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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

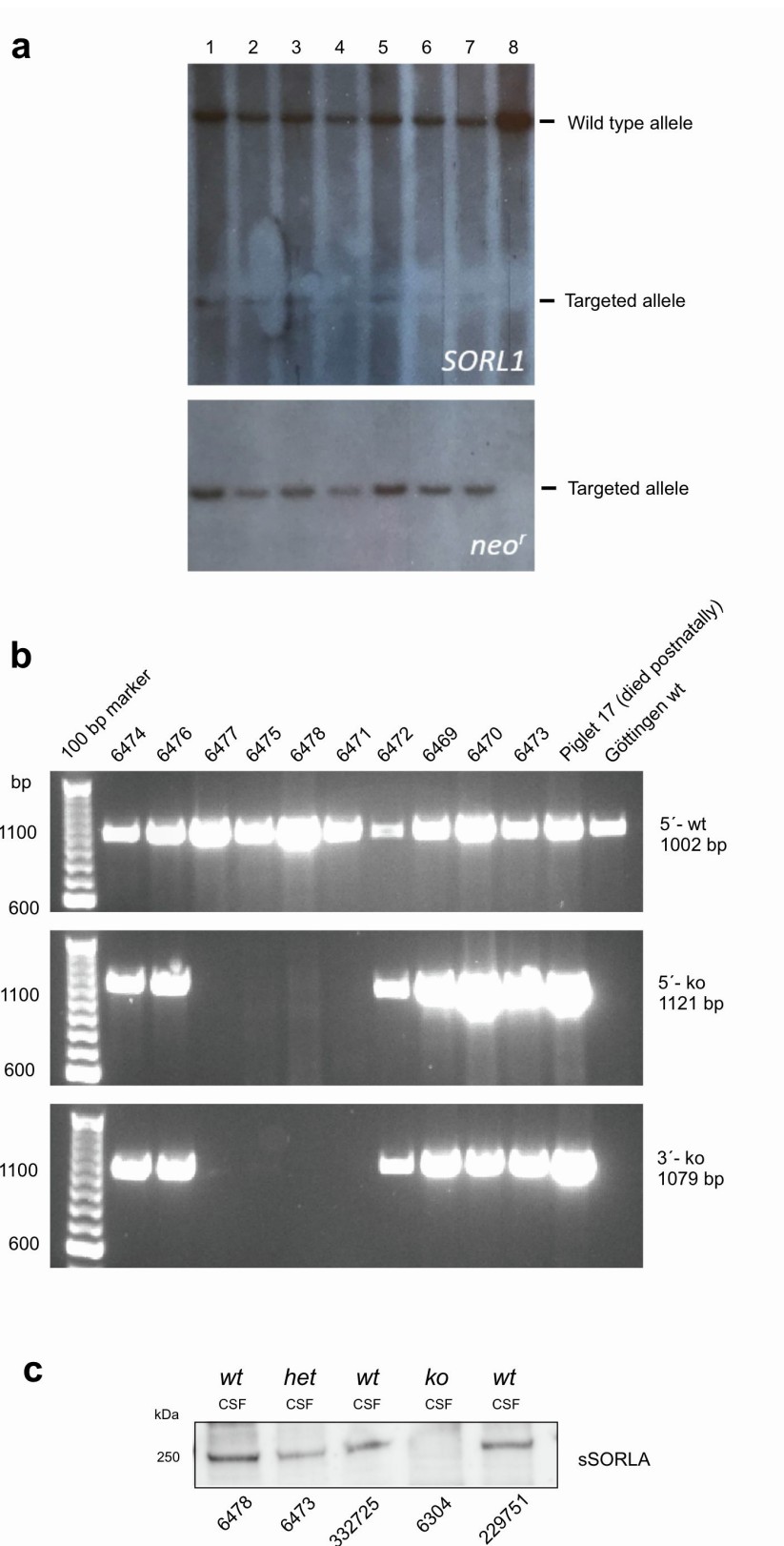
Supplementary table S2. Primer sequences, related to Experimental Procedures

	Forward primer (5'-3')	Reverse primer (5'-3')
rAAV/SORL1 KO vector		
LHA*	<u>atacatac</u> <i>cgggccc</i> CCTCAAAAACCCAGGGTGTGAGTCCAGAGC	<i>gctccogcttttttcccttttag</i> CCCTGGCTGGCGGCTCCTCTGTGCCG
RHA*	<i>cgccctatagtgtgctgtattac</i> GCATCCATCCTTGGCTGCTGCCCTCCAGG	<u>atacatac</u> <i>cgggccc</i> CCAAGGGTTGAACATTTAACTCTGTGATTTCC
3-Fusion*	<u>atacatac</u> <i>cgggccc</i> CCTCAAAAACCCAGGGTGTGAGTCCAGAGC	<u>atacatac</u> <i>cgggccc</i> CCAAGGGTTGAACATTTAACTCTGTGATTTCC
CRISPR sgRNA vectors		
sgRNA1**	accgGCGACACGGAGCAGCAGGA	aaacTCCTGCTGCTCCGTGTCGC
sgRNA2**	accgATGGCGCTGCTGCGCCCGC	aaacCGGGCGGCGAGCAGCGCCAT
C-check vector		
sgRNA1-sgRNA2 target site insert***	<i>gtcggat</i> (GGCGACACGGAGCAGCAGGAGGGATGGCGCTGCTGCCCGGGG) <i>aggt</i>	<i>cggtacct</i> (CCCCGGGCGGCGAGCAGCGCCATCCCTCCTGCTGCTCCGTGTCGCC) <i>atc</i>
PCR screening of donor cells		
5' SORL1 KO PCR (F3+R3)	CTCTAGAAGTAGTCTCTCTTCAGTCCTG	GCGCATGCTCAGACTGCGCTTGG
3' SORL1 KO PCR (F4+R4)	GGTACCCAATTGCGCCATATAGTGAGTC	AATGACACAATAGGCTAAGATGG
Southern blot probes		
SORL1 probe	CTGAGCTCCCCAAAGTTAGAAAGTG	GCCTCTCCAGTTAACAGACCTTCC
neo ^r probe	GAAGCCCGGCATTCTGCACGC	CAGAAGCCATAGACCCACCGCA
Off target analysis		
Chr. 2 (<i>JUNB</i>)	AGCGGTGGCGGCAGCTAC	GTACGAGCTCCGGTACCGAC
Chr. 2 (<i>ARHGAP26</i>)	AGCGCCAGGAGGCCCATG	CAAGGATGCGCTCGTTCCG
Chr. 5 (<i>XRCC6</i>)	CCGTGCCACTCACCATTACC	CAGTTCACCTGCTGACCTGGAGC
Chr. 6 (<i>ISE1</i>)	CTGAATCAGCACATGTCTGGCC	GGACTCGCCGTGAGCAG
Chr. 8 (<i>PCDH7</i>)	GAGTGGGATACGACCGATCTTGC	CGCAACAGCGCGACTCCTATG
Chr. 9 (<i>HEPACAM</i>)	CTCAGGCGCTGATCCACAG	GTGCAGACTCCAGGAGAGGTTCC
Chr. 14 (<i>TXNRD2</i>)	CCTGGAGTTTACAGCAAGGTC	GGTACCCTGAGTGAGCTTTGCTC
Chr. 15 (<i>TWIST2</i>)	AGGCATGACCAGGTATTGAG	TCACGGAGGAGCTCGGC
Plasmid integration analysis		
sgRNA1 plasmid	GGACATAAGCCTGTTGGTTCC	ACGCCACGGAATGATGTCG
hCas9 plasmid	ATGGGCGGTAGGCGGTAC	GATCTCTGCGAGGTAGCAGATC
Genotyping of piglets		
5' SORL1 KO PCR	CTCTAGAAGTAGTCTCTCTTCAGTCCTG	GCGCCTACCGTGGATGTGG
3' SORL1 KO PCR	GGTACCCAATTGCGCCATATAGTGAGTC	AATGACACAATAGGCTAAGATGG
SORL1 WT PCR	CTCTAGAAGTAGTCTCTCTTCAGTCCTG	CTTCGCGCACTTCTCCGCTG
RT-PCR		
5' SORL1 RT-PCR (exon 1-2, F1+R2)	CGGACGAGAAGCGCTCCG	CAAGGCCACGATGACGTTGC
5' SORL1 RT-PCR (exon 1-3, F1+R1)	CGGACGAGAAGCGCTCCG	GCGATCACGGCTTCACTGCTG
3' SORL1 RT-PCR (exon 46-47)	GCCATGAACATCACAGCGTACC	GCTGACACATCCCTACCAGACC
GAPDH RT-PCR	GACTCATGACACCGTCCATG	GTGAGTCCACAACCGACACG
APP RT-PCR	ACCCTATGAAGAGGCCACGG	TGTTACAGAGCACACCTCTCG
ADAM10 RT-PCR	TCCTTGGTCGAACCATCAC	CTGCCCAACAATAAGCCACG
ADAM17 RT-PCR	GCAAAAGGCGTGCTTACTGC	CGAGTGGTCCGTTTCTGTTAC
BACE1 RT-PCR	TTGTGCGGTTGAGATCAAT	CATCTGGGAACCTTCCGTCGAG
PSEN1 RT-PCR	CAGAGAGCCCTGCATCAAT	GCCAGGCATGGATGACCTTAT
PSEN2 RT-PCR	GGCCATCCTCATCGTTTGT	TGTCCATGAAAGGCCGTACC
MAPT RT-PCR	CATGCTCAGGGGACTACAC	CCAAGAGTACCTTCTGCGC
qPCR		
3' SORL1	GCCATGAACATCACAGCGTACC	GCTGACACATCCCTACCAGACC
HPRT1	AAGCTTGCTGGTAAAAGGA	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 *BlnI*, 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). *BspI*-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.

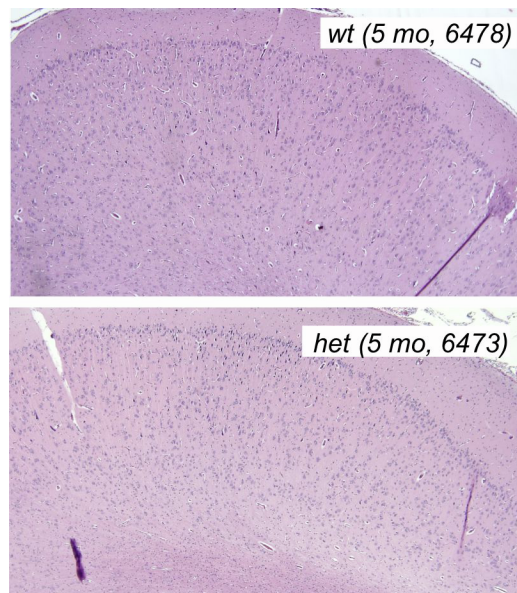
b) Genotyping of F1 *SORL1* piglets. Genomic DNA was isolated from ear biopsies obtained from newborn piglets and used as template in PCR employing primer sets to amplify the wild-type or targeted *SORL1* allele. The *SORL1 KO* allele was detected by amplification of the 5'- and 3'-end of the targeted *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**).

		10	20	30	40	50	60
Pig		MATRSSRRRESRFLFL	LMALLPFGAV	EVVYLLGGRA	PPQDRGLV	LVVGD	PRELRL
Human		MATRSSRRRESRFLFL	LMALLPFGAL	EVVYTORLGGRAF	PPQDRGLV	LVVGD	PRELRL
Pig		70	80	90	100	110	120
Human		GAACRFRG	ADEKPLRRKRS	AALQPEPI	VYGVSLNDSHN	QMVVH	AGEKSNVIVAL
Pig		130	140	150	160	170	180
Human		ARDSL	LARPS	SDVYVSDYGR	SFKRIS	KLNFQ	CNSSEAVIAQFYHSPADNKRYIFA
Pig		190	200	210	220	230	240
Human		DAYAQY	LVVTFDFC	NTLQGF	SIPFRAAD	LLH	SKASNLLGFRDRSHPNQLWKSDDFGQT
Pig		250	260	270	280	290	300
Human		WIMIQEHV	KFSFSG	VDYDKP	NTIY	VERHEP	SGYSTVFRSTDFPQSGREN
Pig		310	320	330	340	350	360
Human		QLRDKY	MFATKVV	HLGSG	SSVOLW	SFGRKPM	RAAQFVTRHPIN
Pig		370	380	390	400	410	420
Human		FVCVSH	NNRNTN	LISEA	BGLF	SLSLEN	VLYSPGGAGSDTLVRYFANEFPADFRHVEG
Pig		430	440	450	460	470	480
Human		LQGVY	IATLINGS	MNEEN	MRSVIT	FDKGGT	WEFLQAPAF
Pig		490	500	510	520	530	540
Human		RLSQ	LLQLRR	PILS	KEA	PGLI	IATG
Pig		550	560	570	580	590	600
Human		YTWGD	HGII	ALAQ	GMET	NELK	YSTN
Pig		610	620	630	640	650	660
Human		FSGN	KEN	HS	WLLQ	VN	TDAL
Pig		670	680	690	700	710	720
Human		CFNG	ED	FR	PVV	NS	CS
Pig		730	740	750	760	770	780
Human		STYR	RR	TR	G	YR	KIS
Pig		790	800	810	820	830	840
Human		EQL	PL	T	G	L	R
Pig		850	860	870	880	890	900
Human		EPL	S	Q	L	L	Y
Pig		910	920	930	940	950	960
Human		PGI	YR	S	N	M	D
Pig		970	980	990	1000	1010	1020
Human		LPH	Y	A	I	A	V
Pig		1030	1040	1050	1060	1070	1080
Human		ACV	R	P	C	S	L
Pig		1090	1100	1110	1120	1130	1140
Human		RN	Q	Y	R	C	S
Pig		1150	1160	1170	1180	1190	1200
Human		LEDD	C	G	N	S	D
Pig		1210	1220	1230	1240	1250	1260
Human		EASN	F	Q	C	N	G
Pig		1270	1280	1290	1300	1310	1320
Human		DC	D	G	S	D	E
Pig		1330	1340	1350	1360	1370	1380
Human		EFH	K	V	C	D	E
Pig		1390	1400	1410	1420	1430	1440
Human		HC	I	P	N	R	K
Pig		1450	1460	1470	1480	1490	1500
Human		G	R	D	C	A	G
Pig		1510	1520	1530	1540	1550	1560
Human		G	D	E	A	N	C
Pig		1570	1580	1590	1600	1610	1620
Human		N	L	Q	W	A	D
Pig		1630	1640	1650	1660	1670	1680
Human		D	T	T	Y	Q	V
Pig		1690	1700	1710	1720	1730	1740
Human		H	G	L	I	R	E
Pig		1750	1760	1770	1780	1790	1800
Human		I	T	T	K	G	V
Pig		1810	1820	1830	1840	1850	1860
Human		G	S	M	L	S	H
Pig		1870	1880	1890	1900	1910	1920
Human		E	C	T	W	G	R
Pig		1930	1940	1950	1960	1970	1980
Human		S	S	Y	V	V	V
Pig		1990	2000	2010	2020	2030	2040
Human		S	Y	K	V	S	R
Pig		2050	2060	2070	2080	2090	2100
Human		V	L	L	F	W	K
Pig		2110	2120	2130	2140	2150	2160
Human		A	C	L	F	G	I
Pig		2170	2180	2190	2200	2210	2220
Human		Y	T	K	H	R	L

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.

		10	20	30	40	50	60
Pig		MLPGLALV	LLAAWTARALEVPTDGNAGLLAE	PQVAMFCGK	LNMHMNVQNGKWE	ESDP	SGTK
Human		MLPGLALL	LLAAWTARALEVPTDGNAGLLAE	PQIAMFCGR	LNMHMNVQNGKWD	ESDP	SGTK
		70	80	90	100	110	120
Pig		TCIGTK	EGILQYCYQEVYPELQITNVVEANQPVTIQNWCKR	SRKQCKTH	THV	VIPYRCLVG	
Human		TCIDTK	EGILQYCYQEVYPELQITNVVEANQPVTIQNWCKR	GRKQCKTH	PHV	VIPYRCLVG	
		130	140	150	160	170	180
Pig		EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR					
Human		EFVSDALLVPDKCKFLHQERMDVCETHLHWHTVAKETCSEKSTNLHDYGMLLPCGIDKFR					
		190	200	210	220	230	240
Pig		GVEFVCCPLAEESDN	IDSADAEEDSDVWVGGADTDYADGSEDKVVEVAEEEEVAD	VEEEE			
Human		GVEFVCCPLAEESDN	VDSADAEEDSDVWVGGADTDYADGSEDKVVEVAEEEEVAE	VEEEE			
		250	260	270	280	290	300
Pig		EA	EDDEDGDEVEEEAEPEYEATER	TTSIATTTTTTTTESV	EEVREVCSEQAETGPC		
Human		EAD	DDDEDGDEVEEEAEPEYEATER	TTSIATTTTTTTTESV	EEVREVCSEQAETGPC		
		310	320	330	340	350	360
Pig		RAMISRWFYFDVTEGKCAPFFYGGCGGNRRNFDTEEYCM	AVCGSVMSQSLLK	TQEHLP	QD		
Human		RAMISRWFYFDVTEGKCAPFFYGGCGGNRRNFDTEEYCM	AVCGSAMSQSLLK	TQEPL	ARD		
		370	380	390	400	410	420
Pig		PVKLP	TAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEERQA				
Human		PVKLP	TAASTPDAVDKYLETPGDENEHAHFQKAKERLEAKHRERMSQVMREWEERQA				
		430	440	450	460	470	480
Pig		KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL					
Human		KNLPKADKKAVIQHFQEKVESLEQEAANERQQLVETHMARVEAMLNDRRRLALENYITAL					
		490	500	510	520	530	540
Pig		QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER					
Human		QAVPPRPRHVFNMLKKYVRAEQKDRQHTLKHFEHVRMVDPKKAAQIRSQVMTHLRVIYER					
		550	560	570	580	590	600
Pig		MNQSL	SLLYNVPAVAEEIQDEVDELLOKEQNYSDVLANMISEPRISYGNDALMPSLTET				
Human		MNQSL	SLLYNVPAVAEEIQDEVDELLOKEQNYSDVLANMISEPRISYGNDALMPSLTET				
		610	620	630	640	650	660
Pig		KTTVELLPVNGEFSLDDLQPW	HFFGVDSVPANTENEVEPVDARPAADRGLTTRPGSGLTN				
Human		KTTVELLPVNGEFSLDDLQPW	HFFGADSVANTENEVEPVDARPAADRGLTTRPGSGLTN				
		670	680	690	700	710	720
Pig		IKTEEI	SEVKMDAEFRHDSGYEVVHHQKLVFFAEDVGSNKGAI	IGLMVG	GVVIA	TVIVITL	
Human		IKTEEI	SEVKMDAEFRHDSGYEVVHHQKLVFFAEDVGSNKGAI	IGLMVG	GVVIA	TVIVITL	
		730	740	750	760	770	
Pig		VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN					
Human		VMLKKKQYTSIHHGVVEVDAAVTPEERHLSKMQQNGYENPTYKFFEQMQN					

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

APP identity 97.8% (753/770)

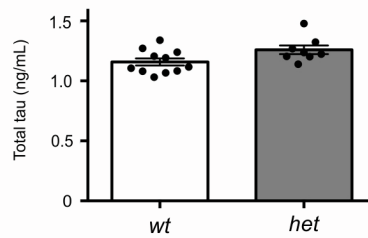
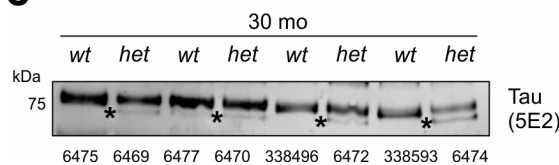
The 42 amino acid sequence of APP corresponding to the A β peptide is 100% conserved (shown in red letters).

a

	10	20	30	40	50	60
Pig	MAEPRQEF	TVMEDHA	-----	QGDTLL	QDHEGD	VDHSLKESPLQTP
Human	MAEPRQEF	TVMEDHAG	TYGLGDRKD	QGGYTM	HQDQEGD	TDAGLKESPLQTP
	70	80	90	100	110	120
Pig	SETSDAKSTPTAE	GDSWWF	PDVTAPLV	DEGAPGE	QAAAQP	TEIPEGTTAEEAGIGDTPN
Human	SETSDAKSTPTAE	-----	DVTAPLV	DEGAPGK	QAAAQP	TEIPEGTTAEEAGIGDTPS
	130	140	150	160	170	180
Pig	LEDQAAGHVTQARMVSK	GKDGTF	DDKK	TKGADGK	PGSKI	ATPRGAAPPQKGFANATRI
Human	LEDEAAGHVTQARMVSK	SKDGTG	SDDKK	AKGADGK	---	TKIATPRGAAPPQKGFANATRI
	190	200	210	220	230	240
Pig	PAKTT	PSPK	TPPG	SGES	CGKSGDR	SGYSSPGSPGTPGSR
Human	PAKTT	PPA	PKTPP	SSGEP	PKSGDR	SGYSSPGSPGTPGSR
	250	260	270	280	290	300
Pig	PPKSPS	AAKSRL	QTAP	CPMPDL	KNVRS	KIGSTENLKHQPGGK
Human	PPKSPS	AAKSRL	QTAP	VPMPDL	KNVRS	KIGSTENLKHQPGGK
	310	320	330	340	350	360
Pig	GSKDNI	KHVP	GGGS	VQIVYK	PVDLS	SKVTSKCGSLGNIH
Human	GSKDNI	KHVP	GGGS	VQIVYK	PVDLS	SKVTSKCGSLGNIH
	370	380	390	400	410	420
Pig	SKIGSL	DNITHV	PGG	GKNIETH	KLTFRE	NAKAKTDHGAEIVYK
Human	SKIGSL	DNITHV	PGG	GKNIETH	KLTFRE	NAKAKTDHGAEIVYK
	430	440	450			
Pig	SSTGS	IDMVD	SPQL	ATL	LADE	VSVASLAKQGL
Human	SSTGS	IDMVD	SPQL	ATL	LADE	VSVASLAKQGL

b

Tau (MSD)
 $P=0.0431$

**c**

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

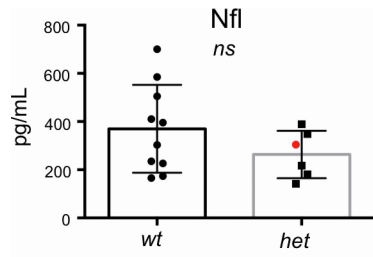
a) Alignment of human and porcine tau protein sequences. Tau identity 89.8%

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 \pm 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 \pm SEM.