Supplementary Data

Materials and Methods

Soft agar colony formation experiment

Single cell suspension was prepared by digestion of 0.25% trypsin-0.02% EDTA in each group at logarithmic growth stage. 1000 cells were resuspended in 1 ml RPMI 1640 complete culture medium containing 0.33% AGAR. rapid inoculation on solid medium containing 0.5% AGAR RPMI 1640 complete medium. observe the formation of cell cloning after continuous culture for 7 days.

Cell proliferation assay and cell cycle analysis

Cell proliferation assays were performed using a CellTiter 96 AQ One Solution Cell Proliferation Assay kit (MTS, Promega, Madison, USA) according to the manufacturer's instructions. Cells were seeded in 96-well plates at 1×10^4 per well. After transfected and untransfected cells were incubated for the indicated times, the absorbance at 490 nm was recorded using an ELISA plate reader. Each assay was performed with 5 replicates. Cell cycle analysis was conducted as follows. Briefly, the transfected and untransfected cells were harvested and resuspended in ice-cold 75% ethanol and fixed for 24 h at 4°C. For subsequent flow cytometry analysis, fixed cells were resuspended in 1 mL of PI (propidium iodide) staining reagent for 30 min. The data were collected using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and analyzed with Verity Winlist Software (Verity Software House, Topsham, ME, USA).

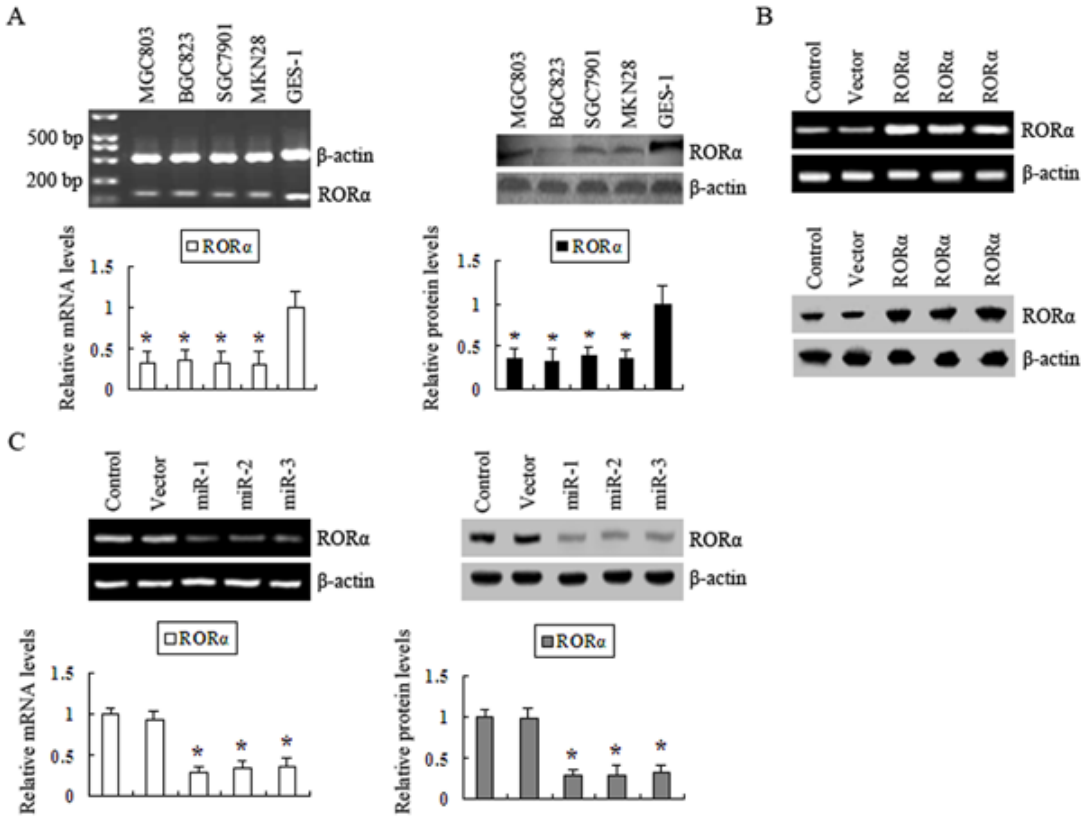

Figure S1. The expression of RORα in gastric cancer cells and cell line establishment. (A) The expression of RORα mRNA and protein was detected by RT-PCR and western blotting in gastric cancer MGC803, BGC823, SGC7901, MKN28 and GES-1 cells. (B) The expression of RORα in MGC803 cells transfected with RORα-expressing plasmid was assessed by RT-PCR and western blotting. (C) The expression of RORα in MGC803 cells transfected with RORα-miR-expressing plasmid was assessed by RT-PCR and western blotting. We chose miR-1, a miR-RORα, for subsequent experiments. The pictures are representatives of three individual experiments. *P<0.05 vs. control.

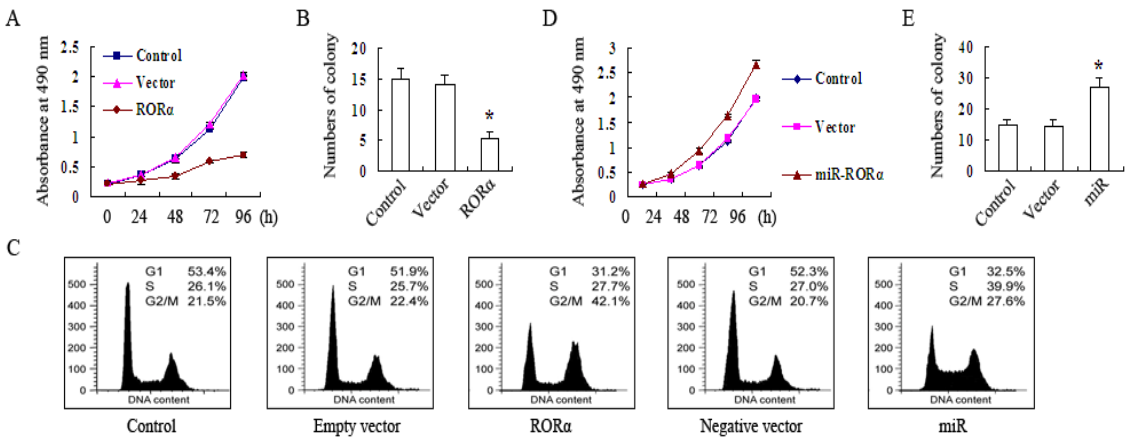

Figure S2. The effect of RORα overexpression and knockdown on GC cell proliferation. (A) Effect of RORα overexpression on proliferation in MGC803 cells was detected by using MTS assay. The proliferation activity of RORα-overexpressing cells was (0.347 ± 0.051), (0.581 ± 0.024) and (0.698 ± 0.046), which was lower than (0.631 ± 0.068), (1.129 ± 0.017) and (1.974 ± 0.045) in the control group and (0.647 ± 0.027), (1.211 ± 0.031) and (2.033 ± 0.038) in the vector group at 48 h, 72 h and 96 h, respectively. (B) Colony formation assays revealed that the colony forming efficiency of RORα-overexpressing cells was 5.2 ± 0.8%, which was lower than those of the control (14.9 ± 1.7%) and vector group (15.2 ± 1.4%). (C) The percentage (42.1%) of RORα-overexpressing cells in G2/M phase was higher than that (21.5%) of control cells and that of (22.4%) of vector cells, and the percentage (39.9%) of miR-RORα cells in S phase was higher than that (26.1) of control cells and that of (27.0%) of negative vector cells. (D) The proliferation activity in miR group was (0.929 ± 0.06), (1.642 ± 0.02) and (2.157 ± 0.07), which was higher than (0.631 ± 0.07), (1.129 ± 0.02) and (1.974±0.05) in the control group and (0.614 ± 0.02), (1.173 ± 0.07 and (1.963 ± 0.04) in the vector group at 48 h, 72 h and 96 h, respectively. (E) The colony forming efficiency of miR group was 18.2 ± 1.8% higher than the 14.9 ± 1.7% in the control group and the 14.7 ± 1.1% in the vector group. The pictures are representatives of three individual experiments. *P<0.05 vs. control.