**Supplementary Material for HMG-2012-W-01291:**

# *Polg2* is essential for mammalian embryogenesis and is required for mtDNA maintenance

**Margaret M. Humble[1], Matthew J. Young[1], Julie F. Foley[2], Arun R. Pandiri[3], Greg S. Travlos[4], and William C. Copeland[1]***

[1]Mitochondrial DNA Replication Group, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709 [2]Special Techniques Group, Cellular and Molecular Pathology Branch, National Toxicology Program, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709 [3]Experimental Pathology Laboratories, Inc. for CMPB and NTP, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709 [4]Cellular and Molecular Pathology Branch, National Toxicology Program, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709

## MATERIALS AND METHODS

### Genotyping PCR

PCR primers for genotyping were designed to target the *loxP*-sites, *Polg2*, *cre*-recombinase, and the shortened *Polg2* gene due to the excision of exons 5 through 7. The 5’ *loxP*-site forward primer located upstream of exon 5 sequence was 5’-TGGTCCACAGAGCAAGTTCCAGG-3’, and the reverse primer sequence was 5’-GCGCGCGTGGATCTTGTGTTTG-3’. The 3’ *loxP*-site forward primer downstream of the NEO cassette sequence was 5’-AAGTACACCGTAGCTGTCCTCAGAC-3’, and reverse primer was 5’-TCACCATGTGGTGGTGGACTGGAT-3’. Exon 5 of *Polg2* was targeted using the forward primer 5’-TTGCGTTCTGTCTGTAAGTGGGGACG-3’, and the reverse primer 5'-TAGCAACTGGTGGCAAGATGGCTCAGAGG-3'. The *cre*-recombinase forward primer was 5'-CGCAGAACCTGAAGATGTTCGCGATTA-3' and reverse primer was 5'-TCTCCCACCGTCAGTACGTGAGATATC-3'. The primer set detecting the knockout of *Polg2* exons 5 - 7 was the 5' *loxP* forward primer and the 3' *loxP* reverse primer. Amplitaq Gold hot start polymerase (Applied Biosystems) was used according to the manufacture's recommendations in a 25 µl reaction with 1 µl of the ear punch lysate. Cycling conditions for all primers sets were: 10 minutes at 95°C, 32 cycles at 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 1 minute, followed by an extension at 72°C for 7 minutes, and hold at 4°C. All PCR products were run on a 1.25% agarose gel stained with ethidium bromide (Invitrogen).

**Clinical Pathology**

The hematopoietic system of the 2 y.o. aging mouse population was evaluated using whole blood collected with EDTA as the anticoagulant that was analyzed for a complete blood count (CBC). CBC's were performed on a Hemavet 1700 (Drew Scientific Inc.) using reagents obtained from the instrument's manufacturer. Blood collected without anticoagulant was allowed to clot, centrifuged and serum harvested for evaluation of several serum biomarkers. Serum chemistries included: alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acids (TBA), cholesterol (Chol), triglycerides (Trig), high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium (Ca), creatinine (Creat) and creatine kinase (CK). All clinical chemistry assays were performed using an Olympus

AU400e Analyzer (Beckman Coulter Inc.). In general, reagents were purchased from the instrument's manufacturer; reagents for TBA, HDL, and LDL analyses were purchased from Sekisui Diagnostics, LLC, and reagents for SDH came from Sigma/Aldrich Inc. Statistical analyses were performed using an exact Mann-Whitney test to compare genotype groups and the p-values are 2-sided.

## Histopathology

E8.5 embryos were used for COXI staining. Embryos were collected intact within the deciduum, embedded in Optimal Cutting Temperature compound (Tissue-Tek) and frozen in the vapor phase of liquid nitrogen. 10 μm cryostat sections were applied to charged slides (Erie Scientific, LLC), and were stained for COXI activity, washed in water, counterstained with hematoxylin, and mounted in Advantage Permanent mounting media (Axell). Staining protocols were adapted from Chen *et al*. (1), and the Washington University Neuromuscular Disease Center website (http://www.neuro.wustl.edu/neuromuscular/index.html).

## Evaluation of mtDNA deletions

Mouse mtDNA deletions were detected using Expand Long Range PCR reagents (Roche), and all amplifications were carried out with a Gene Amp PCR System 9700 (Applied Biosystems). The sequences of the primers and positions correspond to mtDNA sequence submitted by Bibb et al. (2), and primers were designed for the three most prevalent deletions in mice as previously described (3). Primer L1 8858 forward was 5'-TCTATTCATCGTCTCGGAAG-3' 8877, L2 12883 forward was 5'-

TACCATTCCTAACAGGGTTC-3’ 12902, and H 13354 reverse was 5’-TTTATGGGTGTAATGCGGTG-3’ 13335 (3). Cycling conditions were 92°C for 2 minutes, 12 cycles at 92°C for 10 seconds, 56°C for 15 seconds, and 68°C for 5 minutes, followed by a second cycling step consisting of 30 cycles of 92°C for 10 seconds, 56°C for 15 seconds, and 68°C for 5 minutes, 68°C extension for 7 minutes, and a final hold step at 8°C. Primer pair L2/H amplified a 0.47kb fragment from undeleted wild type mtDNA and served as an internal control. L1/H primer pair resulted in three fragments: 1) 262 bp corresponding to a 4236 bp deletion, 2) 630 bp indicative of a 3867 bp deletion, and 3) 772 bp representing a 3726 bp deletion. The wild type band lacking deletions yielded a band of 7.5 kb, but was only seen in 10 month old brain tissue due to a change in stocks of PCR reagents. The deletions in mouse mtDNA are a result of repeats within the sequence as previously described by Tanhauser and Laipis (4).

Mouse mtDNA deletions were quantitated to determine mtDNA heteroplasmy. qPCR primers and the FAM/ TAMRA labeled probes targeting the rarely deleted mitochondrial gene, *Nd1,* and the more frequently deleted mitochondrial gene, *Nd4*, were all designed by Applied Biosystems. qPCR amplifications were carried out on an ABI PRISM 7900HT Sequence Detector (Applied Biosystems) with a cycling protocol consisting of 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. All reactions were done in triplicate, and 1 μl of diluted (1:50) tissue lysate was used in each 25 μl reaction using TaqMan 2X Universal Mix (Applied Biosystems). To calculate the relative copy number ($R$) the following equation was used: $R = 2^{-\Delta\mathrm{Ct}}$ , where $\Delta\mathrm{Ct}$ is the $\mathrm{Ct}_{Nd1} - \mathrm{Ct}_{Nd4}$ (5).

### RNA isolation and cDNA generation

Mouse liver, brain, heart, kidney, and muscle tissues were collected and stored at 4°C in RNA*later* solution (Ambion). Isolation of total RNA was obtained by using the RNAeasy mini-kit (Qiagen) according to manufacture's recommendations. Tissues were disrupted using the TissueLyser apparatus (Qiagen) with the lysis buffer provided in the RNAeasy kit. Reverse transcription was performed using a High Capacity RT (Applied Biosystems) with 150 ng of total RNA in a 60 µl reaction. cDNA generation was performed in a Gene Amp PCR System 9700 (Applied Biosystems) with cycling parameters of 25°C for 10 minutes, 37°C for 90 minutes, 85°C for 5 seconds, and held at 4°C to synthesize cDNA.

### Transcript analysis of the mtDNA replication fork

All primers and fluorescently labeled probes were predesigned and sold as TaqMan Gene Expression Assay 20X mixes (Applied Biosystems), and all sets spanned intron/exon boundaries to avoid DNA amplification. The TaqMan primers and probes targeted the genes: *Polg2, Polg, Twinkle, Tfam, Tfb1, Tfb2, mtRnap, and mtSsb.* All probes were labeled at the 5' end with FAM (reporter dye) and TAMRA (quencher dye) at the 3' end. 1 µl of cDNA was used in each 25 µl reaction with TaqMan 2X Universal Mix with UNG (Applied Biosystems), and all reactions were run in triplicate. QPCR amplifications were carried out on an ABI PRISM 7900HT Sequence Detector (Applied Biosystems) with a cycling protocol consisting of 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The standard Comparative $C_T$ Method ($\Delta\Delta$ $C_T$ ) was used for relative quantification calculations as described in the ABI technical guide on performing relative quantitation of gene expression using real-time PCR (http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042380.pdf), and *Actin* was used as the endogenous reference. Data were expressed as a change from the wild type, and the Mann-Whitney statistical *U*-test was applied to determine statistical significance.

## RESULTS

### Characterization of adult *Polg2* heterozygous mice

### Histopathology results for the aged *Polg2*$^{+/-}$ mice

Aged *Polg2*$^{+/-}$ mice at 2 years were euthanized and comprehensively examined for any alterations in phenotype associated with the *Polg2* heterozygous deletion. There were no differences microscopically between *Polg2*$^{+/-}$ and WT groups at 2 years of age. There was however a moderate to marked microvesicular steatosis within the livers of all the animals (*Polg2*$^{+/-}$ and WT). In addition, several livers also exhibited macrovesicular steatosis with occasional lipid granulomas and minimal to mild inflammatory cell infiltration. Further, there was a sex related effect in the severity of the skeletal muscle lesions. Within the male animals (from both *Polg2*$^{+/-}$ and WT groups), there were moderate to marked degenerative changes within the skeletal muscle (rectus femoris and gastrocnemius) characterized by loss of cross striations, amorphous slightly hypereosinophilic sarcoplasm, nuclear internalization, and non-uniform muscle fiber diameter. In addition, several muscle fibers had a central pale amphophilic amorphous material devoid of cross striations and in some cases the skeletal muscle fibers are replaced by adipose tissue. In comparison, the skeletal muscle lesions within the female mice (from both *Polg2*$^{+/-}$ and WT groups) were minimal to mild. There was a sporadic low incidence of various neoplasms such as lymphoma, histiocytic sarcoma and hepatic adenoma that is most likely attributed to age and strain, but it was not related to the loss of one copy of the *Polg2* gene.

## Heart Electron Microscopy

No differences in mitochondrial ultra-structural features of 1-year old *Polg2*$^{+/-}$ (n=4) and wild-type animals (n=4) were observed. The myofibrils were uniform with evenly spaced striations. The Z-bands of the myofibrils were also evenly spaced. Small lipid droplets, some containing slender myelin figures, were seen randomly scattered throughout the sarcoplasm along with randomly scattered lysosomes, myelin figures, and glycogen particles in both genotypes (Supplemental fig. 2). While few in number, we identified some randomly scattered mitochondria with cristae that were slightly separated indicative of subtle mitochondrial swelling which were found in both genotypes.

## Clinical pathology results for the aged *Polg2*$^{+/-}$ mice

In general, there were no changes in the hematology variables that were considered pathognomonic and attributable to the loss of one copy of the *Polg2* gene in the 2-year old heterozygous population (Supplemental tables 1 - 4). Further, there was no consistent change in the clinical chemistry that would help characterize a phenotype for the *Polg2* $^{+/-}$ genetically modified mice. However, clinical chemistry data of a few individual animals demonstrated changes that suggested pathology in the liver and/or muscle. For example, one female *Polg2*$^{+/-}$ animal (ID 38-8) demonstrated ALT, SDH, AST, and CK activities that were ≥4-fold higher compared to the mean values for the wild-type females

(Supplemental tables 1 and 2). This animal also had an exceptionally low serum calcium concentration. Increases in serum ALT, SDH, and AST activities are consistent with hepatocellular injury resulting in leakage of the enzymes into circulation. While the increases are not exceedingly high, they were high enough to consider the possibility of hepatocellular pathology. Based on previous work showing a high concordance of increases in ALT and SDH to predict histopathology changes in the liver (9), the blood chemistry of this one particular animal may suggest a pathological change in the liver. Although, slight changes in clinical chemistry parameters of other individual mice were observed they were most likely attributable to the increased variability often seen in laboratory animals of advanced age.

## References

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## SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure 1**. The protein alignment of human and mouse POLG2 shows a highly conserved **C-**terminus. Amino acids that are identical are highlighted in yellow, and the green highlighted amino acids indicated a change in the amino acid with a conservation of charge. Exons 5, 6, and 7 are deleted in our Polg2 mouse model. Exon 5 (red) encodes for the dimerization of POLG2, while exons 6 (blue) and 7 (purple**)** both code for the POLG and DNA binding domains.

**Supplemental Figure 2.** Electron microscopy of heart tissue from 1 y.o. wild type (+/+) and Polg2 heterozygous (+/**-**) mice. At 1700X magnification the normal pattern of clustered of mitochondria (darker grey) that are perpendicular to myofibrils of cardiac tissue, and are similar in both the wild type and heterozygous mouse. At 20,500X magnification the mitochondria of the Polg2$_{+/-}$ are similar in size, shape, and cristae pattern compared to the wild type.

**Supplemental Figure 3.** Measuring expression levels of genes encoding proteins important for mtDNA replication. qPCR was used to determine transcript levels of Polg2, Polg, Tfam, mtRnap, Tfb1, Tfb2, Twinkle, and mtSsb. The standard $\Delta\Delta C_T$ method of analysis was used to report data as fold change from the mean levels of wild-type tissues. The transcripts levels were measured in 1-year Polg2$^{+/-}$ tissues (n=10). Tissues included: liver (blue), muscle (red), brain (green), heart (orange), and kidney (light blue). The Mann-Whitney U-test showed no statistical difference ($p>0.05$) in expression from the wild-type. Error bars indicate ± standard deviation.

**Supplemental Figure 4.** Detection of mtDNA deletions in mouse tissue. Mouse mtDNA deletions were detected using Long Range PCR in Polg2$_{+/-}$ (n=3) and age-matched WT (n=3). L1/H primer pair resulted in three fragments: 1) 262 bp corresponding to a 4236 bp deletion, 2) 630 bp indicative of a 3867 bp deletion, and 3) 772 bp representing a 3726 bp deletion. Tissue samples were amplified with an internal control primer set L2/H resulting resulting in a 470 bp fragment. A) mtDNA deletions in 6 month brain (B), liver (L), muscle (M), and heart (H) tissues. Deletions were observed in brain and heart of all 3 WT and 2 Polg2$_{+/-}$ animals. B) mtDNA deletions in 10 month heart samples. The three most common mouse mtDNA deletions were observed in both the wild-type and heterozygous mice at 10 month of age.

**Supplemental Figure 5.** Determing mtDNA heteroplasmy in *Polg2*$^{+/-}$ tissues. mtDNA heteroplasmy was evaluated using qPCR in muscle, brain, heart, and liver tissue of mice 1 year of age. The relative copy number was determined using the following calculation: $R = 2^{-\Delta Ct}$ , where $\Delta Ct$ is the $Ct_{Nd1} - Ct_{Nd4}$. Wild type mice tissues are shown in blue and heterozygous mice tissues in red. There was no significant difference in mtDNA heteroplasmy between the wild type and *Polg2* heterozygous mice.

**Supplemental Figure 6.** The morphology of littermates was analyzed at E8.0-8.5 post coitum. **A)** A sagittal view of a WT embryo enveloped in the amniotic sac with the allantois (a) extending from the embryo, a well formed cardiac sac (c), distinct somites (s), and normal head (h) region. **B)** A sagittal view of a *Polg2*$^{-/-}$ embryo littermate within an intact amniotic sac showing the complete loss of a cardiac sac, abnormally developed head region, and undefined somite architecture.

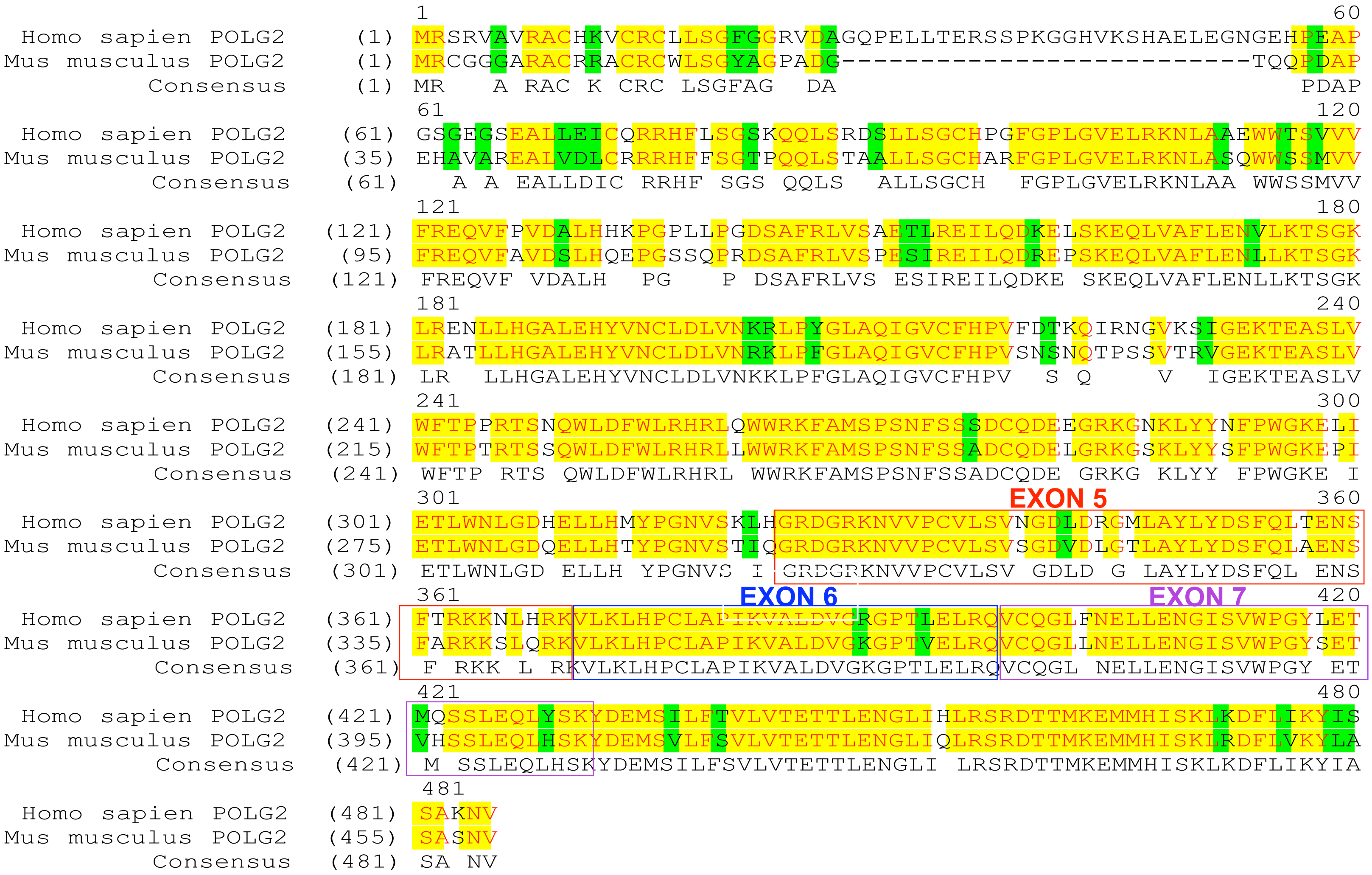
**Supplemental Table 1.** A. The body weights of *Polg2*$^{+/-}$ mice at 40 weeks and 2 years of age. No statistical differences in the total body weight of the wild-type and *Polg2*$^{+/-}$ mice were observed at 40 weeks and 2 years of age. B. The relative liver weights were measured in a population of 2 year old mice. The relative liver weight is calculated based on the ratio of liver weight to the body weight of each mouse. Again, no significant difference was found in the relative liver weights of the wild type in comparison to the *Polg2*$^{+/-}$ mice.

**Supplemental Table 2.** Serum clinical chemistry of 2 y.o. male mice. Serum chemistries included: alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acids (TBA), cholesterol (Chol), triglycerides (Trig), high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium (Ca), creatinine (Creat) and creatine kinase (CK). Changes in individual mice were observed, but they are most likely attributed to the age of the mice, and not the loss of Polg2 on one allele. The Mann-Whitney test was used to determine the significance at $p<0.05$. No sample received (nsr). Quantity not sufficient for assay (qns). Values determined by dilution (*).

**Supplemental Table 3**. Serum clinical chemistry of 2 y.o. female mice. Serum chemistries included: alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acids (TBA), cholesterol (Chol), triglycerides (Trig), high-density lipoprotein (HDL), low-density lipoprotein (LDL), calcium (Ca), creatinine (Creat) and creatine kinase (CK). Changes in individual mice were observed, but they are most likely attributed to the age of the mice, and not the loss of Polg2 on one allele. The Mann-Whitney test was used to determine the significance at $p<0.05$. No sample received (nsr). Quantity not sufficient for assay (qns). Values determined by dilution (*).

**Supplemental Table 4.** Cell blood counts (CBC) were determined on the male mouse aging colony. Parameters measured included: white blood cells (WBC), red blood cells (RBC), neutophils (Neut), lymphocytes (Lym ), monocytes (Mono), eosinophils (Eos), basophils (Baso), hemoglobin (Hgh), hematocrit (Hct), platelet count (Plts), reticulocyte count (Retic), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), percentage of hematocrit (Spun), and large unstained cells (LUC). There were no significant changes observed between the wild type and the heterozygous mice that can be attributed to the loss of one copy of Polg2 at 2 years of age. The Mann-Whitney U-test was used to determine the significance at $p<0.05$.

**Supplemental Table 5.** Cell blood counts (CBC) were determined on the female mouse aging colony. Parameters measured included: white blood cells (WBC), red blood cells (RBC), neutophils (Neut), lymphocytes (Lym ), monocytes (Mono), eosinophils (Eos), basophils (Baso), hemoglobin (Hgh), hematocrit (Hct), platelet count (Plts), reticulocyte count (Retic), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), percentage of hematocrit (Spun), and large unstained cells (LUC). There were no significant changes observed between the wild type and the heterozygous mice that can be attributed to the loss of one copy of Polg2 at 2 years of age. The Mann-Whitney U-test was used to determine the significance at $p<0.05$.



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                           1                                                         60
  Homo sapien POLG2    (1) MRSRVAVRACHKVCRCLLSGFGGRVDAGQPELLTERSSPKGGHVKSHAELEGNGEHPEAP
 Mus musculus POLG2    (1) MRCGGGARACRRACRCWLSGYAGPADG--------------------------TQQPDAP
          Consensus    (1) MR   A RAC K CRC LSGFAG  DA                             PDAP
                           61                                                       120
  Homo sapien POLG2   (61) GSGEGSEALLEICQRRHFLSGSKQQLSRDSLLSGCHPGFGPLGVELRKNLAAEWWTSVVV
 Mus musculus POLG2   (35) EHAVAREALVDLCRRRHFFSGTPQQLSTAALLSGCHARFGPLGVELRKNLASQWWSSMVV
          Consensus   (61)   A A EALLDIC RRHF SGS QQLS  ALLSGCH  FGPLGVELRKNLAA WWSSMVV
                           121                                                      180
  Homo sapien POLG2  (121) FREQVFPVDALHHKPGPLLPGDSAFRLVSAETLREILQDKELSKEQLVAFLENVLKTSGK
 Mus musculus POLG2   (95) FREQVFAVDSLHQEPGSSQPRDSAFRLVSPESIREILQDREPSKEQLVAFLENLLKTSGK
          Consensus  (121) FREQVF VDALH  PG   P DSAFRLVS ESIREILQDKE SKEQLVAFLENLLKTSGK
                           181                                                      240
  Homo sapien POLG2  (181) LRENLLHGALEHYVNCLDLVNKRLPYGLAQIGVCFHPVFDTKQIRNGVKSIGEKTEASLV
 Mus musculus POLG2  (155) LRATLLHGALEHYVNCLDLVNRKLPFGLAQIGVCFHPVSNSNQTPSSVTRVGEKTEASLV
          Consensus  (181) LR  LLHGALEHYVNCLDLVNKKLPFGLAQIGVCFHPV  S Q    V  IGEKTEASLV
                           241                                                      300
  Homo sapien POLG2  (241) WFTPPRTSNQWLDFWLRHRLQWWRKFAMSPSNFSSSDCQDEEGRKGNKLYYNFPWGKELI
 Mus musculus POLG2  (215) WFTPTRTSSQWLDFWLRHRLLWWRKFAMSPSNFSSADCQDELGRKGSKLYYSFPWGKEPI
          Consensus  (241) WFTP RTS QWLDFWLRHRL WWRKFAMSPSNFSSADCQDE GRKG KLYY FPWGKE I
                           301                                                      360
  Homo sapien POLG2  (301) ETLWNLGDHELLHMYPGNVSKLHGRDGRKNVVPCVLSVNGDLDRGMLAYLYDSFQLTENS
 Mus musculus POLG2  (275) ETLWNLGDQELLHTYPGNVSTIQGRDGRKNVVPCVLSVSGDVDLGTLAYLYDSFQLAENS
          Consensus  (301) ETLWNLGD ELLH YPGNVS I GRDGRKNVVPCVLSV GDLD G LAYLYDSFQL ENS
                           361                                                      420
  Homo sapien POLG2  (361) FTRKKNLHRKVLKLHPCLAPIKVALDVGRGPTLELRQVCQGLFNELLENGISVWPGYLET
 Mus musculus POLG2  (335) FARKKSLQRKVLKLHPCLAPIKVALDVGKGPTVELRQVCQGLLNELLENGISVWPGYSET
          Consensus  (361) F RKK L RKVLKLHPCLAPIKVALDVGKGPTLELRQVCQGL NELLENGISVWPGY ET
                           421                                                      480
  Homo sapien POLG2  (421) MQSSLEQLYSKYDEMSILFTVLVTETTLENGLIHLRSRDTTMKEMMHISKLKDFLIKYIS
 Mus musculus POLG2  (395) VHSSLEQLHSKYDEMSVLFSVLVTETTLENGLIQLRSRDTTMKEMMHISKLRDFLVKYLA
          Consensus  (421) M SSLEQLHSKYDEMSILFSVLVTETTLENGLI LRSRDTTMKEMMHISKLKDFLIKYIA
                           481
  Homo sapien POLG2  (481) SAKNV
 Mus musculus POLG2  (455) SASNV
          Consensus  (481) SA NV
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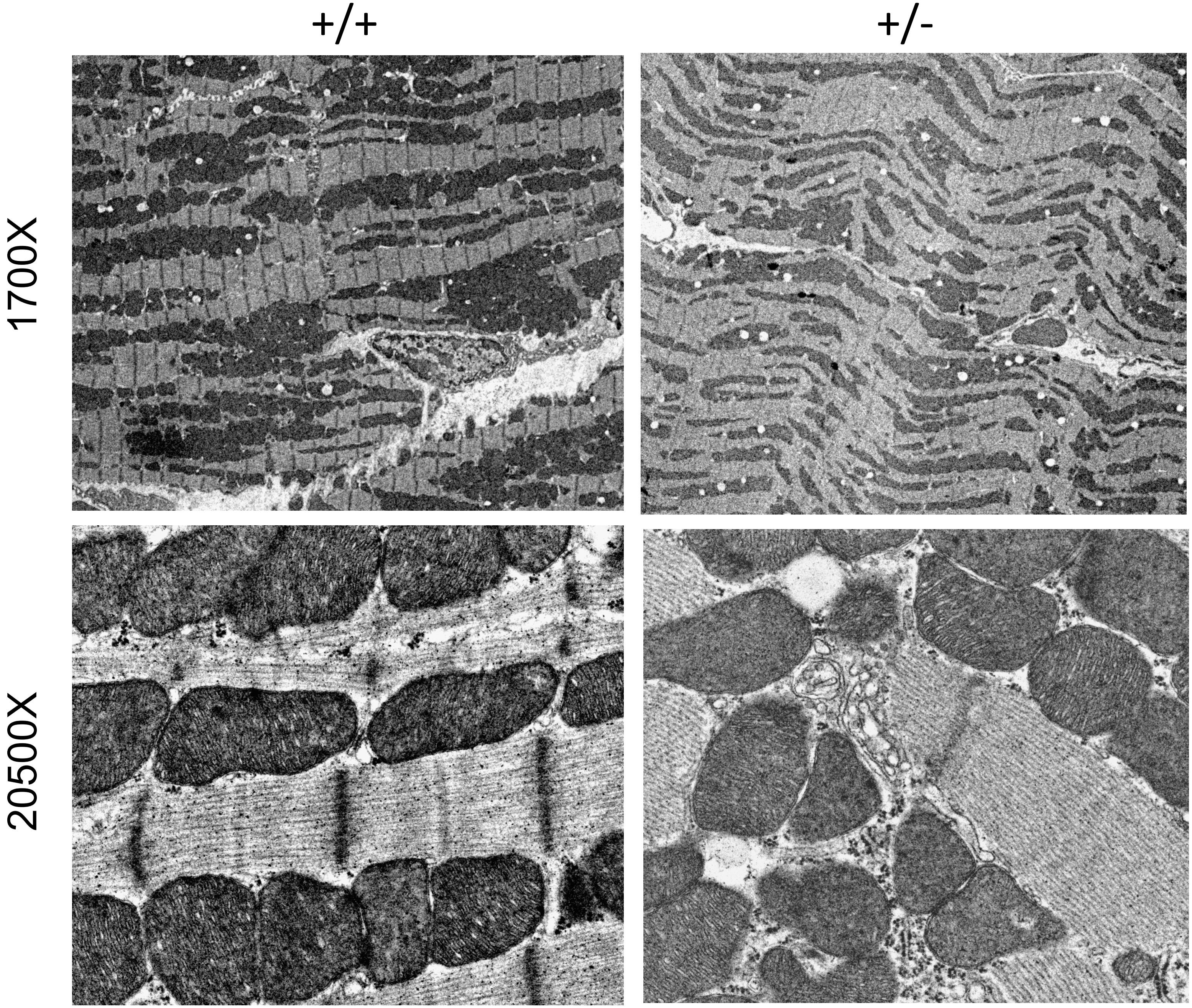
Supplemental figure 1



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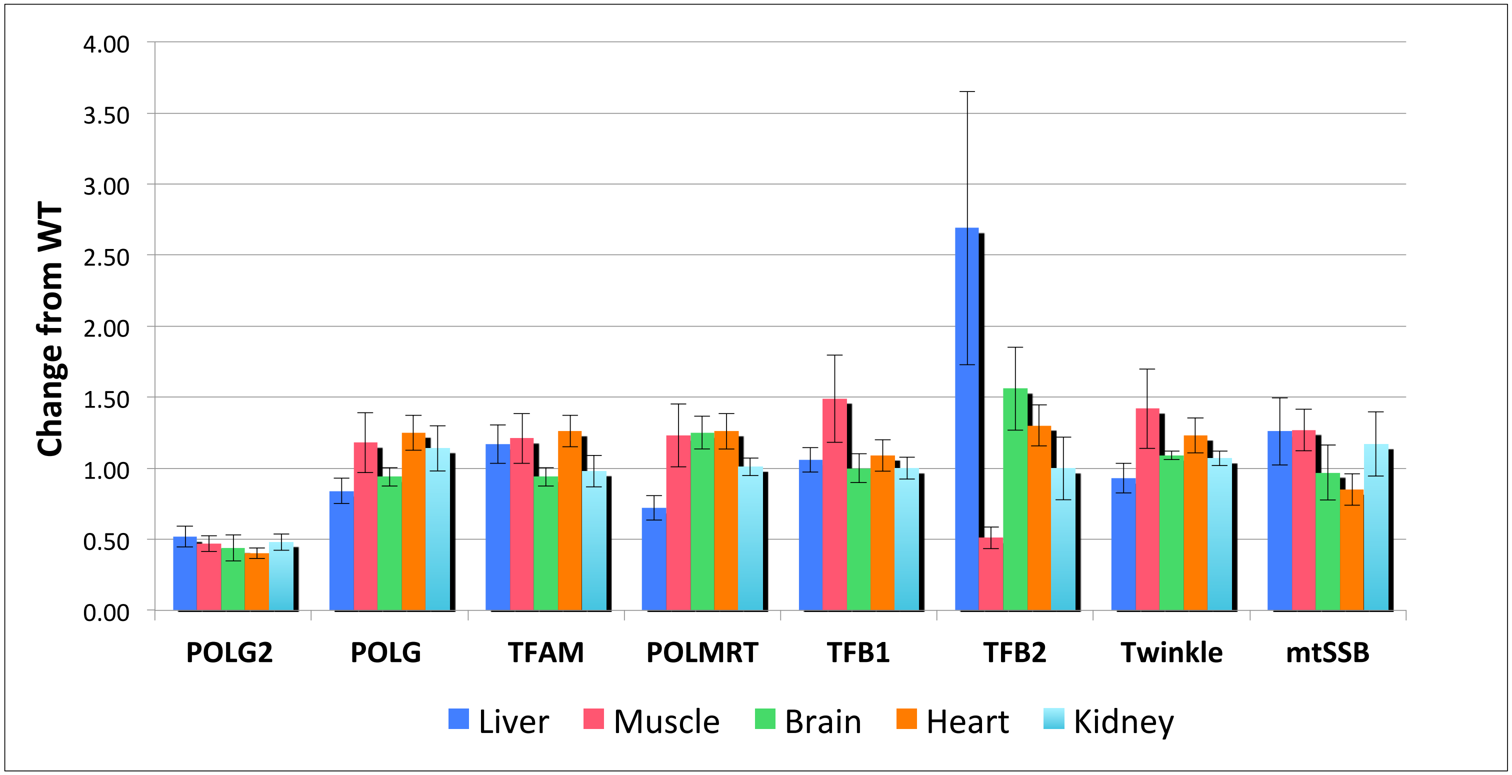

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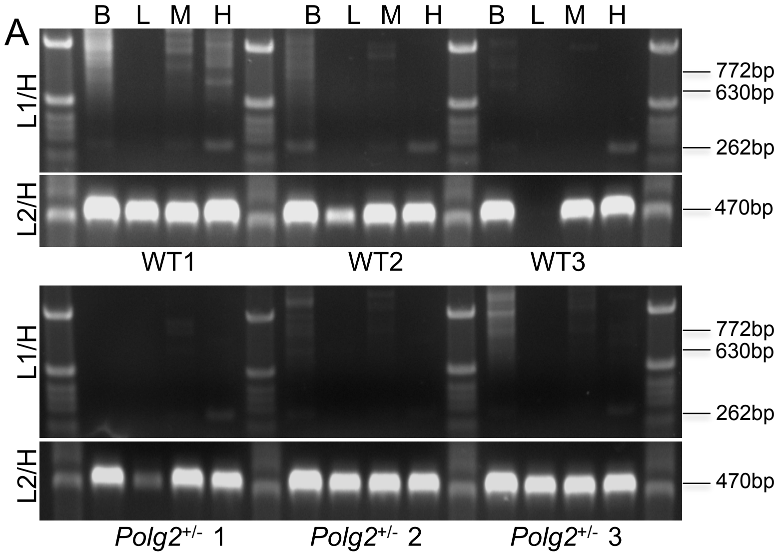
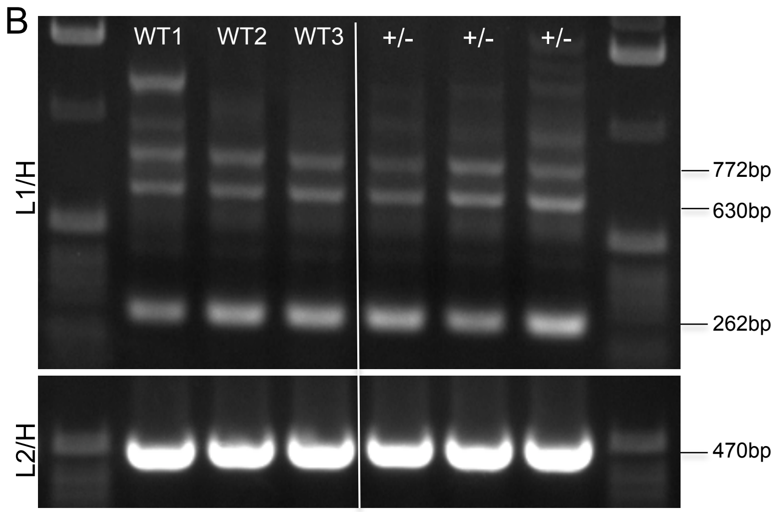
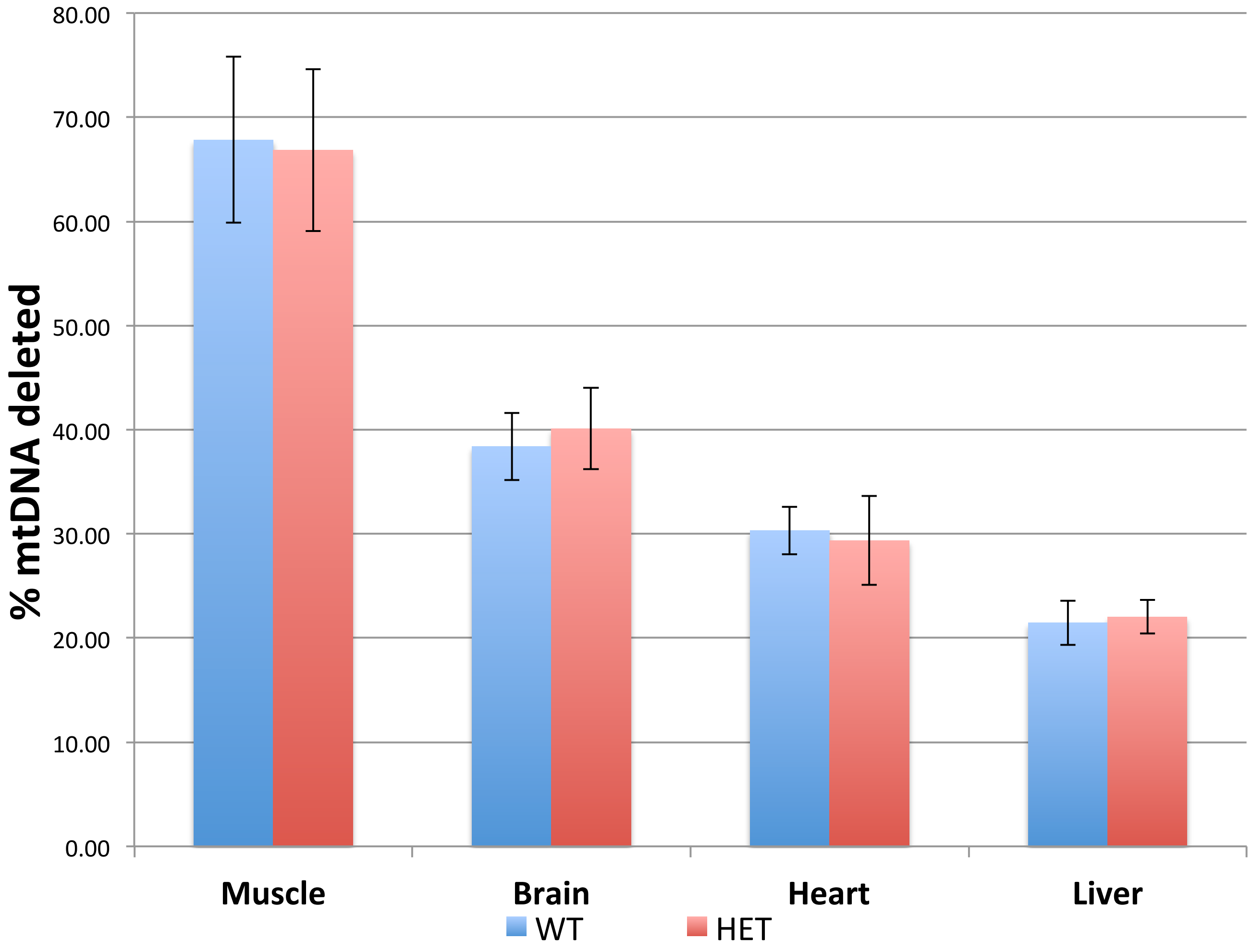


mtDNA deletions at 6 months



mtDNA deletions in heart at 10 months of age

Supplemental figure 4



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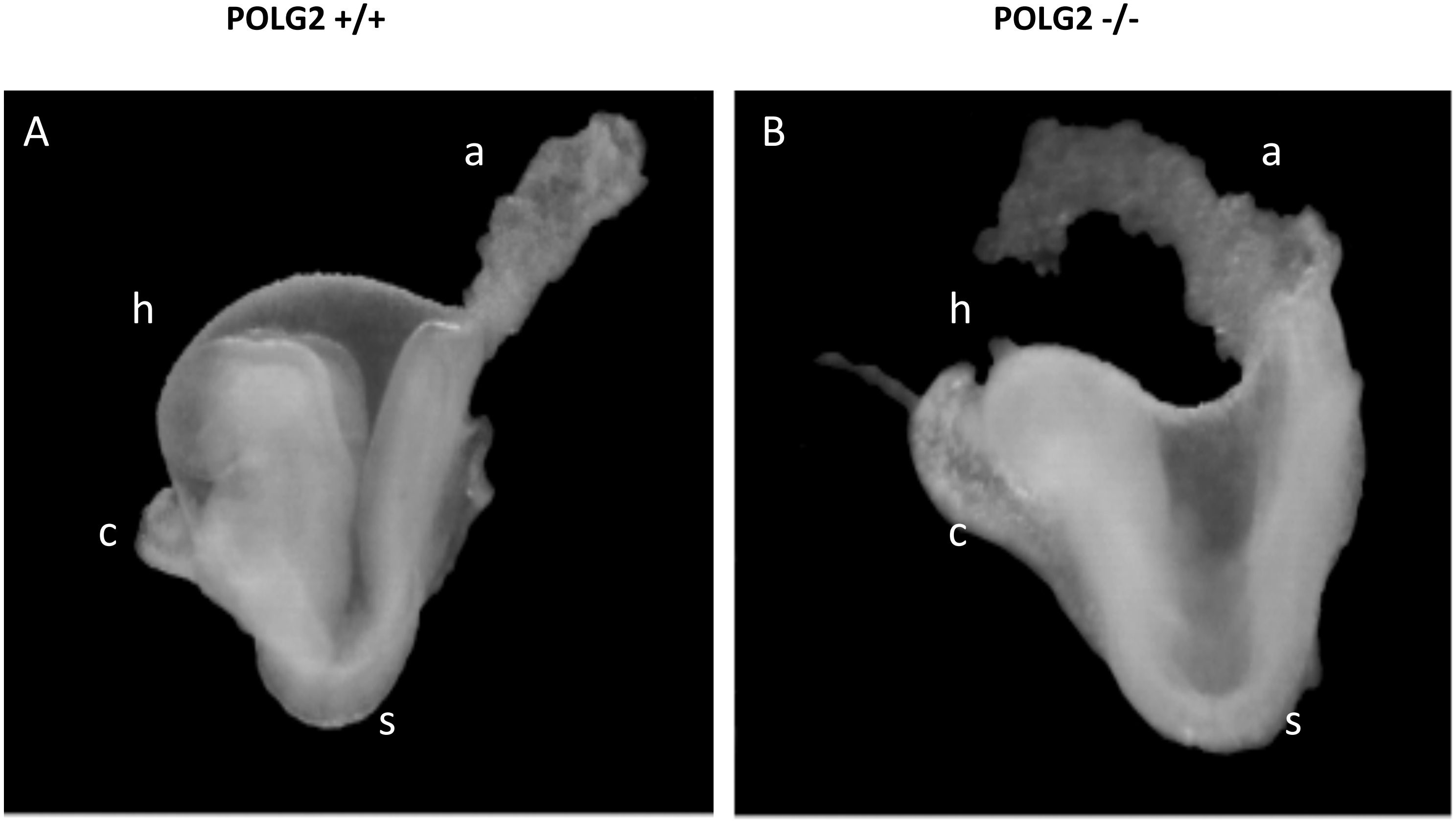

POLG2 +/+

POLG2 -/-

Supplemental figure 6

**A.**

| *Polg2 +/-* | Body Weight (gm) | STD |
|---|---|---|
| **40 wk WT Male** | 36.88 | 5.73 |
| **40 wk HET Male** | 39.48 | 6.34 |
| **40 wk WT Female** | 36.38 | 7.88 |
| **40 wk HET Female** | 26.43 | 3.71 |
| **2 yr WT Male** | 41.55 | 7.88 |
| **2 yr HET Male** | 38.60 | 4.73 |
| **2 yr WT Female** | 34.50 | 10.9 |
| **2 yr HET Female** | 36.27 | 7.03 |

**B.**

| 2 yr *Polg2 +/-* | Liver Weight (gm) | STD | Relative Liver Weight Ratio |
|---|---|---|---|
| **WT Male** | 1.70 | 0.28 | 0.04 – 0.05 |
| **HET Male** | 2.23 | 0.70 | 0.04 – 0.11 |
| **WT Female** | 1.62 | 0.46 | 0.04 – 0.05 |
| **HET Female** | 1.60 | 0.21 | 0.04 – 0.05 |

Supplemental table 1

| Sample# | Group ID | Sex | ALT | SDH | AST | ALP | TBA | Chol | Trig | HDL | LDL | Ca | Creat | CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | U/L | U/L | U/L | U/L | uMol/L | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl | U/L |
| 38-1 | MPOLG2 | M | 483 | 351.2* | 386 | 31 | 3.8 | 69 | 66 | 35 | 6 | 5.8 | 0.1 | 481 |
| 38-2 | MPOLG2 | M | 79 | 65.8 | 75 | 57 | 39.2 | 108 | 68 | 52 | 8 | 7.4 | 0.1 | 83 |
| 38-3 | MPOLG2 | M | 19 | 37.2 | 128 | 22 | 20.8 | 75 | 70 | 30 | 10 | 8.1 | 0.1 | 59 |
| 51-2 | MPOLG2 | M | 133 | 93.2 | 102 | 41 | 6.3 | 99 | 67 | 44 | 8 | 7.4 | 0.1 | 178 |
| 53-1 | MPOLG2 | M | 38 | 57.6 | 53 | 47 | 6.5 | 97 | 57 | 48 | 6 | 7.4 | 0.1 | 54 |
| 54-1 | MPOLG2 | M | 83 | 146* | 77 | 37 | 9.8 | 139 | 47 | 63 | 8 | 6.7 | 0.1 | 33 |
| 54-2 | MPOLG2 | M | 29 | 48.5 | 41 | 42 | 6.7 | 114 | 51 | 60 | 6 | 7.0 | 0.1 | 114 |
| 54-3 | MPOLG2 | M | 27 | 29.2 | 50 | 37 | 6.6 | 110 | 68 | 57 | 5 | 7.3 | 0.1 | 58 |
| 54-4 | MPOLG2 | M | 132 | 285.1* | 127 | 110 | 13.4 | 121 | 51 | 53 | 7 | 8.4 | 0.2 | 79 |
| 57-1 | MPOLG2 | M | 218 | qns | 193 | 45 | 4.3 | qns | 33 | 21 | 6 | 5.7 | qns | qns |
| 62-1 | MPOLG2 | M | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr |
| 62-2 | MPOLG2 | M | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr |
| 67-2 | MPOLG2 | M | 37 | 37.6 | 65 | 37 | 7.2 | 80 | 66 | 38 | 5 | 6.9 | 0.1 | 327 |
| 67-3 | MPOLG2 | M | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr |
| | n = | 14 | 11 | 10 | 11 | 11 | 11 | 10 | 11 | 11 | 11 | 11 | 10 | 10 |
| 52609M1 | WT | M | 29 | 38.9 | 43 | 57 | 4.1 | 73 | 45 | 37 | 6 | 7.4 | 0.9 | 136 |
| 52609M2 | WT | M | 26 | 41.1 | 43 | 53 | 4.2 | 53 | 47 | 25 | 4 | 7.4 | 0.2 | 93 |
| 080309M1 | WT | M | 43 | 64.5 | 63 | 55 | 4.0 | 71 | 61 | 36 | 5 | 7.4 | 0.2 | 71 |
| 080309M2 | WT | M | 34 | 58.6 | 57 | 51 | 4.9 | 109 | 45 | 50 | 5 | 7.2 | 0.1 | 43 |
| | n = | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |

**MEANS**

| Group ID | Sex | ALT | SDH | AST | ALP | TBA | Chol | Trig | HDL | LDL | Ca | Creat | CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MPOLG2 | M | 116 | 115.1 | 118 | 46 | 11.3 | 101 | 59 | 46 | 7 | 7.1 | 0.1 | 147 |
| WT | M | 33 | 50.8 | 52 | 54 | 4.3 | 77 | 50 | 37 | 5 | 7.4 | 0.4 | 86 |

**STANDARD ERRORS**

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MPOLG2 | M | 41 | 35.9 | 30 | 7 | 3.1 | 7 | 4 | 4 | 0 | 0.2 | 0.0 | 46 |
| WT | M | 4 | 6.4 | 5 | 1 | 0.2 | 12 | 4 | 5 | 0 | 0.1 | 0.2 | 20 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mann-Whitney p-value | 0.186 | 0.635 | 0.074 | 0.057 | 0.026 | 0.106 | 0.087 | 0.280 | 0.040 | 0.492 | 0.031 | 0.839 |
| ★Significant at $p < 0.05$ | | | | | ★ | | | | ★ | | ★ | |

Supplemental table 2

| Sample# | Group ID | Sex | ALT | SDH | AST | ALP | TBA | Chol | Trig | HDL | LDL | Ca | Creat | CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  | U/L | U/L | U/L | U/L | uMol/L | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl | mg/dl | U/L |
| 23-5 | MPOLG2 | F | 33 | qns | 87 | 292 | 17.1 | 107 | 50 | 49 | 9 | 9.0 | 0.1 | 956 |
| 23-6 | MPOLG2 | F | 40 | 42.1 | 68 | 163 | 9.8 | 79 | 56 | 40 | 8 | 8.3 | 0.1 | 62 |
| 38-5 | MPOLG2 | F | 48 | 40.5 | 95 | 178 | 12.8 | 65 | 57 | 34 | 5 | 6.9 | 0.04 | 67 |
| 38-6 | MPOLG2 | F | 271 | 191.5* | 273 | 56 | 17.8 | 66 | 60 | 33 | 5 | 6.4 | 0.1 | 88 |
| 38-8 | MPOLG2 | F | 322 | 218.6* | 346 | 32 | 9.5 | 44 | 53 | 22 | 5 | 3.9 | <0.01 | 1366 |
| 53-5 | MPOLG2 | F | 36 | 49.4 | 67 | 54 | 10.4 | 64 | 62 | 32 | 4 | 7.2 | 0.1 | 42 |
| 54-5 | MPOLG2 | F | 39 | 47.1 | 70 | 95 | 5.4 | 82 | 51 | 42 | 5 | 7.6 | 0.1 | 49 |
| 57-2 | MPOLG2 | F | 36 | 48.4 | 79 | 116 | 8.1 | 75 | 55 | 35 | 4 | 7.6 | 0.1 | 46 |
| 62-5 | MPOLG2 | F | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr | nsr |
| 67-8 | MPOLG2 | F | 34 | 34.9 | 61 | 47 | 21.0 | 70 | 60 | 32 | 4 | 7.4 | 0.1 | 107 |
|  | n = | 10 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| 52609F1 | WT | F | 80 | 64.2 | 100 | 38 | 8.0 | 66 | 51 | 34 | 5 | 7.3 | 0.1 | 146 |
| 52609F2 | WT | F | 32 | 26.8 | 67 | 67 | 17.0 | 51 | 41 | 26 | 4 | 7.0 | 0.1 | 110 |
| 52609F3 | WT | F | 70 | 54.4 | 113 | 32 | 8.3 | 92 | 49 | 42 | 6 | 8.1 | 0.1 | 405 |
|  | n = | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

**MEAN VALUES**

| Group ID | Sex | ALT | SDH | AST | ALP | TBA | Chol | Trig | HDL | LDL | Ca | Creat | CK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MPOLG2 | F | 95 | 84.1 | 127 | 115 | 12.4 | 72 | 56 | 35 | 5 | 7.1 | 0.1 | 309 |
| WT | F | 61 | 48.5 | 93 | 46 | 11.1 | 70 | 47 | 34 | 5 | 7.5 | 0.1 | 220 |

**STANDARD ERRORS**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MPOLG2 | F | 38 | 26.8 | 35 | 28 | 1.7 | 6 | 1 | 3 | 1 | 0.5 | 0.0 | 165 |
| WT | F | 15 | 11.2 | 14 | 11 | 3.0 | 12 | 3 | 5 | 1 | 0.3 | 0.0 | 93 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mann-Whitney p-value | 0.973 | 1.000 | 0.759 | 0.155 | 0.482 | 0.896 | 0.027 | 0.973 | 1.000 | 0.973 | 0.796 | 0.209 |
| ★ Significant at p < 0.05 |  |  |  |  |  |  | ★ |  |  |  |  |  |

Supplemental table 3

| Lab Acc | ID # | Sex | WBC $10^3$/ul | RBC $10^6$/ml | Hgb g/dl | Hct % | Spun Hct % | MCV fL | MCH pg | MCHC g/dl | Plts $10^3$/ul | Neut % | Lym % | Mono % | Eos % | Baso % | LUC % | Neut $10^3$/ul | Lymph $10^3$/ul | Mono $10^3$/ul | Eos $10^3$/ul | Baso $10^3$/ul | LUC $10^3$/ul | Retic % | Retic $10^6$/ul |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MPOLG2** | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1687 | 38-1 | M | 3.8 | 8.43 | 12.6 | 37.9 | 37.0 | 44.9 | 14.9 | 33.2 | 959 | 25 | 65 | 7 | 0 | 0 | 3 | 1.0 | 2.5 | 0.3 | 0.0 | 0.0 | 0.1 | 2.5 | 0.2 |
| 1689 | 38-2 | M | 9.4 | 9.71 | 14.7 | 45.7 | 44.0 | 47.1 | 15.1 | 32.2 | 1738 | 11 | 85 | 3 | 0 | 0 | 1 | 1.0 | 8.0 | 0.3 | 0.0 | 0.0 | 0.1 | 1.9 | 0.2 |
| 1688 | 38-3 | M | 3.7 | 5.82 | 8.8 | 27.1 | 27.0 | 46.6 | 15.1 | 32.5 | 1767 | 45 | 50 | 4 | 0 | 0 | 1 | 1.7 | 1.9 | 0.1 | 0.0 | 0.0 | 0.0 | 9.0 | 0.5 |
| 1692 | 51-2 | M | 4.5 | 6.28 | 11.1 | 34.4 | 33.5 | 54.7 | 17.7 | 32.3 | 1841 | 21 | 70 | 8 | 1 | 0 | 0 | 0.9 | 3.2 | 0.4 | 0.0 | 0.0 | 0.0 | 4.1 | 0.3 |
| 1696 | 53-1 | M | 5.9 | 9.30 | 14.5 | 44.9 | 43.5 | 48.3 | 15.6 | 32.3 | 1558 | 17 | 75 | 6 | 0 | 0 | 2 | 1.0 | 4.5 | 0.4 | 0.0 | 0.0 | 0.1 | 2.1 | 0.2 |
| 1698 | 54-1 | M | 4.5 | 9.48 | 14.8 | 43.6 | 43.5 | 46.0 | 15.6 | 33.9 | 1377 | 25 | 66 | 7 | 0 | 0 | 2 | 1.1 | 3.0 | 0.3 | 0.0 | 0.0 | 0.1 | 3.0 | 0.3 |
| 1703 | 54-2 | M | 2.7 | 7.60 | 12.8 | 40.4 | 38.0 | 53.2 | 16.8 | 31.7 | 1468 | 46 | 53 | 1 | 0 | 0 | 0 | 1.3 | 1.4 | 0.0 | 0.0 | 0.0 | 0.0 | 1.2 | 0.1 |
| 1707 | 54-3 | M | 4.1 | 9.27 | 13.4 | 41.6 | 41.5 | 44.9 | 14.5 | 32.2 | 2065 | 37 | 58 | 5 | 0 | 0 | 0 | 1.5 | 2.4 | 0.2 | 0.0 | 0.0 | 0.0 | 2.0 | 0.2 |
| 1702 | 54-4 | M | 3.3 | 7.40 | 12.7 | 35.8 | 37.0 | 48.4 | 17.2 | 35.5 | 1189 | 27 | 68 | 2 | 2 | 0 | 1 | 0.9 | 2.2 | 0.1 | 0.1 | 0.0 | 0.0 | 2.9 | 0.2 |
| 1706 | 57-1 | M | 3.8 | 9.69 | 14.6 | 44.5 | 44.5 | 45.9 | 15.1 | 32.8 | 1382 | 35 | 61 | 2 | 1 | 0 | 1 | 1.3 | 2.3 | 0.1 | 0.0 | 0.0 | 0.0 | 2.2 | 0.2 |
| 1709 | 67-2 | M | 3.8 | 9.63 | 14.5 | 44.6 | 44.0 | 46.3 | 15.1 | 32.5 | 1910 | 33 | 61 | 4 | 0 | 0 | 2 | 1.2 | 2.3 | 0.2 | 0.0 | 0.0 | 0.1 | 2.4 | 0.2 |
| Mean | | | 4.5 | 8.4 | 13.1 | 40.0 | 39.4 | 47.8 | 15.7 | 32.8 | 1568.5<br>45455 | 29.3 | 64.7 | 4.5 | 0.4 | 0.0 | 1.2 | 1.2 | 3.1 | 0.2 | 0.0 | 0.0 | 0.1 | 3.0 | 0.2 |
| SD | | | 1.8 | 1.4 | 1.9 | 5.8 | 5.6 | 3.2 | 1.0 | 1.1 | 332 | 11.1 | 9.9 | 2.3 | 0.7 | 0.0 | 1.0 | 0.3 | 1.8 | 0.1 | 0.0 | 0.0 | 0.0 | 2.1 | 0.1 |
| n | | | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 | 11 |
| **WT** | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1690 | 52609M1 | M | 7.2 | 9.33 | 14.2 | 43.6 | 43.0 | 46.7 | 15.2 | 32.6 | 1929 | 24 | 71 | 5 | 0 | 0 | 0 | 1.7 | 5.1 | 0.4 | 0.0 | 0.0 | 0.0 | 3.0 | 0.3 |
| 1697 | 52609M2 | M | 4.0 | 9.14 | 13.6 | 41.1 | 41.0 | 45.0 | 14.9 | 33.1 | 1472 | 25 | 67 | 5 | 2 | 0 | 1 | 1.0 | 2.7 | 0.2 | 0.1 | 0.0 | 0.0 | 3.4 | 0.3 |
| 1701 | 080309M1 | M | 4.5 | 9.26 | 14.0 | 41.1 | 42.0 | 44.4 | 15.1 | 34.1 | 1246 | 24 | 70 | 4 | 0 | 0 | 2 | 1.1 | 3.2 | 0.2 | 0.0 | 0.0 | 0.1 | 2.9 | 0.3 |
| 1704 | 080309M2 | M | 3.8 | 9.01 | 13.3 | 40.8 | 40.0 | 45.3 | 14.8 | 32.6 | 1194 | 50 | 48 | 0 | 2 | 0 | 0 | 1.9 | 1.8 | 0.0 | 0.1 | 0.0 | 0.0 | 2.8 | 0.3 |
| Mean | | | 4.9 | 9.2 | 13.8 | 41.7 | 41.5 | 45.4 | 15.0 | 33.1 | 1460 | 30.8 | 64.0 | 3.5 | 1.0 | 0.0 | 0.8 | 1.4 | 3.2 | 0.2 | 0.0 | 0.0 | 0.0 | 3.0 | 0.3 |
| SD | | | 1.6 | 0.1 | 0.4 | 1.3 | 1.3 | 1.0 | 0.2 | 0.7 | 335 | 12.8 | 10.8 | 2.4 | 1.2 | 0.0 | 1.0 | 0.5 | 1.4 | 0.1 | 0.0 | 0.0 | 0.0 | 0.3 | 0.0 |
| n | | | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | **MEAN** | | | | | | | | | | | | | | | | | | | | | | | | |
| | **MPOLG2** | | 4.5 | 8.42 | 13.1 | 40.0 | 39.4 | 47.8 | 15.7 | 32.8 | 1569 | 29 | 65 | 4 | 0 | 0 | 1 | 1.2 | 3.1 | 0.2 | 0.0 | 0.0 | 0.1 | 3.0 | 0.2 |
| | **WT** | | 4.9 | 9.19 | 13.8 | 41.7 | 41.5 | 45.4 | 15.0 | 33.1 | 1460 | 31 | 64 | 4 | 1 | 0 | 1 | 1.4 | 3.2 | 0.2 | 0.0 | 0.0 | 0.0 | 3.0 | 0.3 |
| | **STANDARD ERROR** | | | | | | | | | | | | | | | | | | | | | | | | |
| | **MPOLG2** | | 0.5 | 0.43 | 0.6 | 1.7 | 1.7 | 1.0 | 0.3 | 0.3 | 100 | 3 | 3 | 1 | 0 | 0 | 0 | 0.1 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 |
| | **WT** | | 0.8 | 0.07 | 0.2 | 0.7 | 0.6 | 0.5 | 0.1 | 0.4 | 167 | 6 | 5 | 1 | 1 | 0 | 0 | 0.2 | 0.7 | 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| Mann-Whitney p-value | | | 0.431 | 1.000 | 0.928 | 0.974 | 1.000 | 0.131 | 0.223 | 0.218 | 0.753 | 0.929 | 0.776 | 0.634 | 0.330 | 1.000 | 0.528 | 0.343 | 0.641 | 0.851 | 0.264 | 1.000 | 0.498 | 0.213 | 0.078 |

★ Significant at $p < 0.05$

Supplemental table 4

| Lab Acc | ID # | Sex | WBC $10^3$/ul | RBC $10^6$/ml | Hgb g/dl | Hct % | Spun Hct % | MCV fL | MCH pg | MCHC g/dl | Plts $10^3$/ul | Neut % | Lym % | Mono % | Eos % | Baso % | LUC % | Neut $10^3$/ul | Lymph $10^3$/ul | Mono $10^3$/ul | Eos $10^3$/ul | Baso $10^3$/ul | LUC $10^3$/ul | Retic % | Retic $10^6$/ul |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MPOLG2 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1685 | 23-6 | F | 3.2 | 8.37 | 13.4 | 40.4 | 40.0 | 48.3 | 16.0 | 33.2 | 1078 | 15 | 80 | 1 | 0 | 0 | 4 | 0.5 | 2.5 | 0.0 | 0.0 | 0.0 | 0.1 | 4.8 | 0.4 |
| 1686 | 23-5 | F | 2.2 | 6.03 | 11.0 | 31.8 | 32.0 | 52.7 | 18.2 | 34.6 | 854 | 38 | 58 | 4 | 0 | 0 | 0 | 0.8 | 1.3 | 0.1 | 0.0 | 0.0 | 0.0 | 2.7 | 0.2 |
| 1691 | 38-5 | F | 2.4 | 9.28 | 14.8 | 46.1 | 45.0 | 49.7 | 15.9 | 32.1 | 1345 | 32 | 66 | 2 | 0 | 0 | 0 | 0.8 | 1.6 | 0.0 | 0.0 | 0.0 | 0.0 | 1.9 | 0.2 |
| 1693 | 38-6 | F | 3.5 | 7.60 | 12.6 | 37.3 | 37.5 | 49.1 | 16.6 | 33.8 | 776 | 21 | 75 | 3 | 0 | 0 | 1 | 0.7 | 2.7 | 0.1 | 0.0 | 0.0 | 0.0 | 1.9 | 0.1 |
| 1694 | 38-8 | F | 3.7 | 7.70 | 12.7 | 38.2 | 38.0 | 49.6 | 16.5 | 33.2 | 758 | 15 | 82 | 1 | 1 | 0 | 1 | 0.6 | 3.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.1 | 0.2 |
| 1699 | 53-5 | F | 4.3 | 9.28 | 15.1 | 45.2 | 45.0 | 48.7 | 16.3 | 33.4 | 1110 | 33 | 50 | 13 | 0 | 0 | 4 | 1.4 | 2.1 | 0.6 | 0.0 | 0.0 | 0.2 | 3.6 | 0.3 |
| 1700 | 54-5 | F | 2.4 | 8.38 | 14.6 | 44.7 | 43.5 | 53.4 | 17.4 | 32.7 | 884 | 21 | 74 | 3 | 1 | 0 | 1 | 0.5 | 1.8 | 0.1 | 0.0 | 0.0 | 0.0 | 4.0 | 0.3 |
| 1705 | 57-2 | F | 1.9 | 7.93 | 12.9 | 38.7 | 39.0 | 48.8 | 16.3 | 33.3 | 966 | 41 | 55 | 2 | 0 | 0 | 2 | 0.8 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.9 | 0.2 |
| 1710 | 67-8 | F | 4.2 | 9.49 | 14.6 | 44.2 | 44.0 | 46.6 | 15.4 | 33.0 | 2035 | 33 | 61 | 3 | 0 | 0 | 3 | 1.4 | 2.6 | 0.1 | 0.0 | 0.0 | 0.1 | 2.8 | 0.3 |
| Mean | | | 3.1 | 8.2 | 13.5 | 40.7 | 40.4 | 49.7 | 16.5 | 33.3 | 1090 | 27.7 | 66.8 | 3.6 | 0.2 | 0.0 | 1.8 | 0.8 | 2.1 | 0.1 | 0.0 | 0.0 | 0.1 | 3.1 | 0.3 |
| SD | | | 0.9 | 1.1 | 1.4 | 4.7 | 4.4 | 2.1 | 0.8 | 0.7 | 400 | 9.8 | 11.5 | 3.7 | 0.4 | 0.0 | 1.6 | 0.3 | 0.7 | 0.2 | 0.0 | 0.0 | 0.1 | 0.9 | 0.1 |
| n | | | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 |
| **WT** | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1684 | 52609F1 | F | 5.6 | 9.78 | 15.4 | 46.8 | 45.0 | 47.9 | 15.7 | 32.9 | 948 | 25 | 68 | 3 | 1 | 0 | 3 | 1.4 | 3.8 | 0.2 | 0.1 | 0.0 | 0.2 | 2.8 | 0.3 |
| 1695 | 52609F2 | F | 1.8 | 8.23 | 12.9 | 38.2 | 38.0 | 46.4 | 15.7 | 33.8 | 1361 | 51 | 48 | 1 | 0 | 0 | 0 | 0.9 | 0.8 | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 | 0.3 |
| 1708 | 52609F3 | F | 8.2 | 8.65 | 13.5 | 40.6 | 41.0 | 46.9 | 15.6 | 33.3 | 1379 | 22 | 69 | 5 | 0 | 0 | 4 | 1.8 | 5.7 | 0.4 | 0.0 | 0.0 | 0.3 | 2.6 | 0.2 |
| Mean | | | 5.2 | 8.9 | 13.9 | 41.9 | 41.3 | 47.1 | 15.7 | 33.3 | 1229 | 32.7 | 61.7 | 3.0 | 0.3 | 0.0 | 2.3 | 1.4 | 3.4 | 0.2 | 0.0 | 0.0 | 0.2 | 3.2 | 0.3 |
| SD | | | 3.2 | 0.8 | 1.3 | 4.4 | 3.5 | 0.8 | 0.1 | 0.5 | 244 | 15.9 | 11.8 | 2.0 | 0.6 | 0.0 | 2.1 | 0.5 | 2.4 | 0.2 | 0.0 | 0.0 | 0.2 | 0.9 | 0.1 |
| n | | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | **MEAN** | | | | | | | | | | | | | | | | | | | | | | | | |
| | **MPOLG2** | | 3.1 | 8.23 | 13.5 | 40.7 | 40.4 | 49.7 | 16.5 | 33.3 | 1090 | 28 | 67 | 4 | 0 | 0 | 2 | 0.8 | 2.1 | 0.1 | 0.0 | 0.0 | 0.1 | 3.1 | 0.3 |
| | **WT** | | 5.2 | 8.89 | 13.9 | 41.9 | 41.3 | 47.1 | 15.7 | 33.3 | 1229 | 33 | 62 | 3 | 0 | 0 | 2 | 1.4 | 3.4 | 0.2 | 0.0 | 0.0 | 0.2 | 3.2 | 0.3 |
| | **STANDARD ERROR** | | | | | | | | | | | | | | | | | | | | | | | | |
| | **MPOLG2** | | 0.3 | 0.36 | 0.5 | 1.6 | 1.5 | 0.7 | 0.3 | 0.2 | 133 | 3 | 4 | 1 | 0 | 0 | 1 | 0.1 | 0.2 | 0.1 | 0.0 | 0.0 | 0.0 | 0.3 | 0.0 |
| | **WT** | | 1.9 | 0.46 | 0.8 | 2.6 | 2.0 | 0.4 | 0.0 | 0.3 | 141 | 9 | 7 | 1 | 0 | 0 | 1 | 0.3 | 1.4 | 0.1 | 0.0 | 0.0 | 0.1 | 0.5 | 0.0 |
| Mann-Whitney p-value | | | 0.482 | 0.350 | 0.500 | 0.632 | 0.746 | 0.036 | 0.055 | 0.814 | 0.282 | 0.573 | 0.600 | 0.882 | 1.000 | 1.000 | 0.727 | 0.100 | 0.482 | 0.727 | 0.673 | 1.000 | 0.477 | 1.000 | 0.600 |
| ★Significant at $p < 0.05$ | | | | | | | | ★ | | | | | | | | | | | | | | | | | |

Supplemental table 5