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Supplementary Materials for

Vasopressin stimulates the proliferation and differentiation of red blood cell precursors and improves recovery from anemia

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at
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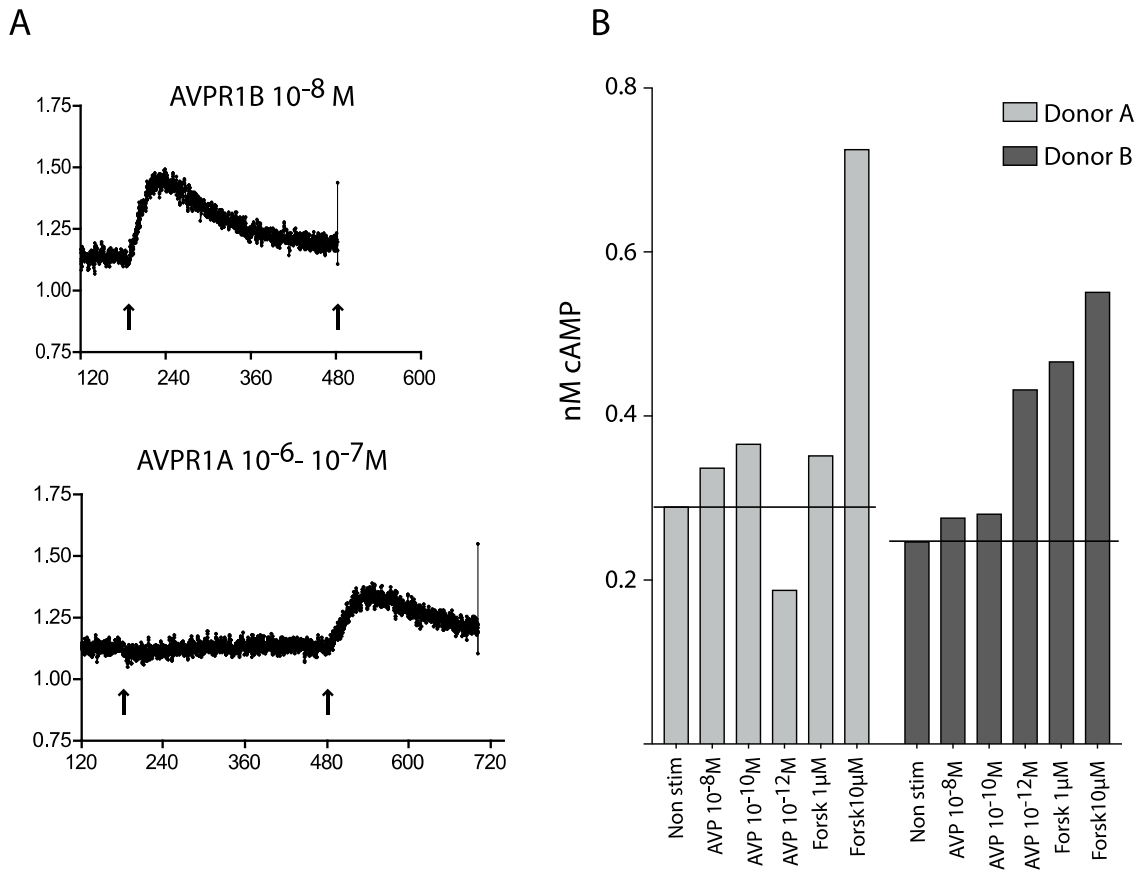

Fig. S1. AVP agonists affect intracellular calcium, and AVP increases cAMP in human progenitors. (A) AVPR1B at 10^{-8} M concentration induces intracellular calcium in HSPCs ($CD34^+$ cells), whereas an AVPR1A agonist has effect at a 100-fold higher dose. **(B)** AVP stimulation also increases cAMP concentrations. In two consecutive experiments, $CD34^+$ cells from two different donors were plated. AVP increased cAMP concentrations in stimulated vs. non-stimulated cells. Forskolin was used as positive control in both experiments in (B).

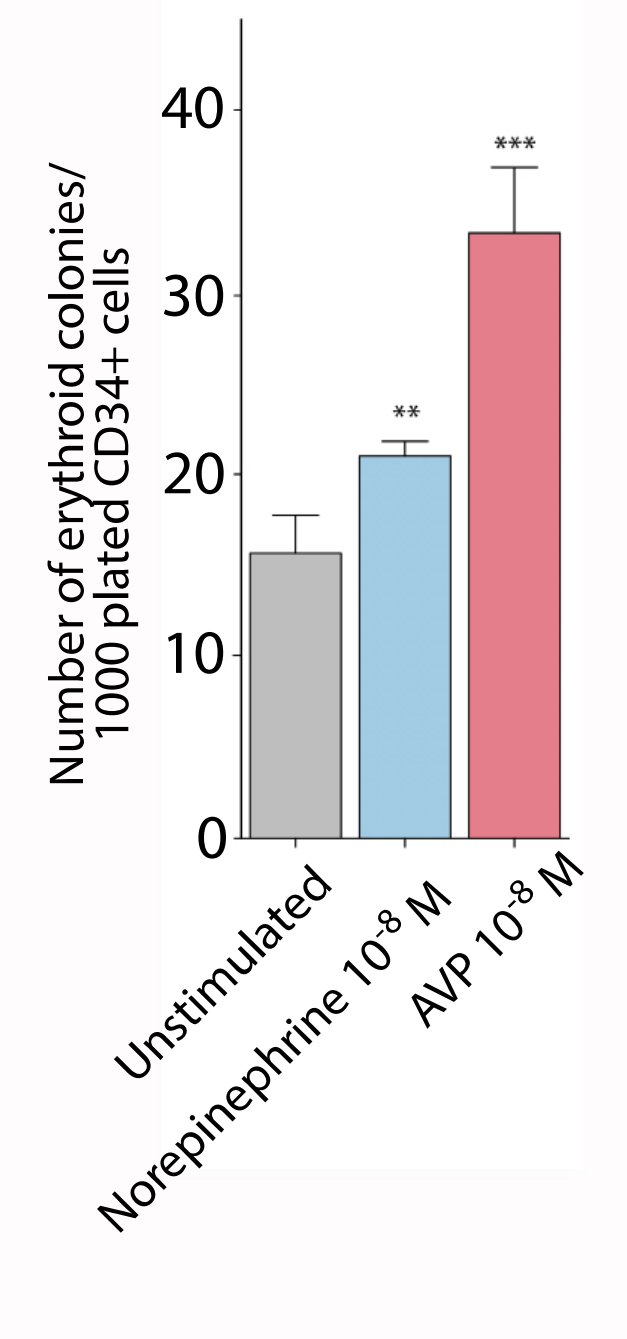

Fig. S2. Norepinephrine and AVP induce an increase in the number of BFU-E colonies. BFU-E colony-forming assay done with norepinephrine as positive control, along with AVP. Error bars represent mean+SD; **p<0.01; ***p<0.001.

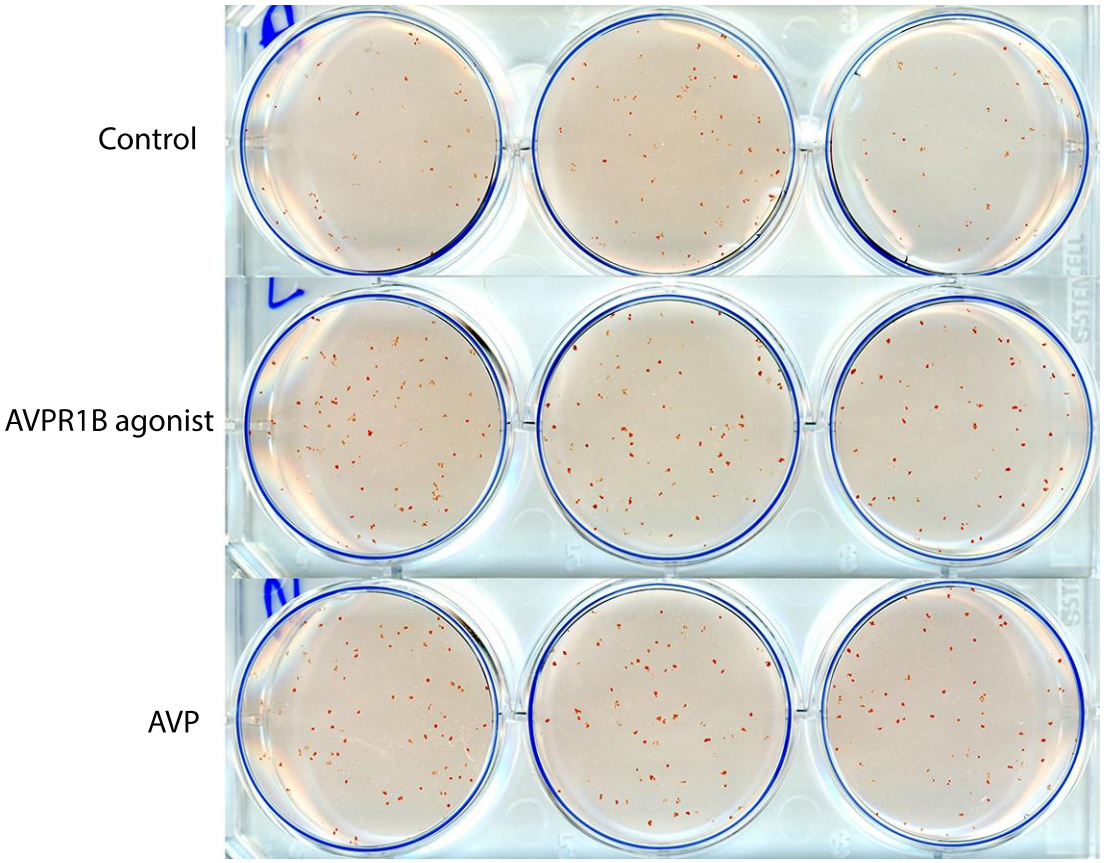

Fig. S3. AVP and AVPR1B agonist [d(Cha^4)-AVP] increase BFU-E formation.
Methylcellulose assay of human CD34$^+$ cells, plates scanned at Day 14 are shown in triplicates. The red dots are BFU-E colonies.

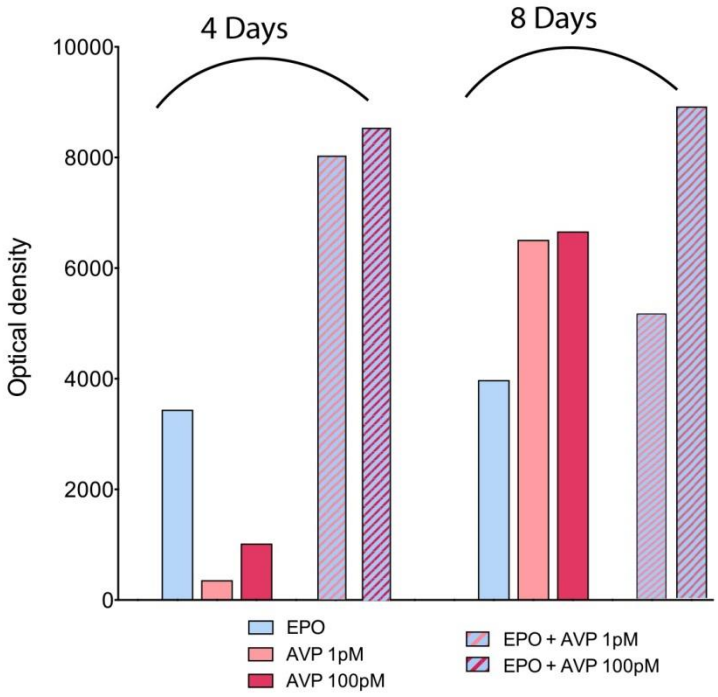

Fig. S4. Band intensities from Western blots were analyzed by ImageJ.
Quantitative evaluation of STAT5 phosphorylation after EPO and/or AVP treatment of developing erythroid progenitors in vitro. Western blots are shown in Fig. 2F. The optical densities of the bands were measured using Image J software.

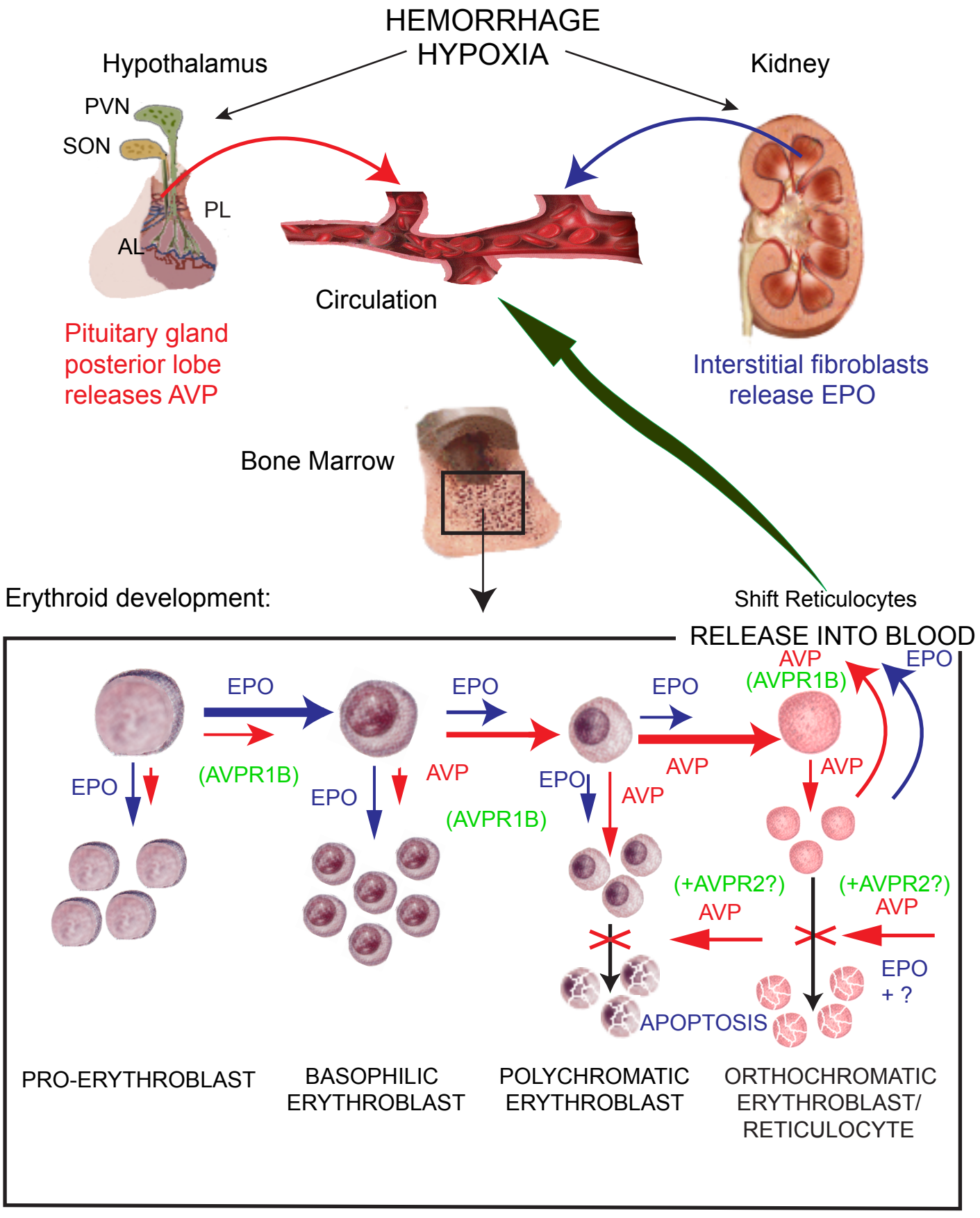
HEMORRHAGE
HYPOXIA
Hypothalamus
Kidney
PVN
SON
PL
AL
Circulation
Pituitary gland
posterior lobe
releases AVP
Interstitial fibroblasts
release EPO
Bone Marrow
Erythroid development:
Shift Reticulocytes
RELEASE INTO BLOOD
EPO
(AVPR1B)
AVP
(+AVPR2?)
APOPTOSIS
EPO
+ ?
PRO-ERYTHROBLAST
BASOPHILIC
ERYTHROBLAST
POLYCHROMATIC
ERYTHROBLAST
ORTHOCHROMATIC
ERYTHROBLAST/
RETICULOCYTE

Fig. S5. Response to anemia is coordinated by AVP and EPO.
In response to blood loss or hypoxia, AVP is released into the blood from the hypothalamo-hypophyseal system, and EPO is released from the specialized interstitial fibroblasts of the kidney. EPO induces proliferation of proerythroblasts and basophilic erythroblasts, as well as their further differentiation. AVP induces proliferation of polychromatic and orthochromatic erythroblasts, and might speed up the differentiation/maturation of young reticulocytes into mature reticulocytes. AVPR1B receptors appear to mediate the effects of AVP on cellular proliferation and differentiation. In addition, AVP might also decrease apoptosis in the most mature population of reticulocytes. Both EPO and AVP participate in releasing cells into the circulation, but AVP may speed the maturation of immature red cells (shift-reticulocytes) and cause their quick release from the bone marrow. This could contribute to a rapid increase in hematocrit. The red arrows suggest roles for AVP, whereas the blue arrows demonstrate well-known roles of EPO.
Abbreviations: AL-anterior lobe of the pituitary gland; PL-posterior (neural) lobe of the pituitary gland; PVN-paraventricular hypothalamic nucleus; SON-supraoptic hypothalamic nucleus

Table S2. Human RT-PCR primers.

Target	Primer sequence	PCR product size	Annealing temp	Cycles
Human *AVPR1A* NM_000706	hu*AVPR1A* for 1: CCGCCTGGGTGCTGAGCTTC hu *AVPR1A* rev 1: TCTTCCCGCGGACGTTGCAC 1 step RT-PCR	234 bp	65°C	45
Human *AVPR1B* NM_000707	hu*AVPR1B*for1: GGCTGCCATCTCGGGTCAGC hu*AVPR1B* rev1: CAGGCAAGGTGACGCAGGGG	283 bp	55°C	45
Human *AVPR2* NM_000054	Hu *AVPR2* for: ATTCATGCCAGTCTGGTGC Hu *AVPR2* rev: TCACGATGAAGTGTCCTTGG[1]	423 bp	58°C	45

Table S3. Mouse RT-PCR primers.

Target	Primer sequence	PCR product size	Annealing temp	Cycles
Mouse *Avpr1a* NM_016847	ms *Avpr1a* F: CAGATGTGGTCAGTCTGGGATA m*Avpr1a* R: CTCATGCTATCCGAGTCATCCT	215 bp	60°C	45
Mouse *Avpr1b* NM_011924	ms *Avpr1b* F: GCTGGCCCAAGTCCTCATCTTCTG *Avpr1b* R: GCGGTGACTCAGGGAACGT	323 bp	60°C	45
Mouse *Avpr2* NM_019404	ms *Avpr2* for 1: CACGTCTGCAGTGCCTGGGG ms *Avpr2* 1: CATGGAAGCGGTCGGTGGCA	302 bp	62°C	45

Table S4. Mouse real-time PCR primers.

Target	Primer sequence
Mouse *Avpr1a* NM_016847	F: TAGGCCTGGTTCGTAAGCAT R: TTCAATCACGGACCAGTTCA
Mouse *Avpr1b* NM_011924	F: ATCCGAACCGTGAAGATGAC R: TCATTAGGGGCATTCTCGTC
Mouse *Avpr2* NM_019404	F: CAAGGGACACCCTGGTTCTA R: CTACGCAACTCCGAGGAGAC
Tbp	Ms_Tbp_1_SG QuantiTect Primer

Table S5. Antibodies used for flow cytometry

Antibodies	***Clones***	***Fluorochromes***	***Source***
anti-mouse			
Gr-1 (Ly-6G)	RB6-8C5	APC-eFluor 780	Ebiosciences
B220 (CD45R)	RA3-6B2	PE-Cy7	Ebiosciences
TER-119	TER-119	APC	Ebiosciences/ BD Biosciences
CD3e	145-2C11	PE	Ebiosciences
CD11b (Mac-1)	M1/70	PerCp-eFluor710	Ebiosciences
CD71	C2	PE	BD Biosciences
CD41	MWReg30	FITC	BD Biosciences
CD61		PE	BD Biosciences
sca1 (Ly-6A/E)	D7	FITC or PE-Cy7	BD Biosciences/ Ebiosciences
c-kit (CD117)	2B8	APC	BD Biosciences
CD34	RAM34	AlexaFluor700	Ebiosciences
CD150	mShad150	PE	Ebiosciences
CD48	HM48-1	PerCp Cy5.5	Ebiosciences
IL-7R (CD127)	A7R34	PerCpCy5.5	Ebiosciences
CD44		APC	BD Biosciences
TER119		FITC	BD Biosciences
CD45		APC-Cy7	BD Biosciences
CD11b		APC-Cy7	BD Biosciences
GR1		APC-Cy7	BD Biosciences
CD16/CD32		Purified Rat anti-mouse	BD Biosciences
CD4		Biotin	Ebiosciences
CD8		Biotin	Ebiosciences
TER119		Biotin	Ebiosciences
Gr-1		Biotin	Ebiosciences
CD11b		Biotin	Ebiosciences
B220		Biotin	Ebiosciences
anti-human			
CD34		PE	BD Biosciences
CD38		FITC	BD Biosciences