Figure S1, related to Figure 3A. Metabolites levels in individual HO mice (n = 6) throughout a 24 hours cycle.

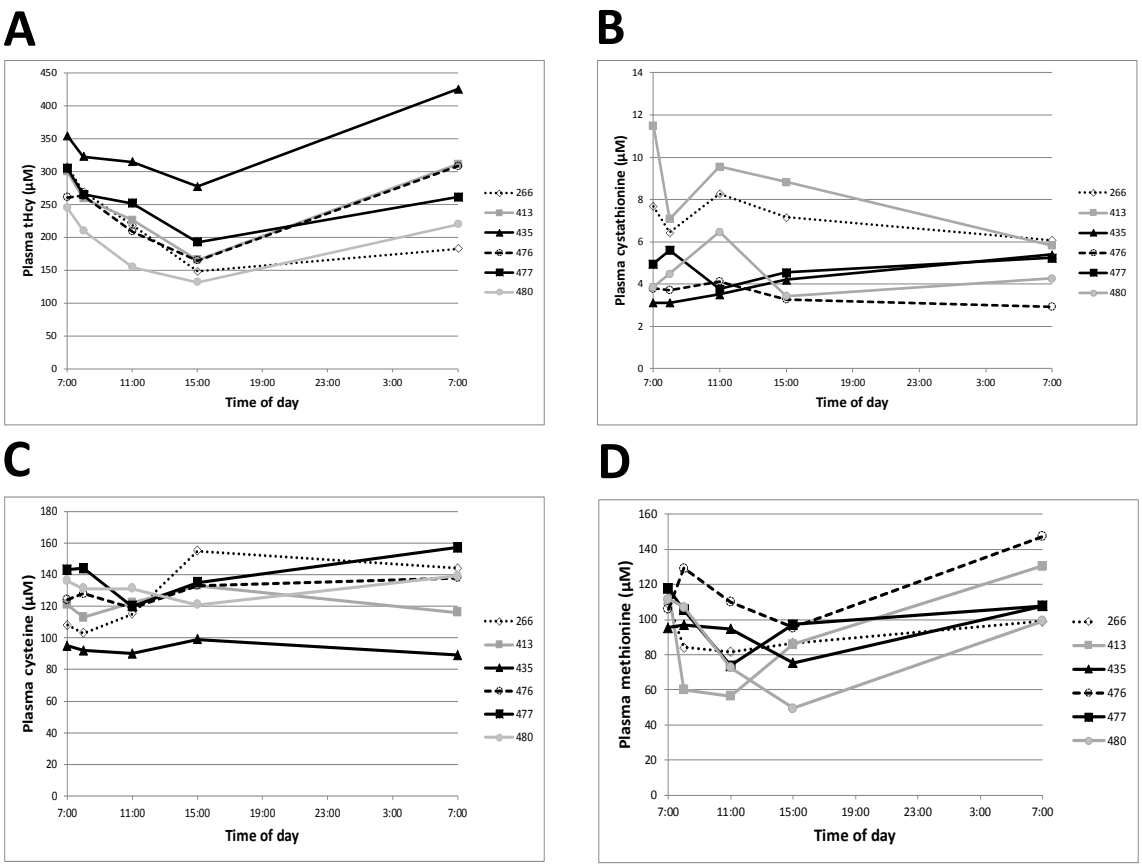
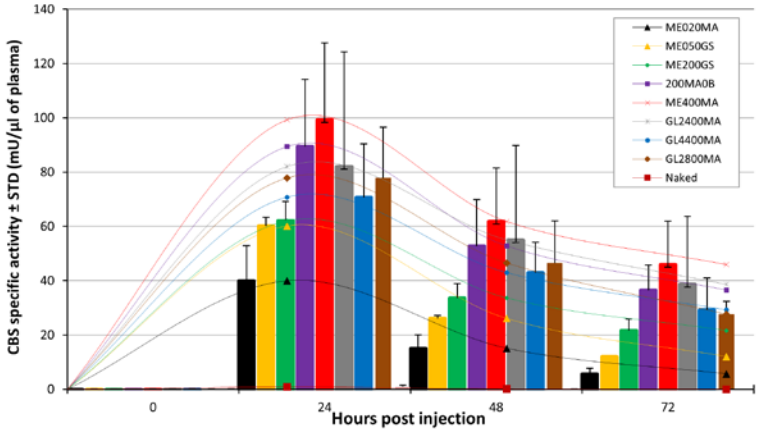


Figure S2, Related to Figure 4. Comparison of the pharmacokinetic properties of the CBS enzyme PEGylated with different PEGs. (A) Twenty seven C57BL/6J mice were divided into 9 experimental groups (n=3). Each experimental group was injected via SQ route with 5 mg/kg body weight of htCBS PEGylated with the indicated PEG molecule (table), or with the non-PEGylated (naked) enzyme. Blood samples were drawn at the indicated time points and the activity of the enzyme was determined using the radiometric activity assay. Data is presented as a histogram with standard deviation (STD), and as a scatter plot. (B) Coomassie-stained SDS-PAGE showing htCBS PEGylation time course. PEGylation reaction was initiated by adding the PEG, and sample were withdrawn from the tube at the indicated time points to be analyzed.

A

PEG molecule	Structure	Target group	Size (kDa)
ME020MA	linear	-SH	2
ME050GS	linear	-NH2, -OH, -SH	5
ME200GS	linear	-NH2, -OH, -SH	20
ME200MA0B	linear	-SH	20
ME400MA	linear	-SH	40
GL2400MA	2 arms	-SH	40
GL4400MA	4 arms	-SH	40
GL2800MA	4 arms	-SH	80



B

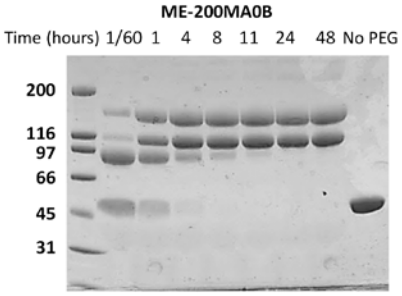


Figure S3, related to Figure 5. Comparison of PEGC15S and PEGhtCBS. The levels of tHcy in HO mice that were injected with 7.5 mg/kg of PEGC15S or PBS (n=4) were measured 24, 48 and 72 hours post injection. Data is presented as mean ± SEM and are compared to time 0 values, using a paired Student's t test (*p ≤ 0.05, **p ≤ 0.01, and ***p ≤ 0.001).

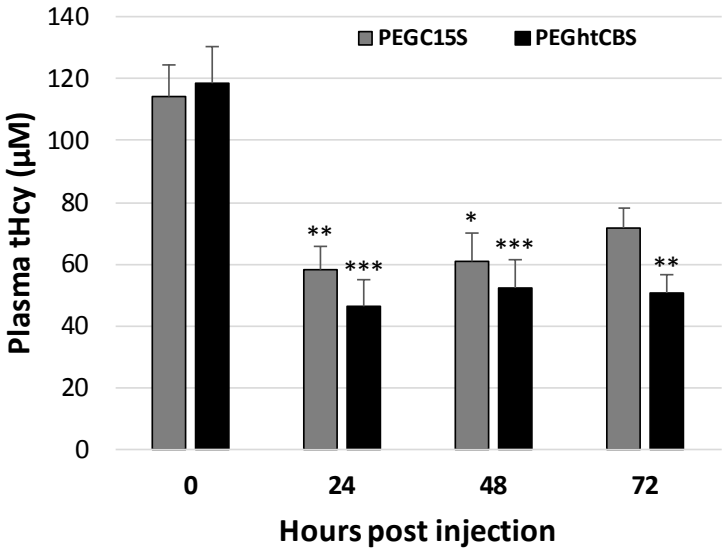


Figure S4, Related to Figure 6. Impact of PEGC15S on metabolites during a 24 hours period. The levels of tHcy, cystathionine, and cysteine in HO mice that were injected with 7.5 mg/kg of PEGC15S or PBS (n=5) 1, 4 and 24 hours post injection. Data is presented as mean ± SEM and each time point is compared between the two groups using unpaired Student's t test (*p=0.05, **p ≤ 0.01, and ***p ≤ 0.001).

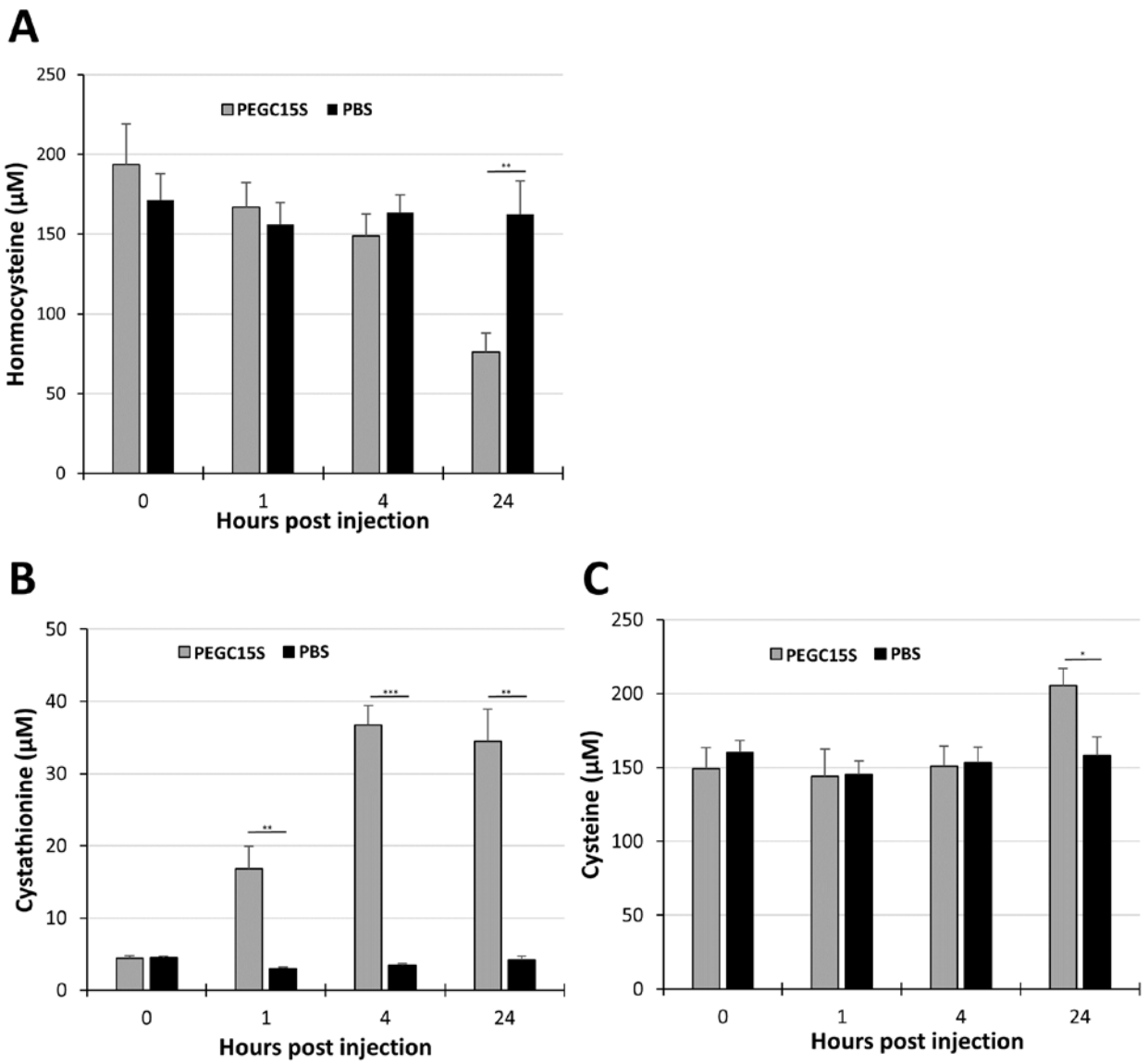


Table S1, Related to Figure 1A

Pharmacokinetic parameter	Unit	IP	SQ	IV
Dose Amount	**mg/kg**	5	5	5
AUC(0-t) (obs area)	**mU-hr/µl**	932.4	500.9	1011
Bioavailability	**%**	92	50	-
E Half-life	**hr**	4.3	6.1	2.7
Cmax (obs)	**mU/µl**	75.9	34.5	-
MRT (area)	**hr**	7.9	9.5	4.9

Table S2, Related to Figure 1D

Pharmacokinetic parameters		PEGylated htCBS ME020MA		PEGylated htCBS GL4-400MA	
Parameter	**Units**	**SQ**	**IV**	**SQ**	**IV**
Dose Amount	**mg/kg**	5	5	5	5
AUC(0-t) (obs area)	**mU-hr/µl**	1637.0	3193.2	2286.3	2836.5
Bioavailability	**%**	51.2	-	80.6	-
E Half-life	**hr**	15.1	16.7	20.1	30.4
Cmax (obs)	**mU/µl**	54.6	-	64.9	-
MRT (area)	**hr**	27.0	25.7	37.0	43.1

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Expression and purification of htCBS

The pET-28a(+) vector, harboring the sequence coding for the truncated human CBS, was transformed into DE3 bacteria, i.e., HMS174(DE3), and bacteria from kanamycin-resistant clones were grown in 5 ml of Luria-Bertani (LB) medium, with 30 μg/ml kanamycin, overnight at 37°C on a rotational shaker at 275 rpm. One ml of the overnight culture was added to a 100 ml Terrific Broth (TB) medium with 30 μg/ml kanamycin and grown overnight. Ten ml of was then added to a 1 liter of TB medium containing 0.001% of thiamine-HCl pH 8.0, 0.0025% of pyridoxine-HCl pH 8.0, 0.3 mM δ-ALA pH 8.0, 150 μM ferric chloride, 30 μg/ml of kanamycin. The culture was then grown at 30°C on a rotational shaker at 275 rpm until OD_{600} reached the value of ~0.6-0.7 and protein expression was induced by addition of 1 mM IPTG. Fermentation was continued for additional 16 hours. Cells were harvested by a 10 minutes, 6,000 rcf centrifugation at 4°C, washed with ice-cold 0.9% NaCl, re-centrifuged as above, and frozen at -80°C. The 4.45 ml of lysis buffer (20 mM NaH_2PO_4, pH 7.2, 40 mM NaCl, 0.1 mM PLP) per 1 gram of pellet was then added to the cell pellet and the latter was homogenized in a Dounce homogenizer and treated with lysozyme (2 mg/ml final), incubated for 1 hour at 4°C on a rocking platform, sonicated to reduce viscosity, and centrifuged at 53,000 rcf. The supernatant, comprising the soluble fraction was then stored at -80°C. The lysate was processed through a multi-step chromatographic procedure. The core process consists of an anion exchange capture column (DEAE Sepharose-FF), followed by an affinity column. This attains purity of approximately 90%. The final purity of > 99% is achieved by the use of one or two polishing chromatography steps. The final column eluate was formulated into PBS or buffer compatible with the PEGylation procedure.

Genotyping

Representative pups were routinely analyzed for homozygosity by qPCR. Tail biopsies were generated using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). DNA quality was monitored by NanoDrop 1000 (Thermo Scientific, DE, USA). Twenty ng samples of DNA were run single-plex in triplicate using Applied Biosystem's (CA, USA) Gene Expression master mix (Item #4369016). Amplification was performed on Applied Biosystem's 7500 Fast Instrument using the standard curve method. Applied Biosystems' Tert (Item #4458366) or Tfrc (Item #4458368) copy number reference assays were used as the homozygous one copy calibrator. Applied Biosystem's assay Mr00299300 was used to detect the *neo* gene.

Enzyme activity assay

CBS activity was determined by a radioisotope assay with ^{14}C-labeled serine as a substrate. Ten µl (total 490 ng) of pure htCBS in dilution buffer (0.1 M Tris-HCl pH 8.6, 1 mM DTT, 10 µM PLP, 0.5 mg/ml BSA) or 10 µl of plasma were added to 85 µl of reaction mixture containing 0.1 M Tris-HCl pH 8.6, 10 mM L-serine, 0.5 mM PLP, 0.5 mg/ml BSA and 0.3 µCi (for pure enzyme) or 0.45 µCi (for plasma) of L-[^{14}C(U)]-Serine. Samples were incubated for 5 min at 37°C and reaction was initiated by addition of 5 µl of 0.2 M Hcy (10 mM final concentration). Following 30 min of incubation at 37°C, a 20 µl aliquot of the assay mixture was applied onto a grade 3 CHR Whatman (NJ, USA) paper. The ^{14}C-cystathionine formed in the reaction was separated from the labeled substrate by an overnight descending paper chromatography in 2-propanol/formic acid/H_2O (75: 5.7: 18.9 v/v). Radioactivity in the area of the marker cystathionine (detected by staining the marker lane with acidic ninhydrin) was determined by cutting the chromatogram into strips that were submerged in 5 ml of Opti-fluor scintillation liquid (PerkinElmer, MA, USA) and

counted in a Beckman LS-3801 scintillation counter. Specific activity values are expressed as µmol of cystathionine produced in 1 hour per 1 mg of CBS or per µl plasma.

Primers

Primers used for cloning and mutagenesis of htCBS expression vector:

Primer A1 – 5'-AGTCGCCCATGGCGTCAGAAACCCCGCAG

Primer A2 – 5'-ATCGCGCTCGAGTTAGCGCAGGTGCCACCAC

Primer B1 – 5'-GGAGATATACCATG**C**CGTCAGAAACCCCGC

Primer B2 – 5'-GCGGGGTTTCTGACG**G**CATGGTATATCTCC

Primer C1 – 5'-TGGGTCCGACGGGT**A**GCCCGCAC

Primer C2 – 5'-GTGCGGGC**T**ACCCGTCGGACCCA

Histology

Livers were removed and were fixed for 24 hours with 4% paraformaldehyde in PBS (pH 7.4). Tissue blocks for histology were trimmed, dehydrated with an ethanol series followed by acetone, acetone-xylene mixture and xylene and then embedded in paraffin. In parallel, small tissue blocks (around 4x2 mm) fixed with paraformaldehyde were rapidly frozen in petrol ether cooled with dry ice, and stored at -50°C for detection of apolar lipids. Paraffin sections 4 µm thick were deparaffinized in xylene and after isopropyl alcohol step rehydrated with ethanol (96%, 70%, 60%). Tissue sections were stained with hematoxylin and eosin (H&E) to evaluate histopathological changes. Masson trichrome staining was performed for

detection of fibrosis. Steatosis was verified using Oil Red O staining for detection of apolar lipids in fixed-frozen sections, 10 µm thick and cut with a Leica CM 1850 Cryomicrotome. The sections were viewed and photographed in a Nikon E800 light microscope equipped with Olympus DP70 digital camera. The pathologist was blinded as far as the treatment regimen of the individual mice.