

CREAX (CDX Regulated Enhancer of Axin2) enhancer sequence:
Chr17:65515956–65516375

GGAACCCGCTGAATGGCTGGGAAACTGTTTTTAATAAACAAGA**GTTCAAACG**CTGAGTCTCCATAGCCAACAGTCACG
CCTTAAACTCTAAACTTTACATGAAAACCTCTCCCAGTC**CCTTTGAAC**TCTCCTTCCCTCTCCGACGGAGCATTCCAGT
GTTTATGCATTTTTTCGAGATTGCTGGCAGGATTGCGAGGC**GCTTTGAAT**ACTTTCCCTCTCTTTAGCAATCTCCTGCC
TTCAGAACCCATTAATGCCCCACTCAGCAACATCAAGGTAAT**GCTTTGAAG**TCCCTTCTGGCTACAGGCCTCTCACAC
TCTTTGGTTACTGTGCAATCAAATAATTAATGGATTCTCAGGGAAAAAAAAAATCATTCTTTTCCCTTGTAATTA
TACTCCAGGTTGGACAGGAGCGCTGTGTTG

c-Myc-335 enhancer sequence:
Chr8:127400835–127401814

CGCTCCATAGAGCCTGCAGAGGGCACTAGACTGCGAATTAGAAAACCTGATTTCCCTTCCAGCTCCACCTCTGACCAA
TTGCCTGACCCTGGTCAAATTGCTTAACCTCTTCCTATCTCAGCTCCCTATCCATAAAACAGAGGGACGAATAAATC
TCCTCCTACCACTAAGAGGTGTAGCCAGAGTTAATACCCTCATCGT**CCTTTGAGC**TCAGCAGATGAAAGGCACTGAG
AAA**GTACAAAGA**ATTTTTATGTGCTATTGACTTTATTTTATTTTATGTGGGGAGGGAGCCGGCCCCAGCTGGAAAGC
TGCTTTCTCTG**AATCAAAGG**GCAGGAACCCAGCAAGTTTCTCAGGATTGGGGCCTTAGACTGGGCTGTGTATACAGAC
AGTGCCAGCCAACCCACAGTTCAGTTTCCCTTTAACCTGGTGCTCCAGGCAATAACTGTGCAACTCTGCAATTTAACA
ATGTGTTCTTTGTCCCACAACCTGTTTCTCGTTTCTCAACTGCCCAGGTAATATGTTTGGGCCTGTAGGAAGAGTCAAAT
AGTTAATAAGGGAAGGGTTTGGCATGCCCTACGTAAGTCTACCAGCAAGTCCCAACAAGAAGGCATTCTGTGTCTCC
TGATTCCCTGACCTACCCCAAATGTACAAATGTACAAGGAATGAGCCCACCTTCCAGCAGGCTGTAATACCAGTTT
GGCCTATATCAATGCATTGGTGAGCTGTGTTTTGTTTATGGTTTTATGCCATCTATTTTCCCATGGATATTATGTTTT
CTAAAGAGCCCTTAAGTTTACGTCAGCTTTTAAAGCTACCAGCAGCACCATTTAGTTCATATTAAGCCCTTAATATG
GTATGAATAGGAGAGCTATTAGACTAAAGAGCCATAATCATCCCTGAGGAAAACATCCATCACCAACATTTATGTGGT
CCCTGAACTTCTAAAAGGTGTCATCTCTCTGGGGTGTATCTGGT

Legend: **TCF sites** **CDX sites** CAG sites

Supplementary Figure 1. Annotated sequences of human CREAX and c-Myc-335 enhancers.
The positions shown are with respect to the GRCh38.p13 primary assembly of the Homo sapiens genome. TCF sites are emboldened and highlighted in red, CDX sites are highlighted in green, and CAG sites are underlined and highlighted in teal.

Position	WT sequence	Mutated sequence
2-11	GAACCCGCTG	TCCAAATAGT
12-21	AATGGCTGGG	CCGTTAGTTT
22-31	AAACTGTTTT	CCCAGTGGGG
32-41	TAATAAACAA	GCCGCCACC
42-52	GAGTTCAAACG	GAGTGAACAG
57-66	GTCTCCATAG	TGAGAACGCT
67-76	CCAACAGTCA	AACCACTGAC
77-86	CGCCTTAAAC	ATAAGGCCCA
87-96	TCTAAACTTT	GAGCCCAGGG
97-106	ACATGAAAAC	CACGTCCCCA
107-116	TCTCCAGTC	GAGAACTGA
117-127	CCTTTGAACTC	CCTGTTCACTC
131-139	TTCCCTCTC	GGAAAGAGA
140-149	CGACGGAGCA	ATCATTCTAC
150-159	TTCCAGTGTT	GGAAGTGTGG
160-169	TATGCATTTT	GCGTACGGGG
170-179	TCGAGATTGC	GATCTCGGTA
180-189	TGGCAGGATT	GTTACTTCGG
190-196	GCGAGGC	TATCTTA
197-207	GCTTTGAATAC	GCTGTTCATAC
209-217	TTCCCTCTC	GGAAAGAGA

Position	WT sequence	Mutated sequence
218-227	TTTAGCAATC	GGGCTACCGA
228-237	TCCTGCCTTC	GAAGTAAGGA
238-247	AGAACCATT	CTCCAAACGG
248-257	AATGCCCCAC	CCGTAAAACA
258-267	TCAGCAACAT	GACTACCACG
268-276	CAAGGTAAT	ACCTTGCCG
277-287	GCTTTGAAGTC	GCTGTTCACTC
288-293	CCTTCT	AAGGAG
294-303	GGCTACAGGC	TTAGCACTTA
304-312	CTCTCACAC	AGAGACACA
313-323	TCTTTGGTTAC	TCTGTTGTTAC
324-329	TGTGCA	GTGTAC
330-337	ATCAAATA	AGAACATA
338-347	ATTAAATGGA	CGGCCCGTTC
348-357	TTCTCAGGGA	GGAGACTTTC
358-367	AAAAAAAAAT	CACACACCG
368-377	CATTCTTTTC	ACGGAGGGGA
378-387	CTTGTAATT	AGGTTGCCGG
388-397	AAATACTCCA	CCCGCAGAAC
398-407	GGTTGGACAG	TTGGTTCACT
408-417	GAGCGCTGTG	TCTATAGTGT

Supplementary table 1. Mutations made in CREAX screening mutagenesis constructs.

Reporter	Position	WT sequence	Mutated sequence
c-Myc-335 TCF mutant	203–211	CCTTTGAGC	CCTgTtcGC
	218–226	GATGAAAGG	GAgtAcAGG
	238–246	GTACAAAGA	GTACAcAtc
	324–332	AATCAAAGG	AAgaAcAGG
c-Myc-335 CAG mutant	29–34	GACTGG	tAaTtG
	212–217	TCAGCA	TaAtaA
	227–232	CACTGA	tAaTtA
	370–380	GACTGGGCTGT	tAaTtGtaTtT
	385–400	ACAGACAGTGCCAGCC	AaAtcaAtTtCaAtaC
	454–460	CTCTGCA	CgaTtCA
	375–380	GGCTGT	GtagtT
c-Myc-335 CDX mutant	130–136	CCATAAA	CCcgAAA
	250–258	TTTATGTGC	TTTcgGTGC
	276–284	TTTATGTGG	TTTcgGTGG
	737–744	TTTATGGT	TTTcgGGT
	745–753	TTTATGCCA	TTTcgGCCA
	925–933	CATTTATGT	CATTTcgGT
CREAX TCF mutant	117–127	CCTTTGAACTC	CCTgTtcACTC
	277–287	GCTTTGAAGTC	GCTgTtcAGTC
CREAX CAG mutant	70–75	ACAGTC	AactTC
	111–116	CCAGTC	CactTC
	152–157	CCAGTG	CACTTG
	236–241	TCAGAA	TACGAA
	258–263	TCAGCA	TACTCA
CREAX CDX mutant	158–164	TTTATGC	TTTcgGC
	246–252	TTAATGC	TTAcgGC

Supplementary table 2. Directed mutations in TCF, CDX, and CAG sites of CREAX and c-Myc-335 reporters.

Gene	shRNA target sequence
CDX1	GTGAAGATCTGGTTCCAAA
CDX2	GAAGGAGTTTCACTACAGT
LEF1	GGTACATAATGATGCCAAA
TCF7	CCAAGAAGCCAACCATCAA
TCF7L2	AAAGTGCGTTCGCTACATA

Supplementary table 3. Targeting sequences of the shRNA constructs used in this study.

Reporter	Primer 1	Primer 2
TCF/CAG	GATCTCCAACAGTCACGGTACCTTTGATCTTGTAAGG GCGTAGTACCAACAGTCACGGTACCTTTGATCTTGTA AGGGCGTAGTACCAACAGTCACGGTACCTTTGATCTT GTAAGGGCGTAC	TCGAGTACGCCCTTACAAGATCAAAGGTACCGTGACT GTTGGTACTACGCCCTTACAAGATCAAAGGTACCGTG ACTGTTGGTACTACGCCCTTACAAGATCAAAGGTACC GTGACTGTTGGA
TCF/CDX	GATCTAACCTCTCACAAGTACCTTTGATCTTGTAAGG ATGCGTAAACCTCTCACAAGTACCTTTGATCTTGTA TTTATGCGTAAACCTCTCACAAGTACCTTTGATCTTG AGTTTATGCC	TCGAGGCATAAACTACAAGATCAAAGGTACTTGTGAG AGGTTTACGCATAAACTACAAGATCAAAGGTACTTGT GAGAGGTTTACGCATAAACTACAAGATCAAAGGTACT TGTGAGAGGTTA
TCF	GATCTAACCTCTCACAAGTACCTTTGATCTTGTAAGG GCGTAGTAAACCTCTCACAAGTACCTTTGATCTTGTA AGGGCGTAGTAAACCTCTCACAAGTACCTTTGATCTT GTAAGGGCGTAC	TCGAGTACGCCCTTACAAGATCAAAGGTACTTGTGAG AGGTTTACTACGCCCTTACAAGATCAAAGGTACTTGT GAGAGGTTTACTACGCCCTTACAAGATCAAAGGTACT TGTGAGAGGTTA
CDX/CAG	GATCTCCAACAGTCACGGTACCTGTTCTTGTAGTT TATGCGTACCAACAGTCACGGTACCTGTTCTTGTGTA GTTTATGCGTACCAACAGTCACGGTACCTGTTCTT GTAGTTTATGCC	TCGAGGCATAAACTACAAGAGAACAGGTACCGTGACT GTTGGTACGCATAAACTACAAGAGAACAGGTACCGTG ACTGTTGGTACGCATAAACTACAAGAGAACAGGTACC GTGACTGTTGGA

Supplementary table 4. Primers used to generate synthetic reporters.

Target site	Oligo sequence
ACTGGAATGCTCCGTCGGAG	CACCGACTGGAATGCTCCGTCGGAG
	AAACCTCCGACGGAGCATTCCAGTC
TAAGGCGTGACTGTTGGCTA	CACCGTAAGGCGTGACTGTTGGCTA
	AAACTAGCCAACAGTCACGCCTTAC
AGATTGCTGGCAGGATTGCG	CACCGAGATTGCTGGCAGGATTGCG
	AAACCGCAATCCTGCCAGCAATCTC
TTAAATACTCCAGGTTGGAC	CACCGTTAAATACTCCAGGTTGGAC
	AAACGTCCAACCTGGAGTATTTAAC

Supplementary table 5. gRNA target sites used to target CREAX for CRISPRi experiments along with the oligonucleotide sequences used to clone them into sgOpti.

UniProt ID	Species	Protein	Position		Sequence
Q9UJU2	Human	LEF1	359	387	HMQLYPGWSARDNYGKKKKRKRREKQLQESA
P36402	Human	TCF7	329	357	HMQLYPGWSARDNYGKKKKRRSREKHQEST
Q9HCS4	Human	TCF711	406	434	HSQLYPTWSARDNYGKKKKRKRREKQLSQT
Q9NQB0	Human	TCF712	410	438	HMQLYPGWSARDNYGKKKKRKRREKQPGET
P27782	Mouse	LEF1	357	385	HMQLYPGWSARDNYGKKKKRKRREKQLQEST
Q00417	Mouse	TCF7	364	392	HMQLYPGWSARDNYGKKKKRRSREKHQEST
Q9Z1J1	Mouse	TCF711	402	430	HAQLYPTWSARDNYGKKKKRKRREKQLSQT
Q924A0	Mouse	TCF712	388	416	HMQLYPGWSARDNYGKKKKRKRREKQPGET
Q9QXN1	Rat	LEF1	357	385	HMQLYPGWSARDNYGKKKKRKRREKQLQEST
D3ZLD0	Rat	TCF7	364	392	HMQLYPGWSARDNYGKKKKRRSREKHQEST
F1M1R9	Rat	TCF711	405	433	HAQLYPTWSARDNYGKKKKRKRREKQLSQT
D4A8X6	Rat	TCF712	319	347	HMQLYPGWSARDNYGKKKKRKRREKQPGET
O93345	Chick	LEF1	329	357	HMQLYPGWSARDNYGKKKKRKRREKQLQESA
Q8JHX2	Chick	TCF7	313	341	HMQLYPGWSARDNYGKKKKRRTREKQQDSN
A0A3Q2TXB3	Chick	TCF711	409	437	HSQLYPTWSARDNYVSVTHAPQRCGGAEC
E1BYU0	Chick	TCF712	387	415	HMQLYPGWSARDNYGKKKKRKRREKQPGET
Q9W7C0	Zebrafish	LEF1	325	353	HMQLYPGWSARDNYGKKKKRKRREKIQEPA
Q5PU93	Zebrafish	TCF7	297	325	HVQLYPSWSARDNYVSALGKKRRRKRDKQ
Q9YHE8	Zebrafish	TCF711a	389	417	HSQLYPGWSARDNYGKRKKRKRREKCKSDSP
Q800Q5	Zebrafish	TCF711b	386	414	HSQLYPGWSARDNYGKRKKRKRREKNDKTDST
Q71G52	Zebrafish	TCF712	393	421	HMQLYPGWSARDNYGKKKKRKRREKQAGEG
P91943	Drosophila	Pangolin	333	361	HMELYPGWSARDNYGYVSKKKRKRKDRST
E2IJ94	Amphimedon queenslandica	TCF	290	318	HMQMPGWSARDNYAAHKKRRKRKRSKQAE
T2M4F7	Hydra	TCF712	250	278	HMQLYPGWSARDNYAQIGRKKKRPRDKNE

Supplementary table 6. Conservation of residues mediating TCF-CDX interactions.

This expanded version of the alignment in Fig. 3B shows the alignment of TCF/LEF family members from several species. The sequence shown consists of the C-terminal end of the HMG domain along with the basic tail. Highlighted in yellow are R350 and K352 in human TCF7 and corresponding residues that are identical in other TCF/LEF family members.

Defa5 (defensin alpha 5) promoter sequence:
Chr8:7057354–7056738

GATGCATTTCAGATCACACCAACTCCTTGAACATAAATCCGAATT **TTTATTT** TAATCTGATAAACTTGGCCTACTATTTT
ACTGAACTCATTTCCTTATAGCCTGATAAGGTCATTGACCTCTCCA TACTGGCA CCAGCG GGAGACTACTCACCTCG
AGATCTCAAAGCCTCCTACATGAGGTTAGTAATATCCCTGAATCCTGCAAT **GAATTAA** CTCTCTACTC CACTGG GTC
CCAGGTCTGCCCCAGAGAGTCATCCAGAGAGTACCAGGGACCATCTTCAGAAAACAAGAGGCATTTGATCCCCAAC
TTCTTGAATGAAAG CGCTGT TGTTTTTCTTTTTTGAATATATAAAAGTAAATACTCAAGCAGATGGGAAACAGAACAG
GATAGTAATACCCTTATCATCATTAAACACCTTGGATCAAGAAGAG **GCATTAA** GCATACAGACTCACG **CTTTGATG** AAA
GCTGGGAGAAAGAGGAG **CATCAAAGG** GATCTTGA **GAACAAAGG** CAGTCCCTCCCCTCCAATCACATGCCACCTCCT
CTCACTGCAGCTTCTGTCTCAGGTCTTCTC CCAGCA GAGCTATAAATCCA GGCTGA CTCTCACTCCCCAC

Defa6 (defensin alpha 6) promoter sequence:
Chr8:6926050–6926680

TCACATGGGTTTCTTG **AAATAAA** TCTTTTGGCTTTAGTTTTACTAACTCTTTACCTAGTATCCCCTGAGTTCTTTTC
CCTTATA GGCTGA TAAGGTCATTATCTTCTCCA CACTGT GCCCCACAAGAGCCTATTACCCCATAAATCTCAAAGCCT
CCTTCATGAGGGCAGAAATGCCCCCTGAATCCTGCAAT **GAATTAA** CTCTCTACTCTAGCGGGATCCAGCTCTGGCCTC
AAGGTCTAGACCTCCAGAGAGTGG CCAGCC CCACCTTCAGAAAATAAGAGGCATTTGATTCTGAAATTATTCATTGA
AAG CACTGT TCTTTTCTTTTTTGAATATTAACAAGTAAATATT CCAGCA GATGGAAAACAGGACAATGTAA CACTGT T
CTTATCATCACTATCAGCTGGGACCAGAACAGACACT **CAATAAACAGCC** TCACA **CTACAATGA** AGCTTGGA **GAACAAA**
GGAG **CATCAAAGG** GACATGGAGGGCAAGGGTAGCTCTTCTGCTCCCCAATCACATGCACTCCCCGTCTCACC GCAACA
TCTGTCCCTGAGCCTTCTC CCAGCA GACCTATAAATCCA GGCTGG CTCTCACTCCCCACACATCTGCTCCTGCTCTC
TCTCCTC

Legend: **TCF sites** **CDX sites** CAG sites

Supplementary Figure 2. Computationally predicted TCF, CDX, and CAG sites in defensin alpha 5/6 promoters.

The positions shown are with respect to the GRCh38.p13 primary assembly of the Homo sapiens genome. Predicted TCF sites are emboldened and highlighted in red, CDX sites are highlighted in green, and CAG sites are underlined and highlighted in teal.