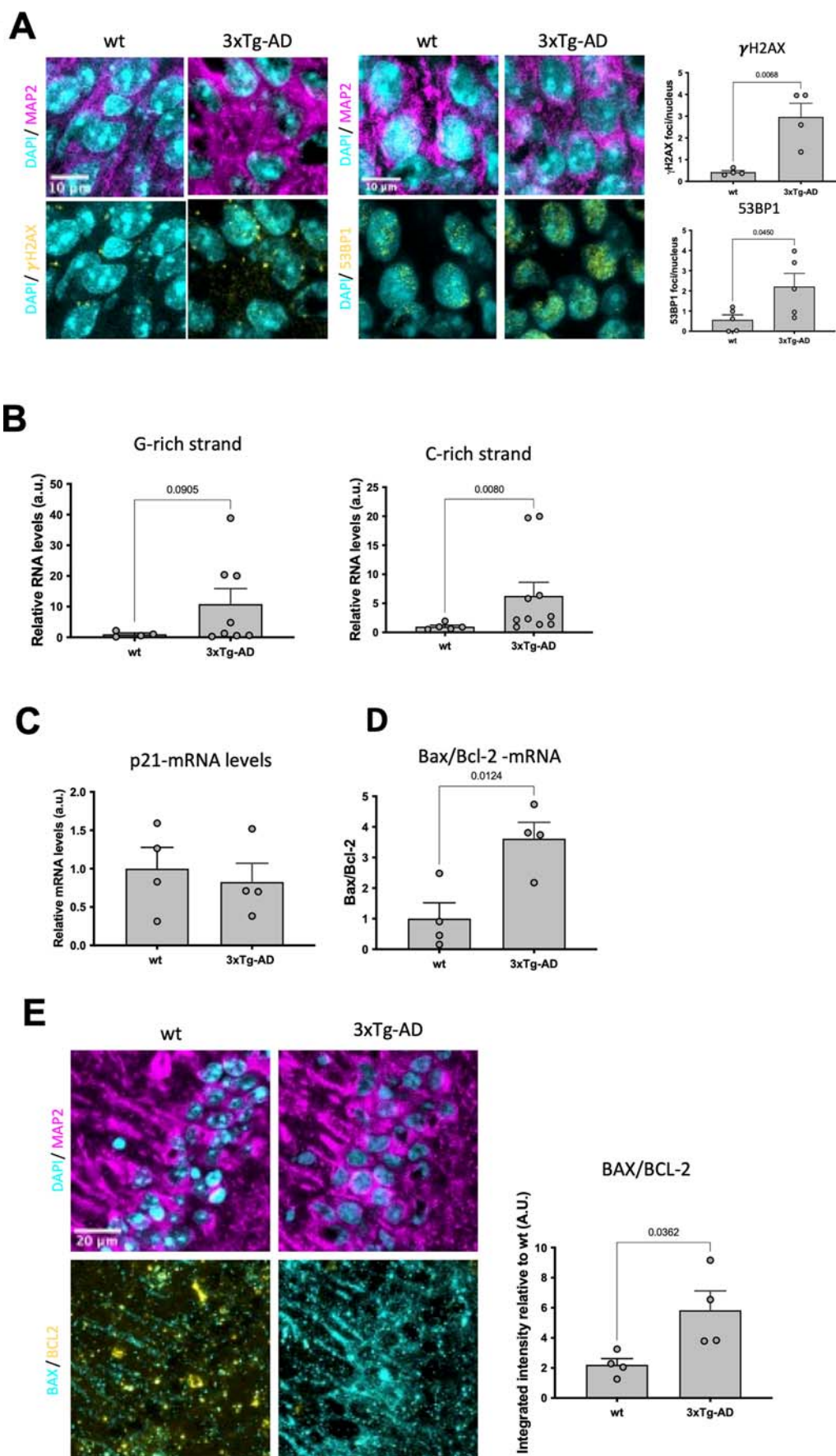


Expanded View Figures

Figure EV1. Markers of tDDR activation and pro-apoptotic gene expression are elevated in the hippocampus of 3xTg-AD mice.

(A–D) Hippocampal regions from 12-month-old wt and 3xTg-AD mice were analyzed. Each circle in the graphs represents one mouse analyzed. (A) Representative confocal images of immunofluorescence staining of hippocampal regions from wt and 3xTg-AD mice with antibodies against γ H2AX, 53BP1, MAP2, nuclei were stained with DAPI. Bar graph shows quantification of the average number of γ H2AX ($n = 4$ wt and $n = 4$ 3xTg-AD), and 53BP1 foci per nucleus ($n = 5$ wt and $n = 5$ 3xTg-AD). At least 50 nuclei per mouse section were scored. (B) Bar graphs show the quantification of G-rich ($n = 4$ wt and $n = 8$ 3xTg-AD) and C-rich ($n = 5$ wt and $n = 10$ 3xTg-AD) tdincRNA levels in 3xTg-AD mice relative to wt samples in brain hippocampus as measured by RT-qPCR. (C) Bar graph shows the quantification of p21 mRNA levels in 3xTg-AD mice relative to wt samples in the hippocampus as measured by RT-qPCR. ($n = 4$ wt and $n = 4$ 3xTg-AD). (D) Bar graph represents the quantification of the ratio between the mRNA levels of Bax over Bcl-2 in 3xTg-AD mice relative to wt samples in the hippocampus as measured by RT-qPCR ($n = 4$ wt and $n = 4$ 3xTg-AD). (E) Representative confocal microscopy images of Immunofluorescence stainings combining antibodies against MAP2, BAX and BCL-2 of sections from wt and 3xTg-AD mice (left panel). Bar graph represents the quantification of the ratio between the signal of BAX over BCL-2 from immunofluorescence in 3xTg-AD mice relative to wt samples in hippocampal regions ($n = 4$ wt and $n = 4$ 3xTg-AD). Unpaired *t* test was applied and data are represented as mean $\pm$ SEM.



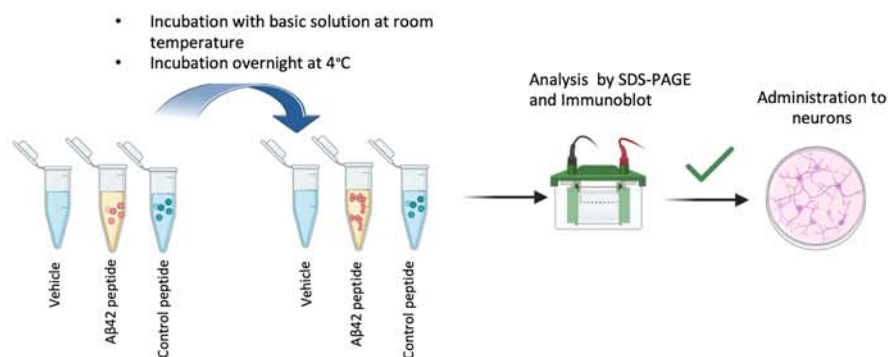
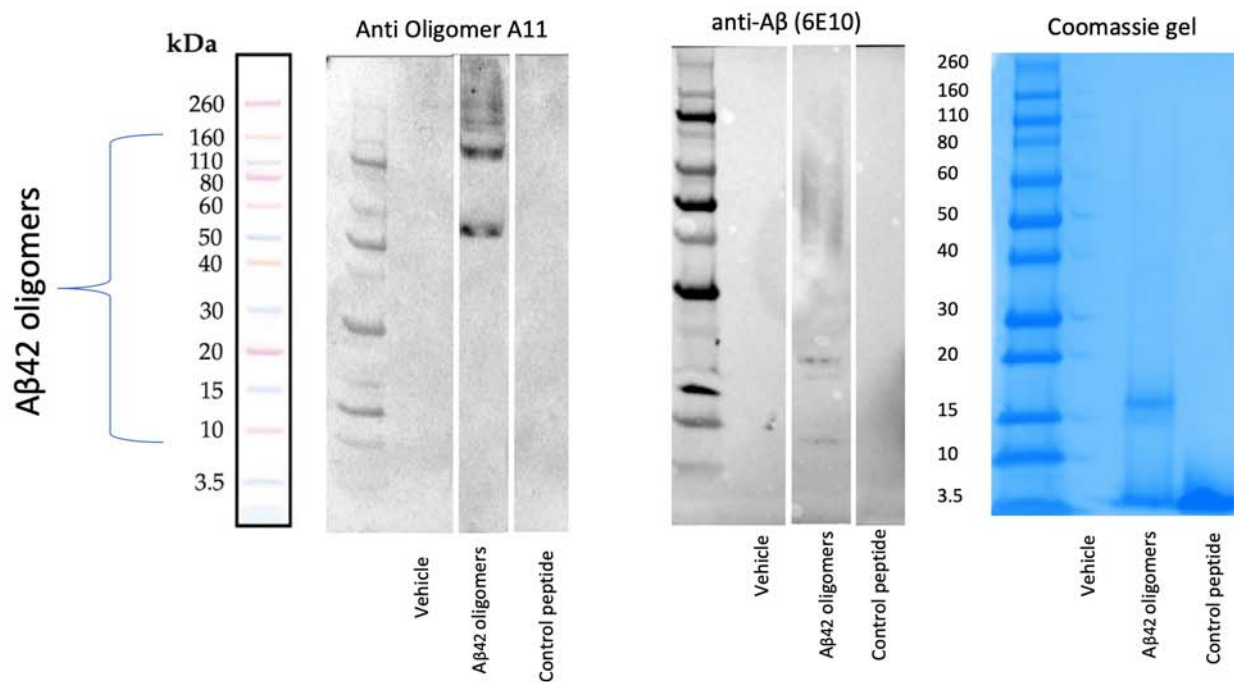
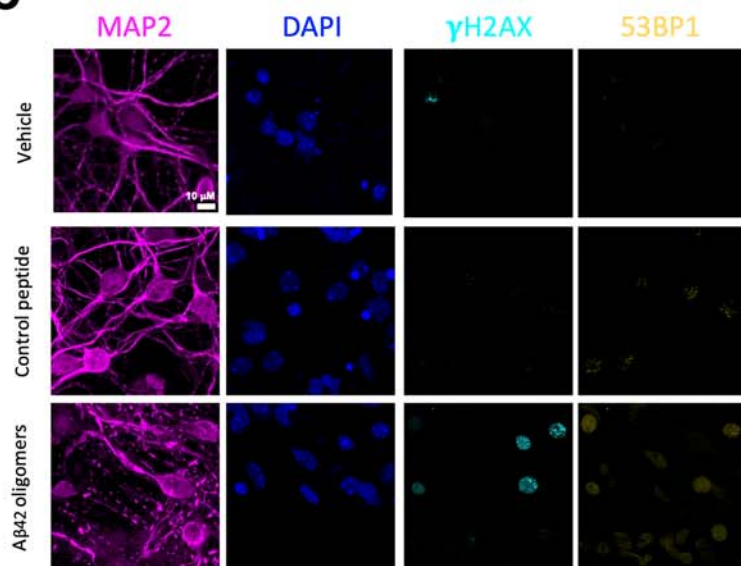
A**B****C**

Figure EV2. Experimental descriptions of A β 42 oligomers preparation and their specificity.

In all experiments in this manuscript, a fresh batch of A β 42 oligomers was prepared as described in the “Methods and protocols” section. (A) Scheme of A β 42 oligomer preparation and analysis of the oligomer’s solution. Each A β 42 oligomers batch was tested by immunoblotting for oligomerization status before use. (B) Representative images of western blots using the Oligomers A11 polyclonal antibody recognizing selectively the oligomeric forms only, or the 6E10 monoclonal antibody recognizing the C terminal fragment (CTF) of the peptide. Control peptide prepared in parallel but unable to undergo oligomerization, was used as negative control. Polyacrylamide gel stained with blue Coomassie-based solution was used to check the A β 42 oligomers and control peptide. (C) Representative images of immunofluorescence staining of mouse primary neurons treated with Vehicle, A β 42 oligomers and solutions containing control peptide and stained with antibodies against MAP2 (magenta) as neuronal marker, γ H2AX (cyan) and 53BP1 (yellow) as DDR markers, nuclei were stained with DAPI. Source data are available online for this figure.

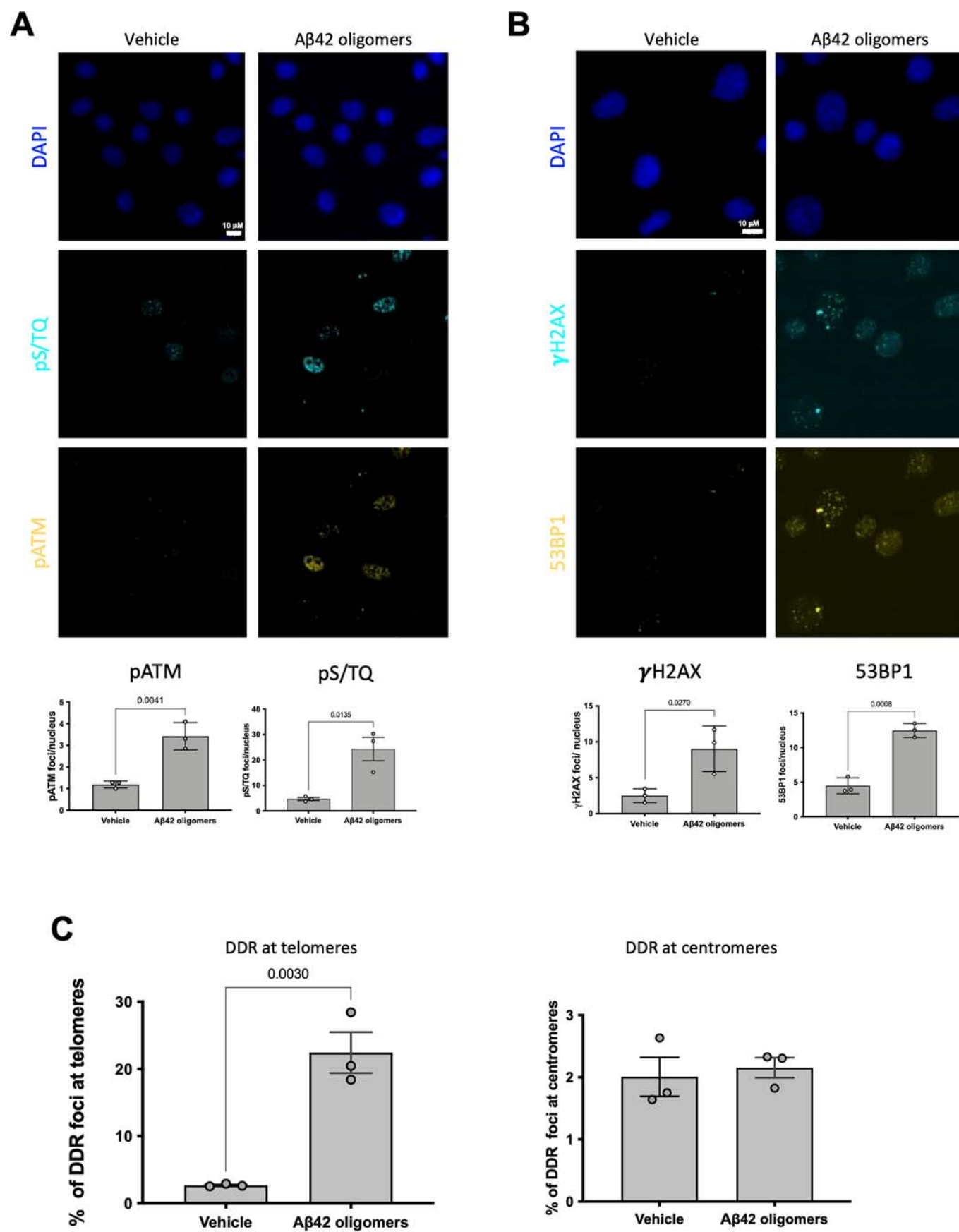


Figure EV3. A β 42 oligomers induce DDR and accumulation at telomeres in HT-22 cells.

(A–C) HT-22 cells were treated with 5 μ M A β 42 oligomers for 24 h or vehicle. Each circle represents an independent biological replicate. (A) Representative confocal image of immunofluorescence staining of HT-22 with antibodies against pS/TQ (cyan) and pATM (yellow) as DDR markers, nuclei were stained with DAPI. Bar graphs show the quantification of average number of DDR foci per nucleus. At least 50 cells for each condition in each replicate were analyzed. ($n = 3$ for each condition) (B) Representative confocal images of immunofluorescence staining of HT-22 with antibodies against γ H2AX (cyan) and 53BP1 (yellow) as DDR markers, nuclei were stained with DAPI. Bar graphs show the average number of DDR foci per nucleus. At least 50 cells for each condition in each replicate were analyzed. ($n = 3$ for each condition). (C) Bar graphs show the percentage of DDR foci that localize at telomeres or centromeres, as detected by immunoFISH combining γ H2AX antibody and FISH for telomeric DNA using a complementary PNA probe and by double immunofluorescence combining antibodies against γ H2AX and the centromeric protein CREST of primary neurons. At least 50 cells for each condition in each replicate were analyzed. ($n = 3$ for each condition). Unpaired t test was applied and data are represented as mean $\pm$ SEM.

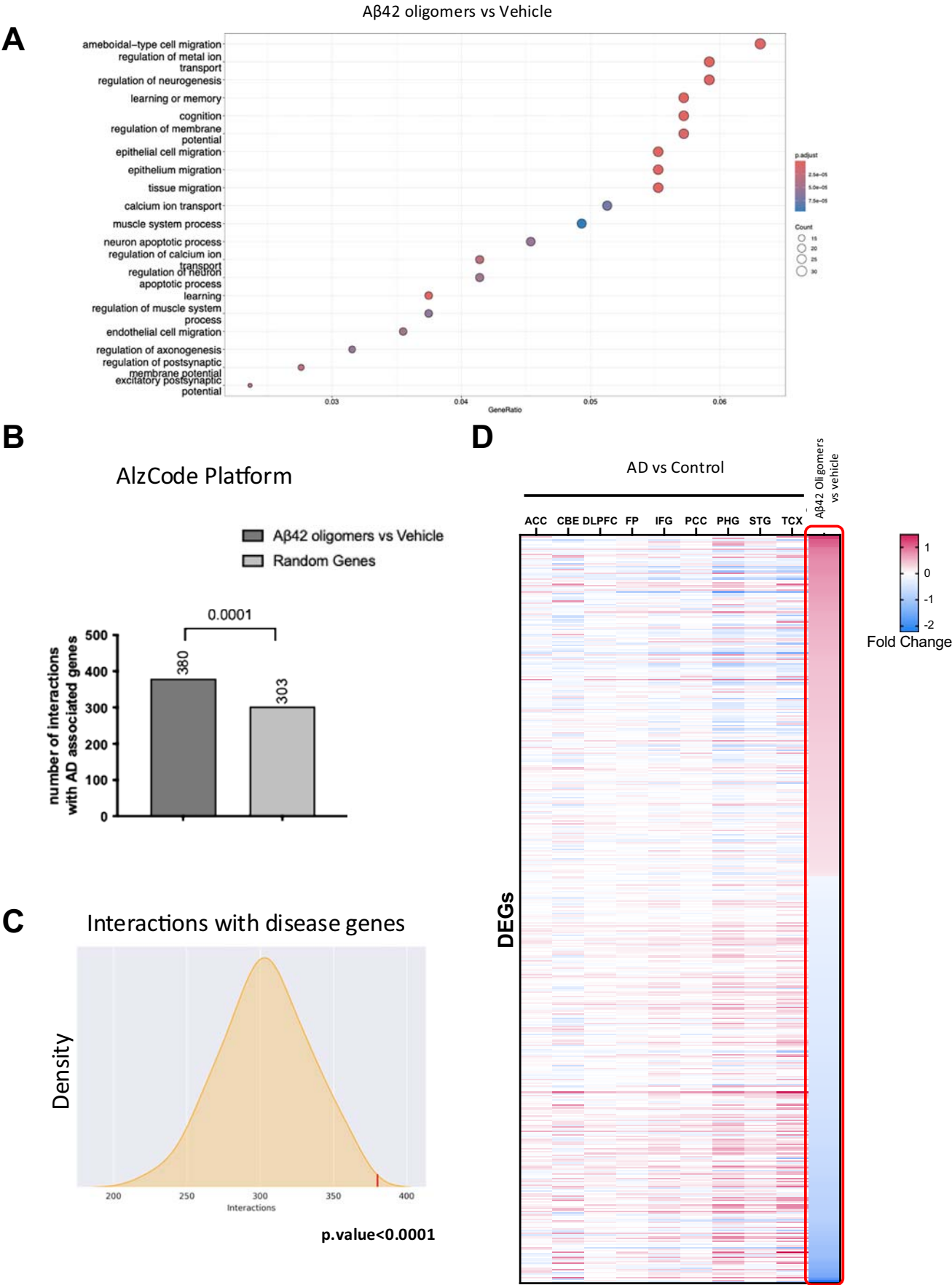



Figure EV4. A β 42 oligomers induce an RNA-signature present in AD-affected areas.

(A) The dotplot shows the significant Gene Ontology Biological processes that emerged from the enrichment analysis of DEGs in A β 42 oligomers versus Vehicle, performed with ClusterProfiler. The name of the pathway is reported on the left of the graph, the x-axis represents the gene ratio, dots size represents the number of DEGs present in the pathway and the color indicates the adjusted *p* value of the pathway enrichment. Statistical analysis was performed with over-representation analysis for Gene Ontology Biological processes on DEGs, performed with ClusterProfiler (Wu et al, 2021). (B) The histogram shows the extent of correlation between DEG in A β 42oligomers vs vehicle and known AD-associated genes when compared to random genes or known AD-associated genes. (C) Plot as generated by <http://alzcode.xyz> showing the number of the interactions of DEGs in A β 42oligomers vs vehicle and known AD-associated genes (red line) compared to the distribution of interactions between random genes and known AD-associated genes. The significance is calculated by permutation test (Lin et al, 2022). (D) Heatmap showing the 477 DEGs in A β 42 oligomers-treated vs vehicle-treated primary neurons and in human AD patients vs control patients in the indicated brain areas. ACC anterior cingulate cortex, CBE cerebellum, DLPFC dorsolateral prefrontal cortex, FP frontal pole, IFG inferior frontal gyrus, PCC posterior cingulate cortex, PHG parahippocampal gyrus, STG superior temporal gyrus, TCX temporal cortex.

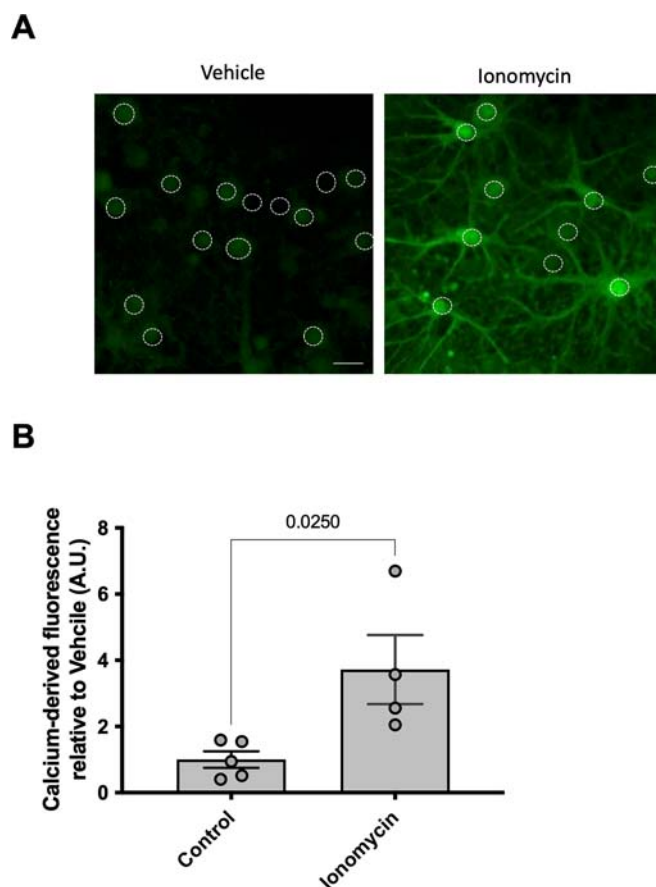


Figure EV5. Positive control for Ca^{2+} measurements in mouse primary neurons.

Treatment of mouse primary neurons with $2\ \mu\text{M}$ ionomycin for 2 h was used as positive control for Ca^{2+} -derived fluorescence signal. Each circle represents an independent biological replicate. (A) Representative confocal microscopy images of mouse primary neurons incubated with Fluo-4 probe, green fluorescence is generated upon Ca^{2+} binding to the Fluo-4 probe. White dashed-line circles indicate nuclei of neurons. White dashed-line circles indicate the nuclei of neurons. Bar correspond to $20\ \mu\text{m}$. (B) Bar graph shows semiquantitative measures of intracellular Ca^{2+} -derived fluorescence. (control, $n = 5$; ionomycin, $n = 4$) Unpaired t test was applied and data are represented as mean $\pm$ SEM.

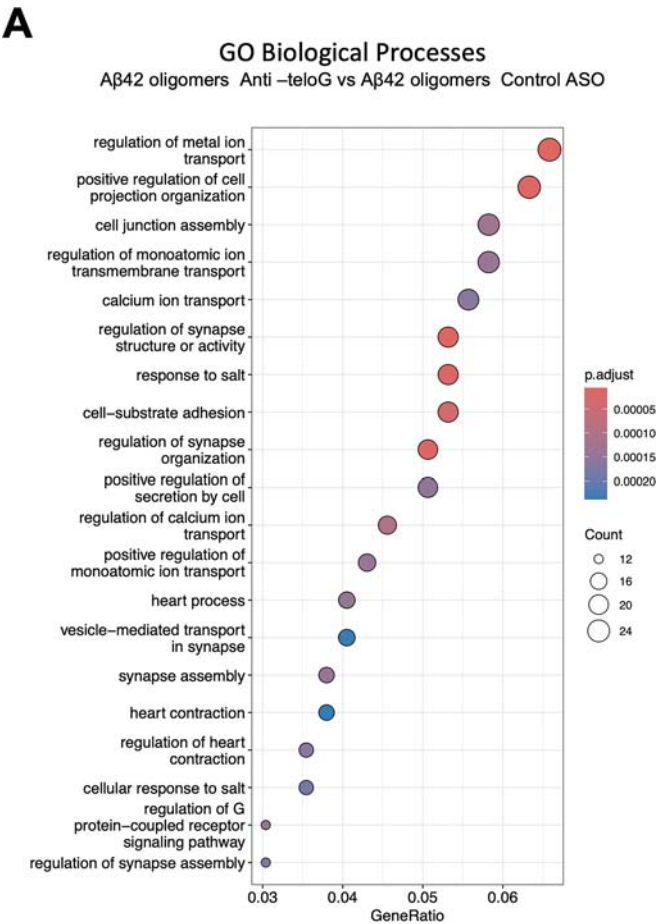


Figure EV6. tASO improves pathways dysregulated by Aβ42 oligomers.

(A) The dotplot shows the significant Gene Ontology Biological processes that emerged from the enrichment analysis of DEGs in Aβ42 oligomers Anti-Telo G-treated vs Aβ42 oligomers Control ASO-treated primary neuron, performed with ClusterProfiler. The name of the pathway is reported on the left of the graph, the x-axis represents the gene ratio, the dot size represents the number of differentially expressed genes present in the pathway and the color indicates the adjusted *p* value of the pathway enrichment. Statistical analysis was performed with over-representation analysis for Gene Ontology Biological processes on DEGs, performed with ClusterProfiler (Wu et al, 2021).