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Supplemental information

Functional, transcriptional, and microbial shifts associated with healthy pulmonary aging in rhesus macaques

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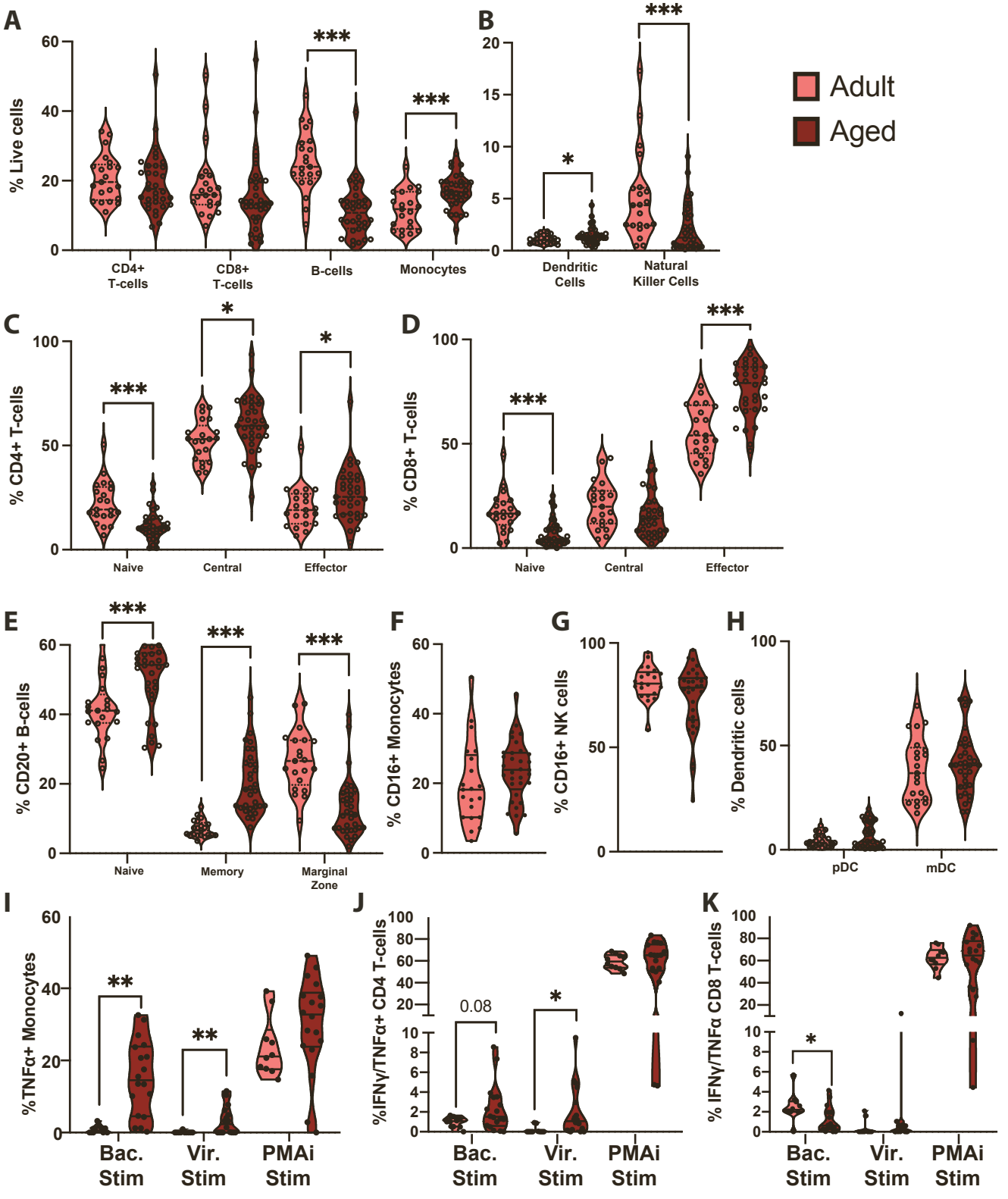


Figure S1: **Age-related differences in circulating immune cell frequency and function.** Related to Figure 1. Violin plots of immune cell frequency as measured by flow cytometry of BAL cells for (A) major immune cell populations (CD4+ T-cells, CD8+ T-cells, B-cells, Monocytes), (B) less abundant immune cell populations (Dendritic cells, Natural killer cells), (C) CD4+ T-cell subsets, (D) CD8+ T-cell subsets, (E) CD20+ B cell subsets, (F) CD16+ Monocytes, (G) CD16+ Natural killer cells, and (H) Plasmacytoid and Myeloid Dendritic cells. (I) Percent abundance of TNF producing monocytes after 16hr PMAi stimulation. Percent abundance of IFN and/or TNF producing (J) CD4+ and (K) CD8+ T-cells after 16hr stimulation. Cytokine producing cells were measured using intra-cellular cytokine staining and flow cytometry. Cells were stimulated with either bacterial ligands (Bac. Sim; LPS, FSL-1, Pam3CSK4), viral ligand (Vir. Stim; ssRNA, Imiquimod, ODN2216), or PMAi. Comparisons were made using an unpaired t-test between age-groups for each cell subset (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$).

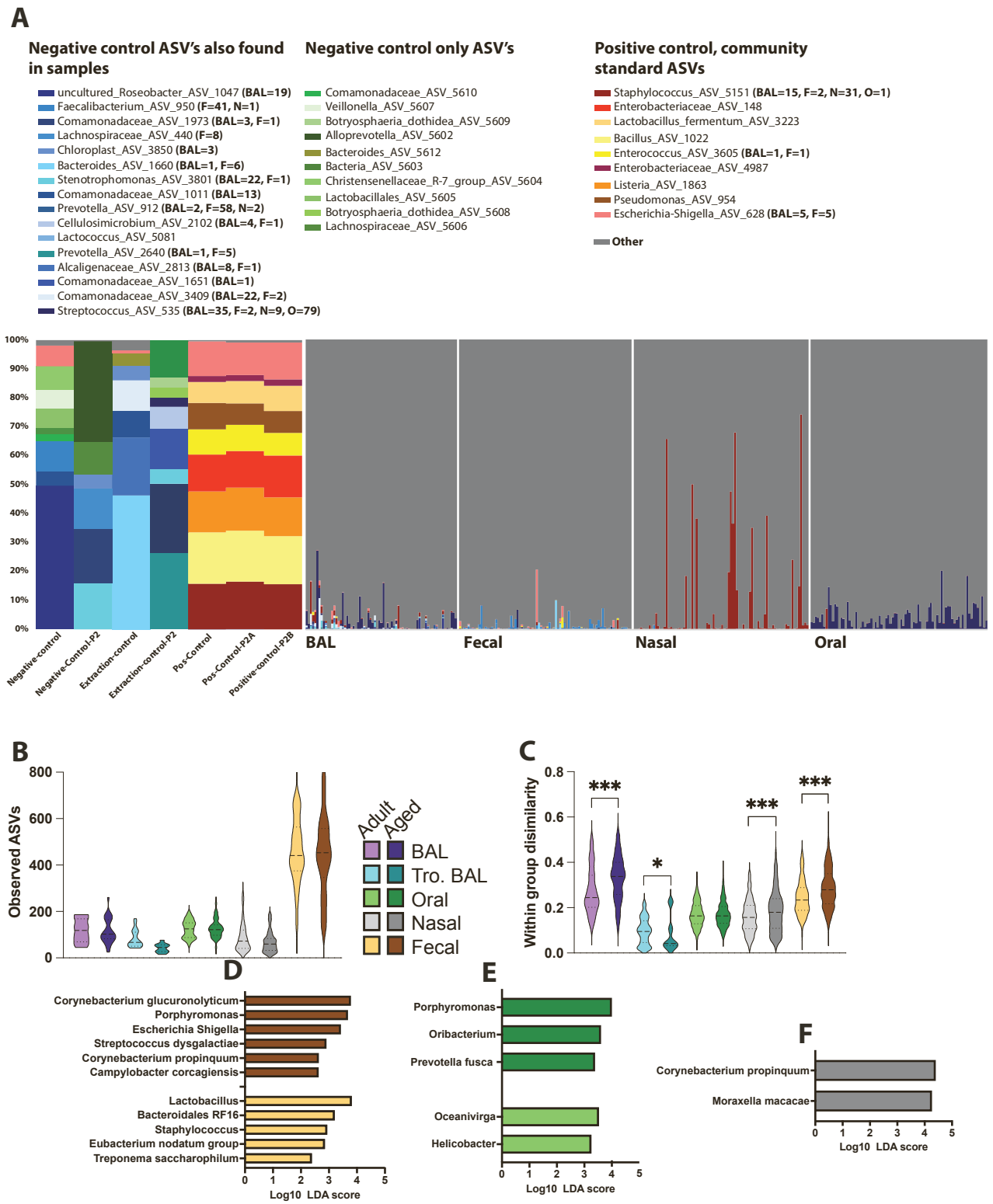
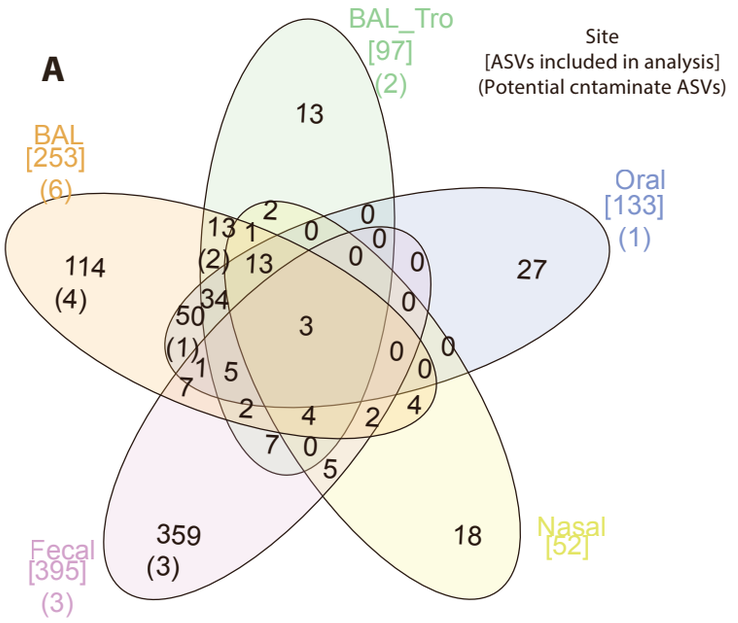
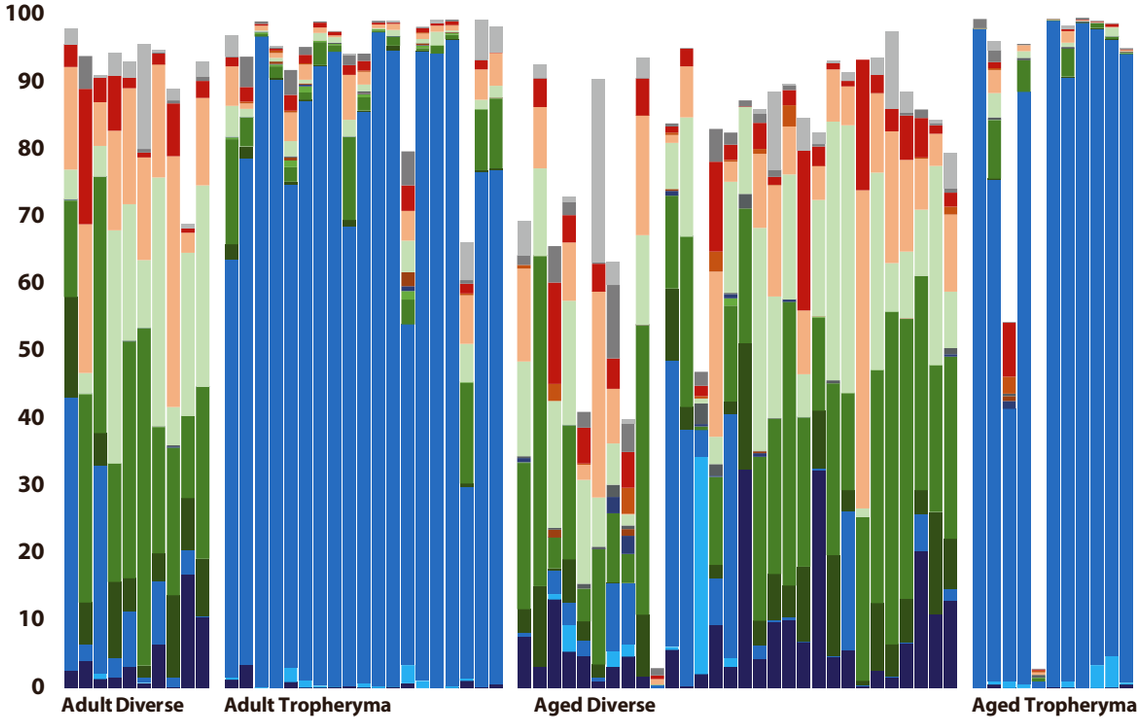


Figure S2: Contribution of environmental contaminates to the lung microbiome and age-related shifts in the rhesus macaque microbiome. Related to Figure 2. (A) Stacked bar plot highlighting taxa found in positive and negative control samples. (B) Violin plots of observed amplicon sequencing variants (ASVs) across sampling sights between age groups. (C) Violin plot illustrating within group weighted UniFrac distances between animals of the same age group within each site. (D-F) Differentially abundant bacterial taxa between age groups at each site (fecal, oral and nasal). Differential abundance was determined using LEFsE (Log10 LDA score > 2) of L7 (species level) taxonomy.





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	Microbiome Compartment	Adult Diverse	Adult Tro.	Aged Diverse	Aged Tro.	(-) Controls
	Diverse BAL only	**4.78**	**0.48**	**7.57**	**0.12**	
	Porphyromonas_canoris_ASV_1309	**0.00**	**0.00**	**0.71**	**0.00**	**0.00**
	Prevotella_ASV_2044	**0.09**	**0.00**	**0.35**	**0.00**	**0.00**
	Lautropia_ASV_2108	**0.03**	**0.00**	**0.35**	**0.00**	**0.00**
	Tro. BAL only	**0.09**	**0.51**	**1.44**	**1.04**	
	Acinetobacter_ASV_4580	**0.00**	**0.03**	**1.12**	**0.77**	**0.00**
	Halomonas_ASV_1847	**0.00**	**0.02**	**0.14**	**0.00**	**0.00**
	Enhydrobacter_ASV_2332	**0.00**	**0.12**	**0.03**	**0.00**	**0.00**
	Diverse BAL & Tro. BAL	**10.03**	**80.52**	**6.32**	**79.03**	
	Tropheryma_ASV_4722	**7.63**	**76.20**	**4.75**	**71.76**	**0.20**
	Tropheryma_ASV_4310	**1.05**	**4.11**	**0.00**	**7.19**	**0.00**
	Diverse BAL & Oral	**7.66**	**0.41**	**5.80**	**0.05**	
	Rodentibacter_ASV_4926	**0.41**	**0.02**	**0.29**	**0.00**	**0.00**
	SR1_bacterium_ASV_3876	**0.54**	**0.01**	**0.33**	**0.01**	**0.00**
	Porphyromonas_ASV_4494	**0.09**	**0.00**	**0.45**	**0.00**	**0.00**
	Diverse BAL & Tro. BAL & Oral	**26.47**	**4.53**	**21.94**	**1.76**	
	Gemella_ASV_3506	**5.71**	**1.40**	**4.62**	**0.22**	**0.00**
	Porphyromonas_ASV_761	**4.03**	**0.50**	**2.60**	**0.24**	**0.00**
	Fusobacterium_ASV_1429	**2.14**	**0.14**	**1.80**	**0.22**	**0.00**
	Tro. BAL & Nasal	**0.03**	**0.31**	**0.05**	**0.02**	
	Diverse BAL & Fecal	**0.04**	**0.08**	**0.30**	**0.12**	
	Tro. BAL & Fecal	**0.00**	**0.18**	**0.09**	**0.07**	
	Diverse BAL & Tro. BAL & Fecal	**0.03**	**0.04**	**0.42**	**0.17**	
	Diverse BAL & Tro. BAL & Oral &Nasal	**17.40**	**1.71**	**14.47**	**0.63**	
	Neisseria_ASV_4242	**4.92**	**0.33**	**3.07**	**0.15**	**0.00**
	Moraxella_ASV_338	**4.00**	**0.42**	**4.30**	**0.22**	**0.00**
	Streptococcus_ASV_2610	**2.21**	**0.42**	**2.07**	**0.13**	**0.00**
	Diverse BAL & Tro. BAL & Oral & Fecal	**16.11**	**2.59**	**10.21**	**0.61**	
	Actinobacillus_ASV_1196	**10.61**	**1.14**	**4.97**	**0.31**	**0.03**
	Rodentibacter_ASV_1435	**3.27**	**0.78**	**2.24**	**0.13**	**0.00**
	Diverse BAL & Tro. BAL & Fecal & Nasal	**0.00**	**0.09**	**0.55**	**0.29**	
	Diverse BAL & Tro. BAL & Oral & Fecal & Nasal	**5.04**	**1.08**	**4.88**	**0.94**	
	Streptococcus_ASV_4404	**4.93**	**0.71**	**3.47**	**0.16**	**0.00**
	Staphylococcus_ASV_5151	**0.05**	**0.26**	**1.00**	**0.77**	**0.00**
	Streptococcus_ASV_4264	**0.06**	**0.11**	**0.41**	**0.01**	**0.00**
	Potential contaminates (-)controls only	**0.70**	**1.04**	**1.47**	**0.38**	
	Comamonadaceae_ASV_3409	**0.23**	**0.04**	**0.86**	**0.05**	**2.63**
	uncultured_Roseobacter_ASV_1047	**0.16**	**0.68**	**0.07**	**0.00**	**12.42**
	Stenotrophomonas_ASV_3801	**0.19**	**0.04**	**0.33**	**0.22**	**5.34**
	ASV found in (-) control, Oral samples, and BAL	**2.89**	**1.02**	**2.85**	**0.16**	
	Streptococcus_ASV_535	**2.89**	**1.02**	**2.85**	**0.16**	**0.62**

Figure S3: Biogeography of the rhesus macaque microbiome in relation to the lungs. Related to Figure 2. (A) Venn diagram of shared and unique microbial ASV's found within and between each sampling sites. To be included in this analysis ASV's had to be detected with >0.01% average relative abundance and present in >10% samples within a site. (B) Stacked bar plot of ASV relative abundance across all samples, colored based on which body sites those ASV's are shared with. (C) Table with the three most abundant ASVs unique to the lung or shared with other body sites averaged for each age group and BAL microbiome phenotype

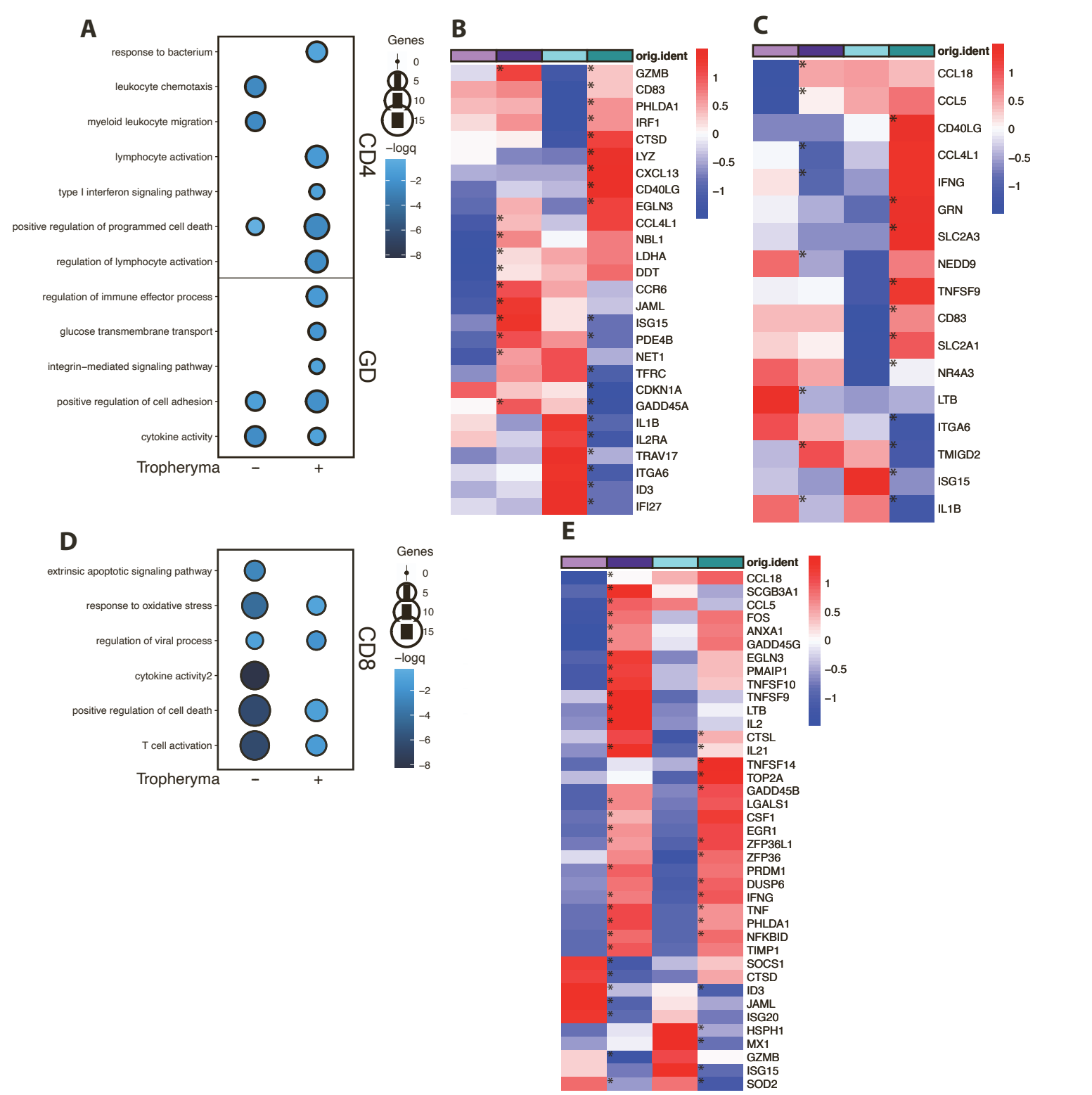

Figure S4: Age- and Tropheryma-related shifts in BAL T-cell expression. Related to Figure 4. (A) Functional enrichment of genes differentially expressed in CD4+ T-cells (clusters 0 and 5) and γδ T-cells (cluster 2) between adult and aged animals in the absence and presence of Tropheryma colonization. Size of the bubble represents statistical significance and number of genes respectively. (B,C) Heatmap of differentially expressed genes within CD4+ T-cells (B) and γδ T-cells (C). (D) Functional enrichment of genes differentially expressed in CD8+ T-cells (clusters 1 and 3) between adult and aged animals in the absence and presence of Tropheryma colonization. Size of the bubble represents statistical significance and number of genes respectively. (E) Heatmap of differentially expressed genes within CD8+ T-cells. For all heatmaps only genes with $p < 0.05$ are included; * indicate the comparison (Adult vs. Aged or Adult Tro. vs. Aged Tro) each DEG was derived from.

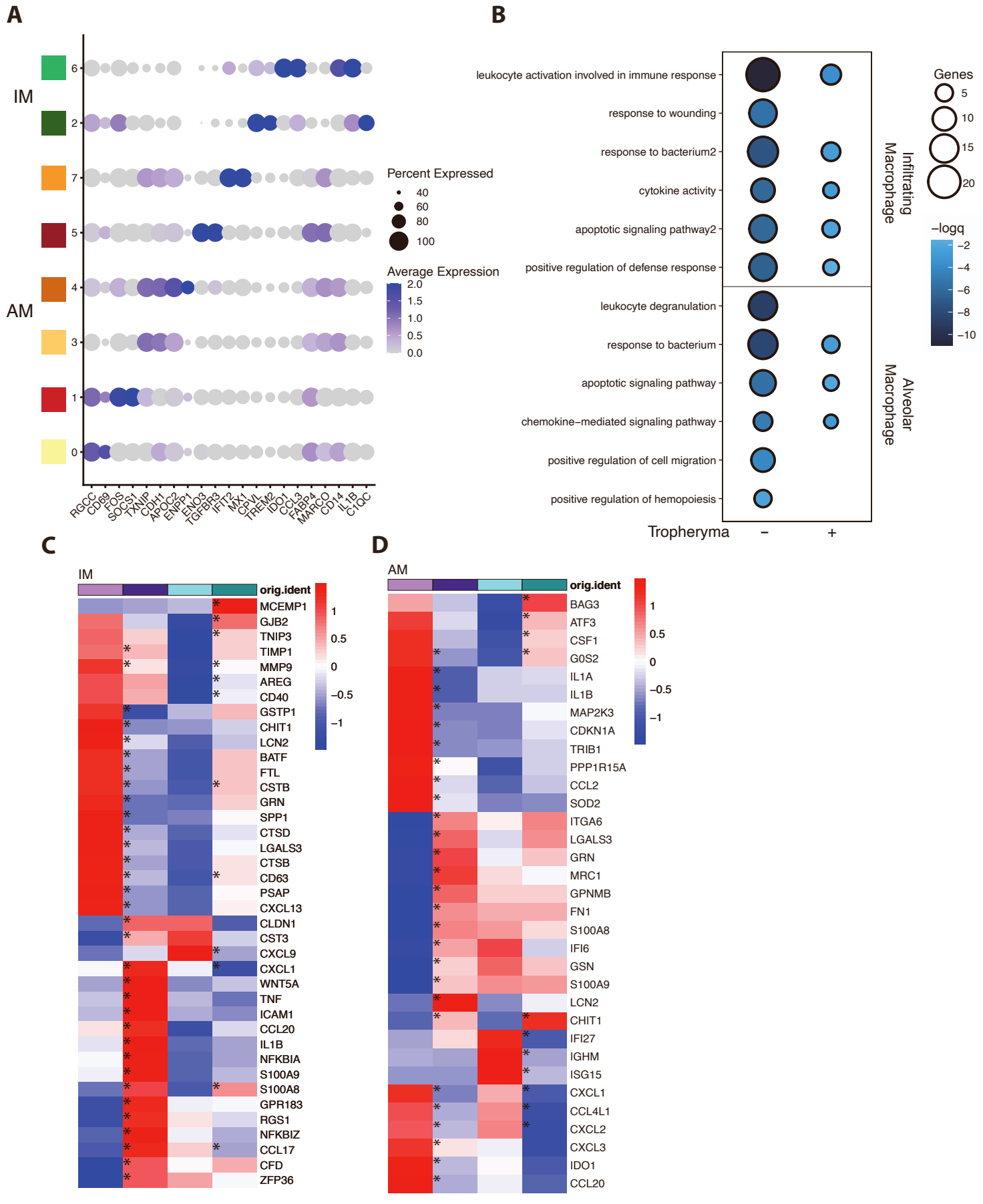

Figure S5: Age and Tropheryma related shifts in BAL AM and IM expression. Related to Figure 5. (A) Bubble plot of identifying genes highly expressed in AM and IM cell clusters determined using the FindMarkers function in Seurat. Level of normalized expression is shown using a color scale ranging from low (white) to high (purple). Size of the bubble is representative of the fraction of cells within each cluster expressing the marker. (B) Functional enrichment of genes differentially expressed in IM (clusters 2 and 6) and AM (clusters 0,1,3,4,5,7) between adult and aged animals in the absence and presence of Tropheryma colonization. Size of the bubble represents statistical significance and number of genes respectively. (C,D) Heatmap of differentially expressed genes within IM (C) and AM (D). For all heatmaps only genes with $p < 0.05$ are included, * indicate for which comparisons (Adult vs. Aged or Adult Tro. vs. Aged Tro) each gene was differentially expressed.