**Supporting Information**

**Title:** Global profiling of cellular substrates of human Dcp2

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**Figure S1** (related to Figure 1-3). Validation of (a) *DCP2* knockout (KO), (b) *XRN1* KO *and XRN1*/*DCP2* double knockout (DKO), (c) *MSI2* KO, and (d) *XRN1*/*MSI2* DKO HEK293T cell lines by Western blot. WT, wild type HEK293T cells.

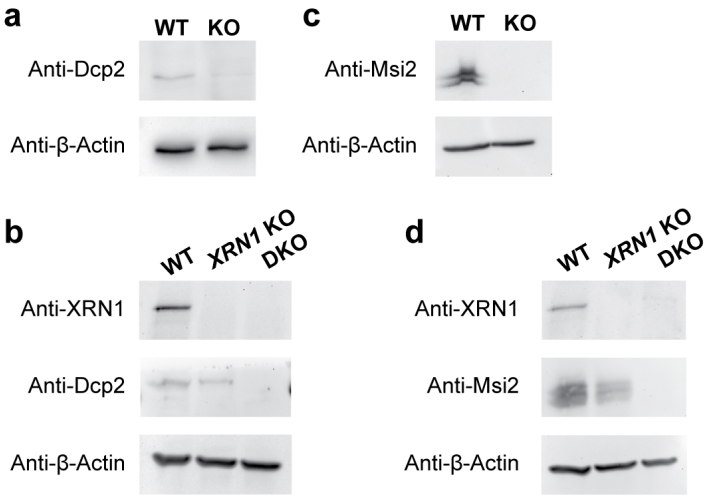
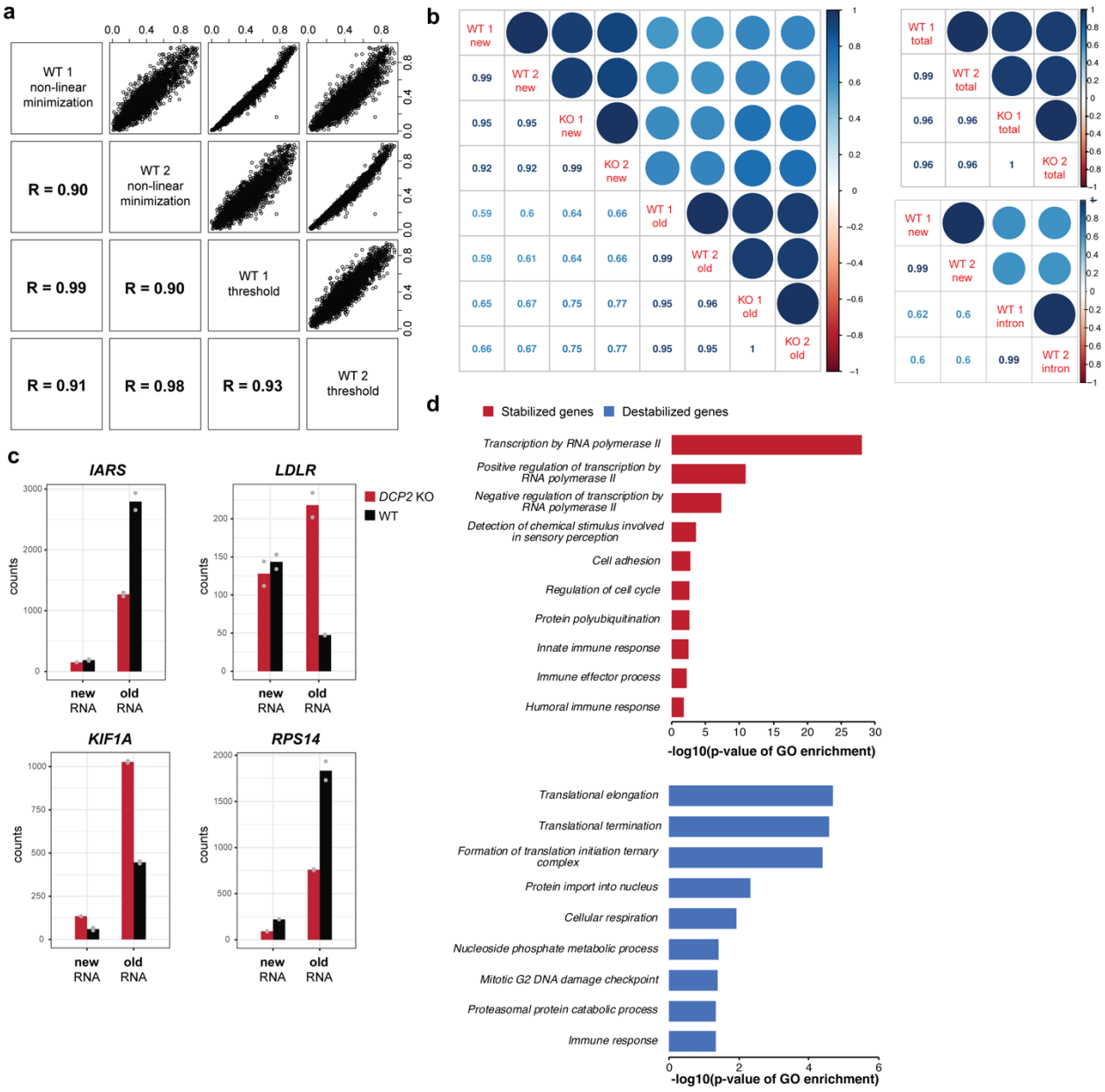


**Figure S2** (related to Figure 1). TimeLapse-seq exhibits high correlation between experimental replicates and identifies gene sets whose stability is altered in *DCP2* KO cells. (a) Correlation matrix of estimated fraction new of mature RNAs by non-linear dynamic modeling (non-linear minimization) and the simpler thresholding approach (threshold) as described in this paper with biological duplicates of wild type HEK293T (WT1, WT2). (b) Correlation matrix of inferred new and old reads (left), total (tot) RNA (top right) and inferred new versus intronic reads (bottom right) between TimeLapse-seq experiments with biological duplicates of wild type HEK293T (WT 1, WT 2) and *DCP2* knockout HEK293T (KO 1, KO 2). (c) Bar plots of new and old RNA reads from representative



genes that are destabilized (*IARS*) or stabilized (*LDLR*) and that are up-regulated (*KIF1A*) or down-regulated (*RPS14*) by changes in RNA synthesis in *DCP2* KO vs. wild type HEK293T cells. (d) Top 10 significant biological process GO-slim terms of stabilized and destabilized genes in *DCP2* KO versus WT HEK293T cells. Fisher's exact test was performed using PANTHER overrepresentation test at FDR<0.05.

**Figure S3** (related to Figure 2). RNA stability measurement in *DCP* KO vs WT HEK293T cells. (a-d) RNA stability of selected genes belonging to the following classes: stabilized (a), upregulated synthesis (b), no change in synthesis or degradation rate (c), destabilized (d), and downregulated synthesis in *DCP2* KO (e) by qRT-PCR after actinomycin D treatment for the indicated times. Number of biological replicates: *n*=3. Half-lives were calculated from a single component decay model as described in the method, and significance was analyzed by two-tailed *t*-test. Error bars represent mean ± s.d. (f) qSL-RT-PCR assay in WT, *XRN1* KO and *XRN1*/*DCP2* DKO HEK293T cell lines for additional Dcp2 targets. Error bars shown are mean ± s.d. *n*=4 biological replicates. Significance at all time points (splint ligation) or the slope of the linear regression (total RNA) was analyzed by one-way ANOVA. P-values are denoted by asterisks; Ns, not significant (p>0.05); *P < 0.05; **P < 0.01; ***P < 0.001; ****P<0.0001.

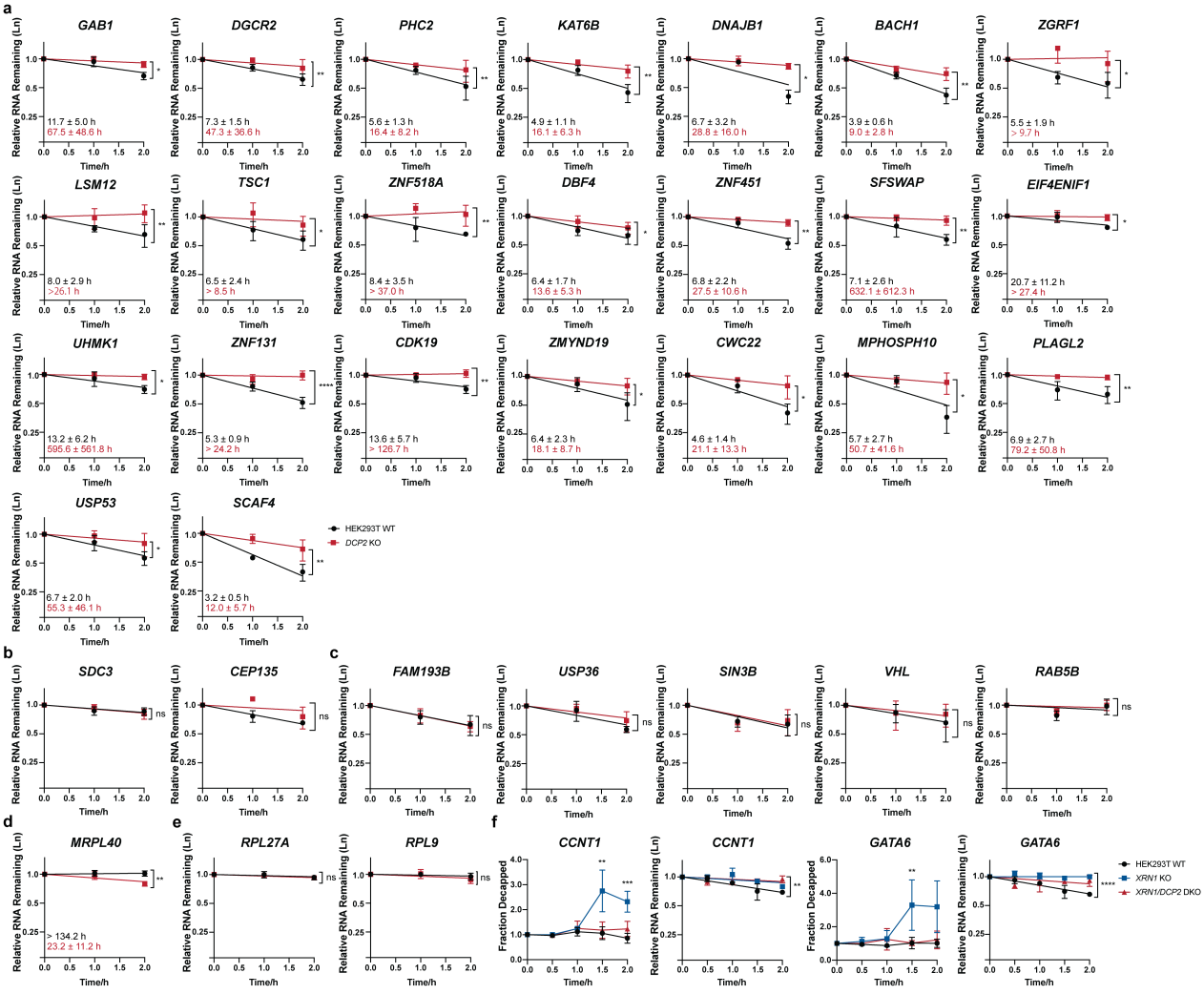


**Figure S4** (related to Figure 3). Human Msi2 and Dcp2 regulate distinct gene sets. (a) Significant biological process GO-slim terms of stabilized and destabilized genes in *MSI2* KO versus WT HEK293T cells. Fisher's exact test was performed using PANTHER overrepresentation test at FDR<0.05. (b) qSL-RT-PCR assay in WT, *XRN1* KO and *XRN1*/*MSI2* DKO HEK293T cell lines suggests that Msi2-dependent decay of a Musashi binding element-containing Dcp2 target, *HOXA13*, proceeds via RNA decapping. Error bars shown are mean ± s.d. *n*=4 biological replicates. Significance at all time points or of the slope of regression lines were analyzed by one-way ANOVA; **P < 0.01; ***P < 0.0001. (c) Msi2 co-localization with P-bodies in HEK293T cells. Dcp1a was used as the P-body marker. Scale bar, 10 μm.

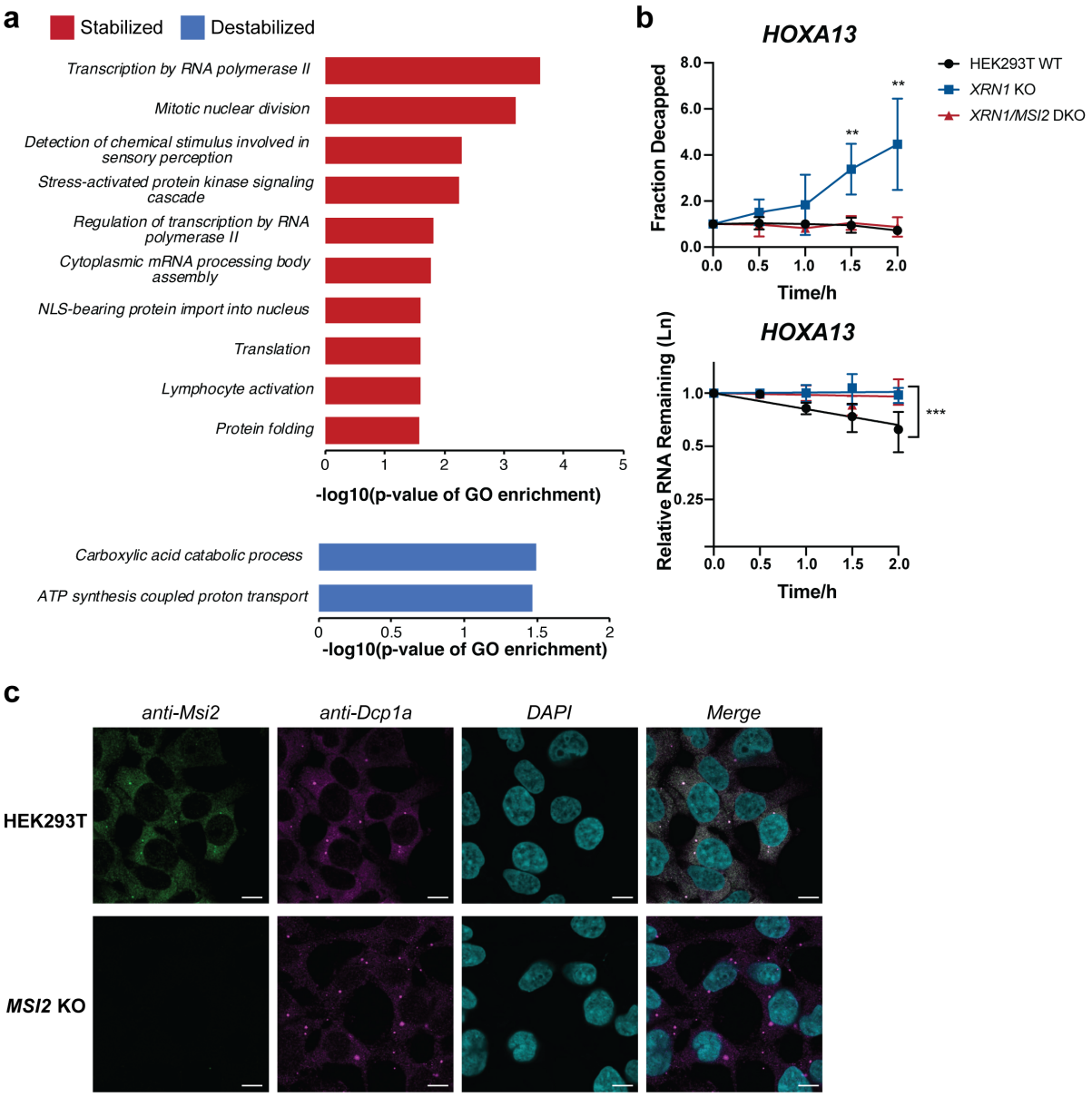


**Figure S5** (related to Figure 4-5). Properties of Dcp2 targets. (a) Venn diagrams summarizing overlap of genes that are stabilized or destabilized in *DCP2* KO cells with P-body enrichment or depletion (adjusted P-value<0.05 and $\log_2$ fold enrichment >0 or <0, respectively) and m$^6$A- or m$^6$A$_m$ modifications (as summarized in Wei et al 2018). (b-d) Boxplots representing RNA transcript length (b), the length of coding sequence (CDS) (c), and GC content (d) (all reference datasets from Khong et al. 2017) for each of the three classes of RNA stability changes in *DCP2* KO versus WT HEK293T cells. Statistical significance is derived from Mann-Whitney *U* test. Ns, not significant (p>0.05); *P < 0.05; ***P < 0.001; ****P < 0.0001.

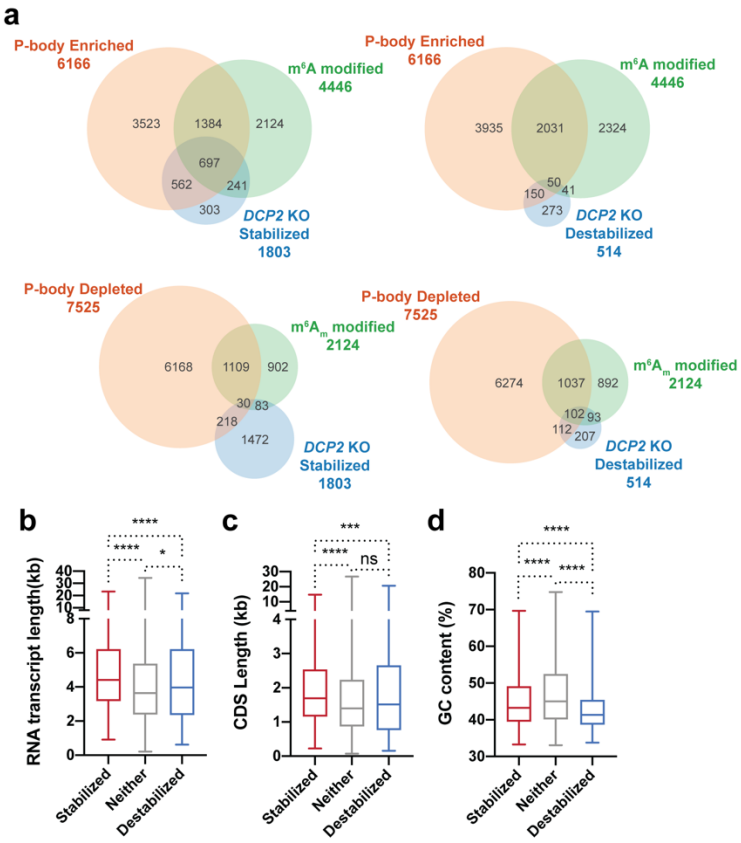


**Table S1** (related to Figure 2). qRT-PCR and qSL-RT-PCR primers used in this study

| Gene/Primer Name | Primer Sequences |
|---|---|
| UHMK1 Fwd/Rev | 5' GCATTGTGCCCGAGATGTTTT 3'<br>5' ATGTTACGTGGTTTGAGGTCC 3' |
| HOXA13 Fwd/Rev | 5' CTATGACAGCCTCCGTGCTC 3'<br>5' CCGCCGTTGTCGTAGAGAAA 3' |
| EPC2 Fwd/Rev | 5' TGACCCTTATGTTGCCTTTCG 3'<br>5' TCACCACCATAGTCTCCCAAAT 3' |
| VGLL4 Fwd/Rev | 5' AACTGCAACCTCTCGCACTG 3'<br>5' GCTCGGGCTCCTTGTAATTCT 3' |
| GFOD1 Fwd/Rev | 5' GACTGACCACATCAAGGGCAT 3'<br>5' CGGGCACGTTGAAGTTGAG 3' |
| SCAF4 Fwd/Rev | 5' CCTCACACACAGAACCAGTATC 3'<br>5' GTGGAGGTGGCACTGTTATAG 3' |
| USP53 Fwd/Rev | 5' CATAGTGCCAAGCAGAGATGC 3'<br>5' CTCCACAGCTACGACACACA 3' |
| MPHOSPH10 Fwd/Rev | 5' AAATTGGATGCCCTCTCAAACTT 3'<br>5' CTCGTTTCTTGTCTGTAGCTGT 3' |
| CWC22 Fwd/Rev | 5' GGAAAAGGTCTCGGAAATCCC 3'<br>5' CCACCAGTGCGAGTAAGAAGA 3' |
| GATA6 Fwd/Rev | 5' CACACCACAACTACCACCTTAT 3'<br>5' TCCTGGTTTGAATTCCCTCTTT 3' |
| ZMYND19 Fwd/Rev | 5' ACCGACTTCAAATTGGGTATCG 3'<br>5' CACTTCCATTCGGGCCTCAA 3' |
| CDK19 Fwd/Rev | 5' TGCCAACAGTAGCCTCATAAA 3'<br>5' GCTTGCTCCGAGGTAATTCT 3' |
| DGCR2 Fwd/Rev | 5' AGGATCCCTGGCTTTGATTAC 3'<br>5' CGATGTCCGGGTACTTGTATG 3' |
| PHC2 Fwd/Rev | 5' AGGGAACGGAAACTCTGCCT 3'<br>5' TCGATAACATGCGTCAGGATTTG 3' |
| ZNF107 Fwd/Rev | 5' TGTGGAGATTATGGCAGAGC 3'<br>5' CCTCGTGTGTGCAGAAAAAGT 3' |
| CCNT1 Fwd/Rev | 5' CGTGTCCCTCATTCGAAACT 3'<br>5' GAGCAGGGAGTGAAGCATATT 3' |
| ZNF451 Fwd/Rev | 5' GGAGGAGCAGCAGTATGTAATC 3'<br>5' GCCACACTGGGTTTCTGTAA 3' |

| | |
|---|---|
| DBF4<br>Fwd/Rev | 5' GGGCAAAAGAGTTGGTAGTGG 3'<br>5' ACTTATCGCCATCTGTTTGGATT 3' |
| ZNF131<br>Fwd/Rev | 5' CCAAGAACCATTGGTGGAGATAG 3'<br>5' TGCTTTCCATACATCATTGGCTT 3' |
| ATXN1<br>Fwd/Rev | 5' TCGTCATGCAATACGCCGAC 3'<br>5' TACGGGTGAGGAACCGACT 3' |
| USP36<br>Fwd/Rev | 5' CACCACCTCTAGCCAACTACC 3'<br>5' GGCGATCTTTTTCAGGTCTCG 3' |
| FAM193B<br>Fwd/Rev | 5' CATGCCAAAGCTCGTCAAGAA 3'<br>5' CTCCATGCTCTTTCGGCAAC 3' |
| LSM12<br>Fwd/Rev | 5' CCAGACCATTCACAAGACCATT 3'<br>5' TTGGCTTTCCACGTCTCTAAAAT 3' |
| SIN3B<br>Fwd/Rev | 5' AGAACGAGCACGACAAGACC 3'<br>5' AGTCCCGTACTTCCCCACTG 3' |
| VHL<br>Fwd/Rev | 5' GCAGGCGTCGAAGAGTACG 3'<br>5' CGGACTGCGATTGCAGAAGA 3' |
| EIF4ENIF1<br>Fwd/Rev | 5' CGTAAGATGTACGAGAGCAAAGA 3'<br>5' CTTCACTGGCCTTCTGAGTATC 3' |
| SFSWAP<br>Fwd/Rev | 5' CCACCGAGAGAAGAAGAGAAAG 3'<br>5' GATAGGCACTGGGAAGAGAATG 3' |
| SDC3<br>Fwd/Rev | 5' CCTCCTGTGTTCTCTGGATTT 3'<br>5' CAGTTTGCGGGCTTCTATTTAC 3' |
| MYO5C<br>Fwd/Rev | 5' GAAGCTGGAAGCAGAACTAGAA 3'<br>5' CTTCCACAGCATCCCTGTATC 3' |
| GAB1<br>Fwd/Rev | 5' GAGCCTTTGGCCCTCTAATA 3'<br>5' CTTACTTGACAGTGGAGGAGAAG 3' |
| GLDC<br>Fwd/Rev | 5' ATTTCTCGTTGATCCCCGTTG 3'<br>5' CACAGGGTAACTTCAGCTCAG 3' |
| RPL9<br>Fwd/Rev | 5' GGCTACCGTTCGGACTATTT 3'<br>5' GAGCATACACAGACCTCATCTT 3' |
| ACAT1<br>Fwd/Rev | 5' CGGGCTAACTGATGTCTACAAT 3'<br>5' GCATAAGCGTCCTGTTCATTTC 3' |
| MRPL40<br>Fwd/Rev | 5' AAGGCTACTCAAGAGCTAATTCC 3'<br>5' CTCTCCTCTCAGTCTCCTCAAA 3' |
| HNRNPU<br>Fwd/Rev | 5' GGCCGTGGTAGTTACTCAAA 3'<br>5' ACGATTGTTTCCTCGTCCTC 3' |
| EIF3B<br>Fwd/Rev | 5' CGGAGACTACTTGTGTGTGAAA 3'<br>5' GGTACCTGTTTCTCCCTCATTC 3' |

| | |
|---|---|
| RPL27A<br>Fwd/Rev | 5' CCTTGACAAATTGTGGACTTTGG 3'<br>5' GCCTTCACGATGACAGGCT 3' |
| MPI<br>Fwd/Rev | 5' TCCATCCAGGCACACCCTAA 3'<br>5' GTCATGGCTCATGGTCTGCTT 3' |
| RAB5B<br>Fwd/Rev | 5' GCCAGTCCTAGCATCGTTATT 3'<br>5' GCCTCTTCATACTCCACCATAC 3' |
| TSC1<br>Fwd/Rev | 5' GCAGTGGGTAGTTCTAAGGATG 3'<br>5' GACTCTGCCCTTAACGCTTAT 3' |
| ZGRF1<br>Fwd/Rev | 5' AGACCTGACTCCTACGGAAA 3'<br>5' GCACAGGTAACTCCAACTACTC 3' |
| ZNF518A<br>Fwd/Rev | 5' CGGGAAGTTAGGGCTAAAGAAA 3'<br>5' CATAAGGCTGGTGGAAGAAGAG 3' |
| PLAGL2<br>Fwd/Rev | 5' ACCATAGCTAGCCAGTCATTTC 3'<br>5' GGGTACTGAGTGCAGGATAAAG 3' |
| BACH1<br>Fwd/Rev | 5' CCGCTTCAGTCTCTACCATATC 3'<br>5' GCCACTGTATTCTGAGTCCTATT 3' |
| KAT6B<br>Fwd/Rev | 5' GAGCCTACCTGTGAGATTGAAG 3'<br>5' CCTTCCTCTTCCTCTGTTTGTC 3' |
| DNAJB1<br>Fwd/Rev | 5' AAGGCATGGACATTGATGACC 3'<br>5' GGCCAAAGTTCACGTTGGT 3' |
| ZNF518A<br>Fwd/Rev | 5' CGGGAAGTTAGGGCTAAAGAAA 3'<br>5' CATAAGGCTGGTGGAAGAAGAG 3' |
| HOXA13<br>Fwd-2/Rev-2 | 5' CTATGACAGCCTCCGTGCTC 3'<br>5' CCGCCGTTGTCGTAGAGAAA 3' |
| HOXA13<br>Splint | 5'<br>GCATGGAGAAGACCCCAGTCATCAAAGCCAGCAAACGCAGTGTTCATTC 3' |
| GATA6<br>Fwd-2/Rev-2 | 5' GAGCGCTGTTTGTTTAGGGC 3'<br>5' TACTGCTCTGCCGGAAAACT 3' |
| GATA6 Splint | 5'<br>CAGGCTGTGGGTCGGAACTCATCAAAGCCAGCAAACGCAGTGTTCATTC 3' |
| CCNT1<br>Fwd-2/Rev-2 | 5' TGCCTTCTGGTTGAAGCACT 3'<br>5' TGAGACGTTAAGACGCTGCC 3' |
| CCNT1 Splint | 5'<br>AAGGCAGCGGCGAAGGCTCATCAAAGCCAGCAAACGCAGTGTTCATTC 3' |
| Anchor Fwd | 5' GCTGATGGCGATGAATGAACACTGC 3' |

**Table S2** (related to Figure 1-2; supplied as individual csv). Dcp2-regulated genes.