Supplemental Material

Single cell RNA-seq analysis reveals that prenatal arsenic exposure results in long-term, adverse effects on immune gene expression in response to Influenza A infection

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Table S1. F_1 offspring and dams used for each experiment.

[1]Number of offspring mice with corresponding number of litter dams in parentheses.

Treatment	Albumin concentration in BALF	Cell number in BALF	Viral Titer Day 3	Viral Titer Day 7	IL-1β in Lung homogenate	IL-10 in BALF	Flow cytometry	Sc-RNA seq
	Female/Male	Female/Male	Female/Male	Female/Male	Female/Male	Female/Male	Female/Male	Female/Male
	n(N)[1]	n(N)[1]	n(N)[1]	n(N)[1]	n(N)[1]	n(N)[1]	n(N)[1]	n(N)[1]
Control (untreated)	14(10)/ 12(10)	13(10) / 12(10)	5(5) / 6(5)	9(9) / 9(9)	10(9) / 10(9)	12(10) / 5(5)	6-10(2-3)/6-8(2-3)	3(2-3) Female only
Arsenic	12(10) / 12(10)	12(10) / 12(10)	5(5) / 6(5)	9(9) / 10(9)	8(9) / 10(9)	12(10) / 8(5)	6-10(2-3)/6-8(2-3)	3(2-3) Female only

Available on Dryad (https://datadryad.org/**) at** doi:10.5061/dryad.vt4b8gtp6

Table S2. Immgen reference annotation.

Samples in the Immgen compendium (original sample names in column 1) were annotated with additional columns for use with the singleR reference-based annotation as follows: Filename_ed: file name edited for typos to allow for computer identification of sample groups; Cell_Description: sample identity description based on markers listed in GEO sample accession viewer (e.g. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1136119); Label_main: generalized cell identity label; Lineage: samples defined as monophagosome system and granulocytes (MPS_GN), Lymphoid, or Other; Include: specifies whether to include sample in singleR annotation. A small subset of samples with treatments difficult to interpret in relation to the present experiment were excluded.

Tables S3-5. Arsenic effect on gene expression in individual monophagosome system and granulocyte cell populations

Genes differentially expressed in mice previously exposed to arsenic vs vehicle control were identified in a cell type specific manner using limma-voom. Tables contain the log2FC (S3), nominal P Value (S4) and FDR-corrected P Value (S5) for the arsenic effect in each cell type (columns) for all genes detected within these populations (rows). Genes that were below detection in an individual cell type are listed as NA.

Tables S6-8. Arsenic effect on gene expression in individual lymphocyte populations

Genes differentially expressed in mice previously exposed to arsenic vs vehicle control were identified in a cell type specific manner using limma-voom. Tables contain the log2FC (S3), nominal P Value (S4) and FDR-corrected P Value (S5) for the arsenic effect in each cell type (columns) for all genes detected within these populations (rows). Genes that were below detection in an individual cell type are listed as NA.

Table S9. Genes differentially expressed in composite analysis of arsenic vs. control cells.

Supplemental Figures

Figure S1. Weight loss of mice after IAV infection. Results of proportion weight loss from original weight of mice were tracked over the course of IAV infection. (n=7-8). Data were analyzed using one-way ANOVA.

Figure S2. Multiplex results of inflammatory cytokines from BALF at day 7 IAV infection. Results from day 7 IAV infection are from one independent experiment. **A)** Female (top) (n=4-5) and male (bottom) (n=5-8) candidate inflammatory cytokines quantified from BALF. Data are presented as means ± standard deviation. Data were analyzed using linear models that accounted for arsenic exposure and batch. * $P < .05$, *** $P < .001$ **B)** Female (top) (n=4) and male (bottom) (n=8) observable cytokines assessed as fold change over control.

Figure S3. UMAP visualization of cells from individual samples. Individual UMAP projections of cells from each sample, with cells colored by cluster. All 22 clusters are present in each sample.

Figure S4. Detection of IAV transcript in individual cells. **A)** Cells with > 0.05% viral transcript were classified as infected and are highlighted in red on the UMAP projection. **B)** Percent of cells infected in each cell type. Bars represent means ± standard deviation, N=3 per treatment group. While higher levels of infection were observed in neutrophils, macrophages, and monocytes, as expected, no significant differences in cell-type specific infection levels were observed between arsenic and control. C) Flow cytometry of HA^+ $CD45^+$ from lung of day 3 IAV infected mice. (Female n=8-10; Male n=7-8) Data were analyzed using linear models that accounted for arsenic exposure and batch.

Figure S1

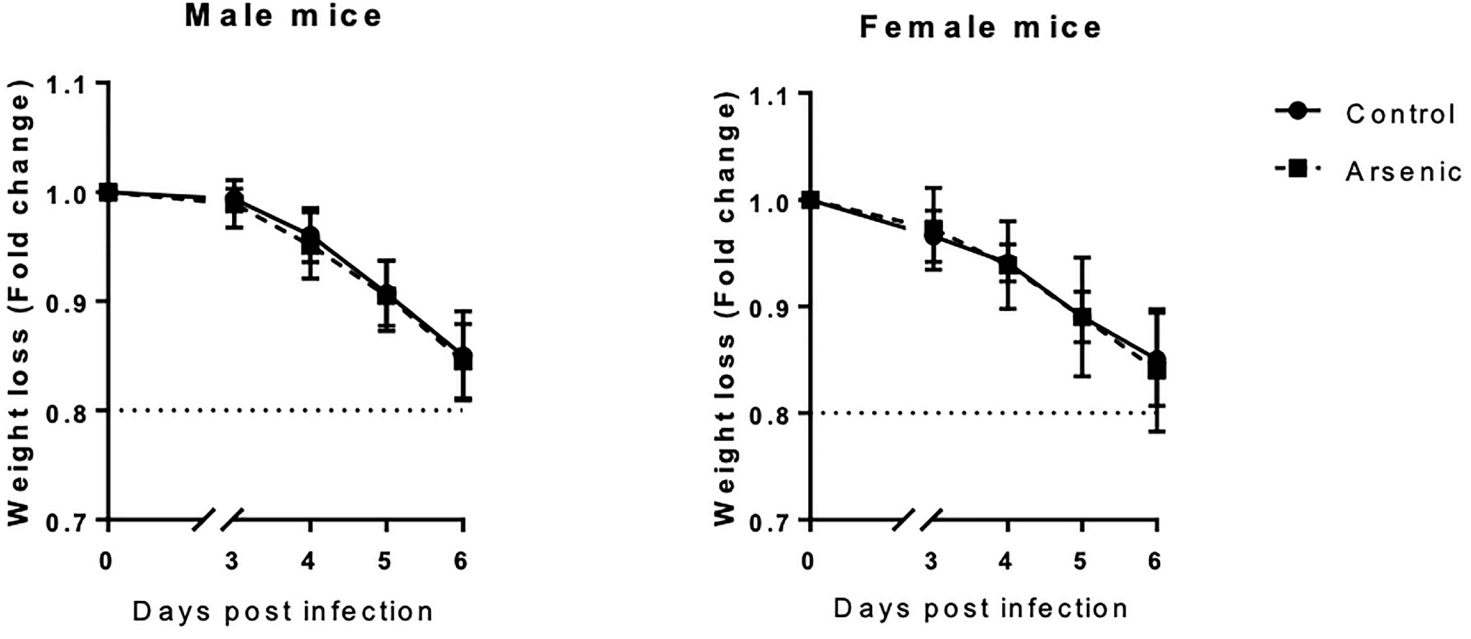
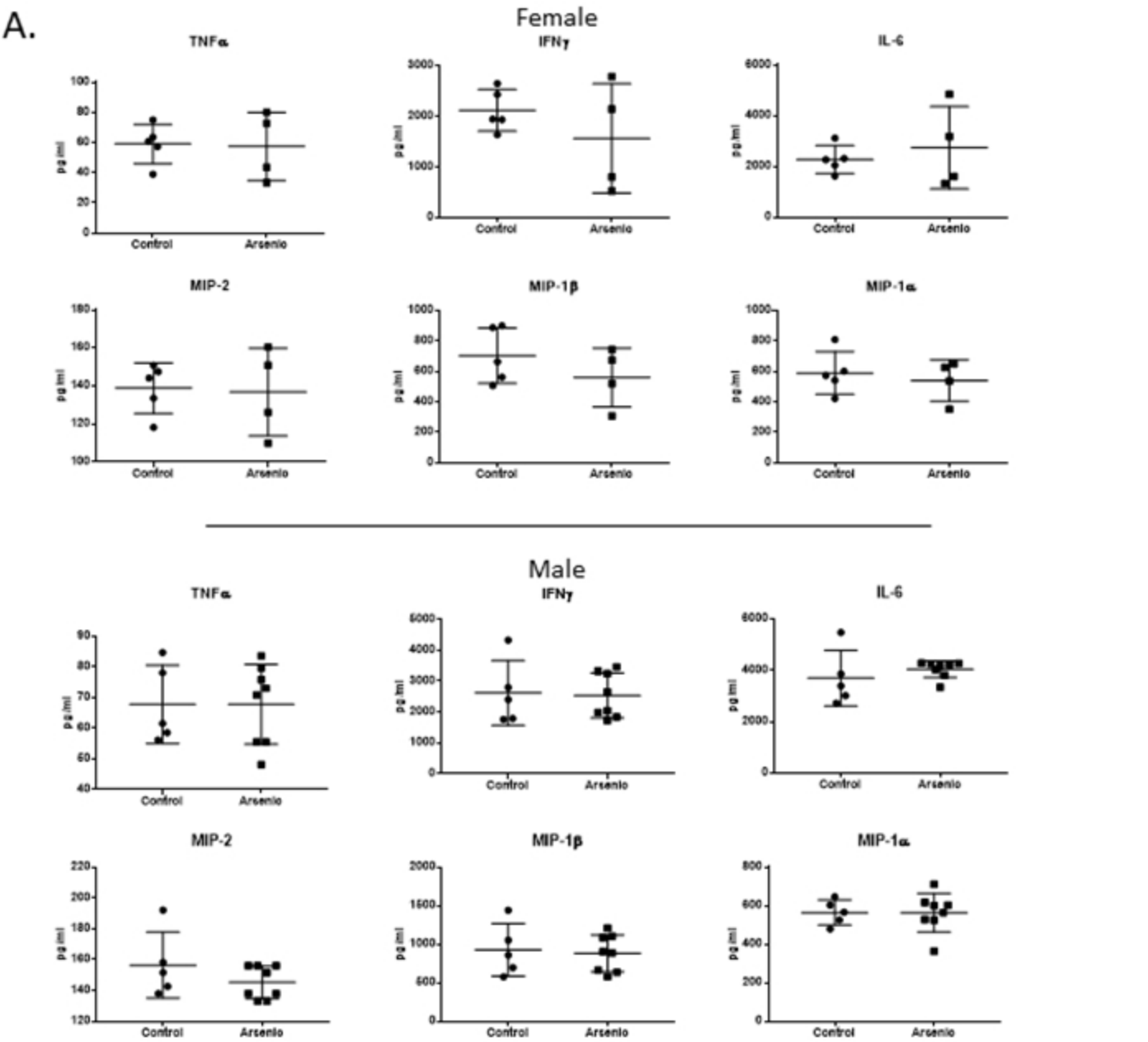

Male mice
Female mice
Weight loss (Fold change)
1.1
1.0
0.9
0.8
0.7
0
3
4
5
6
Days post infection
Control
Arsenic

Figure S2


A.
Female
TNFα
IFNγ
IL-6
MIP-2
MIP-1β
MIP-1α
pg/ml
Control
Arsenic
Male
TNFα
IFNγ
IL-6
MIP-2
MIP-1β
MIP-1α
pg/ml
Control
Arsenic

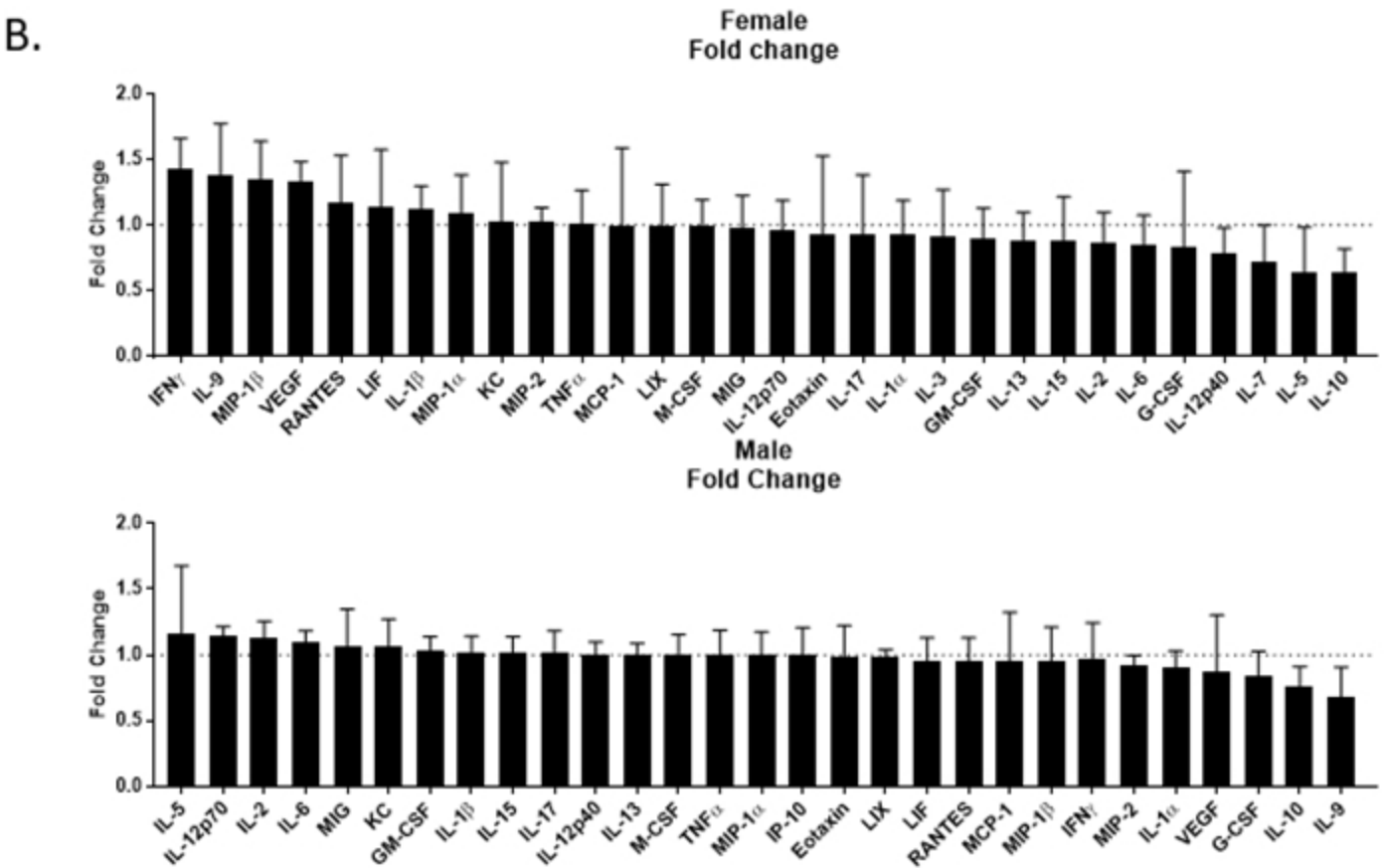

B.
Female
Fold change
Fold Change
IFNγ IL-9 MIP-1β VEGF RANTES LIF IL-1β MIP-1α KC MIP-2 TNFα MCP-1 LIX M-CSF MIG IL-12p70 Eotaxin IL-17 IL-1α IL-3 GM-CSF IL-13 IL-15 IL-2 IL-6 G-CSF IL-12p40 IL-7 IL-5 IL-10
Male
Fold Change
Fold Change
IL-5 IL-12p70 IL-2 IL-6 MIG KC GM-CSF IL-1β IL-15 IL-17 IL-12p40 IL-13 M-CSF TNFα MIP-1α IP-10 Eotaxin LIX LIF RANTES MCP-1 MIP-1β IFNγ MIP-2 IL-1α VEGF G-CSF IL-10 IL-9

Figure S3

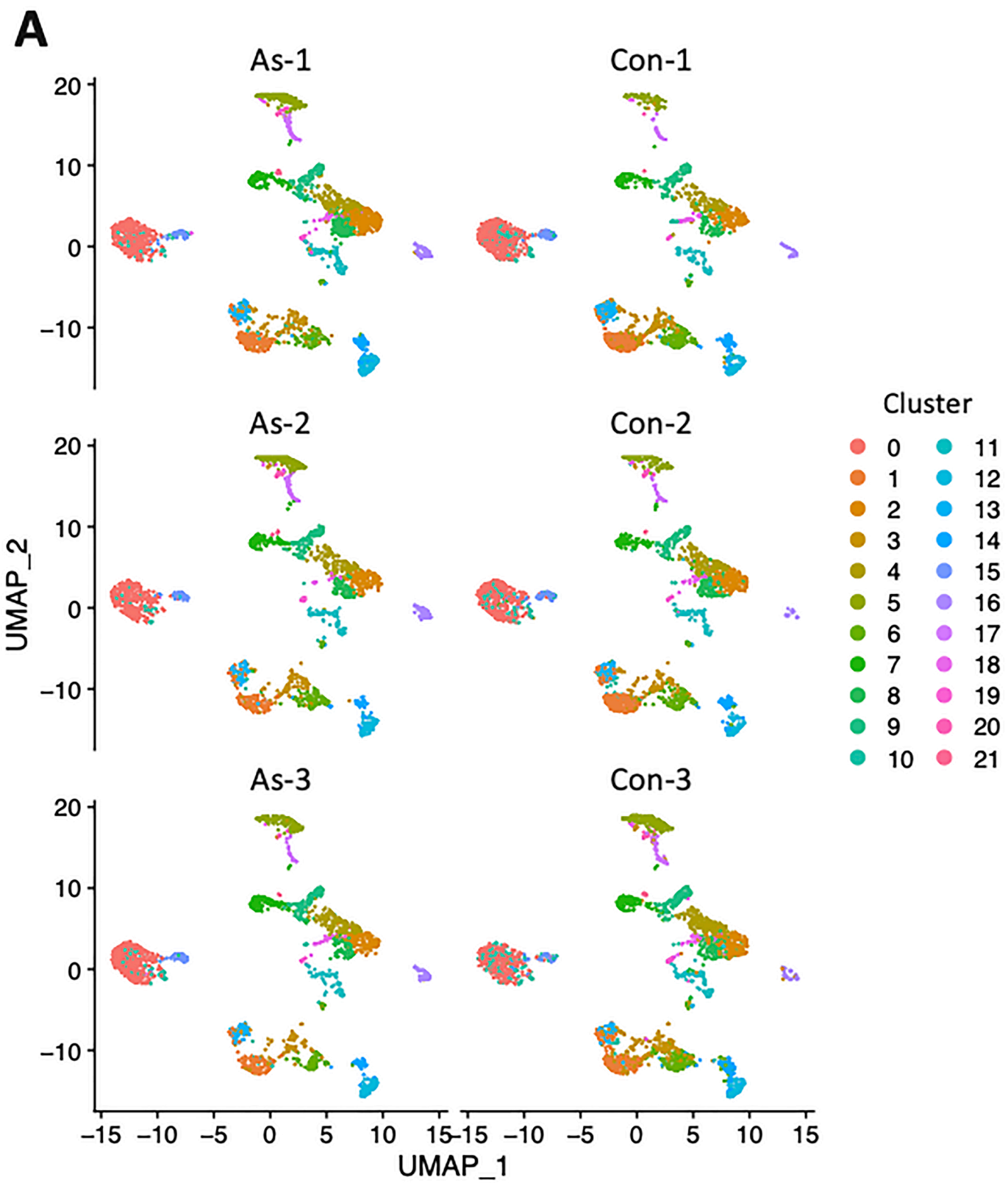
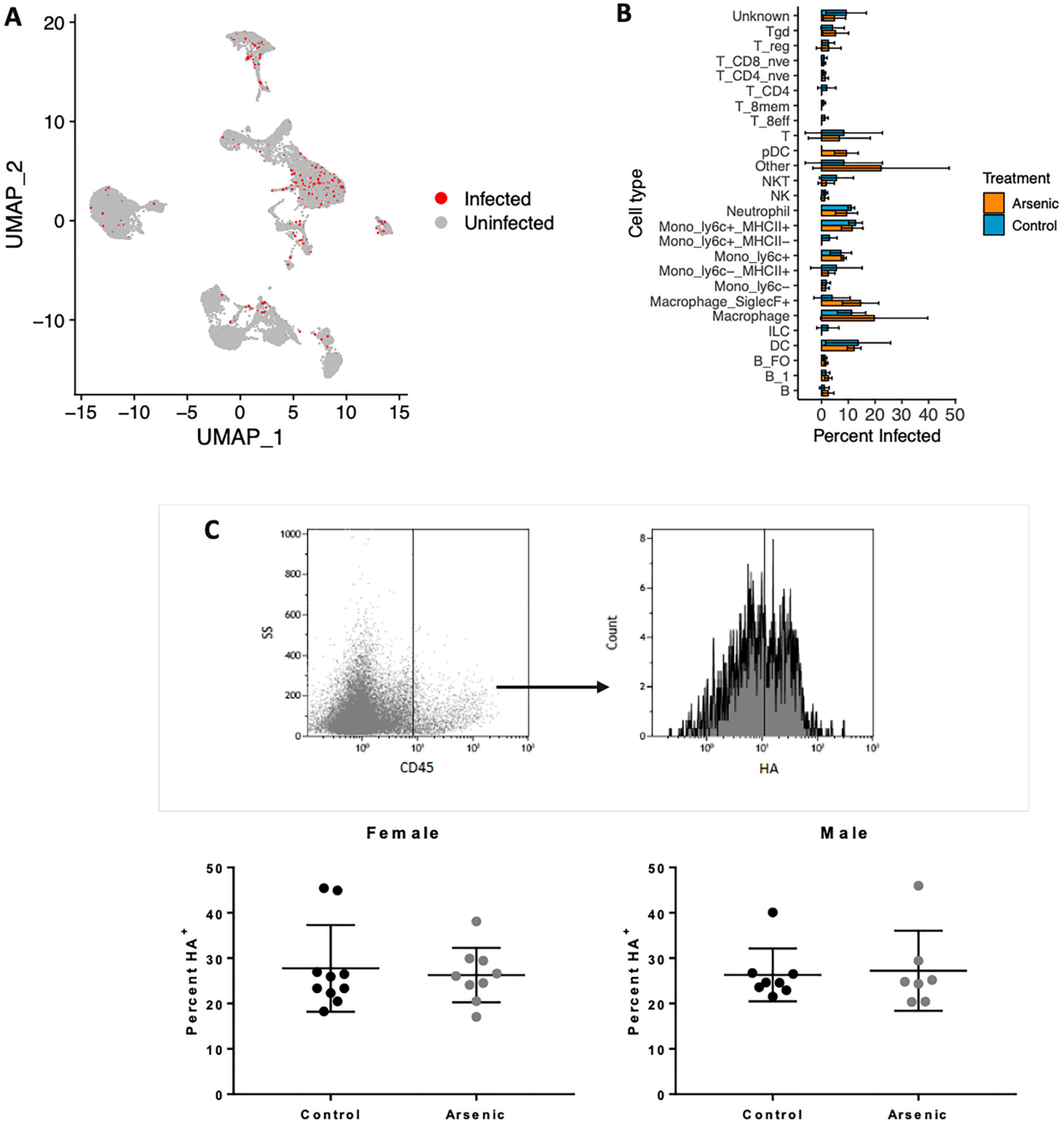

A
As-1
Con-1
As-2
Con-2
As-3
Con-3
UMAP_2
UMAP_1
20
10
0
−10
−15
−10
−5
0
5
10
15
Cluster
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21

Figure S4


A
UMAP_2
UMAP_1
Infected
Uninfected
B
Cell type
Unknown
Tgd
T_reg
T_CD8_nve
T_CD4_nve
T_CD4
T_8mem
T_8eff
T
pDC
Other
NKT
NK
Neutrophil
Mono_ly6c+_MHCII+
Mono_ly6c+_MHCII−
Mono_ly6c+
Mono_ly6c−_MHCII+
Mono_ly6c−
Macrophage_SiglecF+
Macrophage
ILC
DC
B_FO
B_1
B
Percent Infected
Treatment
Arsenic
Control
C
SS
CD45
Count
HA
Female
Male
Percent HA+
Control
Arsenic