Figure Legends for Supplemental Figures

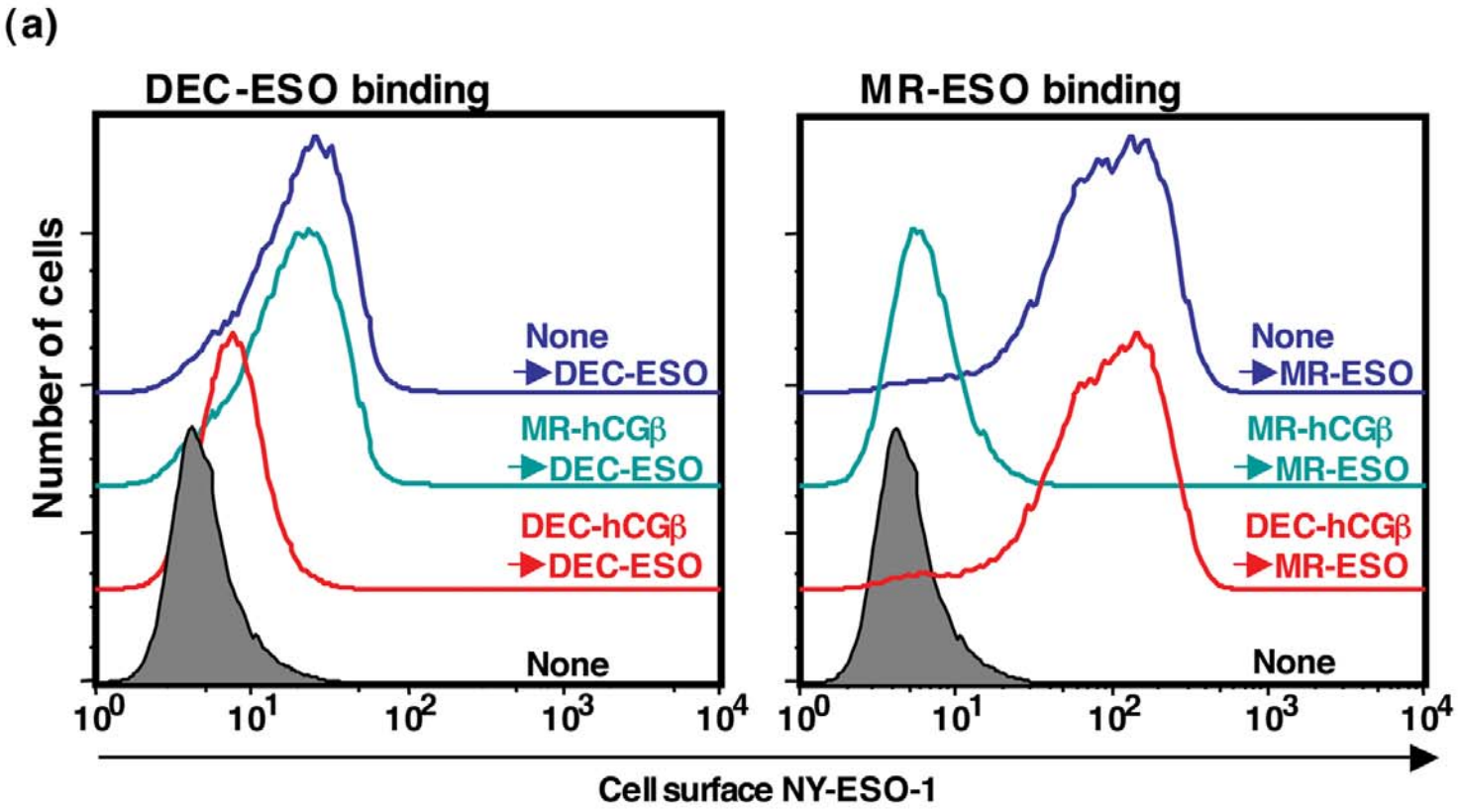
Fig. S1. Blocking of targeting NY-ESO-1 protein binding to the cell surface of mo-DC by competitive antibody. (a) Binding of DEC-ESO and MR-ESO on mo-DC was investigated by flowcytometry. Mo-DC were preincubated with or without 10 μg/ml DEC-hCGβ or MR-hCGβ on ice for 30 minutes and then incubated with 4 μg/ml DEC-ESO (left) or MR-ESO (right). After extensive washes, NY-ESO-1 protein bound on mo-DC was detected by anti-NY-ESO-1 mAb and PE-conjugated anti-mouse IgG1 mAb. Similar results were obtained in mo-DCs from two donors. (b) Inhibition of cross-presentation of DEC-ESO and MR-ESO by blocking their binding to the cell surface molecules. Mo-DCs were preincubated with or without 100 μg/ml DEC-hCGβ or MR-hCGβ on ice for 30 minutes and then incubated with 4 μg/ml DEC-ESO or MR-ESO on ice for 15 minutes. After extensive washes, cells were incubated overnight at 37˚C to allow the antigen processing and used as target cells in ELISPOT assay. Experiments were repeated twice and the result from one experiment was shown as mean + SD from duplicate samples. *, $p<0.05$.

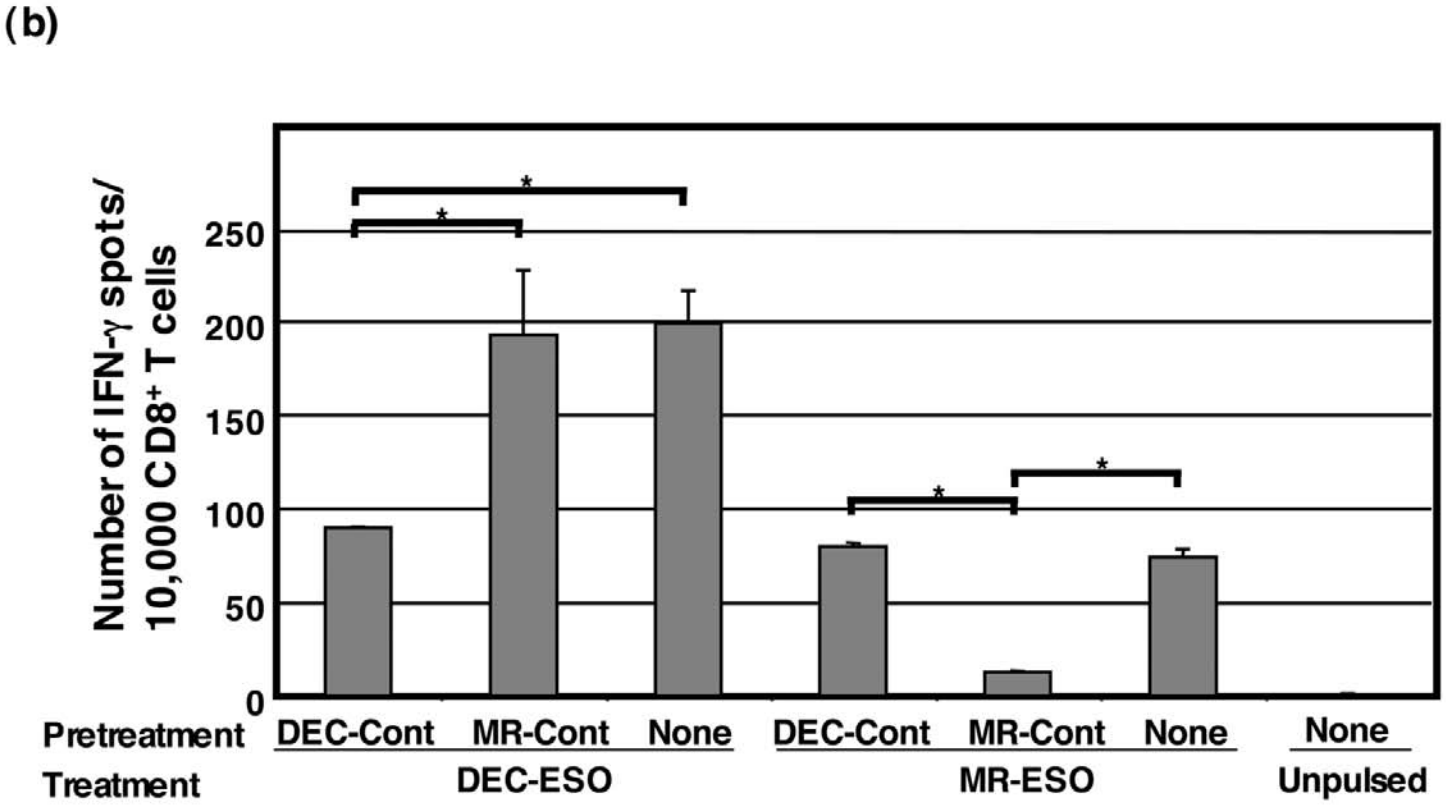
Fig. S2. Absence of stimulatory/costimulatory effect of targeted NY-ESO-1 proteins on monocyte-derived dendritic cells (mo-DCs). Mo-DCs were prepared as described in Materials and Methods. At day 6, mo-DCs were incubated with indicated targeted NY-ESO-1 fusion protein (DEC-ESO or MR-ESO) or recombinant NY-ESO-1 protein for 30 minutes before adding indicated toll like receptor ligands. After 24 hours, cells were harvested and stained with PE-conjugated anti-CD83 and FITC-conjugated anti-CD86 mAbs. Fluorescent intensity was measured and analyzed by a FACSCalibur instrument and CellQuest software, respectively.

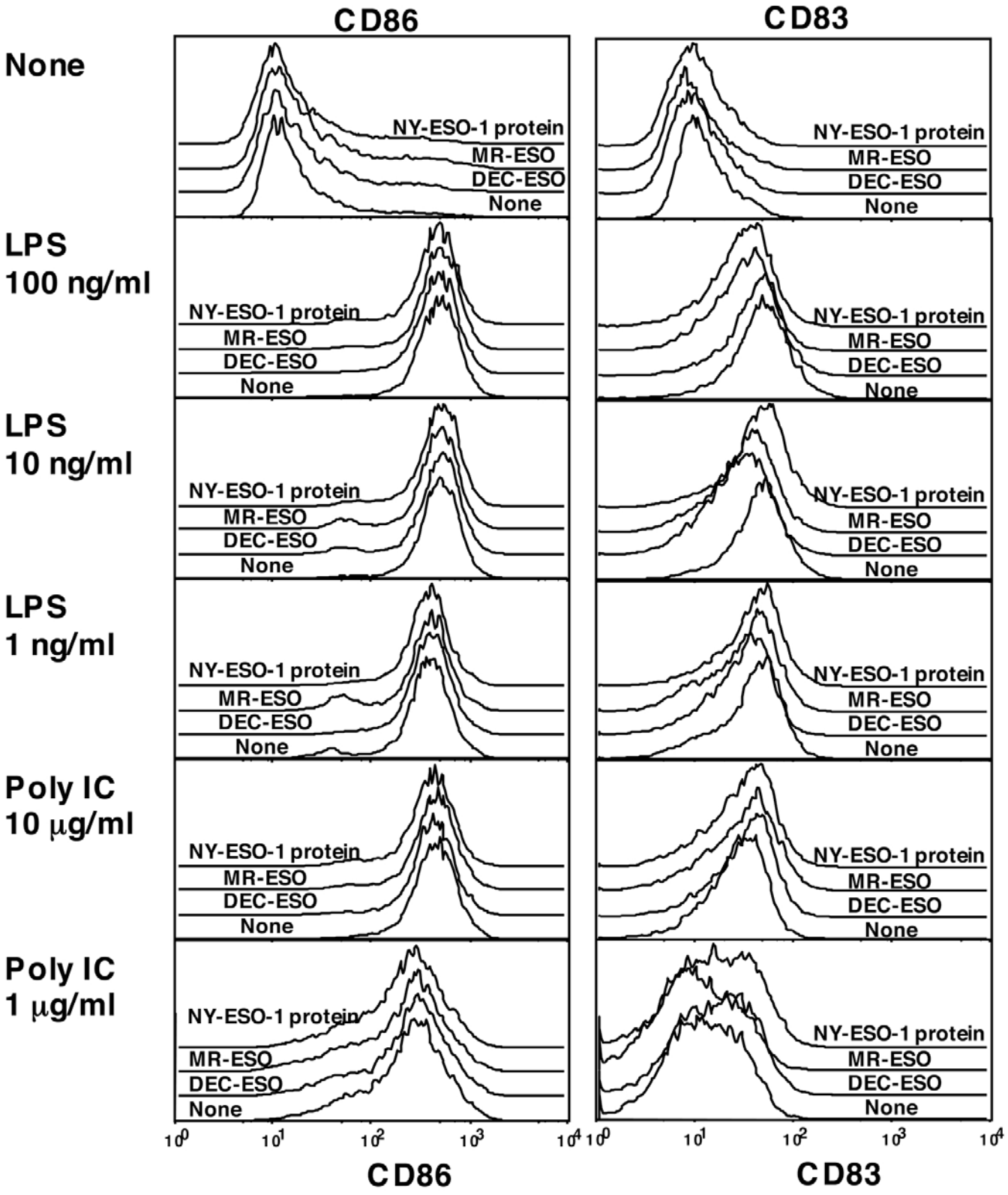
Fig. S3. Absence of stimulatory/costimulatory effect of targeted NY-ESO-1 proteins on monocyte-derived dendritic cells (mo-DCs). Mo-DCs were prepared and stimulated as in Fig. S1. After 24 hours, supernatant was harvested and cytokine levels in the supernatant were measured by enzyme-linked immunosorbent assay (ELISA). The limit of detection in these assays was 78 pg/ml for IL-12, 9.4 pg/ml for IL-10, and 390 pg/ml for TNF. Experiments

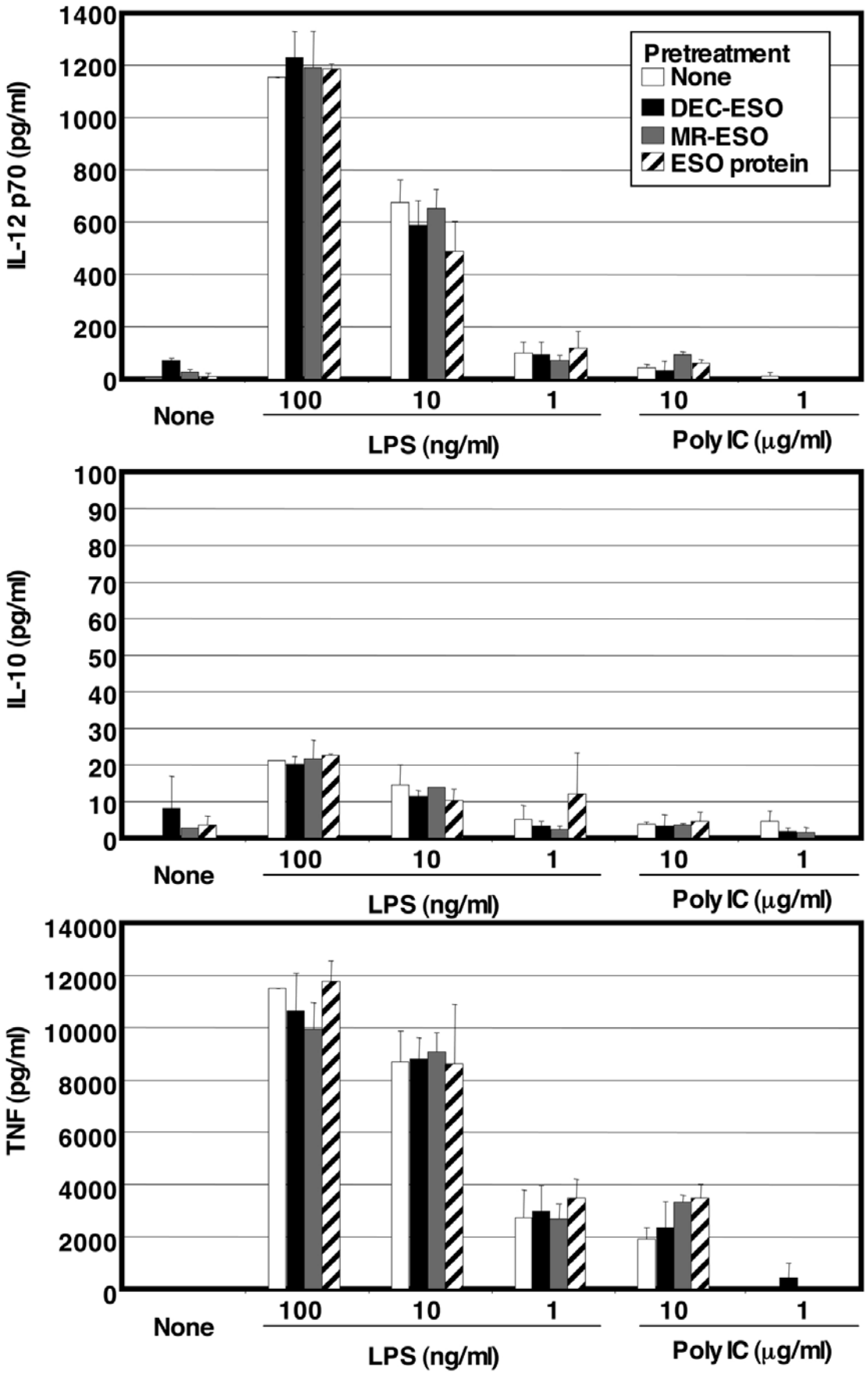
were performed using mo-DCs from two different healthy donors. The results were shown as mean + SD from duplicate samples.

Fig. S4. Characterization of NY-ESO-1-specific $CD8^+$ T cell responses from patient AA. $CD8^+$ T cells from patient AA were presensitized and NY-ESO-1-specific $CD8^+$ T cell line was generated as described in Materials and Methods. (A-B) Peptide specificity of NY-ESO-1-specific $CD8^+$ T cell lines was evaluated using autologous EBV-B cells as APCs by ELISPOT assay. (C) Avidity of $CD8^+$ T cell line against short peptides was evaluated by ELISPOT assay using autologous EBV-B cells pulsed with an indicated concentration of short peptides. (D) HLA-restriction of NY-ESO-$1_{101\text{-}109}$ and NY-ESO-$1_{102\text{-}110}$ was determined as HLA-B*4002 by HLA-compatible allogeneic EBV-B cells. (E) Tumor recognition of HLA-B*4002-restricted and NY-ESO-$1_{101\text{-}109}$-specific $CD8^+$ T cell line. SK-MEL-52: NY-ESO-1^+HLA-B*4002^+; SK-MEL-139: NY-ESO-1^+HLA-$B4002^-$; SK-MEL-149: NY-ESO-1^-HLA-B*4002^+; SK-MEL-37: NY-ESO-1^+HLA-$B4002^-$.

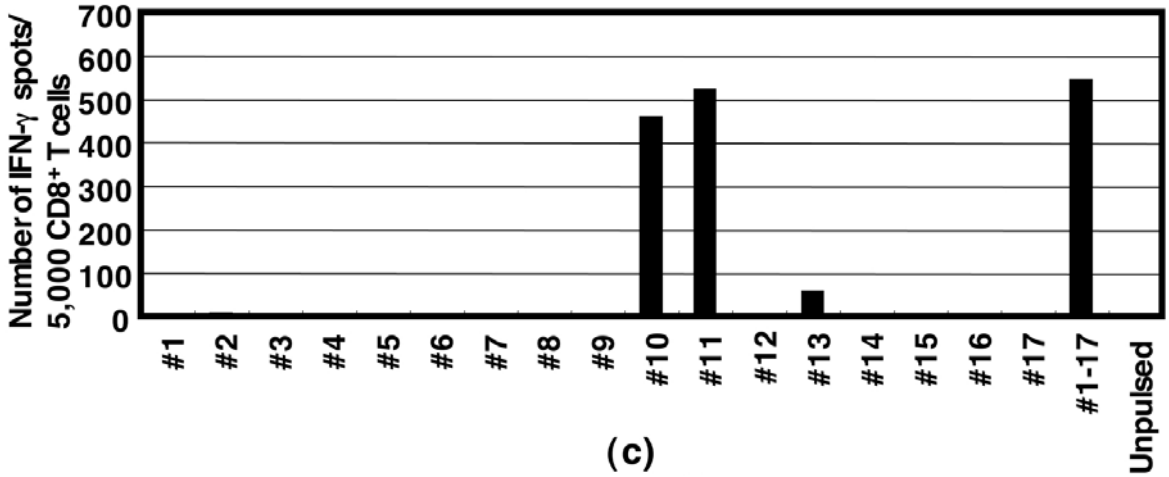








(a)



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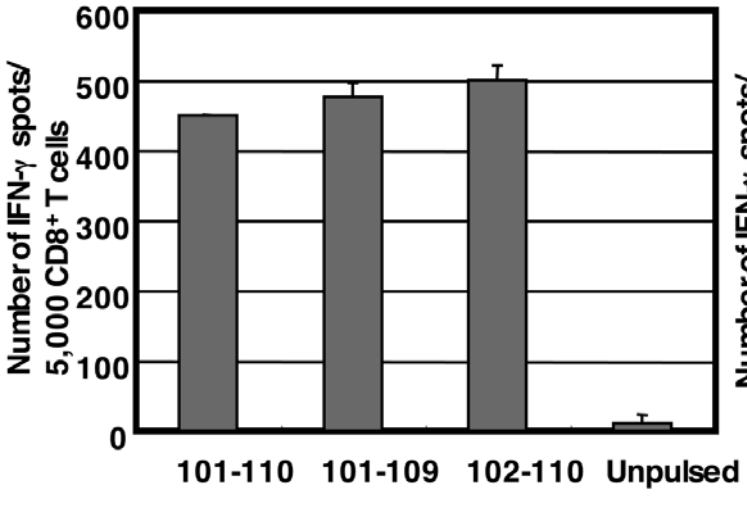

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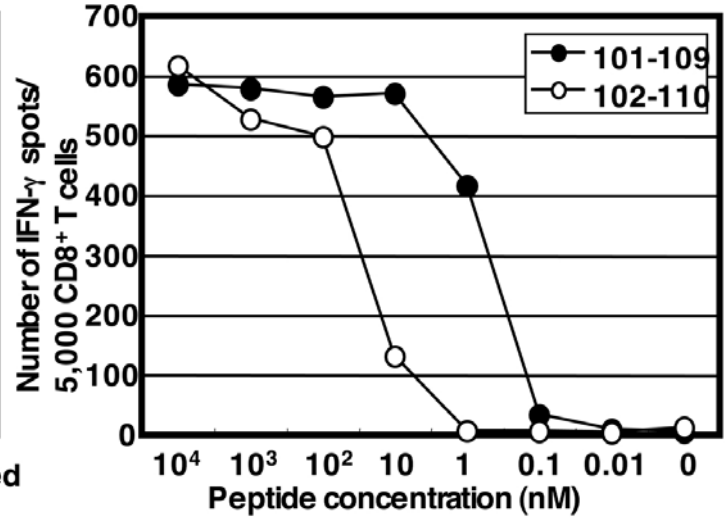
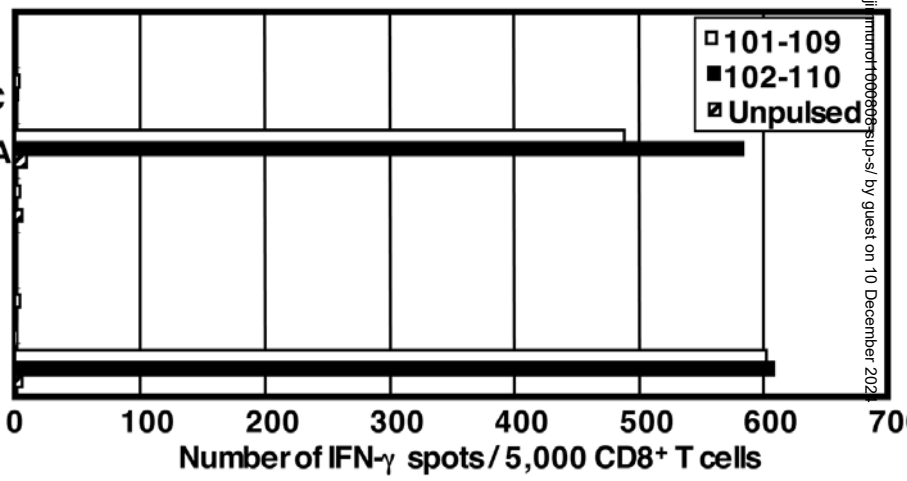


(d)

	HLA-A	HLA-B	HLA-C
NC116	2301 2402	4201 3906	17XX 0702
NC214	26JV 3201	40CCP 5001	0304 06BC
NC158	0301 31AB	3512 4002	0305 04APA
CB	0201 02XX	1801 5101	0202 1202
NC122	1101 2601	3503 3801	1203
NC136	2402	3801 1501	12NP 0304
AA	2402 3201	4002 5101	1203 0202



(e)