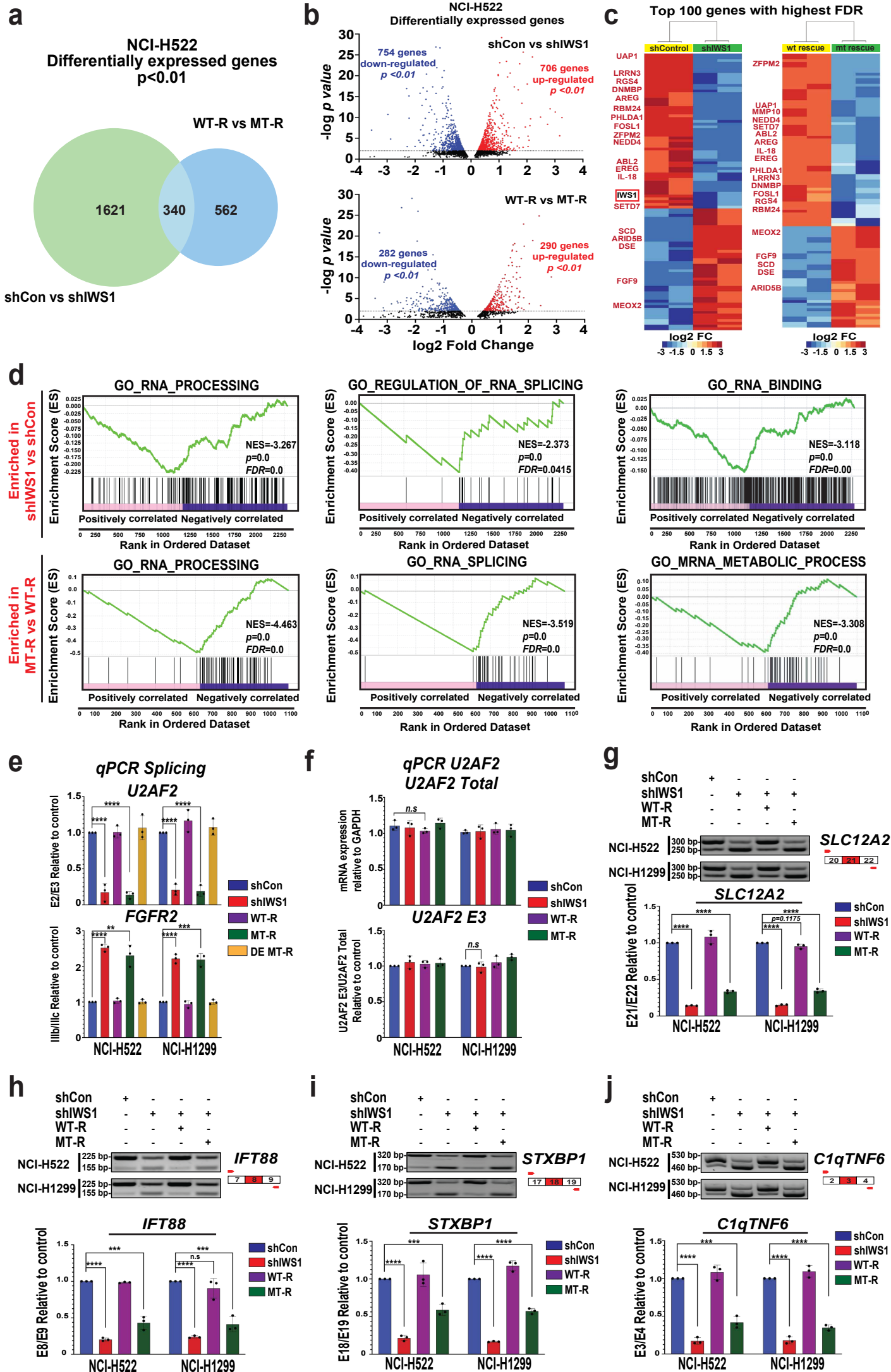


AKT3-mediated IWS1 phosphorylation promotes proliferation of EGFR-mutant lung adenocarcinomas through cell cycle-regulated *U2AF2* RNA splicing.

Laliotis et al.,

- **Supplementary Figures**
- **Supplementary Tables**



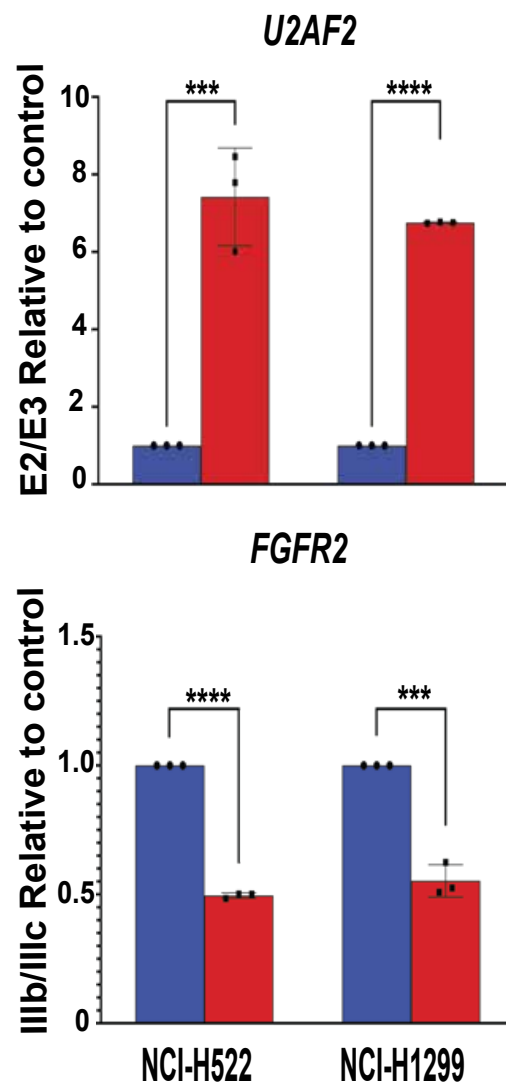
Supplementary Figure 1 (relative to Figure 1). IWS1 expression and/or phosphorylation regulate alternative mRNA splicing

- a. Venn diagram shows the genes that are differentially expressed in shControl versus shIWS1 or shIWS1/WT-R versus shIWS1/MT-R NCI-H522 cells ($q < 0.01$) and the overlap between the two sets.
- b. Volcano plots of all the differentially expressed genes in the indicated groups. Genes upregulated or downregulated with a p value < 0.01 are shown in red and blue, respectively.
- c. Heatmaps of the top 100 differentially expressed genes (lowest FDR) in shIWS1 relative to shControl and in shIWS1/MT-R relative to shIWS1/WT-R NCI-H522 cells. Genes present in both sets are shown in red. The abundance of IWS1 is shown in a red box.
- d. Gene Set Enrichment Analysis (GSEA) of genes differentially expressed in the indicated groups (NES—normalized enrichment score, FDR—false discovery ratio)
- e. Quantitative RT-PCR showing the *E2/E3* and *E8/E9* ratio in the *U2AF2* (upper panel) and *FGFR2* (lower panel) mRNA transcripts in shControl, shIWS1, shIWS1/WT-R, shIWS1/MT-R and shIWS1/DE MT-R NCI-H522 and NCI-H1299 cells. Bars show the *U2AF2 E2/E3* and the *FGFR2 E8/E9* ratio (mean $\pm$ SD) in all the cell lines, normalized relative to the shControl.
- f. (Upper panel) Quantitative RT-PCR, showing the abundance of the total *U2AF2* mRNA, relative to GAPDH ($\pm$ SD) in shControl, shIWS1, shIWS1/WT-R and shIWS1/MT-R NCI-H522 and NCI-H1299 cells. (Lower panel) Quantitative RT-PCR, showing the abundance of *U2AF2* exon 3, relative to the total *U2AF2* RNA ($\pm$ SD) in the same cells.
- g. h. i and i. IWS1 expression and phosphorylation regulate the alternative RNA splicing of *SLC12A2* (exon 21), *IFT88* (exon 8), *STXBP1* (exon 18) and *C1qTNF6* (exon 3). In all cases, IWS1 promotes exon inclusion. Upper panels show the electrophoresed products of the corresponding RT-PCR reactions. These products were quantified and the ratios of the alternatively spliced to non-alternatively spliced adjacent exons were normalized relative to

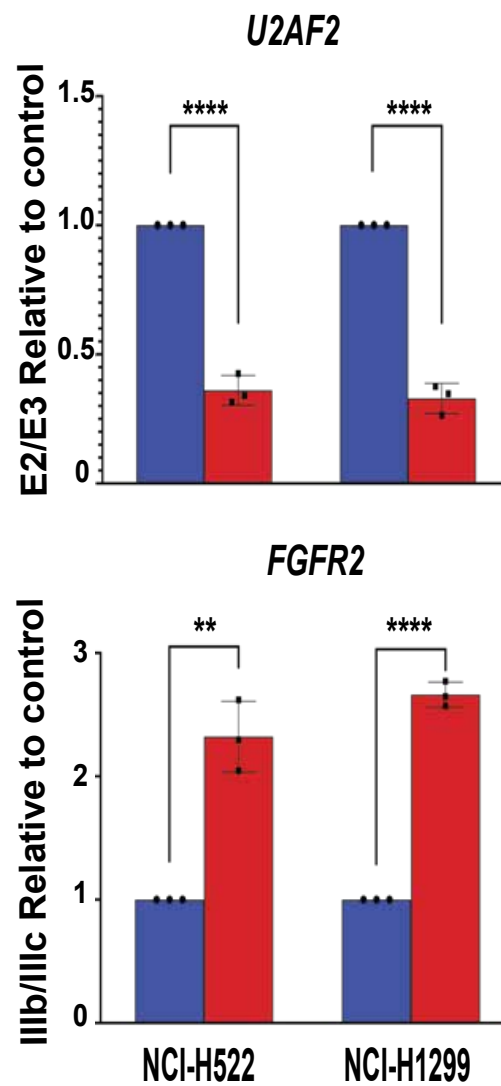
the ratio in the shControl cells. Bars show the relative exon ratios (mean $\pm$ SD) in shControl, shIWS1, shIWS1/WT-R and shIWS1/MT-R NCI-H522 and NCI-H1299 cells. Assays were done in triplicate, on three biological replicates. n.s : non-significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (one-side unpaired t-test)

a*qPCR Splicing*

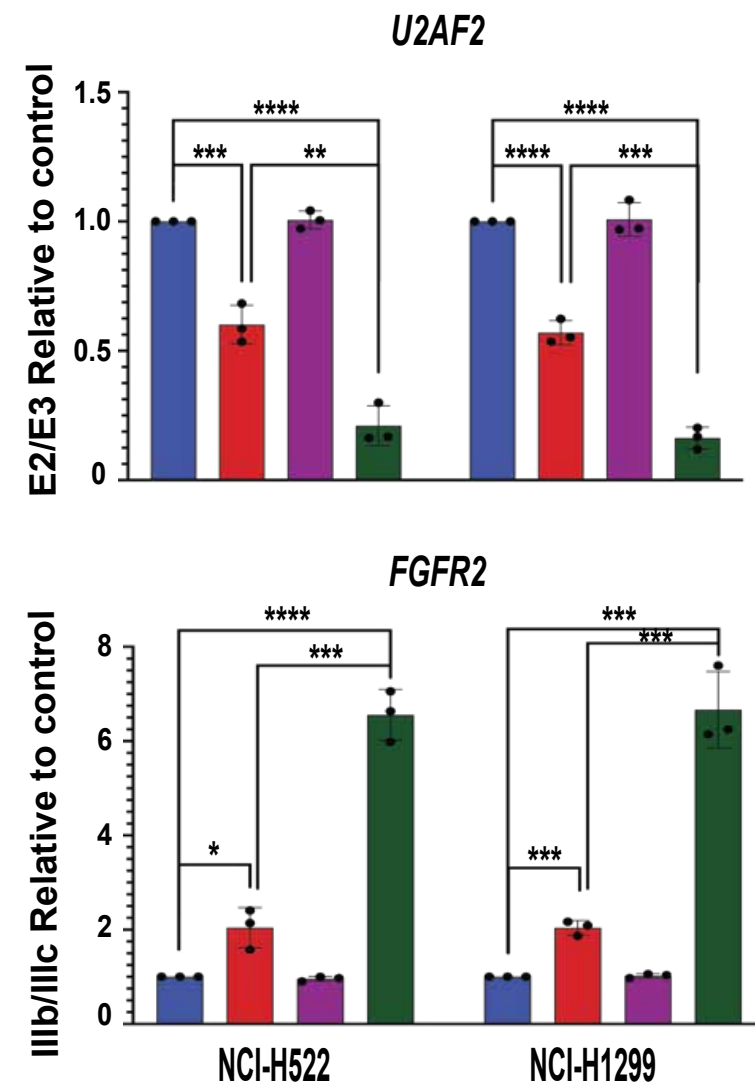
starved IGF-1

**b***qPCR Splicing*

DMSO MK2206

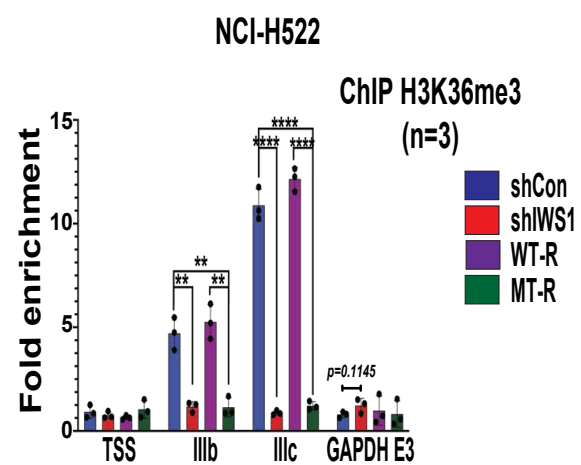
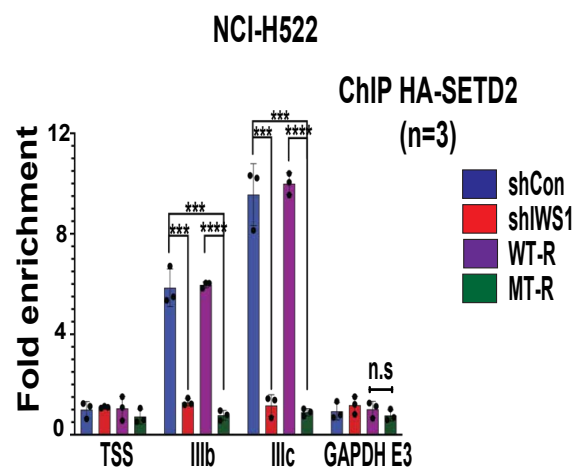
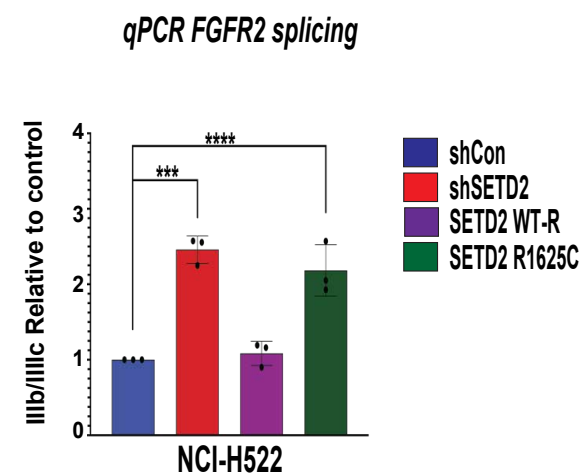
**c***qPCR Splicing*

shCon shAKT1 shAKT2 shAKT3



Supplementary Figure 2 (relative to Figure 2). IWS1 phosphorylation-dependent mRNA splicing of U2AF2 is regulated by serum and IGF-1-induced signals transduced by AKT3

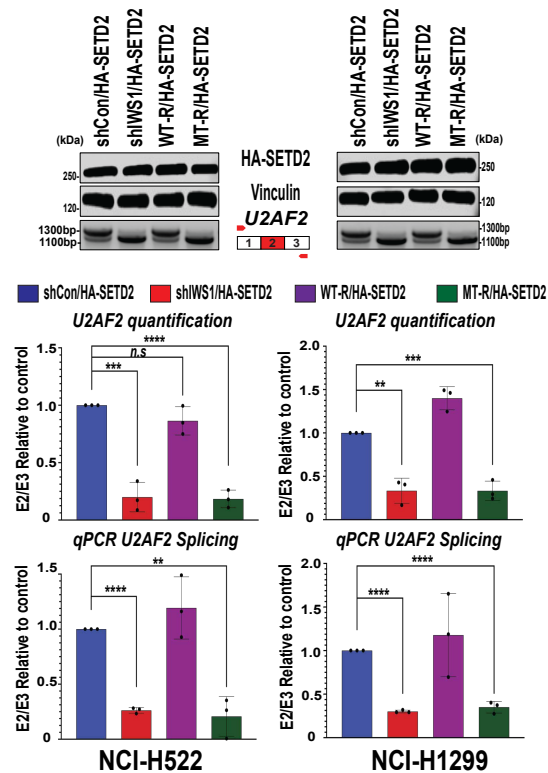
a. b. and c. (Upper panels) The *U2AF2* mRNA *E2/E3* ratio in the cells in figure 2 was measured by quantitative RT-PCR. Bars show the *E2/E3* ratio in IGF-1, MK2206 and shAKT1, shAKT2 and shAKT3 cells relative to the control cells. (Lower panel) The *FGFR2* mRNA *E8/E9* ratio in the same cells was measured, also by quantitative PCR. Bars show the *E8/E9* ratio in IGF-1, MK2206 and shAKT1, shAKT2 and shAKT3 cells relative to the control cells $\pm$ SD. All experiments in this figure were done on three biological replicates, in triplicate. n.s: non-significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (one-side unpaired t-test).

a**b****c**

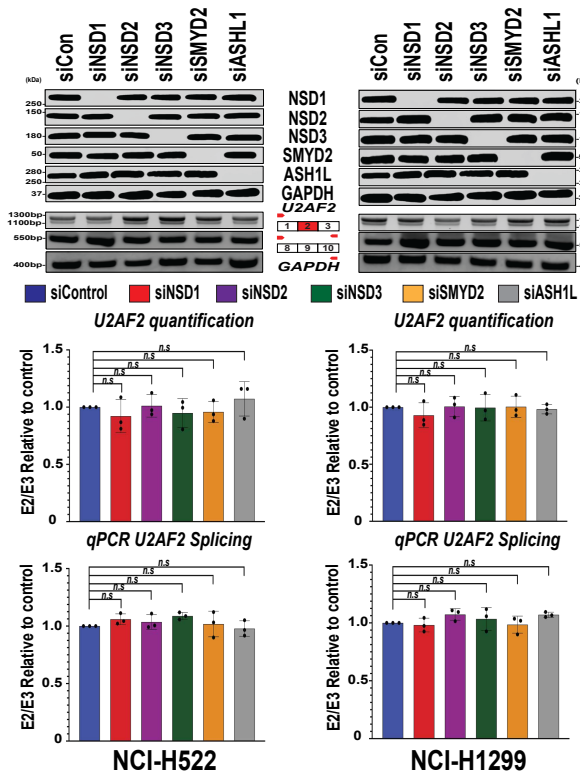
Supplementary Figure 3 (relative to Figure 3). *FGFR2* Exon 8 skipping, induced by IWS1 phosphorylation at Ser720/Thr721, depends on H3K36 trimethylation by SETD2.

- a. ChIP assays showing the abundance of H3K36me3 on the *FGFR2* and *GAPDH* genes in shControl, shIWS1 shIWS1/WT-R and shIWS1/MT-R NCI-H522 cells. Bars show the mean fold enrichment in H3K36me3 (anti-H3K36me3 IP, vs IgG control IP) in the indicated regions of the *FGFR2* gene, in shControl, shIWS1, shIWS1/WT-R and shIWS1/MT-R cells $\pm$ SD. Data were normalized relative to the input (2%).
- b. ChIP assays showing the binding of HA-SETD2 to the indicated regions of the *FGFR2* and *GAPDH* genes, in shControl, shIWS1 shIWS1/WT-R and shIWS1/MT-R NCI-H522, transduced with a lentiviral HA-SETD2 construct. The bars show the mean fold enrichment in SETD2 binding (anti-HA IP, vs IgG control IP) in the indicated regions of the *FGFR2* gene, in shControl, shIWS1, shIWS1/WT-R and shIWS1/MT-R cells $\pm$ SD. Data were normalized relative to the input (2%). The expression of HA-SETD2 is presented in Supplementary Fig. 4a.
- c. *The IWS1-regulated alternative RNA splicing of FGFR2, depends on enzymatically active SETD2.* The ratio of the *FGFR2* exons 8 and 9 was determined by quantitative RT-PCR, using the mRNA isolated from the cells in Figure 3D. Again, bars show the *FGFR2* E8/E9 ratio relative to the shControl. Error bars indicate SD. All experiments in this figure were done on three biological replicates, in triplicate. n.s: non-significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (one-side unpaired t-test)

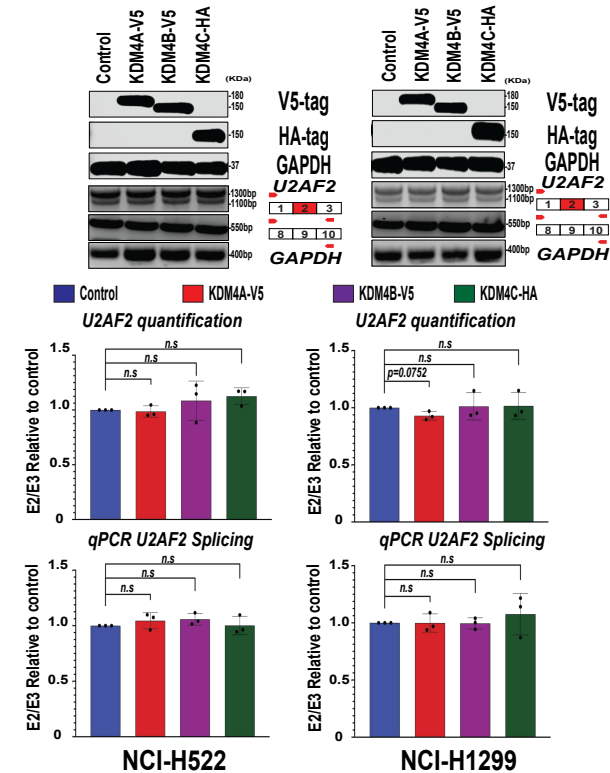
a



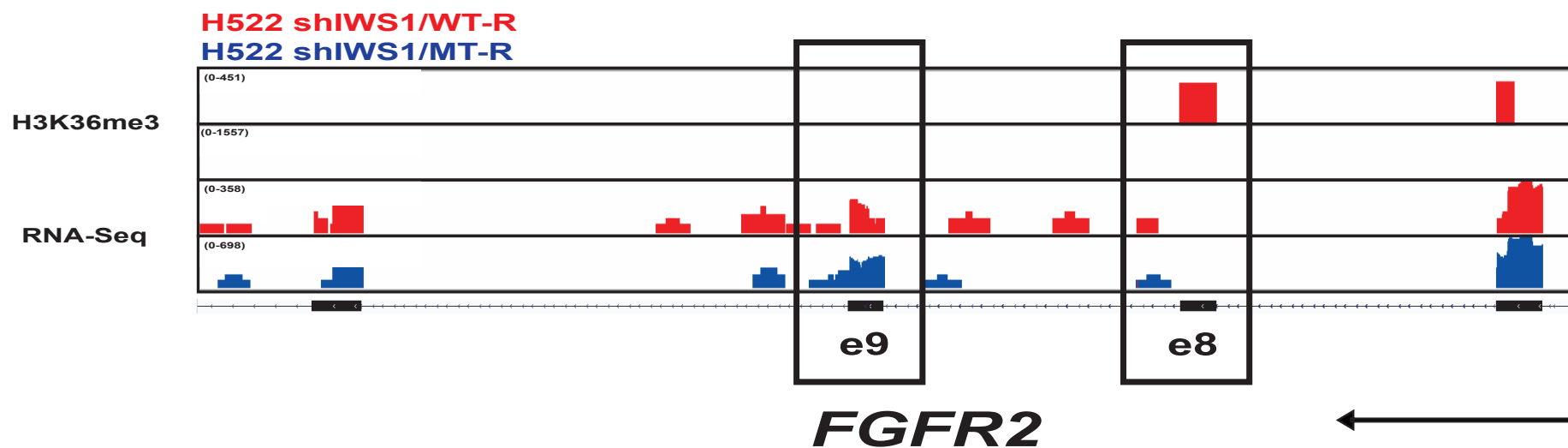
b



c



d

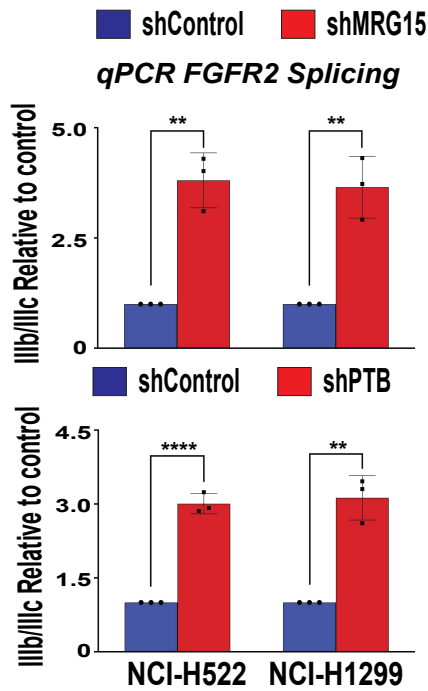
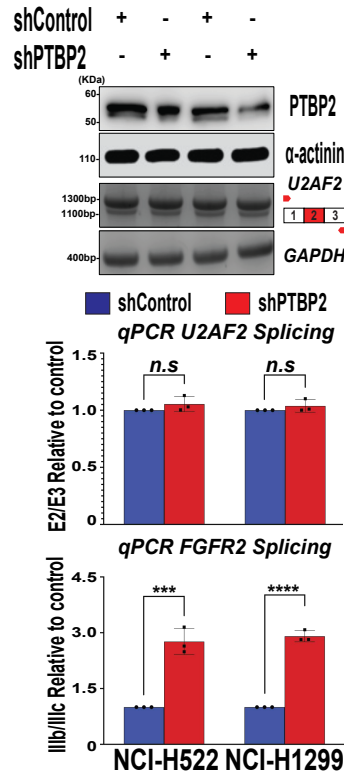
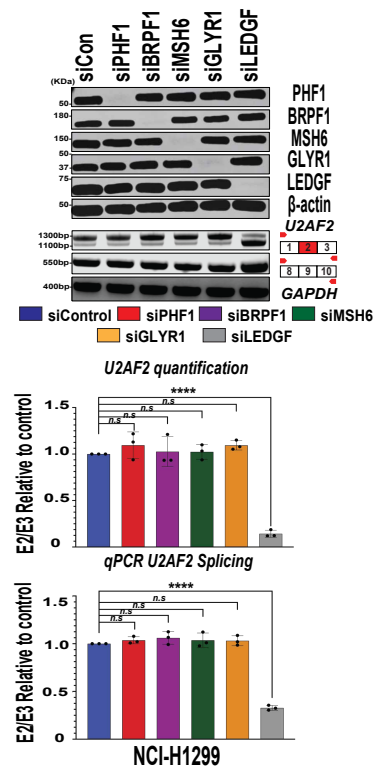
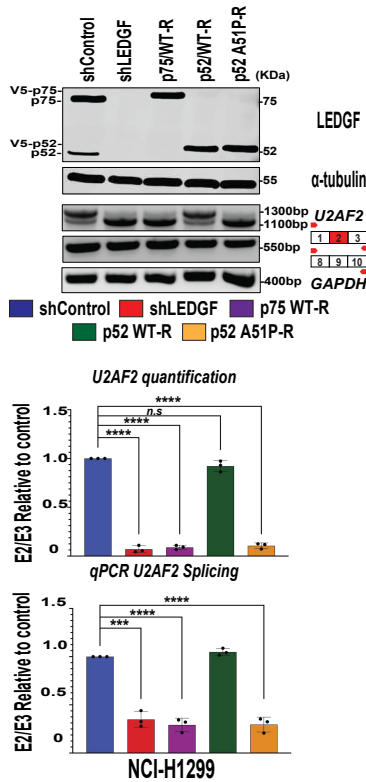
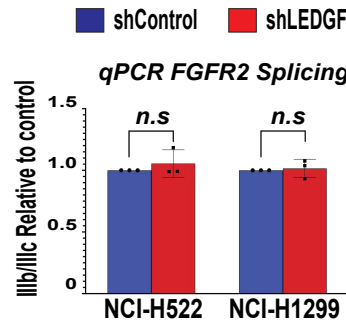
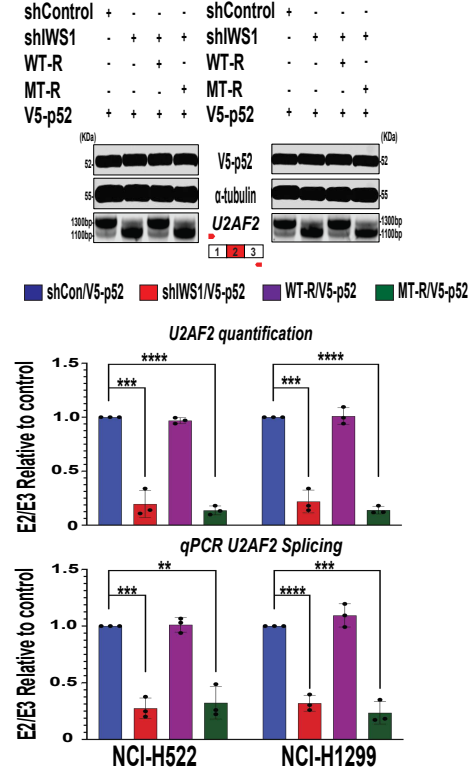
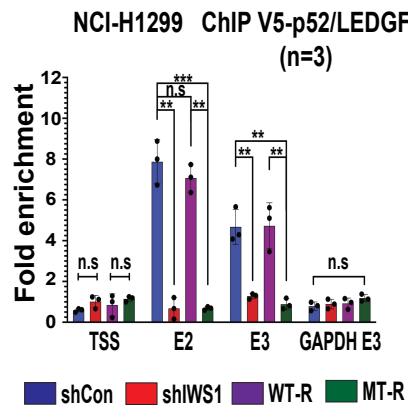


Supplementary Figure 4 (relative to Figure 3). *U2AF2* Exon 2 inclusion, induced by IWS1 phosphorylation at Ser720/Thr721, depends on H3K36 trimethylation by SETD2.

- a. (Upper panels-First two rows) Lysates of the indicated NCI-H522 and NCI-H1299 cells, transduced with a lentiviral construct of wild type HA-SETD2, were probed with the indicated antibodies. (Upper panels-third row) RT-PCR of *U2AF2* exon 2 in the same cells, (Middle panel) Quantification of *U2AF2* E2/E3 ratio based on quantification of the RT-PCR products above, $\pm$ SD. (Lower panel) Quantitative RT-PCR of the *U2AF2* E2/E3 ratio. Bars show again the E2/E3 ratio in the indicated HA-SETD2-transduced cells, relative to HA-SETD2-transduced shControl cells $\pm$ SD.
- b. (Upper panels. first 6 rows) The listed H3K36 methyltransferases were efficiently knocked down in NCI-H522 and NCI-H1299 cells, by siRNA transfection (Upper panels, rows 7 and 9) RT-PCR using *U2AF2*, exon 1 and 3 primers and *U2AF2*, exon 8 and 10 primers (control) and *GAPDH* in the same cells. (Middle panels) *U2AF2* E2/E3 ratio in the siRNA transfected cells, relative to the siControl cells. Data based on quantification of the RT-PCR products above $\pm$ SD. (Lower panels) Quantitative RT-PCR of the *U2AF2* E2/E3 ratio in the same cells, relative to the siControl cells $\pm$ SD.
- c. (Upper Panels, first 3 rows) The indicated H3K36 demethylases were overexpressed in NCI-H522 and NCI-H1299 cells, using viral constructs (pLx304 KDM4A or KDM4B, pBabe-puro KDM4C). Lysates of the transduced cells were probed with the indicated antibodies. (Upper panels, rows 4 and 5) RT-PCR using *U2AF2*, exon 1 and 3 primers and exon 8 and 10 primers (control) and *GAPDH* in the same cells. (Middle panels) *U2AF2* E2/E3 ratio in the demethylase-transduced cells, relative to the vector-transduced cells. Data based on quantification of the RT-PCR data above $\pm$ SD. (Lower panels) Bars show *U2AF2* E2/E3 ratios in the same cells $\pm$ SD, relative to the Control cells, were determined by q-RT-PCR. All assays in this figure were done on three biological

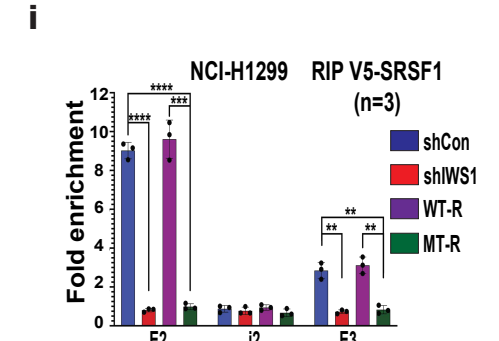
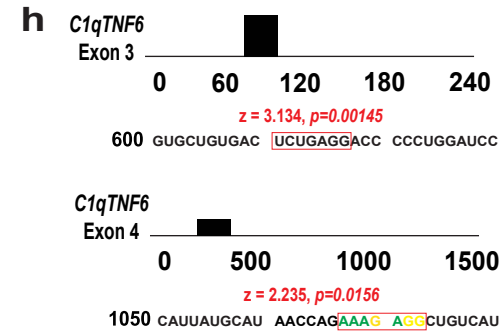
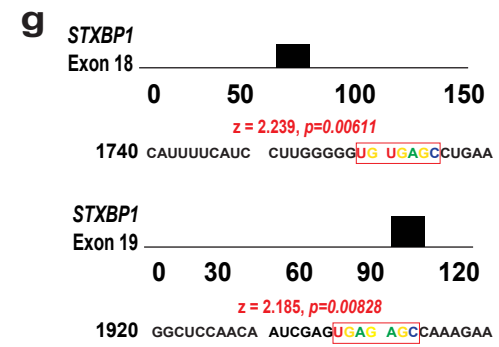
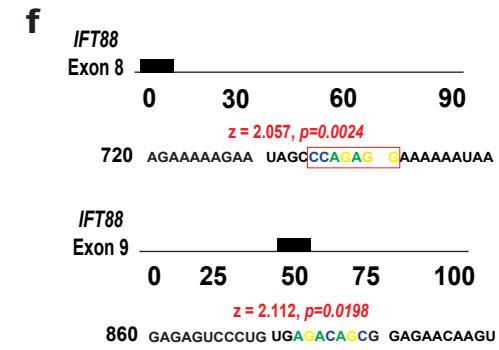
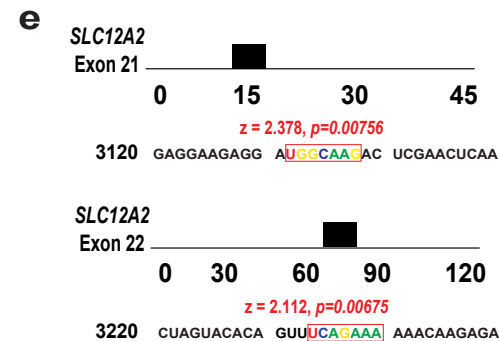
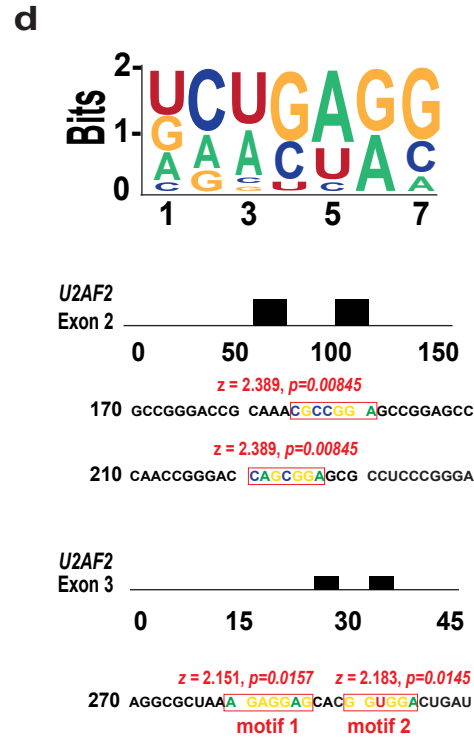
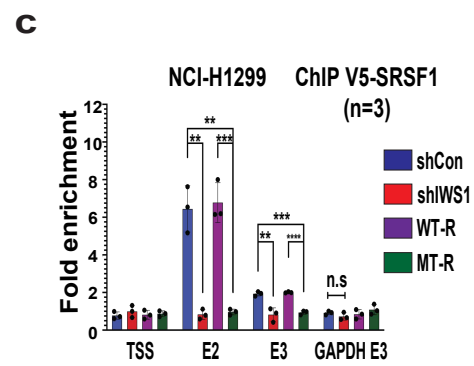
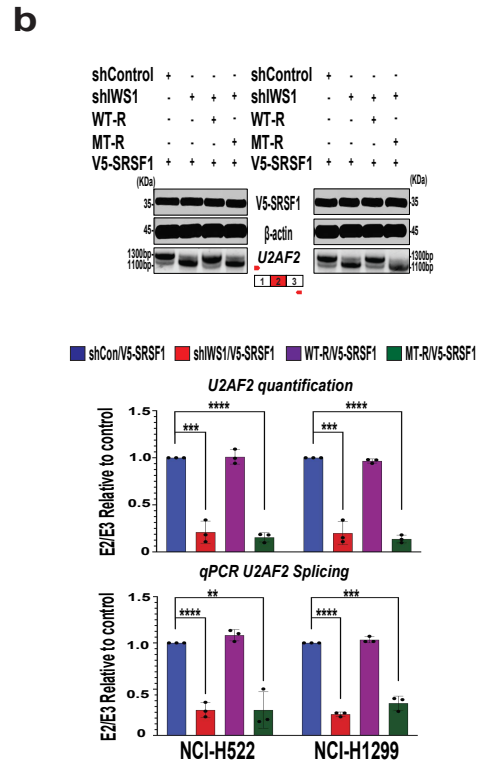
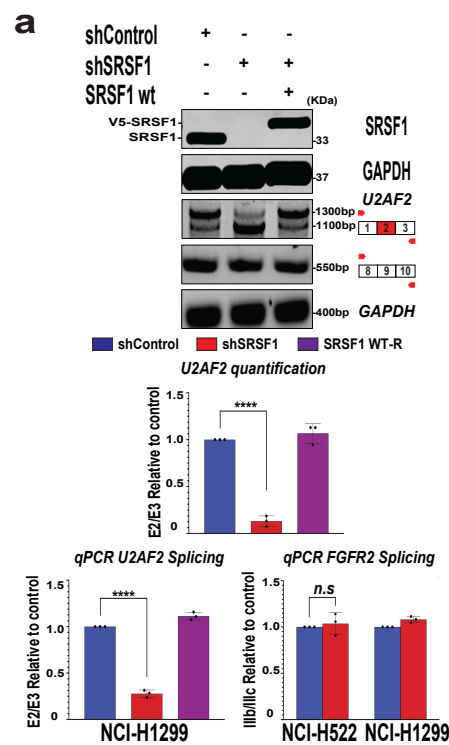
replicates, in triplicate. n.s : nonsignificant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.
(one-side unpaired t-test)

d. Snapshots of the integrative genomic viewer showing the distribution of histone H3K36me3 marks, within the FGFR2 gene, obtained from a ChIP-Seq analysis, along with the distribution of RNA reads obtained from the RNA-seq analysis of shIWS1/WT-R and shIWS1/MTR NCI-H522 cells. Scale represents reads per million (RPM). Snapshots of peaks detected in both biological replicates are shown. The black box outlines FGFR2 exons 8 and 9 and the adjacent regions.

a**b****c****d****e****g****f**

Supplementary Figure 5 (Relative to Figure 4) The regulation of alternative splicing of the *U2AF2* exon 2 by IWS1 and IWS1 phosphorylation, depends on the p52 isoform of the H3K36me3 reader LEDGF and its splicing partner SRSF1.

- a. *FGFR2* E8/E9 ratios in shControl vs shMRG15 and shControl vs shPTB from, NCI-H522 and NCI-H1299 cells $\pm$ SD. Additional data on the same cells in Figure 4a.
- b. (Upper panel) Lysates of shControl and shPTBP2 NCI-H522 and NCI-H1299 cells were probed with the indicated antibodies. RT-PCR, using RNA from the same cells and *U2AF2* exon 1 and 3 primers, in the third row. (Middle and lower panel) *U2AF2* E2/E3 and *FGFR2* E8/E9 ratios in the same cells, relative to the shControl $\pm$ SD, were determined by qRT-PCR.
- c. Repeat of the experiment in figure 4b, in NCI-H1299, as opposed to NCI-H522 cells.
- d. Repeat of the experiment in figure 4c, in NCI-H1299, as opposed to NCI-H522 cells.
- e. *FGFR2* E8/E9 ratios, in shControl and shLEDGF NCI-H522 and NCI-H1299 cells, $\pm$ SD.
- f. Repeat of the experiment in figure 4d, in NCI-H1299, as opposed to NCI-H522 cells.
- g. Ectopic expression of the p52 isoform of LEDGF does not rescue the shIWS1 and shIWS1/MT-R-induced, exon 2 skipping in *U2AF2*. (Upper panel-First two rows) Lysates of shControl, shIWS1, WT-R and MT-R NCI-H522 (left panel) and NCI-H1299 (right panel) cells, transduced with V5-p52/LEDGF, were probed with the indicated antibodies. (Upper panel-Third row) RT-PCR using *U2AF2*, exon 1 and 3 primers on RNA from the same cells (Middle panel) Mean *U2AF2* E2/E3 ratios $\pm$ SD, based on quantification of the RTPCR experiment in the upper panel. (Lower panel) *U2AF2* E2/E3 ratios in the same cells $\pm$ SD, were determined by qRT-PCR. All assays in this figure were done on three biological replicates in triplicate. n.s : non-significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (one-side unpaired t-test).



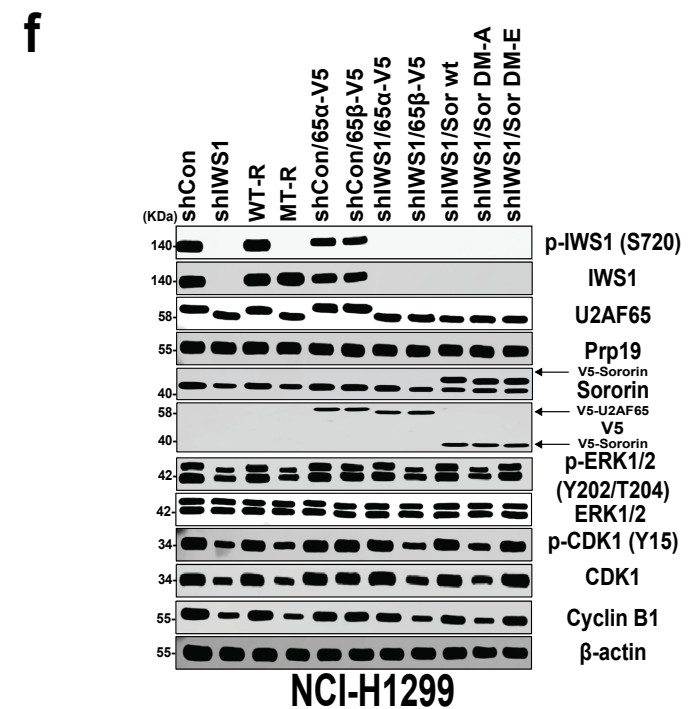
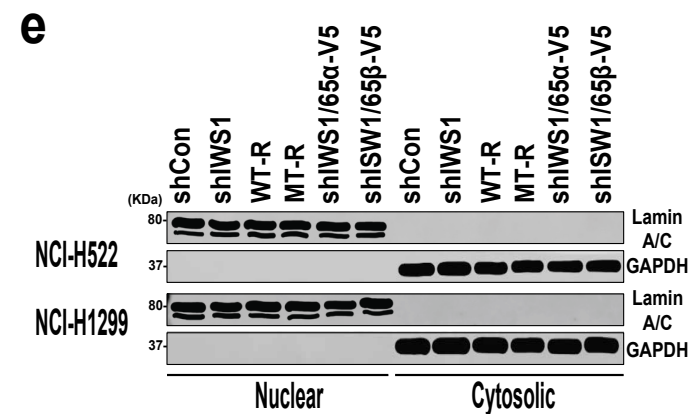
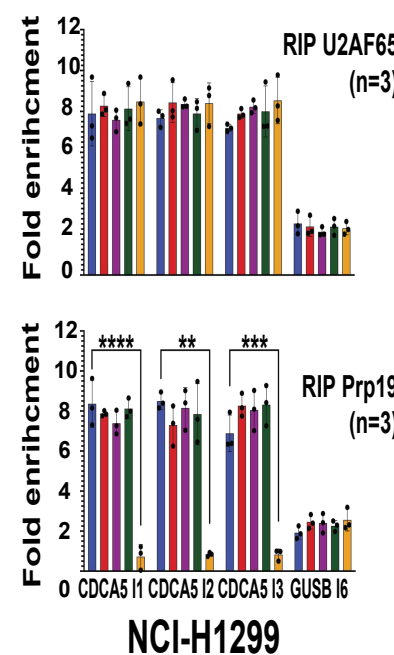
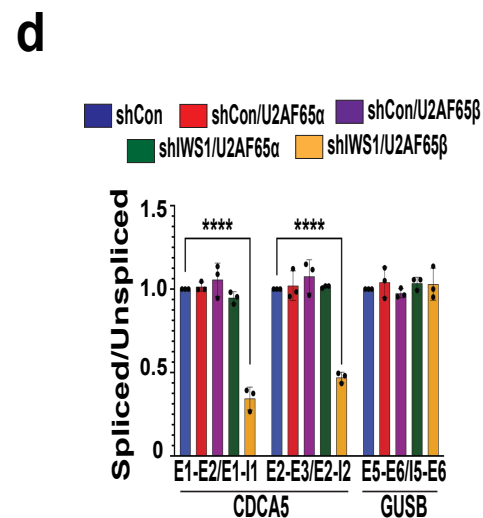
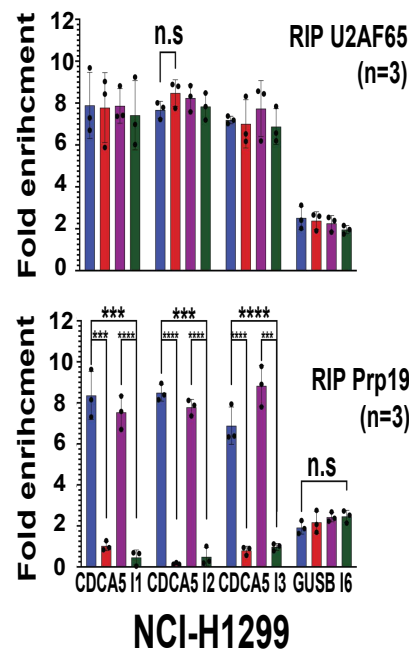
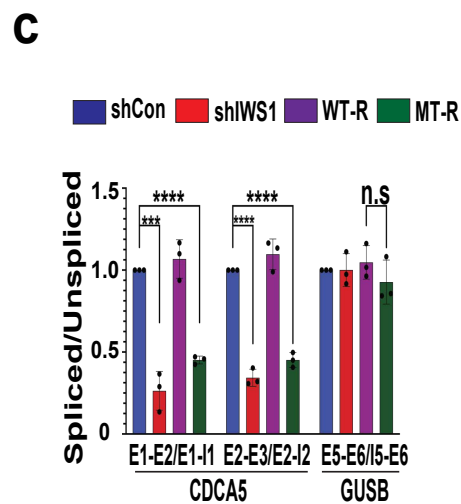
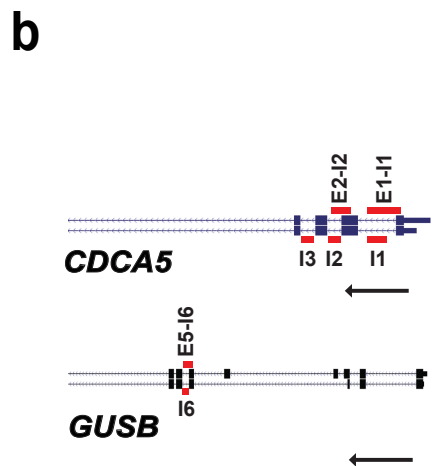
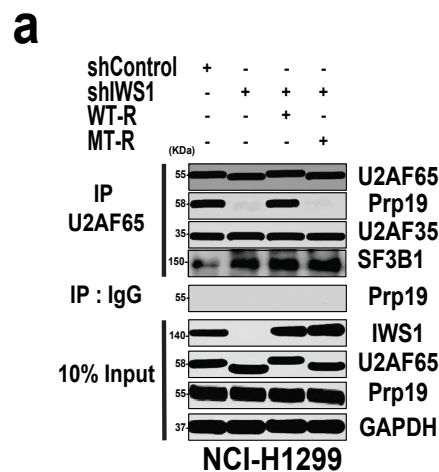
Supplementary Figure 6 (relative to Figure 4). The regulation of the alternative splicing of the *U2AF2* exon 2 by IWS1 and IWS1 phosphorylation, depends on the p52 isoform of the H3K36me3 reader LEDGF and its splicing partner SRSF1.

- a. (Upper panel) Repeat of the experiment in figure 4e, in NCI-H1299, as opposed to NCI-H522 cells. (Middle panel) Mean *U2AF2* E2/E3 ratios $\pm$ SD, based on quantification of the RT-PCR experiment in the upper panel. (Lower panel) *U2AF2* E2/E3 and *FGFR2* E8/E9 ratios in the same cells $\pm$ SD, were determined by qRT-PCR.
- b. Ectopic expression of the SRSF1 does not rescue the shIWS1 and shIWS1/MT-R-induced, exon 2 skipping in *U2AF2*. (Upper panel-First two rows) Lysates of shControl, shIWS1, WT-R and MT-R NCI-H522 (left panel) and NCI-H1299 (right panel) cells, transduced with V5-SRSF1, were probed with the indicated antibodies. (Upper panel-Third row) RT-PCR using *U2AF2*, exon 1 and 3 primers on RNA from the same cells (Middle panel) Mean *U2AF2* E2/E3 ratios $\pm$ SD, based on quantification of the RT-PCR experiment in the upper panel. (Lower panel) *U2AF2* E2/E3 ratios in the same cells $\pm$ SD, were determined by qRT-PCR.
- c. Repeat of the experiment in figure 4f, in NCI-H1299, as opposed to NCI-H522 cells.
- d. (Upper panel) The 7-nt SRSF1 binding motif, involved in the regulation of alternative RNA splicing by SRSF1 (Middle and Lower panels). Boxes show the location of the SRSF1 binding motifs in *U2AF2* exons 2 and 3 and their nucleotide sequence. The height of the boxes is proportional to the $-\log(p\text{-value})$ and it is presented relative to the height of the box mapping between nucleotides 184 and 192. The binding scores (z-scores) and p values are calculated using the RBP map pipeline (<http://rbpmap.technion.ac.il/index.html>)
- e. , f., g., and h. SRSF1 binding sites analysis of the validated splicing targets of IWS1 phosphorylation in Supplementary Fig. 1 (*SLC12A2*, *IFT88*, *STXBP1* and *C1qTNF6*), shows that all harbor such sites.

i. Repeat of the experiment in figure 4g, in NCI-H1299, as opposed to NCI-H522 cells.

All assays in this figure were done on three biological replicates, in triplicate. n.s: non-significant

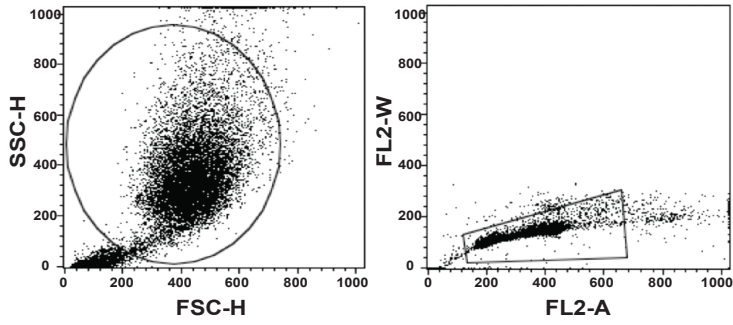
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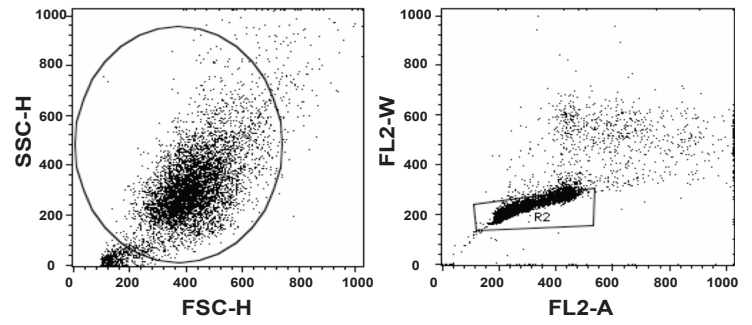
Supplementary Figure 7 (relative to Figure 5). IWS1 phosphorylation controls the CDCA5/ERK phosphorylation feedback loop, through U2AF2 alternative RNA splicing.

- a. Repeat of the experiment in Figure 5c, in NCI-H1299, as opposed to NCI-H522 cells.
- b. UCSC browser snapshot showing the position of the relevant exons of the human *CDCA5* and *GUSB* genes. The map position of the sequences amplified by PCR is indicated with red lines. The arrows indicate the direction of transcription.
- c. Repeat of the experiment in figure 5d in NCI-H1299, as opposed to NCI-H522 cells.
- d. Repeat of the experiment in figure 5e, in NCI-H1299, as opposed to NCI-H522 cells.
- e. Validation of the nucleo-cytoplasmic fractionation of the indicated NCI-H522 and NCI-H1299 cells, by probing the cytosolic and nuclear lysates with the indicated antibodies. The cytoplasmic fractions obtained from this fexperiment were used for the experiment in figure 5f.
- f. Repeat of the experiment in figure 5g, in NCI-H1299, as opposed to NCI-H522 cells. All assays in this figure were done on three biological replicates, in triplicate. n.s non-significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (one-side unpaired t-test).

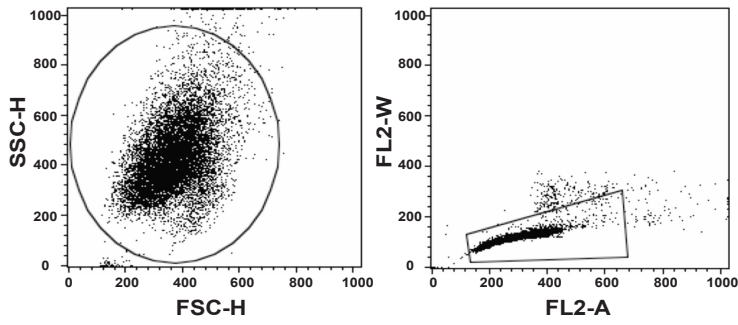
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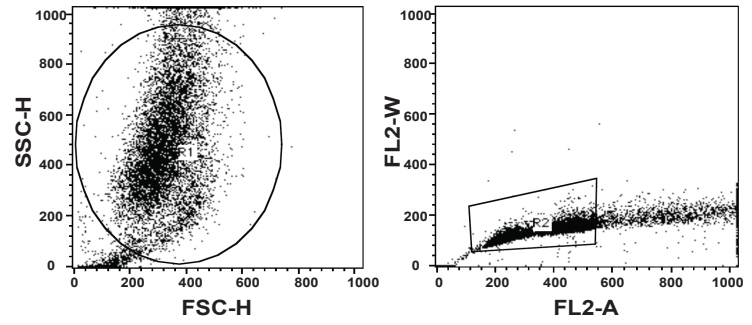
NCI-H1299



A549

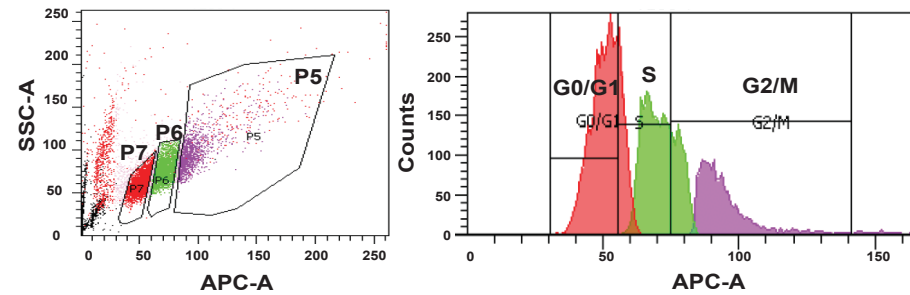
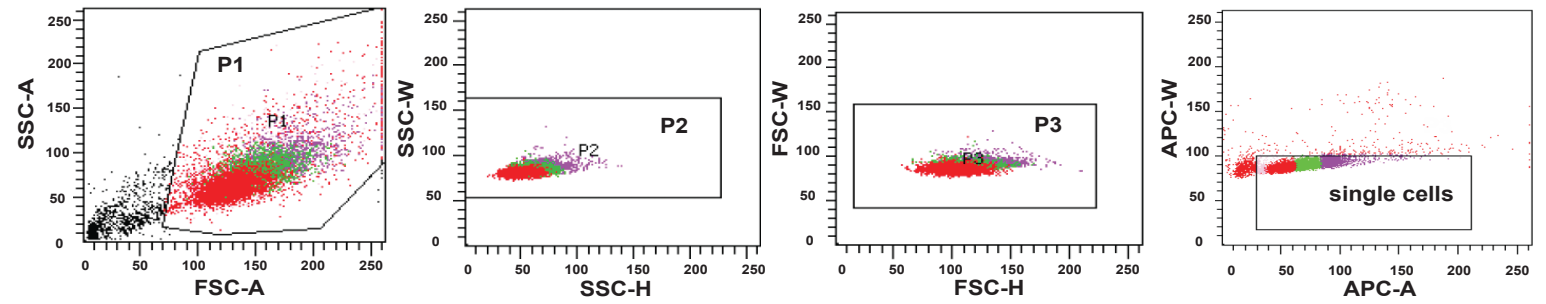


NCI-H1975



b

NCI-H1299

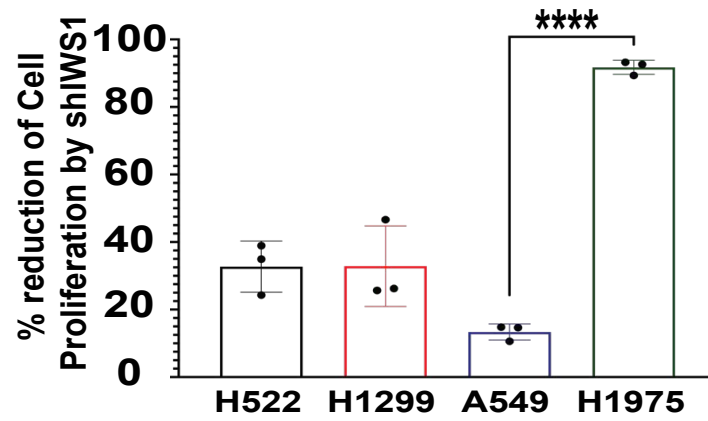
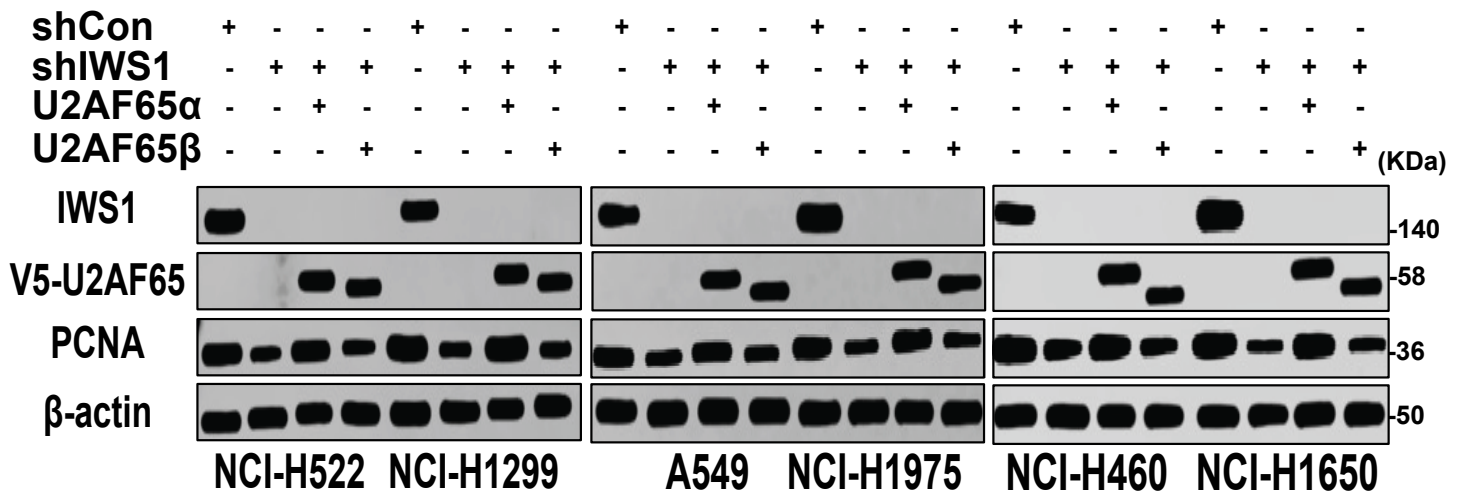
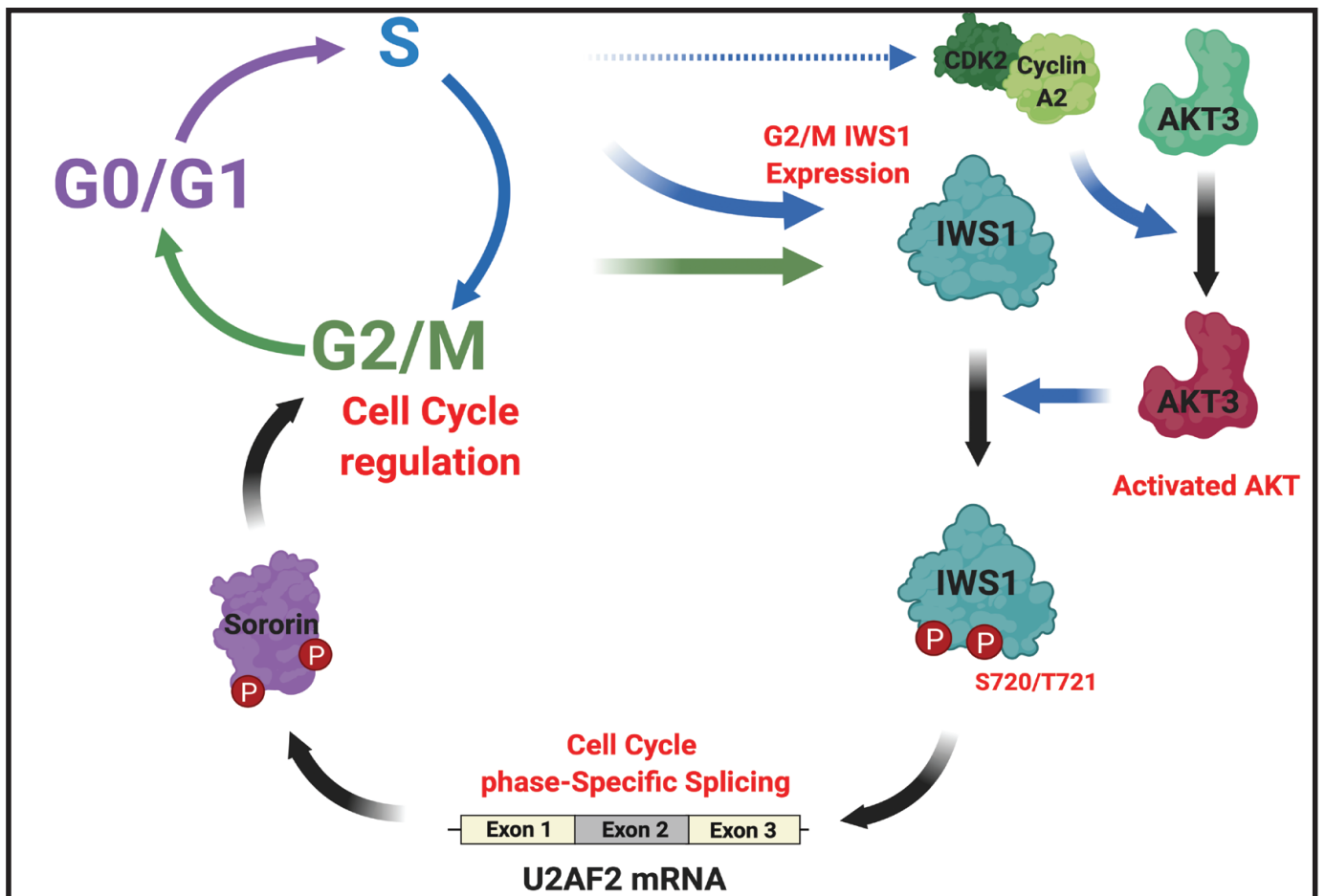


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P3	9,948	99.9
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G0/G1	2,980	32.9
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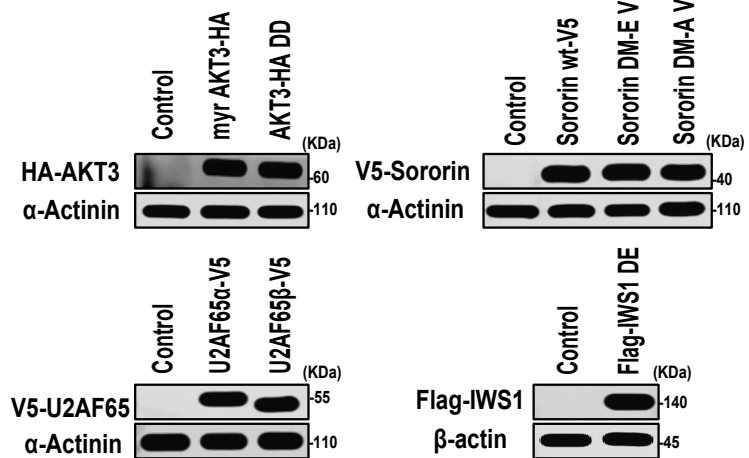
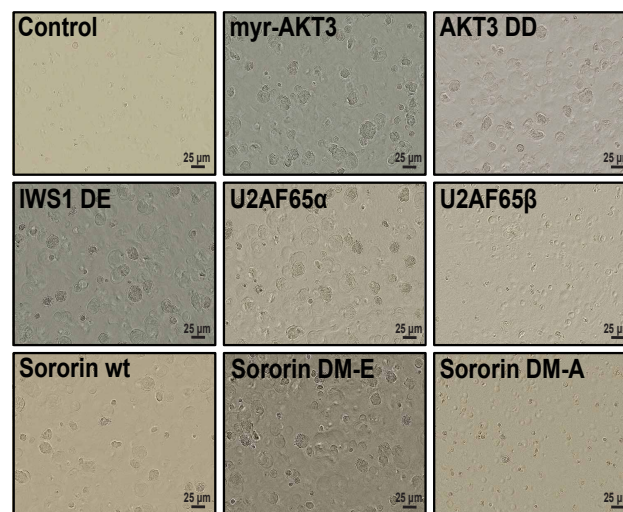
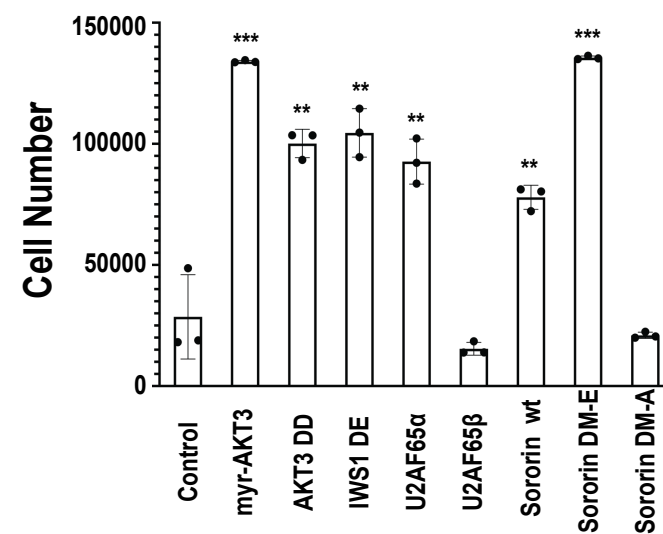
Supplementary Figure 8 (relative to Figure 6). Gating strategy for the FACS Sorting Experiments

- a.** Gating strategy for FACS analysis in the indicated NCI-H522, NCI-H1299, A549 and NCI H1975 parental cells.
- b.** Gating strategy for sorting NCI-H1299 cells stained with Vybrant™ DyeCycle™ Ruby Stain, into fractions, traversing different phases of the cell cycle.

a**b****c**

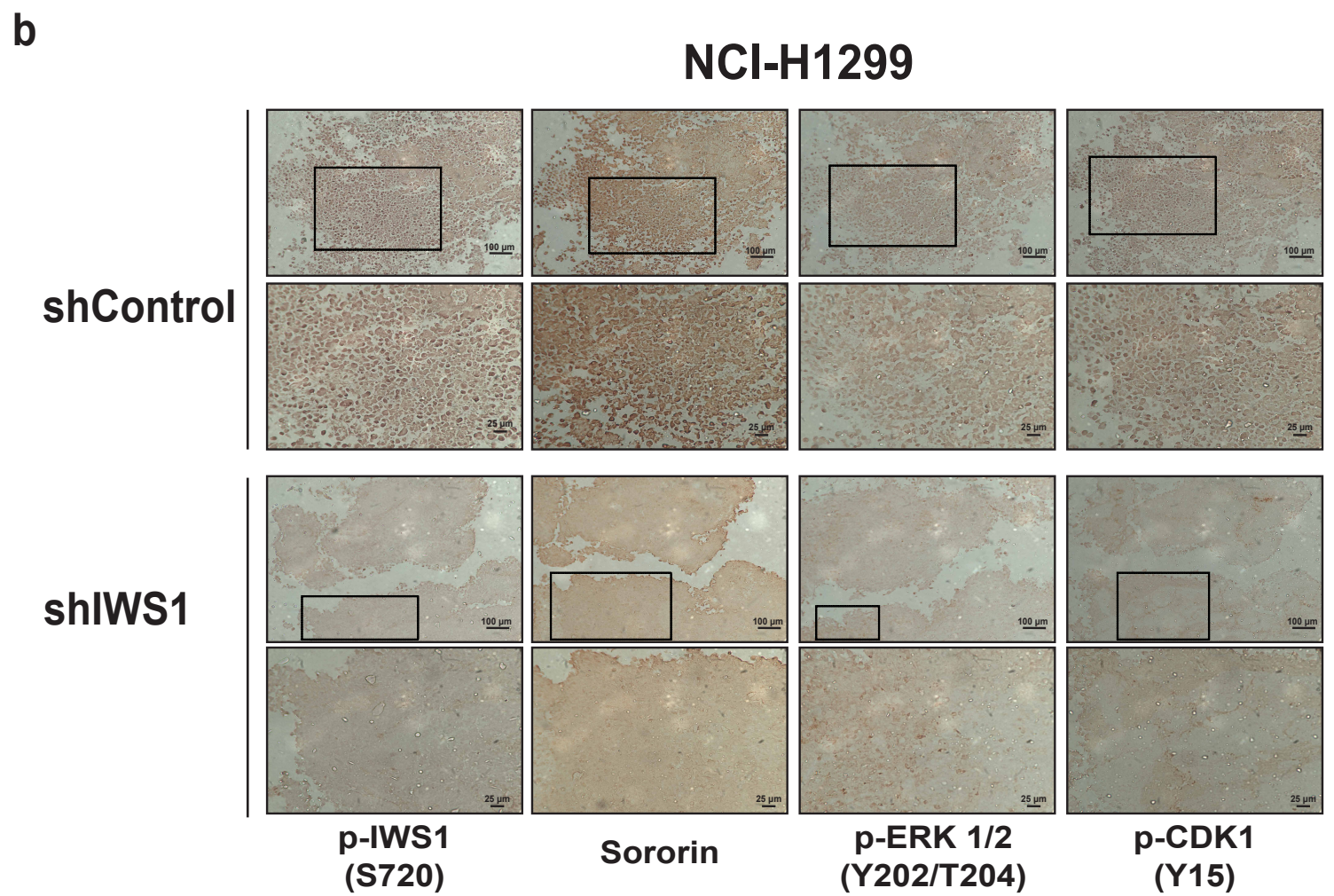
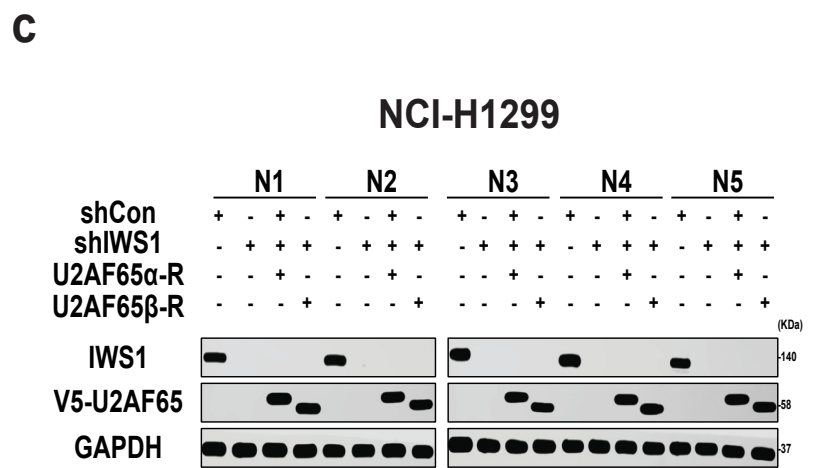
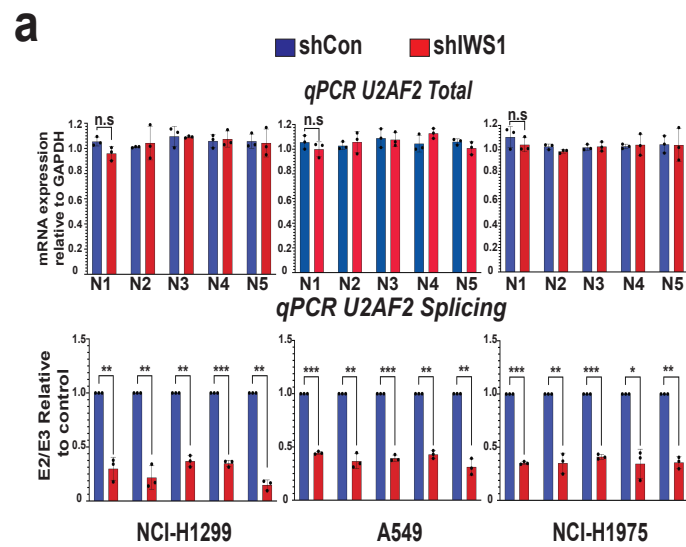
Supplementary Figure 9 (relative to Figure 6). IWS1 phosphorylation promotes cell proliferation by controlling the Sororin/ERK phosphorylation feedback loop, through *U2AF2* RNA splicing.

- a. Percent reduction of cell proliferation, induced by shIWS1 in the indicated cell lines. Bar show the mean reduction of cell proliferation, derived from the end-point data in Figure 6a and 6c. The error bars show the SD of at least 3 biological replicates. The statistics were performed using one-sided unpaired t-test (NCI-H522 vs A549 : $p = 0.0225$, NCI-H1299 vs A549 : $p = 0.0493$, A549 vs NCI-H1975 : $p = 0.0001$, NCI-H522 vs NCI-H1975 : $p = 0.0174$, NCI-H1299 vs NCIH1975 : $p = 0.0011$).
- b. Lysates derived from the indicated cells were probed with antibodies, as shown. PCNA was downregulated by shIWS1 in the four cell lines tested in the cell proliferation experiments in figures 6a and 6c, plus two additional lung adenocarcinoma cell lines (NCI-H460 and NCI H1650). In all cell lines the shIWS1-induced downregulation of PCNA, was rescued by U2AF65 α , but not by U2AF65 β .
- c. *Model of the cell cycle-dependent regulation of U2AF2 alternative RNA splicing, downstream of IWS1 phosphorylation.* IWS1 is expressed during S and G2/M (this report), and according to published data, AKT is activated via phosphorylation by CDK2/Cyclin A2, also as the cells enter S phase (Liu et al., 2014⁷⁶). The expression of IWS1 and its phosphorylation by AKT, promote inclusion of exon 2 in *U2AF2* mRNA. This splice form of *U2AF2*, is required for the splicing and expression of Sororin, which in turn regulates the progression through the G2/M phase of the cell cycle.

a**HBEC hTERT****b****HBEC hTERT****c****HBEC hTERT**

Supplementary Figure 10. The AKT/IWS1/U2AF2/CDCA5/ERK pathway transforms hTert immortalized human bronchial epithelial cells (hTert-HBEC) in culture.

- a.** Lysates of HBEC hTERT cells, transduced with the indicated constructs, were probed with antibodies, as shown.
- b.** The HBEC hTERT cells in **a** were cultured in a soft agar. Cells were plated in triplicate and they were imaged 7 days later, using an Incucyte live cell imager. Scale bars in the right lower corner of each image.
- c.** After imaging, the cells were incubated in WST solution as outlined in the Cell Transformation Assay Kit (Abcam) and the number of live cells was estimated, based on the absorbance at 450 nanometers, 4 hours later. Bars show the number of transformed live cells $\pm$ SD. All assays in this figure were done on three biological replicates, in triplicate, n.s: non-significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. (one-side unpaired t-test).



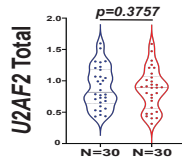
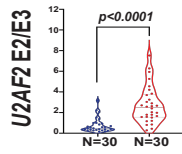
Supplementary Figure 11 (Relative to Figure 7). IWS1 phosphorylation controls tumor growth *in vivo*, by regulating the U2AF2/Sororin/ERK axis.

- a. (Upper panel) The relative abundance of *U2AF2* mRNA in individual tumors in NSG mice inoculated subcutaneously with shControl or shIWS1-transduced NCI-H1299, A549 or NCI-H1975 cells, was determined by q-RT-PCR. Bars show the mean *U2AF2* mRNA abundance relative to the abundance of *GAPDH* $\pm$ SD (Lower panel) The *U2AF2* E2/E3 ratio was determined in the same tumors also by qRT-PCR. Bars show the mean *U2AF2* E2/E3 ratios $\pm$ SD. SD was calculated, based on 3 technical replicates. ** $p < 0.001$, *** $p < 0.001$. (paired t-test), n.s: non-significant (paired t-test)
- b. Phosphor-IWS1 (Ser720), Sororin, phosphor-ERK (Y202/T204) and phospho-CDK1 (Y15) staining, of tumor xenografts induced by subcutaneous inoculation of NSG mice with shControl (upper) or shIWS1 (lower) NCI-H1299 cells. Formalin-fixed, paraffin embedded tumor samples from the experiment in figure 7 were stained with the indicated antibodies. Secondary antibody was HRP-labelled. The staining of individual tumors with different antibodies was done, using sequential tumor sections, so that we could determine whether the expression and/or phosphorylation of proteins of interest spatially overlapped. Boxes delineate the area of higher magnification, shown in the image below. Scale in the right lower corner of each image.
- c. Cell lysates derived from mouse xenograft-induced tumors in NSG mice inoculated subcutaneously with shControl, shIWS1, shIWS1/U2AF65 α or shIWS1/U2AF65 β NCI-H1299 were probed with the indicated antibodies.

a

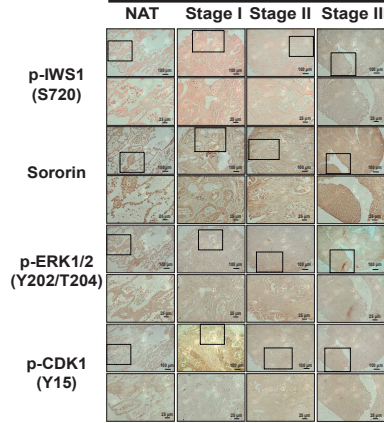
OSU LUAD cohort

■ Normal ■ Tumor



b

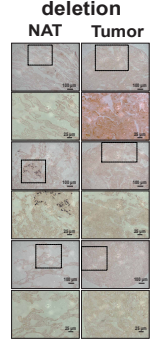
LUAD TMA



c

LUAD TMA

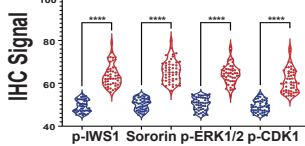
EGFR E746-A750 deletion



d

LUAD TMA

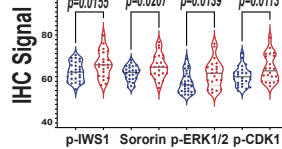
■ Normal (n=50) ■ Tumor (n=50)



e

LUAD TMA

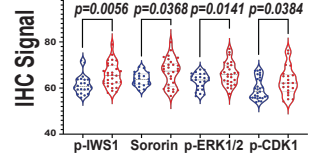
■ Stage I (n=28) ■ Stage II (n=22)



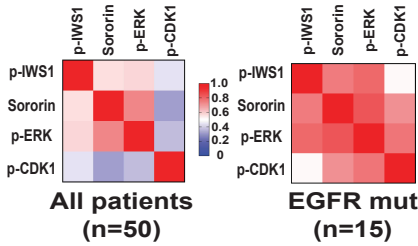
f

LUAD TMA

■ Grade 2 (n=22) ■ Grade 3 (n=28)



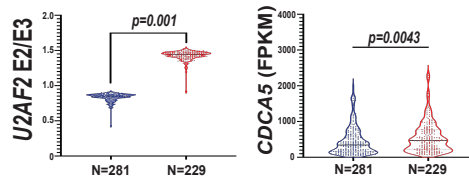
g



h

TCGA LUAD

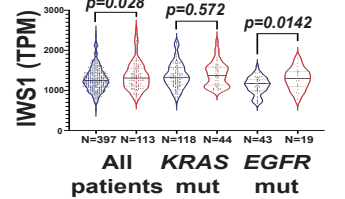
■ Low IWS1 ■ High IWS1



i

TCGA LUAD

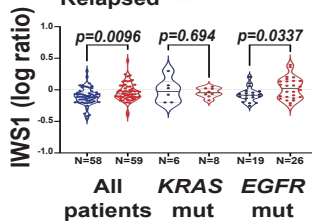
■ Stage I/II ■ Stage III/IV



j

GSE13213

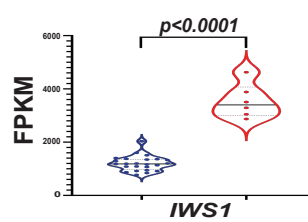
■ Non-Relapsed ■ Relapsed



k

GSE141685

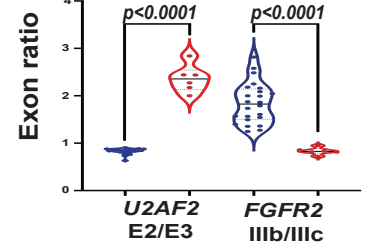
■ Primary Tumor (n=24) ■ Brain Lesion (n=6)



l

GSE141685

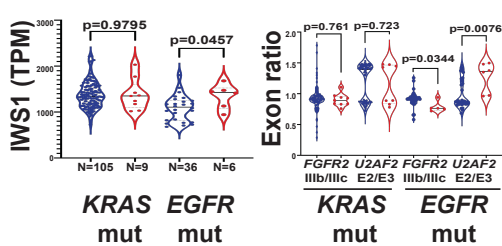
■ Primary Tumor (n=24) ■ Brain Lesion (n=6)



m

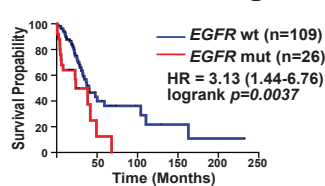
TCGA LUAD

■ M0 ■ M1



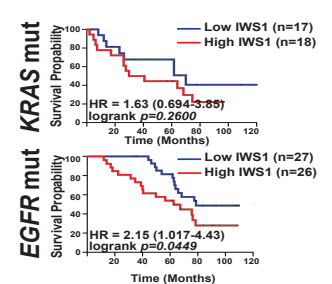
n

TCGA LUAD-High IWS1



o

GSE13213/GSE26939



Supplementary Figure 12 (relative to Figure 8). The AKT/IWS1/U2AF2/CDCA5/ERK pathway is active in human lung adenocarcinomas and impacts tumor grade, stage, metastatic potential and treatment relapse in patients with *EGFR* mutant, but not *KRAS* mutant tumors.

- a. Quantitative RT-PCR showing the *U2AF2* E2/E3 ratio (top) and the total *U2AF2* mRNA (bottom) in lysates derived from the 30 LUAD samples and the paired NAT in figure 8A. The black lines in the violin plots show the mean *U2AF2* E2/E3 ratios and the mean *U2AF2* levels, relative to *GAPDH*. Statistical analyses were done using the paired t-test.
- b. Sequential sections of a commercially available tissue microarray (TMA) of 50 LUAD with paired NAT, were probed with the indicated antibodies. Figure shows the staining of a representative NAT sample and representative tumor samples of Stage I, II and III lung adenocarcinomas (one of each). Boxes delineate areas of higher magnification, which are shown in the images below. Scale in the right lower corner of each image. Data show that pathway activity correlates with tumor stage.
- c. The TMA sections in b were also stained with a monoclonal antibody, which specifically recognizes the EGFR E746-A750 deletion. Figure shows three representative lung adenocarcinomas with EGFR deletion and the corresponding NATs. Boxes delineate areas of higher magnification, which are shown in the image below. Scale in the right lower corner of each image.
- d. Violin plots showing the abundance of IWS1 phosphorylation, along with the abundance of Sororin, phospho-ERK and phospho-CDK1, in the LUAD and NAT samples in the TMA in b and c. The black lines indicates the mean p-IWS1, Sororin, p-ERK and p-CDK1 levels. Statistical analyses were done using the paired t test. Data show that pathway activity is higher in LUADs than in NATs.
- e. Violin plots comparing the phosphorylation of IWS1 and the abundance of Sororin, phospho-ERK and phospho-CDK1 in stage I and Stage II/III lung adenocarcinomas in the

group of 50 tumors sampled in the commercial TMA described above. Statistical analyses were performed using the one-sided unpaired t-test. Data show that pathway activity correlates with tumor stage.

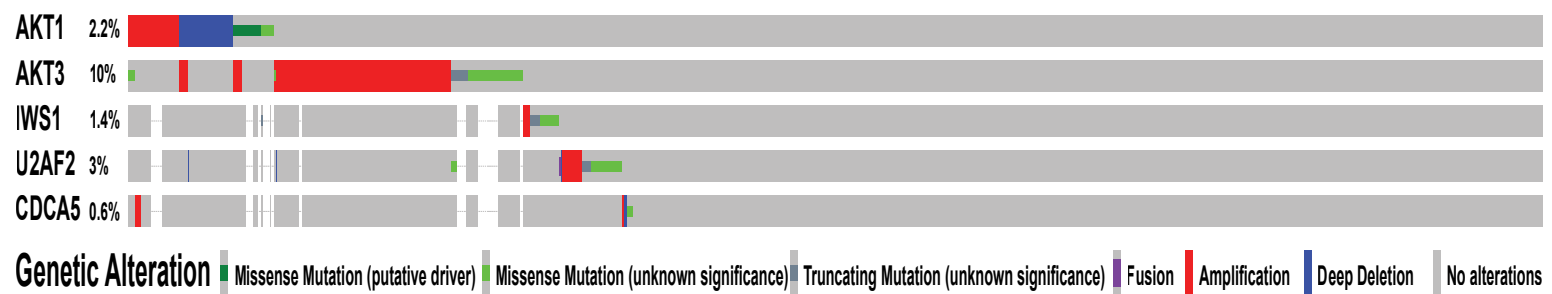
- f. Violin plots comparing the phosphorylation of IWS1 and the abundance of Sororin, phospho-ERK and phospho-CDK1 in histological grade 2 and grade 3 lung adenocarcinomas in the group of 50 tumors in the commercial TMA described above. Statistical analyses were performed using the one-sided unpaired t-test. Data show that pathway activity correlates with tumor grade.
- g. Heatmaps showing the correlation coefficient between the indicated components of the IWS1 phosphorylation pathway in the entire TMA cohort (left) and in the *EGFR* mutant cohort (right). The correlations were calculated using simple linear regression. The statistics and the p values for all the comparisons, can be found in Supplementary Table 5. Data show that the correlation between phosphor-IWS1 and Sororin, phosphor-ERK and phosphor-CDK1 is more robust in *EGFR* mutant tumors.
- h. The expression of IWS1 correlates with Exon 2 inclusion in the *U2AF2* mRNA transcripts (Left panel) and with the expression of *CDCA5* (Right panel) in the TCGA LUAD dataset. The black lines indicate the mean *U2AF2* E2/E3 ratios and *CDCA5* expression. Statistical analyses were performed using the one-sided unpaired t-test.
- i. Violin plots show the abundance of IWS1 in stage I/II and in Stage III/IV LUADs in the TCGA database. Data shown for all tumors, and selectively for *EGFR* or *KRAS* mutant tumors. The horizontal lines indicate mean values of IWS1 expression. Statistical analyses were performed using the one-sided unpaired t-test.
- j. Violin plots showing the abundance of IWS1 in relapsed and non-relapsed tumors of patients in the GSE13213 dataset. Data shown for all tumors, and selectively for *EGFR* or *K-RAS* mutant tumors. The horizontal lines indicate mean values for IWS1. Statistical analyses were performed using the one-sided unpaired t-test.

- k. The expression of IWS1 is higher in brain metastases of lung adenocarcinomas than in primary tumors, in the GSE141685 dataset (see also Figure 8g). The black lines indicate the mean IWS1 expression. Statistical analyses were performed using the one-sided unpaired t-test.
- l. The *U2AF2* E2/E3 ratio is higher and the *FGFR2* E8/E9 ratio is lower in brain metastases of LUADs than in primary LUADs in the GSE141685 dataset (see also figure 8g). The black lines indicate the mean *U2AF2* E2/E3 and *FGFR2* E8/E9 ratios. Statistical analyses were performed using the one-sided unpaired t-test.
- m. (Left panel) Violin plots showing the expression of IWS1 in transcripts per million (TPM) in *KRAS* and *EGFR* mutant, primary and metastatic lung adenocarcinomas in the TCGA LUAD database. IWS1 expression is higher in tumors from patients with metastatic disease (M1) only if the tumors harbor *EGFR* mutations. (Right panel) Exon E2/E3 ratio in the *U2AF2* mRNA and exon E8/E9 ratio in the *FGFR2* mRNA in *KRAS* and *EGFR* mutant lung adenocarcinomas. E2/E3 ratio in the *U2AF2* mRNA is higher and the E8/E9 ratio in the *FGFR2* mRNA is lower in tumors from patients with metastatic disease (M1), only if the tumors harbor *EGFR* mutations. The black lines indicate the mean IWS1 expression, and the mean *U2FA2* E2/E3 and *FGFR2* E8/E9 ratios. Statistical analyses were performed using the one-sided unpaired t-test.
- n. Kaplan-Meier curves showing the impact of *EGFR* mutations on survival, in patients with LUADs expressing high levels of IWS1. Statistical analyses were performed using the one-sided log rank test and Cox's proportional hazards model. Data show that *EGFR* mutations predict poor survival of patients with high IWS1
- o. Kaplan-Meier curves showing the impact of the abundance of IWS1 expression on the survival of patients with lung adenocarcinomas harboring *KRAS* mutations (upper panel) or *EGFR* mutations (lower panel) in the GSE13213/GSE26969 datasets. Statistical analyses were performed using the one-sided log rank test and Cox's proportional hazards

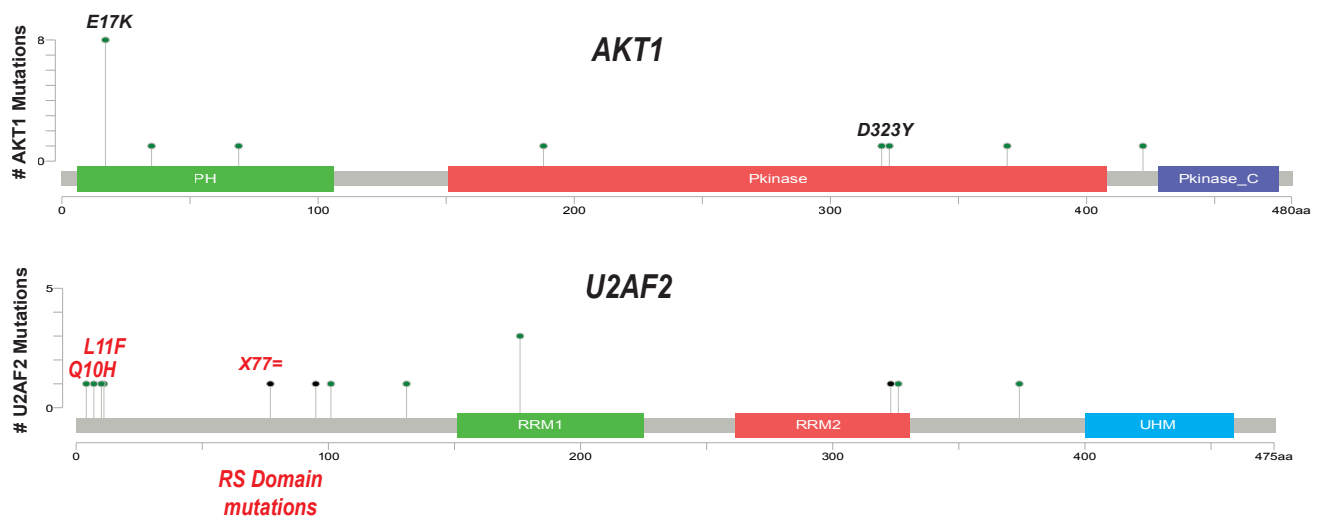
model. Data show that high IWS1 expression predicts poor prognosis in *EGFR*, but not in *KRAS* mutant tumors.

a

LUAD Datasets

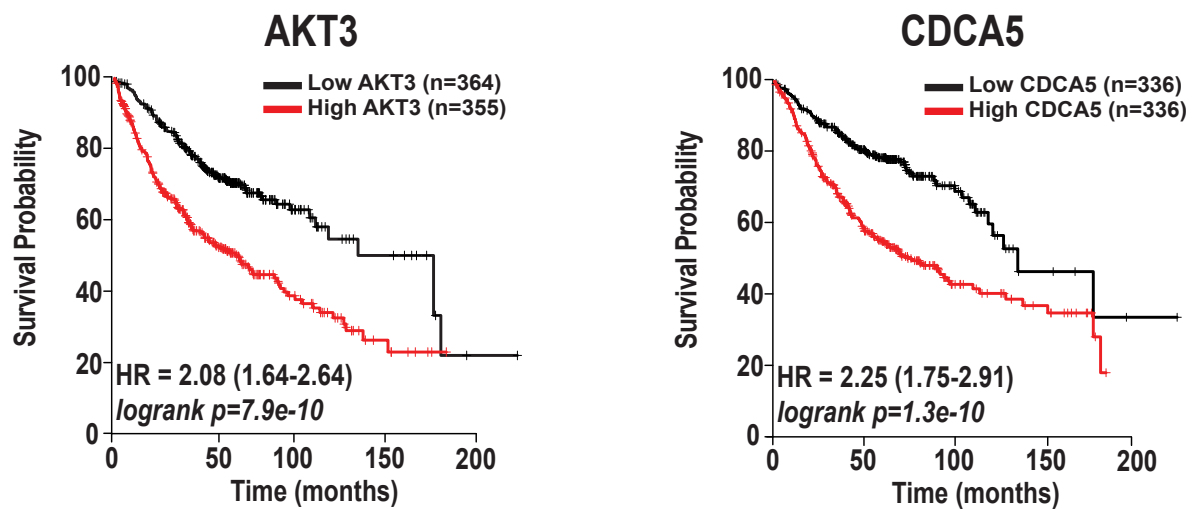


b



c

TCGA LUAD



Supplementary Figure 13 (relative to Figure 8). Genetic changes that may contribute to the activation of the AKT/p-IWS1/U2AF2/CDCA5/ERK pathway in human LUADs

- a.** Genetic changes of genes encoding critical components of the IWS1 phosphorylation pathway, described in this report. Important role in pathway activation is likely to be played by the amplification of AKT1 and AKT3. Alterations were visualized by cBioPortal for Cancer Genomics. Mutations, deletions, amplifications, fusions and other alterations are shown in different colors.
- b.** Mutations of potential significance for pathway activation in the *AKT1* and *U2AF2* genes. *E17K* and *D323Y* mutations are gain of function AKT1 mutations and they may result in IWS1 phosphorylation. The significance of the *L11F* and *Q10H* mutations, immediately upstream of the U2AF65 RS domain and the *X77=* splice site *U2AF2* mutation is not known. The complete list of the mutations in this pathway can be found in Supplementary Data 1.
- c.** Kaplan-Meier curves showing the impact of *AKT3* (left) and *CDCA5* (right) expression on patient survival in patients in the TCGA LUAD database. Statistical analyses were performed using the one-sided log rank test and Cox's proportional hazards model.

Supplementary Table 1 : antibodies used on this report

Target	Company	Cat. No	Application
IWS1	Cell Signaling Technologies	5681	WB (1:1000), ChIP (1:50), ChIC (1:50)
Flag-tag	Cell Signaling Technologies	14793	WB (1:1000), ChIP (1:50)
GAPDH	Cell Signaling Technologies	8884	WB (1:2500)
p-IWS1	Cell Signaling Technologies	Sanidas et al., 2014	WB (1:1000), IHC (1:50)
AKT1	Cell Signaling Technologies	75692	WB (1:1000)
AKT2	Cell Signaling Technologies	2964	WB (1:1000)
AKT3	Cell Signaling Technologies	14982	WB (1:1000)
pan-Akt	Cell Signaling Technologies	4691	WB (1:1000)
p-Akt (T308)	Cell Signaling Technologies	13038	WB (1:1000)
p-Akt (S473)	Cell Signaling Technologies	4060	WB (1:1000)
SETD2	Cell Signaling Technologies	23486	WB (1:1000), ChIC (1:50)
H3K36me3	Cell Signaling Technologies	4909	WB (1:1000), ChIP (1:50), ChIC (1:50)
Vincullin	Cell Signaling Technologies	13901	WB (1:1000)
α-actinin	Cell Signaling Technologies	6487	WB (1:1000)
HA-tag	Cell Signaling Technologies	3724	WB (1:100), ChIP (1:50)
NSD1	AbCam	Ab70732	WB (1:1000)

NSD2	Cell Signaling Technologies	65127	WB (1:1000)
NSD3	Cell Signaling Technologies	92056	WB (1:1000)
SMYD2	AbCam	Ab108217	WB (1:1000)
ASH1L	AbCam	Ab50981	WB (1:1000)
V5-Tag, a-rabbit	Cell Signaling Technologies	13202	WB (1:1000), ChIP (1:50), RIP (1:100)
V5-tag, a-mouse	Cell Signaling Technologies	80076	IP (1:100)
MRG15	Cell Signaling Technologies	14098	WB (1:1000)
β-Actin	Cell Signaling Technologies	4970	WB (1:10000)
PHF1	AbCam	Ab184951	WB (1:1000)
BRPF1	AbCam	Ab168673	WB (1:1000)
GLYR1	AbCam	Ab167155	WB (1:1000)
MSH6	AbCam	Ab92471	WB (1:1000)
LEDGF	Cell Signaling Technologies	2088	WB (1:1000)
α-tubulin	Sigma	T5168	WB (1:10000)
PTB	Cell Signaling Technologies	57246	WB (1:1000)
PTBP2	AbCam	Ab154787	WB (1:1000)
SRSF1	Cell Signaling Technologies	14908	WB (1:1000)
Prp19	GeneTex	GTX111233	WB (1:1000), RIP (1:100)
U2AF65, a-mouse	Sigma	U4758	WB (1:1000), IP (1:100), RIP (1:100)

U2AF65, a-rabbit	Sigma	HPA043562	WB (1:1000)
SF3B1	Cell Signaling Technologies	14434	WB (1:1000)
Sororin	Thermofisher	PA544877	WB (1:1000), IHC (1:50)
p-ERK (T202/Y204)	Cell Signaling Technologies	4370	WB (1:1000), IHC (1:50)
ERK	Cell Signaling Technologies	4695	WB (1:1000)
p-CDK1 (Y15)	Cell Signaling Technologies	4539	WB (1:1000), IHC (1:50)
CDK1	Cell Signaling Technologies	9116	WB (1:1000)
Cyclin B1	Cell Signaling Technologies	12231	WB (1:1000)
Lamin A/C	Cell Signaling Technologies	2032	WB (1:1000)
PCNA	Cell Signaling Technologies	13110	WB (1:1000)
Ki-67	US biological	C100511453	IHC (1:100)
Histone H3	Cell Signaling Technologies	4499	WB (1:1000)
Cyclin E1	Cell Signaling Technologies	20808	WB (1:1000)
CDC25A	Cell Signaling Technologies	3652	WB (1:1000)
phospho-Histone H3 (S10)	Cell Signaling Technologies	53348	WB (1:1000)
RAS (G12V Mutant Specific)	Cell Signaling Technologies	14412	WB (1:1000)
RAS (G12D Mutant Specific)	Cell Signaling Technologies	14429	WB (1:1000)
EGF Receptor (L858R Mutant Specific)	Cell Signaling Technologies	3197	WB (1:1000)
EGF Receptor (E746-A750del Specific)	Cell Signaling Technologies	2085	WB (1:1000)

Supplementary Table 2 : primers used on this report

Alternative Splicing RT-PCR primers			
Gene	Exon Covered	Forward	Reverse
<i>U2AF2</i>	Exon 1- Exon 3	GGA GAG CGC GCC ACA GAA C	ATC AGT CCA CCG TGC TCC T
	Exon 3- Exon 5	CAA ACC TTT GAC CAG AGG CGC T	CTC AGT GAT GCC AAA GGG GAT G
	Exon 8- Exon 10	GGGTTGTGTCCACTGTGGTCC	CTTTCCGCAGCCGGGCACCTC
<i>C1qTNF6</i>	Exon 2- Exon 4	GTC ACC ATG GGG ACA GCC G	TCA CAA AGA CCC TTT CGA AGA GC
	Exon 1- Exon 2	AGT ATT GCA GAC ATG GGC CAA G	CCT TCA GGA TGG TGA TAT TAA TGT
<i>SLC12A2</i>	Exon 20- Exon 22	AAG AAT TAT TGT CAT CAC AAG AGA	CTC CAT CAT CAA AAA GCC ACC AG
	Exon 3- Exon 5	GTA CGT TGT ATG TTA AAC ATT TGG	CTT AAG CAA CTC CAC CAC GGT TTC
<i>STXBP1</i>	Exon 17-19	GAC ACT ATT GAG GAC AAA CTT GAC AC	ACT GCT TAT TTC TTC ATC TGT TTT AT
	Exon 7- Exon 9	GGA ATA CAA GGA CAA TGC CCT GC	TGG GAC ACC TCT GCG ATG TGC
<i>IFT88</i>	Exon 7- Exon 9	GAT GGA GTT ACT AGA CCC ATG ACA GC	CAA TTT TAA GTC TCC ACA ACT ATT
	Exon 10- Exon 12	GAA TAT TGA AAA TGA ATA TGG GAA AT	CTT TCA CGT TCC ATT TGC CTG AG

<i>GAPDH</i>		ACC ACA GTC CAT GCC ATC ACT GC	GTT GAG GGC AAT GCC AGC CC
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Alternative Splicing qPCR Primers		
Gene	Forward	Reverse
<i>U2AF2</i>		
Exon 2	AGC GGG ACA AGG AGA ACC GG	CTG CGT CGT CGC CTG TCC
Exon 3	CAA ACC TTT GAC CAG AGG CG	ATC AGT CCA CCG TGC TCC T
Total	TAC GGG CTT GTC AAG TCC ATC G	CTG GCA GTC AAA CAC AGA GGT G
<i>FGFR2</i> (Sanidas et al., 2014)		
Exon IIIb	AAG TGC TGG CTC TGT TCA ATG T	GTA GTC TGG GGA AGC TGT AAT CTC
Exon IIIc	TGA GGA CGC TGG GGA ATA TAC G	TAG TCT GGG GAA GCT GTA ATC TCC T
<i>IWS1</i>	GAA CAG CAC TGG TGG TCA GAC ACC	AGG CAT TGG GAC CCT TGC ACG
<i>CDCA5</i>	CCA GCG GAA ATC AGG CTC TGA A	CGA TCC TCT TTA AGA CGA TGG GC
18S rRNA	GTA ACC CGT TGA ACC CCA TT	CCA TCC AAT CGG TAG TAG CG

<i>GAPDH</i>	CTG GCC AAG GTC ATC CAT GAC	CTT GCC CAC AGC CTT GGC AG
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<i>U2AF2</i> ChIP/RIP/ChIC Primers		
Gene	Forward	Reverse
<i>U2AF2</i>		
Exon 2	AGC GGG ACA AGG AGA ACC GG	CTG CGT CGT CGC CTG TCC
Exon 3	CAA ACC TTT GAC CAG AGG CG	ATC AGT CCA CCG TGC TCC T
TSS	CGA GAC CCAT CCT GGC TAA CAC	ACT GGA ACC TTC GCC TGC CG
<i>FGFR2</i> (Sanidas et al., 2014)		
Exon IIIb	AGC CCT TTA ATG CCG CTG TTT AGA	TAA CGG CCA ACC AGG AAG GTC TTA G
Exon IIIc	GAA TAT ACG TGC TTG GCG GGT AAT	TAA AAG GGG CCA TTT CTG ATA ACA
TSS	GGC AAC CCT CCC CGC AGT ATC AAG	GCT GGC CAA CGG CTC GCT GAG
<i>GAPDH</i>		
Exon 3	AGG TGG CCT AGG GCT GCT CAC ATA	GGC GCC CAA TAC GAC CAA ATC TAA

CDCA5 Splicing qPCR Primers (Watrin et. al., 2014)

Gene	Region Covered	Forward	Reverse
CDCA5	Exon 1-Intron 1	ACA GGT TCT AGA GAC AGC GAG	AGT TAT GTC TGG GAG GCG AA
	Exon 1- Exon 2	CGT AAG AAG AAG AAA ATG CCA GA	TCA AAC TCG GCA TTC ATG G
	Exon 2- Intron 2	AGA GGG ATG AAC GTG AGC TC	AGG GCC CCA TCT CCT ACT AA
	Exon 2- Exon 3	CAG AAA GCC CAT CGT CTT AAA	GGC TCG TTT TCT TTC TCC AA
GUSB	Exon 5-Intron 6	TTT GGC TGT CAT AGG TGC AG	CCC ACA GGG AGT GTG TAG AAG
	Exon 5- Exon 6	TTT CCG TAC CAG CCA CTA CC	ATC CCA TAG CGG TCA CAC AT

CDCA5 RNA IP Primers			
Gene	Region Covered	Forward	Reverse
CDCA5	Intron 1	GTC GAG CCG TTT GGG GGA CGC AGT	GGG CGC CTC CTC CGG CGG CGG C
	Intron 2	TGA GCC CCA GAT TTC TCA CTT CC	CTG GAG AAG AGG GAT GAA CGT GAG
	Intron 3	AAG GCT AAG GAG AGT GTT GTG GGT	CTG CGG GGG ACA ATA CCA ATT GA
GUSB	Intron 6	TAT GAA AAT TTG CTG GGC ATG GTG	GTG TGA TCT CAG CTC ACT GCA GCC

Mutagenesis Primers		
Gene	Forward	Reverse

SETD2 (R1625C)	AAA GGA AAT TGC TCT TGT TTC ATG AAT CAC AGC	GCT GTG ATT CAT GAA ACA AGA GCA ATT TCC TTT
LEDGF (A51P)	GGA ACT CAT GAG ACT CCT TTT TTA GGA CCA AAG	CTT TGG TCC TAA AAA AGG AGT CTC ATG AGT TCC
CDCA5 (S79A)	CAA TCA CCT CGC AGG GGC CCT AGG ATT TCC TTT	AAA GGA AAT CCT AGG GCC CCT GCG AGG TGA TTG
CDCA5 (S209A)	ACT CTC CCT GGA ATC GCC CCA CCA CCC GAG AAA	TTT CTC GGG TGG TGG GGC GAT TCC AGG GAG AGT
CDCA5 (S79E)	CAA TCA CCT CGC AGG GAG CCT AGG ATT TCC TTT	AAA GGA AAT CCT AGG CTC CCT GCG AGG TGA TTG
CDCA5 (S209E)	ACT CTC CCT GGA ATC GAG CCA CCA CCC GAG AAA	TTT CTC GGG TGG TGG CTC GAT TCC AGG GAG AGT
AKT3 (T305D)	GCA GCC ACC ATG AAG GAC TTC TGT GGC ACT CCA	TGG AGT GCC ACA GAA GTC CTT CAT GGT GGC TGC
AKT3 (S472D)	CAT TTC CCT CAA TTT GAC TAC TCT GCA AGT GGA	TCC ACT TGC AGA GTA GTC AAA TTG AGG GAA ATG
IWS1 (S720/T721AA)	Sanidas et al., 2014	
IWS1 (S720/T721DE)	Sanidas et al., 2014	

Constructs Primers		
Gene	Forward	Reverse
U2AF2 with V5-tag	CAC CAT GTC GGA CTT CGA CGA GTT CG	CCA GAA GTC CCG GCG GTG ATA AGA GTC
p75/LEDGF	CAC CAT GGG AAC TCG CGA TTT CAA A	GTT ATC TAG TGT AGA ATC CTT
pENTR AKT3 HA	CACC ATG GGA AGC GAT GTT ACC ATT GTG	TTA AGC GTA ATC TGG AAC ATC GTA TGG GTA TTC TCG TCC ACT TGC AGA

Supplementary Table 3 : Constructs, siRNA and shRNA used in this report.

Constructs	Company/Source
pLenti CMV Blast DEST Flag-IWS1	Sanidas et. al., 2014
pBabe puro myr-AKT3 HA	Addgene 9019
pDONR201 AKT3	DNAsu Clone : HsCD00005031
pLx304 KDM4A-V5	DNAsu Clone :DmCD00768909
pLx304 KDM4B-V5	DNAsu Clone : HsCD00439656
pBabe puro KDM4C-HA	pBabe puro GASC1-HA, Addgene 24214
pDONR221 p52/LEDGF	DNAsu Clone : HsCD00000034
pLx304 SRSF1-V5	DNAsu Clone : HsCD00434418
pLx304 CDCA5-V5	DNAsu Clone : HsCD00434807

siRNAs	Company
siRNA negative control	Sigma-Millipore SIC001
siNSD1	Sigma-Millipore EHU081541
siNSD2	Sigma-Millipore EHU063351
siNSD3	Sigma-Millipore EHU059551
siSMYD2	Sigma-Millipore EHU049591
siASH1L	Sigma-Millipore EHU137581
siPHF1	Sigma-Millipore EHU092931
siBRPF1	Sigma-Millipore NM_004634 siRNA ID : SASI_Hs01_00189588
siGLYR1	Sigma-Millipore EHU110341
siMSH6	Sigma-Millipore EHU049441
siLEDGF	Sigma-Millipore EHU114381

shRNAs	Company
Non-silencing-GIPZ lentiviral shRNA mir control	Open Biosystems, cat. No RHS4346
pGIPZ shIWS1	Open Biosystems, cat. No RSH4430-101101545, clone ID V3LHS_389019 (<u>shRNA resistant clone, Sanidas et. al., 2014</u>)
pLKO.1 empty vector control	Open Biosystems, cat. No RSH4080

pLKO.1 shAKT1	Sigma, cat. No NM_005163.1-628s1c1, Clone ID: TRCN0000039797
pLKO.1 shAKT2	Sigma, cat. No NM_001626.3-4971s21c1, Clone ID : TRCN0000255916
pLKO.1 shAKT3	Sigma, cat. No NM_005465.3, Clone ID : TRCN0000196520
pLKO.1 shSETD2	Open Biosystems, cat. No. RSH 3979-9571647, clone ID TRCN0000003029
pLKO.1 shMRG15	Sigma, cat. No NM_006791.2-1239s1c1, Clone ID : TRCN0000107580
pLKO.1 shLEDGF	Sigma, cat. No NM_021144.2-1513s1c1, , Clone ID : TRCN0000074818
pLKO.1 shPTBP1	Sigma, cat. No NM_002819.3-3120s21c1, clone ID TRCN0000231421
pLKO.1 shPTBP2	Sigma, cat. No NM_021190.x-762s1c1, Clone ID : TRCN0000001111
pLKO.1 shSRSF1	Sigma, cat. No NM_006924.4-2084s21c1, clone ID TRCN0000272898

Supplementary Table 4 : Results of FACS analysis in all biological replicates.

Cell line	Condition	G0/G1			S			G2/M		
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
NCI-H522	shControl	57.92	59.5	60.77	12.67	9.82	10.55	25.85	28.06	26.11
	shIWS1	37.48	38.89	37.42	7.17	7.52	6.93	49.85	48.15	49.56
	shIWS1/U2AF65 α	58.33	58.39	57.79	7.55	8.2	8.98	29.9	29.21	28.95
	shIWS1/U2AF65 β	36.53	37.73	39.88	7.82	8.13	6.47	49.11	48.29	47.64
NCI-H1299	shControl	61.74	64.23	63.66	22.32	22.23	22.08	14.09	11.63	11.99
	shIWS1	44.91	45.59	48.09	22.02	22.28	23.72	28.82	28.01	24.38
	shIWS1/U2AF65 α	54.12	54.87	51.92	24.85	24.31	25.34	19.07	18.8	19.71
	shIWS1/U2AF65 β	43.21	44.3	43.96	26.83	25.04	24.25	24.97	26.5	28.17
A549	shControl	63.93	64.39	65.87	22.25	20.39	20.48	12.83	14.19	12.15
	shIWS1	53.86	60.18	59.73	19.2	10.14	8.84	22.37	22.16	25.1
	shIWS1/U2AF65 α	69.04	65.23	64.57	11.31	15.44	14.23	12.16	15.21	19.71
	shIWS1/U2AF65 β	57.31	56.97	59.76	11.54	12.14	12.21	24.94	25.52	24.51
NCI-H1975	shControl	46.31	46.89	46.11	17.28	10.94	10.93	28.91	31.35	34.72
	shIWS1	24.48	24.08	25.44	5.38	6.14	5.72	53.15	50.34	50.3
	shIWS1/U2AF65 α	69.04	65.23	64.57	11.31	15.44	14.23	12.16	15.21	19.71
	shIWS1/U2AF65 β	57.31	56.97	59.76	11.54	12.14	12.21	24.94	25.52	24.51

Supplementary Table 5 : Correlations of the IWS1/Sororin pathway in human LUAD**samples.****Figure 8a : OSU LUAD/All Patients Cohort**

The R² coefficient values derived from a simple linear regression are shown. The values in parenthesis describe the p value. The statistics were performed in Graph Pad Prism 8.4

	IWS1	p-IWS1	U2AF2 E2/E3	CDCA5	p-ERK	p-CDK1	CDK1	Cyclin B1	AKT3	p-AKT
IWS1	1	0.7556 (0.0001)	0.8051 (0.0001)	0.7371 (0.0001)	0.4587 (0.0001)	0.5332 (0.0001)	0.2926 (0.0003)	0.254 (0.0009)	0.4021 (0.0001)	0.2671 (0.0007)
p-IWS1	0.7556 (0.0001)	1	0.7206 (0.0001)	0.6668 (0.0001)	0.5418 (0.0001)	0.4812 (0.0001)	0.2501 (0.0010)	0.3171 (0.0002)	0.4321 (0.0001)	0.4127 (0.0001)
U2AF2 E2/E3	0.8051 (0.0001)	0.7206 (0.0001)	1	0.5825 (0.0001)	0.5192 (0.0001)	0.3875 (0.0001)	0.4332 (0.0016)	0.4059 (0.0033)	0.3305 (0.0001)	0.221 (0.0022)
CDCA5	0.7371 (0.0001)	0.6668 (0.0001)	0.5825 (0.0001)	1	0.5985 (0.0001)	0.5582 (0.0001)	0.4749 (0.0001)	0.6392 (0.0001)	0.3787 (0.0001)	0.3503 (0.0001)
p-ERK	0.4587 (0.0001)	0.5418 (0.0001)	0.5192 (0.0001)	0.5985 (0.0001)	1	0.4955 (0.0001)	0.3524 (0.0001)	0.6174 (0.0001)	0.3011 (0.0002)	0.4218 (0.0001)
p-CDK1	0.5332 (0.0001)	0.4812 (0.0001)	0.3875 (0.0001)	0.5582 (0.0001)	0.4955 (0.0001)	1	0.422 (0.0001)	0.471 (0.0001)	0.3074 (0.0002)	0.2314 (0.0017)
CDK1	0.2926 (0.0003)	0.2501 (0.001)	0.4332 (0.0016)	0.4749 (0.0001)	0.3524 (0.0001)	0.422 (0.0001)	1	0.6086 (0.0001) 6	0.1478 (0.0143)	0.1427 (0.0163)
Cyclin B1	0.254 (0.0009)	0.3171 (0.0002)	0.4059 (0.0033)	0.6392 (0.0001)	0.6174 (0.0001)	0.471 (0.0001)	0.6086 (0.0001)	1	0.3853 (0.0001)	0.393 (0.0001)
AKT3	0.4021 (0.0001)	0.4321 (0.0001)	0.3305 (0.0001)	0.3787 (0.0001)	0.3011 (0.0002)	0.3074 (0.0002)	0.1478 (0.043)	0.3853 (0.0001)	1	0.4374 (0.0001)
p-AKT	0.2671 (0.0006)	0.4127 (0.0001)	0.221 (0.0022)	0.3503 (0.0001)	0.4218 (0.0001)	0.2314 (0.0017)	0.1427 (0.0163)	0.393 (0.0001)	0.4374 (0.0001)	1

Figure 8a : OSU LUAD/KRAS Cohort

The R^2 coefficient values derived from a simple linear regression are shown. The values in parenthesis describe the p value. The statistics were performed in Graph Pad Prism 8.4

	IWS1	p-IWS1	U2AF2 E2/E3	CDCA5	p-ERK	p-CDK1	CDK1	Cyclin B1	AKT3	p-AKT
IWS1	1	0.7386 (0.0001)	0.6991 (0.0002)	0.7905 (0.0001)	0.3932 (0.0164)	0.261 (0.0619)	0.2677 (0.0581)	0.2807 (0.0513)	0.3621 (0.0211)	0.214 (0.0958)
p-IWS1	0.7386 (0.0001)	1	0.7046 (0.0002)	0.6312 (0.0007)	0.3017 (0.0419)	0.1973 (0.1115)	0.1207 (0.2236)	0.1421 (0.1839)	0.5512 (0.0024)	0.3901 (0.017)
U2AF2 E2/E3	0.6991 (0.0002)	0.7046 (0.0002)	1	0.6763 (0.0003)	0.4465 (0.009)	0.2381 (0.0767)	0.1153 (0.235)	0.2181 (0.0923)	0.3601 (0.0233)	0.3131 (0.0375)
CDCA5	0.7905 (0.0001)	0.6312 (0.0007)	0.6763 (0.0003)	1	0.3475 (0.0265)	0.19 (0.1193)	0.1863 (0.1233)	0.2313 (0.0817)	0.3077 (0.0395)	0.3963 (0.0159)
p-ERK	0.3932 (0.0164)	0.3017 (0.0419)	0.4465 (0.009)	0.3475 (0.0265)	1	0.1449 (0.1794)	0.1122 (0.2418)	0.1769 (0.1343)	0.195 (0.1139)	0.5205 (0.0054)
p-CDK1	0.261 (0.0619)	0.1973 (0.1115)	0.2381 (0.0767)	0.19 (0.1193)	0.1449 (0.1794)	1	0.2542 (0.066)	0.005 (0.4015)	0.0006 (0.9314)	0.017 (0.6566)
CDK1	0.2677 (0.0581)	0.1207 (0.2236)	0.1153 (0.235)	0.1863 (0.1233)	0.1122 (0.2418)	0.2542 (0.066)	1	0.3852 (0.0236)	0.04317 (0.8389)	0.0002 (0.8691)
Cyclin B1	0.2807 (0.0513)	0.1421 (0.1839)	0.2181 (0.0923)	0.2313 (0.0817)	0.1769 (0.1343)	0.005 (0.4015)	0.3852 (0.0236)	1	0.1615 (0.1544)	0.1439 (0.181)
AKT3	0.3621 (0.0211)	0.5512 (0.0024)	0.3601 (0.0233)	0.3077 (0.0395)	0.195 (0.1139)	0.0006 (0.9314)	0.04317 (0.8389)	0.1615 (0.1544)	1	0.5339 (0.003)
p-AKT	0.214 (0.0958)	0.3901 (0.017)	0.3131 (0.0375)	0.3963 (0.0159)	0.5205 (0.0054)	0.017 (0.6566)	0.0002 (0.8691)	0.1439 (0.181)	0.5339 (0.003)	1

Figure 8a : OSU LUAD/EGFR Cohort

The R^2 coefficient values derived from a simple linear regression are shown. The values in parenthesis describe the p value. The statistics were performed in Graph Pad Prism 8.4

	IWS1	p-IWS1	U2AF2 E2/E3	CDCA5	p-ERK	p-CDK1	CDK1	Cyclin B1	AKT3	p-AKT
IWS1	1	0.9408 (0.0001)	0.7789 (0.0001)	0.7434 (0.0001)	0.5205 (0.0054)	0.6277 (0.0012)	0.4452 (0.0127)	0.7359 (0.0002)	0.7271 (0.0002)	0.6338 (0.0011)
p-IWS1	0.9408 (0.0001)	1	0.8248 (0.0001)	0.7533 (0.0001)	0.6166 (0.0015)	0.5847 (0.0023)	0.6519 (0.0008)	0.6573 (0.008)	0.8205 (0.0001)	0.7777 (0.0001)
U2AF2 E2/E3	0.7789 (0.0001)	0.8248 (0.0001)	1	0.5081 (0.0062)	0.7101 (0.0003)	0.4137 (0.0177)	0.3756 (0.0259)	0.6139 (0.0015)	0.6447 (0.001)	0.6606 (0.0007)
CDCA5	0.7434 (0.0001)	0.7533 (0.0001)	0.5081 (0.0062)	1	0.517 (0.0056)	0.6928 (0.0004)	0.3753 (0.026)	0.7284 (0.0002)	0.7823 (0.0001)	0.7434 (0.0001)
p-ERK	0.5205 (0.0054)	0.6166 (0.0015)	0.7101 (0.0003)	0.517 (0.0056)	1	0.5413 (0.0041)	0.4558 (0.0113)	0.6843 (0.0005)	0.474 (0.0093)	0.6642 (0.0004)
p- CDK1	0.6277 (0.0012)	0.5847 (0.0023)	0.4137 (0.0177)	0.6928 (0.0004)	0.5413 (0.0041)	1	0.3681 (0.0279)	0.7626 (0.0001)	0.6424 (0.001)	0.4683 (0.0099)
CDK1	0.4452 (0.0127)	0.6519 (0.0008)	0.3756 (0.0259)	0.3753 (0.026)	0.4558 (0.0113)	0.3681 (0.0279)	1	0.5497 (0.0024)	0.5622 (0.0032)	0.4099 (0.0184)
Cyclin B1	0.7359 (0.0002)	0.6573 (0.008)	0.6139 (0.0015)	0.7284 (0.0002)	0.6843 (0.0005)	0.7626 (0.0001)	0.5497 (0.0024)	1	0.6626 (0.0007)	0.4479 (0.0124)
AKT3	0.7271 (0.0002)	0.8205 (0.0001)	0.6447 (0.001)	0.7823 (0.0001)	0.474 (0.0093)	0.6424 (0.001)	0.5622 (0.0032)	0.6626 (0.0007)	1	0.6504 (0.0009)
p-AKT	0.6338 (0.0011)	0.7777 (0.0001)	0.6606 (0.0007)	0.7434 (0.0001)	0.6642 (0.0004)	0.4683 (0.0099)	0.4099 (0.0184)	0.4479 (0.0124)	0.6504 (0.0009)	1

Supplementary Figure 12g : TMA LUAD/All patients cohort

The R^2 coefficient values derived from a simple linear regression are shown. The values in parenthesis describe the p value. The statistics were performed in Graph Pad Prism 8.4

	p-IWS1	CDCA5	p-ERK	p-CDK1
p-IWS1	1	0.5614 (<0.0001)	0.5786 (<0.0001)	0.4421 (<0.0001)
CDCA5	0.5614 (<0.0001)	1	0.7288 (<0.0001)	0.315 (<0.0001)
p-ERK	0.5786 (<0.0001)	0.7288 (<0.0001)	1	0.3707 (<0.0001)
p-CDK1	0.4421 (<0.0001)	0.315 (<0.0001)	0.3707 (<0.0001)	1

Supplementary Figure 12g : TMA LUAD/EGFR cohort

The R^2 coefficient values derived from a simple linear regression are shown. The values in parenthesis describe the p value. The statistics were performed in Graph Pad Prism 8.4

	p-IWS1	CDCA5	p-ERK	p-CDK1
p-IWS1	1	0.7513 (<0.0001)	0.7931 (<0.0001)	0.5139 (0.0026)
CDCA5	0.7513 (<0.0001)	1	0.834 (<0.0001)	0.7104 (<0.0001)
p-ERK	0.7931 (<0.0001)	0.834 (<0.0001)	1	0.765 (<0.0001)
p-CDK1	0.5139 (0.0026)	0.7104 (<0.0001)	0.765 (<0.0001)	1

Supplementary Table 6 : TCGA-LUAD Patients used as primary tumors for the GSE141685 dataset

TCGA-LUAD ID	Clinical Stage	EGFR mut	KRAS mut
TCGA-38-4632	Stage IV	No	No
TCGA-55-6968	Stage IV	Yes	No
TCGA-64-1680	Stage IV	No	No
TCGA-78-7145	Stage IV	No	Yes
TCGA-78-7167	Stage IV	No	Yes
TCGA-93-A4JP	Stage IV	Yes	No
TCGA-99-8033	Stage IV	No	Yes
TCGA-05-4398	Stage IIIB	No	No
TCGA-05-5420	Stage IIIA	No	No
TCGA-44-5643	Stage IIIA	No	No
TCGA-49-4512	Stage IIIA	No	No
TCGA-50-5941	Stage IIIA	No	Yes
TCGA-44-8117	Stage IB	No	Yes
TCGA-44-A47A	Stage IB	No	Yes
TCGA-50-6597	Stage IB	No	No
TCGA-55-7570	Stage IA	Yes	No
TCGA-55-8301	Stage IB	No	No
TCGA-62-A46S	Stage IB	No	Yes
TCGA-69-7979	Stage IB	No	No
TCGA-75-7031	Stage IB	No	No

TCGA-91-6828	Stage IA	No	Yes
TCGA-91-8499	Stage IA	No	No
TCGA-97-8175	Stage IB	No	No
TCGA-O1-A52J	Stage IA	No	No