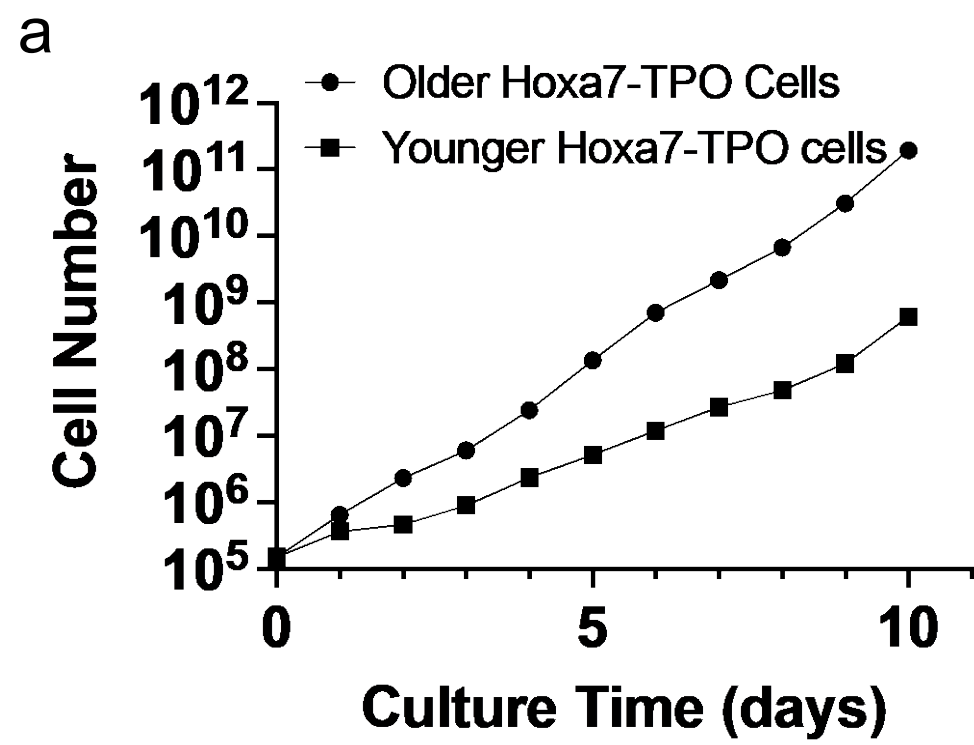
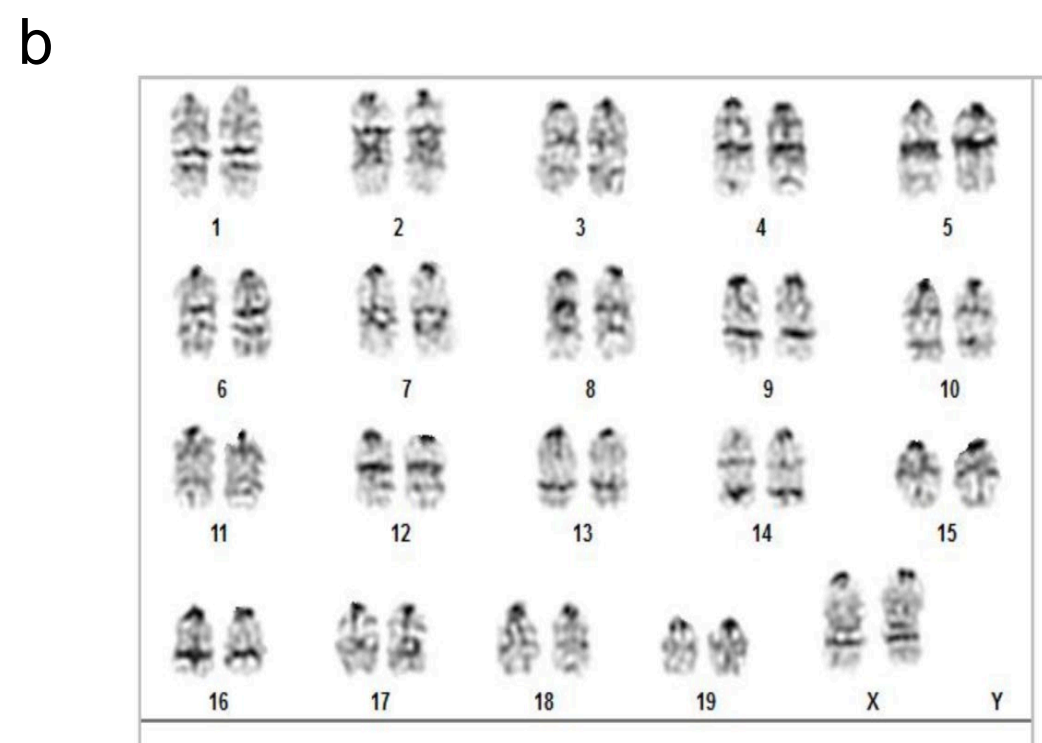
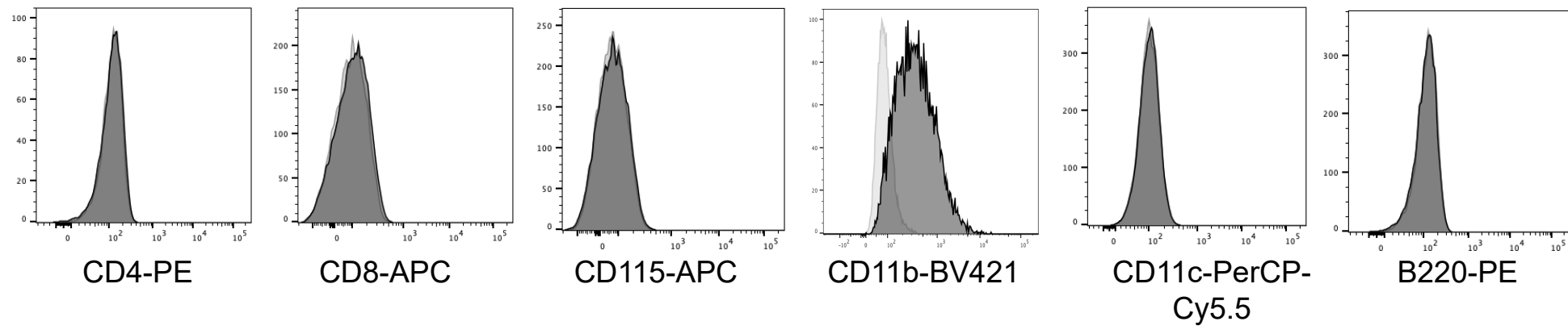




Supplementary Fig. 1 *In vitro* proliferation and karyotype analysis of Hoxa7-TPO cells that were cultured for extended periods of time.

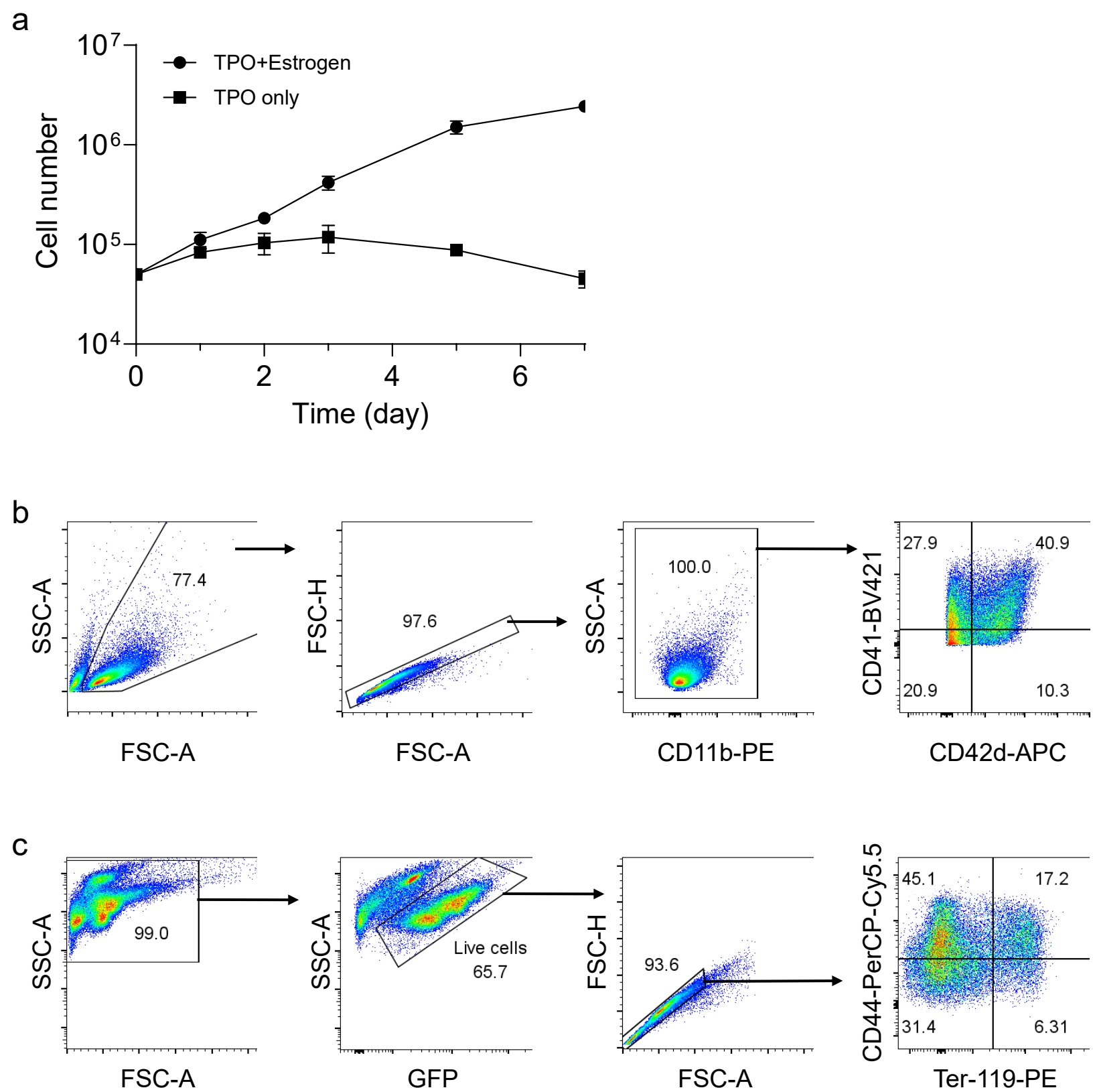
a Comparison of growth characteristics of Hoxa7-TPO cells that were cultured for 4-6 weeks ('younger' Hoxa7-TPO cells) and Hoxa7-TPO cells that were continuously cultured for one year ('older' Hoxa7-TPO cells). Source data are provided as a Source Data file.

b Karyotype analysis of Hoxa7-TPO cells after one year of continuous cell culture.



Supplementary Fig. 2 Flow cytometry analysis of Hoxa7-TPO cells.

Non-differentiated Hoxa7-TPO cells were stained with indicated antibodies and analyzed by flow cytometry. Dark grey, specific antibodies; light grey, isotype controls.

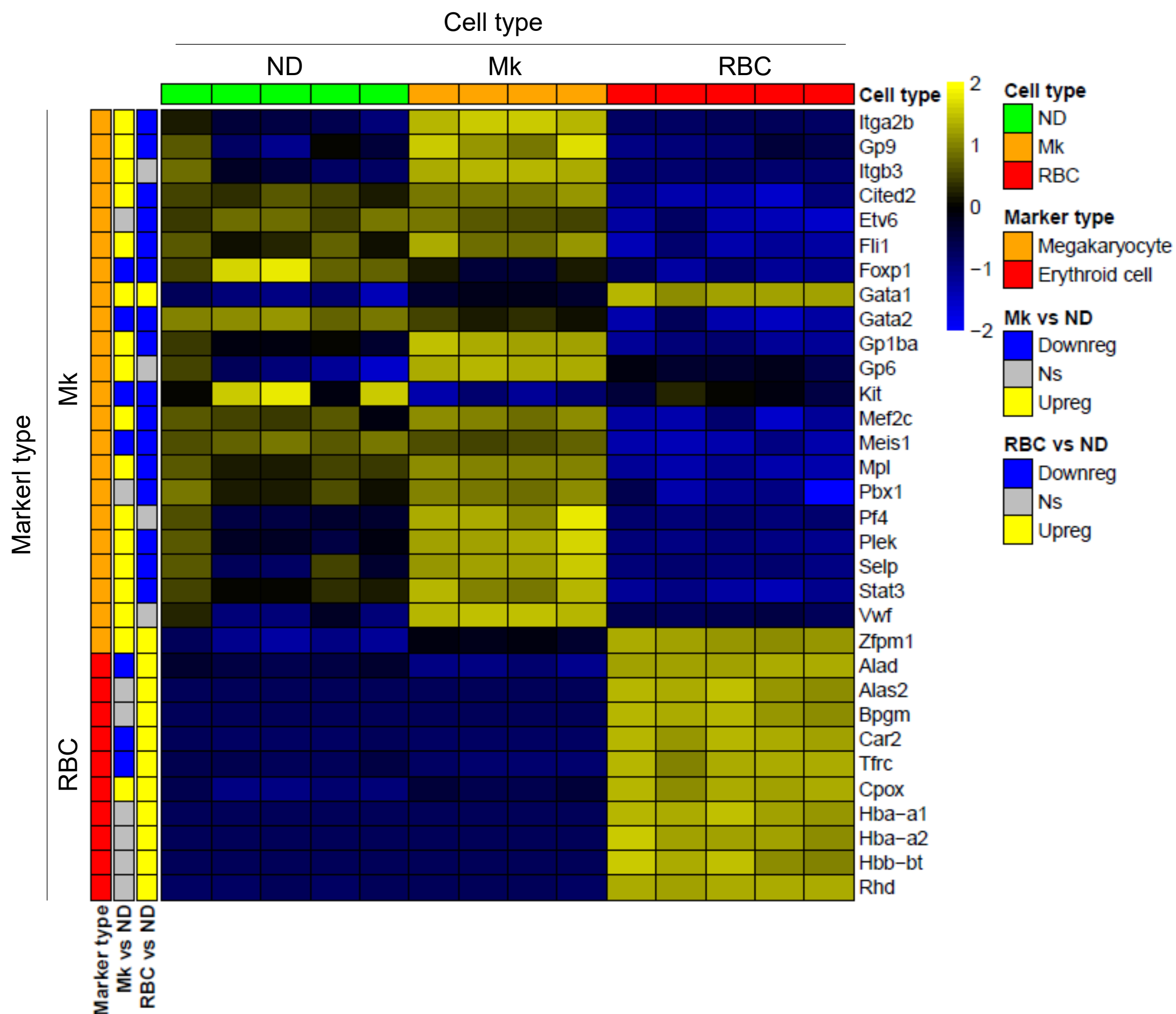


Supplementary Fig. 3 Estrogen-dependent growth characteristics of Hoxa7-TPO cells and flow cytometry-based gating strategy for analysis of *in vitro* differentiated Hoxa7-TPO cells.

a Cell numbers of Hoxa7-TPO cells cultured with TPO (20 ng/ml) in the presence or absence of exogenous estrogen. Data represent mean $\pm$ SD from $n = 5$ technical replicates at each indicated time point. Source data are provided as a Source Data file.

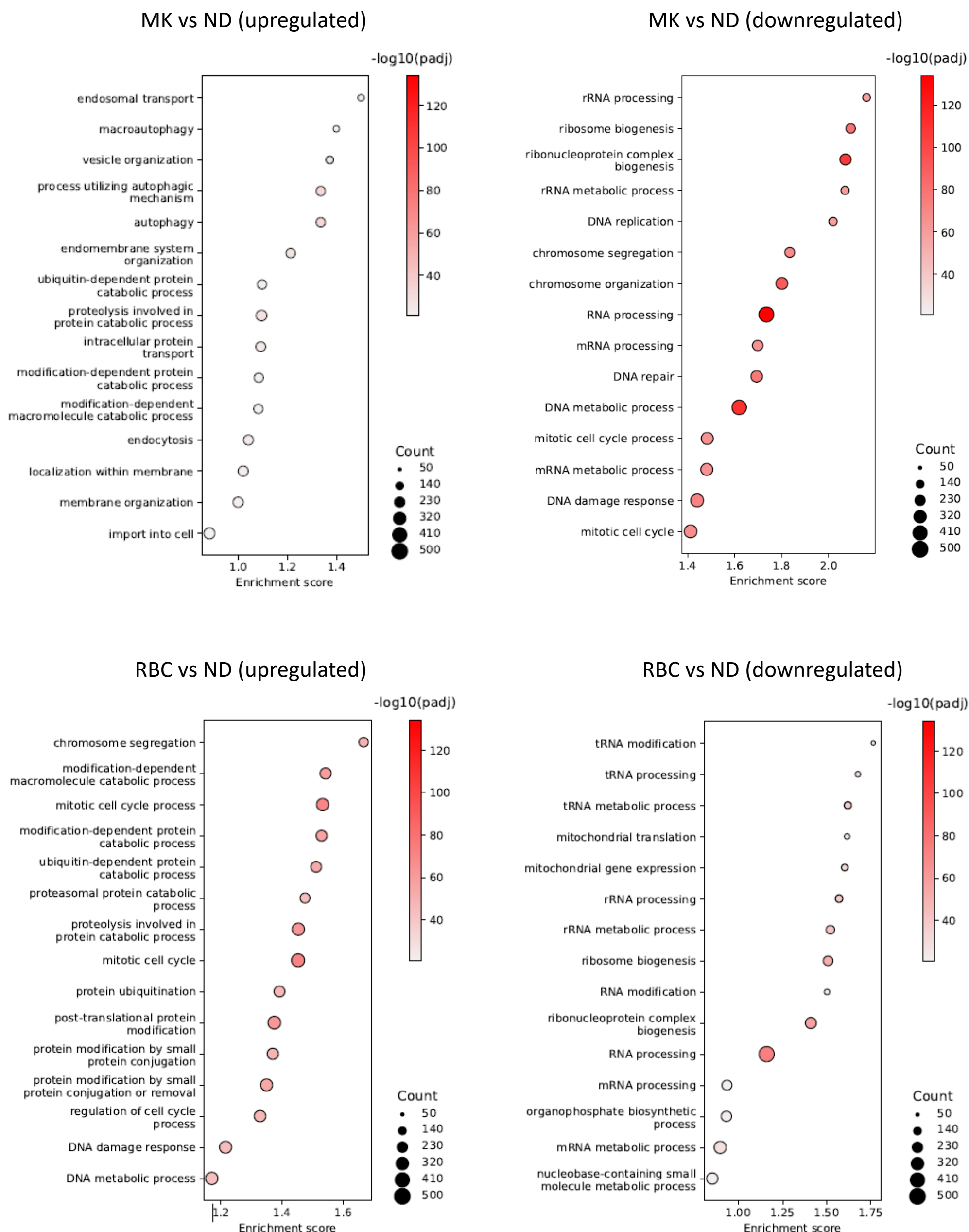
b Gating strategy for analysis of *in vitro* MK cell differentiation of Hoxa7-TPO cells.

c Gating strategy for analysis of *in vitro* erythroid differentiation of Hoxa7-TPO cells.



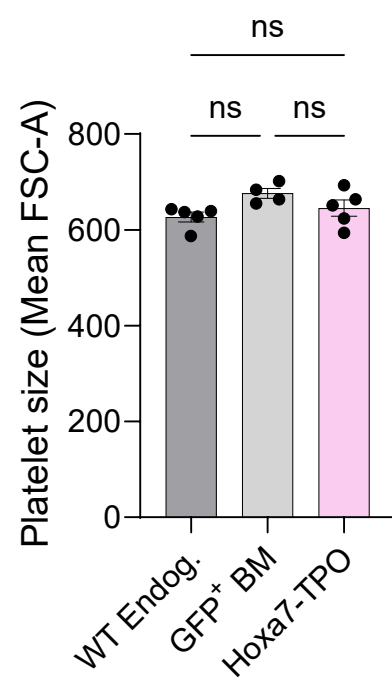
Supplementary Fig. 4 RNAseq-based heat map of marker genes encoding lineage-defining factors.

Analysis is based on RNAseq from non-differentiated Hoxa7-TPO cells, Hoxa7-TPO cells that were differentiated for 4 days *in vitro* in the presence of TPO and Hoxa7-TPO-derived reticulocytes that were isolated from adoptively transferred mice. Experimentally defined marker genes were drawn from the updated, manually curated (scRNA-seq-based) CellMarker 2.0 database (PMID: 36300619).



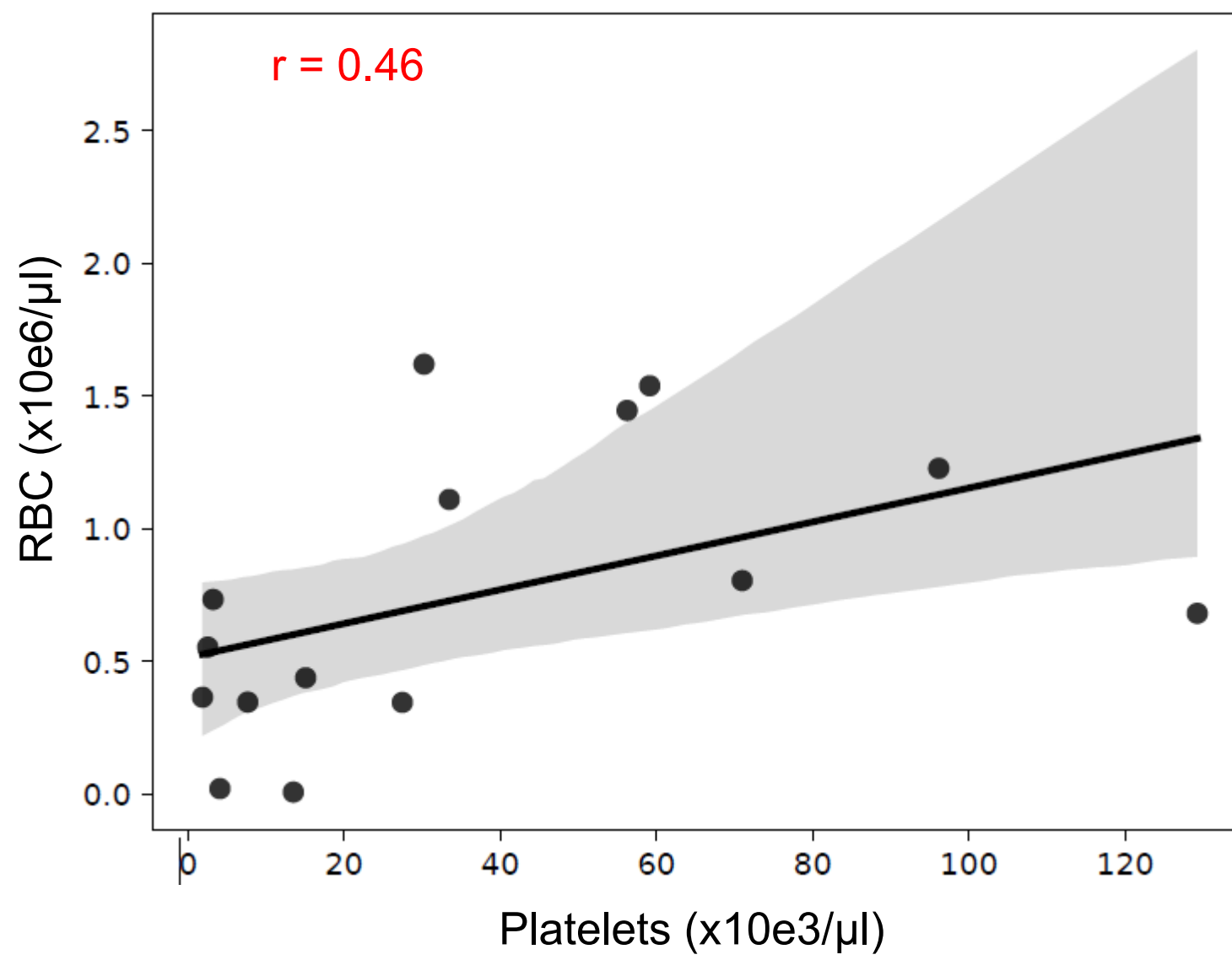
Supplementary Fig. 5 RNAseq-based gene ontology analysis based on up- and down-regulated genes in MK and reticulocytes vs. non-differentiated cells (ND).

For each condition (non-differentiated Hoxa7-TPO cells, Hoxa7-TPO cells that were differentiated for 4 days *in vitro* in the presence of TPO and Hoxa7-TPO-derived reticulocytes that were isolated from adoptively transferred mice), the top 15 most significantly enriched gene ontology “Biological Process” terms are shown (Fisher’s exact test with Benjamini-Hochberg correction), ranked by decreasing enrichment score.



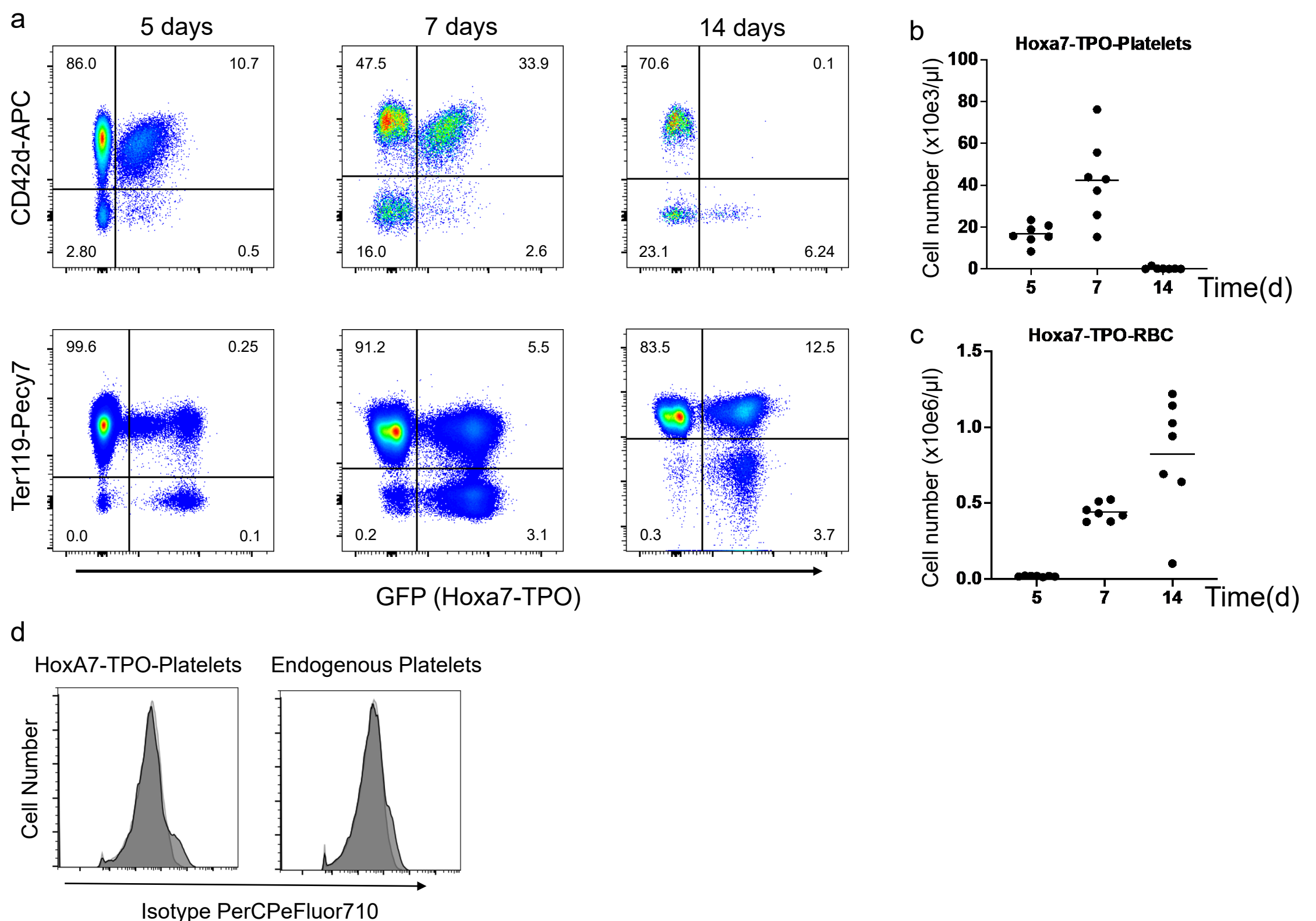
Supplementary Fig. 6 Cell size of Hoxa7-TPO- and BM-derived platelets.

Flow cytometry-based forward scatter analysis of platelets in lethally irradiated mice that were adoptively transferred with BM WT helper cells and Hoxa7-TPO cells (WT endog., Hoxa7-TPO) or GFP⁺ BM cells (GFP⁺ BM) and analyzed 7 days later. Data represent mean $\pm$ S.E. from $n = 5$ independent experiments for WT Endog. BM and Hoxa7-TPO, and $n = 4$ for GFP⁺ BM. Ns, not significant ($P > 0.05$), one-way ANOVA. Source data are provided as a Source Data file.



Supplementary Fig. 7 Correlation analysis of RBC and platelets produced from Hoxa7-TPO clones *in vivo*.

Correlation of RBC and platelet counts from the PB of mice that were adoptively transferred with 15 clones derived from Hoxa7-TPO cells. Each symbol represents data obtained from individual mice transferred with one individual clone. Regression line, 95% confidence interval (shaded) and Pearson correlation index (r , in red) are indicated. Source data are provided as a Source Data file.



Supplementary Fig. 8 *In vivo* potential of Hoxa7-TPO cells after long-term storage in liquid nitrogen and CD62L isotype controls for thrombin-activated platelets.

a Flow cytometry analysis of the PB of mice that were adoptively transferred with GFP-expressing Hoxa7-TPO cells (at indicated time points) that had been stored in liquid nitrogen for four years.

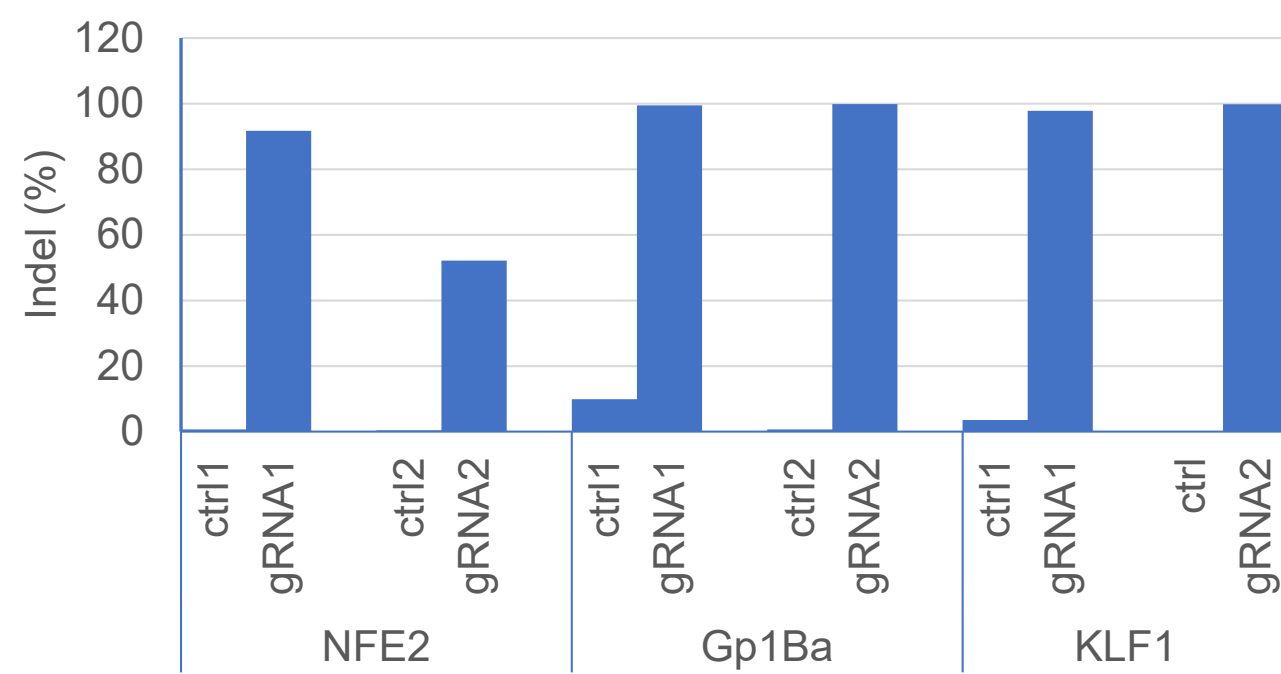
b,c Quantitative analysis of Hoxa7-TPO-derived platelets (b) and RBC (c) described in (a). Symbols represent individually transferred mice (n = 7). Source data are provided as a Source Data file.

d Flow cytometry of thrombin-treated platelets using isotype control antibodies. Platelet-rich plasma was prepared from mice six days after adoptive transfer with GFP+ Hoxa7-TPO cells and BM-derived helper cells, followed by thrombin treatment (2U/ml) for 20 min. Light grey, non-treated cells; dark gray, thrombin-treated cells.

Mice group	% platelets (mean ± SEM)	AUC (mean ± SEM)
Non-treated		
CD41+	5.98 ± 1.09	7,703 ± 200
BM only		
CD41+	3.89 ± 0.40	4,417 ± 184
BM+Hoxa7-TPO		
CD41+	1.60 ± 0.52 (33.7 %)	18,063 ± 917 (44.3%)
CD41+ GFP+	3.15 ± 1.50 (66.3 %)	22,748 ± 929 (55.7 %)

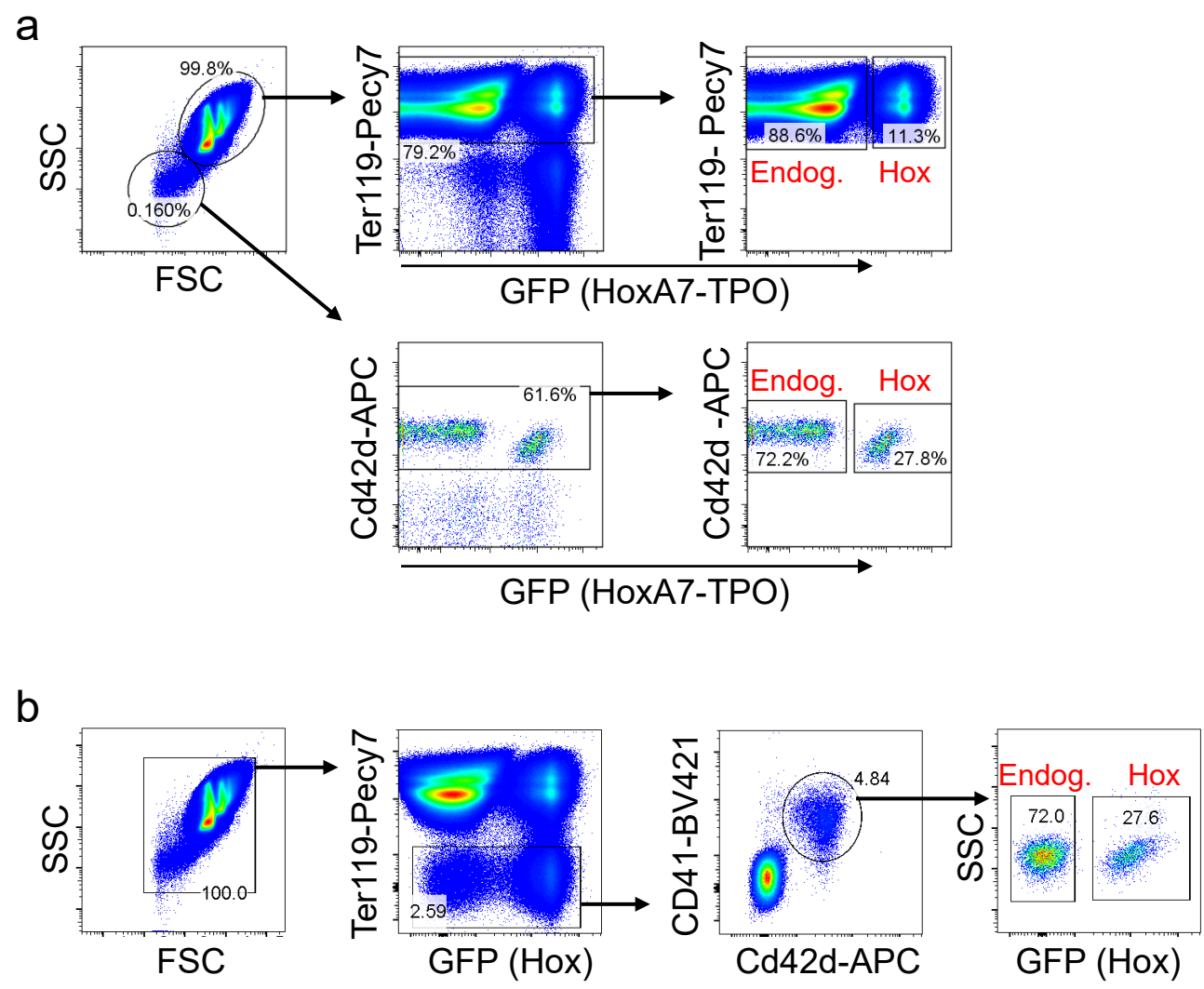
Supplementary Fig. 9 Relative size of cremaster arteriole thrombi and platelet number post laser injury in *Mpl*^{-/-} mice.

Flow cytometry studies determined the percent of cells present in the peripheral blood that are either CD41⁺ or CD41⁺GFP⁺ platelets in the mice at the time of the thrombosis study. The size of the thrombi post-laser injury in cremaster arterioles are shown in relative area-under-the-curve (AUC). Both results are mean ±1 SEM. BM only = *Mpl*^{-/-} mice receiving WT BM marrow transplant only. BM+Hoxa7-TPO = *Mpl*^{-/-} mice transplanted with both WT marrow and non-differentiated Hoxa7-TPO cells. The percentage of endogenous (CD41⁺) and Hoxa7-TPO-derived platelets (CD41⁺ GFP⁺) contributing to thrombi are depicted in red in brackets. All arms had 4 mice studied.



Supplementary Fig. 10 gRNA targeting efficiency

On-target DNA editing efficiency (indel frequency) of sgRNA-transduced Hoxa7-TPO cells was determined by next-generation sequencing. Source data are provided as a Source Data file.



Supplementary Fig. 11 Gating strategy for platelets

a Flow cytometry-based gating strategy for standard assays based on adoptive transfer of Hoxa7-TPO cells.

b Flow cytometry-based gating strategy for analysis of large platelets as obtained upon genetic deletion of GP1BA.

gene-gRNA Name	gRNA target sequence	target-seq-F-primer	target-seq-R-primer
NFE2_g1	TGGTATGGCTACATTACCAT	CACTCTTTCCCTACACGACGCTCTTCCGATCTGCCACTGCCACCTCCAACAT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCCACTGGCAGCCCAATGTCCA
NFE2_g2	AAGTTAACTATCTTGCCGT	CACTCTTTCCCTACACGACGCTCTTCCGATCTCCGGTCCAGTTCGGGCTAA	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCCCACCTTGTTCTTGCCCCGT
Gp1Ba_g1	GACAGGCGCATTGTCCAAGT	CACTCTTTCCCTACACGACGCTCTTCCGATCTCCACGCCAACAGCTGGTACT	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCCTTGTTGGCAGGGACGTCAGG
Gp1Ba1_g2	TGGCTTCGCACAATACCAA	CACTCTTTCCCTACACGACGCTCTTCCGATCTATTGCGTGAGCTGCCCTCTG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGGAGCCAGTGACGGA
KLF1_g1	GCTCGGAGTCGGAGTACGA	GGCTCCGACACTCTTCCCTACACGACGCTCTTCCGATCTGGCTCCGAGGAGCACACAAGGGAGCACACAAG	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCCCCGCGAAAGAGCTGGAAGT
KLF1_g2	GAGGGACGTGACCTGTGCGT	CACTCTTTCCCTACACGACGCTCTTCCGATCTCAAGCAGTACGCTCCCTCCC	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCAGAGACTCAGGCGGCTCGAA

Supplementary Table 1

Sequence information of sgRNA targets and primers used for analysis of targeting efficiency