Supplemental information

IL-1β suppression of *VE-cadherin* transcription underlies sepsis-induced inflammatory lung injury

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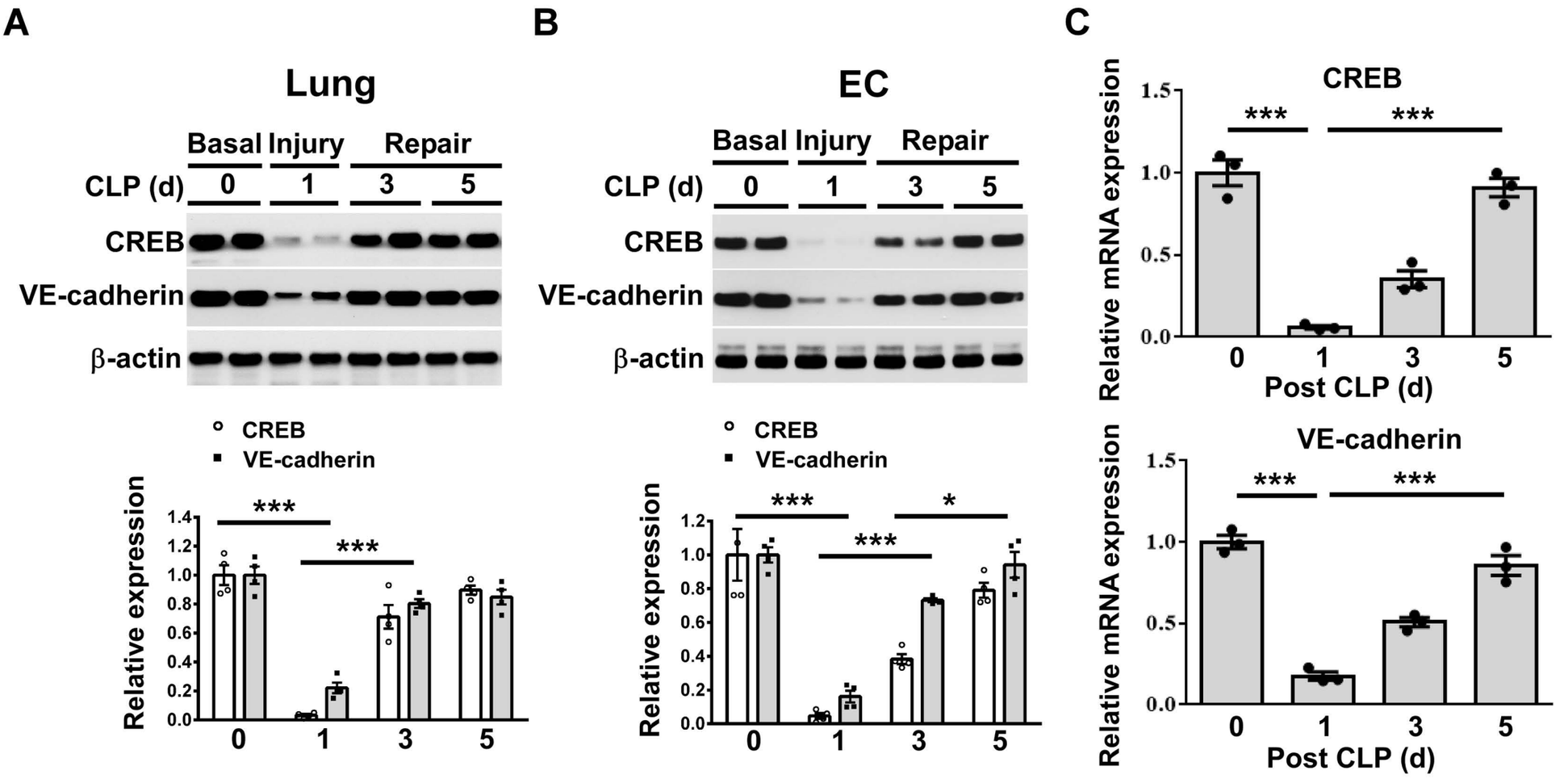
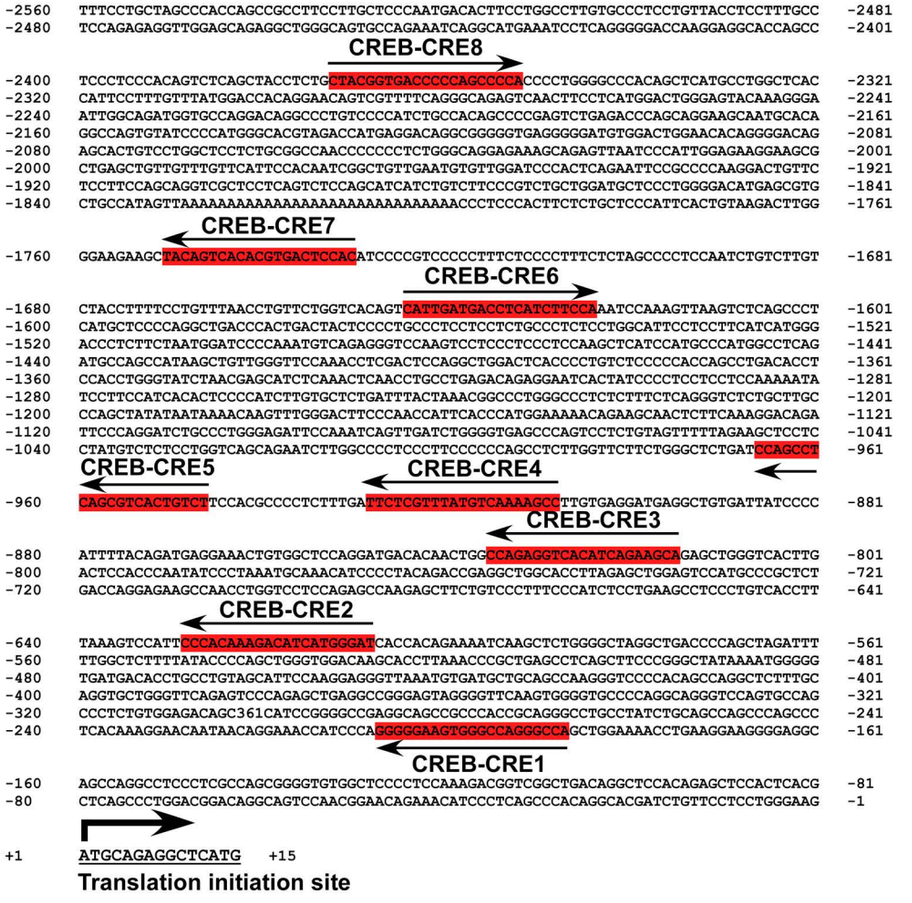


Supplementary Figure 1. Downregulation of CREB-VE-cadherin signaling is involved in CLP-induced lung vascular injury.

C57BL/6J mice (n=3) were subjected to cecal ligation and puncture (**CLP**) surgery for 1, 3, and 5 days. Protein expression was analyzed in whole lung lysates **(A)** and in fresh isolated endothelial cells **(B)** from mice underwent CLP surgery. **(C)** Real-time PCR detection of mRNA levels in fresh isolated endothelial cells was performed (n=3). Representative immunoblots and the bar graph quantification were shown. **P*<0.05, ***P*<0.01, ****P*<0.001.

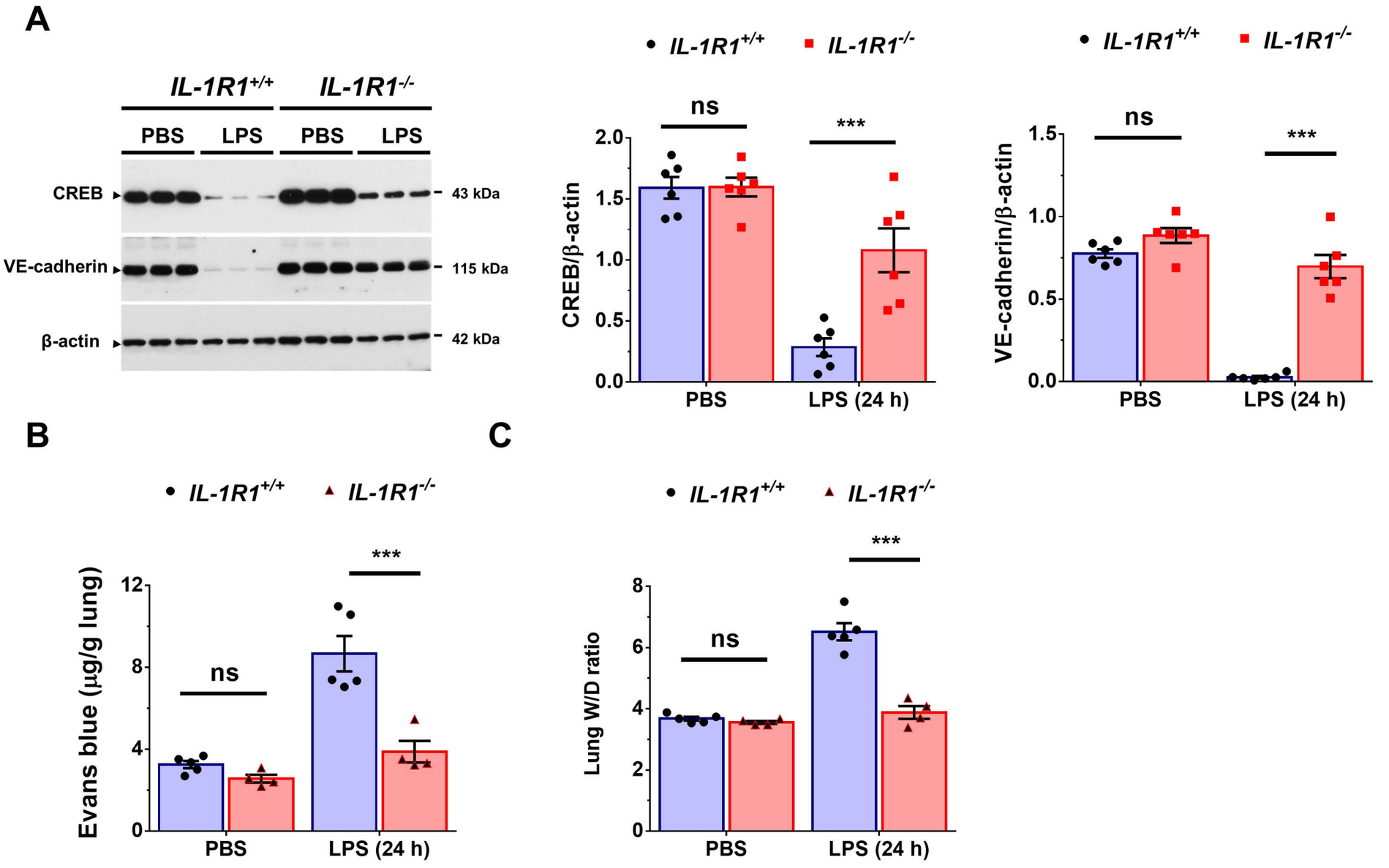
Promoter region of mouse VE-cadherin (NM_009868, 784 aa), Chromosome 8.

Promoter region of human VE-cadherin (NM_001795, 784 aa), Chromosome 16.

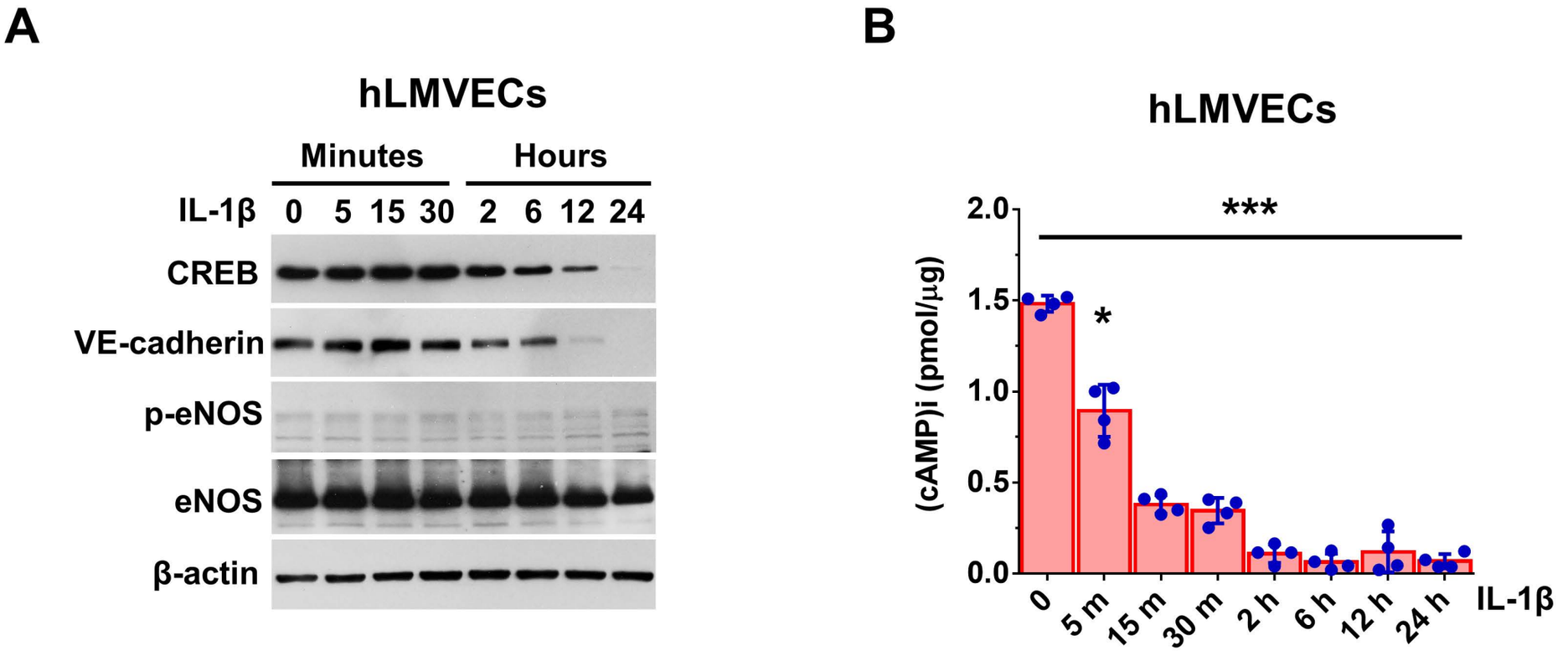


Supplementary Figure 2. Predicted CREB binding CREs within the mouse and human *VE-cadherin* promoter.

The 2.5 kb promoter region of the mouse and human *VE-cadherin* contains multiple highly conserved cyclic-AMP response elements (CREs).

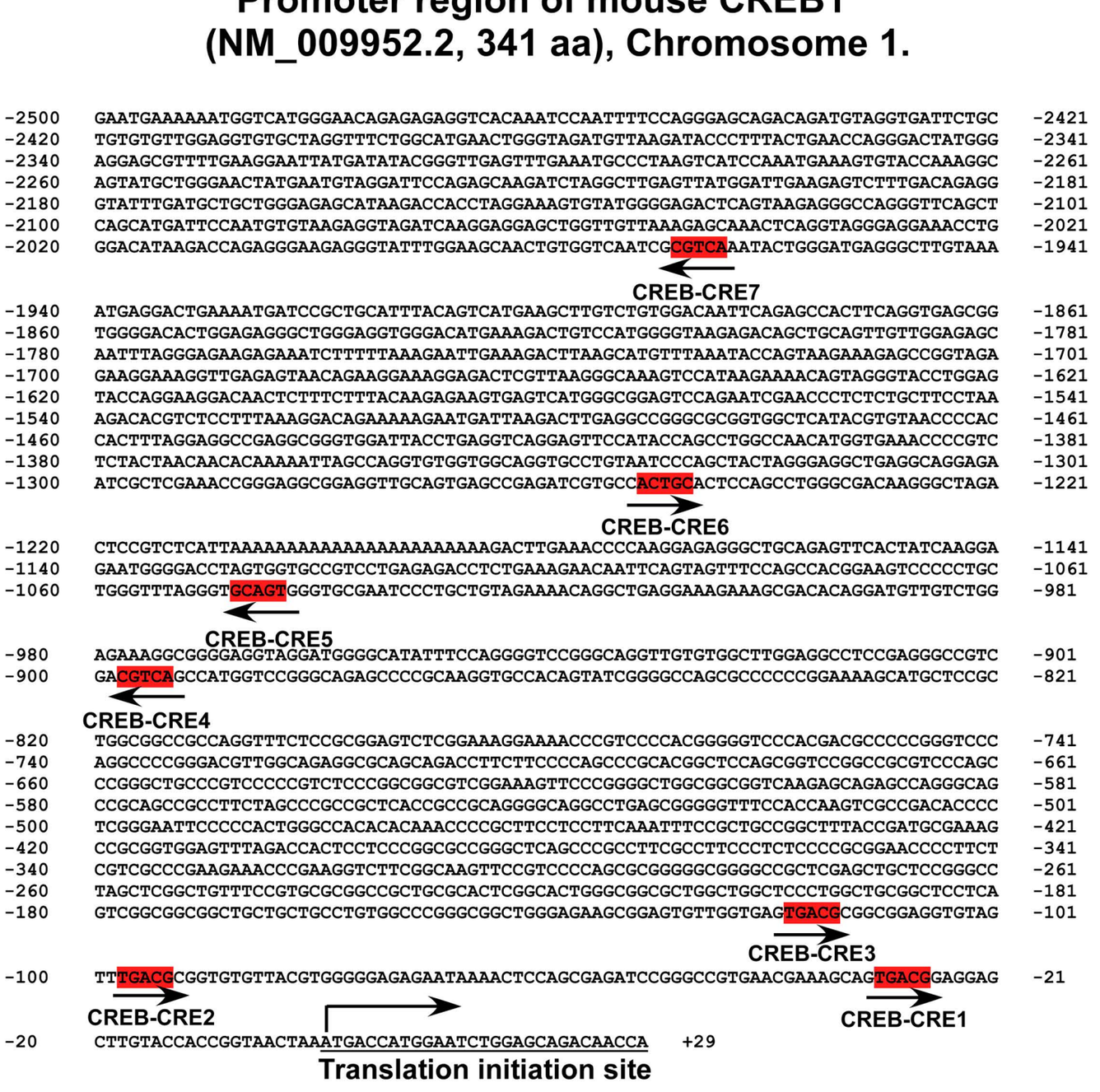


Supplementary Figure 3. IL-1R1 is required for LPS-induced lung vascular leakage.
(A) *IL-1R1*$^{-/-}$ and control mice (n=6) were challenged with LPS (12 mg/kg, i.p.) for 1 day. mLMVECs were freshly isolated using anti-CD31 and magnetic beads. Protein expression in endothelial cells was detected by Western blot using the indicated antibodies. Quantification of protein expression changes in mLMVECs was shown as the ratio of target protein to β-actin using Image J. ***P<0.001; "ns", no significant difference. **(B** and **C)** *IL-1R1*$^{-/-}$ and control mice were challenged with LPS (12 mg/kg, i.p.) for 1 day. (**B**) Lung vascular permeability is detected by Evans Blue dye leakage in the lungs. Extracted dye contents in the formamide extracts were quantified by measuring at 620 nm. (**C**) The ratio of the wet lung to dry lung weight was assessed. Results are shown as mean ± SEM, n=4-5. ***P < 0.001; ns, no significant difference. Statistics obtained from ANOVA.

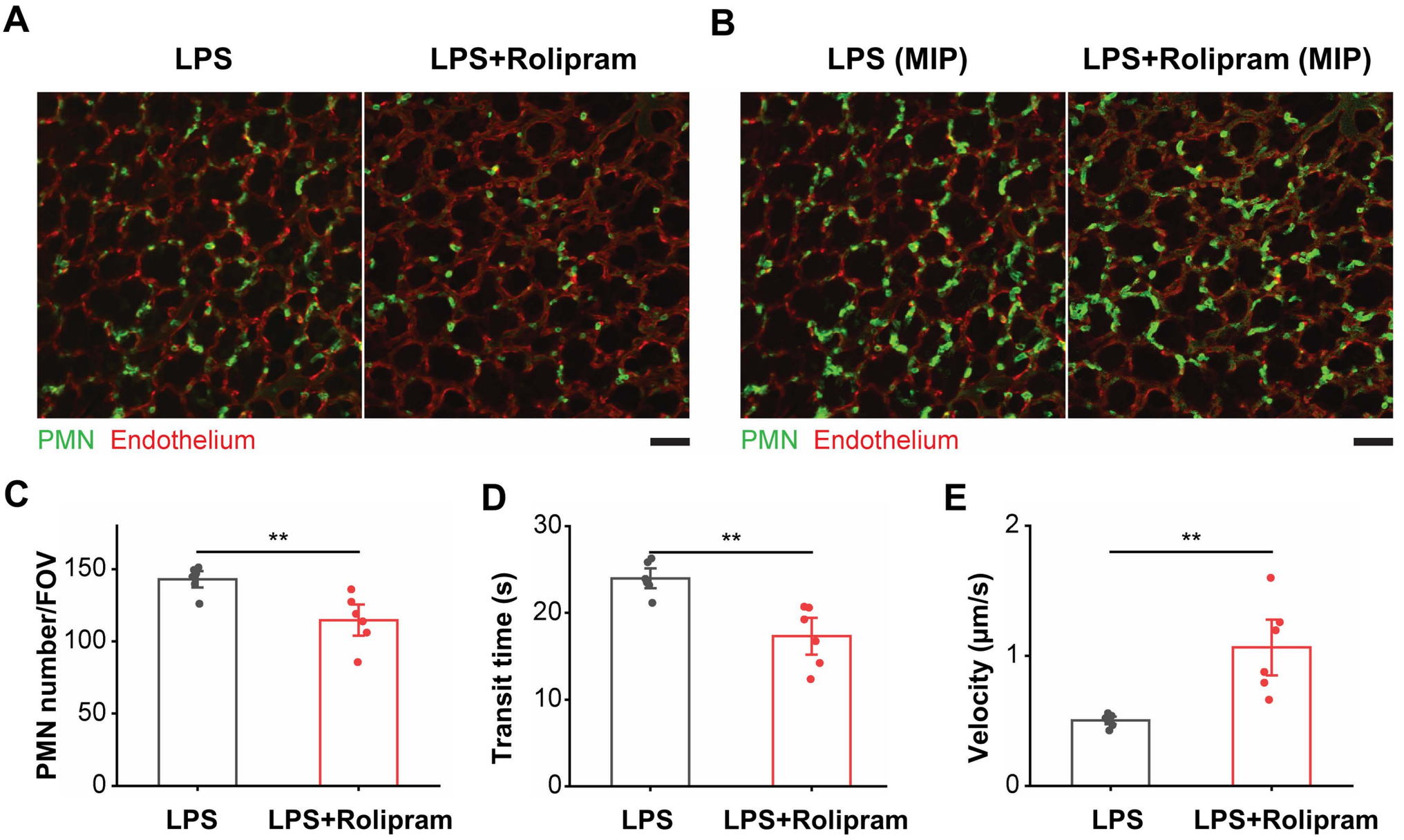


Supplementary Figure 4. IL-1β decreases cAMP-CREB-VE-cadherin signaling in hLMVECs.

hLMVECs were stimulated with IL-1β (5 ng/ml) over time. **(A)** Protein expression and phosphorylation were detected by Western blot using the indicated antibodies. **(B)** Intracellular cAMP levels were measured. Results are shown as mean ± SEM, n=4. **P*<0.05, ***P < 0.001;

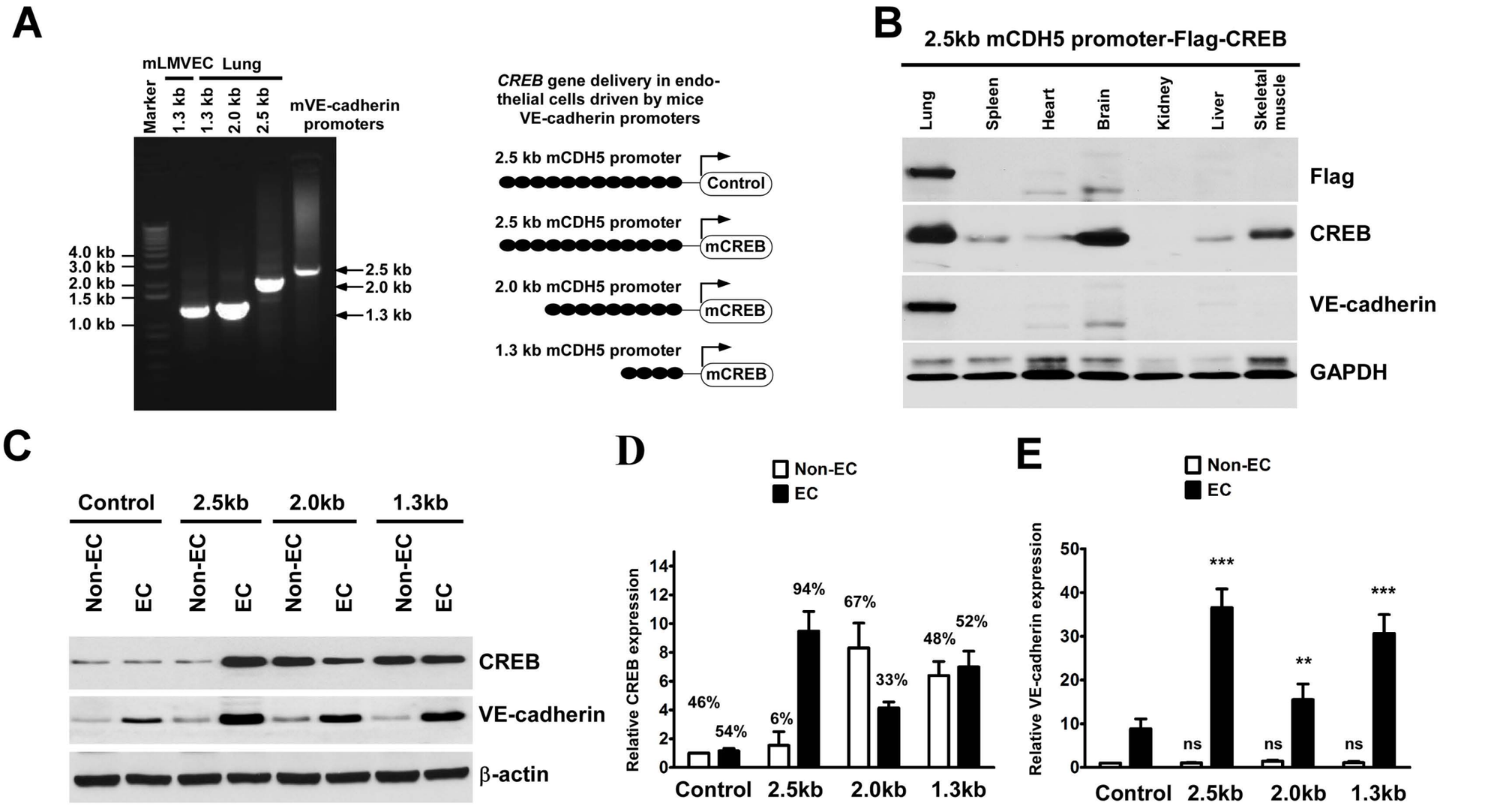


Supplementary Figure 5. Predicted CREB binding CREs within the mice *CREB* promoter. The 2.5 kb promoter region of the mouse *CREB* gene itself contains multiple highly conserved cyclic-AMP response elements (CREs).

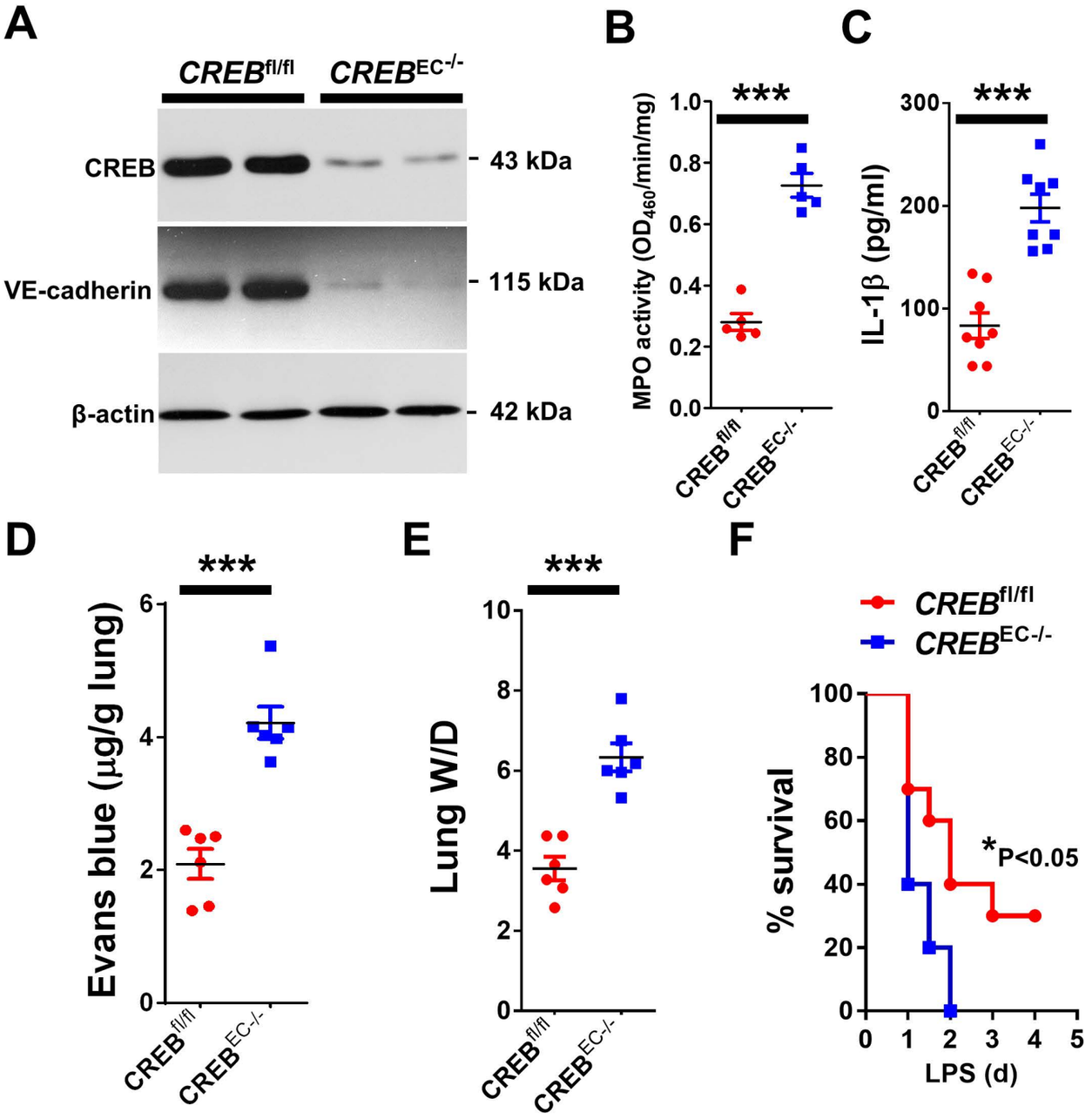


Supplementary Figure 6. Augmentation of cAMP signaling by rolipram alters the dynamics of polymorphonuclear leukocytes (PMNs) through the lung.

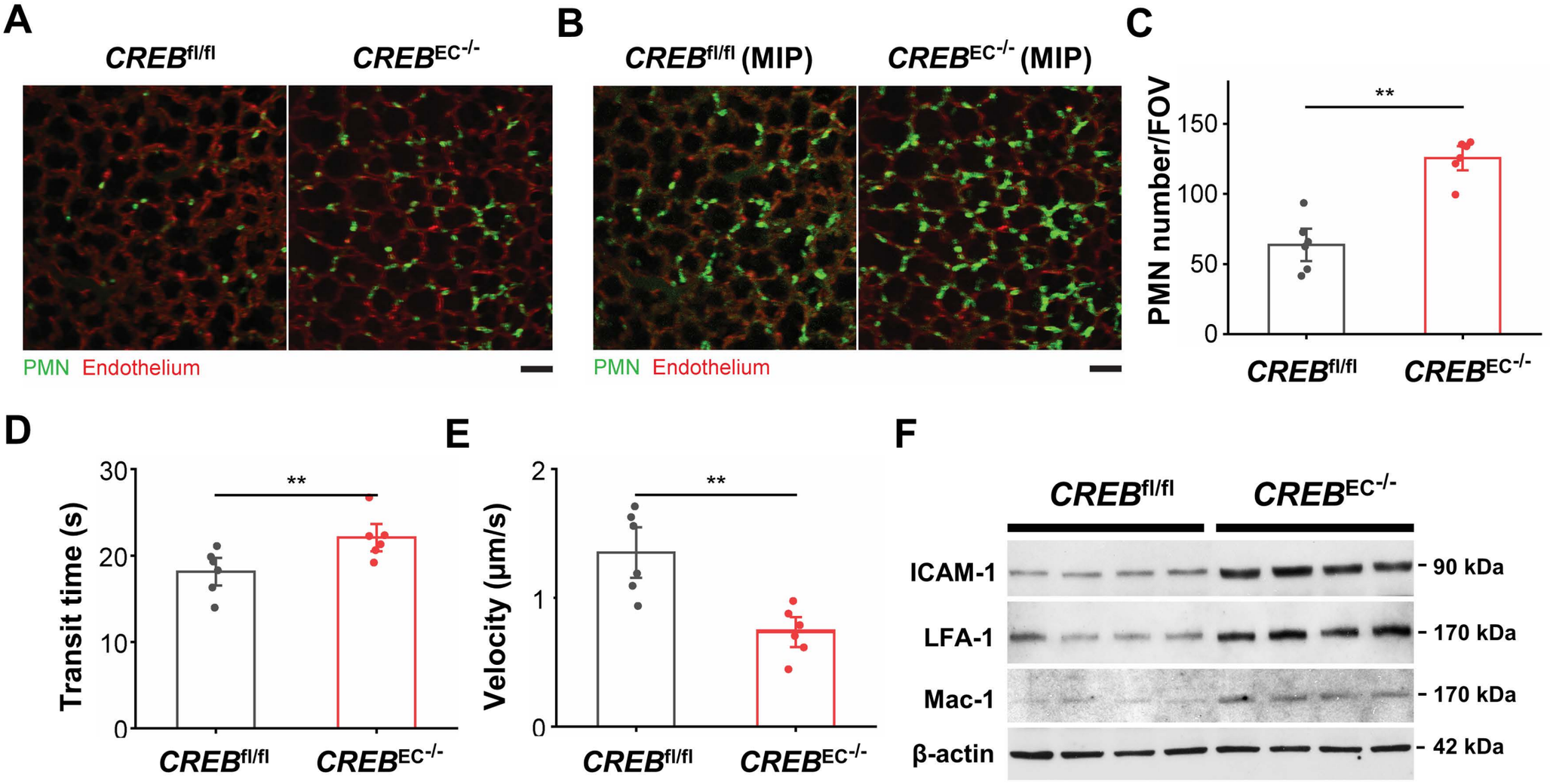
C57BL/6J mice were administrated with rolipram (5 mg/kg) for 1 hour, and injected intraperitoneally with LPS (12 mg/kg) for 1 day. Intravital lung imaging of PMNs and lung microvascular endothelium structures was analyzed. **(A)** Representative two-photon fluorescence images were shown (scale bar, 50 μm). **(B)** PMN trajectory was evaluated. The maximum intensity projection (MIP) images were presented (scale bar, 50 μm). **(C-E)** Quantitative analyses of PMN number **(C),** transit **(D)** and velocity **(E)** were performed. FOV size is 500 x 500 μm. n=6, **P<0.01.



Supplementary Figure 7. Endothelial CREB directly regulates expression of VE-cadherin *In Vivo*. **(A)** Identification of the central *VE-cadherin* promoter regions enabling endothelial specific expression of CREB. Various fragments of mice *VE-cadherin* promoter regions were cloned and inserted into the upstream of *CREB* coding sequence. (**B**) The 2.5 kb-*VE-cadherin* promoter-directed CREB expression constructs were delivered into C57BL/6J mice by liposome-mediated retro-orbital injection for 7 days. Tissue expression distribution was assessed by Western blot. **(C-E**) CREB expression constructs driven by various fragments of *VE-cadherin* promoter were delivered into C57BL/6J mice for 7 days. Protein expression in endothelial cells and non-endothelial cells was analyzed by Western blot and quantified. n=3,***P*<0.01; ****P*<0.001, ns, no significant difference.



Supplementary Figure 8. Loss of endothelial CREB develops lung inflammation and permeability. **(A)** EC-specific *CREB* knockout mice were confirmed by Western blot using the indicated antibodies. **(B)** lung tissue MPO activity was measured (n=5). **(C)** Inflammatory cytokine levels of IL-1β in mice serum were measured by ELISA (n=8). **(D)** Lung vascular permeability is detected in lungs from *CREB*$^{fl/fl}$ and *CREB*$^{EC-/-}$ mice (n=6). **(E)** The ratio of the wet lung to dry lung weight was determined (n=6). (**F**) Survival of mice post 20 mg/kg LPS challenge was monitored and presented as a Kaplan-Meier plot. n=10. ***P<0.001.



Supplementary Figure 9. Loss of endothelial CREB promotes interaction of neutrophils with endothelial cells. (A) Two-photon fluorescence images of PMNs and lung microvessel endothelium structure of *CREB*$^{fl/fl}$ and *CREB*$^{EC-/-}$ mice (scale bar, 50 μm). **(B)** PMN trajectory of *CREB*$^{fl/fl}$ and *CREB*$^{EC-/-}$ mice. The maximum intensity projection (MIP) images were presented (scale bar, 50 μm). **(C-E)** Quantitative analyses of PMN number **(C),** transit **(D)** and velocity **(E)** were performed. FOV size is 500 x 500 μm. n=6, **P<0.01. **(F)** Lung lysates of *CREB*$^{fl/fl}$ and *CREB*$^{EC-/-}$ mice were prepared for expression detection of ICAM-1, LFA-1 and Mac-1 by Western blot.

Table 1: Primers are used for quantitative real-time PCR and RT-PCR

Target gene	GenBank™ accession #	Forward primers	Reverse primers	PCR product size (bps)
mCREB1	NM-001037726.1	CCAGGTCCATGGCGTTATCC	ACTGCCCACTGCTAGTTTGG	353
mVE-cadherin	NM_009868	CAGGCCCTAACTTTCCCCAG	CACAGTGGGGTCATCTGCAT	473
mCTNNB1/ β–cadherin	NM_001165902.1	GTTCTACGCCATCACGACAC	TTCAGCACTCTGCTTGTGGT	279
mGAPDH	NM_001289726.1	CTCAGGAGAGTGTTTCCTCGTC	CTCGCTCCTGGAAGATGGTG	268

Table 2: Primers are used for reporter assay for mice VE-cadherin transcription

Promoter fragments	Forward primers	Reverse primers
0.5 kb (-509/+18)	ACTCGGAGCTCAGAAGCTGAGGTAGCTG	CGATAACTCGAGCTCTGTGAGCCTCTGCAT
0.8 kb (-804/+18)	ACTCGGAGCTCTGCTGACTCAGACCTATGGCTA	CGATAACTCGAGCTCTGTGAGCCTCTGCAT
1.0 kb (-1003/+18)	ACTCGGAGCTCTGGAACACACTCTGTAGACCA	CGATAACTCGAGCTCTGTGAGCCTCTGCAT
1.5 kb (-1512/-24)	ACTCGGAGCTCATGGTCTCAGGACGCTAA	CGACTA CTCGAGCGTGAGCAGAAGGCTCTCTCCA
1.7 kb (-1762/-24)	ACTCGGAGCTCAGCTGTCTATGCTGGGCCAGC	CGACTA CTCGAGCGTGAGCAGAAGGCTCTCTCCA
2.0 kb (-1984/-24)	ACTCGGAGCTCAGGGTTGCTGTCCATCGTTTA	CGACTA CTCGAGCGTGAGCAGAAGGCTCTCTCCA
2.5 kb (-2482/-24)	ACTCGGAGCTCAAGCACACATATTCAG	CGACTA CTCGAGCGTGAGCAGAAGGCTCTCTCCA

Supplementary Table 1 and 2| Sequences of primers for RT-PCR, Real-time PCR and Reporter assay.

Table 3: Primers are used for ChIP assay for mice VE-cadherin promoter

Promoter fragments	Forward primers	Reverse primers
VE-cadherin-CRE1 (-376/-5)	GCTCTGGCAGAAGCTTAGAG	CGTGGAGGAGCTGATCTTGT
VE-cadherin-CRE2 (-803/-524)	GCTGACTCAGACCTATGGCTATC	TCCGTGGCGACTCTGTAGTA
VE-cadherin-CRE3 (-1138/-781)	TTACTCTGAGCCAGAACCCTGA	GATAGCCATAGGTCTGAGTCAG
VE-cadherin-CRE4 (-1465/-1259)	GGGCCTCCGTTCCAGTTCAGTTC	CAGATCTTTCCGGGCATGGAGAA
VE-cadherin-CRE5 (-1679/-1571)	GTTCACAGACACACGCACTCG	GCTGTTCTCAGCTCTGAGCGGAAT
VE-cadherin-CRE6 (-2077/-1902)	AGGGGGTCTGAAGAGCACTA	GTACTCTAGCCCTGGTGATAT

Table 4: Primers are used for ChIP assay for mice CREB promoter

Promoter fragments	Forward primers	Reverse primers
mCREB1-CRE1 (-93/+29)	CGGTGTGTTACGTGGGGGAG	TGGTTGTCTGCTCCAGATTCC
mCREB1-CRE2 (-32/-114)	CGGCGGAGGTGTAGTTTGACG	TTCGTTCACGGCCCGGATCT
mCREB1-CRE3 (-112/-339)	GTCGCCCGAAGAAACCCGAA	CCGCGTCACTCACCAACACT
mCREB1-CRE4 (-780/-1002)	AGCGACACAGGATGTTGTCTGG	TTCCTTTCCGAGACTCCGCGGA
mCREB1-CRE5 (-981/-1171)	AGGAGAGGGCTGCAGAGTTC	CCAGACAACATCCTGTGTCGCT
mCREB1-CRE6 (-1219/-1460)	CACTTTAGGAGGCCGAGGCG	AGTCTAGCCCTTGTCGCCCA
mCREB1-CRE7 (-1837/-2114)	GCCAGGGTTCAGCTCAGCAT	TCCCAGCCCTCTCCAGTGTC

Supplementary Table 3 and 4| Sequences of primers for ChIP assay for mice *VE-cadherin* and *CREB* promoter regions.