Developmental Vitamin D availability impacts hematopoietic stem cell production

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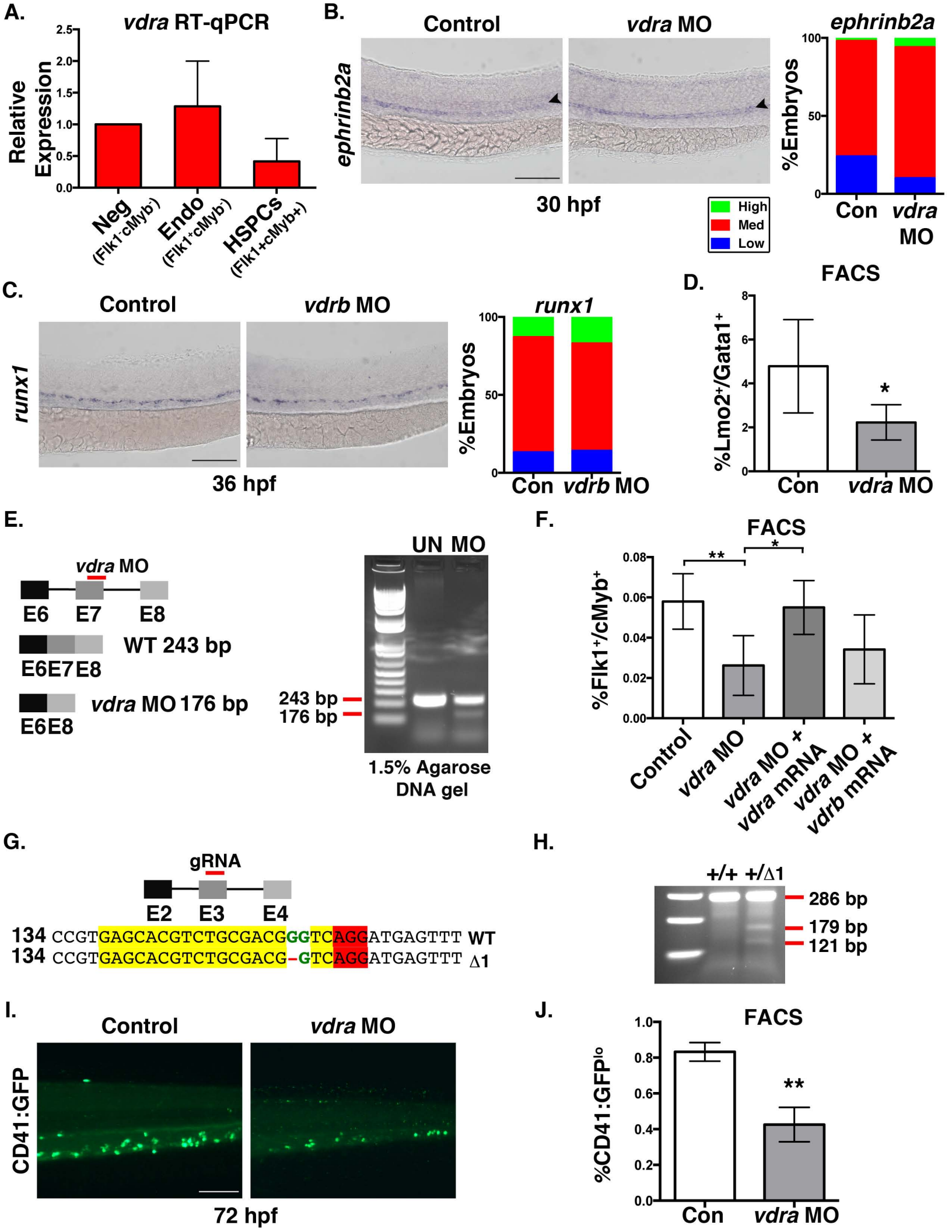
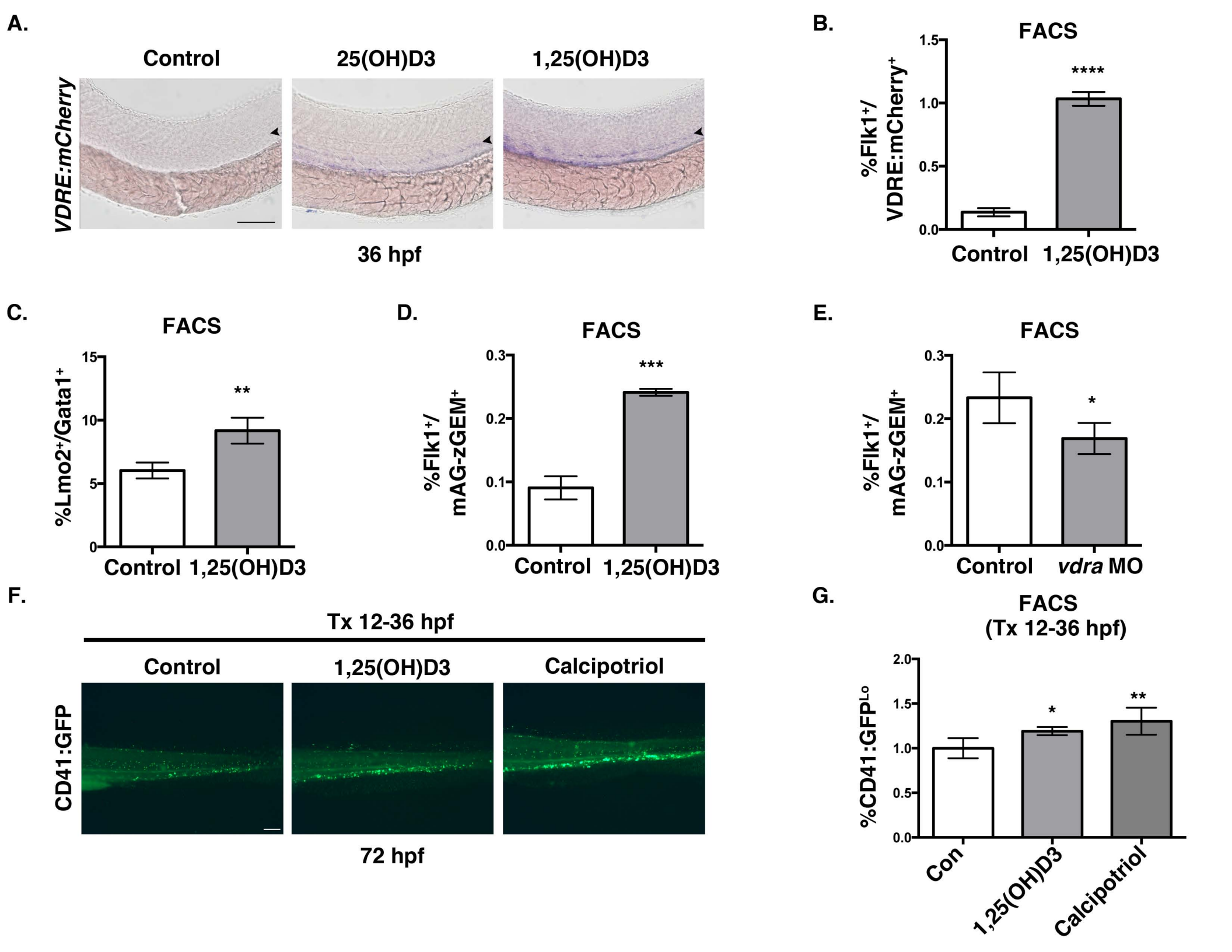
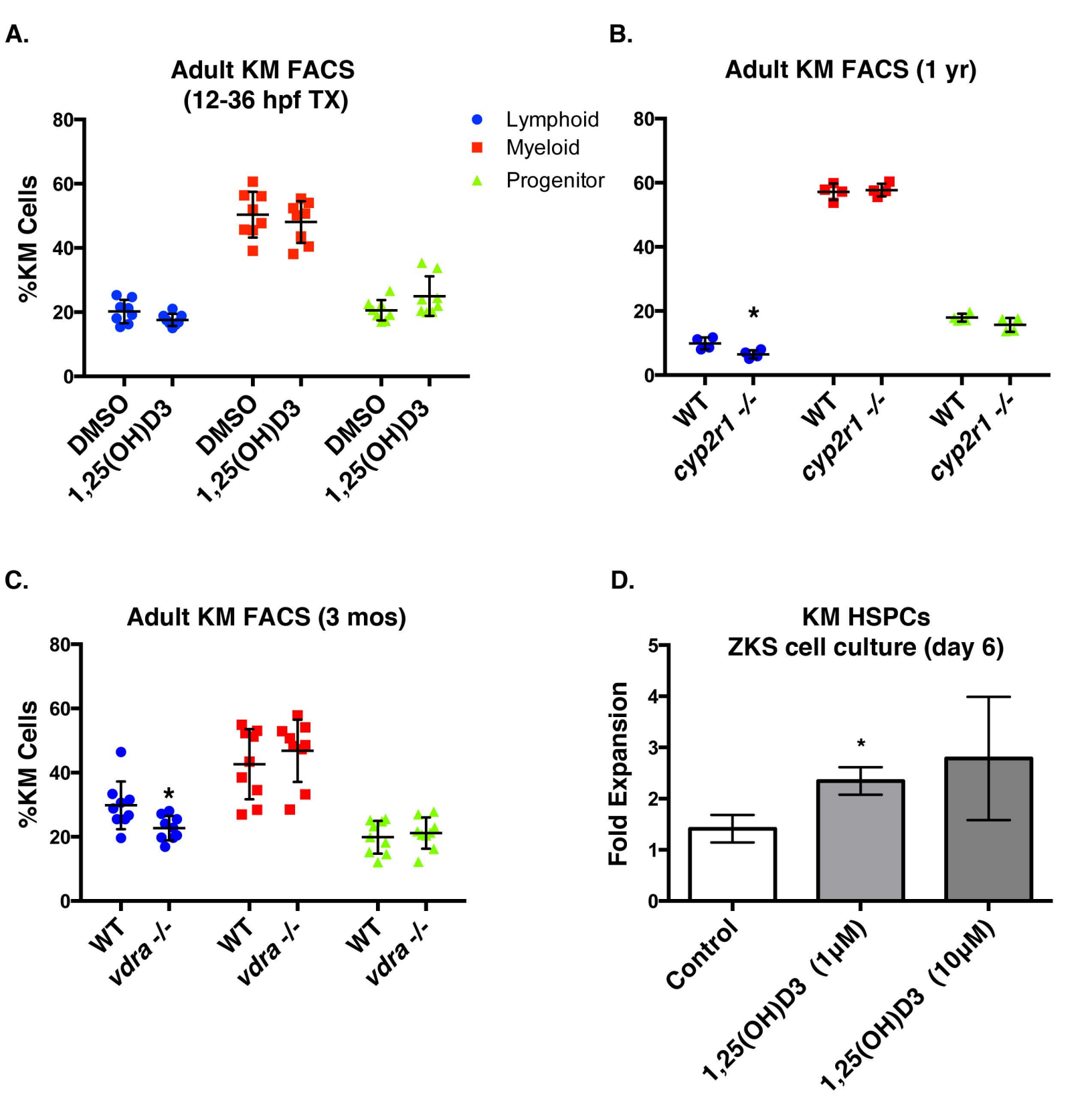
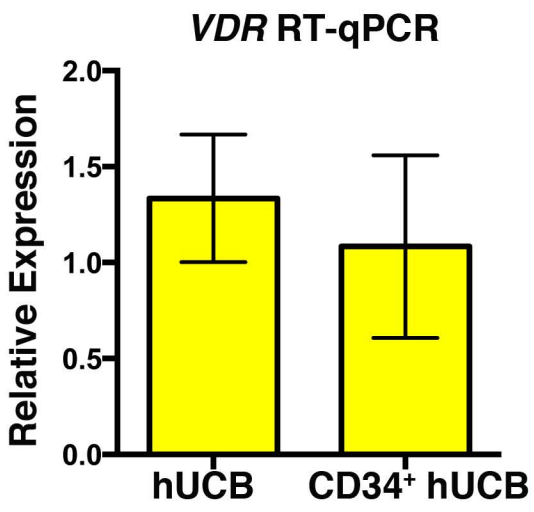

A.
vdra RT-qPCR
Relative Expression
2.5
2.0
1.5
1.0
0.5
0.0
Neg (Flk1-cMyb-)
Endo (Flk1+cMyb-)
HSPCs (Flk1+cMyb+)
B.
Control
vdra MO
ephrinb2a
30 hpf
ephrinb2a
%Embryos
100
50
0
Con
vdra MO
High
Med
Low
C.
Control
vdrb MO
runx1
36 hpf
runx1
%Embryos
100
50
0
Con
vdrb MO
D.
FACS
%Lmo2+/Gata1+
8
6
4
2
0
*
Con
vdra MO
E.
vdra MO
E6
E7
E8
WT 243 bp
E6E7E8
vdra MO 176 bp
E6E8
UN MO
243 bp
176 bp
1.5% Agarose DNA gel
F.
FACS
%Flk1+/cMyb+
0.08
0.06
0.04
0.02
0.00
**
*
Control
vdra MO
vdra MO + vdra mRNA
vdra MO + vdrb mRNA
G.
gRNA
E2
E3
E4
134 CCGTGAGCACGTCTGCGACGGGTCAGGATGAGTTT WT
134 CCGTGAGCACGTCTGCGACG-GTCAGGATGAGTTT Δ1
H.
+/+ +/Δ1
286 bp
179 bp
121 bp
I.
Control
vdra MO
CD41:GFP
72 hpf
J.
FACS
%CD41:GFPlo
1.0
0.8
0.6
0.4
0.2
0.0
**
Con
vdra MO

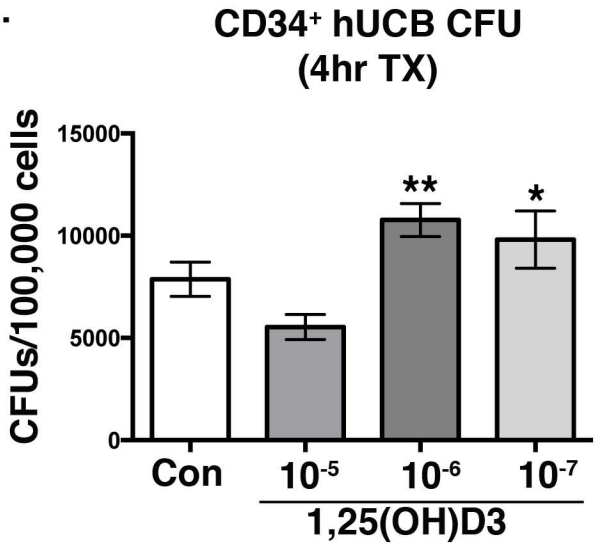

A.
Control
25(OH)D3
1,25(OH)D3
VDRE:mCherry
36 hpf
B.
FACS
%Flk1+/ VDRE:mCherry+
****
Control
1,25(OH)D3
C.
FACS
%Lmo2+/Gata1+
**
Control
1,25(OH)D3
D.
FACS
%Flk1+/ mAG-zGEM+
***
Control
1,25(OH)D3
E.
FACS
%Flk1+/ mAG-zGEM+
*
Control
vdra MO
F.
Tx 12-36 hpf
Control
1,25(OH)D3
Calcipotriol
CD41:GFP
72 hpf
G.
FACS
(Tx 12-36 hpf)
%CD41:GFPLo
*
**
Con
1,25(OH)D3
Calcipotriol


A.
Adult KM FACS
(12-36 hpf TX)
Lymphoid
Myeloid
Progenitor
%KM Cells
80
60
40
20
0
DMSO
1,25(OH)D3
DMSO
1,25(OH)D3
DMSO
1,25(OH)D3
B.
Adult KM FACS (1 yr)
%KM Cells
80
60
40
20
0
*
WT
cyp2r1 -/-
WT
cyp2r1 -/-
WT
cyp2r1 -/-
C.
Adult KM FACS (3 mos)
%KM Cells
80
60
40
20
0
*
WT
vdra -/-
WT
vdra -/-
WT
vdra -/-
D.
KM HSPCs
ZKS cell culture (day 6)
Fold Expansion
5
4
3
2
1
0
*
Control
1,25(OH)D3 (1μM)
1,25(OH)D3 (10μM)

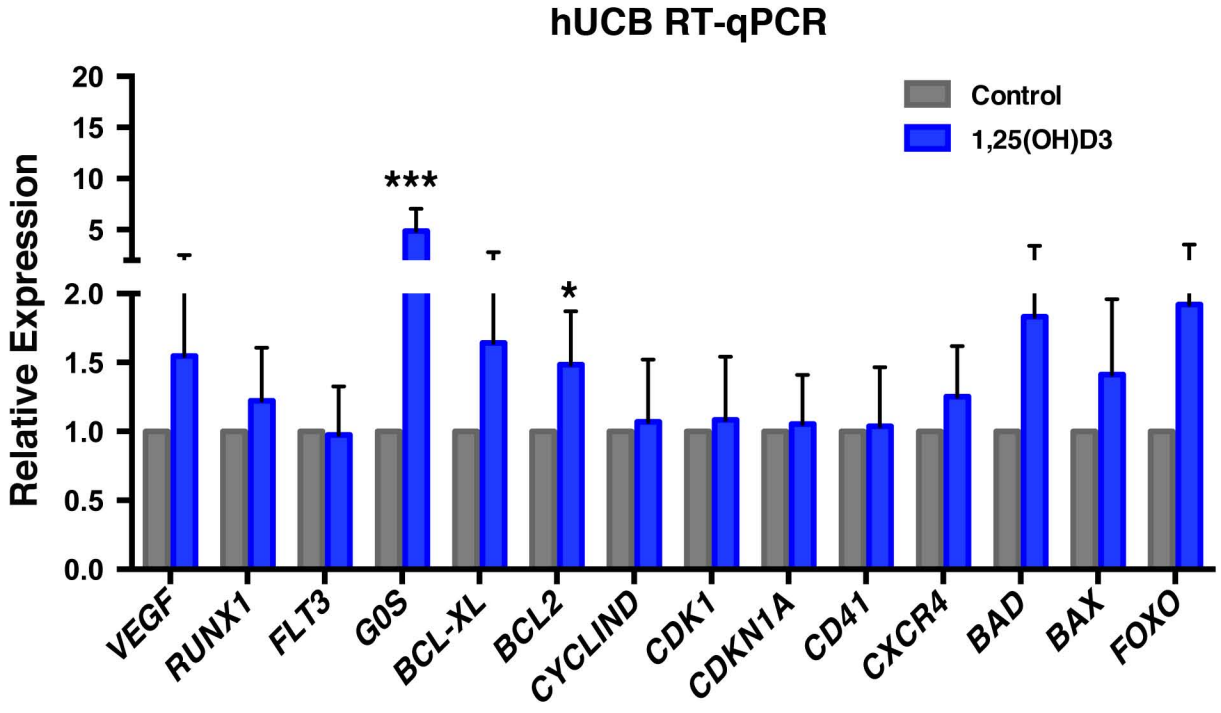
A.



B.



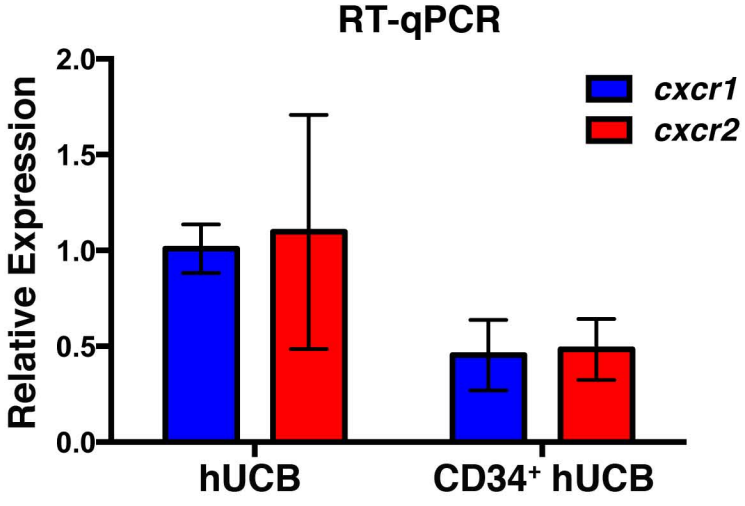
C.

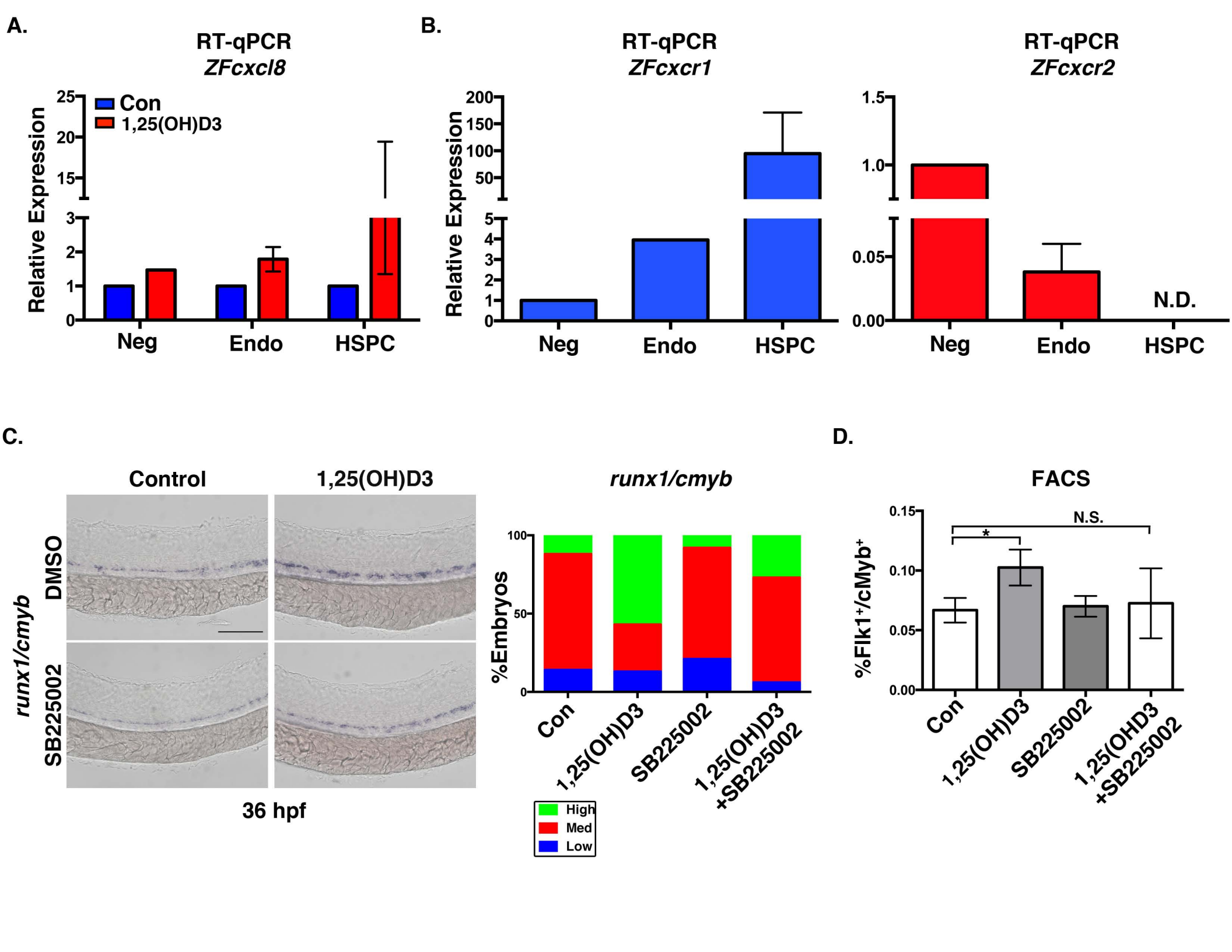


D.

MetaCore Process Networks	-log(p-value)
Translation Elognation-Termination	3.66
Transcription mRNA processing	3.30
Chemotaxis	2.55
Cell adhesion Leucocyte chemotaxis	2.50
Translation Initiation	2.50
Proteolysis Ubiquitin-proteasomal proteolysis	2.05
DNA damage DBS repair	1.78
Inflammation Amphoterin signaling	1.75
Cell cycle Mitosis	1.41
Signal Transduction Cholecystokinin signaling	1.41

E.




A.
RT-qPCR
ZFcxcl8
Relative Expression
Con
1,25(OH)D3
Neg
Endo
HSPC
B.
RT-qPCR
ZFcxcr1
Relative Expression
Neg
Endo
HSPC
RT-qPCR
ZFcxcr2
N.D.
Neg
Endo
HSPC
C.
Control
1,25(OH)D3
runx1/cmyb
DMSO
SB225002
36 hpf
runx1/cmyb
%Embryos
Con
1,25(OH)D3
SB225002
1,25(OH)D3 +SB225002
High
Med
Low
D.
FACS
%Flk1+/cMyb+
N.S.
*
Con
1,25(OH)D3
SB225002
1,25(OHD3 +SB225002

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. *Vdra* knockdown affects HSPCs expansion (*related to figure 1*).

A. qPCR for *vdra* in FACS-sorted Flk1^{-}/cMyb^{-} (double negative), Flk1^{+}/cMyb^{-} (endothelium), and Flk1^{+}/cMyb^{+} (HSPCs) fractions (cells sorted from ≥1000 embryos/condition x 3 replicates) populations at 36hpf.

B. Representative *ephrinb2a* WISH images and qualitative phenotype distribution (High (green)/ Med (red)/ Low (blue); n≥40 embryos/condition) of *vdra* morpholino (MO) injected embryos and sibling controls at 36hpf.

C. WISH and phenotype distribution of *vdrb* MO injected embryos showing no effect on *runx1* expression at 36hpf (n≥35 embryos/condition).

D. FACS analysis of *vdra* morphants showed a significant decrease in Lmo2:GFP^{+}/Gata1:dsRed^{+} EMPs (*p<0.05) at 30hpf (5 embryos/sample x 4 replicates/condition); error bars, mean ± SD.

E. Schematic representation of the predicted exon 7 truncation caused by the *vdra* splice MO, and DNA gel analysis showing the smaller *vdra* band following MO injection (n>20 embryos/condition).

F. FACS analysis of HSPCs at 36hpf for *vdra* morphants (**p<0.01), with and without co-injection of either *vdra* or *vdrb* mRNA; *vdra* mRNA co-injection rescued the reduced number of HSPCs observed with *vdra* MO alone (*vdra* MO vs *vdra* MO + *vdra* mRNA, **p<0.01; *vdra* MO vs *vdra* MO + *vdrb* mRNA, N.S.) (5 embryos/sample x 4 replicates/condition); error bars, mean ± SD.

G. Schematic representation on the CRISPR/cas9-mediated deletion in *vdra.*

H. Gel electrophoresis depicting a mismatched mutation assayed by Surveyor nuclease in *vdra* heterozygote mutants compared to WT sibling controls.

I. Representative fluorescent microscopy example of CD41:GFP expression in the CHT at 72hpf in control and *vdra* morphants.

J. FACS analysis of *vdra* morphants showed a significant decrease (**p<0.01) in the percentage of CD41:GFP^{lo};Gata1:dsRed^{-} HSCs (5 embryos/sample x 3 replicates/ condition); error bars, mean ± SD).

Scale bar 100μm.

Figure S2. Vitamin D treatment expands HSPC number (*related to Figure 2*).

A. Representative *VDRE:mCherry* WISH phenotype of embryos treated with 25(OH)D3 and 1,25(OH)D3 compared to DMSO-treated controls (n≥10).

B. FACS analysis of Tg*(flk1:GFP/VDRE:mCherry)* embryos treated with 1,25(OH)D3 from 12-36hpf showed a significant (****p<0.0001) increase in Flk1^{+} cells responsive to 1,25(OH)D3 (5 embryos/sample x 4 replicates/condition); error bars, mean± SD).

C. FACS analysis of embryos treated with 1,25(OH)D3 (10μM) indicated a 25% increase in EMPs at 30hpf (**p<0.01) compared to controls (n value and error bars as in S2B).

D. FACS analysis of 1,25(OH)D3-treated *Tg(flk1:dsred; EF1:mAG-zGEM(1/100)* embryos at 36hpf showed a 2-fold increase (***p<0.001) in proliferating endothelial cells, inclusive of the hemogenic population (5 embryos/sample x 4 replicates/condition); error bars, mean ± SD.

E. FACS analysis of *vdra* MO-injected *Tg(flk1:dsred; EF1:mAG-zGEM(1/100)* embryos at 36hpf indicated a significant decrease in proliferation (*p<0.05) in the absence of Vitamin D3/VDR signaling (n value and error bars as in S2D).

F. Representative fluorescent microscopy images of Tg*(CD41:GFP)* embryos treated with 1,25(OH)D3 (1μM) or calcipotriol (1μM) between 12-36hpf depicting a sustained increase in CD41:GFP^{+} expression in the CHT at 72hpf.

G. FACS analysis at 72hpf showed a significant increase in the percentage of CD41:GFP^{lo};Gata1:$dsRed^{-}$ HSPCs after both 1,25(OH)D3 (*p<0.05) and calcipotriol (**p<0.01) treatment (5 embryos/sample x 4 replicates/condition); error bars, mean ± SD. Scale bar 100μm.

Figure S3. Vitamin D treatment increases HSPC proliferation (*related to Figure 3*).

A. FACS analysis of adult kidney marrow (KM) at 1 year post transient embryonic exposure (12-36hpf) to 1,25(OH)D3 showed no difference in FSC/SSC profiles in the lymphoid/HSC, myeloid or progenitor gates compared to matched WT sibling controls (n=8 fish/condition).

B. KM FACS of *cyp2r1* adult mutant zebrafish at 1 year showed a significant reduction in the percentage of cells in the lymphoid gate, inclusive of HSCs and lymphoid progenitors, with life-long antagonism of 1,25(OH)D3 synthesis compared to WT sibling controls (lymphoid/HSC, *p<0.05; myeloid, N.S.; progenitor, N.S; n=4 fish/condition).

C. Adult *vdra* mutants displayed a significant decrease in the percentage of cells lymphoid/HSC gate at 3 months of age compared to WT sibling controls by KM FACS analysis (lymphoid/HSC, *p<0.05; myeloid, N.S.; progenitor, N.S; n=9 fish/condition).

D. *In vitro* expansion of adult KM HSPCs cultured on zebrafish kidney stromal (ZKS) cells with 1,25(OH)D3 (1μM) showed a significant increase in total cell number (**p<0.01) (n=4 replicates); error bars, mean ± SD.

Figure S4. 1,25(OH)D3 exposure regulates hUCB expansion and survival (*related to Figure 4*).

A. qPCR analysis indicated expression of the Vitamin D receptor (VDR) in whole hUCB (WCB) and $CD34^+$ hUCB HSPCs (n=3 hUCB samples each).

B. $CD34^+$ hUCB samples were treated *ex-vivo* for 4hrs with increasing concentrations of 1,25(OH)D3 then utilized in a methycellulose colony forming assay: treatment with 10^{-5} M 1,25(OH)D3 caused a refractory response, whereas exposure to 1,25(OH)D3 at 10^{-7} and 10^{-6} M resulted in a 39% (*p<0.05) and 56% (**p<0.01) increase in total CFU number at day 7, compared to DMSO treated controls; error bars, mean ± SD.

C. qPCR gene expression profile of select Vitamin D target genes from whole hUBC split and treated with 1,25(OH)D3 versus DMSO controls (n=6 hUCB samples), showing a significant increase in *G0S2* (***p<0.001), *bcl2* (*p<0.05); error bars, mean ± SD.

D. MetaCore pathway analysis of differentially expressed genes between $CD34^+$ hUCB samples split and treated with 1,25(OH)D3 or DMSO (vehicle control) revealed overlap with networks identified by IPA analysis (see **Figure 4F**); bold type indicates significantly regulated network includes CXCL8 (IL-8).

E. qPCR analysis for *CXCR1* and *CXCR2* expression in WCB and $CD34^+$ hUCB HSPCs (n=3 hUCB samples); error bars, mean ± SD.

Figure S5. Expression of Cxcl8 receptors *cxcr1/cxcr2* in zebrafish HSPCs (*related to Figure 5*).

A. Expression analysis of FACS sorted populations showed enhanced *cxcl8* expression following exposure to 1,25(OH)D3, with the biggest effect in $Flk1^{+}/cMyb^{+}$ HSPCs (cells sorted from ≥1000 embryos/condition x 2 replicates); error bars, mean ± SD.

B. qPCR analysis of ZF*cxcr1* and Zf*cxcr2* expression shows enrichment of ZF*cxcr1* in FACS sorted HSPCs ($Flk1^{+}/cMyb^{+}$) and the $Flk^{-}/cMyb^{-}$ population (n=2 replicates, cells sorted from ≥1000 embryos/condition); N.D. (not detected).

C. WISH analysis and phenotype distributions of embryos treated with 1,25(OH)D3 with and without the CXCL8 inhibitor SB25002 (1μM) showed co-treatment attenuated Vitamin D-mediated increases in *runx1/cmyb* expression (n≥50 embryos/condition).

D. FACS quantification of $Flk1^{+}/cMyb^{+}$ HSPCs after exposure to 1,25(OH)D3 with and without SB25002 (Con vs 1,25(OH)D3, *p<0.01; 1,25(OH)D3 vs SB25002, *p<0.01; Con vs 1,25(OH)D3 + SB25002, N.S.; (5 embryos/sample x 4 replicates/condition; error bars, mean ± SD).

Scale bar 100μm.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Transgenic and mutant zebrafish lines (Refers *to Zebrafish husbandry*)**:**

Official Line Name	**Reference**
Tg(lmo2:eGFP)	(Zhu et al., 2005)
Tg(gata1:dsRed)	(Traver et al., 2003)
Tg(cmyb:eGFP)	(North et al., 2007)
Tg(CD41:dsRed2ex)	(H.-F. Lin et al., 2005)
Tg(Flk1:dsRed2ex)	(Kikuchi et al., 2011)
Tg(EF1:mAG-zGem(1/100))rw0410h	(Sugiyama et al., 2009)
cxcl8 mutant	(Jing et al., 2015)
cyp2r1 mutant	(Cortes et al., 2015)

Vdra CRISPR-Cas9 mutant generation (Refers *to Zebrafish husbandry*)**:**

Guide RNA (gRNA) targeting Zf*vdra* was designed using CRISPRscan (www.crisprscan.org) (Moreno-Mateos et al., 2015). Briefly, gRNA template was generated by annealing a gene specific oligo containing the SP6 promoter followed by the ZF*vdra* gRNA target sequence (5'-GAG CAC GTC TGC GAC GGG TC-3) to a constant oligo as previously described (Gagnon et al., 2014). ZF*vdra* gRNA was *in vitro* transcribed using MEGAscript-SP6 and purified using MEGAclear transcription clean up kit (Thermo-Fisher). Zebrafish embryos were co-injected at the one-cell stage with ZF*vdra* gRNA (40ng/µl) and Cas9 (50ng/µl) protein (New England Biolabs) and raised to adulthood. F1 *vdra* heterozygous mutants were identified using the Surveyor Mutation kit (IDT) by amplification of a 287bp DNA fragment flanking the *vdra* gRNA target sequence with the following primers: vdracrispFwd-5'-TTGAATATGGATGAG CTGTGAGTGT-3',vdracrispRev5'-TGCTGTAATTCGGACTCTAACTGAC-3', and confirmed by DNA sequencing.

Production of the VDRE reporter transgenic zebrafish (Refers *to Zebrafish husbandry*)**:**

A consensus 60-bp vitamin D response element (VDRE): 5'-GATCCACAAGGTTCAC GAGGTTCACGTCCGGATCCACAAGGTTCACGAGGTTCACGTCCG-3'

(Switchgear Genomics) was cloned into the Tol2kit plasmid p5E-Fse-AscI-bas generating: p5E-VDRE-bas. The pDest-Tol2-VDRE:mCherry plasmid was generated by multisite LR Clonase II Plus (Thermo-Fisher) recombination using the following plasmids: p5E-VDRE, pME-mCherry (Kwan et al., 2007), p3EpolyA and pDestTol2AB2 (alpha:crystallin:cerulean) (Zhou et al., 2011). Transgenic zebrafish were produced by co-injection of Tol2-transposase mRNA (Kwan et al., 2007) and pDest-Tol2-VDRE:mCherry plasmid into one-cell stage embryos. Transgenic embryos were identified by lens specific expression of cerulean protein, and confirmed by induction of mCherry protein expression with Vitamin D3 exposure.

Zebrafish morpholino sequences: (Refers to *Morpholino and mRNA injection*)

Target Gene	**Morpholino Sequence**	**Reference**
vdra-1	5'-ACGGCACTATTTTCCGTAAGCATCC3'	(C.-H. Lin et al., 2012)
vdra-2	5'-CTCTGGTGAATGGTTGAATGAGTCT-3'	
vdrb	5'-AGCTGACTCCATCACTGAATATCGC-3'	
cyp27b1	5'-TGAAGCACCATCATCATCCTCCTGC-3'	(Cortes et al., 2015)
cxcl8	5'-CGTATTAGTTTGAAAACTCACATGA-3	(Stoll et al., 2011)

vdra morpholio validation (Refers to *Morpholino and mRNA injection*)**:**

Splicing efficiency of *vdra*-MO2 was determined by qRT-PCR using the following primers: *vdra*ExonFwd: 5'-CGACGACTCGTACTCCGACTTCGTCAGATT-3' and *vdra*ExonRev: 5'-GGCAGC ATGGAGAGCCGGGACTGCTGATCC-3'; specificity was

confirmed by co-injection of *vdra* or *vdrb* mRNA (75ng/ul), as previously described (Cortes et al., 2015).

RT-qPCR primer sequences (Refers to *Expression Analysis*)**:**

Gene	***Forward Primer***	***Reverse Primer***
Dr cyp24a1	GCAGAACACATCAAGAGCATG	CTTACACCGATAGCCTGACTG
Dr vdra	CTCGGATTCTGTGGATGCTT	GGCCTTACGCTTCATACTGC
Dr vdrb	ACACAGCGTGGAGTGGAGT	ACACTCCATGGCAAGAACA
Dr cxcl8	GTCGCTGCATTGAAACAGAA	CTTAACCCATGGAGCAGAGG
Dr cxcr1	CGGCAATGTCGCGTGCAAGC	GGCCACAACCACGGCTACGA
Dr cxcr2	TCCTTGCCCGGAGACCGTGA	ATGGTGCCGAACGGCCAGTG
Dr efnb2a	CAAGGACAGCAAATCGAATG	TGAGCCAATGACTGATGAGG
Dr flk1	AAGGCTTCTTCACTCTTCACG	GAGTGTGAGTGTCCCACCAA
Dr runx1	CGTCTTCACAAACCCTCCTCAA	GCTTTACTGCTTCATCCGGCT
Dr tbp	CGGTGGATCCTGCGAATTA	TGACAGGTTATGAAGCAAAACAACA
Hs CXCL8	CTTGGCAGCCTTCCTGATTT	TTCTTTAGCACTCCTTGGCAAAA
Hs CXCR4	CCTATGCAAGGCAGTCCATGT	GGTAGCGGTCCAGACTGATGA
Hs VEGF	CCAATCGAGACCCTGGTG	CACACAGGATGGCTTGAAGA
Hs CD41	GATGAGACCCGAAATGTAGGC	TCAGTCTTTTCTAGGACGTTCCA
Hs G0S2	GCCACTAAGGTCATTCCCGCCT	CCTTGCGCTTCTGGGCCATCAT
Hs CYP24A1	GATTTTCCGCATGAAGTTGGGT	CCTTCCACGGTTTGATCTCCA
Hs VDR	GTGGACATCGGCATGATGAAG	GGTCGTAGGTCTTATGGTGGG
Hs CXCR1	CTGAGCCCCAAGTGGAACGAGACA	GCACGGAACAGAAGCTTTATTAGGA
Hs CXCR2	CAATGAATGAATGAATGGCTAAG	AAAGTTTTCAAGGTTCGTCCGTGTT
Hs RUNX1	CGTGCACATACATTAGTAGCACTACCTTTG	CTTCCACGAATCTTGCTTGCAGAGGTTAAG
Hs FLT3	TTTACCCCACTTTCCAATCACAT	CGAGTCCGGGTGTATCTGAAC

Hs BCL2	GGTCATGTGTGTGGAGAGC G	GGTGCCGGTTCAGGTACTCA
Hs BCL-XL	AACTGGGTCTCCTGACTTGG	AAGGACAGTGTTGGCAAAGG
Hs CCND1	GTGCTGCGAAGTGGAAACC	ATCCAGGTGGCGACGATCT
Hs CDK1	CCATTTTGCCAGAAATTCGT	TTTTCAGAGCTTTGGGCACT
Hs CDKN1	GTCACTGTCTTGTACCCTTG TG	CGGCGTTTGGAGTGGTAGAAA
Hs BAD	CGAGTGAGCAGGAAGACTC CA	AGGAGTCCACAAACTCGTCACT
Hs BAX	GGCGGCAATCATCCTCTG	TGCTTCAGGGTTTCATCCAG
Hs FOXO1	AGTCTGAGGTGCTGGCGGA G	GGTGGTGGCGTATCAGAGGTG
Hs ACTIN	GCACAGAGCCTCGCCTTT	GGAATCCTTCTGACCCATGC

Zebrafish Western blot (Refers to *Expression Analysis*)**:**

WT and *cxcl8* mutant embryos (60 embryos/condition) at 36 hours post fertilization were dechorionated and deyolked. Deyolked embryos were homogenized in RIPA buffer containing protease and phosphotase inhibitors (Roche). 50ug of total protein was separated on a 10% SDS polyacrylamide gel and transferred to nitrocellulose. Membrane was blocked in 5% nonfat milk in TBST (TBS with 0.1% Tween-20) and incubated overnight at 4 degrees with primary antibody: 1:2000 pAKT, 1:1000 pan AKT (Cell Signaling Technology). Washing several times in TBST, was followed by a 1 hour room temperature incubation with 1:2000 anti-rabbit HRP-conjugated antibody (Cell Signaling Technology). Blot was developed using SuperSignal West Dura ECL substrate (Fisher).

Microarray and data analysis (Refers to *Expression analysis*)**:**

Human $CD34^+$ umbilical cord blood cells (1 million) (Stem Cell Technologies) were split and treated in StemSpan (Stem Cell Technologies) with 1,25(OH)D3 (1μM) or DMSO control for 4hrs at 37 degrees. RNA was extracted using RNAqueous Micro Kit, quality determined using an Agilent Bioanalyzer, and RNA was hybridized to Affimetrix Human

Transcriptome 2.0 GeneChip. Arrays were processed using the 'oligo' BioConductor package (Carvalho and Irizarry, 2010)normalized with RMA (Irizarry et al., 2003), and differentially expressed genes were identified using limma (Smyth et al., 2005). The differentially expressed gene list (FDR<0.05) was used for pathway analysis enrichment using Ingenuity Pathway Analysis (Qiagen) and MetaCore (Thomson Reuters) software.

Zebrafish HSPC CFU assay (Refers to *In vitro functional analysis*)**:**

Adult KM was manually isolated, dissociated, pooled and plated in methylcellulose at a 10,000 cells per ml in duplicate. Complete methylcellulose media included 0.1% carp serum, rZF epo, rZF Gcsfa, and 1% BSA (Stem Cell Technologies), with or without 1,25(OH)D3 ($1x10^{-7}$, and $1x10^{-6}$ M). Cells were incubated for 7-10 days and total hematopoietic colony number quantified with a Zeiss AxioVert microscope. FACS-sorted CD41:eGFP^{+} HSPCs were similarly treated, plated in methylcellulose (1000 cells/ml) and enumerated, then statistically analyzed, as described in the main text.

Zebrafish HSPC in vitro expansion assay (Refers to *In vitro functional analysis*)**:**

$1.2x10^{5}$ leukocytes from WKM were plated into individual wells of a 12-well dish containing 80% confluent zebrafish embryonic stromal trunk (ZEST) cells or zebrafish kidney stromal (ZKS) cells (Campbell et al., 2015). 1,25(OH)D3 (1μM and 10μM) or calcipotriol (1μM), SB225002 (1μM) was added to wells at the time of plating, and total cells were enumerated six days later by trypan blue exclusion.

hUCB CFU assay (Refers to *In vitro functional analysis*)**:**

De-identified commercially available $CD34^+$ hUCB (Stem Cell Technologies) units were freshly thawed according to manufacturers instructions, split and incubated in 2% FCS IMDM at 37 degrees in the presence of 1,25(OH)D3 ($1x10^{-6}$ M) or DMSO for 4hrs. Cells were washed with 10 volumes of 2% FCS IMDM, and plated (2000, 1000, 500 cells/plate) in Methocult H-4434 Classic (Stem Cell Technologies) in triplicate. Colonies were enumerated at day 7 for total colony number and day 10 for lineage analysis (BFU-E, CFU-GM, and CFU-GEMM), with statistics as described above.

hUCB survival/apoptosis and proliferation (Refers to *In vitro functional analysis*)**:**

$CD34^+$ hUCB (Stem Cell Technologies) units were split and treated with 1,25(OH)D3 (1μM) or DMSO control for 4 hrs at 37 degrees, then washed with 10 volumes of Stemspan (Stem Cell Technologies). For viability analysis, after washing in cold PBS, cells were resuspended in AnnexinV-FITC (Trevigen)/ 7-AAD (BD Pharmigen) solution mix, incubated in the dark for 15 minutes, washed and analyzed on BD Canto II. For proliferation analysis, cells were incubated for 1 hr with 10μM EdU (Life Technologies), washed in 1% BSA PBS, incubated with Fc Receptor Binding inhibitor (eBioscience) for 20 minutes, followed by antibody incubation for 2 hrs with: PE anti human CD34 (BD Pharmigen), PerCP-Cy5.5 anti human CD45 (BD Pharmigen). Cells were permeabilized, and labeled using Click-it Plus Edu Alexa Fluor 647 Flow Cytometry Kit (Life Technologies) and analyzed on a BD Canto II, with statistics performed as above.

CXCL8 cytokine analysis (Refers to *In vitro functional analysis*)**:**

$CD34^+$ hUCB (Stem Cell Technologies) units were split and treated with 1,25(OH)D3 (1μM) or DMSO control for 2 hrs at 37 degrees in Stemspan (Stem Cell Technologies). After 2 hrs cells were treated with monensin (ebiosciences) and cultured for an additional 2hrs prior to analysis (4hrs total). Samples were treated with Fc Receptor inhibitor (eBioscience) followed by antibody incubation with PE anti human CD34 (BD Pharmigen). Samples were washed and fixed for 30 minutes in IC Fixation buffer (eBioscience), stained with APC anti human CXCL8 antibody in Permeabilization buffer (eBioscience), and analyzed on BD Canto II.

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