

Fig. S1. Sequence analysis of mutations supported by DIVAC 2-3. Genomic DNA was isolated from (A) IgL(-)GFP2 2-3 and (B) IgL(-)GFP2 cell lines on day 21 post-subcloning. A 1217 bp region of the GFP2 expression cassette was PCR amplified, cloned, and sequenced. The pie chart shows the proportion of sequences containing the indicated number of mutations; the middle circle denotes the number of sequences analyzed. Mutation spectra are depicted in the box diagrams (left, sequences from this analysis; right, published data from (34)). Further information concerning the mutations and mutation frequencies are detailed below the mutation spectra.

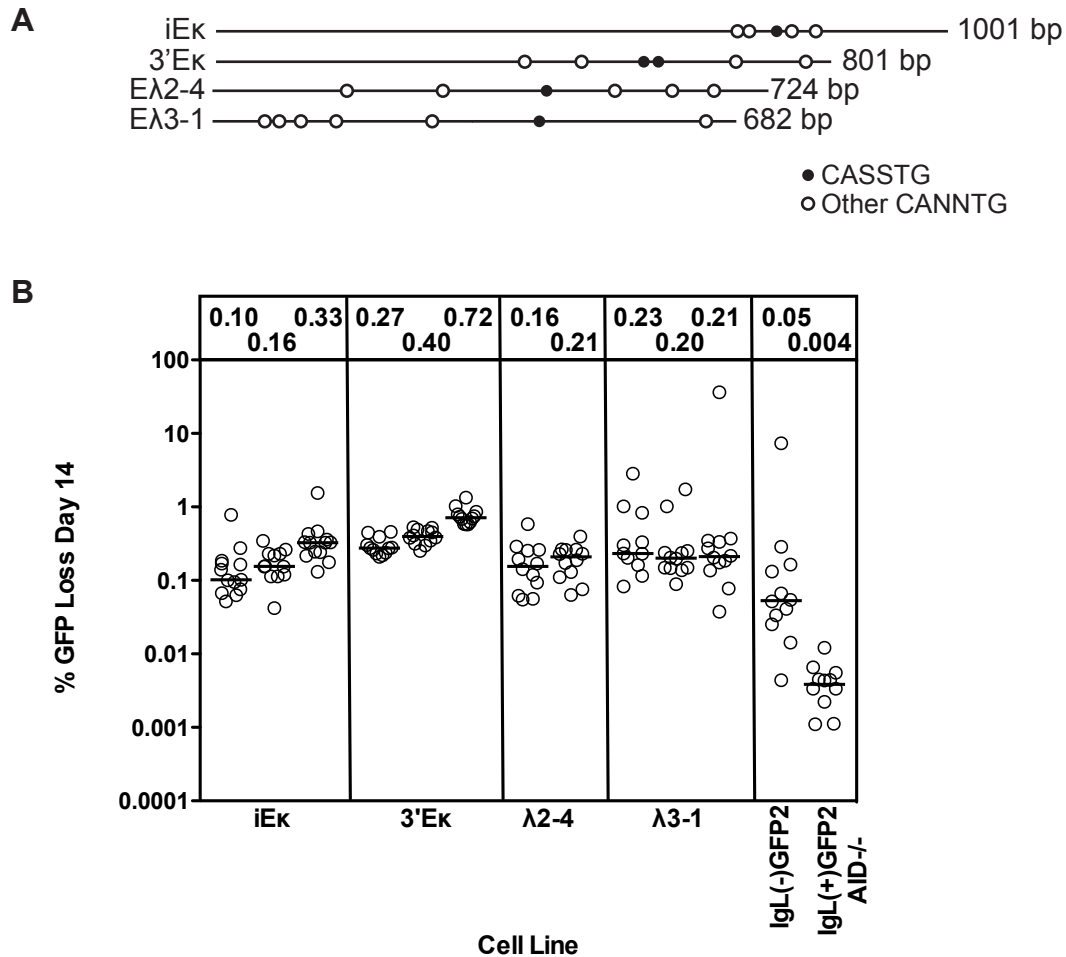


Fig. S2. Analysis of DIVAC function associated with murine Ig light chain enhancers. (A) Schematic diagram of the murine Ig light chain enhancers. The sizes of the fragments containing the intronic Igk enhancer (iEk), 3' Igk enhancer (3'Ek), Igλ 2-4 enhancer (Eλ2-4), and Igλ 3-1 enhancer (Eλ3-1), are indicated, as are the locations of E boxes conforming (closed symbols), or not conforming (open symbols) to the motif CASSTG. (B) Fluctuation analysis of GFP loss in subclones with data depicted as in Fig. 2D.

Fig. S3

A

AGCAGGCCGCATGGTACAGCTCCATCAGCACAGCTGGGGCCACACAAAGAGCTGGGTACTGTGGGCAGCAGGCT
GAAACCCGAAAAACAAGAGCTGGGGGCTCAGAATAGCCCCGGGAGCAGGCAGGGCCTGGGGGAGAGGGCAAGCAC
CAGGCCCAGGGCCACACAGCCCTTCCAGGAAGGCACAGCGCTGTGAGGGTGCAGCACGCTCAGCCCCACCATGCA
GCTGTGCGGCCGGGGCATCCCCAAGCTAAATTTACTTCTCAGTCTCCAATCAGAACTGAAGCTGAGGGGCCACG
CCGGCCAAAAAAGGAAACGAAACAGTCTCCAGAAAGCACTGACGTGTGAAGCAGAGCGAGCGCCCGCAAACCG
GCCGCCATGTACACACCTCAGGTTGGGGCTTTGCCAGACTGAGCTTTGCTGCTGCTCGGGGTGGGTGCCACGGC
CTGGGCACATGGGATGGGTACACACGTACACACACTTGACACCCACACCCCAACACTTCAGGTGATGCTGGTGC
AGATGGGTGCCCCCAGGCTGACCCCGCTTGCAAAGAGGAGAGCATTTCATGGCTGTGGCAAAACAGCAACCGCC
TGTTGTG**CAGCTG**GGATGGTGTATCTGGAAATGTACGCAGCCCAGGAGGGGTAACAGCTCCAACTGAGACCCT
GAGCTTGTCCACAGTTGTAACAGGCTGACATAAACACCTTTGTCCGTGGAAAAATTTATCACCTCAAATATAG
CAGGTTAATAAAATAAACTCCCAACGAGCTTACACCTGCTTTGGAAGGGAAGCAGAC**CACTTG**TTTTCTGCTTGT
GTTGGCTGTAGGAAGCCATGTTTCCGATGCAGGAGGGCCACAAAGCACTGACAACACAATGTGAGCTGAGCTTCG
CCCCTGTTAAGCCCCACCACAGGCTTGTGGCCCTAGAGCAGACAGGGCGCAGGGGTGGCACCAGGGCTGGGTG
ACATGGGCTGGTCTGGGGTGTCTCACTGAGCTCTTTGGGAGGGGTTGGAGCCCTGGGGCAATCACAGCACACAC
AGGAGGTGGGGGGATGCAGCCAG**CAGCTG**CCCTGCACTAAGAAAACCCCATCCGTGGCTTT**CAGATG**GCCTTCC
CATCTCTGCGACCTCTGCATGGGCTGAGCACAAGGTTAAAGTGTCTGCCATGTTTTGGGCATGTTTGGAGGG
GCAGCGTGGGCCCGGGCATAACGGTACCGC**CACGTG**CTGCCAGCCCC**CAGCTG**AGCCTGCACTCTCC**CAGATG**
GCTGACCGCAGCCACGGGGCAACAGTTTCTTTGCTAAAAATTGTAGCCGGGAAGAAAA**CACGTG**GCAACTTCGG
CCAAACAG**CAGCTG**GAGGACAGGAATAGCCGTGGCCACGGCACGCTCTGCTTCTCGGCACAAACATTCCAGTACG
TGGCACCACGAGCGCCGCTGCCCGGCACAGCAGCAAGCAGAGCCAGGAGCAGGAAATGCTGATTTGGGCCCAT
TTGGCCATGGCTGAGAGAAGAGGCTTCCAGGGAGCTGGTCACTTGGTCCCCAAGCTGTGGCTTGGGAAATGATG
GGGAGGGGATTGCCACTGCCACCCTGCAGAGCAGGCTCTGGTCCCATCTCACTGCAGGGCACCAGGGCGTTTGC
ACTGCAGCAATTACAGAAACATTGAAATGGCTCCT

B

AACATCCCACCAGCACACGGGGCTCAGCACGCTGGCGACGTGGCATCAGCAGAGCAGGCCGCATGGTACAGCTC
CATCAGCAC**AGCTG**GGGGCCACACAAAGAGCTGGGTACTGTGGGCAGCAGGCTGAAACCCGAAAAACAAGAGCTGG
GGGCTCAGAATAGCCCCGGGAGCAGGCAGGGCCTGGGGGAGAGGGCAAGCACCAGGCCCAGGGCCACACAGCCC
TTCCAGGAAGGCACAGCGCTGTGAGGGTGCAGCACGCTCAGCCCCACCATG**CAGCTG**TGCGGCCGGGGCATCCCC
AAGCTAAATTTACTTCTCAGTCTCCAATCAGAACTGAAGCTGAGGGGCCACGCCGGCCAAAAAAGGAAACGAAA
CAGTCTCCAGAAAGCACTGACGTGTGAAGCAGAGCGAGCGCCGCGCAAACCGCCGCCATGTACACACCTCAGGT
TGGGGCTTTGCCAGACTGAGCTTTGCTGCTGCTCGGGGTGGGTGCCACGGCCTGGGCACATGGGATGGGGTACA
CACGTACACAC**CACTTG**CACACCCACACCCCAACACTTCAGGTGATGCTGGTGCAGATGGGTGCCCCACAGGCTGAC
CCCCCACGCATGGCCTGGCCACACTGCTCCATCCGTGTCTGACTAGCGGCGGAGCCCC**AGCTG**TAGGAA
GCTCAGAGCCATCACACAGCAATTAAGAGGAATTAATTAATAATAAATGTTTTAGGCGGGCT**CAGCTG**CCAGCA
CCACTGACC GAAACAGCCCGCTTGCAAAGAGGAGAGCATTTCATGGCTGTGGCAAAACAGCAACCGCCTGTTGT
GCAGCTGGGATGGTGTATCTGGAAATGTACGCAGCCCAGGAGGGGTAACAGCTCCAACTGAGACCCTGAGCTT
GTCCACAGGTTGTAACAGGCTGACATAAACACCTTTGTGCCGTGGAAAAATTTATCACCTCAAATATAGCAGGTT
AATAAAATAAACTCCCAACGAGCTT**CACACTG**CTTTGGAAGGGAAGCAGAC**CACTTG**TTTTCTGCTTGTGTTGGC
TGTAGGAAGCCATGTTTCCGATGCAGGAGGGCCACAAAGCACTGACAACACAATGTGAGCTGAGCTTCGCCCTG
TTAAGCCCCACCACAGGGCTTGTGGCCCTAGAGCAGACAGGGCGCAGGGGTGGCACCAGGGCTGGGTGACATGG
GCTGGTCTGGGGTGTCTCACTGAGCTCTTTGGGAGGGGTTGGAGCCCTGGGGCAATCACAGCACACAGAGG
AGGTGGGGGGATGCAGCCAG**CAGCTG**CCCTGCACTAAGAAAACCCCATCCGTGGCTTT**CAGATG**GCCTTCCCATC
TCTCTGCGACCTCTGCATGGGCTGAGCACAAGGTTAAAGTGTCTGCCATGTTTTGGGCATGTTTGGAGGGGAG
CGTGGGCCCGGGCATAACGGTACCGC**CACGTG**CTGCCAGCCCC**CAGCTG**AGCCTGCACTCTCC**CAGATG**TGCTG
ACCGCAGCCACGGGGCAACAGTTTCTTTGCTAAAAATTGTAGCCGGGAAGAAAA**CACGTG**GCAACTTCGGCCAA
ACAG**CAGCTG**GAGGACAGGAATAGCCGTGGCCACGGCACGCTCTGCTTCTCGGCACAAACATTCCAGTACGTGGC
ACCACGAGCGCCGCTGCCCGGCACAGCAGCAAGCAGAGCCAGGAGCAGGAAATGCTGATTTGGGCCCATTTTGG
CCATGGCTGAGAGAAGAGGCTTCCAGGGAGCTGGTCACTTGGTCCCCAAGCTGTGGCTTGGGAAATGATGGGG
AGGGGATTGCCACTGCCACCCTGCAGAGCAGGCTCTGGTCCCATCTCACTGCAGGGCACCAGGGCGTTTGCATG
CAGCAATTACAGAAACATTGAAATGGCTCCT

Fig. S3. Sequences of key DIVAC fragments. (A) DIVAC 1703. (B) DIVAC 1928. In both, the F2 core is the 5' underlined section and the F3 core is 3' underlined section. E boxes are indicated in bold.

Table S1**A**

Construct Name	Primer Sequence	Template DNA	Product Size (bp)
1	F: cccgctagcctgggaacagggggagtctg R: ccactagtcctgtggtcccgtgagt	DT40 Genomic	1493
2	F: cccgctagctgaggagcatgtgctgaatta R: ccactagtgggctgttcggtcaggt	DT40 Genomic	1476
3	F: cccgctagcggcggagccccagctgtagg R: ccactagtgcggtgctccccaggatacca	DT40 Genomic	1633
2a	F: 2 F R: ccactagctctgctgatgccacgtc	2	590
2b	F: cccgctagcaacatcccaccagcacacg R: ccactagtcagagacacggatggagcagt	2	650
2c	F: cccgctagccccccaggctgacccc R: 2 R	2	350
2b-1	F: 2b F R: ccactagtgaagggctgtgtggcctgggcc	2	227
2b-2	F: cccgctagcagccccgggagcaggcagggcct R: ccactagtcaaagctcagtctggcaaagccc	2	316
2b-3	F: cccgctagcagagcgagcgccgcgcaaaccggc R: 2b R	2	244
1-3	F: 1 F R: 3 R	DT40 Genomic	4384
2-3	F: 2 F R: 3 R	DT40 Genomic	2989
2-3Δ2a	F: 2b F R: 3 R	2-3	2452
2-3Δ2c	F: 2 F R: 2b R	2-3 (added to 3)	2640
2-3Δ2a/2c	Digested 2b vector and inserted into 3	--	2103
2-3ΔN	F: 2 F R: ccactagtacaagctcggggctcagttg	2-3	1610
2-3Δ3'N	F: 2 F R: ccactagtaacatggcagaaacacttaaac	2-3	2118
2-3Δ5'N	F1: 2 F R1: tccaaacatgccc aaagggctgtttcgggtca F2: tgaccgaaacagcccttgggcatgtttgga R2: 3 R	2-3	2347
2-3ΔCR1	F: 2 F R: ccactagtaggagccatttcaatgtttctgtga	2-3	2626
2b-2 Δ1-50	F: cccgctagcagccccgggagcaggcag R: 2b-2 R	2b-2	265
2b-2 Δ51-100	F1: 2b-2 F R1: ctgcatggtggggctgactgggctgtgtcctgcc F2: tcagcccaccatgcagc R2: 2b-2 R	2b-2	266
2b-2 Δ101-150	F1: 2b-2 F R1: ctgattggagactgagaaggcgtgctgcaccctgac F2: cccgctagccttctcagctccaatca R2: 2b-2 R	2b-2	266
2b-2	F1: 2b-2 F	2b-2	265

Δ151-201	R1: actgtttcgtttccttttaaatagcttggggat F2: cccgctagcaaaaggaaacgaaacagt R2: 2b-2 R		
2b-2 Δ202-254	F1: 2b-2 F R1: gcggccggtttgcgcggttggccggcggtgggccc F2: cccgctagcgccgcgcaaacggcgccg R2: 2b-2 R	2b-2	263
2b-2 Δ255-316	F: 2b-2 F R: ccactagtgtcgtctgtcttcacac	2b-2	253
2b-2 154	F1: cccgctagctcagcccaccatgcagc R1: ccactagtgtcgtctgtcttcacac	2b-2	154
3a	F: 3 F R: ccactagtgtcagtgtttgtggcctc	3	456
3b	F: cccgctagcccattgttcccagtgagg R: ccactagtggaaaactgttccccggtg	3	471
3c	F: cccgctagcccacagctgagcctgcactc R: ccactagtgtgaacaaggcaggagccat	3	455
3Δa	F: cccgctagcccattgttcccagtgagg R: 3 R	3	888
3Δb	F1: 3 F R1: gctcagctgtggtgtcagtgtttg F2: caaagcactgacaccacagctgagc R2: 3 R	3	1262
3a+b	F: 3 F R: 3b R	3	1216
3c3	F: 3c F R: 3 R	3	807
PG	F1: cccgctagctcagcccaccatgcagc R1: ccactagtgttcagatggaacttctatgttc	W	700
DIVAC 1703	F1: cccgctagcagcaggccgcatggtac R1: ctctctttgcaagcgggtcagcctgggggg F2: ccccccaggctgaccccgttgcaagaggag R2: 2-3ΔCR1 R	DIVAC 2-3	1703
DIVAC 1703 Δ1-200	F: cccgctagccagcacgctcagccc R: 2-3ΔCR1 R	DIVAC 1703	1503
DIVAC 1703 Δ201-400	F1: DIVAC 1703 F1 R1: tctggcaaagccccaccctgacagcgctg F2: cagcgtgtcagggtggggccttgccagac R2: 2-3ΔCR1 R	DIVAC 1703	1503
DIVAC 1703 Δ401-600	F1: DIVAC 1703 F1 R1: cagctgcacaacaggcaacctgaggtgtgtgacatg F2: cacacacctcaggttgctgtgtgcagct R2: 2-3ΔCR1 R	DIVAC 1703	1503
DIVAC 1703 Δ601-800	F1: DIVAC 1703 F1 R1: ctgcttccctccaaggtgtgtttgcc F2: ggcaaaacagcaacctggaagggaagcag R2: 2-3ΔCR1 R	DIVAC 1703	1503
DIVAC	F1: DIVAC 1703 F1	DIVAC 1703	1503

1703 Δ801-1000	R1: cagtgagacaccccaagcaggtgtgtagctc F2: gagctacacacctgctggggtgtctcactg R2: 2-3ΔCR1 R		
DIVAC 1703 Δ1001- 1200	F1: DIVAC 1703 F1 R1: gccctccaaacatgctggaccagcccatgctc F2: gacatgggctggctccgcatgtttggaggggc R2: 2-3ΔCR1 R	DIVAC 1703	1503
DIVAC 1703 Δ1201- 1400	F1: DIVAC 1703 F1 R1: agcagagcgtgccgtgccaaaaacatggcag F2: ctgccatgttttggcacggcacgctctgct R2: 2-3ΔCR1 R	DIVAC 1703	1503
DIVAC 1703 Δ1401- 1703	F: DIVAC 1703 F1 R: cccactagtgccacggctattct	DIVAC 1703	1400
DIVAC 1928	F: 2b F R: 2-3ΔCR1 R	2-3Δ2a/2c	1928
DIVAC 1928 Δ1	F1: 2b F R1: ccttttttggccggcaccctgacagcgtgtg F2: agcgtgtcagggctccggccaaaaaaaggaaac R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ2	F1: 2b F R1: ccttttttggccgggcttgggatgccccggc F2: ggggcatccccaaagcccggccaaaaaaaggaaac R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ3	F1: 2b F R1: ggcgctcgtctgctcgtgggccccctcagcttc F2: gctgagggggcccacgagcagagcagcggcgcg R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ4	F1: 2b F R1: gtctggcaaagcccctcacacgtcagtgcttc F2: agcactgacgtgtgaggggctttgccagactga R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ5	F1: 2b F R1: cctgtgtgtgctgtgggaccagcccatgtcac F2: acatgggctgtcccacagcacacagggagg R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ6	F1: 2b F R1: ggatggggtttctattgccccagggtcca F2: agccctggggcaataagaaaaccccatccgtg R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ7	F1: 2b F R1: ctcagcccatgcagaggagtcagggcagctgctg F2: cagctgccctgcactcctctgcatgggctgagcac R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ8	F1: 2b F R1: cccctccaaacatgcctgcagagagatgggaag F2: cccatctctctgcaggcatgtttggaggggag R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ9	F1: 2b F R1: tggggctggcggccaaaaacatggcagaaa F2: ctgccatgttttggccggcagccccacag R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928	F1: 2b F R1: ctgttgccccctggcagcgtggcggtagccgta	DIVAC 1928	1878

Δ10	F2: gggtagccgcccacgtgccacgggggcaacagtttc R2: 2-3ΔCR1 R		
DIVAC 1928 Δ11	F1: 2b F R1: tggccgaagttgccacgtctgcggtcagcacatctg F2: atgtgctgaccgagacgtggcaactcggcca R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ12	F1: 2b F R1: gaagcagagcgtgccgtggtttctcccggctac F2: gccgggaagaaaaccacggcagctctgcttc R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ13	F1: 2b F R1: ccgggcagcggcgctgccacggctattcctgtc F2: aggaatagccgtggcagcggcctgcccggcac R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ14	F1: 2b F R1: aaatggggcccaaatacctggtgccaatactgg F2: gtatgtggcaccacggattgggccccatttgg R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ15	F1: 2b F R1: tggggaccaagctgaccaagcatttctgctcctgg F2: gagcaggaaatgctgtgacagcttggtcccca R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ16	F1: 2b F R1: gtgggcagtggaagctccctggaagcctcttc F2: aggttccaggagcttgccactgccaccctg R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ17	F1: 2b F R1: caaacgccctggtgctcccctccccatcatttc F2: tgatggggaggggagcaccagggcgtttgac R2: 2-3ΔCR1 R	DIVAC 1928	1878
DIVAC 1928 Δ18	F: 2b F R: cccactagtctgcagtgagatgggac	DIVAC 1928	1878
1-3 ΔF2 core	F1: 1 F R1: tctggcaaagccccaccctgacagcgctg F2: cagcgctgtcaggggtggggctttgccagac R2: 3 R	DIVAC 1-3	4184
1-3 ΔF3 core	F1: 1 F F2: cgggtaccgcccacgtgttgccactgccaccctgcag R1: ggtgggcagtggaacacgtggcggtagccgat R2: 3 R	DIVAC 1-3	4034
1-3 ΔF2/F3 core	F1: 1 F R1: tctggcaaagccccaccctgacagcgctg F2: cagcgctgtcaggggtggggctttgccagac R2: 3 R	DIVAC 1-3 ΔF3 core	3834
2-3 ΔF2 core	F1: 2 F R1: tctggcaaagccccaccctgacagcgctg F2: cagcgctgtcaggggtggggctttgccagac R2: 3 R	DIVAC 2-3	2789
2-3 ΔF3 core	F1: 2 F F2: cgggtaccgcccacgtgttgccactgccaccctgcag R1: ggtgggcagtggaacacgtggcggtagccgat R2: 3 R	DIVAC 2-3	2639
2-3 ΔF2/F3	F1: 2 F R1: tctggcaaagccccaccctgacagcgctg	DIVAC 2-3 ΔF2	2439

core	F2: cagcgctgtcaggggtgggggctttgccagac R2: 3 R		
DIVAC 1928 ΔF2 core	F1: 2b F R1: tctggcaaagccccaccctgacagcgctg F2: cagcgctgtcaggggtgggggctttgccagac R2: 2-3ΔCR1 R	DIVAC 1928	1728
DIVAC 1928 Δ1201-1400	F1: 2b F R1: agcagagcggtgccgtgcaaaaaacatggcag F2: ctgccatgttttggcacggcacgctctgct R2: 2-3ΔCR1 R	DIVAC 1928	1728
DIVAC 751	F1: cccgctagccagcagctcagccc R1: gccctccaaacatgctcagcctggggggcacc F2: ggtgccccccaggtgacgcatgttggaggggc R2: cccactagttcccctcccacatcttc	DIVAC 1928	751
Murine iEk	F: cccgctagccaaggaaaggggtgacttatt R: cccactagtacacaagcacataaggaaga	C57BL/6 Genomic DNA	1001
Murine 3'Ek	F: cccgctagcagctcaaaccagcttaggctac R: cccactagttgtctgggccccatgaaacatc	C57BL/6 Genomic DNA	801
Murine EA2-4	F: cccgctagctggggataactcagtaatcttc R: cccactagttgctctaccaagttgct	C57BL/6 Genomic DNA	724
Murine EA3-1	F: cccgctagctctagttccactatcatctc R: cccactagtcttactcctttgtgctct	C57BL/6 Genomic DNA	682

B

Gene	PCR Fragment	Primer	Ta	Product Size (bp)
γ-actin	--	F: acagcattcgggtggagt R: ttgagcatcctccttcacac Probe: cctcagccctcacctgcacg	60	127
RSV Promoter (in IgLGFP2)	a	F: ttcttcatgcaattgtcggg R: ctgctccctgctgtgtg Probe: tgctcgcgcactactcagcg	60	95
GFP2 (in IgLGFP2)	b	F: tgaccctgaagttcatctgc R: gaagtcgtgctgcttcatgt Probe: cccaccctcgtgaccaccct	60	125
IRES (in IgLGFP2)	c	F: ccttaggaatgctcgtcaag R: cctaacgttactggccgaag Probe: ttccgggcccctcacattgc	60	139
BSR (in IgLGFP2)	d	F: aatggcttctgcacaaacag R: gcgacagagaagattacaatgc Probe: cgaattgccgtcccacatg	60	141
2b	e	F: tccaatcagaaactgaagctg R: cgctctgcttcacacgtc Probe: aggaaacgaaacagtctccagaaagca	60	80
3	f	F: agctggaggacaggaatagc R: ctctgctgctgctgtgc Probe: cgctctgcttctcggcaca	60	109
W fragment, upstream of cores (DIVAC 1)	g	F: gatctgctacagccactcca R: gctggatggagacataggg Probe: cagcacctccagccatcca	60	113

W fragment, between cores (Δ601-800)	h	F: aaacagctccaaactgagacc R: cacggcacaaggtgttat Probe: ccgagctgtccacaggtgtaaaca	60	74
18S	--	F: taaaggaattgacggaaggg R: tgtcaatcctgtccgtgc Probe: cgcaggctccactcctggtg	60	102

Table S1. Primer and probe sequences for experiments. (A) Primers for the creation of all tested DIVAC fragments. (B) qPCR primer pairs and probes for gene expression and ChIP. All sequences are written 5' to 3'.