SUPPLEMENTARY APPENDIX

BET-inhibition by JQ1 promotes proliferation and self-renewal capacity of hematopoietic stem cells

Mark Wroblewski,[1,2*] Marina Scheller-Wendorff,[1,2,3*] Florian Udonta,[1,2] Raimund Bauer,[1,2] Jara Schlichting,[1,2] Lin Zhao,[1,2,4] Isabel Ben Batalla,[1,2] Victoria Gensch,[1,2] Sarina Päsler,[1,2] Lei Wu,[5,6] Marek Wanior,[7] Hanna Taipaleenmäki,[8] Simona Bolamperti,[8] Zeynab Najafova,[9] Klaus Pantel,[2] Carsten Bokemeyer,[1] Jun Qi,[5,6] Eric Hesse,[8] Stefan Knapp,[7,10,11] Steven Johnsen[2,9] and Sonja Loges[1,2]

[1]Department of Hematology and Oncology with Sections BMT and Pneumology, Hubertus Wald Tumorzentrum, University Comprehensive Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Institute of Tumor Biology, Center of Experimental Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [3]Department of Medicine V, Hematology, Oncology and Rheumatology, University of Heidelberg, Germany; [4]Department of Oncology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China; [5]Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA, USA; [6]Department of Medicine, Harvard Medical School, Boston, MA, USA; [7]Institute for Pharmaceutical Chemistry, Johann Wolfgang Goethe-University and Buchmann Institute for Molecular Life Sciences, Frankfurt am Main, Germany; [8]Heisenberg-Group for Molecular Skeletal Biology, Department of Trauma, Hand & Reconstructive Surgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [9]Department of General, Visceral and Pediatric Surgery, University Medical Center Göttingen, Germany; [10]Nuffield Department of Clinical Medicine, Structural Genomics Consortium and Target Discovery Institute, University of Oxford, Old Road Campus Research Building, UK and [11]German Cancer Consortium (DKTK) Frankfurt am Main, Germany

**MWr and MS-W contributed equally to this work.*

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Correspondence: s.loges@uke.de

SUPPLEMENTARY METHODS

Animals

C57BL/6J (Ly5.2) and B6.SJL-Ptprc^{a} Pepc^{b}/BoyJ (Ly5.1) mice were used in this study. Breeding of Ly5.1 mice was performed in homozygosity in our local animal facility (UKE, Hamburg, Germany) and C57BL/6J (Ly5.2) mice were purchased from Charles River Laboratories (Sulzfeld, Germany). Experiments were performed with 4-8 week old female mice as indicated. All experiments were carried out in concordance with the institutional guidelines for the welfare of animals and were approved by the local licensing authority (Behörde für Soziales, Gesundheit, Familie, Verbraucherschutz; Amt für Gesundheit und Verbraucherschutz, Hamburg, Germany, project number G128/16).

Treatments and mixed bone marrow chimeras

For HSC transplantation experiments, Ly5.1 animals received daily i.p injections with 50 mg/kg JQ1 or placebo for 21 days. The BET-inhibitor JQ1 was synthesized and supplied by Prof. Dr. Stefan Knapp and Marek Wanior (Goethe University Frankfurt am Main). To increase aqueous solubility, injection solutions were prepared by diluting DMSO stock solutions dropwise 1:10 in 10 % (2-Hydroxypropyl)-β-cyclodextrin. At day of sacrifice, different cell numbers from bone marrow or spleen of pretreated animals ($2x10^3$, $2x10^4$ or $2x10^5$) were mixed with $2x10^5$ untreated Ly5.2 supporter BM cells and injected i.v. into lethally irradiated Ly5.2 mice (2x 4,5 Gy split dose at an interval of 4 hours). For secondary transplantation experiments, 4×10^6 BM cells from animals originally transplanted with 2×10^5 JQ1- or placebo-treated Ly5.1 bone marrow cells were injected i.v. into lethally irradiated Ly5.2 mice (2x 4,5 Gy split dose at an interval of 4 hours). After each transplantation engraftment was monitored in PB (Hemavet 950, ERBA diagnostics Mannheim GmbH, Mannheim,

Germany) and BM at indicated time points.

Transplantation experiments were performed with total numbers of BM cells per condition and not corrected for equal numbers of HSC.

We therefore normalized the measured CD45.1$^+$ chimerism in the peripheral blood of the secondary recipients to the chimerism of the donor animals (primary recipients). Thereby we corrected for different ratios of transplanted cells (CD45.1 vs. CD45.2) at the time point of secondary transplantation.

In this context, “100 % CD45.1 (% donor CD45.1)” means that secondary recipients harbor the same amount of CD45.1+ cells in peripheral blood as their respective donor animal before secondary transplantation (e.g. primary and secondary recipients both have/had 65 % CD45.1$^+$ cells in peripheral blood). For comparison, the raw data without normalization can be found in the supplementary data (Supplementary Figure 4).

The calculation of repopulating units (RU) was performed with lethally irradiated Ly5.2 mice transplanted with 2x10^5 BM cells from placebo or JQ1 treated Ly5.1 mice together with 2x10^5 supporter BM cells from untreated Ly5.2 mice. RU values are calculated from percentages of donor-derived lymphocytes in the primary recipients 4 months after transplantation using the following formula:

$RU_{donor} = RU_{competitor} \times (\%\ lymphocytes_{donor} / 100 - \%\ lymphocytes_{donor})$

The relative repopulating abilities of 100,000 competitor marrow cells is defined as one RU.

Colony formation assays

Colony formation assays for the quantification of hematopoietic stem and progenitor cells or megakaryocytes were performed using fully supplemented MethoCult GF M3434, H4034 Optimum or MegaCult-C (Stemcell Technologies, Vancouver,

Canada), respectively, according to manufacturer's instructions. Colonies (defined as >100 cells) were scored 7 (M3434 and MegaCult-C) or 14 (H4034 Optimum) days after plating of cells. For serial replating assays, cells were replated and scored every 7 days for four rounds.

ELISA and RT-qPCR

Transcript levels were analyzed using Power SYBR Green RT-qPCR (Applied Biosystems, Foster City, CA, USA) and protein concentrations were determined using ELISA according to manufacturer's instruction (R&D Systems, Minneapolis, MN, USA). All Oligonucleotides that were used for analyzes are listed below.

Oligonuceotides for qRT-PCR

Gene	Primer name	Oligonucleotide sequences
Bcl-2	Bcl-2_fwd Bcl-2_rev	5´- TGG CCT TCT TTG AGT TCG GT -3´ 5´- ATG CCG GTT CAG GTA CTC AG -3´
Bcl2l11 (bim)	Bcl2l11_fwd Bcl2l11_rev	5´- CCA CGT CAA GAT GGC TCA CT -3´ 5´- GTG CAA CGC TGT TTG AGA GG -3´
Mcl1	Mcl1_fwd Mcl1_rev	5´- AAG AGG CTG GGA TGG GTT TGT -3´ 5´- AGT CCC CTA TTG CAC TCA CAA G -3´
Bcl2l1 (bcl-xl)	Bcl2l1_fwd Bcl2l1_rev	5´- GGC CTT TTT CTC CTT TGG CG -3´ 5´- GAT CCA CAA AAG TGT CCC AGC -3´
Bcl2a1a (A1)	Bcl2a1a_fwd Bcl2a1a_rev	5´- TAC GGC AGA ATG GAG GTT GG -3´ 5´- TAA CCA TTC TCG TGG GAG CC -3´
Bax	Bax_fwd Bax_rev	5´- GCC TTT TTG CTA CAG GGT TTC AT -3´ 5´- TTC ATC TCC AAT TCG CCG GA -3´
Bak1	Bak1_fwd Bak1_rev	5´- GGA GCA GAG TCA TTC AGG TGA C -3´ 5´- GTT CCT GCT GGT GGA GGT AAA -3´
Brd2	Brd2_fwd Brd2_rev	5´- GCT TCT GCT CTT GGC CTT CA -3´ 5´- GAA ACT CAA ACA CAT CCT GCA AC -3´
Brd3	Brd3_fwd Brd3_rev	5´- TTT GAG ACC CTG AAG CCA ACC -3´ 5´- CTG AGC CGG ACT TCT CTT TCT TAG -3´
Brd4	Brd4_fwd Brd4_rev	5´- GGA ATT TGG TGC TGA TGT CCG-3´ 5´- GTG GGG CTA CCA CCT TTG TA -3´

Flow cytometry

Different cell populations in Thymus, PB, BM and spleen were stained with antibodies and analyzed in a FACSCanto II flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Murine cells were incubated with TruStain FcX Fc receptor blocking

solution (Biolegend) and the following mouse-specific antibodies were used for staining: Lineage-V450 antibody cocktail kit (BD Bioscience), c-kit-APC (clone 2B8, eBioscience), Sca-1-PE/Cy7 (clone E13-161.7, Biolegend), CD48-APC/Cy7 (clone HM48-1, Biolegend), CD48-FITC (clone HM48-1, eBioscience), CD150-PE (clone mShad150, eBioscience), CD150-PB (clone TC15-12F12.2, Biolegend), CD150-APC (clone mShad150, eBioscience), CD34-FITC (clone Ram34, eBioscience), CD34-eFluor660 (clone Ram34, cBioscience), CD135-PE (clone A2F10, Biolegend), CD45.1-FITC (clone A20, eBioscience), CD45.2-PB (clone 104, Biolegend), FcgRII-PE (clone 93, Biolegend), CD41-PE (clone eBioMWReg30, eBioscience), CD61-FITC (clone 2C9.G3, eBioscience), IgM-APC/Cy7 (clone RMM-1, Biolegend), CD43-PE/Cy7 (clone S7, BDBioscience), IgD-APC (clone 11-26c(11-26), eBioscience), CD71-APC (clone R17217, eBioscience), Ter119-PE (clone TER119, eBioscience), CD11b-PE/Cy7 (clone M1/70, eBioscience), CD3e-APC (clone 145-2C11, Biolegend), CD4-FITC (clone RM4-5, eBioscience), CD8a-eFluor450 (clone 53-6.7, eBioscience), CD25-PE/Cy7 (clone PC61.5, eBioscience), CD44-APC/Cy7 (clone IM7, Biolegend), Gr1-APC/Cy7 (clone RB6-8C5, eBioscience), CD45R-PE (clone RA3-6B2, eBioscience), Annexin V-APC (BD Pharmingen), Brdu-APC flow kit (BD Pharmingen), CD11b-biotin (clone M1/70, eBioscience), Gr1-biotin (clone RB6-8C5, eBioscience), CD3e-biotin (clone 145-2C11, Biolegend), Ter119-biotin (clone TER119, eBioscience), CD4-biotin (clone GK1.5, eBioscience), CD8a-biotin (clone 53-6.7, eBioscience).

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1: Phenotyping of mRNA expression levels in the hematopoietic system.

(A-B) Sorted megakaryocytes (MK), hematopoietic stem cells (HSC), granulocyte-monocyte progenitors (GMP), Gr1+ cells (GR1+) and monocytes (Mo) from BM of mice were analyzed for baseline RNA expression of *Brd2* (A+C) and *Brd3* (B+D) via qRT-PCR (n=2-3; *p<0.05). (E-I) FACS-sorted T cell subpopulations from thymus of placebo or JQ1 treated mice were analyzed for mRNA expression via qRT-PCR (n=5; *p<0.05).

Supplementary Figure 2: Effects of JQ1 on healthy hematopoiesis.

Animals received daily i.p. injections of 50 mg/kg JQ1 for 21 days. (A) Kinetic of body weight over the course of JQ1-treatment (n=5; *p<0.05). (B-I) Analysis of different parameters after 21 days of treatment: Erythrocytes (B) and platelets (C) in peripheral blood (n=8; *p<0.05). (D) Myeloid cells in bone marrow (n=8; *p<0.05). (E) Megakaryocytes in spleen (n=4; *p<0.05). (E) Myeloid-erythroid progenitors (MEP) in bone marrow (n=8; *p<0.05). (F) Granulocyte-monocyte progenitors (GMP) in bone marrow (n=8; *p<0.05). (G-H) After injection of BrdU into mice, the frequency of $BrdU^+$ ST-HSC (G) and LSK cells (H) in bone marrow was determined by flow cytometry (n=5; *p<0.05). (I) LSK from young and old JQ1-treated mice in bone marrow (n=5; *p<0.05). (J) Colony formation assays to quantify hematopoietic progenitors in JQ1-pretreated human $CD34^+$ bone marrow cells *in vitro* (n=3; *p<0.05).

Supplementary Figure 3: JQ1 does not induce lineage priming of HSC.

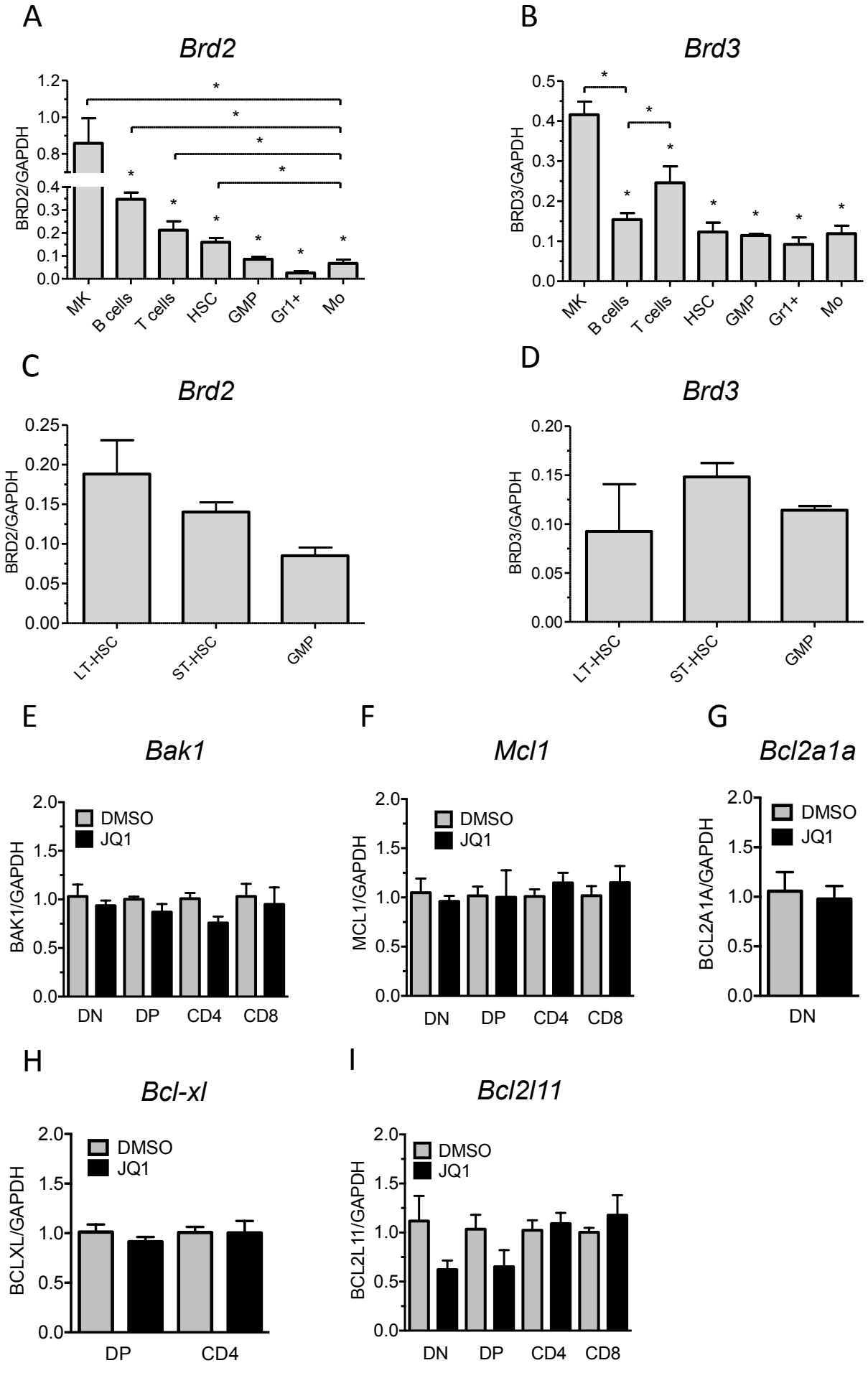
(A) Animals received daily i.p. injections of 50 mg/kg JQ1 for 21 days. Subsequently, the mice were lethally irradiated with 9 Gy and survival was monitored (n=6/8; *p<0.05).

Supplementary Figure 4: JQ1-treated HSC contribute more to hematopoiesis after HSCT into secondary recipients.

CD45.1 mice were treated for 21 days with 50 mg/kg JQ1 or placebo before sacrification. Subsequently, a mix of supporter bone marrow from CD45.2 mice and JQ1- or placebo-treated bone marrow from CD45.1 mice was transplanted into lethally irradiated CD45.2 recipients. Six month after transplantation, bone marrow was retransplanted into secondary recipients and chimerism was monitored by measuring $CD45.1^+$ and $CD45.2^+$ cells in peripheral blood. (A) Kinetic of hematopoietic chimerism following transplantation into secondary recipients (n=18; *p<0.05).

Supplementary Figure 5: Effects of JQ1-treatment on hematopoietic recovery after sublethal irradiation.

Animals received daily i.p. injections of 50 mg/kg JQ1 for 21 days followed by sublethal irradiation with 5 Gy. (A) Cell counts of B cells, T cells, myeloid cells, $Gr1^+$ cells and monocytes were determined via flow cytometry one week after irradiation. Cell numbers were normalized to pre-irradiation values to visualize irradiation-induced myelosuppression (n=4; *p<0.05). (B) Kinetic of the recovery of erythrocyte counts after irradiation in peripheral blood (n=3-5; *p<0.05).



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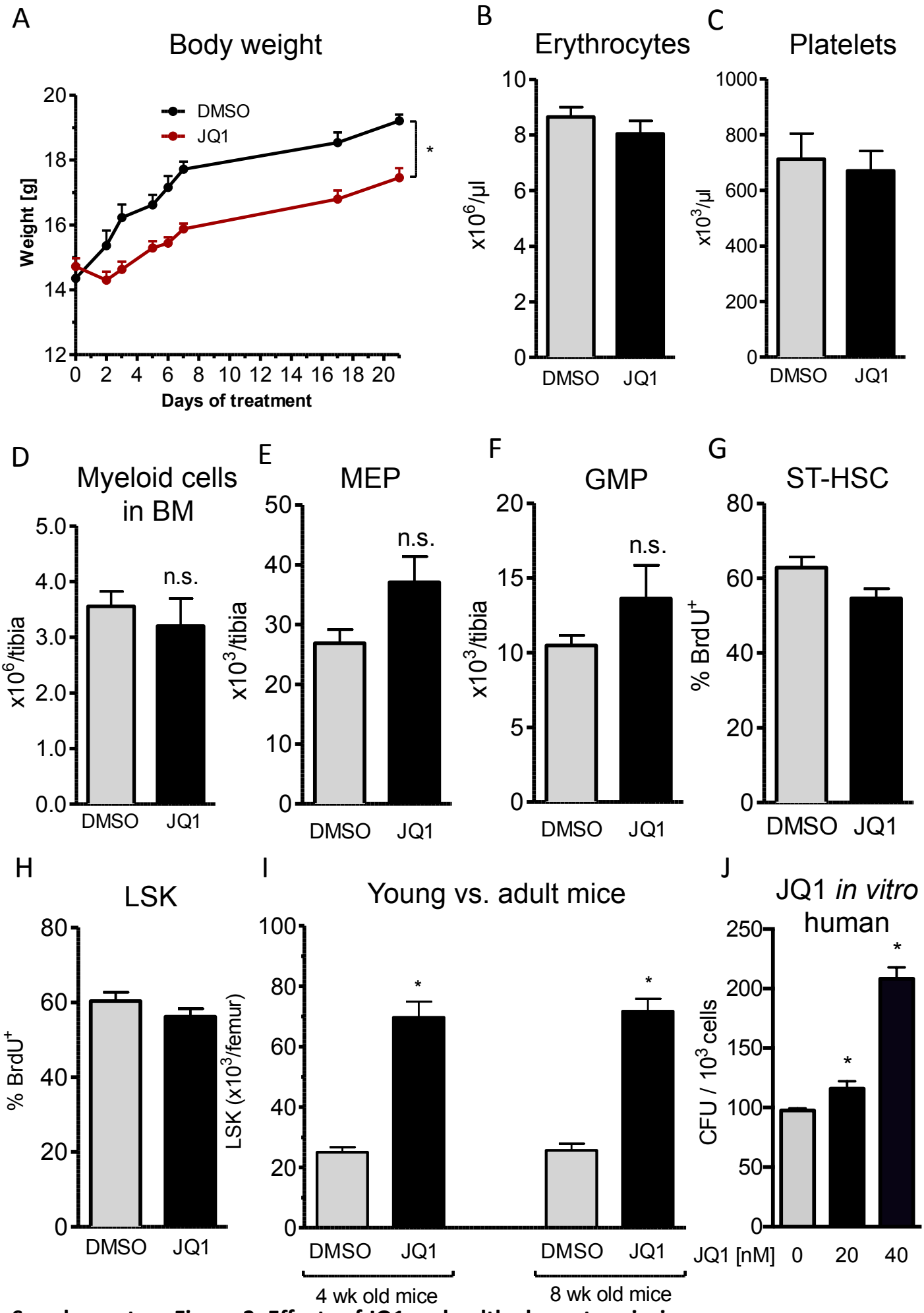
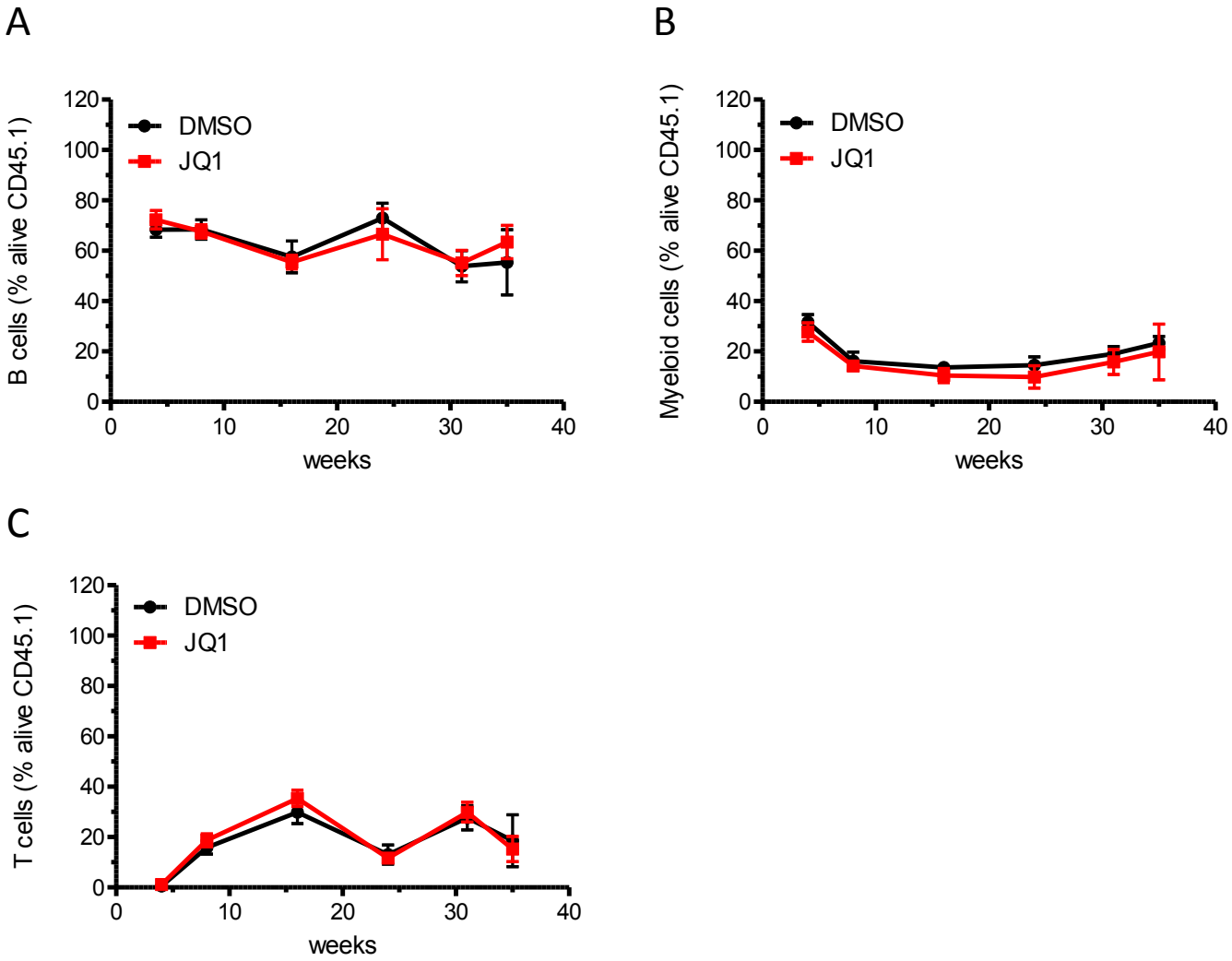
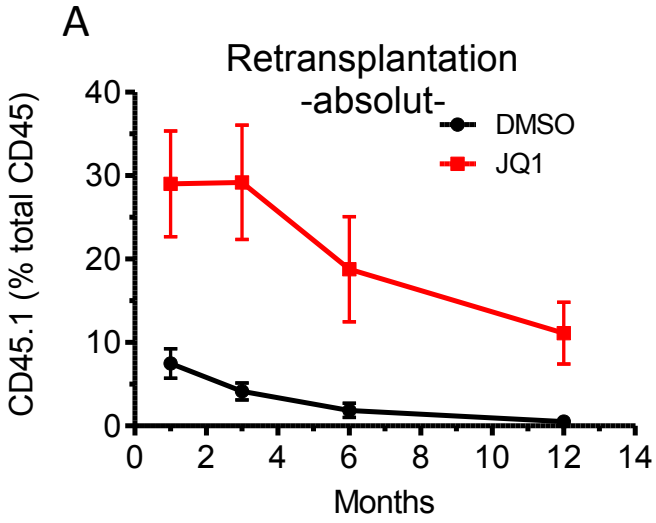


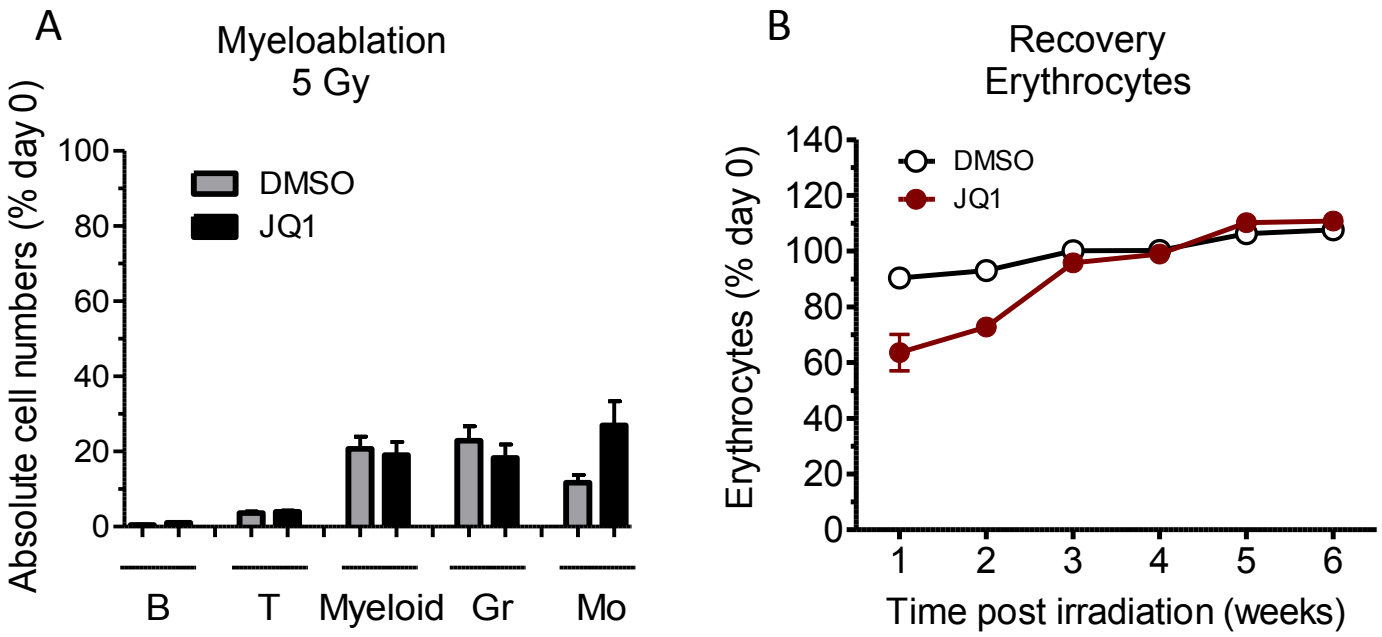
Supplementary Figure 2: Effects of JQ1 on healthy hematopoiesis.



Supplementary Figure 3: JQ1 does not induce lineage priming of HSC.



Supplementary Figure 4: JQ1-treated HSC contribute more to hematopoiesis after HSCT into secondary recipients .



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