Suppl. Fig. 1: Cytokine profile of TGN1412-treated T cells on day 5. 1x10^6 or 2x10^5 freshly isolated human T cells per 24-well or 96-well were left unstimulated, stimulated with 1 μg/ml TGN1412 or 1 μg/ml TGN1412 followed by crosslinking with 2 μg/ml anti-IgG monoclonal antibody (left panel). 8x10^4 HUVECs per 24-well were irradiated and cultured unstimulated for 5 days. Alternatively, HUVECs were stimulated with 200 U/ml TNF-α + 100 U/ml IFN-γ for 3 days. After washing HUVECs with PBS, 1x10^6 freshly isolated human T cells were added to HUVECs and left unstimulated or stimulated with 1 μg/ml TGN1412 (right panel). At day 5 after the indicated stimulations, cell-free supernatant was collected and analyzed for TNF-α, TNF-β, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and IL-12 p70 by FlowCytomix Th1/Th2 11plex analysis. Data shown are from 7-22 independent T cell donors. #, data points are in the saturation area of the assay.
Suppl. Fig. 2: Blocking IL-2 in co-cultures of TGN1412-treated T cells with HUVECs inhibits T cell proliferation. 8x10^4 HUVECs were irradiated and cultured either unstimulated or pre-stimulated with 200 U/ml TNF-α + 100 U/ml IFN-γ for 3 days. After washing HUVECs with PBS, 1x10^6 freshly prepared and PKH26-labeled human T cells were added to HUVECs, treated with 1 µg/ml anti-IL-2 blocking antibody, and stimulated with 1 µg/ml TGN1412 (dotted line). As controls, T cells were co-cultivated with HUVECs without TGN1412 (gray-shaded curves) or additionally treated with TGN1412 without blocking IL-2 (solid line). At day 5 of co-cultivation, PKH26-labeled T cells were harvested, stained with an anti-CD3 antibody, and T cell proliferative responses were measured by flow cytometric analysis. Data shown are representative for 4 different T cell donors.