SUPPLEMENTAL MATERIALS

Cytokine-Mediated Degradation of the Transcription Factor ERG Impacts the Pulmonary Vascular Response to Systemic Inflammatory Challenge

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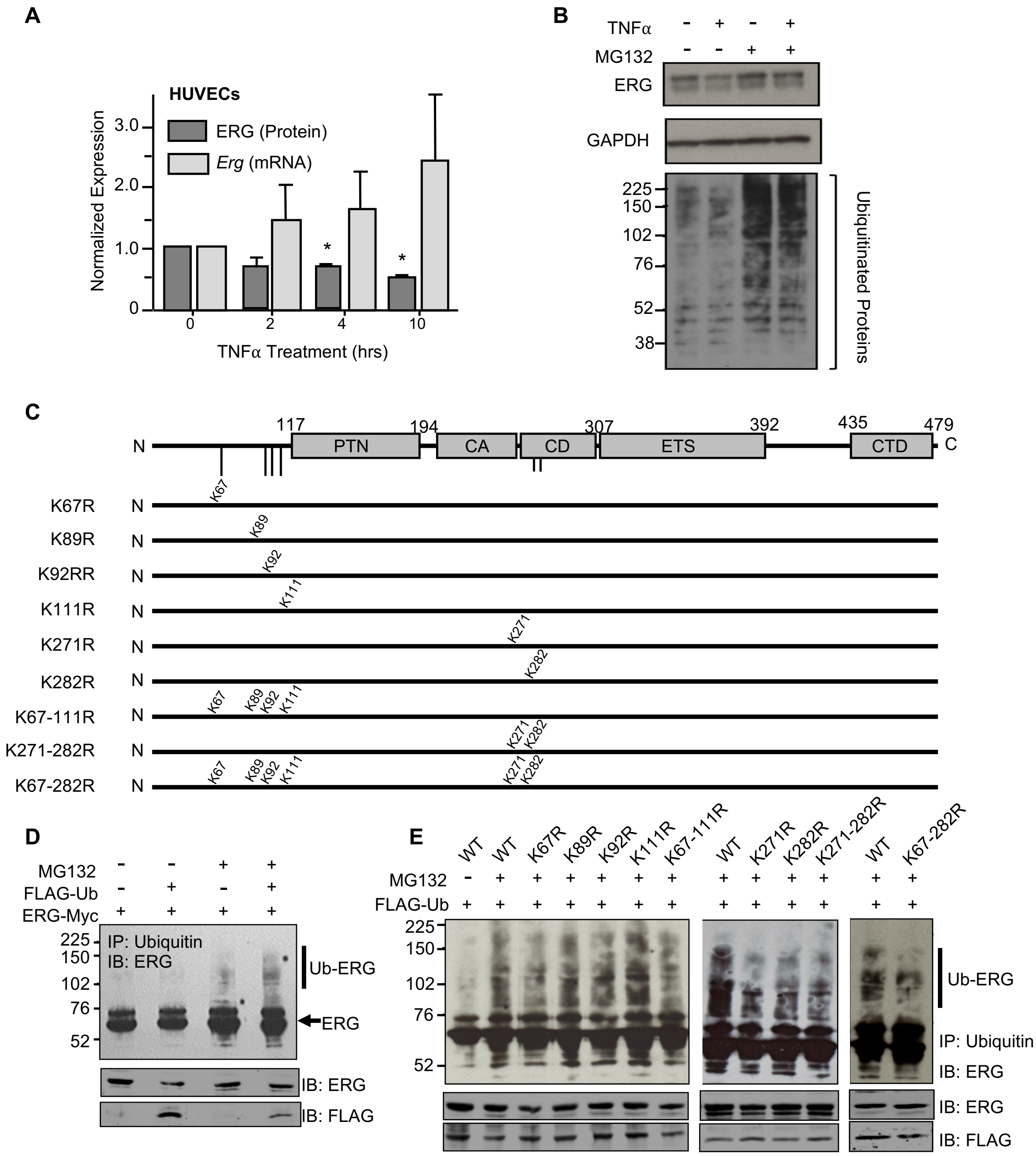
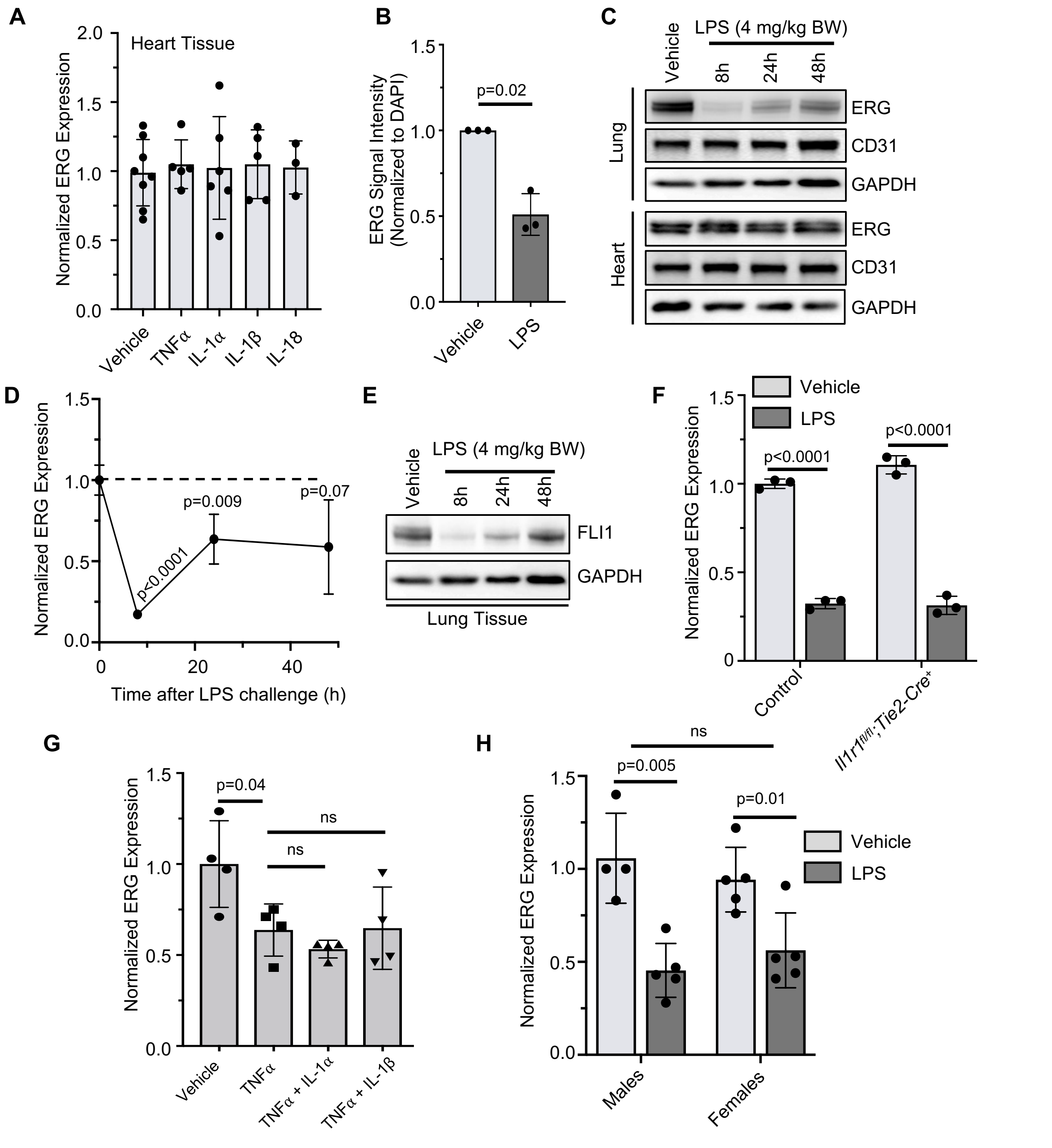
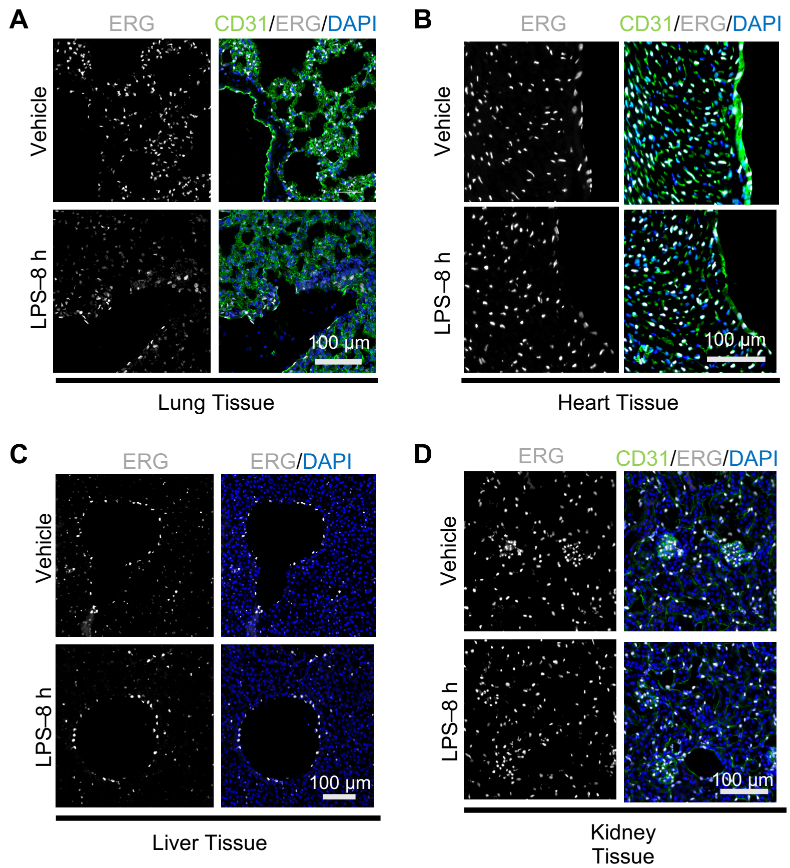


SUPPLEMENTAL FIGURE S1. *TNFα-induced ERG ubiquitination and proteasomal degradation in cultured ECs.* **(A)** HUVECs were treated with TNFα (10 ng/mL) for 0 – 10 h, and ERG protein and transcript expression were assessed by immunoblot and qPCR, respectively. **(B)** Immunoblot of ERG and ubiquitin in HUVECs following a 6 h treatment with TNFα (10 ng/mL) with and without a 30 min MG132 (10 μM) pre-treatment. **(C)** Schematic illustration of each ERG mutant generated for this study. **(D)** Transfection of HeLa Cells with wildtype ERG and a FLAG-tagged ubiquitin led to the accumulation of high MW ERG isoforms that could be isolated by ubiquitin immunoprecipitation. Ubiquitination of ERG was more prominent following proteasomal inhibition by MG132 treatment. **(E)** HeLa cells were transfected with each of the ERG constructs from (C). Cells were then treated with MG132, and immunoblots were used to assess the ERG ubiquitination status, as in (B). *P* values were determined by a 1-way ANOVA followed by a Dunnett's multiple comparison of each timepoint to the 0 h timepoint.

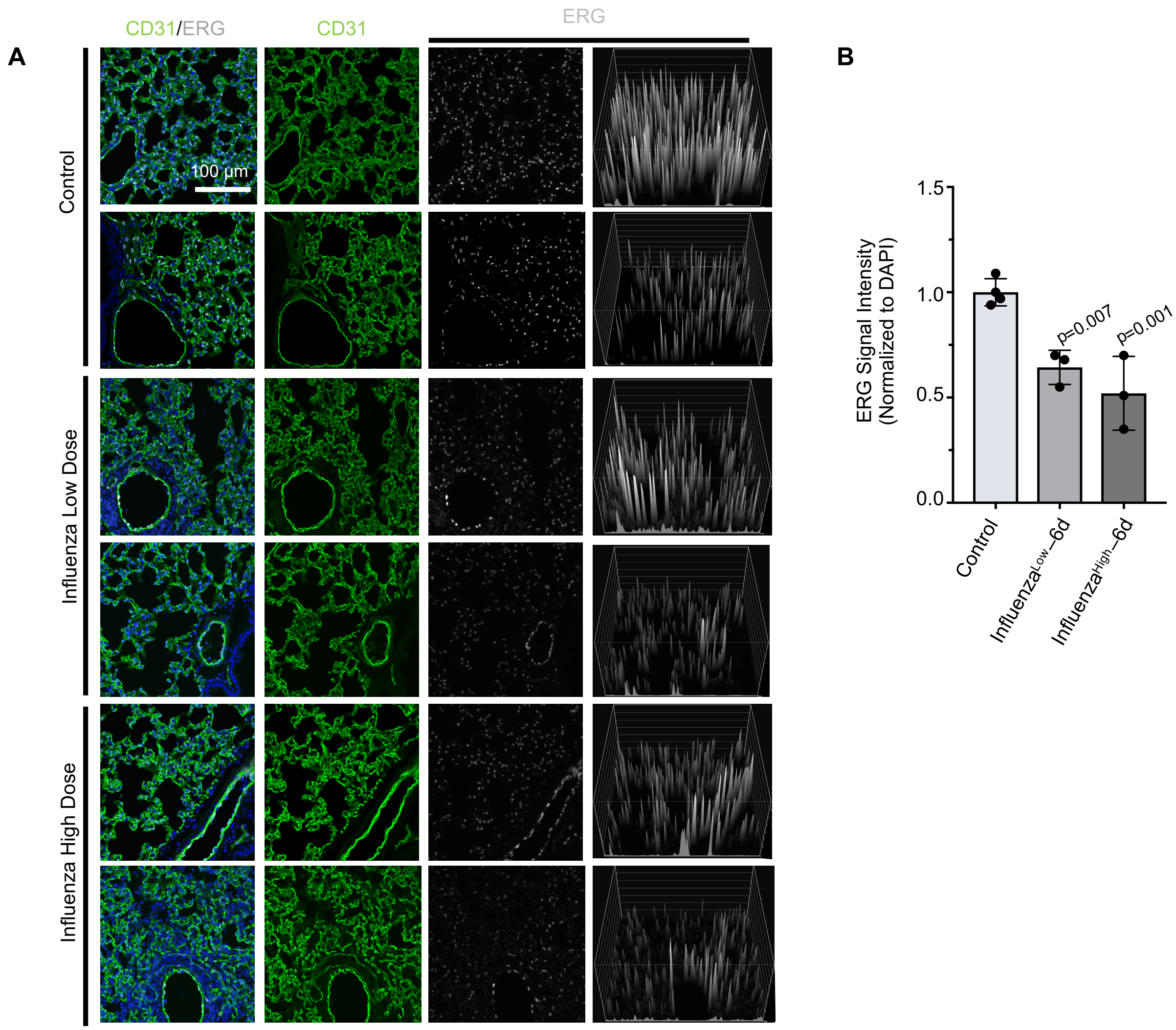


SUPPLEMENTAL FIGURE S2. *Pro-inflammatory cytokines promote lung-specific ERG down-regulation in vivo.* **(A)** Quantification of ERG expression in the heart after intravenous administration of LPS (1 mg/kg BW), TNFα, IL-1α, IL-1β, or IL-18 (50 µg/kg for each cytokine). **(B)** Immunofluorescent ERG intensity in lung tissue samples from vehicle- and LPS (4 mg/kg BW)-

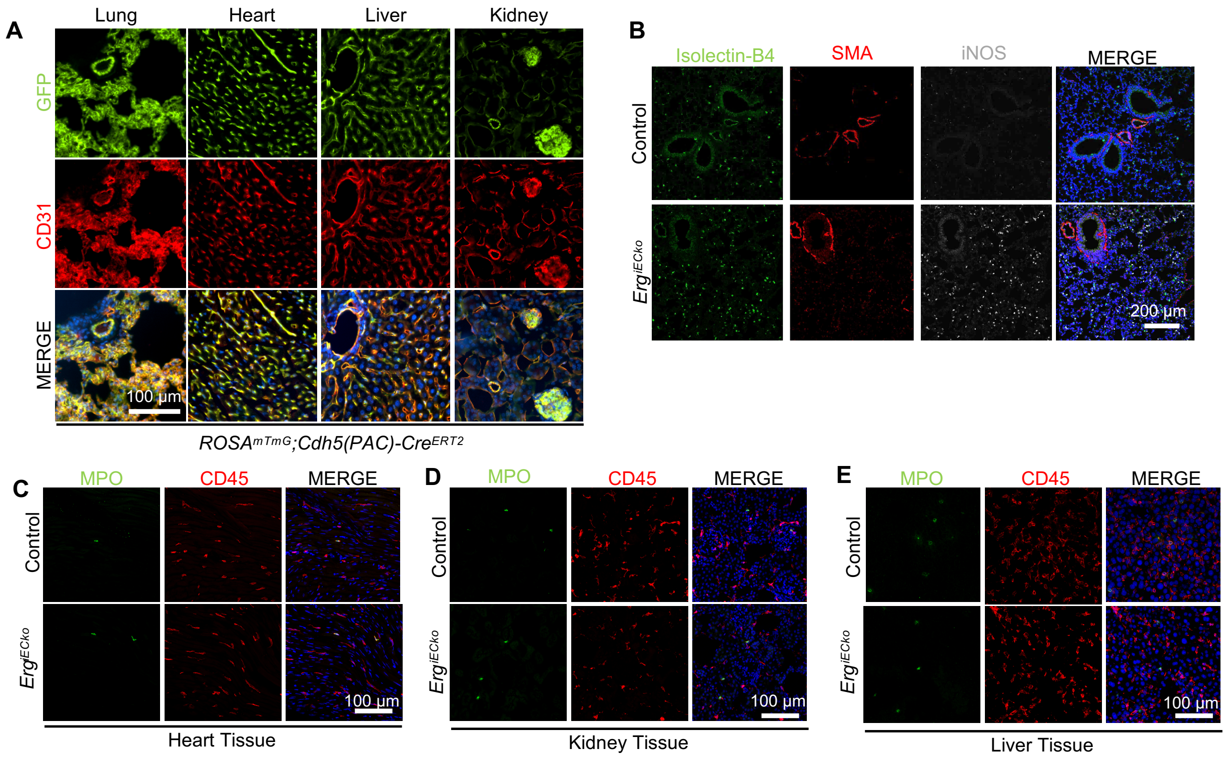
injected mice was quantified and normalized to DAPI (n=4). **(C,D)** ERG expression was assessed by immunoblotting lung and heart lysates at 8-48 h after LPS challenge (B) and pulmonary ERG expression was quantified by densitometry (n=4-5) (C). **(E)** At 8-48 h after LPS challenge, FLI1 expression in the lung was assessed by immunoblot. **(F)** ERG expression was assessed by immunoblot using lung lysates prepared 4 h after intraperitoneal LPS (4 mg/kg BW) injection into control and $Il1r1^{flox/flox}$;*Tie2-Cre*$^{+}$ mice (n=3). **(G)** ERG expression in HUVECs was quantified by immunoblot densitometry after a 10h treatment with vehicle or TNFα (10 ng/mL) in combination with IL-1α (10 ng/mL) or IL-1β (10 ng/mL) (n=4). **(H)** Statistical comparison of LPS-induced ERG downregulation in the lungs of male and female wildtype mice. No statistical difference was observed between genotypes. *P* values were determined by a Welch's t-test (B), a Brown-Forsythe ANOVA followed by a Dunnett's T3 multiple comparison test (D), a 2-way ANOVA followed by a Sidak's multiple comparison test (F), a 1-way ANOVA followed by a Sidak's multiple comparison test (G), or a 2-way ANOVA (H).



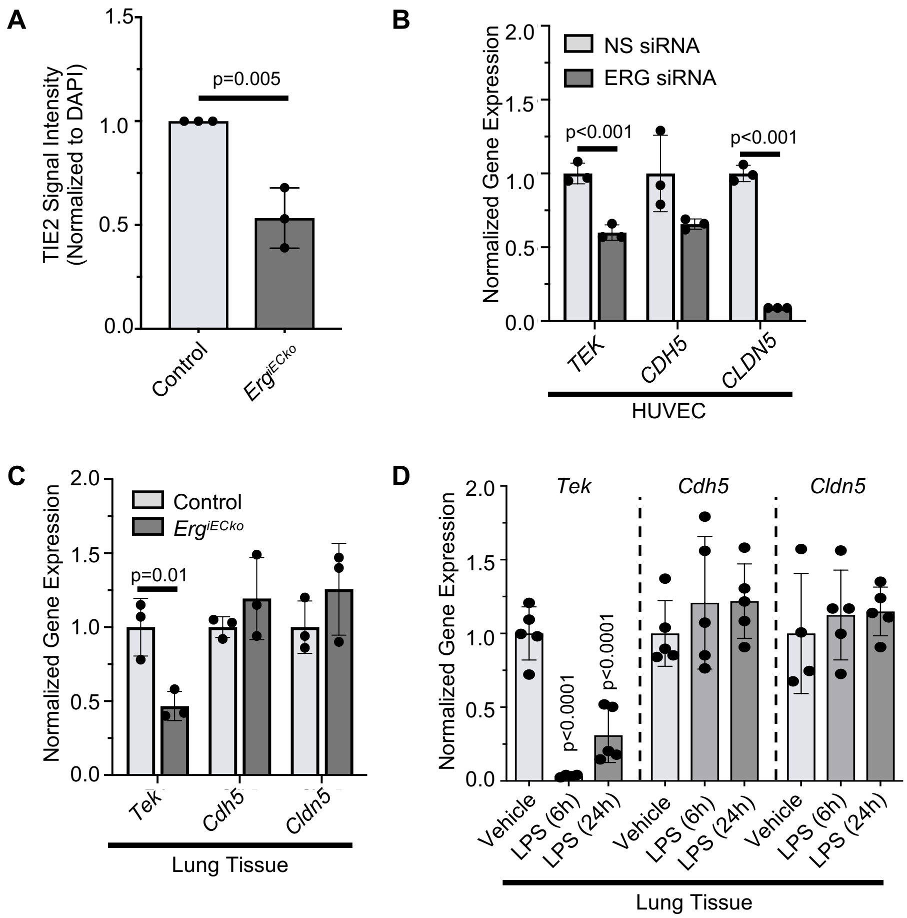
SUPPLEMENTAL FIGURE S3. *Organotypic downregulation of ERG and FLI1 following acute LPS challenge*. **(A-D)** The expression of ERG (grey) in ECs (CD31; green) at 8 h after acute LPS challenge (4 mg/kg BW) was assessed by immunostaining tissue sections of the lung (A), heart (B), kidney (C), and liver (D). DAPI (blue) was used as a nuclear counterstain.



SUPPLEMENTAL FIGURE S4. *Downregulation of ERG in pulmonary capillary ECs following intratracheal influenza infection*. **(A)** Additional images demonstrating the loss of ERG (grey) expression within pulmonary ECs (CD31; green) at 6 d after intratracheal influenza administration. DAPI (blue) was used as a nuclear counterstain. Right panel, the pixel intensity in the ERG channel is plotted as a surface intensity representation demonstrating the selective loss of ERG expression in capillary ECs, relative to larger caliber vessels, following influenza infection. **(B)** Quantification of immunofluorescent ERG signaling, normalized to DAPI, in lung tissue sections collected from vehicle- and influenza-infected mice (n=3). *P* values were determined by 1-way ANOVA followed by a Dunnett's multiple comparison test.



SUPPLEMENTAL FIGURE S5. *Organotypic effects of endothelial Erg deletion of immune cell recruitment*. **(A)** The *ROSA^{mTmG}* reporter line was crossed with the *Cdh5(PAC)-Cre^{ERT2}* line to demonstrate the specificity of Cre activity (GFP; green) to ECs (CD31; red) in the lung, heart, liver, and kidney. **(B)** Lung tissue sections from control and *Erg^{iECko}* mice were immunostained for Isolectin-B4 (green), smooth muscle actin (red), and iNOS (grey) demonstrating elevated immune cell recruitment in the lung following endothelial *Erg* deletion. **(C-E)** Total immune cells (CD45; red) and neutrophils (MPO; green) were visualized by immunostaining heart (C), kidney (D), and liver (E) tissue sections collected from control and *Erg^{iECko}* mice.



SUPPLEMENTAL FIGURE S6. *Differences in the transcriptional regulation of vascular stability by ERG in vitro and in vivo*. **(A)** The expression of TIE2 was quantified in lung tissue sections collected from control and *Erg^iECko* mice. Immunofluorescent TIE2 signal intensity was normalized against DAPI, which was used as a nuclear counter stain (n=3). **(B,C)** qPCR was used to quantify the expression of *TEK, CDH5,* and *CLDN5* in cultured HUVECs treated with non-specific (NS) or *Erg* siRNA (B;n=3) or murine lung tissue collected from control or *Erg^iECko* mice (C; n=3). **(D)** The expression of *Tek, Cdh5,* and *Cldn5* in wildtype murine lung tissue was also quantified following LPS treatment (4 mg/kg BW) for 6 and 24 h (n=4-5). *P* values were determined by a Welch's t-test (A), unpaired t-tests (B,C) or a 1-way ANOVA followed by a Dunnett's multiple comparison test (D).

SUPPLEMENTAL TABLE S1. A comprehensive list of mammalian ubiquitin ligase genes[41] was cross referenced against the gene expression profiles of ECs within the lung, heart, liver, and kidney previously identified by EC-TRAP[40]. mRNA abundance is based on log_2-transformed transcript per million (TPM) read values (n=3) and presented as a range from green (high abundance) to red (low abundance). All ubiquitin ligase genes that are >2-fold more highly expressed in lung ECs relative to the other organs are indicated in red.

	LOG2 (Transcripts per Million)			
E3 Ligases	**Lung**	**Heart**	**Liver**	**Kidney**
RNF7	9.23	8.42	7.93	8.31
RBX1	9.04	8.60	8.38	8.74
ANAPC11	8.66	8.52	8.37	8.60
RNF187	7.64	6.01	6.46	6.57
RNF141	7.36	5.90	5.40	4.72
RNF130	7.34	6.52	7.18	6.73
NSMCE1	7.34	7.14	6.80	7.19
RNF167	7.33	7.17	6.65	7.23
RNF114	7.03	7.37	6.86	7.61
RNF5	6.93	6.02	6.85	6.88
RCHY1	6.91	6.63	6.48	6.91
NOSIP	6.81	7.47	7.30	7.95
RNF181	6.68	7.03	7.03	7.41
RNF220	6.57	5.00	5.30	5.25
SPOP	6.47	6.55	6.11	6.27
RNF125	6.44	6.39	4.44	5.67
IRF2BP2	6.43	5.28	4.63	5.03
TRIM8	6.42	4.23	4.42	4.50
RNF144A	6.40	4.61	3.78	5.09
RNF4	6.18	6.72	6.37	6.39
RNF146	6.16	6.50	6.08	6.28
MKRN1	6.06	4.73	5.01	4.88
NEDD4	6.05	7.35	5.68	6.85
RBCK1	5.97	6.97	6.58	6.83
CYHR1	5.95	5.46	5.39	6.08
TRAF7	5.95	6.55	5.52	6.48
TRIM35	5.92	4.74	4.77	4.96
CGRRF1	5.91	5.95	5.80	6.09
MAEA	5.87	6.56	5.91	6.55
MYLIP	5.84	5.58	6.65	5.37
RNF10	5.84	5.61	5.19	5.65
RNF11	5.83	5.15	4.37	4.99
SMURF2	5.78	4.02	2.96	4.20
PEX2	5.70	5.86	5.76	5.98
RNF145	5.70	4.51	4.82	5.20
XIAP	5.67	4.70	4.25	4.64
MARCH2	5.67	4.91	5.40	4.92
RNF14	5.66	6.17	5.40	6.10
RNF185	5.63	5.71	5.64	5.88
ZNRF1	5.50	4.19	5.13	4.42
RNF44	5.50	5.30	4.75	5.45
BFAR	5.49	5.60	5.44	5.55
PPIL2	5.37	6.27	5.89	6.35

PJA2	5.36	5.76	4.77	5.33
RNF13	5.35	6.27	6.12	6.11
AMFR	5.33	5.32	5.75	5.61
PELI1	5.33	3.99	4.20	4.11
KCMF1	5.31	4.18	3.90	4.08
IRF2BPL	5.28	4.24	3.24	4.11
BIRC3	5.27	5.32	5.16	5.12
RMND5B	5.24	5.20	5.26	5.82
RNF115	5.20	5.03	5.18	4.67
TRIM25	5.18	4.72	4.20	4.15
TRIM28	5.15	5.08	5.31	5.31
ZFP91	5.15	4.26	3.90	4.17
RNF138	5.12	4.31	3.56	3.90
PJA1	5.10	6.21	6.02	6.15
RNF6	5.08	6.24	5.36	6.10
RBBP6	5.08	4.11	3.55	4.18
MARCH5	5.03	4.48	4.53	4.78
MAP3K1	4.99	6.15	5.09	5.40
STUB1	4.94	4.94	4.91	4.49
MARCH8	4.93	4.10	4.07	3.96
RNF2	4.92	4.95	4.59	4.75
RNF38	4.91	4.04	3.03	3.66
RNF19B	4.88	4.05	5.23	4.72
RNF144B	4.86	3.77	3.56	4.33
BIRC2	4.86	4.38	3.70	4.57
MARCH7	4.86	4.07	3.32	3.48
PCGF1	4.86	4.03	4.02	4.38
MGRN1	4.82	6.10	5.30	5.78
RNF41	4.81	5.06	4.78	5.20
RMND5A	4.81	4.32	4.32	4.05
ZNRF2	4.77	3.31	3.41	3.44
MSL2	4.75	3.68	3.31	3.70
RNF20	4.72	4.33	4.11	4.20
UBR7	4.72	4.87	4.28	4.99
RNF25	4.68	4.90	4.70	5.20
RNF215	4.65	3.69	3.54	4.58
UBE3B	4.63	4.36	3.78	4.06
CHFR	4.62	4.11	3.96	4.08
PCGF5	4.60	3.55	2.96	3.19
CNOT4	4.59	4.15	3.44	3.79
BRAP	4.59	3.75	3.73	4.21
TRAF2	4.58	5.73	5.39	6.06
RNF121	4.57	5.03	4.33	4.88
UHRF2	4.55	3.83	3.69	3.46
MKRN2	4.52	5.35	4.40	5.28
Mdm2	4.49	4.65	4.05	4.05
RNF19A	4.49	4.90	3.66	4.77
SYVN1	4.47	3.77	3.95	3.64
TRIM26	4.46	4.66	4.70	4.74
VPS41	4.42	5.36	5.17	5.17
RNF103	4.42	3.20	4.04	3.11
BMI1	4.40	3.52	2.85	3.46
UBE3A	4.37	3.61	3.44	3.66
MARCH6	4.36	3.65	2.63	3.70
MEX3C	4.36	3.52	2.29	2.97
RNF166	4.33	4.17	4.08	4.33
RNF213	4.32	4.75	4.88	4.72
LNX2	4.28	4.02	3.58	4.46
RLIM	4.28	4.12	3.33	4.06

DTX3	4.27	4.90	3.35	4.54
TRAF5	4.26	3.87	2.77	4.13
VPS11	4.23	5.29	4.62	5.32
HECW2	4.21	2.32	2.25	3.01
RNFT1	4.18	4.82	4.33	4.65
MNAT1	4.18	4.14	3.65	4.09
TRIM47	4.17	5.90	5.28	5.79
WWP1	4.16	4.05	2.81	3.28
CBLL1	4.16	3.68	3.16	3.36
TRIM41	4.14	3.22	4.27	3.55
MID2	4.14	2.19	1.65	2.59
RNF31	4.12	4.33	4.70	4.90
NFX1	4.09	5.14	4.16	4.40
SCAF11	4.07	3.66	3.33	3.31
NHLRC1	4.05	0.53	N.D	N.D
RNF216	4.05	4.15	3.92	4.22
RSPRY1	4.04	3.68	3.67	4.05
RFWD2	3.97	3.00	2.57	3.14
RNF168	3.96	3.42	2.90	3.22
TMEM129	3.88	5.20	4.28	4.77
RNF169	3.86	3.51	3.46	3.87
AFF4	3.85	3.04	2.37	2.94
TRIM3	3.81	3.94	3.31	4.17
SH3RF1	3.81	4.19	4.03	3.87
RNF40	3.79	4.40	4.16	4.41
MUL1	3.78	4.71	4.33	4.62
IRF2BP1	3.75	2.91	3.30	3.49
MIB1	3.75	3.93	3.32	3.62
NEURL3	3.73	5.13	6.16	5.57
RNF214	3.72	3.48	3.08	3.51
ARIH1	3.72	3.18	2.60	3.03
PCGF3	3.70	2.72	1.70	1.97
RNF111	3.69	3.49	3.09	3.20
MDM4	3.64	2.49	1.92	2.53
WDSUB1	3.61	4.97	3.99	4.88
RNF139	3.61	3.00	2.92	3.17
RNF26	3.61	3.15	2.87	2.77
ARIH2	3.60	3.31	2.81	2.94
RNF122	3.59	3.61	2.92	4.13
WWP2	3.55	4.99	4.27	4.92
Topors	3.54	3.24	3.27	3.11
RC3H2	3.53	2.60	2.15	2.53
NEURL1B	3.52	2.94	0.71	4.14
ZFPL1	3.48	4.85	4.79	3.75
TRIM32	3.46	4.60	3.94	4.06
TRIM27	3.46	2.46	2.79	2.80
HECTD1	3.45	3.69	2.68	2.87
CBLB	3.43	1.91	0.93	1.52
TRIP12	3.43	4.06	3.67	3.54
UBE4A	3.40	2.77	2.43	2.85
Itch	3.40	3.56	3.35	3.44
PML	3.39	4.65	4.45	4.37
FANCL	3.39	3.28	2.45	3.21
RNF135	3.38	4.27	4.91	4.28
SMURF1	3.38	3.12	2.87	3.51
ANKIB1	3.38	2.50	2.47	2.97
TRIM65	3.35	4.09	3.19	3.18
UBR3	3.32	3.76	2.52	3.35
TTC3	3.31	4.12	2.94	3.81

SIAH2	3.30	2.14	1.72	1.93
TRIM39	3.28	3.51	2.71	4.18
TRIM24	3.25	3.94	2.74	3.84
RNF8	3.25	0.83	2.64	2.28
TRIM2	3.24	2.09	2.04	2.59
DTX2	3.23	3.60	3.11	3.77
TRIM56	3.22	3.18	2.73	3.66
TRAF6	3.21	2.63	1.61	2.22
UBE4B	3.21	3.03	2.52	3.03
RFWD3	3.19	3.77	3.12	3.93
RNF217	3.19	1.56	0.86	1.32
TRIM37	3.16	3.38	2.82	3.62
KMT2D	3.13	3.34	2.91	3.34
TRIM11	3.12	3.25	3.33	2.70
HERC4	3.08	3.20	3.40	3.13
UNK	3.07	3.26	3.08	2.95
RNF149	3.06	2.31	3.90	2.50
RC3H1	3.06	2.97	2.02	3.29
ZNRF3	3.05	3.25	2.81	3.09
PHRF1	3.03	3.53	2.86	3.48
PCGF2	3.03	2.75	1.93	2.51
TRIM23	2.96	3.19	2.58	2.81
UBE3C	2.95	3.63	2.37	3.13
MARCH1	2.92	2.95	3.75	3.31
PEX12	2.91	3.09	2.94	3.14
ZXDC	2.89	2.09	2.00	2.21
PEX10	2.89	3.66	3.29	3.91
VPS18	2.86	3.68	3.47	3.38
VPS8	2.85	2.68	3.23	2.81
AREL1	2.81	3.29	2.71	2.67
RLF	2.80	1.66	1.62	1.43
HACE1	2.79	2.02	1.29	1.77
UBR5	2.78	3.04	2.45	2.80
E4F1	2.75	2.57	2.42	2.67
RNF170	2.73	2.55	1.81	2.36
HLTF	2.70	3.27	3.06	2.80
MECOM	2.68	4.58	3.34	3.61
TRIM59	2.61	2.28	0.45	2.37
UBR2	2.61	2.15	1.86	1.69
PRPF19	2.59	3.97	2.84	2.96
HECTD3	2.59	2.81	3.17	2.98
LTN1	2.58	2.63	1.31	1.94
MYCBP2	2.55	2.51	1.96	2.50
LONRF1	2.52	0.94	1.76	1.07
UBR1	2.51	2.73	1.37	2.26
HUWE1	2.44	2.56	1.71	2.42
TRIM13	2.42	2.59	1.44	2.78
RING1	2.38	1.50	1.82	2.03
RAD18	2.34	2.99	2.90	3.23
NEDD4L	2.29	1.51	1.14	1.64
UBR4	2.29	2.91	2.13	2.31
TRIM68	2.28	2.53	-0.18	2.25
RNF219	2.25	3.25	2.22	2.58
UBOX5	2.22	2.27	2.09	1.92
PELI2	2.19	2.21	0.29	1.87
CBL	2.19	1.71	1.74	2.10
RFFL	2.18	2.28	1.70	1.75
G2E3	2.18	1.89	1.25	2.09
KMT2C	2.17	1.75	1.35	1.87

UHRF1	2.13	4.24	3.45	4.18
TRAF3	2.11	1.19	1.10	1.89
TRIM62	2.07	0.96	1.07	2.41
MEX3D	2.07	0.89	0.17	0.91
NFXL1	1.99	2.41	2.92	2.07
TRIM5	1.95	2.26	2.03	1.75
PHF7	1.95	2.65	2.75	3.15
HERC6	1.88	1.95	2.11	2.60
TRIM33	1.88	1.62	0.98	1.82
TRAF4	1.77	1.85	2.37	2.39
MIB2	1.76	2.50	2.71	2.42
HERC1	1.71	2.33	1.37	1.62
UNKL	1.70	2.44	1.12	2.05
DTX3L	1.69	3.21	1.25	2.70
WDR59	1.68	2.03	2.39	1.47
HERC2	1.64	2.20	1.55	1.71
DTX1	1.61	0.49	1.34	N.D
TRIM45	1.57	1.89	1.99	1.98
SHPRH	1.42	1.09	0.37	0.76
RNF123	1.41	3.28	2.56	2.72
MARCH9	1.41	N.D	N.D	-0.23
DTX4	1.38	2.75	0.12	1.87
RNF126	1.26	N.D	0.07	N.D
TRIM6	1.19	-0.69	-1.44	-0.36
HERC3	1.19	1.90	1.58	0.97
TRIM21	1.17	0.45	0.47	0.85
RNF186	1.15	N.D	2.20	3.18
LRSAM1	1.12	3.02	2.47	2.55
PCGF6	0.95	0.68	0.35	-0.17
TRIM7	0.77	1.07	0.20	2.29
RNF157	0.67	1.56	3.20	2.98
TRIM46	0.59	N.D	1.45	0.10
PLAGL1	0.53	3.90	0.52	-0.88
RNF128	0.44	1.51	2.88	2.60
MID1	0.35	0.96	1.85	0.79
TRIM36	0.32	1.22	-0.34	0.75
RNF150	0.30	1.28	N.D	N.D
RNF180	0.15	2.64	3.16	1.55
CBLC	N.D.	N.D	1.15	-0.61
CUL9	N.D.	1.44	1.25	1.48
DCST1	N.D.	N.D	N.D	1.91
LNX1	N.D.	4.94	-1.14	1.93
PARK2	N.D.	0.75	1.39	0.74
PDZRN3	N.D.	1.32	-0.43	1.12
RAPSN	N.D.	2.71	N.D	1.84
RNF152	N.D.	0.82	-1.03	0.61
RNF183	N.D.	N.D	N.D	2.95
RNF207	N.D.	3.01	N.D	N.D
RNF222	N.D.	N.D	1.29	0.56
RNF43	N.D.	N.D	0.96	-0.69
TRAIP	N.D.	1.43	1.26	1.57
TRIM10	N.D.	1.70	0.63	0.25
TRIM54	N.D.	4.49	N.D	N.D
TRIM55	N.D.	2.60	N.D	N.D
TRIM63	N.D.	4.70	N.D	1.18
TRIM72	N.D.	1.92	N.D	N.D

SUPPLEMENTAL TABLE S2. List of all primers used for genotyping, qPCR, and mutagenesis performed in this study.

Primer Name	Sequence	Application
Il1r1-flox - F	5`-GAAAAGTGCTAGAACATCCTTTGAG	Genotyping (*Il1r1-flox*)
Il1r1-flox - R	5`-GTACCAATGGAGGCCAGAAG	
Tnfa-WT - F	5`-TAGCCAGGAGGGAGAACAGA	Genotyping (TNFa-KO)
Tnfa-WT - R	5`-AGTGCCTCTTCTGCCAGTTC	
Tnfa-MUT - R	5`-CGTTGGCTACCCGTGATATT	
iCdh5-Cre - F	5`-GTACCAATGGAGGCCAGAAG	Genotyping for *Cdh5(PAC)-Cre ERT2*
iCdh5-Cre - R	5`-CGAACCTGGTCGAAATCAGT	
Control - F	5`-CGAACCTGGTCGAAATCAGT	
Control - R	5`-GTAGGTGGAAATTCTAGCATCATCC	
Erg-flox #1 - F	5`-AGAGTCTCTGCACACAGAACTTCC	Genotyping for *Erg-flox*
Erg-flox #1 - R	5`-AATGCTCTGGTAAGGCACACAAGG	
loxP-FWD	5`-GAGATGGCGCAACGCAATTAATG	
Erg-flox #2 - F	5'- AGATTTTGTTCTGGTTAACAAGCCGTGC	Genotyping for *Erg-flox*
Erg-flox #2 - R	5'- AATGAGACAGAGCCATGAGGTAGATGGG	
VE Cad - F	5`-GCAGGCAGCTCACAAAGGAACAAT	Genotyping for *cVE Cad-Cre*
VE Cad - R	5`-TGTCCTTGCTGAGTGACAGTGGAA	
VE Cad Cre - R	5`-ATCACTCGTTGCATCGACCGGTAA	
Tie2-Cre - F	5`-GGGAAGTCGCAAAGTTGTGAGTTG	Genotyping for TIE2-Cre
Tie2-Cre - R	5`-TCCATGAGTGAACGAACCTGGTCG	
Control - F	5`-CGAACCTGGTCGAAATCAGT	
IControl - R	5`-GTAGGTGGAAATTCTAGCATCATCC	
Erg001-F	5'-CCAAGCTTTTGATCGCATTATGGCCAGC	pcDNA3.1-Erg-Myc cloning
Erg-R-Myc	5`CTCTAGATTACAGGTCCTCCTCGCTGATCAGCTTCTGCTCG-TAGTAAGTGCCCAGATGAGAAGG	pcDNA3.1-Erg-Myc clone
K67	5'-AGCCAGGGTCACCATCAGAATGGAATGTAACCCTA	pcDNA3.1-Erg-Myc Mutagenesis
K89	5'-GATGAATGCAGTGTGGCCAGAGGCGGGAAGA	pcDNA3.1-Erg-Myc Mutagenesis
K92	5'-GCTGCCCACCATCCTCCCGCCTTTGGC	pcDNA3.1-Erg-Myc Mutagenesis
K92b	5'-CCAGAGGCGGGAGGATGGTGGGCAG	pcDNA3.1-Erg-Myc Mutagenesis
K111	5'-CGGCAGCTACATGGAGGAGAGGCACATGCCAC	pcDNA3.1-Erg-Myc Mutagenesis
K271	5'-CCACGCCCCAGTCGAGAGCTGCTCAACC	pcDNA3.1-Erg-Myc Mutagenesis
K282	5'-CGCTGGTCTTCAGTTCTGGGCACTGTGGAAG	pcDNA3.1-Erg-Myc Mutagenesis
mActb-F	5`-TGTTACCAACTGGGACGACA	qPCR: Murine Housekeeping Gene
mActb-R	5`-GGGGTGTTGAAGGTCTCAAA	
hActb-F	5`-CTCTTCCAGCCTTCCTTCCT	qPCR: Human Housekeeping Gene
hActb-R	5`-AGCACTGTGTTGGCGTACAG	
mGapdh-F	5`-TCAACGGCACAGTCAAGG	qPCR: Murine Housekeeping Gene
mGapdh-R	5`-ACTCCACGACATACTCAGC	
hGapdh-F #1	5`-GAGTCAACGGATTTGGTCGT	qPCR: Human Housekeeping Gene
hGapdh-R #1	5`-GACAAGCTTCCCGTTCTCAG	
hGapdh-F #2	5'-CAAGGTCATCCATGACAACTTTG	qPCR: Human Housekeeping Gene
hGapdh-R #2	5'-GGGCCATCCACAGTCTTCTG	
hRn18S-F	5`-CCCGAAGCGTTTACTTTGAAA	qPCR: Murine/Human House-keeping Gene
hRn18S-R	5`-CGCGGTCCTATTCCATTATTC	
mErg-F	5`-GCTCAGCCATCTCCCTCTGCAG	qPCR: Murine *Erg* expression
mErg-R	5`-TCGAGCAGGAACTGCCACAGC	

hErg-F	5’-GGAGTGGGCGGTGAAAGA	qPCR: Human *Erg* expression
hErg-R	5’-AAGGATGTCGGCGTTGTAGC	
mTek-F	5`-CTGTGGAGTCAGCTTGCTCCTTT	qPCR: Murine *Tek* expression
mTek-R	5`-ACCTCCAGTGGATCTTGGTGCTG	
hTek-F	5`-TACACCTGCCTCATGCTCAG	qPCR: Human *Erg* expression
hTek-R	5`-TTCACAAGCCTTCTCACACG	

SUPPLEMENTAL TABLE S3. Description of statistical tests used for comparisons.

Figures	Panel	Normality	Equal Variance	Transformed	Statistical Test	Post hoc Test	Comparisons	p value
1	B	YES (Shapiro-Wilk)	NO (Brown-Forsythe)		Kruskal-Wallis	Dunn's	Control vs TNFa	0.0042
							MG132 vs MG132+TNFa	>0.9999
2	B	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	Vehicle vs TNFa	0.0003
							Vehicle vs IL-1a	<0.0001
							Vehicle vs IL-1b	<0.0001
							Vehicle vs IL-18	0.1826
2	C	YES (Shapiro-Wilk)	NO (Brown-Forsythe)		Welch t-test		Lung_Vehicle vs LPS	0.047833
							Heart_Vehicle vs LPS	0.027198
							Liver_Vehicle vs LPS	0.047833
							Kidney_Vehicle vs LPS	0.012966
							Retina_Vehicle vs LPS	0.396898
2	D	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Lung_Vehicle vs LPS	0.000048
							Heart_Vehicle vs LPS	0.489644
							Liver_Vehicle vs LPS	0.489644
							Kidney_Vehicle vs LPS	0.669104
							Retina_Vehicle vs LPS	0.669104
3	A	YES (Shapiro-Wilk)	YES (Spearman's)		Two-way ANOVA	Sidak's	Control: Vehicle vs LPS	<0.0001
							Tnfa-KO: Vehicle vs LPS	0.1771
3	B	YES (Shapiro-Wilk)	NO (Spearman's)	Log	Two-way ANOVA	Sidak's	Control: Vehicle vs LPS	0.0475
							Il1r1-KO: Vehicle vs LPS	0.7742
3	C	YES (Shapiro-Wilk)	NO (Spearman's)	Log	Two-way ANOVA	Sidak's	Control: Vehicle vs LPS	0.0044
							Il1r1-ecKO: Vehicle vs LPS	0.0136
3	F	YES (Shapiro-Wilk)	NO (Spearman's)	Log	Two-way ANOVA	Sidak's	Control vs LPS	0.0007
							MG132 vs LPS+MG132	0.122
4	A	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	Control vs Influenza (Low-2d)	0.995
							Control vs Influenza (Low-6d)	0.0156
							Control vs Influenza (High-6d)	0.0203
5	A	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		ERG: NS vs ERG siRNA	<0.000001
							TEK: NS vs ERG siRNA	0.001371
5	C	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	3'UTR vs Enh	0.0002
5	D	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Lung: Control vs Erg iECko	0.013343
							Heart: Control vs Erg iECko	0.612869
							Liver: Control vs Erg iECko	0.203006
							Kidney: Control vs Erg iECko	0.606031
5	F	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Lung: Control vs Erg iECko	0.000388
							Heart: Control vs Erg iECko	0.553215
							Liver: Control vs Erg iECko	0.525163
							Kidney: Control vs Erg iECko	0.150877
5	H	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	t=0 vs t=6h	<0.0001
							t=0h vs t=24h	<0.0001
							t=0h vs t=48h	<0.0001
							t=0h vs t=72h	0.001
5	I	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	t=0 vs t=6h	<0.0001
							t=0h vs t=24h	0.0446
							t=0h vs t=48h	0.3494
							t=0h vs t=72h	0.6925
6	A	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Lung: Control vs Erg iECko	0.003417
							Heart: Control vs Erg iECko	0.684657
							Liver: Control vs Erg iECko	0.873277
							Kidney: Control vs Erg iECko	0.626458
6	E	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Control vs Erg iECko	0.0066
6	F	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Control vs Erg iECko	0.438
6	I	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Control vs Erg iECko	0.0073
6	J	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Control vs Erg iECko	0.0035
S1	A	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	t=0h vs t=2h	0.0724
							t=0h vs t=4h	0.0074
							t=0h vs t=8h	0.0038
S2	B	YES (Shapiro-Wilk)	NO (Brown-Forsythe)		Welch t-test		Vehicle vs LPS	0.0199
S2	D	NO (Shapiro-Wilk)	YES (Brown-Forsythe)		Brown-Forsythe ANOVA	Dunnett's T3	t=0h vs t=6h	<0.0001
							t=0h vs t=24h	0.0088
							t=0h vs t=48h	0.0716
S2	F	YES (Shapiro-Wilk)	NO (Spearman's)	Log	Two-way ANOVA	Sidak's	Control:Vehicle vs Control:LPS	<0.0001
							ECko:Vehicle vs ECko:LPS	<0.0001
S2	G	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Sidak's	Vehicle vs TNFa	0.0438
							TNFa vs TNFa+IL1a	0.8112
							TNFa vs TNFa+IL1b	0.9996
S2	H	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Two-way ANOVA		Treatment Interaction	<0.0001
							Gender Interaction	0.222
S4	B	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	Control vs Influenza-Low-D6	0.0076
							Control vs Influenza-High-D6	0.0015
S6	A	YES (Shapiro-Wilk)	NO (Brown-Forsythe)		Welch t-test		Control vs Erg iECko	0.005
S6	B	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		TEK: NS vs ERG siRNA	0.0008
							CDH5: NS vs ERG siRNA	0.7486
							CLDN5: NS vs ERG siRNA	0.0006
S6	C	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		Unpaired t-test		Tek: NS vs ERG siRNA	0.0156
							Cdh5: NS vs ERG siRNA	0.8546
							Cldn5: NS vs ERG siRNA	0.7523
S6	D	YES (Shapiro-Wilk)	YES (Brown-Forsythe)		One-way ANOVA	Dunnett's	TEK: t=0h vs t=6h	<0.0001
							TEK: t=0h vs t=24h	<0.0001

SUPPLEMENTAL TABLE S4. List of mouse genders used for the generation of figure data.

Figure	Condition	Males	Females
2B	Vehicle	4	2
	TNFa	4	1
	IL1a	3	2
	IL1b	4	2
	IL-18	2	1
1C	Vehicle	3	
	LPS-2h	3	
1D	Vehicle	4	2
	LPS-8h	4	2
3A	Control+Vehicle	3	2
	Control + LPS	3	3
	Tnfa-KO + Vehicle	2	3
	Tnfa-KO + LPS	4	3
3B	Control + Vehicle	2	2
	Control + LPS	3	2
	Il1r1-GKO + Vehicle	3	1
	Il1r1-GKO + LPS	4	2
3C	Control + Vehicle	4	
	Control + LPS	4	
	Il1r1-ECko + Vehicle	4	
	Il1r1-ECko + LPS	4	
3F	Control	1	2
	LPS	1	2
	MG132	2	2
	LPS + MG132	2	3
4A	Control		3
	Influenza-Low-2d		3
	Influenza-Low-6d		3
	Influenza-High-6d		3
5D	Control		3
	Erg iECko		3
5F	Control		4
	Erg iECko		4
5H	t=0	3	4
	t=6	3	2
	t=24	4	4
	t=48	3	3
	t=72	2	4
5I	t=0	3	4
	t=6	3	2
	t=24	4	4
	t=48	3	3
	t=72	2	4
6A	Control	1	3
	Erg iECko	2	3
6E	Control	3	1
	Erg iECko	3	1
6F	Control	3	1
	Erg iECko	3	1
6I	Control	4	0
	Erg iECko	4	0
6J	Control	4	0
	Erg iECko	4	0

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mouse	Jackson Laboratory	C57Bl/6J	M/F	#000664
Mouse	Jackson Laboratory	*Il1r1^{flox}*	M/F	#028398
Mouse	Jackson Laboratory	*Tnfa$^{-/-}$*	M/F	#003008
Mouse	Taconic	*Cdh5(PAC)-Cre^{ERT2}*	M/F	#13073
Mouse	Jackson Laboratory	*VE-Cadherin-Cre*	M/F	#006137
Mouse	Jackson Laboratory	*Tie2-Cre*	M/F	#008863

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male					
Parent - Female					

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot #	Persistent ID / URL
ERG	Abcam	ab92513	1:200 – 1:1000		RRID:AB_2630401
ERG	Santa Cruz	sc-353	1:500 – 1:2000		RRID:AB_675518
FLI1	Abcam	ab15289	1:1000		RRID:AB_301825
CD31	R&D Systems	AF3628	1:1000		RRID:AB_2161028
Hemagglutinin	BEI Resources	NR-3148	1:200		https://www.beiresources.org/Catalog/BEIPolyclonalAntiserum/NR-3148.aspx
TIE2	R&D Systems	AF762	1:100		RRID:AB_2203220
CD45	R&D Systems	AF114	1:100		RRID:AB_442146
MPO	Epredia	RB373-A0	1:100		https://www.fishersci.com/shop/products/lab-vision-myeloperoxidase-mpo-rabbit-polyclonal-antibody-bsa-azide/RB373A0
FLAG	Sigma	F180	1:1000		RRID:AB_262044
GAPDH	Sigma	G9545	1:1000		RRID:AB_796208
GAPDH	Millipore	MAB374	1:1000		RRID:AB_2107445

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL

Cultured Cells

Name	Vendor or Source	Sex (F, M, or un-known)	Persistent ID / URL
Human Umbilical Vein ECs	ATCC	Unknown	#PCS-100-010

Data & Code Availability

Description	Source / Repository	Persistent ID / URL

Other

Description	Source / Repository	Persistent ID / URL

ARRIVE GUIDELINES

Figure 2B

Groups	Sex	Age	Number (prior to experi-ment)	Number (after ter-mination)	Littermates (Yes/No)	Other description
Group 1 (Control)	M/F	8-12 weeks old	5	5	Yes	
TNFα	M/F	8-12 weeks old	5	5	Yes	
IL-1α	M/F	8-12 weeks old	5	5	Yes	
IL-1β	M/F	8-12 weeks old	5	5	Yes	

Figure 2C and D

Groups	Sex	Age	Number (prior to experi-ment)	Number (after ter-mination)	Littermates (Yes/No)	Other description
Group 1 (Control)	M/F	8-12 weeks old	3-5	3-5	Yes	
LPS	M/F	8-12 weeks old	3-5	3-5	Yes	

Figure 3A-C,F

Groups	Sex	Age	Number (prior to experi-ment)	Number (after ter-mination)	Littermates (Yes/No)	Other description
Group 1 (Control)	M/F	8-12 weeks old	4-6	4-6	Yes	
LPS	M/F	8-12 weeks old	4-6	4-6	Yes	

Figure 4A

Groups	Sex	Age	Number (prior to experi-ment)	Number (after ter-mination)	Littermates (Yes/No)	Other description
Group 1 (Control)	F	6-8 weeks old	3	3	Yes	
Influ-enza_Low Dose_2 day	F	6-8 weeks old	3	3	Yes	

Influ-enza_Low Dose_6 day	F	6-8 weeks old	3	3	Yes	
Influ-enza_High Dose_6 day	F	6-8 weeks old	3	3	Yes	

Figure 5 D-F

Groups	Sex	Age	Number (prior to experi-ment)	Number (after ter-mination)	Littermates (Yes/No)	Other description
Group 1 (Control)	M/F	8-12 weeks old	3-4	3-4	Yes	
Erg iECko	M/F	8-12 weeks old	3-4	3-4	Yes	

Figure 6 A,E,F

Groups	Sex	Age	Number (prior to experi-ment)	Number (after ter-mination)	Littermates (Yes/No)	Other description
Group 1 (Control)	M/F	8-12 weeks old	4-6	4-6	Yes	
Erg iECko	M/F	8-12 weeks old	4-6	4-6	Yes	