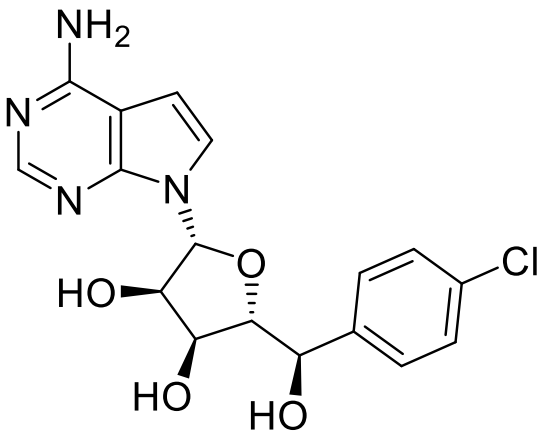
Supplemental Data for PRMT5 regulates T cell interferon response and is a target for acute graft-versus-host disease by Snyder et. al.

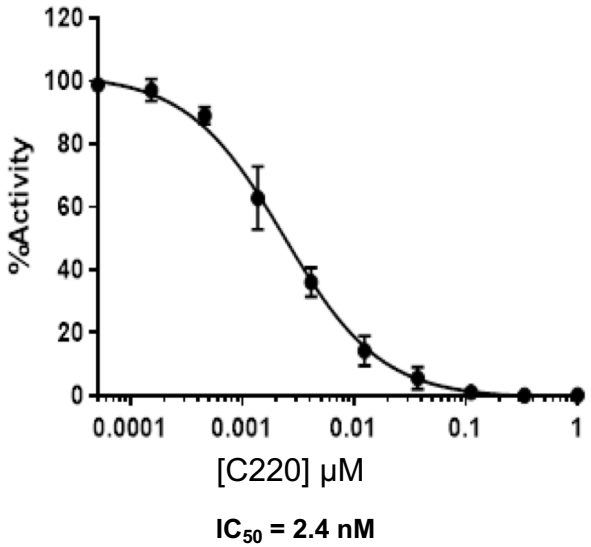
1. Supplemental Figures (1-8).
2. Supplemental Tables (2).

Supplemental Figure 1

A



B



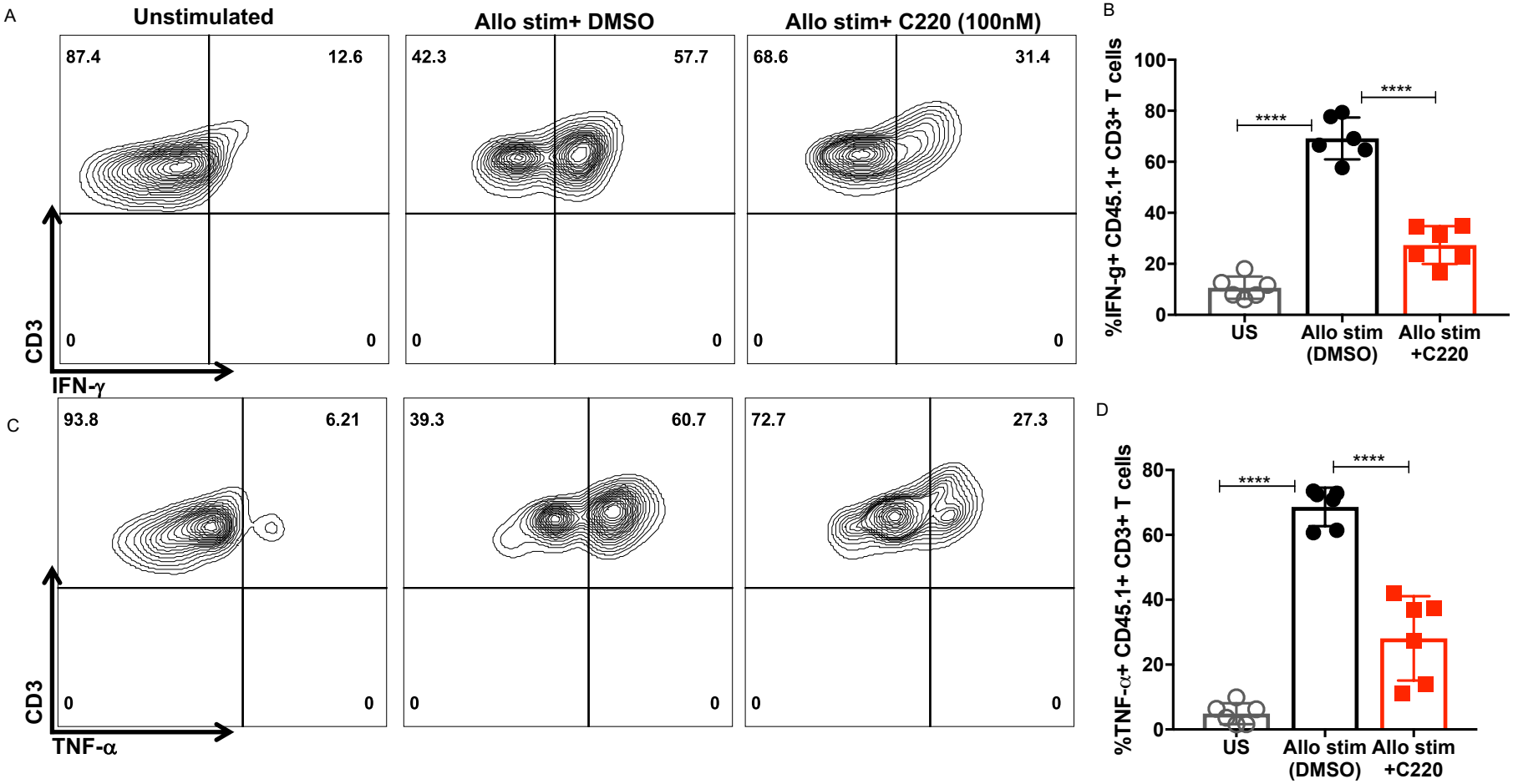
IC_{50} = 2.4 nM

C

Methyltransferases Screened	
NRMT2	METTL21A-GST
SUV420H1TV2	DNMT3b/3L
MLL2 Complex	PRMT4
SMYD1	PRDM9
DNMT3a	DNMT3b
NRMT1	SMYD2
SUV39H2	MLL1 Complex
EZH2 Complex	GLP
SET8	SET7/9
PRMT1	PRMT6
SUV39H1	PRMT8
PRMT3	EZH1 Complex
DNMT1	SETD2
NSD1	ASH1L
G9a	DOT1L
MLL3 Complex	NSD2
NSD3	PRMT7
MLL4 Complex	SET1b Complex

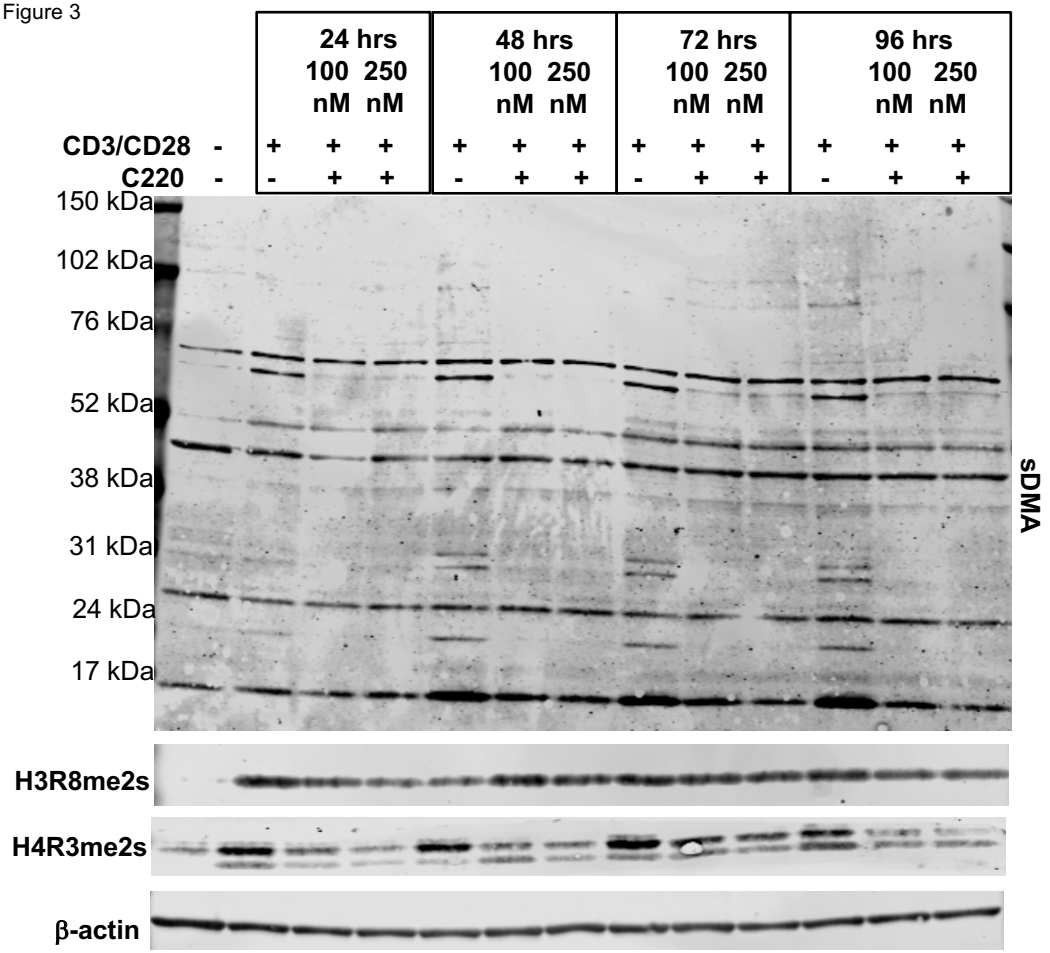
Supplemental Figure 1. (A) Structure of C220. **(B)** In vitro methyltransferase assay: Purified rPRMT5:MEP50 complex (Rection Biology Corp, HMT-22-148) were solubilized in DMSO and further diluted in assay buffer (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.002% Tween20, 1 mM TCEP, 1% DMSO) for 10-dose IC50 mode. Standard reactions were performed in a total volume of 30 µl in assay buffer, with 300 nM histone H4 based AcH4-23 (Anaspec: AS-65002) as substrate. To this was added the PRMT5/MEP50 complex diluted to provide a final assay concentration of 2.5 nM and compounds allowed to preincubate for 20 minutes at 37 °C. The reaction was initiated by adding S-[3 H-methyl]-adenosyl-L-methionine (PerkinElmer: NET155001MC) to final concentration of 1 µM. Following a 30 minutes incubation at 37°C, the reaction was stopped by adding 25 µL of 8M Guanidine HCl. To each reaction, 150 µL of streptavidin YSI SPA bead suspension (Perkinelmer: RPNQ0012 at 0.3 mg/mL in assay buffer) was added and incubated while shaking at room temperature for 30 minutes. The plate was centrifuged at 100 xg for 30 seconds before reading in a scintillation counter. IC50 values were determined by fitting the data to with Hill Slope using GraphPad Prism software. **(C)** List of methyltransferases used in screen.

Supplemental Figure 2

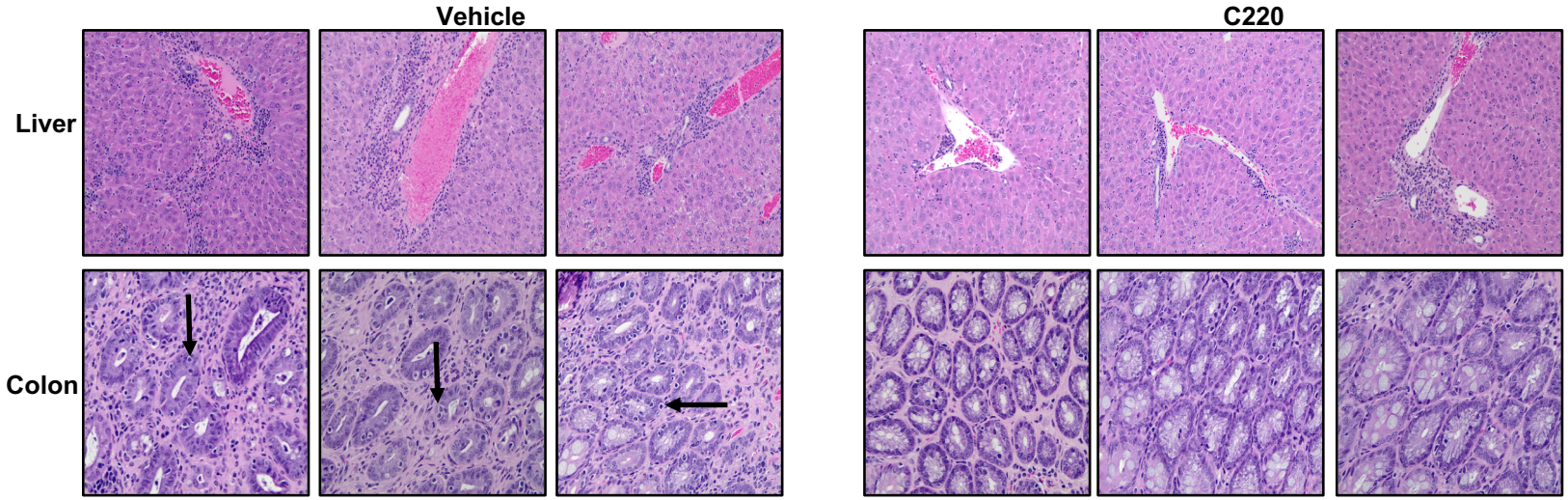


Supplemental Figure 2. CD45.1 B6 T cells were stimulated with allogeneic BALB/c bone-marrow derived dendritic cells (BMDCs) for 4 days in the presence of PRMT5 inhibitor C220, 100nM. Protein transport inhibitor was added for the last 5 hours and intracellular IFN-γ and TNF-α was analyzed by flow cytometry. **(A, C)** Histogram plots of one representative donor is shown. **(B, D)** Percent cytokine producing T cells. ****, $p<0.001$. Results are represented as mean ± SD of biological duplicates of 3 independent experiments.

Supplemental Figure 3

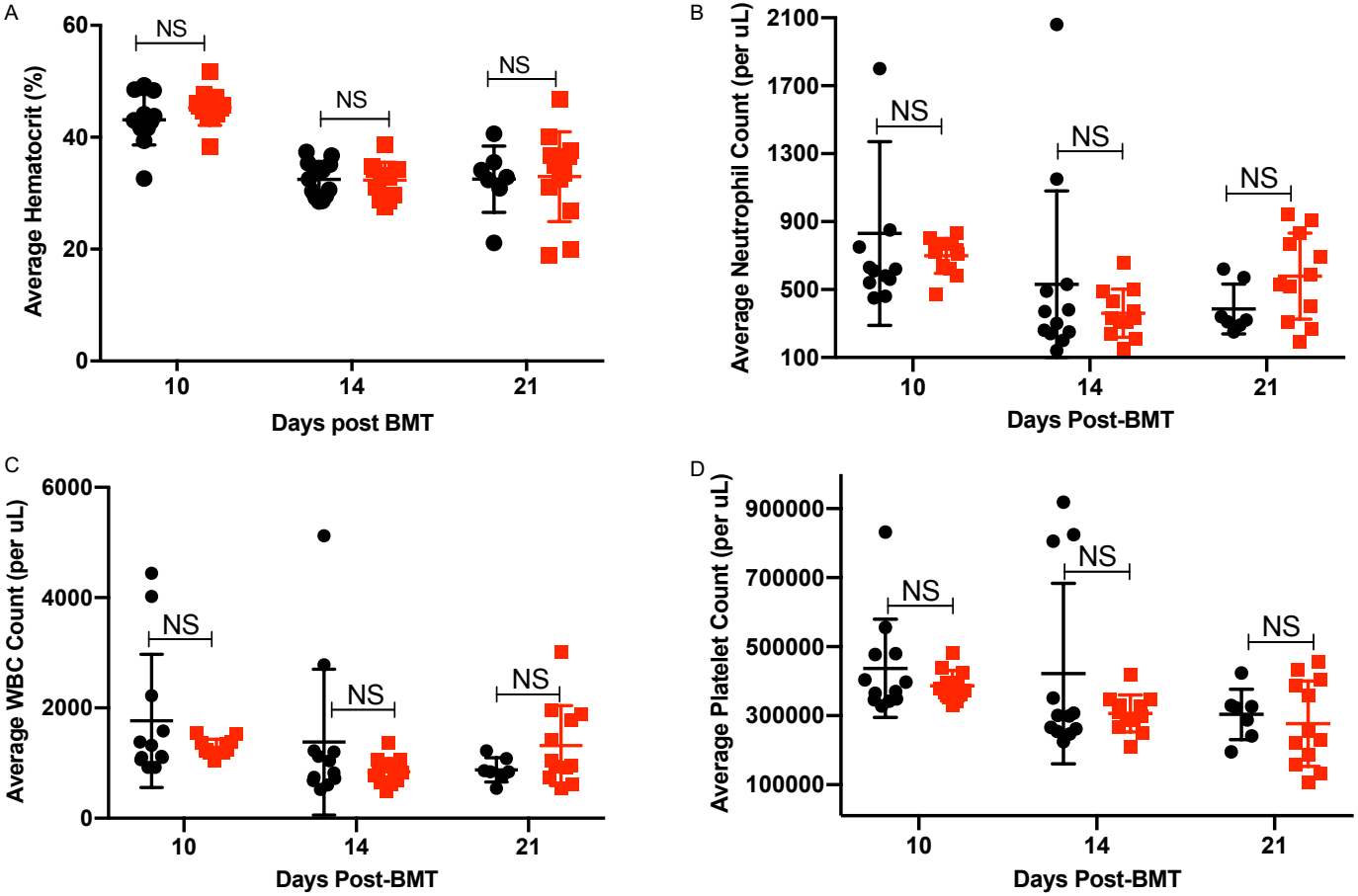


Supplemental Figure 3. T cells were isolated by negative selection from healthy donor PBMCs and stimulated with CD3/CD28 Dynabeads for indicated times in the presence or absence of PRMT5 inhibitor C220 (100nm, 250nM). Day 0 unstimulated T cells were used as control. Immunoblots showing symmetric dimethyl arginine (sDMA), H3R8me2s, H3R4me2s and β-actin.



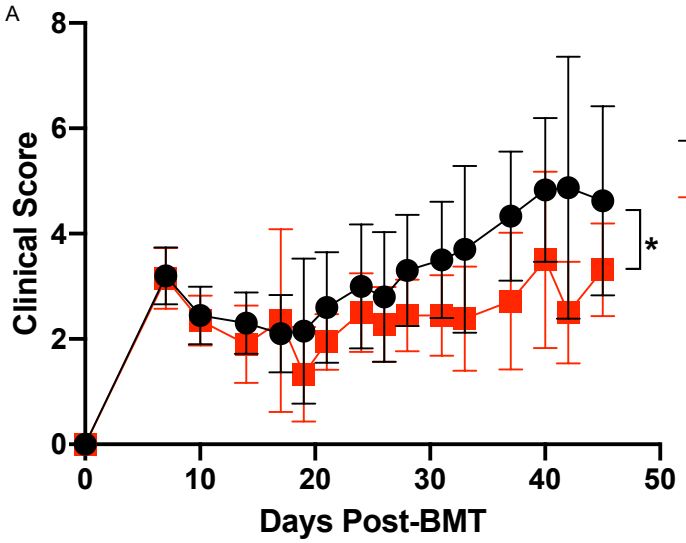
Supplemental Figure 4. B6 into B6D2F1 transplant was performed as described in methods. Mice were sacrificed on day 22 post-transplant, (n=5–6 per group, vehicle and PRMT5 inhibitor C220 groups) and liver and gut tissues isolated, fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, slide-mounted, and stained with H&E for histopathology. Representative histopathologic images from recipient mice (n=3). Arrows highlight areas of tissue inflammation. All images at original magnification X400. Liver – amount of inflammation around vessels (dark purple cells around oblong areas with RBCs). Colon – space and cellularity between crypts, number of apoptotic bodies (arrows; clear circles with dense purple core).

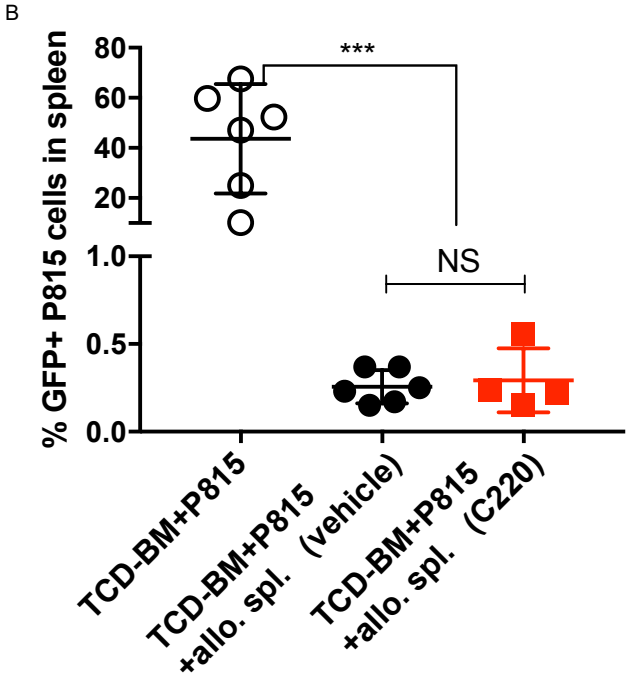
Supplemental Figure 5

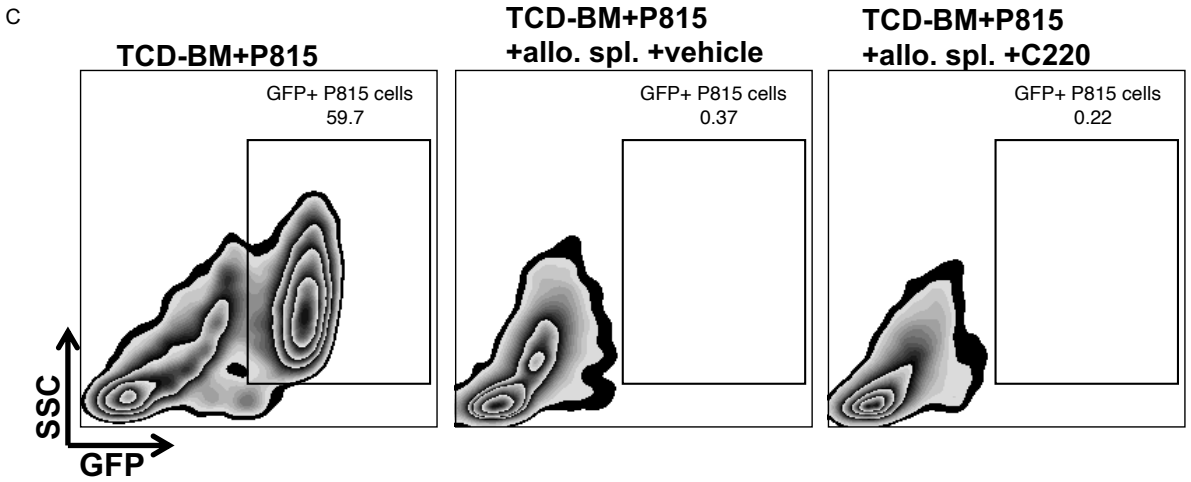


Supplemental Figure 5. PRMT5 inhibition does not adversely impact hematopoiesis. Lethally irradiated B6D2F1 recipients received CD45.1+ B6 T cell depleted bone marrow (TCD-BM, $10x10^6$ cells) or TCD-BM + CD45.1+ B6 splenocytes (15×10^6). Recipients of allogeneic splenocytes were treated with PRT220 (2mg/kg) or vehicle by oral gavage once weekly starting day 7 post-transplant. Mice were bled weekly post-transplant and **(A)** Hematocrit values **(B)** Neutrophil **(C)** WBC and **(D)** Platelet counts were enumerated using a Hemavet counter.

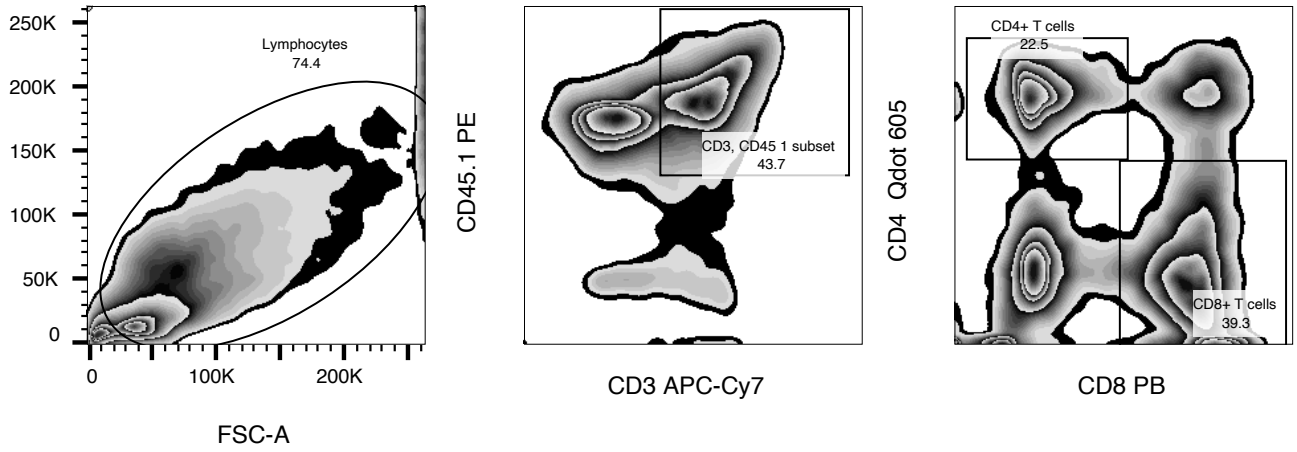
Supplemental Figure 6



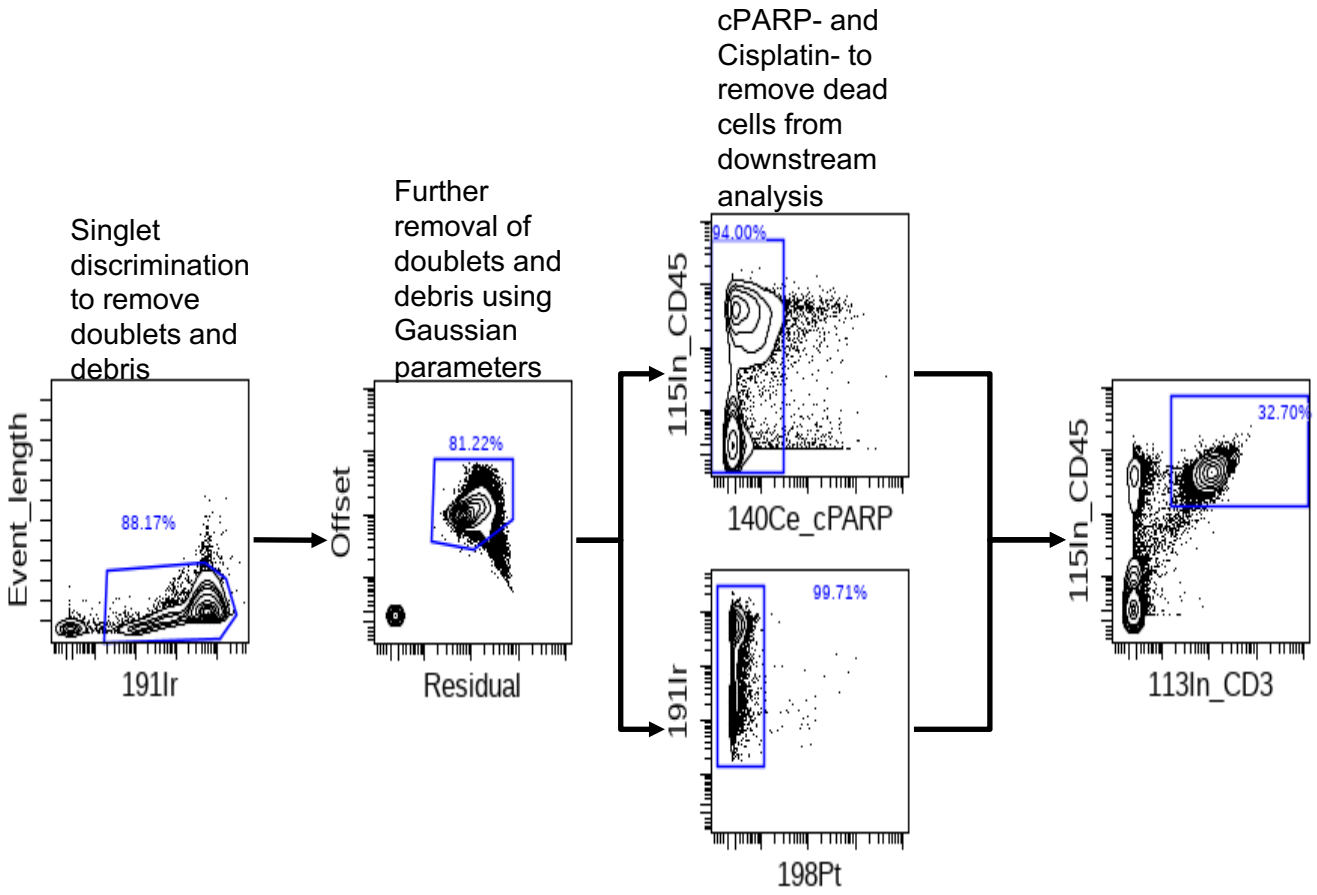




Supplemental Figure 6. PRMT5 inhibition preserves graft versus leukemia effect. Firefly luciferase-transduced GFP+ P815 cells (10,000–15,000 cells) were injected i.v. into lethally irradiated F1 recipients on day 0 along with TCD-BM and B6 donor splenocytes. TCD-BM + P815 cells (leukemia alone) served as the control group. Treatment groups included PRMT5 inhibitor C220 or vehicle control in recipients of allogeneic splenocytes (TCD-BM+ P815+ allo. spl.). **(A)** Clinical scores. *p<0.05, Mann-Whitney test of clinial scores. Splenocytes were isolated at time of death and P815 leukemic burden evaluated by GFP positivity using flow cytometry. **(B)** Percentage GFP positivity representing P815 leukemic cell infiltration in the spleen. **(C)** Representative flow cytometric density plots. Each dot represents a single mouse, data combined from 2 independent GVL transplant experiments.



Supplemental Figure 7. Gating Strategy. B6 into B6D2F1 transplant was performed as described in methods. Mice were sacrificed around day 25 post-transplant and splenocytes isolated for flow cytometric evaluation of donor T cells.



Supplemental Figure 8. Gating Strategy for mass cytometry.

Supplemental Table 1. List of antibodies used in Snyder et al. PRMT5 paper.

Target	Clone	Fluorochrome/Metal	Vendor
CD3	17A2	APC/Cy7	BioLegend
CD3	REA641	APC-Vio770	Miltenyi Biotec
CD3e	145-2C11	eFluor450	eBioscience – Thermo Fisher
CD4	OKT4	Brilliant Violet 421	BioLegend
CD4	RM4-5	Brilliant Violet 605	BioLegend
CD8a	53-6.7	Brilliant Violet 421	BioLegend
CD25	7D4	FITC	BD Biosciences
CD25	REA568	VioBright FITC	Miltenyi Biotec
CD45.1	A20	PE	BioLegend
ERK1/2 p(Thr202/Y204)	6B8B69	PE/APC	BioLegend
ERK1/2 (Total)	137F5		Cell Signaling Technology
FoxP3	REA788	APC	Miltenyi Biotec
IFNγ	XMG1.2	FITC	BD Biosciences
IFNγ	REA638	FITC	Miltenyi Biotec
IL-17F	8F5.1A9	Alexa Fluor 647	BioLegend
Stat1 (pY701)	4a	Alexa Fluor 647	BD Biosciences
Total Stat1 (N-Terminus)	1/Stat1	Alexa Fluor 647/PE	BD Biosciences
β-Actin	13E5	-	Cell Signaling Technology
Dimethyl-Arginine, symmetric	-	-	Millipore Sigma
IRDye® 800CW Goat anti-Rabbit IgG (H + L)	-	-	LI-COR Biosciences
Histone H3R8 Dimethyl Symmetric (H3R8me2s)	-	-	EpiGentek
Histone H4R3 Dimethyl Symmetric (H4R3me2s)	-	-	EpiGentek
Normal Rabbit IgG	-	-	Millipore Sigma
PRMT5	EPR5772	159-Tb	Abcam
Ki67	B56	158-Gd	BD Biosciences
pRb	J112-906	165-Ho	BD Biosciences
cPARP	F21-852	140Ce	BD Biosciences
CD3	UCHT1	113In	BioLegend
CD45	H130	115In	BioLegend

Supplemental Table 2. Patient Characteristics.

Age (yrs)	Gender	Donor source	Transplant Type	Disease	Conditioning	GVHD Prohpylaxis	GVHD grade	CMV status (R/D)	HLA match
Cases									
61	M	PB	Unrelated	CLL	RIC Flu/Cy/TBI post-Cy	post-Cy/Tacro/MMF	2	(+/-)	10/10
55	M	PB	Unrelated	AML	MA Flu/Bu	ATG/Tacro/MTX	2	(-/+)	10/10
66	F	BM	Related	AML	MA Flu/Bu	Tacro/MTG	2	(-/-)	10/10
35	F	dUCB	UCB	HL	RIC Flu/Cy/TBI	Tacro/MMF	2	(-/-)	5/6
32	F	PB	Unrelated	AML	Cy/TBI	ATG/Siro/Tacro	2	(+/+)	12/12
58	F	PB	Related	AML	MA Flu/Bu	Tacro/MTX	3	(-/-)	10/10
65	F	PB	Unrelated	MDS	RIC Flu/Bu	ATG/Tacro/MTX	1	(+/-)	10/10
48	F	PB	Unrelated	Plasma cell leukemia	RIC Flu/Mel	ATG Tac/MTX	3	(+/+)	12/12
60	F	PB	Related	Ph- ALL	Cy/TBI	Tacro/MTX	1	(-/+)	10/10
63	M	PB	Unrelated	CMML	RIC Flu/Bu	Tacro/MTX	2	(-/-)	10/10
58	M	PB	Unrelated	MF	RIC Flu/Bu	ATG/Tacro/MTX	2	(-/-)	10/10
Controls									
46	M	PB	Unrelated	AML	MA Flu/Bu	ATG/Tacro/MTX		(+/-)	10/10
58	M	PB	Unrelated	DLBCL	RIC Flu/Bu	ATG/Tacro/MTG		(+/-)	10/10
60	M	PB	Unrelated	CMML	MA Flu/Bu	ATG/Tacro/MTX		(+/-)	10/10
65	M	PB	Related	MCL	RIC Flu/Bu	Tacro/MTX		(-/-)	10/10
57	M	PB	Unrelated	CTCL	RIC Flu/Bu	ATG Tac/MTX		(-/-)	10/10
74	M	PB	Haplo	AML	RIC Flu/Cy/TBI	post-Cy/Tacro/MMF		(-/-)	5/10
69	M	PB	Unrelated	AML	RIC Flu/Bu	post-Cy/Tacro/MMF		(+/+)	10/10
70	F	PB	Unrelated	AMDS	RIC/Flu/Bu	ATG/Tacro/MTX		(+/-)	12/12