# Supplementary Information

Enhancement of 5-aminolevulinic acid-based fluorescence detection of side population-defined glioma stem cells by iron chelation

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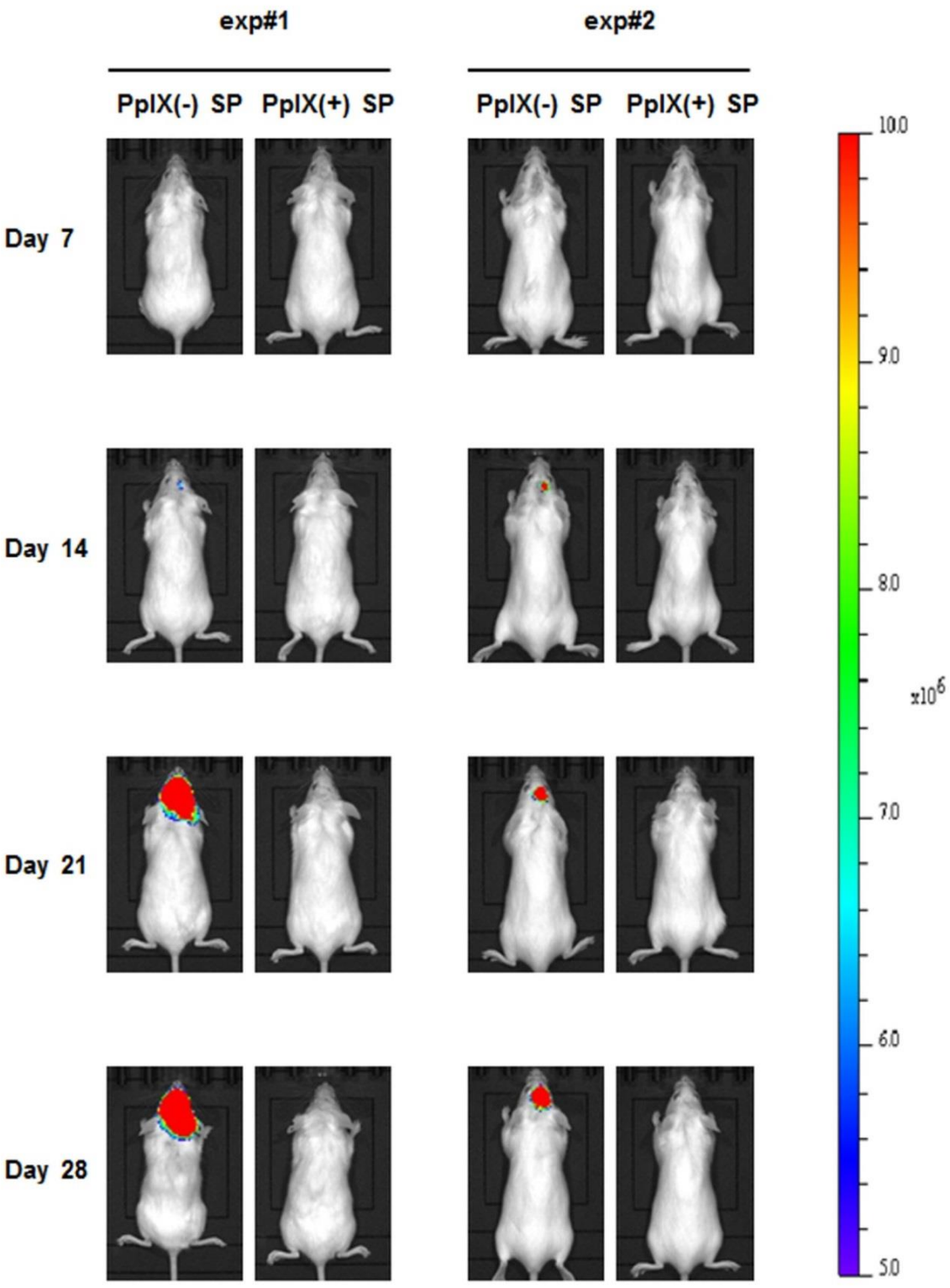
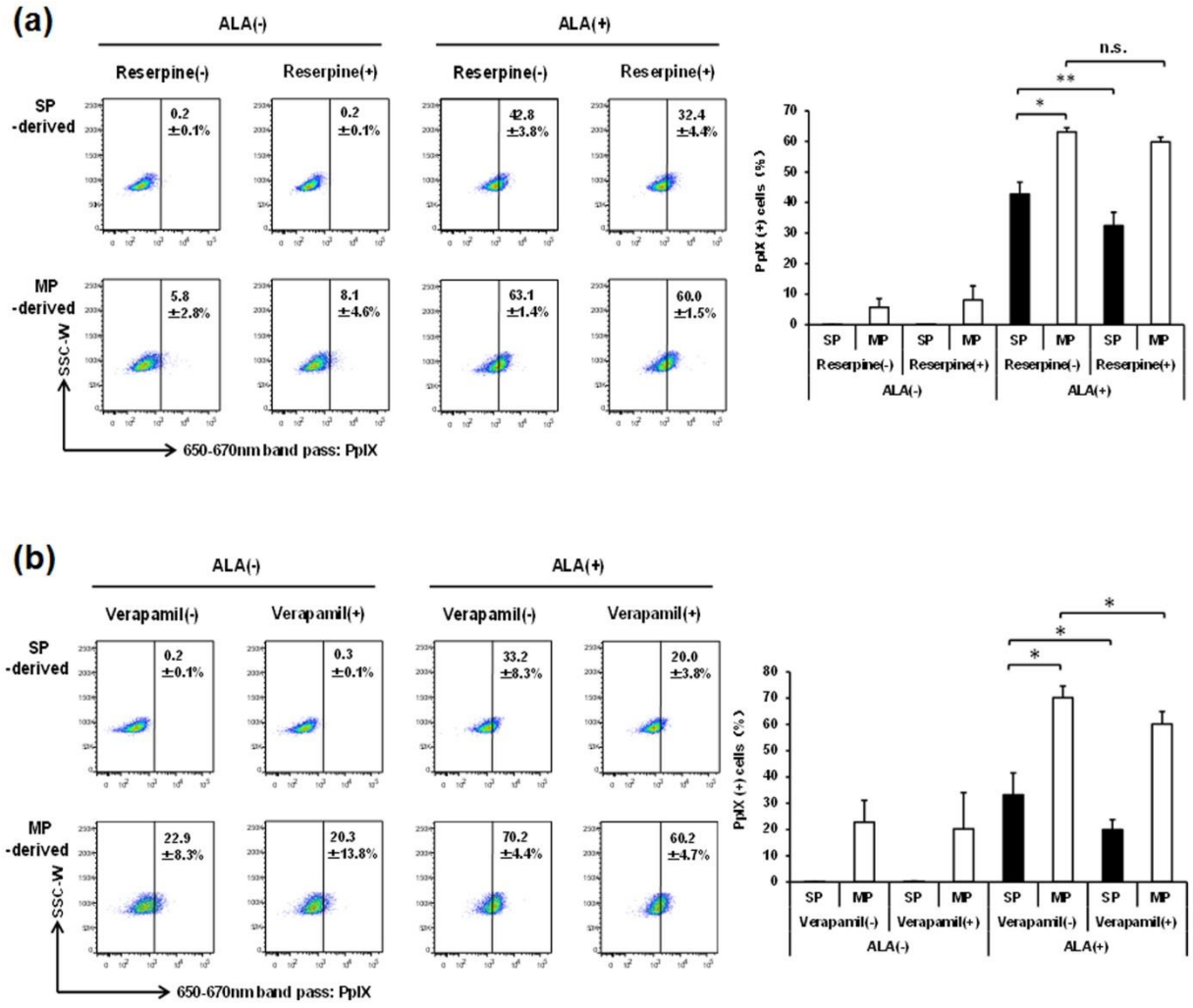
**Supplementary Figures S1-S7**

**Supplementary Table S1**

**Supplementary Methods**

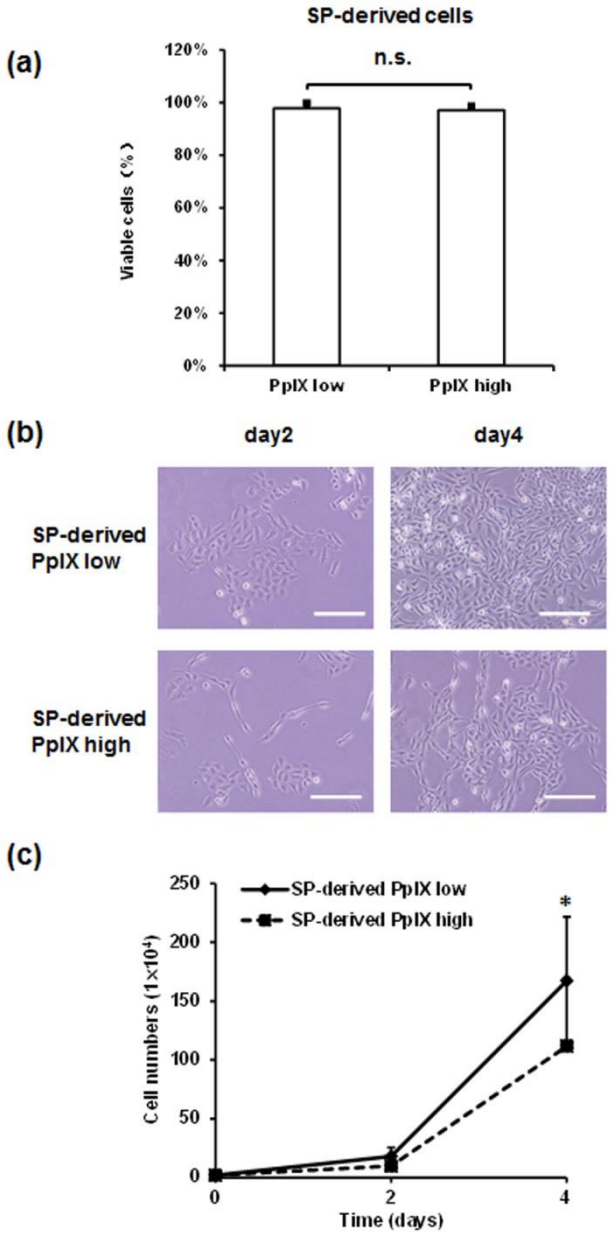



**Supplementary Figure S1. C6 SP-derived PpIX fluorescence low cells have higher tumorigenicity (related to Figure 3)**



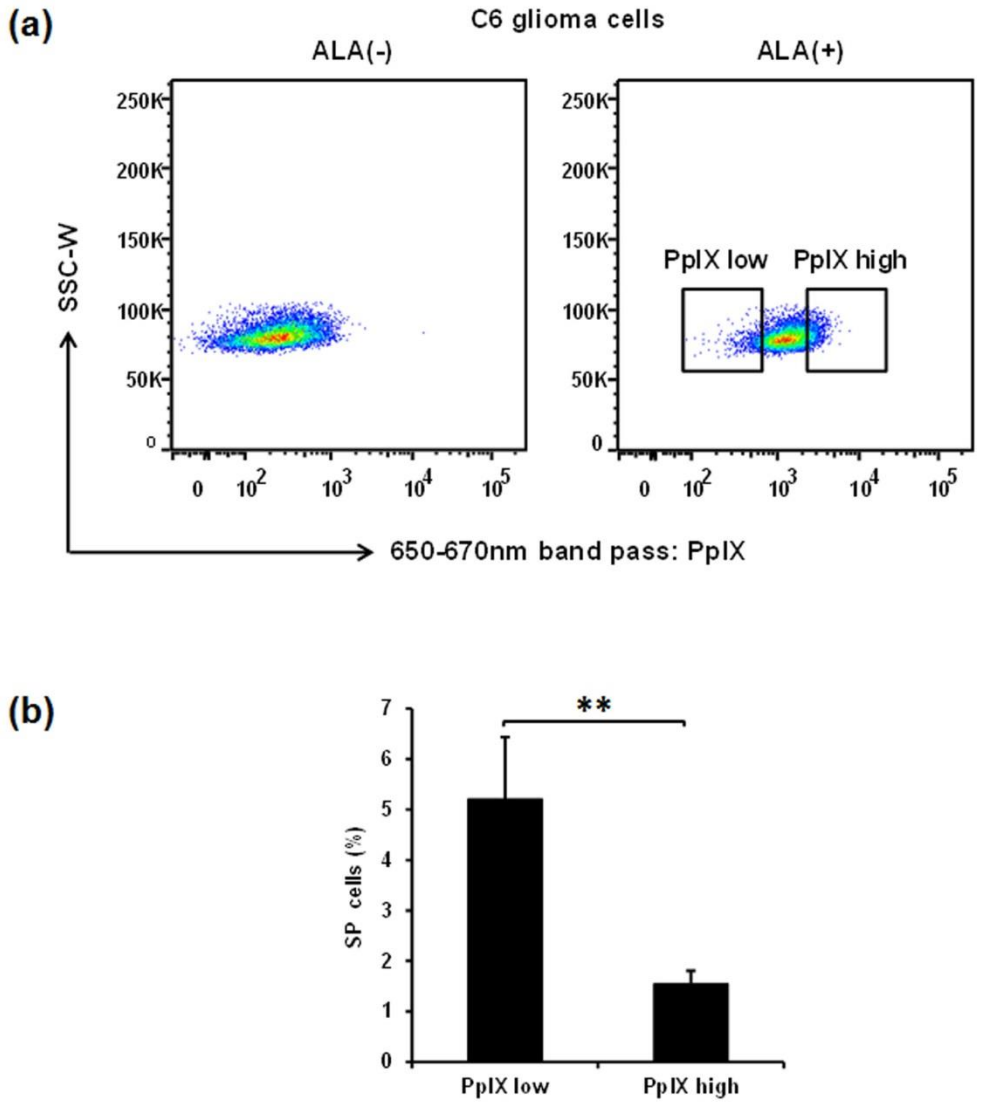

**Supplementary Figure S2. Reserpine and verapamil, inhibitors of ABC transporters, have no effects on low accumulation of PpIX in SP-derived cells**

Intracellular PpIX accumulation in C6 SP- and MP-derived cells treated with or without 5-ALA in the presence or absence of reserpine (a) or verapamil (b). The percentages of PpIX(+) cells are indicated in representative FACS plots (left) and presented in a bar graph (right) as means±SD from three independent experiments. $^{**}P<0.01$.$^{*}P<0.05$. n.s., not significant.



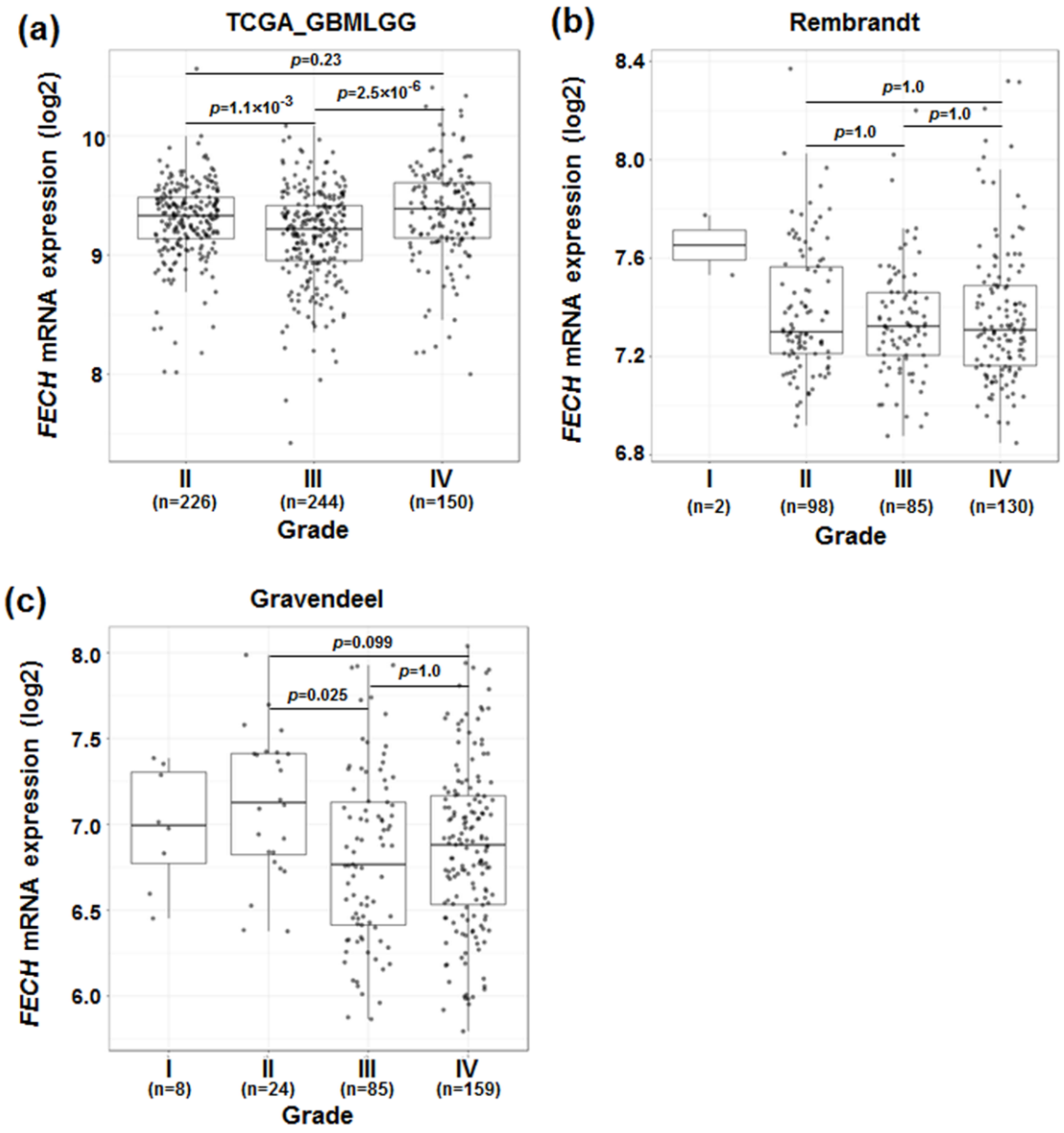

**Supplementary Figure S3. Cell viability and growth of SP-derived PpIX fluorescence low and high cells in vitro**

(a) The percentages of viable cells in PpIX fluorescence low and high fractions are presented in a bar graph as means±SD from three independent experiments. n.s., not significant. (b) Representative images of SP-derived PpIX fluorescence low and high cells are shown on day 2 and 4 after reculturing. Scale bars, 200 μm. (c) The growth curves of SP-derived PpIX fluorescence low and high cells. Data were presented as means±SD from three independent experiments. **P*<0.05.



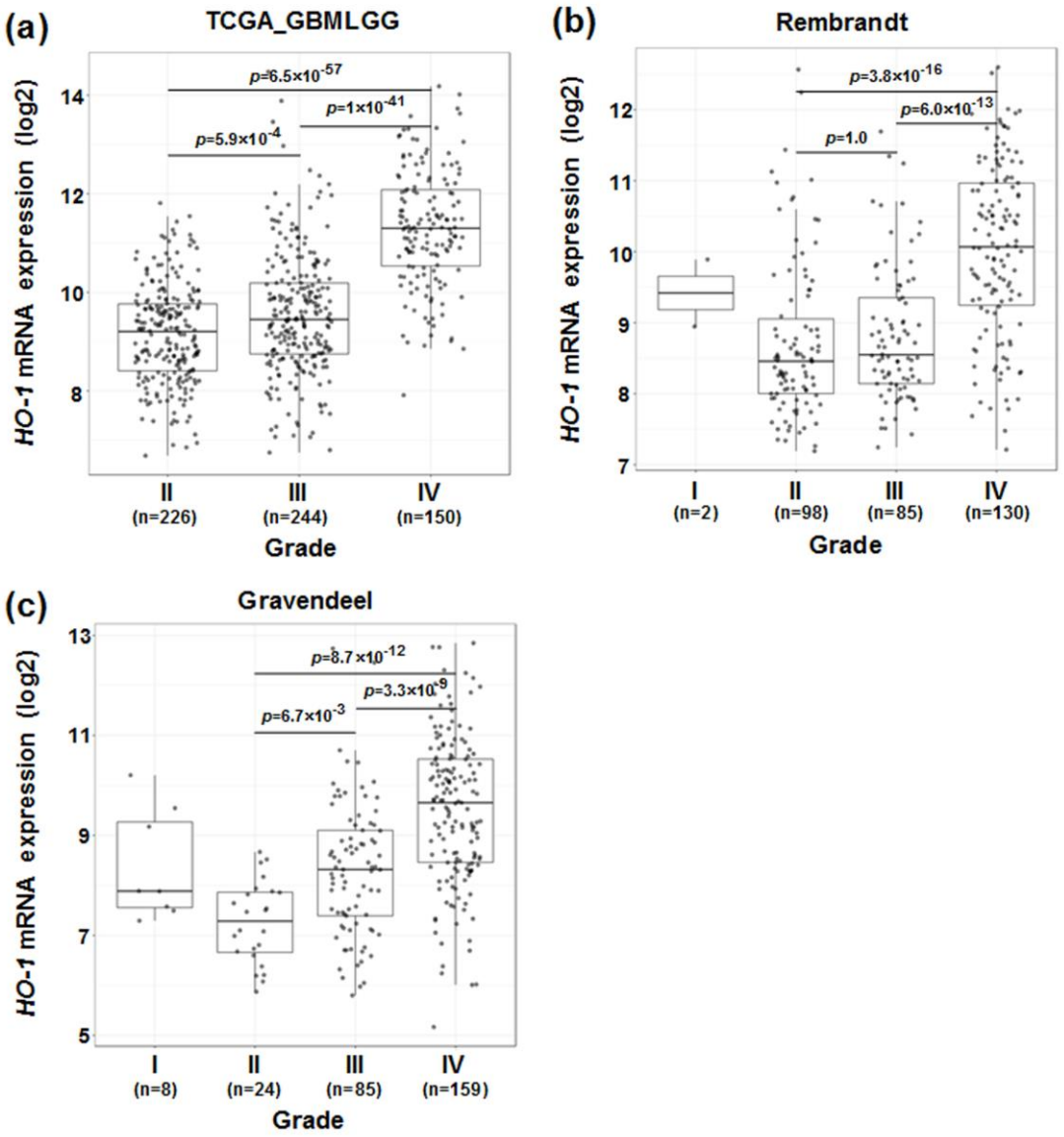

**Supplementary Figure S4. C6 glioma PpIX fluorescence low cells contain higher percentage of SP cells**

(a) FACS plots of PpIX fluorescence low and high cells in C6 glioma cells treated with 5-ALA. (b) The percentages of SP cells in PpIX fluorescence low and high fractions are presented in a bar graph as means±SD from five independent experiments. ***P*<0.01.



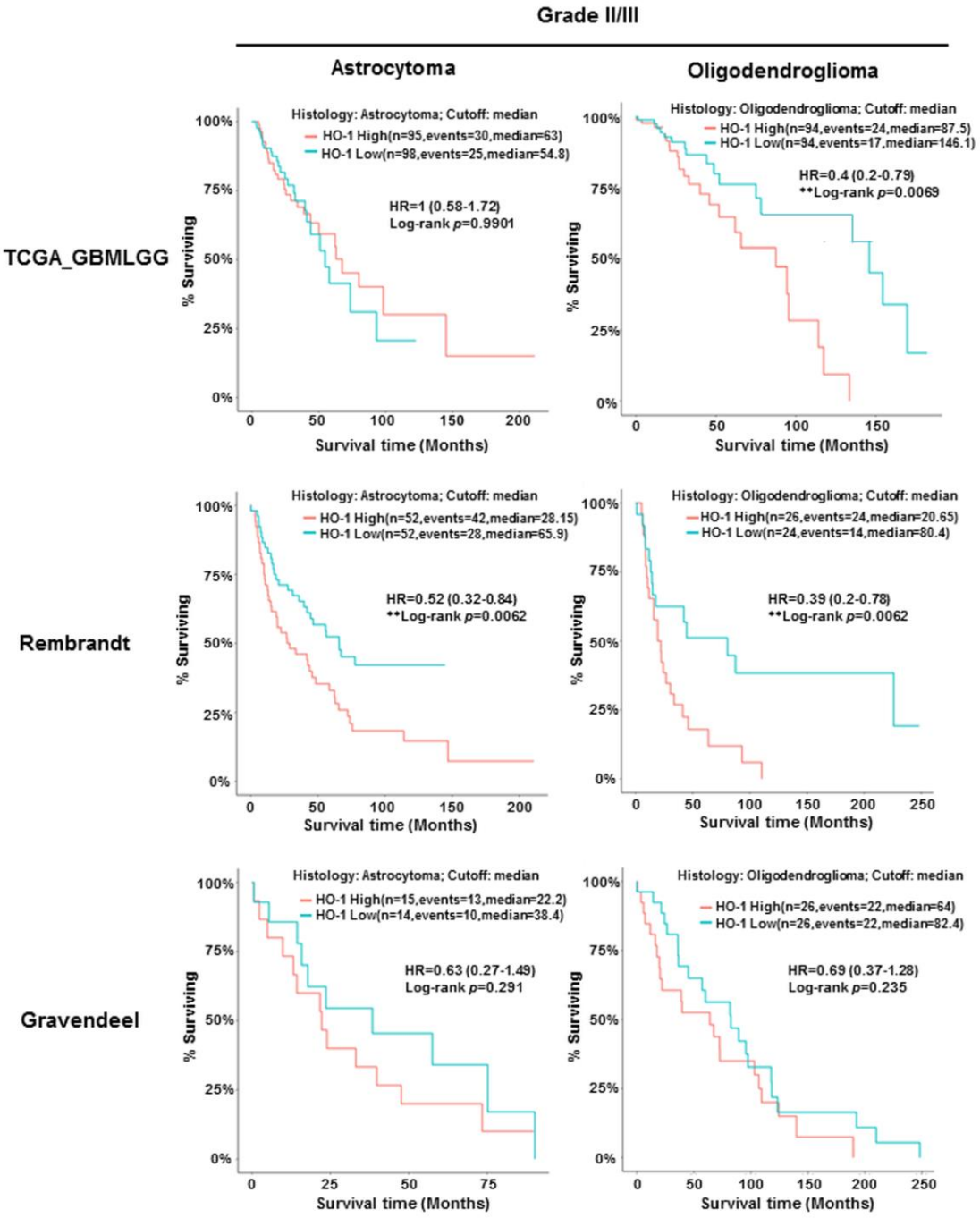

**Supplementary Figure S5. *FECH* expression between different grades of human gliomas**

Box plots for *FECH* mRNA expression in different grades of gliomas from TCGA_GBMLGG (a), Rembrandt (b) and Gravendeel (c) datasets are presented. Data were statistically analyzed by pairwise *t*-tests.




**Supplementary Figure S6. *HO-1* expression is higher in WHO grade IV GBMs than in lower grades of gliomas**

Box plots for *HO-1* (*HMOX1* in the dataset) mRNA expression in different grades of gliomas from TCGA_GBMLGG (a), Rembrandt (b) and Gravendeel (c) datasets are presented. Data were statistically analyzed by pairwise *t*-tests.




**Supplementary Figure S7. Elevated *HO-1* gene expression is correlated with poorer survival in lower grade glioma patients**

Kaplan-Meier survival analysis for human glioma Grade II/III (astrocytoma and oligodendroglioma) patients with higher and lower *HO-1* expression levels from TCGA_GBMLGG, Rembrandt and Gravendeel datasets. Higher and lower expression groups are divided by the median expression value under statistical analysis with the log-rank test.

## Supplementary Table S1. Expression of 5-ALA metabolism enzyme genes in SP and MP cells

| Gene Symbol | GenBank ID | Gene Name | SP | MP | Ratio (SP/MP) |
|---|---|---|---|---|---|
| Hmox1 (Ho-1) | NM_012580 | heme oxygenase (decycling) 1 | 52.363 | 18.989 | 2.757 |
| Alad | NM_012899 | aminolevulinate dehydratase | 33.470 | 43.883 | 0.763 |
| Hmbs | NM_013168 | hydroxymethylbilane synthase (porphobilinogen deaminase) | 4.219 | 4.140 | 1.019 |
| Uros | NM_001012068 | uroporphyrinogen III synthase | 0.174 | 0.185 | 0.944 |
| Urod | NM_019209 | uroporphyrinogen decarboxylase | 20.168 | 20.571 | 0.894 |
| Cpox | NM_001037095 | coproporphyrinogen oxidase | 0.706 | 0.747 | 0.946 |
| Ppox | NM_001105968 | protoporphyrinogen oxidase | 0.687 | 0.761 | 0.902 |
| Fech | NM_001108434 | ferrochelatase | 0.982 | 0.938 | 1.046 |
| Blvra | NM_053850 | biliverdin reductase A | 0.987 | 0.860 | 1.147 |
| Blvrb | NM_001106236 | biliverdin reductase B (flavin reductase) | 16.831 | 21.491 | 0.783 |
| Ugt1a2 | NM_201423 | UDP-glucuronosyl transferase 1 family, polypeptide A2 | 0.783 | 0.738 | 1.061 |
| Ugt1a6 | NM_057105 | UDP-glucuronosyl transferase 1 family, polypeptide A6 | 0.103 | 0.096 | 1.066 |
| Alas1 | NM_024484 | aminolevulinate, delta-, synthase 1 | 26.466 | 16.984 | 1.558 |
| Alas2 | NM_013197 | aminolevulinate, delta-, synthase 2 | 0.027 | 0.025 | 1.084 |

## Supplementary Methods

### Reagents

ABC transporter inhibitors reserpine and verapamil were purchased from Sigma (St Louis, MO, USA).

### FACS analysis of PpIX with inhibitors of ABC transporters

The fluorescence intensity of cellular PpIX was determined by FACS. After SP and MP cells were sorted and re-cultured for 2 days, media were changed to fresh media containing 1 mM 5-ALA, and cells were additionally incubated for 4 hours. In some cases, 10 μM reserpine or 50 μM verapamil was added into media together with 5-ALA. PpIX fluorescence was measured with a FACS Aria II (BD Biosciences) using 488 nm laser and a 660/20 nm band-pass filter.

### Cell viability assay

PpIX fluorescence low and high cells were sorted from cultured SP cells by FACS and collected by centrifugation. The cell suspension was prepared by a 1:1 dilution using a 0.4% Trypan blue solution and examined immediately under a microscope. The numbers of blue staining cells and total cells were counted.

### Bioinformatic analysis

Gene expressions in different grades of gliomas and their correlation with

patients' clinical outcome were analyzed in TCGA_GBMLGG, Rembrandt and Gravendeel datasets by using GlioVis (http://gliovis.bioinfo.cnio.es/).

**Statistical analysis**

All comparisons between experimental groups, except for bioinformatics analysis, were made by Student's *t*-test. Target gene expression in different grade tumors was tested by pairwise *t* tests with corrections for multiple testing (*p* values with Bonferroni correction). For Kaplan-Meier survival curves, *p* values were obtained by using the log-rank test.