**Cell Reports Medicine, Volume *3***

## Supplemental information

# A genetically modified minipig model for Alzheimer's disease with *SORL1* haploinsufficiency

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**SUPPLEMENTAL INFORMATION**

**List of Supplementary information:**

Supplementary Tables S1-S2

Supplementary Figures S1-S6

**Supplementary table S1. Animals included in the study, related to Figure 1**

| Pig (ID no) | Sex | Genotype | Age at CSF sampling (mo) | Age at FDG-PET scan (mo) | Age at PIB-PET scan (mo) | Age at MRI scan (mo) | Age at euthanization (mo) |
|---|---|---|---|---|---|---|---|
| **Cloned animals (F0)** | | | | | | | |
| 6304 | Female | KO | 33 | - | - | - | 33 |
| 6401 | Female | HET | 35 | - | - | - | 35 |
| 6402 | Female | HET | 36* | - | - | - | 36 |
| **F1 offspring** | | | | | | | |
| 6469 | Female | HET | 24 | 21 | 21 | 22 | 29 |
| 6470 | Female | HET | 24 | 21 | 21 | 22 | 29 |
| 6471 | Male | WT | 18 | - | - | - | 18 |
| 6472 | Male | HET | 30 | - | - | 27 | 30 |
| 6473 | Female | HET | 5 | - | - | - | 5 |
| 6474 | Male | HET | 30 | - | - | 27 | 30 |
| 6475 | Female | WT | 24 | 21 | 21 | 22 | 29 |
| 6476 | Male | HET | 18 | - | - | - | 18 |
| 6477 | Female | WT | 24 | 21 | 21 | 22 | 29 |
| 6478 | Female | WT | 5 | - | - | - | 5 |
| **WT control animals** | | | | | | | |
| 334011 | Female | WT | 36 | - | - | - | 36 |
| 230251 | Female | WT | 38 | - | - | - | 38 |
| 339671 | Male | WT | 17 | - | - | - | 17 |
| 339704 | Male | WT | 17 | - | - | - | 17 |
| 338496 | Male | WT | 29 | - | - | 27 | 29 |
| 338593 | Male | WT | 30 | - | - | 27 | 29 |
| **WT breeding boars** | | | | | | | |
| 229751 | Male | WT | 23 | - | - | - | 23 |
| 332725 | Male | WT | 22 | - | - | - | 22 |

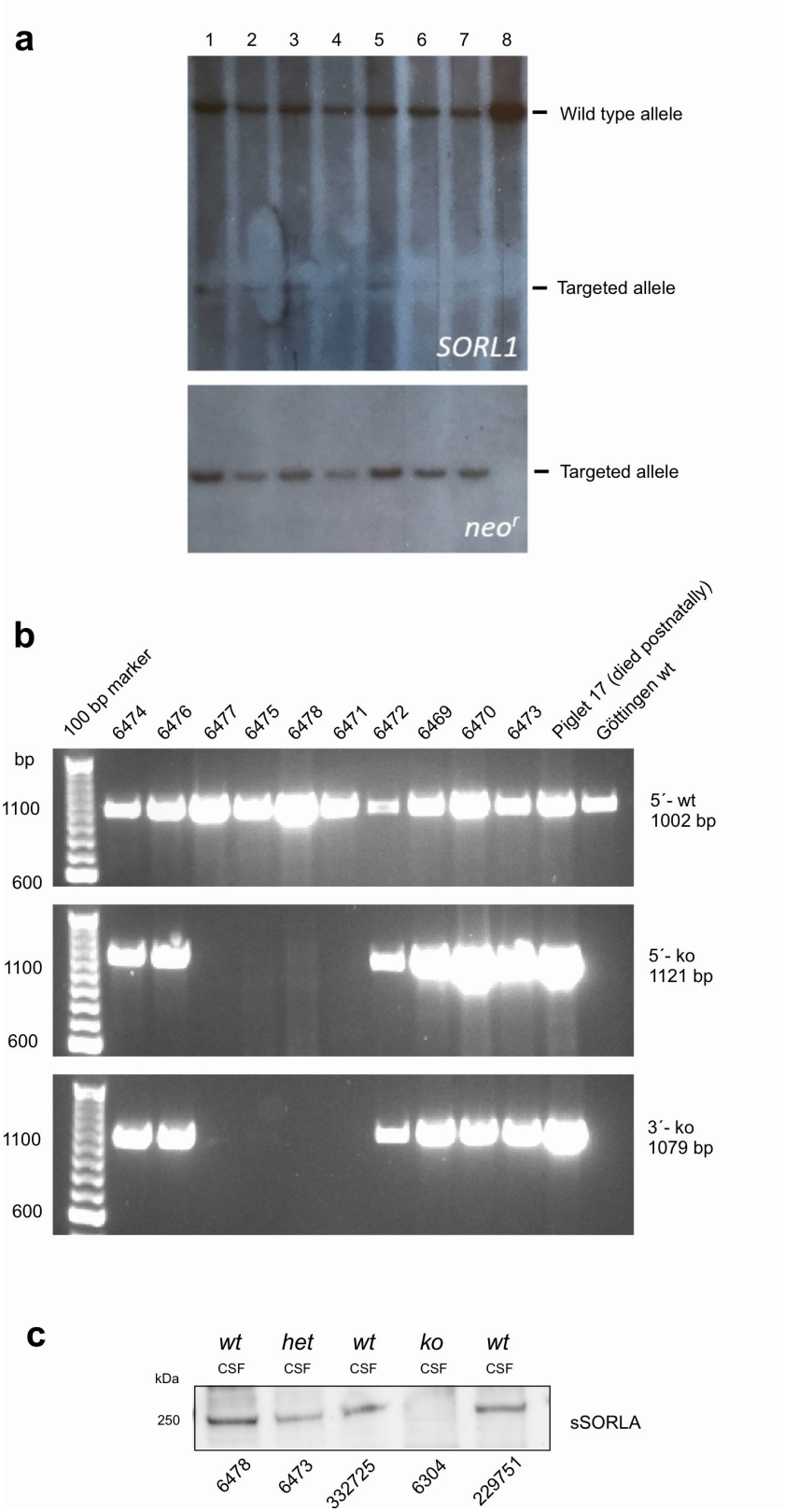
Pigs included in the study listing age in whole months at individual procedures. A dash indicate that the procedure was not performed for the animal in question.

* CSF from animal 6402 was contaminated with blood and not used for any analysis

## Supplementary table S2. Primer sequences, related to Experimental Procedures

| | Forward primer (5´-3´) | Reverse primer (5´-3´) |
|---|---|---|
| **rAAV/*SORL1* KO vector** | | |
| LHA* | atacatac<u>gcggccgc</u>CCTCAAAAACCAGGGTGTGAGTCCAGAGC | *gctccagcttttgttccctttag* CCCTGGCTGGCGCTCCTCCTTGTCCG |
| RHA* | *cgccctatagtgagtcgtattac* GCATCCATCCTTGGCTGTCGCTTCCCAGG | atacatac<u>gcggccgc</u>CCAAGGGTTGAACATTTAACTCTGTGTATTTCC |
| 3-Fusion* | atacatac<u>gcggccgc</u>CCTCAAAAACCAGGGTGTGAGTCCAGAGC | atacatac<u>gcggccgc</u>CCAAGGGTTGAACATTTAACTCTGTGTATTTCC |
| **CRISPR sgRNA vectors** | | |
| sgRNA1** | accgGCGACACGGAGCAGCAGGA | aaacTCCTGCTGCTCCGTGTCGC |
| sgRNA2** | accgATGGCGCTGCTGCCGCCCG | aaacCGGGCGGCAGCAGCGCCAT |
| **C-check vector** | | |
| sgRNA1-sgRNA2 target site insert*** | gtcggat(GGCGACACGGAGCAGCAGGAGGGGATGGCGCTGCTGCCGCCCGGGG)aggt | cggtacct(CCCCGGGCGGCAGCAGCGCCATCCCCTCCTGCTGCTCCGTGTCGCC)atc |
| **PCR screening of donor cells** | | |
| 5´ *SORL1* KO PCR (F3+R3) | CTCTAGAAGTAGTCTCTCCTTCAGTCCTG | GCGCATGCTCCAGACTGCCTTGG |
| 3´ *SORL1* KO PCR (F4+R4) | GGTACCCAATTCGCCCTATAGTGAGTC | AATGACACAATAGGCTAAGATGG |
| **Southern blot probes** | | |
| *SORL1* probe | CTGAGCTCCCCAAAGTTAGAAAGTG | GCCTCTCCAGTTAACAGACCTTCC |
| neo$^r$ probe | GAAGCCCGGCATTCTGCACGC | CAGAAGCCATAGAGCCCACCGCA |
| **Off target analysis** | | |
| Chr. 2 (*JUNB*) | AGCGGTGGCGGCAGCTAC | GTACGAGCTCCCGGTACCGAC |
| Chr. 2 (ARHGAP26) | AGCGCCAGGAGGCCCATG | CAAGGATGCGCTCCGTTCG |
| Chr. 5 (*XRCC6*) | CCGTGCCACTCACCATTCACC | CAGTTCACTCGTGTGACCTGGAGC |
| Chr. 6 (*GSE1*) | CTGAATCAGCACATGTCTGGCC | GGACCTCGCCGTGAGCAG |
| Chr. 8 (*PCDH7*) | GAGTGGGATACGACCGATCTTGC | GCGAACAGCGCGACTCCTATG |
| Chr. 9 (*HEPACAM*) | CTCAGGCGCTGATCTCCACAG | GTGCAGACTTCCAGGAGAGGTCC |
| Chr. 14 (*TXNRD2*) | CCTGGAGGTTCACAGCAAGGTC | GGTACCCTGAGTGAGCTTTGCTC |
| Chr. 15 (*TWIST2*) | AGGCATGACCAGGTCATTCAGG | TCACGGAGGGAGCTCGGC |
| **Plasmid integration analysis** | | |
| sgRNA1 plasmid | GGACATAAGCCTGTTCGGTTCG | ACGCCACGGAATGATGTCG |
| hCas9 plasmid | ATGGGCGGTAGGCGTGTAC | GATCTCCTGCAGGTAGCAGATC |
| **Genotyping of piglets** | | |
| 5´ *SORL1* KO PCR | CTCTAGAAGTAGTCTCTCCTTCAGTCCTG | GCGCCTACCGGTGGATGTGG |
| 3´ *SORL1* KO PCR | GGTACCCAATTCGCCCTATAGTGAGTC | AATGACACAATAGGCTAAGATGG |
| *SORL1* WT PCR | CTCTAGAAGTAGTCTCTCCTTCAGTCCTG | CTTCGCGCACTTTCTCCGCTG |
| **RT-PCR** | | |
| 5´ *SORL1* RT-PCR (exon 1-2, F1+R2) | CGGACGAGAAGCCGCTCCG | CAAGGCCACGATGACGTTGC |
| 5´ *SORL1* RT-PCR (exon 1-3, F1+R1) | CGGACGAGAAGCCGCTCCG | GCGATCACGGCTTCACTGCTG |
| 3´ *SORL1* RT-PCR (exon 46-47) | GCCATGAACATCACAGCGTACC | GCTGACACATCCCTACCAGACC |
| *GAPDH* RT-PCR | GACTCATGACCACGGTCCATG | GTCAGATCCACAACCGACACG |
| *APP* RT-PCR | ACCCTATGAAGAGGCCACGG | TGTTCAGAGCACACCTCTCG |
| *ADAM10* RT-PCR | TCCTTGGTCGAACCATCACC | CTGCCCACCAATAAGCCACG |
| *ADAM17* RT-PCR | GCAAAGGCGTGTCTTACTGC | CGAGTGGTCGGTTTCGTTAC |
| *BACE1* RT-PCR | TTGTGCGGGTGGAGATCAAT | CATCTGGGAACTTCTCCGTCGAG |
| *PSEN1* RT-PCR | CAGAGAGCCCTGCACTCAAT | GCCAGGCATGGATGACCTTAT |
| *PSEN2* RT-PCR | GGCCATCCTCATCGGTTTGT | TGTCCATGAAAGGCCGTACC |
| *MAPT* RT-PCR | CATGCTCAGGGGGACTACAC | CCAAGAGTCACCTTCTGCCG |
| **qPCR** | | |
| 3´ *SORL1* | GCCATGAACATCACAGCGTACC | GCTGACACATCCCTACCAGACC |
| *HPRT1* | AAGCTTGCTGGTGAAAAGGA | GTCAAGGGCATAGCCTACCA |

*Underlined sequence: *Not* I restriction site; sequence in italics: linker for 3-fusion PCR
**Lower case: overhang for BsaI cloning; upper case: porcine *SORL1* target sequence
***Nucleotides in brackets denote the overlapping target site sequences recognized by sgRNA1 and sgRNA2. Nucleotides relevant for cloning are shown in lower case.



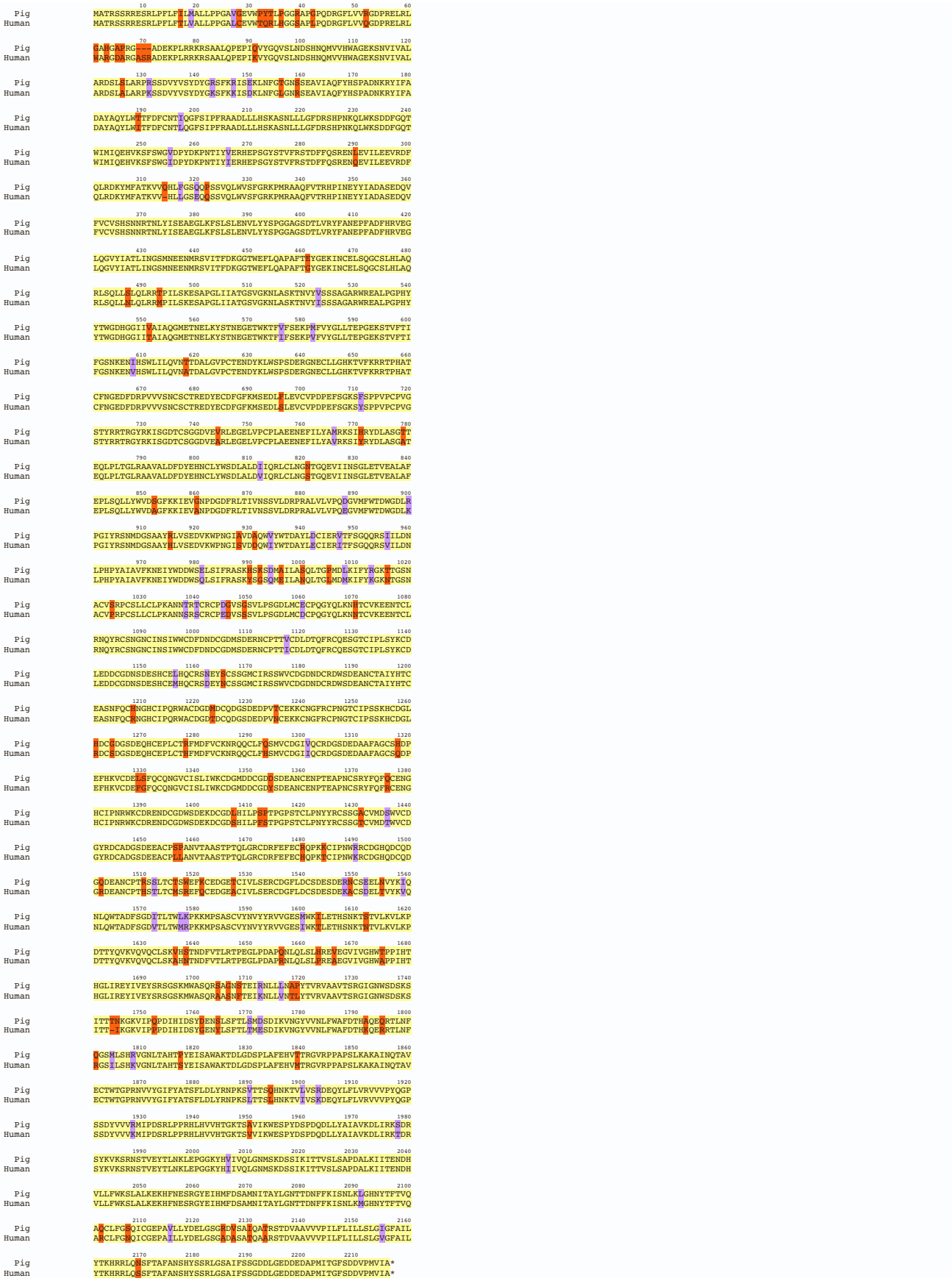

**Supplemental figure S1. Molecular biological and biochemical validation of the *SORL1* Göttingen Minipigs, related to Figure 1 and Figure 2**

a) Representative Southern blot of genomic DNA isolated from re-cloned Göttingen *SORL1 het* minipigs. Genomic DNA was digested with *Blp*I, electrophoresed and blotted onto a nitrocellulose membrane prior to hybridization with

the *SORL1* probe resulting in an upper band (theoretical size 8.5 kb) and lower band (theoretical size 3.2 kb) representing the wild-type and targeted allele, respectively (upper panel). *Blp*I-digested DNA was also hybridized with the *neo*$^{r}$ probe, detecting the *neo*$^{r}$ cassette and yielding a band (theoretical size 6.9 kb) corresponding to the targeted allele (lower panel). Lanes 1-7: Genomic DNA isolated from individual re-cloned *SORL1 het* Göttingen minipigs; Lane 8: Genomic DNA isolated from a Göttingen wild-type minipig.

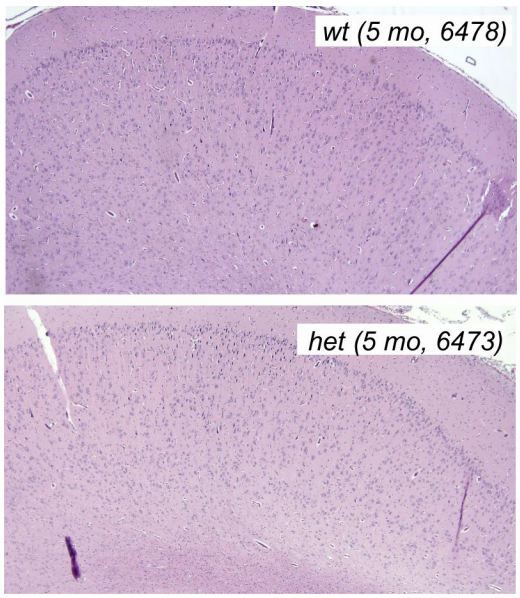
b) Genotyping of F1 *SORL1* piglets. Genomic DNA was isolated from ear biopsies obtained from newborn piglets and used as template in PCR employing primer sets to amplify the wild-type or targeted *SORL1* allele. The *SORL1 KO* allele was detected by amplification of the 5′- and 3′-end of the targeted *SORL1* region, respectively. Genomic DNA, isolated from the pig fibroblasts used for re-cloning as well as from a wild-type Göttingen minipig, were used as positive controls. M; 100 bp DNA marker.

c) WB analysis of cerebrospinal fluid (CSF) isolated from a *SORL1-wt* (6478/5 mo), a *SORL1-het* (6473/5 mo) and the cloned *SORL1-ko* (6304) animal and two older control animals, (*wt* (332725)/22 mo and *wt* (229751)/23 mo), showed absent and reduced sSORLA in the CSF of the *ko* and *het* minipigs, respectively, in accordance with their individual *SORL1* genotypes (see also **Fig. 2a,d**).



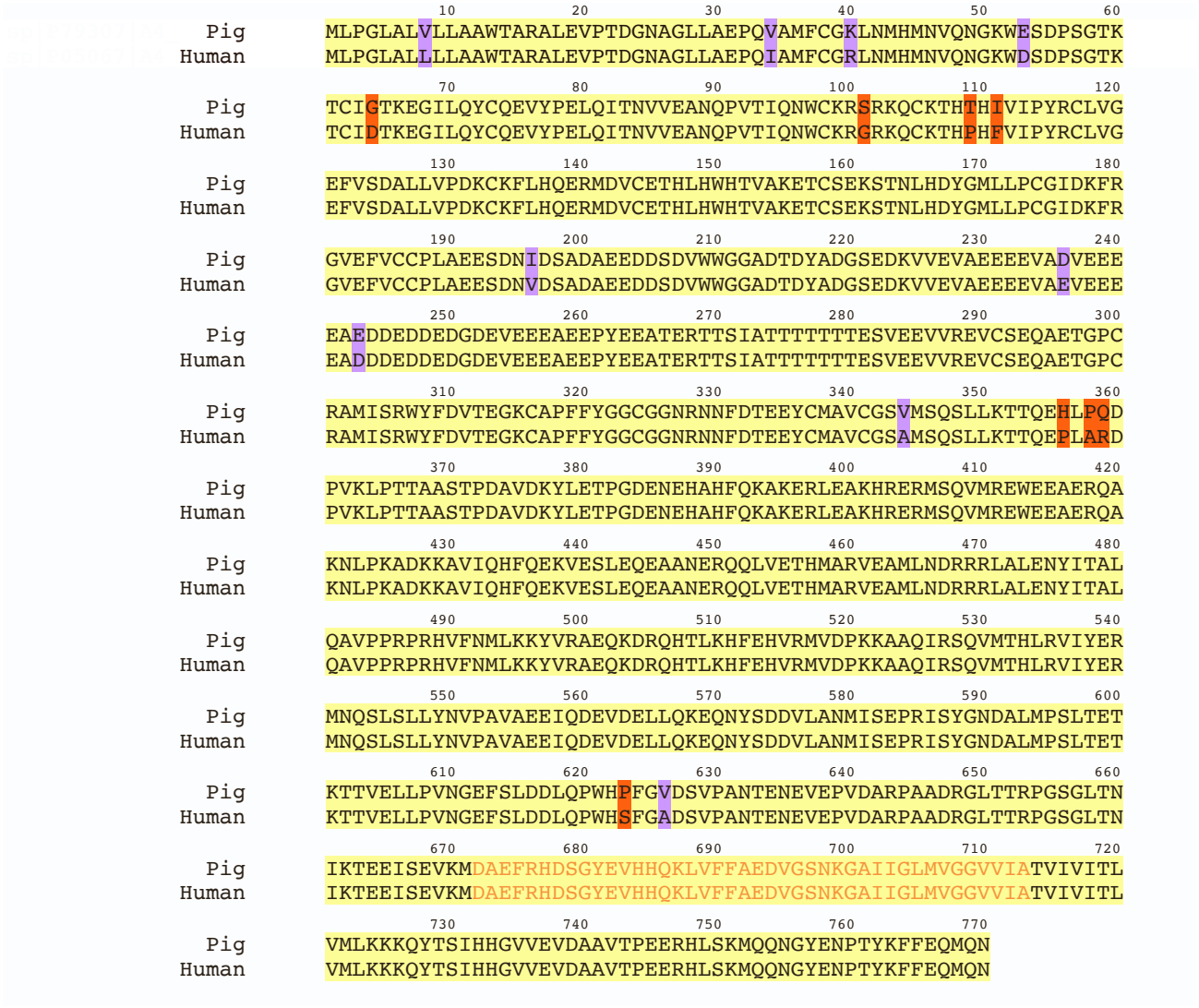
**Supplemental figure S2. Alignment of human and pig SORLA protein sequences, related to Figure 2**

SORLA identity 92.2% (2043/2216)




**Supplemental figure S3. Histopathology of young *SORL1-het* and *SORL1-wt* Göttingen Minipigs, related to Figure 2**

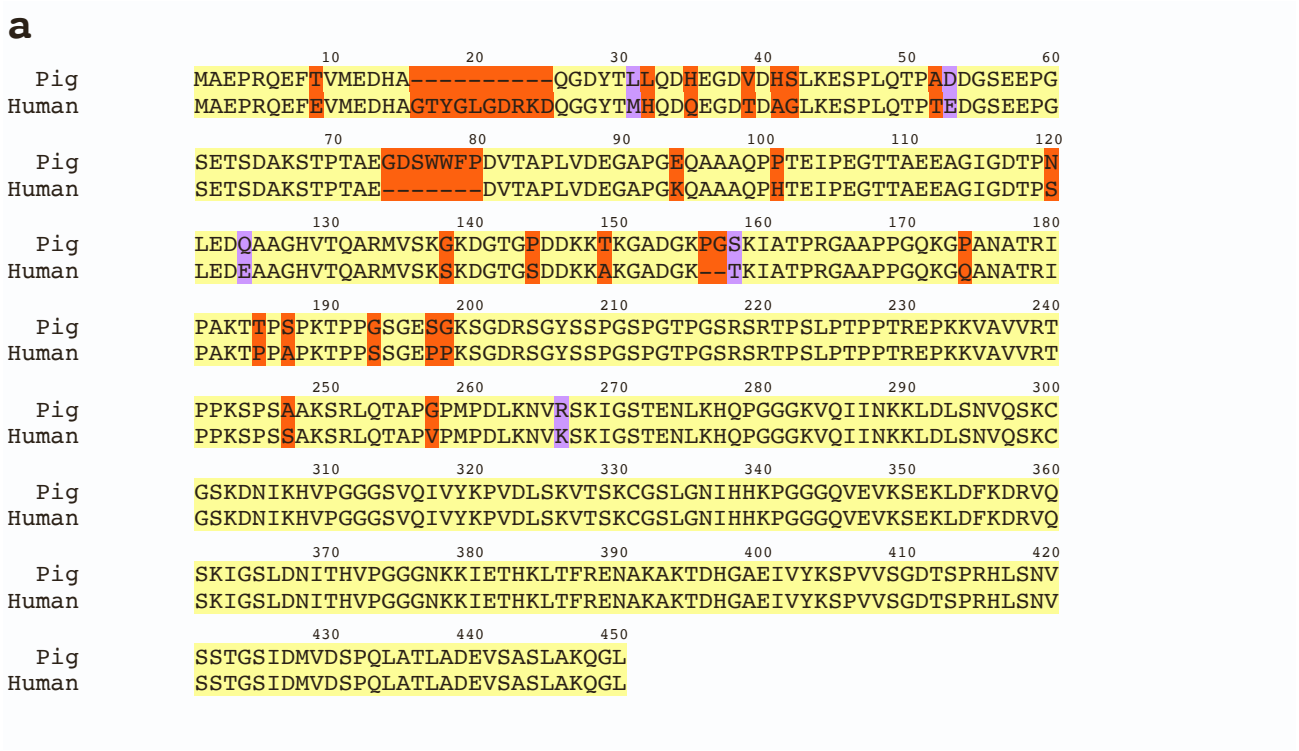
Histological examination of hematoxylin-stained frontal cortex from 5-month old *wt* (6478) and *het* (6473) *SORL1* minipigs showing no detectable difference in neuronal layering and numbers.




**Supplemental figure S4. Alignment of human and porcine APP protein sequences, related to Figure 3**

APP identity 97.8% (753/770)

The 42 amino acid sequence of APP corresponding to the Aβ peptide is 100% conserved (shown in red letters).



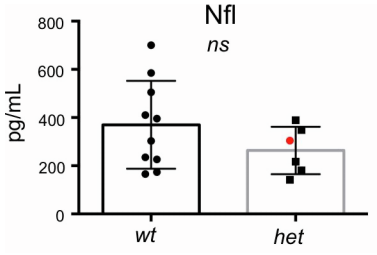

**Supplemental figure S5. Total tau measurements in *SORL1* Göttingen Minipigs, related to Figure 3**

a) Alignment of human and porcine tau protein sequences. Tau identity 89.8%

b) Quantification of total tau using MSD for the mouse tau protein in CSF from *het* (n=8) and age-matched *wt* (n=11) *SORL1* Göttingen Minipigs. Average age of the two groups of pigs were similar. The group of *het* minipigs has higher (*P=0.0431*) total-tau (1.26±0.036 ng/ml) than *wt SORL1* minipigs (1.16±0.030 ng/mL). Two-tailed unpaired Student's t

test was used for the statistical analysis, with *P*-values below 0.05 considered significantly changed. Data are expressed as mean ± SEM.

c) Western blot analysis for CSF isolated from *wt* and *het SORL1* minipigs (29-30 mo old) using the 5E2 anti-tau antibody (as shown in **Fig. 3g**) focusing on a high-molecular tau isoform (band marked by *asterisk*) which seems to appear in the CSF from the *SORL1-het* animals. CSFs for this analysis were isolated from the animals immediately prior to euthanization at the age of 29-30 month. Identification numbers are provided below each lane.




**Supplemental figure S6. Normal neuronal integrity in *SORL1-het* and *SORL1-wt* Göttingen Minipigs, related to Figure 7**

Biochemical assessment of neuronal degradation as measured by neurofilament light chain (NF-L) in collected *SORL1* minipig CSFs (*wt*, n=10; *het*, n=5; *ko*, n=1). The group of *het* minipigs were depicted including data obtained from the *ko* pig 6304 shown in red. Two-tailed unpaired Student's t test was used for the statistical analyses, with *P*-values below 0.05 considered significantly changed. Data are expressed as mean ± SEM.