

## Supplementary Material

# The Pre-Eclampsia Gene *STOX1* Controls a Conserved Pathway in Placenta and Brain Upregulated in Late-Onset Alzheimer's Disease

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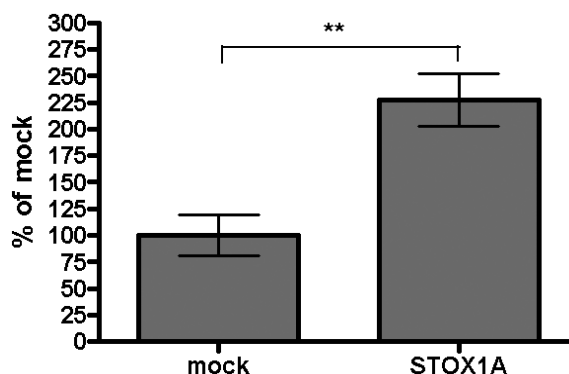
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### SUPPLEMENTARY METHODS

#### *Quantitative analysis of *STOX1* transcripts*

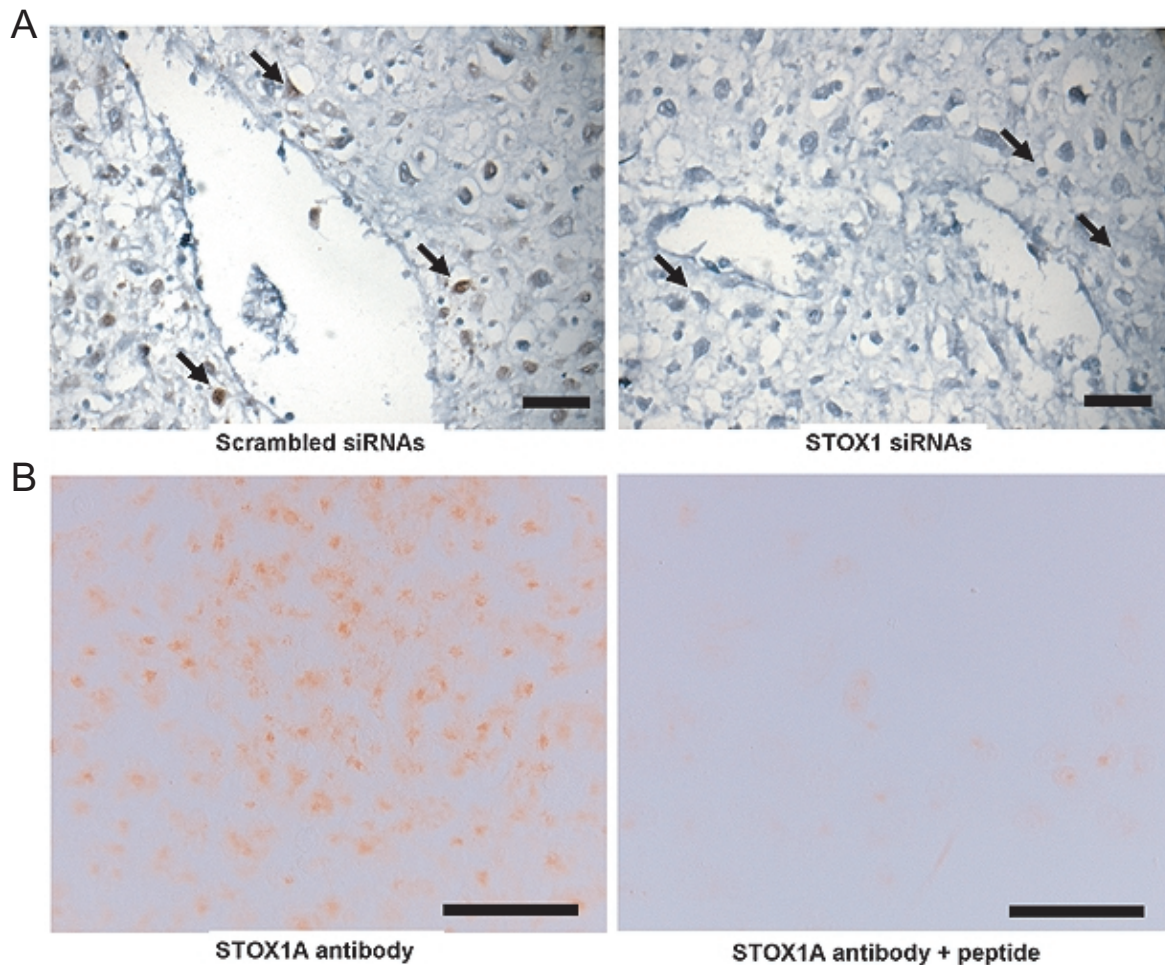
The adult tissue RNA array was obtained from Ambion (FirstChoice Human Total RNA Survey Panel). RNA from human extravillous trophoblast (SGHPL5 cell line) was obtained as described [1,2]. For isolation of RNA from the hippocampal region of non-demented controls ( $n = 2$ , aged 77 and 81) and brains with advanced AD ( $n = 2$ , aged 72 and 85), between 50–100 cryostat sections (20  $\mu\text{m}$  each) were cut from each patient sample (obtained through the Netherlands Brainbank) and immediately mixed with RNABee followed by RNeasy isolation including DNase treatment (Qiagen) according to the manufacturer's instructions. *STOX1* transcript analysis was performed by quantita-

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Supplementary Figure 1. Transactivation of *LRRTM3* by *STOX1*. Quantitative RT-PCR measuring endogenous *LRRTM3* transcription in SGHPL5 cells after challenging with either mock (GFP-only construct) or recombinant GFP-*STOX1* isoform A. Bars are mean  $\pm$  SEM. Connecting bracket with \*\* indicate  $P < 0.01$  compared to mock (t-test).

tive RT-PCR on the ABI 7300 using TaqMan probes and the Superscript II One Step RT-PCR system (In-VitroGen) with the inclusion of 1 M betaine and ROX reference dye. In brief, reactions (50  $\mu\text{l}$ ) containing

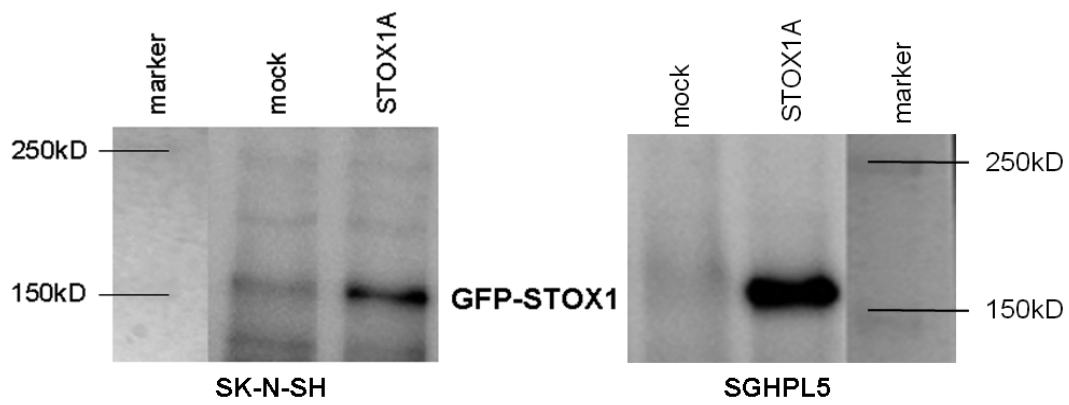


Supplementary Figure 2. STOX1A antibody specificity. The specificity of the STOX1A antibody was tested by downregulating STOX1 using siRNAs in placenta decidua co-cultures ( $n = 4$ ). A clear downregulation of STOX1 positive cells (indicated by arrows) can be detected around transformed spiral arteries (A). A second confirmation of specificity was provided by peptide competition; cells were stained with STOX1 antibody in absence or presence of STOX1 peptide (B). Bars are 40  $\mu\text{m}$ .

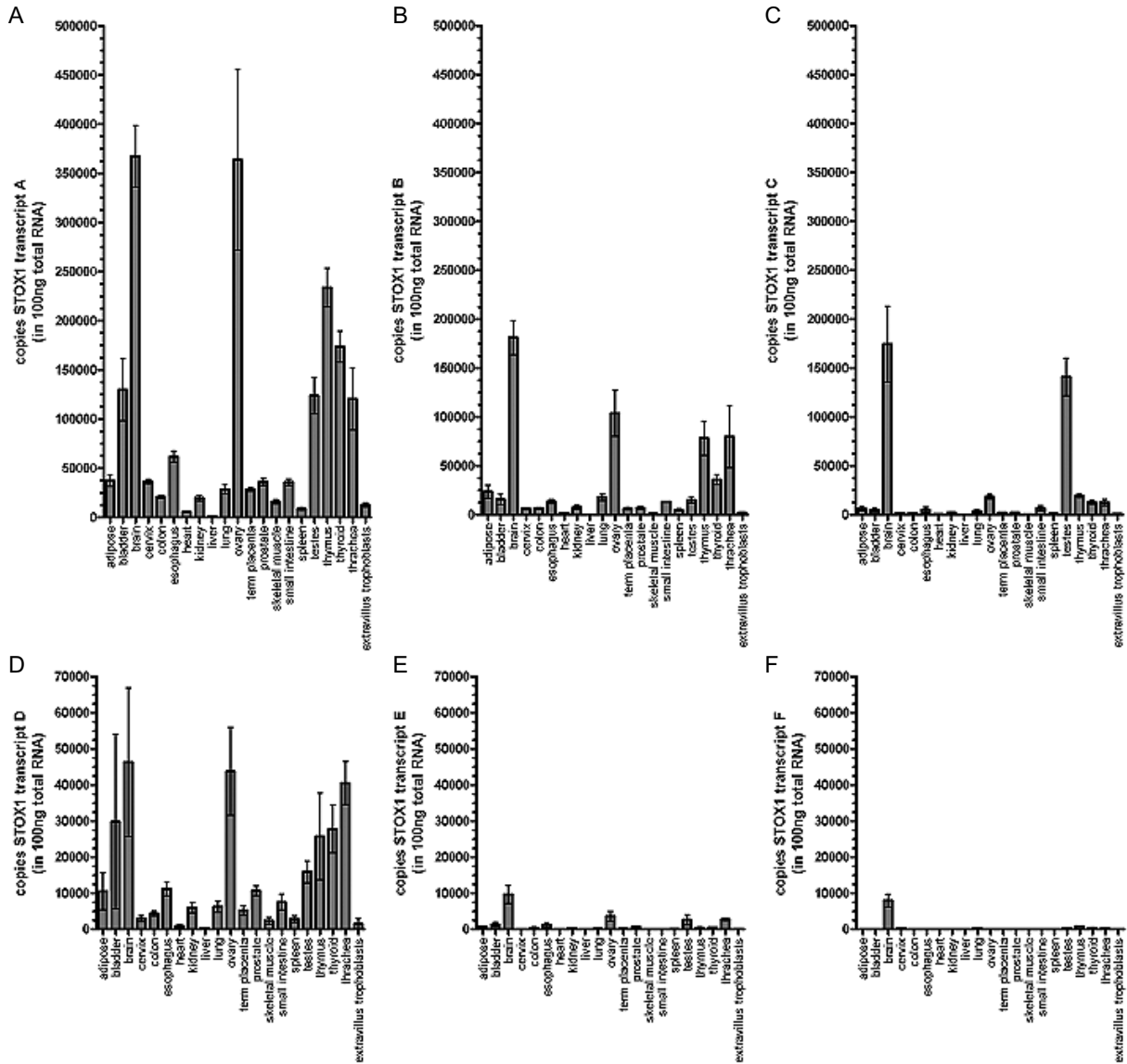
100 ng total RNA were subjected to reverse transcription for 30 min at 50°C, followed by inactivation of the RT enzyme at 95°C for 2 min and PCR consisting of 50 cycles with 1 min at 95°C and 2 min at 60°C. Forward primers for transcripts A and D, B and E, and C and F were localized in exon 3B, junction of exons 3A and 4, and junction of exons 2 and 4, respectively. Reverse primers for A, B and C and for D, E and F were localized in exon 4 and 5, respectively. The TaqMan probe was complementary to a sequence of exon 4 shared by all transcripts and PCR products. Primer and probe sequences are available upon request. Each sample was measured in triplicate.

## REFERENCES

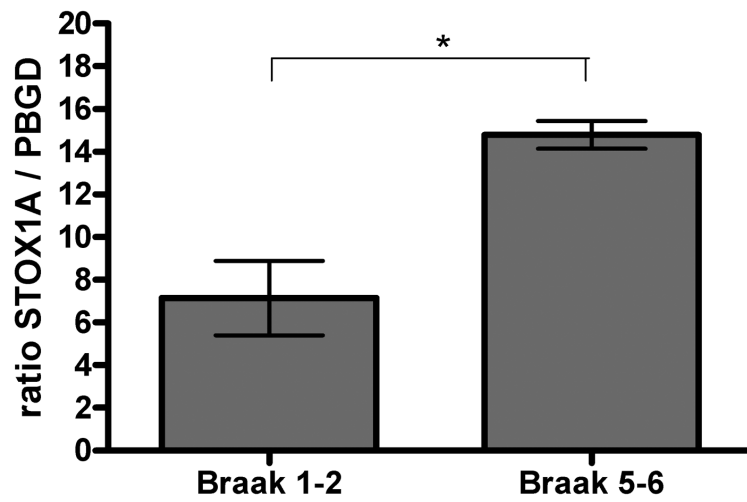
- [1] Oudejans CB, Mulders J, Lachmeijer AM, van Dijk M, Konst AA, Westerman BA, van Wijk IJ, Leegwater PA, Kato HD, Matsuda T, Wake N, Dekker GA, Pals G, ten Kate LP, Blankenstein MA (2004) The parent-of-origin effect of 10q22 in pre-eclamptic females coincides with two regions clustered for genes with down-regulated expression in androgenetic placentas. *Mol Hum Reprod* **10**, 589-598.
- [2] van Dijk M, Mulders J, Poutsma A, Konst AA, Lachmeijer AM, Dekker GA, Blankenstein MA, Oudejans CB (2005) Maternal segregation of the Dutch preeclampsia locus at 10q22 with a new member of the winged helix gene family. *Nat Genet* **37**, 514-519.



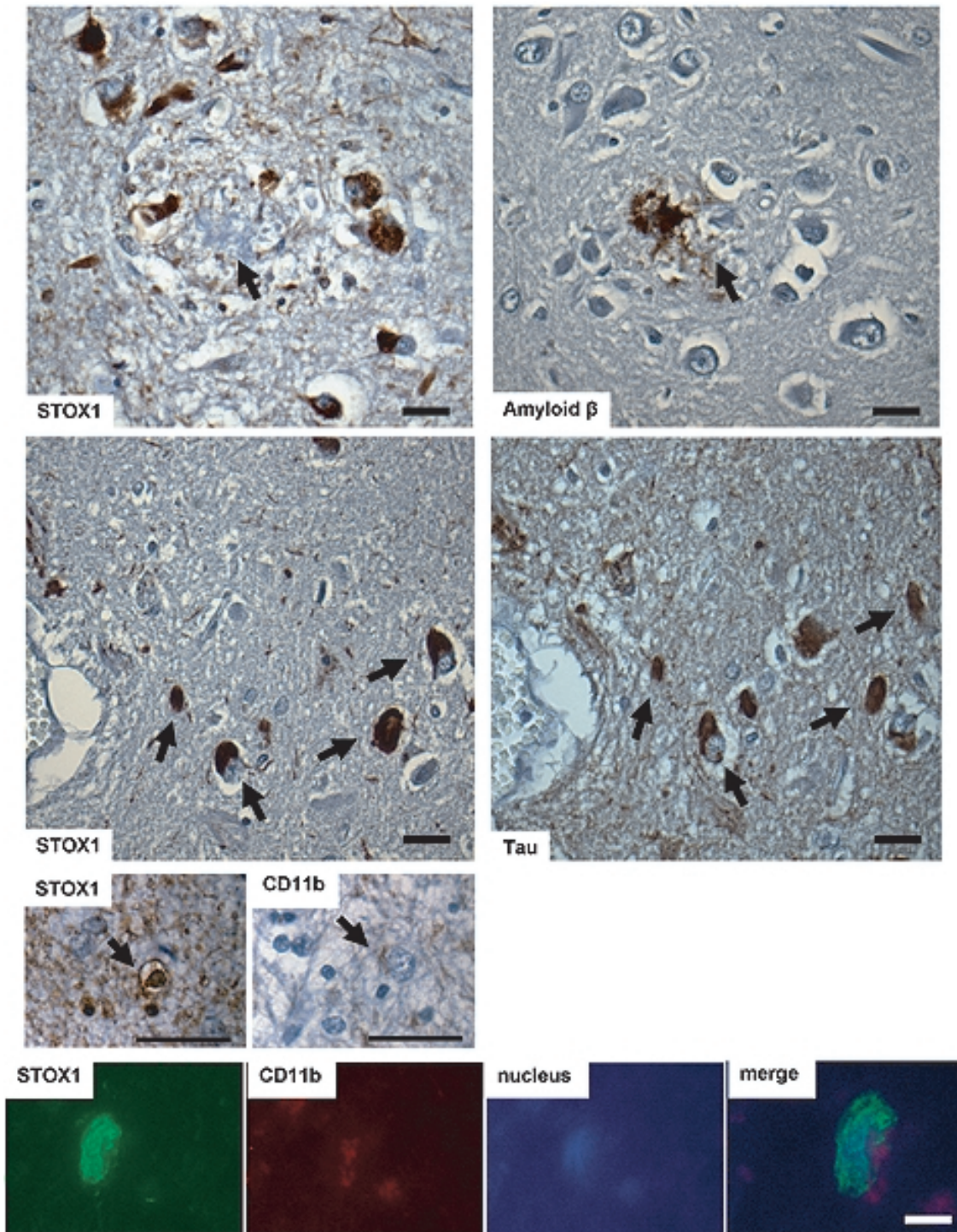
Supplementary Figure 3. STOX1A expression in transfected cells. Western blot analysis of GFP on lysates of SK-N-SH cells stable transfected with A $\beta$ PP and SGHPL5 cells to ensure protein expression of STOX1 after transfection with mock (GFP-only) or STOX1A recombinant protein.



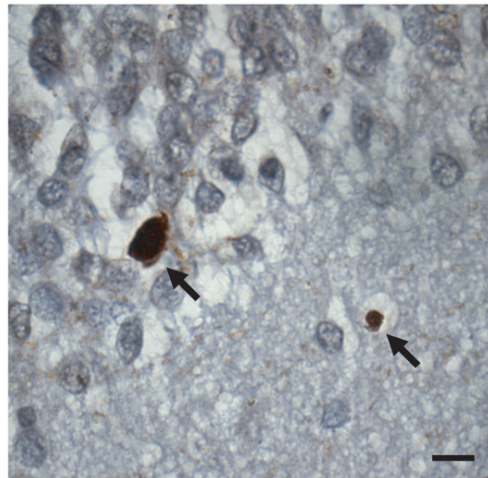
Supplementary Figure 4. High expression of *STOX1* transcripts in brain and reproductive organs. Quantitative RT-PCR performed for six *STOX1* transcripts (A-F) using total RNA from 20 different adult tissues as well as from extravillous trophoblasts. Copy numbers are given in mean  $\pm$  SEM. High expression of transcript A ( $\sim 400,000$  copies/100 ng total RNA) is seen in brain and ovary (A). The expression patterns of transcripts B and C are reciprocal in ovary and testis (B, C). Transcripts D, E, and F, that differ in their 3'-untranslated region from transcripts A, B, and C, but encode the same 3 isoforms, are expressed at background levels (D, E, F).



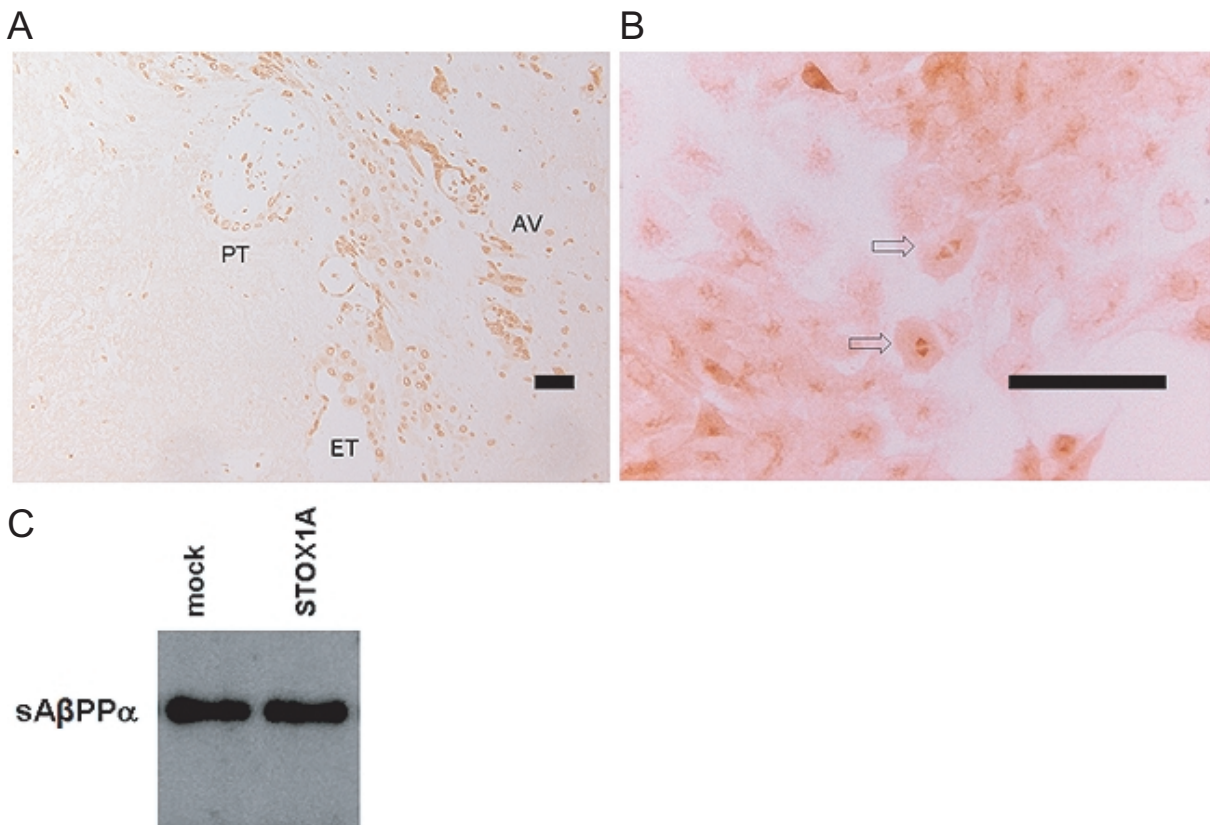
Supplementary Figure 5. Increased *STOX1* mRNA expression in advanced stages of LOAD. The *STOX1A/PBGD* ratio of RNA expression was increased when comparing hippocampal expression in non-demented controls (Braak 1-2) with LOAD patients (Braak 5-6). All bars are mean  $\pm$  SEM. Connecting brackets with \* indicate  $P < 0.05$  (t-test).



Supplementary Figure 6. STOX1 colocalizes in tau tangle positive cells and microglia. STOX1A is expressed around A $\beta$  plaques and within the cytoplasm of tau tangle positive cells. In an area with high CD11b expression identifying the microglial cells, STOX1 can be found in the nuclei. A fluorescent double labeling shows STOX1 in the nucleus with CD11b staining the cytoplasm of the cell. Bars are 150  $\mu\text{m}$ , except the fluorescent labeling in which the bar indicates 5  $\mu\text{m}$ .



Supplementary Figure 7. STOX1 is expressed in the nucleus of granular neurons. STOX1A expression can be found in the nuclei of granular neurons of the dentate gyrus. Bar is 5  $\mu\text{m}$ .



Supplementary Figure 8. STOX1A expression in placenta does not lead to increased A $\beta$ PP processing. STOX1A expression in the nuclei of invasive trophoblasts (the cells primarily affected in pre-eclampsia) of the placenta bed, including trophoblast cells from the anchoring villi (AV), endovascular trophoblasts within (ET) and perivascular trophoblast cells (PT) surrounding the spiral arteries (A). In dividing trophoblast cells endogenous STOX1A is colocalized with the microtubuli network of the mitotic spindle (B). Western blot analysis of sA $\beta$ PP $\alpha$  on supernatant of SGHPL5 trophoblast cells showing no decrease of sA $\beta$ PP $\alpha$  after transfection with GFP-STOX1A constructs compared to mock (GFP only) (C). Bars are 40  $\mu\text{m}$ .