**Supporting Information:**

**Differential antigenic requirements by diverse MR1-restricted T cells**

Rebecca Seneviratna[1,2*], Samuel J. Redmond[1,2*], Hamish E. McWilliam[1,3], Rangsima Reantragoon[1,4,5], Jose A. Villadangos[1,3], James McCluskey[1], Dale I. Godfrey[1,2], Nicholas A. Gherardin[1,2]

[1]Department of Microbiology & Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Victoria 3000, Australia.

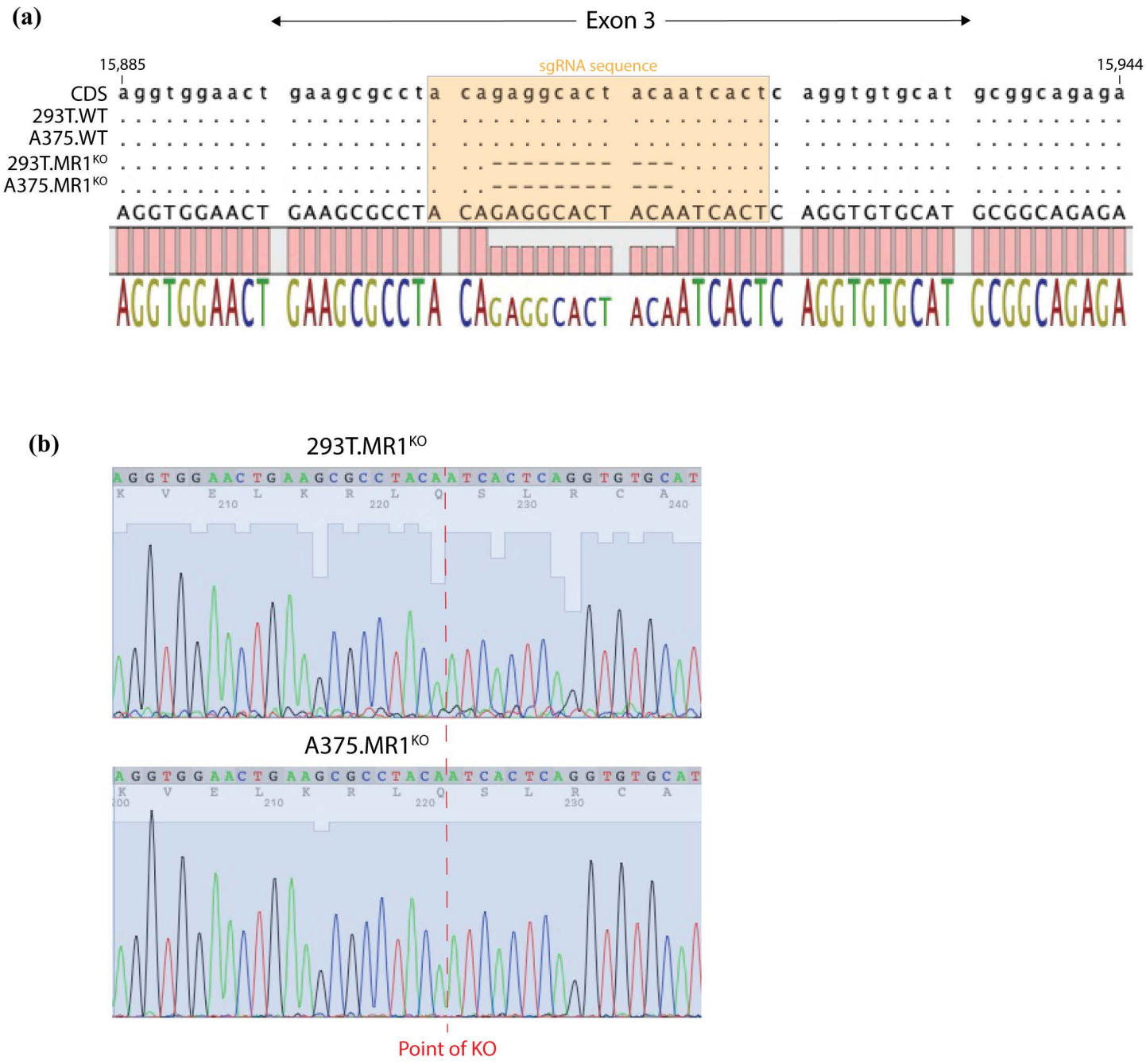
[2]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Melbourne, Parkville, Victoria 3010, Australia.

[3]Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria 3010, Australia.

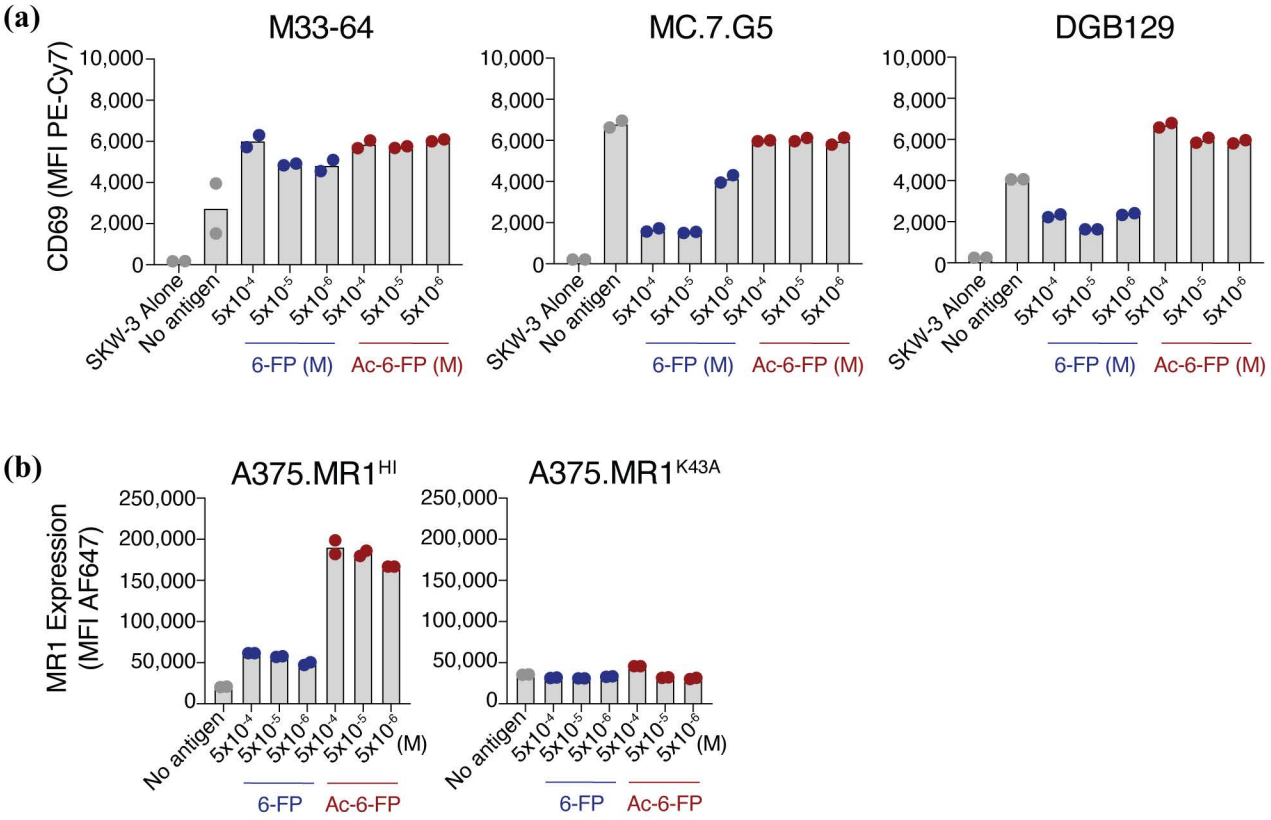
[4]Present address: Immunology Division, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

[5]Present address: Centre of Excellence in Immunology and Immune-mediated Diseases, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

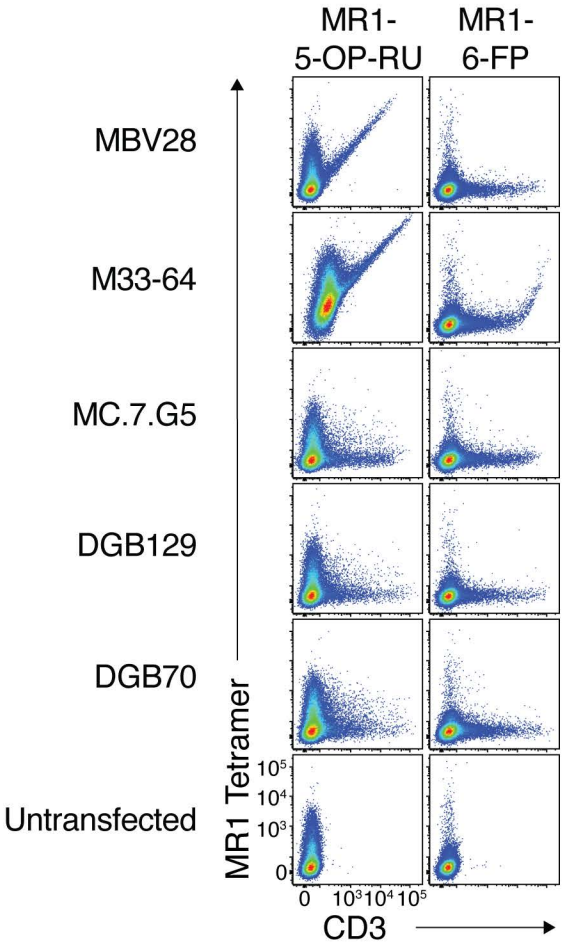
*These authors contributed equally to this work



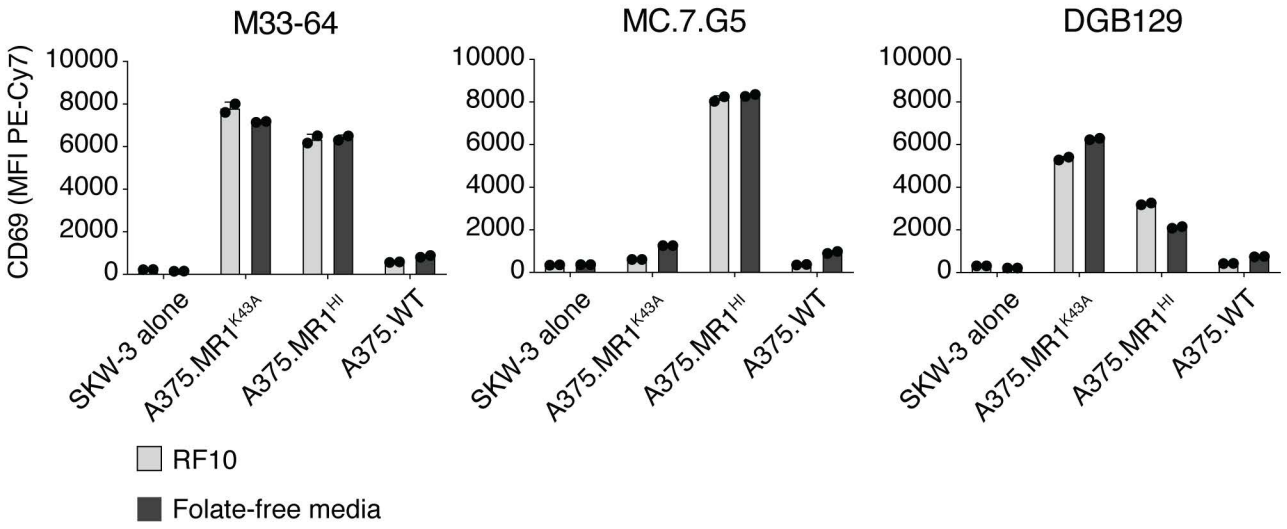

**Supplementary Figure 1: Sequence verification of CRISPR/Cas9-mediated knockout of MR1 in 293T and A375 cells. (a)** Alignment of nucleotide sequences of exon 3 of MR1 from position 15,885 to 15,944 from cDNA derived from 293T.WT, A375.WT, 293T.MR1$^{KO}$ and A375.MR1$^{KO}$ cell lines. The coding sequence (CDS) is shown at the top and is derived from NCBI reference ID NM_001531.3. Dots represent nucleotides identical to that of the CDS. Dashes represent the absence of a nucleotide. The sequence of the sgRNA used during CRISPR/Cas9-mediated knockout is highlighted in orange. Alignment was generated using CLC Main Workbench 8 (Qiagen). **(b)** Sanger Sequencing trace from genomic DNA amplified from 293T.MR1$^{KO}$ and A375.MR1$^{KO}$. The red dotted line indicated where a stretch of DNA has been removed relative to the MR1 CDS. Traces were generated using 4Peaks software.



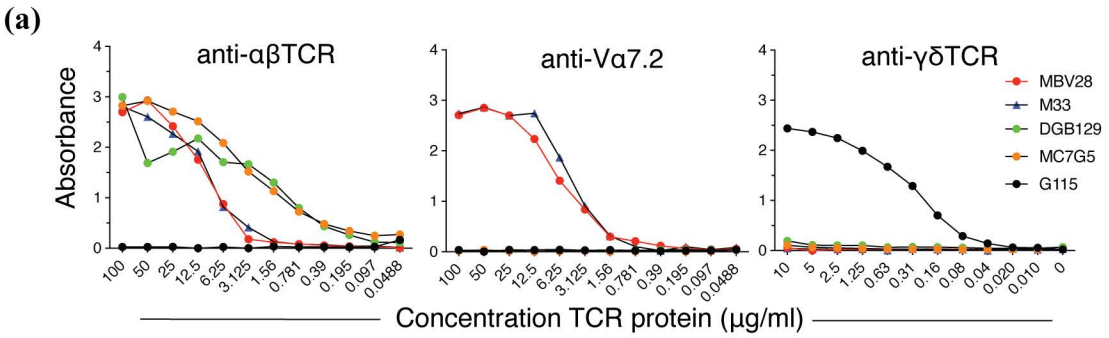

**Supplementary Figure 2: Effects on activation by 6-FP and Ac-6-FP. (a)** Bar graphs showing activation of TCR-expressing SKW-3 cells as measured by CD69, after co-culture with C1R.MR1$^{HI}$ cells **(b)** Bar graphs showing the MFI of MR1 expression on A375.MR1$^{HI}$ or A375.MR1$^{K43A}$ cells when pulsed with titrating concentrations of 6-FP or Ac-6-FP. Points on all plots are replicate wells, and data is representative of n = 3 independent experiments.

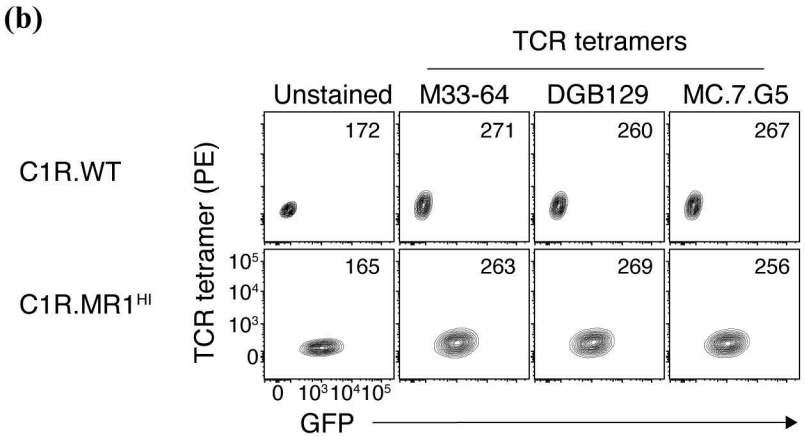


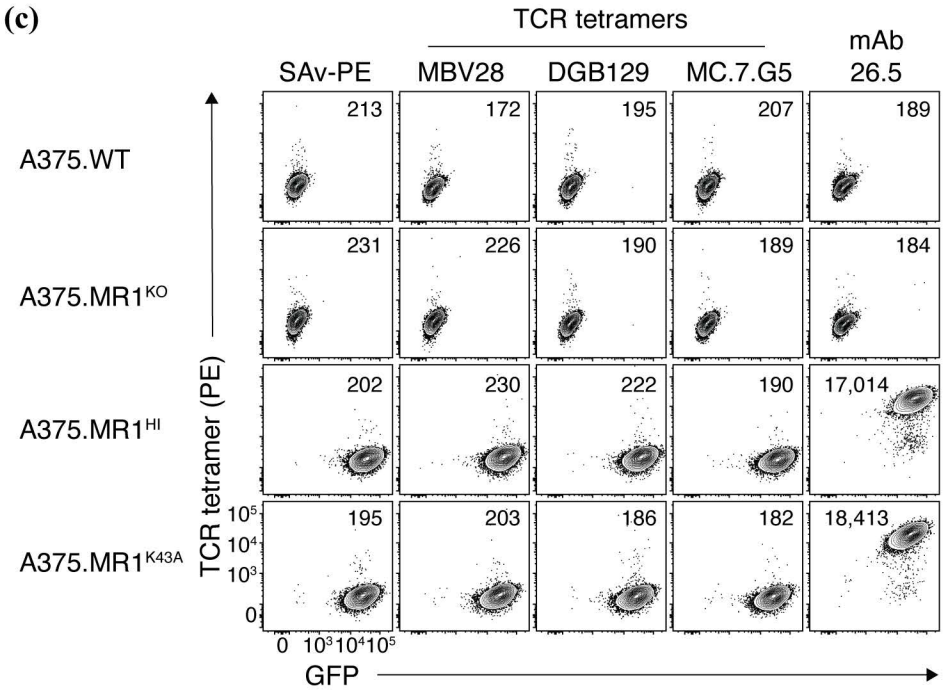

**Supplementary Figure 3: MR1 tetramer staining of MR1-restricted TCRs.** Flow cytometric contour plots showing MR1-5-OP-RU and MR1-6-FP tetramer staining on 293T cells transiently transfected to express diverse MR1-restricted TCRs. Experiment is representative of 2 independent experiments.

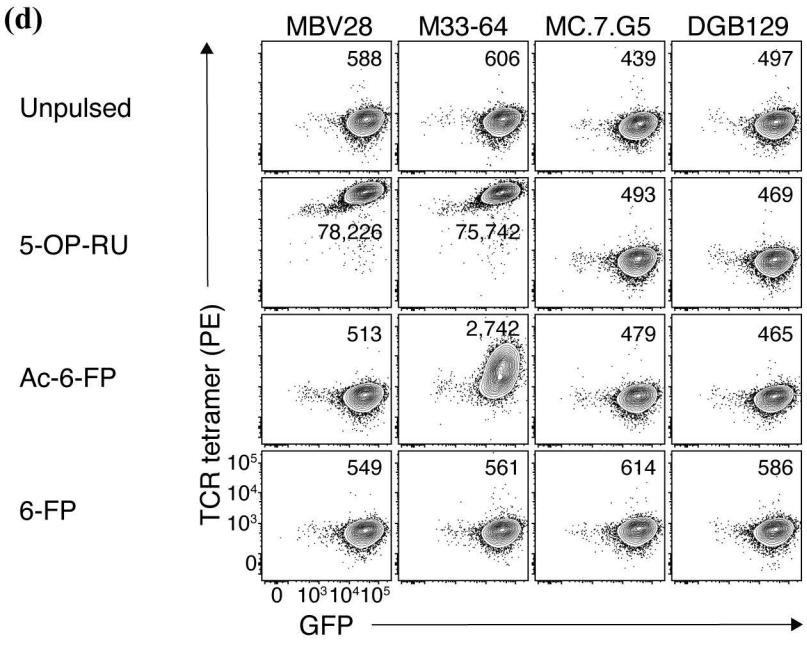



**Supplementary Figure 4: Folate-free media has no effect on activation of MR1-restricted TCRs.** Bar graphs showing activation of TCR-expressing SKW-3 cells as measured by CD69, after co-culture with a panel of antigen-presenting cell lines that had been cultured for 48 hours in normal RF10 media, or RF10 media that used folate-free RPMI-1640 as a base. Points on plots are replicate wells, and data is representative of n = 2 independent experiments.




**Supplementary Figure 5: TCR tetramers fail to stain MR1-overexpressing cell lines.** **(a)** ELISA showing binding of anti-αβTCR, anti-Vα7.2 or anti-γδTCR to soluble TCR proteins. **(b-d).** Flow cytometric contour plots showing TCR tetramer staining on **(b)** C1R cell lines, **(c)** A375 cell lines, or **(d)** A375.MR1$^{HI}$ cell lines pulsed for 4 hours with 10 µM 5-OP-RU, 500 µM Ac-6-FP or 500 µM 6-FP. Plots are representative of n = 2 independent experiments. Numbers in top right of plots indicate MFI of tetramer staining