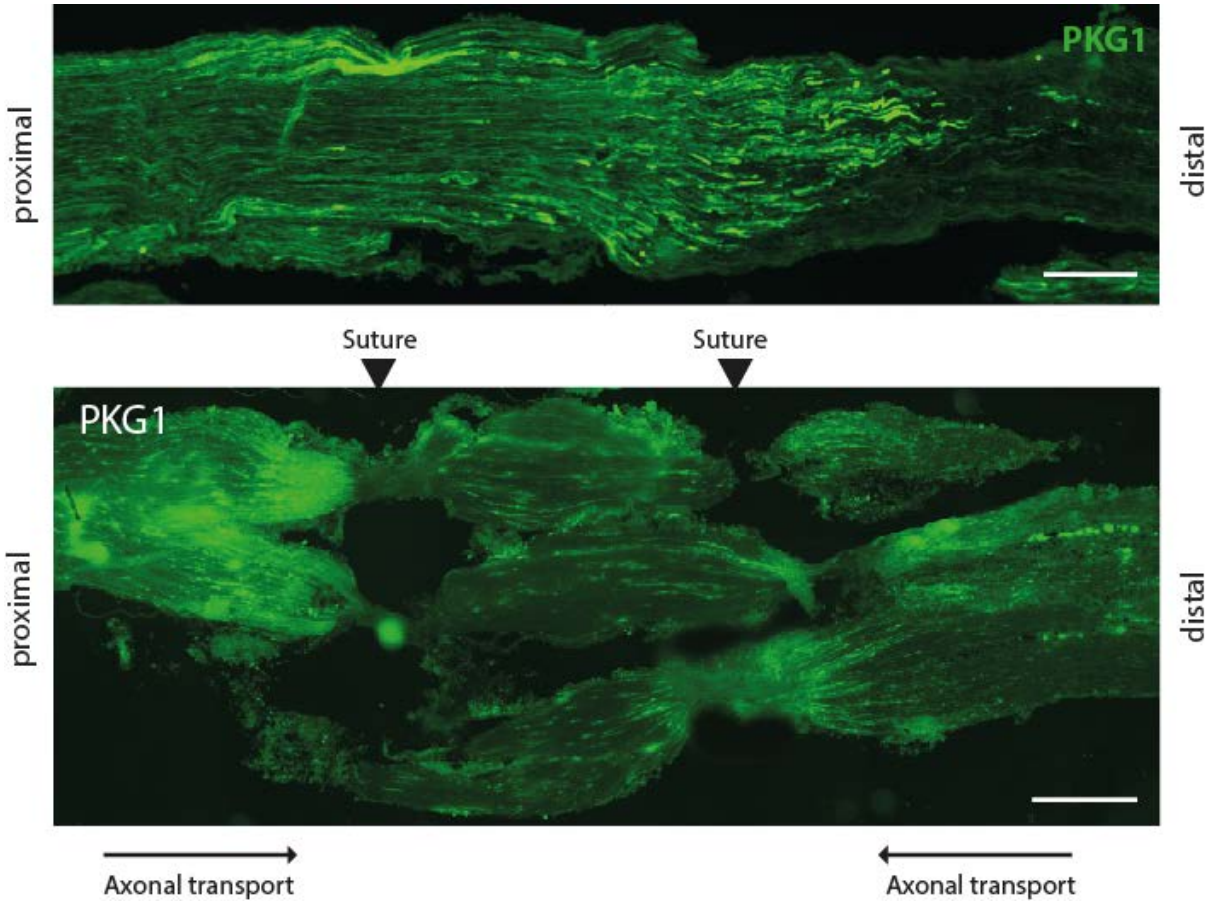
Supplementary Figures, Legends and Tables

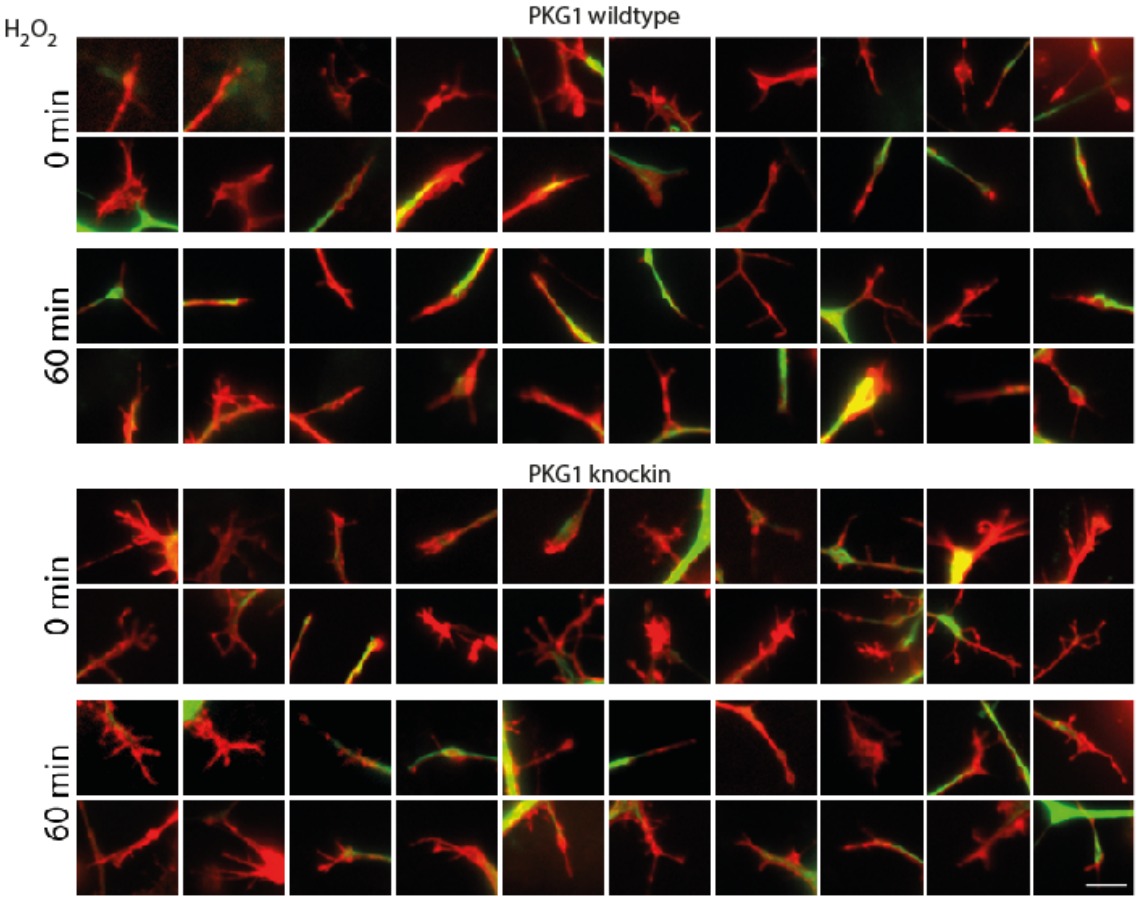
Suppl. Fig. 1



Suppl. Fig. 1: Axonal transport of PKG1

Immunofluorescence analysis of PKG1 in ipsilateral nerves at the site of the crush lesion (top image) showing the accumulation of PKG1 in front of the lesion and in a double-suture model (bottom), where the sciatic nerve was constricted with 2 sutures with a distance of approximately 5 mm. Tiled images were captured on a fluorescence microscope (10x objective lens) and stitched to reconstruct long distances of the sciatic nerve. Proteins which are transported from the DRGs to the periphery accumulate in front of the proximal suture, where most of the PKG1 accumulation occurred. Proteins transported from the periphery back to the DRGs (retrograde) accumulate distal of the 2nd suture. Here, the accumulation of PKG1 was weaker. The analysis suggests that PKG1 is transported in both directions, but mainly antegradely. Scale bars 200 µm (top) and 500 µm (bottom).

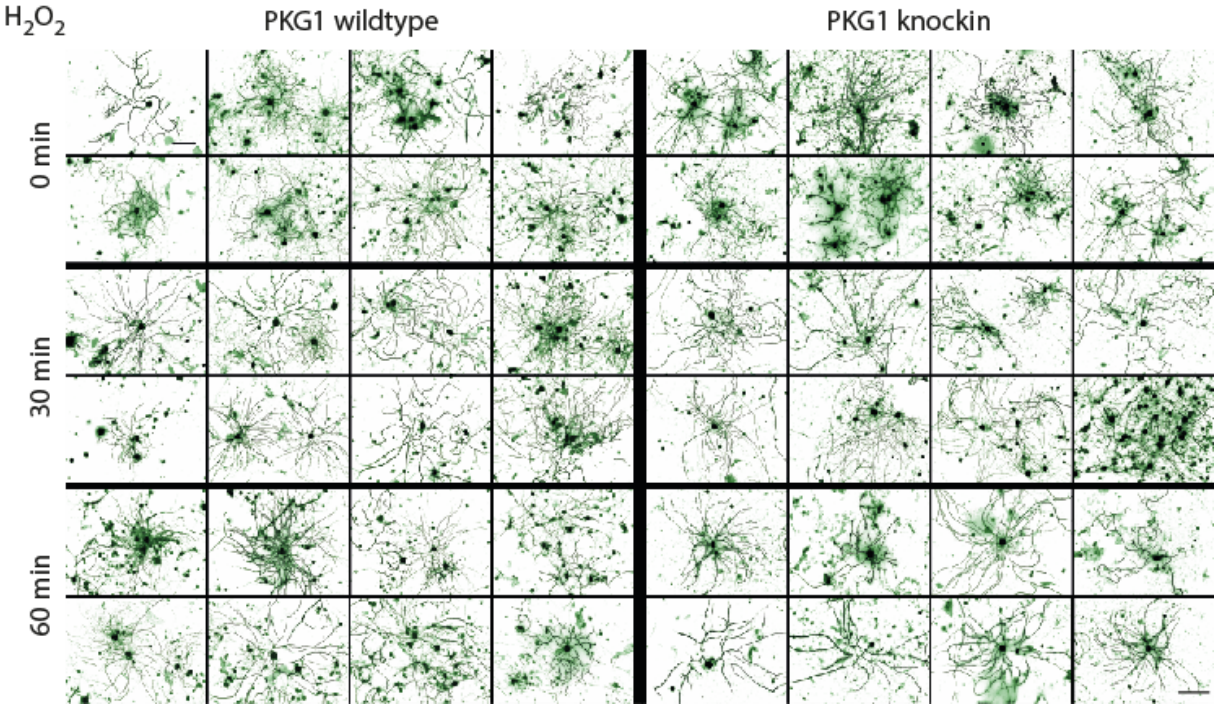
Suppl. Fig. 2



Suppl. Fig. 2: Immunofluorecent analysis of growth cone morphology in DRG neurons of Cys42Ser redox-dead PKG1 mutant mice

The images show immunofluorescence analyses of F-actin (phalloidin-Alexa594) and neurofilament of 200 kDa (NF200, green) in DRG neurons of Cys42Ser PKG1 redox dead mutant versus wild type mice at baseline and 60 min after hydrogen peroxide (H_2O_2 100 µM) stimulation. Exemplary growth cones were enlarged from immunofluorescent images taken with an 20x objective lens. The images were obtained from 4 cultures per condition and genotype. Quantitative analysis is shown in Figure 5 of the main manuscript. Scale bar 10 µm.

Suppl. Fig. 3



Suppl. Fig. 3: Immunofluorescence analysis of neurite outgrowth of DRG neurons of Cys42Ser redox-dead PKG1 mutant mice

The images show exemplary immunofluorescence images of neurofilament of 200 kDa (NF200, green) in DRG neurons of Cys42Ser PKG1 redox dead mutant versus wild type mice at 48h after plating. Neurons were stimulated with vehicle or hydrogen peroxide (H_2O_2, 100 µM) for 30 or 60 min. Quantitative analysis of outgrowth and branching was done with the segmentation plugin of FIJI ImageJ and is presented in Figure 6 D. The binary 8-bit image of the green channel was inverted and is presented as thallium LUT pseudocolor. Scale bar 100 µm.

Suppl. Table 1

Antibodies and stainings

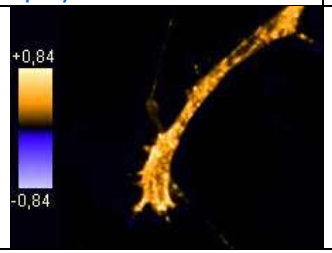
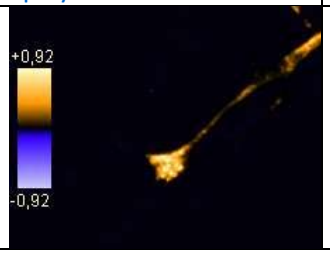
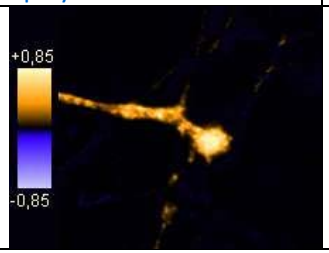
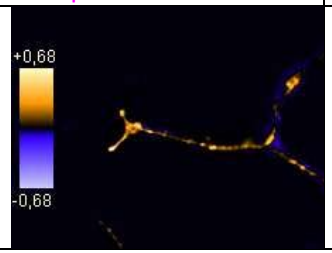
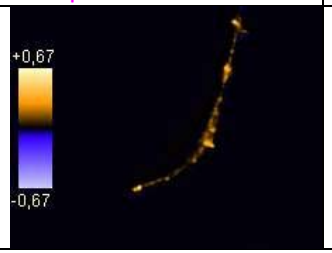
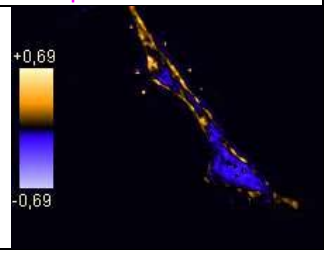
Antibody	Manufacturer	Product #	Dilution	Host	Type	Use
ATF3	Santa Cruz	sc-188	1:500	rabbit	pab	IFL
β-Actin Clone AC-15	Sigma	A5441	1:1000	mouse	mab	Wblot
Cofilin	Santa Cruz	sc-33779	1:200	rabbit	pab	Wblot
ERAB	Abcam	ab52243	1:200	rabbit	pab	IFL
GAP43	Millipore	AB 5220	1:200	rabbit	pab	IFL
GFP-FITC	Abcam	ab6662	1:200	goat	pab	IFL
NeuN	Millipore	MAB377	1:200	mouse	mab	IFL
NF200	Sigma	N-4142	1:200	rabbit	pab	IFL
p-Cofilin	Santa Cruz	sc-12912-R	1:200	rabbit	pab	Wblot
PKG1	Enzo	KAP-PK005-D	1:200	rabbit	pab	Wblot/ IFL
PKG1	Genway	18-272-195676	1:50	rabbit	pab	IFL
JC-1	Lifetechnologies	T3168	1:500			FL
Phalloidin-Alexa-594	Invitrogen	A12380	1:500			FL

IFL, Immunofluorescence; FL, fluorescence; mab, monoclonal antibody; pab, polyclonal antibody; WBlot, Western Blot

Suppl. Table 2

Quantitative analysis of F-actin and PKG1 colocalization in growth cones using JaCoB FIJI ImageJ (Bolte and Cordelieres, 2006) (to Figure 4B)

	Channel A red	Channel B green	Range -1 to 1	Range 0 to 1	Range 0 to 1	Range 0 to 1	Range 0 to 1	Range 0 to 1	Range 0 to 1	Range 0 to 1	Range -0.5 to 0.5
	Phalloidin	PKG1	Pearson's	Manders' Overlap Coefficient (MOC)		Manders' Colocalization Coefficient (M1, M2)		Manders' Colocalization Coefficient (with threshold)		Costes' automatic threshold method	Li's Intensity Correlation Quotient
	Threshold	Threshold	Coefficient	without thr	with threshold	Fraction of A overlapping B	Fraction of B overlapping A	Fraction of A overlapping B	Fraction of B overlapping A	Coefficient	
Image	thr	thr	r	MOC	MOC (thr)	M1	M2	thr-M1	thr-M2	r	ICQ
Spiky-1	138	153	0.802	0.831	0.98	0.999	0.996	0.686	0.518	0.776 thrA 2, thrB 8	0.454
Spiky-2	130	138	0.751	0.771	0.921	0.987	0.918	0.662	0.489	0.640 thrA 2, thrB 5	0.424
Spiky-3	141	141	0.754	0.793	0.924	0.952	0.986	0.536	0.908	0.691 thrA 4, thrB 2	0.351
Collapsed-1	164	94	0.499	0.632	0.95	0.997	0.412	0.072	0.261	0.391 thrA 2, thrB 3	0.087
Collapsed-2	143	107	0.6	0.757	0.914	0.977	0.957	0.139	0.27	0.496 thrA 4, thrB 2	0.334
Collapsed-3	130	146	0.185	0.63	0.937	0.734	0.734	0.086	0.079	0.113 thrA 2, thrB 11	-0.036

	Spiky-1	Spiky-2	Spiky-3	Collapsed-1	Collapsed-2	Collapsed-3
PDM land-scape						

Suppl. Table 1: Quantitative analysis of colocalization

The co-dependent or random nature of apparent colocalizations was tested using the JaCoB plugin of FIJI ImageJ {Bolte, 2006 #16262} that includes the Pearson correlation coefficient (Dunn et al., 2011), Manders' overlap coefficients, Manders' colocalization coefficients, Costes's threshold method and the Intensity Correlation Quotient (ICQ) (Li et al., 2004). After background subtraction growth cone images of PKG1 (green) and F-actin stained with Phalloidin (in red) were subjected to the JaCoP analysis employing auto threshold settings with minor adjustments. Thresholds were used to exclude the surrounding area and restrict the analyses to the cones. Manders' coefficients were calculated with/with thresholds and Pearson's does not employ thresholds.

The Pearson correlation coefficient is a measure for the distribution of the pixel intensity scatter plot of the two channels and ranges from 1 to -1, with 1 representing perfect correlation, 0 = random association and -1 perfect exclusion. Manders' coefficients M1 and M2 measure the overlap of the two channels and ranges from 0 (no overlap, mutually exclusive) to 1 (full overlap). Manders' overlap coefficient (MOC) is closely related to Pearson's but ranges from 0 to 1.

The Intensity Correlation Quotient (ICQ) is a measure of synchrony of red and green pixels and ranges between -0.5 and 0.5; -0.5 refers to segregated staining, zero to random staining and 0.5 to dependent staining. Li's method produces ICA plots, in which colocalized and segregated pixels are found on the right and the left side of the Y-axis, respectively.

The ICA algorithm calculates the 'product of the differences from the mean' (PDM), which is plotted versus the intensity. The landscape of the PDM values is depicted as pseudo-colored yellow-blue image where yellow indicates positive PDM values (high values for both channels). Blue voxels indicate negative PDM values i.e. sites where either one of the channels was below the respective mean.

Suppl. Table 3

Suppl. to Figure 8: Gene names and functions

Name	Gene name	Description	Function (source UniProt)
Arp2/3	arpc1b	Actin-related protein 2/3 complex subunit 1B	Functions as component of the Arp2/3 complex which is involved in regulation of actin polymerization and together with an activating nucleation-promoting factor (NPF) mediates the formation of branched actin networks
Caln	calna	Calcineurin	Protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform; Calcium-dependent, calmodulin-stimulated protein phosphatase. This subunit may have a role in the calmodulin activation of calcineurin. Dephosphorylates HSPB1 and SSH1
Cdc42	cdc42	Cell division control protein 42 homolog	Ccell division cycle 42 (GTP binding protein, 25kDa). Plasma membrane-associated small GTPase which cycles between an active GTP-bound and an inactive GDP-bound state. In active state binds to a variety of effector proteins to regulate cellular responses. Involved in epithelial cell polarization processes. Causes the formation of thin, actin-rich surface projections called filopodia
Cofilin	cfl1	Cofilin 1	Binds to F-actin and exhibits pH-sensitive F-actin depolymerizing activity. Regulates actin cytoskeleton dynamics.
CRMP	crmp1	Collapsin response mediator protein	Dihydropyrimidinase-related protein 1. Necessary for signaling by class 3 semaphorins and subsequent remodeling of the cytoskeleton. Plays a role in axon guidance, invasive growth and cell migration. May participate in cytokinesis
CXCR4	cxcr4	CXC-Motiv-Chemokinrezeptor 4	Chemokine (C-X-C motif) receptor 4; Receptor for the C-X-C chemokine CXCL12/SDF-1. Transduces a signal by increasing the intracellular calcium ions level. Involved in haematopoiesis and in cardiac ventricular septum formation. Plays also an essential role in vascularization of the gastrointestinal tract, probably by regulating vascular branching and/or remodeling processes in endothelial cells. Could be involved in cerebellar development. In the CNS, could mediate hippocampal-neuron survival. Acts as a coreceptor (CD4 being the primary receptor) for HIV-1 X4 isolates
DCC	dcc	Deleted in colon cancer	Deleted in colorectal carcinoma; Receptor for netrin required for axon guidance. Mediates axon attraction of neuronal growth cones in the developing nervous system upon ligand binding. Its association with UNC5 proteins may trigger signaling for axon repulsion. It also acts as a dependence receptor required for apoptosis induction when not associated with netrin ligand. Implicated as a tumor suppressor gene
Eph	epha1	Ephrin type A receptor	EPH receptor A1; Receptor for members of the ephrin-A family. Binds with a low affinity to ephrin-A1
Ephrin	efna1	Ephrin A1	Ephrin-A1; Plays an important role in angiogenesis and tumor neovascularization. The recruitement of VAV2, VAV3 and PI3-kinase p85 subunit by phosphorylated EPHA2 is critical for EFNA1-induced RAC1 GTPase activation and vascular endothelial cell migration and assembly. Exerts anti-oncogenic effects in tumor cells through activation and down-regulation of EPHA2. Activates EPHA2 by inducing tyrosine phosphorylation which leads to its internalization and degradation. Acts as a negative regulator in the tumorigenesis of gliomas by down-regulating EPHA2 and FAK.
Erk2	MAPK1	Mitogen activated protein kinase	Mitogen-activated protein kinase 1; Involved in both the initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors such as ELK1. Phosphorylates EIF4EBP1; required for initiation of translation. Phosphorylates microtubule-associated protein 2 (MAP2). Phosphorylates SPZ1. Phosphorylates heat shock factor protein 4 (HSF4) and ARHGEF2
FAK	ptk2	Focal adhesion kinase 1	PTK2 protein tyrosine kinase 2; Non-receptor protein-tyrosine kinase implicated in signaling pathways involved in cell motility, proliferation and apoptosis. Activated by tyrosine-phosphorylation in response to either integrin clustering induced by cell adhesion or antibody cross-linking, or via G-protein coupled receptor (GPCR) occupancy by ligands such as bombesin or lysophosphatidic

			acid, or via LDL receptor occupancy. Plays a potential role in oncogenic transformations resulting in increased kinase activity
GAP	arhgap1	Rho GTPase-activating protein 1	Rho GTPase activating protein 1; GTPase activator for the Rho, Rac and Cdc42 proteins, converting them to the putatively inactive GDP-bound state. Cdc42 seems to be the preferred substrate
GEF	arhgef1	Rho guanine nucleotide exchange factor gef1	Acts as GTPase-activating protein (GAP) for GNA12 and GNA13, and as guanine nucleotide exchange factor (GEF) for RhoA GTPase.
Gsk3b	gsk3b	Glycogensynthase kinase 3 beta	Glycogen synthase kinase 3 beta; Participates in the Wnt signaling pathway. Implicated in the hormonal control of several regulatory proteins including glycogen synthase, MYB and the transcription factor JUN. Phosphorylates JUN at sites proximal to its DNA-binding domain, thereby reducing its affinity for DNA. Phosphorylates MUC1 in breast cancer cells, and decreases the interaction of MUC1 with CTNNB1/beta-catenin. Phosphorylates CTNNB1/beta-catenin
Integrin a2b1	itga2	Integrin a2b1	Integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor); Integrin alpha-2/beta-1 is a receptor for laminin, collagen, collagen C-propeptides, fibronectin and E-cadherin. It recognizes the proline-hydroxylated sequence G-F-P-G-E-R in collagen. It is responsible for adhesion of platelets and other cells to collagens, modulation of collagen and collagenase gene expression, force generation and organization of newly synthesized extracellular matrix
IP3R	ip3r	Inositoltriphosphate receptor	inositol 1,4,5-triphosphate receptor, type 3; Receptor for inositol 1,4,5-trisphosphate, a second messenger that mediates the release of intracellular calcium
Limk	limk1	LIM domain kinase 1	LIM domain kinase 1; Protein kinase which regulates actin filament dynamics. Phosphorylates and inactivates the actin binding/depolymerizing factor cofilin, thereby stabilizing the actin cytoskeleton. Isoform 3 has a dominant negative effect on actin cytoskeletal changes. May be involved in brain development
Mical	mical1	Protein-methionine sulfoxide oxidase	Microtubule associated monoxygenase, calponin and LIM domain containing 1; May be a cytoskeletal regulator that connects NEDD9 to intermediate filaments
MLC	myl1	myosin light chain	Myosin, light chain 1, alkali; skeletal, fast; Regulatory light chain of myosin. Does not bind calcium
MLCK	mylk	myosin light chain kinase	Myosin light chain kinase; Calcium/calmodulin-dependent enzyme implicated in smooth muscle contraction via phosphorylation of myosin light chains (MLC). Also regulates actin-myosin interaction through a non- kinase activty . Implicated in the regulation of endothelial as well as vascular permeability. In the nervous system it has been shown to control the growth initiation of astrocytic processes in culture and to participate in transmitter release at synapses
Netrin	ntn1	Netrin 1	Netrins control guidance of CNS commissural axons and peripheral motor axons. Its association with either DCC or some UNC5 receptors will lead to axon attraction or repulsion, respectively. It also serve as a survival factor via its association with its receptors which prevent the initiation of apoptosis. Involved in colorectal tumorigenesis by regulating apoptosis
NP1	nrp1	Neuropilin 1	Neuropilin 1; The membrane-bound isoform 1 is a receptor involved in the development of the cardiovascular system, in angiogenesis, in the formation of certain neuronal circuits and in organogenesis outside the nervous system. It mediates the chemorepulsant activity of semaphorins. It binds to semaphorin 3A.
Nppc	nppc	C-type natriuretic peptide	Natriuretic peptide precursor C; Vasorelaxant activity. Has a cGMP-stimulating activity
Npr	npr1	natriuretic peptide receptor 1	Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A); Receptor for atrial natriuretic peptide. Has guanylate cyclase activity on binding of ANF
PAK	pak1	p21-activated serine/threonine kinases	p21 protein (Cdc42/Rac)-activated kinase 1; The activated kinase acts on a variety of targets. Likely to be the GTPase effector that links the Rho-related GTPases to the JNK MAP kinase pathway. Activated by CDC42 and RAC1. Involved in dissolution of stress fibers and reorganization of focal complexes. Involved in regulation of microtubule biogenesis through phosphorylation of TBCB.

PKG1	prkg1	cGMP dependent protein kinase 1 (prkg1)	Protein kinase, cGMP-dependent, type I; Phosphorylates PPP1R12A. Serine/threonine protein kinase that acts as key mediator of the nitric oxide (NO)/cGMP signaling pathway. GMP binding activates PRKG1, which phosphorylates serines and threonines on many cellular proteins. Numerous protein targets for PRKG1 phosphorylation are implicated in modulating cellular calcium, but the contribution of each of these targets may vary substantially among cell types. Proteins that are phosphorylated by PRKG1 regulate platelet activation and adhesion, smooth muscle contraction, cardiac function, gene expression, feedback of the NO-signaling pathway, and other processes involved in several aspects of the CNS like axon guidance, hippocampal and cerebellar learning, circadian rhythm and nociception. Smooth muscle relaxation is mediated through lowering of intracellular free calcium, by desensitization of contractile proteins to calcium, and by decrease in the contractile state of smooth muscle or in platelet activation. Regulates intracellular calcium levels via several pathways: phosphorylates MRVI1/IRAG and inhibits IP3-induced Ca2+ release from intracellular stores, phosphorylation of KCNMA1 (BKCa) channels decreases intracellular Ca2+ levels, which leads to increased opening of this channel. PRKG1 phosphorylates the canonical transient receptor potential channel (TRPC) family which inactivates the associated inward calcium current. Another mode of action of NO/cGMP/PKGI signaling involves PKGI-mediated inactivation of the Ras homolog gene family member A (RhoA). Phosphorylation of RHOA by PRKG1 blocks the action of this protein in myriad processes: regulation of RHOA translocation; decreasing contraction; controlling vesicle trafficking, reduction of myosin light chain phosphorylation resulting in vasorelaxation. Activation of PRKG1 by NO signaling alters also gene expression in a number of tissues. In smooth muscle cells, increased cGMP and PRKG1 activity influence expression of smooth muscle-specific contractile proteins, levels of proteins in the NO/cGMP signaling pathway, down-regulation of the matrix proteins osteopontin and thrombospondin-1 to limit smooth muscle cell migration and phenotype. Regulates vasodilator-stimulated phosphoprotein (VASP) functions in platelets and smooth muscle
Plexin	plxa1	Plexin-A1	Coreceptor for SEMA3A, SEMA3C, SEMA3F and SEMA6D. Necessary for signaling by class 3 semaphorins and subsequent remodeling of the cytoskeleton. Plays a role in axon guidance, invasive growth and cell migration.
Rac	rac1	Ras-related protein	Ras-related C3 botulinum toxin substrate 1 (rho family, small GTP binding protein Rac1); Plasma membrane-associated small GTPase which cycles between active GTP-bound and inactive GDP-bound states. In its active state, binds to a variety of effector proteins to regulate cellular responses such as secretory processes, phagocytosis of apoptotic cells, epithelial cell polarization and growth-factor induced formation of membrane ruffles
RhoA	rho1	Transforming protein RhoA	Regulates a signal transduction pathway linking plasma membrane receptors to the assembly of focal adhesions and actin stress fibers
Robo	robo1	Roundabout homolog 1	Roundabout, axon guidance receptor, homolog 1 (Drosophila); Receptor for SLIT1 and SLIT2 which are thought to act as molecular guidance cue in cellular migration, including axonal navigation at the ventral midline of the neural tube and projection of axons to different regions during neuronal development. In axon growth cones, the silencing of the attractive effect of NTN1 by SLIT2 may require the formation of a ROBO1-DCC complex.
ROCK	rock1	Rho-associated protein kinase 1	Rho-associated, coiled-coil containing protein kinase 1; Phosphorylates and activates DAPK3, which then regulates myosin light chain phosphatase through phosphorylation of MYPT1 thereby regulating the assembly of the actin cytoskeleton, cell migration, invasiveness of tumor cells, smooth muscle contraction and neurite outgrowth. Required for centromere positioning and centromere-dependent exit from mitosis. Necessary for apoptotic membrane blebbing
SDF-1	cxcl12	Stromal cell-derived factor 1	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1); Chemoattractant active on T-lymphocytes, monocytes, but not neutrophils. Activates the C-X-C chemokine receptor CXCR4 to induce a rapid and transient rise in the level of intracellular calcium ions and chemotaxis.
Sema3a	sema3a	Semaphorin 3a	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A; Induces the collapse and paralysis of neuronal growth cones. Could serve as a ligand that guides specific growth cones by a motility-inhibiting mechanism. Binds to the complex neuropilin-1/plexin-1

Sema7	sema7a	Semaphorin 7a	Semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group); May play an imortant role in the nervous system and in modulating immune function
sGC	gucy1a2	soluble guanylate cyclase subunit A1	Guanylate cyclase 1, soluble, alpha 2; Has guanylyl cyclase on binding to the beta-1 subunit
Slit	slit1	Slit homolog 1 protein	Slit homolog 1 (Drosophila); Thought to act as molecular guidance cue in cellular migration, and function appears to be mediated by interaction with roundabout homolog receptors. During neural development involved in axonal navigation at the ventral midline of the neural tube and projection of axons to different regions (By similarity). SLIT1 and SLIT2 together seem to be essential for midline guidance in the forebrain by acting as repulsive signal preventing inappropriate midline crossing by axons projecting from the olfactory bulb
Ssh1	ssh1	Slingshot phosphatase 1	Slingshot homolog 1 (Drosophila); Protein phosphatase which regulates actin filament dynamics. Dephosphorylates and activates the actin binding/depolymerizing factor cofilin, which subsequently binds to actin filaments and stimulates their disassembly. Inhibitory phosphorylation of cofilin is mediated by LIMK1, which may also be dephosphorylated and inactivated by this protein
VASP	vasp	Vasodilator-stimulated phosphoprotein	Ena/VASP proteins are actin-associated proteins involved in a range of processes dependent on cytoskeleton remodeling and cell polarity such as axon guidance, lamellipodial and filopodial dynamics, platelet activation and cell migration
WASP	was	Wiskott-Aldrich syndrome protein	Wiskott-Aldrich syndrome (eczema-thrombocytopenia); Effector protein for Rho-type GTPases, providing a link with the Arp2/3 complex that regulates the structure and dynamics of the actin cytoskeleton. Important for efficient actin polymerization. Possible regulator of lymphocyte and platelet function

Bolte S, Cordelieres FP (2006) A guided tour into subcellular colocalization analysis in light microscopy. J Microsc 224:213-232.

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Li Q, Lau A, Morris TJ, Guo L, Fordyce CB, Stanley EF (2004) A syntaxin 1, Galpha(o), and N-type calcium channel complex at a presynaptic nerve terminal: analysis by quantitative immunocolocalization. J Neurosci 24:4070-4081.