Figure S1

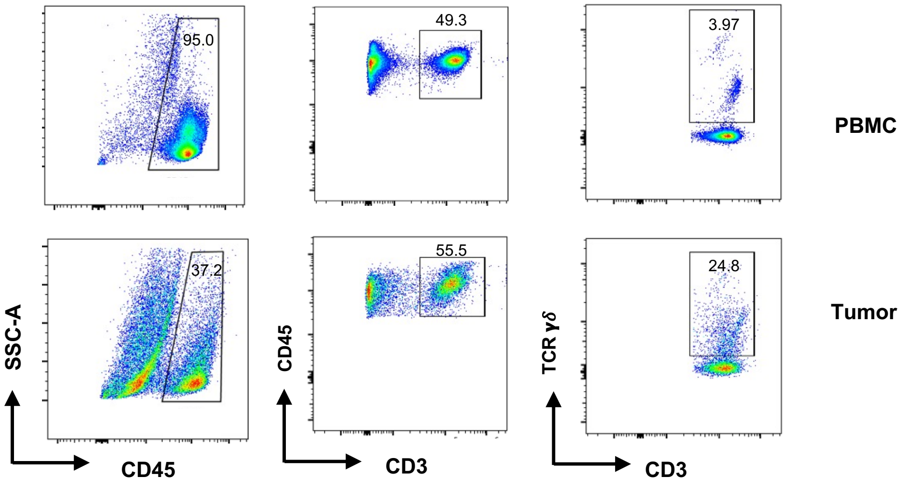

Figure S1: Sorting γδ T cells from PBMC and tumor cell suspensions for scRNA-seq. Flow cytometry showing sorting strategy to isolate live γδ T cells. After sorting, the cells with viability more than 80% were processed for 10x Genomics scRNA-seq.

Figure S2

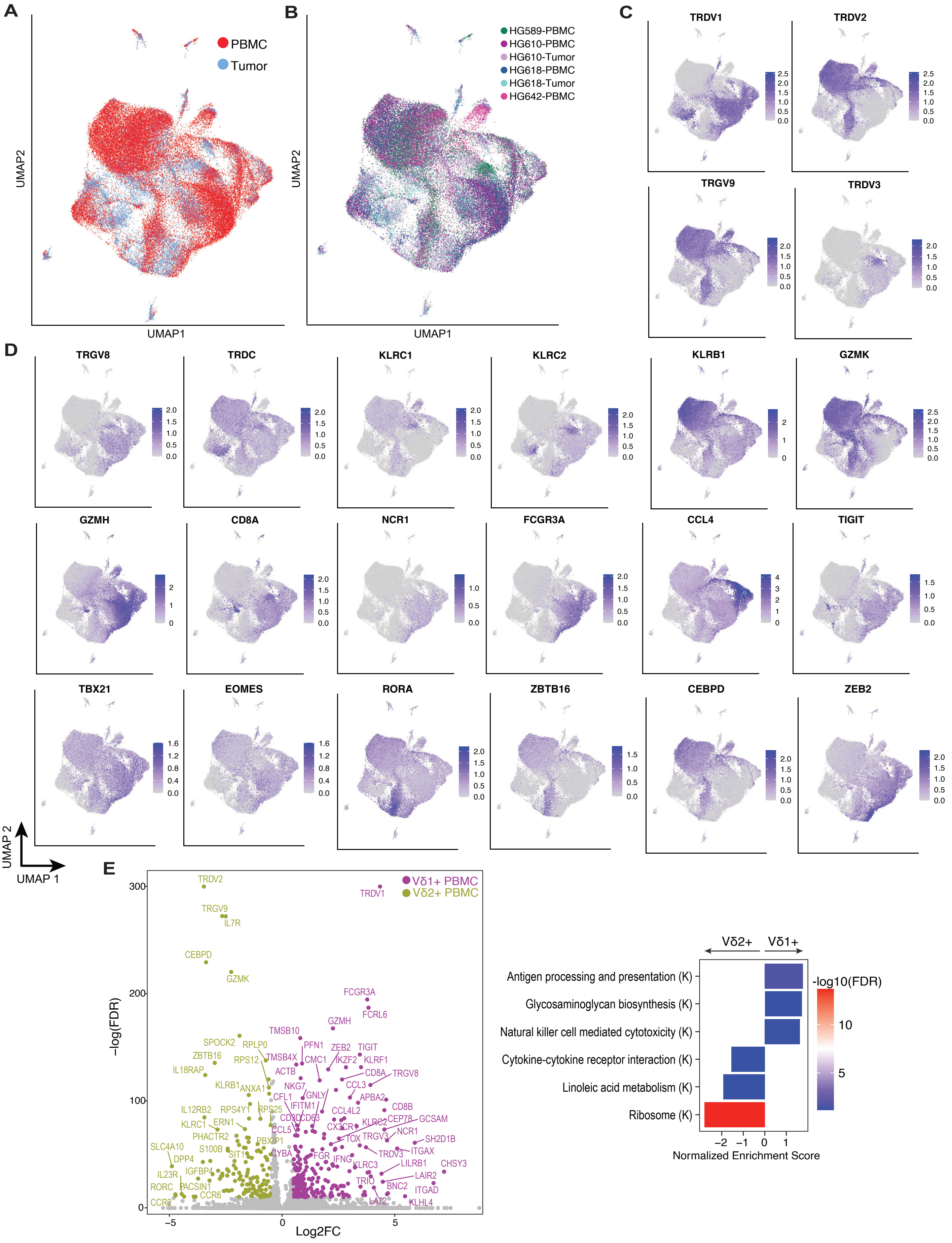
A
PBMC
Tumor
UMAP2
UMAP1
B
HG589-PBMC
HG610-PBMC
HG610-Tumor
HG618-PBMC
HG618-Tumor
HG642-PBMC
UMAP2
UMAP1
C
TRDV1
TRDV2
TRGV9
TRDV3
D
TRGV8
TRDC
KLRC1
KLRC2
KLRB1
GZMK
GZMH
CD8A
NCR1
FCGR3A
CCL4
TIGIT
TBX21
EOMES
RORA
ZBTB16
CEBPD
ZEB2
UMAP 2
UMAP 1
E
Vδ1+ PBMC
Vδ2+ PBMC
-log(FDR)
Log2FC
TRDV2
TRGV9
IL7R
CEBPD
GZMK
TRDV1
FCGR3A
FCRL6
GZMH
TMSB10
SPOCK2
RPLP0
PFN1
ZEB2
TIGIT
ZBTB16
RPS12
TMSB4X
CMC1
IKZF2
KLRF1
IL18RAP
ACTB
CD8A
TRGV8
KLRB1
ANXA1
NKG7
CCL3
CFL1
GNLY
APBA2
IL12RB2
RPS4Y1
RPS25
IFITM1
CD8B
KLRC1
ERN1
CD3D
CD63
CCL4L2
KLRC2
CEP78
GCSAM
PHACTR2
CCL5
CX3CR1
NCR1
SLC4A10
S100B
PBXIP1
TOX
TRGV3
SH2D1B
DPP4
SIT1
CYBA
TRDV3
ITGAX
IL23R
IGFBP4
FGR
IFNG
KLRC3
LILRB1
CHSY3
RORC
PACSIN1
TRIO
LAIR2
CCR6
BNC2
ITGAD
CCR2
LAT2
KLHL4
Vδ2+
Vδ1+
Antigen processing and presentation (K)
Glycosaminoglycan biosynthesis (K)
Natural killer cell mediated cytotoxicity (K)
Cytokine-cytokine receptor interaction (K)
Linoleic acid metabolism (K)
Ribosome (K)
-log10(FDR)
Normalized Enrichment Score

Figure S2: Subsets, features, and pathways of γδ T cells. γδ T cells were sorted from PBMCs and human GIST tumors and processed for 10x Genomics scRNA-seq. **A)** Integrated Uniform Manifold Approximation and Projection (UMAP) of γδ T cells colored by tissue source (PBMC or tumor) and **B)** UMAP of γδ T cells as in (A) colored by sample. **C**) Integrated UMAP plots colored by normalized expression of key marker genes used to delineate cell type populations or **D)** normalized expression of representative immune response genes and transcription factors. **E)** ***left,*** Volcano plot of differentially expressed genes between Vδ1 and Vδ2 cells within PBMC samples. ***right,*** Gene set enrichment analysis (GSEA) of differentially expressed pathways between Vδ1 and Vδ2 cells within PBMC samples. Hallmark (H) and KEGG (K) pathways are indicated in parentheses. Significant pathways (FDR<0.1) are shown. FDR, false discovery rate; NES, normalized enrichment score.

Figure S3

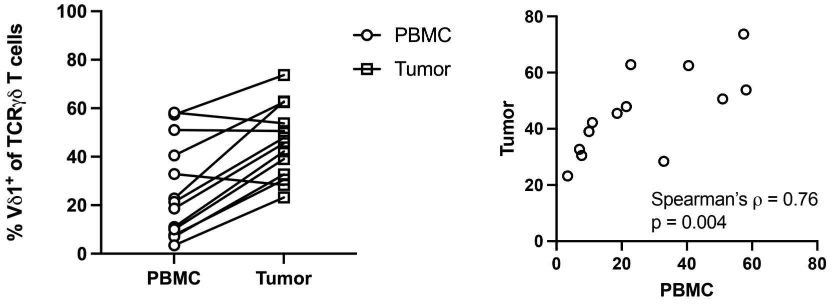

Figure S3: Frequency and correlation of Vδ1 in matched PBMCs and tumors. Frequency of Vδ1 γδ T cells was determined by flow cytometry (left), and Spearman's correlation was analyzed in matched PBMCs and tumors isolated from GIST patients (right), n=13. Spearman's ρ = 0.76, **P* =0.004.

Figure S4

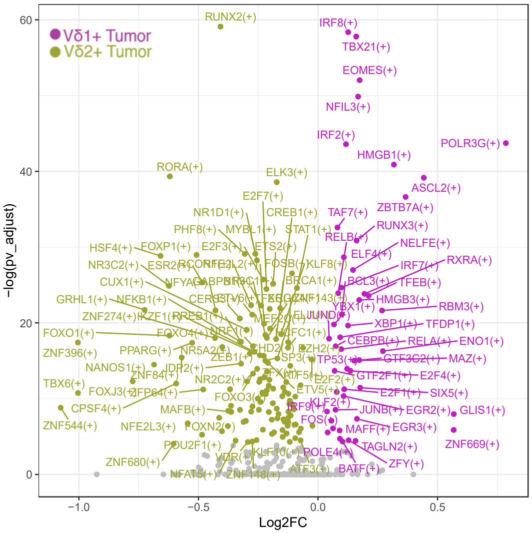

Figure S4: Transcriptional regulons of γδ T cells. γδ T cells were sorted from human PBMCs and GISTs and processed for 10x Genomics scRNA-seq. Differential transcriptional regulons as inferred by SCENIC are shown by volcano plot between tumor Vδ1 and Vδ2 cells.

Figure S5

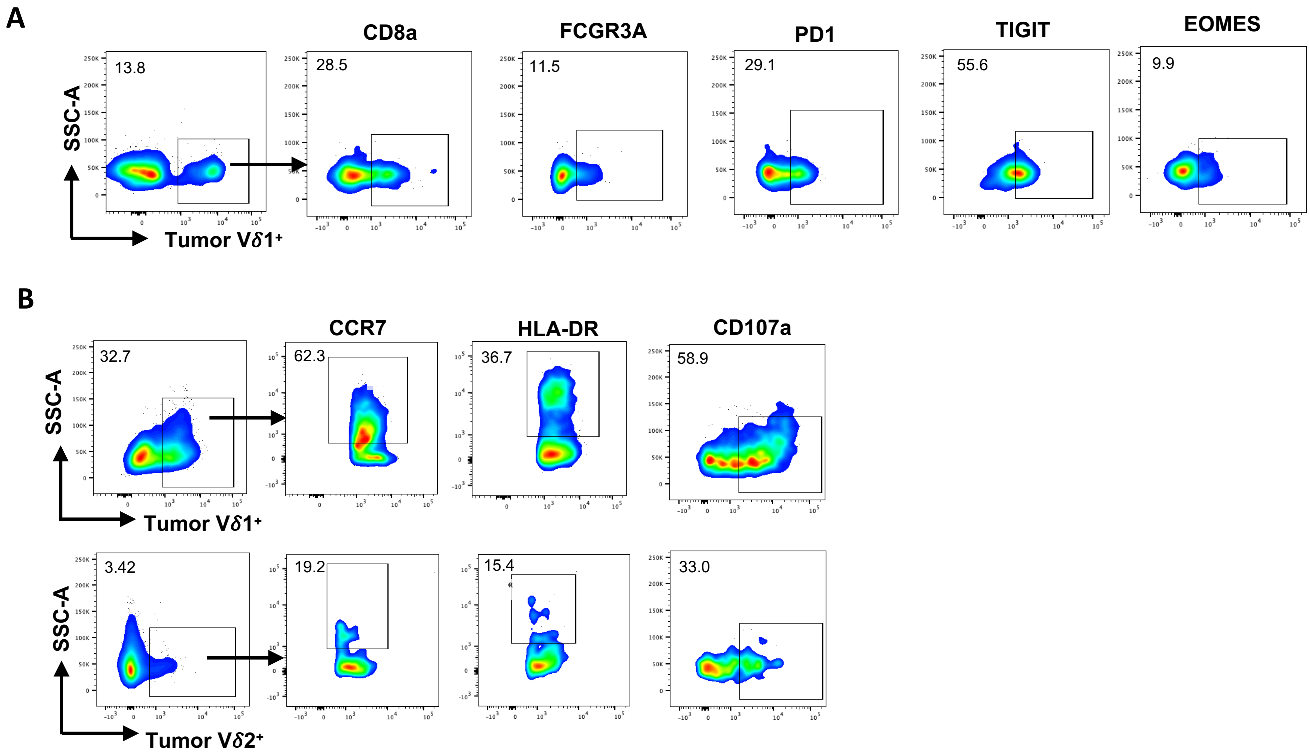

Figure S5: Tumor-infiltrating Vδ1 T cells have distinct function. Tumor single cell suspensions were isolated from untreated GIST patients. **A**) Representative flow plots of selected markers in tumor Vδ1 T cells (n=10). **B)** Representative flow plots showing selected marker expression in tumor Vδ1 T cells versus tumor Vδ2 T cells.

Figure S6

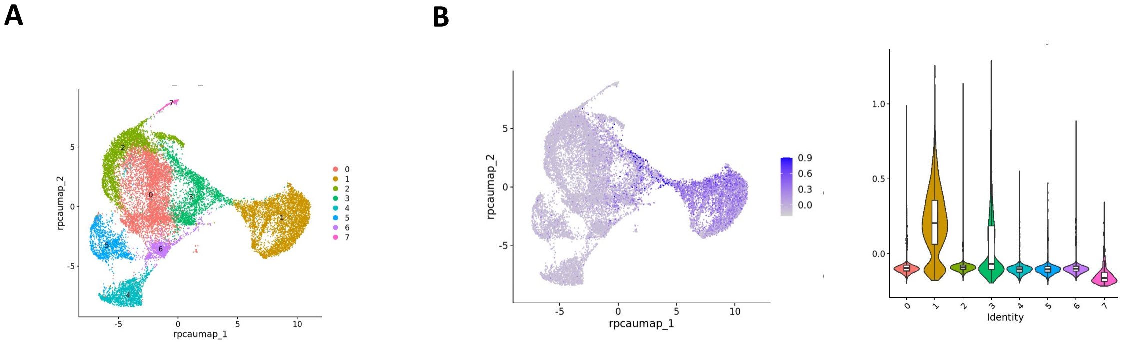

Figure S6: UMAP and subsets of γδ T cells in human GIST tumor scRNA-seq. Human GIST tumor specimens were processed for 10x Genomics scRNA-seq. **A**) UMAP of integrated T cells (n=19,453) identified in scRNA-seq data from untreated (n=3) and resistant (n=2) human GISTs. γδ T cells (n = 6,970 cells) were annotated based on canonical γδ T gene expression (TRDC, TRDV1, TRDV2, TRDV3. **B**) ***left***, γδ T cell module score projected onto UMAP; ***right***, Violin plot of γδ T cell module by cluster, identifying enrichment in clusters 1 and 3.

Figure S7

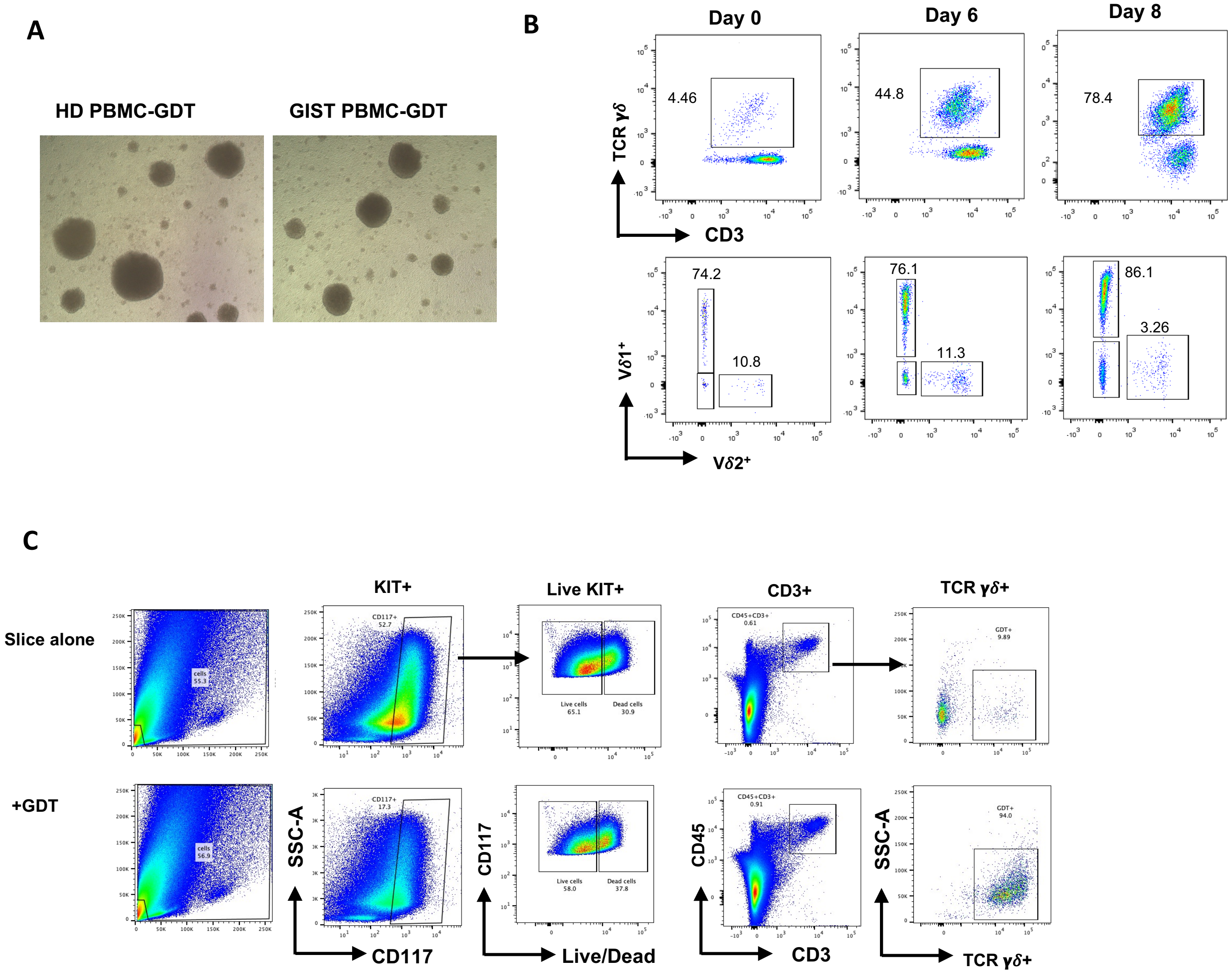

Figure S7: Expansion of γδ T cells (GDT) from PBMCs and cytotoxicity assays in human GIST tissue slices. A) Representative pictures showing γδ T cell expansion on day 6. γδ T cells from PBMCs of 4 healthy donors (HD) and from 3 untreated GISTs were expanded with an anti-γδ TCR antibody supplemented with IL-2 (200IU/ml). **B**) Representative flow plots illustrating the time course of γδ T expansion from PBMCs in a healthy donor. **C**) Gating strategy used to identify KIT⁺ tumor cells and γδ T cells in human GIST tissue slices co-cultured with expanded γδ T cells from a healthy donor at an E:T ratio of 5:1 for 24 hours *in vitro*.

Figure S8

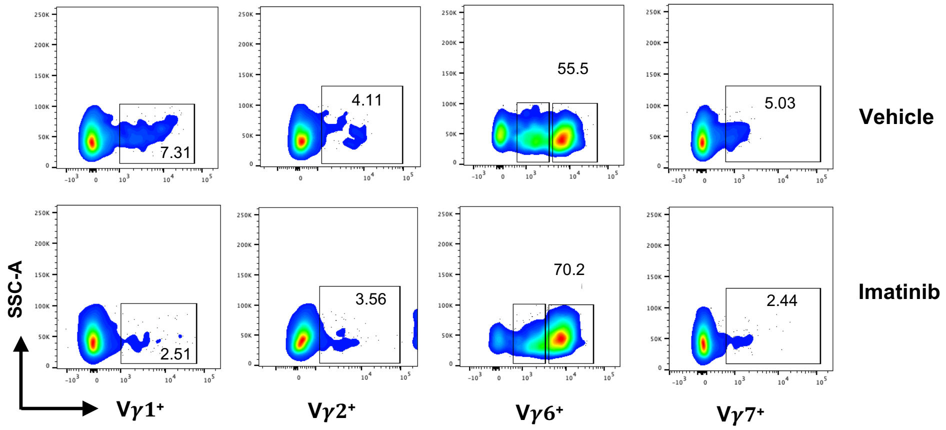

Figure S8: Imatinib changes γδ T cell subsets in *Kit*$^{V558del/+}$ tumors. Representative flow plots and frequency of Vγ1, Vγ2, Vγ6, and Vγ7 among tumor γδ T cells from *Kit*$^{V558del/+}$ mice treated with imatinib at 1 week. n=4-5/group.

Supplementary Table S1: Specific gene module and signature gene list for γδ T-cell clusters

Subtype Annotation	Gene Markers
Vδ1+-CTL response1/ 2	TRDV1, TRDV3, TRGV8, TRGV3,FCGR3A, NCR1, CD244, FCRL6, CD8A, CD63, CX3CR1, HLA-DRB1, GZMH, NKG7, KLRD1, KLRK1, KLRF1, KLRC2, TBX21, EOMES, TYROBP, IKZF2, TIGIT, PDCD1, IFNG
Vδ1+-Naive	TRDV1, TRGV2, CD27, TCF7, LEF1, LTB, XCL1, ZNF683, CCR7, MAL, ANK1, SOX4, CXCR3, KLRC2, IGFBP2
Vδ1+-Inflammatory like	TRDV1, TRGV8, TRGV5, FCGR3A, NCR1, CD244, FCRL6, CX3CR1, CD69, KLRK1 (NKG2D), KLRD1, KLRC2, BTN3A1, IL2RB, TBX21, EOMES, IKZF2, ZEB2, RORA, RUNX1, HAVCR2 (TIM3), TNFRSF9, IFNG, SLAMF6, AOAH, PRKCH, PIK3AP1, TIGIT, ITGAD, LYN
Vδ1+- Innate Activation	TRDV1, TRGV2, CXCR3, CD63, KLRC3, NCR1, CD8A, IL2RB, ZNF683, XCL1, IFNG, IFNG-AS1, HSPA1A, TUBA1A
Vδ1+- Differentiation	TRDV1, TRGV2, TRGV10, CXCR3, CCR7, TIGIT, PDCD1, EOMES, TCF7, MAL, SBK2, GZMK
Vδ1+- Proliferating	MKI67 (Ki67), CDK1, CCNA2, CDC20, AURKB, STMN1, ENTPD1 (CD39), CD1a, PDCD1LG, CXCR3, HLA-DRB1
Vδ3+	TRDV3, TRGV4, TRGV8, CD8A, GZMH, GNLY, KLRC2, TBX21, TYROBP, ZNF683, XCL1, KIR2DL1, KIR3DL3, KIR2DL4, CD101 (IGSF2), CD52
Vδ2+-CTL response	TRDV2, TRGV9, CEBPD, KLRB1, GZMK, KLRC1, IL7R, IL23R, ANXA1, ZBTB16, RORC, IGFBP4, S100B, TNFRSF25, CCR6, CXCR6, IL12RB2, IL18RAP, NR1D1
Vδ2+-Type 3 immunity	TRDV2, TRGV9, RORA, IL7R, IL23R, BTN3A1, DPP4, CCR6, CD44, CD69, CXCR6, AREG,IFNGR1, IL17RA, CAMK4, THEMIS, ZBTB16, SYNE2, CCR7, CDC14A, ANK3, DUSP16, RUNX1, IFNG-AS1, KLRC1, IL12RB1, ITGA1 (CD49a), KLRG1, SLAMF6, NFATC2, IL18RAP, CD28, IGSF9B, MAF
Vδ2+-Naïve	TRDV2, TRGV9, TRGV10, GNLY, KLRC1, IL7R, GPR27, HOXB6, SELL, S100B
Vδ2+-Differentiation	TRDV2, TRGV9, SELL, CCL4, TNF, S100B

Supplementary Table S2: Immune score markers for memory, exhaustion, activation and cytotoxicity by using ImmuneSigDB or other published studies

Immune Profile	Gene Markers
Memory Score	IL7R, CCR7, ZNF683, TCF7, LEF1, CXCR3
Exhaustion Score	PDCD1, HAVCR2, TIGIT, LAG3, CD244, CD160
Proliferation Score	MKI67, STMN1, TYMS, TOP2A, PCNA, CD74
Activation Score	IL2RB, IL2RA, HLA-DRA, HLA-DRB, CD27, CD226, TNFRSF9
Cytotoxicity Score	GZMH, NKG7, KLRD1, GNLY, PRF1

Additional supporting data files in this study:

1. Data file s1: All patient cohort information used in this study (GDT_Patient_data_file_s1)
2. Data file s2: Differentially expressed genes between γδ T cells in untreated (n=3) and resistant (n=2) GISTs based on scRNA-seq data (GDT_untx_v_res_DEG_data_file_s3)
3. Data file s3: Over representation enrichment analysis between γδ T cells in untreated (n=3) and resistant (n=2) GISTs based on scRNA-seq data (GDT_ORA_anlysis_data file_s2)
4. Data file s4: All supporting data for figures (Figure_Supporting_Data_file_s4)