SF3B1^{K700E} Drives Oncogenic Alternative Splicing of Cell Cycle Checkpoint Genes in Cancer

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Keywords: pre-mRNA splicing, SF3B1, cell cycle checkpoint, cancer.

Abstract

Pre-mRNA splicing plays a crucial role in maintaining cellular homeostasis, with strict regulation required for processes such as cell cycle progression. SF3B1, a core component of the spliceosome, has emerged as a key player in alternative splicing regulation and is frequently mutated in cancer. Among these mutations, SF3B1^{K700E} disrupts normal splicing patterns and deregulates cell cycle control. In this study, we investigated the impact of SF3B1 phosphorylation on cell cycle-dependent splicing, with a particular focus on the checkpoint regulators ARPP19 and ENSA, two functionally related proteins that modulate PP2A-B55 activity during mitotic progression. Using RNA-seq analysis, we identified a subset of SF3B1-regulated splicing events that are enriched in cell cycle genes. We

demonstrated that SF3B1 phosphorylation during G2/M promotes the ARPP19-long isoform, a variant associated with poor survival in AML patients and accelerated mitotic exit. Notably, we have shown that inhibition of SF3B1 reverses the splicing changes induced by SF3B1^{K700E}, reducing the levels of ARPP19-long, which suggests a potential therapeutic strategy for cancers with spliceosome mutations. Our findings highlight a direct link between SF3B1-dependent splicing, cell cycle progression, and tumorigenesis, providing new insights into the molecular mechanisms underlying cancer-associated splicing dysregulation.

Background

Pre-mRNA splicing is facilitated by the spliceosome, a dynamic ribonucleoprotein complex composed of U1, U2, U4, U5, and U6 small nuclear ribonucleoproteins (snRNPs) along with numerous accessory proteins. Splicing occurs in a stepwise manner, beginning with the recognition of key sequence elements in the pre-mRNA, including the 5' splice site, 3' splice site, and branch point motif. The spliceosome undergoes conformational changes and catalytic activation to excise the intron and ligate the exons together, generating mature mRNA. SF3B1, a U2 snRNP-associated protein, plays a critical role in this process by stabilizing U2 binding to the branch point motif, ensuring proper recognition of the 3' splice site. During spliceosome activation, SF3B1 undergoes phosphorylation at T313 and T434 by DYRK1A, which may influence its interaction with other spliceosomal components [1-4]. Additional phosphorylation sites at T244 and T248 have been identified through both low- and high-throughput approaches and are thought to promote interaction with the protein phosphatase PPP1R8 (NIPP1), suggesting a potential regulatory mechanism governing SF3B1 function [5, 6].

Recent studies have connected SF3B1 phosphorylation to cell cycle progression, with high phosphorylation levels observed in G2/M due to CDK2 activity, while lower phosphorylation levels are seen in G1/S due to the action of the PP2A and PP1 phosphatases [7], with specific phosphorylation primarily mediated by CDK1 [7, 8]. Additionally, PP1-mediated dephosphorylation of SF3B1 at T142p enables mitotic exit.

The critical role of SF3B1 in proper cell cycle progression is underscored by its knock-down, which leads to G2/M arrest [9-11]. Furthermore, SF3B1 inhibitors, also known as splicing inhibitors, induce cell cycle arrest at S and M phases [12, 13]. Notably, SF3B1 is the most commonly mutated component of the spliceosome in cancer. While the precise role of SF3B1 in cancer is still under investigation, its high prevalence in cancer cases strongly suggests its involvement in cell cycle progression [14, 15].

Non-driver mutations in SF3B1 are frequently observed in myelodysplastic syndromes (MDS) and other neoplasms affecting myeloid and lymphoid cells [16]. The most prevalent mutation is a heterozygous missense substitution known as K700E ($SF3B1^{K700E}$). Additionally, SF3B1 mutations have been identified in various solid tumors, including uveal melanoma, breast cancer, pancreatic cancer, and prostate cancer [17]. This suggests that SF3B1 mutations promote cell cycle progression, in contrast to its knockdown or inhibition. Consequently, SF3B1 inhibitors have been proposed as potential therapeutic agents for

cancer [18], with compounds like H3B-8800 and E7107 exhibiting a pronounced effect on SF3B1^{K700E} cells [19-21].

Phosphorylation of splicing factors has been shown to exert regulatory effects on protein-protein interactions both within the spliceosome and between splicing factors and pre-mRNA molecules [18]. Furthermore, it is well-established that alternative splicing undergoes dynamic regulation across the cell cycle phases [17, 22]. However, the specific mechanism by which SF3B1 regulates the cell cycle is not clear.

In this study, we identified SF3B1-regulated splicing events in cell cycle checkpoint genes and demonstrated that SF3B1 controls their splicing in a cell cycle-dependent manner. Through RNA-seq analysis of SF3B1^{K700E} mutant and wild-type K562 cells, we found and validated a subset of SF3B1 targets enriched in cell cycle regulation. While our results revealed multiple splicing targets with roles in the cell cycle, the mitotic regulator ARPP19 emerges as the most robust target. Our findings show that SF3B1 phosphorylation during the G2/M phase promotes the splicing of ARPP19-long isoform. Furthermore, inhibition of SF3B1 by pladienolide B mimics the effects of SF3B1 knockdown, reducing ARPP19-long levels. Importantly, we show that ARPP19-long correlates with poor survival in acute myeloid leukemia (AML) patients and accelerates mitotic exit, suggesting a potential oncogenic role of this isoform. These findings provide mechanistic insights into how SF3B1 regulates cell cycle progression through alternative splicing, establishing a direct link between spliceosome function, cell cycle control, and cancer progression.

Material and methods

Cell lines

HeLa (ATCC number: CRM-CCL-2) cells were grown in Dulbecco's modified Eagle's medium (DMEM). K562 (ATCC number: CCL-243TM), K562-WT and K562-SF3B1K700E cells were grown in Roswell Park Memorial Institute's medium (RPMI-1640). Media was supplemented with 10% fetal bovine serum (FBS), 2 mM l-glutamine, 0.1 mg/ml penicillin and 0.1 mg/ml streptomycin. Cell lines were maintained at 37°C and 5% CO2 atmosphere.

RNA sequencing and analysis

Total RNA samples for three biological replicates (approximately 1×10^6 cells) from K562-WT and K562-SF3B1 K700E cells were subjected to sequencing. Raw reads were processed for quality trimming and adaptors removal using fastx_toolkit v0.0.14 and cutadapt v2.10. The

processed reads were aligned to the human transcriptome and genome version GRCh37 using TopHat v2.1. Reads were counted with featureCounts version 2.0.1 using GENCODE v47. DESeq2 [23] was used to identify differentially expressed genes using FDR threshold of 0.1. rMATS (version 4.1.2) [24] was used to identify differential alternative splicing events. For each alternative splicing event, we used the calculation on both the reads mapped to the splice junctions and the reads mapped to the exon body [Junction Counts and Exon Counts (JCEC)] and filtered events by FDR threshold of 0.1. Gene set enrichment analysis was performed using the GeneAnalytics [25] online tool. FDR was controlled by the Benjamini-Hochberg procedure.

RNA interference

A pool of four esiRNA oligomers against SF3B1 was purchased from Sigma-Aldrich (EHU131531). HeLa cells were grown to 20–30% confluence and transfected with 20 nM siRNA (esiSF3B1 or esiGFP for control) using TransIT-X2 transfection reagent (Mirus) following the manufacturer's instructions. After 24 h of incubation, cell culture media was replaced and then incubated for additional 48-72 h. Knockdown efficiencies were verified by qPCR.

qRT-PCR

RNA was isolated from cells using the GENEzol TriRNA Pure Kit (GeneAid). cDNA synthesis was carried out with the qScript cDNA Synthesis Kit (QuantaBio). qPCR was performed with the iTaq Universal SYBR Green Supermix (BioRad) on the Biorad iCycler. The comparative Ct method was employed to quantify transcripts, and delta Ct was measured in triplicate. Primers used in this study are provided in Supplementary Table S1.

Cell cycle arrest

For G1/S synchronization, HeLa and K562 cells were cultured in DMEM with thymidine (T1895, Sigma-Aldrich) at a final concentration of 2 mM for 18 h. Cells were then washed with DMEM twice and cultured for 9 h in DMEM alone. Thymidine was then added at the same concentration and the cells were cultured for a further 18 h. For G2/M synchronization, HeLa and K562cells were cultured in DMEM with 40 ng/ml of nocodazole (NCO) (Sigma-Aldrich, M1404). Pharmacological inhibitors were added to G1/S or to G2/M synchronized cells as follows: okadoic acid (protein phosphatases inhibitor, Sigma-Aldrich, 39302), 20 nM for 12 h; purvalanol A (CDK1 inhibitor, Sigma-Aldrich, P4484), 15 μM for 6 h;

INDY (DRKY1A inhibitor, Sigma-Aldrich, SML1011) 5 µM for 6 h. The cell cycle status was checked using flow cytometry.

Immunoblotting

For immunoblotting, cells were harvested and lysed with RIPA lysis buffer supplemented with protease and phosphatase inhibitors, and 20 µg/µl of the extracts were run on a 10% TGX Stain-free FastCast gel (BioRad) and transferred onto a nitrocellulose membrane.

Survival analysis

OncoSplicing [26] online analysis pipeline was used for splicing-based survival analysis and Kaplan-Mayer plot creation. Analysis was performed using SpliceSeq database PSI values and default optimal cutoffs. GEPIA [https://doi.org/10.1093/nar/gkx247] was used for expression-based survival analysis.

Viral infection

ARPP19 and ENSA short and long isoforms were cloned into the p-Lenti-CMV-GFP vector (Addgene, plasmid #17448). Lentiviral or retroviral particles were produced by transfecting HEK293T packaging cells with plasmids expressing ARPP19 and ENSA short or long isoforms and transfections were carried out using TransIT-X2 transfection reagent. HeLa cells were subsequently infected with the viral particles, and stable integrations were selected.

Flow cytometry

Cells were trypsinized, washed with PBS, fixed overnight at −20°C with 70% ethanol in PBS, washed with PBS, and left for 30 min at 4°C. The cell suspension was then incubated with PBS containing 5 µg/mL DNase-free RNase and stained with propidium iodide (PI). Data were acquired using the LSRII Fortessa Analyzer machine at 10,000 events/sample. The percentage of cells in each cell-cycle phase was determined using Flow Jo software.

Pharmacological inhibition of SF3B1

HeLa cells were maintained in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified 5% CO_2 incubator. Cells were seeded at a density of 1–2 × 10^5 cells/mL in culture plates and allowed to grow overnight. Pladienolide B was dissolved in dimethyl sulfoxide (DMSO) to prepare a 10 mM stock solution. For treatment, pladienolide B was diluted in culture medium to the desired working concentration (100

nM). Cells were treated with pladienolide B or an equivalent volume of DMSO (vehicle control) for 6 h.

Results

SF3B1 regulates splicing of cell cycle genes

SF3B1 function is critical for proper cell cycle progression. To search for splicing targets of SF3B1 with a pivotal role during the cell cycle, we analyzed RNA-seq data from WT and SF3B1^{K700E} K562 cells. This comprehensive examination unveiled 22,136 alternative splicing events across 6,738 genes. These events encompassed various types, with 40% involving exon exclusion (8,953 events), 5% alternative 5' splice sites (1,098 events), 16% alternative 3' splice sites (3,583 events), 33% mutually exclusive exons (7,408 events), and 4% intron retention (1,094 events) (Fig. 1A and Supplementary Fig. S1A, FDR < 5%, rMATS, Supplementary Table S2). Our findings validate prior reports of widespread alterations in 3' splice site usage in SF3B1^{K700E}-induced chronic lymphocytic leukemia (CLL) and K562 cells [27].

Gene expression analysis identified 790 differentially expressed genes, with 599 upregulated genes in SF3B1^{K700E} and 191 downregulated genes. Upregulated genes were enriched for Akt signaling (34 genes), angiogenesis (11 genes) and other signaling pathways while downregulated genes showed enrichment for immune and metabolic pathways (Supplementary Fig. S1B). 185 differentially expressed genes are also alternatively spliced ($P < 0.00001$, Fisher's exact test, Fig. 1B, Supplementary Table S3).

From the 6,738 genes regulated in alternative splicing, we found enrichment for genes involved in cell cycle (665 genes), DNA damage response (535 genes) and translation (355 genes) (Fig. 1C). To elucidate the role of SF3B1 during the cell cycle, we selected seven alternative splicing events in cell cycle checkpoint genes for validation (Supplementary Fig. S1C-H. Out of the seven events chosen, we successfully validated five (Fig. 1D and Supplementary Fig. S2A); STAG2, ENSA, and ARPP19, which play roles in the M-phase, and SEPTIN6 and ECT2, which are involved in the S-phase.

To further confirm that SF3B1 regulates the splicing of the five previously validated events, we silenced SF3B1 in HeLa cervical cancer cells using siRNA (Supplementary Fig. S2B). Given that SF3B1^{K700E} is a gain-of-function mutation, we hypothesized that silencing SF3B1 would produce an opposite effect on splicing. Indeed, we observed the opposite effect for

STAG2, ENSA, ARPP19, and ECT2. SEPTIN6 exhibited mild exon 10 inclusion in SF3B1^{K700E} K562 cells, and a similar trend was seen in HeLa cells upon SF3B1 silencing (20% reduction, Fig. 1E). Additionally, we observed a decrease in total mRNA levels for ENSA, ARPP19, ECT2, and SEPTIN6 in HeLa cells, which was not seen in SF3B1^{K700E} (Supplementary Fig. S2C). These findings further support the role of SF3B1 in regulating alternative splicing of key cell cycle genes and note a potential alteration of total mRNA levels by SF3B1.

Phosphorylation of SF3B1 plays a key role in the regulation of splicing for cell cycle checkpoint genes

As SF3B1 undergoes phosphorylation in a cell cycle-dependent manner, we hypothesize that the splicing of its cell cycle checkpoint targets is influenced by its phosphorylation status at different stages of the cycle. To test this hypothesis, we synchronized K562 and HeLa cells in the G1/S and G2/M phases and analyzed the splicing events regulated by SF3B1 (Fig. 2A-H). Results were consistent in both cell lines for ARPP19 exon 2 inclusion, which gives rise to ARPP19-long isoform, with respectively 60% and 40% reduction in G1/S in K562 and Hela cells (Fig. 2A&E). In G2/M, we saw a 40% increase in ARPP19-long in both cell lines (Fig. 2C&G). Total mRNA amount of ARPP19 and ENSA decreased in K562 G2/M cells (Supplementary Fig. S3B). These findings suggest that ARPP19-long isoform is likely regulated by SF3B1 in a cell cycle-dependent manner.

To check whether SF3B1 splicing regulation is dependent on its phosphorylation, we inhibited SF3B1's known kinases and phosphatases and monitored the splicing of its identified checkpoint targets. This analysis encompassed both synchronized and unsynchronized cells to discern the significance of phosphorylation at specific cell cycle stages. We started with the kinase DYRK1A, known to have a role in G1/S and to phosphorylate SF3B1 on T434 [4]. To this end, we arrested K562 and HeLa cells in G1/S and upon release, cells were treated with the DYRK1A/B inhibitor INDY for 6 h. FACS analysis revealed that the treatment did not affect cell cycle progression in naïve cells or following arrest (Fig. 3A&D), a critical observation considering the sensitivity of splicing of certain targets to the cell cycle stage. Our results show that DYRK1A inhibition in naïve cells or in G1/S reduces the amount of ARPP19-long by around 30% in both cell lines (Fig. 3B&E). In addition, we observed a mild reduction in ENSA exon 3 inclusion in HeLa cells (Fig. 3F). This result points at DYRK1A phosphorylation of SF3B1 as important to ARPP19 exon 2 alternative splicing.

We next assessed the role of CDK1 phosphorylation, which is known to phosphorylate SF3B1 during the G2/M phase [7, 8]. To investigate this, we arrested K562 and HeLa cells at G2/M and treated them with the CDK1 inhibitor purvalanol A for 6 h. Monitoring the cell cycle progression revealed no change in the cell cycle stage following CDK1 inhibition (Fig. 4A&D). Our results indicate that CDK1 inhibition in both unsynchronized cells (predominantly in G1) and synchronized cells (predominantly in G2) promotes ARPP19 exon 2 inclusion and ENSA exon 3 exclusion (Fig. 4B-F). These findings suggest that CDK1 phosphorylation of SF3B1 plays a role in regulating alternative splicing events in the cell cycle.

Another factor influencing SF3B1 phosphorylation is PP2A, which dephosphorylates SF3B1 during exit from mitosis, particularly in the M-to-G1 transition. To investigate its effect on splicing, we used its inhibitor, okadaic acid, in both naïve K562 and HeLa cells, as well as following synchronization at G1/S. We began by arresting the cells in G1 phase and, upon release, treated them with okadaic acid for 12 h. Our results show a 70% increase in ARPP19 exon 2 inclusion in unsynchronized cells (predominantly in G1) and a 20% increase in ARPP19 exon 2 inclusion in synchronized cells (enriched for G2/M) (Figure 5A-E). Our investigation aimed to understand the impact of SF3B1 phosphorylation on the regulation of checkpoint factor splicing. Although we measured splicing changes in five genes, our results were particularly robust for ARPP19. These findings suggest a potential correlation between SF3B1 phosphorylation and the alternative splicing of ARPP19.

Alternative splicing of ARPP19 is associated with AML patient survival.

We hypothesize that SF3B1^{K700E} promotes aberrant splicing of checkpoint genes, which contributes to cancer progression. To evaluate whether the isoforms induced by SF3B1 could serve as a prognostic factor for AML, we performed a survival analysis of SF3B1 splicing targets using clinical RNA-seq datasets from TCGA [28, 29]. Our results indicate that ARPP19 isoform-based survival analysis serves as a significant predictor for prognosis while gene expression-based analysis does not (Fig. 6A&B). In contrast, low expression of ENSA serves as a survival predictor, while its exon 3 splicing does not (Fig. 6C&D). Specifically, in AML, increased inclusion of ARPP19 exon 2, resulting in the long isoform (ARPP19-long), correlates with poorer survival compared to ARPP19-short. We observed that ARPP19-long is enriched during G2/M and is promoted by phosphorylated SF3B1. Next, we aimed to investigate the role of this isoform in the cell cycle.

SF3B1 inhibition by pladienolide B promotes the ARPP19-long isoform

Given the correlation between ARPP19-long isoform and poor AML survival, we next examined whether SF3B1 inhibition could influence ARPP19 splicing, as it is speculated to increase survival [13]. Since SF3B1^{K700E} promotes ARPP19 exon 2 inclusion (Fig. 1C), we hypothesized that pharmacological inhibition of SF3B1 would have the opposite effect, leading to a reduction in ARPP19-long isoform expression, similar to SF3B1 knockdown (Fig. 1E). To test this, we treated K562 cells with the well-characterized SF3B1 inhibitor pladienolide B (100 nM) for 6 h, and assessed splicing of STAG2, ENSA, ARPP19, ECT2, and SEPTIN6. Our results show a decrease in ARPP19 exon 2 inclusion (Fig. 6E), meaning less ARPP19-long, which is correlated with decreased AML patient survival and can support the idea that ARPP19-long may serve as a potential therapeutic target in AML.

ARPP19-long isoform facilitates faster progression through mitotic exit

Our findings underscore ARPP19 as a target of SF3B1, with its isoforms playing a critical role in AML survival. To gain further insights, we sought to elucidate the distinct functions of ARPP19 isoforms. ARPP19 operates as a subunit within a complex that exerts inhibitory control over PP2A-B55, with its heterodimeric partner, ENSA, also identified as a target of SF3B1. Interestingly, however, the isoforms of ENSA did not demonstrate significance for AML survival. The cooperative action of ARPP19 and ENSA in regulating substrate phosphorylation during mitotic entry is integral to this process. Although Cyclin B/CDK1 activation initiates mitosis, its full effect depends on the inhibition of PP2A-B55 activity [30]. These findings emphasize the pivotal regulatory functions of ARPP19 and ENSA in ensuring precise control of the mitotic checkpoint.

To understand the differential function of ARPP19 and ENSA isoforms at this stage, we expressed each of the protein isoforms in HeLa cells. We generated stable HeLa cells with GFP-empty, GFP-ARPP19-long, or GFP-ARPP19-short (Fig. 7A), as well as GFP-empty, GFP-ENSA-long, or GFP-ENSA-short (Supplementary S7A). We then synchronized the cells in G2/M following 20 h with nocodazole (Fig 7B and Supplementary S7B). After releasing the cells, we monitored their cell cycle stage after 3 h. Our results demonstrate a differential role for both ARPP19 and ENSA isoforms during mitotic exit (Fig. 7B&C and Supplementary Fig. S7B&C). Specifically, cells overexpressing ARPP19-long progressed to G1 faster than those overexpressing ARPP19-short, while cells overexpressing ENSA-short progressed to G1 more slowly than those overexpressing ENSA-long (Fig. 7C and Supplementary Fig. S7C). This suggests that ARPP19-long and ENSA-short act as more potent isoforms in facilitating mitotic exit compared to ARPP19-short and ENSA-long, respectively. These findings align

with SF3B1^{K700E} promoting ARPP19-long and ENSA-short, as well as the association of ARPP19-long with poor survival in AML. In summary, our findings point to an SF3B1 splicing signature, specifically highlighting ARPP19, as a possible mechanism connecting SF3B1^{K700E} to abnormal cell cycle progression and cancer development.

Discussion

Given that nearly all human genes undergo splicing, this process is indispensable for maintaining cellular homeostasis. Among the many cellular processes dependent on splicing, the cell cycle stands out due to its strict requirement for timely and coordinated gene expression. Alterations in cell cycle regulation drive cancer, and SF3B1, a key spliceosome component, has been implicated in this process. SF3B1 mutations and phosphorylation changes have been linked to aberrant splicing, leading to disrupted cell cycle control and tumorigenesis. Here, we examine the role of SF3B1 phosphorylation in cell cycle-dependent splicing, focusing on its impact on checkpoint genes such as ARPP19 and its potential implications for cancer progression.

Our findings reveal that SF3B1^{K700E}, a cancer-associated mutation, promotes alternative splicing events in key cell cycle regulators, particularly ARPP19 and ENSA. These genes encode proteins that function in a shared complex, controlling mitotic progression through the regulation of PP2A-B55 activity. Notably, SF3B1's phosphorylation state fluctuates across the cell cycle, influencing its splicing activity. We observed that SF3B1 phosphorylation during G2/M enhances the inclusion of ARPP19 exon 2, generating the ARPP19-long isoform, which correlates with poor survival in AML patients. Moreover, CDK1- and DYRK1A-mediated phosphorylation of SF3B1 appears to fine-tune the splicing of these checkpoint genes, reinforcing a feedback loop in which SF3B1-dependent splicing contributes to cell cycle progression, while cell cycle kinases reciprocally modulate SF3B1 activity. This dynamic regulation underscores the intricate interplay between splicing and cell cycle control and highlights how dysregulation of this process can drive oncogenesis.

The functional relationship between ARPP19 and ENSA further supports the idea that SF3B1-mediated splicing contributes to mitotic regulation in a coordinated manner. Both genes likely evolved from a common ancestor, given their structural similarities and overlapping roles in PP2A-B55 inhibition. Interestingly, while ARPP19 alternative splicing correlates with AML patient survival, ENSA splicing does not, despite both genes

functioning within the same PP2A-B55 regulatory complex. This suggests that ARPP19-long may have an additional moonlighting role in cancer progression, potentially acting as a key driver of mitotic exit dysregulation. In addition, our results suggest that phosphorylated SF3B1 and SF3B1^{K700E} promotes ARPP19-long and ENSA-short (Fig. 1D and 3B&E), both of which facilitate faster mitotic exit. This raises the possibility that cancer cells exploit SF3B1 mutations or phosphorylation changes to shift the balance toward isoforms that accelerate cell cycle progression, ultimately supporting tumor growth. Importantly, our findings demonstrate that inhibition of SF3B1, such as treatment with pladienolide B, can reverse these splicing alterations, reducing the levels of ARPP19-long. This suggests that splicing modulation could serve as a therapeutic strategy, particularly for malignancies like AML, where SF3B1-driven splicing patterns correlate with poor prognosis.

Data availability

The data generated in this study are currently in the process of being submitted to GEO. Once the submission is complete, access details will be provided.

Acknowledgments

We thank Liran Shlush for K562-WT and K562-SF3B1^{K700E} cells. The results shown here are in part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga.

Funding

This work was supported by the Israel Science Foundation (ISF 462/22); Israeli Cancer Association; Israel Cancer Research Foundation and the Alon Award from the Israeli Planning and Budgeting Committee (PBC).

Competing interests

The authors declare no conflicts of interest.

Author contribution

MB, EE, AS, MP: Conceptualization, validation, formal analysis, investigation, visualization, methodology, and writing-review-editing. SJH, OG: Validation. MSu, MB: Resources. GK: Visualization and project administration. MS: Conceptualization, funding acquisition, supervision, and writing-original draft.

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Legends

Figure 1. SF3B1 regulates alternative splicing of cell cycle checkpoint genes.

A-C. rMATs analysis was done on RNA-seq data from WT K562 or SF3B1^{K700E}. A volcano plot showing differential splicing events between WT K562 cells and K562^{K700E}. The x-axis

represents the inclusion level difference (ΔPSI), and the y-axis shows statistical significance (-log10 p-value). Significant events (FDR < 0.05) are in black, while non-significant ones are in gray **(A)**. Venn diagram representing the overlap between differentially expressed genes (DE genes) and alternatively spliced genes (AS genes) **(B)**. Gene set enrichment analysis was performed on alternatively spliced genes comparing WT K562 cells and K562^{K700E} cells using the GeneAnalytics tool [26] (**C**). **D.** RNA was extracted from WT K562 and K562^{K700E} cells, converted to cDNA and RT-PCR was performed. RT-PCR analysis of the PSI (percentage splice in) relative to RT-PCR analysis of total mRNA for ARPP19 exon 2 inclusion, ENSA exon 3 inclusion, ECT2 exon 5 inclusion, LRIF1 exon 2 inclusion, FANCD2 exon 22 inclusion, SEPTIN6 exon 10 inclusion and STAG2 exon 31 inclusion. **E**. Cells were transfected with siGFP or siSF3B1 for 72 h. RNA was extracted, converted to cDNA and RT-PCR was performed. RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA for ARPP19 exon 2 inclusion, ENSA exon 3 inclusion, ECT2 exon 5 inclusion, LRIF1 exon 2 inclusion, FANCD2 exon 22 inclusion, SEPTIN6 exon 10 inclusion and STAG2 exon 31 inclusion. Values represent averages of three independent experiments ±SD *p<0.05; **p<0.01; ***p<0.005; ****p<0.001; Student's t-test, compared to PSI and total mRNA values for cells transfected with siGFP (y=1).

Figure 2. Effect of cell cycle stage on alternative splicing.

A-B. K562 cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed then after 9 h treated again with 2 mM thymidine. After 18 h cells were collected and analysed by flow cytometry (**A**) and RT-PCR (**B**). RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA for ARPP19 exon 2 inclusion, ENSA exon 3 inclusion, ECT2 exon 5 inclusion, SEPTIN6 exon 10 inclusion and STAG2 exon 31 inclusion was performed. Values represent averages of three independent experiments ±SD *p<0.05; ***p<0.005; ****p<0.001; Student's t-test, compared to PSI for control cells. **C-D**. K562 cells were treated with 40 ng/ml nocodazole (NCO) for 20 h. Cells were collected and analysed by flow cytometry (**C**) and RT-PCR (**D**). RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA for ARPP19 exon 2 inclusion, ENSA exon 3 inclusion, ECT2 exon 5 inclusion, SEPTIN6 exon 10 inclusion and STAG2 exon 31 inclusion was performed. Values represent averages of three independent experiments ±SD ****p<0.001; Student's t-test, compared to PSI for control cells. **E-F**. HeLa cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed then after 9 h

treated again with 2 mM thymidine. After 18 h cells were collected and analysed by flow cytometry (**E**) and RT-PCR (**F**). RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA for ARPP19 exon 2 inclusion, ENSA exon 3 inclusion, ECT2 exon 5 inclusion, SEPTIN6 exon 10 inclusion and STAG2 exon 31 inclusion was performed. Values represent averages of three independent experiments ±SD *p<0.05; ***p<0.005; ****p<0.001; Student's t-test, compared to PSI for control cells. **G-H**. HeLa cells were treated with 40 ng/ml nocodazole (NCO) for 20 h cells were collected and analysed by flow cytometry (**G**) and RT-PCR (**H**). RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA for ARPP19 exon 2 inclusion, ENSA exon 3 inclusion, ECT2 exon 5 inclusion, SEPTIN6 exon 10 inclusion and STAG2 exon 31 inclusion was performed. Values represent averages of three independent experiments ±SD ****p<0.001; Student's t-test, compared to PSI for control cells.

Figure 3. The effect of inhibition of DYRK1A, following G_1/S synchronization, on alternative splicing. A-C. K562 cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed then after 9 h treated again with 2 mM thymidine. After 18 h cells were washed and then treated with media or the DYRK1A inhibitor INDY at a final concentration of 5 µM for 6 h. Cells were collected for analysis by flow cytometry, using propidium iodide (PI) to measure DNA content. The proportions of cells accumulated in S-phase is shown as a fold-change relative to untreated cells and the results presented as histograms of the various cell treatment groups: non-treated (control) cells (**A**), thymidine-treated cells (**B**), thymidine treated cells, after 6 h further incubation with DMEM alone (**C**), thymidine-treated cells, after 6 h incubation with INDY (**D**). **E-F**. Cells were collected for RT-PCR analysis. RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ARPP19 exon 2 inclusion (**E**), ENSA exon 3 inclusion (**F).** Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test. **G-J.** HeLa cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed then after 9 h treated again with 2 mM thymidine. After 18 h cells were washed and then treated with media or the DYRK1A inhibitor INDY at a final concentration of 5 µM for 6 h. Cells were collected for analysis by flow cytometry, using propidium iodide (PI) to measure DNA content. The proportions of cells accumulated in S-phase is shown as a fold-change relative to untreated cells and the results presented as histograms of the various cell treatment groups: non-treated (control) cells (**G**), thymidine-treated cells (**H**), thymidine treated cells, after 6 h further incubation with DMEM alone (**I**),

thymidine-treated cells, after 6 h incubation with INDY (**J**). **K-L**. Cells were collected for RT-PCR analysis. RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ARPP19 exon 2 inclusion (**K**), ENSA exon 3 inclusion (**L).** Values represent averages of three independent experiments ±SD *p<0.05; ***p<0.005; ****p<0.001; Student's t-test.

Figure 4. The effect of inhibition of CDK1, following G_2/M synchronization, on alternative splicing. A-D. K562 cells were seeded in a six-well plate. Next day, cells were treated with 40 ng/ml nocodazole (NCO) for G2/M arrest, or with DMEM (control). After 20 h, cells were washed with PBS. Control and NCO treated cells were harvested to confirm the arrest. Remaining cells were treated with DMSO or purvalanol A (PA), (15 µM) for 6 h to check G2/M arrest post-PA treatment. Cells were collected for analysis by flow cytometry. The amount of cells accumulated in M-phase is shown as a fold-change relative to untreated cells and the results presented as histograms of the various cell treatment groups: untreated (control) cells (**A)**; NCO treated cells **(B)**; control cells plus 6 h DMSO (**C**); control cells plus PA treatment for 6 h (**D)**. **E-F**. K562 cells were arrested with NCO for 20 h, and treated with either DMSO or PA for 6 h. Cells were collected for RT-PCR. RNA was extracted, converted to cDNA and RT-PCR performed. RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ARPP19 exon 2 inclusion (**E**), ENSA exon 3 inclusion (**F**). Values represent averages of three independent experiments ±SD **p<0.01; Student's t-test. **G-J.** HeLa cells were seeded in DMEM media. Next day, cells were treated with 40 ng/ml nocodazole (NCO) for G2/M arrest, or with DMEM (control). After 20 h, cells were washed with PBS. Control and NCO treated cells were harvested to confirm arrest. Remaining cells were treated with DMSO or PA, (15 µM) for 6 h to check G2/M arrest post-PA treatment. Cells were collected for analysis by flow cytometry. The amount of cells accumulated in M-phase is shown as a fold-change relative to untreated cells and the results presented as histograms of the various cell treatment groups: untreated (control) cells (**G)**; NCO treated cells **(H)**; control cells plus 6 h DMSO (**I**); control cells plus PA treatment for 6 h (**J)**. **K-L**. HeLa cells were arrested with NCO for 20 h, and treated with either DMSO or PA for 6 h. Cells were collected for RT-PCR. RNA was extracted, converted to cDNA and RT-PCR performed. RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ARPP19 exon 2 inclusion (**K**), ENSA exon 3 inclusion (**L**). Values represent averages of three independent experiments ±SD **p<0.01; Student's t-test.

Figure 5. The effect of inhibition of PP2A, following G_1/S synchronization, on alternative splicing. A-F. K562 cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed then after 9 h treated again with 2 mM thymidine. After 18 h cells were washed and then treated with media or the PP2A inhibitor okadaic acid (OA) at a final concentration of 20 nM for 12 h. **A-D.** Cells were collected for analysis by flow cytometry using PI to measure DNA content. The amount of cells accumulated in S-phase is shown as a fold-change relative to untreated cells and the results presented as histograms of the various cell treatment groups: non-treated (control) cells (**A**), OA treatment for 12 h (**B**) thymidine-treated cells (**C**), thymidine-treated cells, plus OA treatment for 12 h (**D**). **E-F**. Cells were collected for RT-PCR. RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ARPP19 exon 2 inclusion (**E**), ENSA exon 3 inclusion (**F**). Values represent averages of three independent experiments ±SD *$p<0.05$; ****$p<0.001$; Student's t-test. **G-M**. HeLa cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed then after 9 h treated again with 2 mM thymidine. After 18 h cells were washed and then treated with media or the PP2A inhibitor okadaic acid (OA) at a final concentration of 20 nM for 12 h. **G-K.** Cells were collected for analysis by flow cytometry using PI to measure DNA content. The amount of cells accumulated in S-phase is shown as a fold-change relative to untreated cells and the results presented as histograms of the various cell treatment groups: non-treated (control) cells (**G**), OA treatment for 12 h (**H**) thymidine-treated cells (**I**), thymidine-treated cells, plus 12 h with DMEM (**J**), thymidine-treated cells, plus OA treatment for 12 h (**K**). **L-M**. Cells were collected for RT-PCR. RNA was extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ARPP19 exon 2 inclusion (**L**), ENSA exon 3 inclusion (**M**). Values represent averages of three independent experiments ±SD *$p<0.05$; ****$p<0.001$; Student's t-test.

Figure 6. **Survival analysis of the SF3B1 splicing targets using clinical RNA-seq datasets from TCGA for AML patients**. **A-B.** Kaplan-Meier curves for total mRNA of ENSA (**A**); and for ENSA exon 3 inclusion (**B**). High exon inclusion level is colored in red and lower inclusion level in blue. Error bars represent 95% confidence intervals of the mean. **C-D**. Kaplan-Meier curves for total mRNA of ARPP19 (**C**); and for ARPP19 exon 2 inclusion (**D**). High exon inclusion level is colored in red and lower inclusion level in blue. Error bars represent 95% confidence intervals of the mean. **E.** K562 cells were treated with pladienolide D, an SF3B1 inhibitor, at 100 nM for 24 h. Cells were collected for RT-PCR. RNA was

extracted, converted to cDNA and RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ENSA exon 3 inclusion, ARPP19 exon 2 inclusion, ECT2 exon 5 inclusion, SEPTIN6 exon 10 inclusion and STAG2 exon 31 inclusion. Values represent averages of three independent experiments ±SD *p<0.05; ****p<0.001; Student's t-test.

Figure 7. ARPP19 isoform function. ARPP19-short/long were cloned to pLenti CMV GFP vector and lentiviral particles were produced by expressing ARPP19 short or long isoform in the HEK293T packaging cell-line using TransIT-X2 transfection reagent. HeLa cells were infected with viral particles to overexpress either the short or long isoform of ARPP19. Protein was collected, and immunoblotting was conducted with the indicated antibodies and quantified relative to the housekeeping protein tubulin (**A**). The stable cell lines were arrested at G2/M using NCO for 20 h, then the cells were released for 3 h, collected and analyzed by flow cytometry using PI to measure DNA content (**B**), and the analysis was blotted in bar graph **(C).**

Supplementary Figures

Sup. Figure 1: **A.** rMATS analysis for the differential alternative splicing was performed on RNA-seq of WT K562 or SF3B1^{K700E}. **B-G** RPKM for each alignment track are shown as sashimi plots. Arcs denote splice junctions, quantified in spanning reads, as specified near each exon junction of ARPP19 **(B)**, ECT2 **(C)**, LRIF1 **(D)**, FANCD2 **(E)**, STAG2 **(F)**, SEPTIN6 **(G)**. **H.** Schematic representation of primer design for detecting alternative splicing events for ARPP19, ENSA, ECT2, SEPTIN6 and STAG2 genes. Primers (black arrows) were positioned in flanking constitutive exons to amplify and distinguish between splice isoforms. Exons are shown as pink boxes, introns as black lines, and alternative splicing events.

Sup. Figure 2. A. RNA was extracted from WT K562 and K562^{K700E} cells, converted to cDNA and RT-PCR was performed. RT-PCR analysis of total mRNA of ARPP19, ENSA, ECT2, LRIF1, FANCD2, SEPTIN6 and STAG2 relative to CycloA and hTBP reference genes. Values represent averages of three independent experiments ±SD *p<0.05; **p<0.01; ***p<0.005; ****p<0.001; Student's t-test. **B-C**. Cells were transfected with siGFP or siSF3B1 for 72 h. RNA was extracted, converted to cDNA and RT-PCR was performed. **(B)** ARPP19, ENSA, ECT2, LRIF1, FANCD2, SEPTIN6 and STAG2 relative to CycloA and hTBP reference genes **(C)**. Values represent averages of three independent experiments ±SD *p<0.05; **p<0.01;

***p<0.005; ****p<0.001; Student's t-test, compared to total mRNA values for cells transfected with siGFP (y=1).

Sup. Figure 3. A. K562 cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed, then after 9 h treated again with 2 mM thymidine. After 18 h cells were collected and analysed by RT-PCR. RNA was extracted, converted to cDNA and RT-PCR analysis of the total mRNA relative to CycloA and hTBP reference genes for ARPP19, ENSA, ECT2, SEPTIN6 and STAG2 was performed. Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test, compared to total mRNA for control cells. **B**. K562 cells were treated with 40 ng/ml nocodazole (NCO) for 20 h cells were collected and analyzed by RT-PCR. RNA was extracted, converted to cDNA and RT-PCR analysis of the total mRNA relative to CycloA and hTBP reference genes for ARPP19, ENSA, ECT2, SEPTIN6 and STAG2 was performed. Values represent averages of three independent experiments ±SD *p<0.01; Student's t-test, compared to total mRNA for control cells. **C.** HeLa cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed, then after 9 h treated again with 2 mM thymidine. After 18 h cells were collected and analyzed by RT-PCR. RNA was extracted, converted to cDNA and RT-PCR analysis of the total mRNA relative to CycloA and hTBP reference genes for ARPP19, ENSA, ECT2, SEPTIN6 and STAG2 was performed. Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test, compared to total mRNA for control cells. **D**. HeLa cells were treated with 40 ng/ml NCO for 20 h, then cells were collected and analyzed by RT-PCR. RNA was extracted, converted to cDNA and RT-PCR analysis of the total mRNA relative to CycloA and hTBP reference genes for ARPP19, ENSA, ECT2, SEPTIN6 and STAG2 was performed. Values represent averages of three independent experiments ±SD *p<0.01; Student's t-test, compared to total mRNA for control cells.

Sup. Figure 4. A-H. K562 cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed, then after 9 h, treated again with 2 mM thymidine. After 18 h cells were washed and then treated with media or the DYRK1A inhibitor INDY at a final concentration of 5 µM for 6 h. Cells were collected for RT-PCR analysis. RNA was extracted, converted to cDNA and total mRNA relative to CycloA and hTBP reference genes is shown for ARPP19 (**A**), ENSA (**B**). RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ECT2 exon 5 inclusion (**C&D**), SEPTIN6 exon 10 inclusion (**E&F**), and STAG2 exon 31 inclusion (**G&H**) was performed. Values represent averages of three independent

experiments ±SD *p<0.05; Student's t-test compared to PSI and total mRNA for control cells. **I-P.** HeLa cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed, then after 9 h, treated again with 2 mM thymidine. After 18 h cells were washed and then treated with media or the DYRK1A inhibitor INDY at a final concentration of 5 µM for 6 h. Cells were collected for RT-PCR analysis. RNA was extracted, converted to cDNA and total mRNA relative to CycloA and hTBP reference genes is shown for ARPP19 (**I**), ENSA (**J**). RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ECT2 exon 5 inclusion (**K&L**), SEPTIN6 exon 10 inclusion (**M&N**), and STAG2 exon 31 inclusion (**O&P**) was performed. Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test compared to PSI and total mRNA for control cells.

Sup. Figure 5. A-H. K562 cells were seeded in a six-well plate. Next day, cells were treated with 40 ng/ml NCO for G2/M arrest, or with DMSO (control). After 20 h, cells were washed with PBS. Control and NCO treated cells were harvested to confirm arrest. Remaining cells were treated with DMSO or purvalanol A (PA) (15 µM) for 6 h to check G2/M arrest post-PA treatment. Cells were collected for RT-PCR. RNA was extracted, converted to cDNA and RT-PCR performed. Total mRNA relative to CycloA and hTBP reference genes is shown for ARPP19 (**A**), ENSA (**B**). RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ECT2 exon 5 inclusion (**C&D**), SEPTIN6 exon 10 inclusion (**E&F**), and STAG2 exon 31 inclusion (**G&H**) was performed. Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test. **I-P.** HeLa cells were treated under the same condition and were harvested for RT-PCR. RNA was extracted, converted to cDNA and RT-PCR performed. Total mRNA relative to CycloA and hTBP reference genes is shown for ARPP19 (**I**), ENSA (**J**). RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ECT2 exon 5 inclusion (**K&L**), SEPTIN6 exon 10 inclusion (**M&N**), and STAG2 exon 31 inclusion (**O&P**) was performed. Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test.

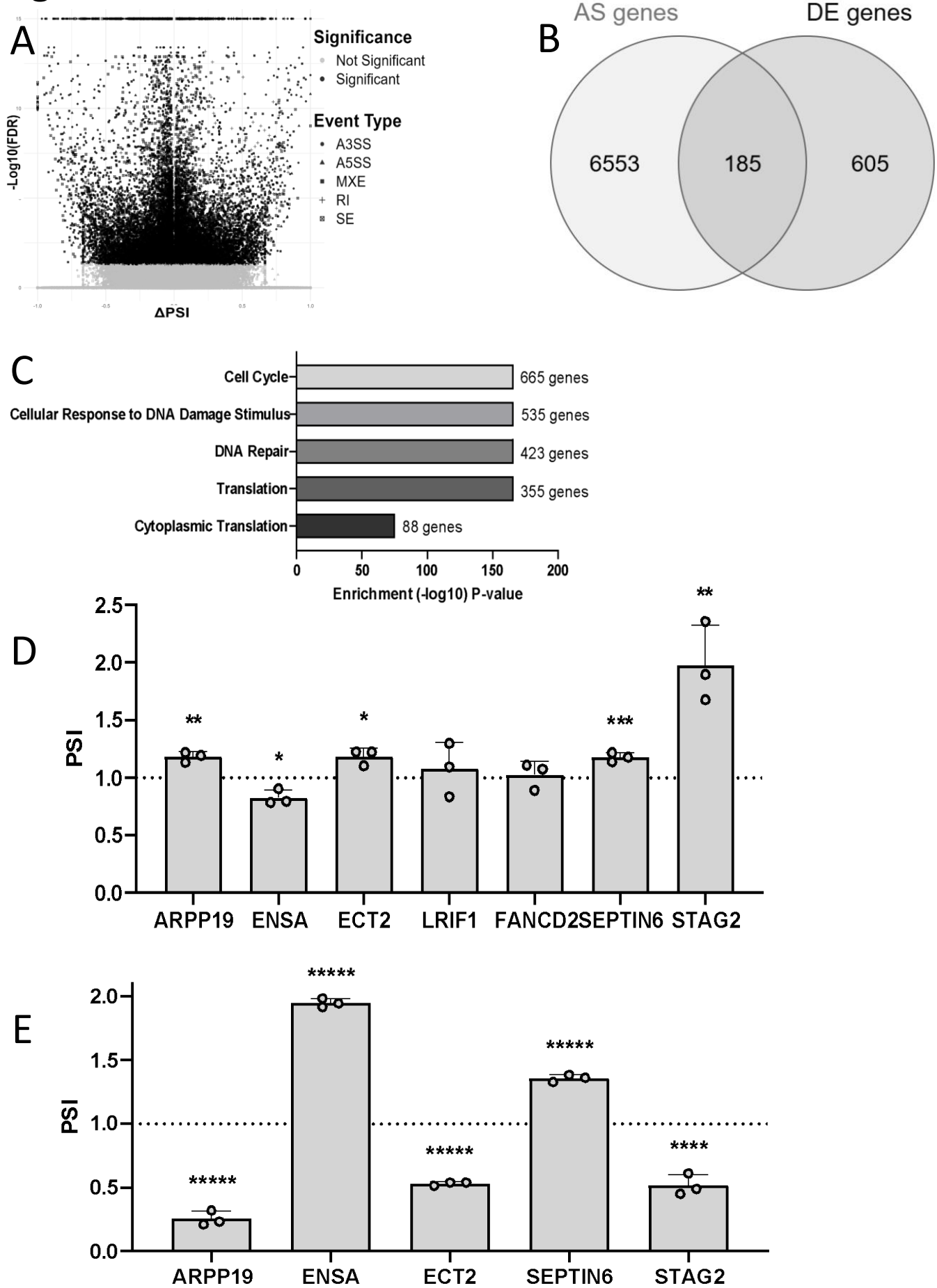
Sub. Figure 6. K562 cells were treated with thymidine at a final concentration of 2 mM, for 18 h. Cells were washed, then after 9 h treated again with 2 mM thymidine. After 18 h cells were washed and then treated with media or the PP2A inhibitor OA at a final concentration of 20 nM for 12 h. Cells were collected for RT-PCR. RNA was extracted, converted to cDNA, and total mRNA relative to CycloA and hTBP reference genes is shown for ARPP19 (**A**), ENSA (**B**). RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ECT2

exon 5 inclusion (**C&D**), SEPTIN6 exon 10 inclusion (**E&F**), and STAG2 exon 31 inclusion (**G&H**) was performed. Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test. **I-P.** HeLa cells were treated under the same conditions and were harvested for RT-PCR. RNA was extracted, converted to cDNA and RT-PCR performed. Total mRNA relative to CycloA and hTBP reference genes is shown for ARPP19 (**I**), ENSA (**J**). RT-PCR analysis of the PSI relative to RT-PCR analysis of total mRNA of ECT2 exon 5 inclusion (**K&L**), SEPTIN6 exon 10 inclusion (**M&N**), and STAG2 exon 31 inclusion (**O&P**) was performed. Values represent averages of three independent experiments ±SD *p<0.05; Student's t-test.

Sup. Figure 7. A-B. Kaplan-Meier curves for ECT2 exon 5 inclusion (**A**); and total mRNA of ECT2 (**B**). **C-D.** Kaplan-Meier curves for STAG2 exon 31 inclusion (**C**); and total mRNA of STAG2 (**D**). **E-F.** Kaplan-Meier curves for SEPTIN6 exon 10 inclusion (**E**); and total mRNA of SEPTIN6 (**F**). PSI>0 (red) and PSI=0 (blue). Error bars represent 95% confidence intervals of the mean.

Sup. Figure 8. A-C. ENSA-short/long were cloned to pLentic CMV GFP vector and lentiviral particles were produced by expressing ENSA short or long isoform in the HEK293T packaging cell-line using TransIT-X2 transfection reagent. HeLa cells were infected with viral particles to overexpress either the short or long isoform of ENSA. Protein was collected, and immunoblotting was conducted with the indicated antibodies and quantified relative to the housekeeping protein tubulin (**A**). The stable cell lines were arrested at G2/M using NCO for 20 h, then the cells were released for 3 h, collected and analyzed by flow cytometry (**B&C**).

Fig. 1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Significant
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185
605
C
Cell Cycle
Cellular Response to DNA Damage Stimulus
DNA Repair
Translation
Cytoplasmic Translation
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423 genes
355 genes
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50
100
150
200
Enrichment (-log10) P-value
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PSI
ARPP19
ENSA
ECT2
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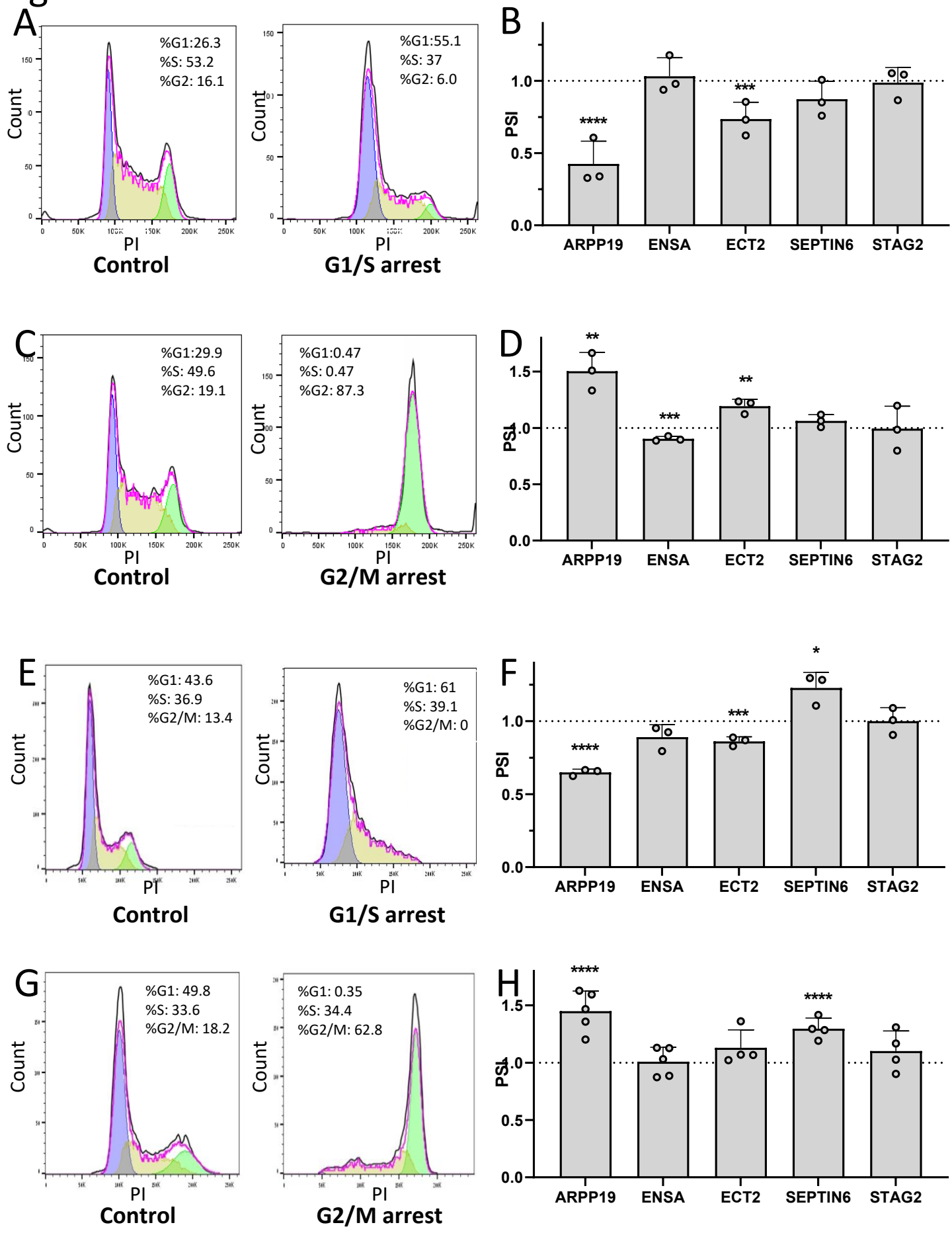
ARPP19
ENSA
ECT2
SEPTIN6
STAG2

Fig. 2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Count
PI
G1/S arrest
B
PSI
****
***
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ENSA
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C
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PI
Control
%G1:0.47
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Count
PI
G2/M arrest
D
PSI
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***
**
ARPP19
ENSA
ECT2
SEPTIN6
STAG2
E
%G1: 43.6
%S: 36.9
%G2/M: 13.4
Count
PI
Control
%G1: 61
%S: 39.1
%G2/M: 0
Count
PI
G1/S arrest
F
PSI
****
***
*
ARPP19
ENSA
ECT2
SEPTIN6
STAG2
G
%G1: 49.8
%S: 33.6
%G2/M: 18.2
Count
PI
Control
%G1: 0.35
%S: 34.4
%G2/M: 62.8
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PSI
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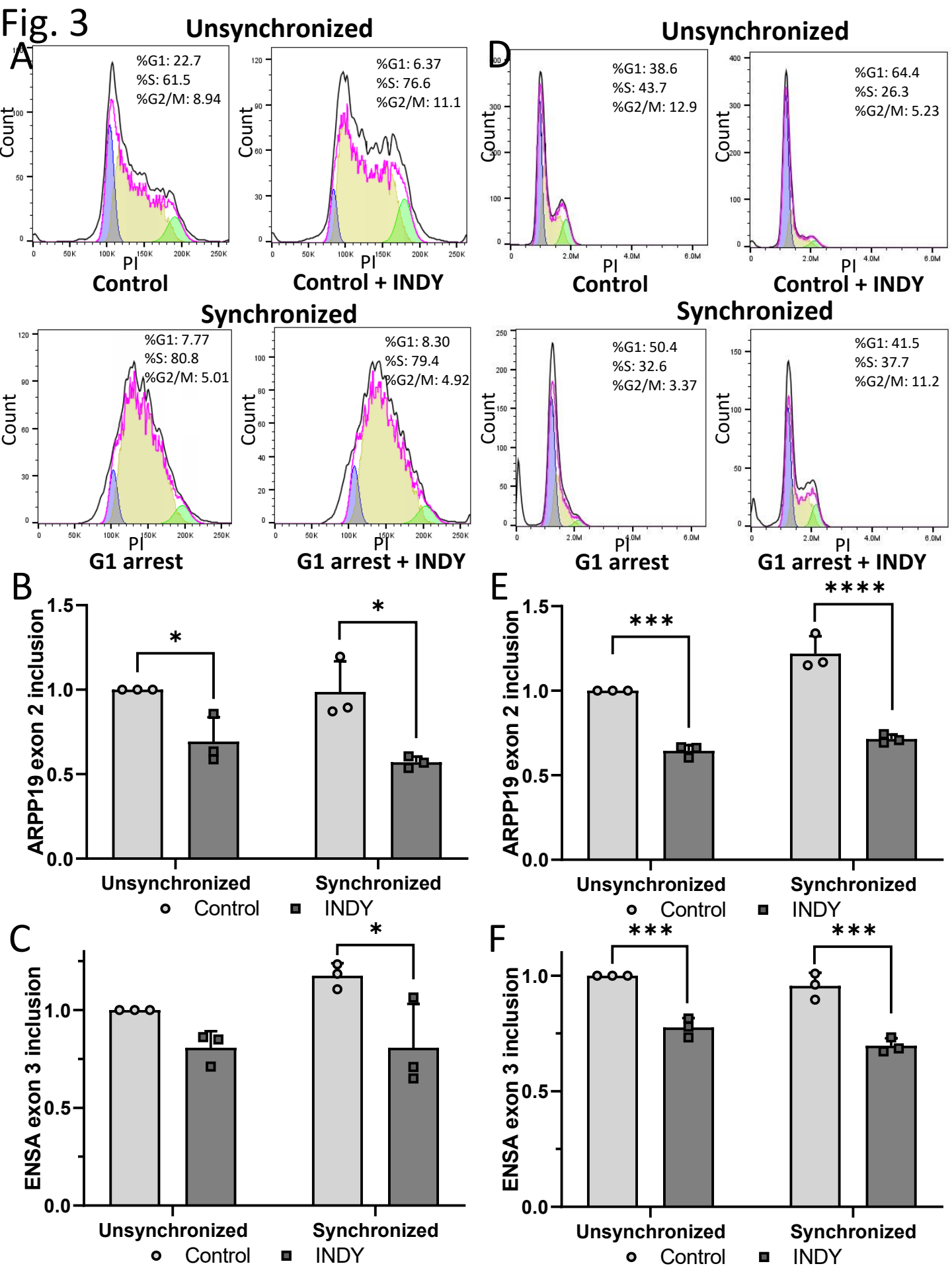
ARPP19
ENSA
ECT2
SEPTIN6
STAG2

Fig. 3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Control + INDY
Synchronized
%G1: 7.77
%S: 80.8
%G2/M: 5.01
G1 arrest
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%S: 79.4
%G2/M: 4.92
G1 arrest + INDY
D
Unsynchronized
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%G2/M: 12.9
Control
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%S: 26.3
%G2/M: 5.23
Control + INDY
Synchronized
%G1: 50.4
%S: 32.6
%G2/M: 3.37
G1 arrest
%G1: 41.5
%S: 37.7
%G2/M: 11.2
G1 arrest + INDY
Count
PI
B
ARPP19 exon 2 inclusion
Unsynchronized
Synchronized
Control
INDY
E
C
ENSA exon 3 inclusion
F

Fig. 4

A

Unsynchronized

%G1: 30.0
%S: 48.9
%G2/M: 18.4

Control

%G1: 30.9
%S: 44.9
%G2/M: 19.8

Control + PA

Synchronized

%G1: 51.1
%S: 21.6
%G2/M: 18.1

G2 arrest

%G1: 38.6
%S: 24.2
%G2/M: 11.4

G2 arrest + PA

D

Unsynchronized

%G1: 50
%S: 38.4
%G2/M: 7.85

Control

%G1: 1.85
%S: 34.3
%G2/M: 50.1

Control + PA

Synchronized

%G1: 50.9
%S: 35.9
%G2/M: 9.41

G2 arrest

%G1: 49.5
%S: 36.5
%G2/M: 9.42

G2 arrest + PA

Count

PI

B

ARPP19 exon 2 inclusion

Unsynchronized

Synchronized

Control

Purvanol A

E

ARPP19 exon 2 inclusion

Unsynchronized

Synchronized

Control

Purvanol A

C

ENSA exon 3 inclusion

Unsynchronized

Synchronized

Control

Purvanol A

F

ENSA exon 3 inclusion

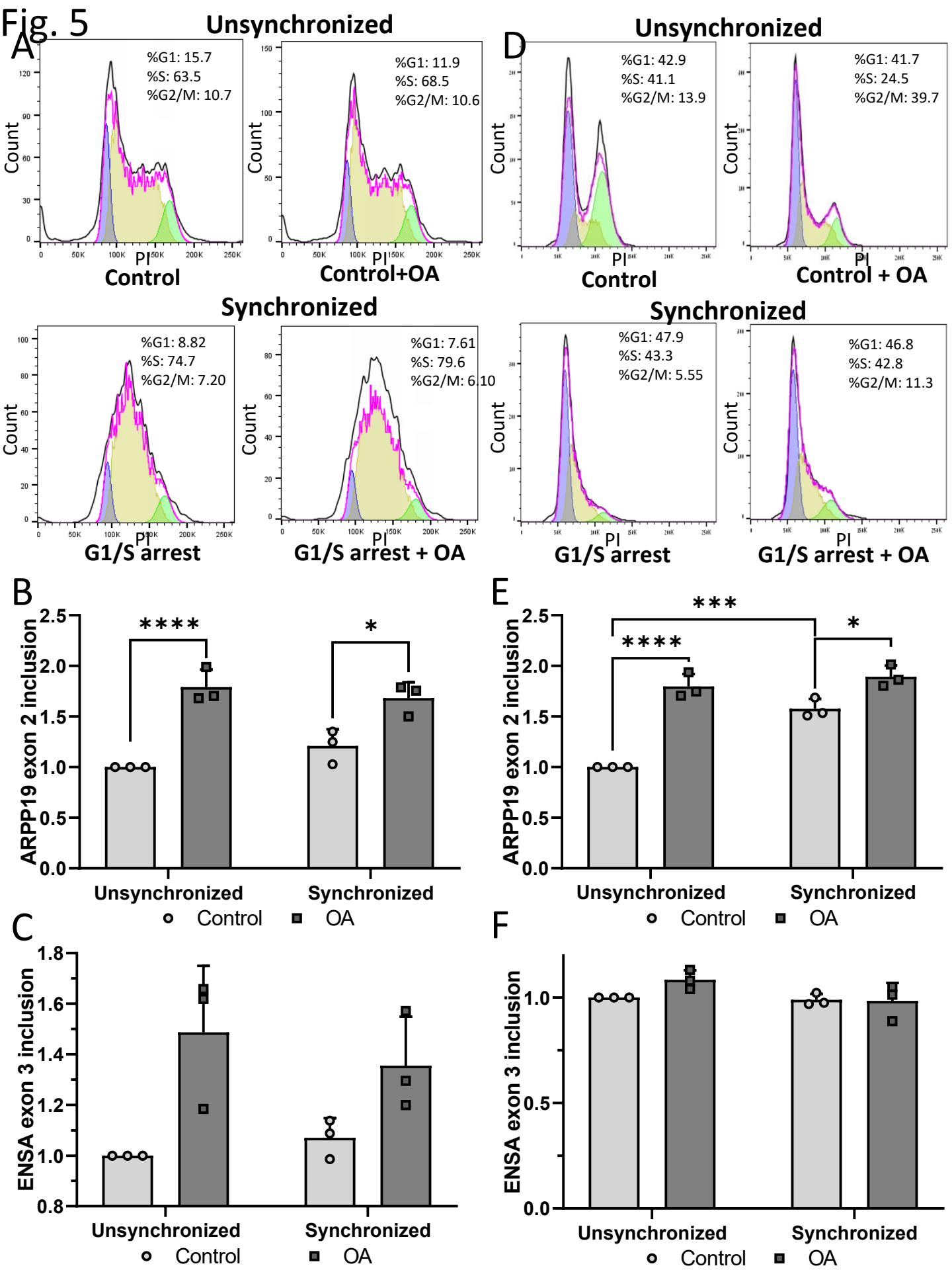
Unsynchronized

Synchronized

Control

Purvanol A

Fig. 5


A
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Control
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Control+OA
Synchronized
%G1: 8.82
%S: 74.7
%G2/M: 7.20
G1/S arrest
%G1: 7.61
%S: 79.6
%G2/M: 6.10
G1/S arrest + OA
D
Unsynchronized
%G1: 42.9
%S: 41.1
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%S: 24.5
%G2/M: 39.7
Control + OA
Synchronized
%G1: 47.9
%S: 43.3
%G2/M: 5.55
G1/S arrest
%G1: 46.8
%S: 42.8
%G2/M: 11.3
G1/S arrest + OA
Count
PI
B
ARPP19 exon 2 inclusion
****
*
Unsynchronized
Synchronized
Control
OA
E
ARPP19 exon 2 inclusion
***
****
*
Unsynchronized
Synchronized
Control
OA
C
ENSA exon 3 inclusion
Unsynchronized
Synchronized
Control
OA
F
ENSA exon 3 inclusion
Unsynchronized
Synchronized
Control
OA

Fig. 6

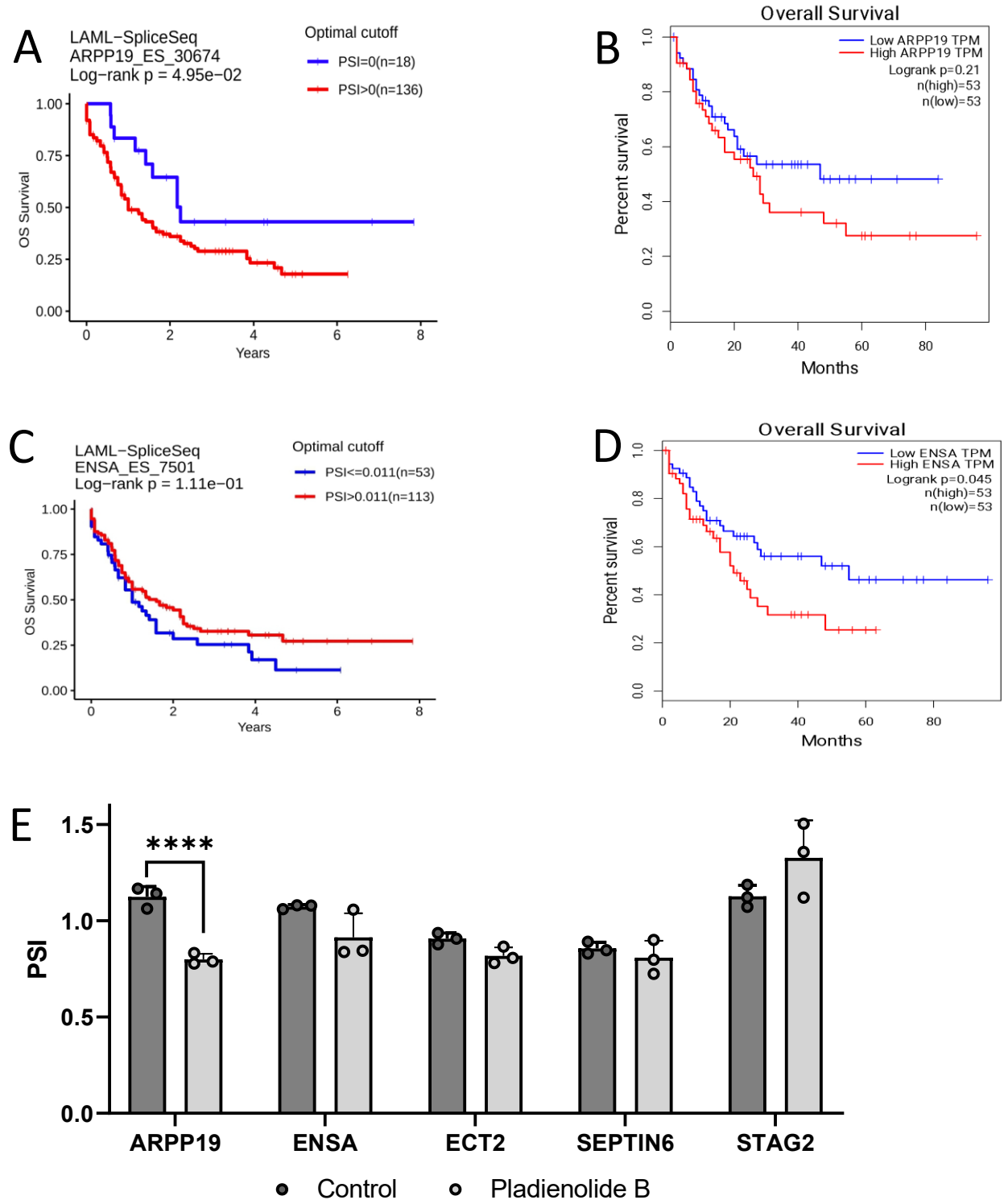
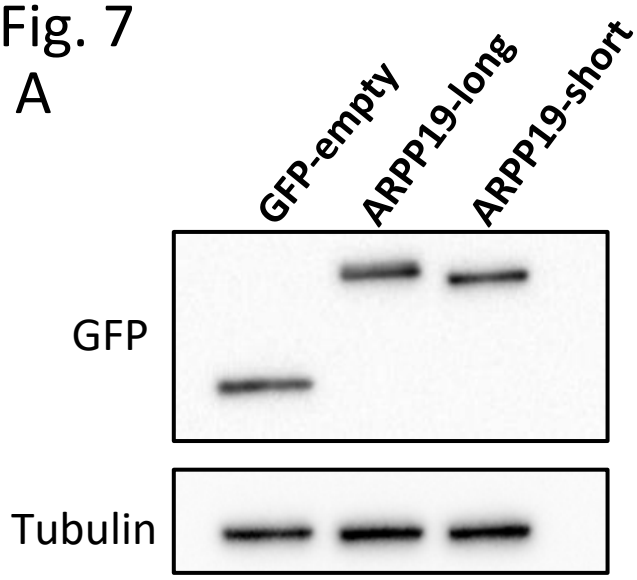
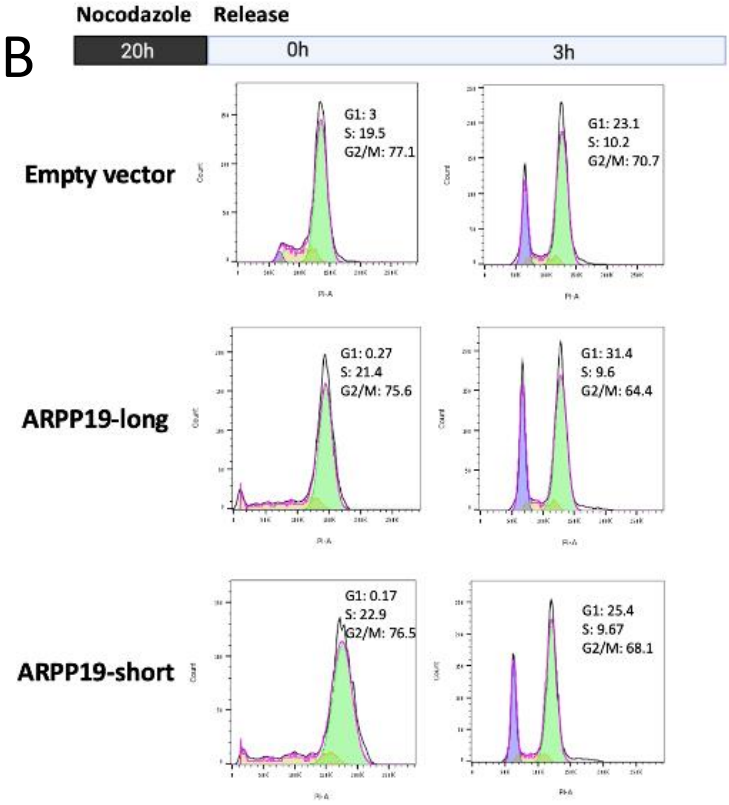

A
LAML−SpliceSeq
ARPP19_ES_30674
Log−rank p = 4.95e−02
Optimal cutoff
PSI=0(n=18)
PSI>0(n=136)
OS Survival
Years
B
Overall Survival
Low ARPP19 TPM
High ARPP19 TPM
Logrank p=0.21
n(high)=53
n(low)=53
Percent survival
Months
C
LAML−SpliceSeq
ENSA_ES_7501
Log−rank p = 1.11e−01
Optimal cutoff
PSI<=0.011(n=53)
PSI>0.011(n=113)
OS Survival
Years
D
Overall Survival
Low ENSA TPM
High ENSA TPM
Logrank p=0.045
n(high)=53
n(low)=53
Percent survival
Months
E
****
PSI
ARPP19
ENSA
ECT2
SEPTIN6
STAG2
Control
Pladienolide B

Fig. 7

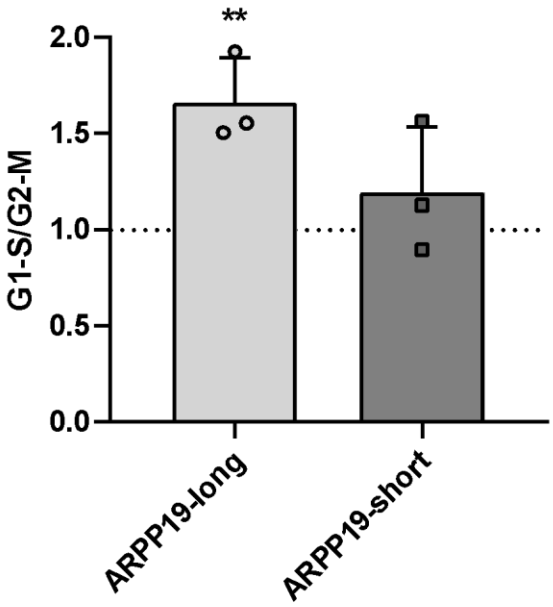
A


GFP-empty
ARPP19-long
ARPP19-short
GFP
Tubulin

B


Nocodazole
Release
20h
0h
3h
Empty vector
G1: 3
S: 19.5
G2/M: 77.1
G1: 23.1
S: 10.2
G2/M: 70.7
ARPP19-long
G1: 0.27
S: 21.4
G2/M: 75.6
G1: 31.4
S: 9.6
G2/M: 64.4
ARPP19-short
G1: 0.17
S: 22.9
G2/M: 76.5
G1: 25.4
S: 9.67
G2/M: 68.1

C


**
2.0
1.5
1.0
0.5
0.0
G1-S/G2-M
ARPP19-long
ARPP19-short