An arrayed CRISPR screen identifies RNA-based regulators of the Alternative Lengthening of Telomeres pathway

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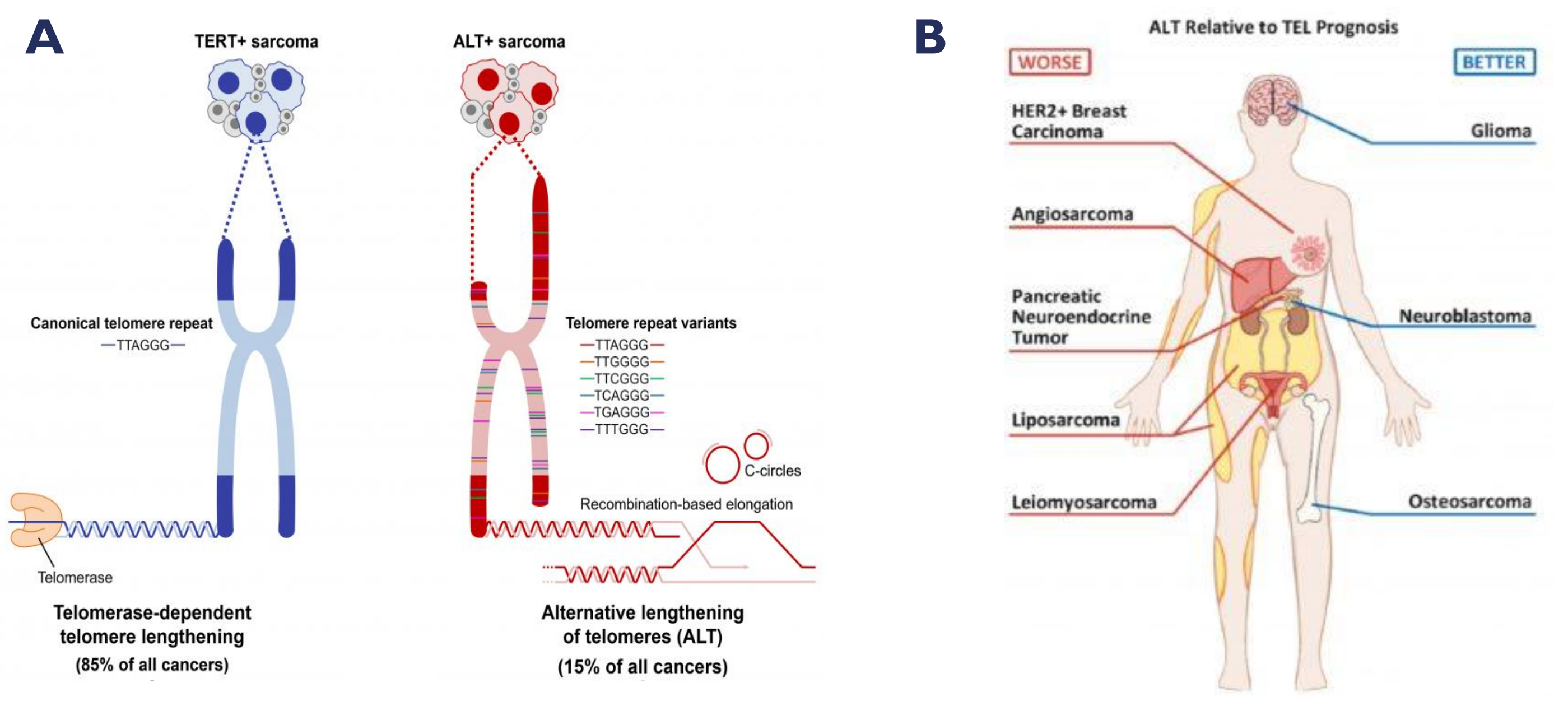
Introduction

Alternative lengthening of telomeres (ALT) is a telomere maintenance mechanism used by 5-10% of cancer cells to achieve replicative immortality and is absent in normal cells. Unlike telomerase (TEL) positive cancers, ALT tumours rely on chronic telomeric replication stress to drive telomere extension. Thus, factors regulating this process serve as promising targets for the development of specific therapeutic inhibitors for oncology. Despite this, no targeted therapies currently exist for ALT cancers. Through collaboration with Nikon Bioimaging lab, we performed a high throughput arrayed CRISPR screen with an imaging output to identifying RNA interacting factors with potential as targets in ALT cancers.



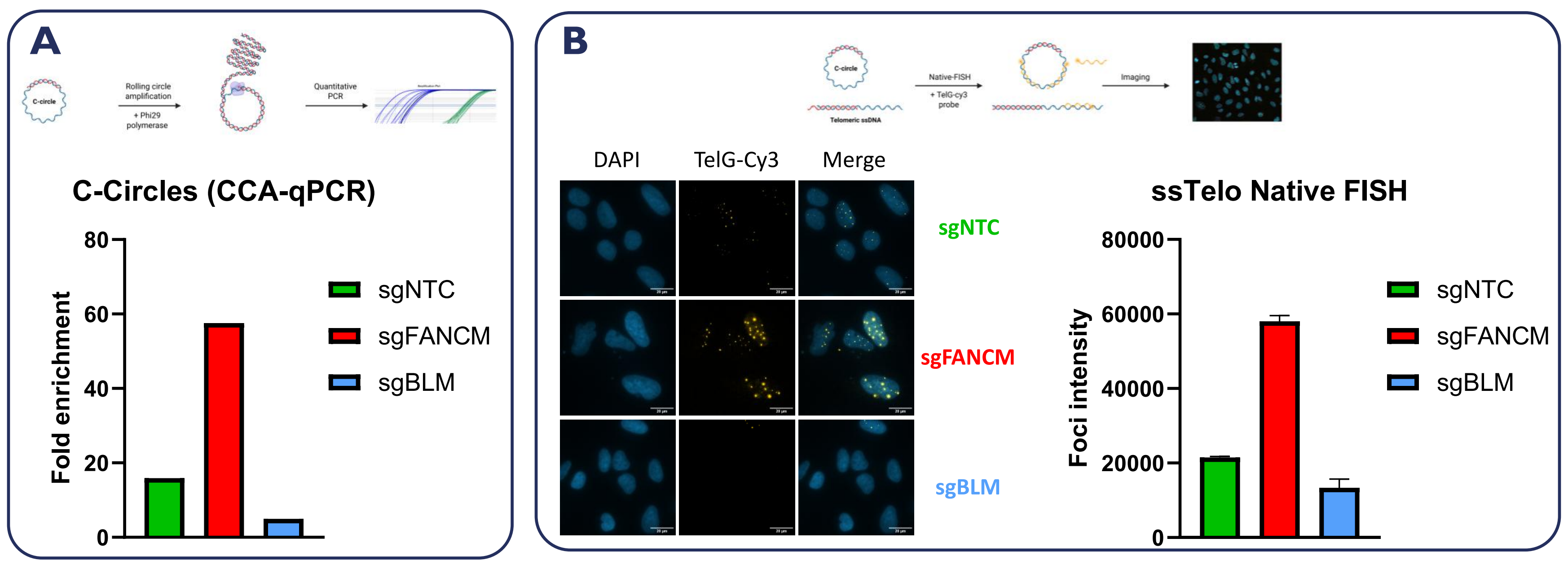
Nucleic acids research, Frank et al 2012

MDPI cancers, Mackenzie Jr et al 2021

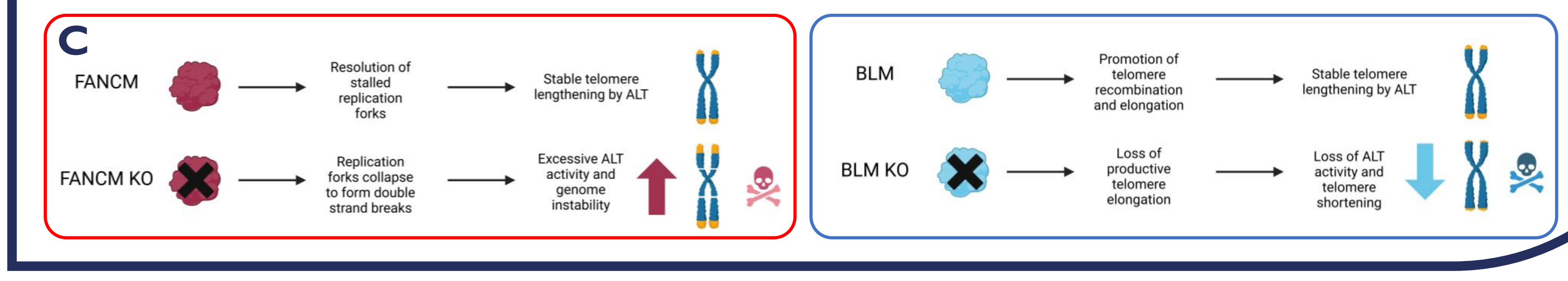
A. Tumours use two predominant mechanisms to maintain telomere length, ALT and TEL.
B. ALT is associated with poor prognosis in a range of different cancer types.

In-house assay development to measure ALT activity

In order to discover and validate therapeutic targets for ALT cancers, we adapted and implemented two assays from published literature, with the ability to detect unique ALT molecular hallmarks; CCA-qPCR for C-circles and ssTelo native FISH for single-stranded telomeric DNA. By performing CRISPR knockout (KO) of literature established control genes, we validate these assays by observing the expected increase (FANCM) or decrease (BLM) in ALT hallmarks associated with loss of either gene.

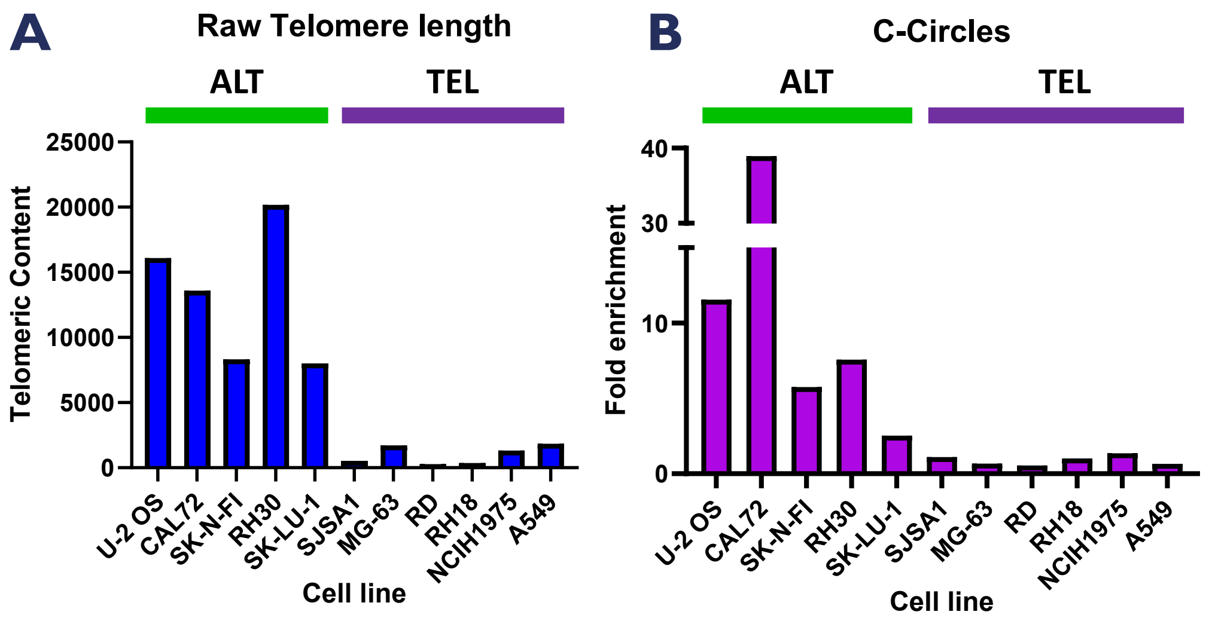


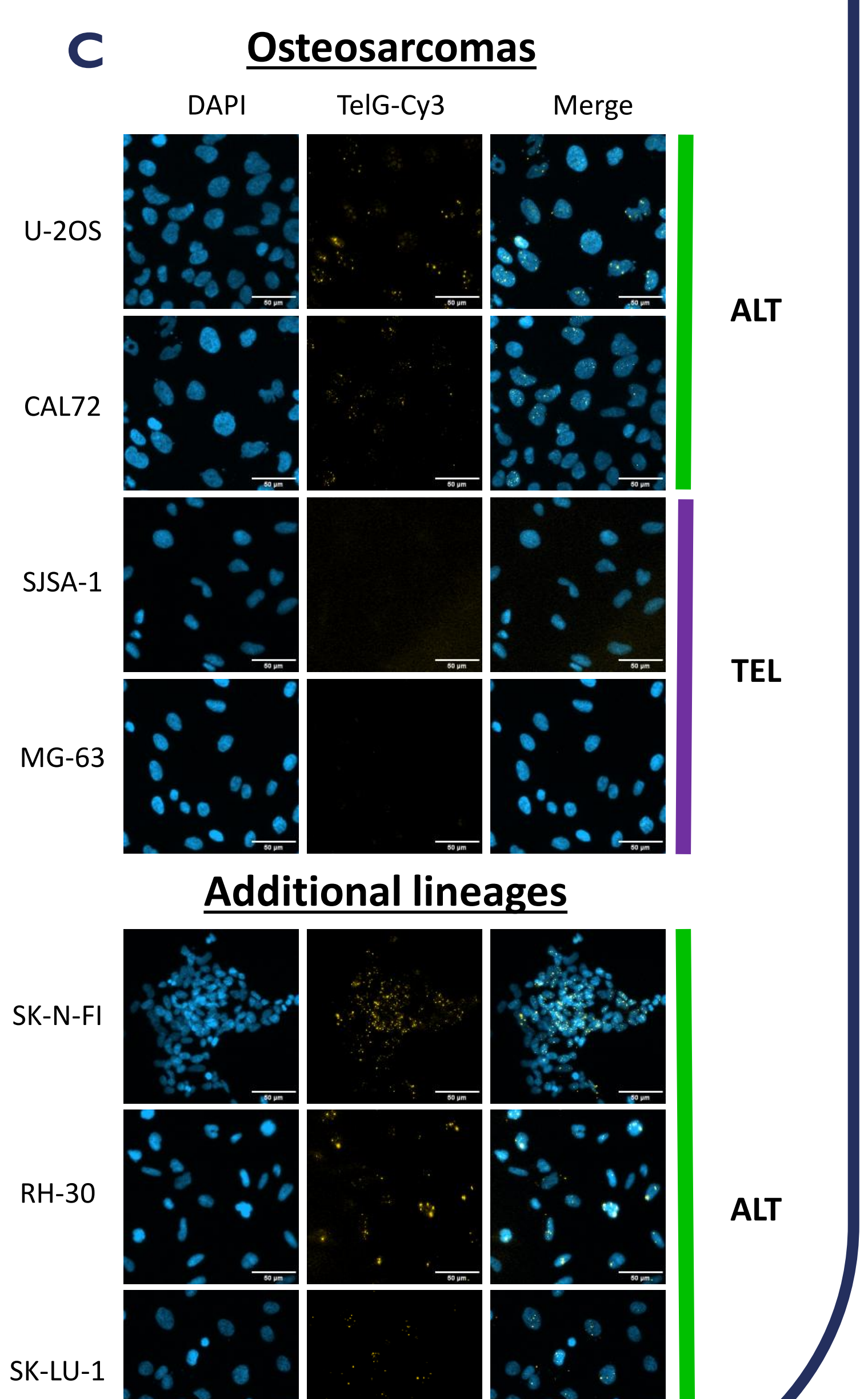
A. CCA-qPCR assay. C-circles are amplified from genomic DNA by rolling circle amplification and fold change detected using telomere specific primers.
B. Representative images from Native-FISH assay where telomeric ssDNA is detected as nuclear foci by a TelG-Cy3 probe in U-2OS cells. Quantification of TelG-Cy3 foci intensity in U-2OS cells treated with control sgRNAs.
C. Schematic depicting function of established ALT control genes FANCM and BLM.



Generation of a cross-lineage ALT/TEL cell line panel

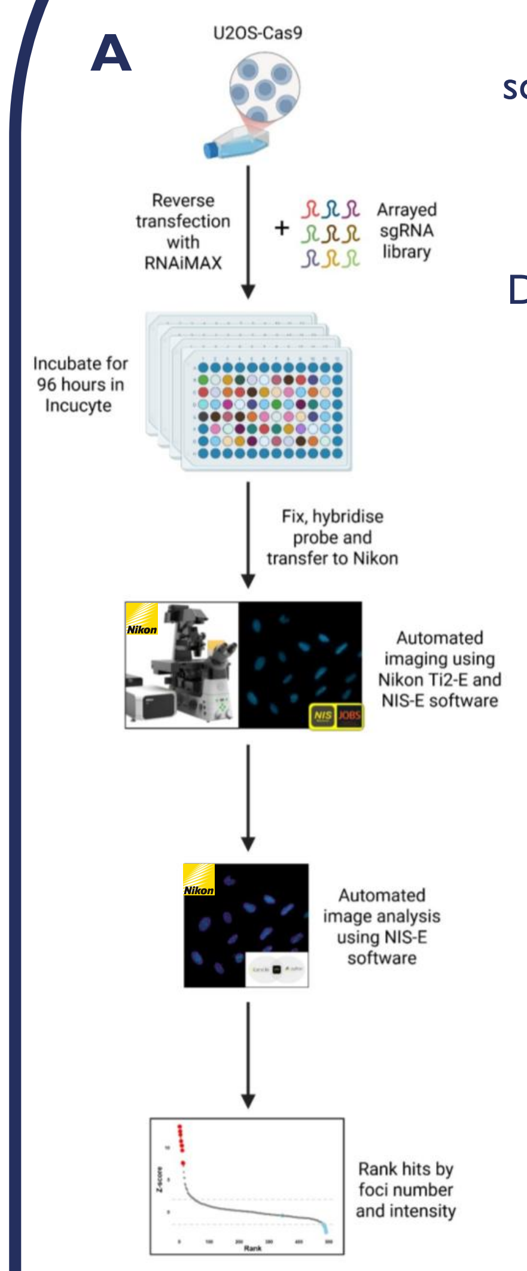
Osteosarcoma models feature heavily in the ALT literature on account of the high prevalence of ALT in clinical samples from this lineage, and the availability and amenability of these cell lines to in vitro manipulation. We use the CCA-qPCR and ssTelo native FISH assays to validate a panel of osteosarcoma cell lines for ALT or TEL status by the presence or absence of the ALT hallmarks. Furthermore, we identify ALT cell lines from brain, soft tissue and lung to expand our ability to identify ALT specific targets across multiple lineages.



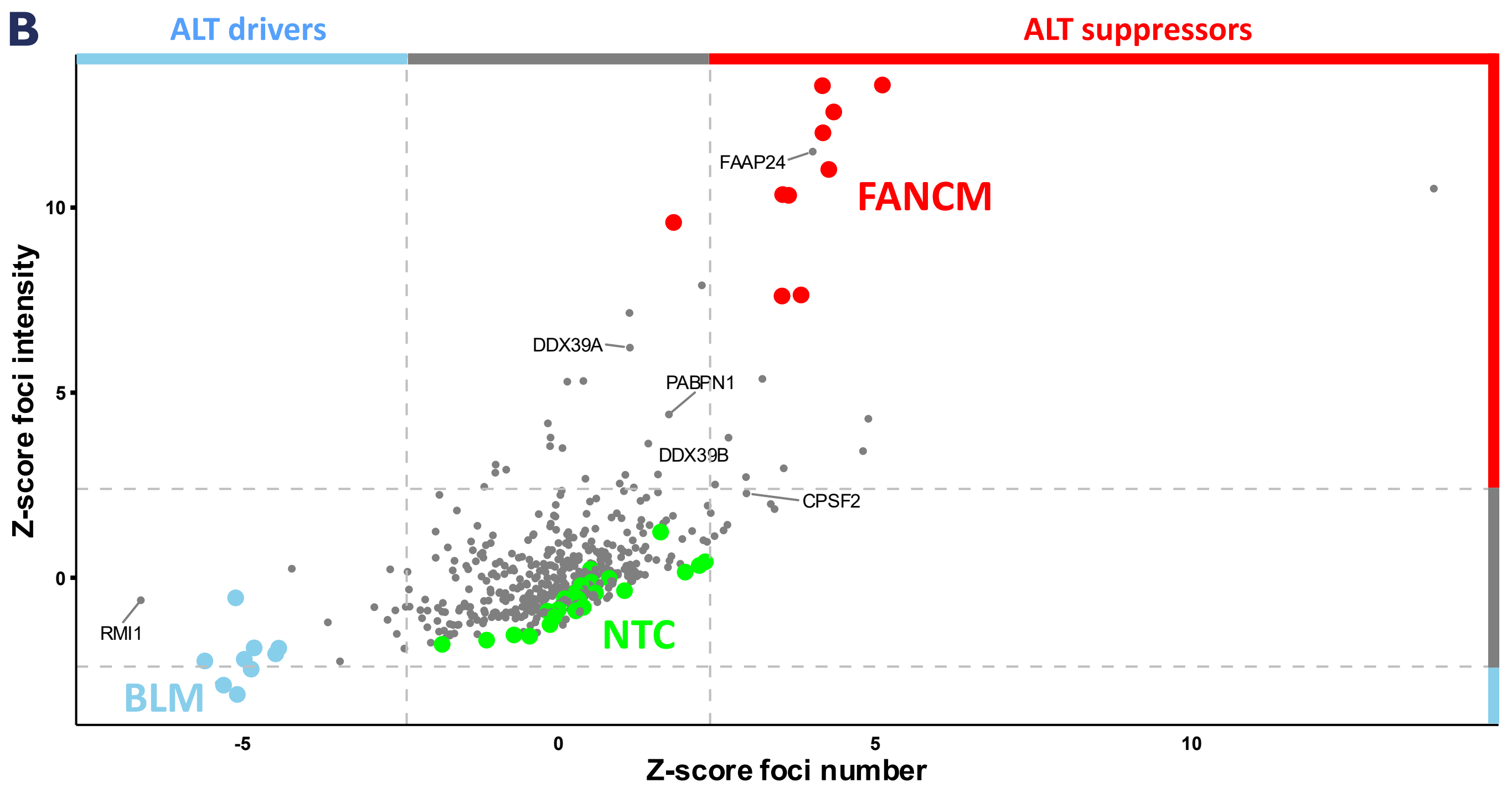


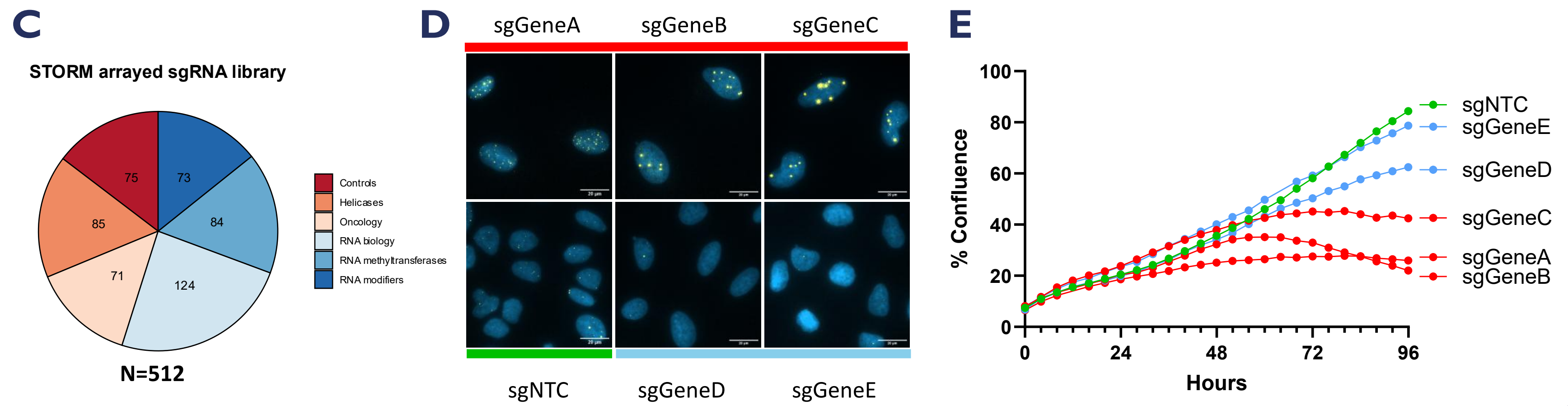
A. qPCR measurement of raw telomere length in an ALT/TEL cell line panel using a telomere specific primer set.
B. CCA-qPCR data highlighting fold enrichment in telomeric DNA after RCA of C-circles.
C. N-FISH images detecting ALT activity in a panel of ALT and TEL cell lines.

A high throughput arrayed KO imaging screen identifies RNA interactors regulating ALT biology



To identify ALT modulators within STORM's target space, we carried out an arrayed KO screen in the ALT-positive osteosarcoma cell line U-2OS and, utilising the imaging and analysis expertise of the Nikon Bioimaging Lab, determined the effects of these KOs on ssTelo positive foci number and intensity. The results of our screen confirmed several known ALT modulators; co-factors of FANCM and BLM, FAAP24 and RMI1, the dead-box helicases DDX39A and DDX39B, and members of the cleavage and polyadenylation machinery CSPF2 and PABPN1. We also identified several novel regulators of ALT activity which could be pursued as potential therapeutic targets for ALT cancers.

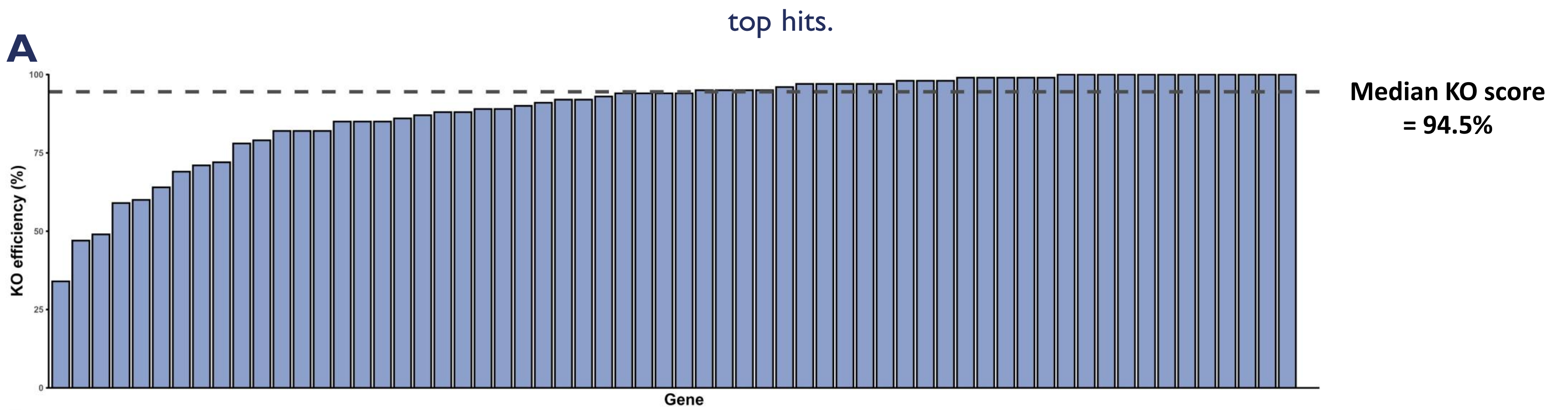


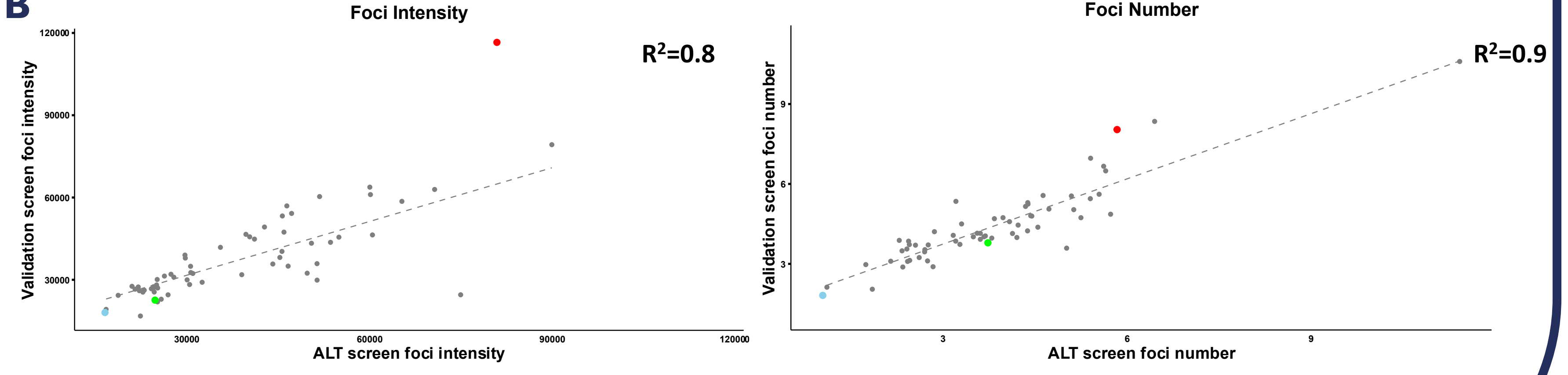


A. Schematic representation of the workflow used to identify modulators of ALT activity.
B. Scatterplot showing quantification of foci intensity and number for 512 gene knockouts.
C. Composition of the STORM sgRNA library.
D. Representative images of ALT suppressors and drivers identified by the screen.
E. Incucyte growth curves showing growth defect for loss of genes in figure D.

Validation screen verifies on target editing and confirms hits from primary screen

We performed a confirmatory screen, focusing on the hits from the first screen along with any associated complex members regardless of their prior performance. We used ICE analysis to demonstrate very high on-target editing efficiency, indicating a low likelihood of false negatives within the primary screen. We observed a strong correlation in the ssTelo FISH results between the primary and secondary screens, confirming the ALT modulatory activity of the top hits.





A. KO scores determined by Inference of CRISPR edits (ICE) software for the entire panel of genes selected for the follow up validation screen.
B. Scatterplots highlighting correlation between ALT and validation screens for foci number and intensity.

Summary

- Through collaboration with the Nikon Bioimaging lab, STORM has run a high throughput arrayed KO screen to identify RNA interacting factors with potential as therapeutic targets for ALT cancers.
- 54 putative ALT modulators were identified with Z score <-2.4 or >2.4. Some of these hits have been described previously but many are novel regulators of ALT biology.
- Hits from the initial ALT screen show strong correlation with a follow up validation screen.

Next steps

- Top hits from our screens will be depleted in a multi-lineage ALT/TEL cell panel to identify ALT-specific dependencies with potential as therapeutic targets.

Harnessing the power of RNA modification