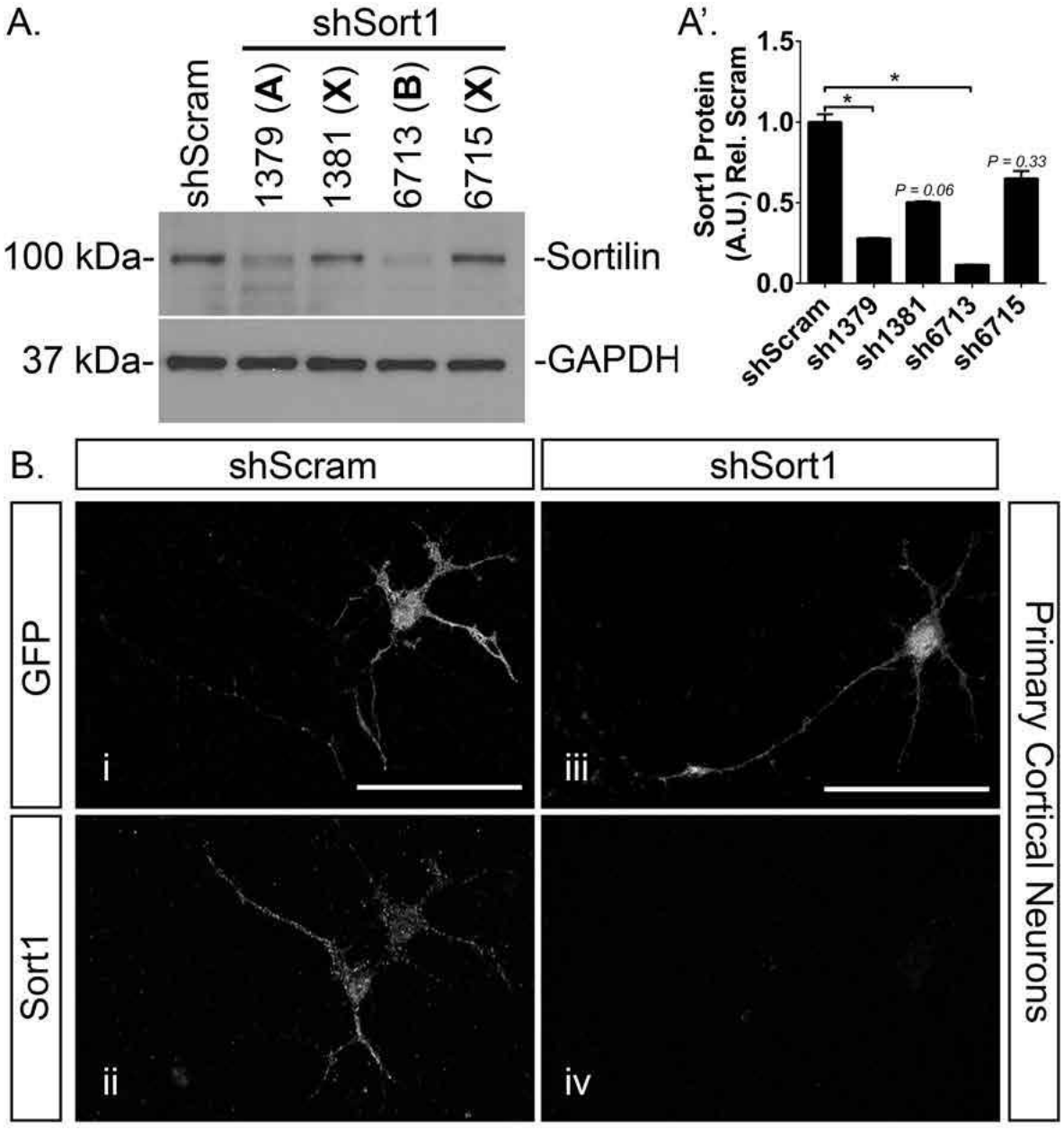
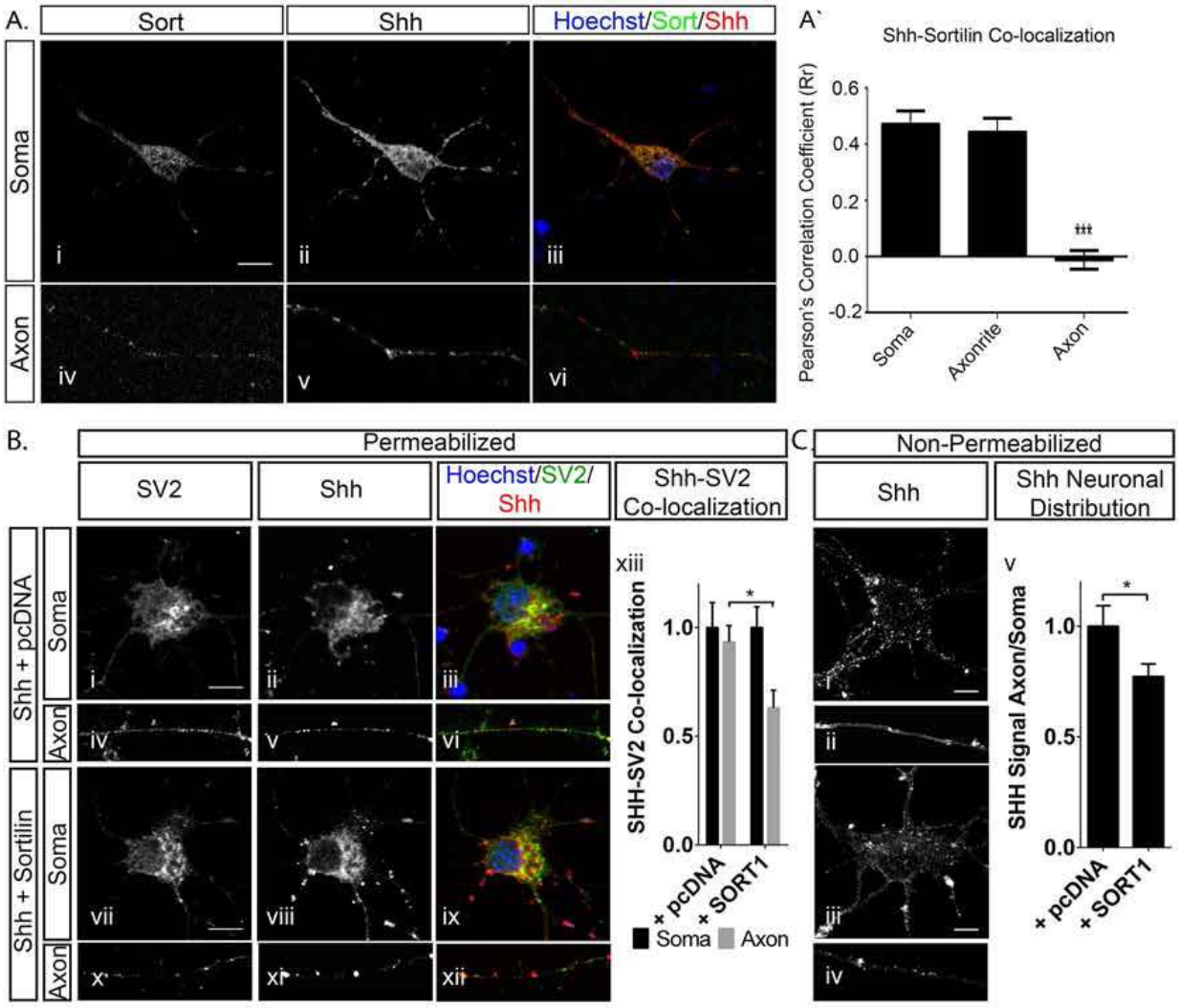
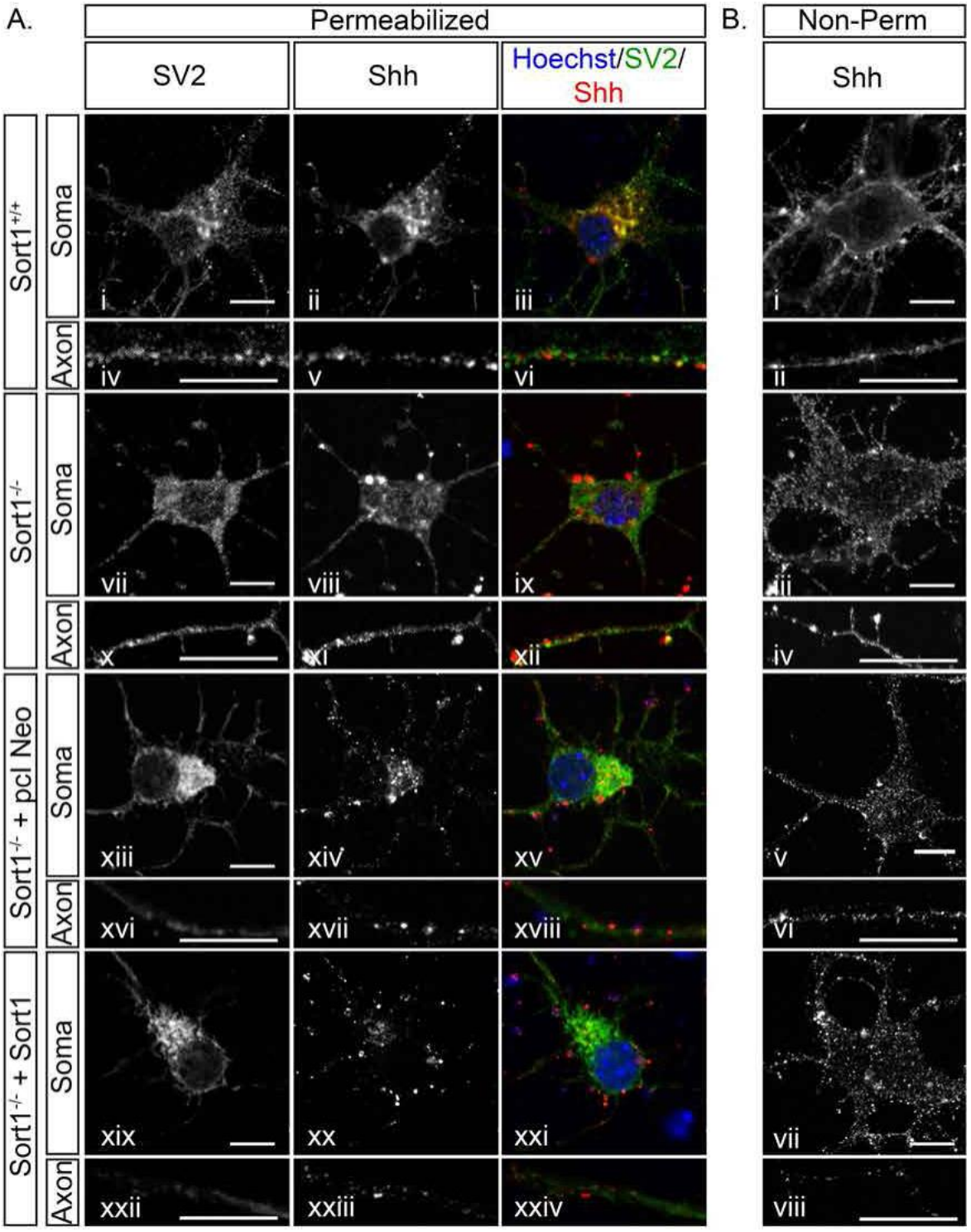

A.
shScram
shSort1
1379 (A)
1381 (X)
6713 (B)
6715 (X)
100 kDa-
-Sortilin
37 kDa-
-GAPDH
A'.
Sort1 Protein (A.U.) Rel. Scram
1.5
1.0
0.5
0.0
*
*
P = 0.06
P = 0.33
shScram
sh1379
sh1381
sh6713
sh6715
B.
shScram
shSort1
GFP
Sort1
i
ii
iii
iv
Primary Cortical Neurons

**Supplemental Figure 1: Validation of short hairpin-mediated Sortilin knockdown. (A, A')** Representative western blots analysis of Sort1 levels in 3T3 cells transiently transfected with a control short hairpin (shScram), or the indicated shSort1 constructs. GAPDH is used as a loading control. Functional short hairpin chosen for further analysis are indicated as "A" and "B", and indicated as shSort1 A or B subsequently in the text. (A') Sort1 protein densitometry was performed on blots in A using the Gels function on ImageJ. Bars indicate mean Sort1 levels from three independent experiments, normalized to GAPDH, relative to scrambled control. Error bars represent S.E.M., * $P < 0.05$, Student's t-test. **(B)** Expression of functional Sort1 KD constructs correlates with a reduction of Sort1 signal in primary neurons. ICC on fixed and permeabilized CNs. GFP indicates transfected cells and (i, iii), Sort1 KD confirmed by staining with α-Sort1 antibody (ii, iv). Scale bars, 10μm.

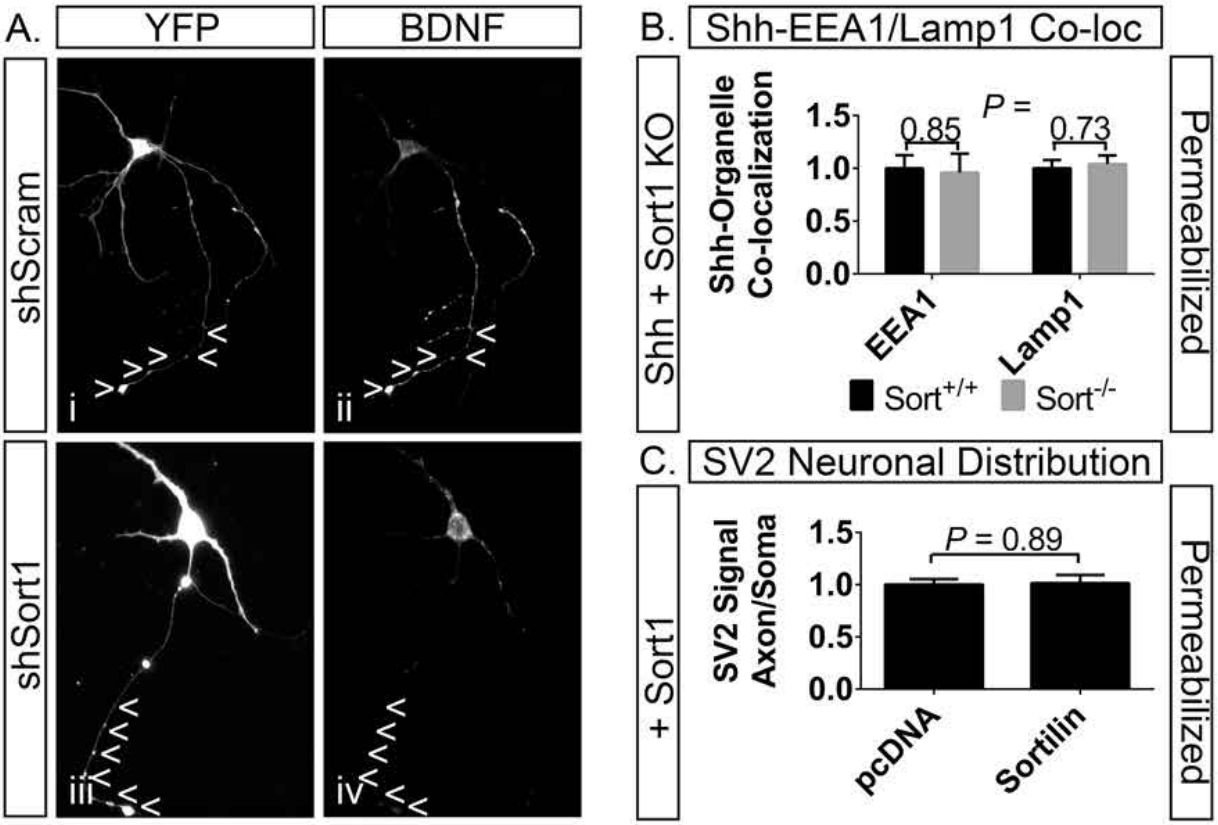



**Supplemental Figure 2: Overexpressed Shh and endogenous Sort1 co-localize extensively in the somatodendritic compartment, but not in axons, in primary CNs.** **(A)** ICC and Hoechst nuclear staining on fixed and permeabilized CNs. Panels show 1μm optic sections in the somatodendritic (upper), or axonal (lower) compartments. Scale bars, 10μm. **(A')** Quantification of the co-localization of Shh and Sort1 in the indicated subcellular compartment in CNs. Bars represent mean Pearson's Correlation Coeffecient (Rr) ($n > 5$ cells per condition). Error bars represent S.E.M., * $p < 0.05$, Student's t-test. **(B)** Overexpression of Sort reduces Shh trafficking to the axon in cortical neurons. Sort1 overexpression reduces the co-localization of Shh with SV2. Representative IHC on fixed and permeabilized primary CNs expressing Shh and pcDNA (i-vi) or Sort1 (vii-xii). Panels show 1μm optic sections in the somatodendritic (upper), or axonal (lower) compartments. Scale bars, 10μm. Co-localization of Shh and SV2 was quantified using

the Intensity Correlation Analysis function in ImageJ (xiii). Bars indicate mean Pearson's Correlation Coeffecient (Rr) (n = 20 neurons per condition) normalized to control conditions. Error bars represent S.E.M., * $p < 0.05$, Student's t-test. **(C)** Sort1 overexpression correlates with a reduction in the ratio of Shh signal on the surface of the axon relative to the soma. Representative ICC on fixed, non-permeabilized primary CNs expressing Shh and shScram (i-ii) or shSort1 (iii-iv). Panels show 1μm optic sections in the somatodendritic (upper), or axonal (lower) panels. Scale bars, 10μm. Shh distribution quantified as the ratio of Shh signal intensity in a distal region of the axon relative to signal intensity in the soma (v). Bars represent mean ratio of axon: soma Shh signal (n = 20 neurons per condition) normalized to control conditions. Error bars represent S.E.M., * $p < 0.05$, Student's t-test.



**Supplemental Figure 3: Sort1 expression negatively correlates with distribution of Shh in axons. (A)** Sort1$^{-/-}$ increased the colocalization of Shh with SV2, Sort1 overexpression in Sort1$^{-/-}$ rescued the phenotype. ICC and Hoechst nuclear staining on fixed and permeabilized primary cortical neurons expressing Shh in Sort1$^{-/-}$ mice (i - xii), or from Sort1$^{-/-}$ mice overexpressing Sort1 (xiii - xxiv). Panels show 1μm optic sections in the somatodendritic (upper), or axonal (lower) compartments. Scale bars, 10μm. Co-localization quantified in Fig 4Di, Ei. **(B)** Sort1$^{-/-}$ correlates with increased ratio of Shh signal on the surface of the axon relative to the soma, Sort1 overexpression in Sort1$^{-/-}$ rescued the phenotype. Sort1 KD correlates with increased ratio of Shh signal on the surface of the axon relative to the soma. ICC on fixed, non-permeabilized primary cortical neurons expressing Shh in Sort1$^{-/-}$ mice (i - iv), or from Sort1$^{-/-}$ mice overexpressing Sort1 (v - viii). Panels show 1μm optic sections in the somatodendritic (upper), or axonal (lower) panels. Scale bars, 10μm. Shh distribution quantified in Fig 4Dii, Eii.




**Supplemental Figure 4: Sort1 perturbation reduces BDNF-HA targeting to the axon, does not affect Shh endosomal or lysosomal targeting, and does not impair SV2+ vesicle biogenesis.** **(A)** Sort1 KD reduced BDNF targeting to axons. ICC on fixed, permeabilized primary cortical neurons transiently transfected with BDNF-HA and shScram (top panels) or shSort1 (bottom panels). Panels show GFP (expressed through an IRES on the sh constructs) and BDNF-HA. ">" denotes axonal compartments, as determined by morphology and YFP staining, Scale bars, 10um. **(B)** Sort1 expression does not correlate with changes in Shh co-localization with EEA1 or Lamp1 + vesicles. Co-localization of Shh and EEA1 or Lamp1 in $Sort1^{+/+}$ and $Sort1^{-/-}$ CNs, quantified using the Intensity Correlation Analysis function in ImageJ. Bars indicate mean Pearson's Correlation Coeffecient (Rr) (n = 20 neurons per condition) normalized to control conditions. Error bars represent S.E.M., * $p < 0.05$, Student's t-test. **(C)** Sort1 overexpression does not correlate with a change in the ratio of SV2 signal in the axon relative to the soma. SV2 neuronal distribution quantified as the ratio of SV2 signal intensity in a distal region of the axon relative to signal intensity in the soma in CNs expressing pcDNA or Sort1-myc his. Bars indicate mean ratio of axon:soma SV2 signal (n = 20 neurons per condition) normalized to control conditions. Error bars represent S.E.M., * $p < 0.05$, Student's t-test.

**Supplemental Table 1: Novel Shh interacting candidates identified in a Shh GST affinity screen.** Interacting candidates identified using a GST affinity screen using ShhN-GST or ShhC-GST as bait, and rat brain microsomal fraction as prey. Candidates were prioritized based on peptide abundance and MASCOT score, with common Sepharose bead artifacts and cytoplasmic localized proteins excluded. Sheet 1 indicates ShhN interactors, Sheet 2 indicates ShhC interactors. Candidates are grouped according to the detergent used to generate the microsomal fraction, either NP40 (top) or CHAPS (bottom), and are represented by name and relevant accession number.

| Bait | Detergent | Interacting Protein | Accession Number |
|---|---|---|---|
| ShhN | NP40 | Agrin | gi\|202799 |
| ShhN | NP40 | Neurexin II | gi\|205715 |
| ShhN | NP40 | Kif1a | gi\|109487519 |
| ShhN | NP40 | myotonic dystrophy kinase-related Cdc42-binding kinase MRCK-beta [Rattus norvegicus] | gi\|2736153 |
| ShhN | NP40 | triple functional domain (PTPRF interacting) | gi\|109464537 |
| ShhN | NP40 | Development and differentiation-enhancing factor 2 | gi\|109478077 |
| ShhN | NP40 | SSTR4 | gi\|7514122 |
| ShhN | CHAPS | low density lipoprotein receptor-related protein | gi\|62652278 |
| ShhN | CHAPS | Agrin | gi\|202799 |
| ShhN | CHAPS | glypican 5 | gi\|109501994 |
| ShhN | CHAPS | podocalyxin-like 2 | gi\|109472343 |
| ShhN | CHAPS | FASN | gi\|55775 |
| ShhN | CHAPS | PI-3-kinase-related kinase SMG-1 | gi\|109462744 |
| ShhN | CHAPS | Neurexin 1/2 | gi\|124106289 |
| ShhN | CHAPS | chondroitin sulfate proteoglycan NG2 | gi\|539947 |
| ShhN | CHAPS | chondroitin sulfate proteoglycan 5 | gi\|41281651 |
| ShhN | CHAPS | Dmx-like 2 | gi\|109483500 |
| ShhN | CHAPS | neuroglycan C | gi\|1585922 |
| ShhN | CHAPS | FAT tumor suppressor homolog 4 | gi\|109464786 |
| ShhN | CHAPS | Kif1a | gi\|109487519 |
| ShhN | CHAPS | Kif1b | gi\|52313412 |
| ShhN | CHAPS | Cdc42-binding protein kinase beta | gi\|76257394 |
| ShhN | CHAPS | Plexin | gi\|109481881 |
| ShhN | CHAPS | neural cell adhesion molecule | gi\|13928706 |
| ShhN | CHAPS | roundabout homolog 1 | gi\|11559953 |
| ShhN | CHAPS | triple functional domain (PTPRF interacting) | gi\|109464537 |
| ShhN | CHAPS | rapamycin and FKBP12 target-1 protein | gi\|9845251 |
| ShhN | CHAPS | acetyl-coenzyme A carboxylase alpha | gi\|11559962 |
| ShhN | CHAPS | similar to CG5937-PA | gi\|109457596 |
| ShhN | CHAPS | odd Oz/ten-m homolog | gi\|109459066 |
| ShhN | CHAPS | neurofibromatosis 1 | gi\|6981264 |
| ShhN | CHAPS | FASN | gi\|56621 |
| ShhN | CHAPS | fat3 [Rattus norvegicus] | gi\|19924085 |
| ShhN | CHAPS | insulin-like growth factor 2 receptor | gi\|6981078 |
| ShhN | CHAPS | Sortilin-related receptor SorLA | gi\|109484566 |
| ShhN | CHAPS | neurestin alpha | gi\|9910320 |
| ShhN | CHAPS | neuropilin | gi\|2407643 |
| ShhC | NP40 | sortilin 1 | gi\|109465375 |
| ShhC | NP40 | glycoprotein, synaptic 2 | gi\|19924091 |
| ShhC | NP40 | SSTR4 | gi\|7514122 |
| ShhC | CHAPS | sortilin 1 | gi\|109465375 |
| ShhC | CHAPS | glycoprotein, synaptic 2 | gi\|19924091 |
| ShhC | CHAPS | collapsin response mediator proteins | gi\|1518520 |
| ShhC | CHAPS | ilvB (bacterial acetolactate synthase)-like | gi\|34862359 |
| ShhC | CHAPS | synaptotagmin P65 - rat | gi\|92791 |
| ShhC | CHAPS | synaptotagmin 2 [Rattus norvegicus] | gi\|6981624 |
| ShhC | CHAPS | copine 7/4 | gi\|109508168 |
| ShhC | CHAPS | FASN | gi\|56133 |
| ShhC | CHAPS | neurestin alpha [Rattus norvegicus] | gi\|9910320 |
| ShhC | CHAPS | neuroligin 3 [Rattus norvegicus] | gi\|19705445 |
| ShhC | CHAPS | Putative alpha-mannosidase C1orf22 | gi\|109498013 |
| ShhC | CHAPS | Exocyst complex component 4 (Exocyst complex component Sec8) (rSec8) | gi\|24418659 |
| ShhC | CHAPS | EH-domain containing 1 [Mus musculus] | gi\|7106303 |

**Supplemental Table 2: List of antibodies used in this study.** Antibodies used in this study listed in alphabetical order of common name, with species, antibody #, source, and application also indicated. WB = western blot, ICC = immunocytochemistry, IHC = immunohistochemistry.

| Antigen | Species | Antibody # | Source | Application | Dilution |
|---|---|---|---|---|---|
| Calnexin | Rabbit | ab22595 | Abcam | ICC | 1:200 |
| c-Myc tag | Mouse | 9E10 (sc-40) | Santa Cruz Biotech | WB/ICC | 1:1000/1:200 |
| EEA1 | Rabbit | ab2900 | Abcam | ICC | 1:200 |
| GAPDH | Mouse | 6C5 (ab8245) | Abcam | WB | 1:5000 |
| GFP | Goat | 600-101-215 | Rockland Inc | WB | 1:1000 |
| GFP | Rabbit | A11122 | Life Technologies | ICC/IHC | 1:1000 |
| HA tag | Rabbit | Y11 (sc-805) | Santa Cruz Biotech | ICC | 1:200 |
| Lamp1 | Rabbit | ab24170 | Abcam | ICC | 1:200 |
| Pax2 | Goat | PRB-276D | Covance | IHC | 1:200 |
| ShhN | Rabbit | H-160 (sc-9024) | Santa Cruz Biotech | WB/ICC | 1:1000/1:200 |
| ShhN (mature) | Mouse | 5E1 | Dev. Studies Hybridoma Bank | ICC | 1:5000 |
| Sortilin | Rabbit | ab16640 | Abcam | WB/ICC | 1:1000/1:200 |
| SV2 | Rabbit | 119002 | Synaptic Systems | ICC | 1:200 |
| Tau | Rabbit | 314002 | Synaptic Systems | ICC | 1:5000 |
| TGN-38 | Mouse | sc-271624 | Santa Cruz Biotech | ICC | 1:200 |
| GRP78 | mouse | 610979 | BD Biosciences | WB | |
| GS28 | mouse | 611184 | BD Biosciences | WB | |
| SHH | rabbit | Sc-9024 | Santa Cruz | WB | |
| Sortilin | mouse | 612101 | BD Biosciences | WB | |
| Vti1b | mouse | 611405 | BD Biosciences | WB | |