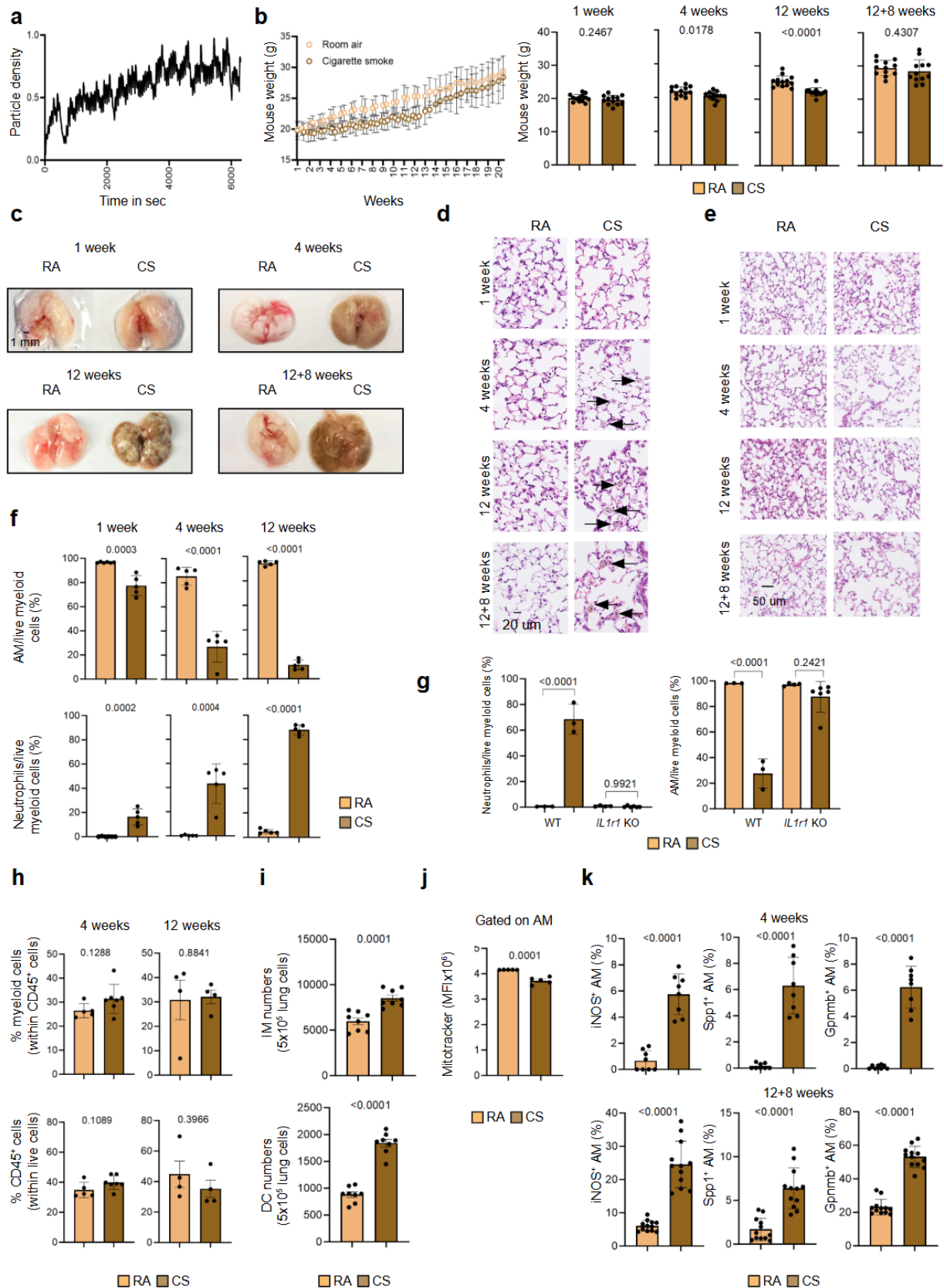
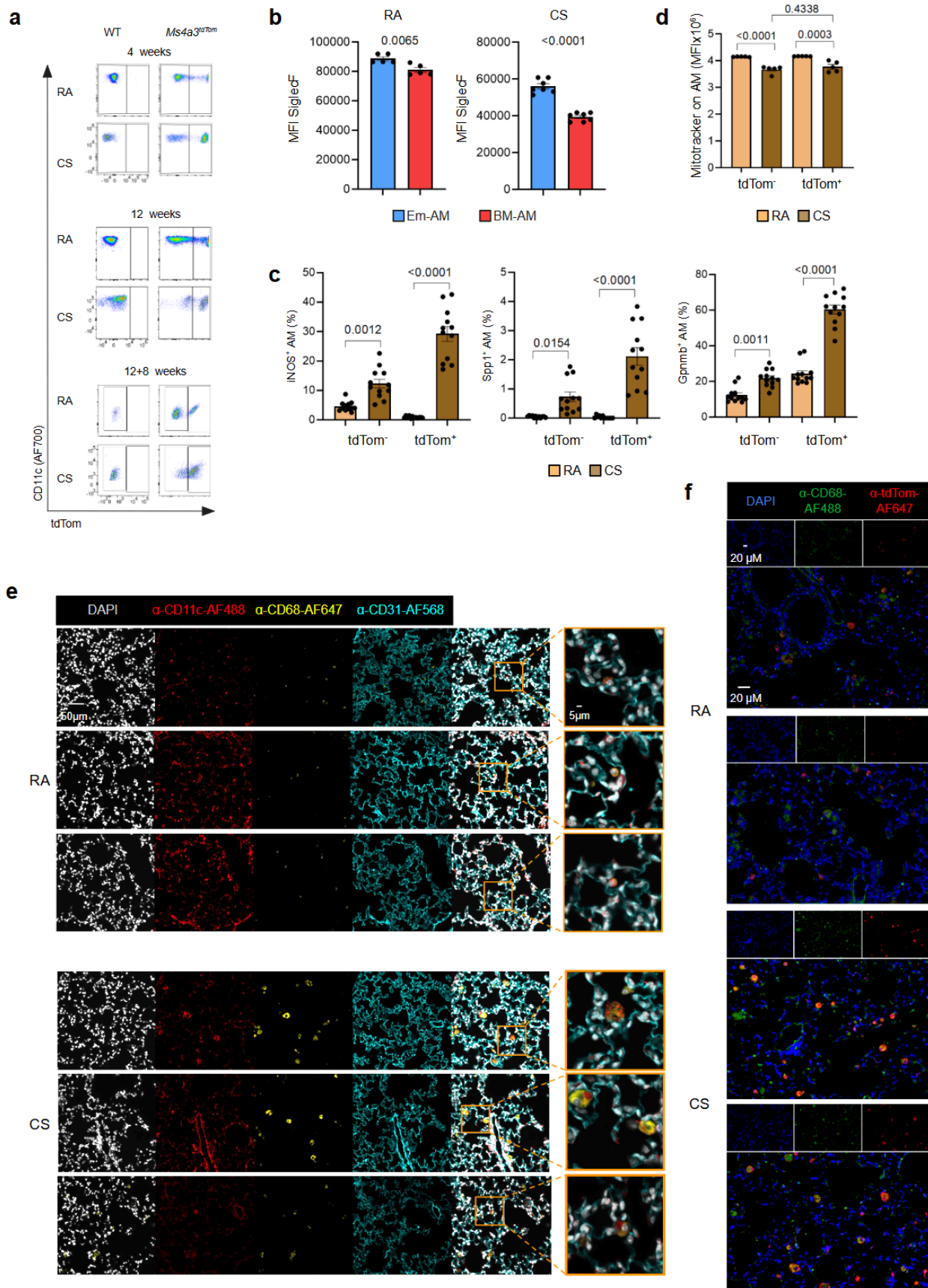


Supplementary information



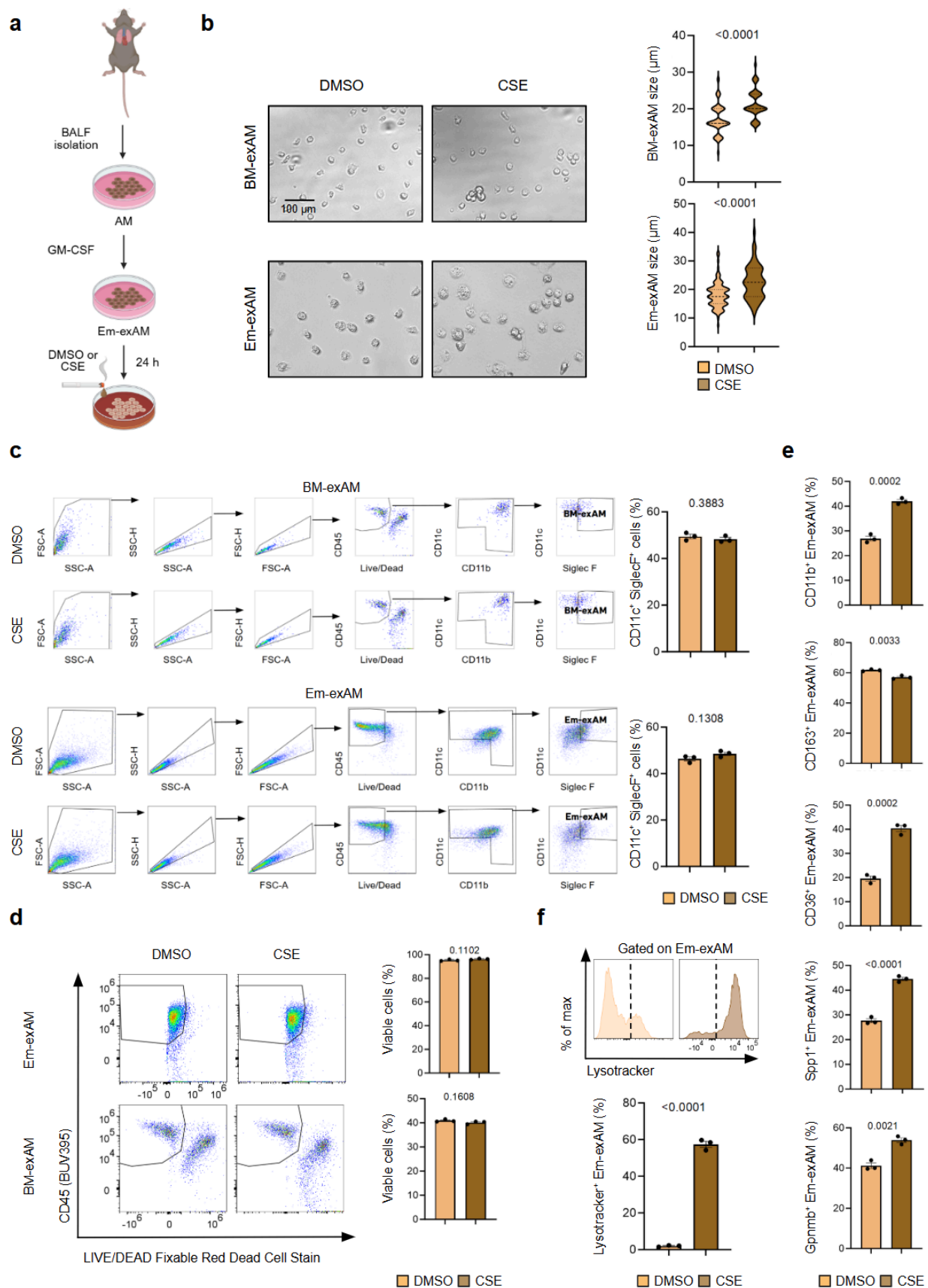
Supplementary Fig. 1 | CS-induced COPD-like mouse model mimics some aspects of human COPD.

- a**, Representative line graph showing particle density recorded during a smoking session.
- b**, Graphs showing mouse weights over 12 weeks of smoking followed by 8 weeks of resting. Weight was recorded every other day prior to the smoking session (n=12-13 biologically independent samples).
- c**, Representative photographs of lungs after CS exposure for 1, 4, 12 or 12+8 weeks.
- d**, Representative enlarged histology analysis of murine lungs after CS exposure for 1, 4, 12 or 12+8 weeks. Arrows indicate large brownish AM.
- e**, Histological images of murine lungs after CS exposure for 1, 4, 12 or 12+8 weeks.
- f**, Flow cytometry analysis of AM and neutrophil frequencies out of all myeloid cells from BALF from mice exposed to CS for 1, 4, 12 or 12+8 weeks (n=5-6 biologically independent samples).
- g**, Flow cytometry analysis of AM and neutrophil frequencies out of all myeloid cells from BALF from WT or *IL1r1* KO mice exposed to CS for 1 week (n=3-6 biologically independent samples).
- h**, Bar graphs showing frequencies of myeloid cells out of all CD45⁺ cells and CD45⁺ cells out of all live cells after 4 and 12 weeks of CS exposure (n=4-7 biologically independent samples).
- i**, Bar graphs showing counts of IM and DC out of 0.5 million lung cells after 4 weeks of CS exposure (n=8 biologically independent samples).
- j**, Bar graph showing Mitotracker MFI on AM after 4 weeks of RA/CS exposure measured by flow cytometry (n=5 biologically independent samples).
- k**, Bar graphs showing iNOS, Spp1 and Gpnmb levels in AM after 4 and 12+8 weeks of CS exposure (n=8-12 biologically independent samples). Data are presented as mean $\pm$ SD (b, f-k). Statistical comparisons were performed with two-tailed unpaired Student's *t*-tests (b, f, h-k; data points were normally distributed) or one-way ANOVA tests (g; for simultaneous comparisons of more than two groups). Source data is provided as a Source Data file.



Supplementary Fig. 2 | AM undergo origin-independent phenotypic changes upon CS exposure.

- a**, Flow cytometry plots showing tdTom signal of WT and *Ms4a3^{tdTom}* mice after 4, 12 or 12+8 weeks of RA/CS exposure.
- b**, Bar graphs showing SiglecF levels of BM-AM and Em-AM after 4 weeks of RA/CS exposure (RA: n=5 biologically independent samples; CS: n=7 biologically independent samples).
- c**, Bar graphs showing iNOS, Spp1 and Gpnmb levels of BM-AM and Em-AM after RA/CS exposure for 12+8 weeks (n=12 biologically independent samples).
- d**, Bar graph showing Mitotracker MFI on AM after 4 weeks of RA/CS exposure measured by flow cytometry (n=5 biologically independent samples).
- e**, Immunofluorescence staining of CD11c and CD68 on AM, and CD31 on endothelial cells in CS lung samples (4 weeks); DAPI was used for nuclear staining (n=3 biologically independent samples).
- f**, Confocal fluorescence microscopy images showing BM-AM (CD68⁺tdTom⁺) and Em-AM (CD68⁺tdTom⁻) in FFPE-embedded lung slices from mice exposed to 4 weeks CS. DAPI was used for nuclear staining (more replicates in Fig. 2d). Data are presented as mean $\pm$ SD (a-d). Statistical comparisons were performed with two-tailed unpaired Student's *t*-tests (b; data points were normally distributed) or one-way ANOVA tests (c-d; for simultaneous comparisons of more than two groups). Source data is provided as a Source Data file.



Supplementary Fig. 3 | *In vitro* exAM exhibit the similar alterations upon CSE activation as observed in the *in vivo* CS-induced COPD-like mouse model.

a, Schematic illustrating experimental layout of the CSE exAM model. AM were isolated from BALF of WT mice and cultured in presence of GM-CSF. The Em-exAM were treated with DMSO or CSE. Created with BioRender.com.

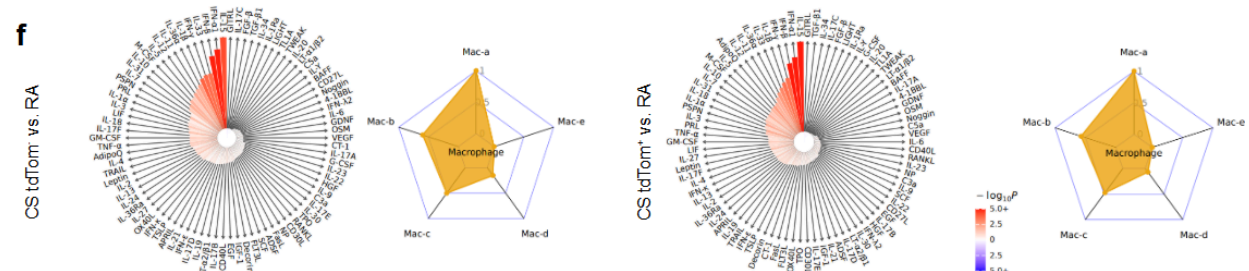
b, Representative microscopy images of DMSO- or CSE-treated Em-exAM and BM-exAM and bar graph showing quantification of cell sizes (BM-exAM: DMSO: n=100 cells; CSE: n=81 cells; Em-exAM: DMSO: n=93 cells; CSE: n=62 cells).

c, Flow cytometry plots and bar graphs illustrate gating strategy and AM percentages of Em-exAM and BM-exAM culture after DMSO and CSE treatment (n=3 biologically independent samples).

d, Representative flow cytometry plots and bar graph showing viability of Em-exAM and BM-exAM after DMSO or CSE treatment (n=3 biologically independent samples).

e, Bar graphs showing expression of CD163, CD11b, CD36, Spp1, and Gpnmb on Em-exAM (n=3 biologically independent samples).

f, Bar graph showing percentage of Lysotracker⁺ Em-exAM (n=3 biologically independent samples). Data are presented as mean $\pm$ SD (b-f). Statistical comparisons were performed with two-tailed unpaired Student's *t*-tests (b-f; data points were normally distributed). Source data is provided as a Source Data file.



Supplementary Fig. 4 | BM-AM and Em-AM share major transcriptomic adaptations upon CS exposure for 4 weeks.

a, Principal component 3 and 4 analysis of AM from 4, 12, and 12+8 weeks CS- and RA-exposed *Ms4a3^{tdTom}* mice (4 and 12 weeks: n=4 biologically independent samples, pooled 2 mice each; 12+8 weeks: n=4 biologically independent samples, pooled 3 mice each).

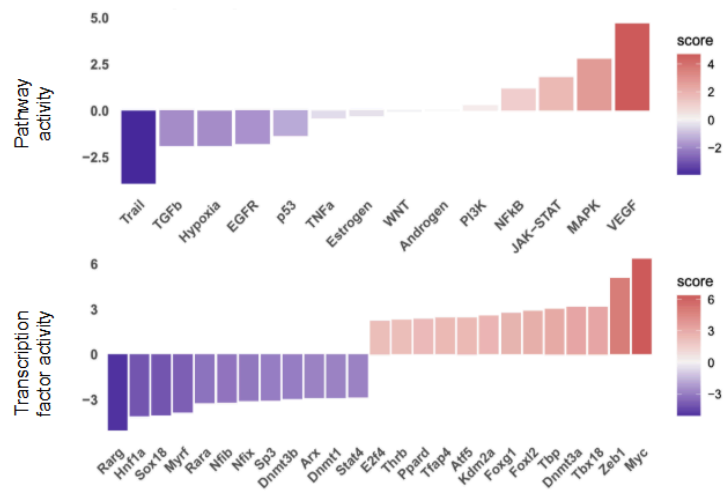
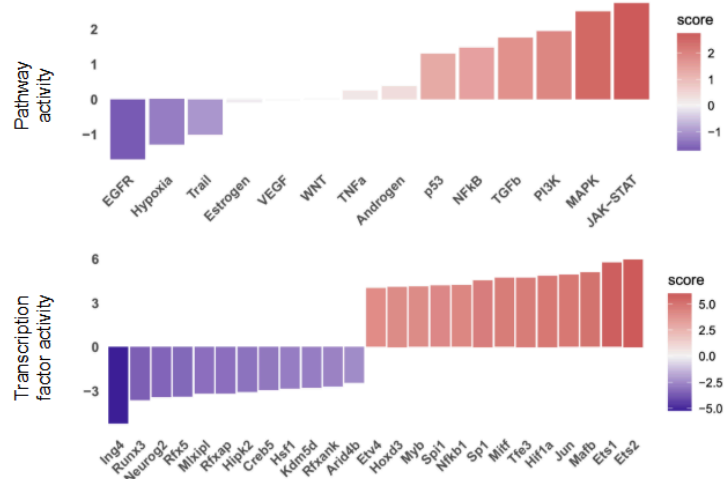
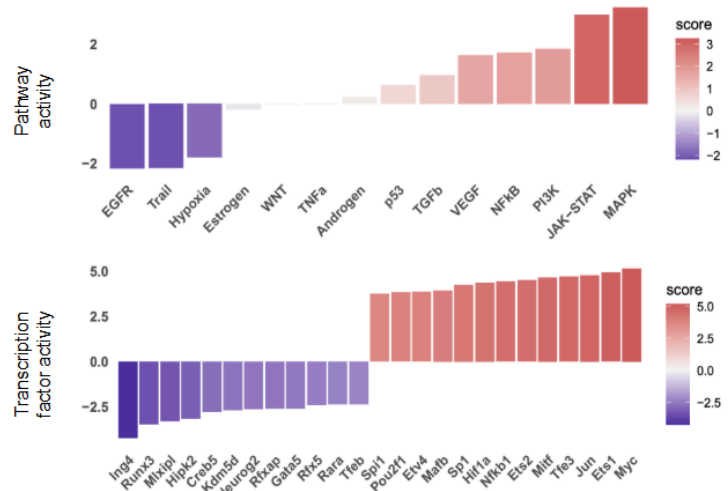
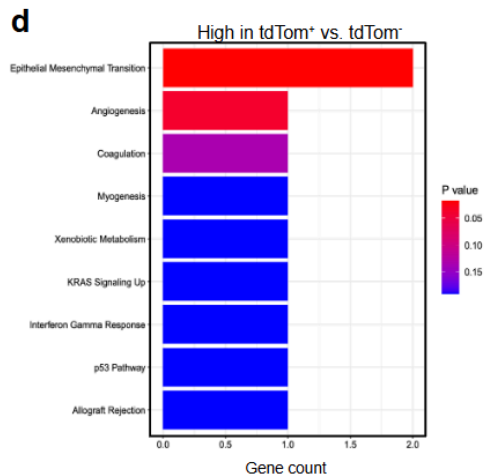
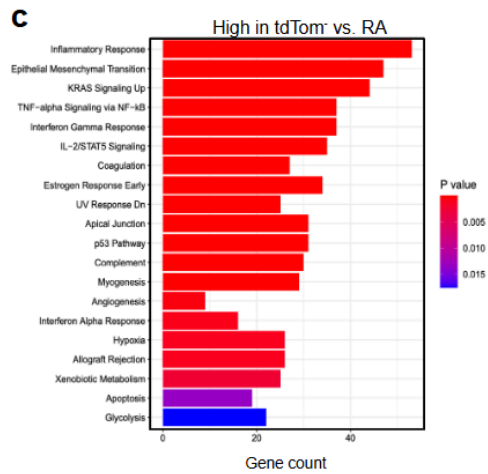
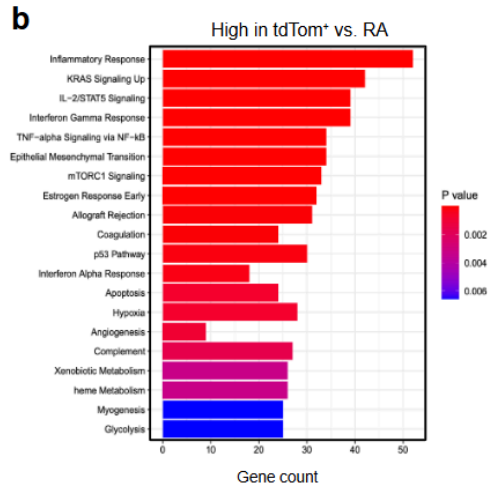
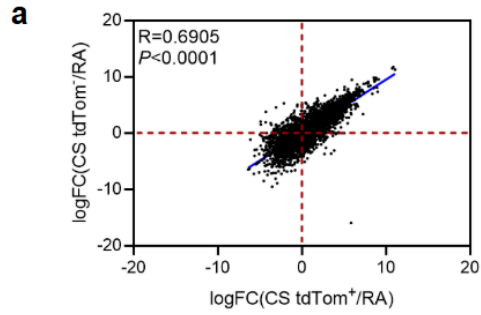
b, Pathway activity, transcription factor activity and gene set enrichment analysis of tdTom⁺ AM of 4 weeks CS-exposed mice vs. RA-AM (n=4 biologically independent samples, pooled 2 mice each).

c, Pathway activity, transcription factor activity and gene set enrichment analysis of tdTom⁻ AM of 4 weeks CS-exposed mice vs. RA-AM (n=4 biologically independent samples, pooled 2 mice each).

d, Pathway activity, transcription factor activity and gene set enrichment analysis of tdTom⁻ AM of CS-exposed mice vs. tdTom⁺ AM of CS-exposed mice (n=4 biologically independent samples, pooled 2 mice each).

e, Heatmap showing RNA expression of *Siglec f* from 4 weeks of RA, tdTom⁻ or tdTom⁺ CS-exposed mice (n=4 biologically independent samples, pooled 2 mice each). *p*-value $\leq 0.001 = ***$

f, IREA for top 100 upregulated genes in *Ms4a3⁻* or *Ms4a3⁺* CS AM vs. RA-AM bulk RNA seq data⁴⁶. Source data is provided as a Source Data file.



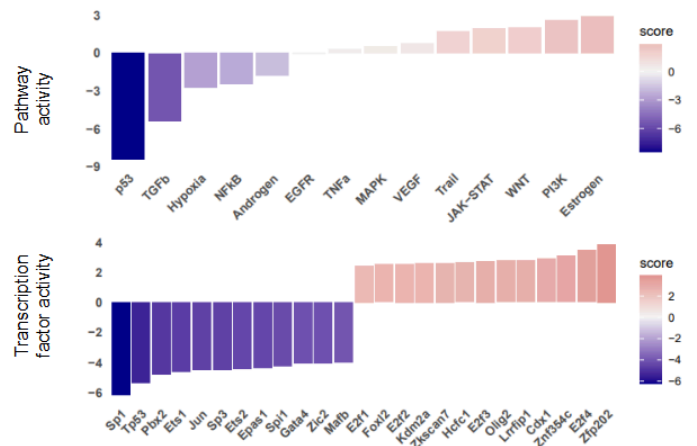
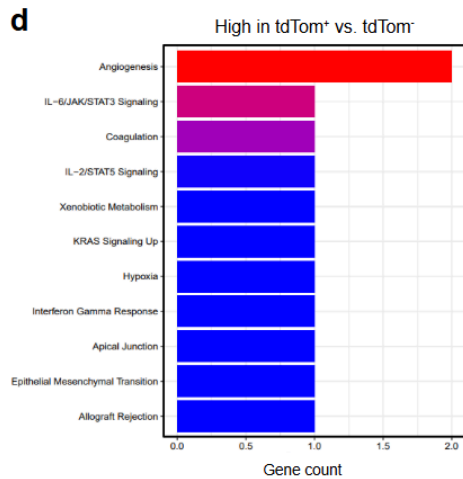
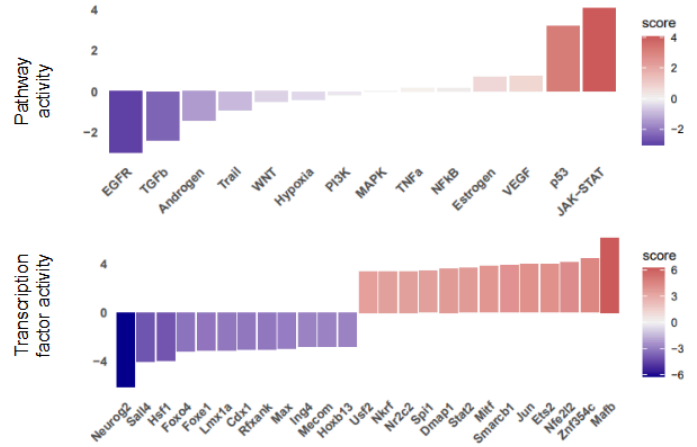
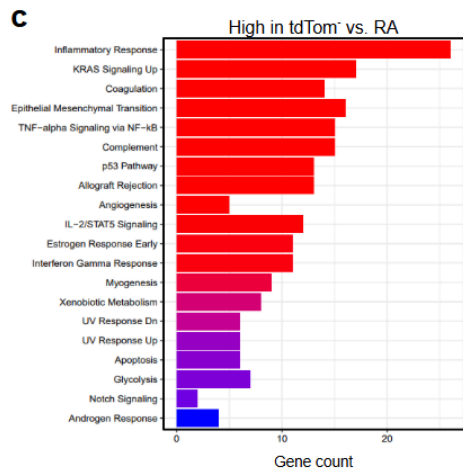
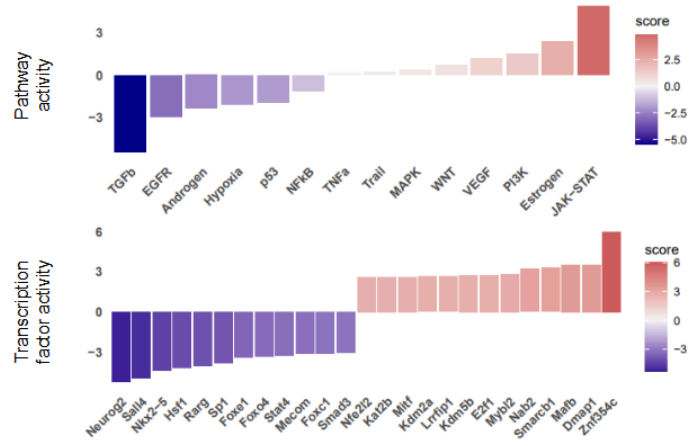
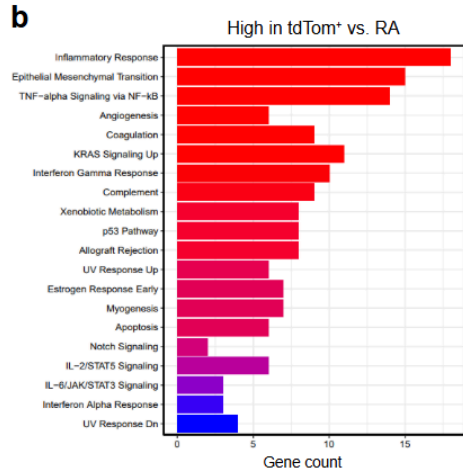
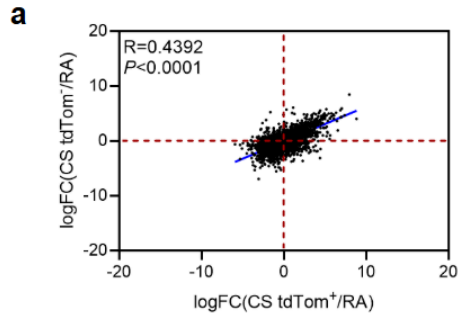
Supplementary Fig. 5 | BM-AM and Em-AM share major transcriptomic adaptations upon CS exposure for 12 weeks.

a, Dot plot shows the correlation between CS-induced changes in gene expression in BM-AM and CS-induced changes in gene expression in Em-AM (n=4 biologically independent samples, pooled 2 mice each; 12 weeks CS exposure).

b, Gene set enrichment, pathway activity and transcription factor activity analysis of tdTom⁺ AM of CS-exposed mice vs. AM of RA-exposed mice (n=4 biologically independent samples, pooled 2 mice each; 12 weeks CS exposure).

c, Gene set enrichment, pathway activity and transcription factor activity analysis of tdTom⁻ AM of CS-exposed mice vs. AM of RA-exposed mice (n=4 biologically independent samples, pooled 2 mice each; 12 weeks CS exposure).

d, Gene set enrichment, pathway activity and transcription factor activity analysis of tdTom⁺ AM of CS-exposed mice vs. tdTom⁻ AM of CS-exposed mice (n=4 biologically independent samples, pooled 2 mice each; 12 weeks CS exposure). Source data is provided as a Source Data file.



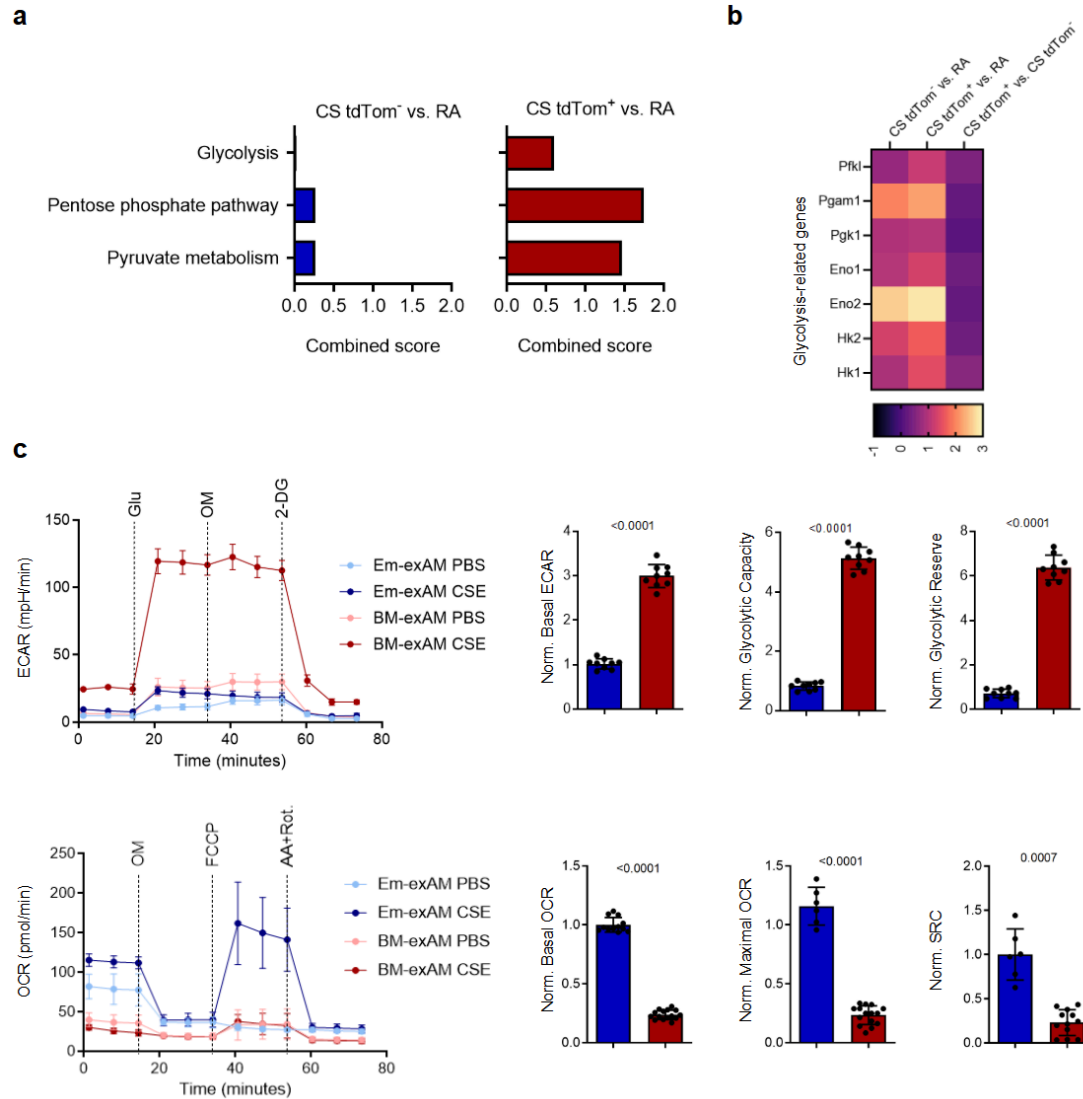
Supplementary Fig. 6 | BM-AM and Em-AM share major transcriptomic adaptations upon CS exposure for 12+8 weeks.

a, Dot plot shows the correlation between CS-induced changes in gene expression in BM-AM and CS-induced changes in gene expression in Em-AM (n=4 biologically independent samples, pooled 3 mice each; 12+8 weeks CS exposure).

b, Gene set enrichment, pathway activity and transcription factor activity analysis of tdTom⁺ AM of CS-exposed mice vs. AM of RA-exposed mice (n=4 biologically independent samples, pooled 3 mice each; 12+8 weeks CS exposure).

c, Gene set enrichment, pathway activity and transcription factor activity analysis of tdTom⁻ AM of CS-exposed mice vs. AM of RA-exposed mice (n=4 biologically independent samples, pooled 3 mice each; 12+8 weeks CS exposure).

d, Gene set enrichment, pathway activity and transcription factor activity analysis of tdTom⁺ AM of CS-exposed mice vs. tdTom⁻ AM of CS-exposed mice (n=4 biologically independent samples, pooled 3 mice each; 12+8 weeks CS exposure). Source data is provided as a Source Data file.

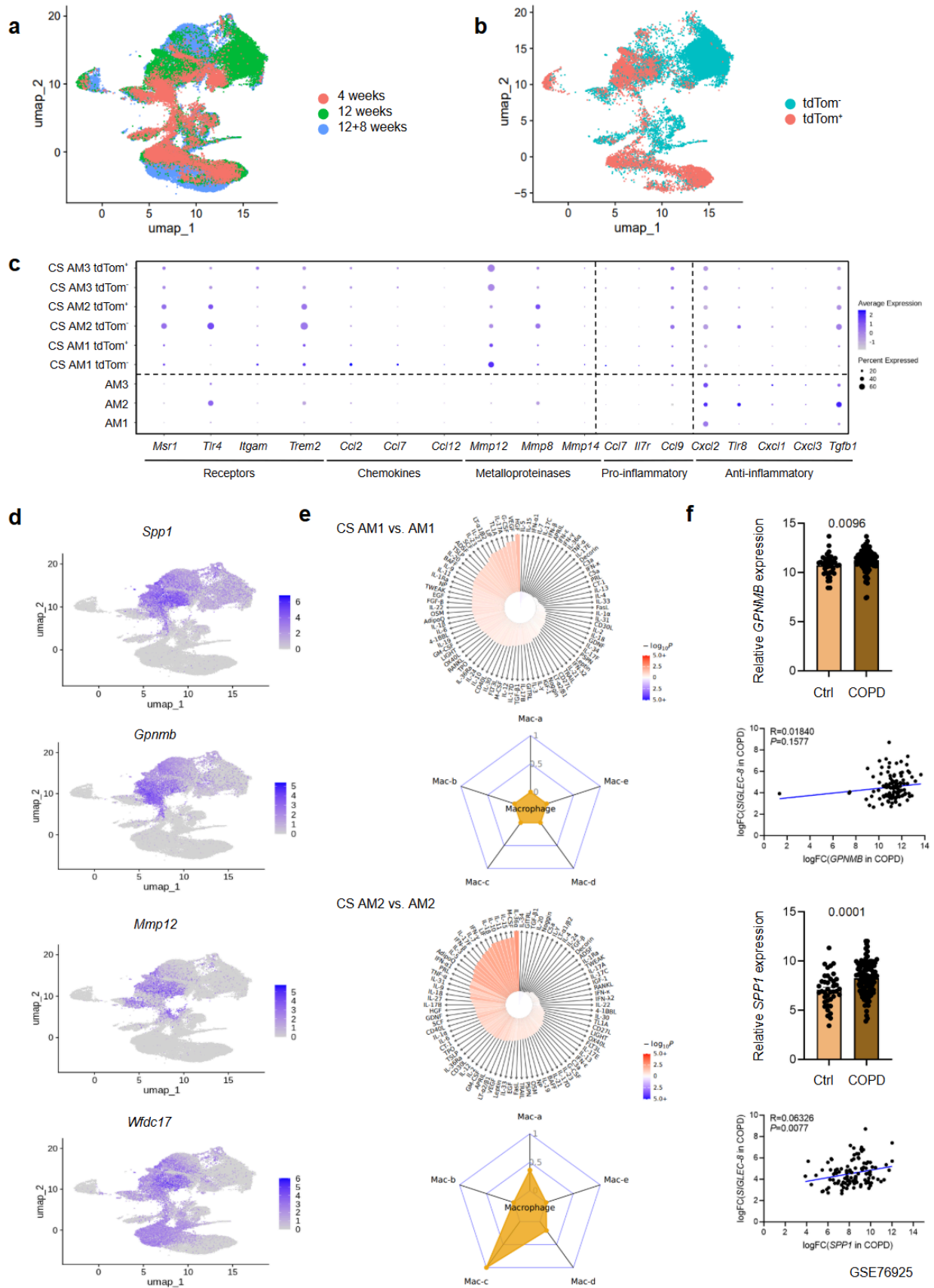


Supplementary Fig. 7 | Metabolic comparison of tdTom⁻ and tdTom⁺ AM.

a, Genes from the bulk RNA seq experiments in Fig. 3 with a log₂FC of 1 or higher in CS tdTom⁻ or CS tdTom⁺ AM vs. RA AM were mapped to metabolic signatures using Enrichr (Panther 2015; Ma'ayan Lab; Computational Systems Biology).

b, Heatmap showing levels of glycolysis-related genes in CS tdTom⁻ or CS tdTom⁺ AM vs. RA AM.

c, Line graphs showing changes in the oxygen consumption rate (OCR), and the extracellular acidification rate (ECAR) of PBS/CSE-activated Em-exAM and BM-exAM. Bar graphs show normalized basal OCR, maximal OCR, and spare respiratory capacity (SRC) and basal ECAR, glycolytic capacity and glycolytic reserve of CSE-activated Em-exAM and BM-exAM (n=9-12 biologically independent samples). OM (Oligomycin), FCCP (Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone), AA (Antimycin A), Rot (Rotenone), 2-DG (2-Deoxyglucose), Glu (Glucose). Data are presented as mean (b) or mean ± SD (c). Statistical comparisons were performed with two-tailed unpaired Student's *t*-tests (c); data points were normally distributed).



Supplementary Fig. 8 | Different AM subtypes induce common gene signatures in an origin-independent manner upon CS exposure.

a, UMAP showing clusters separated by timepoints (4, 12, and 12+8 weeks) from scRNA seq (4 weeks: n=5 biologically independent samples; 12 and 12+8 weeks: n=4 biologically independent samples).

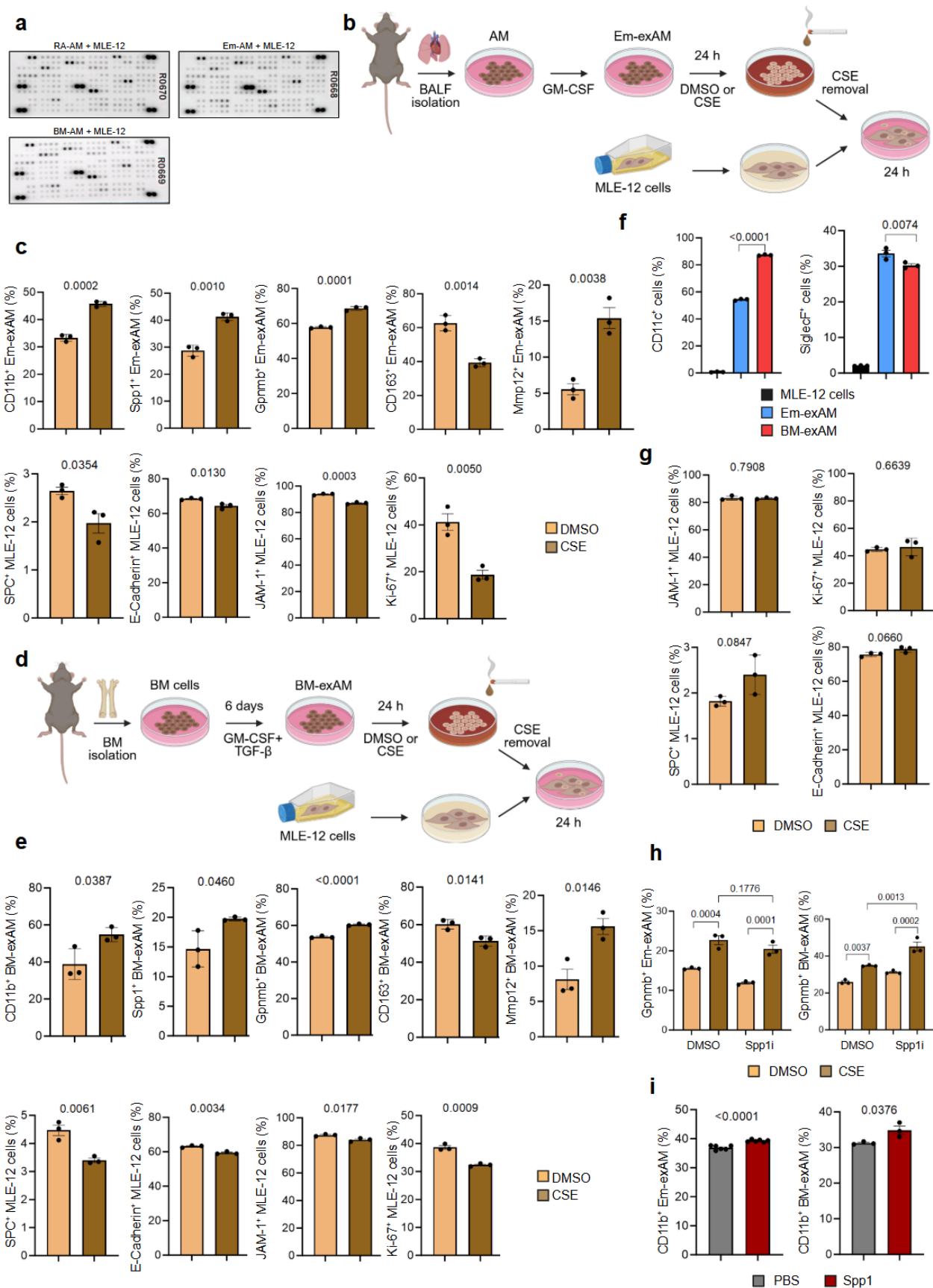
b, UMAP showing clusters separated by myeloid cell origin (12 weeks) from scRNA seq (n=4 biologically independent samples).

c, Dotplot showing expression of the receptors *Msr1*, *Tlr4*, *Itgam*, *Trem2*, the chemokines *Ccl2*, *Ccl7*, *Ccl12*, the metalloproteinases *Mmp12*, *Mmp8*, *Mmp14*, the pro-inflammatory markers *Ccl7*, *Il7r*, *Ccl9*, and the anti-inflammatory markers *Cxcl2*, *Tlr8*, *Cxcl1*, *Cxcl3* and *Tgfb1* in (CS) AM1-3 dependent on AM origin (pooled 12, 12+8 weeks, n=4 biologically independent samples).

d, UMAP showing expression of *Spp1*, *Mmp12*, *Wfdc17* and *Gpnmb* in all myeloid cells from scRNA seq (pooled 4, 12, 12+8 weeks; 4 weeks: n=5 biologically independent samples; 12, 12+8 weeks: n=4 biologically independent samples).

e, IREA for top 100 upregulated genes in CS AM1 and CS AM2 vs. AM1 and AM2 scRNA seq data, respectively⁴⁶.

f, Bar graphs show expression of *SPP1* and *GPNUMB* in human macrophages. Correlation analysis of *SPP1* or *GPNUMB* and *SIGLEC-8*. Data are presented as mean $\pm$ SD (f). Statistical comparisons were performed with two-tailed unpaired Student's *t*-tests (f; data points were normally distributed). Source data is provided as a Source Data file.



Supplementary Fig. 9 | Upon co-culture, CSE-activated BM-exAM and Em-exAM similarly damage lung epithelial cells.

a, Cytokine array membranes probed with supernatants of MLE-12 + RA-AM, Em-AM or BM-AM co-cultures.

b, Schematic illustrating experimental layout in c. Created with BioRender.com.

c, Bar graphs showing expression of CD11b, Spp1, Gpnmb, CD163, and Mmp12 on CSE- or DMSO-treated Em-exAM and SPC, E-Cadherin, JAM-1 and Ki-67 on MLE-12 cells after co-culture (n=3 biologically independent samples).

d, Schematic illustrating experimental layout in e. Created with BioRender.com.

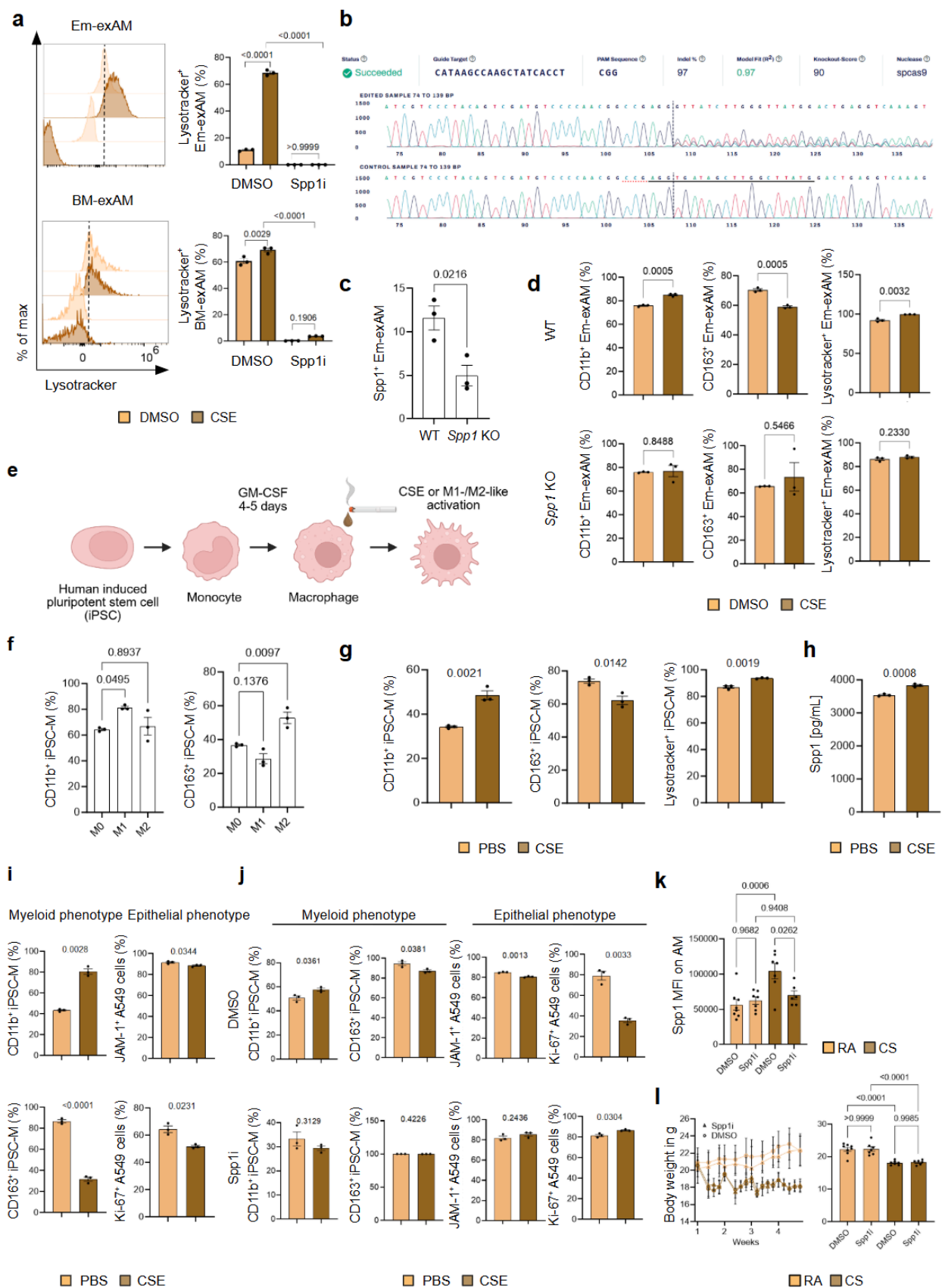
e, Bar graphs showing expression of CD11b, Spp1, Gpnmb, CD163 and Mmp12 on CSE- or DMSO-treated BM-exAM and SPC, E-Cadherin, JAM-1 and Ki-67 on MLE-12 cells after co-culture (n=3 biologically independent samples).

f, Bar graph showing expression of SiglecF and Cd11c on Em-exAM and BM-exAM after CSE exposure (n=3 biologically independent samples).

g, Bar graph showing JAM-1, Ki-67, E-Cadherin, and SPC expression of DMSO- or CSE-treated MLE-12 cells (n=3 biologically independent samples).

h, Bar graph showing expression of Gpnmb in DMSO- or CSE-activated Em-exAM and BM-exAM with or without Spp1i (n=3 biologically independent samples).

i, Bar graph showing CD11b expression of PBS- and Spp1-treated Em-exAM (n=6) and BM-exAM (n=3 biologically independent samples). Data are presented as mean $\pm$ SD (c-e, f-g, h-i). Statistical comparisons were performed with two-tailed unpaired Student's *t*-tests (c-e, g, i; data points were normally distributed) and with one-way ANOVA tests (f, h; for simultaneous comparisons of more than two groups). Source data is provided as a Source Data file.



Supplementary Fig. 10 | Spp1i prevents CS-AM adaptations and epithelial cell damage in human systems.

a, Flow cytometry histogram and bar graph showing percentage of Lysotracker⁺ BM-exAM and Em-exAM with and without Spp1 inhibition (n=3 biologically independent samples).

b, ICE results of control Sanger sequencing after introducing a KO of *Spp1*.

c, Bar graph showing percentage of Spp1⁺ Em-exAM after KO of *Spp1* (n=3 biologically independent samples).

d, Bar graphs showing expression of CD11b, CD163 and Lysotracker on Em-exAM with and without KO of *Spp1* (n=3 biologically independent samples).

e, Schematic illustrating experimental layout in f-j. Created with BioRender.com.

f, Bar graphs showing CD11b and CD163 levels on iPSC-M after M1-like (LPS+IFN γ) and M2-like (IL-4+IL-13) activation (n=3 biologically independent samples).

g, Bar graph showing percentage of CD11b, CD163, and Lysotracker on iPSC-M after CSE activation (n=3 biologically independent samples).

h, Bar graphs showing Spp1 levels in iPSC-M supernatant measured by ELISA (n=3 biologically independent samples).

i, Bar graphs showing expression of CD11b and CD163 on CSE-activated iPSC-M and Ki-67 and JAM-1 in A549 cells after co-culture (n=3 biologically independent samples).

j, Bar graphs showing expression of CD11b on iPSC-M and Ki-67 in A549 cells after co-culture of CSE-activated, Spp1i-treated iPSC-M and A549 cells (n=3 biologically independent samples).

k, Bar graph showing Spp1 MFI on AM in lung lysate of mice after RA/CS with or without Spp1i (n=6-8 biologically independent samples).

l, Graphs showing mouse weights after 4 weeks of RA/CS with or without Spp1i (n=6-8 biologically independent samples). Data are presented as mean $\pm$ SD (a, c-d, f-l). Statistical comparisons were performed with two-tailed unpaired Student's *t*-tests (c-d, g-j; data points were normally distributed) or one-way ANOVA tests (a, f, l; for simultaneous comparisons of more than two groups). Source data is provided as a Source Data file.