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Supplemental information

Comparison of busulfan and total body irradiation conditioning on hematopoietic clonal dynamics following lentiviral gene transfer in rhesus macaques

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Table S1: TBI animal transplantation and engraftment parameters

	TBI Animal ID				
	ZH33	ZG66	ZJ31	ZH19	ZK22
Vector	pCDH-MSCV-T2A- copGFP library11	pCDH-MSCV-T2A- copGFP library 11	pCDH-MSCV-T2A- copGFP library11	pCDH-EF1α-T2A- copGFP library 11	pCDH-MSCV-T2A- copGFP library 19
Transduction MOI	25	25	25	25	25
Transduction condition	FN+ Cytokines + protamine sulfate*				
Transduction efficiency	35%	35%	35%	23%	31%
Number of cells infused(millions)	32	48	23	48	82
Transplantation dose (CD34+ cells millions /kg)	6.9	8.5	4.1	7.1	7.2
Infused GFP+ cells(millions)	11.1	16.7	8	11	25.2

*Fibronectin (FN) coated plate+ % HSA + cytokines (Flt-3, SCF, TPO all at 100ng/mL) + protamine sulfate(4μg/ml)

Table S2: List of antibodies used for flow cytometric analysis and FACS

Antigen	Conjugation	Vendor	Catalog number	Clone
CD3	BV786	BD Pharmingen	557757	SP34-2
CD20	APC-Cy7	BD Pharmingen	335794	L27
CD14	Pacific Blue	Invitrogen	MHCD1428	TuK4
CD16	APC	BioLegend	302012	3G8
CD56	PE-Cy5	BD Pharmingen	555517	B159
NKG2A	PE-Cy7	Beckman Coulter	IM3291U	Z199
CD271	PE	BD Pharmingen	557196	C40-1457

Table S3: List of primers used for barcode retrieval via PCR and sequencing

Table S4: Busulfan pharmacokinetic data

Animal ID	Dose (mg/kg)	C max (ug/mL)	AUC (uM*min)	t 1/2 (h)	Drug Clearance(mL/min/kg)
11021142	5.5	4.433	3675	1.60	6.08
H84D	6.0	5.422	3791	1.39	6.43

Maximum concentration (C max), the total area under the curve (AUC), drug half-life (t 1/2), and drug clearance

Figure S1

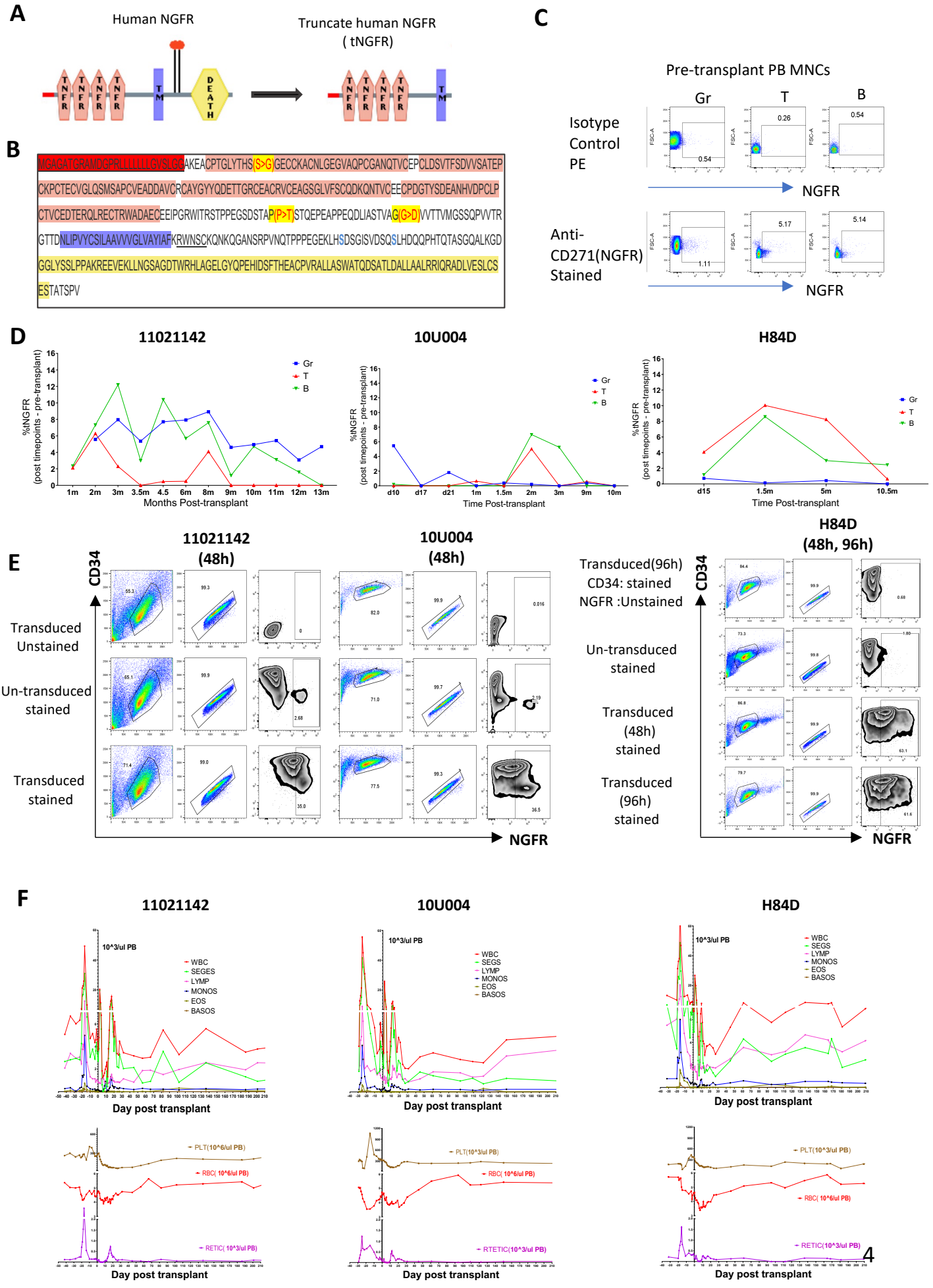
A
Human NGFR
Truncate human NGFR
(tNGFR)
TNFR
TM
DEATH
B
MGAGATGRAMDGPRLLLLLLLGVSLGGAKEACPTGLYTHS(S>G)GECCKACNLGEGVAQPCGANQTVCEPCLDSVTFSDVVSATEP
CKPCTECVGLQSMSAPCVEADDAVCRCAYGYYQDETTGRCEACRVCEAGSGLVFSCQDKQNTVCEECPDGTYSDEANHVDPCLP
CTVCEDTERQLRECTRWADAECEEIPGRWITRSTPPEGSDSTAP(P>T)STQEPEAPPEQDLIASTVAG(G>D)VVTTVMGSSQPVVTR
GTTDNLIPVYCSILAAVVVGLVAYIAFKRWNSCKQNKQGANSRPVNQTPPPEGEKLHSDSGISVDSQSLHDQQPHTQTASGQALKGD
GGLYSSLPPAKREEVEKLLNGSAGDTWRHLAGELGYQPEHIDSFTHEACPVRALLASWATQDSATLDALLAALRRIQRADLVESLCS
ESTATSPV
C
Pre-transplant PB MNCs
Gr
T
B
Isotype Control PE
0.54
0.26
0.54
NGFR
Anti-CD271(NGFR) Stained
1.11
5.17
5.14
NGFR
D
11021142
10U004
H84D
%tNGFR (post timepoints - pre-transplant)
Gr
T
B
Months Post-transplant
Time Post-transplant
E
11021142 (48h)
10U004 (48h)
H84D (48h, 96h)
CD34
Transduced Unstained
Un-transduced stained
Transduced stained
Transduced(96h) CD34: stained NGFR :Unstained
Un-transduced stained
Transduced (48h) stained
Transduced (96h) stained
NGFR
F
11021142
10U004
H84D
10^3/ul PB
WBC
SEGES
LYMP
MONOS
EOS
BASOS
Day post transplant
PLT(10^6/ul PB)
RBC(10^6/ul PB)
RETIC(10^3/ul PB)
PLT(10^3/ul PB)
RTETIC(10^3/ul PB)

Figure S1:
(A): The schema of the truncate human NGFR (tNGFR) used in the lentivector as surface marker gene.
(B): The protein sequence of the human tNGFR, 3 amino acids were different between human and rhesus macaque(human>rhesus)
(C): NGFR expression on the pre-transplanted animal.
(D):NGFR expression follow up on lineage cells post transplantation. The % if the NGFR showing on the Y-axis is the % of NGFR from the flow analysis from the post-transplant samples minus the % of NGFR from the flow analysis from the pre-transplant samples.
(E):NGFR expression on CD34+ HSPCs post in vitro barcode-tNGFR lentivirus transduction at 48h- 96h.
(F): Complete blood counts of 3 busulfan monkeys overtime. Day 0 is the day of transplantation. Top row: WBC, white blood cells, red curve; SEGS, segmented neutrophils, neutrophils, granulocytes, green curve; LYMP, lymphocytes, magenta curve; MONOS, monocytes, blue curve; EOS, eosinophils, light brown curve; BASOS, basophils, brown curve; Bottom row: PLT, platelets, brown curve; RBC, red blood cells, red curve; RETIC, reticulocytes, magenta curve.

Figure S2

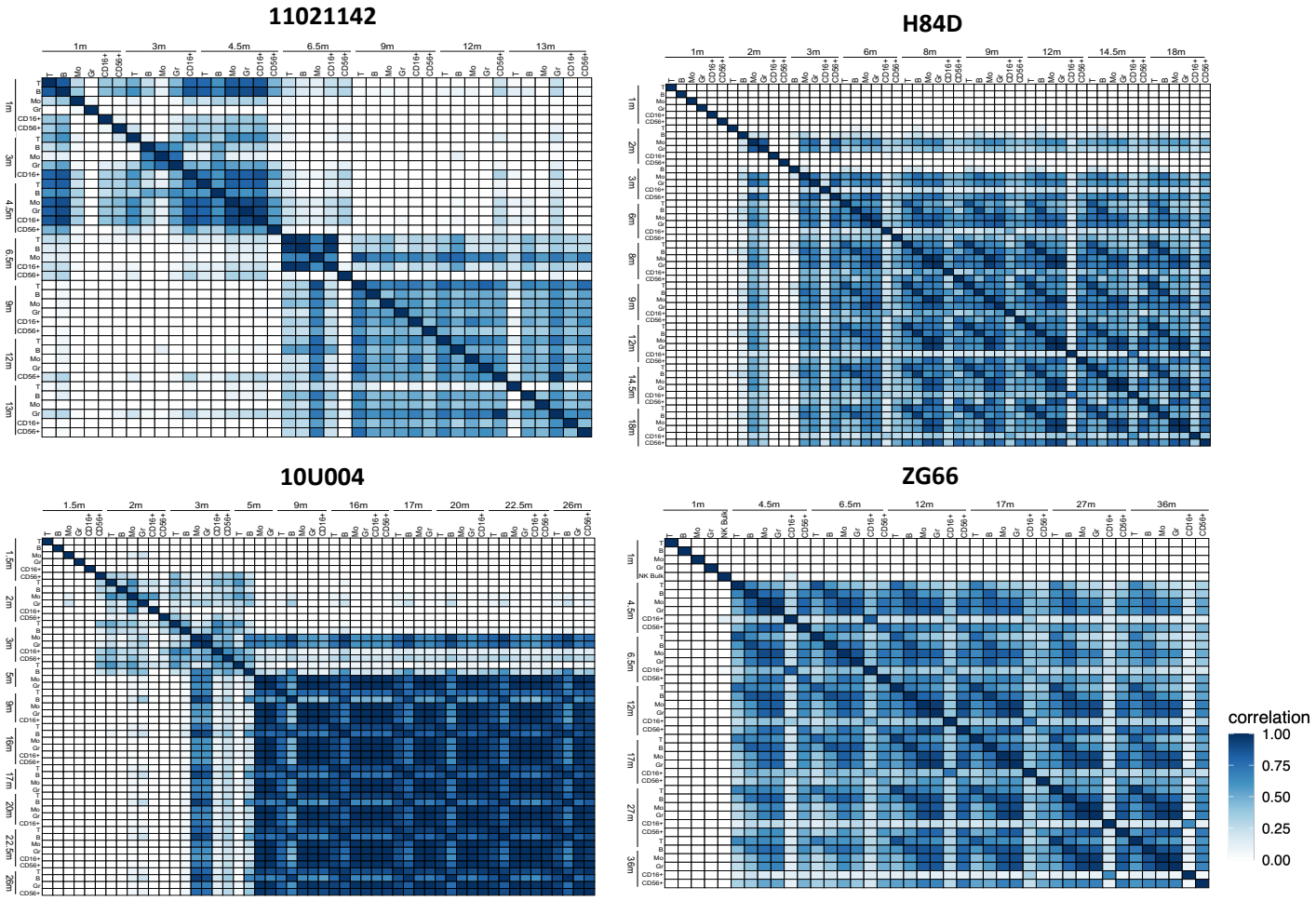

Figure S2: Clonal contributions correlation between PB lineages.

Pearson correlation coefficients plots comparing pairwise fractional contributions between PB lineages (T, B, Mo, Gr, CD16+ NK, and CD56+NK) over time for 11021042, 10U004, H84D, and ZG66. The color scale for correlation values is shown on the right.

Figure S3

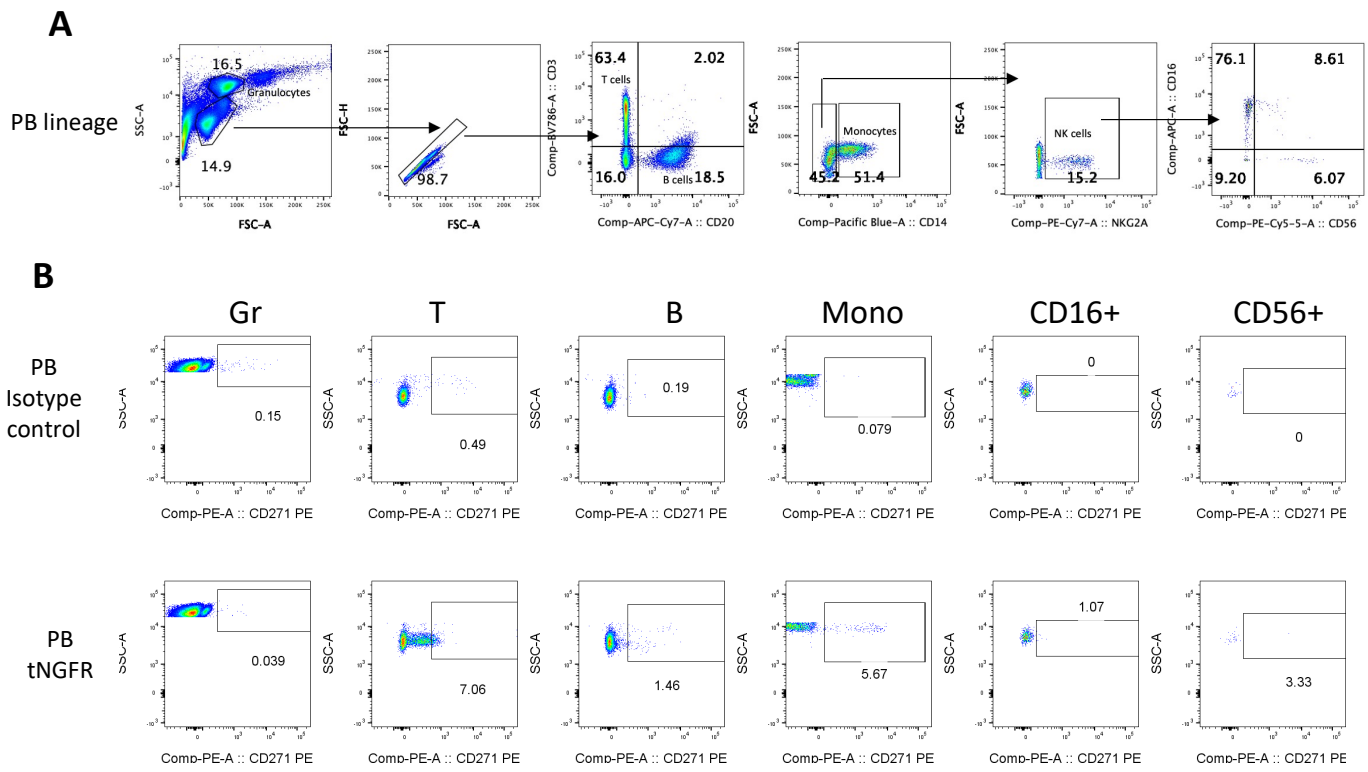

Figure S3: Flow cytometric gating strategy in peripheral blood lineages.

(A): Example gating strategy of PB lineages (Gr, T, B, Mono, CD16+NK, and CD56+NK).

(B): CD271+ detection with an isotype control sample of H84D at 2m after transplantation.

Figure S4

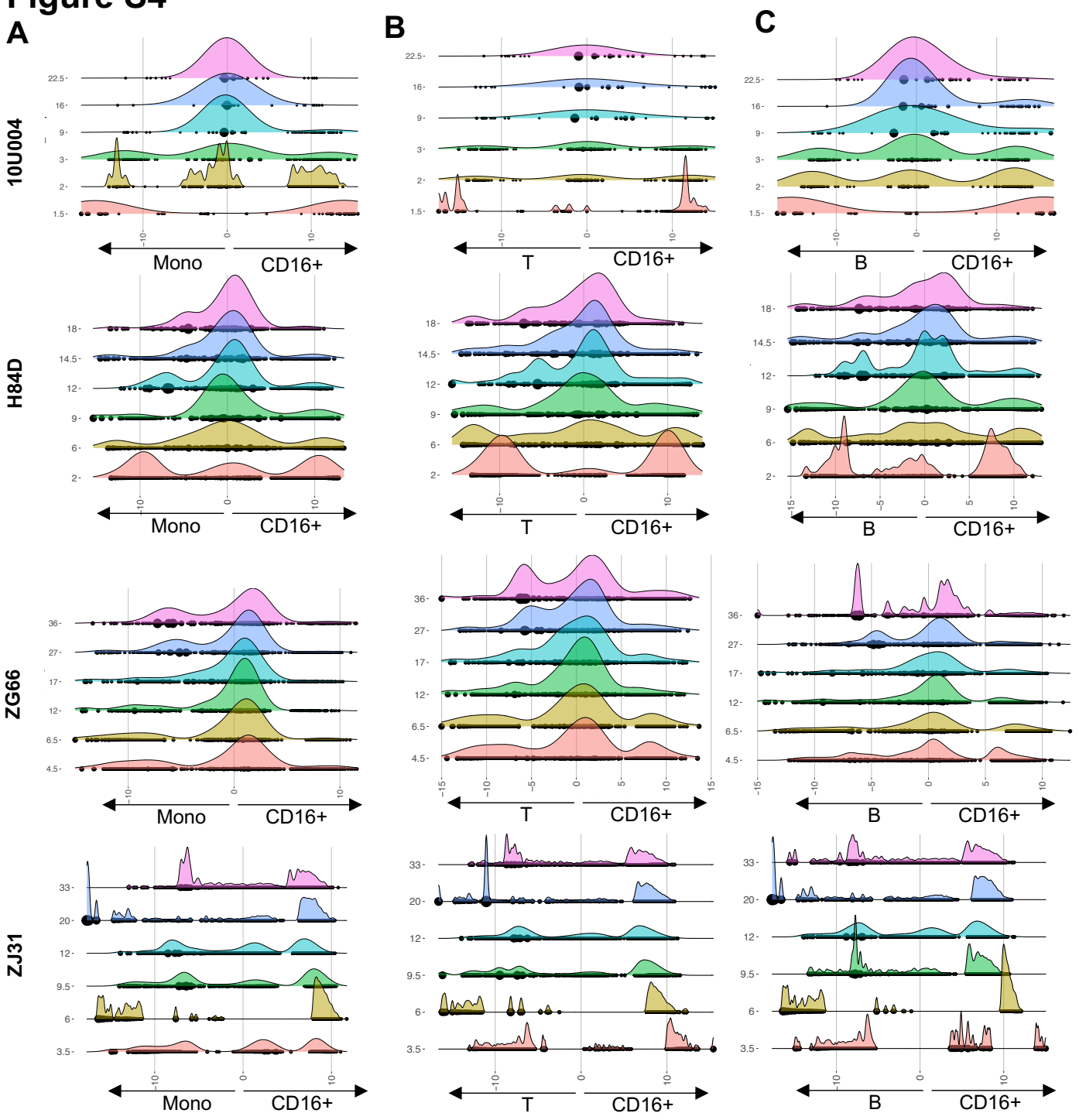

Figure S4: Clonal bias in CD16+ CD56- NK cells versus PB lineages

Ridge plot showing clonal bias between CD16+ NK and (A) Mono, (B) T cells, and (C) B cells over time for busulfan treated animals, 10U004 and H84D, and TBI treated animal, ZG66.