

Supplementary Figures

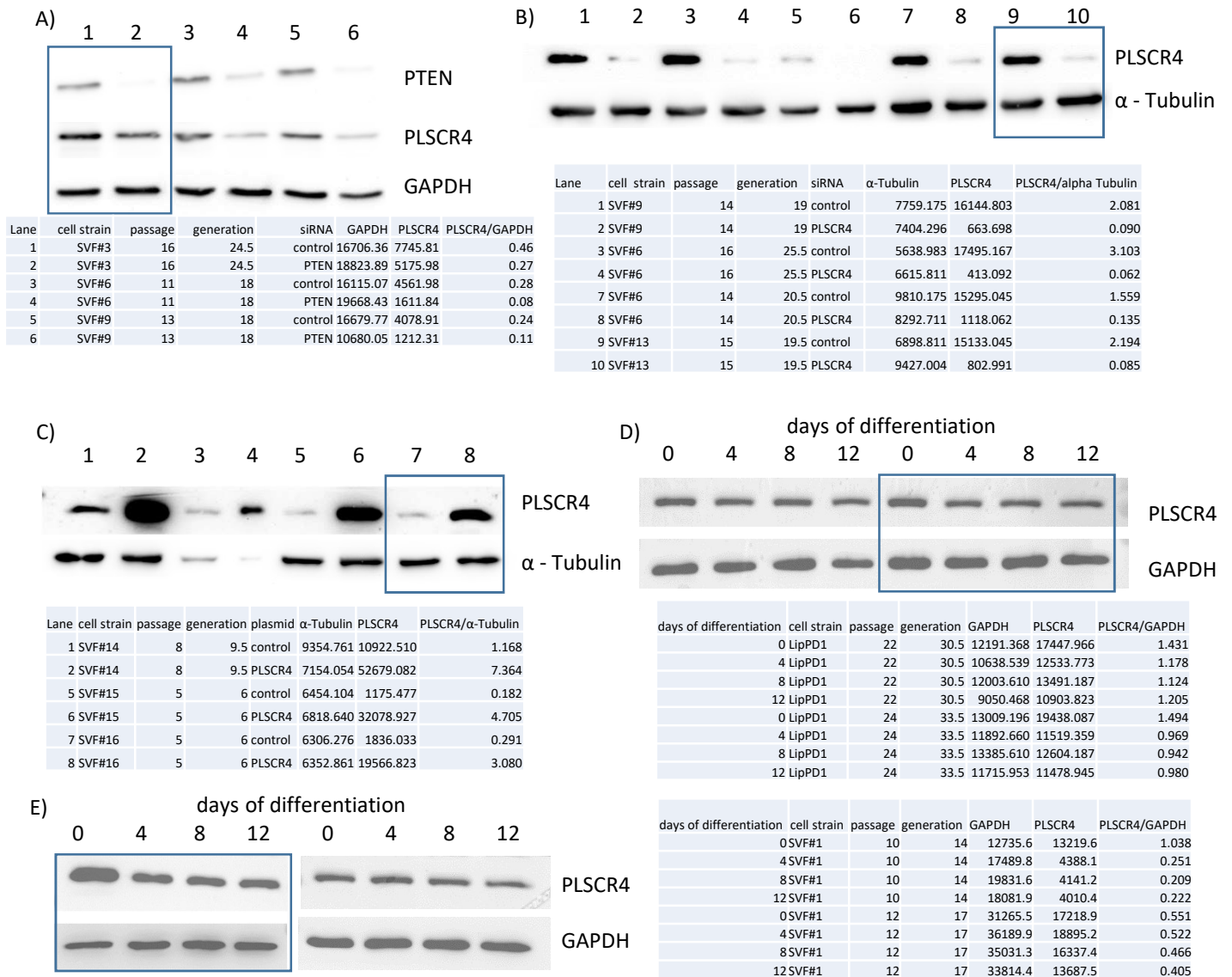


Figure S1: Western blots from PTEN and PLSCR4 knockdown/overexpression adipocyte progenitor cells (APCs). A) Western blots and densitometric analysis of control and PTEN siRNA transfected APCs. PTEN and PLSCR4 protein were normalized to GAPDH. B) Western blots and densitometric analysis of control and PLSCR4 siRNA transfected APCs. PLSCR4 protein was normalized to α -Tubulin. Due to low basal PLSCR4 expression lanes 5 and 6 were excluded from analysis. C) Western blots and densitometric analysis of control and PLSCR4 plasmid (PLSCR4 overexpression) -transfected APCs. PLSCR4 protein was normalized to α -Tubulin. Due to unequal expression of housekeeping gene α - Tubulin lanes 3 and 4 were excluded from analysis. D) Western blots and densitometric analysis of PLSCR4 during adipogenesis in LipPD1 cells. PLSCR4 protein was normalized to GAPDH. E) Western blots and densitometric analysis of PLSCR4 during adipogenesis in APCs. PLSCR4 protein was normalized to GAPDH. Western blot images presented in Figures 2, 3 and 4 were reused (indicated with blue boxes).

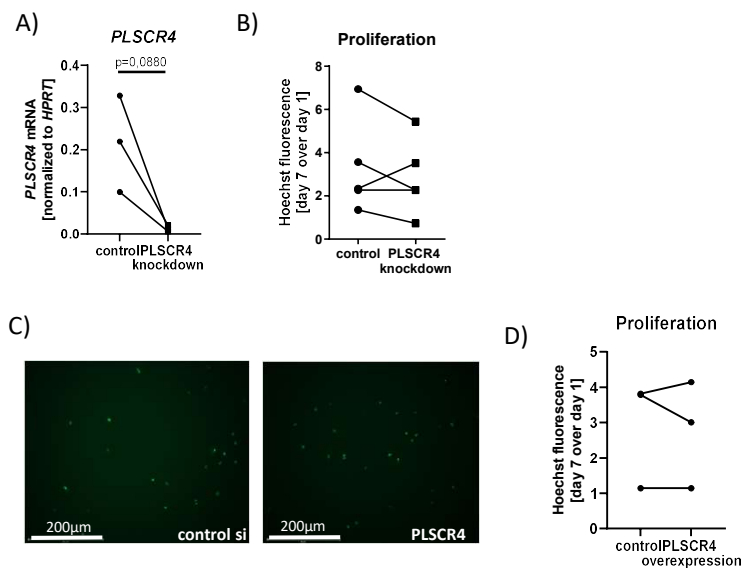
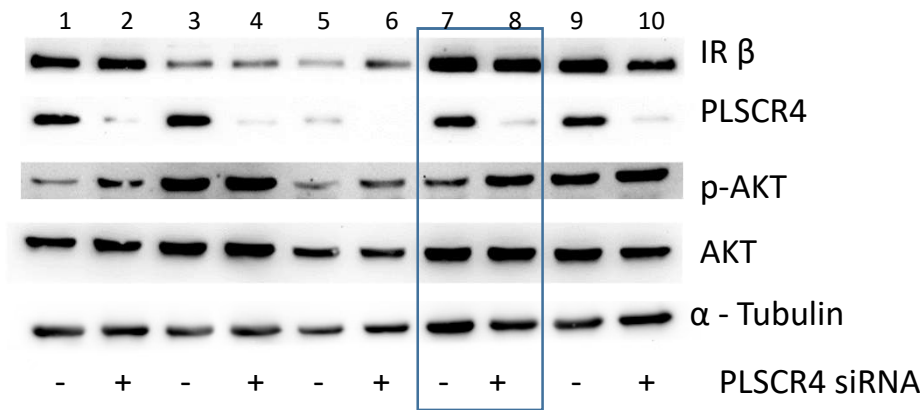


Figure S2. Knockdown/overexpression efficiencies in adipocyte progenitor cells (APCs), and effects on proliferation. A) qPCR of control and PLSCR4 siRNA transfected APCs: *PLSCR4* mRNA expression was downregulated $94.0\% \pm 29.9\%$ (normalized to *HPRT*, $n = 3$, $p = 0.0880$). B) Hoechst nuclei staining of control and PLSCR4 siRNA: transfected APCs 7 days after transfection no significant difference in proliferation of APCs was observed ($13.7\% \pm 14.8\%$, $n = 5$, $p = 0.4044$). C) Proliferation marker Ki-67 (green) immunofluorescence staining in control and PLSCR4 KD SVF cells showed no difference. D) Hoechst nuclei staining of control and PLSCR4 plasmid transfected APCs 7 days after transfection: no significant difference in proliferation of APCs was observed ($5.2\% \pm 11.2\%$, $n = 3$, $p = 0.6910$). Matched results were visualized via lines between data points (control versus knockdown/overexpression). p-values were determined via paired t test ($***p < 0.001$).

A)



B)

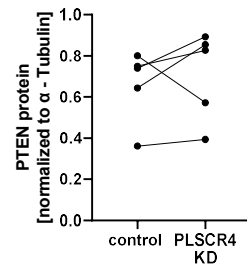
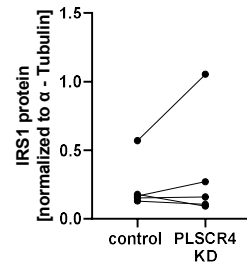
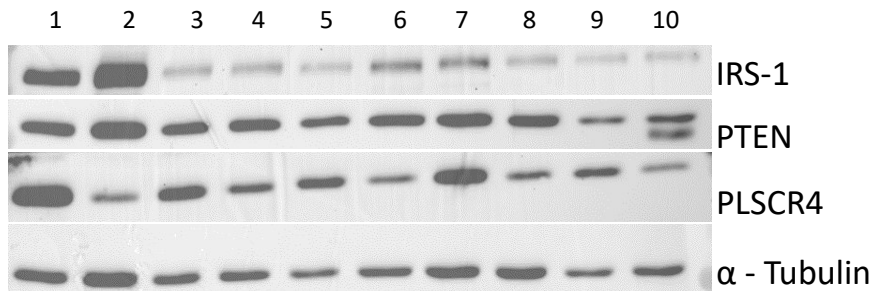
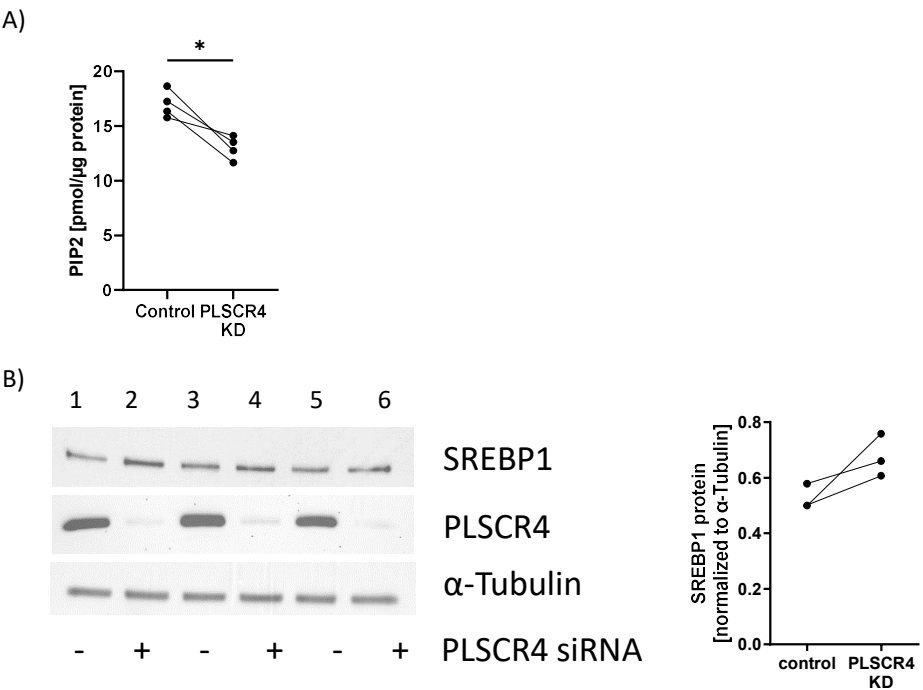


Figure S3. Western blots of PLSCR4 knockdown (KD) adipocyte progenitor cells (APCs). A) Insulin receptor β subunit (IR β), and PLSCR4 were normalized to α -Tubulin. Phosphorylated AKT (pAKT T308) was normalized to total AKT protein. AKT phosphorylation in PLSCR4 KD cells was increased by $52.0\% \pm 13.6\%$ compared to control cells ($n = 5$, $p = 0.0186$). IR β subunit expression was variable between control cells and PLSCR4 KD cells and showed no regulation ($n = 5$, $p = 0.3283$). B) Insulin receptor substrate 1 (IRS-1), PTEN and PLSCR4 were normalized to α -Tubulin. IRS-1 ($n = 5$, $p = 0.3931$) and PTEN ($n = 5$, $p = 0.5500$) expression was not significantly different between control and PLSCR4 KD cells. Western blot images presented in Figure 5 were reused (indicated with blue boxes).



Lane	cell strain	passage	generation	siRNA	α-Tubulin	PLSCR4	SREBP1	PLSCR4/α-Tubulin	SREBP1/α-Tubulin
1	SVF#1	22	29	control	17381.1	28227.7	8693	1.62	0.5
2	SVF#1	22	29	PTEN	18438	1817.7	13988.7	0.1	0.76
3	SVF#9	11	36.5	control	17579.8	25842.3	10168.6	1.47	0.58
4	SVF#9	11	36.5	PTEN	18445.2	2092.1	12164.9	0.11	0.66
5	SVF#10	31	36	control	17358.3	13942.5	8705.4	0.8	0.5
6	SVF#10	31	36	PTEN	18349.9	346.2	11144	0.02	0.61

Figure S4. A) Amount of PIP2 in control and PLSCR4 KD APCs as determined via high pressure liquid chromatography-mass spectrometry (HPLC-MS). PIP2 levels decreased by $23.1 \pm 4.7\%$ in PLSCR4 KD cells ($n = 4$, $p = 0.0215$). B) Western blots of PLSCR4 knockdown (KD) adipocyte progenitor cells (APCs). Sterol regulatory element-binding protein 1 (SREBP1) and PLSCR4 were normalized to α -Tubulin. SREBP1 expression was elevated in PLSCR4 KD cells by $28.9\% \pm 11.6\%$ compared to control cells ($n = 3$, $p = 0.116$). p-values for the experiments were determined via paired t-test ($*p < 0.05$). PLSCR4, phospholipid scramblase 4.

Supplementary Tables

Table S1: Distance matrix of the PLSCR genes from Human, Chimpanzee, and Mouse species.

PLSCR1				PLSCR2				PLSCR3				PLSCR4				PLSCR5			
Human	Mouse	Chimpanzee		Human	Mouse	Chimpanzee		Human	Mouse	Chimpanzee		Human	Mouse	Chimpanzee		Human	Mouse	Chimpanzee	
0		0	0	16	24.68	24.57		46.4	45.8	49.49		51.63	50.5	48.99		40.38	42.96	45.19	PLSCR1
				0	0	0		46.67	49.64	48.71		50	52.88	51.97		38.67	45.98	45.34	PLSCR2
								0	0	0		59.71	61.19	59.15		52.85	53.28	52.94	PLSCR3
												0	0	0		56.93	57.03	60.08	PLSCR4
																0	0	0	PLSCR5

The distance is calculated based on the formula, Distance Score = m/(npos + gaps*gap_penalty), where m = score of matches (1 for an exact match, 0 for no match), npos = the number of positions included in m, gaps = number of gaps in the sequences, and gap_penalty = the score given to a gapped position (value set as Zero). The distances are expressed in terms of the number of substitutions per 100 amino acids and a score of Zero indicates 100 % similarity.

Table S2: Primers used for RT-qPCR

Gene	Forward	Reverse	Probe
<i>hTBP</i>	TTG TAA ACT TGA CCT AAA GAC CAT TGC	TTC GTG GCT CTC TTA TCC TCA TG	AAC GCC GAA TAT AAT CCC AAG CGG TTT G
<i>hHPRT</i>	GGC AGT ATA ATC CAA AGA TGG TCA A	GTC TGG CTT ATA TCC AAC ACT TCG T	CAA GCT TGC TGG TGA AAA GGA CCC C
<i>hPTEN</i>	TGTAAAGCTGGAAAGGGACGA	GGAATAGTTACTCCCTTTTGTCTC	
<i>hPLSCR4</i>	CAGTACAAC TAGACCCGGCG	CCAGGGGGTCCTGGTAAAAA	
<i>mHprt</i>	TCCTCCTCAGACCGCTTTT	CATAACCTGGTTCATCATCGC	
<i>mTbp</i>	GGGTATCTGCTGGCGGTTT	TGAAATAGTGATGCTGGGCACT	
<i>mPTEN</i>	TCCCAGACATGACAGCCATC	TGCTTTGAATCCAAAAACCTTACT	
<i>mPlscr4</i>	AAATGTCAGGTCTGGTCCCCA	AGAAGGAGAGGCAACTGGTC	

Table S3: Western blot (Wb) antibodies

Primary antibody	Dilution	Distributor	Cat.No.
AKT antibody Rabbit polyclonal Ab	1:1000 TBS-T 5%BSA (Wb)	CST	#9272
Phospho-AKT (Thr308) (224F9) Rabbit mAb	1:1000 TBS-T 5%BSA (Wb)	CST	#4056
alpha Tublin (11H10) Rabbit mAb	1:2000 TBS-T 5%BSA (Wb)	CST	#2125
GAPDH (6C5) Mouse mAb	1:50,000 TBS-T 5% milk (Wb)	Merck	MAB374
PTEN (138G6) Rabbit map	1:1000 TBS-T 5%BSA (Wb)	CST	#9559
Insulin Receptor beta (4B8) Rabbit mAb	1:500 TBS-T 5%BSA (Wb)	Cell Signaling	3025S
IRS-1 antibody Rabbit mAb	1:1000 TBS-T 5%BSA (Wb)	Cell Signaling	2382S
Anti PLSCR4 antibody Rabbit polyclonal Ab	1:1000 TBS-T 5%BSA (Wb)	Abcam	ab 233005
Secondary antibody	Dilution	Distributor	Cat.No.
Polyclonal goat anti-rabbit immunoglobulin/HRP	1:2000 TBS-T 5% milk (Wb)	Dako	P0447
Polyclonal goat anti-mouse immunoglobulin/HRP	1:2000 TBS-T 5% milk (Wb)	Dako	P0448