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online data

SUPPLEMENTARY ONLINE DATA

Human ERAL1 is a mitochondrial RNA chaperone involved in the assembly of the 28S small mitochondrial ribosomal subunit

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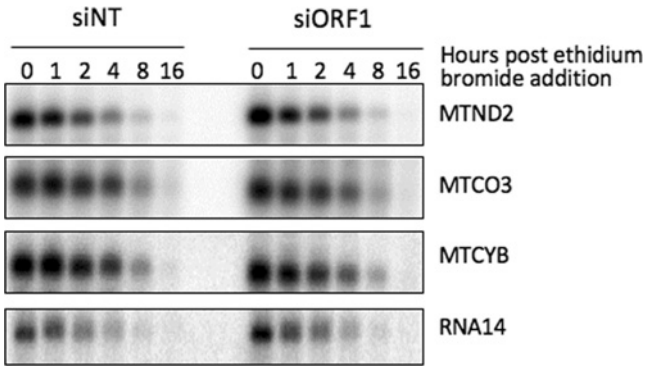

Figure S1 Stability of mitochondrially encoded transcripts is unaffected by loss of ERAL1

HEK-293T cells were transfected with control (siNT) or ERAL1-targeting (siORF1) siRNA for 3 days, after which mitochondrial transcription was inhibited by the addition of ethidium bromide (250 ng/ml). RNA was then isolated at the time points indicated post-addition of transcriptional inhibitor and 4 μg of each sample was separated through 1.2% agarose under denaturing conditions. Northern blot analysis was performed with random hexamer generated DNA probes to the mitochondrial transcripts indicated. MTCO3, COX3; MTCYB; mitochondrially encoded cytochrome *b*.

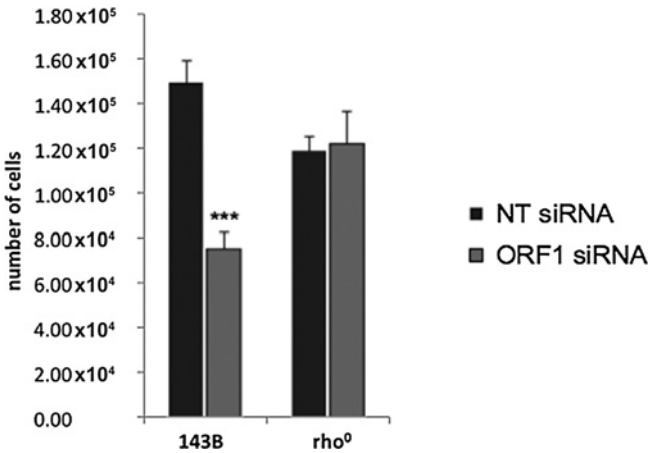

Figure S2 Confirmation that siORF1 that targets *ERAL1* lacks off-target effects

End-point cell proliferation was quantified after 3 days of siRNA depletion of ERAL1 in 143B parental and rho^0 cells with control (NT siRNA) or ERAL1-targeting (ORF1 siRNA). Depletion of ERAL1 with siORF1 showed a significant effect on the proliferation of parental 143B osteosarcoma cells (rho$^+$), that contain mtDNA. In contrast, no effect on cell growth was observed in 143B rho^0 cells (supplemented with 50 μg/ml uridine) that lack mtDNA and therefore are unaffected as they do not require mtDNA gene expression for growth. Results are means + S.D. $P < 0.001$.

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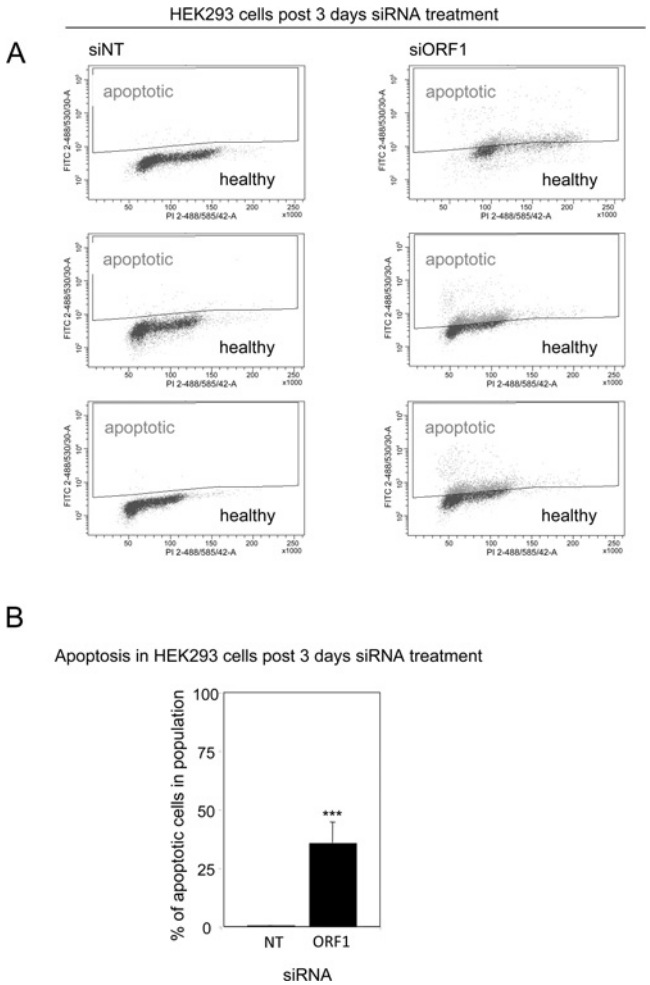

Figure S3 Analysis of apoptosis in HEK-293T cells post-siRNA treatment

HEK-293T cells were treated with control (siNT) or ERAL1-targeting (siORF1) siRNA for 3 days after which the proportion of apoptotic cells was estimated using the APO-Direct kit. (**A**) Representative primary FACS data for the both control and ERAL1-depleted cells. Analyses were performed on independent cultures of siRNA-transfected HEK-293T cells. (**B**) Quantification of the results in (**A**) is presented ($n = 4$) as a percentage of apoptotic cells within the population. Results are means + S.D. $P < 0.001$.

Table S1 Sequences of all 31 short RNA species bound by ERAL1 in the CLIP assay

N, signifies not determined by sequencer; *12S mt-rRNA sequences are portrayed in a linear fashion in Figure 3 in the main paper. TNF, tumour necrosis factor.

CLONE NO.	SEQUENCE	IDENTITY
	……..TCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC 3'term (nt1601)	*MT-RNR2* (12S mt-rRNA) *
1.13	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
1.14	……...CGTAACATGGTAAGTGTACTGGA..GTGCACTTGGACGAA	
1.15	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACA	
1.18	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACA	
1.21	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACA	
1.22	……AGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACG	
1.23	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
1.24	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGA	
1.25	……AGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.10	……AGTCGTAACAT*GTAAGTGTACTGGAAAGTGCACNTGGACGAACAAAG	
2.11	……..…..GTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.13	……...CGTAACATGGTAAGTGTACTGGGAAGTGCACTTGGACGAAC	
2.14	……AGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.3	……AGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.6	……*GGCCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACA	
2.7	……AGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.8	……...CGTAACATGGTAAGTGTACTGGA..GTGCACTTGGACGAA	
2.20	……..……..CATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.22	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACA	
2.24	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.25	……..GTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAAA	
2.26	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.28	……..CAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.29	GAGACAAGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.31	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGNNGANCA	
2.32	……...CGTAACATGGTAAGTGTACTGGAAAGTGCNCTTGGACGA	
2.34	……..GTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
2.36	……...CGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACA	
2.37	……AGTCGTAACATGGTAAGTGTACTGGAAAGTGCACTTGGACGAACA	
2.38	……...CGNAACATGGTAAGTGTACTGGAAAGNNCACNTGGACGAAC	
2.40	……..……..CATGGTAAGTGTACTGGAAAGTGCACTTGGACGAAC	
	GGCGCTAATGGTGGAGTTAAAGACTTTTTCTCTGACCA	*MT-TP* (Mt-tRNAPro)
1.16F	.GGCGCTAATGGTGGAGTTAAAGACTTTTTCTCTGACCA	
1.17R	GGGTGCTAATGGTGGAGTTAAAGACTTTTTCTCTGA	
1.26	.GGTGCTAATGGTGGAGTTAAAGACTTTTTCTCT	
2.16	..GTGCTAATGGTGGAGTTAAAGACTTTTTCTC	
2.9	.GGTGCTAATGGTGGAGTTAAAGACTTTTTCTCTGAC	
2.21	.GGTGCTAATGGTGGAGTTAAAGACTTTTTCTCT	
2.27	GGTTGCTAATGGTGGAGTTAAAGACTTTTTCTCTGACCA	
		E. coli 23S rRNA
1.19	GGGGGTCATCCCGACTTACCAAACCGATGCAAACTCCGAATACCAGCAA	
2.18	ATTATACAAAAGGTACGCAGTCACCGAACAAGTCGGCTCCCAC	
		KNOWN H. sapiens
2.2	CGCACGGGCTACAACCACGACCAATGATATG	HSP70 protein 8 iso 1
2.6	TCTAGATGCATCTTCTAGGAAGGCCTCTGTGGAAGGGACAAGGGACCTGGGACCGGACTGG	TNF receptor family 10B
2.41	AGCGTGTGCCTACCCTACGCCGGCAGGCGCGGGTAACCCGTTGAAC	18S rRNA
		UNKNOWN
2.12	AGGAAGTCGATGTTCAGGCTGTGCTCATGGGCTTCGTCGACAATGATG	
2.15	ACACACTGATTCAGGCTCTGGGCTCCTCCCC	
2.17	GTGATAGCCGCTACACTACGACC	
2.23	GGTAGTCTCGGCCCTAAACGATGATCACTAG	
2.35	ATCACTTTCGCATACGGGGCTGTCACCCGCTATGGCCACACTTTCCAGAGTGTTCTGC	
2.40	TCGTCAGAGAAACAGCAAGCTGTTTCCTGTTACCG	

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