Supplementary material and methods

Cell culture and transfection

SGC7901 cells were cultured in RPMI-1640 medium (Gibco, 11875093) supplemented with 10% fetal calf serum (Gibco, 10099141), and HFF-1 were cultured in DMEM medium (Gibco, 11965092) supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere containing 5% CO_2. For details of confocal microscopy and morphological analysis, see Supplementary Materials and Methods.

Cells were allowed to grow overnight in 6-well plates (5×10^5 cells per well). On the following day, the small interfering RNA (siRNA) against human FOXO1 or HIF-1α and control scrambled siRNA (GenePharma) were individually transfect into cells using Lipofectamine 2000 reagent (Invitrogen, 11668019) according to the manufacturer's protocol and then cultured at 37°C in a humidified atmosphere containing 5% CO_2 for 24-48 h. The siRNA sequences were as follows:

FOXO1 sense: 5'-CCAGGCAUCUCAUAACAAATT-3' and antisense: 5'-UUUGUUAUGAGAUGCCUGGCT-3'; HIF-1α sense: 5'-CCAGUUAUGAUUGUGAAGUUATT-3' and antisense: 5'-UAACUUCACAAUCAUAACUGGTT-3'.

RNA isolation and real-time reverse transcription-polymerase chain reaction

For total RNA of exosomes extraction, RNA was isolated using the Total RNA Purification Kit (Norgen, 17200) according to the manufacturer's instructions from 200 μL of frozen exosome in PBS. The volume of the obtained RNA solution was 60 μL.

Total RNA for quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was extracted from cell lines using Trizol reagent (Invitrogen, 15596026) as specified by the manufacturer's instructions for FOXO1 and HIF-1α messenger RNA (mRNA) expression quantification. cDNA was prepared from 500 ng of total RNA using the Primescript RT Master Kit (Takara RR036A) according to the manufacturer's instructions. A PCR bulk reaction mixture was prepared as described in the SYBR Premix Ex Taq™ IIKit, and the thermal cycling parameters included initial denaturation at 95°C (30 s), followed by 40 cycles at 95°C (5 s) and 60°C (34 s). qRT-PCR was performed in triplicate and was repeated in at least three separate experiments using ABI Prism 7500 (Applied Biosystems). Data analysis was using the $2^{-\Delta\Delta CT}$ method with β-actin serving as the comparator. The results were presented as the fold-change relative to control. The primer sequences for FOXO1 and β-actinwere, respetively:

5'-TGGACATGCTCAGCAGACATC-3' and 5'-TTGGGTCAGGCGGTTCA-3', 5'-ACACCTTCTACAATGAGCTG-3' and 5'-CATGATGGAGTTGAAGGTAG-3'.

Hypoxia induced by $CoCl_2$ solution

25-mM $CoCl_2$ (Amresco, J297) stock solution was prepared in sterile double-distilled water. $CoCl_2$ was used at the final concentration of 12.5 μM-200 μM in culture media to induce hypoxia. $CoCl_2$ containing media was added to cells, and the cultures were incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO_2.

Western blot analysis

Proteins (25 μg/lane) were resolved by 10%-15% SDS-PAGE and electro transferred to a PVDF membrane using standard procedures. After blocking with 5% BSA in TBS-T buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween-20) for 1 h, the blots were probed with primary antibodies at 4°C overnight, reacted with a peroxidase-conjugated secondary antibody for 1 hat room temperature, followed by detection of the proteins with ECL reagents (Pierce). And quantification of protein levels was performed using image J software. The primary antibodies were as follows: Rabbit-anti-FOXO1, 1:1000 (Cell Signaling Technology, 2880), Rabbit-anti-α-SMA, 1:1000 (Abcom, ab5694), Mouse-anti-HIF-1α, 1:1000 (Abcom, ab113642), Mouse-anti-β-actin (Santa Cruz, 1:1000). The secondary antibodies were as follows: goat-anti-mouse IgG and goat-anti-rabbit, 1:500 (Santa Cruz).

Cell survival assay

Cells were seeded in 96-well plates and treated with different treatments. Next, 10 μl of CCK-8 (Dojindo, CK04) was added to each well, and the samples were incubated at 37°C for 4 h in a humidified CO_2 incubator. Absorbance was measured at a wavelength of 450 nm. Omeprazole (AstraZeneca), cisplatin (Haosen, China), paclitaxel (Xiehe, China) and 5-FU (Haixin, China) were used in our research.

Cell apoptosis determination

Cells were seeded at 2×10^6 cells per well in 6-well plates for 24 h. Then, omeprazole or paclitaxel was added at the indicated concentrations following the scheduled groups to culture the cells. Cells were digested by 0.25% pancreatin without EDTA and were collected for analysis subsequently. The determination of cell apoptosis was performed by annexin-V-FITC and propidium iodide (PI) staining following the manufacturer's instructions (BD, 556547), and the samples were analyzed by flow cytometry.

Immunofluorescence

Gastric cancer cells were grown on glass coverslips, washed twice in phosphate-buffered saline and fixed with 4% paraformaldehyde for 20 min. Permeabilization was with 0.1% Triton X-100 for 10 min at room temperature prior to blocking in 5% BSA for 30 min. FOXO1 was detected by reacting with antibody (1:100) overnight at 4°C, followed by Alexa Fluor 488 conjugated (Beyotime, P0176) or Cy3-conjugated (Beyotime, P0193) secondary antibodies at 1:200 for 1 h in the dark. Coverslips were mounted on glass slides using ProLong® Diamond AntifadeMountant with DAPI (Life Technologies, P36971) mounting medium prior to imaging with a fluorescence microscope.

RNA labeling and microRNA microarray hybridization

The Human microRNA Microarray Kit (Agilent Technologies, Agilent-G4870C) was used for labeling and hybridization according to the manufacturer's protocol. In brief, equal amount of small RNA (defined by Agilent bioanalyzer pico assay) was labeled with Cyanine3 (Cy3), re-suspended in hybridization buffer and hybridized to the array platform overnight (20 hours) at 55°C in a rotating Agilent hybridization oven using Agilent's recommended hybridization chamber. Subsequently, the microarrays were washed with the Agilent Gene Expression Wash Buffer 1 for 5 min at room temperature. A second washing step was performed with Agilent Gene Expression Wash Buffer 2 warmed to 37°C for 5 min. Fluorescence signals after hybridization were detected with a DNA microarray scanner G2505C (Agilent Technologies) using one color scan setting for 8 × 60 K array slides (Scan Area 61 × 21.6 mm, Scan resolution 2 μm, Dye channel is set to Green and Green PMT is set to 100%).

Microarray data analysis

In order to obtain background subtracted and outlier rejected signal intensities, the scanned microarray images were analyzed and processed with the Agilent feature extraction software (v10.7.3.1) using default parameters (Grid: 046064_D_20121223). The resulting raw Signal intensities (gMedianSignal) were exported to R software and normalized by Quantile normalization method. The pairwise expression fold change and p value were calculated via Student's paired t-test after merging the spots with same Agilent probe ID. Differential expressed genes were defined when fold change > 1.5 ($|\log2 \text{ ratio}| > 0.585$) and p value < 0.05.

The hybridization protocol, raw and normalized data are provided in NCBI's Gene Expression Omnibus (GEO, Series accession number: GSE87152).

Isolation and identification of SGC-7901 exosomes

All experiments were performed with exosome-free FBS. Exosome-free FBS was prepared by ultracentrifugation at 110,000 × g for 16 h. 10 ml SGC-7901 cells culture media were collected and centrifuged at 3000 g for 15 min. Supernatant was added 2 ml ExoQuick-TC exosome Precipitation Solution (System

Bioscience, EXOTC50A-1). This was mixed well and refrigerated overnight. After that, the mixture was centrifuged at 1500 g for 30 min, and the supernatant was removed. The residual solution was centrifuged at 1500 g for 5 min and aspirated. Due to omeprazole could also affect the proliferation of SGC-7901, CCK8 was used to detect the proliferation of SGC-7901 treated with omeprazole. Absorbance at a wavelength of 450 nm was used to determinate the volume of PBS to resuspend the exosome pellet and the exosome was stored at -80°C for the use in the experiments described later.

By using a BCA protein assay kit (Pierce), the amount of exosome was detected by measuring total protein content. Further, CD63, 1:500 (Santa Cruz, sc-5275) in exosomes was also detected with Western blot analysis.

Internalization of labelled exosomes

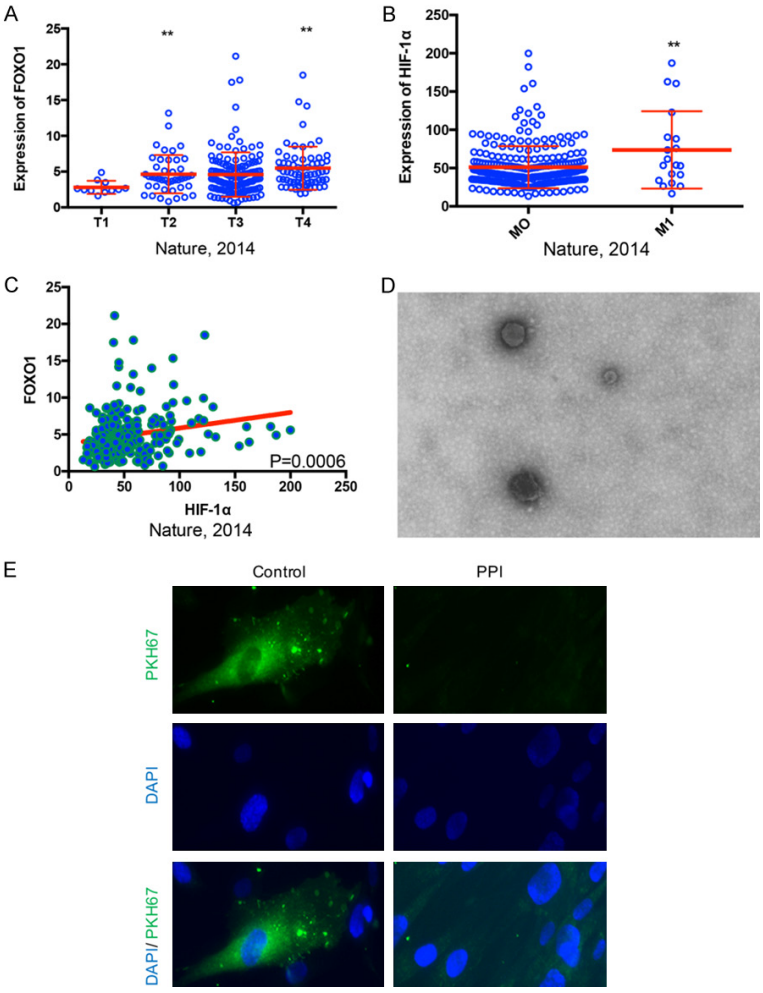
HFF-1 were seeded in a glass coverslips inserted in 12-well plate and cultured overnight. Cells were treated with 30 ul PKH67 (Sigma, miNi67)-labelled SCG-7901 exosome for 3 h at 37°C. After washing, Coverslips were mounted on glass slides using ProLong® Diamond AntifadeMountant with DAPI (Life Technologies, P36971) mounting medium prior to imaging with a fluorescence microscope (Zeiss).

Migration and invasion assays

Cell migratory and invasive abilities were assessed by way of transwell (Corning) and Matrigel invasion (BD Biosciences), respectively. For transwell migration assay, 1×10^5 cells were seeded, whereas 5×10^4 cells were seeded for the invasion assay. Cells that migrated to the underside of the membrane were fixed and stained with 0.1% crystal violet and were enumerated by counting four random fields per transwell. Mean values of migrating or invading cells were expressed as percentages relative to control. Each experiment was performed in replicate inserts, and mean value was calculated from three independent experiments.

Cytokine array

HFF-1 cells were treated with exosome from gastric cancer cells and supernatants were harvested and examined for differential cytokine expression using the Human Cytokine Array G5 (RayBiotech) per the manufacturers using the Imagequant TL software (GE Healthcare Life Sciences).



Supplementary Figure 1. A. Nature, 2014 databases show FOXO1 mRNA has positive correlation with AJCC tumor pathologic in gastric cancer patients. B. Nature, 2014 data bases show that HIF-1α mRNA have positive correlation with AJCC metastasis pathologic in gastric cancer patients. C. Nature, 2014data bases reveal the positive correlation of mRNA between FOXO1 and HIF-1α. D. Typical image of electronic speculum showed the morphology of exosome. E. HFF-1 cells were treated with PKH67 labelled exosome of SGC7901 cells (80 ug/ml of PPI or PBS (control) treated for 24 h) and the uptake of exosome by HFF-1 cells was tested by immunofluorescence, and the nucleus of HFF-1 cells was stained by DAPI (n=3).