SUPPLEMENTAL MATERIALS

SUPPLEMENTAL TABLES

Table S1. Antibodies used for flow cytometric analyses. Conjugated antibodies were purchased from eBioscience (San Diego, CA, U.S.A), BioLegend (San Diego, CA, U.S.A) or BD Biosciences (San Jose, CA, U.S.A.). PE; phycoerythrin. APC; allophycocyanin. Cy7; Cyanine 7. FITC; Fluorescein.

Table S2. Oligonucleotide sequences used for RT-PCR.

Table S3. shRNA sequences used in knockdown studies. PLKO.1 lentivirus plasmids containing these shRNA sequences were purchased from Sigma-Aldrich (St. Louis, MO).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Vdr signaling stimulates the growth of erythroid progenitors from BM. (A) Lin^{neg} cells from adult BM (male mice) were cultured in methylcellulose ($1.6x10^3$ cells/mL) with and without calcitriol (100 nM). CFU-E colonies were scored after 2 or 3 days (n=5). (B) Lin^{neg} BM cells were cultured in methylcellulose ($1.6x10^3$ cells/mL) with calcitriol at the indicated concentrations (n=1). Data for panels A-C were analyzed using an unpaired Student's t test (** $p < 0.01$). Error bars, ± SEM for panel A; ± SD for panel B. (C) A logarithmic regression analysis was performed for the data from panel (B). The increases in CFU-E numbers between untreated and calcitriol treated samples were fitted to the equation shown and an EC_{50} value calculated.

Supplemental Figure 2. Erythroid Maturation of E12.5 Lin^{neg} FL cells. Cells were cultured under progenitor conditions (in PM) for 2 days and then transferred to methylcellulose ($2.0x10^3$ cells/mL) or MM. (A) Photographs of erythroid colonies formed in methylcellulose. A colony of 3-20 CFU-E colonies was scored as multi-CFU-E (mCFU-E); anything larger was scored as BFU-E. A single unite of tightly clustered cells was scored as CFU-E. Scale bars, 100 μm. (B) Growth of Lin^{neg} cells cultured for two days in PM and then for the indicated times in MM (n=5). (C) Frequency of $cKit^+Ter119^{neg}$ and $cKit^{neg}Ter119^+$ cells identified in MM culture for up to 5 days, measured by flow cytometry (n=3). (D) Wright-Giemsa stained cytospin preparations of Lin^{neg} FL cells from culture in MM. Red circle, enucleated erythroblasts (reticulocytes and RBCs); black, extruded nuclei. In this culture system, the frequency of enucleated cells (# enucleated cells/ [total nucleated + enucleated]) is typically 55-70% on day 6. Scale bar, 20 μm. (E) Representative flow cytometry plots of CD44 expression versus FSC for cells cultured in MM for the indicated times. For days 1-6, we gated on $Ter119^+$ cells; for day 0, the entire population of Lin^{neg} cells was analyzed due to the low abundance of $Ter119^+$ cells. (F) Determination of frequency of enucleation of maturing

Ter119^{+} cells. Nucleated erythroblasts, enucleated cells, and expelled nuclei, were FACS sorted, cytospun, and stained with Wright-Giemsa. Scale bar, 20 μm. Error bars (panels B,C), ±SEM.

Supplemental Figure 3. Dose-dependent stimulation of E12.5 Lin^{neg} FL cell proliferation by calcitriol. E12.5 Lin^{neg} FL cells were cultured under progenitor conditions (in PM) for 2 days and then transferred to maturation medium (MM) supplemented with the indicated concentrations of calcitriol. Calcitriol treatment resulted in a dose-dependent increase in cell numbers, up to 100 nM. Doubling the calcitriol concentration to 200 nM was inhibitory (n=2); a representative experiment is shown. Error bars, ±SD.

Supplemental Figure 4. Phenotypic analysis of E12.5 Lin^{neg} cells cultured under maturation conditions with or without calcitriol. Cells were cultured in progenitor medium (PM) for 2 days and then transferred to maturation medium (MM) with or without calcitriol. (A) Representative flow cytometry plots of CD44 versus Ter119 expression on cells in MM culture (n=3). (B) Representative flow cytometry plots of CD44 expression versus FSC for Ter119^{+} cells cultured in MM (n=3). Blue and red boxes gate less mature and more mature erythroblasts, respectively. (C) Representative flow cytometry plots of DRAQ5 staining versus FSC for Ter119^{+} cells cultured in MM (n=3). (D) Photograph of pellets of cells from day 6 of MM culture (n=3). Note the larger size of the pellet of cells cultured in the presence of calcitriol.

Supplemental Figure 5. BFU-E colonies formed from cKit^{+} CD71$^{lo/neg}$ cells in the presence or absence of calcitriol. Analysis of BFU-E colony surface area using ImageJ (30-40 individual colonies) did not reveal significant differences in colony size for the two culture conditions. Error bars, SD.

Supplemental Figure 6. The Lin^{neg} cKit^{+} CD71^{med} population of FL erythroid cells is not a target of signaling through Vdr. (A) Representative flow cytometry plot showing the gating of E12.5 Lin^{neg} cKit^{+} FL cells based on CD71 expression. (B) Distribution of colonies formed from ckit^{+} CD71^{med} cells cultured in methylcellulose (5.0x10^{2} cells/mL) with or without calcitriol (n=3). (C) Proliferation of ckit^{+} CD71^{med} cells cultured in MM with or without calcitriol (n=3). Data were analyzed using an unpaired Student's t test (panel B) or two-way ANOVA (Panel C). Error bars, ± SEM.

Supplemental Figure 7. Phenotype of cKit^{+} CD71$^{lo/neg}$ and cKit^{+} CD71^{hi} cells cultured under maturation conditions with or without calcitriol. (A) Representative flow cytometry plots of cKit versus Ter119 expression on ckit^{+} CD71$^{lo/neg}$ cells cultured in MM (n=3). (B) Representative flow cytometry plots of CD44 expression versus FSC for Ter119^{+} cells cultured in MM (n=3). Blue and red boxes gate less mature and more mature erythroblasts, respectively. (C) Representative flow cytometry plots of DRAQ5 staining versus FSC for Ter119^{+} cells cultured in MM (n=3). (D) Representative flow cytometry plots showing expression of cKit versus Ter119 and expression of CD71

versus Ter119, on ckit^{+} CD71^{hi} cells cultured for 1 day in MM (n=3). (E) Proliferation of ckit^{+} CD71$^{lo/neg}$ cells cultured in MM at the indicated concentrations of calcipotriol (n=3). Data were analyzed using a two-way ANOVA (panel E) (** $p < 0.01$, E). Error bars, ± SEM.

Supplemental Figure 8. Cell cycle analysis of cKit^{+} CD71$^{lo/neg}$ cells cultured under maturation conditions. Cells were subjected to a 30 minute pulse of EdU. Incorporation of EdU and labeling with 7-Aminoactinomycin D (7-AAD) was measured using the Click-iT EDU^{TM} Flow Cytometry Assay Kit (see Supplemental Methods). A transient increase in the numbers of cells in S phase and a decrease in the numbers of cells in G0/G1 in response to calcitriol was observed.

Supplemental Figure 9. Analysis of cKit^{+} CD71$^{lo/neg}$ cells pre-cultured for two days under progenitor conditions and then transferred to maturation medium. (A) Representative flow cytometry plots of cKit versus Ter119 expression on ckit^{+} CD71$^{lo/neg}$ cells cultured in PM for 2 days and then transferred to MM culture (n=3). (B) Representative flow cytometry plots of CD44 expression versus FSC for Ter119^{+} cells cultured in MM culture (n=3). Blue and red boxes gate less mature and more mature erythroblasts, respectively. (C) Representative flow cytometry plots of DRAQ5 staining versus FSC for Ter119^{+} cells cultured in MM for the indicated times (n=3). (D) Proliferation of ckit^{+} CD71$^{lo/neg}$ cells cultured in PM for 2 days and then transferred to MM with or without calcipotriol (100 nM) (n=3). (E) Proliferation of sorted ckit^{+} CD71$^{lo/neg}$ cells cultured in PM for 2 days and then transferred to MM with or without calcitriol or dexamethasone (dex), alone and in combination (100 nM) (n=1). Data were analyzed using a two-way ANOVA (panel D, E) (* $p < .05$, ** $p < 0.01$, panel D). Error bars, ± SEM for panel D and ± SD for panel E.

Supplemental Figure 10. Lin^{neg} cKit^{+} CD71$^{lo/neg}$ progenitors were pre-cultured for two days under progenitor conditions (in PM) with shRNA lentiviruses targeting Vdr (shRNA1 and shRNA2) or luciferase. Mock and untreated cells were included as additional controls. No differences in cell number (A) or frequency of cKit^{+} cells (B) were identified. Data were analyzed using an unpaired Student's t test.

Supplemental Figure 11. Cartoon representing self-renewal of progenitors and maturation to erythrocytes. Limited self-renewal is observed in the absence of vitamin D3 (calcitriol). In the presence of calcitriol, Vdr is activated, translocates into the nucleus, where it binds to VDREs and regulates expression of target genes.

SUPPLEMENTAL METHODS

Preparation of hematopoietic cells

Fetal liver: CD-1 mice (Charles River Laboratories International) were mated overnight. Noon of the day of detection of the copulation plug was taken as day 0.5 of gestation (Embryonic day (E) 0.5). Mice were euthanized by CO_2 asphyxiation at day E12.5, 13.5, or 14.5, as indicated in the Figure Legends. Fetal livers (FL) were dissected from embryos in dissection buffer (2% Fetal Bovine Serum, FBS, and 1% Penicillin/Streptomycin in Phosphate-Buffered Saline, PBS) and transferred to Fetal Liver Buffer (FLB; IMDM containing 15% FBS and 1% Pen/Strep). A single cell suspension was prepared by triturating FL tissue using a 1 mL pipette tip. The cell suspension was filtered through a 40 µm cell strainer (BD Falcon). FL cells were collected by centrifugation (Beckman coulter Allegra X-14R centrifuge, RCF=1000, 5 minutes), suspended in FLB, and counted using a hemocytometer.

Bone marrow: Single cell suspensions were prepared from bone marrow (BM) by dissecting the femora and tibia of 6-to-8 week old ICR mice (female, unless otherwise specified). Femora and tibia were washed with PBS and transferred to a mortar. FLB (10 mL) was added to the mortar and a pestle was used to crush the bones until no red pigmentation was visible in the fragments of bone. The crushed bone and marrow was collected using a 10 mL pipette tip and fresh FLB (10 mL) added to retrieve any remaining BM. Cells were dispersed by trituration, filtered through a 40 µm cell strainer (BD Falcon), and collected by centrifugation (Beckman coulter Allegra X-14R centrifuge at RCF=500, 5 minutes). Cells were suspended in FLB and counted using a hemocytometer.

Enrichment of Lin^{neg} cells: Lin^{neg} cells from FL or BM were enriched from single cell suspensions using a Lineage Cell Depletion Kit (Miltenyi Biotec Inc.) based on the manufacturer's instructions. Lin^{neg} cells were suspended in FLB and counted using a hemocytometer.

Fluorescence activated cell sorting

Lin^{neg} from FL were prepared for sorting as described [1] with modifications. A single cell suspension of FL Lin^{neg} cells ($1.0x10^7$/mL) was incubated with rat serum (10%) in FLB at room temperature (RT) for 10 minutes to limit non-specific binding of targeting antibodies. CD71 and cKit antibody (Supplementary Table S1) were added and the cells were incubated at room temperature for 20 minutes. Cells were diluted to $2.0x10^6$ cells/mL with FLB, 3µM 4',6-diamidino-2-phenylindole (DAPI) added to exclude dead cells, and $ckit^+$ $CD71^{lo/neg}$,$CD71^{med}$, and $CD71^{hi}$ progenitors sorted using a FACSAria III instrument (BD, Franklin lakes,NJ).

Erythroid Progenitor culture

Lin^{neg} cells from FL were plated ($7.5x10^5$/mL) in serum-free progenitor medium (PM): StemSpan SFEM (Stem Cell Technologies Vancouver, BC, Canada) supplemented with human recombinant EPO (0.5 Units/mL; Amgen), mouse SCF (100 ng/mL; Thermo Fisher Scientific), mouse IGF (40 ng/mL; Thermo Fisher Scientific), dexamethasone (10^{-5} M; Sigma D2915), lipid concentrate (1X; Thermo Fisher Scientific), and penicillin/streptomycin (1%; Pen/Strep; Thermo Fisher Scientific). After 24 hours in culture, the cells were collected by centrifugation (RCF=500; 5 minutes; Beckman coulter Allegra X-14R centrifuge), resuspended in 1 mL PBS, and again collected by centrifugation in an Eppendorf 5415D microcentrifuge (RCF=$4.8x10^3$, 5 minutes). The pellet was suspended in PM in which the cells had been growing and then split to a density of $2.5x10^5$ cells/mL using fresh PM so that half the medium was fresh. We found that culturing Lin^{neg} cells in 50% fresh medium resulted in a more consistent maintenance of progenitor potential from one experiment to another but have not examined these conditions systematically. After 48 hours, the cells were washed with PBS (Beckman coulter Allegra X-14R centrifuge, RCF=500, 5 minutes), and transferred to methylcellulose for progenitor assays or to MM (see below) for erythroid maturation culture.

Sorted $CD71^{lo/neg}$ cells were cultured in PM ($1.0x10^5$ cells/mL) for 24 hours, and then split to a density of $1.0x10^5$ cells/mL. After 48 hours, the cells were counted, washed with PBS, and transferred to methylcellulose for progenitor assays or to MM for erythroid maturation culture.

Erythroid maturation culture

Maturation of erythroid progenitors was initiated by transferring cells ($2.5x10^5$/mL) to erythroid maturation medium (MM): IMDM (Corning 10-016-CV) supplemented with recombinant human EPO (2.0 U/mL), mouse SCF (100 ng/mL), Knockout Serum (10%; KO-Serum; Thermo Fisher Scientific), Plasma Derived Serum (5%; Animal Technologies), PFHM-II (10%; Thermo Fisher Scientific), glutamine (1x; Thermo Fisher Scientific), and penicillin/streptomycin (1%) [2]. Cells were harvested at different times for analysis by flow cytometry or for culture in methylcellulose. Cell density was measured daily until cells stopped proliferating. At a density of $4.0x10^6$/mL, the cells were split to $1.0x10^6$/mL. The medium was supplemented with calcitriol (100 nM, or as indicated; Sigma), calcipotriol (100 nM or as indicated; Cayman Chemical), or dexamethasone (100 nM).

Erythroid Progenitor Assays

For BFU-E progenitor assays, cells were cultured (37°C, 5% CO_2) in methylcellulose (Stem Cell Technologies) supplemented with PDS (15%), PFHM-II (5%), SCF (100 ng/mL), mouse IL-3 (20 ng/mL; Peprotech), EPO (2 units/mL), mouse IL-6 (20 ng/mL; Peprotech), and Pen/Strep (1%). BFU-E, multi-CFU-E (late BFU-E), and non-erythroid colonies were scored after 8-9 days. Some CFU-E colonies were also observed at these times and were scored. For specific identification of CFU-E progenitors, cells were

cultured in methylcellulose supplemented with PDS (15%), PFHM-II (5%), SCF (100 ng/mL), and EPO (2 units/mL) and colonies were scored after 2-3 days. Unless otherwise indicated in the figure legends, the BFU-E progenitor assay was used. Colonies were imaged using a Zeiss AxioCam MRC camera mounted on a Zeiss Axiovert 25 microscope (LD A-Plan 5.0x/NA 0.15 or 20x/NA 0.30 objective lenses). Images were processed using Adobe Photoshop software.

Flow cytometry

For analysis of cell surface marker expression, single cell suspensions of Lin^{neg}, sorted, or cultured cells were incubated in FLB containing rat serum (10%) for 10 minutes at room temperature. Cells were incubated with primary conjugated antibodies for 20 minutes. Monoclonal antibodies used in this study are listed in supplemental Table 1**.** To measure enucleation, cells were incubated with DRAQ5 (1:1000 in FLB) at room temperature for 5 minutes and diluted with PBS (150 uL). DAPI was then added (3 µM) to exclude dead cells. Flow cytometry was performed using a BD LSRFortessa 5 laser analyzer. The data were analyzed using FloJo X10 software (TreeStar, Ashland,OR).

Cytospin cytocentrifugation and Wright-Giemsa staining

Cell cytocentrifugation onto glass slides was performed as previously described [3]. The cytospun cells were stained with Hema 3 (ThermoFisher; 23-123869; comparable to Wright-Giemsa) as per the manufacturer's instructions. To detect hemoglobin, cells were incubated (5 minutes; RT) with FLB containing o-dianisidine (Sigma; 0.018% W/V) and hydrogen peroxide (H_2O_2; Sigma). The o-dianisidine (0.2% W/V) was first combined with H_2O_2 (10:1) and then mixed with FLB (1:10). The incubated cells were cytocentrifuged onto glass slides. Hema 3- and o-dianisidine-stained cytospin preparations were imaged using a Zeiss AxioCam MRC camera mounted on a Zeiss Axiovert 25 microscope (LD A-Plan 32x/NA 0.40 or 20x/NA 0.30 objective lenses). Images were processed using Adobe Photoshop software.

Analysis of cell proliferation and apoptosis

Cells were pulsed with EdU (10 µM) for 30 minutes. Incorporation of EdU and labeling with 7-AAD were measured using the Click-iT EDU^{TM} Flow Cytometry Assay Kit (ThermoFisher Scientific) as per the manufacturer's instructions. The percentage of cells synthesizing DNA (cells in S Phase of the cell cycle) was determined using a BD LSRFortessa 5 laser analyzer.

Cell division was tracked using carboxyfluorescein diacetate succinimidyl ester (CFSE; 5 µM; Biolegend). After 4 days in culture in MM, cells were incubated with CFSE for 10 minutes at room temperature, then washed and resuspended in MM. CFSE fluorescence was measured using a BD LSRFortessa 5 laser analyzer for each of the remaining days of maturation.

Apoptosis was measured using an Annexin V apoptosis detection kit (BD Pharmingen 556547) as per the manufacturer's instructions.

Semi-quantitative Real-Time Reverse Transcription Polymerase Chain Reaction
Total RNA was isolated from progenitors using an RNeasy mini kit (Qiagen) as per the manufacturer's instructions. cDNA was synthesized from 1 µg of total RNA in a 20 µL reaction using an iScript^{TM} cDNA synthesis kit (Bio-Rad) as per the manufacturer's instructions. For SYBR Green semi-quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR), the 20 µL reaction contained 1 µL cDNA, 5 µM of each primer, and 10 µL of SYBR Green PCR master mix (Applied Biosystems). The reactions were run in a Biorad CFX384 Touch real-time PCR detection system using the following program: one cycle at 95°C, 10 minutes; 45 cycles at 95°C, 15 seconds; one cycle at 60°C, 15 seconds. The *Ubb* gene served as an internal control, and the relative expression level of each gene was calculated from the C_t value of the gene normalized to that of *Ubb,* as described [4]. The arbitrary value in the formula was set at 100,000. Primer sequences are listed in Supplemental Table 2.

Lentivirus Production
Lentivirus supernatant was prepared as previously described [5], with some modifications. HEK 293FT cells (293FT; 1.8x10^6 cells; Thermo Fisher Scientific) were suspended in DMEM containing 10% FBS and 1% penicillin/streptomycin, plated on Poly-L-Lysine (0.01% in PBS; Sigma) coated petri dishes, and incubated (37°C, 5% CO_2) overnight. The next morning, cells were transfected with 24 µg of plasmid DNA: shRNA lentivirus, Δ8.9 (containing *gag*, *pol*, and *rev* genes), and vesicular stomatitis virus (VSV-g) at a ratio of 4:3:1, respectively. Lipofectamine 2000 (Thermo Fisher Scientific) was included as per the manufacturer's instructions. The shRNA sequences are listed in Supplemental Table 3. After 5 hours, the culture medium was replaced with fresh DMEM containing 10% FBS and 1% penicillin/streptomycin. Supernatant was collected at 48 hours, replaced with fresh medium, and collected again at 96 hours. Lentivirus-containing supernatant was transferred to an Amicon Ultra 15 mL Centrifugal Filter Unit (Millipore) and spun with in a table top centrifuge (Beckman coulter Allegra X-14R centrifuge; RCF=2000) at 20 minute intervals until the volume was ~1.0 mL. The method used to calculate the transduction units (TU)/mL for each lentivirus supernatant preparation was previously described [5]. Viral supernatants were stored at -80°C.

Lentivirus transduction of Lin^{neg} ckit^{+} CD71$^{lo/neg}$ Progenitors
Non-tissue culture treated surfaces (96-well plate; Falcon; 351172 or 24-well plate; Thermo Fisher Scientific 144530) were coated with Retronectin (10µg/mL; Takara) as per the manufacturer's instructions. Lin^{neg} cKit^{+} CD71$^{lo/neg}$ cells were suspended in PM (1x10^5/mL) and plated on Retronectin coated dishes (96- or 24- well plate). Lentivirus supernatant (~7.5x10^7 TU/mL) and 16,16-dimethyl prostaglandin E_2 (DMPGE2, 100µM; Cayman Chemical) were added to the cultures. The plate was then spun in a Beckman coulter Allegra X-14R centrifuge (RCF=800, 1 hour; 32°C) and incubated overnight at 37°C, 5% CO_2. The next morning, cells were detached from the Retronectin coated surface using vigorous pipetting, counted, and diluted to 1x10^5 cells/mL in PM. At 48

hours, the cells were counted and transferred to MM ($2.5x10^5$ cells/mL) in the presence or absence of calcitriol (100 nM).

To determine transduction efficiency, cells were cultured in PM for 2 days and then for an additional day in the presence or absence of puromycin (1.5 µg/mL; Sigma). On day 3, cells were counted and the percentage of transduced cells calculated by dividing the total number of cells cultured with puromycin by the total number cultured without puromycin and then multiplying by 100.

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Table S1. Primary conjugated antibodies used for flow cytometry

Target	Fluorophore	Catalogue #	Vendor	Clone	Dilution
CD117	APC	105811	Biolegend	2B8	1:100
CD71	Pe-cy7	113812	Biolegend	R17217	1:250
CD41	Pe	133917	Biolegend	MWReg30	1:100
CD16/32	Bv510	101333	Biolegend	93	1:100
Ter119	Fitc	11-5921-82	eBioscience	TER-119	1:100
CD44	Pe	12-0441-82	eBioscience	IM7	1:100
Ly6c	Pe	12-5931-82	eBioscience	RB6-8c5	1:100
CD11b	Fitc	557396	Pharmingen	M1/70	1:100

Supplemental Table 1

Table S2. Oligonucleotide sequences used for RT-PCR

Target gene	Acession no.	Primer sequences	Details
Vdr	NM_009504	Forward:5'-ACCCTGGTGACTTTGACCG-3'; Reverse:5'-GGCAATCTCCATTGAAGGGG-3'	Primer bank* ID 31543944a1
Cyp24a1	NM_009996	Forward:5'-CTGCCCCATTGACAAAAGGC-3'; Reverse:5'-CTCACCGTCGGTCATCAGC-3'	Primer bank* ID 6753572a1
Gata1	NM_008089	Forward:5'-CACCGGCAGTGCTTACGGGG-3'; Reverse:5'-ACAAGGGGCCAATGCCAGGC-3'	Custom Designed
Klf1	NM_010635	Forward:5'-AGACTGTCTTACCCTCCATCAG-3'; Reverse:5'-GGTCCTCCGATTTCAGACTCAC-3'	Primer bank* ID 6754454a1
Hbb-b1	NM_001278161	Forward:5'-ACGATCATATTGCCCAGGAG-3'; Reverse:5'-AACGATGGCCTGAATCACTT-3'	(Ross et al., 2012)
Hbb-b2	NM_016956	Forward:5'-AGCACAATCACGATCGCA-3'; Reverse:5'-GCCTGAAAAACCTGGACAAC -3'	Primer bank** qprimer depot
Hba-a1 *Hba-a2*	NM_008218 NM_001083955	Forward:5'-CACCACCAAGACCTACTTTCC-3'; Reverse:5'-CAGTGGCTCAGGAGCTTGA-3'	(Bartman et al., 2012)
ubb	NM_011664	Forward:5'-AGGCTTTGTCCGGTTCGGCG-3'; Reverse:5'-TGCCGGTCAGGGTCTTCACG-3'	Custom Designed
Alas2	NM_009653	Forward:5'-TGGGCTAAGAGCCATTGTCCT-3'; Reverse:5'-GTAGGTGTGGTCCTGTTTCTTC-3'	Primer bank* ID 33859502a1

* From Harvard Primer Bank Database

** From Qprimer Depot Database

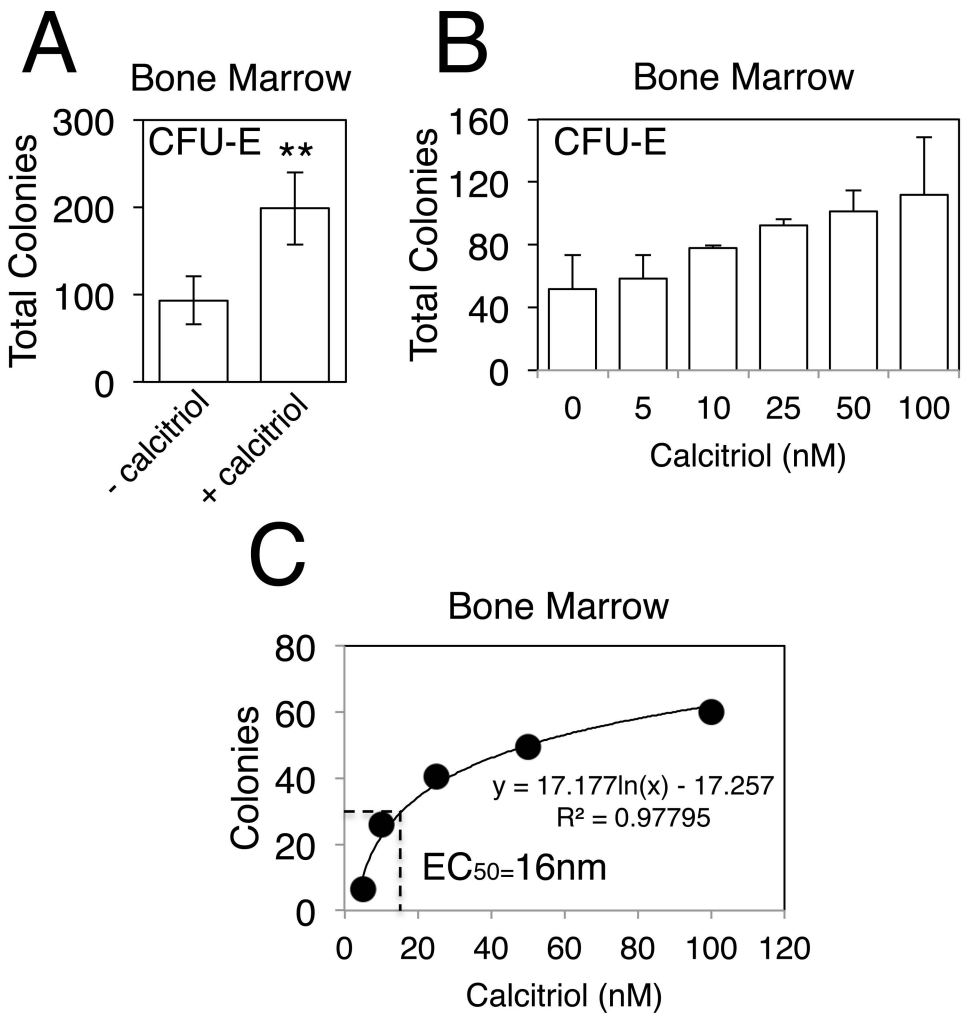
Supplemental Table 2

Table S3. shRNA sequences used in knockdown studies

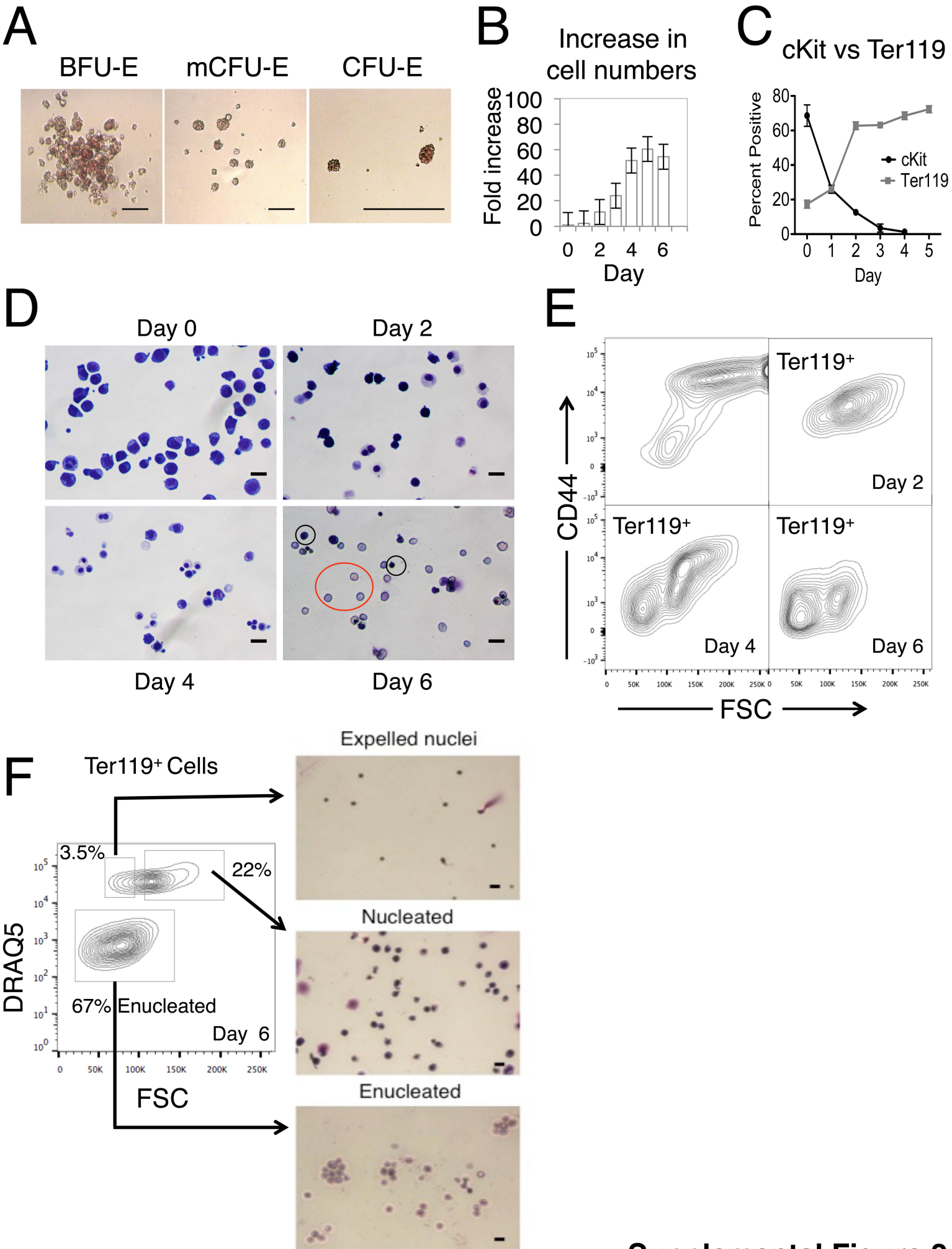
Target gene	Catalogue#	Sequence	Backbone
Luciferase	SHC007	CCGGCGCTGAGTACTTCGAAATGTCCTCGAGGAC ATTTCGAAGTACTCAGCGTTTTT	PLKO.1
Vdr (shRNA1)	TRCN0000027068	CCGGCCTGAGATCAATCACATTTAACTCGAGTTAA ATGTGATTGATCTCAGGTTTTT	PLKO.1
Vdr (shRNA2)	TRCN0000328258	CCGGCCTGGCTGATCTTGTCAGTTACTCGAGTAA CTGACAAGATCAGCCAGGTTTTTG	PLKO.1

Lentivirus shRNAs were purchased from Sigma-Aldrich

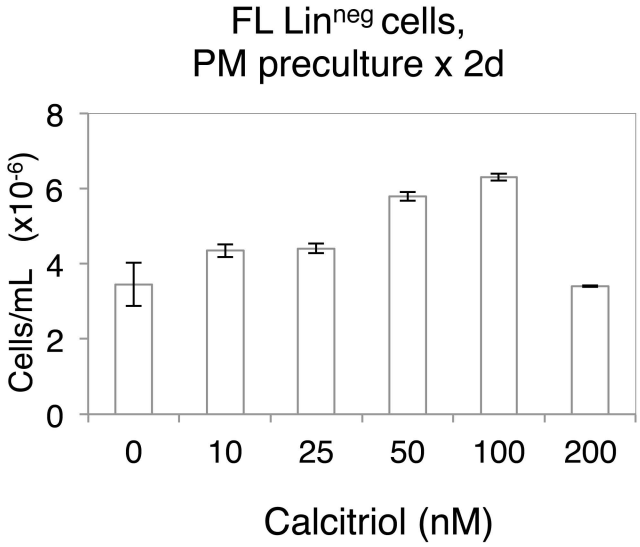
Supplemental Table 3



Supplemental Figure 1



Supplemental Figure 2



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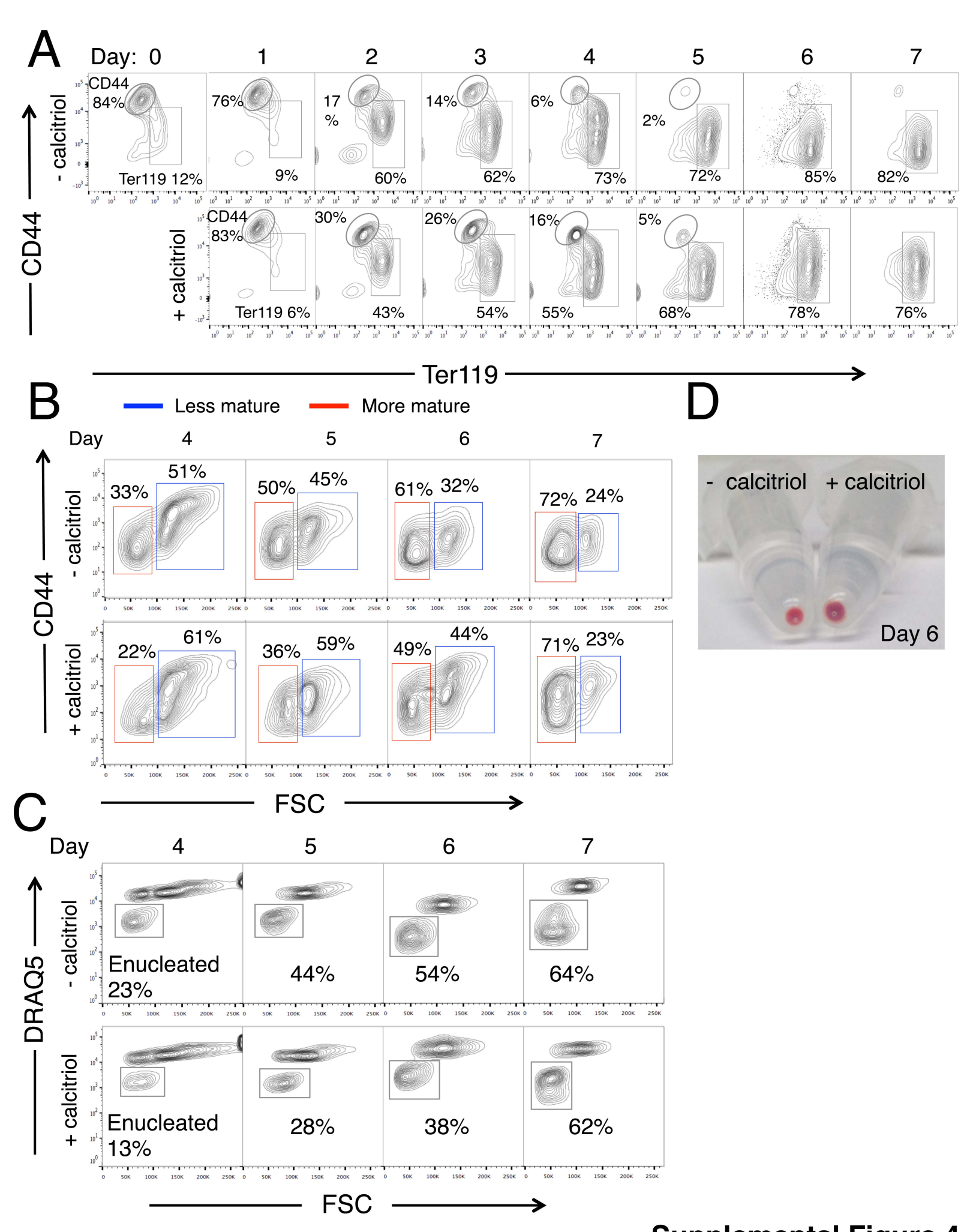

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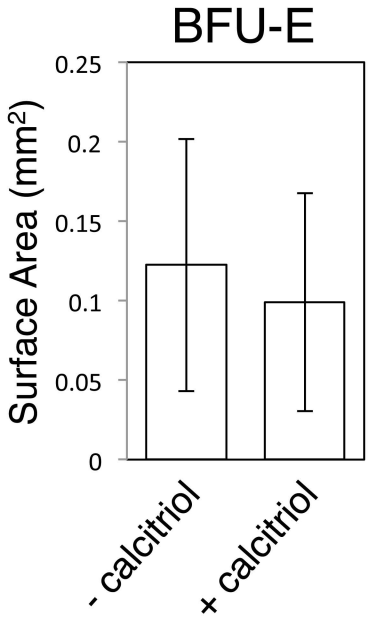
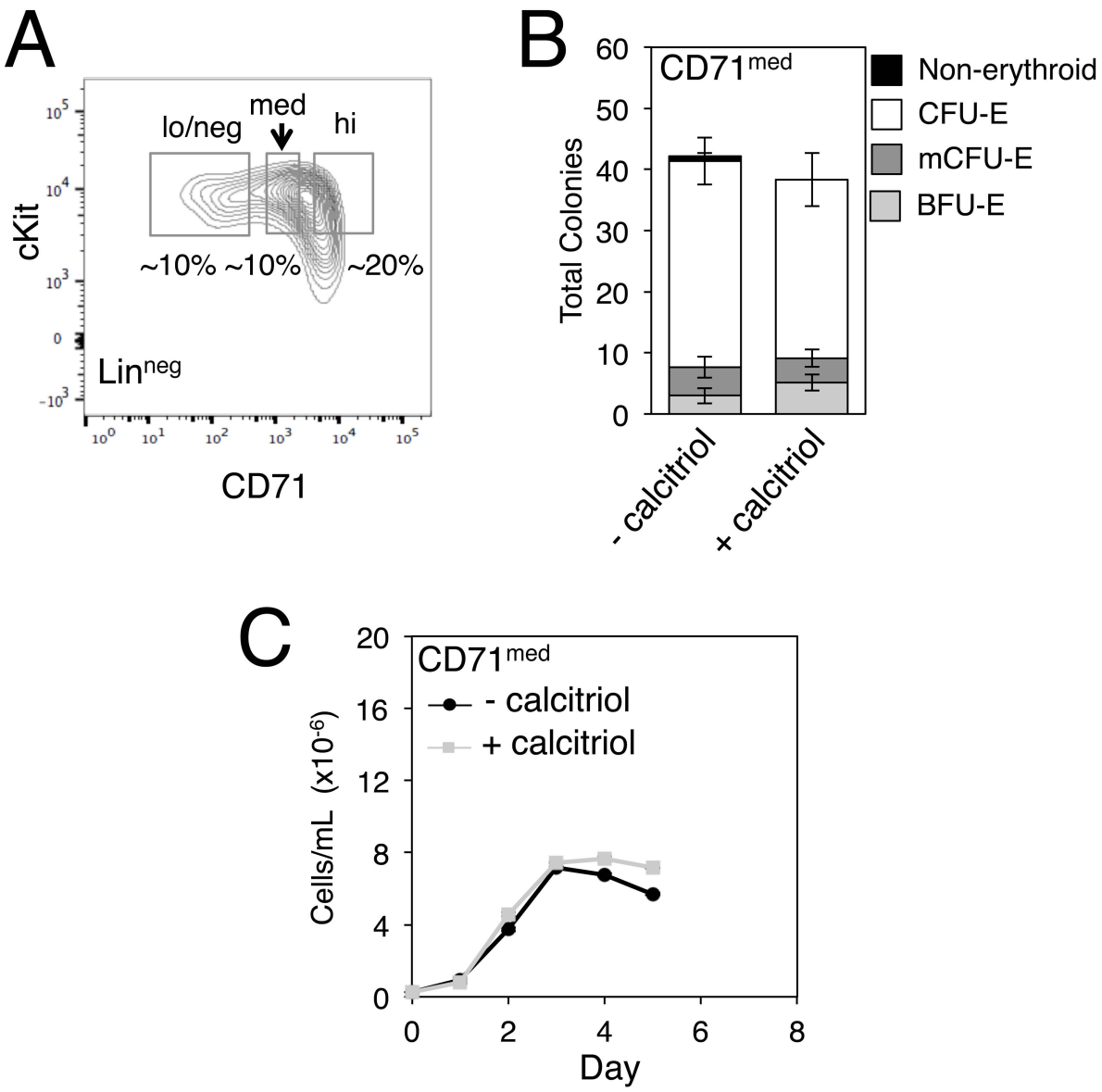
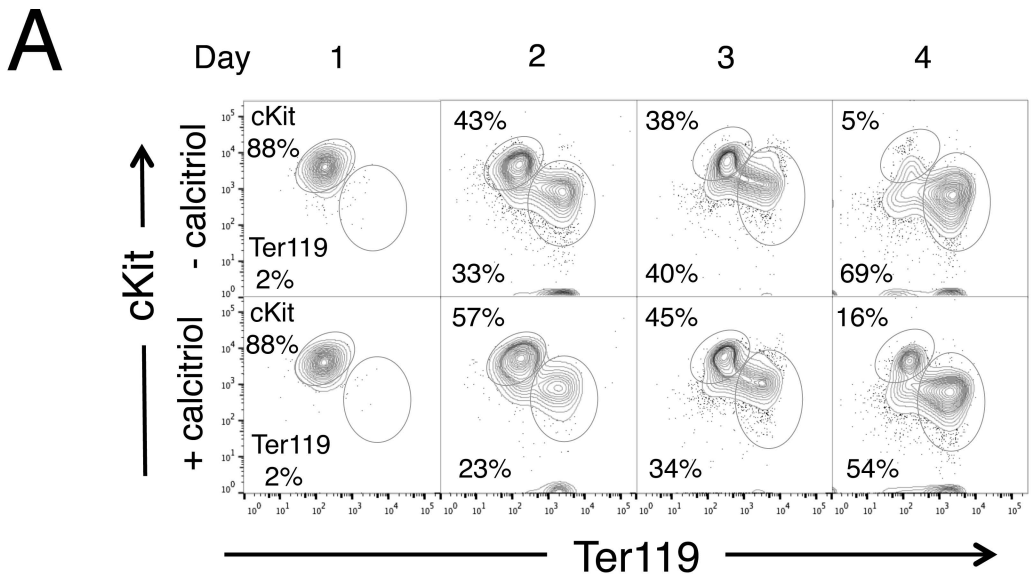
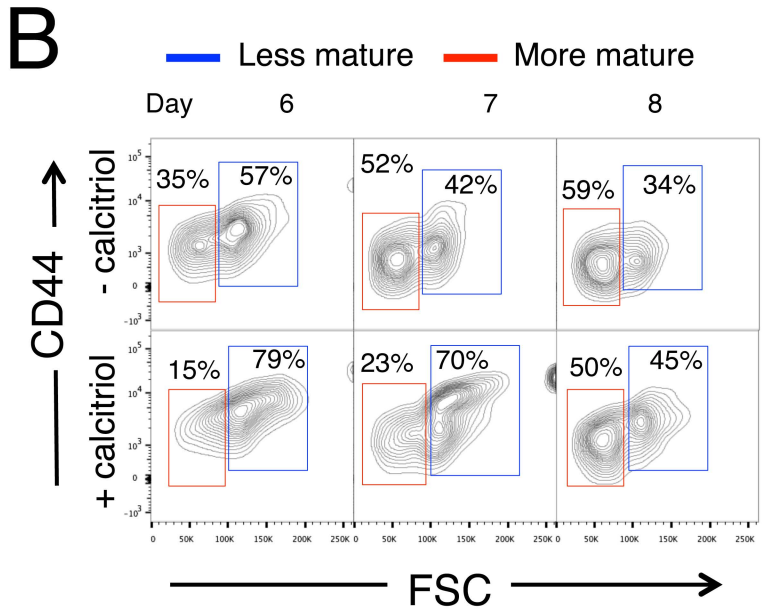


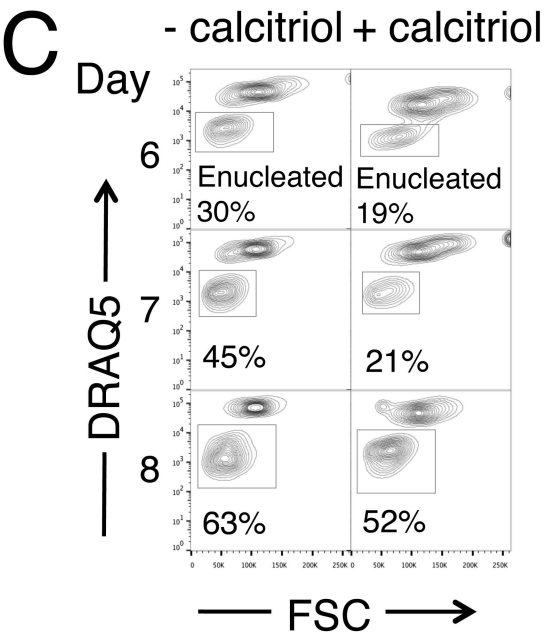
Supplemental Figure 5

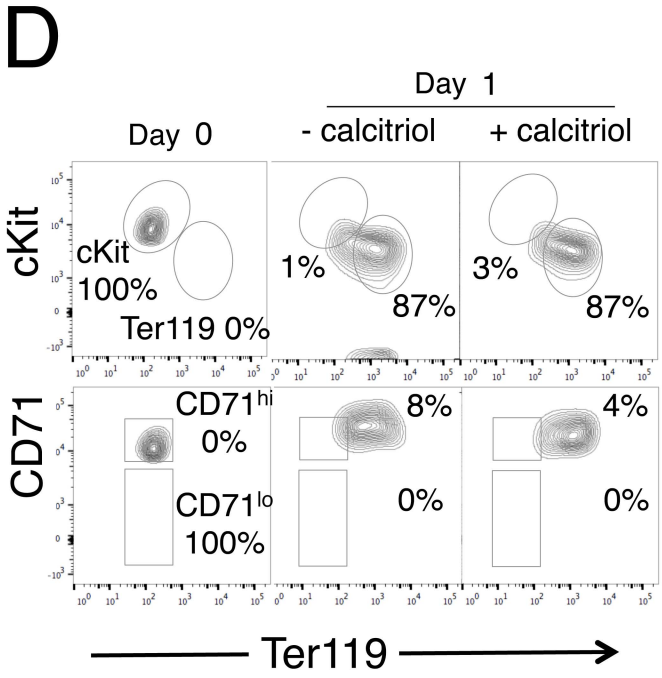


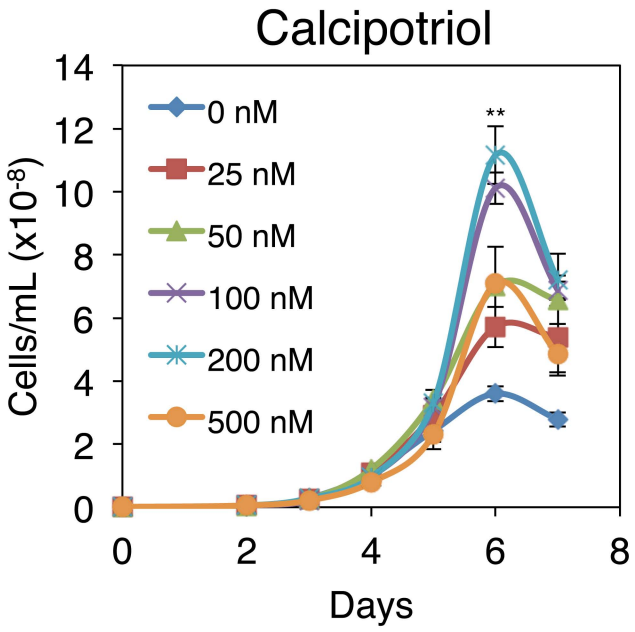
Supplemental Figure 6





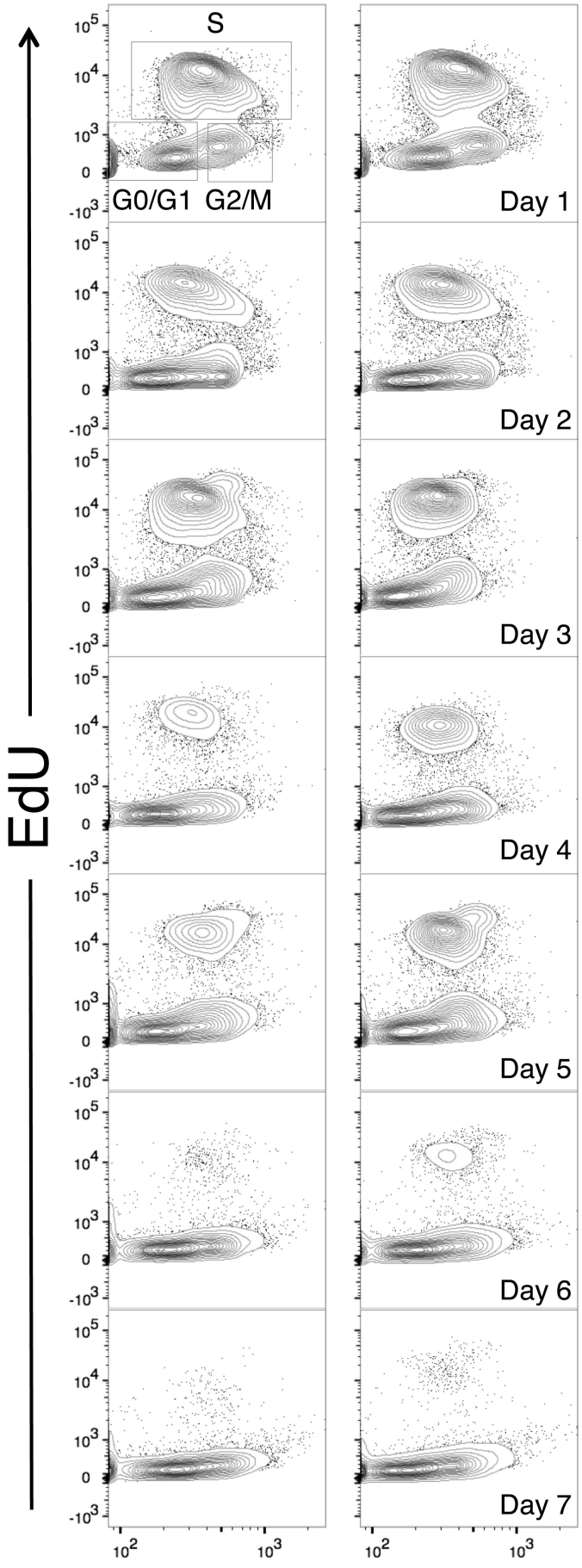






Supplemental Figure 7

A



B

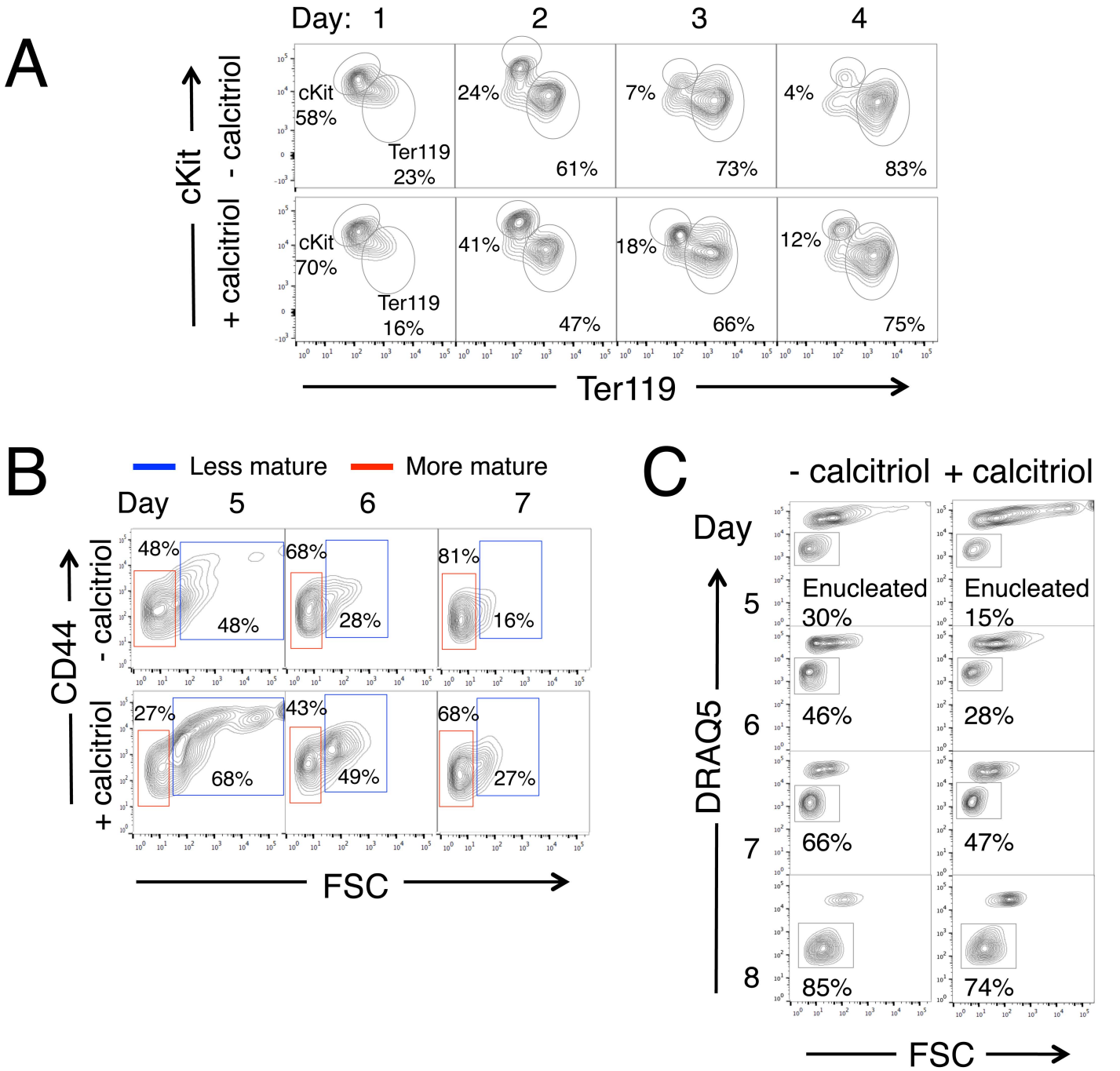
- calcitriol

Day	S	G2/M	G0/G1
1	55	12	18
2	29	8	50
3	39	9	38
4	26	11	51
5	18	12	54
6	4	13	70
7	2	13	66

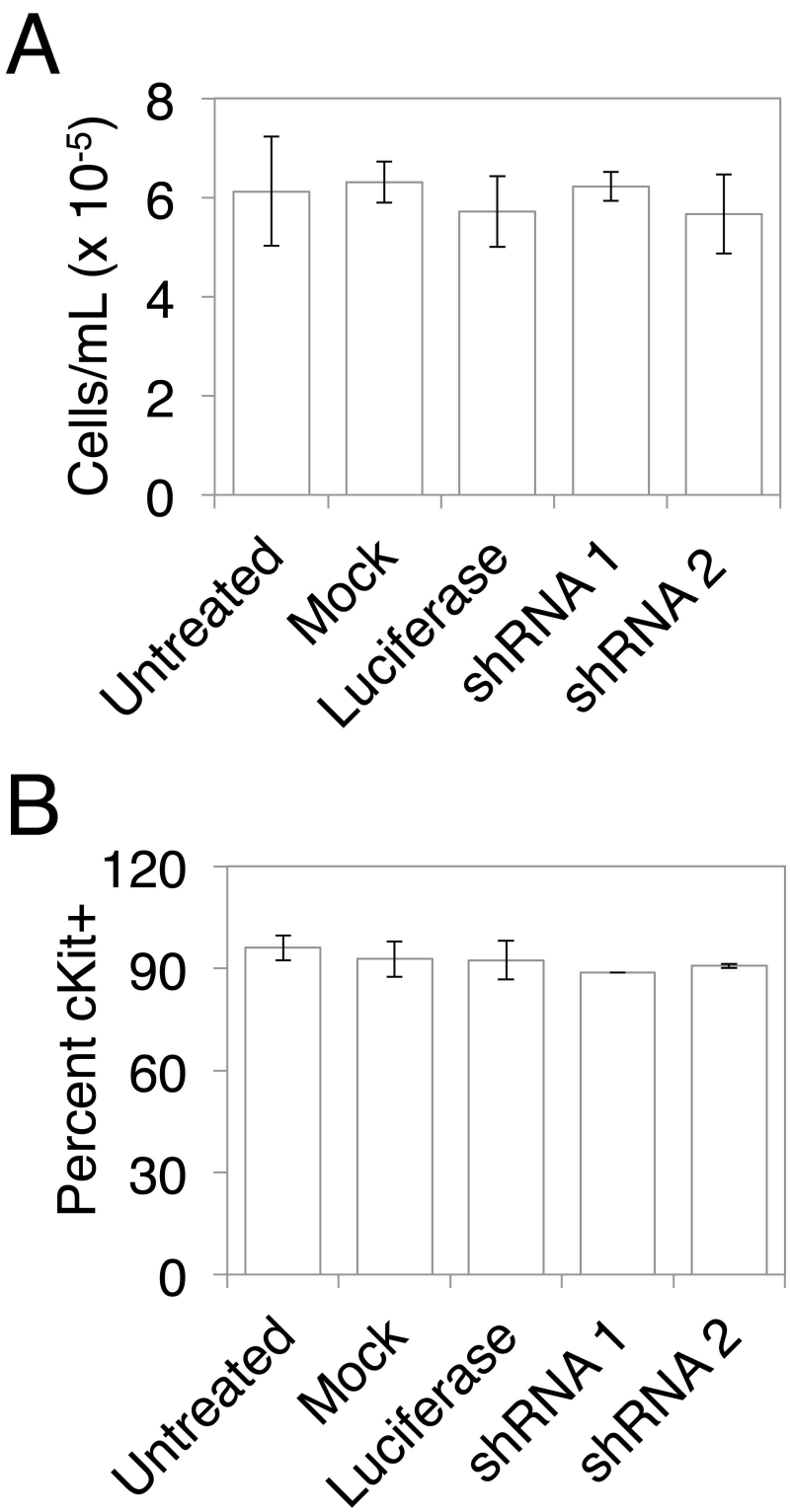
+ calcitriol

Day	S	G2/M	G0/G1
1	54	14	16
2	34	12	40
3	46	8	33
4	31	11	44
5	31	13	35
6	7	12	70
7	3	10	74

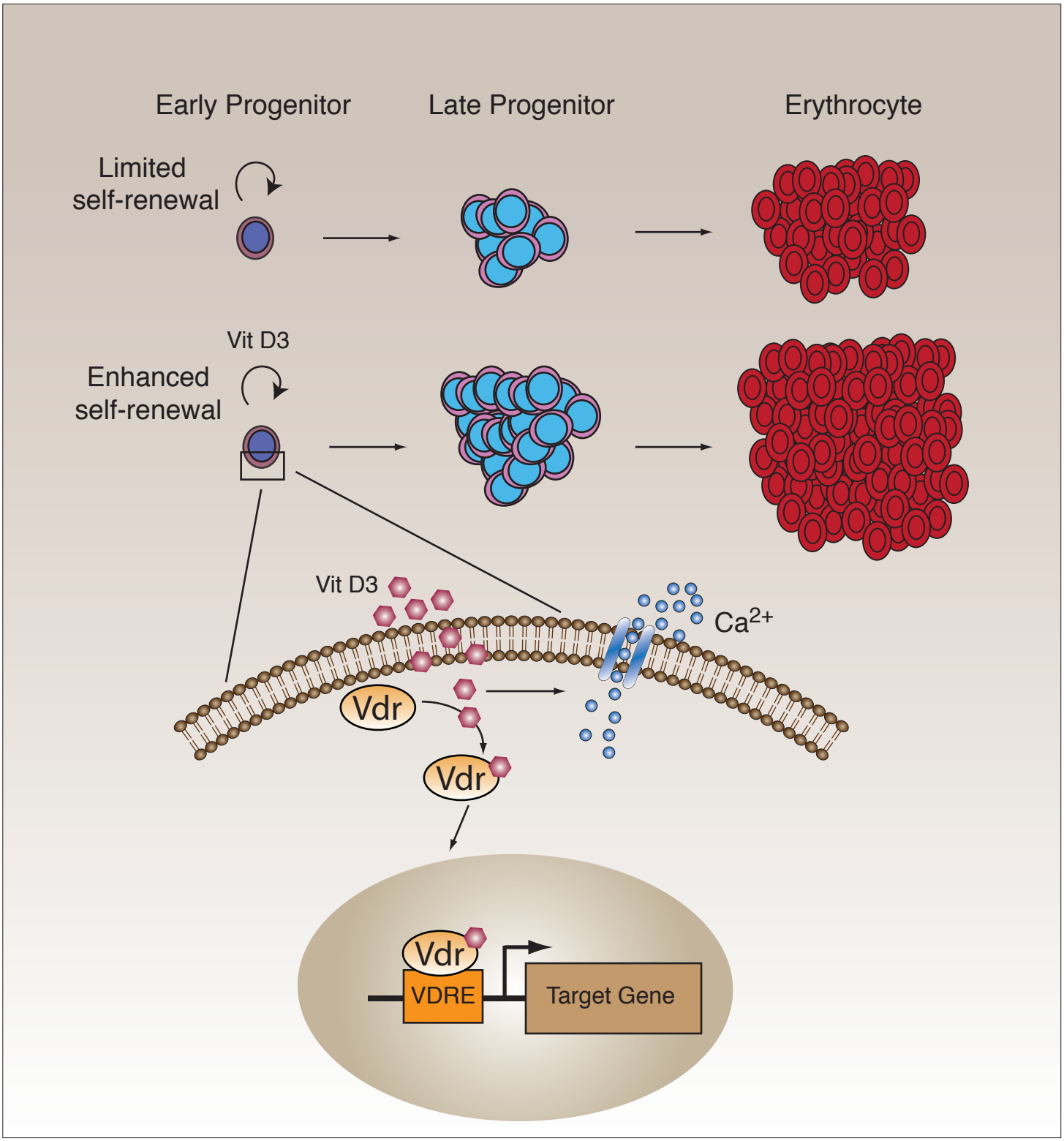
Supplemental Figure 8



Supplemental Figure 9



Supplemental Figure 10



Supplemental Figure 11