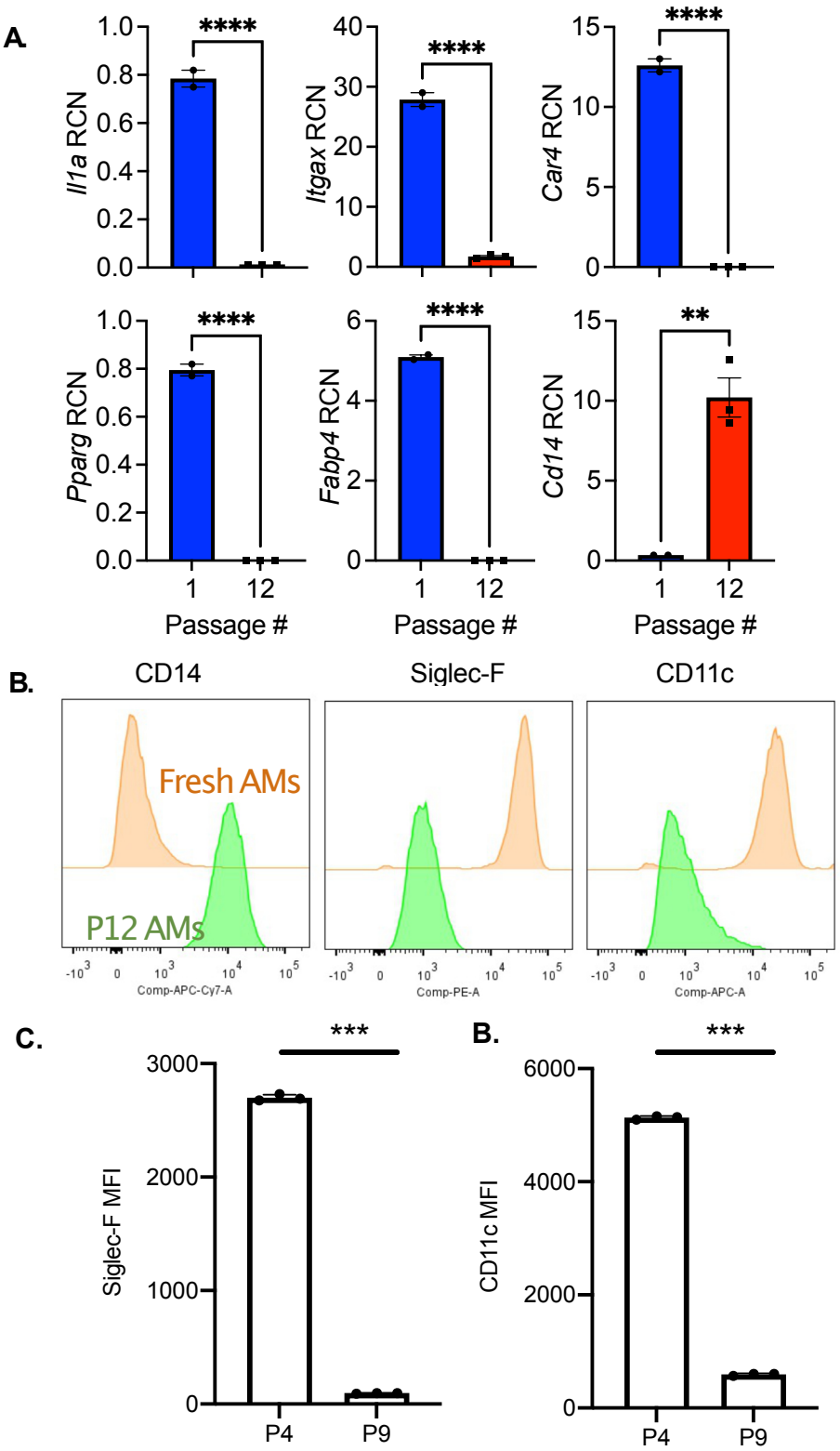

Figure S1. Long term ex vivo culture of AMs leads to loss of AM-defining genes and surface markers. Alveolar macrophages (AM) were isolated and cultured in RPMI with 20 ng/mL GM-CSF for approximately 2 months (12 passages). Gene expression (**A**) and cell surface markers (**B**) were compared between cultured AMs and freshly isolated AMs. Asterisks indicate significant differences (****p<0.0001, **p<0.01) between samples. **(C)** Fetal liver macrophages were cultured for 1 week to allow for development and expression of Siglec-F and CD11c and were then stained and sorted based on Siglec-F expression with over 98% of cells expressing high levels of Siglec-F. Cells were then plated in media containing GM-CSF alone and at the indicated passage cells were lifted and stained for Siglec-F and CD11c Asterisks indicated significant differences between samples (***p<0.001) as determined by a student's T-test. Results are representative of two independent experiments.

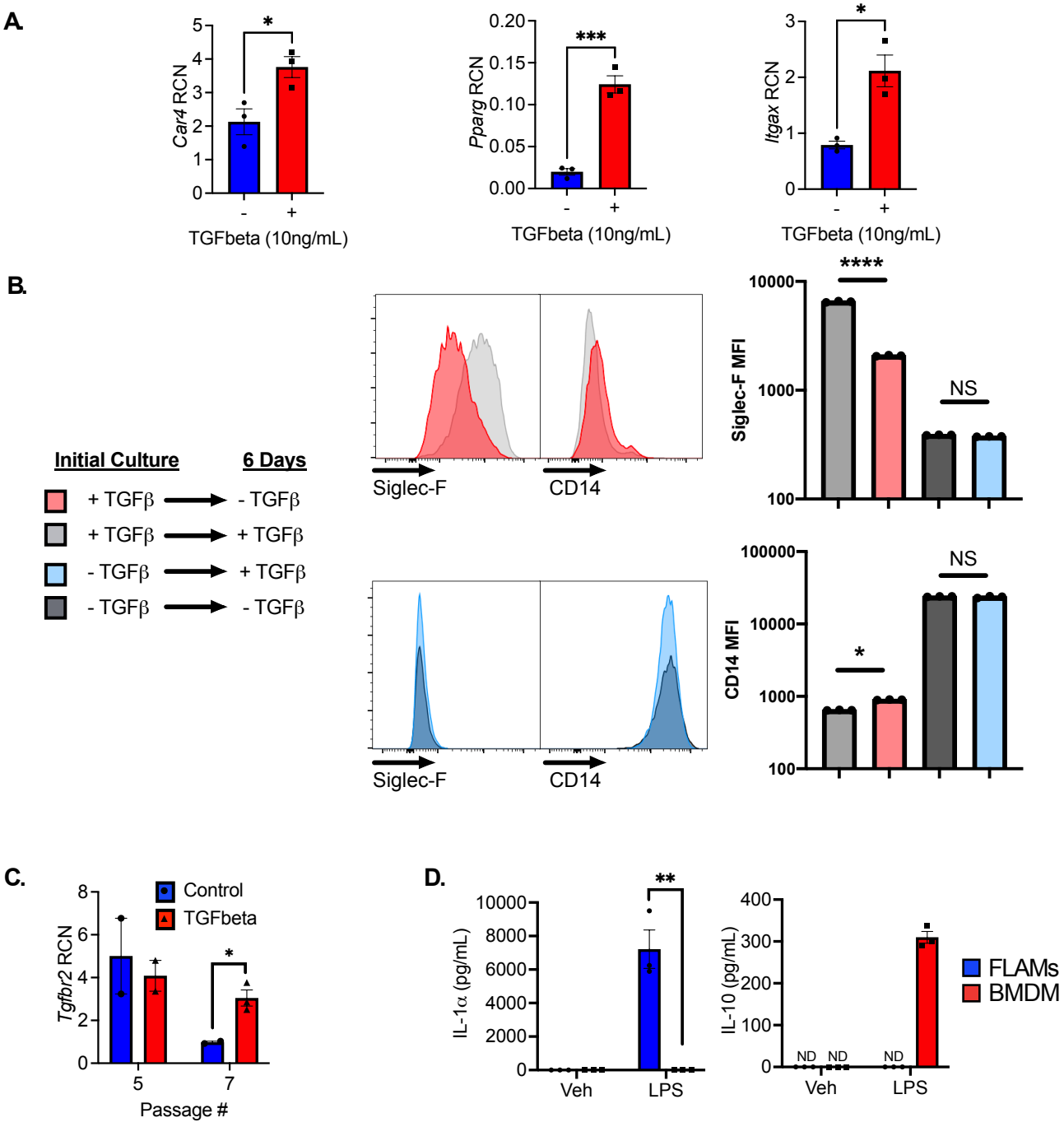

Figure S2. TGFβ induces and maintains AM-like state in early-passage but not older fetal liver macrophages. A) Freshly isolated fetal liver cells were incubated with 10 ng/mL TGFβ for 24 hours prior collecting RNA for qPCR analysis of Car4, Itgax and Pparg gene expression. **B)** Fetal liver macrophages that were low passage (<4) and kept in TGFβ and those that were high-passage (>15) and cultured without TGFβ were cultured for 6 days with and without TGFβ. On day 6, cells were lifted and surface expression of Siglec-F and CD14 was assessed by flow cytometry. **C**) Expression of Tgfbr2 in fetal liver macrophages cultured with and without TGFβ was assessed at P5 and P7. Asterisks indicate significant differences (****p<0.0001, ***p<0.001, *p<0.05) between samples, as determined by Student's t-test. **(D)** Early fetal liver-derived cells and bone marrow-derived macrophages (BMDMs) were treated with 100 ng/mL LPS for 24 hours and cell-free supernatant was analyzed for release of IL-1alpha and IL-10 by ELISA. Asterisks indicate significant differences (**p<0.01) between fetal liver macrophages cells and BMDMs. ND=not detected.