

Supplementary Figure 1. Tungatt et al.

Supplementary Figure 1: Ex vivo staining and detection of T-cells is improved by the addition of an anti-fluorochrome and conjugated secondary antibody to standard pMHC multimer staining protocols. (A) TILs from a HLA-A2⁺ metastatic melanoma patient were left unstained or stained with HLA-A2-ELAGIGILTV (from Melan-A) PE conjugated tetramers ± an anti-PE unconjugated 1° Ab ± a PE conjugated 2° Ab. These TILs did not stain with irrelevant tetramer (e.g. Figure 5). (B) Peripheral blood mononuclear cells (PBMCs) from a healthy donor were stained ex vivo with tetramers or dextramers (HLA-A2-ELAGIGILTV) and Abs as in A. Samples were minimally stained for viability, CD3 and CD8, with CD14 and CD19 also being stained in B. The tetramer⁺ cells are expressed as a percentage of total cells (A) or CD8⁺ cells (B) and the mean fluorescence intensity shown inset. Cells were pre-treated with PKI throughout.