**Fatty acid 2-hydroxylation inhibits tumor growth and increases sensitivity to cisplatin in gastric cancer**

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## Supplement

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**Supplementary Table 1.** FA2H and Gli1 expression of human gastric cancer tumors and matched surrounding normal tissues.

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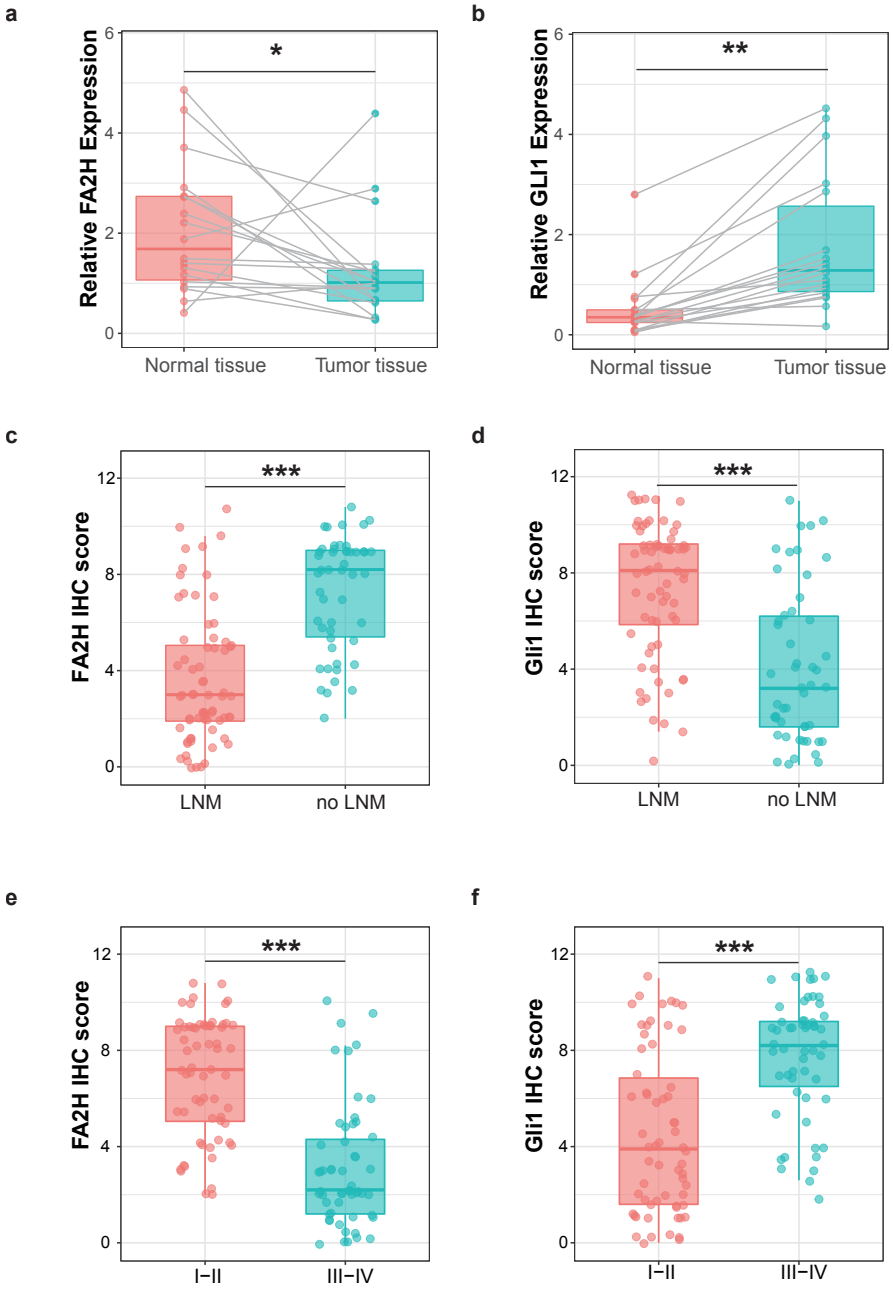
**Supplementary Table 3.** Results of univariate and multivariate analyses of underwent gastrectomy patients' survival by Cox's proportional hazard model.

**Supplementary Table 4.** Antibody information



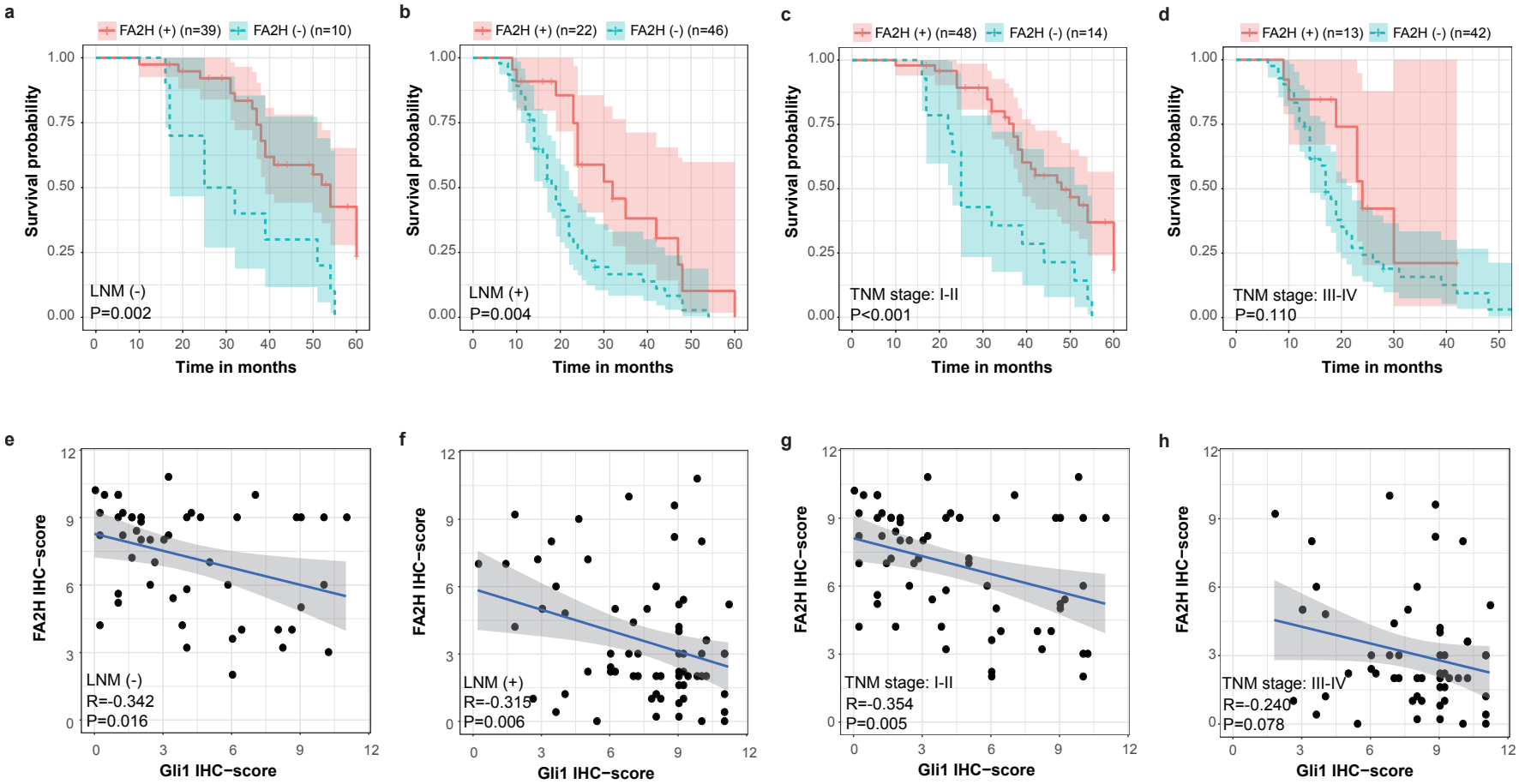

**Supplementary Fig. 1.** Enhanced Gli1 expression was significantly correlated with TNM stage and LNM.

**a-b)** Relative expression of FA2H and Gli1 in 18 paired tissue samples.

**c-d)** The IHC score of FA2H and Gli1 in cancer tissue with or without Lymph node metastasis (LNM).

**e-f)** The IHC score of FA2H and Gli1 in cancer tissue with TNM stage I-II or III-IV.

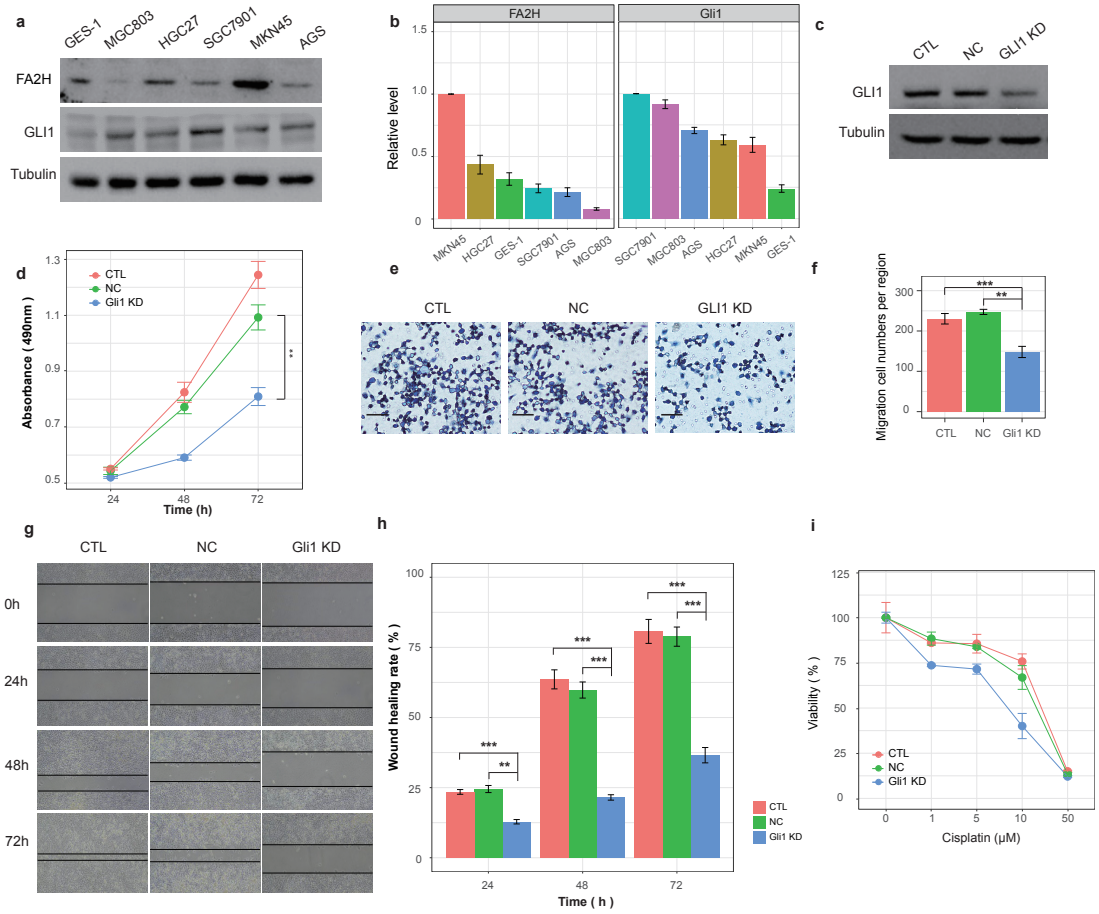
*, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$; ***, $P < 0{\cdot}001$.



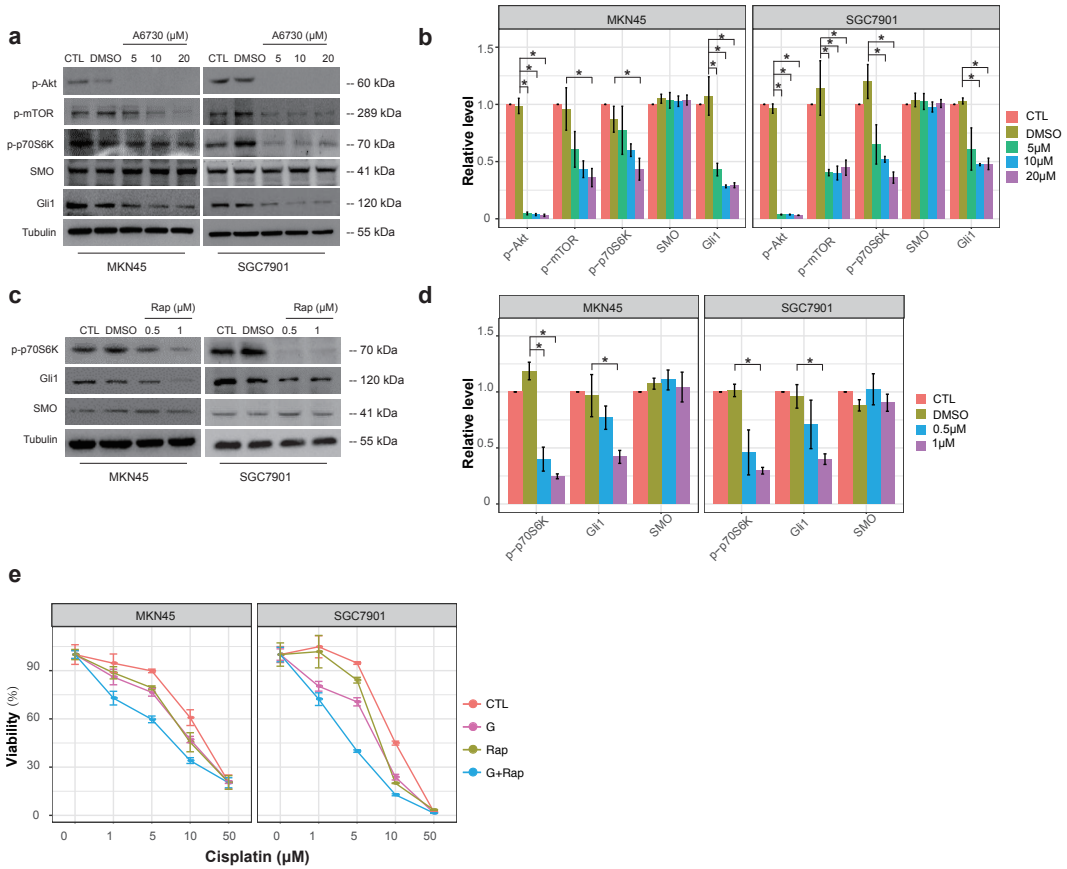

**Supplementary Fig. 2.** The expression of FA2H and Gli1 is in association with LNM and TNM.

**a-d)** Kaplan-Meier curves for overall survival of 49 samples without lymph node metastasis (a), 68 with lymph node metastasis (b), 62 in TNM stage I-II (c), and 55 in TNM stage III-IV (d) according to the expression of FA2H. Cohorts were stratified by the median FA2H expression value of 117 gastric cancer samples (- indicates negative, + indicates positive). The color-shaded areas around the estimated survival curves represent the 95% confidence bands.

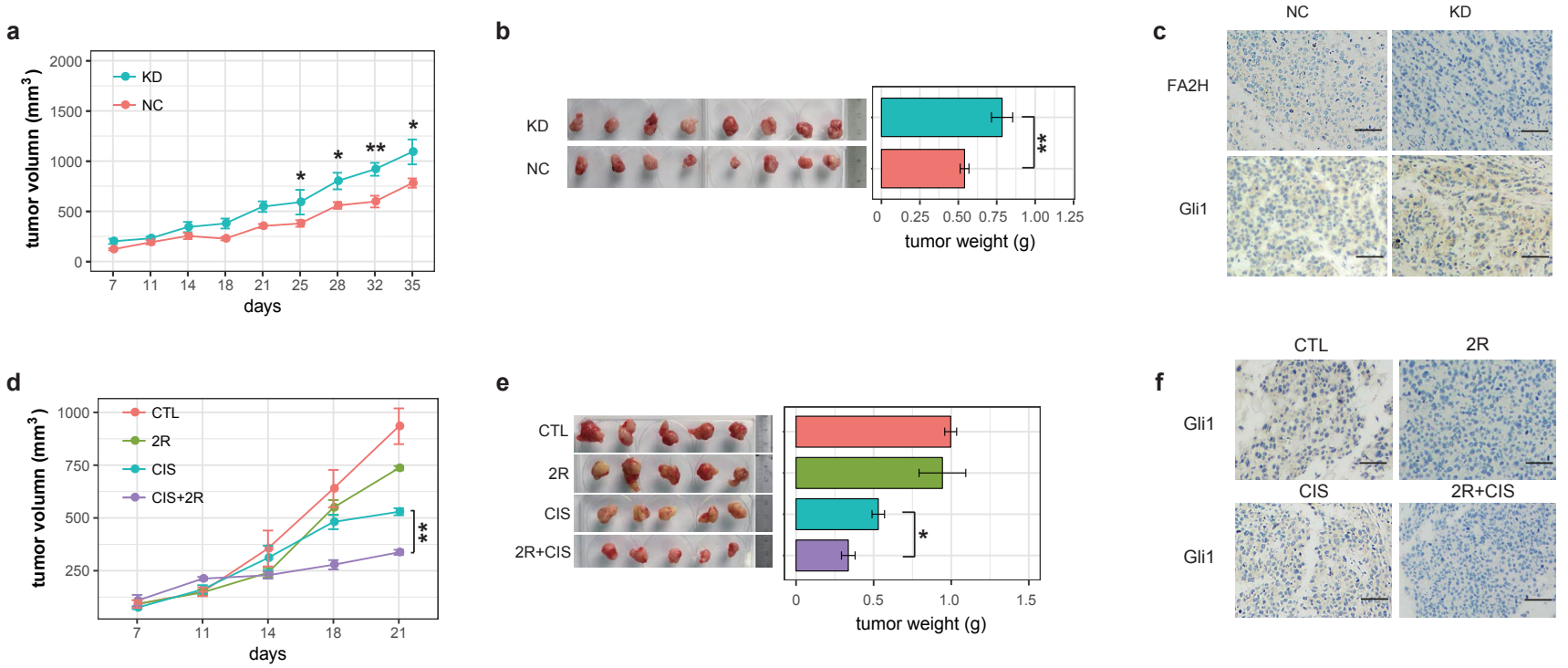
**e-h)** Correlation between Gli1 and FA2H expression levels in 49 samples without lymph node metastasis (e), 68 with lymph node metastasis (f), 62 in TNM stage I-II (g), and 55 in TNM stage III-IV (h). The gray-shaded areas around the estimated regression lines represent the 95% confidence bands.




**Supplementary Fig. 3.** Regulation of proliferation, migration and resistance to chemotherapy by Gli1. **a-b)** The whole cell lysates from different cell lines including 5 GC cell lines (MGC803, HGC27, SGC7901, MKN45, AGS) and a normal gastric cell line (GES-1) were prepared and subjected to Western blot analysis (a). (b) The expression levels of FA2H and Gli1 were quantified as histogram. The bands were quantified and presented as the mean ± SEM (n = 3). **c)** The Western blot of Gli1 expression in SGC7901 cells in untreated (CTL) or transfected with negative control siRNA (NC) or siRNA against Gli1 (Gli1 KD) groups. **d)** MTT assay was used to test the proliferation ability of SGC7901 cells in CTL, NC or Gli1 KD group after incubation for 24 h, 48 h and 72 h. Statistical significance: Gli1 KD group *versus* NC and CTL groups showed $P < 0{\cdot}05$ at least from 48 h to 72 h. **e-f)** Migration assay of SGC7901 cells in CTL, NC or Gli1 KD group after incubation for 24 h. (e) Representative photographs are presented (Scale bar, 100 μm) and (f) the relative number of migratory cells were counted and presented as mean ± SEM (n = 3). **g-h)** Invasive ability of SGC7901 cells in CTL, NC or Gli1 KD group after incubation for 24 h, 48 h and 72 h (g). (h) Wound healing rate (%) was quantified and presented as the mean ± SEM (n=3). **i)** MTT assay of SGC7901 cells in CTL, NC or Gli1 KD group in response to cisplatin, presented as mean ± SEM (n = 3). Statistical significance: GLI1 KD group *versus* NC and CTL groups showed $P < 0{\cdot}05$ at least from 1 μM to 10 μM. *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$; ***, $P < 0{\cdot}001$.




**Supplementary Fig. 4.** Inhibition of Akt or mTOR decreases Gli1 level. **a-b)** MKN45 and SGC7901 were untreated (CTL) or treated with DMSO, 5 μM A6730, 10 μM A6730, 20 μM A6730 for 24 h. (a) The whole cell lysates were prepared and subjected to Western blot analysis with antibodies directed against each specific protein as indicated. (b) The bands were quantified and presented as the mean ± SEM (n = 3). **c-d)** MKN45 and SGC7901 were untreated (CTL) or treated with DMSO, 0·5 μM Rapamycin, 1 μM Rapamycin for 24 h. (c) The whole cell lysates were prepared and subjected to Western blot analysis with antibodies directed against each specific protein as indicated. (d) The bands were quantified and presented as the mean ± SEM (n = 3). **e)** MTT assay of MKN45 and SGC7901 cells untreated (CTL) or pre-treated with 10 μm Gant61 (G), 1 μm Rapamycin (Rap) or 1 μM Rapamycin in combination with 10 μM Gant61 (G+Ra) in response to cisplatin, presented as mean ± SEM (n = 3). Statistical significance for MKN45: G treatment group *versus* control group showed $P <$ 0·01 at 5 μM; R treatment group *versus* control group showed $P < 0{\cdot}01$ at 5 μM; G+R treatment group *versus* control group showed $P < 0{\cdot}05$ at least from 1 μM to 10 μM. Statistical significance for SGC7901: G treatment group *versus* control group showed $P < 0{\cdot}05$ at least from 1μM onwards; R treatment group *versus* control group showed $P < 0{\cdot}05$ at least from 5 μM onwards; G+R treatment group *versus* control group showed $P < 0{\cdot}05$ at least from 1 μM onwards. *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$; ***, $P < 0{\cdot}001$.




**Supplementary Figure 5.** FA2H knockdown promotes while (*R*)-2-OHPA inhibited *in vivo* tumorigenesis of SGC7901 cells.

**a-c)** SGC7901 cells stably expressing FA2H shRNA (KD) or control shRNA (NC) were transplanted into nude mice (n = 8). (a) The volumes of the tumors were measured twice a week during the indicated period. (b)The average tumor mass of each group was also presented. (c) IHC staining of Gli1 and FA2H in representative tumors (Scale bar, 100 μm).

**d-f)** SGC7901 cells were transplanted into nude mice (n = 5) which untreated (CTL), or received 15 μmol/kg (R)-2-OHPA, 5 mg/kg cisplatin (CIS), cisplatin combination with (R)-2-OHPA (CIS+2R). (d) The volumes of the tumors were measured twice a week during the indicated period. (e) The average tumor mass of each group was also presented. (f) IHC staining of Gli1 in representative tumors (Scale bar, 100 μm).

*$P < 0{\cdot}05$, **$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

**Supplementary Table 1.** FA2H and Gli1 expression of human gastric cancer tumors and matched surrounding normal tissues.

| | FA2H | | Gli1 | |
|---|---|---|---|---|
| | positive | negative | positive | negative |
| Tumor tissues | 61 | 56 | 73 | 44 |
| Normal tissues | 98 | 19 | 26 | 91 |
| *P* value | | < 0·001 | | < 0·001 |

**Supplementary Table 2.** Association between Gli1/FA2H and clinic-pathological factors in 117 patients with gastric cancer.

| | Gli1 | | | FA2H | | |
|---|---|---|---|---|---|---|
| | Negative | Positive | *P* value | Negative | Positive | *P* value |
| Age (years) | | | | | | |
| ≤60 | 14 | 30 | 0·316 | 21 | 2 | 0·982 |
| >60 | 30 | 43 | | 35 | 38 | |
| Gender | | | | | | |
| Male | 34 | 56 | 0·944 | 41 | 49 | 0·362 |
| Female | 10 | 17 | | 15 | 12 | |
| Tumor size (cm) | | | | | | |
| ≤5 | 36 | 43 | 0·010* | 29 | 50 | <0·001*** |
| >5 | 8 | 30 | | 27 | 11 | |
| Depth of tumor invasion | | | | | | |
| T1-2 | 19 | 12 | 0·001** | 6 | 25 | <0·001*** |
| T3-4 | 25 | 61 | | 50 | 36 | |
| Lymph node metastasis | | | | | | |
| No | 31 | 18 | <0·001*** | 10 | 39 | <0·001*** |
| Yes | 13 | 55 | | 46 | 22 | |
| Degree of differentiation | | | | | | |
| Well | 24 | 35 | 0·489 | 22 | 37 | 0·021* |
| Poor | 20 | 38 | | 34 | 24 | |
| Venous invasion | | | | | | |
| Negative | 37 | 41 | 0·002** | 31 | 47 | 0·013* |
| Positive | 7 | 32 | | 25 | 14 | |
| Neural invasion | | | | | | |
| Negative | 31 | 47 | 0·500 | 35 | 43 | 0·360 |
| Positive | 13 | 26 | | 21 | 18 | |
| TNM staging | | | | | | |
| I-II | 35 | 27 | <0·001*** | 14 | 48 | <0·001*** |
| III-IV | 9 | 46 | | 42 | 13 | |

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$

**Supplementary Table 3.** Results of univariate and multivariate analyses of underwent gastrectomy patients' survival by Cox's proportional hazard model.

| Varieties | n | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|---|
| | | HR | 95% CI | *P* | HR | 95% CI | *P* |
| Age (≤60 or >60 years) | 44/73 | 0·808 | 0·519-1·257 | 0·344 | | | |
| Gender (Male / Female) | 90/27 | 0·938 | 0·569-1·47 | 0·803 | | | |
| Size of tumor (≤5 or >5 cm) | 79/38 | 0·356 | 0·226-0·560 | <0·001*** | 0·835 | 0·475-1·468 | 0·518 |
| Depth of tumor invasion (T1-2 / T3-4) | 31/86 | 0·250 | 0·148-0·425 | <0·001*** | 0·403 | 0·208-0·781 | 0·007** |
| Lymph node metastasis (negative / positive) | 49/68 | 0·278 | 0·175-0·441 | <0·001*** | 0·472 | 0·228-0·978 | 0·045* |
| Degree of differentiation (differentiated or undifferentiated) | 59/58 | 0·622 | 0·409-0·946 | 0·026* | 0·801 | 0·503-1·275 | 0·333 |
| Venous invasion (negative / positive) | 78/39 | 0·384 | 0·247-0·597 | <0·001*** | 0·723 | 0·426-1·229 | 0·245 |
| Neural invasion (positive / negative) | 78/39 | 0·489 | 0·314-0·761 | 0·002** | 1·072 | 0·626-1·836 | 0·801 |
| TNM staging (I-II / III-IV) | 62/55 | 0·243 | 0·153-0·385 | <0·001*** | 1·426 | 0·625-3·254 | 0·397 |
| FA2H expression (negative / positive) | 56/61 | 3·735 | 2·379-5·862 | <0·001*** | 2·219 | 1·240-3·969 | 0·008** |
| Gli1 expression (negative/ positive) | 44/73 | 0·425 | 0·270-0·668 | <0·001*** | 0·828 | 0·480-1·426 | 0·394 |

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$

**Supplementary Table 4.** Antibody information

| Name | Company | Catalog Number | RRID |
|---|---|---|---|
| Phospho-mTOR (S2448) | Abcam | ab1093 | AB_297588 |
| Gli1 | Abcam | ab151796 | Not available |
| Smoothened (SMO) | Abcam | ab38686 | AB_882615 |
| AMPKα | Cell Signaling Technology | 2532 | AB_330331 |
| Phospho-AMPKα (Thr172) | Cell Signaling Technology | 2535 | AB_331250 |
| Akt | Cell Signaling Technology | 9272 | AB_329827 |
| Phospho-Akt (Ser473) | Cell Signaling Technology | 4058 | AB_331168 |
| Phospho-p70 S6 Kinase (Thr389) | Cell Signaling Technology | 9205 | AB_330944 |
| α-tubilin | Sigma-Aldrich | T6199 | AB_477583 |

Rabbit anti-hFA2H antibody was kindly provided by Dr. Hama Hiroko (University of South Carolina) and rabbit anti-GLUT1 from Dr. Mike Mueckler (Washington University in St. Louis)
*RRID*: Research Resource Identifier.