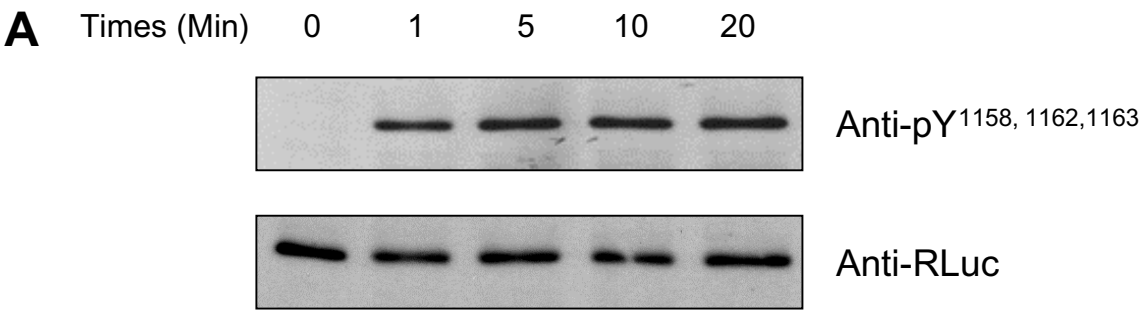
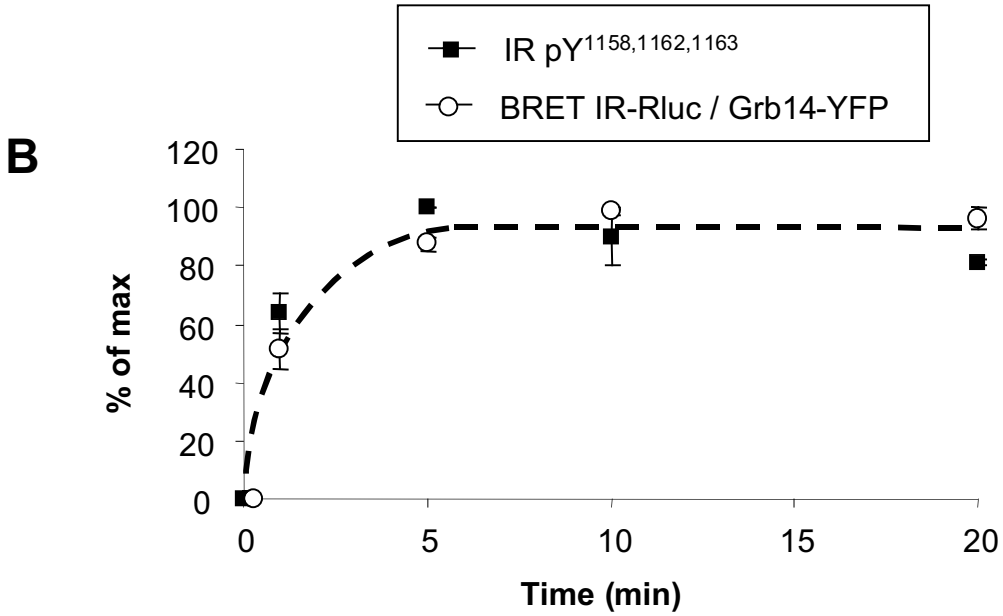
Supplementary information 1





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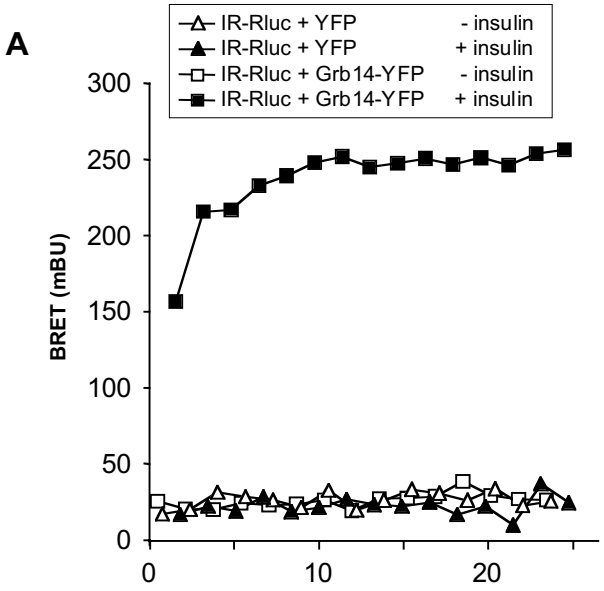

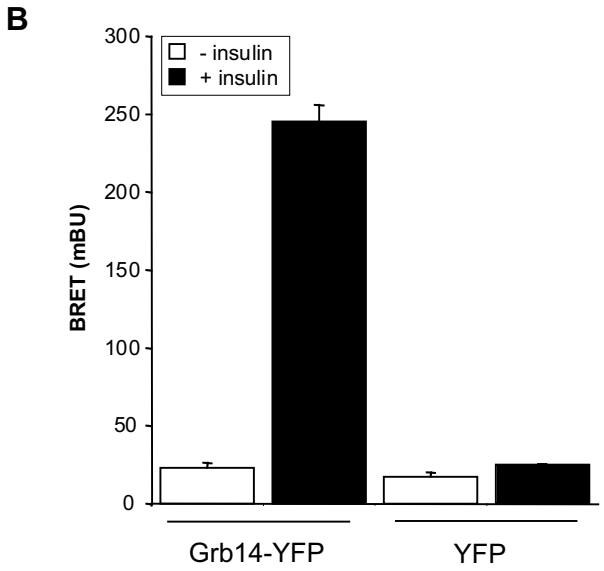

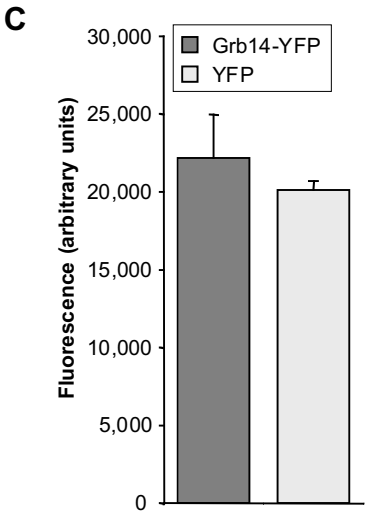

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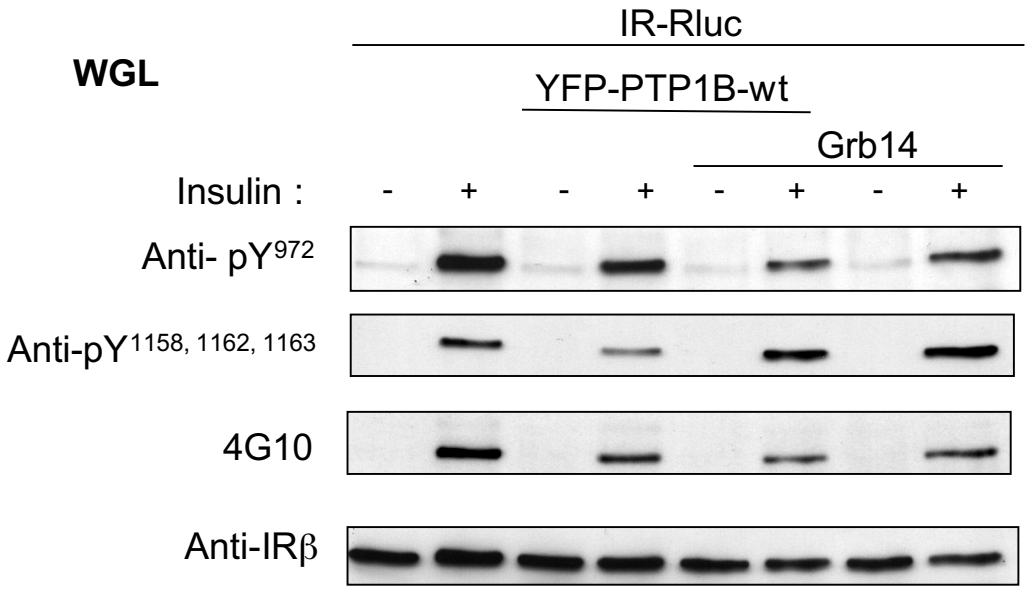

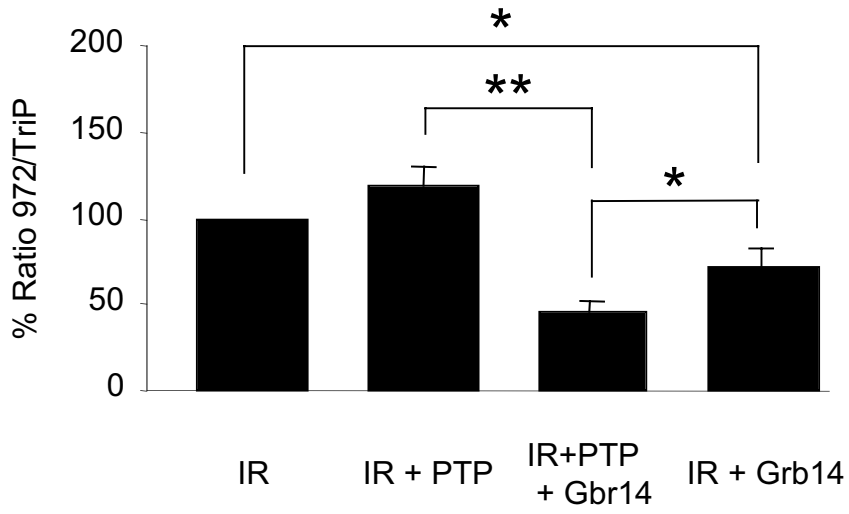

Supplementary information 4

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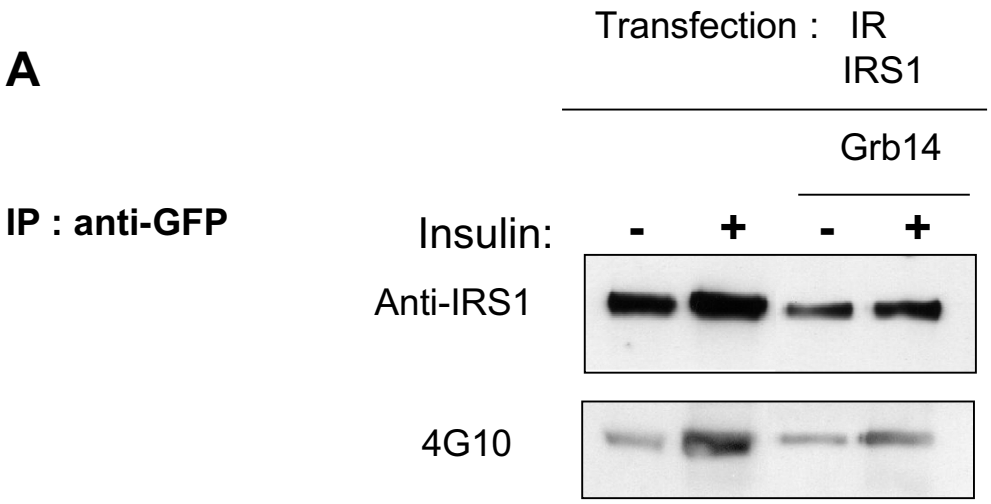

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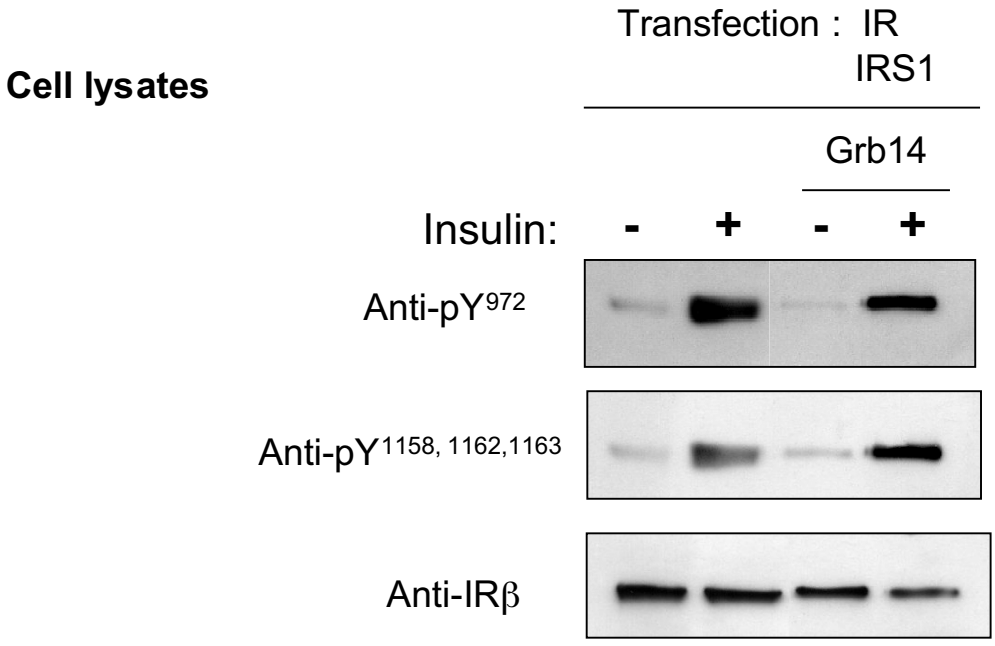

Supplementary information 4

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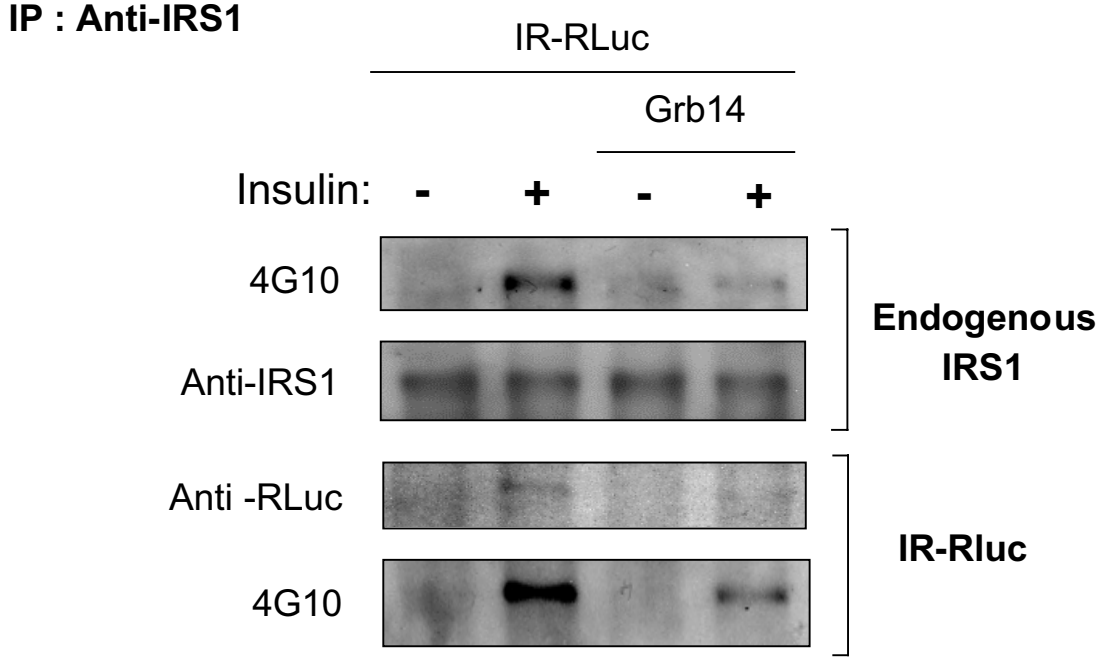

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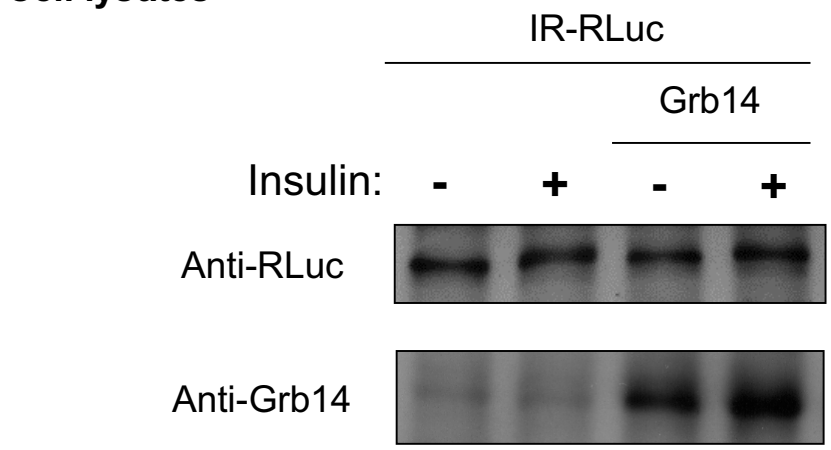
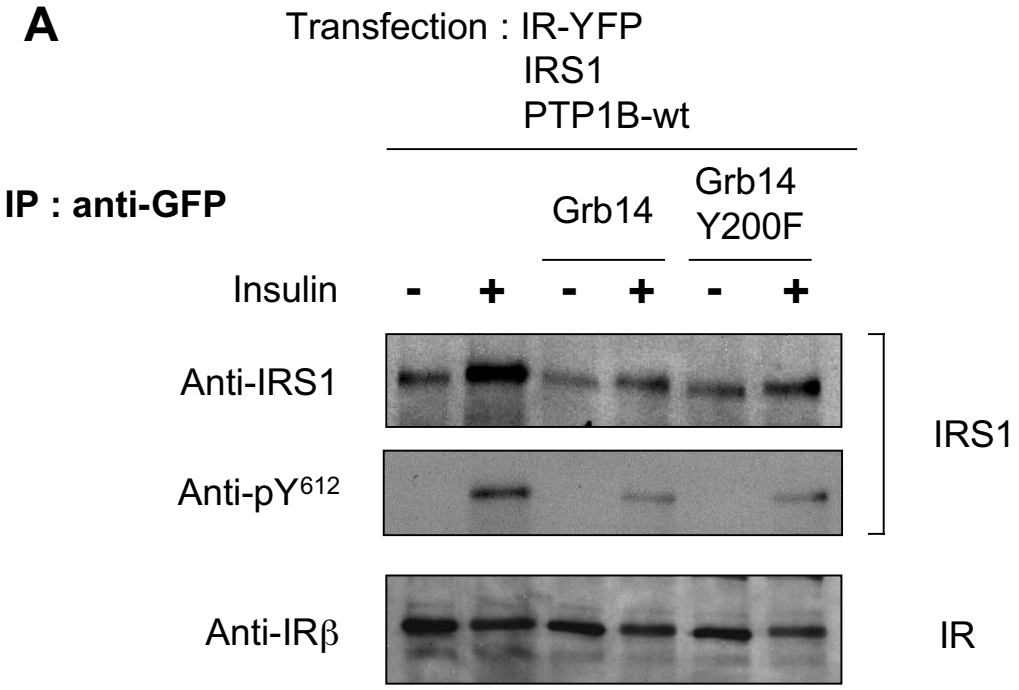
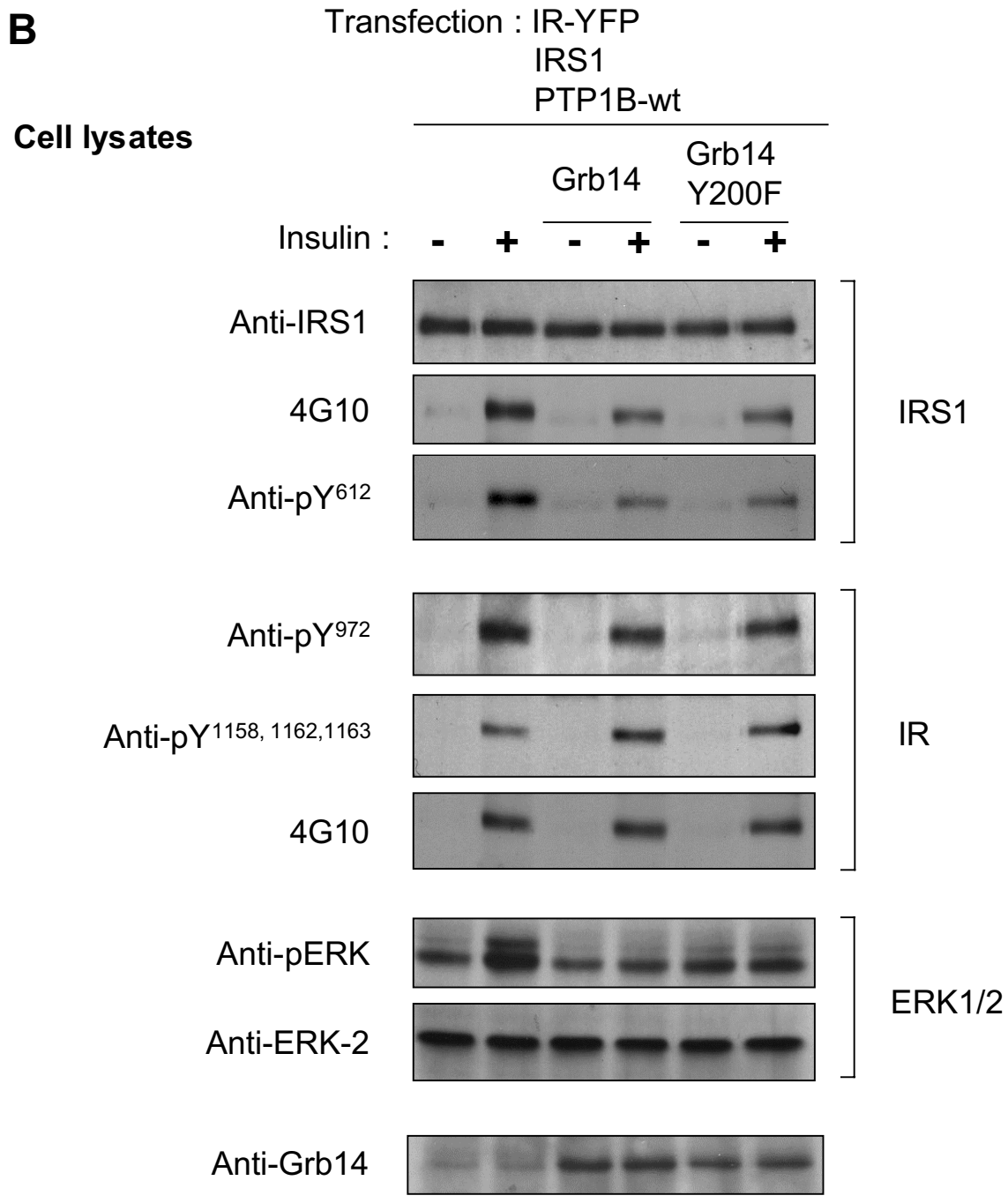


Supplementary information 5



Supplementary information 5

B



Supplementary information 1

Insulin-induced interaction between IR and Grb14 closely follows insulin-induced phosphorylation of the three tyrosines of the activation loop.

Binding of Grb14 to the activated IR is believed to depend on the phosphorylation of tyrosines located in the activation loop of the kinase domain (Y1158, 1162 and 1163). To determine whether insulin-induced BRET signal correlates with insulin-induced phosphorylation of this domain, HEK cells co-transfected with IR-Rluc and Grb14 were incubated with insulin for 1, 5, 10 and 20 min. (A) Insulin receptors were purified on WGL agarose beads and submitted to SDS-PAGE and western-blotting. The phosphorylation level of the receptor was evaluated using an antibody that specifically recognizes the tris-phosphorylated form of the activation loop of the IR. The amount of IR-Rluc present in each well was evaluated using an anti-*Renilla* Luciferase antibody (Chemicon). (B) Densitometric analysis of the phosphorylation of the three tyrosines of the activation loop. Insulin-induced BRET signals at the same time-points were reported on the same graph for comparison. Results are the means of 2 independent experiments.

Supplementary information 2

Specificity of insulin-induced BRET signal between IR and Grb14.

(A) BRET signal was monitored in HEK-293 cells co-transfected with IR-Rluc and either Grb14-YFP (300 ng/well) or YFP (50 ng/well). Cells were pre-incubated for 15 min in presence of coelenterazine (5 μM). Insulin (100 nM) was then added and BRET measurements were started immediately (B) Basal and insulin-stimulated BRET at time 20 min. (C) Fluorescence level of cells transfected with Grb14-YFP or YFP. Results are means ± SEM of 2 independent experiments.

Supplementary information 3

Effect of Grb14 alone on the phosphorylation of the IR in HEK 293 cells

HEK cells were co-transfected with IR-Rluc alone or in combination with YFP-PTP1B-wt, Grb14 or both. Cells were stimulated or not with 100 nM insulin for 10 min and lysed. (A) After partial

purification of IR on WGL, the phosphorylation of the IR was evaluated by western-blotting using a general anti-phosphotyrosine antibody (4G10), or site specific phospho-antibodies (anti-$pY^{1158,1162,1163}$ and anti-pY^{972}). The amount of receptors loaded in each lane was evaluated using an anti-IRβ antibody. (B) After densitometric analysis of the anti-$pY^{1158,1162,1163}$ and anti-pY^{972} phosphotyrosine signals, the $pY^{972}/pY^{1158,1162,1163}$ ratio was calculated. The data presented are means ± SEM of 3 independent experiments (*, $P<0.05$; **, $P<0.01$). We observed that even in the absence of co-transfected PTP1B, Grb14 alone was capable of increasing the phosphorylation of the three tyrosines of the activation loop while decreasing the phosphorylation of Y972. Co-expression of PTP1B further decreases the $pY^{972}/pY^{1158,1162,1163}$ ratio.

Supplementary information 4

Effect of Grb14 alone on the interaction of the IR with IRS-1.

(A) HEK cells were co-transfected with IR-YFP, IRS-1 and either Grb14 or pcDNA3. Cells were stimulated with 100 nM insulin for 10 min and lysed. (A) The IR was immunoprecipitated with an anti-GFP antibody. The level of IRS-1 co-precipitated with the IR was evaluated with an anti-IRS-1 antibody. We observed that even in the absence of co-transfected PTP1B, Grb14 alone was capable of decreasing the association of IRS-1 with the IR. (B) Cell extracts from the same cells were submitted to western blotting and immunodetected with anti-IRS-1, anti-$pY^{1158,1162,1163}$, and anti-pY^{972} antibodies. (C) HEK cells were transfected with IR-Rluc and either Grb14 or pcDNA3. Endogenous IRS-1 was immunoprecipitated and the amount of IR-Rluc co-precipitated with IRS-1 was evaluated using an anti-*Renilla* Luciferase antibody. Association of IR-Rluc with endogenous IRS-1 could be detected with both anti-Rluc and anti-phosphotyrosine antibody. This association was markedly decreased in cells co-transfected with Grb14. (D) IR-Rluc and Grb14 amounts in lysates from the same cells.

Supplementary information 5

Mutation of Y200 in the NPXY motif of Grb14 does not impair the inhibitory effect of Grb14 on the association between IR and IRS-1

The Grb14-Y200F mutation was performed on a pcDNA3-Grb14 plasmid by site directed mutagenesis (QuickChange mutagenesis, Stratagene). HEK cells were co-transfected with IR-YFP, IRS-1, PTP1B, without or with either Grb14 or Grb14-Y200F. Cells were stimulated with 100 nM insulin for 10 min and lysed. (A) The IR was immunoprecipitated with an anti-GFP antibody. The level of IRS-1 co-precipitated with the IR was evaluated using an anti-IRS-1 antibody. (B) Crude cell extracts from the same cells were submitted to western blotting and immunodetected with site specific phospho-antibodies.

Using an anti IRS-1[pY^{612}] antibody (Biosource), we observed that both wild-type and mutated Grb14 markedly decrease the phosphorylation of tyrosine 612, which serves to recruit and activate PI3-kinase. This strongly suggests that an increase in Grb14 expression may also affect the PI-3 kinase pathway.