**Figure S1**

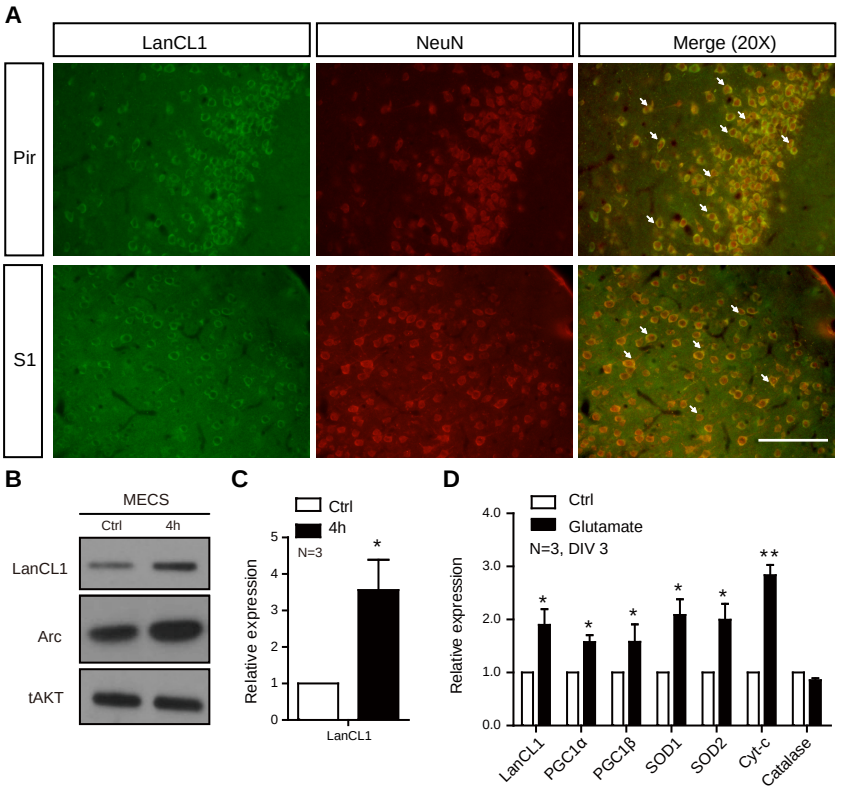

A
LanCL1
NeuN
Merge (20X)
Pir
S1
B
MECS
Ctrl
4h
LanCL1
Arc
tAKT
C
Ctrl
4h
N=3
Relative expression
LanCL1
D
Ctrl
Glutamate
N=3, DIV 3
Relative expression
LanCL1
PGC1α
PGC1β
SOD1
SOD2
Cyt-c
Catalase

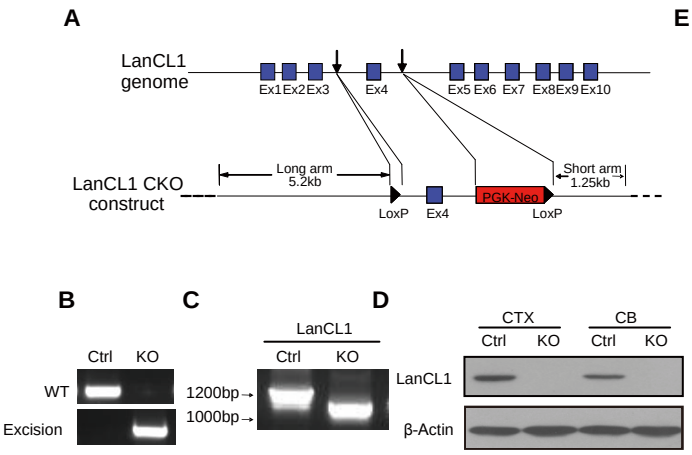
**Figure S1. LanCL1 is a neuronal activity induced protein, related to Figure 1.**

(A) Immunostaining with LanCL1 antibody (green) and NeuN (red) antibody show the neuronal expression of LanCL1 in mice CTX. Bar: 50μm.

(B and C) Western blots and quantification show the induction of LanCL1 protein in WT mice by maximal electroconvulsive seizure (MECS). Error bars indicate SEM, *p<0.05. n=3.

(D) qRT-PCR shows induction of *LanCL1* mRNA along with oxidative defense genes in DIV3 cortical neurons 24hrs after glutamate treatment. The relative fold induction is normalized against non-treatment control (Ctrl). Error bars indicate SEM, *p<0.05 **p<0.01. n=3

**Figure S2**




**E**

LanCL1 +/- (♂) × LanCL1 +/- (♀)

| Mating pair | Litters | Gender | | Genotype | | |
|---|---|---|---|---|---|---|
| | | ♂ | ♀ | +/+ | +/- | -/- |
| 1 | 4 | 2 | 2 | 1 | 3 | 0 |
| 2 | 5 | 2 | 3 | 1 | 2 | 2 |
| 3 | 5 | 4 | 1 | 2 | 3 | 0 |
| 4 | 5 | 3 | 2 | 1 | 2 | 2 |
| 5 | 3 | 1 | 2 | 0 | 2 | 1 |
| 6 | 4 | 2 | 2 | 2 | 1 | 1 |
| 7 | 5 | 2 | 3 | 0 | 4 | 1 |
| 8 | 4 | 4 | 0 | 1 | 3 | 0 |
| 9 | 3 | 1 | 2 | 1 | 2 | 0 |
| 10 | 4 | 3 | 1 | 2 | 1 | 1 |
| 11 | 3 | 1 | 2 | 0 | 2 | 1 |
| 12 | 7 | 3 | 4 | 2 | 3 | 2 |
| Total litters | 52 | 28 | 24 | 13 | 28 | 11 |
| Litters ratio | | 1 : 1 | | 1 : 2 : 1 | | |

**Figure S2. *LanCL1* genetic deletion and embryonic viability, related to Figure 2.**

(A) Illustration of the design of *LanCL1* targeting construct, with floxed exon 4.

(B-D) Validation of *LanCL1* ko. Genomic PCR to confirm the excision of exon 4 (B), RT-PCR to show truncated mRNA products in the ko brains (C), and western blots to show the absence of LanCL1 protein in cortex and cerebellum brain tissues (D).

(E) The number and frequency of offspring of each genotype produced by crossing *LanCL1*+/- mice. *LanCL1*+/+, *LanCL1*+/-, and *LanCL1*-/- pups were born with expected Mendelian frequencies.

**Figure S3**

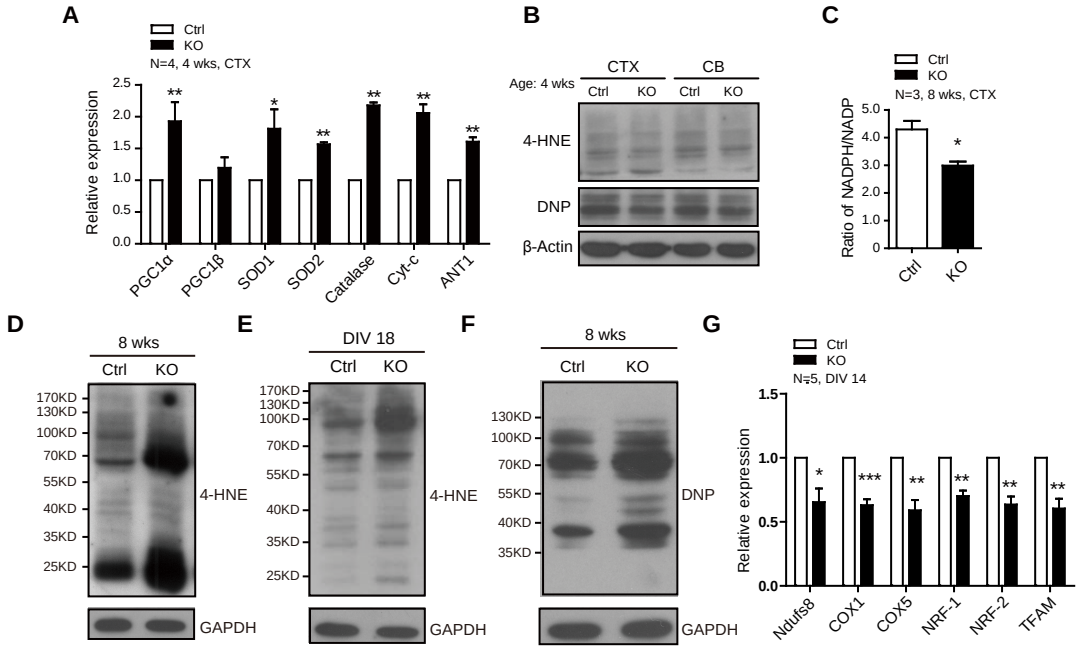

A
Ctrl
KO
N=4, 4 wks, CTX
Relative expression
PGC1α
PGC1β
SOD1
SOD2
Catalase
Cyt-c
ANT1
B
Age: 4 wks
CTX
CB
Ctrl
KO
4-HNE
DNP
β-Actin
C
Ctrl
KO
N=3, 8 wks, CTX
Ratio of NADPH/NADP
D
8 wks
4-HNE
GAPDH
E
DIV 18
4-HNE
GAPDH
F
8 wks
DNP
GAPDH
G
Ctrl
KO
N=5, DIV 14
Relative expression
Ndufs8
COX1
COX5
NRF-1
NRF-2
TFAM

**Figure S3. Oxidative damages in *LanCL1* ko brains, related to Figure 3.**

(A) qRT-PCR shows increases in mRNA levels of antioxidant defense genes in the cortex of 4-week *LanCL1* ko cortex. Error bars indicate SEM, *p<0.05, **p<0.01. n=4.

(B) Western blots show levels of 4-HNE and DNP immunoreactivity in 4-week *LanCL1* ko brains are not different from wt controls.

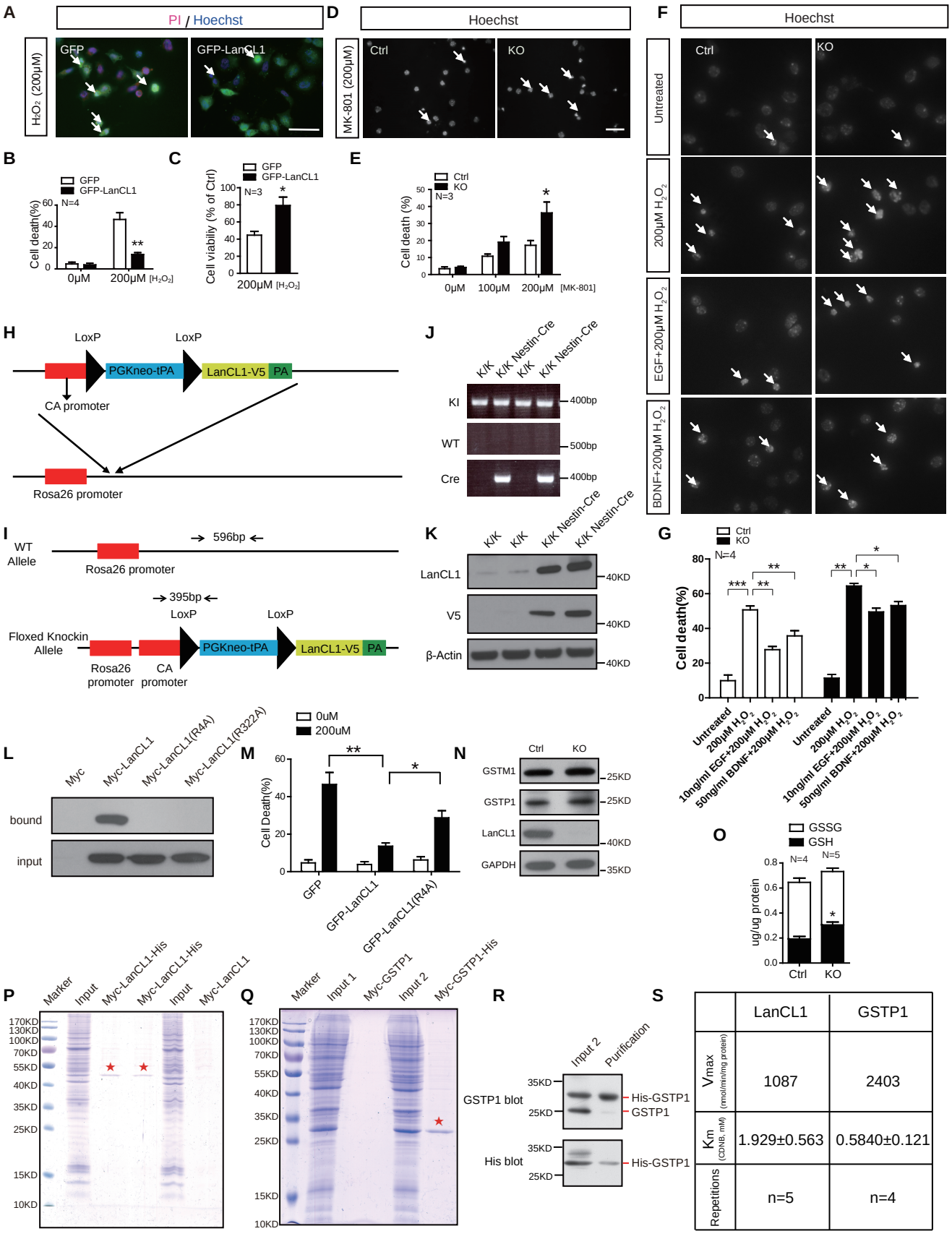
(C) Quantification shows the ratio of NADPH/NADP in 8-week *LanCL1* control and ko cortex. Decreased ratio is indicative of oxidative stress state. Error bars indicate SEM, *p<0.05.

(D, E) Western blots show the increase levels of 4-HNE in 8-week *LanCL1* ko cortex (D) and DIV18 culture neurons (E).

(F) Western blots show the increase levels of DNP in 8-week *LanCL1* ko cortex.

(G) qRT-PCR shows decrease in mRNA levels of mitochondrial related genes in *LanCL1* ko cortical cultures (DIV 14). Error bars indicate SEM, *p<0.05, **p<0.01, ***p<0.001. n=5.

## Figure S4




S

| | LanCL1 | GSTP1 |
|---|---|---|
| Vmax (nmol/min/mg protein) | 1087 | 2403 |
| Km (CDNB, mM) | 1.929±0.563 | 0.5840±0.121 |
| Repetitions | n=5 | n=4 |

**Figure S4. LanCL1 possesses catalytic activity for thioether formation and protects cells against oxidative stress, related to Figure 4.**

(A and B) Propidium iodide (PI, red) /Hoechst (blue) staining shows that overexpression of GFP-tagged LanCL1 in HeLa cells reduces the $H_2O_2$-induced cells death (arrow heads). Green fluorescence shows transfected cells. The data represent the mean ± SEM from three independent experiments, with a total number of 5,000 cells analyzed for each group. Bar 50μm. Error bars indicate SEM, **p=0.0069, n=4.

(C) Quantification shows higher cell viability in LanCL1-overexpressed HeLa cells compared with ctrl cells after 12h treatment of 200μM $H_2O_2$. Error bars indicate SEM, *p=0.038, n=3.

(D and E) Hoechst staining shows *LanCL1* ko cortical neurons (DIV14) are more sensitive to MK-801 treatment (48 hours) to induce cell death (arrow heads). The data represent the mean ± SEM from three independent experiments, with a total number of 2,000 neurons analyzed for each group. Bar, 50μm. Error bars indicate SEM, *p= 0.0362, n=3.

(F and G) Hoechst staining and quantification show impaired protective effect of EGF and BDNF against $H_2O_2$ induced neuronal death (arrow heads) in *LanCL1* ko cortical neurons (DIV9). The data represent the mean ± SEM from four independent experiments, with a total number of 3,000 neurons analyzed for each group. Bar, 50μm. Error bars indicate SEM, *p <0.05, **p<0.01, ***p<0.001, n=4.

(H and I) Illustration of the *Rosa26-LanCL1-V5* targeting construct.

(J) Genomic validation of the Rosa26-*LanCL1*-V5 transgenic mouse by PCR.

(K) Western blots show the cre-dependent expression of LanCL1-V5 transgene.

(L) Cell extracts transfected with *LanCL1* plasmids (wt and point mutants) were loaded onto a GSH–Sepharose4B column to confirm the interaction with GSH. Lanes labeled with input denote preincubated protein samples, and those labeled with bound indicate *LanCL1* protein that bound to the GSH-Sepharose.

(M) Quantification shows that LanCL1 point mutant (R4A), which does not bind GSH, showed impaired cellular protective effect over $H_2O_2$ treatment (200μM, 12h, n=3). Error bars indicate SEM, *p= 0.0457.

(N) Western blots show protein levels of GSTM1 and GSTP1 in 8-week *LanCL1* ko brains that are not different from wt control. GSTM1, p=0.2576; GSTP1, p=0.2560. n=3.

(O) Quantification shows the increase levels of GSH in 8-week LanCL1 ko cortex. Error bars indicate SEM. GSSG, P=0.3287; GSH, *p= 0.0111.

(P) Coomassie brilliant blue staining shows the affinity purification of polyhistidine-tagged LanCL1 expressed in HeLa cells. ★ indicates the LanCL1 band. Input: Myc-LanCL1-His.

(Q and R) Coomassie brilliant blue staining and western blots show the affinity purification of polyhistidine tagged GSTP1 expressed in HeLa cells. ★ indicates the GSTP1 band. Input 1: Myc-GSTP1, Input 2: Myc-GSTP1-His.

(S) The $V_{max}$ and $K_m$ values were determined from the enzymatic kinetic curves of purified Myc-LanCL1-His and Myc-GSTP1-His.