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SUPPLEMENTARY INFORMATION

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Deciphering CD4+ T cell specificity using novel MHC–TCR chimeric receptors

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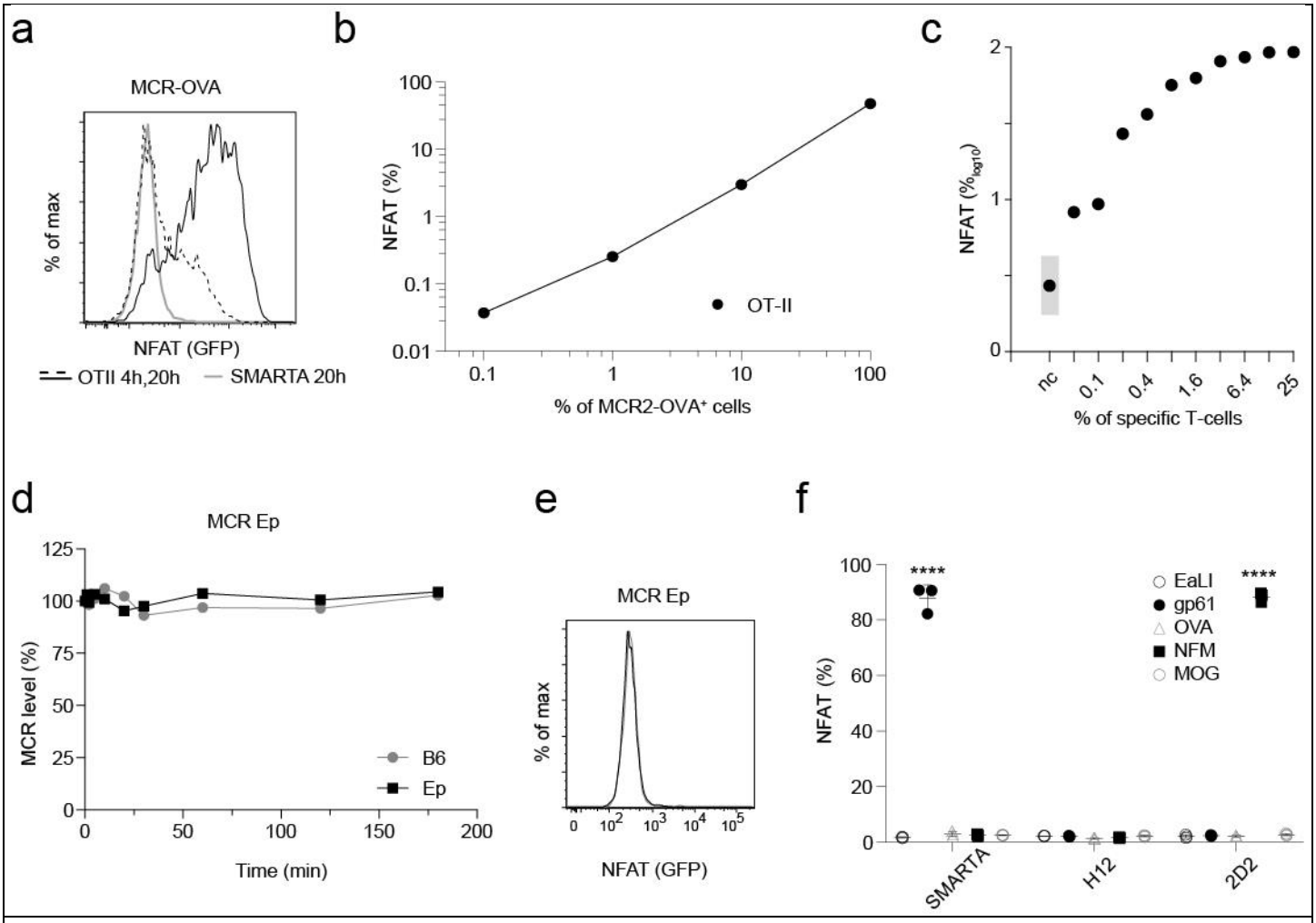
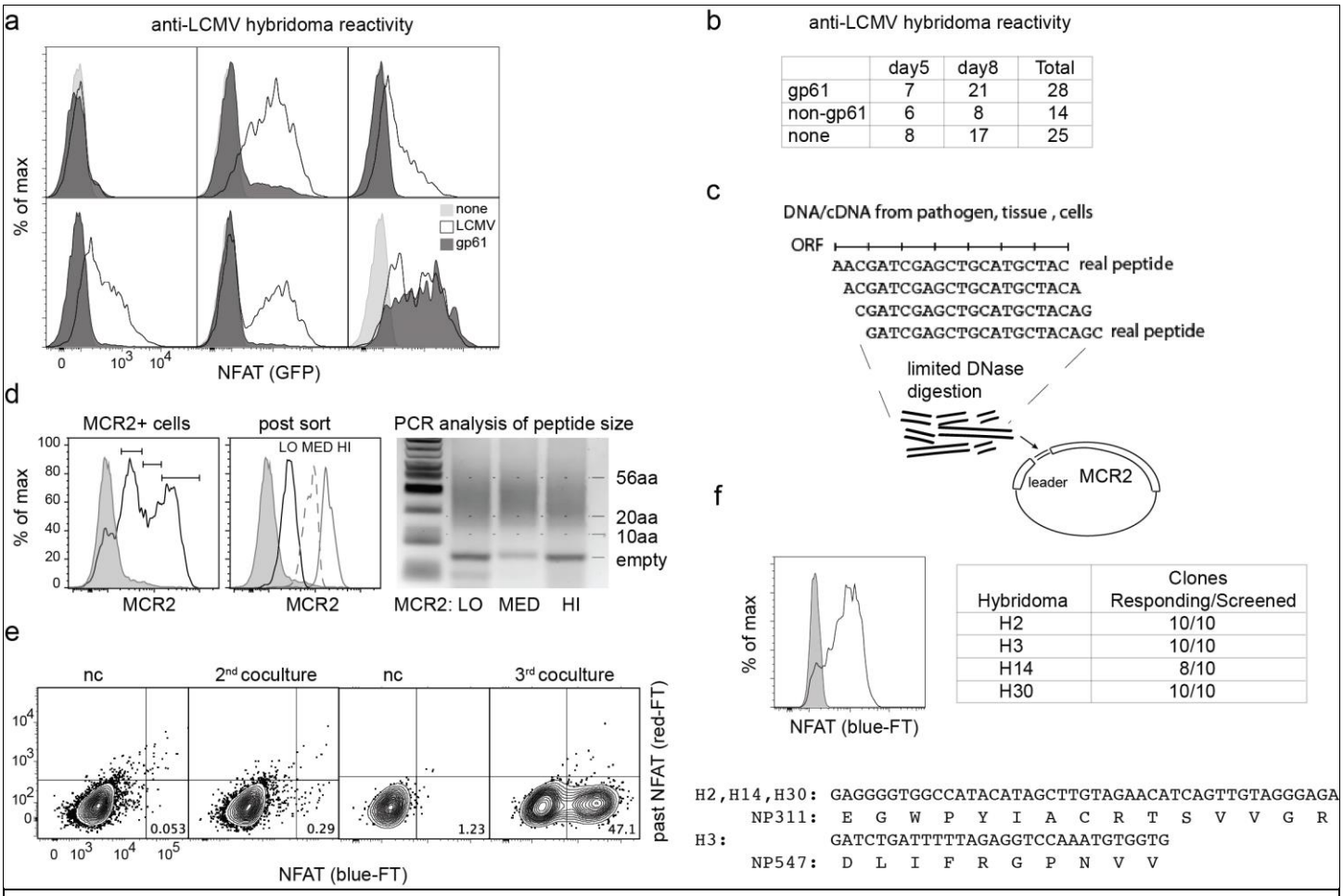


Supplementary Figure 1

Peptide-specific reactivity and sensitivity of the MCR2 sensor.

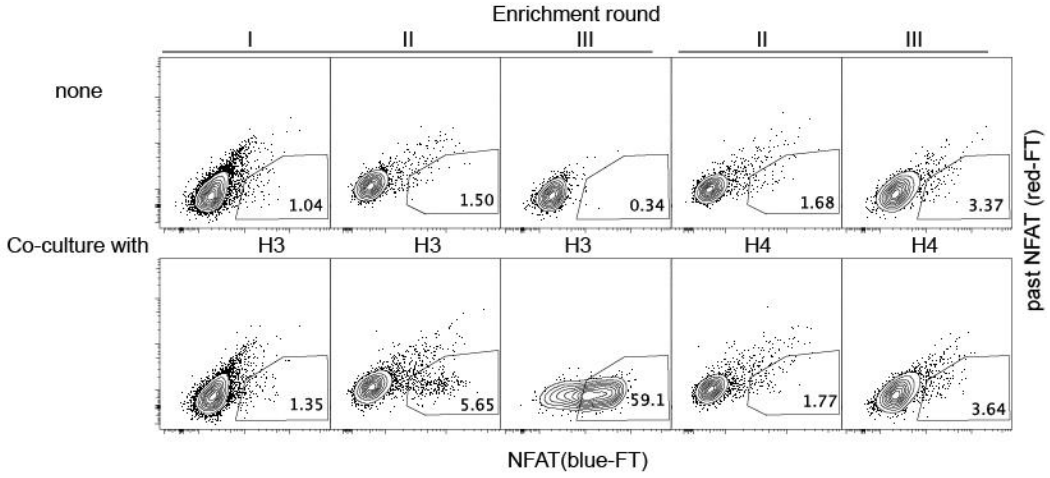
(**a**) Time course of peptide-specific NFAT activation (GFP reporter expression) in MCR2-OVA$^+$H18.3.13 cells co-cultured with OT-II CD4$^+$ T cells or SMARTA CD4$^+$ T cells as controls. Histogram shows examples of NFAT activation measurements in MCR2-OVA$^+$ cells at indicated time points. (**b**) Sensitivity of detection of peptide-specific reporter cells. MCR2-OVA$^+$ H18.3.13 cells were mixed with MCR2-gp61$^+$ H18.3.13 cells at different ratios and NFAT activation was measured after co-culture with OT-II CD4$^+$ T cells. The graph shows a linear (R>0.99) correlation between the percentage of GFP$^+$ H18.3.13 cells and the percentage of cells carrying the MCR2-OVA. (**c**) A standard curve of reporter NFAT activation (blue-FT) in relation to the frequency of specific T cells is shown. The grey box represents standard deviation of the negative control. (**d**,**e**) Time course of MCR2-Ep down-regulation in BEKO cells (**d**) and NFAT activation in MCR2-Ep$^+$ reporter cells (**e**), co-cultured with CD4$^+$ T cells from B6 or single peptide mice(Ep). Values (**d**) show MCR2 levels depicted as percentage of mean fluorescence intensity at the start of co-culture. (**f**) NFAT activation in MCR2$^+$ reporter cells carrying different peptides co-cultured (n = 3 co-cultures, mean ± sd) with hybridomas derived from SMARTA, Ep and 2D2 TCR transgenic mice. Data are representative of one (**b**,**c**,**d**), two (**a**,**e**) and three (**f**) independent experiments. Two-way ANOVA analysis with multiple pair-wise comparisons was done ****$P \leq 0.0001$.



Supplementary Figure 2

Search for viral epitopes.

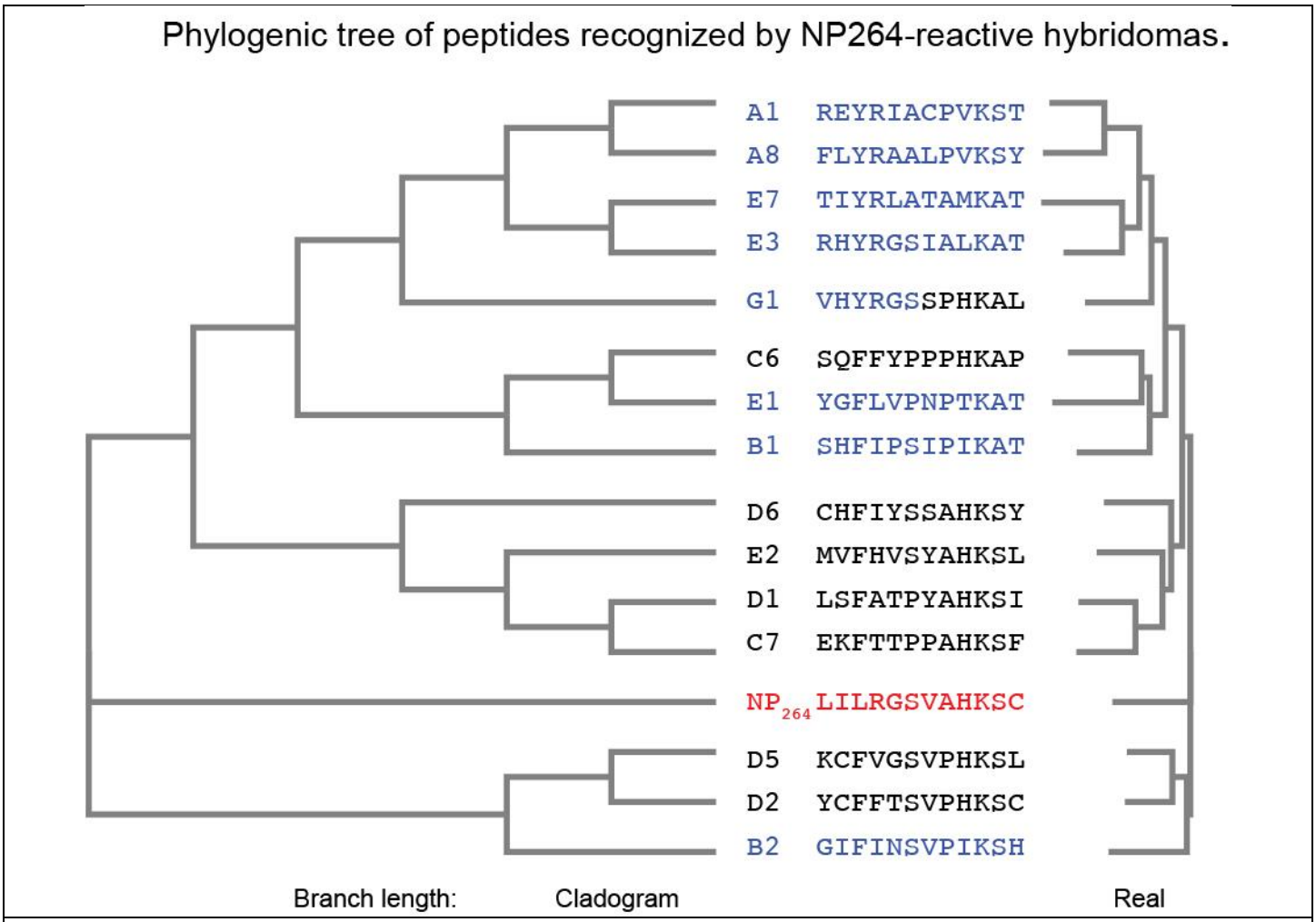
(**a**,**b**) CD4$^+$ T cells from mice infected with LCMV were purified from the spleens 5 and 8 days p.i. and fused with $\alpha^-\beta^-$BW5147 cells carrying an NFAT (GFP) reporter. Reactivity of the resulting hybridomas was determined by NFAT activation after co-culture with LCMV- or gp61-pulsed dendritic cells. Histograms (**a**) show examples of different types of reactivity (LCMV-open or gp61-filled dark grey) which are summarized in **b**. (**c**) A scheme of the cloning strategy for the construction of cDNA-derived peptide (CDP) libraries enriched for natural peptides. ORF – open reading frame dictating the natural protein sequence. (**d**) Cells transduced with the MCR2-CDP library were sorted into MCR2^{low}, MCR2^{med} and MCR2^{hi}, and the length of the MCR-connected peptides was determined by PCR on the isolated DNA. No major difference in the length of the PCR products was observed, indicating that, in all fractions, mainly peptides between 15 and 50aa were present. (**e**) MCR2-LCMV$^+$ 16.2c11 reporter cells were co-cultured with LCMV-reactive hybridomas (H2, H3, H14 and H30) of unknown peptide-specificity. Activated reporter cells were sorted, expanded and co-cultured again with the corresponding hybridomas. Dot plots show example NFAT activation after two or three rounds of co-culture. (**f**) After the 3rd round of enrichment, single cells were sorted, expanded and their reactivity verified by co-culture with hybridomas (histogram shows example NFAT reactivity in reporter cells). The table on the right, shows frequencies of reactive clones. Peptide sequences from the MCR2 constructs, representing the dominant NP311 epitope and the new NP547 epitope are shown below. Figure represents data from one experiment.



Supplementary Figure 3

TCR dependent enrichment of reactive reporter cells.

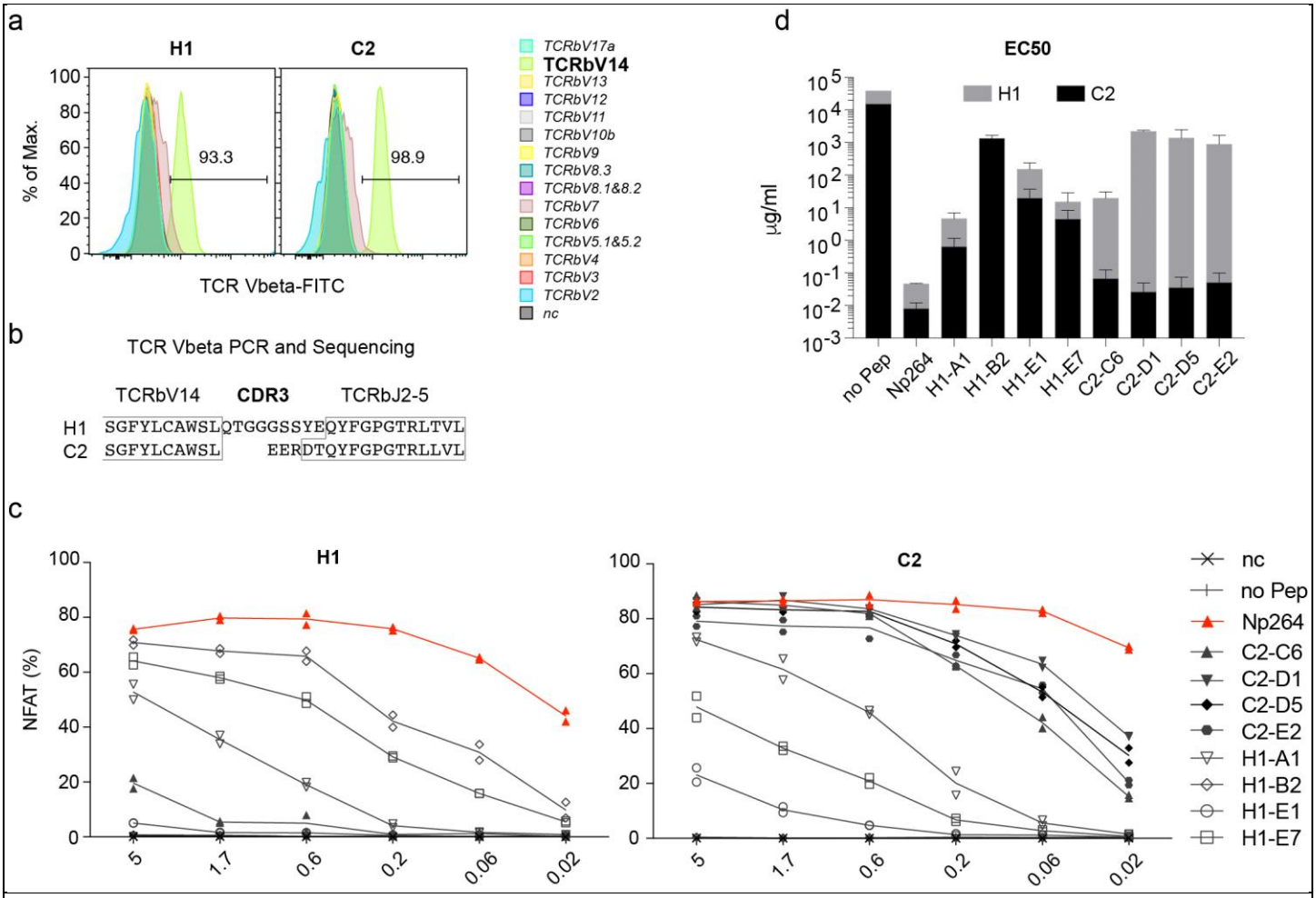
Activation of MCR2-LCMV^{+} 16.2c11 cells after one, two and three rounds of enrichment by co-culture with hybridomas H3 and H4 (H4 is a TCR-negative control). Dot plots show NFAT-activation, numbers indicate % cell in the gate. Figure represents data from one experiment.



Supplementary Figure 4

Clustal analysis of peptide homology.

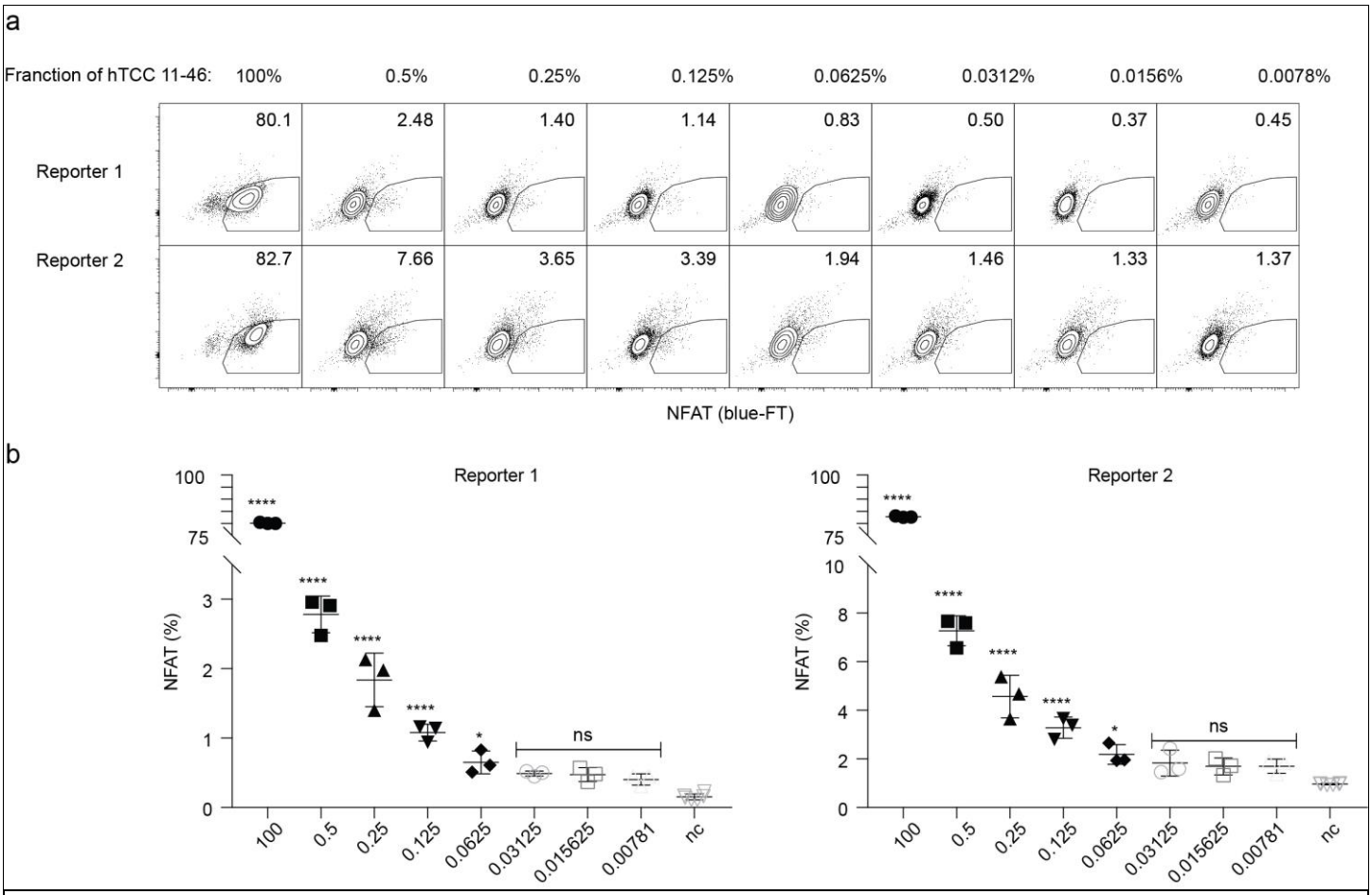
Clustering analysis (done with Clustal Omega) of example H1- and C2-reactive peptide sequences, shown in blue and black, respectively. NP264 sequence is shown in red. Branch length in the right tree is proportional to the amount of inferred change between sequences.



Supplementary Figure 5

Reactivity of H1 and C2 hybridomas.

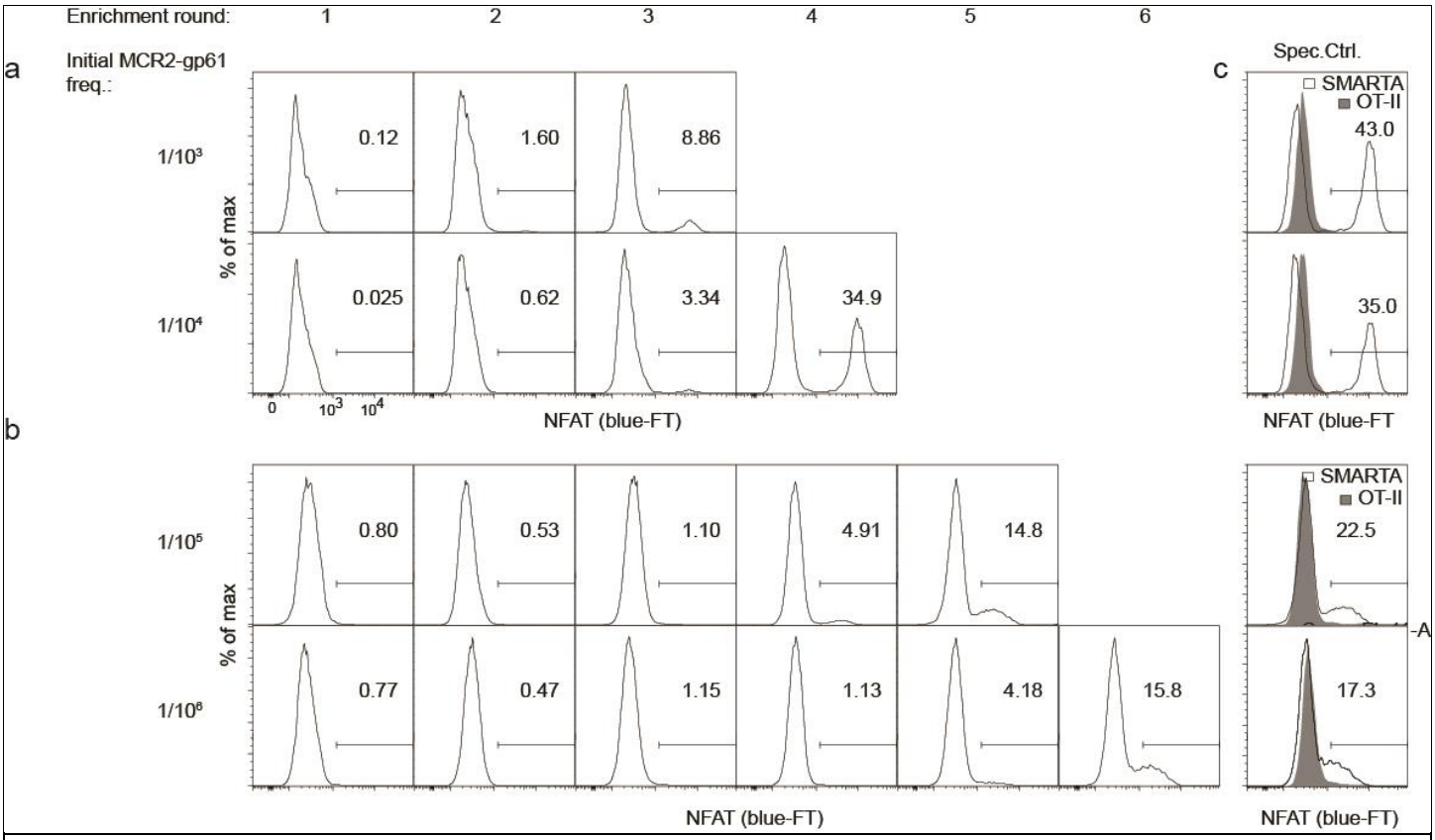
(**a**) T cell hybridomas H1 and C2 were stained with TCR V_β specific antibodies. All cells showed positive staining only with the TCR V_β14 antibody. (**b**) cDNA from hybridomas H1 and C2 was used to PCR and clone the TCRβ chains using oligos specific for different TCR V_β chains. For both hybridomas, apart from the nonfunctional TCRβ chain from the BW fusion partner (not shown) a functional TCRβ containing the TCR V_β14 and TCR J_β2-5 was cloned. A comparison of the protein sequences is shown, highlighting the different CDR3 regions. (**c**) Hybridomas carrying the NFAT-GFP reporter were co-cultured (*n* = 2 cocultures) with BMDCs pulsed with different amounts of the indicated peptides (µg/ml, 90% purity). The graph shows NFAT activation (GFP). Data are representative of two independent experiments. (**d**) Half maximal effective concentration (EC50) was calculated by non-linear regression (curve fit) based on data from two experiments, mean ± sd are shown.



Supplementary Figure 6

Detection limit in a single coculture.

Two hMCR2-DRB1.4-MP1$_{103–120}^{+}$ reporter clones were co-cultured with hTCC11-46 cells mixed with hTCC10-16 cells at different ratios. NFAT activation was measured after 8h (**a**) representative FACS dot plots, numbers indicate % NFAT+ cells and (**b**) statistical analysis of the results, (n = 3 cocultures) mean ± sd are shown. One-way ANOVA analysis with multiple comparisons was done. *P < 0.02, ****P < 0.0001. nc, no co-culture.



Supplementary Figure 7

Sensitivity of the MCR system.

MCR2-gp61^{+} 16.2c11 reporter cells were spiked into MCR2-OVA^{+} 16.2c11 reporter cells at different frequencies and NFAT activation (blue-FT) was measured after subsequent rounds of co-culture/enrichment with SMARTA T cell hybridoma cells (reporter to hybridoma ratio was 1/3 (**a**) and 1/5 (**b**). Last column (**c**) shows NFAT activation in co-cultures with SMARTA or OT-II T cell hybridoma cells after the last round of enrichment. Graphs show all live cells including reporter and hybridoma cells. Figure represents data from one experiment.

Suppl. Table 1

Examples of cross-reactive artificial peptides present in the CDP library. They are mostly derived from UTRs, anti-sense reading frames, frame shifts or intronic sequences, AS- antisense.

mRNA strand	Gene	Peptide Sequence	Comment
+	18srRNA	PRSYSIIPSCGIQAARACFEHSNFFKVNASGPAGHSAKSIEGAPRGKGRG	rRNA
+	Clasp1	PPLSPLLPTSYLNWTFNILPAALQTHMVHCCHSRDCIFETHTLRS	5'UTR
-	Lgals1	SFPRFSTKLLASEATSPRTLRHSPGLRFR	AS 2 exons
-	Smdt1	PPLLPLGPLQRAPGRTATQA	AS exon
-	Wars2	AMFYLHSHEPISFPVLEVHQHANHSNSPSIPSAQRITTGSVTQLANVRKSQELLGP	AS intron
+	Mta1	PINSAAIKAECTARLPEASQSPLVLKQVVRKPLEAV	2 exons
+	Serpinh1	VSNFPEVSWDRQEGVGPAIYRSRARGSELS	5'UTR
+	lncRNA	PSYRQLFDLPISSLRNAGSGFSVSAIWKTQAP	lncRNA
+	Gclc	HPTEVSLMLCPQVPRAHLFNSGMPLLLALS	intron
+	Polr2a	PNLTQLFPHLTKLLPLQPTIYTTVSNLHT	exon fr.shift