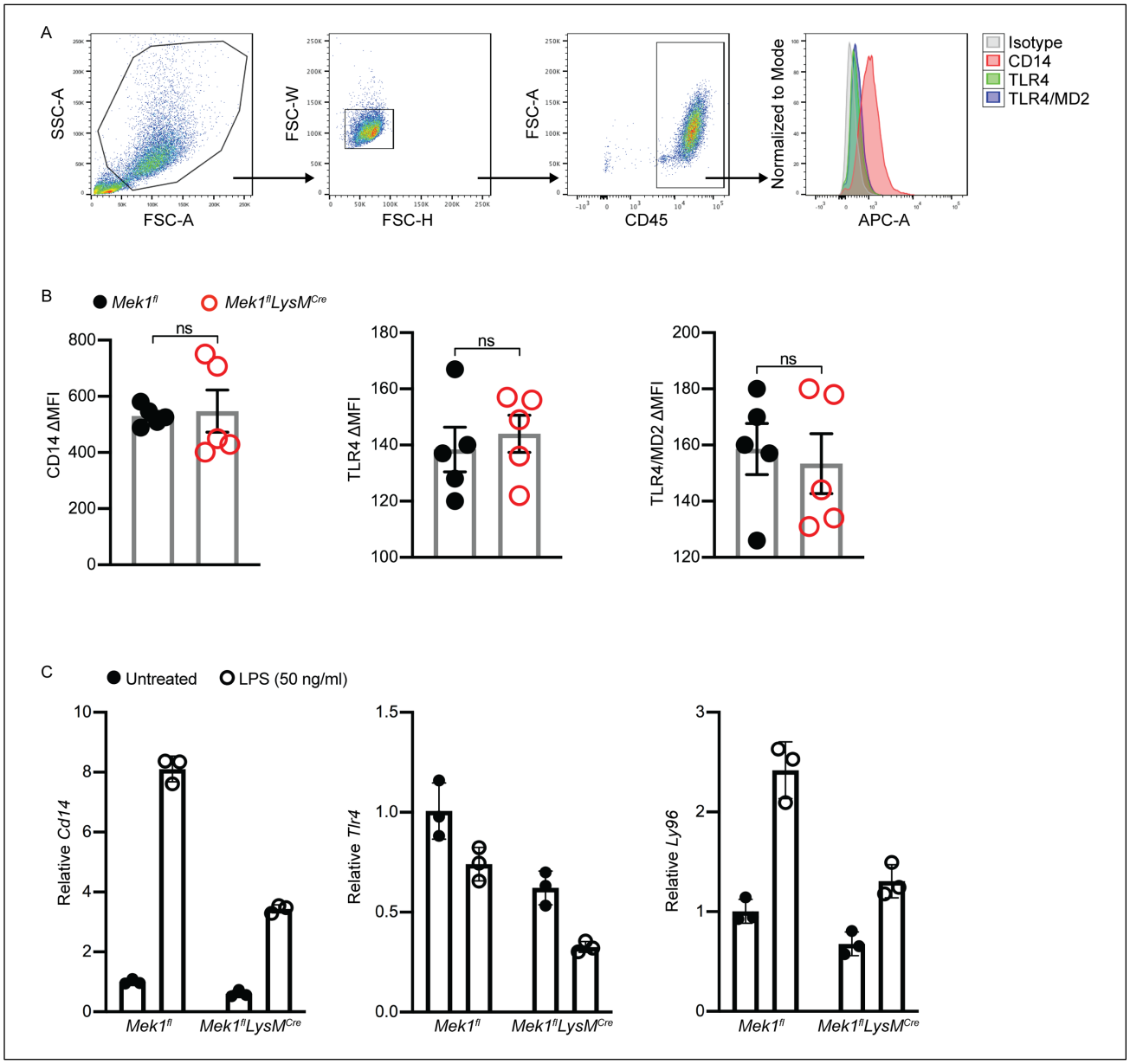
SUPPLEMENTAL MATERIALS

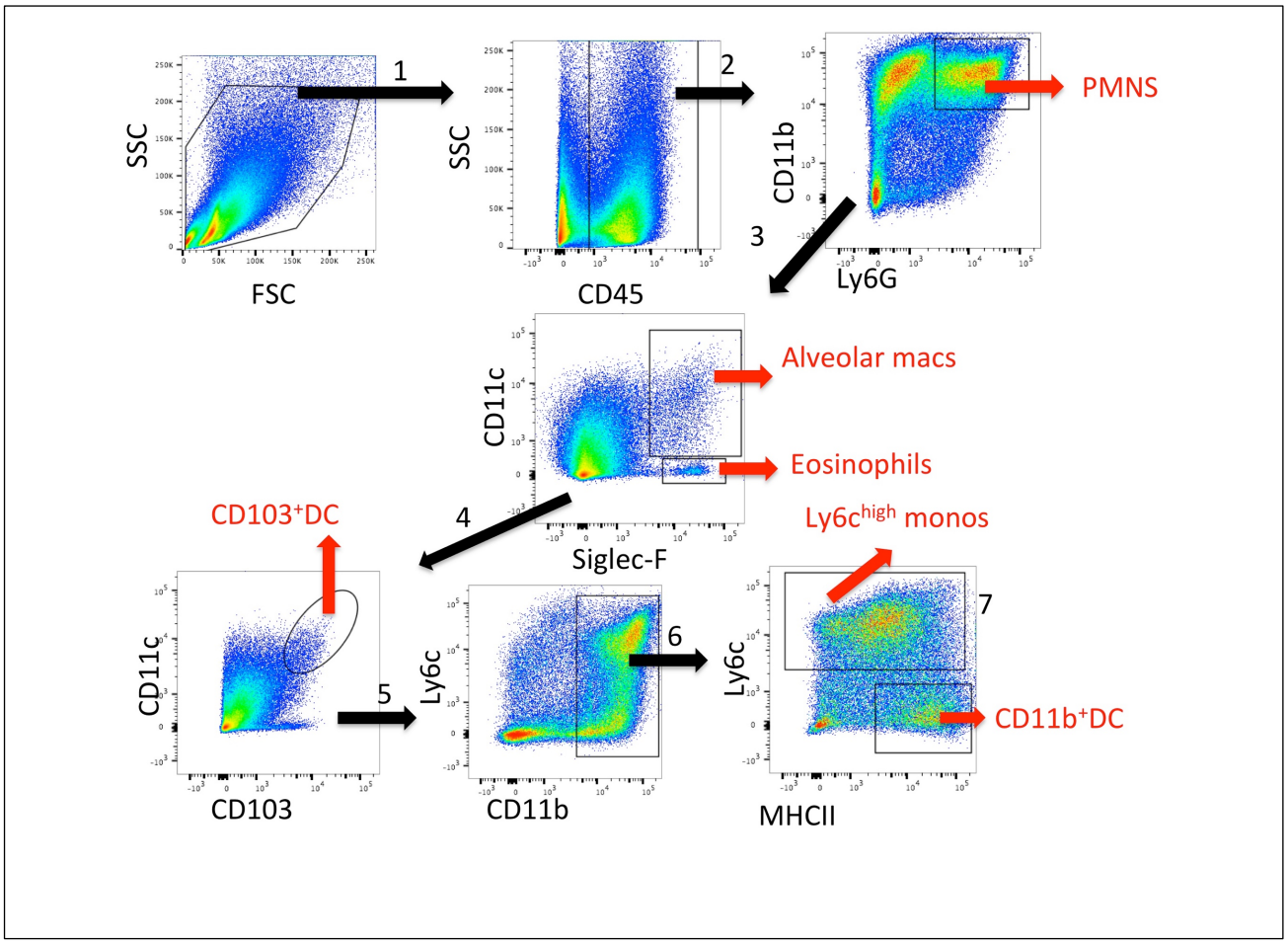
Supplemental Figure 1



Supplemental Figure 1: Alveolar macrophages were obtained from euthanized naïve *Mek1*^{fl} and *Mek1*^{fl}*LysM*^{Cre} mice by performing five broncho-alveolar lavage (BAL) washes. Cells were stained to analyze the surface expression of CD14, TLR4, and TLR4/MD2 complex relative to an isotype stained control. (A) Representative gating strategy used to identify CD45^{+} alveolar macrophages and histogram of representative surface expression relative to an isotype control. (B) Quantitation

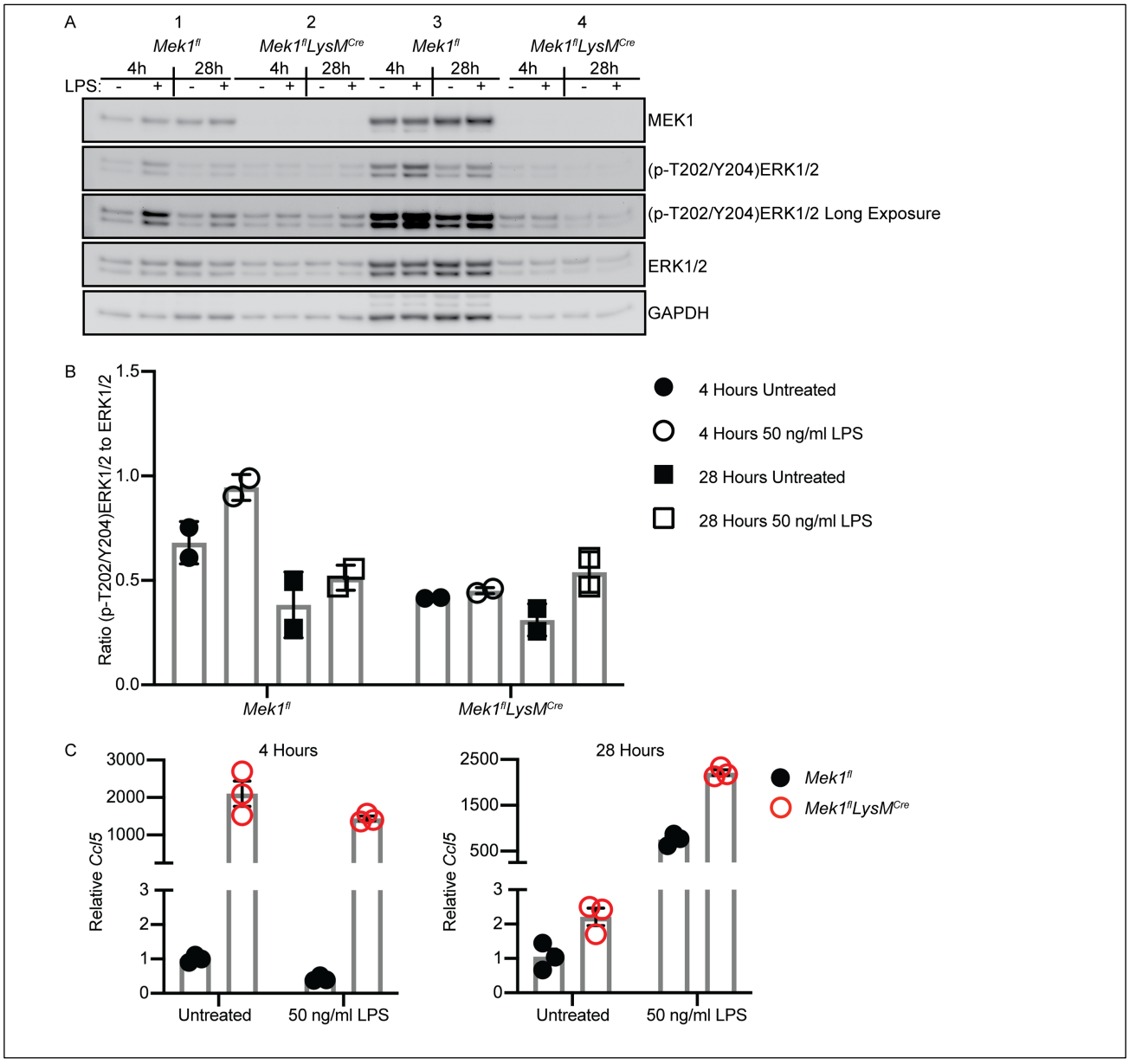
of the ΔMFI of CD14, TLR4, and TLR4/MD2 complex demonstrate no significantly differences between *Mek1*^{fl} and *Mek1*^{fl}*LysM*^{Cre} mice. Data points show values obtained from an individual mouse (n=5 mice per genotype) and the bar is the mean ± SEM. Statistical analyses were performed using unpaired t-test between the genotypes. (C) Naïve alveolar macrophages for each genotype were pooled and seeded at 50,000 cells/well in 96-well plates, with triplicate wells per condition for each genotype. Cells were allowed to adhere for 1 hour at 37°C in RPMI-1640 containing L-glutamine, 10% HI-FBS, 10 mM HEPES, and penicillin/streptomycin. Following adherence, wells were rinsed once with warm PBS and then media (untreated) or media containing 50 ng/ml *E. coli* LPS were added to the wells. RNA was collected after 4 hours and used to make cDNA that was used as the template in qPCR to analyze mRNA levels of *Cd14*, *Tlr4*, and *Ly96* relative to *Hprt*. Samples are normalized to untreated *Mek1*^{fl} at 4 hours. Data show values of technical triplicates from cells that were pooled from n=3 mice per genotype and bars are the mean ± SD.

Supplemental Figure 2



Supplemental Figure 2: Flow gating strategy on lung homogenates from day 4 LPS-induced acute lung injury (LPS-ALI) mice. Cells from whole lung homogenates (1) were used to select $CD45^+$ myeloid cells (2) and PMNs were identified as $Ly6G^+CD11b^+$ cells. Non-PMN myeloid cells (3) identified alveolar macrophages as $CD11c^+Siglec\text{-}F^+$ cells and eosinophils as $Siglec\text{-}F^+$ cells. $CD103^+$ dendritic cells (DC) (4) were identified and (5) Ly6C cells (6) were further identified as (7) $Ly6c^{high}$ monocytes or $CD11b^+$ DC.

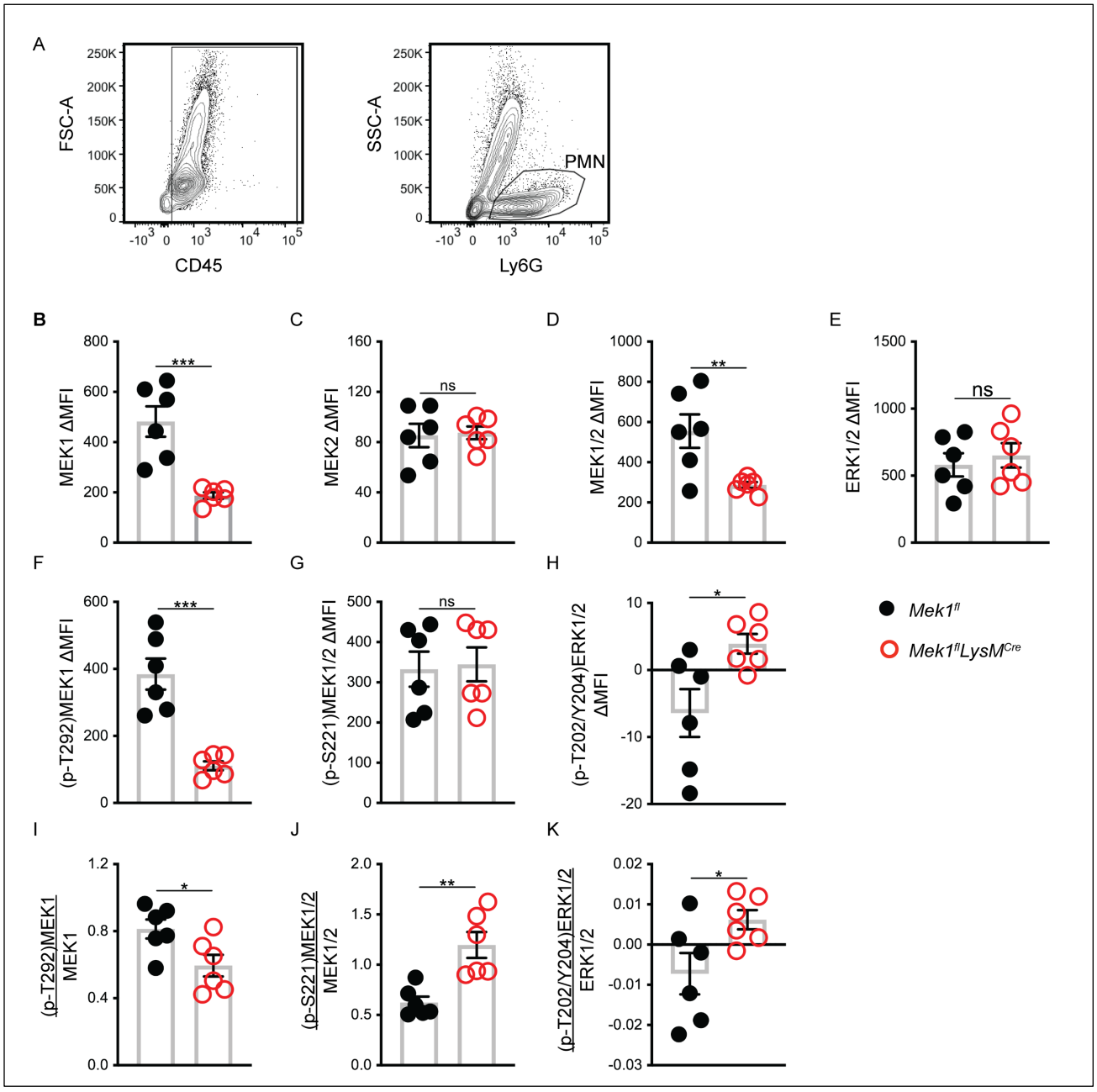
Supplemental Figure 3



Supplemental Figure 3:
Alveolar macrophages were obtained from euthanized naïve *Mek1*^{fl} and *Mek1*^{fl}*LysM*^{Cre} mice by performing five broncho-alveolar lavage (BAL) washes. (A-B) For each sample (A, number 1-4), cells from n=2 mice per genotype were pooled and seeded into 48-well plates. Cells were allowed to adhere for 1 hour at 37°C in RPMI-1640 containing L-glutamine, 10% HI-FBS, 10 mM HEPES, and penicillin/streptomycin. Following adherence, wells were rinsed once with warm PBS and

then media (untreated) or media containing 50 ng/ml *E. coli* LPS were added to the wells. At 4 or 28 hours after stimulation, wells were rinsed with PBS and protein lysates were collected and used for western blots. (B) ImageJ software was used to measure band intensity by densitometry and the ratio of activated to total ERK1/2 is shown. Data represent individual vales from two samples per genotype (each using the pooled cells from n=2 mice), and bar is mean ± SD (C) Naïve alveolar macrophages for each genotype were pooled and seeded at 50,000 cells/well in 96-well plates, with triplicate wells per condition for each genotype. Cells were allowed to adhere for 1 hour at 37°C in RPMI-1640 containing L-glutamine, 10% HI-FBS, 10 mM HEPES, and penicillin/streptomycin. Following adherence, wells were rinsed once with warm PBS and then media (untreated) or media containing 50 ng/ml *E. coli* LPS were added to the wells. RNA was collected after 4 hours or 28 hours and used to make cDNA that was used as the template in qPCR to analyze mRNA levels of *Ccl5*, relative to *Hprt*. Samples are normalized to untreated $Mek1^{fl}$ at either 4 hours or 28 hours. Data show values of technical triplicates from cells that were pooled from n=3 mice per genotype for each timepoint and bars are the mean ± SD.

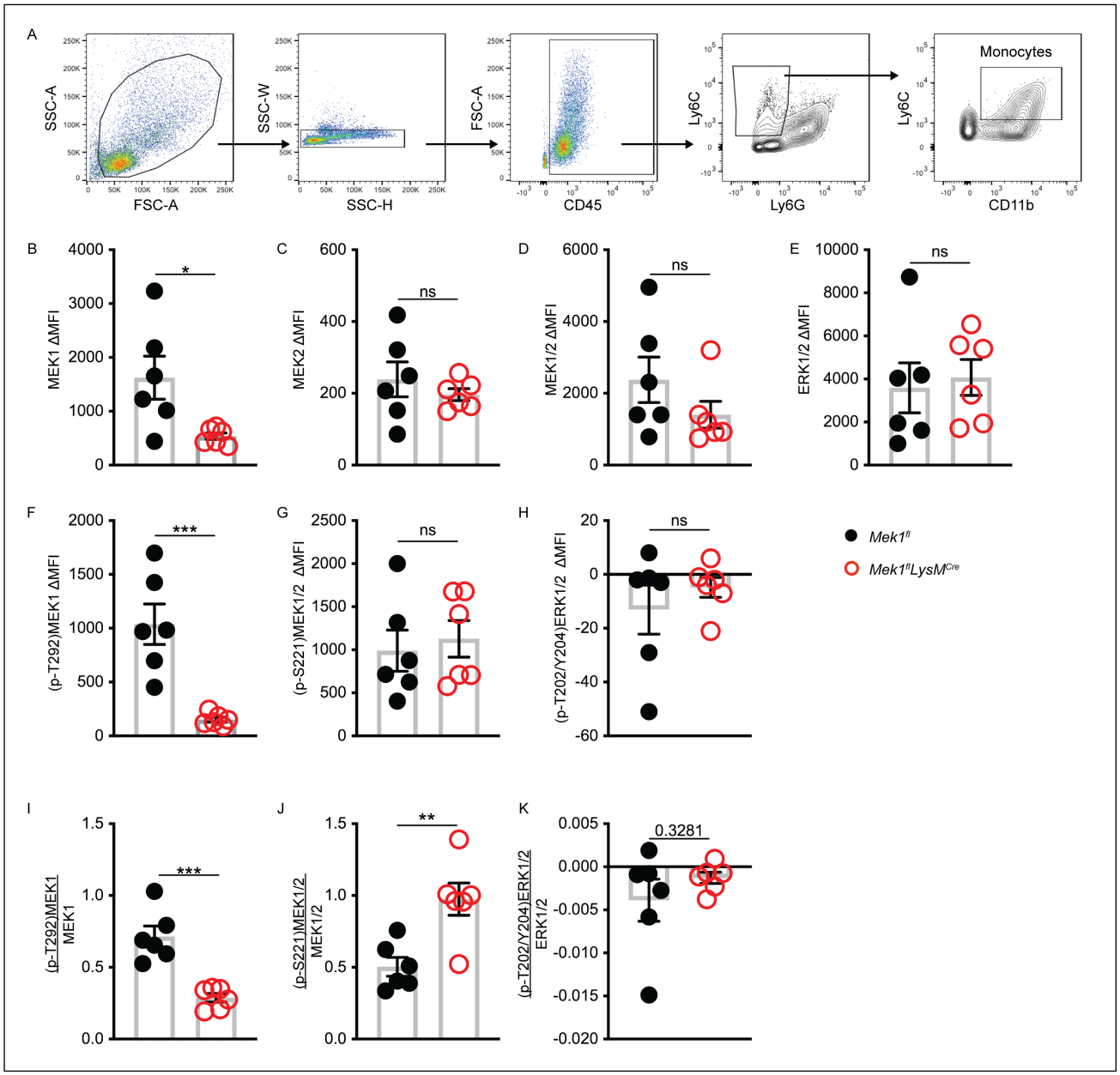
Supplemental Figure 4



Supplemental Figure 4: *Mek1*^{fl} and *Mek1*^{fl}*LysM*^{Cre} mice were subjected to LPS-induced acute lung injury (LPS-ALI) and alveolar cells were collected by broncho-alveolar lavage (BAL) on day 4. BAL cells were and fixed and permeabilized and stained for analysis by flow cytometry. (A) Representative gating strategy to identify CD45^{+} cells and PMNs as Ly6G^{+} cells. (B-H) The ΔMFI

of PMNs for intracellular total proteins (B) MEK1, (C) MEK2, (D) MEK1/2, (E) ERK1/2, (F) (p-T292)MEK1, (G) (p-S22)MEK1/2, and (H) (p-T202/Y204)ERK1/2 were calculated by subtracting background signal from isotype stained control samples. (I-K) The ratio of the ΔMFI of phosphorylated to total protein was calculated for (I) (p-T292)MEK1 to MEK1, (J) (p-S221)MEK1/2 to MEK1/2, and (K) (p-T202/Y204)ERK1/2 to ERK1/2. Dots represent individual mice and the bar is the mean ± SEM. Statistical analyses were performed by unpaired t-tests comparing the two genotypes. *P<0.05, **P<0.01 ***P<0.001, ns is not significant.

Supplemental Figure 5



Supplemental Figure 5: *Mek1*^{fl} and *Mek1*^{fl}*LysM*^{Cre} mice were subjected to LPS-induced acute lung injury (LPS-ALI) and alveolar cells were collected by broncho-alveolar lavage (BAL) on day 4. BAL cells were and fixed and permeabilized and stained for analysis by flow cytometry. (A) Representative gating strategy to identify CD45^{+} monocytes as Ly6G^{-}Ly6C^{+}CD11b^{+} cells. (B-H) The ΔMFI of monocytes for intracellular total proteins (B) MEK1, (C) MEK2, (D) MEK1/2, (E)

ERK1/2, (F) (p-T292)MEK1, (G) (p-S22)MEK1/2, and (H) (p-T202/Y204)ERK1/2 were calculated by subtracting background signal from isotype stained control samples. (I-K) The ratio of the ΔMFI of phosphorylated to total protein was calculated for (I) (p-T292)MEK1 to MEK1, (J) (p-S221)MEK1/2 to MEK1/2, and (K) (p-T202/Y204)ERK1/2 to ERK1/2. Dots represent individual mice and the bar is the mean ± SEM. Statistical analyses were performed by unpaired t-tests comparing the two genotypes. *P<0.05, **P<0.01 ***P<0.001, ns is not significant.

Supplemental Table 1: List of western blot antibodies used in this study.

Target	Company	Species	Catalog #
β-Actin (D6A8)	Cell Signaling	Rabbit	8457
GAPDH (14C10)	Cell Signaling	Rabbit	2118
P44/42 MAPK (ERK1/2) (137F5)	Cell Signaling	Rabbit	4695
p-P44/42 MAPK (ERK1/2) (T202/Y204)	Cell Signaling	Rabbit	9101
MEK1/2 (D1A5)	Cell Signaling	Rabbit	8727
p-MEK1/2 (Ser217/221) (41G9)	Cell Signaling	Rabbit	9154
MEK1 (D2R10)	Cell Signaling	Rabbit	12671
p-MEK1 (T292)	Cell Signaling	Rabbit	51265
MEK2	Cell Signaling	Rabbit	9125

Supplemental Table 2: List of flow cytometry antibodies used in this study.

Target	Company	Fluor(s)	Catalog #
CD3 clone OKT3	Tonbo Biosciences	PerCP Cy5.5	65-0037
CD11b clone M1/70	Biolegend	PE-Cy7	101216
CD11c clone N418	eBioscience eBioscience	e450 APC	48-0114-82 14-0114-82
CD14 clone Sa2-8	eBioscience	APC	17-0141-81
CD45 clone 104 CD45 clone 30-F11 CD45 clone 30-F11 CD45 clone HI30	Biolegend Biolegend Biolegend Tonbo Biosciences	BV650 APC Cy7 Pacific Blue APC Cy7	109835 103116 103126 25-0459
CD103 clone 2E7	Biolegend	AF288	121408
CD169 clone 7-239	Biolegend	APC	346008
CD170 (Siglec-F) clone 1RNM44N	Invitrogen	PerCP e710	46-0114-82
CD206 clone 15-2	Biolegend	BV421	321126
Ly6C clone HK1.4	Biolegend Biolegend	BV785 Pacific Blue	128041 128014
Ly6G clone 1A8	Biolegend Biolegend	APC PerCP Cy5.5	127614 127616
MHCII clone M5/114.15.2	Biolegend	BV510	107635
P44/42 MAPK (ERK1/2) (137F5)	Cell Signaling	PE	8867
ERK1/2 Phospho (Thr202/Tyr204) clone 6B8B69	BioLegend	PE	369506
MEK1/2 (D1A5)	Cell Signaling	PE	28708
p-MEK1/2 (Ser221) (166F8)	Cell Signaling	PE	16211
MEK1 clone 25/MEK1	BD Biosciences	PE	560099
p-MEK1 (T292) (D5L3K)	Cell Signaling	PE	Custom Order PE-Conjugate
MEK2 Clone 96/MEK2	BD Biosciences	PE	560388
TLR4 (CD284) clone SA15-21	BioLegend	APC	145406
TLR4/MD2 Complex clone MTS510	eBioscience	APC	17-9924-82
Rabbit (DA1E) mAb IgG XP isotype control	Cell Signaling	PE	5742
Mouse IgG2a, κ Isotype Ctrl (ICFC) clone MOPC-173	BioLegend	PE	400244
Rat IgG2a Kappa Isotype Control clone eBR2a	eBioscience	APC	17-4321-81

Supplemental Table 3: List of primer probes used in this study.

Target	Company	Species	Catalog #
Ccl5	Life Technologies	Mouse	Mm01302427_m1
Cd14	Life Technologies	Mouse	Mm01158466_g1
Cxcl1	Life Technologies	Mouse	Mm04207460_m1
Cxcl10	Life Technologies	Mouse	Mm00445235_m1
Hprt	Life Technologies	Mouse	Mm01545399_m1
Ifit1	Life Technologies	Mouse	Mm00515153_m1
Il1b	Life Technologies	Mouse	Mm00434228_m1
Ly96	Life Technologies	Mouse	Mm01227593_m1
Nos2	Life Technologies	Mouse	Mm00440502_m1
Tlr4	Life Technologies	Mouse	Mm00445273_m1

Supplemental Table 4: Characteristics of human subjects participating in the study.

Subject	Sex	Age	ARDS Risk Factor	Sample Collection (Days Post ARDS Development)
Healthy Control 1	Female	21	N/A	N/A
Healthy Control 2	Female	34	N/A	N/A
ARDS Patient 1	Male	82	Aspiration	1
ARDS Patient 2	Male	70	Aspiration/Pneumonia	0
ARDS Patient 3	Male	29	Trauma	9
ARDS Patient 4	Male	20	Trauma	12