Supplemental Experimental Procedures

Generation of the *LanCL1* knockout mice

The targeting construct, in which exon 4 of *LanCL1* is flanked by loxP sites (Figure S2A), was made by modifying a BAC clone using recombineering. The excision of exon 4 creates an out-of-frame fusion between exon 3 and exon 5. To facilitate PCR screen to identify ES clones with targeted homologous recombination, we constructed a PCR control vector that contains PGK-neo cassette and the entire short homology arm and genomic sequence beyond the short homology arm. A PCR assay was developed to screen ES clones with the forward primer 5'-ATT GCA TCG CAT TGT CTG AG-3'and the reverse primer 5'- TGC ATG GGA ATC TTA TCA GC-3'. The ES cell work and microinjection of positive ES clones to generate chimeric mice were carried out at the Transgenic Facility of Johns Hopkins University School of Medicine, using standard procedures. Briefly, the targeting construct was electroporated into129/Sv ES cells, which were selected for G418 resistance and screened by PCR assay for homologous recombination. The ES clones with confirmed targeted homologous recombination were injected into blastocysts to generate chimeric mice. The chimeric mice were crossed with C57BL/6 mice to confirm germline transmission and the offspring were bred with Actin-Cre transgenic mice to generate germline deletion of *LanCL1* knockout mice. Knockout mice were genotyped using PCR primers: *LanCL1*-WT-F:5'-CGA ATC GTG TCA TCA TCT GG-3', *LanCL1*-WT-R:5'-TGC ACT AAA AAT GCC GTC TG-3' which amplifies a wt band of 487bp and *LanCL1*-excision-F:5'-CCT TGA GCG TCC TTA CAT CTG C-3',

LanCL1-excision-R: 5'- CAT GTG CAA GTT ATA CAT CCA CC-3', which amplifies a mutant band of 853bp (Figure S2B). The excision of exon 4 was confirmed by RT-PCR (Figure S2C) using the forward primer 5'-GTT CTC ACA TCG CTT GAC CA-3' and the reverse primer 5'-TTT GGT ACG TGG GGA TCA AT-3' and Western blotting with affinity-purified rabbit polyclonal LanCL1 antibody (Figure S2D). All mouse work was done in accordance with the Animal Care and Use Committee guidelines of Johns Hopkins University School of Medicine and Sichuan University West-China Hospital.

Generation of conditional *Rosa26-LanCL1-V5* transgenic mice

The targeting vector to knock a cassette that permits conditional expression of *LanCL1-V5* cDNA into *Rosa26* locus is illustrated by the diagram in Figure S4E. It was made by using components of the Rosa26 targeting system provided by Philippe Soriano (Soriano, 1999). The original PGK-neo sequence was replaced by PGK-EM7-neo expression cassette in plasmid PL452 (Liu et al., 2003) to allow kanamycin and neomycin selection in E. coli and ES cells, respectively. A CMV ß-actin enhancer-promoter (Okada et al., 1999) was placed upstream of the ''floxed'' PGK-EM7-neo-tPA sequence to drive *LanCL1* cDNA transcription once the floxed tPA (transcriptional stop) is removed by Cre recombinase. This CMV-ß-actin enhancer-promoter has been shown to be efficient in driving transgene expression when knocked into Rosa26 locus (Zong et al., 2005 and Zou et al., 2010). The targeting vector was electroporated into ES cells, and ES clones with targeted homologous recombination were identified by two sets of PCR primers coupled with sequencing of PCR products. Confirmed ES clones were injected into blastocyst mouse embryos to generate chimeric

mice. The chimeric mice were crossed with C57BL/6 mice to validate germline transmission. The genotype of the transgenic mice was determined by PCR with the following primers to distinguish wt or knockin (KI) allele and Cre-mediated excision of the ‘‘stop’’ signal: WTF1 (forward), 5’-GCA CTT GCT CTC CCA AAG TC-3’ and WTR1 (reverse), 5’-GCG GGA GAA ATGGAT ATG AA-3’) to amplify wt allele (596 bp); FloxF (forward), 5’-GCA ACG TGC TGG TTA TTG TG-3’ and FloxR (reverse), 5’-GGG GAA CTT CCT GAC TAG GG-3’ to amplify the knockin allele (395 bp) (Figure S4E).

Cell cultures

The protocol for dissecting and culturing neurons is based on the original methods provided by Stefanie Kaech & Gary Banker (Stefanie Kaech & Gary Banker, 2006). Briefly, embryonic day 17.5 (E17.5) mouse cortices were dissected with Papain (Worthington) at 37℃ for 15min. Dissociated neurons were plated in 6-well culture plates with poly-L-lysine–coated (Sigma, 0.5mg/ml) glass coverslips, with a density of $1x10^6$ cells per well. Neurons were cultured with Neurobasal (Gibco) medium with 5% Horse Serum (Hyclone), 2% GlutaMAX (Gibco), 2% B27 (Gibco), 100μg/ml penicillin & streptomycin (Gibco). After 3d in culture, 2.5μM cytosine β-D-arabinofuranoside hydrochloride (Sigma) was added to prevent glial proliferation. Then, the neurons were subsequently maintained with Neurobasal Media (Gibco) containing 1% Horse Serum (Hyclone), 100μg/ml penicillin & streptomycin (Gibco), and 1% GlutaMAX (Gibco), 2% B27 (Gibco). These cultures contained >95% neurons and no detectable microglia.

Astroglia were generated as described (Stefanie Kaech & Gary Banker, 2006). Briefly, cerebral cortex from newborn rats was dissected free of meninges, minced and incubated in a solution containing 0.25% trypsin (Gibco) in PBS at 37°C for 15 minutes. Explants were then triturated, and cells were plated into 75cm2 flasks in MEM (Gibco) with 10% Horse Serum (Hyclone), 1% Pen/Strep/Glutamine (Invitrogen). Feed the culture every 2–3 d. Before removing the medium, slap the flask 5–10 times with hand to dislodge loosely attached cells. Harvest the astroglia when the cells are near confluence (usually within 7–10 d). This procedure routinely yielded cultures of more than 95% positive cells for glial fibrillary acidic protein (GFAP).

Hela cells were maintained with DMEM containing 10% fetal bovine serum.

Induction of LanCL1 expression

LanCL1 induction was examined in neuronal cultures. To examine the induced expression of LanCL1 by neuronal activity, DIV 7 neuronal cultures were treated with 50μM bicuculline (Bic, sigma) for indicated time before cells were harvested for quantitative realtime PCR or Western blotting. To assay the inducibility of LanCL1 in animals, 4-week wt mice were treated with maximal electroconvulsive seizure (MECS) (Brakeman et al., 1997), four hours before the brain cortical extracts were prepared to detect the protein level of LanCL1 in vivo. To induce LanCL1 expression with oxidative stress or growth factors, DIV 7 or DIV 14 neuronal cultures were treated with hydrogen peroxide (H_2O_2, Sigma, 200μM) or each of following growth factor for 5 hours before cell extracts were harvested for preparation of RNA and quantitative realtime PCR and Western blotting. The final concentrations of the grow factors were as follows: insulin

like growth factor (IGF-1, Peprotech), 50ng/ml; epidermal growth factor (EGF, Peprotech), 50ng/ml; platelet-derived growth factor (PDGF, Peprotech), 25ng/ml, and Brain-derived neurotrophic factor (BDNF, Peprotech), 50ng/ml. At the same time, glutamate at 30 mM was used to induce oxidative stress in DIV3 cultures (Ratan, R.R. & Baraban, J.M, 2003). Twenty-four hours after glutamate treatment, cells were harvested for preparation of RNA and quantitative realtime PCR.

Western blotting

Standard Western blotting procedures were carried out with the following antibodies: anti-dinitrophenol (DNP) antibody (Millipore) to detect carbonylation proteins, anti-4-Hydroxy-2-Nonenal (4-HNE) antibody (Abcam) to detect lipid peroxidation, anti-GSTM1 (Abcam) and anti-GSTP1 (Abcam), anti-Bax antibody (Millipore), anti-Prohibitin antibody (Epitomics), anti-Taul antibody (Millipore), anti-Tuj1 antibody (Millipore), anti-β-actin (Millipore), anti-GAPDH (Abcam), anti-NFM antibody (Millipore), anti-MBP antibody (Merck), and anti-GFAP antibody (Millipore). Anti-LanCL1 antibody was generated by immunizing rabbits with full-length GST fusion protein and affinity-purified using maltose bind protein (MBP)-tagged LanCL1 recombinant protein.

Quantitative realtime PCR

Total RNA was extracted from tissues using TRizol reagent (Invitrogen). RNA was subjected to reverse transcription with reverse transcriptase as Manufacturer's instructions (Fermentas). Quantitative real-time PCR was performed using the Bio-Rad

iQ5 system, and the relative gene expression was normalized to internal control as GAPDH. Primer sequences for SYBR Green probes of target genes are as follows:

Gene	Primers
PGC-1α	Fr 5’-CTCCCTGTGGATGAAGACGG-3’
	Rv 5’-GCAAATCACAATCACAGGAT-3’
PGC-1β	Fr 5’-GGCAGGTTCAACCCCGA-3’
	Rv 5’-CTTGCTAACATCACAGAGGATATCTTG-3’
SOD1	Fr 5’-CAAGCGGTGAACCAGTTGTG- 3’
	Rv 5’- TGAGGTCCTGCACTGGTAC-3’
SOD2	Fr 5’-GCCTGCACTGAAGTTCAATG-3’
	Rv 5’-ATCTGTAAGCGACCTTGCTC-3’
Cyt-c	Fr 5’-TTGTTGGCATCTGTGTAAGAGAATC-3’
	Rv 5’-GCAAGCATAAGACTGGACCAAA- 3’
Catalase	Fr 5’-ACCCTCTTATACCAGTTGGC-3’
	Rv 5’-GCATGCACATGGGGCCATCA-3’
Ant	Fr 5’-TTCCTGGCAGGTGGCATCG-3’
	Rv 5’-GGATT CTCACGACACAATCAATG-3’
LanCL1	Fr 5’-CCTTCAGGTGAACCAAGGAA-3’
	Rv 5’-AGATCACGTCAGCACACTGC-3’
IL-1β	Fr 5’-CTGGTGTGTGACGTTCCCATTA-3’
	Rv 5’-CCGACAGCACGAGGCTTT-3’
IL-6	Fr 5’-TTCCATCCAGTTGCCTTCTTG-3’
	Rv 5’-TTGGGAGTGGTATCCTCTGTGA-3’

INF-γ	Fr 5’-TGCTGATGGGAGGAGAGATGTCT-3’
	Rv 5’-TTTCTTTCAGGGACAGCCTGTT-3’
TNF-α	Fr 5’-CATCTTCTCAAAATTCGAGTGACA-3’
	Rv 5’-TGGGAGTAGACAAGGTACAACCC-3’
GSTP1	Fr 5’- ATGCCACCATACACCATTGTC-3’
	Rv 5’- GGGAGCTGCCCATACAGAC-3’
GSTM1	Fr 5’-ATACTGGGATACTGGAACGTCC-3’
	Rv 5’-AGTCAGGGTTGTAACAGAGCAT-3’
GSTA4	Fr 5’-TGATTGCCGTGGCTCCATTTA-3’
	Rv 5’-CAACGAGAAAAGCCTCTCCGT-3’

The amplification efficiency and specificity were confirmed before applying them to realtime PCR assay.

Reactive oxygen species (ROS) detection

Ethidium (Eth, Invitrogen) was prepared as a 1 mg/ml solution in 1% DMSO (vol/vol) and administered at 1 mg/kg of body weight by intraperitoneal injection. Mice were anesthetized with 4% chloral hydrate and perfusion-fixed with 4% formaldehyde (wt/vol) 6h after the Eth injections. Cryostat sections were prepared and photographed with a fluorescent microscope with excitation at 510–550 nm to detect oxidized ethidium species.

NADPH/NADP ratio and Glutathione colorimetric assay

The NADPH/NADP ratio assay was performed on LanCL1 control and KO cortex extracts using the NADP/NADPH assay kit (BioVision). ～20mg samples were extracted in 400µl of the recommended extraction buffer, and 50µl were processed following instructions for each duplicate. OD450 measurements were made on a plate-reader (Thermo), and the data was converted to nmol/sample using a standard curve and values were used for ratio as previously reported (Vera Mugoni et al., 2013).

The GSH and GSSG levels assay were performed on LanCL1 control an KO cortex extracts using the Glutathione Colorimetric Detection Kit (Biovision). ～40mg samples were extracted in 400µl of Glutathione Buffer, and 20µl were processed following instructions for each duplicate. OD405 measurements were made on a plate-reader (Thermo), and the data was converted to ug/ug protein using a standard curve.

JC-1 assay

JC-1 assay was performed according to the instruction provided by the Manufacturer (Beyotime). Briefly, lipophilic fluorescent probe JC-1 was added to cortical culture neurons (DIV14) of wt or *LanCL1* -/- mice and incubated at 37°C for 20 minutes. After washing, fresh neuronal culture medium was added to the cultures and imaged for fluorescence intensity under fluorescence microscope. For each experiment, the mean values of at least 50 representative neurons in wt or *LanCL1* -/- cultures were obtained using Image-Pro Plus software for the calculation of the ratio of fluorescence intensity and the average ratio from three independent experiments were analyzed using GraphPad Prism software.

8-oxoG, 4-HNE, Iba-1, Nissl, TUNEL assay

Mice were anesthetized with 4% chloral hydrate and transcardially perfused with PBS (PH7.4) followed by ice-cold 4% paraformaldehyde (PFA) in phosphate buffer (pH 7.4). The brains were removed and post-fixed overnight in 4% PFA and kept in 30% sucrose at 4°C before sectioning. Standard immunohistochemistry was carried out to examine oxidative damage to DNA using anti-8-oxoguanine antibody (Millipore), oxidative damage to lipids using anti-4-HNE antibody (Abcam), activation of microglia using anti-Iba-1 antibody (Wako) and apoptotic neuronal death using in situ cell death kit (TUNEL, Roche). The apoptotic cells were visualized using colorimetric detection with diaminobenzidine (DAB). Nissl staining was performed according to the instruction provided by the Manufacturer (Beyotime).

Construction and Expression of Poly-histidine Tagged LanCL1 and GSTP1

In order to purify LanCL1 and GSTP1 after expression in mammalian cells, we incorporated the poly-histidine tag into the C terminus of the mice LanCL1 and GSTP1 cDNA using PCR primers (*LanCL1*: Fr 5'- ataGTCGACcATGGCTCAGAGGGCCTTTCC-3'; Rv 5'- attGCGGCCGCTCAGTGGTGGTGGTGGTGGTGGCCGCCGCCGAGTTCAAATGCAGGGAA-3'; GSTP1: Fr 5'- acaGTCGACCATGCCACCATACACCATTGR-3'; Rv 5'- ATAGCGGCCGCCTAATGGTGATGGTGATGATGCTGTTTGCCATTGCC-3'). The final construction was subcloned into the multiple cloning site (SalI–NotI) of the vector PRK5-Myc for overexpression in HeLa cells.

Affinity purification of polyhistidine-tagged LanCL1 protein

HeLa cells were transfected with Myc-LanCL1-His plasmid, or Myc-LanCL1 alone, using Lipofectamine 2000 (Invitrogen). Forty-eight hours after transfection, cells were washed with PBS for three times first, then cold lysis buffer (PBS, 1% Triton X-100, 10mM imidazole, cocktail III, PH 7.4) was added to harvest the cells. Cells were sonicated (Scientz II-D, microtip amplitude = 40%) for 2 sec, 5 times on ice. Centrifugation was performed at 12,000 x g for 10 min, at 4 °C, to remove the cell debris. Supernatant was then transferred to a new centrifuge tube with His Trap HP (GE Healthcare, 17-5248), which was pre-processed with equilibrium buffer (PBS, 1% Triton X-100, PH 7.4). Incubation was performed over night on Vertical Multi-function Rotators at 4 °C, and then centrifuged at 5,000 x g for 5 min to collect precipitates. Precipitates were washed with cold wash buffer (PBS, 1% triton X-100, 15mM imidazole, cocktail III, PH 7.4, three times, 30 min each), before elution buffer (PBS, 150mM imidazole, PH 7.4) was added. Elution was performed on Vertical Multi-function Rotators for 30min, at 4 °C. Finally, after 12,000 x g for 5min centrifugation, supernatant was collected for the following assay.

Assay of glutathione transferase activity

The activity of the glutathione transferase was measured using a standard protocol described by Habig *et al* (Habig *et al*, 1995). The reaction was performed between 0.5mM 1-chloro-2,4-dinitrobenzene and 0.5mM glutathione in a 200 ml reaction system at 25°C containing 0.1 M sodium phosphate (pH 6.5). Activity was measured spectrophotometrically (Thermo) at 340 nm (ϵ= 9600 $M^{-1}\cdot cm^{-1}$), and the reaction was initiated by the addition of enzyme. The enzymatic kinetic assay was performed at

standard condition (25°C, pH 6.5) by fixing the GSH concentration at 5mM, and varying CDNB concentration from 0mM to 6mM. Kinetic data were analyzed by GraphPad Prism with K_{cat} analysis, the V_{max} and the Km values for CDNB were determined from this analysis.

The enzymatic activity with p-Nitrophenyl acetate was performed between 0.2mM p-Nitrophenyl acetate and 0.5mM glutathione in a 200 ml reaction system at 25°C containing 0.1M potassium phosphate (pH 7.0). Activity was measured spectrophotometrically (Thermo) at 400 nm (ϵ= 8790 $M^{-1} \cdot cm^{-1}$)

In vitro cell death and apoptosis assay

HeLa cells were transfected with GFP-LanCL1, or GFP alone plasmid using Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, H_2O_2 was added to the cultures at the final concentration of 200μM. Propidium iodide (PI) and Hoechst staining were performed twelve hours after the H_2O_2 treatment. Cell death was quantified by PI/Hoechst positive cells and statistical analysis was performed by using Student's t test. WT and *LanCL1* ko neuronal cultures were maintained for two weeks before MK801 (200μM) and H_2O_2 (200μM) stimulation. *LanCL1* transgenic neuronal cultures were maintained for 1 week before H_2O_2 (150μM) stimulation. Hoechst staining was performed at forty-eight hours (MK801) and twelve hours (H_2O_2), respectively after stimulation to identify neuronal death. Neurotrophic factors were added 24h hours before H_2O_2 treatment in Fig S4F. Neuronal death was quantified and analyzed as previous described (Sofia Papadia et al, 2008).

Cell Viability Assay

HeLa cells were plated in 96-well plates. Twenty–four hours after transfection with GFP or GFP-LanCL1 plasmid, H_2O_2 treatment was performed for 12 hours. After treatment, cell viability was measured by Cell Counting Kit-8 (CCK-8) system (Dojindo, CK04-11) according to the manufacturer's instructions. Briefly, CCK-8 solution (10μl per 100μl of medium in each well) was added, the plates were then incubated at 37°C for 1 h, and the absorbance of each well was read at 450 nm using a microplate (Thermo) reader.

Statistical analysis

Data represent the mean and standard error of the mean (SEM). Student's t test (one-tailed for western blot, ratio quantification and qRT-PCR, two-tailed for the others) was performed for all statistical significance analysis using GraphPad Prism software. *$p<0.05$, **$p<0.01$