SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIALS AND METHODS

Generation of $TP^{-/-}UP^{-/-}$ mice.

This work performed under a Columbia University Institutional Animal Care and Use Committee approved protocol.

To generate $TP^{-/-}$ (encoded by *Tymp* gene) mice, we initially amplified a 925-bp segment of the mouse *Tymp* transcript by RT-PCR (nts 457-1371 of NM_13802.1). The RT-PCR product served as a template for a α-^{32}P dATP radiolabeled probe to screen hybridization filters spotted with a bacterial artificial chromosome (BAC) 129/SvJ genomic DNA library (Genome System Inc., St. Louis, MO). Two BACs (65d3 and 111d4) containing segments of the mouse *Tymp* genomic sequence were identified. From BAC 65d3 DNA, we subcloned a 5.4 kilobase (kb) fragment containing all 10 exons of *Tymp* and flanked by *Eco*RI and *Bgl*II restriction enzyme sites into a pUC18 vector, which had been digested with *Eco*RI and *Bam*HI, taking advantage of the compatible cohesive ends of *Bgl*II and *Bam*HI (Supplementary Material, Fig. S1).

To knockout the gene, we altered the plasmid targeting construct by introducing a DNA cassette comprised of a phosphoglycerate kinase promoter and neomycin resistance gene flanked by *Lox*P sites (PGK-Neo cassette, LPN) into a unique *Pac*I site (converted from *Not*I) within the *Tymp* consensus sequence in exon 4 of the mouse *Tymp* gene. We electroporated the linearized plasmid DNA targeting construct into 129/SvJ mouse embryonic stem (ES) cells. Neomycin resistant clones were isolated and analyzed by PCR amplifications and Southern Blot analyses for homologous recombinants. To confirm 5´-end *Tymp* recombinations, we performed

PCR amplifications using a primer within PGK-Neo cassette sequence (PN-F5) paired with a primer in genomic DNA segments upstream of the targeting construct (PN-upst-2) (Fig. S1). ES cell clones that produced PCR amplified bands were screened further by Southern blot analyses using DNA digested with *Spe*I and probe B, which recognizes a genomic DNA segment downstream of the 3´-end of the targeting sequence but within fragments generated by the *Spe*I digestion; wild-type DNA produces a 12 kb band while homologous recombinant generate 5.7 kb band (Supplementary Material, Fig. S1). We performed a second PCR to confirm the 3´-end recombination using primers (PN-R4 and M22.2F4). We identified 22 ES cells clones harboring homologous recombinations. We submitted two ES clones (TP33 and TP90) to the Columbia University Transgenic Mouse Facility for injection into C57BL/6J blastocysts and obtained 12 pups (six from each ES clone). Using the previously described Southern blot analyses, we demonstrated that nine mice were chimeric (Supplementary Material, Fig. S1). Homologous recombinant in subsequent mice were confirmed by PCR amplifications; 23 of 47 (49%) F1 mice were heterozygous using PCR screening. We bred four pairs of F1 mice; the first two litters of F2 mice were comprised of four homozygous mutants, seven heterozygous and one wild-type. $TP^{-/-}$ mice did not show embryonic lethality and phenotypically similar to $TP^{+/+}$ mice.

Because murine UP degrades Thd and dUrd, we crossed $UP^{-/-}$ (encoded by *Upp1* gene) mice(1) with $TP^{-/-}$ mice to generate $TP^{+/-}UP^{+/-}$ mice, followed by intercrossing the mice to generate $TP^{-/-}UP^{-/-}$ mice. In all mice studied, the homologous recombination was confirmed by PCR amplifications (Supplementary Material, Fig. S1).

SUPPLEMENTARY TABLES

Table S1. Primers for mtDNA amplification and sequencing in brain

Fragment	Forward Primer	Reverse Primer
1	aggtttggtcctggccttat	gtgaggtagagcggggttta
2	taaaccccgctctacctcac	catctttcccttgcggtact
3	agtaccgcaagggaaagatg	tgtgtatgcctggagaattaga
4	tctaattctccaggcatacaca	agctccatagggtcttctcg
5	aagacgagaagaccctatggag	tgtcctttcgtactgggaga
6	tctcccagtacgaaaggaca	ctgataggctggatgttgct
7	tcccctaccaataccacacc	gtggataggatgctcggatt
8	aatccgagcatcctatccac	gaggctgttgcttgtgtgac
9	acacaagcaacagcctcaat	tggaagacctcctagggatag
10	cctaggaggtcttccaccac	tcggcggtagaagtagattg
11	tctacttctaccgccgaaaa	acaccccagctaaatgaagg
12	ccttcatttagctggggtgt	cgatgtcaagggatgagttg
13	tgggagcagtgtttgctatc	atgtggcgtcttgtagacca
14	tggtctacaagacgccacat	tcctggtcggtttgatgtta
15	taacatcaaaccgaccagga	tagggttcatgtccgtcctt
16	ggacggacatgaaccctaat	gagcaggccaagggttaata
17	tattaacccttggcctgctc	ggcttcaaatccgaagtgat
18	ccgcagcatgatactgacat	ggtgggagcgaaatataagtg
19	ttcgctcccacctaatatcc	ttgggattaaggttgcttca
20	tgaagcaaccttaatcccaac	gtgatgatgtgaggccatgt
21	acatggcctcacatcatcac	caccaaggtttttggttcct
22	aggaaccaaaaaccttggtg	ccgatgcggttatagaggat

23	ctataaccgcatcggagaca	tttttcggatgtcttgttcg
24	ctggcagacgaacaagacat	taatgcggtgaatgatggat
25	atccatcattcaccgcatta	ggatgttggttgtgtttgga
26	tccaaacacaaccaacatcc	ccgacatgaaggaataagca
27	attccttcatgtcggacgag	attggggattgagcgtagaa
28	ccccatattaaacccgaatg	tgcttatatgcttggggaaa
29	ttccccaagcatataagcaa	gccttaggtgattgggtttt
30	cacagtctagacgcacctacg	gtgtggctaggcaaggtgt

Table S2. Primers for mtDNA amplification and sequencing in muscle and small intestine

Fragment	Forward Primer	Reverse Primer
ND3-1	ccgcagcatgatactgacat	tgaattgctcatggtagtgga
ND3-2	ttcgaccctacaagctctgc	aagaaggtagatggcatattgg
ND6-1	caaccaaccaaaaaggctta	gggggatgttggttgtgttt
ND6-2	accaatctcccaaaccatca	gtcgcagttgaatgcgtgt
D-Loop-1	cagcacccaaagctggtatt	caaatggggaaggggatagt
D-Loop-2	actatccccttccccatttg	gccttgacggctatgttgat
D-Loop-3	ttagtccgcaaaacccaatc	ttttggttccggaacatga
D-Loop-4	actatccccttccccatttg	ttttggttccggaacatga
D-Loop-5	cagcacccaaagctggtatt	gccttgacggctatgttgat
Cytb-1	actgcgaccaatgacatgaa	ctcgtccgacatgaaggaat
Cytb-2	acgcaaacggagcctcaata	ctgtttcgtggaggaagagg
Cytb-3	catttattatcgcggcccta	ggcttcgtgctttgaggta
Ciytb-4	aggaggtgtcctagccttaatc	aataccagctttgggtgctg
RO	tctacttctaccgccgaaaa	tggctgagtaagcattagactg

Table S3. Primers for DNA amplification in the SSCP analysis

Fragment	Forward Primer	Reverse Primer	Amplicon size
ND3-1	ccgcagcatgatactgacat	gggggagctagaatgcaac	219 bp
ND3-2	gcattctgactcccccaaat	tgaattgctcatggtagtgga	179 bp
ND3-3	tccactacctgagcaattca	aagaaggtagatggcatattgg	218 bp
ND6-1	caaccaaccaaaaaggctta	tgatggtttgggagattggt	250 bp
ND6-2	accaatctcccaaaccatca	gggggatgttggttgtgttt	188 bp
ND6-3	tccaaacacaaccaacatcc	gtcgcagttgaatgcgtgt	242 bp
D-Loop-1	cagcacccaaagctggtatt	caaatggggaaggggatagt	295 bp
D-Loop-2	actatccccttccccatttg	gtatgggcgataacgcattt	175 bp
D-Loop-3	aaatgcgttatcgcccatac	gattgggttttgcggactaa	211 bp
D-Loop-4	ttagtccgcaaaacccaatc	ttttggttccggaacatga	258 bp
Cytb-1	attgactacctgccccatc	ctcgtccgacatgaaggaat	247 bp
Cytb-2	actgcgaccaatgacatgaa	gatggggcaggtaggtcaat	129 bp
RO	tctacttctaccgccgaaaa	tggctgagtaagcattagactg	164 bp

SUPPLEMENTARY FIGURES AND LEGENDS TO FIGURES

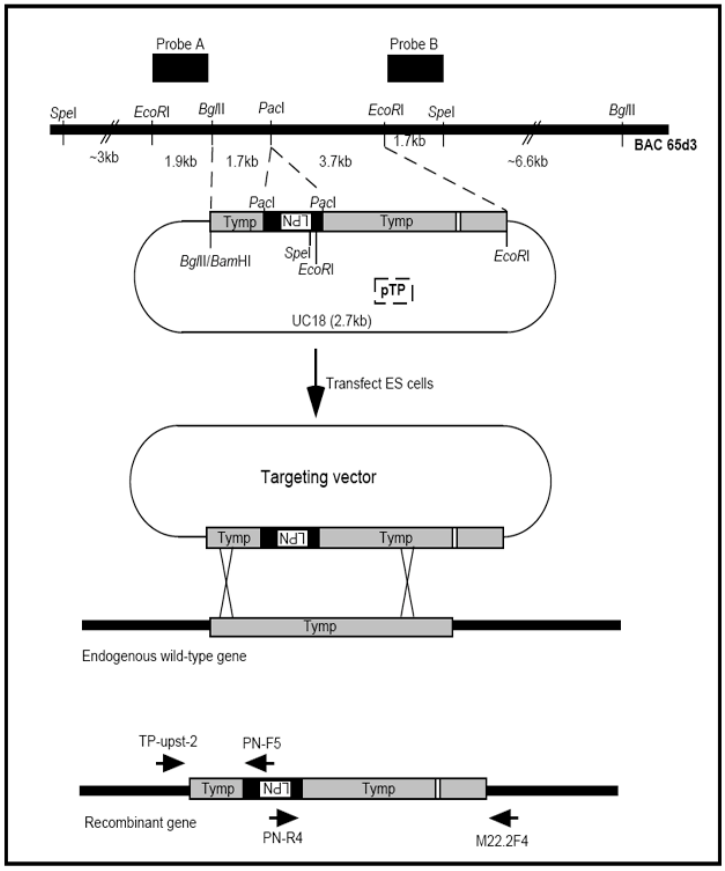

Fig. S1. Strategy to knock-out the mouse *TYMP* gene encoding thymidine phosphorylase. We isolated a 5.4 kb mouse genomic fragment that contains all 10 exons of the *TYMP* gene. This genomic fragment was sub-cloned into pUC18 vector. Within this vector, a unique *Not*I site in the mouse TYMP consensus sequence was identified. The *Not*I site was converted into a *Pac*I site, into which a PGK-neomycin cassette was inserted. We use this vector construct as a replacement vector to insert the disrupted *Tymp* gene into male 129/SvJ ES cells. Recombinants were identified by a combination of PCR and Southern blot screening.

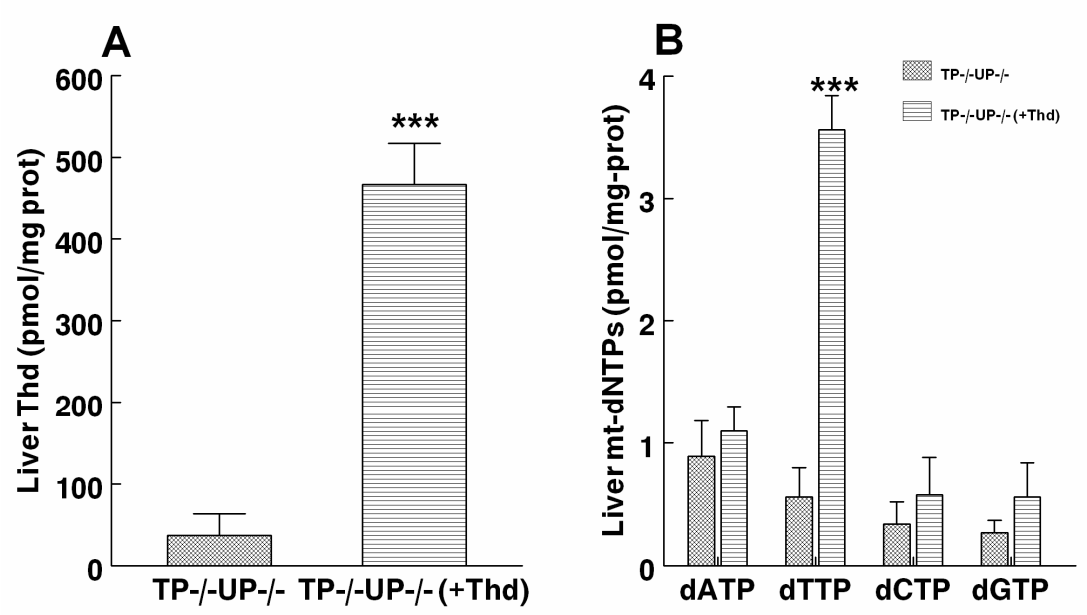

Fig. S2. Contribution of thymidine to the dNTPs pool unbalance. Liver Thd levels (A) and relative amount of dNTPs (B) 20 min after intraperitoneal injection of 100 mg Thd/kg b.w. Thd levels were measured in the whole tissue homogenates after sample deproteinization by HPLC with UV detection as described in the Material and Methods section. Mitochondrial dNTPs were measured in mitochondrial extracts by the radiolabeled polymerase assay described in material and methods section. All mice were sacrificed between 14-18 months old. Data are expressed as mean ± SD of 5 mice per group. *** $P < 0.001$ vs. $TP^{+/+}UP^{+/+}$.

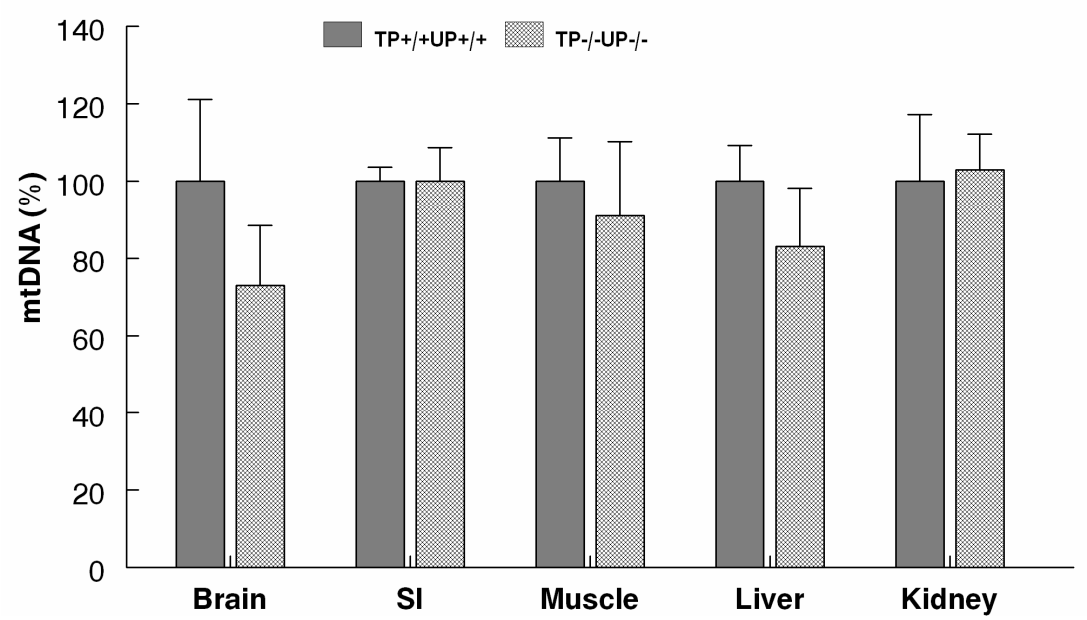

Fig. S3. Levels of mtDNA in 6-month-old relative to wild-type mice. Real time PCR was used to detect mtDNA and nDNA. Data are expressed as mean ± SD of 5 mice per group.

SUPPLEMENTARY REFERENCES

1. Cao, D., Leffert, J. J., McCabe, J., Kim, B. & Pizzorno, G. (2005) *J Biol Chem* **280,** 21169-75.