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Supplemental Information

A high-fidelity RNA-targeting Cas13 restores paternal *Ube3a* expression and improves motor functions in Angelman syndrome mice

Jinhui Li, Zhixin Shen, Yajing Liu, Zixiang Yan, Yuanhua Liu, Xiang Lin, Junjie Tang, Ruimin Lv, Guannan Geng, Zhi-Qi Xiong, Changyang Zhou, and Hui Yang

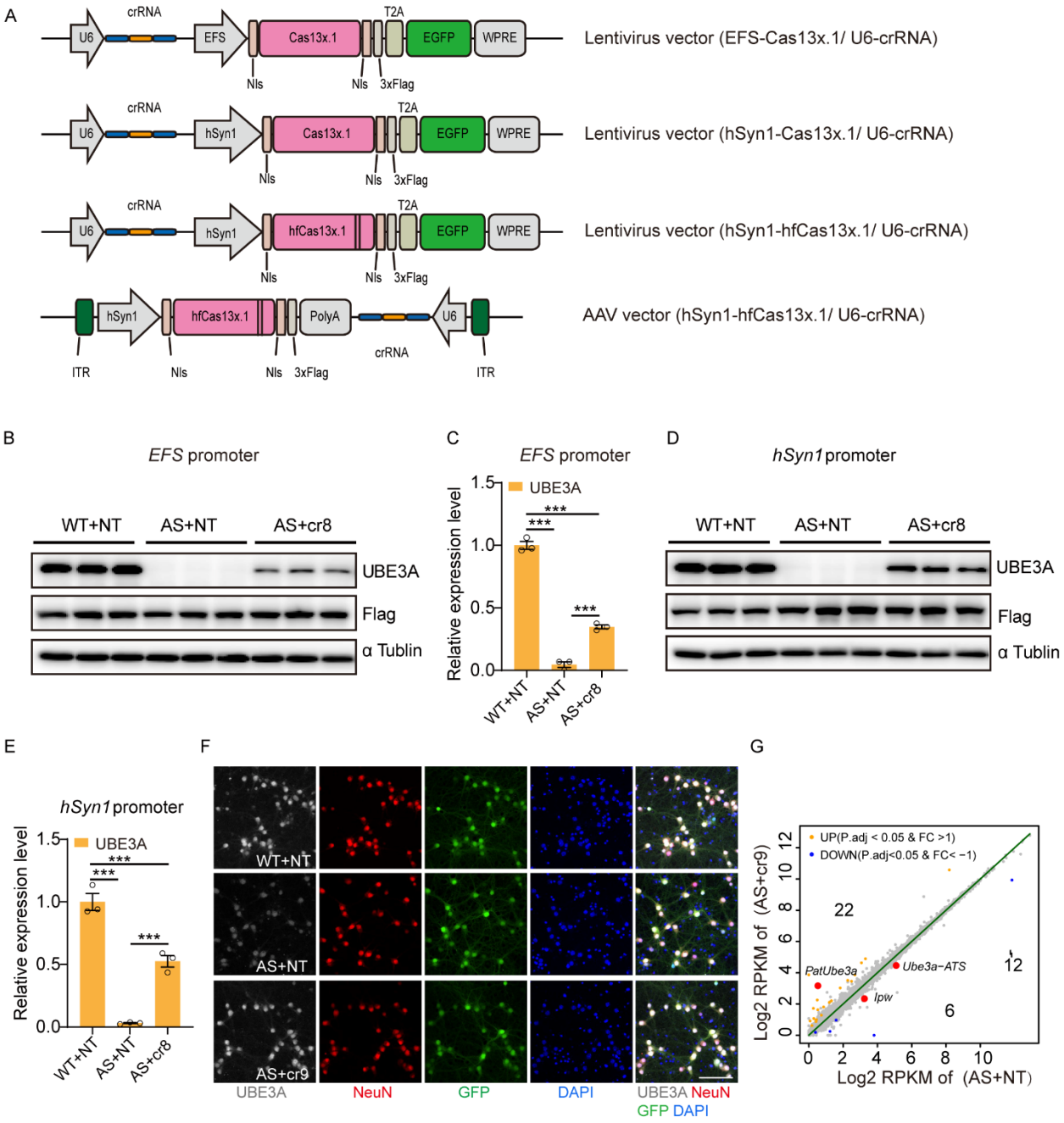

Fig. S1. Development of the Cas13x.1/crRNA system. A, Map of Cas13x.1-crRNA expression cassette in lentivirus or adeno-associated virus (AAV) backbone (not to scale). *U6* promoter, elongation factor 1 alpha short promoter (*EFS*), human synapsin-1 promoter (*hSyn1*), nuclear localization sequence (NLS), 3×Flag tag, T2A self-cleaving peptide, enhanced green fluorescent

protein (EGFP), woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), inverted terminal repeat (ITR), SV40 polyadenylation sequence element (SV40 PolyA), CRISPR RNA (crRNA). **B-E,** Western blot analysis **(B, D)** and band density quantification (**C, E**) of protein expression in WT or primary neurons of AS mice infected with *EFS*-Cas13x.1/*U6*-crRNA (**B, C**) or *hSyn1*-Cas13x.1/*U6*-crRNA (**D, E**) (n = 3 for all groups). **F,** Immunofluorescence staining for indicated proteins in lentivirus-infected primary neurons of WT or AS mice. Primary neurons of WT or AS mice were infected with lentivirus containing *hSyn1*-hfCas13x.1/*U6*-NT or *hSyn1*-hfCas13x.1/*U6*-cr9, scale bar, 100 μm. **G,** Differential expression analysis of total mRNA between *hSyn1*-hfCas13x.1/*U6*-cr9 and *hSyn1*-hfCas13x.1/*U6*-NT infected primary neurons of AS mice (n = 3 for all groups), paternal *Ube3a* (*patUbe3a*) is *Ube3a* mRNA with intact sequence expressed from paternal allele, but not *Ube3a* KO allele. Statistical significance was assessed by one-way ANOVA followed Tukey's multiple comparison test. *P < 0.05; **P < 0.01; ***P < 0.001.

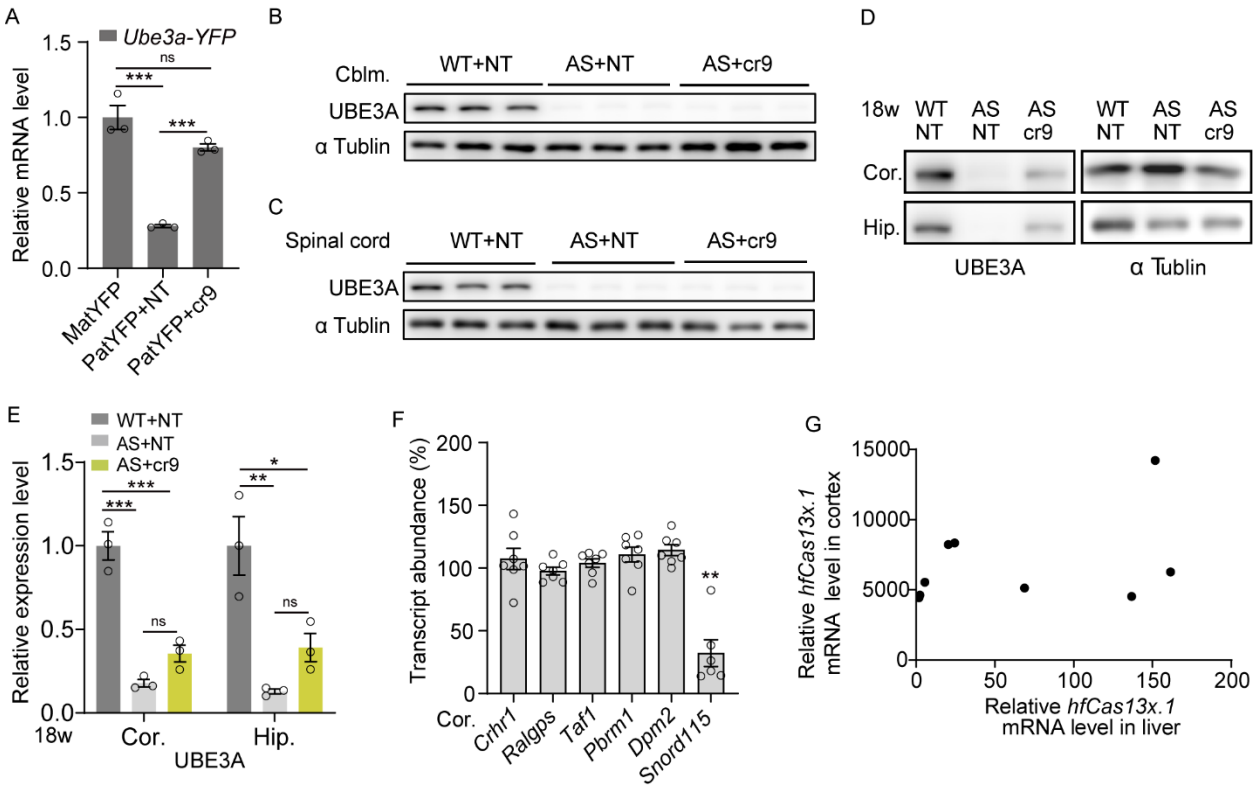

Fig. S2. In vivo detection of *Ube3a* expression and unsilencing efficiency. A, RT-qPCR analysis of mRNA expression of *Ube3a-YFP* in *Ube3a*$^{matYFP/p-}$ or *Ube3a*$^{m-/patYFP}$ mouse primary neurons. *Ube3a*$^{m-/patYFP}$ primary neurons were infected with AAV containing *hSyn1*-hfCas13x.1/*U6*-NT or *hSyn1*-hfCas13x.1/*U6*-cr9. *Ube3a*$^{matYFP/p-}$ primary neurons were infected with AAV containing *hSyn1*-hfCas13x.1/*U6*-NT as a control. **B**, **C**, Western blot analysis of protein expression in the cerebellum (Cblm.) and spinal cord of WT and AS mice at 4 weeks (n = 3 for all groups). **D**, **E**, Western blot (**D**) and quantification (**E**) of protein expression in the cerebral cortex (cor.) and hippocampus (hip.) of WT and AS mice with indicated treatment at 18 weeks (n = 3 for all groups). **F,** mRNA levels of *Snord115* target genes in cortex of AS mice treated with *hSyn1*-hfCas13x.1/*U6*-cr9 at 4 weeks relative to that in AS mice treated with *hSyn1*-hfCas13x.1/*U6*-NT (n = 7 for all groups). **G**, mRNA levels of *hfCas13x.1* in cortex or liver at 18 weeks after treatment (n=9). Statistical significance was assessed by one-way ANOVA followed with

Tukey’s multiple comparison test. *P < 0.05; **P < 0.01; ***P < 0.001.

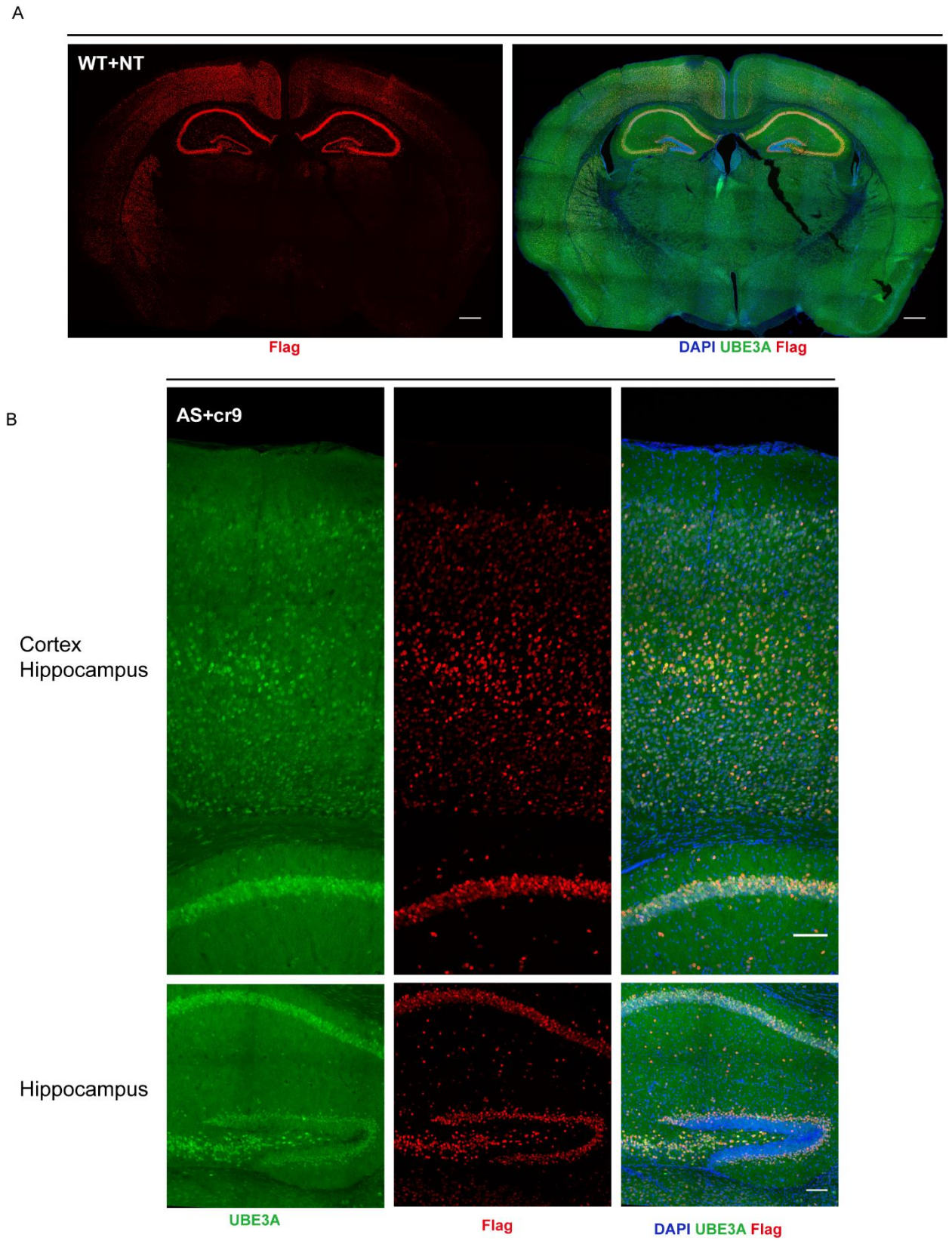

Fig. S3. The expression distribution of hfCax.1-Flag across cortex and hippocampus. A, The

coronal image of immunofluorescence staining for indicated proteins in WT mouse at 4 weeks after I.C.V. injection of AAV-PHP.eb carrying *hSyn1*-hfCas13x.1/*U6*-NT, scale bar, 500 μm. **B**, The enlarged images (for Fig. 2G) of immunofluorescence staining for indicated proteins in cortex and hippocampus of AS mouse at 4 weeks after I.C.V. injection of AAV-PHP.eb carrying *hSyn1*-hfCas13x.1/*U6*-cr9, scale bar, 100 μm.

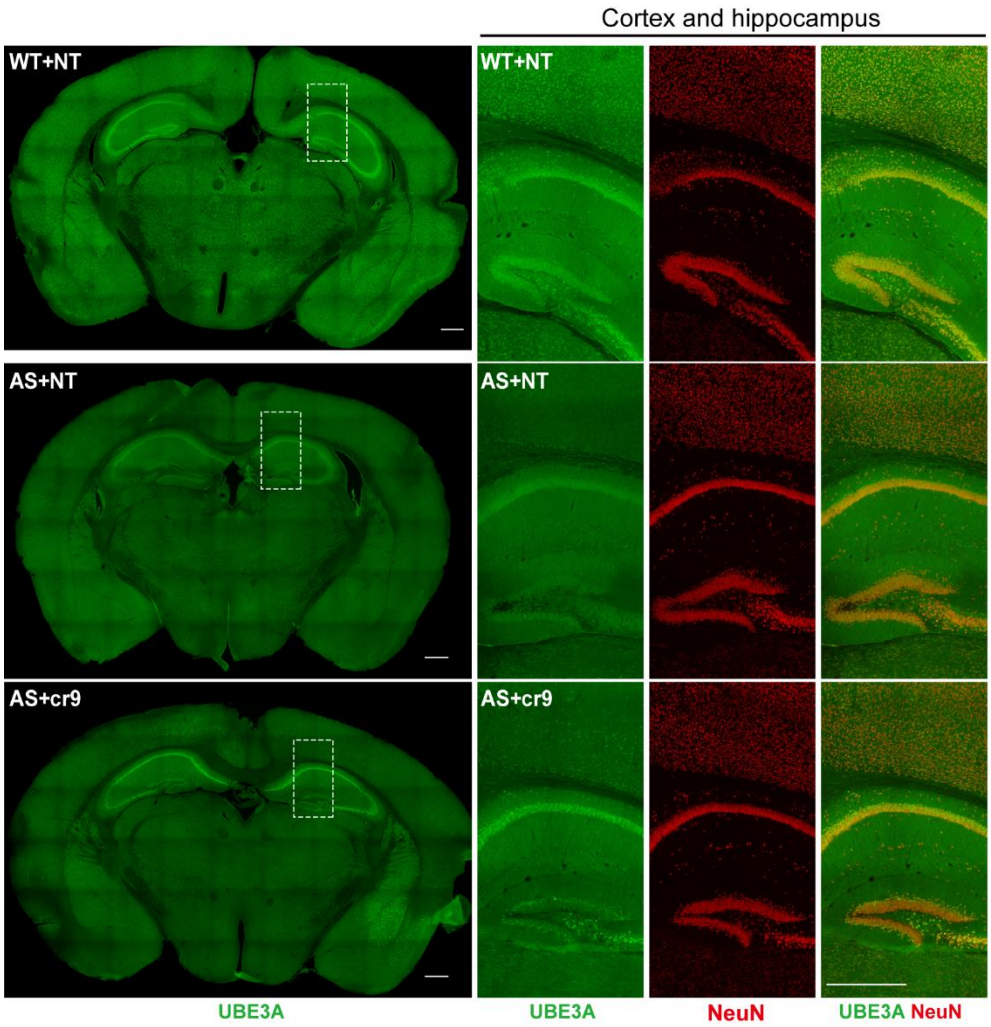

Fig. S4. AAV delivery of the CRISPR-hfCas13x system restores expression of paternal UBE3A in neurons. Representative images of immunofluorescence staining for indicated proteins in cortex and hippocampus of WT or AS mouse at 4 weeks after I.C.V. injection of AAV-PHP.eb carrying *hSyn1*-hfCas13x.1/*U6*-NT or *hSyn1*-hfCas13x.1/*U6*-cr9, scale bar, 500 μm.

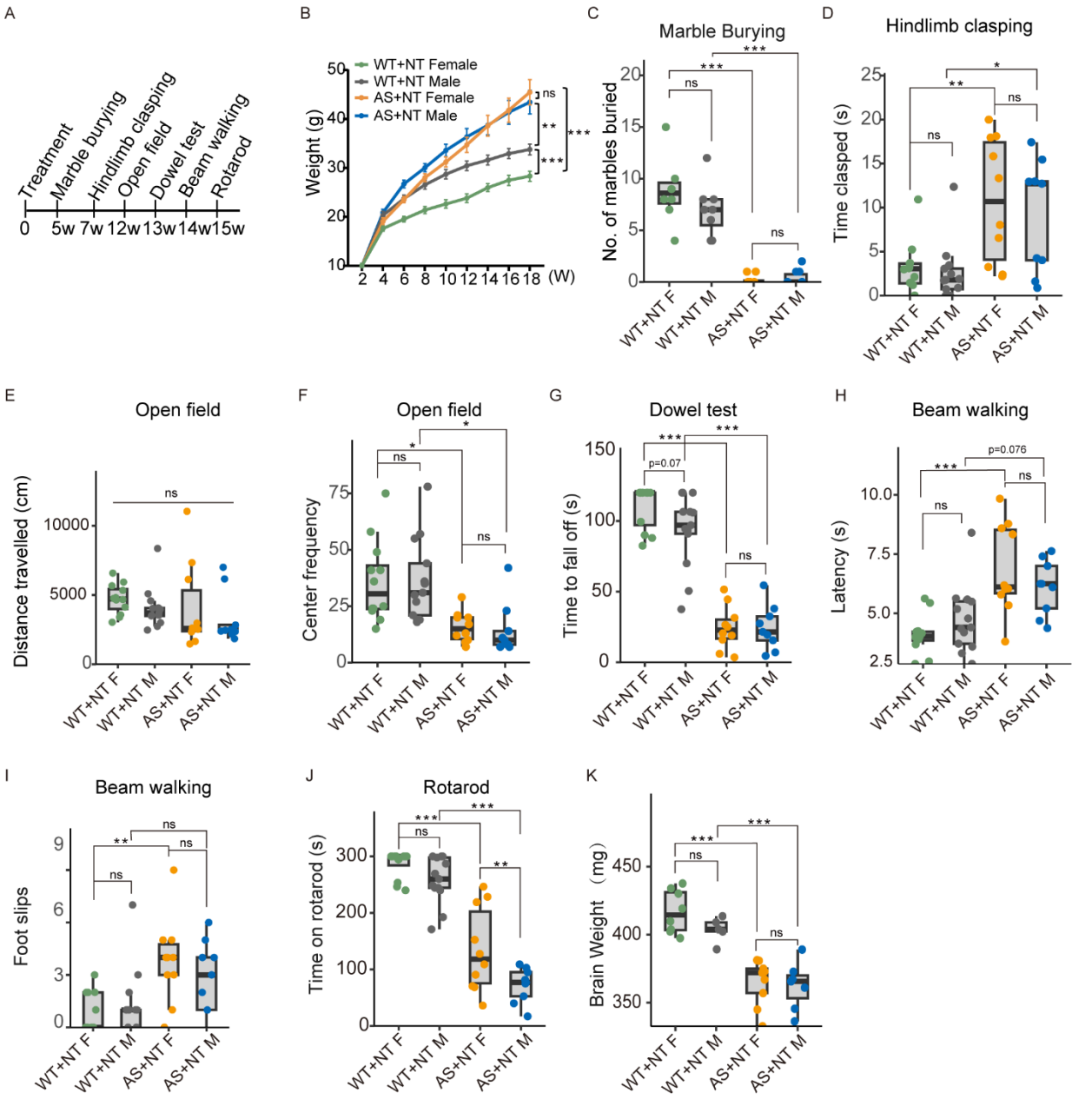

Fig. S5. The behavioral analysis in WT and AS mice injected with hfCas13x.1/NT. A, Timeline of assays performed on WT and AS mice injected I.C.V. bilaterally at P0 with 2 μL of 5 × 10^{13} vg/mL AAV-PHP.eb containing *hSyn1*-hfCas13x.1/*U6*-NT. **B**, Body weight of male and female mice was measured biweekly over 18 weeks (n = 12 for WT+NT Female; n = 10 for AS+NT Female; n = 13 for WT+NT Male; n = 9 for AS+NT Male). **C**, Marble burying test data in 5-week-old mice (n = 8 for WT+NT Female; n = 10 for AS+NT Female; n = 8 for WT+NT Male; n = 10

for AS+NT Male). **D,** Hindlimb clasping assays in 7-week-old mice. **E**, **F**, Open field tests in 12-week-old mice, the distances traveled (**E**) and Center frequency data (**F**) are shown. **G**, Dowel tests in 13-week-old mice. **H**, **I**, Beam walking assays in 14-week-old mice, time to traverse the beam (**H**) and the number of foot slips (**I**) are shown. **J**, Accelerating rotarod test in 15-week-old mice. (**D-J**, n = 12 for WT+NT Female; n = 10 for AS+NT Female; n = 13 for WT+NT Male; n = 9 for AS+NT Male)**. K**, Brain weight measured at 18 weeks of age (n = 8 for WT+NT Female; n = 9 for AS+NT Female; n = 5 for WT+NT Male; n = 7 for AS+NT Male). Statistical significance was assessed by one-way ANOVA followed by holm-sidak comparison test. *P < 0.05; **P < 0.01; ***P < 0.001.

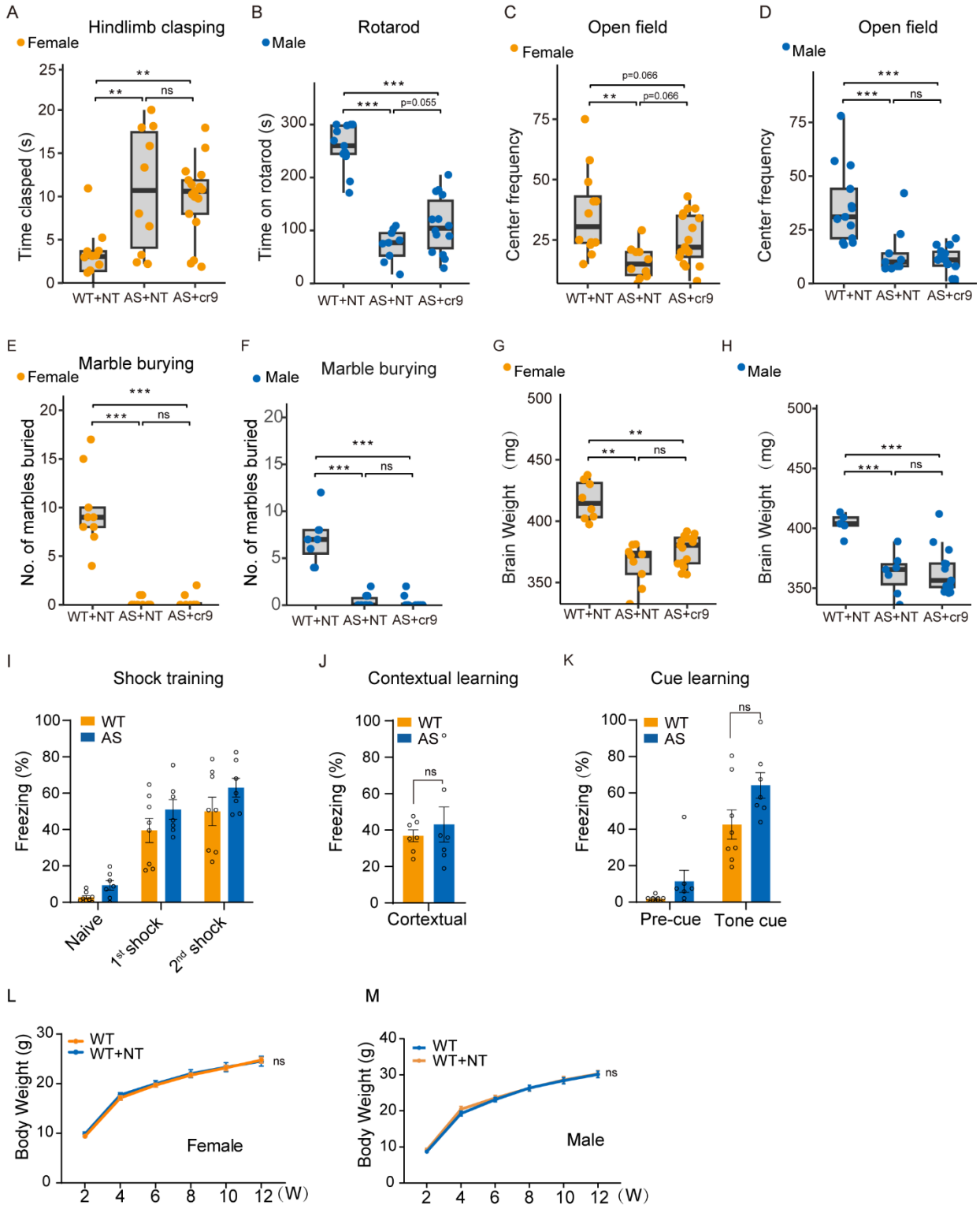

Fig. S6. Additional behavioral tests. A, Hindlimb clasping assays in 7-week-old female mice. **B**, Accelerating rotarod test data in 15-week-old male mice. **C**, **D**, The center frequency of open field

test in 12-week-old female (**C**) or male (**D**) mice. (**A,C**, n = 12 for WT+NT; n = 10 for AS+NT; n = 17 for AS+cr9). (**B**,**D**, n = 13 for WT+NT; n = 9 for AS+NT; n = 14 for AS+cr9) **. E**, **F**, Marble burying test in 5-week-old female mice (**E**) (n = 8 for WT+NT; n = 10 for AS+NT; n = 10 for AS+cr9) and male mice (**F**) (n = 8 for WT+NT; n = 10 for AS+NT; n = 12 for AS+cr9). **G**, **H**, Brain weight measured at 18 weeks of age in female mice (**G**) (n = 8 for WT+NT; n = 9 for AS+NT; n = 15 for AS+cr9) and male mice (**H**) (n = 5 for WT+NT; n = 7 for AS+NT; n = 13 for AS+cr9). (**I-K**), Fear conditioning test in 10-week-old mice. Freezing percent of shock training (**I**), contextual learning (**J**) and cue learning (**K**) were measured during the fear conditioning assay (n = 8 for WT and n = 7 for AS). **L**, **M**, The body weight of WT and WT+NT. Body weight of female mice was measured biweekly over 12 weeks (**L**) (n = 14 for WT; n = 15 for WT+NT). Body weight of male mice was measured biweekly over 12 weeks (**M**) (n = 11 for WT; n = 13 for WT+NT). Statistical significance was assessed by one-way ANOVA followed by holm-sidak comparison test. *P < 0.05; **P < 0.01; ***P < 0.001.

Table S1. CrRNA sequence and the knock-down efficiency.

crRNA	Sequence	Knock-down efficiency of *Ube3a-ATS* in N2a	Number of predicted target sites on pre-mRNA (*Ube3a-ATS*) with 0-2 base pair mismatches			
			with 0 mismatches	with 1 mismatches	with 2 mismatches	Total
cr1	GCUCUGUCCCUUGGGCCUUCUGUGUCAUGG	36.10%	1	0	0	1
cr2	CACAUAAGAAUCCAAGUAUGAGAUCCCAAC	36.80%	1	0	0	1
cr3	AGGCCAGCCUUGUUGGAUAUCAUAGAAUCC	47.60%	1	2	74	77
cr4	GAUCCAUUUGUGUUAAGCUGUAAUGGGUUG	36.90%	1	0	1	2
cr5	UCUCCACAUGGGUGAAUUCCCUGUGGGUUG	29.80%	1	0	0	1
cr6	CCGAAUGUAUAGGCCAUUGUUUCCUCAGUG	63.90%	1	0	0	1
cr7	CUGCUGGAUCAAAUUUGGGCCUUGGUGUCA	46.70%	1	0	0	1
cr8	AUUGCAUGACAGCACUCACUGUGAAAUGUG	74.80%	1	**1**	2	4
cr9	GAUAGGUAUUUCGAGUGUGAUUAAAGUAAC	81.40%	1	95	23	119

Table S2. Differentially expressed genes

Table S3. Predicted off-target sites

Table S4. The behavioural test data

Table S5. RT-qPCR primer list

Primer name	Primer sequence	Species
Ube3a-ATS Q1-F 5-3'	CCAATGACTCATGATTGTCCTG	mouse
Ube3a-ATS Q1-R 5-3'	GTGATGGCCTTCAACAATCTC	mouse
Ube3a-ATS Q3-F 5-3'	GGCACCCTTGTTTGAAACTT	mouse
Ube3a-ATS Q3-R 5-3'	GCTCATGACCCTGTCCTTTC	mouse
Ube3a Q3-F 5-3'	CAAAAGGTGCATCTAACAACTCA	mouse
Ube3a Q3-R 5-3'	GGGGAATAATCCTCACTCTCTC	mouse
Snrpn Q1-F 5-3'	TGTGATTGTGATGAGTTCAGGAAGA	mouse
Snrpn Q1-R 5-3'	ACCAGACCCAAAACCCGTTT	mouse
Snord115 Q1-F 5-3'	CCATGTGACCATTCCTACTCTG	mouse
Snord115 Q1-R 5-3'	AGAATTCGGCTACATCTACTTGG	mouse
Snord116 Q1-F 5-3'	ATTGGTCCCACTGTAATCGG	mouse
Snord116 Q1-R 5-3'	GTTCGATGGAGACTCAGTTGG	mouse
Gapdh Q1-F 5-3'	CTCCCACTCTTCCACCTTCG	mouse
Gapdh Q1-R 5-3'	TAGGGCCTCTCTTGCTCAGT	mouse
Ipw-Q3 F 5-3'	CTGCTGGTAGAAGAAATGGCACC	mouse
Ipw-Q3 R 5-3'	CATGGGCCATGAGTGACATCC	mouse