Iacovino et al., Supplementary material

Supplementary figure 1. Oxa1, Tim17 and Tim23 levels are increased in W327R transformants. (A) W327R cells transformed with the plasmids indicated were analysed by immunoblot for Oxa1 expression. Representative blot shown in (A) and quantification of mean values from two independent experiments are shown in (B), relative to the levels in pRS416 control transformants that was arbitrarily set as 1. The high level of Oxa1 protein in pRS-Oxa1 transformants was due to the strong heterologous promoter used in this plasmid (see Materials and Methods). (C) W327R transformants were analysed by immunoblot for Tim17 and Tim23 expression and quantification of mean values from two independent experiments is shown for Tim17 in (D) and for Tim23 in (E). All cultures were grown overnight in YNBG+cas prior to immunoblot. Expression levels were calculated relative to porin loading control (see Materials and Methods). Averages are shown +/- 1 S.E.M.

Supplementary figure 2. Alignment of *S. cerevisiae* and human Tim17 protein sequences.*TIM17A* is located on chromosome 1 at 1q32.1, while *TIM17B* is located on chromosome X at Xp11.23. Tim17A has a slightly greater identity to *S. cerevisiae* Tim17 than Tim17B. Sequences were aligned using ClustalW2.

Supplementary figure 3. Increased expression of *TIM17A* had no significant effect on mtDNA copy number in 143B osteosarcoma cells. 143B cells were stably transfected with pIRES alone (EV) or pIRES.Tim17A. Mitochondrial DNA levels were measured in three clones of each type. Clones carrying pIRES.Tim17A had a 4-fold (2 clones) or a 29 -fold higher level

of *TIM17A* mRNA than two EV clones based on qPCR analysis. Averages are shown +/- 1 S.D. Endogenous *TIM17A* mRNA levels were very similar in untransfected NT2 and 143B cells (data not shown). The difference between NT2 and 143B cells that makes the former (1) but not the latter (2) prone to mtDNA loss remains unknown. It does not appear to reflect a difference in Tim17, as the level of *TIM17A* mRNA was only 10% higher in 143B than NT2 cells, and *TIM17B* transcripts were less abundant in 143B than NT2 cells, based on qPCR analysis (not shown).

Supplementary figure 4. Gene-silencing of Tim17A does not affect mitochondrial nucleoid size, number or distribution. (A) Human osteosarcoma cells were transfected with or without 10 nM dsRNA-363 targeting Tim17A mRNA (sense strand 5´-UCUCCU-AGCUUUAAUU-GAAGGAGCU-3´), cells were re-transfected after 72 hours and analysed 144 hours after the first transfection. Real-time PCR analysis confirmed that the steady-state level of Tim17A transcript decreased by approximately 70%, relative to GAPDH mRNA (data not shown). The effect of Tim17A siRNA on mtDNA copy number was also determined by real-time PCR analysis, using primers and probes appropriate for cytochrome *b* (mtDNA) and APP (nuclear DNA), as described previously (3). The results were compared to siRNA of ATAD3 (144 hours), and Twinkle DNA helicase (72 hours). Error bars are standard deviations from the mean, n = 7 experiments for ATAD3, n = 3 experiments for Twinkle and Tim17A. (B) Picogreen staining of DNA in living cells was performed as described previously (4, 5) 144 hours after transfection.

Supplementary references

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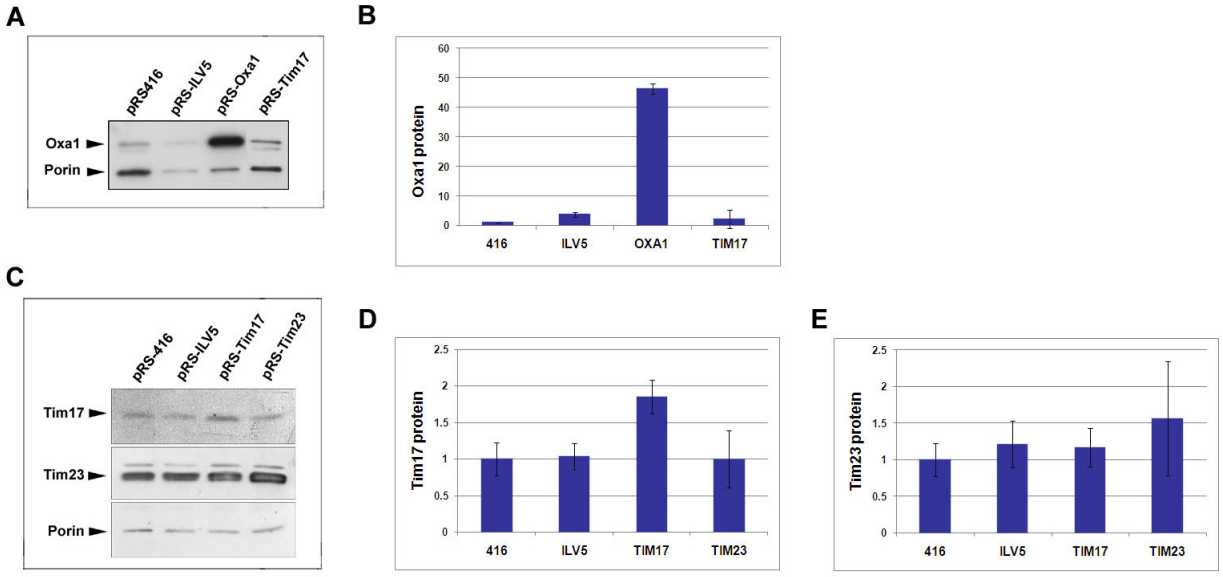
Supplementary Table 1

Clone	Chromosome	Beginning of insert genome position and sequence	End of insert genome position and sequence	Insert size (bp)	No. genes contained on insert	Gene which rescues W327R
2A	XII	833805 GATCCAAAAGAG AGAATAAAAAAA CAATTA	842394 ATGATTTTAATC TTATGTTAACCA ACTTTA	8589	5 (including *ILV5*)	*ILV5*
11A	V	474467 GATCGCGATTCAT CGCCTGGTAGTGC TGGAGG	481463 ATTGGTTGAATA CCTTTAGAAACT TAATGA	6996	3 (including *OXA1*)	*OXA1*
23A	XI	173713 GATCTACATATAC CCGCCATTGTTGC ACTA	181764 GTAAAATCCATT GTTCACTGGTTC CAAATA	8051	7 (including *PET122*)	None
29A	V	468302 GATCTTACTTTCT ACTCAAAAAGAA TCCAA	481463 ATTGGTTGAATA CCTTTAGAAACT TAATGA	13160	7 (including *OXA1*)	*OXA1*
50A	V	468302 GATCTTACTTTCT	481463 ATTGGTTGAATA	13160	7 (including *OXA1*)	*OXA1*

		ACTCAAAAAGAA TCCAA	CCTTTAGAAACT TAATGA			
54A	X	146483 GGATCAATAACC AGCCTACACAGC CTGGCG	154200 CATTTCATCAGC TTCATCTAAGAT GAACAT	7717	6 (including *TIM17*)	*TIM17*

Supplementary Table 1. Details of inserts contained within Yep24 URA3 W327R suppressor plasmids.

Supplementary figure 1



Supplementary figure 2

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Tim17A_HUMAN      -MEEYAREPCPWRIVDDCGGAFTMGTIGGGIFQAIKGFRNSPVGVNHRLRGSLTAIKTRA  59
Tim17B_HUMAN      -MEEYAREPCPWRIVDDCGGAFTMGVIGGGVFQAIKGFRNAPVGIRHRLRGSANAVRIRA  59
Tim17_YEAST       MSADHSRDPCPIVILNDFGGAFAMGAIGGVVWHGIKGFRNSPLGE--RGSGAMSAIKARA  58
                     :::*:***  *::* ****:**.*** :::.******:*:*   *  *: .*:: **
Tim17A_HUMAN      PQLGGSFAVWGGLFSMIDCSMVQVRGKEDPWNSITSGALTGAILAARNGPVAMVGSAAMG  119
Tim17B_HUMAN      PQIGGSFAVWGGLFSTIDCGLVRLRGKEDPWNSITSGALTGAVLAARSGPLAMVGSAMMG  119
Tim17_YEAST       PVLGGNFGVWGGLFSTFDCAVKAVRKREDPWNAIIAGFFTGGALAVRGGWRHTRNSSITC  118
                  * :**.*.******* :**.:  :* :*****:* :* :**. **.*.*     .*:
Tim17A_HUMAN      GILLALIEGAGILLTRFASAQFP-NGPQFAEDPSQLPSTQLPSSP-FGDYRQYQ  171
Tim17B_HUMAN      GILLALIEGVGILLTRYTAQQFR-NAPPFLEDPSQLPPKDGTPAPGYPSYQQYH  172
Tim17_YEAST       ACLLGVIEGVGLMFQRYAAWQAKPMAPPLPEAPSSQPLQA--------------  158
                  . **.:***.*::: *::: *    .* : * **. *
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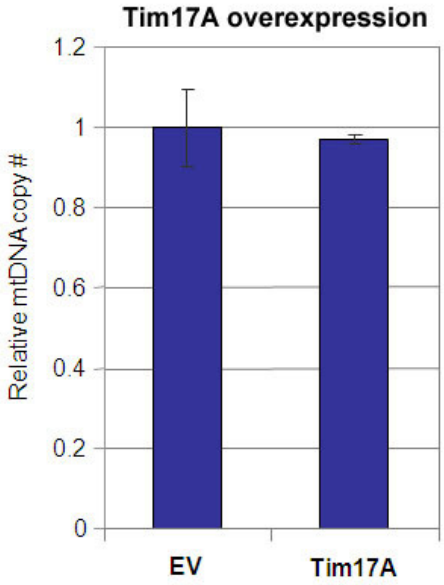
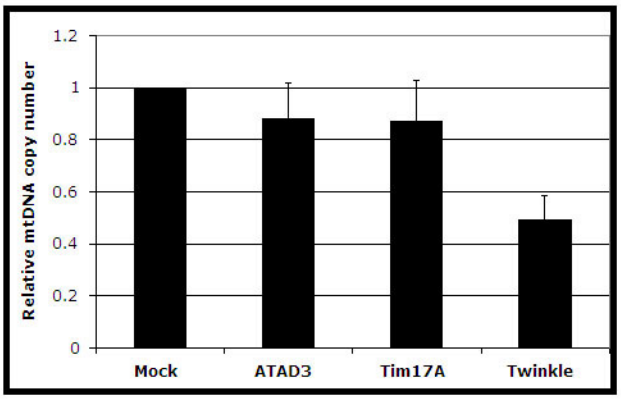
Supplementary figure 3



Supplementary figure 4

A



B