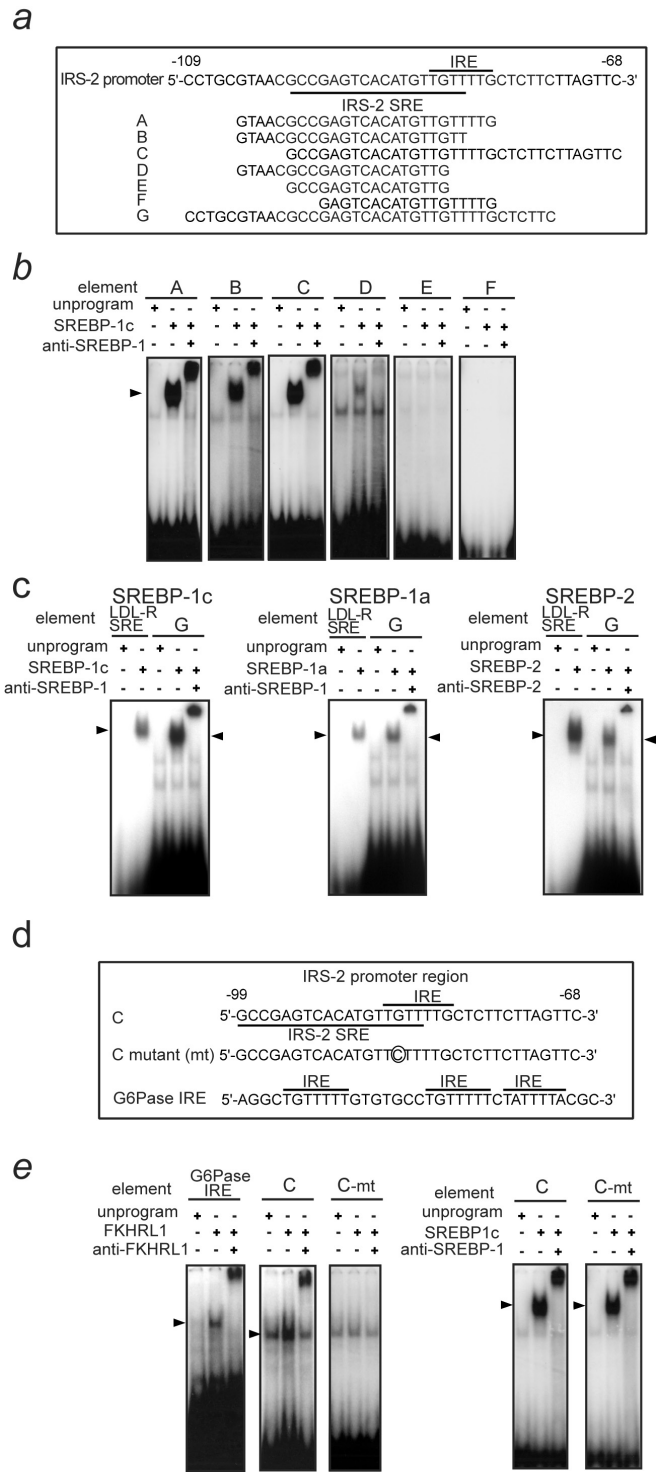

Figure S1. Identification of SREBP binding site (IRS-2-SRE) and FKH binding to IRS-2-IRE in the IRS-2 promoter. ***a,*** Neighboring DNA sequences around a newly identified SREBP binding site (IRS-2-SRE) and insulin response element (IRE) in human IRS-2 promoter and various oligonucleotides used for gel shift assays to determine the IRS-2-SRE (A-F). ***b,*** *Identification of IRS-2-SRE* ***c***, Binding of each SREBP isoform (-1c, -1a, and -2) to IRS-2-SRE (G) and LDLR-SRE. ***d***, A mutation in IRS-2-SRE/IRE (TGTTTTG→ T***C***TTTG) that abolishes FKH binding was introduced into C probe (indicated by an oval in C mt). *e*, DNA binding assays performed with FKHRL1 and SREBP1c by using probes as IRS-2-SRE/IRE (C), IRS-2-SRE/IRE-mutation (C-mt), or G6Pase IRE. ***e,*** IRS-2-SRE as the element responsible for SREBP repression. The indicated IRS-2 promoter constructs with or without IRS-2-SRE were compared in luciferase assays in HepG2 cells. In EMSA assays, each SREBP and FKHRL1 proteins were in vitro-translated. The specific binding of the protein/DNA complex (arrowhead) was confirmed by super-shift induced by the indicated antibodies.

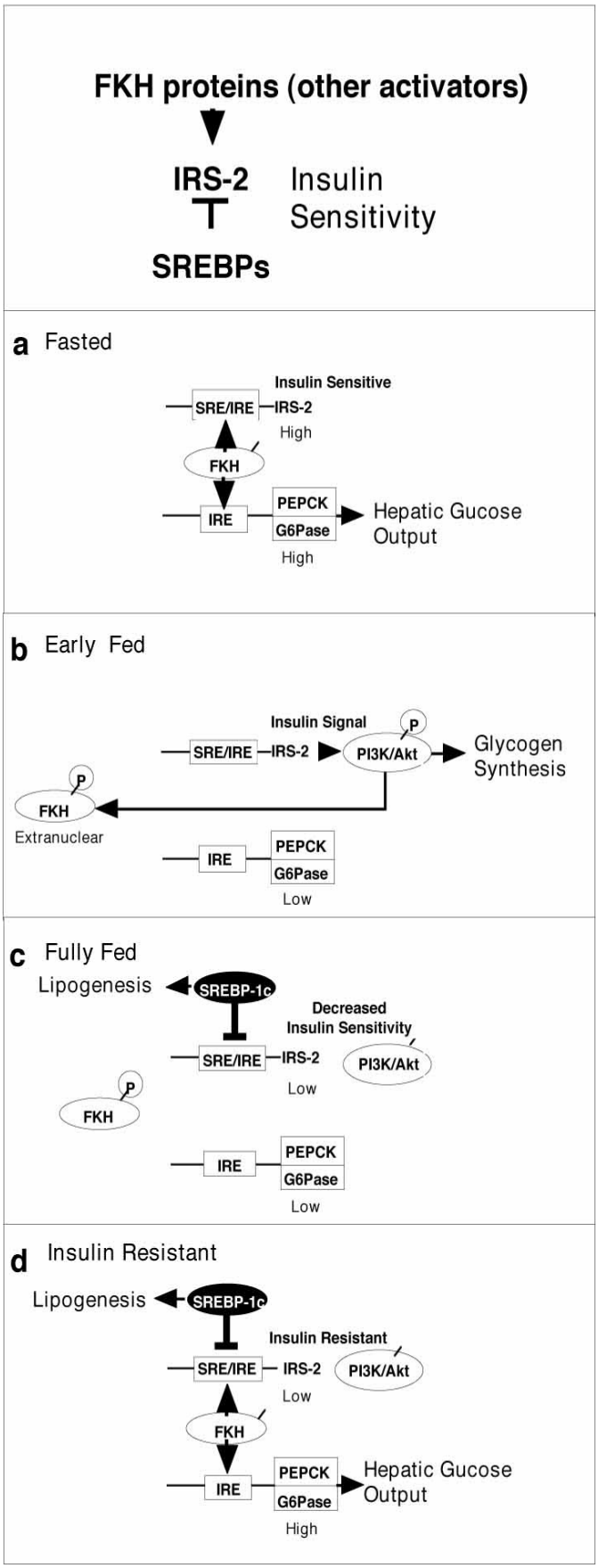

Figure S2. Reciprocal regulation of IRS-2 expression by SREBP-1c and FKHs for hepatic insulin signaling in feeding cycles (a-c) and insulin resistant state (d) Activation by FKHs and inhibition by SREBP-1c of IRS-2 expression could illustrate physiological and pathophysiological regulation of insulin sensitivity, gluconeogenic gene expression, glycogen synthesis, and lipogenic gene expression in fasted (a, insulin-depletion) , early fed (b, insulin action), fully fed (c, chronic insulin action), and insulin resistant (d) states