

Supplementary Materials for
**Identification of potential drug targets for tuberous sclerosis complex
by synthetic screens combining CRISPR-based knockouts with RNAi**

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Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/8/393/rs9/DC1)

- Table S1 (Microsoft Excel format). sgRNA efficiency in relation to GC content data.
- Table S2 (Microsoft Excel format). Phosphorylation changes common to *TSC1* and *TSC2* cell lines.
- Table S3 (Microsoft Excel format). GO analysis of phosphoproteomic data.
- Table S4 (Microsoft Excel format). Synthetic screen results.
- Table S5 (Microsoft Excel format). Conservation of synthetic screen candidate genes.

Table S6 (Microsoft Excel format). Primers used in this study.

Supplementary file 1 (.pdf format). GenBank sequence of CRISPR cell line expression vector (pI018).

Supplementary file 2 (.pdf format). GenBank sequence of the luciferase mutation reporter vector.

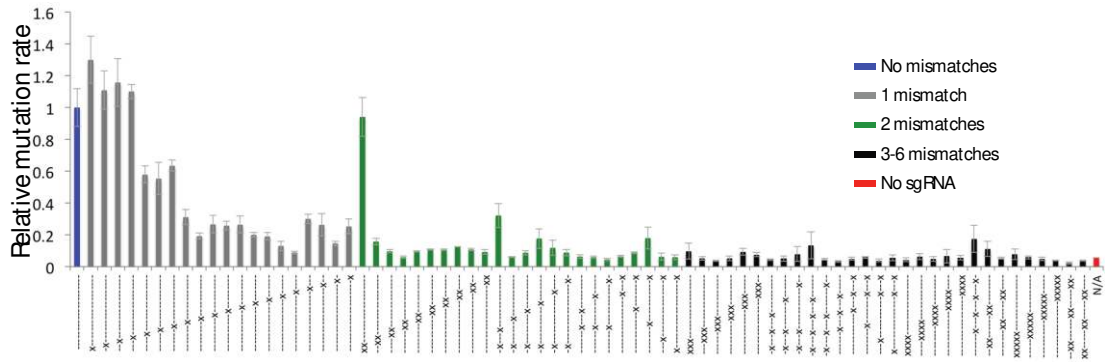


Figure S1: Analysis of CRISPR specificity using quantitative HRM

Graph showing relative mutation rates from 75 sgRNAs used to target a single sequence in the *ye//ow* gene. Mutation rate is calculated as integrated area between each experimental HRM curve and a mean control curve. Each bar represents the mean relative mutation rate from three biological replicates using sgRNAs with 0 mismatches (blue bar), 1 mismatch (grey bars), 2 mismatches (green bars), ≥ 3 mismatches (black bars) or in the absence of sgRNA (red bar). Dashes indicate nucleotides that are matched between sgRNA and the target sequence. Crosses indicate the position of mismatches. Error bars indicate standard error of the mean.

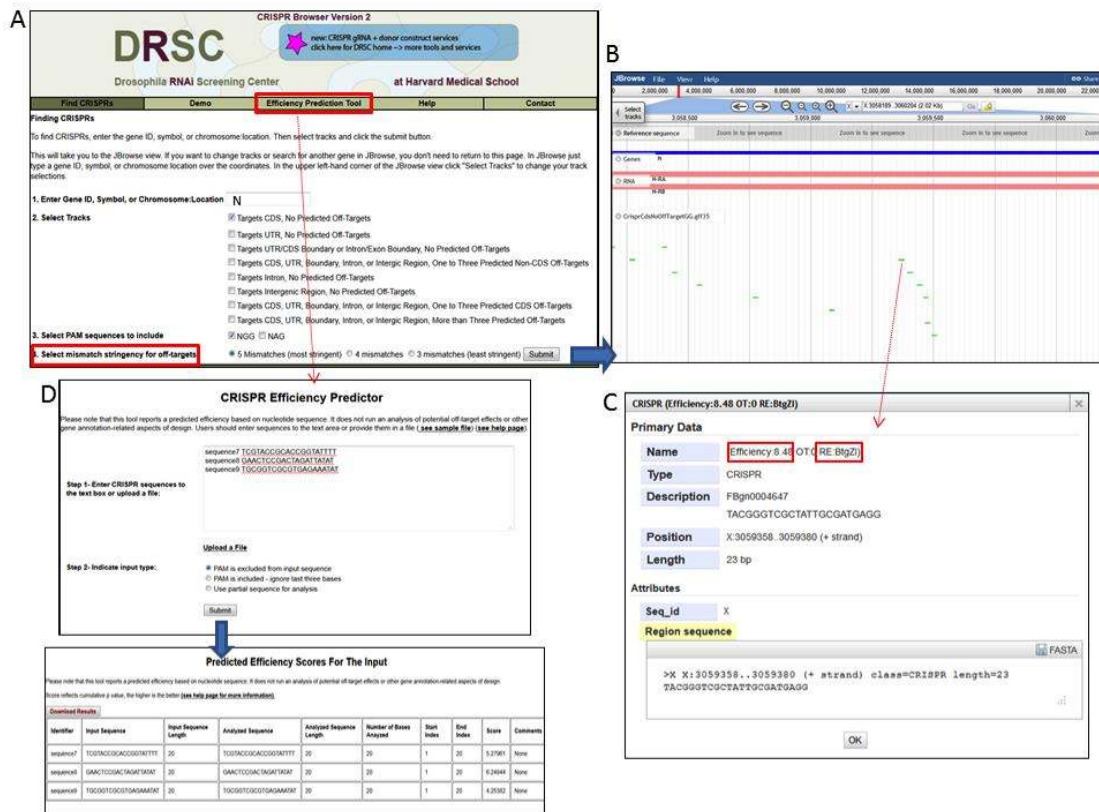


Figure S2: An improved sgRNA design tool

A: Main search page, allowing the user to search by gene symbol, CG number, FBgn as well as genome coordinates. The user also has the option of choosing the off-target threshold as well as the relevant track(s), which are divided based on whether off-targets are predicted and the genomic location of the sgRNA. **B:** JBrowse view of all relevant CRISPR designs. **C:** Detailed information page of each sgRNA design. Efficiency score predictions as well as restriction enzyme annotations are displayed beside the target gene, sgRNA sequence and genome location. **D:** Efficiency prediction tool. This user interface allows calculation of efficiency scores for user-provided sgRNA sequences.

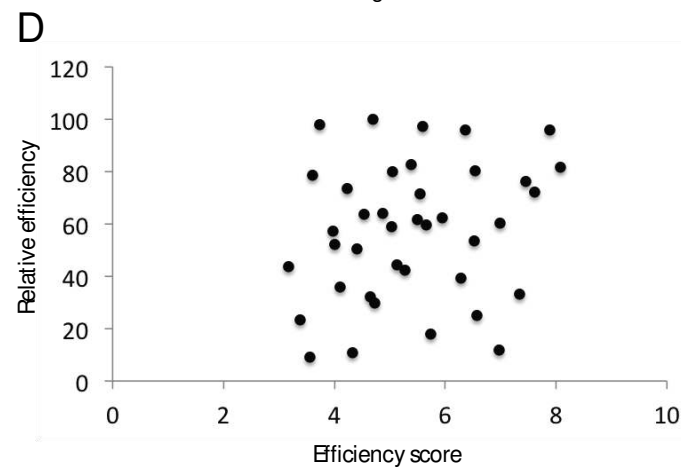
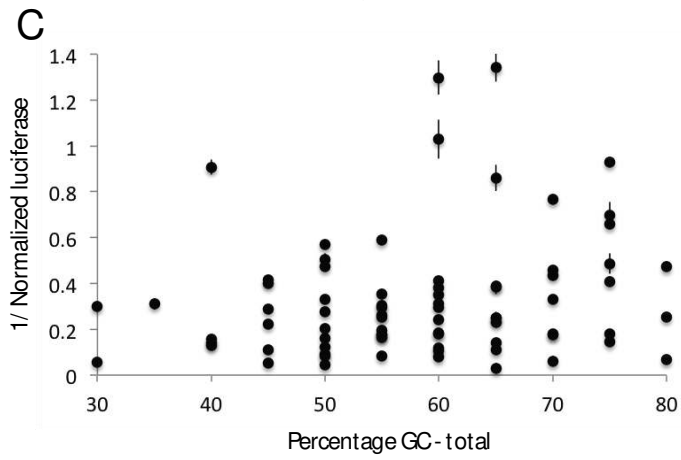
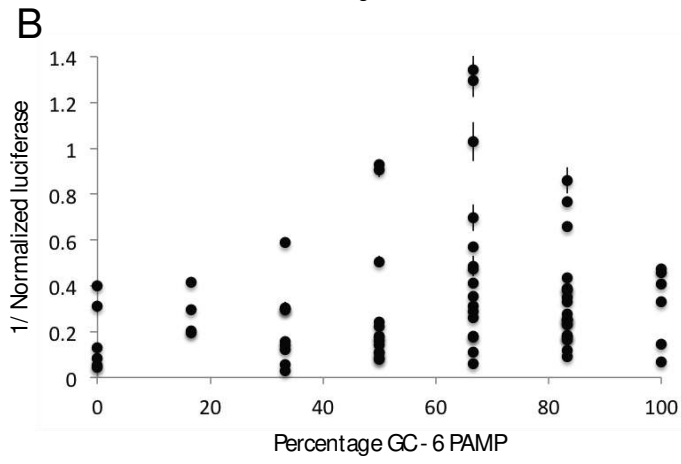
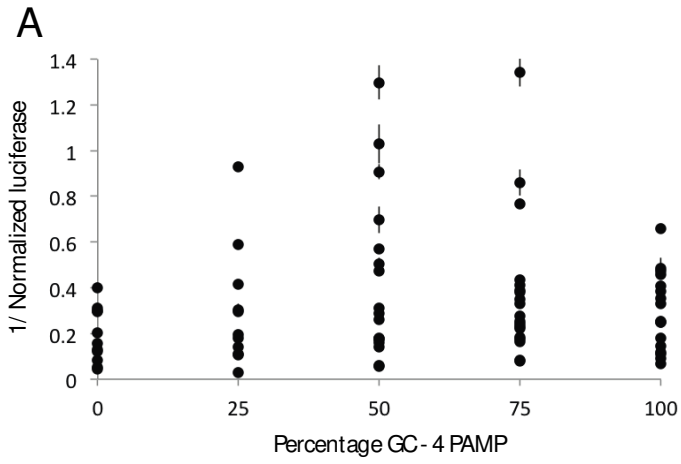


Figure S3: Analysis of GC content in relation to sgRNA efficiency

A-C: Comparison of sgRNA mutagenesis efficiency to GC content considering the final 4 nucleotides (A), the final 6 nucleotides (B) or the whole sgRNA sequence (C). **D:** Comparison of efficiency scores generated using the matrix shown in Figure 1B (horizontal axis) with efficiency (vertical axis) of sgRNAs published by Ren *et al.* (16).

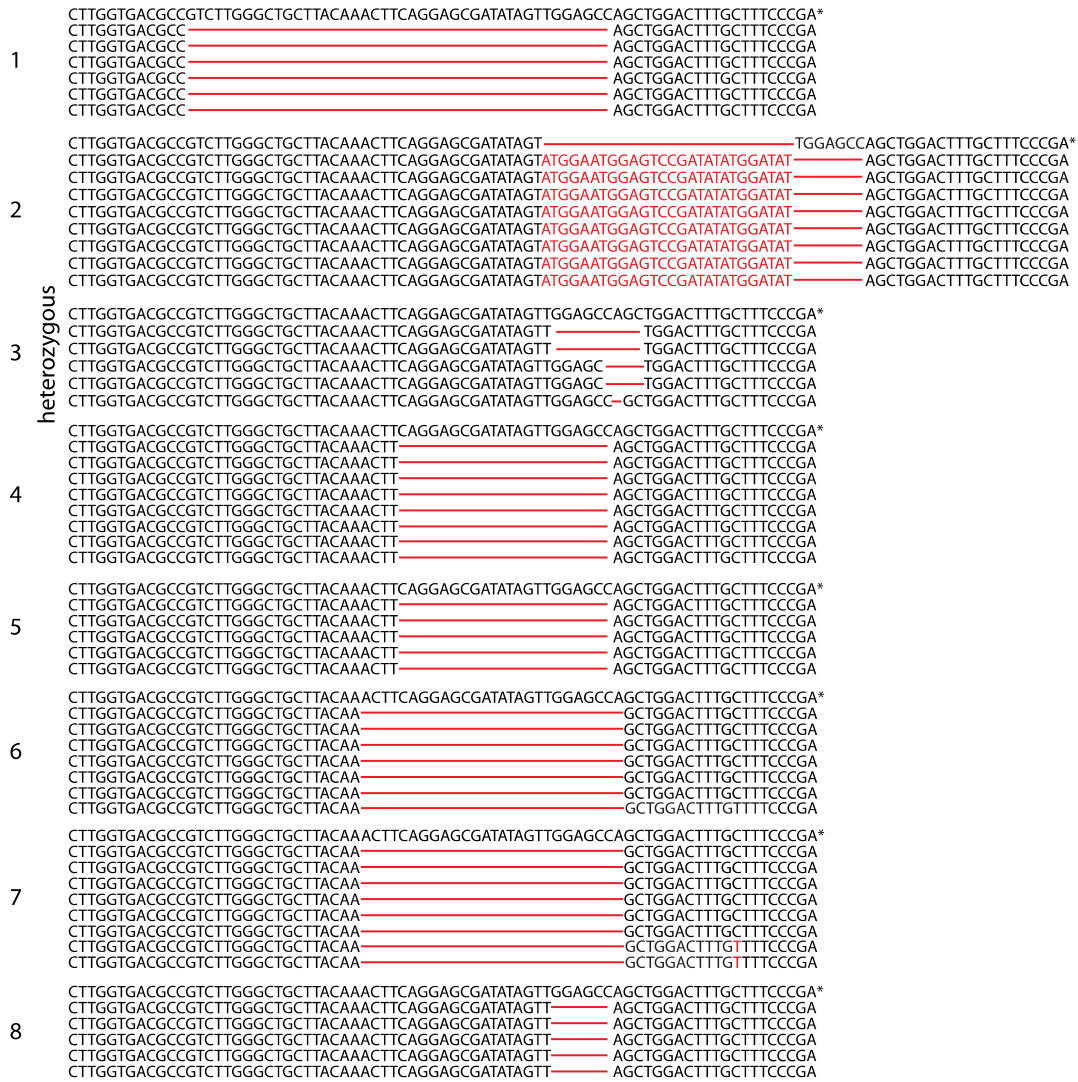


Figure S4: Sequencing of individual CRISPR mutant cells

Sequences are shown from 8 individual cells transfected with CRISPR reagents targeting the *yellow* gene. Samples are numbered 1 to 8, with a minimum of 5 sequence reads shown for each. The top row in each group shows wild-type sequence (marked with asterisks).

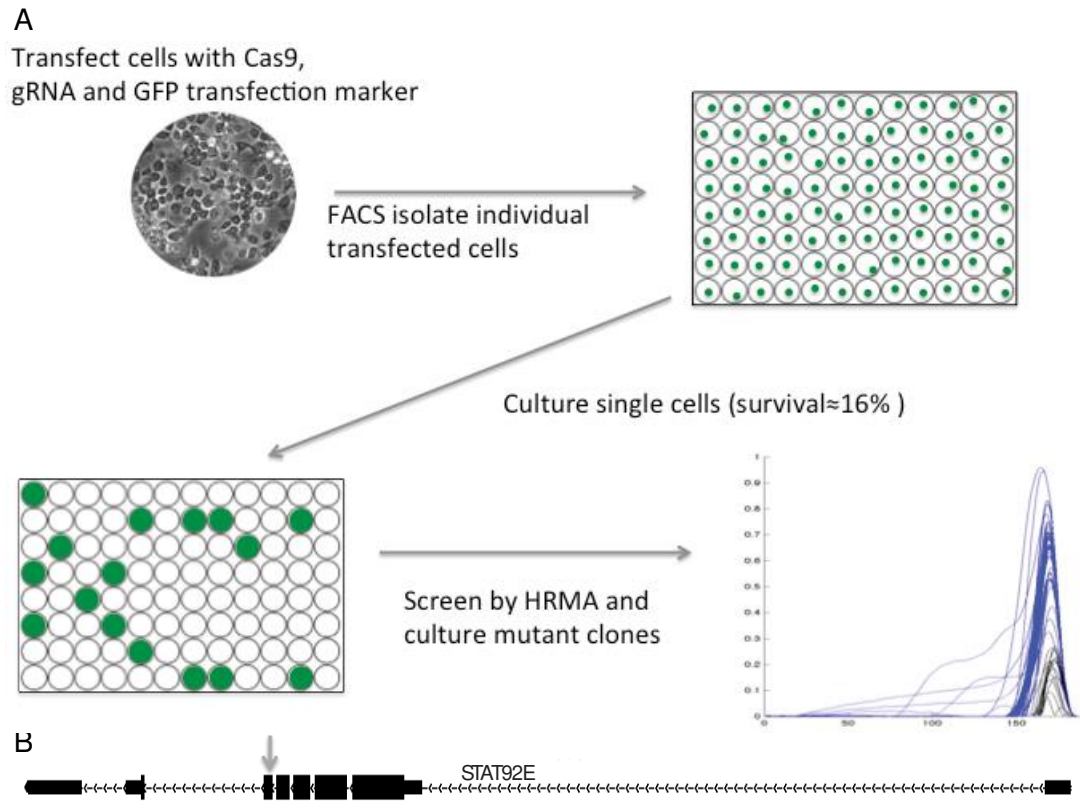


Figure S5: Generation of isogenic mutant cell lines

A: Workflow showing the major steps required to generate mutant cell lines.

B: Schematic of the *STAT92E* gene. UTRs are represented by thin black boxes, coding exons by thick black boxes and introns by black lines. Arrows superimposed on introns indicate the direction of transcription. CRISPR target site is shown by the grey arrow.

Figure S6: Generation of *TSC1* and *TSC2* mutant cell lines

A: Schematics of the *TSC1* and *TSC2* genes. Details are as described for Figure S5A. **B-C:** Sequencing result for at least 20 clones from *TSC1* (B) or *TSC2* (C) mutant cell lines. Asterisks indicate wild-type sequence.

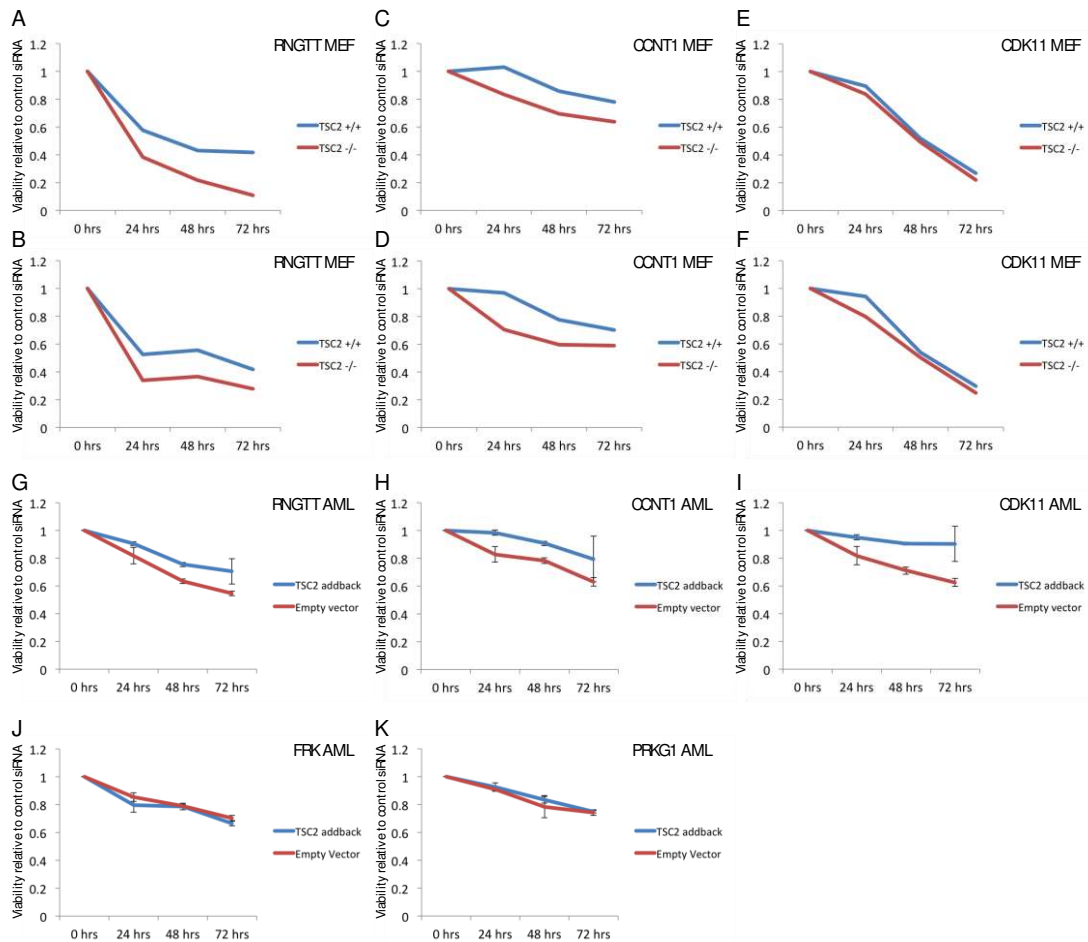


Figure S7: Synthetic effects in MEFs and AML cells

A-K: Viability plots over 72 hours relative to control siRNA treatments in wild type MEFs (A-F – blue lines), TSC2 deficient MEFs (A-F – red lines), AML cells (G-K – red lines) or AML cells with TSC2 addback (G-K – blue lines) treated with siRNA targeting the genes indicated. Results are from two biological replicates for MEFs (shown separately) and three biological replicates for AML cells. Error bars indicate standard error of the mean. See Figure 4C-D for summary plots of these data.

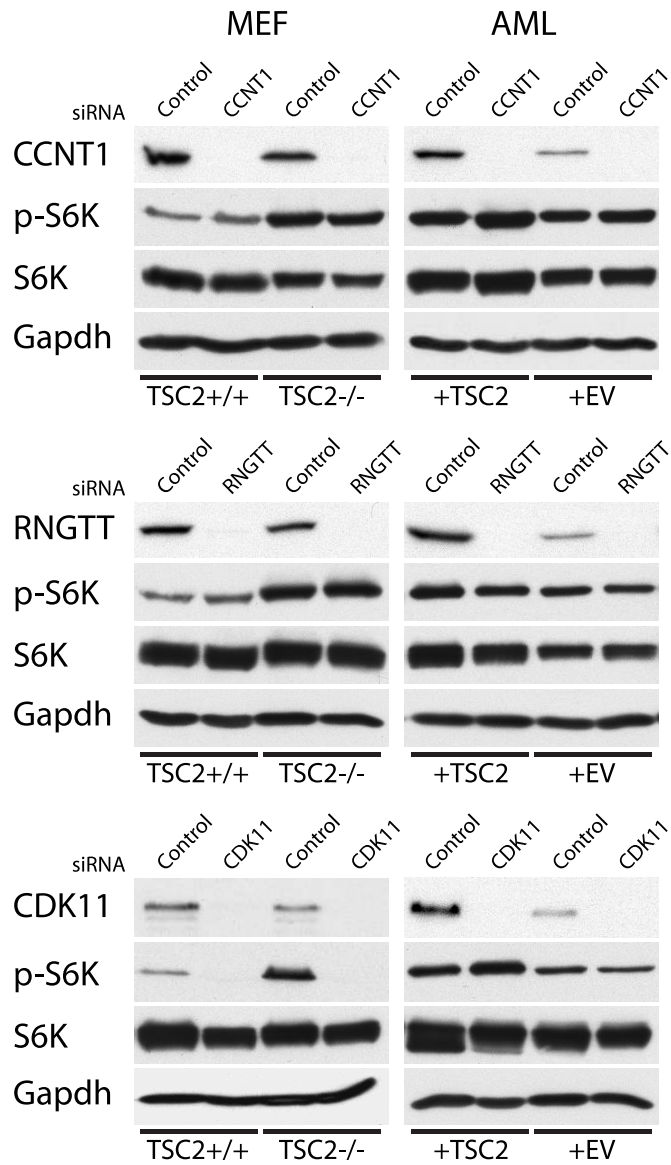


Figure S8: Analysis of knockdown of candidate genes on mTOR signaling
 Western blots for the indicated proteins in wild-type MEFs (*TSC2*^{+/+}), *TSC2* deficient MEFs (*TSC2*^{-/-}), AML cells (+EV) or AML cells with *TSC2* addback (+*TSC2*) in the presence of siRNA targeting *CCNT1*, *RNGTT* or *CDK11* as labeled. All Western Blots are representative of three biological replicates.

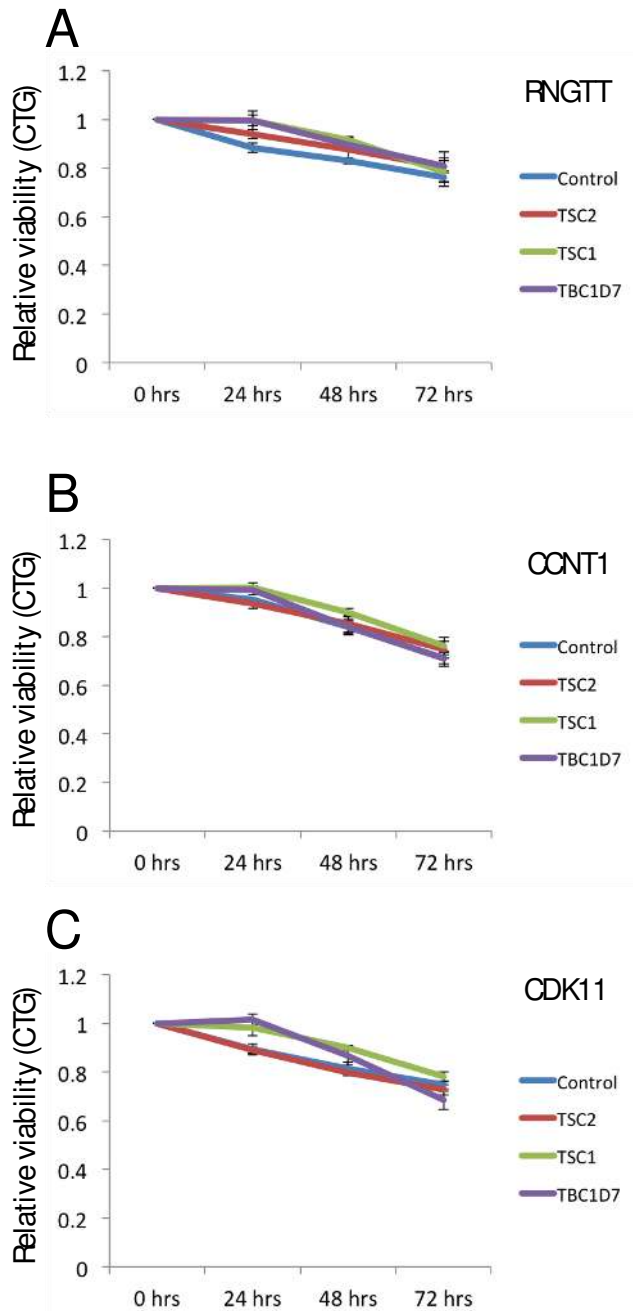


Figure S9: Synthetic effects of candidate genes with *TBC1D7*

A-C: Viability plots over 72 hours relative to control siRNA treatments in wild-type MEFs treated with siRNA targeting the genes indicated in combination with siRNA targeting *TSC1*, *TSC2* or *TBC1D7*. Results represent the average of three biological replicates and error bars indicate standard error of the mean.

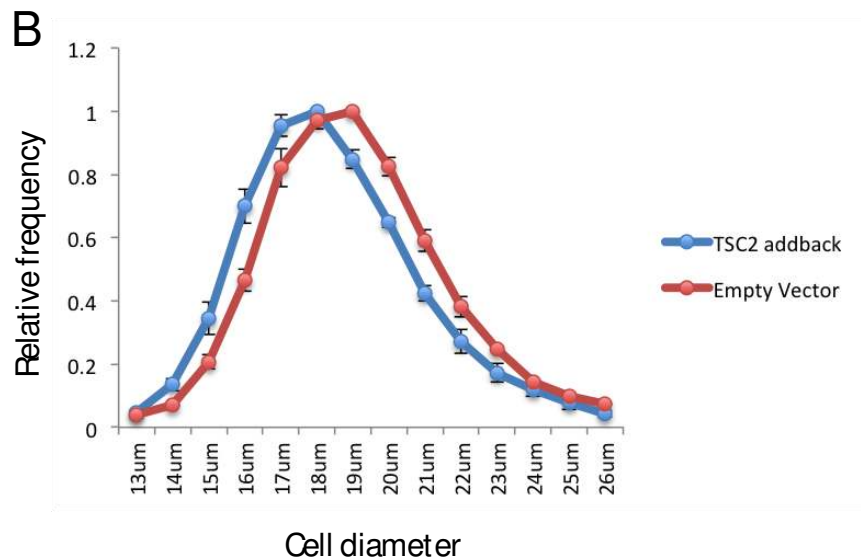
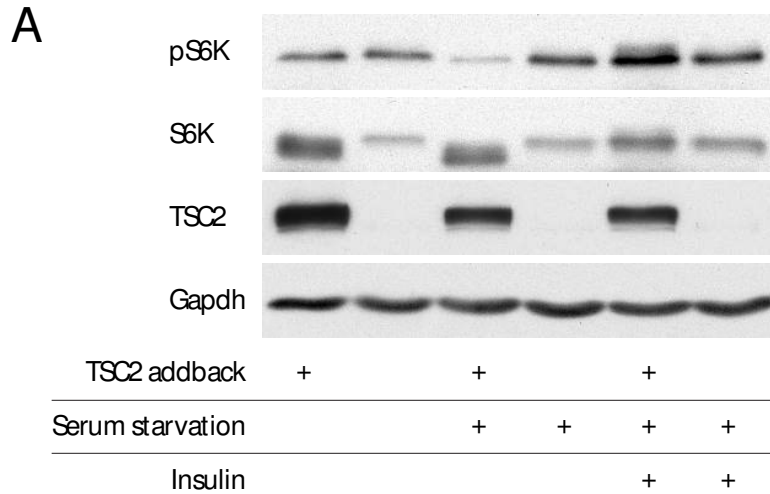


Figure S10: Analysis of *TSC2* addback efficacy in AML cells

A: Western blots showing changes in p-S6K, total S6K, TSC2 or Gapdh in AML cells with or without TSC2 addback. Experiments were performed under standard culture conditions, serum starvation or with insulin stimulation as indicated. Westerns are representative of at least three biological replicates.

B: Plot showing the distribution of cell diameters of AML cells with (blue line) or without (red line) TSC2 addback. Results show the average from three biological replicates and error bars indicate standard error of the mean.