Supplemental Material

A glycoprotein hormones alpha subunit/EGFR/GATA2 positive feedback circuit confers chemoresistance in gastric cancer

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Supplementary Methods

Mass spectrometry

The proteins were reduced by dithiothreitol at 37°C for 2 h and alkylated by iodoacetamide in the dark at RT for 30 min. Then, trypsin was added at a ratio of 1:50 (trypsin/proteins, w/w) and incubated at 37°C overnight. Trypsin was added again at the same weight ratio and incubated for another 4 h. The tryptic digests were desalted by an SPE column (Waters) and dried for further MS analysis. For glycosylation site identification, the CGA glycopeptide enrichment was performed with click maltose-hydrophilic interaction liquid chromatography (HILIC) materials. The prepared materials (5.0 mg) were washed with 80% acetonitrile (ACN) with 1% trifluoroacetic acid, and the digestions of CGA digest were mixed with HILIC materials. After incubation at RT for 40 min, the mixtures were translated into a tip and the supernatant was removed after centrifugation at 4000×g for 10 min. The HILIC tip was further washed three times with 200 µl of loading buffer. Finally, the enriched N-linked glycopeptides were eluted with 100 µl of 30% ACN with 0.1% formic acid (FA). The eluted samples were dried and treated with PNGase F (500 units) in 10 mM NH₄HCO₃ (pH 7.8) at 37°C overnight. The deglycosylated peptides were dried and used for the identification of the glycosylation sites by further MS analysis.

The LC-MS/MS analyses were performed on a Q-Exactive HF mass spectrometer equipped with an Ultimate 3000 system (Thermo) for separation. The LC-MS/MS system contained a C18 capillary trap column (200 µm i.d., C18 AQ beads (1.9 µm, 120 Å)) and a 15 cm C18 capillary analysis column (150 µm i.d., C18 AQ beads (1.9 µm, 120 Å)). Mobile
phases A (98% H₂O, 2% acetonitrile, 0.1% FA) and B (80% acetonitrile, 20% H₂O, 0.1% FA) were used to develop a gradient. For the deglycosylated peptides, the reversed-phase gradient was developed at 600 nl/min as follows: loading sample for 12 min, 4 to 45% buffer B for 25 min, 45 to 90% buffer B for 3 min, 90 to 90% buffer B for 10 min, 90 to 4% buffer B for 0.5 min and 4 to 4% buffer B for 9.5 min. The temperature of the ion transfer capillary was 250°C, and the normalized collision energy was set to 27%. The mass resolution was set to 70,000 for full MS and 15,000 for higher-energy collisional dissociation MS/MS. Survey full scan MS was acquired from m/z 400 to 2,000, and the 15 most intense ions were selected for the MS/MS scan. The dynamic exclusion was set as follows: repeat count, 1; duration, 22 s; exclusion list size, 500; and exclusion duration, 30 s. For glycosylation site analysis, the mass spectrometric data of deglycosylated peptides acquired by MS was directly searched by using pFind (version 3.1.5). The following parameters were used for the search: mass tolerances were 20 ppm for both the precursor and fragments; for trypsin digested samples, enzyme specificity was set to KR/P with up to 2 missed sites; cysteine residues were set as fixed modifications (C, +57.022 Da), and the N-linked glycosylation sites were obtained with sequon (N-X-S/T, X≠P).

**RNA sequencing and data analysis**

Total RNA from cells was extracted with TRIzol and assessed with an Agilent 2100 BioAnalyzer (Agilent Technologies) and Qubit Fluorometer (Invitrogen). All RNA samples that passed quality tests with an RNA integrity number (RIN) > 7.0 and a 28S:18S
ratio > 1.8 were used for RNA sequencing. Sequence libraries were generated and sequenced by CapitalBio Technology. RNA sequencing reads were aligned to the human genome hg38 using HISAT2 with default parameters, and the number of reads mapped to each gene was calculated by HISAT (http://www.ccb.jhu.edu/software/hisat/).

Differentially expressed genes (DEGs) between samples were detected by edgeR based on a negative binomial distribution. The p values were adjusted by Benjamini and Hochberg’s multiple test correction procedures. By searching the ENSEMBL, NCBI, UniProt, GO and KEGG databases, a Basic Local Alignment Search Tool (BLAST) alignment was performed to determine the functional annotation of DEGs, and the best matches were selected. KEGG pathway enrichment analysis was performed for the DGEs using KOBAS 3.0 software.

*Production of miRNA prodrugs*

MSA/miR-708-3p-, MSA/miR-761- and tRNA/MSA-expressing plasmids were transformed into HST08 *E. coli* competent cells. Separation of target RNAs from total bacterial RNA was achieved on an Enrich-Q 10 × 100 column by using the NGC QUEST 10 PLUS fast protein liquid chromatography (FPLC) system (Bio-Rad). The system was first equilibrated with Buffer A (10 mM sodium phosphate, pH 7.0) at a constant flow rate of 2.5 ml/min for 4.4 min and then subjected to the following gradient elution: 64% Buffer B (Buffer A + 1 M sodium chloride, pH 7.0) for 10 min, 64-78% Buffer B for 8 min, and then 100% Buffer B for 3 min. FPLC traces were monitored at 260/280 nm using a UV/Vis
detector. After the confirmation of target RNA by urea-PAGE analyses, fractions were pooled, precipitated by ethanol, desalted and concentrated with centrifugal filters. RNA purities were verified by a high-performance liquid chromatography assay. The purified MSA/miR-708-3p, MSA/miR-761 and tRNA/MSA were formulated with the in vivo-jetPEI (Polyplus Transfection) reagent and administered intratumorally into mice.
Fig. S1. CGA is upregulated in chemoresistant gastric cancer (GC) cells and tissues, related to Fig. 1.

(A) Half maximal inhibitory concentration (IC50) values for indicated chemotherapeutic agents were measured in chemosensitive SGC7901 cells and multi-drug resistant (MDR) SGC7901<sup>ADR</sup> and SGC7901<sup>VCR</sup> cells. Data are presented as mean ± SEM. (B) Functional analysis of the secreted proteins from GC cells (SGC7901) and MDR GC cells (SGC7901<sup>ADR</sup> and SGC7901<sup>VCR</sup>) annotated as blood proteins. The color code represents ten functional classes. (C) IHC staining of CGA in all the six human GC specimens obtained from patients who responded to chemotherapy (scale bar, 50 μm).
**Figure S2**

Fig. S2. CGA is important to maintain chemoresistance in GC cells, related to Fig. 2.

(A) CGA<sup>+</sup> MDR cells were generated by CRISPR/Cas9-mediated genome editing. Sequence of the targeted region and the two knockout alleles (KO-1 and KO-2) are shown.

(B) Immunoblotting of CGA expression in CGA WT and KO MDR cells. (C) Growth
curves of $CGA$ WT and KO MDR cells in the absence of chemotherapy. (D) $CGA$ WT or KO SGC7901$^{VCR}$ cells were treated with fluorouracil (10 μg/ml) or paclitaxel (10 μg/ml). The IC50 values and apoptosis were measured. (E) Growth curves of $CGA$ WT and KO SGC7901$^{VCR}$ cells in the presence of fluorouracil or paclitaxel. (F, G) Viability, apoptosis (F) and growth curves (G) of $CGA$ WT or KO SGC7901$^{VCR}$ cells that were treated with or without recombinant human CGA (rCGA, 20 μg/ml) in the presence of fluorouracil or paclitaxel. Data are presented as mean ± SEM. **p < 0.01 by one-way ANOVA followed by Dunnett’s multiple comparison in (D-G).
Fig. S3. CGA is important to maintain chemoresistance in GC cells both in vitro and in vivo, related to Fig. 2.

(A) Immunoblotting of CGA in a panel of GC cell lines. (B) Immunoblotting of CGA in
BGC823 and MKN45 cells transfected with two independent siRNAs against CGA (siCGA) or a control siRNA (siCtrl). (C) BGC823 and MKN45 cells transfected with siCGA or siCtrl were treated with fluorouracil. The IC50 values and apoptosis were measured with or without rCGA treatment. (D) Xenograft tumors of CGA WT and KO SGC7901ADR cells isolated on day 21 after the indicated treatment. (E-G) CGA WT or KO SGC7901VCR cells were injected subcutaneously into nude mice (n=5). After tumors were palpable, mice received indicated treatment every 3 days (fluorouracil, 20 mg/kg, i.p. injection; paclitaxel, 3 mg/kg, i.p. injection) and tumor size was monitored (E). Tumors were isolated on day 21 after treatment and tumor weight was measured (F). IHC staining of Ki-67 and cleaved Caspase-3 in tumors harvested from each group is shown and the percentages of Ki-67- and cleaved Caspase-3-positive cells were measured (G; scale bar, 50 μm). Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 by one-way ANOVA followed by Dunnett’s multiple comparison in (C), by repeated-measures ANOVA test followed by Dunnett’s multiple comparison in (E) or by Student’s t test in (F and G).
Fig. S4. CGA binds to EGFR and activates EGFR downstream signaling in GC cells, related to Fig. 3.
(A, B) Immunoblotting of CGA in \textit{CGA}^{+/}\textsuperscript{SGC7901}\textsubscript{ADR} cells complemented with FL or ΔSP CGA (A), and viability of indicated cells treated with fluorouracil or adriamycin (B). (C) Immunoblotting of the β-subunits of hCG, FSH, LH and TSH in cell lysates and conditioned media from SGC7901 and MDR cells. (D) Immunoblotting of the β-subunits of hCG, FSH, LH and TSH from SGC7901\textsuperscript{ADR} cells transfected with their corresponding siRNAs or siCtrl. (E) Viability of SGC7901\textsuperscript{ADR} cells transfected with siRNAs against the β-subunits of hCG, FSH, LH and TSH. (F) Immunoblotting of p-EGFR in serum-starved NCI-N87 cells when treated with the indicated concentrations of rCGA (top) or treated with rCGA (20 μg/ml) at different time points (bottom). (G) NCI-N87 cells pretreated with cetuximab (10 μg/ml) followed by rCGA treatment and immunoblotting with indicated antibodies. (H) Total cell lysates were blotted as a control for immunoblotting analysis in Figure 3H. (I) Immunofluorescence (IF) staining of CGA, EGFR and lysosome marker cathepsin D (CathD) in SGC7901 cells after treatment with rCGA at 37°C for a time course (scale bar, 5 μm). (J) Heatmap of differentially expressed genes (fold change > 2 and p < 0.01) in MDR cells after CGA knockdown. (K) KEGG pathway enrichment analysis identified top de-enriched pathways following CGA knockdown. Data are presented as mean ± SEM. **p < 0.01 by one-way ANOVA followed by Dunnett’s multiple comparison in (B). n.s., not significant.
**Fig. S5.** CGA binds to EGFR and activates EGFR downstream signaling in GC cells, related to Fig. 3.

**A** Immunoblotting of CGA in cell lysates and conditioned media from SGC7901 and NCI-N87 cells infected with lentivirus expressing CGA (LV-CGA, MOI=10) or control lentivirus (LV-NC, MOI=10). **B** SGC7901 and NCI-N87 cells infected with LV-CGA or LV-NC were treated with different concentrations of Fluorouracil. The graphs show the relative cell viability and apoptosis rate.

**C** SGC7901 and NCI-N87 cells infected with LV-CGA or LV-NC were treated with different combinations of Fluorouracil, Cetuximab, Erlotinib, and Caspase-3 inhibitors. The graphs show the relative cell viability and the percentage of positive staining cells for Cleaved Caspase-3.
LV-NC were treated with fluorouracil. The IC50 values and apoptosis were measured. (C) Viability of SGC7901\textsuperscript{ADR} and SGC7901\textsuperscript{VCR} cells treated with fluorouracil, cetuximab (10 \(\mu\)g/ml), erlotinib (20 nM) or a combination of them. (D, E) Xenograft tumors on day 24 for Figure 3K (D) and day 27 for Figure 3L (E). IHC staining of Ki-67 and cleaved Caspase-3 in tumors harvested from each group is shown and the percentages of Ki-67- and cleaved Caspase-3-positive cells were measured (scale bar, 50 \(\mu\)m). Data are presented as mean ± SEM. *\(p < 0.05\), **\(p < 0.01\) by Student’s \(t\) test in (B, D and E) or by one-way ANOVA with Bonferroni’s post hoc test in (C).
Fig. S6. N-linked glycosylation is required for CGA-induced chemoresistance, related to Fig. 4.

(A) RT-qPCR analysis of CGA expression in CGA\(^{-/-}\) SGC7901\(^{ADR}\) cells transfected with WT, N52Q, N78Q or DM CGA. Data are presented as mean ± SEM. (B, C) Immunoblotting of CGA in cell lysates and conditioned media from CGA\(^{-/-}\) SGC7901\(^{ADR}\) cells transfected with WT, N52Q, N78Q or DM CGA. (D) Immunoblotting of CGA in CGA\(^{-/-}\) SGC7901\(^{ADR}\) cells transfected with WT, N52Q, N78Q or DM CGA followed by treatment with bafilomycin A1 (BMA, 1 \(\mu\)M) or MG132 (10 \(\mu\)M). (E) Immunoblotting of p-EGFR and EGFR in CGA\(^{-/-}\) SGC7901\(^{VCR}\) cells treated with purified WT, N52Q, N78Q
or DM rCGA. (F) Total cell lysates were blotted as a control for immunoblotting analysis in Figure 4J.
**Figure S7**

(A) Kaplan-Meier analyses of correlations between expression levels of seven transcription factors and survival in gastric cancer.

(B) Relative mRNA expression levels of GATA2 and CGA in SGC7901 and SGC7901VCR cell lines treated with siRNA targeting GATA2.

(C) Relative mRNA expression levels of GATA2 and CGA in NCI-N87 cells transfected with vectors expressing GATA2 or control vector.

(D) Relative cell viability of HEK293T cells treated with CGA and β-actin expression levels.

(E) CGA promoter region analysis using ChIP-seq experiments.

(F) Relative expression levels of GBE1 and GBE2 in HEK293T cells transfected with vectors expressing GATA2 or control vector.

**Fig. S7.** The reciprocal positive regulation between GATA2 and CGA-EGFR signaling, related to Fig. 5.

(A) Kaplan-Meier analyses of correlations between expression levels of seven transcription factors and survival in gastric cancer.
factor candidates and overall survival of GC patients in the KM plotter database. (B, C) RT-qPCR analyses of GATA2 and CGA expression in MDR cells transfected with siGATA2 or siCtrl (B) and in SGC7901 and NCI-N87 cells transfected with either a GATA2 expression vector or an empty vector (C). (D) Immunoblotting of GATA2 and CGA expression (left) and viability (right) in NCI-N87 cells transfected with either a GATA2 expression vector or an empty vector. (E) Multiple alignment of GATA2-binding elements (GBEs) homologues from different species. The GBEs are boxed in red, and the conserved flanking sequences are in orange, cyan and green. (F) The luciferase reporter driven by either the WT, deletion or mutant (MUT) promoter of CGA was transfected into HEK293T cells, and luciferase activity was measured with or without GATA2 co-transfection. (G) Electrophoretic mobility shift assay using nuclear extracts from SGC7901 cells and the indicated probes. Data are presented as mean ± SEM. **p < 0.01 by one-way ANOVA followed by Dunnett’s multiple comparison in (B) or by Student’s t test in (D and F).
Fig. S8. The reciprocal positive regulation between GATA2 and CGA-EGFR signaling, related to Fig. 5.

(A) Immunoblotting of p-EGFR, EGFR and GATA2 in SGC7901 and NCI-N87 cells pretreated with cetuximab followed by EGF (50 ng/ml) treatment. (B) RT-qPCR analyses of GATA2 and CGA expression in SGC7901<sup>ADR</sup> cells treated with the indicated compounds. (C) Immunoblotting of GATA2 and p-GATA2 (Ser192) in SGC7901<sup>ADR</sup> cells treated with the indicated compounds. (D) Viability of SGC7901<sup>ADR</sup> cells treated with PD98059 and/or...
SB202190 in the presence of chemotherapy. (E) RT-qPCR analyses of GATA2 and CGA expression in SGC7901 and NCI-N87 cells treated with low concentrations of adriamycin (0.5 μg/ml) for the indicated time periods. Data are presented as mean ± SEM. **p < 0.01 by one-way ANOVA followed by Dunnett’s multiple comparison in (B and D) or by Student’s t test in (E).
**Figure S9**

(A) IHC staining of CGA, p-EGFR and GATA2 in four PDXs treated with saline control or fluorouracil (scale bar, 50 μm). The CGA images are the same as shown in Figure 1E.

(B, C) Scatter plots showing correlated expression of CGA, GATA2 and EGFR mRNA in

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**Fig. S9.** Elevated CGA and GATA2 expression levels in GC patients who received chemotherapy, related to Fig 6.

(A) IHC staining of CGA, p-EGFR and GATA2 in four PDXs treated with saline control or fluorouracil (scale bar, 50 μm). The CGA images are the same as shown in Figure 1E.

(B, C) Scatter plots showing correlated expression of CGA, GATA2 and EGFR mRNA in
a GC (B) and a colorectal cancer (CRC, C) dataset of GEO. Pearson correlation coefficients $(r)$ and p values are shown.
Fig. S10. miR-708-3p and miR-761 sensitize chemoresistant GC cells by targeting CGA, related to Fig. 7.

(A) Predicted duplex sequences between the WT or MUT 3′-UTR of CGA and the indicated miRNAs. The red portions of the sequences represent the MUT miRNA binding
sites in the 3’-UTR of CGA. (B, C) Viability of SGC7901<sup>ADR</sup> (B) and SGC7901<sup>VCR</sup> (C) cells transfected with miR-708-3p or miR-761 at the indicated concentrations in the presence of chemotherapy. (D) Viability of CGA<sup>-/-</sup> SGC7901<sup>VCR</sup> cells transfected with the indicated CGA vectors in combination with miR-708-3p or miR-761 in the presence of chemotherapy. (E, F) Representative IHC staining of Ki-67 and cleaved Caspase-3 (E) and BIM and BAK (F) in tumors harvested from the indicated groups (scale bar, 50 μm). The percentages of Ki-67- and cleaved Caspase-3-positive cells and the levels of BIM and BAK protein expression were measured. (G) Records of mouse body weights after the indicated treatments. n=7 mice for the saline, fluorouracil and intratumorally injected miRNA prodrugs; 8 mice for fluorouracil in combination with miRNA prodrugs. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01 by one-way ANOVA followed by Dunnett’s multiple comparison in (B-D) or by Student’s t test in (E and F).
Table S1. Secreted proteins identified in the conditioned medium from SGC7901, SGC7901^VCR and SGC7901^ADR cells.

| Cell line | Protein name |
|-----------|--------------|
| SGC7901   | ANXA2, ANXA5, B2M, CALM1, CD59, CDA, CFD, CLIC1, CLU, CMBL, COL5A1, CST3, CTGF, CTSS, CYR61, ECH1, EIF6, FSTL3, GLO1, GLOD4, GSTO1, GSTP1, HBB, HIST1H4A, HLA-A, HNRNPA2B1, HRNR, HSPB1, HSPB2, IFI30, IGFBP1, IGFBP4, IGFBP6, IL18, KRT1, KRT2, KRT5, KRT9, LCN2, LDHA, LDHB, LGALS1, LGALS3, LGALS3BP, LMAN2, MDH1, MDH2, METRN, MFAP5, MIF, MSLN, NME2, PCNA, PFN1, PGAM1, PGLS, PI3, PNP, PPA1, PPIA, PPIB, PPIC, PRDX1, PRDX3, PRDX4, PRDX6, PSMA1, PSMA2, PSMA3, PSMA4, PSMA5, PSMA6, PSMA7, PSMB1, PSMB4, PSMB5, PSMB6, PSMB8, RPS27A, S100A4, SRM, TAGLN2, TIMP1, TIMP2, TP11, TPO4, TXN, TXNDC17, WISP2, YWHAB, YWHAE, YWHAG, YWHAZ, C19orf10, APOA1BP, GNB2L1, DCI |
| SGC7901^VCR | ABHD14B, ACP1, ACTG1, ADAM22, AK2, AMBP, ANXA1, ANXA2, ANXA5, ARHGDI, ARPC2, ARPC4, B2M, BLVRB, BOLA2, C1QBP, C3, CALM1, CAPZB, CBR1, CDA, CFD, CFL1, CGA, CLIC1, CLIC4, CLU, CMBL, CAMPK1, CNBP, CNPY2, COL5A1, COL1, CRIP1, CST3, CSTB, CTGF, CTSS, CTSZ, CTNN, CUTA, CYCS, CYR61, DAG1, DDT, DSTN, DYNL2, ECH1, EC51, EEF1D, EFEMP1, EIF6, ENOPI1, ERH, ERK2, FAM49B, FDX1, FHL2, FHL3, FKBPA, FSTL3, GAPDH, GC5H, GGCT, GLO1, GLO4, GNPA1, GRB2, GSTM1, GSTM3, GSTO1, GSTP1, HADH, HBB, HEBX, HIBADH, HINT1, HIST1H4A, HIST1H4G, HIST2H3A, HLA-A, HLA-B, HNRNPA2B1, HNRNPR, HNRNPAC, HPR1, HRNR, HSD17B10, HSPA5, HSPB1, HSPG2, IFI30, IGFBP1, IGFBP6, IGFBP7, IL6, ISOC1, KIAA0100, KRT1, KRT17, KRT18, KRT2, KRT5, KRT6B, KRT9, LASP1, LDHA, LDHB, LGALS1, LGALS3, LGALS3BP, LMAN2, LMNA, LMS3, LTF, MDH1, MDH2, MFAP2, MIF, MSLN, MYL12A, MYL6, NIT1, NIT2, NME2, NPC2, NQO1, NQPO2, NUDT5, OTUB1, PAFAH1B2, PARK7, PCBD1, PCNA, PDCD6, PEBP1, PFN1, PGAM1, PGK1, PGLS, PI3, PNP, PPA1, PPIA, PPIB, PPIF, PPIH, PPI4C, PRDX1, PRDX2, PRDX3, PRDX4, PRDX6, PROCR, PSMA1, PSMA2, PSMA3, PSMA4, PSMA5, PSMA6, PSMA7, PSMB1, PSMB2, PSMB3, PSMB4, PSMB5, PSMB6, PSMB7, PSMB8, PSMB9, PSMD9, PSME1, PSME2, PSPH, PTMA, RAB1B, RAN, RBP4, RPS12, RPS27A, RPS3, S100A4, S100A6, SFN, SLPI, SNRP, SNRPD1, SNRPD3, SOD1, SOD2, SRI, SRM, STC2, SUMF2, TAGLN2, TBCA, TGFB2, TIMP1, TIMP2, TKT, TMSB10, TPI1, TPO4, TPT1, TRIP6, TSN, TSNAX, TWSG1, TXN, TXNDC17, UBE2I, UBE2M, UBEV1, UCHL3, UFC1, VPS29, WISP2, YWHAB, YWHAE, YWHAG, YWHAO, YWHAZ, C19orf10, APOA1BP, GNB2L1, DCI, C1orf128, THOC4, TCEB1, TUBB2C, RCL |
| Gene Name | Gene Name | Gene Name | Gene Name | Gene Name | Gene Name |
|-----------|-----------|-----------|-----------|-----------|-----------|
| ABHD14B   | ACP1      | ACTG1     | ADAM22    | AHSG      | AK1       |
|            | AK2       | AKR1A1    | AKR1B1    | ALDOA     | ANP32A    |
|            | ANXA1     | ANXA2     | ANXA3     | ANXA5     | APEX1     |
|            | APOA1     | APRT      | ARCN1     | ARF1      | ARHDIA    |
|            | ARPC2     | ARPC3     | ARPC4     | ARPC5     | ATP6AP2    |
|            | B2M       | BLVRB     | BOLA2     | C1QTNF3   | C3        |
|            | C4B       | CALM1     | CAPZB     | CB1R1     | CBX1      |
|            | CD59      | CDKN2A    | CFD       | CFL1      | CFL2      |
|            | CGA       | CHAC2     | CLEC3B    | CLIC1     | CLU       |
|            | CML1      | CMPK1     | CNBP      | CNPY2     | COL1A1    |
|            | COL1A2    | COL3A1    | COL5A1    | COPS6     | COTL1     |
|            | COTX6B1   | CPPED1    | CREG1     | CRIP1     | CRYZ      |
|            | CSRP1     | CST3      | CSTB      | CTGF      | CTSD      |
|            | CTSI      | CUTA      | CYCS      | CYR61     | DAG1      |
|            | DAB1      | DCTPP1    | DDT       | DHX9      | DSTN      |
|            | DUT       | DYNLL2    | ECH1      | ECHS1     | EEF1D     |
|            | EFEMP1    | EIF3G     | EIF3J     | EIF6      | EN01      |
|            | ERH       | ERP29     | ES2D      | EXOSC2    | F5        |
|            | FABP3     | FBN1      | FDPS      | FH3L2     | FKBPIA    |
|            | FKL1      | FSTL3     | GAPDH     | GDF15     | GGCT      |
|            | GGH       | GLO1      | GLOD4     | GRB2      | GSTM1     |
|            | GSTM3     | GSTP1     | H2AFJ     | HADhb     | HBA1      |
|            | HBB       | HIST1H2B  | LID1H4A   | HLA-A     | HNRNPA1   |
|            | HNRNP2A1  | HNRNP2B1  | HNRNPC    | HNRPH1    | HNRNP3    |
|            | HPRH1     | HSD17B10  | HSD17B4   | HSP90AB1  | HSPB1     |
|            | HSPG1     | IAH1      | IFI30     | IGF2      | IGFBP1    |
|            | IGFBP2    | IGFBP4    | IGFBP6    | IGFBP7    | IL6       |
|            | ISG15     | KRT1      | KRT10     | KRT18     | KRT2      |
|            | KRT8      | KRT9      | LDHA      | LDHB      | LGALS1    |
|            | LGALS3    | LMAN2     | LMNA      | LSM3      | LTF       |
|            | MAGOH     | MAGOHB    | MD1H      | MDC2H     | METRN     |
|            | MIF       | MSLN      | MTAP      | MYL12A    | MYL6      |
|            | NEDD8     | NIT2      | NME1      | NME2      | NPM1      |
|            | NQO2      | NT5C      | NUTD21    | NUTD5     | NUTF2     |
|            | NUTF2     | NUTF2     | OAF       | OTUB1     | PAAH1B2   |
|            | PAR7K1    | PCBD1     | PCMT1     | PCNA      | PCSK9     |
|            | PDCD5     | PDXK      | PEBP1     | PFDN2     | PEN1      |
|            | PGAM1     | PGAM4     | PGLS      | PGP       | PI3       |
|            | PNP       | PNPO      | POLR2H    | PPA1      | PPA1      |
|            | PPP1A     | PPP1B     | PPP1CC    | PPP2CA    | PRDX1     |
|            | PRDX2     | PRDX3     | PRDX4     | PRDX6     | PROCR     |
|            | PSMA1     | PSMA2     | PSMA3     | PSMA4     | PSMA5     |
|            | PSMA6     | PSMA7     | PSMB1     | PSMB2     | PSMB3     |
|            | PSMB4     | PSMB5     | PSMB6     | PSMB7     | PSMB8     |
|            | PSMB9     | PSME1     | PSME2     | PSPH      | PXDN      |
|            | PXD10     | RAB11B    | RAB11B    | RAB1B     | RAB7A     |
|            | RAB8A     | RAC1      | RAN       | RBM8A     | RBP4      |
|            | RNASE4    | RPS11     | RPS12     | RPS18     | RPS20     |
|            | RPS21     | RPS27A    | RPS8      | RPS4X     | RPSA      |
|            | RPSA      | SI00A11   | SI00A4    | SI00A6    | SFN       |
|            | SLPI      | SNRPD1    | SNRPD3    | SOD1      | SOD2      |
|            | SOD2      | SPON2     | SPP2      | SRI       | SRM       |
|            | SRP9      | SSBP1     | STC2      | STMN1     | SUMO4     |
|            | TAGL1N2   | TBCA      | TGBF1     | TGBF2     | THBS1     |
|            | TIMP1     | TIMP2     | TNFRSF12A | TPI1      | TPM3      |
|            | TPM4      | TPT1      | TRIP6     | TRMT12    | TSN       |
|            | TUBA1C    | TWSG1     | TXN       | TXNDC17   | UBE21     |
|            | UBE2M     | UBE2V1    | UBE2V2    | UCHL3     | UFC1      |
|            | VPS29     | WISP2     | WYHAB     | WYHAE     | WYHAG     |
|            | WYHAZ     | ZBTB8OS   | C19orf10  | APOA1BP   | GNB2L1    |
|            | THOC4     | TCEB1     | RCL       | C14orf166 | Cxorf26   |
|            | SNRPDL1   | C11orf73  | PLEC1     | CTSL1     |
Table S2. Clinicopathological characteristics of GC patients with paired specimens before and after neoadjuvant chemotherapy.

| No. | Gender | Age | Histology       | Differentiation | TNM stage | Chemotherapy regimen | Response to chemotherapy |
|-----|--------|-----|-----------------|-----------------|-----------|----------------------|--------------------------|
| 1   | male   | 44  | adenocarcinoma  | moderate        | T2N0Mx    | FOLFOX               | SD                       |
| 2   | male   | 50  | adenocarcinoma  | moderate        | T3N0      | FOLFOX               | SD                       |
| 3   | female | 59  | adenocarcinoma  | poor            | T4aN3a    | FOLFOX               | SD                       |
| 4   | male   | 66  | adenocarcinoma  | moderate&poor   | pT3N3     | FOLFOX               | SD                       |
| 5   | male   | 66  | adenocarcinoma  | moderate        | pT3N1     | DCF                  | PR                       |
| 6   | male   | 65  | adenocarcinoma  | poor            | pT3N3M0   | FOLFOX               | PD                       |
| 7   | male   | 60  | adenocarcinoma  | moderate&poor   | pT4N2M0   | FOLFOX               | PD                       |
| 8   | male   | 44  | adenocarcinoma  | poor            | T4bN0     | FOLFOX               | SD                       |
| 9   | male   | 53  | adenocarcinoma  | moderate        | T4aN2     | ECF                  | SD                       |
| 10  | male   | 66  | adenocarcinoma  | well&moderate   | T3N1      | FOLFOX               | SD                       |
| 11  | male   | 68  | adenocarcinoma  | poor            | T4aN3b    | DCF                  | SD                       |
| 12  | male   | 58  | adenocarcinoma  | poor            | T3N1      | ECF                  | SD                       |
| 13  | male   | 57  | adenocarcinoma  | moderate        | rT3N3b    | FOLFOX               | SD                       |
| 14  | male   | 67  | adenocarcinoma  | poor            | T3N3a     | FOLFOX               | SD                       |
| 15  | male   | 56  | adenocarcinoma  | poor            | T3N3a     | XELOX                | SD                       |
| 16  | male   | 48  | adenocarcinoma  | moderate        | T3N0      | FOLFOX               | SD                       |
| 17  | female | 62  | adenocarcinoma  | moderate        | T4aN3a    | DCF                  | SD                       |
| 18  | male   | 58  | adenocarcinoma  | poor            | T4aN1     | FOLFOX               | SD                       |
| 19  | female | 50  | adenocarcinoma  | poor            | T4aN3a    | SOX                  | SD                       |
| 20  | male   | 69  | adenocarcinoma  | poor            | TXN0      | EOX                  | PR                       |
| 21  | female | 57  | adenocarcinoma  | well&moderate   | T3N3a     | FOLFOX               | SD                       |
| 22  | male   | 67  | adenocarcinoma  | poor            | T4aN3a    | DCF                  | PR                       |
| 23  | male   | 51  | adenocarcinoma  | poor            | T3N2      | DCF                  | SD                       |
| 24  | female | 58  | adenocarcinoma  | poor            | T3N0      | SOX                  | SD                       |
| 25 | male | 36 | adenocarcinoma moderate&poor | T3N0 | DCF | PR |
| 26 | male | 35 | adenocarcinoma moderate&poor | T4bN2 | DCF | SD |
| 27 | male | 63 | adenocarcinoma moderate | T4bN2 | SOX | PD |
| 28 | male | 52 | adenocarcinoma moderate&poor | T4aN2 | SOX | SD |
| 29 | male | 55 | adenocarcinoma moderate&poor | T4aN2 | SOX | SD |
| 30 | female | 55 | adenocarcinoma moderate&poor | T1bN3a | DOX | PR |
| 31 | male | 54 | adenocarcinoma poor | T4N1 | DCF | SD |
| 32 | male | 59 | adenocarcinoma well&moderate | T3N0 | DCF | PR |
| 33 | female | 48 | adenocarcinoma moderate | pT4N3M0 | SOX | PD |
| 34 | male | 80 | adenocarcinoma poor | pT0N1M0 | SOX | PD |
| 35 | male | 47 | adenocarcinoma moderate&poor | pT2N1M0 | FOLFIRI | PD |
| 36 | female | 47 | adenocarcinoma moderate&poor | pT1bN1M0 | FOLT | PD |
| 37 | female | 55 | adenocarcinoma poor | pT4N1M1 | FOLT | SD |

1. Patients 1, 2, 3, 4, 5 and 6 in Fig. 1D and Fig. 6A denote No. 12, 13, 29, 33, 35 and 6 in this table, respectively. Patients 1, 2, 3, 4, 5 and 6 in Fig. S1D denote No. 5, 20, 22, 25, 30 and 32 in this table, respectively.

2. Chemotherapy regimens: FOLFOX (fluorouracil, leucovorin and oxaliplatin); EOX (epirubicin, oxaliplatin and capecitabine); DCF (docetaxel, cis-platinum and fluorouracil); ECF (epirubicin, cis-platinum and fluorouracil); XELOX (capecitabine and oxaliplatin); SOX (S-1 and oxaliplatin); FOLFIRI (fluorouracil, leucovorin and irinotecan); FOLT (fluorouracil, leucovorin, oxaliplatin and docetaxel).

3. PR, partial response; SD, stable disease; PD, progressive disease.
**Table S3.** Clinical information of GC tissues used to generate the PDX model.

| PDX No. | Gender | Age | Histology       | Differentiation | AJCC stage | PDX passage |
|---------|--------|-----|-----------------|-----------------|------------|-------------|
| 1       | male   | 47  | adenocarcinoma  | poor&moderate   | 2          | P2          |
| 2       | male   | 68  | adenocarcinoma  | poor            | 4          | P2          |
| 3       | male   | 66  | adenocarcinoma  | moderate        | 4          | P3          |
| 4       | female | 55  | adenocarcinoma  | poor            | 4          | P4          |
Table S4. Docking analysis of CGA and the ECD of EGFR by using the ClusPro server.

| Residues in CGA       | Residues in the ECD of EGFR |
|-----------------------|-----------------------------|
| K44.HZ3               | Y88.OH                      |
| R42.HH12              | N86.OD1                     |
| R42.HH22              | N86.OD1                     |
| Y37.OH                | S262.H                      |
| Y37.HH                | G281.O                      |
| Y37.H                 | C240.O                      |
| T54.O                 | T239.HG1                    |
| S55.HG                | T239.OG1                    |
| S55.OG                | T239.H                      |
| K91.HZ1               | D238.OD1                    |
| Y89.HH                | Q193.O                      |
| D6.OD2                | W584.H                      |
| Q5.OE1                | E578.H                      |
| S85.HG                | E221.OE2                    |
| M29.O                 | N599.HD22                   |
| Q13.HE22              | N599.OD1                    |
| S34.H                 | N580.OD1                    |
| D3.OD1                | T249.H                      |
| D3.OD1                | T249.HG1                    |
**Table S5.** The expression levels of eight transcription factors in SGC7901, SGC7901$^{ADR}$ and SGC7901$^{VCR}$ cells.

| Gene  | Log2 Ratio (SGC7901$^{ADR}$/SGC7901) | $P$ value | FDR  | Log2 Ratio (SGC7901$^{VCR}$/SGC7901) | $P$ value | FDR  |
|-------|-------------------------------------|-----------|------|-------------------------------------|-----------|------|
| CEBPA | 2.921111225                         | 0.000000  | 0.000000 | 2.710206444                        | 0.000000  | 0.000000 |
| EGR1  | 3.049297067                         | 0.000000  | 0.000000 | 3.257048388                        | 0.000000  | 0.000000 |
| FOXD1 | 1.490615045                         | 0.000000  | 0.000000 | 1.753723252                        | 0.000000  | 0.000000 |
| GATA2 | 1.690832402                         | 0.000000  | 0.000000 | 1.64289388                        | 0.000000  | 0.000000 |
| GATA3 | 6.033496871                         | 0.000031  | 0.000053 | 10.34487385                       | 0.000000  | 0.000000 |
| JUN   | 1.322488813                         | 0.000000  | 0.000000 | 1.496223127                       | 0.000000  | 0.000000 |
| NFIL3 | 1.639760573                         | 0.000000  | 0.000000 | 1.675893783                       | 0.000000  | 0.000000 |
| RUNX2 | 7.061719798                         | 0.000000  | 0.000000 | 7.508497903                       | 0.000000  | 0.000000 |

Data came from Chen. et al, *Cancer Lett*, 2014.
| No. | Gender | Age | Histology       | TNM stage     |
|-----|--------|-----|-----------------|---------------|
| 1   | male   | 63  | adenocarcinoma  | T4aN0M0       |
| 2   | female | 64  | adenocarcinoma  | cT4aN3aM0     |
| 3   | male   | 43  | adenocarcinoma  | T3N3aM0       |
| 4   | male   | 66  | adenocarcinoma  | cT4aN0M0      |
| 5   | male   | 49  | adenocarcinoma  | cT4aN3aM0     |
| 6   | male   | 52  | adenocarcinoma  | cT4aN3aM0     |
| 7   | female | 43  | adenocarcinoma  | cT4aN3aM0     |
| 8   | male   | 43  | adenocarcinoma  | T3N3aM0       |
| 9   | male   | 82  | adenocarcinoma  | cT4aN3aM0     |
| 10  | male   | 62  | adenocarcinoma  | T3N1M0        |
| 11  | male   | 62  | adenocarcinoma  | T4aN3M0       |
| 12  | male   | 72  | adenocarcinoma  | T3N3aM0       |
| 13  | male   | 65  | adenocarcinoma  | T4aN3bM0      |
| 14  | male   | 58  | adenocarcinoma  | T3N2M0        |
| 15  | male   | 58  | adenocarcinoma  | T4aN1M0       |
| 16  | male   | 59  | adenocarcinoma  | T4aN3a        |
| 17  | male   | 77  | adenocarcinoma  | T4aN3bM0      |
| 18  | male   | 62  | adenocarcinoma  | T4aN2M0       |
| 19  | male   | 74  | adenocarcinoma  | T3N3M0        |
| 20  | male   | 66  | adenocarcinoma  | T4aNxM0       |
| 21  | male   | 72  | adenocarcinoma  | T4aN3b        |
| 22  | male   | 63  | adenocarcinoma  | T4bN2M1       |
| 23  | male   | 59  | adenocarcinoma  | T4aN3aM0      |
| 24  | male   | 52  | adenocarcinoma  | T4aN3a        |
| 25  | male   | 71  | adenocarcinoma  | T3N1          |
| 26  | male   | 64  | adenocarcinoma  | T3N3M0        |
| 27  | male   | 72  | adenocarcinoma  | T4aN1         |
| 28  | male   | 50  | adenocarcinoma  | T3N2M0        |
| 29  | male   | 50  | adenocarcinoma  | T3N3aM0       |
| 30  | male   | 58  | adenocarcinoma  | T3N3aM0       |
| 31  | male   | 70  | adenocarcinoma  | T3N3b         |
| 32  | male   | 52  | adenocarcinoma  | T2Nx          |
| 33  | female | 59  | adenocarcinoma  | T2Nx          |
| 34  | male   | 49  | adenocarcinoma  | T1bNx         |
| 35  | male   | 66  | adenocarcinoma  | T2Nx          |
| 36  | female | 55  | adenocarcinoma  | T2a1N0        |
| 37  | female | 70  | adenocarcinoma  | T2N           |
| 38  | male   | 50  | adenocarcinoma  | T3N0M0        |
|   |   |   |   |   |
|---|---|---|---|---|
| 39 | female | 47 | adenocarcinoma | T1bN1M0 |
| 40 | female | 53 | adenocarcinoma | T1Nx |
| 41 | male | 68 | adenocarcinoma | T2N0M0 |
| 42 | female | 56 | adenocarcinoma | T2Nx |
Table S7. Clinicopathological characteristics of GC patients who provided plasma samples and received neoadjuvant chemotherapy.

| No. | Gender | Age | Differentiation | TNM stage | Chemotherapy regimen | Response to chemotherapy | Survival time (month) | Status |
|-----|--------|-----|-----------------|-----------|----------------------|--------------------------|----------------------|--------|
| 1   | male   | 50  | moderate        | T3N0      | FOLFOX               | SD                       | 55                   | dead   |
| 2   | male   | 69  | poor            | T3N1      | FOLFOX               | SD                       | 23                   | dead   |
| 3   | female | 46  | poor            | T4aN3a    | FOLFOX               | SD                       | 7                    | dead   |
| 4   | male   | 66  | moderate        | T3N1      | FOLFOX               | PR                       | 84                   | censored |
| 5   | male   | 58  | moderate        | T3N1      | ECF                  | SD                       | 2                    | dead   |
| 6   | male   | 61  | poor            | T4aN3a    | FOLFOX               | PR                       | 17                   | dead   |
| 7   | male   | 57  | moderate        | rT3N3b    | FOLFOX               | SD                       | 8                    | censored |
| 8   | male   | 67  | poor            | T3N3a     | FOLFOX               | SD                       | 5                    | dead   |
| 9   | male   | 65  | poor            | T4aN1     | ECF                  | PR                       | 50                   | censored |
| 10  | male   | 40  | moderate        | T1bN2     | SOX                  | PR                       | 72                   | censored |
| 11  | male   | 38  | poor            | T4aN1     | SOX                  | SD                       | 4                    | dead   |
| 12  | female | 62  | moderate        | T4aN3a    | DCF                  | SD                       | 24                   | dead   |
| 13  | female | 58  | poor            | T4aN1     | FOLFOX               | SD                       | 20                   | dead   |
| 14  | female | 50  | poor            | T4aN3a    | SOX                  | SD                       | 7                    | dead   |
| 15  | male   | 56  | moderate        | T1aN0     | DS                   | -                        | 39                   | censored |
| 16  | female | 57  | moderate        | T3N3a     | FOLFOX               | SD                       | 17                   | dead   |
| 17  | male   | 47  | moderate        | T4aN0     | DCF                  | SD                       | 38                   | censored |
| 18  | male   | 67  | poor            | T4aN3a    | DCF                  | SD                       | 66                   | censored |
| 19  | male   | 51  | poor            | T3N2      | DCF                  | PR                       | 62                   | censored |
| 20  | male   | 48  | moderate        | T2N1      | DCF                  | SD                       | 59                   | censored |
| 21  | male   | 67  | poor            | T3N1      | FOLFOX               | PR                       | 57                   | censored |
| 22  | female | 46  | poor            | T3N0      | DCF                  | SD                       | 40                   | censored |
| 23  | female | 43  | poor            | T4bN3a    | DCF                  | SD                       | 28                   | censored |
| No. | Gender | Age  | Performance | Tumor Stage | Chemotherapy | Response | Status |
|-----|--------|------|-------------|-------------|--------------|----------|--------|
| 24  | male   | 44   | poor        | T3N0        | SOX          | SD       | 17     | dead   |
| 25  | female | 58   | poor        | T3N0        | SOX          | SD       | 52     | censored|
| 26  | male   | 35   | moderate&poor| T4bN2       | DCF          | SD       | 4      | dead   |
| 27  | male   | 56   | poor        | T4aN2       | DF           | SD       | 25     | censored|
| 28  | male   | 63   | moderate    | T3N3a       | SOX          | SD       | 8      | dead   |
| 29  | male   | 52   | moderate&poor| T4aN2       | SOX          | SD       | 50     | censored|
| 30  | female | 63   | poor        | T3N2        | DOX          | SD       | 18     | censored|
| 31  | male   | 62   | moderate    | T3N1        | DCF          | SD       | 10     | censored|
| 32  | male   | 55   | moderate&poor| T4aN2       | SOX          | SD       | 51     | censored|
| 33  | male   | 65   | poor        | T4bN3       | DCF          | SD       | 1      | dead   |
| 34  | male   | 68   | moderate    | T4aN3a      | DCF          | SD       | 10     | censored|
| 35  | male   | 39   | moderate&poor| T4aN3b      | DCF          | SD       | 13     | censored|
| 36  | female | 58   | poor        | T4aN3b      | DCF          | SD       | 5      | dead   |
| 37  | female | 55   | moderate&poor| T1bN3a      | DOX          | PR       | 26     | censored|
| 38  | male   | 59   | moderate    | T3N0        | DCF          | SD       | 50     | censored|
| 39  | male   | 66   | moderate&poor| T4aN0       | XELOX        | PR       | 48     | censored|
| 40  | male   | 57   | well&moderate| T3N1        | S-1          | PR       | 51     | censored|
| 41  | female | 29   | poor        | T4N0        | -            | SD       | 13     | censored|

1. The pre- and post-operative plasma CGA levels of No. 6, 8, 10, 13, 14, 15, 17, 22, 24, 27, 28, 36, 39, 40 and 41 are shown in Fig. 6D.
2. Chemotherapy regimens: DS (docetaxel and S-1); DF (cis-platinum and fluorouracil); DOX (docetaxel, oxaliplatin and capecitabine). Patient 41 was transferred from a nonlocal hospital and her chemotherapy treatment history is not available.
3. PR, partial response; SD, stable disease. The information of response to chemotherapy is not available for No. 15.
Table S8. Clinicopathological characteristics of GC patients who provided plasma samples and received palliative chemotherapy.

| No. | Gender | Age | AJCC stage | Chemotherapy regimen$^1$ | Response to chemotherapy$^2$ | Survival time (month)$^3$ | Status   |
|-----|--------|-----|------------|--------------------------|----------------------------|--------------------------|----------|
| 1   | female | 19  | 4          | DCF                      | SD                         | 2.4                      | censored |
| 2   | male   | 67  | 4          | SOX                      | PD                         | 11                       | dead     |
| 3   | male   | 66  | 4          | FLOT                     | SD                         | -                        | -        |
| 4   | male   | 75  | 4          | SOX                      | SD                         | 14.6                     | dead     |
| 5   | male   | 56  | 4          | FLOT                     | SD                         | 16.6                     | dead     |
| 6   | male   | 57  | 4          | DCF                      | PD                         | -                        | -        |
| 7   | male   | 61  | 4          | SOX                      | PD                         | 11.5                     | censored |
| 8   | female | 66  | 4          | SP                       | PD                         | -                        | -        |
| 9   | female | 35  | 4          | DCF                      | PD                         | -                        | -        |
| 10  | male   | 75  | 4          | SOX                      | PD                         | 21.1                     | dead     |
| 11  | male   | 54  | 4          | SOX                      | PD                         | 21.8                     | dead     |
| 12  | male   | 70  | 4          | DCF                      | SD                         | 7.5                      | dead     |
| 13  | male   | 50  | 4          | DCF                      | SD                         | -                        | -        |
| 14  | male   | 66  | 4          | SOX                      | PD                         | 5.4                      | censored |
| 15  | male   | 56  | 4          | DCF                      | SD                         | 7.9                      | dead     |
| 16  | male   | 55  | 4          | DCF                      | PD                         | -                        | -        |
| 17  | male   | 52  | 4          | DCF                      | SD                         | -                        | -        |
| 18  | male   | 77  | 4          | SOX                      | SD                         | -                        | -        |
| 19  | male   | 54  | 4          | DCF                      | PD                         | 6                        | dead     |
| 20  | male   | 53  | 4          | XELOX                    | PD                         | -                        | -        |
| 21  | male   | 66  | 4          | SOX                      | SD                         | 5                        | dead     |
| 22  | male   | 80  | 4          | SOX                      | PD                         | 6                        | dead     |
| 23  | male   | 65  | 4          | DCF                      | PD                         | -                        | -        |
| 24 | male | 76 | 4 | SOX | PD | - | - |
| 25 | male | 59 | 4 | DCF | PD | - | - |
| 26 | male | 62 | 4 | XELOX | SD | 53.8 | censored |
| 27 | male | 75 | 4 | FOLFOX | SD | - | - |
| 28 | male | 76 | 4 | XELOX | SD | - | - |
| 29 | female | 51 | 4 | XELIRI | PD | - | - |
| 30 | male | 70 | 4 | DS | SD | - | - |
| 31 | female | 51 | 4 | DCF | PD | - | - |
| 32 | female | 47 | 4 | DF | SD | 15.4 | censored |
| 33 | male | 56 | 4 | Fluorouracil | SD | - | - |
| 34 | male | 57 | 4 | DCF | SD | - | - |
| 35 | male | 62 | 4 | FOLFIRI | SD | - | - |
| 36 | male | 65 | 4 | XELOX | SD | - | - |
| 37 | male | 75 | 4 | XELOX | SD | - | - |
| 38 | male | 75 | 3 | DCF | PD | - | - |
| 39 | male | 40 | 4 | DCF | PD | - | - |
| 40 | male | 56 | 4 | DCF | PD | - | - |
| 41 | male | 41 | 4 | DCF | PD | 3.3 | dead |
| 42 | female | 59 | 4 | DCF | PD | - | - |
| 43 | male | 60 | 4 | EOX | SD | 5.9 | dead |
| 44 | male | 30 | 4 | DCF | SD | - | - |
| 45 | female | 31 | 4 | DOX | SD | 5.7 | censored |
| 46 | male | 35 | 4 | DCF | PD | 0.2 | dead |
| 47 | male | 60 | 4 | XELOX | PD | 16.3 | dead |
| 48 | female | 64 | 4 | FLOT | SD | - | - |
| 49 | male | 59 | 4 | EOX | SD | 11.3 | dead |
| 50 | male | 75 | 4 | SOX | SD | 7.3 | dead |
|    | Gender | Age | Cycle | Regimen | Status | Follow-up |
|----|--------|-----|-------|---------|--------|-----------|
| 51 | male   | 51  | 3     | EOX     | PD     | -         |
| 52 | female | 37  | 4     | DCF     | PD     | -         |
| 53 | male   | 61  | 4     | DCF     | PD     | 3.1       |
| 54 | female | 55  | 4     | FOLFOX  | PD     | -         |
| 55 | male   | 61  | 4     | DCF     | PD     | -         |
| 56 | male   | 71  | 4     | FOLFOX  | PD     | -         |

1. Chemotherapy regimens: SP (S-1 and cis-platinum); XELIRI (capecitabine and irinotecan); EOX (epirubicin, oxaliplatin and capecitabine).
2. SD, stable disease; PD, progressive disease.
3. Twenty-three patients received survival follow-up.
Table S9. miRNAs targeting CGA predicted by the overlap between miRWalk (version 2.0) and TargetScan (version 7.2).

| miRNA           | Gene symbol | Position in 3'-UTR of CGA | Context++ score |
|-----------------|-------------|----------------------------|-----------------|
| hsa-miR-7843-3p | CGA         | 121-127                    | 84              |
| hsa-miR-7706    | CGA         | 120-126                    | 89              |
| hsa-miR-766-5p  | CGA         | 151-157                    | 96              |
| hsa-miR-761     | CGA         | 28-34                      | 94              |
| hsa-miR-708-3p  | CGA         | 181-187                    | 97              |
| hsa-miR-6891-5p | CGA         | 153-160                    | 99              |
| hsa-miR-6876-5p | CGA         | 149-155                    | 97              |
| hsa-miR-6516-5p | CGA         | 226-233                    | 99              |
| hsa-miR-651-3p  | CGA         | 96-103                     | 99              |
| hsa-miR-630     | CGA         | 203-209                    | 89              |
| hsa-miR-6124    | CGA         | 94-100                     | 98              |
| hsa-miR-525-5p  | CGA         | 38-44                      | 94              |
| hsa-miR-5192    | CGA         | 88-94                      | 99              |
| hsa-miR-501-5p  | CGA         | 126-132                    | 86              |
| hsa-miR-4999-5p | CGA         | 205-212                    | 99              |
| hsa-miR-4742-3p | CGA         | 204-210                    | 90              |
| hsa-miR-449c-5p | CGA         | 145-151                    | 99              |
| hsa-miR-4499    | CGA         | 178-184                    | 98              |
| hsa-miR-4476    | CGA         | 149-155                    | 96              |
| hsa-miR-4463    | CGA         | 178-184                    | 96              |
| hsa-miR-4428    | CGA         | 90-96                      | 98              |
| hsa-miR-3714    | CGA         | 146-152                    | 98              |
| hsa-miR-362-5p  | CGA         | 125-132                    | 99              |
| hsa-miR-3619-5p | CGA         | 28-34                      | 92              |
| hsa-miR-34b-5p  | CGA         | 145-151                    | 99              |
| hsa-miR-342-3p  | CGA         | 77-83                      | 94              |
| hsa-miR-320c    | CGA         | 138-145                    | 99              |
| hsa-miR-3180-5p | CGA         | 38-44                      | 97              |
| hsa-miR-3173-3p | CGA         | 153-160                    | 99              |
| hsa-miR-2682-5p | CGA         | 145-151                    | 99              |
| hsa-miR-214-3p  | CGA         | 28-34                      | 97              |
| hsa-miR-17-3p   | CGA         | 134-140                    | 98              |
| hsa-miR-1298-5p | CGA         | 199-205                    | 97              |
| hsa-miR-1285-5p | CGA         | 78-85                      | 99              |
| hsa-miR-127-5p  | CGA         | 121-127                    | 88              |
| hsa-miR-1183    | CGA         | 163-170                    | 99              |
### Table S10. Antibodies used in this study.

| Antibodies                                      | Source                     | Catalog #   |
|------------------------------------------------|----------------------------|-------------|
| Rabbit anti-human CGA                          | Abcam                      | ab92738     |
| Mouse anti-human α-Tubulin                     | Sigma-Aldrich              | T5168       |
| Rabbit anti-human EGFR (for IB)                | Abcam                      | 06-847      |
| Rabbit anti-human EGFR (for IP)                | Proteintech                | 51071-2-AP  |
| Mouse anti-human EGFR (for IF)                 | Abcam                      | ab30        |
| Rabbit anti-human p-EGFR (Tyr1068)             | Cell Signaling Technology  | 3777        |
| Rabbit anti-human ERK                          | Cell Signaling Technology  | 4695        |
| Rabbit anti-human p-ERK (Thr202/Tyr204)        | Cell Signaling Technology  | 4370        |
| Rabbit anti-human AKT                          | Cell Signaling Technology  | 4691        |
| Rabbit anti-human p-AKT (Ser473)               | Cell Signaling Technology  | 4060        |
| Rabbit anti-human β-actin                      | Cell Signaling Technology  | 4970        |
| Rabbit anti-Flag                               | Sigma-Aldrich              | F7425       |
| Rabbit anti-human Ki-67                        | Abcam                      | ab15580     |
| Rabbit anti-human cleaved Caspase-3            | Abcam                      | ab2302      |
| Rabbit anti-human CGB                         | Proteintech                | 11615-1-AP  |
| Mouse anti-human FSHB                         | RD Systems                 | MAB4310     |
| Sheep anti-human LHB                          | RD Systems                 | AF8016      |
| Mouse anti-human TSHB                         | RD Systems                 | MAB57941    |
| Rabbit anti-human GATA2                       | Cell Signaling Technology  | 4595        |
| anti-human p-GATA2 (Ser192)                    | Thermo Fisher Scientific   | PA5-105538  |
| anti-human p-p38 (Thr180/Tyr182)               | Cell Signaling Technology  | 4511        |
| anti-human p-JNK (Thr183/Tyr185)               | Cell Signaling Technology  | 4668        |
| Donkey polyclonal anti-Rabbit IgG, HRP conjugated | GE Healthcare Life Sciences | NA934      |
| Sheep polyclonal anti-Mouse IgG, HRP conjugated | GE Healthcare Life Sciences | NA931      |
| Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed, Alexa Fluor 488 conjugated | Thermo Fisher Scientific | A32766     |
| Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed, Alexa Fluor Plus 594 conjugated | Thermo Fisher Scientific | A32754     |
| Rabbit IgG                                     | Proteintech                | B900610     |
| Antibody Description         | Vendor                        | Catalog Number |
|-----------------------------|-------------------------------|----------------|
| Rabbit anti-human BIM       | Cell Signaling Technology    | 2933           |
| Rabbit anti-human BAK       | Cell Signaling Technology    | 12105          |
| Mouse anti-human EEA1       | Abcam                        | ab75852        |
| Mouse anti-human CathD      | Santa Cruz Biotechnology     | sc377299       |
| Donkey anti-Mouse IgG (H+L), Alexa Fluor 405 conjugated | Abcam | Ab175658 |
| Rabbit anti-human EGFR, Alexa Fluor 488 conjugated | Cell Signaling Technology | 5616 |
Table S11. Oligonucleotides used in this study.

| Oligonucleotide                          | Sequence                                      |
|------------------------------------------|-----------------------------------------------|
| qPCR primers for CGA                     | F: GCCCTGAACACATCCTGCAA                       |
|                                          | R: GCCCTGAACACATCCTGCAA                      |
| qPCR primers for GATA2                   | F: CAAGGCTCGTTCCTGTTTCAG                     |
|                                          | R: TGCCCATTCATCTTGTGGTAG                      |
| qPCR primers for GAPDH                   | F: GCACCCTCAAGGCTGAGAC                      |
|                                          | R: TGGTGAAGACGCCAGTGGG                      |
| qPCR primers for U6                      | F: TGGAAACGCTTCCAGAATTTGCG                   |
|                                          | R: GGAACGATACAGAGAAGATTAGC                   |
| qPCR primers for GBE1 of CGA promoter    | F: CAACGTTAAAAGGGCTGTACCT                    |
|                                          | R: AGCCATAGCCAGCAAGCTGG                     |
| qPCR primers for GBE2 of CGA promoter    | F: ATGCATACTGGAAGGAACAC                     |
|                                          | R: TTTGCAACACAGAGATTAGC                     |
| qPCR primers for distal region of CGA promoter | F: CCAGCTCTGGGAAGAAACTCTC                  |
|                                          | R: TTATGAATGAGCCATTAGCC                     |
| qPCR primers for −77 kb of GATA2 locus   | F: TGTCTACTCTCAACAAAGATG                    |
|                                          | R: CTTTAATGGCCACAGATAG                       |
| qPCR primers for −3.9 kb of GATA2 locus  | F: TTGACCTGCTGGAGATGAG                      |
|                                          | R: CCCATGGCGACGCAGATG                       |
| qPCR primers for −3.0 kb of GATA2 locus  | F: GCAGAGATAAGGAACACCATT                    |
|                                          | R: GAAGATAATGAGCCAGATCG                     |
| qPCR primers for −1.8 kb of GATA2 locus  | F: AGAGCAGTTATCCAGCATG                      |
|                                          | R: ACCATCCAGACCTTCTTACAAC                   |
| qPCR primers for +9.5 kb of GATA2 locus  | F: TGTTTTCTCTCACAGGCATCT                    |
|                                          | R: GACAAGCCCTTGCCTTATG                      |
| qPCR primers for +14.5 kb of GATA2 locus | F: AGTGACAAAAATCCAGCCAC                    |
|                                          | R: CATGCAAGCTTATCAAAAAAGCA                   |
| Biotin-labeled probe for GBE1 of CGA promoter | F: AATCATTCAGATAAAAAAGAAAATAAA              |
|                                          | R: TTTATTTCTTTTTTATCTGAAATGATT              |
| Competitor probe for GBE1 of CGA promoter | F: AATCATTCAGATAAAAAAGAAAATAAA              |
|                                          | R: TTTATTTCTTTTTTATCTGAAATGATT              |
| Mutant probe for GBE1 of CGA promoter    | F: AATCATGAGAGCGGGGGAGAATAAA                |
|                                          | R: TTTATTTCTTTTTTTATCTGAAATGATT             |
| Biotin-labeled probe for GBE2 of CGA     | F: AATCATTCAGATAAAAAAGAAAATAAA              |
| promoter                                | R: TTTATTTCTTTTTTATCTGAAATGATT              |
|                                          | R: ATTTTTCTGTGATAAAAAACAAATTTGTGT          |
| Competitor probe for GBE2 of CGA promoter | F: AAACAAATTTGTTTATCACAGAAAAT  
|                                             | R: ATTTTCTGTGATAAACAATTTTGT    |
| Mutant probe for GBE2 of CGA promoter       | F: AAACAAAGCCCAACCGCTGGGAAAAT  
|                                             | R: ATTTTCCACAGCGGTTGGGCTTTTGT  |
| scrambled siRNA control                     | UUCUCCGAACGUGACGUTT            |
| siRNA#1 targeting CGA                       | GCAAGCUAUCUUUCUGUCACAC        |
| siRNA#2 targeting CGA                       | GAUUACAGAUUGCCAGAAUG           |
| siRNA#1 targeting CGB                       | ACCGUCAACACCACCAUCUTT         |
| siRNA#2 targeting CGB                       | ACAUGUCUCUCUUAGCGTT           |
| siRNA#1 targeting LHB                       | ACCGUCAACACCACCAUCUTT         |
| siRNA#2 targeting LHB                       | ACAUGUCUCUCUUAGCGTT           |
| siRNA#1 targeting FSHB                      | GAAAGCAUAUCUGUGCAATT          |
| siRNA#2 targeting FSHB                      | GAAUGUCGUUUCUGCAUAATT         |
| siRNA#1 targeting TSHB                      | GCUUAUUGCCUAACCAUCATT         |
| siRNA#2 targeting TSHB                      | CCUGUUGCUUUAAGCUGUATT         |
| siRNA#1 targeting GATA2                     | ACUACAGCAGCGGACUCUUTT         |
| siRNA#2 targeting GATA2                     | CCUGUGGCCUCUACCACAAATT        |