Supplementary Data

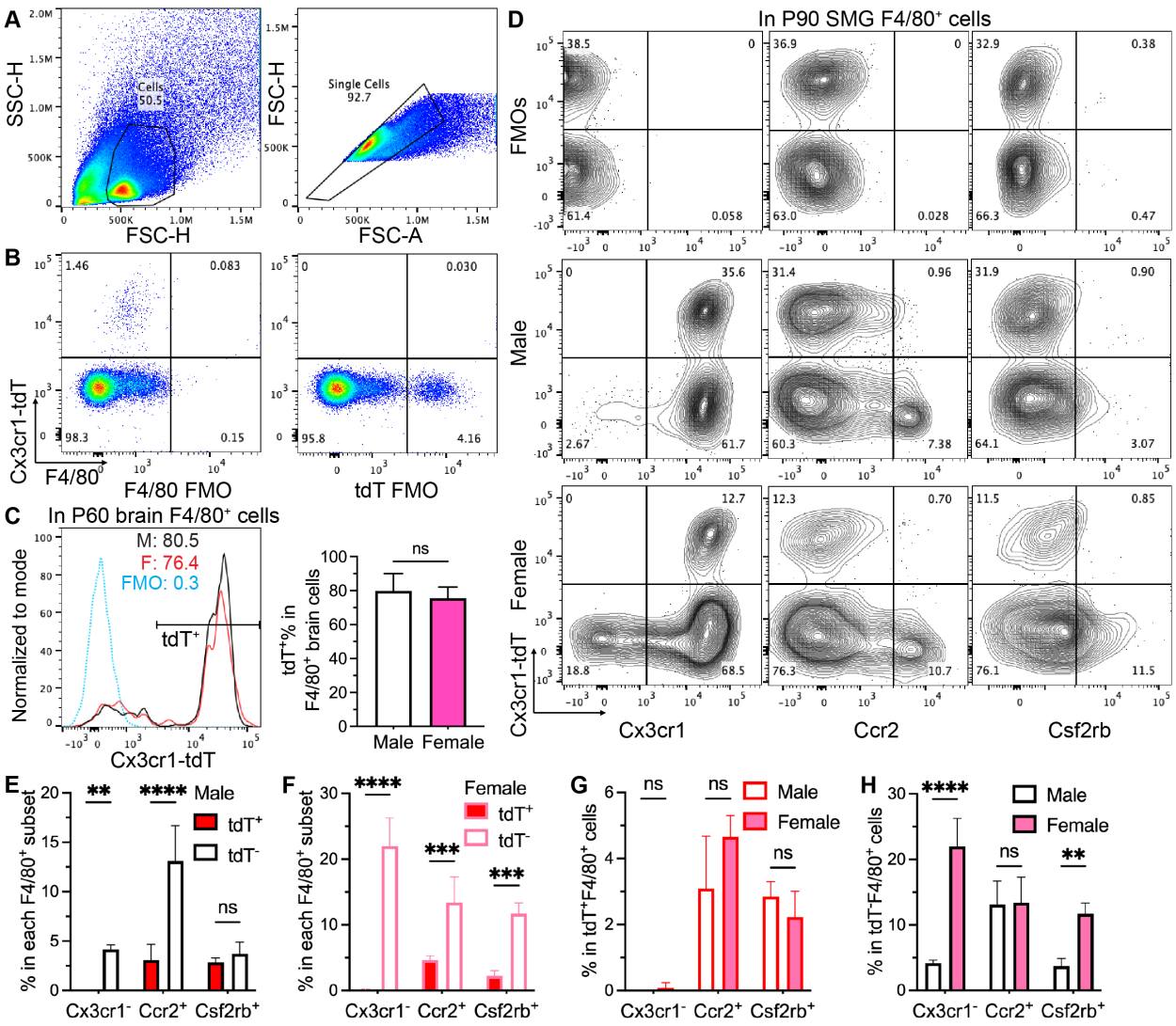

Figure S1. Supplementary data for *Cx3cr1-CreER^{T2};Ai9* mice induced at E8.5.

A: Gating strategies of live and single SMG cells for flow cytometry analyses of surface markers.

B: The gate for tdT and F4/80 was determined with Fluorescence minus one (FMO) controls.

C. In P60 mice of both gender, percentages of tdT$^+$ cells in F4/80$^+$ cells from brain and spleen were examined with flow cytometry.

D: F4/80$^+$ cells were gated in flow cytometry of SMGs from P90 mice of both genders and further analyzed for the expression of tdT vs. Cx3cr1, Ccr2, or Csf2rb. The gates of these markers were determined with corresponding FMOs, and representative contour plots were shown.

E-F: Percentages of Cx3cr1$^+$, Ccr2$^+$, or Csf2rb$^+$ were compared between tdT$^+$F4/80$^+$ cells (yolk sac derived primitive macrophages) and tdT$^-$ F4/80$^+$ cells in SMGs from P90 mice.

G-H: Percentages of Cx3cr1$^+$, Ccr2$^+$, or Csf2rb$^+$ in SMG tdT$^+$F4/80$^+$ cells (primitive macrophages) were compared between male and female mice at P90.

All quantified data are shown as Mean±SD. N = 4, ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

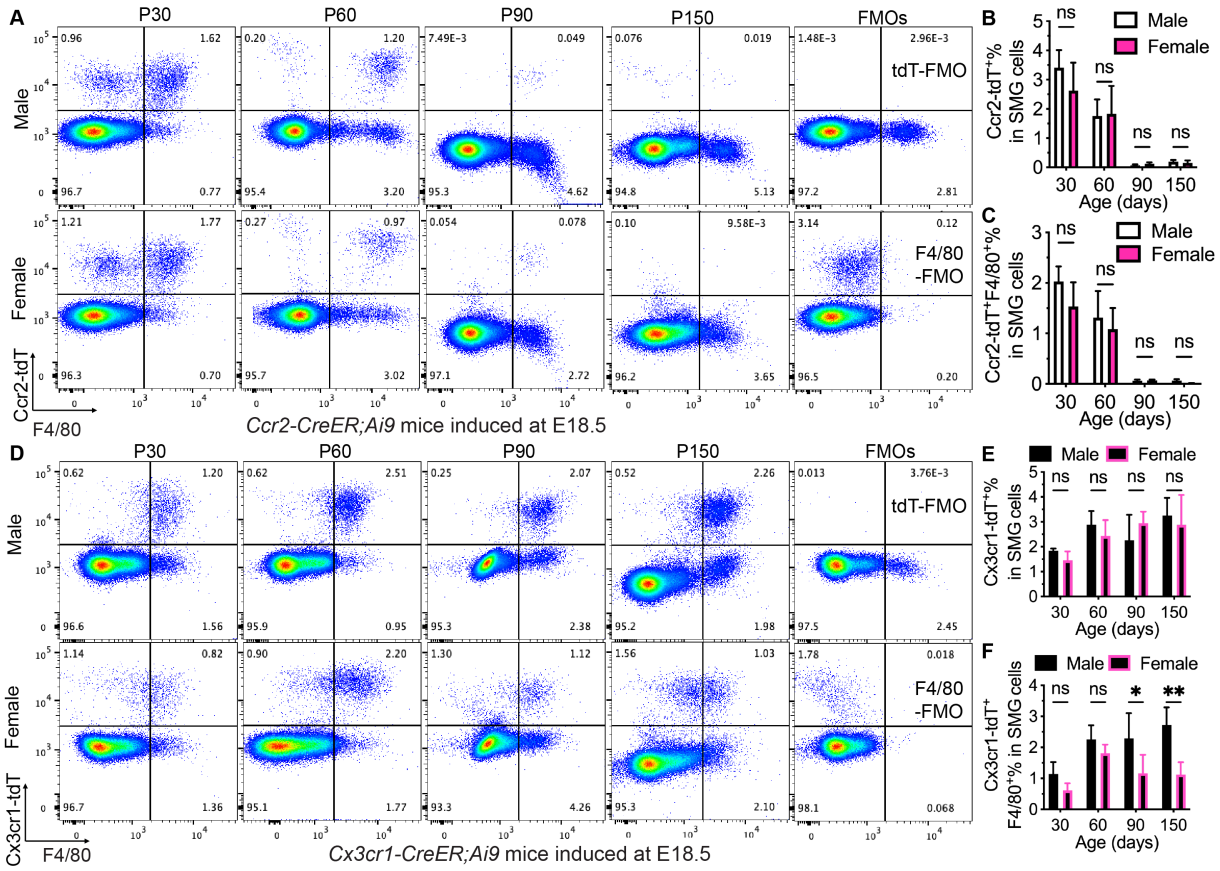

Figure S2. Supplementary data for Ccr2- or *Cx3cr1-CreER^{T2};Ai9* mice induced at E18.5.

A-C: Male and female *Ccr2-CreER;Ai9* mice were induced at E18.5, and their SMGs were collected at P30, 60, 90 and 150 for flow cytometry analyses. Representative pseudo color plots of F4/80 and tdT expression were shown (A), and percentages of tdT$^+$ of and F4/80$^+$tdT$^+$ in SMG cells were compared between males and females (B-C).

D-F: Parallel analyses of *Cx3cr1-CreER;Ai9* mice.

All quantified data are shown as Mean±SD. N = 4, ns: not significant, *: $p < 0.05$, **: $p < 0.01$.

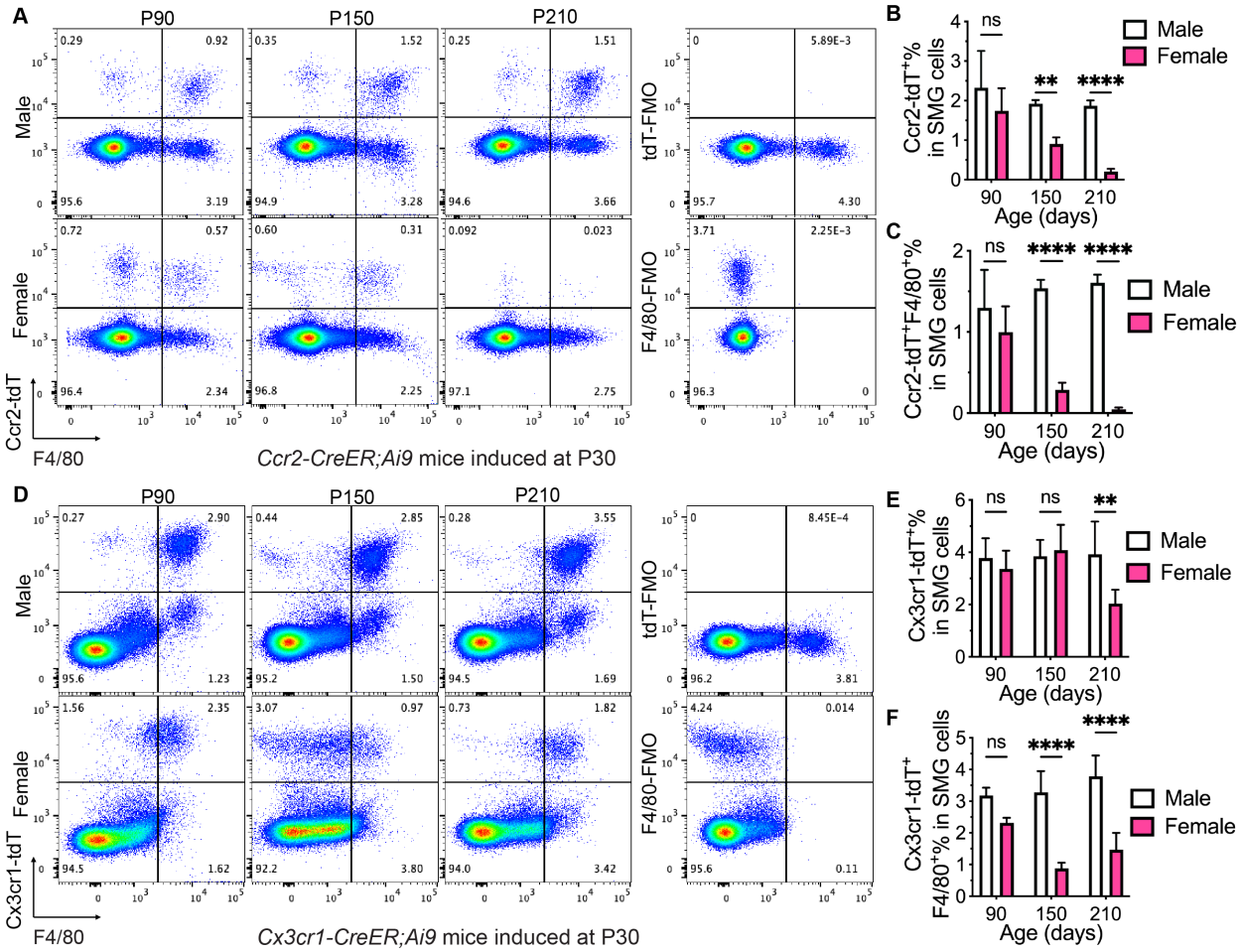

Figure S3. Supplementary data for Ccr2- or *Cx3cr1-CreER^{T2};Ai9* mice induced at P30.

A-C: Male and female *Ccr2-CreER;Ai9* mice were induced at E18.5, and their SMGs were collected at P90, 150 and 210 for flow cytometry analyses. Representative pseudo color plots of F4/80 and tdT expression were shown (A), and percentages of tdT$^+$ of and F4/80$^+$tdT$^+$ in SMG cells were compared between males and females (B-C).

D-F: Parallel analyses of *Cx3cr1-CreER;Ai9* mice induced at P30.

All quantified data are shown as Mean±SD. N = 4, ns: not significant, *: $p < 0.05$, ***: $p < 0.001$, ****: $p < 0.0001$.

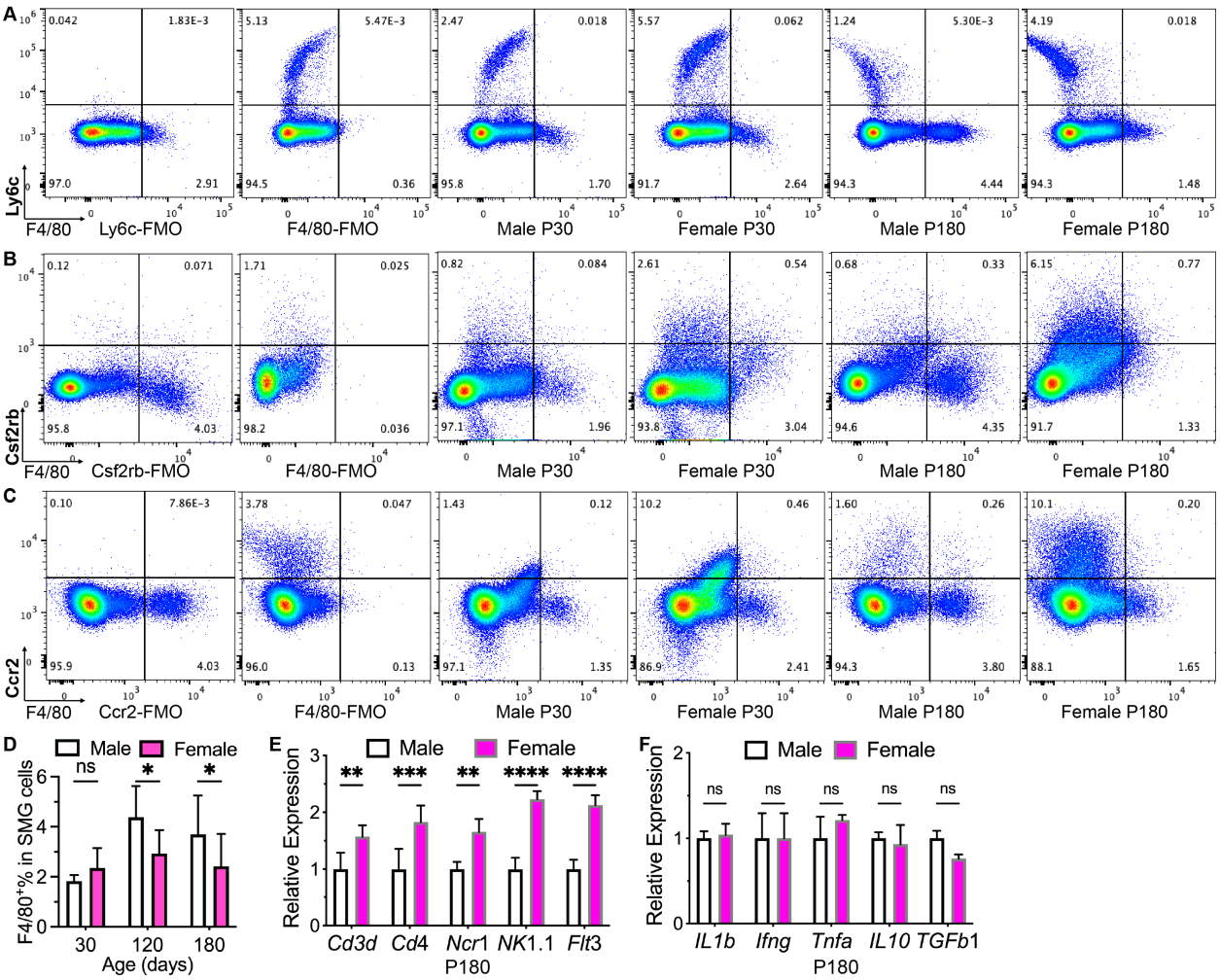

Figure S4. Data from C57BL/6 mice related to Figure 4.

A-C: Representative pseudo color plots of flow cytometric analyses of Ly6c vs. F4/80 (A), Csf2rb vs. F4/80 (B), and Ccr2 vs. F4/80 (C) in SMG cells from wild type P60 and P180 C57BL/6 mice of both genders.

D: Percentages of F4/80$^+$ cells in SMGs at different ages were compared between male and female C57BL/6 mice.

E-F: SMGs from wildtype male and female P180 C57BL/6 mice were examined with qRT-PCR for markers of T cell, innate lymphoid cells, and dendritic cells (E) and immune modulatory cytokines (F).

N = 4, ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

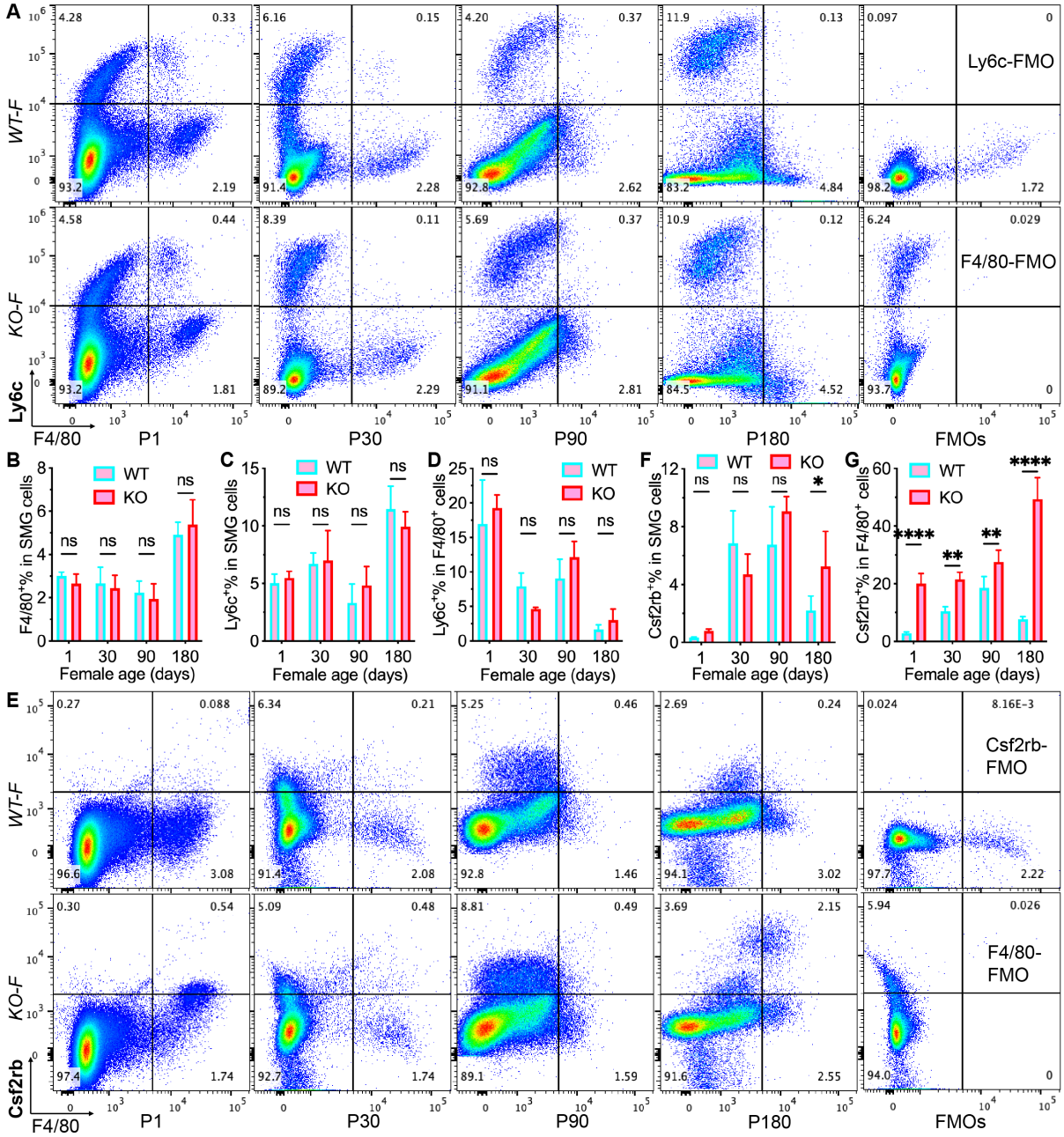

Figure S5. Effects of *Cx3cr1* knockout in female SMGs.

A-B: Representative pseudo color plots of flow cytometric analyses of Ly6c vs. F4/80 (A) and Csf2rb vs. F4/80 (B) in SMG cells from female wildtype (WT) and Cx3cr1 knockout (KO) mice at P30, 90, and 180.

C-G: Percentages of F4/80$^+$, Ly6c$^+$, and Csf2rb$^+$ cells in SMGs and Ly6c$^+$ or Csf2rb$^+$ cells in F4/80$^+$ cells were compared between female WT and KO mice at P30, 90, and 180.

N = 4, ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

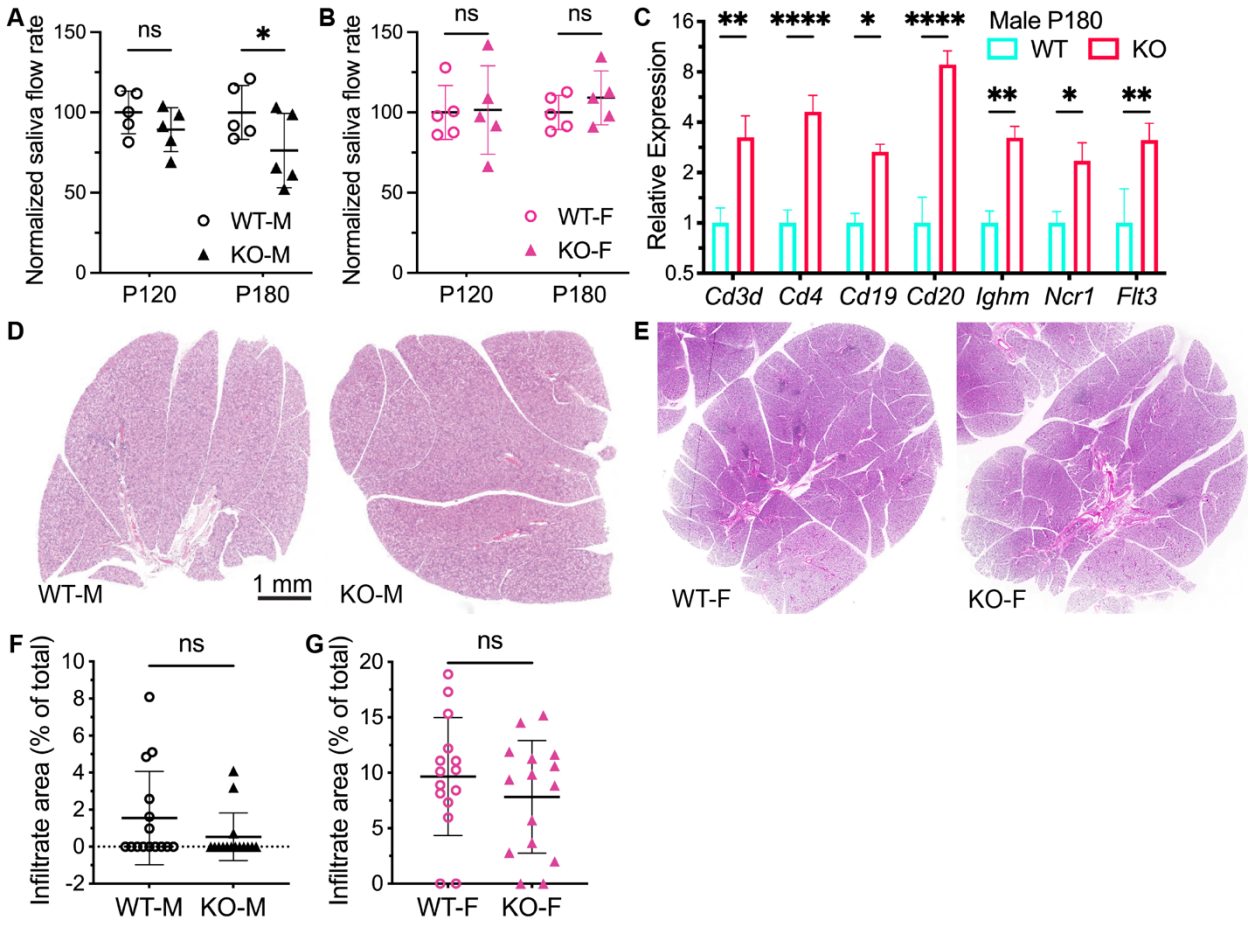

Figure S6. Effects of *Cx3cr1* knockout on saliva secretion and SMG histology.

A-B: Stimulated saliva flow rates were measured at P120 and 180 male and female wildtype (WT) and Cx3cr1 knockout (KO) mice. Data shown were normalized to body weight and corresponding rates of WT mice.

C: SMGs from male P180 WT and KO mice were examined with qRT-PCR for markers of T cells (*Cd3d, Cd4*), B cells (*Cd19, Cd20, Ighm*), innate lymphoid cells (*Ncr1*), and dendritic cells (*Flt3*).

D-E: Representative H&E staining of SMGs from P180 male and female wildtype (WT) and Cx3cr1 knockout (KO) mice.

F-G: The areas of leukocyte infiltrate were quantified from three H&E stained sections from 3 fields of each of 5 SMGs.

N = 5. ns: not significant.