

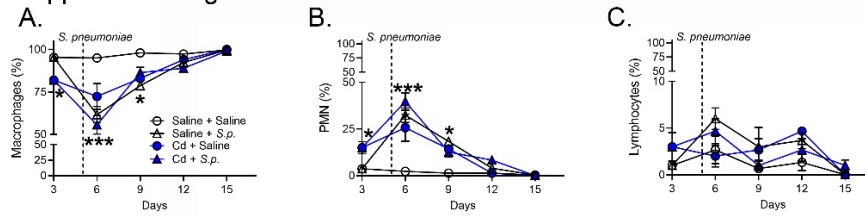
Supplemental Information

Impaired PPAR γ activation by cadmium exacerbates infection-induced lung injury

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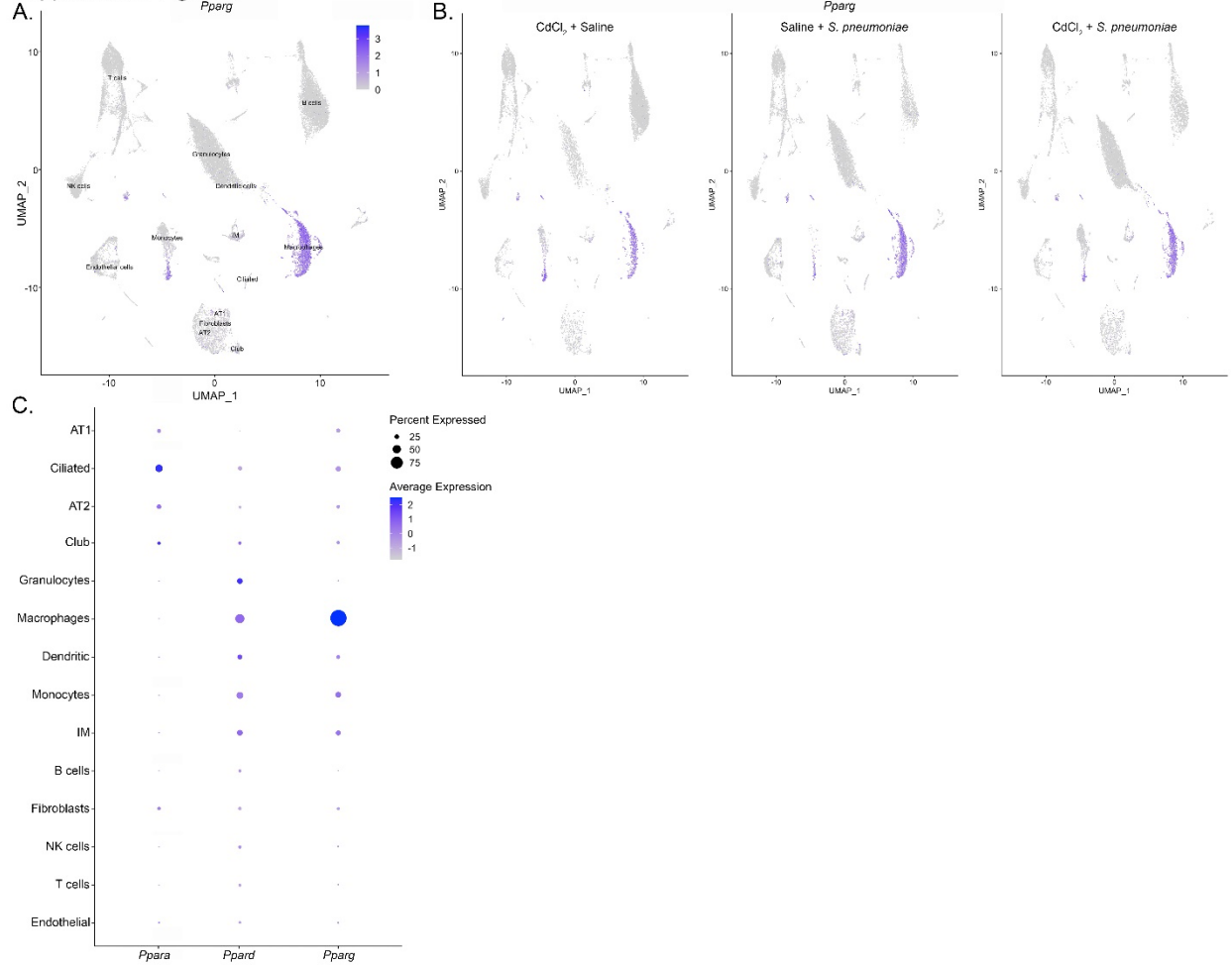
Supplemental Figures and Legends:

Supplemental Figure 1



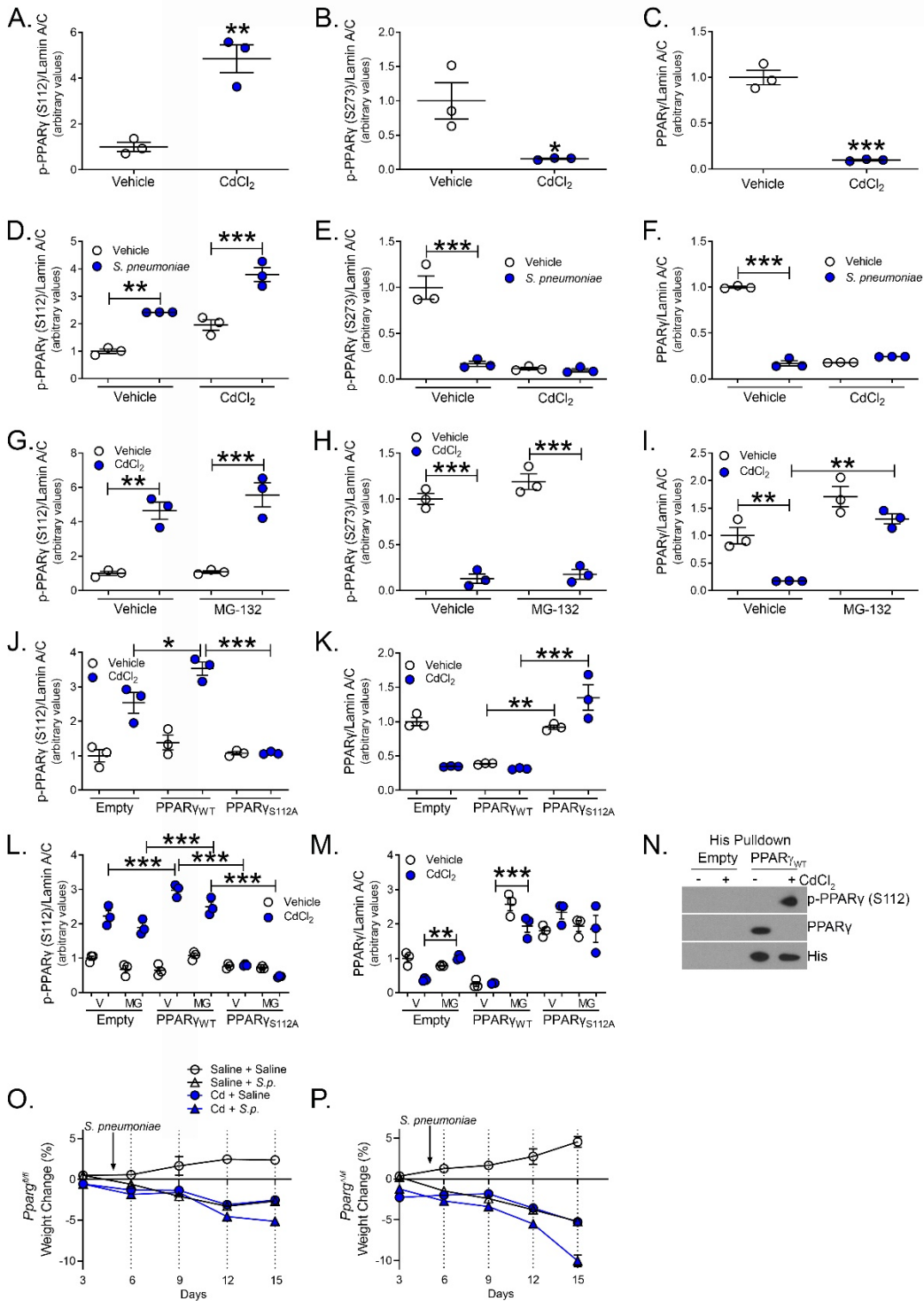
Supplemental Figure 1. Cell differentials from exposed mice. Cell differential to identify (A) macrophages, (B) neutrophils (PMN), and (C) lymphocytes. $n = 3$. *, $p < 0.05$; ***, $p < 0.0001$ vs. Saline + Saline. Values shown as mean \pm S.E.M. Two-way AVOVA, Tukey's post hoc.

Supplemental Figure 2



Supplemental Figure 2. Cell clusters expressing *Pparg* from exposed mice. *Pparg* expression on UMAP plot of (A) all cells and (B) after individual exposure. (C) Dot plots of *Ppara*, *Ppard*, and *Pparg* expression in all cell clusters.

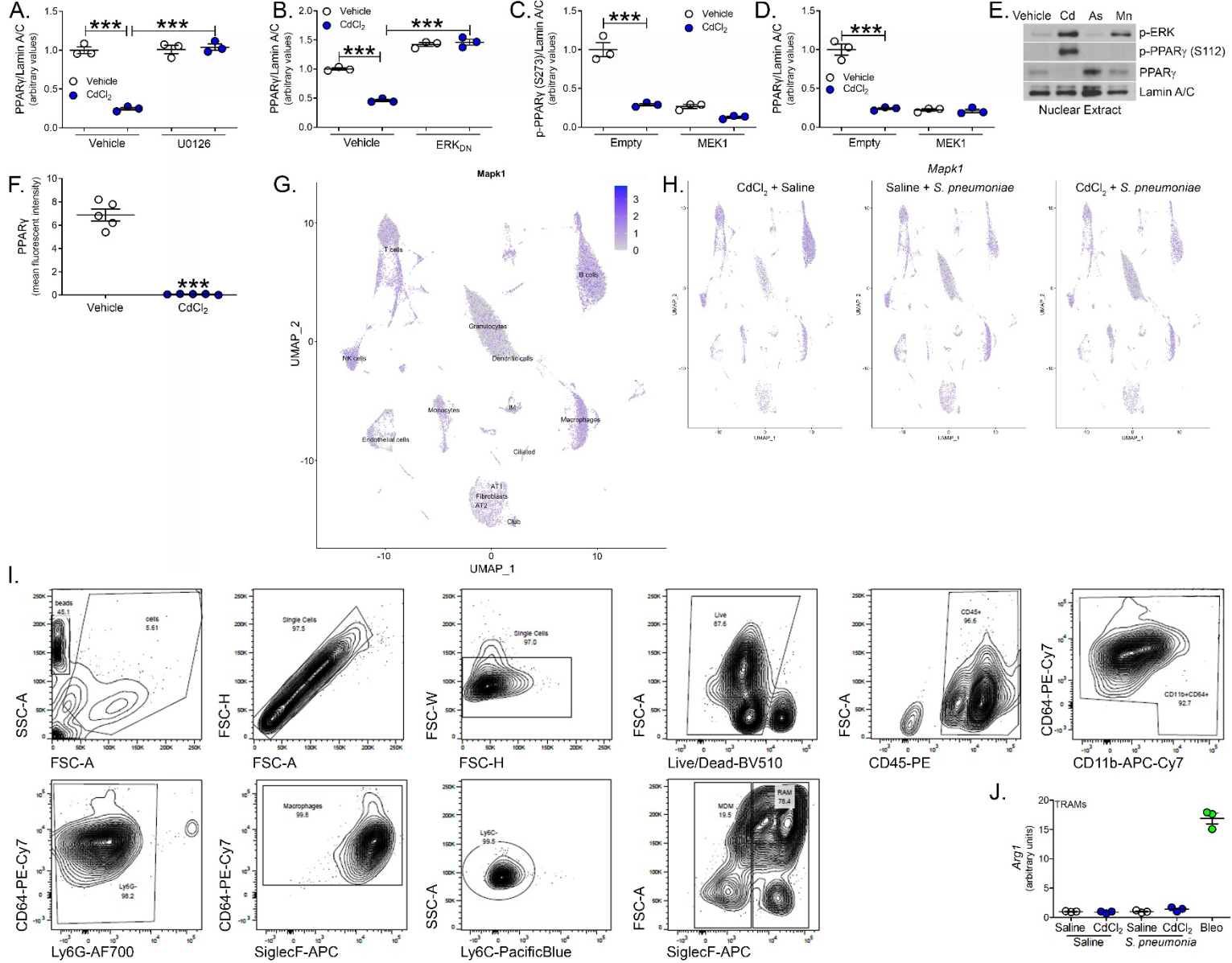
Supplemental Figure 3



Supplemental Figure 3. Cadmium regulates PPAR γ phosphorylation at Ser¹¹² resulting in greater lung injury. Statistical quantification of (A) p-PPAR γ (S112), (B) p-PPAR γ (S273), and (C) PPAR γ from the nuclear immunoblot analysis of THP-1 cells exposed to CdCl₂ (50 μ M, 3h)

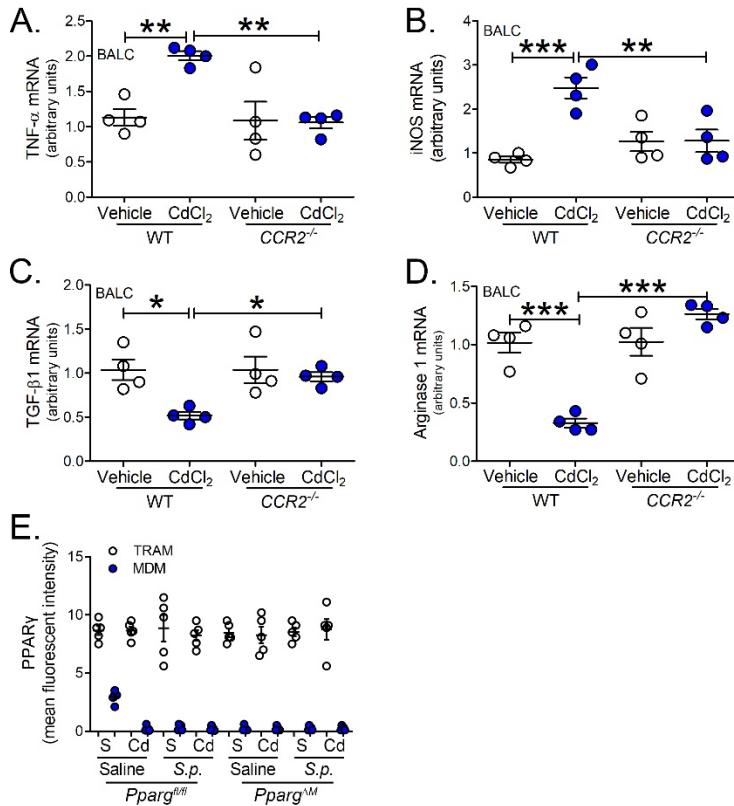
n = 3. Statistical quantification of **(D)** p-PPAR γ (S112), **(E)** p-PPAR γ (S273), and **(F)** PPAR γ from the nuclear immunoblot analysis from BAL cells from exposed WT mice *n* = 3. Statistical quantification of **(G)** p-PPAR γ (S112), **(H)** p-PPAR γ (S273), and **(I)** PPAR γ from immunoblot analysis in isolated nuclear extract of THP-1 cells treated with vehicle or MG-132 (20 μ M, 6h) and saline or CdCl $_2$ (50 μ M, 3h) *n* = 3. Statistical quantification of **(J)** p-PPAR γ (S112) and **(K)** PPAR γ from the nuclear immunoblot analysis of THP-1 cells expressing empty, PPAR γ_{WT} , or PPAR γ_{S112A} treated with saline or CdCl $_2$ *n* = 3. Statistical quantification of **(L)** p-PPAR γ (S112) and **(M)** PPAR γ from the nuclear immunoblot analysis of THP-1 cells expressing empty, PPAR γ_{WT} , or PPAR γ_{S112A} treated with saline or CdCl $_2$ and vehicle or MG-132. *n* = 3. **(N)** THP-1 cells transfected with empty or PPAR γ_{WT} -His and treated with saline or CdCl $_2$ (50 μ M, 3h). PPAR γ -His was purified by pull down and immunoprecipitation for PPAR γ . Percent weight change of exposed **(O)** *Pparg*^{*fl/fl*} and **(P)** *Pparg*^{*ΔM*} mice. *n* = 3-5. *, *p* < 0.05; **, *p* < 0.001; ***, *p* < 0.0001. Values shown as mean \pm S.E.M. Student's *t*-test used in A-C. One-way AVOVA, Tukey's post hoc.

Supplemental Figure 4



Supplemental Figure 4. Cell clusters expressing *Mapk1* from exposed mice. (A) Statistical quantification of PPAR γ from the nuclear immunoblot analysis of THP-1 cells exposed to vehicle or U0126 (10 μ M, 1h) and CdCl $_2$ (50 μ M, 3h) $n = 3$. (B) Statistical quantification of PPAR γ from the nuclear immunoblot analysis of THP-1 cells transfected with empty or ERK $_{DN}$ and exposed to saline or CdCl $_2$ $n = 3$. Statistical quantification of (C) p-PPAR γ (S273) and (D) PPAR γ from the nuclear immunoblot analysis of THP-1 cells transfected with empty or MEK1 and exposed to saline or CdCl $_2$ $n = 3$. (E) Immunoblot analysis of THP-1 cells exposed to CdCl $_2$ (50 μ M, 3h), NaAsO $_3$ (100 μ M, 3h), or MnCl $_2$ (200 μ M, 3h). (F) Statistical quantification of representative confocal imaging of exposed MH-S cells for PPAR γ staining $n = 5$. *Mapk1* expression on UMAP plot of (G) all cells and (H) after individual exposure. (I) Representative flow gating strategy from isolated BAL after cadmium exposure. Tissue resident alveolar macrophages (TRAM, CD45 $^{+}$ CD11b $^{+/-}$ Ly6G $^{-}$ CD64 $^{+}$ Ly6c $^{-}$ Siglec F hi) and monocyte-derived macrophages (MDM, CD45 $^{+}$ CD11b $^{+/-}$ Ly6G $^{-}$ CD64 $^{+}$ Ly6c $^{-}$ Siglec F low). (J) Arginine 1 mRNA expression in TRAMs from exposed WT mice, bleomycin (bleo) BAL cells used as positive control. $n = 3$. ***, $p < 0.0001$. Values shown as mean \pm S.E.M. Student's t -test used in F. One-way AVOVA, Tukey's post hoc.

Supplemental Figure 5

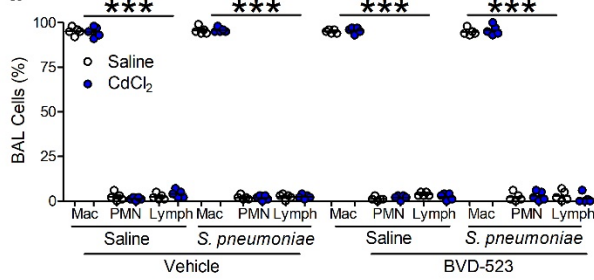


Supplemental Figure 5. *CCR2*^{-/-} mice show inhibition of cadmium-mediated inflammation.

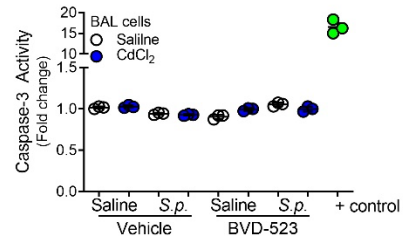
WT and *CCR2*^{-/-} mice were exposed to saline or CdCl₂ (100ng/kg) by i.t. administration. mRNA expression in isolated BAL cells of (A) TNF- α , (B) iNOS, (C) TGF- β 1, and (D) Arg1. $n = 4$. Statistical quantification of confocal imaging from (E) PPAR γ staining $n = 5$. *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean \pm S.E.M. One-way AVOVA, Tukey's post hoc.

Supplemental Figure 6

A.



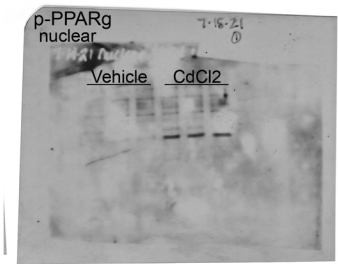
B.



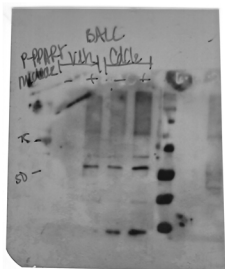
Supplemental Figure 6. ERK inhibition does not alter cell type present in BAL fluid. (A) Cell differential to identify macrophages (mac), neutrophils (PMN), and lymphocytes (lymph). $n = 5$. **(B)** Caspase-3 activity in BAL cells from exposed mice. $n = 3$. Positive control = 5 μ M Rhodamine 110 reference standard. ***, $p < 0.0001$. Values shown as mean \pm S.E.M. One-way AVOVA, Tukey's post hoc.

Figure 3

A.



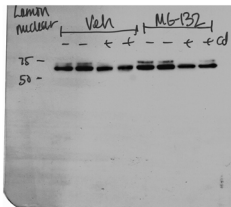
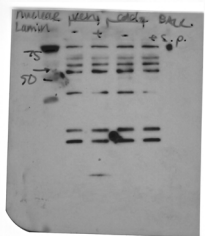
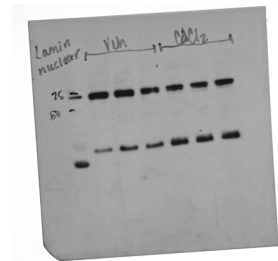
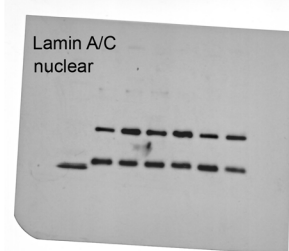
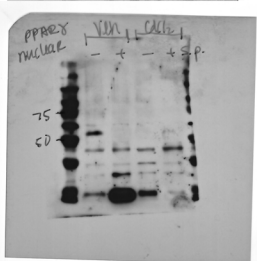
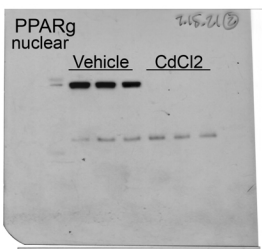
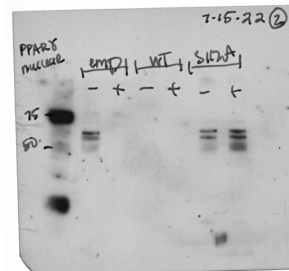
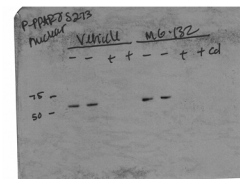
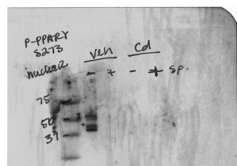
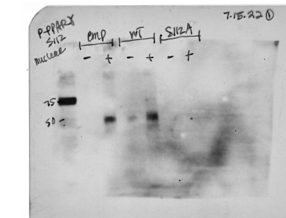
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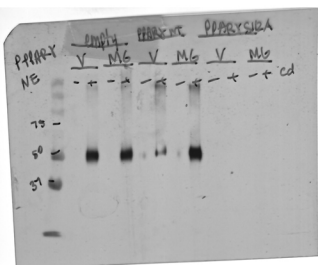
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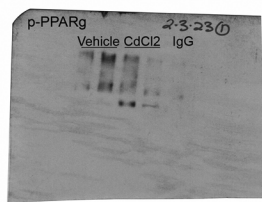
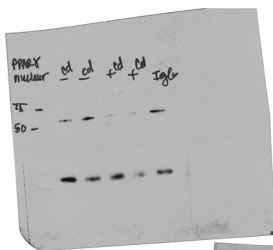
D.



E.



G.



I.

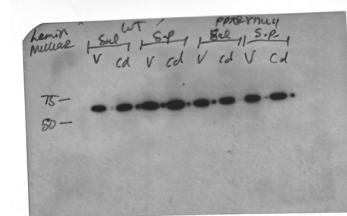
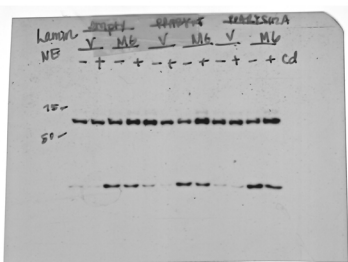
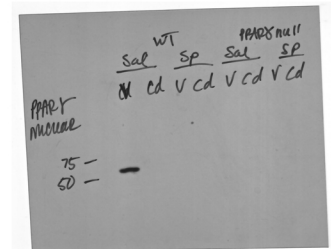
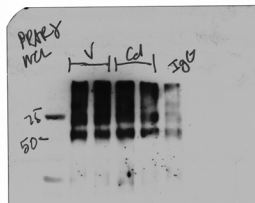
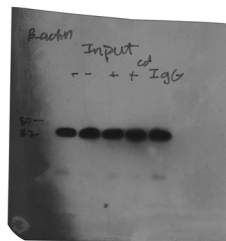
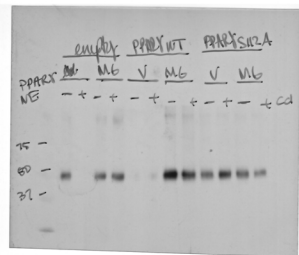
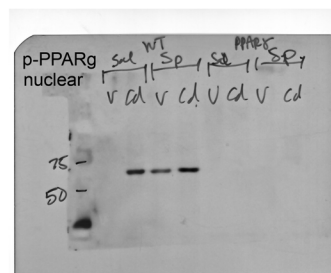
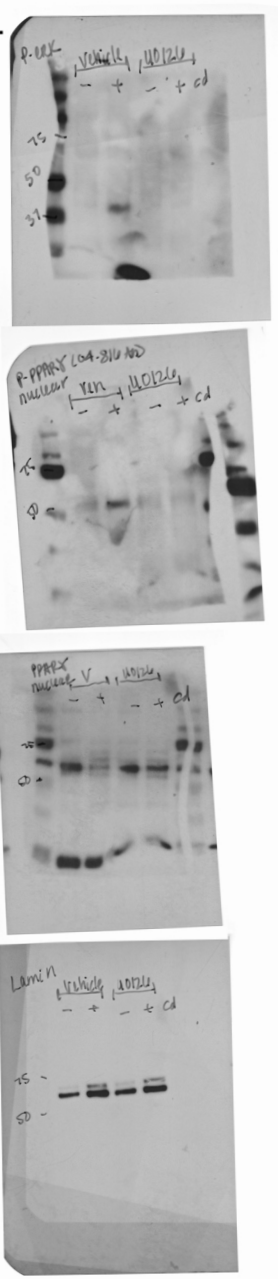
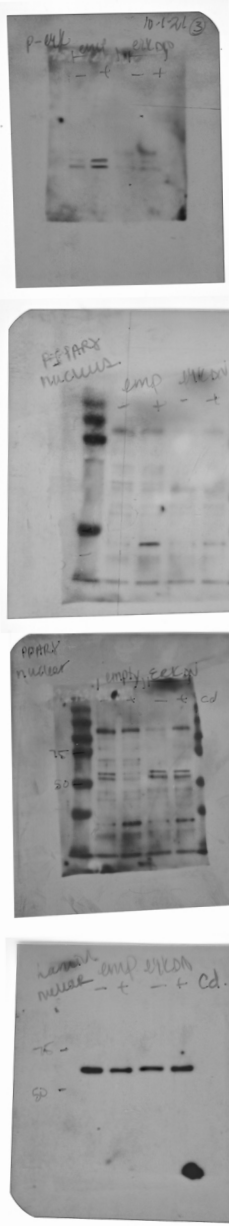


Figure 4

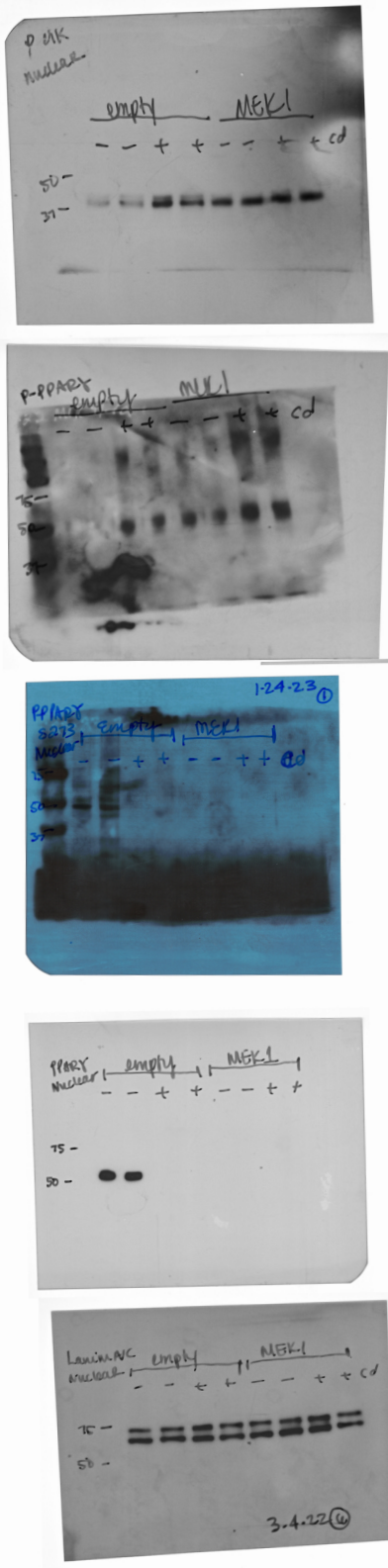
A.



D.



G.



N.

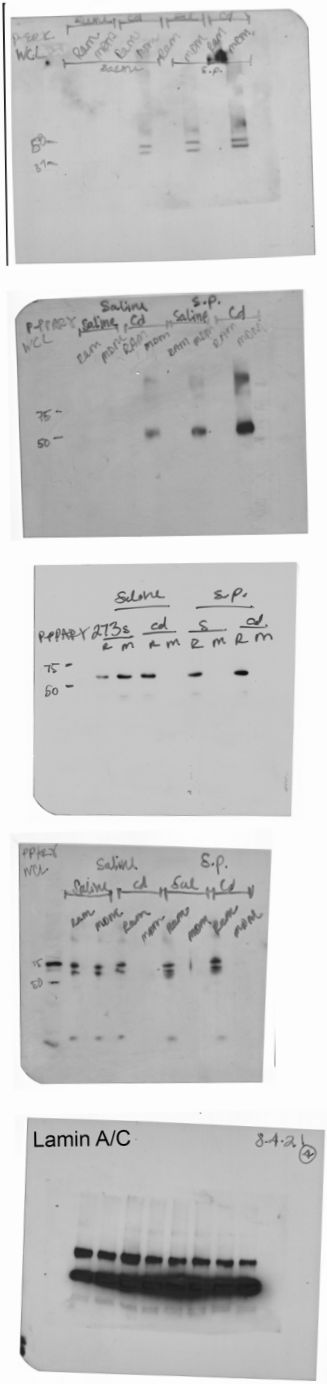


Figure 6

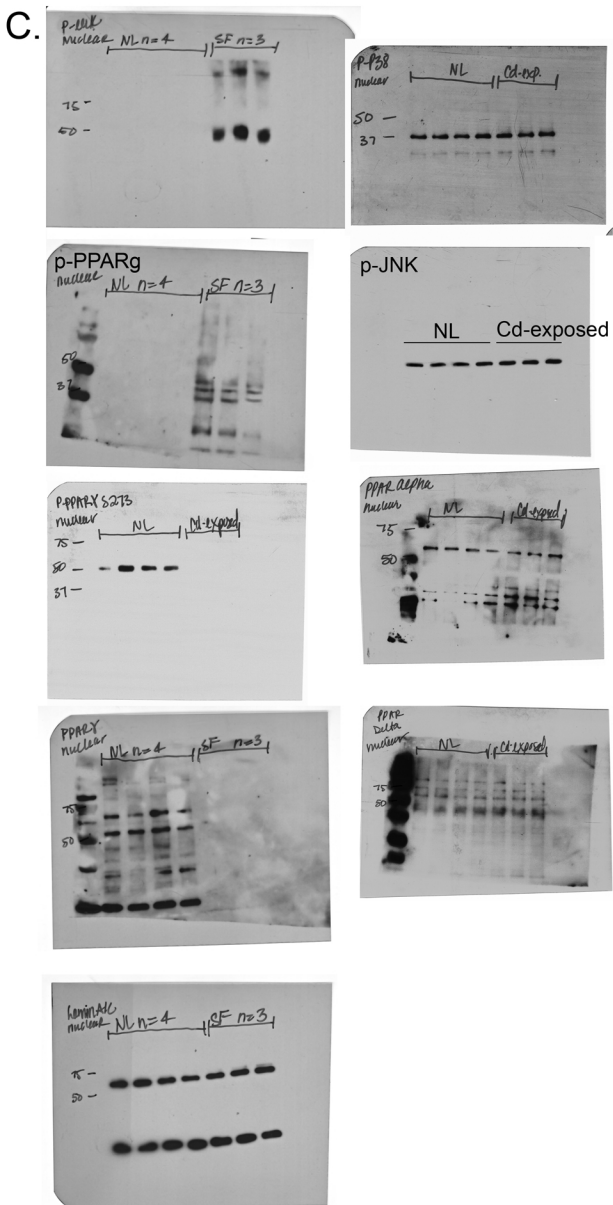
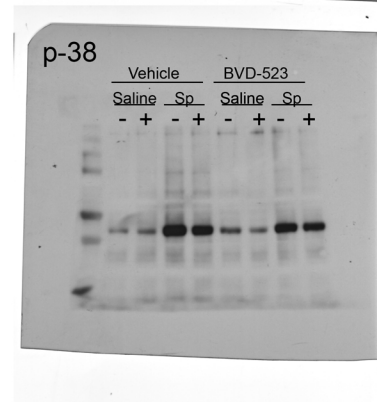
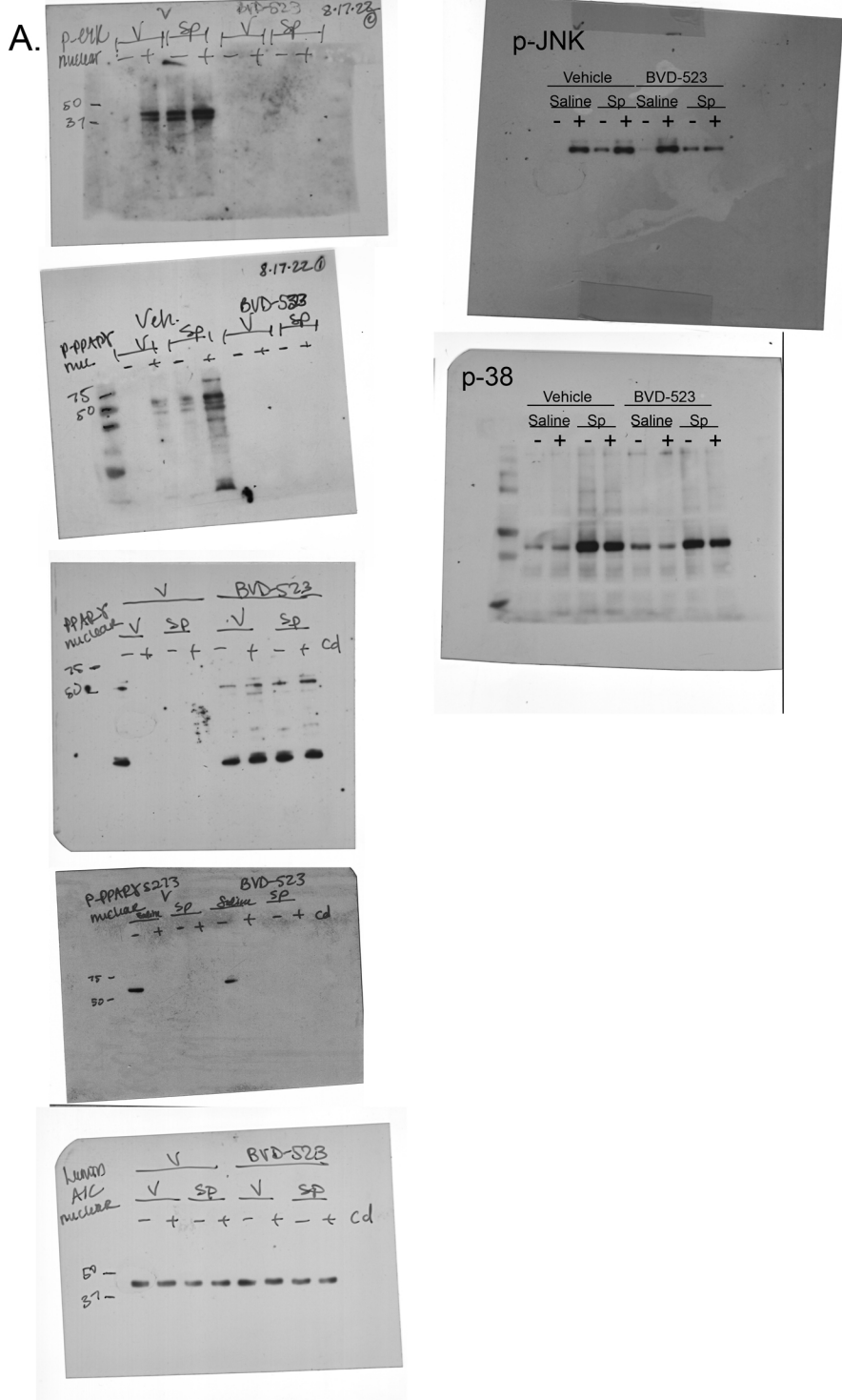
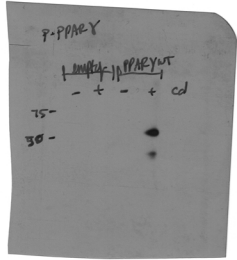
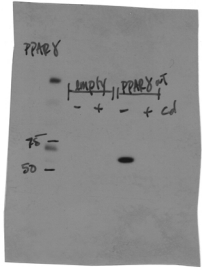


Figure 7



Supplemental Figure 3

N.



Supplemental Figure 4

E.

