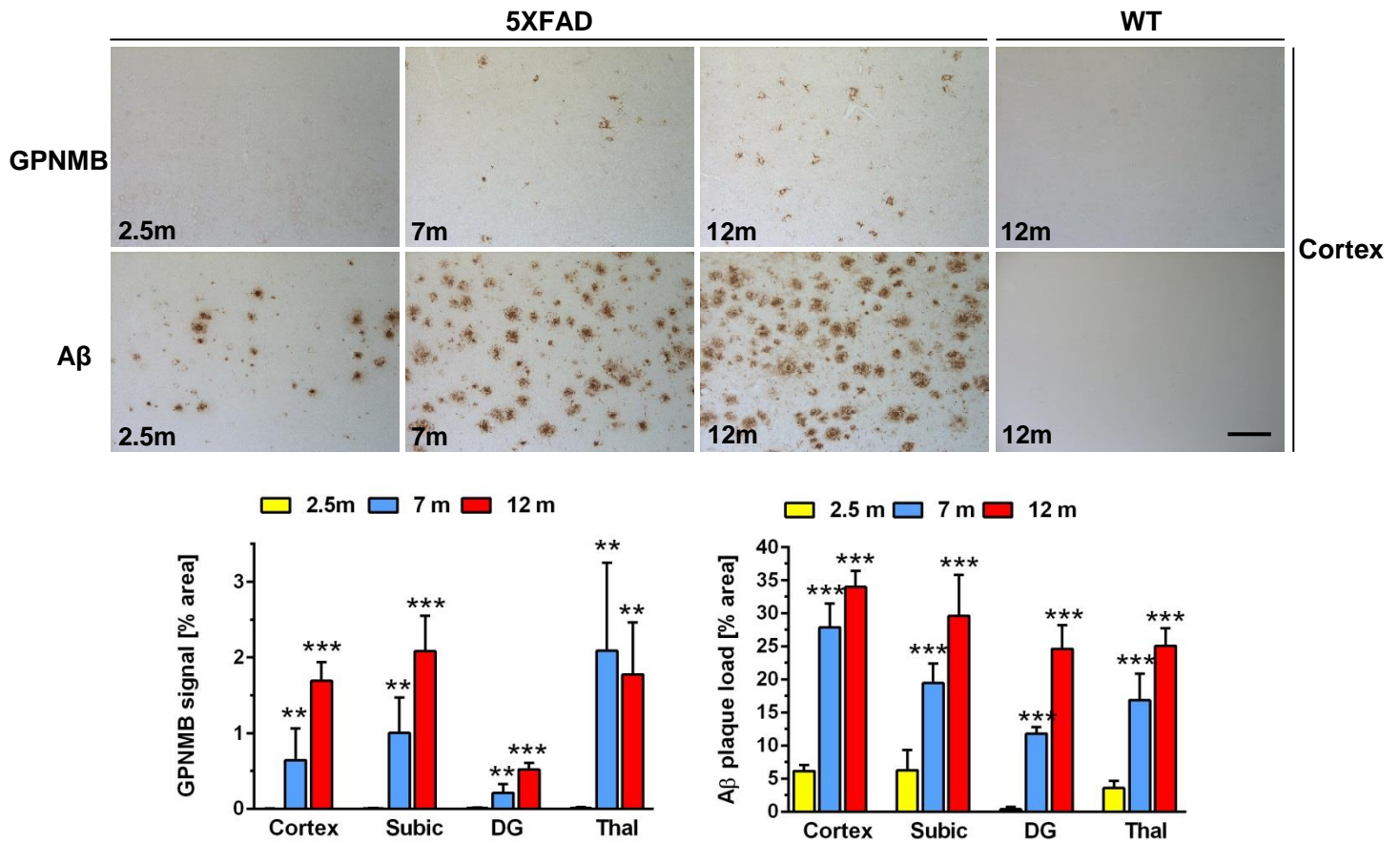


### Additional File 3:

#### Quantification of A $\beta$ plaque load and GPNMB immunoreactivity

A $\beta$  plaque load and GPNMB immunoreactivity were quantified using 2.5-, 7- and 12-month-old 5XFAD mice ( $n = 5$  per time point). In brief, three paraffin sections per animal, which were at least 30  $\mu\text{m}$  distant from each other, were stained simultaneously with DAB. The relative A $\beta$  plaque load and GPNMB signals in cortex, subiculum, thalamus and dentate gyrus were evaluated using a BX51 microscope (Olympus, Center Valley, PA, USA) equipped with a Moticam Pro 282 camera (Motic, Wetzlar, Germany) and the ImageJ software package (V1.41, NIH, USA). For each section, representative images from respective brain areas were captured, binarized to 8-bit black-and-white images using a fixed intensity threshold and the percentage covered by DAB was measured.



**GPNMB expression increases in parallel with amyloid deposition in 5XFAD mice.** (A) Representative pictures of cortical GPNMB- and A $\beta$  immunoreactivity in 2.5-, 7- and 12-month-old 5XFAD mice and 12-month-old WT mice as a negative control. Quantification of GPNMB immunoreactivity (A) and extracellular A $\beta$  plaque load (B) in 2.5-, 7- and 12-month-old 5XFAD mice in cortex, subiculum (Subic), dentate gyrus (DG) and thalamus (Thal). All data were given as mean  $\pm$  standard deviation (SD). \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ . Scale bar = 50  $\mu\text{m}$ .