

Supp. Figure S1 - Flow cytometry gating strategy T cell from human blood and tumor samples. Lymphocytes are gated based on FSC-A versus SSC-A, followed by doublet removal using FSC-A and FSC-h. Dead cells (LD NIR+), B cells (CD19+) and monocytes (CD14+) are then removed in a cocktailed dump channel, $T$ cells are then gated as CD45+CD3+ cells. T cells were then backgated to ensure the original gates captured all T cells.

5'-CTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA_Insert-GTACTCTGCGTTGATACCACTGCTT-3' 3'-GATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA Insert-CATGAGACGCAACTATGGTGACGAA-5'

## ii)

## Enrichment 1

Human T-Cell External Mix 5'-AATGATACGGcGaccaccga-gatctacactctttccctacacgacgctc-3'

Reverse External primers: Cy external primer and Cס 5'-CTTCATATTTACCAAGCTTGACAG-3' (Сठ. , a.k.a. TRDC external) 5'-GGtGttсссстсстGG-3' (Cү. , a.k.a. TRGC external)

## Enrichment 2

## Forward primer: FTC2

5'-AATGATACGGCGACCACCGA-GATCT-3'

Reverse Internal primers: Cy internal primer and Cס 5'-GATGACAATAGCAGGATCAAAC-3' (Cס. , a.k.a. TRDC internal) 5'-CCCAGAATCGTGTTGCT-3' (Cү. , a.k.a. TRGC internal)

5’-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-NNNNNNNNNNNNNNNNN-NNNNNNNNNN-TTTCTTATATGGG-cDNA_Insert-Inner_Primer-3' 3'-TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA_Insert-Inner_Primer-5'

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\text { Adaptor } \begin{aligned}
& \text { 5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3' } \\
& \text { 3'-TCTAGCCTTCTCG-5' }
\end{aligned}
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 3'-TTACTATGCCGCTGGTGGCT-CTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-NNNNNNNNNNNNNNNN-NNNNNNNNNN-AAAGAATATACCC-cDNA_Insert-TCTAGCCTTCTCG-5'

## P5 Forward Primer P7 Reverse Primer (Sample Index)

5'-AATGATACGGCGACCACCGA-GATCTACACTCTTTCCCTACACGACGCTC-3'
iii)



Supp. Figure S2-Schematic for primer design for the amplification and enrichment of full length TCR transcripts for scTCR-seq. i) Primers comprising GEM-formation and cell barcoding for cDNA production. ii) Primers utilised in the enrichment of full-length TCRs via PCR amplification of the TCR constant regions. P5 and P7 primers added as priming sites used in Illumina sequencing. iii) Final sample index and PCR product sequences.


Supp. Figure S3 - Distribution of mean PD-L1+ Merkel cells per ROI. For box and whisker plots, boxes display lower quartile, median and upper quartile. Whiskers display the minimum and maximum values within the lower and upper IQRs respectively $+I Q R^{* 1} .5$, "+" refers to mean.


Supp. Figure S4-mIHC/IF infiltrate distribution in paired primary and secondary sites. A shared dominant infiltrate subset found in 10 of 17 (59\%) paired tumor samples.


Supp. Figure S5 - Analysis of NKT and MAIT cells in MCC tumors. A) Representative FACS plots showing gating of NKT and MAIT cells in healthy blood and MCC tumor. Healthy blood was included to validate tetramer reagents. Plots are gated on total CD3+ T cells. B) Box and whisker plot showing proportion of CD3+ T cells that are MAIT cells from $n=11$ MCC tumors. 3 data points on X-axis has undetected MAIT cells. C) FACS plots showing analysis of MCC liver metastasis with high proportion of MAIT cells. Right plot is gated on MAIT cells and shows CD4 and CD8 co-receptor distribution


Supp. Figure S6-TCR repertoire and specificity of tumor infiltrating $\gamma \delta$ T cells. A) Representative FACS plots showing MFI CD69 expression on Jurkat.TCR cells after co-culture with C1R antigen presenting cells expressing monomorphic antigen-presenting molecules. MFI is depicted in top right corner of each plot. B) Bar graphs showing cumulative data from $\mathrm{n}=3$ independent experiments as per A . presented as fold-change in CD69 expression from unstimulated cells. C) Bar graphs showing MFI of CD69 expression on Jurkat.MG1 or Jurkat. MG2 cells after co-culture with C1R. CD1c or C1R. MR1 cells respectively, in the presence of CD1c and MR1 blocking mAb. Data is derived from 3 independent experiments and depicted as fold-change from co-culture with C1R cells with no blocking mAb. Error bars denote SD.


Supp. Figure $\mathrm{S7}$ - scTCR-seq of CD8 ${ }^{+}$and CD4 ${ }^{+} \alpha \beta$ T cells from a single dissociated MCC tumor (P055), illustrating the top 20 CDR3 sequences and their corresponding $\alpha \beta$ TCR variable regions. A lack of a dominant clone within the cohort suggests a polyclonal population of $\alpha \beta$ T cells.


Supp. Figure S8-TCR gene regulation in an RNA-seq set of cell-sorted PBMCs. Scaled logCPM values used to illustrate expression of $ү \delta$ variable region and constant region genes. Immune cells comprise: NK cells ( $n=11$ ), V $\delta 1^{+} ү \delta \mathrm{~T}$ cells ( $n=10$ ), V $\delta 2^{+}$Y $\delta$ T cells $(n=12)$, NKT cells $(n=12)$, CD8 ${ }^{+}$T cells $(n=11)$, MAIT cells $(n=11)$ and CD4 ${ }^{+}$T cells $(n=12)$. 79 enriched samples in total, derived from 6 healthy individuals (Sourced from GSE125731).


Supp. Figure S9 - Enrichment of the 13-gene signature in the internally produced scRNA-seq dataset. ү $\bar{\delta}$-enriched clusters show greater expression than adjacent $\alpha \beta$ T cell clusters.


Supp. Figure S10-GSVA enrichment of the $\overline{\boldsymbol{\gamma}} \mathbf{\delta}$ gene-signature in an RNA-seq cohort of enriched FACS-sorted PBMC immune cells comprising: NK cells $(n=11)$, V $\delta 1^{+} \gamma \delta \mathrm{T}$ cells $(n=10)$, V $\delta 2^{+} \gamma \delta \mathrm{T}$ cells ( $n=12$ ), NKT cells ( $n=12$ ), CD8 ${ }^{+}$T cells $(n=11)$, MAIT cells $(n=11)$ and CD4 ${ }^{+}$T cells $(n=12)$ - for a total of 79 enirched samples derived from 6 healthy individuals (Sourced from GSE124731). Boxplot - Whiskers display the minimum and maximum values within the lower and upper IQRs respectively + IQR*1.5. V $1 / 2$ display a greater enrichment than sorted innate-like T cells and $\alpha \beta$ T cells, but less enrichment than NK populations. Dotplot represents the average logCPM values for each of the 13 signature genes in each cell population. The dot size and intensity of red is relative to the average logCPM value.


Supp. Figure S11-GSVA enrichment of the $ү \delta$ gene-signature and the individual signature gene scaled logCPM expression values (RNA-Seq) and z-score transformed RMA-normalized expression values (Microarray) across 2 internal RNA-seq MCC cohorts and 1 externally sourced MCC microarray set (GSE22396): RNA-Access $(n=9)$, NEB-Next $(n=21)$ and Microarray $(n=29)$.

