

1 **Title: Differential antigenic requirements by diverse MR1-restricted T cells**

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7 **List of supplementary items:**

8 **Figure S1:** Sequence verification of CRISPR/Cas9-mediated knockout of MR1 in 293T and  
9 A375 cells

10 **Figure S2:** Effects on activation by 6-FP and Ac-6-FP

11 **Figure S3:** MR1 tetramer staining of MR1-restricted TCRs

12 **Figure S4:** Folate-free media has no effect on activation of MR1-restricted TCRs

13 **Figure S5:** TCR tetramers fail to stain MR1-overexpressing cell lines.

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15 **Figure S1: Sequence verification of CRISPR/Cas9-mediated knockout of MR1 in 293T**

16 **and A375 cells. A.** Alignment of nucleotide sequences of exon 3 of MR1 from position 15,885  
17 to 15,944 from genomic DNA derived from 293T.<sup>WT</sup>, A375.<sup>WT</sup>, 293T.MR1<sup>KO</sup> and

18 A375.MR1<sup>KO</sup> cell lines. The coding sequence (CDS) is shown at the top and is derived from  
19 NCBI reference ID NM\_001531.3. Dots represent nucleotides identical to that of the CDS.

20 Dashes represent the absence of a nucleotide. The sequence of the sgRNA used during

21 CRISPR/Cas9-mediated knockout is highlighted in orange. Alignment was generated using

22 CLC Main Workbench 8 (Qiagen). **B.** Sanger Sequencing trace from genomic DNA amplified

23 from 293T.MR1<sup>KO</sup> and A375.MR1<sup>KO</sup>. The red dotted line indicated where a stretch of DNA

24 has been removed relative to the MR1 CDS. Traces were generated using 4Peaks software.

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1 **Figure S2: Effects on activation by 6-FP and Ac-6-FP.** A. Bar graphs showing activation of  
2 TCR-expressing SKW-3 cells as measured by CD69, after co-culture with C1R.MR1<sup>HI</sup> cells  
3 B. Bar graphs showing the MFI of MR1 expression on A375.MR1<sup>HI</sup> or A375.MR1<sup>K43A</sup> cells  
4 when pulsed with titrating concentrations of 6-FP or Ac-6-FP. Points on all plots are replicate  
5 wells, and data is representative of n=3 independent experiments.

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7 **Figure S3: MR1 tetramer staining of MR1-restricted TCRs.** Flow cytometric contour plots  
8 showing MR1-5-OP-RU and MR1-6-FP tetramer staining on 293T cells transiently transfected  
9 to express diverse MR1-restricted TCRs. Experiment is representative of 2 independent  
10 experiments.

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12 **Figure S4: Folate-free media has no effect on activation of MR1-restricted TCRs.** Bar  
13 graphs showing activation of TCR-expressing SKW-3 cells as measured by CD69, after co-  
14 culture with a panel of antigen-presenting cell lines that had been cultured for 48 hours in  
15 normal RF10 media, or RF10 media that used folate-free RPMI-1640 as a base. Points on plots  
16 are replicate wells, and data is representative of n=2 independent experiments.

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18 **Figure S5: TCR tetramers fail to stain MR1-overexpressing cell lines.** A. ELISA showing  
19 binding of anti- $\alpha\beta$ TCR, anti-V $\alpha$ 7.2 or anti- $\gamma\delta$ TCR to soluble TCR proteins. **B-D.** Flow  
20 cytometric contour plots showing TCR tetramer staining on **B.** C1R cell lines, **C.** A375 cell  
21 lines, or **D.** A375.MR1 cell lines pulsed for 4 hours with 10  $\mu$ M 5-OP-RU, 500  $\mu$ M Ac-6-FP  
22 or 500  $\mu$ M 6-FP. Plots are representative of n=2 independent experiments. Numbers in top  
23 right of plots indicate MFI of tetramer staining.

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