Supporting Online Material for:

Zinc finger nuclease-mediated CCR5 knockout hematopoietic stem cell transplantation controls HIV-1 in vivo.

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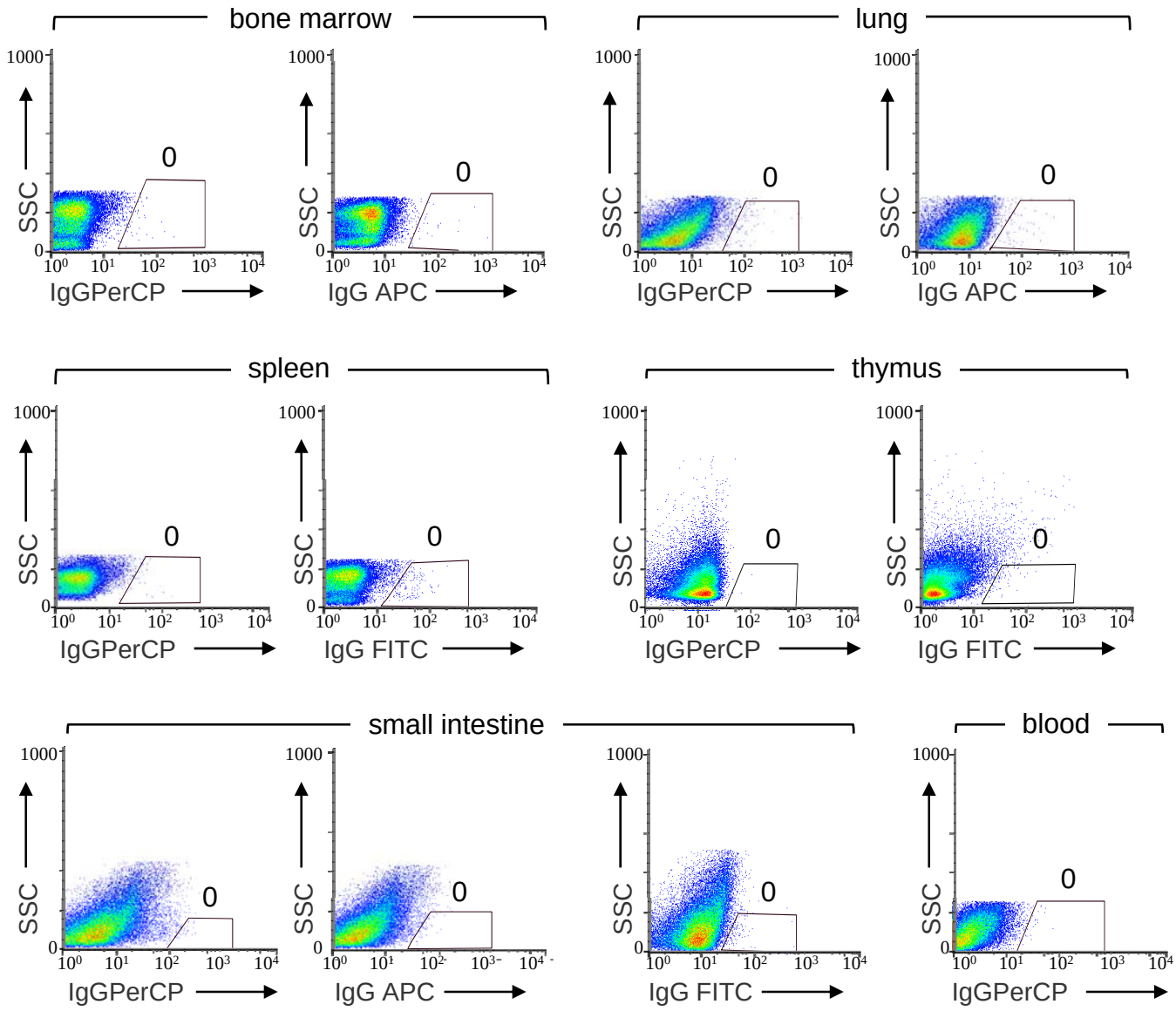
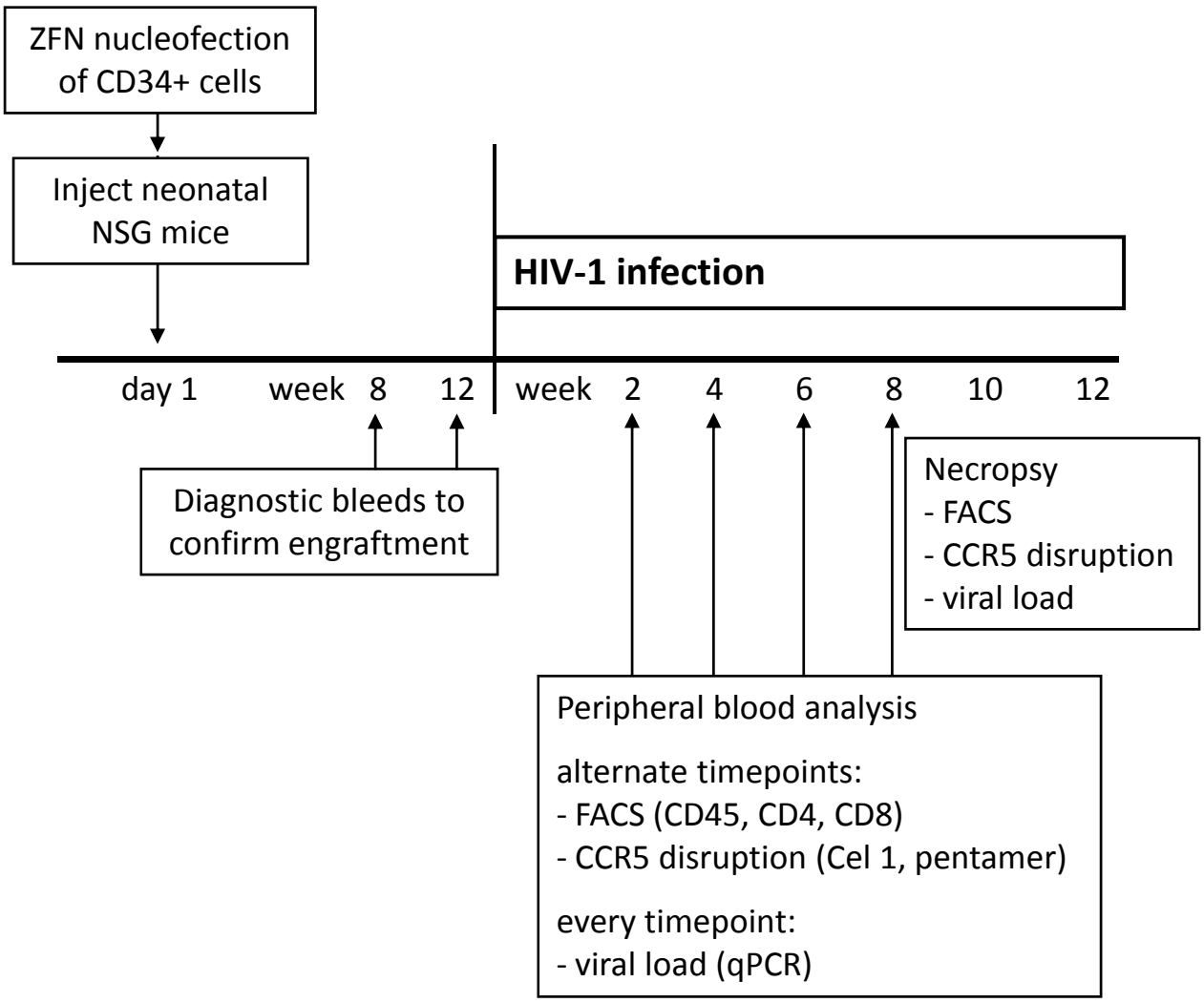
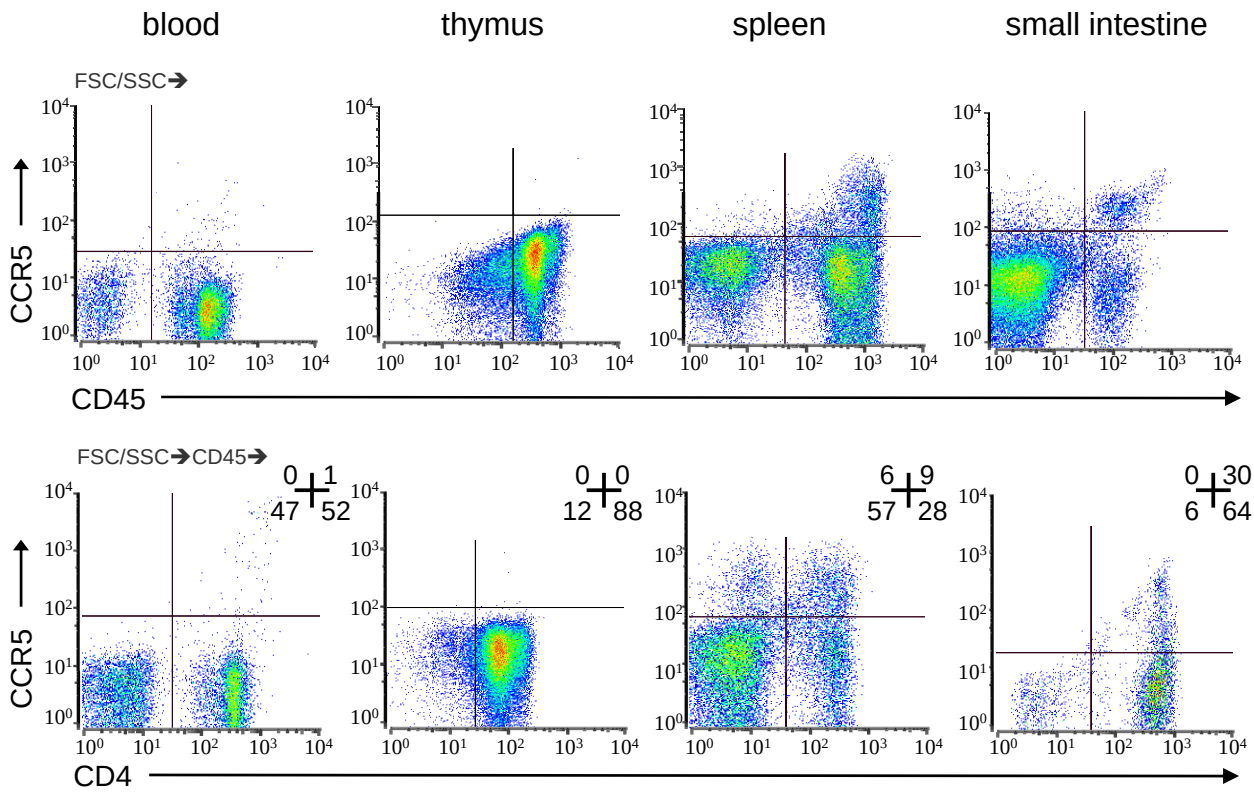


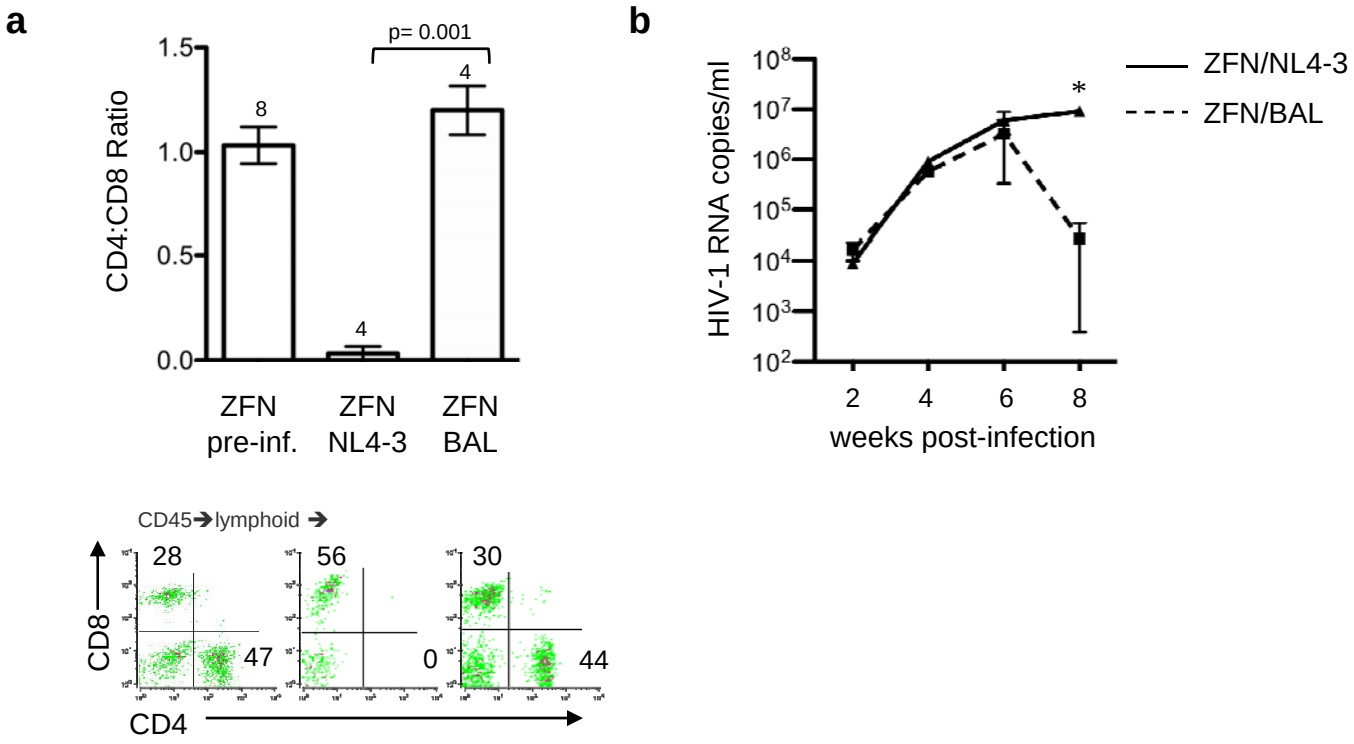
Supplementary Figure 1 Isotype controls for FACS analysis of human cells in mouse tissues. Staining by mouse IgG1 isotype controls is shown for each fluorochrome used: IgG1-PerCP for anti-human CD45; IgG1- APC for anti-human CD19, CD14, CD3 and CCR5; IgG1-FITC for anti-human CD4. Cells were gated on FSC/SSC to remove debris.



Supplementary Figure 2 Experimental outline and timeline for HSC engraftment of NSG mice, HIV-1 infection and analyses performed.



Supplementary Figure 3 Levels of CCR5 expression on human cells in HSC engrafted NSG mice. FACS analysis of blood, thymus, spleen, and small intestine lamina propria tissues are shown from one representative mouse transplanted with untreated (neg.) HSC and necropsied at 20 weeks post-engraftment. In an ungated analysis (top panel), a significant population of human (CD45+) CCR5+ cells (upper right quadrant) can be seen in only the spleen and small intestine samples. The lower panel shows the percentage of human cells (CD45+ gate) in each tissue expressing CD4 or CCR5, and is indicated for each quadrant. All cells were first gated on FSC/SSC to remove debris. No staining was observed with isotype-matched control antibodies, or for animals receiving no human graft (data not shown).



Supplementary Figure 4 Effects of CXCR4- and CCR5-tropic HIV-1 infection. A cohort of 8 mice received ZFN-treated HSC. At 8 weeks post-transplantation, blood was harvested (pre-infection samples) and 4 mice each were infected with either CXCR4-tropic HIV-1_{NL4-3}, or with CCR5-tropic HIV-1_{BAL}. (**a**) Mean +/- SD ratio of human CD4 to CD8 T cells in peripheral blood of mice, pre-infection (n=8) or at 8 weeks post-infection (n=4 for each virus) and below, representative FACS plots from each group. Cells were gated on FSC/SSC to remove debris, on human CD45, and a lymphoid gate was applied before analysis of CD4 and CD8 subsets. (**b**) Mean +/- SD levels of HIV-1 RNA in blood of mice infected with HIV-1_{NL4-3} (n=4) or HIV-1_{BAL} (n=4), at indicated timepoints. Asterisk indicates statistically significant difference between the two groups (p=0.03).

Supplementary Table 1 Human hematopoietic cells in mouse tissues

tissue/cells	untreated (neg.) HSC (n=3)	ZFN-treated HSC (n=3)
thymus		
% CD45+/total[a]	99 +/- 10	96 +/- 14
% CD4+CD8+/human[b]	95 +/- 10	91 +/- 16
bone marrow		
% CD45+/total	64 +/- 22	70 +/- 19
% CD19+/human	66 +/- 19	72 +/- 15
spleen		
% CD45+/total	55 +/- 38	59 +/- 47
% CD4+/human	33 +/- 17	39 +/- 14
% CD8+/human	19 +/- 7	21 +/- 10
SE		
% CD45+/total	17 +/- 6	17 +/- 5
% CD3+/human	64 +/- 26	71 +/- 29
SP		
% CD45+/total	14 +/- 3	15 +/- 4
% CD3+/human	59 +/- 21	70 +/- 11
LE		
% CD45+/total	14 +/- 6	12 +/- 6
% CD3+/human	68 +/- 24	61 +/- 18
LP		
% CD45+/total	18 +/- 7	19 +/- 3
% CD3+/human	91 +/- 30	95 +/- 37

[a] Mean +/- SEM percentage of human CD45+ cells in 50,000 total cells analyzed from the indicated mouse tissues. [b] Mean +/- SEM percentage of indicated human subsets within the human CD45+ population. Cells were harvested from adult NSG mice previously engrafted with either untreated (neg.) HSC or ZFN-treated HSC. Student's t-tests demonstrated no significant differences ($p>0.05$) in the human cell populations between the neg. and ZFN groups. Abbr. S, small intestine; L, large intestine; E, intraepithelial layer; P, lamina propria.

Supplementary Table 2 HSC treatment and HIV infection in mouse cohorts[a]

ID #	HSC treatment	HIV status
1	neg	uninf.
2	neg	uninf.
3	neg	uninf.
4	neg	uninf.
5	ZFN	uninf.
6*	ZFN	uninf.
7	ZFN	uninf.
8	ZFN	uninf.
9	mock	uninf.
10	mock	uninf.
11	mock	uninf.
12	mock	uninf.
13	mock	uninf.
14*	neg	HIV+
15*	neg	HIV+
16*	neg	HIV+
17*	neg	HIV+
18*	neg	HIV+
19*	neg	HIV+
20	neg	HIV+
21	neg	HIV+
22	neg	HIV+
23*	ZFN	HIV+
24	ZFN	HIV+
25*	ZFN	HIV+
26*	ZFN	HIV+
27	ZFN	HIV+
28	ZFN	HIV+
29	ZFN	HIV+
30	ZFN	HIV+
31	ZFN	HIV+
32	ZFN	HIV+
33	ZFN	HIV+
34	ZFN	HIV+
35	ZFN	HIV+
36*	ZFN	HIV+
37*	ZFN	HIV+
38	ZFN	HIV+
39	ZFN	HIV+
40	ZFN	HIV+
41	ZFN	HIV+
42	ZFN	HIV+
43	ZFN	HIV+
44	ZFN	HIV+
45	ZFN	HIV+
46	no graft	uninf.
47	no graft	uninf.

Fig./Table	assay	Mice ID #s
1b	FACS	1-2, 5-6, 9-13, 14-16, 23-25
1c	FACS	7
2a	FACS	3, 7-8, 20-22, 27-35
2b	FACS	14-35
3a	FACS	3, 7-8, 20-22, 27-35
3b	IHC	3-4, 7-8, 20-22, 27-35, 45-46
4a	Cel 1/ pentamer	23-25, 27-29
4b	Cel-1	6-7, 25, 33-34
4c	FACS	3, 7-8, 20-22, 27-35
4d	FACS	3, 7-8, 20-22, 27-35
5	CCR5 sequence	24
6a	FACS/qPCR	20-22, 27-35
6b	qPCR	14-37
Supp 1	FACS	3-4, 7-8
Supp 3	FACS	3-4
Supp 4	FACS/qPCR	38-45
Supp Table 1	FACS	1-3, 5, 7-8

[a] HSC treatment and HIV-1 infection status of individual mice (left table), and the specific animals included in each data set (right table). Animals were infected with HIV-1 at 8-12 weeks post-engraftment and were followed up to 12 weeks post-infection. Animals marked with an asterisk (*) were necropsied at earlier time points, including any found dead due to environmental contamination. Only 12 week post-infection survivors were analyzed for Figs 2a, 3, 4c, 4d and 6a. Blood analyses (FACS, Cel I and qPCR) were performed on alternating samples due to limiting sample volumes. Cel I and pentamer assays were only performed on samples that generated human CCR5 PCR fragments.