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Supplemental Information

Reprogramming of the heavy-chain CDR3 regions of a human antibody repertoire

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Off-target analysis by iGUIDE assay. (A) Target specificities of Mb2Cas12a RNP in comparison with AsCas12a. iGUIDE assay was used to determine genome-wide off-target activities when targeting the IgM and JH4 region in Jeko-1 cells. In each panel the top sequence indicates the on-target sequence. Each panel of a representative result of three replicates. Complete list of off targets is shown in Table S1. (**B**) The mean of off-target percentage of Mb2Cas12a and AsCas12a was shown in bar graph, with each dot representing one experiment.

Figure S2. Mb2Cas12a and SpCas9 target regions. (**A**) A graphical representation of the Jeko-1-cell heavy-chain locus with VH, DH, JH, CH, and HCDR3 regions represented in blue, yellow, purple, gray, and green, respectively. The Jeko-1-cell heavy-chain derives from VH2-70 and JH4 genes, as indicated. Black bar and arrow indicate HCDR3 region whose nucleotide and amino-acids are shown below. Four distinct Mb2Cas12a (orange) and SpCas9 (cyan) PAM and matching cleavage sites, used in Figures 2 and 3, are indicated. Note that Mb2Cas12a leaves 5' overhangs and that SpCas9 creates blunt ends. For each cut site, each DNA strand is labelled according to whether it is the target (T) or non-target (NT) strand of the indicated CRISPR protein and gRNA. (**B**) Summary of HA knock-in efficiency at different cut sites. Sense-strand (red) and anti-sense (blue) HDRT could be either NT or T depending on the cut site. Top panel used HDRT-A, and bottom panel used HDRT-B. At each site, SpCas9 AND Mb2Cas12a always preferred the same strand regardless of it being sense/anti-sense or NT/T. (**C**) Mb2Cas12a RNP targeting the GTTC PAM of Site 4 in Jeko-1 cells with the HDRT of different homology arms. Number indicates length of the 5' and 3' homology arm, respectively. Editing efficiency was by flow cytometry with fluorescently labeled PSG2. (**D**) HCDR3 derived from other HIV bNAb (CAP256 and CH01) were tested in a similar way in Figure 4B. Specificity of edited Jeko-1 was determined by fluorescent labeled HIV SOSIP trimers. One representative from three independent experiments is present in panel C and D.

Figure S3. Mb2Cas12a-mediated HDR using the HDRT with consensus sequences of the 3' VH regions. (**A**) 5' homology arms of HDRT used to edit primary human B cells, as shown in Figures 5 and 6, were designed based on the intrafamily conservation of the 3' of the indicated VH genes, as shown. Sequence logo presents conserved residues, full height indicates conserved within the indicated family and smaller letters indicated less conservation. The 5' homology arms used in the figures are shown in black. (**B**) Comparison of IgM knockout efficient by SpCas9 and Mb2Cas12a in primary human B cells targeting IgM or JH4, respectively. (**C**) Comparison of the efficiency of HA-insertion to the HCDR3 region by SpCas9 and Mb2Cas12a in primary human B cells targeting the JH4 region.

Figure S4. Inserting the CH01 HCDR3 to primary human B cells and neutralization of PG9 HCDR3 pairing with multiple VH genes. (**A**) HCDR3 derived from HIV bNAb CH01 was inserted to human primary B cells and sorted by HIV SOSIP trimers for NGS analysis, similarly described in Figure 6A. Specificity of edited Jeko-1 was determined by fluorescent labeled HIV

SOSIP trimers. (**B**) The IC_{50} values of soluble forms of the antibodies characterized in Figure 7A against indicated HIV-1 isolates is represented.

Figure S5. Autoreactivity assay. Autoreactivity of antibodies bearing the PG9 HCDR3 paring with various VH and VL genes to human epithelial HEp-2 cells was determined by indirect immunofluorescence on HEp-2 slides using FITC-conjugated goat anti-human IgG. The anti-HIV-1 antibody 2F5 is used as a positive control. All antibodies samples were tested at 150 μg/mL.

Table S1. Off targets identified by iGUIDE assay. All off-targets identified from the three replicates are shown.

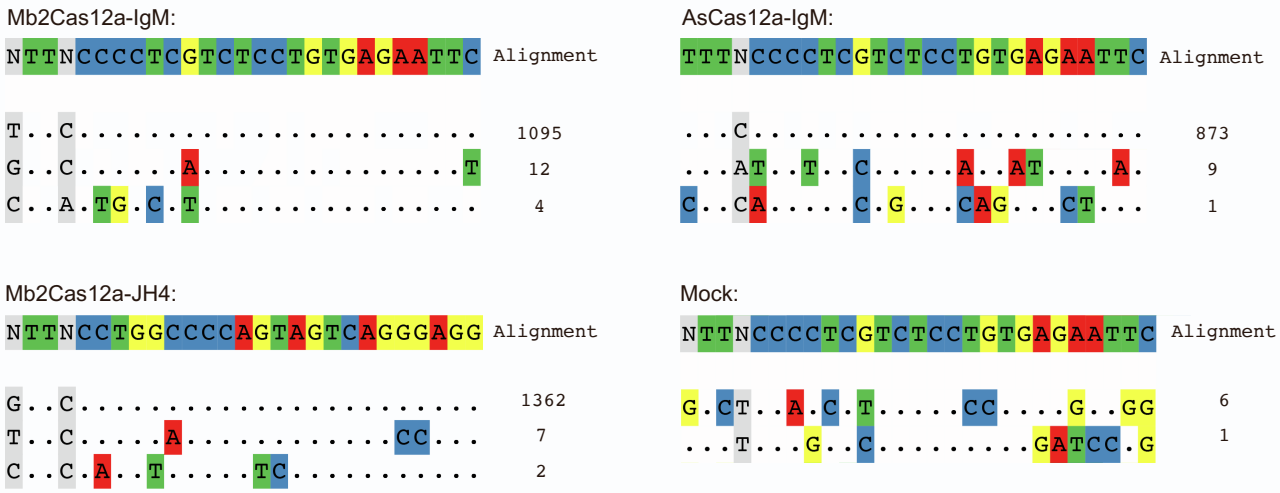
Table S2. The length of 3'mismatch tail. Actual DNA sequences of the target region and HDRT-B from the study of Figure 2C and D,
and indicates how the length of the 3' mismatch tail was calculated.

Table S3. gRNA and HDRT sequences. All gRNA and ssDNA HDRT were ordered from IDT.

Table S4. Primers used for NGS.

Figure S1

A


Mb2Cas12a-IgM:
NTTNCCCCTCGTCTCCTGTGAGAATTC Alignment
T..C.....
G..C.....A.....T 1095 12
C..A.TG.C.T..... 4
AsCas12a-IgM:
TTTNCCCCTCGTCTCCTGTGAGAATTC Alignment
...C.....
...AT..T..C.....A..AT....A. 873 9
C..CA.....C.G...CAG...CT... 1
Mb2Cas12a-JH4:
NTTNCCTGGCCCCAGTAGTCAGGGAGG Alignment
G..C.....
T..C.....A.....CC... 1362 7
C..C.A..T.....TC..... 2
Mock:
NTTNCCCCTCGTCTCCTGTGAGAATTC Alignment
G.CT..A.C.T.....CC....G..GG 6
...T...G..C.....GATCC.G 1

B

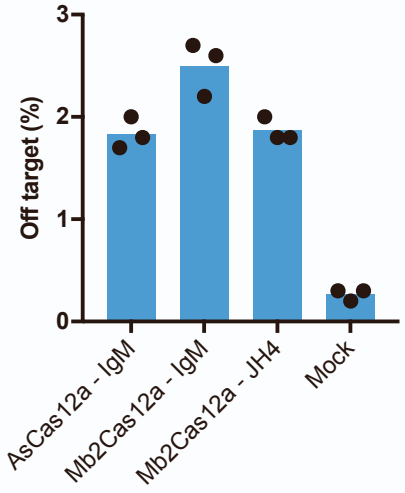

Off target (%)
3
2
1
0
AsCas12a - IgM
Mb2Cas12a - IgM
Mb2Cas12a - JH4
Mock

Figure S2

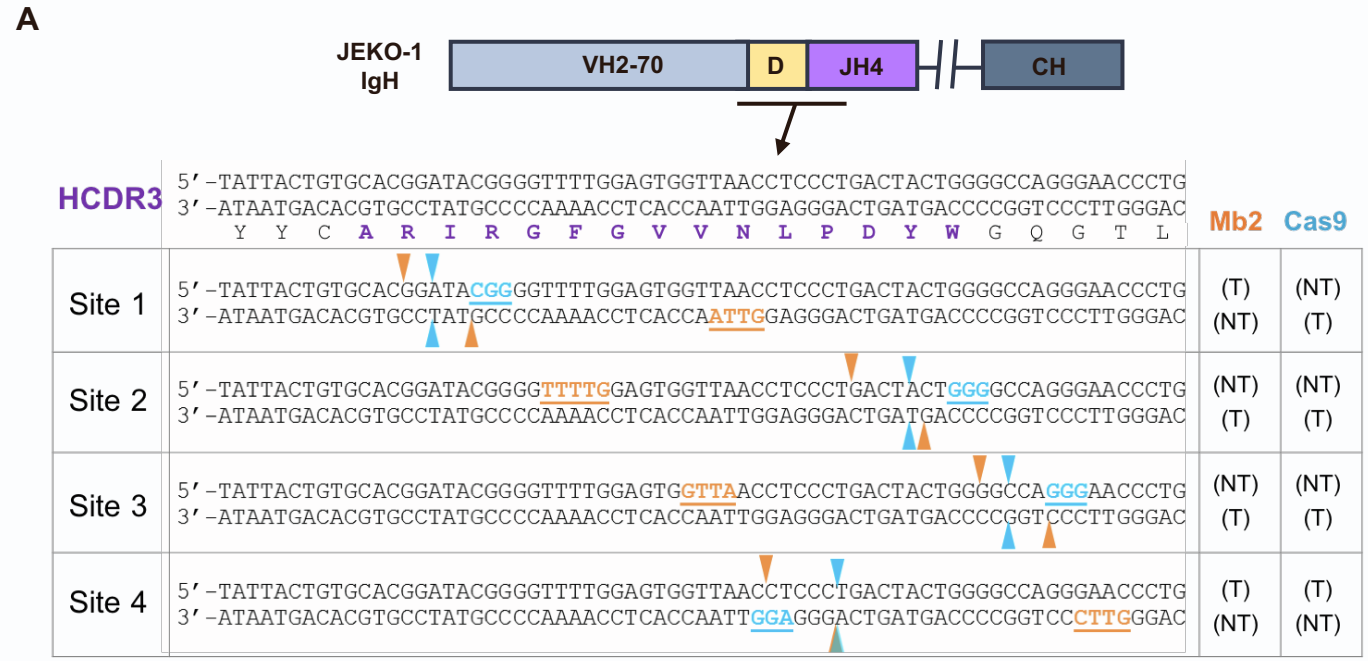
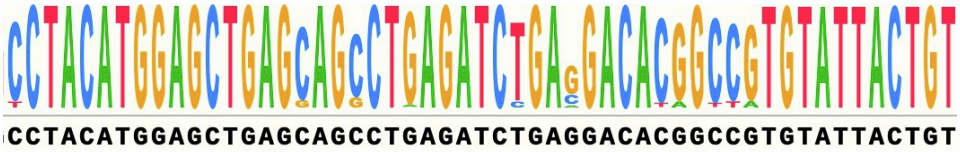

A
JEKO-1 IgH
VH2-70
D
JH4
CH
HCDR3
5′-TATTACTGTGCACGGATACGGGGTTTTGGAGTGGTTAACCTCCCTGACTACTGGGGCCAGGGAACCCTG
3′-ATAATGACACGTGCCTATGCCCCAAAACCTCACCAATTGGAGGGACTGATGACCCCGGTCCCTTGGGAC
Y Y C A R I R G F G V V N L P D Y W G Q G T L
Mb2
Cas9
Site 1
5′-TATTACTGTGCACGGATACGGGGTTTTGGAGTGGTTAACCTCCCTGACTACTGGGGCCAGGGAACCCTG
3′-ATAATGACACGTGCCTATGCCCCAAAACCTCACCAATTGGAGGGACTGATGACCCCGGTCCCTTGGGAC
(T) (NT)
(NT) (T)
Site 2
5′-TATTACTGTGCACGGATACGGGGTTTTGGAGTGGTTAACCTCCCTGACTACTGGGGCCAGGGAACCCTG
3′-ATAATGACACGTGCCTATGCCCCAAAACCTCACCAATTGGAGGGACTGATGACCCCGGTCCCTTGGGAC
(NT) (T)
(NT) (T)
Site 3
5′-TATTACTGTGCACGGATACGGGGTTTTGGAGTGGTTAACCTCCCTGACTACTGGGGCCAGGGAACCCTG
3′-ATAATGACACGTGCCTATGCCCCAAAACCTCACCAATTGGAGGGACTGATGACCCCGGTCCCTTGGGAC
(NT) (T)
(NT) (T)
Site 4
5′-TATTACTGTGCACGGATACGGGGTTTTGGAGTGGTTAACCTCCCTGACTACTGGGGCCAGGGAACCCTG
3′-ATAATGACACGTGCCTATGCCCCAAAACCTCACCAATTGGAGGGACTGATGACCCCGGTCCCTTGGGAC
(T) (NT)
(T) (NT)
B
Site 1
Site 2
Site 3
Site 4
sense
anti-sense
%HA knockin
T/NT:
Nuclease:
Mb2
Cas9
C
PG9 50/50
PG9 30/70
PG9 70/30
HA (control)
9.24
4.21
7.85
0.22
PSG2
IgM
D
PG9
CAP256
CH01
HA (control)
7.21
1.48
1.12
0.057
SOSIP
IgM

Figure S3

A

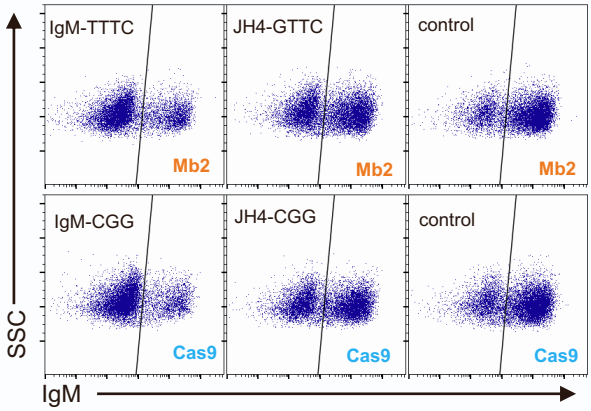
Consensus sequence of VH1-FR3:



Consensus sequence of VH3-FR3:

Consensus sequence of VH4-FR3:

B



C

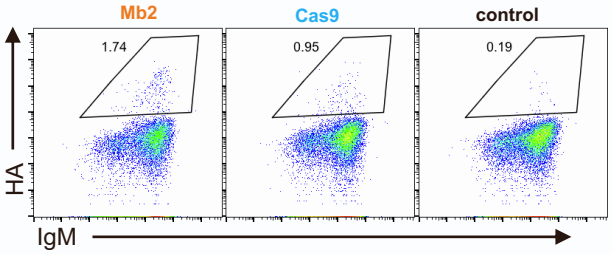
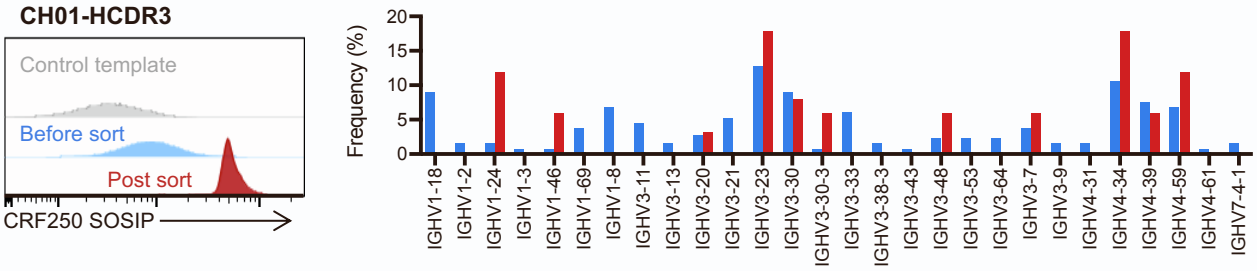


Figure S4

A

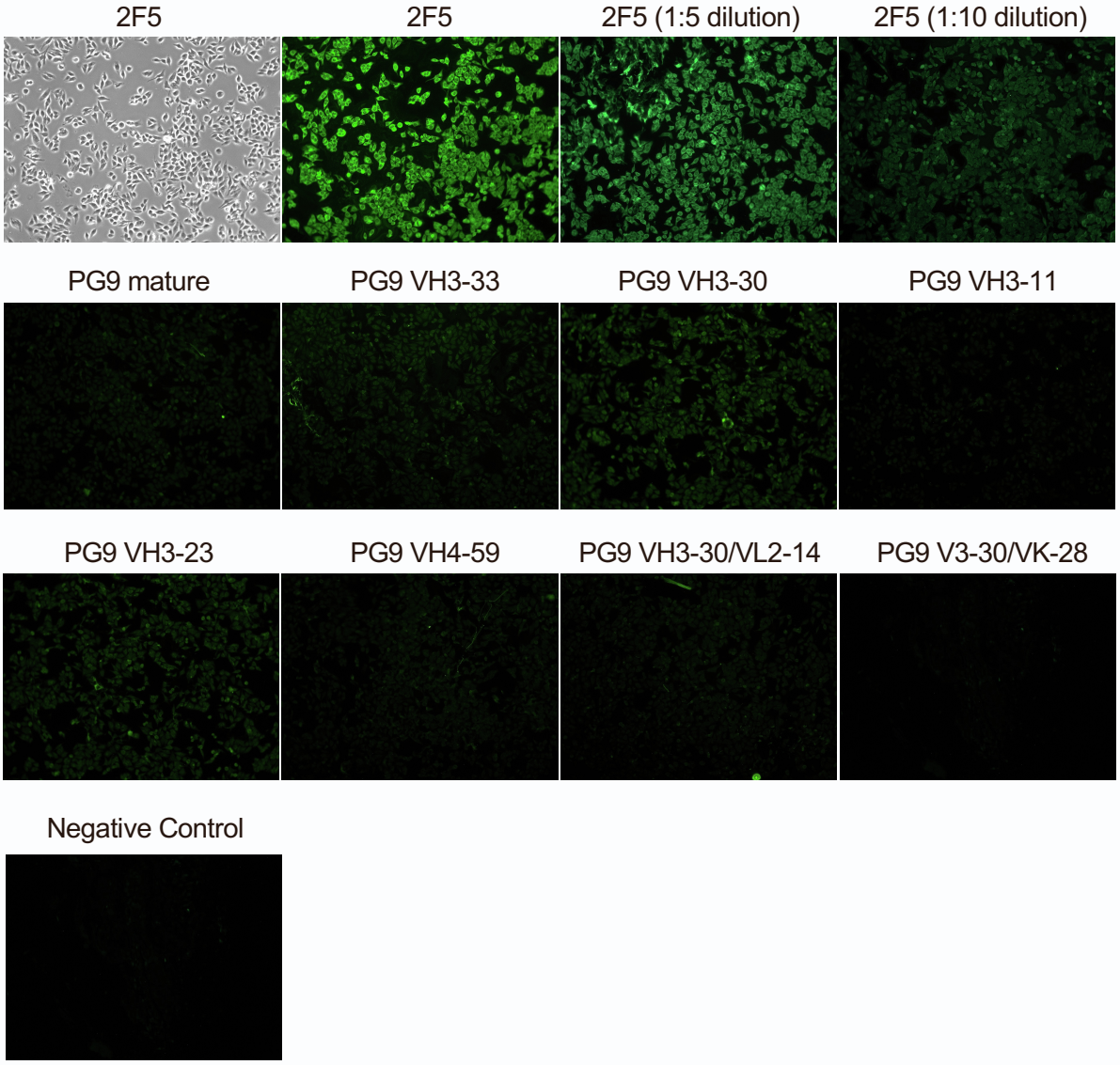


B

PG9-HCDR3

HIV isolate	Clade	VH3-33	VH3-30	VH3-23	VH3-11	VH4-59
16055	C	1.81	0.95	1.67	3.99	3.15
25710	C	34.94	4.89	>50	>50	>50
Bal.26	B	>50	2.30	>50	>50	>50
BG505	A	8.02	2.29	32.22	>50	>50
CRF_AG_250	AG	0.57	0.20	0.86	4.20	1.49

Figure S5


2F5
2F5
2F5 (1:5 dilution)
2F5 (1:10 dilution)
PG9 mature
PG9 VH3-33
PG9 VH3-30
PG9 VH3-11
PG9 VH3-23
PG9 VH4-59
PG9 VH3-30/VL2-14
PG9 V3-30/VK-28
Negative Control