

CASE STUDY



Advancing Cancer Research with EditCo's XDel Knockout Cell Pools:

Unveiling SURF2's Role in Nucleolar Stress and Its Potential as a Therapeutic Target



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Overview



Introduction

Simon Lebaron, a researcher at the Molecular, Cellular and Developmental Biology Unit (MCD) within the Centre de Biologie Intégrative (CBI) at the Université de Toulouse in France, is investigating the nucleolar stress response, a critical pathway in cancer biology. This research identified SURF2 as a significant regulator of nucleolar stress and a potential therapeutic target in cancer treatment, offering promising avenues for intervention ([Nature Communications publication](#)).

Background

Unlike normal cellular function, cancer cells have an increase in ribosome synthesis, allowing them to increase cellular proliferation. Many cancer treatments, such as chemotherapies, induce nucleolar stress (NS) that corresponds to ribosome synthesis inhibition, leading to 5S RNP activation outside the ribosome. This resulting free 5S RNP notably promotes the stabilisation of the tumor suppressor, p53. However, despite these treatments, frequently patients continue to show progressive disease, indicating possible resistance mechanisms to NS. Identifying and understanding additional elements responsible for NS resistance, likely associated with free 5S RNP, will elucidate therapy combinations able to effectively treat more aggressive tumor proliferation.

Challenge

One clear interactant of free 5S RNP the team identified was the protein SURF2. Additionally, SURF2 was shown to be overexpressed in most cancers using RNAseq. Furthermore, its knock-down using siRNA technology promotes efficacy of cancer treatment. However, its role in phenotypic traits such as cell migration under nucleolar stress conditions remained unclear. Addressing this gap required the development of robust knockout models and tools to evaluate the impact of both SURF2 deletion and overexpression.

Solution

To address these challenges, the researchers turned to CRISPR technology. [EditCo's XDel Knockout Cells](#) provided complete and reliable knockout models, offering the robust and permanent genetic disruption required for cell migration phenotypic assays, such as wound healing experiments. Simon Lebaron's lab, which had no prior CRISPR expertise, leveraged EditCo's fast, efficient knockout cell lines to accelerate their research. This enabled them to focus on characterizing SURF2's role in nucleolar stress and its implications in cancer biology, skipping the need to spend the time and resources required to optimize and generate the CRISPR cell lines first.



While siRNA strategies initially yielded promising results, we sought a more robust and long-term solution to avoid potential biases caused by transfection-induced stress responses. Lacking the time and expertise to develop a CRISPR strategy within our team, we turned to EditCo for assistance in generating knockout pools for these cell lines.



Introduction



Ribosome synthesis, a key driver of cellular growth and proliferation, is frequently upregulated in cancer, contributing to aggressive tumor phenotypes. The nucleolar stress response plays a central role in cellular homeostasis and tumor suppression through p53 activation, mediated by free 5S ribonucleoprotein (RNP) complexes, with its dysregulation implicated in various cancers. In this study, SURF2 has been identified as a critical factor within the nuclear stress pathway, functioning as an MDM2 antagonist to regulate p53 activation under nucleolar stress conditions. Moreover, SURF2 is overexpressed in most cancers, suggesting its involvement in promoting cancer cell phenotypes.

Simon Lebaron's team at the Centre de Biologie Intégrative (CBI) whose team [focuses on ribosomes in normal and pathological conditions](#), aimed to elucidate the role of SURF2 in cancer biology. His group was particularly interested in its impact on phenotypic traits such as cell migration. Initial siRNA-based knockdown studies suggested SURF2's regulatory role in nucleolar stress. However, the absence of permanent and robust genetic disruption methods hindered deeper characterization. To overcome this limitation, the team utilized EditCo's XDel Knockout Cell Pools, employing CRISPR-mediated SURF2 knockouts in two different cell lines (U2OS and HepG2). XDel Knockout Cell Pools provided constitutive and, in this instance, 100% efficient SURF2 knockouts, accelerating their research by enabling comprehensive functional characterization of SURF2 without the requirements of creating the knockout cell lines themselves.

Background



SURF2 siRNA Gene Knockdown and Validation

Researchers began by employing siRNA to generate SURF2 knockdowns and assess its initial impact on nucleolar stress sensitivity. This approach provided preliminary data confirming SURF2's significance in the nucleolar stress pathway.

SURF2 CRISPR Knockout Cell Lines

To achieve permanent, reproducible and robust genetic disruption necessary for the phenotypic characterization of SURF2, CRISPR technology was employed using EditCo's XDel Knockout Cell Pools. The cell pools guarantee over > 80% knockout efficiency and assay-ready models in less than three weeks. For the cell lines provided to the MCD team, the cells had 100% knockout efficiency, which they maintained over time ensuring robust validation of SURF2's role.

Functional characterization: Phenotypic Assays

Wound healing assays were performed to evaluate the impact of SURF2 deletion and overexpression on cancer cell migration. The wound healing assay involved creating a scratch in a monolayer of U2OS cells and monitoring cell migration into the wound area over time, with and without nucleolar stress induced by Actinomycin D.

U2OS cells were used for both knockout and SURF2-Flag overexpression studies.

Migration assays were quantified using imaging software, comparing control and SURF2-modified cells. The results were statistically analyzed to confirm significance.

Within a remarkably short time, we received pools with 100% homozygous deletions. After verifying the results ourselves, we were impressed by the efficiency and quality, even isolating clones to further refine our analyses.

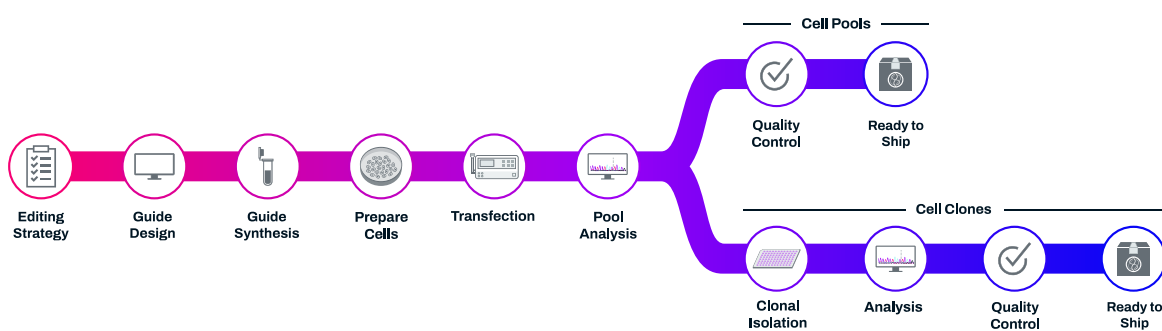


Figure 1. EditCo's Automated Immortalized Cell Line Editing Process.

Results



Validation of SURF2 CRISPR Knockout

The generation of SURF2 knockout U2OS cells was successfully achieved using EditCo's XDel CRISPR technology, confirming that SURF2 is non-essential for cellular viability, consistent with DepMap data. This robust model provided an ideal platform for detailed phenotypic analysis.

Table 1. Knockout (KO) Score assessed by EditCo's ICE CRISPR Analysis Tool.

Cell line	U2OS	HepG2
Gene	SURF2	SURF2
Knockout (KO) Score	100	99



Figure 2. SURF2 depletion in U2OS and HepG2 cell lines. Western blot analyses for SURF2 in U2OS and HepG2 cell lines relative to the negative controls.

As researchers studying SURF2's role in cancer resistance to chemotherapy, we investigated its depletion in U2OS and HepG2 cell lines. While siRNA yielded initial insights, we sought a more robust approach and partnered with Editco to generate CRISPR knockout pools. They provided 100% homozygous deletions quickly, allowing us to validate results, isolate clones, and assess SURF2 knockout effects. This work led to [our publication in Nature Communications](#) and continues to drive our research. Editco's expertise and efficiency were instrumental, and we look forward to future collaborations.

Results, Cont.



SURF2 and Cell Migration Characterization

Knockout Results: Wound healing assays revealed that SURF2-deficient cells exhibited a significant reduction in migratory capacity, underscoring its role in promoting cell movement and oncogenic potential (Fig. 3a & 3b).

Overexpression Results: Conversely, cells overexpressing SURF2 showed enhanced migratory capacity, particularly under nucleolar stress conditions induced by Actinomycin D. These results reinforce SURF2's function in modulating cancer cell behavior and its interplay with stress pathways (Fig. 3c & 3d).

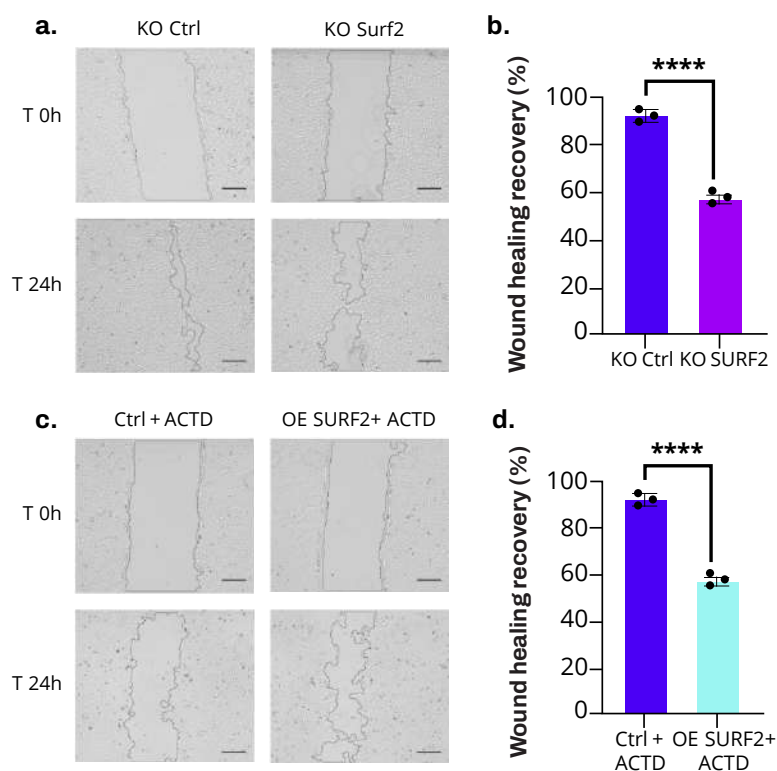


Figure 3. SURF2 expression levels affect U2OS phenotypic traits. Analysis of U2OS KO SURF2 or U2OS overexpressing SURF2-Flag cell migration by wound healing assay. **a., c.** Images were taken with Cell Imaging EVOS (Gx40) at 0 h and 24 h ($n = 3$) (scale bar = 0.1 mm). **b., d.** Quantification of wound healing by measuring the percentage of persistent scar area after 24 h from three independent biological replicates ($n = 3$). Use of an unpaired two-tailed t test for statistical tests. Data are presented as mean values $\pm$ SD, and significant differences are indicated by stars as follows p -value $< 0.05^*$; 0.01^{**} 0.001^{***} and 0.0001^{****}) or with the precise p -value on the graph (p). Source data are provided as a Source Data file.

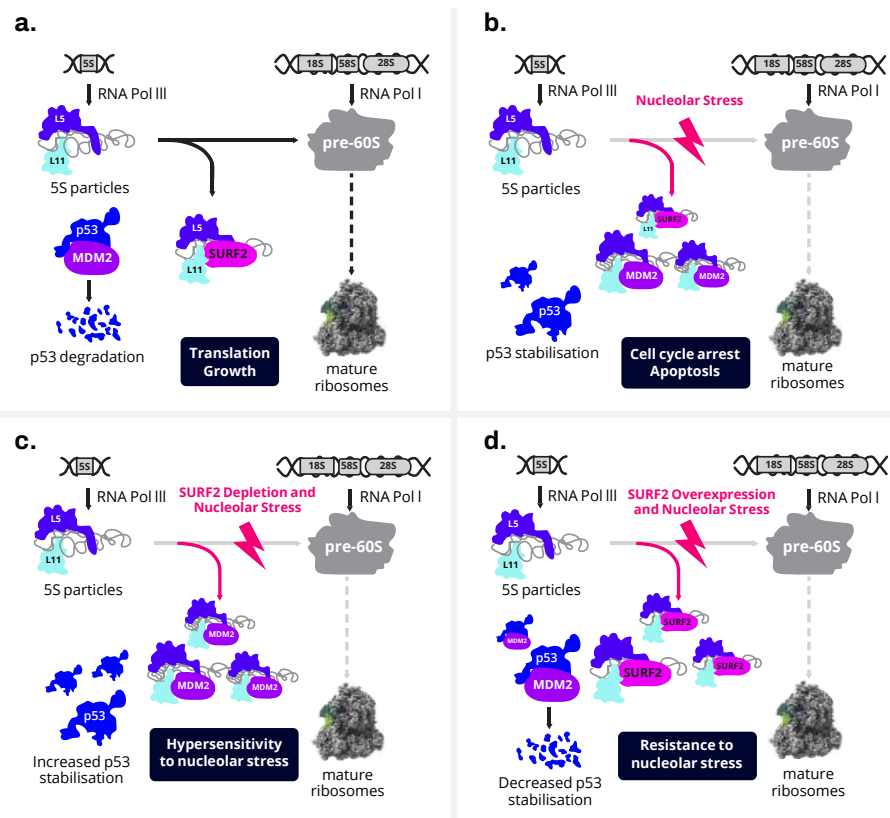
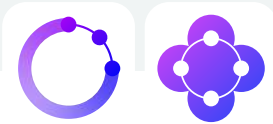


Figure 4. Model of SURF2 function in free 5S RNP regulation. Schematic representation of free 5S regulation by SURF2 in different conditions.

a. In normal cells, both 5S and 47S rDNAs are transcribed by RNA polymerase III and I, respectively. Ribosome synthesis is producing pre-60S ribosomes and 5S particles, constituted by the association of RPL5 and RPL11 to 5S rRNA, are incorporated into these large pre-ribosomes. The remaining overproduced free 5S are bound by SURF2 to avoid MDM2-Free 5S interaction, which would induce p53 stabilization and cell cycle arrest. At the same time, p53 is constantly ubiquitinated by MDM2 to promote its degradation by the proteasome. **b.** After nucleolar stress (drug-induced or caused by genetic mutations/ribosomopathies), ribosome synthesis is impaired and a larger amount of free 5S particles accumulate in the nucleoplasm. The extra free 5S particles can then be recognized by MDM2, which can no longer ubiquitinate p53, thereby stabilizing p53 and promoting cell cycle arrest. **c.** In cells lacking SURF2, nucleolar stress still impairs ribosome synthesis, but this time, even more free 5S RNPs are able to bind to MDM2, inducing stronger stabilization and activation of p53, followed by more cell cycle arrest. **d.** In contrast, in cells overexpressing SURF2, nucleolar stress promotes free 5S RNP accumulation in the nucleoplasm, all of which are recognized by SURF2, which competes with MDM2 for binding. As a result, MDM2 is free and ubiquitinates p53, conferring to these cells a capacity to resist nucleolar stress.

Conclusion



Implications for Cancer Research

SURF2 plays a critical role in regulating nucleolar stress by buffering free 5S ribonucleoprotein (RNP) particles and modulating their interaction with MDM2 and hence the stabilisation of p53. Its depletion enhances sensitivity to nucleolar stress, promoting stronger p53 activation, cell cycle arrest, and cell death, particularly under chemotherapeutic treatments. These findings position SURF2 as a promising therapeutic target to improve cancer treatment efficacy in wild-type TP53 cancers.

Moreover, SURF2 overexpression in cancers such as adrenocortical carcinoma highlights its potential as a prognostic marker and therapeutic target. Targeting SURF2 could overcome resistance to stress-inducing therapies, offering a new avenue for cancer treatment.

In summary

Dr. Simon Lebaron's group highlights the critical role of SURF2 in regulating nucleolar stress and cancer cell migration, positioning it as a potential therapeutic target in cancer treatment. By using [EditCo's XDel Knockout Cell Pools](#), the research team efficiently created robust knockout models, revealing that SURF2 depletion reduces cell migration while overexpression enhances it.

Given that CRISPR gene editing can be challenging and requires significant time and resources to create and validate knockout cell lines, especially in labs without prior CRISPR experience, using EditCo's XDel Knockout Cell Pools to further confirm their siRNA depletion findings allowed a rapid and convenient avenue to get to their phenotypic assays immediately. The confirmation of 100% editing efficiency of the SURF2 knockout cell lines allowed Dr. Lebaron's team to avoid both the time and labor associated with CRISPR editing, but also avoided the additional weeks required to isolate clonal cells since the cell pools provided sufficient confidence in the reliability and repeatability of their assay results.

Overall, identifying new strategies for cancer therapies, especially in instances where current therapeutic options have not been sufficient to block unchecked cell proliferation characteristic to particularly aggressive cancer variants, will continue to be vital in the fight toward greater cancer survival rates. The ability for teams like Simon Lebaron's group at the MCD to rapidly test new therapeutic options with the ease and confidence exemplified in this study, will be increasingly important as we move forward in the fight against cancer.