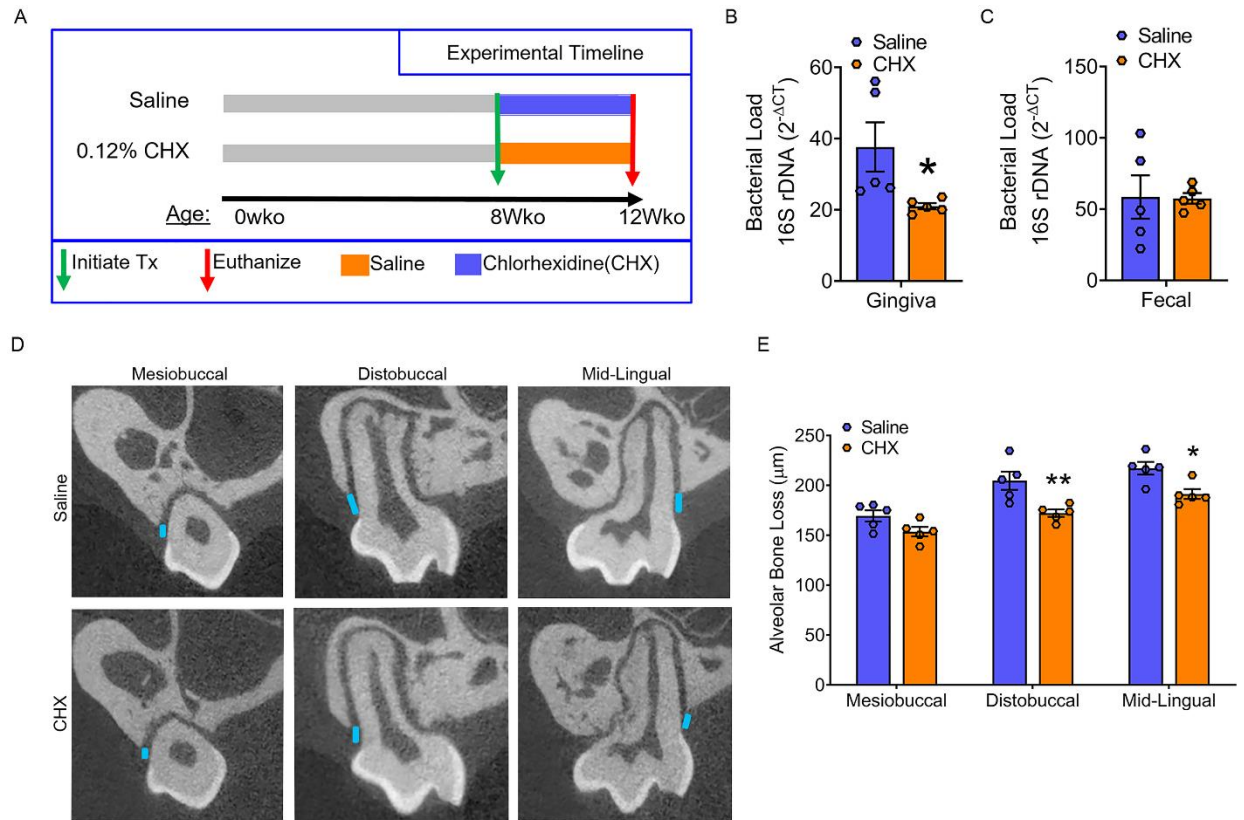


Supplemental Table 1: Flow Cytometry Antibodies

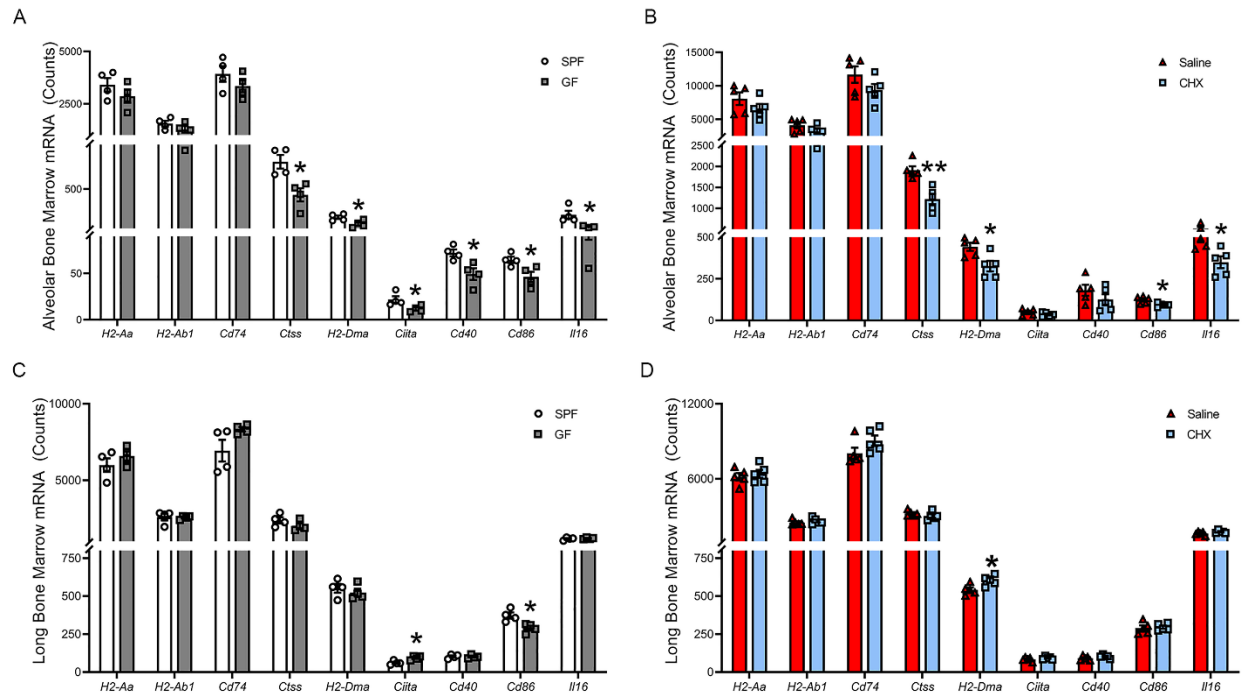
| Cell Type | Tissue | Cell Marker | Fluorescent Tag | Vendor | Clone |
|------------------------------------|---------------|------------------------|----------------------------|---------------|---------------|
| <i>Monocytes / Neutrophils</i> | CLNs | CD11b | APC | Miltenyi | M1/70.15.11.5 |
| | | Ly6G | PacB | Biolegend | 18A |
| | | Ly6C | FITC | Novus Bio. | HK1.4 |
| | | F4/80 | PE | eBioscience | BM8 |
| <i>Macrophages (M1s, M2s)</i> | CLNs | CD11b | APC | Miltenyi | REA592 |
| | | CD68 | PE-Vio770 | Miltenyi | REA835 |
| | | MHC II | FITC | Miltenyi | REA528 |
| | | CD64 | PE | eBioscience | MR6F3 |
| | | CD206 | APC-Vio770 | Miltenyi | REA286 |
| <i>T-cells</i> | ABM, LBM | CD3 | APC-Vio770 | Miltenyi | REA641 |
| | | CD4 | VioBlue | Miltenyi | REA604 |
| | | CD8 | VioGreen | Miltenyi | REA601 |
| | | CD62L | PerCP-Cy5.5 | Thermofisher | MEL-14 |
| | | CD45 | AF700 | Miltenyi | 30-F11 |
| | | CD44 | FITC | Miltenyi | REA664 |
| | | CD28 | PE | Miltenyi | REA806 |
| <i>Dendritic cells</i> | ABM, LBM | CD45 | AF700 | Thermofisher | 30-F11 |
| | | CD11b | PE-Vio770 | Miltenyi | REA592 |
| | | CD11c | VioBlue | Miltenyi | REA754 |
| | | B220 | PerCP-Cy5.5 | Thermofisher | RA3-6B2 |
| | | CD8 | APC-Vio770 | Miltenyi | REA601 |

| | | | | | |
|-----------------------------------|----------|----------|-------------|--------------|---------|
| | | MHC II | VioGreen | Miltenyi | REA813 |
| | | CD86 | APC | Miltenyi | REA1190 |
| | | Siglec-H | FITC | Miltenyi | REA819 |
| <i>B-cells</i> | ABM, LBM | CD45 | PE-Vio770 | Miltenyi | REA737 |
| | | CD19 | VioBlue | Miltenyi | REA749 |
| | | B220 | PerCP-Cy5.5 | Thermofisher | RA3-6B2 |
| | | MHC II | VioGreen | Miltenyi | REA813 |
| | | IgM | FITC | Miltenyi | REA979 |
| | | CD3 | APC-Vio770 | Miltenyi | REA641 |
| | | CD86 | APC | Miltenyi | REA1190 |
| <i>Macrophages (M1s, M2s)</i> | ABM, LBM | CD45 | APC-Vio770 | Miltenyi | REA737 |
| | | CD11b | PE-Vio770 | Miltenyi | REA592 |
| | | CD11c | VioBlue | Miltenyi | REA754 |
| | | F4/80 | PE-Cy5 | Thermofisher | BM8 |
| | | MHC II | VioGreen | Miltenyi | REA813 |
| | | CD64 | PE | Miltenyi | REA286 |
| | | CD86 | APC | Miltenyi | REA1190 |
| | | CD206 | AF700 | Thermofisher | MR6F3 |
| <i>T_H1 cells</i> | ABM, LBM | CD3 | APC-Vio770 | Miltenyi | REA641 |
| | | CD4 | VioBlue | Miltenyi | REA604 |
| | | CD8 | VioGreen | Miltenyi | REA601 |
| | | CD183 | FITC | Miltenyi | REA724 |
| | | Tbet | APC | Miltenyi | REA102 |
| <i>T_H17 cells</i> | ABM, LBM | CD3 | APC-Vio770 | Miltenyi | REA641 |

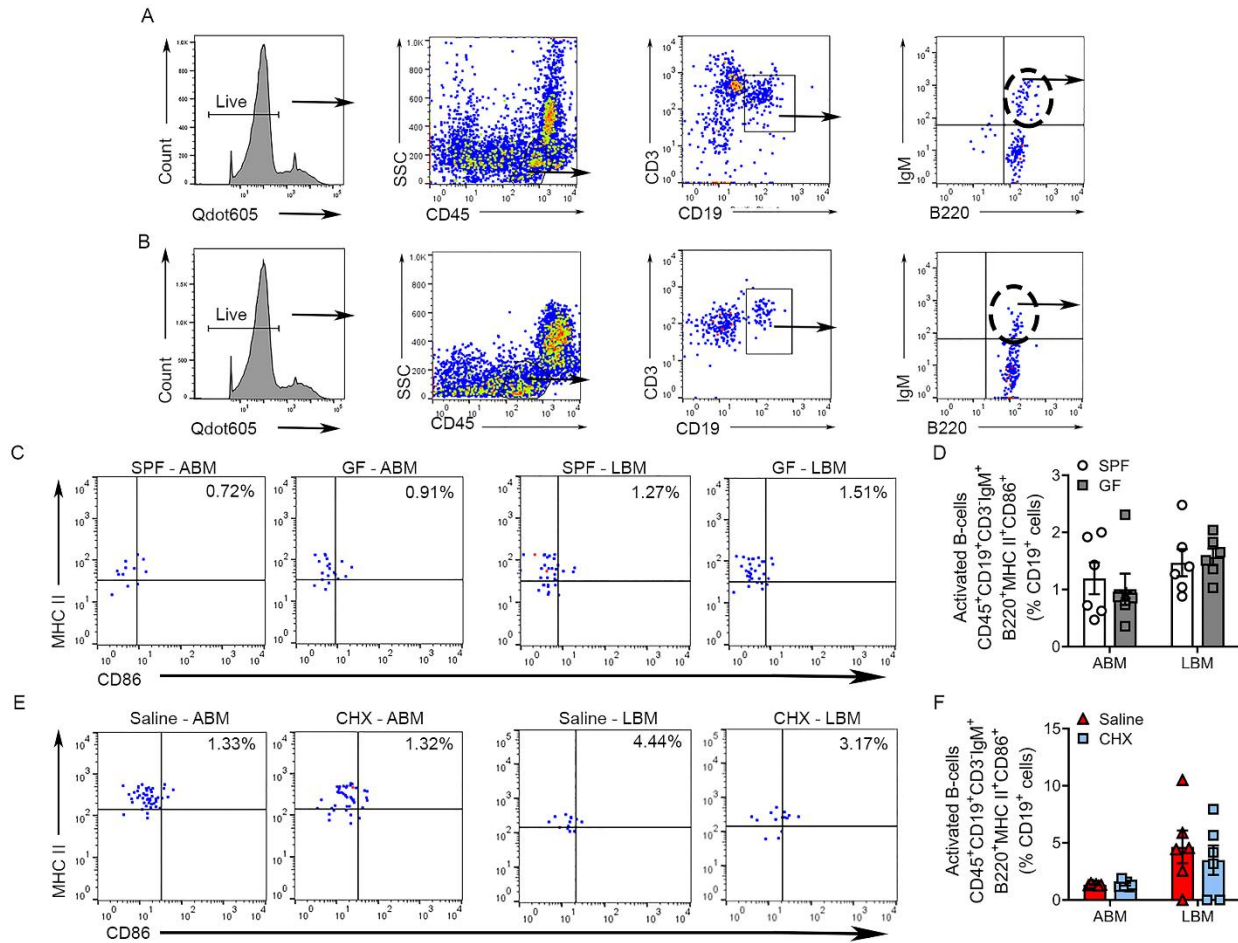
| | | | | | |
|------------------------------|------------------|-----------|------------|--------------|---------|
| | | CD4 | VioBlue | Miltenyi | REA604 |
| | | CD196 | PE | Miltenyi | REA227 |
| | | RORgT | APC | Miltenyi | REA278 |
| <i>T_H2 cells</i> | ABM, LBM | CD3 | APC-Vio770 | Miltenyi | REA641 |
| | | CD4 | VioBlue | Miltenyi | REA604 |
| | | GATA3 | AF700 | Thermofisher | TWAJ |
| | | IRF4 | PE-Vio770 | Miltenyi | REA201 |
| <i>T_{REG} cells</i> | ABM, LBM | CD3 | APC-Vio770 | Miltenyi | REA641 |
| | | CD4 | VioBlue | Miltenyi | REA604 |
| | | CD8 | VioGreen | Miltenyi | REA601 |
| | | CD25 | PE-Vio770 | Miltenyi | REA570 |
| | | FoxP3 | PE | Miltenyi | REA788 |
| <i>Dendritic cells</i> | In vitro studies | CD11c | VioBlue | Miltenyi | REA754 |
| | | MHC II | VioGreen | Miltenyi | REA813 |
| | | CD86 | APC | Miltenyi | REA1190 |
| <i>T-cells</i> | In vitro studies | CD3 | APC-Vio770 | Miltenyi | REA641 |
| | | CD4 | VioBlue | Miltenyi | REA604 |
| | | CD28 | PE | Miltenyi | REA806 |
| Live/Dead fixable stain | All studies | Live/dead | Qdot605 | Thermofisher | N/A |



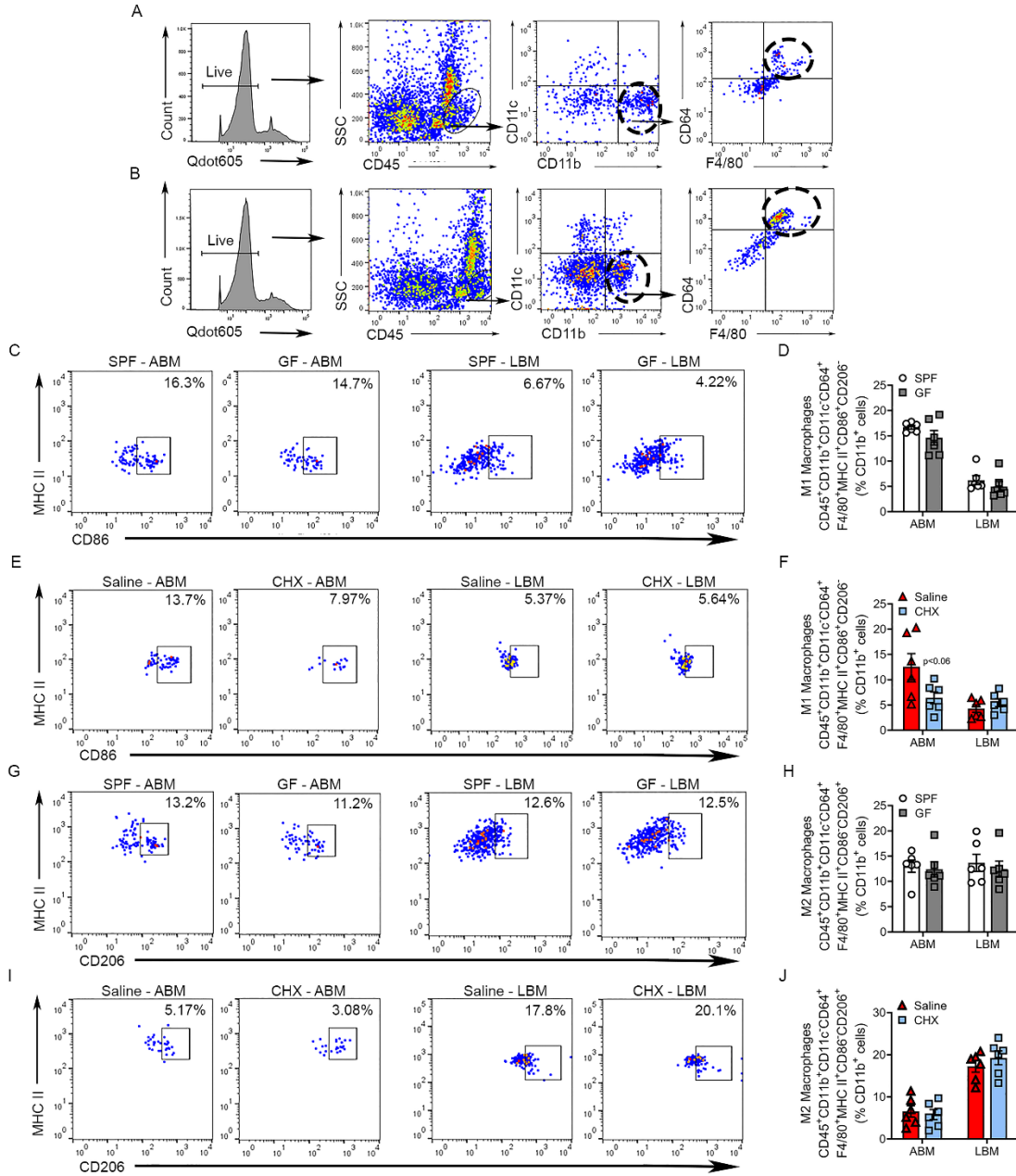
Supplemental Figure 1. Four-week treatment with CHX oral rinse suppresses commensal oral microbiota and protects from alveolar bone loss. (A) Experimental timeline of SPF mice treated with CHX vs. Saline oral rinse from age 8 to 12 weeks. (B-C) 16S rDNA analysis of bacterial load for (B) maxillary gingiva and (C) fecal pellets; data reported as $2^{-\Delta C_T}$; $n=5/gp$. (D-E) Alveolar bone loss was assessed by evaluating the linear distance between the cemento-enamel junction (CEJ) and alveolar bone crest (ABC) at the maxillary first molar in reconstructed micro-CT images. (D) Representative micro-CT images displaying CEJ-ABC linear distance (blue line) at the mesiobuccal, distobuccal, and mid-lingual aspect of the maxillary first molar. (E) Quantitative measures of CEJ-ABC linear distance at the mesiobuccal, distobuccal, and mid-lingual aspect of the maxillary first molar; $n=5/gp$. Unpaired t -test; data presented as mean \pm SEM; * $p<0.05$, ** $p<0.01$.



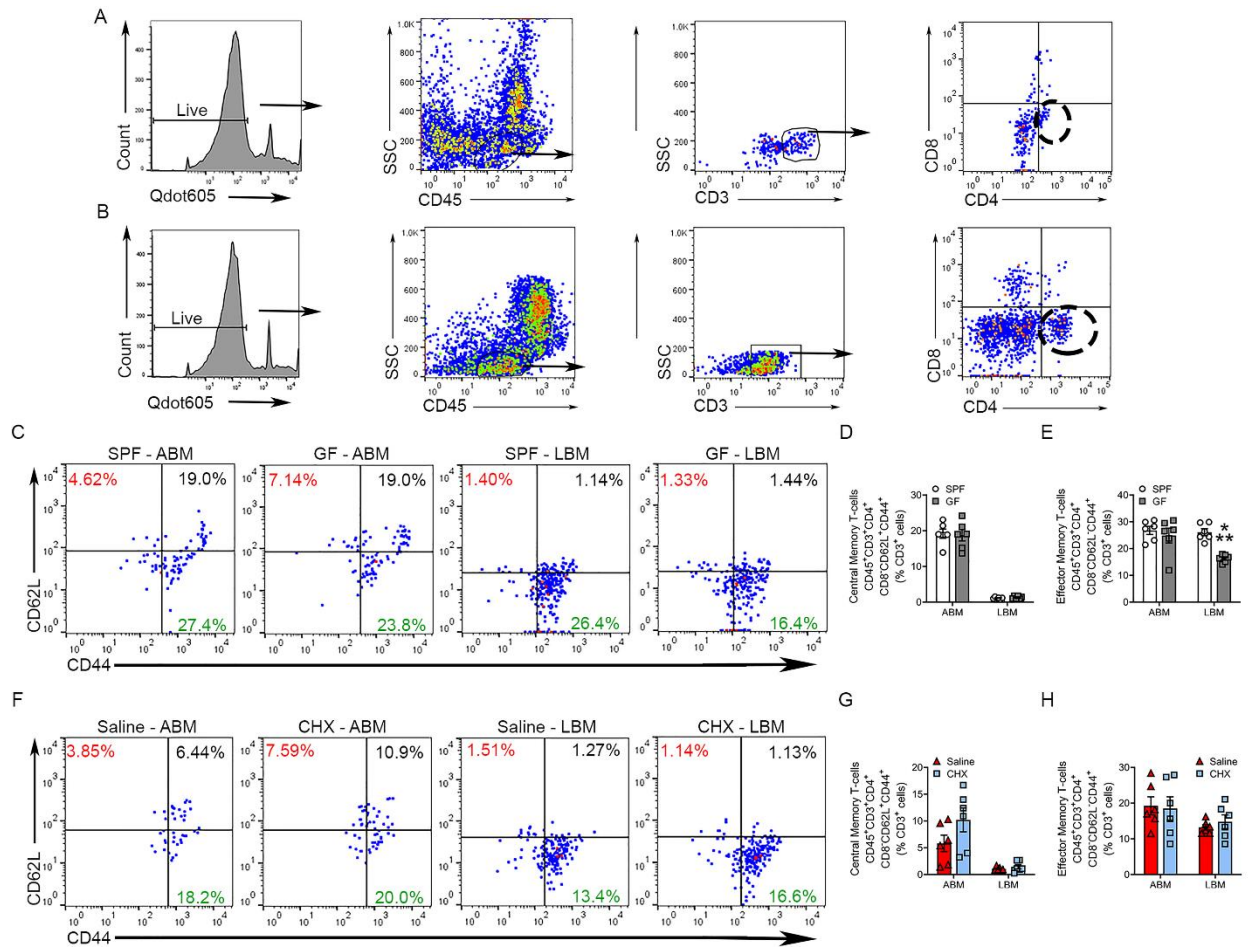
Supplemental Figure 2. Commensal oral microbiota has immunomodulatory effects to induce MHC II antigen presentation genes in alveolar bone marrow. (A-B) nCounter analysis was performed to assess MHC II antigen processing and presentation genes in alveolar bone marrow from (A) SPF vs. GF mice (n=4/gp) and (B) Saline vs. CHX mice (n=6/gp). Data presented as mRNA counts. (C-D) nCounter analysis evaluated MHC II antigen processing and presentation genes in long bone marrow from (C) SPF vs. GF mice (n=4/gp) and (D) Saline vs. CHX mice (n=6/gp). Data presented as mRNA counts. Unpaired *t*-test; data reported as mean \pm SEM; **p*<0.05, ***p*<0.01.



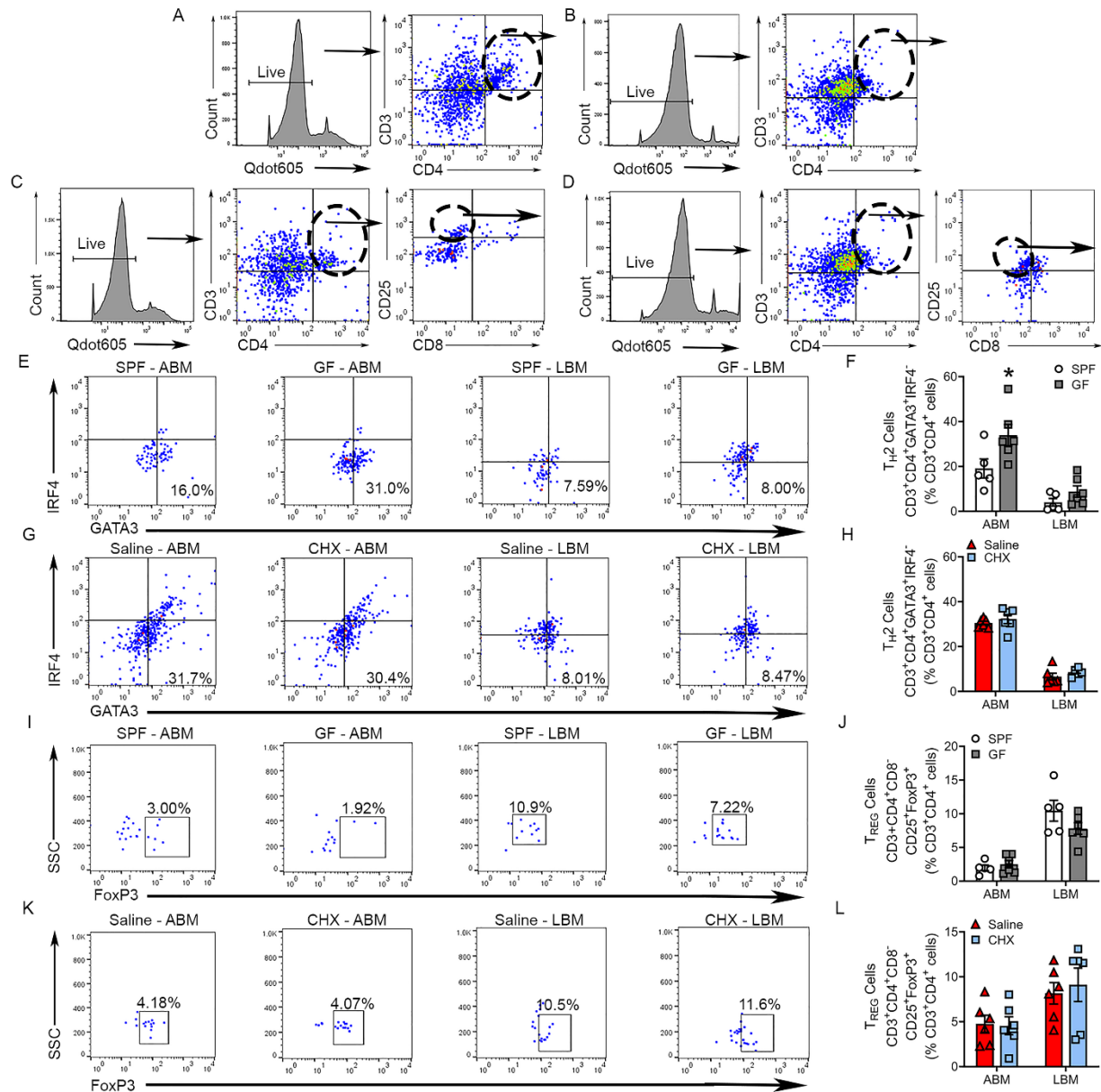
Supplemental Figure 3. Commensal oral microbiota did not alter B-cell activation in alveolar bone marrow. Flow cytometric analysis of activated B-cells in the alveolar bone marrow (ABM) and long bone marrow (LBM), reported as % CD19⁺ cells; n=6/gp. **(A-B)** Representative gating strategy for activated B-cells in **(A)** ABM and **(B)** LBM. **(C-D)** Representative dot plots and **(D)** quantitation for CD45⁺CD19⁺CD3⁺IgM⁺B220⁺MHC II⁺CD86⁺ activated B-cells in ABM and LBM of SPF vs. GF mice. **(E)** Representative dot plots and **(F)** quantitation for CD45⁺CD19⁺CD3⁺IgM⁺B220⁺MHC II⁺CD86⁺ activated B-cells in ABM and LBM of Saline vs. CHX mice. Unpaired *t*-test; data are presented as mean ± SEM.



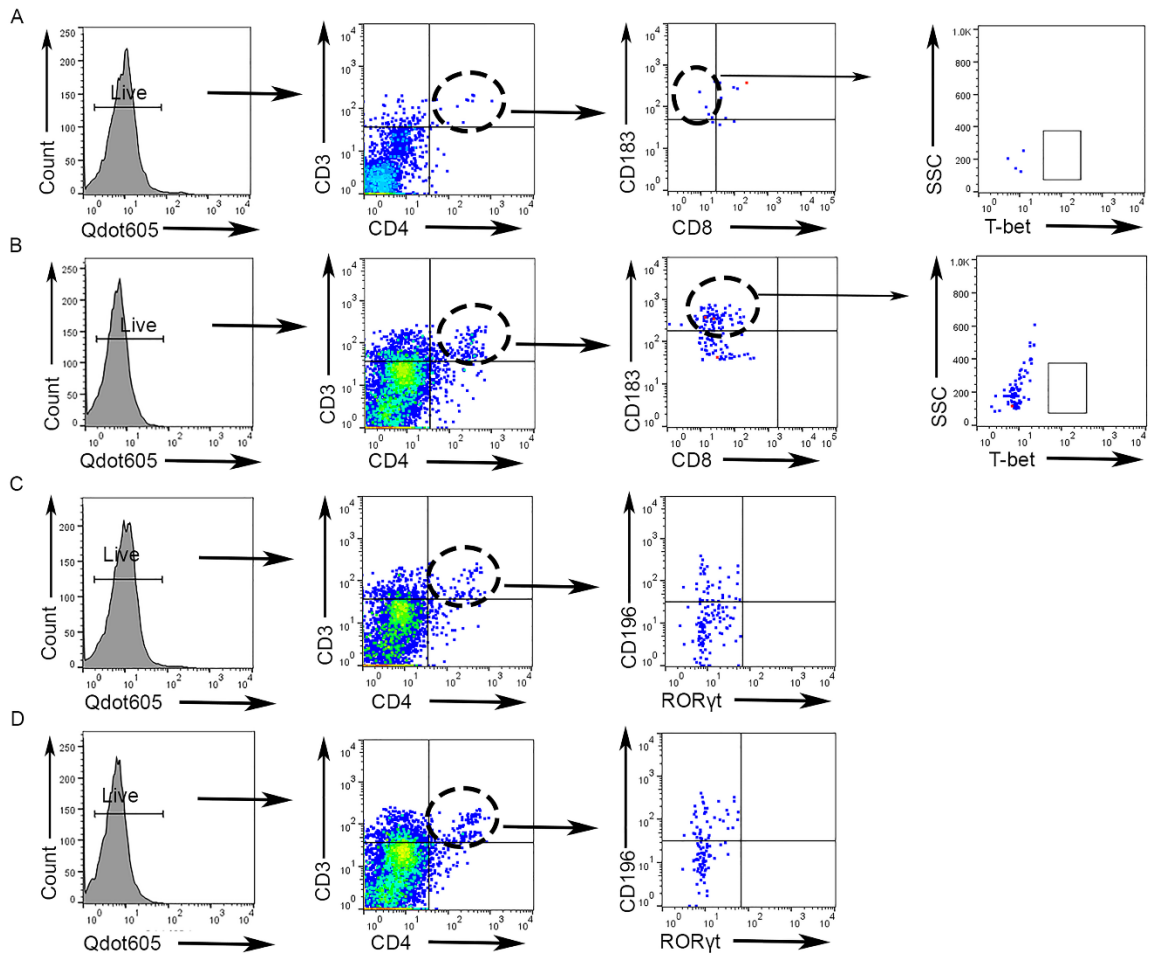
Supplemental Figure 4. Commensal microbiota effects on M1/M2 macrophages in oral and non-oral bone marrow. Flow cytometric analysis of M1 and M2 macrophages in the alveolar bone marrow (ABM) and long bone marrow (LBM), reported as % CD11b⁺ cells; n=6/gp. (A-B) Representative gating strategy for macrophages in (A) ABM and (B) LBM. (C) Representative dot plots and (D) quantitation for CD45⁺CD11b⁺CD11c⁻CD64⁺F4/80⁺MHC II⁺CD86⁺CD206⁻ M1 macrophages in ABM and LBM of SPF vs. GF mice. (E) Representative dot plots and (F) quantitation for CD45⁺CD11b⁺CD11c⁻CD64⁺F4/80⁺MHC II⁺CD86⁺CD206⁻ M1 macrophages in ABM and LBM of Saline vs. CHX mice. (G) Representative dot plots and (H) quantitation for CD45⁺CD11b⁺CD11c⁻CD64⁺F4/80⁺MHC II⁺CD86⁺CD206⁺ M2 macrophages in ABM and LBM of SPF vs. GF mice. (I) Representative dot plots and (J) quantitation for CD45⁺CD11b⁺CD11c⁻CD64⁺F4/80⁺MHC II⁺CD86⁺CD206⁺ M2 macrophages in ABM and LBM of Saline vs. CHX mice. Unpaired *t*-test; data presented as mean ± SEM.



Supplemental Figure 5. Commensal microbiota effects on central and effector memory CD4⁺ T-cells in oral and non-oral bone marrow. Flow cytometric analysis of CD4⁺ helper T-cell cells in the alveolar bone marrow (ABM) and long bone marrow (LBM), reported as % CD3⁺ cells; n=6/gp. **(A-B)** Representative gating strategy for CD4⁺ T-cells in **(A)** ABM and **(B)** LBM. **(C)** Representative dot plots and quantitation for **(D)** CD45⁺CD3⁺CD4⁺CD8⁻CD62L⁺CD44⁺ central memory T-cells (Upper right; black %) and **(E)** CD45⁺CD3⁺CD4⁺CD8⁻CD62L⁻CD44⁺ effector memory T-cells (Lower right; green %) in ABM and LBM of SPF vs. GF mice. **(F)** Representative dot plots and quantitation for **(G)** CD45⁺CD3⁺CD4⁺CD8⁻CD62L⁺CD44⁺ central memory T-cells (Upper right; black %) and **(H)** CD45⁺CD3⁺CD4⁺CD8⁻CD62L⁻CD44⁺ effector memory T-cells (Lower right; green %) in ABM and LBM of Saline vs. CHX mice. Unpaired *t*-test; data presented as mean ± SEM; ****p*<0.001.



Supplemental Figure 6. Commensal microbiota effects on TH2 and TREG cells in oral and non-oral bone marrow. Flow cytometric analysis of CD4⁺ helper T-cell subsets in the alveolar bone marrow (ABM) and long bone marrow (LBM), reported as % CD3⁺CD4⁺ cells; n=5-6/gp. (A-B) Representative gating strategy for TH2 cells in (A) ABM and (B) LBM. (C-D) Representative gating strategy for TREG cells in (C) ABM and (D) LBM. (E) Representative dot plots and (F) quantitation for CD3⁺CD4⁺GATA3⁺IRF4⁺ TH2 cells in ABM and LBM of SPF vs. GF mice. (G) Representative dot plots and (H) quantitation for CD3⁺CD4⁺GATA3⁺IRF4⁺ TH2 cells in ABM and LBM of Saline vs. CHX mice. (I) Representative dot plots and (J) quantitation for CD3⁺CD4⁺CD8⁻CD25⁺FoxP3⁺ TREG cells in ABM and LBM of SPF vs. GF mice. (K) Representative dot plots and (L) quantitation for CD3⁺CD4⁺CD8⁻CD25⁺FoxP3⁺ TREG cells in ABM and LBM of Saline vs. CHX mice. Unpaired *t*-test; data presented as mean ± SEM; **p*<0.05.



Supplemental Figure 7. Fluorescence Minus One (FMO) controls for T-bet and RORyt. (A-B) Flow cytometric analysis of TH1 antibody markers without staining for T-bet. Representative gating strategy for TH1 cells in (A) ABM and (B) LBM. (C-D) Flow cytometric analysis of TH17 antibody markers without staining for RORyt. Representative gating strategy for TH17 cells in (A) ABM and (B) LBM.