

Wnt/ β -Catenin-Signaling Modulates Megakaryopoiesis at the Megakaryocyte-Erythrocyte Progenitor Stage in the Hematopoietic System

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Supplementary Figure Legends

Suppl. Fig. 1:

Exemplary gating strategy for leukocytes. Single (A2), debris free (A3) blood cells were counted using leukocyte-specific markers such as Ly6G for granulocytes or F4/80 for monocytes. For the lymphoid compartment CD4⁺ T cells or CD19⁺ B cells were quantified. 1x10⁵ events were counted and analyzed using FACS Canto TM II.

Suppl. Fig. 2:

Gating of GFP⁺ cells from MKPs in BM recipients after tamoxifen treatment. (A) Flow-cytometry gating strategy for the detection of MKPs in BM cells. In first line, BM cells (A1) were gated for CD150 (A2) and then gated for CD41⁻ (A3) and CD41⁺ (A4). CD41⁺ cells were gated for GFP (A5), showing 91.0% of all CD41⁺ cells. These cells exhibit high granularity and size (A6). 1x10⁶ were counted on the FACS CantoTM II.

(B) FMO control gating for GFP expressing cells from BM. 1x10⁶ were counted on the FACS CantoTM II.

Suppl. Fig. 3:

Flow-cytometry gating strategy for analyzing BM engraftment in recipient mice from peripheral blood. The blood cells (A1) were gated for the newly engrafted CD45.2⁺ cells (A3) after irradiation and the pre-existing recipient CD45.1⁺ cells (A2) after irradiation and BMT. To track the hematopoiesis of mature blood cell types by newly engrafted HSCs, the blood cells (A1) were gated for CD19⁺ B cells (A4), CD3⁺ T cells (A5) and CD11b⁺ (A6) cells in combination with the engraftment indicator markers CD45.1 and CD45.2 (A4.1, A5.1, A5.2, A6.1, A6.2). 1x10⁵ events were counted on the FACS Canto TMII.

Suppl. Fig. 4:

Transgene GFP-reporter analysis of MKPs in BMT recipients after tamoxifen treatment. (A) Flow-cytometry gating strategy for the detection of MKPs in BMT animals. In first line, BM cells (A1) were checked whether they have origin of donor (A2) or recipient mice (A3). MKPs (A4, A5) were gated out of CD45.2⁺ donor cells and plotted for GFP presence (A6). 1x10⁶ were counted on the FACS CantoTM II.

(B) Gated MKPs (A4) did rise from newly engrafted donor BM (CD45.2⁺). BMT CTL MKPs were not expressing the transgene GFP-reporter (red box), whereas the mutants (Ctnnb1^{BM-GOFwt/fl}) expressed the GFP-reporter (green box).

Suppl. Fig. 5:

Analysis of the skin from Ctnnb1^{BM-GOFwt/fl} and Ctnnb1^{BM-CTL} mice. (A) HE staining on skin cryosection samples revealed normal formation of hair follicles (asterisk) in Ctnnb1^{BM-CTL} animals. In contrast, Ctnnb1^{BM-GOFwt/fl} mice developed hyperkeratosis with abnormalities in the formation of hair follicles (asterisk). Scale bar: 250µm

(B) KI67 staining on paraffin embedded skin sections. In Ctnnb1^{BM-CTL} animals, the KI67 staining occurred mainly in hair follicles (asterisk) and at lower level in the epidermal layer (arrowheads). In the Ctnnb1^{BM-GOFwt/fl} samples, epidermal cells were strongly positive for Ki67 (arrowheads). Furthermore, hyperplastic hair follicles (asterisk) were strongly Ki67 positive.

The dermal layer showed several individual stained parenchymal cells (arrows). Scale bar: 250 μ m

Suppl. Fig. 6:

Analysis of lung tissue from *Ctnnb1*^{BM-GOFwt/fl} and *Ctnnb1*^{BM-CTL} mice. (A) Analysis of lung cryosections using different staining methods: there was no apparent difference between *Ctnnb1*^{BM-CTL} and *Ctnnb1*^{BM-GOFwt/fl} animals with regard to the parenchyma of the lung (HE staining). (B) Furthermore, no anomalies could be seen in the collagen content (Picro-Sirius staining) or (C) in the inflammation status by the staining of T cells (CD3, arrows in CTL and *Ctnnb1*^{BM-GOFwt/fl}). Scale bar: 50 μ m

Suppl. Fig. 7:

CBC assay from PB from *Ctnnb1*^{BM-GOFwt/fl} and *Ctnnb1*^{BM-CTL} mice, showing hemoglobin (Hb, g/dL), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), red cell distribution width (RDW, %) for of CTL (n=12), *Ctnnb1*^{BM-GOFwt/fl} (n=6) and *Ctnnb1*^{BM-GOFfl/fl} (n=7) mice.

Suppl. Fig. 8:

Blood analysis by FACS from PB after BMT from *Ctnnb1*^{BM-GOFwt/fl} and *Ctnnb1*^{BM-CTL} mice. (A-D) BM engraftment analysis in PB cells. Using the gating strategy shown in Fig. 4 allowed to control for lineage specific engraftment, such as myeloid cells and lymphoid cells (T cells and B cells). The BMT was successful since most PB cells had CD45.2 positive origins. n=3

(E-G) CBC assay analysis of BMT (peripheral blood) PB samples. There were no significant alterations in the erythroid compartment or the thrombocytes. However, erythrocytes in BMT_ *Ctnnb1*^{BM-GOFwt/fl} were slightly reduced. The WBCs were decreased in BMT_ *Ctnnb1*^{BM-GOFwt/fl} but remaining in the normal range though. Normal range WBCs: 1.8-10.7 K/ μ l, normal range erythrocytes: 6.36-9.42 M/ μ l, normal range thrombocytes: 592-2972 K/ μ l.

Suppl. Fig. 9:

Analysis of HSCs and Progenitors in the BM of BMT animals. After the successful engraftment of newly transplanted BM and tamoxifen application, HSCs and several hematopoietic progenitors were counted using FACS. The activation of the Wnt/ β -catenin pathway in BMT *Ctnnb1*^{BM-GOF} did not lead to changes in the differentiation of HSCs, CMPs, MEPs and GMPs in comparison to the CTL. Gating strategy shown in Suppl. Fig. 2 was applied and 1x10⁶ events were recorded.

Suppl. Fig. 10:

***Ctnnb1*^{EC-GOFwt/fl} transgene analysis of MKPs in the BM after tamoxifen treatment.** (A) Scheme of *Cdh5*(PAC)-CreERT2 mice crossed with *Ctnnb1*^{lox(ex3)/(ex3)} mice to achieve endothelial-specific activation of β -catenin (*Ctnnb1*^{EC-GOFwt/fl}). (B) Flow-cytometry gating strategy for the detection of MKPs in BM of control (CTL) animals. At first, the BM was gated

for CD150⁺ cells, followed by gating for the CD150⁺ and CD41⁺ double positive MKPs. 1x10⁶ were counted on the FACS CantoTM II.

(C) Detection of CD150⁺ and CD41⁺ double positive MKPs in BM of Ctnnb1^{EC-GOFwt/fl} animals.

(D) Quantification of MKPs as % of bone marrow (BM) cells. (E) Fluorescence minus one (FMO) control for CD41-PE/Cy7. (F) Fluorescence minus one (FMO) control for CD150-PE.

Suppl. Fig. 11:

Ctnnb1^{EC-GOFwt/fl} transgene analysis of MKPs in the spleen after tamoxifen treatment. (A)

Flow-cytometry gating strategy for the detection of MKPs in spleen of control (CTL) animals. At first, the BM was gated for CD150⁺ cells, followed by gating for the CD150⁺ and CD41⁺ double positive MKPs. 1x10⁶ were counted on the FACS CantoTM II.

(B) Detection of CD150⁺ and CD41⁺ double positive MKPs in the spleen of Ctnnb1^{EC-GOFwt/fl} animals. (C) Quantification of MKPs as % of spleen (SP) cells. (D) Fluorescence minus one (FMO) control for CD41-PE/Cy7. (E) Fluorescence minus one (FMO) control for CD150-PE.

Suppl. Fig. 12:

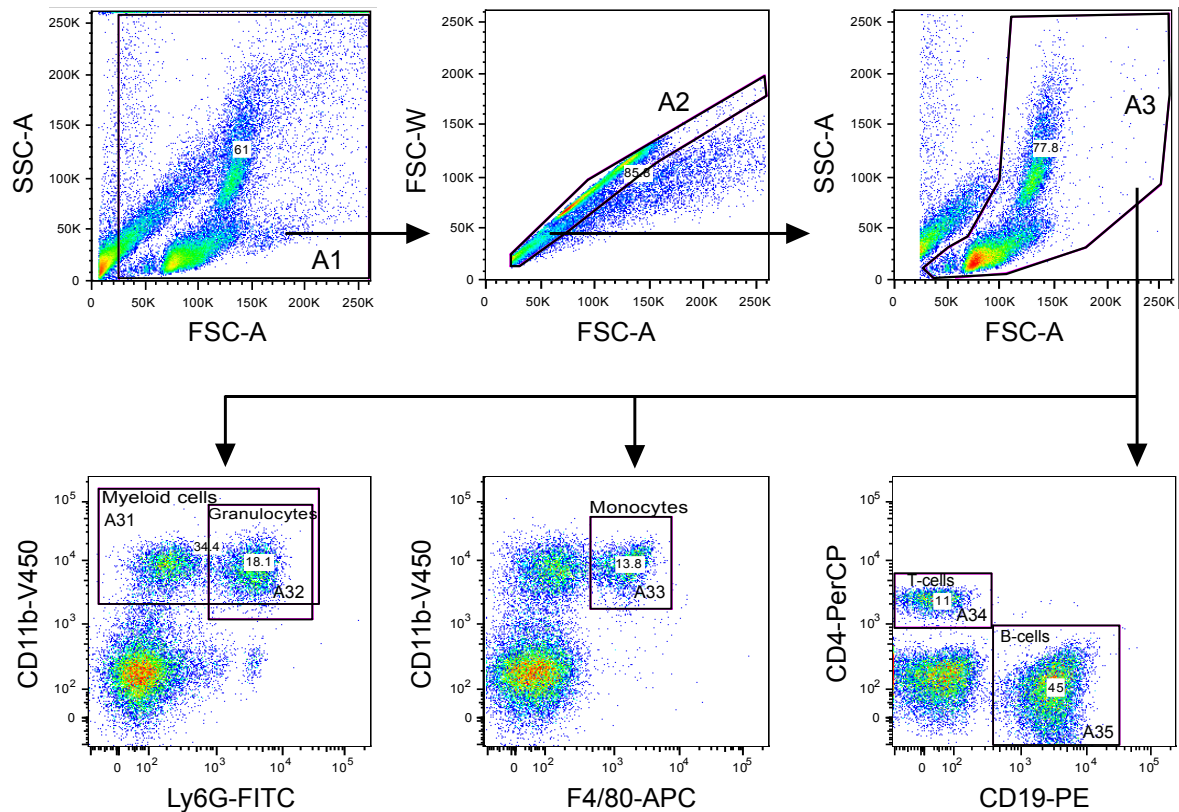
Ctnnb1^{EC-GOFwt/fl} transgene is recombined and Wnt/β-catenin target is activated upon 4-OHT treatment in vitro.

(A) Isolated bone marrow mononucleated cells from PDGFb-iCreERT2^{wt/wt}:Ctnnb1^{wt/lox(Ex3)} (Ctrl) PDGFb-iCreERT2^{wt/cre}:Ctnnb1^{wt/lox(Ex3)} (GOF) mice were cultured for 7 days in StemSpanTM SFEM media (STEMCELL #09650) with stem cell factor (m-SCF, 100 ng/ml) and thrombopoietin (TPO, 100 ng/ml), treated every second day with 25 μM 4-hydroxy-tamoxifen (4-OHT) and 7 days.

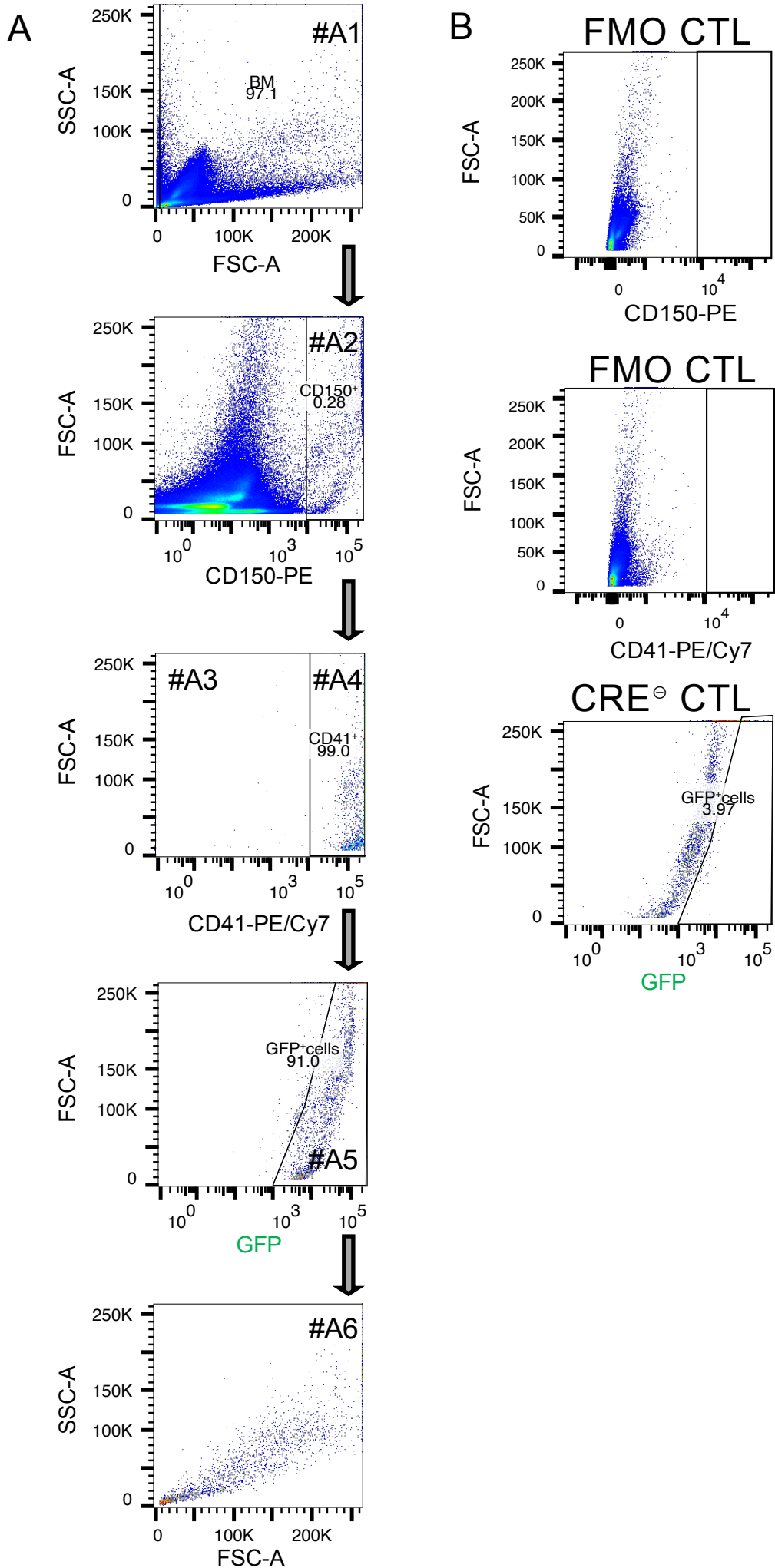
(B) Pdgfb-expressing brain microvascular endothelial cells (MBMECs) from the same mice were isolated, cultured and harvested after 7 days.

From both cells types gDNA and total RNA was isolated and recombination of the transgene and induction of the Wnt/β-catenin target Axin2 was analyzed by PCR and qRT-PCR, respectively (n=1). The data confirming recombination as well as Axin2 induction in GOF cells.

Suppl. Fig. 1, Yalcin et al.

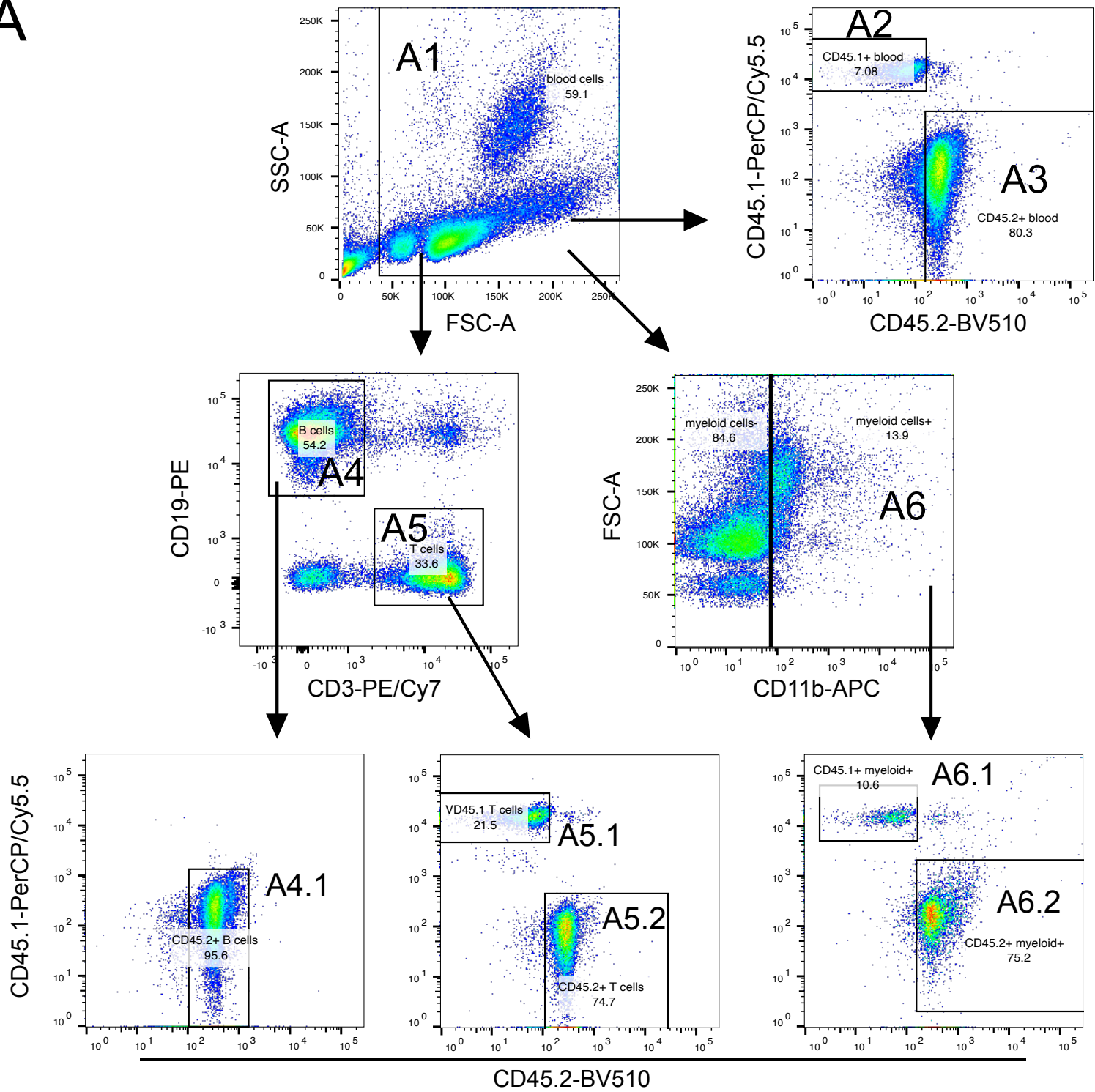


Suppl. Figure 2, Yalcin et al.

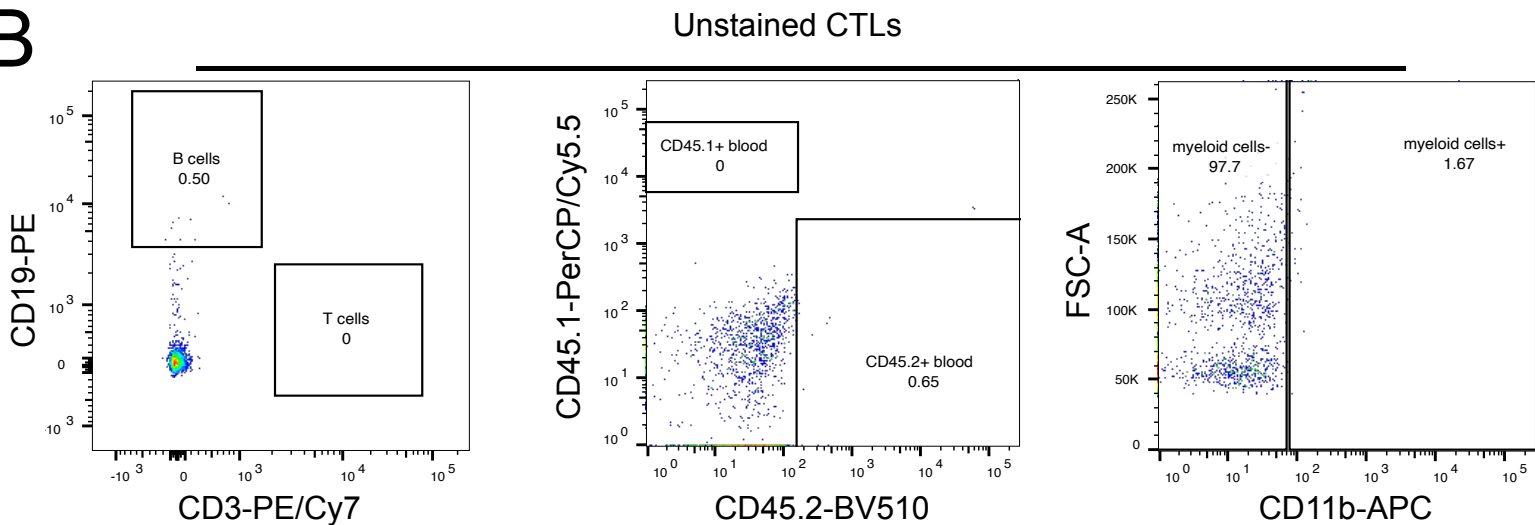


Suppl. Fig. 3, Yalcin et al.

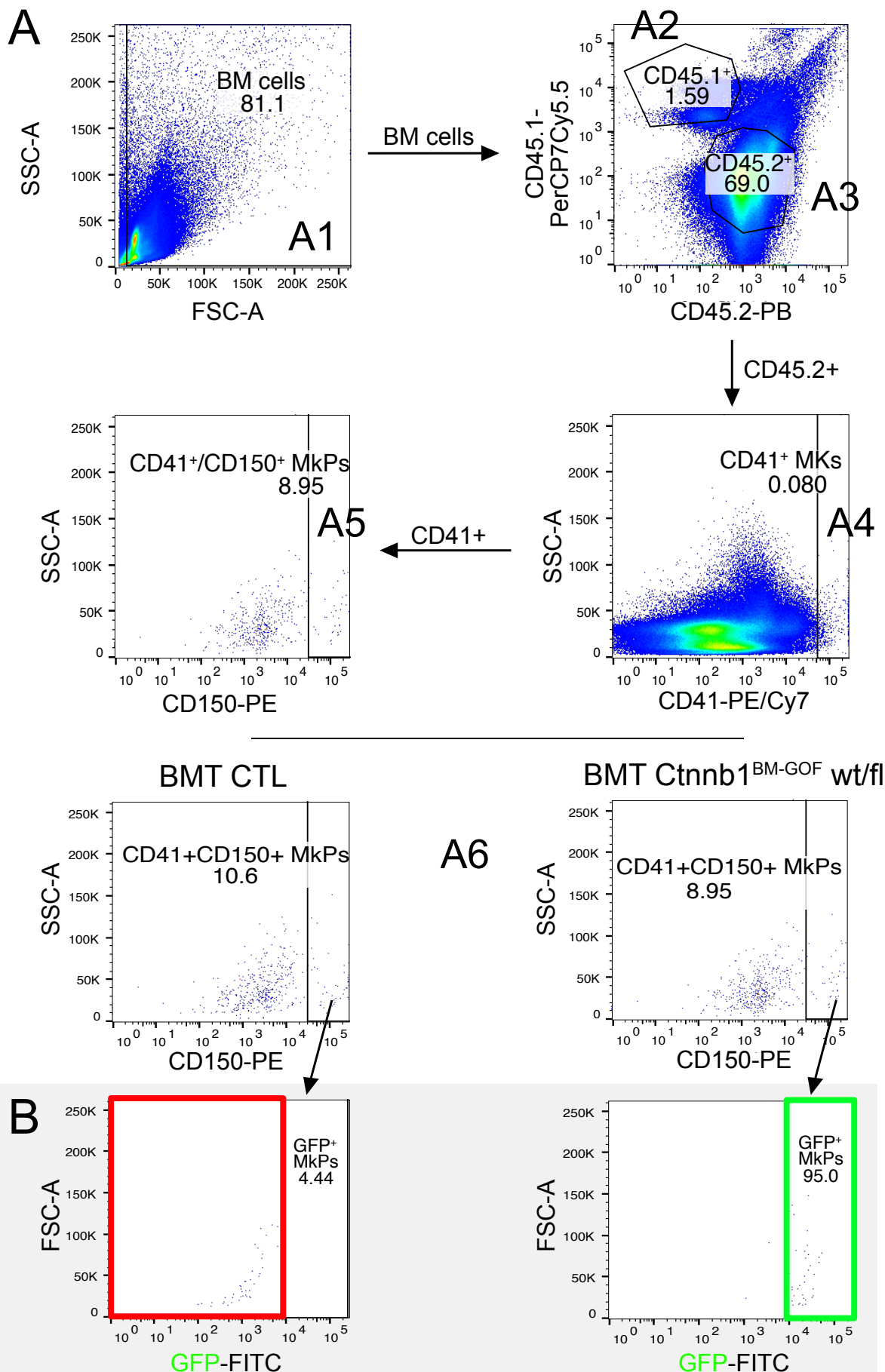
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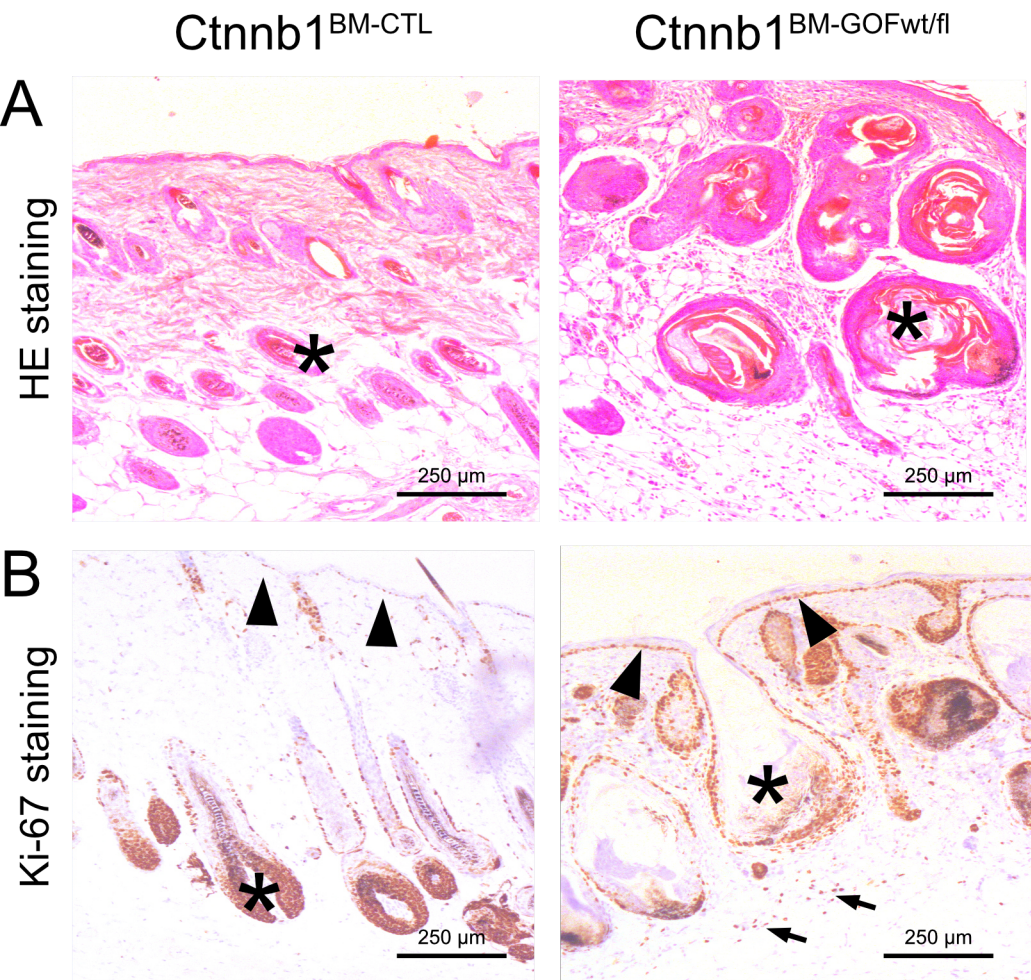
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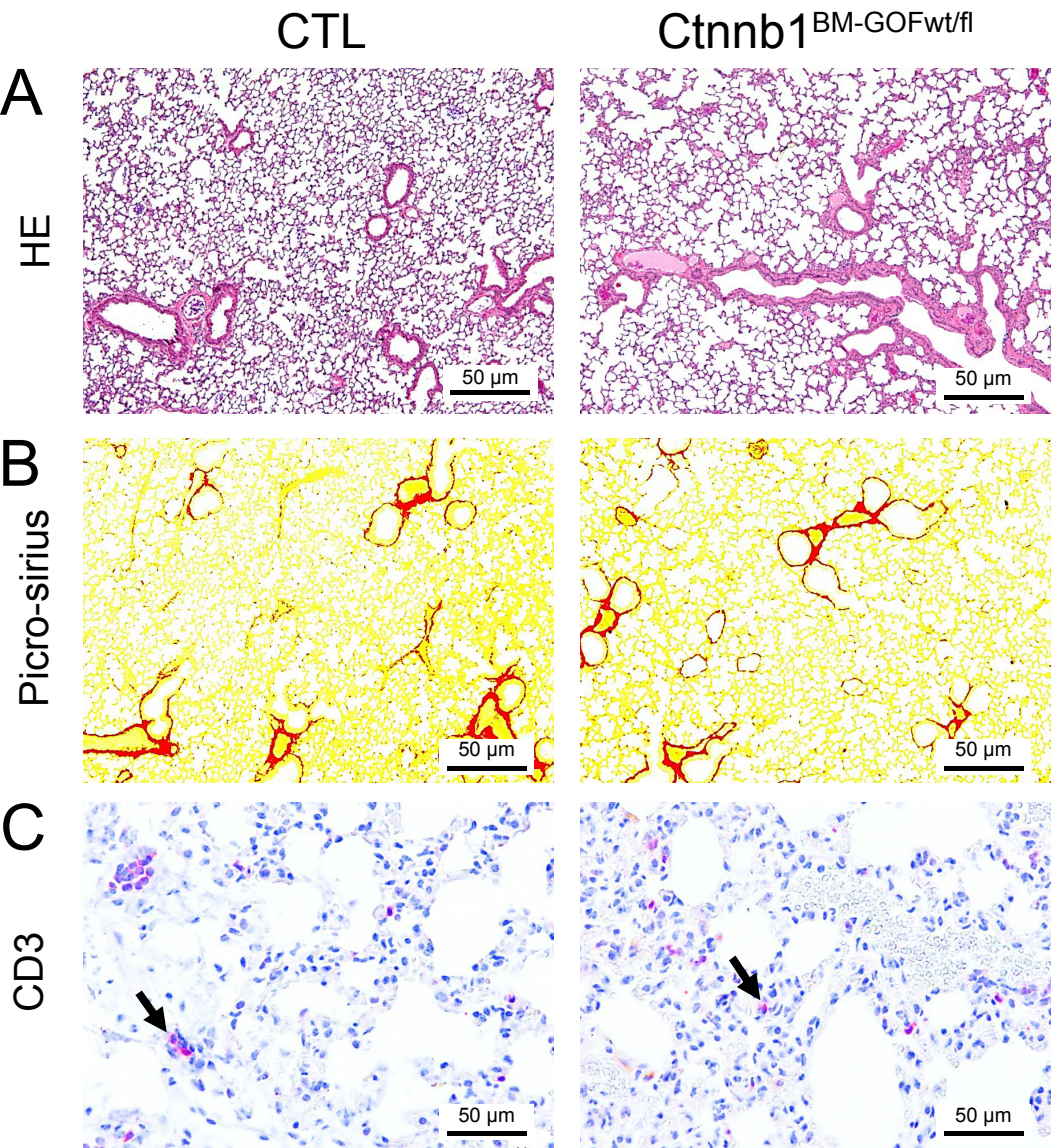
Suppl. Fig. 4, Yalcin et al.



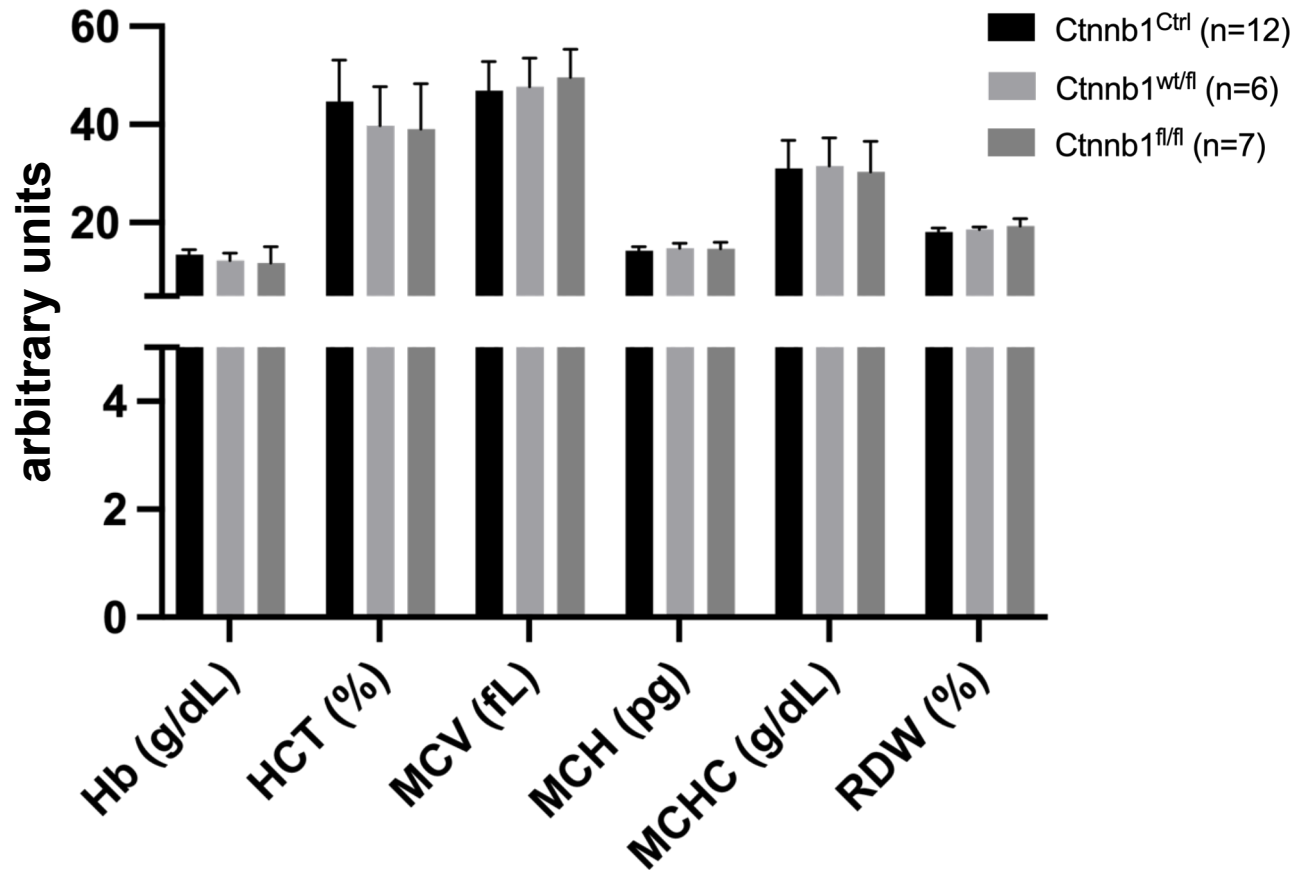
Suppl. Fig. 5, Yalcin et al.



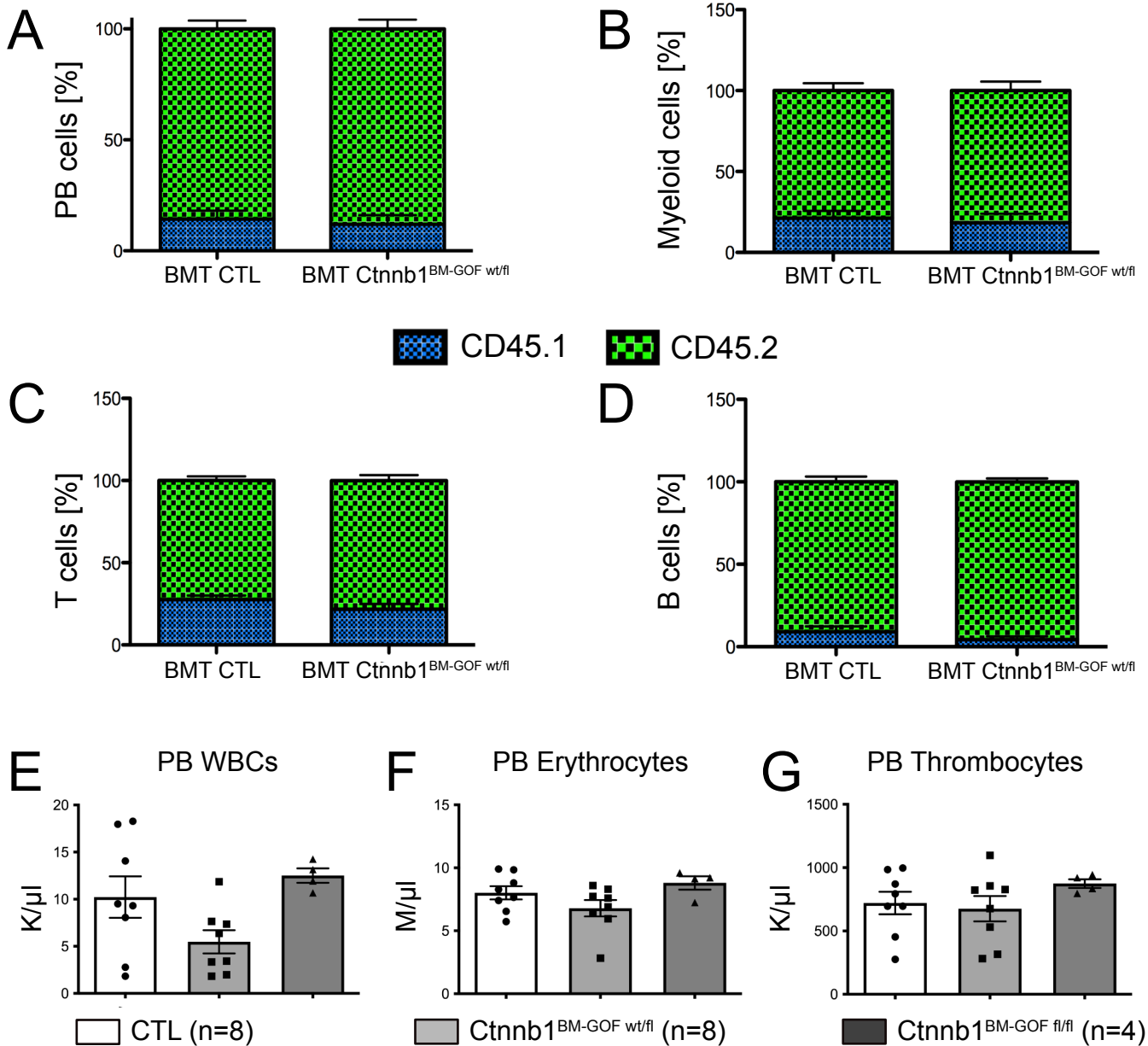
Suppl. Fig. 6, Yalcin et al.



Suppl. Figure 7, Yalcin et al.

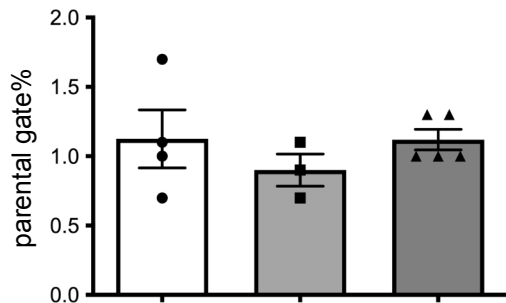


Suppl. Figure 8, Yalcin et al.

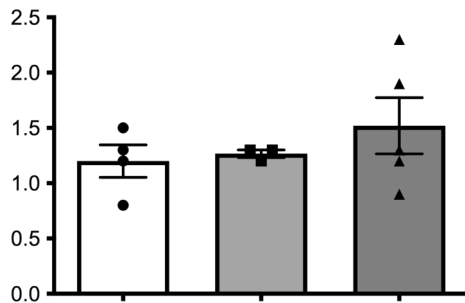


Suppl. Fig. 9, Yalcin et al.

KSL

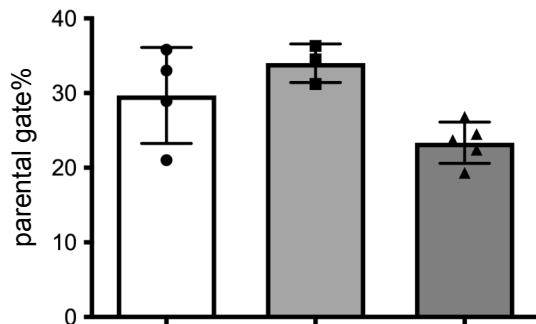


CMPs

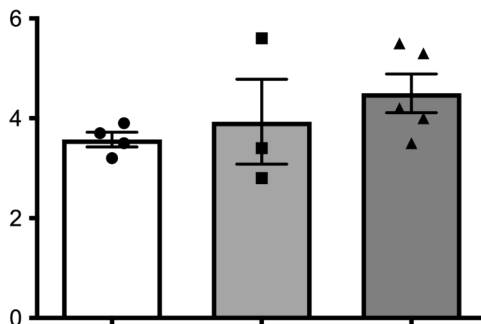


CTL (n=4) Ctnnb1^{BM-GOF wt/fl} (n=3) Ctnnb1^{BM-GOF fl/fl} (n=5)

MEPs

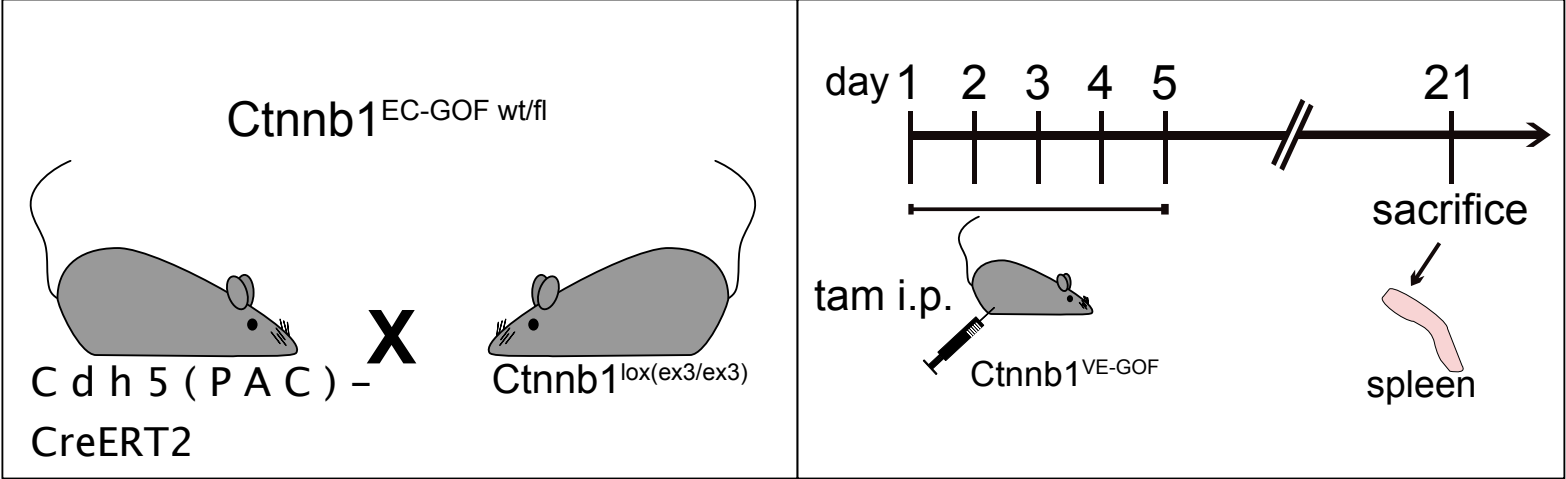


GMPs

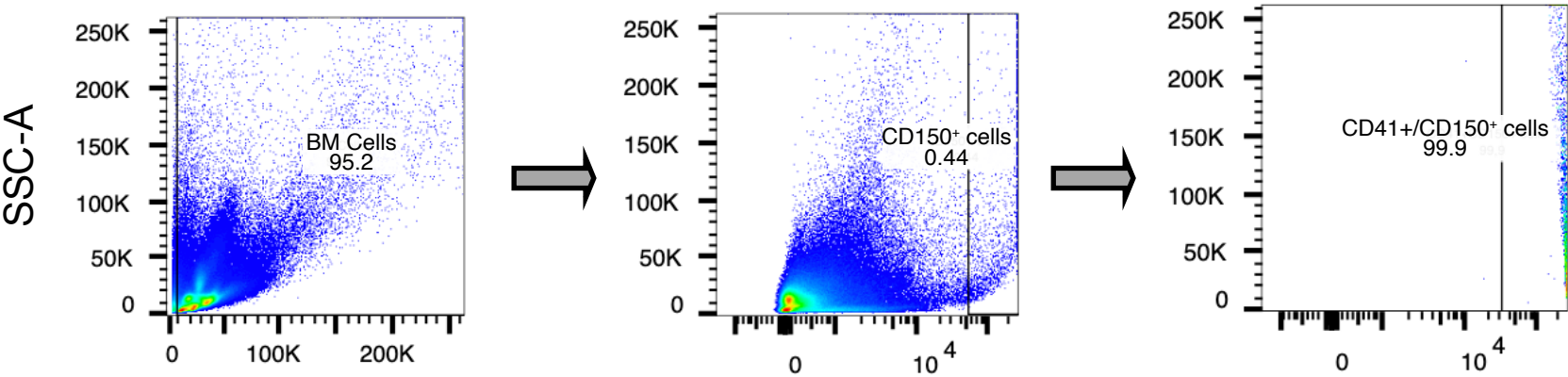


Suppl. Fig. 10, Yalcin et al.

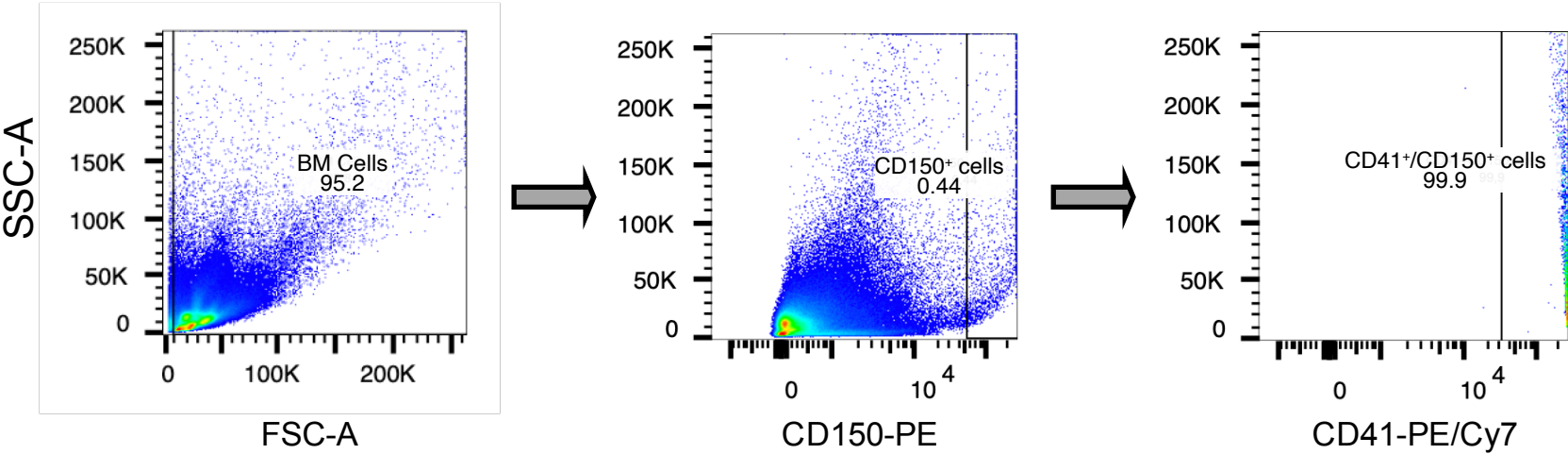
A



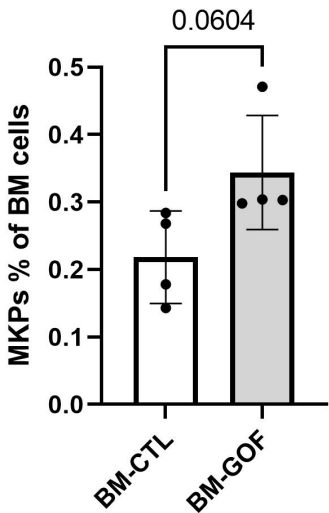
B CTL



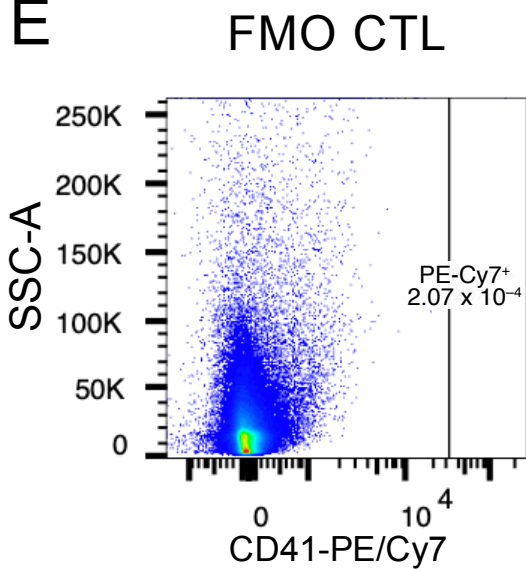
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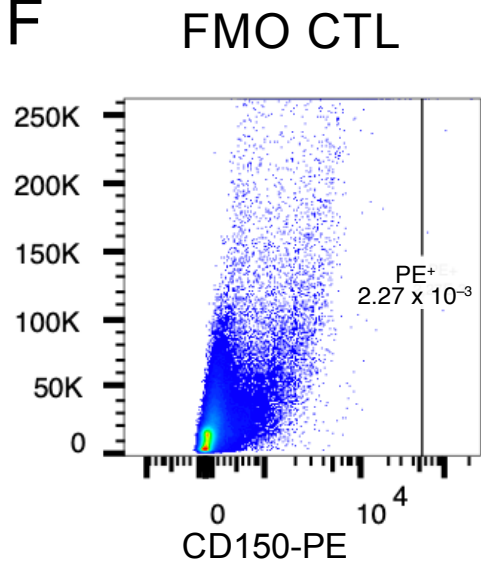
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E

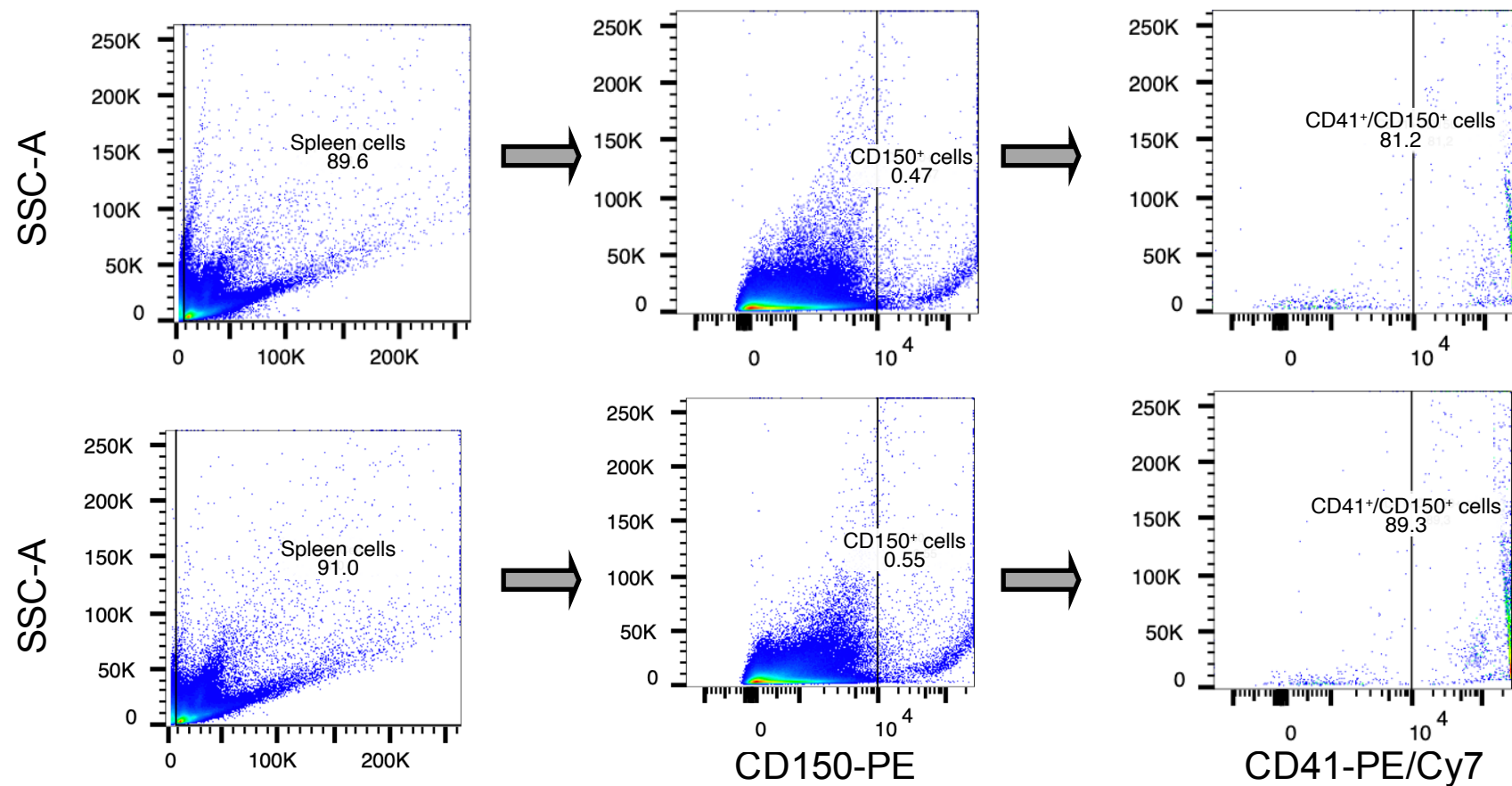


F

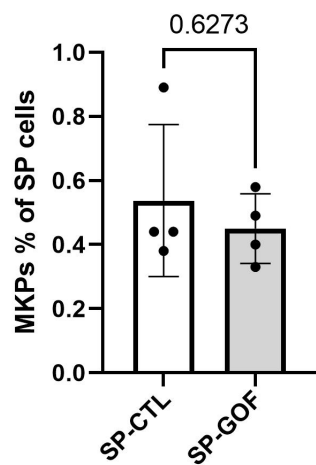


Suppl. Fig. 11, Yalcin et al.

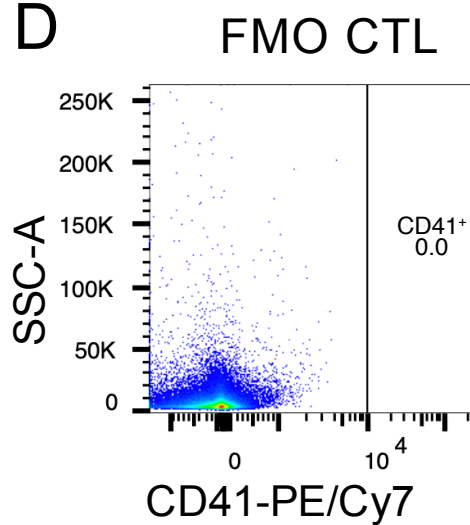
A CTL



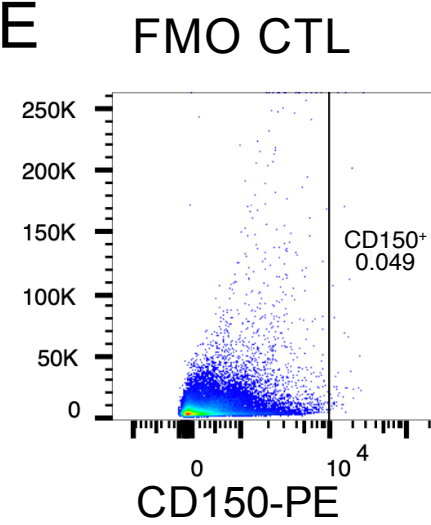
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D



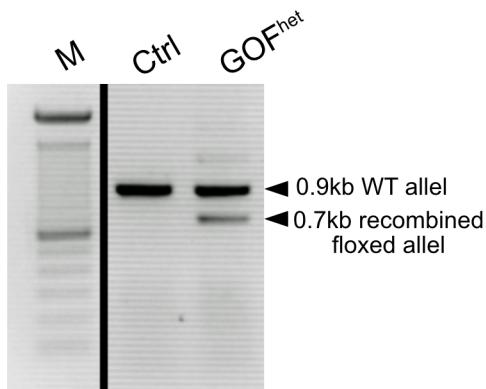
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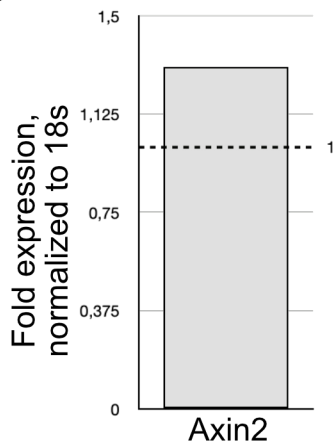
Suppl. Figure 12, Yalcin et al.

A

BM culture 7d

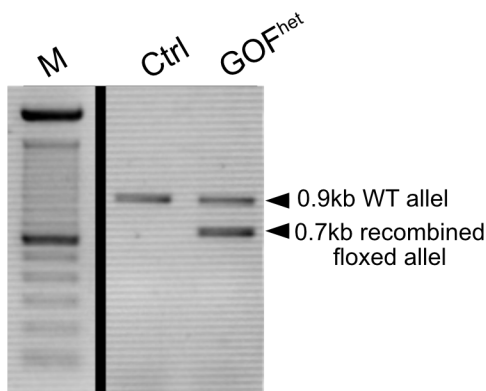


B



C

MBMEC culture 7d



D

