Supplementary Figure S1. Overlay of PD-L1 and LAG-3 IHC demonstrates close geographic association of these cell-surface markers in a melanoma lymph node metastasis. PD-L1 membranous staining (light blue cells, white arrows) is observed on melanoma cells and macrophages at the interface of tumor (middle and right side of image) and background lymph node (left side of image). LAG-3 is present on TILs (red cells, yellow arrows). Original magnification 400X.
Supplementary Figure S2. Comparison of immunohistochemistry (IHC) and in situ hybridization (ISH) for LAG-3 expression in a melanoma lymph node metastasis. A similar density and distribution of LAG-3+ lymphocytes is identified at the interface of tumor (upper left quadrant of each panel, asterisk) and background lymph node, by both IHC (A) and ISH (B). Negative and positive controls for LAG3 ISH were dapB and PPIB, shown in panels (C) and (D) respectively. Original magnification 400X.
Supplementary Figure S3. IL-32-γ activates CD3+ T cells and induces their expression of PD-L1. Unseparated PBMCs were cultured for 3 days with IL-32-α or IL-32-γ (100 ng/ml). CD3+ gated events were assessed for expression of PD-L1 or CD69, an early T cell activation marker, using flow cytometry. Percent positive cells and mean fluorescence intensity (parentheses) are indicated. Results are representative of 2 donors tested.