SUPPLEMENTARY MATERIALS

Protein Delivery of Artificial Transcription Factor Restores Widespread Ube3a Expression in an Angelman Syndrome Mouse Brain

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Figure S1: Sequence of proteins and reporters used in this study.

A) Sequence of the TAT-S1 ATF (922 aa)
Maltose binding protein-TAT-mCherry-HA-tag-SV40-NLS-**Zinc fingers (recognition helices F1 -> F6 underlined)**-KRAB-A box

Protein
MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFW
AHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNP
PKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGA
KAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFK
GQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKD
PRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNN
NNNLGIEGRISEFGSGAPGRKKRRQRRRVDMVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFE
IEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGF
KWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPED
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EGRHSTGGMDELYKSRYPYDVPDYANSPGIPGMGPKKKRKVGRLE**PGEKPYMCAECGKSFSRS
DDLVRHQRTHTGEKPYKCPECGKSFSDCRDLARHQRTHTGEKPYKCPECGKSFSQRAHLERHQ
RTHTGEKPYKCPECGKSFSREDNLHTHQRTHTGEKPYKCPECGKSFSRSDDLVRHQRTHTGEK
PYKCPECGKSFSTSGNLTEHQRTHTG**AAATLVTFKDVFVDFTREEWKLLDTAQQIVYRNVMLE
KYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHP*

DNA
ATGAAAATCGAAGAAGGTAAACTGGTAATCTGGATTAACGGCGATAAAGGCTATAACGGTCTCGCTGAAG
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TACGCTCAATCTGGCCTGTTGGCTGAAATCACCCCGGACAAAGCGTTCCAGGACAAGCTGTATCCGTTTA
CCTGGGATGCCGTACGTTACAACGGCAAGCTGATTGCTTACCCGATCGCTGTTGAAGCGTTATCGCTGAT
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GAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTCTAGATACCCATACGATGTCCCAGACT
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GGAGAAACCATATAAGTGTCCAGAGTGTGGTAAATCCTTTAGCAGAAGCGATGATCTGGTGAGACATCAA
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CTGGTGGAGAGAGAAATTCACCAAGAGACCCATCCTTAGCTGCAGAAGCTT

B) SR-KRAB and AT-KRAB ATF luciferase reporter:
This segment was inserted NheI/XhoI into pGL3-ctrl (Promega).
GCTAGCAGAGTGTGAGCACTGGGCAAAGTTGCCCACCCTGGAGGGCGCGGAGGGACTGGTGGGC
TGGGACGGGGAGGAGCCGGCCAAGAGGGCGGGATGGAATAGGAAAATACAGGTGAGGGGGAGGG
TGTTGTAAAGGGGGAGCAGGAAAAGGAGGGTACAGAAGCAGGGGTCAAAGTGGGAGCTCCTAGG
GTGGATGTGACAGCAGGACCTGCTGCACTGGGGGTTGGGAGTGTGATTCTCGAG

C) SNF-KRAB ATF luciferase reporter:
This segment was inserted NheI/XhoI into pGL3-ctrl (Promega).
S1 site is underlined.
GCTAGCTCCATTGCGTTGCAAATCACTCCTCAGAACCAAGCGTCTGGCATCTCCGGCTCCCTCT
CCTCTCTGCGCTAGTCTTGCCGCAATGGCTCAGGTTTGTCGCGCGGCTCCCTACGCATGCGTCC
CAGGCAATGGCTGCACATGCGCACATTTTTGCCGCAATGCAGGGGTCTCTGTCCCTCTGACCGG
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CAATTTGTAACAATATCTAAAAACAAAAACAAAAACATTTTGTAGATTGTGGATACATATATAA
GAATTTATTTCAGTTACTTTTGACCTATTAATTTCCTGTCTGCCTACTCGAG

D) Ube3a-VP64 ATF luciferase reporter:
This segment was inserted XhoI/HindIII into pGL3-ctrl (Promega),
replacing the SV40 promoter.
CTCGAGGTGGGTTCCAGGACAGCCAGGGCTTCACAGAGAAATCTGTCTTGAAAAACCAATAATA
AAAATATTTTTTATAGTGGGGAGATCTTTCATATATATATGATGTGTATATATATATACACACA
CATACACACACATTATAGGAGCTGTAATAAACAGAAAAGCACATTAATACATGTGATTGTTACA
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TTTATAATAATTTAGTGTCAAAATCATTAACAACAAACCTAAGTTAAACCCTCACTCCGGTACC

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GGCTTTTACCTACTAGTCTGGTAAGCCAGTGACATATCATGTTCTGGCAGAGGCTGATGGCCTT
GTTTGCTGAAAGGTTGCCCAATCTCAACGCCTGAATCCTTTACTCGGACAGTTCGGATGAAAAG
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CGCGAGTACACCGAAGCACACTGGTCTATAGATTGAGTAAAAGGAGCAGAGGCTAAAAACACCA
AGCCTCTCTCAGCCCAGTCTCAAGATGGCGACGATGCTGGCCAGCTTCCACCCAGCCCAAGGCC
ATCTCCCCTTCCCAAAGGCCCCTGCGCCGCAGGCGAAGGCGAGCTAGAGGAGGGCCCCACAGAG
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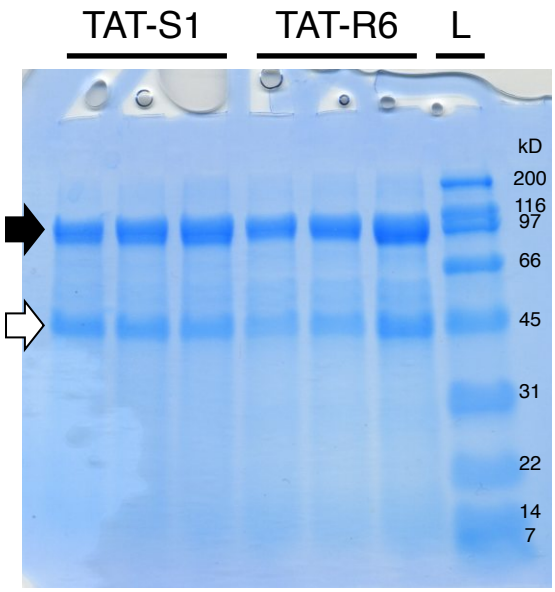

Figure S2: TAT-ATFs protein purification. Coomassie-stained gel demonstrating the purity of three separate preparations of TAT-S1 and TAT-R6 protein. Filled arrow, 100 kD full-length protein band is actually two close bands representing proteins with and without an attached MBP domain. Open arrow, 44 kD maltose binding protein.

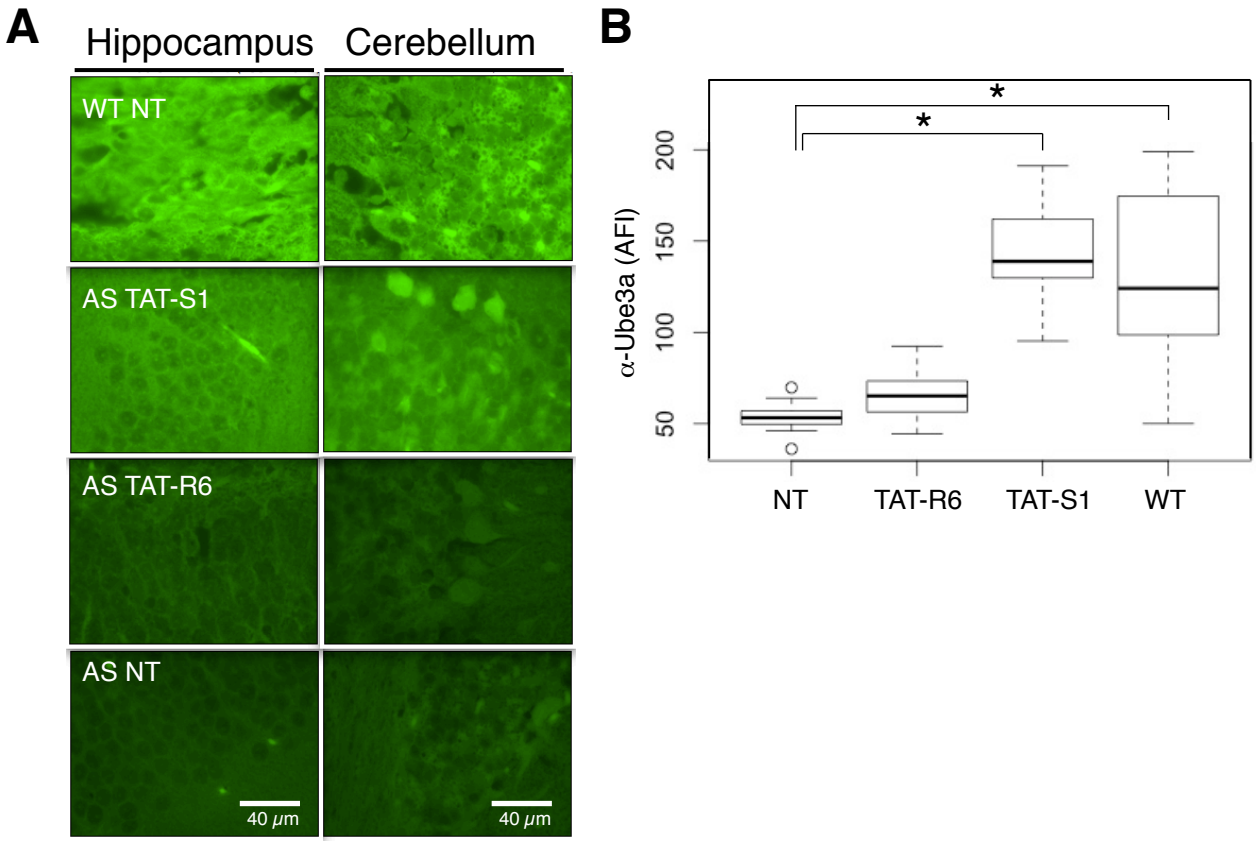

Figure S3: High resolution images of Ube3a activation by TAT-S1 but not TAT-R6. A mouse model of Angelman Syndrome (AS) was injected with TAT-S1 or TAT-R6 (160–200 mg/kg, intraperitoneal [IP]) three times per week for 7.5 weeks. **A**) High-resolution imaging of Ube3a protein expression in brain slices of the hippocampus hilus region of the dentate gyrus and the cerebellum Purkinje cell layer (α-Ube3a [Atlas HPA039410], 5 μm). Sections from no treatment (NT) wild type and AS mice are shown as controls. All images are unaltered, with no adjustment for autofloresence. **B**) Quantification of Ube3a from unaltered images of the same regions in different mice. One-way ANOVA found significant difference between groups [$F(3,22)=16.7$, $p<0.0001$], n = 3-4 mice. *, $p < 0.001$, post-hoc Tukey-Kramer HSD; AFI, average fluorescence intensity.

Table S1: DNA binding sequence (5' -> 3') and recognition helices (positions -1, 1, 2, 3, 4, 5, & 6 for fingers F6 -> F1) of the zinc finger arrays used in this study.

ZF Name	F6	F5	F4	F3	F2	F1
A4	CCA	GAC	TAG	TAG	GTA	AAA
helices	TSHSLTE	DPGNLVR	REDNLHT	REDNLHT	QSSSLVR	QRANLRA
A6	GGC	CAA	GAG	GGT	GTA	GCC
helices	DPGHLVR	QSGNLTE	RSDNLVR	TSGHLVR	QSSSLVR	DCRDLAR
A7	ATA	GAT	TGA	GTA	AAA	GGA
helices	QKSSLIA	TSGNLVR	QAGHLAS	QSSSLVR	QRANLRA	QRAHLER
A8	GCC	GCA	GGC	GAA	GGC	GAG
helices	DCRDLAR	QSGDLRR	DPGHLVR	QSSNLVR	DPGHLVR	RSDNLVR
A9	AGG	GCT	CGG	AGT	CCG	GAG
helices	RSDHLTN	TSGELVR	RSDKLTE	HRTTLTN	RNDTLTE	RSDNLVR
AT56	GGT	GAG	GGG	GAG	GGT	GTT
helices	TSGHLVR	RSDNLVR	RSDKLVR	RSDNLVR	TSGHLVR	TSGSLVR
AT74	ATG	GAA	TAG	GAA	AAT	ACA
helices	RRDELNV	QSSNLVR	REDNLHT	QSSNLVR	TTGNLTV	SPADLTR
S1	CAT	GCG	TAG	GGA	GCC	GCG
helices	TSGNLTE	RSDDLVR	REDNLHT	QRAHLER	DCRDLAR	RSDDLVR
S2	GCA	ATG	GCT	GCA	CAT	GCG
helices	QSGDLRR	RRDELNV	TSGELVR	QSGDLRR	TSGNLTE	RSDDLVR
S3	GAT	CTG	GAG	GAA	ATA	GTT
helices	TSGNLVR	RNDALTE	RSDNLVR	QSSNLVR	QKSSLIA	TSGSLVR
SR71	GCA	GGA	CCT	GCT	GCA	CTG
helices	QSGDLRR	QRAHLER	TKNSLTE	TSGELVR	QSGDLRR	RNDALTE
SR115	CCT	AGG	GTG	GAT	GTG	ACA
helices	TKNSLTE	RSDHLTN	RSDELVR	TSGNLVR	RSDELVR	SPADLTR
R6 (neg ctrl)	AAA	GTT	GCC	CAC	CCT	GGA
helices	QRANLRA	TSGSLVR	DCRDLAR	SKKALTE	TKNSLTE	QRAHLER

Table S2: Primers used in this study

Name	Sequence
	Primers for ChIP-PCR
Snurf-F	5’-CTCTCCTCTCTGCGCTAGTC-3’
Snurf-R	5’-AGAGACCCCTGCATTGCG-3’
mmchr4-F	5’-GAGCTATGGCCCATTGATGT-3’
mmchr4-R	5’-AATAGTGGGATGGTGGGAGA-3’
	Sequencing primers for Luciferase plasmids
RVprimer3-f	5'CTAGCAAATAGGCTGTCC3'
GLprimer2-r	5'CTTTATGTTTTTGGCGTCTTCC3'
	EMSA of purified ATFs
Biotinylated f	/5Biosg/CCTCTTCGCTATTACGCCAGC
Primer r	5'CACCCTGACTCGAGTACGATCGAACGTTC
S1 target site	5'-CCTCTTCGCTATTACGCCAGC CATGCGTAGGGAGCCGCG GAACGTTCGATCGTACTCGAGTCAGGGTG-3'
	AS mice genotyping primers
R1965	5'GCTCAAGGTTGTATGCCTTGGTGCT3'
WTF1966	5'AGTTCTCAAGGTAAGCTGAGCTTGC3'
ASF1967	5'TGCATCGCATTGTCTGAGTAGGTGTC3'