

Supplementary to: Gain-of-function analysis of cis-acting diversification elements in DT40 cells
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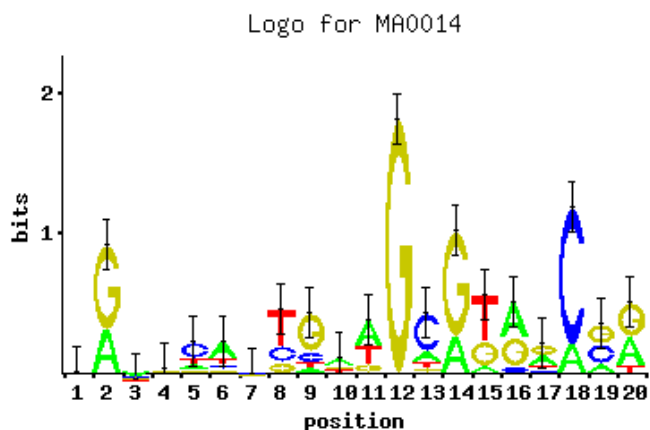
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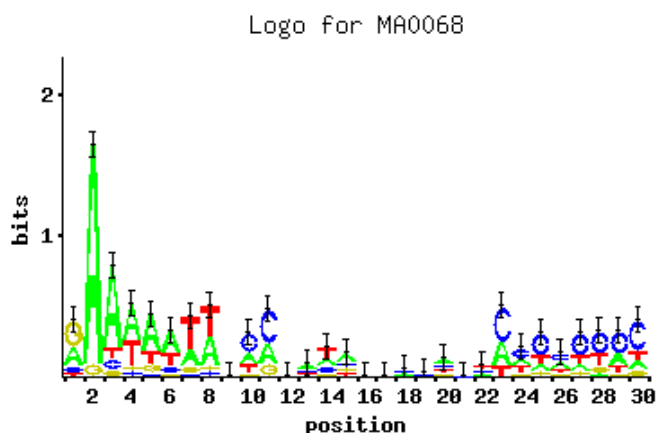
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Supplementary figure 1. Brief over-view of supervised search for areas of interest.

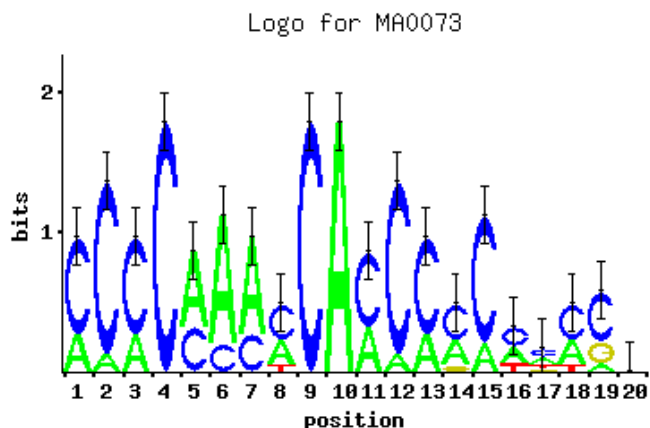
(a) MEME (Multiple Em for Motif Elicitation)³¹ and MAST (Motif Alignment & Search Tool)³² high probability motifs in WebLogo format (ID refers to the JASPAR database).



IDMA0014
Supergroupvertebrate
NamePax5
Protein ClassPAIRED
SpeciesMus musculus

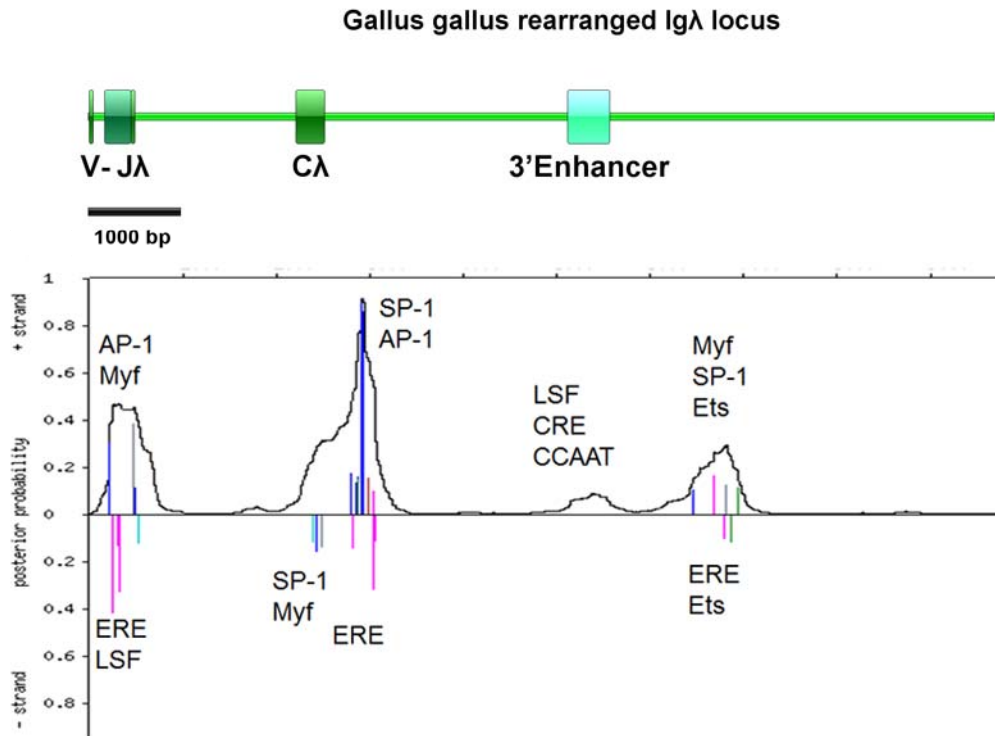


IDMA0068
Supergroupvertebrate
NamePax4
ClassPAIRED-HOMEO
SpeciesMus musculus

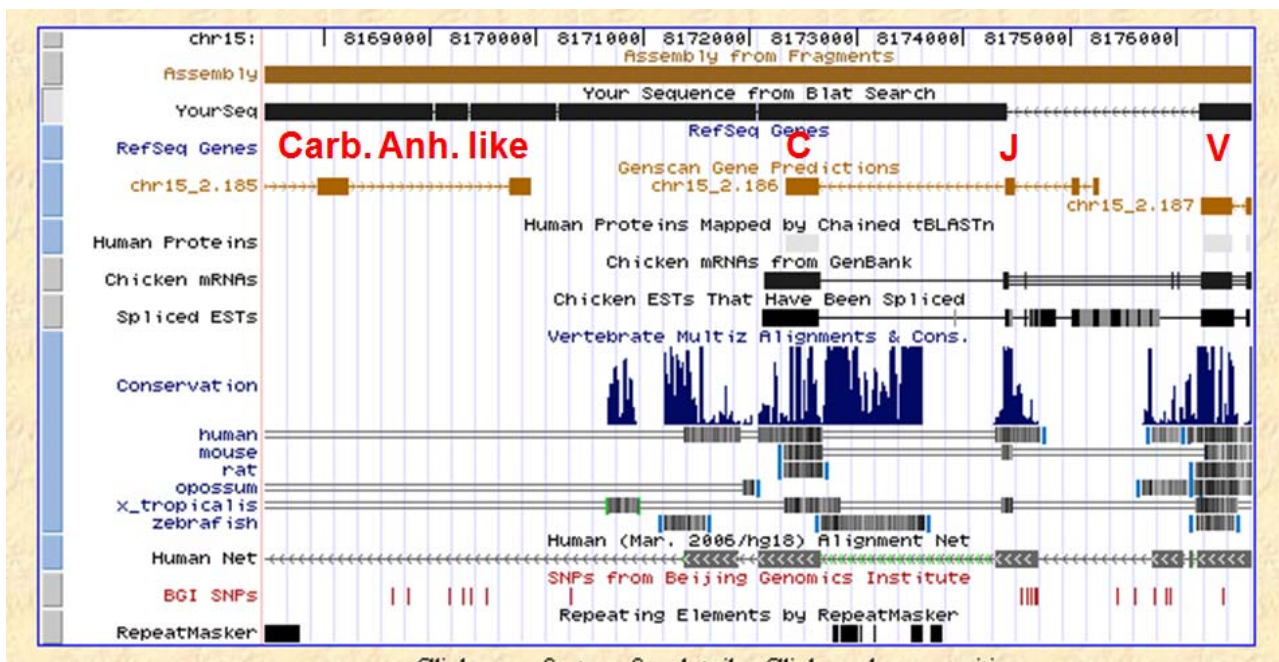


IDMA0073
Supergroupvertebrate
NameRREB1
ClassZN-FINGER, C2H2
SpeciesHomo sapiens

(b) Cister²⁸ generated plot of DT40 Igλ rearranged locus showing selected high scoring transcription factor motifs.



(c) BLAT map showing sequence conservation using the ~9.8kb rearranged DT40 Igλ sequence as input.



(D) Mapping of DT40's Ig λ rearranged locus.

IgA -L, R, S, U & N (respectively in order) underlined, conserved sequence text in italics, 3' hypersensitive sites in green text, select putative motifs are highlighted yellow or blue or shown in bold text (may overlap), CpG rich highlighted in grey, and the putative silencer is highlighted in red.

[illegible]

ccacgcccggcca~~aaaaaaggaa~~acgaaacag~~gtctccag~~aaagca~~ctgacgtgtg~~aagcagagcgagcgccgcgcaaacgggcccgcacatgtcacacacctcaggttggggctt
tgccagactgagctttgctgctgctcggtgggtgccacggcctgggcacatgggatgggttacacacgtacacacacttgacaccccacaccccaacacttcaggtgat
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ctcaaataatagcaggttaataaaaataaaactcccaacgg~~agactaca~~~~cacctgctttggaagggaagcagacacttggtttctgct~~tgatgttggtgttaggaagccatgttt
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tgagcatcctgctggacagcaggtggtgtgagccaccggtgtgccctggtggccaagaaggccagtggtatcctggggagcaccgcaatgaga~~gtgggcagcagggcgag~~
~~ggaggtgaggtgcatttggagc~~accgtgccagtttctgggtcctcagttcaaggcagacagggaaactgctggagagagcccagcagaggggctgcaatgatgatggggt
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~~cacggctttggccagacggggcccttccatccca~~gctagtgtagt

Supplementary table 1. Primers used

Primer	Sequence	Target
rbc261	gctagcatggtactcgttgcgcctgtgctgggagctggtggcaggggctctgtccattctgcgc	L-region forward oligo
rbc262	ggcgagggctgtgctgctggggcgctcactgattgctgcttctccctctctccctctccagactagta	L-region forward oligo
rbc263	actagctgtggaggggagagggagaggggagaaacggcaatcagtgcacgccccgcacgcacag	L-region reverse oligo
rbc264	ccctgccgcgcagcaatgggacagagcccgtgccacagtccccgaccagggcgcaacgagtaccatgtagca	L-region reverse oligo
rbc557	ttacctaggtctagaaactagctgtgggatgcaatgtgaggacagt	R-region forward
rbc558	ttacctagggctagcactagtcccaaatcaccatttctgtacca	R-region reverse
rbc559	ttaactagtcataatggcacagcgctgcagggtgcagcac	S-region forward
rbc561	ttagctagccatatgcgtgtgtacccatcccatgtgcc	S-region reverse
rbc563	ttaactagtgccgccacactgcttgggaaggaagcagac	U-region forward
rbc564	ttagctagcggcgccggcgagcgcgctcgtgtgtgccac	U-region reverse
rbc565	ttaactagtagcatccatccggcgacccacccaccctc	N-region forward
rbc566	ttagctagcatgcatactagctgggatggaaggcgctgc	N-region reverse
rbc583	catggattggacgaaccactgaattccgcatggca	RSV forward
rbc585	tatagacaaacgcacaccggcctta	IRES reverse
rbc586	gcaatatggtggaaaataaca	IRES reverse
rbc587	tccgggcctcacattgccaaaagacg	IRES reverse
rbc598	aatgctagcgcgcgctggtgagcaaggcgaggagctgtcac	eGFP forward
rbc600	taagctagcattactagatgtgactcgttgcgcctggtcggggactgtg	L-region forward
rbc601	gccgctagcctggagggagagggagggaggggagaaaaac	L-region reverse
rbc602	gccagatctattagagtcgggactgtacagctcgtccatgc	eGFP reverse
rbc734	ctagctcgatataataaacgccatttgaccattca	RSV forward
rbc735	taagtgcctagctcgatataataaacgccatttga	RSV forward
rbc775	ctctgtccattgctgcgcgggcag	L-region forward
rbc776	gcgtgcggggccgtcactgattgc	L-region forward
rbc813	gcctattccaagcggcttcggccagtaac	IRES reverse
rbc843	ttctcaatcccgcatgcc	GFP qPCR forward
rbc844	aagtcgatgcccttcagctc	GFP qPCR reverse
PU5	cccaccgactctagaggatcataatcagcc	Puro/BSR reverse
PS169	ccagctaattgaggacgatgtgaaagtag	IgA-Pro targeting forward
PS31	tctgagggaaaaggacgcgtgaattgca	IgA-KO targeting forward

Supplementary table 2. Over-view of clones used and experiments performed. For the fluctuation analysis, up to 36 subclones for each primary clone was used. For sequencing, up to 32 sequences were obtained from each primary clone. For RT-qPCR, two independent element test clones and technical replicates of the control clones were used.

Clones used / generated	AID status	Cis status <i>IgA VJ to C.Anh.</i>	Element tested	Flucuation Analysis - primary clones tested	RT-qPCR	Sequencing
AID / genome status experiment performed						
Aidr1 ψ - FG2GFP	AID reconstituted	ψ -, Cis native	Pos control	C14, C19, C111	C14, C19	C14, C19, C111
Aidr1 ψ - FG2GFP-L	AID reconstituted	ψ -, Cis native	L	C14, C16, C111	C14, C16	C14, C16, C111
Aidr1 ψ - Δ PC4 FG2GFP-L	AID reconstituted	ψ -, Cis native	L in Δ PC4 background	C13, C14, C15	C13, C14	C13, C14, C15
Aidr1 IgA- CiskGFP2	AID reconstituted	Cis excised	Neg control	3X C19	2X C19	
Aidr1 IgA- CiskGFP2 - W	AID reconstituted	Cis excised	W (cis reconstituted)	C13	2X C13	
Aidr1 IgA- CiskGFP2 - L	AID reconstituted	Cis excised	L	C13, C14, C15	C13, C14	
Aidr1 IgA- CiskGFP2 - R	AID reconstituted	Cis excised	R	C13, C15	C13, C15	
Aidr1 IgA- CiskGFP2 - Я	AID reconstituted	Cis excised	Я	C12, C14		
Aidr1 IgA- CiskGFP2 - S	AID reconstituted	Cis excised	S	C13, C14	C13, C14	
Aidr1 IgA- CiskGFP2 - Z	AID reconstituted	Cis excised	Z	C13, C110		
Aidr1 IgA- CiskGFP2 - SS	AID reconstituted	Cis excised	SS	C11, C14		
Aidr1 IgA- CiskGFP2 - ZZ	AID reconstituted	Cis excised	ZZ	C12, C14		
Aidr1 IgA- CiskGFP2 - ZZZ	AID reconstituted	Cis excised	ZZZ	C12, C13	C12, C13	
Aidr1 IgA- CiskGFP2 - U	AID reconstituted	Cis excised	U	C12, C13	C12, C13	
Aidr1 IgA- CiskGFP2 - N	AID reconstituted	Cis excised	N	C11, C12, C13	C11, C12	
Aidr1 IgA- CiskGFP2 - RS	AID reconstituted	Cis excised	RS	C11, C12		
Aidr1 IgA- CiskGFP2 - RZ	AID reconstituted	Cis excised	RZ	C11, C17		
Aidr1 IgA- CiskGFP2 - RSS	AID reconstituted	Cis excised	RSS	C11, C14, C17		
Aidr1 IgA- CiskGFP2 - RZZ	AID reconstituted	Cis excised	RZZ	C11, C13		
Aidr1 IgA- CiskGFP2 - RU	AID reconstituted	Cis excised	RU	C11, C12		
Aidr1 IgA- CiskGFP2 - RN	AID reconstituted	Cis excised	RN	C11, C14, C18	C14, C18	
Aidr1 IgA- CiskGFP2 - SN	AID reconstituted	Cis excised	SN	C11, C12		
Aidr1 IgA- CiskGFP2 - UN	AID reconstituted	Cis excised	UN	C12, C14		
Aidr1 IgA- CiskGFP2 - RUN	AID reconstituted	Cis excised	RUN	C12, C16		
Aidr1 IgA- CiskGFP2 - RSN	AID reconstituted	Cis excised	RSN	C11, C13		
Aidr1 IgA- CiskGFP2 - RSSN	AID reconstituted	Cis excised	RSSN	C12, C13		
Aidr1 IgA- CiskGFP2 - SU	AID reconstituted	Cis excised	SU	C11, C12		
Aidr1 IgA- CiskGFP2 - SSU	AID reconstituted	Cis excised	SSU	C11, C13		
Aidr1 IgA- CiskGFP2 - SUN	AID reconstituted	Cis excised	SUN	C11, C12, C13		
Aidr1 IgA- CiskGFP2 - SSUN	AID reconstituted	Cis excised	SSUN	C11, C12	C11, C12	
Aidr1 IgA- CiskGFP2 - RSU	AID reconstituted	Cis excised	RSU	C12, C13		
Aidr1 IgA- CiskGFP2 - RSSU	AID reconstituted	Cis excised	RSSU	C16, C18	C16, C18	
Aidr1 IgA- CiskGFP2 - RSUN	AID reconstituted	Cis excised	RSUN	C11, C12, C13		
Aidr1 IgA- CiskGFP2 - RSSUN	AID reconstituted	Cis excised	RSSUN	C12, C13, C17	C12, C13	
Aidr1 IgA- pFG3 - L	AID reconstituted	Cis excised	L	C12, C15	C12, C15	
Aide IgA- pFG3 - L	AID excised	Cis excised	L	C12		
Aidr1 IgA- pFG3 - RSSUN (+)	AID reconstituted	Cis excised	RSSUN (+)	C11, C12, C13	C11, C12	
Aidr1 IgA- pFG3 - RSSUN (-)	AID reconstituted	Cis excised	RSSUN (-)	C11, C12, C13, C14	C11, C12	
Aidr1 IgA- pFG3 - LRSSUN (+)	AID reconstituted	Cis excised	L, RSSUN (+)	C12, C13	C12, C13	
Aide IgA- pFG3 - LRSSUN (+)	AID excised	Cis excised	L, RSSUN (+)	C11		
Aidr1 IgA- pFG3 - LRSSUN (-)	AID reconstituted	Cis excised	L, RSSUN (-)	C12, C14	C12, C14	
Aide IgA- pFG3 - LRSSUN (-)	AID excised	Cis excised	L, RSSUN (-)	C11		