**Supplementary Information**

**Materials and Methods**

*Transient and Stable Transfections*

The cloning process of cyr61 expression plasmid was described previously (10). Briefly, total RNA was extracted and Cyr61 complementary DNA was cloned and amplified by RT-PCR with the primer with cloning sites 5'-TATAGGATCCGAATTCATGAGCTCCCGCATCGCC-3' (forward) and 5'-TATAGCGGCCGCTTAGTGGTGGTGGTGGTGGTGGTGGTCCCTAAATT TGTGAATG-3' (reverse) subcloned into a pcDNA 3.1 (Invitrogen, San Diego, CA) in forward (sense) or reverse (anti-sense) direction. pCMV-Cyr61, a constitutive expression vector, carries 1.14-kb full-length human *Cyr61* cDNA under control of the CMV promoter/enhancer sequence. AGS cells were transfected with pCMV-Cyr61 or control pcDNA3 vector (GIBCO Invitrogen, Grand Island, NY, USA) containing a CMV promoter and a neomycin selection marker, using the TransFast$^{TM}$ transfection reagent (Promega, Madison, WI, USA). Twenty-four hours after transfection, the cells were serum-starved for 16 h and lysed for RT-PCR and Western blot. For stable cell population selection, twenty-four hours after transfection, cells were replated in RPMI-1640 (GibcoBRL, Rockville, MD, USA) with 10% (vol/vol) fetal calf serum (FCS) and 800 μg/ml G418 (Sigma, St. Louis, MO, USA). G418-resistant clones were

selected and expanded. The mRNA and protein levels of Cyr61 in these cells were checked by RT-PCR and Western blot analysis. AGS cells transfected with control vector (AGS/pcDNA3) served as control. AGS/Cyr61 and AGS/pcDNA3 cells were grown at 37°C and 5% $CO_2$ in RPMI-1640 medium supplemented with 10% FCS and 100 μg/ml G418.

*RNA Isolation and Reverse Transcriptase-PCR*

Total RNA was isolated by using Trizol reagent (Invitrogen Life Tech) according to the manufacturer's instructions. Total RNA (3 μg) was reverse transcribed into single-stranded cDNA using a Moloney murine leukemia-virus reverse transcriptase and random hexamers (Promega, Madison, WI). The cDNAs were amplified with the forward (F) and reverse (R) primers by PCR as described. The primer sequences for CYR61were 5'- CAG GGT GGA GTT GAC GAG AAA C -3' (F) and 5'- AGG ACT GGA TAT CAT GAC GTT CT -3' (R). The primer sequences for HIF-1α were 5'- CAG CTA TTT GCG TGT GAG GA -3' (F) and 5'- CCA AGC AGG TCA TAG GTG GT -3' (R). The primer sequences for PAI-1 were 5'- TCG TCC AGC GGG ATC TGA -3' (F) and 5'- CCT GGT CAT GTT GCC TTT C -3' (R). The primer sequences for c-MET were 5'- GGA AAC ACC CAT CCA GAA TGT CAT T -3' (F) and 5'- TGA TAT CGA ATG CAA TGG ATG ATC T -3' (R). The primer sequences

for AMF were 5'- ATG GCC AGC ATG CTT TTT AC -3' (F) and 5'- GGT AGA AGC GTC GTG AGA GG -3'(R). The primer sequences for HGF were 5'- TGG ATG CAC AAT TCC TGA AA -3' (F) and 5'- TTG TAT TGG TGG GTG CTT CA -3' (R). The primer sequences for ADM were 5'- GGG TAG CTG CTG GAC ATC CG -3' (F) and 5'- GTA GCC CTG GGG GCT GAT CT -3'(R). Primers were used at a final concentration of 0.5 µM. Reaction mixture was first denatured at 95°C for 10 min. The PCR conditions applied were 30 cycles of 95°C for 1 min, 52°C for 1 min, and 72°C for 1 min, followed by 72°C for 10 min. PCR products were visualized by ethidium bromide staining after agarose gel electrophoresis.

*Western Blot Analysis*

Cells were harvested and lysed in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 1 mM $Na_3VO_4$, 1 mM NaF, 1 mM EGTA, 1 mM PMSF, 1 µg/ml leupeptin, and aprotinin), cleared by centrifugation for 20 min at 4 °C, and the supernatant collected. Protein (20–50 µg) was loaded onto 8–12% gradient SDS-PAGE gels, separated, and transferred onto polyvinylidene difluoride Immobilon membranes. The membranes were blocked with 5% milk and incubated with the appropriate primary antibody. After washing, the membranes were

stained with the correct secondary antibody. Protein bands were visualized by chemiluminescent detection (ECL) (Amersham Biosciences).

*HIF-1α reporter Activity Assay*

For transfections, cells were seeded in 6-well plates. After reaching about 70% confluence, the cells were transfected with pGL2 vector, Hypoxia response element (HRE), using TransFast$^{TM}$ (Promega). After transfection, the medium was replaced by fresh normal growth medium, and the cells were incubated for 24 h. After starvation in serum-free medium for 16 h, the cells were harvested, and the luciferase activity was measured with the Promega Dual-Luciferase Reporter Assay system (Promega) as described by the manufacturer, using a Turner Designs model TD-70/20 Luminometer (Jimenez et al., 1999).

*Immunofluorescence Staining*

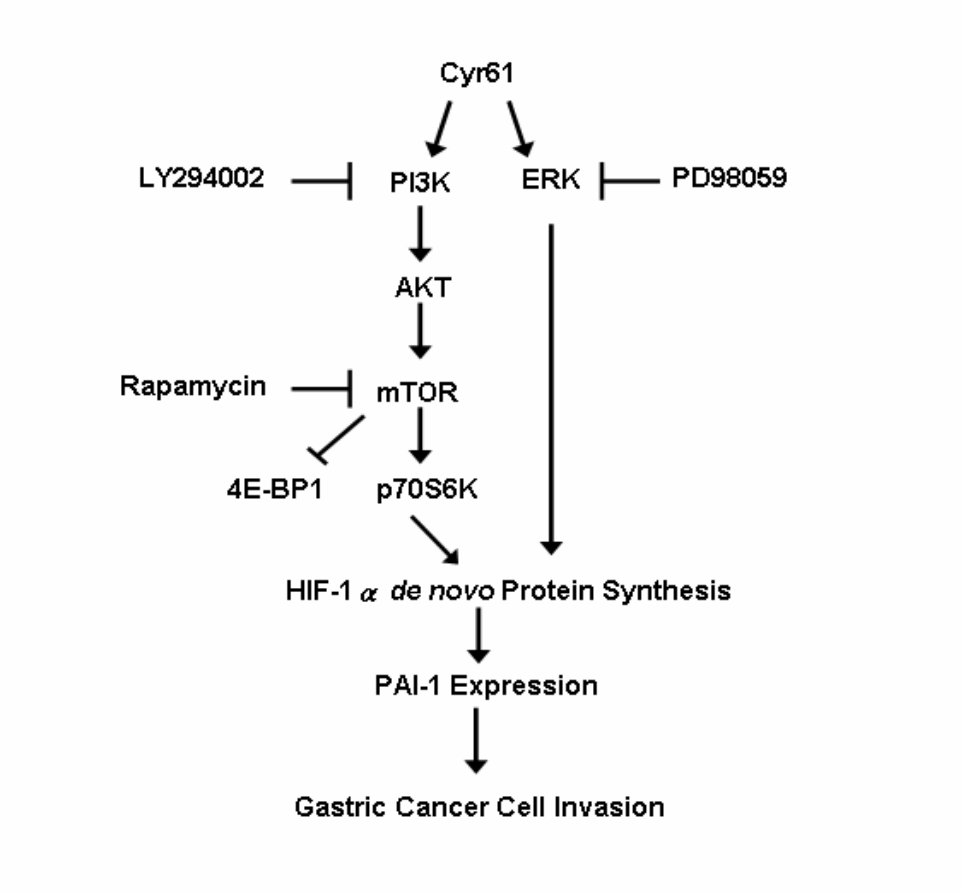
Cells were cultured to 60 to 80% confluence on degreased glass coverslips in regular culture medium and then subjected to starvation for 16 h, after which cells were treated with Cyr61 (40µg/mL) for a period of 8 hr and 24 hr. Cells were then fixed in methanol/acetic acid [3:1 (v/v)] for 30 min at 4°C. After this, these cells were then rinsed and blocked for one hour in 5% fetal bovine serum at room temperature. The

cells were then incubated with anti-HIF-1α monolyclonal antibody (BD Biosciences) at 4°C overnight, after which cells were washed in PBS and then incubated with a secondary fluorescein isothiocyanate-conjugate antibody (1:100, Sigma) and DAPI (1:10000) for 1h at room temperature. After extensive washing, the coverslips were inverted onto glass slides using Mowiol (Calbiochem) as a mounting medium. The slides were examined with a fluorescent microscope.

*Boyden chamber assay*

Invasion assays were done using modified Boyden chambers with filter inserts for 24-well dishes containing 8-µm pores (Nucleopore Corp., Pleasanton, CA). Matrigel (30 µg, Collaborative Biomedical, Becton Dickinson Labware, San Jose, CA)–coated filters were used for invasion assays. Cells ($2.5 \times 10^4$) were plated into 100 µL of complete RPMI in the upper chamber, and the lower chamber was filled with 1 mL of RPMI. After 48 hours in culture, cells were fixed in methanol for 15 minutes and stained with 0.05% crystal violet in PBS for 15 minutes. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed in PBS. Cells on the underside of the filters were viewed and counted under a microscope (type 090-135.001, Leica Microsystems, Wetzlar, Germany). Each clone was plated in triplicate in each experiment and each experiment was repeated at least thrice.

**Supplementary Figure 1**




Proposed invasive molecular mechanisms of HIF-1α-mediated PAI-1 expression in Cyr61-overexpressing cells.

Supplementary Fig 2

In order to verify the specificity of reducing PAI-1 or Cyr61 activity, a siRNA approach was done as the following:

Fig 2A. the sequence of siRNA to PAI-1 or Cyr61

siRNA sequence

siCyr61
(RNA) UAA AGG GUU GUA UAG GAU GCG AGG C
(RNA) GCC UCG CAU CCU AUA CAA CCC UUU A

siPAI-1
(RNA) AUA GCU GCU UGA AUC UGC UGC UGG G
(RNA) CCC AGC AGC AGA UUC AAG CAG CUA U

Fig 2B. Transient transfection of scramble or siRNA to Cyr61 into TSGH gastric cancer cells, which constitutively expressing Cyr61. The protein expression of Cyr61 and its downstreaming target HIF-1α were specifically reduced by siRNA to Cyr61 (Lane 4).

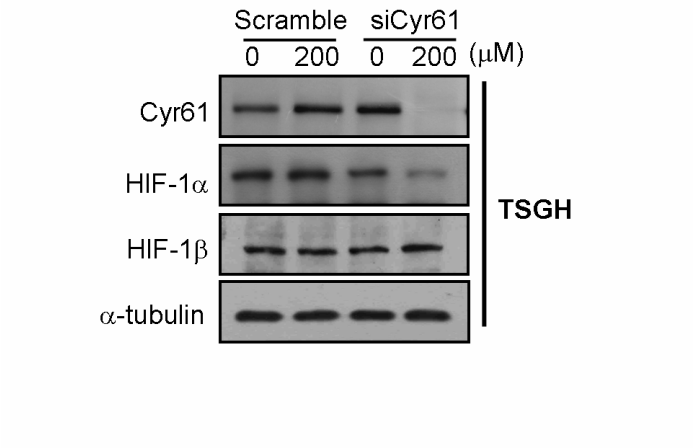


Fig 2C. Transient transfection of scramble or siRNA to PAI-1 into TSGH gastric cancer cells, which constitutively expressing PAI-1. (*Upper*) The protein expression of PAI-1 was specifically reduced by SiRNA to PAI-1 (Lane 4) and (*lower*) the invasiveness of TSGH was also attenuated significantly (※ *P* values of <0.05).

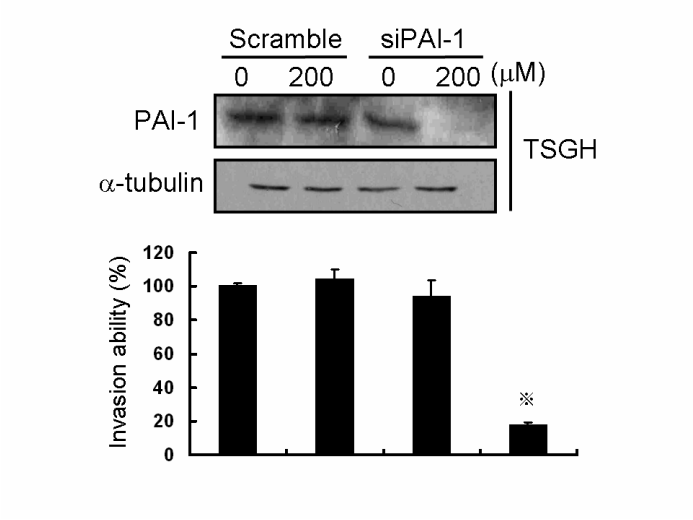



Fig 2D. Transient transfection of scramble or siRNA to PAI-1 into AGS/Cyr61 gastric cancer cells, which constitutively expressing PAI-1. (*Upper*) The protein expression of PAI-1 was specifically reduced by SiRNA to PAI-1 (Lane 4) and (*lower*) the invasiveness of AGS/Cyr61 was also attenuated significantly (※ *P* values of <0.05).