Supplementary Figure 1. Phenotypic characterization of hematopoietic and early T cells derived from CD34+CD38-/low Notch signaling-induced differentiation cultures. A) Flow cytometric analysis of CD34 and CD38 and **B)** CD1a and CD7 expression on FSC vs SSC gated cells at day 16 of culture with OP9-DL1 conditioned medium in the presence (2.5 μg/mL or 5 μg/mL) or absence (negative control)

of immobilized human Fc-DLL1 Notch ligand . **C)** Flow cytometric analysis of CD4 and CD8 expression at day 22 on FSC vs SSC gated cells. PBMC-derived lymphocytes were included in the FACS staining as a positive control for CD4, CD8 and CD3 staining. **D)** Histogram showing CD3 expression on CD4+CD8+ DP cells derived from Fc-DLL1-induced cultures at day 22 of differentiation (3), compared to DP cells from no ligand control cultures (2) and isotype staining (1) (bottom panel). Representative contour plots show that about 25.3% of CD4+CD8+ cells from Fc-DLL1 (2.5 μg/mL) cultures express CD3, compared to CD4-CD8- cells from the same cultures, which express minimal CD3. Isotype staining controls were included in each experiment to determine quadrant gates (top panel).

Supplementary Figure 2. **Phenotypic characterization of myeloid cells derived from CD34+CD38-/low Notch signaling-induced differentiation cultures.** Flow cytometric analysis of CD11c, HLA-ABC and HLA-DR expression on CD34+CD38-/low HSCs cultured for 16 days with OP9-DL1 conditioned medium in the presence of immobilized human Fc-DLL1 Notch ligand (2.5 μg/mL and 5 μg/mL) or absence of Notch ligand (negative control). Isotype staining control shown was included in each experiment to determine the quadrant gates.

Supplementary Figure 3. CMV antigen-specific T cells generated in the presence of CMV tetramer or CMV peptide are CD8+CD4- T cells. CD8+CD4-CMV+ cells were generated when CMV tetramers or CMV peptide were added to the differentiation cultures. Cultures with no tetramer incubation show non-specific background staining. Differentiated cells from both culture conditions (CMV tetramer and CMV peptide) were stained with an irrelevant tetramer (GIL tetramer-APC) as a negative control (right panels).

Supplementary Figure 4. **Generation of GIL antigen-specific CD8+ T cells in the presence of GIL tetramer.** CD8+GIL+ cells were generated when GIL M1 tetramers were added to the differentiation cultures. Some of these cells (48.9%) expressed CD107a in a CTL assay (Functional CD8+GIL+ cells). CD107a was also expressed on non-antigen specific CD8+ T cells that were probably activated (non-specific CD107a staining) by co-stimulatory molecules used during differentiation. Cultures incubated with an irrelevant tetramer (CMV tetramer) show a non-specific background staining (GIL tetramer-APC binds non-specifically to some CMV+ cells).