Supplementary Material and Methods

Cell lines and culture conditions
SGC7901, BGC823, MKN28, MKN45, HGC27, and N87 cell lines were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). AGS cells were purchased from American Type Culture Collection (ATCC). GES-1 cells were described as before. HGC27 cells were maintained in minimum essential medium and other cell lines were maintained in RPMI 1640 medium. All cells were supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in a humidified atmosphere with 5% CO₂. The cells were allowed to reach 80% confluency before passage. The culture medium was replenished with fresh medium every 2 or 3 days.

Bio-Plex assay
Bio-Plex System (Bio-Rad, USA) allows the simultaneous measurement of different cytokines. In our experiment, we custom-tailored four cytokines (IL-6, IL-8, VEGF, and IL-1β) that are associated with gastric cancer progression. Supernatants were harvested at 1, 3, 6, 24 h after being stimulated with increasing concentrations of NE (0, 0.1, 1, 10 μM). The levels (pg/mL) of these cytokines were analyzed by the Bio-Plex assay kit following the manufacturer’s protocol. The detectable limit ranges from 1.95 to 32,000 pg/mL.

Western blot
Cells were lysed in sample solution. Proteins were separated on 10% SDS-PAGE gels, transferred to nitrocellulose membranes, and bands were detected using specific antibodies as indicated. The membranes were incubated with the primary antibodies at 4°C overnight and with horseradish peroxidase-conjugated secondary antibodies (KPL Inc., USA) for 2 h at room temperature before detection using an enhanced chemiluminescence system (Pierce Biotechnology, USA). The rabbit polyclonal anti-β₁-adrenoreceptor antibody was from GeneTex (USA), rabbit polyclonal anti-β₂-adrenoreceptor antibody was from Abcam (USA), and rabbit polyclonal anti-GAPDH antibody was from PTG Inc. (USA).
Supplementary Figures

Figure S1. Norepinephrine (NE) differentially modified the levels of VEGF, IL-8, and IL-6 proteins in culture supernatants of four gastric cancer cell lines and GES-1 cells. Supernatants were harvested at 1, 3, 6, 24 h after being stimulated with increasing concentrations of NE (0, 0.1, 1, 10 μM). The levels (pg/mL) of these cytokines (IL-1β, IL-6, IL-8, and VEGF) were analyzed by Bio-Plex system (Bio-Rad). NE modified the levels of VEGF, IL-8, and IL-6 proteins in culture supernatants of AGS (A-C), MKN45 (D-F), GES-1 (G-I), MKN28 (J and K) cells, and HGC27 (L) after treatment with NE. IL-6 secretion of MKN28 cells and IL-6, IL-8 secretion of HGC27 cells were not detectable. Furthermore, none of the cell lines produced detectable levels of the IL-1β protein in untreated and NE-treated culture supernatants.
Figure S2. Western blot analysis of β₁-AR (ADRB1) and β₂-AR (ADRB2) protein expression in seven gastric cancer cells and GES-1 cells. A, A band at 51 kDa was expressed in GES-1 cells, consistent with the weight of the unglycosylated protein, and bands migrating around 55-72 kDa were due to other post-translational modifications. B, A band at 47 kDa was expressed in GES-1 cells, consistent with the weight of the unglycosylated protein, and bands migrating around 47-170 kDa were due to other post-translational modifications.