

Fig. 2: IR immunoprecipitation and mass spectrometry

(A) The G/E fraction was prepared as depicted for Figure 2 and methods except that rats were preinjected with the PTP inhibitor bpV(phen) (intraperitonally, 0.5 mg/100g b.w., 30 minutes before the experiments). The membranes were analyzed using the anti-IR and anti-phosphotyrosine (pY-95 kDa) antibodies. (B) Membranes were solubilized and submitted to the immunoprecipitation procedures (0.3% EmpigenBB, Fiset, A. et al. Cell Signal 23, 911-919, 2011). Protein bands that were stained with SYPRO Ruby (Bio-Rad, Hercules, CA) were excised and subjected to tryptic digestion. The resulting peptides were separated and analyzed by tandem mass spectrometry (Online Table 2). (C) Electron microscopy of the G/E fraction shows typical tubulovesicular structures as well as lipoprotein-filled vesicles of 70-200 nm.