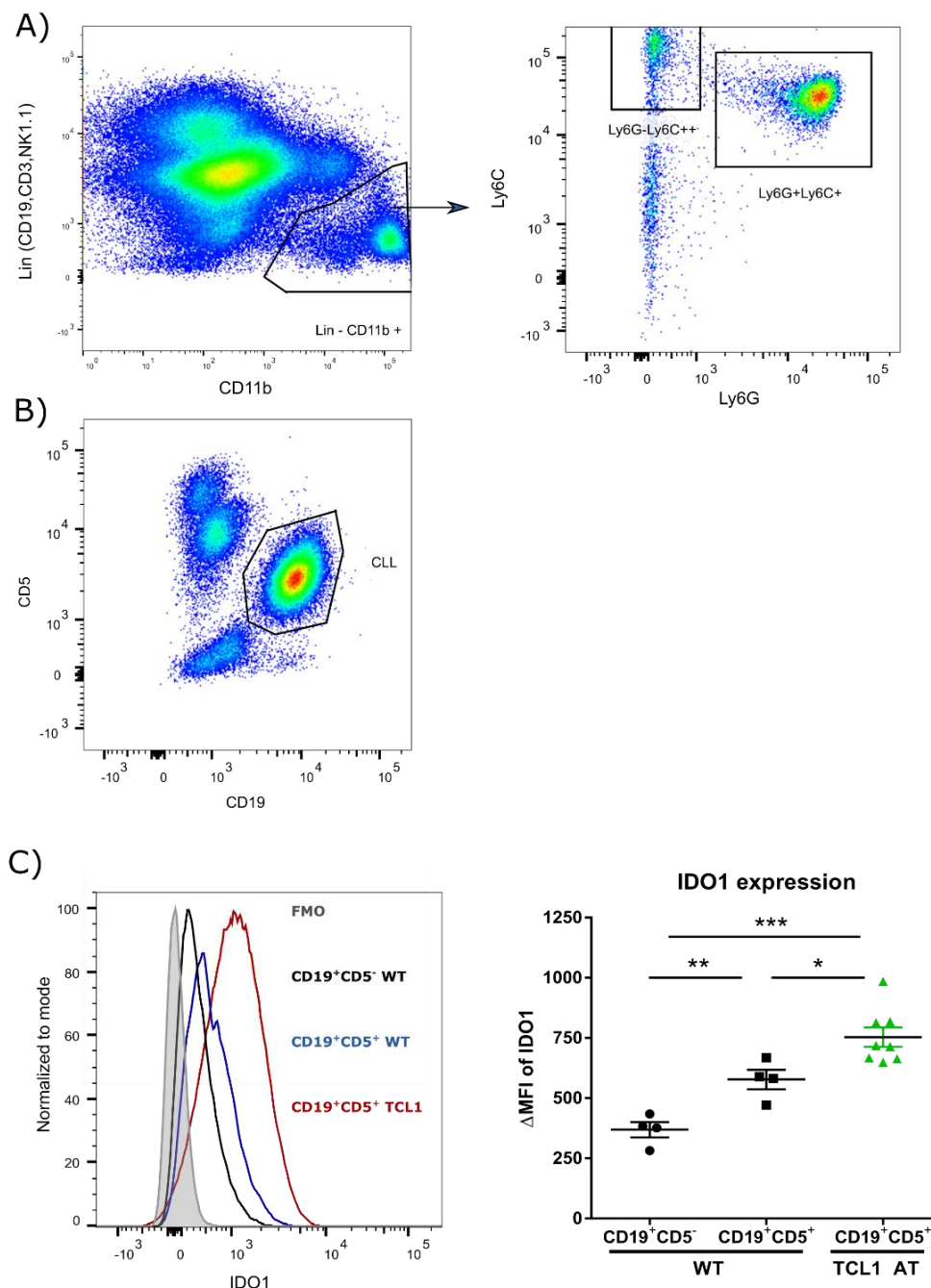
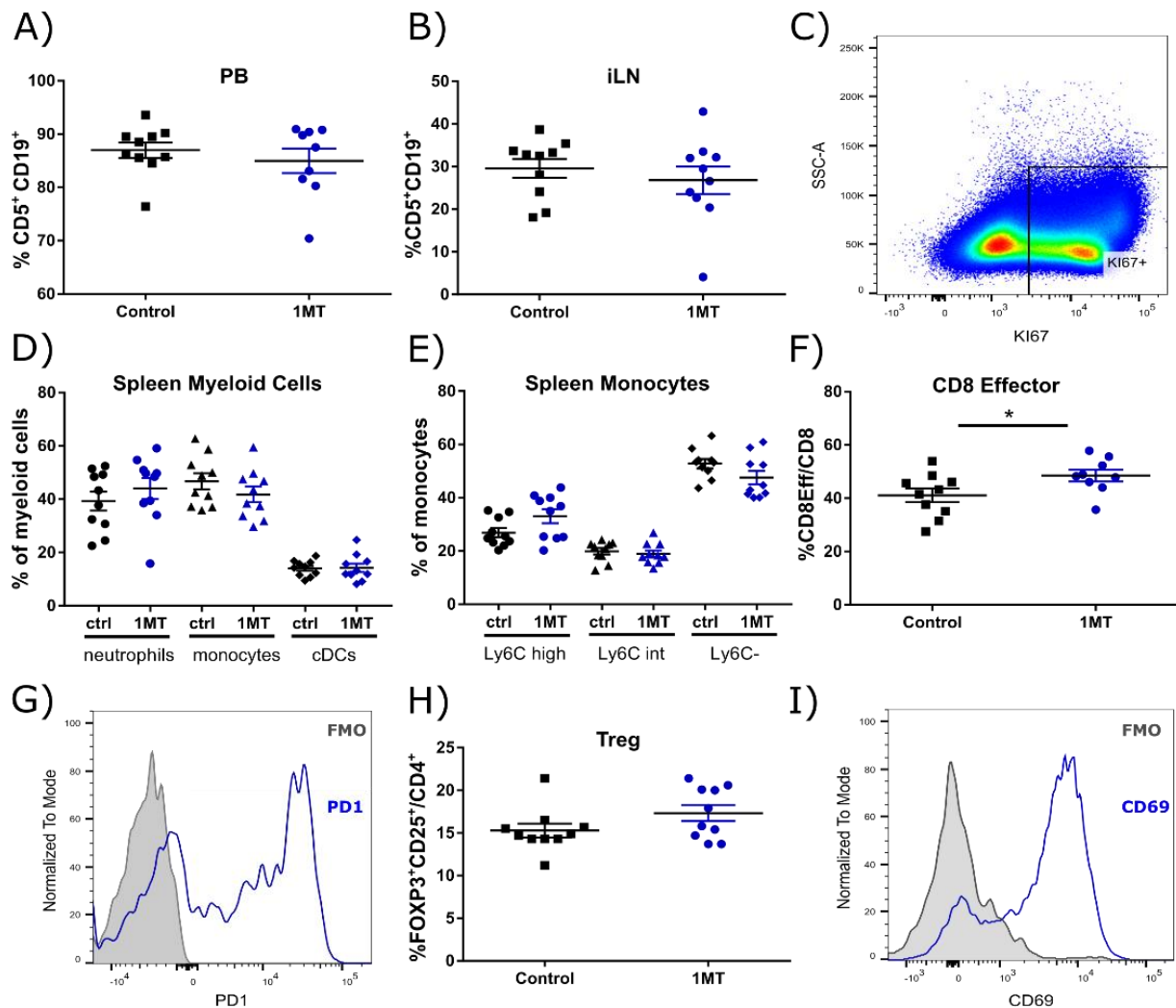


# Supplementary Materials: IDO1-Targeted Therapy Does Not Control Disease Development in the E $\mu$ -TCL1 Mouse Model of Chronic Lymphocytic Leukemia

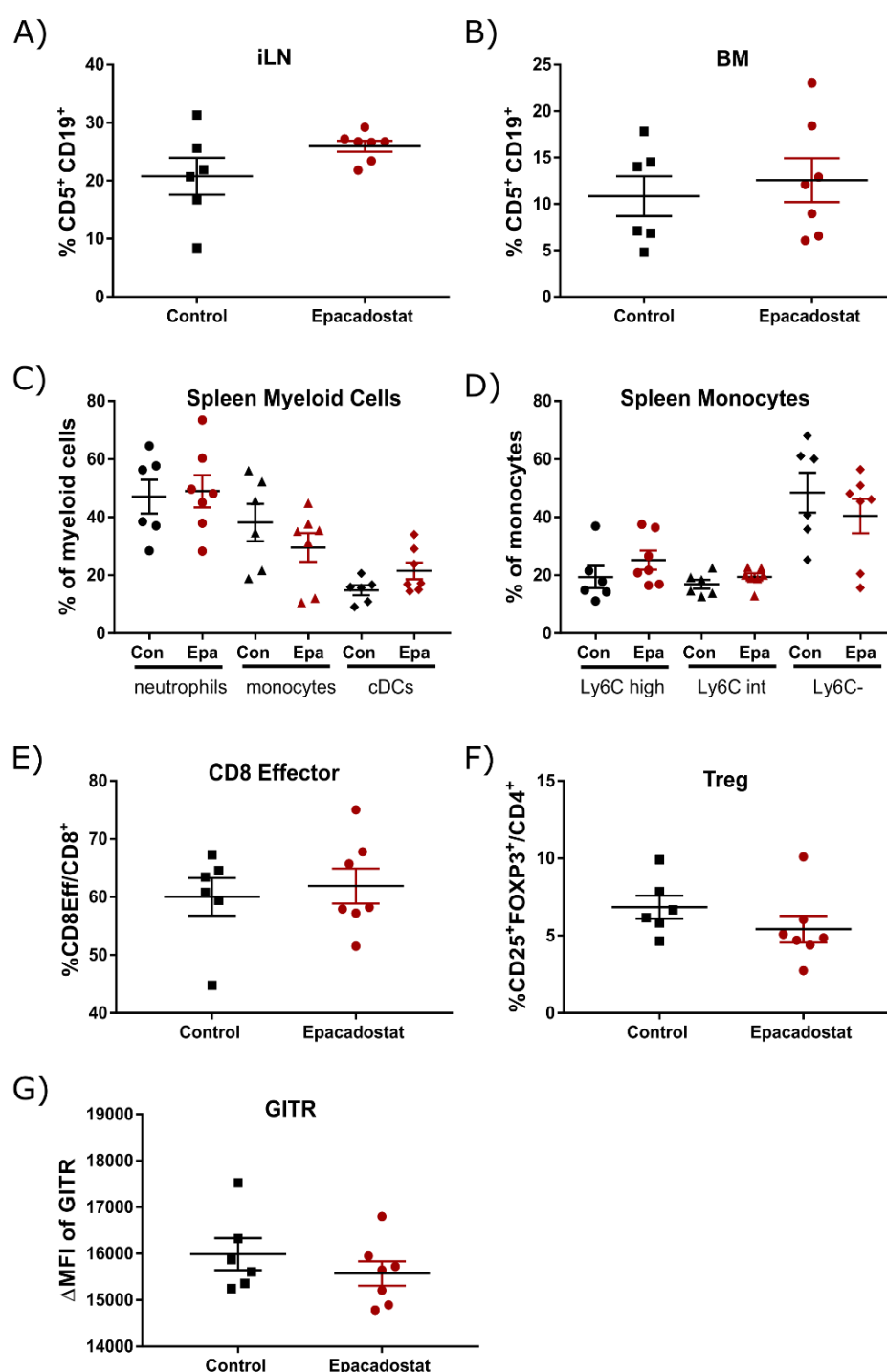
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**Figure S1.** Gating strategies related to Fig. 1 and IDO1 expression in B cell subsets. **A)** Gating strategy for flow cytometry analysis of myeloid cells depicted in Figure 1A-D. **B)** Gating strategy for CLL cells. **C)** Representative histogram and quantification of IDO1 expression in CD5+CD19+ and CD5-CD19+ B cells from tumor-free wild-type (WT) mice, as well as CD5+CD19+ tumor cells in mice after adoptive transfer of TCL1 leukemia cells (TCL1 AT). All graphs show means  $\pm$  SEM. Comparison of groups was performed with Welch's t-test. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001. MFI = normalized median fluorescence intensity.



**Figure S2. 1MT treatment has minor effects on CLL development and tumor-associated immune cells.** Wild-type mice were transplanted i.p. with  $2 \times 10^7$  TCL1 tumor cells and were divided into 2 groups (n=10) directly after transplantation. Mice of the 1MT group had continuous access to the drug via drinking water. Mice of the control group (Con) received vehicle in the drinking water. Flow cytometry analysis was performed 6 weeks after tumor transplantation. **A–B**) Percentages of CD5<sup>+</sup>CD19<sup>+</sup> CLL cells in peripheral blood (PB) and inguinal lymph nodes (iLN) at the endpoint. **C**) Representative dot plot showing the gating strategy for KI67<sup>+</sup> cells. **D**) After exclusion of cells expressing CD19, CD3 or NK1.1, neutrophils were defined as CD11b<sup>+</sup>Ly6G<sup>+</sup>, conventional dendritic cells (cDCs) as CD11c<sup>high</sup>MHC II<sup>+</sup>, and monocytes as CD11b<sup>+</sup>F4/80<sup>+</sup>CD11c<sup>low</sup> cells. Frequencies of these populations in the spleen out of total myeloid cells are depicted. **E**) Splenic monocyte subsets were further analyzed by their Ly6C expression and frequencies of cells with high, intermediate (int) and no Ly6C expression are depicted. **F**) CD8<sup>+</sup> effector T cells in the spleen were quantified as CD127<sup>low</sup>CD44<sup>+</sup> cells. **G**) Representative histogram showing PD-1 staining on splenic CD8<sup>+</sup> effector T cells. **H**) Quantification of CD25<sup>+</sup>FOXP3<sup>+</sup> Tregs in the spleen. **I**) Representative histogram showing CD69 staining on splenic Tregs. All graphs show means ± SEM. Comparison of groups was performed with Welch's t-test. Statistics were only indicated when significant. \*p<0.05. FMO = fluorescence-minus-one control.



**Figure S3. Epacadostat treatment has minor effects on CLL development and tumor-associated immune cells.** Wild-type mice were transplanted i.p. with  $2 \times 10^7$  TCL1 tumor cells and were divided into 2 groups 2 weeks post tumor transplantation. Mice were treated with epacadostat (Epa) or vehicle (Control) by oral gavage once daily and flow cytometry analysis was performed 5 weeks after tumor transplantation (Control,  $n=6$ ; Epacadostat,  $n=7$ ). **A-B**) Percentages of CD5<sup>+</sup>CD19<sup>+</sup> CLL cells in inguinal lymph nodes (iLN) and bone marrow (BM) at the endpoint. **C**) After exclusion of cells expressing CD19, CD3 or NK1.1, neutrophils were defined as CD11b<sup>+</sup>Ly6G<sup>+</sup>, conventional dendritic cells (cDCs) as CD11c<sup>high</sup>MHC II<sup>+</sup>, and monocytes as CD11b<sup>+</sup>F4/80<sup>+</sup>CD11c<sup>low</sup> cells. Frequencies of these populations in the spleen out of total myeloid cells are depicted. **D**) Monocyte subsets were further analyzed by their Ly6C expression and frequencies of cells with high, intermediate (int) and no Ly6C expression are depicted. **E**) CD8<sup>+</sup> effector T cells in the spleen were quantified as CD127<sup>low</sup>CD44<sup>+</sup> cells. **F**) Quantification of CD25<sup>+</sup>FOXP3<sup>+</sup> Tregs in the spleen. **G**) Quantification of GITR expression on splenic Tregs. All graphs show means  $\pm$  SEM. Comparison of groups was performed with Welch's t-test. Statistics were only indicated when significant. MFI = normalized median fluorescence intensity.

**Table S1.** Antibodies used in this study.

| ANTIBODY                                       | SOURCE       | IDENTIFIER  |
|--|--------------|---|
| Anti-mouse CD11b (clone M1/70)                 | ThermoFisher | Cat# 25-0112-81, RRID:AB_469587   |
| Anti-mouse CD11c (clone N418)                  | Biolegend    | Cat# 117334, RRID: AB_2562415   |
| Anti-mouse CD127 (clone A7R34)                 | Biolegend    | Cat# 135014, RRID:AB_1937265  |
| Anti-mouse CD19 (clone eBio1D3)                | ThermoFisher | Cat# 11-0193-82, RRID:AB_657666, Cat# 12-0193-81, RRID:AB_657661, Cat# 48-0193-82,RRID:AB_2734905 |
| Anti-mouse CD25 (clone PC61)                   | Biolegend    | Cat# 102036, RRID:AB_2563059  |
| Anti-mouse CD3e (clone 145-2C11)               | ThermoFisher | Cat# 11-0031-82, RRID:AB_464882   |
| Anti-mouse CD3e (clone 500A2)                  | BD           | Cat# 560801, RRID:AB_2034005  |
| Anti-mouse CD4 (clone GK1.5)                   | Biolegend    | Cat# 100414, RRID:AB_312699   |
| Anti-mouse CD44 (clone IM7)                    | ThermoFisher | Cat# 56-0441-82, RRID:AB_494011   |
| Anti-mouse CD5 (clone 53-7.3)                  | ThermoFisher | Cat# 17-0051-81, RRID:AB_469330   |
| Anti-mouse CD5 (clone 53-7.3)                  | BD           | Cat# 563194, RRID:AB_2738061  |
| Anti-mouse CD69 (clone H1.2F3)                 | Biolegend    | Cat# 104508, RRID:AB_313111   |
| Anti-mouse CD8a (clone 53-6.7)                 | Biolegend    | Cat# 100744, RRID:AB_2562609  |
| Anti-mouse F4/80 (clone BM8)                   | Biolegend    | Cat# 123110, RRID:AB_893486   |
| Anti-mouse Foxp3 (clone FJK-16s)               | ThermoFisher | Cat# 56-5773-82, RRID:AB_1210557  |
| Anti-mouse GITR (clone DTA-1)                  | Biolegend    | Cat# 126316, RRID:AB_2563384  |
| Anti-mouse IDO1 (clone mIDO-48)                | ThermoFisher | Cat# 50-9473-82, RRID:AB_2574335  |
| Anti-mouse Ki67 (clone 16A8)                   | Biolegend    | Cat# 652404, RRID:AB_2561525  |
| Anti-mouse KLRG1 (clone 2F1/KLRG1)             | Biolegend    | Cat# 138424, RRID:AB_2564051  |
| Anti-mouse Ly6G (clone 1A8)                    | ThermoFisher | Cat# 48-9668-82, RRID:AB_2637124  |
| Anti-mouse Ly6C (clone HK1.4)                  | Biolegend    | Cat# 128026, RRID:AB_10640120   |
| Anti-mouse MHC-II I-A/I-E (clone M5/114.15.2 ) | ThermoFisher | Cat# 56-5321-82, RRID:AB_494009   |
| Anti-mouse NK1.1 (clone PK136 )                | Biolegend    | Cat# 108706, RRID:AB_313393   |
| Anti-mouse PD-1 (clone RMP1-30 )               | Biolegend    | Cat# 109116, RRID:AB_2566548  |