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Supplemental Information

Augmentation of NK Cell Proliferation and Anti-tumor Immunity by Transgenic Expression of Receptors for EPO or TPO

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Supplemental Figures

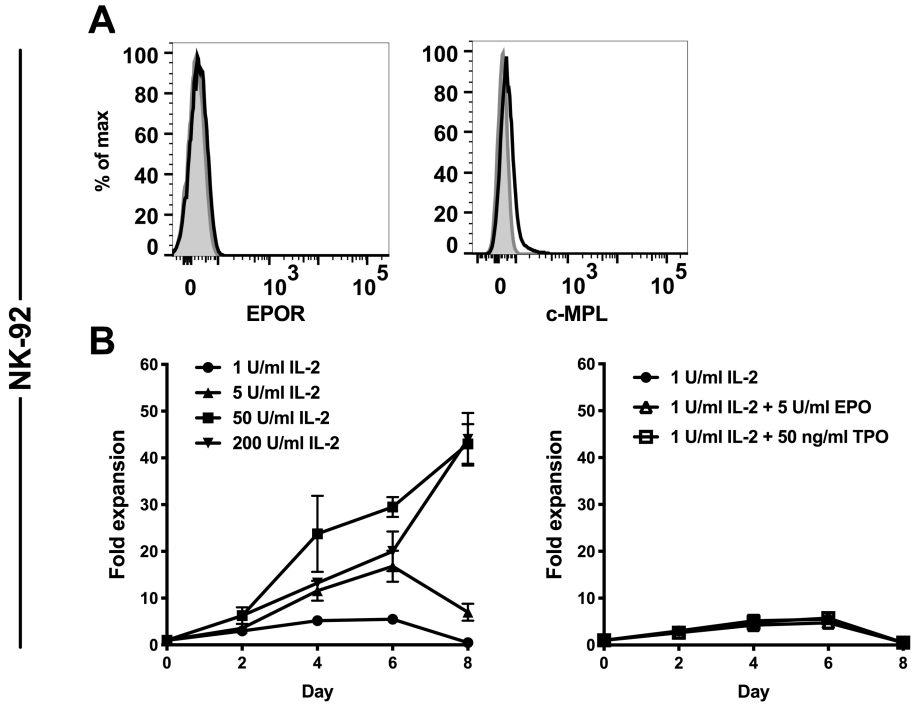

Figure S1. EPO and TPO do not affect proliferation of the unmodified NK-92 cell line. (A) Histograms show EPOR and c-MPL expression on parental NK-92 cells. (B) The fold expansion of parental NK-92 cells when cultured with the indicated concentrations of IL-2/EPO/TPO are shown. Data in (B) represent mean and SD of 3 independent experiments.

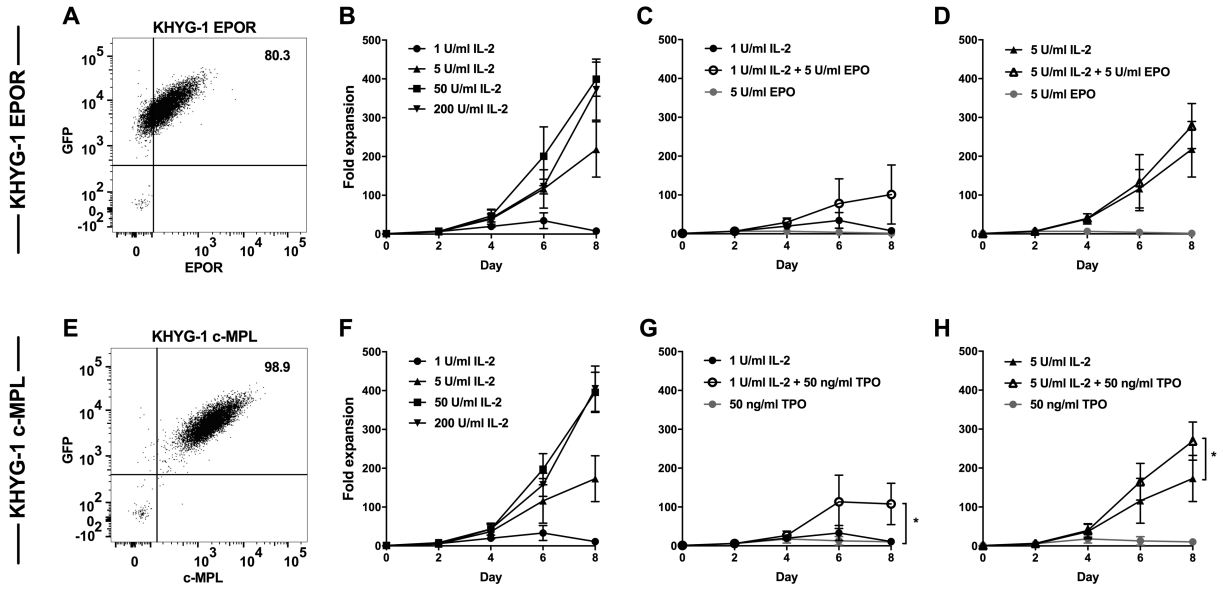

Figure S2. Exogenous expression of EPOR or c-MPL on KHYG-1 NK cell line facilitates proliferation in the presence of EPO or TPO. (A) EPOR and GFP or (E) c-MPL and GFP expression on KHYG-1 cell lines after fluorescence-activated cell sorting of transduced cells. Fold expansion of (B-D) EPOR$^+$ KHYG-1 or (F-H) c-MPL$^+$ KHYG-1 cells in the presence of IL-2, EPO, or TPO supplemented into culture medium at varying concentrations as indicated. Mean and SD from 3-4 independent experiments are shown. Significance was analyzed at day 8 by unpaired t test. (* $p<0.05$).

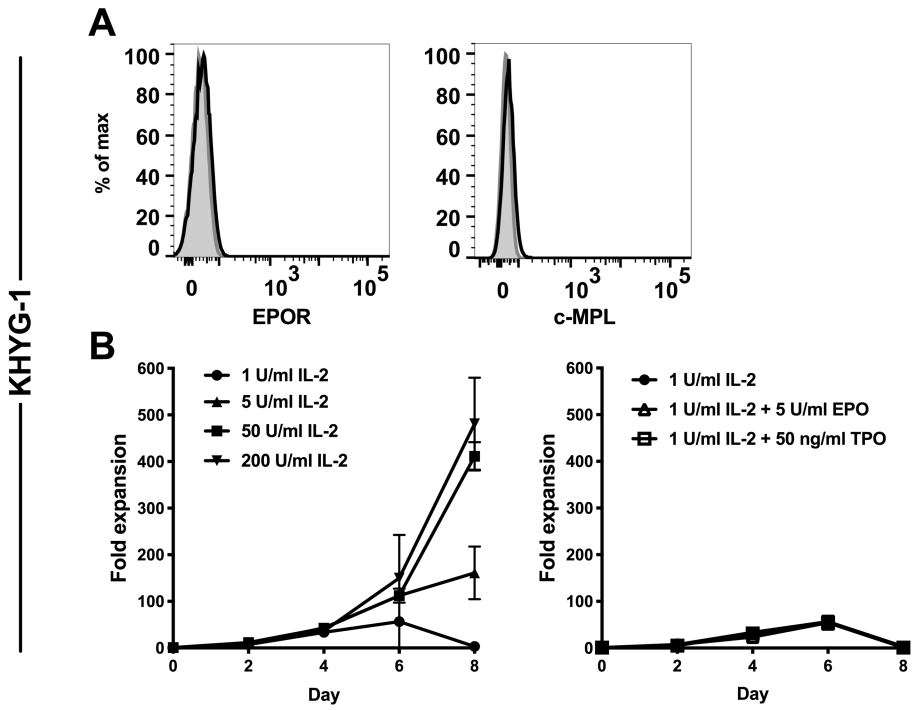

Figure S3. EPO and TPO do not affect proliferation of the unmodified KHYG-1 cell line. (A) Histograms show EPOR and c-MPL expression on parental KHYG-1 cells. (B) The fold expansion of parental KHYG-1 cells when cultured with the indicated concentrations of IL-2/EPO/TPO are shown. Data in (B) represent mean and SD of 3 independent experiments.

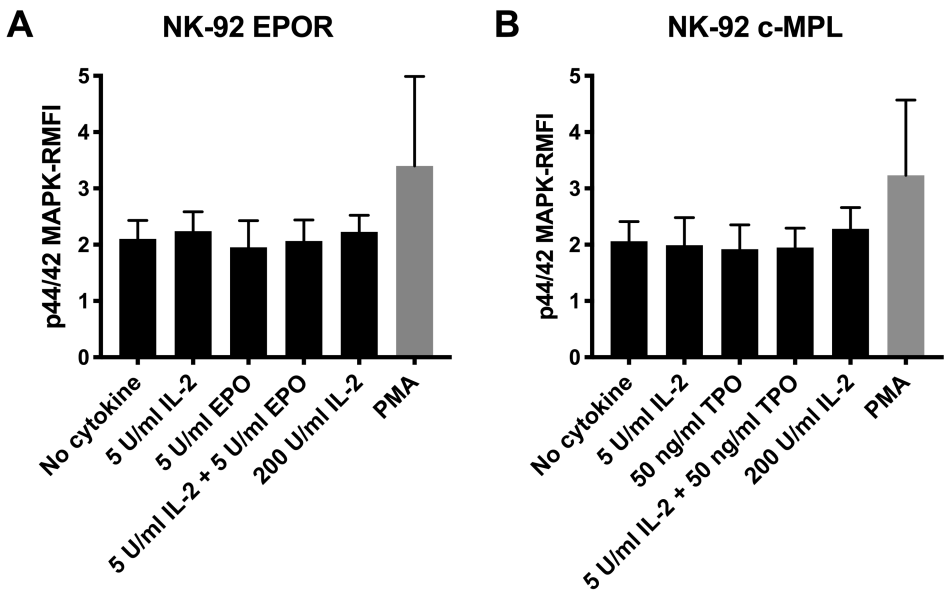

Figure S4. Phosphorylated p44/p42 MAPK did not increase in cytokine treated NK-92 cell lines. Flow cytometry measurements of p-p44/42 MAPK expression in (A) $EPOR^+$ NK-92 and (B) $c\text{-}MPL^+$ NK-92 are presented as RMFI (normalized to isotype control) separately for each type of activation listed. Mean and SD are shown. (PMA, phorbol 12-myristate 13-acetate)

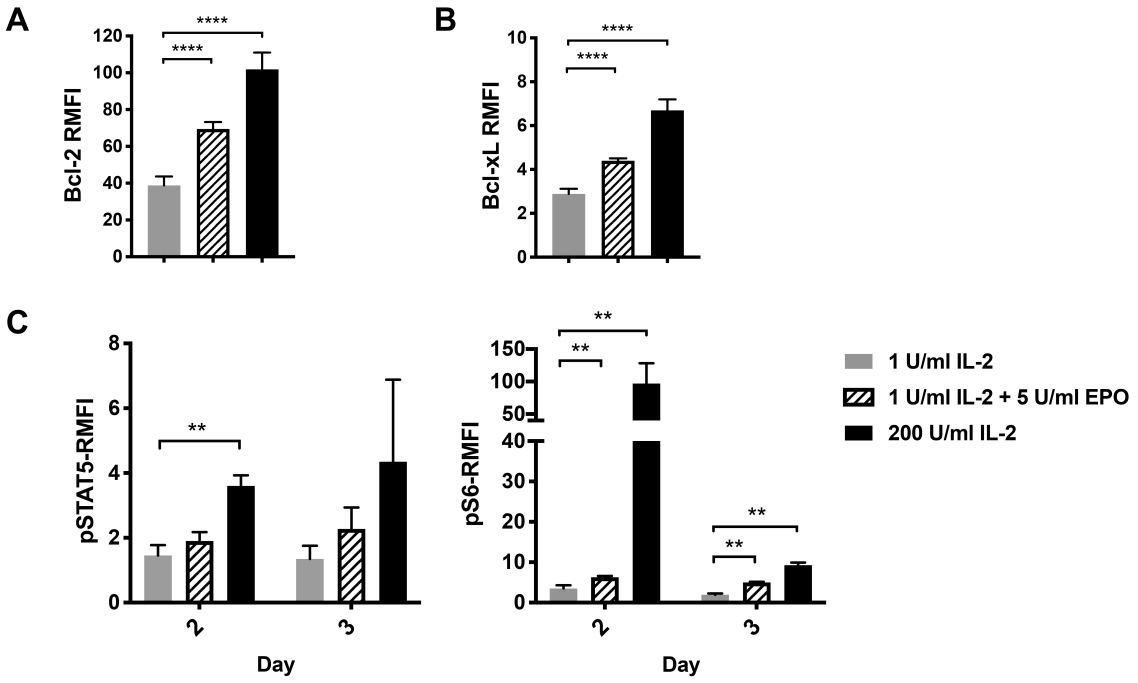

Figure S5. Evaluation of signaling pathways and anti-apoptotic proteins in EPO-treated EPOR$^+$ NK-92 cells. Further data from the experiment depicted in Figure 2. On day 2, cells were analyzed for (A) Bcl-2 and (B) Bcl-xL expression (RMFI) by flow cytometry. (C) In parallel, the pSTAT5 and pS6 expression were determined in EPOR$^+$ NK-92 cells from day 2 and 3 of the experiment. Mean and SD are shown in each case. (** $p < 0.01$, **** $p < 0.0001$ by unpaired t test).

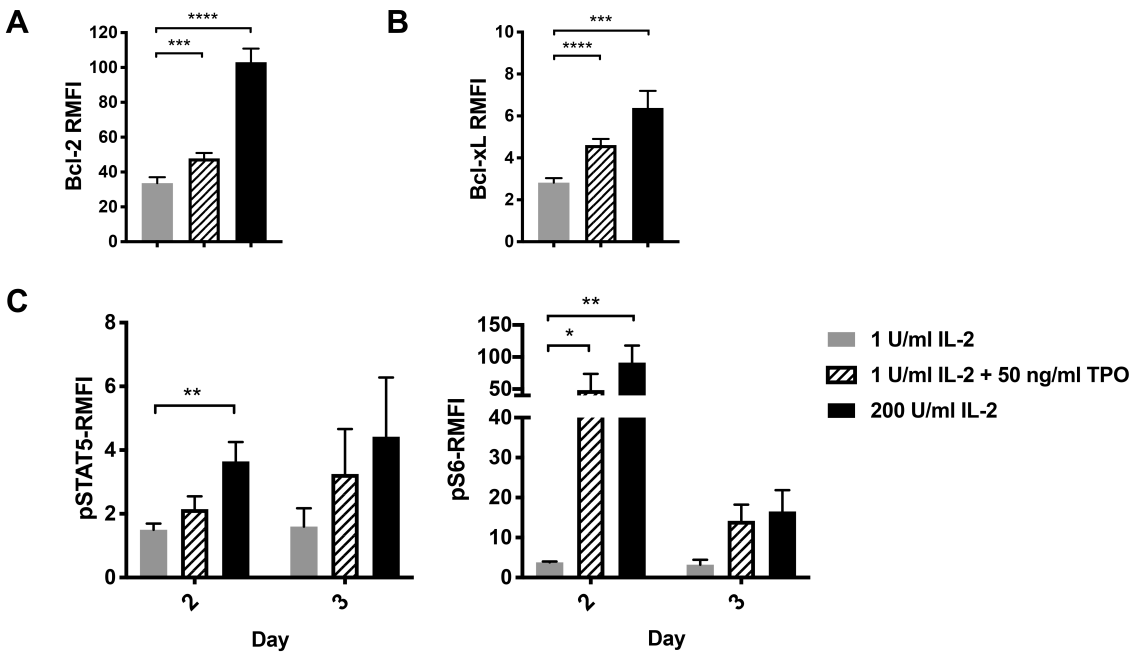

Figure S6. Evaluation of signaling pathways and anti-apoptotic proteins in TPO-treated c-MPL$^+$ NK-92 cells. Further data from the same experiment depicted in Figure 2. On day 2, the cells were analyzed for (A) Bcl-2 and (B) Bcl-xL expression (RMFI). (C) In parallel, the pSTAT5 and pS6 expression were determined in c-MPL$^+$ NK-92 cells from day 2 and 3 of the experiment. Mean and SD are shown. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ by unpaired t test).

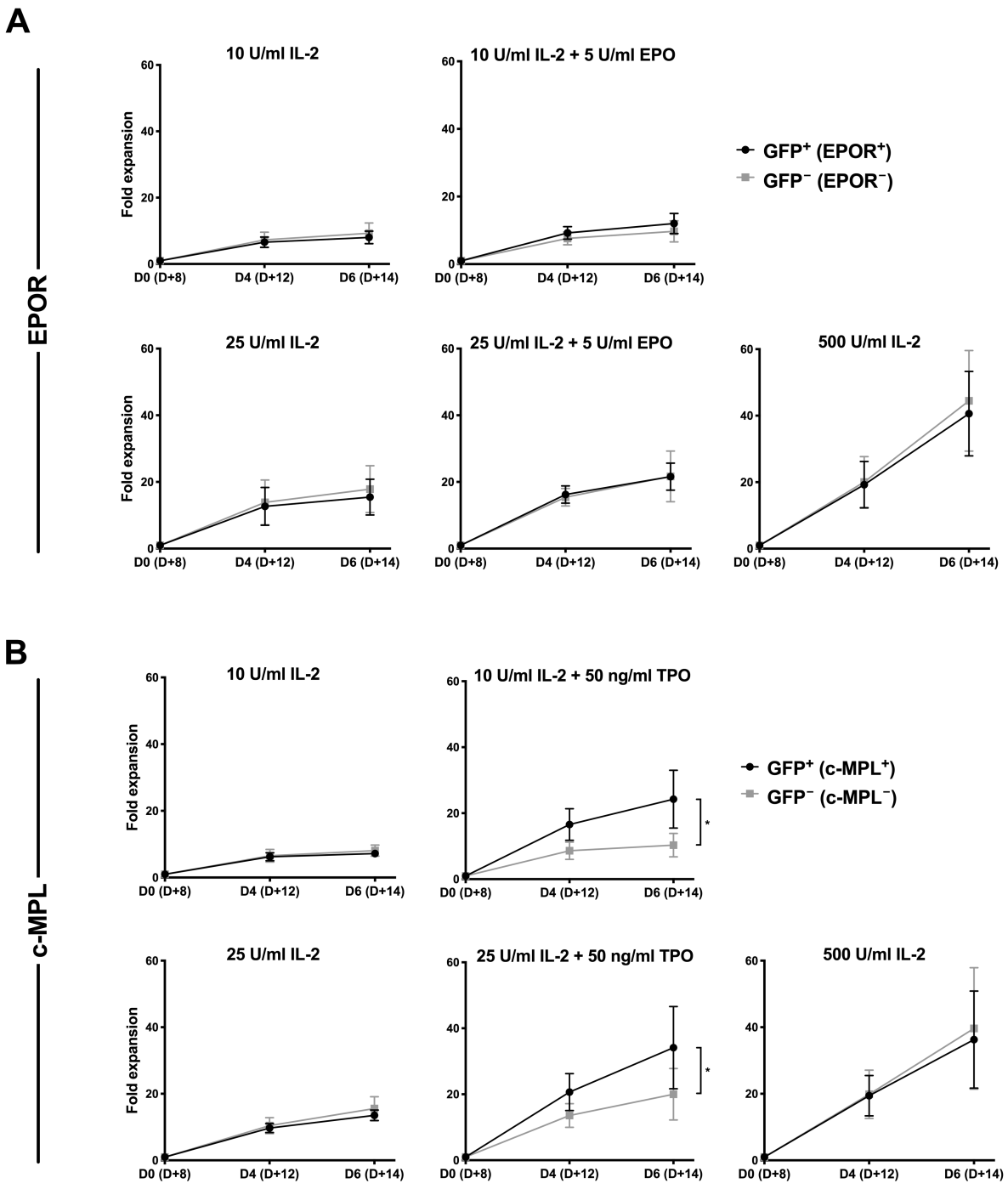

Figure S7. Transgenic expression of c-MPL in primary NK cells promotes proliferation in presence of TPO. Further data from the same experiment depicted in Figure 5. Proliferation of GFP^+ (transduced) either (A) $EPOR^+$ or (B) c-MPL^+ NK cells was contrasted with proliferation of the GFP^- (un-transduced) NK cell population in each case under the culture conditions listed. Mean fold expansion values were calculated using overall cell counts and GFP percentages obtained by flow cytometry and displayed with SD. (* $p<0.05$ by paired t test on $\log_{10}$ transformed data).

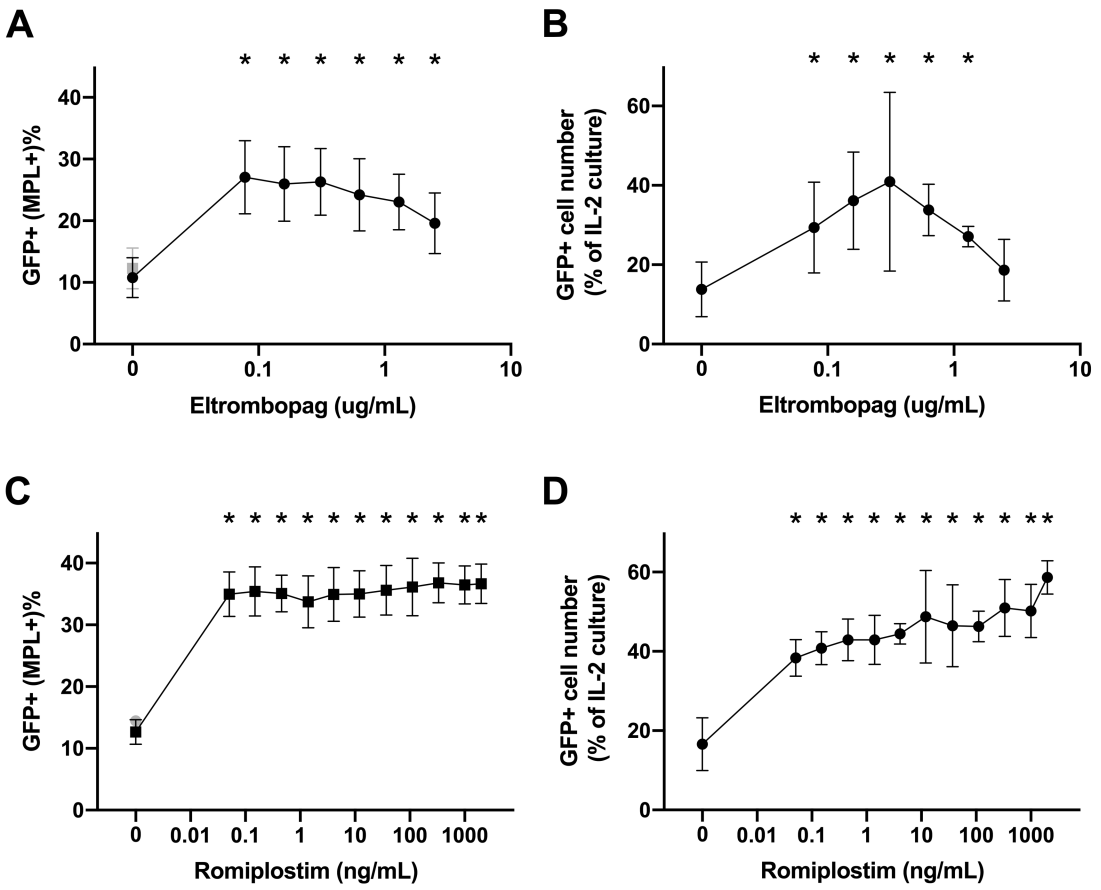

Figure S8. The TPO-mimetics eltrombopag and romiplostim increase numbers of c-MPL-expressing human NK cells *in vitro.* Blood NK cells were stimulated and transduced with c-MPL/GFP lentiviral vectors. 2-3 days after transduction, cells were subdivided into cultures containing eltrombopag or romiplostim at the indicated concentrations. (A,C) Percentage of GFP^+ (MPL^+) cells was evaluated after an additonal 4 days. (B,D) The number of GFP^+ cells after 4 days was expressed as a percentage of results in parallel cultures supplemented with 500U/mL IL-2 lacking a TPO-mimetic drug. * $p<0.05$ by unpaired t test compared to (B,D) culture medium alone or (A,C) parallel IL-2-containing culture without a TPO-mimetic drug (recessed grey symbol at 0). Mean and SD from (A,B) 9 cultures from 3 blood donors or (C,D) 6 cultures from 2 donors are shown.

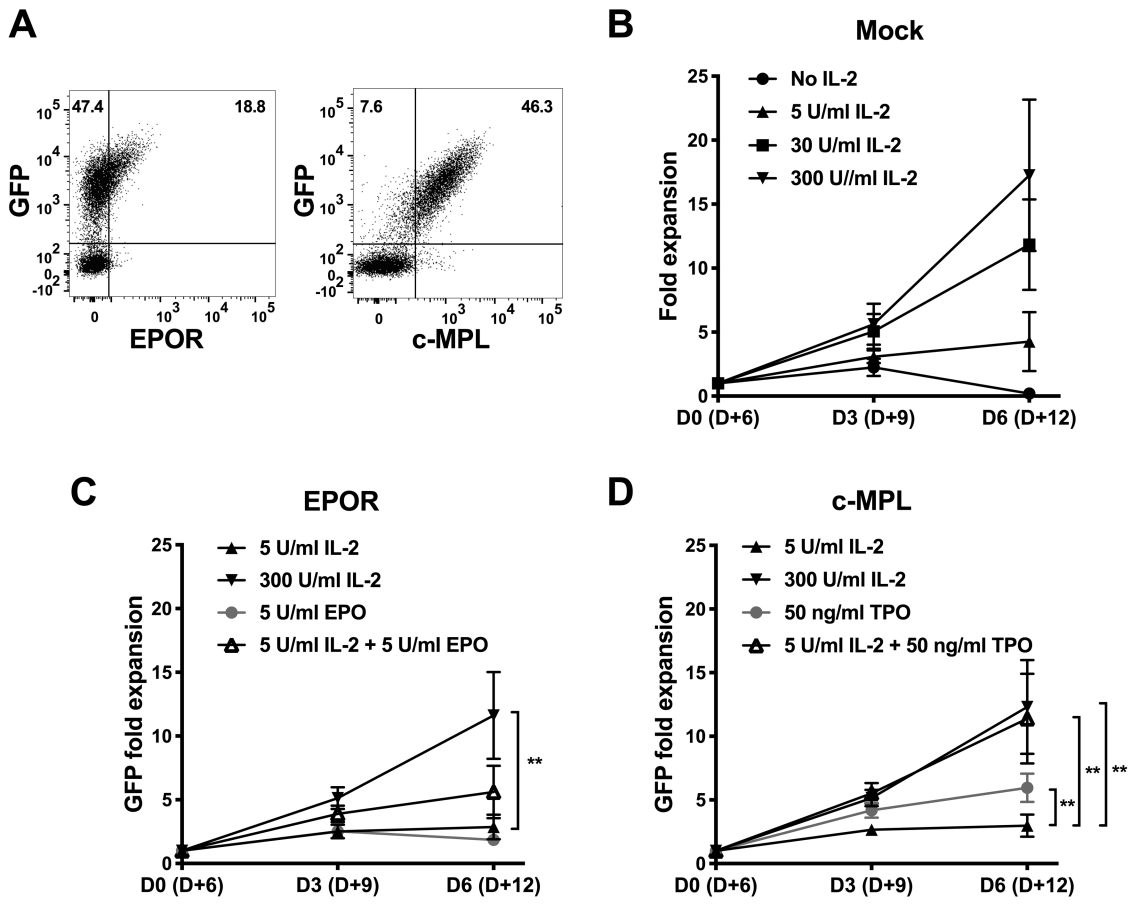

Figure S9. Effects on proliferation of primary human T cells genetically modified to express EPOR or c-MPL. PBMC were transduced (MOI=10) after 2 days of stimulation with anti-CD3 (OKT3) coated on plates and soluble anti-CD28 (CD28.2) antibodies and maintained initially in RPMI with 10% FBS and 300U/ml IL-2. (A) Cells were examined for the transduced gene expression by flow cytometry (data from one donor). In each case, greater than 95% of resulting cells were $CD3^+$; 40-60% of cells were GFP^+. Beginning 4 days after transduction, mixed GFP^+ and GFP^- populations were cultured with varying concentrations of the indicated growth factors, replacing medium and growth factors after 3 additional days, while tracking cell numbers. (C,D) The fold-expansion of GFP^+ (virally-transduced) T cells expressing (C) EPOR and (D) c-MPL is shown (mean and SD). (B) Similarly activated but un-transduced (Mock) T cells expanded in an IL-2 dependent manner. Statistical significance with samples cultured in varying media was measured with unpaired Student's t-test using data from 4 healthy donors on day 6 of culture (equivalent to day 12 from the initial T-cell isolation (D+12). (** $p<0.01$).

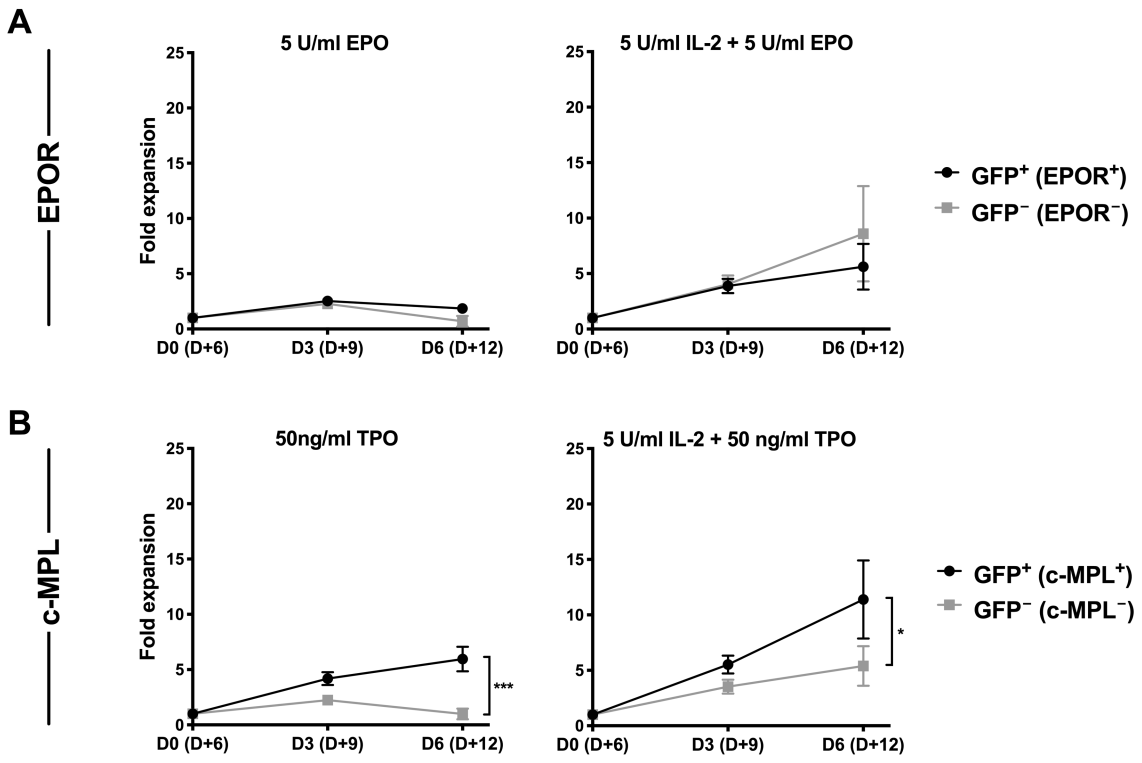

Figure S10. Transgenic expression of c-MPL in primary T cells promotes proliferation in presence of TPO. Further data from the same experiment depicted in Figure S9. Proliferation of GFP^+ (transduced) either (A) $EPOR^+$ or (B) $c\text{-}MPL^+$ T cells was contrasted with proliferation of the GFP^- (un-transduced) T cell population in each case under the culture conditions listed. Mean fold expansion values were calculated using overall cell counts and GFP percentages obtained by flow cytometry and displayed with SD. (* $p<0.05$ by unpaired t test).