Table S1. Co-occurrence analysis of *PPP1R1B* and IGF-1R transcripts

PPP1R1B[a]	***IGF-1R***[a]	p-Value	Log odds ratio[c]	Tendency
Up[b]	Up	0.001	-1.75	Mutually exclusive
Up	Down	<0.001	1.63	Co-occurrence
Down	Up	0.01	1.016	Co-occurrence
Down	Down	0.097	-0.733	Mutually exclusive

[a]Gene name
[b]Expression trend (Up, ≥2 S.D. above mean; Down, ≤2 S.D. below mean).
[c]The effect of *PPP1R1B* over/under-expression on the likelihood of IGF-1R being over/under expressed (1, no effect on outcome; >1, increased likelihood of outcome; <1, decreased likelihood of outcome)

Table S2. Hazard ratios associated with PPP1R1B and IGF-1R expression

Gene	Expression[a]	HR[b]	95% CI[c]	p-Value
PPP1R1B	>2.5 S.D.	0.675	0.4994 to 0.9124	<0.0001
PPP1R1B	>2 S.D.	0.677	0.5593 to 0.8196	<0.0001
PPP1R1B	>1.5 S.D.	0.8813	0.7606 to 1.021	0.01
PPP1R1B	<-1.5 S.D.	0.9821	0.781 to 1.235	0.4998
IGF1R	>2.5 S.D.	1.005	0.6887 to 1.454	0.552
IGF1R	>2 S.D.	1.156	0.8798 to 1.52	0.52
IGF1R	>1.5 S.D.	1.16	0.9732 to 1.382	0.083
IGF1R	<-1.5 S.D.	0.8352	0.7242 to 0.9633	<0.0001
IGF1R	<-2 S.D.	0.9331	0.7472 to 1.16	0.032

[a]Magnitude of expression above or below the mean expression for each gene. S.D., standard deviation.
[b]HR, hazard ratio for 20 year overall survival
[c]CI, confidence interval

Table S3. Multivariate analysis of PPP1R1B and IGF-1R with HER2 for OS

Gene	Cohort	p-Value	HR[a]
PPP1R1B	All patients	0.028	1.62
IGF-1R	All patients	0.0002	0.41

[a]HR, hazard ratio for 5 year OS The correlation between gene expression and overall survival was calculated while controlling for HER2 expression in each sample.

Supplemental methods

Molecular activation of IGF-1R signaling using recombinant t-Darpp. L6 cells (ATCC® CRL1458™) were grown and maintained in Dulbecco's Modification Eagle's Medium (DMEM) containing 10% FBS in a 5% CO_2 incubator. For Western analysis (below), cells were seeded at 7 x 10^4 cells per well in a 6-well plate. Once the cells reached confluence, medium was changed to DMEM containing 2% horse serum (HS) (Atlanta Biologicals). The medium was changed every two days until the cells became fully differentiated. For the stimulation trials myotubes were grown in serum-deficient media (0.05% HS) overnight (16 hours) prior to acute treatment with t-Darpp, IGF-1 or insulin. The SK-BR-3-derived cell lines were seeded in 12-well plates at 20,000 cells/well. The following day the cells were washed with PBS and placed in serum free media (SFM) for 6 hours. The media was then aspirated and replaced with fresh SFM containing the indicated concentrations of recombinant t-Darpp for 15 minutes (dose-response assay). The preparation of cell lysates and the analysis of protein lysates by SDS-PAGE and western analyses were performed as described for tumor cells and myocytes. Cells were lysed using modified RIPA, the lysate was quantified using BCA protein assay kit (ThermoFisher Scientific) and equal amounts of protein were separated on a gradient 4%-15% SDS-PAGE and transferred to a nitrocellulose membrane. For Western analysis, 40µg of protein was denatured by boiling with SDS loading buffer and resolved by SDS-PAGE. Gels were transferred to nitrocellulose membranes by electroblotting with Towbin transfer buffer. For immunoblotting, membranes were blocked for 1 hour with 3%-5% non-fat milk and incubated with phospho-specific Akt antibody (Cell Signaling, Ser473 D9E Rabbit mAb #4060) phospho-specific IGF-1R antibody (Cell Signaling, Rabbit mAB #3204), anti-αTubulin (abcam, ab4074) or anti-Akt1 antibody (Cell Signaling, C6E7 Rabbit mAb #4691) overnight at 4°C. Membranes were incubated with horseradish peroxidase secondary antibody (Jackson Immunoresearch, goat anti-rabbit IgG H+L) and detected with enhanced chemiluminescent substrate according to the

manufacturer's protocol (ThermoFisher Scientific, Pierce ECL). Band intensity was quantified using ImageJ software.

Proteomic analysis of t-Darpp interacting proteins. SK-BR-3 and BT474 cells were grown to 80% confluence in T75 cell culture flasks, the cells were then washed twice with PBS and treated with 0.25% trypsin to detach cells. The detached cells were transferred to a 15ml tube and centrifuged at 500xg for 3 minutes. The pellet was washed with PBS and the centrifugation was repeated. The pelleted cells were solubilized by addition of 500µl of modified RIPA buffer (50mM Tris, 1%NP-40, 150mM NaCl, 0.5mM EDTA, pH 8.0) and incubation at 4°C for 30 minutes and centrifuged at 6000xg for 15 minutes to remove cell debris. The lysate was than incubated with 50µl Ni-NTA His bind resin (QIAGEN) to remove non-specific interactions and spun for 1 minute at 1000xg. The supernatant was then moved to a fresh tube and incubated with 50µl of 50µM His-tagged recombinant t-Darpp overnight at 4°C on a roller. The following day 50µl of Ni-NTA His bind resin was added for 1 hour and the cells were spun at 1000xg for 1 minute, supernatant was collected as flow through. Cells were then washed 3 times with modified RIPA containing 5mM imidazole and eluted in 500mM imidazole. Fractions were boiled with Laemmli buffer at 99°C for 5 minutes, separated on a gradient SDS-PAGE (Novex) and stained with Commassie blue. The gels were then divided into 3 parts of equal size. The proteins were digested on the gel and the peptides were analyzed by tandem mass spectrometry.

Figure S1

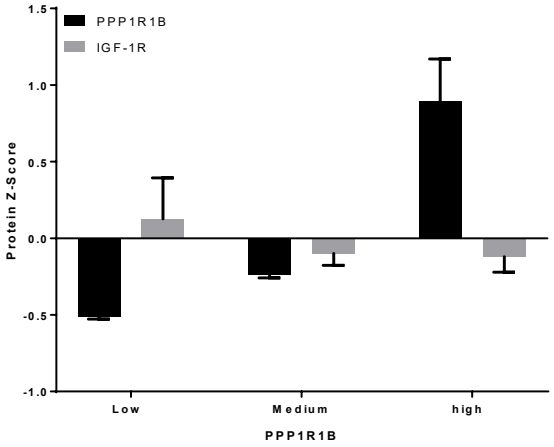


Figure S1. Protein levels of PPP1R1B and IGF-1R. Protein expression levels were obtained from the METABRICK data set. We divided all breast cancer patients into three bins of equal size according to PPP1R1B expression level (Low, Medium and High) and compared PPP1R1B and IGF-1R protein expression between the groups.

Figure S2

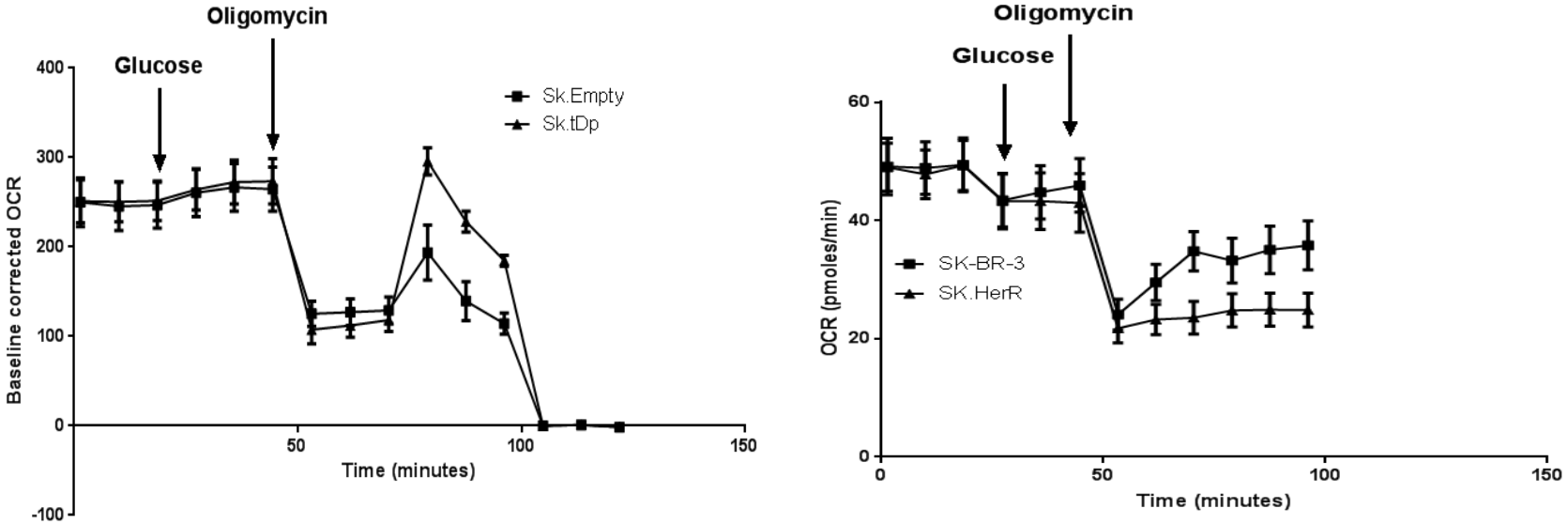


Figure S2. Comparison of respiration rates in SK-BR-3 parental and derived cell lines. Oxygen consumption rates (OCR) of SK.tDrp compared to SK.empty (left) and SK.Her^{R} compared to SK-BR-3 (right) following glucose injection.

Figure S3

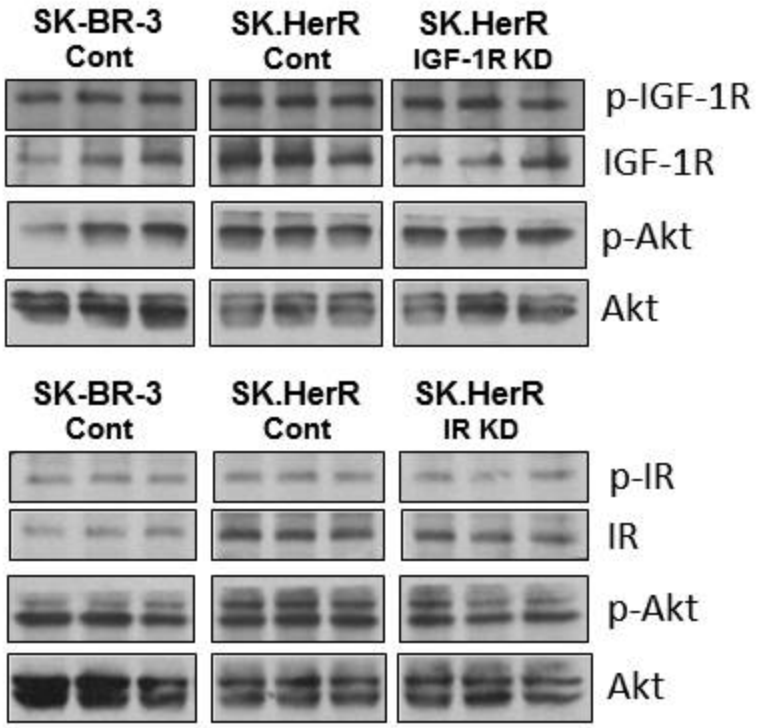


Figure S3. siRNA knockdown of IGF-1R and IR.
Western blot of total cell lysates from IGF-1R and IR in SK.HerR cells following 48 hour treatment with the indicated siRNAs

Figure S4

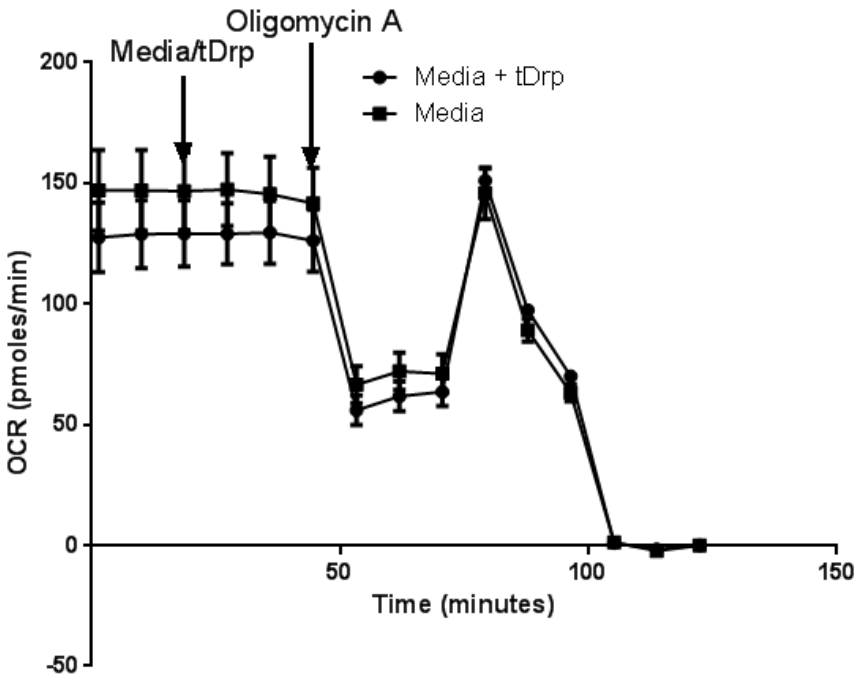
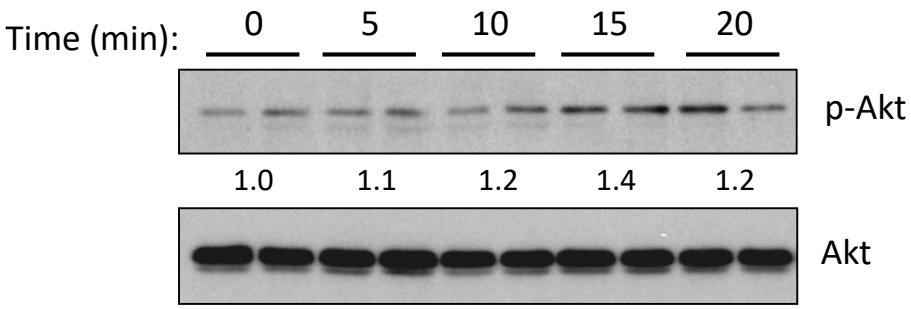
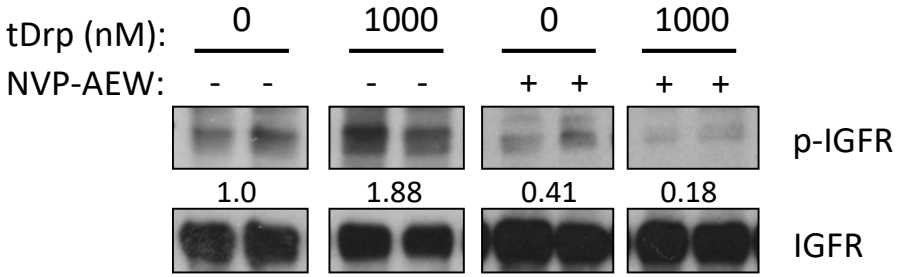
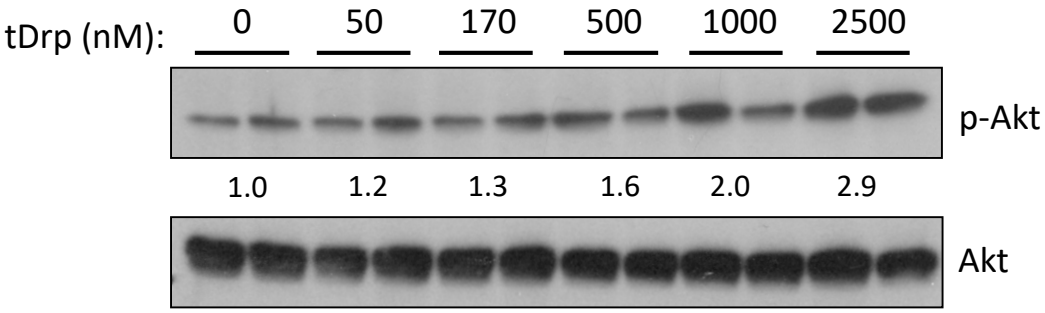


Figure S4. The effect of recombinant t-Darpp on oxygen consumption rate (OCR) of SK-BR-3 cells. Changes in OCR following injection of media (squares) or media supplemented with 500nM t-Darpp (circles). Arrows indicate the time of injection of indicated compounds during the trace.

Figure S5







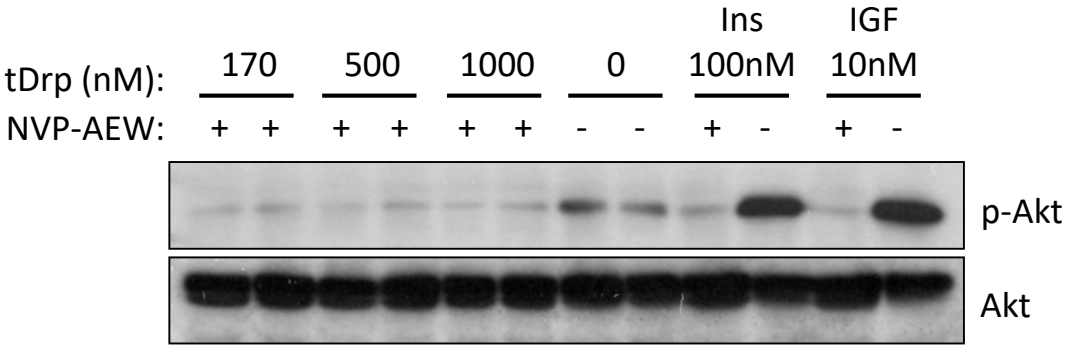


Figure S5. Western analysis of IGF-1R and Akt phosphorylation in L6 myotubes by treatment with recombinant t-Darpp. (Top left) Time course of Akt phosphorylation following treatment with 500nM recombinant t-Darpp. (Middle) Dose response for t-Darpp stimulation of Akt phosphorylation following 15 minute treatment with indicated concentrations. (Bottom) Phosphorylation of Akt following 15 minute treatment with recombinant t-Darpp at the indicated concentrations after pre-treatment with 2uM NVP-AEW. (Top right) IGF-1R phosphorylation following treatment with recombinant t-Darpp protein -/+ pre-treatment with 2uM NVP-AEW.

Figure S6

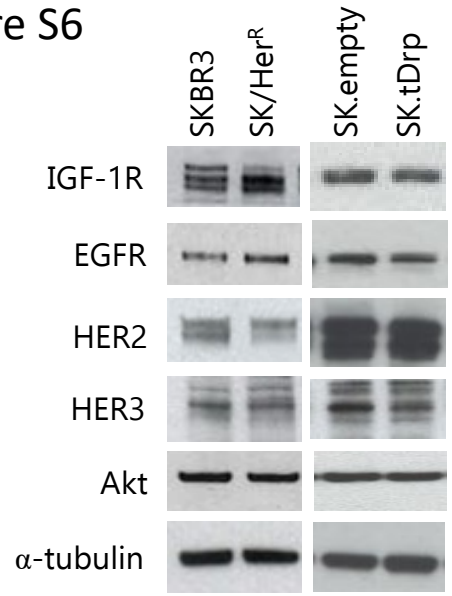


Figure S6. Relative endogenous receptor levels in SK-BR-3 and derived cell lines. Western analysis of IGF-1R, EGFR, HER2, HER3 and Akt in the indicated cell lines.