Supplemental tables 1-3

Table 1. Summary of antibodies used for western immunoblots

	primary antibody	company	dilution	secondary antibody	company	dilution
nitrotyrosine	mouse anti-nitrotyrosine	Upstate, Temecula, CA	1:2000	Alexa Fluor 680 conjugated goat anti-mouse antibody	Invitrogen	1:2500
GPx-1	rabbit anti-GPx-1	LabFrontier Co., Korea	1:1000	IRDye 800CW conjugated goat anti-rabbit antibody	Rockland Immunochemical	1:5000
CuZnSOD	rabbit anti-CuZnSOD	LabFrontier Co., Korea	1:2000	IRDye 800CW conjugated goat anti-rabbit antibody	Rockland Immunochemical	1:5000
MnSOD	rabbit anti-MnSOD	Stressgen	1:4000	IRDye 800CW conjugated goat anti-rabbit antibody	Rockland Immunochemical	1:5000
caspase-3	rabbit anti-caspase-3	Cell Signaling, Danvers, MA	1:1000	IRDye 800CW conjugated goat anti-rabbit antibody	Rockland Immunochemical	1:5000
MAP2	mouse anti-MAP2 (clone HM-2)	Sigma, St. Louis, MO	1:1000	Alexa Fluor 680 conjugated goat anti-mouse antibody	Invitrogen	1:2500
Actin	rabbit anti-actin	Sigma, St. Louis, MO	1:400	IRDye 800CW conjugated goat anti-rabbit antibody	Rockland Immunochemical	1:5000
_-actin	mouse anti-b-actin	Sigma, St. Louis, MO	1:20000	Alexa Fluor 680 conjugated goat anti-mouse antibody	Invitrogen	1:2500
protein carbonyl	rabbit anti-dinitrophenylhydrazone	Chemicon, Temecula, CA	1:1000	IRDye 800CW conjugated goat anti-rabbit antibody	Rockland Immunochemical	1:5000

Table 2. Summary of western immunoblot analyses in the hippocampus

	WT						GPx						2way ANOVA (p values)		
	Sham		3h		24h		sham		3h		24h		inter-action	effect of time	effect of genotype
	mean	SEM	mean	SEM	mean	SEM	Mean	SEM	mean	SEM	mean	SEM			
Antioxidant enzymes															
GPx	0.43	0.35	0.51	0.41	0.38	0.18	2.49	0.48	3.25	1.41	5.14	2.72	0.59	0.63	0.01
CuZnSOD	0.32	0.04	0.27	0.01	0.32	0.06	0.19	0.03	0.20	0.03	0.27	0.03	0.49	0.24	0.01
MnSOD	1.41	0.53	0.85	0.25	1.31	0.48	1.41	0.83	1.88	0.65	1.55	0.62	0.66	0.99	0.39
Oxidative stress markers															
Nitrotyrosine	0.01	0.00	0.03	0.01	0.04	0.01	0.01	0.00	0.03	0.01	0.02	0.00	0.05	0.01	0.16
Protein carbonyls	0.64	0.33	0.63	0.23	0.69	0.23	0.31	0.09	0.35	0.03	0.42	0.08	0.99	0.89	0.06
Injury markers															
pro-caspase3	0.31	0.18	0.19	0.06	0.13	0.04	0.22	0.07	0.28	0.11	0.17	0.07	0.64	0.51	0.87
cleaved-caspase3	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.96	0.65	0.46
MAP2a	1.66	0.52	1.35	0.32	1.84	0.55	1.81	0.52	1.51	0.23	2.06	0.99	1.00	0.67	0.71
MAP2a+b+c	2.52	0.69	2.10	0.45	2.60	0.71	2.58	0.70	2.12	0.21	2.87	1.30	0.98	0.71	0.86

Table 3. Statistic values of obtained sterology parameters

Subregion	group	Sampling sites		Section thickness		Number of cells counted		CE (Gunder sen)	CV
		mean	SEM	Mean	SEM	mean	SEM	mean	
CA1									
	WT-sham	280.00	24.62	19.78	0.42	374.00	27.55	0.07	0.16
	WT-injured	224.20	23.62	19.60	0.38	227.60	14.85	0.09	0.13
	GPx-sham	249.50	11.55	18.63	0.46	322.00	22.66	0.08	0.15
	GPx-injured	221.00	35.39	19.95	0.66	279.30	48.80	0.08	0.34
CA3									
	WT-sham	203.80	11.34	19.25	0.58	334.00	13.62	0.08	0.08
	WT-injured	164.80	27.30	19.44	0.55	183.80	37.00	0.09	0.44
	GPx-sham	269.00	10.02	18.58	0.45	422.80	24.82	0.07	0.09
	GPx-injured	153.00	9.83	19.93	0.31	212.00	20.41	0.08	0.17
DG									
	WT-sham	185.50	14.53	19.13	0.40	373.00	23.12	0.07	0.12
	WT-injured	99.80	13.13	19.24	0.44	113.00	10.62	0.10	0.22
	GPx-sham	171.00	21.37	18.70	0.37	339.30	36.65	0.07	0.21
	GPx-injured	110.80	14.86	19.45	0.42	167.80	22.98	0.09	0.24

Supplemental Figure Legends

Supplemental Figure 1. TUNEL positive nuclei in the hippocampus at 24 hours postinjury.

A-D) Representative photomicrographs of TUNEL staining, an indicator of irreversible cell injury. TUNEL positive cells are prominent in the granule cell layer of the dentate gyrus (arrows) in both genotypes.

Scale bar, 100μm.

E) The percentage of TUNEL-positive cells [(number of TUNEL-positive nuclei/number of Hoechst-positive nuclei) × 100%] was determined within 5 regions of the hippocampus for each genotype. The numbers of labeled nuclei are more prominent in caudal hippocampus relative to rostral. However, no differences in TUNEL labeling are noted between genotypes.

Supplemental Figure 2. Temporal changes in the cortical lesion as assessed by magnetic resonance imaging.

A) Representative T2WI and DWI from a WT injured animal. At 1 day postinjury, the site of injury exhibits relative high signal intensity on T2 images, suggesting vasogenic edema, and high signal intensity on DWI. Calculated ADCs are abnormally low for the injured region, suggesting cytotoxic edema was also present. At 7 days postinjury the lesion consists of both a hypointense region on T2 (arrows), consistent with aged clot, and hyperintense regions on T2 whose signal are almost nullified on DWI (not present in selected case) consistent with liquified tissue. At 14 and 24 days postinjury, the lesion consists of liquified tissue.

B) Time course of signal increase in injured brain over 30 min after contrast administration at 1 day postinjury. This graph shows a relatively steady signal increase over time after contrast administration consistent with leakage of contrast material into the injured tissue. Minor but non-significant differences between WT and GPxTg groups can be ascribed to somewhat smaller injury among the GPxTg animals examined.

Supplemental Figure 3. Exploratory activity and anxiety levels after TBI or sham surgery.

Exploratory activity and anxiety levels were assessed in the open field.

A) GPxTg animals spend more time in the center of the open field (# $p<0.05$), indicating lower levels of anxiety than WT mice.

B) There is no difference in total distance moved, an indication of total activity levels.

C, D) Within the GPxTg group, injured mice enter the center less (C, * $p<0.05$) and move less in the center (D, * $p<0.05$).

Supplemental Figure 4. Sensorimotor learning after TBI or sham surgery.

Sensorimotor function was assessed using the rotorod.

A) All groups exhibit improvement with training.

B) The GPxTg animals that received TBI show significantly less improvement in rotorod performance with training than GPxTg sham mice (difference in time between trial 9 and trial 1).

* $p<0.05$

Supplemental Figure 5. Cortical and hippocampal lesion volumes at 4 months postinjury.

A, B) Cresyl violet staining demonstrates how a cortical cavitation replaces the frontal and parietal gray matter and subcortical white matter.

Scale bars, 10μm.

C, D) Regional volumes of ipsilateral cortex and hippocampus, estimated by the Cavalieri method, are reduced after TBI. However, this reduction is similar between genotypes (unpaired T-tests; p=0.622 and P=0.512 for the cortex and hippocampus, respectively).

Supplemental methods

Detection of protein carbonyls

Protein samples (10μg) were derivatized with 20mM 2,4-dinitrophenylhydrazine. Nonderivatized controls were run for each sample. Samples were separated by 12% SDS-PAGE and transferred to a PVDF membrane. Membranes were incubated with a rabbit anti-dinitrophenylhydrazone (DNP) antibody (1:1000) and mouse anti-β-actin (1:20000) followed by incubation in IRDye 800 conjugated goat anti-rabbit antibody (1:5000) and Alexa Fluor 680 conjugated goat anti-mouse antibody (1:2500).

Semiquantification of Fluorojade-labeled sections.

Five coronal sections, prepared from the hippocampus and each separated by 400μm, were selected for semiquantification. The first section from each animal was taken from the same anatomical plane (Bregma -1.46) in order to include the site of maximal injury. The following scale was used to assess Fluorojade labeled structures in the hippocampus:

0 = no Fluoro-Jade C positive cells;

1 = a few Fluoro-Jade C positive cells or fibers (less than approximately 10% of the total neurons);

2 = a moderate number of Fluoro-Jade C positive cells or fibers (approximately 10-30% of total neurons);

3 = robust number of Fluoro-Jade C positive cells or fibers (more than 30% of total neurons).

Quantification of TUNEL-positive cells in the hippocampus at 24 hours post injury.

Following TUNEL staining, sections were labeled with Hoechst 33342 (Molecular Probes, Invitrogen) to label nuclei. Sections treated with DNase I served as positive controls. The TdT enzyme was omitted in negative controls.

Images were captured on a fluorescent microscope coupled to a CCD camera with Spot Software (Diagnostic Instruments Inc.). Each captured image corresponded to 0.25mm^2, including the hilus and granule cell layer of the dentate gyrus or the pyramidal cell layer of CA3. Hoechst staining was imaged within the same field. For each image, a fluorescent threshold value was determined by measuring the average grayscale value of 4 background (negatively-stained) regions within each image. The total number of nuclei exhibiting fluorescence intensity higher than threshold was calculated. Relative TUNEL fluorescence was determined by calculating the proportion of TUNEL-positive nuclei to Hoechst-positive nuclei expressed.

Stereologic analysis of hippocampal numbers

At the completion of the behavioral studies, a stack of MAC 5000 controller modules was configured to interface to a Nikon E600W microscope with a motorized stage and a DVC color digital camera (DVC Co., Austin, TX) with a PC workstation. From each brain, approximately 70 sections contained a uniform hippocampus with well discernable subregions in the ipsilateral hemisphere. The hippocampus was divided into three subregions (DG, CA3, and CA1)[24]. Every 12th section was selected for analysis (5-6 sections per brain). The first of these sections was randomly selected from the first twelve sections with a uniform hippocampus. A counting frame was used to count neurons at predetermined regular x–y intervals within volume samples (optical dissectors). Selection criteria for counting an object (neuron) within the optical dissector frame were implemented[44]. Inclusion and exclusion counting criteria were followed by an observer

who recorded counts only when a single neuronal nucleolus was brought into focus within the dissector frame. Because the area (a) of the counting frame is known relative to the regular stage-stepping intervals over the section, one can calculate the area sampling fraction (asf) as follows:

$$asf = a\ (frame)/a\ (step).$$

The height (h) of the optical dissector is known relative to the thickness (t) of the section. With these parameters, it is possible to estimate the number of neurons (N) with the formula

$$N=\sum Q^{-}\_t/h\_1/asf\_1/ssf,$$

in which $\sum Q^{-}$ is the total number of neurons counted in the region of interest. To analyze this sampling scheme for every animal, the relative variance of the neuronal number estimate and the coefficient of error (CE) were calculated[45].

Estimation of brain volumes.

To estimate cortical mantle and hippocampal volumes, every12th coronal section was stained with cresyl violet and used for quantification. The areas of the ipsilateral and contralateral cortical mantle and hippocampus were contoured and volumes were computed using the Cavalieri method[46].

MRI

T1WI were obtained with TR/TE of 500/12ms, acquisition time of 4.27 minutes, while T2WI and DWI had a TR/TE set to 2500/70ms and acquisition time of 21.33 minutes. Diffusion weighting b-factor was 1030 s/mm^2 along the z-axis. Seven mice were administered 1.0mmol/kg GdDTPA-BMA (Omniscan, GE Healthcare, United Kingdom) by intraperitoneal injection. The

mice were briefly removed from the magnet for contrast administration then reinserted. A set of 6 post-contrast images was obtained, beginning within 1-2 minutes and continuing for 30 minutes after administration of contrast. All images were analyzed using MRvision software (The MRVision Co., Winchester, MA).

Apparent diffusion coefficient (ADC) maps were calculated from T2- and diffusion-weighted images using the equation:

$$ADC = -ln[SI(DW)/SI(T2)]/b,$$

where SI is pixel signal intensity of the indicated image. Size of injury was determined by summing pixels that exhibited abnormal signal intensity on T2 and DW images. Contrast-enhanced images were evaluated by determining the rate of signal change over 30 minutes after contrast administration. Regions of interest were defined on subtraction images (6^{th} image - 1^{st} *image) and applied to all post contrast images. Rate of signal change was defined as:*

$$(SI(n)-SI(1))/(SI(1)*\Delta t).$$

It was assumed that adsorbtion of contrast media into the blood from the peritoneal fluid was the same for all animals.

Behavioral Evaluations

The mice were kept on a 12:12 hr light-dark schedule (lights on at 6AM) with lab chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO) and water given *ad libitum*. All procedures were according to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of the Oregon Health and Science University (OHSU). OHSU has an Association for the Assessment and Accreditation of Laboratory Animal Care approved animal facility. Mice were

housed singly starting 3 days prior to the first behavioral test. The person testing was blinded to the genotype and treatment of the mice. Mice were tested in the open field and subsequently on the rotorod in week 1, and in the water maze in week 2, as described below.

Open field

To evaluate exploratory behavior and measures of anxiety, mice were placed in a 50.8 cm X 50.8 cm (45.7 cm high) brightly lit open arena equipped for a single ten-minute session. The behavioral performance of the mice was videotaped with a Panasonic AG 185 video camera. The videotapes were fed through the Noldus Ethovision video tracking system (Noldus Information Technology, Sterling, VA), set at six samples/second and the behavioral performance was analyzed. In the open-field, the center zone (25.4 x 25.4 cm) is more anxiety-provoking than the peripheral zone. Mice that are more anxious in the open-field spend less time in the center. Therefore, to assess measures of anxiety in the open field, the data were also analyzed for only the center or peripheral zone.

Rotorod

To assess sensorimotor ability, mice were tested using a rotorod apparatus (Hugo Basile Accelerated Rotorod (Jones and Roberts for mice 76450). Mice were placed on an elevated rod initially rotating at 4rpm. Every 15 sec, the rod was accelerated by 15 rpm. Fall latency was recorded by timers, which stopped when the mouse broke the photobeams at the bottom of the chamber. Mice received three trials per day for three subsequent days.

Water maze

The water maze was used to assess spatial learning and memory. A circular pool (diameter 140 cm) was filled with opaque water (24ºC) and mice were trained to locate a submerged platform in order to escape from the water (luminescence: 40 lux). First they were trained to locate a clearly marked platform (days 1 and 2). Subsequently, they were trained with to locate a platform hidden beneath the surface of water made opaque using white chalk (days 3-5). During the training with the hidden platform, the mice had to navigate to it using the available spatial cues. There were two daily sessions 3.5 hr apart, each consisting of three 60 sec trials (with 10-15 min inter-trial intervals). Mice that failed to find the platform within 60 seconds were led to the platform by the experimenter and allowed to stay on the platform for 3 seconds. During the visible platform training, the platform was moved to a different quadrant of the pool for each session. For the hidden platform training, the platform location was kept constant for each group of mice. The mice were assigned to four groups using a randomized block design to avoid any potential quadrant bias. Mice were placed into the water facing the wall at the side of the pool in 9 different locations around the pool circumference, and the starting location was changed for each trial. The swimming patterns of the mice were videotaped as described for the open field. The video tapes were fed through the Noldus Ethovision video tracking system, set at six samples/second. Time to locate the platform (latency), distance moved, and swim speeds, were analyzed.

Probe trials (no platform present) were used to assess spatial memory retention. Probe trials were conducted 1 h after the last hidden trial on each day of hidden training (total of 3 probe trials). The time mice spent swimming in the target quadrant (where the platform was located during hidden platform training), and in the three non-target quadrants was measured over a 60 second trial. For the probe trials, mice were dropped in the quadrant opposite from the

target quadrant.