

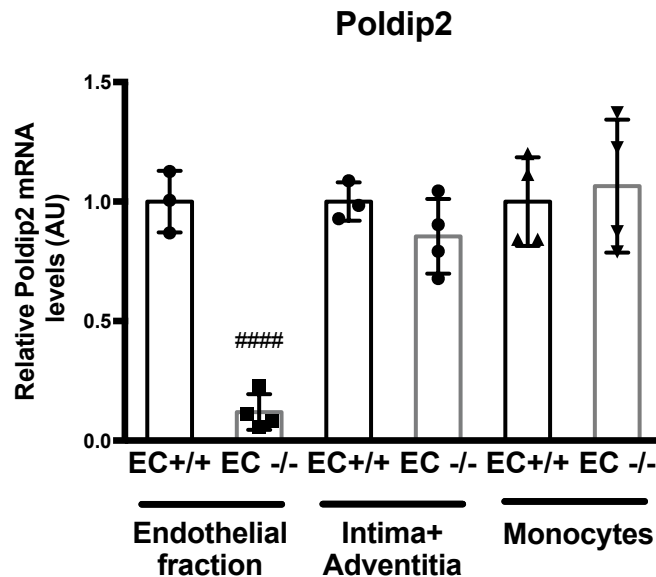
Supplementary Material

Endothelial Poldip2 Regulates Sepsis-induced Lung Injury via Rho Pathway Activation

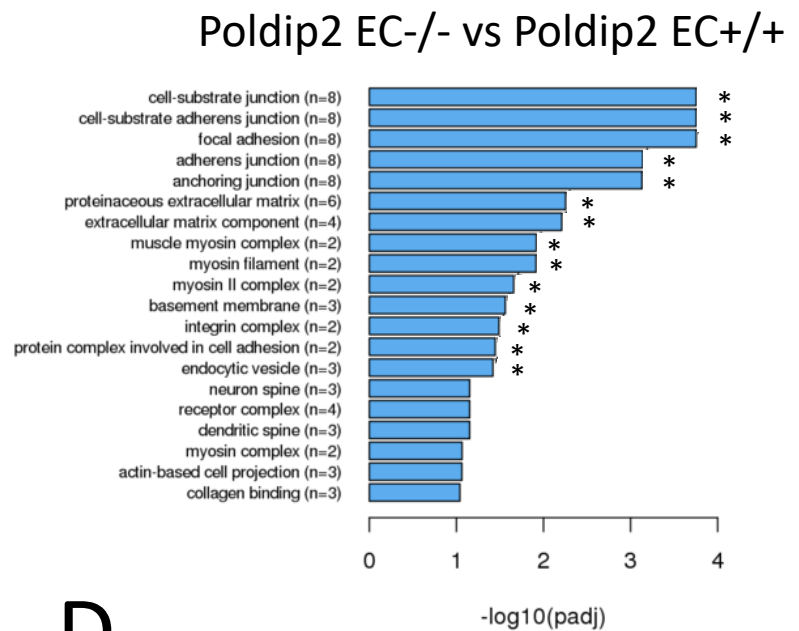
Dolmatova EV, Forrester SJ, Wang K, Ou Z, Williams HC, Joseph G, Kumar S, Valdivia A, Kowalczyk A, Qu H, Jo H, Lassègue B, Hernandez MS, Griendling KK

Supplementary Figure 1

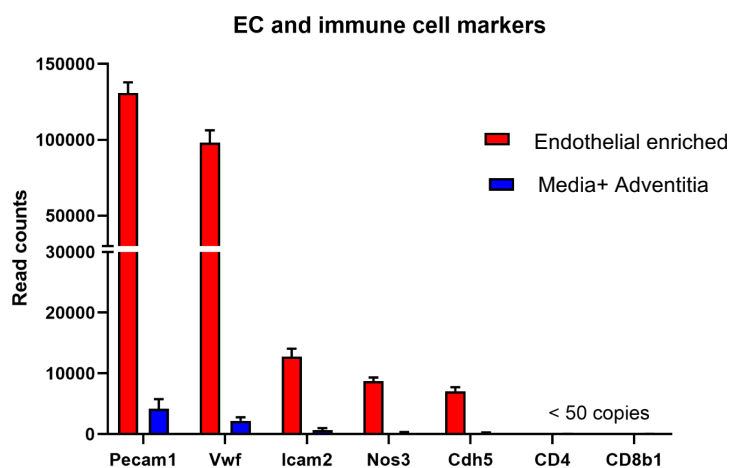
A



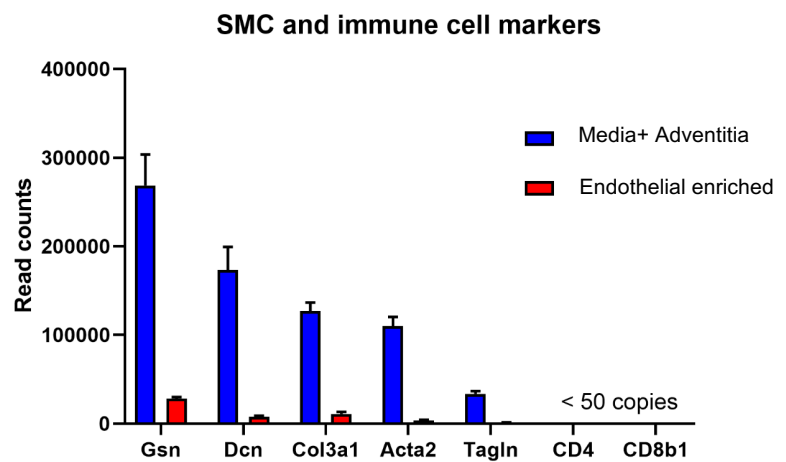
B



C

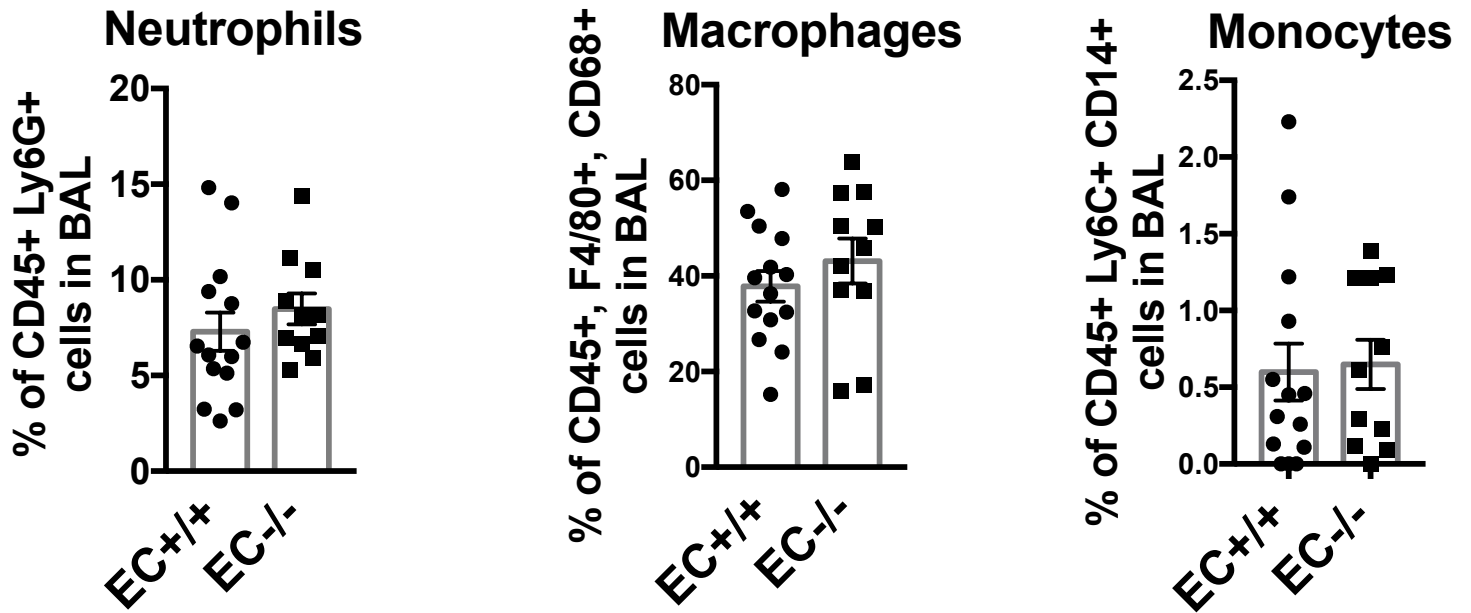


D



- Supplementary Figure 1.** A. Relative Poldip2 expression in endothelial, media+adventitia-enriched fraction and isolated monocytes of Poldip2 EC-/- and Poldip2 EC+/+ mice(####-p<0.0001 compared to Poldip2 EC+/+, (two-tailed T-test)). B. Gene ontology analysis of RNASeq data of endothelial fraction from Poldip2 EC-/- and Poldip2 EC+/+ mice showing enrichment for cell substrate and adherens junction genes, *- FDR<0.05. C and D. Comparison of the expression profiles of endothelial and media + adventitia-enriched fractions from Poldip2 EC-/. The endothelial-enriched fraction had significantly higher expression of endothelial-specific genes and had lower expression of smooth muscle specific genes. The opposite was true for the media + adventitia-enriched fraction. (n=3-4)

Supplementary Figure 2

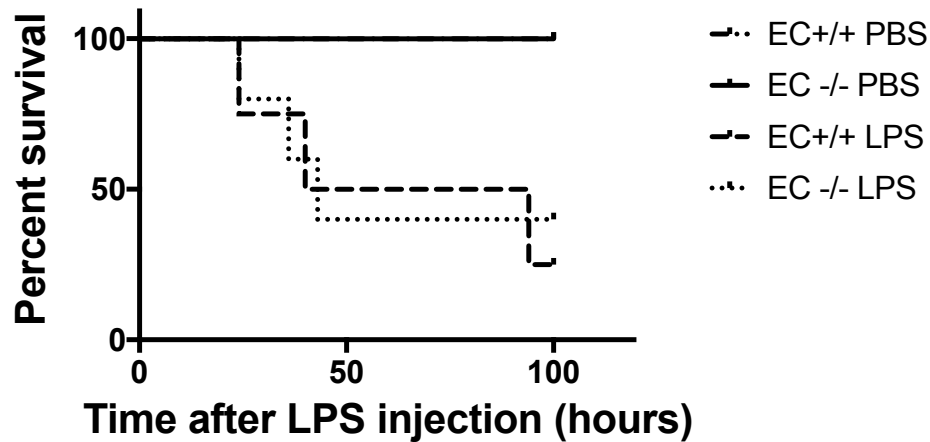


- **Supplementary Figure 2.** Composition of the cells in BAL after LPS injection showing highest percentage of macrophages, followed by neutrophils and monocytes. No significant difference in cellular composition of BAL between Poldip2 EC-/- (n=11) and Poldip2 EC+/+ (n=14) mice was noted (Two-tailed T-test).

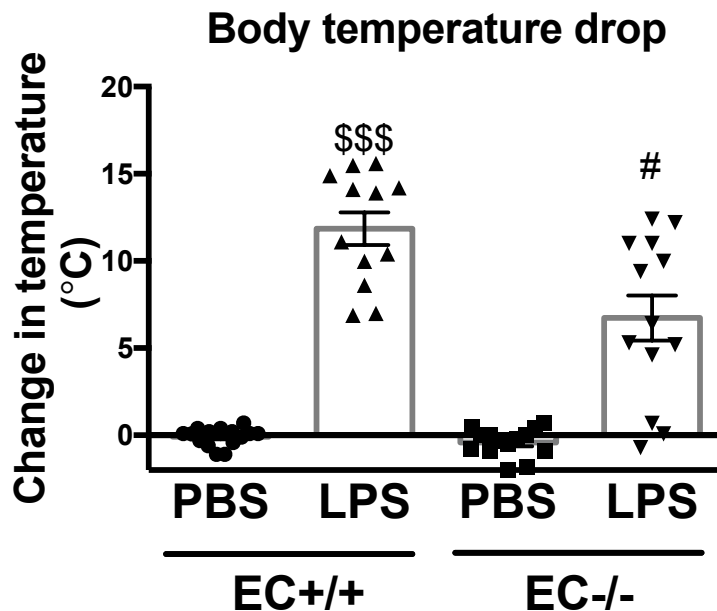
Supplementary Figure 3

A

Survival proportions after LPS injection

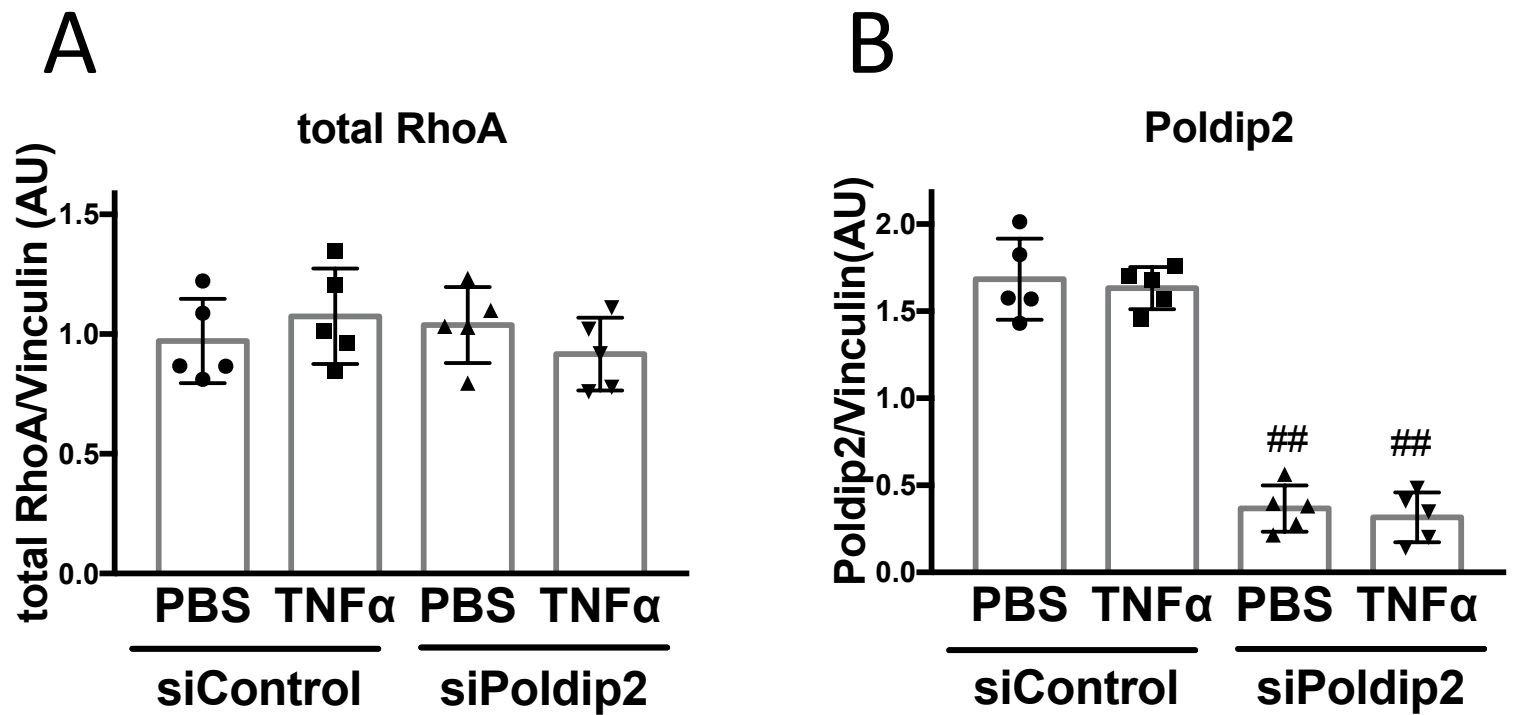


B



- Supplementary Figure 3.** A. Survival curves showing no significant difference in survival between Poldip2 EC+/+ and EC-/- mice (n=5). B. Sepsis severity assessment by core body temperature measurement showing significantly larger temperature drop in Poldip2 EC+/+ vs EC-/- mice after LPS injection (n=12-13 for LPS treated groups, n=14-15 for PBS treated groups). (\$\$\$- p<0.001 compared to PBS injected Poldip2 EC+/+ mice, #- p<0.05 compared to LPS injected Poldip2 EC+/+ mice, two-way ANOVA, with Tukey's correction)

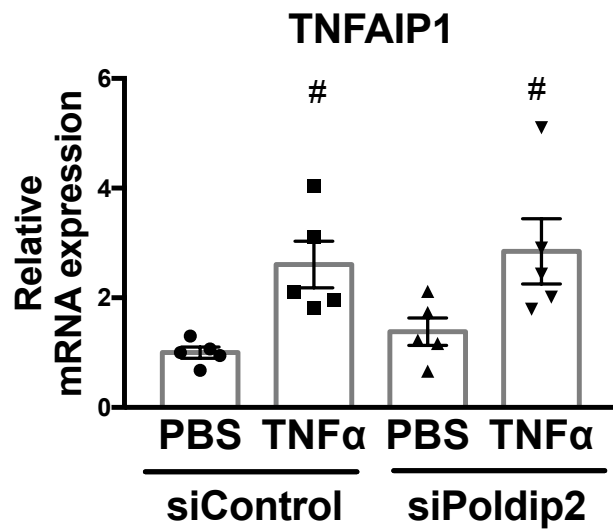
Supplementary Figure 4



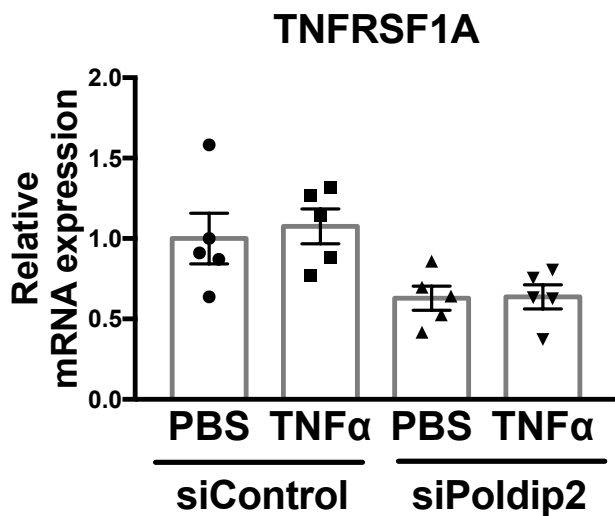
- **Supplementary Figure 4.** A. Quantification of total RhoA levels by Western Blotting in siControl and siPoldip2 treated RBMECs with and without TNF α stimulation. B. Quantification of Poldip2 levels and confirmation of Poldip2 knock-down in RBMECs by Western Blotting (n=5, ##- p<0.01 as compared to corresponding siControl treated cells, two-way ANOVA, with Tukey's correction)

Supplementary Figure 5

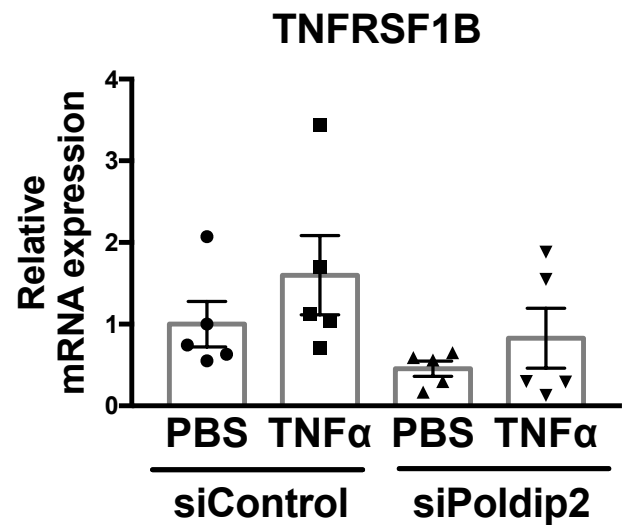
A



B

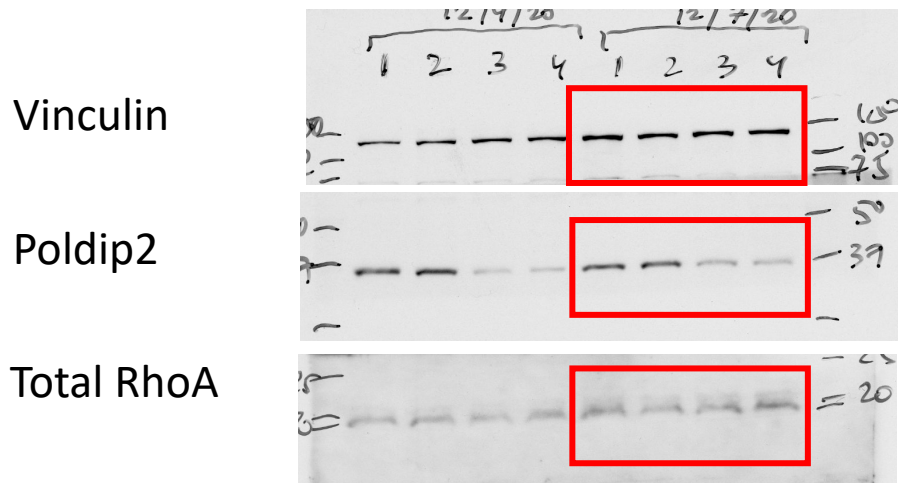


C

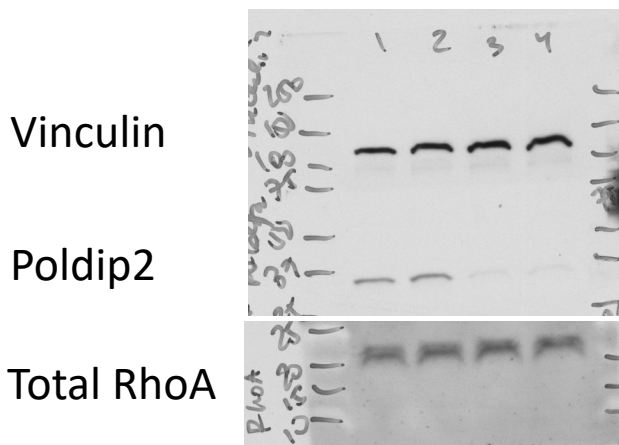
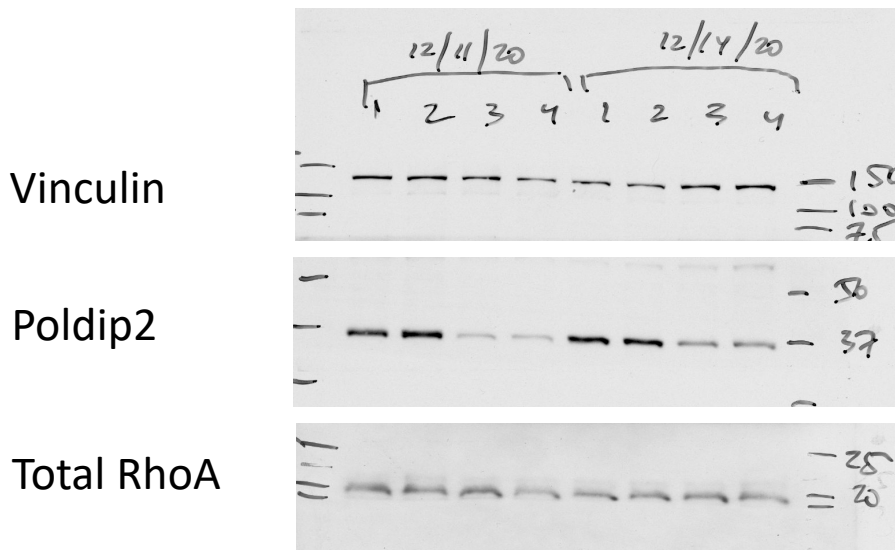


- **Supplementary Figure 5.** qPCR analysis of the expression levels of TNFAIP1(A), TNFRSF1A (B) and TNFRSF1B (C) after Poldip2 knockdown with and without TNF α treatment (n=5, #- p<0.05 as compared to corresponding PBS treated cells, two-way ANOVA, with Tukey's correction)

Supplementary Figure 6 (Unedited Gels)



1. siControl+ vehicle
2. siControl +TNF
3. siPoldip2 + vehicle
4. siPoldip2+ TNF



- **Supplementary Figure 6.** Unedited films of the Western blots mentioned in the paper (n=5).
Membranes were cut to enable probing for multiple proteins simultaneously. Representative blot presented in the main manuscript is highlighted with the red frame.

Supplementary table 1.

qPCR primer sequences

Gene	Forward primer	Reverse primer
Mouse		
Ikbα	5'-GTTTCGCTCTTGTTGAAATG-3'	5'-CCACTTATAATGTCAGACGC-3'
Tnfα	5'-CTATGTCTCAGCCTCTTCTC-3'	5'-GGCCATTTGGGAACCTTCTCA-3'
Cxcl1	5'-AAAGATGCTAAAAGGTGTCC-3'	5'-GTATAGTGTTGTCAGAAGCC-3'
Cxcl2	5'-GTTGACTTCAAGAACATCCAG-3'	5'-CTTTCTCTTTGGTTCTTCCG-3'
Vcam1	5'-GAGTGTACAGCCTCTTTATG-3'	5'-CTGCAGTTCCCCATTATTTAG-3'
Hprt	5'-GCTGACCTGCTGGATTACAT-3'	5'-GGTCCTTTTCACCAGCAAGCT-3'
Ppia	5'-AGCTCTGAGCACTGGAGAGA-3'	5'-GCCAGGACCTGTATGCTTTA-3'
Human		
TNFAIP	5'- ACCCTACATATGACAGAACC-3'	5'- TTAGGGTAGAAAGACATGGG- 3'
TNFRSF1A	5'- CCCCTGGTCATTTTCTTTG- 3'	5'- ATTTCCCACAAACAATGGAG- 3'
TNFRSF1B	5'- AGCACTGGCGACTTC- 3'	5'- ACAAGGGCTTCTTTTTCAC- 3'