The NUCKS1-SKP2-p21/p27 axis controls S phase entry

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Abstract

Efficient entry into S phase of the cell cycle is necessary for embryonic development and tissue homeostasis. However, unscheduled S phase entry triggers DNA damage and promotes oncogenesis, underlining the requirement for strict control. Here, we identify the NUCKS1-SKP2-p21/p27 axis as a checkpoint pathway for the G1/S transition. In response to mitogenic stimulation, NUCKS1, a transcription factor, is recruited to chromatin to activate expression of *SKP2*, the F-box component of the SCF^{SKP2} ubiquitin ligase, leading to degradation of p21 and p27 and promoting progression into S phase. In contrast, DNA damage induces p53-dependent transcriptional repression of *NUCKS1*, leading to SKP2 downregulation, p21/p27 upregulation, and cell cycle arrest. We propose that the NUCKS1-SKP2-p21/p27 axis integrates mitogenic and DNA damage signalling to control S phase entry. TCGA data reveal that this mechanism is hijacked in cancer, potentially allowing cancer cells to sustain uncontrolled proliferation.

Introduction

Entry into S phase of the cell cycle is essential to sustain the proliferation that permits embryonic development and tissue repair[1], but unscheduled S phase entry induces replication stress, DNA damage, and oncogenesis[2–5]. G1/S progression must therefore be strictly controlled[6–8]. S phase entry is driven by mitogens, which increase the ratio of G1/S cyclins: cyclin-dependent kinase (CDK) inhibitors and activate G1/S CDKs as a result. In contrast, DNA damage inhibits S phase entry, stimulating p53 signalling to reduce the G1/S cyclin: CDK inhibitor ratio and prevent G1/S CDK activity[9]. Only cells whose mitogenic signalling outcompetes their DNA damage load are permitted to enter S phase[6,10–14], which must be achieved through integration of these antagonistic stimuli by signalling hubs. However, signalling hubs that achieve this goal are not well-characterised[6].

The transcription factor Nuclear Ubiquitous Casein kinase and cyclin-dependent Kinase Substrate 1 (NUCKS1) has emerged in the light of recent studies as a promising candidate for one such signalling hub. NUCKS1, a member of the high mobility group family of proteins[15], increases chromatin accessibility at target promoters to enable recruitment of RNA polymerase II[16]. So far, the only direct transcriptional targets identified for NUCKS1 regulate insulin receptor signalling[16]. However, NUCKS1 is known to affect cell cycle progression and proliferation in mammary epithelial cells[17] and gastric cancer cells[18], and also plays a role in the protection of replication fidelity by regulating double-strand break (DSB) repair[19–22]. In addition, NUCKS1 is a phosphorylation substrate for CDK2 and CDK1, the major kinases controlling the G1/S and G2/M transitions[23–27], and for the DNA damage response (DDR) kinases ATM and DNA-PK[28]. Furthermore, Rb-E2F1[29] and p53[30] have been detected in the proximity of the *NUCKS1* promoter by genome wide ChIP-

Seq, suggesting that *NUCKS1* expression might be regulated by the cell cycle or by DNA damage.

NUCKS1 also exhibits oncogenic properties, and its overexpression, correlating with poor patient prognosis, has been reported in a number of cancers[31–36]. Furthermore, NUCKS1 depletion inhibits - while its overexpression promotes - xenograft tumour growth[18,37,38], suggesting a direct role in tumourigenesis.

Altogether, these studies suggest a potentially important role for NUCKS1 in cell cycle progression. However, mechanistic details explaining how NUCKS1 does this are unknown. In particular, whether NUCKS1 employs transcriptional control of the cell cycle – and which putative targets of NUCKS1 are involved – has not been established. The precise cell cycle phase affected by NUCKS1 is also not known, and how NUCKS1 is regulated throughout the cell cycle, by mitogens, or following DNA damage, has not been explored.

Here, we show that S phase Kinase-associated Protein 2 (*SKP2*) is a transcriptional target for NUCKS1 in late G1 phase, and identify the SKP2-p21/p27 axis as a pathway controlled by NUCKS1. SKP2 is a substrate-recruiting F-box protein, which forms, along with SKP1, CUL1, and RBX1, the SCF^{SKP2} ubiquitin ligase complex[39]. During the G1/S transition, SKP2 directs SCF^{SKP2} for degradation of the CDK inhibitors p21 and p27, relieving p21/p27-mediated inhibition of cyclin E-CDK2[12,14,40–42]. In this way, SCF^{SKP2} controls cell cycle and cancer progression[43–45].

We find that the SKP2-p21/p27 axis acts through NUCKS1 to integrate mitogenic and DNA damage signalling at the G1/S transition. We show that NUCKS1 is stimulated by mitogens to promote *SKP2* expression and consequent p21/p27 degradation, enabling S phase entry. In contrast, DNA damage inhibits NUCKS1 through p53,

reducing SKP2 levels, increasing p21/p27 levels, and blocking S phase entry. In this way, the NUCKS1-SKP2-p21/p27 axis acts as a checkpoint pathway for the G1/S transition, only permitting S phase entry for cells whose mitogenic signalling outcompetes their load of DNA damage.

Results

NUCKS1 transcriptionally controls the SKP2-p21/p27 axis

To investigate whether NUCKS1 regulates the transcription of genes involved in cell cycle progression, we cross-compared a list of genes whose expression correlates with *NUCKS1* mRNA in tumour samples and cell lines[46], with genes whose promoter NUCKS1 binds in genome-wide ChIP-Seq[16]. This generated a list of 232 putative NUCKS1 transcriptional target genes. Gene Ontology (GO) Biological processes enrichment analysis for the top hits, showing the best correlation with *NUCKS1*, reveals significant enrichment for genes associated with cell cycle progression (Fig. 1A). Among them, we found several genes regulating the G1/S transition (e.g. *SKP2, E2F3, CDK6, CCND1*), DNA replication (e.g. *PCNA*), and the p53 pathway (e.g. *MDM2*).

In a panel of the putative cell cycle targets, *SKP2* displays the strongest and most reproducible downregulation upon NUCKS1 depletion (Supplementary Fig. 1A), and, given its role in cell cycle progression, DNA replication, and the DDR[43,45,47,48], we focused on *SKP2*. To gain a more comprehensive understanding of *NUCKS1*'s correlation with *SKP2*, we interrogated samples from The Cancer Genome Atlas (TCGA) database. Across a range of cancer types, mRNAs encoding *NUCKS1* and *SKP2* display significant positive correlation (Fig. 1B). There is no such correlation between *NUCKS1* and the housekeeping genes used as negative controls, *B2M* and

GAPDH (Supplementary Fig. 1B). In particular, the correlation between *NUCKS1* and *SKP2* is most striking in glioblastoma, kidney renal papillary cell carcinoma, skin cutaneous melanoma, and uveal melanoma (Supplementary Fig. 1C).

To confirm binding of NUCKS1 at the *SKP2* promoter[16], and to map the binding site, we designed ChIP-qPCR assays employing a panel of 10 primer sets spanning sequential regions of the *SKP2* promoter (Fig. 1C). In these assays, we found that NUCKS1 displays specificity for the chromatin directly upstream of the *SKP2* transcription start site (TSS), consistent with its role as a transcription factor (Fig. 1D).

Next, we tested the effect of NUCKS1 loss by siRNA-mediated depletion or CRISPR/Cas9-mediated deletion on *SKP2* mRNA levels (Fig. 1E, Supplementary Fig. 1D). We found that loss of NUCKS1 reduces *SKP2* gene expression across a cell line panel comprising three non-transformed cell lines (hTERT-immortalised bronchial epithelial cells: NBE1-hTERT; normal primary embryonic fibroblasts: TIG-1; hTERT-immortalised retinal epithelial cells: RPE1-hTERT), and six cancer cell lines (five colorectal cancer cell lines: HCT116, RKO, HT29, DLD1, CACO2; and osteosarcoma cells: U2OS) (Fig. 1E, Supplementary Fig. 1D). Loss of *SKP2* occurs independently of the status of p53, Rb, mitogen-activated protein kinase (MAPK) or microsatellite instability (MSI) (Fig. 1E). Furthermore, NUCKS1 depletion reduces SKP2 protein levels and increases levels of SKP2's degradation targets, p21 and p27, confirming loss of SKP2 activity (Fig. 1F), and this is independent of p53 (Supplementary Fig. 1E, F). Consistent with reduction in SKP2 levels, loss of NUCKS1 increases the stability of both p21 and p27, measured using cycloheximide chase assays (Supplementary Fig. 1G).

SKP2 mRNA levels are low in early G1 and increase during the G1/S transition[49]. To test G1 cell cycle enrichment in NUCKS1-depleted cells (demonstrated in Figure 3) as an indirect mechanism for *SKP2* downregulation, we measured *SKP2* levels in cells synchronised to G0/G1 before NUCKS1 depletion (Supplementary Fig. 1H). Under these conditions, loss of NUCKS1 still reduces *SKP2* mRNA levels (Supplementary Fig. 1I), comparable with NUCKS1 depletion from asynchronous cells. These results indicate that indirect cell cycle changes do not account for reduced levels of *SKP2* in NUCKS1-depleted cells.

Altogether, these data identify *SKP2* as a transcriptional target of NUCKS1, and show that NUCKS1 regulates *SKP2* expression independently of genetic background, and in multiple cellular contexts.

NUCKS1 levels and chromatin-binding are induced in late G1 to promote *SKP2* expression and G1/S progression

To determine whether NUCKS1 itself is subject to cell cycle-dependent regulation, and to determine the point in the cell cycle during which NUCKS1 regulates *SKP2*, we measured protein levels over the course of the cell cycle after release from G0/G1 synchronisation by contact inhibition. Using cyclin A2 as a marker for the onset of S phase[50], we found that levels of NUCKS1 are low at the start of G1, increasing as cells progress into S phase (Fig. 2A). The upregulation of SKP2 (but not NUCKS1) is driven partially by an increase in its mRNA levels, which is NUCKS1-dependent (Fig. 2B). Furthermore, we detected recruitment of NUCKS1 to chromatin following release from contact inhibition-mediated G0/G1 arrest, using PCNA and MLH1 - both of which are recruited to chromatin once S phase has started[51,52] - as controls (Supplementary Fig. 2A). The major positive stimulus for S phase entry is provided by mitogens, which

activate growth factor signalling[53]. We found that stimulation of cells with mitogens following 48 h of their withdrawal triggers the recruitment of NUCKS1 to chromatin, demonstrating a potential activation of NUCKS1 by mitogenic signalling (Fig. 2C).

Stimulation of NUCKS1 during G1 progression and by mitogen stimulation suggests an active role in S phase entry. To test this, we released control, NUCKS1-, or SKP2-depleted cells from G0/G1, and measured their ability to enter S phase. We found that siRNA-mediated NUCKS1 depletion substantially delays S phase entry following G0/G1 release, phenocopying SKP2 loss (Fig. 2D, E; Supplementary Fig. 2B). Similarly, deletion of *NUCKS1* from U2OS cells impairs S phase entry (Supplementary Fig. 2C).

Together, these results demonstrate that NUCKS1 is recruited to chromatin during G1 progression and by mitogenic stimulation, where NUCKS1 is required for induction of *SKP2* transcription and S phase entry.

NUCKS1 controls S phase entry through the SKP2-p21/p27 axis

Next, we investigated the phenotypic impact of control of the SKP2-p21/p27 axis by NUCKS1. We found that CRISPR/Cas9-mediated deletion of *NUCKS1* enriches cells in G0/G1 phase of the cell cycle, with concomitant reduction in replicating cells (Fig. 3A). This phenotype is reversed through overexpression of wildtype NUCKS1, but not by a DNA-binding defective mutant of NUCKS1 (in which the GRP motif is mutated to AAA), confirming that NUCKS1's DNA-binding activity is important for its role in cell cycle progression (Fig. 3B, Supplementary Fig. 3A). Furthermore, overexpression of NUCKS1 rescues cell cycle progression in NUCKS1-depleted HCT116 cells (Supplementary Fig. 3B, C), and NUCKS1 depletion delays cell cycle progression in TIG-1, NBE1-hTERT, and RPE1-hTERT cells (Supplementary Fig. 3D-F).

As a consequence, *NUCKS1* deletion from U2OS cells (Fig. 3C), and NUCKS1 depletion from TIG-1 or NBE1-hTERT cells (Supplementary Fig. 3G, H), considerably reduce cellular proliferation. NUCKS1 depletion does not cause DNA damage, measured by alkaline comet assay (which detects single strand DNA breaks (SSBs) and DSBs) (Supplementary Fig. 3I) or γH2AX/53BP1 immunofluorescence (Supplementary Fig. 3J), demonstrating that these phenotypes are not explained by DNA damage-induced quiescence.

We then tested whether accumulation of p21/p27 in NUCKS1-depleted cells is due to loss of SKP2. We found that overexpression of SKP2 in NUCKS1-depleted HCT116 (Fig. 3D) and A549 cells (Supplementary Fig. 3K) mostly induces degradation of the p21/p27 that accumulate in these cells. Consequently, overexpression of SKP2 in NUCKS1-depleted HCT116 (Fig. 3E, H) or A549 cells (Fig. 3F, I) largely rescues S phase entry. Similarly, co-depletion of SKP2's degradation targets, p21 or p27 (Fig. 3G, J; Supplementary Fig. 3L, M, N), largely reverses cell cycle arrest.

Exploring these phenotypes further through proliferation assays, we found that SKP2 overexpression (Fig. 3K) or p21/p27 co-depletion (Fig. 3L) partially rescues the proliferation defects of NUCKS1-depleted cells. Finally, depletion of NUCKS1 from SKP2-depleted cells has no additional effect on proliferation, supporting the idea that SKP2 is a major determinant of NUCKS1's effect on proliferation (Supplementary Fig. 3O).

These results demonstrate that NUCKS1 controls p21/p27 levels, cell cycle progression, and proliferation through its transcriptional stimulation of the *SKP2* gene, and identify the NUCKS1-SKP2-p21/p27 axis as a driving pathway for the G1/S transition.

Analysis of NUCKS1 binding at the *SKP2* promoter

To more comprehensively understand the regulation of *SKP2* by NUCKS1, we employed the electrophoretic mobility shift assay (EMSA), using a fluorescent *SKP2* promoter probe (Fig. 4A). To do this, we started by purifying NUCKS1 from Sf9 insect cells, which preserves NUCKS1's post-translational modifications (Fig. 4B). We found that in-tact, phosphorylated NUCKS1 displays low affinity for the *SKP2* probe. However, dephosphorylation of NUCKS1 (using lambda phosphatase) increases the affinity of NUCKS1 for the *SKP2* probe almost 10-fold (Fig. 4C, D). Since a previous publication reported a GC-box as a potential NUCKS1 binding site[16], and since our *SKP2* EMSA probe contains a GC-box, we mutated this sequence and performed competition EMSAs to investigate whether NUCKS1 exhibits specificity for this site. We found that the interaction of NUCKS1 with the *SKP2* probe is readily outcompeted by a 100-fold excess of unlabelled WT *SKP2* probe, but not by a mutant of the *SKP2* probe with no GC-box (Fig. 4E, F). Finally, we performed EMSAs using WT and *NUCKS1*-KO nuclear extract, and found that nuclear extract from WT cells displays much higher affinity for the *SKP2* probe than extract from *NUCKS1*-KO cells (Fig. 4G, H).

Altogether, these results demonstrate that NUCKS1 directly interacts with the *SKP2* promoter's DNA, that this may be via a GC-box in the *SKP2* promoter, and that this may be regulated by the phosphorylation status of NUCKS1.

DNA damage inhibits the NUCKS1-SKP2 axis through p53-dependent transcriptional repression

DNA damage activates an ATM/p53-dependent pathway to instigate cell cycle arrest, delay DNA replication, and accomplish DNA repair[54]. To determine whether this

response involves NUCKS1 or SKP2, we analysed the NUCKS1-SKP2 axis following induction of DNA damage. In U2OS cells (which express WT *TP53*, encoding p53), treatment with the chemotherapeutic drug 5-fluorouracil (5-FU) markedly reduces NUCKS1 and SKP2 protein levels, with concomitant upregulation of p21 (controlled by both p53 and SKP2), and p27 (controlled by SKP2) (Fig. 5A). 5-FU treatment also abolishes occupancy of NUCKS1 at the *SKP2* promoter (Fig. 5B), suggesting that downregulation of SKP2 is due to loss of NUCKS1 binding at its promoter. WT RPE1-hTERT cells treated with 5-FU similarly downregulate NUCKS1 and SKP2, and upregulate p21 and p27. However, this response is absent in *TP53*-KO RPE1-hTERT cells, suggesting a role for p53 in DNA damage-mediated NUCKS1/SKP2 downregulation (Fig. 5C). Consistent with loss of SKP2, the stability of p21 and p27 is extended in 5-FU-treated cells, revealed through chase assays with the translation inhibitor cycloheximide (Supplementary Fig. 4A).

To investigate this putative role for p53, we used RT-qPCR to measure the mRNA levels of *NUCKS1* and *SKP2* after treatment with 5-FU, IR, hydrogen peroxide (H_2O_2), and camptothecin (CPT), DNA-damaging agents which induce distinct DNA lesions. Upregulation of *CDKN1A* mRNA, encoding p21, was used as a control for p53 activation. We found that *NUCKS1* and *SKP2* transcripts are substantially reduced in response to all tested DNA-damaging agents (Fig. 5D). Consistent with Western blotting data, this is largely dependent on p53 (Fig. 5E-H). Induction of DNA damage also induces cell cycle changes which similarly depend on *TP53* status (Supplementary Fig. 4B). These results demonstrate that DNA damage induces a p53 response, involving downregulation of NUCKS1 and SKP2, upregulation of p21 and p27, and cell cycle arrest.

Next, we sought to understand the mechanism underpinning p53-dependent downregulation of NUCKS1 and SKP2. Much p53-mediated transcriptional repression relies on activation of the DREAM transcriptional repression complex by p53-induced p21[55]. To investigate whether this pathway is involved in the downregulation of NUCKS1 or SKP2, we treated WT and *CDKN1A*-knockout cells with 5-FU. As expected, transcripts of the p21-DREAM target *CCNB1*[56], used as a positive control, are only reliably downregulated upon DNA damage in WT cells. However, transcripts of *NUCKS1* and *SKP2* are downregulated both in WT and *CDKN1A*-knockout cells, suggesting that *NUCKS1* and *SKP2* are not targets of the p21-DREAM pathway (Supplementary Fig. 4C-E).

Finally, we found that RNAi-mediated p53 depletion in TIG-1 cells, which express high endogenous p53 levels[57] (Supplementary Fig. 4F), as well as deletion of *TP53* from RPE1-hTERT cells (Supplementary Fig. 4G), increases *NUCKS1* levels, suggesting that p53 may regulate *NUCKS1*/*SKP2* expression both under basal conditions, as well as following p53 activation.

We propose that the p53-NUCKS1-SKP2-p21/p27 axis constitutes a checkpoint pathway for the G1/S transition, which may respond to DNA damage to prevent the replication of damaged DNA.

Copy number gain and p53 loss contribute to *NUCKS1* and *SKP2* overexpression in cancer

Transcriptional overexpression of *NUCKS1* and *SKP2* have been reported in numerous cancer types[18,31–34,36,45,58–60]. Although some reports, focused on specific cancer types, attribute this to increased copy number[31,35,37,45,59,61], no pan-cancer analyses have been performed, and the full mechanisms underlying the upregulation

remain poorly defined. Seeking to explore this further, we analysed *NUCKS1* and *SKP2* expression in TCGA datasets. *NUCKS1* and *SKP2* are overexpressed in most TCGA datasets, including many shared cancer types (Fig. 6A, B). Consistent with oncogenic functions for *NUCKS1*[37] and *SKP2*[60], both genes are subjected to copy number increase in many cancers, while deletions are rare (Fig. 6C, D). These results confirm that increased copy number of *NUCKS1* and *SKP2* can contribute to their overexpression in cancer.

Because we found that p53 negatively regulates levels of *NUCKS1* and *SKP2* (Fig. 5), we investigated the effect of p53 mutation in cancer. To do so, we used p53-proficient vs. -deficient HCT116 cells, and expressed WT p53 as well as its DNA-binding mutants, R175H, R248W, and R273H, which frequently drive cancer[62]. We found that p53-deficient HCT116 cells have increased levels of both *NUCKS1* and *SKP2*. Notably, overexpression of WT p53 - but not its DNA-binding mutants - substantially reduces *NUCKS1*/*SKP2* levels (Fig. 6E). These results further support our finding that p53 negatively regulates levels of *NUCKS1* and *SKP2*, and demonstrate that p53 mutants defective for DNA-binding lose the ability to repress *NUCKS1*/*SKP2*.

Finally, we asked whether p53 mutations also affect *NUCKS1*/*SKP2* expression in cancer patients, using TCGA datasets. Consistent with our *in vitro* data, we found that mutation of p53 correlates with overexpression of *NUCKS1*/*SKP2* in several cancer types (Fig. 6F).

Together, these results show that increased copy number, as well as p53 mutation, contribute to the overexpression of *NUCKS1* and *SKP2* in many cancers. This may enable cancer cells to proliferate in the absence of mitogenic stimulation, or in the presence of DNA damage.

Discussion

Here, we identify the NUCKS1-SKP2-p21/p27 axis as a cell cycle checkpoint pathway, which responds antagonistically to mitogen and DNA damage input to control S phase entry. In early G1 cells and in the absence of mitogens, NUCKS1 protein levels and chromatin retention are low, ensuring its inhibition in non-replicating cells. NUCKS1 is upregulated and recruited to chromatin during G1/S progression, permitting NUCKS1 to stimulate the expression of *SKP2*, the F-box component of the SCF^{SKP2} ubiquitin ligase, leading to the degradation of p21/p27 and S phase entry. In contrast, DNA damage induces p53-dependent transcriptional repression of *NUCKS1*, leading to loss of SKP2 and upregulation of p21/p27 for cell cycle arrest. Some cancer cells hijack this mechanism, increasing *NUCKS1/SKP2* copy numbers and transcriptionally upregulating *NUCKS1* and *SKP2* through p53 mutation. We propose that this may enable cancer cells to sustain proliferation, even in the absence of mitogens or in the presence of DNA damage (Fig. 7).

Our study identifies the SKP2-p21/p27 pathway as the second pathway transcriptionally controlled by NUCKS1, after the insulin receptor pathway[16]. However, the question of precisely how NUCKS1 regulates transcription remains unanswered. NUCKS1 is known to bind chromatin with higher affinity than naked DNA, does not bind ssDNA, and binds D-loops better than dsDNA[19] (although whether NUCKS1 binds R-loops has not been tested). NUCKS1 does not have a transcription activation domain, but promotes chromatin accessibility at - and recruits RNAPII to – its target promoters[16]. It is possible that NUCKS1 cooperates with other transcription factors to direct transcription; for example, NUCKS1 has been reported as an activator of NF-κB[63]. Since NF-κB regulates *SKP2* levels[64], NUCKS1 may cooperate with NF-κB to

control *SKP2* expression. Nevertheless, future work will focus on characterising NUCKS1's interactome, and investigate its mechanism for transcriptional regulation.

Our EMSA data (Fig. 4) and others' ChIP-Seq data[16] reveal that NUCKS1 displays affinity for the GC-box target sequence. The sequence we identify, GGCGGG, is present twice within the 600 nucleotide *SKP2* promoter, but is absent from the remaining ~45,000 nucleotides of the *SKP2* gene, which may explain the specificity of NUCKS1 for the *SKP2* promoter *in vivo*, and for other NUCKS1 targets more broadly. Going forward, research should focus on the structural basis of NUCKS1's interaction with the GC-box, and investigate whether NUCKS1 has multiple target DNA-binding sequences.

We show that p53 mediates the transcriptional downregulation of *NUCKS1* in response to DNA damage (Figure 5), but we do not fully characterise the mechanism. Binding of p53 at the *NUCKS1* promoter, with enrichment following DNA damage, has been detected as part of genome-wide ChIP-Seq studies[30], and our data showing that the downregulation of *NUCKS1* following DNA damage is independent of p21-DREAM (Supplementary Fig. 4) suggest that *NUCKS1*'s repression may be a direct result of p53 binding. To explore this further, it would be useful to measure the rate of synthesis of new *NUCKS1* transcripts, as well as the stability of *NUCKS1* transcripts, to determine whether p53 controls *NUCKS1*'s transcription itself or the stability of its mRNA. Furthermore, would mutation of p53's putative binding site in the *NUCKS1* promoter alter *NUCKS1*'s expression, DNA damage resistance, cell cycle progression, and proliferation? These experiments will form part of future studies.

NUCKS1 is the most post-translationally modified protein in the human proteome (for its size) and its major modification is phosphorylation[23]. NUCKS1 is phosphorylated

by the G2/M cell cycle kinase CDK1 at S181, reducing NUCKS1 binding to DNA[26,27], although the *in vivo* function of this phosphorylation is unknown. Interestingly, S181 phosphorylation of NUCKS1 could act to reset the level of chromatin-bound NUCKS1 for the daughter G1 phase, during which CDK1 activity is low, and explain the G1/S chromatin recruitment of NUCKS1 that we observe (Supplementary Fig. 2). Furthermore, the DDR kinase ATM promotes the indirect phosphorylation of NUCKS1 at S181 following DNA damage[19,28,65]. Therefore, ATM-dependent NUCKS1 phosphorylation could provide a secondary mechanism to p53-dependent transcriptional repression, to ensure NUCKS1's removal from cell cycle promoters after DNA damage, and warrants investigation in the future. Notably, phosphorylation of NUCKS1 at S181 may also explain our EMSA data, which reveal a significant increase in DNA-binding affinity following NUCKS1 dephosphorylation (Figure 4).

By stimulating the activity of RAD54, NUCKS1 promotes HR, the S/G2-specific DSB repair pathway[19,22], demonstrating that NUCKS1 acts to maintain the fidelity of DNA replication. Consistent with this, we show that NUCKS1 levels remain high throughout S phase and into G2 (Fig. 2). These findings raise a model in which NUCKS1 stimulates entry into S phase and promotes the fidelity of the ensuing DNA replication through HR, after its role in S phase entry is achieved. Notably, this function would mirror that of other G1/S factors, which boost both S phase entry and DNA repair, including E2F1[66] and SKP2 itself[48].

In summary, our study identifies NUCKS1 as an important factor for the G1/S transition, placing NUCKS1 within the SKP2-p21/p27 axis. Future studies will investigate NUCKS1's mechanism of transcriptional regulation, mechanisms for its regulation by posttranslational modification, and delve deeper into its roles in oncogenesis.

Figure legends

Fig. 1: NUCKS1 transcriptionally controls the SKP2-p21/p27 axis

A) Schematic of approach to identify NUCKS1's transcriptional targets, and the GO biological processes enrichment of the top hits, using NUCKS1 ChIP-Seq[16], SEEK[46], and GeneCodis[67].
B) Pearson's correlation of *NUCKS1* and *SKP2* mRNAs from TCGA datasets, made using data from GEPIA2[68].
C) Map of human *SKP2* promoter annotated with sequence positions of ChIP-qPCR primers.
D) ChIP-qPCR of NUCKS1 on the *SKP2* promoter in U2OS cells. N=3 +/- SEM. Ordinary one-way ANOVA with Dunnett's multiple comparisons test, using -25 - +148 as a reference.
E) Left: RT-qPCR after control or siRNA-mediated NUCKS1 depletion, in 9 cell lines. Dotted line denotes mRNA levels in siCtrl-treated cells. n=1-4 +/- SEM. Right: RT-qPCR in two different clones of *NUCKS1*-KO U2OS cells. Dotted line denotes mRNA levels in WT U2OS cells. n=2 +/- SEM. Left: One-way ANOVA with Sidak post-test. Right: One-way ANOVA with Dunnett's multiple comparisons test.
F) Western blot in control- or NUCKS1-depleted RPE1-hTERT cells.

Fig. 2: NUCKS1 levels and chromatin-binding are induced in late G1 to promote *SKP2* expression and G1/S progression

A) Western blot in whole cell extracts of RPE1-hTERT cells synchronised to G0/G1 by 72 h contact inhibition (*t*=0) followed by re-plating at low density to release cells into S phase.
B) RT-qPCR in NBE1-hTERT cells treated as in A.
C) Western blot in the chromatin fraction of NBE1-hTERT cells starved of serum for 48 h (*t*=0) followed by mitogenic stimulation (15% FBS) for the indicated periods of time.
D) PI cell cycle profiles of control, NUCKS1- or SKP2-depleted RPE1-hTERT cells treated as in A.
E) Quantification of D. N=3 +/- SEM.

Fig. 3: NUCKS1 controls S phase entry through the SKP2-p21/p27 axis

A) EdU/PI cell cycle profiles of WT U2OS cells and three different clones of *NUCKS1*-KO cells (left) and corresponding quantifications (right). Ordinary one-way ANOVA with Dunnett multiple comparisons test on S phase population. N=3 +/- SEM.

B) Left: EdU/PI cell cycle profiles of WT and *NUCKS1*-KO U2OS cells expressing the indicated variants of NUCKS1. Right: Quantification. N=2 +/- SEM.
C) Proliferation assay in WT U2OS cells and three different clones of *NUCKS1*-KO cells. Ordinary one-way ANOVA with Dunnett multiple comparisons test at day 8. N=2 +/- SEM.
D) SKP2 overexpression largely rescues p21/p27 accumulation in NUCKS1-depleted HCT116 cells, measured by Western blot.
E) SKP2 overexpression largely rescues HCT116 EdU/PI cell cycle profiles following treatment with NUCKS1 siRNA.
F) SKP2 overexpression largely rescues EdU/PI cell cycle profiles in A549 cells treated with control or NUCKS1 siRNA.
G) EdU/PI cell cycle profiles of HCT116 cells treated with control, p21, p27, or NUCKS1 siRNAs.
H) Quantification of HCT116 SKP2 cell cycle profiles in E. N=2 +/- SD. Ordinary one-way ANOVA with Sidak multiple comparisons test on S phase populations.
I) Quantification of A549 SKP2 cell cycle profiles in F. N=2 +/- SEM.
J) Quantification of HCT116 p21/p27 cell cycle profiles in G. N=2 +/- SD. Ordinary one-way ANOVA with Sidak multiple comparisons test on S phase populations.
K) SKP2 overexpression partially rescues proliferation following NUCKS1 depletion in A549 cells. N=2 +/- SEM. Ordinary one-way ANOVA with Sidak multiple comparisons test at day 8.
L) Proliferation assay in RPE1-hTERT cells treated with control, NUCKS1, p21 or p27 siRNAs. N=2 +/- SD. Ordinary one-way ANOVA with Sidak multiple comparisons test at day 7.

Fig. 4: Analysis of NUCKS1 binding at the *SKP2* promoter.

a) Schematic showing sequence positions of the EMSA probe in relation to *SKP2*'s transcription start site (TSS) and the region giving peak binding in our ChIP-qPCR assays.
b) Coomassie gel demonstrating NUCKS1 purification. Treatment with lambda phosphatase removes NUCKS1 phosphorylation and reduces its molecular weight.
c) Titration of phosphorylated or dephosphorylated NUCKS1 (10.24, 25.6, 64, 160, 400, 1000, 2500 nM) with the *SKP2* promoter probe.
d) Quantification of C. n=3 +/- SEM.
e) Titration of phosphorylated or dephosphorylated NUCKS1 with the *SKP2* promoter probe. In lanes 6/12 and 7/13, respectively, 100 X molar quantity of unlabelled WT or mutant *SKP2* probe were added as competition in binding reactions.
f) Quantification of E. n=3 +/- SEM.

g) Titration of WT or *NUCKS1*-KO U2OS nuclear extract with the *SKP2* promoter probe. In lanes 6 and 11, 100 X molar quantity of unlabelled WT probe was added as competition in binding reactions.
h) Quantification of G. n=3 +/- SEM.

Fig. 5: DNA damage inhibits the NUCKS1-SKP2 axis through p53-mediated transcriptional repression

a) Western blot in WT U2OS cells treated with 50 µM 5-FU for 24 or 48 h.
b) ChIP-qPCR of NUCKS1 on the *SKP2* promoter in U2OS cells after 50 µM 5-FU for 48 h. N=3 +/- SEM.
c) Western blot in RPE1-hTERT WT and *TP53*-KO cells treated with 10 µM 5-FU for 24 or 48 h.
d) RT-qPCR in WT RPE1-hTERT cells treated with 5-FU (10 µM), IR (4 Gy), H_2O_2 (200 µM), or CPT (100 nM) for 24 or 48 h. N=3-5 +/- SEM. Ordinary two-way ANOVA with Dunnett's multiple comparisons test.
e) RT-qPCR after 5-FU (10 µM) in RPE1-hTERT WT or *TP53*-KO cells. N=2 +/- SEM.
f) RT-qPCR after IR (4 Gy) in RPE1-hTERT WT or *TP53*-KO cells. N=2 +/- SEM.
g) RT-qPCR after H_2O_2 (200 µM) in RPE1-hTERT WT or *TP53*-KO cells. N=2 +/- SEM.
h) RT-qPCR after CPT (100 nM) in RPE1-hTERT WT or *TP53*-KO cells. N=3 +/- SEM.

Fig. 6: Copy number gain and p53 loss contribute to *NUCKS1* and *SKP2* overexpression in cancer

A) *NUCKS1* expression in normal vs. tumour tissue, using data from UCSC Xena[69]. In A and B, box plots show median values along with 25/75% (box) and 10/90% (whiskers). Statistics were analysed using Kruskal Wallis with Dunn's post test.
B) *SKP2* expression in normal vs. tumour tissue, using data from UCSC Xena[69].
C) *NUCKS1* copy number changes in cancer, using data from TCGA PanCancer Atlas datasets in CBioPortal[70,71].
D) *SKP2* copy number changes in cancer, using data from TCGA PanCancer Atlas datasets in CBioPortal[70,71].
E) Western blot and RT-qPCR in p53 proficient vs. deficient HCT116 cells expressing indicated variants of p53. n=3 +/- SEM. Ordinary one-way ANOVA with Tukey's multiple comparisons test.

F) Analysis of *TP53*, *NUCKS1*, *SKP2* or *XPC* (used as a positive control for p53 activity) mRNA levels in WT vs. mutant tumours, using PanCancer Atlas or METABRIC datasets in CBioPortal[70,71]. Box plots show median values along with 25/75% (box) and 10/90% (whiskers) and outliers. Mann-Whitney test.

Fig. 7: Model depicting the role for NUCKS1 in the S phase entry decision.

The NUCKS1-SKP2-p21/p27 axis constitutes a signalling hub which integrates the opposing cell cycle signals, mitogens and DNA damage.

Mitogens stimulate binding of NUCKS1 to the *SKP2* promoter, *SKP2* expression, p21/p27 degradation, and S phase entry.

DNA damage induces p53-dependent repression of *NUCKS1*, leading to *SKP2*'s transcriptional downregulation, upregulation of p21/p27, and cell cycle arrest.

Some cancer cells increase *NUCKS1/SKP2* copy number and mutate p53, leading to *NUCKS1* and *SKP2* overexpression.

Supplementary Fig. 1: NUCKS1 transcriptionally controls the SKP2-p21/p27 axis (related to figure 1)

A) RT-qPCR in WT RPE1-hTERT cells treated with control or NUCKS1 siRNA. n=4 +/- SEM.
B) Pearson's correlation of *B2M*/*GAPDH* (used as control, housekeeping genes) and *NUCKS1* mRNAs in cancer, using data from Gepia2[68].
C) Pearson's correlation of *NUCKS1* and *SKP2*/*B2M*/*GAPDH* mRNAs in cancer, made using Gepia2[68]. GBM = Glioblastoma, KIRP = Kidney renal papillary cell carcinoma, SKCM = Skin Cutaneous Melanoma, UVM = Uveal melanoma.
D) Western blot validating CRISPR/Cas9-mediated *NUCKS1* deletion in U2OS cells.
E) Western blot in control- or NUCKS1-depleted WT or *TP53*-KO RPE1-hTERT cells.
F) Western blot in control, NUCKS1-, or SKP2-depleted WT and *TP53*-KO RPE1-hTERT cells.
G) Western blot from WT RPE1-hTERT cells treated with control or NUCKS1 siRNA followed by treatment with 10 µg/ml cycloheximide (CHX) for the indicated periods of time.
H) Cell cycle profiles of NBE1-hTERT cells synchronised to G0/G1 by contact inhibition for 72 h followed by control or NUCKS1 depletion for 72 further hours.
I) RT-qPCR of NBE1-hTERT cells treated as in J. N=2 +/- SEM. Ordinary one-way ANOVA with Dunnett's multiple comparisons test.

Supplementary Fig. 2: NUCKS1 levels and chromatin-binding are induced in late G1 to promote *SKP2* expression and G1/S progression (related to figure 2)

A) Western blot in NBE1-hTERT cells synchronised to G0/G1 by contact inhibition for 72 h (t=0) and released to enter the cell cycle by re-plating at low density.
B) PI profiles of NBE1-hTERT cells synchronised to G0/G1 by contact inhibition for 72 h (*t*=0), treated with control or NUCKS1 siRNA for a further 72 h, and released to enter the cell cycle by re-plating at low density.
C) Cell cycle quantification of WT or *NUCKS1*-KO U2OS cells synchronised to G0/G1 by treating with 40 µM lovastatin for 48 h (*t*=0) followed by 4 mM mevalonate to release cells into S phase. N=2 +-/ SEM.

Supplementary Fig. 3: NUCKS1 controls G1/S entry through the SKP2-p21/p27 axis (related to figure 3)

A) Western blot in WT or *NUCKS1*-KO U2OS cells expressing indicated variants of NUCKS1.
B) Western blot validating NUCKS1 depletion and overexpression in HCT116 cells.
C) EdU/PI cell cycle profiles of control, NUCKS1-depleted, and NUCKS1-overexpressing HCT116 cells (left) and corresponding quantifications (right). Ordinary one-way ANOVA with Sidak multiple comparisons test on S phase population. N=3 +/- SEM.
D) Cell cycle quantification of control and NUCKS1-depleted TIG-1 cells. N=3 +/- SEM.
E) Cell cycle quantification of control and NUCKS1-depleted NBE1-hTERT cells. N=4 +/- SEM.
F) Cell cycle quantification of control and NUCKS1-depleted RPE1-hTERT cells. N=6 +/- SEM. For D-F, ordinary one-way ANOVAs with Dunnett multiple comparisons test on the S phase populations were performed.
G) Proliferation assay in control and NUCKS1-depleted TIG-1 cells. One-way ANOVA with Dunnett multiple comparisons test at day 6. N=3 +/- SD.
H) Proliferation assay in control and NUCKS1-depleted NBE1-hTERT cells. One-way ANOVA with Dunnett multiple comparisons test at day 8. N=2 +/- SD.
I) DNA damage measured using the alkaline comet assay after NUCKS1 depletion or 10 m 50 µM H_2O_2 treatment (used as a positive control) in TIG-1 and U2OS cells. Box plots show median values along with 25/75% (box) and 10/90% (whiskers) and outliers. Kruskal-Wallis with Dunn's multiple comparisons test.
J) DNA damage measured using yH2AX or 53BP1 immunofluorescence in control-, NUCKS1-, or SKP2-depleted RPE1-hTERT cells. N=203, 152, 189, and 250 for control, NUCKS1 (sequence 1/ 2) and SKP2 depletions, respectively.

K) Overexpression of SKP2 partially rescues p21/p27 accumulation in NUCKS1-depleted A549 cells, measured by Western blot. Asterisk indicates a non-specific band.
L) Western blot validating p21 depletion by siRNA.
M) Western blot validating p27 depletion by siRNA.
N) Cell cycle quantification of TIG-1 cells treated with control, NUCKS1, p21, or p27 siRNAs, as indicated. N=2 +/- SEM. One-way ANOVA with Sidak multiple comparisons test.
O) Fold proliferation increase at day 4 after siRNA in RPE1-hTERT cells treated with NUCKS1 or SKP2 siRNAs, or combinations. N=2 +/- SD. One-way ANOVA with Tukey multiple comparisons test.

Supplementary Fig. 4: DNA damage inhibits the NUCKS1-SKP2 axis through p53-mediated transcriptional repression (related to figure 5)

A) Western blot in control or 5-FU (10 µM, 48 h)-treated WT RPE1-hTERT cells chased with cycloheximide (10 µg/ml) for the indicated periods of time.
B) Cell cycle profiles of WT and *TP53*-KO RPE1-hTERT cells treated with 10 µM 5-FU, 4 Gy IR, 200 µM H_2O_2, or 100 nM CPT for the indicated periods of time.
C) Western blot validation of *CDKN1A*-KO RPE1-hTERT cells.
D) RT-qPCR in RPE1-hTERT WT and *CDKN1A*-KO cells treated with 10 µM 5-FU for 24 h. N=3 +/- SEM.
E) Data from D: mRNA levels in 5-FU-treated *CDKN1A*-knockout cells were divided by mRNA levels in 5-FU-treated WT cells.
F) RT-qPCR after p53 siRNA in TIG-1 cells. N=2 +/- SEM. Student's t test.
G) RT-qPCR in untreated RPE1-hTERT WT and *TP53*-KO cells. N=18 +/- SEM. Student's t test.

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
NUCKS1	Prof. Anne Østvold[25]	
NUCKS1	ProteinTech	12023-2-AP
SKP2	ThermoFisher Scientific	32-3300
p21	Cell signalling	#2947
p27	Cell signalling	#2552
p53	Santa Cruz	sc-126
p53	Santa Cruz	sc-126 X
PCNA	Santa Cruz	sc-56
MLH1	Abcam	ab92312
Beta-Actin	Abcam	ab6276
Alpha-Tubulin	Sigma-Aldrich	T6199
Alpha-Tubulin	Abcam	Ab4074
Vinculin	Santa Cruz	sc-73614
Histone H3	Abcam	ab201456
Cyclin A2	Abcam	ab181591
IRDye® 800CW Goat anti-Rabbit IgG (H + L)	Li-Cor	925-32211
IRDye® 680RD Goat anti-Mouse IgG (H + L)	Li-Cor	925-68070
IRDye® 680RD Goat anti-Rabbit IgG Secondary Antibody	Li-Cor	925-68071
IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody	Li-Cor	926-32210
Normal rabbit IgG	Santa Cruz	sc-2027
Normal mouse IgG	Santa Cruz	sc-2025
γH2AX	Millipore	05-636
53BP1	Santa Cruz	sc-22760
Alexa Fluor 488, Goat Anti-Rabbit IgG (H + L)	Thermo Fisher Scientific	A-11034
Alexa Fluor 555, Goat Anti-Mouse IgG (H + L)	Thermo Fisher Scientific	A-32727
Experimental Models: Cell Lines		
TIG-1, primary embryonic fibroblasts	Coriell Institute Cell Repository	AG06173
NBE1-hTERT, hTERT-immortalised normal bronchial epithelial cells	Prof. Anderson Ryan[72]	
U2OS, Osteosarcoma	American Type Culture Collection (ATCC)	ATCC® HTB-96™
U2OS *NUCKS1*-KO	This study	

RPE1-hTERT WT, hTERT-immortalised retinal pigment epithelial cells	ATCC	ATCC® CRL-4000™
RPE1-hTERT *TP53*-KO	Dr. Ross Chapman	
RPE1-hTERT *CDKN1A*-KO	Prof. Iain Cheeseman[73]	
RKO, colorectal cancer	ATCC	ATCC® CRL-2577™
HCT116, colorectal cancer	ATCC	ATCC® CCL-247™
HCT116 *TP53*-KO	Prof. Vogelstein[74]	
CACO2, colorectal cancer	ATCC	ATCC® HTB-37™
HT29, colorectal cancer	ATCC	ATCC® HTB-38™
Doxycycline-inducible SKP2 A549, lung adenocarcinoma	Prof. Shyamala Maheswaran[75]	
DLD1, colorectal cancer	ATCC	ATCC® CCL-221™
Sf9 cells	ThermoFisher Scientific	11496015
Recombinant DNA		
SKP2	Dr. Vincenzo D'Angiolella	
p53 WT	Prof. Eric O'Neill	
p53 R175H	Prof. Eric O'Neill	
p53 R248W	Prof. Eric O'Neill	
p53 R273H	Prof. Eric O'Neill	
NUCKS1 CRISPR/Cas9 KO plasmid	SantaCruz	sc-413018
NUCKS1 HDR plasmid	SantaCruz	sc-413018-HDR
NUCKS1 WT	Abgent (Generon)	
NUCKS1 DNA-binding mutant (GRP to AAA)	This study	
pDEST53-NUCKS1	Thermofisher Scientific	12288015

Cell culture

Cell lines were cultured in DMEM (Life Technologies) with 15% (TIG-1, NBE1-hTERT) or 10% (U2OS, RPE1-hTERT, HT29, A549, DLD1, RKO, HCT116, CACO2) FBS, at 37°C in a humidified atmosphere with 5% CO_2. All cells tested negative for mycoplasma. For ionising radiation, treatments were performed using a GSR-D1 137Cs γ-irradiator (RPS Services) at a dose rate of 1.8 Gy/min.

siRNA and plasmid transfections

siRNA transfections were performed using Lipofectamine RNAiMAX, according to the manufacturer's instructions. Cells were transfected with 30 nM siRNA for 3-7 days. siRNA sequences used are as follows:

siCtrl: Eurogentec, SR-CL000-005; siNUCKS1 (1): GAGGCGAUCUGGAAAGAAU; siNUCKS1 (2): GGCAUCUAAAGCAGCUUCU; siNUCKS1 (3' UTR): GCAGGAGGGACUAGAGAAAUU; siSKP2: GCUUCACGUGGGGAUGGGA; sip21: GAUGGAACUUCGACUUUGU; sip27: AAGGUUGCAUACUGAGCCAAG; sip53: AAGACUCCAGUGGUAAUCUAC.

Plasmid transfections were performed using Lipofectamine 3000, according to the manufacturer's instructions. Assays were performed 48 h after plasmid transfection. Plasmids used in the study are listed in the Reagent or Resource table.

CRISPR-Cas9 genome editing

NUCKS1 CRISPR/Cas9 KO plasmid (sc-413018) and *NUCKS1* HDR plasmid (sc-413018-HDR) were co-transfected into early passage U2OS cells. Cells were treated with 5 µg/ml puromycin for 3 days to select successfully-transfected cells, and seeded as single cells. Colonies were expanded and successful clones were confirmed using RT-qPCR and Western blotting.

Western blotting

Whole cell extracts were prepared using a modified version of Tanaka's method, as described previously[76,77]. Nuclear/chromatin fractionations were performed as described previously[78]. Proteins were resolved using SDS-PAGE and transferred onto Immobilon-FL PVDF membranes (Millipore) and blotted using the antibodies indicated

in the Key Resources table. Western blot detection was performed using the Odyssey image analysis system (Li-Cor Biosciences).

RT-qPCR

Total RNA was extracted using the RNeasy kit. Reverse transcription was performed using the SuperScript II Reverse Transcriptase kit. RT-qPCR was performed using Fast SYBR Green Master Mix using the 7500 Fast Real-Time PCR System (Applied Biosystems), using the comparative CT method for quantification. Reference genes used for RT-qPCR were *B2M*/*GAPDH*/*TBP*. Primer sequences are listed in supplementary information.

Protein expression and purification

Production of baculoviral particles, infection of Sf9 cells and expression of recombinant protein was performed as described previously[79]. Mid log phase *Spodoptera frugiperda* (Sf9) cells ($2x10^6$/ml) were transfected with pDEST53-*NUCKS1* bacmid using Cellfectin II transfection reagent in a 6-well plate format, according to the manufacturer's protocol. Following incubation for 5 days at 27 °C, medium containing P0 baculovirus was collected and stored at 4 °C, protected from light. Two sequential rounds of virus amplification were performed to generate higher titer P2 baculovirus stocks. Sf9 cells were infected with P2 virus (120 µg/50 ml Sf9), and incubated at 27 °C for 3 days on an orbital shaker. Cells were harvested by centrifugation (900 g, 20 min, 4 °C), washed with PBS, pelleted again, and then stored at -80 °C. Cell pellets were resuspended in buffer A (50 mM HEPES pH 7.5, 300 mM NaCl, 5% (w/v) glycerol) supplemented with 1 mM TCEP, and 1:500 (v/v) protease inhibitor cocktail (Sigma-Aldrich, P8849) (12 ml buffer A per 100-ml culture cell pellet), and lysed by sonication, followed by incubation with Benzonase (20 U/µl) for 30 min on ice. Cell lysate were clarified by centrifugation, and supernatant passed through a 0.45-µm

syringe filter. Supernatant was then supplemented with 5 mM imidazole prior to loading onto a 1-ml HisTrap column (GE Healthcare) attached to an AKTA system at 1 ml/min. After sample loading, column was washed with buffer A containing 5 mM (10 column volumes (CV)) and 50 mM imidazole (10CV). NUCKS1 was eluted with a linear 50-250 mM imidazole gradient (20CV) and 0.5 ml fractions were collected. His_6-tagged NUCKS1-containing fractions were pooled, and dialysed against storage buffer (50 mM Tris pH 7.5, 150 mM NaCl, 5% (w/v) glycerol, 0.5 mM DTT). To improve purity, His_6-tagged NUCKS1 was further purified by size-exclusion chromatography. 1.5 mg of HisTrap-purified NUCKS1 was diluted to 200 µl in storage buffer and loaded onto a Superdex 200 HR 10/30 column (GE Healthcare, Little Chalfont, UK), and 0.5 ml fractions collected.

Immunofluorescence

Cells seeded on coverslips were subjected to pre-extraction in a buffer containing 10 mM HEPES, 100 mM NaCl, 0.3 M sucrose, 3 mM $MgCl_2$, and 0.1% Triton X-100 for two minutes, washed twice using a buffer containing 10 mM HEPES, 100 mM NaCl, 0.3 M sucrose, and 3 mM $MgCl_2$, and then fixed in 4% formaldehyde for 15 minutes on ice. Cells were blocked overnight in 5% BSA at 4°C, and primary antibodies (indicated in Reagent or Resource table) were diluted in 2.5% BSA and incubations performed for 1 h at RT. Cells were washed in PBS, incubated in secondary antibodies (indicated in Reagent or Resource table) for 1 h at RT, and stained with DAPI. Microscopy was performed using the Nikon NiE.

Site-directed mutagenesis

NUCKS1's GRP DNA-binding motif was mutated to AAA using the Phusion Site-Directed Mutagenesis Kit (Thermofisher), with primers as follows:

gcctttgaagctgtggcggcagccactttccctttgcc/ggcaaagggaaagtggctgccgccacagcttcaaaggc. Mutant NUCKS1 was validated by sequencing.

Comet assays

Alkaline comet assays were performed as described previously[80].

Proliferation assays

50,000 cells (TIG-1) or 100,000 cells (all other cell lines) were seeded at day 0, treated as indicated, and viable cells were counted at indicated days, using Trypan Blue staining (Life Technologies) and the Countess™ Automated Cell Counter (Thermo Fisher Scientific).

EMSAs

For NUCKS1 EMSAs, NUCKS1 was dephosphorylated using lambda phosphatase (100 units/1 µg of recombinant NUCKS1), in the presence of Protein MetalloPhosphatases buffer and $MnCl_2$ (1 mM), for 90 min at 30 °C, followed by addition of phosphatase inhibitor cocktail (50 x, Merck Millipore). Consequently, binding reactions using indicated quantities of intact or dephosphorylated NUCKS1, or WT or *NUCKS1*-KO nuclear extract, were set up in the presence of binding buffer (20 mM Tris-HCl pH 7.5, 100 mM KCl, 0.2% NP-40, 20% glycerol, 2 mM DTT) supplemented with 50 ng or 1 µg salmon sperm DNA (for pure protein and nuclear extract, respectively). 25 nM double-stranded probes were added, and reactions were incubated for 15 min at 37 °C before loading on native 6% PAGE gels at 150 V for 50 min. Gels were imaged using the Odyssey image analysis system (LiCor Biosciences). The double-stranded sequence of the *SKP2* probes used in EMSAs were Gccgaccagtcccgctcccgcgggggttgtgggtatctcgaaggcgggtaaagctgca (WT *SKP2* probe) and GccgaccagtcccgctcccgcgggggttgtgggtatctcgaaAAAAAAtaaagctgca (mutant *SKP2* probe). The WT probe was IRDye-800 fluorescence-labelled. In competition

assays, unlabelled probes were included in binding reactions at 100-times the concentration of labelled probes.

ChIP-qPCR

ChIP was performed as previously described[57], using U2OS cells fixed in 1% formaldehyde for 15 min, ensuring sonication fragments between 100 and 500 bp, and using 5 µg anti-NUCKS1 antibody (ProteinTech 12023-2-AP) or 5 µg normal rabbit IgG (SantaCruz sc2027). Primers used for ChIP-qPCR are listed in supplementary materials.

Flow cytometry

For propidium iodide staining, trypsinised cells were fixed in cold 70% ethanol for 30 min on ice. Cells were then centrifuged at 250 g for 5 min and resuspended in PBS with 0.5 µg/ml RNAseA and 10 µg/ml propidium iodide, before incubation for 15 min at 37 °C.

For EdU/PI staining, the Click-iT™ Plus EdU Alexa Fluor™ 488 Flow Cytometry Assay Kit was used, according to the manufacturer's instructions.

The BD FACSCalibur™ (BD Biosciences) was used for sample acquisition, and FlowJo v10.6.1 and ModFit were used for analysis.

Bioinformatics

Bioinformatics screens for putative transcriptional targets of NUCKS1, as outlined in Fig. 1A, were performed using the SEEK Princeton database[46]. The SEEK database was used to generate lists of the 1000 genes correlating most positively with *NUCKS1* across 15 different cancer types, with three sample types per cancer (cancer tissue, tumour tissue, or cell line). This generated 45 lists of 1,000 genes, which were subsequently, independently, filtered through NUCKS1-interacting promoters in ChIP-

Seq data[16] (Fig. 1A). The GO Biological processes enrichment presented in Fig. 1A was generated using Genecodis3[67]. Fig. 1B and supplementary Fig. 1B and 1C were generated using data from GEPIA2[68]. Fig. 6A and B were generated using data from UCSC XENA, using RSEM norm count values from GTEX/TCGA normal datasets, and TCGA tumour datasets[69]. Fig. 6C, D and G were generated using data from CBioPortal[70,71].

Statistical analyses

Statistical tests, indicated in figure legends, were performed using GraphPad Prism 8.

Supplementary materials

Primers for RT-qPCR
NUCKS1-F; GCCCGAATCTCTTCCATAATC
NUCKS1-R; TTCTCGACTCGGTCCTGTTT
SKP2-F; GGAAGGGAGTCCCATGAAA
SKP2-R; GCTGAAGAGCAAAGGGAGTG
CDKN1A – F; CATGGGTTCTGACGGACATC
CDKN1A – R; TGCCGAAGTCAGTTCCTTGT
CDKN1B – F; TTCATCAAGCAGTGATGTATCTGA
CDKN1B – R; AAGAAGCCTGGCCTCAGAAG
B2M-F; ATGTCTCGCTCCGTGGCCTTA
B2M-R; ATCTTGGGCTGTGACAAAGTC
GAPDH-F; AGCCACATCGCTCAGACAC
GAPDH-R; GCCCAATACGACCAAATCC
TBP-F; CGGTTTGCTGCGGTAATCAT
TBP-R; TTTCTTGCTGCCAGTCTGGAC
CCNB1-F; CGGGAAGTCACTGGAAACAT
CCNB1-R; AAACATGGCAGTGACACCAA
BBC3-F; GTAAGGGCAGGAGTCCCAT
BBC3-R; GACGACCTCAACGCACAGTA
MDM2-F; GCAGTGAATCTACAGGGACGC
MDM2-R; ATCCTGATCCAACCAATCACC
TP53-F; TGTTTCCTGACTCAGAGGGG
TP53-R; GAGCGTGCTTTCCACGAC
Primers for ChIP-qPCR
SKP2 promoter F: -9806 - -9695 TCTGAGATAGGCTGAGAGGAAA
SKP2 promoter R: -9806 - -9695 CTTCAGTAGACGGTAGTGAGAAAC
SKP2 promoter F: -1448 - -1298 CAATCTTCAGGGAAAGGACGTAG
SKP2 promoter R: -1448 - -1298 CTAGGCTAAGCCGTTCATCAAA
SKP2 promoter F: -855 - -673 CAGCAGAGATAGGCTGAAAG
SKP2 promoter R: -855 - -673 GCCTTGACAGGTCTCATAAA
SKP2 promoter F: -417 - -266 GCTACTGTCACCACTCAGCA
SKP2 promoter R: -417 - -266 GATCGGACGGTGAGCCTAAG
SKP2 promoter F: -346 - -197 CTCTCTTTCCGCCTGCTCTC
SKP2 promoter R: -346 - -197 GTGGATCCCAGAACCTGGAC
SKP2 promoter F: -25 - +148 GTATCTCGAAGGCGGGTAAAG
SKP2 promoter R: -25 - +148 TAAGCCTAGCAACGTTCCATC
SKP2 promoter F: +420 - +575 GTGAATGGATGGATGCCTTAG
SKP2 promoter R: +420 - +575 GCAGAAGGCCTCAAGAATTT
SKP2 promoter F: +843 - +1001 TGGGATTCCAGCAAGACTTC
SKP2 promoter R: +843 - +1001 GTCACTCCCTTTGCTCTTCA
SKP2 promoter F: +1455 - +1592 TATCAGTGGACTGCGAAGTGA
SKP2 promoter R: +1455 - +1592 CGGGTTGGGTGATTAAACAGATAG
SKP2 promoter F: +10467 - +10629 CTCTCCCTTGCCCTGTTTAAT
SKP2 promoter R: +10467 - +10629 AGGTGTTGGGAAGGAGTAGTA

Author contributions

G.L.D. and A.J.L. conceived the study and were in charge of overall direction and planning. S.H., C.P.G., P.L. and A.J.L. performed experiments. All authors designed and analysed experiments. V.D. gave critical suggestions and provided reagents. A.J.L., K.R. and G.L.D. supervised the project. S.H., A.J.L., K.R. and G.L.D. wrote the manuscript. All authors read and approved the manuscript.

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Competing interests

The authors declare no competing interests.

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References

1. Neganova, I. & Lako, M. G1 to S phase cell cycle transition in somatic and embryonic stem cells. *J. Anat.* **213**, 30–44 (2008).

2. Khoronenkova, S. V & Dianov, G. L. ATM prevents DSB formation by coordinating SSB repair and cell cycle progression. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 3997–4002 (2015).

3. Macheret, M. & Halazonetis, T. D. Intragenic origins due to short G1 phases underlie oncogene-induced DNA replication stress. *Nature* **555**, 112–116 (2018).

4. Halazonetis, T. D., Gorgoulis, V. G. & Bartek, J. An oncogene-induced DNA damage model for cancer development. *Science* **319**, 1352–1355 (2008).

5. Hills, S. A. & Diffley, J. F. X. DNA replication and oncogene-induced replicative stress. *Curr. Biol.* **24**, R435-44 (2014).

6. Hume, S., Dianov, G. L. & Ramadan, K. A unified model for the G1/S cell cycle transition. *Nucleic Acids Res.* **48**, 12483–12501 (2020).

7. Pennycook, B. R. & Barr, A. R. Restriction point regulation at the crossroads between quiescence and cell proliferation. *FEBS Lett.* (2020). doi:10.1002/1873-3468.13867

8. Rubin, S. M., Sage, J. & Skotheim, J. M. Integrating Old and New Paradigms of G1/S Control. *Mol. Cell* **80**, 183–192 (2020).

9. Bartek, J. & Lukas, J. Pathways governing G1/S transition and their response to DNA damage. *FEBS Lett.* **490**, 117–122 (2001).

10. Spencer, S. L. *et al.* The proliferation-quiescence decision is controlled by a bifurcation in CDK2 activity at mitotic exit. *Cell* **155**, 369–383 (2013).

11. Yang, H. W., Chung, M., Kudo, T. & Meyer, T. Competing memories of mitogen and p53 signalling control cell-cycle entry. *Nature* (2017). doi:10.1038/nature23880

12. Heldt, F. S., Barr, A. R., Cooper, S., Bakal, C. & Novak, B. A comprehensive model for the proliferation-quiescence decision in response to endogenous DNA damage in human cells. *Proc. Natl. Acad. Sci. U. S. A.* **115**, 2532–2537 (2018).

13. Barr, A. R. *et al.* DNA damage during S-phase mediates the proliferation-quiescence decision in the subsequent G1 via p21 expression. *Nat. Commun.* (2017). doi:10.1038/ncomms14728

14. Barr, A. R., Heldt, F. S., Zhang, T., Bakal, C. & Novák, B. A Dynamical Framework for the All-or-None G1/S Transition. *Cell Syst.* **2**, 27–37 (2016).

15. Bianchi, M. E. & Agresti, A. HMG proteins: dynamic players in gene regulation and differentiation. *Curr. Opin. Genet. Dev.* **15**, 496–506 (2005).

16. Qiu, B. *et al.* NUCKS is a positive transcriptional regulator of insulin signaling. *Cell Rep.* **7**, 1876–1886 (2014).

17. Yuan, X. *et al.* NUCKS1 is a novel regulator of milk synthesis in and proliferation of mammary epithelial cells via the mTOR signaling pathway. *J. Cell. Physiol.* (2019). doi:10.1002/jcp.28240

18. Huang, Y.-K. *et al.* NUCKS1 promotes gastric cancer cell aggressiveness by upregulating IGF-1R and subsequently activating the PI3K/Akt/mTOR signaling pathway. *Carcinogenesis* **40**, 370–379 (2019).

19. Parplys, A. C. *et al.* NUCKS1 is a novel RAD51AP1 paralog important for homologous recombination and genome stability. *Nucleic Acids Res.* **43**, 9817–9834 (2015).

20. Yue, Y. *et al.* Nucks1 synergizes with Trp53 to promote radiation lymphomagenesis in mice. *Oncotarget* **7**, 61874–61889 (2016).

21. De Angelis, P. M. *et al.* Nondysplastic Ulcerative Colitis Has High Levels of the Homologous Recombination Repair Protein NUCKS1 and Low Levels of the DNA Damage Marker Gamma-H2AX. *Inflamm. Bowel Dis.* **24**, 593–600 (2018).

22. Maranon, D. G. *et al.* NUCKS1 promotes RAD54 activity in homologous recombination DNA repair. *J. Cell Biol.* **219**, (2020).

23. Wisniewski, J. R. *et al.* Constitutive and dynamic phosphorylation and acetylation sites on NUCKS, a hypermodified nuclear protein, studied by quantitative proteomics. *Proteins* **73**, 710–718 (2008).

24. Malumbres, M. & Barbacid, M. Cell cycle, CDKs and cancer: a changing paradigm. *Nat. Rev. Cancer* **9**, 153–166 (2009).

25. Ostvold, A. C., Holtlund, J. & Laland, S. G. A novel, highly phosphorylated protein, of the high-mobility group type, present in a variety of proliferating and non-proliferating mammalian cells. *Eur. J. Biochem.* **153**, 469–475 (1985).

26. Grundt, K. *et al.* A putative DNA-binding domain in the NUCKS protein. *Arch. Biochem. Biophys.* **407**, 168–175 (2002).

27. Grundt, K., Haga, I. V, Huitfeldt, H. S. & Ostvold, A. C. Identification and characterization of two putative nuclear localization signals (NLS) in the DNA-binding protein NUCKS. *Biochim. Biophys. Acta* **1773**, 1398–1406 (2007).

28. Matsuoka, S. *et al.* ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* **316**, 1160–1166 (2007).

29. Chicas, A. *et al.* Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence. *Cancer Cell* **17**, 376–387 (2010).

30. Nikulenkov, F. *et al.* Insights into p53 transcriptional function via genome-wide chromatin occupancy and gene expression analysis. *Cell Death Differ.* **19**, 1992–2002 (2012).

31. Qiu, B., Han, W. & Tergaonkar, V. NUCKS: a potential biomarker in cancer and metabolic disease. *Clin. Sci. (Lond).* **128**, 715–721 (2015).

32. Shi, C. *et al.* NUCKS nuclear elevated expression indicates progression and prognosis of ovarian cancer. *Tumour Biol.* **39**, 1010428317714631 (2017).

33. Liu, T. *et al.* Increased NUCKS expression is a risk factor for poor prognosis and recurrence in endometrial cancer. *Am. J. Cancer Res.* **5**, 3659–3667 (2015).

34. Drosos, Y. *et al.* NUCKS overexpression in breast cancer. *Cancer Cell Int.* **9**, 19 (2009).

35. Kikuchi, A. *et al.* Identification of NUCKS1 as a colorectal cancer prognostic marker through integrated expression and copy number analysis. *Int. J. cancer* **132**, 2295–2302 (2013).

36. Gu, L. *et al.* NUCKS1 overexpression is a novel biomarker for recurrence-free survival in cervical squamous cell carcinoma. *Tumour Biol.* **35**, 7831–7836 (2014).

37. Cheong, J. Y. *et al.* Identification of NUCKS1 as a putative oncogene and immunodiagnostic marker of hepatocellular carcinoma. *Gene* **584**, 47–53 (2016).

38. Zhao, S. *et al.* NUCKS1 Promotes Proliferation, Invasion and Migration of Non-Small Cell Lung Cancer by Upregulating CDK1 Expression. *Cancer Manag. Res.* **12**, 13311–13323 (2020).

39. Cardozo, T. & Pagano, M. The SCF ubiquitin ligase: insights into a molecular machine. *Nat. Rev. Mol. Cell Biol.* **5**, 739–751 (2004).

40. Moldovan, G.-L., Pfander, B. & Jentsch, S. PCNA, the maestro of the replication fork. *Cell* **129**, 665–679 (2007).

41. Abbas, T. & Dutta, A. p21 in cancer: intricate networks and multiple activities. *Nat. Rev. Cancer* **9**, 400–414 (2009).

42. Chu, I. M., Hengst, L. & Slingerland, J. M. The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. *Nat. Rev. Cancer* **8**, 253–267 (2008).

43. Carrano, A. C., Eytan, E., Hershko, A. & Pagano, M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat. Cell Biol.* **1**, 193–199 (1999).

44. Bornstein, G. *et al.* Role of the SCFSkp2ubiquitin ligase in the degradation of p21Cip1in S phase. *J. Biol. Chem.* (2003). doi:10.1074/jbc.M301774200

45. Frescas, D. & Pagano, M. Deregulated proteolysis by the F-box proteins SKP2 and beta-TrCP: tipping the scales of cancer. *Nat. Rev. Cancer* **8**, 438–449 (2008).

46. Zhu, Q. *et al.* Targeted exploration and analysis of large cross-platform human transcriptomic compendia. *Nat. Methods* **12**, 211–4, 3 p following 214 (2015).

47. Kitagawa, M., Lee, S. H. & McCormick, F. Skp2 suppresses p53-dependent apoptosis by inhibiting p300. *Mol. Cell* **29**, 217–231 (2008).

48. Wu, J. *et al.* Skp2 E3 ligase integrates ATM activation and homologous recombination repair by ubiquitinating NBS1. *Mol. Cell* **46**, 351–361 (2012).

49. Imaki, H. *et al.* Cell cycle-dependent regulation of the Skp2 promoter by GA-binding protein. *Cancer Res.* **63**, 4607–4613 (2003).

50. Yam, C. H., Fung, T. K. & Poon, R. Y. C. Cyclin A in cell cycle control and cancer. *Cell. Mol. Life Sci.* **59**, 1317–1326 (2002).

51. Johnson, C., Gali, V. K., Takahashi, T. S. & Kubota, T. PCNA Retention on DNA into G2/M Phase Causes Genome Instability in Cells Lacking Elg1. *Cell Rep.* **16**, 684–695 (2016).

52. Schroering, A. G., Edelbrock, M. A., Richards, T. J. & Williams, K. J. The cell cycle and DNA mismatch repair. *Exp. Cell Res.* **313**, 292–304 (2007).

53. Meloche, S. & Pouysségur, J. The ERK1/2 mitogen-activated protein kinase pathway as a master regulator of the G1- to S-phase transition. *Oncogene* **26**, 3227–3239 (2007).

54. Vousden, K. H. & Prives, C. Blinded by the Light: The Growing Complexity of p53. *Cell* **137**, 413–431 (2009).

55. Engeland, K. Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. *Cell Death Differ.* **25**, 114–132 (2018).

56. Fischer, M., Quaas, M., Steiner, L. & Engeland, K. The p53-p21-DREAM-CDE/CHR pathway regulates G2/M cell cycle genes. *Nucleic Acids Res.* **44**, 164–174 (2016).

57. Poletto, M., Legrand, A. J., Fletcher, S. C. & Dianov, G. L. p53 coordinates base excision repair to prevent genomic instability. *Nucleic Acids Res.* **44**, 3165–3175 (2016).

58. Yokoi, S. *et al.* Amplification and overexpression of SKP2 are associated with metastasis of non-small-cell lung cancers to lymph nodes. *Am. J. Pathol.* **165**, 175–180 (2004).

59. Zhu, C. Q. *et al.* Skp2 gene copy number aberrations are common in non-small cell lung carcinoma, and its overexpression in tumors with ras mutation is a poor prognostic marker. *Clin. cancer Res. an Off. J. Am. Assoc. Cancer Res.* **10**, 1984–1991 (2004).

60. Gstaiger, M. *et al.* Skp2 is oncogenic and overexpressed in human cancers. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 5043–5048 (2001).

61. Rose, A. E. *et al.* Clinical relevance of SKP2 alterations in metastatic melanoma. *Pigment Cell Melanoma Res.* **24**, 197–206 (2011).

62. Muller, P. A. J. & Vousden, K. H. p53 mutations in cancer. *Nat. Cell Biol.* **15**, 2–8 (2013).

63. Poon, M.-W. *et al.* Inhibition of NUCKS Facilitates Corneal Recovery Following Alkali Burn. *Sci. Rep.* **7**, 41224 (2017).

64. Schneider, G. *et al.* IKKalpha controls p52/RelB at the skp2 gene promoter to regulate G1- to S-phase progression. *EMBO J.* **25**, 3801–3812 (2006).

65. Bensimon, A. *et al.* ATM-dependent and -independent dynamics of the nuclear phosphoproteome after DNA damage. *Sci. Signal.* **3**, rs3 (2010).

66. Ren, B. *et al.* E2F integrates cell cycle progression with DNA repair, replication, and G(2)/M checkpoints. *Genes Dev.* **16**, 245–256 (2002).

67. Tabas-Madrid, D., Nogales-Cadenas, R. & Pascual-Montano, A. GeneCodis3: a non-redundant and modular enrichment analysis tool for functional genomics. *Nucleic Acids Res.* **40**, W478-83 (2012).

68. Tang, Z., Kang, B., Li, C., Chen, T. & Zhang, Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res.* (2019). doi:10.1093/nar/gkz430

69. Goldman, M. *et al.* The UCSC Xena platform for public and private cancer genomics data visualization and interpretation. *bioRxiv* 326470 (2019). doi:10.1101/326470

70. Cerami, E. *et al.* The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2**, 401–404 (2012).

71. Gao, J. *et al.* Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **6**, pl1 (2013).

72. Stead, L. F. *et al.* The transcriptional consequences of somatic amplifications, deletions, and rearrangements in a human lung squamous cell carcinoma. *Neoplasia* **14**, 1075–1086 (2012).

73. McKinley, K. L. & Cheeseman, I. M. Large-Scale Analysis of CRISPR/Cas9 Cell-Cycle Knockouts Reveals the Diversity of p53-Dependent Responses to Cell-Cycle Defects. *Dev. Cell* **40**, 405-420.e2 (2017).

74. Bunz, F. *et al.* Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science (80-.).* (1998). doi:10.1126/science.282.5393.1497

75. Tajima, K. *et al.* SETD1A protects from senescence through regulation of the mitotic gene expression program. *Nat. Commun.* **10**, 2854 (2019).

76. Orlando, G., Khoronenkova, S. V, Dianova, I. I., Parsons, J. L. & Dianov, G. L. ARF induction in response to DNA strand breaks is regulated by PARP1. *Nucleic Acids Res.* **42**, 2320–2329 (2014).

77. Tanaka, M., Lai, J. S. & Herr, W. Promoter-selective activation domains in Oct-1 and Oct-2 direct differential activation of an snRNA and mRNA promoter. *Cell* **68**, 755–767 (1992).

78. Halder, S. *et al.* SPRTN protease and checkpoint kinase 1 cross-activation loop safeguards DNA replication. *Nat. Commun.* **10**, 3142 (2019).

79. Bushell, S. R. *et al.* The structural basis of lipid scrambling and inactivation in the endoplasmic reticulum scramblase TMEM16K. *Nat. Commun.* **10**, 3956 (2019).

80. Burdova, K. *et al.* E2F1 proteolysis via SCF-cyclin F underlies synthetic lethality between cyclin F loss and Chk1 inhibition. *EMBO J.* e101443 (2019). doi:10.15252/embj.2018101443

Figure 1

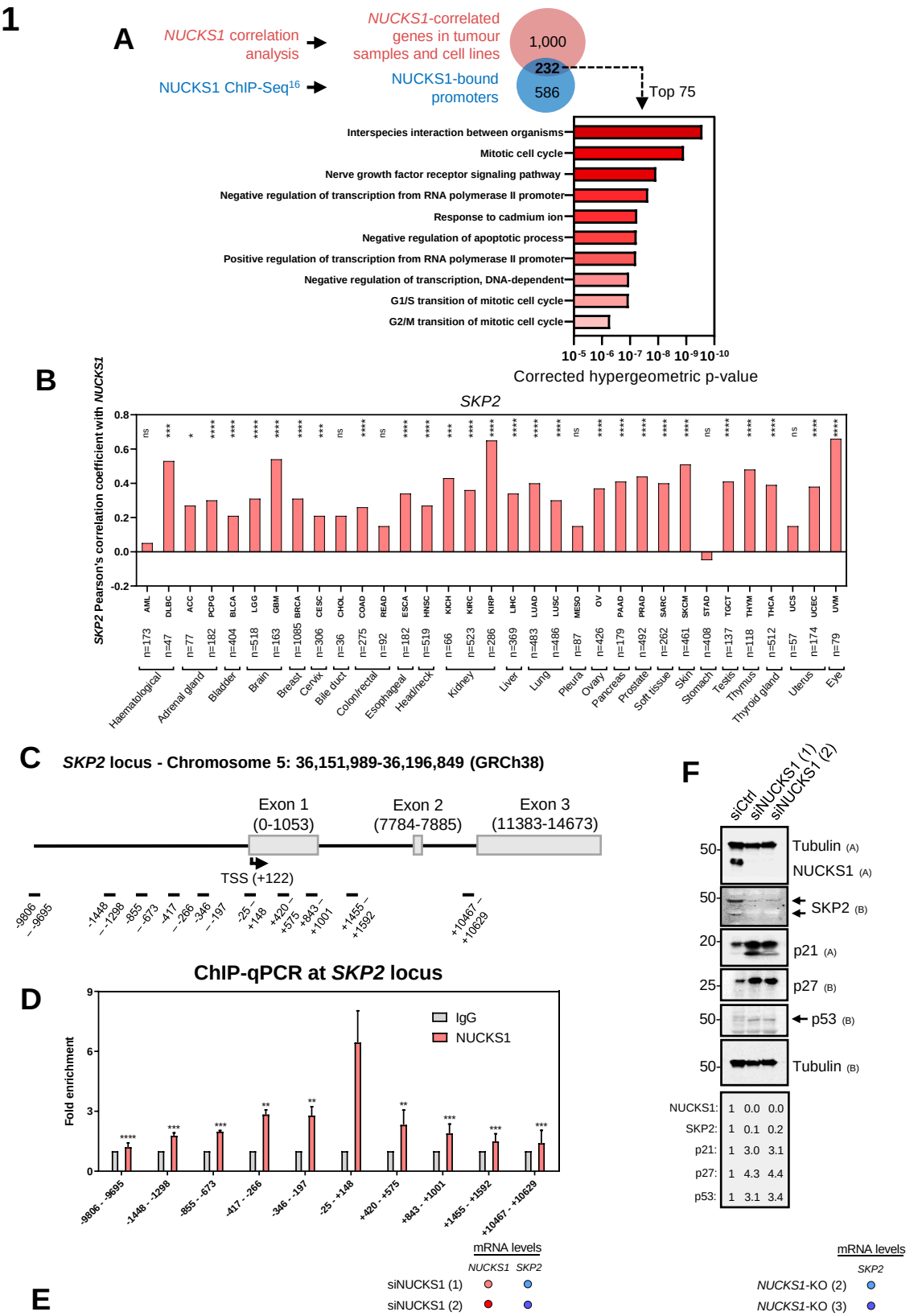

A
NUCKS1 correlation analysis
NUCKS1 ChIP-Seq16
NUCKS1-correlated genes in tumour samples and cell lines
NUCKS1-bound promoters
1,000
232
586
Top 75
Interspecies interaction between organisms
Mitotic cell cycle
Nerve growth factor receptor signaling pathway
Negative regulation of transcription from RNA polymerase II promoter
Response to cadmium ion
Negative regulation of apoptotic process
Positive regulation of transcription from RNA polymerase II promoter
Negative regulation of transcription, DNA-dependent
G1/S transition of mitotic cell cycle
G2/M transition of mitotic cell cycle
Corrected hypergeometric p-value
B
SKP2
SKP2 Pearson's correlation coefficient with NUCKS1
AML DLBC ACC PCPG BLCA LGG GBM BRCA CESC CHOL COAD READ ESCA HNSC KICH KIRC KIRP LIHC LUAD LUSC MESO OV PAAD PRAD SARC SKCM STAD TGCT THYM THCA UCS UCEC UVM
Haematological Adrenal gland Bladder Brain Breast Cervix Bile duct Colon/rectal Esophageal Head/neck Kidney Liver Lung Pleura Ovary Pancreas Prostate Soft tissue Skin Stomach Testis Thymus Thyroid gland Uterus Eye
C
SKP2 locus - Chromosome 5: 36,151,989-36,196,849 (GRCh38)
Exon 1 (0-1053)
Exon 2 (7784-7885)
Exon 3 (11383-14673)
TSS (+122)
D
ChIP-qPCR at SKP2 locus
Fold enrichment
IgG
NUCKS1
-9806 - -9695
-1448 - -1298
-855 - -673
-417 - -266
-346 - -197
-25 - +148
+420 - +575
+843 - +1001
+1455 - +1592
+10467 - +10629
F
siCtrl
siNUCKS1 (1)
siNUCKS1 (2)
Tubulin (A)
NUCKS1 (A)
SKP2 (B)
p21 (A)
p27 (B)
p53 (B)
Tubulin (B)
NUCKS1: 1 0.0 0.0
SKP2: 1 0.1 0.2
p21: 1 3.0 3.1
p27: 1 4.3 4.4
p53: 1 3.1 3.4
mRNA levels
NUCKS1 SKP2
siNUCKS1 (1)
siNUCKS1 (2)
mRNA levels
SKP2
NUCKS1-KO (2)
NUCKS1-KO (3)
E
mRNA levels relative to control
RPE1-hTERT
NBE1-hTERT
TIG-1
WT
TP53-KO
HCT116
RKO
HT29
DLD1
CACO2
U2OS
p53 pathway + + + - + + - - - +
Rb pathway + + + + - - - - - -
MAPK pathway + + + + +++ + + +++ + +
MSI - - - - + + - + - -

Figure 2

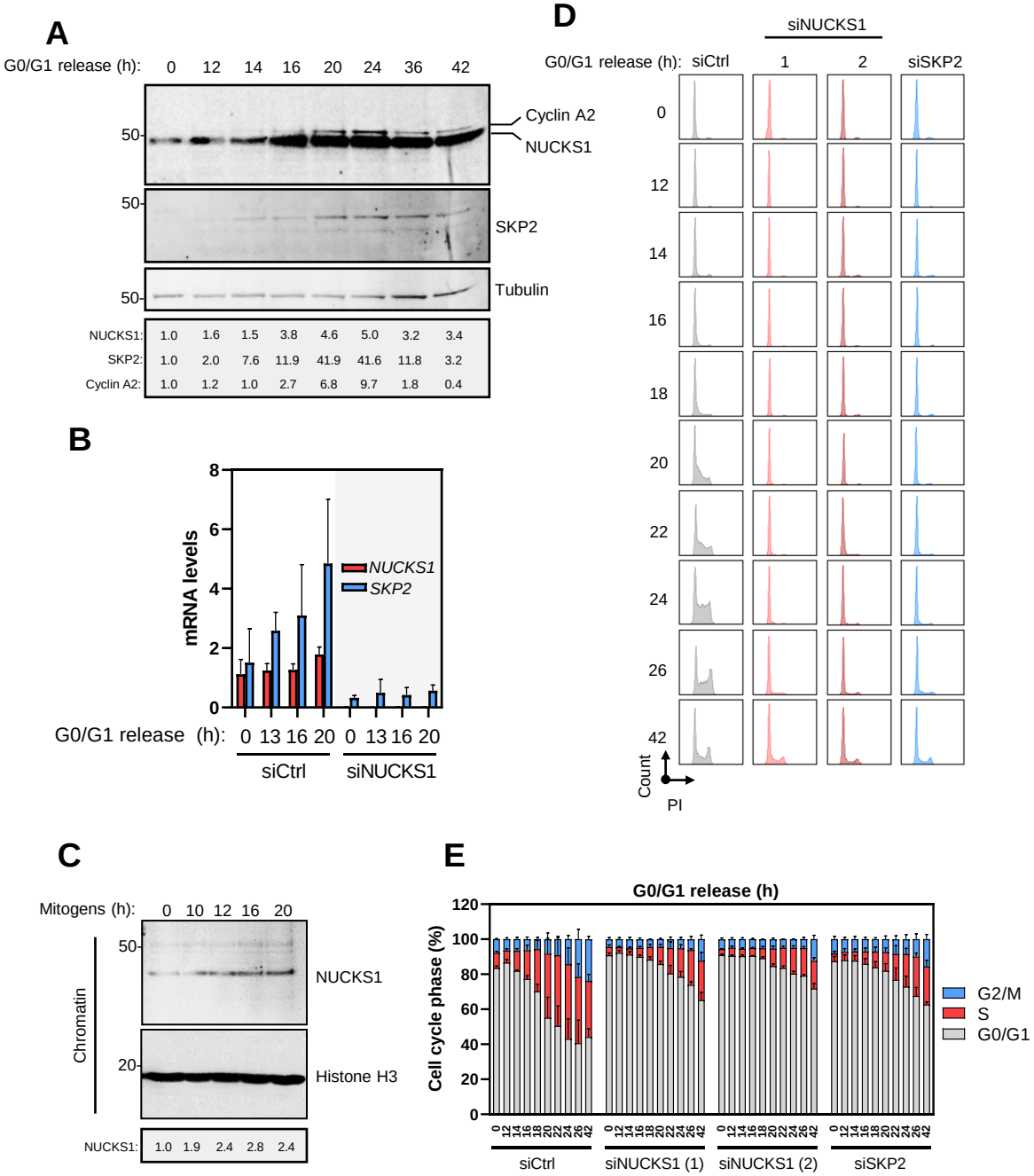

A
G0/G1 release (h): 0 12 14 16 20 24 36 42
50
Cyclin A2
NUCKS1
50
SKP2
50
Tubulin
NUCKS1: 1.0 1.6 1.5 3.8 4.6 5.0 3.2 3.4
SKP2: 1.0 2.0 7.6 11.9 41.9 41.6 11.8 3.2
Cyclin A2: 1.0 1.2 1.0 2.7 6.8 9.7 1.8 0.4
B
mRNA levels
NUCKS1
SKP2
G0/G1 release (h): 0 13 16 20 0 13 16 20
siCtrl
siNUCKS1
C
Mitogens (h): 0 10 12 16 20
Chromatin
50
NUCKS1
20
Histone H3
NUCKS1: 1.0 1.9 2.4 2.8 2.4
D
siNUCKS1
G0/G1 release (h): siCtrl 1 2 siSKP2
0
12
14
16
18
20
22
24
26
42
Count
PI
E
G0/G1 release (h)
Cell cycle phase (%)
G2/M
S
G0/G1
siCtrl
siNUCKS1 (1)
siNUCKS1 (2)
siSKP2

Figure 3

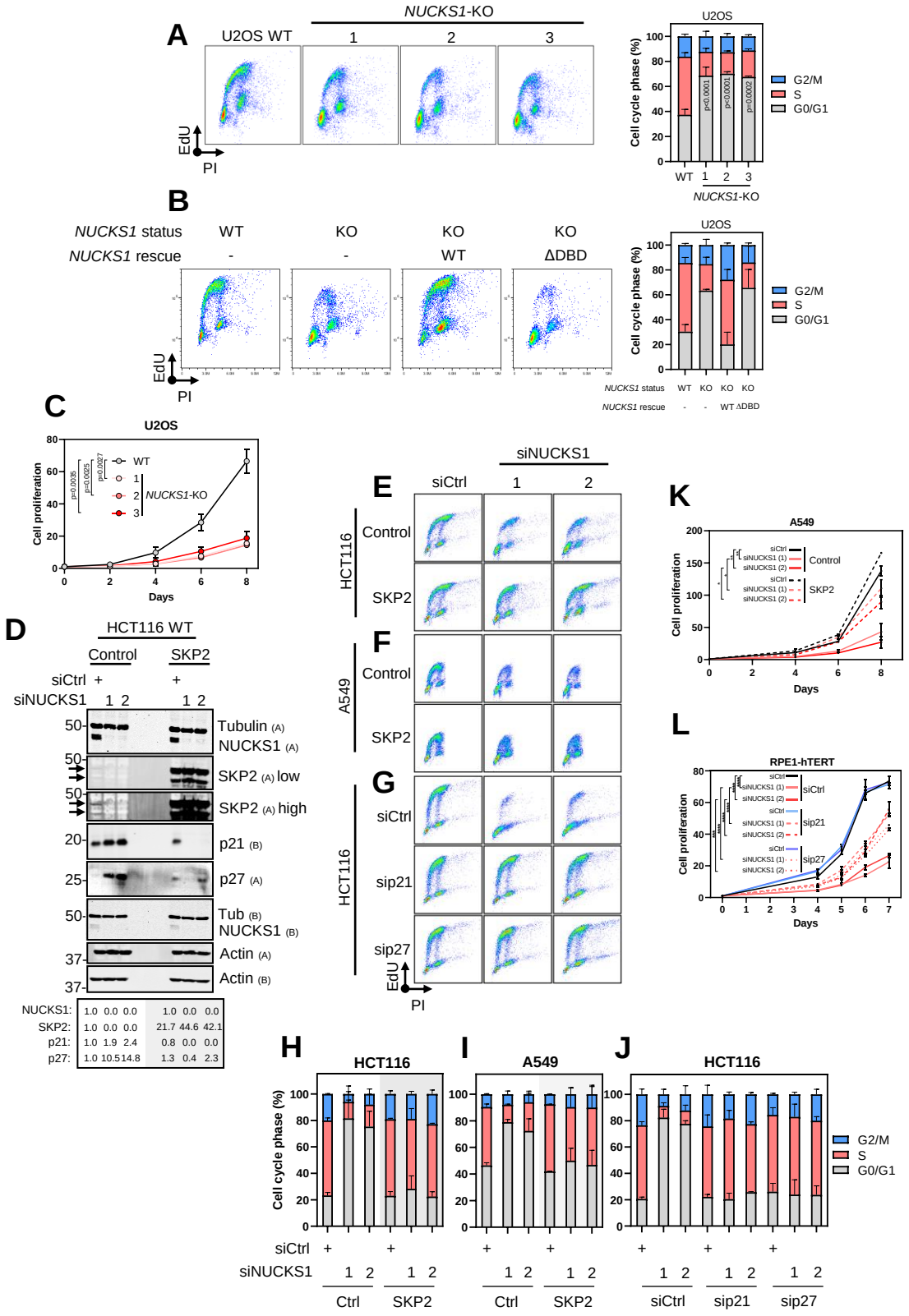

A
U2OS WT
NUCKS1-KO
1
2
3
EdU
PI
U2OS
Cell cycle phase (%)
p=0.0001
p=0.0001
p=0.0002
G2/M
S
G0/G1
WT 1 2 3
NUCKS1-KO
B
NUCKS1 status WT KO KO KO
NUCKS1 rescue - - WT ΔDBD
C
U2OS
Cell proliferation
Days
WT
NUCKS1-KO
D
HCT116 WT
Control
SKP2
siCtrl
siNUCKS1
Tubulin (A)
NUCKS1 (A)
SKP2 (A) low
SKP2 (A) high
p21 (B)
p27 (A)
Tub (B)
NUCKS1 (B)
Actin (A)
Actin (B)
NUCKS1: 1.0 0.0 0.0 1.0 0.0 0.0
SKP2: 1.0 0.0 0.0 21.7 44.6 42.1
p21: 1.0 1.9 2.4 0.8 0.0 0.0
p27: 1.0 10.5 14.8 1.3 0.4 2.3
siNUCKS1
siCtrl
E
HCT116
Control
SKP2
F
A549
G
siCtrl
sip21
sip27
K
A549
L
RPE1-hTERT
H
HCT116
I
A549
J
HCT116
Ctrl
SKP2
siCtrl
sip21
sip27

Figure 4

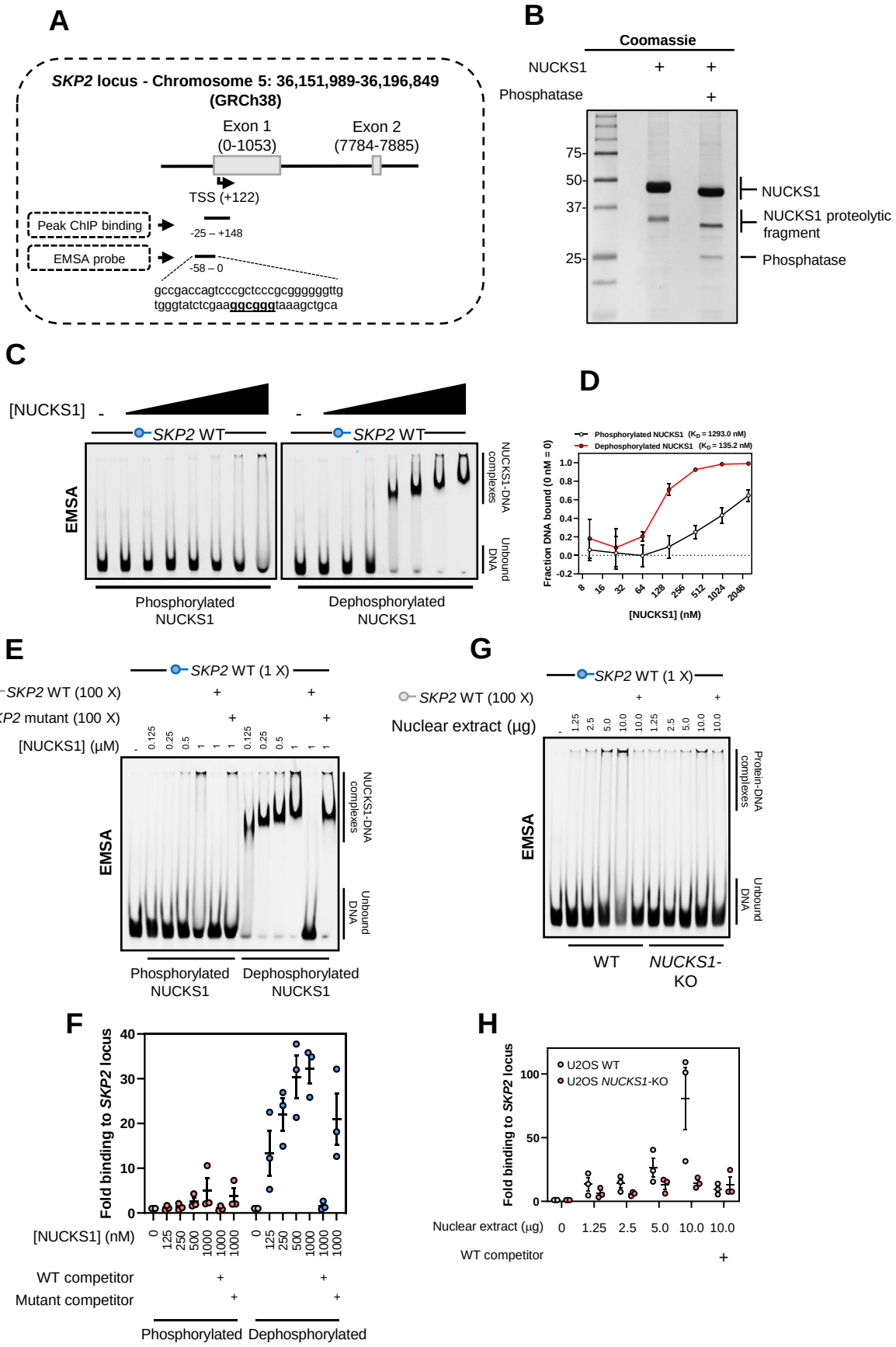

A
SKP2 locus - Chromosome 5: 36,151,989-36,196,849 (GRCh38)
Exon 1 (0-1053)
Exon 2 (7784-7885)
TSS (+122)
Peak ChIP binding
-25 – +148
EMSA probe
-58 – 0
gccgaccagtcccgctcccgcgggggttg
tgggtatctcgaaggcgggtaaagctgca
B
Coomassie
NUCKS1
Phosphatase
75-
50-
37-
25-
NUCKS1
NUCKS1 proteolytic fragment
Phosphatase
C
[NUCKS1]
SKP2 WT
EMSA
NUCKS1-DNA complexes
Unbound DNA
Phosphorylated NUCKS1
Dephosphorylated NUCKS1
D
Phosphorylated NUCKS1 (KD = 1293.0 nM)
Dephosphorylated NUCKS1 (KD = 135.2 nM)
Fraction DNA bound (0 nM = 0)
[NUCKS1] (nM)
E
SKP2 WT (1 X)
SKP2 WT (100 X)
SKP2 mutant (100 X)
[NUCKS1] (µM)
EMSA
NUCKS1-DNA complexes
Unbound DNA
Phosphorylated NUCKS1
Dephosphorylated NUCKS1
G
SKP2 WT (1 X)
SKP2 WT (100 X)
Nuclear extract (µg)
EMSA
Protein-DNA complexes
Unbound DNA
WT
NUCKS1-KO
F
Fold binding to SKP2 locus
[NUCKS1] (nM)
WT competitor
Mutant competitor
Phosphorylated
Dephosphorylated
H
U2OS WT
U2OS NUCKS1-KO
Fold binding to SKP2 locus
Nuclear extract (µg)
WT competitor

Figure 5

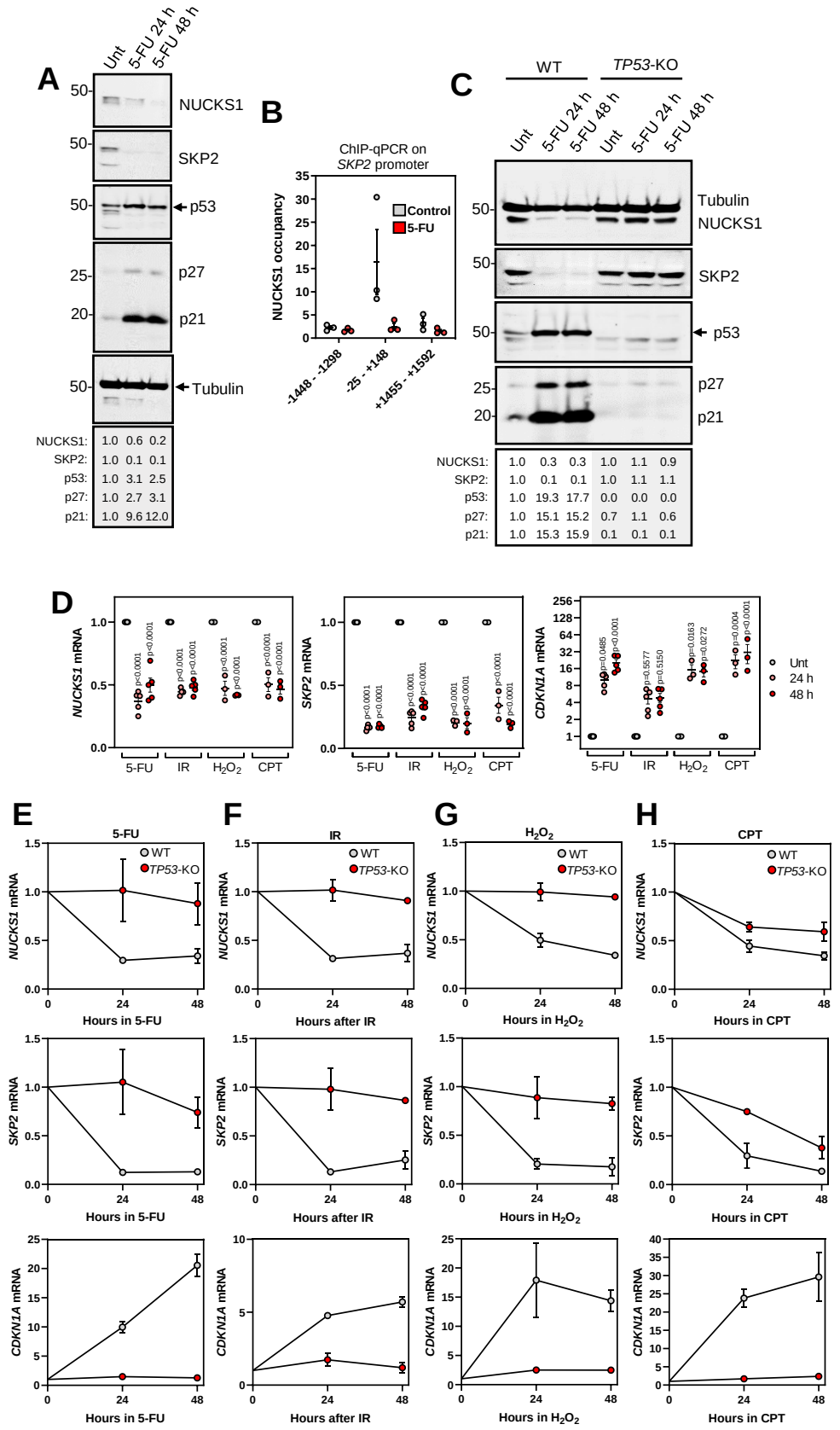
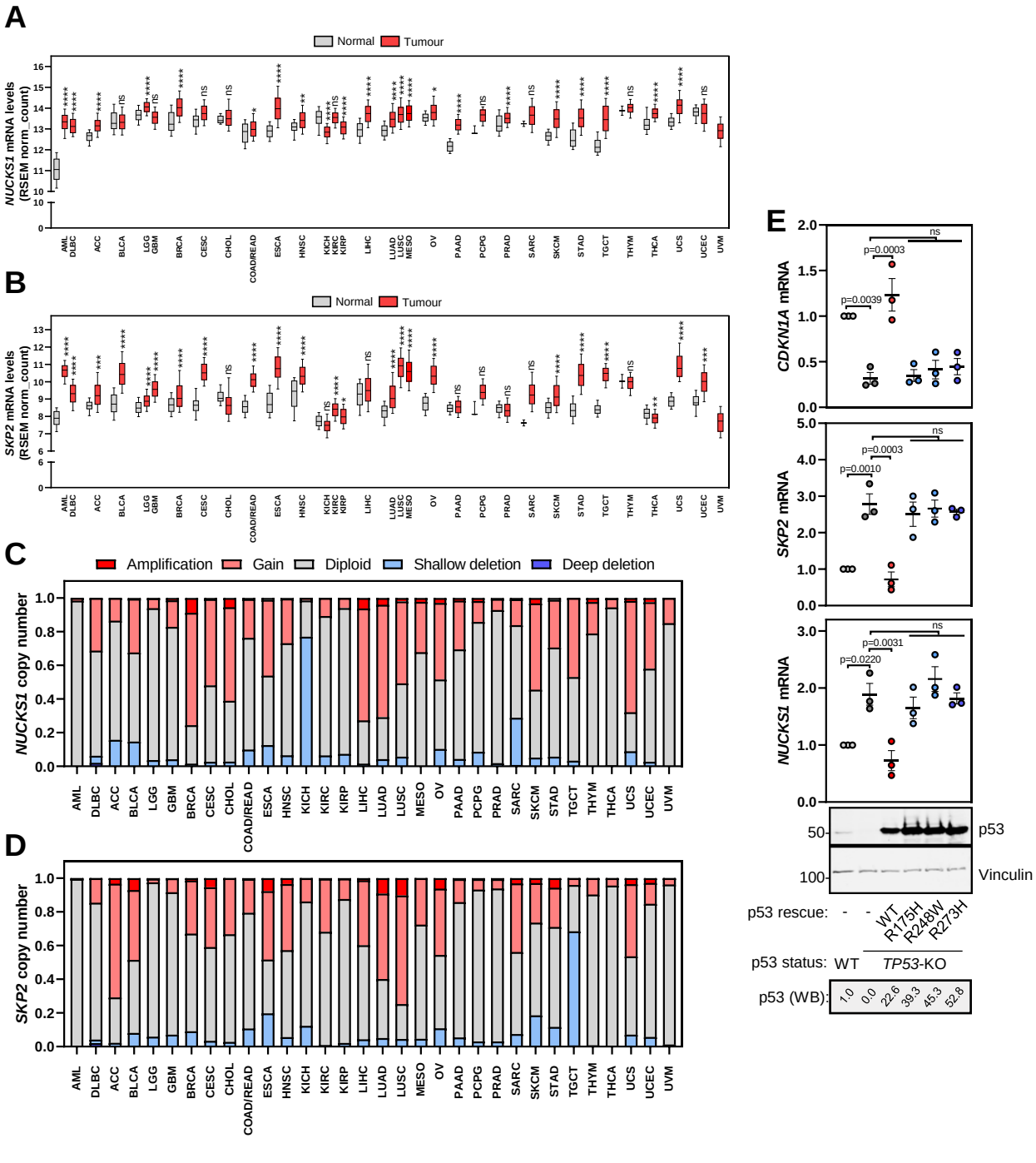

A
Unt
5-FU 24 h
5-FU 48 h
NUCKS1
SKP2
p53
p27
p21
Tubulin
NUCKS1: 1.0 0.6 0.2
SKP2: 1.0 0.1 0.1
p53: 1.0 3.1 2.5
p27: 1.0 2.7 3.1
p21: 1.0 9.6 12.0
B
ChIP-qPCR on SKP2 promoter
NUCKS1 occupancy
Control
5-FU
-1448 - -1298
-25 - +148
+1455 - +1592
C
WT
TP53-KO
Unt
5-FU 24 h
5-FU 48 h
Unt
5-FU 24 h
5-FU 48 h
Tubulin
NUCKS1
SKP2
p53
p27
p21
NUCKS1: 1.0 0.3 0.3 1.0 1.1 0.9
SKP2: 1.0 0.1 0.1 1.0 1.1 1.1
p53: 1.0 19.3 17.7 0.0 0.0 0.0
p27: 1.0 15.1 15.2 0.7 1.1 0.6
p21: 1.0 15.3 15.9 0.1 0.1 0.1
D
NUCKS1 mRNA
SKP2 mRNA
CDKN1A mRNA
5-FU
IR
H2O2
CPT
Unt
24 h
48 h
E
F
G
H
5-FU
IR
H2O2
CPT
WT
TP53-KO
NUCKS1 mRNA
SKP2 mRNA
CDKN1A mRNA
Hours in 5-FU
Hours after IR
Hours in H2O2
Hours in CPT

Figure 6


A
Normal
Tumour
NUCKS1 mRNA levels (RSEM norm. count)
B
SKP2 mRNA levels (RSEM norm. count)
C
Amplification
Gain
Diploid
Shallow deletion
Deep deletion
NUCKS1 copy number
D
SKP2 copy number
AML DLBC ACC BLCA LGG GBM BRCA CESC CHOL COAD/READ ESCA HNSC KICH KIRC KIRP LIHC LUAD LUSC MESO OV PAAD PCPG PRAD SARC SKCM STAD TGCT THYM THCA UCS UCEC UVM
E
CDKN1A mRNA
SKP2 mRNA
NUCKS1 mRNA
p=0.0039
p=0.0003
p=0.0010
p=0.0031
p=0.0220
ns
p53
Vinculin
p53 rescue: - - WT R175H R248W R273H
p53 status: WT TP53-KO
p53 (WB): 1.0 0.0 22.6 39.3 45.3 52.8

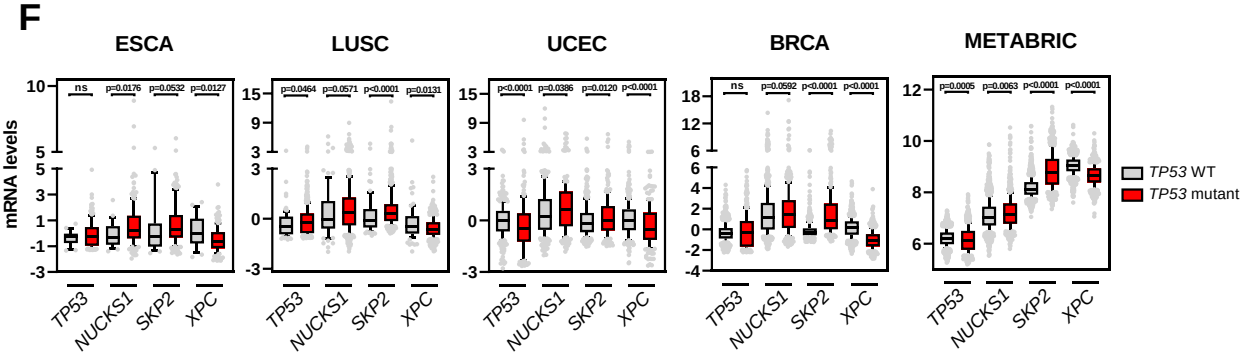
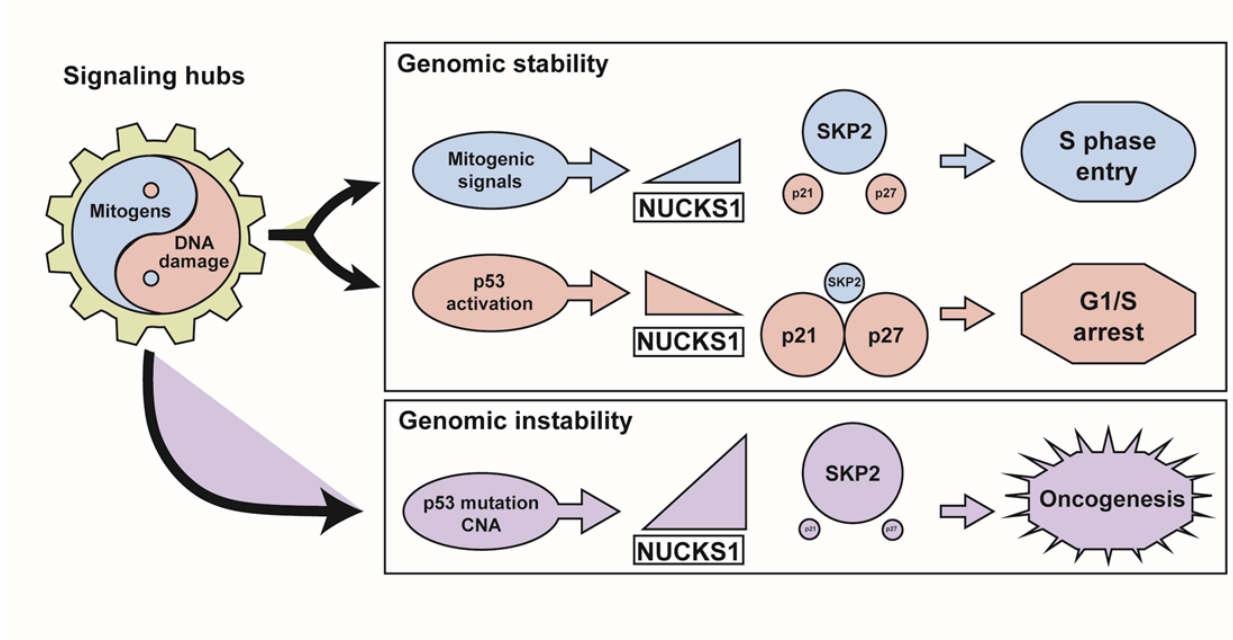
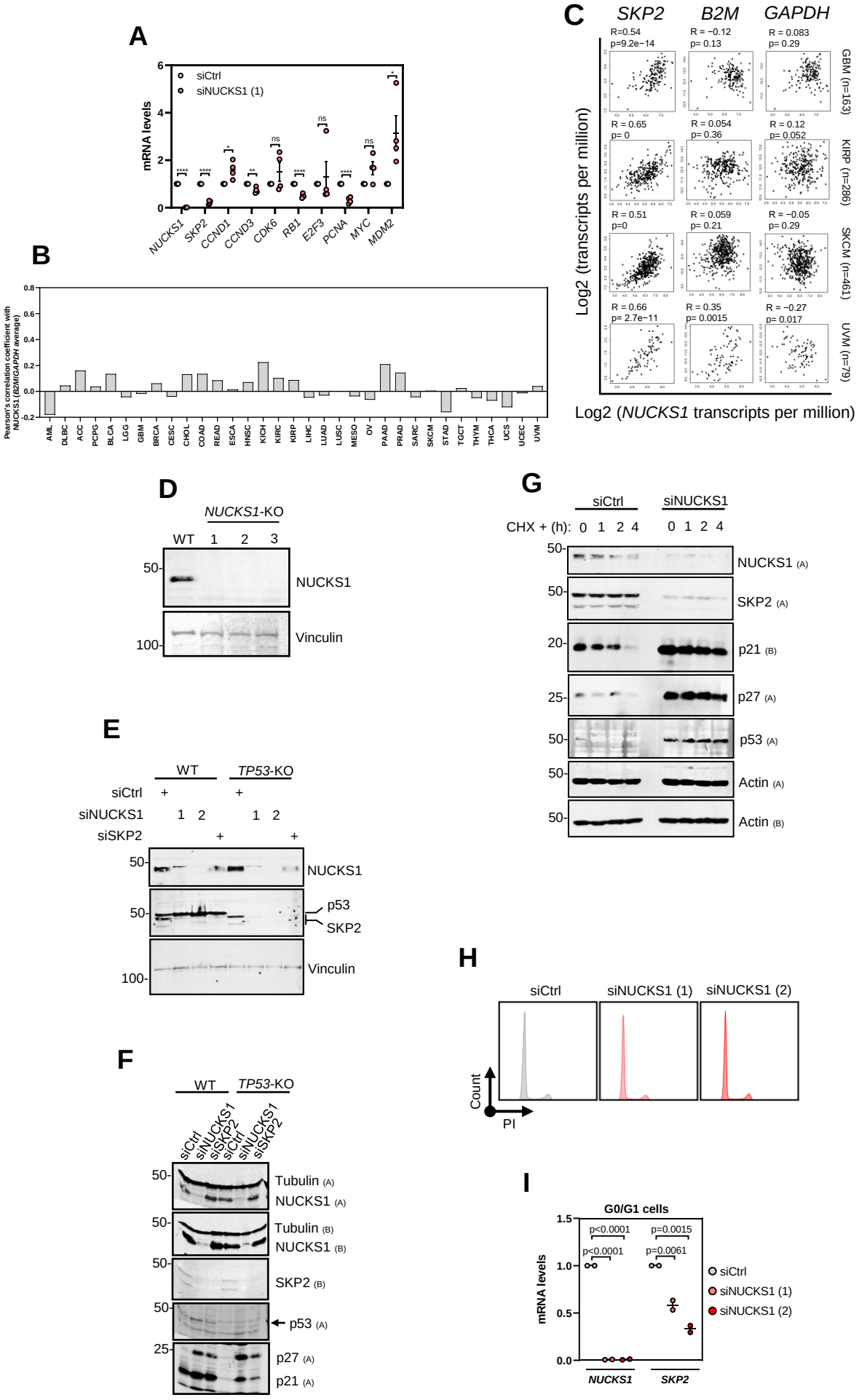

F
ESCA
LUSC
UCEC
BRCA
METABRIC
mRNA levels
TP53
NUCKS1
SKP2
XPC
TP53 WT
TP53 mutant

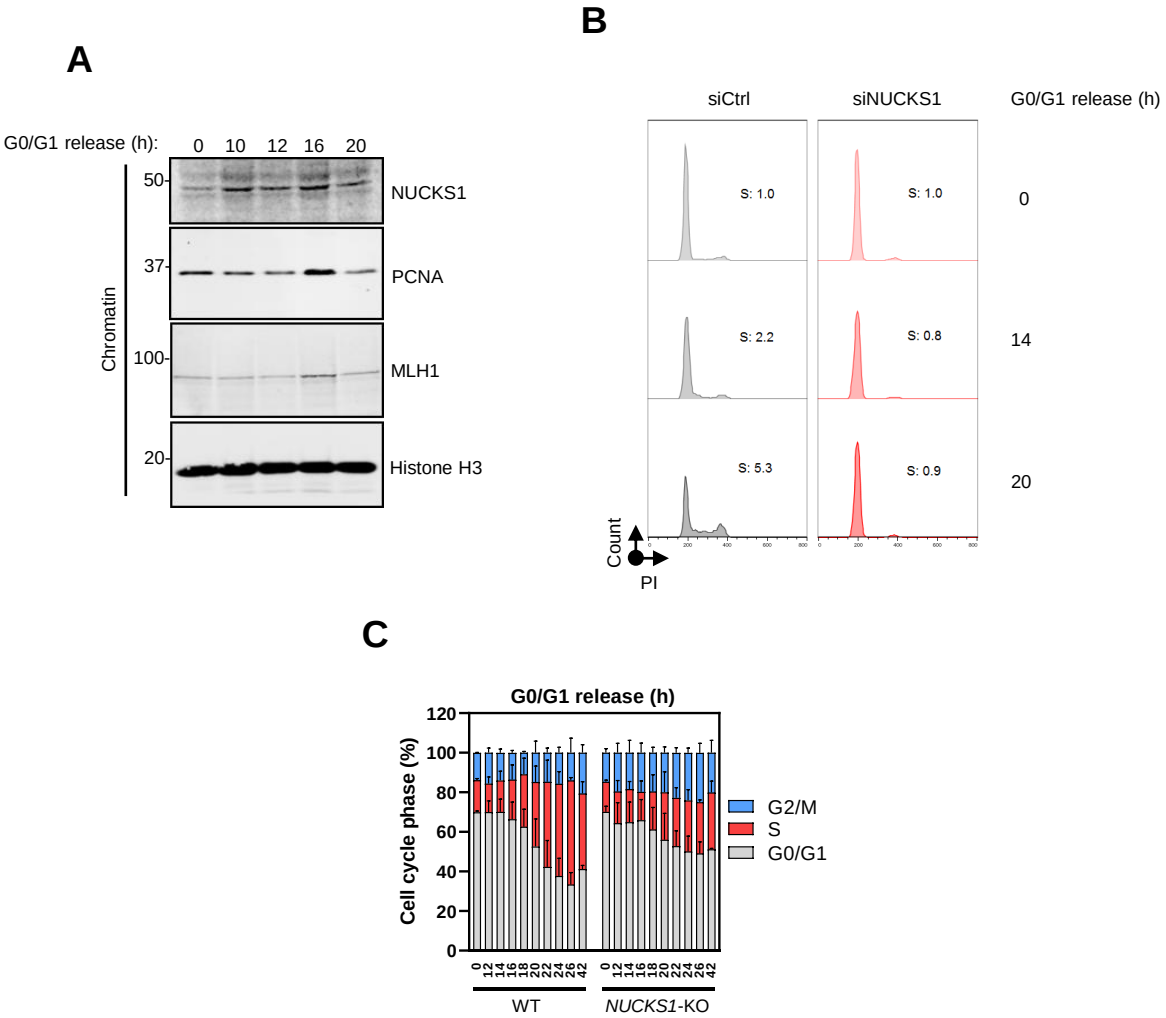
Figure 7


Signaling hubs
Mitogens
DNA damage
Genomic stability
Mitogenic signals
NUCKS1
SKP2
p21
p27
S phase entry
p53 activation
NUCKS1
SKP2
p21
p27
G1/S arrest
Genomic instability
p53 mutation CNA
NUCKS1
SKP2
Oncogenesis

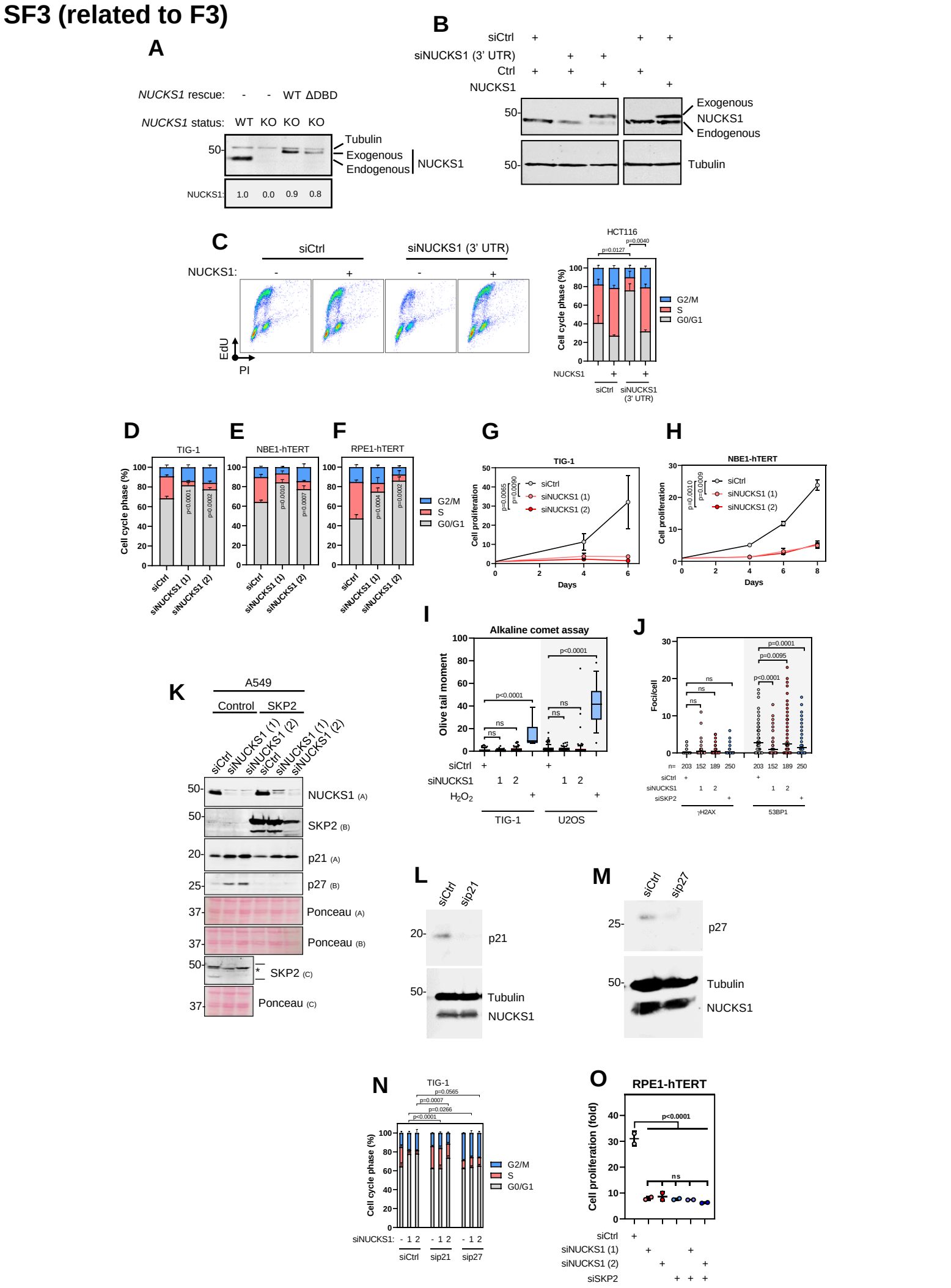
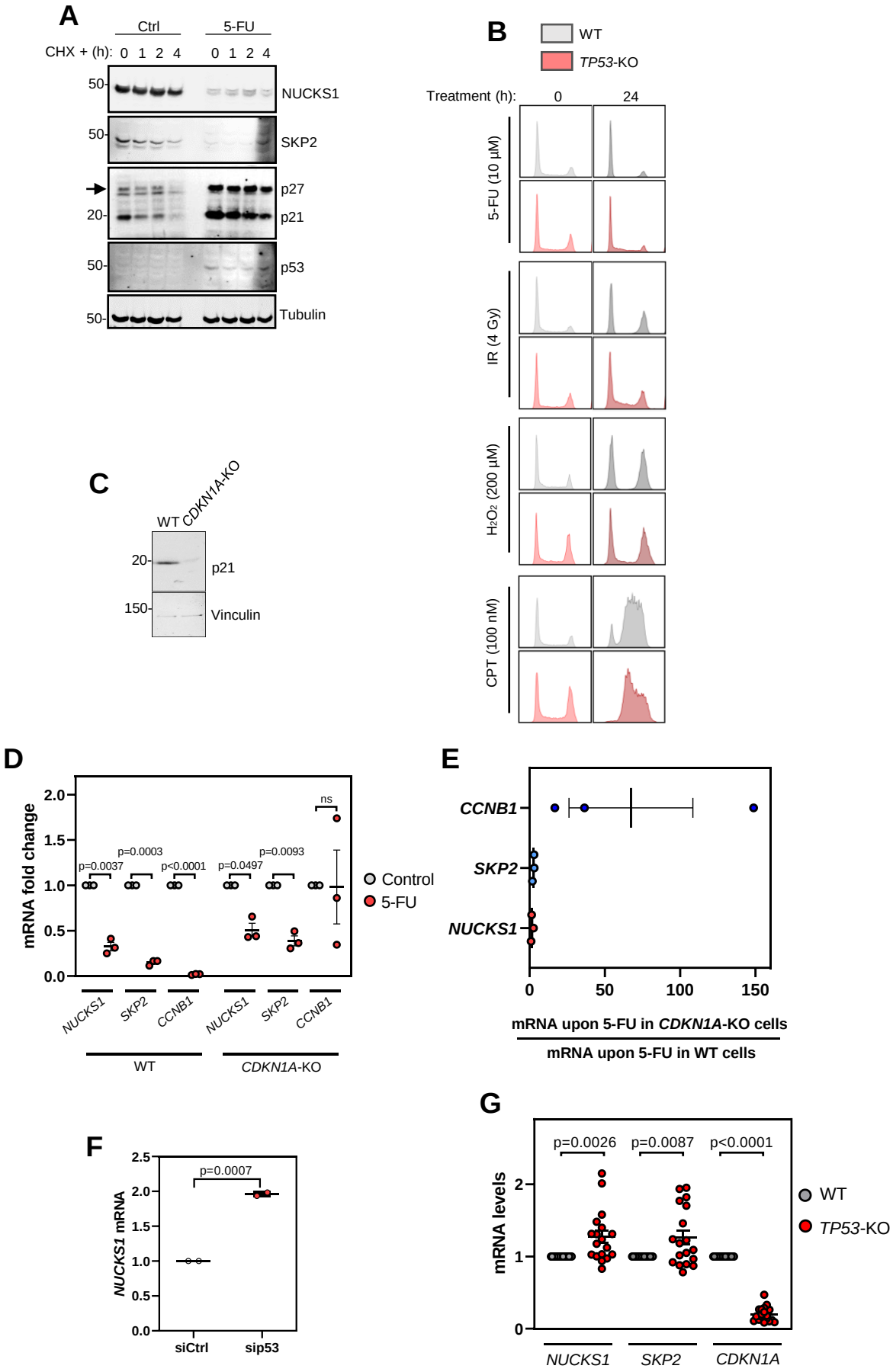
SF1 (related to F1)



SF2 (related to F2)



SF3 (related to F3)



SF4 (related to F5)