

Figure S1. Validation of TRAILshort antibody

(A) HEK293T cells were transfected with plasmids encoding either HA-tag, HA-tagged TRAILshort or HA-tagged TRAIL_{FL}, lysed, separated on SDS-PAGE, blotted, then probed with anti-HA (top panel). Blots were stripped and reprobed with an anti-TRAILshort monoclonal antibody (middle panel). Probing with anti-actin confirmed equal loading of lysates (bottom pane). (B) HEK293T cells were transfected with plasmids encoding GFP, GFP-TRAILshort, or GFP-TRAILFL, stained with anti TRAILshort antibody (and secondary goat anti-mouse PE), and DAPI. Cells were analyzed by confocal microscopy for PE label (red), GFP (green) and DAPI stained nuclei (blue), and images were superimposed (merge). (C) Sequential flow cytometry sorting of whole blood PBMCs and subsequent flow cytometric analysis for expression of TRAILshort in T-cell subpopulations, monocytes and B-cells. Whole blood PBMCs (upper left corner panel) were sorted by gating as indicated for mid levels of side-scatter (SSC) and forward scatter (FSC). Then as blue down arrow indicates, these were further sorted into quadrants according to their CD3 and CD56 expression (staining with anti-CD3 and CD56 antibodies): CD3_{low}/CD56_{low}; CD3_{high}/CD56_{low}; CD3_{high}/CD56_{high}; CD3_{high}/CD53_{low}. Then as indicated by subsequent blue arrows, each of these quadrants were resorted after gating on double staining with pertinent pairs of anti-T cell (CD4, CD8, CD3), anti-NK cell (CD56), anti-monocyte (CD14) or anti-B cell (CD20) markers, and analyzed to confirm their separation into CD4+ T cells, CD8+ T cells, NK cells, monocytes and B-cells. Finally, the expression of TRAILshort in each purified subpopulation of cells was confirmed after staining with labeled anti-TRAILshort antibody with an Ig3a isotype matched mAb control. The numbers in each upper right hand quadrant of indicates the relative percentages of cells expressing TRAILshort.

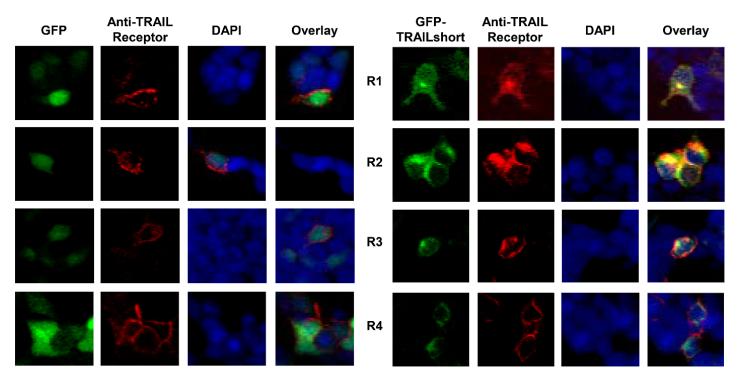


Figure S2. TRAILshort colocalizes with TRAIL R1 and TRAIL R2, and less so with TRAIL R3 and TRAIL R4

293T cells were transfected with GFP (left) or TRAILshort (right) and either TRAIL R1, R2, R3 or R4. After transfection, cells were surfaced stained with the appropriate anti-TRAIL receptor antibody (conjugated to AlexaFluor 594) and colocalization determined by confocal microscopy.