The c-Jun NH_2-terminal protein kinases (JNKs) are members of the mitogen-activated protein kinase (MAPK) family. Map2k4 is a MAPK kinase that directly activates JNKs in response to extracellular and intracellular stresses, and its deficiency leads to abnormal hepatogenesis, resulting in loss of HBs [20,27-29]. In situ hybridization shows that expression of *Map2k4* transcripts is detected in FL by 12.5 dpc and then up-regulated until 16.5 dpc, at which time expression is down-regulated [30]. When *Map2k4*$^{-/-}$ FL cells were transplanted into *rag1*$^{-/-}$ adult mice, both B cells and T cells were generated, demonstrating that *Map2k4*$^{-/-}$ FL cells contain HSCs and/or HPCs [28]. In addition, normal hematopoiesis reportedly occurs in the *Map2k4*$^{-/-}$ YS at both 9.5 and 10.5 dpcs, based on erythroid, myeloid and mixed colony formation [28]. Taken together, hematopoietic potential of *Map2k4*$^{-/-}$ FL looked normal compared to wild-type mice. Therefore, the decrease of hematopoietic cells were likely due to lack of HBs, but not due to alteration of hematopoietic potential in *Map2k4*$^{-/-}$ FL. Recently, Hikita et al. reported a novel mouse model lacking both HBs and hepatocytes using an *Alb* Cre driver [31]. This mutant mouse, generated by crossing *Alb Cre* mice with both *bcl-xl*$^{flox/flox}$ mice and *mcl-1*$^{flox/flox}$ mice, showed a decreased number of hepatocytes at 18.5 dpc. Future studies will be required to address the function of HBs and hepatocytes in FL erythropoiesis using this model.

Conclusion

Hepatoblasts comprise a niche for erythropoiesis through cytokine secretion.

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Authorship contributions

D. Sugiyama designed the research, performed research, analyzed data and wrote the paper. K. Kulkeaw performed research and analyzed data. C. Mizuochi, Y. Horio and S. Okayama performed research.

Conflict of Interest Disclosures

The authors have no conflict of interest to declare.

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Figure Legends

Fig. 1. Cytokine gene expression in fetal liver. (A) *FMS-like tyrosine kinase 3 ligand (Flt3l), thrombopoietin (TPO), eryrhropoietin (EPO), stem cell factor (SCF), interleukin-3 (IL-3), interleukin-6 (IL-6), interleukin-11 (IL-11), granulocyte-colony stimulating factor (G-CSF)* and *granulocyte/macrophage-colony stimulating factor (GM-CSF)* mRNAs were examined in FL samples at 12.5 and 14.5 dpc by real-time PCR. Note high expression of *Flt3l, TPO, EPO* and *IL-6* in FL. (B) A single cell suspension was obtained from FL at 12.5 dpc and expression of CD45/Ter119, Dlk-1, Lyve-1 and CD31 was analyzed by flow cytometry. (1) CD45–/Ter119–/Dlk-1+ defines HBs; (2) CD45–/Ter119–/Lyve-1+/CD31+ defines SECs; and (3) CD45+/Ter119+ defines HCs. (C) Expression of *Flt3l, TPO, EPO* and *IL-6* was examined by real-time PCR in HBs, SECs and HCs sorted by flow cytometry, according to gates defined in Figure 1B. *EPO* and *TPO* expression was high in HBs at both 12.5 dpc and 14.5 dpc. *SCF* expression was higher in HBs than in SECs. Expression of *Flt3l* was high in HCs. Expression of *IL-6* was detected only in HCs.

Fig. 2. Expression of EPO and SCF protein in fetal liver. (A) EPO protein in sorted cells was assayed by ELISA. EPO was detected in HBs (63.8 pg/mL/10000 cells) but not in SECs and HCs at 12.5 dpc. (B) SCF protein in sorted cells was assayed by ELISA. SCF was detected in HBs (7.01 pg/mL/10000 cells), SECs (2.38 pg/mL/10000

cells) and HCs (0.025 pg/mL/10000 cells) at 12.5 dpc. (C) The data from Figure 2A with 2B were combined to compare EPO and SFC protein expression in each fraction sorted from fetal liver. EPO protein expression was highest in HBs. (D-F) Liver sections were prepared from ICR mouse embryos at 12.5 dpc and stained with Dlk-1 (green), EPO (red) and TOTO-3 (blue) (D); Lyve-1 (green), SCF (red) and TOTO-3 (blue) (E); and Dlk-1 (green), SCF (red) and TOTO-3 (blue) (F). Arrowheads show co-localization of the antigens, respectively. Samples were observed under confocal microscopy. EPO was primarily expressed in HBs expressing Dlk-1, whereas SCF was more widely expressed in FL. Original magnification is 40x. (D-F)

Fig. 3. Phenotypic analysis of FL in *Map2k4*$^{-/-}$ mouse embryos. (A) Expression of *EPO* and *SCF* was examined by real-time PCR in *Map2k4*$^{-/-}$ fetal liver (FL). Expression levels of both *EPO* and *SCF* were down-regulated in *Map2k4*$^{-/-}$ FL relative to wild-type FL. (B) Liver sections were made from both *Map2k4*$^{-/-}$ and wild-type mouse embryos at 12.5 dpc, stained with c-Kit (green), Ki-67 (red) and TOTO-3 (blue), and observed under confocal microscopy. The number of Ki-67 positive cells in a field decreased in *Map2k4*$^{-/-}$ FL compared to wild-type FL. Original magnification is 40x. (C) Single cell suspensions were obtained from *Map2k4*$^{-/-}$ and wild-type FL at 12.5 dpc and the number of living cells was counted after Trypan Blue staining. Significant decreases in the number of *Map2k4*$^{-/-}$ FL cells were observed compared to wild-type FL cells.

Figure 1A

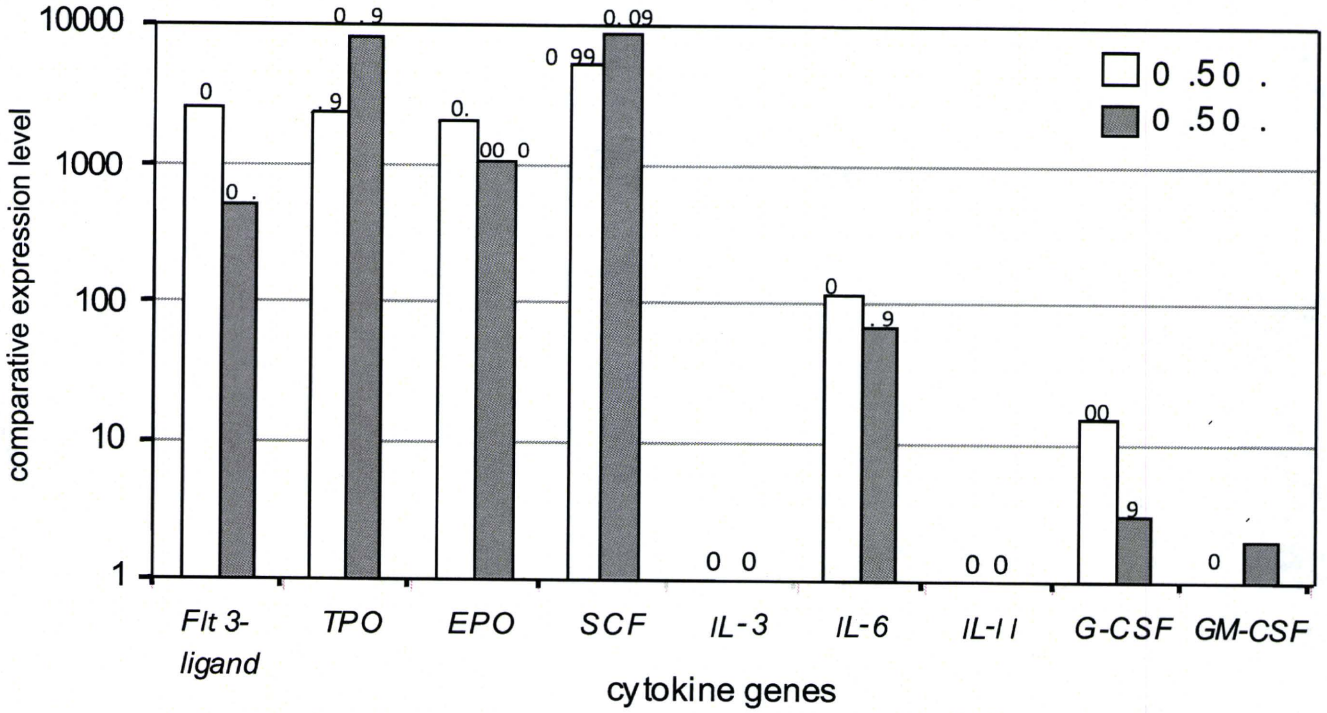


Figure 1B

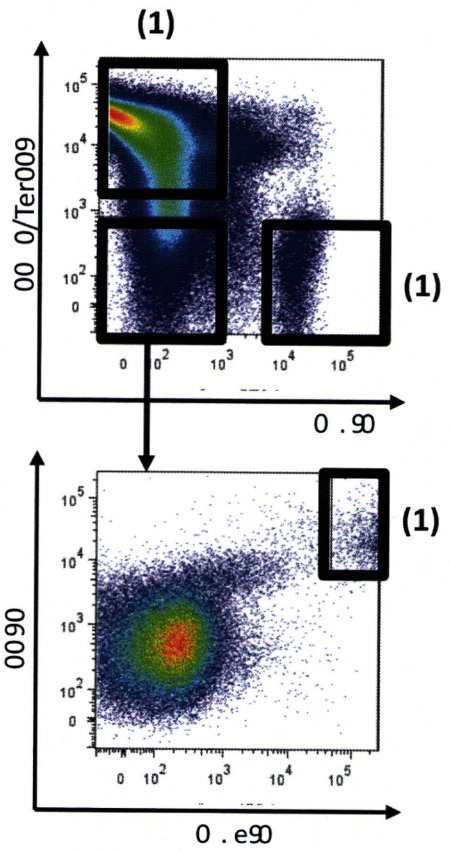


Figure 1C

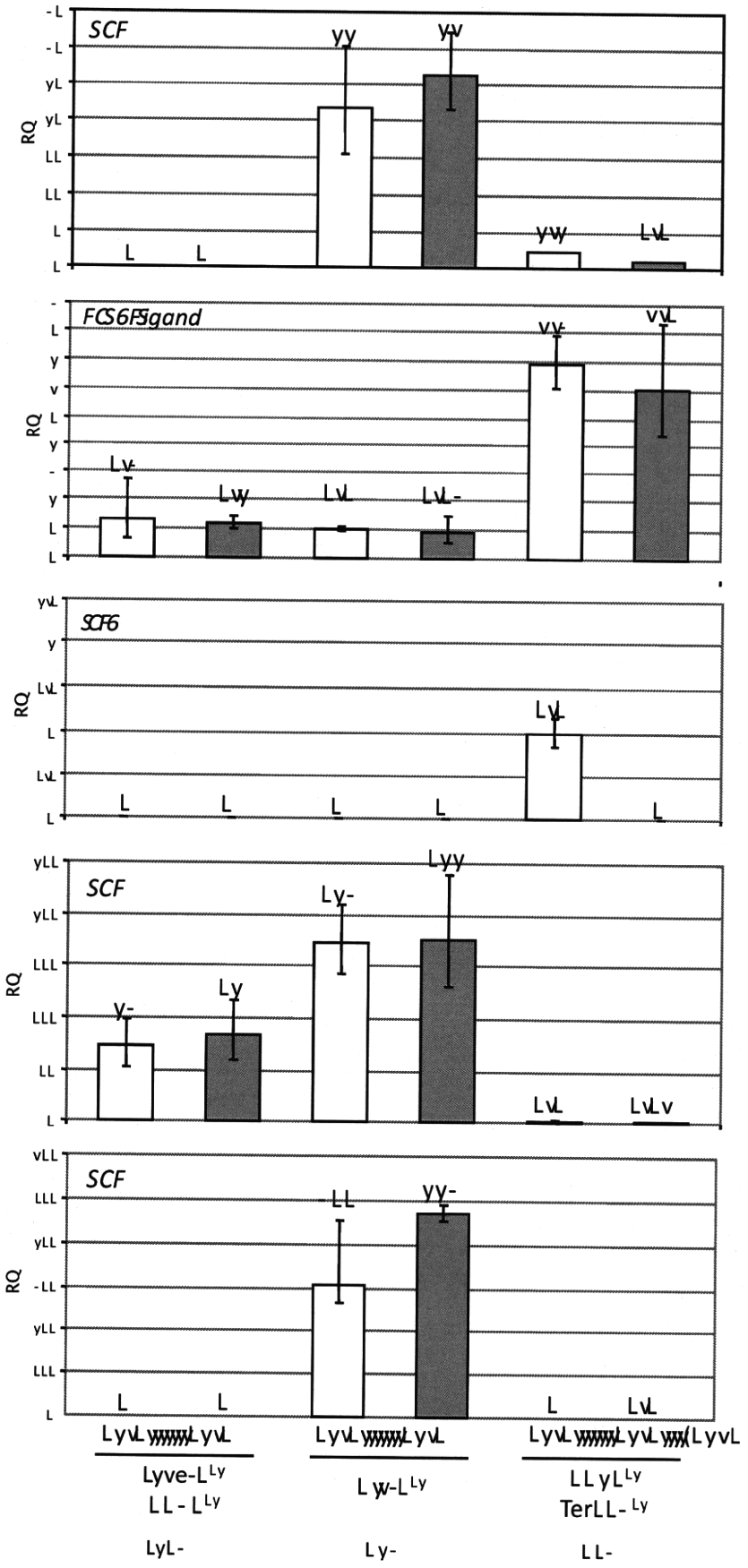
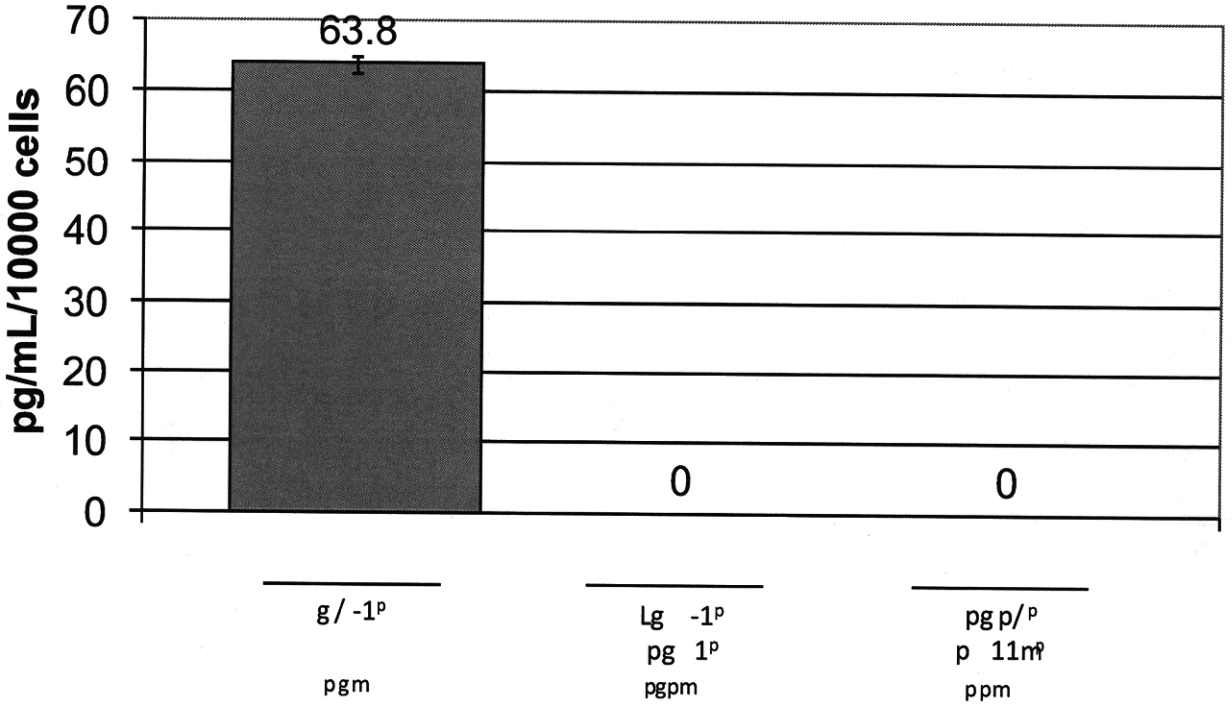


Figure 2A



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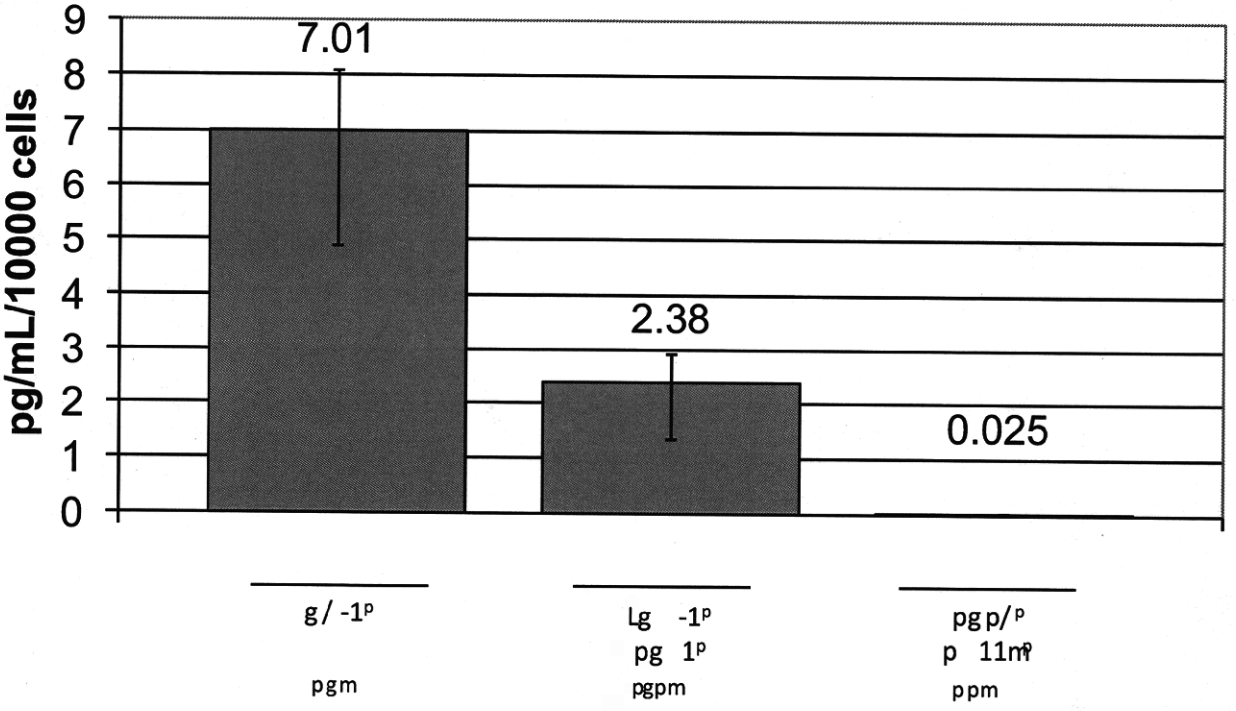

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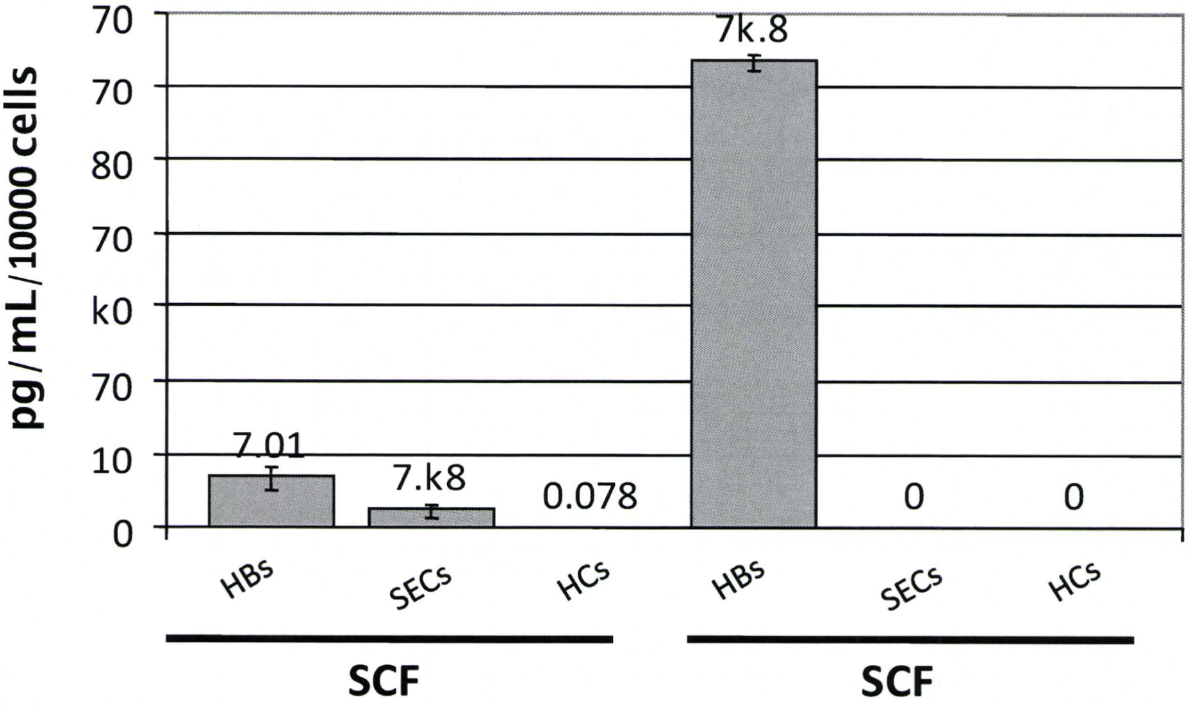

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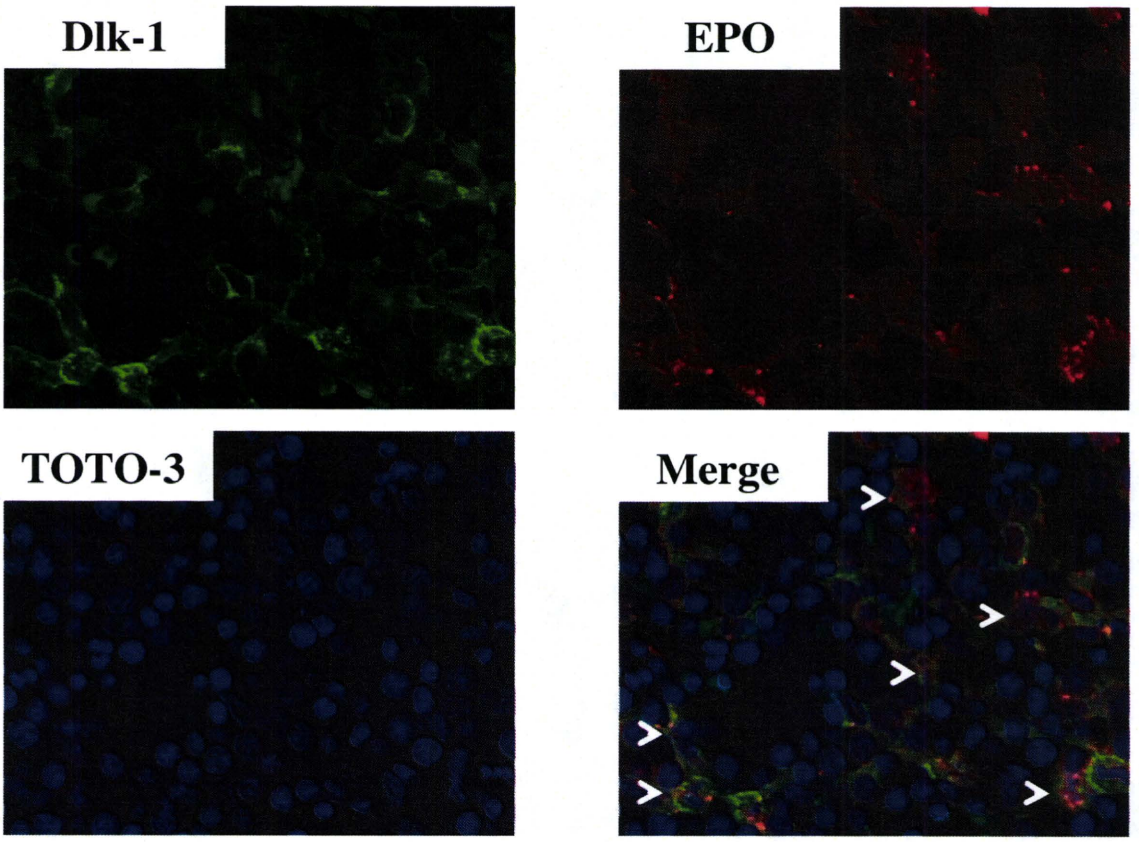

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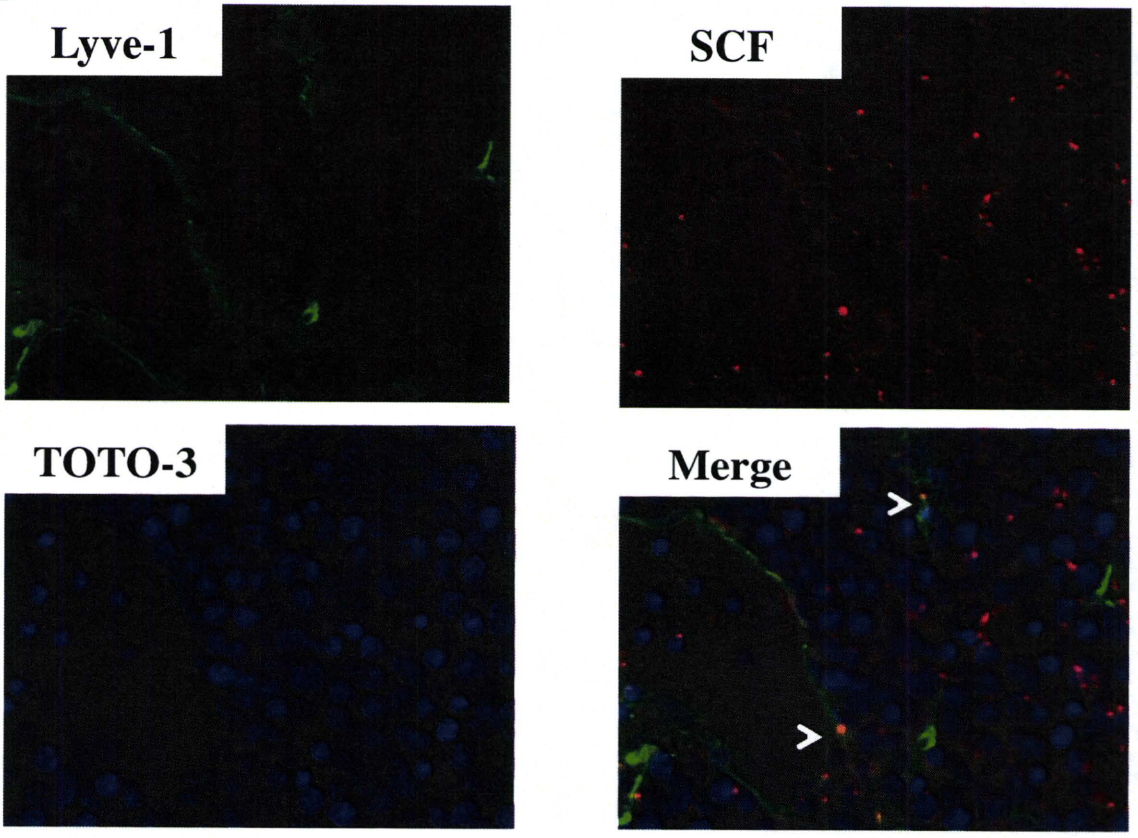

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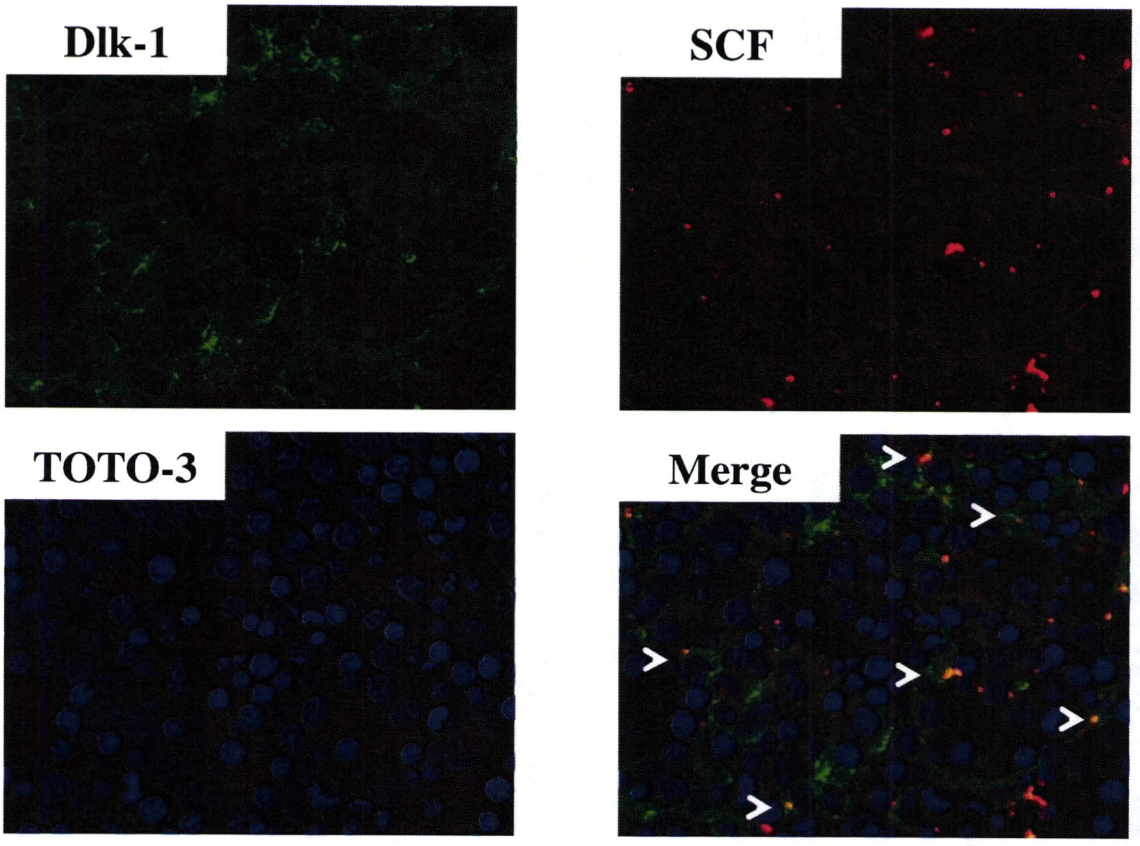
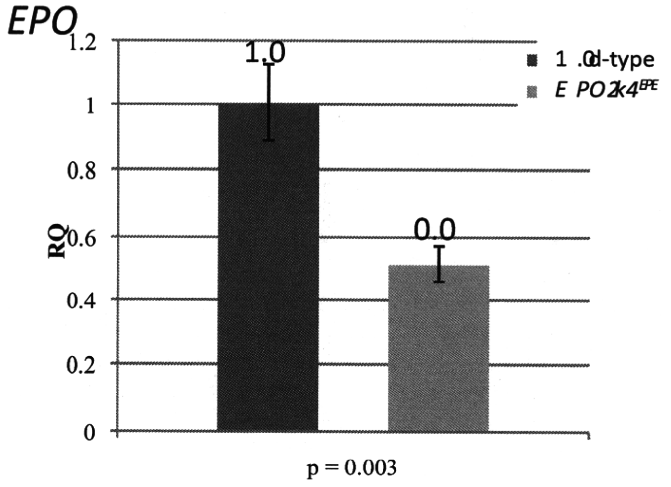


Figure 3A


EPO
1.2
1
0.8
0.6
0.4
0.2
0
RQ
1.0
0.0
1.0 d-type
EPO2k4BPE
p = 0.003

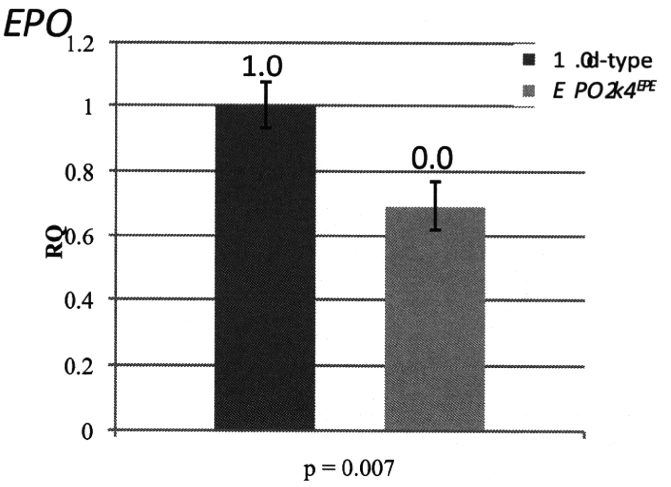

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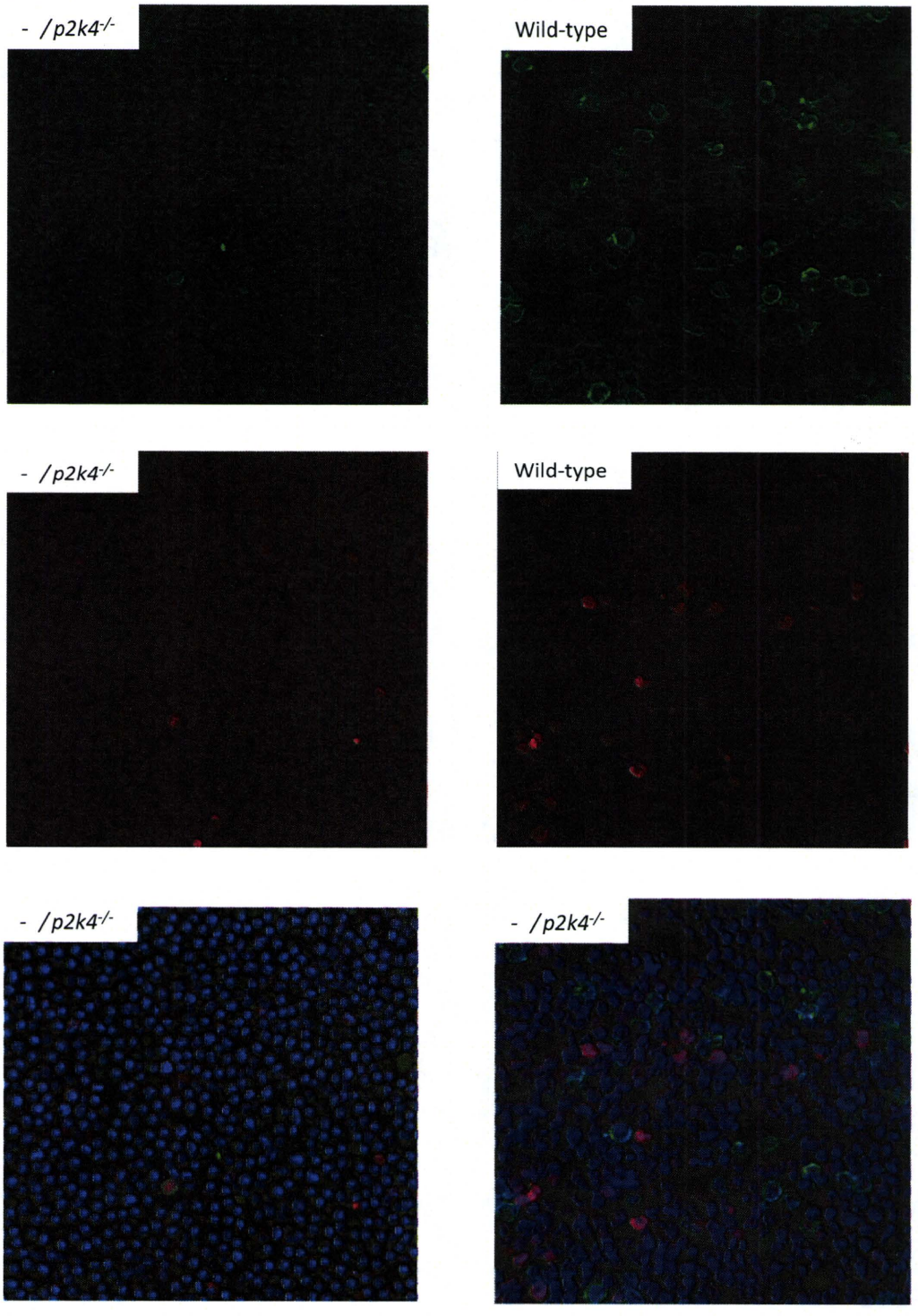
Figure 3B



c-Kit Ki-67 TOTO-3

Figure 3C