

Supplemental Information

**Transcriptional reprogramming restores UBE3A
brain-wide and rescues behavioral phenotypes
in an Angelman syndrome mouse model**

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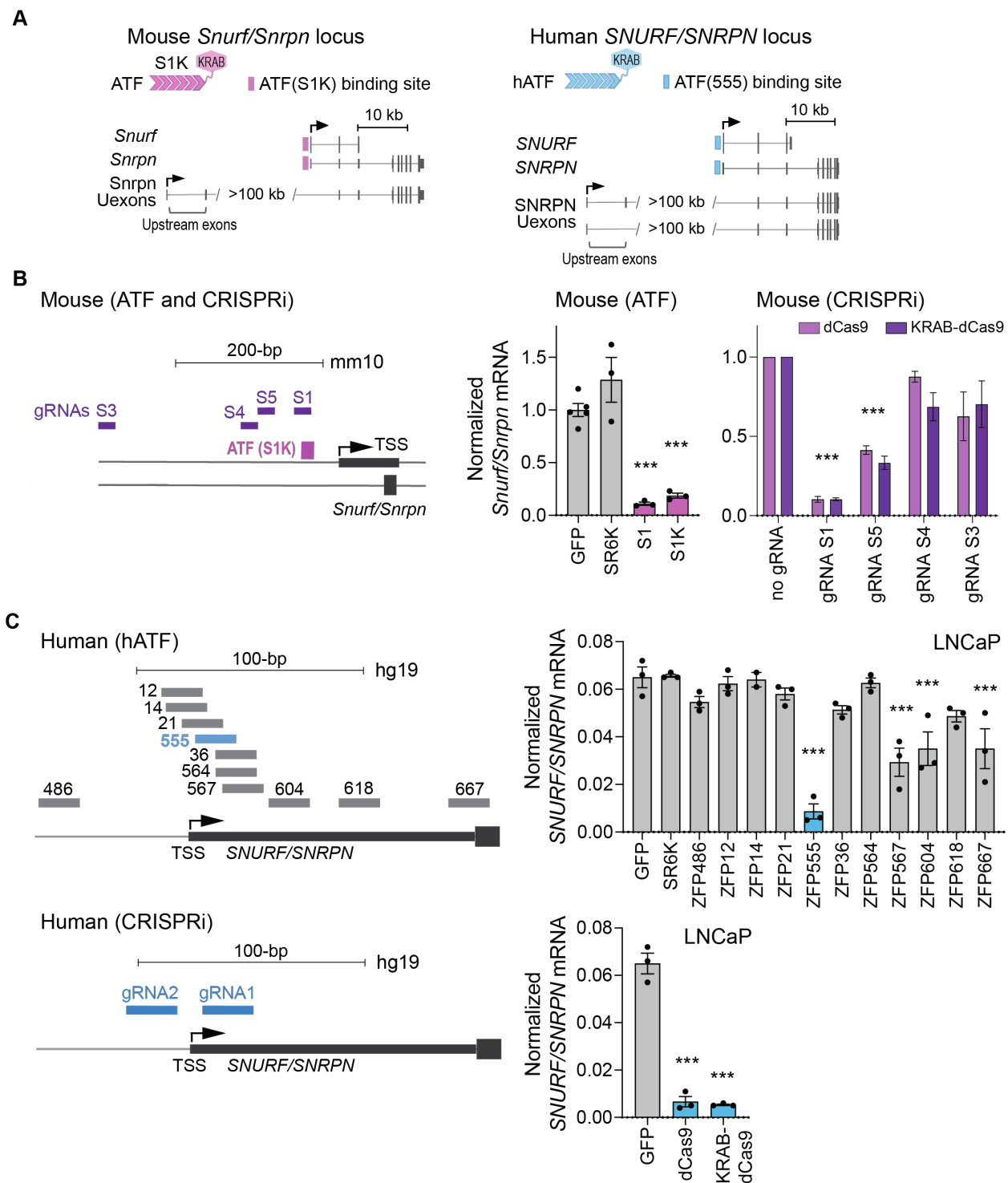
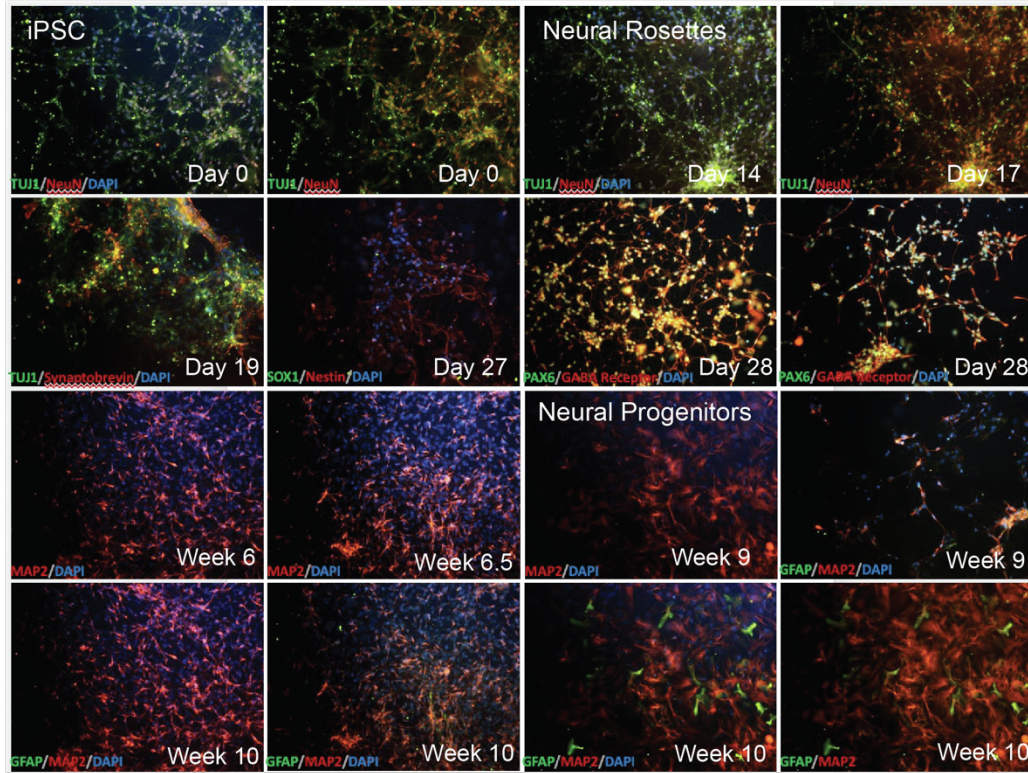


Fig. S1. Screening of artificial zinc finger transcription factors and CRISPR/KRAB-dCas9 for transcriptional reprogramming of *SNURF/SNRPN* in mouse and human cells. A. Diagram depicting the architecture of *Snurf/Snrpn* locus in mouse (left) and human (right). Exons are represented by bars and connected by introns (lines). Arrows highlight the transcriptional start

site (TSS). Pink and blue bars indicate binding sites for mouse and human ATFs (S1K and hATF-555, respectively). **B.** Efficiency of *Snurf/Snrpn* downregulation was evaluated in mouse Neuro-2A cells 5 days after transfection with plasmids expressing artificial transcription factors (ATFs) or CRISPR/KRAB-dCas9. The S1 zinc finger protein without effector domain was included to determine its effect on *Snurf/Snrpn* transcription. Diagram on the left indicates ATF-S1K binding site (pink) and target sites for CRISPR/KRAB-dCas9 (purple). *Snurf/Snrpn* expression was measured by RT-qPCR, normalized to GAPDH and compared to GFP control for ATFs or a no-guide control for CRISPR/KRAB-dCas9 ($n \geq 2$, average \pm SEM, Dunnett's multiple comparisons tests; $***p < 0.001$). **C.** Human *SNURF/SNRPN* expression was measured by RT-qPCR in LNCaP cells. Horizontal bars (gray and blue) depict target sites of human ATF screen with the lead candidate hATF-555 highlighted in blue. LNCaP cells were also transfected with plasmids expressing KRAB-dCas9 or dCas9 with two guide RNAs (gRNA1 and gRNA2). Normalized *SNURF/SNRPN* expression was compared to GFP control ($n \geq 2$, average \pm SEM, Dunnett's multiple comparisons tests; $***p < 0.001$)

A



B

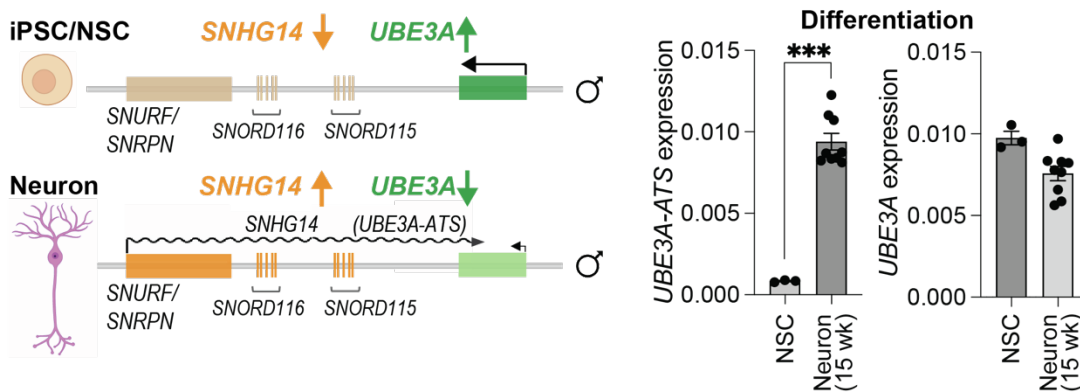
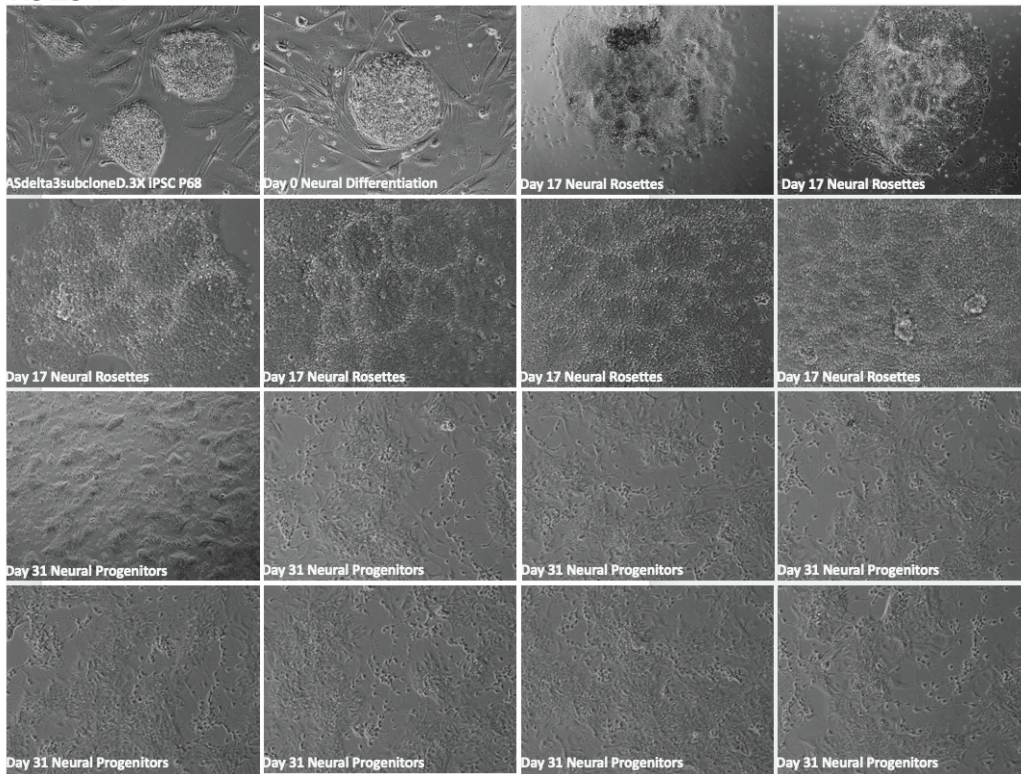


Fig. S2. Neuronal differentiation of AS patient derived iPSC cell line. A. Neuronal differentiation of the patient derived AS iPSC line (ASdel1-0, AGI-0) was evaluated by immunohistochemistry with antibodies as indicated in each image (TUJ1, NeuN, SOX1, PAX6, GABA receptor, MAP2, GFAP) and cells were labeled with DAPI to visualize nuclei. Neuronal differentiation was confirmed by the presence of NeuN and MAP2 markers and a low abundance of GFAP+ cells. **B.** RT-qPCR assays were performed to measure *UBE3A-ATS* and *UBE3A* expression in iPSC-derived neurons at 10 weeks following neuronal differentiation from neuronal stem cells (NSCs). Expression of *UBE3A-ATS* is upregulated (two-tailed unpaired t-test, $***p < 0.001$) during neuronal differentiation leading to *UBE3A* downregulation in mature neurons.

A AS ΔS-115



B

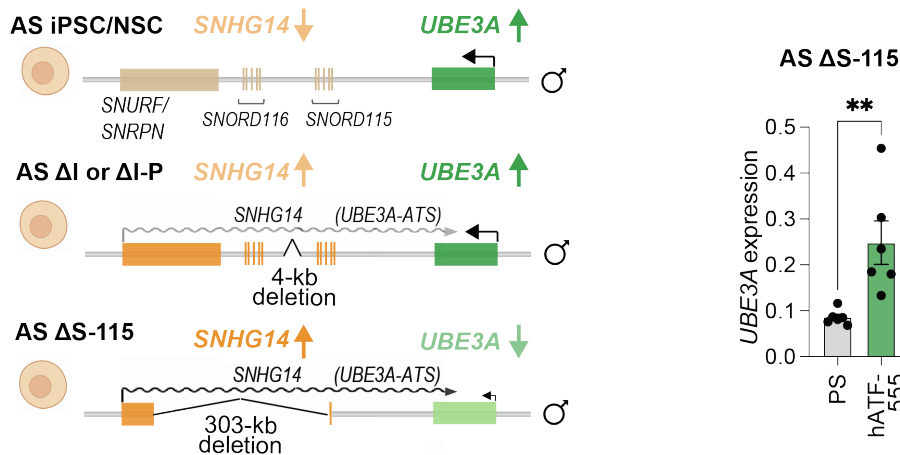


Fig. S3. hATF-555 increases *UBE3A* Expression in the constitutively active *UBE3A-ATS* cell line AS ΔS-115 iPSC. A. The iPSC cell line AS ΔS-115 was engineered to carry a 303-kb deletion spanning from *SNRPN* intron 1 to SNORD115-45 on the paternal allele resulting in constitutive expression of *UBE3A-ATS* and downregulation of paternal *UBE3A* even in non-neurons such as iPSCs and NSCs. AS ΔS-115 iPSCs were differentiated into neural progenitor cells over a 31-day period. Phase images display morphologically distinct NSCs. Scale bar = 100 μm. **B.** hATF-555 partially rescues *UBE3A* expression in AS ΔS-115 NSCs. hATF-555 transduced AS ΔS-115 NSCs showed increased *UBE3A* expression relative to protamine sulfate controls (two-tailed unpaired t-test, $p < 0.01$) 3 days after transduction.

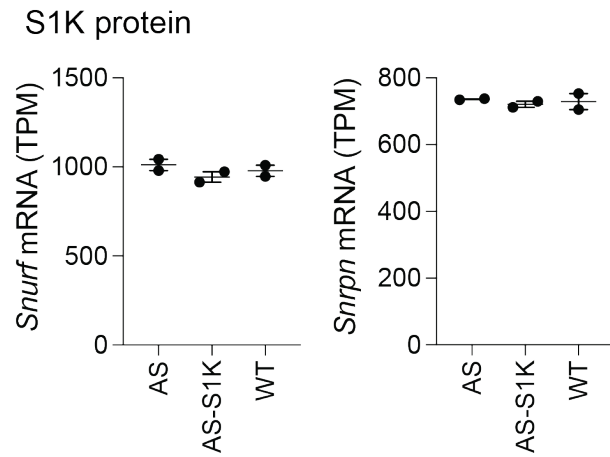


Fig. S4. Low efficiency of injectable S1K protein treatment *in vivo*. AS mice were injected with purified S1K protein (AS-S1K) every 2-3 days as previously reported (26). At ~11 weeks of age, RNA-seq was performed on brains from treated AS animals (AS-S1K) and from control AS and WT animals. Normalized *Snurf* and *Snrpn* expression is shown as transcripts per million reads (TPM) (n=2, average \pm SEM).

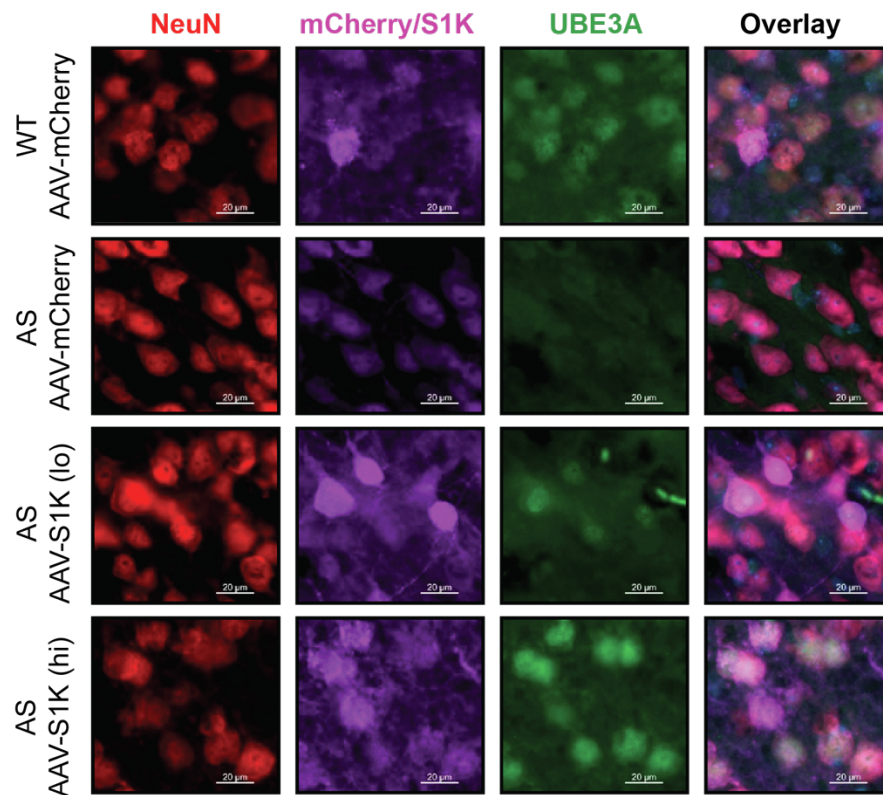


Fig. S5. Brain-wide restoration of UBE3A in mature AS neurons. A single AAV-S1K injection into the tail vein leads to brain-wide restoration of UBE3A in mature neurons. Immunostaining of cortex isolated regions (Scale bar: 20 μ m) was performed 5 weeks after AAV-S1K treatment. Mice were evaluated at 11 weeks of age. Representatives are shown for wild type (top row), AS mice (second row), and AAV-S1K treated AS mice (bottom two rows). AS mice with low mCherry intensity are labeled AAV-S1K (lo), while AS mice with mid to high mCherry intensity are labeled AAV-S1K (hi). Brain tissue was labeled for mature neurons (NeuN+, red), ATF-S1K (mCherry+, violet) and Ube3a (green). Merged images are shown in the last column.

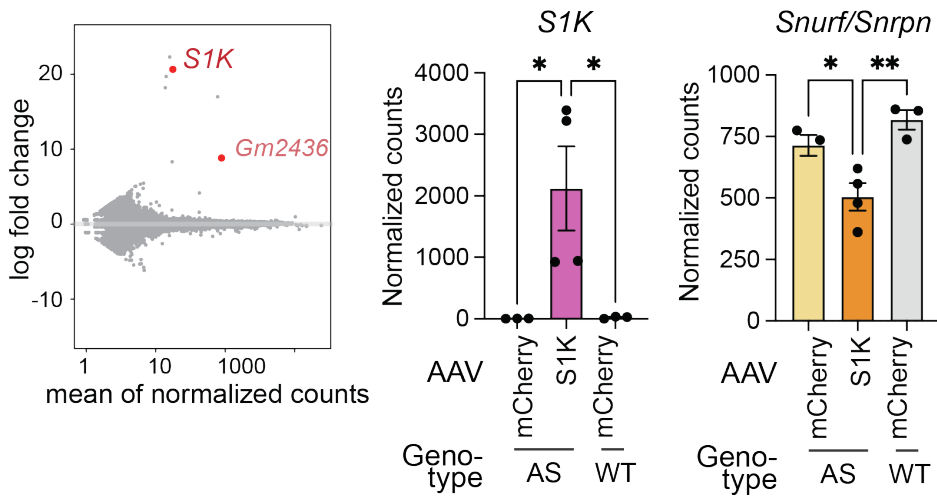
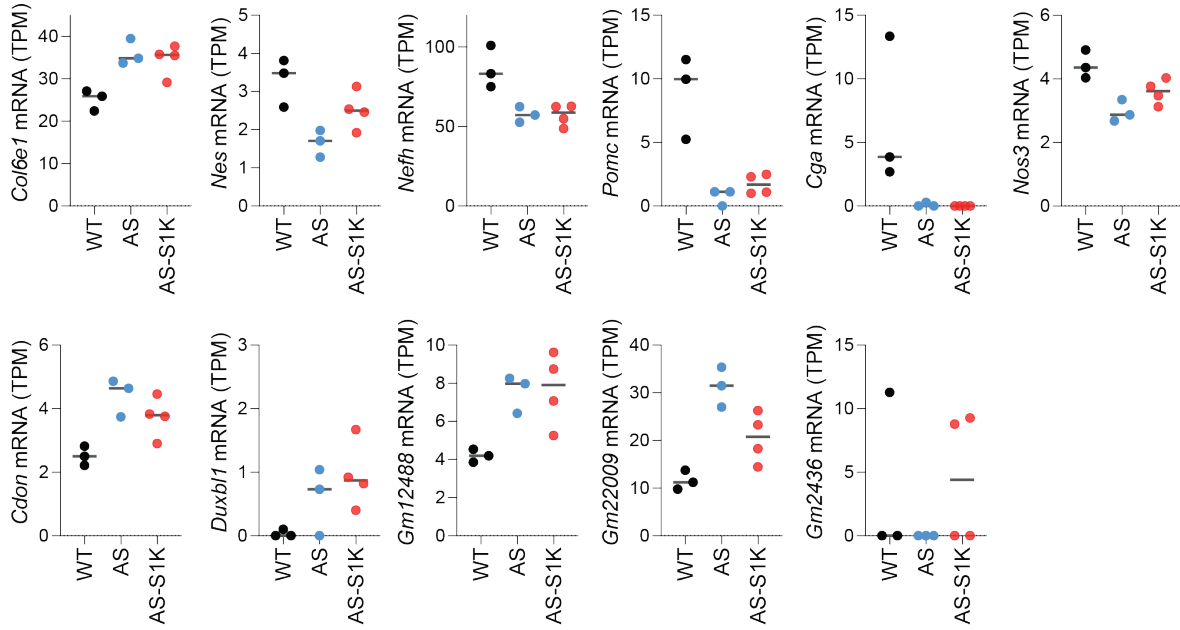
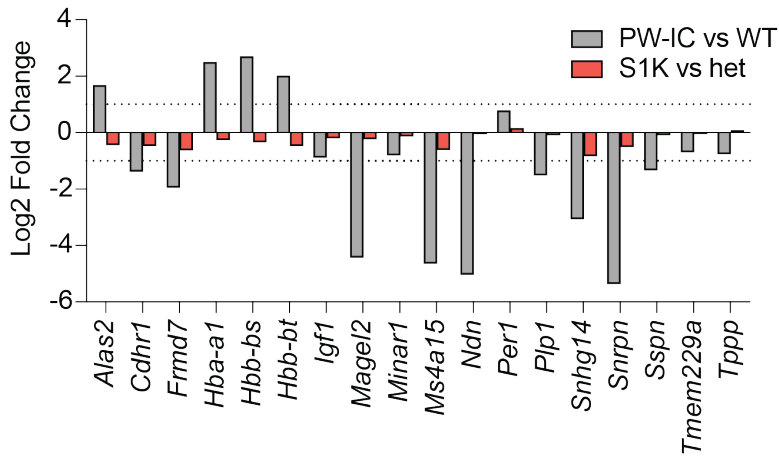
A**B****C**

Fig. S6. RNA-seq evaluates expression changes of Prader-Willi syndrome (PWS) related transcripts after AAV-S1K treatment. **A.** Differential RNA-seq analysis of brain samples 5 weeks after a single AAV-S1K treatment compares AAV-S1K treated AS mice to control AS mice treated with AAV-mCherry. ATF-S1K expression was detected in AAV-S1K treated animals along with an increase in the pseudogene *Gm2436* (adjusted p-value < 0.05). No other transcriptomic changes were observed demonstrating specificity of the treatment. Normalized counts are shown for S1K and *Snurf/Snrpn* expression ($n \geq 2$, average \pm SEM, Dunnett's multiple comparisons tests; * $p < 0.05$, ** $p < 0.01$). **B.** Transcript levels of genes differentially expressed between WT and AS mice treated with control AAV (AAV-mCherry). Normalized expression of gene transcripts (TPM, transcripts per million) is shown on the y-axis for WT and AS control mice and AAV-S1K treated AS mice. **C.** Differential gene expression analysis of PWS mouse model showed expected loss of expression of genes from the PWS locus (*Snhg14*, *Snrpn*, *Magel2*), and notably the circadian clock regulator *Per1* and the insulin growth factor *Igf1*⁴¹. PWS related transcripts were obtained from Zahova *et al.*⁴¹. Predicted genes of unknown function and low expression (FPKM < 1) were excluded from the graph. The y axis on this graph shows log₂ fold change of expression. Grey bars represent gene expression changes of the PWS mouse model (PW-IC) compared to wild type (WT). Red bars show gene expression changes of AAV-S1K treated AS mice compared to AS control mice. We did not observe changes in PWS-related transcripts as a result of AAV-S1K treatment.

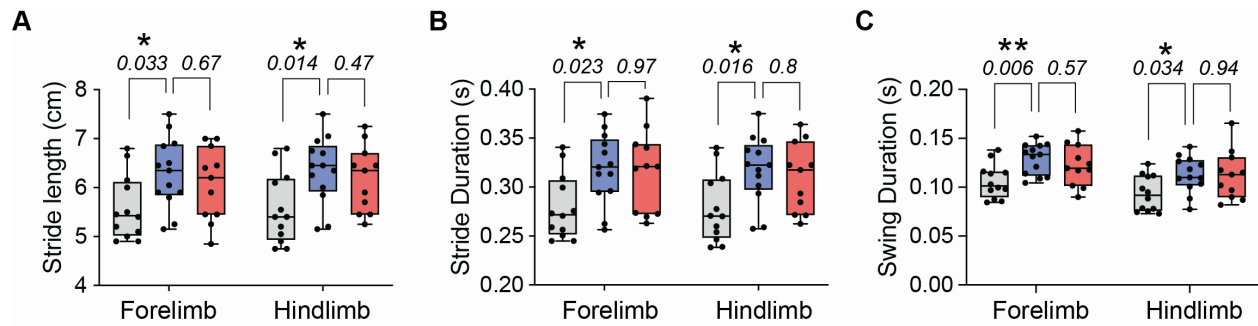


Fig. S7. Evaluation of motor deficits after AAV-S1K treatment of an AS mouse model. AAV-S1K treated AS mice and control mice treated with AAV-mCherry were subjected to treadmill walking. DigiGait™ analysis showed **A)** longer stride length, **B)** stride duration and **C)** swing duration in the AS group compared to WT. Both control (AAV-mCherry) and AAV-S1K treated AS mice showed AS gait deficits (Dunnett's multiple comparisons tests; * $p < 0.05$, ** $p < 0.01$).

Table S1. List of sequences for gRNA targets and RT-qPCR primers.

Target gene	gRNA name	Target site (PAM blue)
mouse Snurf	S1	CTCCCTACGCATGCCGTCCCAGG
	S3	ATGGCTCAGGTTTGTGCGCGGG
	S4	GGCAGGACATTCCGGTCAGAGG
	S5	GACAGAGACCCCTGCATTGCGG
	hSNURF gRNA1	GCTGGCGCGCATGCTCAGGCGG
human SNURF	hSNURF gRNA2	GCAAACAAGCACGCCTGCGCGG

Gene	Forward Oligo (RT-qPCR)	Reverse Oligo (RT-qPCR)
S1K	AGCAGAAGCGATGATCTGGT	CAGTGTGCGTCCTCTGATGT
Snurf [mouse]	TTGGTTCTGAGGAGTGATTTGC	CCTTGAATTCACCACCTTG
Snord115 [mouse]	CTGGGTCAATGATGACAAC	TTGGGCTCAGCGTAATCC
Snord116 [mouse]	GGATCTATGATGATTCCCAG	GGACCTCAGTTCCGATGA
Ube3a-ATS [mouse]	CCAATGACTCATGATTGTCCTG	GTGATGGCCTTCAACAATCTC
Ube3a [mouse]	GCACCTGTTGGAGGACTAGG	GTGATGGCCTTCAACAATCTC
Gapdh [mouse]	TGACCACAGTCCATGCCATC	GACGGACACATTGGGGGTAG
hSNURF	CTGTCTGAGGAGCGGTCAGT	CAGGTAATGCTGCTGCTGA
hUBE3A-ATS	GCACTGAAAATGTGGCATCCAGTC	GGTGTGTCAGCTGTGCTGGTGTCA
hUBE3A	ATGACGGTGGCTATACCAGG	CCTTTCTGTGCTGGGCATTTTTGG
GAPDH	AATCCATCACCATCTTCCA	CTCCATGGTGGTGAAGACG

Table S2. Differentially expressed genes identified by strand-specific mRNA-seq analysis.

Table S3. Statistical analysis of behavior assays.