

**Supplemental Figure 1. (a)** Representative flow cytometry plots showing gating strategy used to sort CD4+ (top two rows) and CD8+ (bottom two rows) memory cells T cells. **(b)** Frequency of MTC subsets in resting and stimulated samples for CD4+ (left) and CD8+ (right) T cells (\*p < 0.05, paired t-test). **(c)** Post-sort analysis, including overlaid CCR7 by CD45RA scatter (last column), of MTC subsets depicting purity of sorted populations.



**Supplemental Figure 2. (a)** Bar plot showing FDR-corrected p-value of enriched gene ontology pathways representing CD4+ specific, CD8+ specific and shared DEGs for TCM. Significant enrichment level marked with dotted line. **(b)** Same as A for TEM.





**Supplemental Figure 4.** (a) Scatter plot showing intersection of DEG and DAR for CD4+ TCM. Dotted lines represent ±1 log2FC. (b) Same as (a) for CD4+ TEM. (c-f) Genome plots showing the average accessibility levels in each of the memory subsets and naïve T cells at the indicated locus. Data represent the mean of each cell type. DAR are highlighted by black boxes at the top of plots.



**Supplemental Figure 5.** (a-f) tSNE plot from Figure 5b colored by variability around indicated binding motifs. (g-i) Scatter plots showing log2 fold changes (log2FC) of RNA expression (x-axis) and PageR-ank statistic log2FC (y-axis) between memory and naïve samples for labeled CD8+ memory subsets. (j-k) Scatter plots of log2FC PageRank statistic (vs Naïve) for TEM (x-axis) vs TCM (y-axis) in CD4+ (j) and CD8+ (k). Solid line represents unity line. Dotted lines represent 1 log2FC away from unity.



**Supplemental Figure 6. (a)** Median fluorescence intensity (MFI) of corresponding transcription factors in unstimulated and stimulated MTC measured by intracellular staining and flow cytometry. Isotype controls substracted as background. N=9, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. Paired t-test was performed. **(b)** RNA expression data of the transcription factors for MTC subsets unstimulated and stimulated expressed as reads per kilobase million (rpkm). Statistical analyses were performed using DEseq2. N=4,  $\blacktriangle$ , FDR < 0.05 and log2FC > 1.



**Supplemental Figure 7.** Cytokines expression was measured in stimulated T cells by intracellular staining and flow cytometry. Left, representative flow cytometry plots showing data and gating strategy. Right, bar plots showing the frequency of the cells that express the corresponding cytokine. N=9, \*P<0.05, \*\*P<0.01, \*\*\*P<0.001. One way ANOVA, multiple comparisons was performed to determine significant differences.



**Supplemental Figure 8. (a)** Histograms of counts for PAR categories for indicated MTC subsets. **(b)** Pie charts for indicated PAR categories showing percentages of genomic annotations associated with each.



Supplemental Figure 9. (a) Scatter plot showing log2-transformed fold changes (log2FC) for DEG found to be significant in either unstimulated memory vs unstimulated naïve T cells (x-axis) or stimulated memory vs stimulated naïve T cells (y-axis). Dots are colored in respective colors if there is at least one PAR mapped to differential gene locus. (b) Empirical cumulative distribution function plots for unstimulated DEG (left) and stimulated DEG (right) colored by presence of different PAR categories.

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Rat IgG2a, κ Isotype Ctrl PE/Cyanine7 400521 Biolegend 0.5   IL-5 eFluour 450 48-7052-82 Thermofisher 4   Rat IgG1, κ Isotype Ctrl eFluour 450 48-4301-80 Thermofisher 4   IFN-γ BV711 502539 Biolegend 1   Mouse IgG1, κ Isotype Ctrl BV711 400167 Biolegend 1   TNF-α BV650 502937 Biolegend 2	IL-2	PE/Cyanine7	500326	Biolegend	0.5
IL-5 eFluour 450 48-7052-82 Thermofisher 4   Rat IgG1, κ Isotype Ctrl eFluour 450 48-4301-80 Thermofisher 4   IFN-γ BV711 502539 Biolegend 1   Mouse IgG1, κ Isotype Ctrl BV711 400167 Biolegend 1   TNF-α BV650 502937 Biolegend 2	Rat IgG2a, ĸ Isotype Ctrl	PE/Cyanine7	400521	Biolegend	0.5
Rat IgG1, $\kappa$ Isotype CtrleFluour 45048-4301-80Thermofisher4IFN- $\gamma$ BV711502539Biolegend1Mouse IgG1, $\kappa$ Isotype CtrlBV711400167Biolegend1TNF- $\alpha$ BV650502937Biolegend2	IL-5	eFluour 450	48-7052-82	Thermofisher	4
IFN-γBV711502539Biolegend1Mouse IgG1, κ Isotype CtrlBV711400167Biolegend1TNF- $\alpha$ BV650502937Biolegend2	Rat IgG1, κ Isotype Ctrl	eFluour 450	48-4301-80	Thermofisher	4
Mouse IgG1, κ Isotype Ctrl BV711 400167 Biolegend 1   TNF-α BV650 502937 Biolegend 2	IFN-γ	BV711	502539	Biolegend	1
TNF-a BV650 502937 Biolegend 2	Mouse IgG1, κ Isotype Ctrl	BV711	400167	Biolegend	1
	TNF-α	BV650	502937	Biolegend	2
Mouse IgG1, K Isotype Ctrl BV650 400163 Biolegend 2	Mouse IgG1, K Isotype Ctrl	BV650	400163	Biolegend	2
IL-22 BUV737 367-7229-42 Thermofisher 1	IL-22	BUV737	367-7229-42	Thermofisher	1
Mouse IgG1, κ Isotype Ctrl BUV737 367-4714-81 Thermofisher 1	Mouse IgG1, K Isotype Ctrl	BUV737	367-4714-81	Thermofisher	1
II17/II17A PerCP IC3171C-025 R&D systems 1	IL-17/IL-17A	PerCP	IC3171C-025	R&D systems	1
Mouse IgG1. Isotype Ctrl PerCP IC002C R&D systems 1	Mouse IgG1, Isotype Ctrl	PerCP	IC002C	R&D systems	1