

Supplementary Data 1

Localisation of IR and PTP1B fusion proteins by confocal microscopy.

48h-transfected HEK cells were labelled with either anti-calnexin antibody (1:200) and a Cy3-conjugated anti-rabbit antibody (1:150) or 83-14 anti-IR antibody (1:200) and a Cy5-conjugated anti-mouse antibody (1:150). Immunofluorescence images were collected in a scanning confocal microscope (MRC 1000, Bio-Rad). (A) YFP-PTP1B-wt and YFP-PTP1B-D181A are mainly co-localized with an endoplasmic reticulum marker (calnexin), although some YFP-PTP1B-D181A also appears to concentrate at cell-cell contacts. (B) In the basal state, a substantial amount of insulin receptors co-localized with YFP-PTP1B-D181A in the endoplasmic reticulum.

Supplementary Data 2:

Inhibitory effect of H₂O₂ on the interaction between the insulin receptor and PTP1B-D181A.

A common characteristic of protein tyrosine-phosphatases is the presence of a cysteine residue in the catalytic site of the enzymes. Oxidation of this cysteine by reactive oxygen species, such as H₂O₂, induces the formation of sulfenyl groups. As a consequence, the modified cysteine can no longer function as a phosphate acceptor. We observed that 20 min treatment with 0.5 mM H₂O₂ markedly inhibits the effect of 100 nM insulin on BRET signal. Results are representative of at least 3 independent experiments.

Supplementary Data 3:

Effect of the tyrphostin AG1024 on the interaction between the IR and the substrate-trapping mutant of PTP1B. HEK cells co-expressing IR-Rluc and YFP-PTP1B-D181A were pre-incubated for 1h in the absence or the presence of 100 μM AG1024. Cells were then stimulated with 100 nM insulin. (A) Cell extracts were analyzed by

western blot using anti-phosphotyrosine antibody (4G10). (B) AG1024 inhibits basal and insulin-stimulated BRET signal. Results are representative of at least four independent experiments.

Supplementary Data 4:

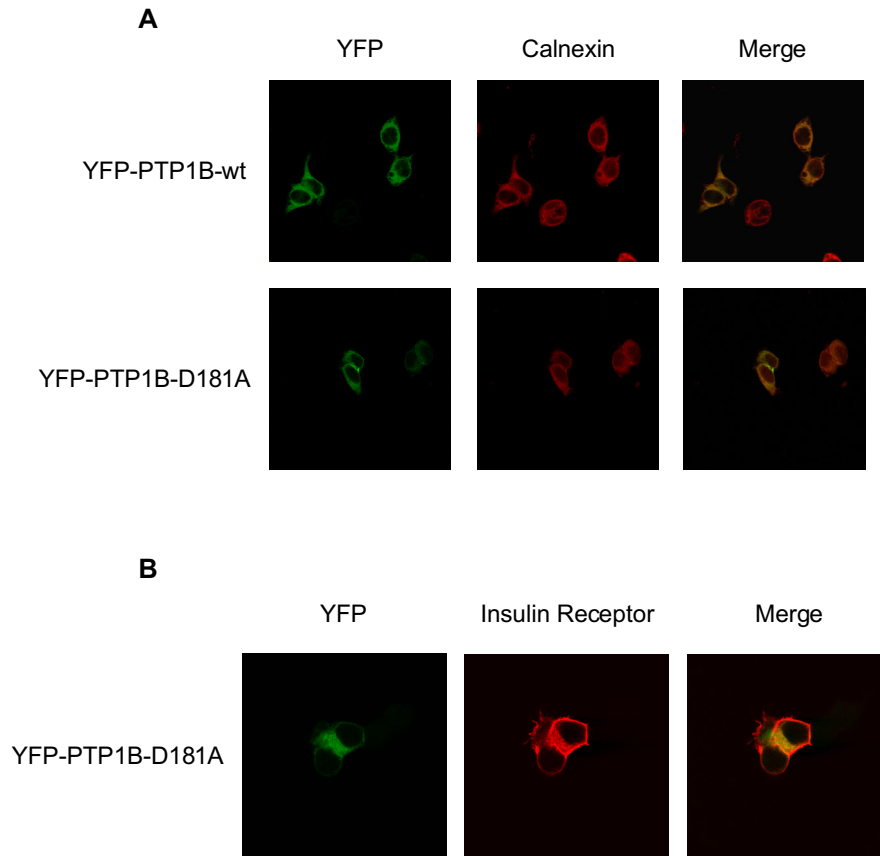
BRET measurements in subcellular fractions of HEK cells expressing IR-Rluc and YFP-PTP1B-D181A or YFP-PTP1B-D181A-Cter.

HEK-293 cells were co-transfected with IR-Rluc and YFP-PTP1B-D181A or YFP-PTP1B-D181A-Cter cDNA. 48 h after transfection, cells were lysed in ice-cold hypotonic buffer (20 mM Hepes, pH 7.4, 2 mM EDTA, 2 mM EGTA, 6 mM magnesium chloride, 1 mM AEBSF, and 1mg/ml each aprotinin, pepstatin, antipain and leupeptin) and homogenized by eight passes through a ball-bearing homogenizer (Cell craker EMBL). The lysates were layered on continuous 0.25 - 2 M sucrose gradients. The samples were centrifuged at 48 000 rpm in a Beckman SW55 rotor for 6 h at 4°C. The fractions were collected from the top of the tube. BRET measurements were performed in a final volume of 50 μ l containing 20 μ l of fraction and 5 μ l of coelenterazine.

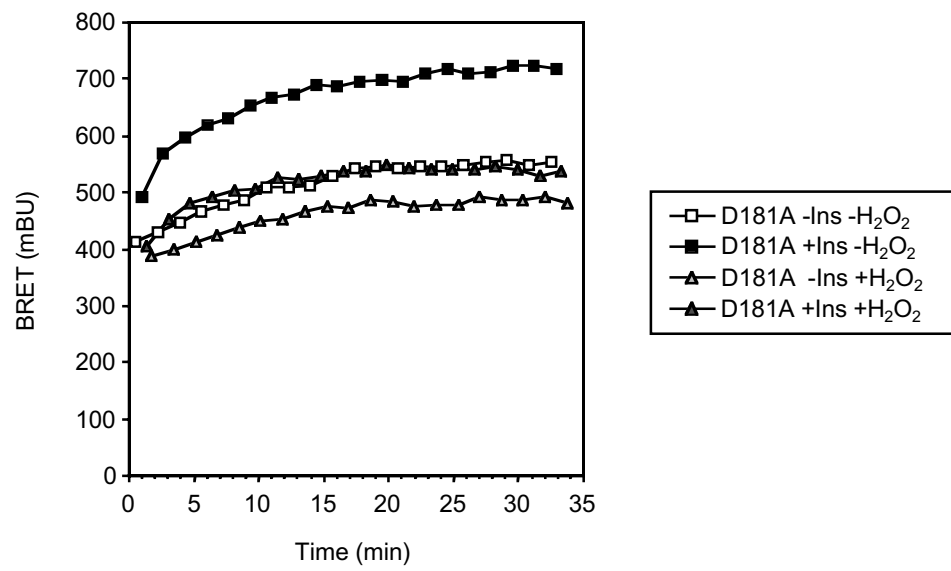
The presence of plasma membrane (5'-nucleotidase activity) and endoplasmic reticulum (calnexin) markers was assessed in each fraction. 5' nucleotidase activity was assessed using a kit from Sigma Diagnostic. The presence of calnexin was detected by western-blotting using a rabbit polyclonal antibody.

The data are representative of two independent experiments.

Supplementary Data 1

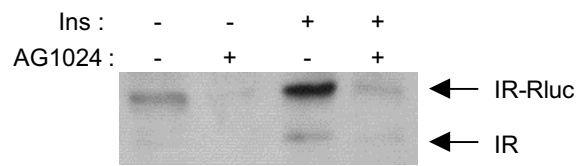


Supplementary data 2

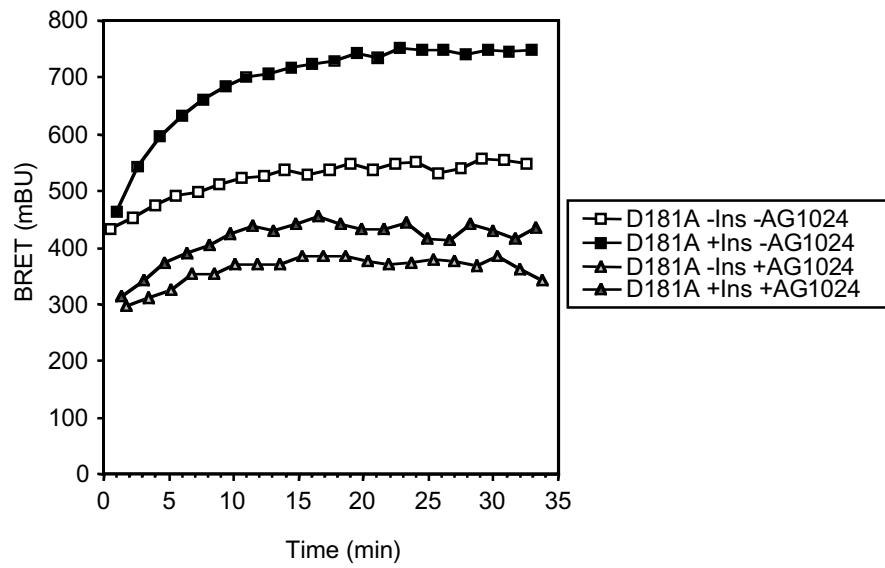


Supplementary Data 3

A



B



Supplementary Data 4

