**Description of experimental procedure and corresponding quantification**

**Cell treatment**

* All cells were cultured in DMEM with 4.5 g/L glucose, 10% FCS and L-glutamine
  + 6x 6 cm plates were seeded with MCF-7 cells
  + 6x 6 cm plates were seeded with T47D cells
* Cells were washed 2x with 3 mL of PBS and then starved overnight with 3 mL HBSS (begin around 16:00)
* Cell confluence at beginning of the experiment varied between 60% – 85%
* Cells were stimulated with amino acids and insulin by replacing the HBSS with DMEM with 1.0 g/L glucose and L-glutamine (without FCS), and 100 nM insulin.
* Cells were stimulated for 0, 15, 30, 60, 90 and 120 minutes.
* For the 0 min stimulation the HBSS was refreshed at the same time as the longest time point.
* Lysis starts in the morning around 10:30.

**Lysis protocol**

* All steps were performed on ice.
* Plates were washed 3x with ice cold PBS and PBS was removed
* 150-200 µl Lysis buffer was added (plus phosphatase (Cock. 2&3 1:100) and protease (complete +EDTA 1:25) inhibitors) per 6 cm plate (amount is depending on the confluency of the cells).
* Scrape the adherent cells
* Cells were lysed by pipetting up and down
* Cell lysate was transferred to eppi tube and and lysate was incubated for 5 min on ice
* Centrifuge 10 min, 6000 g, 4°C
* White pellet was discarded and supernatant was transferred to fresh eppi tube
* For each sample a bradford OD measurement was performed at 595 nm:
  + Bradford reagent was diluted 1:5 with mQ water
  + 1 ml diluted bradford reagent per sample (plus 1 reference sample) was used
  + 4 µl sample was used
* Samples were adjusted to the same protein level after OD measurement with and ¼ 5x Lämmli buffer in relation to sample volume was added.
* Samples were frozen at -20°C or directly loaded on gel after boiling the samples for 5 min at 95C.

**Western Blot**

* 3x 8 %, 3x 10 % and 2x 14 % gels were used
* Heat samples for 5 min at 95°C in heatblock
* Vortex shortly and spin down
* Load samples: marker All Blue 4 µL, sample 12µL and marker dual color 2µL
* Let samples run in at 90 V until samples reach the lower gel, then switch to max. 190 V
  + One 10% gel was used for total protein staining with coomassie blue:
    - Gel was washed 3x for 5 min with milliQ while shaking.
    - SimplyBlue SafeStain was added for 1 hour and for reducing the background milliQ was added while shaking overnight at 4°C.
    - After the first hour the milliQ was refreshed.
    - Staining was detected with the camera system (Amersham Imager 600).
* Activate PVDF membrane in methanol
* Blot the gel on the short plate (otherwise detection will be mirror inverted) and assemble the gel sandwish in new blotting buffer
* Blot the gel using blotting buffer at 45 V per blotting chamber for 1 h and 50 minutes
* Block membranes for 20 min in 5% BSA in TBST at RT
* Let 1st antibody shake overnight, 4°C
* Wash membranes 3x shortly with TBST and 3x 10 min with TBST shaking at RT
* Incubate membrane with 2nd antibody in 2.5% BSA in TBST shaking for > 2h at RT
* Wash membranes 3x shortly with TBST and 3x 10 min with TBST shaking at RT
* Detect proteins with Pierce ECL and SUPER-FEMTO Western Blotting Substrate and camera system (Amersham Imager 600).
* The raw images were exported as RGB color TIFF files using ImageJ and further processed with Adobe Photoshop CS6

**Quantification**

The quantifications of the immunoblots were obtained by quantifying the raw image files using ImageQuant TL, GE Healthcare.

All protein levels were normalized to the average intensity. Afterwards to each sample was normalized to the respective lane in a coomassie blue staining. Coomassie blue stains all proteins in a sample.

The normalized data were statistically analyzed using GraphPad Prism 5.00:

A two-way-ANOVA test was used for the comparison of the multiple groups in the time course experiments. The test was followed by Bonferroni's multiple comparison tests.

When the p-value was below 0.05, the data was considered significant.