S1

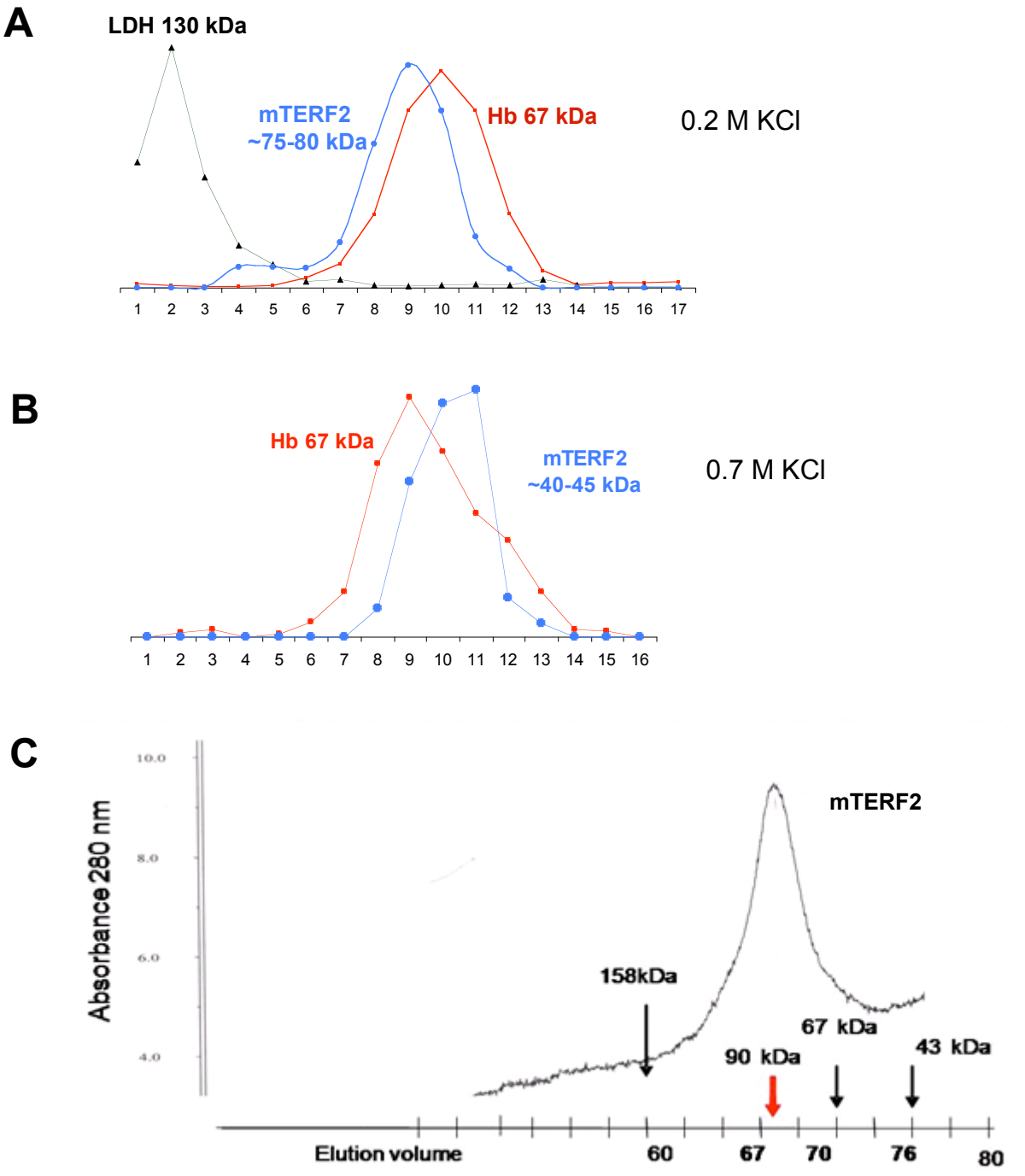

Figure S1. mTERF2 is able to form oligomers *in vitro and in vivo*.
(A) and **(B)** Differential centrifugation of mTERF2 extracted with mild salt condition (0.2M KCl) and high salt condition (0.7 M KCl) from heart mitochondria followed by immunobloting with anti-mTERF2 antibody. Lactate dehydrogenase and hemoglobin were used as molecular weight markers. **(C)** Gel filtration chromatography of recombinant mTERF2 shows a molecular weight of ~ 90 kDa.

S2

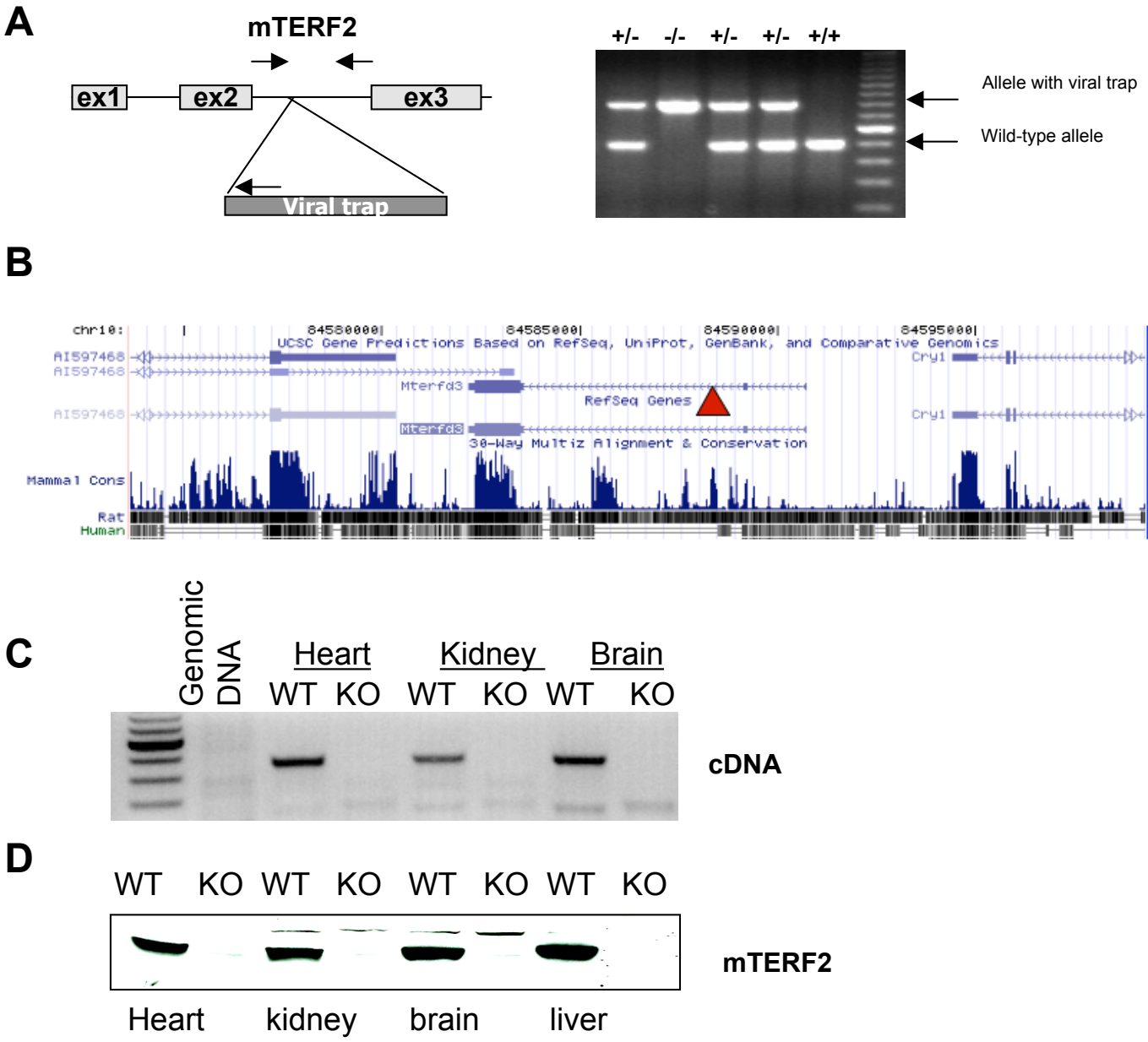

Figure S2. Gene trapping strategy for the generation of mTERF2 deficient mice
(A) Position of the viral trap in the mTERF2 genomic structure. Primers used for genotyping are shown by black arrows. Primers are located in the viral trap and flank the insertion site. Multiplex-PCR using these three primers yields a band of ~ 500 bp for the wild-type allele and ~ 800 bp for the allele containing the viral trap. **(B)** UCSC browser view of the mTERF2 (mTERFD3) genomic region. The trap is shown as a red triangle. **(C)** Reverse transcriptase PCR of cDNA from isolated from different tissues of mTERF2 knockout and wild-type mice were tested for mTERF2 cDNA. **(D)** Western blotting of protein extracted from different tissues of mTERF2 knockout and wild-type mice using a mTERF2 specific antibody.

S3

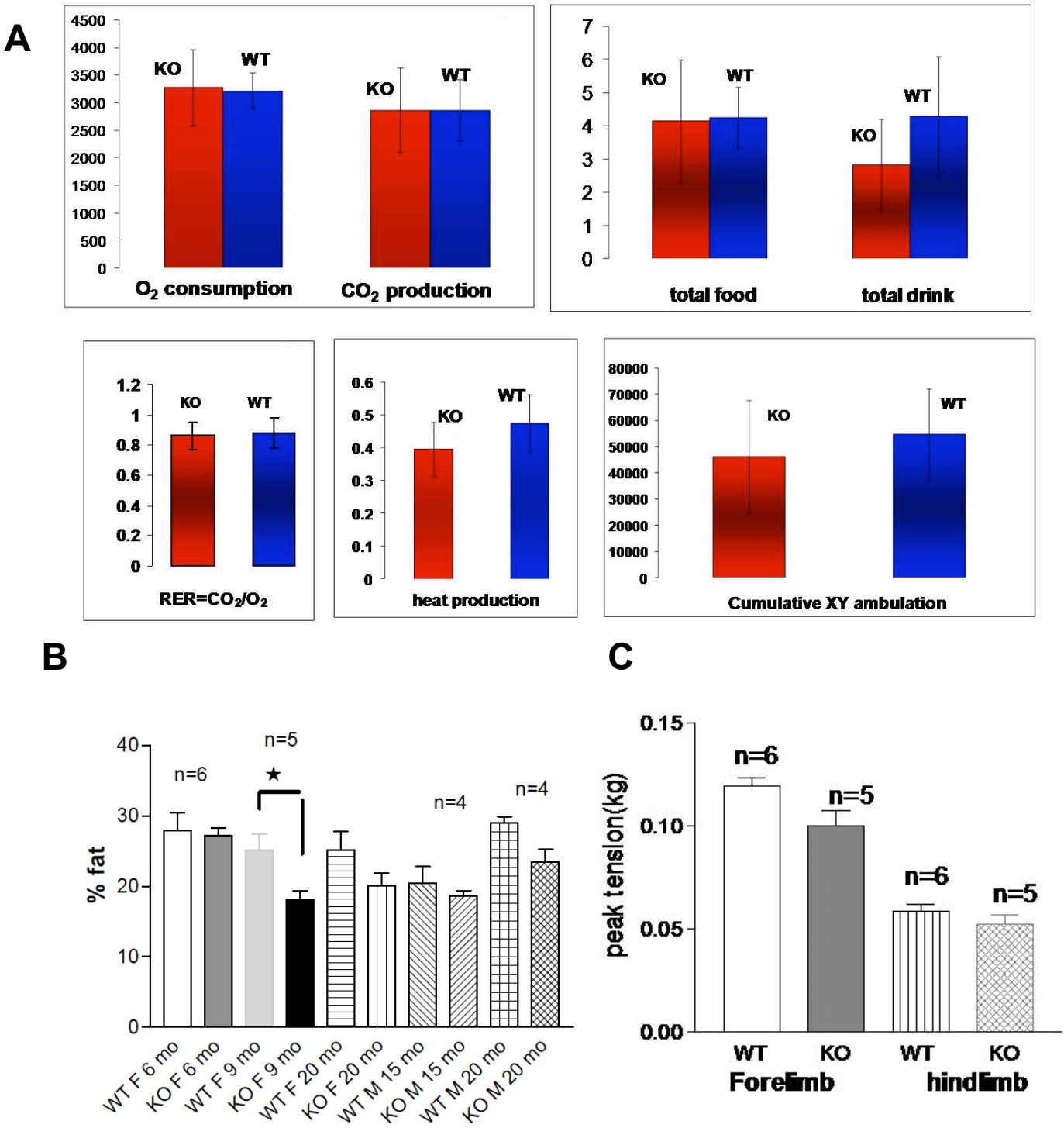

Figure S3. Phenotype analysis of mTERF2 knockout mice compared to wild-type mice.

(A) 9 months old mTERF2 knockout and wild-type mice were analyzed for basic metabolic parameters (N=6 each). **(B)** Total body fat content was measured using Dual Energy X-ray Absorption (DEXA) in animals of different ages. *P=0.019 **(C)** Grip strength test of forelimb and hind limb of mTERF2 knockout mice (N=5) and wild-type controls (N=6) at 9 months of age.

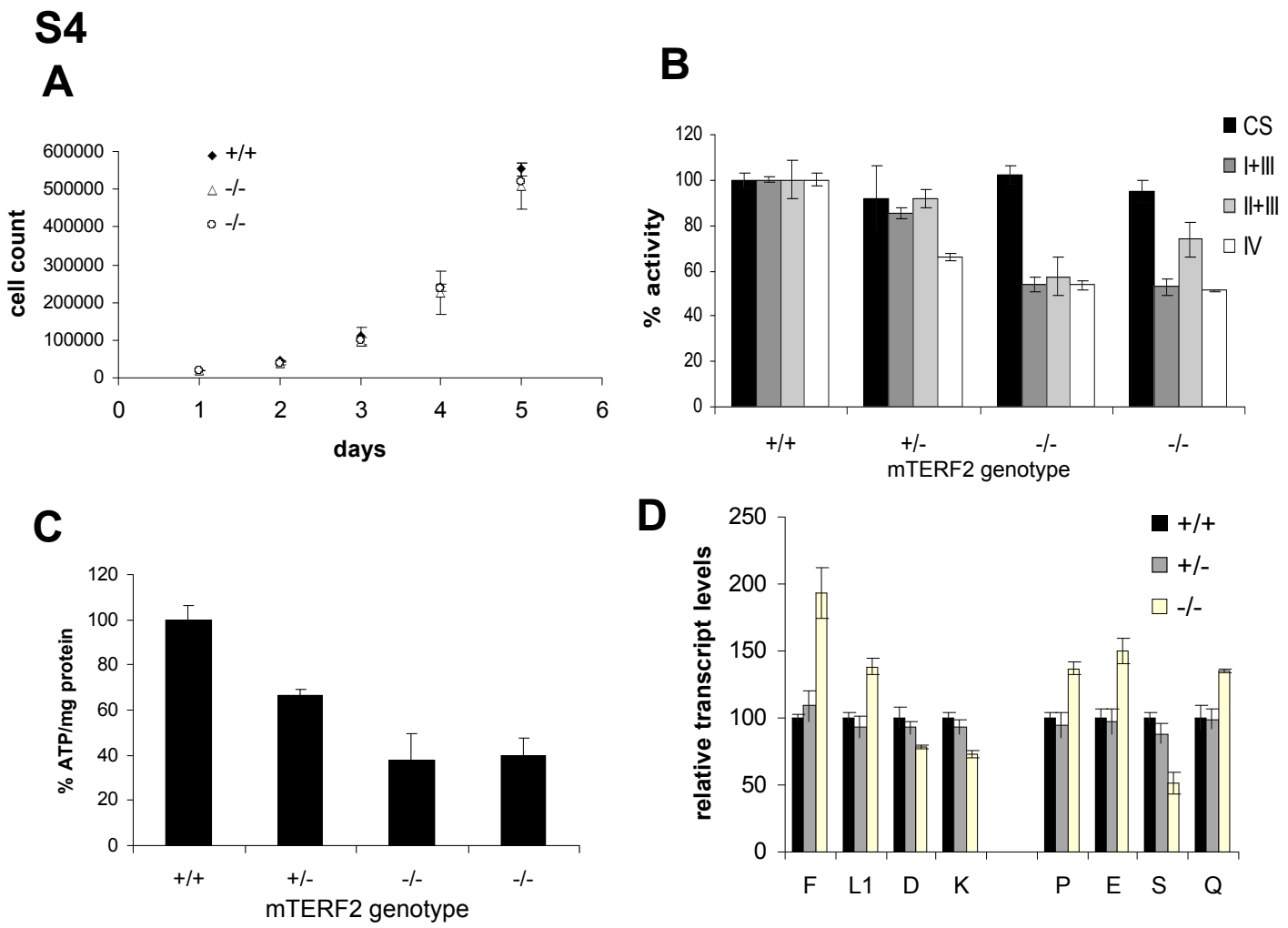

Figure S4. mTERF2 deficient fibroblasts have an OXPHOS deficiency

(A) Growth rate of control and mTERF2 knockout fibroblasts were measured in media supplemented with glucose (N=3 for each time point).

(B) Citrate synthase (CS), complex I+III (I+III), complex II+III(II+III) and cytochrome *c* oxidase (IV) activity in fibroblast mitochondria of deficient in mTERF2 knockout, heterozygous and controls (N=2 for each group). **(C)** Quantification of ATP in fibroblasts of deficient in mTERF2 knockout, heterozygous and controls (N=2). **(D)** Steady-state levels of different mitochondrial tRNAs on the HSP and LSP transcript in mTERF2 deficient and control fibroblast (N=3).

S5

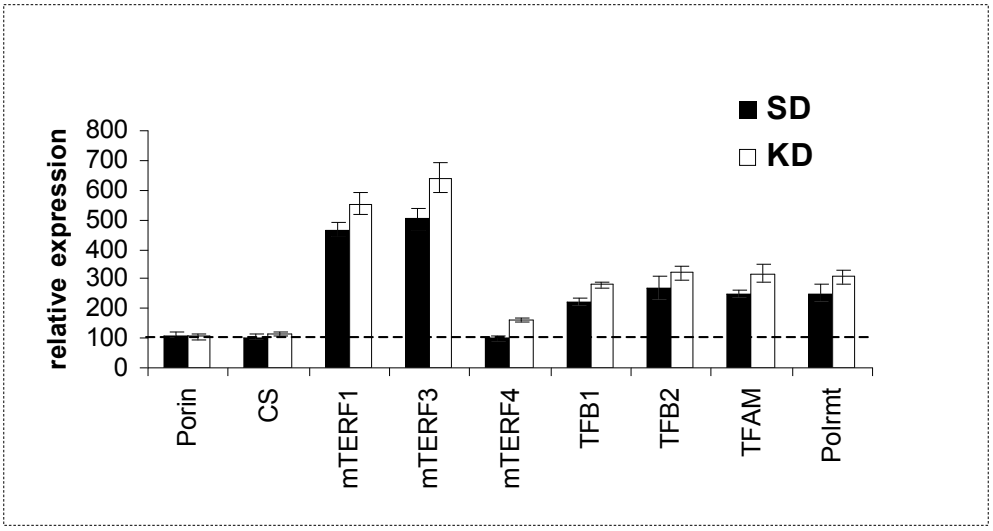

Figure S5. mTERF2 deficiency in the heart is associated with upregulation of mTERF homologues

Relative expression of mTERF1, mTERF3, mTERF4, mitochondrial RNA polymerase (POLR), transcription factor B1 (TFB1) and B2 (TFB2), mitochondrial transcription factor A (TFAM) and the mitochondrial proteins porin and citrate synthase (CS) in heart of 6 months old mTERF2 knockout and control mice (N=3). The dashed line indicates the SD or KD-fed control.

S6

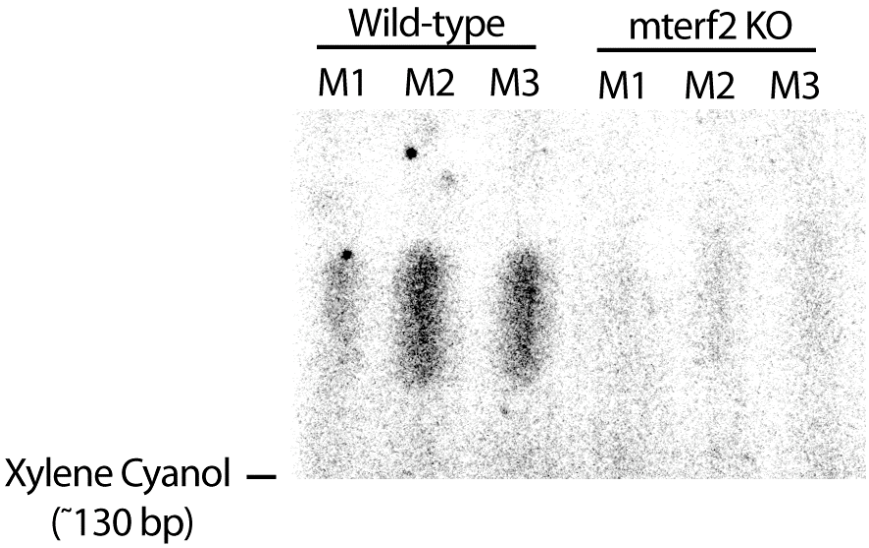

Figure S6. mTERF2 deficiency decreases mitochondrial transcription *in vitro*

Audioradiogram of labeled RNA transcripts from an in vitro mitochondrial transcription assay using mitochondrial extracts from skeletal muscle of wild-type and mTERF2 KO animals.