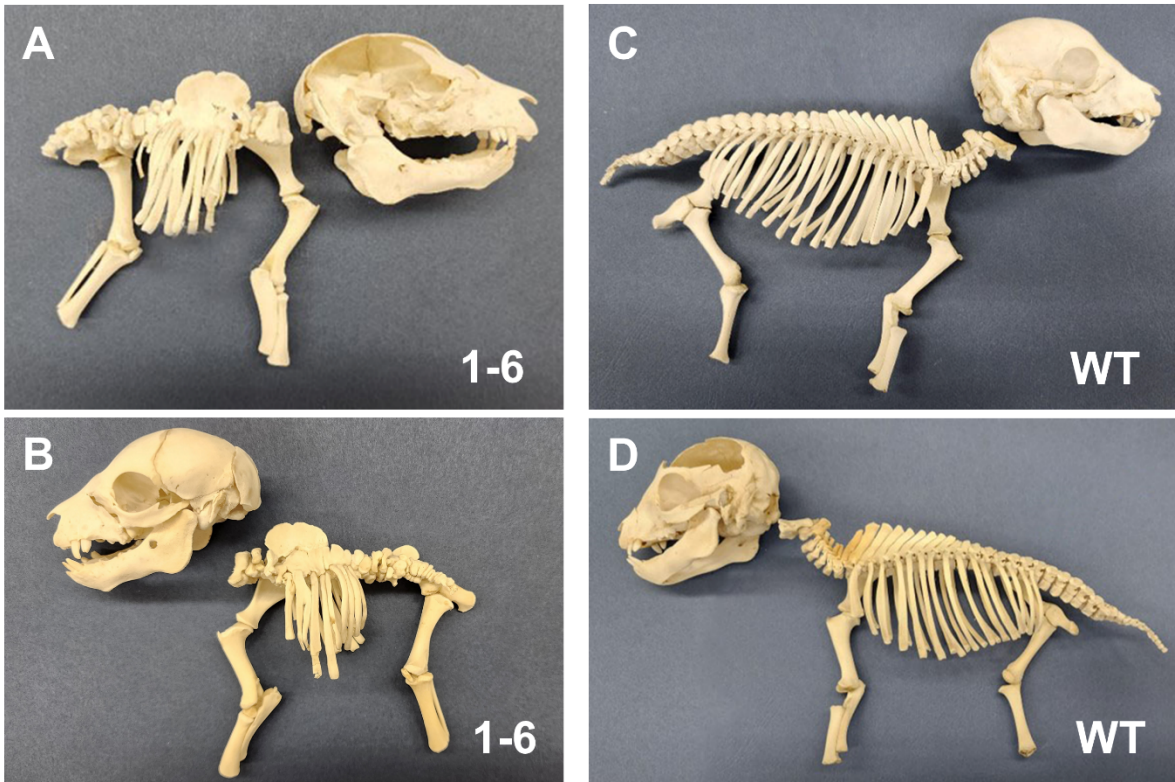
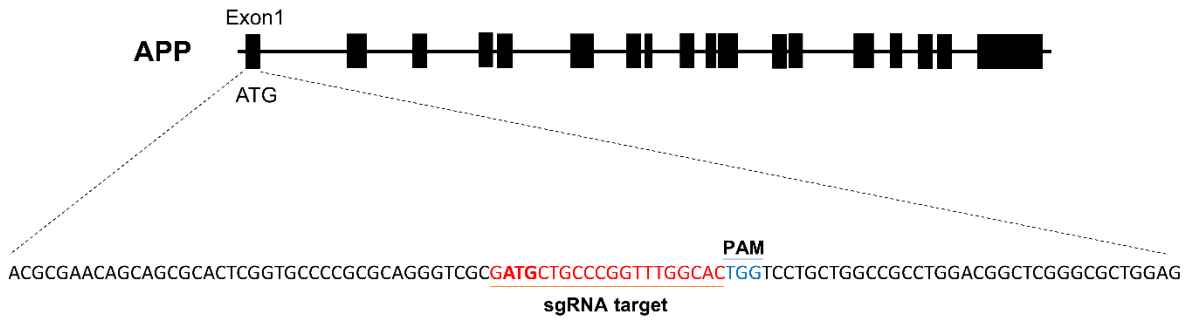


Supplementary Material

Impaired Skeletal Development by Disruption of Presenilin-1 in Pigs and Generation of Novel Pig Models for Alzheimer's Disease



Supplementary Figure 1. Skeletal defects in *PSEN1* knockout piglets. A) Skeleton of *PSEN1*-targeted stillborn piglet 1-6 after clearing tissues (right side). Defects were found in all vertebrae and nine partially fused ribs were present on the right side. Thoracic vertebrae were fused while lumbar, sacral, and caudal vertebrae were absent or crushed together. The skull had no abnormalities; damaged portions were from brain sampling. B) Skeleton of *PSEN1* 1-6 stillborn. The left side rib cage contained ten ribs with fused bones present. Vertebral defects were comparable to the right side. C) Normal skeletal development in a wildtype piglet at birth (right side). D) Normal skeletal development in a wildtype piglet on left side. No abnormalities were detected in either animal in the leg or foot structures.

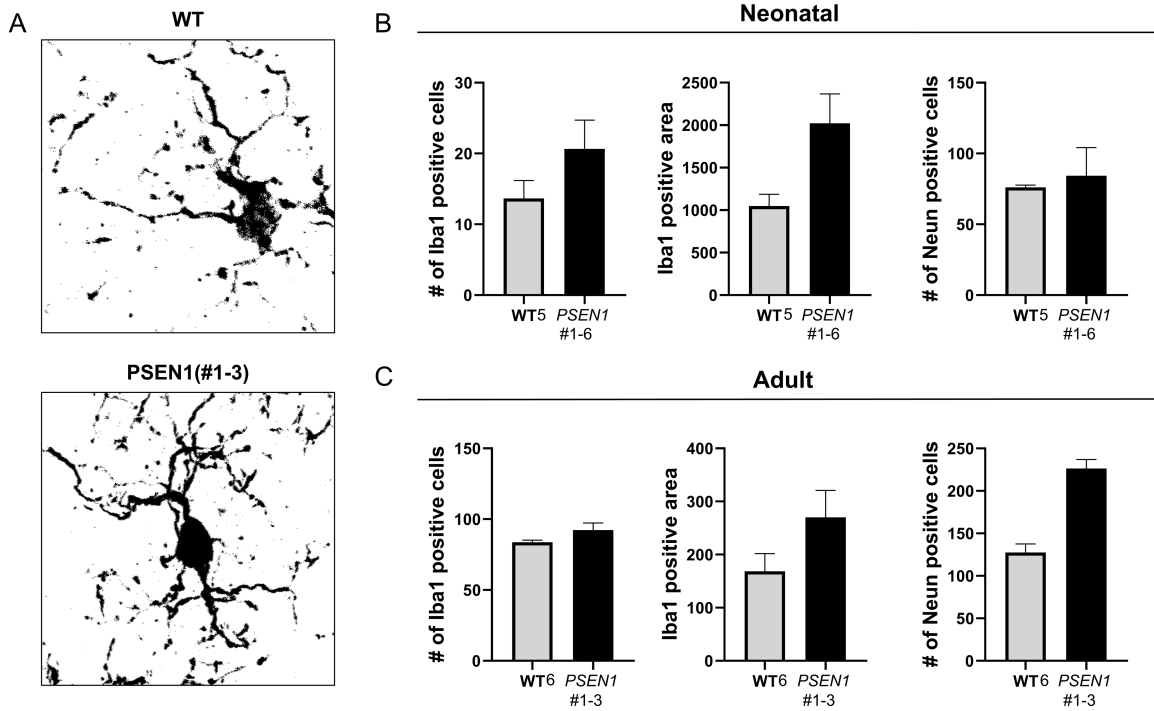


Fetuses	Mutations in gDNA
1	Mosaic w/o wild
2	Biallelic
3	Mosaic w/o wild
4	N/D
5	Biallelic
6	Biallelic
7	Mosaic w/o wild
8	Biallelic

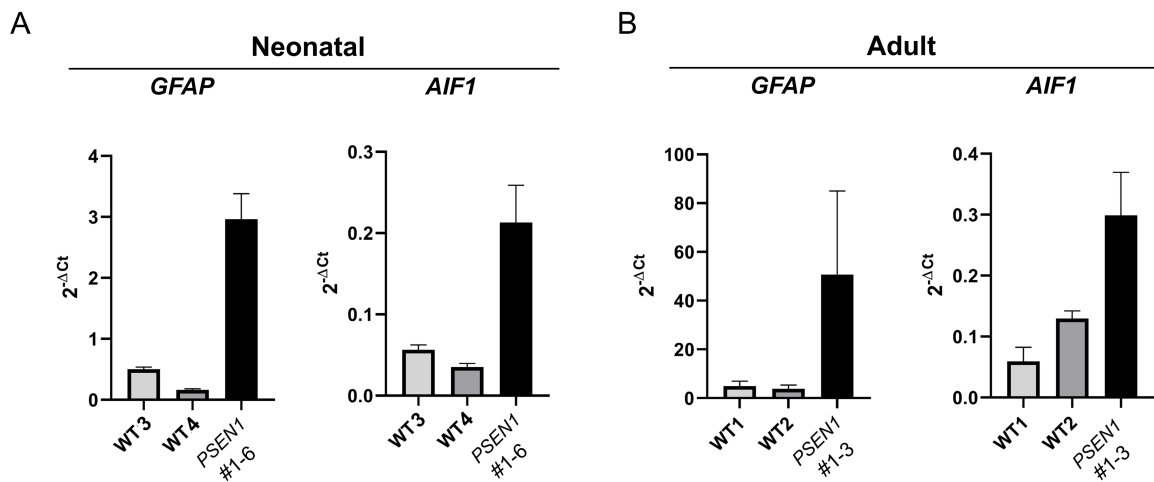
Supplementary Figure 2. *APP* knockout strategies using the CRISPR/Cas9 system and genotyping results. Mutations in the *APP* gene were detected in all *APP/PSEN1* double-targeted fetuses, except for fetus 4. N/D indicates that the genotype could not be determined due to incomplete PCR amplification – no PCR amplification may indicate a large deletion of *APP*.



Supplementary Figure 3. Image of an *APP* knockout pig at 4 weeks old.



Supplementary Figure 4. A) Representative images used to assess microglial activity in the brain. Quantitative comparison of immunohistochemical staining of Iba1-positive cell, Iba1-positive area within microglia, and NeuN-positive neuron cells between wild-type and PSEN1 pigs in (B) neonates and (C) adults.



Supplementary Figure 5. Transcript levels of *GFAP* and *AIF1* were increased in the brain of both (A) neonatal and (B) adult *PSEN1* pigs compared to wildtype pigs.



Supplementary Figure 6. Image of a *PSENI*^{AE9/+} half-NIH mini boar at six months old.

Supplementary Table 1. Primers used in this study.

RT-qPCR Primers	Sequence (5' - 3')
<i>AIFI</i> Forward	GCTCTGGGGAGACTTTCAGC
<i>AIFI</i> Reverse	GACCCGTTGGCTTTTCCTGT
<i>GFAP</i> Forward	GAGAGGGACAACCTGGCG
<i>GFAP</i> Reverse	CTTCCTCTCCAGATCCAGCC
<i>GAPDH</i> Forward	ATGACATCAAGAAGGTGGTGAAGC
<i>GAPDH</i> Reverse	CCAGCATCAAAAGTGGAAAGAGTGA
Genotyping Primers – DNA	Sequence (5' - 3')
<i>PSENI</i> Forward	ATTGTTGCTGGCGTGAACAC
<i>PSENI</i> Reverse	CTTGGCTGTACGAACTGGGT
Genotyping Primers – RNA	Sequence (5' - 3')
<i>PSENI</i> Forward	TTGAAACAGCTCAGGAGAGAAA
<i>PSENI</i> Reverse	ACCAACCAGAACACTGTAGAAA