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Supplemental Data

Argininosuccinate Lyase Deficiency Causes an Endothelial-Dependent Form of Hypertension

Jordan Kho, Xiaoyu Tian, Wing-Tak Wong, Terry Bertin, Ming-Ming Jiang, Shan Chen, Zixue Jin, Oleg A. Shchelochkov, Lindsay C. Burrage, Anilkumar K. Reddy, Hong Jiang, Reem Abo-Zahrah, Shuangtao Ma, Ping Zhang, Karl-Dimiter Bissig, Jean J. Kim, Sridevi Devaraj, George G. Rodney, Ayelet Erez, Nathan S. Bryan, Sandesh C.S. Nagamani, and Brendan H. Lee

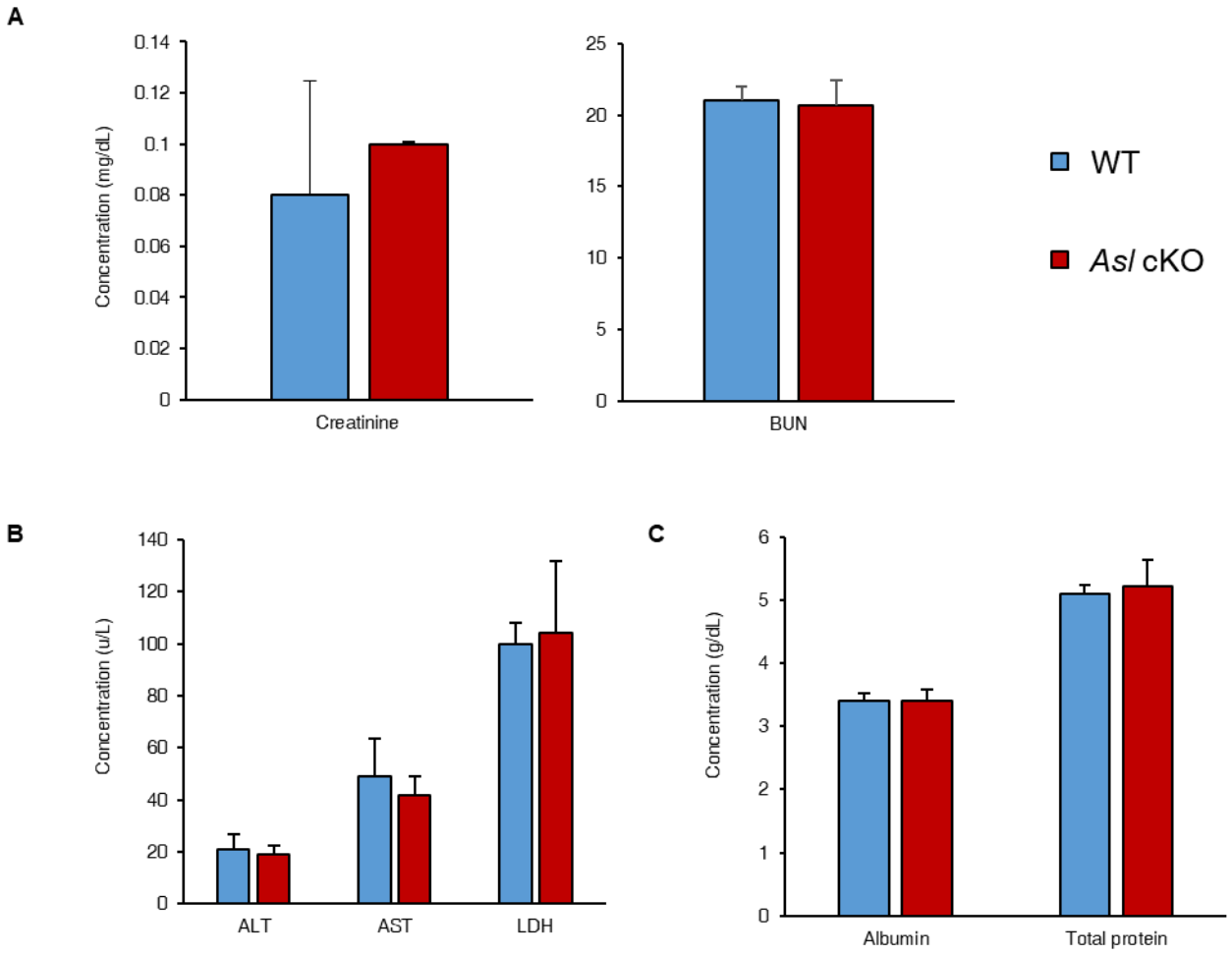

Figure S1. ***Asl*** **cKO mice display normal serum level of biomarkers for kidney and liver functions**

Serum level of (**A**) Creatinine, BUN, (**B**) ALT, AST, LDH, (**C**) albumin, and total protein in *Asl* cKO mice as compared to their wild-type littermates (n = 5, age = 12 weeks). Bar graphs represent mean values while error bars represent the standard deviation. Student's t-test.

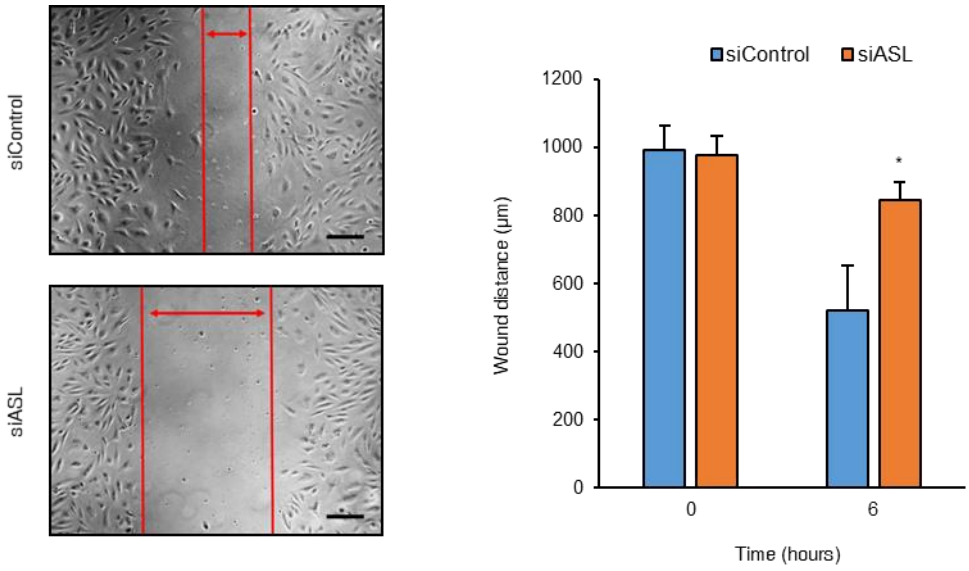

Figure S2. Loss of ASL in HAECs impairs cell migration
Wound healing assay performed on siASL-transfected HAECs. Wound distance was measured at t=0 h and t=8 h after wounding (n = 3). Scale bar, 200 µm. Bar graphs represent mean values while error bars represent the standard deviation. *P<0.05. Student's t-test.

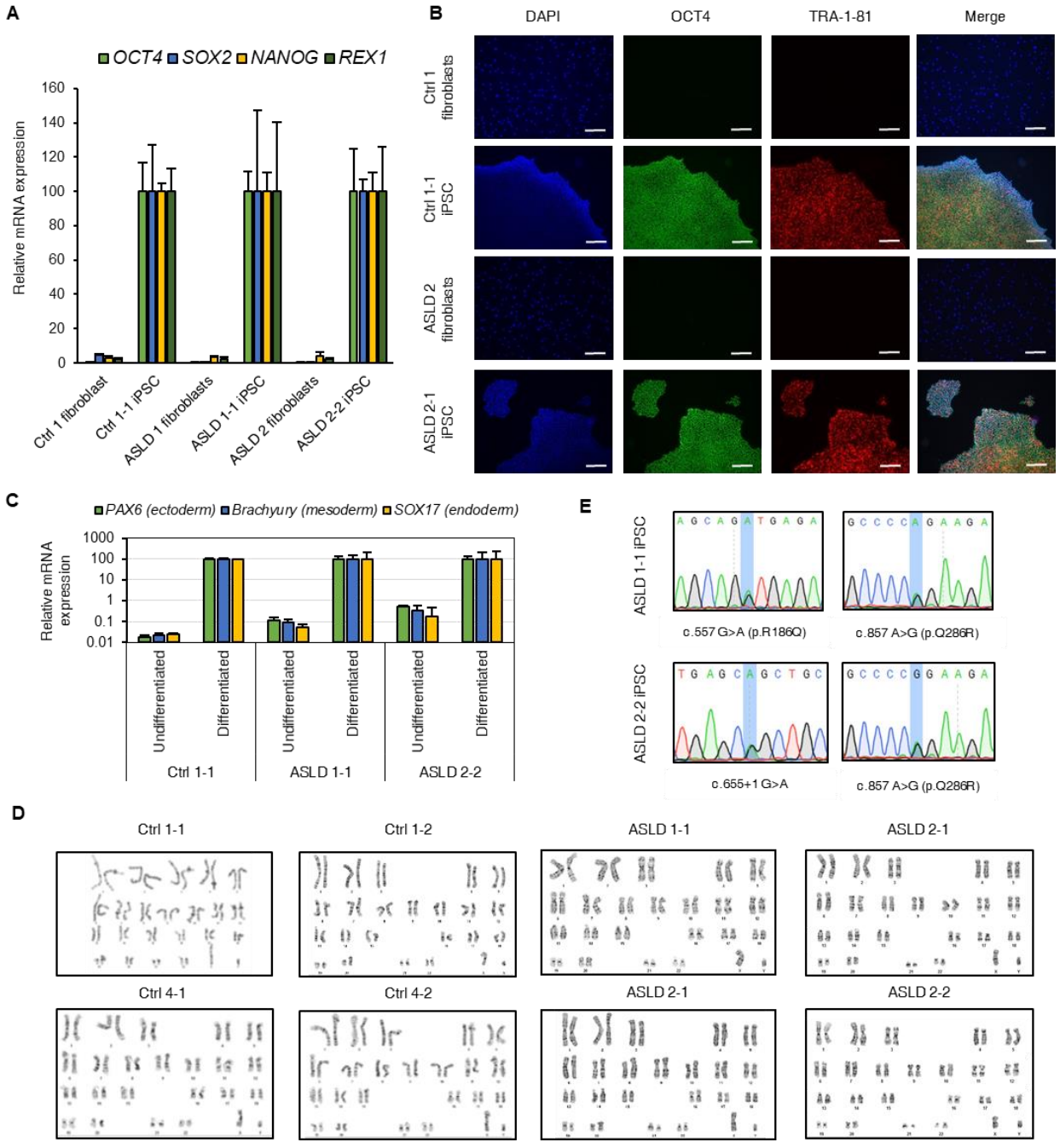

Figure S3. Characterization of iPSC lines from healthy individuals and subjects with ASLD
(**A**) Relative mRNA expression of pluripotency markers in representative fibroblasts and iPSC lines (n = 3). (**B**) Immunocytochemistry of pluripotency markers in representative control and ASLD iPSC lines. Scale bar, 200 µm. (**C**) Relative mRNA expression of endodermal, mesodermal, and ectodermal markers in representative iPSC lines upon differentiation into each respective germ layer (n = 3). (**D**) Karyotypes of control and ASLD iPSC lines. (**E**) Sanger sequencing showing that the ASLD iPSC lines continue to harbor the mutations present in the patient fibroblasts (GenBank: NM_001024943.1). Error bars represent the standard deviation.

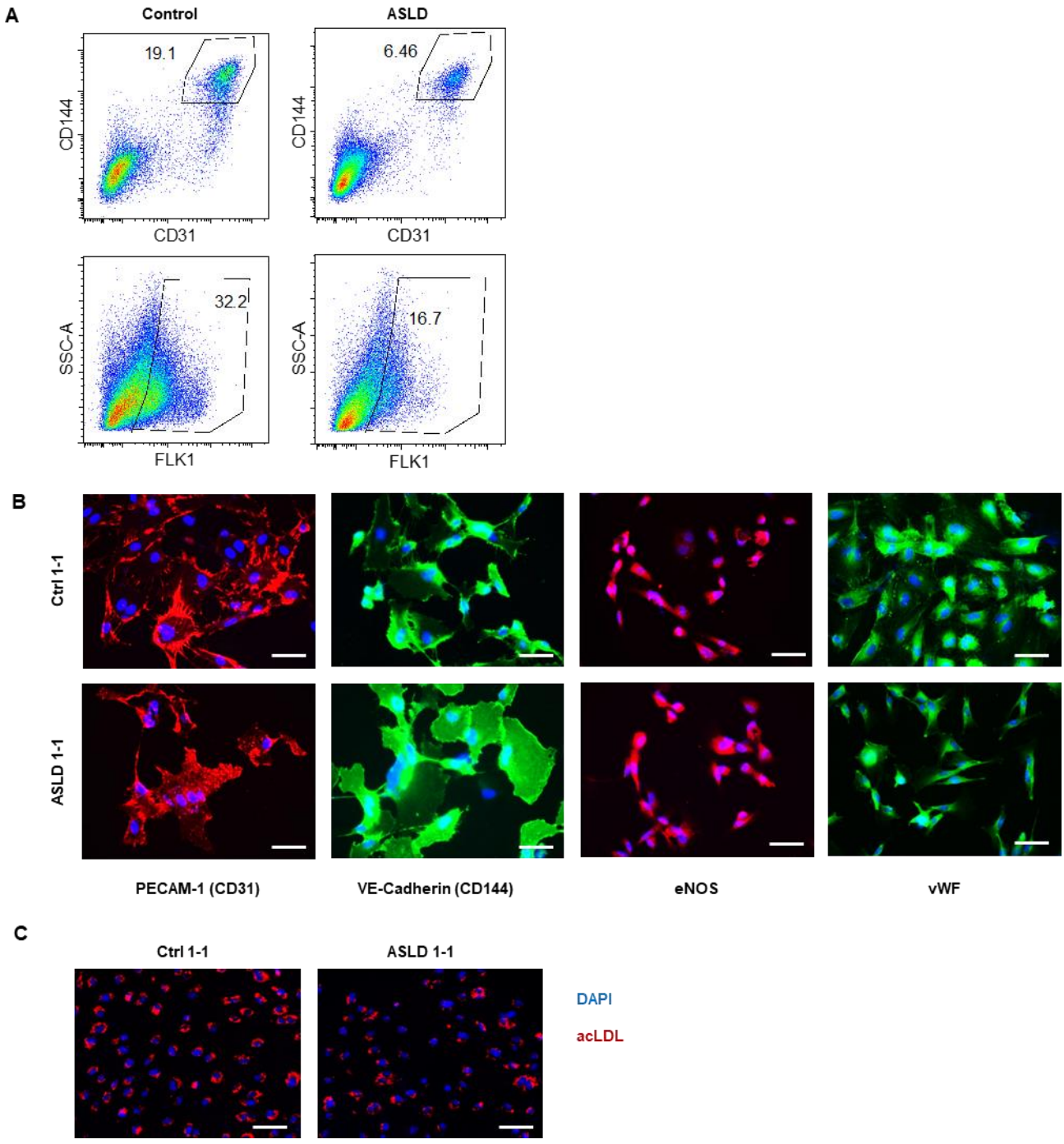

Figure S4. Generation of hiPSC-derived endothelial cells
(**A**) Representative FACS data obtained by sorting of CD31+/CD144+ and Flk1+ cells from differentiated control and ASLD iPSC. (**B**) Immunocytochemistry of endothelial markers in representative control and ASLD iPSC-ECs. Scale bar, 50 µm. (**C**) Representative images of uptake of acetylated LDL (acLDL) by iPSC-ECs. Scale bar, 100 µm.

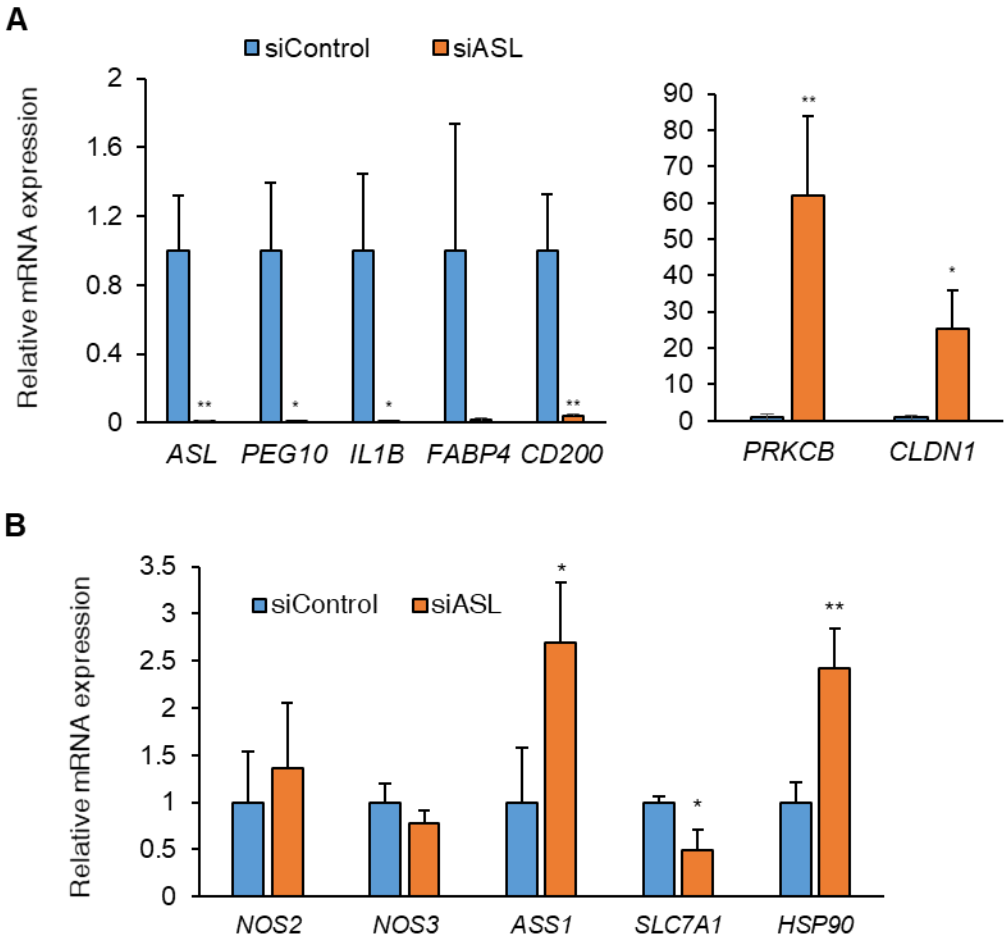

Figure S5. qRT-PCR confirmation of RNA-Sequencing gene expression patterns in ASL-deficient HAECs

The mRNA expression level of (**A**) top dysregulated and (**B**) NO synthesis complex genes in siASL-transfected HAECs were quantified by qRT–PCR (n = 3 biological replicates of siControl and siASL). *NOS1* expression in HAECs was undetectable by qRT-PCR. Bar graphs represent mean values while error bars represent the standard deviation. *P<0.05 and **P<0.01. Student's t-test.

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Subject	Age (years)	Sex	Height (cm)	Weight (kg)	BMI
100006	13.8	M	169	63	22.06
101893	13.0	M	141	43	21.90
100551	4.5	M	98	18	18.74
101880	10.1	F	130	29	17.16
102635	9.1	F	131	27	15.73
102747	11.2	F	127	23	14.26
104591	13.4	F	155	38	15.82
106088	13.8	F	160	51	19.92

Table S1. Demographic characteristics of individuals with ASLD enrolled in the trial NCT00345605

iPSC line	Sex	Diagnosis	Age at biopsy (years)	Pathogenic variants in ASL	Source of cells	Method of iPSC derivation	Reprogramming factors	Notes
Ctrl1-1	Male	Healthy control	22	N/A	Skin fibroblasts	Sendai virus	OCT4, SOX2, KLF4, c-MYC	Provided by BCM-Human Stem Cell Core (HSCC-003iPS)
Ctrl1-2								
Ctrl 2	Male	Healthy control	61	N/A	Skin fibroblasts	Gamma-retrovirus	OCT4, SOX2, KLF4, c-MYC	N/A
Ctrl 3	Male	Healthy control	N/A	N/A	Cord blood cells	Episomal vector	OCT4, SOX2, KLF4, c-MYC	Purchased from Applied StemCell
Ctrl 4-1	Male	Healthy control	16	N/A	Skin fibroblasts	Sendai virus	OCT4, SOX2, KLF4, c-MYC	Provided by BCM-Human Stem Cell Core (HSCC-022iPS)
Ctrl 4-2								
ASLD 1-1	Male	ASLD	16	c.557 G>A (p.R186Q) c.857 A>G (p.Q286R)	Skin fibroblasts	Sendai virus	OCT4, SOX2, KLF4, c-MYC	N/A
ASLD 1-2								
ASLD 2-1	Male	ASLD	10	c.655+1 G>A c.857 A>G (p.Q286R)	Skin fibroblasts	Sendai virus	OCT4, SOX2, KLF4, c-MYC	N/A
ASLD 2-2								

Table S2. List of iPSC lines and methods of derivation

	Gene		FPKM		log2(fold change)
			siControl	siASL	
Top downregulated genes	SNORD116-4	small nucleolar RNA, C/D box 116-4	31.750	0	
	LINC01173	long intergenic non-protein coding RNA 1173	0.773	0	
	PEG10	Paternally expressed 10	22.079	0.171	-7.011
	IL1B	Interleukin-1 beta	22.901	0.533	-5.425
	CD200	OX-2 membrane glycoprotein	14.678	0.463	-4.988
	ASL	Argininosuccinate lyase	15.056	0.520	-4.856
	FABP4	Fatty acid-binding protein, adipocyte	16.898	0.610	-4.793
	GNG2	Guanine nucleotide-binding protein G(I)/G(S)/G(O) subunit gamma-2	1.261	0.049	-4.687
	CEND1	Cell cycle exit and neuronal differentiation protein 1	1.934	0.089	-4.448
	CORO1A	Coronin-1A	3.524	0.173	-4.347
Top upregulated genes	KIAA1644	Uncharacterized protein KIAA1644	0.059	1.641	4.794
	PRKCB	Protein kinase C beta type	0.017	0.433	4.633
	CLDN1	Claudin-1	0.222	5.105	4.522
	GHR	Growth hormone receptor	0.034	0.485	3.839
	PLSCR4	Phospholipid scramblase 4	3.593	46.199	3.685
	NTSR1	Neurotensin receptor type 1	0.101	1.302	3.683
	P2RY6	P2Y purinoceptor 6	0.174	2.185	3.648
	SRRM3	Serine/arginine repetitive matrix protein 3	0.059	0.736	3.631
	SCN1B	Sodium channel subunit beta-1	2.373	27.276	3.523
	TGFB2	Transforming growth factor beta-2	0.246	2.778	3.500

Table S3. List of top dysregulated genes in ASL-deficient HAECs

Gene		FPKM		log2(fold change)	Significant
		siControl	siASL		
NOS1 (nNOS)	Nitric oxide synthase 1	0.001	0.003	1.221	no
NOS2 (iNOS)	Nitric oxide synthase 2	0	0.007		no
NOS3 (eNOS)	Nitric oxide synthase 3	33.186	33.496	0.013	no
ASS1	Argininosuccinate synthase	0.315	1.701	2.430	yes
SLC7A1	High affinity cationic amino acid transporter 1	20.571	7.026	-1.550	yes
HSP90AA1	Heat shock protein HSP 90-alpha	369.379	437.263	0.243	yes

Table S4. Effects of ASL deficiency on expression of components of NO synthesis complex in HAECs