Online Supplement

Sex-specific alterations in pulmonary immune, metabolic and lipid signaling pathways after e-cigarette aerosol exposure during adolescence in mice

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Methods

RT-qPCR

Total mRNA was extracted from whole lung tissue using the Aurum miniKit (Bio-Rad, Mississauga, ON, CA) and reverse transcribed into cDNA using the iScript cDNA synthesis kit (Bio-Rad, Mississauga, ON, CA). The expression levels of select genes of interest were measured via qPCR using the SSoFast EvaGreen Supermix (Bio-Rad, Mississauga, ON, CA) and custom primers synthesized from Integrated DNA Technologies. Primer sequences are listed in Table 2. All genes were normalized to 18s rRNA and expressed as relative fold-change using the $2^{-\Delta\Delta Ct}$ method.

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Supplemental Table 1. Fluorochrome-conjugate antibodies used for the identification of immune cells in the BAL and lung tissue.

Innate immune cell panel					
Fluorochrome	**Marker**	**Company**	**Cat#**	**Clone**	**Volume**
APC-Cy7	CD45	BD Bioscience	557659	30-F11	0.5 µL
APC	CD11b	BD Bioscience	553312	M1/70	0.5 µL
BV711	CD11c	BD Bioscience	563048	HL3	0.5 µL
PE	Ly6C	BioLegend	128007	HK1.4	0.5 µL
PE/Cy7	Ly6G	BioLegend	127618	1A8	0.5 µL
AlexaFluor 700	mhcII	Invitrogen	56-5321-82	M5/114.15.2	0.5 µL
BV421	merTK	BioLegend	151510	2B10C42	0.5 µL
BV605	Siglec-F	BD Bioscience	740388	E50-2440	0.5 µL
PerCP/Cy5.5	CD64	BioLegend	139308	X54-5/7.1	0.5 µL
Adaptive immune cell panel					
Fluorochrome	**Marker**	**Company**	**Cat#**	**Clone**	**Volume**
APC-Cy7	CD45	BD Bioscience	557659	30-F11	0.5 µL
BV421	CD19	BioLegend	115538	HIB19	0.5 µL
FITC	CD3	Invitrogen	11-0032-82	17A2	0.5 µL
PE	CD4	BioLegend	100408	GK1.5	0.5 µL
APC	CD8	BioLegend	100712	53-6.7	0.5 µL

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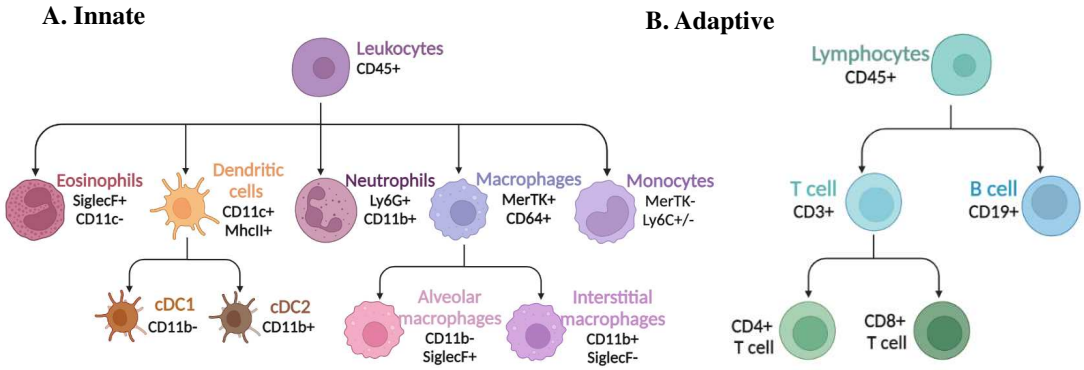
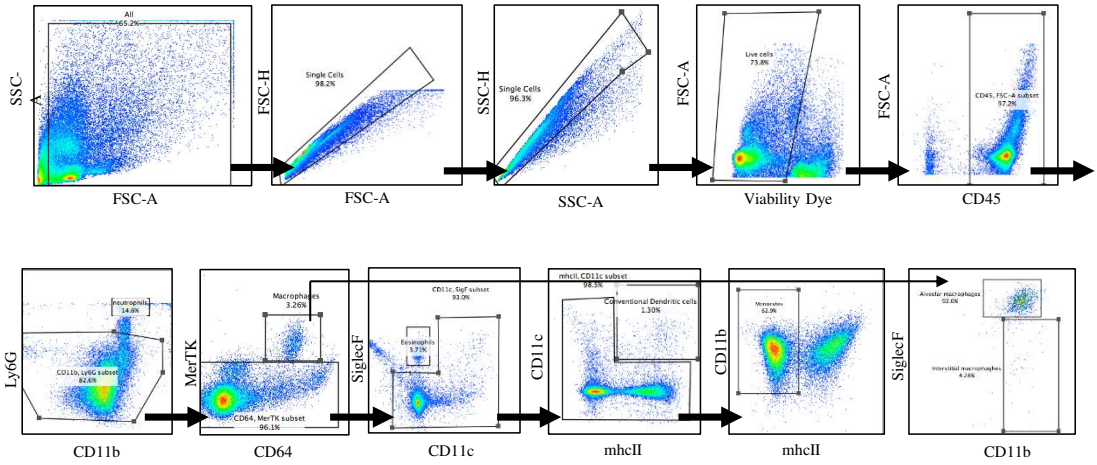
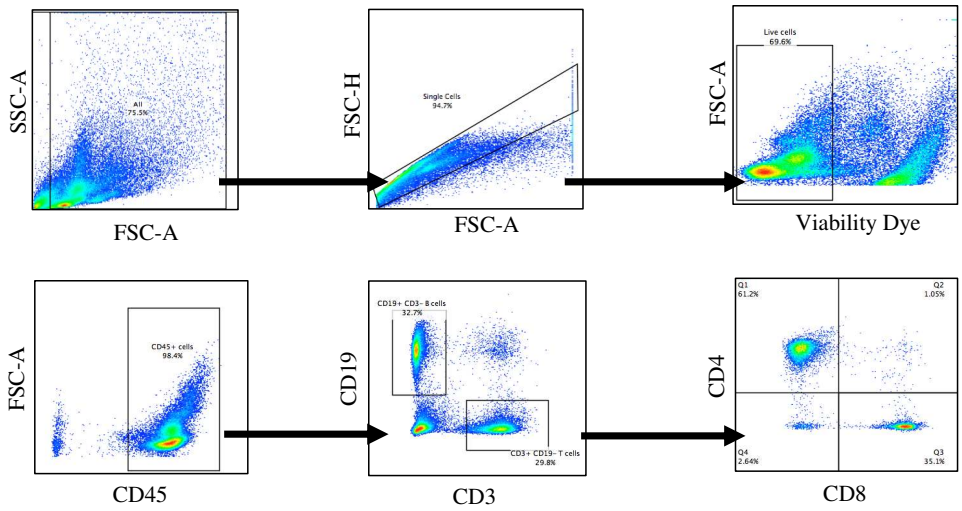


Supplemental Figure 1. Immune cells identified with flow cytometry. To identify innate and adaptive immune cells in the lung tissue and BAL fluid, we designed two panels of fluorochrome-conjugated antibodies specific for mouse cell-surface markers. (A) Innate: Innate immune cells were identified as follows: all immune cells ($CD45^+$), eosinophils ($Siglec\text{-}F^+$, $CD11c^-$), dendritic cells ($CD11c^+$, $MhcII^+$), neutrophils ($Ly6G^+$, $CD11b^+$), macrophage ($MerTK^+$, $CD6^+$), alveolar macrophages ($CD11b^-$, $Siglec\text{-}F^+$), interstitial macrophages ($CD11b^+$ $Siglec\text{-}F^-$), monocytes ($MerTK^+$, Ly6C+/-). (B) Adaptive: Adaptive immune cells were identified as follows: all immune cells ($CD45^+$), T cells ($CD3^+$), $CD4^+$ T cells ($CD3^+$, $CD4^+$), $CD8^+$ T cells ($CD3^+$, $CD8^+$), and B cells ($CD19^+$).

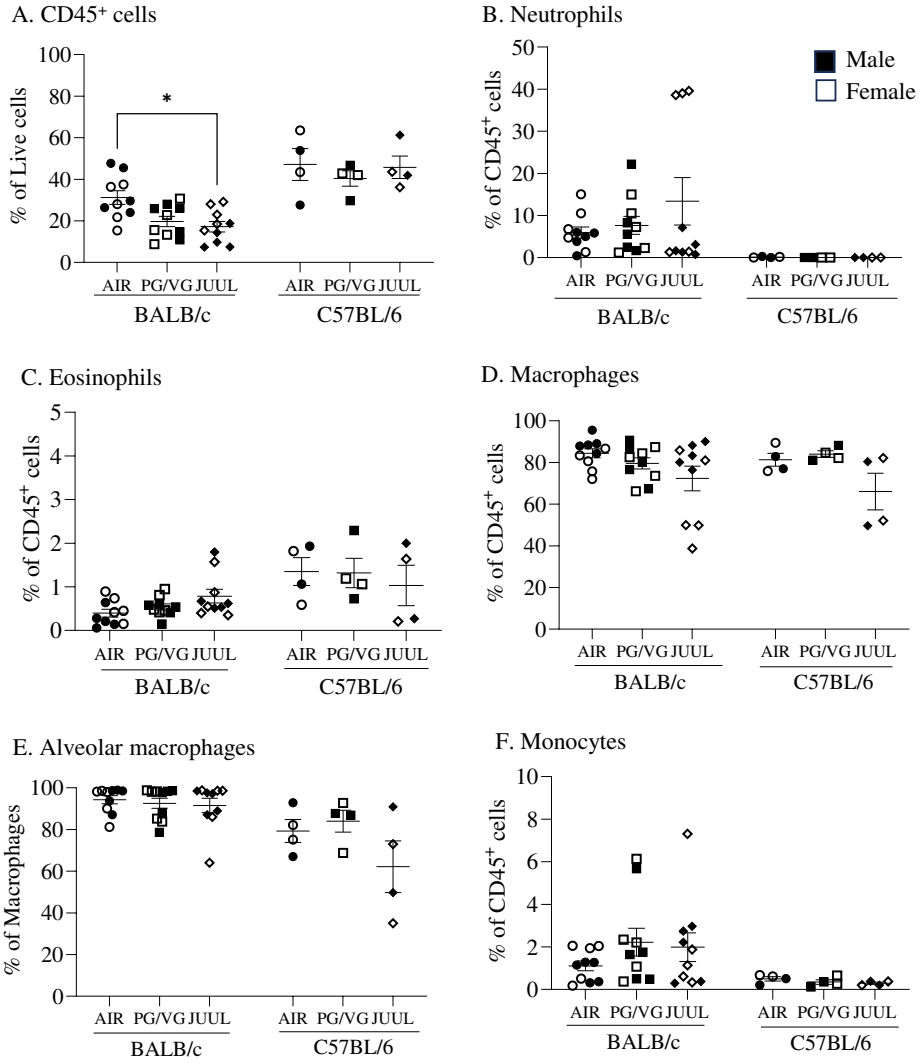
A. Innate



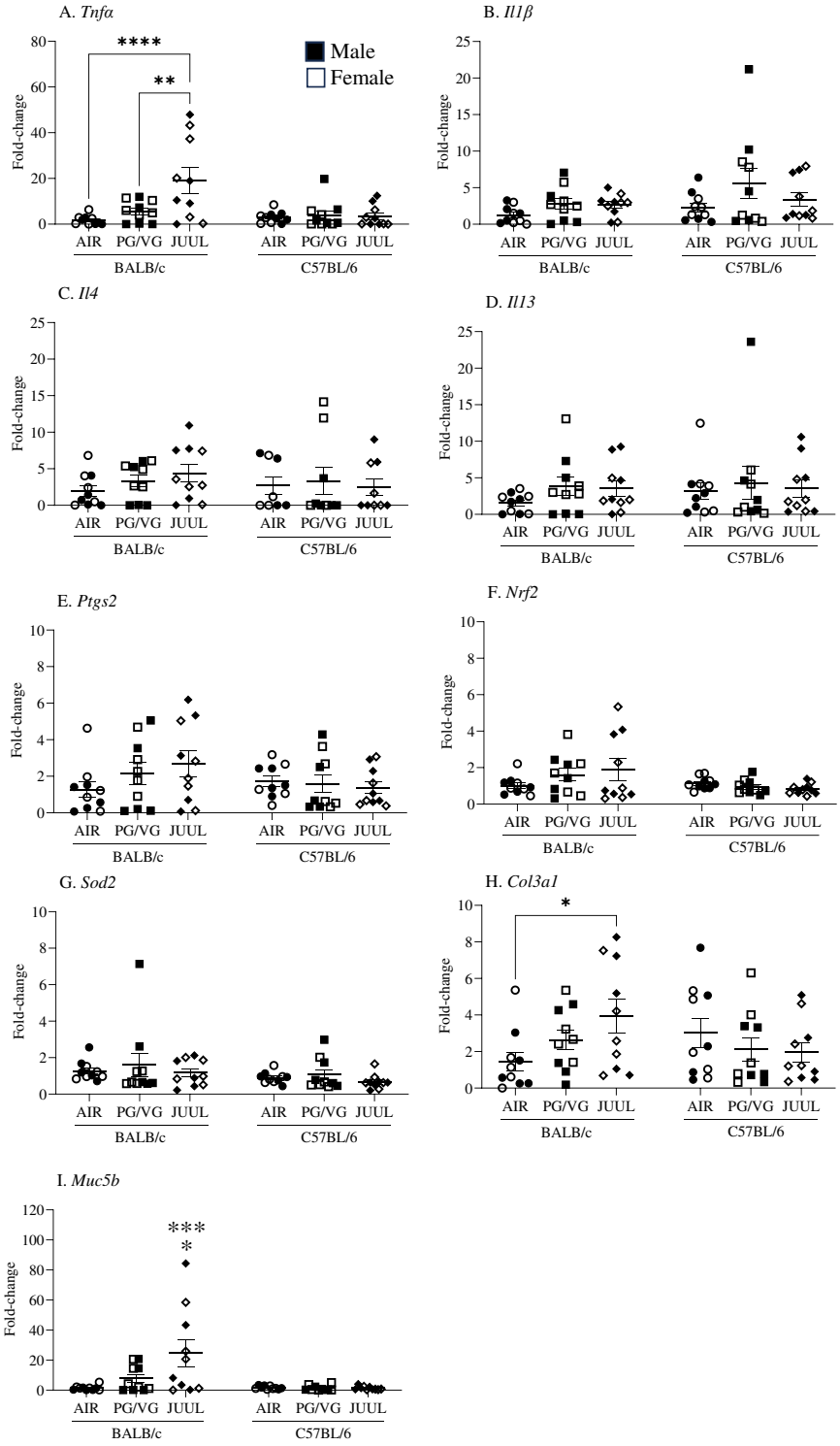
B. Adaptive



Supplemental Figure 2. Gating strategy for the identification of immune cells in BAL fluid and lung tissue. The gating strategies for innate immune cells (A) and adaptive immune cells (B) are shown.



Supplemental Figure 3. There are minimal changes in the percentages of BAL immune cells in BALB/c and C57BL/6 mice after JUUL exposure. The frequency of $CD45^+$ cells (A), neutrophils (B), eosinophils (C), macrophages (D), alveolar macrophages (E), and monocytes (F) are shown. JUUL exposure decreased the frequency of total immune cells only in BALB/c mice. Data represent pooled samples from two independent experiments and are expressed as mean ± SEM. Differences were analyzed by two-way ANOVA (* $p \leq 0.05$).


A. *Tnfα*
B. *Il1β*
C. *Il4*
D. *Il13*
E. *Ptgs2*
F. *Nrf2*
G. *Sod2*
H. *Col3a1*
I. *Muc5b*
Male
Female
Fold-change
AIR PG/VG JUUL
BALB/c
C57BL/6
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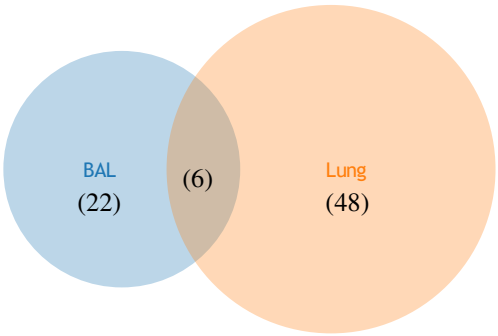
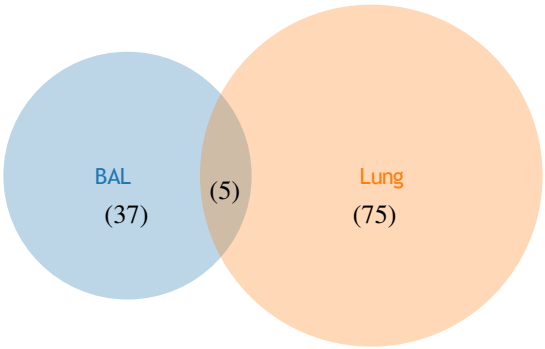
Supplemental Figure 4. Effects of JUUL exposure on gene expression in the lung tissue of BALB/c and C57BL/6 mice. Expression of *Tnfα* (A), *Il1β* (B), *Il4* (C), *Il13* (D), *Ptgs2* (E), *Nrf2* (F), *Sod2* (G), *Col3a1* (H), *Muc5b* (I) was evaluated. There was significant upregulation of *Tnfα*, *Col3a1*, and *Muc5b* mRNA only in JUUL-exposed BALB/c mice. There were no significant changes in the expression of these genes in C57BL/6 mice. Gene expression was normalized to 18s rRNA. Data represent pooled samples from two independent experiments and are expressed as mean ± SEM. Differences were analyzed by two-way ANOVA (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

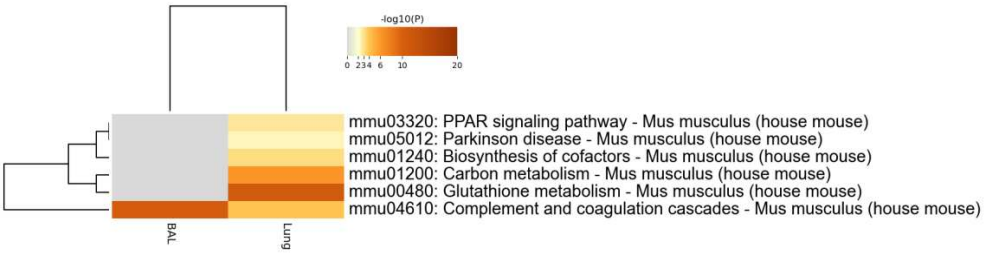
A. Male versus Female C57BL/6



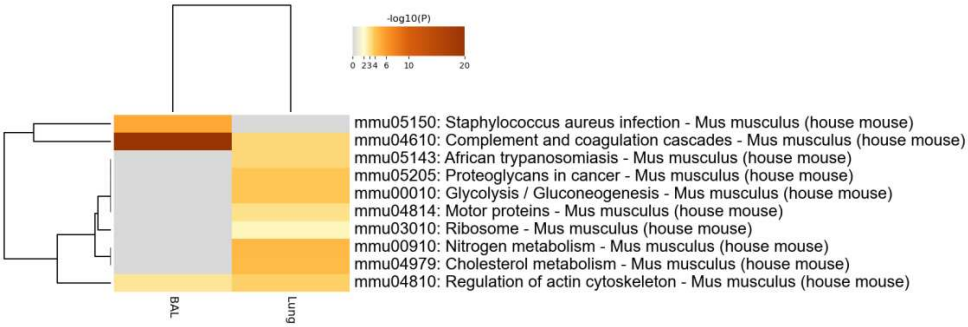
B. Male versus Female BALB/c



C. Male versus Female C57BL/6- KEGG



D. Male versus Female BALB/c- KEGG



Supplemental Figure 5. Sex-specific changes in the expression of proteins in naïve mice. (A) Male versus Female C57BL/6. Numbers on the Venn diagram indicates DEPs between male versus female air-exposed mice. Note that there was minimal overlap in DEPs between the lungs and BAL. (B) Male versus Female BALB/c. Numbers on the Venn diagram indicates DEPs between male versus female air-exposed mice. Note that there was minimal overlap in DEPs between the lungs and BA. (C). Male versus Female C57BL/6- KEGG: KEGG pathways enrichment revealed minimal overlap in pathways between the lungs and the BAL. Note the enrichment of metabolic pathways in the lungs. (D) Male versus Female BALBc- KEGG: KEGG pathways enrichment

revealed minimal overlap in pathways between the lungs and the BAL. Note the enrichment of metabolic pathways in the lungs.