Supplementary Methods

Plasmids and transfections. The si-Id1 and si-CON, purchased from Dharmacon (Chicago, IL), were transiently transfected into KYSE150 cells respectively (1). Id1 overexpressing plasmid pBabe-puro-Id1 and Id1 shRNA in pRetroSuper were kindly provided by Joan Massague (Memorial Slocan-Kettering Cancer Center, New York) (2). The pRS-shIGF-II plasmid expressing the shRNA against IGF-II (5'-CGTGCTCGCCAAGGAGCTCGAGGCGTTCA-3'), purchased from OriGene Technologies (Rockville, MD), and the non-effective plasmid (pRS-shCON; catalog number TR30003) serving as irrelevant control were transfected into KYSE150-CON and KYSE150-Id1. The stable cell lines KYSE150-CON-shCON, KYSE150-Id1-shCON, and KYSE150-Id1-shIGF-II were obtained after selection with puromycin (1 mg/mL; Invitrogen, Gaithersburg, MD) for two weeks. A luciferase expression vector generated by subcloning the luciferase coding region into pLenti/V5-D-TOPO (Invitrogen) was used to generate the luciferase-expressing stable cell line KYSE150-Luc.

Antibodies used in Western blotting and immunohistochemistry. Primary antibodies used in Western blotting and immunostaining included phospho-AKT (Ser^{473}), AKT, phospho-GSK3β (Ser^{9}), GSK3β, thymidylate synthase, caspase-3 and cleaved caspase-3 obtained from Cell Signaling Technology (Beverly, MA), IGF-II (R&D Systems, Minneapolis, MN), Id1, actin and CD31 from Santa Cruz Biotechnology (Santa Cruz, CA), Ki-67 (DAKO Diagnostics, Mississauga, ON), and human-specific CK-8 (Epitomics, Burlingame, CA).

Quantitative real-time PCR

Total RNA was isolated using Trizol reagent according to the manufacture's protocol (Invitrogen), and cDNA was synthesized using the SuperScript First-Strand Synthesis System

(Invitrogen). The mRNA expression levels of IGF-II, and of GAPDH as internal control, were detected by real-time PCR using IQ SYBR Green Supermix (Bio-Rad, Hercules, CA). The primers used were: 5'-TGGCATCGTTGAGGAGTGCTGT-3' (forward) and 5'-ACGGGGTATCTGGGGAAGTTGT-3' (reverse) for IGF-II; 5'-AAGGTGAAGGTCGGAGTCAA-3' (forward) and 5'-GACAAGCTTCCCGTTCTCAG-3' (reverse) for GAPDH.

***In vivo* tumorigenesis experiments**

All the animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. Esophageal cancer cells (1 x 10^6 KYSE150 or 5 x 10^5 KYSE270 cells) were subcutaneously into the flank of nude mice. For the treatment experiments, when the tumors reached ~5 mm diameter, the animals were given intratumoral injections of neutralizing antibody against human IGF-II (10 μg /cm^3 tumor; R&D Systems), or intraperitoneal injections of cixutumumab (25 mg/kg or 50 mg/kg; ImClone Systems Incorporated, New York), 5-FU (20 mg/kg; Calbiochem, Darmstadt, Germany) or cisplatin (2 mg/kg; Calbiochem) twice a week. Additional groups of animals received cixutumumab (25 mg/kg) combined with 5-FU (20 mg/kg) or cisplatin (2 mg/kg) twice weekly. Control groups received the corresponding isotype IgG.

***In vivo* metastasis experiments**

KYSE150-Luc cells (1 x 10^6) were injected intravenously into nude mice through the lateral tail vein. For the treatment experiments, seven days after injection of cancer cells, the animals were treated twice weekly with cixutumumab or isotype IgG as described above. Metastatic activity in the lung area was assessed by bioluminescent imaging with an IVIS Imaging System (Xenogen, Alameda, CA) after intraperitoneal injection of D-Luciferin (Gold

Biotechnology, St Louis, MO).

Immunohistochemistry and evaluation of staining

After antigen retrieval and blocking with normal serum, the slides were incubated overnight at 4 ºC with primary antibodies followed by biotinylated secondary antibodies and peroxidase-conjugated avidin-biotin complex. Immunostaining was visualized using 3, 3'-diaminobenzidine (DAKO) as chromogen. The sections were counterstained with hematoxylin. For assessment of Id1, IGF-II, and p-AKT immunostaining in the TMA, both cores of each case were individually scored, and the highest score selected for statistical analysis. A scale of 0 to +++, representing negative (0), weak (+), moderate (++) and strong (+++) staining, respectively, was used to grade the intensity of staining. The stained sections were reviewed independently by two observers. For those tumors for which there was disagreement, another review was performed to obtain a consensus. The Ki-67 proliferative index was calculated as the percentage of positive tumor nuclei divided by the total number of tumor cells in a minimum of six randomly selected fields from representative tumor sections. Microvessel density (MVD) was determined by immunostaining of the slides for the endothelial cell marker CD31, and was expressed as the mean number of vessels stained in six random fields of representative tumor sections.

TUNEL assay

Apoptotic cells in the tumor xenografts were determined by incubating the tumor sections in TUNEL reaction mixture (*In situ* Cell Death Detection kit Fluorescein, Roche Diagnostics, Mannheim, Germany) for 1 h, then counterstained with DAPI. Eight representative areas were randomly selected under a fluorescence microscope, then TUNEL- and DAPI-positive cells were scored. The apoptotic index was determined by dividing the number of TUNEL-

positive cells by the total number of cells (DAPI-positive cells).

Supplementary References

1. Li B, Cheung PY, Wang X, Tsao SW, Ling MT, Wong YC, et al. Id-1 activation of PI3K/Akt/NFkappaB signaling pathway and its significance in promoting survival of esophageal cancer cells. Carcinogenesis 2007;28:2313-20.

2. Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, et al. Genes that mediate breast cancer metastasis to lung. Nature 2005;436:518-24.