Fig. S1

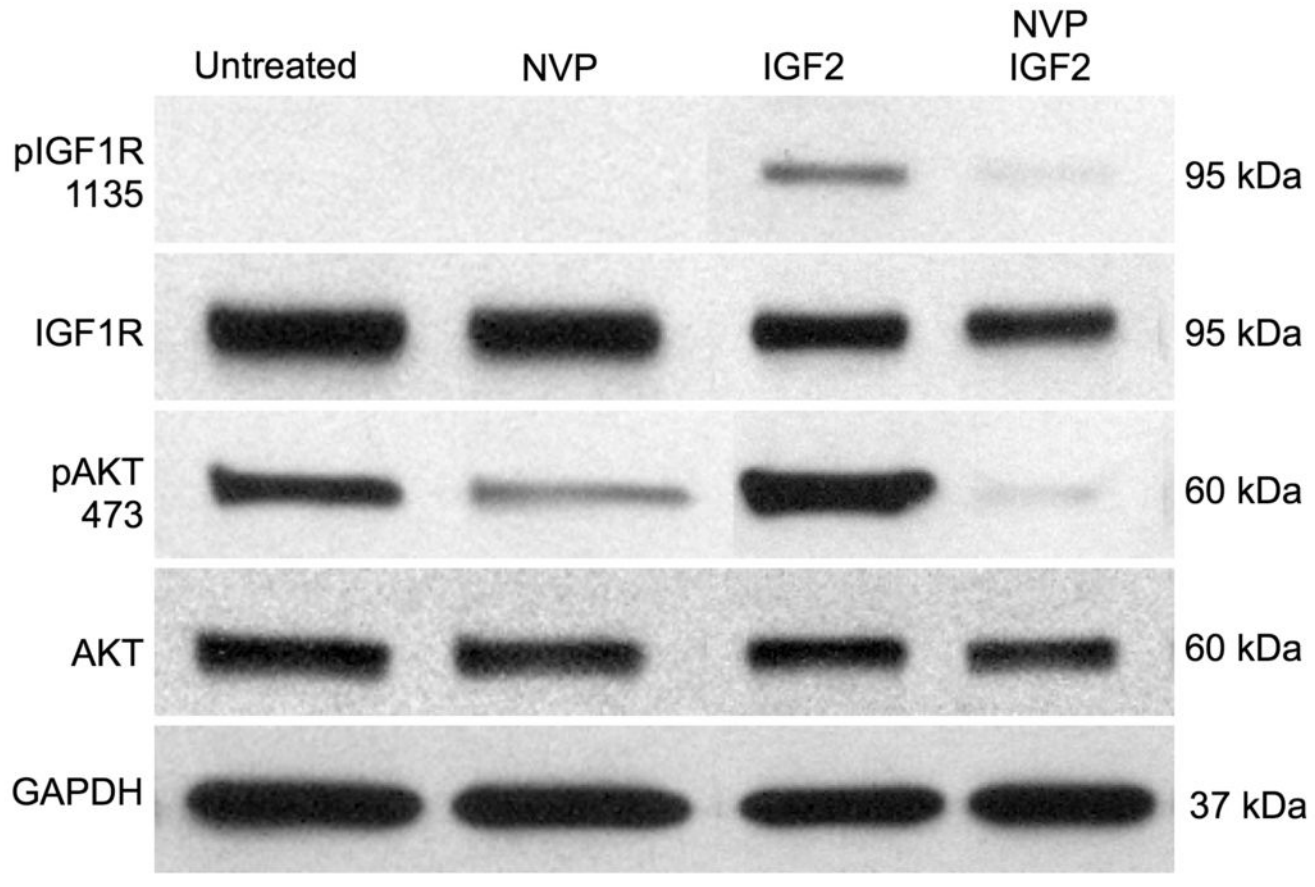

Untreated
NVP
IGF2
NVP
IGF2
pIGF1R
1135
95 kDa
IGF1R
95 kDa
pAKT
473
60 kDa
AKT
60 kDa
GAPDH
37 kDa

Fig. S2

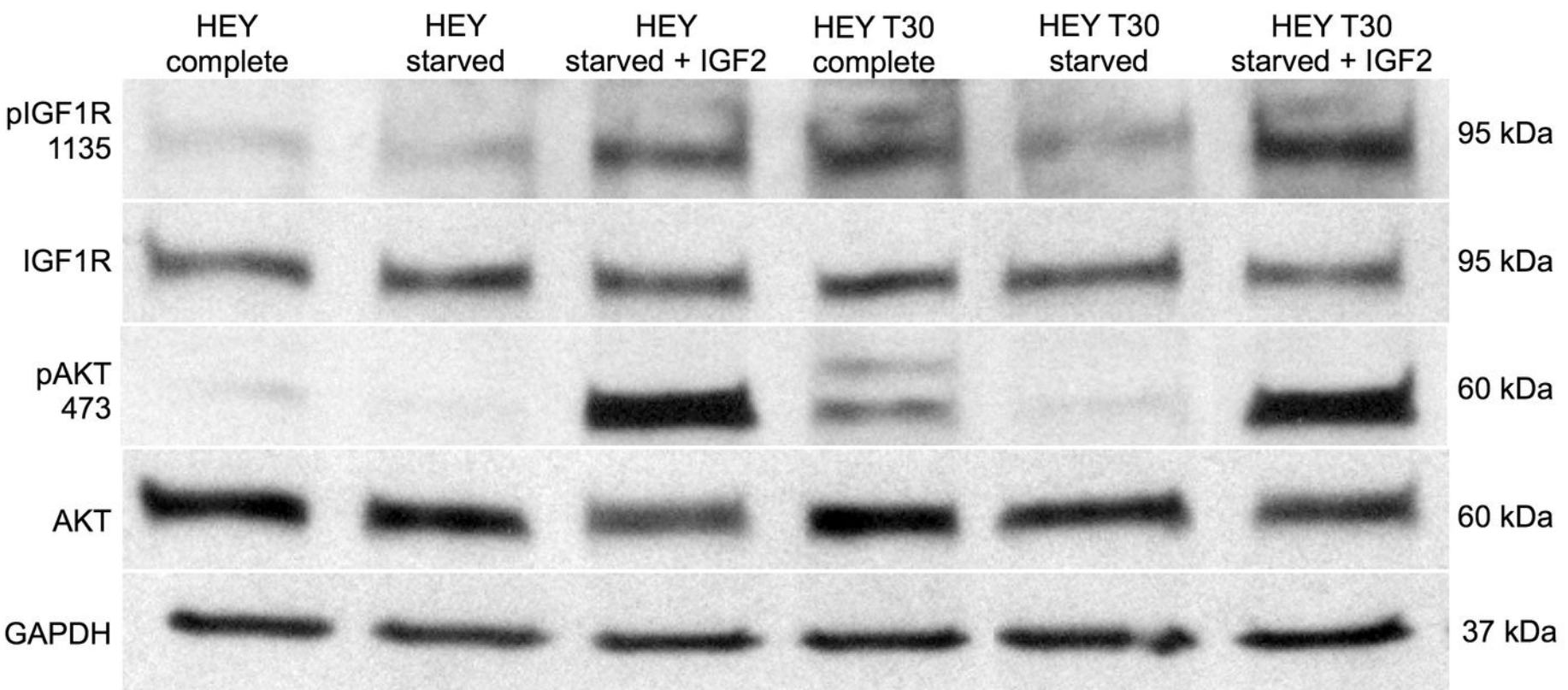
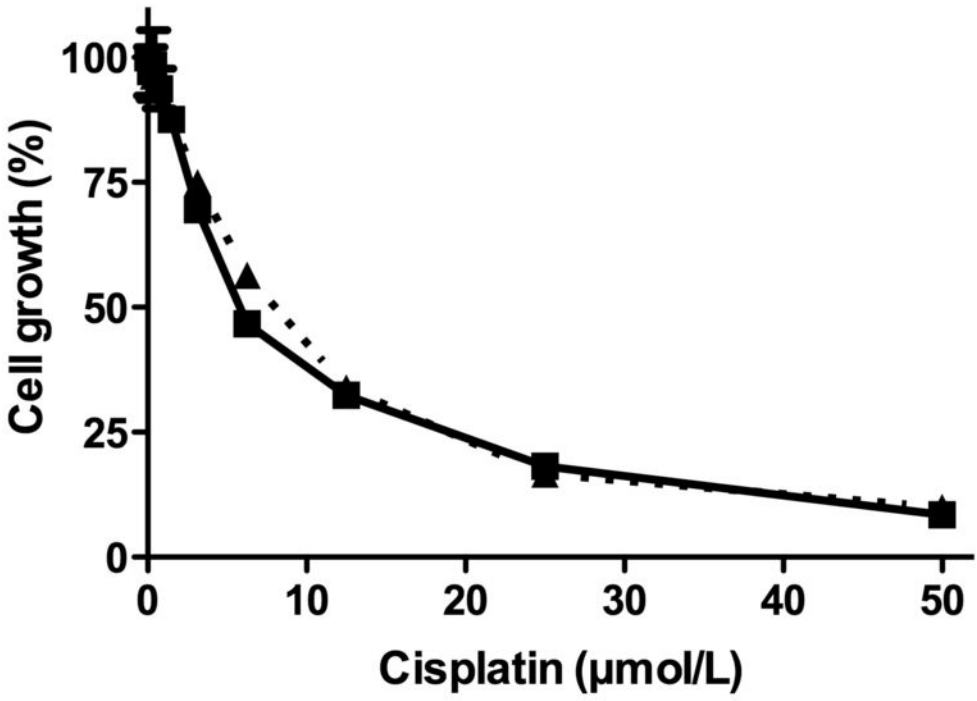

HEY complete
HEY starved
HEY starved + IGF2
HEY T30 complete
HEY T30 starved
HEY T30 starved + IGF2
pIGF1R 1135
IGF1R
pAKT 473
AKT
GAPDH
95 kDa
95 kDa
60 kDa
60 kDa
37 kDa

Fig. S3 A


100
75
50
25
0
Cell growth (%)
0
10
20
30
40
50
Cisplatin (µmol/L)

B

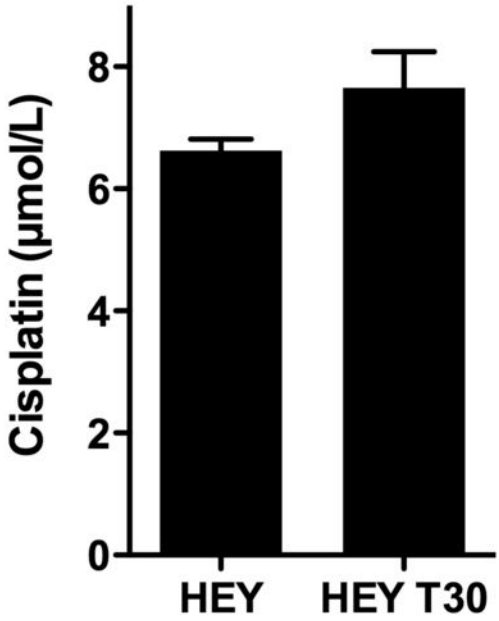
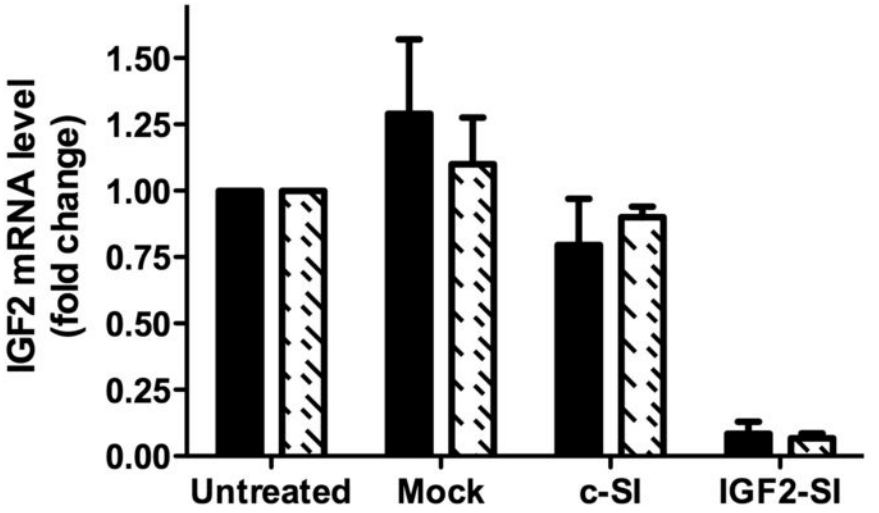
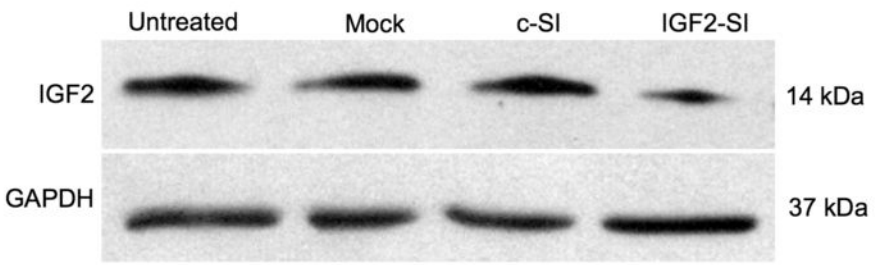

8
6
4
2
0
Cisplatin (µmol/L)
HEY
HEY T30

Fig. S4 A


IGF2 mRNA level
(fold change)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Untreated
Mock
c-SI
IGF2-SI

B


Untreated
Mock
c-SI
IGF2-SI
IGF2
14 kDa
GAPDH
37 kDa

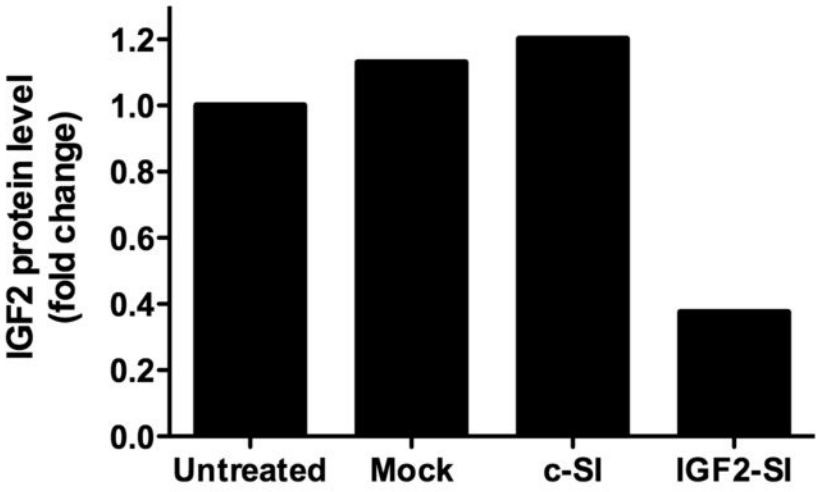
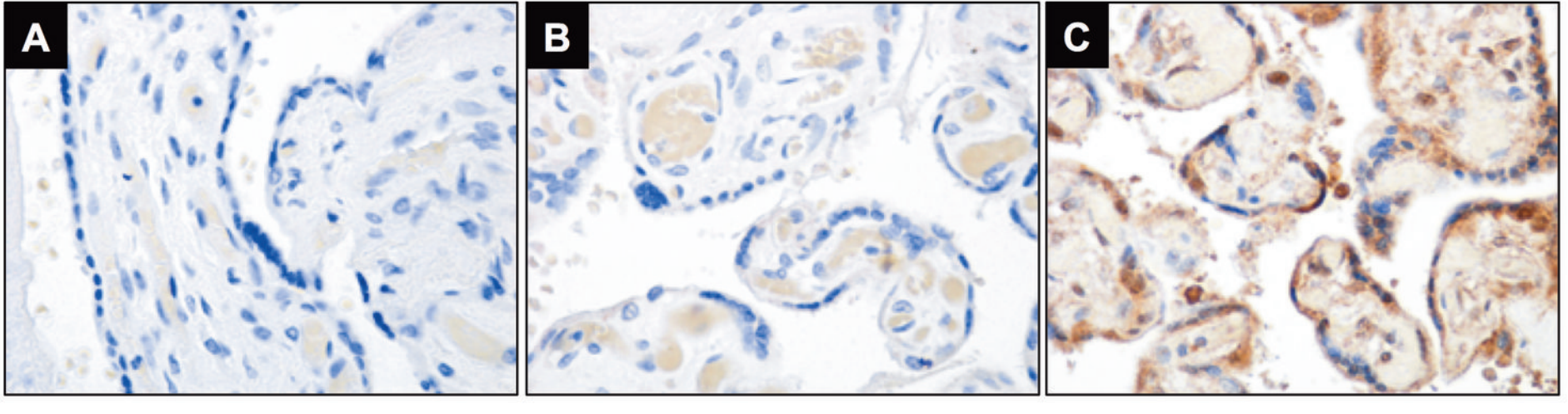

IGF2 protein level
(fold change)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Untreated
Mock
c-SI
IGF2-SI

Fig. S5



Supplemental Figure Legends

Figure S1. Inhibition of IGF1R by NVP-AEW541

A2780 cells were serum-deprived for 24 hours then treated as indicated prior to preparation of cell lysates as described in the methods. Lane 1: untreated, unstimulated cells; Lane 2: cells were treated with NVP-AEW541 (1 μmol/L) 45 minutes prior to harvesting; Lane 3: cells were stimulated with IGF2 (50 ng/mL) 15 minutes prior to cell lysate preparation; Lane 4: cells were treated with NVP-AEW541 (1 μmol/L) 45 minutes prior to harvesting and stimulated with IGF2 (50 ng/mL) 15 minutes prior to harvesting. Immunoblotting was performed as described in the methods. As shown in the representative immunoblots, NVP-AEW541 (1 μmol/L) abrogates IGF2-stimulated IGF1R phosphorylation and AKT phosphorylation.

Figure S2. Constitutive IGF1R activation in HEY-T30 cells

HEY and HEY-T30 cell lysates were prepared under conditions of growth in complete media, serum-deprivation for 24 hours, or serum-deprivation followed by IGF2 (50 ng/mL) stimulation for 10 minutes prior to harvesting. Preparation of cell lysates and immunoblotting are described in the methods. As shown in the representative immunoblots, phosphorylation of IGF1R and AKT are increased in the Taxol-resistant HEY-T30 cells compared to HEY parental cells.

Figure S3. Evaluation of cisplatin cytotoxicity in HEY and HEY-T30 cells

A. Cytotoxicity assays were performed using the sulforhodamine B method to determine the effect of cisplatin treatment in HEY (squares; solid connecting lines) and HEY-T30 cells (triangles; dotted connecting line). The cell number

relative to untreated cells, expressed as cell growth (%), is plotted over a range of cisplatin concentrations.

B. The IC50 concentrations of cisplatin in HEY and HEY-T30 cells Taxol are shown in bar graph (mean ±SE; 3 independent experiments with 6 replicates each).

Figure S4. IGF2 depletion by siRNA transfection in HEY-T30 cells

A. As determined by quantitative real-time PCR, IGF2 mRNA levels at 48 hours (solid bar) and 96 hours (cross-hatched bar) are shown for mock-transfected, control siRNA-transfected, and IGF2-siRNA transfected HEY-T30 cells. Results are expressed as fold-change relative to untreated HEY-T30 cells (mean ±SE; 3 independent experiments).

B. The IGF2 protein level was determined by immunoblotting. A representative experiment is shown, and the expression level determined by densitometry is shown in the accompanying bar graph.

Figure S5. Quality control assays for IGF2 immunohistochemistry

A. Negative control placenta section: Primary antibody is omitted (antibody diluent is applied), followed by the identical staining procedure as used for the positive control section.

B. Negative control placenta section: The primary antibody to IGF2 (Abcam, AB9574) is pre-absorbed with recombinant IGF2 (Abcam, AB9575) at a 1:1 concentration, followed by the identical staining procedure as used for the positive control section.

C. Positive control placenta section: The primary antibody to IGF2 (Abcam, AB9574) is used at a 1:100 dilution (5 µg/mL) as described in the methods.

Positive and negative control sections are stained concurrently with experimental sections, as described in the methods.