Competition between end maturation and degradation drives human snRNA 3' end

quality control

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Supplemental Material Titles

Supplemental Figure S1. Related to Figure 1. Characterization of TOE1-degron cell lines.

Supplemental Figure S2. Related to Figure 2. snRNAs are tailed and trimmed at early and late steps of snRNA biogenesis.

Supplemental Figure S3. Related to Figure 3. TOE1 depletion causes accumulation of unprocessed adenylated snRNAs with PHAX.

Supplemental Figure S4. Related to Figure 4. snRNAs become targets of the nuclear exosome in the absence of TOE1.

Supplemental Figure S5. Related to Figure 5. TOE1 selectively processes regular U1 snRNA over U1 snRNA variants.

Supplemental Figure S6. Related to Figure 6. U1 variant snRNAs are targets of the nuclear exosome.

Supplemental Table S1. List of DNA and RNA oligos used.

U2

TOE1: +

U5

TOE1: +

-

0.1 0

-



0.1

0 Γ

-

TOE1: +

Supplemental Figure S1 related to Figure 1. Characterization of TOE1-degron cell lines. (A) Western blot analysis of endogenous TOE1 and FLAG-TOE1-mAID levels in TOE1-degron cell lines treated with doxycycline (Dox) or auxin for 48 hours. Western blotting for Upf1 served as a loading control. Wt: parental cell line; Het: cell line expressing both endogenous TOE1 and exogenous FLAG-TOE1mAID; Degron 1 and 2: cell lines expressing exogenous FLAG-TOE1mAID with endogenous TOE1 knocked out. Auxin treatment resulted in levels of FLAG-TOE1mAID below the limit of detection. (B) Cumulative plot of 3' ends of U1 snRNA harvested at steady state from wt, het, degron 1 and 2 cell lines described in (A), treated with doxycycline (black line) or auxin (gray line) for 48 hours. Position '0' refers to the mature 3' end of snRNAs indicated by the border between gray and white backgrounds. Only reads terminating at or downstream of position -5 are represented. Solid lines represent actual 3' end positions of snRNAs including any post-transcriptional nucleotides, while dotted lines represent the predicted 3' end of genome-encoded sequences with post-transcriptionally added nucleotides indicated by the shading between the lines. (C) Left panels: Sequence logo plots representing the percent of U1 snRNA with post-transcriptionally added nucleotides, broken down by nucleotide composition, for each cell line treated with doxycycline (black) or auxin (gray) for 48 hours. Right panels: average number of post-transcriptional adenosines and uridines per U1 snRNA transcript monitored by snRNA 3' end sequencing from indicated cell lines treated with doxycycline or auxin as indicated. (D) Average number of post-transcriptional uridines per snRNA transcript monitored by snRNA 3' end sequencing when TOE1 is present (black) or depleted (red) from the degron 1 cell line. Error bars: SEM from at least three independent experiments and p-values (Student's two-tailed t-test) **:p<0.05; ***:p <0.01.



Supplemental Figure S2 related to Figure 2. snRNAs are tailed and trimmed at early and late steps of snRNA biogenesis. (A) Enrichment of U1, U4 and U4atac snRNAs in crosslinking and immunoprecipitation experiments with antibodies against PHAX, SMN, SNUPN, and BRR2 as measured by RT-qPCR and normalized to immunoprecipitation reactions with no antibody, with the latter condition set to 1. (B) Average number of post-transcriptionally added adenosines and uridines per snRNA transcript immunoprecipitated with indicated biogenesis factors. Input: snRNAs from cell lysates prior to immunoprecipitation. Error bars: SEM from at least three independent experiments. (C) Enrichment of U1, U4 and U4atac snRNAs in crosslinking and

immunoprecipitation experiments with antibodies against PHAX from HeLa cells as measured by RT-qPCR and normalized to immunoprecipitation reactions with no antibody, with the latter condition set to 1. **(D)** Cumulative plots of 3' end positions for snRNAs associated with snRNA biogenesis factor PHAX (red) from HeLa cells monitored by crosslinking and immunoprecipitation followed by snRNA 3' end sequencing. Input samples are shown in black. Only reads terminating at or downstream of position -5 are represented.



Supplemental Figure S3 related to Figure 3. TOE1 depletion causes accumulation of unprocessed adenylated snRNAs with PHAX. (A) Cumulative plots of 3' end positions of U1, U4 U4atac snRNAs associated with SMN, SNUPN and and BRR2 after crosslinking/immunoprecipitation and 3' end sequencing from cells with TOE1 present (black) or depleted (red). The average of three independent experiments is plotted. (B) Sequence logo plots representing the percent of snRNAs associated with SMN, SNUPN and BRR2 containing post-transcriptionally added tails, broken down by nucleotide composition, in the presence or absence of TOE1. The average of three independent experiments is plotted. (C) Enrichment of U1, U4, U4atac, U3 and U8 sn/snoRNAs in crosslinking and immunoprecipitation experiments using antibodies against PHAX, SMN, SNUPN, and BRR2 as measured by RT-gPCR and normalized to immunoprecipitation with no antibody, with the latter condition set to 1. Experiments were performed from cell lines with TOE1 present (black) or depleted (red). (D) Relative levels of U1, U4 and U4atac snRNAs associated with PHAX when TOE1 is present (black) or depleted (red) as measured by RT-qPCR assays normalized to the TOE1 non-target control U8 snoRNA, with averages of normalized U1, U4 and U4atac snRNA levels when TOE1 is present set to 1. (E) Relative levels of nascent U1, U4 and U4atac snRNAs in cells +/- TOE1, measured by RT-qPCR. Error bars: SEM from at least three independent experiments and pvalues (Student's two-tailed t-test) *:p<0.1; **:p <0.05; ***:p <0.01.

Supplemental Fig. S4



Supplemental Figure S4 related to Figure 4. snRNAs become targets of the nuclear exosome in the absence of TOE1. Representative Western blots showing co-depletions of MTR4, ZCCHC8, DIS3, and ZC3H18 with TOE1. Upf1 serves as an internal control for calculating percent remaining protein indicated below panels.



Supplemental Figure S5 related to Figure 5. TOE1 selectively processes regular U1 snRNA over U1 snRNA variants. (A) Cumulative plots of 3' ends of U1 variant snRNAs from RNA harvested at steady state when TOE1 is present (black) or depleted (red). The average of four independent experiments is plotted. (B) Sequence logo plots representing the percent U1 snRNA variants with post-transcriptionally added nucleotides when TOE1 is present or depleted, broken down by nucleotide composition. The average of four independent experiments is plotted. (C) Relative enrichment of snRNA variants with PHAX monitored by crosslinking/immunoprecipitation followed by RT-gPCR as normalized to immunoprecipitations with no antibody which was set to 1. (D) Relative levels of association of U1 variants with PHAX Lardelli and Lykke-Andersen

when TOE1 is present (black) or depleted (red) as measured by RT-qPCR assays normalized to the TOE1 non-target control U8 snoRNA, with averages of normalized U1 variant snRNA levels when TOE1 is present set to 1. Error bars: SEM from three independent experiments and p-values (Student's two-tailed t-test) ***:p <0.01.





Supplemental Figure S6 related to Figure 6. U1 variant snRNAs are targets of the nuclear exosome. (A) Relative levels of variant U1 snRNAs upon siRNA-mediated co-depletion of MTR4 and ZCCHC8 as measured by RT-qPCR from total RNA and normalized to levels from control-treated (siCtrl) cells and to averages of 12S and 7SK RNA levels as internal controls. Error bars: SEM from four independent experiments and p-values (Student's two-tailed t-test) *:p<0.1; **:p<0.05; ***:p<0.01. (B) Representative western blots showing siRNA-medicated co-depletion of MTR4 and ZCCHC8. Upf1 is used an internal control for calculating percent remaining protein. (C) Relative levels of U1 variant snRNAs upon TOE1 depletion and siRNA-mediated depletion of Ctrl and MTR4, ZCCHC8, ZC3H18, or DIS3 as measured by RT-qPCR from total RNA and normalized to levels in the Ctrl siRNA/TOE1+ condition shown in Figure 6 and to the averages of mitochondrial 12S and 7SK RNA levels as internal controls. Error bars: SEM from at least

three independent experiments and p-values (Student's two-tailed t-test) *:p<0.1; **:p <0.05;

***:p <0.01.

Supplemental Table S1: RNA and DNA oligos used.

RNA adapter	RNA sequence
AG-10N	/5Phos/AGNNNNNNNNAGAUCGGAAGAGCGUCGUG/3SpC3/
AG-11N	/5Phos/AGNNNNNNNNNNAGAUCGGAAGAGCGUCGUG/3SpC3/
gene specific primers for 3' end library p	reparation
FU1Vs-01	
FU1Vs-02	
FU11/e_05	
FU11/e_06	
FU1_\/s_07	
FU1-Ve-08	
FU1-Vs-09	
FU1-Vs-10	CAGACGTGTGCTCTTCCGATCT GTCAAGTGCTAGC ATGATCACGAAGGTGGTTTT
FU1-Vs-11	CAGACGTGTGCTCTTCCGATCT CAATGTACGAACGT ATGATCACGAAGGTGGTTTT
FU1-Vs-12	CAGACGTGTGCTCTTCCGATCT TAGC ATGATCACGAAGGTGGTTTT
FU1-Vs-13	CAGACGTGTGCTCTTCCGATCT CTTGC ATGATCACGAAGGTGGTTTT
FU1-Vs-14	CAGACGTGTGCTCTTCCGATCT CAACAT ATGATCACGAAGGTGGTTTT
FU1-Vs-15	CAGACGTGTGCTCTTCCGATCT GATGGTA ATGATCACGAAGGTGGTTTT
FU1-Vs-16	CAGACGTGTGCTCTTCCGATCT ACCGAAGT ATGATCACGAAGGTGGTTTT
FU1-Vs-17	CAGACGTGTGCTCTTCCGATCT TGATGCACA ATGATCACGAAGGTGGTTTT
FU1-Vs-18	CAGACGTGTGCTCTTCCGATCT AGCATGTGTC ATGATCACGAAGGTGGTTTT
FU1-Vs-19	CAGACGTGTGCTCTTCCGATCT GTGTCATACTG ATGATCACGAAGGTGGTTTT
FU1-Vs-20	CAGACGTGTGCTCTTCCGATCT TACGACTGTTCA ATGATCACGAAGGTGGTTTT
FU1-Vs-21	CAGACGTGTGCTCTTCCGATCT CAGTTCACGATCG ATGATCACGAAGGTGGTTTT
FU1-Vs-22	CAGACGTGTGCTCTTCCGATCT GTTACATGCTTGCA ATGATCACGAAGGTGGTTTT
FU1-Vs-23	
FU1-VS-24	
FU1-Vs-25	
FU1-VS-26	
FU1-VS-27	
FU I-VS-20	
FUI-VS-29 FUI-VS-30	
FU1_\/c_31	
FU1_\/c_32	
FU1-Vs-33	
FU1-Vs-34	CAGACGTGTGCTCTTCCGATCT GTTGGAATC ATGATCACGAAGGTGGTTTT
FU1-Vs-35	CAGACGTGTGCTCTTCCGATCT CCGGA ATGATCACGAAGGTGGTTTT
FU1-Vs-36	CAGACGTGTGCTCTTCCGATCT TCTCTA ATGATCACGAAGGTGGTTTT
FU4-01	CAGACGTGTGCTCTTCCGATCT CGTA GCAGTATCGTAGCCAATGAGG
FU4-02	CAGACGTGTGCTCTTCCGATCT CGTTC GCAGTATCGTAGCCAATGAGG
FU4-03	CAGACGTGTGCTCTTCCGATCT TACAAC GCAGTATCGTAGCCAATGAGG
FU4-04	CAGACGTGTGCTCTTCCGATCT ATGGTAG GCAGTATCGTAGCCAATGAGG
FU4-05	CAGACGTGTGCTCTTCCGATCT TGATGGCA GCAGTATCGTAGCCAATGAGG
FU4-06	CAGACGTGTGCTCTTCCGATCT ACACGTTGT GCAGTATCGTAGCCAATGAGG
FU4atac-01	CAGACGTGTGCTCTTCCGATCT ACCAC CCATCTTTTTCTTGAGGTTGCAC
FU4atac-02	CAGACGTGTGCTCTTCCGATCT GCACTA CCATCTTTTCTTGAGGTTGCAC
FU4atac-03	CAGACGTGTGCTCTTCCGATCT GTGACTA CCATCTTTTTCTTGAGGTTGCAC
FU4atac-04	CAGACGTGTGCTCTTCCGATCT TTCTCTA CCATCTTTTTCTTGAGGTTGCAC
FU4atac-05	
FU4atac-06	
FU2-01	
FU2-02	
FU2-03	
FU2-04	
FU2-06	
FU2-07	CAGACGTGTGCTCTTCCGATCT TCGG GGAGATGGAATAGGAGCTTGC
FU2-08	CAGACGTGTGCTCTTCCGATCT CGACA GGAGATGGAATAGGAGCTTGC
FU2-09	CAGACGTGTGCTCTTCCGATCT CATTGA GGAGATGGAATAGGAGCTTGC
FU2-10	CAGACGTGTGCTCTTCCGATCT GCGAAGT GGAGATGGAATAGGAGCTTGC
FU2-11	CAGACGTGTGCTCTTCCGATCT ACTAGTCA GGAGATGGAATAGGAGCTTGC
FU2-12	CAGACGTGTGCTCTTCCGATCT TGAAGCATA GGAGATGGAATAGGAGCTTGC
FU5-04	CAGACGTGTGCTCTTCCGATCT GATGGTA ATACTCTGGTTTCTCTTCAGATCG
FU5-05	CAGACGTGTGCTCTTCCGATCT ACCGAAGT ATACTCTGGTTTCTCTTCAGATCG
FU5-06	CAGACGTGTGCTCTTCCGATCT TGATGCACA ATACTCTGGTTTCTCTTCAGATCG
FU5-07	CAGACGTGTGCTCTTCCGATCT GACATTCGTA ATACTCTGGTTTCTCTTCAGATCG
FU5-08	CAGACGTGTGCTCTTCCGATCT ATGGACTAGTA ATACTCTGGTTTCTCTTCAGATCG
FU5-09	
FU5-10	
FUT1-UT	
FU11_03	
FU11_04	
FU11_05	
FU11-06	
FU12-01	CAGACGTGTGCTCTTCCGATCT CTAG CGAATCCTCACTGCTAATGTGAG
FU12-02	CAGACGTGTGCTCTTCCGATCT ACTTC CGAATCCTCACTGCTAATGTGAG
FU12-03	CAGACGTGTGCTCTTCCGATCT GATATG CGAATCCTCACTGCTAATGTGAG
FU12-04	CAGACGTGTGCTCTTCCGATCT TGCTGAC CGAATCCTCACTGCTAATGTGAG
FU12-05	CAGACGTGTGCTCTTCCGATCT AGTACTGT CGAATCCTCACTGCTAATGTGAG
FU12-06	CAGACGTGTGCTCTTCCGATCT TCAGTCAAC CGAATCCTCACTGCTAATGTGAG
FU3-01	CAGACGTGTGCTCTTCCGATCT TACA CTCTGAACGTGTAGAGCAC
FU3-02	CAGACGTGTGCTCTTCCGATCT ATGTA CTCTGAACGTGTAGAGCAC
FU3-03	CAGACGTGTGCTCTTCCGATCT GCACGA CTCTGAACGTGTAGAGCAC

Supplemental Table S1: RNA and DNA oligos used.

E11.5-04	
FU0 05	
FU3-05	CAGACGIGIGCICTICCGATCICAACTCGA CICIGAACGIGIAGAGCAC
FU3-06	CAGACGTGTGCTCTTCCGATCT TCTTGGATA CTCTGAACGTGTAGAGCAC
FU8-01	
EU8 02	
FU0.00	
FU8-03	
FU8-04	CAGACGTGTGCTCTTCCGATCT GGTTTTG GGATAATCCTTACCTGTTCCTCC
FU1V68-01	CAGACGTGTGCTCTTCCGATCT ATGC TTCCCCAAATGTGGGAA
FU11/68-02	
T 0 T V 00-02	
FU1V68-03	CAGACGIGIGCICTICCGATCIGITAGITICCCCAAATGIGGGAA
FU1V68-04	CAGACGTGTGCTCTTCCGATCT CATCTAC TTCCCCAAATGTGGGAA
FU1V68-05	CAGACGTGTGCTCTTCCGATCT TCGGTATC TTCCCCCAAATGTGGGGAA
EU11//68.06	
FUv1-6-01	CAGACGIGIGCICIICCGAICIAGGA IGGCAGGAGAGAIACCCIGG
FUv1-6-02	CAGACGTGTGCTCTTCCGATCT TGGT TGGCAGGAGAGATACCCTGG
FUv1-6-03	CAGACGTGTGCTCTTCCGATCT ACACA TGGCAGGAGAGATACCCTGG
EUv1 6 04	
TUV1-0-04	
FUV1-6-05	CAGACGIGIGCICTICCGATCICCAATTIGGCAGGAGAGATACCCTGG
FUv1-6-06	CAGACGTGTGCTCTTCCGATCT GGAATA TGGCAGGAGAGATACCCTGG
FUv1-3-01	CAGACGTGTGCTCTTCCGATCT TGTG ATACTTATGTTTATCTGGCAGAAGAA
FUv1-3-02	CAGACGTGTGCTCTTCCGATCT CACG ATACTTATGTTATCTGGCAGAAGAA
FU:1 2 02	
FUV1-3-03	CAGACGIGIGCICITICCGATCI GCCATATACITATGITIATCIGGCAGAAGAA
FUv1-3-04	CAGACGTGTGCTCTTCCGATCT ATACG ATACTTATGTTTATCTGGCAGAAGAA
FUv1-3-05	CAGACGTGTGCTCTTCCGATCT TTACCG ATACTTATGTTTATCTGGCAGAAGAA
FUv1-3-06	CAGACGTGTGCTCTTCCGATCT AATGGC ATACTTATGTTTATCTGGCAGAAGAA
EUv1 15 01	
	CAGACGTGTGCTCTTCCGATCT AAGGATCCGTATGTTCCGGATGTA
FUv1-15-02	CAGACGTGTGCTCTTCCGATCT TTCC ATCCGTTATGTTCCGGATGTA
FUv1-15-03	CAGACGTGTGCTCTTCCGATCT GGTTA ATCCGTTATGTTCCGGATGTA
EUv1 15 04	
FUVI-13-04	CAGACGIGIGCICITCCGATCICCAATATCCGITATGTTCCGGATGTA
FUv1-15-05	CAGACGTGTGCTCTTCCGATCT GAGTCT ATCCGTTATGTTCCGGATGTA
FUv1-15-06	CAGACGTGTGCTCTTCCGATCT ACAGTG ATCCGTTATGTTCCGGATGTA
EUv1 9.02	
TU 1001	
FUv1-8-04	CAGACGIGIGCICTICCGATCTICCAAATCCTAGCAGAAGAAAATCGIGTTIACG
FUv1-8-05	CAGACGTGTGCTCTTCCGATCT ATTCCAAT CCTAGCAGAAGAAAATCGTGTTTACG
FUv1-8-06	CAGACGTGTGCTCTTCCGATCT GATTGGCCT CCTAGCAGAAGAAATCGTGTTTACG
library prep and sequencing	
PCR_F_D501	AATGATACGCGACCACCGAGATCTACACTATAGCCTACACTCTTTCCCTACACGACGCTCTT
PCR_F_D502	AATGATACGGCGACCACCGAGATCTACACATAGAGGCACACTCTTTCCCTACACGACGCTCT
PCR F D503	AATGATACGGCGACCACCGAGATCTACACCCTATCCTACACTCTTTCCCTACACGACGCTCT
PCR F D701	
PCR_F_D702	CAAGCAGAAGACGGCATACGAGATTCTCCGGAGTGACTGGAGTTCAGACGTGTGCTCTTCCG
PCR_F_D703	CAAGCAGAAGACGGCATACGAGATAATGAGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCG
PCR F D704	CAAGCAGAAGACGGCATACGAGATGGAATCTCGTGACTGGAGTTCAGACGTGTGCTCTTCCG
gRNAs	
gRNAs CAF1Z_gRNA1	GACACCACCTGGGGGTTAGG
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA2	GACACCACCTGGGGGTTAGG GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCACCT
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA3	GACACCACCTGGGGGTTAGG GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout	GACACCACCTGGGGGTTAGG GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA2 CAF1Z_gRNA3 primers - endogeous knockout caf1z_seq_F1	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2	GACACCACCTGGGGGTTAGG GACACCACCTGGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGGCACACGGTGAGAGGT ACGTGGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA3 primers - endogeous knockout caf1z_seq_F1 caf1z_seq_F2 caf1z_seq_F1	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCGGCGAAAAGCTACCA
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA3 primers - endogeous knockout caf1z_seq_F1 caf1z_seq_F2 caf1z_seq_F2 caf1z_seq_F2	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGGCACACGGTGAGAGT ACGTGGGGTTACAAAGGCTT GCTTGTGGGGAAAAGCTACCA TTCAGCAAACCTTTCCTGTCC ACGTGGGGAAAAGCTACCA
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R1 caf12_seq_R2	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGGT ACGTGGGACACGGTGAGAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA3 primers - endogeous knockout caf1z_seq_F1 caf1z_seq_F2 caf1z_seq_R1 caf1z_seq_R2 siRNAs	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R1 caf12_seq_R2 siRNAs siControl (siLuciferase)	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGGCACCACGGTGAGAGT ACGTGGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA2 CAF1Z_gRNA3 primers - endogeous knockout caf1z_seq_F1 caf1z_seq_F2 caf1z_seq_F2 caf1z_seq_R1 caf1z_seq_R2 siRNAs siControl (siLuciferase) siMTR4	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGGAAUACUUCGAUU
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R1 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACCTCTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGUACCUCAGGAUAAUU
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA3 primers - endogeous knockout caf1z_seq_F1 caf1z_seq_F2 caf1z_seq_R1 caf1z_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZC3H18	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACCTCTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGUACUCAGGAUAAUU GGAAUGAAUUGUAGGUUUAUU
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZC3H18 siDIS3	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCGTGAGAGT ACGTGGGACACGGTGAGAGGT ACGTGGGACACGGTGAGAGGT TCCAGCAACGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGUACUCAGGUUGUAGUAAUU GGAAUGAACUUGGAGUAAUU
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf1z_seq_F1 caf12_seq_F2 caf12_seq_F2 caf12_seq_R1 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZCCHC8 siZC3H18 siDIS3 gPCB primers	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGAACUCUCAGGAUAAUU GGAAUGAAUUGUAGGUUUAUU AGGUAGAGUUGUAGGAUAUU
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R1 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZC3H18 siDIS3 qPCR primers	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGAACUCAGGAUAAUU GGAAUGAACUCAGGAUAAUU
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZCCH78 siZC3H18 siDIS3 qPCR primers U1-forward	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGUACCUCAGGAUAAUU GGAAUGUACCUCAGGAUAAUU GGAAUGAAUUGUAGGUUUAUU AGGUAGAGUUGUAGGAUAUU GCACTCCGGATGTGCTGACCC
gRNAs CAF1Z_gRNA1 CAF1Z_gRNA2 CAF1Z_gRNA2 CAF1Z_gRNA3 primers - endogeous knockout caf1z_seq_F1 caf1z_seq_F2 caf1z_seq_R1 caf1z_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZC3H18 siDS3 qPCR primers U1-forward U1-reverse	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACCTCTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGAACUCUCAGGAUAAUU GGAAUGAACUCUCAGGAUAAUU GGAAUGAACUCUCAGGAUAAUU GGAAUGAACUCUCAGGAUAAUU GGAAUGAACUGUGUAGGAUUAUU AGGUAGAGUUGUAGGAUUAUU
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gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_R1 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZC3H18 siDIS3 qPCR primers U1-forward U4-reverse U4-forward U4-reverse U4atac-forward U4atac-reverse 7sk-reverse 40-foreuric Choreuric	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGUACCUCAGGAUAAUU GGAAUGUACCUCAGGAUAAUU GGAAUGUACCUCAGGAUAAUU GGAAUGAAUUGUAGGUUUAUU AGGUAGAGUUGUAGGAAUAUU GCACTCCGGATGTGCTGACCC CAGGGGAAAGCGCGAACGCAG GCGCGATTATTGCTAATTGAAA AAAATTGCCAATGCCGACTA GCACCAAAATAAAGCAAAGC
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gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_R1 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZC3H18 siDIS3 qPCR primers U1-forward U1-reverse U44aco-forward U4-acoreverse 7sk-reverse 12s-reverse 12s-reverse	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACGGTGACGACT GCTTGTGGGAAAAGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGAACUCUCAGGAUAAUU GGAAUGAACUCUCAGGAUAAUU GGAAUGAAUUGUAGGUUUAUU AGGUAGAGUUGUAGGAUAUU GCACTCCGGATGTGCTGACCC CAGGGGAAAGCGCGAACGCAG GCGCGATTATTGCTAATTGAAA AAAAATTGCCAATGCCGACAT GCACCAAAATAAAGCAAAGC
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gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZC3H18 siDIS3 qPCR primers U1-forward U1-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-reverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse U4-forward U4-feverse	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACCGGTGAGAGT ACGTGGGTTACAAAGGCTACCA TTCAGCAAACTCTTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGUACCUCAGGAUAAUU GGAAUGAACUCTGCGAGUAAUU GGAAUGAACUGUAGGAUUAUU AGGUAGAGUUGUAGGAAUAUU GGAAUGAAU
gRNAs CAF12_gRNA1 CAF12_gRNA2 CAF12_gRNA2 CAF12_gRNA3 primers - endogeous knockout caf12_seq_F1 caf12_seq_F2 caf12_seq_R2 siRNAs siControl (siLuciferase) siMTR4 siZCCHC8 siZC3H18 siDIS3 qPCR primers U1-forward U1-reverse U4-forward U1-reverse U4-forward U4-reverse U4-forward	GACACCACCTGGGGGTTAGG TGCTTTTGCTGACACCACCT GCTGTGGACACGGTGAGAGT ACGTGGGTTACAAAGGCTT GCTTGTGGGAAAAGCTCTCCTGTCC ACCTACCCACAGGAGCTGAG CGUACGCGGAAUACUUCGAUU CAAUUAAGGCUCUGAGUAAUU GGAAUGAUUGUAGGUUUAUU AGGUAGAUUGUAGGUUUAUU AGGUAGAUUGUAGGUUUAUU GGAAUGAAU
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