Supplementary Figures:

Supplementary Figure 1. Derivation of induced pluripotent stem cells (iPSCs) cells from human fibroblasts. (**a**) Experimental design**.** Human dermal fibroblasts were transduced three times (on day 0, day 1 and day 2) with three pSIN4-based lentiviral reprograming plasmids. Six days post transduction (DPT), the fibroblasts were collected and seeded with iMEFs. The fibroblast culture medium was replaced with human ESC medium supplemented with the stem molecules Y-27632, thiazovivin and PD0325901. (**b**) At 10 DPT, the fibroblasts started changing morphology. At 30 DPT, human ESC-like colonies were manually detached from the plate using a rounded tip glass Pasteur pipette, collected and partially disaggregated. (**c**) iPSCs derived from familiar Alzheimer's disease (fAD), Down syndrome (DS) and healthy control (HC) patients were positive for pluripotency markers alkaline phosphatase (AP), Oct4, Tra-1-60, Sox2 and SSEA4. Nuclei were stained with DAPI (blue). (**d**) The expression of pluripotency genes in the iPSC lines was evaluated by RT-PCR using primers specific for the following endogenous markers: *OCT3/4*, *SOX2*, *NANOG*, *c-MYC, KLF4, growth and differentiation factor 3* (*GDF3*), *reduced expression 1*(*REX1*), *fibroblast growth factor 4 (FGF4)* and *telomerase reverse transcriptase (TERT).* The level of expression was equivalent or higher compared with human ESC. (**e**) *In vitro* differentiation of iPSCs. iPSC lines formed compact EBs under feeder-free culture conditions (upper-left picture). The EBs differentiated during 3 weeks generated cells positive for markers of the three germ layers of the embryo including the ectoderm marker microtubule-associated protein 2 (MAP2), the mesoderm marker brachyury and the endoderm

marker α-fetoprotein (AFP). Nuclei were stained with DAPI (blue). Scale bars: B,C: 25μm; E: 50μm.

Supplementary Figure 2. Early development of COs. (**a**) 9000 cells were plated per well (upper insert, 0 DIV), which undergo compaction over time (lower insert, 7 DIV). Bright and homogeneous structures emerged over the surface (arrows) around 12 DIV. (**b**) A general view of COs at 30 DIV stained with hematoxylin, reveals peripheral areas with high cellular density and small cavities surrounded by proliferative neuroepithelial-like cells (∗), many of which are positive for phosphorylated histone H3 (H3, left inset) and display mitotic figures (right inset, arrows). These cells express neuroepithelial markers including (**c**) nestin and (**d**) SOX2. (**e**) The neuroepithelial cells region (∗) are surrounded by doublecortin (DCX) positive young neurons. Scale bars: 100 μm.

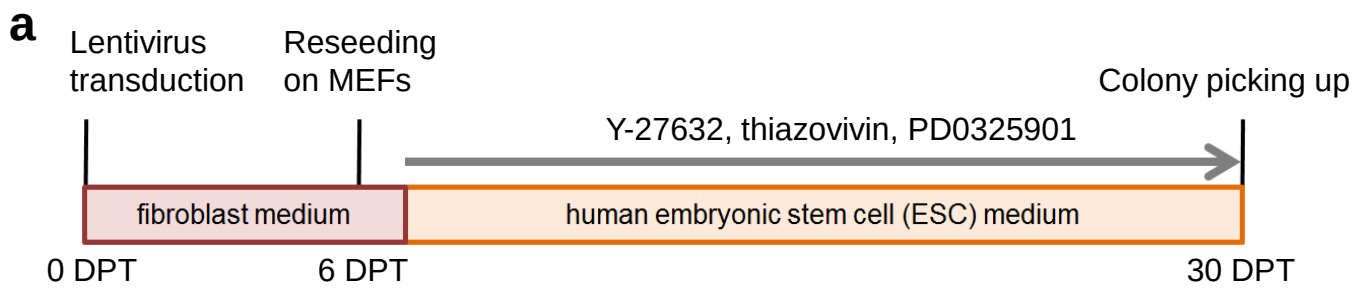
Supplementary Figure 3. Amyloid plaques and phosphorylated tau pathology in DS COs. COs prepared from DS iPSCs were cultivated for 110 days and processed for histological analysis as described in Methods. (**a**) Representative image of COs co-stained with DAPI (blue), 6E10 (green) and MAP-2 (red) antibodies. (**b**) Representative image of COs co-stained with DAPI (blue), AT8 (red) and MAP-2 (green) antibodies. After staining, slides were visualized by confocal microscopy. Scale bar: 10 μm.

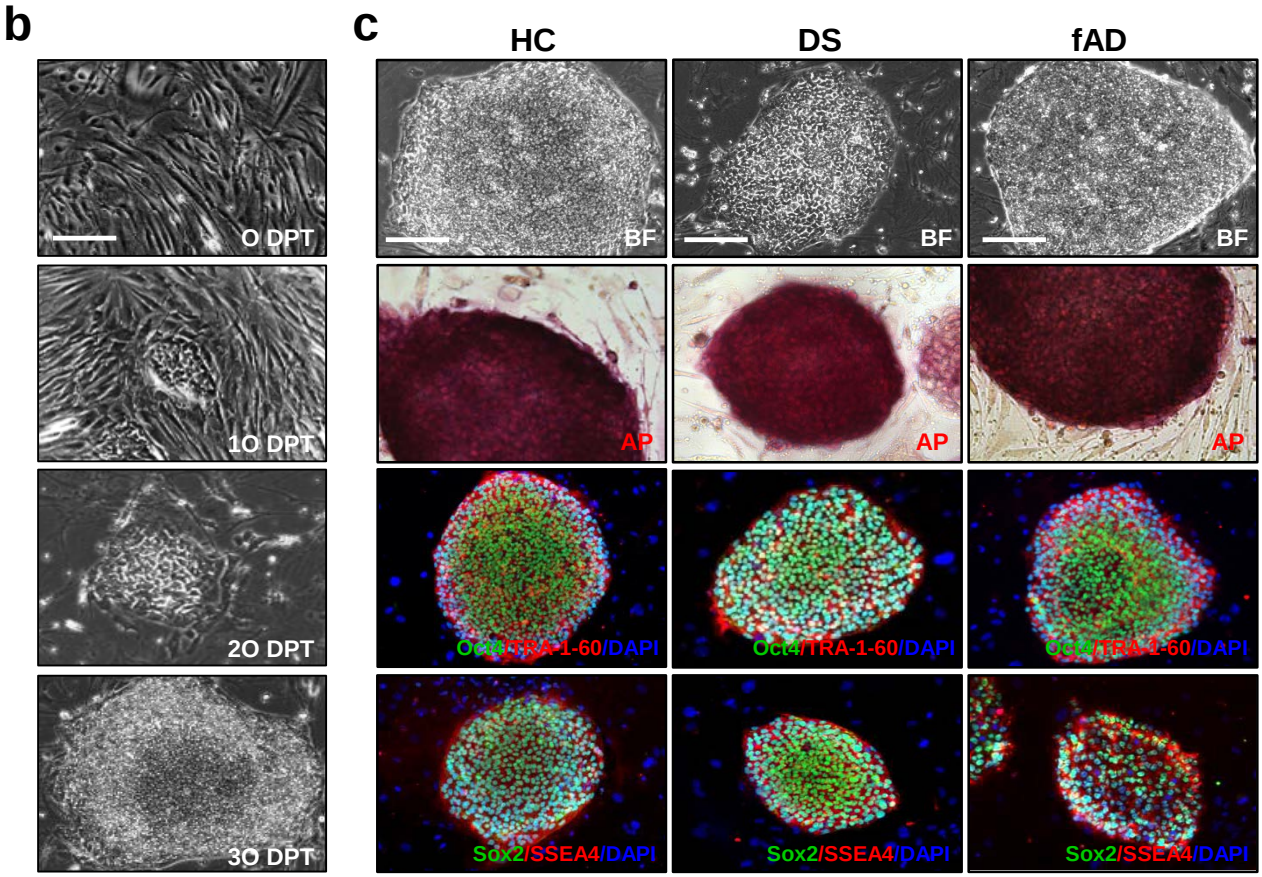
Supplementary Figure 4. Analysis of amyloid-beta and phosphorylated tau pathology on COs generated from mouse iPSCs and ESCs. COs prepared from mouse ESCs (**a, b, g,** and **h**) or mouse iPSCs (**c, d, i,** and **j**) were cultivated for 110 days and processed for histological analysis as described in Methods. (**e, f, k,** and **l**) As positive control, we analyzed COs derived from DS iPSCs. Amyloid deposits were

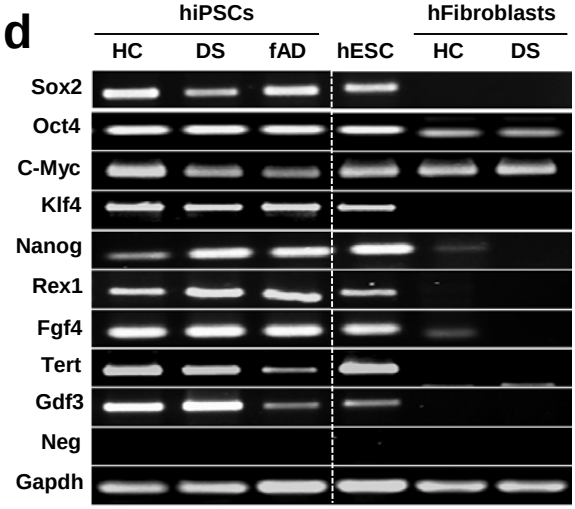
stained with the 4G8 (**a, c,** and **e**) or 6E10 antibodies, while phosphorylated tau was stained with the AT8 (**g, i,** and **k**) or PHF-1 (**h, j,** and **l**) antibodies. Scale bars: 25 μm.

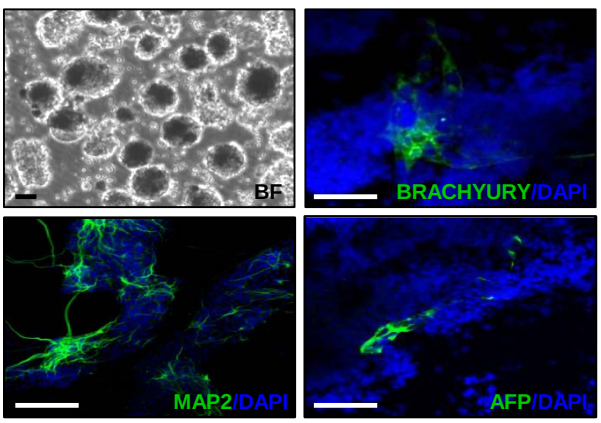
Supplementary Figure 5. **Correlation between tau phosphorylated at different phospho-epitopes.** The levels of p-tau at Thr181 or Ser396 correlated directly with phosphorylation at Ser231, suggesting that tau protein is hyper-phosphorylated in those COs. For this graph, the level of p-tau in COs samples coming from fAD was used.

Supplementary Figure 1

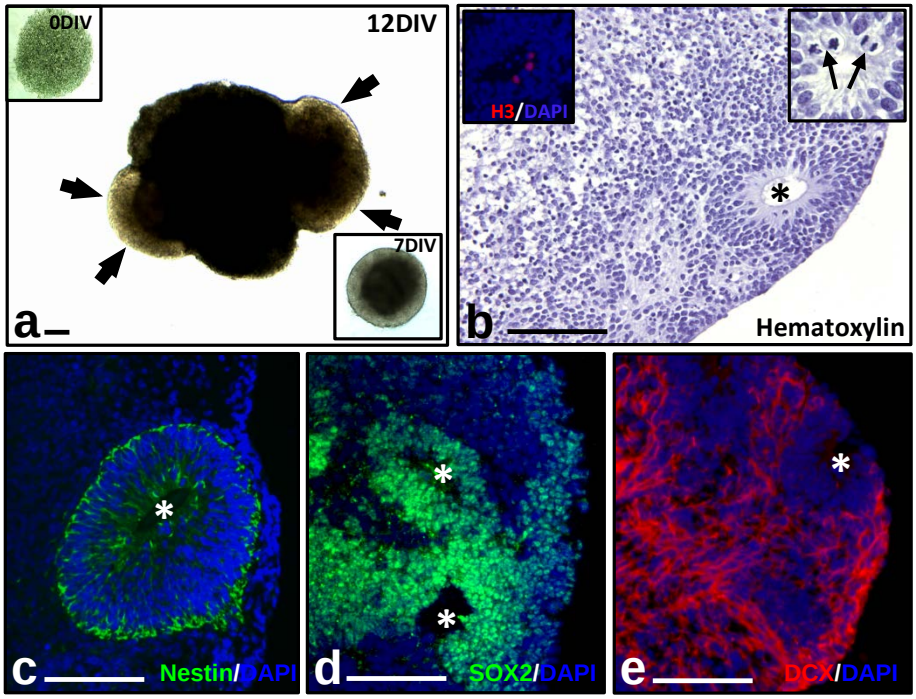




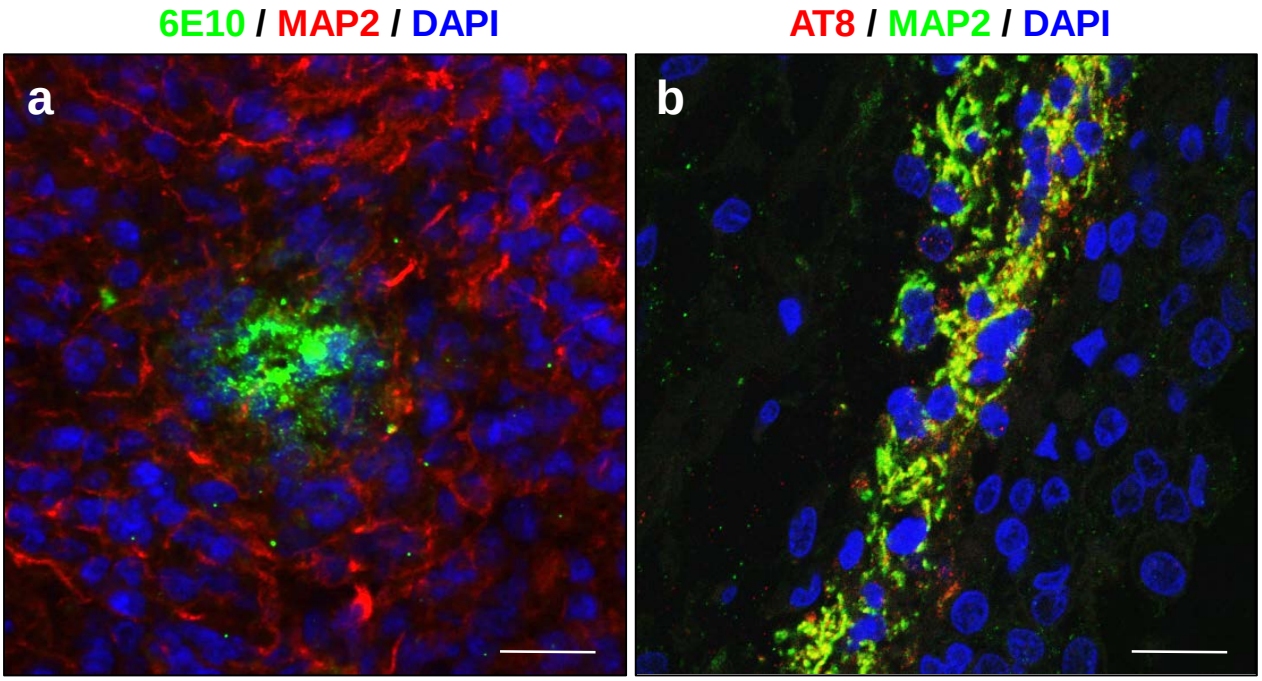




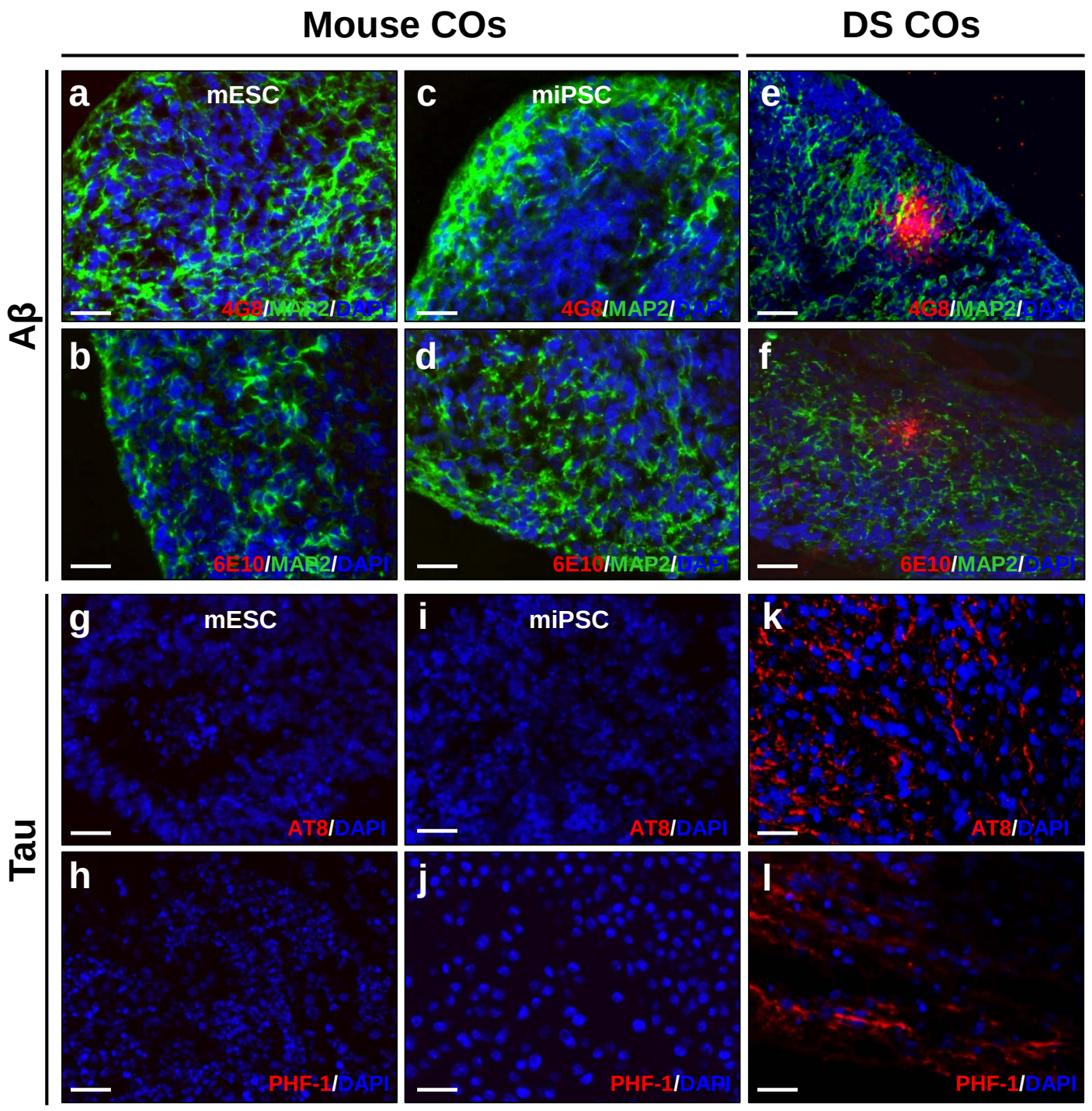
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5