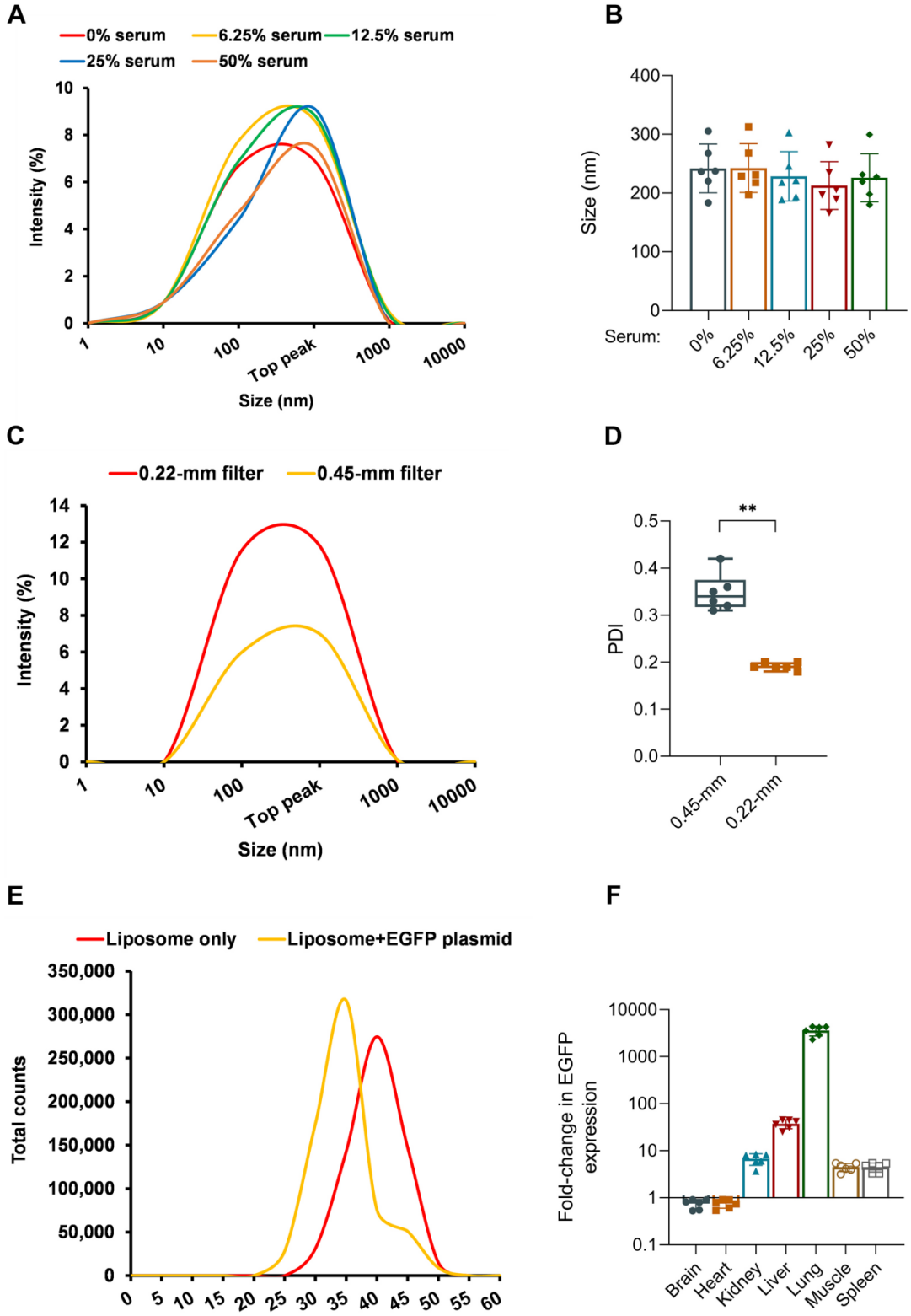
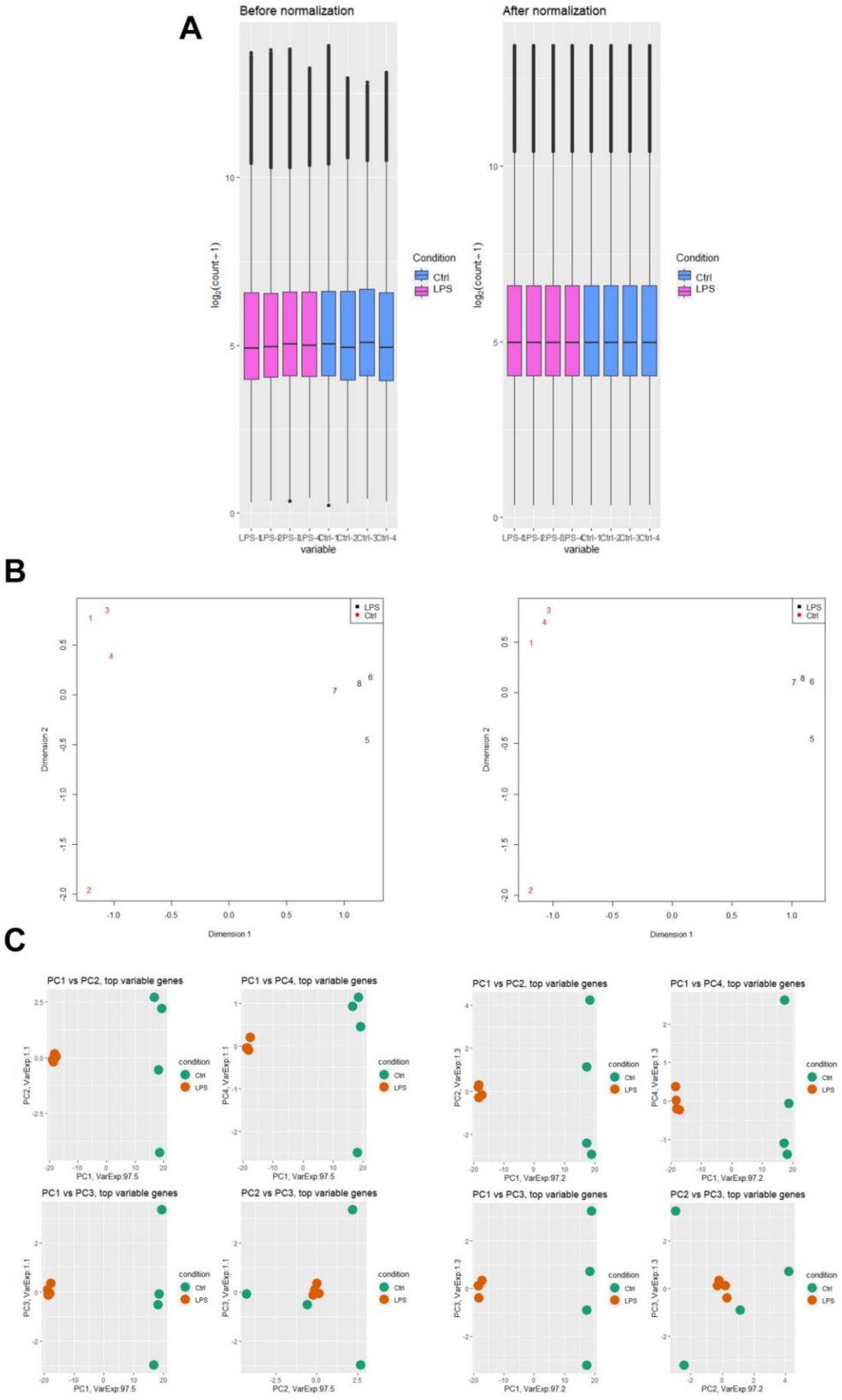
SUPPLEMENTARY FIGURES

Supplementary Figure S1



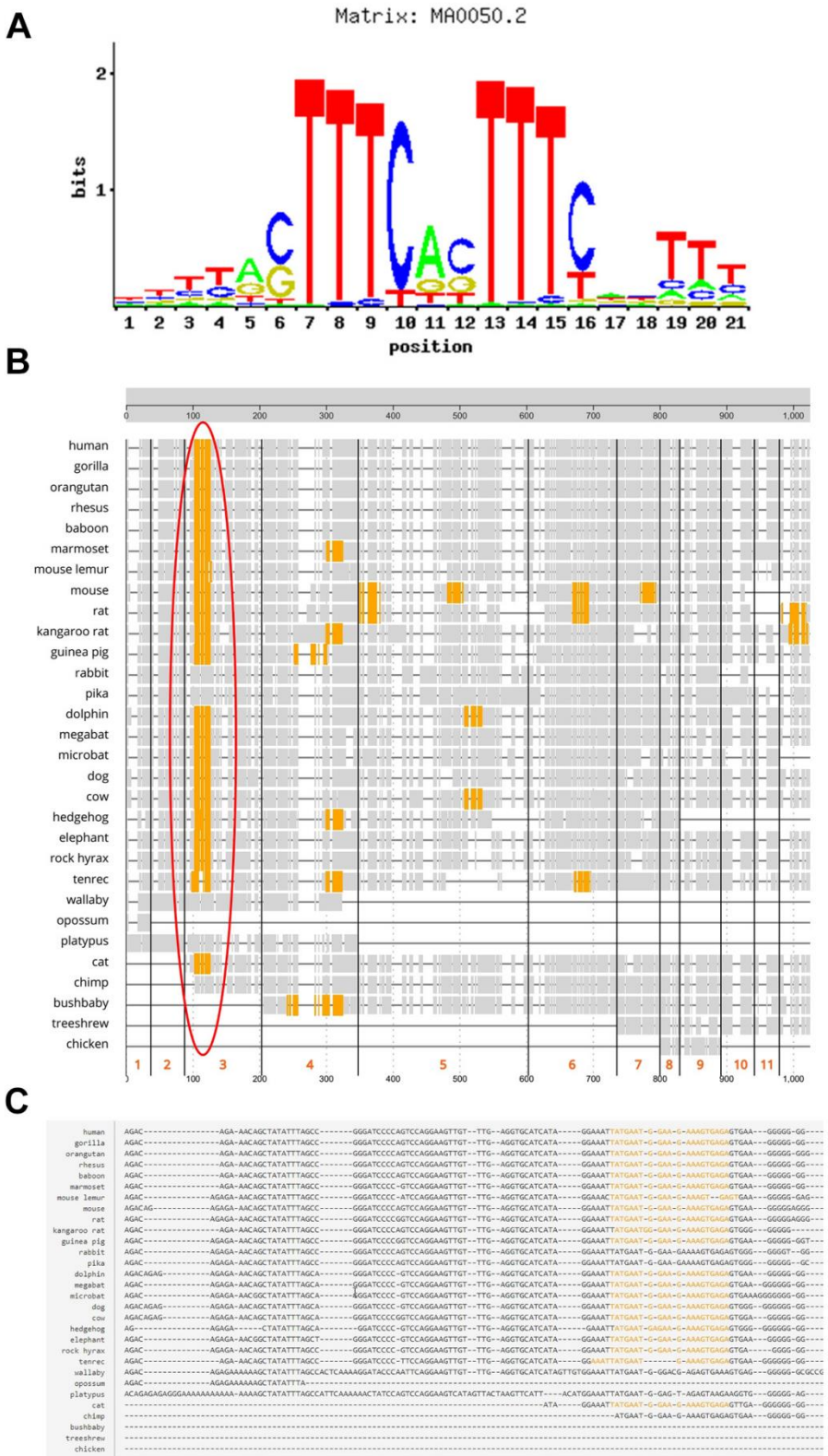
Supplementary Figure S1. Characterization of liposomes. (A) Following a 2 hour ultracentrifugation, extracellular vesicles were removed from serum and measured for size. Liposomes were prepared through combining liposomes (100 µl) and 900-µl serum fractions that had been serially-diluted to 50%, 25%, 12.5%, and 6.25% serum. **(B)** Following a 5-minute incubation, the liposome size was determined by dynamic light scattering. **(C)** Liposome hydrodynamic diameter was determined using dynamic light scattering following preparation with either a 0.45-mm or 0.22-mm filter and **(D)** polydispersity index (PDI) distributions of liposomes prepared using a 0.45-mm or 0.22-mm filter. There was a clear narrowing of the PDI distribution when using the 0.22-mm filter. **(E)** Liposome characterization of zeta potential indicated their cationic nature. The liposome zeta potential reduced following the introduction of plasmid DNA. **(F)** *EGFP* transgene organ biodistribution following *in vivo* delivery of liposomes. Organs were harvested 23 hours post-liposomal injection. *EGFP* mRNA expression levels assessed by qPCR showed that the lung was the organ with the greatest *EGFP* transgene expression. Data represented as means ± SDs. **P*<0.05, ***P*<0.01 [one-way ANOVA with Dunnett's post-hoc tests].

Supplementary Figure S2



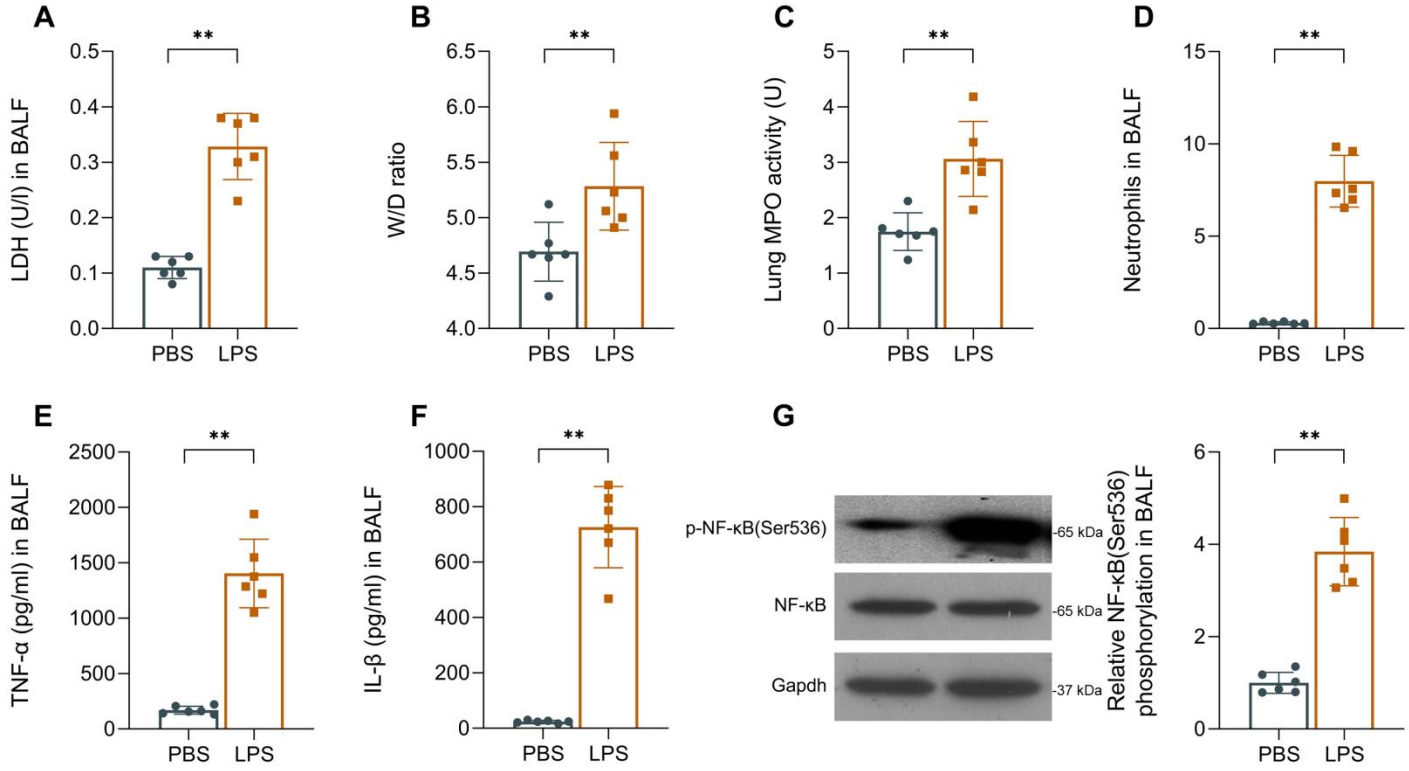
Supplementary Figure S2. R-based filtering and normalization. (A) Boxplots of expression data before and after normalization. The post-normalization boxplots distribute in the same intervals with the same median, indicating successful normalization. **(B)** Multi-dimensional scaling (MDS) plots before and after normalization and **(C)** principal components analysis (PCA) plots before and after normalization showing clear separation of the experimental groups.

Supplementary Figure S3



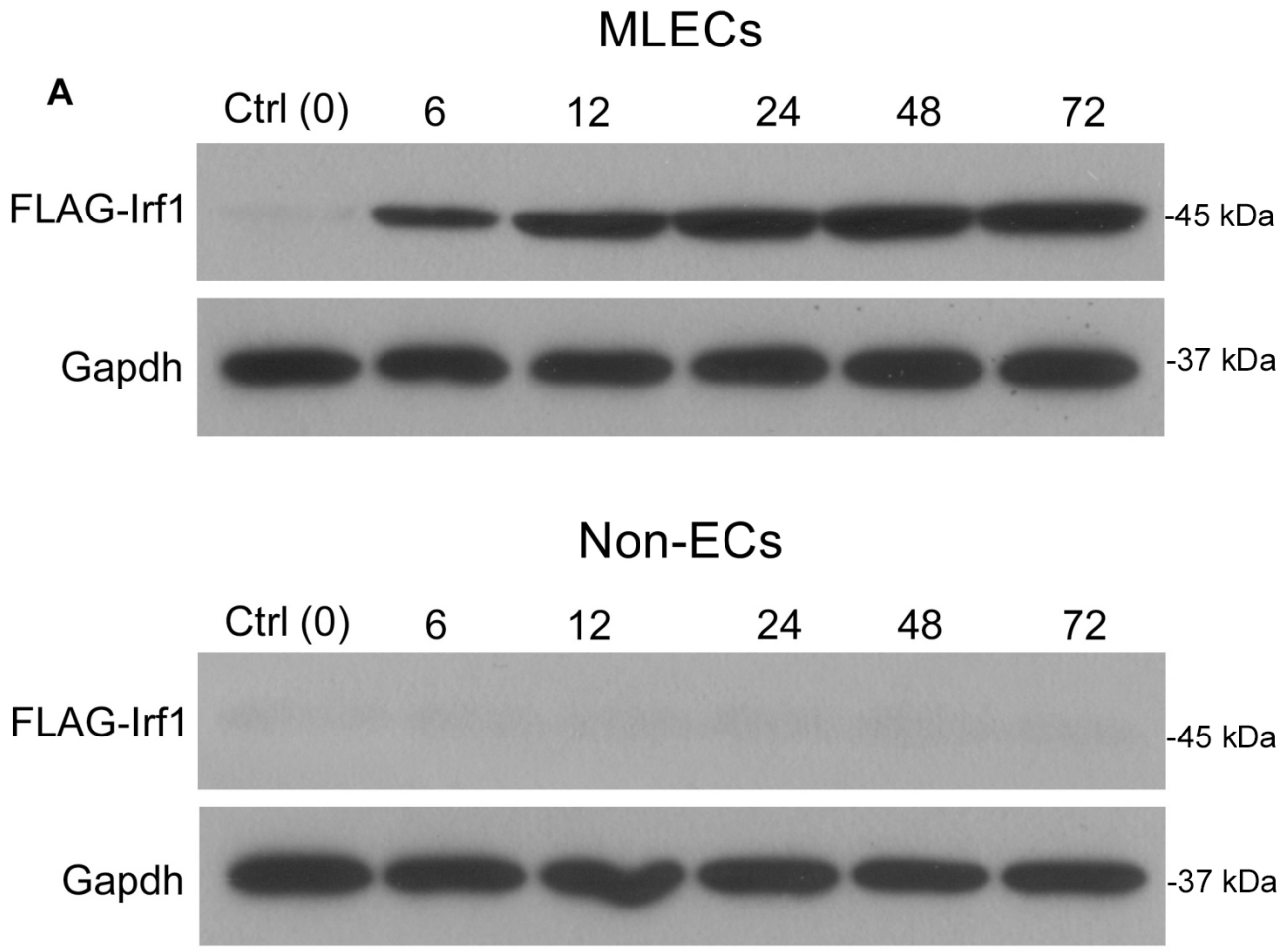
Supplementary Figure S3. *LIF* promoter analysis. (A) Schematic of the JASPAR CORE 2016 IRF1 ISRE motif employed in the *LIF* promoter analysis. **(B)** The 1000-bp promoter segment immediately upstream of the human *LIF* transcription start site **(**TSS) was analyzed using the ConTra v3 tool to identify putative IRF1 binding sites. One IRF1 ISRE binding site (circled in red) was conserved across multiple species. **(C)** Detailed sequence of the IRF1 ISRE binding site.

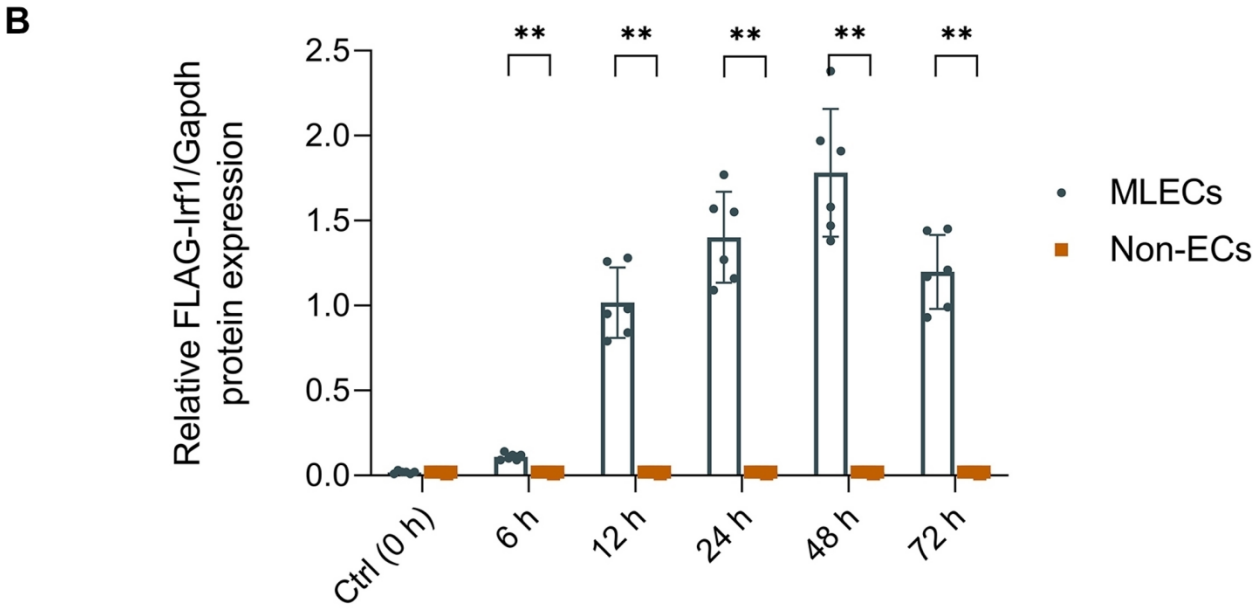
Supplementary Figure S4



Supplementary Figure S4. Assessment of lung inflammation in ALI model mice. Mice were randomly divided into two cohorts (*n*=6 mice/cohort) and subjected to either i.p. injection of LPS or PBS vehicle control. The following studies were performed 24 h post-injection. Assessment of **(A)** BALF LDH by ELISA, **(B)** lung W/D ratio; **(C)** lung homogenate MPO activity, **(D)** BALF neutrophil count, **(E)** BALF TNF-α by ELISA, **(F)** BALF IL-1β by ELISA, and **(G)** NF-κB(Ser536) phosphorylation by Western blotting. Data represented as means ± SDs. **P*<0.05, ***P*<0.01 [Student's *t*-test].

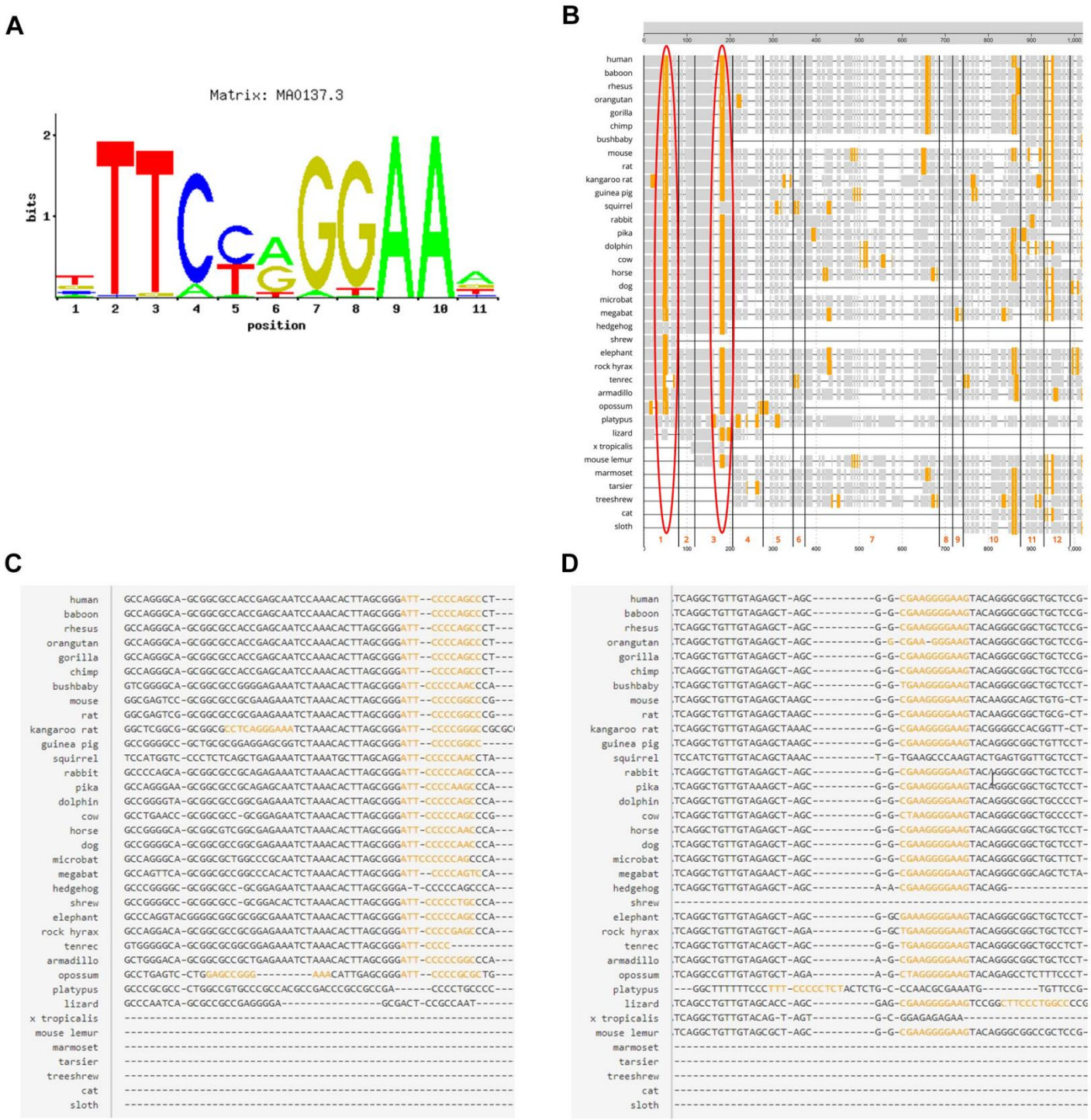
Supplementary Figure S5





Supplementary Figure S5. Liposomal delivery of endothelial cell-specific FLAG-*Irf1*. Representative immunoblots and densitometric quantification of FLAG-Irf1 expression in lung endothelial cells and non-endothelial cells post-LPS at various time points indicated. Data represented as means ± SDs. **P*<0.05, ***P*<0.01 [two-way ANOVA].

Supplementary Figure S6



Supplementary Figure S6. *IRF1* promoter analysis. (A) Schematic of the JASPAR CORE 2016 STAT1 GAS motif employed in the *IRF1* promoter analysis. **(B)** The promoter sequence of the human *IRF1* gene was analyzed using the ConTra v3 tool to identify putative STAT1 GAS binding sites. Two STAT1 GAS binding sites (circled in red) were conserved across multiple species. **(C, D)** Detailed sequences of **(C)** the STAT1 GAS1 binding site and **(D)** the STAT1 GAS2 binding site.

SUPPLEMENTARY TABLES

Supplementary Table S1. qPCR primer sequences.

Target	Forward primer (5′→3′)	Reverse primer (5′→3′)
Lif (murine)	TCAACTGGCACAGCTCAATGGC	GGAAGTCTGTCATGTTAGGCGC
LIF (human)	AGATCAGGAGCCAACTGGCACA	GCCACATAGCTTGTCCAGGTTG
Irf1 (murine)	TCCAAGTCCAGCCGAGACACTA	ACTGCTGTGGTCATCAGGTAGG
IRF1 (human)	GAGGAGGTGAAAGACCAGAGCA	TAGCATCTCGGCTGGACTTCGA
IRF1 ISRE site on *LIF* promoter (human)	GTCTGTTCTCCCCACCCTC	ACTGGAGCCTGTGTTGTAAGA
STAT1 GAS1 site on *IRF1* promoter (human)	GAACAGCCGCGTCTAATTGG	GAAAGCGAAACTACCCGGC
STAT1 GAS2 site on *IRF1* promoter (human)	CGTCCTTCTGATCGTTCTCTAAG	GTATTTCCGCCGGCTTCC

Supplementary Table S2. Results from the CemiTool gene set enrichment analysis (GSEA).

Module	LPS net enrichment score (NES)	Adj. *p*-value
M1	3.95	0.00069**
M2	4.89	0.00069**
M3	0.88	0.73851

*$P<0.05$, **$P<0.01$

Supplementary Table S3. Total body weights for mouse cohorts in the pulmonary transvascular permeability assays (means ± SDs).

(A)	*Irf1*$^{fl/fl}$ Ctrl	*Irf1*$^{EC-/-}$	*P*-value
PBS	23.5 ± 1.7	22.1 ± 1.9	0.21
LPS (24 h)	22.1 ± 1.3	21.5 ± 1.5	0.41
LPS (72 h)	20.3 ± 0.8	20.0 ± 0.7	0.44
LPS (120 h)	20.6 ± 1.2	20.3 ± 1.6	0.74
(B)	*Irf1*$^{EC-/-}$ pcDNA-Ctrl	*Irf1*$^{EC-/-}$ pcDNA-*Irf1*	*P*-value
PBS	23.6 ± 2.7	21.7 ± 1.6	0.18
LPS (72 h)	18.7 ± 1.3	19.5 ± 1.0	0.29
LPS (120 h)	21.7 ± 1.9	19.5 ± 2.8	0.15

(C)	pcDNA-Ctrl	pcDNA-*Irf1*	pcDNA-*Irf1*/shLif	*P*-value
PBS	21.5 ± 2.1	20.8 ± 1.6	21.1 ± 1.7	0.80
LPS (24 h)	22.8 ± 2.0	22.0 ± 2.7	23.0 ± 2.4	0.75
LPS (72 h)	20.1 ± 1.7	19.4 ± 1.7	20.0 ± 1.3	0.71
LPS (120 h)	23.8 ± 2.7	22.1 ± 2.2	23.5 ± 1.9	0.41