SUPPLEMENTARY INFORMATION

Figure S1. Recombinant tagged C4orf14 co-fractionates with the mitochondrial 28S subunit. Proteins derived from sucrose gradient fractions equivalent to those shown in Figure 2B from HEK293T cells expressing recombinant (HA or FLAG-StrepII tagged) C4orf14 were separated by SDS-PAGE and immunoblotted with antibodies to the HA and FLAG tags and antibodies recognising MRPS6, MRPS10, MRPS29, MRPL11, and MRPL15.

Figure S2. Double-strand RNAs targeting C4orf14 repress its expression, and in one case decreases mitochondrial mRNA levels. A; C4orf14 mRNA levels were measured via Q-PCR in RNA samples isolated from 143B osteosarcoma cells treated with or without one of two dsRNAs targeting C4orf14. Results are expressed as a proportion of the values for the control (mock-transfected cells). B; Mitochondrial mRNA levels for three transcripts, NADH dehygrogenase 1 (ND1), cytochrome *c* oxidase subunit II (COX2) and cytochrome *b*, were assayed by Q-RT-PCR from untransfected 143B cells, or cells transfected with a scrambled RNA, or dsRNAs c3 or c6 targeting C4orf14, or mock transfected. Values are expressed as a proportion of the mRNA level in the untransfected 143B cells. N = 3 independent experiments. ** $p < 0.01$, *** $p < 0.001$.

Figure S3. Proteins associated with the 28S subunit are much less abundant in affinity-purified preparations of C4orf14 with a disabled GTPase, compared to the wild-type protein. Functional (wt) and GTPase disabled (m3), recombinant forms of C4orf14 were affinity purified from HEK cells, as described in the methods. 30 μg of input protein (mitochondrial 1,000 g_{max} supernatant - see methods) was

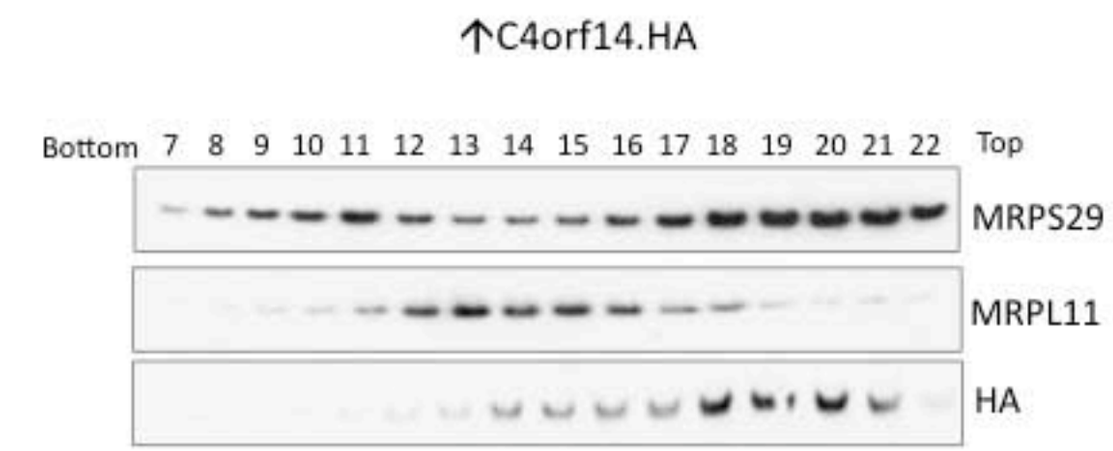
separated by SDS-PAGE together with eluate 3 (the latter adjusted to give an equal signal for the bait protein).

Figure S4. C4orf14 co-sediments with the 28S subunit only if its GTPase activity is preserved. Mitochondria lysed with n-dodecyl-β-D-maltoside, from A) control HEK293T cells (without a transgene); B) HEK293T cells expressing GTPase active C4orf14 tagged with HA; C) HEK293T cells expressing GTPase disabled (M3) C4orf14 with flag and strepII tags, were fractionated on 10-30% sucrose gradients, and proteins from fractions 2-17 (collected manually from the base of the tube) were separated by SDS-PAGE and immunoblotted with antibodies to C4orf14, and 55S ribosome components MRPS2 and 27, and MRPL11.

Figure S5. Conversion of supercoiled to relaxed plasmid DNA increases Picogreen signal. 250 ng lots of plasmid DNA were incubated with, 2 or 4 Units of S1 nuclease for 2 min at 37 ^{o}C. After DNA extraction, half the material was separated by 1 % agarose gel electrophoresis, while a quarter was incubated with a 1/300 dilution of Picogreen for 10 min in the dark. The DNA/Picogreen mixture was excited at 485 nm and the emissions at 535 nM quantified in a SPECTRAmax plate reader (Molecular Devices).

Figure S6. GTPase inactive C4orf14 co-purifies with human mtDNA. Single-step affinity purification of StrepII tagged C4orf14.m3 was carried out as described in the methods and other figures, supernatant (S), flow-through (F), washes (w) and eluted fractions (e) were loaded and separated on a 0.8 % agarose gel and *Pvu*II digested mtDNA detected by Southern hybridization after transfer to solid support.

Sucrose gradient fractionation



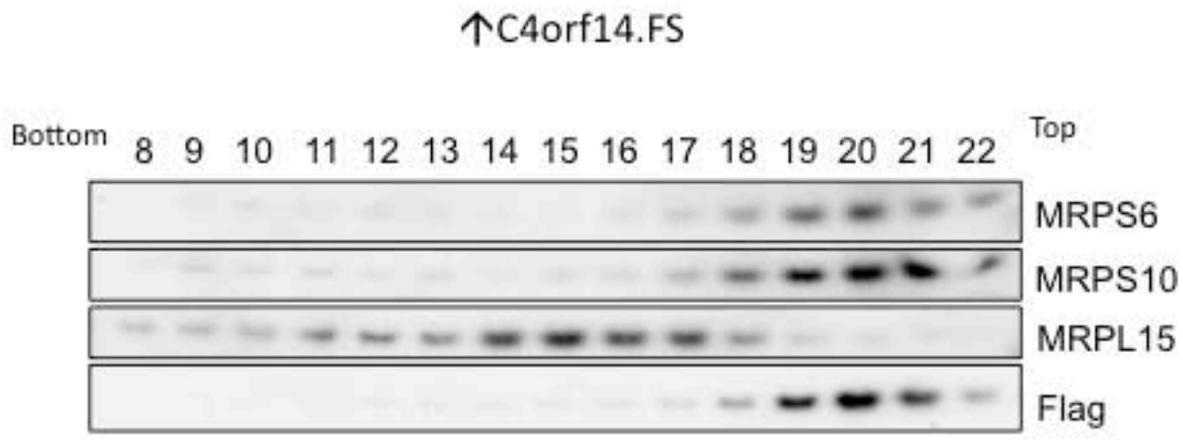
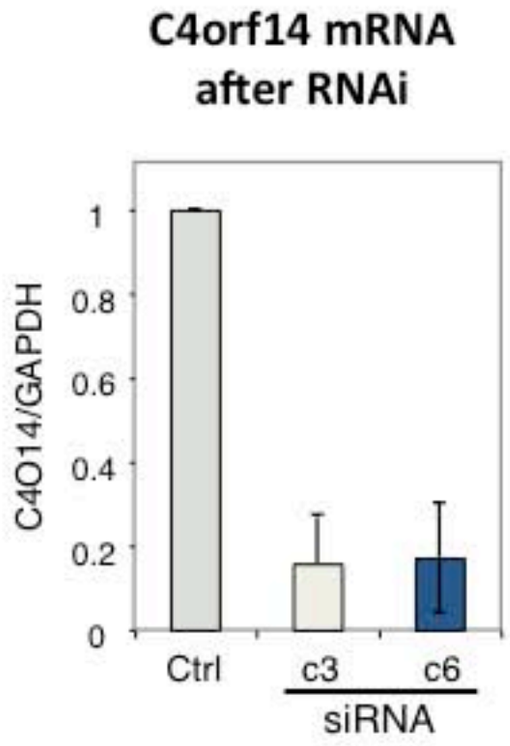


Figure S1

A


C4orf14 mRNA
after RNAi
C4O14/GAPDH
1
0.8
0.6
0.4
0.2
0
Ctrl
c3
c6
siRNA

B

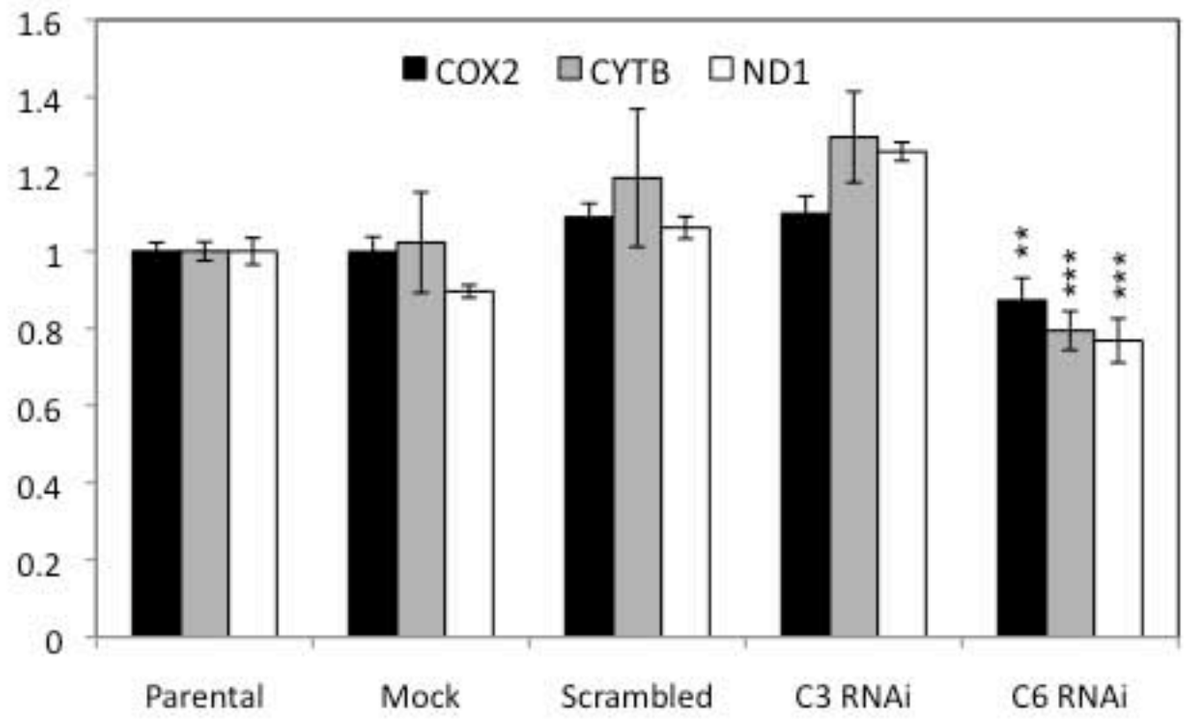

Mitochondria RNAs after C4orf14 RNAi
COX2
CYTB
ND1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
***
***
Parental
Mock
Scrambled
C3 RNAi
C6 RNAi

Figure S2

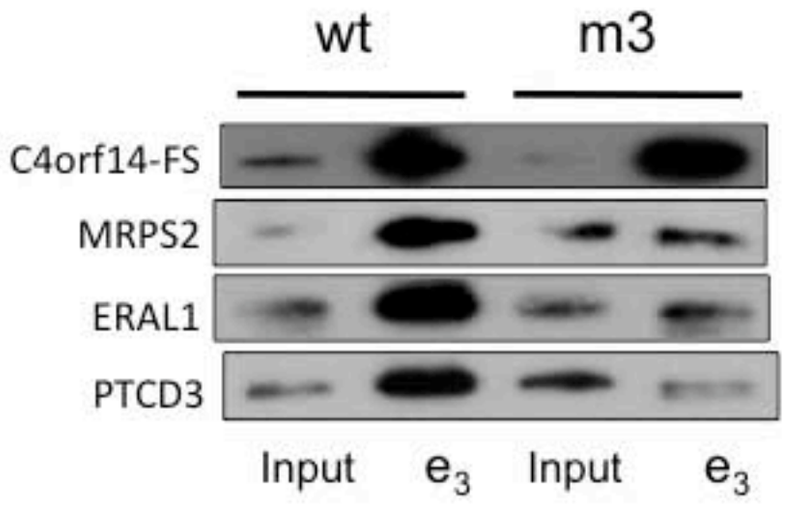


Figure S3

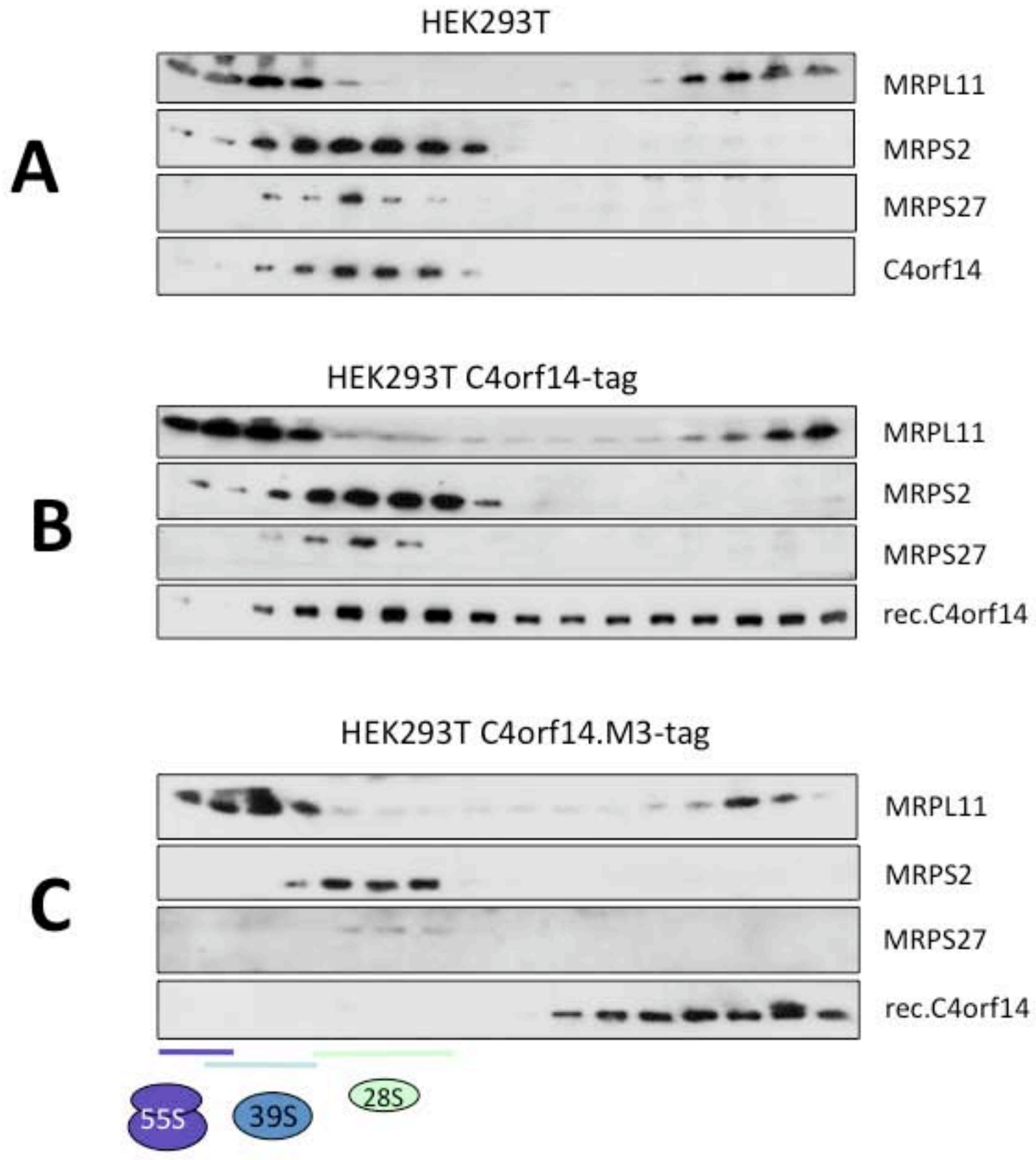
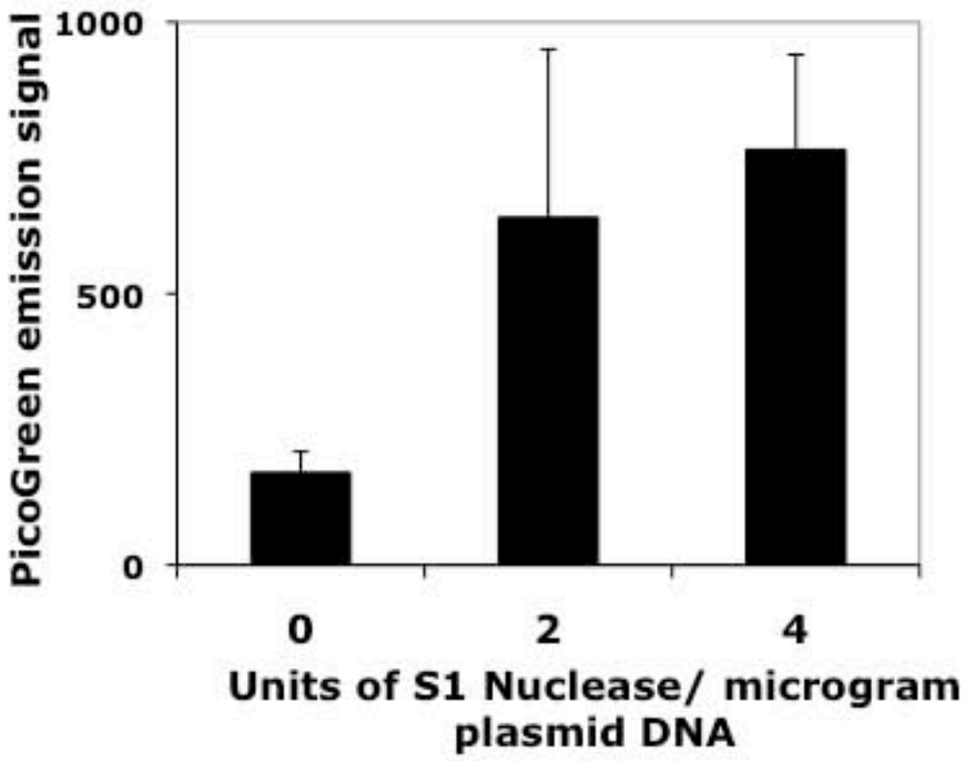

A
HEK293T
MRPL11
MRPS2
MRPS27
C4orf14
B
HEK293T C4orf14-tag
MRPL11
MRPS2
MRPS27
rec.C4orf14
C
HEK293T C4orf14.M3-tag
MRPL11
MRPS2
MRPS27
rec.C4orf14
55S
39S
28S

Figure S4


PicoGreen emission signal
1000
500
0
0
2
4
Units of S1 Nuclease/ microgram plasmid DNA

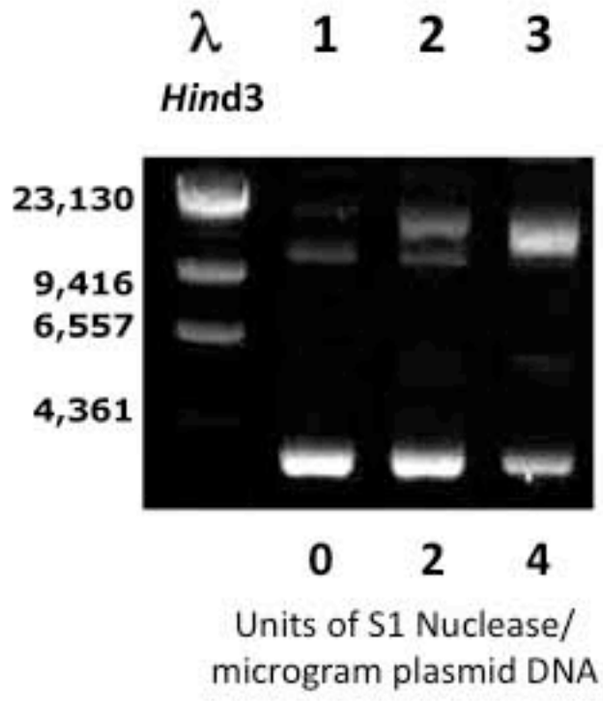

λ
1
2
3
Hind3
23,130
9,416
6,557
4,361
0
2
4
Units of S1 Nuclease/
microgram plasmid DNA

Figure S5

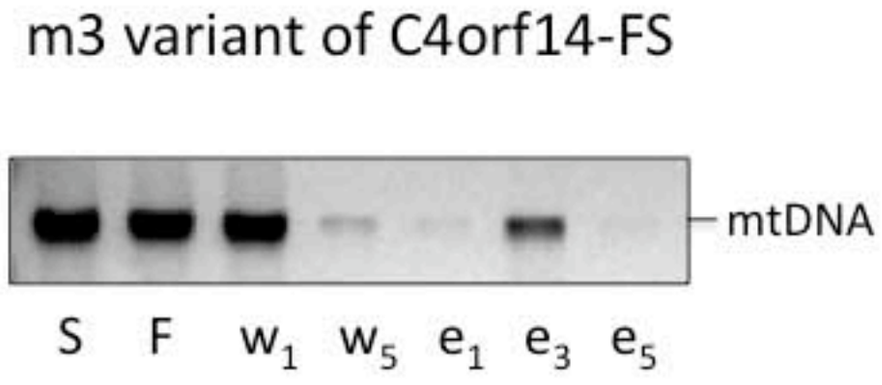


Figure S6