

Figure S3: Differences in protein expression and phosphorylation of insulin-treated healthy (h) and diabetic (ff) hearts.

Panel A: Global GLUT4 protein levels (measured in total tissue extracts) are significantly reduced in the diabetic insulintreated hearts. Panel B: Insulin receptor substrate-1 (IRS-1) Ser307 phosphorylation is increased in diabetic hearts as compared to healthy hearts. Panel C: Global JNK protein levels (measured in total tissue extracts) are significantly increased in the diabetic heart. Panel D: Protein levels of novel PKC0 are significantly increased in the total tissue fraction (normalized to tubulin) and in the membrane fraction (normalized to Na,K-ATPAse) of diabetic hearts as compared to healthy hearts. Protein levels of novel PKC0 in the cytosolic fraction are unchanged.

Abbreviations are as follows: h, healthy (hearts from control rats); ff, hearts from fructose-fed rats: INS, treatment with insulin; IRS-1, Insulin receptor substrate-1; JNK, Jun amino-terminal kinase; PKC  $\theta$ , protein kinase C,  $\theta$  isoform. Results are presented as means  $\pm$  SD. \*p<0.05, significantly different from healthy. N=4.