Online Supplementary Materials

Table S6. Characteristics of mutant INSR and patient phenotypes

Mutation	INSR subunit	Phenotype	Plasma insulin pmol/L	INSR defect characteristics				Cell types used to characterise mutant
				Cell surface expression	Insulin binding	Insulin-stimulated autophosphorylation	Internalisation, dissociation, degradation	
L62P	α	TA-IR	600 – 3000*(1)	✖	↓(1)	↓(1)	NA	RBC(1), HEK293(2)
R118C	α	TA-IR, RMS	70 - 3000(3)	N(3)	↓(3)	↓(3)	NA	CHO(3)
I119M	α	TA-IR, RMS	2000 – 20000*(4)	N(4)	N(4)	N(4)	Dissociation ↓(4)	EBVL(4), CHO(4)
P193L	α	RMS	1000 – 2000*(5)	↓(6)	↓(5)	NA	NA	EBVL(5), Rat-1(6)
F248C	α	DS	7000	⬩	⬩	⬩	⬩	⬩
R252C	α	TA-IR	NR	N(7)	↓(7)	N(7)	Internalisation ↓(7)	CHO(7)
S323L	α	DS, RMS	2000 – 8000(8,9)	N(8, 10)	↓(8, 10, 11)	↓(8, 10, 11)	NA	PBMC(8), NIH-3T3(8, 10), CHO(11)
F382V	α	TA-IR	NR	↓(12)	N(12, 13)	↓(13)	NA	NIH-3T3(12, 13)
K460E	α	DS, RMS	1000 – 70000(14, 15)	N(15)	↑(16)	N/↑(14)	Int. ↑(14), diss.↓ (17), deg. ↑(14)	EBVL(16, 17), PBMC(15), PDF(15), NIH-3T3(14)
D707A	α	DS	2000 – 3000*(18)	N(18)	↓(18)	✖(18)	Internalisation ↓(18)	PDF(18), CHO(18)
P1178L	β	TA-IR	2000 - >4000(9)	N(19, 20)	N(19, 20)	✖(19, 20)	NA	CHO(19, 20)

Previously published characteristics of naturally occurring INSR mutations used in the current study. Mutations are numbered as per the mature INSR B isoform (exon 11+). TA-IR, Type-A Insulin Resistance; RMS, Rabson Mendenhall Syndrome; DS, Donohue Syndrome; *, reported fasting insulin levels; NR, not reported; ✖, absent; N, normal; ↓, decreased compared to WT receptor; ↑, increased compared to WT receptor; ⬩, not previously described; NA, not assessed; RBC, red blood cells; EBVL, Epstein Barr virus transformed lymphoblasts; PBMC, peripheral blood mononucleocytes; CHO, Chinese Hamster Ovary; Rat-1, Rat-1 fibroblasts; NIH-3T3, NIH-3T3 murine fibroblasts; HEK293, human embryonic kidney 293.

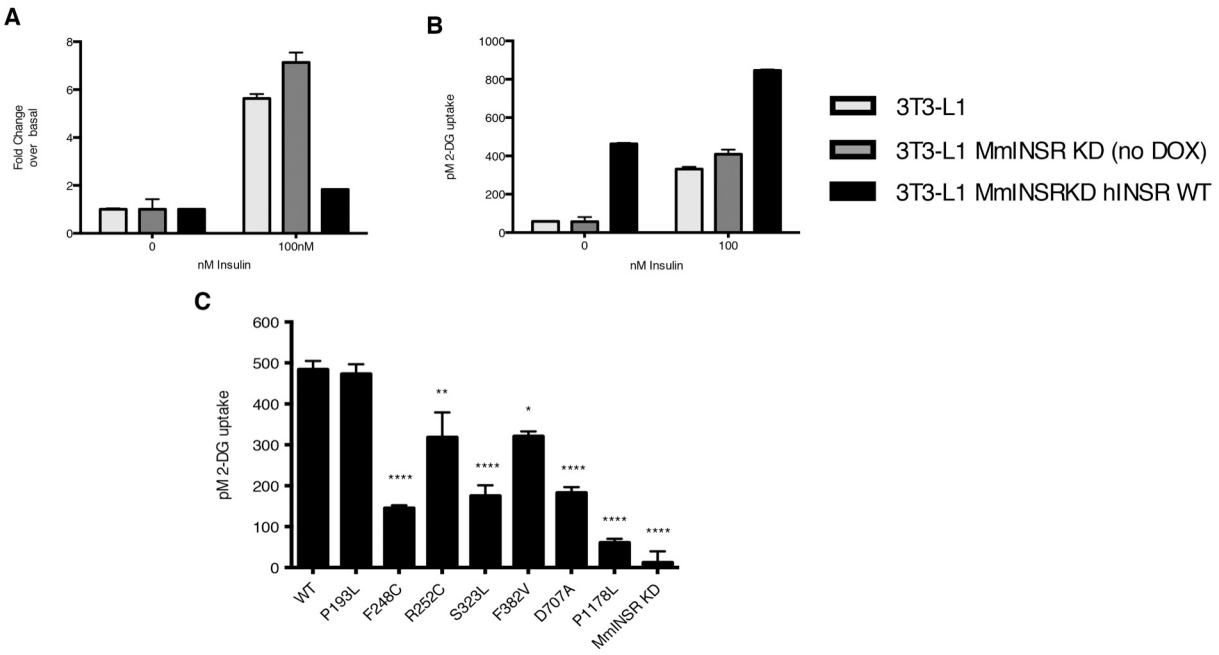

Fig S1. Basal glucose uptake differs between each of the 3T3-L1 MmINSRKD cell lines overexpressing mutant INSR. (**A** & **B**) Insulin stimulated glucose uptake in parent 3T3-L1 cells that were used to generate 3T3-L1 MmINSRKD cells, 3T3-L1 MmINSRKD cells cultured without DOX induction of knockdown of endogenous mouse INSR, and 3T3-L1 MmINSRKD hINSR WT cells cultured in the presence of DOX to induce knockdown of endogenous mouse INSR and expression of hINSR WT. (**C**) 3T3-L1 MmINSRKD hINSR (mutant as indicated) adipocytes were grown in the presence of 1μg/ml DOX for 10 days prior to overnight serum-starvation on day 15 of differentiation. Cells were then glucose starved for 30 minutes prior to the addition of 2-Deoxy-D-glucose for 5 minutes. Cells were then washed, lysed and assessed for 2-Deoxy-D-glucose uptake. Data is the mean ± SEM from three independent experiments. The statistical significance between non-stimulated basal 2-Deoxy-D-glucose uptake of each of the mutant receptor expressing cell lines was determined by one-way ANOVA with Tukey's multiple comparison test. Statistical significance between WT expressing cells and mutant INSR expressing cells denoted by * $p<0.05$; ** $p<0.01$, and **** $p<0.0001$.

Table S1: Composition of cell culture medium

Cell Line	Media Name	Base Medium	Supplements
CHO FlpIN	CHO Media	F12	10% (v/v) foetal calf serum (FCS), 50 units/ml penicillin, 50units/ml streptomycin, 4mM L-glutamine
3T3-L1 Preadipocytes	Preadipocyte Media	DMEM	10% (v/v) newborn calf serum (NCS), 50 units/ml penicillin, 50units/ml streptomycin, 4mM L-glutamine
3T3-L1 Adipocytes	Adipocyte Media	DMEM	10% (v/v) TET-approved FCS (Clontech), 50 units/ml penicillin, 50units/ml streptomycin, 4mM L-glutamine
3T3-L1 Preadipocytes	Differentiation media 1	DMEM	Same as Adipocyte media with the addition of 1µM insulin, 200nM rosiglitazone, 500µM methylisobutylxanthine, 1µM dexamethasone
3T3-L1 Adipocytes	Differentiation media 2	DMEM	Same as Adipocyte media with the addition of 1µM insulin and 200nM rosiglitazone

Table S2: Vectors and sub-cloning steps used in generation of CHO FlpIn hINSR cells

Vector	Source/Reference	Use
pCR_Blunt_II_TOPO	Invitrogen	TOPO clone hINSR PCR product from pDNR-Dual
pCDNA/5/FRT/TO	Invitrogen	hINSR expression vector ApaI/HindIII hINSR fragment from pCR_Blunt_II_TOPO
pOG44	Invitrogen	Expression of Flp recombinase

Table S3: Target sequences, primers, vectors and sub-cloning steps used in the generation of lentiviruses

Sequence/vector	Source/Reference	Use
CGGATCCCATATCAGTTTCTAA	Open Biosystems	Target sequence for murine INSR miR-shRNA
AAGACCAGACCCGAAGATTTCC	Seibler *et al (2007)* *Nucleic Acids Res.* **35**, e54	Target sequence for murine INSR miR-shRNA
pEN-TGmiRC3	Shin *et al (2006)* PNAS **103**, 13759–13764 (2006)	miR-shRNAs concatenated into this entry vector by SpeI, XbaI, PstI directional cloning as described by Shin *et al*
pSLIK-Hygro	Shin *et al (2006)* PNAS **103**, 13759–13764 (2006)	miR-shRNAs gateway cloned into this lentiviral expression vector by gateway cloning with LR clonase
pMDLg/pRRE, pRSVREV, pVSV-G	Shin *et al (2006)* PNAS **103**, 13759–13764 (2006)	Third-generation lentivirus packaging and pseudotyping plasmids
pEN_Tmcs	Shin *et al (2006)* PNAS **103**, 13759–13764 (2006)	Entry vector
pEN_TmcsMCS2	N/A	Oligonucleotide linker encoding NotI-BamHI-ScaI-SphI-HindIII-NcoI-PmeI-KpnI-ApaI-XhoI was cloned into pEN_Tmcs between NotI and XhoI sites. PCR amplified hINSR cloned into SpeI and HindIII sites.
GGGGACTACTTCCACCATGGCCACCG	Sigma-Aldrich	Fwd 5'-3' primer to amplify myc-tagged hINSR mutants from pCDNA5/FRT/TO
GCATGCAAGCTTCTACAGATCCTCTTC TGAGATGAG	Sigma-Aldrich	Rev 5'-3' primer to amplify myc-tagged hINSR mutants from pCDNA5/FRT/TO
pSLIK-NEO	Shin *et al (2006)* PNAS **103**, 13759–13764 (2006)	Mutant hINSR cloned into this expression vector by gateway cloning from pEN_TmcsMCS2

Table S4: Buffer Composition

Buffer Name	Composition
FACS Buffer	PBS, 0.5% BSA, 0.1% sodium azide
Lysis Buffer	20mM HEPES, 150mM NaCl, 1.2mM $MgCl_2$, 1mM EGTA, 1mM PMSF, 1mM Na_3VO_4, 10% (v/v) glycerol, 1% (v/v) Triton-X-100, complete-EDTA-free protease inhibitors (Roche), phosSTOP (Roche)
KRPH Buffer	120mM NaCl, 5mM KCl, 1.2mM $MgCl_2$, 10mM $NaHCO_3$, 1.3mM $CaCl_2$, 1.2mM KH_2PO_4, 20mM HEPES

Table S5: Antibodies used during Western blotting

Target	Dilution	Catalogue #	Manufacturer
INSRβ	1:200	SC-711	Santa Cruz Biotechnology
INSRβ	1:1000	3025	Cell Signalling Technology
Phospho-INSRβ (Tyr1162/Tyr1163)	1:1000	44804G	Life Technologies
Myc-tag	1:1000	05-724	Millipore
Calnexin	1:1000	Ab22595	Abcam
Phospho-AKT (Thr308)	1:1000	2965	Cell Signalling Technology
Phospho-AKT (Ser473)	1:1000	4060	Cell Signalling Technology
AKT	1:1000	2920	Cell Signalling Technology
Phospho-ERK1/2 (Tyr204/Tyr187)	1:1000	5726	Cell Signalling Technology
ERK1/2	1:1000	4695	Cell Signalling Technology
Phospho-GSK3α/β (Ser21/Ser9)	1:1000	9331	Cell Signalling Technology
GSK3α/β	1:1000	5676	Cell Signalling Technology
Phospho-p70S6K (Thr389)	1:1000	9205	Cell Signalling Technology
p70S6K	1:1000	2708	Cell Signalling Technology
Phospho-AS160	1:1000	4288	Cell Signalling Technology
Anti-mouse IgG HRP conjugated	1:10,000	7076	Cell Signalling Technology
Anti-rabbit IgG HRP-conjugated	1:5,000	7074	Cell Signalling Technology