Supplementary Information for

Genome-wide screens identify specific drivers of mutant hTERT promoters
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Supplementary Methods

Generation of firefly luciferase reporter cell lines
pLenti CMV Puro LUC (w168-1) was a gift from Eric Campeau & Paul Kaufman (Addgene plasmid # 17477, RRID: Addgene_17477). The firefly luciferase expression constructs and ViraSafe™ lentiviral packaging system (Cell Biolabs) were co-transfected into HEK293T cells using Lipofectamine 3000. After 24 hrs, the medium was replenished with the full growth medium and incubated for another 24 hrs to harvest the viral particles. The viral particles were collected and target cells were infected in the presence of 8 µg/mL of polybrene (Santa Cruz Biotechnology). Firefly luciferase expressing nano-luciferase (NLuc) reporter cells were selected with Puromycin (Gibco).

Whole genome siRNA screening
Silencer™ Select Human whole-genome siRNA library (Invitrogen) was used for whole-genome siRNA screening against the reporter cell lines. This library targets >21,000 human genes arrayed in microplates with each well containing a pool of 3 siRNAs targeting a particular gene. The siRNAs were first dispensed into white, 384-well plates (Corning) at a concentration of 200 nM and 5 µL per well. Silencer™ Select Negative Control No. 1 and No. 2 siRNAs (Invitrogen) were included as negative controls. Silencer™ KIF11 siRNA (Invitrogen), Silencer™ Firefly Luciferase siRNA (Invitrogen) and a customized Silencer™ Select nano-
luciferase siRNA (Invitrogen) were also included as positive controls. Each gene was tested in triplicate.

Reverse transfection was carried out by first dispensing 5 µL of Opti-MEM™ Reduced Serum Medium (Gibco) containing 0.1 µL of Lipofectamine™ RNAiMAX Transfection Reagent (Invitrogen) into each well containing siRNA. The transfection reagent and siRNAs were allowed to complex for 20 min at room temperature before addition of 50 µL per well of cells in DMEM (Gibco) containing 12% FBS (Gibco) to yield final transfection mixtures containing about 17 nM siRNA in DMEM and 10% FBS. The cell numbers used for each cell line were 2,000 cells per well for DLD-1-Mut-NLuc and HCT116-Mut-NLuc, and 4,000 cells per well for LN382-Mut-NLuc. Cell viability and nano-luciferase reporter activity were assayed 72 hr after reverse transfection. For DLD-1-Mut-NLuc cells, cell viability was measured with PrestoBlue cell viability reagent (Invitrogen) and nano-luciferase reporter activity was measured with Nano-Glo Luciferase assay system (Promega) and following the manufacturer’s protocols. For HCT116-Mut-NLuc and LN382-Mut-NLuc cells, cell viability and nano-luciferase reporter activity were measured with Nano-Glo Dual-Luciferase assay system (Promega), and following the manufacturer’s protocols. Signals were detected using Tecan Infinite M1000 or Perkin Elmer EnSpire multimode plate readers.

**siRNA screening data analysis**

Readouts from the whole genome siRNA screens were uploaded onto the CHiP-GIS database portal (https://www.chip-phenomics.org/portal.pl). Percentage toxicity and percentage inhibition was calculated using the following formula:
%Toxicity or %Inhibition = \left(1 - \frac{(RFU or RLU)x}{(RFU or RLU)y}\right) \times 100\%

Where x represents the signal from each well containing siRNA and y represents the median signal of the negative control siRNAs in the same plate. A cumulative plot of all the %Inhibition scores for all the siRNAs investigated was then obtained for each cell line and a %Inhibition cutoff was determined by identifying the inflection point. Genes for which the siRNAs resulted in %Inhibition score greater than or equal to the cutoff were plotted in a second cumulative plot based on their %Toxicity scores and a cutoff for %Toxicity score was determined similarly as for the %Inhibition cutoff.

Simultaneously, the readouts from the whole genome siRNA screens were analysed using GUItars, a siRNA screen analysis tool based on the SSMD method. Wells containing negative control siRNAs were selected as negative controls while wells containing KIF11 siRNAs were selected as positive controls for both cell viability and reporter inhibition. For HCT116-Mut-NLuc and LN382-Mut-NLuc cells, wells containing firefly luciferase siRNAs were also selected as a second positive control. Hits were identified based on a Toxicity cutoff of SSMD ≥ -3 and an Inhibition cutoff of SSMD < -3. Overlapping hits identified from both the analysis methods were then selected as the final hits for each cell line.

**siRNA secondary screening**

The siRNA secondary screening was carried out in a similar fashion to the primary whole-genome screen. The difference between the secondary screen and the
primary screen was that in the secondary screen, siRNAs against hit genes identified from the primary screen were cherrypicked and arrayed in white, 384-well plates. Cell lines used for the secondary screen include DLD-1 WT-NLuc (2,000 cells per well), and DLD-1 Mut-NLuc (2,000 cells per well). Data analysis for the secondary screen was carried out by calculating the percentage toxicity and percentage inhibition scores as per the primary screen.

**Lentivirus production of sgRNA library**

The Toronto KnockOut Library v3 (TKOv3) (Addgene plasmid #90294, a gift from Jason Moffat) was purchased. The library of sgRNAs were amplified followed by lentiviral preparation of the sgRNA library in 293FT cells as described (1). Briefly, 293FT cells were seeded at a density of $3 \times 10^6$ cells/10cm$^2$ plate. The next day, cells were transfected with a mixture of pRSV-Rev (2.5 µg), pMDG.2 (2.5 µg), pMDLg/pRRE (7.5 µg), TKOv3 plasmid library (10 µg), and Lipofectamine LTX (42 µl), following the manufacturer’s protocol. At 8 hr after transfection, the medium was changed to the growth medium (DMEM, 10% FBS, 1% penicillin/streptomycin). Virus-containing medium was harvested 24 hr and 48 hr after transfection and centrifuged at 1200 rpm for 5 min. Viral titters in HCT116 cells were determined by infecting cells with a titration of TKOv3 lentiviral library in the presence of polybrene (8 µg/ml). 24 hr post infection, puromycin (2 µg/ml) containing medium was added onto cells to select the transduced cells. After 72 hours, the multiplicity of infection (MOI) of the titrated virus was determined by comparing the percent survival of infected cells to non-infected control cells.
Generation of CRISPR cell lines and screening

Target cell lines (HCT116-WT-GFP, HCT116-Mut-GFP and U251-Mut-GFP) were grown in 15cm plates and a total of \(40 \times 10^6\) cells were infected with TKOv3 lentiviral library (71,090 gRNAs) at an MOI of \(\sim 0.5\) to achieve \(\sim 200\)-fold coverage of the library after selection. Polybrene at 8 µg/ml was added to increase transduction efficiency. All screenings were performed in duplicates. After 24 hr, the virus-containing medium was removed and replaced with growth medium (DMEM, 10% FBS, 1% penicillin/streptomycin) supplemented with puromycin (1 µg/ mL) and cultured for 5 days. The cells were maintained at 20 \(\times\) 10^6 cells at each passage every 3–4 d to have \(\sim 200\)-fold coverage. Post selection, a total of 20 \(\times\) 10^6 cells were collected for genomic DNA extraction at day 0 and day 14. 20 \(\times\) 10^6 cells were fluorescence sorted by FACS (BD FACSAria II) on day14 to GFP low and GFP high. The cells were collected and grown until they reach a 20 \(\times\) 10^6 cells and were harvested for genomic DNA isolation. 20 million cells post-CRISPR screen were used to isolate genomic DNA using QIAGEN DNA Maxi kit (Qiagen), according to the manufacturer’s instruction. Concentration of the isolated DNA was quantified using Qubit 2.0 fluorometer (Thermo Fisher Scientific).

Library preparation and sequencing of CRISPR knockout libraries

To generate Illumina libraries for deep sequencing, 2 PCR reactions were performed. (1) enrich guide-RNA regions in the genome and (2) amplify guide-RNA with i5 and i7 indices. Both PCR reactions were performed using Q5 Hot Start High-Fidelity 2 × Master Mix and forward/reverse primers pair (v2.1-F1 and v2.1-
R1, Supplementary Table 2) in a 50 μl reaction. For PCR1, 40 independent PCR reactions were set up for each sample using 2.5 μg of the genomic DNA. Using this concentration, the library is maintained at 200-fold coverage. The PCR conditions were as follows: 98°C for 30 s, 98°C for 10 s, 66°C for 30 s and 72°C for 15 s (X25 cycles), and the final extension, 72°C for 2 min. The PCR products (600bp) were resolved on 1% agarose gel and individual reactions were pooled. About 5 μl of pooled PCR products from all 40 reactions and purified using QIAquick PCR purification kit (QIAGEN). The concentration of purified PCR products were quantified using the Qubit 2.0 fluorometer (Thermo Fisher Scientific). 5 μl of the PCR pooled purified product was used in a second PCR performed with 2x NEBNext Ultra II Q5 Master Mix. 2.5 μl of different i5 primer and 2.5 μl of different i7 primers (Supplementary Table 2) were used. The PCR conditions were as follows: 98°C for 30 s, 98°C for 10 s, 55°C for 30 s and 65°C for 15 s (10–12 cycles), and the final extension, 65°C for 5 min. The PCR product (200bp) was excised after running on 2% agarose gel. Finally, QIAquick Gel Extraction Kits (Qiagen) were used to purify the PCR products. Purified libraries were resuspended in 50 μl nuclease-free water and analysed using Agilent High Sensitivity DNA kit (Agilent Technologies) on Agilent 2100 Bioanalyzer (Agilent Technologies).

Purified libraries of the duplicate screens tagged with different i5 and i7 tags were pooled and sequenced at about 300x coverage on Illumina HiSeq 2500. About 30–40 million reads were obtained for each replicate.
CRISPR data processing

Quality control step was performed on sequencing reads by using Trimmomatic v0.32 to filter low-quality bases and adapter sequences. Reads that passed quality control were used as input in downstream analysis using MAGeCK v0.5.9 tool. Read counts corresponding to single-guided RNAs (sgRNAs) were first quantified and normalized using median normalization. Subsequently, the relationship of replicates was learned by mean-variance modeling and the statistical significance of each sgRNA was calculated using the learned mean-variance model. Essential genes were then positively and negatively selected (p<0.01) using robust rank aggregation (RRA) method. Essential genes identified in different cell lines and screens (CRISPR and RNAi) were compared to identify common genes. Finally, by reference to KEGG (Kyoto Encyclopedia of Genes and Genomes) database, enriched pathways (p<0.05) were identified by applying the gene set enrichment analysis (GSEA) algorithm to the ranked list of genes.

RNA isolation and gene expression analysis

Total RNA was isolated from cells after 36h of transfection by Trizol method followed by column purification (2). cDNA synthesis was performed from 1µg RNA using iScript cDNA synthesis kit, BioRad. RT-qPCR analysis was performed using diluted cDNAs (5ng/reaction) by SSO-Sybr Greener qPCR master mix (BioRAD). Expression values were normalized to actin and relative expression was calculated by delta-delta Ct method.
Chromatin Immunoprecipitation (ChIP) Assays

Cells were harvested 36h post-transfection and cross-linked for 10 minutes with 1% Formaldehyde. After isolation of nuclei fraction, sonication was performed by 30 sec on 30 sec off intervals to obtain DNA smear between 150bp - 750 bp. Soluble chromatin fraction was pre-cleared with Protein-A/G beads and incubated over-night with IgG (Sant Cruz), anti-MED12 (Bethyl A300-774A) and anti-Gabpa (Santa Cruz Biotechnology; sc-22810) antibodies. ChIP-qPCR was performed with hTERT promoter-specific primers (forward: GCGGCGCGAGTTTCAG, reverse: AGCACCTCGCGGTAGTG)

TRF Assay

TRF assay was performed in isogenic T98G cell line using TeloTAGGG telomere length assay kit (Sigma, Cat No: 12209136001) following manufacturer instruction. Briefly, the cells were harvested and counted. 2X 10^6 cell were used to lyse and extract pure genomic DNA using The Wizard® Genomic DNA Purification Kit (Promega, Cat No: A1120). The extracted DNA was quantified using Nanodrop. 4µg of total DNA was used in the assay. The telomere specific probes were used to bind to the digested DNA after they are transferred to the nylon membrane and UV-crosslinked. The DEG labelled probes are visualised using HRP substrate and the signal intensity was quantified using image J software.

Cell cycle analysis

Cell cycle analysis was performed by DNA content analysis using propidium iodide (PI)/RNase staining. Briefly, the cells were transfected with siRNA using RNAiMAX reagent. After specific time point, the cells were harvested and fixed with absolute
ethanol overnight. Next day, the cells were pelleted and washed 3 times. PI was used at 1:5000 dilution in PBS and incubated with cells for 30-60 mins. After the incubation, the cells were analysed using FACS with specific gates using 532-nm excitation with a 585/42-nm bandpass filter.

**3C- chromatin interaction assay**

Cells were fixed with 2% formaldehyde for 10 mins and lysed with cold lysis buffer (50mM Tris-HCL pH7.5, 150mM NaCl, 5mM EDTA, 0.5% NP-40, 1% Triton X-100) on ice for 20 mins. Isolated nuclei were resuspended in DNase-free water and chromatin was digested using HindIII enzyme for 36 hours. Fragmented chromatin was ligated overnight at 16C and after phenol-chloroform extraction, 3C template DNAs were quantified using Qubit dsDNA High Sensitivity Kit (Thermo Fischer). 3C-qPCR was performed as described previously using primers specific to hTERT promoter and the T-INT1 region (3).
Supplementary Figures

A

B

C

D

E
Supplementary Figure 1: A) Schematics of CRISPR genome editing in hTERT gene to insert GFP or NanoLuc gene. Either GFP or NanoLuc gene is inserted under the control of endogenous hTERT promoter in exon 1 with in-frame fusion. B) Gene expression of hTERT and C) telomerase activity was measured in all our reporter cell lines after successfully inserting promoter mutation (C228T/C250T) and reporter gene (GFP/NLuc). Actin was used as a control gene to normalise hTERT expression. D) Chromatogram images showing the mutated residues in the promoter of hTERT gene in GFP reporter lines (top) and NanoLuc reporter lines (bottom). E) Chromatogram images showing in-frame fusion of GFP in reporter lines HCT116-Mut-GFP and U251-Mut-GFP (top); NanoLuc gene in reporter lines LN382-Mut-NLuc, DLD-1-Mut-NLuc and HCT116-Mut-NLuc (bottom).
Supplementary Figure 2: A) Scatter plot showing signals from PrestoBlue in 3 different replicates of DLD-1-Mut-NLuc cells treated with siRNA library. B) Scatter plot showing luminescence-based NanoLuc readings (obtained using Nano-Glo luciferase assay system) from different replicates from DLD-1-Mut-NLuc cells treated with siRNA library. C-D) Scatter plot showing FLuc and NLuc activity from LN382-Mut-NLuc cells after siRNA treatment. FLuc activity (left) is a measure of cell viability while NanoLuc activity (right) is a measure of the hTERT promoter activation. E-F) Scatter plot showing FLuc and NLuc activity from HCT116-Mut-NLuc cells after siRNA treatment. FLuc activity (left) is a measure of cell viability while NanoLuc activity (right) is a measure of the hTERT promoter activation.
**Supplementary Figure 3:**

**A)** Box plot showing the sgRNA count (in log2 scale) across various conditions and harvesting days from HCT116-WT-GFP cells (left) and HCT116-Mut-GFP cells (right).

**B)** Box plot showing the sgRNA count (in log2 scale) across various conditions and harvesting days from U251-Mut-GFP cells (right). The sgRNA representation is equal across various days and conditions used in both cell lines.

**C)** Venn diagram showing the overlap of hits obtained from cell lines with the same hTERT mutation status (C228T). The common overlapped genes are listed in a yellow box. The known regulators are shown in green font while the novel hits are shown in blue.

**D)** Venn diagram showing the overlap of hits obtained from cell lines with different hTERT mutation statuses (C250T and C228T). The common overlap genes are listed in a yellow box. The known regulators are shown in green font while the novel hits are shown in red font.

**E)** Cell cycle analysis was performed in T98G isogenic cell lines after transient knockdown (with 2 siRNAs) of MED12. X-axis represents PI staining and y-axis represents cell count. For Mut-hTERT promoter cell line (T98G-C250T), the analysis was performed at early (p1) and late passage (p10) with continuous knockdown (with 2 siRNAs) of MED12.

**F)** Telomere length assay was performed in T98G isogenic cell lines after continuous transient knockdown (with 2 siRNAs) of MED12 for two months. The mean telomere length of each sample is indicated below (TRF: Telomere restriction fragment).
Supplementary Figure 4: A) qPCR data showing the expression of GABPA in non-reporter T98G and U251 cell lines with Mut hTERT promoter after transient knockdown (with 2 siRNAs) of MED12. B) Gene expression analysis was performed for the MED12 gene by qPCR in T98G-WT and T98G-Mut after stable MED12 knockdown (with 2 shRNAs). Ct values were normalized to actin gene. C) Western blot image shows the protein levels of MED12 in T98G-Mut lines after stable MED12 knockdown (with 2 shRNAs) and rescued with MED12 overexpression. HSP90 was used as a loading control. D) Telomerase activity assay was measured in T98G-Mut lines after stable MED12 knockdown (with 2 shRNAs) and rescued with MED12 overexpression. Error bars indicate the mean ± SD of three independent experiments. P values were calculated by Student's t-test method (*, p<0.05; **, p<0.01; ***, p<0.001). E) Image showing the colony formation by crystal violet staining of T98G-WT and T98G-Mut cells after MED12 knockdown (with 2 shRNAs) and rescued with MED12 overexpression.
Supplementary Figure 5: A-B) ChIP-qPCR was performed for MED12 and GABPA in T98G-WT (A) and BLM-WT (B) cells transfected with siControl and siMED12. Enrichment in the proximal hTERT promoter was calculated by using % input method. C) CoIP was performed in isogenic T98G lines against endogenous MED12 and IgG. The fractions were separated on SDS-PAGE and probed with anti-MED12 and anti-GABPA. D-E) ChIP-qPCR was performed for MED12, GABPA and IgG in T98G-Mut (D) and BLM-Mut (E) cells transfected with siControl and siGABPA. Enrichment in the proximal hTERT promoter was calculated by using % input method. F) Chromatin interaction frequency between Mut-hTERT promoter and T-INT1 region was analysed by 3C-qPCR in WT hTERT promoter lines transfected with siControl and siMED12. Error bars indicate the mean ± SD of three independent experiments. P values were calculated by Student's t-test method (*, p<0.05; **, p<0.01; ***, p<0.001).
Supplementary Table 1. List of hits across different cell lines used in genome wide screens

| siRNA screen | CRISPR knockout screen |
|--------------|------------------------|
| DLD-1-Mut-Nluc | LN382-Mut-Nluc | HCT116-Mut-Nluc | U251-Mut-GFP | HCT116-Mut-GFP |
| ALDOA | ABCC10 | BCL9 | ABCB10 | ABHD5 |
| AMD1 | ABHD9 | CACNA1S | ACOT9 | ACBD5 |
| APEX1 | ANKAR | S100G | ADAM19 | ACCSL |
| CCND1 | APOL4 | CFL1 | AGRP | ACOX1 |
| BCL9 | ARHGAP22 | DPYD | ALDH5A1 | ACSBG2 |
| BGLAP | ASB14 | EIF2S3 | ALG2 | ACSF3 |
| BOK | ASTN2 | EPHB2 | API5 | ACTN2 |
| ZFP36L1 | ATG3 | ETU2 | ARL6IP1 | ACVR2B |
| MPPED2 | ATG7 | GABPA | ARMCX1 | AIM1L |
| C11orf10 | B3GAT2 | GABPB1 | ARMCX2 | AKAP6 |
| S100G | BCL9 | GZMK | ARMCX3 | AKT1 |
| CAPN3 | BGLAP | HRG | ARMCX6 | AKTIP |
| CAPN6 | C19orf18 | IL17A | ASCL4 | APOBEC1 |
| CAPS | C19orf39 | PDX1 | ASGR2 | ARL13B |
| RUNX1T1 | C19orf44 | LHX1 | ATG4A | ATP13A4 |
| CCND2 | C19orf47 | SMAD2 | ATP1B4 | B9D1 |
| CDH8 | C1orf70 | MCM2 | ATP6V1B2 | BCL7A |
| CDK4 | C3orf62 | MYC | AWAT2 | BCL7C |
| CDK7 | C5orf22 | RPL10A | BEX4 | BIRC2 |
| CDR2 | C9orf135 | NTF4 | BEX5 | C17orf82 |
| CDX1 | C9orf9 | ODC1 | C11orf83 | C1orf137 |
| CFL1 | CACNA1S | OR1F1 | C1GALT1C1 | C21orf59 |
| CHD4 | CACUL1 | PDK2 | C3orf55 | C7orf55 |
| CNN1 | CALML4 | PFDN4 | C9orf50 | CALCRL |
| COL4A2 | CARD8 | PLN | CABIN1 | CAMTA2 |
| CPA | CASTOR1 | PPP2R3A | CAPN6 | CBLN1 |
| COXI | CCDC181 | CCL4 | CAPRIN1 | CCDC146 |
| CSNK1A1 | CCDC67 | SGCD | CAST | CCDC6 |
| CSNK1G2 | CCDC88A | SLC15A2 | CCDC115 | CCND3 |
| CTNNB1 | CCDC92 | SNRPD2 | CCDC22 | CD180 |
| DAB1 | CDR2L | ZNF19 | CCDC83 | CD4 |
| DAZL | CHCHD5 | ZNF79 | CDC42BP | CD96 |
| DBI | CHL1 | CCDC6 | CDC45 | CDC37L1 |
| DIO1 | CHST5 | PPRF18 | CDC4A8 | CDC4A |
| DPYD | CHTF18 | SART1 | CDX4 | CDH6 |
| DSC3 | CLEC19A | NCR2 | CHIC1 | CHRNA6 |
| DUSP8 | COL6A6 | MED27 | CHRD1 | CLSTN2 |
| Gene 1 | Gene 2 | Gene 3 | Gene 4 | Gene 5 |
|--------|--------|--------|--------|--------|
| E4F1   | COPG1  | MED7   | CINP   | CMIP   |
| EEF1A1 | CPLX3  | SH3BP5 | CITED1 | CPXM1  |
| EIF1AX | CPSF6  | ZNF432 | CLEC5A | CREG1  |
| EIF2S1 | CTRC   | SDC3   | COPA   | CSTF3  |
| EIF2S3 | CXorf22| SART3  | COPS5  | CYP4F3 |
| EIF5   | CYBASC3| IQSEC1 | CPXCR1 | DAK    |
| ELK3   | DDX19B | MED6   | CRB3   | DAPK2  |
| ENDOG  | DDX21  | AVIL   | CT47B1 | DEAF1  |
| STOM   | DDX43  | DDX19B | CTAG2  | DEPDC4 |
| EPHB2  | DEFB112| PUF60  | CTDSPL2| DPYS15 |
| ETF1   | DENND1B| COTL1  | CTHRC1 | EPS15  |
| EZH1   | DIO1   | BACE2  | CUL4B  | FAM111A|
| FAAH   | DLX4   | SOSTDC1| CXCRR3 | FAM122A|
| FAH    | DNAJC7 | VSX1   | CXorf65| FAM221A|
| FBN1   | DOCK11 | ATP6V0A4| CXorf66| FAM69A |
| FGB    | DOK4   | RNF138 | DACH2  | FCR1G  |
| FGFR2  | DPP9   | RIN2   | DCAP12L2| FLAD1 |
| FOXF1  | DPYD   | CCER2  | DIX    | FNBP1L |
| FLII   | DPYS12 | RRN3   | DDX39B | FNDC1  |
| FLNA   | EHF11  | ZNF586 | DDX55  | FNIP2  |
| GABPA  | EIF4G3 | TRPM7  | DGAT2L3| FOXL1  |
| GABPB1 | EP400  | SVOP   | DOCK11 | FRAS1  |
| GBA    | EPC1   | ZNF83  | DPPA3  | FSD1L  |
| GDF2   | EPHB2  | SLC22A11| DRP2  | GAB1   |
| GPC1   | ESPNL  | UBQLN4 | EDA    | GABBR1 |
| HELLS  | EZH1   | PRDM8  | EDDM3B | GDPGP1 |
| HLA-DQB2| F11    | ACN9   | EFN1B  | GFI1B  |
| HRM2PH1| FAAH   | C16orf62| EIF3B  | GLRA4  |
| PRMT1  | FABP2  | NAT14  | EP300  | GORASP1|
| HSPA5  | FAM120AOS| ZNF250| ERCC3  | GPR108 |
| HSPA9  | FAM98B | HERPUD2| EXOSC5 | GPR135 |
| IL5    | FBXO11 | MRPL36 | TAM199X| GVQW1  |
| IL18   | FGFR9  | ACD    | FAM19A2| H2AFV  |
| IMPDH1 | FKB9   | PPDPF  | FAM19A3| HHEX   |
| ITG6   | FOLR4  | FCRL2  | FAM26F | HN1L   |
| JARID2 | GABPA  | TMCO7  | FAM46D | HOXB5  |
| KCNJ15 | GABPB1| SEL1L2 | FBXL14 | HOXD12 |
| KRAS   | GFOD2  | OR2B2  | FDFT1  | HSPB6  |
| KTN1   | GLYATL1| SF3B5  | FITM2  | HYI    |
| LLGL1  | GPC1   | HS6ST2 | FOXK2  | IKBKG  |
| SMAD2  | GPR112 | ER11   | FRK    | IPO4   |
| MCM2   | GPT8   | TMEM88 | FRMD7  | JPH1   |
| MPST   | GTF3C4 | RG9MTD2| GABRR2 | JUP    |
| MYC | H1FOO | PLCD3 | GGCX | KATNAL2 |
|-----|-------|-------|------|---------|
| NCK1| HAND1 | LRR1C5| GGCX | KCNA7   |
| RPL10A| HCC1 | DOCK11| GK   | KCNA1B  |
| NONO| HCN1 | MDP1  | GLRX3| KCTD14  |
| NTN3| HEY1 | CCDC17| GLUD2| KIAA1211L |
| NUP98| HEYL| FAAH2 | GPC3 | KIF3C   |
| OPRK1| HIST2H3A| ZNF836| GPR174| KLHL7   |
| P2RX3| HTT | DENND1B| GREM2| KLRG2   |
| FURIN| IKBKG | C1orf177| GRIA3| KRIT1   |
| PDGFRB| INPP5F| C1QL2 | GRWD1| KRTAP10-4|
| PDK2| ISM2 | OR5A51 | GTF2E2| LAMB2   |
| PGF| ISO1 | RNF169 | GTF2H5| LCA5    |
| PML| ITGB6 | WDR51B | H2BFM| LRFN4   |
| POLR2C| ITSN2| NKA1N3 | H2BFWT| LRRC46  |
| POLR2E| JARID2| VN1R5 | HIF1A | LRRK1   |
| POLR2G| KCNA3 | TCEAL5 | HINFP| LZIC    |
| POLR2I| KCNH2 | PALM3 | HIRA  | MAFB    |
| PPP2R3A| KIAA2013| OTOP3| HNRNPH2| MAPKAPK5|
| PRIM1| KLC4 | TRIM77 | HTR2C | MCOLN2  |
| PRKAR2A| L1TD1| OR5AU1 | HTR3E | MESC2   |
| PRSS1| LAMA3| RPL18P4| IL13RA1| MEX3B   |
| PSMA1| LCE1F | TEX19  | IL13RA2| MORN3   |
| PSMA5| LGALS9B| C14orf53| IRS4  | MPND    |
| PSMA6| LHX9 | LOC646570| ITGB1BP2| MSX1    |
| PSMA7| LIME1 | LOC646872| ITM2A | MTR     |
| PSMB3| LINC01501| RPL7P9| KAT6A | MTRR    |
| PSMB6| LOC401442| BCL9 | KCNN10| NCAN    |
| PSMB7| LOC646570| CACNA1S| KHSRP | NDST4   |
| PSMC1| LOC649975| S100G | KIAA2022| NFKBIB  |
| PSMC2| LOC727894| CFL1  | KLHL22| NOL7    |
| PSMC3| LOC728685| DYPD  | KLHL4 | OAF     |
| PSMC5| LOC729296| EIF2S3| KRT13 | ODF3    |
| PSMC6| LOC729421| EPHB2 | LAMP2 | OMP     |
| PSMC2| LOC729823| ETV2  | LDLR  | OR51A7  |
| PSMC3| LOC729827| GABPA | LMAN1 | OR7D2   |
| PSMC7| LOC730271| GABPB1| LPCAT1| OTOA    |
| PSMC11| LOC732028| GZMK | LRRTM4| PAQR9   |
| PSMC12| LOXL4| HRG   | LSMEM2| PDS5A   |
| PTPRB| LYZL4| IL17A | LTV1  | PIM1    |
| RFC2| MCM2 | PDX1  | LYPD6| PJA1    |
| BRD2| MDS1 | LHX1  | MAPKAPK3| PLCE1   |
| RPLPL1| MED6 | SMAD2 | MATR3 | PLEKH2  |
| RPLPL2| MPND | MCM2  | MAVS  | PLOD2   |
| RPS3  | MYF5  | MYC  | MCEE  | POU2F3  |
|-------|-------|------|-------|---------|
| RPS3A | NAA10 | RPL10A | MED12 | PPFIA3  |
| RPS4X | NAT14 | NTF4  | MED19 | PPM1D   |
| RPS5  | NCOA2 | ODC1  | MED27 | PRICKLE3|
| RPS6  | NDRG3 | OR1F1 | METTL23 | PROM2  |
| RPS7  | NDUFS7 | PDK2  | MFSD12 | PRPH    |
| RPS8  | NGEF  | PFDN4 | MPL   | PSD4    |
| RPS9  | NKAIN3 | PLN  | MRPL41 | PTGS1   |
| RPS10 | NPIPB7 | PPP2R3A | MSN  | PTPN22  |
| RPS11 | NRSN1 | CCL4  | MTMR8 | PTPRZ1  |
| RPS13 | OR13G1 | SGCD  | MX1   | RAB39B  |
| RPS14 | OR51T1 | SLC15A2 | NABP2 | RBP3    |
| RPS15 | OR52E6 | SNRPD2 | NAGK  | REPS2   |
| RPS27 | OR8B2 | ZNF19 | NDP   | RGL4    |
| RPS27A | OTOP3 | ZNF79 | NHSL2 | RGS12   |
| SDC4  | PABPC4L | CCDC6 | NSMCE2 | RGS18   |
| ST3GAL1 | PAK4 | PRPF18 | NUTF2 | RHNO1   |
| SLC1A3 | PALM3 | SART1 | NXF5  | RND3    |
| SLC4A2 | PALMD | NCR2  | NXT1  | RPL12   |
| SLC8A3 | PCNT  | MED27 | OPHN1 | S100Z   |
| SLC15A2 | PCSK2 | MED7  | OTOS  | SAT2    |
| SLC16A1 | PHF16 | SH3BP5 | OTUD6A | SEMA4C  |
| SMARCB1 | PHF21A | ZNF432 | P2RY10 | SGPP2   |
| SNRPD1 | PHKB  | SDC3  | P2RY4 | SKIL    |
| SNRPD2 | PIWIL2 | SART3 | PATL2 | SLC25A43|
| SNRPD3 | PMVK  | IQSEC1 | PCDH19 | SLC5A5  |
| SNRPF | PPWD1 | MED6  | PCDHGB1 | SNAPC4   |
| SON   | PRC1  | AVIL  | PCGF6 | SPATA24 |
| SUPT5H | PRDM8 | DDX19B | PIAS1 | SPDYA   |
| TAT   | PRKAR2A | PUF60 | PIGO  | SPNS2   |
| TBX1  | PRPF18 | COTL1 | PIGV  | SRSF2   |
| TKT   | PRR12  | BACE2 | PIN1  | ST8SIA4 |
| TPR   | PRR17  | SOSTDC1 | PIN4  | STRIP2  |
| UBTF  | PRR4  | VSX1  | PLA2G12A | SVOPL   |
| UPK1B | PRRX2 | ATP6V0A4 | PLEKHM1 | TBL2    |
| UQCRC2 | PRSS1 | RNF138 | PLP1  | TCHHL1  |
| WEE1  | PSG1  | RIN2  | PNMA5 | THBS3   |
| ZNF19 | PSMA7 | CCSER2 | PNPLA4 | TICRR   |
| ZNF79 | PTPRB | RRN3  | POIF1B | TLR1    |
| ZNF84 | RAD50  | ZNF586 | POLR2I | TMEM220  |
| ZNF193 | RAPGEF4 | TRPM7 | POU3F2 | TMEM235  |
| MLL2  | RASGRP1 | SVOP  | PRPS1 | TMPPE   |
| AP3B2 | RBPMS2 | ZNF83 | PRR7  | TNNT2   |
| Gene A | Gene B | Gene C | Gene D | Gene E |
|--------|--------|--------|--------|--------|
| SMC1A  | RILP   | SLC22A11 | PRRG3  | TSKU   |
| SLC10A3 | RNF138 | UBQLN4  | PSMD10 | TTTL10 |
| HIST3H3 | RPLP2  | PRDM8   | RAB40A | UCK2   |
| HIST1H3J | RXRG   | ACN9    | RAB9B  | VWA3A  |
| MAD1L1  | S100G  | C16orf62 | RBM41  | WDR41  |
| NIPSNAP1 | S100PBP | NAT14  | RBMXL3 | WDR86  |
| IFITM1  | SAMD13 | ZNF250  | RIPPLY1 | XYLT1  |
| CAMK1   | SART3  | HERPUD2 | RNF128 | YOD1   |
| BARX2   | SEC22B | MRPL36  | RNPS1  | ZBTB10 |
| CGGBP1  | SH3TC2-DT | ACD | RPL30  | ZBTB47 |
| CDC14B  | SIGLEC12 | PPDPF | RPS19  | ZFP92  |
| PRPF18  | SLC43A1 | FCRL2   | RRN3   | ZNF197 |
| PDLIM4  | SMAD2  | TMCO7   | RTP3   | ZNF268 |
| RUVBL1  | SON    | SEL1L2  | S1PR2  | ZNF384 |
| NCOA1   | SPINT2 | OR2B2   | SAPCD2 | ZNF575 |
| DDX3Y   | SRMS   | SF3B5   | SATL1  | ABCA2  |
| EIF3A   | SSTR1  | HS6ST2  | SEC11A | ABHD2  |
| EIF3B   | STOM   | ER1I    | SEC63  | ACAN   |
| EIF3C   | SUCLG1 | TMEM88  | SEMA5B | AHNAK  |
| EIF3G   | SYNGR1 | RG9MTD2 | SH3BGRL | AHR |
| EIF3I   | TCEAL6 | PLCD3   | SLC15A4 | ALDH6A1 |
| GALNT4  | TFAP2E | LRRC15  | SLC16A2 | ALG14 |
| SUCLG1  | TFPI2  | DOCK11  | SLC25A53 | ALOXE3 |
| CES2    | TMCC2  | MDP1    | SLC29A2 | ANAPC13 |
| TOP3B   | TMEM98 | CCDC17  | SLC33A1 | ANTXRL |
| HIST1H2BJ | TMEM9B | FAAH2   | SLC38A10 | AP1G2 |
| SYNGR1  | TMSB15A | ZNF836 | SLC7A3 | AP2S1 |
| DDX21   | TMTC3  | DENND1B | SLK    | APOO   |
| MED14   | TOMM40 | C1orf177 | SNRPB2 | ARMC1 |
| NDST3   | TRAK1  | C1QL2   | SNRPD2 | ASB6   |
| MED21   | TRAP1  | OR5AS1  | SPATA6 | ATF7IP |
| DDX23   | TRIM50 | RNF169  | SPINK2 | ATP5A1 |
| HAND1   | TRIM68 | WDR51B  | SPTLC1 | ATP5SL |
| MED17   | TRPM7  | NKAIR3  | SRPX2  | ATP6AP2 |
| MED7    | TTC22  | VN1R5   | STX17  | BACH2  |
| STX8    | TTPAL  | TCEAL5  | SYMPK  | BCAP29 |
| CTR9    | TUBB4B | PALM3   | SYTL4  | BRD2   |
| MDC1    | TUBGCP4 | OTOP3  | TAC1   | C11orf91 |
| ZNF432  | TXLNB  | TRIM77  | TADA1  | C16orf92 |
| NUP93   | UBXN11 | OR5AU1  | TAF5L  | C18orf21 |
| RAPGEF2 | UGT2B15 | RPL18P4 | TAF9B  | C19orf70 |
| SART3   | UPF1   | TEX19   | TBC1D12 | C2orf68 |
| CKAP5   | VWF    | C14orf53 | TBL1X  | C7orf26 |
| Gene    | Gene    | Gene    | Gene    | Gene    |
|---------|---------|---------|---------|---------|
| NUPL1   | WDR51B  | LOC645670 | TBPL1   | C8orf74 |
| TMCC2   | WFIKKN1 | LOC648672 | TBX22   | CABP1   |
| MED12   | WIPF1   | RPL7P9  | TCEAL1  | CAD     |
| MED6    | WNK2    | TCEAL7  | CAP1    |         |
| CHAF1A  | YWHAB   | TERT    | CCDC106 |         |
| PDIA6   | ZNF187  | TEX11   | CCDC39  |         |
| PSMD14  | ZNF250  | THOC6   | CCT7    |         |
| PEMT    | ZNF418  | TIMM8A  | CD6     |         |
| TOMM40  | ZNF432  | TMED2   | CDC37   |         |
| HAX1    | ZNF552  | TMEM230 | CDC5L   |         |
| NXF1    | ZNF778  | TMEM259 | CDIPT   |         |
| IPO8    | ZNF799  | TMEM31  | CDT1    |         |
| TNFSF13B| ZNF836  | TMEM35  | CEP57   |         |
| AVIL    |         |         |         |         |
| TOB2    |         |         |         |         |
| RUVBL2  |         |         |         |         |
| GPR83   |         |         |         |         |
| RAB10   |         |         |         |         |
| ERP29   |         |         |         |         |
| CPSF6   |         |         |         |         |
| OGFR    |         |         |         |         |
| RAPGEF4 |         |         |         |         |
| TOPBP1  |         |         |         |         |
| CLASRP  |         |         |         |         |
| RPL35   |         |         |         |         |
| DDX19B  |         |         |         |         |
| COPZ1   |         |         |         |         |
| CARD8   |         |         |         |         |
| TRAK1   |         |         |         |         |
| SEPHS1  |         |         |         |         |
| CDK19   |         |         |         |         |
| WDR43   |         |         |         |         |
| NUP205  |         |         |         |         |
| SF3B3   |         |         |         |         |
| SF3B1   |         |         |         |         |
| TMEM50A |         |         |         |         |
| NUP62   |         |         |         |         |
| PLD3    |         |         |         |         |
| IL17RA  |         |         |         |         |
| BRD1    |         |         |         |         |
| MTCH2   |         |         |         |         |
| FBXO7   |         |         |         |         |
| SPDEF   |         |         |         |         |
|         |         |         |         |         |
| Gene 1 | Gene 2 | Gene 3 |
|--------|--------|--------|
| TXN2   | ZCCHC16 | FBXO11 |
| DAK    | ZNF280C | FERD3L |
| ACOT11 | ZNF414  | GABRB1 |
| ATRNL1 | ZRANB2  | GABRB3 |
| SLITRK5| GDAP1L1 |        |
| GIGYF2 |        |        |
| CCDC9  | GNB2L1  |        |
| GTPBP5 | GNPDA1  |        |
| OR2F1  | GRB10   |        |
| HEY1   | H2AFX   |        |
| MTBP   | HBZ     |        |
| CECR6  | HDGF    |        |
| TRIB2  |        |        |
| CCDC113| HIRIP3  |        |
| NOSIP  | HOXA9   |        |
| CUTC   | HSPB9   |        |
| FCF1   | IFNBI   |        |
| TUBD1  | IGLL1   |        |
| THEM6  | IL17D   |        |
| POMP   | IL27    |        |
| AIG1   | INO80   |        |
| ANAPC5 | IPO11   |        |
| RNF138 | ISCA1   |        |
| LARS   | ISCU    |        |
| C14orf100|       |        |
| AZIN1  | KARS    |        |
| PCF11  | KCNQ4   |        |
| ZNF44  | KDM5C   |        |
| LUC7L3 | KLF17   |        |
| SSH1   | L3MBTL2 |        |
| STAG3L1| LARP4B  |        |
| CCSER2 | LAT2    |        |
| SMCR7L | LCE3C   |        |
| APBB1IP| LDB3    |        |
| HES2   | LECT1   |        |
| GATAD2A| MADCAM1 |        |
| TRPM7  | MAML1   |        |
| C1orf26| MAP2K1  |        |
| ASPN   | MCCD1   |        |
| WDR55  | MDM2    |        |
| COMMMD8| MED10   |        |
| MED9   | MERTK   |        |
| AKIRIN2| METTL14 |        |
| Gene 1     | Gene 2      |
|-----------|-------------|
| RBM23     | MKI67IP     |
| CEP55     | MKS1        |
| TYW1      | MMP26       |
| TMEM100   | MORF4L1     |
| DDX43     | MRPL21      |
| SUPT20H   | MRPL34      |
| RAB20     | MRPL51      |
| DENND4C   | MRPS23      |
| FRMD4A    | NCL         |
| DOK4      | NDUFA1      |
| MUDENG    | NEK11       |
| SLC22A11  | NID2        |
| PCDHGA1   | NOL9        |
| BEX4      | NUP133      |
| CLDND1    | OR5AC2      |
| TMEM9B    | PAFAH1B3    |
| UBQLN4    | PALB2       |
| MCCC1     | PARD6B      |
| DUSP22    | PCDHA3      |
| XAB2      | PDCL        |
| OTUD7B    | PGD         |
| KIF15     | PHYH        |
| C16orf62  | PIGL        |
| ADAMTSL3  | PLIN1       |
| NHSL1     | PLK1        |
| SMAGP     | PLXNB2      |
| NDRG3     | PMVK        |
| GATAD2B   | PNMAL2      |
| KIDINS220 | POLD2       |
| STARD9    | POLD3       |
| TMEM181   | POLR1E      |
| WDR19     | POP5        |
| ZNF250    | POP7        |
| ARHGAP22  | PPA1        |
| GNB4      | PPP1R11     |
| PBOV1     | PROM2       |
| AASDHPPT  | PRPS2       |
| ELAC2     | PSMD12      |
| RIC8A     | RAB11B      |
| ZFAND3    | RAN         |
| MRPL17    | RASGRP1     |
| SH2D4A    | RASSF8      |
| CARD9     | RBM23       |
| EDDM3B | RHOST2 |
|-------|--------|
| LPIN3 | RIOK3  |
| UBE2Z | RNGTT  |
| CYP4F12 | RPL11 |
| LYNX1 | RPL23  |
| GID4  | RPL35  |
| PDCL3 | RPS2   |
| PPDPF | RSU1   |
| IRX1  | RTCA   |
| SAP130 | SAE1  |
| TMEM53 | SAE1  |
| ZNF552 | SCO1  |
| ARHGAP28 | SERPINB2 |
| PIP4K2C | SLC25A47 |
| RMI1  | SLC38A2 |
| CCDC92 | SLC50A1 |
| ABTB1 | SLX4   |
| KCNIP4 | SMG9  |
| NIPA2 | SMAD2  |
| OR2B2 | SMUG1  |
| TTC25 | SNRPD3 |
| PARP9 | SNX12  |
| WDR87 | SNX22  |
| ING5  | SPC25  |
| C14orf142 | SRPRB |
| C6orf168 | SRXN1 |
| CYorf15B | STK40 |
| MYO18B | SV2B   |
| CBX2  | TAF7   |
| MPV17L2 | TECPR1|
| PARP10 | TFAM   |
| LRP11 | TFB1M  |
| MAEL  | TLN1   |
| C12orf62 | TMC5  |
| STON2 | TNNT2  |
| WNT3A | TNPO3  |
| LMLN  | TP53   |
| NAV2  | TPI1   |
| C9orf140 | TPT1  |
| KIAA2013 | TRAIP |
| DCAF15 | TRIB1  |
| ZNF799 | TRMT10C|
| ANKRD40 | TRPM1 |
| Gene  | Gene  | Gene  |
|-------|-------|-------|
| RFT1  | TRUB2 |       |
| IMP4  | TSFM  |       |
| ZBTB47| TTC30A|       |
| C13orf27| TWIST1|       |
| RG9MTD2| USP34 |       |
| SERHL | WARS2 |       |
| NACC1 | WDR3  |       |
| PANX3 | WDR33 |       |
| OR51A7| WDR66 |       |
| AK7   | WRAP53|       |
| C19orf47| XPO1 |       |
| TPRG1L| YRDC  |       |
| C1orf216| YWHAE|       |
| EDARADD| ZNF280C|     |
| IQGAP3|       |       |
| C1orf88|       |       |
| CST9LP1|       |       |
| CIB4  |       |       |
| SCLT1 |       |       |
| C9orf135|       |       |
| UPRT  |       |       |
| GPR119|       |       |
| DOCK11|       |       |
| CACUL1|       |       |
| RPL14L|       |       |
| C13orf31|      |       |
| HAPLN3|       |       |
| C17orf77|       |       |
| AMAC1 |       |       |
| C19orf84|       |       |
| COMMD7|       |       |
| CAMSAP1|       |       |
| TTL11 |       |       |
| KIAA2026|       |       |
| C9orf84|       |       |
| PATE1 |       |       |
| ZNF600|       |       |
| FAM47B|       |       |
| CLEC4C|       |       |
| GPHA2 |       |       |
| DAND5 |       |       |
| DHRS7C|       |       |
| TMEM192|       |       |
| Gene      |
|-----------|
| CDHR3     |
| NEGR1     |
| NALCN     |
| TAS2R50   |
| MDGA1     |
| WDR51B    |
| C11orf72  |
| MORN3     |
| LGALS9B   |
| BOD1P     |
| C19orf54  |
| TPRX1     |
| OR22Z1    |
| ZNF844    |
| FAM19A3   |
| ERICH2    |
| RPS26P40  |
| PRSS38    |
| LINC01139 |
| ESPNL     |
| KLHL38    |
| POTEA     |
| STAC2     |
| ZNF404    |
| BARHL2    |
| RSHL3     |
| ZNF322B   |
| TMEM212   |
| GRXCR1    |
| USP27X    |
| OR5B12    |
| LOC390335 |
| THA1P     |
| PRAMEF8   |
| RPS12P4   |
| RPS15AP17 |
| LOC391722 |
| LINC01061 |
| RPS26P10  |
| LOC401677 |
| RPL15P11  |
| OR4A47    |
| LOC440055 |
| LOC729036 |   |   |
| LOC729398 |   |   |
| LOC729592 |   |   |
| LOC729622 |   |   |
| LOC729720 |   |   |
| LOC729807 |   |   |
| LOC729833 |   |   |
| RPSAP19   |   |   |
| LOC730271 |   |   |
| LOC731517 |   |   |
| HGC6.3    |   |   |
| FAM196B   |   |   |
| TIMM23    |   |   |
| THEGL     |   |   |
### Supplementary Table 2. Primers used in CRISPR screen library amplification.

| Primer name | Sequence                                                                 |
|-------------|--------------------------------------------------------------------------|
| v2.1-F1     | GAGGGCCTATTCCCCATGATTC                                                   |
| v2.1-R1     | GTTGCAGAAAAAGAGCTTTCAGGG                                                |
| D501        | AATGATACGGCCACCAGGAGATCTACATACTATAGCCTACACCTTCTTCCTACCGATCTTTGTGGAAAGGACGAA CACCG |
| D502        | AATGATACGGCCACCAGGAGATCTACATACAGGACACAGAGTCTGCAAGGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D503        | AATGATACGGCCACCAGGAGATCTACACAGGACACAGAGTCTGCAAGGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D504        | AATGATACGGCCACCAGGAGATCTACAGGCGAAGGACACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D505        | AATGATACGGCCACCAGGAGATCTACACAGGACACAGAGTCTGCAAGGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D506        | AATGATACGGCCACCAGGAGATCTACACATAGGCGAAGGACACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D701        | CAAGCAGAAGACGGCATACGAGATCGAAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D702        | CAAGCAGAAGACGGCATACGAGATCGAAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D703        | CAAGCAGAAGACGGCATACGAGATCGAAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D704        | CAAGCAGAAGACGGCATACGAGATCGAAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D705        | CAAGCAGAAGACGGCATACGAGATTTTCTGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D706        | CAAGCAGAAGACGGCATACGAGATTTTCTGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
| D707        | CAAGCAGAAGACGGCATACGAGATTTTCTGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGCTATTTCTAGCTCTAA AAC |
Supplementary Table 3. List of secondary siRNA screen hits

| Gene Symbol | Gene ID | WT Toxicity | WT Inhibition | C228T Toxicity | C228T Inhibition | Mutant specific inhibition score |
|-------------|--------|-------------|---------------|----------------|-----------------|----------------------------------|
| C7orf26     | 79034  | -34.43      | -259.95       | -44.88         | -52.58          | -207.36                         |
| MED12       | 9968   | 86.98       | -32.70        | 78.53          | 57.02           | 89.71                           |
| CHD4        | 1108   | 31.35       | -35.91        | 31.09          | 46.79           | 82.70                           |
| TNRC18P1    | 644962 | 40.19       | -13.51        | 50.87          | 47.58           | 61.09                           |
| SNRPD2      | 6633   | -4.68       | -3.61         | -34.28         | 51.72           | 55.33                           |
| TAF7        | 6879   | -35.29      | -52.09        | -8.28          | 1.12            | 50.96                           |
| EIF3C       | 8663   | 75.56       | 4.95          | 70.98          | 54.48           | 49.53                           |
| GNB2L1      | 10399  | 28.93       | -22.97        | 47.22          | 19.45           | 42.42                           |
| RG9MTD1     | 54931  | -33.14      | -45.36        | -5.80          | -7.80           | 37.57                           |
| DPP9        | 91039  | 36.92       | -19.52        | 35.01          | 17.86           | 37.38                           |
| RPL35       | 11224  | 78.22       | 18.38         | 81.01          | 54.56           | 36.17                           |
| PSMD12      | 5718   | 65.63       | 9.45          | 60.18          | 42.68           | 33.23                           |
| Loc         | Luc    | 98.62       | -35.60        | 97.38          | -3.64           | 31.96                           |
| LOC730271   | 730271 | 60.12       | -5.36         | 37.69          | 25.12           | 30.48                           |
| SNRPD3      | 6634   | 28.23       | 25.87         | -18.81         | 51.24           | 25.36                           |
| RAPGEF4     | 11069  | 68.29       | 24.99         | 73.93          | 48.80           | 23.81                           |
| PMVK        | 10654  | -65.37      | 2.62          | -32.55         | 25.08           | 22.46                           |
| MORRN3      | 283385 | 16.19       | 27.04         | 16.76          | 49.42           | 22.38                           |
| Neg1        | Neg1   | 17.70       | -13.55        | -3.75          | 8.04            | 21.59                           |
| KIAA2013    | 90231  | 33.66       | 24.43         | 32.01          | 45.24           | 20.81                           |
| FAM19A3     | 284467 | 66.49       | 31.86         | 56.92          | 51.96           | 20.11                           |
| GABPA       | 2551   | 21.04       | 28.36         | -4.95          | 48.42           | 20.07                           |
| CYP4F3      | 4051   | -1.36       | -7.75         | -2.73          | 12.12           | 19.87                           |
| EDDM3B      | 64184  | 10.67       | 18.38         | 12.29          | 37.81           | 19.43                           |
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2. Akincilar SC, et al. (2021) NAIL: an evolutionarily conserved lncRNA essential for licensing coordinated activation of p38 and NFkappaB in colitis. Gut 70(10):1857-1871.

3. Akincilar SC, et al. (2016) Long-Range Chromatin Interactions Drive Mutant TERT Promoter Activation. Cancer Discov 6(11):1276-1291.