Supplemental Methods

pcDNA3-IRS2^{WT} The construction of pcDNA3-IRS2^{WT} was performed in two steps. First the N-terminus coding region 1-1770bp was excised from pCMV-His with BamH1 and XbaI and was ligated into pcDNA3 using the same restriction sites. In the second step, the coding region of the core and the carboxy terminal of the IRS-2 protein was excised by Xba-Xba and ligated into the pcDNA3-N terminus using the same enzymes.

Generation of IRS-2^{5A} Site-directed mutagenesis was performed with the primers given below using a Quick-Change site-directed mutagenesis kit (Stratagene) according to manufacturer's instructions. pcDNA3-IRS-2 served as a template. Mutated amino acid codons are shown in italic type. The restriction sites introduced are underlined. The mutations were verified by restriction mapping and sequencing. 1) The S303A primers were 5' GCC TCG CAG CAA G*GC* TCA GTC GTC CGG *A*TC GTC AGC CAC GC 3' (sense) and 5' GCG TGG CTG ACG ATC CGG ACG ACT GAG CCT TGC TGC GAG GC 3' (Anti sense). A BspEI was introduced. 2) The S343A primers were 5' CGC TCG CGC ACT GAC *GCG T*TG GCG GCC ACC CCC CC 3' (sense) and 5' GGG GGG GTG GCC GCC AAC GCG TCA GTG CGC GAG CG 3' (anti sense). An MluI site was introduced. 3) The S362A 5' GCC GGG TTC GTA CC*G* CC*G C*CG AGG GCG ACG GCG G3' (Sense). 5' CCG CCG TCG CCC TCG GCG GCG GTA CGA ACC CGG C 3' (Anti sense). A BsiWI site was eliminated. 4) The S381A 5' GGC AGG AGG CAG GC*C CAT GG*C GGT GGC AGG GAG CCC 3' (Sense). 5' GGG CTC CCT GCC ACC GCC ATG GGC CTG CCT CCT GCC 3' (Anti sense). An NcoI site was introduced. 5) The S480A 5' GGC CAG CGT CCG T*CC GCG* GGT AGT GCC TCC GCC 3' (sense). 5' GGC GGA GGC ACT ACC CGC GGA CGG ACG CTG GCC 3'. A SacII site was introduced.

Construction of Myc-tagged IRS-2^{WT}and IRS-2^{5A} The generation of full length Myc-IRS-2 WT or 5A was carried out using the same method we applied to generate Myc-tagged IRS-1 proteins [Liu, 2004 #2094]. In brief, pcDNA3 IRS-2 was digested with BamH1 and NheI and a synthetic oligonucleotide encoding Myc was ligated into the plasmid.