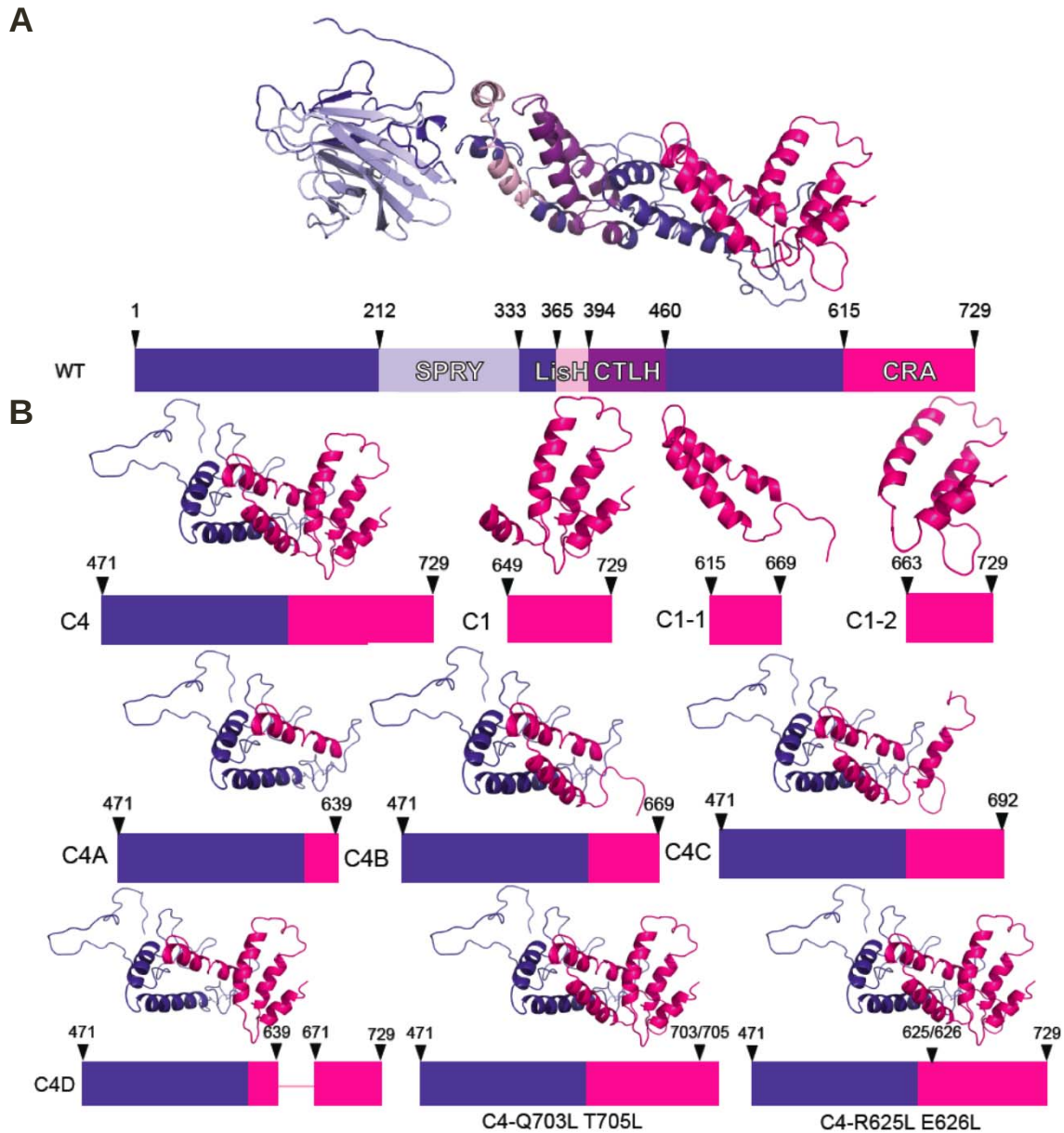
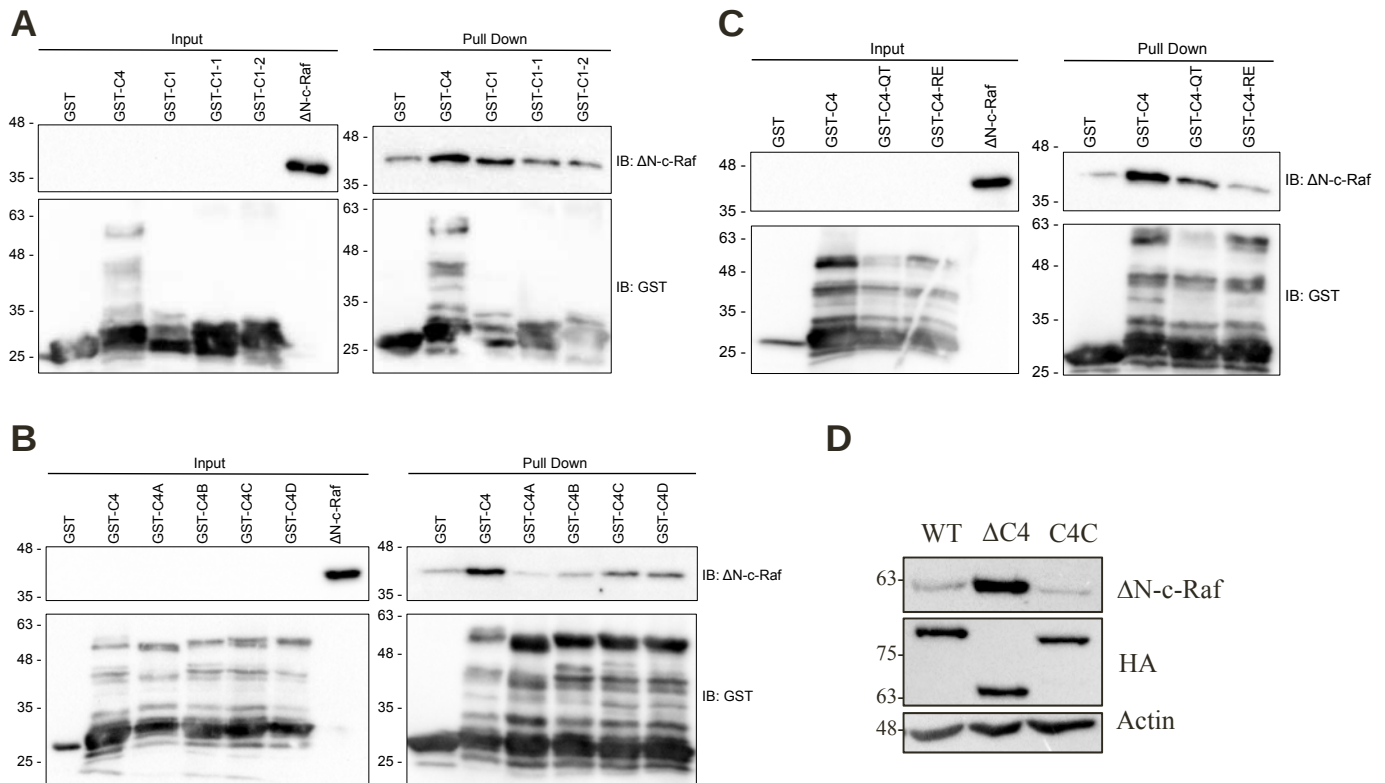
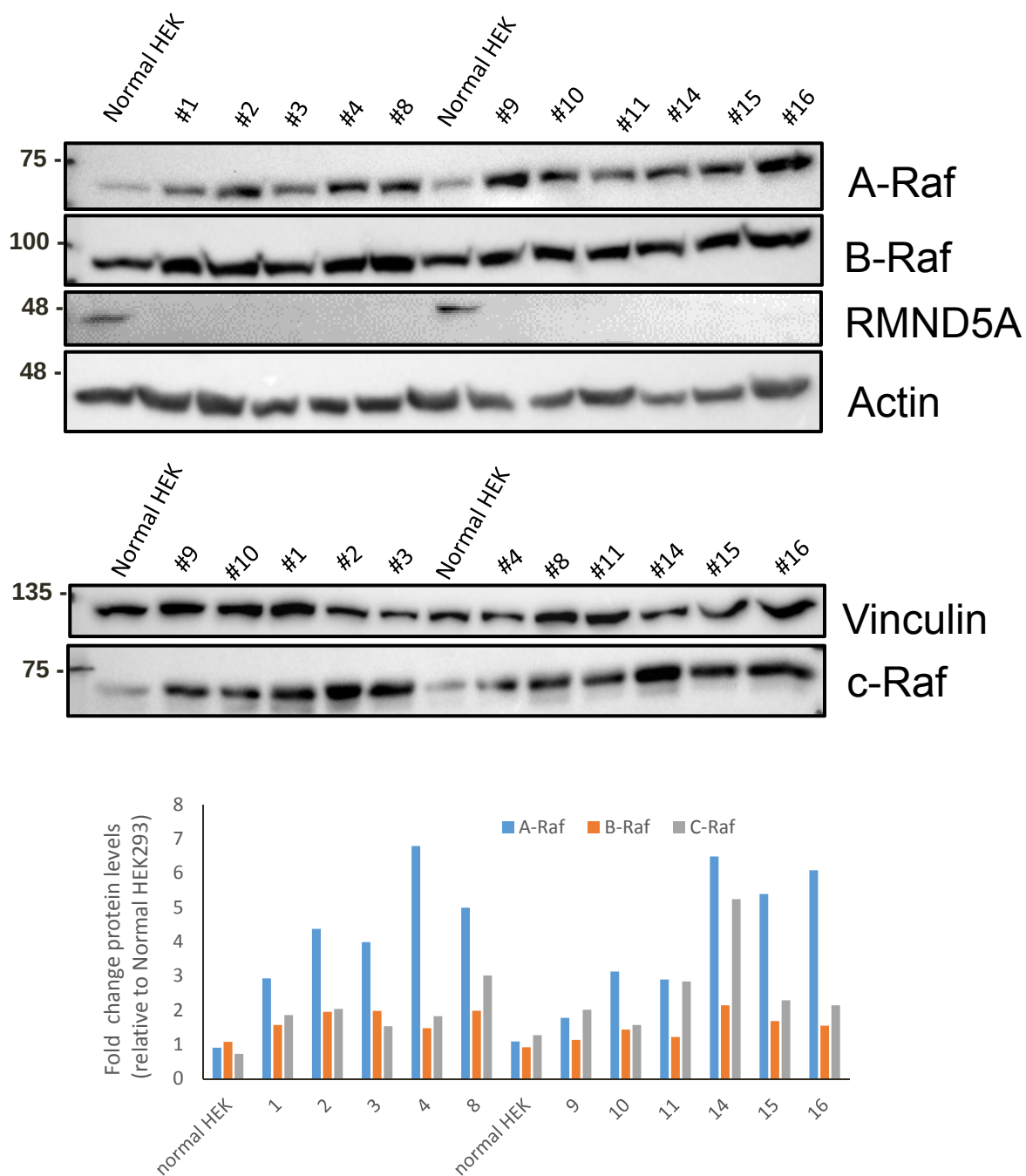




Supplementary Figure 1 Schematics and predicted structures of RanBPM and the C-terminal CRA domain mutants of RanBPM. **A.** Schematics of RanBPM with the domain indicated and predicted structure shown above. **B.** Schematics of the RanBPM mutants subcloned in the pGEX-4T-1 vector with the predicted structure shown above.



Supplementary Figure 2. The CRA domain of RanBPM interacts directly with Δ N-c-Raf. GST pull-down assays were performed using GST, GST-C4, GST-C1, GST-C1-1 and GST-C1-2 (**A**); GST-C4A, GST-C4B, GST-C4C, and GST-C4D (**B**); and GST-C4-Q703L/T705L, and GST-C4-R625L/E626L (**C**) *E. coli* extracts as well as Δ N-c-Raf *E. coli* extracts. Pull-downs were analyzed by Western blot with C-Raf and GST antibodies to detect Δ N-c-Raf and GST-fusion proteins, respectively. **D.** Analysis of RanBPM C4C mutant in mammalian cells. HeLa RanBPM shRNA cells were transfected with pEBG-GST- Δ N-c-Raf and either pCMV-HA RanBPM, RanBPM- Δ C4, or RanBPM- Δ C4C, and whole cell extracts were prepared 24 h post-transfection and analyzed by Western blot. HA, c-Raf and β -actin antibodies were used to detect HA-RanBPM constructs, Δ N-c-Raf and β -actin proteins, respectively.



Supplementary Figure 3. Western Blot analysis of WT HEK293 or RMND5 KO clonal derivatives. Blots were hybridized as indicated in the figure and materials and methods. Same extracts were analyzed on two separate blots as shown. Quantification of band intensity normalized to Actin (a-Raf, b-Raf) or vinculin (c-Raf) is shown below.

Supplementary Figure 4

Analysis of on-target and off-target sites of RMND5A HEK293 KO clone #1

Guide design by benchling.com

Guide sequence: 5'GTGGAGCACTTCTTTCGACA PAM: AGG

On-target Score = 65.7

Off-target score = 86.0

Targets exon 3 of RMND5A (guide sequence shaded)

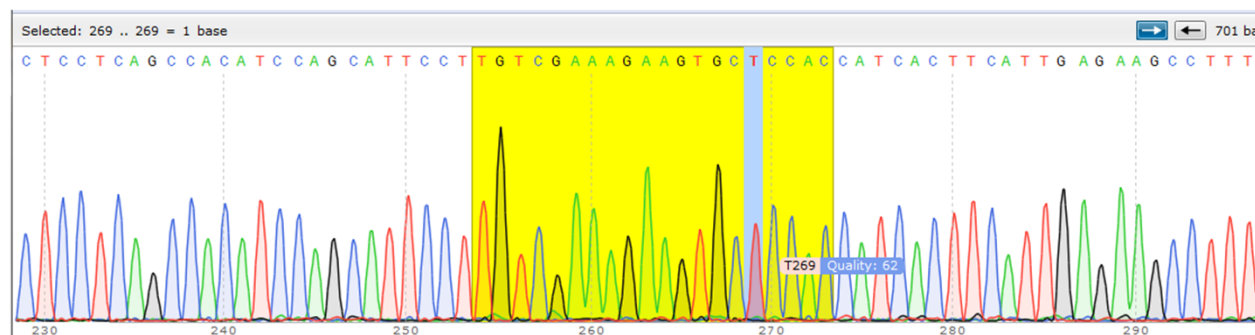


Analysis of Indels in RMND5A KO cells (guide sequence underlined):

On-target

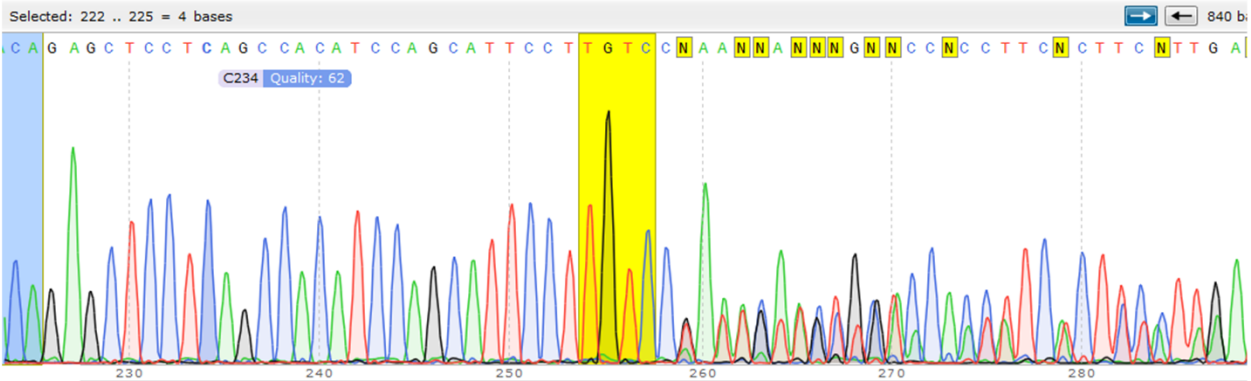
Reverse sequencing of control cells:

NNNNNNNNNNNCTGGNNACAGTTCTAGACTATAATTTTGGCACTTAAAAAATCCCCAAAATACTCCTAGTAAA
ATGAGGTCATCTCTGATAATTTAAATATTTAAAAATCTATCTATAGCTAGAGGAAGACTCAAAACCCACCCCTGCAA
ATACTATAAACTCCAAGGAGTTCCCTTGTTTCTTTTCATATCCTCCCAGTTGGCACACTGTTACCTGACAGAGCT
CCTCAGCCACATCCAGCATTCCCTTGTCTCNAAGAAGTGCTCCACCATCACCTCATTGAGAAGCCTTTGGCTGTCTG
CCTGCCANCANCCATCTATTCCCACACTGCTAATGTCANAATCAAAATTCTGGGGAAAAGAGAAAGGGAAT
CATAAATAGATTATTTTACCCCCACTTAACAGGAAAAAATGGTCTTTGTTTCAATCTGANATACCCCAATAAGAAC
CCAAGTGAATTTATANCGTCCCAAAGGAAGGAATTTTCCACTGCTCCTTTCTAAAACCTGTCATGCTANAANAN
TAAATANGTATATTTTAGATAATGTTTTTTAAAGTAGATTTAATAATGGTGTCCATGAATAAAATAAAATCAAGCN
AATGAAGTATTTCTANGNNTTATATGCANCAATATTTTAGAGGAATGNNNTGANNANNANNAANCAATGCNC
ACANNANNNAAN



Reverse sequencing of RMND5A KO cells:

NNNNNNNNNNNNNNCTGGNNANNNNTTCTAGAACTATAATTTTTGCCACTTAAAAAATTCGCCAAAATACTCCTAGTA
AAATGAGGTCATCTCTGATAATTTAAATATTAATAATCTATCTATAGCTAGAGGAAGACTCAAAACCCACCCCTGC
AAATACTATAAACTCCAAGGAGTTCCTTGTTCCTTTTCATATCCTCCCAGTTGGCACACTGTTACCTGACAGAG
CTCCTCAGCCACATCCAGCATTCTTGTCCNAANNANNGNCCNCCTTCNCTTCNTTGANAANNCNTNNNCTG
NANGNNAGCNNGCNCCATCTATTCCCACNCTGCTAATGTCNGAATCNNAATTCNGGGGAAAAANAANNANAANG
GNAATCATAANTANATTATTTTACCCCGCTTANCNNGAAAAAATGGNCTTTGTTTCAGTCTGANATACCCCTNT
ANNANCCNACTGCNNNTTATANCGTACCAAAGGANGGAATTTTTCCNCTGNTCCTTTCNAAAANTTGNNTTNT
NNAANANTAAATANGTNTATTTNNNNNTAANGNTTTTTTAAAGTANATTTAATAATGGNGTNNNTNANNNNNNNNN
NNNCAANNNNNNNNNNCGGNTTCTAGGNATTANATGCANNNTATTTTTAGAGNANTGNAATGNNNNNNNNNNNN
NNGNNTNNGNNNNANNANANNAANNNNCGAATGAAGTATTCTAGGCATTATATGCANCAATATTTTTAGAGGA
NTGCAATGATGACNTGNN
NNNNNNNNNNNNNNNNNN



Off-target

Sequence	PAM	Score	Gene	Chromosome	Strand	Position	Mismatches	On-target
GTGGAGCACTTCTTTTCGACA	AGG	100	ENSG00000153561	chr2	1	86979117	0	TRUE
CAGAAGCATTCTTTTCGACA	TAG	0.934201		chr8	-1	1.35E+08	4	FALSE
GTGGGGGACTTCTTTCTACA	GGG	0.881154		chr19	-1	53219258	3	FALSE
GTGGGGGACTTCTTTCTACA	GGG	0.881154		chr19	1	53046429	3	FALSE
CTGGAGCACTGCTTTCAACA	CAG	0.861959		chr10	1	1.06E+08	3	FALSE

Analysis of top predicted off target site:

Forward sequencing of control cells:

NNNNNNNNNNNNNNNNNNNNNNCCCTTGACTGCATTCTTTAGAAATAGAGGAAAGATTGTATCCCTTGATGATGTC
TGTGAGCTTGAGAGAGCTATGAATCACAGCAAAATGGCATTATATTTAGTTATTCATTTCCCATTCAAATCCAG
GTGACAAACCTTGTGCAAAACAGCTCCGTATGACTCACATCAACTTTTAAAGATGTTGGTAACGAAGTCTGACGA
ATCGTTATTGACCCAGCTTTGAGAGTGACACAGTTTATCTCAGCTATTAATAAGATATGCAATCTTCATCTGGTT
CAGAACATAATTATTATTATTTTCTCCCTCGTCATGACTCTCATGACAAGAGGGAAGATGTATTCCTGACTTAACA
GAGCGTCTGTGAGCTATGTCAAAGAAATGCTTCTGGATTTTACCACATCTAGAAGTCTTGGAAATGGATTCTAGA
TATAAGACAAAAGTGAGGAACACATAAAAAATGGGCATGTTACAATAATAATAATTTTATTATTAATAGATCAATAAT
TTATTAATGTTAAATTTATATAATAAAAAAGTGCAACAGGTTGAAGAGATGCTAAACATACAGGTGGTTCCAGAATC
ACCCCATTTGTTGTTTCTTGTGGCTCAAGCCTGGGTTTTTTCTATCATCCCCTCTGATTGGTTGTGCCAAATCTGA
CCTTTGTTATTTAATTAGTTAACTCATTTTTTGAATGGATAGGTATTCAGAAAACATATNANNTGCTGGAATATGA
AGCTGGNATNAAGGNNNN

Forward sequencing of KO cells:

NNNNNNNNNNNNNNNNNNNNNGNNTTCTGACTGCATTCTTTAGAAATAGAGGAAAGATTGTATCCCTTGATGATGTCTGTCACTTGA
AGAGCTATGAATCACAGCAAATGGCATTATATTTAGTTATTCATTTCCCATTCAAATCCAGGTGACAAATCTTGCAAAACAGCTCCGTA
TGACTCACATCAACTTTTAAAGATGTTGGTAACGAAGTCTGACGAATCGTTATTGACCCAGCTTTGAGAGTGTACACAGTTTATCTCAGCTATT
AATAAGATATGCAATCTTCATCTGGTTCAGAACAATAATTATTATTTTTCTCCCTCGCATGACTCTCATGACAAGAGGGAAGATGTATTCTGT
ACTTAACAGAGCGTCTGTGAGCTATGTAAAAAGAAATGCTTCTGGATTTTACCACATCTAGAAGTCTTGGAAATGGATTCTAGATATAAGACAA
AAGTGAGGAACACATAAAAATGGGCATGTTACAATAATAAATTTTATTATTAATAGATCAATAATTTATTAATGTTAAATTTATATAATAA
AAAGTGCAACAGGTTGAAGAGATGCTAAACATACAGGTGGTTCAGAATCACCCCATTTGTTGTTCTTGTTGGCTCAAGCCTGGGTTTTTCTAT
CATCCCCCTGATTGGTTGTGCCAAATCTGACCTTGTATTATTAATTAGTTAACTCATTTTTTGAATGGATAGGTATTCAGAAAACATNTNANGT
GCTGGAATATGAAGCTGGATAAGGCNNNNNNNNNNNNNNNNNNNTGCCCTNNNCANNNNNNNNNNNNNNTGNTNNNNNNNNNNNNNN
NNGNNNNNNNTGNNTTCT

NCBI blast search control cells (query) versus KO cells (subject):

Download v Graphics

Sequence ID: Query_59419 Length: 856 Number of Matches: 1

Range 1: 27 to 767 Graphics

Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
1312 bits(1454)	0.0	738/742(99%)	1/742(0%)	Plus/Plus
Query 24	CTTGACTGCATTCCTTAGAAAATAGAGGAAAGATTGTATCCCTTGATGATGTCGTGAGCT	83		
Sbjct 27	CTTGACTGCATTCCTTAGAAAATAGAGGAAAGATTGTATCCCTTGATGATGTCGTGAGCT	86		
Query 84	TGAGAGGCTATGAATCACAGCAAAATGGCATTTTATATTTAGTTATTCAATTCGCCATTC	143		
Sbjct 87	TGAGAGGCTATGAATCACAGCAAAATGGCATTTTATATTTAGTTATTCAATTCGCCATTC	146		
Query 144	AAATCCAGGTGACAAACCTTTGTGCAAAACAGCTCCGATGACTCACATCAACTTTTAAAG	203		
Sbjct 147	AAATCCAGGTGACAAACCTTTGTGCAAAACAGCTCCGATGACTCACATCAACTTTTAAAG	206		
Query 204	ATGTTGGTAAACGAAGCTGACGAATCGTTATTGACCCAGCTTTGAGAGGTACACAGTTT	263		
Sbjct 207	ATGTTGGTAAACGAAGCTGACGAATCGTTATTGACCCAGCTTTGAGAGGTACACAGTTT	266		
Query 264	ATCTCAGCTATTAATAAGATATGCAATCTTCATCGGTTGAGAACATAAATTATTATTATT	323		
Sbjct 267	ATCTCAGCTATTAATAAGATATGCAATCTTCATCGGTTGAGAACATAAATTATTATTATT	326		
Query 324	TTCTCCCTCGTCATGACTCTCATGACAAGAGGGAAGATGTTCTTCACTTAACAGAGCG	383		
Sbjct 327	TTCTCCCTCGTCATGACTCTCATGACAAGAGGGAAGATGTTCTTCACTTAACAGAGCG	386		
Query 384	TCTGTGAGCTATGTCAAAAGAAATGCTTCGGATTTTACCACATCAGAAAGCTTGGAAAT	443		
Sbjct 387	TCTGTGAGCTATGTCAAAAGAAATGCTTCGGATTTTACCACATCAGAAAGCTTGGAAAT	446		
Query 444	GGATTCTAGATATAAGACAAAAGTGAGGAACACATAAAAAATGGGCTAGTTACAataataa	503		
Sbjct 447	GGATTCTAGATATAAGACAAAAGTGAGGAACACATAAAAAATGGGCTAGTTACAATAATAA	506		
Query 504	taattttattattataatagatcaataattttattaatgttaaaatttatataataaaaaGTG	563		
Sbjct 507	TAATTTTATTATTAAATAGATCAATAATTTTAAATGTTAAATTTATATAATAAAAAAGTG	566		
Query 564	CAACAGGTTGAAGAGATGCTAAACATACAGGTGGTTGAGAATCACCCATTGTTGTTCT	623		
Sbjct 567	CAACAGGTTGAAGAGATGCTAAACATACAGGTGGTTGAGAATCACCCATTGTTGTTCT	626		
Query 624	TGTGGCTCAAGCTGGGTTTTTTCTATCATCCCCCTGATTGGTTGTGCCAAATCTGAC	683		
Sbjct 627	TGTGGCTCAAGCTGGGTTTTTTCTATCATCCCCCTGATTGGTTGTGCCAAATCTGAC	686		
Query 684	CTTTGTTATTAAATAGTTAACTCATTTTTTGAATGGATAGGTATTGAGAAAACATATNA	743		
Sbjct 687	CTTTGTTATTAAATAGTTAACTCATTTTTTGAATGGATAGGTATTGAGAAAACATATNA	746		
Query 744	NNNTGCTGGAATATGAAGCTGG	765		
Sbjct 747	-NGTGTGGAATATGAAGCTGG	767		

Note: base 160 in control cells may potentially be a C, see trace: