Supplemental Figure S1: Inhibition of insulin receptor autophosphorylation by AG1024

HEK-293 cells were transfected with IR-Rluc and YFP-PTP1B-D181A-Y46F. 48h after transfection, cells were pre-incubated for 1 hour in absence or presence of 100 μM AG1024 and then incubated for 5 minutes in the absence or presence of 100 nM insulin. Cells were extracted and receptors were partially purified on WGL-agarose beads. Phosphorylation on tyrosine residues was detected by immunoblotting using an anti-phosphotyrosine antibody (4G10). AG1024 markedly inhibited the tyrosine-phosphorylation of the β-subunit of the IR (β-Rluc). AG1024 also inhibited the tyrosine-phospohrylation of a band with high-molecular weight corresponding to the αβ-Rluc precursor. Results are representative of 3 independent experiments.

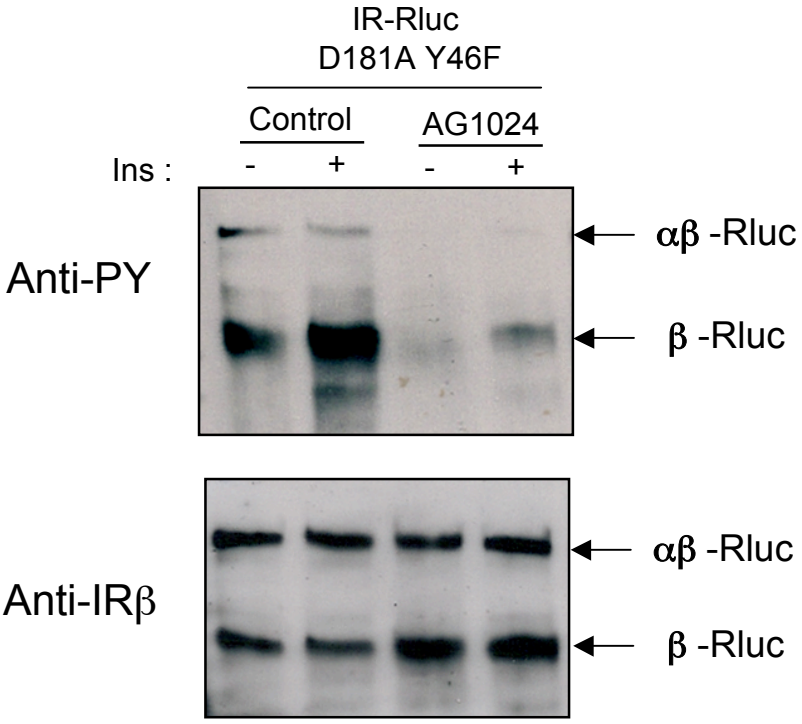
Supplemental Figure S2: Basal BRET signal and insulin-induced BRET depend on the amount of PTP1B-D181A-Y46F transfected in the cells

HEK-293 cells were transfected with 300 ng of IR-Rluc and either 150 ng or 600 ng of PTP1B-D181A-Y46F cDNA/well. 48h after transfection, BRET experiments were performed as described in the method section. (A) Basal and insulin-stimulated BRET were measured in real time during more than 25 min. Basal BRET signal was higher whereas insulin effect was lower in cells transfected with 600 ng/well than in cells transfected with 150 ng/well of PTP1B-D181A-Y46F cDNA, suggesting trapping of the IR precursor in the endoplasmic reticulum and reduction of expression of insulin-responsive mature receptor at the cell surface. (B) Basal and (C) insulin-induced BRET signal at time 20 min. Results are the mean of 3 independent experiments. *** $P < 0.001$.

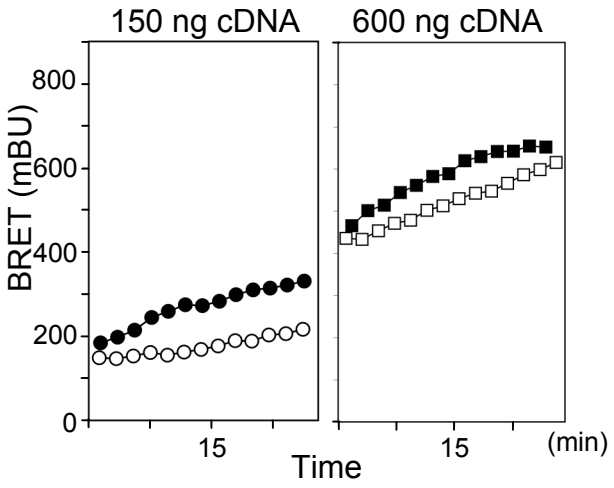
Supplemental Figure S3: The dynamics of interaction with PTP1B is similar for IR_A and IR_B isoforms. HEK-293 cells were transfected with 300 ng of IR_A-Rluc or IR_B-Rluc and 600 ng of either PTP1B-D181A or PTP1B-D181A-Y46F cDNA/well. 48h after transfection, BRET experiments were performed as described in the method section. Basal and insulin-stimulated BRET were measured in real time during more than 25 min. (A) Dynamics of interaction between IR_A and PTP1B mutants. (B) Dynamics of interaction between IR_B and PTP1B mutants. (C) Comparison of basal BRET signal at time 20 min in cells expressing either IR_A and IR_B. Results are the mean of 2 experiments.

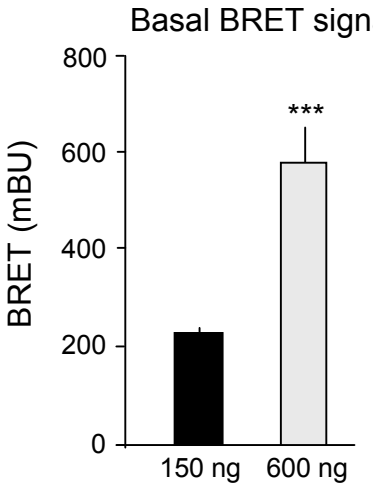
Supplemental Figure S4: Similar amounts of uncleaved IR_A and IR_B precursors are recovered in the fully activated, Tris-phospohorylated form. HEK-293 cells were transfected with either IR_A-YFP or IR_B-YFP and untagged versions of PTP1B-D181A or PTP1B-D181A-Y46F. 48h after transfection, cells were incubated for 5 min in presence of 100 nM insulin and lysed. The IR were immuno-precipitated using an anti-GFP antibody and immunoblotted with an antibody directed against the tris-phosphorylated form of the activation loop of the kinase domain. The membranes were then reprobed with an anti-GFP antibody. Results are representative of 2 independent experiments.

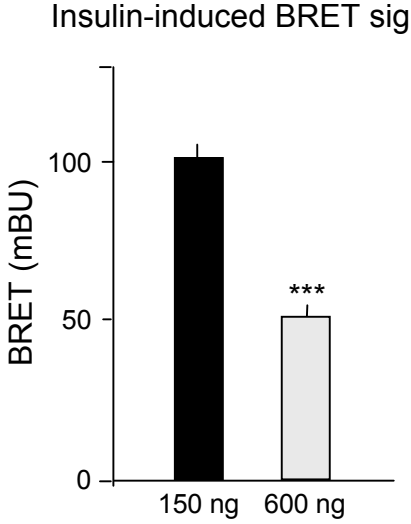
Supplemental Figure S1



Supplemental Figure S2

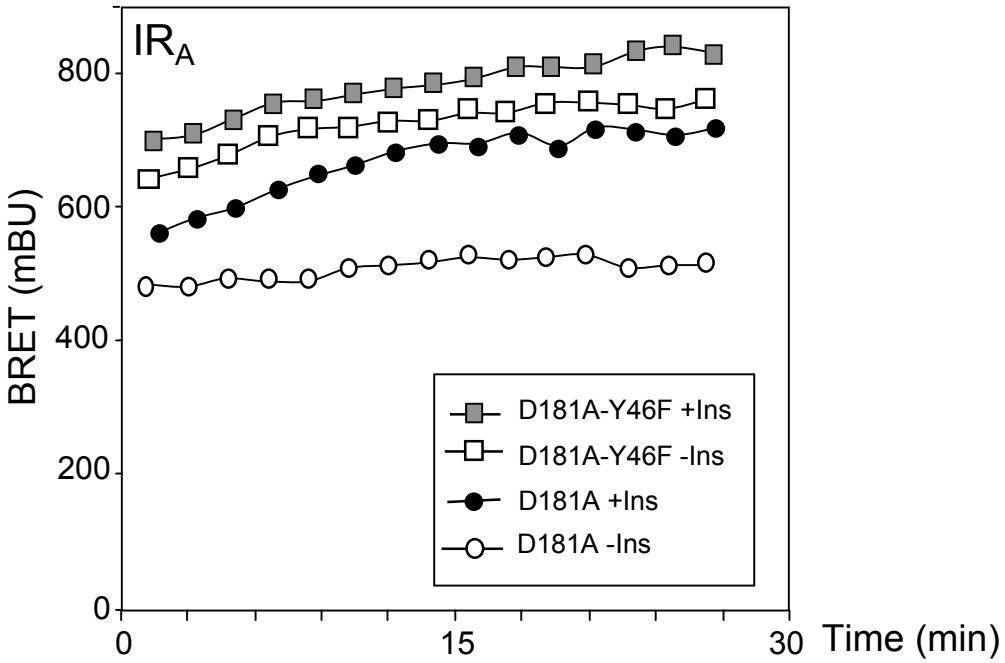




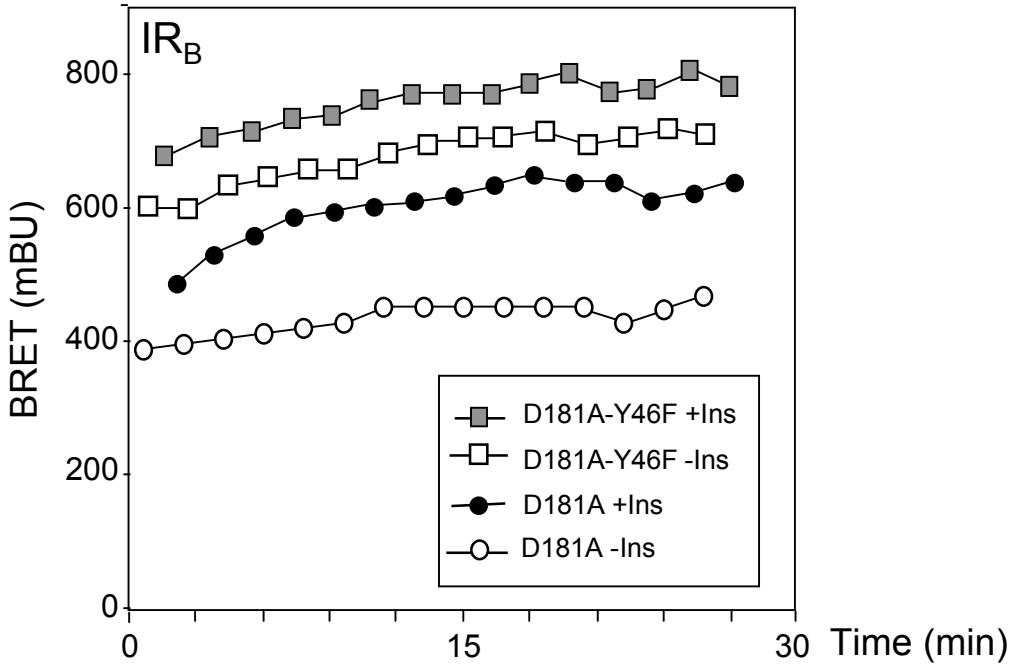


Supplemental Figure S3

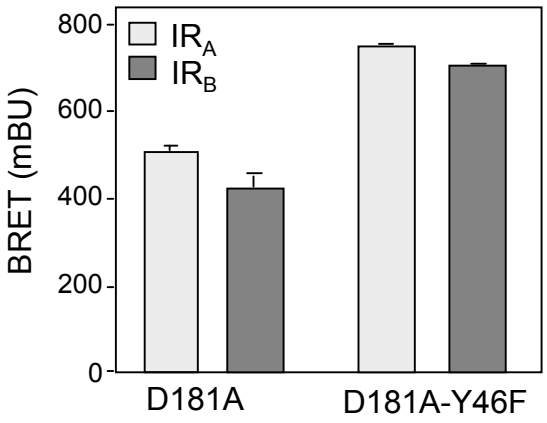
A



B



C



Supplemental Figure S4