SUPPLEMENTARY DATA

Role of AP-1 in rosiglitazone mediated downregulation of follistatin. The follistatin promoter contains binding sites for AP-1 in the proximal region (Fig. 6). To address the importance of the Ap-1 in mediating the observed inhibitory effects of PPARγ, we first blocked AP-1 signaling with SP600125, a specific inhibitor of JNK. SP600125 was able to efficiently block anisomycin induced JNK phosphorylation (p54 JNK; Suppl. Fig. 1A) but had no significant effect on rosiglitazone-mediated downregulation of follistatin in RIE-PPARγ cells (Suppl. Fig. 1B). As regulation of AP-1 can occur by mechanisms downstream and independent of JNK such as direct protein-protein interaction with AP-1, we further investigated the role of AP-1 by targeting the complex with TAM67, and TAM67, a dominant negative form of c-Jun. A RIE-PPARγ cell line was generated that stably expresses TAM67 (Suppl. Fig. 1C). Expression of TAM67 effectively inhibited AP-1 activity as illustrated by its ability to block PMA-induced AP-1 transcription activation of an AP-1 responsive reporter gene in RIE-PPARγ-TAM67 cells (Suppl. Fig. 1D). However, TAM67 expression had no effect on rosiglitazone-mediated downregulation of follistatin mRNA expression (Suppl. Fig. 1E). Taken together, these data indicate that AP-1 signaling does not mediate PPARγ inhibition of follistatin expression.

Suppl. Fig.1. **Downregulation of follistatin expression by rosiglitazone is not mediated through AP-1 signaling** *A.* The efficacy of SP600125 was shown by treating RIE-PPARγ cells with ± anisomycin (10 μM) and ± SP600125 for 1 h. Western blot of total cell lysate (10 μg) stained for total JNK, β-actin and the three forms of phosph-JNK (p54, p46, and p42) using PhosphoPlus SAPK/JNK (Thr183,Tyr185) antibody kit (Cell Signaling). Analysis of the p46 and p42 phospho-JNK forms is obscured by cross-reactivity of the phospho-JNK antibody with p44 and p42 forms of MAP kinase. *B.* RIE-PPARγ cells were pretreated for 1 h with an inhibitor of JNK (SP600125, 10 μM) and subsequently exposed to 6 h ± rosiglitazone. qPCR was used to measure follistatin mRNA abundance from each sample. *C.* Western blot of 20 μg of total cell lysate from RIE-PPARγ and RIE-PPARγ-TAM67 cell lines stained for c-jun expression (Santa Cruz). Expression of c-jun and the truncated dominate negative c-jun, TAM67, are shown. *E*. Transcriptional activity of an AP-1 responsive reporter gene, $(AP1)_3$ p54 IL8 Luc, in RIE-PPARγ and RIE-PPARγ-TAM67 cells in response to PMA (100 nM) for 6 h. *F*. Follistatin mRNA abundance was measured by qPCR in RIE-PPARγ and RIE-PPARγ-TAM67 cell lines treated with rosiglitazone (1 μM) for 6 h. Follistatin mRNA was normalized to GAPDH and expressed as fold change relative to the control. Data represent mean ± S.D., n=3.

Supplementary Figure 1