SUPPLEMENTARY DATA

TRF2-mediated ORC recruitment underlies telomere stability upon DNA replication stress

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Supplementary Figure Legends

Supplementary Figure S1. Representative images of TRF2 (45-244)-LacI and ORC1 at *lacO* arrays in U2OS 2-6-3 cells.

U2OS 2–6-3 cells were transfected with HA-LacI or TRF2 (45-244) mutant-LacI expression vectors for 24 h and then double-immunostained with anti-LacI (red) and anti-ORC1 (green) antibodies, followed by DAPI staining. Representative images of two independent experiments are shown. White arrows indicate colocalization of ORC1 with the foci of LacI proteins. The colocalization frequencies are shown in Figure 1B. Scale bar, 10 μm.

Supplementary Figure S2. Source data and supplemental analysis of ChIP experiments.

(A) % of input values used for the calculation in Figure 3E are shown.

(B) The relative specific ChIP values (mean values; Fig. 3E) normalized to the TRF2/CBB values (mean values; Fig. 3D) are shown.

Supplementary Figure S3. The ratio of telomere-free micronuclei/nucleus in the HeLa clones.

In the experiments shown in Figure 4D, the number of telomere-free micronuclei were also counted. The means ± SDs are shown (n = 3). n.s., not significant (two-tailed Student's t-test).

Supplementary Figure S4. Co-localization frequencies of wild-type and L229A ORC1 with TRF2ΔMyb-LacI.

After co-transfection with the indicated ORC1 and LacI expression vectors, U2OS 2-6-3 cells were subjected to co-immunostaining with anti-LacI and anti-FLAG antibodies. Co-localization frequency was examined. The values represent the score from two independent experiments. [a] Total number of LacI foci. [b] Number of LacI foci co-localizing with FLAG foci. [c] Co-localization frequency of FLAG foci with LacI foci. [d] Results of the χ^2 test in comparison with HA-LacI. ***, $P < 0.001$.

TRF2ΔMyb, consisting of the amino acids 1-445 but lacking the Myb domain, has been reported to recruit ORC1 to the *lacO* array when fused to LacI (Higa et al. 2017 BBA Mol Cell Res 1864: 191-201). The data presented show that the co-localization frequency of ORC1 (L229A)-3×FLAG with TRF2ΔMyb-LacI was ~48%, which was comparable to that of wild-type ORC1-3×FLAG (~45%).

Supplementary Figure S5. Overexpression of FLAG-ORC1 (244-511) fragment reduces ORC1-3xFLAG co-immunoprecipitation with HA-TRF2.

The signal intensities of the bands shown in Figure 6A were quantitated and relative ORC1-3xFLAG co-immunoprecipitation values were calculated by normalizing the signals of co-precipitated ORC1-3xFLAG to those of the precipitated TRF2-LacI. The mean and the individual data from two independent experiments are shown with the value with the empty vector set as 1.

Supplementary Figure S6. Overexpression of FLAG-ORC1 (244-511) fragment does not affect co-immunoprecipitation of RAP1 and SLX4 with HA-TRF2.

(A) At 42 h post-transfection with the indicated vectors, HCT116 cells were harvested and immunoprecipitated with an anti-RAP1 antibody or normal rabbit IgG (Control IP). IPs and 3% of input were analyzed by immunoblotting with the indicated antibodies. The representative data of two independent experiments were shown.

(B) At 42 h post-transfection with the indicated vectors, HEK293T cells were harvested and

immunoprecipitated with an anti-HA antibody or normal rat IgG (Control IP). IPs and 0.5% of input were analyzed by immunoblotting with the indicated antibodies. The representative data of two independent experiments were shown.

Supplementary Figure S7. Re-replication induced by co-expression of Cdt1 + ORC1 is dependent on the ORC1 Walker B motif.

HEK293T cells were co-transfected with a mixture of the expression vectors (T7-Cdt, ORC1 (WT)-3×FLAG, and ORC1 (D620A)-3×FLAG) or their empty vectors (-), as indicated for 48 h. *Top,* DNA content was analyzed by flow cytometry. The percentage of re-replicated cells was calculated as in Figure 6D. *Bottom,* whole-cell lysates were subjected to immunoblotting with the indicated antibodies. CBB staining serves as the loading control. The results of two independent experiments are shown. These data show that the disruption of ORC1 Walker B motif (D620A) abolishes re-replication induced by co-expression of Cdt1 + ORC1.

Supplementary Figure S8. Overexpression of an HA-ORC1 (244-411) fragment does not affect HeLa cell proliferation.

(A) HeLa cells stably expressing HA-ORC1 (244-411) fragment were established by retroviral infection. Expression of the introduced protein was analyzed by immunoblotting with anti-HA antibody. CBB serves as a loading control. **(B)** The cell growth was investigated for five days. The means ± SDs are shown (n = 2). **(C)** The cell cycle was analyzed by flow cytometry. The means ±SDs are shown (n = 2).

Supplementary Figure S9. Source data of ChIP experiments.

% of input values used for the calculation in Figure 7B are shown.

Supplementary Figure S10. Schematics of ORC1 domain architecture showing the predicted intrinsically disordered region.

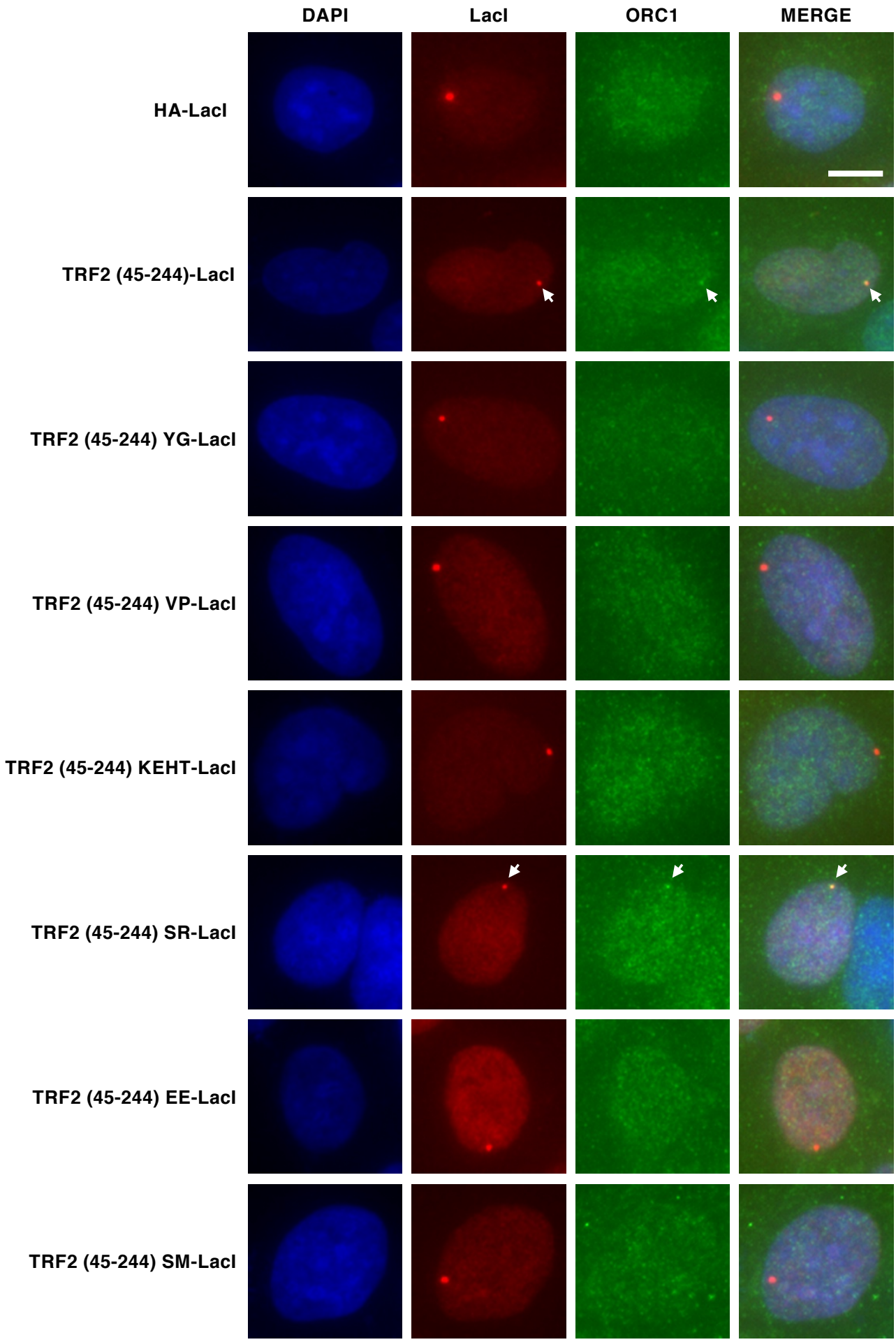
(A-C) Intrinsically disordered region in ORC1 was predicted by (A) PrDOS (Ishida and

Kinoshita, 2007) or (B) IUPred2A (Mészáros *et al*., 2018). Predicted disorder probability is plotted. Red horizontal line represents the disorder/order threshold (a cutoff value 0.5) and residues scored above the line are predicted to be disordered. **(C)** Schematics of the intrinsically disordered region in ORC1. Purple rectangles represent overlapping regions of disorder predicted by each server. **(D)** Schematics of the full-length human ORC and ORC1 (244-511) fragments used in this study. BAH, bromo adjacent homology domain; AAA+, ATPases associated with diverse cellular activities domain; WH, winged-helix domain.

References:

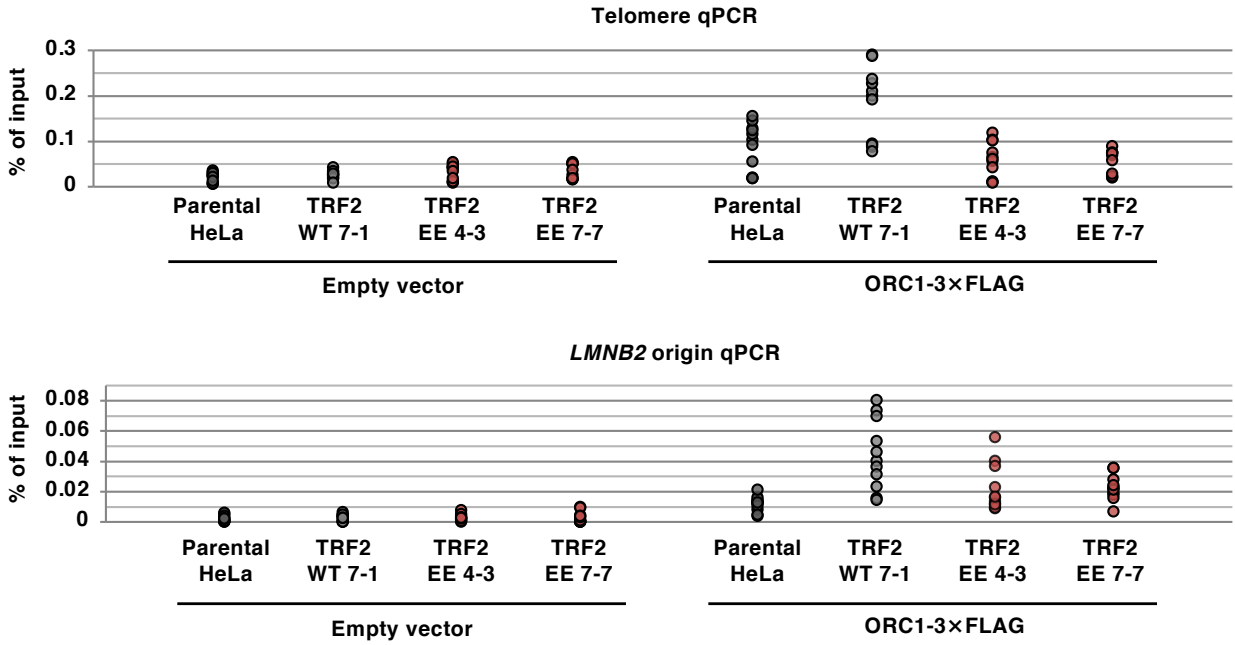
1. T. Ishida and K. Kinoshita (2007) PrDOS: Prediction of disordered protein regions from amino acid sequence. Nucleic Acids Res. 35: W460-W464.

2. B. Mészáros, G. Erdős, and Z. Dosztányi (2018) IUPred2A: Context-dependent prediction of protein disorder as a function of redox state and protein binding. Nucleic Acids Res. 46: W329–W337.



Supplementary Figure S1. Higa et al.

A



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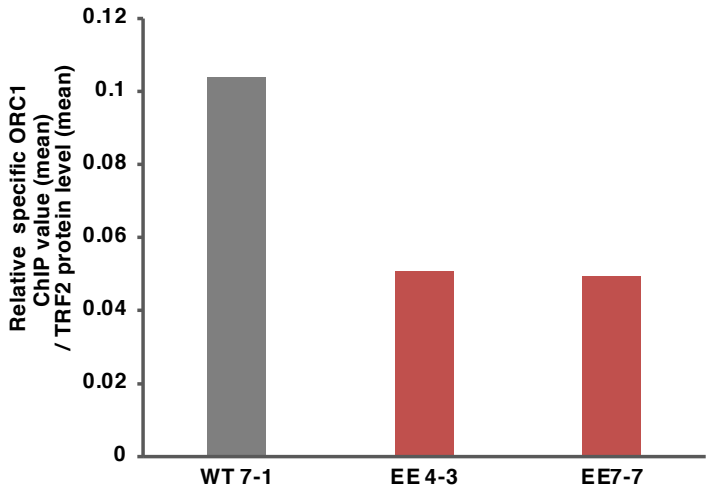
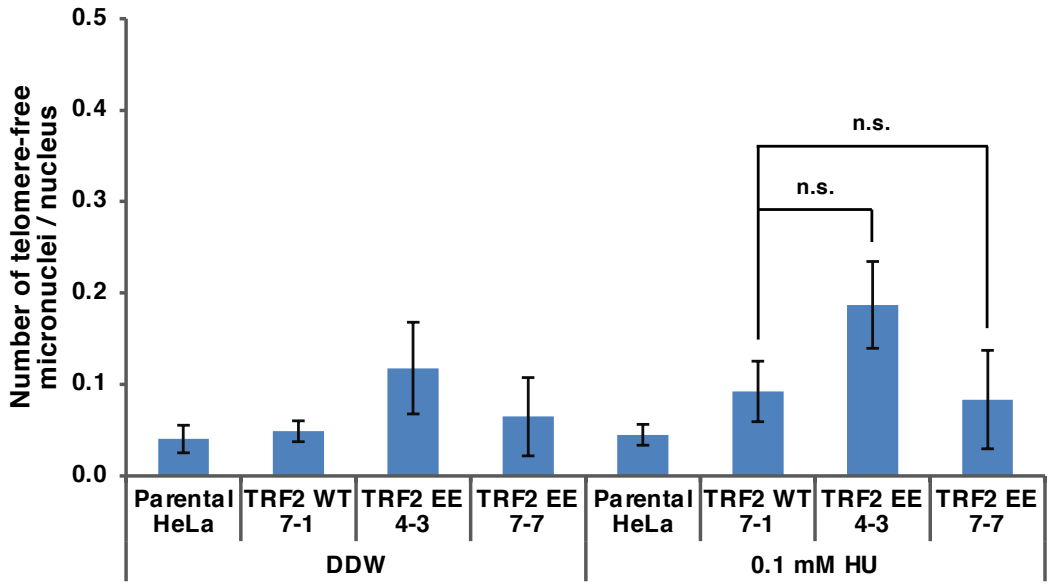


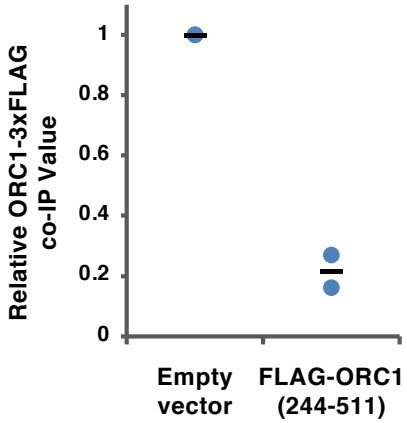
Supplementary Figure S2. Higa et al.



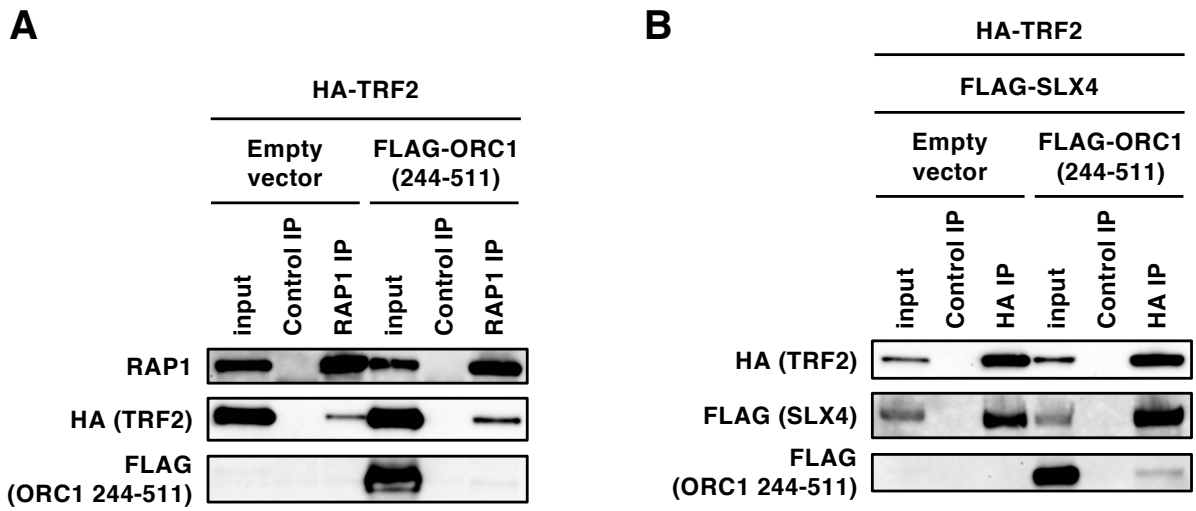
Supplementary Figure S3. Higa et al.

Prey	Bait	LacI foci [a]	Co-loc. foci [b]	Co-loc. freq. (%) [c]	χ^2-test [d]
ORC1-3xFLAG	HA-LacI	198	4	2.0	
	TRF2 ΔMyb-LacI	172	78	45.3	***
ORC1 (L229A)-3xFLAG	HA-LacI	145	6	4.1	
	TRF2 ΔMyb-LacI	154	72	47.8	***

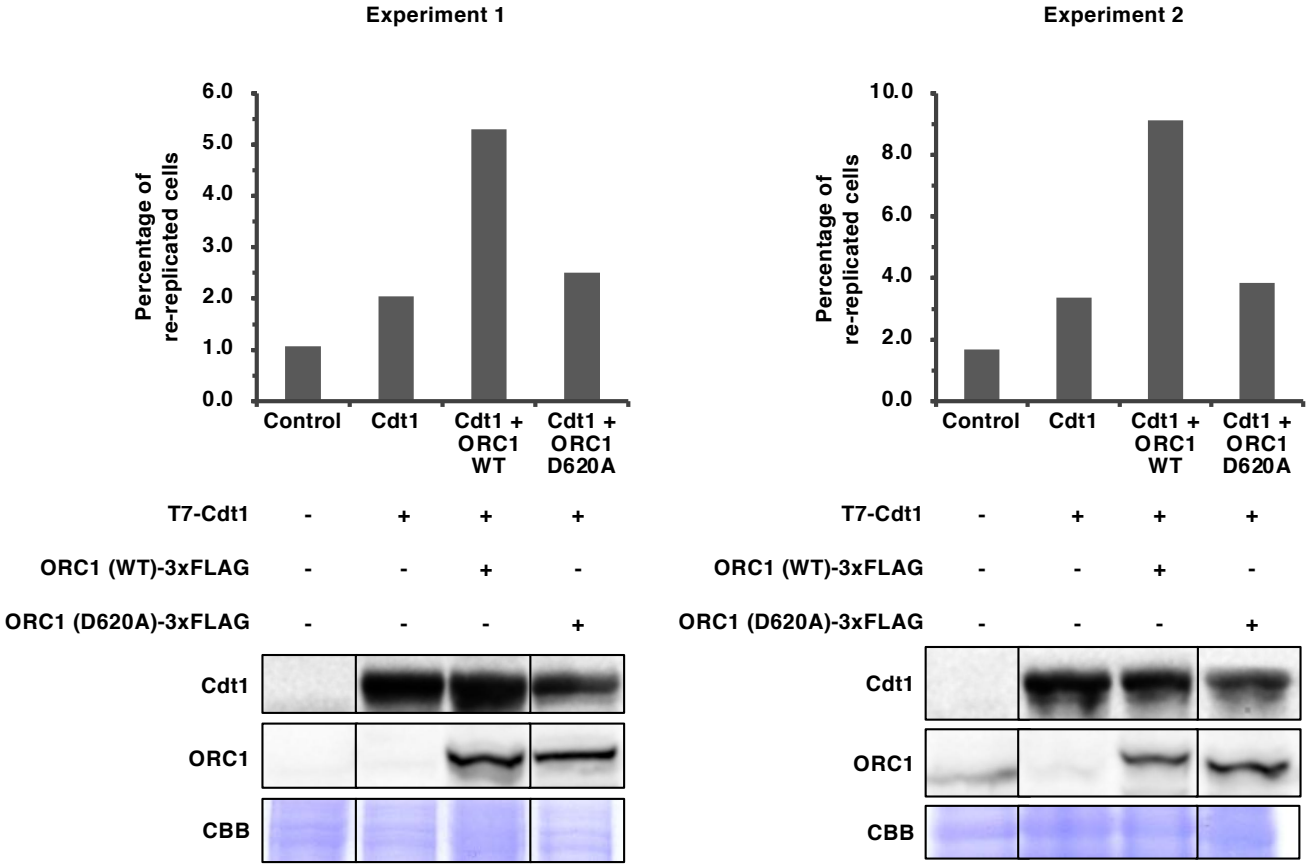
Supplementary Figure S4. Higa et al.



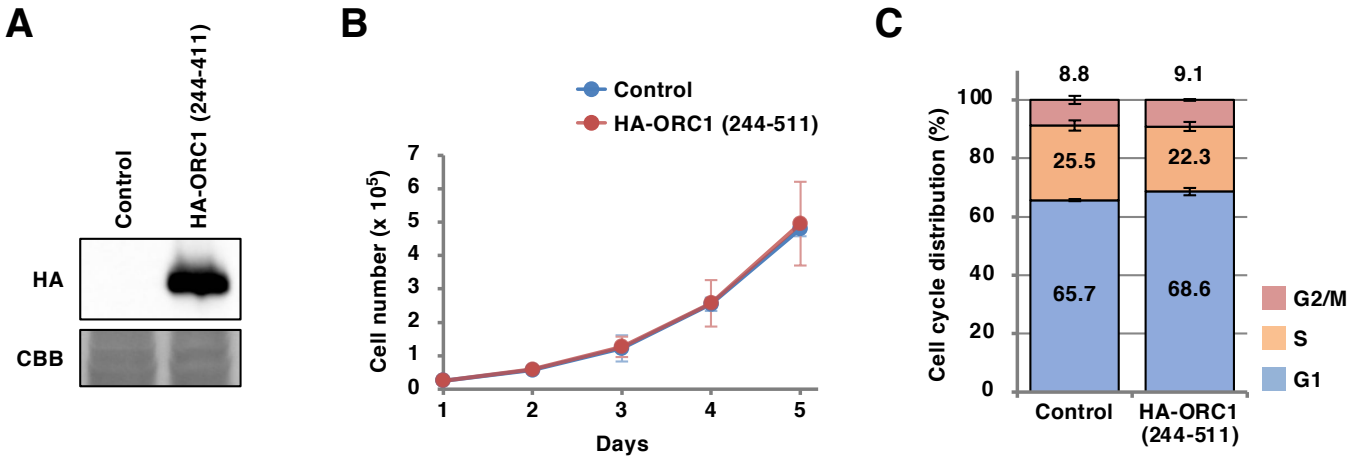
Supplementary Figure S5. Higa et al.



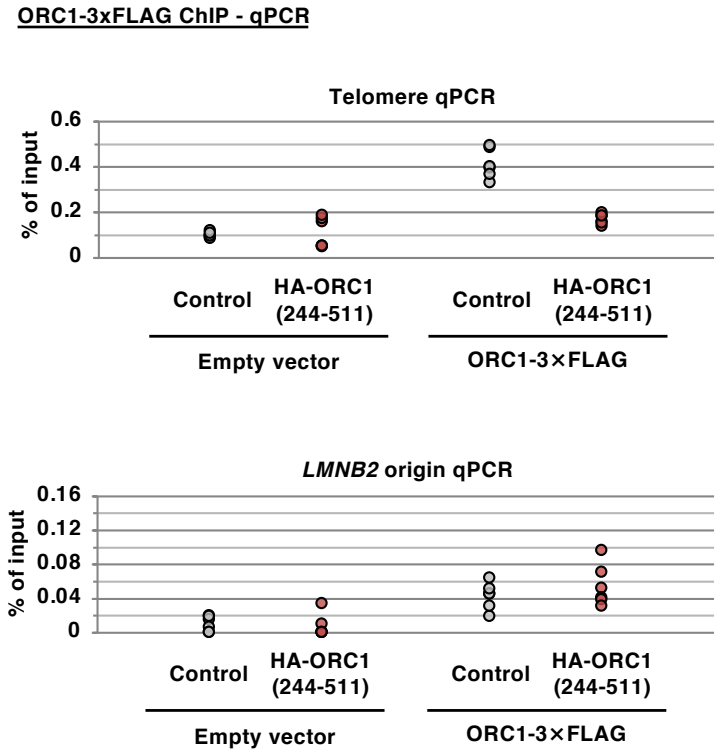
Supplementary Figure S6. Higa et al.



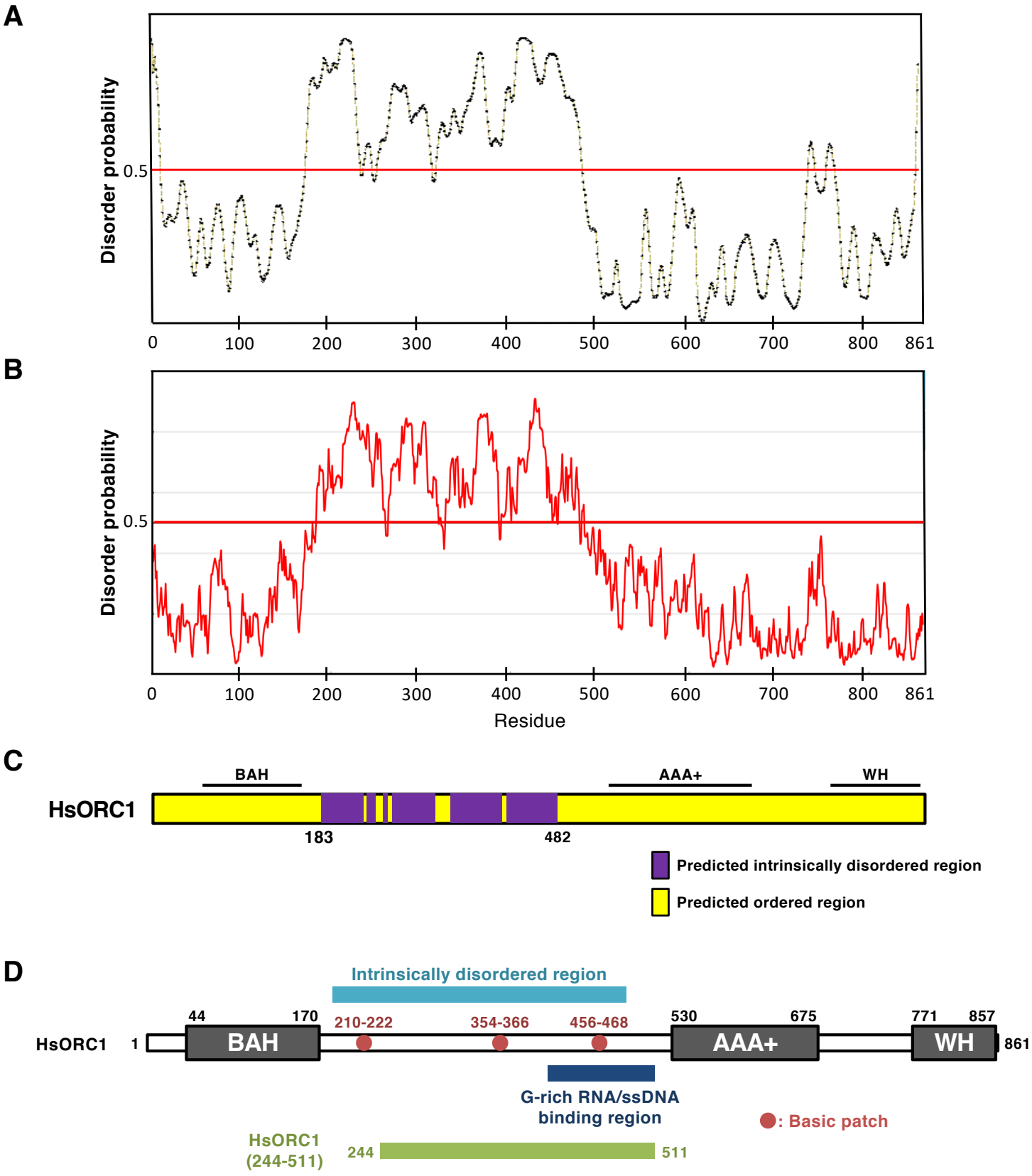
Supplementary Figure S7. Higa et al.



Supplementary Figure S8. Higa et al.



Supplementary Figure S9. Higa et al.



Supplementary Figure S10. Higa et al.

Supplementary Table S1. Compliance of qPCR experiments with the MIQE guidelines.

ITEM TO CHECK	IMPORTANCE	CHECKLIST	COMMENTS
EXPERIMENTAL DESIGN			
Definition of experimental and control groups	**E**	✓	**Figs 3E, 7B, S2 and S9: Experimental groups:** ORC1-3×FLAG ChIP; **Control groups:** empty vector ChIP. **Figs 3H and 7C: Experimental groups:** anti-MCM7 ChIP; **Control groups:** Rabbit IgG ChIP.
Number within each group	**E**	✓	**Figs 3E and S2:** n=9; **Fig 3H:** n=4; **Figs 7B, C, and S9:** n=6.
Assay carried out by core lab or investigator's lab?	D	✓	Investigator's lab
Acknowledgement of authors' contributions	D	✓	MH, YM, and JF performed qPCR.
SAMPLE			
Description	**E**	✓	Tissue culture cells
Volume/mass of sample processed	D	✓	6×10^6 cells / antibody
Microdissection or macrodissection	**E**	✓	Not applicable
Processing procedure	**E**	✓	Not applicable
If frozen - how and how quickly?	**E**	✓	Not applicable
If fixed - with what, how quickly?	**E**	✓	Not applicable
Sample storage conditions and duration (especially for FFPE samples)	**E**	✓	Not applicable
NUCLEIC ACID EXTRACTION			
Procedure and/or instrumentation	**E**	✓	After formaldehyde reversal, the samples were serially treated with DNase-free RNase A (final 5 µg, Invitrogen) and Proteinase K (final 50 µg/ml, Roche). The DNA was phenol:chloroform (Nacalai tesque) extracted, ethanol (Nacalai tesque) precipitated in the presence of 1 µl of glycogen (Nacalai tesque), and dissolved in Tris-EDTA buffer.
Name of kit and details of any modifications	**E**	✓	We did not use any kit.
Source of additional reagents used	D	✓	Not applicable
Details of DNase or RNAse treatment	**E**	✓	RNase A (5 µg, Invitrogen) was added and incubated at 37°C for 30 min.
Contamination assessment (DNA or RNA)	**E**	✓	Not performed
Nucleic acid quantification	**E**	✓	Using absorbance at 260 nm
Instrument and method	**E**	✓	Nanodrop (Thermo Scientific)
Purity (A260/A280)	D	✓	1.8 - 1.9
Yield	D	✓	The mean yield of input DNA was 170 µg/ml.
RNA integrity method/instrument	**E**	✓	Not applicable
RIN/RQI or Cq of 3' and 5' transcripts	**E**	✓	Not applicable
Electrophoresis traces	D	✓	Not applicable
Inhibition testing (Cq dilutions, spike or other)	**E**	✓	Not applicable
REVERSE TRANSCRIPTION			
Complete reaction conditions	**E**	✓	Not applicable
Amount of RNA and reaction volume	**E**	✓	Not applicable
Priming oligonucleotide (if using GSP) and concentration	**E**	✓	Not applicable
Reverse transcriptase and concentration	**E**	✓	Not applicable
Temperature and time	**E**	✓	Not applicable
Manufacturer of reagents and catalogue numbers	D	✓	Not applicable
Cqs with and without RT	D	✓	Not applicable
Storage conditions of cDNA	D	✓	Not applicable
qPCR TARGET INFORMATION			
If multiplex, efficiency and LOD of each assay.	**E**	✓	Not applicable
Sequence accession number	**E**	✓	**LMNB2:** NM_032737.3
Location of amplicon	D	✓	See Cawthon, Nucleic Acids Research, 30: e47, 2002; Sugimoto et al, Nucleic Acids Research, 43: 5898-5911, 2015.
Amplicon length	**E**	✓	**Telomere:** 76 bp; **LMNB2 ori:** 232 bp.
In silico specificity screen (BLAST, etc)	**E**	✓	We confirmed all primers by BLAST.
Pseudogenes, retropseudogenes or other homologs?	D		
Sequence alignment	D		
Secondary structure analysis of amplicon	D		
Location of each primer by exon or intron (if applicable)	**E**	✓	Not applicable
What splice variants are targeted?	**E**	✓	Not applicable
qPCR OLIGONUCLEOTIDES			
Primer sequences	**E**	✓	**Telomere Forward:** GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT, **Reverse:** TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA; **LMNB2 ori Forward:** GGCTGGCATGGACTTTCATTTCAG, **Reverse:** GTGGAGGGATCTTTCTTAGACATC.

RTPrimerDB Identification Number	D	✓	Not applicable
Probe sequences	D	✓	Not applicable
Location and identity of any modifications	**E**	✓	Not applicable
Manufacturer of oligonucleotides	D	✓	**Telomere:** SIGMA; **LMNB2 ori:** FASMAC
Purification method	D	✓	**Telomere:** OPC purification; **LMNB2 ori:** Reversed-phase chromatography
qPCR PROTOCOL			
Complete reaction conditions	**E**	✓	PCR reactions were performed in a CFX96 Touch Real-Time PCR Detection System (BIO-RAD) using SYBR or TB Green Premix EX Taq II (Takara) in final volume of 25 µl. Reaction mix consisted of sterile H_2O MilliQ, 12.5 µl 2× SYBR or TB Green Premix EX Taq II, 1 µl 5 µM each forward and reverse primers and 1 µl ChIPed DNA.
Reaction volume and amount of cDNA/DNA	**E**	✓	Reaction volume: 25 µl; amount of ChIPed DNA: 1 µl
Primer, (probe), Mg++ and dNTP concentrations	**E**	✓	200 nM primers
Polymerase identity and concentration	**E**	✓	Manufacturer's proprietary
Buffer/kit identity and manufacturer	**E**	✓	SYBR or TB Green Premix EX Taq II (Takara)
Exact chemical constitution of the buffer	D	✓	Manufacturer's proprietary
Additives (SYBR Green I, DMSO, etc.)	**E**	✓	No additives
Manufacturer of plates/tubes and catalog number	D	✓	96-well plates (SKPCR96C) and PCR String Caps (SKPCR8FC), both provided by SEIKO Co. LTD (Japan).
Complete thermocycling parameters	**E**	✓	**Telomere:** 30 sec at 95 °C; and 40 cycles at 95 °C for 15 sec and at 54 °C for 2 min. **LMNB2 ori:** 1 min at 95 °C; five cycles at 95 °C for 30 sec, at 66.9 °C for 30 sec, and at 72 °C for 30 sec, five cycles at 95 °C for 30 sec, at 64.9 °C for 30 sec, and at 72 °C for 30 sec, and 50 cycles at 95 °C for 30 sec, at 62.9 °C for 30 sec, and at 72 °C for 30 sec.
Reaction setup (manual/robotic)	D	✓	Manual setup
Manufacturer of qPCR instrument	**E**	✓	CFX96 Touch Real-Time PCR Detection System (BIO-RAD)
qPCR VALIDATION			
Evidence of optimisation (from gradients)	D	✓	None
Specificity (gel, sequence, melt, or digest)	**E**	✓	Melting curve analysis, ramping from 72 °C to 95 °C in step of 0.5 °C, where fluorescence data are measured continuously. Specific amplification was confirmed in 3% agarose gel electrophoresis stained with ethidium bromide. No template controls were run for each amplicon to detect unspecific amplification and primer dimerization.
For SYBR Green I, Cq of the NTC	**E**	✓	**Telomere:** > 25; **LMNB2 ori:** > 40 or no amplification
Standard curves with slope and y-intercept	**E**	✓	Representative values are shown. **Telomere:** y = -3.317x + 18.583 **LMNB2 ori:** y = -3.449x + 18.262
PCR efficiency calculated from slope	**E**	✓	**Telomere:** 79.9 - 155.2%; **LMNB2 ori:** 76.8 - 125.9%
Confidence interval for PCR efficiency or standard error	D		
r2 of standard curve	**E**	✓	**Telomere:** 0.807 - 1.0; **LMNB2 ori:** 0.984 - 1.0
Linear dynamic range	**E**	✓	The Cq values of unknown samples fell within the linear range.
Cq variation at lower limit	**E**	✓	Not performed
Confidence intervals throughout range	D	✓	Not performed
Evidence for limit of detection	**E**	✓	Not performed
If multiplex, efficiency and LOD of each assay.	**E**	✓	Not applicable
DATA ANALYSIS			
qPCR analysis program (source, version)	**E**	✓	CFX Manager Software (BIO-RAD), version 3.1
Cq method determination	**E**	✓	The CFX software Single Threshold mode was used to determine Cq for each locus.
Outlier identification and disposition	**E**	✓	None of the Cq values was discarded.
Results of NTCs	**E**	✓	**Telomere:** > 25; **LMNB2 ori:** > 40 or no amplification
Justification of number and choice of reference genes	**E**	✓	Not applicable
Description of normalisation method	**E**	✓	Standard curve quantification
Number and concordance of biological replicates	D	✓	**Figs 3E and S2:** n=3; **Fig 3H:** n=4; **Figs 7B, C and S9**: n=2
Number and stage (RT or qPCR) of technical replicates	**E**	✓	qPCR reactions were perfomed in triplicate.
Repeatability (intra-assay variation)	**E**	✓	Standard deviation of replicates: **Experimental groups:** 0.019 - 1.643 Cq; **Control groups:** 0.016 - 10.955 Cq
Reproducibility (inter-assay variation, %CV)	D		
Power analysis	D		
Statistical methods for result significance	**E**	✓	Two-tailed Student's t-test
Software (source, version)	**E**	✓	Microsoft Excel for Mac, version 16.43
Cq or raw data submission using RDML	D		

E: essential information; D: desirable information.

Fig1_Source_data

Figure 1B	Experiment 1			Experiment 2		
ORC1	# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.
HA-LacI	94	6	6.4	88	1	1.1
TRF2 (45-244)-LacI	64	24	37.5	88	29	33.0
TRF2 (45-244) YG-LacI	65	8	12.3	127	19	15.0
TRF2 (45-244) VP-LacI	65	9	13.8	93	10	10.8
TRF2 (45-244) KEHT-LacI	61	16	26.2	148	25	16.7
TRF2 (45-244) SR-LacI	59	27	45.8	150	39	26.0
TRF2 (45-244) EE-LacI	64	5	7.8	138	7	5.0
TRF2 (45-244) SM-LacI	70	13	18.8	121	11	9.1

Figure 1C	Experiment 1			Experiment 2		
ORC2	# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.
HA-LacI	102	0	0	96	4	4.2
TRF2 (45-244)-LacI	72	9	12.5	66	12	18.2
TRF2 (45-244) EE-LacI	75	2	2.7	80	5	6.3
ORC3	# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.
HA-LacI	69	5	7.2	85	4	4.7
TRF2 (45-244)-LacI	69	15	21.7	79	17	21.5
TRF2 (45-244) EE-LacI	72	5	6.9	76	9	11.8

Figure 1D	Experiment 1	Experiment 2
GST	0.112	0.186
GST-TRF2 WT	1.000	1.000
GST-TRF2 YG	0.233	0.175
GST-TRF2 VP	0.063	0.230
GST-TRF2 EE	0.216	0.251
GST-TRF2 SM	0.400	0.183

Figure 1E	Experiment 1	Experiment 2
HA-TRF2	1.000	1.000
HA-TRF2 EE	0.013	0.310

Fig2_Source_data

Figure 2B	Experiment 1			Experiment 2			Experiment 3		
	# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.
HA-LacI	71	0	0.0	52	1	1.9	46	2	4.3
TRF2 (45-244)-LacI	58	35	60.3	61	51	83.6	30	30	100.0
TRF2 (45-244) YG-LacI				69	54	78.3	76	64	84.2
TRF2 (45-244) VP-LacI				64	48	75.0	81	68	84.0
TRF2 (45-244) EE-LacI	55	29	52.7	35	30	85.7	83	73	88.0
TRF2 (45-244) SM-LacI				81	63	77.8	73	64	87.7

Figure 2E	Experiment 1	Experiment 2
HA-TRF2 WT	1.000	1.000
HA-TRF2 YG	0.157	0.502
HA-TRF2 VP	0.086	0.073
HA-TRF2 EE	0.225	0.579
HA-TRF2 SM	0.867	0.881

Fig3_Source_data

Figure 3E	1	2	3	4	5	6	7	8	9
Parental HeLa	7.4972	8.7753	9.9652	7.6438	9.3334	10.1390	1.8399	2.1614	1.8486
TRF2 WT 7-1	4.1533	4.3894	4.8020	3.6273	3.5761	2.8797	3.8329	4.2363	4.0794
TRF2 EE 4-3	2.1040	3.0287	1.2714	1.7510	1.3854	1.3354	0.0000	0.1098	0.0000
TRF2 EE 7-7	1.4140	1.7200	1.7182	1.5237	0.9500	0.9214	0.1417	0.2378	0.2806

Figure 3G	1			2			3		
	G1	S	G2/M	G1	S	G2/M	G1	S	G2/M
Parental HeLa	62.45	24.38	13.17	56.83	27.36	15.81	69.33	20.33	10.34
TRF2 WT 7-1	64.36	24.76	10.88	60.07	24.98	14.95	70.77	18.49	10.74
TRF2 EE 4-3	57.84	29.38	12.78	57.11	29.18	13.71	66.67	21.64	11.69
TRF2 EE 7-7	54	32.44	13.56	55.68	28.21	16.11	64.39	25.09	10.53

Figure 3H		1	2	3	4
IgG	Parental HeLa	0.003069	0.004394	0.010902	0.002736
	TRF2 WT 7-1	0.003873	0.003773	0.011045	0.006595
	TRF2 EE 4-3	0.006433	0.004397	0.004250	0.002143
	TRF2 EE 7-7	0.005340	0.002159	0.000890	0.004575
MCM7 IP	Parental HeLa	0.099381	0.088621	0.086882	0.077056
	TRF2 WT 7-1	0.105150	0.080518	0.069455	0.132258
	TRF2 EE 4-3	0.044869	0.070257	0.069518	0.043968
	TRF2 EE 7-7	0.060003	0.045300	0.043766	0.066415

Figure 3I		1	2	3	4
IgG	Parental HeLa	0.005945	0.002547	0.040813	0.075808
	TRF2 WT 7-1	0.000000	0.001086	0.050608	0.007092
	TRF2 EE 4-3	0.002298	0.007292	0.034525	0.023157
	TRF2 EE 7-7	0.010966	0.011411	0.042890	0.026624
MCM7 IP	Parental HeLa	0.076269	0.130012	0.145099	0.141218
	TRF2 WT 7-1	0.055259	0.077773	0.079508	0.142851
	TRF2 EE 4-3	0.044368	0.078830	0.088061	0.052661
	TRF2 EE 7-7	0.077290	0.098867	0.065466	0.067369

Fig4_Source_data

Figure 4B		Cells with ≥ 10 TIFs	
		1	2
Parental HeLa	DDW	0	0
	0.1 mM HU	1	3
TRF2 WT 7-1	DDW	7	4
	0.1 mM HU	4	12
TRF2 EE 4-3	DDW	1	7
	0.1 mM HU	12	15
TRF2 EE 7-7	DDW	7	7
	0.1 mM HU	14	17

Figure 4D		1	2	3
DDW	Parental HeLa	0.0515	0.1341	0.0906
	TRF2 WT 7-1	0.1373	0.0750	0.0383
	TRF2 EE 4-3	0.1051	0.1307	0.1594
	TRF2 EE 7-7	0.1309	0.0569	0.1327
0.1 mM HU	Parental HeLa	0.1555	0.2655	0.2059
	TRF2 WT 7-1	0.2849	0.2348	0.1484
	TRF2 EE 4-3	0.4640	0.3880	0.3418
	TRF2 EE 7-7	0.3761	0.3537	0.3271

Fig5_Source_data

Figure 5B		Experiment 1			Experiment 2			Experiment 3		
		# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.
HA-LacI	FLAG-ORC1 (2-511)	51	5	9.8	56	5	8.9	50	2	4.0
TRF2 (45-244)-LacI	FLAG-ORC1 (2-511)	43	30	70.0	31	24	77.4	38	28	73.7
	FLAG-ORC1 (2-325)	41	3	7.3				72	4	5.6
	FLAG-ORC1 (2-244)	40	4	10				79	2	2.5
	FLAG-ORC1 (2-85)	62	1	1.6				91	1	1.1
	FLAG-ORC1 (244-511)				63	52	82.5	62	60	96.8
	FLAG-ORC1 (325-511)				71	32	45.1	90	43	47.8

Figure 5D		Experiment 1			Experiment 2			Experiment 3		
		# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.
HA-LacI	FLAG-ORC1 (full-length)	137	13	9.5	90	5	5.6	91	12	13.2
TRF2 (45-244)-LacI	FLAG-ORC1 (full-length)	136	33	24.0	63	15	24.0	91	28	31.0
	FLAG-ORC1 (Δ326-510)	112	2	1.8	65	6	9.2	78	12	15.0
	FLAG-ORC1 (Δ386-510)	58	13	22.0	58	6	10.0	104	6	5.8
	FLAG-ORC1 (Δ411-510)				68	6	8.8	110	6	5.5
	FLAG-ORC1 (Δ446-510)	64	16	25.0	140	25	18.0			
	FLAG-ORC1 (Δ411-445)				125	18	14.0	113	19	17.0

Fig6_Source_data

Figure 6B		Experiment 1			Experiment 2		
	HA-ORC1 (244-511)	# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc foci	Co-loc. freq.
TRF2 (45-244)-LacI + FLAG-ORC1	-	99	40	40.4	107	28	26.2
	+	112	20	17.8	98	12	12.2

Figure 6E	1	2	3
Control	1.00	1.00	1.00
Cdt1	1.36	1.70	1.64
Cdt1 + ORC1	3.18	3.67	3.07
Cdt1 + ORC1 + FLAG-ORC1(244-511)	3.20	3.03	2.54

Fig7_Source_data

Figure 7B	1	2	3	4	5	6
Control	17.20	17.60	10.50	6.79	6.93	6.09
HA-ORC1(244-511)	0.00	0.18	0.41	2.66	3.05	3.98

Figure 7C		1	2	3	4	5	6
IgG	Control	0.006126	0.006405	0.006505	0.010926	0.012694	0.013055
	HA-ORC1 (244-511)	0.005103	0.004818	0.003669	0.002426	0.001971	0.001538
MCM7 IP	Control	0.098947	0.132881	0.127861	0.148822	0.103051	0.111091
	HA-ORC1 (244-511)	0.036151	0.047339	0.045867	0.048472	0.044881	0.044714

Figure 7D		1	2
IgG	Control	0.033255	0.114720
	HA-ORC1 (244-511)	0.025677	0.030497
MCM7 IP	Control	0.211553	0.458258
	HA-ORC1 (244-511)	0.198998	0.340009

Figure 7E		Cells with ≥ 5 TIFs		
		1	2	3
Control	DDW	0	0	1
	0.1 mM HU	2	1	1
HA-ORC1 (244-511)	DDW	0	0	1
	0.1 mM HU	5	4	4

FigS2_Source_data

Supplementary Figure S2A										
Telomere		1	2	3	4	5	6	7	8	9
Empty vector	Parental HeLa	0.023172	0.024908	0.022977	0.033663	0.029927	0.028749	0.004756	0.005121	0.004957
	TRF2 WT 7-1	0.041229	0.031583	0.033020	0.025275	0.024721	0.024082	0.020525	0.017232	0.017583
	TRF2 EE 4-3	0.040073	0.039101	0.041975	0.048434	0.051786	0.042468	0.007675	0.008522	0.009430
	TRF2 EE 7-7	0.024052	0.052235	0.050519	0.050002	0.047797	0.049040	0.015771	0.015629	0.014241
ORC1-3xFLAG	Parental HeLa	0.101396	0.114644	0.126977	0.123031	0.143423	0.153145	0.016492	0.018509	0.016546
	TRF2 WT 7-1	0.199148	0.208464	0.224745	0.289229	0.285493	0.234704	0.085762	0.092846	0.090091
	TRF2 EE 4-3	0.063529	0.073703	0.054370	0.116376	0.102010	0.100045	0.008098	0.009488	0.007438
	TRF2 EE 7-7	0.067755	0.073269	0.073237	0.086894	0.072606	0.071894	0.018155	0.020149	0.021038
LMNB2 origin		1	2	3	4	5	6	7	8	9
Empty vector	Parental HeLa	0.005058	0.000179	0.000000	0.000000	0.005693	0.003786	0.000659	0.000482	0.001805
	TRF2 WT 7-1	0.006110	0.001156	0.003596	0.000004	0.004148	0.000000	0.000003	0.003802	0.004407
	TRF2 EE 4-3	0.000000	0.001527	0.003811	0.002338	0.007457	0.004728	0.001014	0.000477	0.002097
	TRF2 EE 7-7	0.009444	0.004088	0.009058	0.000049	0.000000	0.000000	0.000000	0.000627	0.000765
ORC1-3xFLAG	Parental HeLa	0.011971	0.014320	0.012660	0.013494	0.016038	0.020894	0.007979	0.010267	0.003528
	TRF2 WT 7-1	0.039951	0.053167	0.036111	0.073241	0.069610	0.080086	0.031060	0.015481	0.014359
	TRF2 EE 4-3	0.013441	0.015785	0.011785	0.055761	0.040160	0.036503	0.008902	0.009187	0.011322
	TRF2 EE 7-7	0.022457	0.019063	0.035143	0.035332	0.017634	0.021896	0.015631	0.020973	0.027755

Supplementary Figure S2B	
TRF2 WT 7-1	0.1039
TRF2 EE 4-3	0.0508
TRF2 EE7-7	0.0495

FigS3_Source_data

Supplementary Figure S3				
Number of telomere-free micronuclei / nucleus		1	2	3
DDW	Parental HeLa	0.02	0.04	0.06
	TRF2 WT 7-1	0.03	0.05	0.06
	TRF2 EE 4-3	0.05	0.16	0.15
	TRF2 EE 7-7	0.12	0.02	0.06
0.1 mM HU	Parental HeLa	0.03	0.06	0.05
	TRF2 WT 7-1	0.07	0.14	0.07
	TRF2 EE 4-3	0.14	0.16	0.25
	TRF2 EE 7-7	0.06	0.04	0.16

FigS4_Source_data

Supplementary Figure S4		Experiment 1			Experiment 2		
		# LacI foci	# Co-loc. foci	Co-loc. freq.	# LacI foci	# Co-loc. foci	Co-loc. freq.
ORC1-3xFLAG	HA-LacI	125	1	0.8	73	3	4.1
	TRF2 ΔMyb-LacI	98	54	55.1	74	24	32.4
ORC1 (L229A)-3xFLAG	HA-LacI	71	5	7.0	74	1	1.4
	TRF2 ΔMyb-LacI	82	44	43.7	72	28	38.9

FigS5_Source_data

Supplementary Figure S5	Experiment 1	Experiment 2
Empty vector	1	1
FLAG-ORC1 (244-511)	0.27	0.16

FigS7_Source_data

Supplementary Figure S7	Experiment 1	Experiment 2
Control	1.07	1.69
Cdt1	2.04	3.36
Cdt1 + ORC1 WT	5.3	9.13
Cdt1 + ORC1 D620A	2.51	3.85

FigS8_Source_data

Supplementary Figure S8B	Days	1	2
Control	1	0.250	0.250
	2	0.525	0.616
	3	1.614	0.829
	4	2.740	2.342
	5	5.025	4.579
HA-ORC1 (244-511)	1	0.250	0.250
	2	0.690	0.486
	3	1.573	0.962
	4	3.266	1.875
	5	6.210	3.703

Supplementary Figure S8C	1			2		
	G1	S	G2/M	G1	S	G2/M
Control	65.35	27.3	7.36	66.07	23.79	10.14
HA-ORC1 (244-511)	67.37	23.85	8.78	69.87	20.76	9.37

FigS9_Source_data

Supplementary Figure S9							
Telomere		1	2	3	4	5	6
Empty vector	Control	0.095468	0.085217	0.099072	0.097818	0.121524	0.107805
	HA-ORC1 (244-511)	0.159064	0.173862	0.187148	0.053795	0.049745	0.052083
ORC1-3xFLAG	Control	0.485382	0.494429	0.332596	0.396963	0.403065	0.367355
	HA-ORC1 (244-511)	0.158685	0.184199	0.198534	0.141315	0.154423	0.185552
LMNB2 origin		1	2	3	4	5	6
Empty vector	Control	0.020041	0.014918	0.006336	0.018960	0.000000	0.000100
	HA-ORC1 (244-511)	0.000000	0.000000	0.034150	0.000000	0.009791	0.000000
ORC1-3xFLAG	Control	0.045274	0.045132	0.019274	0.064199	0.051114	0.030957
	HA-ORC1 (244-511)	0.071192	0.096277	0.051757	0.040642	0.038818	0.031088