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WT1 activates transcription of the splice factor kinase *SRPK1* gene in PC3 and K562 cancer cells in the absence of corepressor BASP1

Tareg Belali[1&], Chigeru Wodi[1%], Bethany Clark[1], Man-Kim Cheung[1], Tim J. Craig[1], Gabrielle Wheway[1$], Nicole Wagner[2], Kay-Dietrich Wagner[2], Stefan Roberts[3], Sean Porazinski[1#*], Michael Ladomery[1*]

[1]Centre for Research in Bioscience, Faculty of Health and Applied Sciences, University of the West of England, Coldharbour Lane, Frenchay, Bristol BS16 1QY, United Kingdom

[2]Université Côte d'Azur, CNRS, INSERM, iBV, 06107 Nice, France

[3]Biomedical Sciences Building, University of Bristol, University Walk, Clifton, Bristol BS8 1TD, United Kingdom

[&]Present address: Faculty of Applied Medical Sciences, University of Bisha, 255, Al Nakhil, Bisha 67714, Saudi Arabia

[%]Present address: Department of Medical Laboratory Science, Ebonyi State University, P.M.B. 53 Abakaliki, Nigeria

[$]Present address: Faculty of Medicine, Duthie Building, University of Southampton, Southampton SO17 1BJ, United Kingdom

[#]Present address: Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, Sydney NSW 2010, Australia

[*]Joint senior and corresponding authors

Correspondence to: Michael Ladomery (Michael.Ladomery@uwe.ac.uk, +44(0) 117 3283531, University of the West of England (UWE Bristol), Frenchay Campus, Coldharbour Lane, Bristol, BS16 1QY, United Kingdom. Sean Porazinski (s.porazinski@garvan.org.au, +61(0) 2 9355 5895, Faculty of Medicine, St Vincent's Clinical School, University of NSW, Darlinghurst, Sydney 2010, Australia).

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ABSTRACT

Dysregulated alternative splicing plays a prominent role in all hallmarks of cancer. The splice factor kinase SRPK1 drives the activity of oncogenic splice factors such as SRSF1. SRSF1 in turn promotes the expression of splice isoforms that favour tumour growth, including proangiogenic VEGF. Knockdown (with siRNA) or chemical inhibition (using SPHINX) of SRPK1 in K562 leukemia and PC3 prostate cancer cell lines reduced cell proliferation, invasion and migration. In glomerular podocytes, the Wilms tumour suppressor zinc-finger transcription factor WT1 represses *SRPK1* transcription. Here we show that in cancer cells WT1 activates *SRPK1* transcription, unless a canonical WT1 binding site adjacent to the transcription start site is mutated. The ability of WT1 to activate *SRPK1* transcription was reversed by the transcriptional corepressor BASP1. Both WT1 and BASP1 co-precipitated with the *SRPK1* promoter, and BASP1 significantly increased the expression of the antiangiogenic $VEGF_{165}b$ splice isoform. We propose that by upregulating *SRPK1* transcription WT1 can direct an alternative splicing landscape that facilitates tumour growth.

1. Introduction

Up to 94% of human multi-exon genes are alternatively spliced [41], enabling genes to express multiple splice isoforms, often exhibiting antagonistic properties. Not surprisingly, alternative splicing affects genes involved in all hallmarks of cancer and the factors that regulate alternative splicing are often deregulated [32]. There is increasing interest in understanding how aberrant alternative splicing contributes to cancer hallmarks, and to explore ways in which the manipulation of splicing could be used therapeutically [6].

Of particular interest to cancer biologists is the alternative splicing of *VEGFA*, encoding the widely studied vascular endothelial growth factor (VEGF). The *VEGFA* transcript is comprised of eight exons and the alternative splicing of exons 5-7 results in the expression of splice isoforms with different activities and bioavailabilities [42]. An additional splice isoform was discovered arising from an alternative, distal 3' splice site in terminal exon 8 which results in six different amino-acids at the C-terminus. This new splice isoform was called $VEGF_{xxx}b$ (where xxx represents the total number of amino-acids in the isoform). $VEGF_{xxx}b$ isoforms are antiangiogenic and downregulated in cancer, suggesting a therapeutic benefit in modifying their expression [21,42]. We previously demonstrated that the oncogenic splice factor SRSF1 promotes proangiogenic VEGF splice isoform expression by enhancing the use of the proximal 3' splice site in exon 8 [30,31]. There are physiological contexts in which antiangiogenic $VEGF_{xxx}b$ expression is more appropriate, for example in glomerular podocytes. To gain further insights into regulation, we previously investigated the cause of severely reduced $VEGF_{xxx}b$ expression in podocytes derived from children with Denys-Drash Syndrome (DDS) in which the WT1 (Wilms' tumour suppressor) zinc-finger transcription factor is mutated with impaired DNA-binding ability [1]. In DDS podocytes with mutant WT1 the expression of the

serine-arginine splice factor protein kinase (SRPK1) was abnormally high. In wildtype podocytes WT1 acts as a transcriptional repressor of *SRPK1* and binds to a GC-rich sequence proximal to the transcription start site. However, in DDS, SRPK1 is derepressed; as a result, high SRPK1 levels lead to increased phosphorylation, nuclear localisation and activity of SRSF1 resulting in higher expression of proangiogenic VEGF [1].

SRSF1 was the first splice factor to be described as a proto-oncogene [2,20]. The splice factor kinase SRPK1 is now also considered to exhibit oncogenic properties. SRPK1 is upregulated in prostate cancer, correlating with increased invasion and angiogenesis [8]. High SRPK1 expression is a poor prognostic indicator in colorectal cancer [44]. SRPK1 promotes tumour cell migration and metastasis [38] and inhibits apoptosis in breast cancer [23]. SRPK1 targeting with kinase inhibitors is a potential novel antiangiogenic strategy in melanoma [13] and prostate cancer [27].

WT1 can both activate and repress target genes depending on the presence of cofactors [34,36]. Brain acid soluble protein 1 (BASP1) promotes WT1's transcriptional repression activity through PIP2-dependent recruitment of histone deacetylase [9,35]. BASP1 expression is evident in developing nephron structures in the embryonic kidney and in adult podocytes. In a conditionally-immortalised human podocyte cell line model that can differentiate *in vitro*, WT1 and BASP1 co-precipitate with the podocyte-specific *PODXL* gene promoter. *PODXL* encodes podocalyxin, a podocyte-specific cell adhesion sialoglycoprotein. Upon differentiation, the association of BASP1 with the *PODXL* promoter is reduced, allowing WT1 to activate its transcription [16]. The myelogenous leukemia cell line K562 expresses WT1, but not BASP1. When BASP1 is expressed in genetically engineered K562 cells, WT1 can induce differentiation to a neuronal-like phenotype, characterised by an arborized morphology and by the expression of genes associated with neurite outgrowth and synapse formation [15].

The proteins work together in other contexts: murine Wt1 and Basp1 repress Wnt4 in epicardial cells [12] and maintain the differentiated state of human taste receptor cells [14]. Despite *WT1*'s initial characterisation as a classic tumour suppressor gene, it is increasingly evident that *WT1* can also act as an oncogene in the context of several cancers including leukemia [24,43], breast cancer [45] and neuroblastoma [33]. WT1 promotes tumour angiogenesis and metastasis, and inhibits the anti-tumoral immune response [39]. At a molecular level WT1 can exert its oncogenic effects by activating transcription of the *MYC* proto-oncogene [17]; by repressing *CDH1* (encoding E-cadherin) transcription [7]; and by interacting with the mitochondrial protease HtrA, promoting antiapoptotic activity [11]. Given the importance of dysregulated alternative splicing in cancer, we reasoned that WT1 might also exert its oncogenic effects through the regulation of transcription of the oncogenic splice factor kinase gene *SRPK1*. We hypothesized that in leukemia and prostate cancer cells WT1 activates *SRPK1* transcription in the absence of BASP1.

2. Materials and Methods

2.1 Cell culture and SPHINX treatments

The K562 cell line (ATCC CRL-3344) was cultured using RPMI-1640 culture medium with L-glutamine (Sigma). V-K562 (stably transfected with empty vector (EV) pcDNA3) and B-K562 cells (stably transfected with BASP1-expressing pcDNA3, prepared in Prof. Stefan Roberts' laboratory as previously described [36]) were grown in similar conditions with 2mg/ml G418 (Sigma) to maintain selection for BASP1-expressing cells. Human prostate cancer cells PC3 (ATCC CRL-1435) were cultured in DMEM (Sigma). Both cell lines are guaranteed as authentic by the commercial supplier (ATCC). All culture media were supplemented with 10% fetal

bovine serum (FBS) and 0.1mg/ml penicillin/streptomycin (Sigma). For SRPK1 inhibition, K562-derived or PC3 cell lines, up to $2x10^6$ cells, were treated with 5-methyl-N-[2-(morpholin-4-yl)-5-(tri-fluoromethyl) phenyl] furan-2-carboxamide (SPHINX), purchased from Enamine (Ukraine).

2.2 Cloning and the Dual-Luciferase Renilla (DLR) assays

The *SRPK1* promoter was cloned into a pGL3 luciferase vector (Promega) for *in vitro* transcription assays [1]. The WT1 binding site (GGGGCGGGGG) located adjacent to the transcription start site was mutated to GAATTCAAAA. Transcription was measured using the Dual-Luciferase Renilla (DLR) assay (Promega). PC3, wildtype V-K562 and B-K562 cells were seeded in 24 well plates at 5×10^4 cells per well, 24h before transfection. Cells were transfected with transfection reagent (Lipofectamine 2000, ThermoFisher Scientific) using 2µg plasmid/100µl) using equal amounts (0.8µg for 24 well, 1.6 µg for 12 well and 4.0 µg per 6 well plates) of Renilla-expressing plasmid and *SRPK1* promoter: luciferase pGL3 constructs, wild-type and mutant. For experiments involving BASP1, two different concentrations (0.5 and 1.5µg) of BASP1-expressing plasmid were used (pcDNA3) [9].

2.3 SiRNA-mediated knockdown of WT1 and SRPK1

SiRNAs targeting WT1, SRPK1 and a negative control (firefly luciferase GL2) were as described [4,10] (Eurofins Genomics). Cells were transfected at 80% confluence 24hrs after seeding with 10nM, 50nM or 100nM final siRNA concentrations using the Lipofectamine RNAiMAX transfection reagent (ThermoFisher Scientific) and cells were harvested 48h post-transfection. In the DLR assays, cells were co-transfected with 100nM siRNA and 2.5µg pGL3 plasmid constructs in Optimem medium (Thermofisher Scientific).

2.4 Western Blotting

Cells lysates were prepared using RIPA buffer (10 mM Tris-Cl (pH 8.0), 1 mM EDTA, 1% (v/v) Triton X-100, 0.1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS and 140 mM NaCl) supplemented with protease inhibitor tablets (ThermoFisher Scientific). Equal protein samples, 20 μg protein, were separated on 10% (v/v) SDS polyacrylamide gels and transferred to PVDF membranes (Sigma) which were blocked and probed overnight at 4 °C with primary antibodies: anti- WT1 (ab89901, Abcam; 1:1000), anti-β-actin (ab8226, Abcam; 1:5000), anti-BASP1 (ab214322, Abcam; 1:1000), anti-SRPK1 (EE13, Santa Cruz; 1:1000), anti-VEGF (AF-293-NA, R&D Systems; 1:1000), anti VEGFxxxb (MAB3045-100, R&D Systems; 1:1000). Membranes were incubated in HRP-linked anti-rabbit or anti-mouse IgG secondary antibody (Cell Signalling; 1:1500) for 1h at room temperature. Membranes were incubated in Luminata Forte Western HRP substrate (Millipore) for chemiluminescent detection prior to image acquisition using a Li-Cor Odyssey imaging system. When required, western blots were stripped in order to re-probe them with the β-actin loading control antibody. Band densities were exported and analysed using GraphPad Prism software. Statistical differences between treatments groups were compared using ANOVAs and t-tests.

2.5 Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) was performed on PC3, K562 and B-K562 cells using the Imprint Chromatin Immunoprecipitation Kit (Sigma). Cells were incubated for 10 minutes with 1% (w/v) formaldehyde at room temperature to cross-link DNA and protein. Cells were lysed and DNA was sheared to about 1000bp using an ultrasonic probe sonicator. The DNA-

protein mixture was incubated with 3µg of WT1 and BASP1 antibodies or with 1µg of RNA Polymerase II antibody (positive control) or 1µg non-specific mouse IgG (negative control). DNA was eluted (GeneElute columns, Sigma) and used in PCR to detect *SRPK1* and *GAPDH* promoter sequences that were immunoprecipitated. PCR was performed at 95°C initially for 2 minutes followed by up to 35 cycles: 95°C for 1 minute, annealing for 1 minute at 55°C and extension at 72°C for 1 minute followed by a final elongation at 72°C for 5 minutes. The identity of the amplicons was verified by sequencing (Eurofins Genomics).

2.6 MTT assay for cell proliferation and viability

$1x10^5$ cells per well were seeded in 96-well plates. Following treatments and transfections, media was removed and a mixture of serum-free media and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (ab211091, Abcam) was added to each well. After incubation at 37°C for 3 h, MTT solvent was added and absorbance at 590 nm was measured. Cell proliferation was determined using the corrected absorbance value (test sample - culture medium background) of the formazan precipitate which is proportional to cell number. Cell viability was calculated: cell viability % = (control - absorbance of treatment wells) / absorbance of control well × 100.

2.7 Transwell cell migration and invasion assays

For cell migration, PC3 cells were re-suspended in serum-free medium and transferred to the top chamber of transwell inserts. For the invasion assay, inserts were pre-coated with 50µl of Matrigel (Corning) and allowed to solidify overnight. Complete medium containing 10% FBS was added to the lower chamber of the 24-well plate and cells were allowed to migrate for 24h. Cells that migrated to the bottom side of the inserts were fixed in 4% (w/v)

paraformaldehyde and stained with 0.2% (w/v) crystal violet in 2% methanol. To quantify the migration of cells, 0.1% (w/v) SDS in PBS was added to the lower chambers and absorbance intensity measured on a plate reader at 590 nm.

3. Results

3.1 WT1 activates SRPK1 transcription in K562 leukemia and PC3 prostate cancer cell lines

We previously showed that WT1 represses transcription of the splice factor kinase gene *SRPK1* in conditionally-immortalised wild-type glomerular podocytes. This activity is lost in DDS podocytes in which several, independent zinc-finger domain mutations impair the ability of WT1 to bind to its DNA targets [1]. We fused the *SRPK1* promoter to a luciferase reporter, both wild-type and with a mutation in the WT1 binding site (GGGGCGGGGG to GAATTCAAAA) [1]. We transfected these reporters into two independent, well-characterised WT1-expressing cancer cell lines; PC3, derived from a prostate cancer bone metastasis, and K562, derived from chronic myelogenous leukemia [9,19]. In both cell lines the wild-type *SRPK1* promoter was clearly active, whereas the mutant *SRPK1* promoter was not (Fig. 1). Co-transfection of an siRNA directed against WT1 strongly reduced the activity of the wild-type *SRPK1* promoter. We also measured the effect of siRNA-mediated WT1 knockdown on the expression of endogenous SRPK1 in K652 (Fig. 2A,B) and PC3 cells (Fig. 2C,D). In both cell lines the knockdown of WT1 resulted in a significant reduction in endogenous SRPK1 protein levels. To confirm the oncogenic properties of SRPK1, we transfected both cell lines with siRNA directed against SRPK1 and treated cells with SPHINX, a 3-(trifluoromethyl)anilide scaffold highly specific SRPK1 inhibitor [4]. SRPK1 knockdown (with 100 nM siRNA) and inhibition (with

SPHINX) significantly reduced cell proliferation and viability in both cell lines (Fig. 3). We performed transwell assays with adherent PC3 cells, and observed that SPHINX treatment reduced cell migration and invasion, confirming the oncogenic properties of SRPK1.

We previously co-precipitated WT1 with the endogenous *SRPK1* promoter using nuclear extracts from glomerular podocytes [1]. We tested whether WT1 similarly interacts with the *SRPK1* promoter in K562 and PC3 cells using ChIP. WT1 co-precipitated with the *SRPK1* promoter in both K562 (Fig. 4A,B) and PC3 (Fig. 4C,D) cells. The sequence amplified from the ChIP comprises the WT1 binding and transcription start sites and its identity was confirmed by sequencing (Fig. 4E). A positive control (antibody against RNA polymerase II) and a negative control (mouse IgG) confirmed the specificity of the pull-down. As a further control, the *GAPDH* promoter co-precipitated with the RNA polymerase II, but not with the WT1 antibody. Taken together these experiments suggest that in both leukemia and prostate cancer cells, WT1 interacts with the *SRPK1* promoter through its cognate WT1 binding site located proximal to the transcription start site. However instead of repressing (as in glomerular podocytes), WT1 activates *SRPK1* transcription in these two cancer cell lines. Given that the transcriptional corepressor BASP1 is expressed in glomerular podocytes [9], we next examined BASP1's effect on the ability of WT1 to activate *SRPK1* transcription.

3.2 In the presence of BASP1, WT1 fails to activate SRPK1 transcription in K562 cells

K562 cells do not express detectable levels of BASP1. A K562 cell line subclone that stably expresses BASP1, called B-K562 is available [15]. We transfected the *SRPK1* promoter:luciferase reporter into both K562 and B-K562 cells and observed reduced *SRPK1* promoter activity in B-K562 cells (Fig. 5A). To confirm the effect of BASP1 on SRPK1 expression we performed transient transfections combining the *SRPK1* promoter:luciferase construct

with a BASP1-expressing plasmid. Co-transfection of the *SRPK1* promoter:luciferase construct with empty vector (EV) had no effect, whereas the BASP1-expressing plasmid significantly reduced activity (Fig. 5B), confirming the observation in B-K562 cells (Fig. 5A). Next we examined the expression of endogenous SRPK1 in K562 and B-K562 cells. As expected, BASP1 was undetectable in parental K562 cells, but highly expressed in B-K562 cells (Fig. 5C,D). SRPK1 levels were significantly reduced in B-K562 cells. We also observed a reduction in endogenous WT1 (Fig. 5C,D). Next, we performed ChIP analysis on B-K562 cells, and confirmed that BASP1, like WT1, associates with the *SRPK1* promoter (Fig. 5E,F).

3.3 BASP1 increases the expression of antiangiogenic VEGF splice isoforms in PC3 cells

Having established that WT1 activates the transcription of *SRPK1* in cancer cells in the absence of BASP1, we next examined a previously characterised downstream effect of *SRPK1* transcriptional regulation by WT1. BASP1 is expressed in glomerular podocytes in which WT1 represses *SRPK1* [1,9]. Lower *SRPK1* expression in wild-type podocytes results in reduced phosphorylation of SRSF1, therefore less active nuclear SRSF1. This favours the use of the distal alternative 3′ splice site in VEGF exon 8 resulting in higher expression of the antiangiogenic $VEGF_{xxx}$b splice isoform. We previously showed that the inhibition of SRPK1 with SPHINX in PC3 cells in orthotopic mouse xenografts drastically reduces tumour growth, correlating with increased expression of antiangiogenic $VEGF_{xxx}$b [27]. The overexpression of proangiogenic VEGF in PC3 cells in which SRPK1 is stably knocked down restores xenograft growth [27]. We therefore reasoned that increased levels of BASP1 in PC3 cells would result in a similar shift in VEGF alternative splicing. Firstly we confirmed that the inhibition of SRPK1 in PC3 cells with SPHINX significantly increased expression of the antiangiogenic splice isoform $VEGF_{165}$b relative to total $VEGF_{165}$ (Fig. 6A). Next we transfected a BASP1-expressing plasmid

into PC3 cells. The expression of BASP1 in PC3 cells mirrored the effect of SRPK1 inhibition by SPHINX, increasing the expression of $VEGF_{165}b$ relative to total $VEGF_{165}$ (Fig. 6B,C).

4. Discussion

We had previously shown that WT1 acts as a transcriptional repressor of *SRPK1* in glomerular podocytes in which the corepressor BASP1 is expressed [1,9]. WT1's ability to activate or repress *SRPK1* transcription depends on the presence of specific cofactors such as BASP1 with which it physically interacts [9,16]. We propose a model for the regulation of *SRPK1* transcription by WT1 (Figure 7). WT1 binds to its cognate GC-rich binding site proximal to the transcription start site. In proliferating cells (and cancer cells), in the absence of BASP1, WT1 recruits transcriptional co-activators (CBP and associated histone acetyl transferase activity). In cells that are differentiated and non-proliferative, such as glomerular podocytes, BASP1 is expressed. This converts WT1 to a transcriptional repressor by recruiting histone deacetylase in the presence of the co-factor $PI4,5P_2$.The ability of transcription factors to activate and repress target genes is well established and WT1 is not unusual in having this dual ability [22]. WT1 both activates and represses *Wnt4* transcription through a chromatin structure switching mechanism [12].

We also observed a reduction in WT1 levels in B-K562 cells. WT1's ability to autoregulate its own expression has previously been reported in K562 cells [3,25]. Thus the expression of BASP1 in B-K562 cells also reduces WT1 expression, contributing to the downregulation of SRPK1.

WT1 is a highly multifunctional protein and its zinc-fingers can interact with both DNA and RNA. WT1 interacts with several splice factors including U2AF65, RBM4 and WTAP [29,37],and

we have previously observed that WT1 is present in mRNP complexes in K562 cells [28]. Bharathavikru and colleagues identified RNA targets of WT1 in ES cells [5]. They showed that WT1 binds preferentially to the 3' untranslated regions of developmentally expressed mRNAs contributing to the regulation of mRNA turnover. Whether or not WT1 also regulates SRPK1 expression post-transcriptionally remains to be investigated.

A recent study by Wagner and colleagues [40] examined the expression of Wt1, Srpk1 and Srsf1 in tumours compared to normal murine lung endothelium. All were highly expressed in tumour endothelium compared to healthy tissue, together with proangiogenic VEGF splice isoforms. This pattern correlated with a stronger nuclear localisation of the splice factor SRSF1, consistent with the effects of high SRPK1 activity, and similar to what had previously been described in DDS podocytes in which WT1 is mutated and SRPK1 de-repressed [1]. The authors then used an inducible conditional vessel-specific knockout of Wt1, and observed a drop in the expression of Srpk1, and a resulting increase in the expression of antiangiogenic VEGF. They also showed that Wt1 binds to and activates the *Srsf1* and *Srpk1* promoters in murine endothelial cells [40], consistent with our findings. We have not examined the effect of WT1 on the transcription of *SRSF1* in PC3 and K562 cells within the scope of the present study. It is reasonable to speculate that WT1 upregulates the transcription of *SRSF1*, and potentially of other oncogenic splice factors and splice factor kinases in cancer as well as *SRPK1*.

WT1's role in cancer, either as an oncogene or tumour suppressor, is now well established [24,43]. Here we provide further evidence that confirms the critical role of BASP1 in modulating WT1's oncogenic activity. There is increasing evidence that BASP1 has a prominent role in oncogenic processes. BASP1 can counteract the oncogenic role of MYC by interfering with MYC's ability to interact with calmodulin [18]. BASP1 interacts with oestrogen

receptor α and enhances the antitumourigenic effects of tamoxifen, and its expression is associated with better prognosis in breast cancer [26]. In acute myeloid leukemia, the oncogenic AML1-ETO fusion protein resulting from the t(8;21) translocation recruits DNA methyltransferase 3a (DNMT3a) to the *BASP1* promoter, causing its silencing. Re-expression of BASP1 reduces the proliferative capacity of the leukemic cells [47]. Consistent with its tumour suppressor properties in pancreatic cancer tissue, a proteomic screen identified BASP1 as a potential prognostic biomarker. Immunohistochemical analysis suggested that BASP1 expression is associated with better survival and favourable response to adjuvant chemotherapy [46].

5. Conclusion

In summary, we have shown that WT1 activates *SRPK1* transcription in two independent cancer cell lines, K562 and PC3; and that WT1's ability to activate *SRPK1* transcription is prevented by the transcriptional corepressor BASP1. The ability of WT1 to activate *SRPK1* transcription is likely to contribute to oncogenic processes in many types of cancer. We suggest that through the activation of *SRPK1* transcription, WT1 stimulates the activity of oncogenic splice factors such as SRSF1, altering the alternative splicing landscape to favour tumour growth including the expression of proangiogenic VEGF. These findings add a novel dimension to WT1's oncogenic functions. We suggest that there are opportunities to develop novel cancer therapies focused on blocking both WT1 activity and that of its downstream target, the splice factor kinase gene *SRPK1*.

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Figure Legends

Fig. 1. The *SRPK1* promoter fused to a luciferase reporter is active in WT1-expressing (A) PC3 and (B) K562 cells (Dual Luciferase-Renilla (DLR) assay). A mutated *SRPK1* promoter in which the WT1 binding site was removed (mutSRPK1) is not active. The knockdown of WT1 with siRNA reduces the activity of the wild-type *SRPK1* promoter in both cell lines. The control siRNA (Ctrl) is the firefly luciferase GL2. RLU, relative luciferase units normalised to Renilla. Data are means ± S.E. *** $p<0.01$; ** $p<0.001$, one-way ANOVA (n=3). SiRNA-mediated WT1 knock-down in the DLR experiments was verified by western blotting in PC3 (C) and K562 (D) cell lines; 20μg total protein was loaded in each lane.

Fig. 2. Effect of WT1 knockdown on endogenous SRPK1 expression in K652 and PC3 cells. SiRNA directed against WT1 reduces the expression of WT1 and SRPK1. (A) Western blot of transfected K562 cells, quantified in (B) relative to β-actin; (C) and (D) in PC3 cells; 20μg total protein was loaded in each lane. **** $p<0.0001$, *** $p=0.0002$ ** $p<0.01$, * $p<0.05$, two-way ANOVA (n=3).

Fig. 3. Effect of SRPK1 knockdown and inhibition on cell proliferation, migration and invasion. The MTT assay was used to analyse the effect of SRPK1 knockdown *via* both 100nM siRNA (for 48hrs) and SRPK1 inhibition with SPHINX (for 72hrs) on cellular proliferation (A,C) and viability (B,D) in K562 and PC3 cells. Data represent mean ± S.E. **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$, two-way ANOVA (n=3). Effect of SRPK1 inhibition through SPHINX on PC3

cell migration and invasion. PC3 cells were treated with 10μM SPHINX for 48hrs and examined for cell migration (E) and invasion (F) in a transwell assay. ** p=0.0089, * p=0.0211, unpaired t test (n=3). The siRNA-mediated SRPK1 knock-down was verified by western blot in K562 (G) and PC3 (H) cells; 20μg total protein was loaded in each lane.

Fig. 4. WT1 co-immunoprecipitates with the *SRPK1* but not the *GAPDH* promoter in (A) K562 and (C) PC3 cells, respectively quantified in (B) and (D). RNA polymerase II (positive control) and mouse IgG (negative control) are included. Data are expressed as means ± S.E. **** p<0.0001, one-way ANOVA (n=3). (E) Verification of SRPK1 ChIP. The PCR product amplified in the WT1 and BASP1 ChIP experiments was sequenced (Eurofins Genomics). The forward primer lies just upstream of the transcription start site and the canonical WT1 binding site (in bold); the reverse primer is in the SRPK1 5'UTR. Partial SRPK1 mRNA (accession NM_003137) and SRPK1 promoter sequences (Eukaryotic Promoter Database, https://epd.epfl.ch/) are shown. The transcription start site (+1) is indicated.

Fig. 5. The presence of BASP1 prevents *SRPK1* transcription activation by WT1. (A) The wildtype *SRPK1* promoter is active in K562 cells, but significantly less active in B-K562 cells which overexpress BASP1. (B) Transfection of a BASP1-expressing plasmid reduces the activity of the SRPK1 promoter, as per the removal of the WT1 binding site (mutSRPK1). (C) Western blot comparing the expression of BASP1, SRPK1, and WT1 in K562 cells and B-K562 cells that overexpress BASP1, quantified in (D). 20μg total protein was loaded per lane; the bar-graph represents three independent experiments. BASP1 co-immunoprecipitates with the SRPK1 but not the GAPDH promoter in (E) B-K562 cells, quantified in (F). RNA polymerase II (positive

control) and mouse IgG (negative control) are included. Data expressed as means ± S.E. For (a) and (b): **** $p<0.0001$, *** $p<0.001$, one-way ANOVA (n=3). For (d) and (f): **** $p<0.0001$, * $p<0.05$, two-way ANOVA (n=3).

Fig. 6. Effect of SRPK1 inhibition and BASP1 expression on the expression of antiangiogenic $VEGF_{165}b$. (A) PC3 cells were treated for 48 h with 10μM of the SRPK1 inhibitor SPHINX. Western blot showing the expression of $VEGF_{165}b$ relative to total $VEGF_{165}$. 20μg total protein was loaded per lane. (B) BASP1-expressing plasmid was transiently transfected into PC3 cells; elevated BASP1 expression was confirmed by western blotting relative to β-actin. (C) The effect of BASP1 on $VEGF_{165}b$ expression in PC3 cells transfected with BASP1-expressing plasmid. 20μg total protein was loaded in each lane. ** $p<0.01$, * $p<0.05$, unpaired t test (n=3).

Fig. 7. Proposed model for the regulation of *SRPK1* transcription by WT1. (A) WT1 binds to a GC-rich target sequence adjacent to the transcription start site and activates *SRPK1* transcription (e.g. in cancer cells) by recruiting co-factors that open up chromatin (p300-CBP) together with histone acetyltransferases (HAT).(B) WT1 represses (e.g. in glomerular podocytes) *SRPK1* transcription in the presence of the transcriptional co-repressor BASP1, its co-factor (PIP2) and histone deacetylases (HDAC).

Figure

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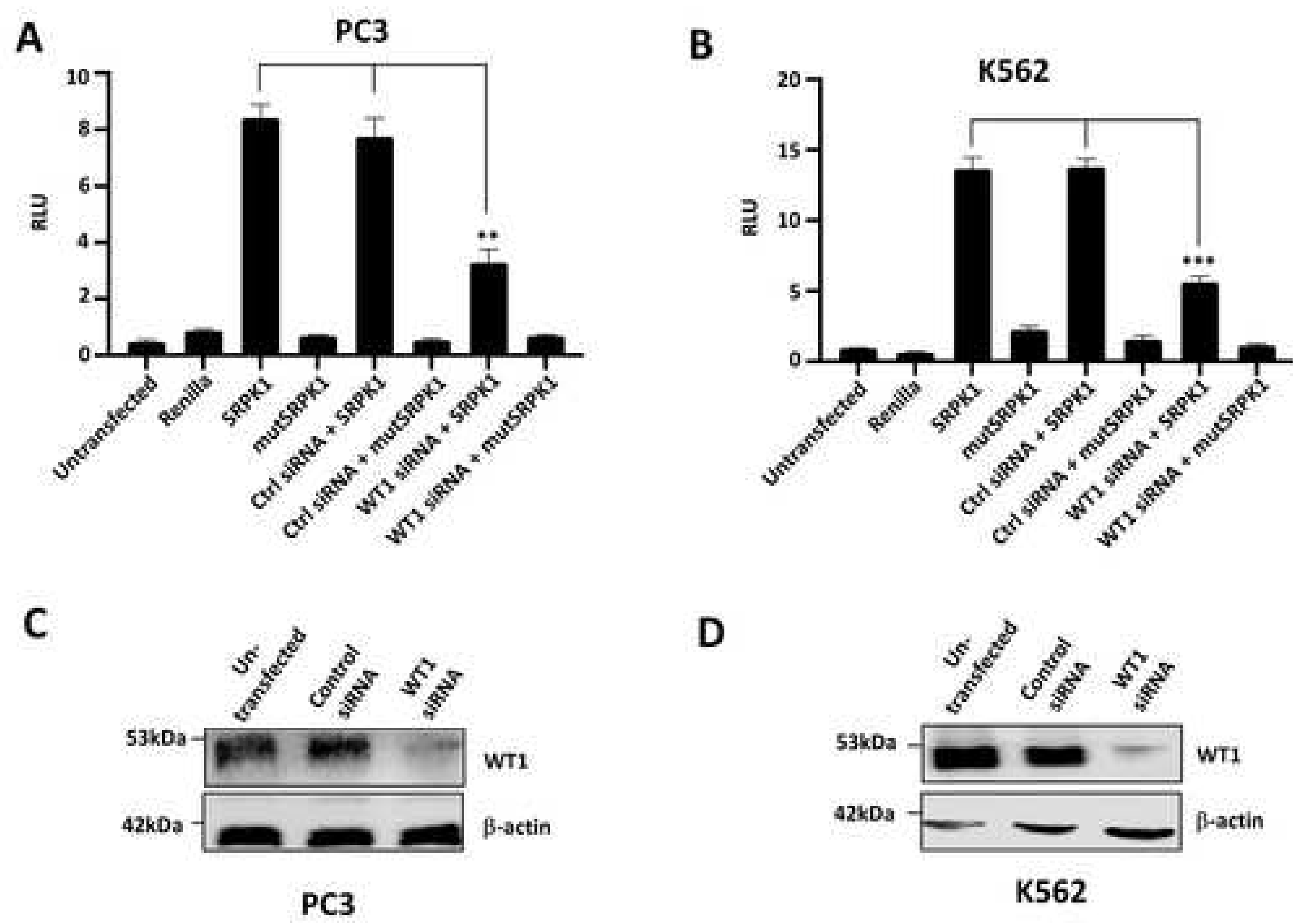

Figure 1

Figure

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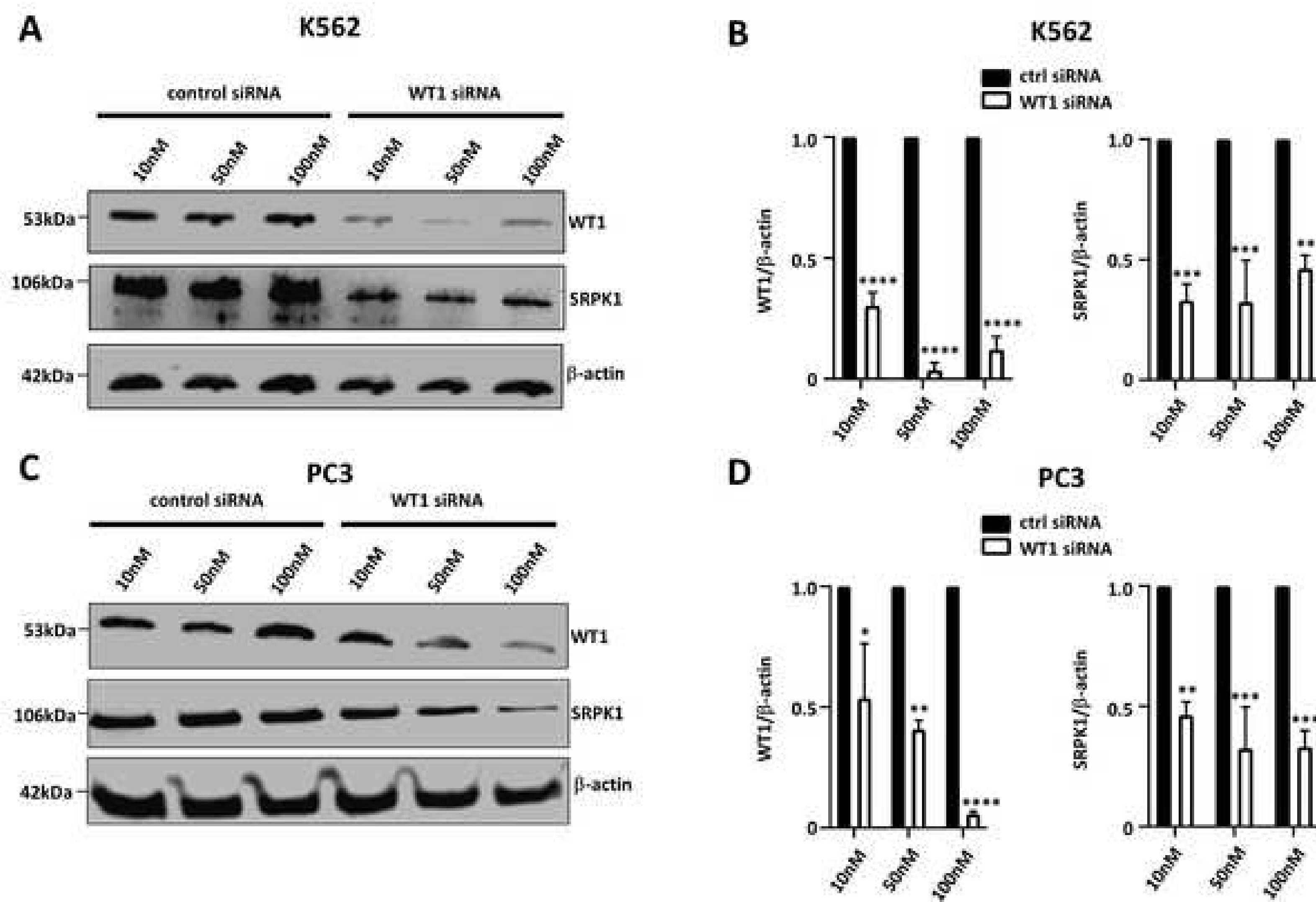

Figure 2

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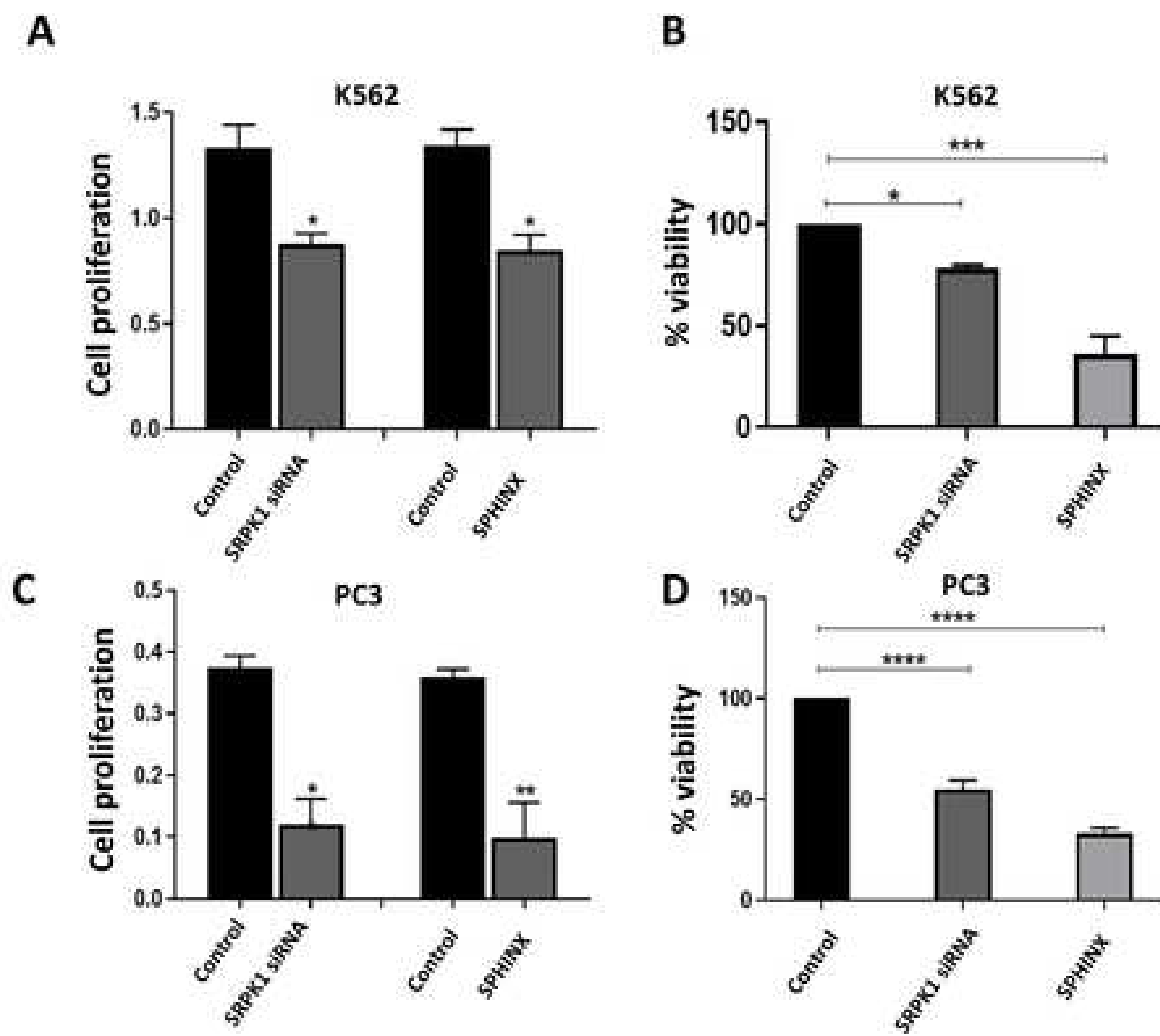

Figure 3

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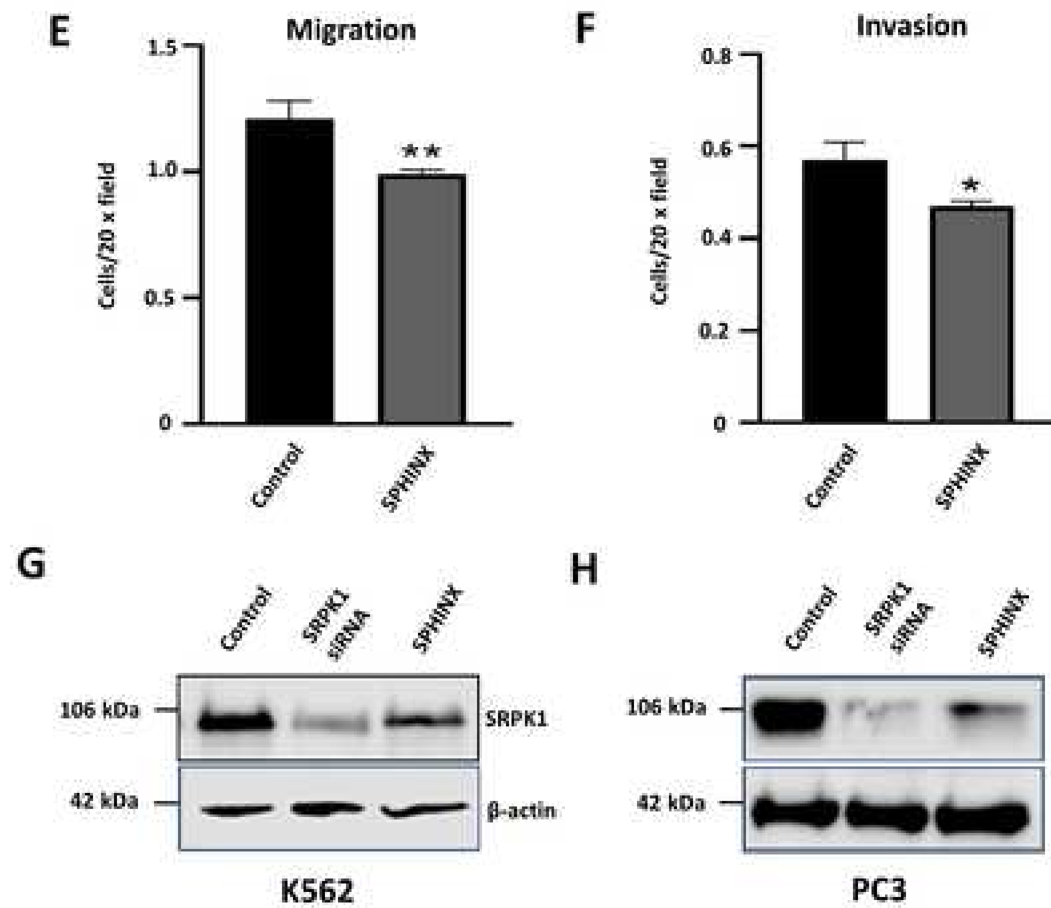

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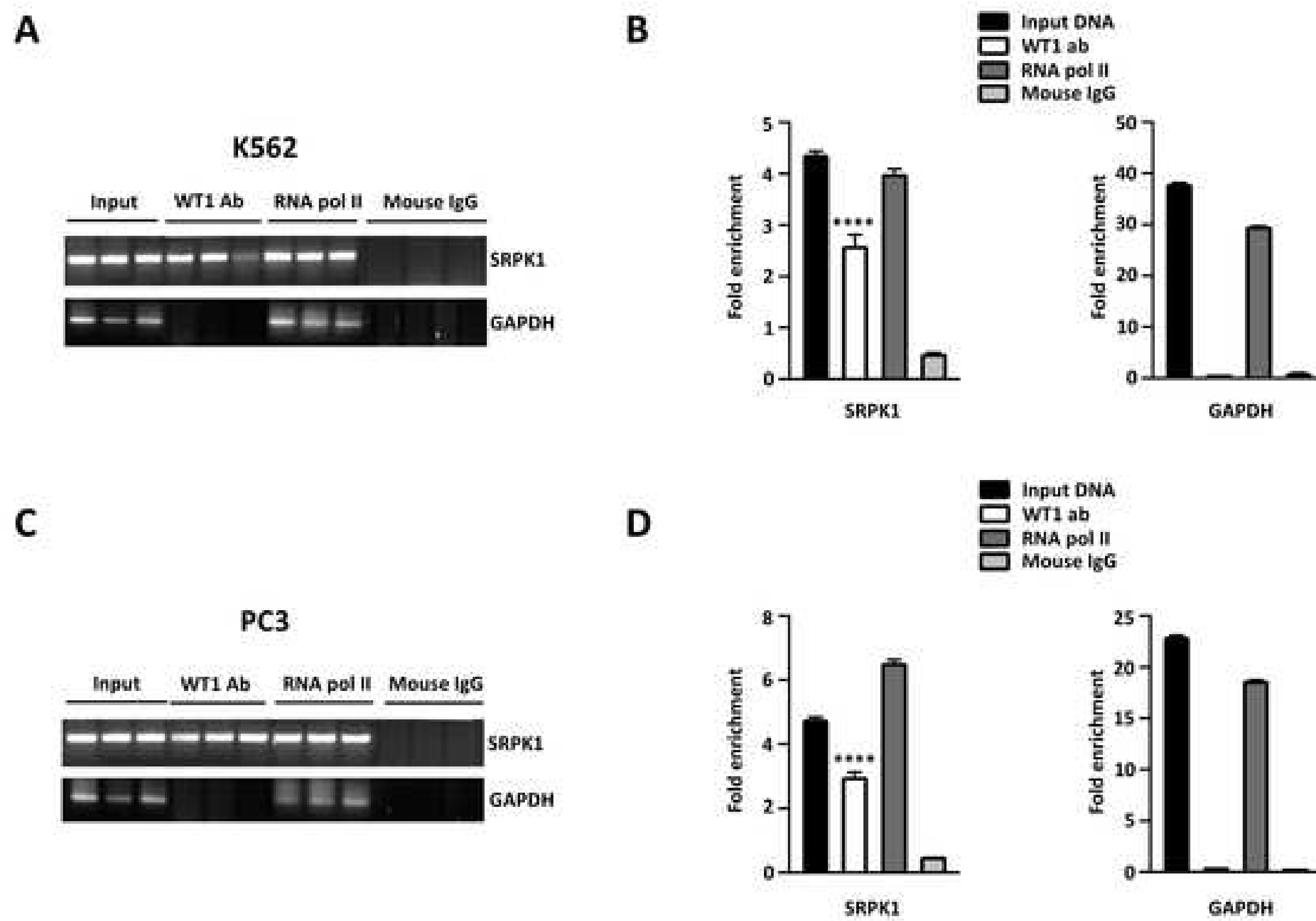

Figure 4

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E

```
SRPK1 mRNA 5'UTR    ----------------------------------------------------ATCGT
Forward primer      --------------------------------------CGCGCAAGCGCGGCATCGT
SRPK1 promoter      CCCCCTCCTCGGGAGCAGGTGGTAGGCTCCGCGCCCAACGCGCAAGCGCGGCATCGT
                                                                      +1

Sequencing data     -----------------GGACTGAGGGCGGAGTGTGAGCGGGCTCGGTTTTGGGCCGCG
SRPK1 mRNA 5'UTR    GGGGCGGGGGTGGGGCGGGACTGAGGGCGGAGTGTGAGCGGGCTCGGTTTTGGGCCGCG
WT1 binding site    GGGGCGGGGG-------------------------------------------------
SRPK1 promoter      GGGGCGGGGGTGGGGCGGGACTGAGGGCGGAGTGTGAGCGGGCTCGGTTTTGGGCCGCG

Sequencing data     GCGGGAGCGGGAGTCGCCGCCACTCGAGTGCGCAG------------------------
SRPK1 mRNA 5'UTR    GCGGGAGCGGGAGTCGCCGCCACTCGAGTGCGCAGGCGCCTGGCGATTACCGGTCTCAC
Reverse primer      --------------CGCCGCCACTCGAGTGCGCAG------------------------
SRPK1 promoter      GCGGGAGCGGGAGTCGCCGCCACTCGAGTGCGCAGGCGCCTGGCGATTACCGGTCTCAC
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In the WT1 binding site and second SRPK1 promoter rows, the GGGGCGGGGG motif is printed in bold.

Figure 4 contd

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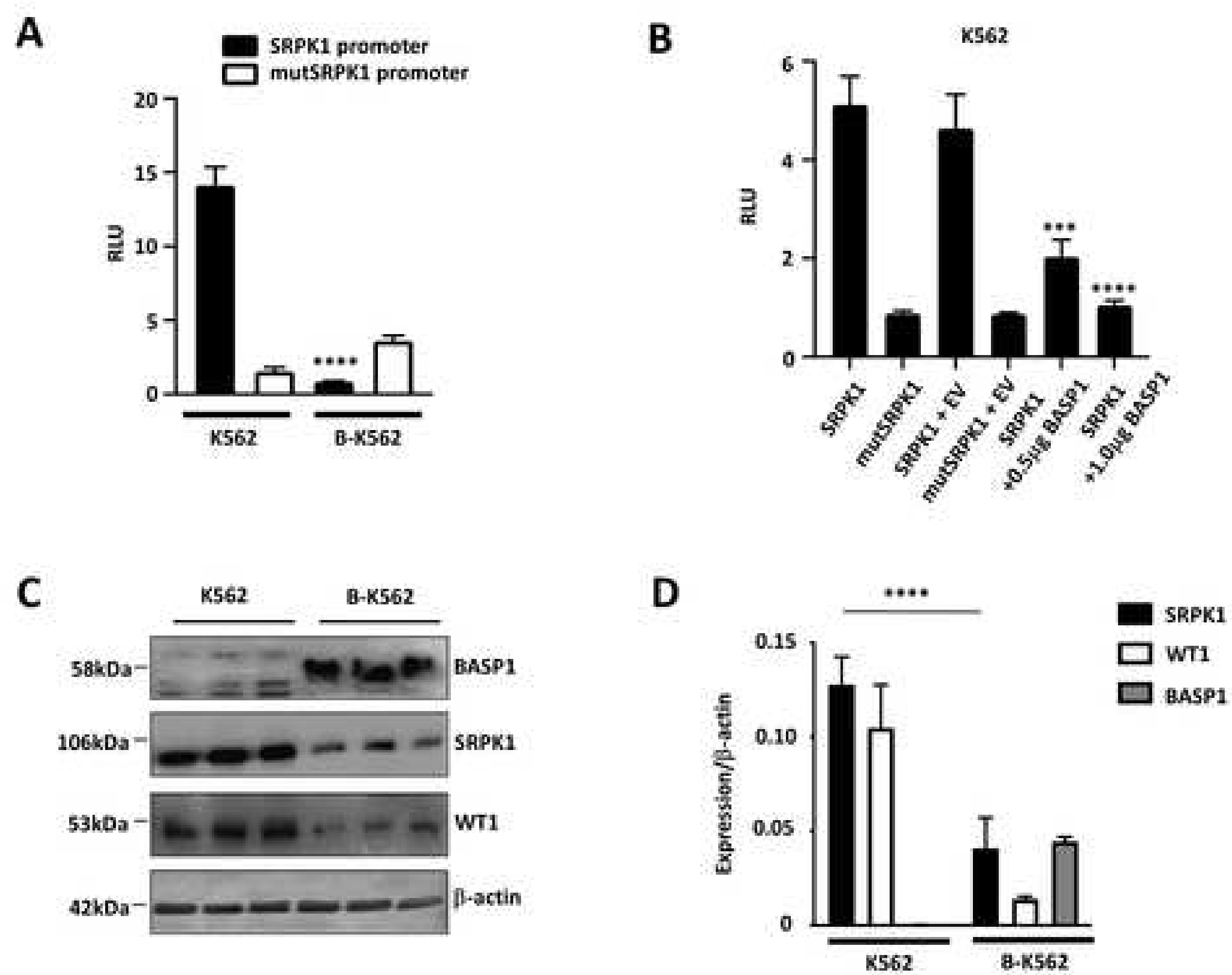

Figure 5

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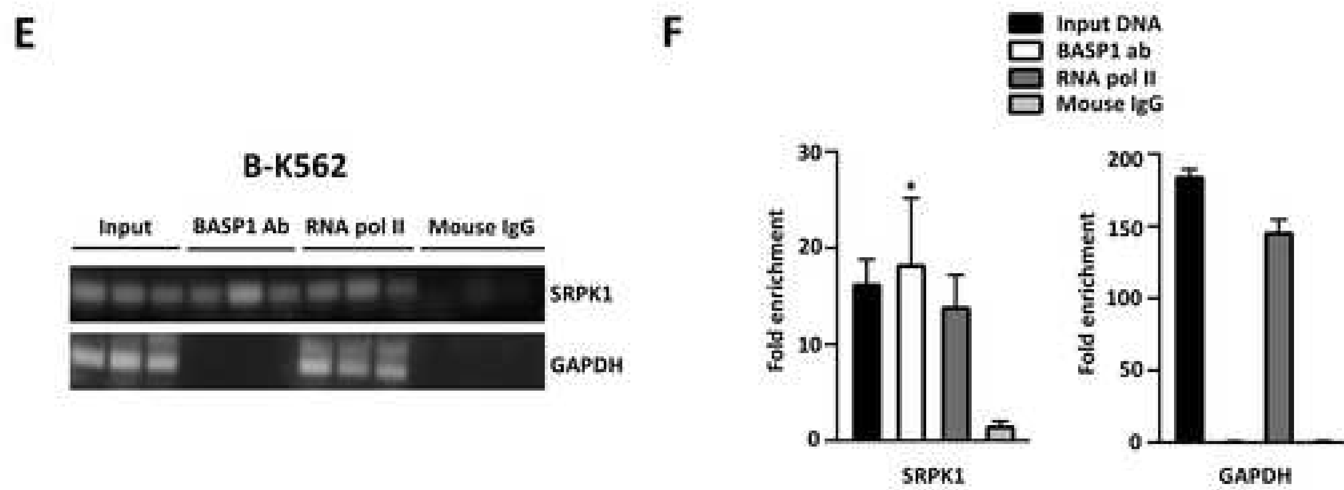

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Figure

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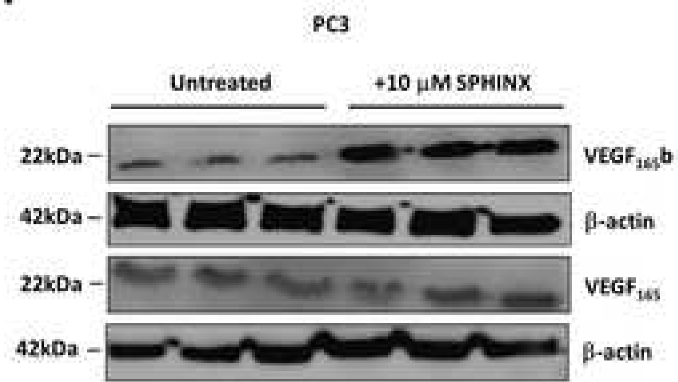

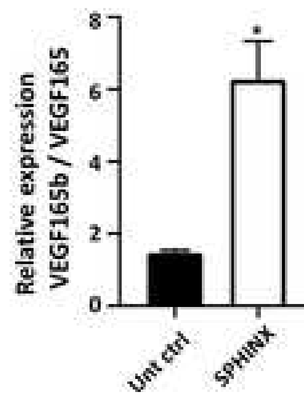

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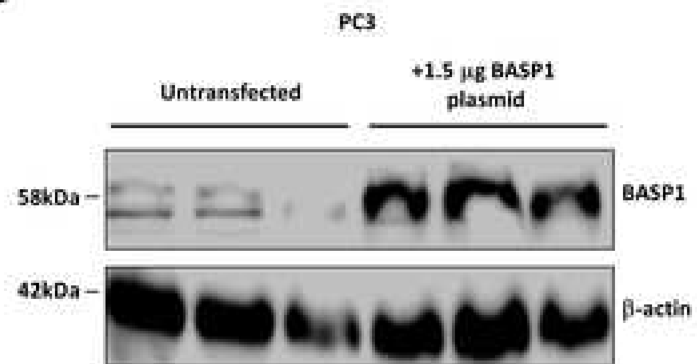

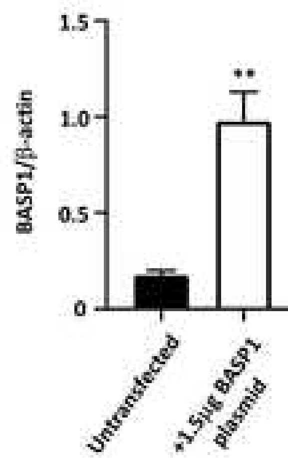

Figure 6

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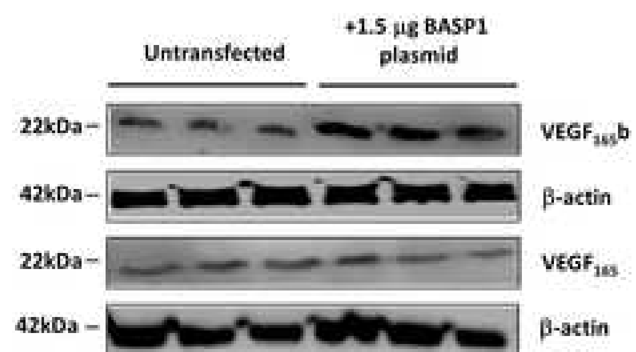

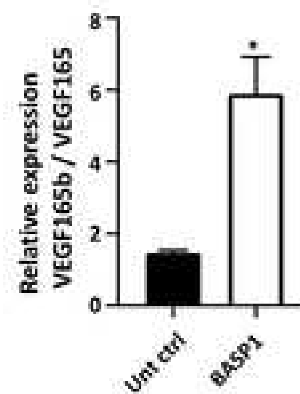

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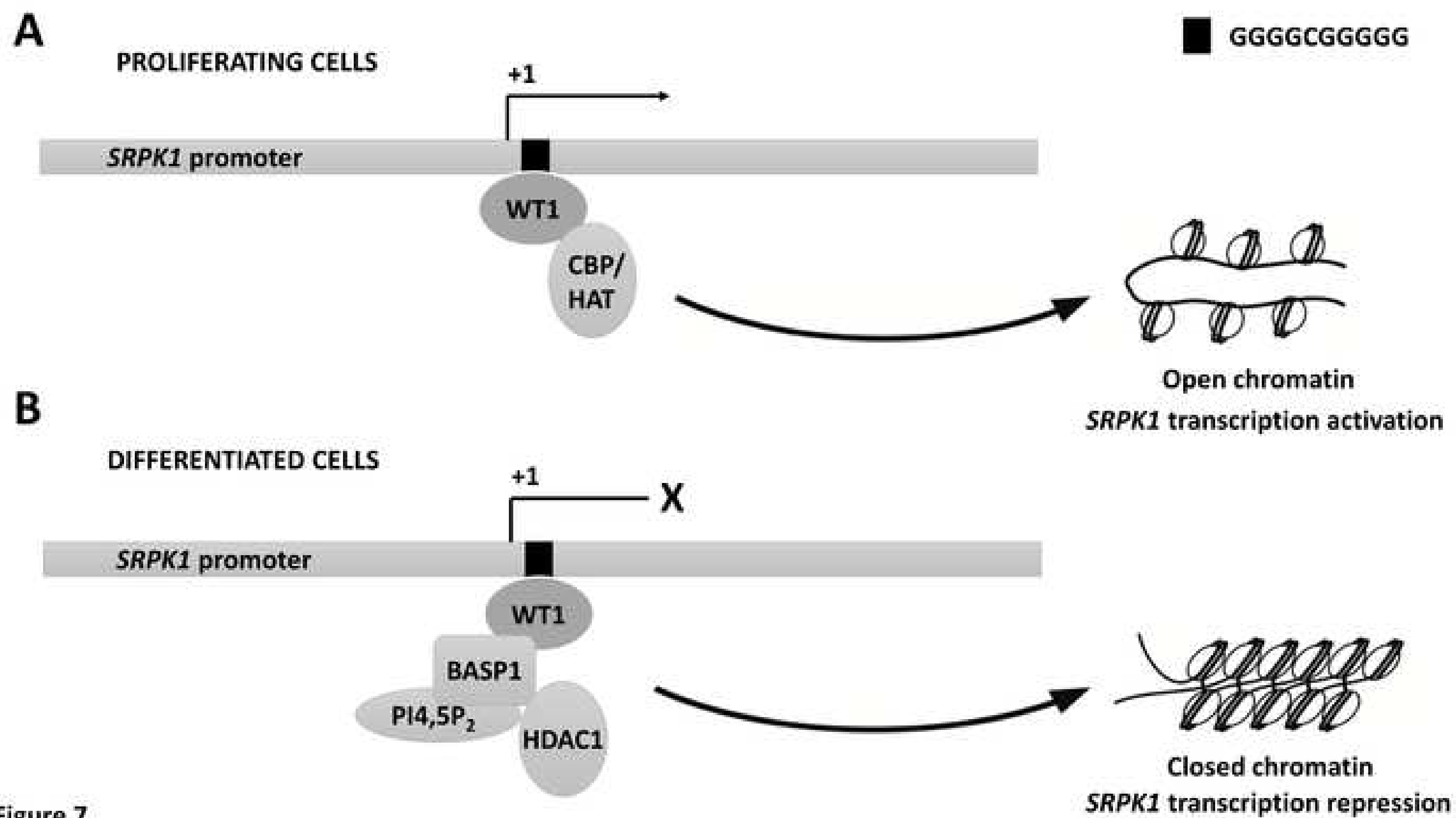

Figure 7