1 SUPPLEMENTARY INFORMATION

2 The upstream 5' splice site remains associated to the transcription

3 machinery during intron synthesis

4

6 Hussein¹, Ofir Hameiri¹, Luna Tammer¹, Jonathan Zonszain¹, Ifat Keydar¹, Dror

Yodfat Leader^{1*}, Galit Lev Maor^{1*†}, Matan Sorek^{2*}, Ronna Shayevitch¹, Maram

- 6 Hussein', Offir Hameiri', Luna Tammer', Jonathan Zonszain', Ifat Keydar', Dro
- 7 Hollander¹, Eran Meshorer², and Gil Ast¹[†]

8

5

- ¹Department of Human Molecular Genetics and Biochemistry, Sackler Faculty of
- 10 Medicine, Tel-Aviv University, Ramat Aviv 69978, Israel.
- ²Department of Genetics, The Institute of Life Sciences, and The Edmond and Lily
- 12 Center for Brain Sciences (ELSC). The Hebrew University of Jerusalem, Edmond J.
- 13 Safra Campus, Jerusalem 91904, Israel.

14

*These authors contributed equally to this work.

16

- [†]Corresponding authors. Contact information: Gil Ast, gilast@post.tau.ac.il. Galit Lev
- 18 Maor, galitlm@tauex.tau.ac.il

19

20

21

22

23

24

25

28

29

31

32

33

34

35

36

37

38

39

40

41

42

43

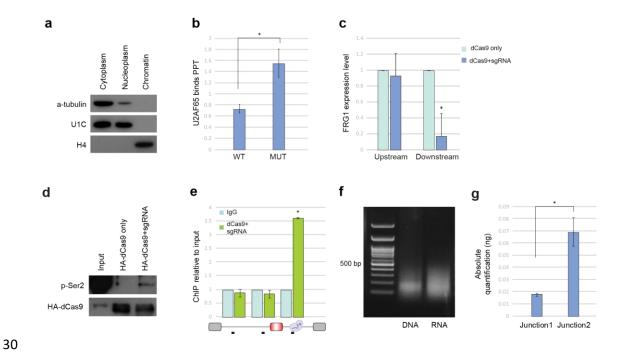
44

45

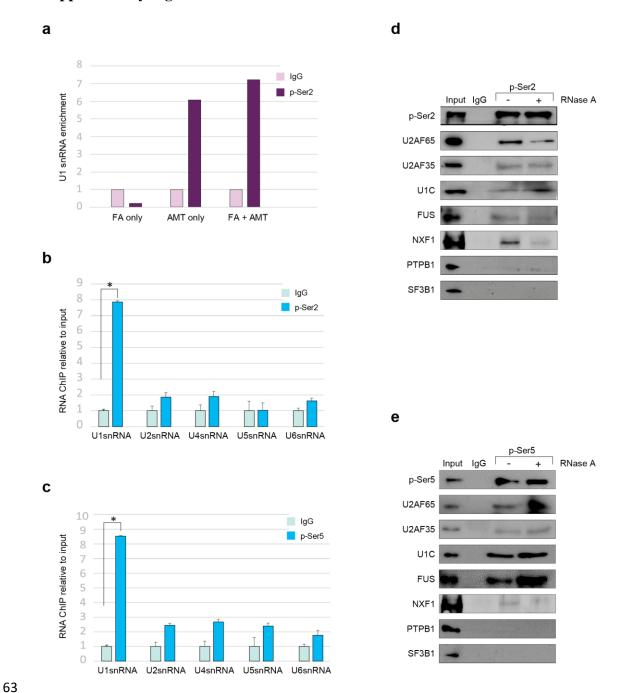
46

Supplementary Figures

Supplementary Figure 1



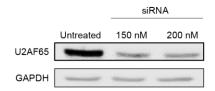
Supplementary Figure 1. CRISPR- interference analyses. a. HEK293 cells fractionation. a western blot was done with anti-α-tubulin, anti-U1C, and anti-Histone 4 antibodies detected in the cytoplasmic, nucleoplasmic, and chromatin fractions, respectively. Twenty-five micrograms of total protein from each fraction was loaded per lane. Source data are provided as a Source Data file. b. FRG1 WT and MUT cells were crosslinked with FA and nuclei were isolated. RNA was fragmented with MNase and sonication. Immunoprecipitation was performed using U2AF65 antibody, the RNA was extracted, and qRT-PCR was performed to examine the enrichment of the PPT of intron 1 FRG1 minigene. N=3 independent experiments. Error bars show mean values +/- SEM. Asterisk indicates P = 0.037 two-tailed t-test. **c.** FRG1 WT cells were transfected with a plasmid for expression of HA-dCas9 with or without the plasmid for expression of sgRNA targeted against a location in the middle of FRG1 intron 2. After 48 h RNA was extracted and RT was done using hexamers primer. Relative FRG1 RNA level were measured using qRT-PCR with primers specific for nascent FRG1 transcript. The primers were located near the start and end of the transcript. N=3 independent experiments. Error bars show mean values + SEM. Asterisk indicates P = 0.037 twotailed t-test. **d.** The CRISPR interference-based protocol was performed. After HAdCas9 IP, a western blot was done with anti-p-Ser2 pol II and anti-HA antibodies. Source data are provided as a Source Data file. **e.** CRISPR interference-based protocol targeting the middle intron of the FRGI minigene was performed, and mean DNA levels were measured by qPCR. N=3 independent experiments. Error bars show mean values +/- SEM. Asterisk indicates P=0.020 two-tailed t-test. Each bar corresponds to the amplified segment marked in the gene diagram below the graph. The sgRNA binding is indicated as a purple shape. **f.** Agarose gel of purified DNA and RNA after MNase and sonication treatment. **g.** RNA extraction from WT cells CRISPR interference-based protocol. RNA was quantified by absolute qRT-PCR. N=3 independent experiments. Error bars show mean values +/- SEM. Asterisk indicates P=0.032 two-tailed t-test. Junction-1 denotes the exon 1- intron 1 junction, and Junction-2 denotes the exon 2- intron 2 junction.



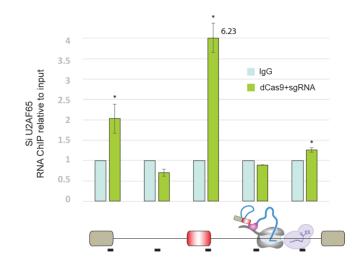
Supplementary Figure 2. U1 snRNP associates with pol II. a. HEK293 cells were either crosslinked or not with FA and AMT, followed by purification of nuclei, sonication, and RNA-ChIP using p-Ser2 pol II antibody or IgG antibody as a control. Total RNA was extracted and reverse transcribed using RT-FLEX with hexamer primers. U1 snRNA was quantified by qRT-PCR in one experiment. **b, c.** HEK293 cells were treated with FA and AMT, followed by UV irradiation, purification of nuclei, and

DNA and RNA fragmentation, and RNA-ChIP with antibodies against: p-Ser2 (b) and p-Ser5 (c) pol II CTD. snRNA levels were measured by qRT-PCR. N=4 for b and N=3 for c independent experiments. Error bars show mean values + SD. Asterisk indicates for panel b $P = 1.23 \times 10^{-6}$, for panel c $P = 4.7 \times 10^{-4}$, two-tailed t-test. **d, e.** Nuclear extracts of HEK293 cells were treated or not with RNase A and subjected to immunoprecipitation with antibodies against: p-Ser2 (d) and p-Ser5 (e) pol II CTD. Western blotting was performed with the indicated antibodies. Source data are provided as a Source Data file.

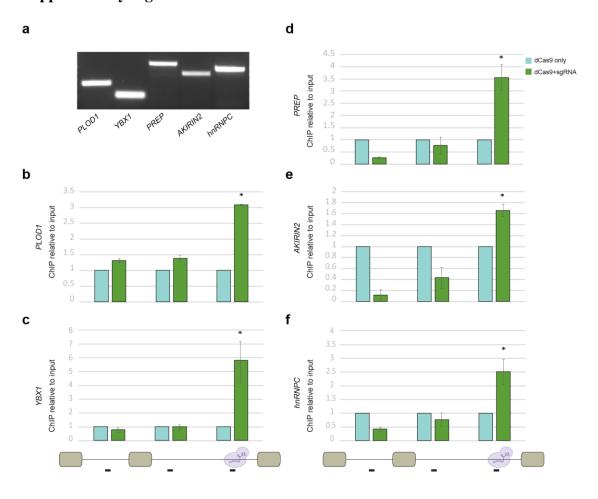
а



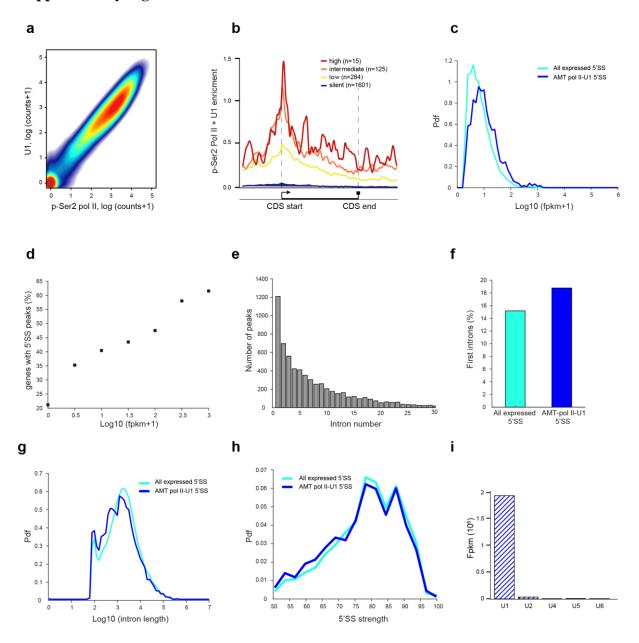
b



Supplementary Figure 3. U2AF2 and the PPT are not essential for 5'SS association with pol II. a. Western blot analysis of total protein extracted 48 h after U2AF2 KD using 150 nM or 200 nM of siRNA. Source data are provided as a Source Data file. b. CRISPR interference-based experiment was performed after U2AF65 siRNA to evaluate the association of various transcript regions with pol II located mid-intron 2 of WT *FRG1* minigene. Mean RNA levels were measured. N=3 independent experiments. Each bar corresponds to the amplified segment marked in the gene diagram below the graph. Error bars show mean values +/- SEM. From left to right asterisk indicates P=0.05, P=0.007, P=0.002, two-tailed t-test.



Supplementary Figure 4. Splicing pattern of the endogenous genes, and sgRNA binding efficient. a. Total RNA was extracted from HEK293 cell line. RT-PCR was performed to examine splicing pattern of the selected three exons. Source data are provided as a Source Data file. b-f. Binding specificity of the sgRNAs. CRISPR interference-based protocol targeting the middle intron of the indicated genes was performed, and mean DNA levels were measured by qPCR. N=3 independent experiments. Error bars show mean values +/- SEM. Asterisk indicates for panel b $P=4.4 \times 10^{-8}$, for panel c P=0.016, for panel d P=0.008, for panel e P=0.004, for panel f P=0.03, two-tailed t-test. Each bar corresponds to the amplified segment marked in the gene diagram below the graph. The sgRNA binding is indicated as a purple shape.



Supplementary Figure 5. p-Ser2 pol II and U1 travel together on expressed genes. a. p-Ser2 pol II and U1 ChIP signals are highly correlated. Pearson coefficient = 0.9887, $P = 2.2 \times 10^{-16}$, one-tailed correlation test. b. p-Ser2 pol II:U1C occupancy over intronless genes surrounded by 500 bp flanking sequences around the coding regions. Genes were divided based on expression (fpkm) into high, intermediate, low, and silent. Coding regions were scaled to 1000 bp. c. Expression distribution of pol II-U1-5'SS peaks in expressed genes compared to all 5'SS in expressed genes. d. Percentage of genes with pol II-U1-5'SS peaks among expressed genes

with different expression level thresholds. **e.** Intron number distribution of the pol II-U1-5'SS peaks in expressed genes. **f.** The fraction of first introns among pol II-U1-5'SS peaks in expressed genes compared to all 5'SS in expressed genes. One-tailed population proportion test z is -7.8407, $P < 10^{-10}$. N=126,770 expressed junctions. **g.** Intron length and **h.** 5'SS strength of pol II-U1-5'SS peaks in expressed genes compared to all 5'SS in expressed genes. **i.** Spliceosomal snRNA levels (fpkm) in double RNA-ChIP sample, Pdf – probability density function.

Supplementary Tables

RNAs. Each sgRNA oligonucleotide was annealed to its complementary oligonucleotide before cloned into pX552 sgRNA expression plasmid. All sgRNAs used in this work are listed.

| Name | Sequence (5' - 3') | Notes |
|------------------|----------------------|--|
| FRG1- intron 1-1 | ATCCCAGGAGATTCTCGGTA | Targets intron 1 of FRG1 minigene |
| FRG1- intron 1-2 | TTACCTTACCGAGAATCTCC | Targets intron 1 of FRG1 minigene |
| FRG1- intron 2-1 | GGCACAGGTTTAAATCGTCT | Targets intron 2 of FRG1 minigene |
| FRG1- intron 2-2 | AGTATCTCATCCTAACTAGT | Targets intron 2 of FRG1 minigene |
| PLOD1-1 | GTTCCCGGAAACAATTACC | Targets intron 17 of <i>PLOD1</i> gene |
| PLOD1-2 | GAATTGTGAGAATTAACCG | Targets intron 17 of <i>PLOD1</i> gene |
| <i>YBX1</i> -1 | AACCCTTAAGGGTTGCCAAC | Targets intron 3 of YBX1 gene |
| <i>YBX1-</i> 2 | CTGCTATCCCCTATGACTGC | Targets intron 3 of YBX1 gene |
| AKIRIN2-1 | ATGTTTCAACCACTAATACC | Targets intron 2 of AKIRIN2 gene |
| AKIRIN2-2 | GGGCACTAGACTGTATTAGC | Targets intron 2 of AKIRIN2 gene |
| hnRNPC-1 | GCCTTAGACAGTTCCTAATT | Targets intron 2 of hnRNPC gene |
| hnRNPC-2 | TAGAGTTCTAATGCTAGATT | Targets intron 2 of hnRNPC gene |
| PREP-1 | TCACCGGTTCAACCACTAAG | Targets intron 9 of <i>PREP</i> gene |
| PREP-2 | GCGGCAAACTTTGTAACCCA | Targets intron 9 of <i>PREP</i> gene |

143

144

145

| Name | Sequence (5' - 3') | Assay |
|-----------------|-------------------------------------|--|
| FRG1 minigene-F | AAAGGTACCTTGATGAGGGCCCTAGTCCT CC | Used in FRG1 minigene construction |
| FRG1 minigene-R | AAAGGATCCCTTGATCATTTCTTCTCCC | Used in FRG1 minigene construction |
| U1-F-1 | CCATGATCACGAAGGTGGTTT | Used in RNA ChIP-qPCR to amplify U1snRNA |
| U1-R-1 | ATGCAGTCGAGTTTCCCACAT | Used in RNA ChIP-qPCR to amplify U1snRNA |
| U2-F | TCCCAGGGCGAGGCTTATCCATT | Used in RNA ChIP-qPCR to amplify U2snRNA |
| U2-R | GAACGCAGTCCCCCACTACCACAAAT | Used in RNA ChIP-qPCR to amplify U2snRNA |
| U4-F | CTCGGCCTTTTGGCTAAGAT | Used in RNA ChIP-qPCR to amplify U4snRNA |
| U4-R | TATTCCATCTCCCTGCTCCA | Used in RNA ChIP-qPCR to amplify U4snRNA |
| U5-F | GATCATTTCTATAGTGTGTTACTAGA | Used in RNA ChIP-qPCR to amplify U5snRNA |
| U5-R | CAATACGGAGAGAAGAACGATC | Used in RNA ChIP-qPCR to amplify U5snRNA |
| U6-F | GCAGTATCGTAGCCAATGAGG | Used in RNA ChIP-qPCR to amplify U6snRNA |
| U6-R | CTGTCAAAAATTGCCAGTGCC | Used in RNA ChIP-qPCR to amplify U6snRNA |

| FRT Fw | GTTTTGACCTCCATAGAAGACAC | Used in PCR to check FRG1 splicing and in qPCR to amplify spliced transcript ("spliced 1") |
|------------------------|-----------------------------|--|
| FRT Rv | CTGATCAGCGGGTTTAAACGT | Used in PCR to check FRG1 splicing and in qPCR to amplify spliced transcript ("spliced 2") |
| R-juncEX2_EX1- FRG1 | TCA GGG CGA TTC TGG AAT CAG | Used in qPCR to amplify spliced cotranscriptional FRG1 transcripts ("spliced 1") |
| F-uniqex1 | TAGTCCTCCAGAGCAGTTTAC | Used in qPCR to amplify unspliced cotranscriptional FRG1 transcripts ("spliced 1") |
| FRG-in1-R | CTGTGGGCTTAAGACAGATAC | Used in qPCR to amplify unspliced cotranscriptional FRG1 transcripts ("spliced 1") |
| F-juncEX2-EX3-FRG1 | GTCTTTCAAAATGGGAAAATGGC | Used in qPCR to amplify spliced cotranscriptional FRG1 transcripts ("spliced 2") |
| FRG1_ex2 splicing_F | GAACAATGGGAACCAGTCTTTC | Used in qPCR to amplify unspliced cotranscriptional FRG1 transcripts ("spliced 2") |
| FRG-in2-R | TATGATTGACTGTTAACAGACTTTCT | Used in qPCR to amplify unspliced cotranscriptional FRG1 transcripts ("spliced 2") |
| F-uniq in1 BS | ACCACTCAGCCACATGCAG | Used in qPCR to amplify brance site sequence of <i>FRG1</i> intron 1. Used for U2 and U2AF65 RNA ChIP experiments. |
| R-uniq in1 BS | GGACATCAAAACAGGACATGC | Used in qPCR to amplify brance site sequence of <i>FRG1</i> intron 1. Used for U2 and U2AF65 RNA ChIP experiments. |

| FRG1-in2gRNAcheck- | | Used in PCR for checking sgRNA |
|--------------------|----------------------------|--|
| F | CCAGCACACATGTATAACTGAAG | efficient. |
| in2gRNAcheck-R | GCTTAACTCCCTTAACAAATATAATG | Used in PCR for checking sgRNA efficient. |
| FRT_Fw | GTTTTGACCTCCATAGAAGACAC | Used in qPCR for transcription inhibition by dCas9 in <i>FRG1</i> minigene experiment ("Upstream"). |
| FRG-EX1-R | ACTGCTCTGGAGGACTAGG | Used in qPCR for transcription inhibition by dCas9 in <i>FRG1</i> minigene experiment ("Upstream"). |
| FRG-in2near 3ss-F | CACAAGAAGTATCCTCATGGC | Used in qPCR for transcription inhibition by dCas9 in <i>FRG1</i> minigene experiment ("Downstream"). |
| FRT_Rv | CTGATCAGCGGGTTTAAACGT | Used in qPCR for transcription inhibition by dCas9 in <i>FRG1</i> minigene experiment ("Downstream"). |
| F-uniqex1 | TAGTCCTCCAGAGCAGTTTAC | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (first 5'SS). |
| FRG-in1-R | CTGTGGGCTTAAGACAGATAC | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (first 5'SS). |
| F-uniqin1upsg | CAGAGAATGTTACAGAATCATAACG | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (negative control 1 intron 1 for sgRNA intron 2 or positive control for sgRNA intron 1). |
| R-uniqin1upsg | CTATACTTTTGCACAAGGTGAGA | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (negative control 1 intron 1 for sgRNA intron 2 or positive control for sgRNA intron 1). |

| FRG1-mid INTRON1-F | TGCGAACAATCAGATCTCAATAGA | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (negative control 2 intron 1). |
|-------------------------|-------------------------------|--|
| R-FRG1-mid INTRON1 | AGTATCCTATAAATTTGCATTTTAGTAGT | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (negative control 2 intron 1). |
| FRG1-JUNCTION- WT-F | CAATGGGAACCAGTCTTTCAAAATG | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (second 5'SS WT). |
| FRG1-JUNCTION- MUT-F | CAATGGGAACCAGTCTTTCAAAATA | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (second 5'SS MUT). |
| FRG-in2-R | TATGATTGACTGTTAACAGACTTTCT | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (second 5'SS). |
| FRG1-middle intron2-F | CAGTAAGGACCAATGGGCAC | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (negative control 3 intron 2). |
| R-FRG1-middle intron2 | CTTAGAAAACACTGCTGAGTTCC | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (negative control 3 intron 2). |
| F-FRGIN2-near-sg | GACTTAGATAGTACTAATAATACAACA | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (positive control for sgRNA intron 2). |
| R-FRGIN2-near-sg | GCAATAGTTTTAAGACCCTCTTTC | Used in qPCR for CRISPR interference protocol to amplify <i>FRG1</i> minigene regions (positive control for sgRNA intron 2). |
| PLOD1_5ss_up | GAGGAGATGGAGCACTTTGG | Used in PCR to check <i>PLOD1</i> splicing |
| PLOD1_splice_R | GAGGGCTGCTCATCAGGC | Used in PCR to check <i>PLOD1</i> splicing |
| YBX1-ex2-F | CAACGAAGGTTTTGGGAACAG | Used in PCR to check YBX1 splicing |

| YBX1_splice_R | CACAGTCTCTCCATCTCCTAC | Used in PCR to check YBX1 splicing |
|-------------------|----------------------------|--|
| F_PREPex7 | TACAGCAGGAATCCAGTGGC | Used in PCR to check PREP splicing |
| R_PREPex10 | TGGAATCTTCGTACCATCCTTG | Used in PCR to check PREP splicing |
| AKIRIN2_ex1_F | GTATCTCCGAATGGAGCCATC | Used in PCR to check AKIRIN2 splicing |
| AKIRIN2_ex4_R | CATTATTTGATCATGCGTAAACTTCA | Used in PCR to check AKIRIN2 splicing |
| HNRNPC_ex1_F | ACGAAGACTGAGCGGTTGTG | Used in PCR to check hnRNPC splicing |
| HNRNPC_ex4_R | CGCTGCAGATCGTTTCACAC | Used in PCR to check hnRNPC splicing |
| PLOD1_5ss_up | GAGGAGATGGAGCACTTTGG | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (first 5'SS). |
| PLOD1_5ss_up-R | AGAAGACCCCAGACAGTGAG | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (first 5'SS). |
| PLOD1_neg.cont1-F | CACTGTACTTAAAGGCAACATTAC | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (negative control 1 intron 1). |
| PLOD1_neg.cont1-R | GAGGTGGGTTGGGGCTTG | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (negative control 1 intron 1). |
| PLOD1_5ss _down-F | CAAATTCCTGCTGGAGTACATTG | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (second 5'SS). |
| PLOD1_5ss_down-R | CCCAGCCACTCCACTGAC | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (second 5'SS). |

| PLOD1_neg.cont3-F | AGTACGGGTGGAGGAACATC | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (negative control 2 intron 2). |
|-------------------|--------------------------|--|
| PLOD1_neg.cont3-R | CACACGCTACTAGATGGAGG | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (negative control 2 intron 2). |
| PLOD1_pos.cont-F | GCCCTGCTTCGTCCGAAG | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (positive control). |
| PLOD1_pos.cont-R | GCATTCAAGGGATGAATGACAAC | Used in qPCR for CRISPR interference protocol to amplify <i>PLOD1</i> regions (positive control). |
| YBX1_5ss_up-F | GGAACGGATATGGTTTCATCAAC | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (first 5'SS). |
| YBX1_5ss_up-R | CGCCTTTCAGAACAGAGCTG | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (first 5'SS). |
| YBX1_neg.cont1-F | GTGTCAGACTGTGCAGGTG | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (negative control 1 intron 1). |
| YBX1_neg.cont1-R | CAAGCTGTCAAGTTCCTATGG | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (negative control 1 intron 1). |
| YBX1_5ss_down-F | CCAAGGAAGATGTATTTGTACAC | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (second 5'SS). |
| YBX1_5ss_down-R | GCTGAGAACATATCTAACCTTTTC | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (second 5'SS). |
| YBX1_neg.cont2-F | CTGATGAGTACTGCATAGTGAAC | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (negative control 2 intron 2). |

| YBX1_neg.cont2-R | CACTTGGCAGTGGTTTACATTAG | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (negative control 2 intron 2). |
|------------------|--------------------------|---|
| YBX1 pos.cnt-2 F | GTATTTGATGACAAGGAGGAAGC | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (positive control). |
| YBX1 pos.cnt-2 R | AACATTTTGTCTGAAGTAGTCCTG | Used in qPCR for CRISPR interference protocol to amplify <i>YBX1</i> regions (positive control). |
| F_junc1_PREP | GTACTTGTTCCTGAGCATGAG | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (first 5'SS). |
| R_junc1_PREP | ACTAGAGAATGAGAGACGCAC | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (first 5'SS). |
| F-mid1 new2_PREP | TCTTCCTGCACCTCATCCAG | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (negative control 1 intron 1). |
| R-mid1 new2_PREP | GACTGGTGGGTTGAGGATC | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (negative control 1 intron 1). |
| F_junc2_PREP | AAGGACACTGAAATCTTCTATCAG | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (second 5'SS). |
| R_junc2_PREP | CATAGCATTATACTTCCATTTCCC | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (second 5'SS). |
| F_mid2_PREP | AACCTGGAGTACAGCAGCAG | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (negative control 2 intron 2). |
| R_mid2_PREP | ACTTCTGTACCCAGTGTAAATTTG | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (negative control 2 intron 2). |

| F_nSG_PREP | GACATTTGATAACTCTGGCAGG | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (positive control). |
|-------------------|---------------------------|--|
| R_nSG_PREP | GTAAGTCATAAATGGCTTGGGC | Used in qPCR for CRISPR interference protocol to amplify <i>PREP</i> regions (positive control). |
| F junc1 Akir | CTCACCACAGGTGGGACC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (first 5'SS). |
| R junc1 Akir | TGCTAAGAACAAGAACCACACAGTC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (first 5'SS). |
| AKIRIN2_int1F | TGCCCAGTTGTGAGTATCTTTC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (negative control 1 intron 1). |
| AKIRIN2_int1R | CAACTATTCAAGAGGCCCAAAC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (negative control 1 intron 1). |
| AKIRIN2_ex2int2F | ACCAGCTTCACCAGGTAATAAC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (second 5'SS). |
| AKIRIN2_ex2int2R | GAGGAGATACAGTAGCTTAAATGAC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (second 5'SS). |
| AKIRIN2_int2F3 | GTACCTTGTCATGACTGAACAC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (negative control 2 intron 2). |
| AKIRIN2_int2R3 | ATAAGACAAGTGTGTGAGGCAG | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (negative control 2 intron 2). |
| AKIRIN2int2_seq_F | AAATCAAAGACCATAACATAGTC | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (positive control). |

| R near guide Akir | CAATGGGCATGGCAGTATTTAG | Used in qPCR for CRISPR interference protocol to amplify <i>AKIRIN2</i> regions (positive control). |
|-------------------|-------------------------|---|
| Fjunc1_HNRNPC | CCCCTTCTTGGTAAGTGGAG | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (first 5'SS). |
| HNRNPC_int1R | ACAAGCAGAATAAATGAGGGCC | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (first 5'SS). |
| HNRNPC_int1F | GAATGGCTGTGCGAAACAAGC | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (negative control 1 intron 1). |
| HNRNPC_int1R2 | AACTCCTAGGCAAACTTACTCC | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (negative control 1 intron 1). |
| HNRNPC_ex2int2F | TTTTGATCTTCAGCTACAGTAAG | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (second 5'SS). |
| HNRNPC_int2R4 | GTGTCATTATCATCATCATCATC | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (second 5'SS). |
| HNRNPC_int2F3 | CTGAAAGCCATCTTCCTCCTG | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (negative control 2 intron 2). |
| HNRNPC_int2R3 | CTGGGCATCCATGTTGCTG | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (negative control 2 intron 2). |
| F_nSG3_HNRNPC | AAGAGTCAAACCATAGGAGATAC | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (positive control). |
| R_nSG3_HNRNPC | ATTTCCCTGCTTTGTGAGGTC | Used in qPCR for CRISPR interference protocol to amplify <i>hnRNPC</i> regions (positive control). |

| FRG_mutx2_R | | Used in qPCR for CRISPR interference |
|-------------|------------------------------|--------------------------------------|
| | GAAGTTTTTATAAACAATAACAGCTCTC | protocol to amplify FRG1 minigene |
| | | regions (second 5'SS MUTx2). |
| | | |