SUPPLEMENTARY APPENDIX

In vitro and *in vivo* evaluation of possible pro-survival activities of PGE2, EGF, TPO and FLT3L on human hematopoiesis

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 doi:10.3324/haematol.2018.191569

Received: February 18, 2018.

Accepted: November 14, 2018.

Pre-published: November 15, 2018.

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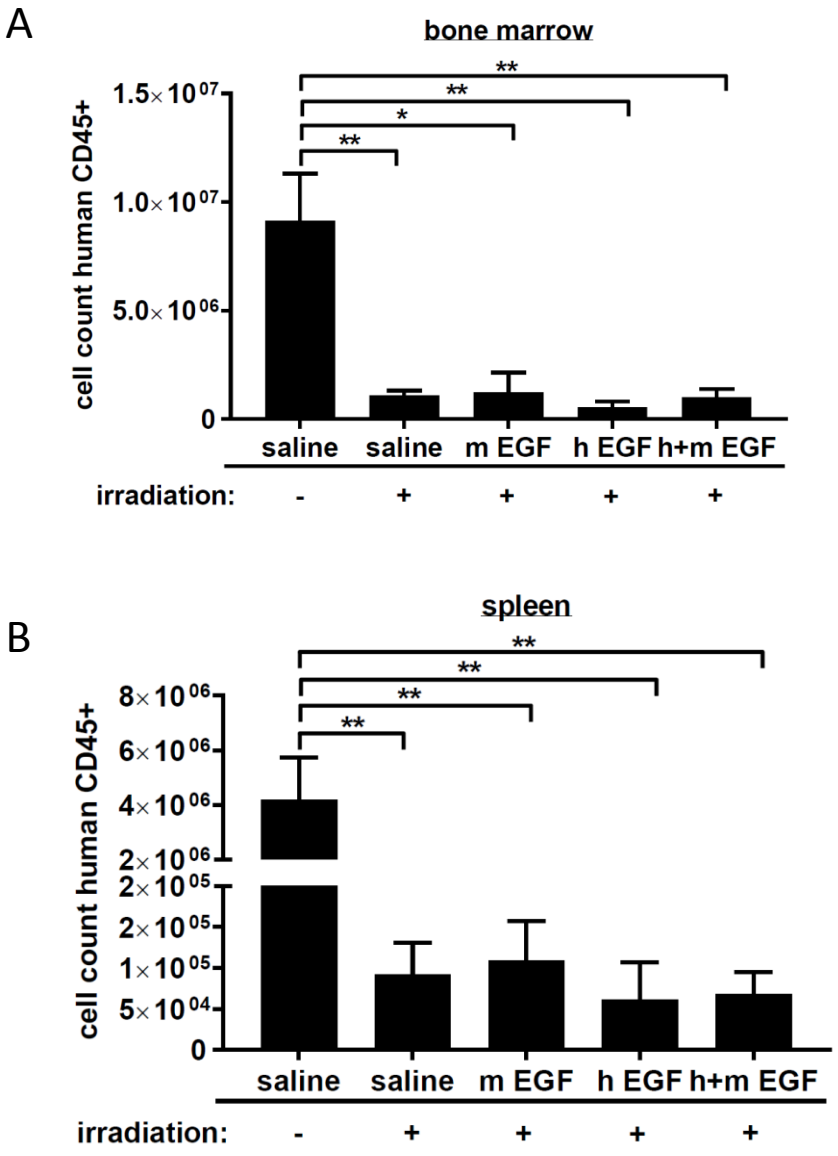

Supplemental Figure 1: Cell count of human CD45+ cells 7 days after irradiation

Human CD34+ cells were transplanted into sublethally irradiated *Rag2*$^{-/-}\gamma c^{-/-}$ mice. Four weeks later, mice were irradiated with 3 Gy or left untreated. Mice that were irradiated received daily injections of human and/or murine EGF. Eight days after irradiation, mice were sacrificed and cell count of human CD45+ cells was calculated in bone marrow **(A)** and spleen **(B)**. Bars represent means ± SEM of n=4-7 animals from 3 independent experiments. P-values were determined using the Mann-Whitney test (* $p \leq 0.05$; ** $p \leq 0.01$).

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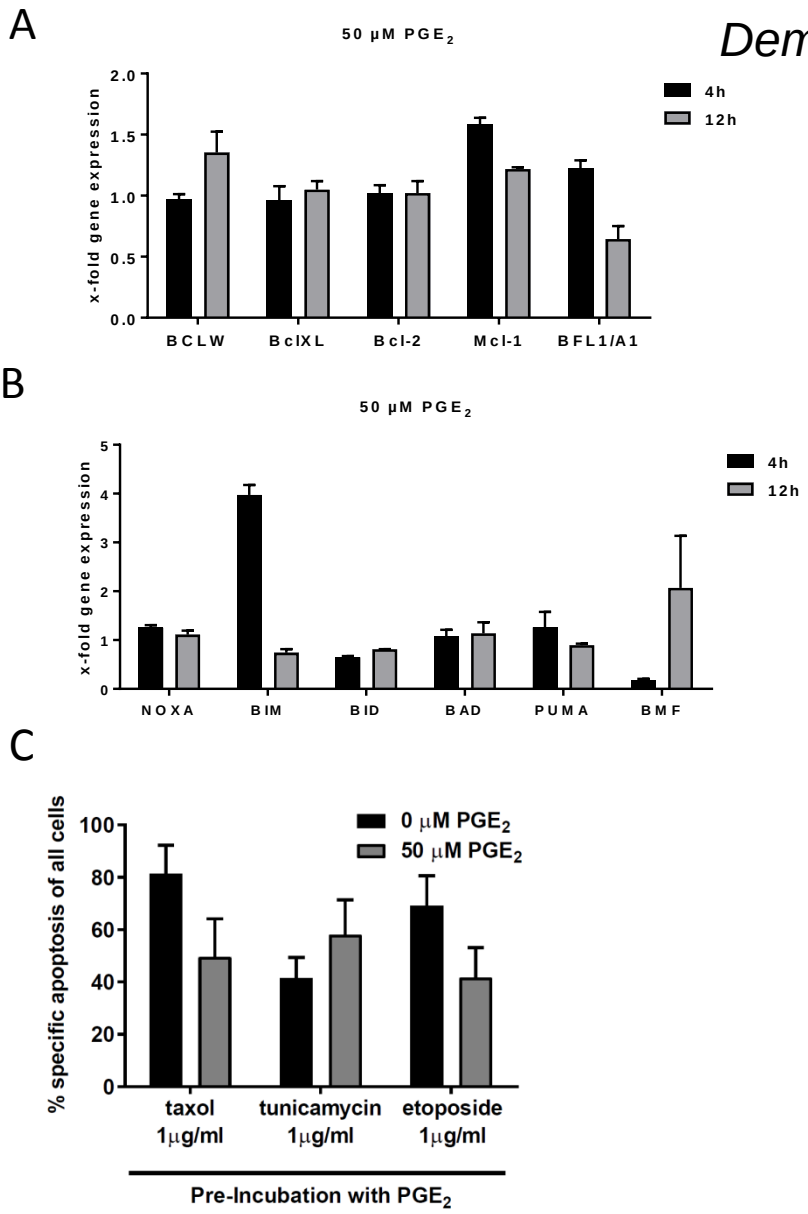
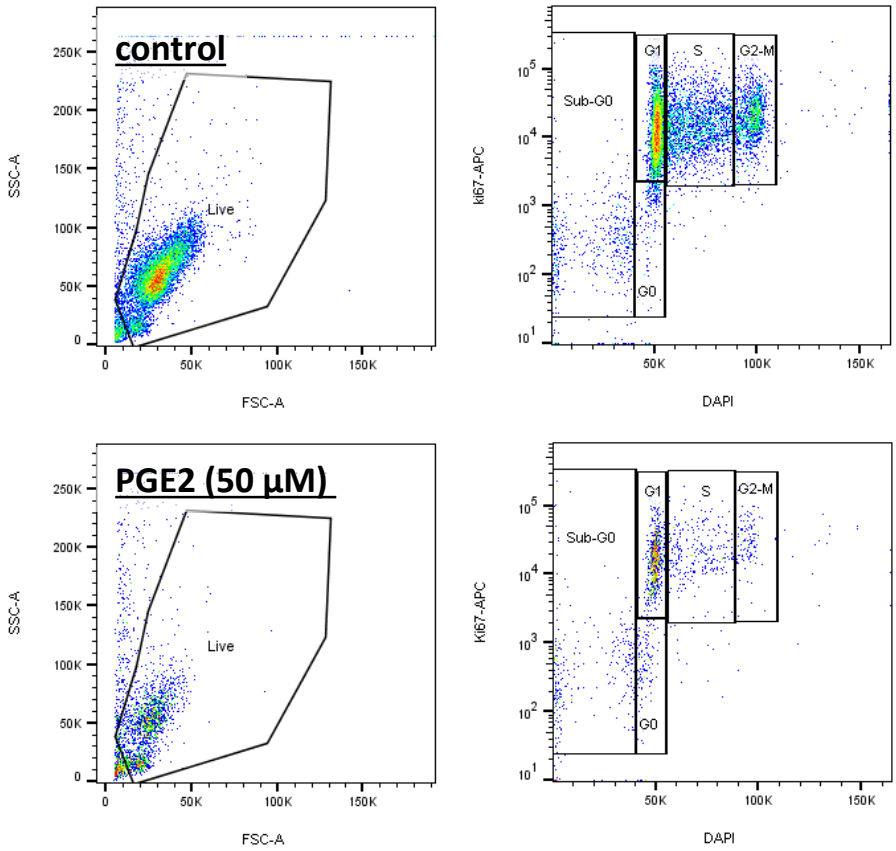
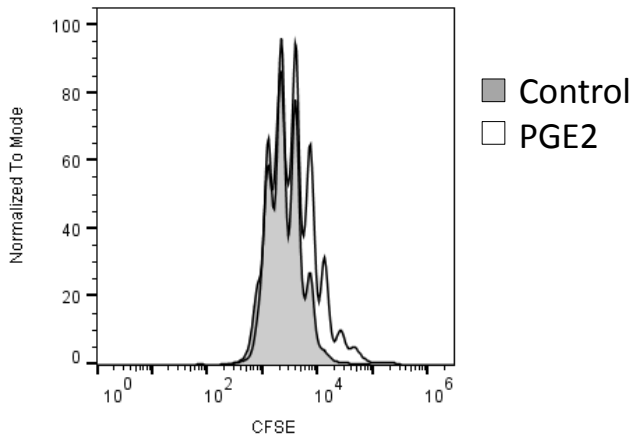


Supplemental Figure 2:
(A+B)PGE2-induced regulation of BCL-2 proteins
CD34+ cells were treated with PGE2 for 4 hours. RT-MLPA was performed to analyze mRNA levels of the antiapoptotic BCL-2 proteins (upper panel) and their antagonists, the pro-apoptotic BH3-only proteins (lower panel). Graphs represent means of n=3 independent experiments. Mann-Whitney test did not reveal significant differences.
(C) PGE2 pre-incubation Cord blood-derived CD34+ cells were pre-incubated with or without PGE2 for 24 hours and then subjected to different cytotoxic agents. Control cells were treated with serum, FLT3L, SCF, TPO and IL3. PGE2 was added at indicated concentration. After 72 hours, cells were stained with AnnexinV/7AAD and specific apoptosis was determined. Bars represent means ± SEM of n=4 from 4 independent experiments.

A

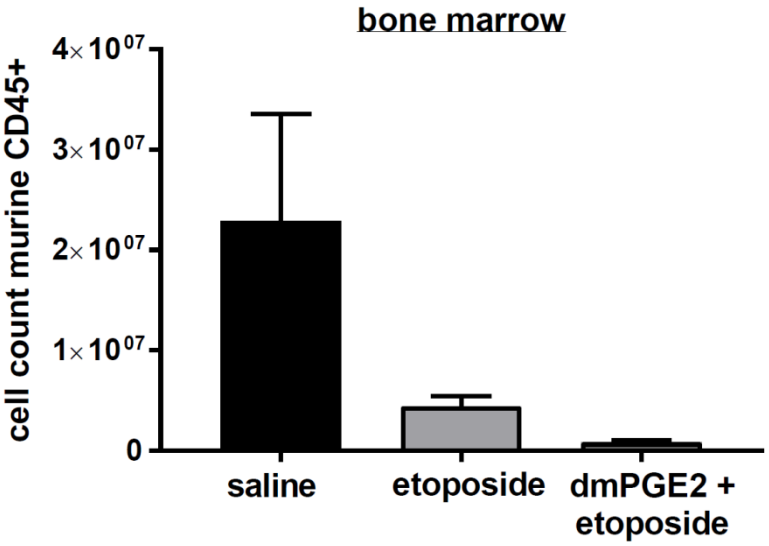


B



Supplemental Figure 3: Antiproliferative effects of PGE2 on human HSPCs
CD34+ cells were cultured in the presence or absence of PGE2 (50 µM). Medium contained 10% serum and TPO (50 ng/ml), FLT3L, SCF and IL3 (100 ng/ml each).
(A) After 3 days of culture, CD34+ cells were stained with Ki67 and DAPI.
(B) CD34+ cells were cultured in the presence of CFSE, and CFSE content was determined by flow cytometry 4 days later.

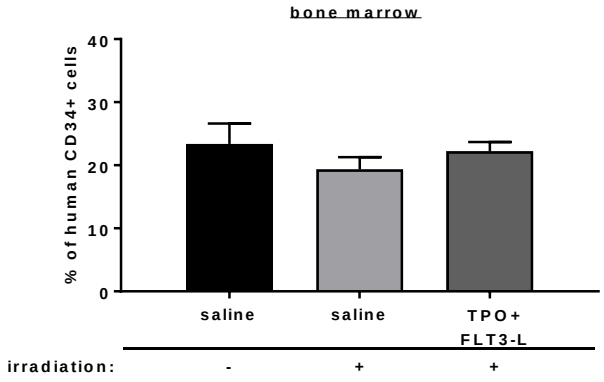
A



Supplemental Figure 4: Cell numbers of murine CD45+ cells after etoposide treatment

Human CD34+ cells were transplanted into sublethally irradiated *Rag2*$^{-/-}\gamma c^{-/-}$ mice. Four weeks later, mice were treated for 7 days once daily with etoposide (20 mg/kg) or left untreated. One group additionally received daily injections of dmPGE2 (2 µg/g). Eight days after start of treatment, mice were sacrificed and cell numbers of murine CD45+ cells were determined in bone marrow (A). Bars represent means ± SEM of n=2-4 animals from 2 independent experiments.

A



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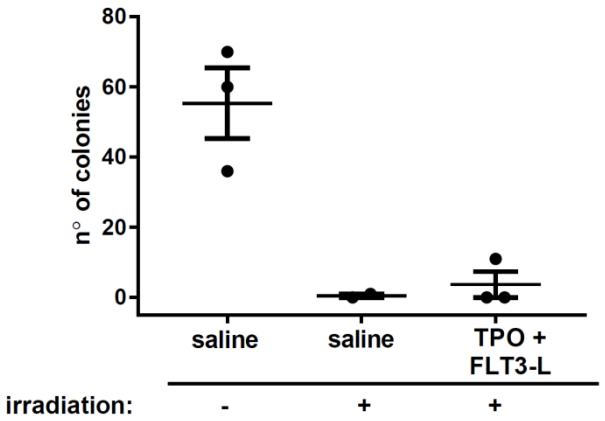

Supplemental Figure 5: Effects of FLT3-L and TPO on human hematopoiesis *in vivo*

Human CD34+ cells were transplanted into sublethally irradiated *Rag2*$^{-/-}\gamma c^{-/-}$ mice. Four weeks later, mice were irradiated with 3 Gy or left untreated. Mice that were irradiated received daily injections (7 or 14 days) of human TPO and/or FLT3L. Eight days after irradiation, mice were sacrificed and the proportion of CD34+ immature cells was determined within the human cell population. Bars represent means ± SEM of n=4-7 animals from at least 3 independent experiments. (A)

After 14 days of cytokine treatment mice were sacrificed and human CD45+ cells were isolated from the bone marrow. Human cells were cultured in colony forming assays for 11 days. Colony numbers were determined by light microscopy. Bars represent means ± SEM from n=3 (B).