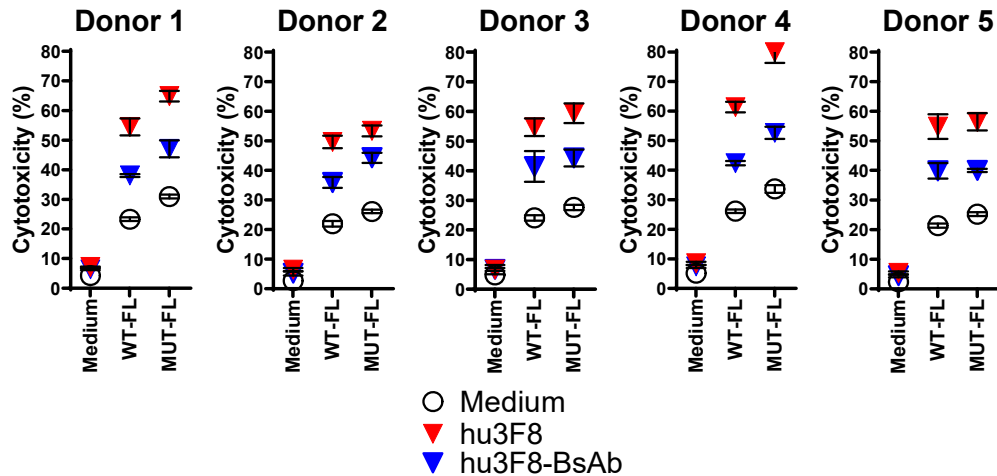
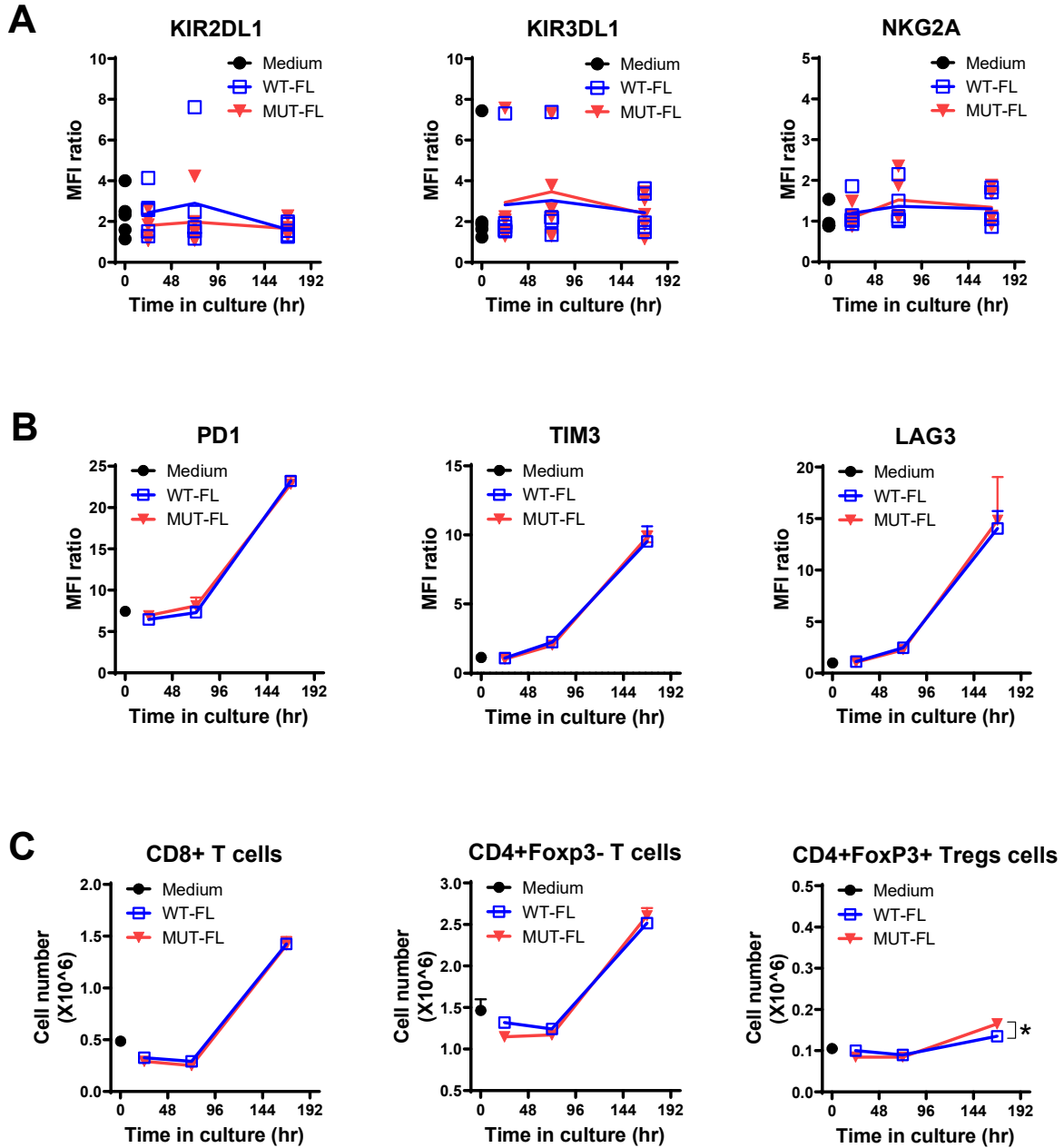


Supplemental Figure S1



Supplemental Figure S1. Comparison of cytotoxicity by WT-FL vs MUT-FL stimulated PBMCs obtained from different donors. PBMCs from five healthy donors were cultured *in vitro* in medium either without (Medium) or with 1 nM WT-FL or MUT-FL complex. After 72 hrs of culture, the PBMCs were harvested, re-adjusted in numbers and tested at 5:1 E:T ratio in an *in vitro* cytotoxicity assay against M14 cells, either in the absence (Medium) or presence of 0.01 ug/mL hu3F8 or hu3F8-BsAb. Results are presented as percent of tumor cell lysis (Mean \pm SEM, n=3).

Supplemental Figure S2



Supplemental Figure S2. Effect of WT-FL vs. MUT-FL stimulation on expression of inhibitory markers on NK cells or T cells. (A) Surface expression of inhibitory markers on NK cells (KIR2DL1, KIR3DL1 and NKG2A). PBMCs from healthy donors (n=5) were cultured *in vitro* in medium either without (Medium) or with 1 nM WT-FL or MUT-FL complex. At 24, 72 and 168 hr cells were tested by flow cytometry for expression of different surface markers. Analysis is based on gating on T cells (CD3⁺CD56⁻ lymphocytes) or NK cells (CD3⁻CD56⁺ lymphocytes). Results are presented as geo-MFI ratio of the marker of interest, individual for each donor. Lines represent Mean (n=5) of those 5 individual values. (B) Surface expression of inhibitory/exhaustion markers on T cells (PD1, TIM3 and LAG3). Same condition as in panel (A) except one healthy donor was used in this case, with each data point was done in duplicates (Mean + SD, n=2). (C) Tregs proliferation assay. Same condition as in panel (A) except one healthy donor was used in this case, with each data point was done in duplicates (Mean + SD, n=2). Cell numbers were calculated by multiplying the total live cell count in each well (8×10^6 cells per well at time 0) and percentage of each gated population from FACS. * $p < 0.01$ when WT-FL treatment groups were compared with MUT-FL treatment groups at indicated time point, respectively.