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MDPI

*Supplementary data*

# Star-PAP RNA Binding Landscape Reveals Novel Role of Star-PAP in mRNA Metabolism That Requires RBM10-RNA Association

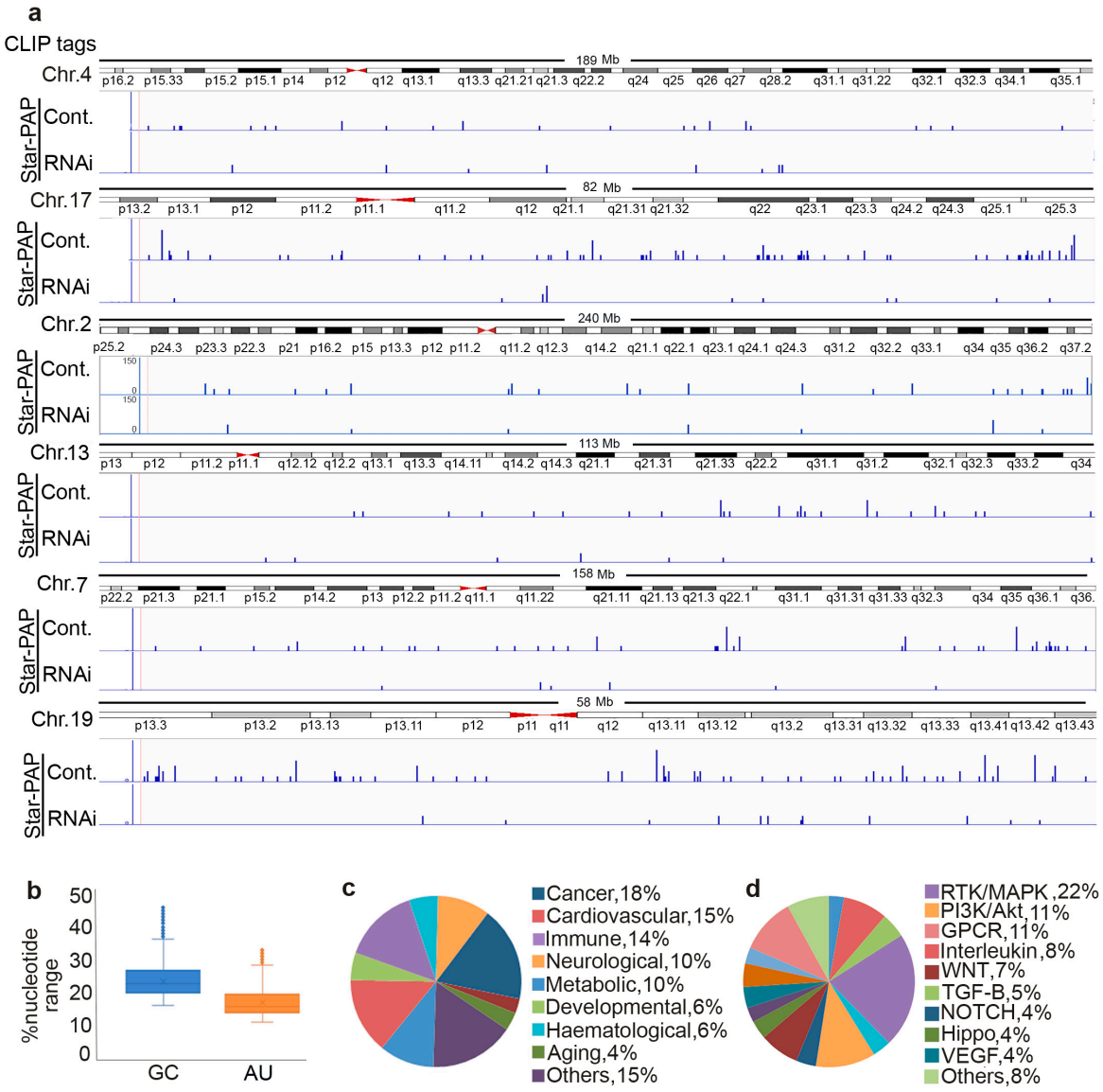


**Figure S1.** (**a**) Star-PAP HITS-CLIP reads cluster distribution and the loss of the mapped tags on siStar-PAP on different chromosomes as indicated. (**b**) Box plot showing %GC and AU nucleotide composition across Star-PAP mapped regions in the human genome. (**c**) A schematic pie chart showing functional pathway analysis of Star-PAP target mRNAs obtained from HITS-CLIP experiment. (A list of Star-PAP associated mRNAs is provided in Supplementary Table S1). (**d**) Signalling pathway analysis of Star-PAP target mRNAs that are detected in HITS-CLIP experiment. Percentage of genes in different signaling pathways is indicated.

**a**

| Chr. No. | %reads from Star-PAP | %loss on siStar-PAP | Chr. No. | %reads from Star-PAP | %loss on siStar-PAP |
|---|---|---|---|---|---|
| Chr1 | 6.5 | 80.9 | Chr12 | 2.9 | 73.8 |
| Chr2 | 4.2 | 60.8 | Chr13 | 2.2 | 60.83 |
| Chr3 | 5.5 | 90.6 | Chr14 | 2.5 | 59.2 |
| Chr4 | 2.8 | 53.8 | Chr15 | 3.8 | 57.3 |
| Chr5 | 2.9 | 52.3 | Chr16 | 4.3 | 75.6 |
| Chr6 | 2.5 | 40.4 | Chr17 | 6.4 | 78.8 |
| Chr7 | 5.5 | 80.2 | Chr18 | 5.8 | 72.4 |
| Chr8 | 6.2 | 5.20 | Chr19 | 6.1 | 68.5 |
| Chr9 | 3.8 | 67.7 | Chr20 | 5.4 | 76.3 |
| Chr10 | 5.0 | 70.4 | Chr21 | 4.2 | 38.7 |
| Chr11 | 4.4 | 69.3 | Chr22 | 2.4 | 65.3 |
| ChrX | 4.6 | 42.5 | ChrY | 0.8 | 2.02 |

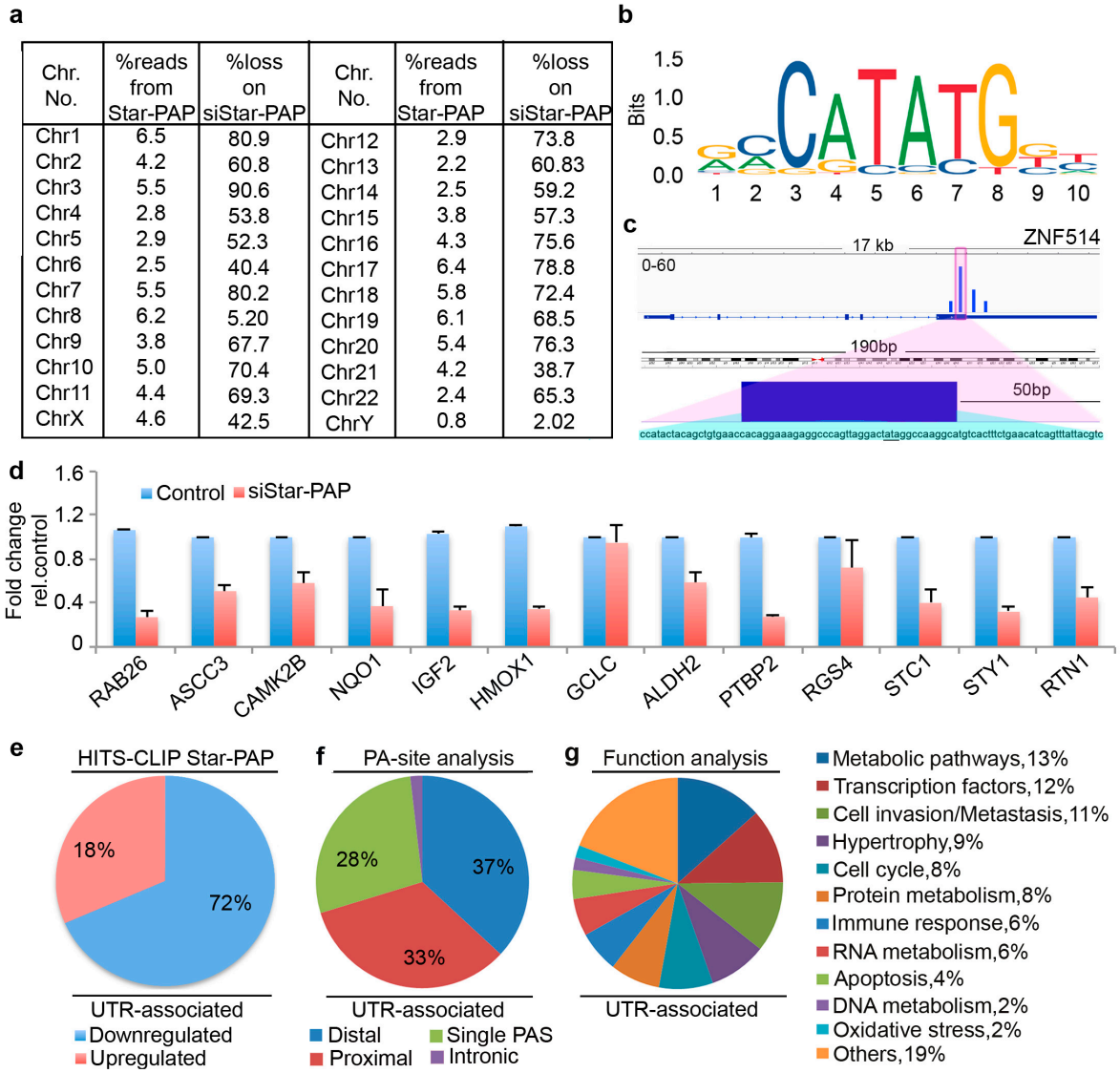


**Figure S2.** (**a**) % Star-PAP HITS-CLIP tags mapped in different chromosomes and % loss of the associated reads on Star-PAP knockdown on each chromosome. (**b**) In vivo Star-PAP binding motif obtained with MEME-Chip software using 20 nucleotide sequences surrounding the top 1000 Star-PAP associated cross-linked positions in the 3′-UTR and CDS regions. (**c**) Star-PAP HITS-CLIP association on select mRNA with distinct associations in 3′-UTR. An enlarged region at the peak area showing the associated nucleotide composition is shown below. (**d**) qRT-PCR analysis of Star-PAP target mRNAs from HEK 293 cells with control or Star-PAP specific siRNAs as indicated. (**e**) Comparisons of UTR associated Star-PAP target mRNAs with that of siRNA Star-PAP microarray analysis showing percentage of mRNAs down regulated or up regulated on siStar-PAP. (**f**) Analysis of PA-site choice on Star-PAP UTR associated mRNAs from Star-PAP HITS-CLIP. (**g**) Functional pathway analysis of same Star-PAP target mRNAs in f.

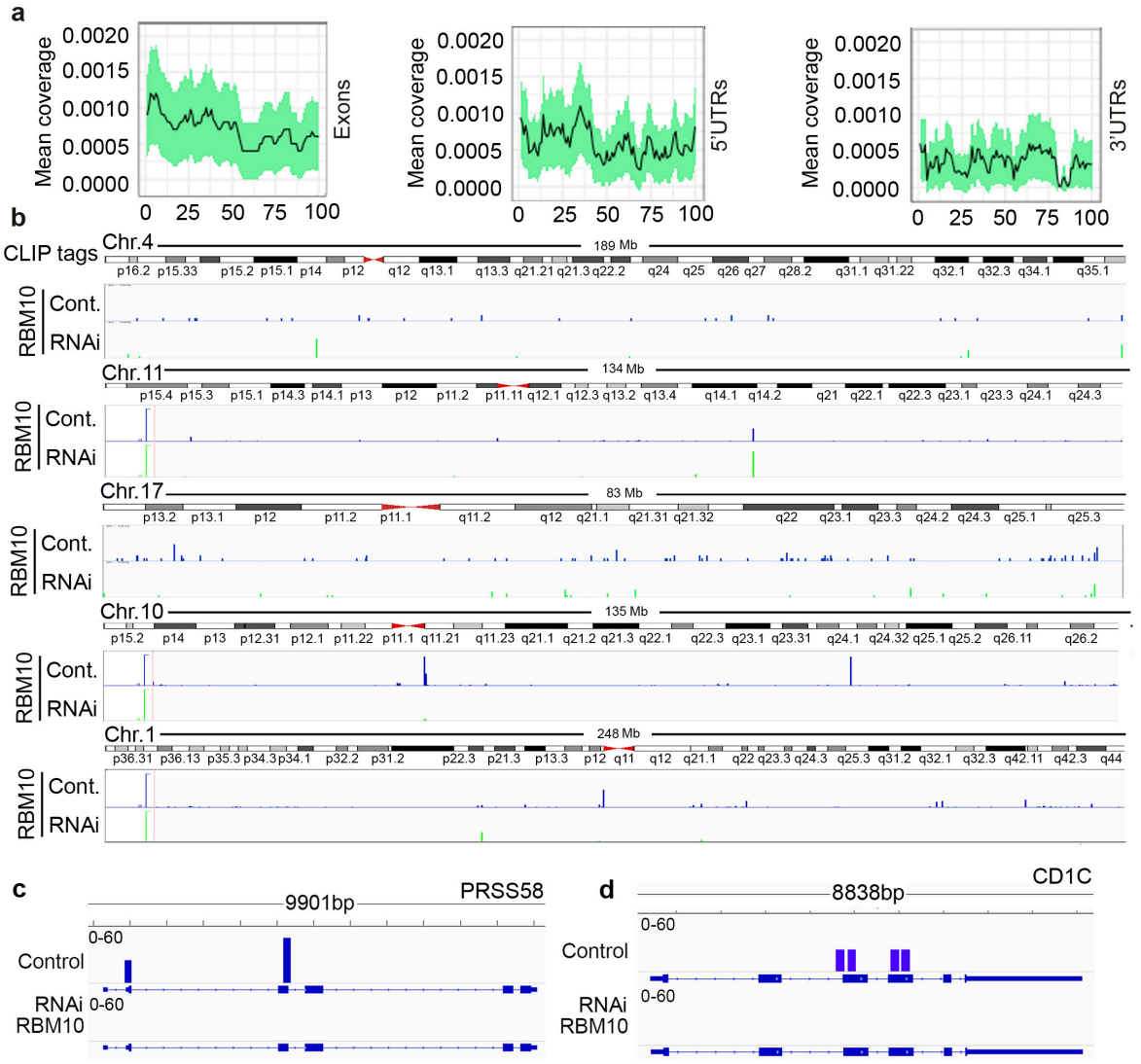


**Figure S3. (a)** Ribbon area plot showing mean coverage association of Star-PAP in 3′-UTR, 5′-UTR and exonic regions among the Star-PAP targets that are upregulated on siStar-PAP in microarray analysis. (**b**) Star-PAP HITS-CLIP tag distribution and the loss of the mapped read cluster on siRBM10 on different chromosomes as indicated. (**c-d**) Star-PAP HITS-CLIP association on select mRNAs in the presence and absence of RBM10 knockdown as indicated (distinct associations in the CDS or 5′-UTR regions are represented).

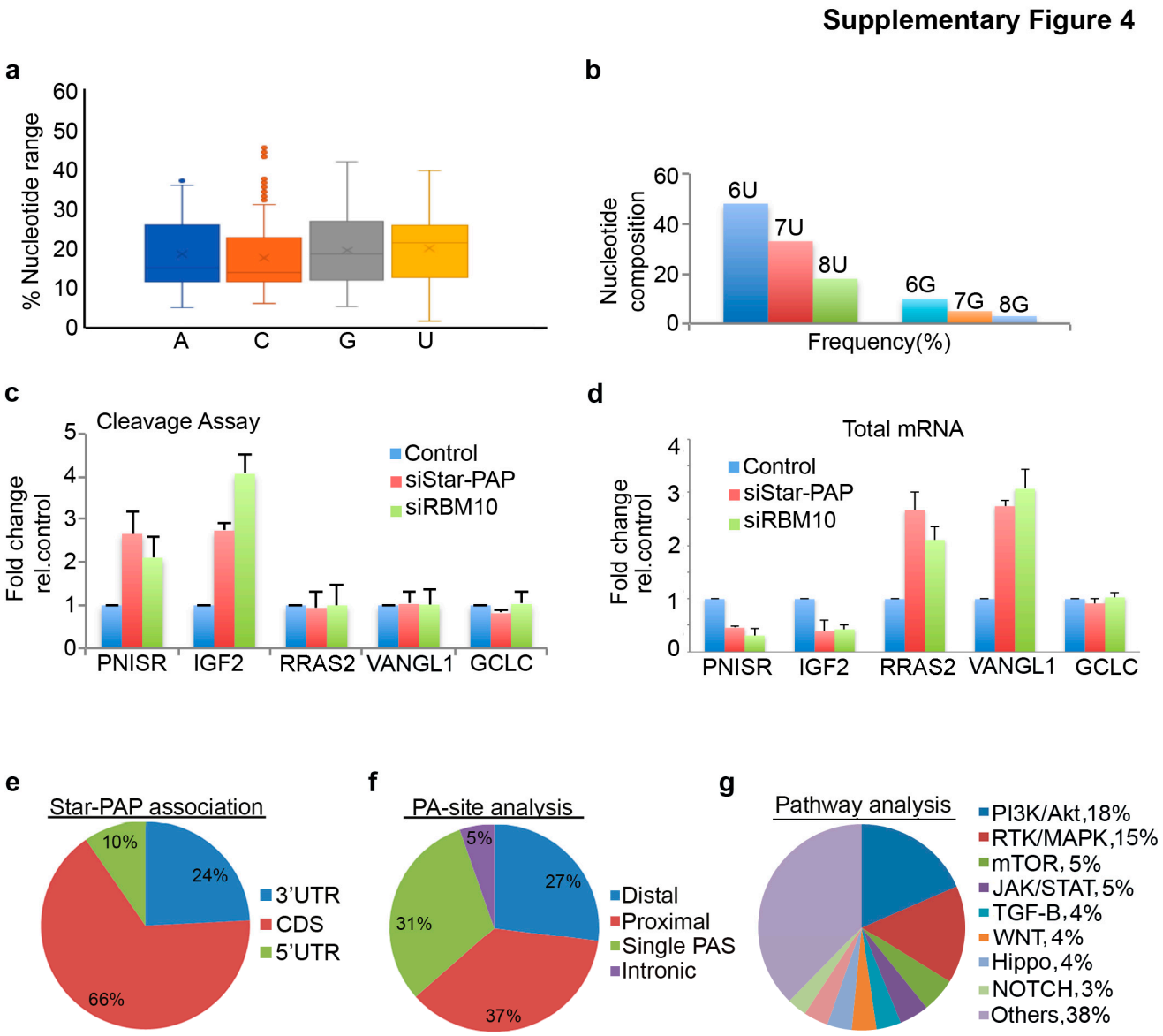


**Figure S4.** (**a**) Box plot showing percentage nucleotide composition (G, C, A and U) across Star-PAP mapped regions at various genes that are lost on RBM10 knockdown. (**b**) Motif analysis using Centrimo (MEME) software on the nucleotide sequence at the Star-PAP mapped regions on mRNAs that were lost on RBM10 knockdown. Frequency of occurrence of 6U, 7U, 8U and 6G, 7G and 8G indicated. (**c**,**d**) Measurement of uncleaved pre-mRNA levels (**c**) expressed relative to total mRNA (**d**) after Star-PAP knockdown or RBM10 knockdown as indicated. (**e**) Distribution of Star-PAP associated nucleotide positions in coding region and 3′-UTR region of RBM10-independent Star-PAP associated mRNAs. (**f**) Analysis of PA-site choice on Star-PAP associated mRNAs that are independent of RBM10 depletion. (**g**) Signalling pathway analysis of same Star-PAP target mRNAs in (**f**).

## List of antibodies

Mouse monoclonal anti-HMOX1 (A-3) antibody (Santacruz, TX, USA SC-136960), Mouse monoclonal β-actin (Santacruz SC-47778), Rabbit anti-RBM10 (Bethyl A301-006A), Rabbit anti-RNA polymerase II antibody (Bethyl, TX, USA A300-653A), Mouse monoclonal E-Cadherin (CDH1) antibody (Cell Signalling Technology, MA, USA 24E10), Mouse monoclonal CDK6 antibody (Cell Signalling Technology, MA, USA DCS83), Rabbit polyclonal anti-Star-PAP antibody (Mohan et. al. 2015).

**List of primers employed in the study**

*qRT-PCR analysis primers*

| Gene | Forward (5′ to 3′ Direction) | Reverse (5′ to 3′ Direction) |
|---|---|---|
| *KCNMA1* | GAAGGTGATGACGCAATCTG | CTGCCATGTGTCTTCCTCAA |
| *COL5A1* | ATTCAAGCGTGGGAAACTG | TAGCAGTGGTAGGTGACGTTCT |
| *NQO-1* | GAACTTCAATCCCATCATTTCCAG | CAGCTTCTTTTGTTCAGCCACAAT |
| *FEZ1* | AACTTCCTCCCAGGCAGACT | CTGCTGCACCAGCTCCTC |
| *RAB26* | ATTCAAGGATGGTGCTTTCC | CACATCTGCAGCTTCACCTT |
| *BPNT1* | TGGGAGGCAAGTTAACCGAT | GCCCGGCCAAATGAAACTTT |
| *CEP57* | GGCATTAGTGGGAAGGATGG | CTGCTGCTGTTTCTTTCTTCA |
| *COQ2* | TGGTCTTAAGTCAACGGCTCT | GAGTCAGATGGGCTCCTACA |
| *CTSO* | GATTGTGCGGAATTCCTGGG | CTGCCCAACATGTCACACAA |
| *DNAJC* | AGCCAATACGAAGGGGAAGA | GGTGGAAGTGGGAGGAAAGA |
| *DPH5* | GCAGTTACCGAGGAGACACT | TTGGATGTATGCTGCCTCCT |
| *GAD1* | CTGAGCACACAAACGTCTGT | AATGTCAGACTGGGTAGCGG |
| *POLR3F* | GCAAAAGAAGGCACAGTTGG | TTCGAGCCACTCTGTCATGT |
| *SSX21P* | GTACTTCACCATCAACGGCG | GCCTTCTTGCCACCTTTCAG |
| *RGS4* | AAGTACGCTCAAAGCCGAAG | GTTGTGGGAAGAATTGTGTTCA |
| *AGTR2* | TGTGAACATCTCTGGCAACA | CCACAGCGAGGTTGAAGA |
| *ASCC3* | AGCCTTTGCCTTCCCAATAC | TTGGTAAAGGCTGAAGATCCA |
| *CAMK2B* | TCCTGAAGCCAAAAACCTCA | ATCATGGATGCTACCGTGGA |
| *LHX9* | CATCAACCACAACCCGGATG | AGTGGGATTGGTCAGGTCTG |
| *TP73* | TGAAGATCCCCGAGCAGTAC | GGTTGGGGATGGTGATGGT |
| *RRAS2* | AAGGACAACAGTTAGCACGG | TGGCCGTTGGTAGCTAAAAC |
| *CYB5A* | GAACTTTGAGGATGTCGGGC | TAGGCGATACATCAAGGCGA |
| *PNISR* | AGCAGCAAACAGAAAGGGTT | CTTCCCGACCTACTCCTTCC |
| *WIF1* | CAAGTTGGTTTCCCATGTCT | TTAAAGAAGATAGCATTTTGAGGTG |
| *RTN1* | CATCGTGTTTGGGAGTTTCC | AAGGGTGATCTCAAGCTCCA |
| *SYT* | TGACCAGGGACATCATCAAA | ACCGGTGATATCCATCTTCG |
| *STC1* | TTGCATGCCTGGAAAACTC | TCAGCAATCATCCTTTGGAA |
| *PTBP2* | GGCACCACAGTTAGCGAGAGTGCA | CTGGAGCAAAGCTTGAAACTGGTT |
| *ALDH2* | ACCTTCGTGCAGGAGGACAT | CGTGTTGATGTAGCCGAGGA |
| *GCLC* | AAGTTCTTGAAACTCTGCAAGAGAAGG | GCCTCAACTGTATTGAACTCGGAC |
| *HO-1* | CATGCAGCGCTATGTGAA | ATGTTGGGGAAGGTGAAGA |
| *IGF2* | TGGTGCTTCTCACCTTCTTG | GAAACAGCACTCCTCAACGA |
| *BRCA1* | TGTGCTTTTCAGCTTGACACAGG | CGTCTTTTGAGGTTGTATCCGCTG |
| *NOS2* | CTCAGCTCATCCGCTATGCT | TCAGGTGGGATTTCGAAGAG |
| *AGTR1* | TTGCCAGCTATAATCCATCG | CCTTCTTTAGGGCCTTCCAA |
| *PAK1* | AGTGTGGGCGATCCTAAGAA | TCCCTCATGACCAGGATCTC |
| *LMNB1* | GCTCTTGCTACTGCACTTGGT | GTAAGGCTCTGACAACGATTCTC |
| *NGEF* | TCACCTACGTCAGCAATCAG | TCTGGAAAGGCAGGATGA |
| *ANKRD1* | TGGATGTGCCTACGTTTCTG | GGCTCCAGCTTCCATTAACTT |
| *ZEB1* | GGCAGAGAATGAGGGAGAAG | CTTCAGACACTTGCTCACTACTC |
| *GAPDH* | GAAGGTCGGAGTCAACGGATTT | GAATTTGCCATGGGTGGAAT |

*RNA Immunoprecipitation andCDS/UTR binding analysis*

| Gene | Forward (5′ to 3′ Direction) | Reverse (5′ to 3′ Direction) |
|---|---|---|
| *KCNMA1* | GAAGGTGATGACGCAATCTG | CTGCCATGTGTCTTCCTCAA |
| *COL5A1* | ATTCAAGCGTGGGAAACTG | TAGCAGTGGTAGGTGACGTTCT |
| *RRAS* | GGTGTTCGCCATTAACGA | TGACTCCAGATCTGCCTTGT |
| *VEGF* | CTACCTCCACCATGCCAAGT | CACAGGATGGCTTGAAGATG |
| *BIK* | TCTTGATGGAGACCCTCCTG | GTCCTCCATAGGGTCCAGGT |
| *AGTR1* | TTGCCAGCTATAATCCATCG | CCTTCTTTAGGGCCTTCCAA |
| *PTEN* | AAGCTGGAAAGGGACGAACT | ACACATAGCGCCTCTGACTG |
| *ZEB1* | GGCAGAGAATGAGGGAGAAG | CTTCAGACACTTGCTCACTACTC |
| *NQO-1* | GAACTTCAATCCCATCATTTCCAG | CAGCTTCTTTTGTTCAGCCACAA T |
| *FEZ1* | AACTTCCTCCCAGGCAGACT | CTGCTGCACCAGCTCCTC |
| *RAB26* | ATTCAAGGATGGTGCTTTCC | CACATCTGCAGCTTCACCTT |
| *BPNT1* | TGGGAGGCAAGTTAACCGAT | GCCCGGCCAAATGAAACTTT |
| *PNISR* | AGCAGCAAACAGAAAGGGTT | CTTCCCGACCTACTCCTTCC |
| *IFRD1* | | |
| *GCLC* | AAGTTCTTGAAACTCTGCAAGAGAA GG | GCCTCAACTGTATTGAACTCGGA C |
| *PAK* | AGTGTGGGCGATCCTAAGAA | TCCCTCATGACCAGGATCTC |
| *NOS2* | CTCAGCTCATCCGCTATGCT | TCAGGTGGGATTTCGAAGAG |
| *GAPDH* | GAAGGTCGGAGTCAACGGATTT | GAATTTGCCATGGGTGGAAT |
| *KCNMA1* UTR | AAGTTACGCCCTCAGAACATTT | TTGGCCCATTCTATTCATCC |
| *KCNMA1* CDS | TAGCAATATCCACGCGAACC | TGATGAGCGCATCCATCTT |
| *NQO-1* UTR | TGCCTTCATCTTCACTGCAA | TTGTCAAGCCAGTCACCAAG |
| *NQO-1* CDS | AGCCCAGATATTGTGGCTGA | TTGTCATACATGGCAGCGTAA |
| *COL5A1* UTR | CTGTAATGCCCAGGAAAGGA | TTACGGTGGAAACACCCAAC |
| *COL5A1* CDS | ATTCAAGCGTGGGAAACTG | TAGCAGTGGTAGGTGACGTTCT |
| *WIF1* UTR | GAACTTTTTGCATTGGCTTGA | GTGCAAAGATCACCTCCACA |
| *WIF1* CDS | CAAGTTGGTTTCCCATGTCT | TTAAAGAAGATAGCATTTTGAGG TG |
| *GCLC* UTR | ATGCCTGGTTTTCGTTTGCA | AGCTGTGGAACTCACACACACTC A |
| *GCLC* CDS | CATGATTGAAGGGACACCAG | TTGTGCAAAGAGCCTGATTT |
| *FEZ1* UTR | CTGGCTACAGCCCTCAAAAA | AGAATGCTATACAGCCCGTCTC |
| *FEZ1* CDS | AGCCCCAGTGTTTCTATGGTT | TTCCGAAAGCAGACATTGAG |
| *RRAS2* UTR | ACAAAAGTGAACCATCTCAGACC | GAGTAGGAGTAGTAACACAGGG T |
| *RRAS2* CDS | AAGGACAACAGTTAGCACGG | TGGCCGTTGGTAGCTAAAAC |
| *VANGL1* UTR | CCCTTCCTCTTTCTCCCAGT | ACATTGTTCCCACTGCAAAAG |
| *VANGL1* CDS | CAGAAGTACCTGCGCATCAC | TCATCACTGACAAGCCTCCA |
| *LHX9* UTR | AGTGGTCTAGCAAAATTGTCCA | GCTGGAACACACTAGTACGG |
| *LHX9* CDS | CATCAACCACAACCCGGATG | AGTGGGATTGGTCAGGTCTG |
| *TP73* UTR | AGTTCAACCTAGCGCCCAT | AAGGAAAGCAATGCCCTGTG |
| *TP73* CDS | TGAAGATCCCCGAGCAGTAC | GGTTGGGGATGGTGATGGT |
| *PNISR* UTR | AACATGCCATTCTCTTTCAGC | ACAAAATTCCAGCTGCCTTTT |
| *PNISR* CDS | AGCAGCAAACAGAAAGGGTT | CTTCCCGACCTACTCCTTCC |
| *IFRD1* UTR | AGTGGTCTAGCAAAATTGTCCA | GCTGGAACACACTAGTACGG |
| *IFRD1* CDS | GGAGGTTCTTGGATCAGGGA | GCTTTGAAGGCTGCAGAGTT |
| *PTEN* UTR | CCACAGGGTTTTGACACTTGT | ACACATCAGTCTGTCTCCACT |
| *PTEN* CDS | AAGCTGGAAAGGGACGAACT | ACACATAGCGCCTCTGACTG |

*Cleavage assay primers*

| Gene | Forward (5′ to 3′ Direction) | Reverse (5′ to 3′ Direction) |
|---|---|---|
| *GCLC* unclv | ATGCCTGGTTTTCGTTTGCA | AGCTGTGGAACTCACACACACTCA |
| *GCLC* total | CATGATTGAAGGGACACCAG | TTGTGCAAAGAGCCTGATTT |
| *LHX9* unclv | AGTGGTCTAGCAAAATTGTCCA | GCTGGAACACACTAGTACGG |
| *LHX9* total | CATCAACCACAACCCGGATG | AGTGGGATTGGTCAGGTCTG |
| *TP73* unclv | AGTTCAACCTAGCGCCCAT | AAGGAAAGCAATGCCCTGTG |
| *TP73* total | TGAAGATCCCCGAGCAGTAC | GGTTGGGGATGGTGATGGT |
| *RRAS* unclv | ATAAATGTCACTGCCAACGTCA | CTGGTCAAAAACTCCTGGACTC |
| *RRAS* total | AAGCAACCACCACAAGCTCT | AGGAAGTAAGGGTGGGTATGTG |
| *ZEB1* unclv | GCCTGAACTGCTGTCATTCT | CCTCTCCAGAAACATTTAGCCA |
| *ZEB1* total | GGCAGAGAATGAGGGAGAAG | CTTCAGACACTTGCTCACTACTC |
| *NQO-1* UTR | TGCCTTCATCTTCACTGCAA | TTGTCAAGCCAGTCACCAAG |
| *NQO-1* CDS | AGCCCAGATATTGTGGCTGA | TTGTCATACATGGCAGCGTAA |
| *COL5A1* UTR | CTGTAATGCCCAGGAAAGGA | TTACGGTGGAAACACCCAAC |
| *COL5A1* CDS | ATTCAAGCGTGGGAAACTG | TAGCAGTGGTAGGTGACGTTCT |
| *WIF1* UTR | GAACTTTTTGCATTGGCTTGA | GTGCAAAGATCACCTCCACA |
| *WIF1* CDS | CAAGTTGGTTTCCCATGTCT | TTAAAGAAGATAGCATTTTGAGGTG |
| *CYB5A* unclv | CTGTCCTTTCTGCGCTCAAA | CCAAACAGGCAAACACGGT |
| *CYB5A* total | GAACTTTGAGGATGTCGGGC | TAGGCGATACATCAAGGCGA |
| *PNISR* unclv | AACATGCCATTCTCTTTCAGC | ACAAAATTCCAGCTGCCTTTT |
| *PNISR* total | AGCAGCAAACAGAAAGGGTT | CTTCCCGACCTACTCCTTCC |
| *IGF2* unclv | CATGCTTGGGTTTTGTCTTT | TTCCCCTTCCCTCTTCTATC |
| *IGF2* total | TGACCTCATTTCCCGATACC | GGGGGAGAATTCGTCTGATT |
| *VANGL1* cpFP | CCCTTCCTCTTTCTCCCAGT | ACATTGTTCCCACTGCAAAAG |
| *VANGL1* total | CAGAAGTACCTGCGCATCAC | TCATCACTGACAAGCCTCCA |
| *GAPDH* unclv | CACACTGAATCTCCCCTCCT | TTGACACAAGCCCAGCTTC |
| *GAPDH* total | AGAACATCATCCCTGCCTCT | GCTTCACCACCTTCTTGATG |

*Gene specific 3′-RACE assay primers*

| Gene | Forward (5′ to 3′ Direction) |
|---|---|
| *BIK1* | GCTGGAACACTGCTGAGGTT |
| *KCNMA1* | AAGTTACGCCCTCAGAACATTT |
| *NQO1* | TGCTCAGAGAAGGAAAAGAC |
| *HMOX1* | CTTACACTCAGCTTTCTGGTG |
| *BPNT1* | TGGGAGGCAAGTTAACCGAT |
| *VANGL1* | CCCTTCCTCTTTCTCCCAGT |
| *FEZ1* | CTGGCTACAGCCCTCAAAAA |
| *IGF2* | CATGCTTGGGTTTTGTCTTT |
| *AGTR1* | TTGCCAGCTATAATCCATCG |
| *RRAS* | GGTGTTCGCCATTAACGA |
| *COL5A1* | CTGTAATGCCCAGGAAAGGA |
| Adapter primer | GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTT |
| AUAP RP | GGCCACGCGTCGACTAGTAC |

*mRNA half life assay primers*

| Gene | Forward (5′ to 3′ Direction) | Reverse (5′ to 3′ Direction) |
|---|---|---|
| *BPNT1* | TGGGAGGCAAGTTAACCGAT | GCCCGGCCAAATGAAACTTT |
| *COQ2* | TGGTCTTAAGTCAACGGCTCT | GAGTCAGATGGGCTCCTACA |

| | | |
|---|---|---|
| *IGF2* | TGGTGCTTCTCACCTTCTTG | GAAACAGCACTCCTCAACGA |
| *DNAJC* | AGCCAATACGAAGGGGAAGA | GGTGGAAGTGGGAGGAAAGA |
| *GAD1* | CTGAGCACACAAACGTCTGT | AATGTCAGACTGGGTAGCGG |
| *GCLC* | AAGTTCTTGAAACTCTGCAAGAGAAGG | GCCTCAACTGTATTGAACTCGGAC |
| *GAPDH* | GAAGGTCGGAGTCAACGGATTT | GAATTTGCCATGGGTGGAAT |