

Cockayne syndrome mice reflect human kidney disease and are defective in *de novo* NAD biosynthesis

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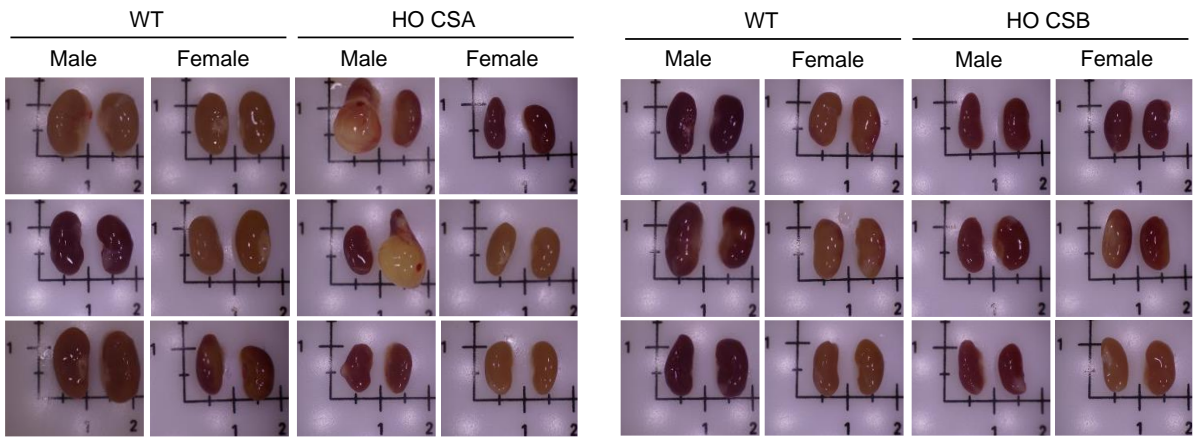
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⁶Danish Center for Healthy Aging, University of Copenhagen, 2200 Copenhagen, Denmark;

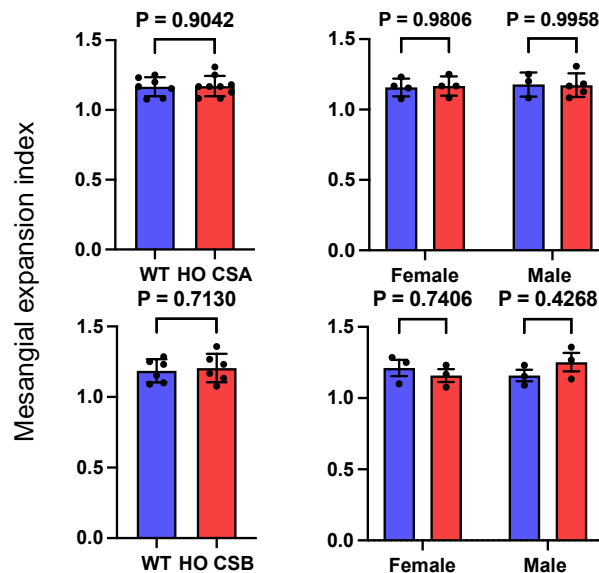
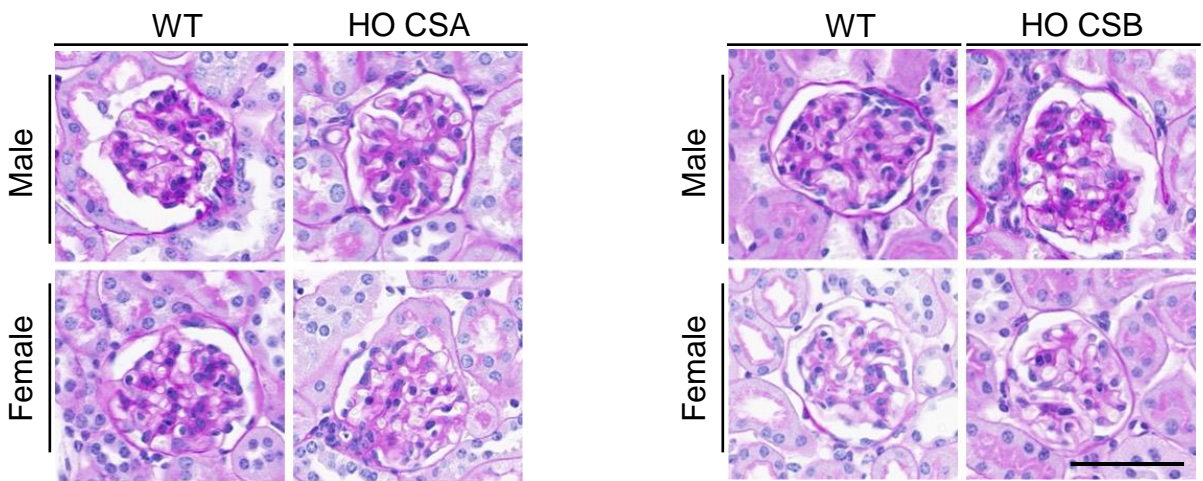
⁷Lead Contact

Supplementary Data

A



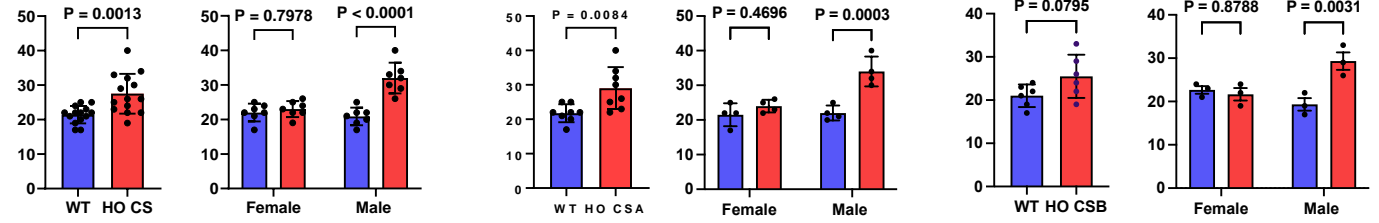
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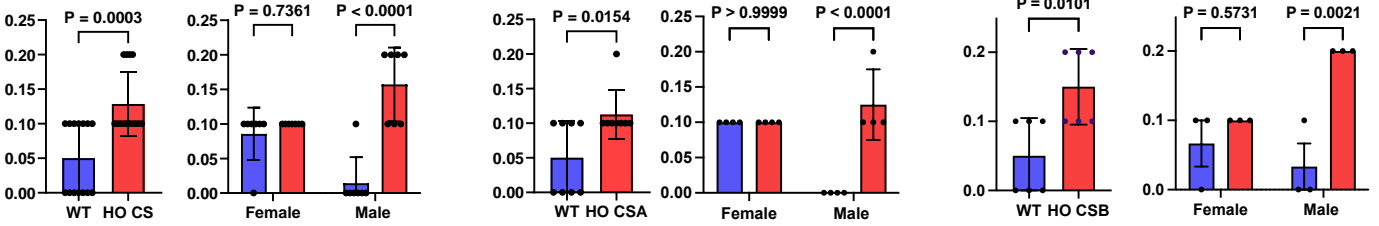
Supplementary Figure S1

(A) Kidneys were removed from 45-86 weeks-old mice at termination and directly compared. (WT= wildtype; HO=Homozygous; M= Male; F= Female) Background scale $\times 10$ mm. (B) Representative image of PAS staining in CS mice. Scale bar, 50 μ m. The mesangial expansion index is defined as the ratio of the mesangial area to the glomerular tuft area. The mesangial area is measured by assessing the PAS-positive, nucleus-free regions within the mesangium. Data are presented as mean \pm SD ($n \geq 3$). An unpaired t-test was used for comparing CS with WT. A two-way ANOVA Sidak test was used for the comparisons between males and females. p-values indicated (Blue bar, WT; Red bar, HO CSA/CSB).

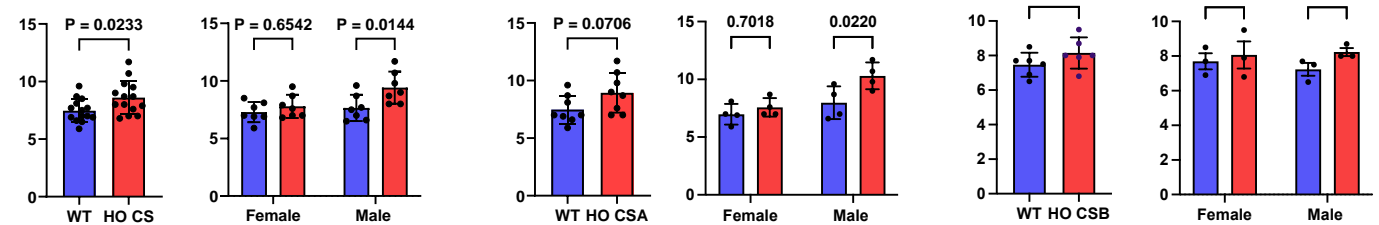
Blood urea nitrogen (mg/dL)



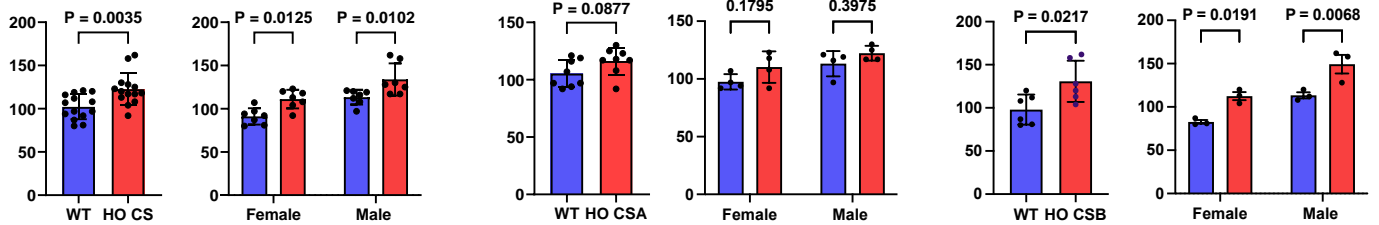
Serum creatinine (mg/dL)



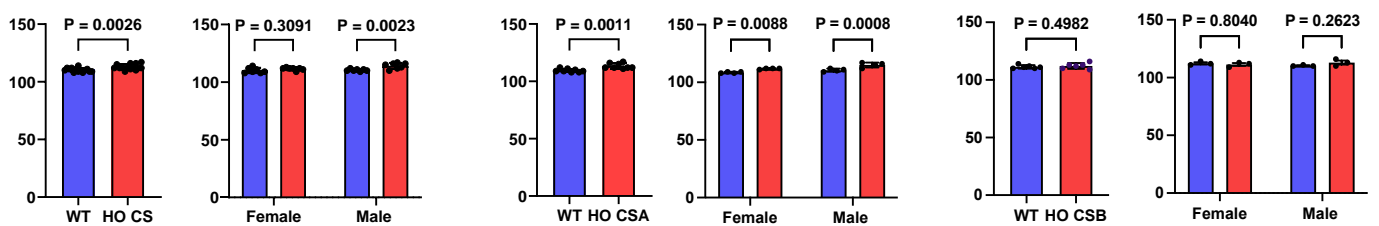
Serum phosphorous (mg/dL)



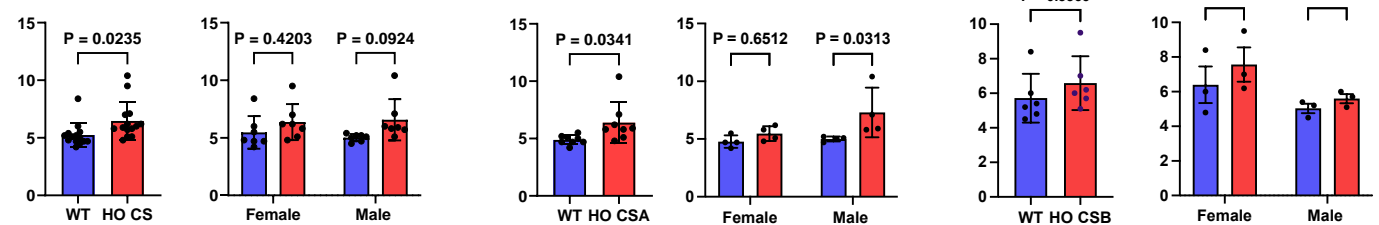
Serum cholesterol (mg/dL)



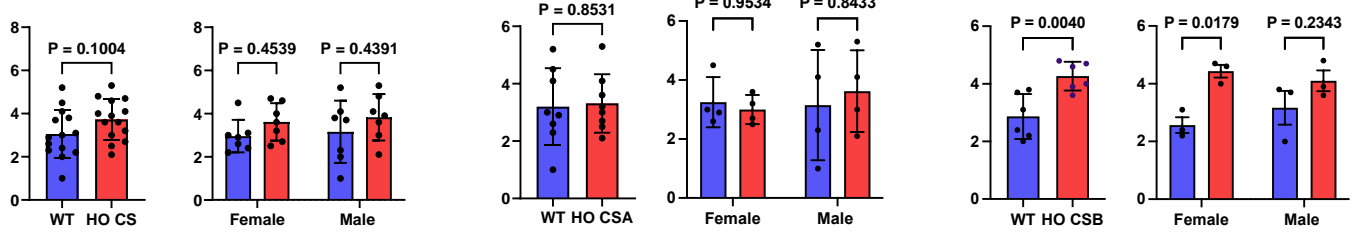
Serum chloride (mmol/L)



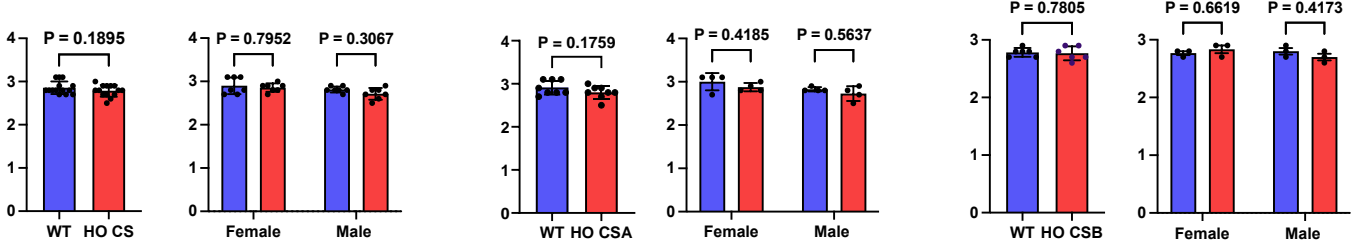
Serum potassium (mmol/L)



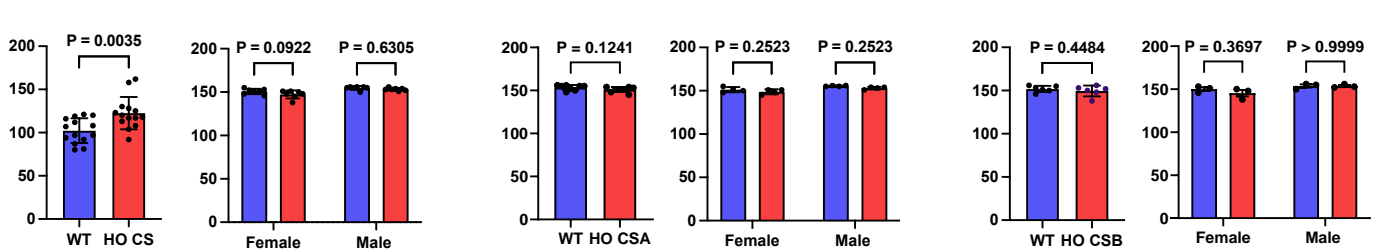
Serum uric acid (mg/dL)



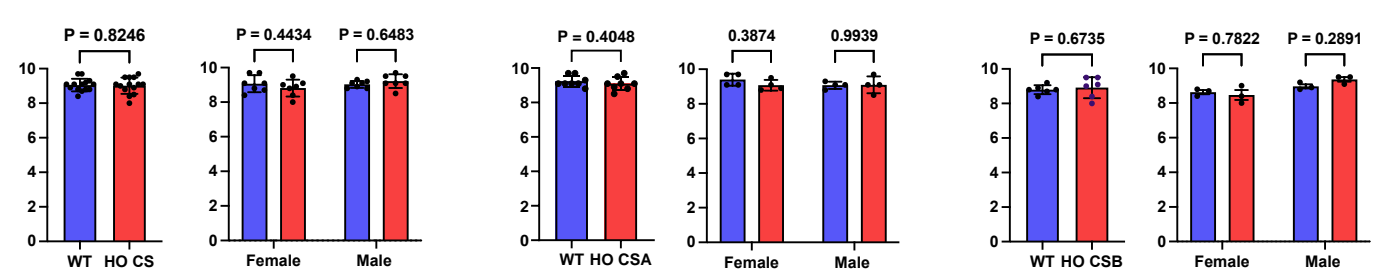
Serum albumin (g/dL)



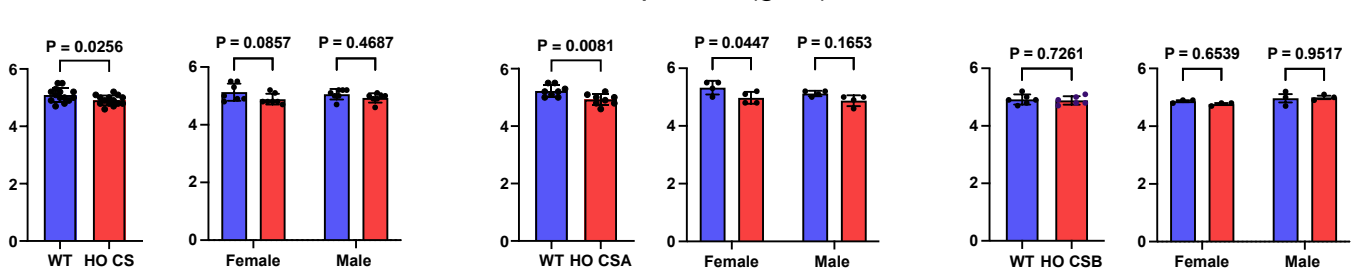
Serum sodium (mmol/L)



Serum calcium (mg/dL)



Serum total protein (g/dL)

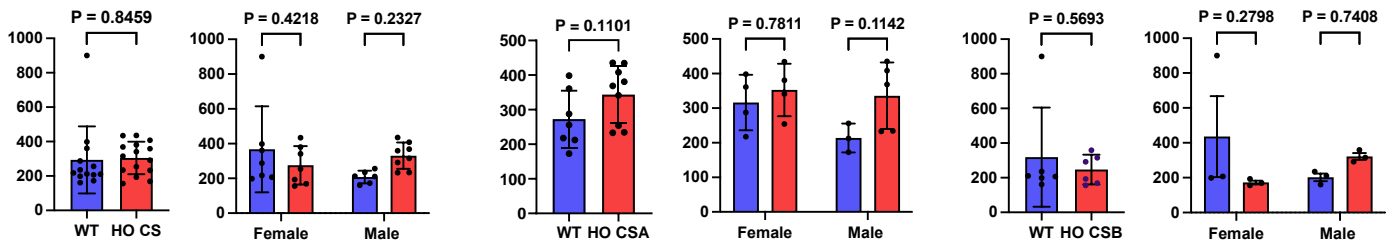


Supplementary Figure S2

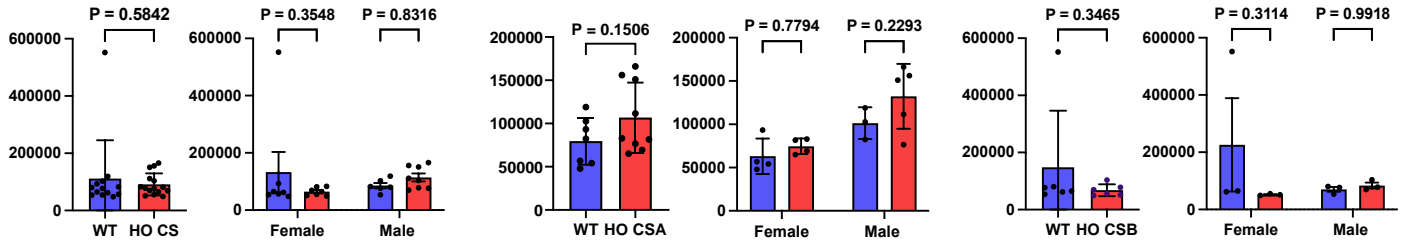
Serum isolated from the whole blood samples of the WT and CS mice were collected and analyzed for the parameters indicated. Data presented as mean \pm SD ($N \geq 3$), Unpaired t-test was used for comparing CS with WT. Two-way ANOVA test used for male vs female comparison. P-values indicated (Blue bar, WT; Red bar, HO CSA/CSB).

A

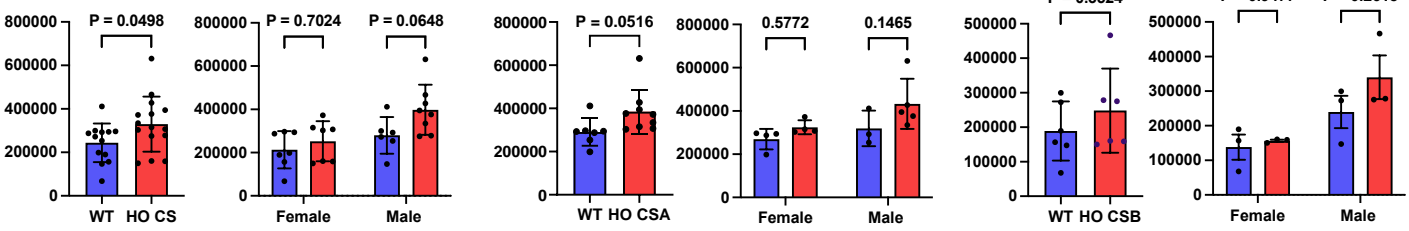
Serum Havcr1 (pg/ml)



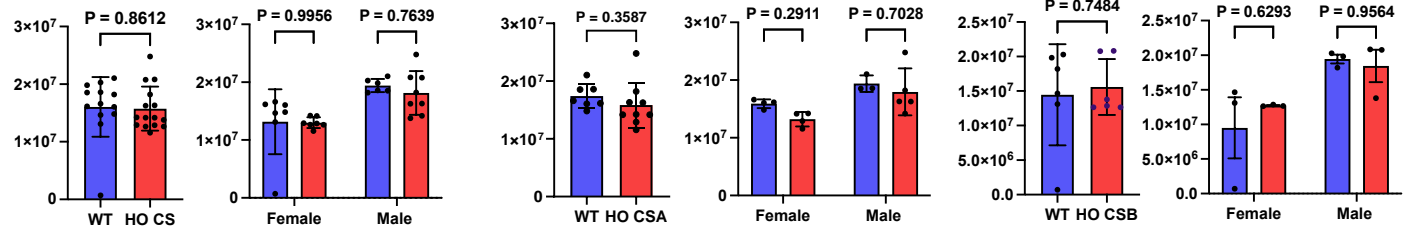
Serum Lcn2 (pg/ml)



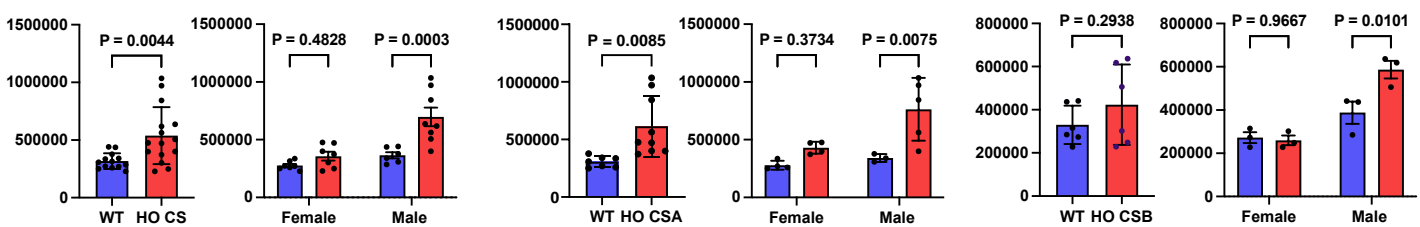
Serum Cst3 (pg/ml)



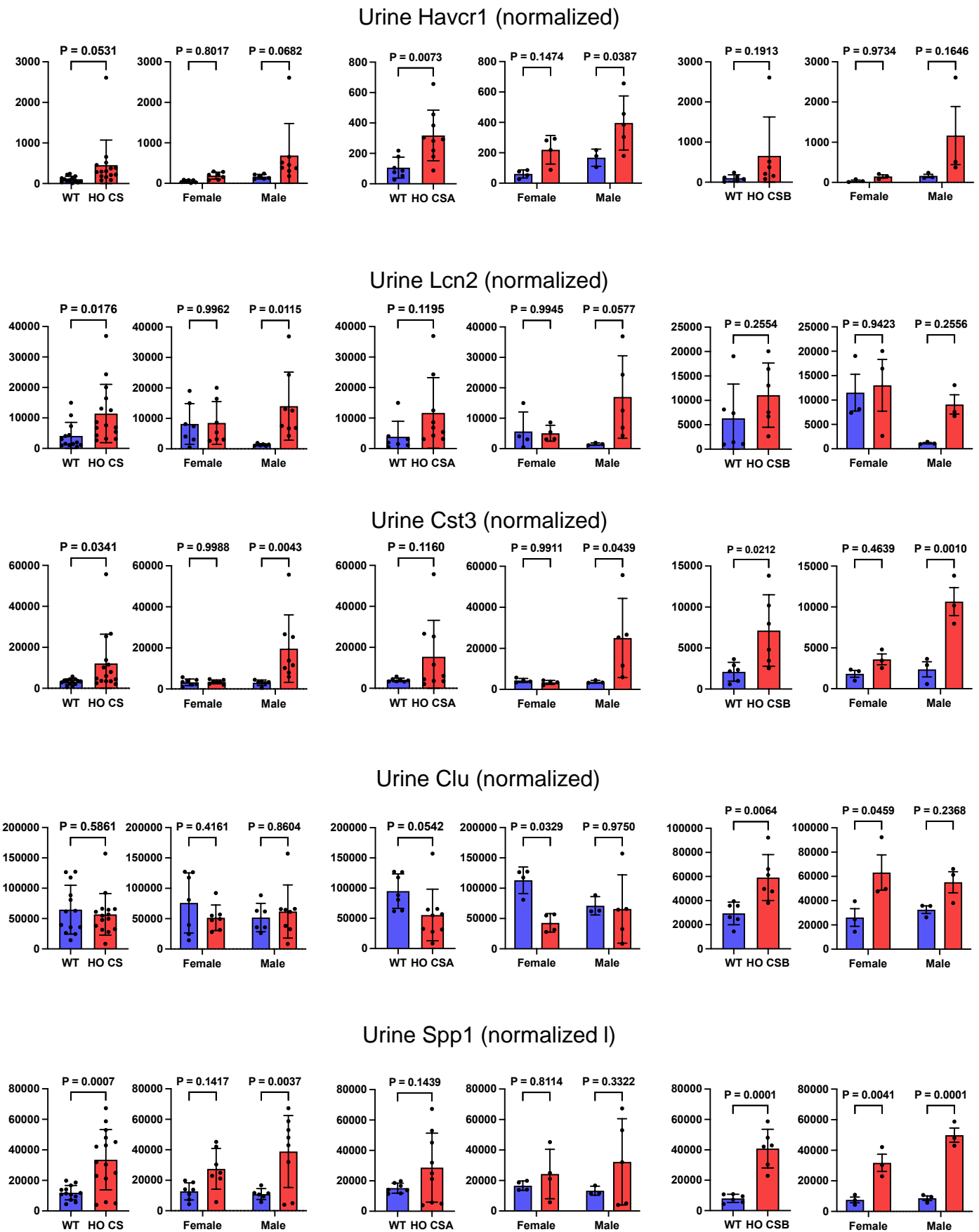
Serum Clu (pg/ml)



Serum Spp1 (pg/ml)

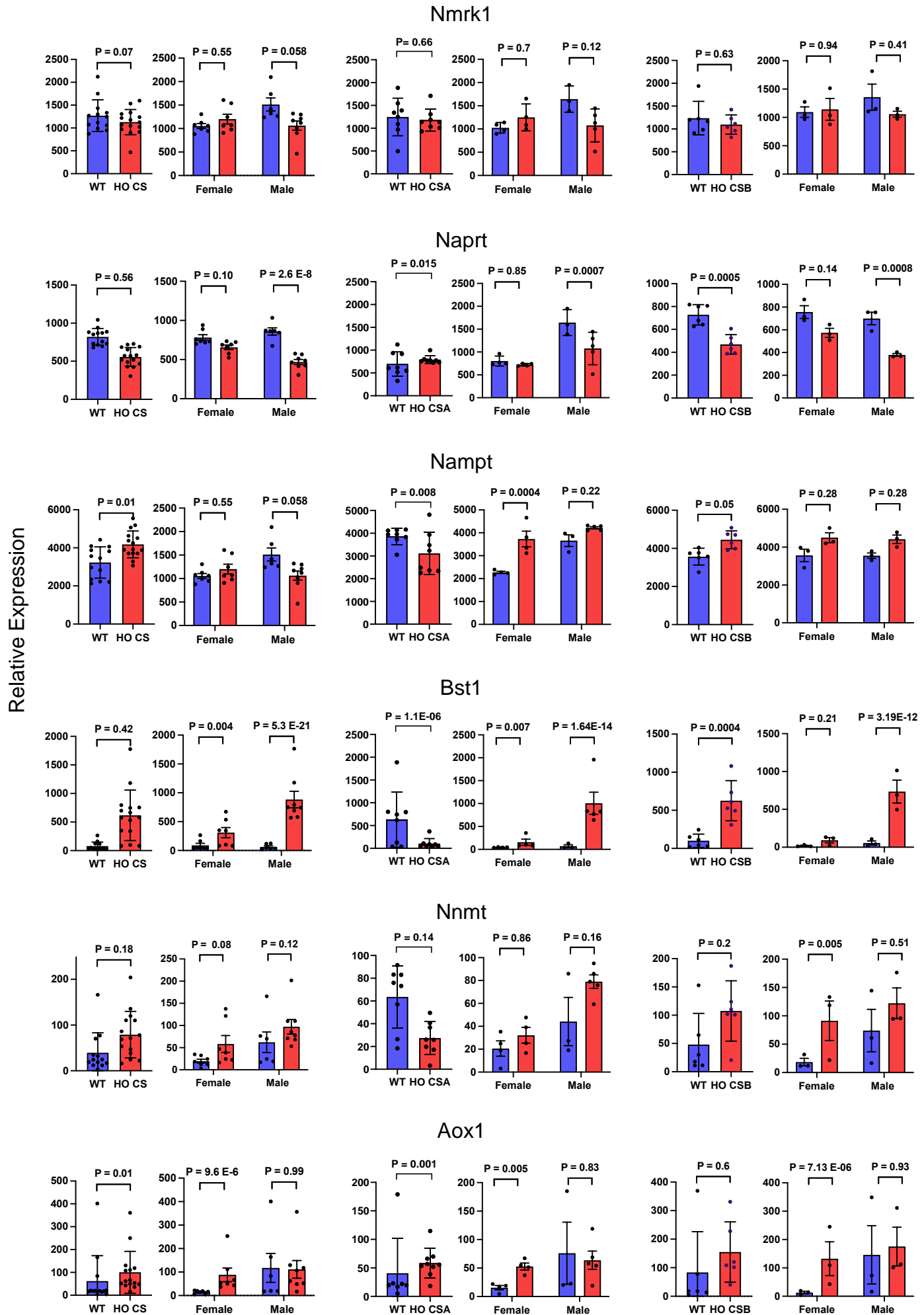


B

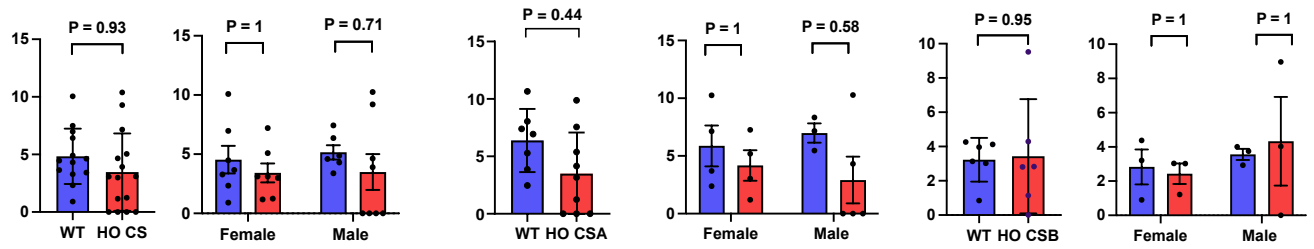


Supplementary Figure S3

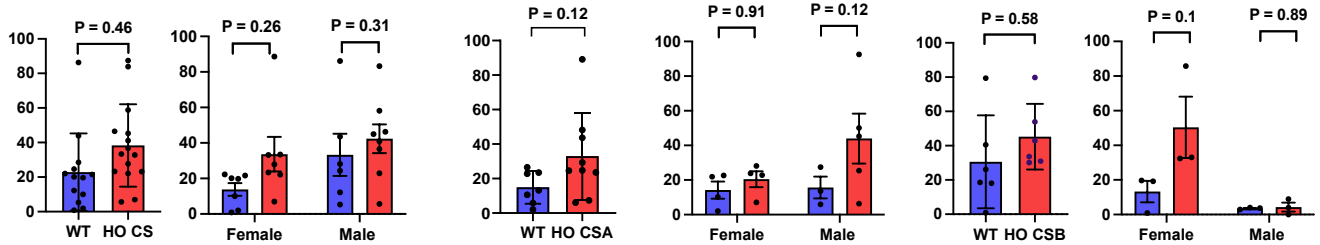
Measurement of (A) kidney toxicity markers in serum and (B) Creatinine normalized urine values of CS mice. Data shown as mean \pm SD ($N \geq 3$), Unpaired t-test was used for comparing CS with WT. Two-way ANOVA test used for male vs female comparison. P-values (Blue bar, WT; Red bar, HO CSA/CSB).



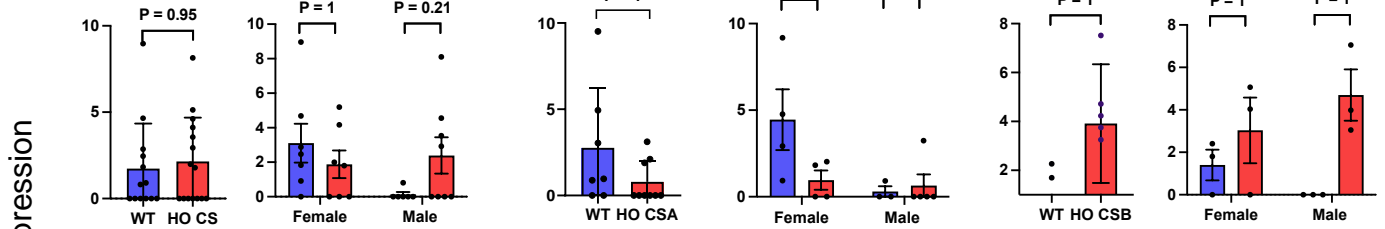
Aox2



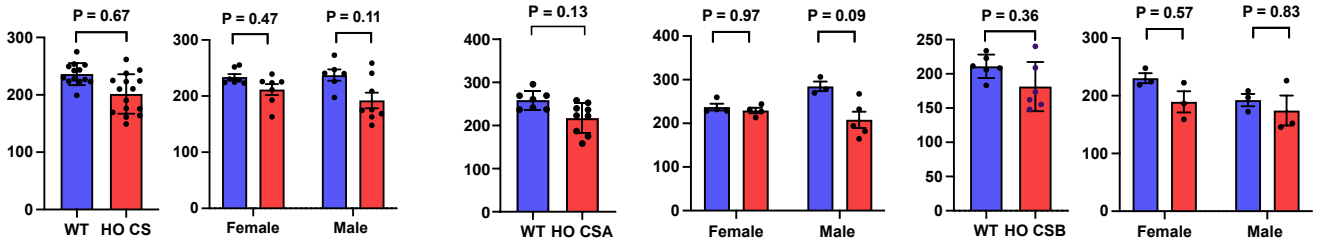
Aox3



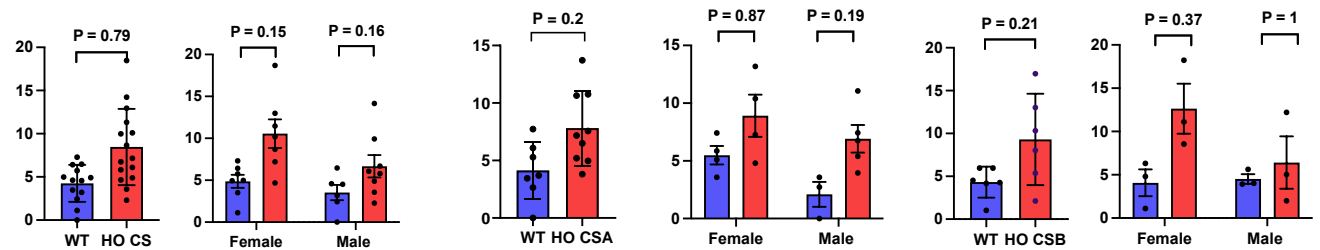
Aox4



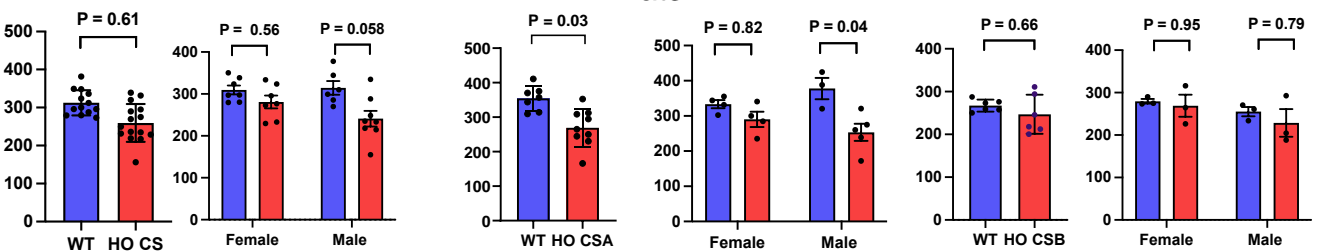
Nmnat1



Nmnat2

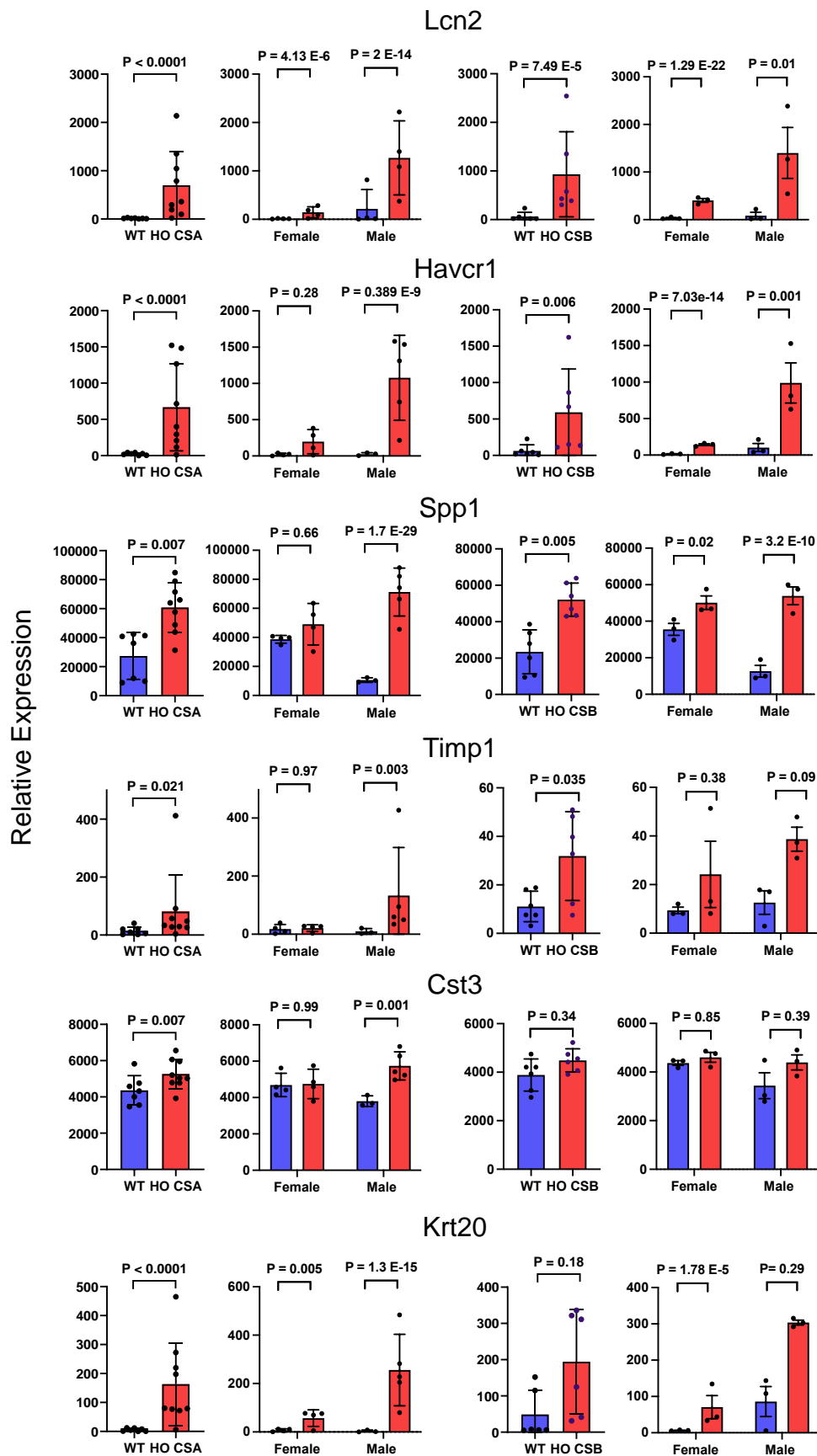


Nmnat3



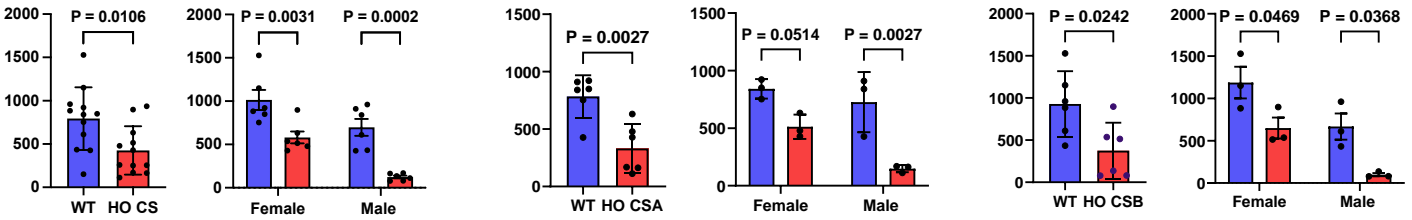
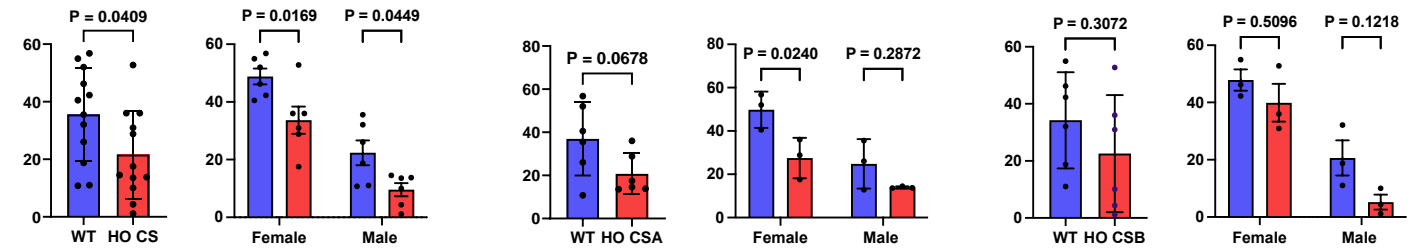
Supplementary Figure S4

Expression of genes in 'Nicotinate and Nicotinamide metabolism' panel from DEGs of WT and CS mice kidney RNA-sequencing analysis. P-adjusted values indicated (Blue bar, WT; Red bar, HO CSA/CSB).



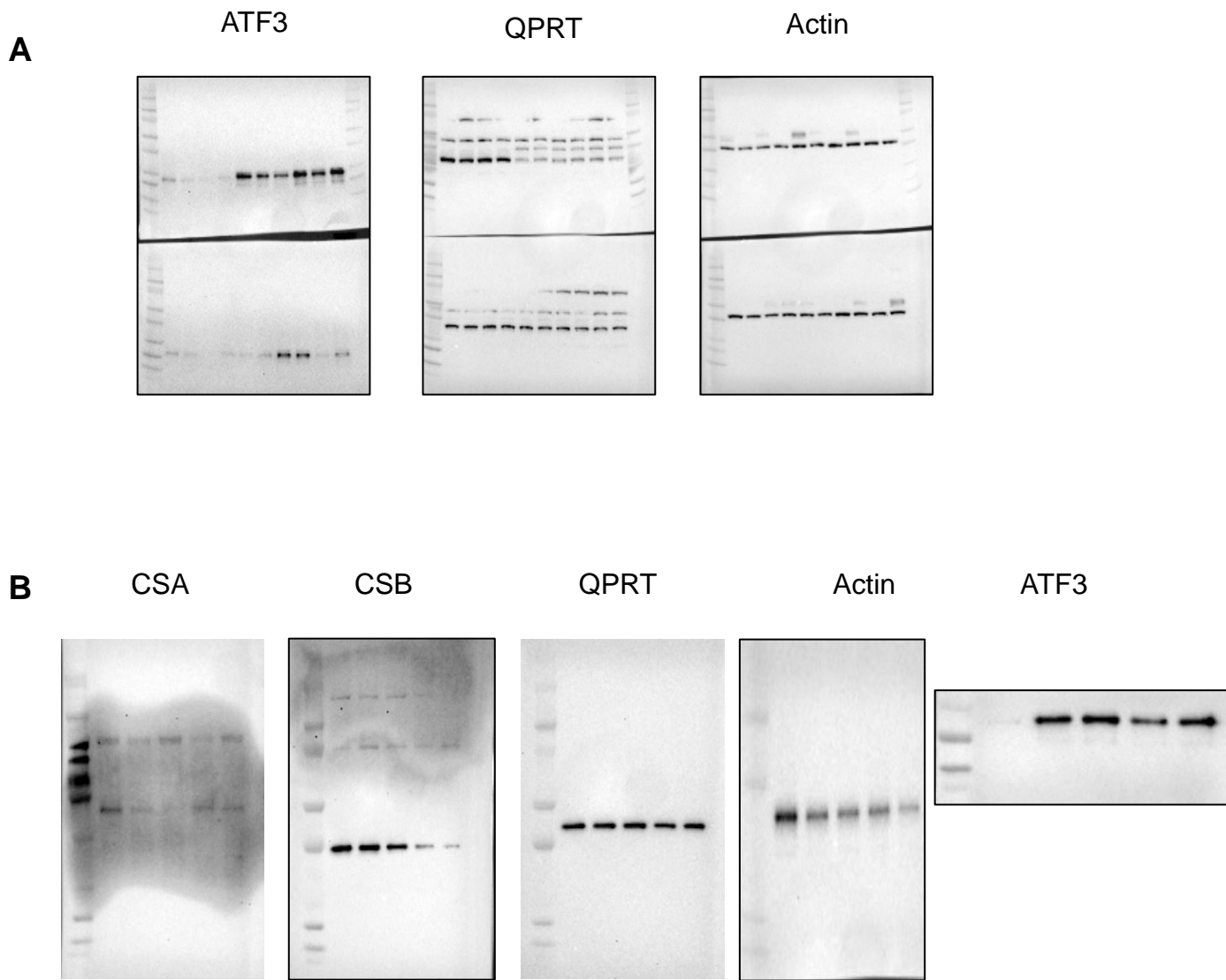
Supplementary Figure S5

Expression of kidney toxicity markers in kidney from DEGs of WT and CS mice kidney RNA-sequencing analysis. P adjusted-values indicated (Blue bar, WT; Red bar, HO CSA/CSB).

NAD⁺ (ng/mg protein)NAD⁺/NADH (ratio)

Supplementary Figure S6

Measurement of NAD⁺ and NAD/NADH levels in WT and CS mice kidneys. Data are shown as mean \pm SD (N \geq 3), Unpaired t-test was used for comparing CS with WT. Two-way ANOVA test used for male vs female comparison. P-values indicated (Blue bar, WT; Red bar, HO CSA/CSB).

**Supplementary Figure S7**

Uncropped images of western blot for figure 6 (A) Mouse Kidney tissues (B) HK-2 cells

Supplementary Table S1

Primers

Primers	Sequences (5'to 3')
CSA Forward	GACCTTGAGAACTCCAGCAG
CSA Reverse	GTGTCATGAGGATACTACTGT
CSB Forward	TCTTGGCAGGTCTGAGCTA
CSB Reverse	TGCATCACTGTTGTTGGACA
mACT Forward	GATTACTGCTCTGGCTCCTAG
mACT Reverse	GACTCATCGTACTCCTGCTTG
mATF3 Forward	AGATGTCAGTCACCAAGTCTG
mATF3 Reverse	TGTCTTCTCCTTTTTCTTGTTCG
mQPRT Forward	CATGCCACCGCTACGAC
mQPRT Reverse	GCTACATTCCACCTCTACCTTC
mTIMP1 Forward	AGACAGCCTTCTGCAACTC
mTIMP1 Reverse	CAGCCTTGAATCCTTTTAGCATC
mHAVCR1 Forward	CTGCTACTGCTCCTTGTGAG
mHAVCR1 Reverse	GCGTTCGCAAAGCTTCAATC
mLCN2 Forward	CTACAATGTCACCTCCATCCTG
mLCN2 Reverse	CCTGTGCATATTTCCAGAGT
hATF3 Forward	GCTGGAATCAGTCACTGTCAG
hATF3 Reverse	CTTATTTCTTTCTCGTCGCCTCT
hGAPDH Forward	ACATCGCTCAGACACCATG
hGAPDH Reverse	TGTAGTTGAGGTCAATGAAGGG
ChIP Primer 1 Forward	TCTCAGGAGTGGTGTGTT
ChIP Primer 1 Reverse	AGAAGGTCAATATTTCTGTGACA
ChIP Primer 2 Forward	GGGAGGGCAGAGCTATTA
ChIP Primer 2 Reverse	CCTGGAGCTGTTGTGAAG
ChIP Primer 3 Forward	GTGGCTCACACCTGTAAT
ChIP Primer 3 Reverse	GACGAGGTTTCTCCATGTT
ChIP Primer 4 Forward	CTGGTGATGGTGAAGGATTAAC
ChIP Primer 4 Reverse	GCTGCTGCATTGCACTT
ChIP Primer 5 Forward	ATCACCTGGACAACCT
ChIP Primer 5 Reverse	AACAGCTTGAGGGAGAAATC
ChIP Primer 6 Forward	GCTTGAGCCCAACTCTG
ChIP Primer 6 Reverse	CCCATGTGGTTGATCCTTTA
ChIP Primer 7 Forward	AGCATTGATTCTTGATCCTCAT
ChIP Primer 7 Reverse	TTTCCAGGGATCCAGGTT
ChIP Primer 8 Forward	TACCGATGGACCACAGTC
ChIP Primer 8 Reverse	GGACAGAGCCACCAGAG
ChIP Primer 9 Forward	CCTGTAATCCCAGCACTTTG
ChIP Primer 9 Reverse	TTTCGCCATGTTGGTCAG
ChIP Primer 10 Forward	CCCGTCTACTAAACATACCA
ChIP Primer 10 Reverse	AACTCCTGACCTCATGATCC