SUPPLEMENTARY FIGURES

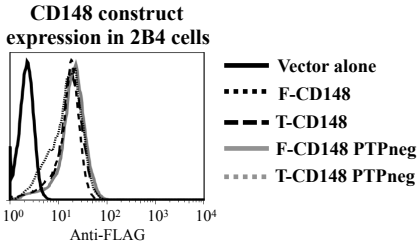

FIGURE S1

Expression levels of CD148 constructs in 2B4 cells. 2B4 T-cells expressing the indicated CD148 constructs were stained with anti-FLAG antibody. Overlayed histogram plots of anti-FLAG staining is shown.

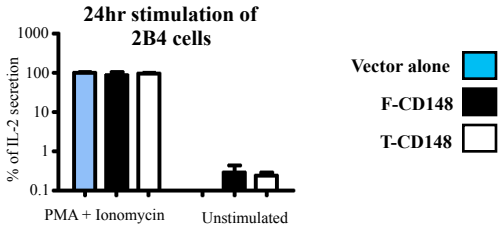

FIGURE S2

PMA and Ionomycin stimulation produces equal responses in sorted 2B4 cells. The indicated 2B4 cells were stimulated with 50 ng/mL PMA and 670 nM Ionomycin for 24 hrs and IL-2 secretion measured. Error bars represent the SD of the mean from 3 replicates. Data were normalised to vector transduced controls.

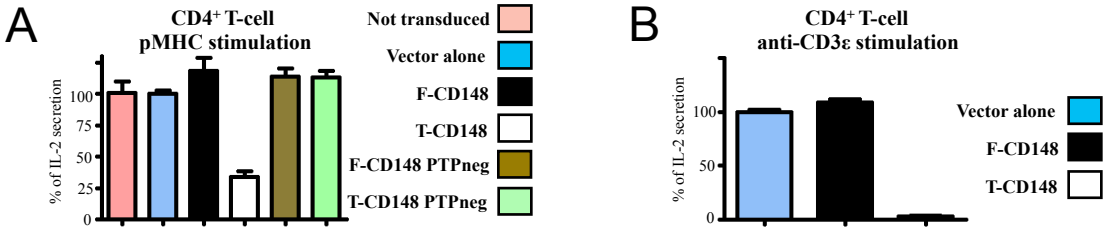

FIGURE S3

Expression of truncated CD148 lacking the C-terminal GFP tag abrogates IL-2 secretion in T cells. Control (untransduced or vector transduced) 2B4 T cells or 2B4 T cells expressing the indicated CD148 construct (Fig. 1A) without a C-terminal GFP tag were stimulated with either CHO cells expressing I-E^k presenting the cognate MCC peptide (A) or plate-immobilised anti-mouse CD3ε (B) and IL-2 secretion analysed after 14-18 hrs. Error bars represent the SD of the mean from at least 3 replicates. Data were normalised to vector transduced controls.

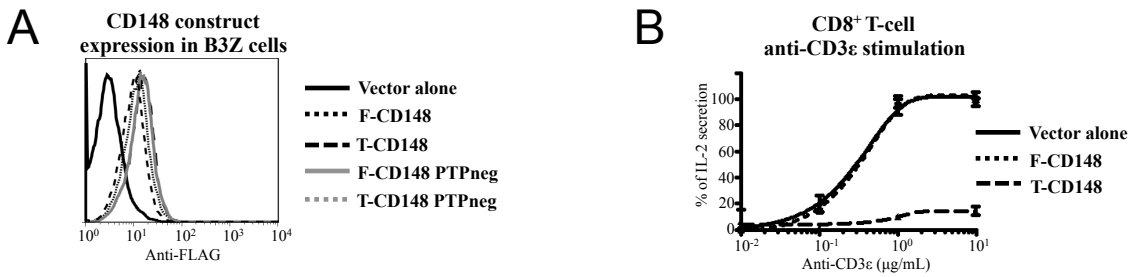

FIGURE S4

Expression levels of CD148 constructs in B3Z cells. (A) B3Z T-cells expressing the indicated CD148 constructs were stained with anti-FLAG antibody. Overlayed histogram plots of anti-FLAG staining is shown. (B) IL-2 secretion analysed after 14-18 hrs stimulation on plate-immobilised anti-mouse CD3ε. Error bars represent the SD of the mean from at least 3 replicates. Data were normalised to vector transduced controls.

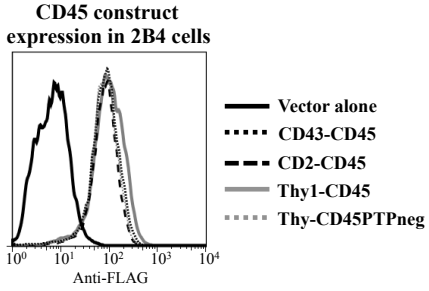

FIGURE S5

Expression levels of CD45 constructs in 2B4 cells. 2B4 T-cells expressing the indicated CD45 constructs were stained with anti-FLAG antibody. Overlayed histogram plots of anti-FLAG staining is shown.

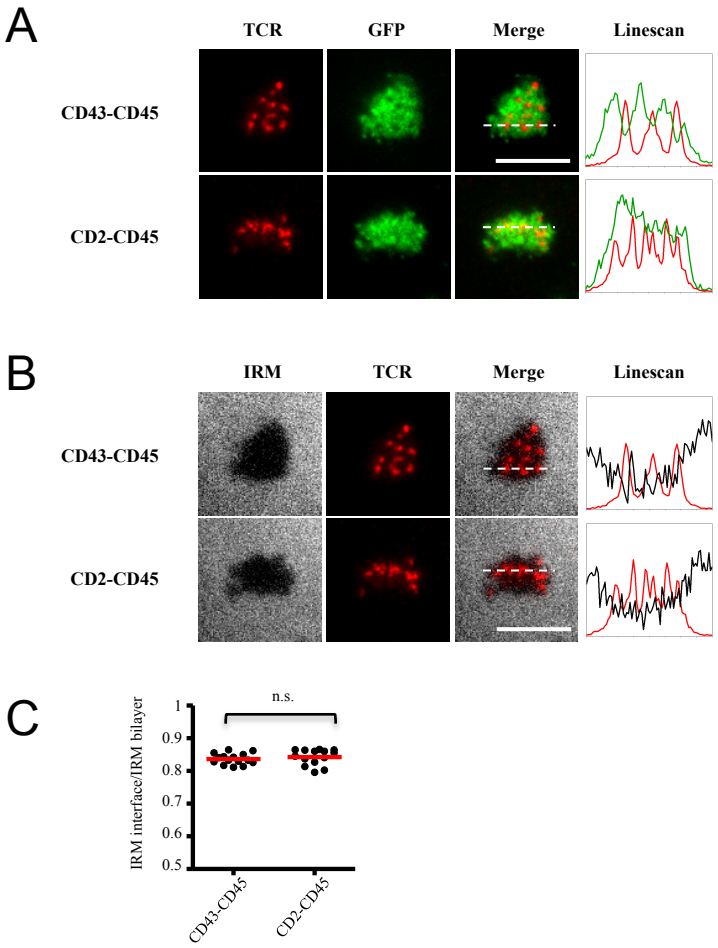

FIGURE S6

T cells expressing CD45 phosphatase with a long or short ectodomain form comparable contacts with planar bilayers. T cells expressing CD45-GFP chimeras with a long (CD43-CD45) or short (CD2-CD45) ectodomain were imaged by TIRFM within 5 minutes of contact with planar bilayers containing anti-CD3ε antibody 2C11 and ICAM-1. TCR was detected using fluorescently labelled anti-TCRβ antibody (H57) Fab' fragments. (A) Right image panels show overlays of GFP and TCR TIRFM fluorescence at contact interfaces of T cells expressing CD45 with large (CD43-CD45) or small (CD2-CD45) ectodomains. Right panels show TCR (red) and GFP (green) fluorescence intensity profiles along dashed white line in right left panels. (B) IRM, interference reflection (microscopy) image of the cells in FigS6A. Darker regions represent closer contact of cells with supported planar bilayers. Right image panels shows overlays of TCR fluorescence, imaged by TIRF, and reflection images of the contact interface. Lines scans represent TCR (red) and reflection gray-value along dashed white lines. (C) Comparison of contact proximity (calculated as a ratio of the mean gray value for interfaces divided by that of an adjacent non-interface region) between cells expressing CD43-CD45 (N = 15) and CD2-CD45 (N= 14). Dots represent individual interfaces, red bars represent means, n.s. t-test $P > 0.05$. Scale bar, 10 μm.

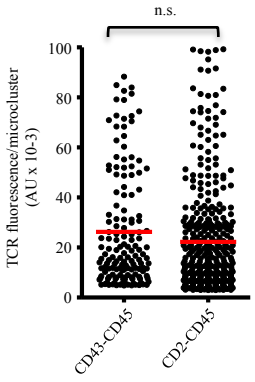

FIGURE S7

Binding-induced TCR clustering is similar in T cells expressing CD45 phosphatase with a long or short ectodomains. Total (integrated) fluorescence of individual TCR microclusters at contact interfaces of cells expressing CD43-CD45 (N = 149 from 8 cells) and CD2-CD45 (N = 381 from 15 cells), detected using fluorescently labelled anti-TCRβ antibody (H57) Fab' fragments was measured by applying a threshold of +2S.D. above the non-interface value in background-subtracted TCR channels. Dots represent individual microclusters and red bars represent mean fluorescence. n.s. $P > 0.05$ for comparison of means (t-test) and variance (F-test).