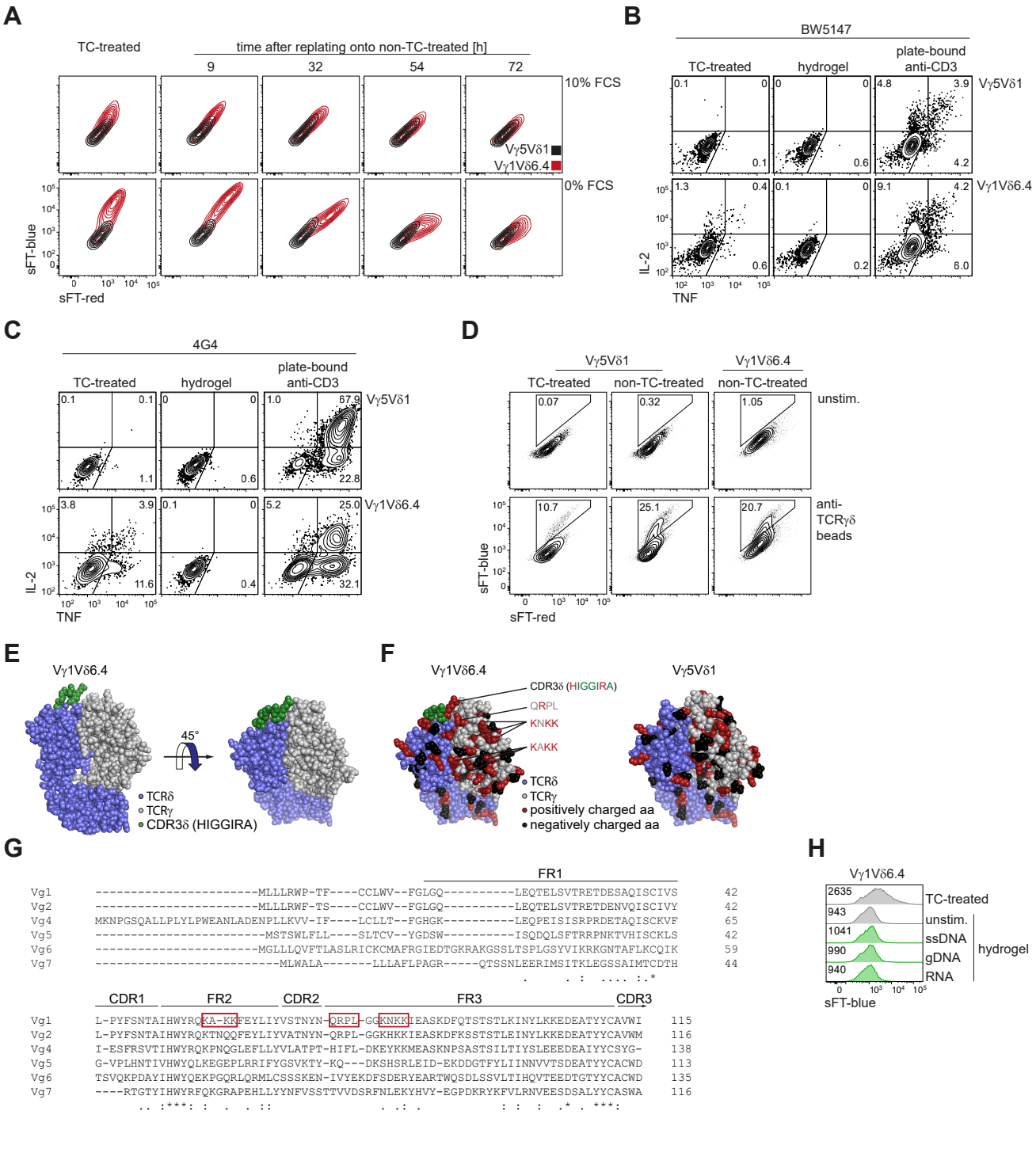
Dunst *et al.*, Supplementary Figure 1



Supplementary figure 1. Recognition of synthetic polyanionic ligands underlies 'spontaneous' reactivity of Vγ1 γδTCRs.
(**A**) Flow cytometric analysis of sFT-blue and sFT-red expression by Vγ5Vδ1- and Vγ1Vδ6.4-expressing reporter cells after overnight culture on TC-treated culture surface and replating onto non-stimulating (non-TC-treated) surface at indicated time points; cells were cultured in complete medium (10% FCS) or under serum starvation (0% FCS). (**B-C**) Analysis of intracellular cytokines IL-2 and TNF in Vγ5Vδ1- and Vγ1Vδ6.4-expressing BW5147 (**B**) and 4G4 cells (**C**) after 8h of culture on the indicated culture surfaces in the presence of BrefeldinA. (**D**) Flow cytometric analysis of sFT-blue and sFT-red expression by the reporter cells transduced with indicated TCRs upon stimulation with anti-TCRγδ-coated beads or non-coated beads (unstimulated) on negatively charged (TC-treated) or non-charged (non-TC-treated) cell culture surfaces. (**E**) Visualization of Vγ1Vδ6.4 structure modeled by SWISS model in two indicated projections. (**F**) Visualization of the distribution of positively and negatively charged amino acids on models of Vγ1Vδ6.4 (left) and Vγ5Vδ1 (right) TCRs. (**G**) Alignment of all functional murine Vγ segments, framework regions (FR) and complementarity determining regions (CDR) are indicated and clusters of positively charged amino acids within Vγ1 are highlighted (boxes). (**H**) Flow cytometric analysis of sFT-blue signal in Vγ1Vδ6.4-expressing reporter cells upon stimulation with ssDNA (15μM), genomic DNA (gDNA, 15μg/m), or RNA (15μg/ml).