Supplementary information

Supplemental Figures Legends

Supplementary fig. 1. Molecular identification and sequence analysis of a novel mTOR splicing variant. (**A**) Schematic representation of mTOR and the localization of primers used for the identification of mTOR splicing variants. (**B** and **C**) PCR amplification of a potential mTOR splicing variant. Total RNA was purified from Hep2G, HEK293 and MCF-7 cell lines as described in "Methods". A set of mTOR specific primers was used in RT-PCR reactions to search for potential splicing variants. Amplified fragments were resolved by electrophoresis in agarose gel. The most prominent bands were excised from the gel, cloned and sequence identified. One set of primers (S1 and AS2) reproducibly amplified a 100bp fragment from three cell lines. Specific fragments of glyceraldehyde-3-phosphate dehydrogenase (GADPH) and β-actin were amplified and used as loading and quality controls (**B**). The 2.1kb DNA fragment was amplified with the second set of primers (S1, AS1) and found to contain the full length coding sequence of mTORβ. (**D**) Sequence alignment of 100bp PCR fragments, amplified with S1 and AS2 mTOR specific primers. Amplified fragments were sequenced and the sequences aligned by ClustalW program. The position of the splice junction sequences is indicated by an arrow. (**E**) Amino acid sequence of a 100bp PCR product, amplified with S1 and AS2 mTOR specific primers. The arrow indicates the position of a potential splice junction. The sequences, corresponding to the N-terminal region and the FATN domain of mTOR are underlined. (F) Western blot analysis of mTORα and mTORβ expression in human tissues. The membrane, containing SDS-PAGE separated proteins from human tissues was from ProSci Incorporated (15 μg of total protein in each lane). The membrane was initially immunoblotted with the CS-mTOR antibody, then stripped and re-probed F11 mTOR monoclonal antibody.

Supplementary fig 2. Regulation and subcellular localization of mTORβ (**A**) The 80kDa mTOR immunoreactive protein is not the product of proteolytic degradation. The extracts of HEK293 and MCF7 cells were immunoprecipitated with the C-terminal mTOR antibody (mTOR-CS) and the immune complexes probed with the N-terminal mTOR antibody (N19, Santa Cruz) and mTOR F11 antibody. (**B**) Amino acid sufficiency leads to mTORβ phosphorylation in MCF-7 cells. Exponentially growing MCF-7 cells were incubated in serum-free medium for 24hrs and then in Dulbecco/PBS for 2 hrs. A cocktail of amino acids was added to starved cells for 1hr. Total cell lysates were probed with mTOR-CS (upper panel) and pS2448 mTOR (lower panel) antibodies. (**C**) Kinase activity does not affect mTORβ binding to Raptor and Rictor *in vitro*. HEK293 cells were transfected with pcDNA3.1/FLAG-mTORβWT or pcDNA3.1/FLAG-mTORβKD together with pcRK/HA-Raptor or pRK/Myc-Rictor. Two days later, cell lysates were immunoprecipitated with anti-

FLAG or nonspecific antibodies. Immune complexes were resolved by SDS-PAGE and immunobloted with anti-mTOR, anti-HA or anti-Rictor antibodies. (**D**) Analysis of mTORβ subcellular localization. (Left panel) Exponentially growing MCF-7 cells were fractionated using ProteoExtract Extraction kit (Calbiochem). mTORα and mTORβ were immunoprecipitated from all fractions using mTOR-CS antibody and immune complexes resolved by SDS-PAGE and immunoblotted with mTOR-SC antibody. Anti-4E-BP1, HSP60, and c-Jun antibodies were used as controls for cytoplasm, membrane, and nuclear fractions, respectively. (Right panel) Analysis of subcellular localization of Myc-mTORα or Myc-mTORβ transiently expressed in HEK293 cells. 36 h after transfection with corresponding plasmids cells were fixed and incubated with primary anti-Myc antibody and secondary fluorescein isothiocyanate-labeled anti-mouse immunoglobulin G and analyzed by immunofluorescence. (**E**) mTORβ does not dimerize with mTORα *in vivo*. HEK293 cells were transfected with pcDNA3.1/Myc-mTORα or pcDNA3.1/Myc-mTORβ together with pcDNA3.1/FLAG-mTORβ. Cells were lysed in buffer with CHAPS (see "Experimental Procedures") and ectopically expressed mTOR isoforms were immunoprecipitated with anti-Myc tag antibody. The immune complexes were resolved by SDS-PAGE and probed in Western blotting with anti-FLAG antibody followed by Western blotting with anti-mTOR (CS) antibody.

Supplementary fig 3.

Transient overexpression of mTORβ, but not mTORα, in HEK293 cells induces cell proliferation. Proliferation assay was performed as described in "Experimental Procedures". Growth curves for each pool of transfected HEK293 cells were plotted. Immunoblotting of total cell lysates was carried out with antibodies to FLAG-tag to check the levels of FLAG-mTOR α and FLAG-mTORβ 36h after transfection.

Supplementary fig 4. Immunoblot analysis of mTORβ expression in cell lines prior to injection and grafted tumors from Fig. 4B. Total protein extracts from stable cell lines and tumors were separated by SDS-PAGE and immunoblotted with the mTOR-CS and β-actin antibodies.