TCL1: A shared tumor-associated antigen for immunotherapy against B-cell lymphomas

Weng et al.

Supplementary Figure legends

Supplementary Figure 1. TCL1 expression in B cell progenitors, mature B cell subsets, and immature T cells. (A) TCL1 expression in hematopoietic stem cells (HSC - $CD19^{-}CD20^{-}CD10^{-}CD34^{+}$), pro-B cells ($CD19^{+}CD20^{-}CD10^{+}CD34^{+}$), pre-B cells ($CD19^{+}CD20^{-}CD10^{+}CD34^{-}$), and immature B cells ($CD19^{+}CD20^{+}CD10^{-}CD34^{-}$) in normal human bone marrow samples, and primary mantle cell lymphoma tumor cells was determined by flow cytometry and compared to autologous non-B cells or isotype antibody staining as control. Mean fluorescence intensity of each gated subset is indicated on right. Representative data from one of three bone marrow samples tested is shown. (B) TCL1 expression in naïve B cells ($CD19^{+}IgD^{+}CD10^{-}CD27^{-}CD38^{-}$), germinal center (GC) B cells ($CD19^{+}IgD^{-}CD10^{+}CD27^{-}CD38^{int}$), memory B cells ($CD19^{+}IgD^{-}CD10^{-}CD27^{+}CD38^{-}$), and plasma cells ($CD19^{+}IgD^{-}CD10^{-}CD27^{+}CD38^{hi}$) in normal tonsils was determined by flow cytometry and compared to autologous non-B cells as control. Mean fluorescence intensity of each gated subset is indicated on right. Representative data from one of three tonsil samples tested is shown. (C) TCL1 expression was determined by flow cytometry in 18-week-old human fetal thymocytes. *Top panel*. Gating strategy for Double Negative (DN), Double Positive (DP), and Single Positive (SP) CD4 and CD8 thymocytes is shown. *Bottom panel*. TCL1 expression (open histogram) in thymocyte subsets as compared with isotype control antibody (grey

histogram) is shown. Representative data from one of two fetal thymus tissues tested is shown.

Supplementary Figure 2. Percentage of TCL1-specific T cells in T cell lines generated from normal donors. T cell lines generated from three HLA-$A2^+$ normal donors using $TCL1_{65-79}$ peptide were incubated with autologous CD3-depleted PBMC as antigen-presenting cells in the presence or absence of the $TCL1_{65-79}$ peptide, and intracellular cytokine assay was performed. The percentage of $CD4^+$ and $CD8^+$ T cells producing IFN-γ above the background are shown.

Supplementary Figure 3. Avidity of TCL1-specific CTL. $TCL1_{71-78}$ tetramer positive T cells were isolated from a TCL1-specific T cell line by MACS using $TCL1_{71-78}$ tetramer PE and anti-PE microbeads. The isolated T cells were incubated with T2 cells pulsed with various concentrations of $TCL1_{65-79}$ peptide and IFN-γ production was determined in the supernatants by ELISA after 18 hours. Dot plot shows purity of sorted T cells. Representative data from one of two experiments.

Supplementary Figure 4. Phenotype and specificity of TCL1-specific CTL. (A) Phenotypic analysis of $CD8^+$ T cells purified from $TCL1_{65-79}$ peptide-specific T cell lines was performed with CD16 PE, CD56 PE, CD3 PerCP-Cy5.5, and CD8 FITC antibodies. *Top panel*. The percentage of T cells (CD3+), NK cells (CD3-CD16+CD56+), and CD8 T cells is shown. Representative data from one of three lines is shown. *Bottom* panel. Normal donor (ND) PBMC were used as control. (B) $CD8^+$ T cells purified from a $TCL1_{65-79}$ peptide-specific T cell line were incubated with HLA-$A2^+$ normal human bone marrow-derived $CD34^+$ hematopoietic stem cells; normal tonsil-derived naïve B cells, germinal center (GC) B cells, or memory B cells; Jeko-1 cells; and primary follicular

lymphoma tumor cells (FL5). A 4-hour ^{51}Cr-release cytotoxicity assay was performed. The percent specific lysis is shown. Representative data from one of three lines is shown.

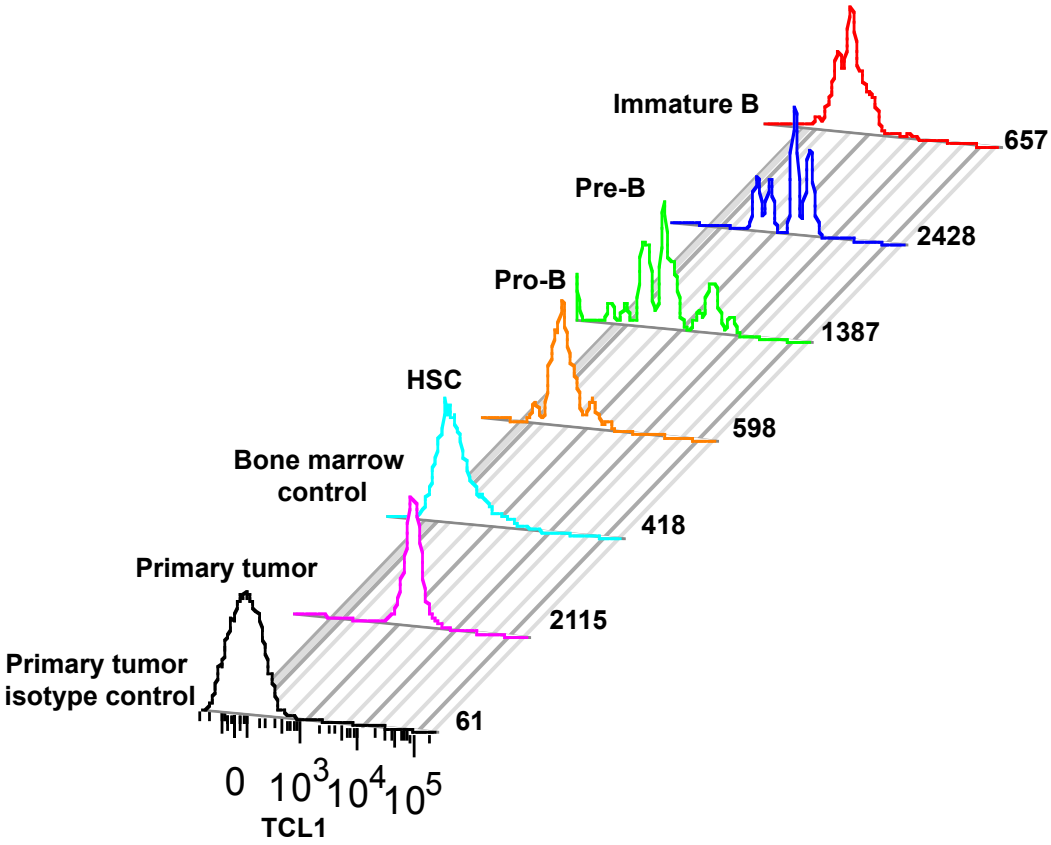
Supplementary Figure 5. Expression of coinhibitory molecules on TCL1-specific CTL derived from lymphoma patients. Expression of coinhibitory molecules was determined by flow cytometry on $TCL1_{71-78}$ tetramer^{+} $CD8^{+}$ T cells (open histogram) and compared with $TCL1_{71-78}$ tetramer^{-} $CD8^{+}$ T cells (grey histogram). Representative data from one of three lymphoma patients is shown.

Supplementary Table 1. Overlapping peptides derived from TCL1

Peptide name	Position	Sequence
P1	1-15	MAECPTLGEAVTDHP
P2	5-20	PTLGEAVTDHPDRLW
P3	9-24	EAVTDHPDRLWAWEK
P4	13-28	DHPDRLWAWEKFVYL
P5	17-32	RLWAWEKFVYLDEKQ
P6	21-36	WEKFVYLDEKQHAWL
P7	25-40	VYLDEKQHAWLPLTI
P8	29-44	EKQHAWLPLTIEIKD
P9	33-48	AWLPLTIEIKDRLQL
P10	37-52	LTIEIKDRLQLRVLL
P11	41-56	IKDRLQLRVLLRRED
P12	45-60	LQLRVLLRREDVVLG
P13	49-64	VLLRREDVVLGRPMT
P14	53-68	REDVVLGRPMTPTQI
P15	57-72	VLGRPMTPTQIGPSL
P16	61-76	PMTPTQIGPSLLPIM
P17	65-80	TQIGPSLLPIMWQLY
P18	69-84	PSLLPIMWQLYPDGR
P19	73-88	PIMWQLYPDGRYRSS
P20	77-92	QLYPDGRYRSSDSSF
P21	81-96	DGRYRSSDSSFWRLV
P22	85-100	RSSDSSFWRLVYHIK
P23	89-104	SSFWRLVYHIKIDGV
P24	93-108	RLVYHIKIDGVEDML
P25	97-112	HIKIDGVEDMLLELL
P26	101-116	DGVEDMLLELLPDD

Supplementary Figure1

A



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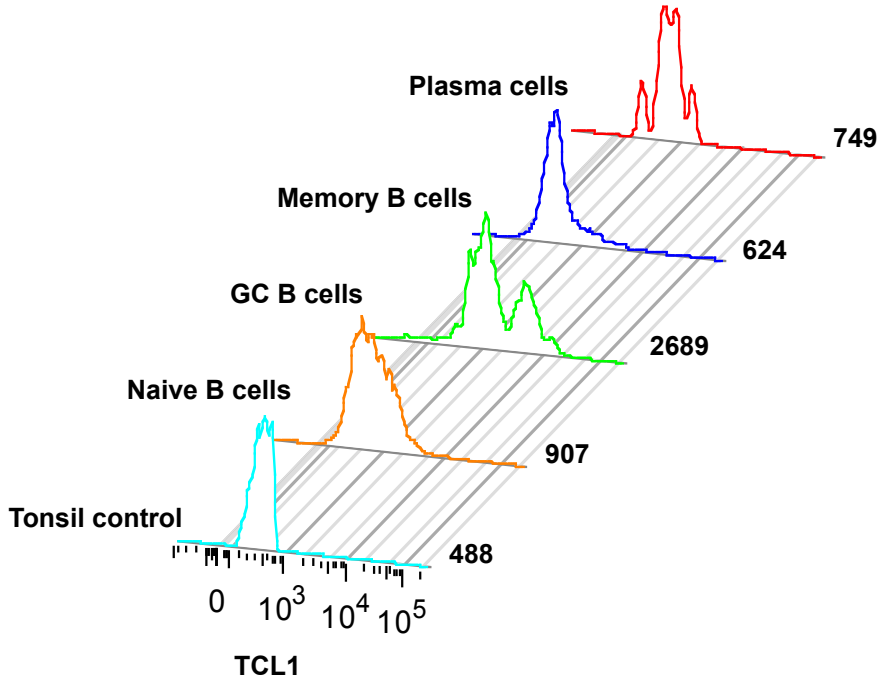

Supplementary Figure1 (contd.)

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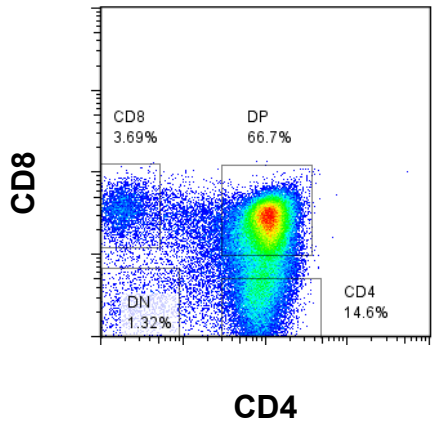
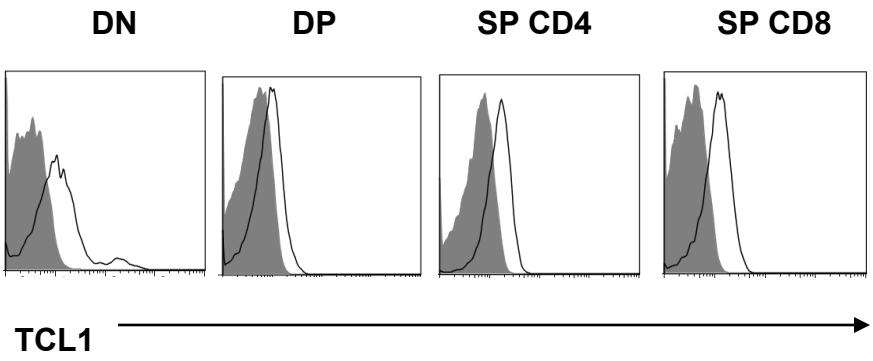
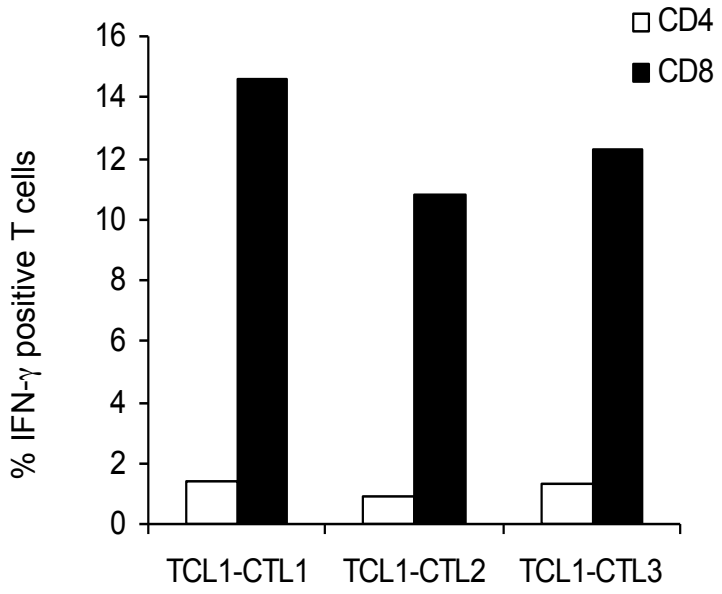
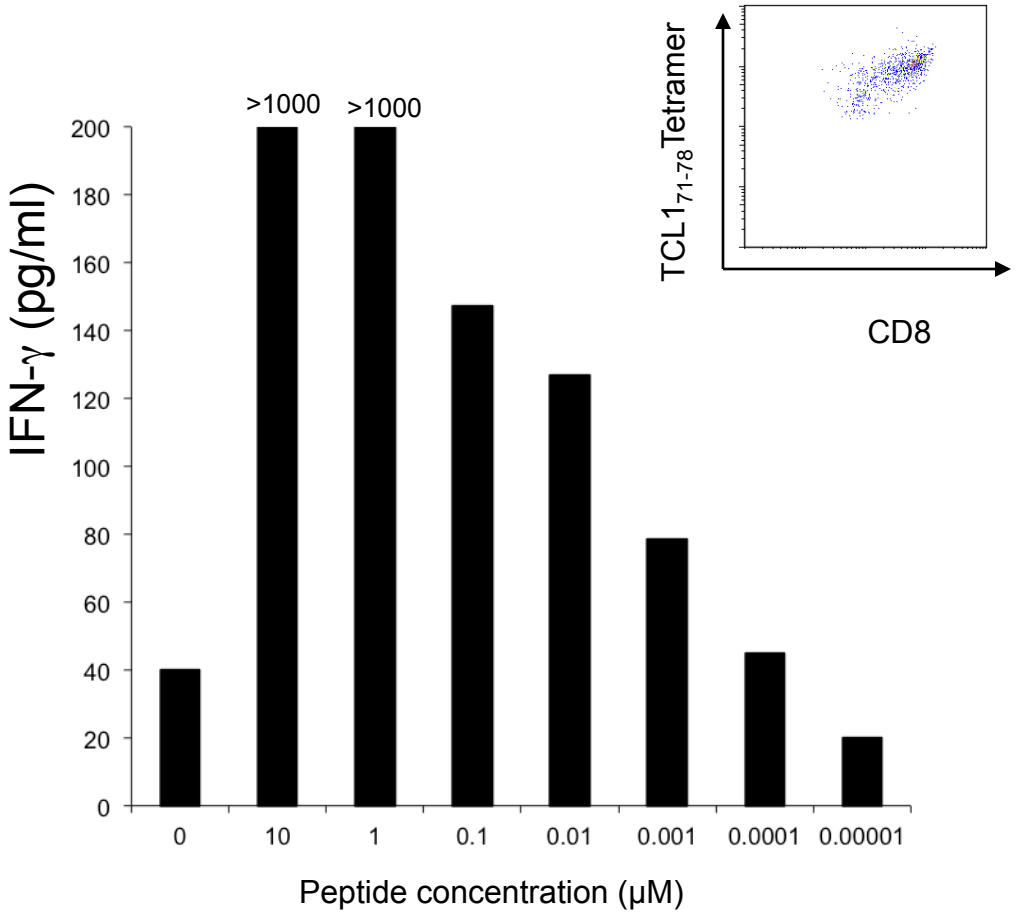




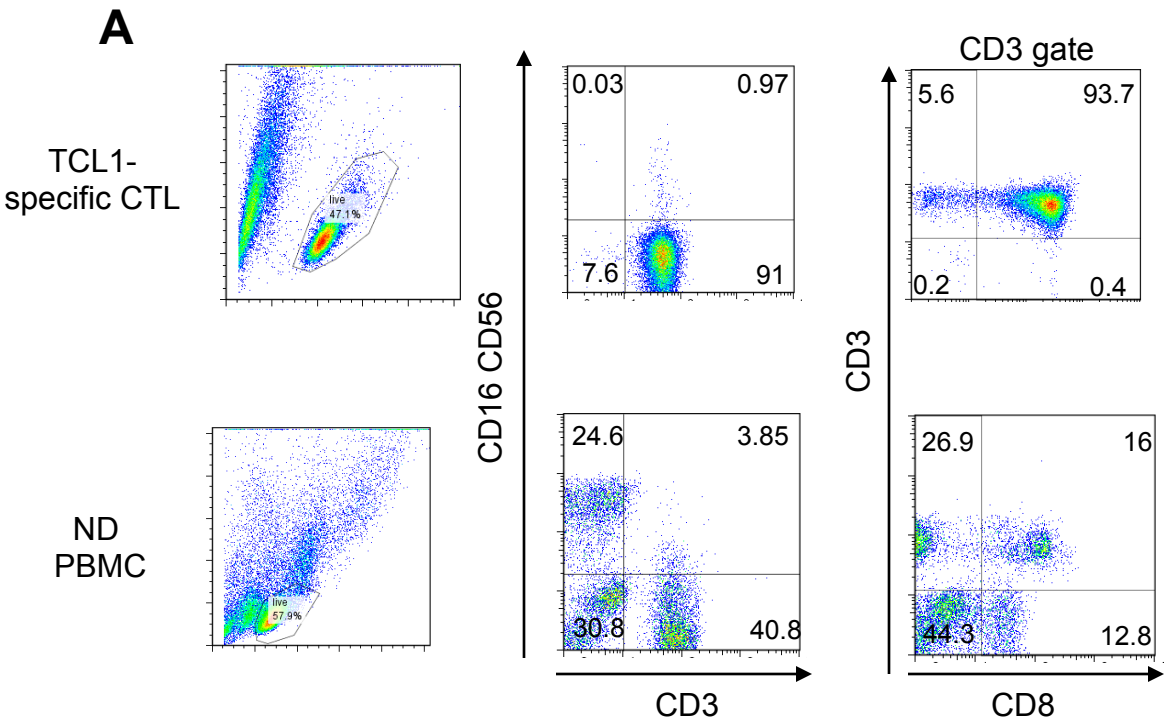
Supplementary Figure 2

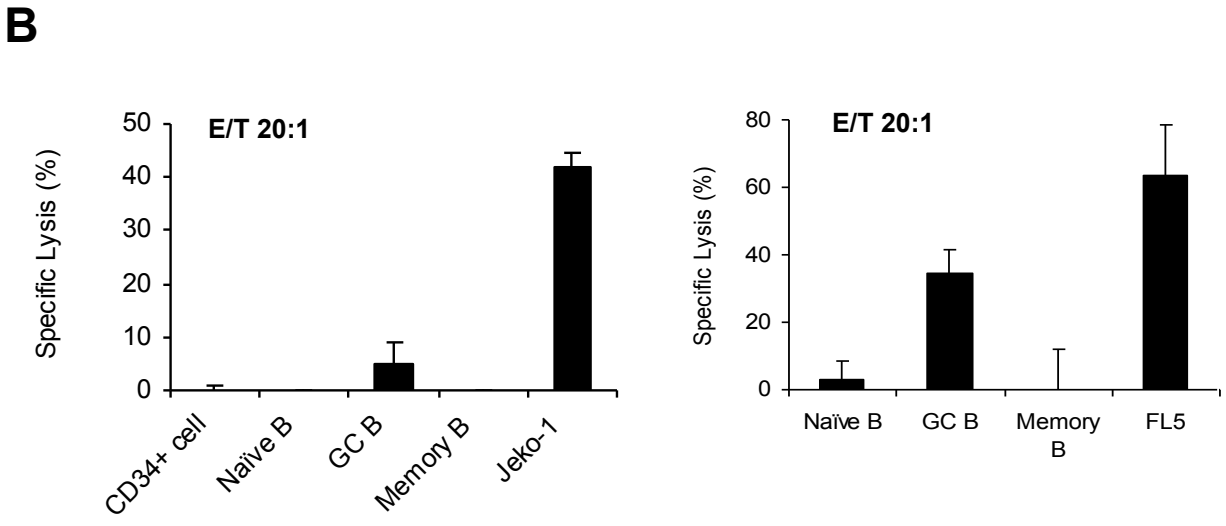


Supplementary Figure 3



Supplementary Figure 4





Supplementary Figure 5