LETTER TO THE EDITOR

# Targeting cytokine-induced leukemic stem cell persistence in chronic myeloid leukemia by IKK2-inhibition

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Supplementary Appendix

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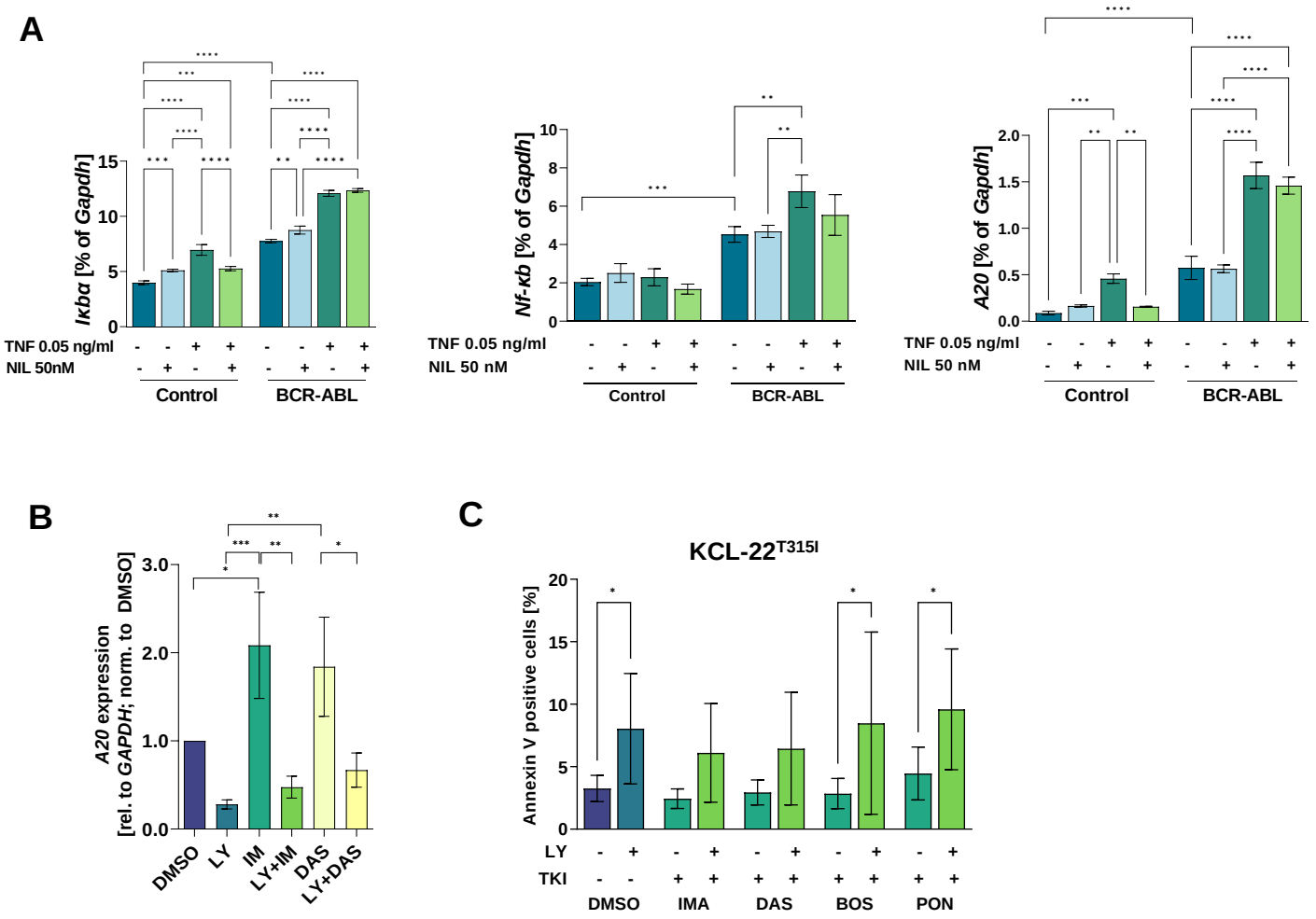
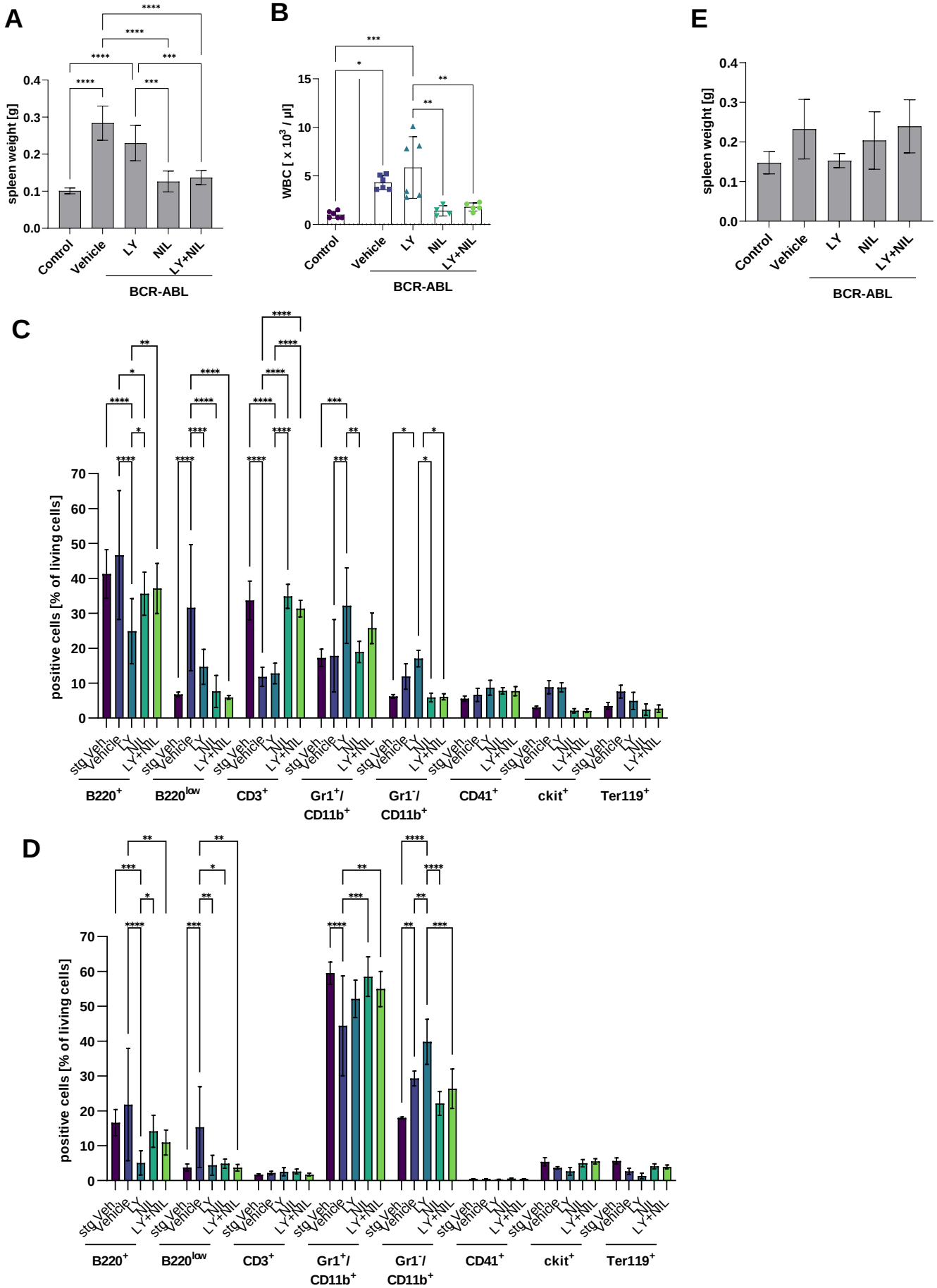


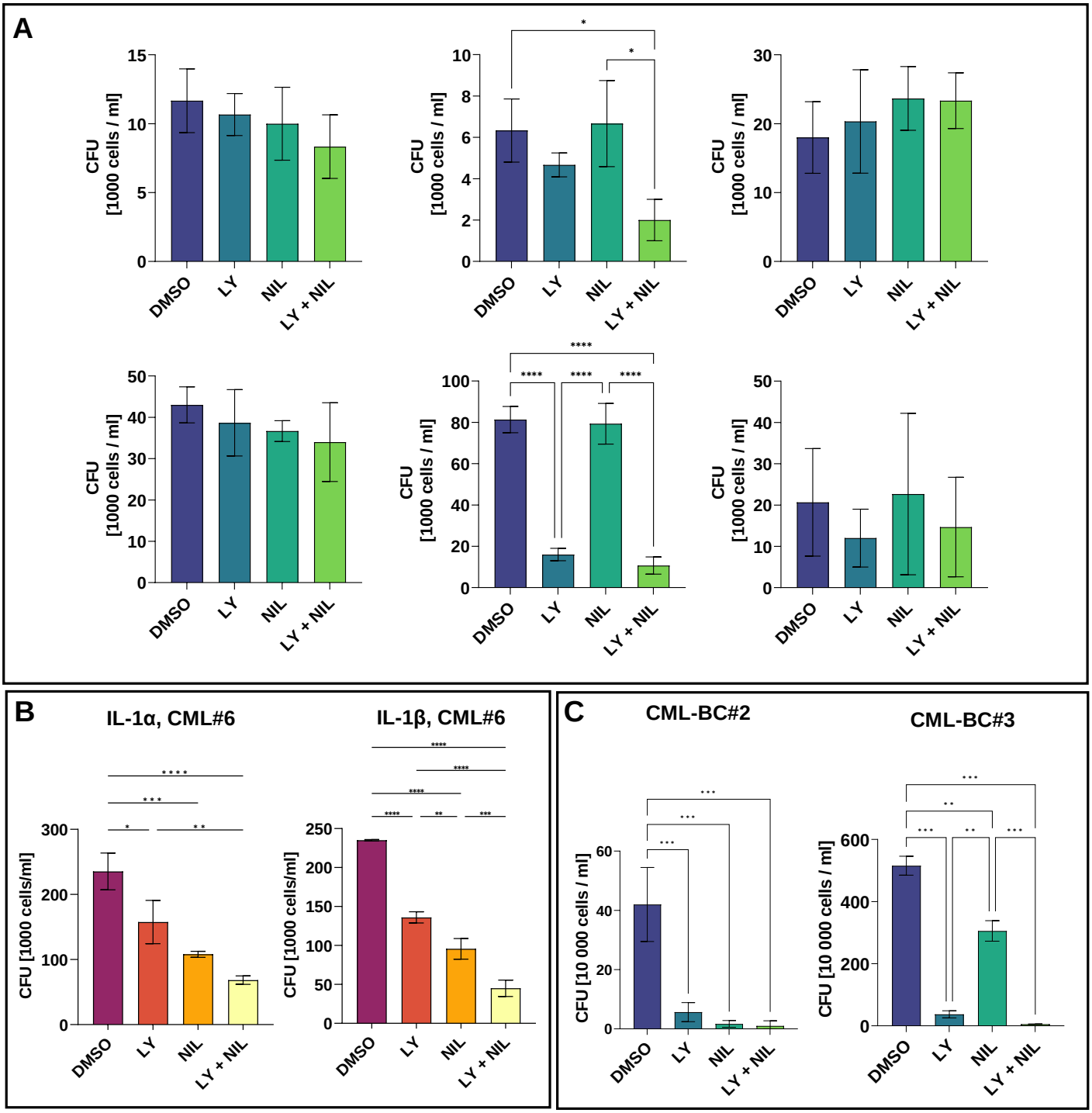

**Supplemental Figure S1**

**S1A** Expression of *IκBα, NF-κB,* and *A20*-mRNA in BCR-ABL-negative control- and BCR-ABL-positive murine myeloid progenitor 32Dcl3 cells is shown as % of *Gapdh.* Cells were treated using 0.05 ng/ml TNF and/or 50 nM NIL for 30 min. One representative of n=3 is shown. **S1B** Human CML KCL-22 cells were treated using LY (10 µM), IM (1 µM), DAS (10 nM) or LY+IM, and LY+DAS, in the presence of 1 ng/ml TNF for 16h. Expression of *NF-κB*-target gene *A20* was analyzed via qRT-PCR. Shown are mean values of n=3, normalized to DMSO control. **S1C** Flow cytometry analyses of AnnexinV-positive KCL-22$^{T315I}$ cells upon 48h of treatment using LY (5 µM), IM (1 µM), LY+IM, DAS (10 nM), LY+DAS, BOS (5 nM), LY+BOS, PON (5 nM), LY+PON in the presence of TNF (1 ng/ml; n=3). Mean ± SD, significances were calculated using one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001




**Supplemental Figure S2**

**S1A** Spleen weight [g] upon final analysis of primary recipients, that were treated using the indicated drugs. **S1B** White blood cell count (WBC) upon final analysis. **S1C** FACS-positive cells in spleen and BM **(S1D)**, shown as % of living cells for B-cells (B220$^{+}$), immature B-cells (B220$^{low}$), T-cells (CD3$^{+}$), granulocytes (Gr1$^{+}$/CD11b$^{+}$), immature granulocytes (Gr1$^{-}$/CD11b$^{+}$), megakaryocytes (CD41$^{+}$), ckit$^{+}$-positive cells, and erythrocytes (Ter119$^{+}$). **S1E** Spleen weight [g] upon final analysis of secondary recipients. Mean ± SD, level of significance was calculated using one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001




**Supplemental Figure S3**

**S3A** Healthy donor CD34+ cells were isolated from femoral heads and were treated for 72 h using LY (10 µM), NIL (50 nM), LY+NIL, or DMSO control, in the presence of 1 ng/ml TNF. Subsequently, cells were seeded in methylcellulose and colonies were counted on day 10. **S3B** CFU-assays using CML-CP patient derived BM CD34+ cells after treatment using LY (10 µM), NIL (50 nM), LY+NIL, or DMSO control, in the presence of IL-1α (2.5 ng/ml, left panel) or IL-1β (3.5 ng/ml, right panel) for 72 h. One patient is presented as an example out of n=3. **S3C** CFU-assays of BC patient derived mononucleated BM being treated for 72 h using LY (10 µM), NIL (50 nM), LY+NIL, or DMSO control, in the presence of TNF (1 ng/ml). Mean ± SD, level of significance was calculated using one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001