Supplemental Figures and Tables

Supplemental Figure 1. Pre-treatment with anti-CD117 antibodies 2D12, 4F7, and 5F11 do not inhibit binding of fluorescently-labeled SCF to human CD117. A mixture of CD117-expressing and untransfected fibroblasts were stained with SCF-A488 alone (left), or first stained with the anti-CD117 mAb indicated, then stained A647-labeled goat anti-mouse secondary antibody, and subsequently stained with SCF-A488 (right).

Supplemental Figure 2. SR-1 mAb but not 4F7 mAb inhibits normal UCB-derived human HSC proliferation *in vitro.*

A. SR-1 but not 4F7 inhibits proliferation of FACS-purified human UCB-derived HSCs ($Lin^-CD34^+CD38^-CD90^+CD45RA^-$) plated 10 cells/well in StemSpan media supplemented with SCF, TPO, FLT3L, IL-3, and IL-6, and treated with varying concentrations of SR-1 and 4F7, with cell counts performed daily on days 1-6. * $p<0.05$ SR-1 was compared to each 4F7 control group and no antibody control group (Student's t-test). Error bars indicate one standard error of the mean.
B. Representative images of the colonies formed by cultured UCB HSCs at the indicated time points are shown (for untreated and 1 μg/ml SR-1).
C. SR-1 inhibits proliferation of FACS-purified human UCB-derived hematopoietic progenitors (Lin^-CD34^+) plated 10 cells/well in StemSpan media supplemented with SCF, TPO, FLT3L, IL-3, and IL-6, and treated with indicated concentrations of SR-1 and 4F7, with cell counts performed daily on days 1-5. * $p<0.05$ SR-1 was compared

to no antibody control (Student's t-test). Error bars indicate one standard error of the mean.

Supplemental Figure 3. SR-1 does not cause cell death of normal UCB-derived human HSCs *in vitro*

A. Incubation with SR-1 does not increase annexin V+ or propidium iodide (PI) staining on human UCB HSCs after 3 days or 7 days of *in vitro* liquid culture, but incubation with staurosporin, a known apoptosis inducer, causes increased annexin V+ staining on cultured human HSCs.
B. Addition of Z-VAD-FMK, a caspase inhibitor, to *in vitro* liquid cultures of human UCB HSCs with SR-1 does not abrogate the inhibition of proliferation by SR-1. * $p<0.05$ compared to control caspase inhibitor (Student's t-test). Error bars indicate one standard error of the mean.

Supplemental Figure 4. Only human B cells and T cells remain among the human cells detected in humanized mice after treatment with SR-1.

A. Baseline level of total human chimerism (human $CD45^+$) as well as distribution of myeloid (human $CD45^+CD13/33^+$), B cell (human $CD45^+CD19^+$), and T cell (human $CD45^+CD3^+$) chimerism in BM of human UCB HSC xenografted mice measured ~12 weeks after transplant. (n=3)
B. SR-1 depletes normal human UCB-derived HSCs *in vivo*, as shown by chimerism of human myeloid ($hCD13/CD33^+$) cells on pre-treatment d0 and on d8 and 8 weeks

after completion of SR-1 treatment administered IV on day 1, day 3, day 5, and day 7. * $p<0.001$ compared to IgG control at same time point (Student's t-test). (n=3)

C. Distribution of myeloid (human $CD45^+CD13/33^+$), B cell (human $CD45^+CD19^+$), and T cell (human $CD45^+CD3^+$) chimerism in BM among persisting human cells in human UCB HSC xenografted mice treated with SR-1, measured 8 weeks after completion of SR-1 treatment. (n=3)

Supplemental Figure 5. Engraftment of low risk MDS HSCs and high risk MDS HSCs transplanted into NSG newborn mice

A. >12 weeks human $CD45^+$ chimerism per 500 MDS HSCs transplanted. (n=6 low risk MDS, n=4 high risk MDS)
B. Cytogenetically abnormal MDS cells with clonal cytogenetic markers (-Y, del(5q), del(20q), -7, +8) were found at high frequency among human $CD45^+$ cells isolated from human xenografts, which correlates with the high frequency of cytogenetically abnormal HSCs found in the MDS patients from which the xenografts were derived. (n=6 low risk MDS, n=4 high risk MDS)
C. Baseline level of total human chimerism (human $CD45^+$) as well as distribution of myeloid (human $CD45^+CD13/33^+$), B cell (human $CD45^+CD19^+$), and T cell (human $CD45^+CD3^+$) chimerism in BM of MDS HSC xenografted mice measured ~12 weeks after transplant. (n=4)
D. Blasts, as assessed by percentage of $CD34^+$ cells among human $CD45^+$ cells, were not increased in low risk MDS HSC engrafted mice. (n=4)

Supplemental Figure 6. SR-1 depletes human blood cells derived from low risk MDS HSCs *in vivo*

A. SR-1 depletes low risk MDS HSCs *in vivo* up to 8 weeks, as shown by chimerism of human myeloid (hCD13/CD33$^+$) cells on pre-treatment d0 and on d8 and 8 weeks after completion of SR-1 treatment administered IV on day 1, day 3, day 5, and day 7. * $p<0.001$ compared to IgG control at same time point (Student's t-test). (n=4)
B. SR-1 depletes low risk MDS HSCs *in vivo* up to 8 months, as shown by chimerism of human myeloid (hCD13/CD33$^+$) cells on pre-treatment d0 and on d8 and 8 months after completion of treatment with 500 μg SR-1 administered IV on day 1, 3, 5, and 7. * $p<0.001$ compared to d0 (Student's t-test). (n=3)
C. MDS cells remained reduced in low risk MDS xenografted mice treated with SR-1 and ACK2, without transplantation with an allogeneic normal UCB HSC graft, 8 months after completion of antibody treatment. (n=2)

Supplemental Figure 7. Recovery of high risk MDS clones in xenografts 8 weeks after completion of treatment with SR-1

A. IgG control antibody treatment does not deplete human blood cells derived from high risk MDS HSCs *in vivo*, as shown by chimerism of human myeloid (hCD13/CD33$^+$) cells on pre-treatment d0 and on d8 and 8 months after completion of treatment with 500 μg IgG control antibody administered IV on day 1, 3, 5, and 7. (n=2)

B. Cytogenetically abnormal MDS cells with clonal cytogenetic markers (del(5q), -7, +8) were found at high frequency among human $CD45^+$ cells isolated from high risk MDS xenografts, 8 weeks after completion of treatment with SR-1. (n=4)
C. Blasts, as assessed by percentage of $CD34^+$ cells among human $CD45^+$ cells, were depleted by d8, and recovered by 8 weeks following exposure to SR-1. (n=4)

Supplemental Figure 8. MDS clones remained reduced in high risk MDS xenografted mice treated with SR-1 and then transplanted with an allogeneic normal UCB HSC graft, even after 18 weeks post-second transplantation. (n=4)

Supplemental Figure 9. MDS clones persist in high risk MDS xenografted mice treated with IgG control antibody and then transplanted with an allogeneic normal UCB HSC graft. Cells were analyzed 12 weeks after second normal human UCB HSC transplant. (n=2)

Supplemental Table 1. MDS patient characteristics, including IPSS-R score and cytogenetics for MDS BM samples used in the present study.

Supplemental Table 2. Percent of mutant clones detected among human $CD45^+$ cells in human xenografts. Frequency of cytogenetically abnormal clones (-Y, del(20q), or +8), as detected by FISH within human cells FACS-isolated from BM of low risk MDS xenografted mice treated with IgG control antibody or SR-1 mAb on days 1, 3, 5, and 7 and then transplanted on day 14 with a second normal human UCB HSC graft. Cells were analyzed 12 weeks after second normal human UCB HSC transplant.

Supplemental Table 3. Percent of mutant clones detected among human $CD45^+$ cells in human xenografts. Frequency of cytogenetically abnormal clone (-7, del(5q), or +8), as detected by FISH within human cells FACS-isolated from BM of high risk MDS xenografted mice treated with SR-1 mAb on days 1, 3, 5, and 7 and then transplanted on day 14 with a second normal human UCB HSC graft. Cells were analyzed 12 weeks after second normal human UCB HSC transplant.

Supplemental Methods

Human BM samples

Human BM samples from MDS patients were collected from the Stanford Department of Pathology or Stanford Hematology Tissue Bank, according to IRB-approved protocols, with informed consent, where applicable. Stanford Department of Pathology samples were selected from submitted cases solely based on the ability of investigators to obtain and either use or freeze the samples within 72 h after collection. MDS patient samples represented both primary diagnostic samples and those with previously known diagnoses. All high risk and very high risk MDS samples also belonged to World Health Organization (WHO) refractory anemia with excess blasts (RAEB)-1 or RAEB-2 categories. Mononuclear cells were cryopreserved in 90% FBS/10% (vol/vol) DMSO. Cryopreserved samples were thawed and washed with IMDM containing 10% (vol/vol) FBS and DNase I.

Generation of human CD117-expressing fibroblasts

Tail tip fibroblasts were generated from newborn BALB/c mice by mincing tail tip tissue from 1-3 day old pups and placing into RPMI media with 10% FBS. Cells were passaged for several rounds until only immortalized fibroblasts remained in culture. These BALB/c fibroblasts were then infected with a lentivirus containing human CD117 cDNA. This was generated using the pCDH cDNA Cloning and Expression Lentivector System (SBI System Biosciences; Palo Alto, CA), Human CD117 cDNA was generated by the Stanford Protein and Nucleic Acid Core based upon the following sequence:

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actgcagccccaaccgacagaagcccgtggtagaccattctgtgcggatcaattctgtcggcagcaccgcttcctcctcccagcctct

gcttgtgcacgacgatgtctga

Cloning was done using the following primers:

hCD117.1Fk.nhe: ggcggctagcgccaccATGAGAGGCGCTCGCGGCGCCTG

hCD117.1R.sal: ggcggtcgacTCAGACATCGTCGTGCACAAGCAGAGGC

Once human CD117$^+$ expressing tail-tip fibroblast cells were generated, they were enriched by fluorescence activated cell sorting (FACS) based upon binding of commercial anti-hCD117 mAb Clone 104D2 (BioLegend; San Diego, CA).

Generation of anti-human-CD117 mAbs, including 4F7

To generate an anti-human CD117 mAb that paralleled the antagonistic anti-mouse CD117 ACK2 mAb and potentially could deplete normal human HSCs, we immunized BALB/c mice with HMC-1 cells, a human mast-cell tumor line expressing high levels of human CD117. In brief, BALB/c mice were immunized through 6 serial rounds of 1x10^6 HMC-1 cells via footpad injection. Popliteal lymph nodes were harvested and fused to SP2/0 mouse multiple myeloma cells using PEG 1500. Clones were propagated in HAT-media and then switched to HT-media. Hybridoma cells were then sub-cloned to segregate individual monoclonal lines. Specificity for human CD117 was established by screening hybridoma sera on human CD117$^+$ expressing BALB/c fibroblasts and normal non-human CD117$^-$ expressing BALB/c fibroblasts, using anti-mouse A647 for detection of binding. The hybridoma cell lines were expanded and sub-cloned to establish high producing hybridoma cell lines. We generated 10 hybridoma cell lines producing specific mouse anti-human CD117 mAbs, including clone 4F7. However, none of these antibodies were antagonistic as none blocked binding of fluorescently-labeled human SCF (Supplemental Figure 1). In parallel efforts, we identified a mouse anti-human CD117 mAb, SR-1, that was reported to specifically recognize human CD117 and block binding of ^{125}I-human SCF.[23]

FISH Analysis

Nuclei from MDS patient BM cells with monosomy 7 contain one orange and one green signal. Nuclei from MDS patient BM cells with trisomy 8 contain three green signals. Nuclei from MDS patient BM cells with del(5q) contain two orange signals and one green signal. Nuclei from MDS patient BM cells with –Y contain one orange signal and no green signal. Nuclei from MDS patient BM cells with del(20q) contain one orange signal and two green signals.

SR-1 Purification

Hybridomas were grown in the Integra flask system (Integra Biosciences; Chur, Switzerland) and media containing antibody was collected. SR-1 was purified on an IgG purification column by binding the SR-1 to the column and eluting with 100mM Glycine and 5mM NaN3. The eluted positive fractions (OD280 >0.2) were combined, dialyzed for 12 hours in PBS, and concentrated using a Vivaspin concentrator (Sartorius AG, Goettingen, Germany).

Assessment of apoptosis of in vitro cultured human HSCs and hematopoietic progenitor cells

To determine the role of apoptosis, cells were also incubated in presence of 100μM Z-VAD-FMK General Caspase Inhibitor (Sequence: Z-V-A-D(OMe)-FMK, R&D Systems, Minneapolis, MN) as per manufacturer's instructions. Additionally, apoptosis was assessed using a FITC Annexin V Apoptosis Detection Kit II (BD Biosciences; San Jose, CA). Cells were washed twice with cold PBS, and then resuspended in 100μl of 1x Binding buffer. 5μl of FITC

Annexin was added and 5µl of 50µg/ml PI. Cells were gently vortexed and incubated for 15 min at 25°C in the dark. Immediately after 200µl of 1x Annexin Binding Buffer was added to each tube and cells were analyzed by flow cytometry.

AMG 191

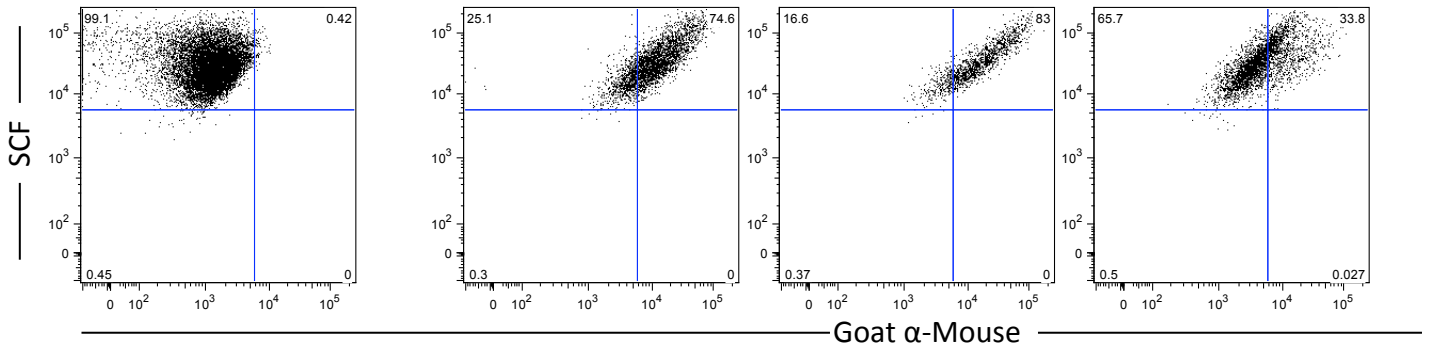
AMG 191 is a humanized anti-human CD117 IgG1 monoclonal antibody based on complementarity determining region (CDR) segments from SR-1.[31]

Anti-CD117 antibody and cellular dosing

Dosage of SR-1 in the experiments was selected to attempt to maximize effect seen. Full dose titration was not possible due to scarcity of patient samples. AMG 191 is a humanized anti-human mAb and dosage was selected based on available pharmacokinetic data on studies performed in mice and non-human primates.[30] SR-1 is an IgG2a mouse antibody, with an estimated half-life of 6-8 days. *In vivo* pharmacokinetic data, including half-life, were not obtained. The cell numbers chosen for the transplantation studies were determined based on the availability of UCB and MDS HSCs at the time of the experiments, with the intention of maximizing human chimerism using the available human samples and xenografted mice at any given time. Human chimerism levels correlate directly with numbers of donor HSCs transplanted. Not all mice transplanted with human cells showed engraftment and only mice with human engraftment were selected for antibody treatment and further experimentation. Secondary transplantations following SR1 treatment were performed one week after infusion to minimize depletion of donor cells by residual antibody, while maximizing depletion of target cells. One week was selected based on the

estimated half-life of SR-1 and our previous published experience with anti-mouse CD117 antibody studies.[17,18] Selection of xenografted mice/MDS cases for a given experiment were made based on the availability of xenografted mice.

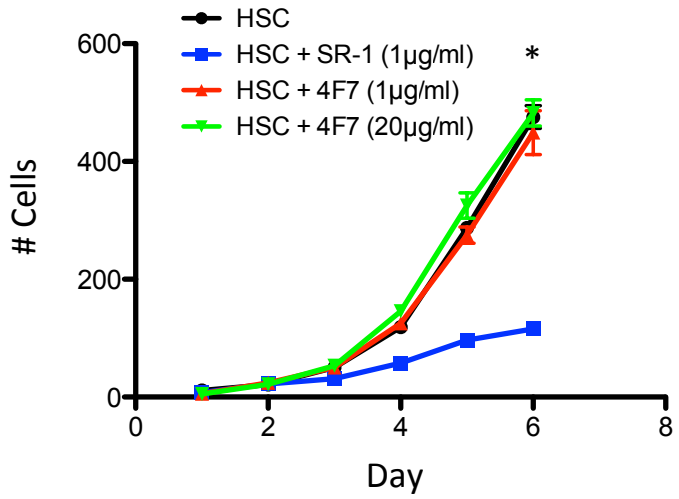
Supplemental Figure 1



SCF Alone 2D12+SCF 4F7+SCF 5F11+SCF

Supplemental Figure 2

A



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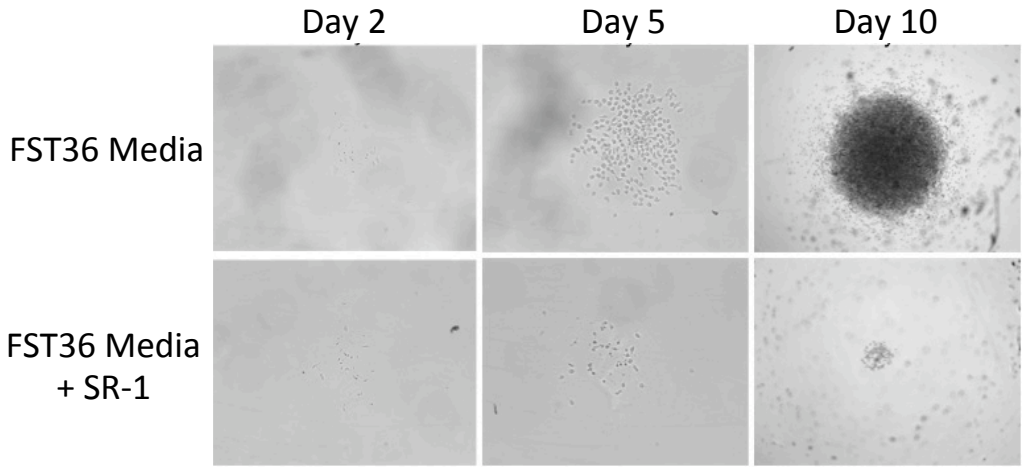

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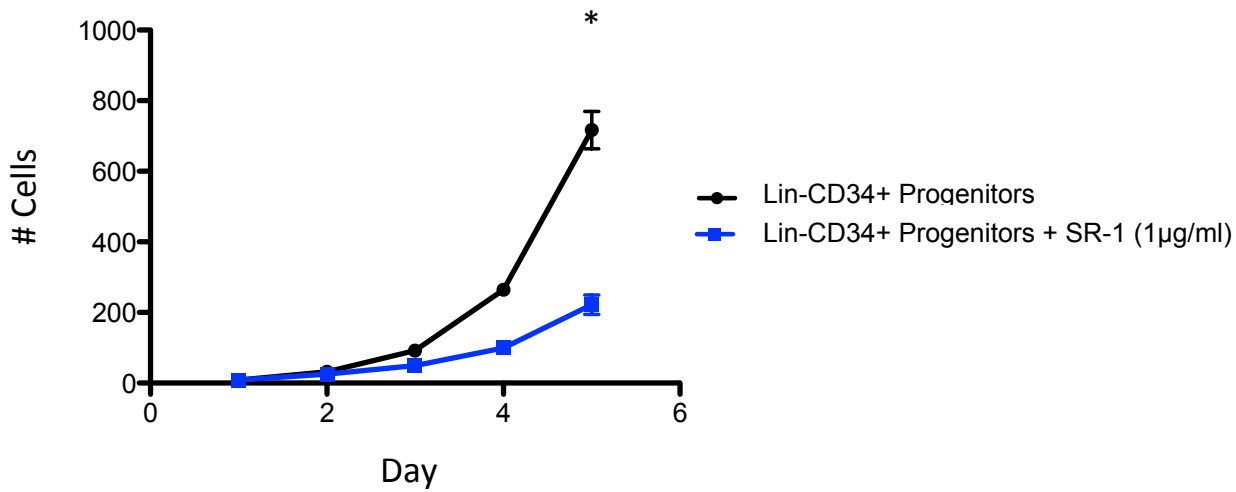

Supplemental Figure 3

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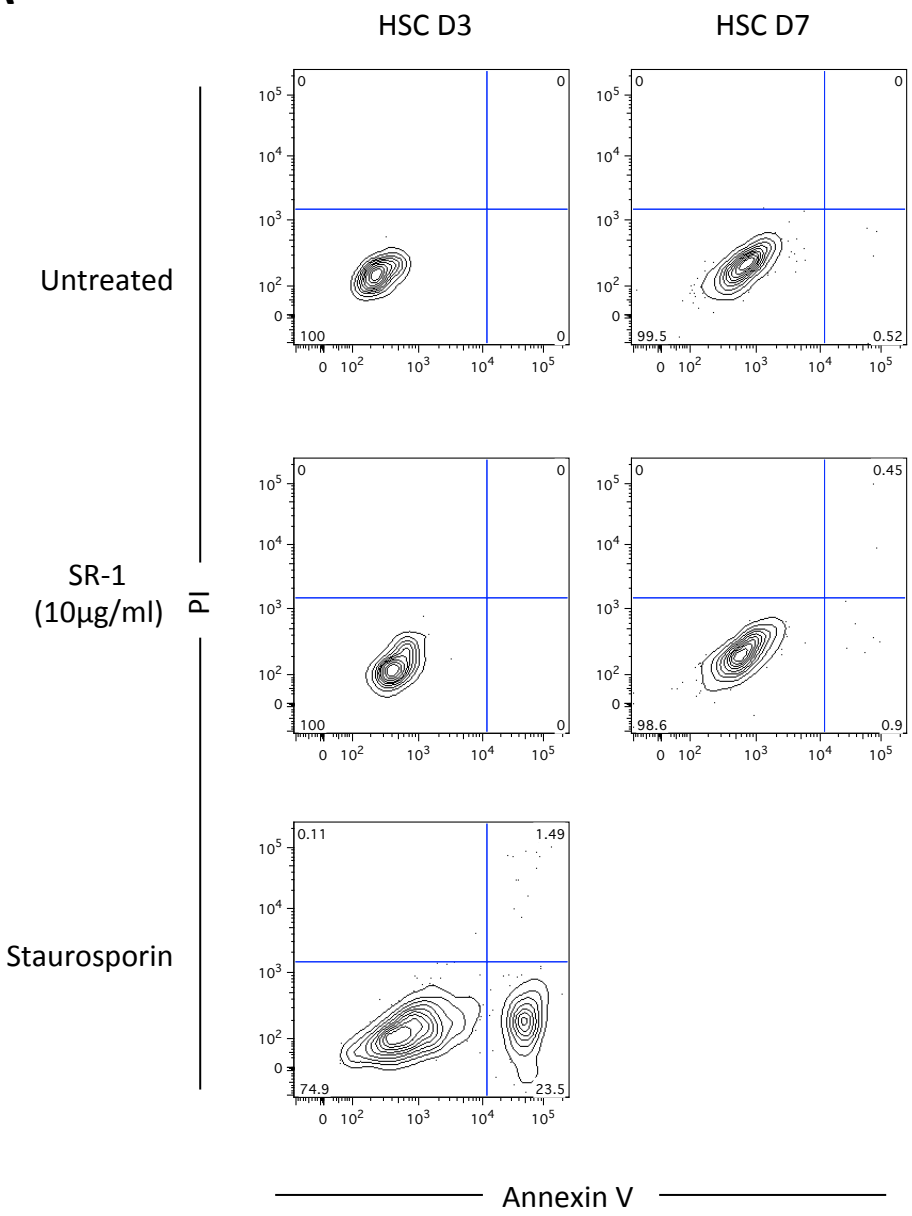

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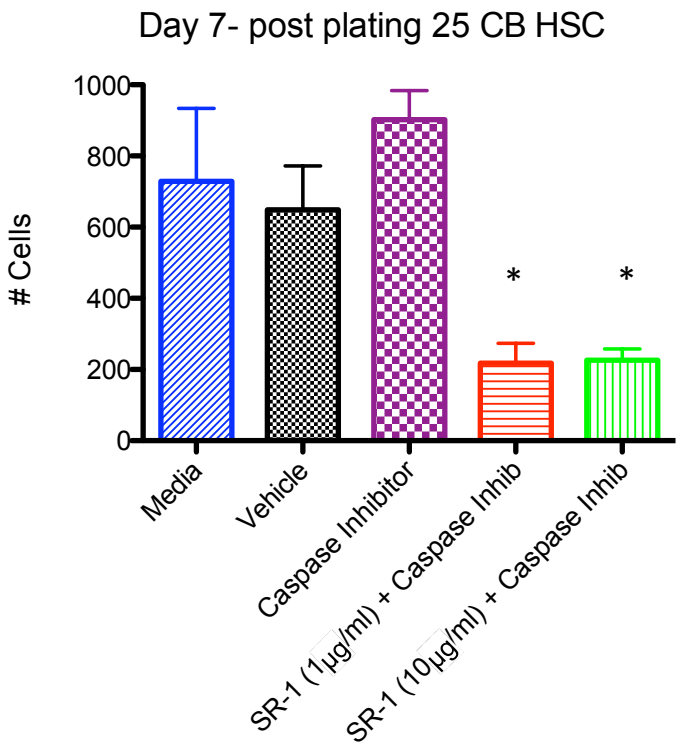

Supplemental Figure 4

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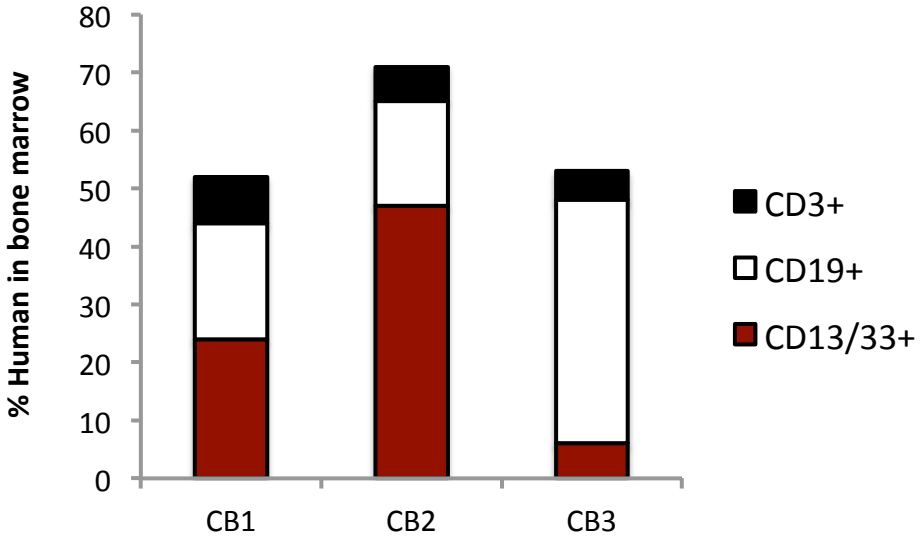

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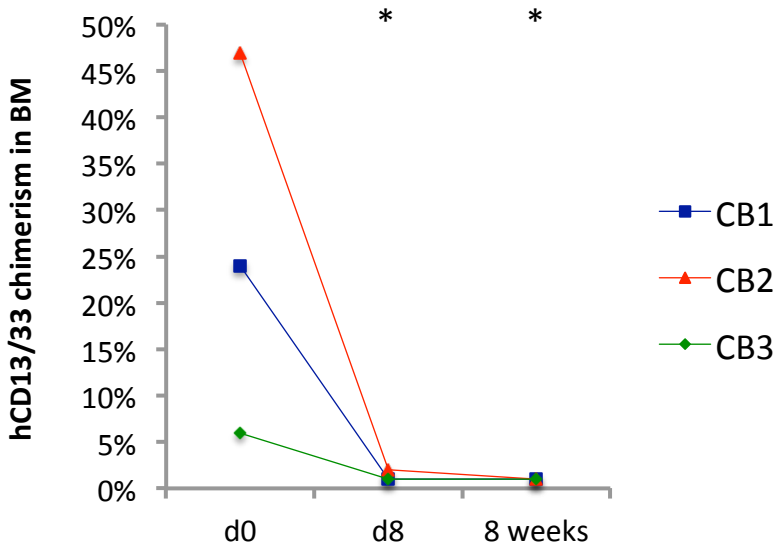

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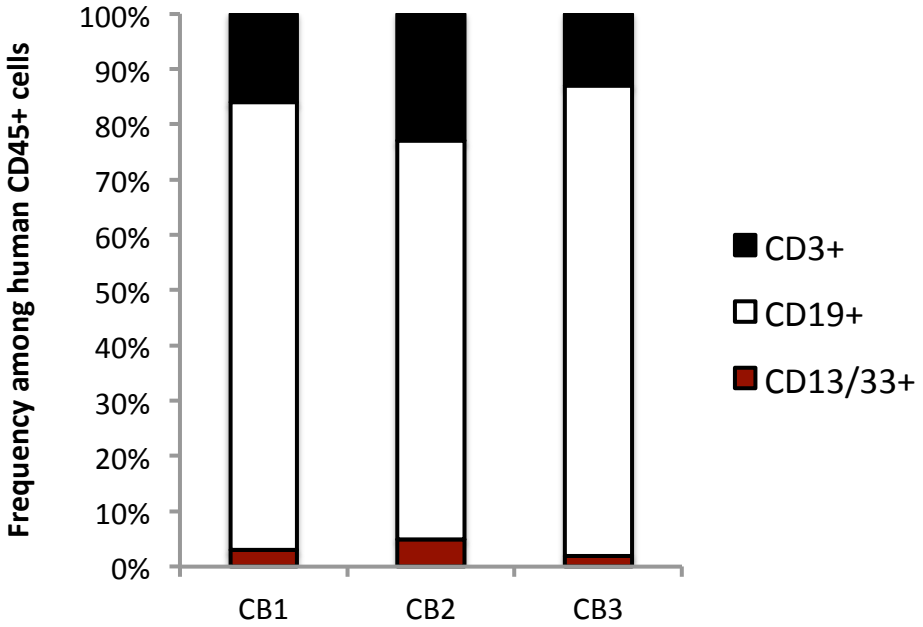
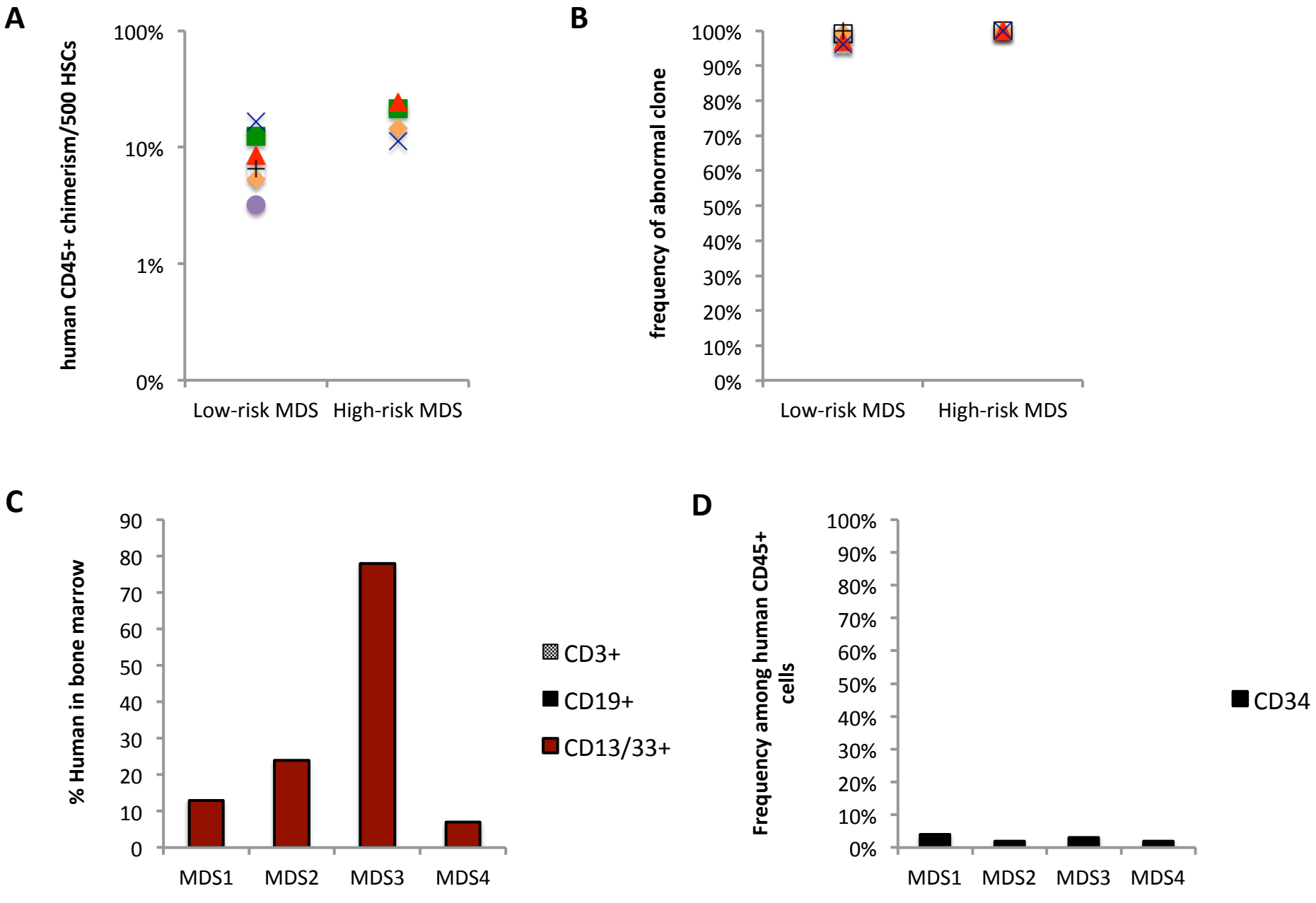


Supplemental Figure 5



Supplemental Figure 6

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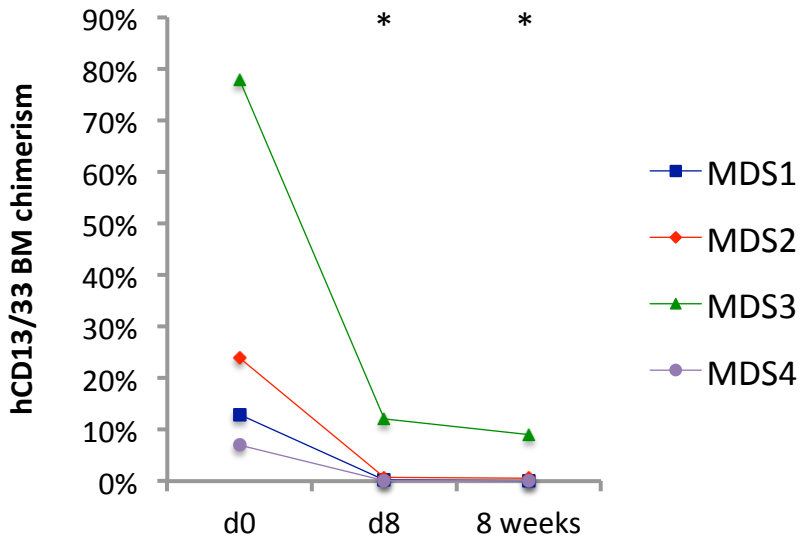

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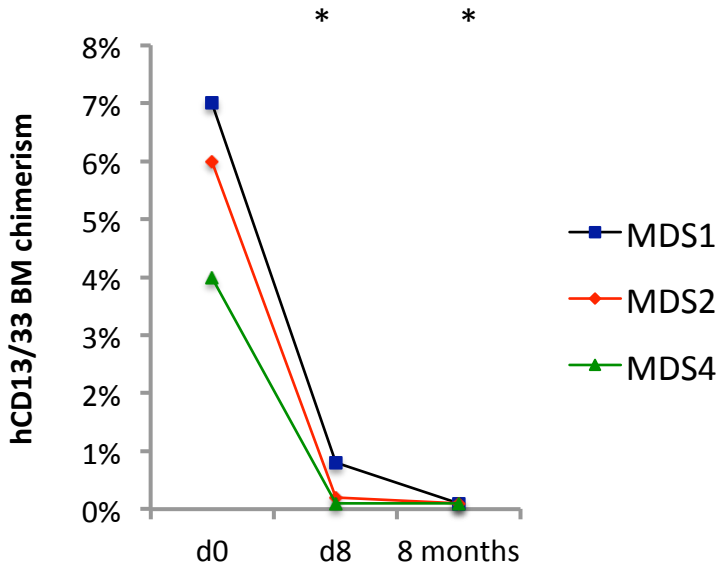

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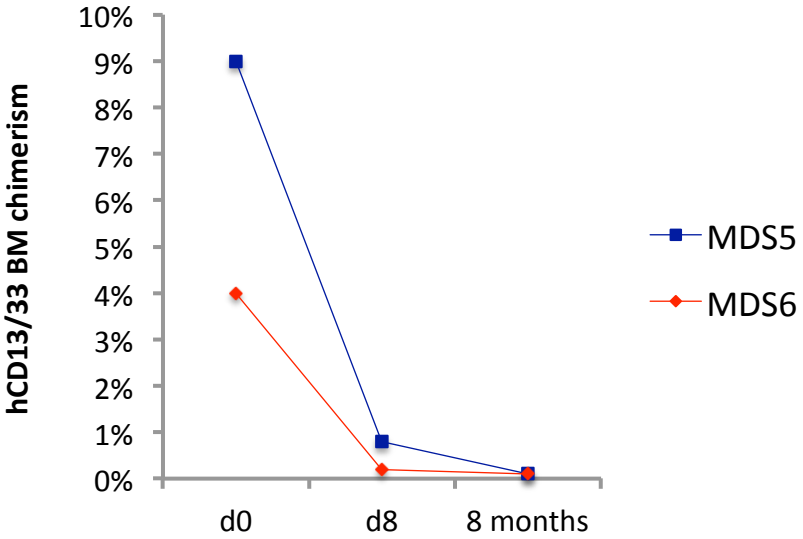
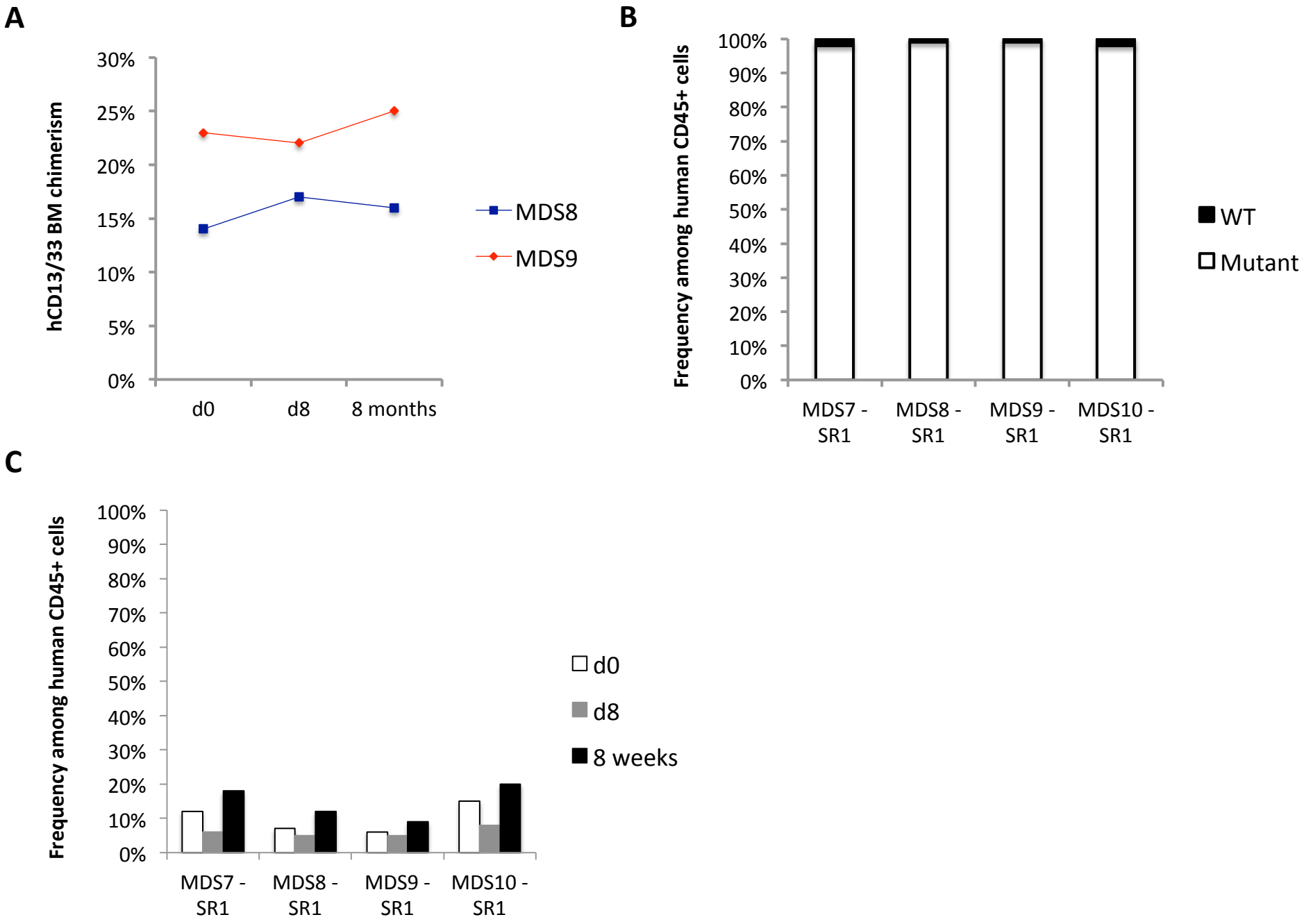
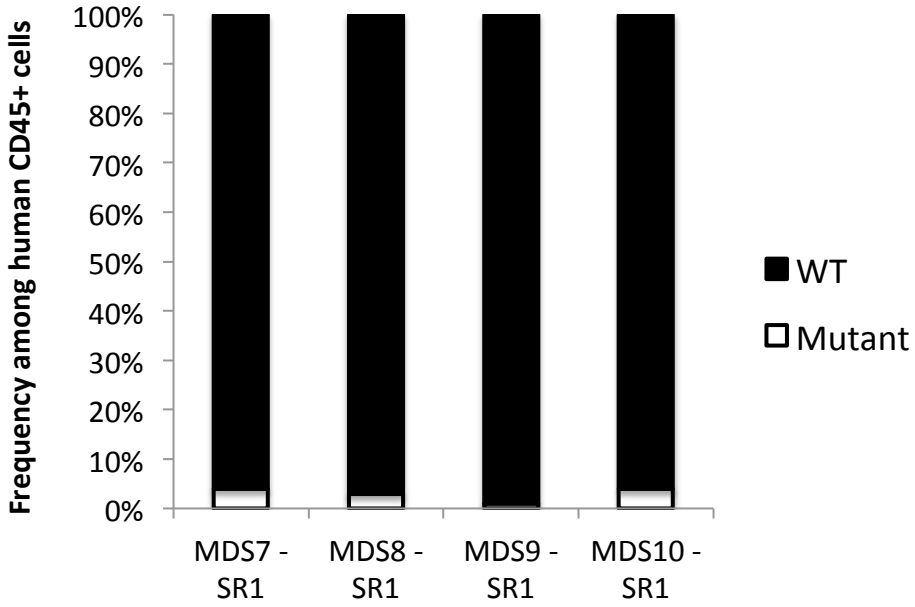


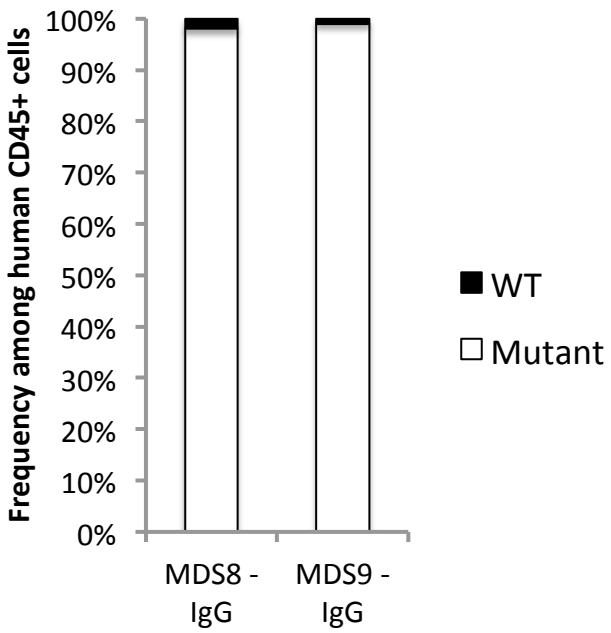
Supplemental Figure 7



Supplemental Figure 8



Supplemental Figure 9



Supplemental Table 1

	IPSS-R	Cytogenetics
MDS1	Low	del(20q)
MDS2	Very Low	+8
MDS3	Very Low	-Y
MDS4	Low	del(20q)
MDS5	Low	+8
MDS6	Low	+8
MDS7	High	+8
MDS8	High	del(5q)
MDS9	Very High	del(5q)
MDS10	Very High	-7

Supplemental Table 2

	% Mutant Clone
MDS1 - IgG	98
MDS1 - SR1	1
MDS2 - IgG	92
MDS2 - SR1	2
MDS5 - IgG	98
MDS5 - SR1	0
MDS6 - IgG	96
MDS6 - SR1	0

Supplemental Table 3

	% Mutant Clone
MDS7 - SR1	6
MDS8 - SR1	4
MDS9 - SR1	1
MDS10 - SR1	3