

Engineering B cells to Express Fully Customizable Antibodies with Enhanced Fc Functions

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Supplementary Tables

Supplementary Table 1. gRNA sequences and corresponding ssODN HDR donors.

Supplementary Table 2. HDR donors to insert HCABs at *IGHG1* CH2 intron or *IGHG4* CH3 exon.

Supplementary Table 3. PCR primer sets.

Supplementary Table 4. Flow cytometry staining panels.

Supplementary Table 5. Sequences of HCAB expression plasmids.

Supplementary Table 6. Primers and probes used for ddPCR.

Supplementary Table 1. gRNA sequences and corresponding ssODN HDR donors.

a. gRNAs targeting *IGHG1* CH2 intron

gRNA	Sequence PAM	Matched ssODN HDR template (lowercase is XhoI restriction site)
CH2-g1	ATGTGGCCCTCG CACCCAC GGG	ATCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACCCGTGctcgagGGGTGCGAG GGCCACATGGACAGAGGCCGGCTCGGCCAC
CH2-g2	AAGCCAAAGGTG GGACCCGT GGG	GCCGAGCCGGCCTCTGTCCATGTGGCCCTCGCACCCACGctcgagGGTCCCAC CTTTGGCTTTGGAGATGGTTTTCTCGATGGG
CH2-g3	AGCCAAAGGTGG GACCCGTG GGG	GGCCGAGCCGGCCTCTGTCCATGTGGCCCTCGCACCCACctcgagGGGTCCCA CCTTTGGCTTTGGAGATGGTTTTCTCGATGGG
CH2-g4	GTGGGACCCGTG GGGTGCGA GGG	AGAGGGTGGGCCGAGCCGGCCTCTGTCCATGTGGCCCTCGctcgagCACCCAC GGGTCCCACCTTTGGCTTTGGAGATGGTTTTCT
CH2-g5	CATGTGGCCCTC GCACCCA CGG	TCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACCCGTGGctcgagGGTGCGAGG GCCACATGGACAGAGGCCGGCTCGGCCAC

b. gRNAs targeting *IGHG4* CH3 exon

gRNA	Sequence PAM	Matched ssODN HDR template (lowercase is XhoI restriction site)
G4-g1	TCTCTGGGTAAAT GAGTGCC AGG	ACCCCGAGAGCCCGGGGAGCGGGGGCTTGCCGGCCCTGGCctcgagACTCATTT ACCCAGAGACAGGGAGAGGCTCTTCTGTGTGT
G4-g2	AAGAGCCTCTCCC TGTCTCT GGG	GGAGCGGGGGCTTGCCGGCCCTGGCACTCATTTACCCAGActcgagGACAGGGA GAGGCTCTTCTGTGTGTAGTGGTTGTGCAGAG
G4-g3	CGTGGACAAGAG CAGGTGGC AGG	TCATGCATCACGGAGCATGAGAAGACATTCCCCTCCTGCCctcgagACCTGCTCTT GTCCACGGTGAGCCTGCTGTAGAGGAAGAA
G4-g4	GACAAGAGCAGG TGGCAGGA GGG	AGCCTCATGCATCACGGAGCATGAGAAGACATTCCCCTCCctcgagTGCCACCTG CTCTTGTCCACGGTGAGCCTGCTGTAGAGGA
G4-g5	GGACAAGAGCAG GTGGCAGG AGG	GCCTCATGCATCACGGAGCATGAGAAGACATTCCCCTCCTctcgagGCCACCTGCT CTTGTCCACGGTGAGCCTGCTGTAGAGGA

Supplementary Table 2. HDR donors to insert HCAs at *IGHG1* CH2 intron or *IGHG4* CH3 exon. (a-c) HDR donor sequences to create J3-CH2 HCAs at the CH2-g1 target site in *IGHG1*, containing codon-wobbled IgG1 Hinge (Hi) and CH2 exons and a modified CH2 splice donor (modSD; underlined, with specific base change in lower case), and with additional (b) AE or (c) LA mutations (bold, underlined). (d-f) HDR donor sequences to create J3-IgG1 HCAs at the G4-g2 target site in *IGHG4*, containing codon-wobbled IgG1 Hinge, CH2 and CH3 exons, and with additional (e) LS mutations (bold, underlined) or (f) DKD mutations (bold, underlined). (g) HDR donor for insertion of GFP domain at the C-terminus of CH3, using the G4-g2 gRNA target site in *IGHG4*, and inserting a J3-IgG1-GFP cassette containing a modified CH3-SD (modSD; underlined, with specific base change in lower case), an SGGG linker between CH3 and the GFP domain (italicized, underlined) and a modified GFP sequence (mGFP) with a cryptic SD at the 3' end removed (specific base change in lower case). (h) HDR for insertion of eCD4-IgG1 at the G4-g2 gRNA target site in *IGHG4*. The insert contains CD4 domains 1 and 3 as an ARD and a C-terminal CCR5 mimetic peptide (R5m), both derived from plasmid CMVR-eCD4-IgG2-v26, and an SGGG linker (italicized, underlined) between the CH3 and R5m sequences. Sequences are colored throughout to indicate specific regions, as noted in construct names, with multiple cloning sites between segments in black text. Additional abbreviations: LHA, left homology arm; RHA, right homology arm; EEK, EEK promoter; J3, anti-HIV VHH domain.

a. LHA-EEK-J3-HiCH2-modSD-RHA

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b. LHA-~~EEK~~-J3-HiCH2(AE)-modSD-RHA

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c. LHA-EEK-J3-HiCH2(LA)-modSD-RHA

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d. LHA-EEK-J3-HiCH2CH3-RHA

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e. LHA-EEK-J3-HiCH₂CH₃(LS)-RHA

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f. LHA-EEK-J3-HiCH2CH3(DKD)-RHA

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g. LHA-EEK-J3-HiCH₂CH₃-modSD-mGFP-RHA

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h. LHA-EEK-CD4d1d2-HiCH2CH3-R5m-RHA

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Supplementary Table 3. PCR primer sets. Primers for detecting on- and off-target editing at (a) the *IGHG1* CH2 intron, and (b) the *IGHG4* CH3 exon. (c) Primer sets for nested in-out PCR, to confirm J3-CH2 insertion at *IGHG1* CH2 intron.

a. For *IGHG1* CH2 intron targeting gRNAs

Targets	Locus	Primer name	Sequence
On-target	IGHG1	IGHG1-CH2-Fwd	ACACCTTCTCTCCTCCCAGATTCC
		IGHG1-CH2-Rev	CGGCGATGTCGCTGGGA
Off-target	IGHG2	IGHG2-CH2-Fwd	CGACCCCAAAGGCCAAACTG
		IGHG2-CH2-Rev	GGAGTCCAGCATGGGAGGT
	IGHG3	IGHG3-CH2-Fwd	GTCGGGTGCTGACACATCTG
		IGHG3-CH2-Rev	GAGGCTCTTCTGCGTGAAGC
	IGHG4	IGHG4-CH2-Fwd	GGGGGACCATCAGTCTTCCTG
		IGHG4-CH2-Rev	GGAGCATGAGAAGACATTCCCCTC
	IGHGP	IGHGP-CH2-Fwd	ACACATGCCCACCATGTGCAA
		IGHGP-CH2-Rev	GCTATAGAGGAAGAAGGAGCCGTT
Sequencing primer		CH2-Uniseq	TGGAGGTGCATAATGCCAAGAC

b. For *IGHG4* CH3 exon targeting gRNAs

Targets	Locus	Primer name	Sequence
Off-target	IGHG1	IGHG1-CH2-Fwd	ACACCTTCTCTCCTCCCAGATTCC
		IGHG1-end-Rev	CAGGGACGTGACGTGGT
	IGHG2	IGHG2-CH2-Fwd	CGACCCCAAAGGCCAAACTG
		IGHG2-end-Rev	GCCAGTGTGGGGACAGTGGA
	IGHG3	IGHG3-CH2-Fwd	GTCGGGTGCTGACACATCTG
		IGHG3-end-Rev	AGTTGCAGCTCTGGACAGGAAGA
On-target	IGHG4	IGHG4-CH2-Fwd	GGGGGACCATCAGTCTTCCTG
		IGHG4-end-Rev	CAGGAGGATGGTGAAACCCACC
Off-target	IGHGP	IGHGP-CH2-Fwd	ACACATGCCCAACCATGTGCAA
		IGHGP-end-Rev	AGCATCCTCGTGCGACCG
Sequencing primer		end-Uniseq	CCTGCCTGGTCAAAGGCTTC

c. For nested in-out PCR

Purpose	Primer name	Sequence
1st PCR	IGHG1-HA-Fwd2	GTCAGGGGGCTTCAGGGGG
	EEK-Beg-Rev	CTTGACCAAAGCAGTGTGACG
2nd PCR	IGHG1-F1	CCTGGCACCCCTCCTCAA
	IO-EEK-In-6	GACAGTTGCTATCTTGGTCCCTG
Sequencing primer	IO-EEK-In-6	GACAGTTGCTATCTTGGTCCCTG

Supplementary Table 4. Flow cytometry staining panels. (a) Panel for tonsil organoid or PBMC edited human B cells expressing J3 HCAb. (b) Panel for ADCC assay (c) Panel for J3 HCAb or eCD4-Ig edited Raji cells and eCD4-Ig edited PBMC B cells.

(a)

Antibodies	Fluorescence	Source	Cat. num.	Intracellular stain
IgG	BV421	Biolegend	410704	Yes
His	PE	Miltenyi	130-120-718	
VHH	APC	GeneScript	A01994	Yes
CD27	BV510	BioLegend	302836	
CD38	PerCP-eF710	Thermo Fisher	46-0389-42	
CD138	BV711	Biolegend	356522	
CD3	PE-eF610	Thermo Fisher	61-0038-42	
CD19	SB600	Thermo Fisher	63-0198-42	
IgM	FITC	BD	555782	
IgD	PE-Cy7	BD	561314	
Viability dye	Near-IR	Thermo Fisher	L34976	

(b)

Antibodies	Fluorescence	Source	Cat. num.	Intracellular stain
CD107A	Alexa Fluor 647	BD	562622	Yes
CD56	BB-700	BD	566400	
p24	RD1 (PE)	Beckman Coulter	6604667	Yes
Viability dye	Near-IR	Thermo Fisher	L34976	

(c)

Antibodies	Fluorescence	Source	Cat. Num.
His	APC	Miltenyi	130-119-782
IgG	PE	Biolegend	410708
Viability dye	Near-IR	Thermo Fisher	L34976

Supplementary Table 5. Sequences of HCAb expression plasmids. (a, b) Expression plasmid for HCAb J3-IgG1, with PGK promoter and (a) without and (b) with LS mutations (bolded and underlined) in IgG1 CH3. (c) Expression plasmid for HCAb eCD4-IgG1, with hybrid CMV/R promoter from plasmid CMVR-eCD4-IgG2-v26. A GGGG linker is present between CH3 and the R5m domain (italicized, underlined). (d) Expression plasmid for HCAb CD4-IgG1. The sequences are colored to indicate specific regions, as noted in construct name, with multiple cloning sites between segments in black text.

a. PGK-J3-HiCH2CH3-polyA

GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGG
GAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGTCCGTTTCGCAGCGTCACCCGGATCTTCGCCGCT
ACCCTTGTGGGCCCCCGGCGACGCTTCTGCTCCGCCCTAAGTCGGGAAGGTTCTTGCGGTTTCGCGGCGTGC
CGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGACAGCGCCAGGGAGCAATGGCAGCG
CGCCGACCGCGATGGGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGC
GGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCTTGCCGCGCGGTGTTCCGCATTCTGCAAGCC
TCCGGAGCGCACGTTCGGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAGGGGGATCCACTAGTC
CAGTGTGGTGAATTACCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTTGGCTATTTTAAAAGGTGTCCAGTGTGA
GGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGGCCGGGGGTTCTGAGACTCTCCTGTGAGCTGAGGGG
AAGCATCTTTAACCAGTATGCCATGGCCTGGTTCGCCAGGCTCCAGGGAAGGAGAGGGAGTTTCGTCGCCGGCATG
GGCGCCGTGCCCCACTACGGCGAGTTTCGTGAAGGGCCGTTTACCATCTCCAGAGACAATGCCAAGAGCACGGTG
TATCTGCAAATGAGCAGCCTGAAGCCCGAGGACACGGCCATCTATTTCTGTGCCAGGAGCAAGAGCACCTACATCAG
CTACAACAGCAACGGCTACGACTACTGGGGCAGGGGAACCCAGGTCACCGTGAGCAGCGAGCCGAAGAGCTGTGA
CAAAACACATACATGCCCCCCTGCCCGCGCCCGAGCTGCTTGCGGTCCAAGCGTGTTCCTGTTCCCCCCTAAG
CCCAAGGACACCCTGATGATCAGCAGAACCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCC
GAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTAGAGAGGAGCAGTAC
AACAGCACCTACAGAGTGGTGGAGCGTGTGACCGTGTGACCAAGACTGGCTGAACGGCAAGGAGTACAAGTGCA
AGGTGTCCAACAAGGCCCTGCCCGCCCCCATCGAGAAGACCATCAGCAAGGCCAAGGGCAGCCTAGAGAGCCCC
AAGTGTACACCCTGCCCCCTAGCAGAGACGAGCTGACCAAGAACCAAGTGAGCCTGACCTGCCTGGTGAAGGGCTT
CTACCCTAGCGACATCGCCGTGGAGTGGGAGAGCAACGGGCAGCCCGAGAACAACCTACAAGACCACCCCCCGT
GCTGGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCAGATGGCAGCAAGGCAACGTG
TTCAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACACAGAAGAGCCTGAGCCTGAGCCCGGGTAAAT
GAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCC
CTCCCCCGTGCTTCTTGACCCTGGAAGGTGCCACTCCACTGTCTTTCTAATAAAATGAGGAAATTGCATCGCA
TTGTCTGAGTAGGTGTATTCTATTCTGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAAT
AGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG

b. PGK-J3-HiCH2CH3(LS)-polyA

GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTTGCGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGG
GAAACGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGTCCGTTTCGCAGCGTCACCCGGATCTTCGCCGCT
ACCCTTGTGGGCCCCCGGCGACGCTTCTGCTCCGCCCTAAGTCGGGAAGGTTCTTGCGGTTTCGCGGCGTGC
CGGACGTGACAAACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGACAGCGCCAGGGAGCAATGGCAGCG
CGCCGACCGCGATGGGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCAGCGGCCGGGAAGGGGC
GGTGCGGGAGGCGGGGTGTGGGGCGGTAGTGTGGGCCCTGTTCTTGCCGCGCGGTGTTCCGCATTCTGCAAGCC
TCCGGAGCGCACGTTCGGCAGTCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCAGGGGGATCCACTAGTC
CAGTGTGGTGAATTACCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTTGGCTATTTTAAAAGGTGTCCAGTGTGA
GGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGGCCGGGGGTTCTGAGACTCTCCTGTGAGCTGAGGGG
AAGCATCTTTAACCAGTATGCCATGGCCTGGTTCGCCAGGCTCCAGGGAAGGAGAGGGAGTTTCGTCGCCGGCATG
GGCGCCGTGCCCCACTACGGCGAGTTTCGTGAAGGGCCGTTTACCATCTCCAGAGACAATGCCAAGAGCACGGTG
TATCTGCAAATGAGCAGCCTGAAGCCCGAGGACACGGCCATCTATTTCTGTGCCAGGAGCAAGAGCACCTACATCAG
CTACAACAGCAACGGCTACGACTACTGGGGCAGGGGAACCCAGGTCACCGTGAGCAGCGAGCCGAAGAGCTGTGA
CAAAACACATACATGCCCCCCTGCCCGCGCCCGAGCTGCTTGCGGTCCAAGCGTGTTCCTGTTCCCCCCTAAG
CCCAAGGACACCCTGATGATCAGCAGAACCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGCCACGAGGACCCC

GAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAGCCTAGAGAGGAGCAGTAC
AACAGCACCTACAGAGTGGTGAGCGTGCTGACCGTGCTGCACCAAGACTGGCTGAACGGCAAGGAGTACAAGTGCA
AGGTGTCCAACAAGGCCCTGCCCCGCCCCCATCGAGAAGACCATCAGCAAGGCCAAAGGGCAGCCTAGAGAGCCCC
AAGTGTACACCCTGCCCCCTAGCAGAGACGAGCTGACCAAGAACCAAGTGAGCCTGACCTGCCTGGTGAAGGGCTT
CTACCCTAGCGACATCGCCGTGGAGTGGGAGAGCAACGGGCAGCCCCGAGAACAACCTACAAGACCACCCCCCGT
GCTGGACAGCGACGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGAGACAAGAGCAGATGGCAGCAAGGCAACGTG
TTCAGCTGCAGCGTGCTGCACGAGGCCCTGCACTCCCACTACACACAGAAGAGCCTGAGCCTGAGCCCCGGGTAAAT
GAGTCTAGAGGGGCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCC
CTCCCCCGTGCTTCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCTAATAAAATGAGGAAATTGCATCGCA
TTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGGAGGATTGGGAAGACAAT
AGCAGGCATGCTGGGGATGCGGTGGGCTCTATGG

c. CMV/R-CD4d1d2-HiCH2CH3-R5m-polyA

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGT
TACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATG
TTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAG
TACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCC
CAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGTATGCGG
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAA
TGGGAGTTTGTGGTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGG
GCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCA
TCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCATCGGCTCGCATCTCTCCTTCACGCGC
CCGCCGCCCTACCTGAGGCCGCCATCCACGCCGTTGAGTCGCGTTCTGCCGCTCCCGCCTGTGGTGCCTCCTG
AACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTA
CCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTAGTTAACGGTGGAGGGCAGTGTAGT
CTGAGCAGTACTCGTTGCTGCCGCGCGCGCCACCAGACATAATAGCTGACAGACTAACAGACTGTTCTTTCCATGG
GTCTTTTCTGCAGTCACCGTCGTCGACACGTGTGATCAGATATCGCGGCCGCTCTAGACCACCATGCCCATGGGGTC
TCTGCAACCGCTGGCCACCTTGACCTGCTGGGGATGCTGGTGCCTTCCGTGCTAGCGAAGAAGGTGGTGGTGGGG
AAGAAGGGCGACACCGTGAGCTGACCTGCACCGCCTCCAGAGAAGAGCATCCAGTTCCACTGGAAGAACAGCA
ACCAGATCAAGATCCTGGGAAATCAGGGGAGCTTCTGACCAAAAGGCCCTCTAAACTGAACGACCGGGCTGACTC
CCGCCGATCCCTGTGGGATCAGGGCAACTTCCCTTTGATCATCAAAAACCTGAAGATCGAGGACTCCGACACCTACA
TCTGCGAGGTGGAGGATCAAAGGAGGAGGTGCAACTGCTGGTGTTCGGGCTGACCGCTAACAGCGATACCCACCT
GCTGCAAGGCCAGTCCCTGACCCTGACCCTGGAGAGCCCACCAGTTCCAGCCCTTCCGTGCAGTGCCGGTCTCCC
GGGGGCAAGAATATCCAGGGAGGCAAGACCCTGTCTGTGTCCAGCTGGAGCTCCAGGACAGCGGGACCTGGACC
TGTACCGTGCTGCAGGACCAGAAGACCGTGAGTTCAAGATCGACATCGTGGTGGTGGCTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCACCGTGCCACGACCTGAACCTCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTG
AGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA
CAGCAGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
GTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGG
TGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTA
TCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCT
GGACTCCGATGGCAGCTTCTTCTCTATTCAAAGCTGACAGTGGACAAATCCAGATGGCAGCAGGGGAACGTCTTTA
GCTGCTCTGTGATGCACGAGGCCCTGCACAATCATTACCCAGAAGAGTCTCTCACTGTCCCCCGGAAAGGGCGG
TGCGGGTGCGGATTATTACGATTACGACGGTGGCTATTACTATGATGGCGACTGAGGATCCAGATCTGCTGTGCCTT
CTAGTTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCTTCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTT
TCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGA
CAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGG

d. CMV/R-CD4dd2-HiCH2CH3-polyA

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGT
TACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCATTGACGTCAATAATGACGTATG

TTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAG
TACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCC
CAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGG
TTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAA
TGGGAGTTTGTGGTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGG
GCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCA
TCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCATCGGCTCGCATCTCTCCTTACGCGC
CCGCCGCCCTACCTGAGGCCGCCATCCACGCCGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTG
AACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTA
CCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTAGTTAACGGTGGAGGGCAGTGTAGT
CTGAGCAGTACTCGTTGCTGCCGCGCGCGCCACCAGACATAATAGCTGACAGACTAACAGACTGTTCTTTCCATGG
GTCTTTTCTGCAGTCACCGTCGTCGACACGTGTGATCAGATATCGCGGCCGCTCTAGACCACCATGCCCCATGGGGTC
TCTGCAACCGCTGGCCACCTTGTACCTGCTGGGGATGCTGGTGCCTTCCGTGCTAGCGAAGAAGGTGGTGGTGGGG
AAGAAGGGCGACACCGTGGAGCTGACCTGCACCGCCTCCCAGAAGAAGAGCATCCAGTTCCACTGGAAGAACAGCA
ACCAGATCAAGATCCTGGGAAATCAGGGGAGCTTCTGACCAAAGGCCCTCTAAACTGAACGACCGGGCTGACTC
CCGCCGATCCCTGTGGGATCAGGGCAACTTCCCTTTGATCATCAAAAACCTGAAGATCGAGGACTCCGACACCTACA
TCTGCGAGGTGGAGGATCAAAGGAGGAGGTGCAACTGCTGGTGTTCGGGCTGACCGCTAACAGCGATACCCACCT
GCTGCAAGGCCAGTCCCTGACCCTGACCCTGGAGAGCCCACCAGGTTCCAGCCCTTCCGTGCAGTGCCGGTCTCCC
GGGGGCAAGAATATCCAGGGAGGCAAGACCCTGTCTGTGTCCCAGCTGGAGCTCCAGGACAGCGGGACCTGGACC
TGTACCGTGCTGCAGGACCAGAAGACCGTGGAGTTCAAGATCGACATCGTGGTGGTGGCTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTG
AGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAA
CAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGG
TGACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTA
TCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCT
GGACTCCGATGGCAGCTTCTTCTCTATTCAAAGCTGACAGTGGACAAATCCAGATGGCAGCAGGGGAACGTCTTTA
GCTGCTCTGTGATGCACGAGGCCCTGCACAATCATTACCCAGAAGAGTCTCTCACTGTCCCCCGGAAAGTGAGGA
TCCAGATCTGCTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCTTCTTGACCCTGGAAGG
TGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGG
GGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCT
CTATGG

Supplementary Table 6. Primers and probes used for ddPCR.

Detection	Primer/ probe	Sequence
Total J3 HCAb or eCD4-IgG1 mRNA	dd-optHiCH2-F1	GGACACCCTGATGATCAGCAGA
	dd-optHiCH2-R1	CCTTTGGCCTTGCTGATGGTC
	dd-optHiCH2-probe (HEX)	/5HEX/CAGTACAAC/ZEN/AGCACCTACAGAGTGG/3IABkFQ/
J3 HCAb BCR or eCD4-IgG1 BCR mRNA	dd-optCH3M1-F1	CTGAGCCTGAGCCCGGAG
	dd-G4BCR-R2	GGCCCCCTGCCTTATCATGT
	dd-BCR-probe	/56-FAM/TCTTCTCCT/ZEN/CAGTGGTGGACCTGA/3IABkFQ/
J3 HCAb secreted mRNA	dd-optCH3-F1	CCTGCCCCCTAGCAGAGAC
	dd-optCH3-sec-R1	GGCACTCATTTACCCGGGCT
	dd-sec-probe	/56-FAM/TGACCAAGA/ZEN/ACCAAGTGAGCCTGA/3IABkFQ/
eCD4-IgG1 secreted mRNA	dd-optCH3-F1	CCTGCCCCCTAGCAGAGAC
	dd-CCR5m-R1	CGTCGTAATCGTAATAATCGCCACC
	dd-sec-probe	/56-FAM/TGACCAAGA/ZEN/ACCAAGTGAGCCTGA/3IABkFQ/