Title: RTF2 controls replication repriming and ribonucleotide excision at the replisome

Authors:

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Supplementary Information includes:

Supplementary table 1. Mouse strains

MOUSE STRAIN	SOURCE	IDENTIFIER
Rtf2 $^{tm1a(KOMP)wtsi}$	This paper	
B6.Cg Tg(ACTFLPe)9205Dym/J	Jackson Laboratories	005703
B6.FVB/N-Tg(Ella-cre)C5379Lmgd/J	Jackson Laboratories	003314
C57BL/6J	Jackson Laboratories	000664

Supplementary table 2. Mammalian cell lines

CELL LINE	**SOURCE**	**IDENTIFIER**
Human: HEK 293T	ATCC	
Human: HEK 293T endogenously GFP-tagged RTF2	This paper	N/A
Human: BJ-hTERT-E6/7, male	Smogorzewska Lab	
Human: U2OS, female	ATCC	HTB-96
Human: RPE *p53*$^{-/-}$, *pRb*$^{-/-}$, PRIM1-AID-mClover	de Lange Lab	
Human: HeLa (HeLa Parental, HeLa WT Clone, HeLa RNASEH2A KO Clone), female	Durocher and Jackson Labs	
Human: HCT-116 *p53*$^{-/-}$ (HCT-116 *p53*$^{-/-}$ WT Clone, HCT-116 *p53*$^{-/-}$ RNASEH2A KO Clone)	Durocher and Jackson Labs	
Mouse: RTF2 MEFs (*Rtf2*$^{+/+}$, *Rtf2*$^{+/lox}$, *Rtf2*$^{-/lox}$, and *Rtf2*$^{lox/lox}$, SV40-immortalized *Rtf2*$^{-/-}$ clone, *p53*$^{-/-}$;*Rtf2*$^{+/lox}$, *p53*$^{-/-}$;*Rtf2*$^{-/lox}$)	This paper	N/A
KOMP mES cells Rtf2$^{tm1a(KOMP)wtsi}$	KOMP	MGI code: 1913654

Supplementary table 3. Primers

mESC long range genotyping primers		
Oligonucleotide	**VENDOR**	**IDENTIFIER**
mESCs Cassette 3' Universal Forward cacacctccccctgaacctgaaac	IDT	KOMP
mESCs Cassette 5' Universal Reverse ggtggtgtgggaaagggttcgaag	IDT	KOMP
mESCs Cassette GF3 gccgaagaaggtcgagaaggtcag	IDT	KOMP
mESCs Cassette GR3 cgaatctctccacctgctcaatccag	IDT	KOMP

RTF2 mouse genotyping primers		
Oligonucleotide	**VENDOR**	**IDENTIFIER**
Genotyping_PCR1_Fwd gcctgtgagcttggcaggta	IDT	This Paper
Genotyping_PCR1_Rev aggggaagacctgactgtgt	IDT	This Paper
Genotyping_PCR2_Fwd gcctgtgagcttggcaggta	IDT	This Paper
Genotyping_PCR2_Rev agcctgagctctgtcacatt	IDT	This Paper
Genotyping_PCR3_Fwd gatattgctgaagagcttgg	IDT	This Paper
Genotyping_PCR3_Rev gaagttatctcgacgaagttc	IDT	This Paper

RT-qPCR primers		
Oligonucleotide	**VENDOR**	**IDENTIFIER**
Mouse *Rtf2* RT-qPCR Forward gaagtgtgtcacacgtgtgg	IDT	This Paper
Mouse *Rtf2* RT-qPCR Reverse tttcttttccagcttggccc	IDT	This Paper
Human *RTF2* RT-qPCR Forward tgctgaagacaaggatggag	IDT	Kottemann et al
Human *RTF2* RT-qPCR Reverse tgaaacagactctgctgcct	IDT	Kottemann et al.
Mouse *Rnaseh2a* RT-qPCR Forward gcatctttgccaaggtggcc	IDT	This Paper
Mouse *Rnaseh2a* RT-qPCR Reverse ggtcttgggatcattgggat	IDT	This Paper
Human *RNASEH2A* RT-qPCR Forward gctgaaagtggcagactcaa	IDT	This Paper
Human *RNASEH2A* RT-qPCR Reverse caggttgtatttgacccgcc	IDT	This Paper
Human *RNASEH1* RT-qPCR Forward aggaatcggcgtttactggg	IDT	This Paper
Human *RNASEH1* RT-qPCR Reverse aggctgcatgaatttccgc	IDT	This Paper
Human *DDI1* RT-qPCR Forward tggaacacaacgtgctacct	IDT	Kottemann et al.
Human *DDI1* RT-qPCR Reverse atctgtctgggggctgtct	IDT	Kottemann et al.
Human *DDI2* RT-qPCR Forward cgatgtagtgtgtgtgtactgc	IDT	Kottemann et al.
Human *DDI2* RT-qPCR Reverse ccagtgaggtagattctttaccactt	IDT	Kottemann et al.
Human *GAPDH* RT-qPCR Forward ggtcggagtcaacggattt	IDT	This Paper
Human *GAPDH* RT-qPCR Reverse gccccacttgattttggag	IDT	This Paper
Mouse b-actin RT-qPCR Forward ctaaggccaaccgtgaaaag	IDT	Thongthip et al.
Mouse b-actin RT-qPCR Reverse accagaggcatacagggaca	IDT	Thongthip et al.
Human *PRIM1* Forward RT-qPCR gacagagcattgaaggagga	IDT	This Paper
Human *PRIM1* Reverse RT-qPCR cgtcttgaccacccttaca	IDT	This Paper
Human *PRIMPOL* Forward RT-qPCR ggcacttcagtagaaaccat	IDT	This Paper

Human *PRIMPOL* Reverse RT-qPCR cgccgaattcctcctttaat	IDT	This Paper

Gateway cloning primers		
Oligonucleotide	**VENDOR**	**IDENTIFIER**
attB human RNASEH2A Forward ggggacaagtttgtacaaaaaagcaggcttcatggatctcagcgagctgga	IDT	This Paper
attB human RNASEH2A Reverse ggggaccactttgtacaagaaagctgggtctagaggctggttgctgact	IDT	This Paper
attB mouse RTF2 Forward ggggacaagtttgtacaaaaaagcaggcttcatgggttgcgacggaggcac	IDT	This Paper
attB mouse RTF2 Reverse ggggaccactttgtacaagaaagctgggttcagaagcagtaggatgtgt	IDT	This Paper
attB human PRIM1 Forward ggggacaagtttgtacaaaaaagcaggcttcatggagacgtttgaccccac	IDT	This Paper
attB human PRIM1 Reverse ggggaccactttgtacaagaaagctgggtttatttctcaaggaaaattt	IDT	This Paper

Mutagenesis cloning primers		
Oligonucleotide	**VENDOR**	**IDENTIFIER**
human RNASEH2B_F300A;F301A Forward ccaatttttttttattttttaccccagcagcggtatcaatactttttcattccactcttgtcaactttagcca	IDT	This Paper
human RNASEH2B_F300A;F301A Reverse tggctaaagttgacaagagtggaatgaaaagtattgataccgctgctggggtaaaaaataaaaaaaaaattgg	IDT	This Paper
human RNASEH2A_D34A cctgggcgtcgctgaggcgggca	IDT	This Paper
human RNASEH2A_D169A caaggccaaagcagctgccctctacccgg	IDT	This Paper
human RNASEH2A_P40D cgggcaggggcgacgtgctgggcc	IDT	This Paper
human RNASEH2A_R210A actgattatggctcaggcgccccccaatgatcccaagac	IDT	This Paper

CRISPR cloning primers		
Oligonucleotide	**VENDOR**	**IDENTIFIER**
RTF2 5'BamHI aaaaggatcccatgggttgcgacgggggga	IDT	This paper
RTF2 3'NotI aaaagcggccgctcagaagcagtaggacgtgtgg	IDT	This paper
hRTF 5' UTR Fwd INFUSION accatgattacgccaagcttactcttggaacgggcatggc	IDT	This paper
hRTF 5' UTR Rev INFUSION tcctcgcccttgctcaccatcgcaggacgggagtcagagc	IDT	This paper

GFP Fwd INFUSION atggtgagcaagggcgagga	IDT	This paper
hRTF rev INF gagcggtggcagtgcgggcttcagaagcagtaggacgtgt	IDT	This paper
hRTF 3' UTR Fwd INF agcccgcactgccaccgctc	IDT	This paper
hRTF 3' UTR Rev INF aacgacggccagtgaattctaacttataggcagataaaat	IDT	This paper
mouse sgTrp53 exon 5 Fwd caccgaagtcacagcacatgacgg	IDT	This paper
mouse sgTrp53 exon 5 Rev aaacccgtcatgtgctgtgacttc	IDT	This paper

Supplementary table 4. siRNAs and shRNAs

Oligonucleotide	**VENDOR**	**IDENTIFIER**
Luciferase siRNA (siCtrl)	Thermo Fischer	12935146
hRNASEH1 siRNA 1 gggaaagaggugaucaacatt	Ambion	s48356
hRNASEH1 siRNA 2 cagacaguauguuuacgautt	Ambion	s48357
hRNASEH1 siRNA 3 cgggauuuauaggcaauatt	Ambion	s48358
hRNASEH2A siRNA 1 caaugaucccaagacaaatt	Ambion	s20656
hRNASEH2A siRNA 2 ccaccgauauuuccuggaatt	Ambion	s20657
hRTF2 siRNA 1 caaagaugccgucauugaatt	Ambion	s226737
hPrim1 siRNA 1 gaaccagagauuauaagaatt	Ambion	s11050
hPrim1 siRNA 2 caucgucucuggguauauutt	Ambion	s11051
hPrim1 siRNA 3 caacuacgguggagugauatt	Ambion	s10052
hPrimpol siRNA 1 ggcuauugauagaguuaaatt	Ambion	s11053
hPrimpol siRNA 2 ccacgaagaagagaucauatt	Ambion	s11054
hPrimpol siRNA 3 ggaucuccuucgauuuagatt	Ambion	s11055
hDDI1 siRNA1 ccggagacaucaauguuccaucgat	ThermoFisher	HSS181016
hDDI1 siRNA 2 ggaaauuacacauucagucauggat	ThermoFisher	HSS140552
hDDI1 siRNA 3 ccggagacaucaauguuccaucgat	ThermoFisher	HSS140553
hDDI2 shRNA uggaaaucgauacagcuca	Open Biosystems	V3LHS_328065
mRNASEH2A shRNA #1 ccgggctcgattacaacagcactttctcgagaaagtgctgttgtaatcgagcttttttg	MilliporeSigma	TRC0000119585
mRNASEH2A shRNA #2 ccggcgggtcgttgtcgtctgagttctcgagaactcagacgacaacgaccgtttttg	MilliporeSigma	TRC0000119584

Supplementary Table 5. Plasmids

Plasmid	VENDOR	IDENTIFIER
Gateway Entry Vectors		
pDONOR233; spectinomycin resistant; Gateway	Hill and Vidal Labs	pDONOR233
pENTR223-EV; spectinomycin resistant; Gateway	Smogorzewska Lab	AS183
pENTR223-mRTF2; spectinomycin resistant; Gateway	This paper	BC153
pENTR223-RNASEH2A; spectinomycin resistant; Gateway	This paper	NJB2
pENTR223-RNASEH2A^{CD}_D34A;D169A; spectinomycin; Gateway	This paper	NJB6
pENTR223-RNASEH2A^{IOF}_P40D;Y210A; spectinomycin; Gateway	This paper	NJB11
pENTR223-RNASEH2B; spectinomycin resistant; Gateway	This paper	NJB27
pENTR223-RNASEH2B^{PIPm}_ F300A;F301A; spectinomycin resistant; Gateway	This paper	NJB29
pENTR223-hPRIM1; spectinomycin resistant; Gateway	This paper	CB35
Retroviral expression vectors		
PEA59-GFP-EV-dest (destination vector); chloramphenicol and ampicillin resistant; Gateway	Smogorzewska Lab	AS769
PEA59-GFP-EV-puro; ampicillin resistant; retroviral; Gateway	Smogorzewska Lab	AS1050
PEA59-GFP-mRTF2-puro; retroviral; ampicillin resistant; Gateway	This paper	BC123
pMSCVpuro-DEST (destination vector); chloramphenicol and ampicillin resistant; Gateway	Addgene	Plasmid# 119745
pMSCVpuro-EV; ampicillin; Gateway	This paper	NJB22
pMSCVpuro-RNASEH2A; ampicillin; Gateway	This paper	NJB18
pMSCVpuro-RNASEH2A-siRNA Resistant; ampicillin; Gateway	This paper	NJB19
pMSCVpuro-RNASEH2A^{CD}_D34A;D169A -siRNA Resistant; ampicillin; Gateway	This paper	NJB20
pMSCVpuro- RNASEH2A^{IOF}_P40D;Y210A-siRNA Resistant; ampicillin; Gateway	This paper	NJB20
pMSCV_PM_shRNA_Control_puro	Elledge lab	BC75
pMSCV_PM_shRNA_shDDI2_puro	Smogorzewska Lab	MK59-63
pMSCV-GFP-H2B-hygro	Smogorzewska Lab	YK77
pEGFP-RNASEH2B; kanamycin resistant; Gateway	Addgene	Plasmid #108697
pMMP Hit & Run Cre; retroviral; self-excising	Livingston Lab	NA
pWZL Cre-hygro; retroviral	de Lange Lab	NA

pMSCV-HA-FLAG-Dest: destination vector; chloramphenicol and ampicillin resistant; Gateway	Smogorzewska Lab	AMS157
pMSCV-HA-FLAG-EV: ampicillin resistant; Gateway	Smogorzewska Lab	AMS184
pMSCV-HA-FLAG-hPRIM1: ampicillin resistant; Gateway	This paper	CB36
Lentiviral expression vectors		
pLKO.1 shRNASEH2A #1_puro; ampicillin resistant	MilliporeSigma	SHCLNG-NM_027187, TRC0000119585
pLKO.1 shRNASEH2A #2_puro; ampicillin resistant	MilliporeSigma	SHCLNG-NM_027187, TRC0000119584
pLKO.1 shRNA Control Plasmid puro; ampicillin resistant;	MilliporeSigma	SHC002
pLVpuro-CMV-N-EGFP; destination vector; chloramphenicol and ampicillin resistant; Gateway	Addgene	Plasmid #122848
pLVpuro-CMV-N-EGFP- RNASEH2B; ampicillin resistant; Gateway	This paper	PR3
pLVpuro-CMV-N-EGFP-RNASEH2B^{PIPm}_F300A;F301A; ampicillin resistant; Gateway	This paper	PR5
Other expression vectors		
ppyCAG_RNaseH1_WT; ampicillin resistant	Addgene	Plasmid #111906
ppyCAG_RNaseH1_D210N; ampicillin resistant	Addgene	Plasmid #11904
CRISPR generation of GFP-AID-RTF2 HEK 293Ts		
pcDNA5-FRT-TO-EGFP-AID	Addgene	Plasmid #80075
MLM3636	Addgene	Plasmid #43860
MLM3636 5' UTR_1 sequence acgctaggcgcggcgtagcg	This paper	BC201, 202
px330	Addgene	Plasmid #42230
px330 3'UTR_1 atgtgaggcgtgtcggttcc	This paper	BC204, 206
pUC19-5UTR-GFP-AID-hRTF2-3UTR Homology Donor Construct	This paper	BC219
CRISPR generation of p53-/-;Rtf2$^{+/lox}$ and p53-/-;Rtf2$^{-/lox}$ MEFs		
pSpCas9(BB)-2A-Puro (PX459) V2.0	Addgene	Plasmid #62988
pX459-sgTRP53	This paper	NJB92
Protein Biochemistry		
PSKA002 HIS14-SUMO-MCS Expression Vector	Klinge Lab	
PSKA002 HIS14-SUMO-RTF2	This paper	BC142

PSKA008 HIS14-GFP-MCS-Expression Vector	Klinge Lab	
PSKA008 HIS14-GFP-RTF2	This paper	BC138
pGEX6P1-hsRNASEH2BCA	Addgene	Plasmid #108692
Protein Biochemistry		
VSV-G; retroviral packaging	Mulligan lab	AMS166
Gagpol; retroviral packaging	Mulligan lab	AMS167
pMD2.G (VSV-G envelope expressing plasmid); lentiviral packaging	Addgene	Plasmid #12259
psPAX2; lentiviral packaging	Addgene	Plasmid #12260

Supplementary Table 6. Antibodies

ANTIBODY	SOURCE	IDENTIFIER	LOT (if known)
Primary Antibodies			
Mouse IgG	Santa Cruz	Cat# sc-2025, RRID:AB_737182	D2022
Rabbit IgG	Santa Cruz	Cat# sc-2027, RRID:AB_737197	
Rabbit IgG	Cell Signaling Technology	Cat# 2729, RRID:AB_1031062	10
Mouse monoclonal anti-α-tubulin (clone DM1A), WB:1:5000	MilliporeSigma	Cat# T9026, RRID:AB_477593	0000137585
Mouse monoclonal anti-γH2AX Ser139 (clone JBW301), IF 1:2000	MilliporeSigma	Cat# 05-636, RRID:AB_309864	3782118
Mouse monoclonal anti-biotin, IF: 1:2000	Jackson ImmunoResearch	Cat# 200-002-211, RRID:AB_2339006	151728
Rabbit monoclonal anti-biotin, IF: 1:2000	Bethyl	A150-109A	11
Mouse monoclonal anti-BrdU (B44), combing: 1:10	BD Biosciences	Cat# 347580, RRID:AB_400326	9172603
Mouse monoclonal anti-Poly (ADP-Ribose) Polymer antibody [10H], WB:1:100	Abcam	Cat # ab14459, RRID:AB_301239	
Mouse monoclonal anti-PCNA (PC10), WB: 1:1000	Santa Cruz	Cat# sc-56, RRID:AB_628110	K1121
Mouse Monoclonal anti-vinculin, Unconjugated, Clone hVIN-1	MilliporeSigma	Cat# V9131, RRID:AB_477629	018M4779V
Rat monoclonal anti-BrdU [BU1/75 (ICR1)], combing: 1:20	Abcam	Cat# ab6326, RRID:AB_305426	
Rabbit monoclonal anti-MCM7 (D10A11) XP, WB: 1:1000	Cell Signaling Technology	Cat# 3735S, RRID:AB_2142705	3
Rabbit polyclonal anti-c20orf43 (RTF2), WB: 1:500	Novus	Cat# NBP2-30645	R72074
Rabbit polyclonal anti-RTF2, WB: 1:500	Proteintech	Cat# 16633-1-AP, RRID: AB_2256547	00074586

Mouse monoclonal anti-RTF2 (clone OTI1E8), WB: 1:1000	LS Bio	Cat# LS-C340588	75213
Rabbit polyclonal anti-GFP	Smogorzewska Lab	Kotteman et al.	
Rabbit polyclonal anti-GFP	Abcam	Cat# ab290 RRIDL AB_303395	GR3251545-1
Mouse monoclonal Anti-GFP, WB: 1:3000	Roche	Cat# 11814460001	
Rabbit polyclonal anti-RNASEH2A, WB: 1:500	Proteintech	Cat# 16132-1-AP, RRID:AB_2269729	00023264
Rabbit polyclonal anti-RNASEH2A, WB: 1:500	Abcam	Cat# ab83943, RRID:AB_1861175	GR3212381-12
Mouse monoclonal anti-RNASEH2A, WB:1:500	Santa Cruz	Cat# sc-515475	
Rabbit polyclonal anti-RNASEH2C, WB: 1:500	AbClonal	Cat# A13884, RRID:AB_2760737	0067820201
Rabbit polyclonal anti-RNASEH2C, WB: 1:500	Abcam	Cat# ab89726, RRID:AB_2042815	GR74866-1
Rabbit polyclonal anti-RNASEH2B, WB: 1:1000	Thermo Fisher Scientific	Cat# PA5-59059, RRID:AB_2646610	V13088599
Rabbit polyclonal anti-RNASEH2B, WB: 1:1000	Atlas Antibodies	Cat# HPA041469, RRID:AB_2677496	000043725
Rabbit polyclonal anti-phospho-RPA32 (S4/8), WB:1:1000	Bethyl	Cat# A300-245A, RRID:AB_210547	7
Rabbit polyclonal anti-RPA32, WB 1:2000	Bethyl	Cat# A300-244A, RRID:AB_185548	3
Rabbit polyclonal anti-PRIM1, WB:1000	Proteintech	Cat # 10773-1-AP RRID:AB_2237549	00009142
Mouse monoclonal anti-HA.11 Epitope Tag, WB: 1:3000	BioLegend	Cat# 901514, RRID:AB_2565336	B272772

Mouse monoclonal anti-V5 tag antibody [SV5-PK1], WB: 1:5000	Abcam	(Abcam Cat# ab27671, RRID:AB_471093)	GR3337308-16
Sheep polyclonal anti-human RNase H2 complex, WB: 1:500	Jackson Lab		
Secondary Antibodies			
Goat Anti-Mouse IgG H&L Cross-Absorbed (Alexa Fluor® 488), IF:1:1000, combing:1:100	ThermoFisher	Cat# A-11001, RRID:AB_2534069	632115
Goat Anti-Mouse IgG H&L Cross-Absorbed (Alexa Fluor® 647), combing:1:100	ThermoFisher	Cat# A-21235, RRID:AB_2535804	1837146
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)	ThermoFisher	Cat# A-11008, RRID:AB_143165	645151
Goat Anti-Rat IgG H&L Cross-Absorbed (Alexa Fluor® 594), IF: 1:1000, combing: 1:100	ThermoFisher	Cat# A-11007, RRID:AB_10561522	2107787
Peroxidase-AffiniPure Goat Anti-Mouse IgG (H + L) antibody, WB: 1:5000	Jackson ImmunoResearch Labs	Cat# 115-035-003, RRID:AB_10015289	
Peroxidase-AffiniPure Donkey Anti-Sheep IgG (H + L) antibody, WB: 1:5000	Jackson ImmunoResearch Labs	Cat# 713-035-003, RRID:AB_2340709	
Peroxidase-AffiniPure Goat Anti-Rabbit IgG (H + L) antibody, WB: 1:5000	Jackson ImmunoResearch Labs	Cat# 115-035-144, RRID:AB_2307391	