Supplementary Figure 1
Supplementary Figure 1. Confocal microscopic images showing the effect of BMOV on the phosphorylation of total tyrosines in IM9 cells. (A) Dose-and time-dependent induction of tyr-phosphorylation by BMOV in cells receiving insulin post-treatment. Cells were incubated with 50 or 100 µM of BMOV for the indicated time periods and co-treated with 100 nM insulin for 15 min prior to sampling. The corresponding controls received the treatment of BMOV vehicle accordingly in addition to 15 min exposure to 100 nM insulin. After desired treatments, cells were fixed, permeabilized, and blocked as described under Materials and Methods and stained for cellular phosphorylated tyrosines using anti-phosphotyrosine monoclonal primary and CY3-conjugated anti-mouse monoclonal IgG secondary antibodies and subsequently visualized on a confocal microscope. The image marked with ‘Control’ represents the typical staining pattern of cells serving as controls for different BMOV treatments. (B) The impact of insulin on the BMOV-induced tyr-phosphorylation of IM9 cells was assessed separately in another experiment. For this, cells were incubated with 0, 50 or 100 µM of BMOV for 18 h with (+) or without (-) being subjected to insulin post-treatment and processed as above to determine the level of cellular tyr-phosphorylation.
Supplementary Figure 2
Supplementary Figure 2. Confocal microscopic images showing the effect of dexamethasone on the level of IRβ in IM9 cells. After 18 h incubation with the indicated doses of dexamethasone, the cells were fixed and blocked as described under Materials and Methods and stained for IRβ using affinity purified anti-IRβ polyclonal primary and CY3-conjugated anti-rabbit monoclonal IgG secondary antibodies. Immunofluorescence images were acquired using a confocal microscope. The intensity of peripheral staining of cells marking membrane-bound IRβ was increased in response to BMOV and was brightest at 10 µM dose of the drug.