Supplementary Methods.

Oligos used to generate the 5’TL fusions to the LP/SP reporter.

CAMKK1 V1/3	5'-GATCTGAATTCCTGGGCCCCAGCGAGGCGGT-3'
	5'-CCTCCATGGCTTCAGTCAAGGGGGT-3'
CAMKK1 V2	5'-GATCTGAATTCAGCAGAACAGAGTATGCAAT-3'
	5'-CCCCTCCATGGCTTCAGTCAAGG-3'
CCND3 V1	5'-GATCTGAATTCACCTATGCCGCGTGGGGCGG-3'
	5'-CCAGGTAGTCCATGGCCAGGGGGAAC-3'
CCND3 V2/3	5'-GATCTGAATTCATTCCACGGTTGCTACATCG-3'
	5'-AGCAGCTCCATGGTCGGGCAGCGAAC-3'
CLDN7 V1/3	5'-GATCTGAATTCGGAAAAAAAAGGAGAGCAGC-3'
	5'-TTGGCCATGGCCGCCCTCAGAAAAC-3'
CLDN7 V2	5'-GATCTGAATTCTCCAGTTAGGAGCCTTGATG-3'
	5'-TTGGCCATGGCCGCCCTCAGAAAAC-3'
53BP1 V1/2	5'-GATCTGAATTCGAGTTCGCGGCCGGTGGCGG-3'
	5'-CCCAGCCATGGCGGCGGGAGGT-3'
53BP1 V3	5'-GATCTGAATTCGTTTTTTGTCACTGCCTGCC-3'
	5'-AGTAGGGTCCATGGGCTCCCCAG-3'

WNT5BV1 5-’GATCTGAATTCGACCATTAGCAGGCACCCAGGCCTGTCTTTGGCTCGG
AAACGGTGGCCCCCAATGTAGCCTAGTTTGAACCTAGGAACTGCAGGACCAGAGAGAT
TCCACTGGAGCCTGATGGACGGGT-3’

5-’GAGCTCCATGGTCGGCCTCAGCCCTCCCCAGTGCCCTGGGACT
GACAGTTTCCAGAGTAGGGTTCCCTCTGTCACCCGTCCATCAGGCTCCAGTGGAATCTC
TCTGGTCCTGCCAGTTCTAGGTTCAAACTAGGCTAC-3’

WNT5B V2 5’-GATCTGAATTCTATTCTTCCAAATGGAAACTGCTAATTTTTGAAGC
AGAAGGTTGACAGCTTCAGTAAGATCTCAAGAGAGCGAGAAGACTGGAATCAGGG-3’

5’-GAGCTCCATGGTCGGCCTCAGCCCTCCCCAGTGCCCTGGGACTGAC
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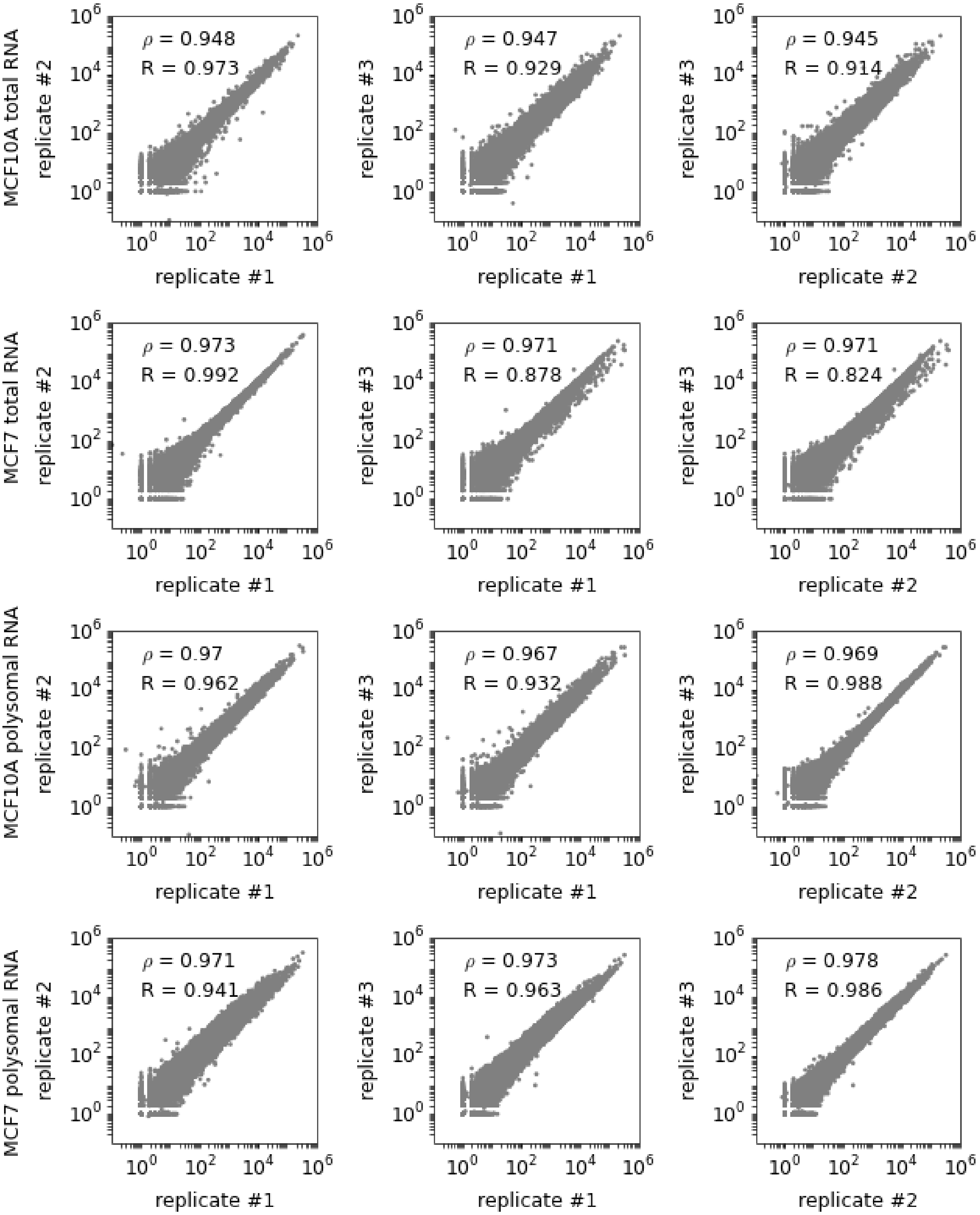


Figure S1: Reproducibility of the total and polysomal RNAseq experiments across all replicas. In each scatter plot the Spearman correlation and Pearson correlation coefficient between samples are indicated.

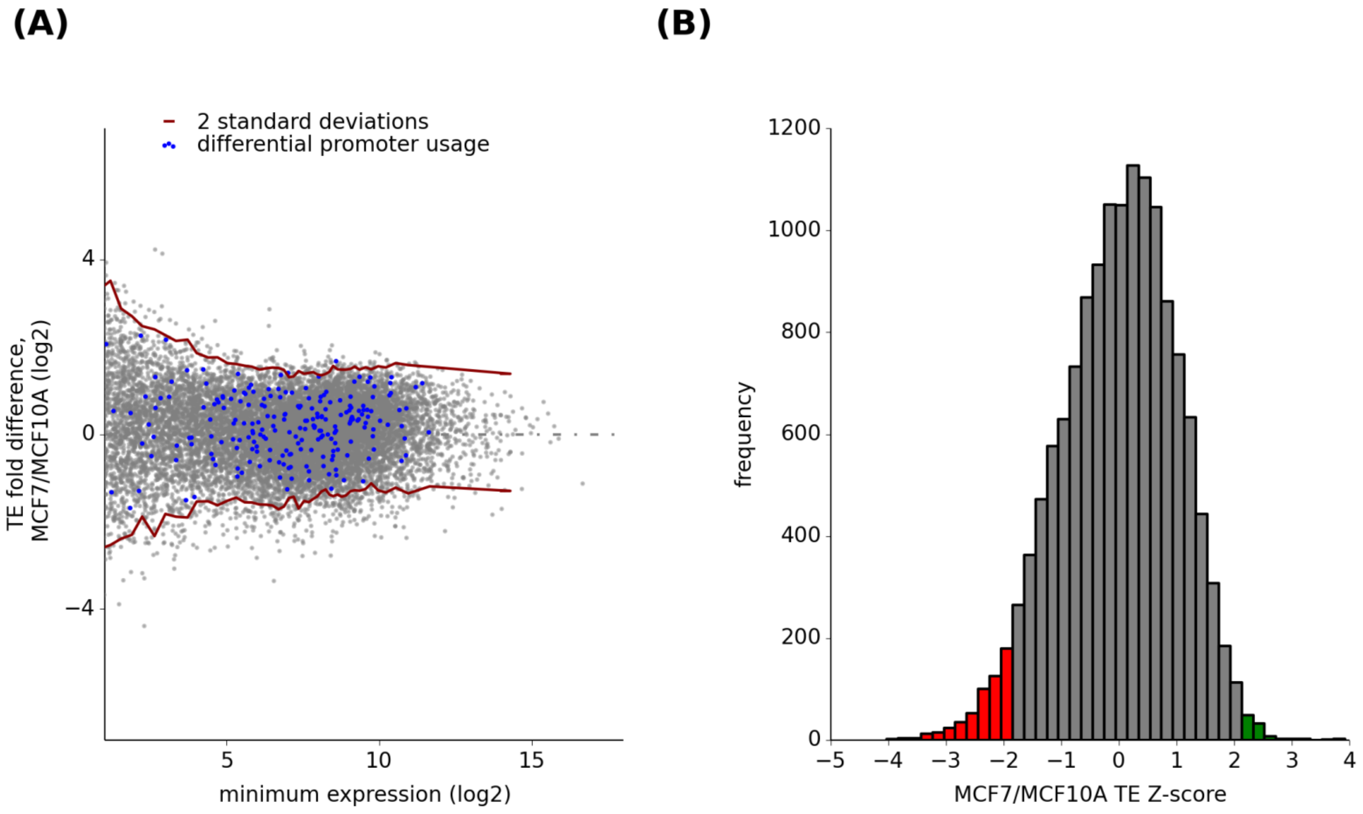


Figure S2: (A) MA plot used to carry out Z-score calculation for translation efficiency changes between MCF7 and MCF10A. The level of expression for each gene is obtained from an average of three replicates. Genes identified to have differential promoter usage are shown in blue. (B) Histogram of Z-score obtained for all genes. Those with an absolute Z-score greater than 2 were taken to be differentially translated.

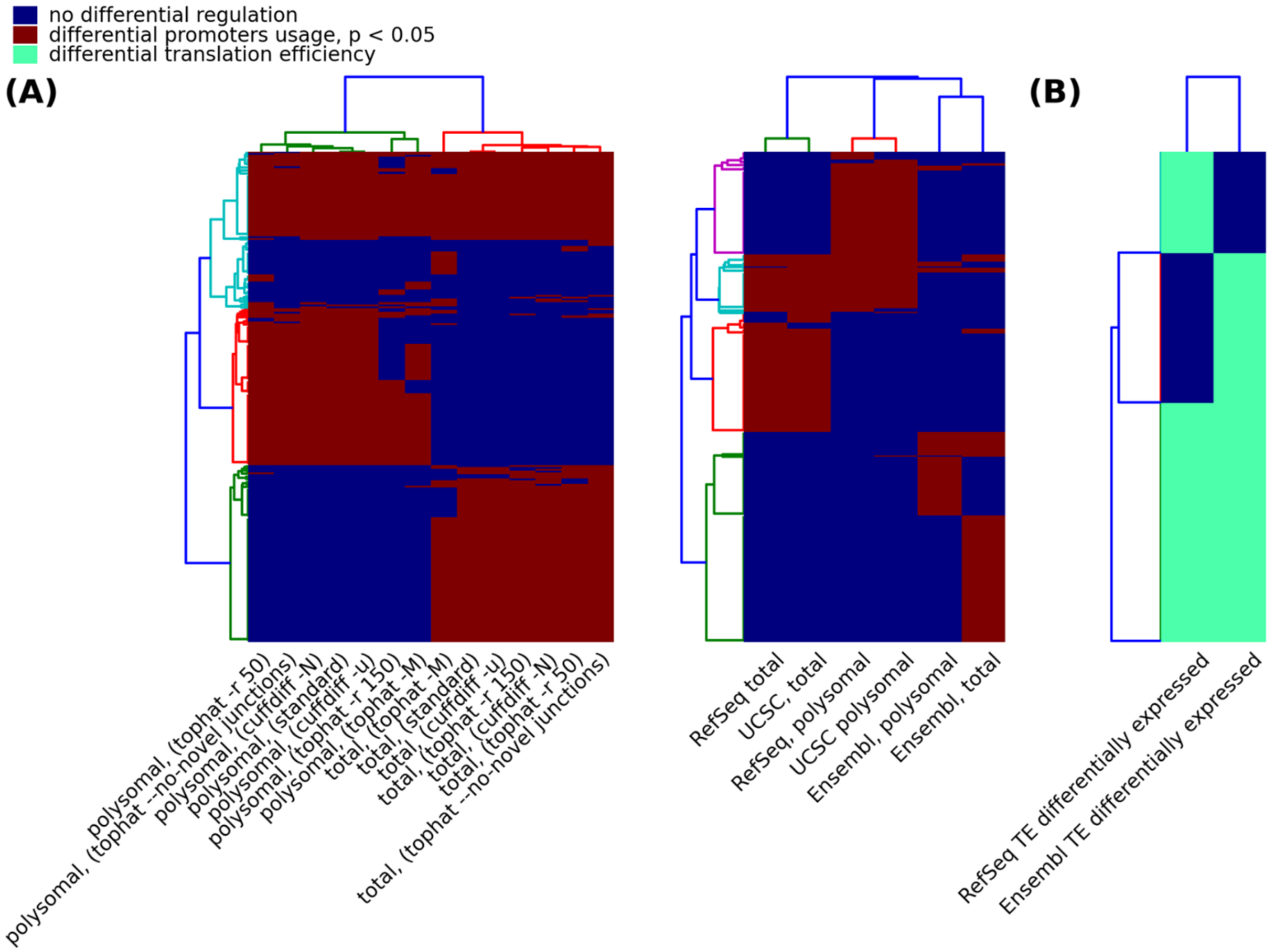
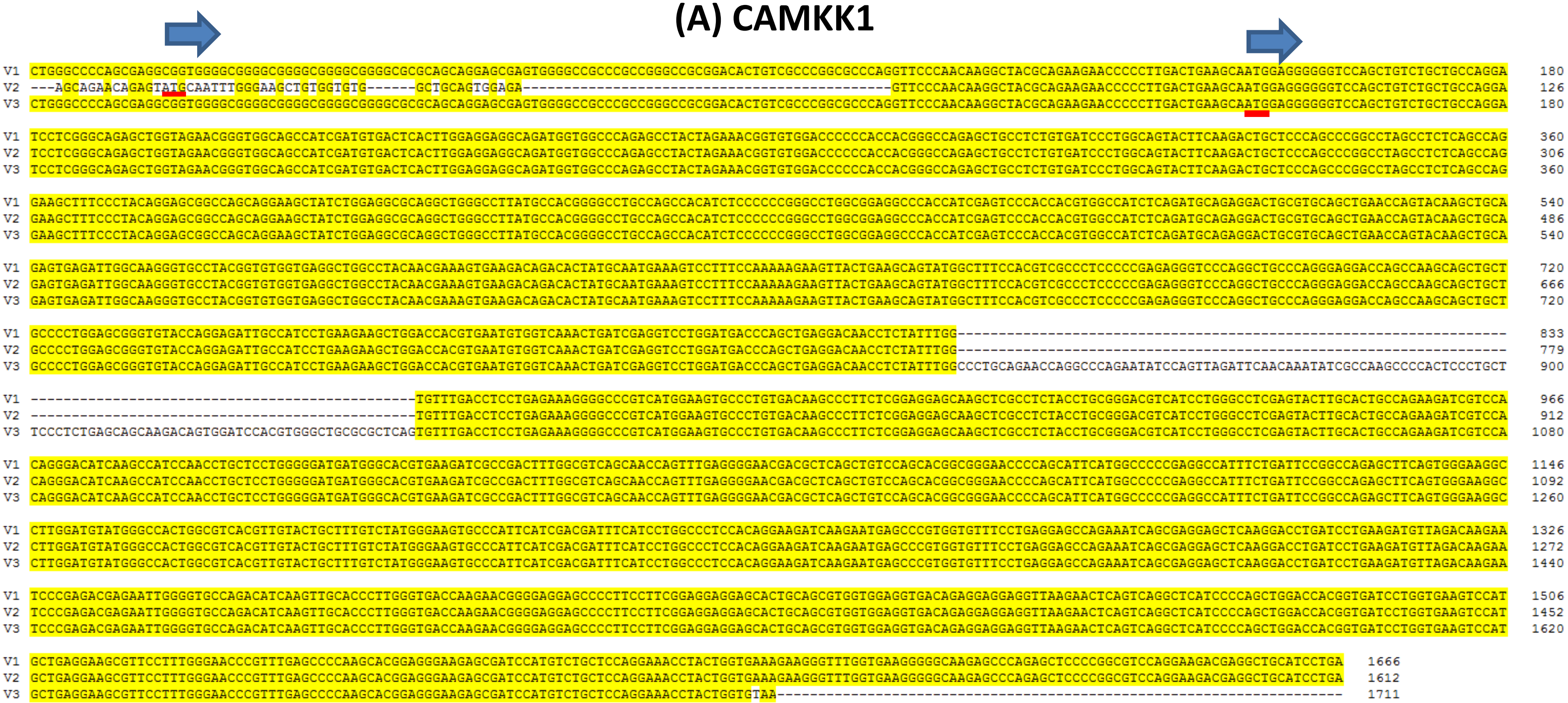


Figure S3: Identification of differential expressed genes under various input parameters. (A) Heatmaps indicating conditions that genes are identified to have differentially expressed promoter usage in polysomal and total RNAseq using altered parameters for Tophat and Cuffdiff (left) and when reads are aligned with different gene annotations (right). Details of the alignment parameters can be found in the Additional File 4. The genes are found to group together according to the RNAseq sample. (B) A heatmap indicating conditions when genes are identified to be TE differentially expressed with RefSeq and Ensembl gene annotations. The samples are clustered with the cityblock distance metric and "average" linkage criteria.

(A) CAMKK1



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V2  ---AGCAGAACAGAGTATGCAATTTGGGAAGCTGTGGTGTG------GCTGCAGTGGAGA---------------------------------------------GTTCCCAACAAGGCTACGCAGAAGAACCCCCTTGACTGAAGCAATGGAGGGGGGTCCAGCTGTCTGCTGCCAGGA   126
V3  CTGGGCCCCAGCGAGGCGGTGGGGCGGGGCGGGGCGGGGCGGGGCGCGCAGCAGGAGCGAGTGGGGCCGCCCGCCGGGCCGCGGACACTGTCGCCCGGCGCCCAGGTTCCCAACAAGGCTACGCAGAAGAACCCCCTTGACTGAAGCAATGGAGGGGGGTCCAGCTGTCTGCTGCCAGGA   180

V1  TCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCATCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCCACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCCTCTCAGCCAG   360
V2  TCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCATCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCCACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCCTCTCAGCCAG   306
V3  TCCTCGGGCAGAGCTGGTAGAACGGGTGGCAGCCATCGATGTGACTCACTTGGAGGAGGCAGATGGTGGCCCAGAGCCTACTAGAAACGGTGTGGACCCCCCACCACGGGCCAGAGCTGCCTCTGTGATCCCTGGCAGTACTTCAAGACTGCTCCCAGCCCGGCCTAGCCTCTCAGCCAG   360

V1  GAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCCTTATGCCACGGGGCCTGCCAGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCACCATCGAGTCCCACCACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCA   540
V2  GAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCCTTATGCCACGGGGCCTGCCAGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCACCATCGAGTCCCACCACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCA   486
V3  GAAGCTTTCCCTACAGGAGCGGCCAGCAGGAAGCTATCTGGAGGCGCAGGCTGGGCCTTATGCCACGGGGCCTGCCAGCCACATCTCCCCCCGGGCCTGGCGGAGGCCCACCATCGAGTCCCACCACGTGGCCATCTCAGATGCAGAGGACTGCGTGCAGCTGAACCAGTACAAGCTGCA   540

V1  GAGTGAGATTGGCAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAATGAAAGTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCAGGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCT   720
V2  GAGTGAGATTGGCAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAATGAAAGTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCAGGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCT   666
V3  GAGTGAGATTGGCAAGGGTGCCTACGGTGTGGTGAGGCTGGCCTACAACGAAAGTGAAGACAGACACTATGCAATGAAAGTCCTTTCCAAAAAGAAGTTACTGAAGCAGTATGGCTTTCCACGTCGCCCTCCCCCGAGAGGGTCCCAGGCTGCCCAGGGAGGACCAGCCAAGCAGCTGCT   720

V1  GCCCCTGGAGCGGGTGTACCAGGAGATTGCCATCCTGAAGAAGCTGGACCACGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACAACCTCTATTTGG-------------------------------------------------------------------   833
V2  GCCCCTGGAGCGGGTGTACCAGGAGATTGCCATCCTGAAGAAGCTGGACCACGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACAACCTCTATTTGG-------------------------------------------------------------------   779
V3  GCCCCTGGAGCGGGTGTACCAGGAGATTGCCATCCTGAAGAAGCTGGACCACGTGAATGTGGTCAAACTGATCGAGGTCCTGGATGACCCAGCTGAGGACAACCTCTATTTGGCCCTGCAGAACCAGGCCCAGAATATCCAGTTAGATTCAACAAATATCGCCAAGCCCCACTCCCTGCT   900

V1  -----------------------------------------------TGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTGTGACAAGCCCTTCTCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAAGATCGTCCA   966
V2  -----------------------------------------------TGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTGTGACAAGCCCTTCTCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAAGATCGTCCA   912
V3  TCCCTCTGAGCAGCAAGACAGTGGATCCACGTGGGCTGCGCGCTCAGTGTTTGACCTCCTGAGAAAGGGGCCCGTCATGGAAGTGCCCTGTGACAAGCCCTTCTCGGAGGAGCAAGCTCGCCTCTACCTGCGGGACGTCATCCTGGGCCTCGAGTACTTGCACTGCCAGAAGATCGTCCA  1080

V1  CAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCCGACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAGCATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGC  1146
V2  CAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCCGACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAGCATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGC  1092
V3  CAGGGACATCAAGCCATCCAACCTGCTCCTGGGGGATGATGGGCACGTGAAGATCGCCGACTTTGGCGTCAGCAACCAGTTTGAGGGGAACGACGCTCAGCTGTCCAGCACGGCGGGAACCCCAGCATTCATGGCCCCCGAGGCCATTTCTGATTCCGGCCAGAGCTTCAGTGGGAAGGC  1260

V1  CTTGGATGTATGGGCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCCCTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCAAGGACCTGATCCTGAAGATGTTAGACAAGAA  1326
V2  CTTGGATGTATGGGCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCCCTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCAAGGACCTGATCCTGAAGATGTTAGACAAGAA  1272
V3  CTTGGATGTATGGGCCACTGGCGTCACGTTGTACTGCTTTGTCTATGGGAAGTGCCCATTCATCGACGATTTCATCCTGGCCCTCCACAGGAAGATCAAGAATGAGCCCGTGGTGTTTCCTGAGGAGCCAGAAATCAGCGAGGAGCTCAAGGACCTGATCCTGAAGATGTTAGACAAGAA  1440

V1  TCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTTGCACCCTTGGGTGACCAAGAACGGGGAGGAGCCCCTTCCTTCGGAGGAGGAGCACTGCAGCGTGGTGGAGGTGACAGAGGAGGAGGTTAAGAACTCAGTCAGGCTCATCCCCAGCTGGACCACGGTGATCCTGGTGAAGTCCAT  1506
V2  TCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTTGCACCCTTGGGTGACCAAGAACGGGGAGGAGCCCCTTCCTTCGGAGGAGGAGCACTGCAGCGTGGTGGAGGTGACAGAGGAGGAGGTTAAGAACTCAGTCAGGCTCATCCCCAGCTGGACCACGGTGATCCTGGTGAAGTCCAT  1452
V3  TCCCGAGACGAGAATTGGGGTGCCAGACATCAAGTTGCACCCTTGGGTGACCAAGAACGGGGAGGAGCCCCTTCCTTCGGAGGAGGAGCACTGCAGCGTGGTGGAGGTGACAGAGGAGGAGGTTAAGAACTCAGTCAGGCTCATCCCCAGCTGGACCACGGTGATCCTGGTGAAGTCCAT  1620

V1  GCTGAGGAAGCGTTCCTTTGGGAACCCGTTTGAGCCCCAAGCACGGAGGGAAGAGCGATCCATGTCTGCTCCAGGAAACCTACTGGTGAAAGAAGGGTTTGGTGAAGGGGGCAAGAGCCCAGAGCTCCCCGGCGTCCAGGAAGACGAGGCTGCATCCTGA   1666
V2  GCTGAGGAAGCGTTCCTTTGGGAACCCGTTTGAGCCCCAAGCACGGAGGGAAGAGCGATCCATGTCTGCTCCAGGAAACCTACTGGTGAAAGAAGGGTTTGGTGAAGGGGGCAAGAGCCCAGAGCTCCCCGGCGTCCAGGAAGACGAGGCTGCATCCTGA   1612
V3  GCTGAGGAAGCGTTCCTTTGGGAACCCGTTTGAGCCCCAAGCACGGAGGGAAGAGCGATCCATGTCTGCTCCAGGAAACCTACTGGTGTAA---------------------------------------------------------------------   1711
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Supplementary Figure 4

(B) CCND3

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V1 ACCTATGCCGCGTGGGCGGGGCTGCGGGCGGGGTGCGCGGCGCCGGATGAGCCTCCTGGCCTCGCCGAGGGTGTACAGGGGGTGGGAGAGTCAGGCCGGGGACCGAAACTTGGCTGAGCAG----AGCACCAGCCCCTTTGTCTC-CTCCGCCCCCTCTTCCCCACTTCCTGCCCAG   175
V2 ----ATTCCAC----GGTTGCTACATCGTCGCGAGGGGCGGGGCGCCTG-------TCAGGGAAGCGGCGCGCGCGCGGGCGGCGGG-----CGGGCTGGGGA-------TCCGCCGCGCAGTGCCAGCGCCAGCGCCAGACCCGCGCCCCGCGCTCTCCGGCCCGTCGCCTGCC---   150
V3 ----ATTCCAC----GGTTGCTACATCGTCGCGAGGGGCGGGGCGCCTG-------TCAGGGAAGCGGCGCGCGCGCGGGCGGCGGG-----CGGGCTGGGGA-------TCCGCCGCGCAGTGCCAGCGCCAGCGCCAGACCCGCGCCCCGCGCTCTCCGGCCCGTCGCCTGCC---   150

V1 CTCTGGATCGGCGGCGCG-GCGCGGACTTTGTAAACACTTCGCCACTGCAGGGGTGGAGACTGGCTCTGTTCGGATGCCGGCCGGGGG-GAGAGGTGCAATCCTCTCCTCGCGGCTGGTGGTTGCGACCACCCCCACTCCCCAAAGGCAGGCTCCGGA-GGCGGCGGG------ACAGA   346
V2 ------TTGGGACTCGCGAGCCCGCACTCCCGCCCTGCCTGTTCGCTGCCCGAGTATGGAGCTGCTGTGTTGCGAAGGCACCCGGCACGCGCCCCGGGCCGGGCCGGACCCGCGGCTGCTGG--GGGACCA--GCGTGTCCTGCAGAGCCTGCTCCGCCTGGAGGAGCGCTACGTACCCC   320
V3 ------TTGGGACTCGCG-AGCCCG----------CACTCCCGCCCTGC-----------------CTGTTCG-----CTGCCCGAGTA-TGGAGCTGCTGT-------------------GTTGCGAAGGCACCC----------GGCACGCGCCCCG-GGC--CGGG------CCGGA   252

V1 GCGCCTGCGACC-CCAGTCGGTGCTCCGGGGAGGTCA-------CCTGACGA----------------------GGAGGTATGTGAGGAGCAGCGCTGTGAGGAGGAAGTCTTCCCCCTGGCCATGAACTACCTGGATCGCTACCTGTCTTGCGTCCCCACCCGAAAGGCGCAGTTGCAG   496
V2 GCGCCTCCTACTTCCAGTGCGTGCAGCG-GGAGATCAAGCCGCACATGCGGAAGATGCTGGCTTACTGGATGCTGGAGGTATGTGAGGAGCAGCGCTGTGAGGAGGAAGTCTTCCCCCTGGCCATGAACTACCTGGATCGCTACCTGTCTTGCGTCCCCACCCGAAAGGCGCAGTTGCAG   499
V3 ---CCCGCG-------------GCTGCTGGG-------------------------------------------------------GGACCAGCGTGTCCTGCAGAGCCTGCTCCGCCTGG------------AGGAGCGCTACGTACCCCGCGCCTCC------------TACTTCCAG   337

V1 CTCCTGGGTGCGGTCTGCATGCTGCTGGCCTCCAAGCTGCGCGAGACCACGCCCCTGACCATCGAAAAACTGTGCATCTACACCGACCACGCTGTCTCTCCCCGCCAGTTGCGGGACTGGGAGGTGCTGGTCCTAGGGAAGCTCAAGTGGGACCTGGCTGCTGTGATTGCACATGATTTC   676
V2 CTCCTGGGTGCGGTCTGCATGCTGCTGGCCTCCAAGCTGCGCGAGACCACGCCCCTGACCATCGAAAAACTGTGCATCTACACCGACCACGCTGTCTCTCCCCGCCAGTTGCGGGACTGGGAGGTGCTGGTCCTAGGGAAGCTCAAGTGGGACCTGGCTGCTGTGATTGCACATGATTTC   679
V3 ---------------TGCGTGCAGCGGGAGATCAAGC------------CGCACATG-------------------------CGGAAGATGCTG--GCTTACTGGATGCTGGAGGACTGGGAGGTGCTGGTCCTAGGGAAGCTCAAGTGGGACCTGGCTGCTGTGATTGCACATGATTTC   463

V1 CTGGCCTTCATTCTGCACCGGCTCTCTCTGCCCCGTGACCGACAGGCCTTGGTCAAAAAGCATGCCCAGACCTTTTTGGCCCTCTGTGCTACAGATTATACCTTTGCCATGTACCCGCCATCCATGATCGCCACGGGCAGCATTGGGGCTGCAGTGCAAGGCCTGGGTGCCTGCTCCATG   856
V2 CTGGCCTTCATTCTGCACCGGCTCTCTCTGCCCCGTGACCGACAGGCCTTGGTCAAAAAGCATGCCCAGACCTTTTTGGCCCTCTGTGCTACAGATTATACCTTTGCCATGTACCCGCCATCCATGATCGCCACGGGCAGCATTGGGGCTGCAGTGCAAGGCCTGGGTGCCTGCTCCATG   859
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V1 TCCGGGGATGAGCTCACAGAGCTGCTGGCAGGGATCACTGGCACTGAAGTGGACTGCCTGCGGGCCTGTCAGGAGCAGATCGAAGCTGCACTCAGGGAGAGCCTCAGGGAAGCCTCTCAGACCAGCTCCAGCCCAGCGCCCAAAGCCCCCCGGGGCTCCAGCAGCCAAGGGCCCAGCCAG   1036
V2 TCCGGGGATGAGCTCACAGAGCTGCTGGCAGGGATCACTGGCACTGAAGTGGACTGCCTGCGGGCCTGTCAGGAGCAGATCGAAGCTGCACTCAGGGAGAGCCTCAGGGAAGCCTCTCAGACCAGCTCCAGCCCAGCGCCCAAAGCCCCCCGGGGCTCCAGCAGCCAAGGGCCCAGCCAG   1039
V3 TCCGGGGATGAGCTCACAGAGCTGCTGGCAGGGATCACTGGCACTGAAGTGGACTGCCTGCGGGCCTGTCAGGAGCAGATCGAAGCTGCACTCAGGGAGAGCCTCAGGGAAGCCTCTCAGACCAGCTCCAGCCCAGCGCCCAAAGCCCCCCGGGGCTCCAGCAGCCAAGGGCCCAGCCAG   823

V1 ACCAGCACTCCTACAGATGTCACAGCCATACACCTGTAG   1075
V2 ACCAGCACTCCTACAGATGTCACAGCCATACACCTGTAG   1078
V3 ACCAGCACTCCTACAGATGTCACAGCCATACACCTGTAG    862
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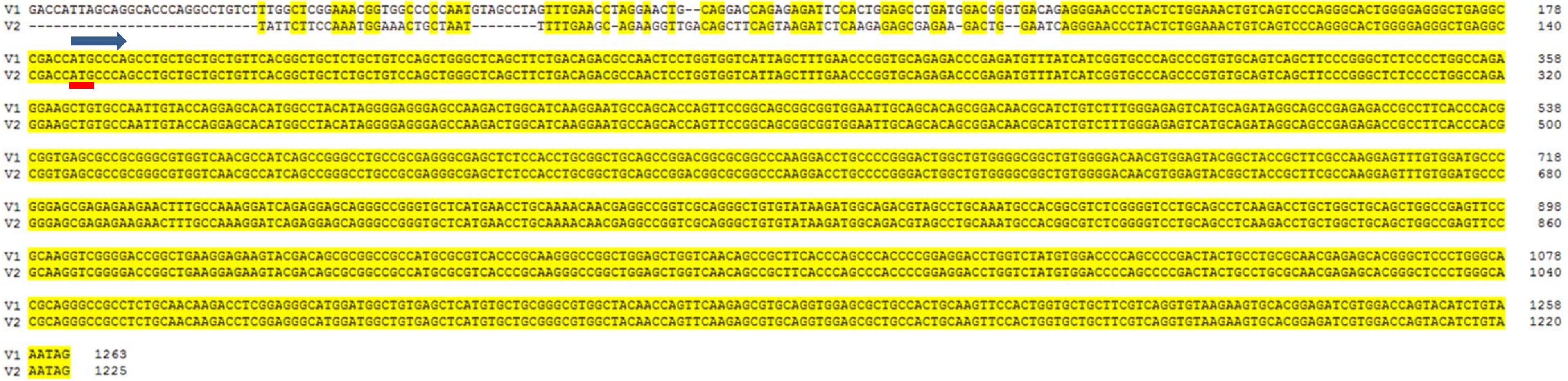
(C) WNT5B



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V2 ----------------------------TATTCTTCCAAATGGAAACTGCTAAT--------TTTTGAAGC-AGAAGGTTGACAGCTTCAGTAAGATCTCAAGAGAGCGAGAA-GACTG--GAATCAGGGAACCCTACTCTGGAAACTGTCAGTCCCAGGGCACTGGGGAGGGCTGAGGC   140

V1 CGACCATGCCCAGCCTGCTGCTGCTGTTCACGGCTGCTCTGCTGTCCAGCTGGGCTCAGCTTCTGACAGACGCCAACTCCTGGTGGTCATTAGCTTTGAACCCGGTGCAGAGACCCGAGATGTTTATCATCGGTGCCCAGCCCGTGTGCAGTCAGCTTCCCGGGCTCTCCCCTGGCCAGA   358
V2 CGACCATGCCCAGCCTGCTGCTGCTGTTCACGGCTGCTCTGCTGTCCAGCTGGGCTCAGCTTCTGACAGACGCCAACTCCTGGTGGTCATTAGCTTTGAACCCGGTGCAGAGACCCGAGATGTTTATCATCGGTGCCCAGCCCGTGTGCAGTCAGCTTCCCGGGCTCTCCCCTGGCCAGA   320

V1 GGAAGCTGTGCCAATTGTACCAGGAGCACATGGCCTACATAGGGGAGGGAGCCAAGACTGGCATCAAGGAATGCCAGCACCAGTTCCGGCAGCGGCGGTGGAATTGCAGCACAGCGGACAACGCATCTGTCTTTGGGAGAGTCATGCAGATAGGCAGCCGAGAGACCGCCTTCACCCACG   538
V2 GGAAGCTGTGCCAATTGTACCAGGAGCACATGGCCTACATAGGGGAGGGAGCCAAGACTGGCATCAAGGAATGCCAGCACCAGTTCCGGCAGCGGCGGTGGAATTGCAGCACAGCGGACAACGCATCTGTCTTTGGGAGAGTCATGCAGATAGGCAGCCGAGAGACCGCCTTCACCCACG   500

V1 CGGTGAGCGCCGCGGGCGTGGTCAACGCCATCAGCCGGGCCTGCCGCGAGGGCGAGCTCTCCACCTGCGGCTGCAGCCGGACGGCGCGGCCCAAGGACCTGCCCCGGGACTGGCTGTGGGGCGGCTGTGGGGACAACGTGGAGTACGGCTACCGCTTCGCCAAGGAGTTTGTGGATGCCC   718
V2 CGGTGAGCGCCGCGGGCGTGGTCAACGCCATCAGCCGGGCCTGCCGCGAGGGCGAGCTCTCCACCTGCGGCTGCAGCCGGACGGCGCGGCCCAAGGACCTGCCCCGGGACTGGCTGTGGGGCGGCTGTGGGGACAACGTGGAGTACGGCTACCGCTTCGCCAAGGAGTTTGTGGATGCCC   680

V1 GGGAGCGAGAGAAGAACTTTGCCAAAGGATCAGAGGAGCAGGGCCGGGTGCTCATGAACCTGCAAAACAACGAGGCCGGTCGCAGGGCTGTGTATAAGATGGCAGACGTAGCCTGCAAATGCCACGGCGTCTCGGGGTCCTGCAGCCTCAAGACCTGCTGGCTGCAGCTGGCCGAGTTCC   898
V2 GGGAGCGAGAGAAGAACTTTGCCAAAGGATCAGAGGAGCAGGGCCGGGTGCTCATGAACCTGCAAAACAACGAGGCCGGTCGCAGGGCTGTGTATAAGATGGCAGACGTAGCCTGCAAATGCCACGGCGTCTCGGGGTCCTGCAGCCTCAAGACCTGCTGGCTGCAGCTGGCCGAGTTCC   860

V1 GCAAGGTCGGGGACCGGCTGAAGGAGAAGTACGACAGCGCGGCCGCCATGCGCGTCACCCGCAAGGGCCGGCTGGAGCTGGTCAACAGCCGCTTCACCCAGCCCACCCCGGAGGACCTGGTCTATGTGGACCCCAGCCCCGACTACTGCCTGCGCAACGAGAGCACGGGCTCCCTGGGCA   1078
V2 GCAAGGTCGGGGACCGGCTGAAGGAGAAGTACGACAGCGCGGCCGCCATGCGCGTCACCCGCAAGGGCCGGCTGGAGCTGGTCAACAGCCGCTTCACCCAGCCCACCCCGGAGGACCTGGTCTATGTGGACCCCAGCCCCGACTACTGCCTGCGCAACGAGAGCACGGGCTCCCTGGGCA   1040

V1 CGCAGGGCCGCCTCTGCAACAAGACCTCGGAGGGCATGGATGGCTGTGAGCTCATGTGCTGCGGGCGTGGCTACAACCAGTTCAAGAGCGTGCAGGTGGAGCGCTGCCACTGCAAGTTCCACTGGTGCTGCTTCGTCAGGTGTAAGAAGTGCACGGAGATCGTGGACCAGTACATCTGTA   1258
V2 CGCAGGGCCGCCTCTGCAACAAGACCTCGGAGGGCATGGATGGCTGTGAGCTCATGTGCTGCGGGCGTGGCTACAACCAGTTCAAGAGCGTGCAGGTGGAGCGCTGCCACTGCAAGTTCCACTGGTGCTGCTTCGTCAGGTGTAAGAAGTGCACGGAGATCGTGGACCAGTACATCTGTA   1220

V1 AATAG   1263
V2 AATAG   1225
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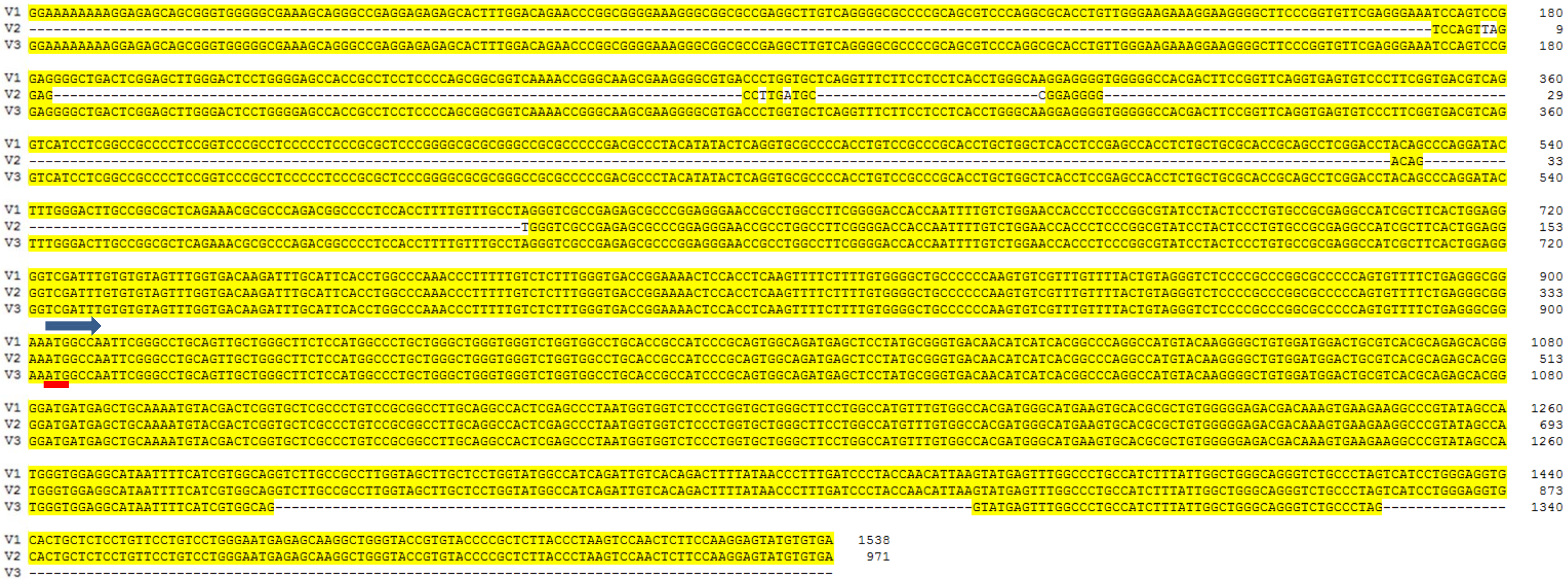
Supplementary Figure 4

(D) CLDN7



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V2 ---------------------------------------------------------------------------------------------------------------------------------------------------------------------------TCCAGTTAG    9
V3 GGAAAAAAAAGGAGAGCAGCGGGTGGGGGCGAAAGCAGGGCCGAGGAGAGAGCACTTTGGACAGAACCCGGCGGGGAAAGGGCGGCGCCGAGGCTTGTCAGGGGCGCCCCGCAGCGTCCCAGGCGCACCTGTTGGGAAGAAAGGAAGGGGCTTCCCGGTGTTCGAGGGAAATCCAGTCCG  180

V1 GAGGGGCTGACTCGGAGCTTGGGACTCCTGGGGAGCCACCGCCTCCTCCCCAGCGGCGGTCAAAACCGGGCAAGCGAAGGGGCGTGACCCTGGTGCTCAGGTTTCTTCCTCCTCACCTGGGCAAGGAGGGGTGGGGGCCACGACTTCCGGTTCAGGTGAGTGTCCCTTCGGTGACGTCAG  360
V2 GAG------------------------------------------------------------------------------------CCTTGATGC---------------------------CGGAGGGG-------------------------------------------------   29
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V1 GTCATCCTCGGCCGCCCCTCCGGTCCCGCCTCCCCCTCCCGCGCTCCCGGGGCGCGCGGGCCGCGCCCCCGACGCCCTACATATACTCAGGTGCGCCCCACCTGTCCGCCCGCACCTGCTGGCTCACCTCCGAGCCACCTCTGCTGCGCACCGCAGCCTCGGACCTACAGCCCAGGATAC  540
V2 ----------------------------------------------------------------------------------------------------------------------------------------------------------------------ACAG----------   33
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V1 GGTCGATTTGTGTGTAGTTTGGTGACAAGATTTGCATTCACCTGGCCCAAACCCTTTTTGTCTCTTTGGGTGACCGGAAAACTCCACCTCAAGTTTTCTTTTGTGGGGCTGCCCCCCAAGTGTCGTTTGTTTTACTGTAGGGTCTCCCCGCCCGGCGCCCCCAGTGTTTTCTGAGGGCGG  900
V2 GGTCGATTTGTGTGTAGTTTGGTGACAAGATTTGCATTCACCTGGCCCAAACCCTTTTTGTCTCTTTGGGTGACCGGAAAACTCCACCTCAAGTTTTCTTTTGTGGGGCTGCCCCCCAAGTGTCGTTTGTTTTACTGTAGGGTCTCCCCGCCCGGCGCCCCCAGTGTTTTCTGAGGGCGG  333
V3 GGTCGATTTGTGTGTAGTTTGGTGACAAGATTTGCATTCACCTGGCCCAAACCCTTTTTGTCTCTTTGGGTGACCGGAAAACTCCACCTCAAGTTTTCTTTTGTGGGGCTGCCCCCCAAGTGTCGTTTGTTTTACTGTAGGGTCTCCCCGCCCGGCGCCCCCAGTGTTTTCTGAGGGCGG  900

V1 AAATGGCCAATTCGGGCCTGCAGTTGCTGGGCTTCTCCATGGCCCTGCTGGGCTGGGTGGGTCTGGTGGCCTGCACCGCCATCCCGCAGTGGCAGATGAGCTCCTATGCGGGTGACAACATCATCACGGCCCAGGCCATGTACAAGGGGCTGTGGATGGACTGCGTCACGCAGAGCACGG 1080
V2 AAATGGCCAATTCGGGCCTGCAGTTGCTGGGCTTCTCCATGGCCCTGCTGGGCTGGGTGGGTCTGGTGGCCTGCACCGCCATCCCGCAGTGGCAGATGAGCTCCTATGCGGGTGACAACATCATCACGGCCCAGGCCATGTACAAGGGGCTGTGGATGGACTGCGTCACGCAGAGCACGG  513
V3 AAATGGCCAATTCGGGCCTGCAGTTGCTGGGCTTCTCCATGGCCCTGCTGGGCTGGGTGGGTCTGGTGGCCTGCACCGCCATCCCGCAGTGGCAGATGAGCTCCTATGCGGGTGACAACATCATCACGGCCCAGGCCATGTACAAGGGGCTGTGGATGGACTGCGTCACGCAGAGCACGG 1080

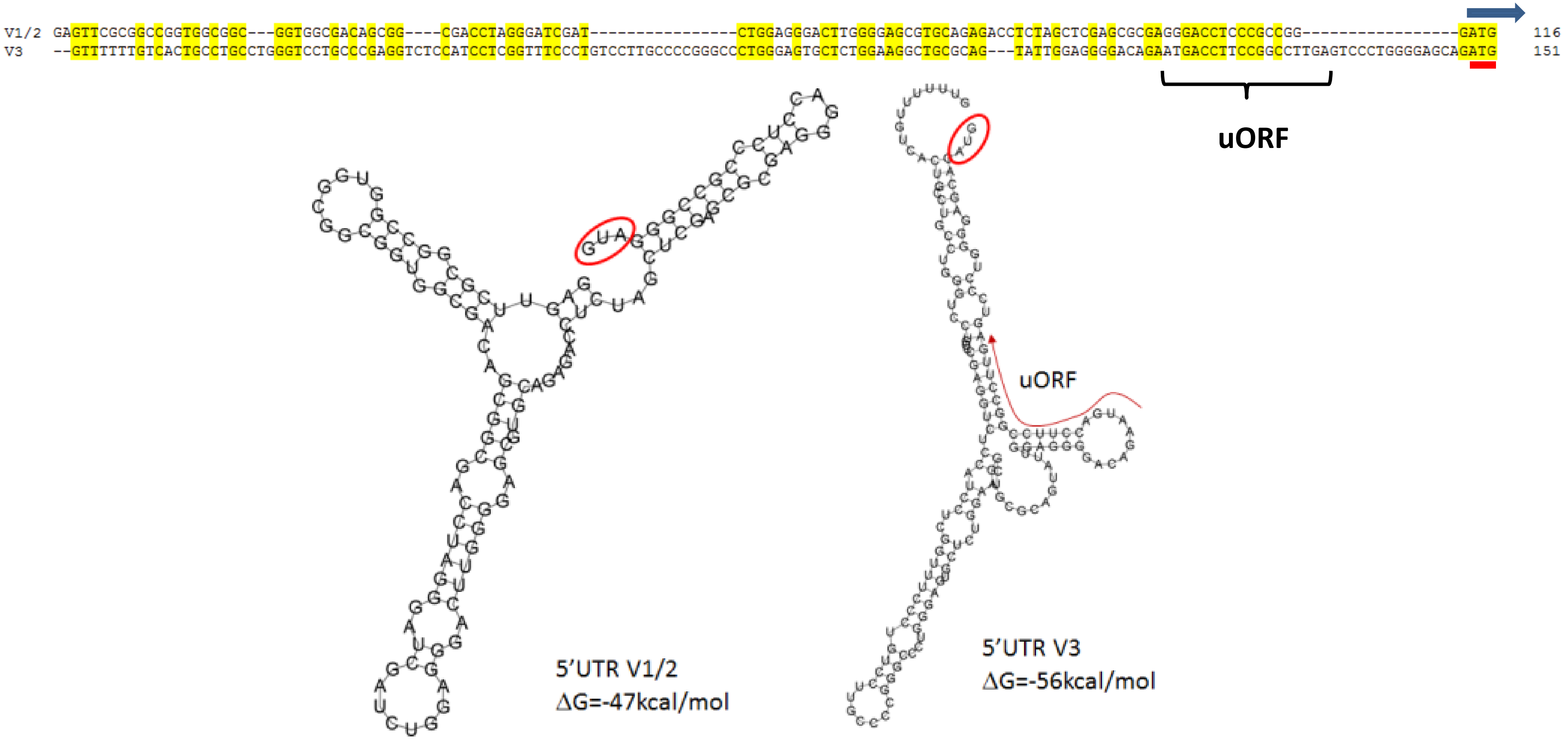
V1 GGATGATGAGCTGCAAAATGTACGACTCGGTGCTCGCCCTGTCCGCGGCCTTGCAGGCCACTCGAGCCCTAATGGTGGTCTCCCTGGTGCTGGGCTTCCTGGCCATGTTTGTGGCCACGATGGGCATGAAGTGCACGCGCTGTGGGGGAGACGACAAAGTGAAGAAGGCCCGTATAGCCA 1260
V2 GGATGATGAGCTGCAAAATGTACGACTCGGTGCTCGCCCTGTCCGCGGCCTTGCAGGCCACTCGAGCCCTAATGGTGGTCTCCCTGGTGCTGGGCTTCCTGGCCATGTTTGTGGCCACGATGGGCATGAAGTGCACGCGCTGTGGGGGAGACGACAAAGTGAAGAAGGCCCGTATAGCCA  693
V3 GGATGATGAGCTGCAAAATGTACGACTCGGTGCTCGCCCTGTCCGCGGCCTTGCAGGCCACTCGAGCCCTAATGGTGGTCTCCCTGGTGCTGGGCTTCCTGGCCATGTTTGTGGCCACGATGGGCATGAAGTGCACGCGCTGTGGGGGAGACGACAAAGTGAAGAAGGCCCGTATAGCCA 1260

V1 TGGGTGGAGGCATAATTTTCATCGTGGCAGGTCTTGCCGCCTTGGTAGCTTGCTCCTGGTATGGCCATCAGATTGTCACAGACTTTTATAACCCTTTGATCCCTACCAACATTAAGTATGAGTTTGGCCCTGCCATCTTTATTGGCTGGGCAGGTCTGCCCTAGTCATCCTGGGAGGTG 1440
V2 TGGGTGGAGGCATAATTTTCATCGTGGCAGGTCTTGCCGCCTTGGTAGCTTGCTCCTGGTATGGCCATCAGATTGTCACAGACTTTTATAACCCTTTGATCCCTACCAACATTAAGTATGAGTTTGGCCCTGCCATCTTTATTGGCTGGGCAGGTCTGCCCTAGTCATCCTGGGAGGTG  873
V3 TGGGTGGAGGCATAATTTTCATCGTGGCAG-------------------------------------------------------------------------------------GTATGAGTTTGGCCCTGCCATCTTTATTGGCTGGGCAGGTCTGCCCTAG--------------- 1340

V1 CACTGCTCTCCTGTTCCTGTCCTGGGAATGAGAGCAAGGCTGGGTACCGTGTACCCCGCTCTTACCCTAAGTCCAACTCTTCCAAGGAGTATGTGTGA 1538
V2 CACTGCTCTCCTGTTCCTGTCCTGGGAATGAGAGCAAGGCTGGGTACCGTGTACCCCGCTCTTACCCTAAGTCCAACTCTTCCAAGGAGTATGTGTGA  971
V3 --------------------------------------------------------------------------------------------------
```

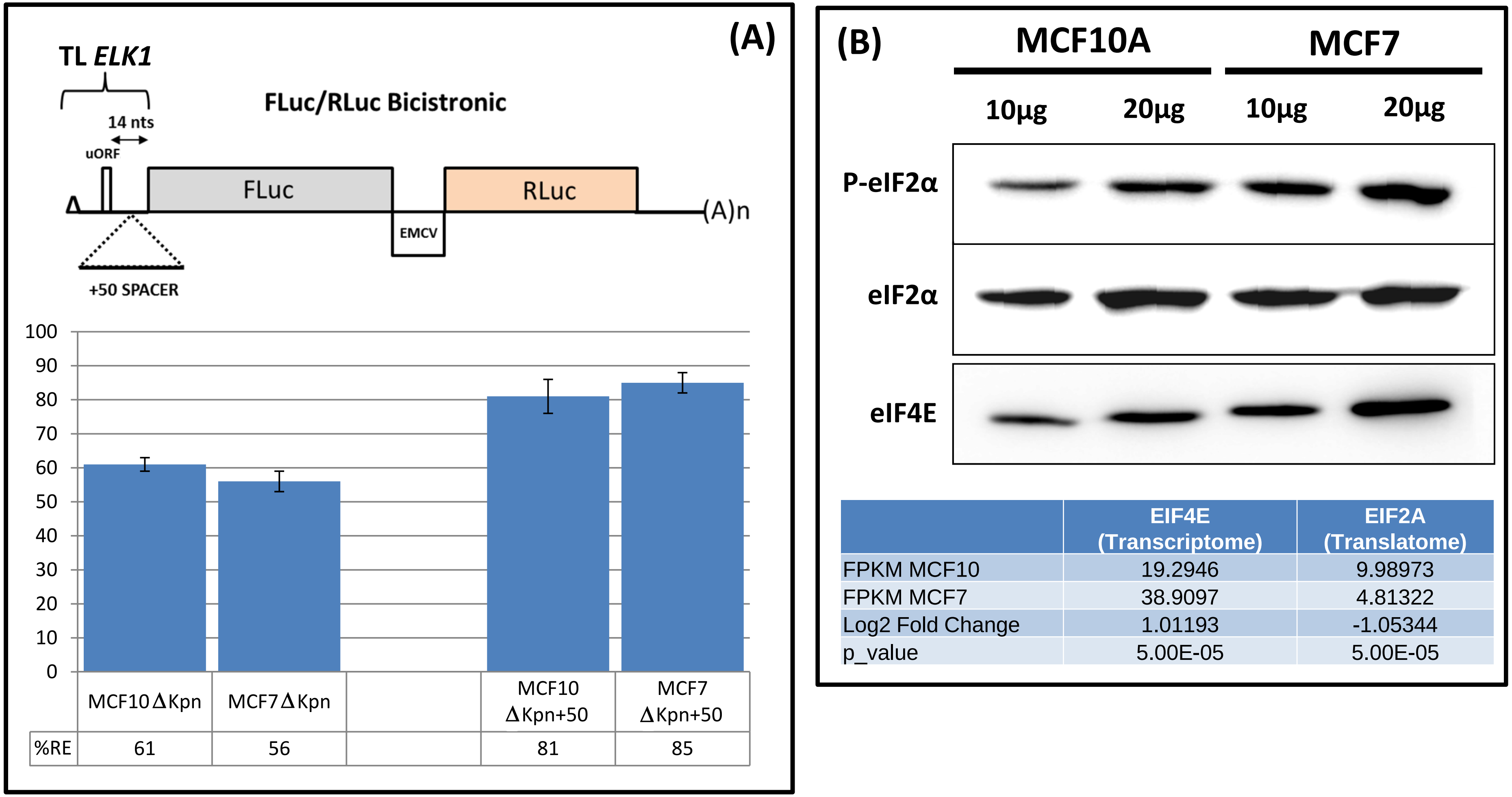
Supplementary Figure 4

(E) 53BP1



```
V1/2 GAGTTCGCGGCCGGTGGCGGC---GGTGGCGACAGCGG----CGACCTAGGGATCGAT----------------CTGGAGGGACTTGGGGAGCGTGCAGAGACCTCTAGCTCGAGCGCGAGGGACCTCCCGCCGG-----------------GATG   116
V3   --GTTTTTTGTCACTGCCTGCCTGGGTCCTGCCCGAGGTCTCCATCCTCGGTTTCCCTGTCCTTGCCCCGGGCCCTGGGAGTGCTCTGGAAGGCTGCGCAG---TATTGGAGGGGACAGAATGACCTTCCGGCCTTGAGTCCCTGGGGAGCAGATG   151
```

Figure S4: **A-D.** Sequence alignments of the different transcript variants for the genes *CAMKK1*, *CCND3*, *WNT5B* and *CLDN7*. For clarity only the 5'TLs and ORFs have ben included in the alignments. The AUG start codons are highlighted (red line and blue arrow). Gaps in the alignment within the principle ORF arise due to alternative splicing. **E**. Alignment of the two TLs for the gene 53BP1. The uORF in V3 is indicated. In the lower image the two TLs have been folded. The AUG start codon is circled, the position of the uORF within the RNA structure and the minimum free energies are indicated



	MCF10ΔKpn	MCF7ΔKpn		MCF10 ΔKpn+50	MCF7 ΔKpn+50
%RE	61	56		81	85

	EIF4E (Transcriptome)	EIF2A (Translatome)
FPKM MCF10	19.2946	9.98973
FPKM MCF7	38.9097	4.81322
Log2 Fold Change	1.01193	-1.05344
p_value	5.00E-05	5.00E-05

Figure S5: A. The upper image is a schematic representation of a Fluc-EMCV-Rluc bicistronic vector carrying the TL region of the *ELK1* gene. This carries a short uORF (2 codons) 14 nts upstream of the AUG^{Fluc}. In a second vector the UGA stop codon in the uORF was changed to UGC. As a consequence the uORF now overlaps that of Fluc. These constructs were transfected into both MCF7 and MCF10A cells. Comparison of the normalised Fluc/Rluc values for the UGA and UGC constructs gives a measure of reinitiation events (RE) occurring at the AUG^{Fluc} (lower image). The frequency of RE events was significantly increased by the insertion of a 50nts spacer between the uORF and the AUG^{Fluc} (for further details see Rahim et al[23]). **B**. Immunoblots showing steady-state levels of eIF4E, eIF2α and phospho-eIF2α in MCF7 and MCF10A cells. Two quantities of cell extract have been loaded onto the SDS-PAGE gel. In the lower panel, the FPKM values for both genes, extracted from the transcriptome and translatome analysis, are indicated.

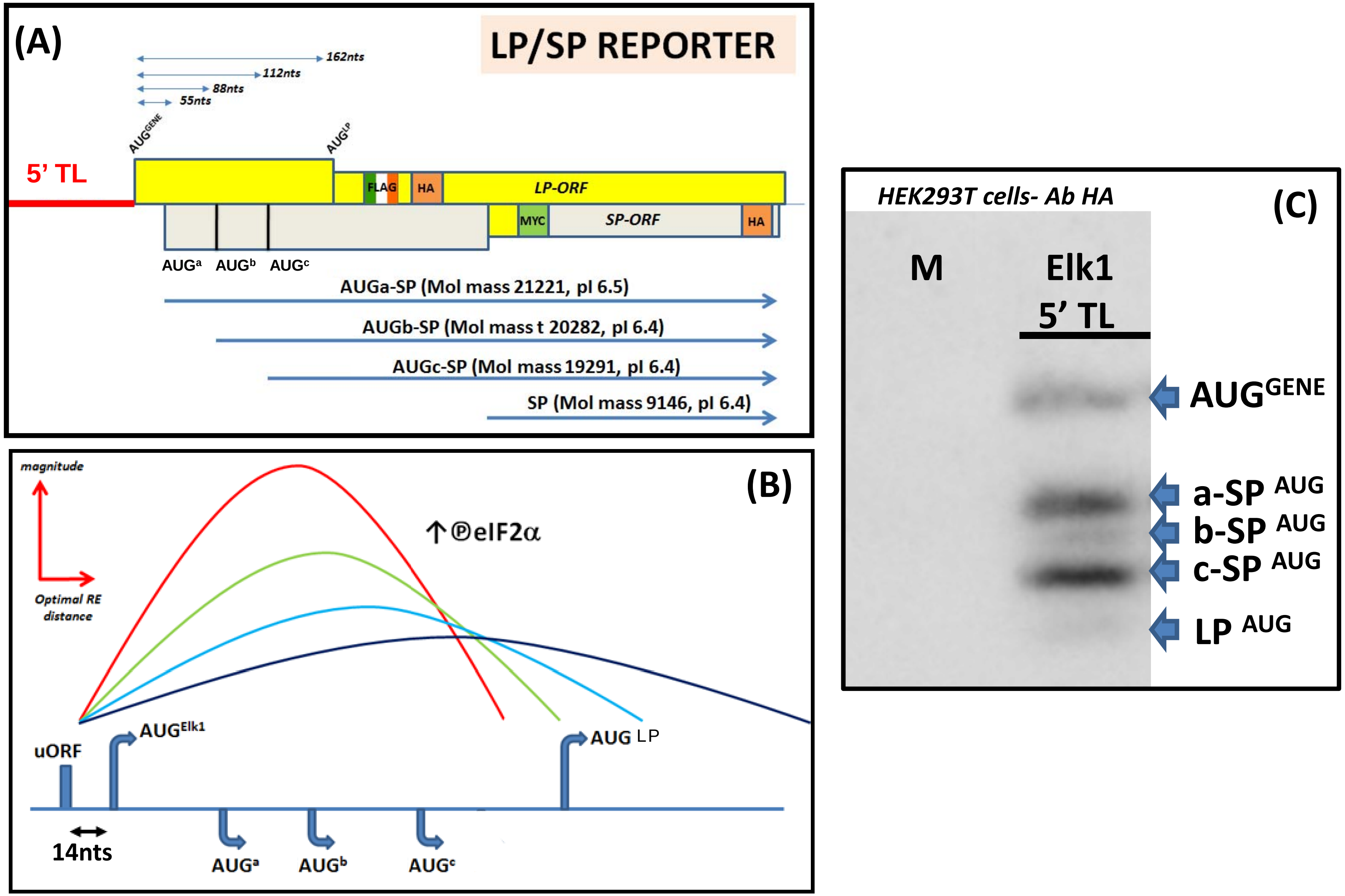


Figure S6: A. Detailed schematic representation of the LP/SP reporter system. The fused TL is indicated in red. The positions of the various AUG codons, the molecular mass of the protein products and the positioning of the epitope tags are all indicated. **B**. A simple model describing what occurs as phospho-eIF2α levels rise, and consequently TC levels fall, with regards to the readout from the LP/SP reporter. Reduced TC levels globally down-regulate translation but in parallel they displace reinitiation events mediated by the small uORF to the 3'. **C**. An immunoblot developed using the anti-HA Ab. The gel was loaded with a HEK293T cell extract in which an LP/SP reporter carrying the TL of the gene ELK1 was expressed. The protein products are indicated. M indicates a mock transfected control (see Rahim et al[23])

GROWTH CONTROL, ONCOGENES, TUMOUR SUPPRESSORS, APOPTOSIS	
UP REGULATED	
TGFBR3L	Transforming Growth Factor-Beta Receptor Type III-Like Protein . Binds to various members of the TGF-beta superfamily of ligands via its core protein, and bFGF via its heparan sulfate chains.
MAP3K14	It participates in an NF-kappaB-inducing signalling cascade
FGFRL1	Fibroblast Growth Factor Receptor-Like 1. A marked difference between this gene product and the other family members is its lack of a cytoplasmic tyrosine kinase domain.
SHB	Src Homology 2 Domain Containing Adaptor Protein B: May play a role in apoptosis.
IKBKE	Inducible I Kappa-B Kinase. IKBKE has also been identified as a breast cancer (MIM 114480) oncogene and is amplified and overexpressed in over 30% of breast carcinomas and breast cancer cell lines.
ARAF	v-raf murine sarcoma oncogene homolog: May also regulate the TOR signaling cascade.
PDLIM2	PDZ And LIM Domain 2: The encoded protein is also a putative tumor suppressor protein.
DOWN REGULATED	
FAM60A	Family With Sequence Similarity 60, Member A: Subunit of the Sin3 deacetylase complex (Sin3/HDAC), this subunit is important for the repression of genes encoding components of the TGF-beta signaling pathway
CD109	CD109 Molecule: Modulates negatively TGFB1 signaling in keratinocytes (see Chen et al., 2001)
LIN9	Lin-9 Homolog: This gene encodes a tumor suppressor protein that inhibits DNA synthesis and oncogenic transformation through association with the retinoblastoma 1 protein.
CDC73	Cell Division Cycle 73: Tumor suppressor probably involved in transcriptional and post-transcriptional control pathways.
DNA REPAIR, CELL CYCLE	
DOWN REGULATED	
XRCC5	DNA repair protein- implicated in breast cancer
XRCC4	DNA repair protein
CKS2	CDC28 Protein Kinase Regulatory Subunit 2: binds to the catalytic subunit of the cyclin dependent kinases and is essential for their biological function.
RAD21	RAD21 Homolog: may play a role in spindle pole assembly during mitosis. Also plays a role in apoptosis, via its cleavage by caspase-3/CASP3 or caspase-7/CASP7.
CCNC	Cyclin C: Component of the Mediator complex (see MED23). Binds to and activates CDK8 that phosphorylates the CTD (C-terminal domain) of the large subunit of RNA Pol II
NPEPPS	Aminopeptidase Puromycin Sensitive: involved in proteolytic events regulating the cell cycle.
POLK	DNA Polymerase Kappa: DNA polymerase specifically involved in DNA repair.
CENPK	Centromere Protein K: involved in assembly of kinetochore proteins, mitotic progression and chromosome segregation.
CENPQ	Centromere Protein Q: involved in assembly of kinetochore proteins, mitotic progression and chromosome segregation.
NBN	Nijmegen Breakage Syndrome 1 (Nibrin): Component of the MRE11-RAD50-NBN (MRN complex) which plays a critical role in the cellular response to DNA damage and the maintenance of chromosome integrity.
CDK1	Cyclin-Dependent Kinase 1: Plays a key role in the control of the eukaryotic cell cycle.
MNAT1	CDK-Activating Kinase Assembly Factor MAT1: Involved in cell cycle control and in RNA transcription by RNA polymerase II.
NSMCE2	Non-SMC Element 2, MMS21 Homolog: Required for sister chromatid cohesion during prometaphase and mitotic progression.
SMC3	Structural Maintenance Of Chromosomes 3: Central component of cohesin, a complex required for chromosome cohesion during the cell cycle. Cohesion is coupled to DNA replication and is involved in DNA repair.
TTK	TTK Protein Kinase: found to be a critical mitotic checkpoint protein for accurate segregation of chromosomes during mitosis.
SUPT16H	Suppressor Of Ty 16 Homolog: involved in multiple processes that require DNA as a template such as mRNA elongation, DNA replication and DNA repair.
HAT1	Histone Acetyltransferase 1: May play a role in DNA repair in response to free radical damage.
PRKDC	Protein Kinase, DNA-Activated, Catalytic Polypeptide: functions with the Ku70/Ku80 heterodimer protein in DNA double strand break repair and recombination.
RAD51AP1	RAD51-Associated Protein 1: May participate in a common DNA damage response pathway associated with the activation of homologous recombination and double-strand break repair.
POLE2	DNA Polymerase II Subunit 2: Participates in DNA repair and in chromosomal DNA replication
ASCC3	Activating Signal Cointegrator 1 Complex Subunit 3: 3'-5' DNA helicase involved in repair of alkylated DNA.
GTF3C3	General transcription factor
RAD51AP1	RAD51-Associated Protein 1. May participate in a common DNA damage respomse associtaed with double strand break repair
MSH2	MutS Homolog 2: Component of the post-replicative DNA mismatch repair system (MMR).
LIG4	Ligase IV, DNA, ATP-Dependent: The LIG4-XRCC4 complex is responsible for the NHEJ ligation step
PRIM1	DNA Primase 49 KDa Subunit: DNA primase is the polymerase that synthesizes small RNA primers for the Okazaki fragments during DNA replication.
	UP REGULATED
XRCC3	member of the RecA/Rad51-related protein family that participates in homologous recombination to maintain chromosome stability and repair DNA damage. Implicated in breast cancer.

Table S1: List of genes showing differential translational regulation (scored in both the RefSeq and Ensembl annotations) that are associated with growth control/oncogenesis/apoptosis (top panel) and DNA repair/cell cycle (lower panel).