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Supplemental Information

Negative Regulation of BOK Expression by Recruitment of TRIM28 to Regulatory Elements in Its 3′ Untranslated Region

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SUPPLEMENTAL FIGURES

Figure S1 related to Figure 3. Occurrence of AU-, GU- and U-rich elements within the 3'UTR of selected BCL2 family members. The presence of regulatory elements within the 3'UTR of the indicated human genes were predicted using the publicly available tool ARE site2 (http://rna.tbi.univie.ac.at/ARE site2) and their absolute frequency plotted (left panel). The positions of the predicted motifs were color-coded according to the individual class and mapped on a normalized length version of each 3'UTR using the genomic coordinates as follows: $Motif\ position = \frac{|Motif_{Start} - 3'UTR_{Start}|}{|3'UTR_{Start} - 3'UTR_{End}|}$ (right panel). Nucleotides are designated according to IUPAC convention; K=G or T, N= any base, W= A or T. Original data available online at the Mendeley Database; http://dx.doi.org/10.17632/9dfn8m6sgd.1.

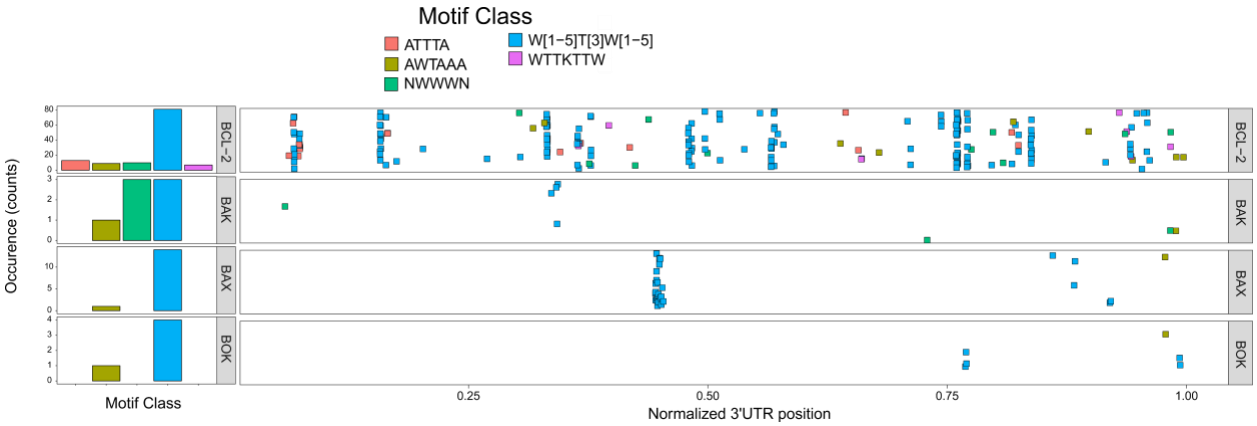
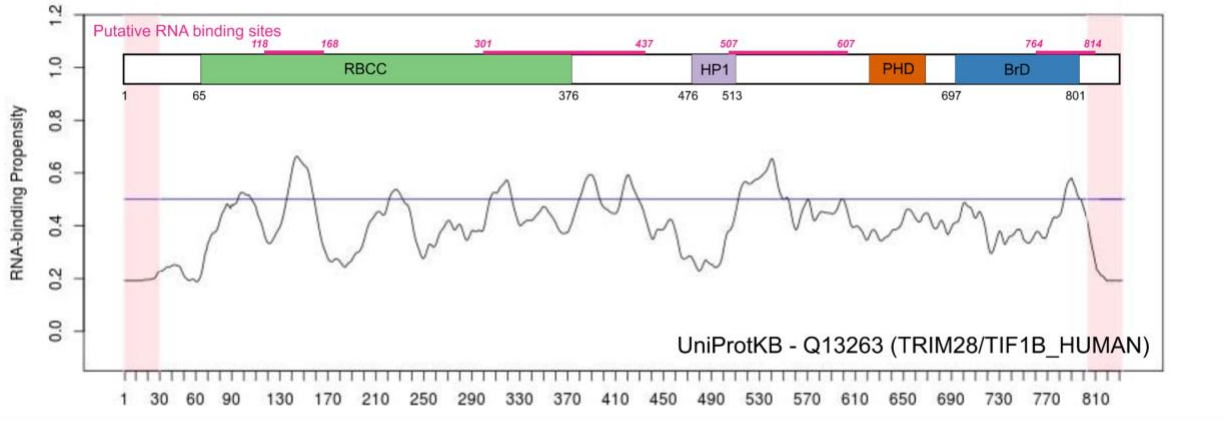


Figure S2 related to Figure 5. Theoretical occurrence of RNA binding modules in the TRIM28 sequence. Amino acid sequence of human TRIM28 protein (Q13263) was used for the analysis for RNA binding propensity in the public web server http://s.tartaglialab.com/page/catrapid_group. Likelihood of binding above 0.5 indicates potential RNA-binding. The predicted modules are horizontally highlighted in pink on a superimposed cartoon depicting the functional domains of TRIM28. Original data available online at the Mendeley Database; http://dx.doi.org/10.17632/9dfn8m6sgd.1.



RBCC: Ring Finger B-Box Coiled-Coil Tripartite Motif
HP1: HP1 Binding Box
PHD: Plant Homeodomain type Zinc Finger
BrD: Bromodomain

Figure S3 related to Figure 5. (A) SV40 WT MEF were transiently co-transfected with 2 µg of plasmid mix (ratio 1:9; pcDNA3.EGFP/pcDNA3.humanTRIM28) and the endogenous BOK protein levels analyzed by immunoblotting after 48 hours. (B, C) Quantification of BOK abundance at both protein (B) and transcript (C) levels in the lung cancer cell lines NCI-H1299 and LFX-289 stably transduced with HA-tagged human TRIM28. *TRIM28* mRNA levels are much lower in the LFX-289 line, correlating with decreased mean fluorescence of IRES-EGFP. The data is presented as mean ± SD, N=3. See Figure 5E for representative western blot.

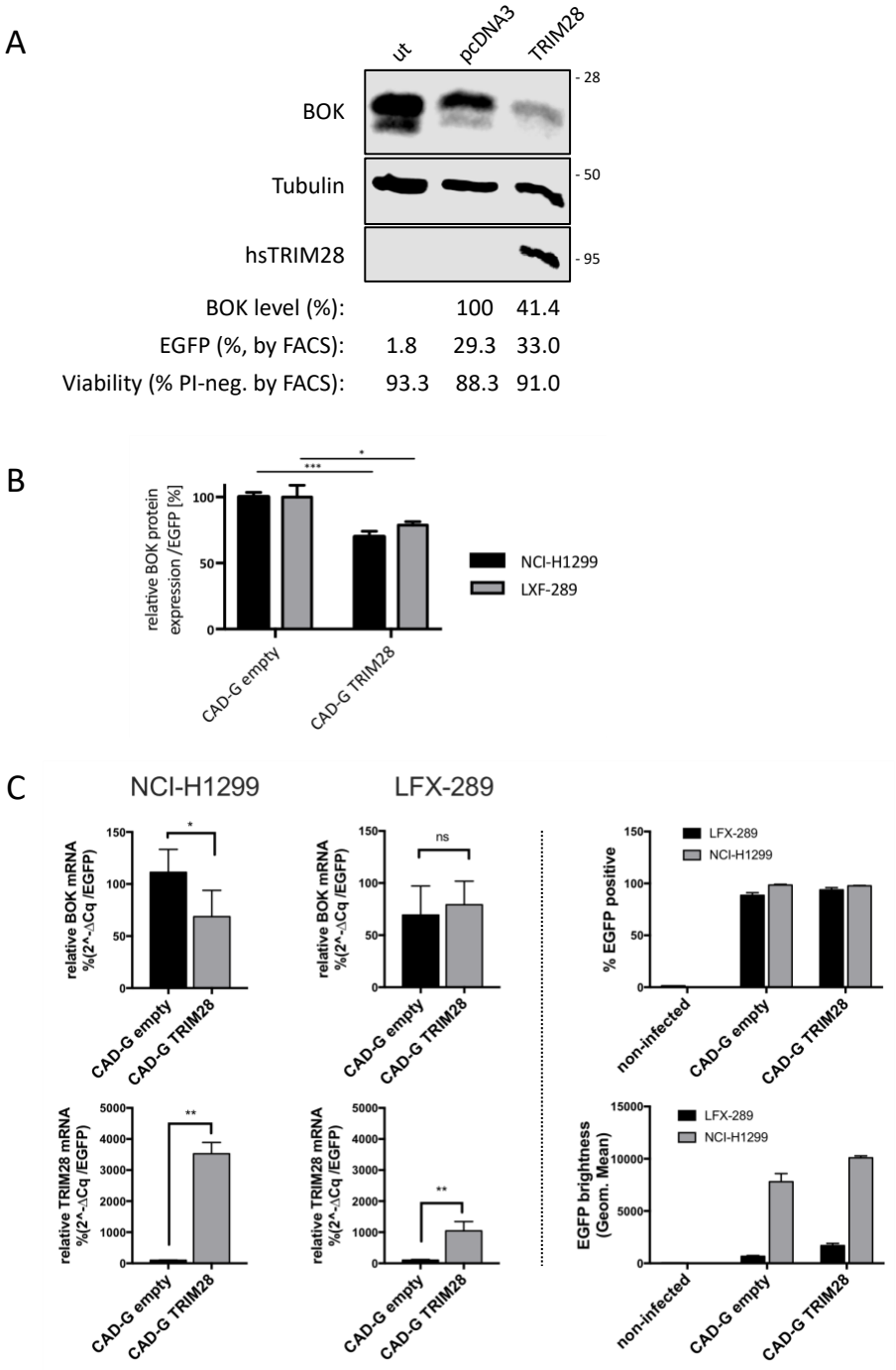


Figure S4 related to Figure 6. Estimation of risk associated with *BOK* and *TRIM28* mRNA levels in several cancer types. Multiparametric Cox hazard ratio models using expression of *TRIM28* and *BOK* mRNA levels as continuous variables were prepared for thirty-three cancer types. 95% confidence interval and number of patients used in the analysis are included in the graph. Significant covariates are colour coded (red). See Table S2 for original data, http://dx.doi.org/10.17632/9dfn8m6sgd.1#file-08fd9021-e239-4c81-906e-66bf80b92d1d.

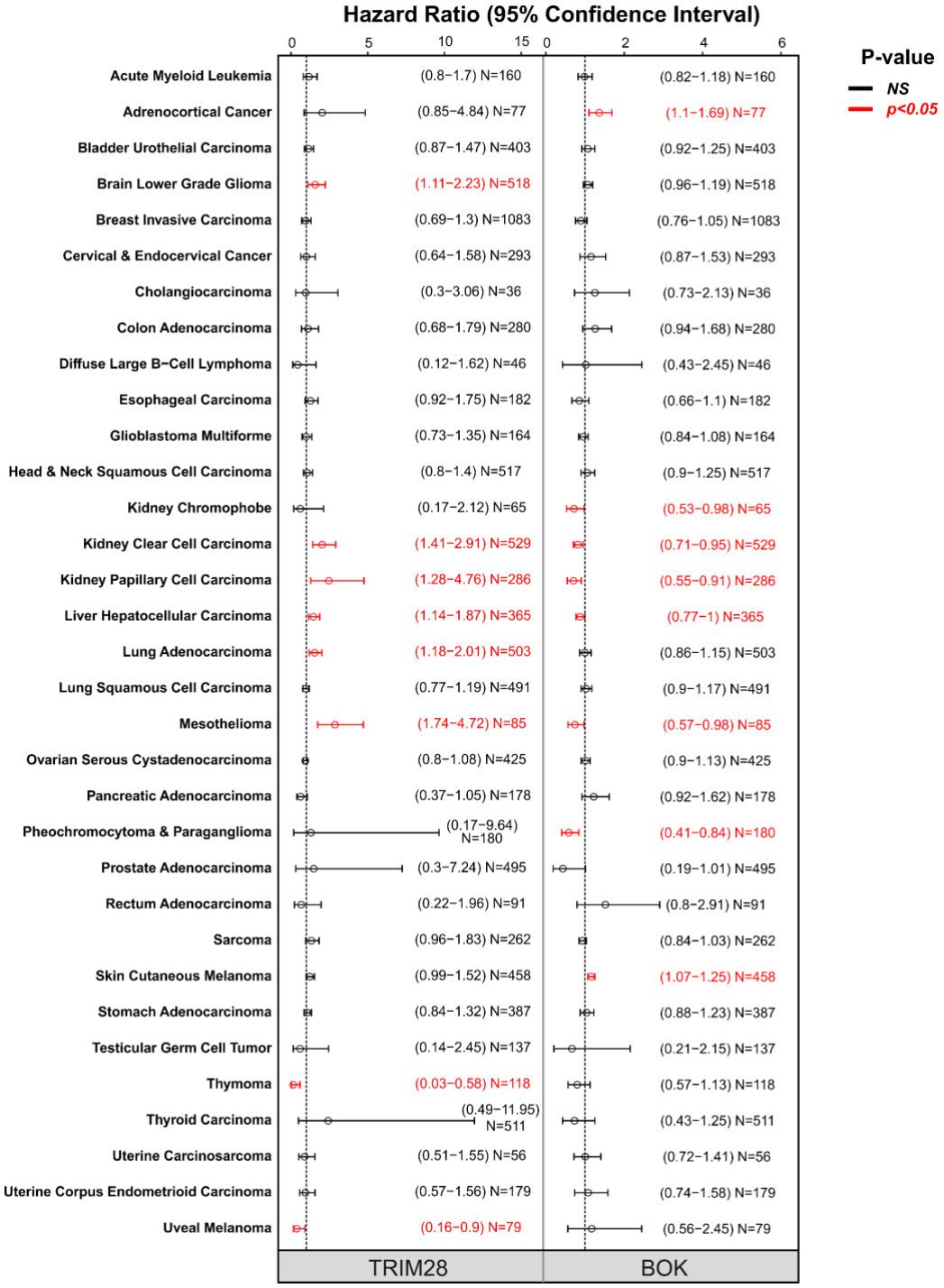
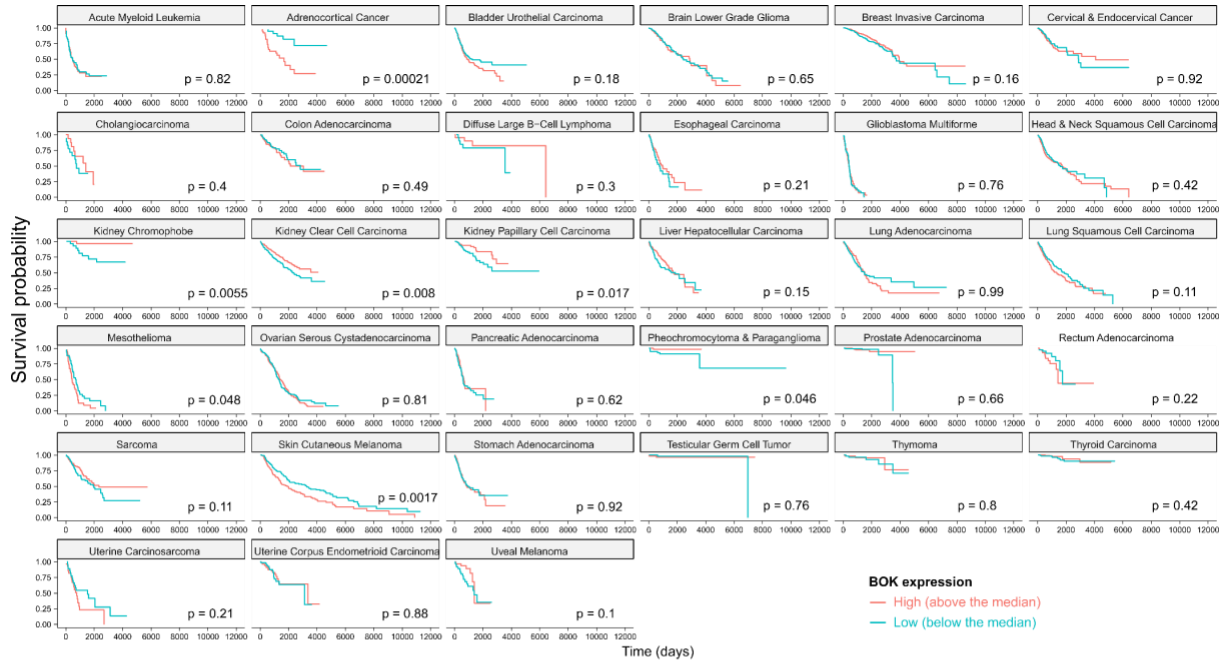
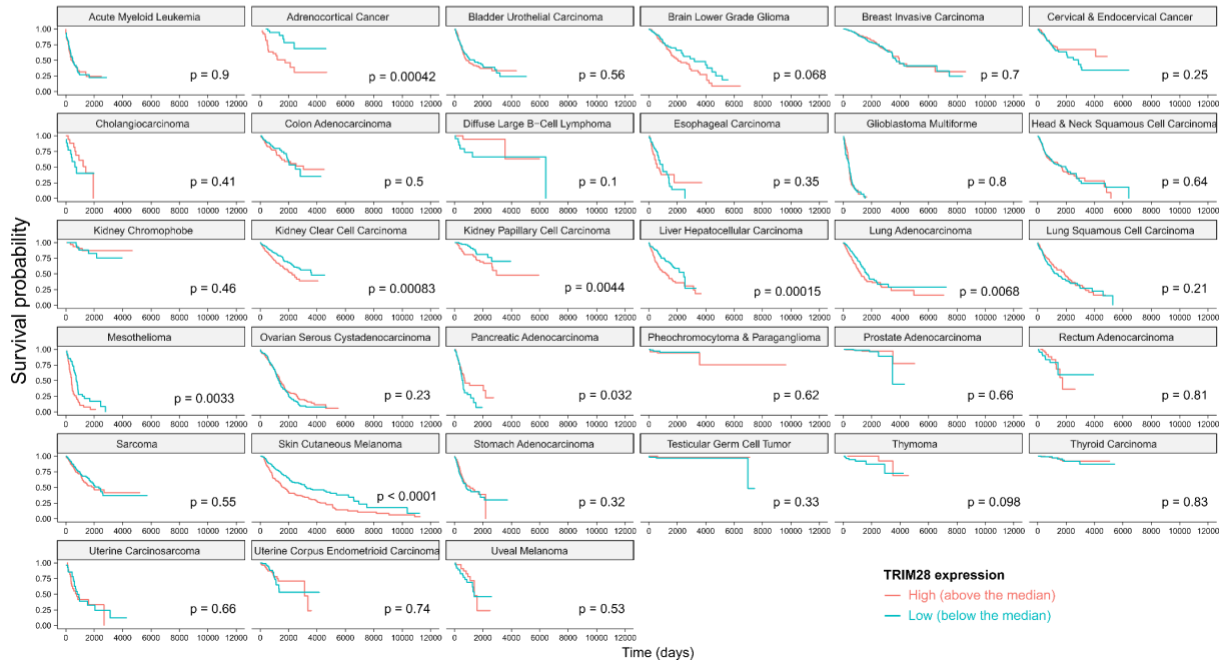


Figure S5 related to Figure 6. Overall survival of patients from different cancer types according to TRIM28 or BOK expression levels. Patients were classified into "high" or "low" strata according to the population median of *BOK* (upper panel) or *TRIM28* (lower panel) and the overall survival plotted as a function of each strata. Differences in survival were detected with the Log-Rank test. Significant differences were declared for p values below 0.05. See Table S2 for original data, http://dx.doi.org/10.17632/9dfn8m6sgd.1#file-08fd9021-e239-4c81-906e-66bf80b92d1d.





TRANSPARENT METHODS

Cell lines and cell culture

Dulbecco's Modified Eagle's Medium-high glucose (DMEM), RPMI 1640 AQmedia™ and 100 mM cell culture grade sodium pyruvate were purchased from Sigma-Aldrich Chemie GmbH (Buchs, CH); penicillin/streptomycin (P/S) and trypsin-EDTA were purchased from Gibco (Thermo Fisher Scientific Switzerland, Reinach, CH). Ultra-low endotoxin fetal calf serum (FCS) was purchased from Pan Biotech (Aidenbach, DE). SV40 LT-immortalized mouse embryo fibroblasts (SV40 MEF) derived from *Bok*$^{-/-}$ animals have been previously described (Echeverry et al., 2013). The human embryonal kidney cell line containing the temperature sensitive mutant of the SV-40 large T-antigen (HEK293T) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, DE). The non-small cell lung cancer cell line LXF-289 and the large-cell lung carcinoma cell line NCI-H1299 were obtained from the European Collection of Authenticated Cell Culture (ECACC, Salisbury, UK) and the DSMZ respectively. The colon colorectal carcinoma cell line HCT-116 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). *Bok*$^{-/-}$ SV40 MEF and HEK293T cells were cultured in DMEM supplemented with 1 mM sodium pyruvate, 100 U/mL penicillin, 100 µg/mL streptomycin and 5 % FCS while cancer cell lines were cultured in RPMI 1640 supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin and 10 % FCS. All cells were kept in a humidified incubator at 37°C with 5 % CO_2. Human *BOK* cDNA harboring wildtype or URE mutated 3'UTR were cloned into pcDNA3.1 (see below), transfected into HEK293T cells and selected with 2.3 mg/mL G-418 (Gibco, Thermo Fisher Scientific Switzerland, Reinach, CH).

cDNA

Mouse *Bok* cDNA (ENSMUST00000027499.12, Cat#: MC206561, cloned into a pCMV6-Kan/Neo vector) was purchased from Origene (Rockville, MD, USA). Human *BOK* cDNA (ENST00000318407.4) was synthetized from isolated RNA derived from human immortalized hepatocytes (IHH cell line kindly provided by Prof. Thomas Brunner, University of Konstanz, DE). Briefly, 10^7 cells were collected washed with saline and the RNA extracted using the SV Total RNA extraction kit followed by reverse transcription of 1 µg of total RNA using the M-MLV reverse transcriptase according to the manufacturer's instructions (Promega, Dübendorf, CH). Human *BOK* cDNA was then amplified with the primers forward 5'- GTAGAATTCCTCGCTGCCCAGGCCCCCGACG-3' and reverse 5'-CATGCGGCCGCTCTGTCTAAAAT GACAACGA TCG-3' using the Phusion Hot Start Flex DNA Polymerase (New England Biolabs, Ipswich, MA, US), digested with *EcoRI*/*NotI* and cloned into the pcDNA3.1 vector.

PCR based subcloning and site directed mutagenesis

Mouse *Bok* constructs devoid of, or bearing combination of its 5' and/or 3'UTRs were amplified from the pCMV6-*Bok*(cDNA) plasmid and subcloned via the restriction sites *EcoRI/NotI* into the pcDNA3.1 vector using the following set of primers: *mm*cDNA forward 5'-TACGACTCACTATAGGGCG-3' reverse 5'-GTACGCGTAAGCTTGGGC-3'; mmCDS forward 5'-GTAGAATTCATGGAGGTGCTGCGGCGCTCTTCTG-3', reverse 5'-CTGGCGGCCGCTCATCTCT CTGGCAACAACAGGAAG-3'; *5'UTR-CDS* forward 5'-TACGACTCACTATAGGGCG-3', reverse 5'-CTGGCGGCCGCTCATCTCTCTGGCA ACAACAGGAAG-3'; *mmCDS-3'UTR* forward 5'-GTAGAATTCATGGAGGTGCTGCGGC GCTCTTCTG-3', reverse 5'-GTACGCGTAAGCTTGGGC-3'. Truncated versions of the *Bok* 3'UTR were created by amplifying the whole pcDNA3/mmBokCDS-3'UTR construct except the desired area to delete using the following primers per construct: *mmCDS-3'UTR∆1* (deletion between positions 951-1134) forward 5'-CATTCCTTTGTGGACC CTGG-3', reverse 5'-TCATCTCTCTGGCAACAACAGG-3'; *mmCDS-3'UTR∆2* (deletion between positions 1134-1341) forward 5'-GCTAATGCAGGAGAAGCCAG-3', reverse 5'-CAGACTCTGGCTTTCTCC-3'; *mmCDS-3'UTR∆3* (deletion between positions 1341-1575) forward 5'-GAAGAAGGTATCCCTAAGCC-3', reverse 5'-CAGAGTCCCCTTCA GGTCAA-3'. PCR-based mutagenesis of the ARE site (position 1049, AUUUA→GACGU) located in the mouse *Bok* 3'UTR was created using the pcDNA3/mmBokCDS-3'UTR as template and complementary primers carrying the desired sequence: forward 5'-AATTG GGAGCGACGTGCCCCTGGG-3', reverse 5'-TGAGTGAGGGAGGTGCTTTG-3'.

To create an expression construct of EGFP flanked by the 5'- and 3'-UTRs of *Bok,* or its ARE site mutated version, respectively, the EGFP ORF was amplified by PCR from the pEGFP-C3 (Clontech, USA) template using the primers forward 5'-ATGGTGAGCAAGGGCGAG-3' and reverse 5'-TCAGTTATCTAGATCCGGTGG-3' and cloned into pcDNA3/mmBOK-cDNA or pcDNA3/mmBok-cDNA-3'UTRAREmut in exchange for the *Bok* CDS (removed by PCR: forward primer 5'- GCTGGCCA CCAGGGCAG -3', reverse primer 5'-GGCCGAAGCGCGGGCGA-3').

The human *BOK* coding sequence devoid of (hsCDS) or containing the 3'UTR (hsCDS-3'UTR) was amplified from the isolated cDNA (described above) using the following primers: hsCDS forward 5'-GTAGAATTCATGGAGGTGCTGCGGCGCTCTTCTG-3', reverse 5'-CATGCGGCCGCTCATCTCTCTGGCAGCAGCACGAAG-3'; hsCDS-3'UTR forward 5'-GTAGAATTCATGGAGGTGCTGCGGCGCTCTTCTG-3', reverse 5'- CATG CGGCCGCTCTGTCTAAAATGACAACGATCG -3' and subcloned using *EcoRI/NotI* into the pcDNA3.1 vector. Constructs with mutations in the predicted URE sites of the human *BOK* 3'UTR were created using pcDNA3.1/hsBOK-CDS-3'UTR as template and pairs of complementary primers as follows: *hsCDS-3'UTR URE mut1* (position 2224, AUUUUU→GCAACG) forward 5'-CCCATCTGCTGCAACGGTGCTCATCC-3', reverse 5'-GTC ACCCTCACCCTCCACTG-3'; *hsCDS-3'UTR URE mut2* (position 2635, AUUUUA→GCAACG) forward 5'-GATCGTTGTCGCAACGGACAGAGCGG-3', reverse 5'-GTTTGGAGAAGTTTATTA CCACCC-3', *hsCDS-3'UTR URE mut3* (position 2608, AAUAAA→GCAUGC) forward 5'-GTAG GGGTGGTGCATGCCTTCTCCAAAC-3', reverse 5'-CCTCCAGTGGGATCTCAATG-3'. Sequential mutagenesis PCRs were performed with the previous oligos to create double (*hsCDS-3'UTR UREmut1+2*) or triple (*hsCDS-3'UTR UREmut1-3*) URE mutants. Correct sequences of all generated constructs were confirmed by sequencing (Microsynth, Balgach, CH).

Transient transfection, quantitative real time RT-PCR analysis and immunoblotting

1 x 10^6 HEK293T cells were seeded in 3 mL of media in a 6 well plate (Greiner Bio-One, Frickenhause, DE) and transfected with 2.5 µg of DNA using X-tremeGENE™ HP DNA Transfection Reagent (Roche, Rotkreuz, CH) according to the manufacturer's instructions. The transient transfection efficiency of BOK expression constructs was normalized by co-transfecting a second plasmid encoding an internal reporter, pEGFP or pcDNA3.1/FLAG-MCL-1, at a ratio of 1:10 of the target construct. Cells were harvested after 24 h an divided for RNA isolation and quantitative RT-PCR (qPCR) analysis or immunoblotting, respectively. RNA isolation, reverse transcription and PCR amplification were performed as described elsewhere (Echeverry et al., 2013). For qPCR analysis, *BOK* cDNA was amplified using the following specie specific primers: human forward 5'-CAGTCTGAGCCTGTGGTGAC-3', reverse 5'-TGATGCCTGCAGAGAAGATG-3'; mouse forward 5'-TTCTCAGCAGGTATCACATGG-3', reverse 5'-TAGCCAAGGTC TTGCGTACA-3'. Human β-actin was used as reference gene using the primers: forward 5'-CTGGCACCCAGCACAATG-3', reverse: 5'-GGGCCGGACTCGTCATAC-3'. Mouse *Hprt* was used as reference gene in MEF: forward 5'-TGGATACAGGCCAGACTTTGTT 3', reverse 5'-CAGATTCAACTTGCGCTCATC-3'. For immunoblotting, unless otherwise indicated, all lysates were prepared in pre-heated H8 buffer (20 mM Tris-HCl pH 7.5, 2 mM EDTA, 2 mM EGTA, 1 % SDS, 100 mM DTT) during 5 min at 95°C followed by mechanical shearing with a 25G needle, centrifugation at 15'000 rpm and protein concentration estimation using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Reinach, CH). Equal amounts of lysates were prepared with reducing Laemmli buffer, separated on 12.5 % SDS-PAGE gels and blotted to Immobilon-FL membranes (0.45 µm pore PVDF, Merck Millipore, Schaffhausen CH). Membranes were probed with the following antibodies: rabbit monoclonal anti-BOK clone 1-5 (Echeverry et al., 2013), mouse anti-FLAG antibody (clone M5) and mouse anti-tubulin (clone B-5-1-2) from Sigma-Aldrich (Buchs, CH), rabbit anti-GFP (D5.1) from Cell Signaling Technology (Danvers, MA, US), rabbit anti-BAX (N-20: sc-493), mouse anti-TIF1β (D-7: sc-515790), mouse anti-SAFB2 (C-5: sc-514963), mouse anti-LSM5 (G-5: sc-398656) and mouse anti-TIAR (TIAL-1) (G-6: sc-398372) from Santa Cruz Biotechnologies (Dallas, TX, USA), rabbit anti-BAK (BAK-NT) from Merck Millipore (Schaffhausen, CH) and rat anti-HA (clone 3F10) from Roche (Rotkreuz, CH). Commercial secondary antibodies specific for mouse, rabbit or rat IgG coupled to the infrared fluorophores IRDye®680CW or IRDye®800CW were purchased from LI-COR Biosciences (Bad Homburg, DE). Immunoblot signals were acquired in an Odyssey Fc (LI-COR Biosciences) and quantified using the Image Studio Lite Software version 5.25 (LI-COR Biosciences).

BOK 3'UTR RNA probe synthesis and pulldown of URE-binding proteins

Biotinylated RNA probes encoding the human *BOK* CDS-3'UTR and its URE triple mutant counterpart (UREmut1-3) were synthesized using the commercial AmpliScribe T7-Flash Biotin-RNA Transcription kit (Lucigen, WI, USA) following the manufacturer's instructions. Cytosolic or nuclear lysates were prepared using a manual Dounce homogenizer from 3x10^7 HEK393T cells which were harvested, washed with saline and resuspended in hypotonic buffer (10 mM Tris-HCl pH 7.4, 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, complete

protease inhibitor cocktail (Roche, Rotkreuz, CH) or nuclear isolation buffer (25 mM Tris-HCl pH 7.4, 150 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.5 % NP-40, complete protease inhibitor cocktail), respectively. Three independent pulldowns of proteins binding to the probes was performed as described elsewhere (Bai et al., 2016). Briefly, the RNA biotinylated probes were folded by sequential thermal stress (2 min at 95 °C, 2 min at 4 °C, 30 min at 25 °C) in 2x RNA structure buffer (20 mM Tris-HCl pH 7.4, 0.2 M KCl, 20 mM $MgCl_2$, 2 mM DTT, 0.8 IU of recombinant RNasin ribonuclease inhibitor (Promega, Dübendorf, cH). 10 µg of each probe was incubated with a total of 250 µg of cytosolic and nuclear lysates mix (1:1 w/w) in the presence of RNAse inhibitor during 30 min at room temperature, then supplemented with 2.5 mg/mL of heparin (Sigma-Aldrich) and further incubated for 10 min at room temperature, mixed with 50 µL of 50 % slurry of Streptavidin-Sepharose High Performance beads (GE Healthcare, Glattbrugg, CH) and incubated overnight at 4°C on a rotating wheel. The beads were then washed three times with hypotonic buffer and 10 % of each sample was used to confirm the presence of proteins by SDS-PAGE and silver staining and the rest was air-dried and send for shotgun mass spectrometry analysis.

Mass spectrometry

Proteins bound to the streptavidin beads were directly digested on the beads with 100 ng sequencing grade trypsin (Promega, Dübendorf, CH) for 6 h at 37°C after the following treatment: the dry beads were suspended in 30 µL of 8 M urea in 50 mM Tris-HCl, pH 8.0, followed by reduction of the proteins with 3 µL 0.1 M DTT for 30 min at 37 °C and alkylation by addition of 3 µL 0.5 M iodoacetamide for 30 min at 37 °C in the dark. The urea concentration was then diluted to 2 M by addition of 20 mM Tris-HCl pH 8.0 containing 2 mM $CaCl_2$. Digestion was stopped by adding 1/20 volume of 20 % (v/v) trifluoroacetic acid. An aliquot of 5 µL of each digest was analysed three times by LC-MS/MS on an EASY-nLC1000 chromatograph connected to a QExactive mass spectrometer (Thermo Fisher Scientific, Reinach, CH). Peptides were trapped on a Precolumn (C18 PepMap100, 5 µm, 100 Å, 300 µm × 5 mm, Thermo Fisher Scientific, Reinach, CH) and separated in backflush mode on a C18 column (3 µm, 100 Å, 75µm x 15 cm, Nikkyo Technos, Tokyo, Japan) by applying a 40-minutes gradient of 5-40 % acetonitrile in water, 0.1 % formic acid, at a flow rate of 300 nL/min. The Full Scan method was set to a resolution of 70’000 with an automatic gain control (AGC) target of $1x10^6$ and maximum ion injection time of 50 ms. The data-dependent method for ten precursor ion fragmentations per cycle was applied with the following settings: resolution 17’500, AGC of $1x10^5$, maximum ion time of 110 milliseconds, mass window 2 m/z, collision energy 27, under fill ratio 1 %, charge exclusion of unassigned and 1^+ ions, and peptide match preferred, respectively.
LC-MS/MS data was processed with MaxQuant (version 1.5.4.1) using default settings for peak detection, trypsin cleavage disregarding the proline rule, allowing up to three missed cleavages, variable oxidation on methionine, acetylation of protein N-termini, and strict carbamidomethylation of cysteines, using the SwissProt human protein sequence database released in April 2016, respectively. Match between runs was used within each sample group with a retention time window of 0.7 min. Protein identifications were

accepted only if at least two razor peptides at a 1 % false discovery rate (FDR) cut-off were identified.

Mass spectrometry data processing and visualization

For label-free protein quantification, we relied on the MaxQuant built-in label-free quantification (LFQ) algorithm (Cox et al., 2014) and applied a top3 peptide approach (Braga-Lagache et al., 2016). For the latter, all peptide identifications in the evidence output file from MaxQuant were median normalized before imputation of missing values from the normal distribution of log_2-transformed peptides using a down shift of 1.8 and a width of 0.3 standard deviations (default settings in Perseus software, version 1.5.5.3) (Tyanova et al., 2016). Missing values imputation was carried out, when there was at least one peptide identification in the nine replicates from the same sample set, otherwise the intensity was set to zero according to recommendations by Lazar and colleagues (Lazar et al., 2016). The three most intense peptide intensities were then summed to the protein group intensity. The LFQ values were also log_2-transformed, and missing values imputed as described above if there was at least one LFQ in a sample group, otherwise a zero was set. Fake protein group intensities were imputed (down shift of 2.5 and a width of 0.3 standard deviations) in each sample group where no protein intensity was available before performing Student's two-sample t tests. Statistical significance of differentially pulled down proteins was assigned with a 1 % permutation-based FDR adjustment and if there was at least a two-fold abundance change between sample groups.
The proteins detected on each sample were explored and visualized using a Venn diagram (Bardou et al., 2014) and the package *ggplot2* (Wickham, 2009) within the R environment (R Core Team, 2017).

Functional enrichment analysis

Genes having an expression ratio wild-type-to-UREmut probe greater than 1.5 were used to perform a gene ontology statistical overrepresentation tests using the Panther Tool (http://pantherdb.org/, version 13.1 Released 2018-02-03) (Mi et al., 2017). The results of the analysis were restricted to GO terms contained into the Molecular Function, Biological Process, Cellular Component and Reactome Pathways categories with terms showing significant enrichment scores (Fisher's exact test with false discovery rate corrected for multiple comparison, adjusted p-value ≤ 0.05) using the human genome as background.

RNA interference experiments and TRIM28 overexpression

The selected candidates TIAL1, TRIM28, SAFB2 and LSM5 were validated using *in vitro* transcribed endoribonuclease-prepared short interfering RNA pools, with a luciferase specific control RLUC (MISSION esiRNA, Sigma-Aldrich). $2x10^5$ HEK293T cells stably expressing either human BOK CDS-3'UTR or CDS-3'UTR(URE mut1-3), respectively, were seeded in a 24 well plate, allowed to attach for 7 h and transfected with 0.5 µg of the desired esiRNA using the X-tremeGENE siRNA Transfection Reagent (Roche,

Rotkreuz, cH) following the provider's instructions. Cells were harvested after 72 h and target gene expression was evaluated by western blot.
The pKH3-TRIM28 was a gift from Fanxiu Zhu (Addgene plasmid # 45569) (Liang et al., 2011) and was used for transient expression of a C-terminally HA-tagged TRIM28 in the target cells HCT-116, NCI-H1299, HEK293T and MEF. For stable expression, *TRIM28* CDS was extracted by PCR using the oligonucleotides 5'-GACCGAATTCATGTACC CATACGATGTTC-3' and 5'-GATTGAATTCTCAGGGGCCATCAC-3' and subcloned using *EcoRI* into the lentiviral CAD-G-Whiz vector (Rizzi et al., 2007). The sequence and correct orientation of the expression cassette was confirmed by sequencing. The resulting plasmid was co-transfected with the pMD2GVSV-G envelope and psPAX2 packaging plasmids into HEK293T cells using X-tremeGENE™ HP DNA Transfection Reagent (Roche, Rotkreuz, CH) in order to produce lentiviral particles. Particles were harvested from the supernatant, filtered through a 0.22 µm disc filter (Merck Millipore, Schaffhausen CH), mixed with polybrene at a final concentration of 8 µg/mL and used directly to transduce target cell lines. Transduction efficiency was checked by the presence of the IRES-translated EGFP reporter included in the CAD-G-Whiz plasmid.

Patient datasets

Clinical and gene expression data of 9639 samples from 33 cancer types included in the cohort TCGA-TARGET-GTEx (Vivian et al., 2017) were retrieved through the UCSC Xena browser (http://xena.ucsc.edu). The clinical data matrix contains 41 descriptors (https://xenabrowser.net/datapages/?cohort=TCGA%20TARGET%20GTEx, version 17.10.2017) while the gene expression corresponds to the transformed normalized transcript count (RNAseq, ILLUMINA platform, $\log_2$(norm_count+1), version 12.04.2016). Cox proportional-hazard models were recreated in R software using the package *survival* (Therneau, 2015) by modelling the overall survival as a function of the expression levels of *BOK* and *TRIM28* (used as continuous variables). The patients from the selected cancer types were classified into "high" and "low" strata according to the median expression of *BOK* and *TRIM28* genes. Spearman's correlation between *BOK* and *TRIM28* was calculated on the strata and Kaplan-Meier curves were prepared using the package *survminer* (Kassambara and Kosinski, 2018). Differences in overall survival was determined using a Log-rank test, declaring significant differences for p-values ≤ 0.05.

Statistical analysis

Unless otherwise indicated, the data from at least three independent experiments is presented as mean ± SD using the Prism Software v6 (GraphPad, La Jolla, CA, US). Potential differences between treatments were detected using a one-way ANOVA followed by a Tukey's *post-hoc* test. Significant values were represented according to the following convention: $p \geq 0.05^{ns}$, $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$*** and $p \leq 0.0001$****.

SUPPLEMENTAL TABLES

Table S1 related to Figure 4. Results from shotgun mass spectrometry analysis using mRNA biotinylated probes. Details the identified proteins, peptide numbers, principal component analysis, clustered analysis, t-test and FDR correction for each independent replicate pulled down with the BOK-3'UTR wild type or URE-mutated RNA probes. Available online at the Mendeley Database; http://dx.doi.org/10.17632/9dfn8m6sgd.1#file-5706e58e-b2c5-4eea-925e-09073d7ff8d5.

Table S2 related to Figure 6. Clinical and expression matrix used for Cox regression, survival and correlation analysis. Dataset containing *TRIM28* and *BOK* gene expression matched to clinical parameters in 33 cancer types by patient ID following the TCGA nomenclature. Include detailed description of the original data source as well as the manipulations performed in this work to stratified patients according to the selected gene expression. Available online at the Mendeley Database; http://dx.doi.org/10.17632/9dfn8m6sgd.1#file-08fd9021-e239-4c81-906e-66bf80b92d1d.

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