

**Supplementary Table 4a| B2a Selected Sequences**

<b>B2a-1</b>						<b>B2a-2</b>						
<b>1405</b>	<b>1407</b>	<b>1494</b>	<b>1495</b>	<b>1496</b>	<b>No.</b>	<b>1407</b>	<b>1408</b>	<b>1409</b>	<b>1491</b>	<b>1494</b>	<b>No.</b>	
<b>G</b>	<b>C</b>	<b>G</b>	<b>T</b>	<b>C</b>		<b>C</b>	<b>A</b>	<b>C</b>	<b>G</b>	<b>G</b>		
G	C	G	T	C	32	C	A	C	G	G	33	
T	T	T	T	A	2	G	C	A	G	A	1	
G	C	G	T	T	2	C	A	C	A	G	13	
G	G	T	G	G	1	C	G	A	T	G	2	
T	T	C	A	C	1	C	A	A	T	G	1	
G	T	C	T	T	1	G	C	G	G	A	1	
T	A	T	G	G	1	C	G	A	C	G	1	
G	G	T	A	A	1	C	A	T	G	G	2	
G	A	G	T	C	1	C	G	T	C	G	4	
G	C	G	A	C	1	C	A	T	T	G	4	
T	T	A	C	G	1	A	C	T	C	A	1	
A	A	G	T	C	1	C	G	A	C	G	1	
C	G	G	C	T	1	G	A	T	T	T	1	
C	G	G	T	C	1	C	A	A	C	G	1	
G	C	A	G	T	1	T	C	C	A	G	1	
						A	T	T	G	T	1	
						C	A	T	A	G	1	
						C	G	T	G	G	1	
						C	G	C	T	G	1	
<b>Freq</b>	40	36	39	40	37	48	65	56	48	39	66	71
<b>Z-score</b>	10.8	8	9.9	10.8	8.6		20.2	11.12	7.7	5.1	22.4	

**Supplementary Table 4b** B2b Selected Sequences

B2b-1				B2b-2						B2b-3		
791	792	793	No.	1514	1515	1516	1520	1521	No.	1517	1518	no.
G	A	T		G	G	G	C	C		G	A	
G	A	T	9	G	T	G	C	T	1	G	A	68
G	A	A	15	A	T	G	G	T	2	G	G	2
G	G	A	4 <sup>1</sup>	C	C	G	G	A	1	A	A	6
G	T	G	10	A	T	C	T	T	1			
G	G	T	13	C	G	G	G	G	1			
G	C	G	4	A	G	G	C	G	1			
G	G	C	2	T	T	C	G	G	1			
G	A	G	1	T	T	G	C	G	1			
G	A	C	2	C	G	G	T	T	4			
T	C	T	1	G	T	G	T	T	2			
G	C	T	2	A	T	C	G	T	1			
A	T	A	1	T	T	G	C	T	1			
G	T	A	2	G	C	G	T	G	1			
G	T	T	1	C	T	G	G	T	1			
T	A	T	1	G	C	A	G	C	1			
G	T	C	1	C	T	G	A	C	1			
				G	T	G	G	A	1			
				T	T	G	G	T	1			
				C	G	G	A	A	1			
				G	C	T	G	G	1			
				G	G	G	G	C	1			
				G	G	G	C	G	1			
				G	T	T	G	C	1			
				T	T	T	T	T	1			
				G	T	G	G	G	1			
				T	C	T	G	G	1			
				G	T	G	A	G	1			
				T	G	C	T	T	1			
				C	T	G	G	T	1			
				G	G	A	T	T	4			
				G	T	C	T	T	1			
				T	T	G	G	A	1			
				G	T	C	G	C	1			
				T	G	T	T	T	6			
				G	A	C	T	T	1			
				G	C	G	T	C	1			
				C	T	T	T	G	1			
				T	T	G	T	T	3			
				A	C	G	T	T	1			
				T	T	G	T	G	2			
				C	G	G	C	C	1			
				G	T	G	T	C	1			
				A	T	T	G	T	2			
				G	G	G	T	A	1			
				C	C	T	G	T	1			
				C	A	T	T	G	2			
				T	A	G	T	T	1			
				C	C	T	G	A	1			
				G	G	G	A	T	2			
				G	G	G	A	G	1			
				T	G	T	T	T	1			
				T	T	G	A	G	1			
				A	C	T	A	G	1			

					C	T	G	A	G	1			
					C	A	G	T	T	1			
					G	A	G	G	T	1			
					T	C	G	T	G	1			
					A	G	G	G	T	1			
<b>Freq</b>	66	28	27	69	26	27	46	6	8	77	70	74	76
<b>Z-score</b>	28.8	2.6	2.4		1.6	1.9	6.2	-5.6	-4.2		21.7	32.2	

**Supplementary Table 4c** B2c Selected Sequences

B2c-1							B2c-2					
770	771	808	809	899	900	No.	772	773	806	807	No.	
C	G	C	G	C	A		T	G	C	A		
C	G	C	G	C	A	5	T	G	C	A	0	
G	C	T	T	T	A	1	C	A	C	T	1	
G	T	A	G	T	C	1	C	C	C	G	3	
C	T	T	T	C	A	3	T	C	T	A	1	
C	C	G	G	A	A	3	A	G	G	G	3	
C	T	C	T	T	T	1	C	C	G	C	2	
C	C	A	G	C	A	1	C	A	G	G	3	
T	A	T	T	C	A	1	A	A	T	C	1	
A	A	T	G	C	A	2	T	A	G	G	10	
T	T	C	A	C	A	1	C	C	T	G	7	
C	A	C	G	A	A	2	T	T	G	A	1	
C	T	T	T	C	A	3	T	T	C	A	1	
G	C	G	T	C	A	1	C	C	G	T	4	
T	C	T	A	C	A	1	A	T	G	A	1	
C	C	C	G	C	A	2	C	C	G	G	7	
C	C	G	A	C	A	3	A	C	A	T	1	
G	T	G	C	C	A	1	C	T	T	T	3	
A	A	T	G	T	G	1	C	A	G	G	2	
C	G	T	G	C	A	1	T	A	A	A	1	
A	C	G	C	G	T	1	T	C	G	T	1	
G	T	T	C	C	A	10	C	A	C	G	1	
C	C	G	G	C	A	1	T	C	G	A	2	
T	C	G	A	C	A	1	C	C	A	G	2	
G	C	A	C	C	A	1	T	C	T	G	1	
T	C	T	T	T	C	1	T	A	G	A	5	
C	C	T	G	C	A	3	T	G	A	G	1	
T	C	G	T	C	A	1	C	T	G	G	3	
A	G	C	C	T	C	1	C	T	A	T	2	
T	A	T	G	C	A	1	T	T	T	G	1	
G	C	T	A	T	C	1	A	C	G	G	1	
G	C	C	G	A	T	1	T	A	T	T	1	
T	A	T	A	T	G	1	C	A	T	T	2	
T	G	T	T	A	T	1	T	C	G	G	1	
T	C	T	T	C	A	2						
C	G	A	T	G	G	1						
A	A	T	C	T	A	1						
G	T	C	C	C	A	1						
G	T	A	G	G	C	1						
A	C	A	G	A	C	1						
A	T	C	T	T	G	1						
T	G	A	T	C	C	1						
T	C	G	C	A	A	1						
A	C	C	A	C	T	1						
T	T	A	A	T	G	1						
<b>Freq</b>	29	10	16	26	48	54	71	27	4	6	12	76
<b>Z-score</b>	2.7	-2.6	-0.5	2.0	7.7	10.1		1.9	-7.1	-5.0	-1.8	

**Supplementary Table 4d** B3 Selected Sequences

<b>B3-1</b>						<b>B3-2</b>					
<b>1415</b>	<b>1416</b>	<b>1483</b>	<b>1484</b>	<b>1485</b>	<b>No.</b>	<b>1417</b>	<b>1418</b>	<b>1482</b>	<b>1483</b>	<b>No.</b>	
<b>G</b>	<b>G</b>	<b>A</b>	<b>C</b>	<b>T</b>		<b>G</b>	<b>A</b>	<b>G</b>	<b>A</b>		
G	G	A	C	T	3	G	A	G	A	45	
T	A	A	A	T	1	A	A	G	A	16	
G	G	C	A	T	1	T	A	G	C	2	
C	T	A	G	G	1	G	A	A	A	1	
T	G	A	T	G	6	C	G	G	A	1	
T	G	A	C	G	2	G	C	A	A	3	
A	T	A	G	C	1						
A	A	A	G	T	1	G	A	G	G	1	
T	C	G	G	C	1	A	A	A	A	1	
G	C	C	G	G	1	G	G	A	A	1	
T	C	A	G	G	2	A	T	G	A	2	
T	G	A	C	T	1						
T	A	C	T	A	1						
T	T	C	A	G	1						
A	G	A	C	G	1						
T	G	C	C	G	1						
G	T	A	A	G	1						
A	G	A	G	G	1						
G	A	A	T	A	3						
T	C	A	G	C	1						
C	T	G	G	G	1						
C	C	A	G	G	1						
T	G	T	C	G	1						
T	G	C	T	A	1						
C	G	G	C	G	1						
T	A	A	T	G	3						
T	T	A	G	G	2						
C	A	A	C	G	7						
G	G	C	C	C	4						
G	T	A	A	A	1						
G	C	A	G	G	4						
T	C	A	G	A	1						
T	C	G	G	A	1						
G	G	A	T	C	1						
T	T	A	A	A	1						
A	C	A	G	T	1						
C	G	A	C	G	1						
G	T	A	A	C	1						
G	T	A	A	T	2						
G	T	C	T	C	1						
T	G	A	T	A	1						
G	A	A	T	C	1						
<b>Freq.</b>	24	26	53	22	10	69	51	66	67	70	73
<b>Z-score</b>	1.7	2.2	10.2	1.2	-2.5		9.3	28.8	35.7	30.5	

Supplementary Table 4e| B5 Selected Sequences

B3-3						B6-3				
1418	1419	1420	1480	1481	No.	1424	1425	1475	1476	No.
A	G	T	A	T		T	T	G	A	
A	G	T	A	T	1	T	T	G	A	3
T	A	G	G	T	1	C	T	T	T	1
T	G	A	T	T	1	A	T	T	T	1
T	G	G	A	T	1	C	A	T	T	1
T	A	T	A	A	1	C	T	G	G	2
T	G	G	A	A	1	T	G	A	T	5
A	C	C	G	G	1	C	C	T	A	2
A	T	G	T	G	1	T	C	G	A	1
A	G	C	T	G	1	T	T	C	C	1
G	G	T	A	A	1	T	C	A	G	1
G	C	T	C	T	1	C	T	A	T	1
T	T	T	G	T	1	C	T	G	T	1
G	A	A	G	G	1	A	G	T	T	2
G	G	C	T	G	1	G	T	C	A	1
C	A	T	A	G	1	A	A	T	C	3
T	G	G	A	G	1	A	A	A	T	1
G	G	A	A	G	1	A	A	T	G	1
T	A	C	A	G	1	A	A	T	T	2 <sup>1</sup>
T	T	C	A	G	1	A	G	G	G	1
G	C	A	G	G	1	A	G	G	T	2
C	T	T	G	T	1	A	T	G	T	3
G	A	A	A	T	1	C	C	C	C	1
G	C	G	T	T	1	C	C	T	G	2
T	G	G	G	G	1	T	T	T	A	1
A	G	C	T	C	1	T	A	A	T	1
C	C	T	G	T	1	T	A	T	A	1
C	C	T	G	A	1	C	C	C	T	2
G	C	C	G	G	1	G	A	G	T	1
A	T	C	T	T	1	C	T	C	A	1
G	T	T	C	A	1	G	G	G	G	1
G	C	G	C	T	1	A	A	C	T	2
C	A	C	G	A	1	A	T	G	T	2
C	A	T	T	T	1	T	G	A	T	4
T	G	G	T	T	2	T	T	G	T	2
C	A	G	A	A	1	G	T	G	A	2
T	G	T	G	A	2	T	T	T	T	1
T	G	T	T	C	6	G	C	T	T	1
C	A	G	C	G	1	A	C	G	C	1
A	A	T	G	G	1	T	G	T	C	1
						T	G	G	C	1
						T	T	G	A	2
						C	A	C	C	2
						C	G	G	A	1
						C	C	C	T	1
						C	C	T	A	1
						C	C	G	C	1
						A	G	C	C	1
						G	A	G	G	1
						C	C	T	G	1
						C	T	T	C	1
						C	G	T	C	1
						C	A	G	G	1
						A	T	G	G	1

						C	C	G	T	1	
						A	A	G	T	2	
						G	C	T	A	1	
						G	G	T	G	1	
						C	G	G	C	1	
						T	T	G	G	1	
						A	A	A	A	1	
						G	G	A	G	1	
						A	T	A	A	1	
						A	A	T	C	2	
<b>Freq</b>	7	21	20	12	15	46	26	29	35	19	91
<b>Z-score</b>	-1.8	2.8	2.5	0.2	1.1		0.8	1.4	2.6	-1.0	

Supplementary Table 4f| B6 Selected Sequences

B6-1							B6-2				
1426	1427	1428	1472	1473	1474	No.	1429	1430	1470	1471	No.
G	C	A	T	G	T		A	A	T	T	
G	C	A	T	G	T	3	A	A	T	T	4
T	A	A	C	A	G	1	A	C	A	T	1
G	C	A	C	C	G	1	C	C	T	T	1
T	C	A	T	A	G	1	A	T	G	T	1
T	A	A	G	T	T	1	A	C	G	C	1
C	T	A	C	C	A	1	A	A	G	G	1
A	A	C	A	G	T	1	C	A	C	G	1
C	A	C	G	G	C	1	A	A	C	T	1
A	C	G	G	A	T	1	C	T	A	C	1
G	A	C	C	G	T	1	A	T	T	T	1
A	A	A	A	T	C	1	A	C	T	T	6
G	C	C	T	A	T	1	A	C	C	T	2
A	A	A	T	C	A	1	C	G	A	T	1
C	A	T	C	C	A	1	A	G	G	G	2
C	G	A	T	C	A	1	C	G	C	T	1
A	T	A	T	C	T	1	A	G	C	T	1
C	C	A	G	G	G	1	A	T	G	T	1
C	A	A	G	C	T	1	A	G	C	G	1
A	T	G	A	G	G	1	C	A	C	A	1
G	T	A	C	T	T	1	A	T	C	C	2
G	G	A	C	T	T	1	C	T	A	A	1
C	C	A	T	T	T	1	C	C	A	A	1
G	C	A	T	C	G	1 <sup>1</sup>	A	C	G	A	1
T	C	A	C	T	G	1	G	C	G	T	2
G	G	A	C	C	T	1	A	C	A	G	1
G	G	A	G	C	A	1	C	C	C	T	2
T	A	C	C	A	C	1	C	C	T	G	2
G	G	A	C	A	C	1	A	G	C	C	1
C	T	A	G	G	T	1	C	C	T	T	3
A	C	A	C	A	T	1	A	T	T	T	2
G	A	A	C	G	G	1	A	G	T	A	5
T	C	A	A	T	G	1	A	C	G	G	1
A	T	G	A	T	G	1	C	C	T	A	1
T	T	A	G	G	T	1	T	T	A	G	1
A	T	A	C	A	G	1	A	C	C	A	1
G	C	C	T	C	T	1	C	T	G	A	1
A	C	C	A	T	C	1	T	A	A	G	1
A	T	C	T	A	G	1	G	C	T	T	1
A	A	G	T	T	A	1	C	C	C	A	1
G	G	C	C	G	A	1	G	T	C	T	1
G	T	A	G	G	A	1	T	C	C	T	1
T	A	C	T	C	T	1	A	A	T	G	2
A	G	A	T	A	T	1	G	G	T	T	1
A	A	A	A	T	T	9	C	C	A	T	1
G	A	A	G	C	T	1	T	C	A	T	1
G	A	A	C	C	G	2	C	A	A	G	1
C	G	A	C	A	T	1	G	C	G	G	1
T	C	C	A	G	G	1	G	A	T	T	1
C	T	A	A	A	G	1	A	T	C	A	1
A	T	A	G	G	T	2	A	A	A	T	1
A	A	T	A	C	G	1					
T	A	A	C	C	T	1					
G	G	C	T	A	T	1					





T	A	A	G	C	G	1						
A	C	A	G	C	A	1						
C	C	A	T	A	G	2						
T	T	A	G	C	G	2						
G	G	A	C	G	T	1						
A	C	C	G	A	G	1						
G	A	T	T	A	T	1						
A	A	A	T	A	T	1						
G	T	C	G	G	G	1						
G	T	A	A	T	T	1						
T	A	A	A	T	C	1						
G	A	C	G	A	G	1						
G	A	C	A	C	T	1						
C	A	A	C	C	T	1						
T	G	C	A	T	G	1						
C	G	T	G	T	A	1						
G	A	A	C	T	G	1						
A	A	A	G	T	A	1						
A	A	A	G	T	A	1						
C	A	T	C	A	A	1						
A	G	A	A	G	T	1						
C	A	A	A	C	G	1						
A	C	A	G	G	T	1						
T	C	G	T	G	C	1						
A	A	A	T	G	T	1						
G	A	A	T	G	A	1						
A	A	A	A	C	A	1						
<b>Freq.</b>	47	34	116	35	49	81	174	40	13	29	37	72
<b>Z-score</b>	0.6	-1.8	11.7	-1.6	0.9	5.7		5.2	-1.5	2.6	4.5	

**Supplementary Table 4g| B7a Selected Sequences**

	<b>700</b>	<b>701</b>	<b>702</b>	<b>703</b>	
	<b>G</b>	<b>T</b>	<b>A</b>	<b>G</b>	<b>No.</b>
G	T	A	G	9	
G	T	T	T	3	
T	T	A	C	1	
T	A	A	T	1	
C	T	A	A	1	
G	T	A	C	5	
G	C	G	G	1	
G	C	A	T	1	
C	T	G	T	1	
G	C	T	A	1	
T	A	C	A	1	
G	C	G	A	1	
G	A	A	T	1	
G	C	T	T	1	
A	T	A	G	1	
A	A	A	T	2	
G	G	A	T	2	
A	T	C	T	1	
G	G	T	G	2	
G	A	T	T	3	
T	A	C	T	1	
G	C	C	A	1	
G	C	T	G	1	
A	T	G	T	1	
G	T	A	T	5	
C	T	T	G	1	
T	T	A	T	1	
G	T	T	A	1	
A	T	A	A	1	
C	C	T	G	1	
A	C	A	T	1	
T	T	T	C	4	
G	G	A	A	1	
T	C	T	T	1	
G	C	G	T	1	
T	G	G	G	1	
C	T	T	T	2	
G	T	G	G	1	
T	A	T	A	1	
T	T	T	A	1	
T	T	T	G	1	
A	T	T	C	1	
A	T	T	G	1	
<b>Freq</b>	41	43	33	20	70
<b>Z-score</b>	5.7	6.3	3.7	0.7	

**Supplementary Table 4h** Enrichment of Single Mutants

	<b>702A</b>	<b>1514G</b>	<b>1515G</b>	<b>807A</b>
% pre-seln.	31.5	25.8	26.6	25.3
$\sigma$	1.5	1.8	2.6	1.5
% post-seln.	53.8	43.1	62.7	93.5
$\sigma$	3.9	4.4	4.3	1.3
Fold enrichment	1.7	1.7	2.4	3.7
$\sigma$	0.1	0.2	0.2	0.1

**Supplementary table 4.** From Poisson sampling the diversity of B6-1 is ~500 unique clones, 8% of starting library is active. For other potentially diverse libraries the statistics are B6-2 ~100 clones, 40 %; B6-3 ~ 120 clones , 46%; B3-3 ~140 clones, 10%; B2b2 ~121 clones, 47%. In Table 4e and 4d position 1418 has different *Z-scores* due to different mutations in surrounding sequence. In all analysis we have used the higher *Z-score*. **B.** 1) 2 of 4 have A780T. **F.** 1) Has A1429C. **E.** 1) Has 1426 deleted. **4h.** 702 is a positive control. All enrichments correspond to *Z-scores* greater than 3.