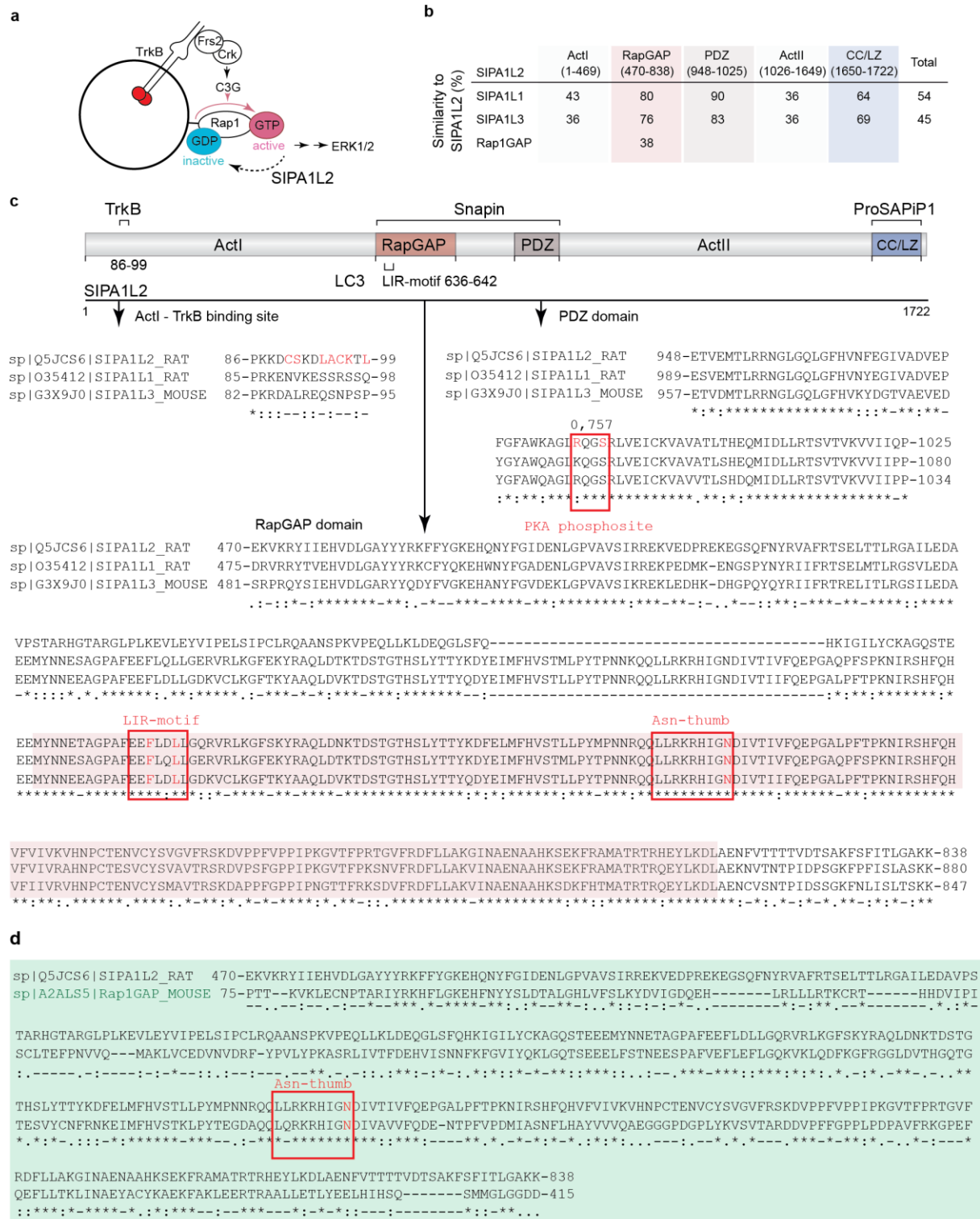


## **Supplementary Information**

**SIPA1L2 controls trafficking and local signaling of TrkB-containing amphisomes at presynaptic terminals**

**Andres-Alonso et al.**

## Supplementary Figures



**Supplementary Figure 1.** SIPA1L2 shows high degree of similarity with other SIPA1L family members and Rap1GAP.

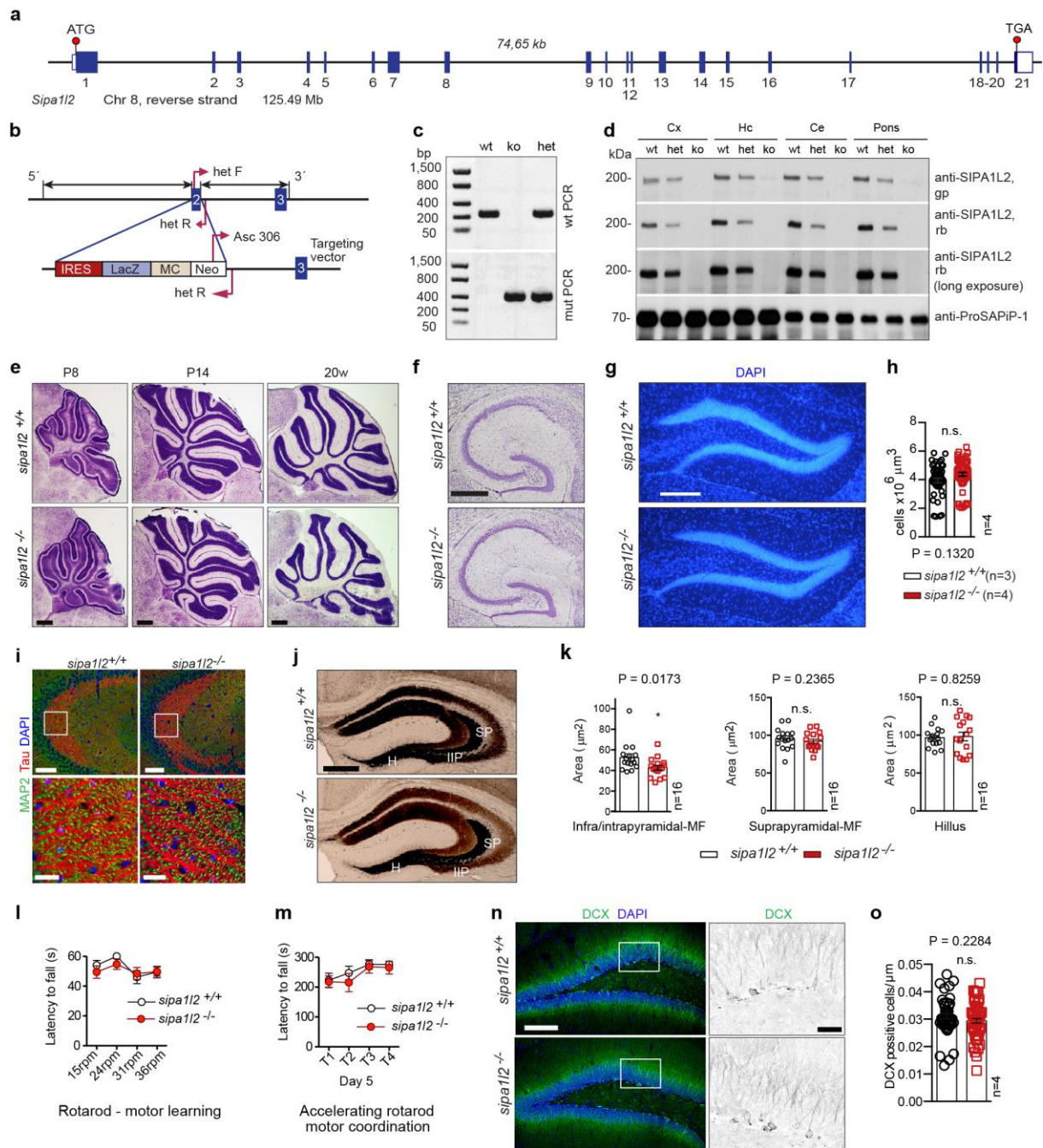
**a.** Cartoon depicting the molecular cascade leading to the TrkB-dependent ERK1/2 activation. Upon BDNF binding, sustained ERK1/2 is achieved after TrkB activation by recruitment of the protein adaptor fibroblast growth factor receptor substrate-2 (Frs2) that provides the binding for Crk. This association promotes the activation of C3G, the guanyl nucleotide exchange factor (GEF) for Rap1 that triggers the conversion of the inactive, GDP-bound to the active GTP-bound Rap1. Rap1-GTP stimulates B-Raf and activates the ERK cascade (see [7] for a

detailed revision). SIPA1L2 is a RapGAP for Rap1/2 that stimulates the GTPase activity of Rap and promotes the inactivation of the pathway.

**b.** Table showing the similarity of SIPA1L2 with SIPA1L1 and SIPA1L3 as well as the RapGAP domain of Rap1GAP. Comparisons were made according to the SIPA1L2 domains defined by the amino acids depicted in the heading. The RapGAP and PDZ domains are highly conserved among SIPA1L family members.

**c.** Scheme showing the domain organization and interaction motifs of SIPA1L2 with TrkB, Snapin, LC3 and ProSAPiP1 [9]. Depicted is the sequence comparison of the ActI, RapGAP and PDZ domains between SIPA1L family members. Boxes within the RapGAP domain indicate the LIR-motif and the Asparagine thumb where amino acids in the so-called LIR-mutant (F638A-L641A) and RapGAP dead mutant (N705A) are indicated in red. Unless otherwise stated, interaction assays performed in this study involving the RapGAP or PDZ domain were done with SIPA1L2-624-813 (SIPA1L2-RapGAP; red shaded box in the figure) and SIPA1L2-948-1025 (SIPA1L2-PDZ). The alignment for the RapGAP domain is done with a longer sequence stretch (470-838) defined by comparison with a published, catalytically-active fragment of Rap1GAP (aa 75-415; 23). The fragment SIPA1L2-470-838 was used in RapGAP activity assays. Symbols represent: fully conserved residues (\*), groups with strongly similar properties (:) and groups with weakly similar properties (.) according to ClustalW2.

**d.** Sequence comparison of SIPA1L2 with the catalytically active RapGAP domain of Rap1GAP. Symbols represent: fully conserved residues (\*), aa with strong similarity (:), aa with no similarity (.) according to ClustalW2.



**Supplementary Figure 2.** Generation and characterization of *sipa1l2* knockout mice.

- Schematic representation of the *sipa1l2* genomic structure comprising 21 exons located on mouse chromosome 8 with one alternatively spliced exon (no. 18).
- Scheme depicting the strategy used for generation of the constitutive *sipa1l2* knockout mice. The targeting vector replaces exon 2 with an IRES-LacZ sequence. The positions of primers for genotyping analysis (hetF, hetR and Asc306) are indicated by pink arrows.
- Representative gel of mouse genotyping by PCR with primers indicated in **b**.
- Western blot on mouse brain tissue from different areas of wt, *sipa1l2* het and ko mice (Cx, Cortex; Hc, hippocampus; Ce, cerebellum; Pons). Specific SIPA1L2 antibodies from different species (guinea pig and rabbit) were used. The expression of ProSAPiP-1 was not altered.

**e.** Nissl-stained sagittal cerebellar sections from *ko* and *wt* mice at different developmental stages. Scale bar = 500  $\mu$ m.

**f.** Nissl staining from mouse *wt* and *ko* hippocampal sections (scale bar = 500  $\mu$ m).

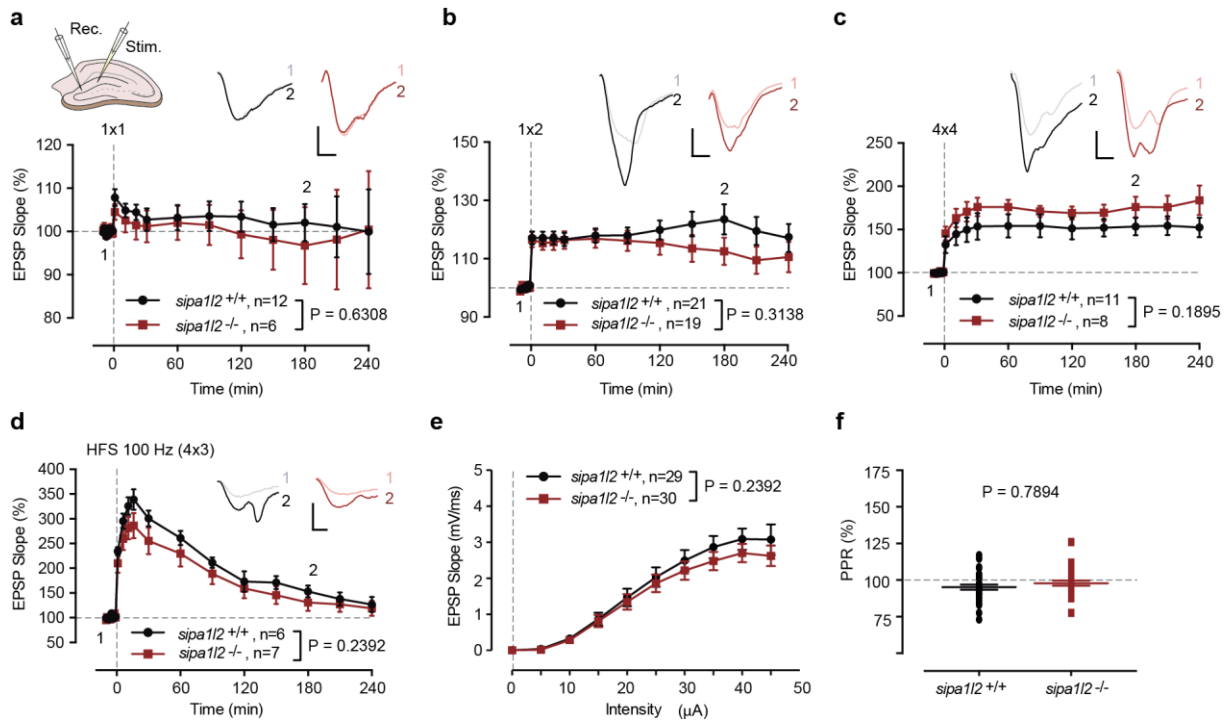
**g-h.** Representative DG images (**g**) and quantification (**h**) from *wt* and *ko* slices stained with DAPI. Scale bar =150  $\mu$ m.

**i-k.** Tau (**i**) and Timm staining (**j**) performed in *sipa1l2 ko* and *wt* slices. In (**j**) H stands for Hilus, SP for suprapyramidal MF and IIP for infra-and intrapyramidal MF (Mann Whitney U test for infra/intrapyramidal and suprapyramidal regions and Student's t test for quantification in the Hillus). Scale bar =100  $\mu$ m and 20  $\mu$ m (inset). Scale bar for Timm staining is 400  $\mu$ m.

**l-m.** In **l**, quantification of the latency to fall of *ko* and *wt* mice submitted to 4 days of rotarod task with increased speeds each day (maximum latency of 60 sec). In **m**, animals were submitted on day 5 to the accelerating rotarod task 4 times (maximum latency 300 sec). (Two-way ANOVA, n animals=9).

**n-o.** Representative images (**n**) from hippocampal sections from *wt* and *ko* mice stained with doublecortin (DCX) for adult-born granule cells and DAPI and quantification (**o**). Scale bar =100  $\mu$ m and 20  $\mu$ m (inset).

Data is show as mean  $\pm$  S.E.M. n.s. stands for not significant.



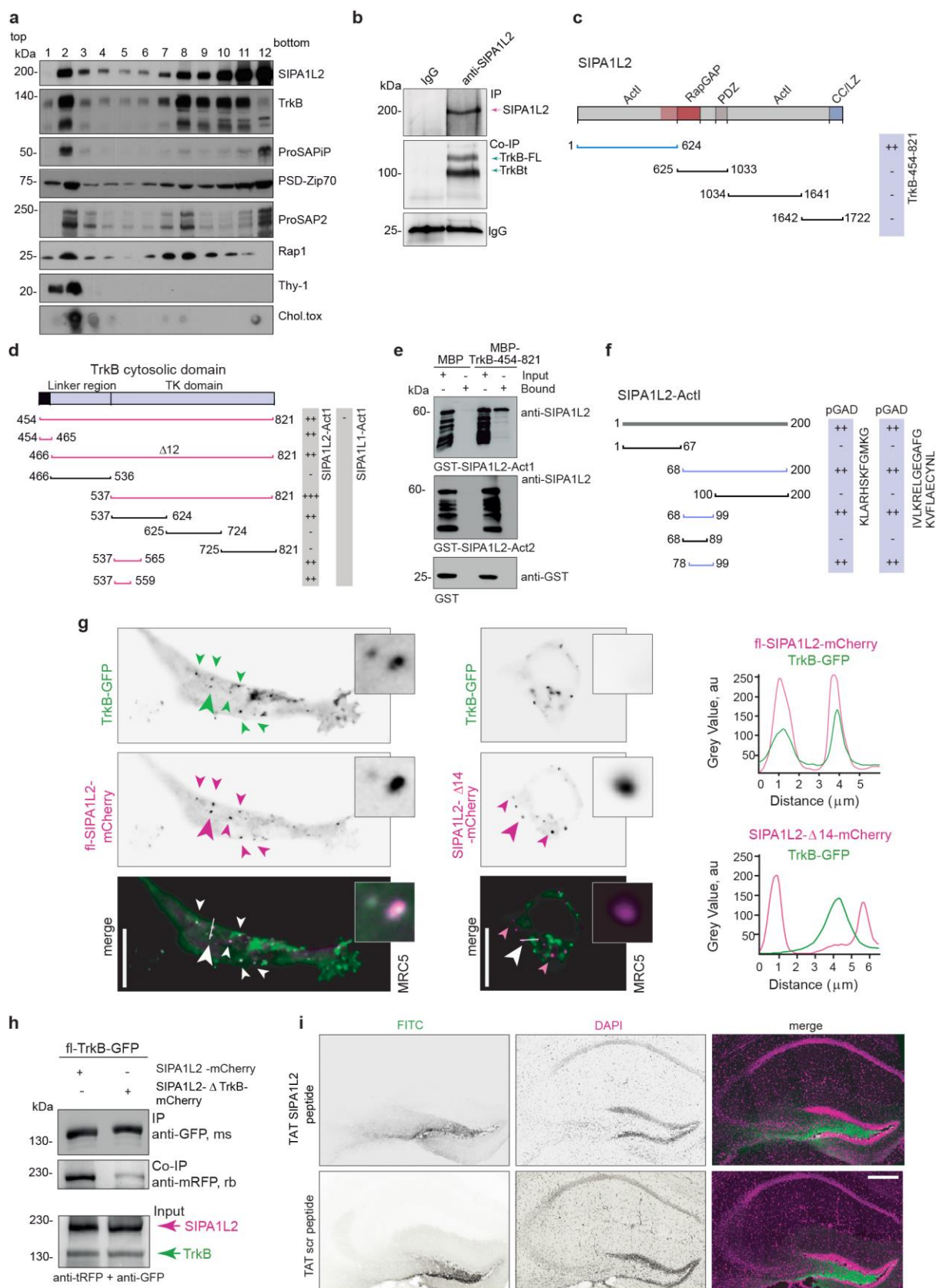
**Supplementary Figure 3.** Basal synaptic transmission and synaptic plasticity in the DG of *sipa1l2* ko animals was similar to wt mice.

**a-b.** EPSP slopes after theta burst stimulation (TBS) of different strengths delivered on acute slices from wt and ko mice. Delivery of one brief presynaptic burst of 10 pulses at 10 Hz - 0.2ms per half-wave duration - repeated 10 times at 5Hz (**a**) or two of these episodes (inter-episode interval 10s) (**b**) resulted in similar responses in both experimental groups (two-way ANOVA).

**c-d.** EPSP slopes upon delivery of a stronger TBS protocol consisting of 4 episodes (inter-episode interval 10s) repeated 4 times every 5min (**c**) or a high-frequency stimulation protocol (4x100Hz 1s, every 5min) (**d**) (two-way ANOVA).

**e-f.** No differences in input-output curve of the field excitatory postsynaptic potential (**e**) and paired-pulse facilitation (**f**) between *sipa1l2*<sup>+/+</sup> and *sipa1l2*<sup>-/-</sup> were observed (Student's t test).



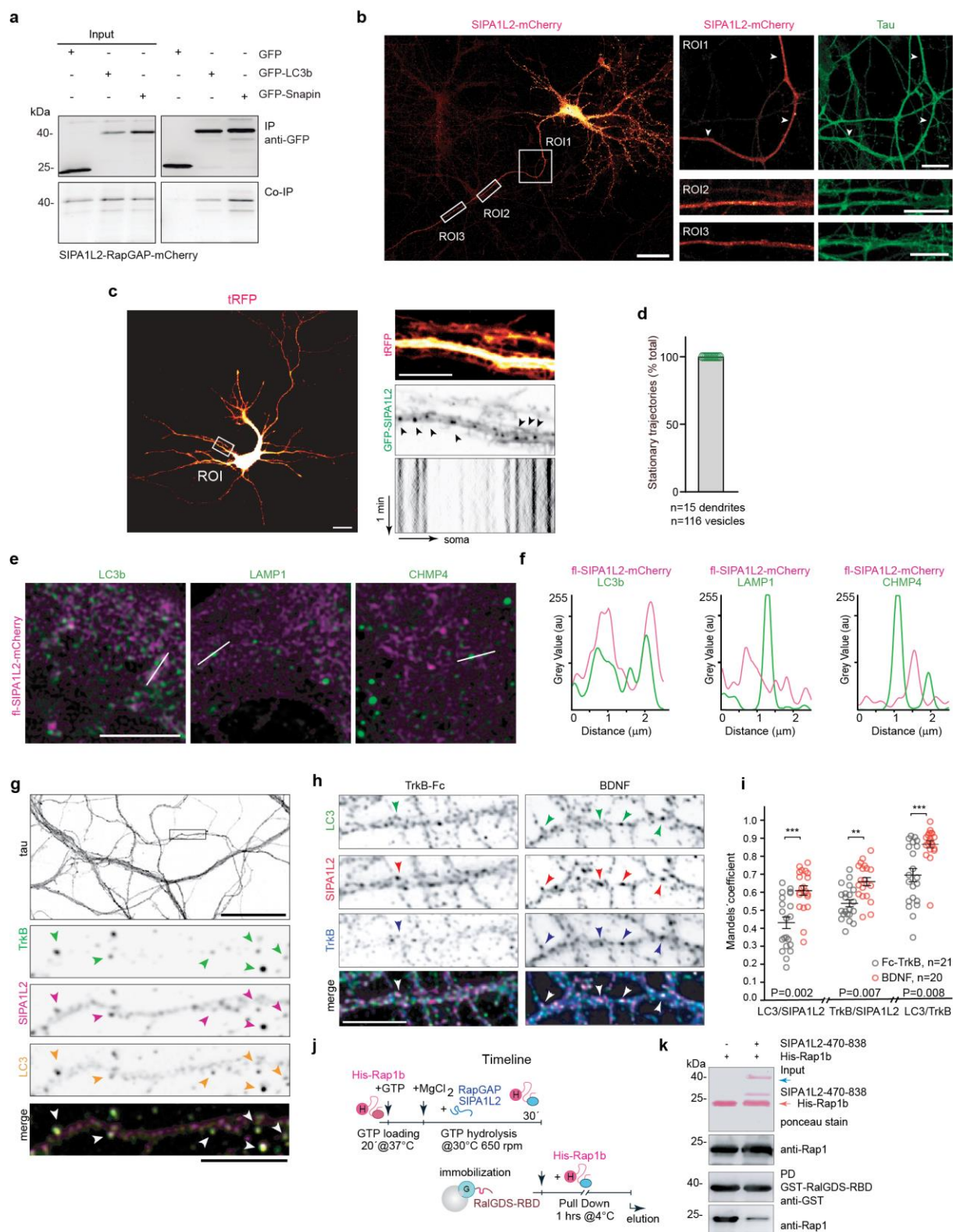


**Supplementary figure 4. SIPA1L2 interacts with TrkB.**

**a.** Lipid raft isolation from rat cerebellum shows that SIPA1L2 distribution overlaps with TrkB and Rap1 and associates with lipid rafts identified by Thy-1. Lipid raft fractions were also identified by sphingolipids-dot blot assay using Cholera toxin (lower panel). Twelve fractions (from top to bottom of the gradient) were immunoblotted for indicated proteins.

- b.** TrkB co-immunoprecipitates with endogenous SIPA1L2 from rat hippocampus. SIPA1L2 was immunoprecipitated with anti-SIPA1L2 rabbit antibodies (IP) and rabbit IgG was used as a control. Western blot of precipitates was detected anti-TrkB goat antibodies.
- c.** Schematic representation of SIPA1L2 domains and the corresponding fragments used in YTH. Results are depicted in the light-blue box on the right. Numbers indicate amino acid (aa) residue position in each protein according to the rat sequence. The intensity of the binding is represented as: “+++” <60 min, “++” 60-120 min, “+” 120-180 min, and – for no interaction
- d.** Scheme depicting the fragments used in YTH and corresponding interactions. Interacting fragments are shown in pink. Two binding interfaces in TrkB were found to interact with ActI-SIPA1L2: 12aa within the juxtamembranous region of TrkB (TrkB-454-465) and the first 23aa the tyrosine kinase (TK) domain (TrkB-538-560).
- e.** MBP-pull-down assays confirmed the direct interaction of the ActI domain in SIPA1L2 with the cytosolic part of TrkB (MBP-TrkB-454-821). A band at about 60kDa is visualized in the WB using anti-SIPA1L2 (rb) antibodies. The ActII domain of SIPA1L2 (68 kDa; GST-SIPA1L2-1026-1650) as well as GST alone does not interact with the cytosolic TrkB.
- f.** Identification of the TrkB-binding interface within the Act1 domain of SIPA1L2 by YTH. The first 200 aa of the ActI domain were chosen as bait and the interaction with the juxtamembranous region of TrkB (aa 454-465) as well as with the first 23 aa of TK domain (TrkB-537-559) was verified.
- g.** Representative images and line profiles showing the co-localization between TrkB-GFP with SIPA1L2-mCherry, but not SIPA1L2-Δ14-mCherry
- h.** Heterologous co-immunoprecipitation experiments between GFP-SIPA1L2-Δ86-99 or fl-SIPA1L2 and TrkB.
- i.** Representative images show coronal brain sections from injected mice after behavioral testing. Scale bar is 200 μm.





**Supplementary figure 5.** SIPA1L2 associates with Snapin, TrkB and LC3b, but not with LAMP1 and CHMP4.

**a.** RapGAP domain of SIPA1L2 (aa 624-813) co-immunoprecipitates with both GFP-Snapin as well as with GFP-LC3 from HEK293T cell extracts.

**b.** Confocal tile scanned image of neuron overexpressing fl-SIPA1L2-mCherry co-stained with Tau showing representative distal axonal regions used for co-trafficking assays. Scale bars are 50  $\mu$ m and 10  $\mu$ m within the ROIs.

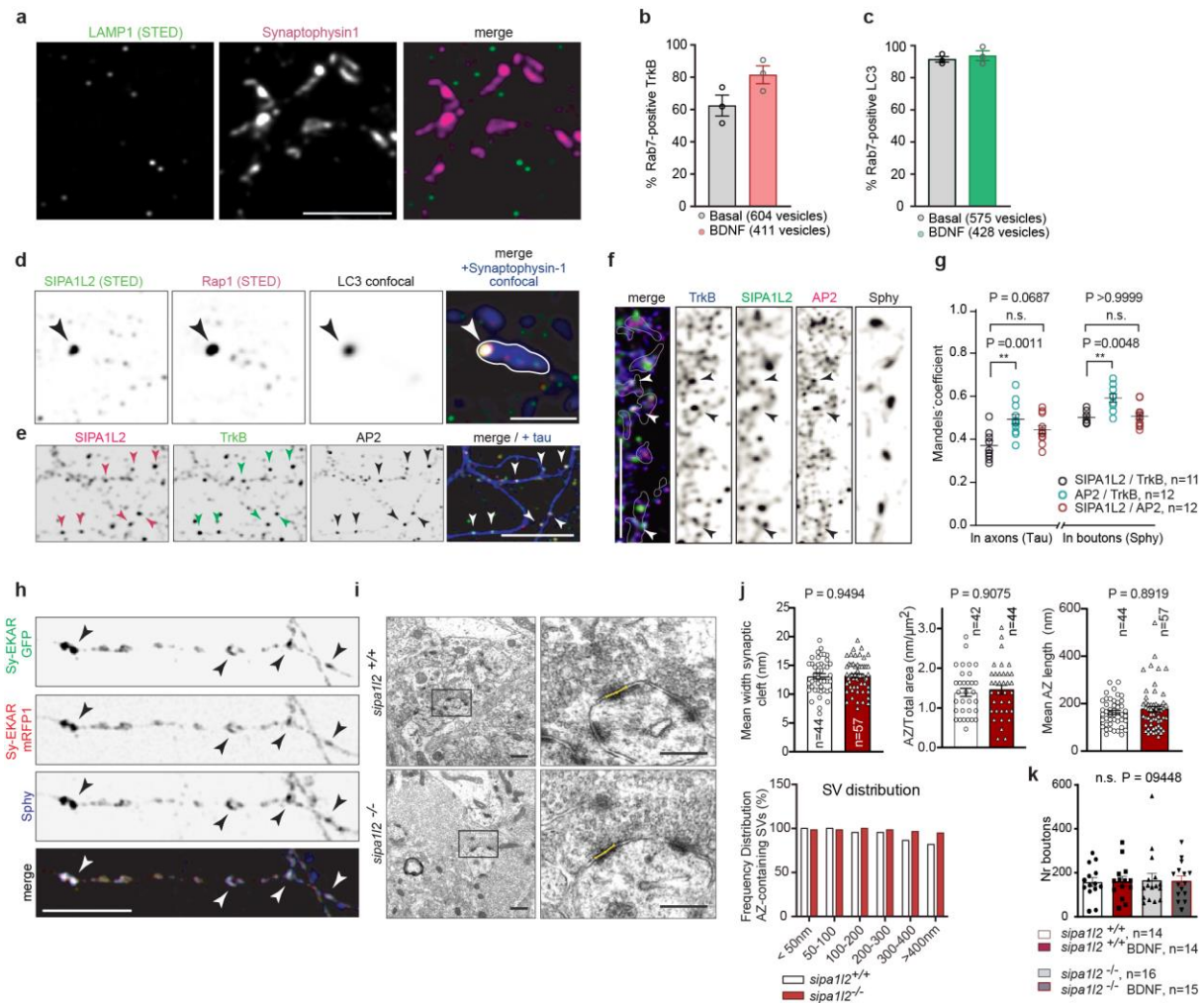
**c-d.** Confocal images showing a neuron (DIV 10) overexpressing tRFP and GFP-SIPA1L2. The depicted ROI shows the dendritic region live-imaged for 5 minutes to assess the dendritic trafficking of SIPA1L2. Kymograph shows stationary particles and the bar graph (**d**) shows the percentage of stationary particles quantified per dendritic segment. Scale bars are 20  $\mu\text{m}$  in the overview image and 10  $\mu\text{m}$  in the ROI.

**e-f.** Representative confocal images (**e**) of MRC5 cells overexpressing fl-SIPA1L2-mCherry and stained against endogenous LC3b, LAMP1 or CHMP4 (multivesicular bodies marker) and corresponding line profile (**f**). Scale bar is 5  $\mu\text{m}$ .

**g.** Confocal images from rat hippocampal cultures immunostained for Tau, TrkB, SIPA1L2 and LC3 showing the colocalization of the later three in axons. Scale bar = 20  $\mu\text{m}$  in overview image, 5  $\mu\text{m}$  in insert.

**h-i.** Representative confocal images (**h**) from rat primary cultures immunostained for LC3, SIPA1L2 and TrkB after treatment with TrkB-Fc bodies or BDNF and corresponding Manders' colocalization coefficient (**i**). Circles represent single analyzed images. Black bars are mean $\pm$ S.E.M. (Mann-Whitney U test). Scale bar = 10  $\mu\text{m}$ .

**j-k.** Time line (**j**) of the Rap1b loading with GTP and conditions for Rap1b-GTP hydrolysis in the RapGAP assay (**k**) after addition of SIPA1L2. SIPA1L2-(470-838) is generated based on sequence alignment with the catalytically active RapGAP-domain of Rap1GAP.



**Supplementary Figure 6.** SIPA1L2/TrkB/LC3 signaling amphisomes are positive for AP2 and absence of SIPA1L2 does not result in changes of MF ultrastructure.

**a.** Representative images of rat hippocampal neurons stained against LAMP1 and Synaptophysin1 and imaged using STED and confocal microscopy revealed no staining for LAMP1 at presynaptic boutons labeled with Synaptophysin1. Scale bar is 2,5  $\mu$ m.

**b-c.** Quantification of the percentage of TrkB (**b**) and LC3 (**c**) organelles that are positive for Rab7 from the experiment in Figure 7a in which primary hippocampal neurons were stained against SIPA1L2, TrkB, LC3 and Rab7. Circles represent averaged data per imaged quantified. Number of vesicle in the legend represent total particles analyzed from the three images.

**d.** Quadruple immunofluorescence combining confocal and super-resolution STED imaging depicting association of Rap1 with SIPA1L2/LC3 positive amphisomes in the presynaptic boutons from hippocampal primary neurons. Scale bar is 1  $\mu$ m

**e-g.** Immunostaining of primary hippocampal cultures showing the colocalization of SIPA1L2/TrkB/AP2 in axons labeled with anti-tau (**e**) and boutons (**f**) labeled with anti-Synaptophysin-1 antibodies and corresponding Manders' co-localization coefficient (**g**). Circles in the graph represent average Manders' coefficient per image and "n" numbers of analysed images (One-way ANOVA). Scale bars are 5  $\mu$ m.

**h.** Representative confocal images from rat hippocampal neurons transfected with Sy-EKAR (GFP-mRFP1) and immunostained for Synaptophysin-1 (Sph1) showing the localization of the ERK sensor in presynaptic terminals. Scale bar is 10  $\mu$ m.

**i-j.** Representative EM images (**i**) and quantification (**j**) of mean width of the synaptic cleft, mean active zone length and frequency distribution of AZ-containing synaptic vesicles. Representative active zones are shown in yellow. “N” numbers are within columns represent the number of images analyzed. Scale bars – 500 nm (overview) and 300 nm (insert).

**k.** Number of boutons detected by immunofluorescence against Syn in *wt* and *sipa1l2*<sup>-/-</sup> neurons after treatment with BDNF as compared to control, non-treated cells. Data shown as mean±S.E.M. “n” numbers represent independent cells. n.s.=p>0.05 (One-way ANOVA with Bonferroni posthoc test).

## Supplementary tables

Supplementary table 1. Description of the antibodies, constructs and oligonucleotides used in this study and corresponding sources.

| Antibodies                                           | Source                       | Identifier / Dilution                                |
|------------------------------------------------------|------------------------------|------------------------------------------------------|
| Rabbit anti-SIPA1L2                                  | 1                            | N/A; WB: 1:1000                                      |
| Guinea pig anti-SIPA1L2                              | 1                            | N/A; IF/STED-1:150                                   |
| Goat anti-TrkB (cys32-His429)                        | R&D Systems                  | Cat#AF1494-SP;<br>RRID:AB_2155264;<br>IF/STED-1:200  |
| Rabbit anti-pTrkA (Tyr490)<br>(pTrkBY515 )           | Cell Signaling               | Cat#9141S;<br>RRID:AB_2298805; IF-<br>1:200          |
| Mouse anti-Rab7 (clone Rab7-117)                     | Abcam                        | Cat#ab50533;<br>RRID:AB_882241; IF-1:300             |
| Rabbit anti-Rab7                                     | Cell Signaling<br>Technology | Cat#2094;<br>RRID:AB_2300652                         |
| Mouse anti-Synaptotagmin 1<br>(cytoplasmic tail)     | Synaptic Systems             | Cat#105011;<br>RRID:AB_887832; IF-1:400              |
| Mouse anti-Synaptotagmin 1 labeled<br>with Oyster650 | Synaptic Systems             | Cat#105311C5;<br>RRID:AB_2199308; IF-<br>1:300       |
| Guinea pig anti-Synaptophysin 1                      | Synaptic Systems             | Cat#101004;<br>RRID:AB_1210382; IF-<br>1:400         |
| Mouse anti-Synaptophysin 1 labeled<br>with Oyster488 | Synaptic Systems             | Cat#101011C2;<br>RRID:AB_10890165<br>IF-1:300        |
| Mouse anti-LC3 (clone5F10)                           | NanoTools                    | Cat#0231-<br>100;RRID:AB_2722733                     |
| Mouse anti-LC3 (human)                               | MBL                          | Cat#M152-3;<br>RRID:AB_1279144;<br>IF/STED-1:250     |
| Rabbit anti-LC3b                                     | Abcam                        | Cat#ab48394;<br>RRID:AB881433;<br>IF-1:300           |
| Rabbit anti-LAMP1                                    | Abcam                        | Cat#ab24170;<br>RRID:AB_775978; IF-<br>1:1000        |
| Mouse anti-LAMP1 (LY1C6)                             | ThermoFisher Scientific      | Cat#MA1-164;<br>RRID:AB_2536869;<br>IF/STED-1:300    |
| Mouse anti-Dynein intermediate<br>chain              | Merck Millipore              | Cat #MAB1618;<br>RRID:AB_2246059; WB-<br>1:4000      |
| Rabbit anti-Rap1 mAb                                 | Cell signaling               | Cat#8825;RRID:N/A;<br>WB/IF-1:500/1:300              |
| Rabbit anti-Snapin                                   | Synaptic Systems             | Cat#148 002,<br>RRID:AB_887796;<br>WB/IF-1:500;1:300 |
| Guinea pig anti-Synapsin 1/2                         | Synaptic Systems             | Cat#106004;<br>RRID:AB_1106784; IF-<br>1:1500        |
| Mouse anti-mCherry                                   | Novus Biologicals            | Cat#NBP1-96752;<br>RRID:AB_11034849; IF-<br>1:100    |
| Rabbit anti-pSynapsin (pS62)                         | ThermoFisher Scientific      | Cat#PA5-38336;<br>RRID:AB_2554937; IF-<br>1:1000     |

|                                    |                                                |                                                  |
|------------------------------------|------------------------------------------------|--------------------------------------------------|
| Rabbit anti-CHMP4b                 | Abcam                                          | Cat#ab105767;<br>RRID:AB_10858466; IF:<br>1:1000 |
| Mouse anti-Tau1 (PC1C6)            | Merck Millipore                                | Cat#MAB3420;<br>RRID:AB_94855; IF-1:300          |
| Rabbit anti-Tau                    | Synaptic Systems                               | Cat#314002;<br>RRID:AB_993042; IF-1:300          |
| Anti-doublecortin (DCX)            | Abcam                                          | Cat#ab18723;<br>RRID:AB_732011; IF-1:500         |
| Mouse anti-AP2                     | BD Biosciences                                 | Cat#610501;<br>RRID:AB_397867; IF-1:200          |
| Mouse anti-MAP2 (HM-2)             | Sigma Aldrich                                  | Cat#M-4403;<br>RRID:AB_477193; IF-1:500          |
| Mouse anti-GM130 clone 35/GM130    | BD Bioscience                                  | Cat#610822;<br>RRID:AB_10015242; WB-<br>1:500    |
| Rabbit sera anti-ProSAP2           | kind gift from Prof.<br>Böckers <sup>2</sup>   | N/A; WB-1:500                                    |
| Rabbit sera anti-ProSAP1P          | kind gift from Prof.<br>Böckers <sup>3</sup>   | N/A; WB-1:500                                    |
| Rabbit anti-PSD-Zip70              | kind gift from Dr. Kenji<br>Sobue <sup>4</sup> | N/A; WB-1:1000                                   |
| Mouse anti-Rap1 and Rap2           | BD Bioscience                                  | Cat#610196;<br>RRID:AB_397595; WB-<br>1:1000     |
| Mouse anti-Thy1                    | BD Bioscience                                  | Cat#550571;<br>RRID:AB_2201306; WB-<br>1:1000    |
| Mouse anti-Intein (CBD)            | NE BioLabs                                     | Cat#E8034S; WB-1:100                             |
| Rabbit anti-RFP                    | Rockland                                       | Cat#600-401-379;<br>RRID:AB_2209751              |
| Mouse anti-GFP (3E6)               | Molecular probes                               | Cat#A11120;<br>RRID:AB_221568                    |
| Rabbit anti-His-tag                | Cell signaling                                 | Cat#2365;<br>RRID:AB_2115720; WB-<br>1:500       |
| Mouse anti-His-tag (27E8)          | Cell signaling                                 | Cat#2366S;<br>RRID:AB_10698449; WB-<br>1:500     |
| Mouse anti- $\beta$ -actin (AC-15) | Sigma                                          | Cat#A-5441;<br>RRID:AB_476744;<br>WB-1:500       |
| Mouse anti-MBP                     | NE BioLabs                                     | Cat#E8032S;WB-1:500                              |
| Rabbit anti-GST                    | Sigma                                          | Cat#G7781;<br>RRID:AB_259965;WB-<br>1:500        |
| Anti-mouse-AlexaFluor 405          | ThermoFisher Scientific                        | Cat#A-31553;<br>RRID:AB_221604; IF-1:300         |
| Anti-mouse-AlexaFluor 488          | ThermoFisher Scientific                        | Cat#A-11001; RRID:AB<br>2534069; IF-1:300        |
| Anti-mouse-AlexaFluor 568          | ThermoFisher Scientific                        | Cat#A-11004;<br>RRID:AB_2534072; IF-<br>1:300    |
| Anti-mouse-AlexaFluor 647          | ThermoFisher Scientific                        | Cat#A-21235;<br>RRID:AB_2535804; IF-<br>1:300    |
| Anti-rabbit-AlexaFluor 488         | ThermoFisher Scientific                        | Cat#A-11034;<br>RRID:AB_2576217; IF-<br>1:300    |
| Anti-rabbit-AlexaFluor 568         | ThermoFisher Scientific                        | Cat#A-11036;<br>RRID:AB_10563566; IF-            |



|                                                                           |                         |                                                        |
|---------------------------------------------------------------------------|-------------------------|--------------------------------------------------------|
|                                                                           |                         | 1:300                                                  |
| Anti-rabbit-AlexaFluor 647                                                | ThermoFisher Scientific | Cat#A-21236;<br>RRID:AB_2535805; IF-1:300              |
| Anti-guinea pig-AlexaFluor 488                                            | ThermoFisher Scientific | Cat#A-11073;<br>RRID:AB_2534117; IF-1:300              |
| Anti-goat-AlexaFluor 568                                                  | ThermoFisher Scientific | Cat#A-11057;RRID:AB_2534104;<br>IF/STED -1:200         |
| Anti-guinea pig-AlexaFluor 568                                            | ThermoFisher Scientific | Cat#A-11075;<br>RRID:AB_2534119; IF-1:300              |
| Anti-guinea pig-AlexaFluor 647                                            | ThermoFisher Scientific | Cat#A-21450;<br>RRID:AB_2535867; IF-1:300              |
| Anti-rabbit-Abberior STAR 580                                             | Abberior                | Cat#2-0012-005-8;<br>IF/STED-1:200; IF-1:200           |
| Anti-mouse-Abberior STAR 580                                              | Abberior                | Cat#2-0002-005-1;<br>RRID:AB_2620153;<br>IF/STED-1:250 |
| Anti-mouse-Abberior STAR 635P                                             | Abberior                | Cat#2-0002-007-5;<br>IF/STED-1:250                     |
| Anti-guinea pig-Abberior STAR 635P                                        | Abberior                | Cat#2-0112-007-1;<br>IF/STED-1:250                     |
| Anit-goat-IgG-HRP                                                         | Dianova                 | Cat#705-035-147;<br>RRID:AB_2337022<br>1:1000          |
| Anti-rabbit-IgG-HRP                                                       | Dianova                 | Cat#111-035-114; 1:1000                                |
| Anti-mouse-IgG-HRP                                                        | Dianova                 | Cat#115-035-146; 1:1000                                |
| Anti-guinea pig-IgG-HRP                                                   | Dianova                 | Cat#705-035-148; 1:1000                                |
| 4',6-diamidino-2-phenylindole (DAPI)                                      | Biozol                  | Cat#BCL-BCFA-211;<br>1:1000                            |
| <b>Peptides and oligonucleotides</b>                                      |                         | <b>Source</b>                                          |
| TAT-SIPA1L2- YGRKKRRQRRRPKKDCSKDLACKTLGGK                                 |                         | Genosphere<br>biotechnologies                          |
| TAT- Scr- YGRKKRRQRRRPALKLSKTDCKKGGK                                      |                         | Genosphere<br>biotechnologies                          |
| Generation of sipa1l2 mutant mice:<br>3armF, GAGGTAAGAACTGCTGTTGGTCACTG   |                         | This paper                                             |
| Generation of sipa1l2 mutant mice:<br>3armR, ACACACTGTACATACCCCTTGTTTCATC |                         | This paper                                             |
| Generation of sipa1l2 mutant mice:<br>5armF: CTCCCTCCATCCCTTGATGCCATCAC   |                         | This paper                                             |
| Genotyping: musHetR<br>ATGGGACCACATAACCGCCTCTCAGTG                        |                         | This paper                                             |
| Genotyping: Asc306 AATGGCCGCTTTTCTGGATTCATCGAC                            |                         | This paper                                             |
| Sy_EKAR cloning: SIPA_52AAGCGGCCGAGATACT                                  |                         | This paper                                             |
| Sy_EKAR cloning: SIPA_51:<br>CGGATCCA(GGTGGCGGTGGA) ATGGCCTCCTCCGAGGAC    |                         | This paper                                             |
| shRNA targeting sequence for Snapin KD<br>GATCCGTGACAACCTAGCTACAGAA       |                         | 5                                                      |
| shRNA targeting sequence for Snapin scrambled<br>ATCCGCTGACCTCCAGAAATAAGA |                         | This paper                                             |
| <b>Recombinant DNA</b>                                                    |                         | <b>Source</b>                                          |
| Mouse Syp-(Myc-DDK-tagged)-synaptophysin                                  |                         | Origene<br>MR204364;NM009305                           |
| EKAR-GFT/RFP (ERK-activity FRET sensor)                                   |                         | Addgene #18680                                         |
| Rat SyGCaMP2                                                              |                         | Addgene #26124                                         |



|                                                                            |                                      |
|----------------------------------------------------------------------------|--------------------------------------|
| Rat EGFP-LC3b                                                              | Addgene #21073                       |
| Mouse GFP-fl-SIPA1L2-1-1722 (pEGFP-C1, Clontech)                           | 1                                    |
| Human His-LC3b                                                             | Addgene #73949                       |
| Mouse GFP-fl-SIPA1L2-N705A                                                 | This paper                           |
| Mouse fl-SIPA1L2-F638A-L641A-mCherry (LIR-motif mutant: EEExxLL to EEAxAL) | This paper                           |
| Mouse SIPA1L2-1-86-100-1722-mCherry ( $\Delta 14$ )                        | This paper                           |
| Mouse SIPA1L2-RapGAP-470-838-tRFP (pTag-RFP-N1, EVROGEN)                   | This paper                           |
| Mouse SIPA1L2-PDZ-948-1025-tRFP                                            | This paper                           |
| Mouse SIPA1L2-RapGAP+PDZ-470-1025-tRFP                                     | This paper                           |
| Mouse Intein-SIPA1L2-470-1025 (PMXB10)                                     | This paper                           |
| Mouse Intein-SIPA1L2-470-1025-S990D (PMXB10)                               | This paper                           |
| Mouse Intein-SIPA1L2-RapGAP-470-838 (PMXB10)                               | This paper                           |
| Rat fl-TrkB-GFP                                                            | Kind gift from Prof. Ursula Wyneken  |
| Mouse GFP-fl-Snapin                                                        | Snapin was cloned from cDNA library. |
| Rat 6xHis-Rap1b (pET28)                                                    | This paper                           |

Supplementary table 2. Sequence and names of the primers used in this study and the corresponding constructs. In white boxes are forward primers and in blue reverse primers.

| Construct                              | Sequence (5' to 3')                                   | Primer name |
|----------------------------------------|-------------------------------------------------------|-------------|
| SNAPf-fl-SIPA1L2-1-1722                | GAGCTAGCGCCACCATGGACAAAGACTGCG                        | SIPA_01     |
|                                        | CGTTCGGACCCGACCCAGGAGCTCTG                            | SIPA_02     |
| fl-SIPA1L2-1-1722-mCherry              | CGCTAGCGCTACCGGACTCAGATCATGAGTGATCCAAGGCCATC<br>A     | SIPA_03     |
|                                        | GATCTAGAGTCGCGGCCGCTTTACTTGTACAGCTCGTCCATGCC          | SIPA_04     |
| GFP-fl-SIPA1L2-N705A                   | GCACATCGGGGCCGACATCGTC                                | SIPA_05     |
|                                        | CCGTCGTGCGAAGACTCCTTCTC                               | SIPA_06     |
| fl-SIPA1L2-F638A-L641A-mCherry         | GGCCTTCGAGGAGGCCCTGGACGCCCTCGGCCAGCGA                 | SIPA_07     |
|                                        | TCGCTGGCCGAGGGCGTCCAGGGCCTCCTCGAAGGCC                 | SIPA_08     |
| GFP-RapGAP-625-813                     | GAGAATTCATGTACAACAACGAGACAG                           | SIPA_09     |
|                                        | ACGGATCCCGTAAATCCTTTAAGTATTCAT                        | SIPA_10     |
| SIPA1L2-RapGAP-470-838-tRFP            | GATCTCGAGCTCAAGCTTCTGCCACC ATG<br>GAGAAGGTCAAACGTTAC  | SIPA_11     |
|                                        | CTCTTCGCCCTTAGACACTGGATCCACCTTCTTCGCCCCCAGGG<br>TG    | SIPA_12     |
| SIPA1L2-PDZ-948-1025-tRFP              | GATCTCGAGCTCAAGCTTCTGCCACC ATG<br>GAGACTGTGGAAATG     | SIPA_13     |
|                                        | CTCTTCGCCCTTAGACACTGGATCCACCTCATGGGGCTGGATG           | SIPA_14     |
| SIPA1L2-RapGAP+PDZ-470-1025-tRFP       | 5GATCTCGAGCTCAAGCTTCTGCCACC ATG<br>GAGAAGGTCAAACGTTAC | SIPA_15     |
|                                        | CTCTTCGCCCTTAGACACTGGATCCACCTCATGGGGCTGGATG           | SIPA_16     |
| SIPA1L2-Actl-1-164-delta 88-99-mCherry | TGGGAGAGCCGTTCTCAGTCC                                 | SIPA_17     |

|                               |                                              |             |
|-------------------------------|----------------------------------------------|-------------|
|                               | AGGCCACTCAGACACTCTGGC                        | SIPA_1<br>8 |
| Intein-SIPA1L2-RapGAP-470-838 | TTATATCATATGGAGAAGGTCAAACGTTACA              | SIPA_1<br>9 |
|                               | AAATATCTCGAGCTTCTTCGCCCCC                    | SIPA_2<br>0 |
| Intein-SIPA1L2-470-1025       | TTATATCATATGGAGAAGGTCAAACGTTACA              | SIPA_2<br>1 |
|                               | AAATATCTCGAGGGGCTGGATGATAACCACTTT            | SIPA_2<br>2 |
| Intein-SIPA1L2-470-1025-S990D | GATCGGCTTGTGGAGATCTGCAAG                     | SIPA_2<br>3 |
|                               | CCCTTGGCGAAGGCCTGCCTTC                       | SIPA_2<br>4 |
| MBP-TrkB-454-821              | GATCGAGGGAAGGATTTCAAGAATTCAAGTTGGCGAGACATTCC | SIPA_2<br>5 |
|                               | GCTGAAAATCTTATCTCAGCCTAGGATGTCCAGG           | SIPA_2<br>6 |
| MBP-TrkB-454-465              | GATCGAGGGAAGGATTTCAAGAATTCAAGTTGGCGAGACATTCC | SIPA_2<br>7 |
|                               | GCTGAAAATCTTATCTCAGCCTTTCATGCCAAAC           | SIPA_2<br>8 |
| fl-TrkB-SNAPf                 | CGGGATCCACCATGGACAAAGACTGC                   | SIPA_2<br>9 |
|                               | CCGTTCGGACCCGACCCAAGTAGATCTCG                | SIPA_3<br>0 |
| GFP-fl-Snapin-1-136           | GAGAATTCATGGCCGCGGCTGGTTC                    | SIPA_3<br>1 |
|                               | CCAAGAGGTTTCGTTTATTCCTAGGCG                  | SIPA_3<br>2 |
| GFP-Snapin-S50D               | GTCAGAGAAGATCAAGTAGAG                        | SIPA_3<br>3 |
|                               | CTCTACTTGATCTTCTCTGAC                        | SIPA_3<br>4 |
| GFP-Snapin-S50A               | GTCAGAGAA GCG CAAGTAGAG                      | SIPA_3<br>5 |
|                               | CTCTACTTGCGCTTCTCTGAC                        | SIPA_3<br>6 |
| 6xHIS-Snapin                  | GGTCGCGGATCCGATGGCGGGGGCTGGTTC               | SIPA_3<br>7 |
|                               | GAGCTCGAATTCTTGCCTGGGGAGCCAG                 | SIPA_3<br>8 |
| SNAPf-fl-LC3b-1-142           | GCGGATCCATGCCGTCCGAGAAGAC                    | SIPA_3<br>9 |
|                               | CTCGGTACGAACACTGAGCTCGC                      | SIPA_4<br>0 |
| 6xHis-Rap1b                   | TTCTGCCATATGCGTGAGTATAAGCTAGTCGTTC           | SIPA_4<br>1 |

|                                                  |                                                      |             |
|--------------------------------------------------|------------------------------------------------------|-------------|
|                                                  | TATGCAGGATCCTTAAAGCAGCTGACATGATGA                    | SIPA_4<br>2 |
| GST-RapGAP                                       | AGCTTAGAATTCCGGTACTGCAAAGCCGGGCAG                    | SIPA_4<br>3 |
|                                                  | GAATTGGATCCGTAAATCCTTTAAGTATTCATGCCTT                | SIPA_4<br>4 |
| GST-SIPA1L2-PDZ                                  | AGCTTAGAATTCCGGGAAATGACCCTGAGAAGGAAT                 | SIPA_4<br>5 |
|                                                  | GAATTGGATCCGCTCATGGGGCTGGATGATA                      | SIPA_4<br>6 |
| GST-ActII<br>GST-SIPA1L2-<br>ActII-1026-<br>1650 | GGTCGTGGGATCCCCGAATTCGCCCTTCACCATGAGGATGGCTC<br>TCCC | SIPA_4<br>7 |
|                                                  | CGTCAGTCAGTCACGATGCGGCCGCTCTATGATCGCTCCCCTGG<br>TG-  | SIPA_4<br>8 |
| GST-SIPA1L2-<br>ActI- 1-624                      | GGTCGTGGGATCCCCGAATTCGCCCTTCAC<br>ATGAGTGATCCAAGGCC  | SIPA_4<br>9 |
|                                                  | CGTCAGTCAGTCACGATGCGGCCGCTCTATTCTCTTCCGTGCT<br>CTG   | SIPA_5<br>0 |
| Sy-EKAR                                          | CGGATCCAGGTGGCGGTGGAATGGCCTCCTCCGAGGAC               | SIPA_5<br>1 |
|                                                  | AAGCGGCCGCAGATACT                                    | SIPA_5<br>2 |
| musHet                                           | TGAAAATCTTGGCCCTGTGGCGGTCAG                          | SIPA_5<br>3 |
|                                                  | ATGGGACCACATAACCGCCTCTCAGTG                          | SIPA_5<br>4 |
| Asc306                                           | AATGGCCGCTTTTCTGGATTCATCGAC                          | SIPA_5<br>5 |
| 3arm                                             | GAGGTAAGAACTGCTGTTGGTCACTG                           | SIPA_5<br>6 |
|                                                  | ACACACTGTACATACCCCTTGTTTCATC                         | SIPA_5<br>7 |
| 5armF                                            | CTCCCTCCATCCCTTGATGCCATCAC                           | SIPA_5<br>8 |

## Supplementary references

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