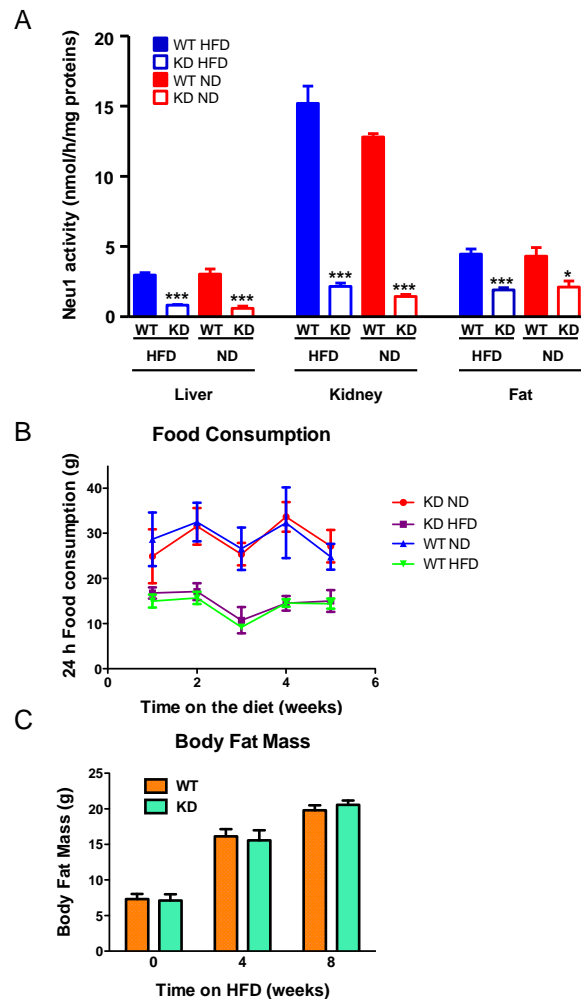


SUPPLEMENTARY DATA

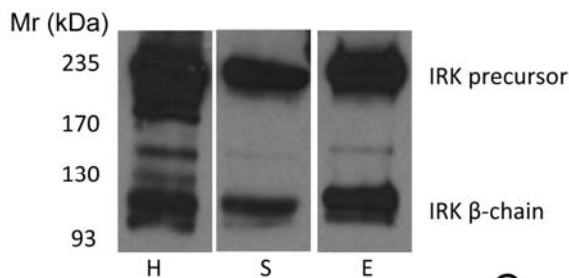
Supplementary Figure 1. Homozygous *Catha*^{S190A-Neo} mice (KD) have reduced tissue sialidase activity as compared with their wild type (WT) counterparts (A) but have similar food consumption (B) and fat body mass (C). Four-month-old (~25 g BW) male mice received ad libitum either a normal chow (5% fat, 57% carbohydrate, ND) or a high-fat diet with an elevated ratio of fat (35% fat, 36% carbohydrate, HFD). (A) Mice fed normal diet or HFD for 4 weeks were sacrificed by cervical dislocation. Livers, kidneys, and abdominal fat were immediately removed, frozen and kept at -80°C. Sialidase activity in tissue homogenates was measured against 4MU-NeuAc [2'-(4-methylumbelliferyl)- α -D-N-acetylneuraminic acid] in 50 mM sodium acetate buffer, pH 4.2 for 30 min at 37°C. Reaction was stopped with 0.4 M sodium glycine buffer, pH 10.4 and fluorescence (ex. 365 nm, em. 450 nm) was measured using a spectrofluorophotometer RF-5301PC (Shimadzu). Protein concentration was measured according to the Bradford method. Data represent mean values \pm S.D. of three independent experiments. (B) Body weight and total food consumption were measured weekly. (C) At 0, 4 and 8 weeks, body and lean masses were measured by dual-energy X-ray absorptiometry (DEXA) scanning (Lunar PIXImus).



SUPPLEMENTARY DATA

Supplementary Figure 2. Affinity purification of TAP-tagged recombinant human IRK expressed in HEK293 cells and its identification by mass spectrometry. (A) Purification of IRK was performed as described in Experimental Procedures and aliquots of cell homogenate (H), supernatant of cell extract (S) and CBP-agarose eluate (E) were analyzed by Western blot using anti-CBP antibody. (B) Sequence of the β -chain of human IRK showing peptides identified by LC-MS/MS (bold red). Phosphorylated residues with validated MS/MS spectra are circled. MS/MS spectra of peptide 1183 DIYETDYR 1191 with phosphorylated Y 1205 (C) and Y 1209 (D) residues. Sequence segments with y-type fragment ion series are shown above the spectra. Affinity purified IRK protein was resolved on SDS-PAGE, stained with Coomassie blue, and protein bands corresponding to the MW of 98 kDa were excised and digested with trypsin. Peptides were analyzed by MS/MS as described in Experimental Procedures. Peptides were identified with Mascot search engine v 2.10 (Matrix Science, UK) against the human IRK sequence using trypsin and semi-tryptic cleavage sites. Figure 3B summarizes the results of 3 independent purifications. Assignments of Tyr phosphorylation sites were validated through manual inspection of relevant MS/MS spectra.

A



B

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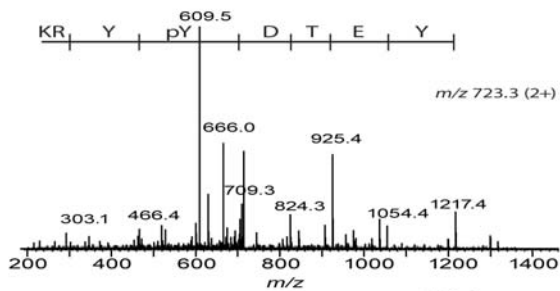
1 MTPAGQRRAP DPRRPAPAA MGTGGRGAA AAPLLVAVAA LLLGAAGHLY
51 PGEVCPGMDI RNNLRLHEL ENCSVIEGHL QILLMFKTRP EDFRDLSFFK
101 LIMITDYLLL FRVYGLSELK DLFPNLTVIR GSRLFFNYAL VIFEMVHLKE
151 LGLYNLMMIT RGSVRIEKN ELCYLATIDW SRILDSVEDN HIVLNKDDNE
201 ECGDICPGTA KGKTNCPATV INGQFVERCW THSHCQKVCV TICKSHGCTA
251 EGLCCHSECL GNCSPDDPT KCVACRNFYL DGRCVETCVP PYYHFQDWRC
301 VNFSPCQDLH HKCKNSRRQG CHQYVIHNNK CIPECPSTGYT MNSNLLCTP
351 CLGQPCPKVCH LLEGEKTIDS VTSAQELRGC TVINGSLIIN IRGGNNLAE
401 LEANLGLIEE ISGYLKIRRS YALVLSLFFR KLRLIRGETL EIGNYSFYAL
451 DNQNLRLQMD WSKHNLTQTQ GKLFPHYNPK LCLSEIHKME EVSGTKGRQE
501 RNDIALKTNG DKASCENELL KFSYIRTSFD KILLRWEPYW PPDFRDLDFG
551 MLFYKEAPYQ NVTEFDGQDA CGSNSWTVD IDPPLRSNDP KSQNHGWLML
601 RGLKPWTQYA IFVKTIVTFS DERRTYGAKS DIIVVQTDAT NPSVPLDPIS
651 VSNSSQIIL KWKPPSPDNG NITHYLVFWE RQAEDELFE LDYCLGKGLK
701 PSRTWSPFFE SEDSQKHNSQ EYEDSAGECC SCPKTDSQLL KELEESSFRK
751 TFEDYLNHV FVPRKTSSTG GAEDPRPSRK RRLSGDVGNV TVAVPTVAAF
801 PNTSSTSVPT SPEEHRPFKE VVNKESLVIS GLRHFTGYRI ELQACNQDTP
851 EERCVAAYV SARTMPEAKA DDIVGPTVE IFENNVMHLM WQEPKPEPGL
901 IVLYEVSYR YGDEELHLCV SRKHFALEGR CRLRGLSPGN YSVRIRATSL
951 AGNGSWTEPT YFYVTDYLDV PSNIAKIIG PLIFVLFSPN VIGSIYLFRL
1001 KRQPDGLPG LYASSNPEYL SASDVFPCSV YVPDEWEVSR EKITLLRELG
1051 QGSFGMVEG NARDIIRGEA ETRVAVKTVN ESASLRERE FLNEASVMKG
1101 FTCHHVRL GVVSKGQPTL VMELMAHGD LKSYLRSRLE EAENNPGRFP
1151 PTLQEMIQMA AEIADGMAYL NAKKEVHRDL AARNCMVAHD FTVIGDFGM
1201 TRD RRGGKGLLPV RWMAPESLKD GVFTTSSDMW SFGVVLWEIT
1251 SLAEQPYQGL SNEQVLRKFM DGGYLDQPDN CPERVTDLMR MCWQFNPKMR
1301 PTFLEIVNLL KDDLHPSFPE VSFHSEENK APESSELEME FEDMENVPID
1351 RSSHCRQEEA GGRDGGSSLG FK EEHIP YTHMNGGKKN GRILTLPRSN
1401 PS
    
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Bold red indicates identified peptides

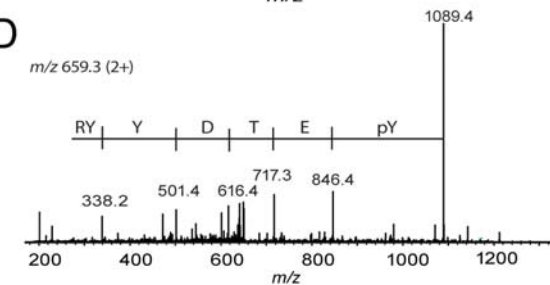
○ Phosphorylated residues

Sequence Coverage: 43%

C



D



SUPPLEMENTARY DATA

Supplementary Figure 3. Neu1 fused at the C-terminus with YFP protein shows full enzymatic activity (A) and interacts with its binding partner, SHC protein (B). (A) HEK293 cells were co-transfected with plasmids coding for Neu1-YFP and CathA or Neu1 and CathA, sialidase activity was measured in cell homogenates against 4MU-NeuAc [2'-(4-methylumbelliferyl)- α -D-N-acetylneuraminic acid] at pH 4.75. The control shows sialidase activity in homogenate of non-transfected cells. Data represent mean values \pm S.D. of three independent experiments. (B) HEK293 cells co-expressing IR-hRluc and SHC-YFP were incubated for 15 min with coelenterazine H and then stimulated with insulin at 5 or 50 nM concentration. In the presence of insulin the SHC protein interacts with IRK which results in appearance of a BRET signal.

