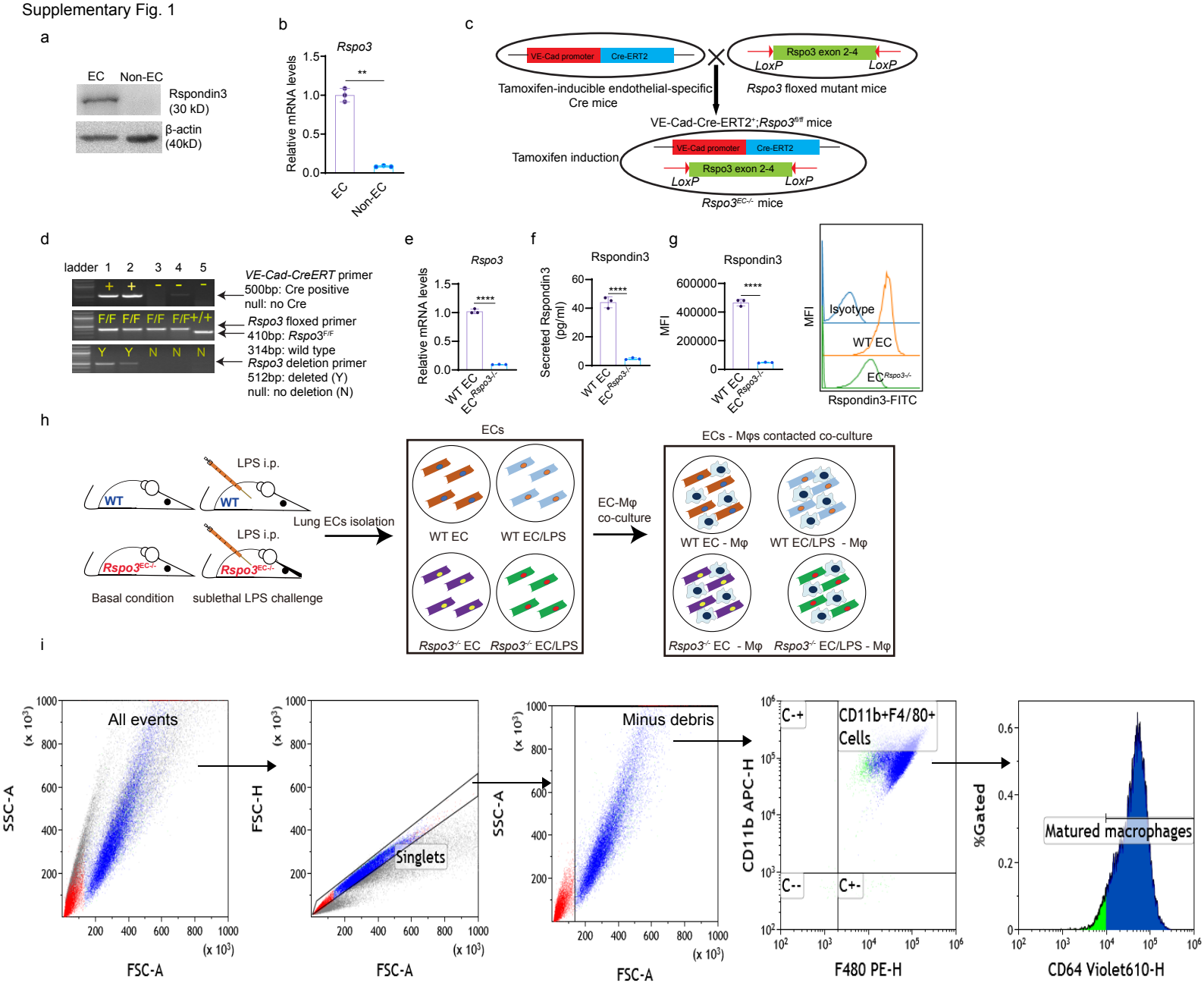
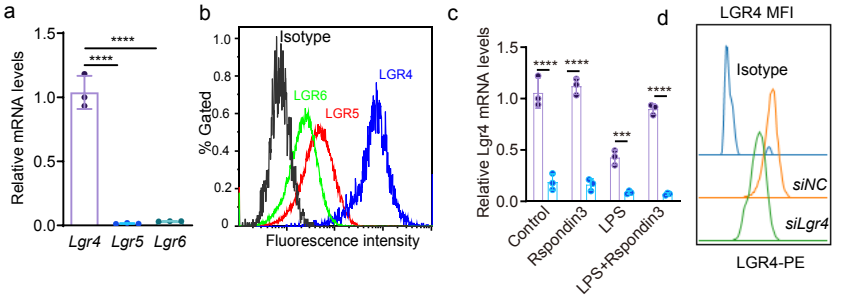
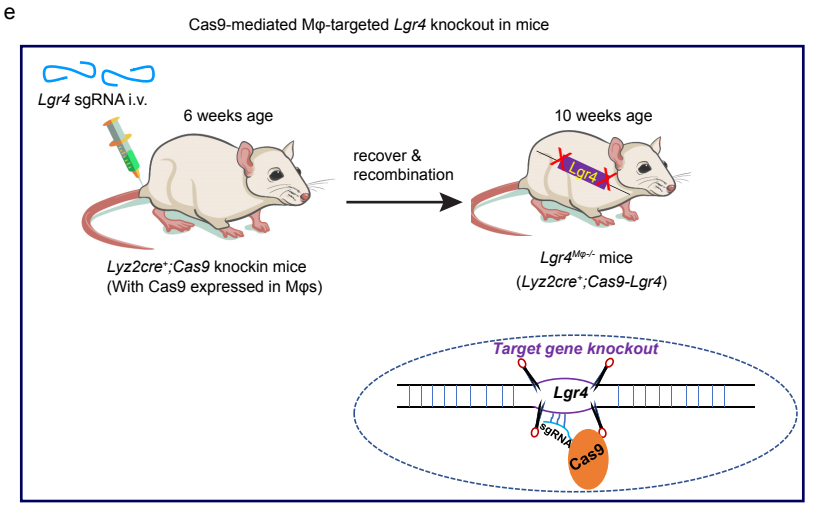
Supplementary Fig. 1



Supplementary Fig. 1: *Rspo3* is expressed in EC and generation of the *Rspo3*$^{EC-/-}$ mice. (a) Rspondin3 protein levels detected by western blot for mouse EC and non-EC samples purified from mouse lungs (Representative cropped images from three independent experiments are shown); (b) *Rspo3* mRNA levels measured by qPCR for mouse EC and non-EC purified from mouse lungs; n=3 independent cell samples from three individual mice per group (mean ± sd), two-sided paired t-test was determined using GraphPad Prism. **P=0.0030. (c) Schematic of generation of the inducible endothelial *Rspo3* knockout mouse model (*VE-cadherin-CreERT2+;Rspo3*$^{fl/fl}$, abbreviated as *Rspo3*$^{EC-/-}$); (d) Representative gel images for the genotyping PCR using mouse tails and isolated ECs (for *Rspo3*-deleted band detection) were shown (three repeats for each mouse), which indicated the *VE-cadherin-CreERT2* positive bands, *Rspo3*-floxed bands, and *Rspo3*-deleted bands; (e) qPCR measuring the mRNA levels of *Rspo3* in ECs from wild type mice (WT EC) and endothelial *Rspo3* knockout mice (EC$^{Rspo3-/-}$); n=3 independent EC samples from three mice per group. Graphs show the mean ± s.d, with each dot representing an individual sample. Two-sided unpaired Student's t-test was determined using GraphPad Prism. ****P<0.0001. (f) Secreted Rspondin3 levels in WT ECs and *Rspo3*$^{EC-/-}$ ECs supernatants measured by ELISA; n=3 independent EC samples from three mice per group. Graphs show the mean ± s.d, with each dot representing an individual sample. Two-sided unpaired Student's t-test was determined using GraphPad Prism. ****P<0.0001. (g) Rspondin3 levels in ECs from WT mice and *Rspo3*$^{EC-/-}$ mice measured by flow cytometry; n=3 independent EC samples from three mice per group. Graphs show the mean ± s.d, with each dot representing an individual sample. Two-sided unpaired Student's t-test was determined using GraphPad Prism, ****P<0.0001. (h) Schematic of EC-Mφ in-contact co-culture: lung ECs were isolated from WT mice and *Rspo3*$^{EC-/-}$ mice at basal conditions or sublethal LPS challenge for 24h, and purified ECs were mixed cultured with BMDMs at a ratio of 1:1; (i) Gating strategy for gating the mature macrophages ($CD11b^+F4/80^+CD64^+$) from BMDMs, which were used for *in vitro* experiments as shown in Fig. 1a, Fig. 3a, Fig. 4a, Extended Data Fig. 1d-e, and Extended Data Fig. 2a-b.

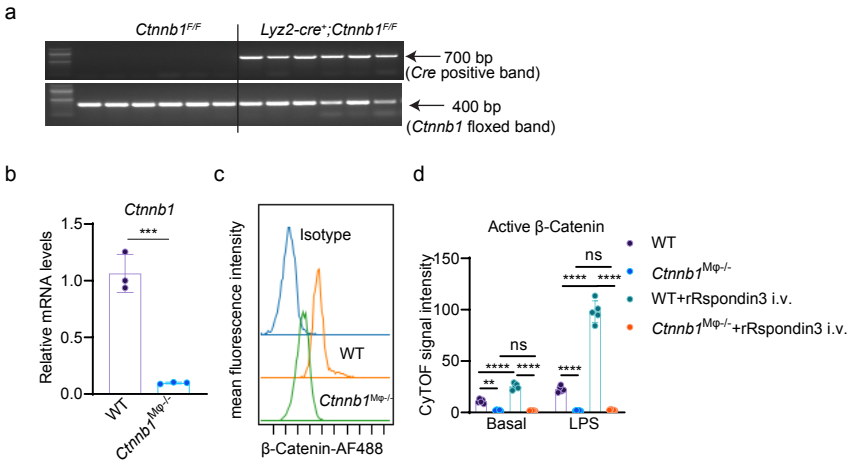
Supplementary Fig. 2





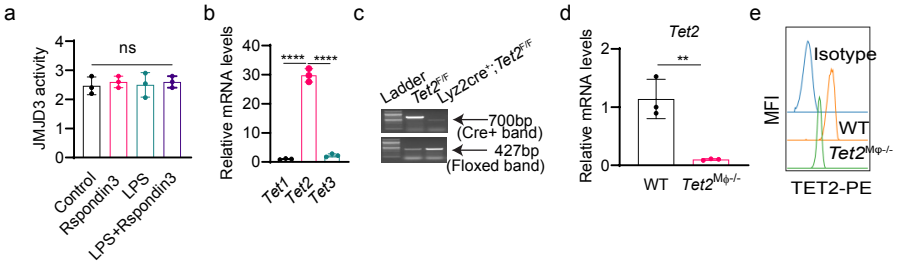
Supplementary Fig. 2: Detection of *Lgr4* expression and generation of the *Lgr4*$^{Mφ-/-}$ mice. (a) *Lgr4*, *Lgr5*, and *Lgr6* gene expression levels in BMDMs as measured by qPCR; n=3 independent cell samples prepared from three individual mice each group (mean ± sd), statistical significance was determined by ordinary one-way ANOVA multiple comparisons using GraphPad Prism. P values are ****P<0.0001, ****P<0.0001. (b) Flow cytometry overlay histogram for MFI of LGR4, LGR5, and LGR6 expression in BMDMs; (c) *Lgr4* mRNA levels by qPCR in BMDMs with or without *Lgr4* depleted; n=3 independent cell samples from three individual mice per group (mean ± sd), two-way ANOVA with Sidak's multiple comparisons test was determined using GraphPad Prism. ****P<0.0001, ****P<0.0001, ***P=0.0002, ****P<0.0001. (d) Overlaid flow cytometry histograms showing mean fluorescence intensity of LGR4 in BMDMs with or without *Lgr4* depleted; (e) Schematic of the generation of *Lgr4*$^{Mφ-/-}$ mice model: a Mφ-specific expressed Cas9 mice were i.v. injected with *Lgr4* sgRNA (10 nM/mice) prepared in liposomes at 6 weeks old, followed with a 4 weeks recover and recombination to deplete *Lgr4* in macrophages *in vivo*.

Supplementary Fig. 3



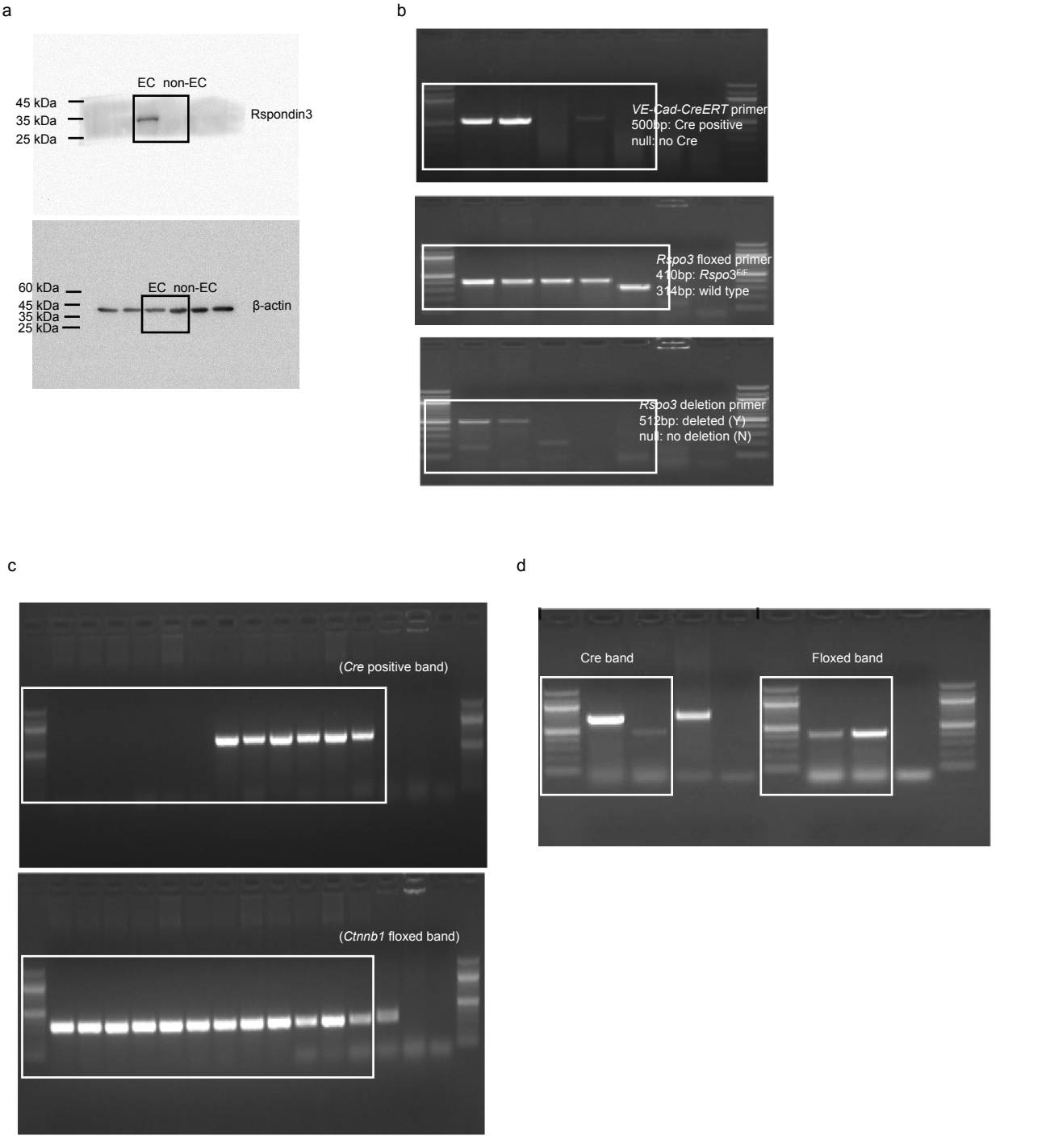
Supplementary Fig. 3: Generation and verification of the *Ctnnb1*$^{Mφ-/-}$ mice. (a) Representative gel images for the genotyping PCR to verify the genotype of *Ctnnb1*$^{Mφ-/-}$ mice (three repeats for each mouse). (b) mRNA levels in BMDMs from *Ctnnb1*$^{Mφ-/-}$ mice and littermate control mice; n=3 independent cell samples from three individual mice per group (mean ± sd), two-sided unpaired t-test was determined using GraphPad Prism. ***P=0.0006. (c) Flow cytometry overlay histograms showing β-catenin levels by mean fluorescence intensity for BMDMs from *Ctnnb1*$^{Mφ-/-}$ mice and littermate control mice; (d) Active β-Catenin (Non-phospho) levels in IM in WT and *Ctnnb1*$^{Mφ-/-}$ mice with or without rRspondin3 i.v. under basal conditions and post sublethal LPS challenge for 24h measured by CyTOF (data are representative of three independent experiments with five mice per group). Graphs show the mean ± s.d, with each dot representing an individual mouse. Statistical significance was determined by two-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism with individual P values (left to right) are: **P=0.0091, ****P<0.0001, ns P=0.9998, ****P<0.0001, ****P<0.0001, ****P<0.0001, ns P=0.9942, ****P<0.0001.

Supplementary Fig. 4



Supplementary Fig. 4: Detection of *Tet2* expression and verification of the *Tet2*$^{Mφ-/-}$ mice. (a) JMJD3 demethylase activity in BMDMs stimulated with Rspondin3, LPS alone or combination of both were measured; n=3 independent cell samples from three individual mice per group (mean ± sd); Statistical significance was determined by ordinary one-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism. ns P>0.9435. (b) Relative gene expression of *Tet1*, *Tet2* and *Tet3* in BMDMs measured by qPCR; n=3 independent cell samples from three individual mice per group (mean ± sd); Statistical significance was determined by ordinary one-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism. P values (left to right) are ****P<0.0001, ****P<0.0001. (c) Representative gel images for the genotyping PCR to verify the genotype of *Tet2*$^{Mφ-/-}$ mice (three repeats for each mouse); (d qPCR for *Tet2* in BMDMs prepared from the *Tet2*$^{Mφ-/-}$ and WT mice; n=3 independent cell samples from three individual mice per group (mean ± sd); Statistical significance was determined by two-sided unpaired t test using GraphPad Prism. ns P=0.0060. (e) Overlaid flow cytometry histograms showing TET2 intensity (MFI) in BMDMs from *Tet2*$^{Mφ-/-}$ mice compared to WT mice.

Supplementary Fig. 5



Supplementary Fig. 5: Source Data for western blot and DNA gels. (a) Unprocessed source images for western blot in Supple Fig. 1a; (b) Unprocessed source images for DNA gel in Supple Fig. 1d; (c) Unprocessed source images for DNA gel in Supple Fig. 3a; (d) Unprocessed source images for DNA gel in Supple Fig. 4c.