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Supplemental information

Multi-hallmark long noncoding RNA maps reveal non-small cell lung cancer vulnerabilities

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Figure S1

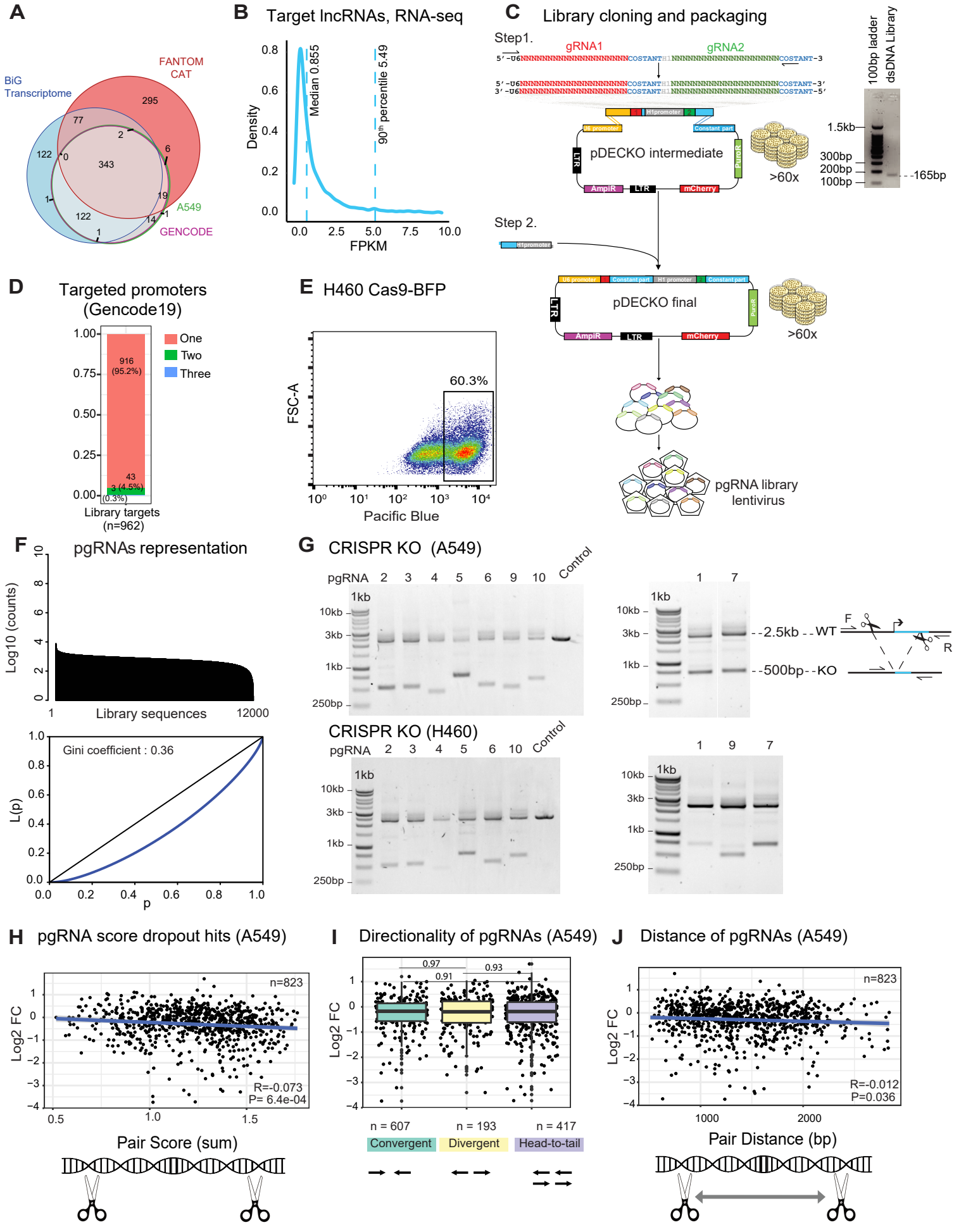
A
BiG Transcriptome
FANTOM CAT
GENCODE
A549
295
77
2
6
122
0
343
19
1
122
14
1
1
B
Target lncRNAs, RNA-seq
Density
Median 0.855
90th percentile 5.49
FPKM
C
Library cloning and packaging
Step1.
gRNA1
gRNA2
pDECKO intermediate
>60x
100bp ladder
dsDNA Library
1.5kb
300bp
200bp
100bp
165bp
Step 2.
pDECKO final
>60x
pgRNA library lentivirus
D
Targeted promoters (Gencode19)
One
Two
Three
916 (95.2%)
43
3 (4.5%)
(0.3%)
Library targets (n=962)
E
H460 Cas9-BFP
60.3%
FSC-A
Pacific Blue
F
pgRNAs representation
Log10 (counts)
Library sequences
Gini coefficient : 0.36
L(p)
p
G
CRISPR KO (A549)
pgRNA
Control
2.5kb
WT
500bp
KO
CRISPR KO (H460)
H
pgRNA score dropout hits (A549)
n=823
Log2 FC
R=-0.073
P= 6.4e-04
Pair Score (sum)
I
Directionality of pgRNAs (A549)
0.97
0.91
0.93
n = 607
n = 193
n = 417
Convergent
Divergent
Head-to-tail
J
Distance of pgRNAs (A549)
n=823
R=-0.012
P=0.036
Pair Distance (bp)

Figure S1. libDECKO-NSCLC1 library creation. Related to Figure 1.

(A) Library design pipeline. LncRNAs from indicated annotations are merged and filtered by TSS proximity to protein-coding genes (<2 kb excluded) and by expression in A549 (<0.1 FPKM excluded). TSS are clustered together if closer than 300bp, then selected based on three evidence sources: CAGE, ChromHMM and DNAseI hypersensitivity. TSS candidates are targeted by 10 paired guide RNA (pgRNA) designs using CRISPETa.

(B) Expression of targeted lncRNAs in A549 cells.

(C) Library cloning. Oligonucleotide library of pgRNAs is amplified by PCR. Two steps of cloning to insert the amplified fragment and the constant part into the pDECKO backbone. Coverage of clones to library sequences was estimated to be >60x. The plasmid library is packaged into viral particles.

(D) Number of independent annotated genes' TSS/promoters targeted by each candidate region. 95.2% of the library targets single lncRNA genes, 4.8% of candidate regions contain two or three independent TSS. Note that classical "bidirectional promoters" comprise TSS from two independent lncRNA genes (green), while a small number of targeted regions (3, blue) contain TSS from three independent lncRNA genes.

(E) Fluorescence activated cell sorting (FACS) was used to sort stable Cas9-expressing H460 cells based on expression of a Blue Fluorescent Protein (BFP) marker. The box indicates the sorted cell populations used in screens.

(F) Library representation. Upper panel: y-axis: Number of reads per pgRNA sequence; x-axis: ranked pgRNAs from library. Less than 0.5% of the total pgRNAs constructs dropped out with no reads. The ratio of 10th – 90th percentile is 4.6-fold. Lower panel: Lorenz curve, depicting library read coverage. Equality is represented by the diagonal.

(G) DNMBP-AS1 locus was targeted by CRISPR-deletion in A549 and H460 cells with multiple pgRNAs. The gene locus as well as the genotyping PCR primers (F, R) are shown in Figure 1E. Agarose gels showing template genomic DNA (gDNA) from cells transfected with non-targeting pgRNA (Control) or DNMBP-AS1 TSS pgRNAs (KO).

(H) Figure depicts pgRNAs targeting lncRNA candidates defined as hits (A549 dropout, FDR<0.2). y-axis: log2 FC (fold change) in abundance; x-axis: sum of individual sgRNA scores for each pair from RuleSet2 algorithm. Significance estimated using linear model.

(I) as for (H), but separating pgRNAs according to the orientation of their individual sgRNAs.
(J) as for (H), but now for the genomic distance (bp) between the sgRNAs of each pgRNA.

Figure S2

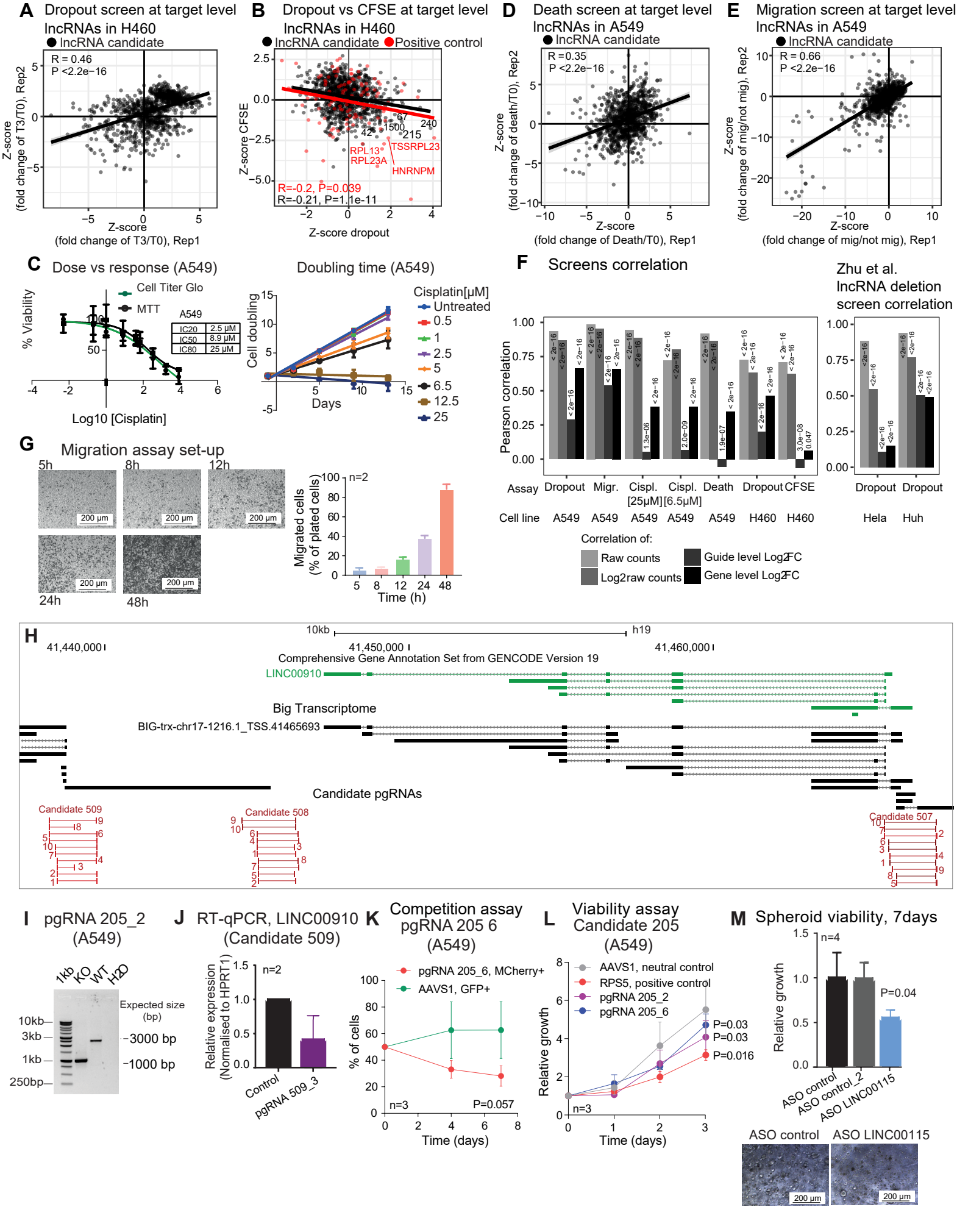
A
Dropout screen at target level
lncRNAs in H460
lncRNA candidate
R = 0.46
P <2.2e−16
Z-score (fold change of T3/T0), Rep2
Z-score (fold change of T3/T0), Rep1
B
Dropout vs CFSE at target level
lncRNAs in H460
lncRNA candidate
Positive control
Z-score CFSE
Z-score dropout
R=-0.2, P=0.039
R=-0.21, P=1.1e-11
RPL13*
RPL23A
TSSRPL23
HNRNPM
D
Death screen at target level
lncRNAs in A549
lncRNA candidate
R =0.35
P <2.2e−16
Z-score (fold change of death/T0), Rep2
Z-score (fold change of Death/T0), Rep1
E
Migration screen at target level
lncRNAs in A549
lncRNA candidate
R = 0.66
P <2.2e−16
Z-score (fold change of mig/not mig), Rep2
Z-score (fold change of mig/not mig), Rep1
C
Dose vs response (A549)
Cell Titer Glo
MTT
A549
IC20 2.5 µM
IC50 8.9 µM
IC80 25 µM
% Viability
Log10 [Cisplatin]
Doubling time (A549)
Cell doubling
Days
Cisplatin[µM]
Untreated
0.5
1
2.5
5
6.5
12.5
25
F
Screens correlation
Zhu et al.
lncRNA deletion
screen correlation
Pearson correlation
Assay Dropout Migr. Cispl. [25µM] Cispl. [6.5µM] Death Dropout CFSE
Cell line A549 A549 A549 A549 A549 H460 H460
Hela Huh
Correlation of:
Raw counts
Log2raw counts
Guide level Log2FC
Gene level Log2FC
G
Migration assay set-up
5h
8h
12h
24h
48h
200 µm
Migrated cells (% of plated cells)
n=2
Time (h)
H
10kb
h19
41,440,000
41,450,000
41,460,000
Comprehensive Gene Annotation Set from GENCODE Version 19
LINC00910
Big Transcriptome
BIG-trx-chr17-1216.1_TSS.41465693
Candidate pgRNAs
Candidate 509
Candidate 508
Candidate 507
I
pgRNA 205_2
(A549)
1kb
KO
WT
H2O
Expected size (bp)
10kb
3kb
1kb
250bp
3000 bp
1000 bp
J
RT-qPCR, LINC00910
(Candidate 509)
Relative expression (Normalised to HPRT1)
n=2
Control
pgRNA 509_3
K
Competition assay
pgRNA 205 6
(A549)
pgRNA 205_6, MCherry+
AAVS1, GFP+
% of cells
n=3
P=0.057
Time (days)
L
Viability assay
Candidate 205
(A549)
AAVS1, neutral control
RPS5, positive control
pgRNA 205_2
pgRNA 205_6
Relative growth
P=0.03
P=0.03
P=0.016
n=3
Time (days)
M
Spheroid viability, 7days
n=4
Relative growth
P=0.04
ASO control
ASO control_2
ASO LINC00115
ASO control
ASO LINC00115
200 µm

Figure S2. Assessing screen accuracy. Related to Figure 2.

(A) LncRNA candidates (black) correlation between biological replicates of the dropout screen in H460 cells. Data presented as Z-score transformed log2 FC values. T3: time point three weeks. T0: time point zero. Statistical significance: Pearson correlation.

(B) LncRNA candidates (black) correlation distinct from the positive controls (red) correlation in negative dropout and positive CFSE screens in H460 cells. Data presented as Z-score transformed log2 FC values. Statistical significance: Pearson correlation.

(C) (Left) Cisplatin dose-response curves in A549 and H460 cells. Error bars: standard deviation. (Right) Cell doublings calculated at indicated cisplatin concentrations. Error bars: standard deviation.

(D) LncRNA candidates (black) correlation between biological replicates of the death screen in A549 cells. Data presented as Z-score transformed log2 FC values. Statistical significance: Pearson correlation.

(E) LncRNA candidates (black) correlation between biological replicates of the migration screen in A549 cells. Data presented as Z-score transformed log2 FC values. Statistical significance: Pearson correlation.

(F) Replicates correlation is shown at three levels (raw reads: invariably display high correlations but provide little insight into replicability; pgRNA: typically display lower correlation and may be affected by stochastic effects when library coverage is low; gene levels: integrate the fold changes from the multiple individual pgRNAs. The latter is the most biologically-meaningful readout where technical noise may be overcome through sgRNA integration. (Left) Summary of the screens' correlation. (Right) Zhu et al. 2016[49] deletion screen correlation. Only lncRNA candidates are taken into account.

(G) Migration screen set-up. A549 cells (0.5M cells/well) were seeded onto transwell inserts and allowed to migrate for the indicated times. After crystal violet staining, cells that migrated through the membrane counted in five randomly selected fields. Results are expressed as means ± standard deviation (n=2).

(H) Genomic locus of Candidate 507, 508 and 509. i) Assaying genomic deletion with pgRNAs for Candidate 205: figure shows agarose gel electrophoresis of PCR product with primers (Supplementary File 5) amplifying the Candidate 205 target region.

(J) RT-qPCR with primers for Candidate 509 RNA (Supplementary File 5). Error bars: standard deviation.

(K) Competition assay with an additional pgRNA targeting Candidate 205. The plot shows the percentage of mCherry+ or GFP+ cells at indicated times (n=3 biological replicates; error bar: standard deviation; two-tailed Student's *t* test).

(I) Cell viability assay with the two pgRNAs targeting Candidate 205 (n=3 biological replicates; error bar: standard deviation; two-tailed Student's *t* test). The cell viability was measured by using the CellTiter-Glo® 2D.

(M) Cell viability in 3D spheroids grown from H441 cells upon ASO knockdown of *LINC00115*. The viability was measured with CellTiter-Glo® 3D, seven days after ASO transfection (n=4 biological replicates; error bars: standard deviation; statistical significance: one-tailed Student's *t* test).

Figure S3

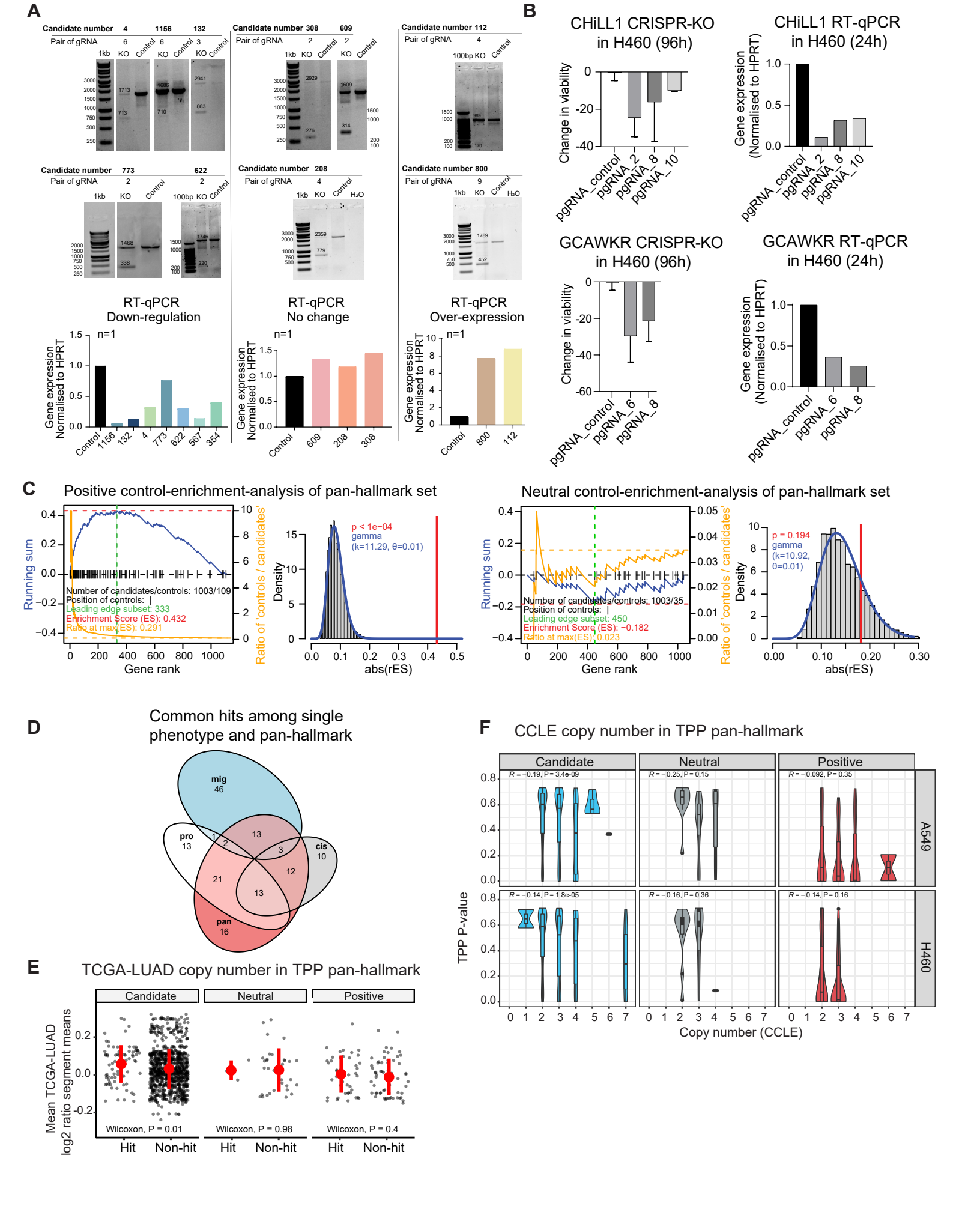
A
Candidate number 4 1156 132
Pair of gRNA 6 6 3
1kb KO Control KO Control KO Control
Candidate number 308 609
Pair of gRNA 2 2
1kb KO Control KO Control
Candidate number 112
Pair of gRNA 4
100bp KO Control
Candidate number 773 622
Pair of gRNA 2 2
1kb KO Control 100bp KO Control
Candidate number 208
Pair of gRNA 4
1kb KO Control H2O
Candidate number 800
Pair of gRNA 9
1kb KO Control H2O
RT-qPCR Down-regulation
RT-qPCR No change
RT-qPCR Over-expression
Gene expression Normalised to HPRT
n=1
Control 1156 132 4 773 622 567 354
Control 609 208 308
Control 800 112
B
CHiLL1 CRISPR-KO in H460 (96h)
Change in viability
pgRNA_control pgRNA_2 pgRNA_8 pgRNA_10
CHiLL1 RT-qPCR in H460 (24h)
Gene expression (Normalised to HPRT)
GCAWKR CRISPR-KO in H460 (96h)
pgRNA_control pgRNA_6 pgRNA_8
GCAWKR RT-qPCR in H460 (24h)
C
Positive control-enrichment-analysis of pan-hallmark set
Running sum
Ratio of 'controls / candidates'
Number of candidates/controls: 1003/109
Position of controls: |
Leading edge subset: 333
Enrichment Score (ES): 0.432
Ratio at max(ES): 0.291
Gene rank
Density
p < 1e−04
gamma
(k=11.29, θ=0.01)
abs(rES)
Neutral control-enrichment-analysis of pan-hallmark set
Number of candidates/controls: 1003/35
Position of controls: |
Leading edge subset: 450
Enrichment Score (ES): −0.182
Ratio at max(ES): 0.023
p = 0.194
gamma
(k=10.92,
θ=0.01)
D
Common hits among single phenotype and pan-hallmark
mig 46
pro 13
cis 10
pan 16
1
2
13
3
12
21
13
E
TCGA-LUAD copy number in TPP pan-hallmark
Candidate
Neutral
Positive
Mean TCGA-LUAD log2 ratio segment means
Wilcoxon, P = 0.01
Wilcoxon, P = 0.98
Wilcoxon, P = 0.4
Hit
Non-hit
F
CCLE copy number in TPP pan-hallmark
R = −0.19, P = 3.4e-09
R = −0.25, P = 0.15
R = −0.092, P = 0.35
R = −0.14, P = 1.8e-05
R = −0.16, P = 0.36
R = −0.14, P = 0.16
A549
H460
TPP P-value
Copy number (CCLE)

Figure S3. Validation of the method, TPP quality assessment and CNV analysis. Related to figure 3.

(A) Randomly picked pgRNAs show intended genomic deletion and expression changes in A549-Cas9-BFP. The genomic DNA (gDNA) and RNA were extracted 7 days after transfection. (Top) PCR products from the template genomic DNA (gDNA) of cells transfected with non-targeting pgRNA (Control) or targeting pgRNAs (KO). The expected lengths for Control, and deletion amplicons are indicated. (Bottom) RT-qPCR measurements of the selected genes. 7/12 downregulated genes (left), 3/12 genes with no change in expression (centre), 2/12 upregulated (right).

(B) (Left) Cell viability assay with the three pgRNAs targeting *CHiLL1*, and two pgRNAs targeting *GCAWKR* in H460-Cas9-BFP cells (n=2 biological replicates; error bar: standard deviation). Cells were transfected, and selected for 7 days with puromycin, then plated for the assay. The cell viability was measured by using the CellTiter-Glo® 2D. (Right) RT-qPCR measurements of the *CHiLL1* and *GCAWKR* expression upon CRISPR-KO with different pgRNAs. RNA extracted 24h after the start of the viability assay.

(C) (Left) Enrichment of positive controls in the top of the TPP ranking results by gene set enrichment analysis (GSEA). The running sum (blue) is calculated by iterating through the ranks, increasing with positive controls, decreasing otherwise. The maximum absolute value of the running sum is the Enrichment Score (ES). Black ticks on the x-axis represent the location of positive controls. The significance of the enrichment is nominally evaluated by simulating 10,000 perturbations of the labelling of the genes. (Right) The same analysis with neutral controls.

(D) The intersection of hits identified by Target Prioritisation Pipeline (TPP) in the indicated datasets. Pro: proliferation; Cis: cisplatin; Mig: migration; Pan: pan-hallmarks, integration of all the screens together.

(E) Copy number status in TCGA-LUAD samples for pan-hallmark lncRNA hits. For each candidate or control, the log2 (ratio segment means) of each TCGA-LUAD sample was retrieved for the library target region. Then, the log2 ratio segment means across TCGA-LUAD were averaged for each candidate or control. Red dots and lines display the mean and the standard deviation, respectively. Statistical significance: Wilcoxon test

(F) Relationship between pan-hallmark TPP scores (uncorrected P-values) (y-axis) and copy number status (x-axis) in A549 or H460. Copy number status of the library

target regions was retrieved from the Cancer Cell Line Encyclopedia (CCLE). Statistical significance: Pearson correlation.

Figure S4

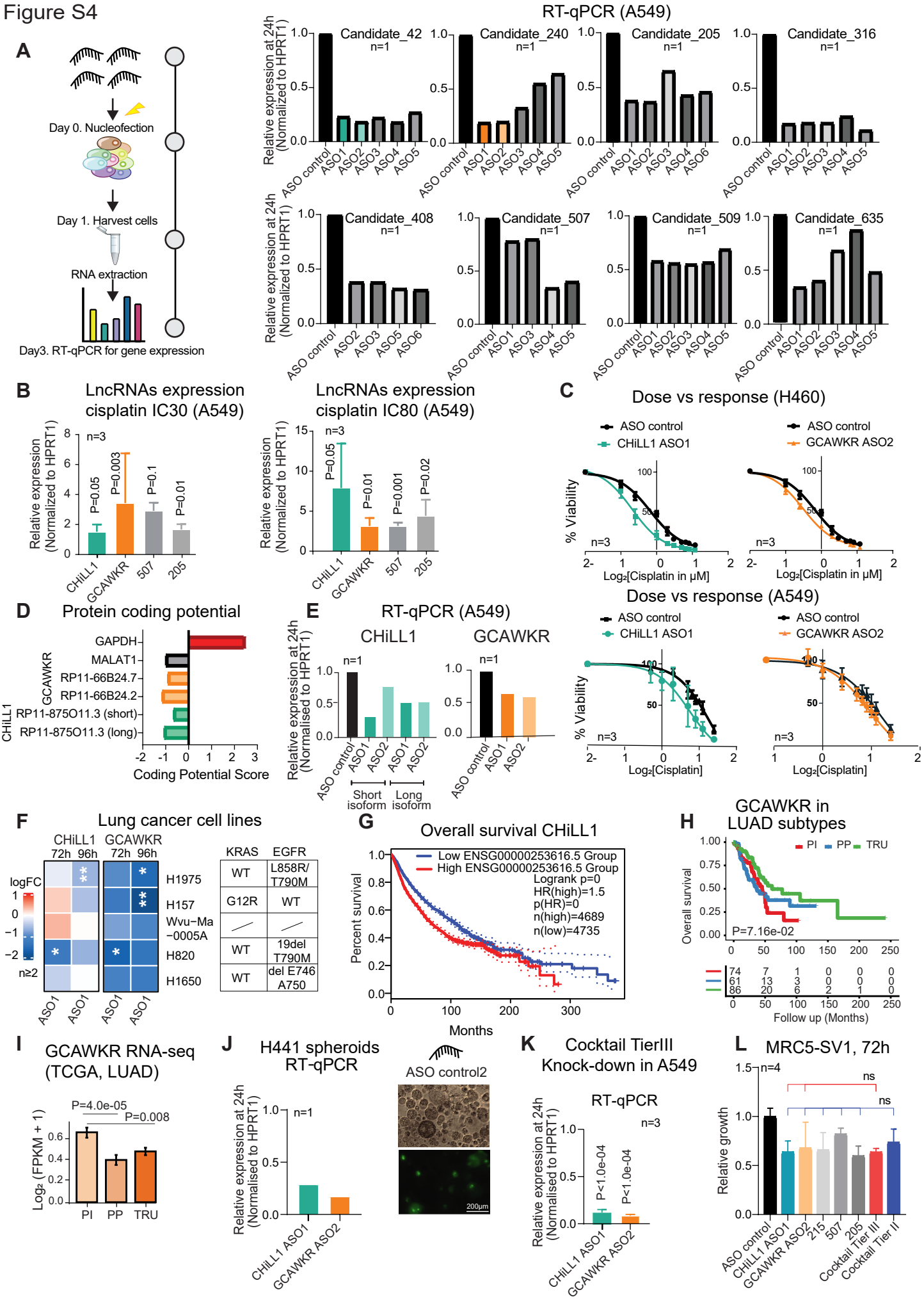
A
Day 0. Nucleofection
Day 1. Harvest cells
RNA extraction
Day3. RT-qPCR for gene expression
RT-qPCR (A549)
Relative expression at 24h (Normalized to HPRT1)
Candidate_42 n=1
Candidate_240 n=1
Candidate_205 n=1
Candidate_316 n=1
Candidate_408 n=1
Candidate_507 n=1
Candidate_509 n=1
Candidate_635 n=1
ASO control
ASO1
ASO2
ASO3
ASO4
ASO5
ASO6
B
LncRNAs expression cisplatin IC30 (A549)
LncRNAs expression cisplatin IC80 (A549)
Relative expression (Normalized to HPRT1)
n=3
P=0.05
P=0.003
P=0.1
P=0.01
P=0.05
P=0.01
P=0.001
P=0.02
CHiLL1
GCAWKR
507
205
C
Dose vs response (H460)
ASO control
CHiLL1 ASO1
GCAWKR ASO2
% Viability
Log2[Cisplatin in µM]
Dose vs response (A549)
Log2[Cisplatin]
D
Protein coding potential
GAPDH
MALAT1
RP11-66B24.7
RP11-66B24.2
RP11-875O11.3 (short)
RP11-875O11.3 (long)
GCAWKR
CHiLL1
Coding Potential Score
E
RT-qPCR (A549)
CHiLL1
GCAWKR
Short isoform
Long isoform
F
Lung cancer cell lines
CHiLL1 72h 96h
GCAWKR 72h 96h
logFC
n≥2
H1975
H157
Wvu−Ma −0005A
H820
H1650
KRAS
EGFR
WT
L858R/ T790M
G12R
19del T790M
del E746 A750
G
Overall survival CHiLL1
Low ENSG00000253616.5 Group
High ENSG00000253616.5 Group
Logrank p=0
HR(high)=1.5
p(HR)=0
n(high)=4689
n(low)=4735
Percent survival
Months
H
GCAWKR in LUAD subtypes
PI
PP
TRU
Overall survival
P=7.16e-02
Follow up (Months)
I
GCAWKR RNA-seq (TCGA, LUAD)
P=4.0e-05
P=0.008
Log2 (FPKM + 1)
J
H441 spheroids RT-qPCR
ASO control2
200µm
K
Cocktail TierIII Knock-down in A549
RT-qPCR
P<1.0e-04
P<1.0e-04
L
MRC5-SV1, 72h
Relative growth
ns
ns
Cocktail Tier III
Cocktail Tier II

Figure S4. Tier 2 candidates and cancer hallmarks. Related to Figure 4.

(A) (Left) Experimental strategy to test ASO knockdown effectiveness. (Right) Measured gene knockdown by RT-qPCR in response to ASOs.

(B) RT-qPCR of Tier 2 candidates in A549 cells treated with cisplatin IC30 (left) and IC80 (right panel) for 72 h. The control represents the reference expression of the genes in untreated conditions. (n=3, error bars: standard deviation; statistical significance: one-tailed Student's *t* test).

(C) Dose response curves in H460 (top) and A549 (bottom) in cells transfected with ASO control and the best performing ASOs for *CHiLL1* (left) and *GCAWKR* (right). Error bars: standard deviation.

(D) Coding Potential Scores (CPS) of *CHiLL1* and *GCAWKR* transcripts using the algorithm Coding Potential Calculator[120]. *GAPDH* and *MALAT1* transcripts were used as reference for the protein-coding and non-protein coding, respectively.

(E) *CHiLL1* and *GCAWKR* knockdown efficiency measured by RT-qPCR, using two independent ASOs.

(F) The effect of *CHiLL1* and *GCAWKR* ASOs in additional NSCLC cell lines. Mutational status is indicated on the right. Columns: time points for the cell viability measurements. Values reflect the mean log2 FC in viability following ASO transfection, with respect to a control non-targeting ASO, from at least two independent biological replicates. One-tailed Student's *t* test. * indicate P< 0.05; ** indicate P<0.01.

(G) Kaplan–Meier survival analysis of *CHiLL1*. Analysis was performed using GEPIA2 tool with default settings[121], including all the tumours types available in the TCGA dataset.

(H) Kaplan–Meier survival analysis of *GCAWKR* in TCGA data within transcriptional subtypes. Proximal-inflammatory (PI), proximal-proliferative (PP) and terminal respiratory unit (TRU).

(I) *GCAWKR* expression in transcriptional sub-types in TCGA dataset (n=513, Wilcoxon signed-rank tests). Proximal-inflammatory (PI), proximal-proliferative (PP) and terminal respiratory unit (TRU).

(J) Knockdown efficiency measured by RT-qPCR in 3D spheroids with *CHiLL1* and *GCAWKR* ASOs relative to ASO control. Error bars: standard deviation. On the right: representative pictures of spheroids transfected with green fluorescent-labelled ASOs.

(K) Knockdown efficiency of *CHiLL1* and *GCAWKR* measured in the cocktail experiment in A549 cells with *CHiLL1* and *GCAWKR* ASOs relative to ASO control

(n=3 biological replicates; error bars: standard deviation; statistical significance: two-tailed Student's *t* test).

(L) Growth of MRC5-SV1 cells in response to indicate ASO transfections (n=4 biological replicates; error bars: standard deviation; statistical significance: one-tailed Student's *t* test).

Figure S5

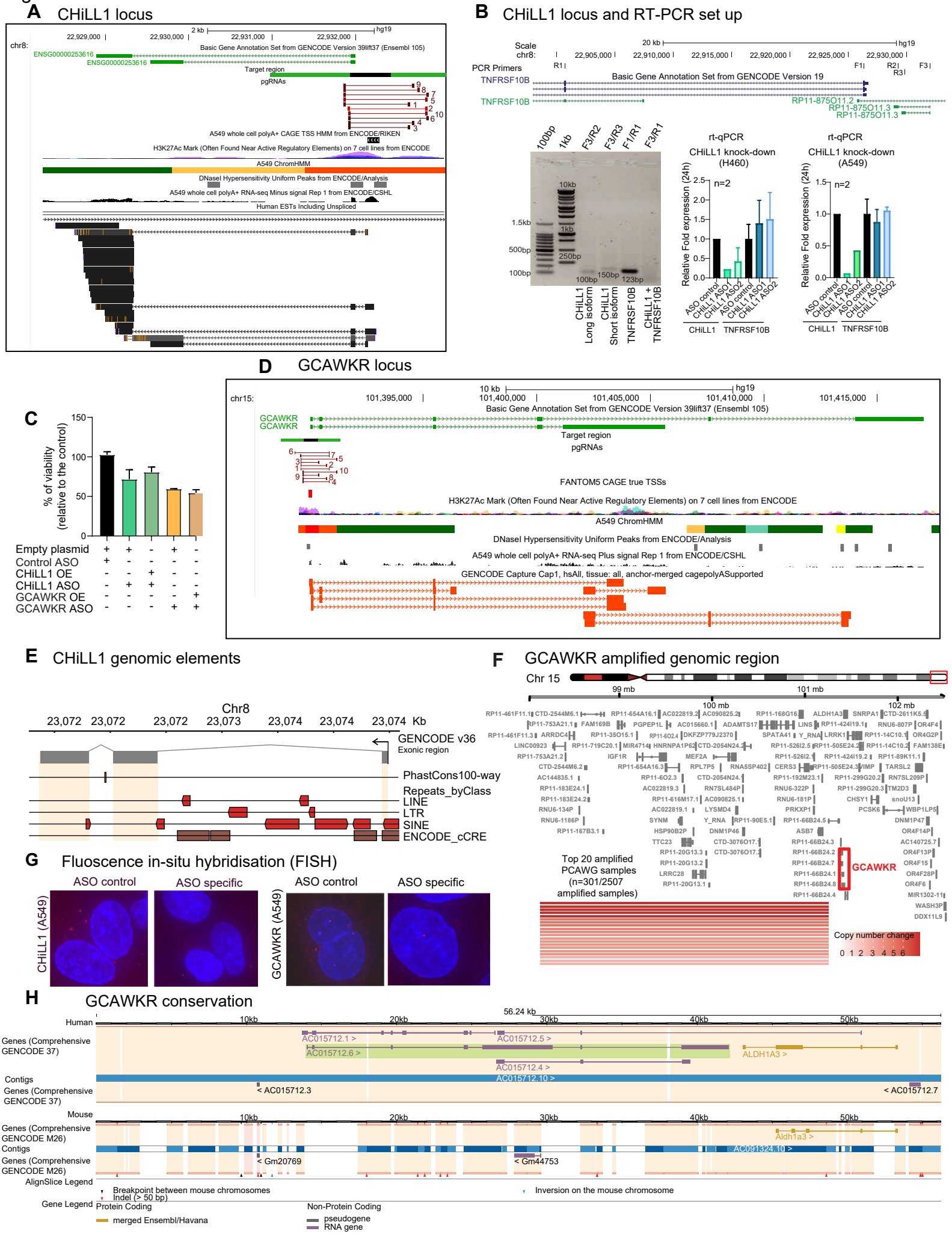
A CHiLL1 locus
B CHiLL1 locus and RT-PCR set up
C
D GCAWKR locus
E CHiLL1 genomic elements
F GCAWKR amplified genomic region
G Fluoscence in-situ hybridisation (FISH)
H GCAWKR conservation

Figure S5. Further information on CHiLL1 and GCAWKR. Related to Figure 5.

(A) Tracks to support for CHiLL1 gene structure, and the lack of evidence for read through transcription between CHiLL1 and TNFRSF10B, from expressed sequence tags (ESTs).

(B) Searching for evidence of read through transcription between *CHiLL1* and *TNFRSF10B. (*Left) Agarose gel electrophoresis of RT-qPCR products using indicated primers with A549 cDNA. *CHiLL1* + *TNFRSF10B* indicates a PCR performed with the forward primer (F3 in the figure) in the lncRNA exon1 and reverse primer (R1) in the second exon of TNFRSF10B. (Right) Expression of RNA from CHiLL1 locus, and primers used.

(C) Rescue experiment. Cell viability after transfection with CHiLL1 transgene + *ChiLL1* ASO, and GCAWKR + GCAWKR ASO. n=3.

(D) GCAWKR genomic locus. The GCAWKR annotated TSS is supported by multiple evidence, including FANTOM CAGE, and it is shown the annotation of the full-length transcript with Capture Long-Read Sequencing[122].

(E) Genomic elements in the CHiLL1 locus. GENCODE v36 exons were merged into a single transcript and queried against a set of genome-wide element annotations using ezTracks[94].

(F) Recurrently amplified genomic region that encompasses GCAWKR according to Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium. Ensembl 75 (hg19) annotation was used. For each gene, the longest transcript is shown. GCAWKR locus is highlighted. The copy number gain for the top 20 amplified samples in PCAWG is shown.

(G) Representative confocal microscopy images of RNA-FISH performed with *CHiLL1*, *GCAWKR* probe sets upon treatment with targeting ASOs or non-targeting ASO control in A549 cells. Selected lncRNA foci in the treated sample and in the control are arrowed.

(H) GCAWKR orthology between human annotation (GENCODE 37) and mouse (GENCODE M26).

Figure S6

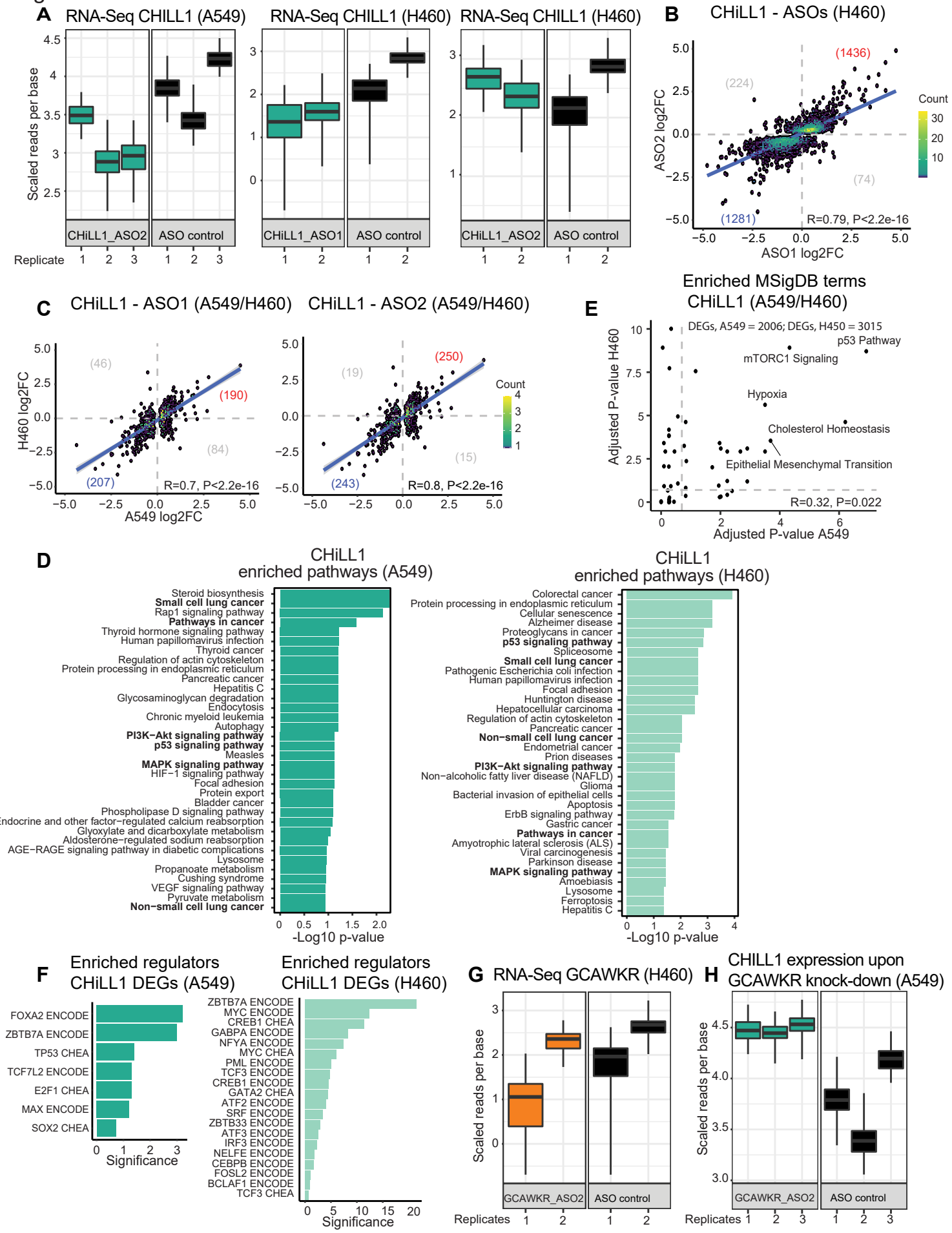
A
RNA-Seq CHILL1 (A549)
RNA-Seq CHILL1 (H460)
RNA-Seq CHILL1 (H460)
Scaled reads per base
CHiLL1_ASO2
ASO control
CHiLL1_ASO1
ASO control
CHiLL1_ASO2
ASO control
Replicate
B
CHiLL1 - ASOs (H460)
(1436)
(224)
(74)
(1281)
R=0.79, P<2.2e-16
ASO2 log2FC
ASO1 log2FC
Count
C
CHiLL1 - ASO1 (A549/H460)
CHiLL1 - ASO2 (A549/H460)
(46)
(190)
(84)
(207)
R=0.7, P<2.2e-16
(19)
(250)
(15)
(243)
R=0.8, P<2.2e-16
H460 log2FC
A549 log2FC
Count
E
Enriched MSigDB terms
CHiLL1 (A549/H460)
DEGs, A549 = 2006; DEGs, H450 = 3015
p53 Pathway
mTORC1 Signaling
Hypoxia
Cholesterol Homeostasis
Epithelial Mesenchymal Transition
R=0.32, P=0.022
Adjusted P-value H460
Adjusted P-value A549
D
CHiLL1
enriched pathways (A549)
Steroid biosynthesis
Small cell lung cancer
Rap1 signaling pathway
Pathways in cancer
Thyroid hormone signaling pathway
Human papillomavirus infection
Thyroid cancer
Regulation of actin cytoskeleton
Protein processing in endoplasmic reticulum
Pancreatic cancer
Hepatitis C
Glycosaminoglycan degradation
Endocytosis
Chronic myeloid leukemia
Autophagy
PI3K−Akt signaling pathway
p53 signaling pathway
Measles
MAPK signaling pathway
HIF−1 signaling pathway
Focal adhesion
Protein export
Bladder cancer
Phospholipase D signaling pathway
Endocrine and other factor−regulated calcium reabsorption
Glyoxylate and dicarboxylate metabolism
Aldosterone−regulated sodium reabsorption
AGE−RAGE signaling pathway in diabetic complications
Lysosome
Propanoate metabolism
Cushing syndrome
VEGF signaling pathway
Pyruvate metabolism
Non−small cell lung cancer
-Log10 p-value
CHiLL1
enriched pathways (H460)
Colorectal cancer
Protein processing in endoplasmic reticulum
Cellular senescence
Alzheimer disease
Proteoglycans in cancer
p53 signaling pathway
Spliceosome
Small cell lung cancer
Pathogenic Escherichia coli infection
Human papillomavirus infection
Focal adhesion
Huntington disease
Hepatocellular carcinoma
Regulation of actin cytoskeleton
Pancreatic cancer
Non−small cell lung cancer
Endometrial cancer
Prion diseases
PI3K−Akt signaling pathway
Non−alcoholic fatty liver disease (NAFLD)
Glioma
Bacterial invasion of epithelial cells
Apoptosis
ErbB signaling pathway
Gastric cancer
Pathways in cancer
Amyotrophic lateral sclerosis (ALS)
Viral carcinogenesis
Parkinson disease
MAPK signaling pathway
Amoebiasis
Lysosome
Ferroptosis
Hepatitis C
-Log10 p-value
F
Enriched regulators
CHiLL1 DEGs (A549)
FOXA2 ENCODE
ZBTB7A ENCODE
TP53 CHEA
TCF7L2 ENCODE
E2F1 CHEA
MAX ENCODE
SOX2 CHEA
Significance
Enriched regulators
CHiLL1 DEGs (H460)
ZBTB7A ENCODE
MYC ENCODE
CREB1 CHEA
GABPA ENCODE
NFYA ENCODE
MYC CHEA
PML ENCODE
TCF3 ENCODE
CREB1 ENCODE
GATA2 CHEA
ATF2 ENCODE
SRF ENCODE
ZBTB33 ENCODE
ATF3 ENCODE
IRF3 ENCODE
NELFE ENCODE
CEBPB ENCODE
FOSL2 ENCODE
BCLAF1 ENCODE
TCF3 CHEA
Significance
G
RNA-Seq GCAWKR (H460)
Scaled reads per base
GCAWKR_ASO2
ASO control
Replicates
H
CHILL1 expression upon
GCAWKR knock-down (A549)
Scaled reads per base
GCAWKR_ASO2
ASO control
Replicates

Figure S6. CHiLL1 and GCAWKR perturbation impacts disease transcriptome. Related to Figure 6. (A) Response of *CHiLL1* expression to ASO transfection, as measured by RNA-seq. The y-axis represents the normalised expression (counts) per nucleotide and the boxplots show the variance of inference using bootstraps from Kallisto.

(B) Changes in gene expression (log2 FC) with two different *CHiLL1* ASOs in H460. Numbers indicate the differentially expressed genes in each part of the scatter plot. Statistical significance: Pearson correlation.

(C) as for (B), but comparing effects of the same *CHiLL1* ASO in two cell backgrounds (left: ASO1; right: ASO2).

(D) KEGG pathways enriched for *CHiLL1* target genes, for A549 (left) and H460 (right) cells. Analysis was performed using common differentially expressed genes between the ASO1 and ASO2 knockdown.

(E) Gene ontology analysis performed using the Molecular Signatures Database (MSigDB). Shown are enriched terms between A549 and H460 cells by using the set of common differentially-expressed genes of ASO1&2 for *CHiLL1*. Statistical significance: Pearson correlation.

(F) Binding sites[115,123] enriched in the intersection of differentially expressed genes (DEGs) from ASO1&2 knockdown of *CHiLL1*.

(G) Expression of *GCAWKR* in H460 in response to *GCAWKR* ASOs. The y-axis represents the normalised expression (counts) per nucleotide and the boxplots show the variance of inference using the bootstraps of Kallisto.

(H) Expression of CHiLL1 (RNA-seq) upon ASO knockdown of *GCAWKR*. The y-axis represents the normalised expression (counts) per nucleotide and the boxplots show the variance of inference using the bootstraps of Kallisto.

Data S4. Related to Star Methods.

CRISPETA runs. Related to Methods (Library design).

	Run1	Run2	Run3		Run4	Run5	Run6	Run7	Run8
-eu	0								
-ed	0								
-du	1000		1200						
-dd	1000		1200						
-si	0.2								0
-t	0,0,0,x,x	0,0,1,x,x			0,0,5,x,x	0,1,5,x,x	0,x,x,x,x	1,x,x,x,x	1,x,x,x,x

PCR reaction ssDNA to dsDNA. Related to Methods (Library cloning).

Component	Amount per reaction
5x Buffer	20 ul
dNTPs 10 mM	3 ul
Forward oligo 100uM	2 ul
Reverse oligo 100 uM	2 ul
Template DNA 10ng/ul	2 ul
Phusion Polymerase High Fidelity 2U/ul	2ul
UltraPure water	Up to 100 ul

pDECKO_backbone plasmid digestion. Related to Methods (Library cloning).

Component	Amount per reaction
pDECKO plasmid	5 ug
Tango 10x Buffer	5 ul
DTT (20mM)	2.5 ul
Esp3I (BsmBI)	1 ul
UltraPure H2O	Up to 50 ul

Long oligos to generate pDECKO constant part (HPLC purification). Related to Methods (Library cloning).

Oligos	Sequences (5'-3')
Forward_1 (F1) 125 bp	TAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAA AAAGTGGCACCGAGTCGGTGCTTTTTTGAACGCTGACGTCATCA ACCCGCTCCAAGGAATCGCGGGCCCAGTGTCACTAG
Forward_2 (F2) 148 bp	GCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCT GTGAGGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCT ATGTGTTCTGGGAAATCACCATAAACGTGAAATGTCTTTGGATTT GGGAGTCTTATAAGTT
Reverse_1 (R1) 146 bp	GCGCACGCGCGCTGGGTGTTCCCGCCTAGTGACACTGGGCCCG CGATTCCTTGGAGCGGGTTGATGACGTCAGCGTTCAAAAAAGCA CCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTAT TTTAACTTGCTATT
Reverse_2 (R2) 127 bp	ACAGAACTTATAAGACTCCCAAATCCAAAGACATTTCACGTTTATG GTGATTTCCCAGAACACATAGCGACATGCAAATATTGCAGGGCG CCACTCCCCTGTCCCTCACAGCCATCTTCCTGCCAGG

Colony PCR primers. Related to Methods (Library cloning).

Primer	Sequences (5'-3')
Oligo_colony_Fw	GTACAAAATACGTGACGTAG
Oligo_colony_Rv	ATGTCTACTATTCTTTCCCC

Colony PCR reaction. Related to Methods (Library cloning).

95ºC 2', 95ºC 30'', 60ºC 40'', 72ºC 2' for 29 cycles, 72ºC 2', 4ºC forever	
Component	Amount per reaction
5x Buffer	10 ul
dNTPs 10 mM	1 ul
Oligo_colony_Fw 10 uM	1 ul
Oligo_colony_Rv 10 uM	1 ul
Template colony	2 ul
Go Taq G2 polimerase (Promega)	0.25 ul
Nuclease-free water	Up to 50 ul

Primers for the genomic deletion. Related to Methods (PCR amplification from genomic DNA).

gDNA_Candidate_331_For	TTGCAACCCCCAAACAGACT
gDNA_Candidate_331_Rev	GGGGCACCATTTTGGACCTA
gDNA_Candidate_205_For	AGCCTGTCACAAACTGATTCTTA
gDNA_Candidate_205_Rev	TTGTTGACCCGGAAACGGAT

Composition of lung cancer organoids medium. Related to Methods (Patient-derived xenograft organoids)

Substances	Final Conc.	Supplier	Catalogue number
DMEM:F-12 HAM medium	-	Sigma-Aldrich	D6421
EGF	50 ng/mL	Thermo Fisher Scientific	PHG6045
FGF	20 ng/mL	Thermo Fisher Scientific	PHG6015
L-Glutamine	2 mM	Sigma-Aldrich	G7513-100mL
OmniPur HEPES	10 mM	Sigma-Aldrich	5320-500GM
Penicillin/Streptomycin	100 U/mL	Sigma-Aldrich	P3032-10MU S6501-25G
N-2 supplement	1x	Thermo Fisher Scientific	17502-048
B-27 supplement	1x	Thermo Fisher Scientific	17504-044
Noggin	100 ng/mL	Prospec	CYT-475
ROCK-inhibitor (Y-27632)	10 µM	Stemcell	72304

Primers RT-qPCR. Related to Methods (RNA isolation and RT–qPCR).

HPRT1_For	ATGACCAGTCAACAGGGGACAT
HPRT1_Rev	CAACACTTCGTGGGGTCCTTTTCA
Candidate_331_For	CAGGGAGCAGGGACTATCAA
Candidate_331_Rev	TGGTCTTCCAACATGGGCTTG
Candidate_205_For (LINC00115)	CCTAGTTCTCTTCACCGTCCG
Candidate_205_Rev (LINC00115)	AAGACAAGCCACATGCCGAA
Candidate_205_For (LINC01128)	AGAGGTTAAAAGTCACAAGGGTGT
Candidate_205_Rev (LINC01128)	GCCTTGACAGCAAGCCTAGA
Candidate_42_For (ENST00000520840.2)	GCAGTGACCCAGAATGAGGAAG
Candidate_42_Rev (ENST00000520840.2)	TACTGAAATTGGAGGCTGTGGA
Candidate_42_For (ENST00000523806.1)	GCAGTGACCCAGAATGAGGT
Candidate_42_Rev (ENST00000523806.1)	GCTCTAGCTTCCAGGTTGGG
Candidate_240_For	CTCACGGCAGCTATGAGACT
Candidate_240_Rev	GCTCCAAGATGCCACTCACA
Candidate_448_For	GAAACCTCCTCGACACTCCG
Candidate_448_Rev	AGTCTTCGAACAGGCTGCAA
Candidate_215_For	GCAATTGTACCTGAGGACCCA
Candidate_215_Rev	TGGCATATGGTGGATGTTCCC
Candidate_489_For	AAGCGCTCATTCAAGGTTGC
Candidate_489_Rev	GGTTCAGTCTGGGCCCTTTT
Candidate_408_For	GCGATGGAAGAAGTTTCGCC
Candidate_408_Rev	GGAACTCAGGTAACAGGAATTTCAC
Candidate_316_For	GACCAACTCCGTTTCCCGAT

Candidate_316_Rev	TCAAGGGCCCAGCCTTATTC
Candidate_635_For	AATTCCACCCACGCACCTAT
Candidate_635_Rev	GAGCCACCGTTAATTCAGCC
Candidate_507_For	TCCTTGCTAACCACACGGAC
Candidate_507_Rev	ATGTGGGTCCCAGTATCCGA
Candidate_509_For	TTGGCACACTCAGATGCGAT
Candidate_509_Rev	AAAACAGTCCCGCTTGGGAT
GAPDH_For	GCACCGTCAAGGCTGAGAAC
GAPDH_Rev	TGGTGAAGACGCCAGTGGA
MALAT1_For	GATTGAGGCGTTTTCCAAGA
MALAT1_Rev	ACTTTCTCCCCCAACTGCTT

pgRNA guides for CRISPR deletion. Related to Methods (DECKO and lentiviral production).

Gene Target	**Candidate**	**sgRNA1**	**sgRNA2**
DNMBP-AS1	331_1	GTAATATTCAGGAAGGGCAA	GCTCTAAACAAAACTAGAGA
DNMBP-AS1	331_2	GCTGGTTTCAGGGAGATCAG	GCTCTAAACAAAACTAGAGA
DNMBP-AS1	331_3	TGATCTCCCTGAAACCAGCT	GCTCTAAACAAAACTAGAGA
DNMBP-AS1	331_4	ACACTTTGATTACAGTTGGG	GCTCTAAACAAAACTAGAGA
DNMBP-AS1	331_5	GTAATATTCAGGAAGGGCAA	GAAAACTATGAAACTAGAGA
DNMBP-AS1	331_6	GCTGGTTTCAGGGAGATCAG	GAAAACTATGAAACTAGAGA
DNMBP-AS1	331_7	GTAATATTCAGGAAGGGCAA	GCTAAAGGTTCAGGGTTGGA

DNMBP-AS1	331_9	GTAATATTCAGGAAGGGCAA	GCACTTGCTAATAACATACA
DNMBP-AS1	331_10	GCTGGTTTCAGGGAGATCAG	GCTAAAGGTTCAGGGTTGGA
LINC01128; LINC00115	205_2	CCTCCTCTATATGAACTCCA	GCGGACGGTGAAGAGAACTA
LINC01128; LINC00115	205_2	CCTCCTCTATATGAACTCCA	GCAAACCCCACGCTTCGGGG
CHiLL1	42_2	GAGACATTAGTGGACATAGA	GGATGGGTGAGCCAACCTCA
CHiLL1	42_8	ATAGTAAGTCAAAGTAATGG	GAGCAAATTCTCCCTTGGGAA
CHiLL1	42_10	ATTGTTTGCCCAATGCAGAG	GCTTCTGCTGCCTTTCCCAA
GCAWKR	240_6	GTACCACAGACACTACAGTT	TCTGAGCACAGTGATTCCGG
GCAWKR	240_8	TGCACTTTCCCAGATAACAC	GCTAGTAAACCCACAGCGGAC
GCAWKR	240_9	TCGAATGTTCCATTTCCTCA	GCTAGTAAACCCACAGCGGAC
GCAWKR	240_10	TTTGTTAGTTCCCTTTTAGC	GCTAGTAAACCCACAGCGGAC
lncRNA not annotated in GENCODE	509_3	ACAGGCGCTTTTCTGTTCAG	GTTACTTCTACGCGTTCACGT
AASV1	targeting control	ACTCTTTAAGGAAAGAAGGA	CCTCAGTCTGAAGAGCAGAGC