Supplementary Information

In-Depth Determination and Analysis of the Human Paired Heavy and Light Chain Antibody Repertoire

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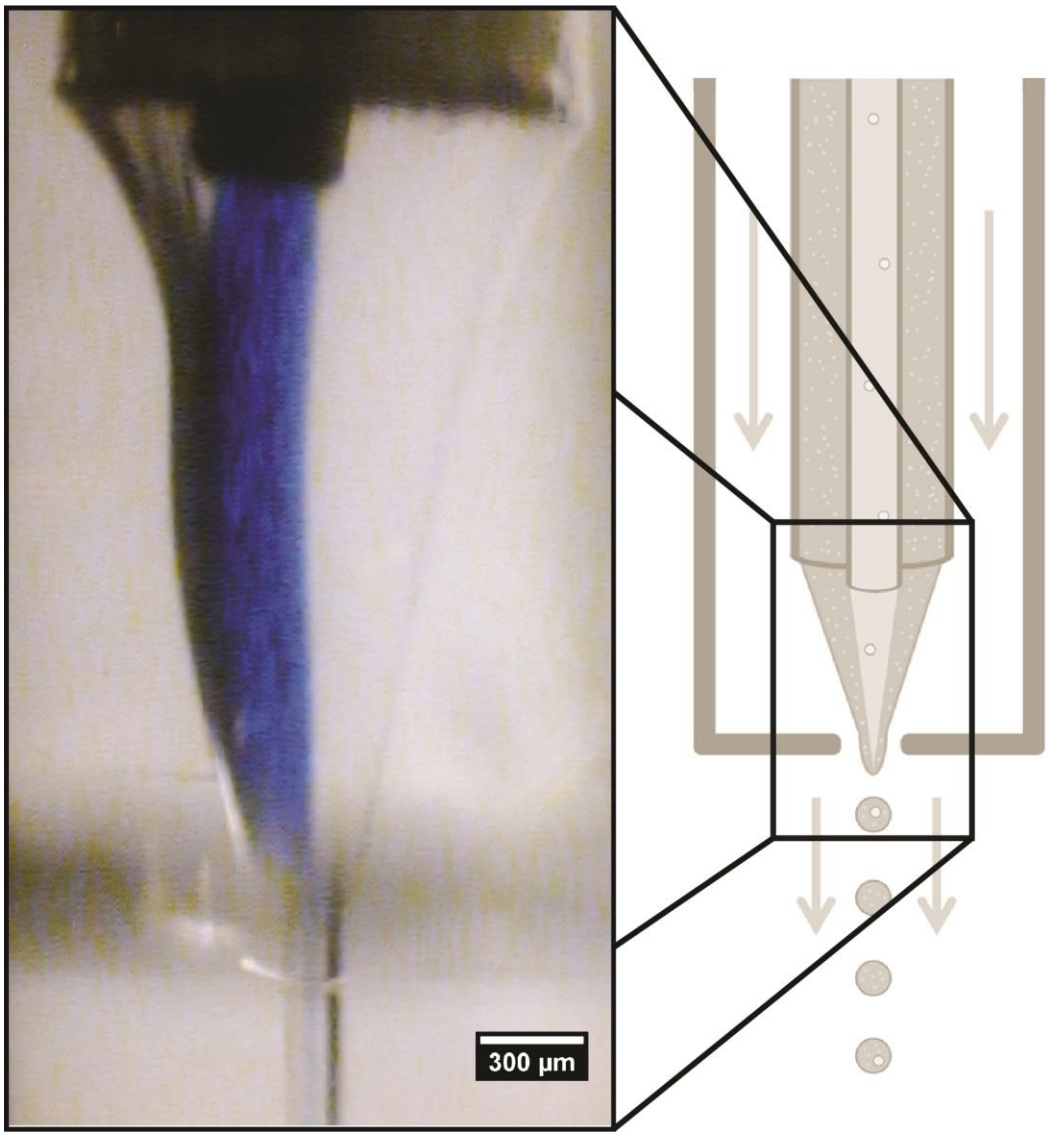

Supplementary Figure 1 A micrograph of the axisymmetric flow-focusing nozzle during emulsion generation (left), placed in context of the diagram from Figure 1a (right), where PBS/0.4% Trypan blue exits the inner needle and cell lysis buffer exits the outer needle.

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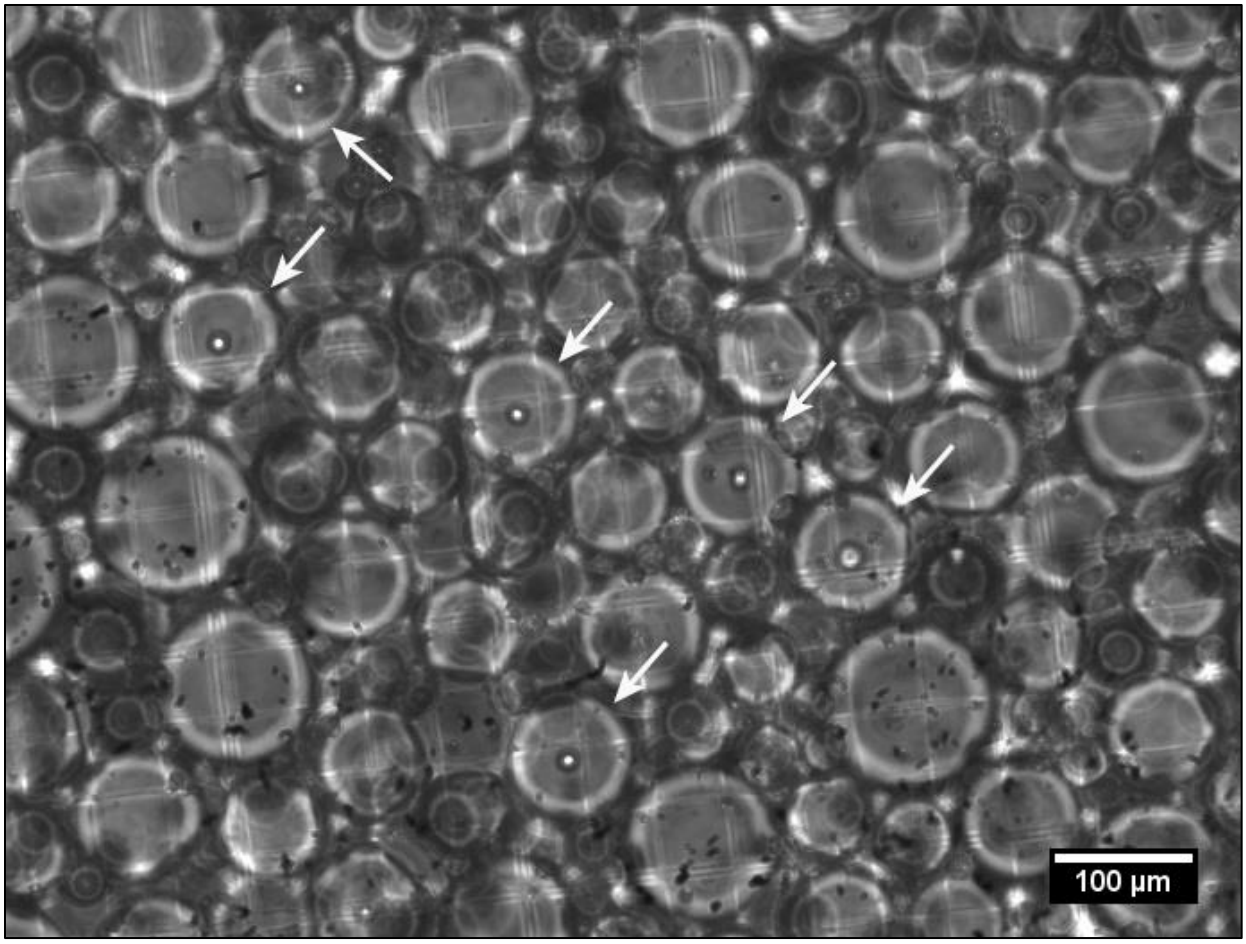

Supplementary Figure 2 MOPC-21 immortalized B cells encapsulated in emulsion droplets. The outer aqueous stream that normally contains cell lysis buffer (Fig. 1a, gray solution) was replaced with 0.4% Trypan blue in PBS to examine cell viability throughout the flow focusing and emulsification process. Emulsified cell viability was approximately 90% and cell viability did not differ substantially from non-emulsified controls.

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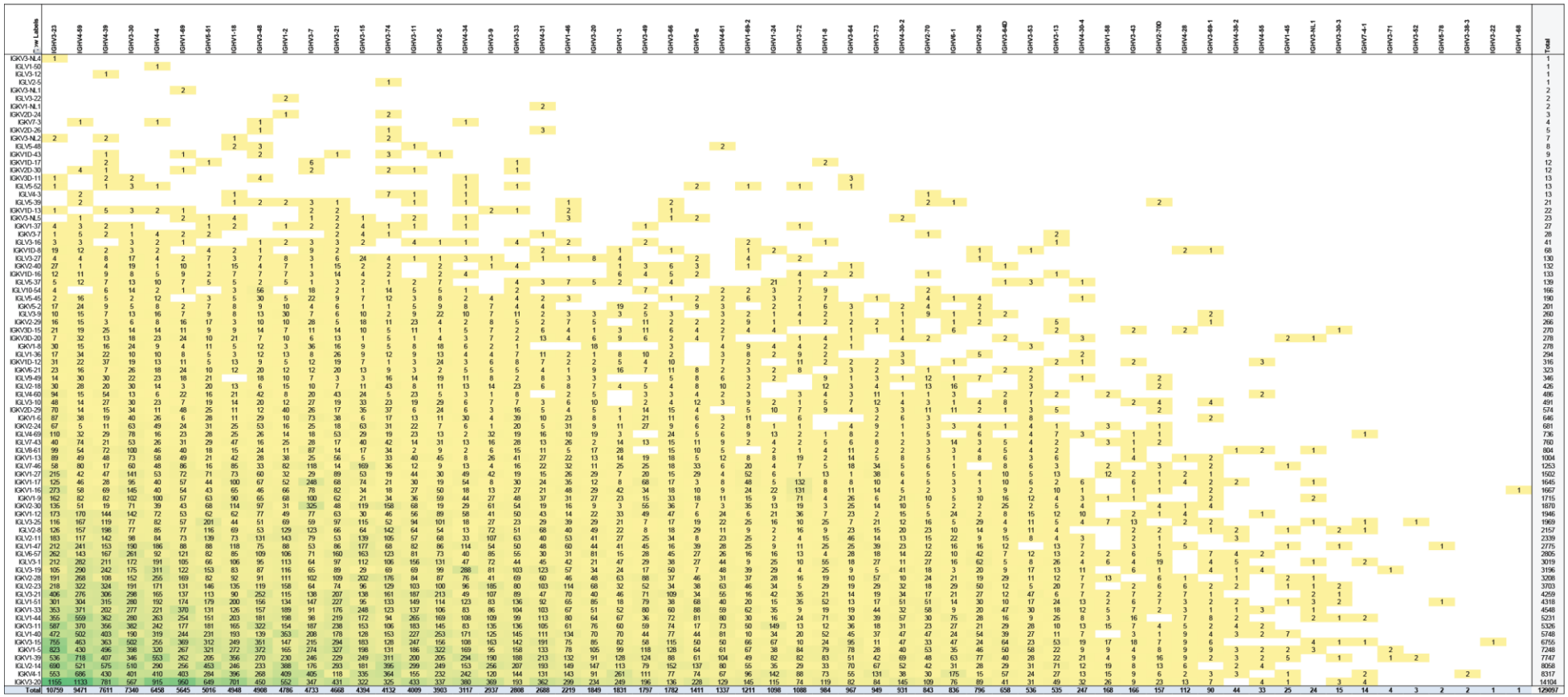

Supplementary Figure 3 Heat map of V-gene usage for 129,097 VH:VL clusters recovered from Donor 1. Sequences were collected using primers targeting the framework 1 region; raw data is available in the online supplement.

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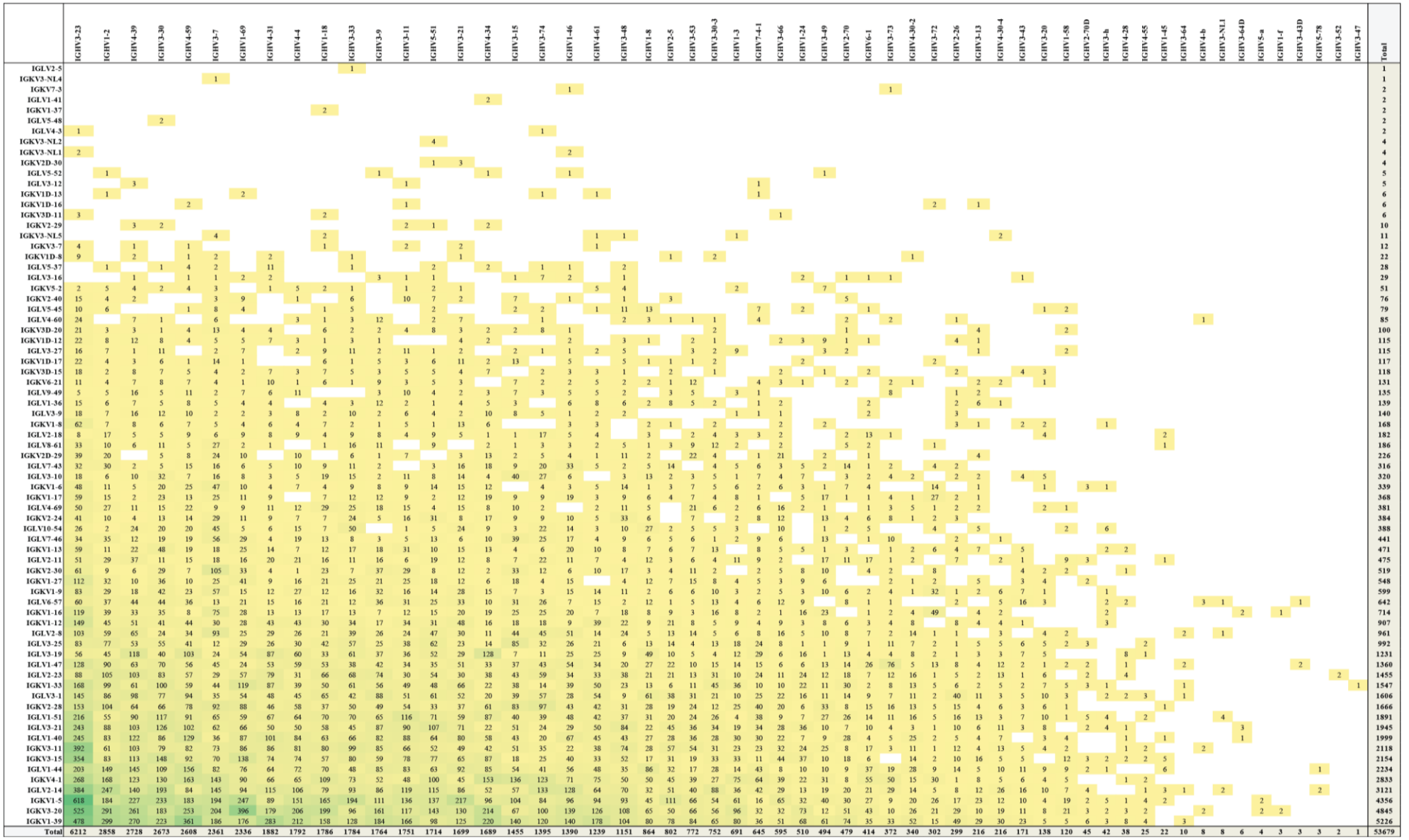

Supplementary Figure 4 Heat map of V-gene usage for 53,679 VH:VL clusters recovered from Donor 2. Sequences were collected using primers targeting the framework 1 region; raw data is available in the online supplement.

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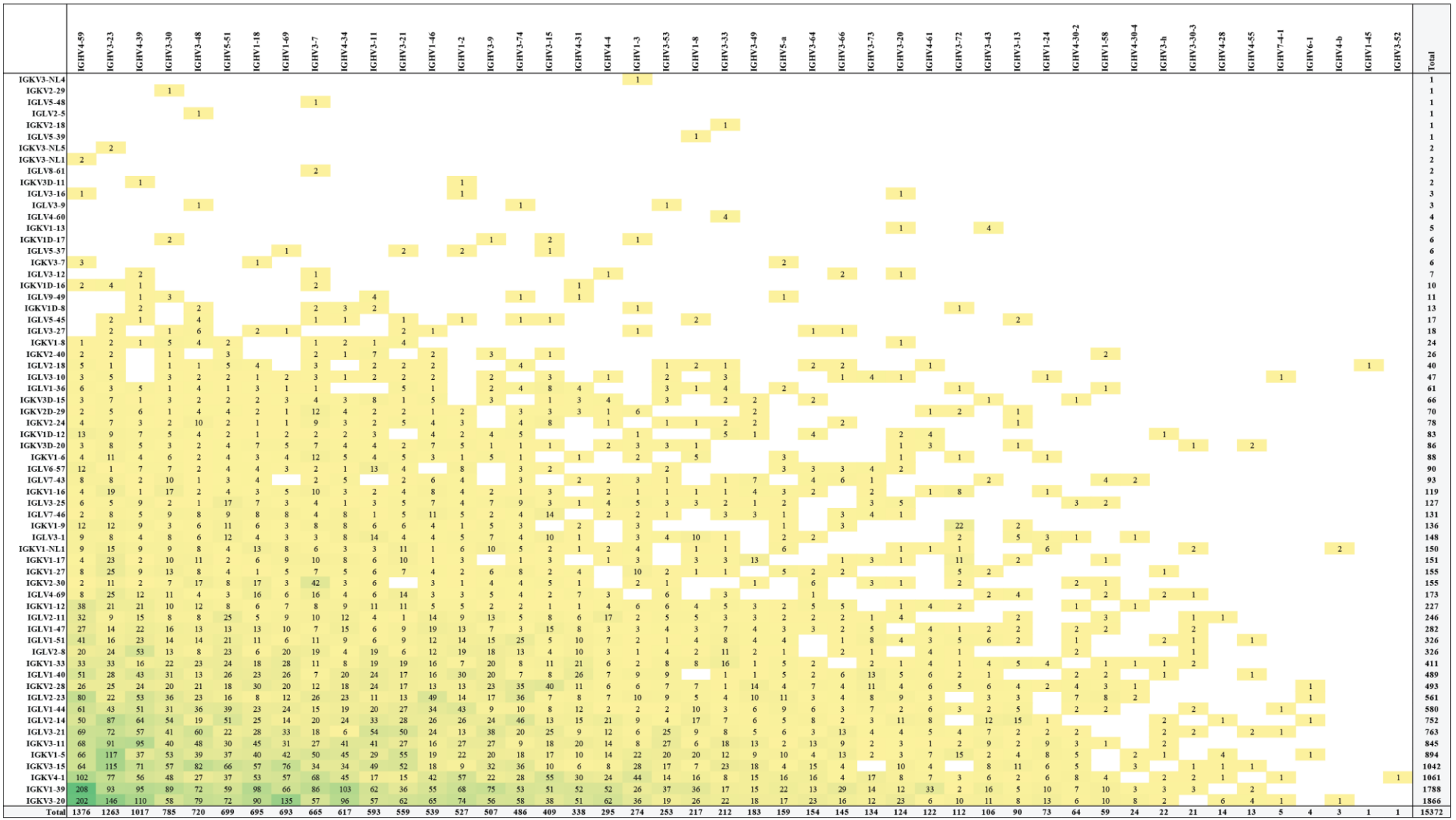

Supplementary Figure 5 Heat map of V-gene usage for 15,372 VH:VL clusters recovered from Donor 3. Sequences were collected using primers targeting the leader peptide region; raw data is available in the online supplement.

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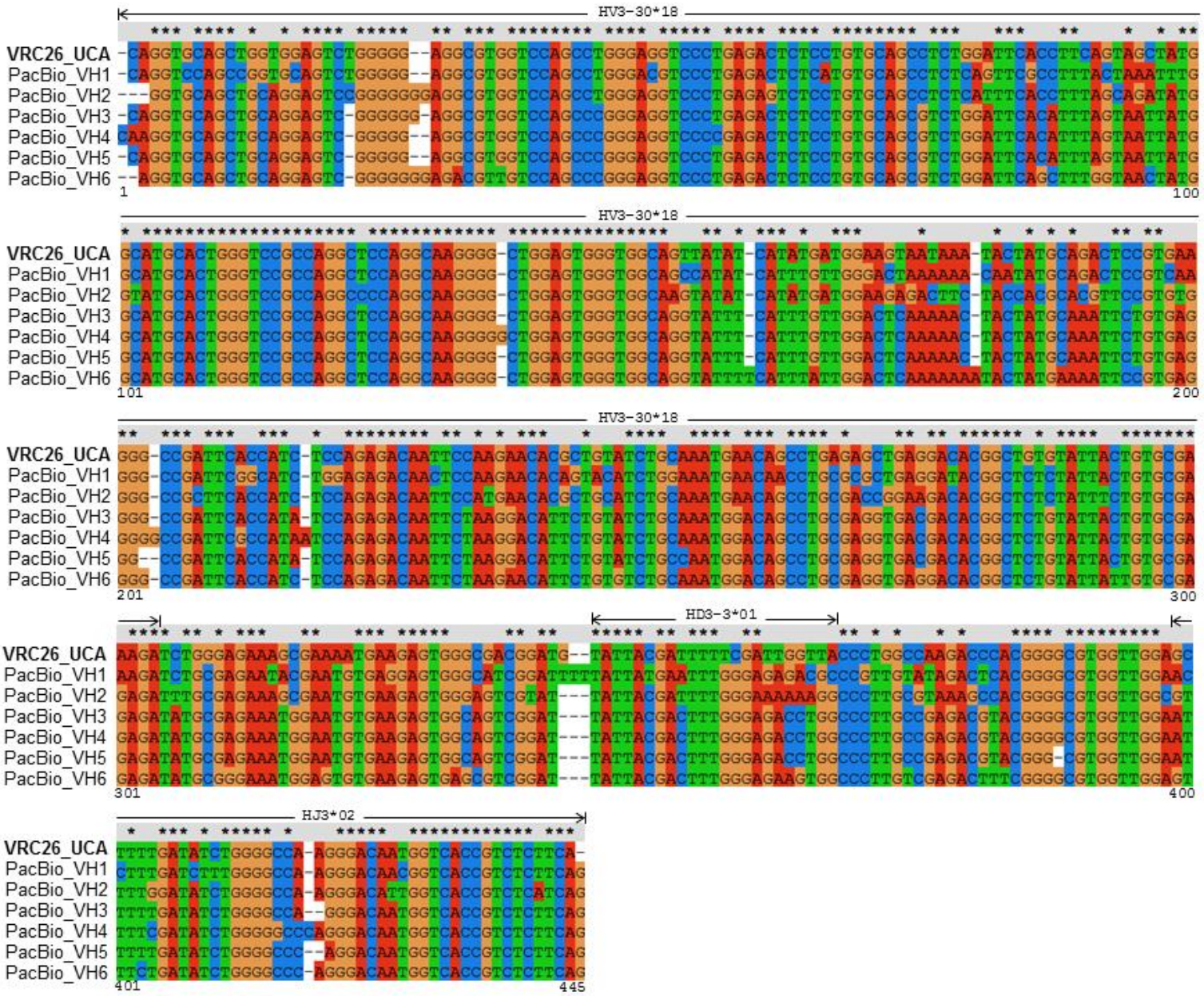

Supplementary Figure 6 VH alignment of the six VRC26 HIV broadly neutralizing antibody variants recovered by PacBio sequencing of complete ~850bp VH:VL amplicons. Sequences were recovered from CD27^{+} peripheral B cells of the CAP256 donor and aligned to the VRC26 VH unmutated common ancestor (UCA, Doria-Rose et al., *Nature* 2014). Corresponding light chain variants are shown in Supplementary Figure 7.

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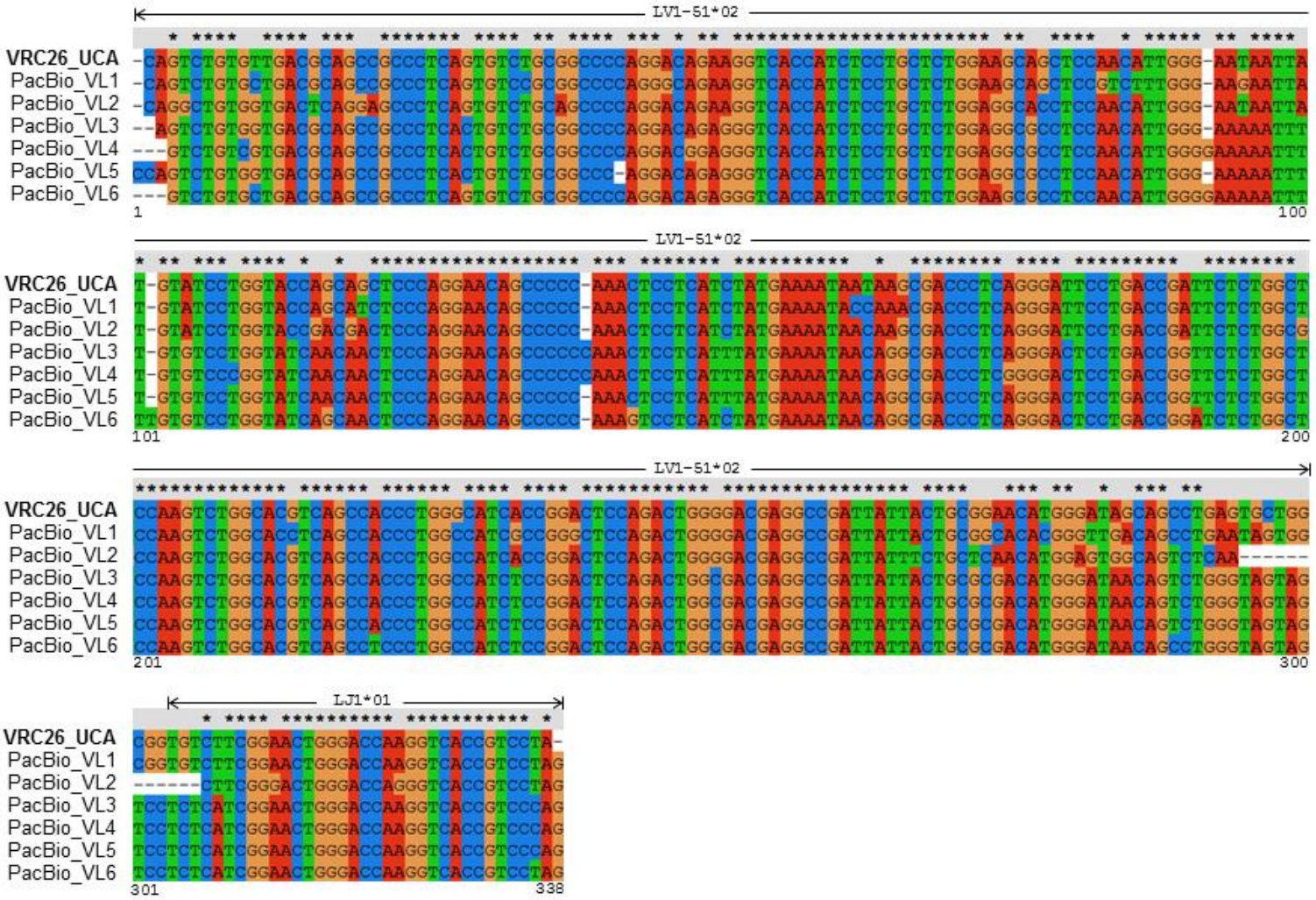

Supplementary Figure 7 VL alignment of the six VRC26 HIV broadly neutralizing antibody variants recovered by PacBio sequencing of complete ~850bp VH:VL amplicons. Sequences were recovered from $CD27^{+}$ peripheral B cells of the CAP256 donor and aligned to the VRC26 VL unmutated common ancestor (UCA, Doria-Rose et al., *Nature* 2014). Corresponding heavy chain variants are shown in Supplementary Figure 6.

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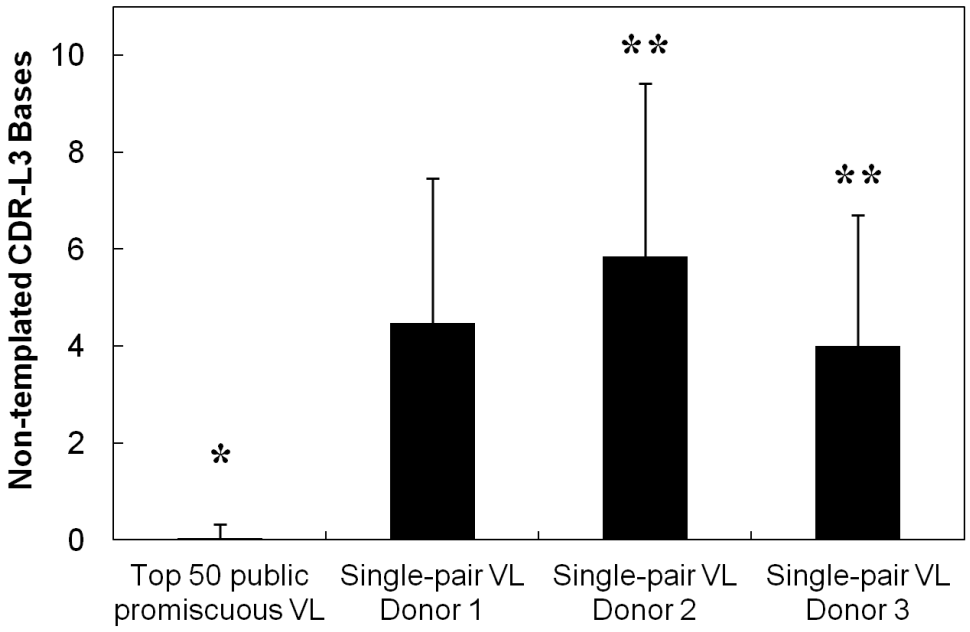

Supplementary Figure 8 Comparison of the number of non-templated bases (sum of somatic mutations and non-templated insertions) in the top 50 public, promiscuous VL nucleotide junctions shared by Donors 1, 2, and 3 to 50 randomly selected VL junctions paired with only a single heavy chain in the Donor 1, Donor 2, or Donor 3 repertoires (mean±s.d.). Statistical significance noted where $p < 0.05$ (* $p < 10^{-10}$ compared to all other groups, ** $p = 0.0043$).

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Supplementary Table 1 VH:VL pairing analysis of a mixture of HEK293 cells transfected with 11 different known antibodies. The maximum read count for each row and column is highlighted; 11/11 antibodies were identified and paired correctly in this control experiment. Read count variation was expected due to varying transfection & expression efficiency for the 22 distinct heavy and light chain plasmids, and antibody clones #10 and 11 exhibited notable VH-VL imbalance by total read counts. The signal:topVLnoise ratio (the relevant parameter for native pair assignment, see Supplementary Table 2) averaged 35:1 overall and 87:1 if noise from light chains 10 and 11 (which showed VH-VL imbalance, see total VH and VL reads) was excluded.

		Heavy Chain											
		1H	**2H**	**3H**	**4H**	**5H**	**6H**	**7H**	**8H**	**9H**	**10H**	**11H**	**Total**
Light Chain	**1L**	**1,842**	4	20	13	18	16	39	20	49	6	4	**2,031**
	2L	0	**4,916**	34	31	59	41	102	127	146	28	8	**5,492**
	3L	0	2	**6,251**	9	38	25	116	60	118	13	2	**6,634**
	4L	21	27	75	**14,592**	81	158	348	189	397	75	51	**16,014**
	5L	5	15	97	41	**16,204**	99	192	231	277	86	19	**17,266**
	6L	2	12	92	37	64	**16,427**	358	180	404	62	23	**17,661**
	7L	9	13	218	72	112	180	**21,315**	203	1,320	78	45	**23,565**
	8L	4	39	85	71	242	145	365	**32,393**	506	79	72	**34,001**
	9L	4	29	182	105	116	186	1,335	323	**35,391**	109	46	**37,826**
	10L	12	24	944	189	1,597	1,080	3,519	1,898	4,291	**8,535**	98	**22,187**
	11L	32	66	1,153	272	1,258	1,655	6,405	6,567	6,185	555	**14,126**	**38,274**
	Total	**1,931**	**5,147**	**9,151**	**15,432**	**19,789**	**20,012**	**34,094**	**42,191**	**49,084**	**9,626**	**14,494**	**220,951**

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Supplementary Table 2 Accuracy statistics for human VH:VL paired analysis with an ARH-77 immortalized cell line control spike.

Estimated input human B cells	20,000
Estimated ARH-77 spiked cells	260
VH:VL Reads after CDR3 clustering	403,897
Recovered CDR-H3:CDR-L3 Clusters	1,751
Correct ARH-77 VH:VL Reads (Signal)	2,604
ARH-77 Top Incorrect VL Reads (topVLnoise)	27
ARH-77 2nd-Ranked Incorrect VL Reads	19
ARH-77 3rd-Ranked Incorrect VL Reads	16
ARH-77 Signal:topVLnoise Ratio*	96.4

* The key metric for VH:VL pair assignment (see main text)

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Supplementary Table 3 Memory B cell counts before and after *in vitro* activation. Values must be considered rough estimates due to varying contributions of hemocytometer sampling, centrifugation/recovery cell loss, and cell death, stasis, and expansion over four days *in vitro*.

Sample	*FACS Count* **Fresh Bmems**	*Hemocytometer Count* **After 4d Activation**
Donor 1	1.8 million	1.6 million viable
Donor 2	1.1 million	1.3 million viable
Donor 3	347k	300k viable
ARH-77 spike experiment	87k	20k viable

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Supplementary Table 4 Leader peptide overlap extension primers.

Conc (nM)	Primer ID	Primer Sequence
40	VH1_LP	tattcccatcgcggcgcACAGGTGCCCACTCCCAGGTGCAG
40	VH3_LP	tattcccatcgcggcgcAAGGTGTCCAGTGTGARGTGCAG
40	VH4/6_LP	tattcccatcgcggcgcCCCAGATGGGTCCTGTCCCAGGTGCAG
40	VH5_LP	tattcccatcgcggcgcCAAGGAGTCTGTTCCGAGGTGCAG
40	hVλ1for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAGGTCCTGGGCCCAGTCTGTGCTG
40	hVλ2for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAGGTCCTGGGCCCAGTCTGCCCTG
40	hVλ3for-2_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAYWCTGCACAGGCTCTGTGACCTCCTAT
40	hVλ4/5for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAGGTCTCTCTCSCAGCYTGTGCTG
40	hVλ6for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAGTTCTTGGGCCAATTTTATGCTG
40	hVλ7for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAGGTCCAATTCYCAGGCTGTGGTG
40	hVλ8for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAGAGTGGATTCTCAGACTGTGGTG
40	hVκ1/2for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAATGAGGSTCCCYGCTCAGCTGCTGG
40	hVκ3for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCACTCTTCCTCCTGCTACTCTGGCTCCCAG
40	hVκ4for_LP	gcgccgcgatgggaataNNNNNNNNNNNNNNNNTCTGCTCGAGTTCGGTCAATTTCTCTGTTGCTCTGGATCTCTG

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