Supplementary Material and Methods, including Supplementary Figures 1-7

Constructs

For WNT-luciferase reporter assays, the plasmid M50 Super 8x TOPFlash (#12456, AddGene) was used. It contains TCF/LEF sites for beta-catenin-mediated transcriptional activation upstream of a firefly luciferase gene. Co-transfection was performed with pRL-TK (#E2241, Promega), and firefly luciferase luminescence was normalized to renilla luciferase.

For GNB1 3'UTR reporter assays, the plasmid pmirGLO (#E1330) was used. Complete or parts of GNB1 gene 3'UTR were cloned downstream of the Firefly luciferase open reading frame using primers:

FF_short: ATACAAGCTAGCCGCCAGTAGCATGTGGATGC;

Rev_short: GATGGCCTCGAGTCAAGTTTACCTTCTGGTTA;

FF_long: ATACAAGCTAGCGTAAACTTGAGTGTAATTGT;

Rev_long: GATGGCCTCGAGGTCCCTCATGTCAAACTGCT

A set of constructs containing specific shRNA sequences to target human PCF11 mRNA was designed by Sigma-Aldrich based on pLKO-puro-IPTG-3xLacO backbone. Target sequences of PCF11 were: ATCGAAATCGAAATCGAAATC, AGTAGCCTCCCACTGATTAAA, AGATCCTGCTTGGCCTATTAA (was used throughout all functional experiments), CAATCAGACTGGTCCATATAA, TTTGCCATCGGTCTTATC.

For overexpression experiments, the coding sequence of human PCF11 protein was cloned into pCI-neo vector backbone (#E1841, Promega). The cDNA was cloned by fusion of two fragments. Amplicons were synthesized using 2 primer pairs

FF1: AAGCCACCGCTCGAGTCAGAGCAGACGCCGGCC,

Rev1: AAGCCACCGCTCGAGATGTCAGAGCAGACGCCGGCC;

FF2: GTGTGCGAGAAGAGCAGAGA,

Rev2: CGGGTCGACTCTAGATTAAACTGACTCGACTGTGTCAT

for 5’ and 3’ parts respectively. Both parts were inserted into pCI-neo with a Flag peptide-coding sequence in the multiple-cloning site. Ligation was performed using the InFusion cloning kit (Clontech® Laboratories) according to manufacturer’s instructions.

Mammalian cell lines

Neuroblastoma cell lines (CHP-134, LAN-6, SH-SY5Y) were purchased from Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Culture. The BE(2)-C cell line was a generous gift from Prof. Olaf Witt (DKFZ, Heidelberg). All cells were tested negative for mycoplasma, and propagated in monolayer culture in DMEM medium with 10% FBS (10-30 passages). BE(2)-C were differentiated using 5 μM ATRA.

Transfection of mammalian cell culture

Cells were plated (60-70% confluence) 12 hours prior to the transfection procedure. A master mix for transfection included RotiFect RNAi Lipo (Roth, 2 μl per 1 ml of total transfection volume), siRNA (50 nM final) or plasmid (0.5 μg/ml final) mixed in OptiMEM (ThermoFisher Scientific) 20% of final volume according to the manufacturer's instruction. Cells were assayed 48-72 hours after transfection.

Northern blotting

Total RNA was extracted from cells using PeqGold TriFast (VWR). Northern blotting analysis (in an agarose gel system) was performed as described previously[1].

For a higher resolution (in the range of 200-2000 nt) polyacrylamide gel systems were used. Specifically, total RNA was deadenylated in presence of 200 pmol of oligo-$(dT)_{25}$ and 5 units of RNase H. Purified RNA (1-3 µg) was heat denatured in 50% formamide in TAE and separated in a polyacrylamide gel consisting of 3.5% polymerized acrylamide-methylenebisacrylamide mix (37.5:1), 7 M urea. Electrophoresis was performed in TAE buffer for 4 hours at 140 V. RNA was transferred to a nylon membrane (ThermoFisher Scientific) by semi-dry electroblotting. Membranes were UV cross-linked and hybridized with specific probes in analogy to the standard procedure[1].

Non-radioactive DNA probe synthesis and detection

For specific DNA probe synthesis (for Northern blotting), RNA was reverse transcribed. PCR amplicons (of about 450-550 base pairs long) were generated using OneTaq Master Mix (New England BioLabs). Purified PCR products (1-3 ng) were used in the next round of asymmetric PCR (reverse to forward primers ratio 100:1) to generate biotin-labelled probes (50 PCR cycles). Probes were purified by extraction from agarose gels by using NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel). For hybridization, 50-100 ng of probe was used in 5 mL of Church buffer in analogy to procedures described previously[1]. For the detection of biotinylated probes, membranes were washed and blocked for 1 hour in solution containing 1x TBS, 0.5% SDS and 0.1% of Aurora™ Blocking Reagent (#04821548, MP Biomedicals). Streptavidin−Peroxidase Polymer (#S2438, Sigma-Aldrich) was used next (diluted 1/7000) for 1 hour, following 3 cycles of 10 minutes washing (with

1x TBS, 0.5% SDS). Signal detection was carried out by using ECL Select Western Blotting Detection Reagent (GE Healthcare). Blots were scanned using ChemiDoc™ MP System (Bio-Rad) and bands were quantified (Image Lab™ software; Bio-Rad).

Western blotting

Protein lysates were generated as described previously [1] and separated using Criterion™ TGX™ Stain-Free™ gel (Bio-Rad Laboratories) followed by the transfer onto nitrocellulose membrane (GE Healthcare). Equal sample loading was assayed by in-gel fluorescent detection or Ponceau S staining of the membrane. Membranes were blocked by TBS buffer with 5% milk for 1 hour and probed with specific antibodies. All antibodies used were purchased at Bethyl Laboratories with exception for: IGF1R, EIF3AK, pEIF3AK, ATF4, GNB1 and MYCN (Santa Cruz Biotechnology); AKT and pAKT (Cell signalling Technology); pEIF2S1 (Abcam); TUBB3 (BioLegend); AES (Novus Biologicals). Blots were scanned using ChemiDoc™ MP System (Bio-Rad) and bands were quantified Image Lab™ software (Bio-Rad).

Neuroblastoma TREND annotation assembly

3′READS was carried out as previously described [2]. Briefly, total RNA obtained from differentiated and undifferentiated neuroblastoma cell line (BE(2)-C) was subjected to 1 round of poly(A) selection using oligo(dT) beads (NEB), followed by fragmentation on-bead with RNase III (NEB). Poly(A)-containing RNA fragments were isolated using the MyOne streptavidin C1 beads (Invitrogen) coated with a 5' biotinylated chimeric dT_{45} U_5 oligo (Sigma), followed by washing and elution through digestion of the poly(A) tail with RNase H. The part of the poly(A)-tail annealed to the U residues of the oligo was refractory to digestion and was thus used as evidence of the poly(A)

tail. Eluted RNA fragments were purified by phenol-chloroform extraction and ethanol precipitation, followed by sequential ligation to a 5'-adenylated 3'-adapter (5'-rApp/NNNNGATCGTCGGACTGTAGAACTCTGAAC/3ddC) with the truncated T4 RNA ligase II (NEB) and to a 5' adapter (5'-GUUCAGAGUUCUACAGUCCGACGAUC) by T4 RNA ligase I (NEB). The resultant RNA was reverse-transcribed with Superscript III (Invitrogen), followed by 12 cycles of PCR amplification with Phusion high fidelity polymerase (NEB). cDNA libraries were sequenced on an Illumina HiSeq 2500.

Filtered poly(A) site-supporting (PASS) reads were used to construct peaks using the Cufflinks software. Obtained peaks were associated with the UCSC assembly of human genes based on the peaks position using the closest-features command of BEDOPS toolkit. PASS mapped within the 5,000 nucleotide region downstream of the annotated gene were considered as novel 3' UTR isoforms. This annotation was used to exclude TRENDseq reads originating from internal priming on the genome encoded adenosine-rich regions.

Nucleic acid quality assurance

RNA integrity was assayed with Agilent RNA 6000 Nano Kit (Agilent Technologies) according to manufacturer's instructions, and a threshold of minimal RNA integrity number (RIN) of 9.5 was applied for total RNA. Homogeneity and size of DNA libraries for Illumina sequencing were analysed using Agilent High Sensitivity DNA Kit (Agilent Technologies) following the manufacturer's instructions. Qubit® 2.0 Fluorometer in combination with dsDNA HS Assay Kit (ThermoFisher Scientific) was used to assay cDNA library concentration.

TRENDseq. Library preparation and sequencing

Total RNA (100 ng) was reverse transcribed in presence of oligonucleotide (RT) primer containing T7 promoter, Illumina 5' adapter, individual in-lane barcode and an anchored oligo-dT stretch, as described previously[3]. For the cDNA and aRNA synthesis MessageAMp II aRNA Amplification Kit (ThermoFisher Scientific) was used according to the manufacturer's recommendations with modifications. Specifically, for the first and second cDNA strands synthesis for each individual RNA input sample 1/10 of full reaction size was used. Up to 25 samples were pooled after second cDNA strand synthesis reaction. *In vitro* transcription (aRNA synthesis) was performed in 40 µl reaction format according to manufacturer's protocol with 14 hours of incubation at 37° C. Purified aRNA was sheared using Covaris M220 Focused-Ultrasonicator™ (Peak incident power 50 Watt, Duty Factor 20% and 200 Cycles per Burst (cbp) for 420 seconds at 7° C) and size selected on the 6% PAGE in denaturing conditions (7 M urea). The gel region corresponding to 100 nucleotides was excised, and RNA was eluted from gel by 2 minutes incubation in 50-100 µl of buffer containing 100 mM Tris-HCl (pH 8.0), 500 mM NaCl and 1% SDS at room temperature. Size-selected RNA was purified using miRNeasy Kit (Qiagen).

Illumina platform compatible cDNA library was synthesized as described previously [3] with a number of PCR cycles reduced down to 9. Each pooled library (up to 25 samples) was labelled with Illumina indexing barcode and up to 3 libraries were pooled together adding up to 75 samples per sequencing run. The libraries were sequenced on the Illumina HiSeq or NextSeq platform with addition of 30% PhiX Sequencing control (Illumina) in the paired-end setup. Read 1 (9 nt) sequenced individual sample in-lane barcode (introduced in the first reverse transcription-step), Read 2 (50 nt) sequenced the RNA insert to be mapped to the genome (**Fig. 2a**). Illumina indexes were sequenced as a dedicated read.

Generation of stable cell lines

To generate clones with IPTG-inducible expression of specific shRNA against PCF11 or firefly luciferase (as negative control respectively), BE(2)-C and CHP-134 were transfected with pLKO-puro-IPTG-3xLacO constructs (Sigma-Aldrich). Antibiotic selection was performed with 3 µg/ml of puromycin (ThermoFisher Scientific).

Stable cell lines were generated by transfection of the wild type BE(2)-C and CHP-134 neuroblastomas. For stable overexpression a pCI-neo plasmid containing a full length PCF11 coding sequence with N-terminal Flag peptide was transfected. An empty pCI-neo vector was used to generate a control cell line. 1 mg/ml of Geneticin® G-418 (ThermoFisher Scientific) was used for selection of clones with the stable genome integration of transfected constructs.

Minimal concentrations of selection antibiotics with 100% effect on cell death were determined during 10 days treatment of wild-type neuroblastoma cells. For both types of constructs G-418-selection was initiated 48 hours after transfection and carried out for the following next 10 days. Individual clones were then propagated and tested for target protein overexpression or depletion using western blotting.

Immunofluorescent micrographs

Cells were plated onto sterile microscopy cover slips and propagated under the experimental conditions (detailed above). Fixation was performed for 10 minutes in PBS solution containing 4% paraformaldehyde and 10 mM NH4Cl. Cells were permeabilised with 0.2% Triton X-100 in PBS at room temperature for 10 minutes. Cells were blocked with 2.5% Normal Horse Serum Blocking Solution (Vector Laboratories) for 1 hour and stained with a primary antibody directed against TUBB3 in PBS solution containing 1% BSA and 0.05% Triton X-100. After staining with Cy3-labeled secondary antibody (anti-rabbit) samples were mount onto microscope slides

with Vectashield Antifade Mounting Medium containing the DAPI stain (Vector Laboratories).

Apoptosis assay

Apoptosis measurements were performed using the Cellular DNA Fragmentation ELISA kit (Sigma-Aldrich). BE(2)-C cells were plated in black 96-well plate with transparent bottom in duplicates (5.000 cells per well). 1 mM IPTG was added to the cells to induce PCF11-depletion (see above). 72 hours after plating, cells were labeled with 10 μM bromodeoxyuridine (BrdU) for 24 hours. After BrdU withdrawal, cells were kept in culture for another 48 hours. For cell lines with stable PCF11 overexpression, 3 μg/ml of puromycin or 1 μM lometrexol (Sigma-Aldrich) were applied to trigger apoptotic response for the same period after BrdU withdrawal. After 48 hours and prior to the harvesting procedure, cells were labelled with NucBlue® Live ReadyProbes® Reagent (ThermoFisher Scientific) for 30 minutes (2 droplets of reagent per 1 ml of media). After washing with PBS, cells were imaged with fluorescence microplate reader (Fluoroskan Ascent FL, ThermoFisher Scientific), and the signal at the wavelength ~ 461 nm was used as a measure of cell number in the well (as normalization control). Thereafter the ELISA procedure was performed according to the manufacturer's instructions (Cellular DNA Fragmentation ELISA kit protocol). The colorimetric signal was normalized to the cell number in each well.

Cell cycle analysis

PCF11 knockdown in BE(2)-C cells was performed by siRNA transfection as described above. 48 hours after transfection, cells were synchronized with 2 mM hydroxyurea (HU) for 16 hours. Cells were harvested at 0, 3, 6, 9 and 12 hours after HU-withdrawal. Cells were treated with 30-50 μg/ml of propidium Iodide

(ThermoFisher Scientific) solution in 0.1 % Triton-X100 in PBS in presence of 2 mg of DNase-free RNase A (ThermoFisher Scientific). After staining for 15 minutes at 37°C, the fluorescent signal was measured with a LSR II Flow Cytometer (BD Biosciences) using a 670 nm long pass filter. Cell doublet discrimination was performed using FSC-H/FSC-A, SSC-H/SSC-A, and PI-H/PI-A gates.

Proliferation assay

Cells were plated in black 96-well plates with clear bottom in 10 replicates (3,000 cells per well). PCF11-knockdown was performed by addition of 1 mM IPTG to the culture medium (see above). After 48 hours of incubation, 2 replicates were stained for 30 minutes with NucBlue® Live ReadyProbes® Reagent (ThermoFisher Scientific) according to manufacturer's recommendations. Cells were washed in PBS, and the signal was measured with a fluorescence microplate reader (Fluoroskan Ascent FL, ThermoFisher Scientific, ~ 460 nm wavelength). The procedure was repeated over 5 days with 2 fresh replicates to assay proliferation kinetics.

Colony formation assay

For colony formation assays, 200 BE(2)-C cells (see above) were plated into each well of a 6-well plate for 10 days without and with addition of IPTG. Thereafter the cells were washed and fixed, and colonies were stained with 0.5% crystal violet for 10 min at room temperature.

Matrigel invasion assay

To assess the invasive properties upon PCF11-depletion, 80.000 BE(2)-C cells (see above) were seeded into the insert of a growth factor reduced 24-well Matrigel™ invasion chamber assay plate (BD BioCoat™) without and with addition of IPTG (in

0.5 ml serum free media); the inserts were transferred into wells containing 0.75 ml culture medium with 10% FCS with and without IPTG. After 72h, non-invading cells were removed from the upper surface of the membrane with a cotton swab, and invading cells were fixed and stained with Diff-Quick® (Medion Diagnostics) and quantified by counting invaded cells in four independent areas in pentaplicates. All experiments were performed in accordance with the manufacturer's protocols.

Mouse tumour xenotransplantation

Female athymic nude mice (Crl:NU(NCr)-Foxn1^{nu}, Charles River) were used to assess tumour progression of BE(2)-C cells in response to the PCF11 expression status. To that end, 12,5x10^{6} cells each in 0.2 ml PBS were subcutaneously injected into the right/left flank of 30 nude mice (aged 6 weeks, weighting ~20-25 grams). For PCF11-depletion, half of the animals were randomly assigned to a cohort (on day 3 after tumour transplantation), which received IPTG-injections (1.95 M, Roth, Germany) every second or third day (injection of PBS served as negative control), and tumour dimensions were measured with callipers every 2 or 3 days. Tumour volume was calculated by modified ellipsoid formula ($\frac{1}{2} \times (\text{Length} \times \text{Width}^2)$); mice were sacrificed after a follow-up of ~20 days after tumour cell injection and tumours were removed and weighted.

Luciferase reporter assay

For the WNT reporter assays wild type BE(2)-C or clones with inducible shRNAs directed against PCF11 (see above) were plated 12 hours prior to the procedure in 24-well plates with or without 1mM IPTG. Transfection with 1.6 μg of TOPFlash plasmid and 0.4 μg of pRL-TK (control) was performed in antibiotic-free medium. Modulators of WNT and IGF1R pathways were added in serum-free OptiMEM

medium 48 hours after reporter transfection. WNT pathway activation was induced by the recombinant WNT-3a (canonical) or WNT-5a (non-canonical WNT ligand; each 250 ng/ml, R&D Systems®, diluted in 0.1% BSA-PBS). WNT pathway inhibition was carried out by addition of 0.2 mM BML-286 (Enzo Life Sciences) or 1 mM NSC668036 (Sigma-Aldrich). IGF1R was inhibited by adding 25 - 50 µM Tyrphostin AG1024 (Enzo Life Sciences). DMSO was used as a solvent control for inhibitors of the WNT and IGF1R pathways.

For monitoring WNT activity, luciferase assays were carried out 24 hours after compound addition (see above). Cells were lysed in 12-well plates with Passive Lysis buffer (Promega) for 15 minutes at room temperature. Firefly and Renilla luciferase luminescence was assayed in reactions with Bright-Glo™ reagent (Promega) and coelenterazine (Promega), respectively. Firefly luciferase was used as a readout for WNT-signalling pathway activation and normalized to the luminescence of Renilla luciferase (vector delivery control).

For analysis of the effect of GNB1 3'UTR isoforms on luciferase expression, pmir GLO-constructs were transfected into wild type BE(2)-C in 12-well plates. Luciferase activity was assayed 24 hours later as described above.

Inducible PCF11-RNAi mouse model

For reversible depletion of PCF11, an inducible knock-down allele of the PCF11 gene was generated via targeted transgenesis of a doxycycline-inducible shRNA cassette into the ROSA26 locus (Gt(ROSA)26Sor) as described earlier[4].

In vitro differentiation of murine neurons

To assay the effect of PCF11 on neuronal differentiation, primary murine neurons were harvested from PCF11 KD (animals) and litter control embryos (E18) by neuronal tissue dissociation kit (Miltenyi Biotec). Thereafter 5,000 cells were seeded onto coverslips with primary murine astrocytes as feeder cell (plated 24h before) in 24-well plates in duplicates. The effect of PCF11-depletion on neurodifferentiation was assessed 4 days after addition of doxycycline (1 µM) to neuronal precursors obtained from PCF11 KD and litter control embryos. To that end, cells were fixed, permeabilised and stained with a primary- (TUBB3) and a Cy3-labeled secondary antibody (as above). Finally the samples were mount onto microscope slides with Vectashield Antifade Mounting Medium (Vectashield) and the extent of neuronal differentiation was calculated by applying the Neurite Tracer plugin of ImageJ.

Extraction of TREND-signatures from microarray data

In order to assay the relative proportion of transcript isoforms with shortened or elongated 3' ends in the GEO GSE49710 dataset[5] we selected probes of the relevant microarray platform that could distinguish different TREND isoforms of the same gene. To do so sequences of all probes of Agilent-020382 Human Custom Microarray 44k were mapped to the human genome (version GRCh38/hg38) using STAR[6]. Resulting mappings were overlapped exclusively with tandem sites obtained from the TREND annotation (see Materials and Methods) to associate probes with TREND-isoforms detected in our experimental setup. Raw microarray data was downloaded from GEO (GSE49710[5]), background-corrected and quantile-normalized using bioconductor limma package[7]. Only genes with probes that could detect at least two different TREND-isoforms were used for further analysis. Lengthening index was calculated by dividing expression levels of any detectable long isoform by

the levels of the shortest isoform. Lengthening index was used for Student’s *t*-test (**Fig. 6b,c**), ROC curves and AUC calculations (**Fig. 7a,b**). AUCs (Area Under Curve) were compared using statistical tests based on bootstrapping and DeLong's method[8].

Programming and packages

TRENDseq data analysis and visualization was performed in the R environment (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). Packages used: gplots, igraph, ggplot2, pROC, limma.

Methods Proteomics

SDS-PAGE and protein digestion

40 µg of protein was mixed with NuPAGE LDS buffer (Novex) and loaded onto a 4–12% NuPage gel (Invitrogen). Gels were run at 180V and stained with Instant Blue Coomassie (expedeon). Each lane was cut into 10 slices per lane, which were de-stained, alkylated with 2-iodoacetamide and digested with trypsin, as previously described[9]. Peptides were extracted from the gel pieces with acetonitrile, loaded onto STAGE tips for storage, and eluted from the tips shortly before MS analysis[9].

Mass Spectrometry

By using an EASY- nLC 1000 (Thermo Scientific) LC system, peptides were separated at a flow rate of 400 nL/min on a self-packed column (75 µm ID, 1.9 µm Reprosil-Pur 120 C-18AQ beads, Dr Maisch Germany) housed in a custom-built column oven at 45°C. Peptides were separated using gradient of buffers A (0.1% formic acid) and B (80% acetonitrile, 0.1% formic acid): 0-10 min 10% B, 10-55 min 10% to 38% B, 55-60 min 38% to 60% B, 60-65 min 60% to 95% B, 65-70 min 95%

B, 70-73 min 95% to 3% B, 73-75 min 3% B. The column was interfaced with a Nanospray Flex Ion Source (Thermo Scientific) to a Q-Exactive HF mass spectrometer (Thermo Scientific). MS instrument settings were: 1.5 kV spray voltage, Full MS at 60K resolution, AGC target 3e6, range of 300-1750 m/z, max injection time 20 ms; Top 15 MS/MS at 15K resolution, AGC target 1e5, max injection time 25 ms, isolation width 2.2 m/z, charge exclusion +1 and unassigned, peptide match preferred, exclude isotope on, dynamic exclusion for 20s.

Protein identification and analysis

Mass spectra were recorded with Xcalibur software 3.1.66.10 (Thermo Scientific). Proteins were identified with Andromeda by searching against human proteome database (71985 proteins including isoforms) downloaded from UniProt and were quantified with the LFQ algorithm embedded in MaxQuant version 1.5.3.17[62]. The following parameters were used: main search max. peptide mass error of 4.5 ppm, tryptic peptides of min. 6 amino acid length with max. two missed cleavages, variable oxidation of methionine, protein N-terminal acetylation, fixed cysteine carbamidomethylation, LFQ min. ratio count of 2, matching between runs enabled, PSM and (Razor) protein FDR of 0.01, advanced ratio estimation and second peptides enabled. Protein-protein interaction network analysis of validated TREND-affected candidates was carried out with String-DB (http://string-db.org/).

Supplementary References

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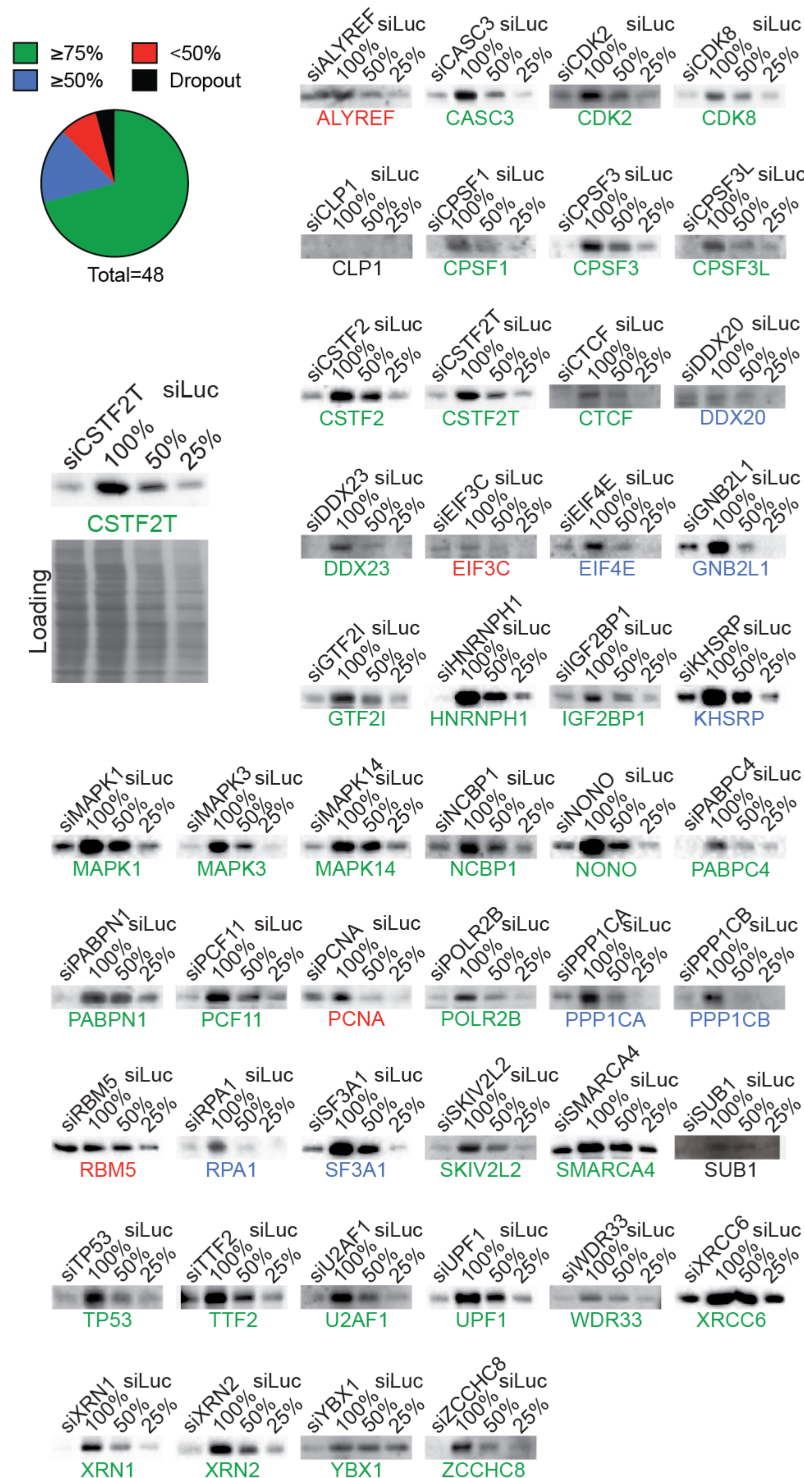
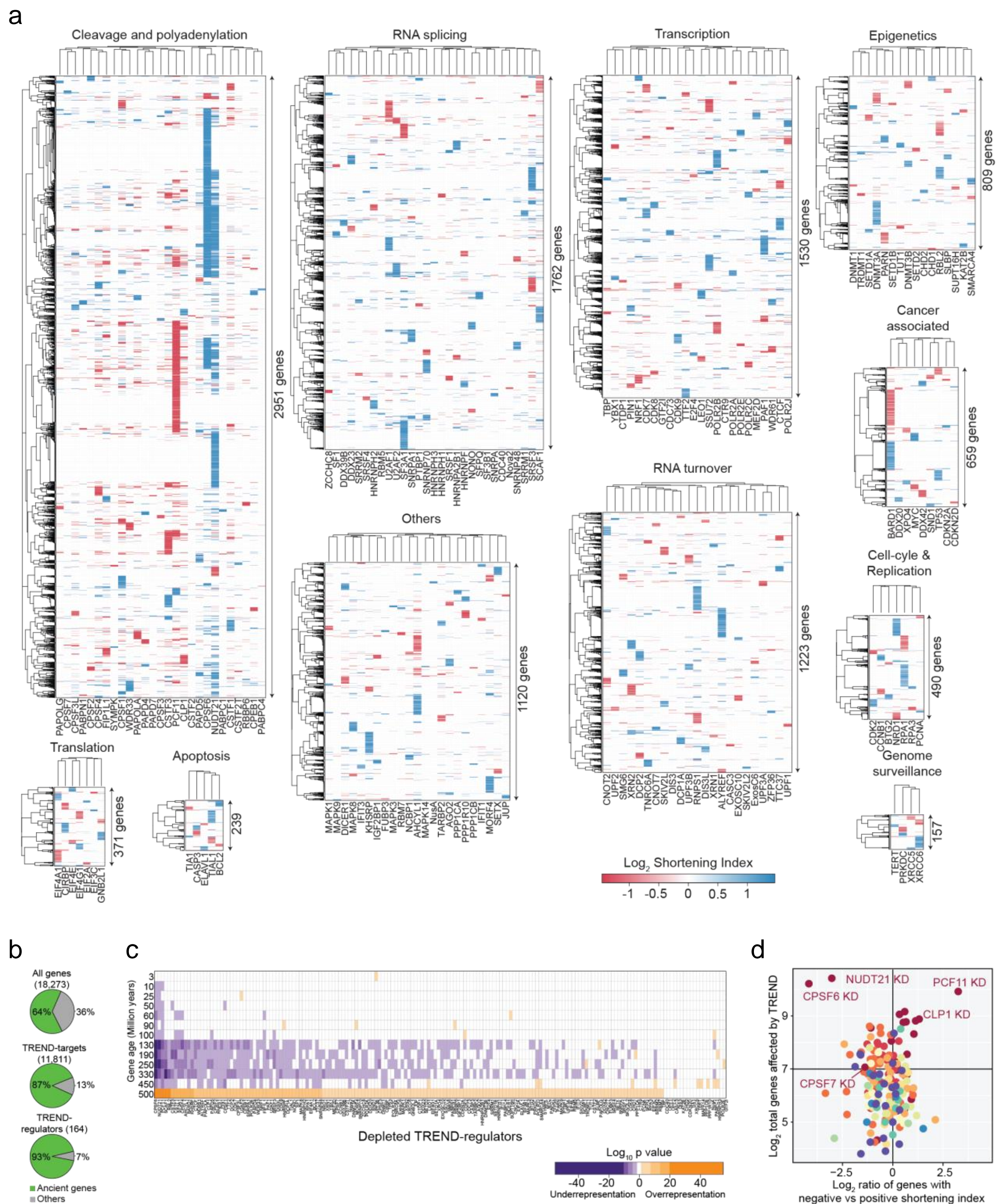
Supplementary Figures 1-7:

Supplementary Figure 1



Supplementary Figure 1. Targeting drivers of transcriptome 3'end diversity (TREND) in a model of neuroblastoma.
Depletion of 174 putative TREND-regulators with a custom siRNA library in BE(2)-C cells. Knockdown efficiency for 48 randomly selected candidates (western blotting, right panels) confirming a successful depletion (down to at least 25%) for more than 70% of the putative TREND-regulators (shown loading control applies to all knockdowns, whole-protein stain).

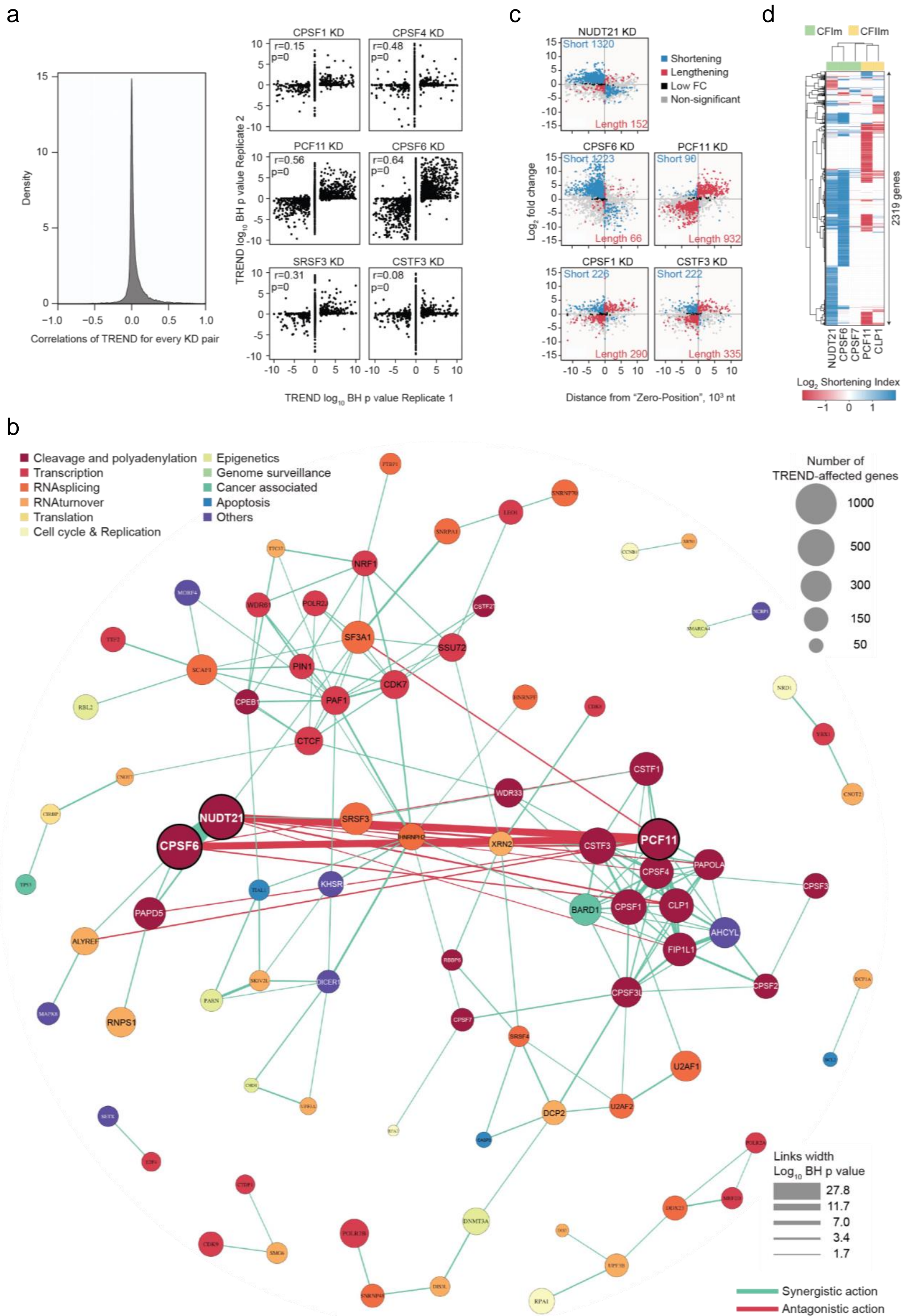
Supplementary Figure 2



Supplementary Figure 2. TREND is phylogenetically conserved and controlled by various mechanisms affecting RNA life.
a Heat maps of clustered TREND-affected genes (number on the right, y-axis, scaling corresponds to number of TREND-affected genes) grouped per functional category of depleted TREND-regulators (x-axes; hierarchical clustering according to shortening index is based on Pearson's correlation coefficient and complete linkage method). Identity of genes and TREND-signatures are displayed in detail in the TREND-DB web explorer (http://shiny.imbei.uni-mainz.de:3838/trend-db, see also **Supplementary Table 2**). **b** Age indexing of genes with discovered TREND-isoforms (middle panel) and screened TREND-

regulators (lower panel) implying a high conservation of TREND (human gene age assignments obtained from[10]; ‘ancient genes’ are genes with gene age >450 million years). **c** Enrichment analysis of age index of TREND-affected genes upon siRNA-depletion of 174 TREND-regulators revealing that dynamic changes at the RNA 3’end are mostly found among ancient genes (orange and purple depict significant over- and underrepresentation, respectively, of genes of a particular age group (y-axis); depleted TREND-regulators are shown on the x-axis; hyper-geometric test enrichment p-values; only p-values below 0.05 are coloured). **d** Components of the CFIm (NUDT21 and CPSF6) and CFIIm complexes (PCF11) pervasively regulate TREND in neuroblastoma in a unidirectional manner (total number of genes affected by TREND (y-axis), **Supplementary Table 2**).

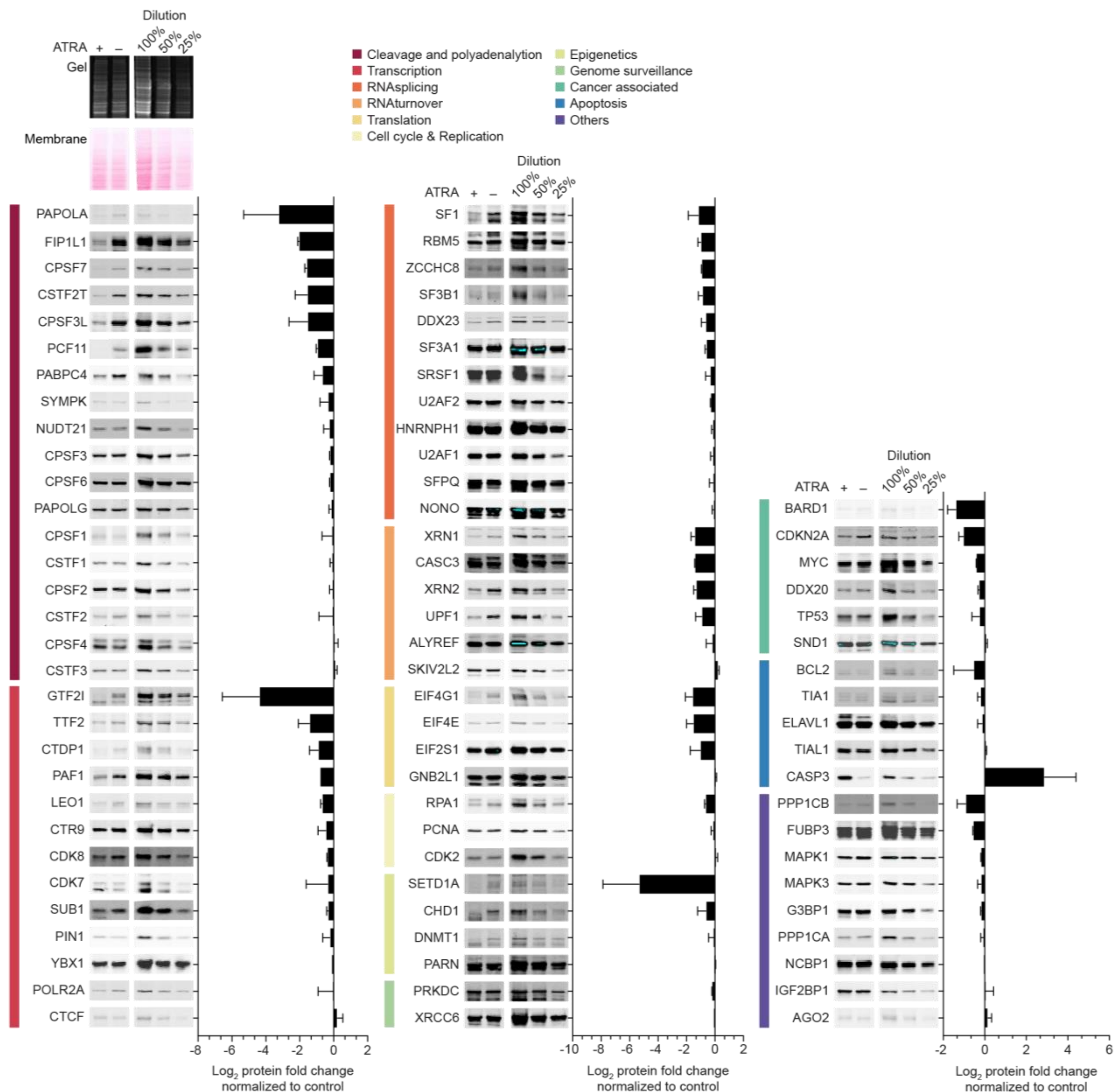
Supplementary Figure 3



Supplementary Figure 3. Global TREND-patterns uncover central regulatory hubs and reveal synergistic and antagonistic TREND-regulation.

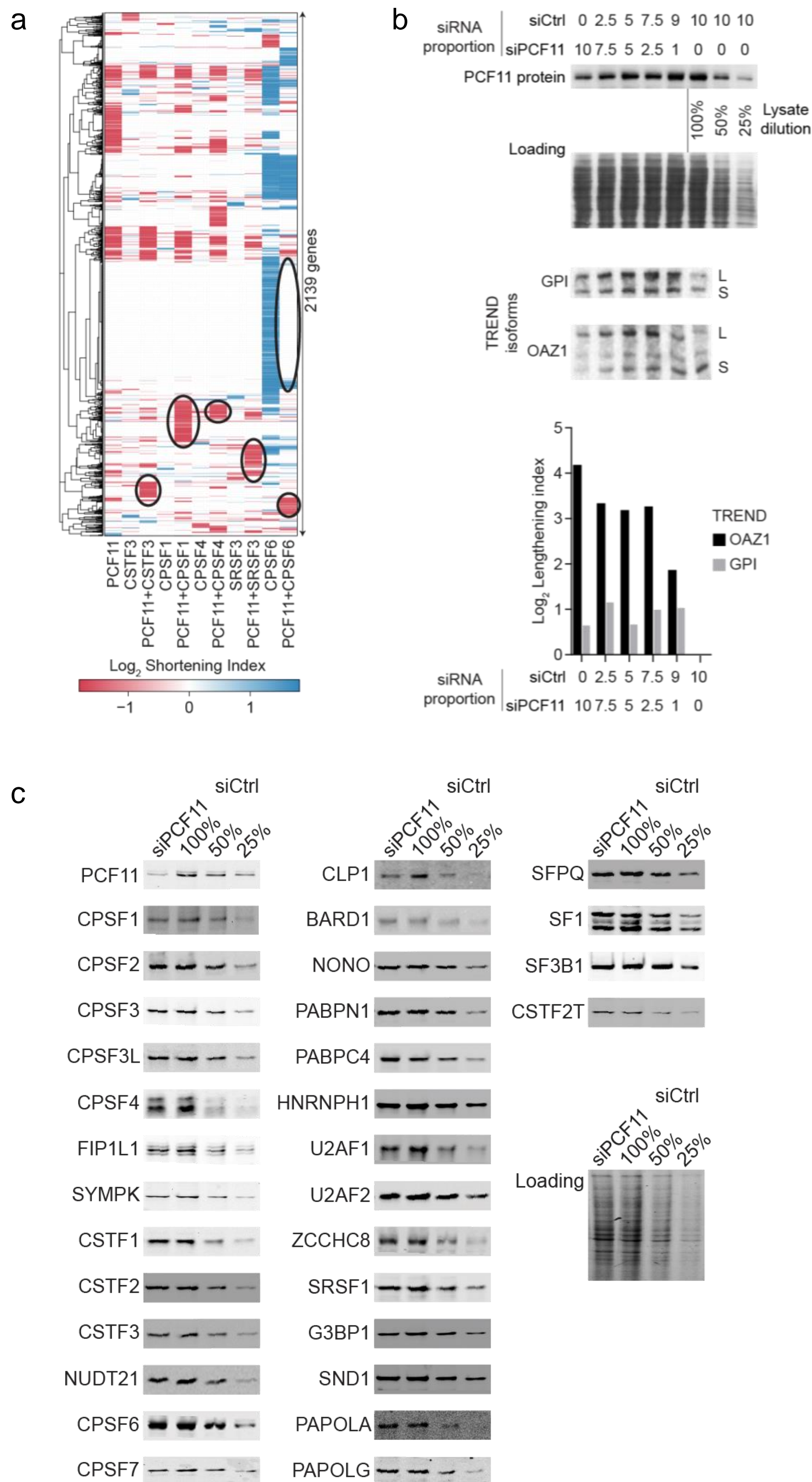
a High technical and biological reproducibility of TRENDseq replicates. Pearson's correlation (*r* coefficient and *t*-test p-value) of TREND-profiles for all pairwise permutations between 174 individual depletions showing an overall poor correlation for most of the random pairs (density plot, left panel). Correlation for two independent depletion- and sequencing replicates of TREND-regulators indicated (scatter plots on the right; Pearson's correlation for most replicates is positive and exceeds more than two standard deviations from the mean, compare to density plot). **b** TREND-network analysis ('APA-network map') illustrating cooperative (and antagonistic) interactions between TREND-regulators (affecting TREND of identical genes). The diameter of the nodes reflects the number of TREND-affected genes. The links between a pair of nodes depict synergism (green, i.e. unidirectional lengthening or shortening) or antagonism (red, i.e. reciprocal lengthening or shortening) of regulation of the common pool of genes (being affected and shared upon depletion of the respective TREND-regulators). The width indicates the BH-adjusted p-value (Fisher's exact test) reflecting overrepresentation of genes being synergistically or antagonistically regulated (further details see Materials and Methods). **c** Effects of TREND on individual transcript isoforms for top 5 TREND-regulators among 174 depletions (KD=knockdown). Each dot represents a transcript isoform and the corresponding fold change (y-axis) relative to the position of the longest annotated (and significantly TREND-regulated) transcript isoform per gene ("zero-position", x-axis, for further definition see Materials and Methods). For example, PCF11-depletion up-modulates a significant proportion of transcript isoforms with 3'ends exceeding the annotated gene length (red dots in the upper right quadrant). **d** Heat map of TREND-regulation for CFIm and CFIIm complexes illustrating an overall reciprocal regulation (i.e. lengthening (red) versus shortening (blue) phenotype) with partially overlapping clusters reflecting antagonistic effects on TREND for a substantial number of identical target genes.

Supplementary Figure 4



Supplementary Figure 4. Protein profiling revealing protein abundance changes of TREND regulators during neuronal differentiation.
Total BE(2)-C protein lysate after 7 days of ATRA differentiation analysed by western blotting (equal loading is represented by in-gel and Ponceau S staining on the membrane, top panel on the left). A merge integrating the fold-regulation of protein abundance and global effect on TREND is depicted in **Fig. 3a** (error bars show SEM for 2 independent replicates).

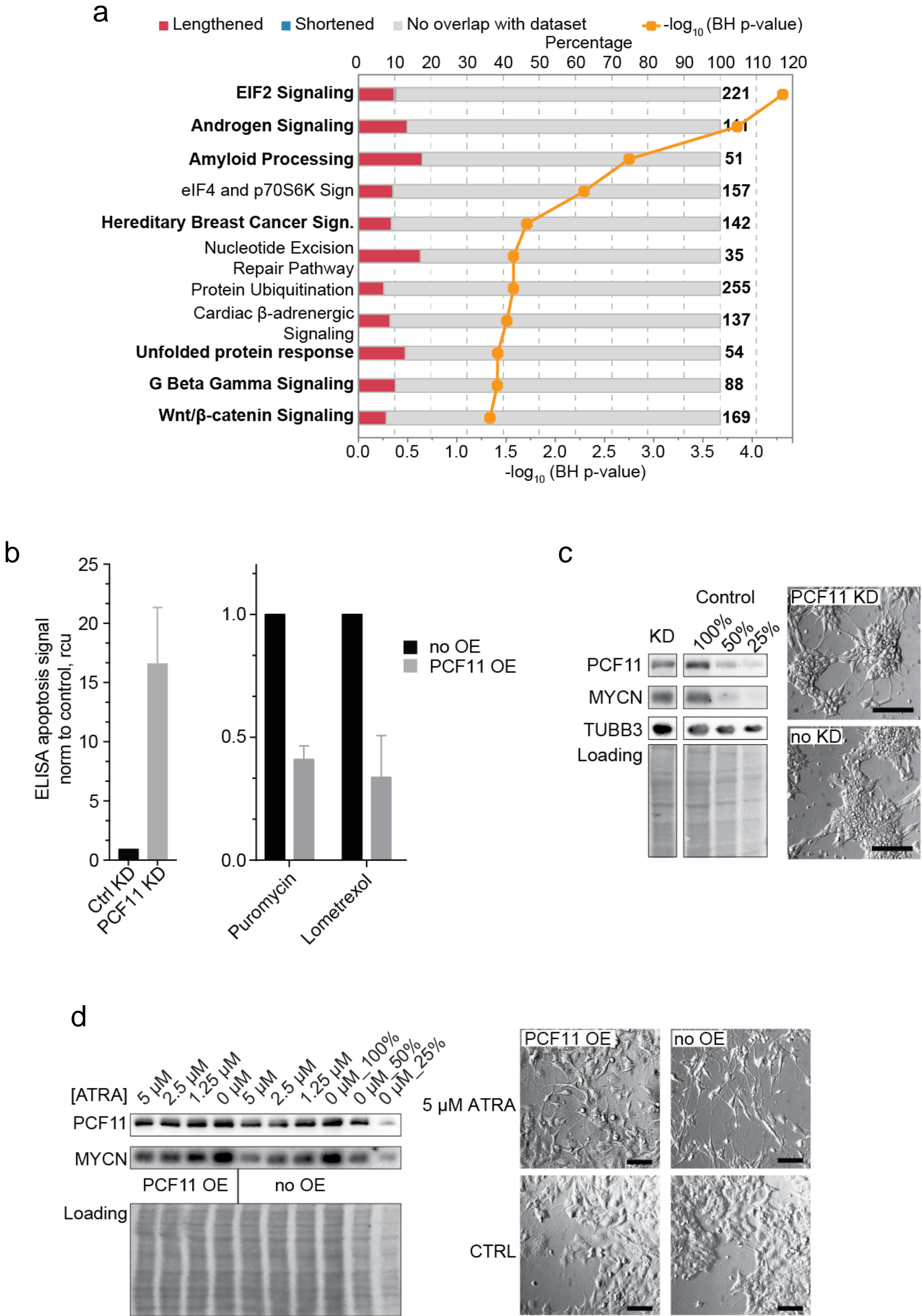
Supplementary Figure 5



Supplementary Figure 5. PCF11 is a key driver of TREND in neuroblastoma.
a Depletion of top TREND-regulators alone and in combination with PCF11 reveals a key role for PCF11 in the hierarchy regulating TREND. The heat map reflecting TREND-changes per gene shows substantial TREND-lengthening (red) and/or abrogation of TREND-shortening (blue; 'native' CPSF6-phenotype) upon co-depletion of PCF11. Of note, big clusters of genes showing TREND-lengthening are unique to dual depletions (highlighted)

indicating that 3'elongated transcript isoforms (upon PCF11-depletion) harbour cis-elements responsive to the depletion of the co-depleted processing factor. This 'TREND-facilitating' role of PCF11 may reflect its known function in RNA Pol II pausing and/or termination control[11-13] thereby regulating the exposition of weak or strong polyadenylation signals to the trans-acting 3'end processing machinery (see discussion). **b** Minimal PCF11-alterations affect TREND most significantly (reflected by substantial lengthening of representative indicator transcripts OAZ1 and GPI, compare quantity of long (L) and short (S) transcript isoforms in northern blotting and lengthening index depicted in the lower panel; reduction of the specific PCF11 siRNA concentration by dilution down to 1:9, specific versus unspecific control siRNA, respectively, is shown in the lanes 1-5). **c** Lack of protein abundance changes of other core 3'end processing components upon PCF11-depletion suggest a direct TREND-regulation in neuroblastoma via PCF11 (representative loading control is shown in the lower right panel).

Supplementary Figure 6

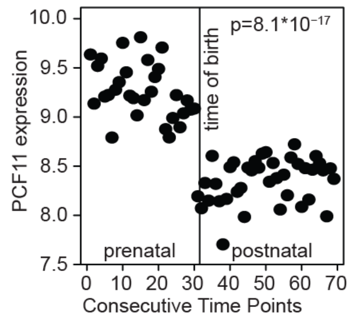


Supplementary Figure 6. PCF11 regulates critical programs impinging on WNT linking TREND to tumorigenesis and neurodifferentiation.
a PCF11-mediated TREND-regulation significantly affects various signalling pathways including WNT (Ingenuity Pathway Analysis, BH-adjusted Fisher's Exact test p-value<0.05). Highlighted are pathways involved in tumorigenesis and neurodifferentiation (647 TREND-affected genes in at least 3 out of 5 independent PCF11-depletion replicates, **Supplementary Table 4**). **b** ELISA-DNA fragmentation assay showing increased apoptosis in cells upon PCF11-depletion (left panel, n = 3). In contrast, constitutive PCF11-overexpression (OE) increases resistance towards pro-apoptotic drugs such as puromycin (n

= 3) and lometrexol (n = 2) (right panel, error bars show SEM for replicates). **c** PCF11-depletion in a complementary CHP-134 neuroblastoma model leads to down-regulation of MYCN and up-regulation of TUBB3 (left) and results in neurodifferentiation (micrographs on the right, scale bar 100 µm; see also main Figures e.g. Fig. 4). **d** PCF11 overexpression (OE, stably expressing cell line) antagonizes ATRA induced neurodifferentiation (i.e. MYCN down-regulation on the left and inhibited morphological changes on the right; micrographs scale bar 100 µm).

Supplementary Figure 7

a



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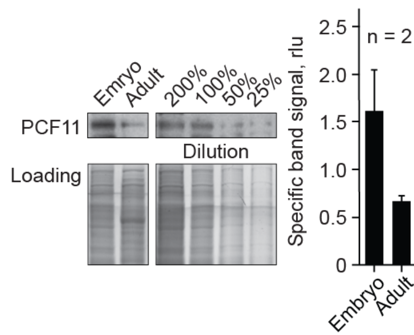
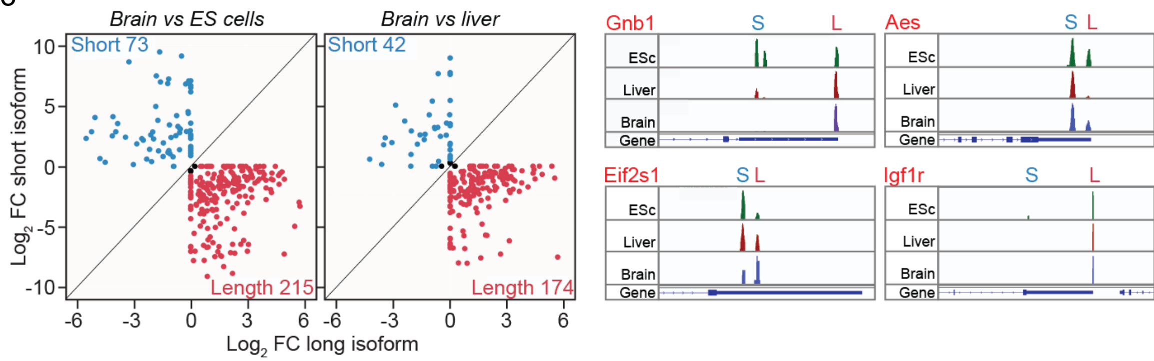
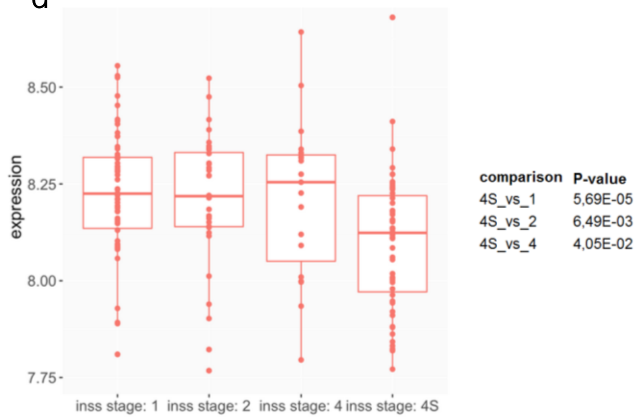


c

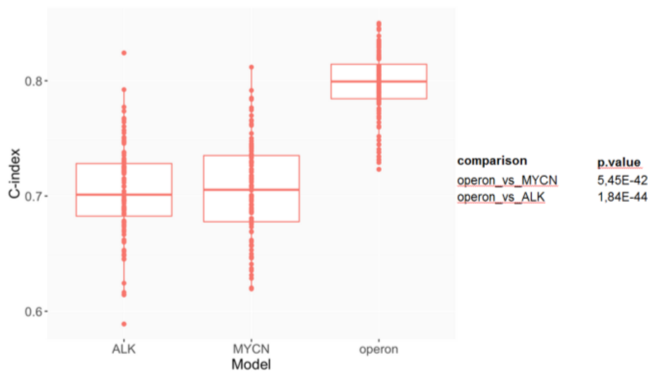


d



comparison	P-value
4S_vs_1	5,69E-05
4S_vs_2	6,49E-03
4S_vs_4	4,05E-02

e



comparison	p.value
operon_vs_MYCN	5,45E-42
operon_vs_ALK	1,84E-44

Supplementary Figure 7. Down-regulation of PCF11 during brain development is associated with a TREND-lengthening phenotype *in vivo* and spontaneous neuroblastoma regression in patients, with PCF11-dependent TREND alterations having superior prognostic value compared to established stratification markers.

a PCF11 mRNA expression in prenatal human brain samples (n=31) is significantly higher than in postnatal samples (n=38; two-sided *t*-test). **b** PCF11 protein abundance in murine embryos and adult mice. **c** Global (unidirectional) TREND-lengthening phenotype in murine brain compared to murine embryonic stem cells or liver (scatter plots on the left). Shortening and lengthening of representative PCF11-derived TREND-operon transcripts in murine ES cells, liver and brain (right panels; Igf1r, Aes, Eif2s1 and Gnb1, for further details see **Fig. 4a,b**). **d** PCF11 expression in tumor samples from age-matched patients at different neuroblastoma stages, indicating that low PCF11-expression is specific for spontaneously regressing neuroblastoma (stage 4S) as compared to metastatic (stage 4) or localized low risk (stage 1 or 2) tumors (gene expression obtained from GEO GSE49711; pairwise comparisons between stages were tested by t-test with pooled standard deviations). **e** Cox modeling showing a superior prediction of the PCF11-dependent neurodifferentiation operon ('operon') for survival compared to established risk markers, MYCN and ALK, respectively (cox proportion hazard model was built with 0.7 of training data, using MYCN or ALK expression as independent variables. Alternatively, ratios of proximal-to-distal (APA) isoforms of PCF11-dependent neurodifferentiation operon" were used in multivariate Cox model. Modeling and validation was bootstrapped 100 times, C-index (concordance) for model validations were plotted. To assay statistical difference between models, two-sided t-test was used).