Supporting Information

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SI Materials and Methods

Generation of His_6-HA-SUMO1 KI Mice. A 15-kbp SV129 mouse genomic fragment containing exon 1 of the SUMO1 gene and flanking sequences was isolated from a λFIXII genomic library (Stratagene) (Fig. S1*A*). A 3-kbp fragment containing exon 1 of the SUMO1 gene was subcloned into pBluescript. The His_6-HA double tag was inserted in frame 3′ of the start codon using PCR with engineered primers. The mutated fragment was then excised with XbaI and inserted into the NheI site of the targeting vector pTKNeoLox. For the long arm, a SmaI/BamHI fragment of 5 kbp was excised from the genomic clone and inserted into the NsiI/BamHI sites of the targeting vector. The final construct contained two copies of the HSV thymidine kinase gene, a short arm containing the tagged exon 1, a neomycin resistance cassette flanked by two loxP sites, and a long arm (Fig. S1*B*) and was used to transfect mouse embryo-derived stem (ES) cells (SV129/ola). Southern blot analyses using EcoRI and a probe located 5′ of the targeting vector (Fig. S1*B*) revealed that 1% of G418/gancyclovir-resistant ES cell clones were carrying the His_6-HA-SUMO1 mutation as a result of homologous recombination. One clone was injected into blastocysts of C57 mice to obtain chimeric mice that transmitted the mutation via the germ line as assessed by PCR (Fig. S1*C*). Heterozygous mice were crossed with EIIa-cre mice that carry the *cre* transgene under the control of the adenovirus EIIa promoter and express Cre recombinase in early embryonic stages (1). Germ-line transmission of the Cre recombined gene was assessed by PCR (Fig. S1 *A* and *B*). Heterozygous mutants were crossed to generate WT and KI littermates that were used to generate separate WT and KI lines. For genotyping, DNA was prepared from tail tips using a commercial genomic DNA isolation kit (Nextec).

Southern Blot and PCR Analyses. Twenty micrograms of DNA were digested overnight (EcoRI), separated on an agarose gel, and transferred onto nylon membranes. After cross-linking, membranes were radiolabeled using a ^{32}P-labeled probe, which represents bp −4,595 to −5,113 upstream of the start codon of the SUMO1 gene (Fig. S1 *A* and *B*). For PCR analyses, we used the following primers: forward primer, 5′-CCCGGGTGAATCCACGTCA-3′; reverse primer for Cre recombined DNA, 5′-CTGGGCGCCGTCGAGAG-3′; reverse primer for non-Cre recombined DNA, 5′-TGAAAACCACACTGCTCGACC-3′. The PCR generated a DNA fragment of 287 bp in the WT case, of 410 bp in KI mice after Cre recombination, and of 354 bp for KI mice before Cre recombination.

Monoclonal Anti-SUMO Antibodies. The anti-SUMO1(21C7)– and anti-SUMO2(8A2)–expressing hybridoma cells were developed by M. Matunis and were obtained from the Developmental Studies Hybridoma Bank, which has been developed under the auspices of the National Institute of Child Health and Human Development and maintained by the Department of Biology, University of Iowa, Iowa City, IA.

Biochemistry. Subcellular fractionations were prepared essentially as described previously (2). They were designated as follows: H, homogenate; P1, nuclear pellet; S1, supernatant after P1 sedimentation; P2, crude synaptosomal pellet; S2, supernatant after P2 sedimentantion; LP1, lysed synaptosomal membranes; LS1, supernatant after LP1 sedimentation; and SPM, synaptic plasma membranes. Total homogenates of mouse brains were generated by homogenization in 320-mM sucrose containing 4 mM Hepes (pH 7.4), protease inhibitors (1 μg/mL aprotinin, 0.5 μg/mL leupeptine, and 17.4 μg/mL PMSF), and 20 mM N-ethylmaleimide (NEM).

Immunoblotting and Quantitative Western Blot Analysis. SDS/PAGE was performed with standard discontinuous gels or with commercially available 4–12% (wt/vol) Bis-Tris gradient gels (Invitrogen). Western blots were probed using primary and secondary antibodies as indicated in Tables S1 and S2. Blots were routinely developed using enhanced chemiluminescence (GE Healthcare). For quantitative Western blotting, transferred proteins were visualized by Fast Green FCF (Sigma) staining of the membrane. Corresponding images were taken with an Odyssey reader (LI-COR) using the 700-nm channel and used for the normalization of protein loading. After standard Western blot analyses using secondary antibodies conjugated to IRDye800 (Biomol), signals were measured using an Odyssey reader and corresponding software (LI-COR).

Immunohistochemistry. Mice were anesthetized and transcardially perfused with 4% (wt/vol) paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB) (pH 7.4) at 4 °C for 10 min. Brains were removed and postfixed for 1 h at 4 °C. The tissue was cryoprotected in 30% (wt/vol) sucrose in PBS. Sagittal 35-μm sections were prepared with a cryostat and collected free-floating in PBS. For immunohistochemistry, sections were preincubated for 1 h in PB containing 5% (vol/vol) normal goat serum (NGS) and 0.3% (vol/vol) Triton X-100 and then incubated for 24 h at 4 °C in primary antibodies diluted in PBS containing 2% (vol/vol) NGS and 0.3% (vol/vol) Triton X-100. After washing repeatedly in PBS, sections were incubated for 2 h in dye-coupled secondary antibodies, repeatedly washed, and mounted on slides with Aquapolymount (Polysciences). The antibodies used are listed in Tables S1 and S2. For Nissl staining, sections were washed once with PBS, mounted on SuperFrost Plus slides (Menzel GmbH), and air-dried before staining with 0.1% (wt/vol) thionin.

Immunocytochemistry. Hippocampal neurons were prepared as described (3, 4). Primary neuron cultures were fixed for 20 min in cold PB containing 4% (wt/vol) PFA (pH 7.4). Coverslips were then washed repeatedly in PBS. Blocking and permeabilization were performed for 1 h at room temperature in PBS containing 3% (vol/vol) FBS and 0.3% (vol/vol) Triton X-100. Primary antibodies were applied for 2 h at room temperature in PBS containing 3% (vol/vol) FBS. Coverslips were then washed repeatedly in PBS and incubated with secondary antibodies for 1 h at room temperature in PBS containing 3% (vol/vol) FBS. Coverslips were then washed repeatedly in PBS and mounted on slides with Aquapolymount (Polysciences). The antibodies used are listed in Tables S1 and S2.

Image Acquisition. Serial confocal images were captured at a magnification of 63× (sections) or 100× (cultures) on a confocal laser-scanning microscope (LSM SP2 and SP5; Leica). All images were acquired as single optical sections. During acquisition, imaging parameters (gain and offset) were kept constant for a given labeling and/or genotype to allow for fluorescence intensity comparisons.

Immunoaffinity Purification. For anti-HA immunoaffinity purification, frozen brains were reduced to powder in a liquid nitrogen bath using a porcelain mortar and pestle. The powder was resuspended in cold RIPA buffer [150 mM NaCl, 1% (vol/vol)

Triton X-100, 0.5% (wt/vol) sodium deoxycholate, 0.1% (wt/vol) SDS, and 10 mM Tris (pH 7.6)] containing protease inhibitors (1 μg/mL aprotinin, 0.5 μg/mL leupeptine, and 17.4 μg/mL PMSF) and 20 mM NEM, sonicated, and ultracentrifuged at 100,000 × *g* for 1 h at 4 °C. The resulting supernatant was passed over a column containing 0.4 mL of anti-HA beads (Roche) for 12 h at a flow rate of 1 mL/min. The column was washed with 100 column volumes of RIPA buffer, and bound material was eluted twice, once at 30 °C and once at 37 °C, with three column volumes of RIPA buffer containing HA peptide (0.5 mg/mL). Eluates were pooled and proteins were precipitated as described (5). The precipitates were dissolved in SDS/PAGE sample buffer. For immunoprecipitation of specific proteins, whole-brain extracts were prepared as described above. The resulting extract was preincubated with either Protein A or Protein G Sepharose beads (GE Healthcare). The beads were then removed by centrifugation, and antibodies (specific IgG or control IgG from the same species) were added to the supernatant, along with new Protein A or Protein G Sepharose beads (GE Healthcare). After incubation for 2 h at 4 °C on a rotating wheel, the beads were pelleted and washed repeatedly in RIPA buffer. Bound material was eluted directly into SDS/PAGE sample buffer.

Mass Spectrometry and Data Analysis. Proteins were separated by one-dimensional SDS/PAGE [4–12% (wt/vol) Bis-Tris gradient gels; Invitrogen] and the entire lane of the Coomassie blue-stained gel was cut into 23 slices. All slices were reduced with 10 mM DTT for 55 min at 56 °C, alkylated with 55 mM iodoacetamide for 20 min at 26 °C, and digested with modified trypsin (Promega) overnight at 37 °C. Tryptic peptides were injected into a C_{18} precolumn (1.5 cm, 360 μm o.d., 150 μm i.d., Reprosil-Pur 120A°, 5 μm, C_{18}-AQ; Dr. Maisch GmbH) at a flow rate of 10 μL/min. Bound peptides were eluted and separated on a C_{18} capillary column (15 cm, 360 μm o.d., 75 μm i.d., Reprosil-Pur 120A°, 5 μm, C_{18}-AQ; Dr. Maisch GmbH) at a flow rate of 300 nL/min, with a 7.5–37.5% (vol/vol) acetonitrile gradient in 0.1% (vol/vol) formic acid for 50 min using an Agilent 1100 nano-flow LC system (Agilent Technologies) coupled to an LTQ-Orbitrap XL or Velos hybrid mass spectrometer (Thermo Fisher Scientific). Mass spectrometry (MS) conditions were as follows: spray voltage, 1.5 kV; normalized collision-induced dissociation (CID) collision energy 37.5% for MS/MS in LTQ. An activation q = 0.25 and activation time of 30 ms were used. The mass spectrometer was operated in the data-dependent mode to automatically switch between MS and MS/MS acquisition. Survey MS spectra were acquired in the Orbitrap (*m/z* 350–1,600) with the resolution set to 30,000 at *m/z* 400 and automatic gain control target at 5×10^5. The five most intense ions were sequentially isolated for CID MS/MS fragmentation and detection in LTQ for Orbitrap XL MS. The 15 most intense ions were used for Orbitrap Velos MS. Ions with single and unrecognized charge states were excluded. Raw data were analyzed with MaxQuant software version 1.2.2.5 (6, 7) or the Mascot search engine for peptide and protein identifications (version 2.3.02; Matrix Science). The International Protein Index (IPI) mouse database (version 3.87) was used as the sequence database. The MS mass tolerance was set to 7 ppm and MS/MS mass tolerance was set to 0.6 Da. Up to two missed cleavages of trypsin were allowed. Methionine oxidation and cysteine carboxyamidomethylation were searched as variable modifications. The false-positive rate was set to 1% at the peptide level and the protein level. Proteins were filtered using the following requirements: at least one unique peptide in two out of four KI samples, and no identified peptides in the WT samples. Because some SUMOylated proteins that also bind nonspecifically to the affinity matrix would be filtered with these settings, we quantified the abundance of the remaining proteins in the KI and WT samples using the robust statistical framework QSpec (8). Proteins were quantified based on their spectral count across all experiments. Flagged highly abundant proteins with an overestimated Bayes factor were excluded. Proteins enriched in the KI sample with a Bayes factor of at least 5 were included in the final list (Tables S1 and S2).

Identification of SUMOylated Lysines. Identification of SUMOylated lysine residues was performed as described (9). In brief, we used the ChopNSpice software (www.chopnspice.gwdg.de) to generate a concatenated Rangap1 sequence to identify the actual SUMO-sites with Mascot as a search engine. For this purpose, the FASTA sequence of RanGAP1 was chopped into tryptic fragments allowing zero, one, two, or three missed cleavages. The tryptic peptide sequence of SUMO1 that is putatively attached to any lysine residue within RanGAP1 is attached to the N terminus of each tryptic peptide of RanGAP1 that contains a lysine residue at a missed cleavage site. To avoid the generation of nonnatural peptides, a virtual amino acid J is attached to the C terminus of each tryptic fragment derived from RanGAP1 and SUMO1. The modified tryptic fragments are concatenated to yield a novel large FASTA sequence that is submitted to a database search. Upon database search with the search engine Mascot, cleavage with an artificial endoproteinase was allowed that specifically recognizes N- and C-terminal J and a user-defined number of missed cleavages. The search engine then compares the *in silico* generated and concatenated tryptic peptides derived from RanGAP1 attached to a tryptic peptide derived from SUMO1 with the fragment spectra obtained experimentally by LC-MS/MS. The following parameters were used with the ChopNSpice software: spice species was *Mus musculus*; spice sequence was SUMO1; spice site was KX; spice mode was once per fragment; include unmodified fragments in output; enzyme was trypsin; allow up to three protein miscleavages; allow up to one miscleavage in the spice sequence; output formatting was FASTA (single protein sequence); mark all cleaved sites J; retain comments in FASTA format without line breaks in FASTA output. For SUMOylated site identification with Mascot, all MS/MS spectra were searched against a new FASTA file that was created by ChopNSpice with the following parameters: mass tolerance of 7 ppm in MS mode and 0.8 Da in MS/MS mode; allow zero missed cleavages; consider methionine oxidation and cysteine carboxyamidomethylation as variable modifications; enzyme cleaved at J at N and C termini for Mascot.

Functional and Network Analysis. For functional and network analyses, the IPI ID numbers of all proteins were mapped to UniProtKB accession numbers. The Ingenuity pathway analysis software (Ingenuity Systems) was used to identify enriched biological functions related to the identified proteins. Uniprot Knowledgebase (UniProtKB) accession numbers were mapped to the Ingenuity database and statistically enriched (Fisher's exact test, $P < 0.05$) molecular and cellular functions were identified and plotted as a bar chart. The known protein–protein interactions within each dataset were obtained from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (10). Interactions derived from experimental and curated database evidence with a confidence level higher than 0.7 were imported in Cytoscape (11). The proteins without any known interactions within the datasets were imported as individual nodes and the average clustering coefficient [C(p)] (12) of each network was calculated using the NetworkAnalyzer plug-in (13). Only the proteins with known interactions within the datasets were exported and visualized (Fig. S9).

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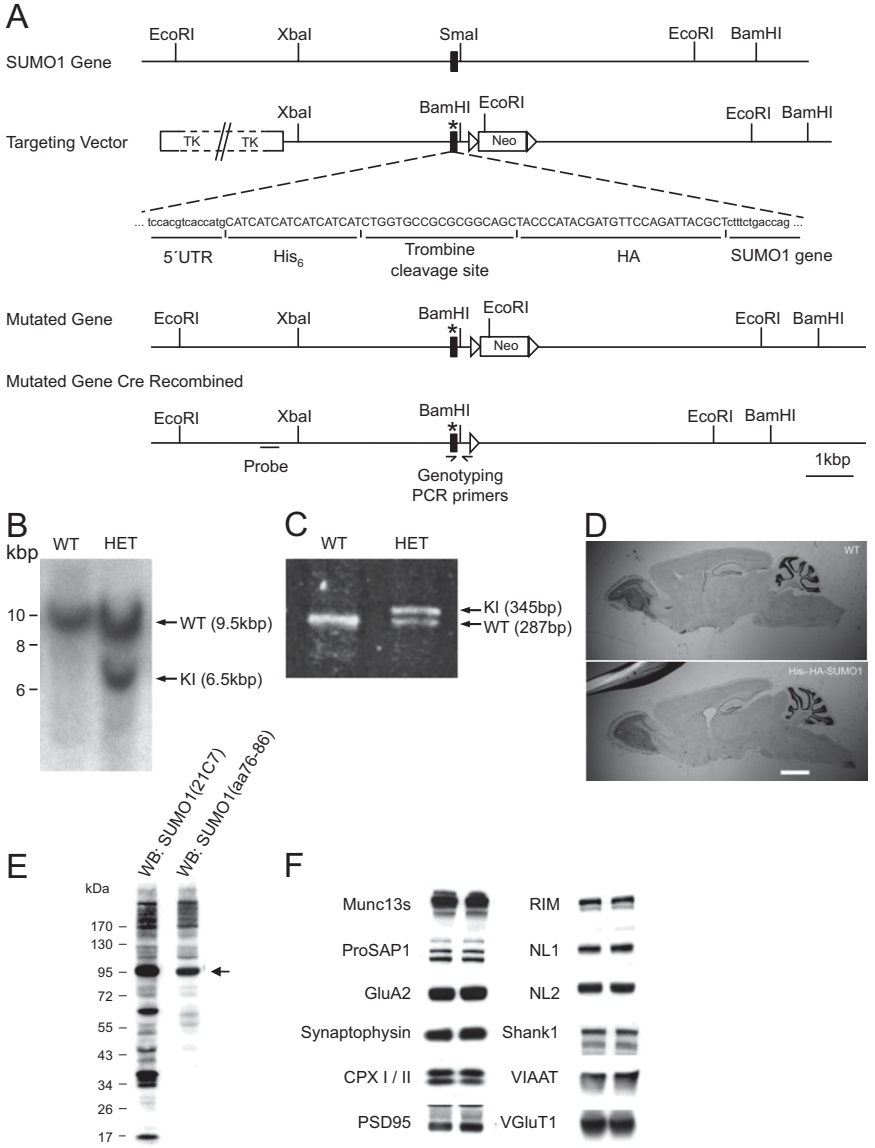

Fig. S1. Generation of His_6-HA-SUMO1 KI mice. (*A*) Structure of the SUMO1 gene, targeting vector, mutated gene after homologous recombination, and mutated gene after homologous recombination and Cre recombination. The first coding exon is indicated by a black box, and an asterisk marks the insertion of the His_6-HA tag, whose coding sequence and insertion location are shown beneath the targeting vector. loxP sites are indicated by triangles. The probe used for Southern blot analysis and the PCR primers used for genotyping are shown below the structure of the mutated and Cre recombined gene. Neo, neomycin resistance gene; TK, herpes simplex virus thymidine kinase. (*B*) Southern blot analysis of mutated gene using EcoRI-digested stem cell DNA and the probe indicated in *A*. HET, heterozygous. (*C*) PCR analysis of WT and of mutated and Cre recombined gene using mouse-tail DNA and the PCR primers indicated in *A*. (*D*) Nissl-stained sagittal sections of WT and KI mouse brain. (Scale bar, 2 mm.) (*E*) Western blot analysis of total mouse brain homogenates using monoclonal antibodies anti-SUMO1(21C7) and anti-SUMO1(aa76–86). Arrows indicate SUMOylated RanGAP1. (*F*) Western blot analysis of selected synaptic marker proteins in brain homogenates of adult WT and KI mice. The panels show representative data from three independent experiments.

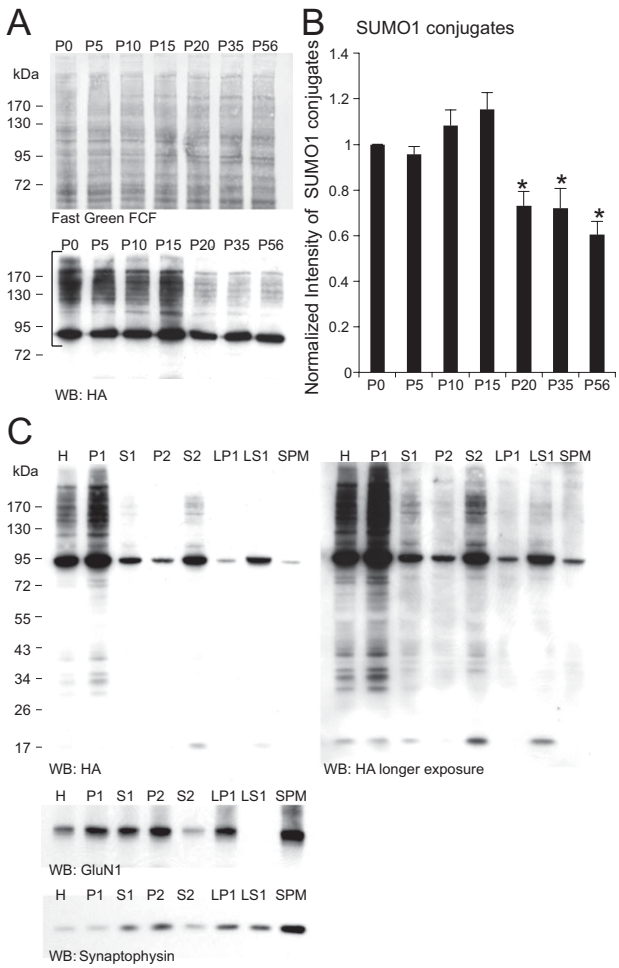

Fig. S2. Developmental profile and subcellular distribution of SUMO1-conjugated proteins in His_6-HA-SUMO1 KI mouse brain. (*A*) Whole-brain homogenates of KI mice of the indicated ages (in days postnatally) were analyzed by SDS/PAGE and blotted to nitrocellulose membranes. Membranes were stained with Fast Green FCF (*Upper*) to label total protein load and then analyzed by probing with anti-HA antibodies. The bracket indicates SUMO1 conjugates quantified in *B*. (*B*) Odyssey-based quantification of SUMO1 conjugates (see bracket in *A*). Data were normalized to the P0 levels; data are expressed as mean ± SEM ($n = 3$; the asterisk indicates a significant difference from the P0 level as assessed by Student *t* test; P20, $P = 0.029$; P35, $P = 0.014$; P56, $P = 0.008$). (*C*) Subcellular fractions of adult KI mouse brain were analyzed by Western blotting using anti-HA antibodies (upper two panels) and antibodies to GluN1 and Synaptophysin to validate the fractionation procedure (lower two panels). H, homogenate; P1, nuclear pellet; S1, supernatant after P1 sedimentation; P2, crude synaptosomal pellet; S2, supernatant after P2 sedimentantion; LP1, lysed synaptosomal membranes; LS1, supernatant after LP1 sedimentation; SPM, synaptic plasma membranes.

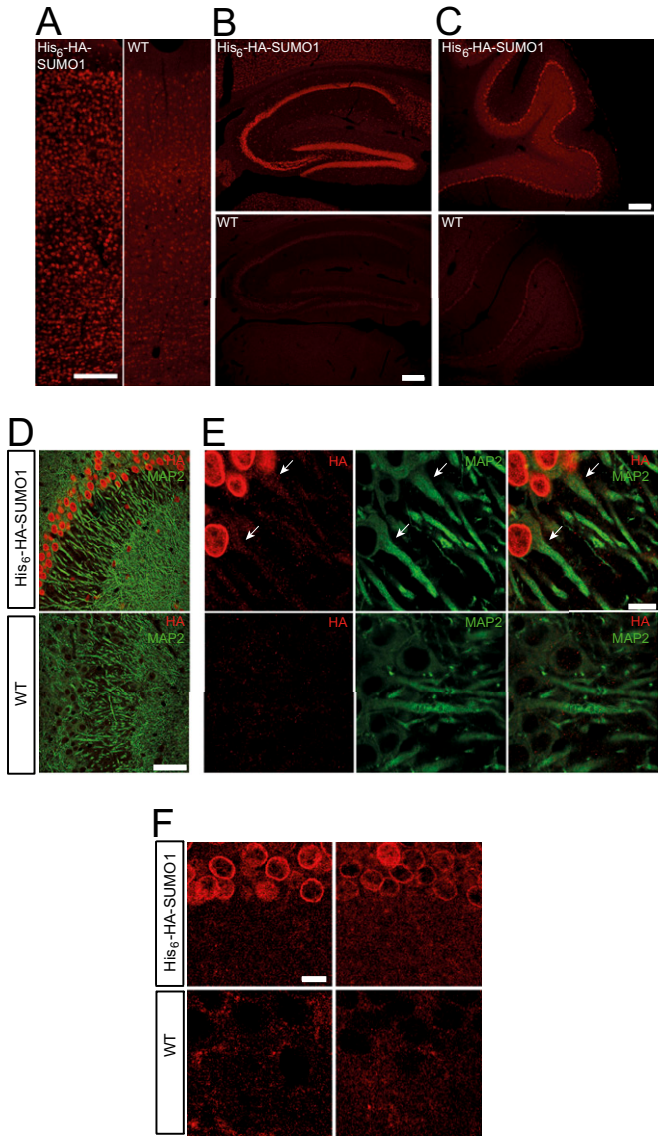

Fig. S3. Regional, cellular, and subcellular localization of SUMO1-conjugated proteins in His_6-HA-SUMO1 KI mouse brain. Sagittal brain sections were stained using antibodies to HA (red) and MAP2 (green), which labels neuronal somata and dendrites. Images are representative of three independent experiments. (*A*) Motor cortex of a KI and a WT control mouse. (Scale bar, 200 μm.) (*B*) Hippocampal formation of a KI and a WT mouse. (Scale bar, 200 μm.) (*C*) Cerebellum of a KI and a WT mouse. (Scale bar, 200 μm.) (*D*) CA3 region of hippocampus of a KI and a WT mouse. (Scale bar, 50 μm.) (*E*) Enlarged region of the CA3 region of the hippocampus (stratum pyramidale and stratum lucidum) of a KI and a WT mouse. The white arrows point at pyramidal cell dendrites containing punctate and weak diffuse HA-immunopositive structures in the KI. (Scale bar, 10 μm.) (*F*) Enlarged regions of the CA1 region of the hippocampus (*Left*, stratum pyramidale and adjacent stratum radiatum) and of the dentate gyrus (*Right*, granule cell layer and adjacent molecular layer) in a KI (*Upper*) and a WT (*Lower*) mouse. No dendritic HA-positive structures were detected in these regions of the KI brain. (Scale bar, 10 μm.)

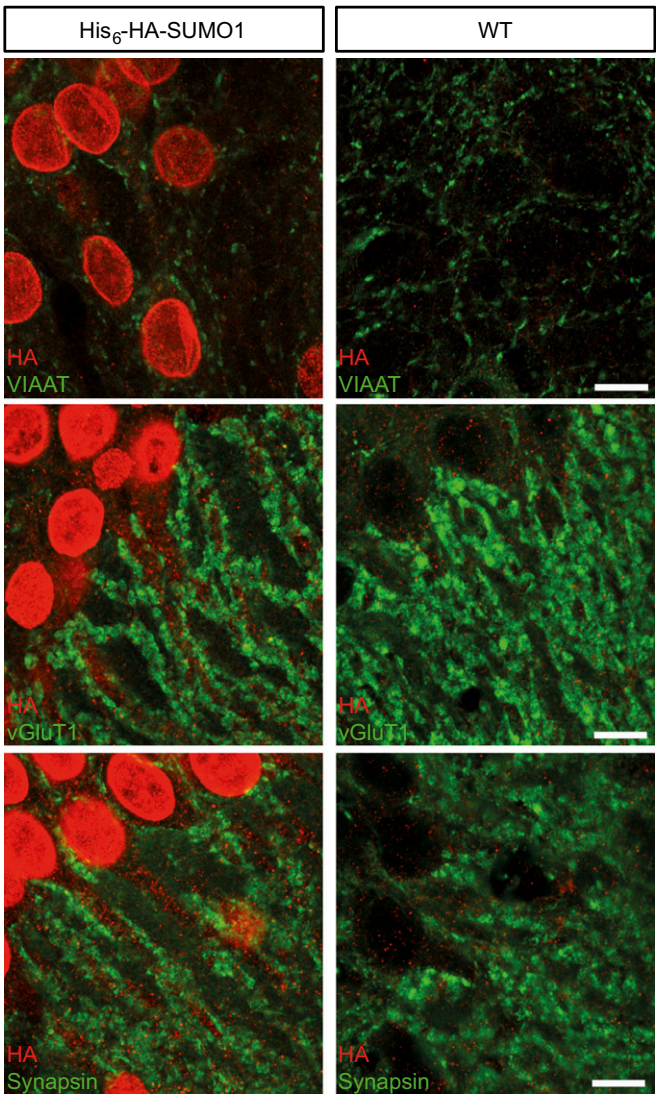

Fig. S4. Lack of colocalization of SUMO1-conjugated proteins with synaptic markers in His_6-HA-SUMO1 KI mouse brain. Sagittal brain sections were stained using antibodies to HA (red) and the inhibitory presynapse marker VIAAT (green, *Top*), the excitatory presynapse marker VGluT1 (green, *Middle*), or the general presynapse marker Synapsin (green, *Bottom*). The images show the CA3 region of the hippocampus (stratum pyramidale and stratum lucidum) of WT and KI animals. Note that HA-immunopositive puncta in the dendrites of KI neurons are not apposed to presynapses. Images are representative of three or more independent experiments. (Scale bar, 10 μm.)

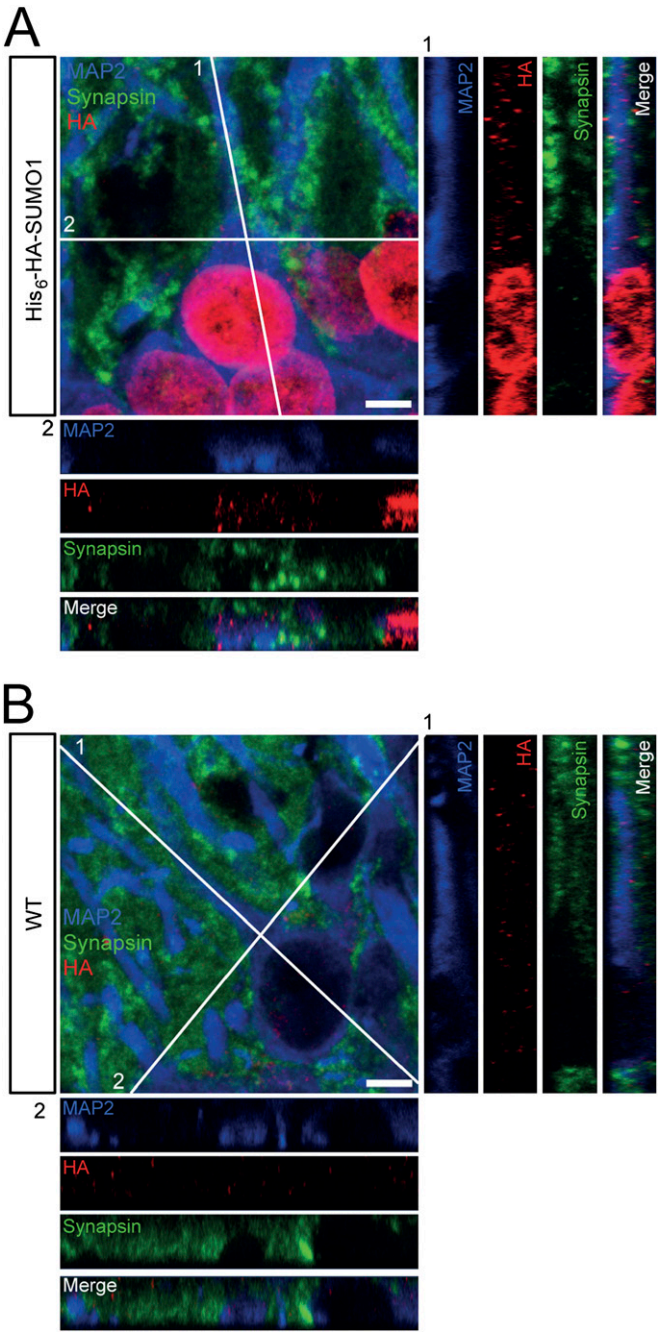

Fig. S5. Nuclear and cytoplasmic localization of SUMO1-conjugated proteins in CA3 neurons of His_6-HA-SUMO1 KI hippocampus. Sagittal brain sections were stained using antibodies to HA (red), MAP2 (blue), which labels neuronal somata and dendrites, and Synapsin (green), which labels presynapses. The images on the left in *A* and *B* show triple-labeled CA3 pyramidal cells and proximal apical dendrites of a KI and a WT mouse. The white lines show the orientation of the line scans used to generate the image stacks shown in side view on the right and bottom. Images are representative of three or more independent experiments. (*A*) KI sample. (Scale bar, 10 μm.) (*B*) WT sample. (Scale bar, 10 μm.) Note that HA-immunopositive puncta in KI neurons overlap with MAP2 staining, indicating that they are located in the cytosplasm of neuronal somata and dendrites. WT cells in sagittal sections show rather strong background staining.

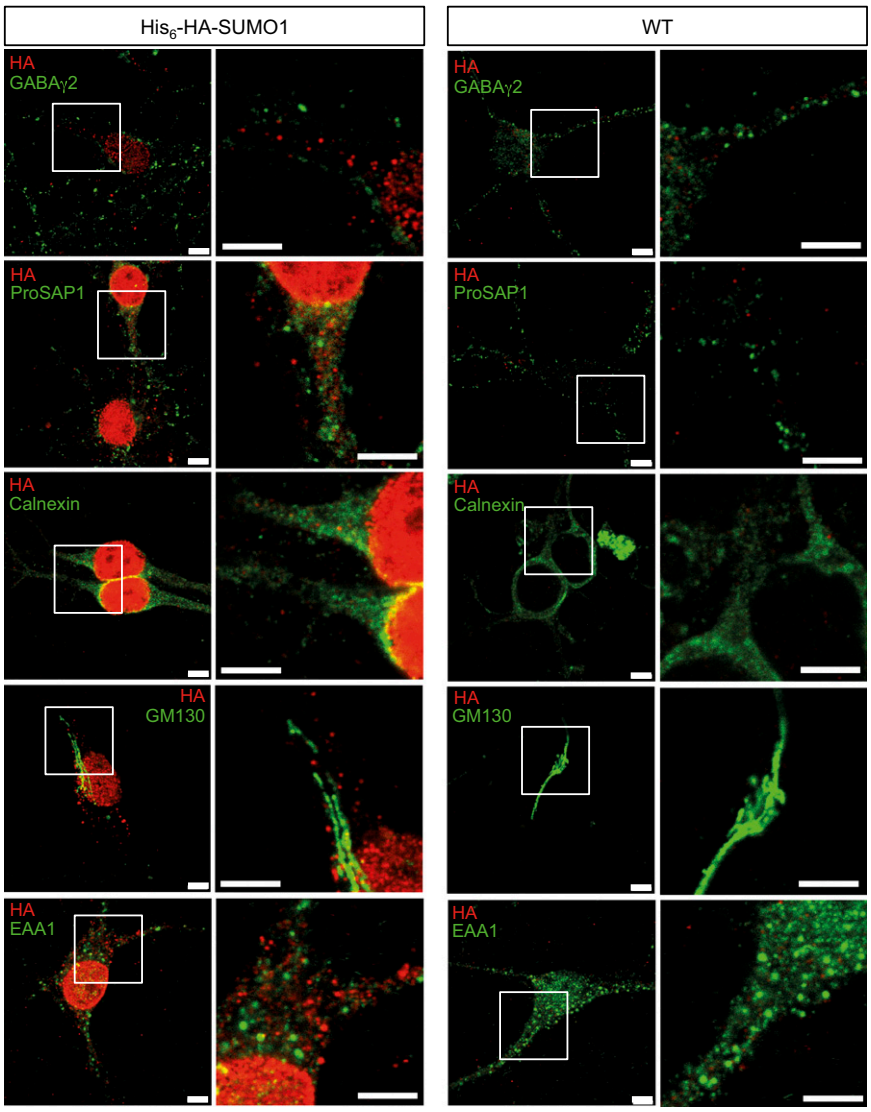

Fig. S6. Lack of colocalization of SUMO1-conjugated proteins with markers of inhibitory postsynapses, excitatory postsynapses, endoplasmic reticulum, Golgi apparatus, or endosomes in cultured hippocampal His_6-HA-SUMO1 KI neurons. Cultured hippocampal neurons were stained using antibodies to HA (red) and to markers for inhibitory postsynapses ($GABA_A\gamma2$, green), excitatory postsynapses (ProSAP1, green), endoplasmic reticulum (Calnexin, green), Golgi apparatus (GM130, green), or endosomes (EAA1, green). Note that HA-immunopositive structures do not specifically colocalize with any of the markers tested. Images are representative of three independent experiments. (Scale bars, 5 μm.)

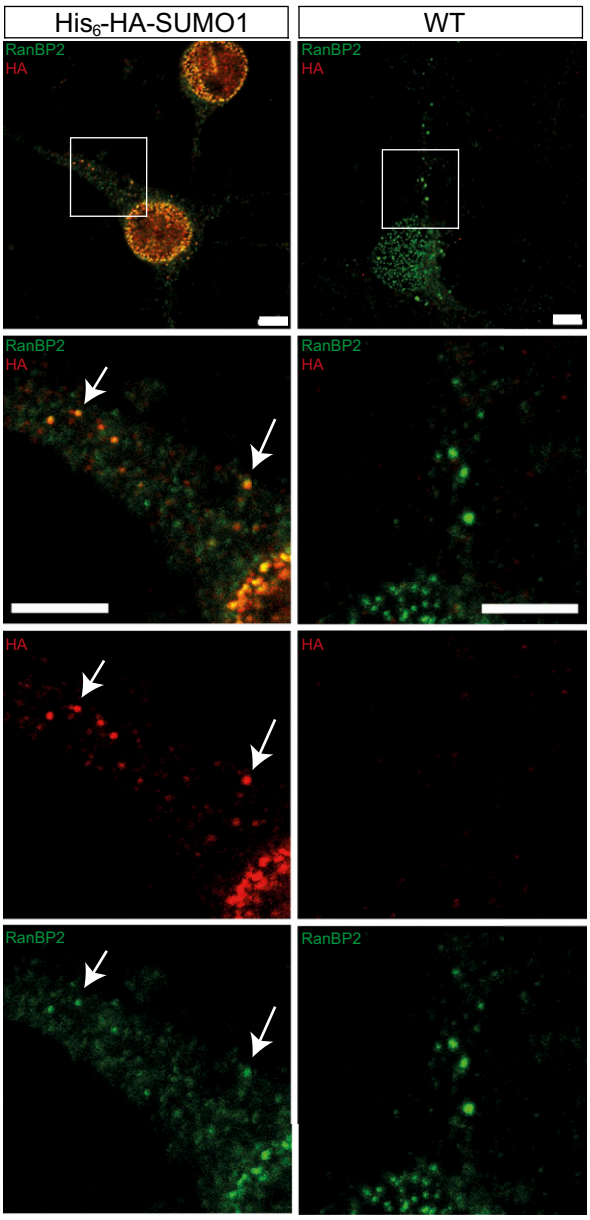

Fig. S7. Colocalization of extranuclear HA-immunopositive signals with markers of annulate lamellae in cultured hippocampal neurons of His_6-HA-SUMO1 KI mice. Cultured hippocampal neurons were stained using antibodies to HA (red) and RanBP2 (green). Images are representative of three independent experiments. KI (*Left*) and WT (*Right*) cells stained for HA (red) and RanBP2 (green). The bottom panels in each column represent an enlargement of the areas boxed in white in the top panels of each column. White arrows indicate colocalization of HA-immunopositive signals with RanBP2-immunopositive signals in the nuclear envelope and cytoplasm. (Scale bar, 5 μm.)

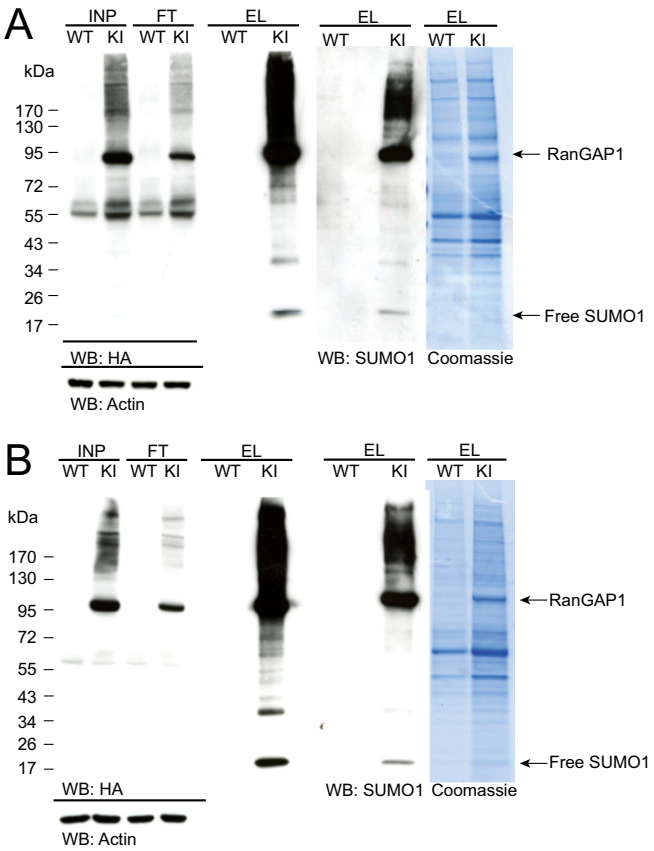

Fig. S8. Anti-HA affinity purification of SUMO1-conjugated proteins from His_6-HA-SUMO1 KI mouse brain. Detergent extract input samples (INP) of WT and KI mouse brains, corresponding flow through samples (FT) after HA affinity chromatography, and specific HA-peptide eluates (EL) were analyzed by SDS/PAGE and Coomassie staining (*Right*) or Western blotting (WB) using antibodies directed against the indicated antigens. (*A*) Data from adult brain extracts. (*B*) Data from P10 brain extracts. Note that HA-positive proteins are partially depleted in the FT fraction and strongly enriched in the EL fraction of the KI sample, and that SUMO1-positive proteins are enriched specifically in the EL fraction obtained from the KI sample.

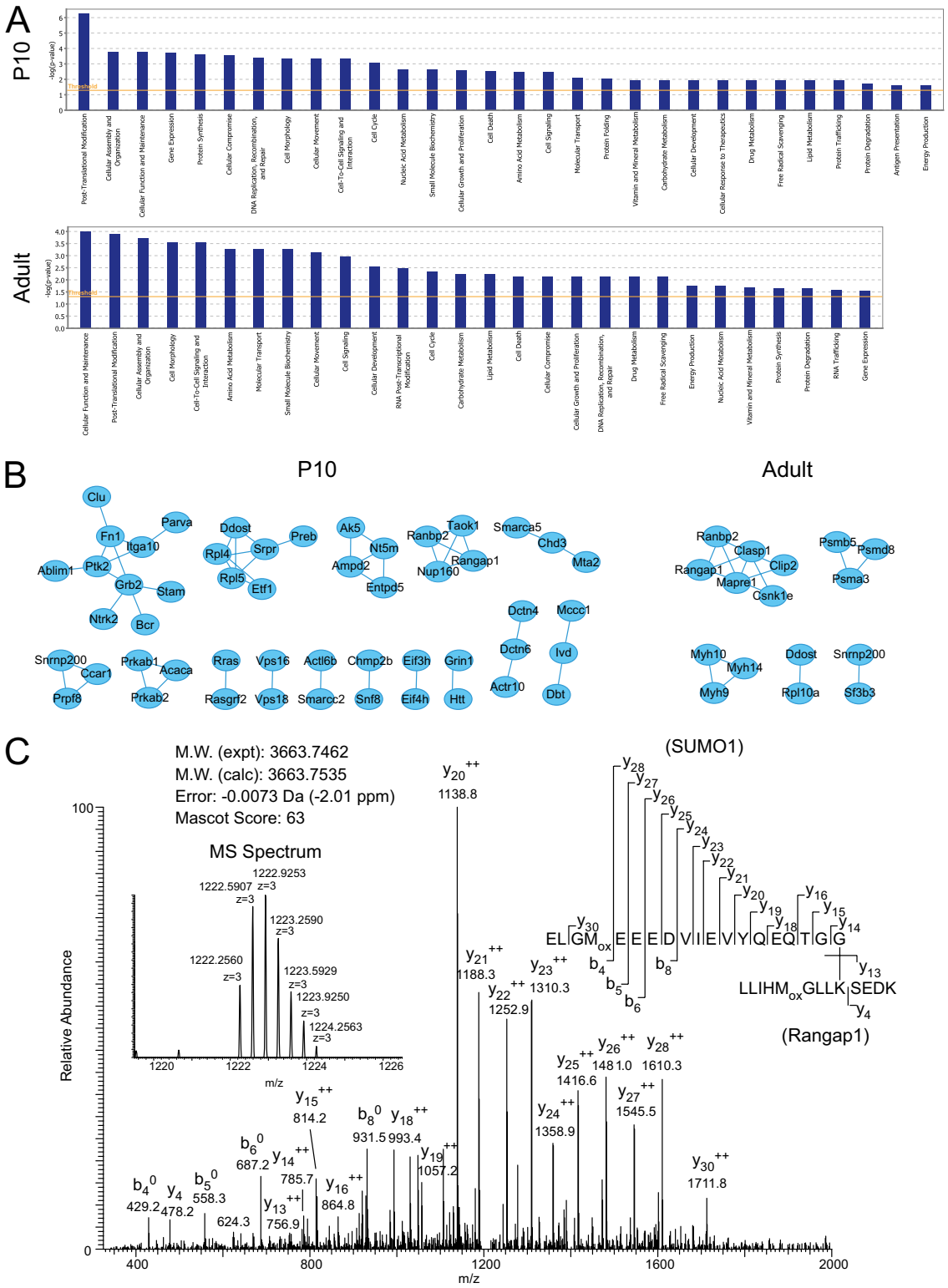

Fig. S9. Analysis of mass spectrometric data. (*A*) Ingenuity IPA functional analysis. The graph shows the significantly enriched biological functions related to the identified proteins in each dataset on the x-axis. The significance of the enrichment (Fisher's exact test) is represented as $-\log_{10} P$ values with a threshold of 0.05 (orange line) on the *y* axis. The analysis shows a wide variety of molecular and cellular functions in both datasets with no particular bias. (*B*) Protein-protein interactions within the datasets. High-confidence STRING interactome network of the proteins with known interactions within each dataset shows low connectivity with average clustering coefficients C(p) for the complete datasets of 0.077 (P10 brain) and 0.085 (adult brain). The values are in the range of the expected connectivity from a random interactome network of mouse brain proteins [C(p) ~0.07], indicating that the majority of the proteins were not isolated

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as a result of secondary interactions. Nodes are labeled with official gene names as reported by STRING. (*C*) Nanoliquid chromatography-electrospray ionization mass spectrometry (nanoLC-ESI MS) analysis of tryptic RanGAP1 and SUMO1-conjugated peptide. MS/MS spectrum of a SUMO1-conjugated peptide at *m/z* 1222.2560 derived from RanGAP1 encompassing positions 518–530 (LLIHM$_{ox}$GLLKSEDK) with fragment ions recorded in the LTQ analyzer of the LTQ-Orbitrap Velos MS. In combination with the Mascot database, searches of the modified RanGAP1 sequence by using ChopNSpice software confirmed the known Lys526 as the actual SUMOylation site. Ions (y- and b-type) are shown in the spectrum and at their respective positions in the conjugated peptide. The MS spectrum (*Inset*) indicates the experimental *m/z*, theoretical *m/z*, mass error, and Mascot score.

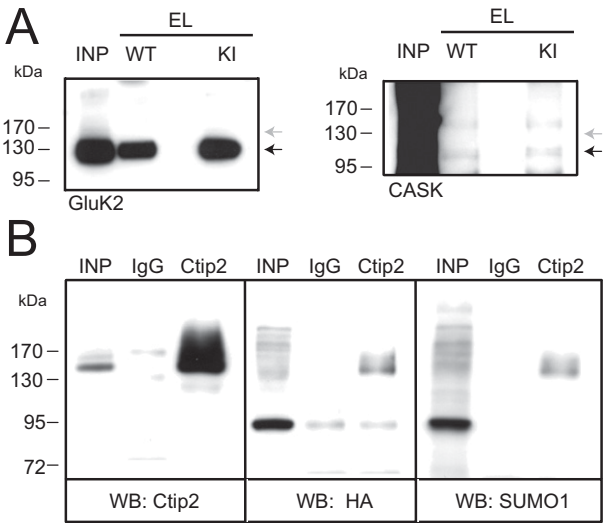

Fig. S10. Western blot analysis of candidate SUMO1-conjugated proteins. (*A*) Representative detergent extract input sample (INP, from WT) and specific HA-peptide eluates (EL) of HA-immunoaffinity purified samples from adult WT and KI brains were analyzed by SDS/PAGE and Western blotting (WB) with antibodies to GluK2 (*Left*) and CASK (*Right*). The presence of GluK2 (black arrow) in both WT and KI samples indicates that GluK2 binds nonspecifically to the affinity matrix. Note the absence of SUMO1-conjugated GluK2 of the expected molecular weight (gray arrow) in the KI sample. Even very long exposures of the anti-CASK Western blots showed only trace amounts of CASK in the eluate samples (black arrow). They were present at similar levels in the HA peptide eluates from WT and KI samples. Note the absence of SUMO1-conjugated CASK of the expected molecular weight (gray arrow) in the KI sample. (*B*) SUMOylation of Ctip2. Ctip2 was immunoprecipitated from KI brain. Control experiments were conducted with a nonrelated IgG. Input (INP) and immunoprecipitates were analyzed by SDS/PAGE and Western blotting (WB) with antibodies to the indicated proteins. Images are representative of three independent experiments.

Table S1. Primary antibodies used in the present study

Antigen	Species	Sources	Applications	Dilution
Actin	Rabbit	Sigma	WB	1:7,500
Bcl11A/Ctip1 (B-cell lymphoma/leukemia 11A)	Mouse	Abcam	WB	1:1,000
Bcl11B/Ctip2 (B-cell lymphoma/leukemia 11B)	Rat	Abcam	WB/IP	1:500
Calnexin	Rabbit	Abcam	ICC	1:1,000
CPX I/II (Complexin I/II)	Rabbit	Synaptic Systems	WB	1:2,500
GABAγ2	Guinea pig	Gift from J. M. Fritschy (University of Zurich, Switzerland)	ICC	1:1,000
GluA2 (AMPA receptor 2)	Mouse	Chemicon	WB	1:500
GluN1 (NMDA receptor 1)	Mouse	Synaptic Systems	WB	1:1,000
GM130 (Golgi matrix protein 130)	Rabbit	Abcam	ICC	1:1,000
HA (hemagglutinin)	Mouse	Covance	WB/IHC/ICC	1:1,000
KAP1 (KRAB-associated protein 1)	Mouse	Abcam	WB	1:1,000
MEF2A (myocyte specific-enhancer factor 2A)	Rabbit	Santa Cruz	WB	1:1,000
NL1 (Neuroligin 1)	Mouse	Synaptic Systems	WB	1:5,000
NL2 (Neuroligin 2)	Rabbit	Varoqueaux et al. (1)	WB	1:1,000
Munc13s (mam. homologs of *Caenorhabditis elegans* unc-13)	Mouse	BD Biosciences	WB	1:1,000
ProSAP1 (proline-rich synapse-associated protein 1)	Rabbit	Gift from T. Böckers (University of Ulm, Germany)	WB/IHC	1:500/1:1,000
PSD95 (postsynaptic density protein 95)	Mouse	Abcam	WB	1:1,000
RanBP2 (Ran binding protein 2)	Goat	Gift from F.M.	WB/ICC	1:1,000
RanGAP1 (Ran GTPase activating protein 1)	Goat	Gift from F.M.	WB/ICC	1:2,000/1:1,000
RIM (Rab3a-interacting molecule)	Mouse	BD Biosciences	WB	1:500
Shank1 (SH3 and multiple ankyrin repeat domain protein 1)	Rabbit	Synaptic Systems	WB	1:500
Sip1 (Smad-interacting protein 1)	Rabbit	Gift from V. Tarabykin (Charité, Berlin, Germany)	WB	1:1,000
Smchd1 (structural maintenance of chromosome flexible hinge-domain containing protein 1)	Mouse	BD Biosciences	WB	1:2,500
SUMO1 (small ubiquitin-like modifier 1) SUMO1 (aa76–86)	Mouse	Developmental Studies Hybridoma Bank; gift from F.M. (University of Heidelberg, Germany)	WB	1:1,000
SUMO2 (small ubiquitin-like modifier 2)	Mouse	Developmental Studies Hybridoma Bank	WB	1:1,000
Synapsin	Rabbit	Synaptic Systems	WB/IHC/ICC	1:1,000
Synaptophysin	Mouse	Synaptic Systems	WB	1:10,000
TIF1γ/TRIM33 (transcriptional intermediary factor 1 γ)	Goat	Novus	WB	1:1,000
VIAAT (vesicular inhibitory amino acid transporter)	Rabbit	Synaptic Systems	WB/IHC	1:500/1:1,000
VGluT1 (vesicular glutamate transporter 1)	Rabbit	Synaptic Systems	WB/IHC	1:1,000
Wiz (widely interspaced zinc finger-containing protein)	Rabbit	Novus	WB	1:250
Zbtb20 (zinc finger and BTB domain 20)	Rabbit	Abcam	WB	1:500

Table S2. Secondary antibodies used in the present study

Name	Application	Supplier	Dilution
Goat anti-mouse HRP conjugated	WB	Jackson Immunolaboratory	1:10,000
Goat anti-rabbit HRP conjugated	WB	Jackson Immunolaboratory	1:10,000
Mouse anti-goat HRP conjugated	WB	Millipore	1:10,000
Goat anti-rat HRP conjugated	WB	Jackson Immunolaboratory	1:10,000
Goat anti-guinea pig Alexa Fluo-488	ICC	Invitrogen	1:2,000
Goat anti-mouse IRDye 800	Odyssey	Biomol	1:2,000
Goat anti-mouse Alexa Fluo-555	IHC/ICC	Invitrogen	1:2,000
Goat anti-rabbit Alexa Fluo-488	IHC/ICC	Invitrogen	1:2,000
Goat anti-chicken Alexa Fluo-633	IHC/ICC	Invitrogen	1:2,000
Goat anti-rat HRP conjugated	IHC/ICC	Mobitec	1:2,000

ICC, immunocytochemistry; IHC, immunohistochemistry; IP, immunoprecipitation; WB, Western blot.

1. Varoqueaux F, Jamain S, Brose N (2004) Neuroligin 2 is exclusively localized to inhibitory synapses. *Eur J Cell Biol* 83(9):449–456.

Dataset S1. Candidate SUMO1-conjugated proteins in adult and P10 mouse brain identified by proteomic analyses of anti-HA affinity purified material from His_6-HA-SUMO1 KI mouse brain

Dataset S1

Green, novel candidate SUMO1-conjugated protein, validated by Western blotting in the present study. Orange, known SUMO1-conjugated protein, validated by Western blotting in the present study. Red, novel candidate SUMO1-conjugated protein, negative in Western blotting validation experiments in the present study. Blue, known SUMO1-conjugated protein, not tested in Western blotting validation experiments in the present study.