Towards a therapy for Angelman syndrome by targeting a long non-coding RNA

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Angelman syndrome is a single-gene disorder characterized by intellectual disability, developmental delay, behavioural uniqueness, speech impairment, seizures and ataxia1-2. It is caused by maternal deficiency of the imprinted gene UBE3A, encoding an E3 ubiquitin ligase3-6. All patients carry at least one copy of paternal UBE3A, which is intact but silenced by a nuclear-localized long non-coding RNA, UBE3A antisense transcript (UBE3A-ATS)7,8. Murine Ube3a-ATS reduction by either transcription termination or topoisomerase I inhibition has been shown to increase paternal Ube3a expression4,5,9. Despite a clear understanding of the disease-causing event in Angelman syndrome and the potential to harness the intact paternal allele to correct the disease, no gene-specific treatment exists for patients. Here we developed a potential therapeutic intervention for Angelman syndrome by reducing Ube3a-ATS with antisense oligonucleotides (ASOs). ASO treatment achieved specific reduction of Ube3a-ATS and sustained unsilencing of paternal Ube3a in neurons in vitro and in vivo. Partial restoration of UBE3A protein in an Angelman syndrome mouse model ameliorated some cognitive deficits associated with the disease. Although additional studies of phenotypic correction are needed, we have developed a sequence-specific and clinically feasible method to activate expression of the paternal Ube3a allele.

Phosphorothioate-modified chimeric 2'-O-methoxylated (2'-MOE) DNA ASOs (n = 240) were designed complementary to a 113 kilobase pair (kb) region of mouse Ube3a-ATS downstream of the Snord115 cluster of small nuclear RNAs (snRNAs) (Fig. 1a). After nuclear hybridization of the ASO to the target RNA, RNase H cleaves the RNA strand of the ASO–RNA heteroduplex, resulting in subsequent RNA degradation by exonucleases1. A high-throughput imaging screen identified ASOs that unsilenced the paternal allele. Primary neurons from Ube3a-ATS knock-in mice11 were cultured and treated with ASO (15 μM, 72 h), and we determined the fold increase of paternal Ube3a-YFP fluorescence of 2.11 ± 0.03, respectively (Fig. 1c). ASOs modulated RNA expression in a dose-dependent manner with greater than 90% reduction of Ube3a-YFP-ATS (Fig. 1d, top) within 48 h of treatment (Fig. 1d, bottom).

Snrpn, Snord116 and Snord115 are processed from the same precur-
rson transcript as Ube3a-ATS (Fig. 1a) and are critical genes in Prader–Willi Syndrome (PWS)11. Their expression was not affected by increasing the dose or time of ASO treatment (Fig. 1d, e). The ability to down-regulate Ube3a-ATS without affecting Snord16 expression can be attributed to a fast rate of Snord16 splicing (approximately 30 min) relative to the length of time required for transcription of the 332 kb region between Snord16 and the ASO-binding site (approximately 80 min) (Extended Data Fig. 1). While Ube3a-ATS ASOs did not affect expression of mature Snord16 or its precursor, ASOs designed directly to Snord16 strongly reduced Snord116 and the entire Ube3a-ATS precursor transcript (Extended Data Fig. 1).

ASO treatment (10 μM, 24 h) specifically reduced Ube3a-ATS (1,000 kb) without affecting expression of five other long genes (Nrxn3, 1,612 kb; Astn2, 1,024 kb; Pcdh15, 828 kb; Csmdl1, 1,643 kb; H1rapl1, 1,368 kb), whereas topotecan (300 nM, 24 h), which acts by impairing transcription elongation4, strongly inhibited their expression (Fig. 1f).

Primary neurons from PatYFP mice treated with ASO (10 μM, 72 h) or topotecan (300 nM, 72 h) resulted in biallelic UBE3A protein expression due to unsilencing of the paternal allele (Fig. 1g). Additionally, ASO treatment of primary neurons from Ube3aKO-/- (Angelman syndrome) mice13 achieved 66–90% of wild-type levels of UBE3A protein (Fig. 1h). ASO treatment (10 μM) did not affect DNA methylation at the PWS imprinting centre (Fig. 1i). A sequence-matched ASO that was rendered unresponsive to RNase H by complete modification with 2’-MOE nucleotides (ASO, inactive) did not affect paternal UBE3A expression, indicating that reduction of the antisense transcript is required for paternal Ube3a unsilencing (Fig. 1g).

Although reduction of the antisense transcript was required, additional studies indicated that it was not sufficient for paternal Ube3a unsilencing. ASOs complementary to the region of Ube3a-ATS upstream of Ube3a (non-overlapping ASOs, n = 15) upregulated Ube3aYFP RNA 7.4 ± 0.6 fold relative to untreated control neurons (Extended Data Fig. 2). ASOs complementary to the region of Ube3aYFP-ATS located within the Ube3a gene body (overlapping ASOs, n = 12) only upregulated Ube3aYFP RNA 1.7 ± 0.2 fold. Because both non-overlapping and overlapping ASOs reduced Ube3aYFP-ATS to a similar level, a mechanism independent of the presence of the long non-coding RNA may have a role in Ube3a silencing.

Next, we tested whether central nervous system (CNS) administration of Ube3a-ATS ASOs unsilenced paternal Ube3a in vivo. A single intracerebroventricular (ICV) injection of ASO was administered into the lateral ventricle of adult PatYFP mice. The ASO treatment was generally well tolerated, despite transient sedation after surgery. No significant changes in body weight, expression of A1F1 (marker for microgliosis), or expression of GFAP (marker for astrocytosis) were observed 1 month after treatment (Extended Data Fig. 3). Four weeks after treatment, ASO A and ASO B reduced Ube3a-ATS RNA by 60–70% and upregulated paternal Ube3aYFP RNA two- to five-fold in the brain and spinal cord (Fig. 2a). However, compared with Ube3aYFP+ (MatYFP) mice, ASO treatment did not fully unsilence the paternal allele. Ube3aYFP RNA in ASO-treated PatYFP mice was 30–40% of the level in MatYFP mice (Fig. 2a).

Western blot quantification showed that UBE3A–YFP protein was upregulated in the cortex (82 ± 7%), hippocampus (33 ± 3%) and thora
cic spinal cord (73 ± 33%) in ASO-A-treated PatYFP mice compared with MatYFP mice (Fig. 2b). No significant downregulation of Snrpn, Snord116, Snord115, or the sentinel long genes was observed, including any Snord16 reduction in the hypothalamus (Fig. 2a, c and Extended Data Fig. 4).

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After a single ASO dose, Ube3a-ATS reduction was sustained for 16 weeks in the CNS, and returned to basal expression by 20 weeks after treatment (Fig. 2d). Both the RNA and protein levels of paternal UBE3A–YFP were significantly higher than in PBS-treated mice at 2 to 16 weeks after treatment, and returned to the silenced state 20 weeks after treatment (Fig. 2d, e). No significant changes in Snrpn, Snord115 or Snord116 expression were observed (Fig. 2d). Immunostaining on brain sections 16 weeks after treatment further confirmed the long stability of the ASO and duration of paternal UBE3A protein expression (Extended Data Fig. 5). This result is consistent with the long stability of other centrally administered ASOs that are chemically modified to resist intracellular nuclease degradation16,17.

After ICV delivery, the ASO displayed widespread bilateral distribution throughout the brain, as demonstrated by immunostaining (Fig. 3a), and in situ hybridization confirmed the in vivo downregulation of Ube3a-ATS (Fig. 3b). UBE3A–YFP protein was expressed in ASO-positive cells (Fig. 3c). Increased UBE3A–YFP signal was detected in NeuN-positive cells throughout the brain, as demonstrated by immunostaining (Fig. 3a), and spinal cord (Sp. cord) 4 weeks after ICV injection (Fig. 3b). UBE3A–YFP protein (e) in ASO-treated PatYFP mice 2–20 weeks after treatment. *P < 0.05, **P < 0.005, two-tailed t-test, n = 3–4 per group, mean ± standard error of the mean (s.e.m.). For western blot quantification, YFP signal intensity was calculated relative to α-tubulin.
However, paternal unsilencing was not complete compared with the maternal UBE3A–YFP level, consistent with the western blot analysis. To further increase the concentration of ASO in the brain, intrahippocampal delivery of ASO A was performed in PatYFP mice and complete unsilencing of UBE3A–YFP was observed near the injection site (Extended Data Fig. 8).

On the basis of the ability of ASO A to upregulate UBE3A, it was chosen for assessment of phenotypic correction in Angelman syndrome mice. Angelman syndrome mice phenocopy the impaired motor coordination and memory deficit observed in patients with the disease\textsuperscript{15}. They have additional phenotypes including obesity, hypoactivity and decreased marble burying behaviour\textsuperscript{18–20}. Sex-matched Angelman syndrome littermates at 2–4 months of age were treated with ASO A or non-targeting control ASO (Ctl ASO). To determine the ability of ASO A to correct expression and behaviours relative to wild-type levels, a group of PBS-treated wild-type mice was included. After a single ICV injection, Angelman syndrome mice treated with ASO A showed reduction of Ube3a-ATS and partial restoration of UBE3A protein in the cortex (35 ± 6\%), hippocampus (35 ± 15\%) and cerebellum (47 ± 7\%) compared with wild-type mice (Fig. 4b and Extended Data Fig. 9). UBE3A immunofluorescence also showed partial restoration of UBE3A protein in these brain regions (Fig. 4c and Extended Data Fig. 9). Four weeks after treatment, the mice were subjected to behavioural tests. A reversal of contextual freezing comparable to normal behaviour was observed in ASO-A-treated Angelman syndrome mice (analysis of variance (ANOVA), F(2,39) = 5.242, P < 0.01), indicating that the memory impairment was reversed (Fig. 4d and Extended Data Fig. 9). However, there was no difference between mice treated with ASO A or Ctl ASO in open field, marble burying and accelerating rotarod tests (Extended Data Fig. 9). Complete phenotypic reversal may require treatment before a critical developmental window, a longer recovery time for rewiring of neural circuits, or a higher UBE3A induction level. Body weight was measured in a set of female mice that were injected at 3 months of age and followed for 5 months (Fig. 4e and Extended Data Fig. 9). The obesity phenotype in Angelman syndrome mice was corrected 1 month after treatment, and body weight remained significantly decreased compared with control ASO-treated mice for 5 months.

The genomic organization and regulation at the imprinting control centre is highly conserved between mouse and human. Therefore, ASO-mediated reduction of UBE3A-ATS is expected to restore UBE3A messenger RNA and protein in Angelman syndrome patient neurons. It is believed that maternal deficiency of UBE3A causes the majority of phenotypic findings in Angelman syndrome, and it is reasonable to expect that all patients with the condition, regardless of exact genotype, would benefit enormously from restored UBE3A expression. ASO therapy has been tested for neurological diseases in non-human primates and human clinical trials via intrathecal administration, with no serious adverse events\textsuperscript{16,21–23}. The well-tolerated delivery, broad tissue distribution and long duration of action indicate that ASOs may be a viable therapeutic strategy for CNS diseases and highlights the potential of an ASO drug for Angelman syndrome.
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Author Contributions L.M. and A.J.W. designed and performed experiments, analysed data, and wrote the paper (equal contribution). S.C. performed ASO delivery for Figure 2. C.F.B., A.L.B. and F.R. supervised the project. All authors discussed the experimental results.

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METHODS

Animals. All the animals of wild-type, Ube3aKO+/− (ref. 12), and Ube3aKO−/− (ref. 12) genotypes were kept on C57BL/6 background and housed under standard conditions in a pathogen-free mouse facility. All animal procedures were performed in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine and Isis Pharmaceuticals. To generate Angelman syndrome mice, Ube3aKO+− mice were born to mothers who carry the mutation on their paternal chromosome. Wild-type and Angelman syndrome littermates were housed in the same possible cage.

Oligonucleotide synthesis. Synthesis and purification of all chemically modified oligonucleotides was performed as previously described8. The 5′-MOE gapmer ASOs are 20 nucleotides in length, wherein the central gap segment comprising ten 2′-deoxyxynucleotides is flanked on the 5′ and 3′ wings by five 2′-MOE modified oxy nucleotides. Internal nucleoside linkages are purely phosphorothioate (ASO B) or interspersed with phosphodiester (ASO A), and all cytosine residues are 5′-methylcytosines. The RNase H inactive ASO consists of 20 2′-MOE modified nucleotides. The sequences of the ASOs are as follows: Ctl ASO (in vitro), 5′-CTTCTCTTGTAGAGGTTCCTC-3′; Ctrl ASO (in vivo), 5′-CTCAAGTACATGACACCCAC-3′ (ref. 25); ASO A, 5′-GATCCATTTGTGTTAAGCTG-3′; ASO B, 5′-CAGGCTCTTGTGTTATCACT-3′; ASO116, 5′-CAGGATTTGACATTTGTC-3′.

Primary neuron culture and ASO treatment. Primary cultures of hippocampal and cortical neurons were established as previously described8 from P0–P2 offspring of wild-type C57BL/6 or Ube3aKO−/− mice. Four days after plating, half of the medium was replaced and the ASO (10 μM) or topotecan (300 nM) was added to the culture medium for 72 h, unless otherwise noted. Arabinofuranosyl cytidine (Sigma) was used to inhibit glial proliferation.

Immunofluorescence. Primary neurons were fixed with 4% paraformaldehyde (PFA) for 1 h and washed in PBS. For in vivo samples, mice were anaesthetized and perfused with PBS and 4% PFA. Brain tissue was fixed with PFA overnight and dehydrated in 30% sucrose. Coronal sections of 35 μm were prepared and stained as previously described8. The following antibodies were used: anti-GFP (ab13970, Abcam, 1:1,000), anti-NeuN (MAB377, Millipore, 1:1,000 dilution), anti-ASO (Iis, 1:10,000)9. For the high-throughput in vitro ASO screen, the plates were imaged with ImageXpress®confocal system (Molecular Device) and then further processed with the MetaXpress software ( Molecular Device). Typically 200–800 cells were scored per well and the signal intensities were averaged and normalized to untreated control cells. For tissue sections, images were taken using a confocal microscope (Leica).

qRT–PCR. Total cellular RNA was isolated from cultured neurons and mouse tissue using the RNAeasy kit (Qiagen). For preparation of mouse tissue, samples were first lysed using FastPrep Lysing Matrix Tubes (MP-Biomedicals) in RLT buffer containing 1% β-mercaptoethanol. On-column DNase digestion was performed for all samples. For qRT–PCR, approximately 10 ng RNA was added to EXPRESS One-Step SuperScript qRT–PCR Kit (Life Technologies) with Taqman primer and probe sets or EXPRESS One-Step SYBR GreenER Kit (Life Technologies) with SYBR primer sets (see Extended Data Table 1 for sequences). All quantification was performed by the relative standard curve method and normalized to total RNA by Ribogreen or to the housekeeping genes Gapdh.

DNA methylation analysis. Primary neuron cultures were derived from the F1 hybrid of CAST, cd7 male and C57BL/6 female mice and treated with ASO (10 μM, 72 h). Genomic DNA was then extracted and processed for bisulphite sequencing of the PWS imprinting centre at the Srrpn DMR1 region (Srrpn promoter and exon 1) as previously described9.

Northern blot. Total RNA was isolated from ASO-treated primary neurons (10 μM, 72 h) by TRIzol (Life Technologies) according to the manufacturer’s protocol. Three micrograms total RNA was separated on an 8% polyacrylamide–7M urea gel, and then transferred by semi-dry transfer (12 V, 30 min) to GeneScreen plus hybridization transfer membrane (Perkin Elmer). The northern probes were 5′-end labelled with ATP Gamma S (P) (Perkin Elmer) using T4 polynucleotide kinase (New England Biolabs), and then hybridized to the membrane at 42 °C for 30 min. After washing membranes in wash buffer (2× SSC containing 0.1% SDS), the membrane was exposed to PhosphorImager and quantified. The oligonucleotide probe sequences used were Snord1165′-TTCCGTGAGTAGGTGGCTGAGACA-3′ and 5′-TCTCCTGAATTTCAATTTCTGGCCACTGAC-3′.

Western blot. Cultured neurons and mouse tissue were homogenized and lysed in RIPA buffer (Sigma–Aldrich) containing EDTA–free Complete Protease Inhibitor Cocktail (Roche). Protein concentration of the supernatant was determined by the DC protein assay (Bio-Rad). Ten to forty micrograms of protein lysate was separated on a precast 4–20% Bis-Tris gel (Life Technologies) and transferred by iBlot (Life Technologies). The following primary antibodies were diluted in Odyssey blocking buffer: anti-UBE3A (611416, BD Biosciences, 1:500), anti-GFP (NB600-308, Novus Biologicals, 1:500), anti-β-tubulin (T9026, Sigma, 1:20,000) and anti-α-tubulin (T5168, Sigma, 1:8,000). Following primary antibody incubation, membranes were probed with goat anti-rabbit IRDye680LT (LiCor) or goat anti-mouse IRDye800CW (LiCor) and imaged and quantified using the LiCor Odyssey system.

ASO in vivo administration. Lyophilized ASOs were dissolved in sterile PBS without calcium or magnesium and quantified by ultraviolet spectrometry. The ASOs were then diluted to the desired concentration required for dosing mice and sterilized through a 0.2 μm filter. Mice were anaesthetized with 2% isoflurane and placed in a stereotaxic frame (David Kopf Instruments). After exposing the skull, a needle (Hamilton, 1701 RN 10 μl micro syringe, needle 26 n°/2 ) was used to penetrate the skull to 0.2 mm posterior and 1.0 mm lateral to the bregma, and lowered to a depth of 3.0 mm, to deliver PBS or ASO (ASO A, 700 μg; ASO B, 500 μg) at a rate of approximately 1 μl per 30 s. The needle was left in place for 5 min, slowly withdrawn and the incision was sutured. For intrahippocampal injection, the coordinate of −2.0 mm anterior, 1.5 mm lateral and −2.0 mm dorsal to the bregma was used.

Fluorescence in situ hybridization. Tissue preparation and RNA fluorescence in situ hybridization (FISH) was carried out by the RNA In-Situ Hybridization Core at Baylor College of Medicine, as previously described9. Primers for DNA template synthesis are 5′-AGATTAGTGCACTATAGGAAGGATGTCAGTTT-3′ and 5′-TTAATACGACTCAGTATAGGAGTTCTGCTCT-3′. The T7 promoter was used to generate the Ube3a-ATS probe.

Behavioural tests. Three groups of age- and sex-matched littermates were generated, and mice were randomly assigned to each treatment group. At 12 to 14 months of age, Angelman syndrome mice received a single 700 μg dose of non-targeting control ASO or ASO A. Wild-type mice injected with an equal volume of PBS were included as controls. Four weeks after treatment, a battery of behavioural tests was performed by an experimenter blind to the genotype and treatment group using a protocol previously described9 in the Neurobehavioural Core at Baylor College of Medicine. The open field and marble burying tests were performed on day 1, the accelerating rotarod test was performed on day 2 and 3, and the fear conditioning test was performed on day 4 and 5. Mice were acclimated to the test room for 30 min before each behaviour test.

For the open field assay each mouse was placed in the centre of a clear Plexiglas (40 × 40 × 30 cm) open-field arena (Versamax Animal Activity Monitor, AccuScan Instruments) and allowed 30 min to explore. Overhead lighting was ~800 lux inside the field, and the white noise was at ~60 dB. Mouse activity was recorded and quantified.

For the marble burying test, each mouse was placed in a standard mouse cage containing 20 small (1.5–2 cm) clean black marbles on top of 4 inches of corn cob bedding, forming 4 rows of 5 columns. After a period of 30 min exploration, the mouse was removed from the cage and the number of marbles buried at least 50% was recorded.

For the accelerating rotarod, the test was performed with a rotating rod system that rotates from 4 to 40 r.p.m. within 5 min (model 7650 Rota-rod, Ugo Basile). Mice were placed on the rotating rod and the time until falling off or losing balance (mice not walking on the rod for two consecutive turns) was recorded. For two consecutive days, four trials were performed per day with at least a 30 min interval between trials.

For contextual fear conditioning, on the training day, each mouse was placed in a test chamber. After 2 min of free exploration (baseline/pre-shock freezing), the mouse received an auditory tone (2,800 Hz, 85 dB, 30 s) followed by a foot-shock (0.7 mA, 2 s). The training was repeated once. The mouse remained in the chamber for one additional min (post-shock freezing) and then was returned to the home cage. Twenty-four hours after training, mice were returned to the same test chamber for 5 min and tested for freezing in response to the training context (contextual freezing). Afterwards, the environmental settings of the test chamber were drastically altered and the mice were placed back in the modified context. They were allowed 3 min of free exploration, and then the auditory tone was presented for 3 min to test the fear response to the cue (cued freezing). Freezing frequency was analysed with FreezeFrame software (San Diego Instruments).

Isolation of nascent RNA. Nascent RNA was isolated using the Click-IT Nascent RNA Capture Kit (Life Technologies), according to the manufacturer’s protocol. In brief, wild-type primary neurons were incubated with 5-ethynyl uridine (EU, 0.5 mM) for 0 to 150 min at which time total RNA was isolated by TRIzol. Five micrograms total RNA was biotinylated with 0.5 mM biotin azide, and 500 ng biotinylated RNA was precipitated on streptavidin beads. Nascent EU–containing RNA captured on the beads was used for SuperScript VILO cDNA synthesis (Life Technologies) followed by qPCR.

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Extended Data Figure 1 | ASOs targeting Snord116 reduced Ube3a-ATS pre-mRNA. a, Top, schematic of the ASO-binding sites and location of qRT–PCR primer and probe sets. Bottom, qRT–PCR from wild-type primary neurons treated with ASO A or ASO116 (72 h) using primer and probe sets to the indicated regions of Ube3a-ATS pre-mRNA and mRNA. b, Nascent transcripts were isolated from wild-type primary neurons incubated with 5-ethynyl uridine (see Methods) for the indicated time. qRT–PCR for pre-mRNA and mature mRNA within the Snord116 region. The red line indicates the 30 min delay between the appearance of pre-mRNA and mature mRNA. Assuming a transcription elongation rate of 4 kb min$^{-1}$, it would take RNAPII 80 min to transcribe the 332 kb distance from the last copy of Snord116 to the ASO-binding site. HG, host gene. $n = 2$ per group, mean ± absolute deviation.
Extended Data Figure 2 | ASOs complementary to two regions of Ube3a-ATS differed in their ability to unsilence paternal Ube3a. PatYFP primary neurons were treated with ASOs that bind Ube3a-ATS 5’ of Ube3a (non-overlap ASOs, n = 15) or that bind to the gene body region (overlap ASOs, n = 12) for 72 h. The level of Ube3a<sup>YFP</sup>-ATS reduction and Ube3a<sup>YFP</sup> upregulation was analysed by qRT–PCR and normalized to untreated control (UTC) neurons. Data are shown as mean ± s.e.m.
Extended Data Figure 3 | In vivo ASO administration was well tolerated.

a. Left, body weight of individual wild-type C57BL/6 female mice (2 months old) treated with PBS or ASO measured weekly for 4 weeks after treatment. Right, change in body weight at each time point relative to body weight at time of treatment. n = 4 per group, mean ± s.e.m.

b. Per cent change in body weight of PatYFP mice 4 weeks after treatment relative to pre-treatment. n = 3–4, mean ± s.e.m.

c. Microglial activation was measured by Aif1 qRT–PCR 4 weeks after treatment. CTX, cortex; HIP, hippocampus; SC, thoracic spinal cord. *P < 0.05, two-tailed t-test, n = 3–4 per group, mean ± s.e.m.

d. Immunohistochemistry for AIF1 and GFAP on sagittal brain sections from wild-type C57BL/6 female mice treated with PBS or ASO for 2 weeks.

n = 3–4, mean ± s.e.m. c. Microglial activation was measured by Aif1 qRT–PCR 4 weeks after treatment. CTX, cortex; HIP, hippocampus; SC, thoracic spinal cord. *P < 0.05, two-tailed t-test, n = 3–4 per group, mean ± s.e.m. d. Immunohistochemistry for AIF1 and GFAP on sagittal brain sections from wild-type C57BL/6 female mice treated with PBS or ASO for 2 weeks.
Extended Data Figure 4  |  Snord116 was not reduced in the hypothalamus. qRT–PCR on RNA isolated from Pat<sup>YFP</sup> mice 4 weeks after treatment with PBS or ASO B.
Extended Data Figure 5 | UBE3A unsilencing persisted 4 months after treatment. ASO and YFP immunofluorescence on brain sections of cortex and cerebellum in PatYFP mice 4 months after treatment with ASO A.
Extended Data Figure 6 UBE3A–YFP was upregulated throughout the brain. Whole-brain image of YFP fluorescence in Pat<sup>YFP</sup> mice treated with PBS or ASO 4 weeks after treatment.
Extended Data Figure 7 | Imaging of unsilenced UBE3A–YFP in specific brain regions. a–l, Immunofluorescence for ASO, UBE3A–YFP and NeuN 4 weeks after treatment in MatYFP or PatYFP mice of the amygdala (a), hippocampus CA2 and CA3 layers (b), dentate gyrus (c), striatum (d), thalamus (e), hypothalamus (f), medulla (g), third ventricle (h), motor cortex (i), somatosensory cortex (j), auditory cortex (k) and visual cortex (l).
Extended Data Figure 8 | Intrahippocampal injection of ASO A in Pat\textsuperscript{YFP} mice resulted in near complete unsilencing of paternal UBE3A–YFP. YFP immunofluorescence on brain sections from Pat\textsuperscript{YFP} mice treated with Ctl ASO, 100 µg ASO A via intrahippocampal injection, or 700 µg ASO A via ICV injection. A Mat\textsuperscript{YFP} mouse treated with PBS was included for comparison.
Extended Data Figure 9 | ASO treatment in Angelman syndrome mice upregulated Ube3a. a, RNA levels of Ube3a-ATS and Ube3a were determined by qRT–PCR in wild-type (WT) mice treated with PBS and Angelman syndrome (AS) mice treated with Ctl ASO or ASO A. \( n = 2\)–3 per group, mean ± s.e.m. b, UBE3A immunofluorescence on brain sections was performed 2 to 8 weeks after treatment. c–h, ASO treatment in adult Angelman syndrome mice did not reverse some disease-associated phenotypes. c, Total distance travelled in the open field assay. d, Vertical activity in the open field assay. e, Stereotype activity in the open field assay. f, Marble burying test. The y axis represents the number of marbles at least 50% buried. g, Accelerating rotarod test during eight trials. h, Post-shock and cued response measured during the fear conditioning assay. \( n = 13\)–15 per group *\( P < 0.05\), ***\( P < 0.001\) (one-way ANOVA with Newman–Keuls post-hoc analysis). NS, not significant. i, Growth curve of age-matched female mice. Each line represents weight measurements of a single mouse over a 5-month time course post-injection, \( n = 5\) per group. Tx age, age of mouse at time of treatment.
## Extended Data Table 1 | qRT–PCR primer sequences

| Target          | Forward Sequence | Reverse Sequence | Probe Sequence (Taqman) |
|-----------------|------------------|------------------|------------------------|
| *Ube3a*-ATS     | CCAATGACTCATGATTTGCTTG | GGTACC CGGGATCCTCTAG |                          |
| *Ube3a*-FP      | TGGAGGACTAGGAAAATGAGATG | GCCTCTGTCTCAGAGATG | CCAAAAAAGCCGACAGACAGAG |
| *Ube3a*-ATS     | CCAATGACTCATGATTTGCTTG | GTTACGGTCTCAGACCATCT |                          |
| *Ube3a*         | GCACCTGTGGAGGACTAGT | GTTACGGTCTCAGACCATCT |                          |
| Snrpn           | TGTGATTTGATGAGTTTGCAGGAAGA | ACCAGACC AAACCCCCTTTT | CAAGCCAAAGAAGACCCAGAA |
| Snord116        | GGATCTATGTATTTGCTCCCCAG | GGACCTCACTTCCGATGA |                          |
| Snord116HG      | TGGTGCTGACTTTGCCCCCTAG | GTTGAGTGGAGACTCAGTTTG | AACATGCGAGAAATGGCCCC |
| Snord116pre     | ATTTGATCTGGATTTGCTTTTG | GTTGAGTGGAGACTCAGTTTG | AACATGCGAGAAATGGCCCC |
| Snord115        | CTGGGCTCAGCTCAGATACCC |                          |                          |
| Snord115HG      | CAGCAATCCCCTCCTCCAGTTC | AAGGTGCGATGTGAGATGAC | TGTGACCATCTCCTACTGAGGAG |
| Snord115pre     | CCAATGCTGGCTCTCTCTCTCTG | AGATCCTCGATACATCTTCTTGG | TGGAAAAAGGTAAGTGTGGATAGG |
| Ipw             | GCTGATAACATTCACTCCTCGAGA | AAGTGACCTCGAGAACCTCATCC | TTGGAACACCTCTTGGCAAGAGAAT |
| Gapdh           | GGCAATCCTACGCGACAGT | GGCTCTCGCTCTGGAGAT | AAGGCCGAGAATGGGAAGTTCATC |
| Pgk1            | ATGTCGCTTTTCCAAAAGCTG | GCTCCATTGCTCAGGACAGAT |                          |
| Nrxn3           | GATGAAGACCTTTGTGAATGTA | CCCTGATTTTCTGGCTCGTG | GACACATCCCTGTTGACTGC |
| Astn2           | CAGCACCACCTACACTCTCAGC | TACCTACCTGCAGACAGACATCAGCA | CCGAAGAATCGAGTACCTT |
| Pchd15          | CGGGCAAGTCTTGTTGGAAC | ACCAATGATCATCTTTTTTCTGGCCAC | GTTGTGCTCAGCTCAGACT |
| Csm1            | GCTGCCATTTCTTTTGGCTCTCCT | ACTTCTTTCTGTGTTGTTGGTAGGT | TCAAATGAGCTTTGAGAATGACT |
| Il1rap1         | CTTGATATCTCCTCGTGTATGCT | CCACTCGGACACATGAGTGGCTG | CCCCTTACTTTGGAGTAGAG |
| Aif1            | TGGGCCCCACGCAAGA | CCCACCTGATGCTACCCA | AGCTACTGGAGCTGGTTTGGG |

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