SUPPLEMENTARY DATA

Age-related metabolic changes limit efficacy of deoxynucleoside-based therapy in TK2-deficient mice

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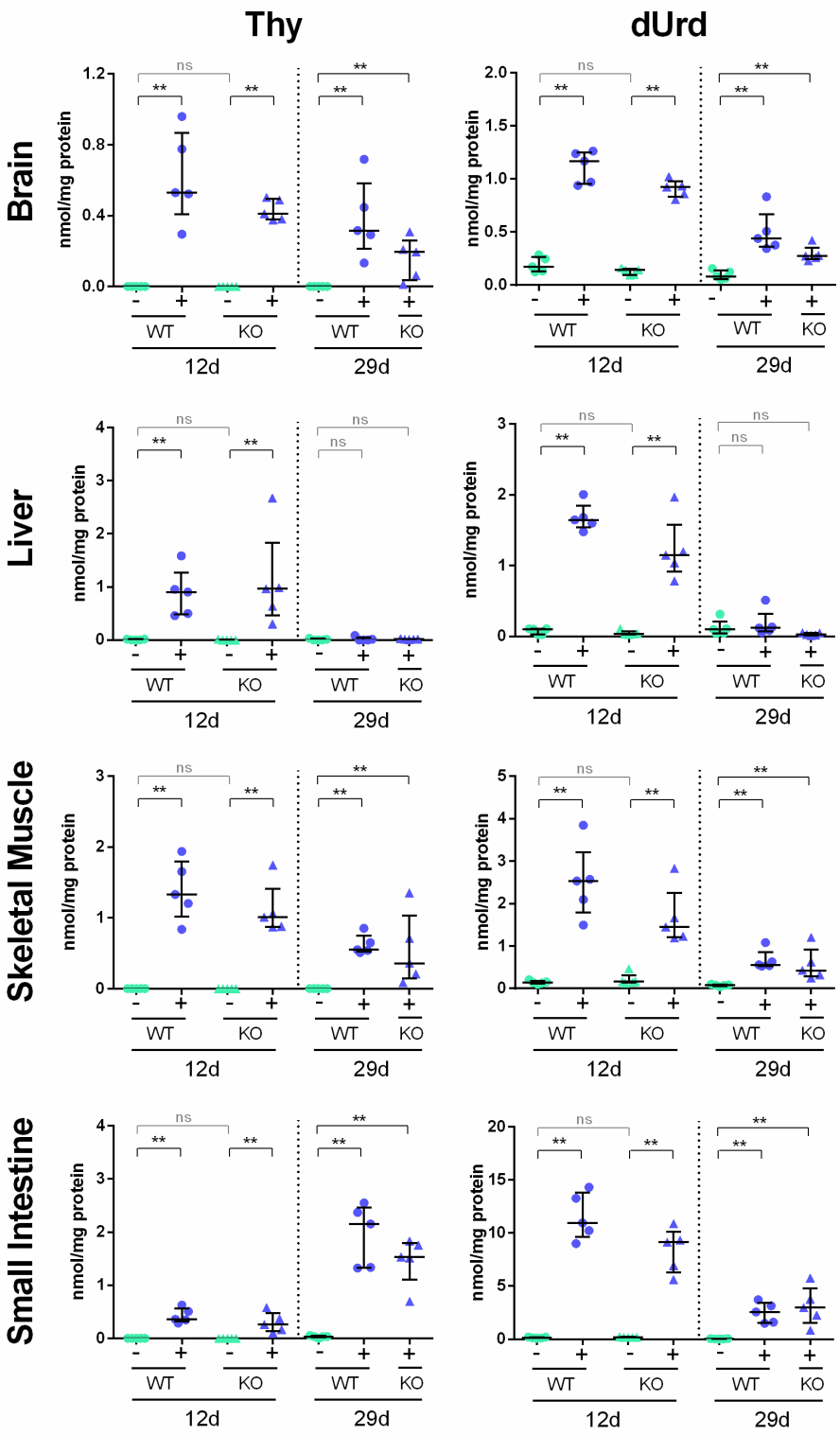
SUPPLEMENTARY METHODS:

SUPPLEMENTARY FIGURES:

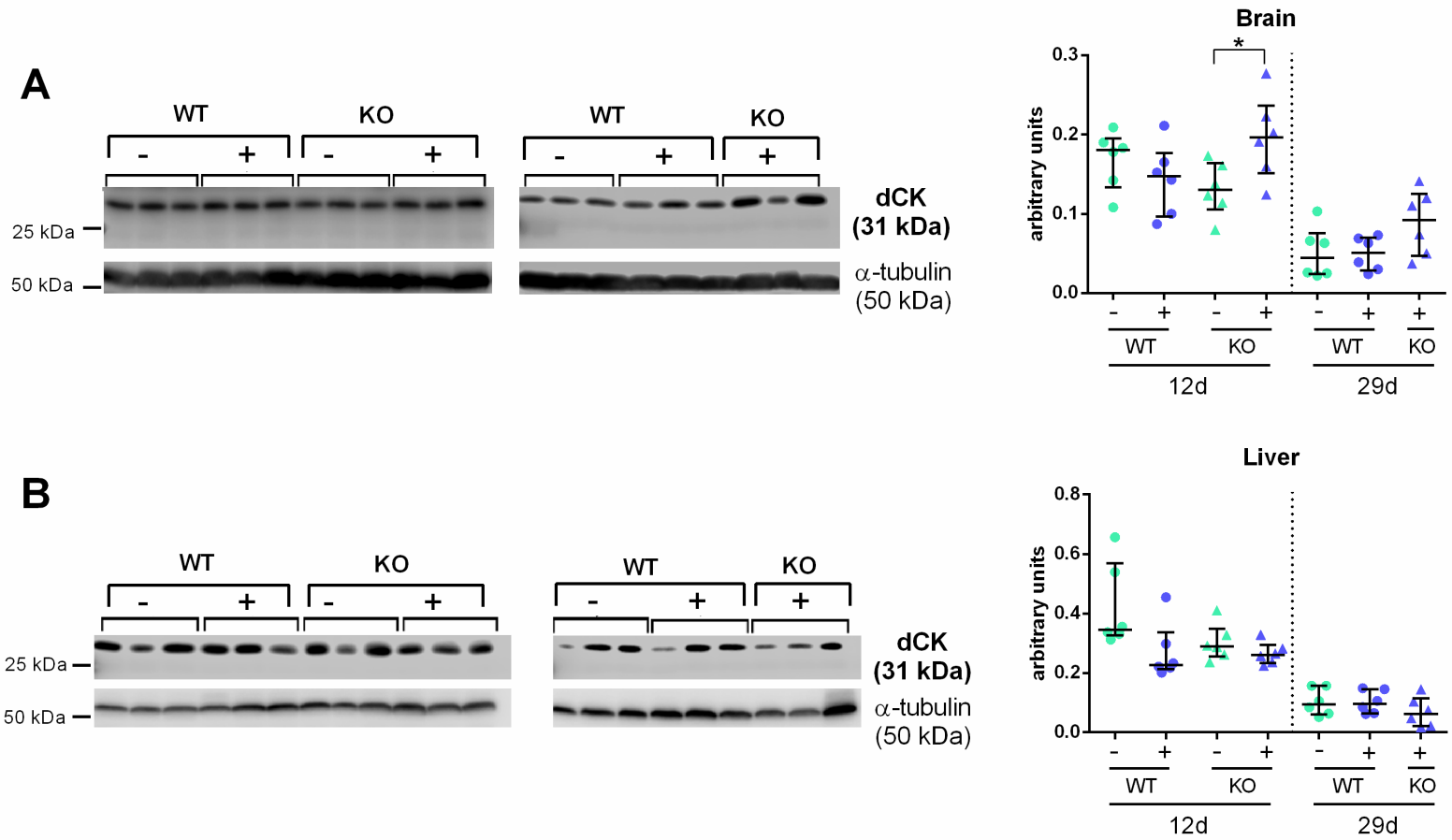
SUPPLEMENTARY TABLE:

dCK protein level determination

We determined steady-state levels of dCK protein in brain and liver by western blot. Protein homogenates were obtained as described for dCK activity determinations, supplemented with antiproteases cocktail (Complete, Roche) and stored at -80ºC until further use. 50 µg of protein were resolved on 12% SDS-PAGE gels and wet-transferred to PVDF membranes (Immunblot-PVDF, Bio-Rad). Membranes were decorated with primary specific antibody against dCK (Abcam, ab186128) and α-tubulin (ab184613). Peroxidase-conjugated anti-rabbit and anti-mouse antibodies (Dako) were used for immunodetection. The chemiluminescent signal was obtained after incubation with Immobilon reagent (Millipore) and captured on an Odissey Fc system (Li-Cor Biosciences). Densitometric analysis of the signal was performed with Image Studio (Li-Cor Biosciences) software.



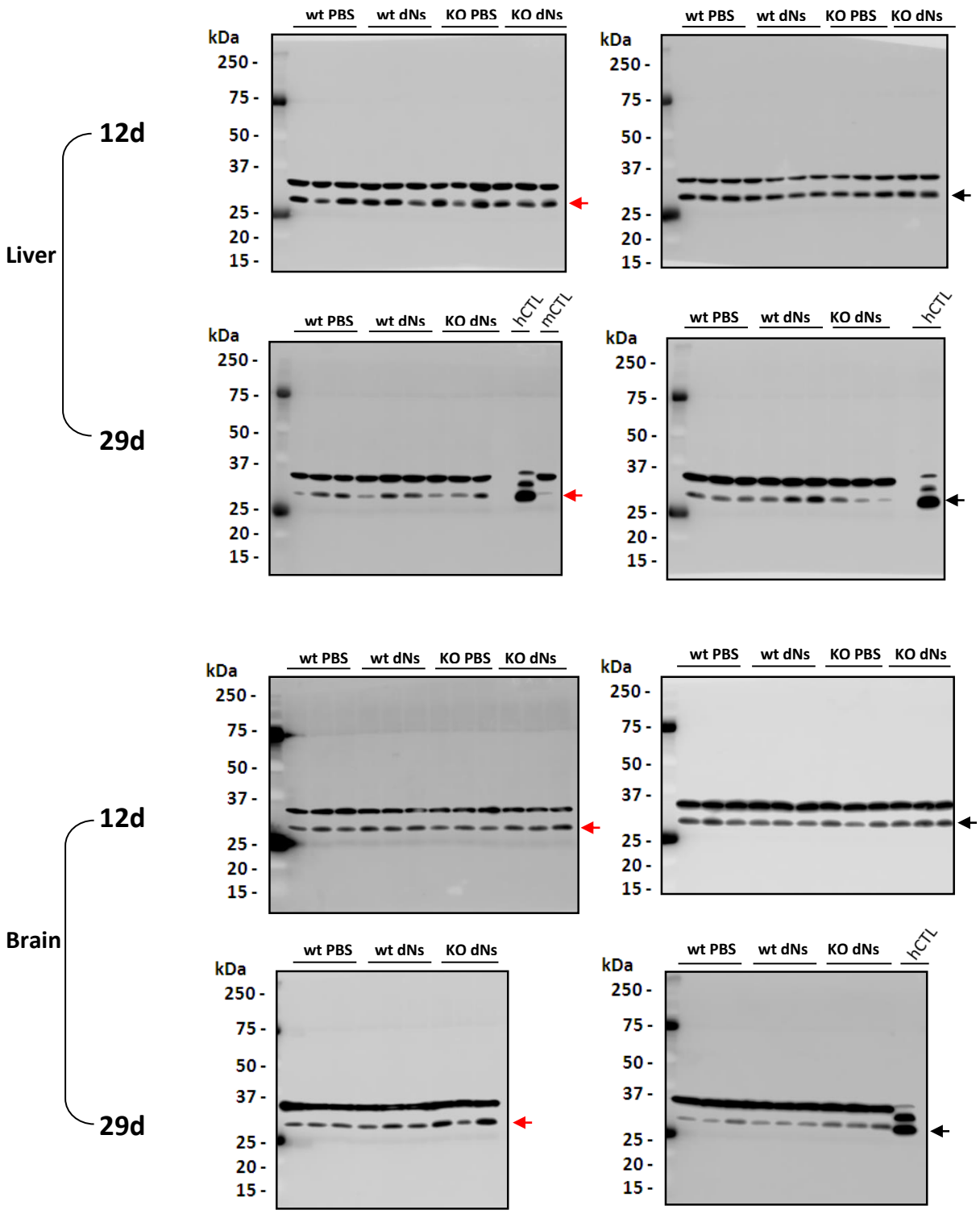
Supplementary Fig 1: Thy and dUrd levels in tissues of mice treated with dThd+dCtd. Concentrations of Thy and dUrd, the first degradation products of dThd and dCtd, respectively, in brain, liver, skeletal muscle (gastrocnemius), and small intestine by LC-MS/MS. Results indicate nmols of metabolite per mg of protein in tissues from mice aged 12 (12d) or 29 days (29d). *TK2*^{KO} (KO; triangles) and *TK2*^{WT} (WT; dots) mice, treated with either 400 mg/kg/day of dThd+dCtd (+; blue) or PBS (-; green). Tissues were collected 1 h after oral gavage. Scatter plots represent the median (horizontal line) with interquartile range. Mice in each group, n=5. P-values obtained with the Mann-Whitney U test: *p<0.05; **p<0.01; ns, non-significant differences.

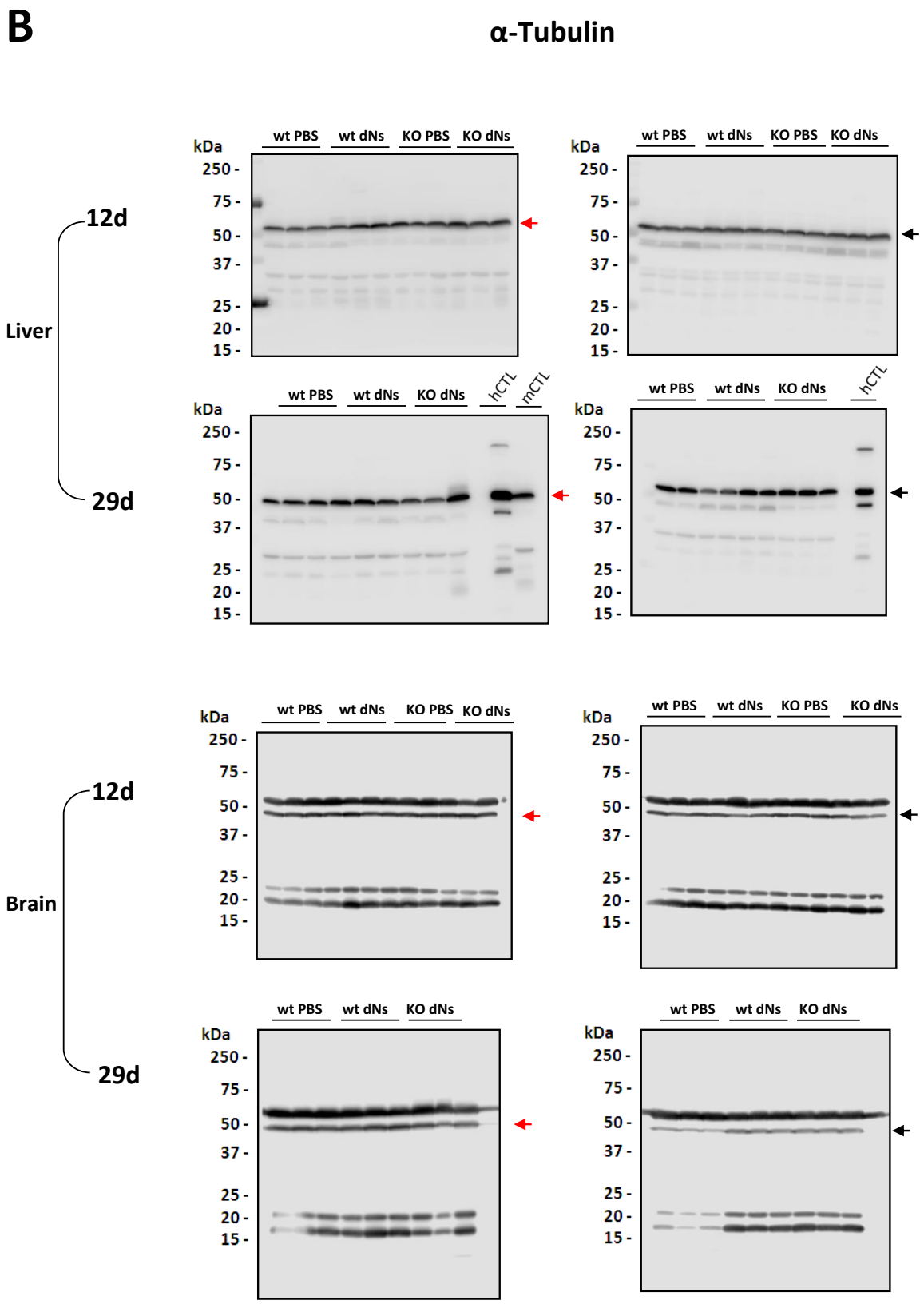


Supplementary Fig 2: dCK protein levels in dThd+dCtd treated mice. Western blot analysis of dCK steady-state protein levels in brain (**A**) and liver (**B**) homogenates. α-tubulin levels were used to normalize data among different samples. Representative western-blots (left panels) and quantification corresponding to 2 different blots (right panels) for each tissue. We plotted samples from 3 different animals from each experimental group in each blot (n=6 from each experimental group). Scatter plots represent the median (horizontal line) with interquartile range. dCK protein levels are decreased with age (12d-*TK2*^{WT}-PBS vs 29d-*TK2*^{WT}-PBS $p<0.01$ in both tissues; Mann-Whitney U test). MW of resolved bands was stated according to migration of Precision Plus Protein™ Dual Color standard (Bio-Rad). P-values in the graph obtained with the Mann-Whitney U test: *$p<0.05$. Full immunoblots from all samples in this analysis are included in Supplementary Fig. 3.

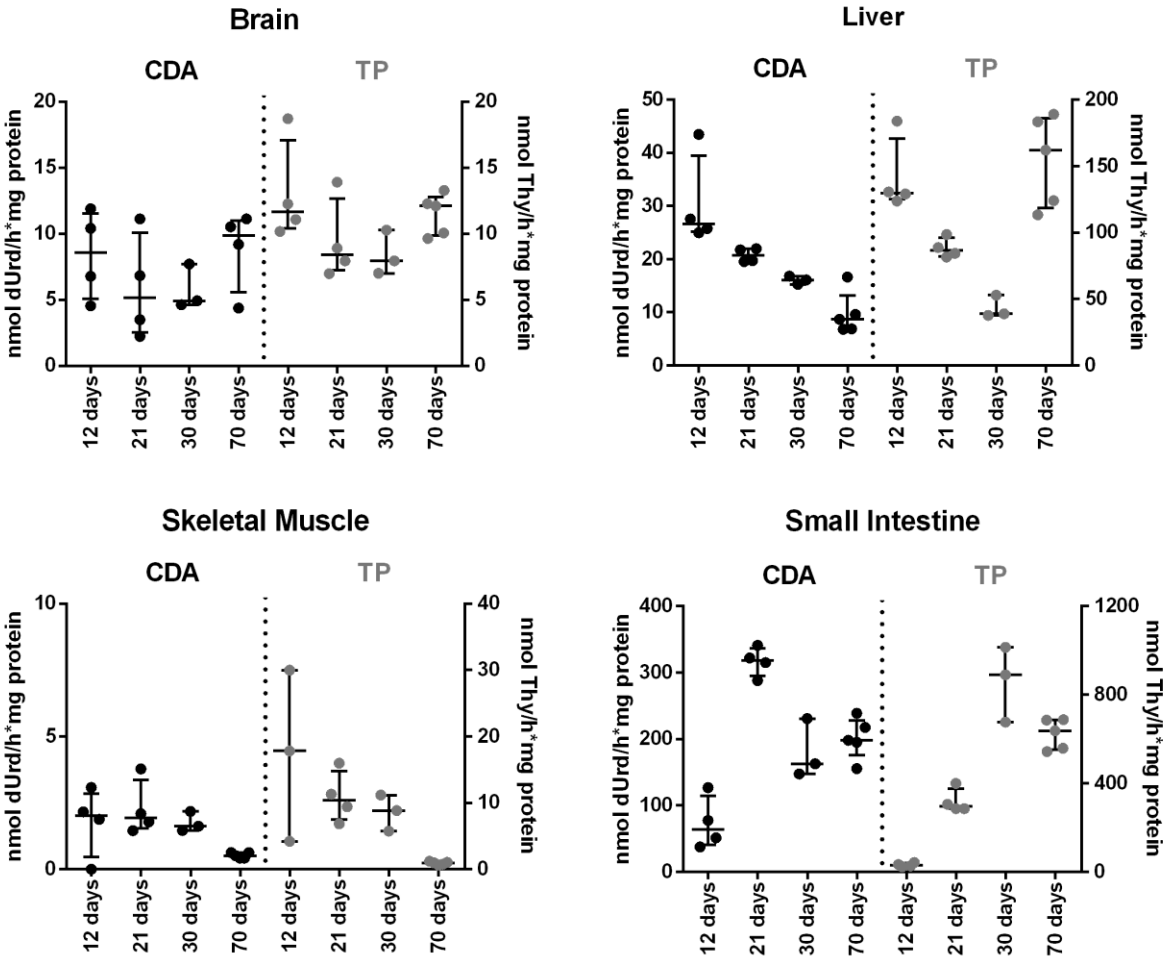
A

dCK

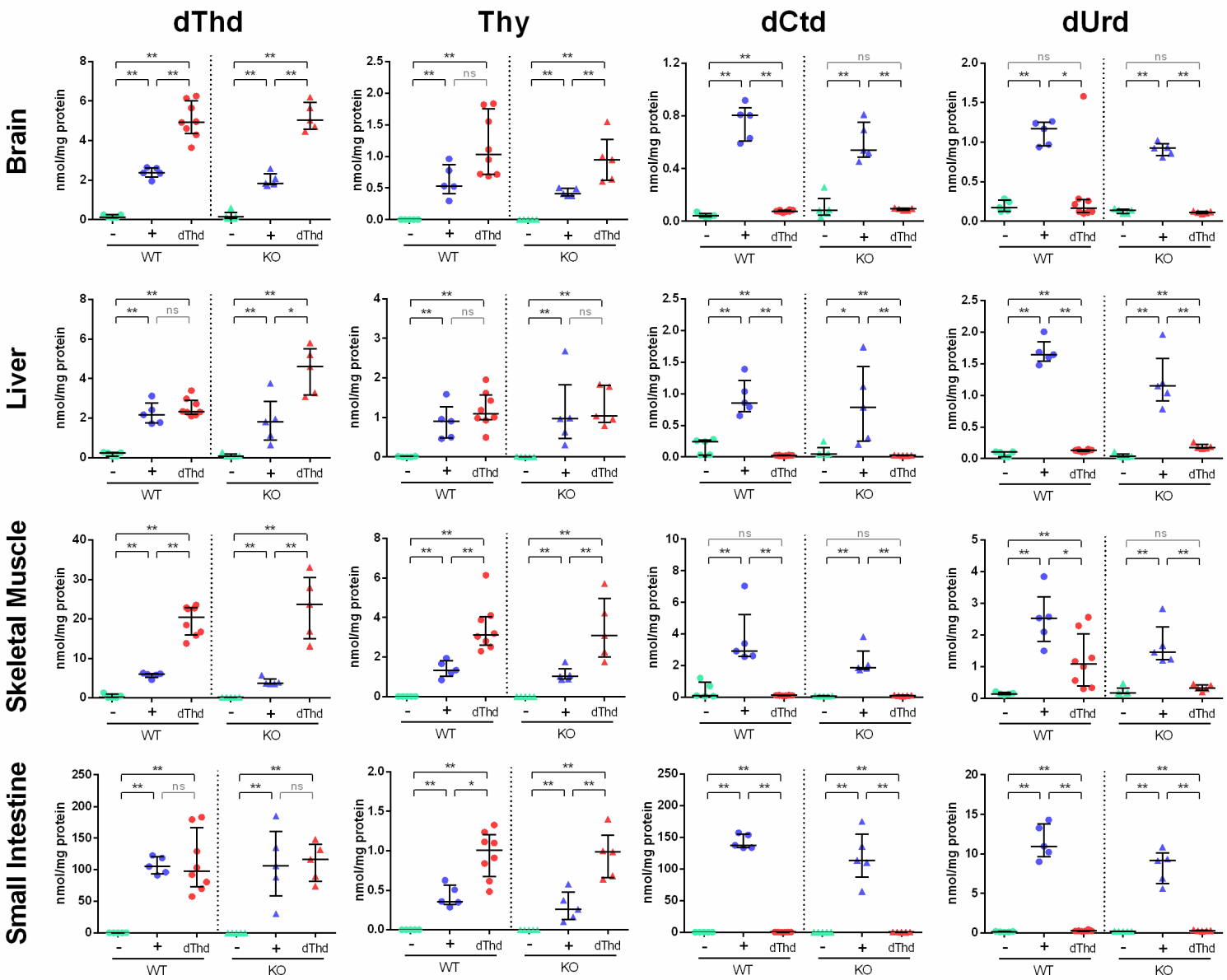

Liver
12d
29d
Brain
12d
29d
wt PBS
wt dNs
KO PBS
KO dNs
hCTL
mCTL
kDa
250 -
75 -
50 -
37 -
25 -
20 -
15 -



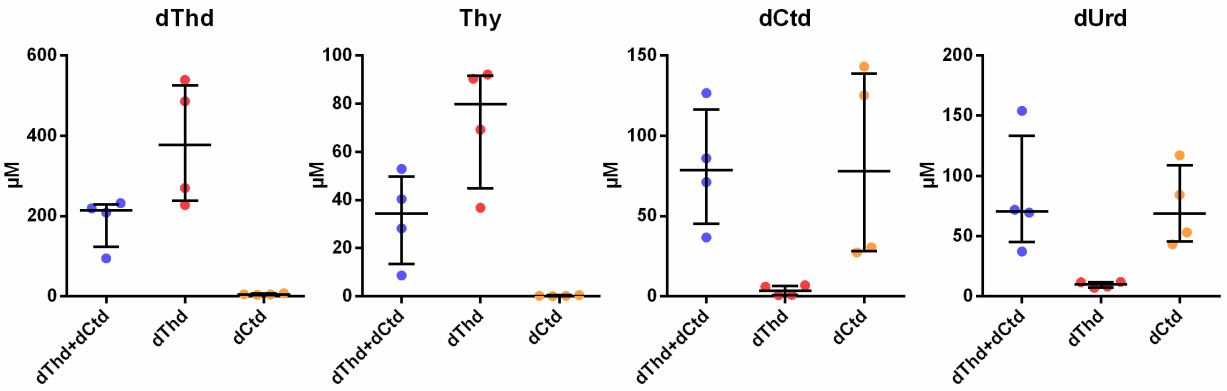
Supplementary Fig 3: Full-length Western blots for Supplementary Fig. 2. Full-length blots decorated with specific antibodies recognizing dCK (**A**) and α-tubulin (**B**). Specific bands corresponding to the protein of interest are depicted with an arrow (red arrow in **r**epresentative full blots corresponding to images in Supplementary Fig. 2). All blots were analysed and used for quantification graphs in Supplementary Fig. 2. Whole cell extracts from HEK-293 cells (hCTL) and liver extracts from 31-week-old wild-type mice (mCTL) were used as controls in different membranes. dNs: treated with dThd+dCtd.



Supplementary Fig 4: TP and CDA activities in wild-type mice. Enzyme activity from CDA (black) and TP (grey) in brain, liver, skeletal muscle (gastrocnemius) and small intestine from wild-type (C57BL/6) mice aged 12, 21, 30 and 70 days. Scatter plots represent the median (horizontal line) with interquartile range. Results are represented as nmol of product formed in 1 h from 1 mg of protein in the tissue homogenate. Mice in each group, n=3-4.



Supplementary Fig 5: dThd, dCtd, dUrd and Thy levels in tissues of mice treated with dThd alone. Concentrations of dThd, dCtd, dUrd, and Thy in brain, liver, skeletal muscle (gastrocnemius), and small intestine by LC-MS/MS. Results indicate nmols of the metabolite per mg of protein in tissues of mice aged 12 days. *TK2*^{KO} (KO; triangles) and *TK2*^{WT} (WT; dots) mice, treated with either 400 mg/kg/day of dThd+dCtd (+; blue), 400 mg/kg/day of dThd (dThd; red), or PBS (-; green). Tissues were collected 1 h after oral gavage. Scatter plots represent the median (horizontal line) with interquartile range. Mice in each group, n=5-8. P-values obtained with the Mann-Whitney U test: *p<0.05; **p<0.01; ns, non-significant differences.



Supplementary Fig 6: dThd and dCtd plasma levels after acute administration of each compound. Concentrations (µM) of dThd, dCtd and their first degradation products (dUrd and Thy) in plasma of wild-type (C57BL/6) pups aged 11-16 days by LC-MS/MS. Blood was collected 1h after oral gavage of a single acute dose (400 mg/kg) of dThd+dCtd (blue), dThd (red) or dCtd (orange). Scatter plots represent the median (horizontal line) with interquartile range (n=4).

	Number of rows	# of blank lines	# censored subjects	# deaths	Median survival
WT PBS	270	223	46	1	Undefined
KO PBS	270	212	38	20	16
WT dThd+dCtd (400mg/kg/day)	270	215	54	1	Undefined
KO dThd+dCtd (400mg/kg/day)	270	222	36	12	34
WT dTMP+dCMP (620mg/kg/day)	270	256	14	0	Undefined
KO dTMP+dCMP (620mg/kg/day)	270	259	0	11	32
WT dThd (400mg/kg/day)	270	260	10	0	Undefined
KO dThd (400mg/kg/day)	270	258	0	12	33
WT dCtd (400mg/kg/day)	270	261	9	0	Undefined
KO dCtd (400mg/kg/day)	270	264	0	6	15

Supplementary Table 1: Survival data summary. Mice shown in Kaplan-Meier curves of figure 2A and 8A are the ones included in this table.