iScience, Volume *26*

Supplemental information

Characterizing the differential distribution and targets of Sumo1 and Sumo2 in the mouse brain

Terry R. Suk, Trina T. Nguyen, Zoe A. Fisk, Miso Mitkovski, Haley M. Geertsma, Jean-Louis A. Parmasad, Meghan M. Heer, Steve M. Callaghan, Fritz Benseler, Nils Brose, Marilyn Tirard, and Maxime W.C. Rousseaux

Figure S1, related to Figure 1.

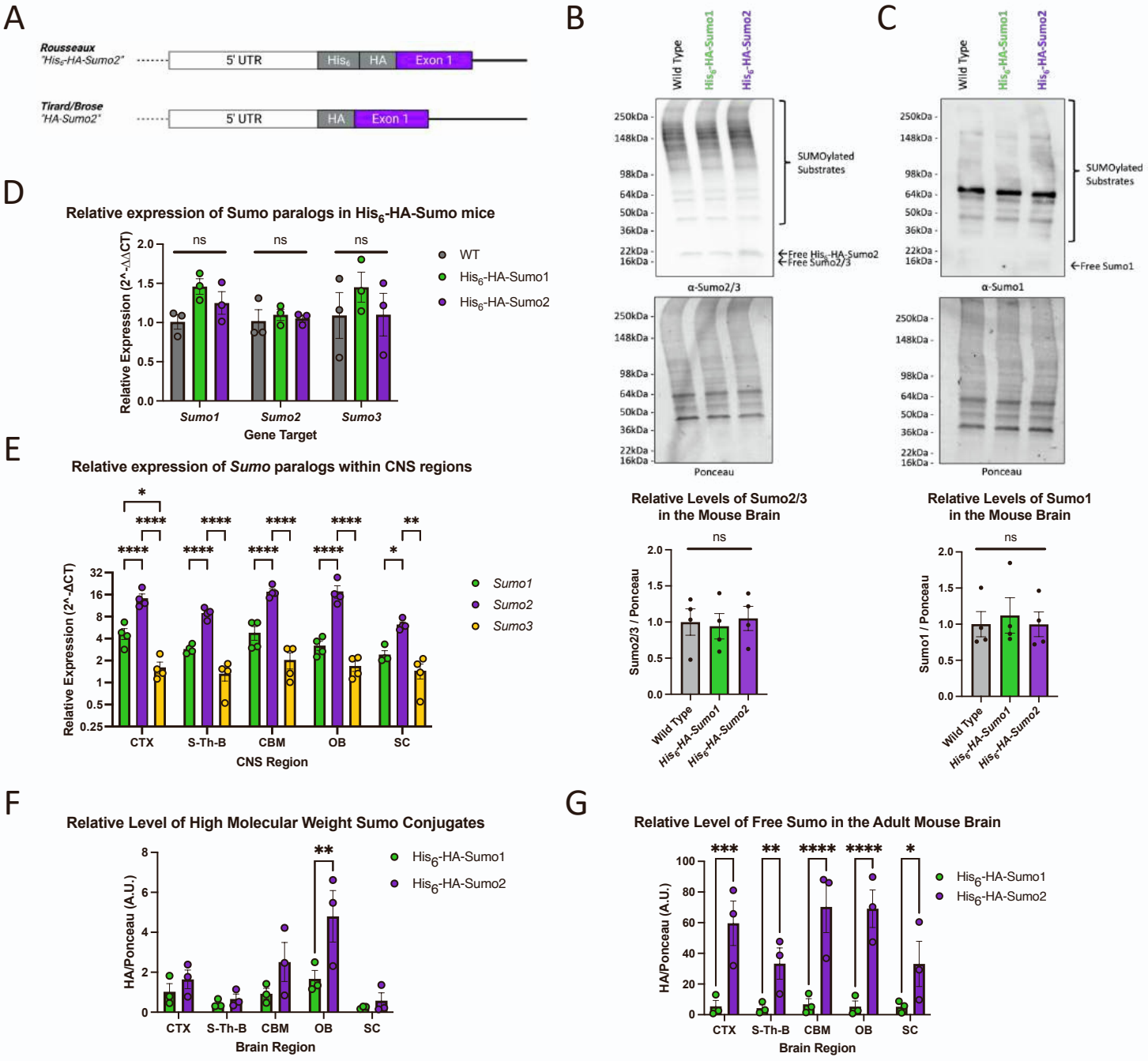
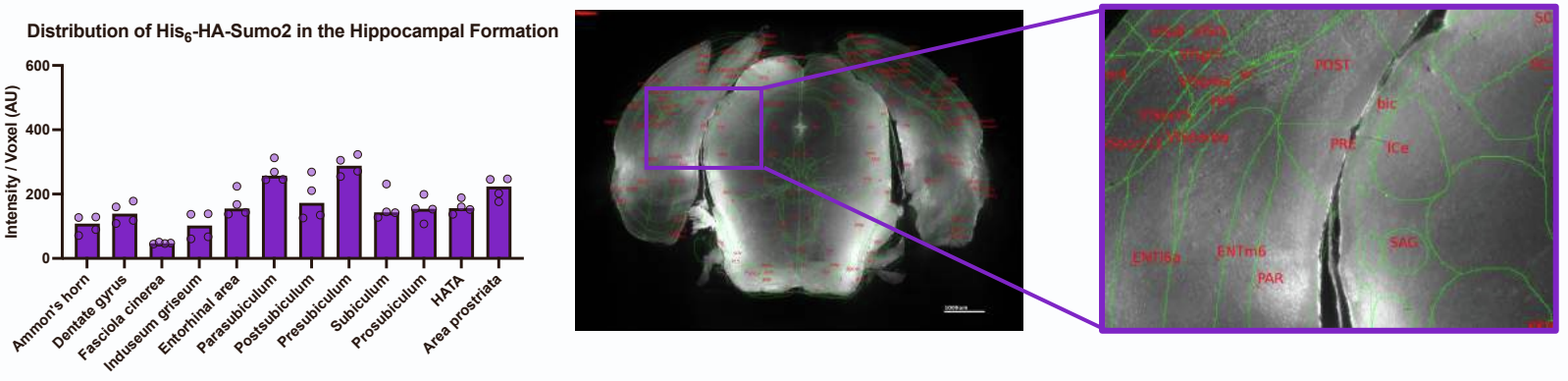


Figure S1: Heterozygous His_6-HA-Sumo2 and His_6-HA-Sumo1 mice exhibit normal Sumo levels. Related to Figure 1.

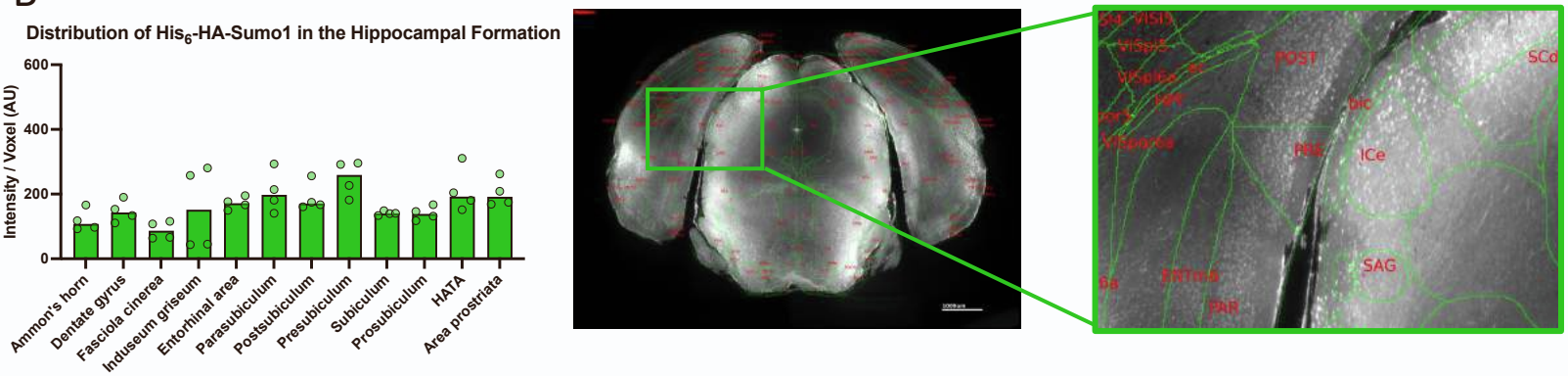
(A). Schematic of His_6-HA-Sumo2 and HA-Sumo2 knock-in mice. (B). Anti-Sumo2/3 Western blot analysis of total brain homogenates from heterozygous His_6-HA-Sumo1, His_6-HA-Sumo2 mice and WT controls. Anti-Sumo2/3 signal (bracket on the right side of the top left panel) was analyzed by densitometry. Signal from either heterozygous His_6-HA-Sumo1 or His_6-HA-Sumo2 mice was normalized to ponceau signal (N=4). (C). Anti-Sumo1 Western blot analysis of total brain homogenates from heterozygous His_6-HA-Sumo1, His_6-HA-Sumo2 mice and WT controls. Anti-Sumo1 signal (bracket on the right side of the top left panel) was analyzed by densitometry. Signal from heterozygous His_6-HA-Sumo1 and His_6-HA-Sumo2 mice was normalized to ponceau signal (N=4). (D). RT-qPCR analysis of *Sumo1*, *Sumo2,* and *Sumo3* transcript levels in whole brain from heterozygous His_6-HA-Sumo1, His_6-HA-Sumo2 mice and WT controls (N=3). (E). Alternative comparison/data presentation for regional *Sumo1*, *Sumo2*, or *Sumo3* expression displaying changes in *Sumo* paralog expression per region (N=4). (F). Alternative comparison/data presentation of Western blot analysis of free Sumo levels per region. (G). Alternative comparison/data presentation of Western blot analysis of conjugated Sumo levels per region. All statistical tests were analyzed using an ordinary One or Two-way ANOVAs with Tukey's multiple comparisons tests. Data are presented as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure S2, related to Figure 2

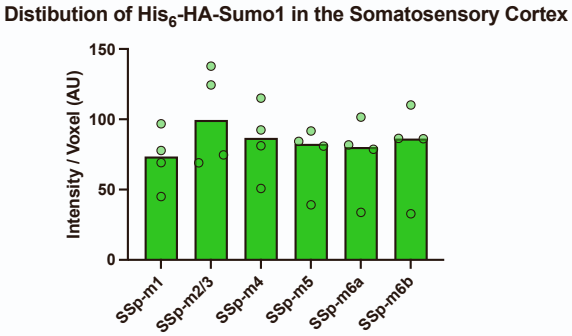
A

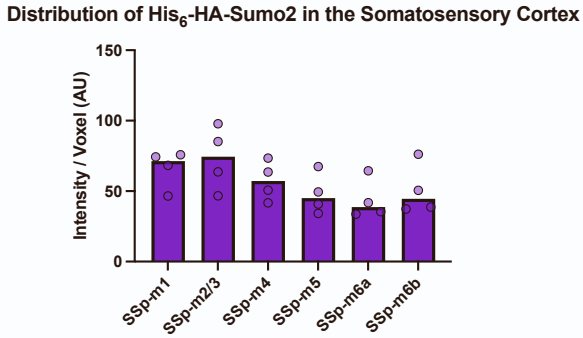


B

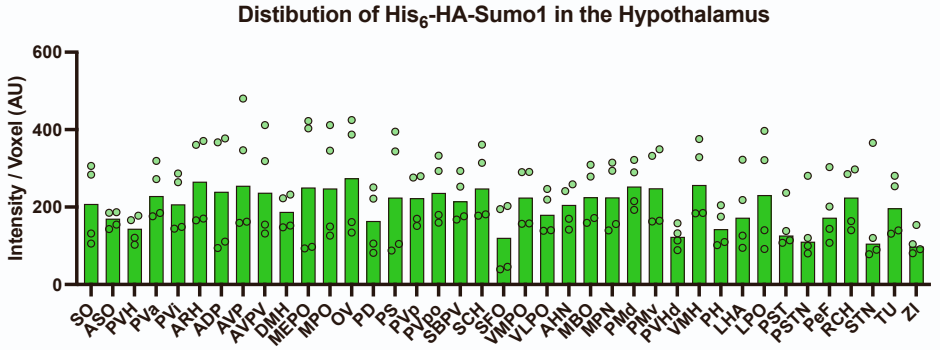


C





D



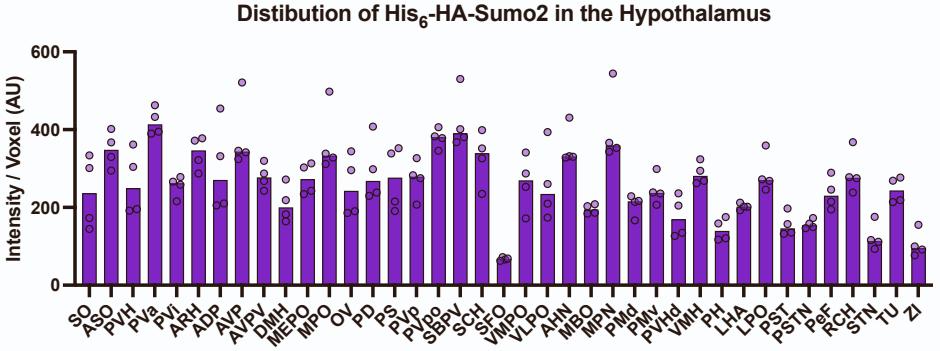


Figure S2: Detailed examination of regional anatomical Sumo paralog distribution. Related to Figure 2. (A). Bar plot depicting the relative levels of anti-HA immunosignal in the regions of His_6-HA-Sumo2 hippocampus. Each datapoint is the mean intensity from a single hemisphere (N=2, two hemispheres/replicate). Middle and Left panels depict a representative image and zoom of the hippocampal formation overlayed with a mask depicting anatomical regions defined by the Allen Brain Atlas. Scale Bar: 1000 μm. (B). Bar plot depicting the relative levels of anti-HA immunosignal across His_6-HA-Sumo1 brain regions. Middle and Left panels depict a representative image and zoom of the hippocampal formation overlayed with a mask depicting anatomical regions defined by the Allen Brain Atlas. Scale Bar: 1000 μm. (C). Bar plot depicting relative anti-HA immunosignal levels in the primary somatosensory area–mouth (SSp-m) layers of His_6-HA-Sumo1 (Top) and His_6-HA-Sumo2 (Bottom) brain (D). Bar plot depicting relative anti-HA immunosignal levels in regions of the hypothalamus of His_6-HA-Sumo1 (Top) and His_6-HA-Sumo2 (Bottom). Each datapoint from the brain atlases depict the mean intensity from a single hemisphere (N=2, two hemispheres/replicate). For each abbreviated brain region, refer to Table S1 for legend. Data are presented as mean ±SEM.

Figure S3, related to Figure 2.

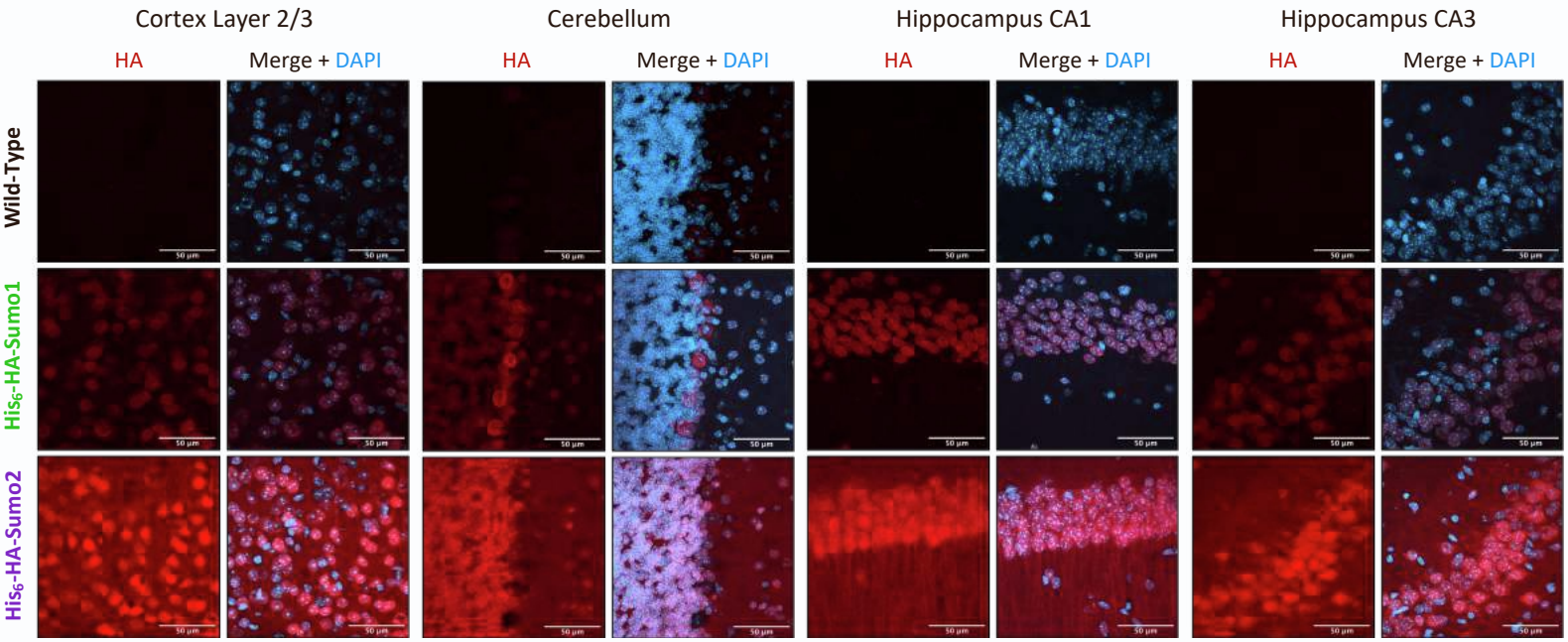


Figure S3: Sumo1 and Sumo2 share similar and distinct anatomical locations and subcellular compartments. Related to Figure 2.

Confocal microscopy analysis of anti-HA (red) and DAPI (blue) nuclear labeling of wild-type (untagged, top lane), His_6-HA-Sumo1 (middle lane) and His_6-HA-Sumo2 (bottom lane) cortex layer 2/3, cerebellum, and hippocampal CA1 and CA3 regions. Z-projection images are representative of three independent replicates. Scale bar: 50 μm

Figure S4, related to Figure 3.

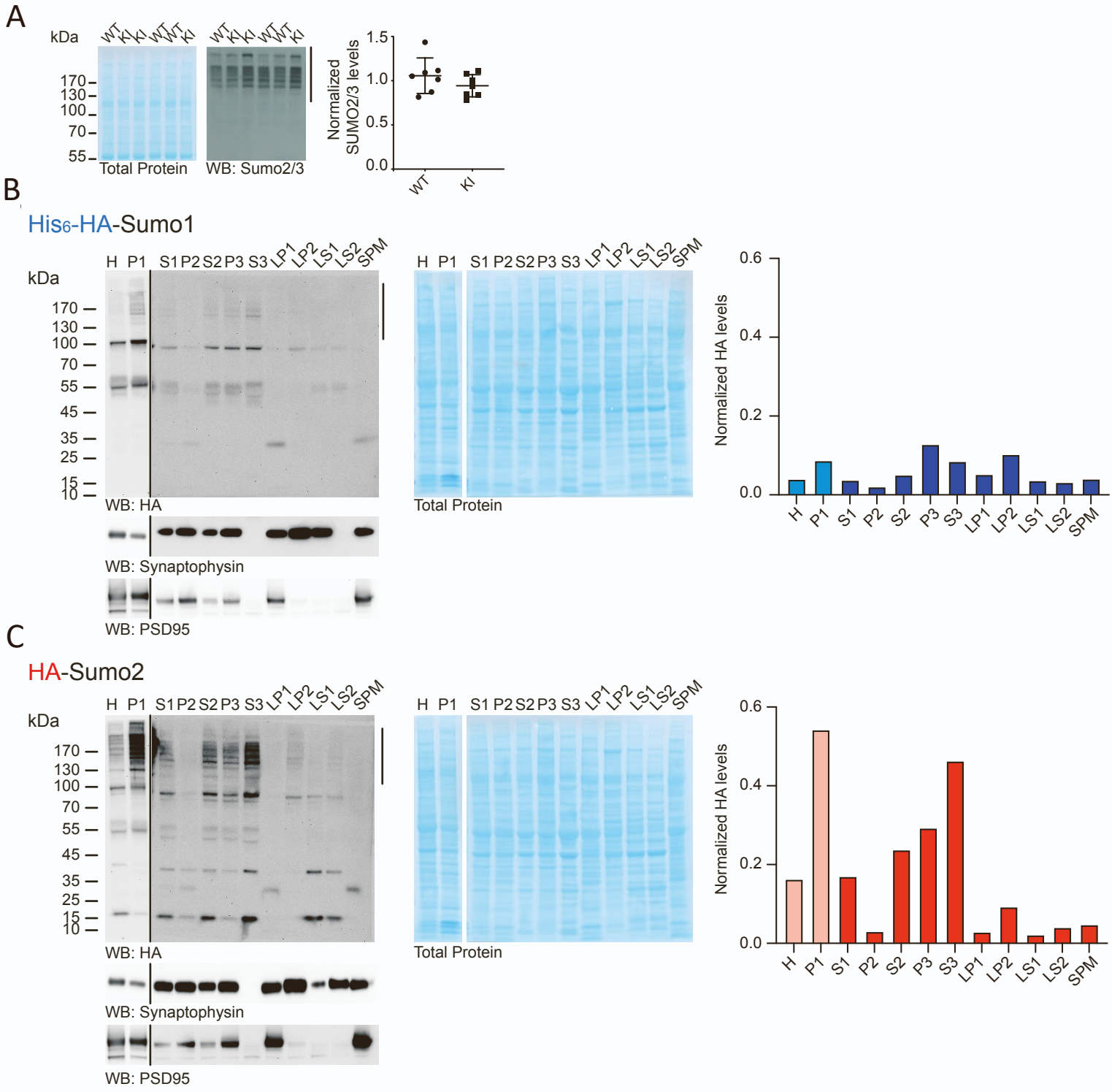


Figure S4: Differential levels and sub-cellular distribution of Sumo1 and Sumo2 conjugates in whole mouse brain fractions. Related to Figure 3.

(A). Total Protein stain (left panel) and anti-HA Western blot (right panel) analysis of whole brain lysates from homozygous HA-Sumo2 and corresponding WT littermates. Anti-HA signal (black bar on the right) indicates Sumo2 signal was normalized to the total protein stain and quantification is shown by the dot plot (right panel).

(B and C). Total protein stain (right panel) and anti-HA (left panel) Western blot analysis of subcellular fractions from homozygous His_6-HA-Sumo1 (B) and HA-Sumo2 KI (C). Western blot analysis using anti-Synaptophysin and PSD95 (bottom panels) validates the subcellular fractionation procedure. Anti-HA signal (black line on the right of each blot) was normalized to the total protein stain and quantification is depicted as a bar plot (right panel). The black line between the HA panel indicates that different exposure times are depicted, as the HA signal in the P1 fraction saturates faster than in the other fractions. Thus, the color of the first two bars is lighter to indicate that the quantification was done after different exposure time. Subcellular fractions are designated as follows: *H*, whole brain homogenate; *P1*, nuclear pellet; *P2*, crude synaptosomal pellet; *P3*, light membrane pellet; *S3*, cytosolic fraction; *LP1*, lysed synaptosomal membranes; *LP2*, crude synaptic vesicle fraction; *LS2*, cytosolic synaptosomal fraction; *SPM*, synaptic plasma membranes. Bar plots (Right) depict the quantification of anti-HA signal as indicated by the black line on the right side of each anti-HA Western blot in C and D, relative to the total protein stain. Data are presented as mean ±SEM.

Figure S5, related to Figure 4.

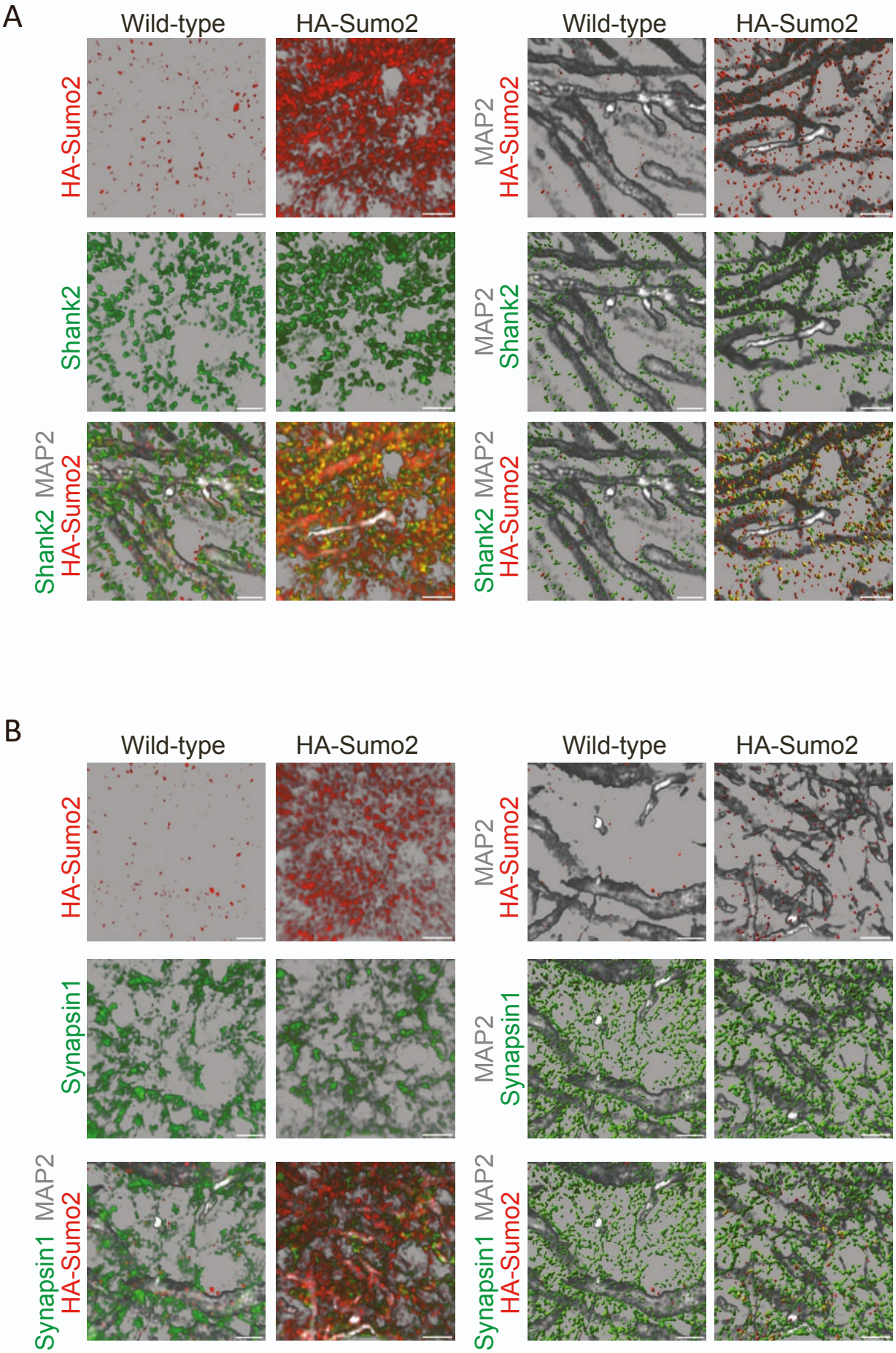
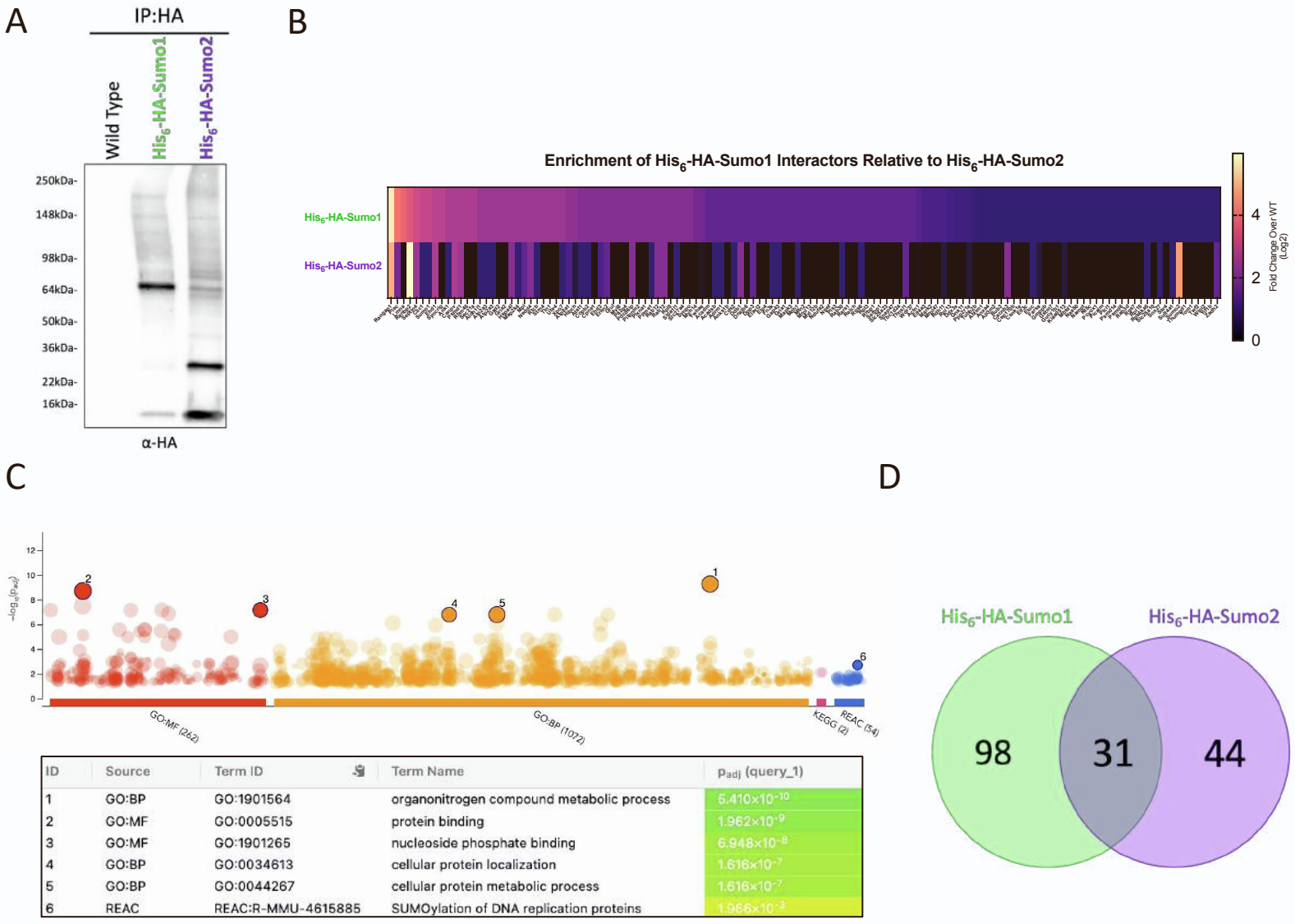


Figure S5: Imaris-based representation of the synaptic Sumo2 co-localization with Shank2 and Synapsin1. Related to Figure 4.

Blended representation generated in Imaris using images from Figure 4B and depicting the colocalization between HA-Sumo2 and Shank2 (A) and Synapsin 1 (B). The two left panels depict the individual HA-Sumo2 (red, top row A and B), Shank2 (green, middle row A) and Synapsin1 (green, middle row B) raw signal, as well as an overlay thereof (bottom rows, A and B), which additionally contains the MAP2 (grey) raw signal. In all cases, the lookup table of the respective channel was scaled equally to enable direct visual comparison of channel intensity between WT and HA-Sumo2 KI. The right two panels depict the raw MAP2 signal (grey). Instead of the raw signal, Imaris-generated, 3D surface objects based on HA-Sumo2 (red surfaces, top row A and B), Shank2 (green surfaces, middle row A) or Synapsin1 (green surfaces, middle row B) signals are shown. Yellow, surface objects representing colocalization with HA-Sumo2 are shown in the respective bottom rows of A and B, along with non-colocalizing Shank2 (green, bottom row A) or Synapsin1 (green, bottom row B) or non-colocalizing HA-Sumo2 surfaced (red, bottom rows A and B). Scale bar: 5 μm.

Figure S6, related to Figure 5.



ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	GO:BP	GO:1901564	organonitrogen compound metabolic process	5.410×10^{-10}
2	GO:MF	GO:0005515	protein binding	1.962×10^{-9}
3	GO:MF	GO:1901265	nucleoside phosphate binding	6.948×10^{-8}
4	GO:BP	GO:0034613	cellular protein localization	1.616×10^{-7}
5	GO:BP	GO:0044267	cellular protein metabolic process	1.616×10^{-7}
6	REAC	REAC:R-MMU-4615885	SUMOylation of DNA replication proteins	1.966×10^{-3}

Figure S6: Neuronal Sumo1 has shared and distinct substrates compared to Sumo2 *in vivo.* Related to Figure 5.

(A). Anti-HA Western blot analysis of eluates from anti-HA affinity immunoprecipitation (IP: HA) from WT, His_6-HA-Sumo1, and His_6-HA-Sumo2 mouse brain lysates demonstrating HA signal patterns of Sumo1 and Sumo2 and their corresponding conjugates (B). Heat map depicting the relative peptide abundance of His_6-HA-Sumo1 interactors relative to levels in His_6-HA-Sumo2 immunoprecipitation. (C). gProfiler2 Gene Ontology analysis for His_6-HA-Sumo1 interactors. (D). Venn diagram depicting unique and common His_6-HA-Sumo1 and His_6-HA-Sumo2 interactors.

Figure S7, related to Figure 6.

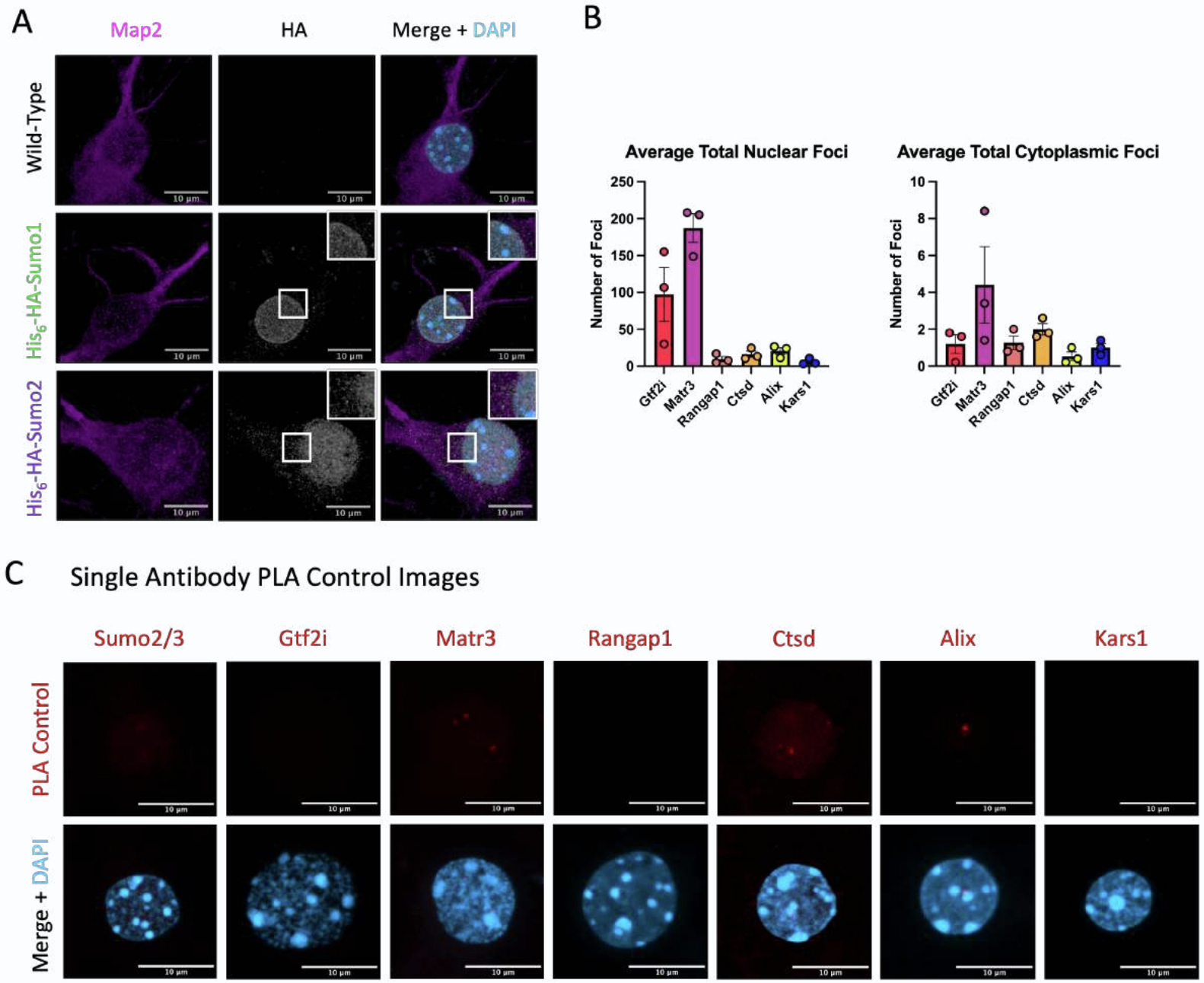


Figure S7: Localization of Sumo2 to extranuclear compartments and proof of PLA assay specificity. Related to Figure 6.

(A). Confocal microscopy analysis of anti-Map2 (purple) and anti-HA (grey) immunolabeling of WT (top lanes), His_6-HA-Sumo1 (middle lanes), and His_6-HA-Sumo2 primary cortical neurons (bottom lanes). Scale bar: 10 µm. N=3 (B). Bar plot depicting the total number of nuclear (left plot) and cytoplasmic (right plot) PLA foci for the indicated antibody (N=3). Data are presented as a mean ± S.E.M. (C). Representative Z-projected images of proximity ligation assays performed with only a single antibody (indicated on top, top lanes) merged with DAPI (bottom panels). Scale bar: 10 µm. Data are presented as mean ±SEM.