**Supplementary figures and tables for:**

# DNA polymerase beta participates in mitochondrial DNA repair

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Running title: A novel function for DNA polymerase beta in the mitochondria

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Figure S1

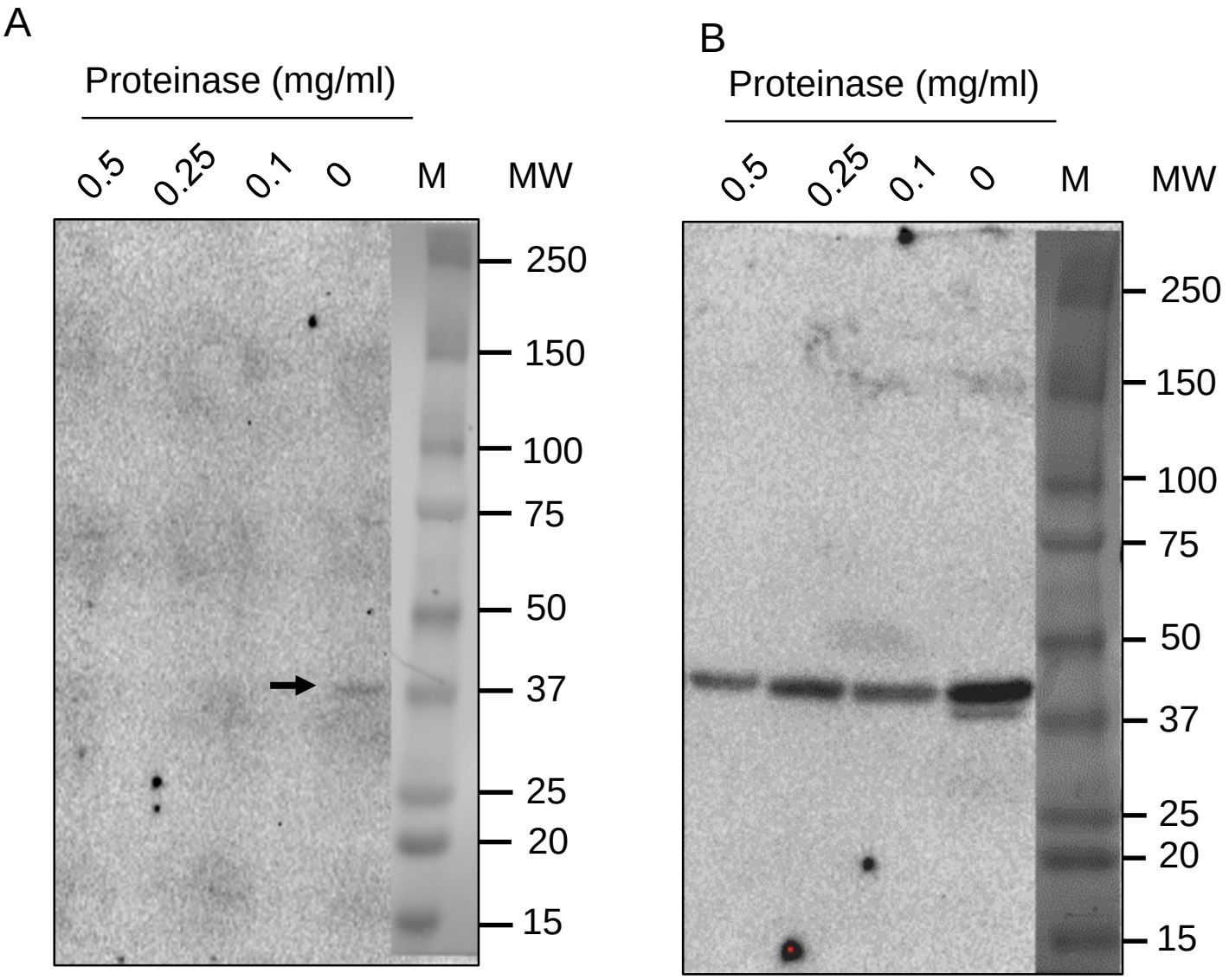



**Figure S1A-B to accompany Figure 1: (A)** Full western blot of liver mitochondrial extract after probing with Polβ specific antibody. (Arrow)This band is consistent with the size expected for nuclear Polβ and is only in the undigested lane (0 mg/ml) . **(B)** Mitochondrial extracts derived from the mouse brain. Mitochondrial band is higher than nuclear as seen in undigested lane. Full blot shows that antibody is specific for Polβ only. 4 -15% gradient gel. M denotes broad range marker has been superimposed on image from the same blot. Molecular Weight (MW) in kDa. Both liver and brain samples have been loaded on the same gel. Liver image has been overexposed to show residual nuclear Polβ band. 50 µg of mitochondrial lysate was loaded per well.

Figure S1

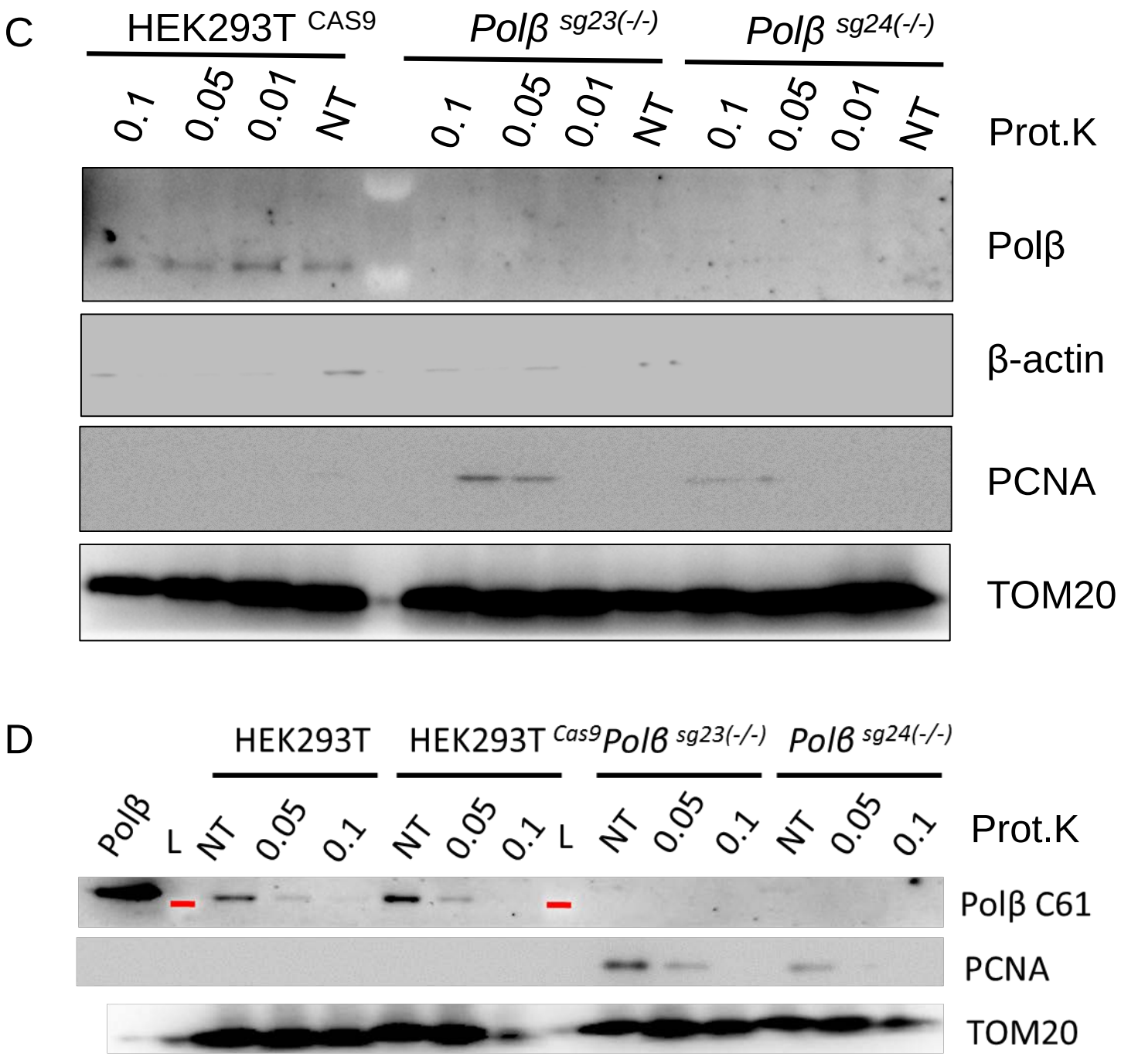



**Figure S1C/D to accompany Figure 1D: Alternate loading amounts and extractions of mitochondrial protein**. Western analysis from the HEK293T Polβ deficient lines. **(C)** 50 µg mitochondrial extract loaded. **(D)** 70 µg of mitochondrial extract loaded. Note that the very faint higher MW band seen in the Polβ KO C61 lanes is Aprataxin antibody from previous western analysis. See figure 1D for further information about these blots.

# Figure S1

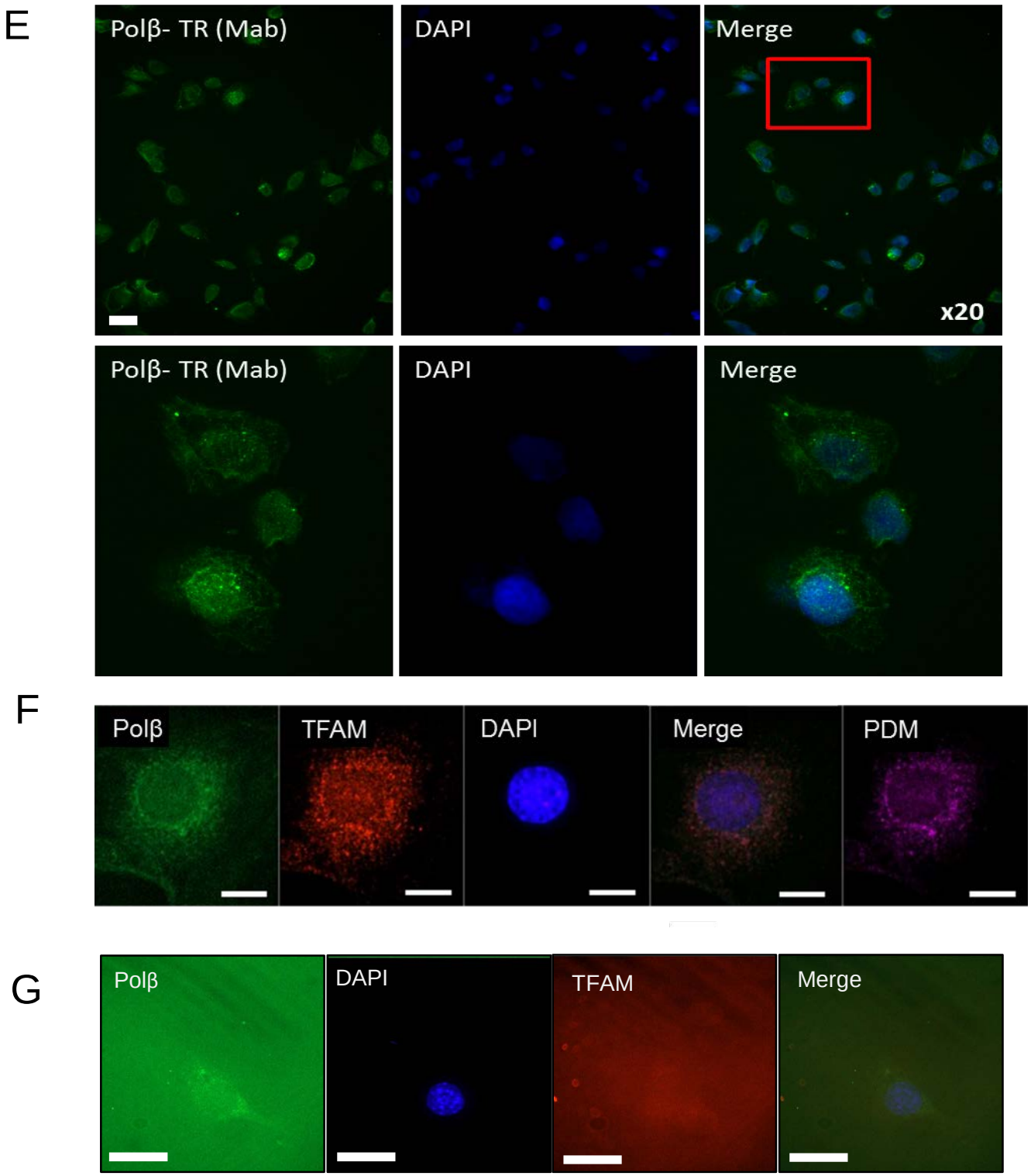



**Figure S1E-G to accompany Figure 1: (E)** Intracellular localization was tested using Polβ antibody on transformed mouse fibroblasts. Antibody detected protein in the nucleus and cytoplasm consistent with previous reports. DAPI was used to stain nucleus. Scale bar is 40 µm **(F)** The antibody signal was compared to anti-TFAM signal. PDM represents a channel of co-localization between Polβ and TFAM signal and shows distinct regions of col-localization outside of the nucleus **(G)** Antibody was also tested for specificity on Polβ KO MEF cells. Antibody detected a slight amount of non-specific binding only visible with image overexposure. Scale bar is 50 µm.

Figure S1

H

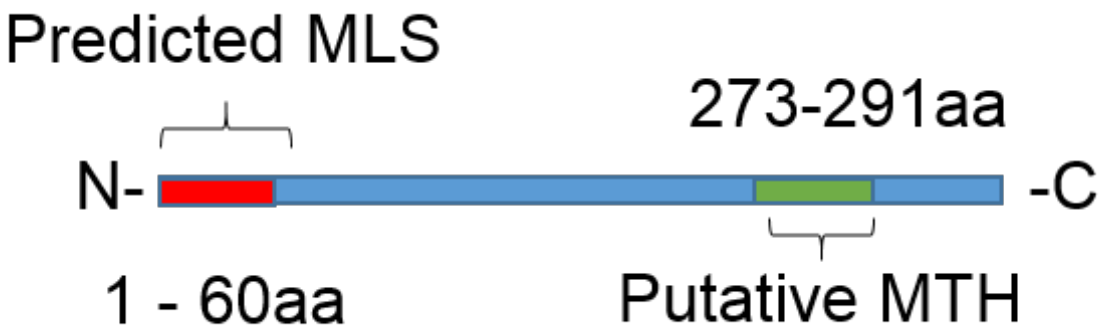



**Figure S1H to accompany Figure 1:** A predicted mitochondrial localization sequence (MLS) was detected in the N-terminal (first 60 aa) domain with a predicted mitochondrial probability of 0.85. Deletion of the first 17 aa in this N-terminal MLS sequence brings this probability down to 0.07. The protein also contains a putative mitochondrial targeting helix (MTH) located at the carboxyl domain between amino acid residues 273-291.

# Figure S1

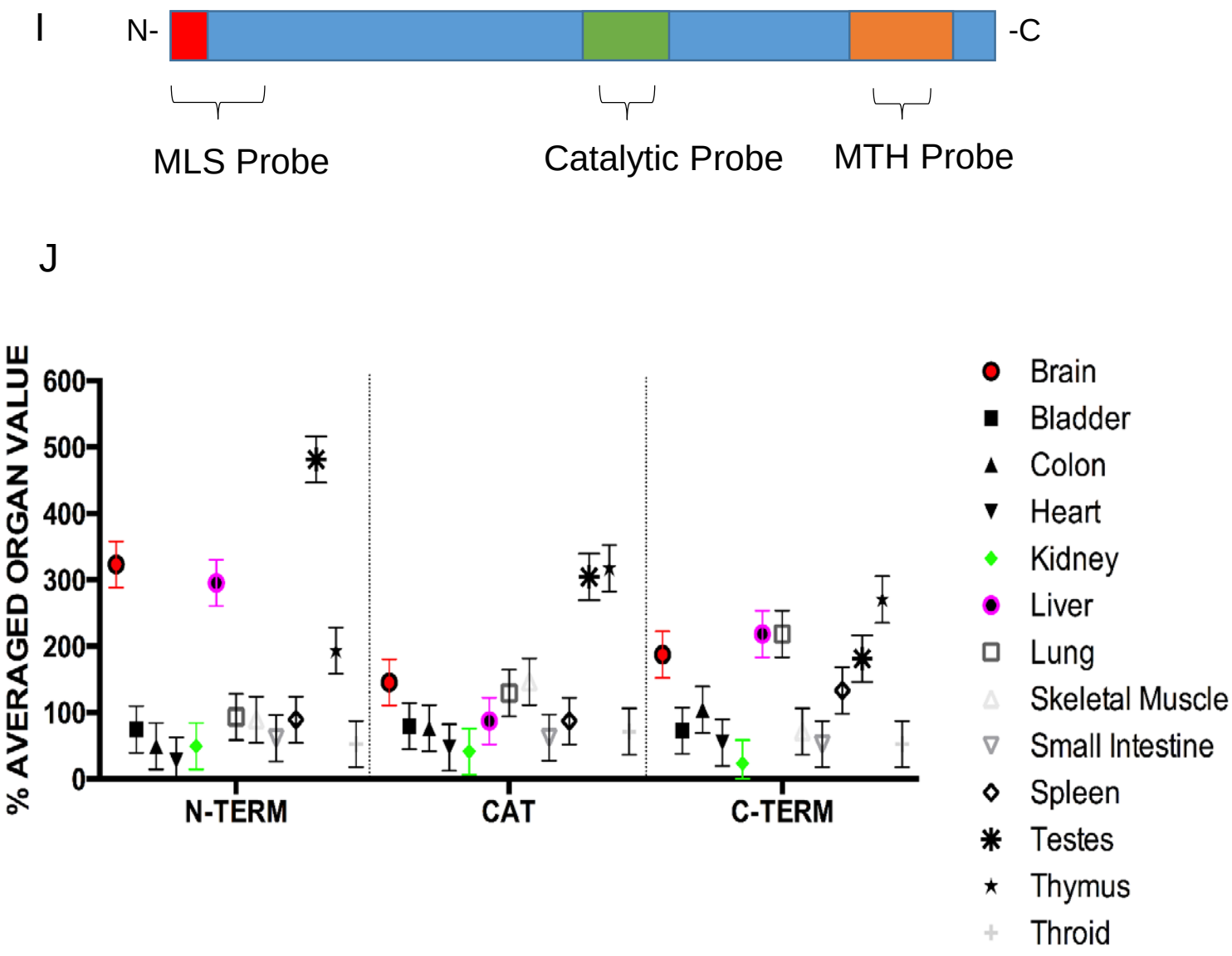



**Figure S1I/J to accompany Figure 1.** RT-PCR was used to measure the mRNA expression pattern of Polβ isoforms in human tissue. The investigation focused on using three different probe sets **(I)** An N-terminal specific probe based on the first 20 amino acids. A C-terminal probe which crosses the sequence responsible for the putative MTH and a catalytic sequence probe which is found in all the isoforms. **(J)** The results of the 13 tissues were pooled and averaged to achieve a base line average expression value in all tissues (100%). Assessment of the expression of N and C terminals showed that specific tissues had elevated levels of N and C terminal Polβ isoforms however this did not clearly correlate with the mitochondrial Polβ protein levels suggesting mRNA isoform levels of Polβ alone could not predict the amount of Polβ in the mitochondria. Each point is the average of between 4 and 5 technical replicates with GAPDH used as an internal house keeping gene. Error bars represent average SD in the assay set at ± 35%.

Figure S1

K

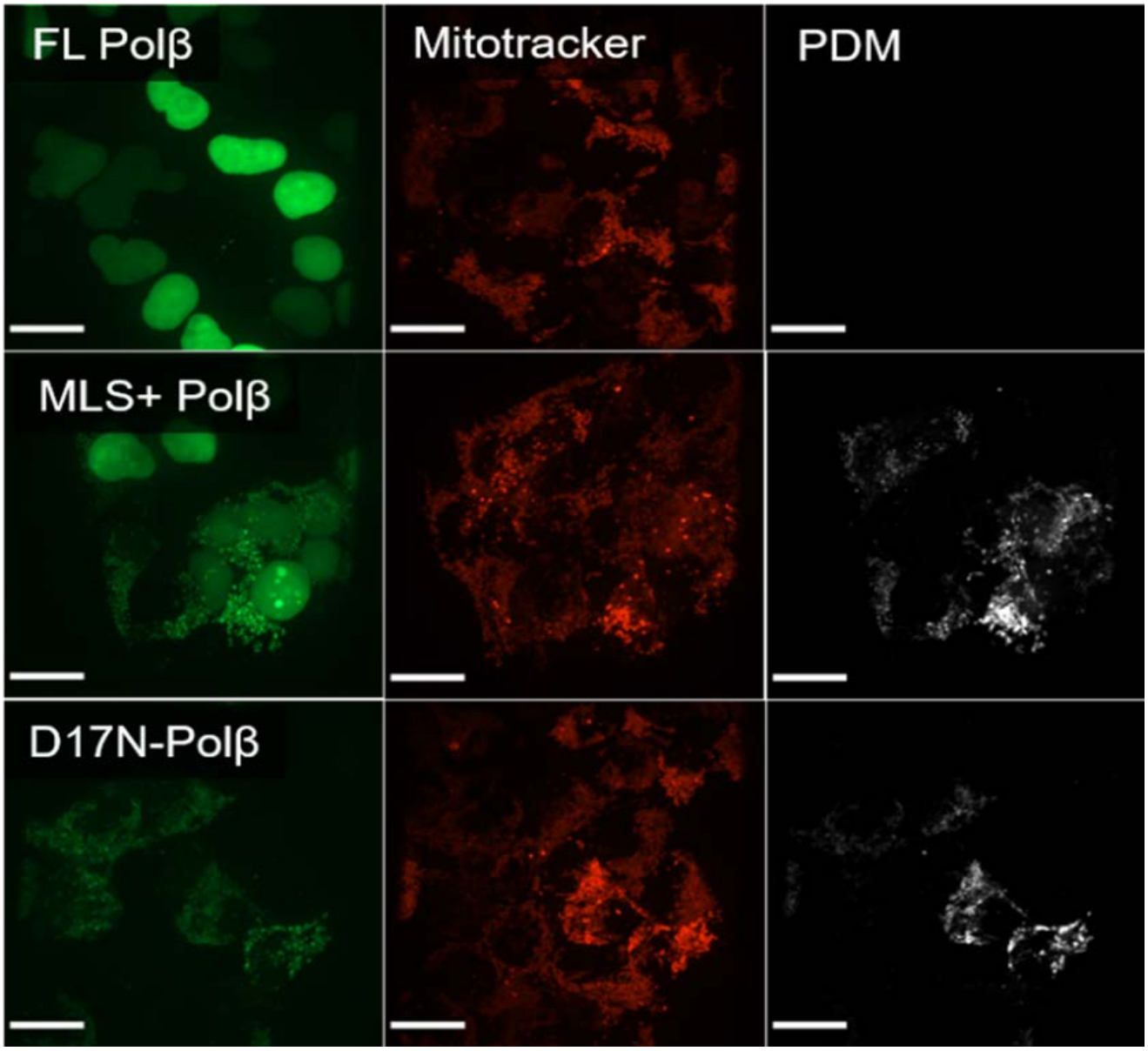



**Figure S1K to accompany Figure 1:** Live cell microscopy using vectors illustrated in diagram (Figure 1E). Polβ has a strong nuclear localization signal at the N-terminal. FL Polβ-GFP cells show minimal col-localization (PDM channel) between GFP and MitoTracker (Far red) signal. Addition of SOD2 MLS signal enhanced the PDM signal, however, only with the deletion of the 17 aa N-terminal region (D17N) is there complete loss of nuclear localization and enhanced mitochondrial localization.( x40, z-stack, scale bar = 40 μM).

Figure S2

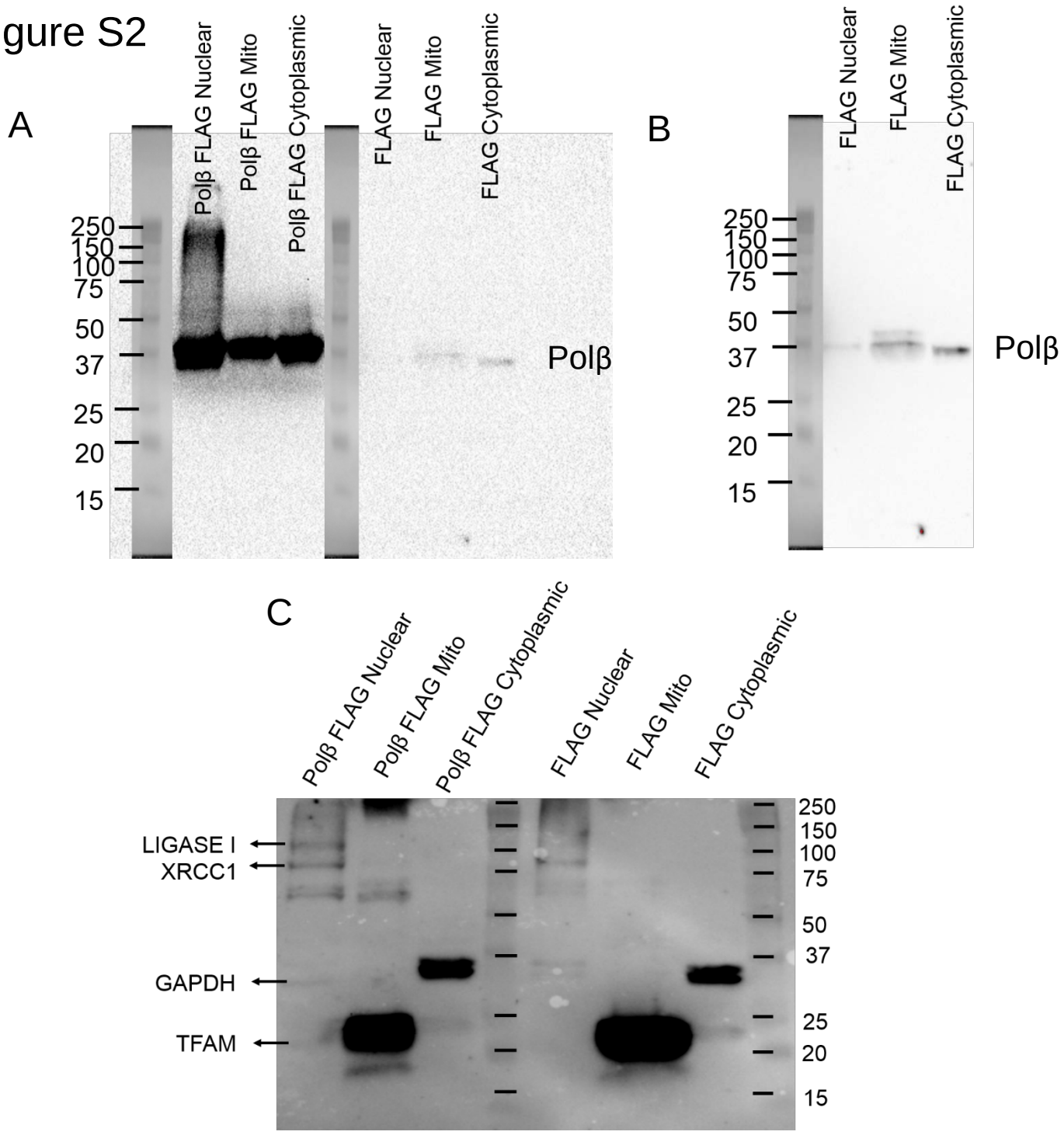



**Figure S2A-C to accompany Figure 2.** HEK293T cells were stably transfected with Polβ – FLAG vector or FLAG vector alone. Cell pellets were extracted for mitochondrial and nuclear or cytoplasmic fractions and used in mass spectrometry and associated immunoblots. **(A)** Flag-tagged Polβ was present at high levels in all fractions tested with protein levels many folds that of the endogenous Polβ levels. **(B)** same as (A) blot with longer exposure to show Polβ bands in the vector samples. This pertinent blot to the research shows clearly, nuclear Polβ with a single band, Mitochondrial Polβ with two bands and the cytoplasmic Polβ with a single band that appears marginally smaller than the nuclear or bottom mitochondrial band. **(C)** Control proteins for blot shown in (A): TFAM was used to measure mitochondrial enrichment, GAPDH for cytoplasmic content. Ligase I and XRCC1 are nuclear DNA repair proteins which were not detected in the cytoplasmic or mitochondrial fractions.12% gel with 10 µg of each fraction loaded.

# Figure S2

D

| Macro Function | - N-terminal | - C-terminal |
|---|---|---|
| | | |
| DNA maintenance | | |
| | (1,2) PARP1 | (15) PARP1 |
| | (3) Lig3, TOP1 | (16) Lig3 |
| | (12) TFAM | (17) TDP1 |
| | (14) SSBP1 | (18) PNKP |
| Stress response/ import | | |
| | (5) HSPA9 | |
| | (6) HSPD1 | |
| | (12) GRPEL1 | |
| | | |
| RNA processing | | |
| | (4) PTCD3 | |
| | (9) MRPS27 | |
| | (10) MRPS22 | |
| | (13) MRPL12 | |
| | | |
| Metabolism | | |
| | (8) BCKDHA | |
| | (11) BCKDHB | |
| | (7) ATP5A1 | |

**Figure S2D to accompany Figure 2B and Table 1**. Complete table of proteins numbered in Figure 2B. Proteins are segregated into four major groups. Of note, Branched Chain Keto Acid Dehydrogenase E1 (BCKDHA) and associated complex protein BCKDHB are both identified, these proteins play a principle role in the conversion of alpha-keto acids to acyl-CoA in the inner mitochondrial membrane

Figure S3

A

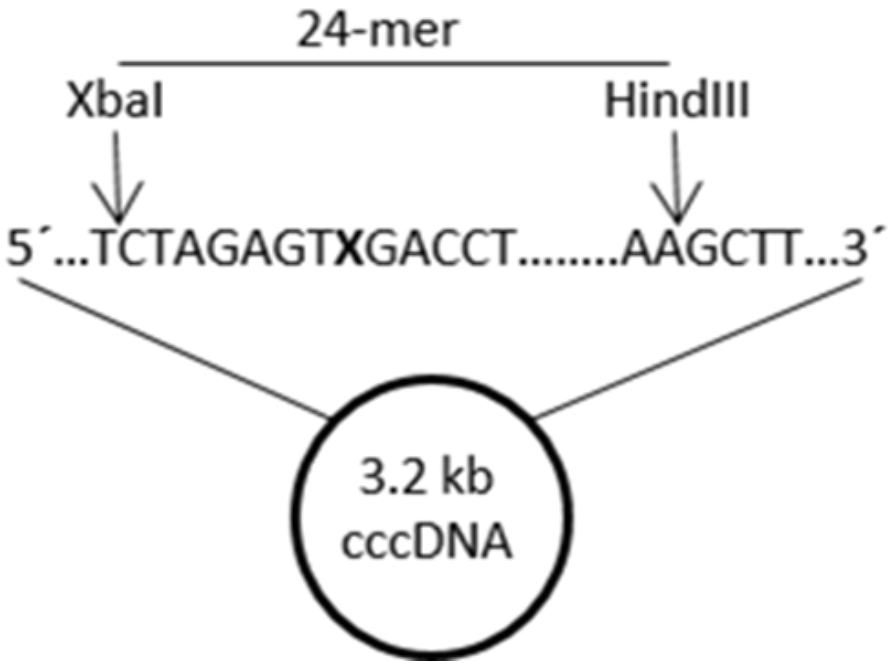



**Figure S3A to accompany Figure 3:** A plasmid substrate with an AP site analog (THF labelled as X) was used to measure in vitro DNA repair capacity in mitochondrial extracts.

Figure S3

B

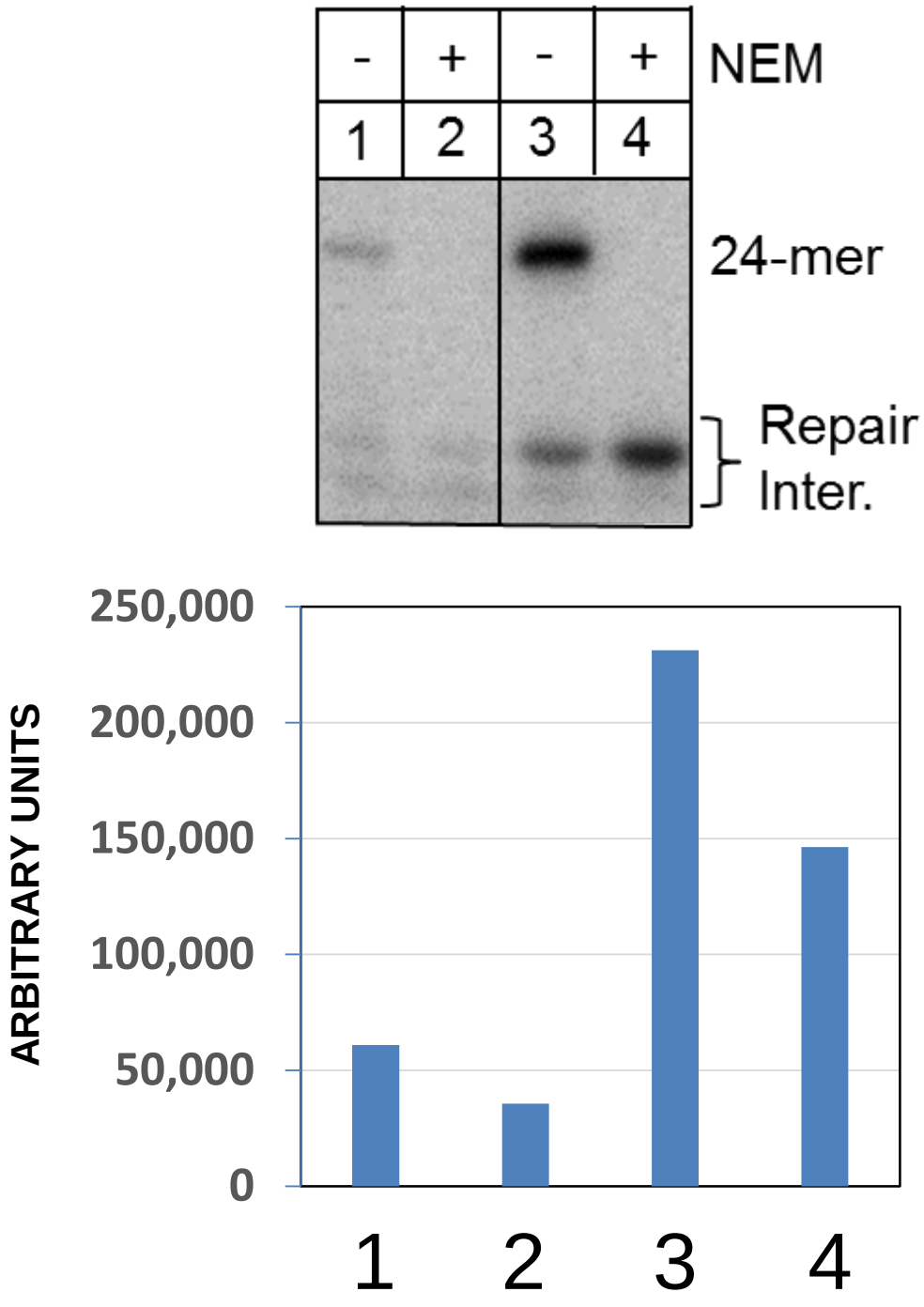



**Figure S3B to accompany Figure 3. (Upper)** HEK extracts were exposed to the Polγ inhibitor, NEM. With inhibitor, an incorporation band is still present in Lanes 2 and 4. Ligation is also completely inhibited. Lane 1: HEK293T vector control mitochondria, Lane 2: HEK vector mitochondria + 5mM NEM, Lane 3: HEK293T+ Polβ mitochondria, Lane 4: HEK293T+Polβ mitochondria + 5mM NEM. All activity assays (15 µg protein input) have been conducted at least in duplicate. (**Lower)** The amount of incorporation was quantitated and graphed. The result shows that the NEM inhibitor does reduce the amount of nucleotide incorporation in both HEK+ vector (compare Lane 1 to Lane 2) and HEK+ Polβ (compare Lane 3 to Lane 4).

Figure S3

C

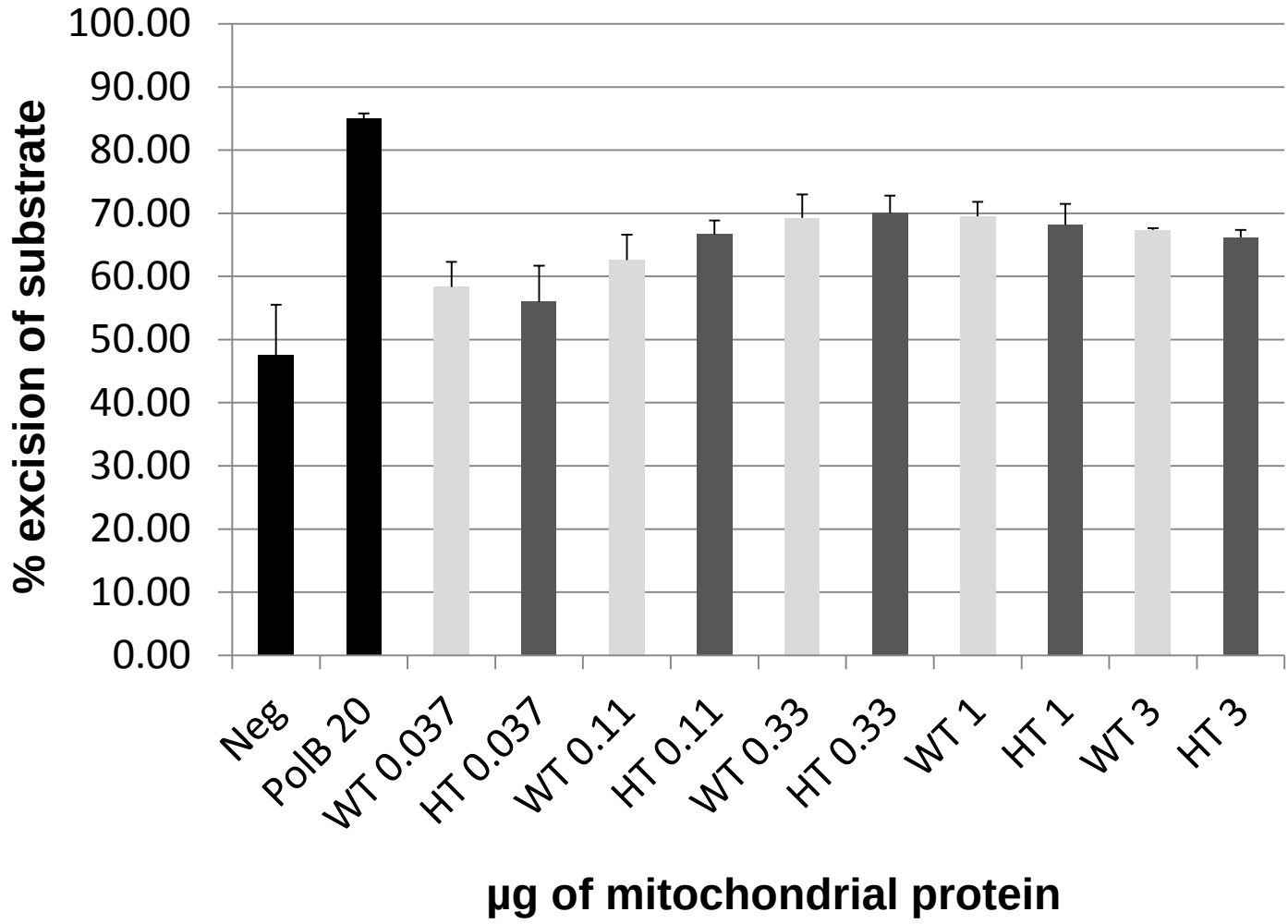



**Figure S3C to accompany Figure 3**. Graphed results from 5'-dRP lyase activity assay using previously described brain mitochondrial extracts. Assay showed no difference in any of the extracts tested. Average of n=3 technical replicates, Error bar are ± standard deviation.

# Figure S4

A

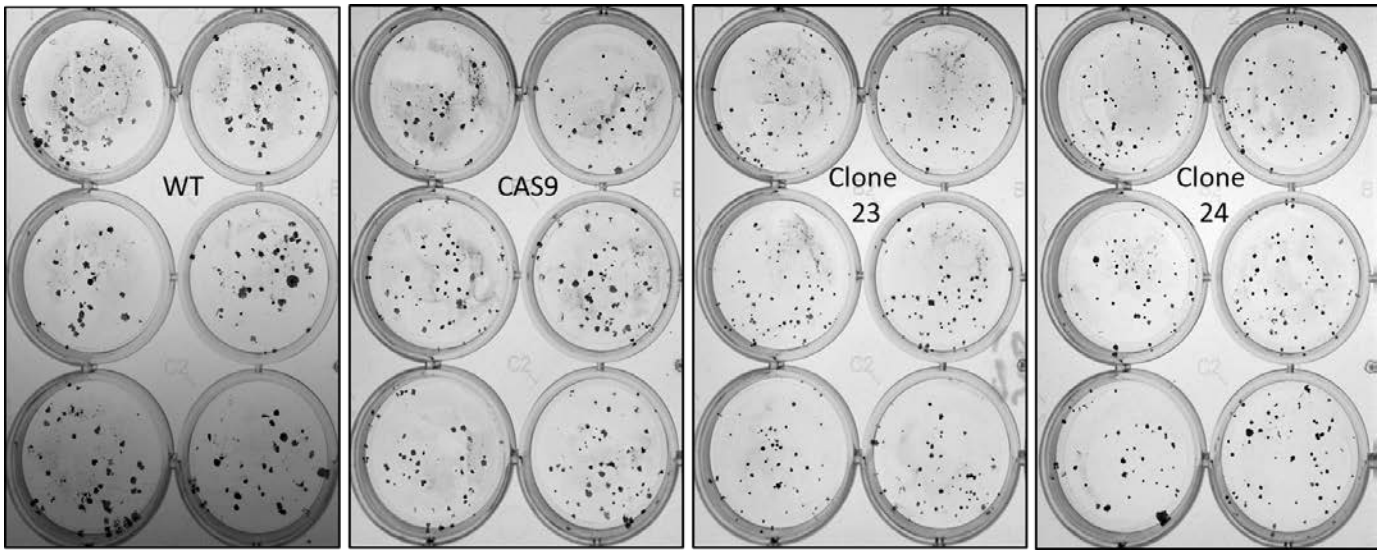



**Figure S4A to accompany Figure 4**. 100 cells were plated from each of the four HEK293T cell lines and allowed to grow for 10 days as per materials and methods. Plating efficiency was comparable across all groups with no statistical difference. (n=6).

# Figure S4

B

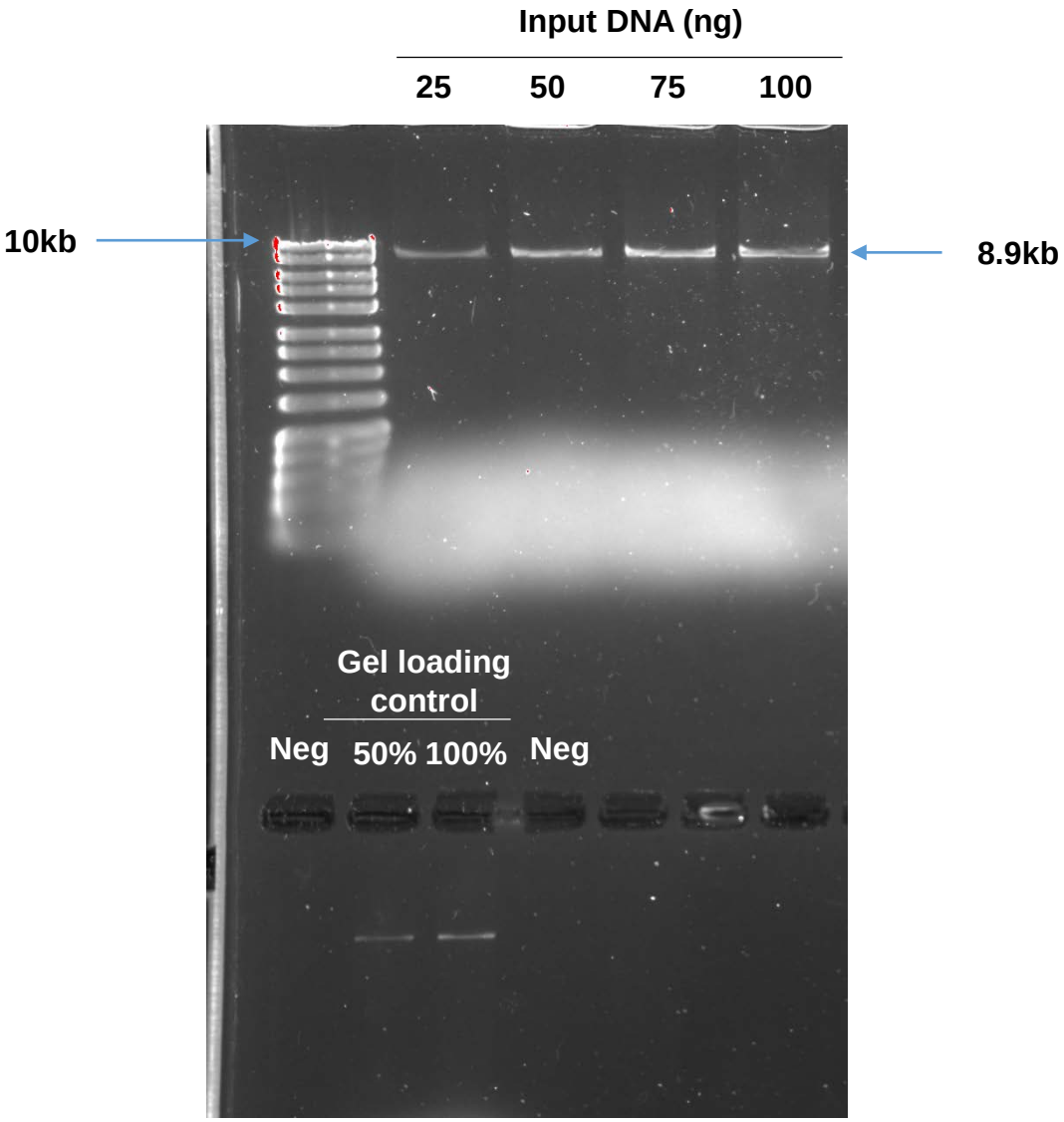
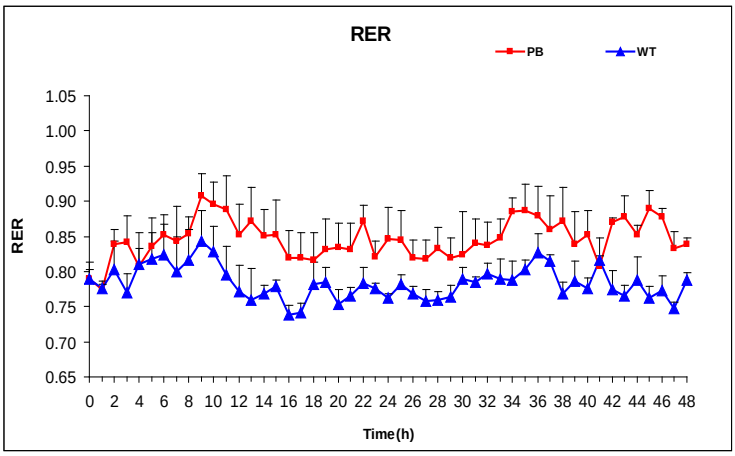
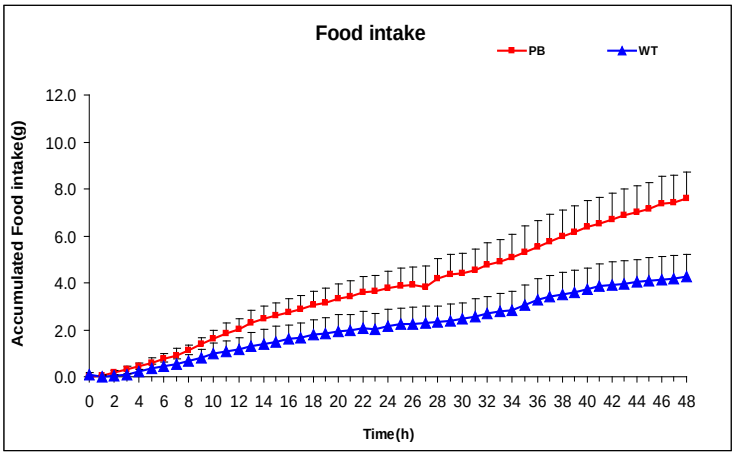


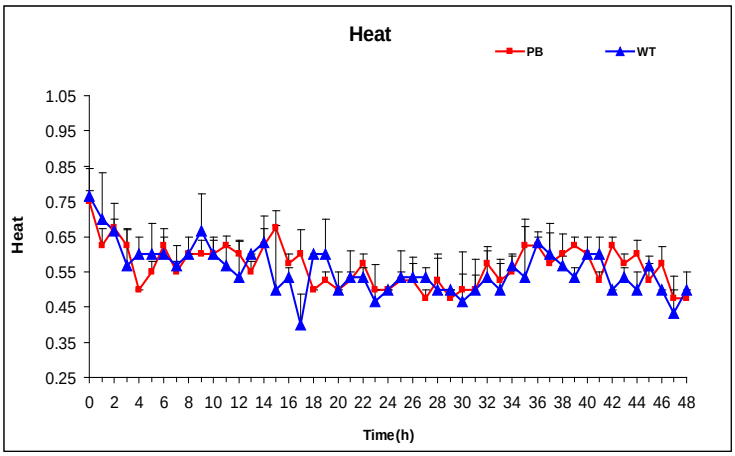

**Figure S4B to accompany Figure 4D:** LA-PCR validation experiment shows that the assay is linear when using input DNA between 25-75ng, but not higher. Amplification results in a single band and there is no residual large fragment DNA in the wells. Representative image of three experiments. Ladder shows 1kb increments. Top band in ladder is 10kb. mtDNA band is 8.9kb. Also shown is the gel loading control to confirm that the analysis is linear and negative PCR controls that show no amplification.

# Figure S4

C Polβ (-/+) mice (PB) Wildtype (WT) mice



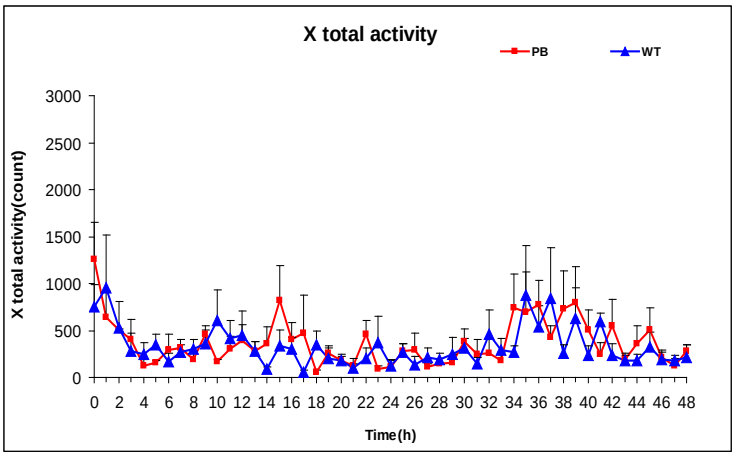




**Figure S4C companion to Figure 4** : Metabolic data from age and weight matched Polβ HT (PB) and WT mice. Respiratory exchange rate (RER) is higher in Polβ deficient animals due to elevated use of glycolysis pathway compared to oxidative phosphorylation. Elevated food intake without weight gain suggests higher energy demand in Polβ HT mice. Heat output and activity is not affected. Please refer to Sykora *et al* (2015), for further information and analysis of this data.

Figure S4

D

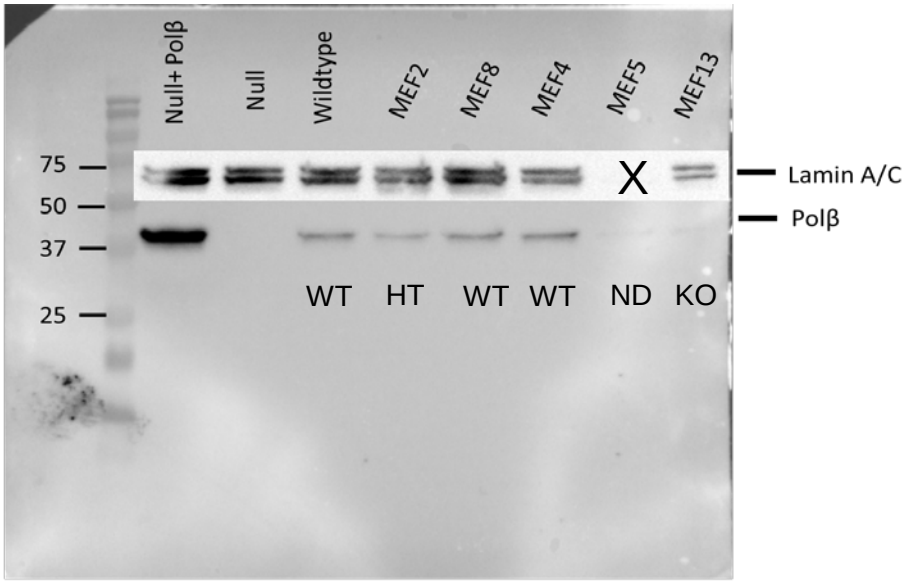



**Figure S4D to accompany Figure 4**: Example of Polβ genotyping to confirm RT-PCR analysis. Protein sample were extracted from embryo tails, 50 µg total protein each well. Sampled were compared to known WT and KO transformed MEFS and also to an over expressing line. Westerns not only confirms RT-PCR genotyping but also shows that the antibody (Trevigen) is specific for only Polβ. Lamin A/C was used to normalize samples. (WT= wildtype, HT= heterozygous, ND= No data, KO= knockout)

E

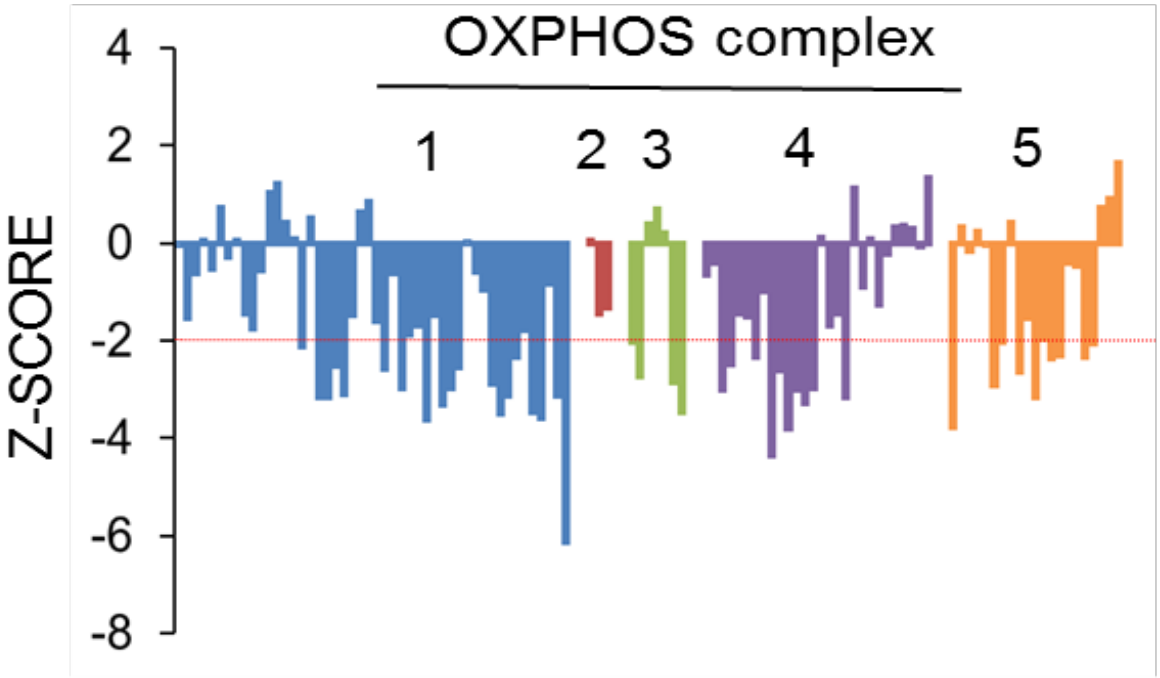



**Figure S4E to accompany Figure 4:** Microarray profiling of 10 primary MEF cells lines (5 Polβ KO/ 5 WT). OXPHOS gene expression was significantly reduced in complex 1, 3, 4 and 5 but not in the nuclear gene derived complex 2. Red line marks -2 fold z-score cut off

# Figure S4

F

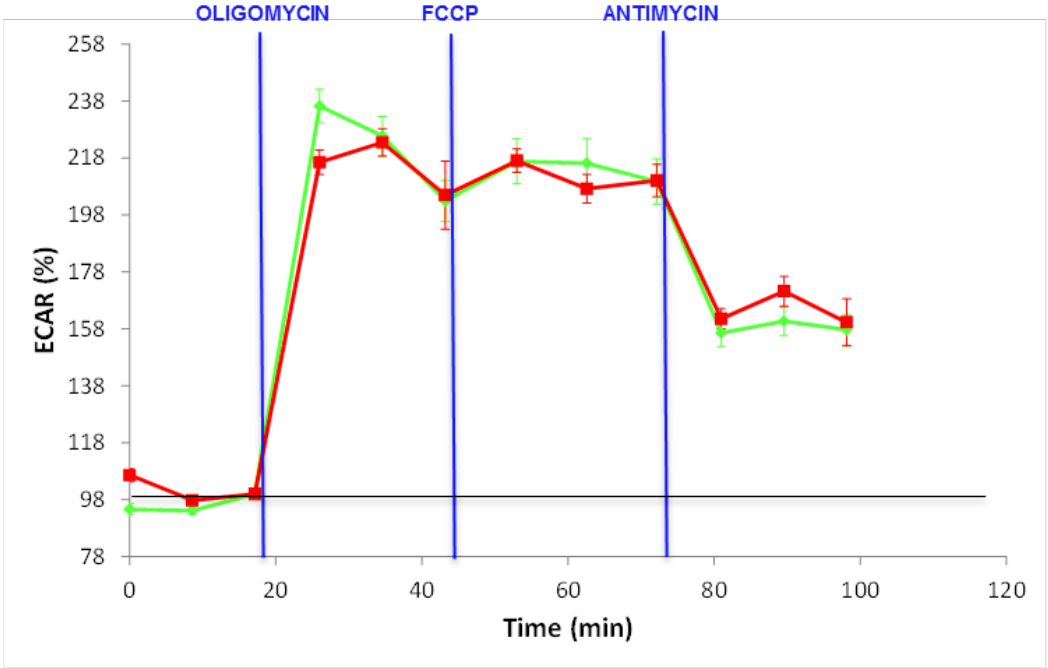



**Figure S4F to accompany Figure 4**: Extracellular acidification rate (ECAR) does not show any significant change between Polβ KO fibroblasts (red line) and passaged matched wildtype fibroblasts (green line). As measured by seahorse apparatus.

**Table S1:** Measurement of mitochondrial parameters in MEF Polβ deficient cells.

| Parameter tested | Transformed MEF Polβ KO |
|---|---|
| | |
| **Mitochondrial Content** | Decreased (Fig.4F) |
| **Membrane Potential** | Decreased (Fig. 4F) |
| **ROS levels** | Unaffected [a] (Fig. 4F) |
| **Network integrity** | Affected (Fig. 4E) |
| **mtDNA copy number** | Decreased – 40% WT |
| **Cell Morphology** | Unaffected |

[a] This was not normalized to mitochondrial content.