Supplementary Table 1 – Antibodies for immunophenotyping

Marker	Clone	Fluorochrome	Supplier	Catalogue
Checkpoint				
CD45	HI30	BUV496	BD Biosciences	750179
CD366 (TIM-3)	7D3	BUV737	BD Biosciences	748820
CD3	UCHT1	BUV805	BD Biosciences	612895
CD152 (CTLA-4)	BNI3	BV421	BD Biosciences	562743
CD25	M-A251	BV480	BD Biosciences	566102
CD4	SK3	BV650	BD Biosciences	563875
TIGIT	741182	BV711	BD Biosciences	747839
CD279 (PD-1)	EH12.1	BB660-P	BD Biosciences	624295
CD56	B159	BB790-P	BD Biosciences	624296
FoxP3	236A/E7	PE-CF594	BD Biosciences	563955
CD127	HIL-7R-M21	PE-Cy7	BD Biosciences	560822
CD223 (LAG3)	T47-530	APC-R700	BD Biosciences	565774
CD8	SK1	APC-H7	BD Biosciences	560179

Supplementary Table 2 – Gene expression datasets used for analysis of TIM-3 in CML LSCs and normal HSCs

GEO accession number	Phenotype	Phases	Condition	No. of CML patients vs Control	Platform	Reference	PMID
GSE47927	CD34+CD38-lin-CD45RA-CD90+	CP, AP and BC	diagnosis	12 vs 3	Affymetrix Human Gene 1.0 ST Array	Cramer-Morales K et al, 2013	23836560
GSE43754	CD34+CD38-ALDH high	CP	diagnosis	5 vs 5	Affymetrix Human Exon 1.0 ST Array	Gerber JM et al, 2013	23651669
GSE97562	CD34+CD38-lin-	CP	diagnosis	5 vs 5	Affymetrix Human Gene 1.0 ST Array	Avilés-Vázquez Set al,2017	29030909
GSE76312	Lin-CD34+ CD38-	CP	diagnosis	18 (477 single cells) vs 6 (232 single cells)	scRNA-seq HiSeq2000 & 4000	Giustacchini A et al 2017	28504724

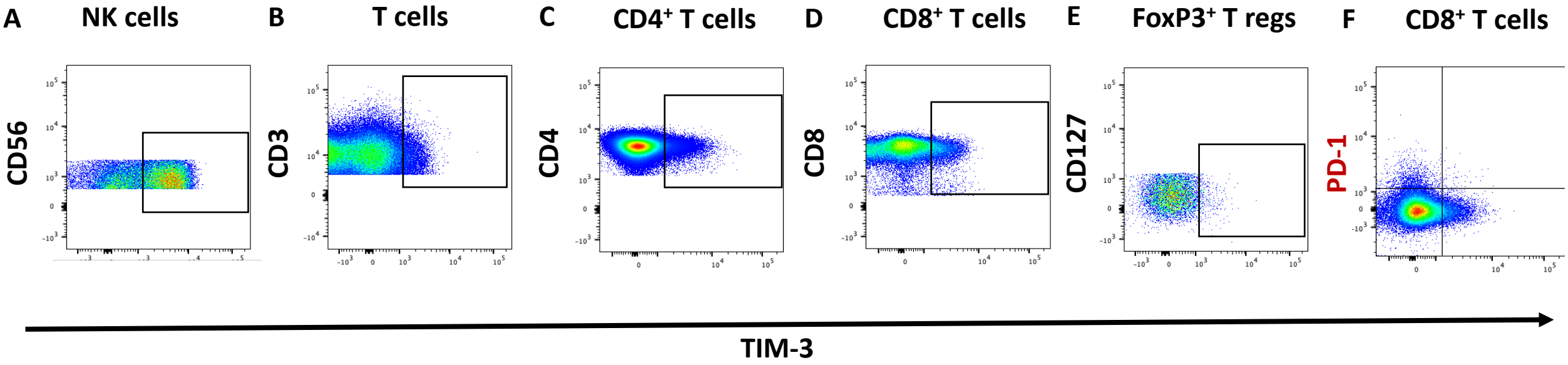
Reference

[12] Cramer-Morales, K. *et al.* Personalized synthetic lethality induced by targeting RAD52 in leukemias identified by gene mutation and expression profile. *Blood* **122**, 1293-1304 (2013).

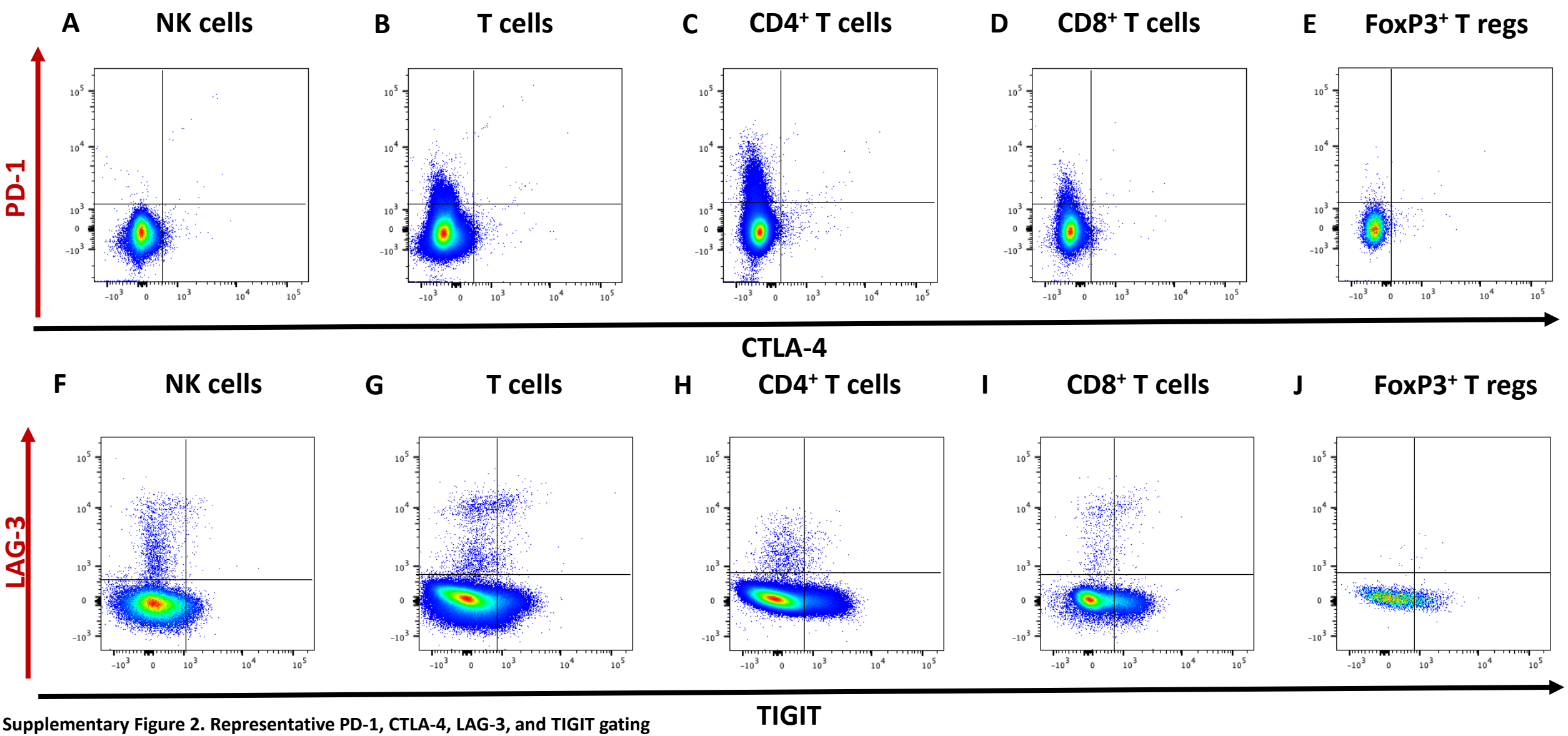
[13] Gerber, J. M. *et al.* Genome-wide comparison of the transcriptomes of highly enriched normal and chronic myeloid leukemia stem and progenitor cell populations. *Oncotarget* **4**, 715-728 (2013).

[14] Aviles-Vazquez, S. *et al.* Global gene expression profiles of hematopoietic stem and progenitor cells from patients with chronic myeloid leukemia: the effect of in vitro culture with or without imatinib. *Cancer Med* **6**, 2942-2956 (2017).

[15] Giustacchini, A. *et al.* Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. *Nature medicine* **23**, 692-702 (2017).

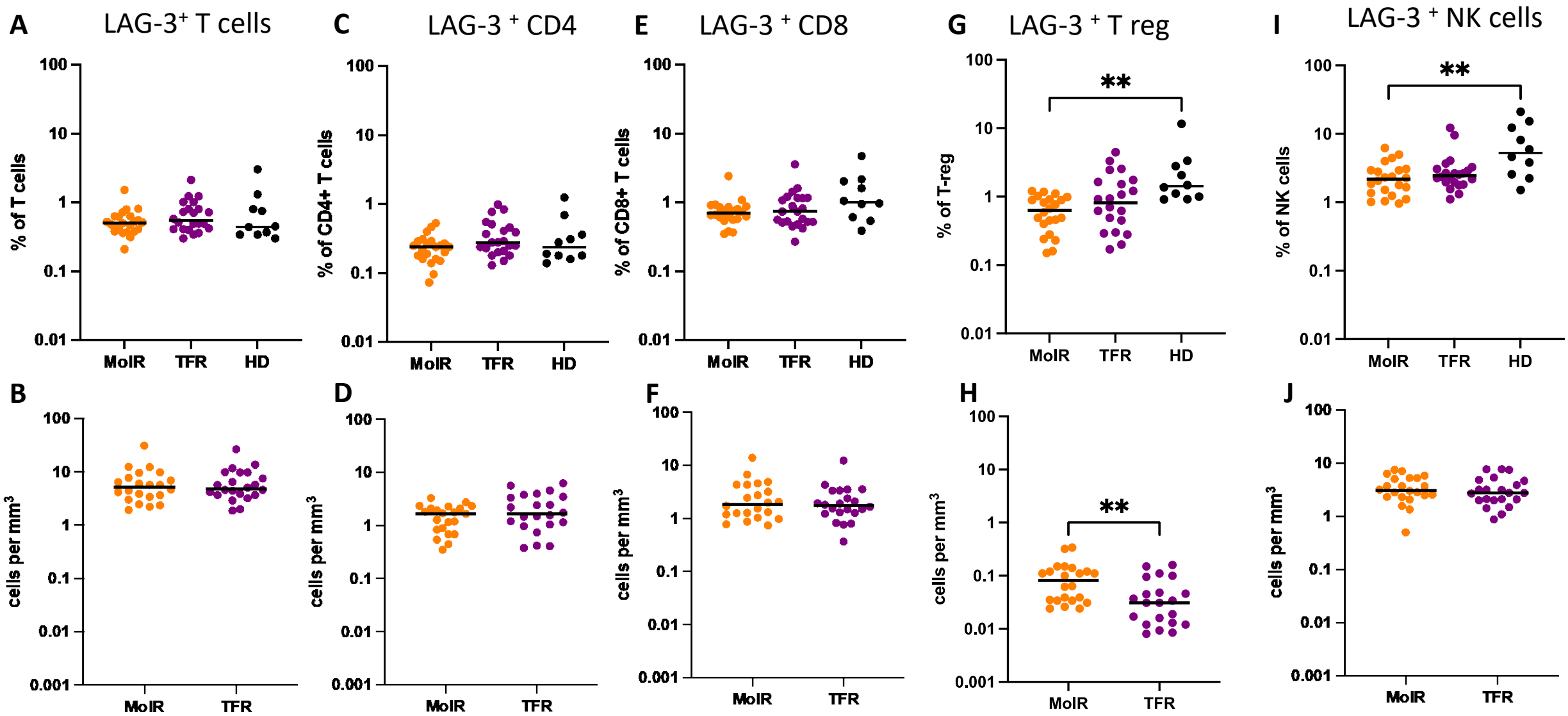


Supplementary Figure 1. Representative gating for TIM-3
Lymphocytes were gated from the forward and side scatter characteristics. Single cells were gated from forward scatter height vs forward scatter area, and viable cells were then gated using the fixable viability stain FVS575V, this population was used for subsequent gating. Plots show representative TIM-3 expression in **(A)** NK cells ($CD3^{-}$ $CD56^{dim}$), **(B)** $CD3^{+}$ T-cells, **(C)** CD4+ T-cells, **(D)** CD8+ T-cells and **(E)** regulatory T-cells ($CD3^{+}$ $CD4^{+}$ $CD25^{+}$ $CD127^{-}$ $FoxP3^{+}$), **(F)** PD-1 and TIM-3 co-expression in $CD8^{+}$ T cells.



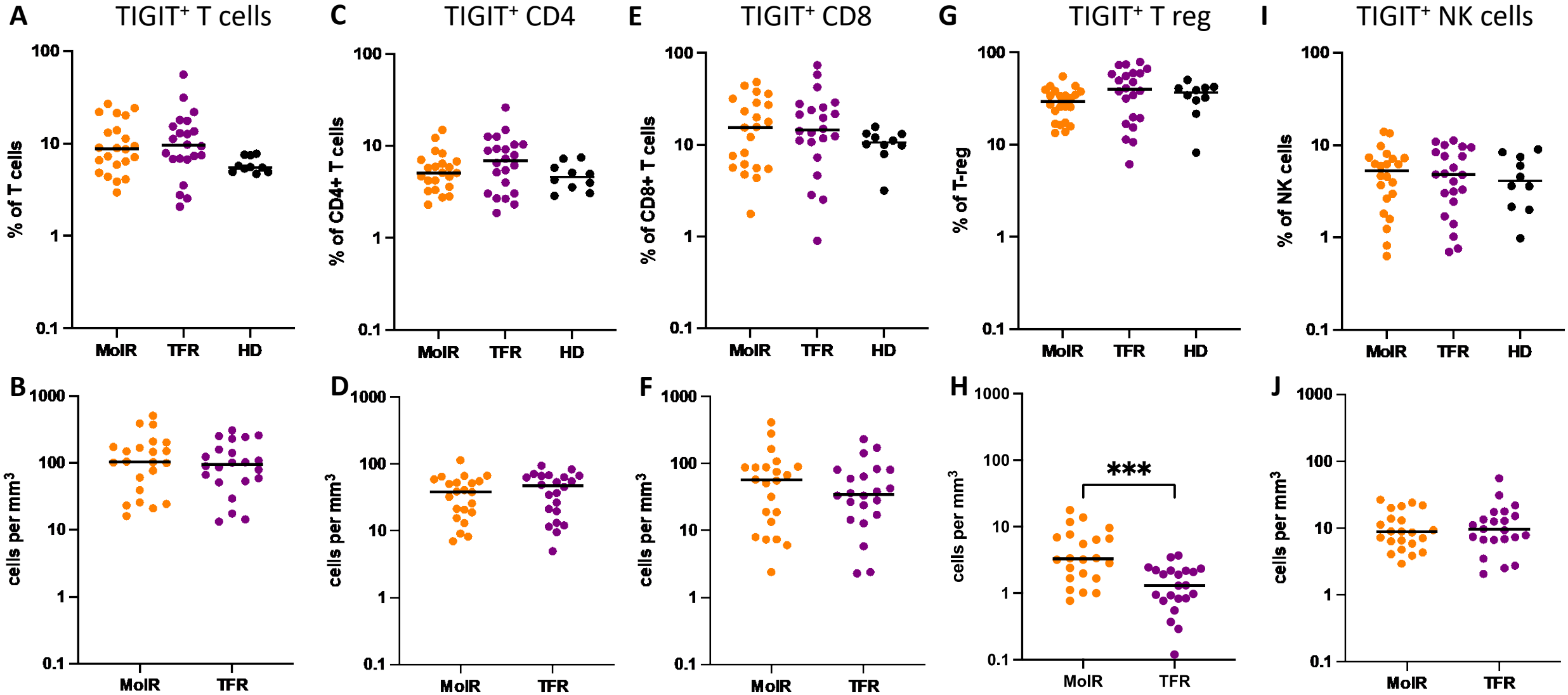
Supplementary Figure 2. Representative PD-1, CTLA-4, LAG-3, and TIGIT gating

Lymphocytes were gated from the forward and side scatter characteristics. Single cells were gated from forward scatter height vs forward scatter area, and viable cells were then gated using the fixable viability stain FVS575V, this population was used for subsequent gating. Plots show representative PD-1 (y-axis) and CTLA-4 (x-axis) expression in **(A)** NK cells ($CD3^-$ $CD56^{dim}$), **(B)** $CD3^+$ T-cells, **(C)** CD4+ T-cells, **(D)** CD8+ T-cells and **(E)** regulatory T-cells ($CD3^+$ $CD4^+$ $CD25^+$ $CD127^-$ $FoxP3^+$), and representative LAG-3 (y-axis) and TIGIT (x-axis) expression in **(F)** NK cells ($CD3^-$ $CD56^{dim}$), **(G)** $CD3^+$ T-cells, **(H)** CD4+ T-cells, **(I)** CD8+ T-cells and **(J)** regulatory T-cells ($CD3^+$ $CD4^+$ $CD25^+$ $CD127^-$ $FoxP3^+$).



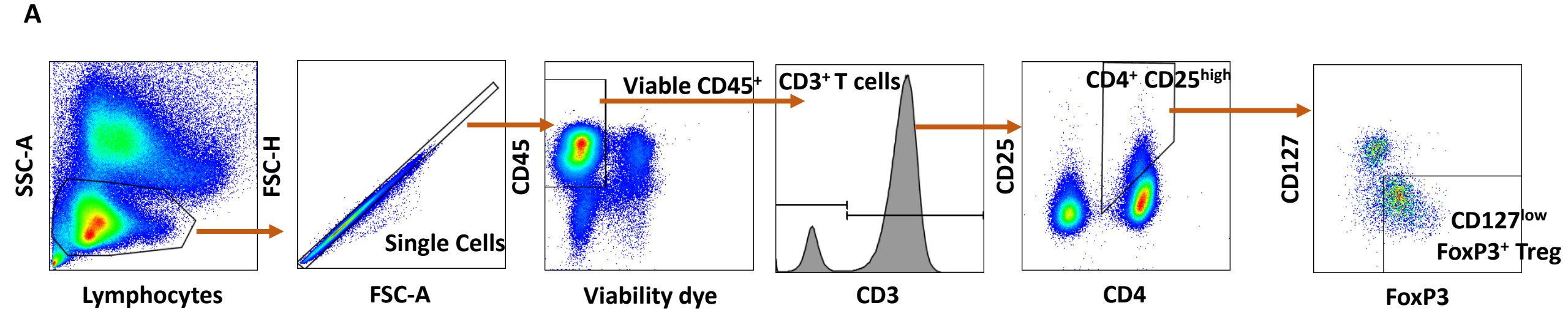
Supplementary Figure 3. LAG-3 expression in MoIR and TFR patients at the time of TKI cessation

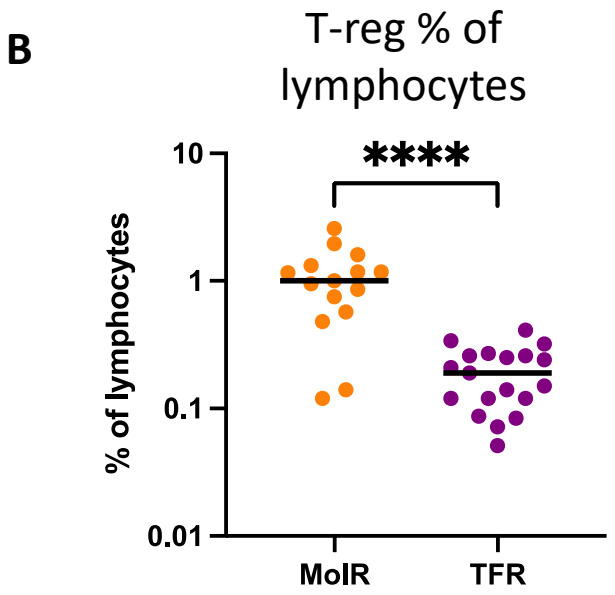
There was no statistically significant difference in LAG-3 expression between MoIR, TFR patients, or healthy donors (HD) **(A)** as a percentage of T cells or **(B)** absolute count of LAG-3$^+$ T cells, **(C)** percentage of CD4$^+$ T-cells or **(D)** absolute count of LAG-3$^+$ CD4$^+$ T-cells, **(E)** percentage of CD8$^+$ T-cells or **(F)** absolute count of LAG-3$^+$ CD8$^+$ T-cells. **(G)** LAG3$^+$ T-regs as a percentage of T-regs were significantly higher in HD compared with MoIR patients. **(H)** The absolute count of LAG-3$^+$ T-regs was significantly higher in the MoIR group compared with the TFR group, most likely due to significantly higher absolute counts of T-regs in this group. **(I)** LAG-3 expression as a percentage of NK cells was significantly higher in HD compared with MoIR patients and **(J)** absolute count of LAG-3$^+$ NK cells was not different between MoIR and TFR patients. The Kruskal-Wallis test was used to compare 3 groups and the Mann-Whitney U test was used to compare two groups, alpha was set at 0.05, **p<0.01.

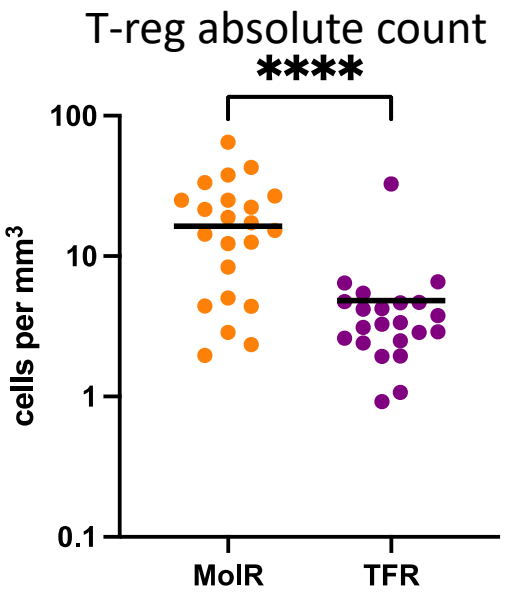


Supplementary Figure 4. TIGIT expression in MolR and TFR patients at the time of TKI cessation

There was no statistically significant difference in TIGIT expression between MolR, TFR patients, or healthy donors (HD) **(A)** as a percentage of T cells or **(B)** absolute count of TIGIT+ T cells, **(C)** percentage of CD4+ T-cells or **(D)** absolute count of TIGIT+ CD4+ T-cells, **(E)** percentage of CD8+ T-cells or **(F)** absolute count of TIGIT+ CD8+ T-cells, **(G)** percentage of T-regs. **(H)** The absolute count of TIGIT+ T-regs was significantly higher in the MolR group compared with the TFR group, most likely due to significantly higher absolute counts of T-regs in this group. **(I)** TIGIT expression as a percentage of NK cells or **(J)** absolute count of TIGIT+ NK cells was not different between MolR and TFR patients. The Kruskal-Wallis test was used to compare 3 groups and the Mann-Whitney U test was used to compare two groups, alpha was set at 0.05, ***p<0.001.







Supplementary Figure 5. Representative gating of regulatory T-cells and association with relapse

(A) Representative gating of T-regs, lymphocytes were gated from the forward and side scatter characteristics. Single cells were gated from forward scatter height vs forward scatter area, and viable cells were then gated using the fixable viability dye FVS575V. $CD3^+$ T-cells were gated followed by $CD4^+CD25^{high}$. Finally, $CD127^{neg/low}$ and $FoxP3^+$ regulatory T-cells were gated. **(B)** T-regs were significantly increased in the molR group both as a percentage of lymphocytes as well as absolute counts. The Mann-Whitney U test was used to compare group, alpha was set at 0.05, ****p<0.0001