 / IMAGE:4723725	Golgi	Golgi
Surfeit 4	O15260	BX647961 / DKFZp686P1751	Golgi	Golgi + vesicles
Ras-related protein Rab-6C (control)	Q9H0N0	AL136727 / DKFZp566K144	Golgi	Golgi
Ras-related protein Rab-33B (control)	Q9H082	AL136904 / DKFZp434G099	Golgi	Golgi
Coatomer beta subunit (control)	P53618	AL136593 / DKFZp761K102	Golgi	Golgi
Ras-related protein Rab-1B (control)	Q9H0U4	AL136635 / DKFZp564I172	Golgi	Golgi
Coatomer epsilon subunit	O14579	AL136928 / DKFZp586A1622	Golgi	Golgi
Biglycan	P21810	BC002416 / IMAGE:3162633	Golgi + ER	Golgi
Vesicle docking protein p115, VDP	Q86TB8	AL832010 / DKFZp451D234	Golgi	Golgi
ADP-ribosylation factor-like protein 1, ARL1	P40616	BX537387 / DKFZp686O2082	Golgi	Golgi
KIAA1040 Hypothetical protein	Q7Z3U7	BX537415 / DKFZp686F0546	Golgi	Golgi
Zinc transporter ZnT-7, Solute carrier family 30	Q8NEW0	BX537375 / DKFZp686D18111	Golgi + endosomes, lysosomes	Golgi + neurite tips
Serine protease 23 precursor	O95084	AL136914 / DKFZp586B0719	Golgi	neurite tips
Hypothetical protein	Q9Y4S1	AL080121	Golgi + endosomes	Golgi + neurite tips

Secreted phosphoprotein 1, SPP-1	Q96IZ1	BC007016 / IMAGE:4052438	Golgi	Golgi + neurite tips
SACM1L	Q9NTJ5	AL136831 / DKFZp434O1328	Golgi + ER	Golgi
GTP-binding protein SAR1 (COPII-associated small GTPase) (control)	Q9NR31		not tested	Golgi + cytoplasm
Vesicle-associated membrane protein 2 (Synaptobrevin 2), VAMP2 (control)	Q9BUC2		not tested	Golgi + neurite tips
GTP restricted dominant negative mutant of Sar1p GTPase (Sar1p ^{H79G}) (control)			not tested	Golgi

Cytoskeleton

Protein Name	Swissprot ID	Accession Number / clone ID	localisation in Vero cells	localisation in PC12 cells
Hypothetical protein	Q9H0T6	AL136646 / DKFZp564B1162	cytoskeleton - focal adhesions	plasma membrane
Epithelial protein lost in neoplasm, EPLIN	Q9UHB6	AL136911 / DKFZp586I1918	cytoskeleton - actin	cytoskeleton- actin
Hypothetical protein, DNA polymerase-transactivated protein 6	Q9NTW4	AL110124 / DKFZp564A2416	cytoskeleton - microtubules	cytoskeleton - microtubules
Hypothetical protein	Q9H0M7	AL136730 / DKFZp434H2010	cytoskeleton - focal adhesions	plasma membrane
Gamma-1-syntrophin, G1SYN, Syntrophin 4	Q9NSN8	AL161971 / DKFZp761I2312	cytoskeleton - filaments	cytoskeleton + cytoplasm + nucleus
Hypothetical protein		BI828924 / IMAGE:5166884	cytoskeleton - microtubules	cytoskeleton - microtubules
Rho/rac guanine nucleotide exchange factor (GEF) 2	Q9H023	AL512715 / DKFZp547L106	cytoskeleton - microtubules	cytoskeleton - microtubules + cytoplasm
Neuronal-specific septin 3	Q8N3P3	AL833942 / DKFZp761P16121	cytoskeleton	unknown + neurite tips
intracellular membrane- associated calcium- independent phospholipase A2 gamma variant	Q8N3I3	AL834147 / DKFZp761P18121	cytoskeleton	unknown + neurite tips

Kinesin-like protein KIF3C	O14782		not tested	cytoplasm + neurite tips
Microtubule-associated protein tau	P10636	BC000558 / cytoskeleton - microtubules		cytoskeleton - microtubules
Kinesin family member 23, KIF23	Q8WVP0	BC017705 / IMAGE:4420248	cytoskeleton - microtubules	cytoskeleton - microtubules + neurite tips
Angiomotin-like 1	Q8IY63	BX648729 / DKFZp451H0710	cytoskeleton	unknown
Hypothetical protein		AL832686 / DKFZp313C0434	cytoskeleton - actin	cytoskeleton
Leiomodin 3, LMOD3	Q5JPG6	AL832033 / DKFZp451P024	AL832033 / DKFZp451P024 cytoskeleton - actin + cytoplasm	
Hypothetical protein		AL833438 / DKFZp313E0842	cytoskeleton - actin	cytoskeleton
Hypothetical protein		AL832031 / DKFZp451O084	cytoskeleton - actin	cytoskeleton + aggregates
Myopalladin, MYPN	Q86T37	AL832379 / cytoskeleton - DKFZp451K027 actin		aggregates
Spermatogenesis associated protein 7, Spermatogenesis associated protein HSD3	Q9P0W8	AL136604 / cytoskeleton - DKFZp564F2122 microtubules		cytoskeleton - microtubules
G2/mitotic-specific cyclin B2	O95067	AL080146 / DKFZp434B174	cytoskeleton - microtubules + aggregates	cytoskeleton + cytoplasm
Hypothetical protein	Q9BQI0	AL833896 / DKFZp761N011	cytoskeleton - actin	cytoskeleton + cytoplasm
Synaptopodin	Q8N3V7	AL831818 / DKFZp451G172	cytoskeleton - actin	cytoskeleton
Microtubule-associated protein, RP/EB family, member 3	Q9UPY8		not tested	cytoskeleton - microtubules
Microtubule-associated protein, RP/EB family, member 2	Q15555	BC007318 / IMAGE:2989643	cytoskeleton - microtubules	cytoskeleton - microtubules + cytoplasm
Hypothetical protein	Q9BQI0	AL833896 / DKFZp761N011	cytoskeleton - actin	cytoskeleton + Golgi + cytoplasm

Sorbin and SH3 domain- containing protein 1, SH3P12	Q9UFT2	AL117472 / DKFZp586P1422	cytoskeleton - focal adhesions	cytoskeleton + neurite tips
Hypothetical protein	Q9Y4P9	AL080154 / DKFZp434I114	cytoskeleton - microtubules	cytoskeleton - microtubules + nucleus
Hypothetical protein	Q9H0Q7	AL136694 / DKFZp564J047	cytoskeleton - microtubules	cytoskeleton - microtubules
CRIB-containing BORG2 protein	Q9UKI2	AL136842 / DKFZp434A0530	cytoskeleton - actin	cytoskeleton + cytoplasm
Kinesin family member 2C, KIF2C	Q99661	BC014924 / IMAGE:3909438	cytoskeleton - microtubules	cytoplasm
Synaptopodin-2, SYP-2	Q9UMS6	AL833547 / DKFZp686M023	cytoskeleton - actin	cytoskeleton - microtubules
Kinesin-associated protein 3, KAP3	Q5VXW0		not tested	cytoplasm
Tubulin alpha 2 , TUBA2 (control)	Q13748		not tested	cytoskeleton - microtubules
actin beta, ACTB (control)	P60709		not tested	cytoskeleton - actin
Microtubule-associated protein, RP/EB family, member 1 (control)	Q15691		not tested	cytoskeleton - microtubules
Cell division cycle 42, CDC42 (control)	P60953		not tested	cytoplasm
Transforming protein RhoA (control)	P61586		not tested	cytoplasm

Negative controls						
Protein name (SwissPROT ID)	Localization in PC12 cells	Function/citation describing how overexpression effects neurite outgrowth	Overexpression effect on neurite outgrowth			
GFP	cytoplasm	/	no effect			
Tubulin alpha (Q13748)	microtubules	cytoskeletal component	no effect			
Actin beta (P60709)	actin	cytoskeletal component	no effect			
RB/EB family member 1 (Q15691)	microtubules	cytoskeletal component	no effect			
Sar1 (Q9NR31)	Golgi, cytoplasm	membrane traffic	no effect			
Rab6 (Q9H0N0)	Golgi	membrane traffic	no effect			
Rab33 (Q9H082)	Golgi	membrane traffic	no effect			
Rab1 (Q9H0U4)	Golgi	membrane traffic	no effect			
Coatomer beta subunit (P53618)	Golgi	membrane traffic	no effect			
Synaptotagmin-3 (Q9BQG1)	Golgi, neurite tips	synaptic exocytosis/ (Nonet et al., 1993)	no effect			
Synaptoporin (Q8TBG9)	Golgi, neurite tips	synaptic vesicles maturation/ (Dai et al., 2003)	no effect			
Annexin A4 (P09525)	cytoplasm, plasma membrane	synaptic exocytosis/ (Willshaw et al., 2004)	no effect			
Positive controls						
SNAP-25 (P60880)	Golgi, plasma membrane	vesicle fusion/ (Shirasu et al., 2000)	increases the number of neurites per cell (from 2 to 2.7 neurites per cell on average)			
Cdc42 (P60953)	Golgi, plasma membrane	actin cytoskeleton remodelling/ (da Silva and Dotti, 2002)	induces sprouting of numerous filopodia-like structures, constitutively active form of Cdc42 increases the number of neurites per cell.			
small chemical Y-27632 (ROCK inhibitor)	/	actin cytoskeleton remodelling/ (da Silva and Dotti, 2002; Hirose et al., 1998)	increases the number of neurites per cell (from 2 to 2.7 neurites per cell on average)			
VAMP2 (Q9BUC2)	Golgi, neurite tips	vesicle fusion/ (Shirasu et al., 2000)	increases the neurite length by 30%			
CaMK-II beta (Q13554)	plasma membrane, cytoplasm	prominent kinase involved in the many aspects of neurite outgrowth/ (Nomura et al., 1997)	increases the neurite length by 25%			
RhoA (P61586)	cytoplasm, plasma membrane	actin cytoskeleton remodelling/ (Nusser et al., 2002)	blocks neurite outgrowth in 81% of the cells			
GTP restricted dominant negative mutant of Sar1	Golgi	blocks ER to golgi transport	blocks neurite outgrowth in 65% of the cells			

Supplementary Table 2. Controls used in the establishment of the screen

medbared by rear time quar					
Protein Name	SwissPROT ID	primer pair used	fold change +/- S.D.		
Proteins whose overexpression affects the number of neurites per cell					
Coiled-coil domain containing 8, CCDC8	Q9H0W5	5' gttgggggaacccaactt 3' 5' cagtgtcgcctccctgtaat 3'	3.59 +/- 0.54		
Frizzled homologue 7, FZD7	Q96B74	5' cgcctttcactgcgatgt 3' 5' gcaagagaaggggaaagacc 3'	1.31 +/- 0.21		
Hypothetical protein, new isoform of m2 receptor	Q4VBK6	5' gcatccccaatacagtatggac 3' 5' cggattgatggtgctattga 3'	1.19 +/- 0.14		
Hypothetical protein	Q69YW2	5' cacaaagacgccgagacc 3' 5' cttctcgcgaacctgcac 3'	0.99 +/- 0.01		
RP/EB family member 3	Q9UPY8	5'aaaggattacaaccctctgctg 3' 5'ggacgtcctctgtggaactg 3'	2.7 +/- 0.96		
Protein	s whose overexpress	ion affects neurite length			
Gamma-BAR	Q9H0V0	5' gaatgacgacagcacctcct 3' 5' ggcagcatcctattcattcc 3'	1.65 +/- 0.13		
SACMIL	Q9NTJ5	5' agtttattagctcggcgttcc 3' 5' tcccacatgcaaaactccta 3'	1.61 +/- 0.05		
Proteins v	vhose overexpression	blocked neurite outgrowth			
Transmembrane 4 superfamily member 10	Q9BQJ4	5' gttgctgtcatgctttttgc 3' 5' ttttcaagctcacagtttcaataaa 3'	2.04 +/- 0.16		
Hypothetical protein, zinc and ring finger 1	Q9H083	5' tggaggagctgcttcagg 3' 5' tcacttcaaaccatgagtctatgc 3'	1.4 +/- 0.34		
Hypothetical protein	Q9BQI0	5' tcctaccgagactttgtgaacat 3' 5' ttccttcaaacatcatgaccag 3'	0.58 +/- 0.02		
Hypothetical protein	Q9Y4P9	5' acctetecegggatttea 3' 5' gggacataattgtgeatetee 3'	1.33 +/- 0.14		
CRIB-containing BORG2 protein	Q9UKI2	5' gaatccettteegaeetgae 3' 5'aaaagtgagggeeeeaga 3'	0.69 +/- 0.05		
Kinesin family member 2C, KIF2C	Q99661	5' gccatttcccattcacaca 3' 5' catcaacaacggtaattctgtca 3'	0.26 +/- 0.1		
RP/EB family member 2	Q15555	5' tcctacagttggggaatggc 3' 5'acccatgcaatgatgtcatg 3'	0.53 +/- 0.18		
Proteins used as controls and others					
Adenomatous polyposis coli protein (APC protein)	P25054	5' aaggcatggaccaggaca 3' 5' cagggcagatctgatgctc 3'	2.79 +/- 0.36		
Glycogen synthase-kinase 3 beta (Gsk3 beta)	P49481	5' cacatcettggaegaaggte 3' 5' aggagaeggetaeaeagtge 3'	1.38 +/- 0.36		
Cell division cycle 42, Cdc42	P60953	5'ggctgtcaagtatgtggagtgt 3' 5' ggctettetteggttetgg 3'	1.14 +/- 0.09		
Ras-related protein RAB5A	P20339	5'gctaagacatcaatgaatgtaaatgaa 3' 5' tgtgcaggctcagtaaggtc 3'	2.32 +/- 0.45		
RP/EB family member 1	Q15691	5' atgacgaagctgctgaattg 3' 5' ctccttctccaagtcttcaacag 3'	1.17 +/- 0.2		
Kinesin-like protein Kif3C	O14782	5' gaagatgctggaggatctgaa 3' 5' ccccaatgagtagcttgctt 3'	2.04 +/- 0.03		
neurofilament triplet M protein (NF- M) (positive control)	P07197	5' cgtcatttgcgagaatacca 3' 5' tcttcaccctccagtagtttcc 3'	4.65 +/- 0.05		

Supplementary Table 3. Changes in gene expression 24 hours after NGF treatment measured by real-time quantitative RT-PCR

Supplementary references:

da Silva, J. S., and Dotti, C. G. (2002). Breaking the neuronal sphere: regulation of the actin cytoskeleton in neuritogenesis. Nat Rev Neurosci *3*, 694-704.

Dai, J., Ji, C., Gu, S., Wu, Q., Wang, L., Xu, J., Zeng, L., Ye, X., Yin, G., Xie, Y., and Mao, Y. (2003). Cloning and sequence analysis of the human cDNA encoding the synaptoporin (delta), a highly conservative synaptic vesicle protein. Mol Biol Rep *30*, 185-191.

Hirose, M., Ishizaki, T., Watanabe, N., Uehata, M., Kranenburg, O., Moolenaar, W. H., Matsumura, F., Maekawa, M., Bito, H., and Narumiya, S. (1998). Molecular dissection of the Rho-associated protein kinase (p160ROCK)-regulated neurite remodeling in neuroblastoma N1E-115 cells. J Cell Biol *141*, 1625-1636.

Nomura, T., Kumatoriya, K., Yoshimura, Y., and Yamauchi, T. (1997). Overexpression of alpha and beta isoforms of Ca2+/calmodulin-dependent protein kinase II in neuroblastoma cells -- H-7 promotes neurite outgrowth. Brain Res 766, 129-141.

Nonet, M. L., Grundahl, K., Meyer, B. J., and Rand, J. B. (1993). Synaptic function is impaired but not eliminated in C. elegans mutants lacking synaptotagmin. Cell *73*, 1291-1305.

Nusser, N., Gosmanova, E., Zheng, Y., and Tigyi, G. (2002). Nerve growth factor signals through TrkA, phosphatidylinositol 3-kinase, and Rac1 to inactivate RhoA during the initiation of neuronal differentiation of PC12 cells. J Biol Chem 277, 35840-35846.

Shirasu, M., Kimura, K., Kataoka, M., Takahashi, M., Okajima, S., Kawaguchi, S., Hirasawa, Y., Ide, C., and Mizoguchi, A. (2000). VAMP-2 promotes neurite elongation and SNAP-25A increases neurite sprouting in PC12 cells. Neurosci Res *37*, 265-275.

Willshaw, A., Grant, K., Yan, J., Rockliffe, N., Ambavarapu, S., Burdyga, G., Varro, A., Fukuoka, S., and Gawler, D. (2004). Identification of a novel protein complex containing annexin A4, rabphilin and synaptotagmin. FEBS Lett *559*, 13-21.