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## Supplemental Data

# *OTUD7A* Regulates Neurodevelopmental Phenotypes in the 15q13.3 Microdeletion Syndrome

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# SUPPLEMENTAL FIGURES

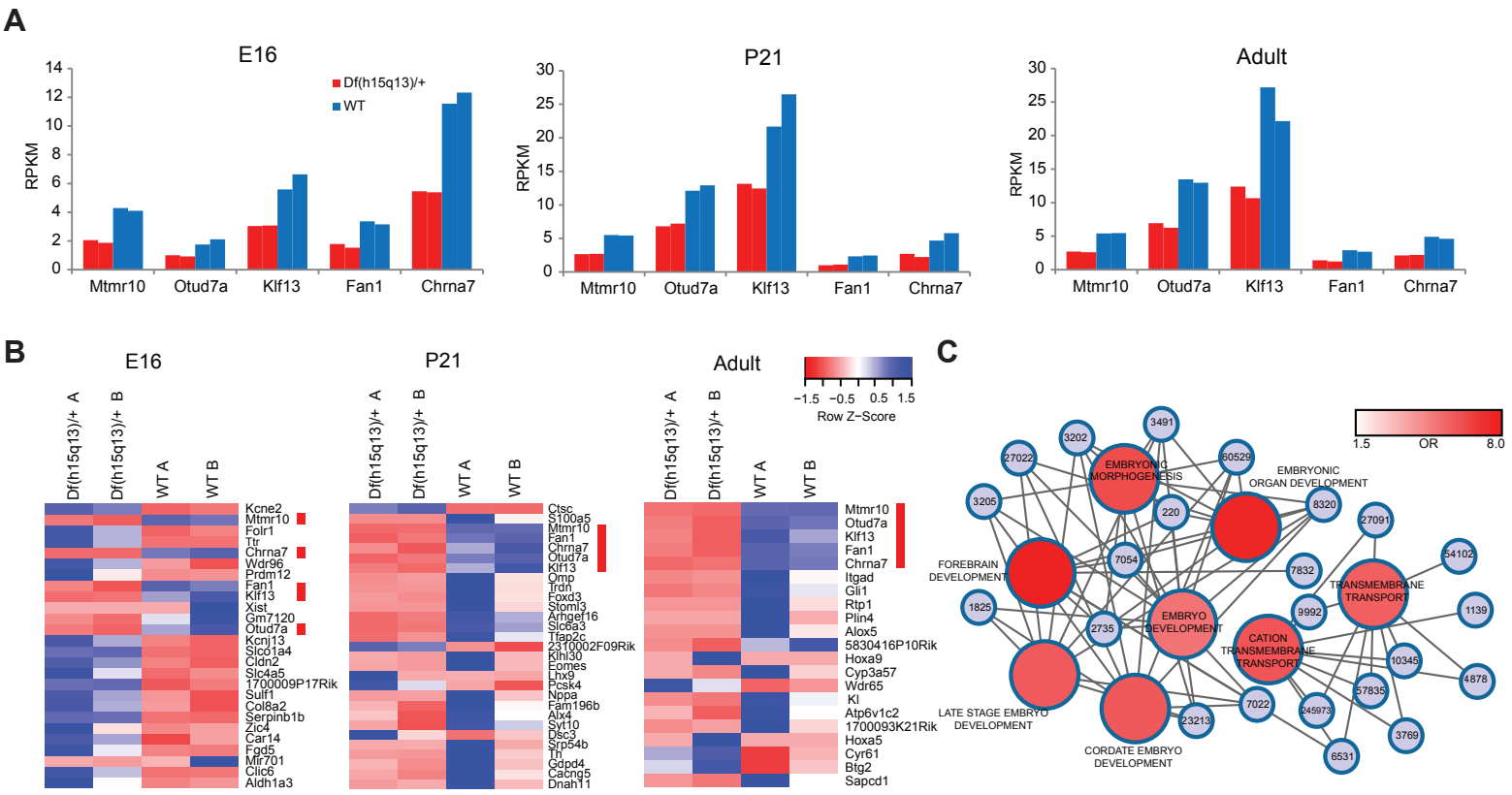


**Figure S1** A 15q13.3 mouse model (Df(h15q13)/+) has defects in forebrain signaling pathways. (**A**) RNA sequencing analysis of cortical brain tissue from E16, P21, and adult WT and Df(h15q13)/+ mice (n = 2 brains per condition). All 5 brain expressed genes within the deletion are downregulated by ~50%. (**B**) Differential expression analysis and (**C**) gene enrichment analysis revealed that forebrain development is the most significantly altered biological process across all ages.

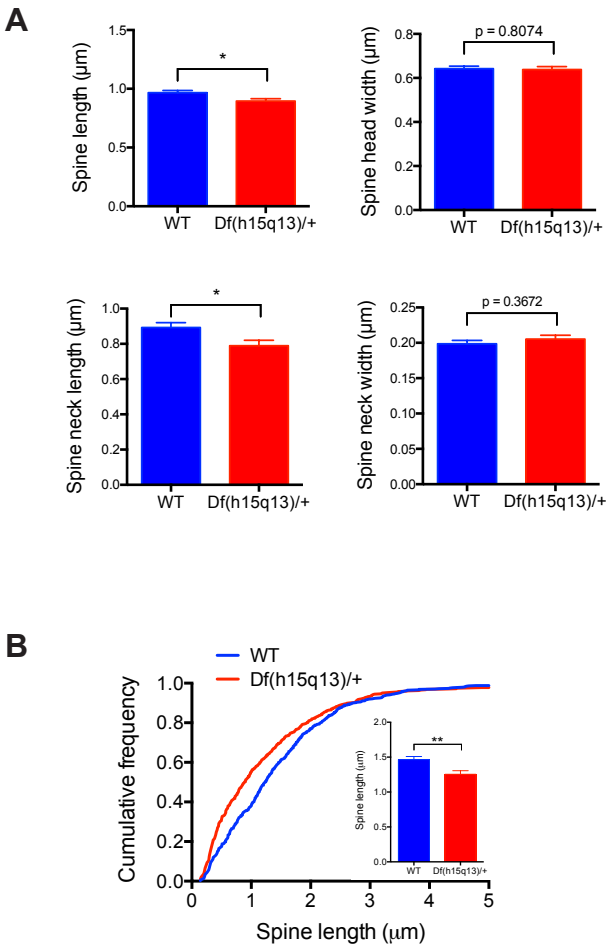


**Figure S2** Df(h15q13)/+ mice have defects in dendritic spine development. (**A**) Df(h15q13)/+ show a decrease in spine length (left), and neck length (right) (WT, n = 962 spines; Df(h15q13)/+, n = 775 spines, 4 mice per condition, student's t test, *p < 0.05, t(1735) = 2.566, t(544) = 0.2439, t(544) = 2.469, t(544) = 0.9025). 15q13.3 mouse model (Df(h15q13)/+) has defects in forebrain signaling pathways. (**B**) Analysis of dendritic spine length in WT and Df(h15q13)/+ neurons. Df(h15q13) cultured cortical neurons show a decrease in spine length. (WT, n = 645 spines; Df(h15q13)/+, n = 543 spines, 3 cultures, student's t test, **p < 0.01, t(1186) = 3.109). Error bars represent s.e.m.

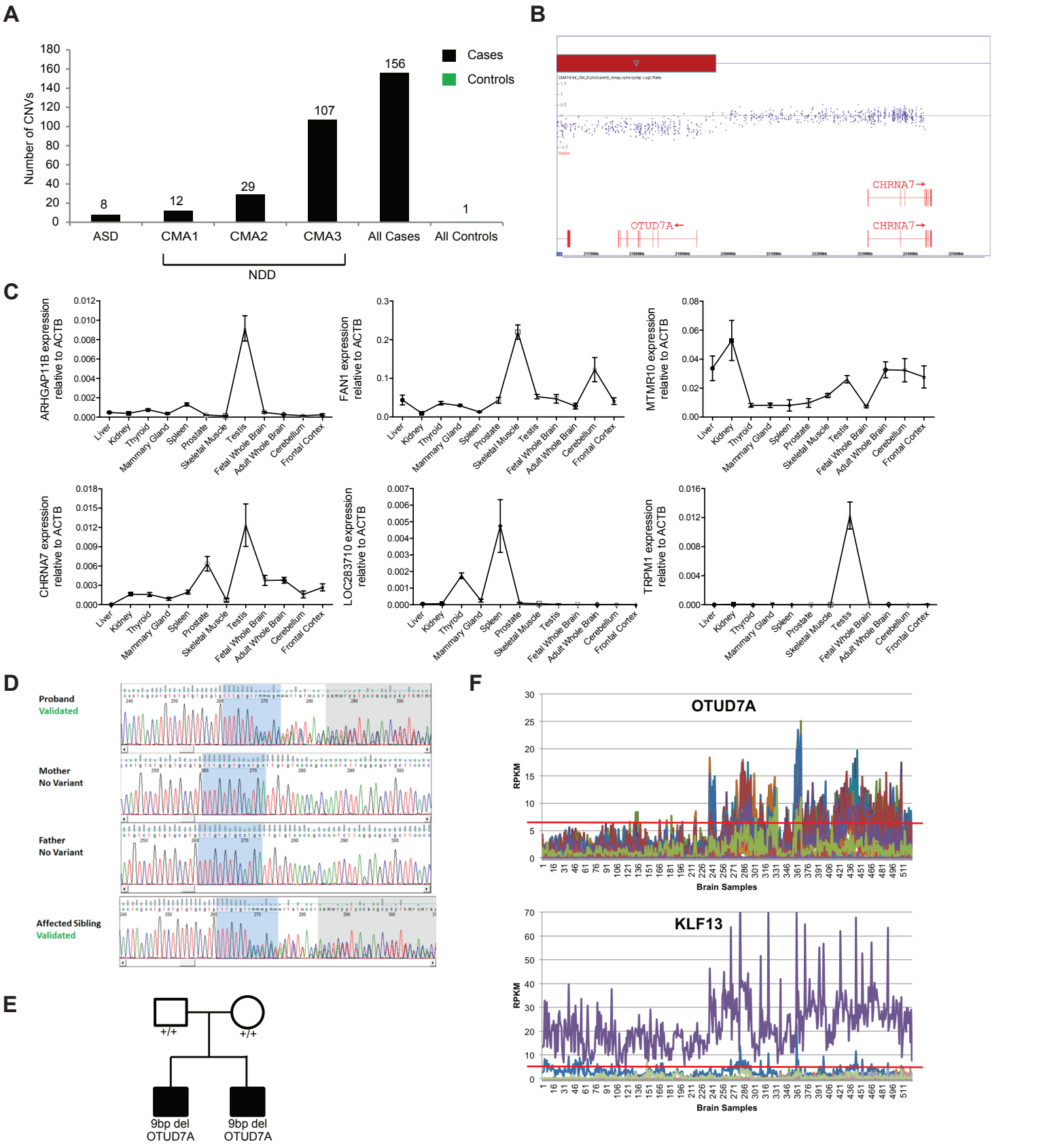


**Figure S3** Analysis of overlapping deletions and gene expression in the human 15q13.3 region. (**A**) Number of typical 15q13.3 microdeletions identified within an autism spectrum disorder (ASD) cohort, three independent developmental delay (DD) cohorts (shown in black bars) and in controls (green bar). (**B**) An atypical deletion breakpoint showing the breakpoint (CNV call from CHAS algorithm) probe intensities (log2 ratio) that clearly impacts *OTUD7A* and excludes *CHRNA7*. (**C**) Relative RNA expression (2^(-dCt)) from quantitative real-time PCR (qRT-PCR) relative to *ACTB* (replicated with another housekeeping gene *MED13*) shown for *ARHGAP11B, FAN1, MTMR10, CHRNA7, LOC283710*, and *TRMP1* within 12 different human tissues. (**D**) Sanger sequencing chromatogram validating the *OTUD7A de novo* indel in Proband and affected sibling (Proband is case 3 in Table 1), which is not present in the mother and father. (**E**) Pedigree showing the unaffected father and mother and the two affected offspring diagnosed with ASD and harboring the de novo N492_K494 deletion in OTUD7A. (**F**) Spatio-temporal expression from 524 brain regions from Brain Span for *OTUD7A* and *KLF13*. The X-axis defines the brain region, and the Y-axis is the level of expression, and each colored line represents the level of expression for an individual exon. The red line defines the 75th percentile line of expression from the entire BrainSpan RNA-seq data. *KLF13* shows only one exon is highly expressed.

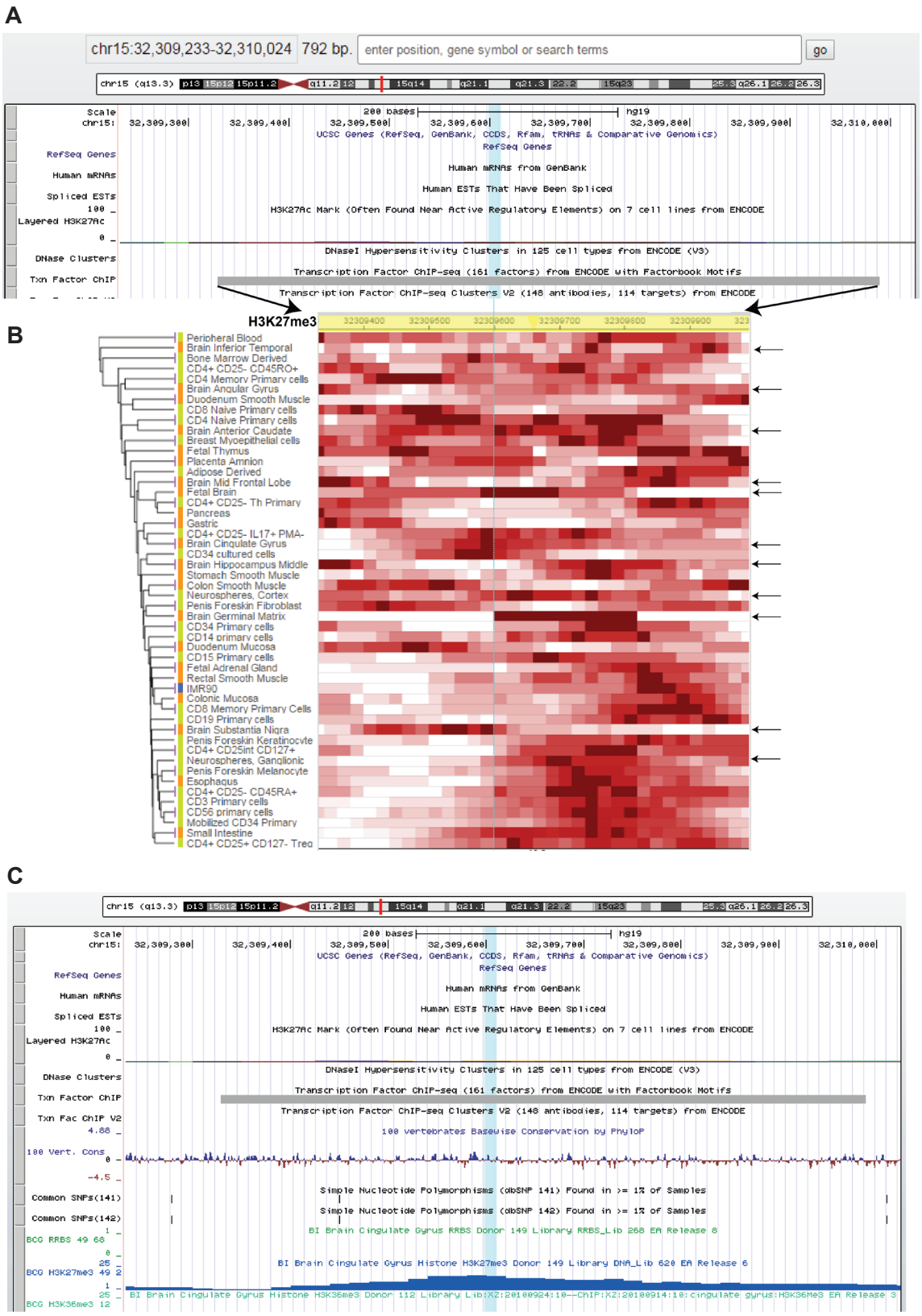


**Figure S4** *In silico* analysis reveals a *de novo* ASD-linked mutation near *OTUD7A* occurs in the binding region of the transcription factor *EZH2*. (**A**) *De novo* mutation (chr15:32309598-32309610) impacts (blue vertical line) a known transcription factor *EZH2* (grey horizontal box) but the functional consequences is unknown. (**B**) Roadmap data on 50 different tissue types for the histone marker H3K27me3 and the color code represents the peak intensities (light to dark red) for different tissue types. (**C**) *De novo* mutation impacting a high peak from chip-seq experiment obtained from Cingulate Gyrus tissue type.

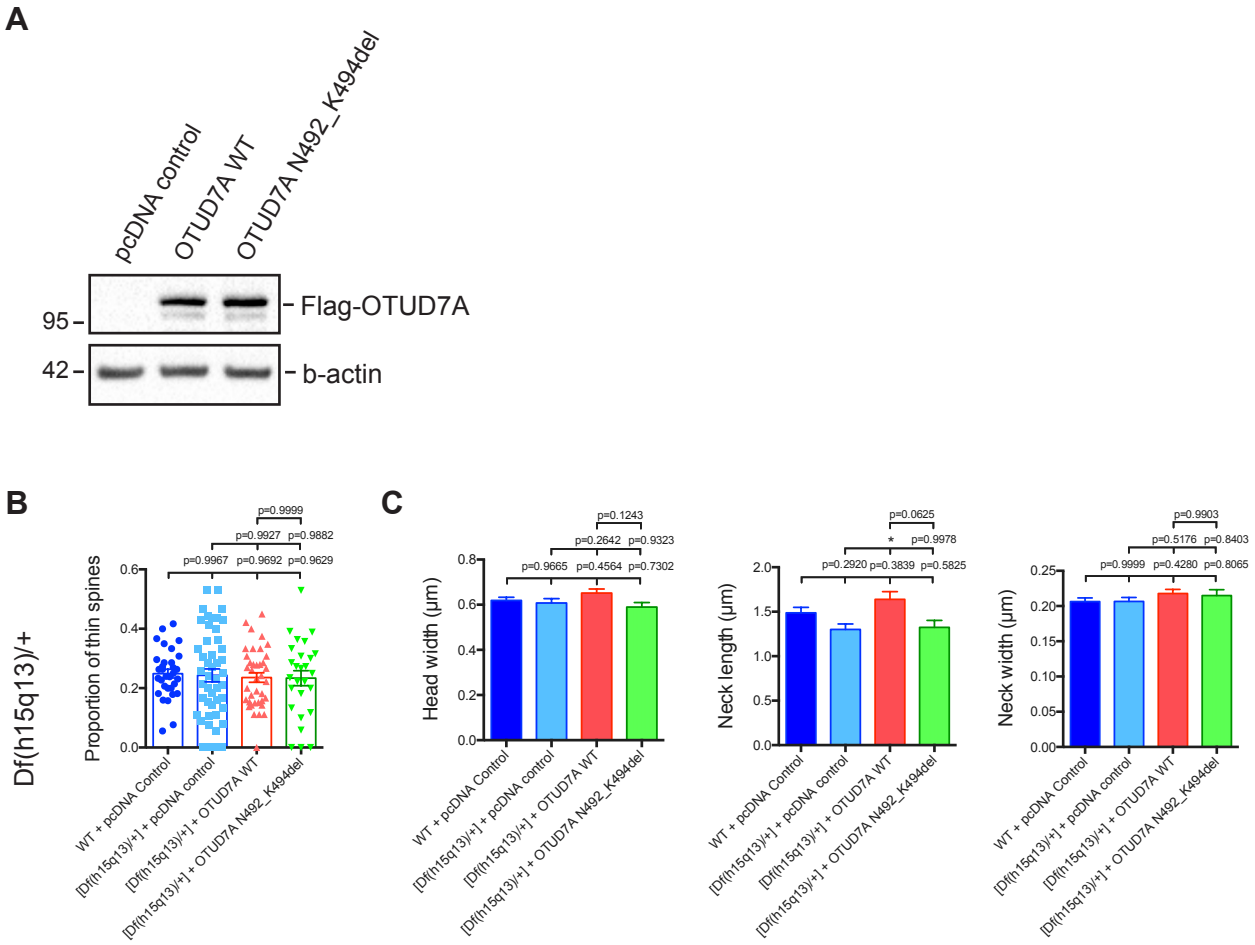


**Figure S5** Dendritic spine analysis in Df(h15q13)/+ cultured neurons expressing OTUD7A WT or the de novo OTUD7A Asn492_Lys494del mutation. (**A**) Overexpression of pcDNA control vector, FLAG-OTUD7A-WT, or FLAG-OTUD7A N492_K494del vectors in HEK293 cells show the presence of OTUD7A (isoform 1), detected by anti-Flag. (**B**) Overexpression of OTUD7A WT or OTUD7A N492_K494del in Df(h15q13)/+ neurons does not show changes in thin spines (WT + pcDNA, n = 32 dendritic segments, 18 neurons; pcDNA control, n = 48 dendritic segments, 30 neurons; OTUD7A WT, n = 38 dendritic segments, 21 neurons; OTUD7A N492_K494del, n = 28 dendritic segments, 20 neurons, 5 cultures, one-way ANOVA followed by Tukey's post hoc test, $F(3, 141) = 0.1061$). (**C**) Overexpression of OTUD7A WT in Df(h15q13)/+ neurons showed an increase in spine neck length. Overexpression of OTUD7A WT or OTUD7A N492_K494del does not show any changes in spine head width or spine neck width (WT + pcDNA control, n = 244 spines; [Df(h15q13)/+] + pcDNA control, n = 180 spines; [Df(h15q13)/+] + OTUD7A WT, n = 247 spines; [Df(h15q13)/+] + OTUD7A N492_K494del, n = 112 spines, 5 cultures, one-way ANOVA followed by Tukey's post hoc test, **p < 0.01, $F(3, 779) = 2.039$, $F(3, 768) = 4.209$, $F(3, 779) = 1.044$). Error bars represent s.e.m.

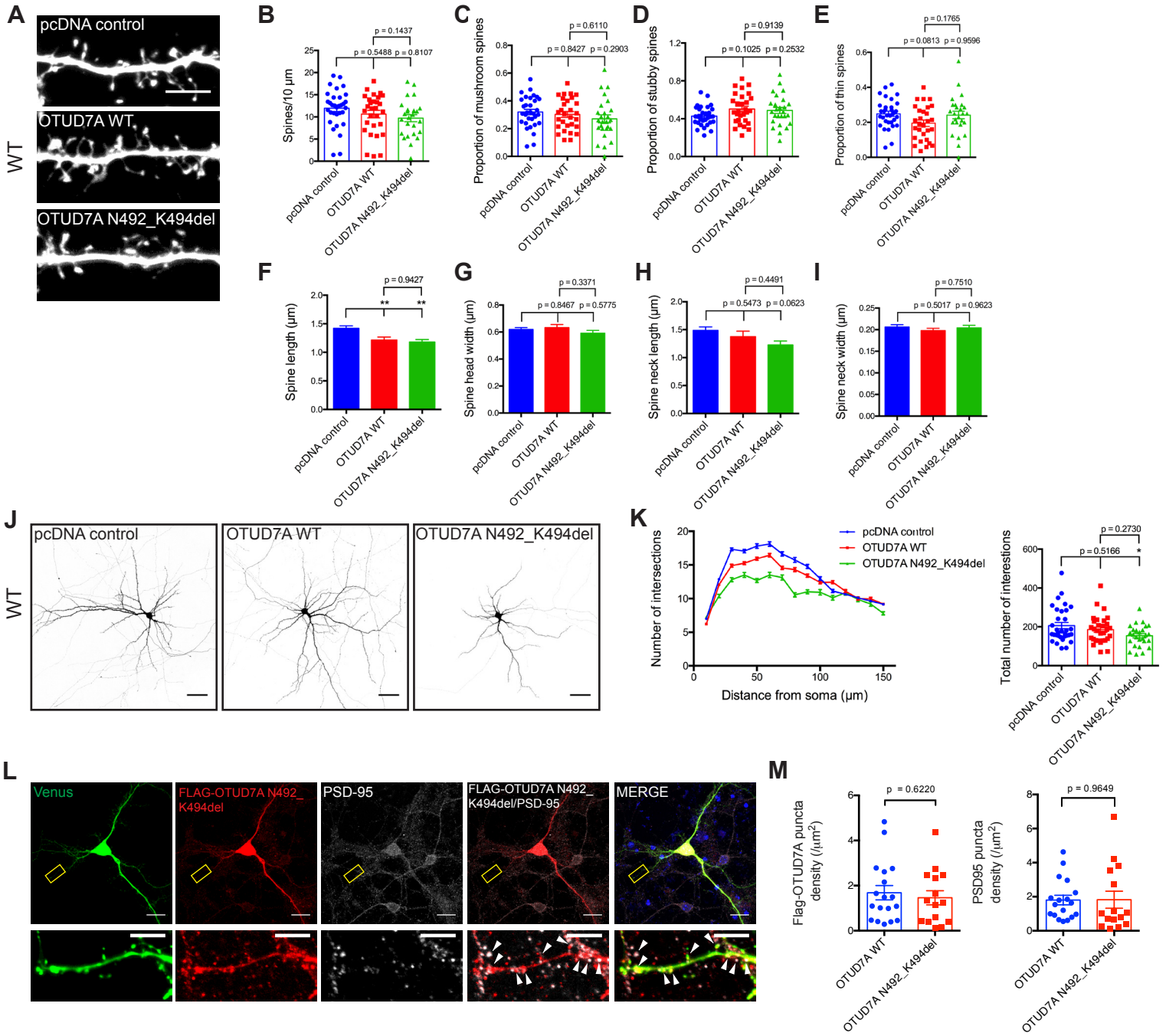


**Figure S6** The OTUD7A Asn492_Lys494del de novo mutation exhibits dominant negative defects in WT cortical neurons. (**A**) Dendritic spines from DIV14 WT cultured cortical neurons co-expressing Venus, and pcDNA control, FLAG-OTUD7A WT, or FLAG-OTUD7A N492_K494 del (scale bar = 5µm). (**B**) In WT cultured cortical neurons, expression of OTUD7A WT and OTUD7A N492_K494 did not have an effect on spine density (pcDNA control, n = 32 dendritic segments, 18 neurons; OTUD7A WT, n = 29 dendritic segments, 15 neurons; OTUD7A N492_K494del, n = 26 dendritic segments, 13 neurons, 5 cultures, one-way ANOVA followed by Tukey's post hoc test, $F(2, 84) = 2.022$). (**C**, **D**, **E**) Overexpression of OTUD7A WT or OTUD7A N492_K494del in WT neurons did not show any changes in mushroom (**C**), stubby (**D**) or thin (**E**) spines (pcDNA control, n = 32 dendritic segments, 18 neurons; OTUD7A WT, n = 29 dendritic segments, 15 neurons; OTUD7A N492_K494del, n = 26 dendritic segments, 13 neurons, 5 cultures, one-way ANOVA followed by Tukey's post hoc test, $F(2, 84) = 1.156$), $F(2, 84) = 2.401$, $F(2, 84) = 2.706$). (**F**, **G**) Spine length was significantly decreased in neurons expressing OTUD7A WT or OTUD7A N492_494del compared to control (**F**), however no changes in spine head width were detected (**G**) (pcDNA control, n = 653 spines; OTUD7A WT, n = 522 spines; OTUD7A N492_K494del, n = 427 spines, 5 cultures, one-way ANOVA, $^{**}P < 0.01$, $F(2, 1599) = 8.275$, $F(2, 548) = 1.010$). (**H**, **I**) Overexpression of OTUD7A WT or OTUD7A N492_K494del in Df(h15q13)/+ neurons does not show any changes in spine neck length or spine neck width (pcDNA control, n = 244 spines; OTUD7A WT, n = 173 spines; OTUD7A N492_K494del, n = 134 spines, 5 cultures, one-way ANOVA followed by Tukey's post hoc test, $F(2, 548) = 2.574$, $F(2, 548) = 0.6441$). (**J**) DIV14 WT cortical neurons co-expressing Venus and pcDNA control, FLAG-OTUD7A WT, or FLAG-OTUD7A N492_K494del (scale bar = 50µm). (**K**) In WT neurons, expression of OTUD7A N492_494del caused a significant decrease in dendritic arborization (pcDNA control, n = 31 neurons; OTUD7A WT, n = 27 neurons; OTUD7A N492_K393del, n = 16 neurons, 5 cultures, one-way ANOVA followed by Tukey's post hoc test, $^{*}p < 0.05$, $F(28, 1065) = 14.65$, $F(2, 86) = 3.498$). (**L**) DIV14 WT mouse cortical neurons co-expressing Venus and FLAG-OTUD7A N492_K494del, and co-stained for FLAG and PSD95. OTUD7A N492_K494del is co-localized with PSD95 in dendrites and dendritic spines. Arrows indicate co-localized puncta located in dendritic spines (scale bars = 20 µm, top; 5 µm, bottom). (**M**) Quantification of FLAG-OTUD7A (left) and PSD95 (right) puncta localization showed no changes between OTUD7A WT and OTUD7A N492_K494del overexpression (OTUD7A WT, n = 18 neurons; OTUD7A N492_K494del, n = 15 neurons, 2 cultures, student's t test, $t(31) = 0.4980$, $t(31) = 0.04441$). Error bars represent s.e.m.

**Table S3.** Number of cases from each cohort, phenotypic ascertainment and the controls.

| Microarray Dataset | Phenotype Ascertainment | # of cases | 15q13.3 microdeletion |
|---|---|---|---|
| ASD | Strictly Autism | 1026 | 8 |
| Clinical Microarray 1 (CMA 1)* | Broader neurodevelopmental disorders | 9,322 | 12 |
| Clinical Microarray 2 (CMA 2)* | Broader neurodevelopmental disorders | 10,619 | 29 |
| Clinical Microarray 3 (CMA 3) | Broader neurodevelopmental disorders | 17358 | 107 |
| All_cases | | 38325 | 156 |
| All_controls | | 22,241 | 1 |

* New unpublished cohort analyzed for 15q13.3 microdeletion syndrome.