Supporting Information

Qiao *et al* 10.1073/pnas.0711861105

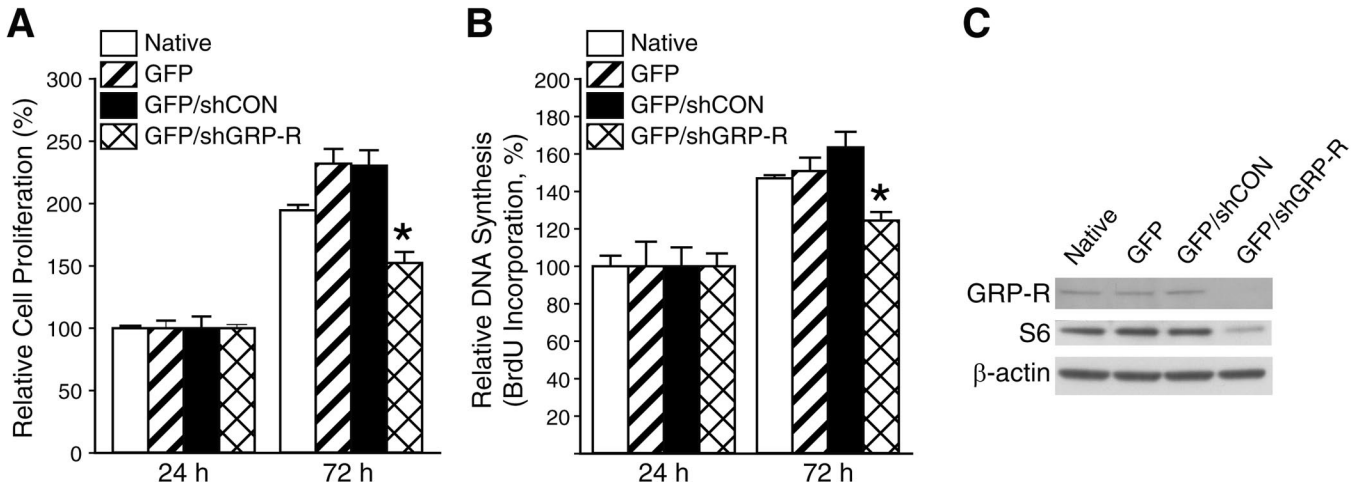

Fig. S1. Comparison of transfected versus native BE(2)-C cells. (*A*) BE(2)-C cells (native, expressing GFP, shGRP-R, or shCON) were plated 4×10^3 cells/well and cell proliferation was measured (data represent mean ± SEM; $*P < 0.02$ vs. native, GFP, or shCON). (*B*) DNA synthesis was analyzed by measuring the BrdU incorporation (data represent mean ± SEM; $*P < 0.02$ vs. native, GFP, or shCON). (*C*) Western blot analysis was performed with GRP-R and S6 antibodies; β-actin was used as a loading control.

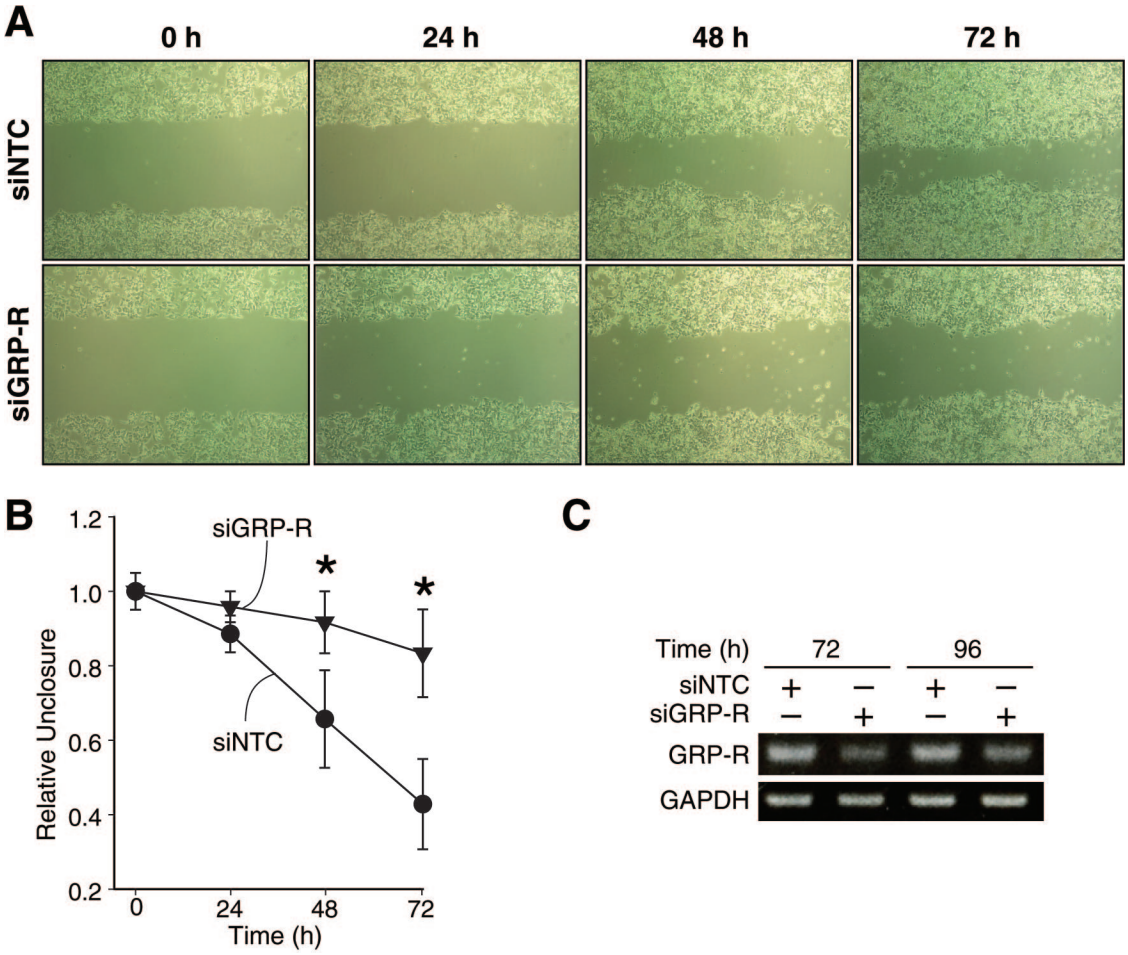

Fig. S2. GRP-R silencing inhibits wound closure. (*A*) BE(2)-C cells transfected with siNTC or siGRP-R were grown to confluence on 6-well plates in complete culture media and then serum starved overnight. Wounds, or defects, were generated in confluent cell monolayers using a modified rubber scraper at 48 h after transfection. Serum-free medium was changed to 1% FBS/RPMI 1640 after wounding. Representative photographs of wound closure, captured with an inverted microscope (Nikon, ECLIPSE TS100), are shown for the indicated time points. (*B*) Quantitative analysis of wound-healing assay (*$P < 0.05$ vs. siNTC). Data are representative of the mean unclosure distance of three independent experiments. (*C*) GRP-R knockdown was confirmed by RT-PCR analysis 72 to 96 h after transfection.

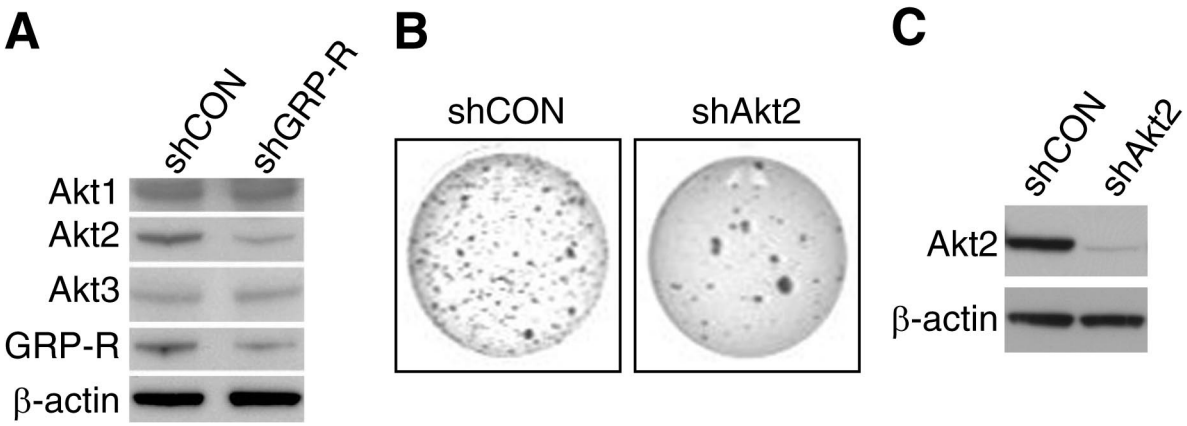

Fig. S3. Akt2 is regulated by GRP-R and shAkt2 decreases anchorage-independent growth. (*A*) Western blot analysis of Akt isoforms, Akt1, Akt2, and Akt3 in BE(2)-C cells expressing shCON or shGRP-R. Akt2 expression alone was down-regulated by shGRP-R. (*B*) BE(2)-C cells expressing either shAkt2 or shCON were plated in soft agar (2.5×10^3 cells/well) for 3 weeks. (*C*) Western blots confirm inhibition of Akt2 protein levels after stable transfection.