

**Mechanisms underlying the lack of endogenous processing and CLIP-mediated binding of the invariant chain by HLA-DP<sup>84Gly</sup>**

Mark Anczurowski<sup>1,2</sup>, Yuki Yamashita<sup>1</sup>, Munehide Nakatsugawa<sup>1</sup>, Toshiki Ochi<sup>1</sup>, Yuki Kagoya<sup>1</sup>, Tingxi Guo<sup>1,2</sup>, Chung-Hsi Wang<sup>1,2</sup>, Muhammed A. Rahman<sup>1</sup>, Kayoko Saso<sup>1</sup>, Marcus O. Butler<sup>1,2,3</sup>, Naoto Hirano<sup>\*1,2</sup>

<sup>1</sup>Tumor Immunotherapy Program, Campbell Family Institute for Breast Cancer Research, Campbell Family Cancer Research Institute, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada M5G 2M9 <sup>2</sup>Department of Immunology, University of Toronto, Toronto, Ontario, Canada M5S 1A8; <sup>3</sup>Department of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A8

\*Correspondence should be addressed to N.H.

**Contact Information**

Correspondence and requests for materials should be addressed to N.H. ([naoto.hirano@uhnresearch.ca](mailto:naoto.hirano@uhnresearch.ca)).

Naoto Hirano, MD, PhD

Princess Margaret Cancer Centre

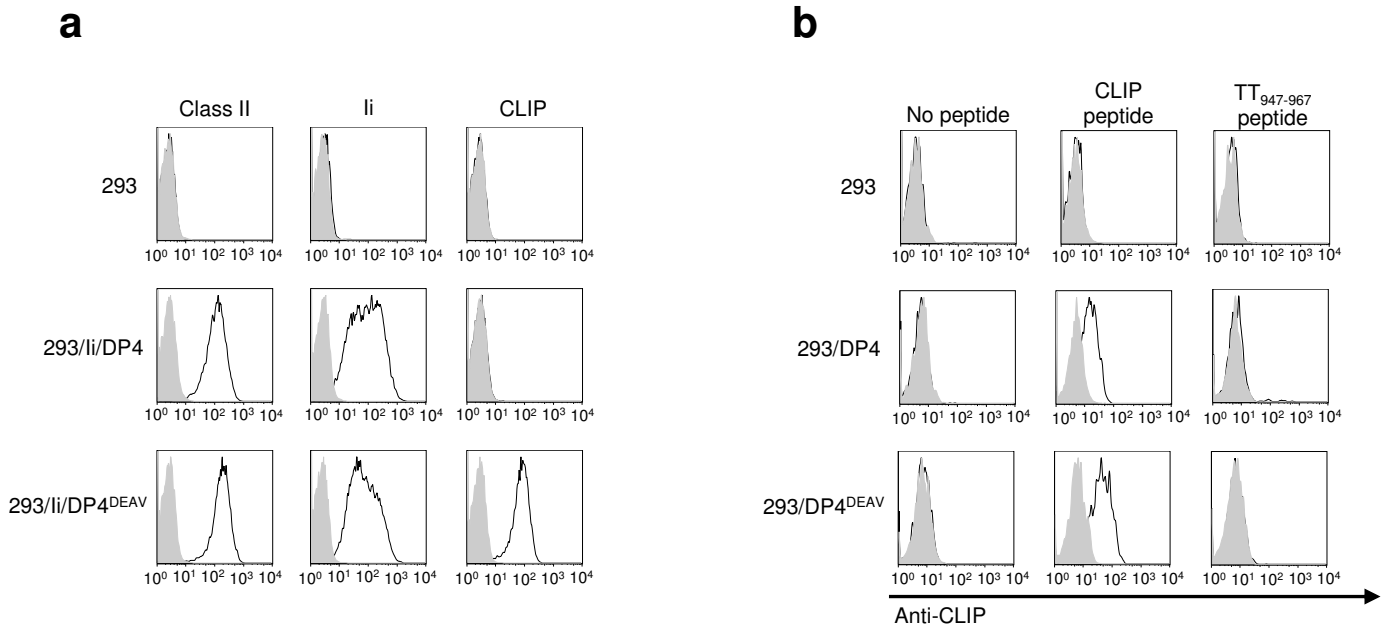
610 University Avenue, Toronto, ON M5G 2M9, Canada

Phone: (416) 946-2190

Fax: (416) 946-6529

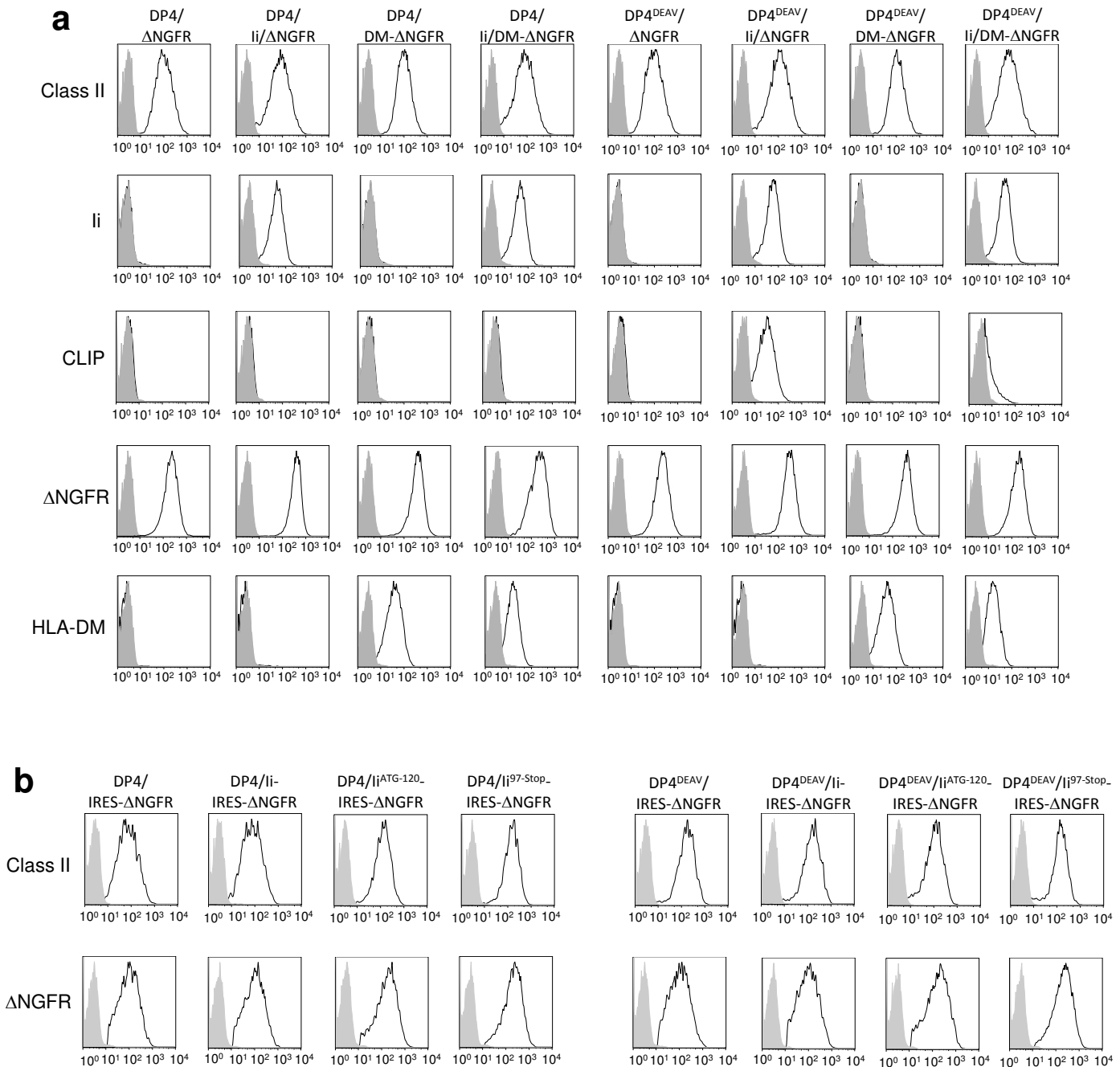
E-mail: [naoto.hirano@uhnresearch.ca](mailto:naoto.hirano@uhnresearch.ca)

# Supplementary Figure 1



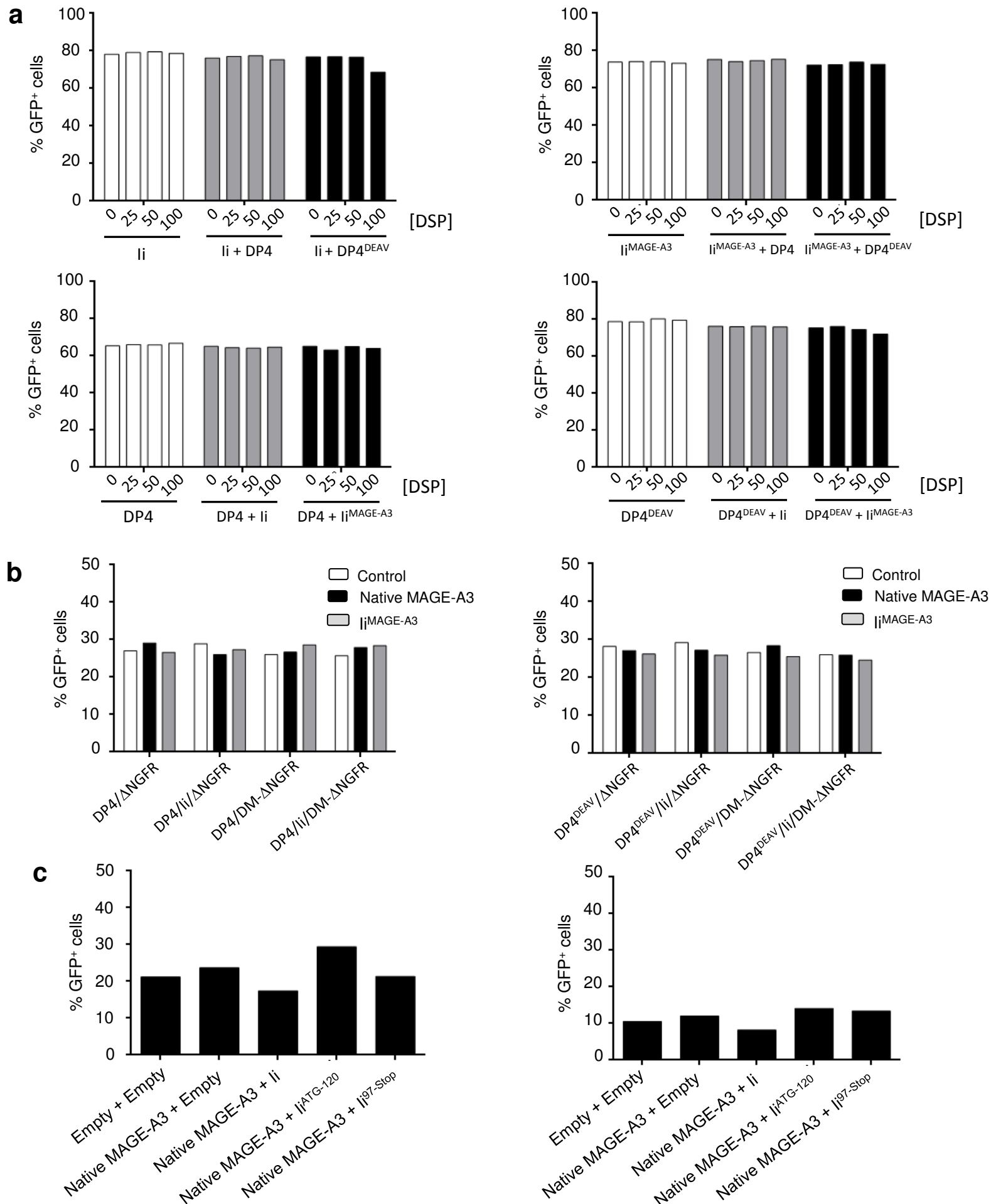
**Supplementary Figure 1:** CLIP derived from endogenous li is presented by HLA-DP4<sup>84DEAV87</sup>, but not DP4, yet can associate with both DP4 and DP4<sup>84DEAV87</sup>-expressing HEK293 cells when exogenously pulsed to the cell surface as a synthetic peptide. **(a)** Surface class II and CLIP expression, along with intracellular li expression, were analyzed on HEK293 transfectants by flow cytometry following staining with specific mAbs. HEK293 cells were retrovirally transduced with *DPA1\*01:03* (DPA1) and either wild-type or mutated *DPB1\*0401* (DPB4) with substitution mutations of the 84-87 region of the DPβ chain made as indicated. **(b)** Parental HEK293 or HEK293 cells stably expressing DP4 or DP4<sup>84DEAV87</sup> were pulsed with 100 μM of CLIP, TT<sub>947-967</sub>, or an equivalent volume of DMSO for 18 hrs. Surface CLIP expression was then analyzed by flow cytometry on the indicated HEK293 transfected cells following staining with CLIP-specific mAb.

## Supplementary Figure 2



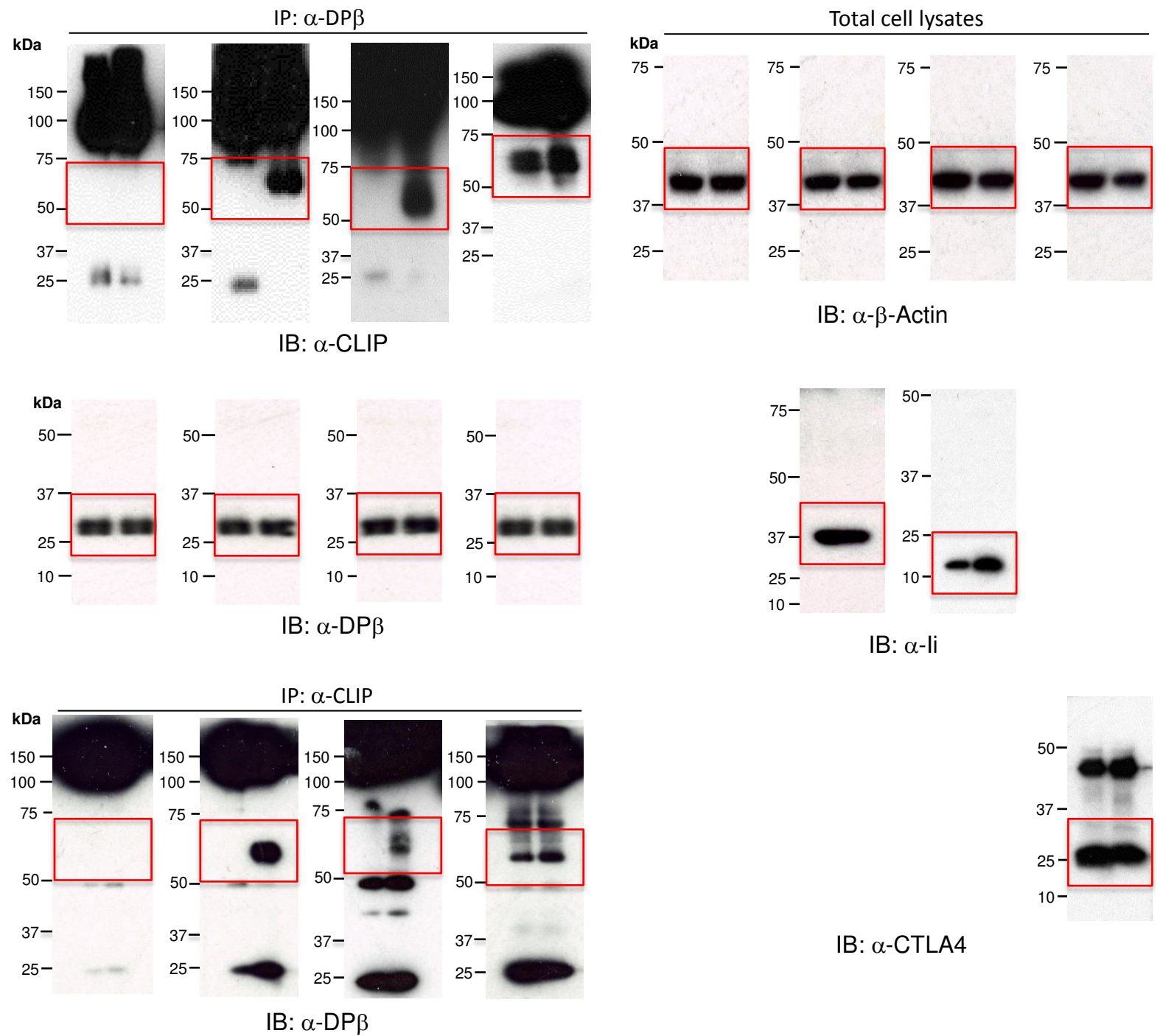
**Supplementary Figure 2:** Expression data for cells used in T cell activation (**Fig. 4**) (**a**) and flow cytometry experiments (**Fig. 5d**) (**b**). Surface class II, CLIP and  $\Delta$ NGFR expression, along with intracellular li and HLA-DM expression as indicated, were analyzed on the indicated K562 transfectants by flow cytometry following staining with specific mAbs. K562 cells were retrovirally transduced with *DPA1\*01:03* (*DPA1*) and either wild type or mutated *DPB1\*04:01* (*DPB4*) with substitution mutations of the 84-87 region of the *DP $\beta$*  chain made as indicated to generate *DP4* or *DP4*<sup>84<sup>DEAV</sup>87</sup> respectively.

# Supplementary Figure 3



**Supplementary Figure 3:** Transfection efficiencies for cross-linked cells lysed in Figure 3b and 3c (a), as well as for stimulator cells used in ELISPOT analysis in Figure 4 (b) and Figure 6 (c). Transduction efficiencies are measured by GFP expression in cells by flow cytometry.

## Supplementary Figure 4



**Supplementary Figure 4:** Uncropped western blotting images from Figure 5c. Outlined regions represent portion of each image displayed in the main figures.