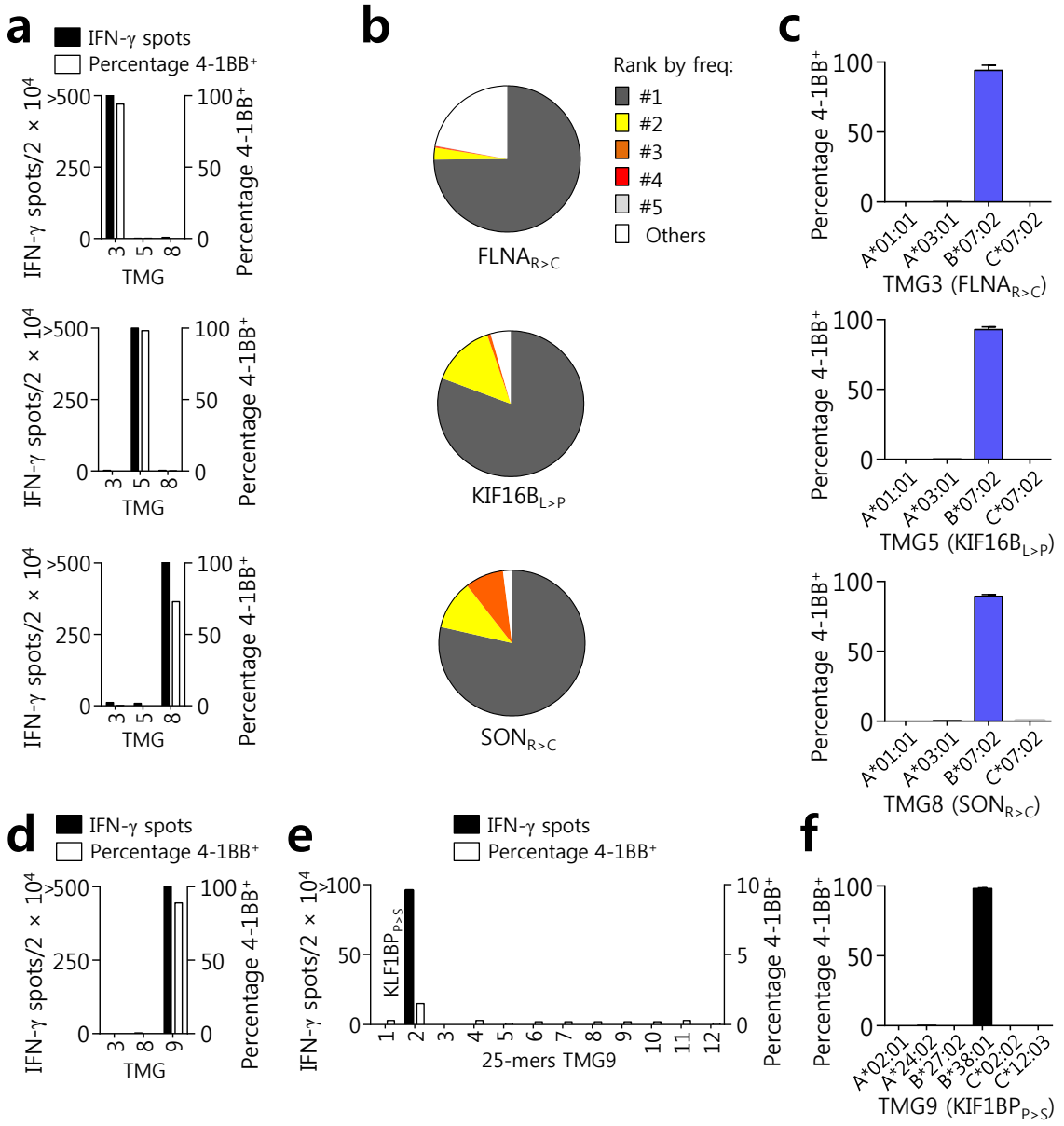

Supplementary Figure 3 Characterization of neoantigen-reactive lymphocytes isolated from the circulating $CD8^+PD1^+$ subset of subjects NCI-3784, and 3903. Enriched populations of TMG-reactive cells were generated by selecting $4\text{-}1BB^+$ lymphocytes and expanding the cells *in vitro* for 15 d. (**a–c**) NCI-3784 TMG3- (top), TMG5- (middle), and TMG8-reactive (bottom) lymphocytes, recognizing $FLNA_{R>C}$, $KIF16B_{L>P}$, or $SON_{R>C}$, respectively, were cocultured with DCs transfected with the indicated TMGs (**a**). IFN-γ ELISPOT assay and flow cytometry detection of 4-1BB upregulation at 20 h are shown. Data are gated on $CD3^+CD8^+$ cells. (**b**) TRB deep sequencing of the TMG-enriched populations. Data show the frequency of the top five TRB clonotypes (unique V-D-J sequences) for each population. (**c**) TMG3-, TMG5- and TMG8-reactive lymphocytes were co-incubated with COS7 cotransfected with the corresponding constructs expressing the indicated TMG and individual *HLA* alleles encoding the HLA class I molecules indicated on the *x* axis (such as HLA-A*01:01). Flow cytometry detection of 4-1BB at 20 h after the coculture is shown. Data are gated on $CD3^+CD8^+$. (**d–f**) NCI-3903 TMG9-reactive cells were cocultured with DCs transfected with the indicated TMGs (**d**), DCs pulsed with the individual 25-mers encoded by 3903 TMG9 (**e**), or COS7 cells co-transfected with TMG9 and DNA constructs encoding for the HLA-I molecules indicated in the x axis (**f**). IFN-γ ELISPOT assay and or flow cytometry detection of 4-1BB up-regulation are shown. Data are gated on $CD3^+CD8^+$ cells. "$>$" denotes greater than 500 spots /2 × 10^4 cells. Data are representative of at least two independent experiments.

Nature Medicine: doi:10.1038/nm.4051