Supplementary Materials and Methods

Generations of mutations in IGF1R gene: The pGR 15 vector, containing full length human IGF-1R cDNA was a gift from Dr Renato Baserga (Kimmel Cancer Center, PA, USA). We subcloned the entire IGF1R coding region into pENTR/D-TOPO Vector (Invitrogen) by using primers IGF1R F (CAC CAT GAA GTC TGG CTC CGG AGG A) and IGF1R R (GCA GGT CGA AGA CTG GGG CAG CG). We used this vector to generate mutations by QuikChange XL Site-Directed Mutagenesis Kit (Stratagene). The longevity associated IGF1R mutations (A37T and R407H) were introduced by using primers A37T F (5'- CCT GCT CAT CTC CAA GAC CGA GGA CTA CC -3') and A37T R (5'- GGT AGT CCT CGG TCT TGG AGA TGA GCA GG -3'); R407H F (5'- CTG GGA CCA CCA CAA CCT GAC CAT CAA AGC -3') and R407H R (5'- GCT TTG ATG GTC AGG TTG TGG TGG TCC CAG -3'). Nucleotide sequencing of the entire cDNA confirmed the presence of the introduced mutations but no other changes.

Production of lentiviral vectors expressing human IGF1R gene variants: The lentiviral vectors expressing human IGF1R gene variants were generated through recombination between pENTR vectors bearing the IGF1R coding region (wild type or the gene variants), with pLenti6/Ubc/V5 -DEST using the Gateway LR recombination clonase (Invitrogen). Ubc promoter enable recombinant protein expressed close to physiological level. Positive clones were identified by restriction digest and subsequent sequencing. The 293FT cell line was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. All recombinant lentiviruses were produced by transient transfection of 293T cells according to the manufacturer`s protocols. Briefly, subconfluent 293FT cells were cotransfected with 16 µg of plasmid vector, 4 µg pLenti6/Ubc/V5-DEST vector and 12 µg ViraPower Packaging Mix (Invitrogen) by using 36 µl Lipofectamine 2000 (Invitrogen) in 3 ml OptiMEM transfection medium (Invitrogen) to the total 8 ml of media, in a 100 mm dish. The medium was changed next morning and the lentivirus were harvested 48 h later

Stable lentiviral transduction in Igf1r$^{-/-}$ MEFs: Early passage Igf1r$^{-/-}$ MEFs were kind gifts of Dr Renato Baserga. Cells were grown to 80% confluency in 24 well plates. Cells were infected with 2 ml lentiviral supernatant, and polybrene hexadimethrine bromide) at a final concentration of 6 µg/ml. Cells were infected for 6 h in this media and then another 2 ml of cell culture media was added. Positive stable transformations were selected and cultured in presence of 3 µg/ml Blasticidin for 10–14 day. The expression of respective proteins was verified via Western blot analysis.

Cell Culture: Cells were maintained in Dulbecco's modified Eagle's medium with high glucose (Gibco/BRL) with 10% fetal bovine serum (FBS) (Gibco/BRL) supplemented with penicillin/streptomycin antibiotics (Gibco/BRL). Igf1r$^{-/-}$ MEFs cells were maintained in media containing 50 µg/ml G418 (Sigma). The engineered MEFs expressing human IGF1R variants cells were maintained in the

presence of 50 µg/ml G418 and 3 µg/ml Blasticidin.

Flow cytometry: Cells were grown in serum-free medium without or with 10nM IGF1 for 24 hours. Incubation was stopped by washing with PBS. Trypsinized cells were washed in cold PBS and fixed in 70% ethanol at −20 °C. After a wash in cold PBS, cells were incubated in PBS with RNase A (100 µg/ml) at 37 °C for 30 min, washed in PBS, and resuspended in PBS containing 20 µg/ml propidium iodide. A minimum of 20,000 cells/sample were analyzed with cytometry using a Becton Dickinson FacScan (Becton Dickinson, Cowley, Oxford, UK). Cell cycle analysis was performed using Modifit Software (Verity Software House Inc.)

Western Blot: Antibodies against AKT, Phospho-AKT (Ser 473) and IGF1R, Phospho-IGF1R (Tyr1135/1136) were purchased from Cell Signaling Technology. Cells were lysed in RIPA buffer (1×PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 100 µg/ml phenylmethylsulfonyl fluoride, 50 kallikrein-inactivating units/ml aprotinin, and 1 mM sodium orthovanadate). Protein concentration of each sample was determined by BCA Protein Assay Reagent (Pierce). 40 µg of proteins was loaded on 15% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride filters (Millipore). The filter was blocked and then incubated with the primary antibody (1:2000) in 5% nonfat dry milk in 0.05% TBST (10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) overnight at 4°C. After washing, the blots were incubated with secondary antibody (1 µg/ml) conjugated to horseradish peroxidase at 1:4000 in 5% nonfat dry milk in 0.05% TBST for 1 hr at room temperature. Proteins were visualized with Chemiluminescent Substrate (Pierce) according to the manufacturer's instruction. Bands were quantified with Image J.

RT-PCR: Expression of known IGF-1 induced genes was measured by real-time PCR. Total RNA was purified from the engineered MEFs expressing different human IGF1R gene variants (Qiagen) and cDNA was synthesized from total RNA with SuperScript III kit (Invitrogen) according to the manufacturers` protocols. Real time PCRs were performed by using Fast SYBR green master mix (Applied Biosystems) on StepOnePlus Real-Time PCR systems (Applied Biosystems). Total of 8 ng cDNA (1ng/µl) was used for each reaction in 20 µl final reaction volume with 200 nm primers.