# Figure S1 (Related to Figure 1)

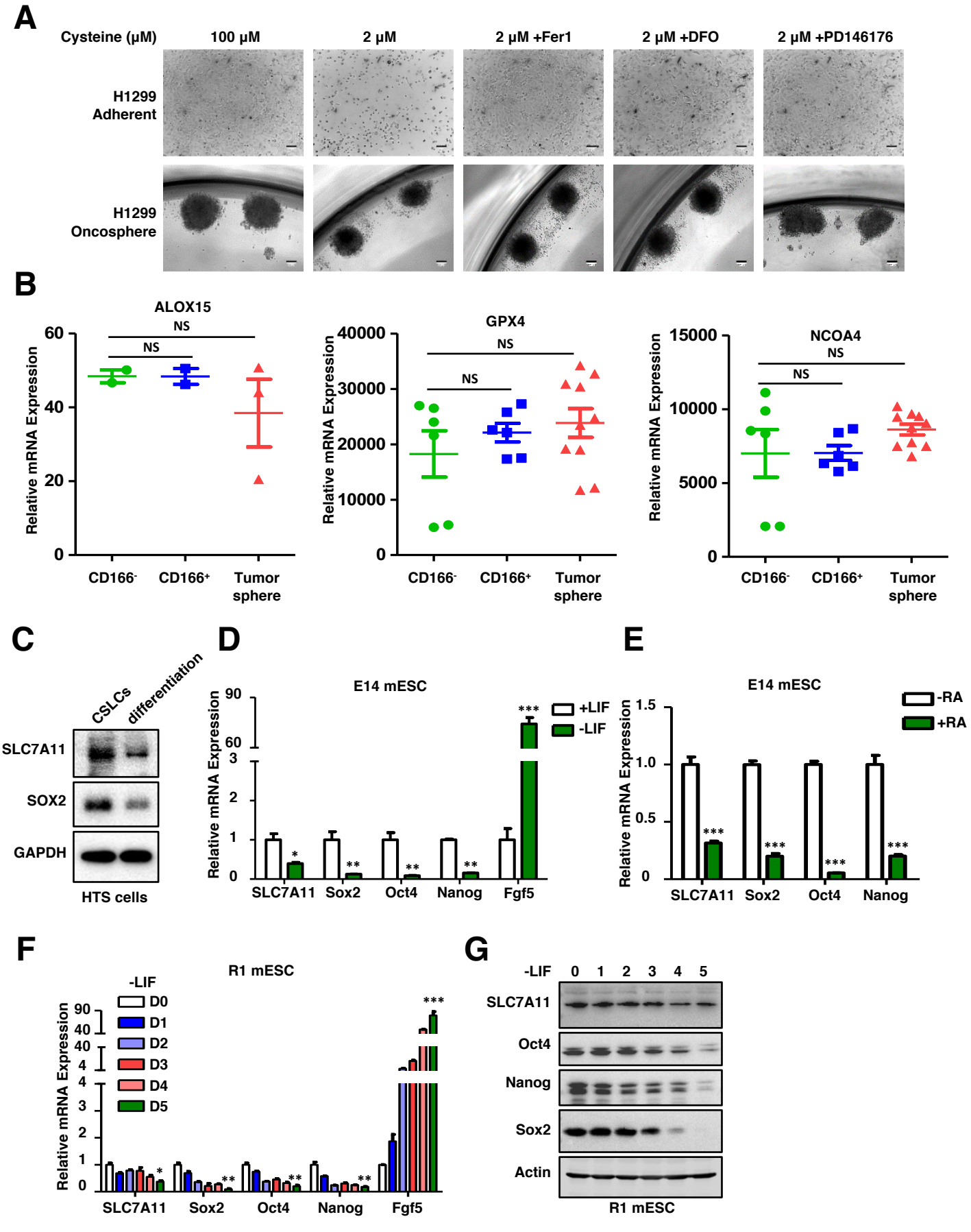


**Figure S1 (Related to Figure 1)**

A. Representative images of H1299 adherent and oncosphere cells treated with cysteine deprivation, with or without ferroptosis inhibitors Fer-1 (1 μM), DFO (100 μM) or PD146176 (1 μM) for 24 hours. Scale bar: 100 μm.
B. Expression levels of ALOX15, GPX4 and NCOA4 in $CD166^-$ control cells, $CD166^+$ lung CSLCs and lung tumor sphere were showed (NS, not significant, Student's *t*-test).
C. The protein levels of SLC7A11 and SOX2 in HTS cells and serum withdrawal induced differentiation cells were analyzed by WB.
D. Relative gene mRNA expression levels in E14 mESC and LIF withdrawal induced differentiation cells were analyzed by qRT-PCR, **P*<0.05, ***P*<0.01, ****P*<0.001 (Student's *t*-test).
E. Relative gene mRNA expression levels in E14 mESC and retinoic acid (RA) induced differentiation cells were analyzed by qRT-PCR, ****P*<0.001 (Student's *t*-test).
F. Relative gene mRNA expression levels in R1 mESC and LIF withdrawal induced differentiation cells were analyzed by qRT-PCR, **P*<0.05, ***P*<0.01, ****P*<0.001 (two-way ANOVA test).
G. The protein levels of SLC7A11 in R1 mESC and LIF withdrawal induced differentiation cells were analyzed by WB.

# Figure S2 (Related to Figure 2)

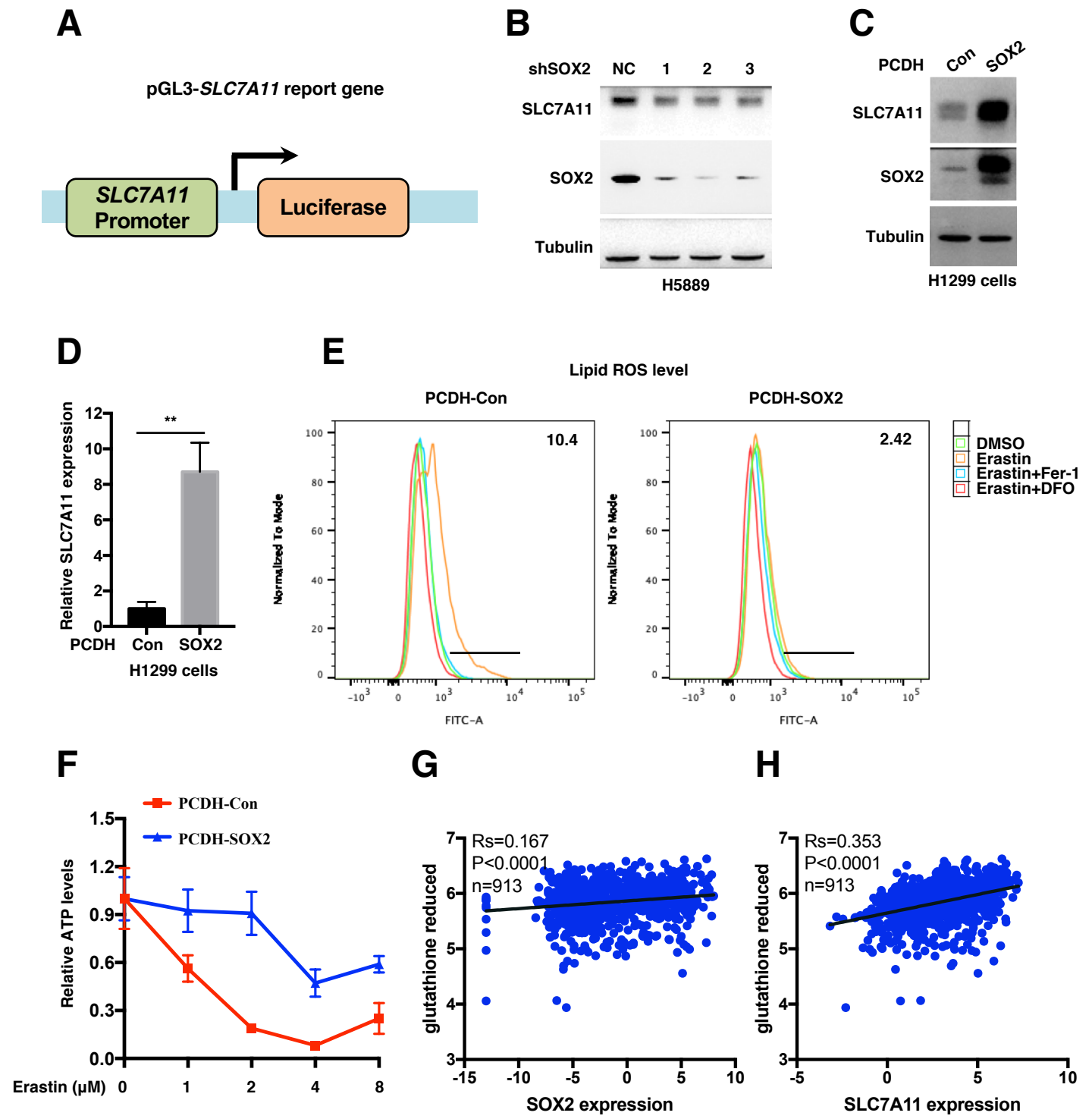
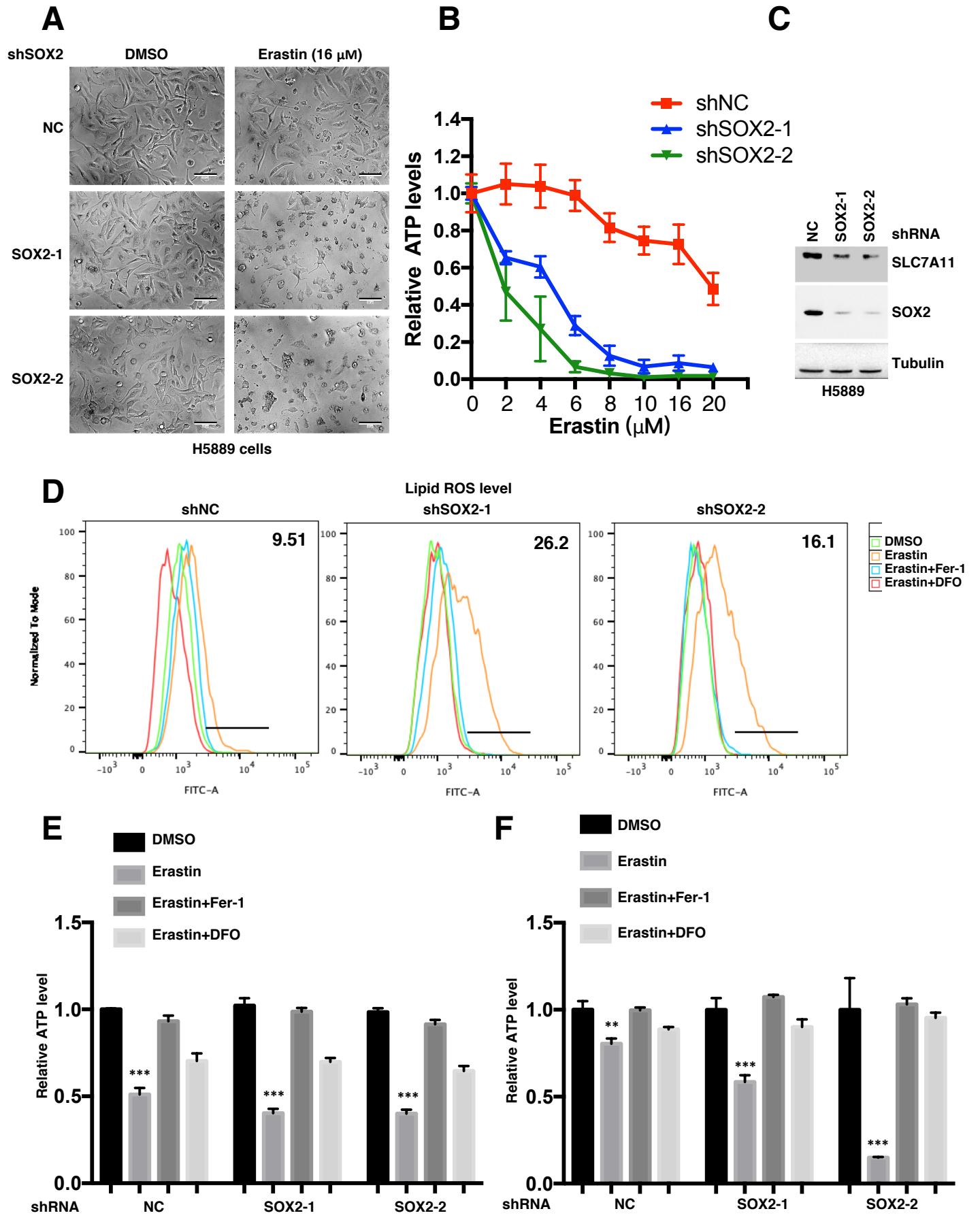


**Figure S2 (Related to Figure 2)**

A. Schematic diagram of pGL3-*SLC7A11* promoter reporter gene.
B. shRNA mediated SOX2 knock down resulted in decreased expression of SLC7A11 in H5889 cells, protein levels of SLC7A11 and SOX2 were analyzed by WB.
C. The protein levels of SLC7A11 and SOX2 in indicated H1299 cells were analyzed by WB.
D. Relative mRNA expression levels of SLC7A11 in indicated H1299 cells were analyzed by qRT-PCR, $^{**}P<0.01$ (Student's *t*-test).
E. Lipid ROS levels of indicated H1299 cells treated with 5 μM Erastin for 10 hours. 1 μM Fer-1 and 50 μM DFO treatment could rescue Erastin-induced lipid peroxidation.
F. Indicated H1299 cells were treated with different dose of Erastin for 20 hours and cell viability of indicated cells was measured. The data was representative of three independent experiments.
G. The positive correlation between reduced glutathione level and SOX2 gene expression in CCLE was showed in dot plots, the correlation was assessed by the Spearman test.
H. The positive correlation between reduced glutathione level and SLC7A11 gene expression in CCLE was showed in dot plots, the correlation was assessed by the Spearman test.

# Figure S3 (Related to Figure 3)



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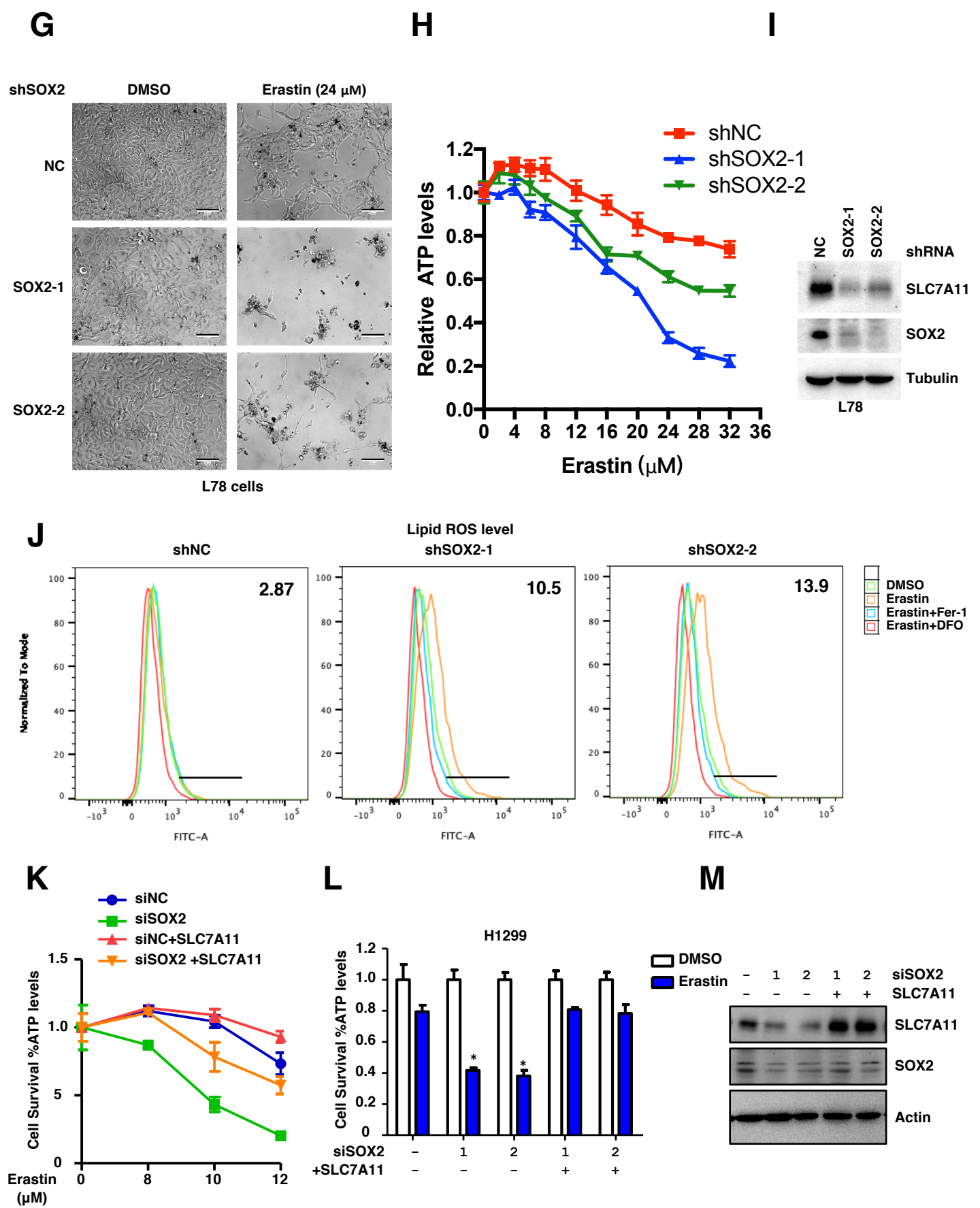

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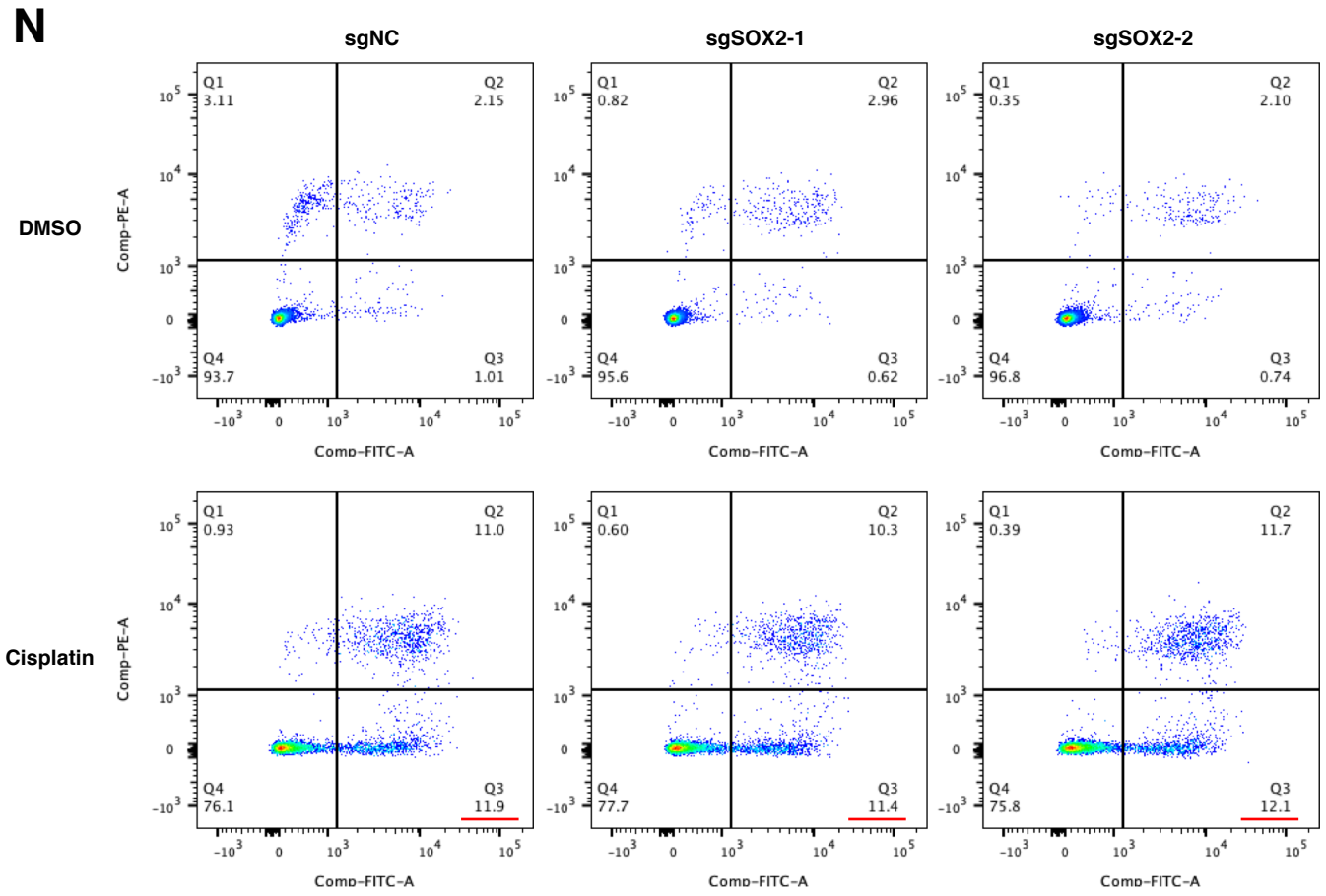



## Figure S3 (Related to Figure 3)

A. The indicated H5889 cell lines were treated with 16 μM Erastin for 36 hours and representative images from each condition were shown. Scale bar: 100 μm. The data was representative of three independent experiments.
B. Cell viability of indicated cells was measured (related to Fig. S3A). The data was representative of three independent experiments.
C. Protein levels of SLC7A11 and SOX2 (related to Fig. S3A) were analyzed by WB.
D. Lipid ROS levels of indicated H5889 cells treated with 16 μM Erastin for 18 hours. 1 μM Fer-1 and 50 μM DFO treatment could rescue Erastin-induced lipid peroxidation.

**Figure S3 (Related to Figure 3)**

E. Indicated H5889 cells were treated with 16 μM Erastin for 36 hours, 1 μM Fer-1 and 50 μM DFO treatment could rescue Erastin-induced cell death. Cell viability of indicated cells was measured. ***$P$<0.001 (two-way ANOVA test).

F. Indicated L78 cells were treated with 24 μM Erastin for 36 hours, 1 μM Fer-1 and 50 μM DFO treatment could rescue Erastin-induced cell death. Cell viability of indicated cells was measured. **$P$<0.01, ***$P$<0.001 (two-way ANOVA test).

G. The indicated L78 cell lines were treated with 24 μM Erastin for 36 hours and representative images from each condition were shown. Scale bar: 100 μm. The data was representative of three independent experiments.

H. Cell viability of indicated cells was measured (related to Fig. S3G). The data was representative of three independent experiments.

I. Protein levels of SLC7A11 and SOX2 (related to Fig. S3G) were analyzed by WB.

J. Lipid ROS levels of indicated L78 cells treated with 24 μM Erastin for 18 hours. 1 μM Fer-1 and 50 μM DFO treatment could rescue Erastin-induced lipid peroxidation.

K. H1299 cells were transfected with indicated siRNAs and plasmids for 48 hours, the cells were then treated with different dose of Erastin for 20 hours and cell viability of indicated cells was measured. The data was representative of three independent experiments.

L. H1299 cells were transfected with indicated siRNAs and plasmids for 48 hours, the cells were then treated with 12 μM Erastin for 20 hours and cell viability of indicated cells was measured, *$P$<0.05 (Student's $t$-test).

M. Protein levels (related to Fig. S3K) were analyzed by WB.

N. The indicated H1299 cells were treated with 50 μM Cisplatin for 18 hours to induce apoptosis, and measured ANNEXIN V (FITC) and PI (PE) via FACS. SOX2 KO had little effect on apoptosis.

# Figure S4 (Related to Figure 4)

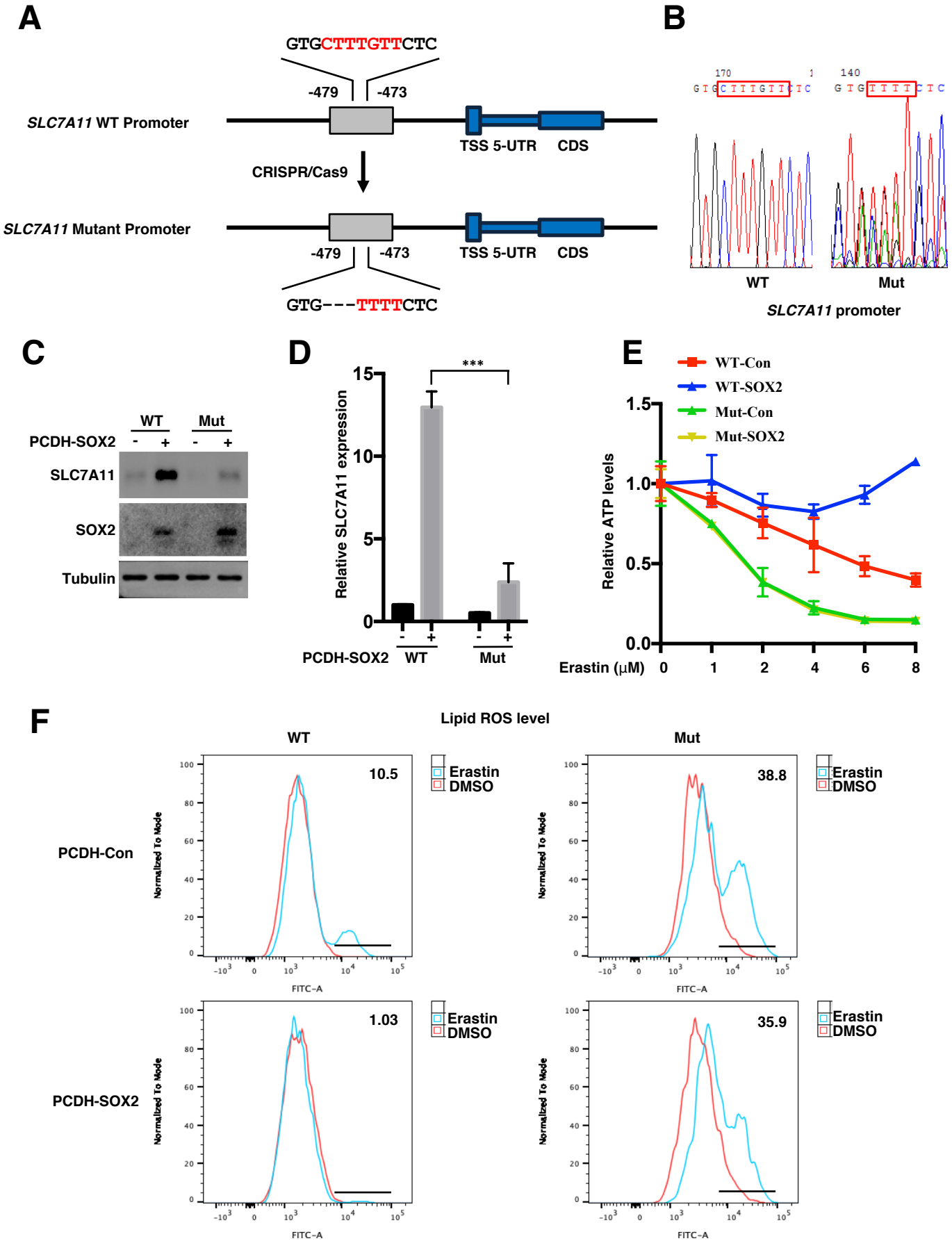


**Figure S4 (Related to Figure 4)**

A. Scheme of CRISPR-Cas9–mediated mutation in the endogenous *SLC7A11* promoter of H1299 cells.
B. DNA Sanger sequencing data showed that SOX2 binding site on *SLC7A11* promoter was mutated in the mutant cells (from CTTTGTT to TTTT).
C. The protein levels of SLC7A11 and SOX2 in indicated H1299 cells were analyzed by WB. *SLC7A11* promoter mutation largely attenuated SOX2's effect on SLC7A11 expression.
D. Relative mRNA expression levels of SLC7A11 in indicated H1299 cells were analyzed by qRT-PCR, ***$P$<0.001 (two-way ANOVA test).
E. Indicated H1299 cells were treated with different dose of Erastin for 20 hours and cell viability of indicated cells was measured. The data was representative of three independent experiments.
F. Lipid ROS levels of indicated H1299 cells treated with 5 μM Erastin for 10 hours. 1 μM Fer-1 and 50 μM DFO treatment could rescue Erastin-induced lipid peroxidation. *SLC7A11* promoter mutation largely attenuated SOX2's effect on ferroptosis resistance.

# Figure S5 (Related to Figure 5)

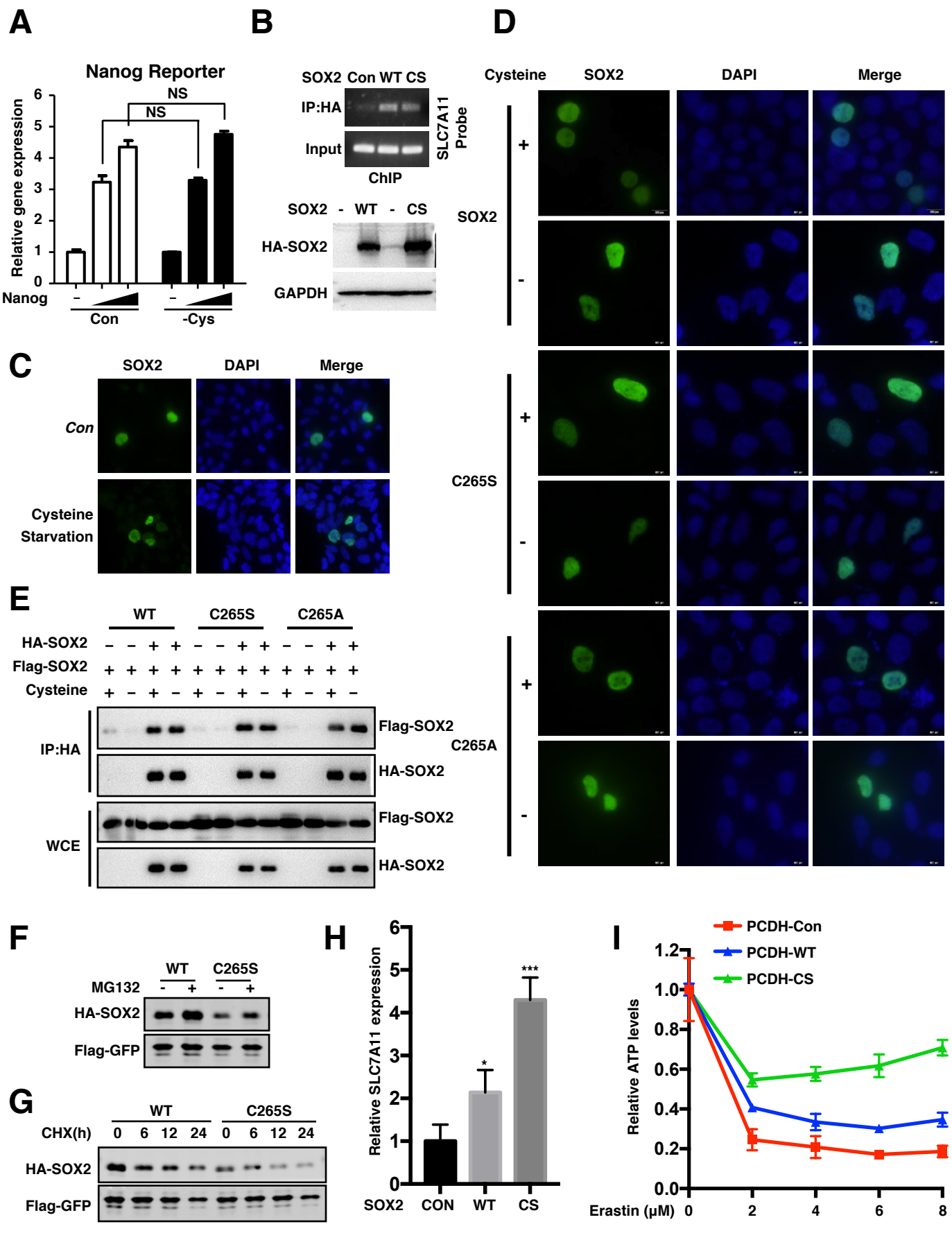
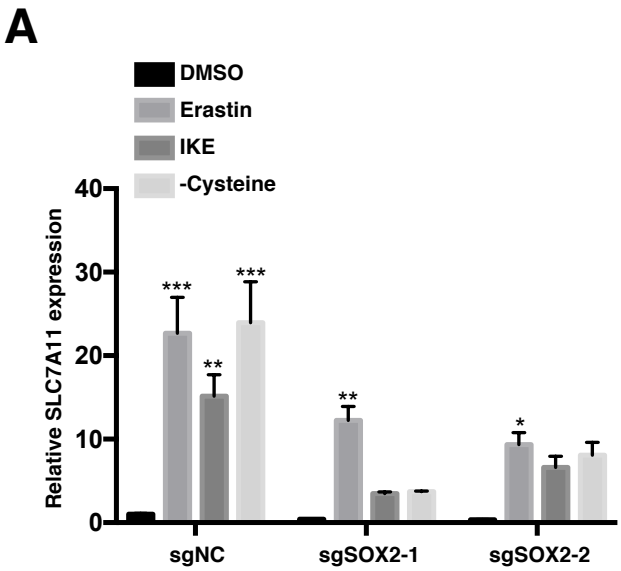
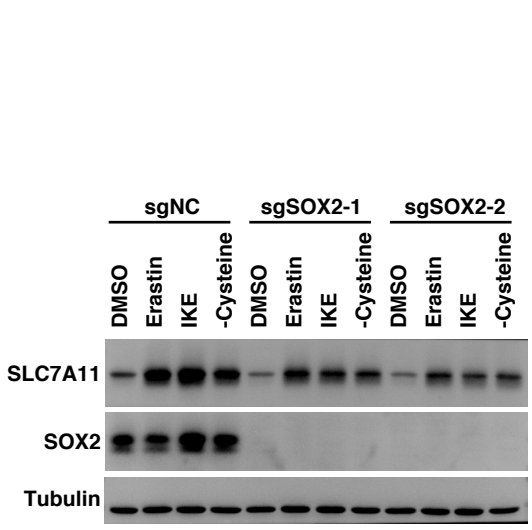


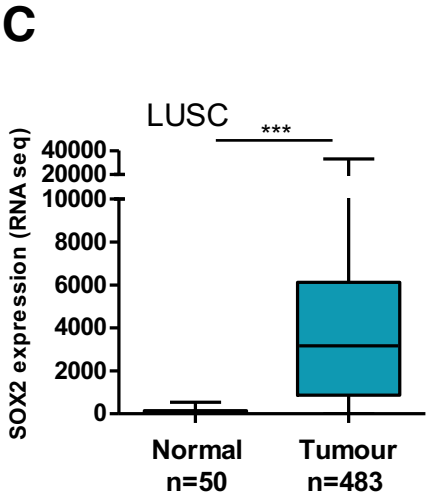
**Figure S5 (Related to Figure 5)**

A. Nanog activity reporter gene was co-expressed with different dose of Nanog in HEK293T cells for 24 hours, then the cells were treated with cysteine deprivation for 6 hours or not, and the expression of Luciferase was measured and normalized to Renilla. (NS, not significant, two-way ANOVA test)
B. ChIP results of SOX2 WT and CS binding on *SLC7A11* promoter in H1299 cells.
C. HA-SOX2 was transfected in HEK293T cells and treated with cysteine deprivation for 6 hours or not, immunofluorescent staining analysis for SOX2 was shown. Cysteine deprivation had no effect on SOX2 subcellular location in nuclear.
D. HEK293T cells were transfected with indicated plasmids for 24 hours and treated with cysteine deprivation for 6 hours or not. Immunofluorescent staining analysis for SOX2 was shown. There was no significant difference between subcellular location of SOX2 WT and C265S mutant.
E. HEK293T cells were transfected with indicated plasmids for 24 hours and treated with cysteine deprivation for 6 hours or not, cell lysates were prepared for co-IP and WB. Cysteine deprivation did not affect dimerization of SOX2 WT or mutants.
F. HEK293T cells were transfected with indicated plasmids for 24 hours and treated with proteasome inhibitor MG132 (10 μM) for 6 hours or not before harvesting. MG132 could stabilize both the protein expression of SOX2 WT and C265S mutant to similar extent.
G. HA-SOX2 and C265S mutant plasmids were transfected in HEK293T cells. After treating cells with CHX (10 μg/mL) for indicated time intervals, protein level of SOX2 was analyzed by WB. There was no significant difference between the protein half life of SOX2 WT and C265S mutant.
H. Relative mRNA expression levels of SLC7A11 in indicated H1299 cells were analyzed by qRT-PCR, $*P<0.05$, $***P<0.001$ (one-way ANOVA test).
I. Indicated H1299 cells were treated with different dose of Erastin for 20 hours and cell viability of indicated cells was measured. The data was representative of three independent experiments.

# Figure S6 (Related to Figure 6)



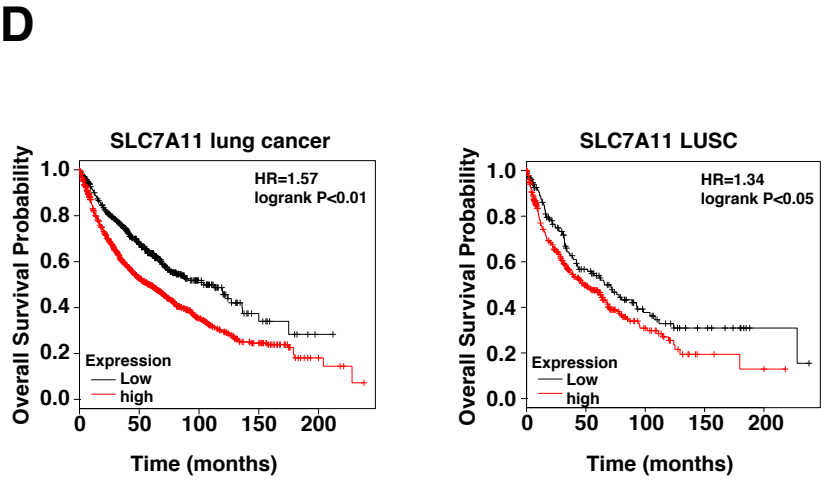



**Figure S6 (Related to Figure 6)**

A. SOX2 WT and KO H1299 cells were treated with 5 μM Erastin, 5 μM IKE and cysteine-deprivation for 12 hours, the treatment upregulated the mRNA expression of SLC7A11 in both sgNC and sgSOX2 cells. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to DMSO group (two-way ANOVA test).
B. The protein levels of SLC7A11 and SOX2 in indicated H1299 cells were analyzed by WB. All treatment upregulated the protein expression levels of SLC7A11 in both sgNC and sgSOX2 cells.
C. SOX2 mRNA expression levels in normal and tumor samples were compared using box-and-whisker plots in LUSC. The boxes showed the median and the interquartile range, and the whiskers represented the minimum and the maximum. Significance was assessed by the Wilcoxon test and ****P*<0.001 compared between the indicated two groups. SOX2 was highly expressed in human lung squamous cell carcinoma.
D. Kaplan-Meier analysis of the overall survival in lung cancer and lung squamous cell carcinoma (LUSC). Data were obtained from http://kmplot.com/analysis/index. Statistical significance was determined by the log-rank test.

# Figure S7 (Related to Figure 7)

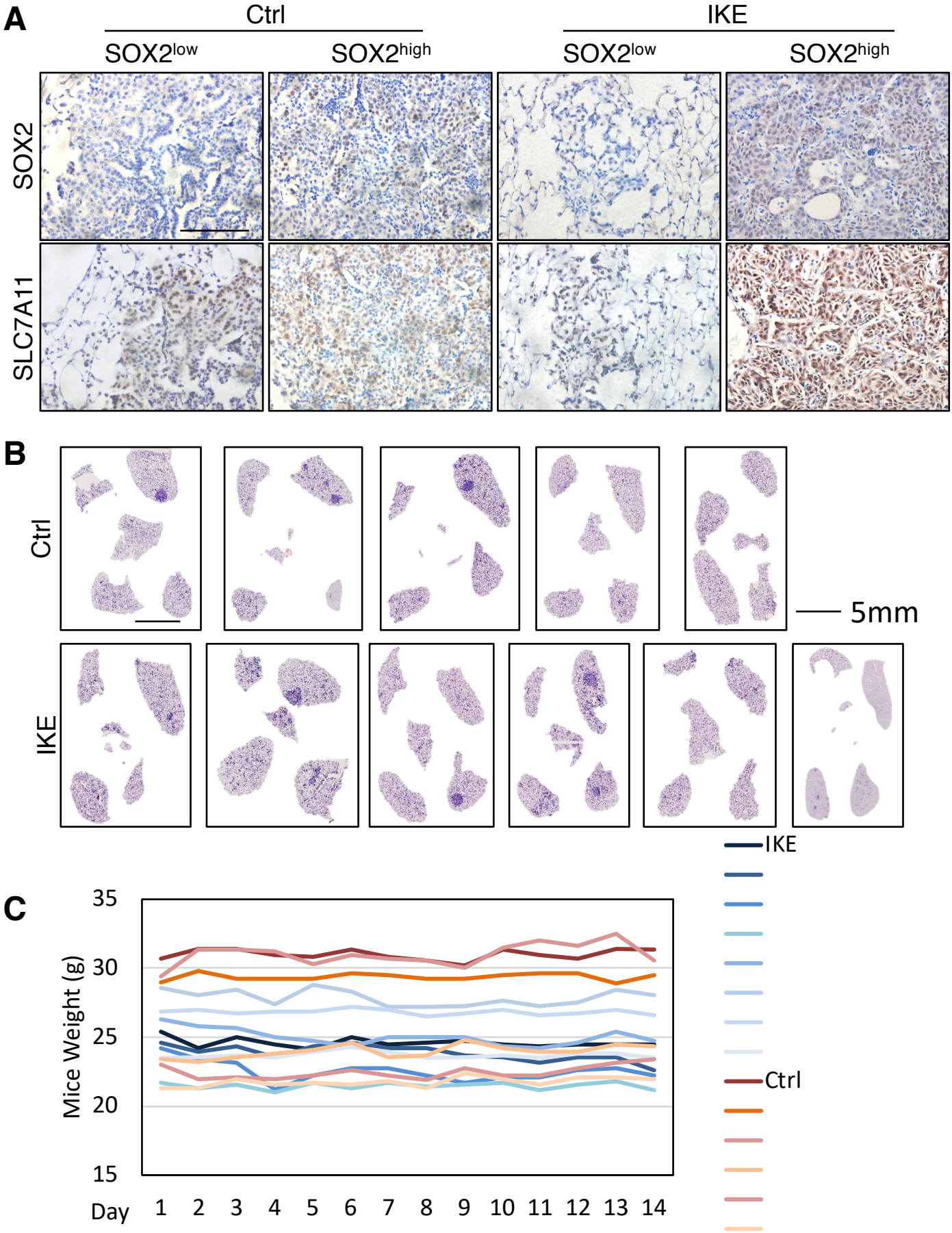
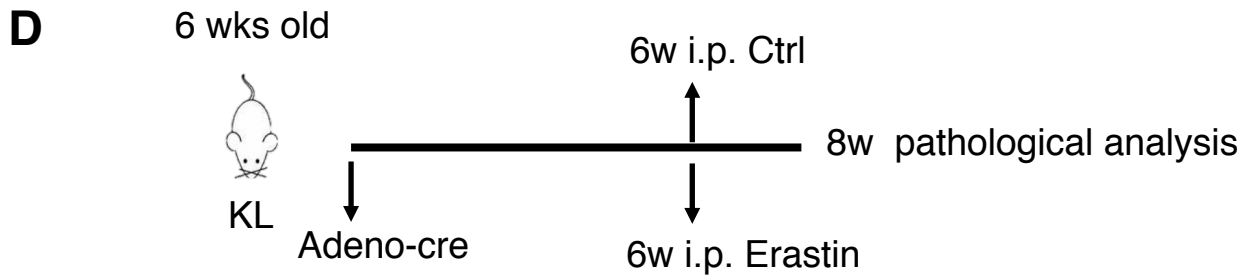
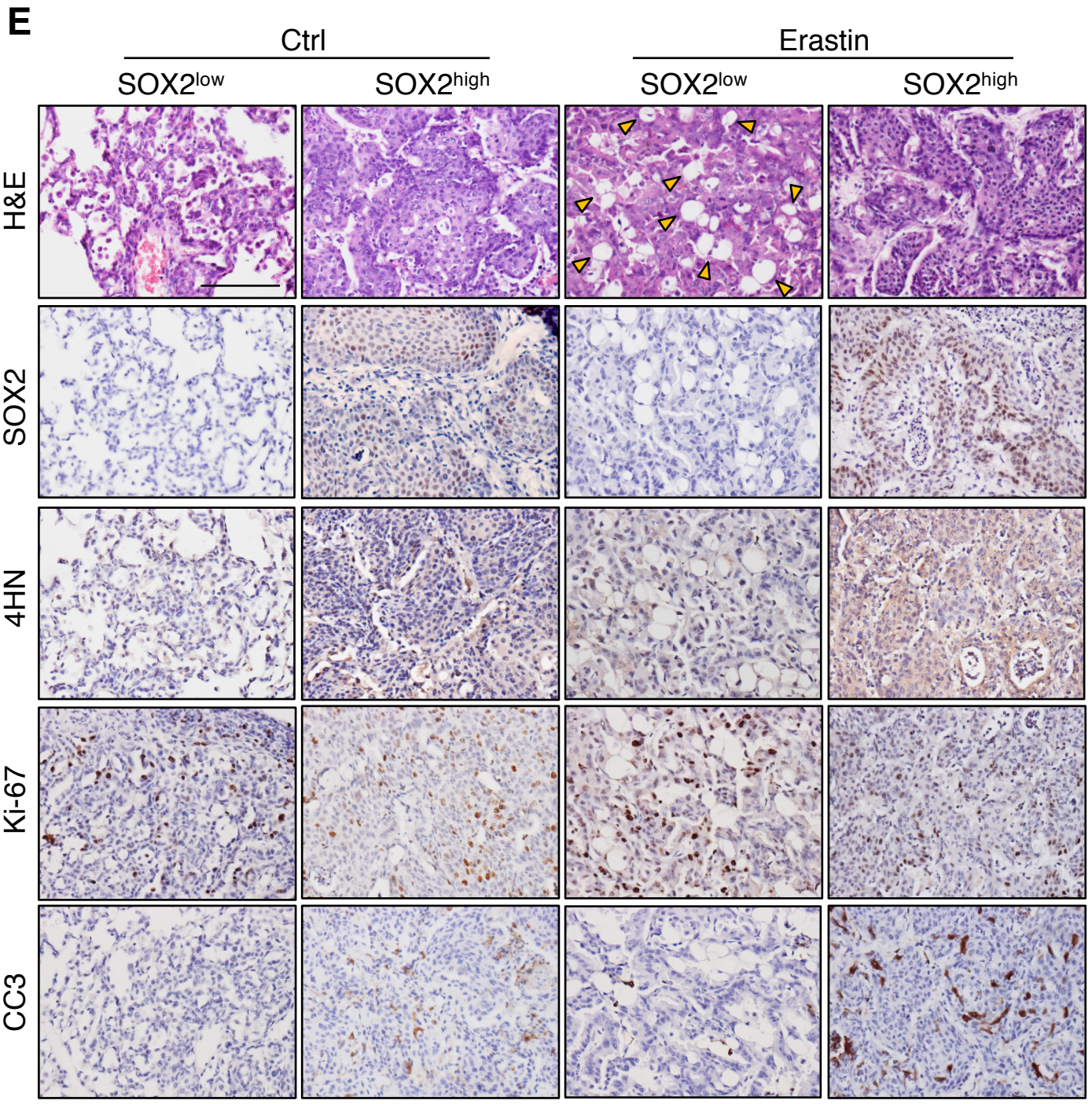


**Figure S7 (Related to Figure 7)**



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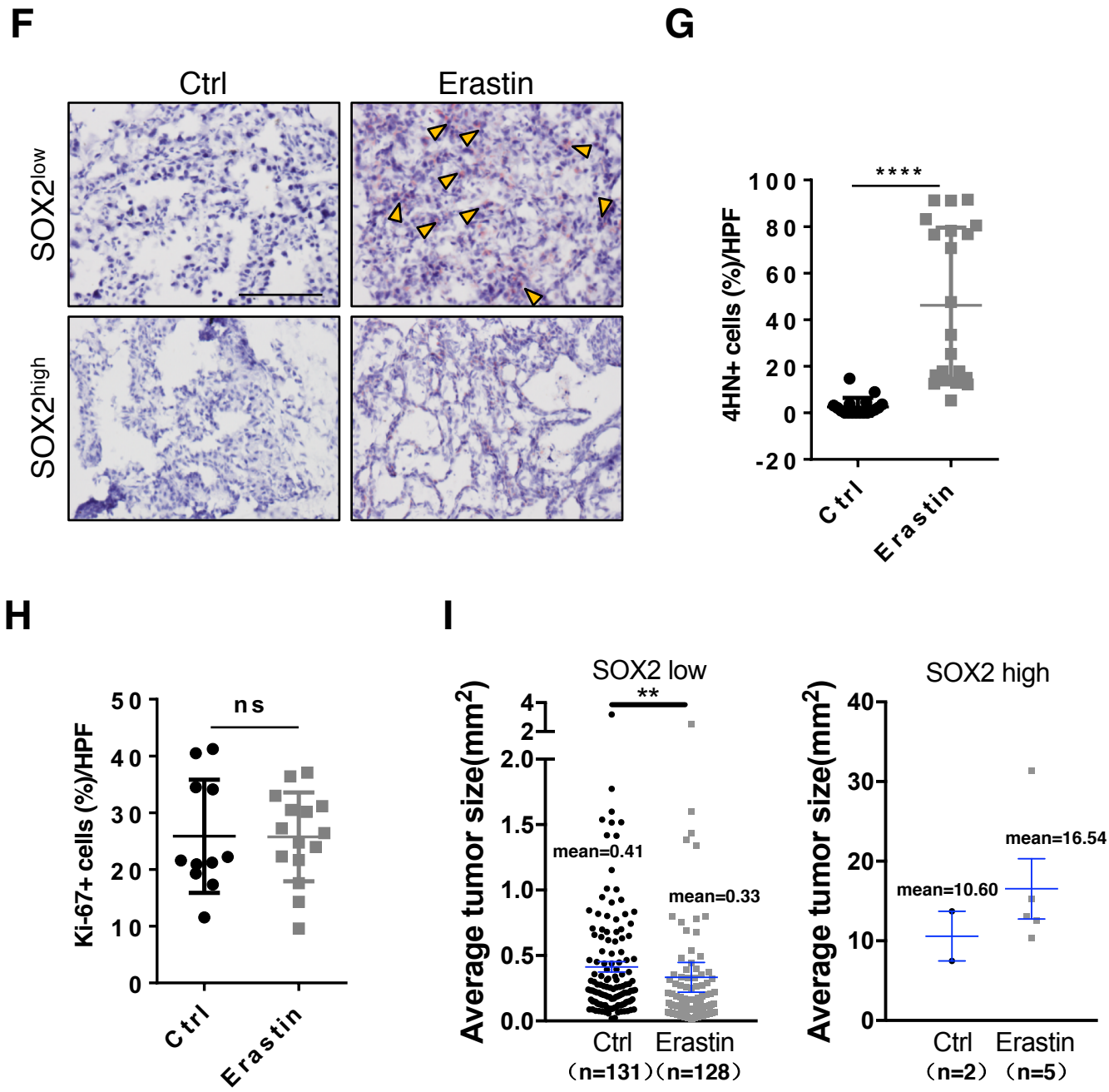

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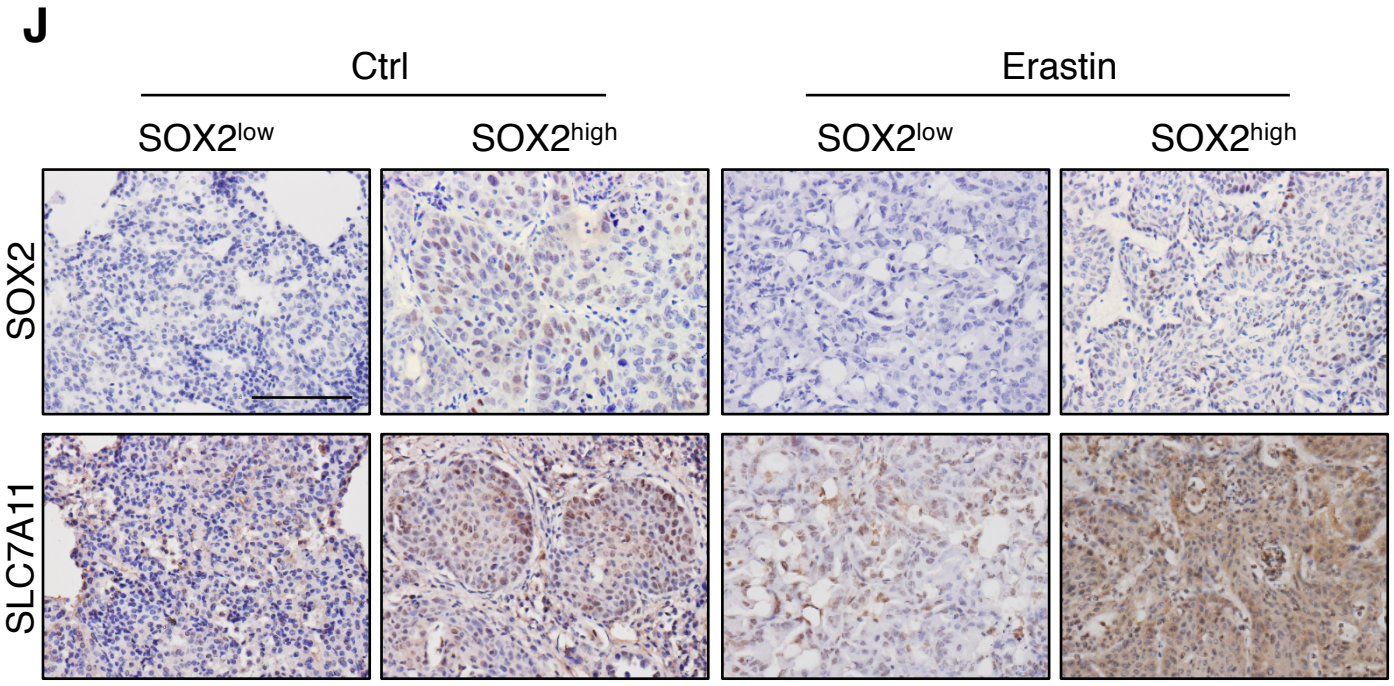


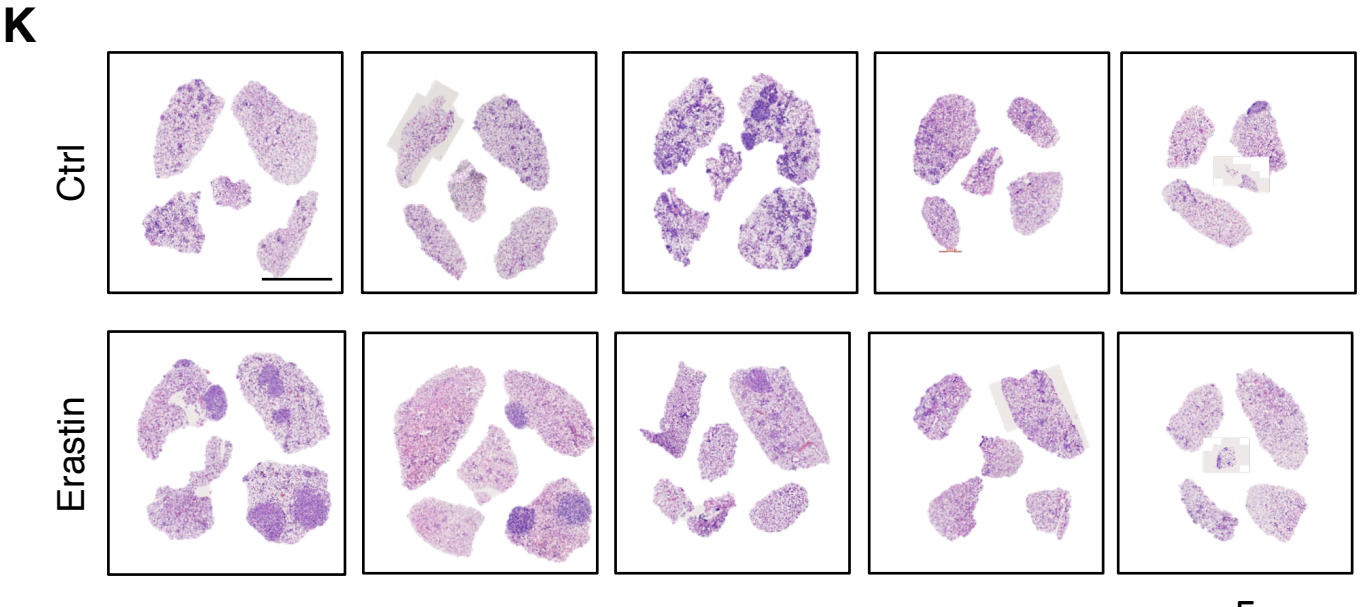


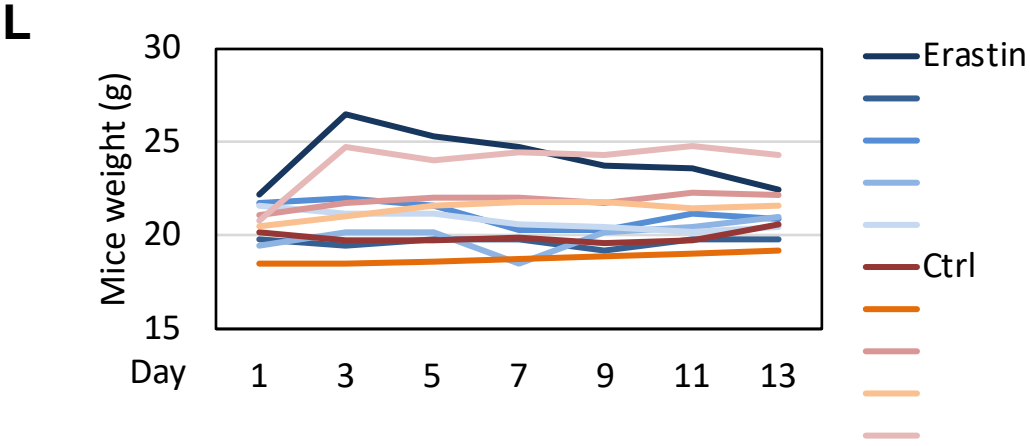

**Figure S7 (Related to Figure 7)**

A. Representative H&E and IHC staining of SOX2, and SLC7A11 of Ad-Cre-infected KL mice from control and IKE groups. Scale bar: 100 μm.
B. Lung sections of Ad-Cre-infected KL mice from control and IKE groups. Scale bar: 5 mm.
C. Analysis of growth curve of body weight in control and IKE treated mice.
D. The scheme of Erastin treatment. The KL mice at 6 weeks post Ad-Cre infection were treated with Erastin for 2 weeks, followed by tumor analysis.
E. Representative H&E and IHC staining of 4HN, Ki-67, cleaved caspase3 (CC3) and SOX2 of Ad-Cre-infected KL mice from control and Erastin groups, yellow arrowheads indicated lipid droplets. Scale bar: 100 μm.
F. Oil Red O staining of Erastin treated tumors showing lipid droplets (in red) of large size. Scale bar: 100 μm.
G. Statistical analysis of the percentage of $4HN^+$ cells in the lung tumors control and Erastin groups in Ad-Cre-infected KL mice. Data was shown as mean ± SEM. Significance was assessed by the Wilcoxon test and ****$P < 0.0001$.
H. Statistical analysis of the percentage of $Ki\text{-}67^+$ cells in the lung tumors control and Erastin groups in Ad-Cre-infected KL mice. Data was shown as mean ± SEM. Significance was assessed by the Wilcoxon test.
I. Quantification of individual tumor size of Ad-Cre-infected KL mice from control and Erastin groups. Data was shown as mean ± SEM. Significance was assessed by the Wilcoxon test and **$P<0.01$ compared between the indicated two groups. Erastin treatment decreased the tumor size of SOX2 low but not SOX2 high tumors.
J. Representative H&E and IHC staining of SOX2, and SLC7A11 of Ad-Cre-infected KL mice from control and Erastin groups. Scale bar: 100 μm.
K. Lung sections of Ad-Cre-infected KL mice from control and Erastin groups. Scale bar: 5 mm.
L. Analysis of growth curve of body weight in control and Erastin treated mice.