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Supplemental Information

This PDF file includes:

Figures S1 to S6
Figure S1. FKBP10 expression negatively correlates with survival of patients with other cancer-types. Related to Figure 1

(A), FKBP10 expression negatively correlates with survival of male and female patients with lung adenocarcinoma. Data were obtained from KMPLOT (high and low values of expression were split by median expression of FKBP10). (B), In the same cohort as in Figure 1G, expression of FKBP10 in tumors with KRAS or EGFR mutations vs non mutants, respectively (analysis performed in CANCERTOOL). In the box plots, centre line is median; box limits are the upper and lower quartiles; whiskers are Tukey's 1.5× interquartile range; and points are outliers. Comparisons were performed by two-tailed unpaired Student’s t test. (C), FKBP10 expression negatively correlates with survival of patients with other cancer-types. Data were obtained from KMPLOT (high and low values of expression were split by median expression of FKBP10).
Figure S2. FKBPI0 ablation suppresses tumorigenesis after tumor onset, Related to Figure 3 (A), Schematic representation of the breeding pairs used to generate KFU mice. (B), Graphs representing tumor growth evolution (mm$^3$) by micro-CT scanning obtained at week 0, 6 and 12 after tumorigenesis induction of individual mice.
Figure S3. FKBP10 promotes cancer stem-like traits via its PPIase activity. Related to Figure 4 (A), Tumorsphere-formation capacity of indicated human cancer cells harbouring empty vector or shFKBP10. (B), Tumorsphere-formation capacity of A549 cells (harbouring empty vector or shFKBP10) isolated cells with ALDH\textsuperscript{high} and ALDH\textsuperscript{low} activity. (C), Kaplan-Meier curves comparing percentage of tumor-free mice at different time points after subcutaneous injection of A549 and H1650 cells harboring empty vector or shFKBP10 (at concentrations $1\times10^4$ and $5\times10^4$). Data are represented as mean ± SEM. Comparisons were performed by two-tailed unpaired Student’s t test, (A, B) or long-rank test (C); *p<0.05, **p<0.01 and ****p<0.0001. Scale bar: 50 μm (A); n=6 mice per group (C).
Figure S4. FKBP10 does not impact on protein translation initiation, Related to Figure 5

(A), Immunoblot showing expression of FKBP10 in A549 cells harbouring an empty vector or shFKBP10 (in duplicates) in both whole cellular lysate (input) and immunoprecipitated (IP) proteins from whole cellular lysate using an antibody against FKBP10. (B), The levels of p-eIF2α and tubulin as a control were evaluated by Western blotting from the A549 cells harbouring empty vector or shFKBP10. Histogram is showing the quantification of the protein content (normalization over Tubulin). (C), The levels of total and phosphorylated eIF2 in tumors of Kras<sup>G12D</sup> and Kras<sup>G12D; Fkbp10<sup>+/−</sup></sup> mice were evaluated by Western blotting, and quantified (p-eIF2α/eIF2α). (D), Representative figure of immunostaining of A549 cells using an antibody recognizing the cytotoxic granule associated RNA binding protein TIA1 (arrow indicates a cytoplasmic TIA1 positive granule; Φ represents a nucleus). Exposure of cells at 44°C for 20 minutes was used to induce stress granules and hence as a positive control. Data are represented as mean ± SEM. Statistical analyses were done using two-tailed unpaired Student’s t test (B, C).
**Figure S5. Ribosome profiling quality control, Related to Figure 6**

(A), Pairwise scatterplots of logged PPKMs for Ribo-Seq were produced for each sample pair. Pearson correlation coefficients were calculated for each pairing. (B), A histogram of ribosome footprint lengths was plotted for each sample. (C), A barplot of the total number of P-site counts occupying each reading frame was plotted for each sample. (D), All fold changes generated by edgeR differential expression analysis comparing P site counts for each CDS in empty vector and shFKBP 10 were plotted against the proline content of the CDS. Significantly upregulated genes were overlaid in red, downregulated in blue. (E), Ribosome footprint P site counts per thousand were evaluated per codon at the genome wide level before and after FKBP10 knockdown and ordered by mean occupancy.
Cancer cells with FKBP10

Cancer cells lacking FKBP10

FKBP10

E P A

Pro

RPS15

20kDa

RPS27

15kDa

10kDa

β-actin

37kDa

RPS13

E P A

Pro

RPS15

20kDa

RPS27

15kDa

10kDa

β-actin

37kDa
Figure S6. RP mRNA levels are not significantly different between control and FKBP10 knockdown cells, Related to Figure 6
(A), Pairwise scatterplots of logged RPKMs for RNA-Seq were produced for each sample pair. Pearson correlation coefficients were calculated for each pairing. (B), For all RP genes, mean RPKMs from the empty vector and shFKPB10 RNA-Seq were plotted against each other. Selected RP genes are highlighted in green and the equality line is shown in red. (C), Barplots showing the levels of the selected RP mRNAs by RT-qPCR, normalized over beta-actin, in A549 cells harbouring empty vector or shFKBP10. (D), Comparison of the total RNA levels in empty vector versus FKBP10 knockdown (x axis) relative to ribosome footprint RNA in empty vector versus FKBP10 knockdown (y axis). RP genes are indicated in green. (E) Levels of selected RPs (RPS27 and RPS15) were evaluated in A549 cells harboring empty vector and shFKBP10 by Western blotting. (F) A model illustrating the role of FKBP10 in cancer cells. According to this model, in lung cancer cells FKBP10 can access the ribosome catalytic centre mostly during the early phase of translation elongation and accelerates translation elongation in particular upon insertion of prolines (pro). Hence, lack of FKBP10 impairs translation elongation at proline codons. The E-site (exit), P-site (peptidyl) and A-site (aminoacyl) are shown. Data are shown as mean ± SEM. Statistical analyses were done using two-tailed unpaired Student’s t test (C).