

High-Content Optical Pooled CRISPR Screening with Multimodal Single-Cell Readouts to Map NF- κ B Subcellular Dynamics

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1) Myllia Biotechnology
2) Element Biosciences

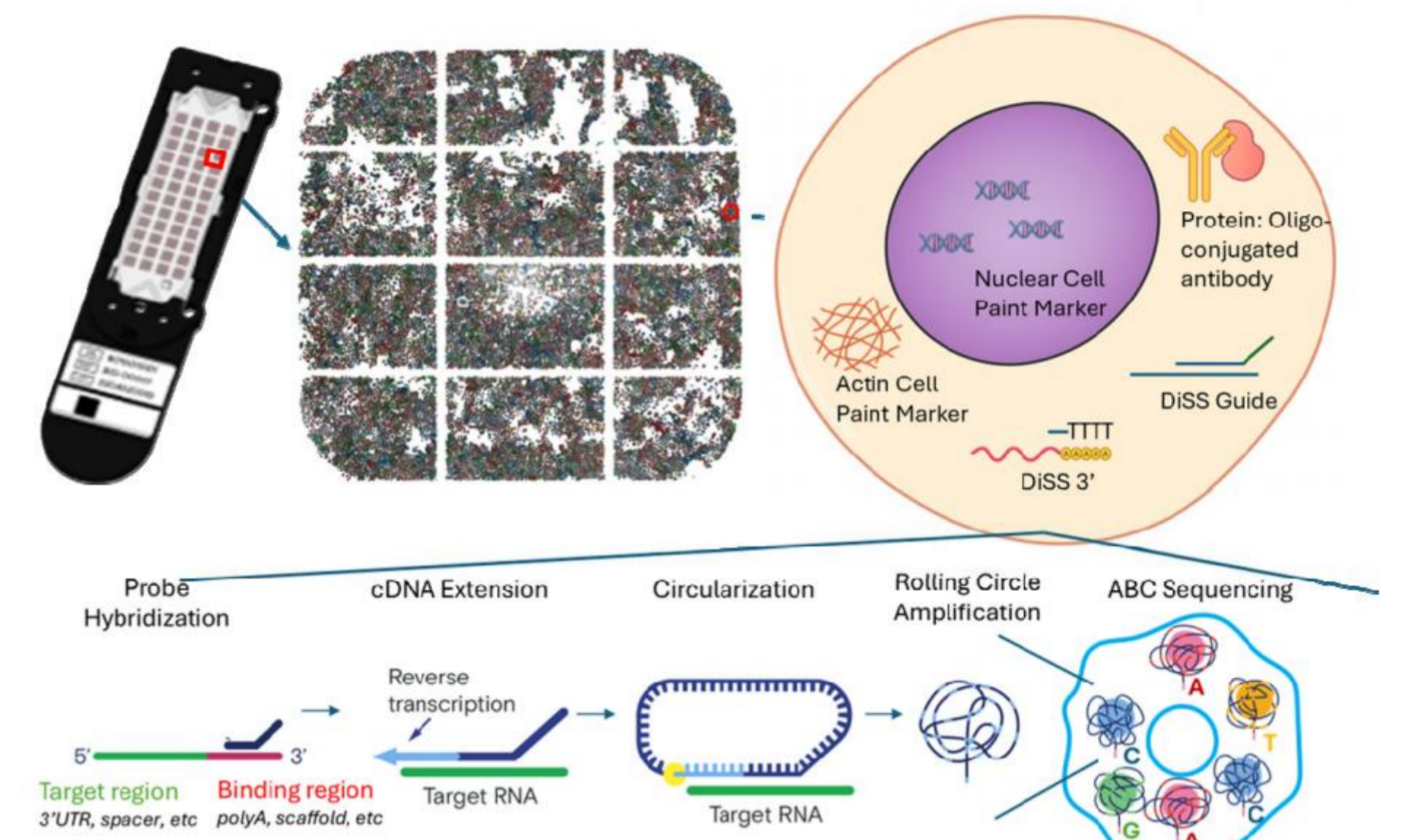
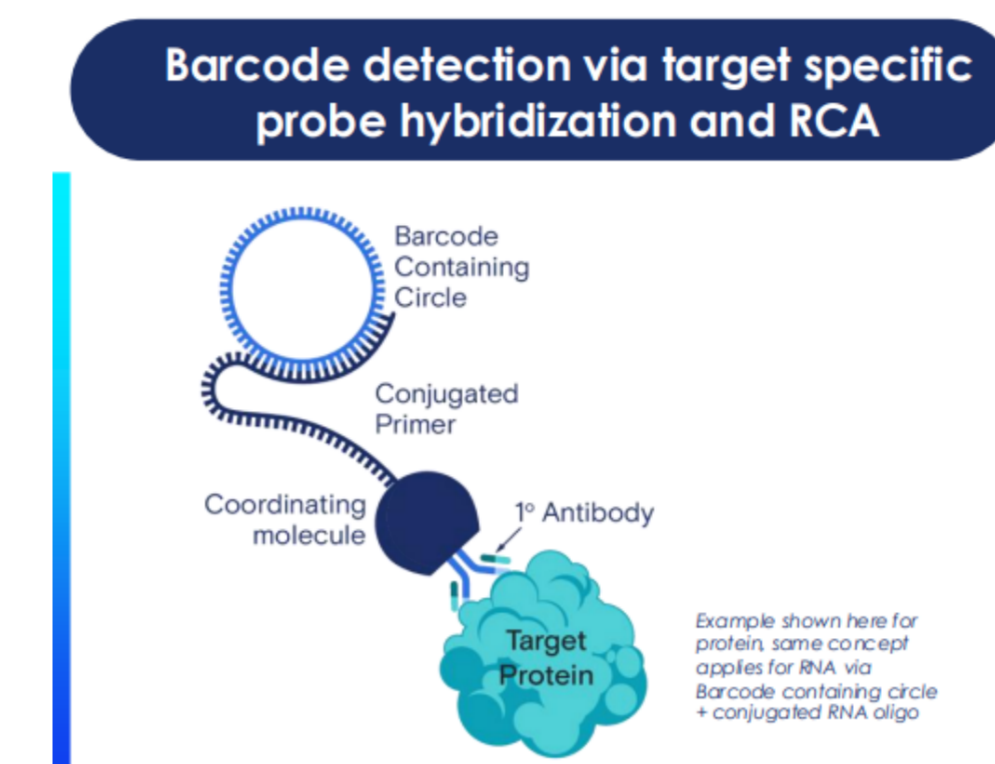
Introduction

NF- κ B is a family of transcription factor complexes that regulate transcription, cytokine production, and cell survival. Upon stimulation with pro-inflammatory cytokines such as TNF- α , active NF- κ B (p50/p65 heterodimer) rapidly translocates from the cytoplasm to the nucleus. While core pathway components are well characterized, additional chromatin and transcriptional regulators have recently been implicated in shaping NF- κ B dynamics. To systematically identify genes affecting NF- κ B nuclear accumulation, we performed a pooled CRISPR knockout screen targeting 195 genes and quantified stimulus-dependent p65 translocation using Element Biosciences' AVITI24 platform.

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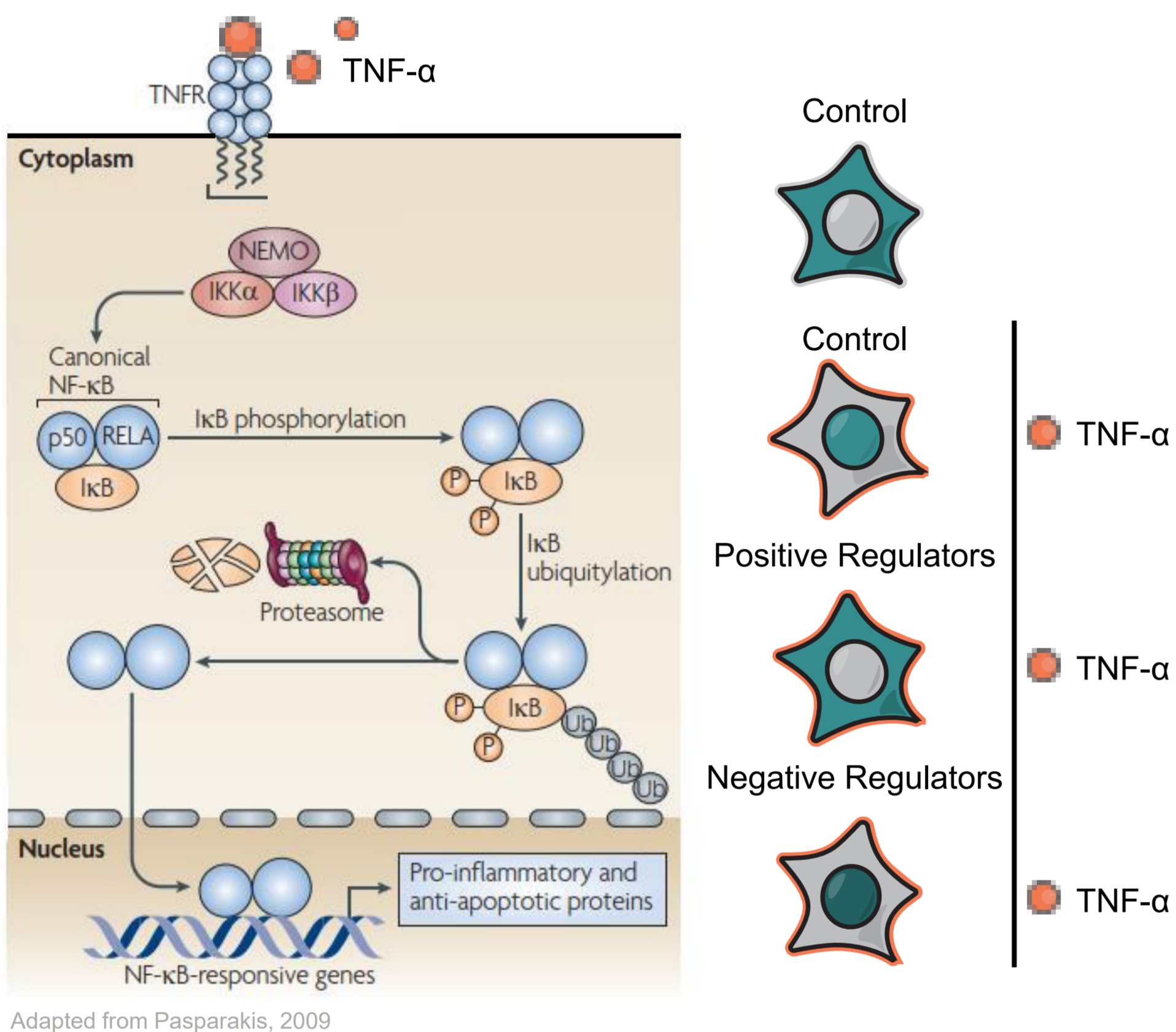
The AVITI24 Platform

Element Biosciences

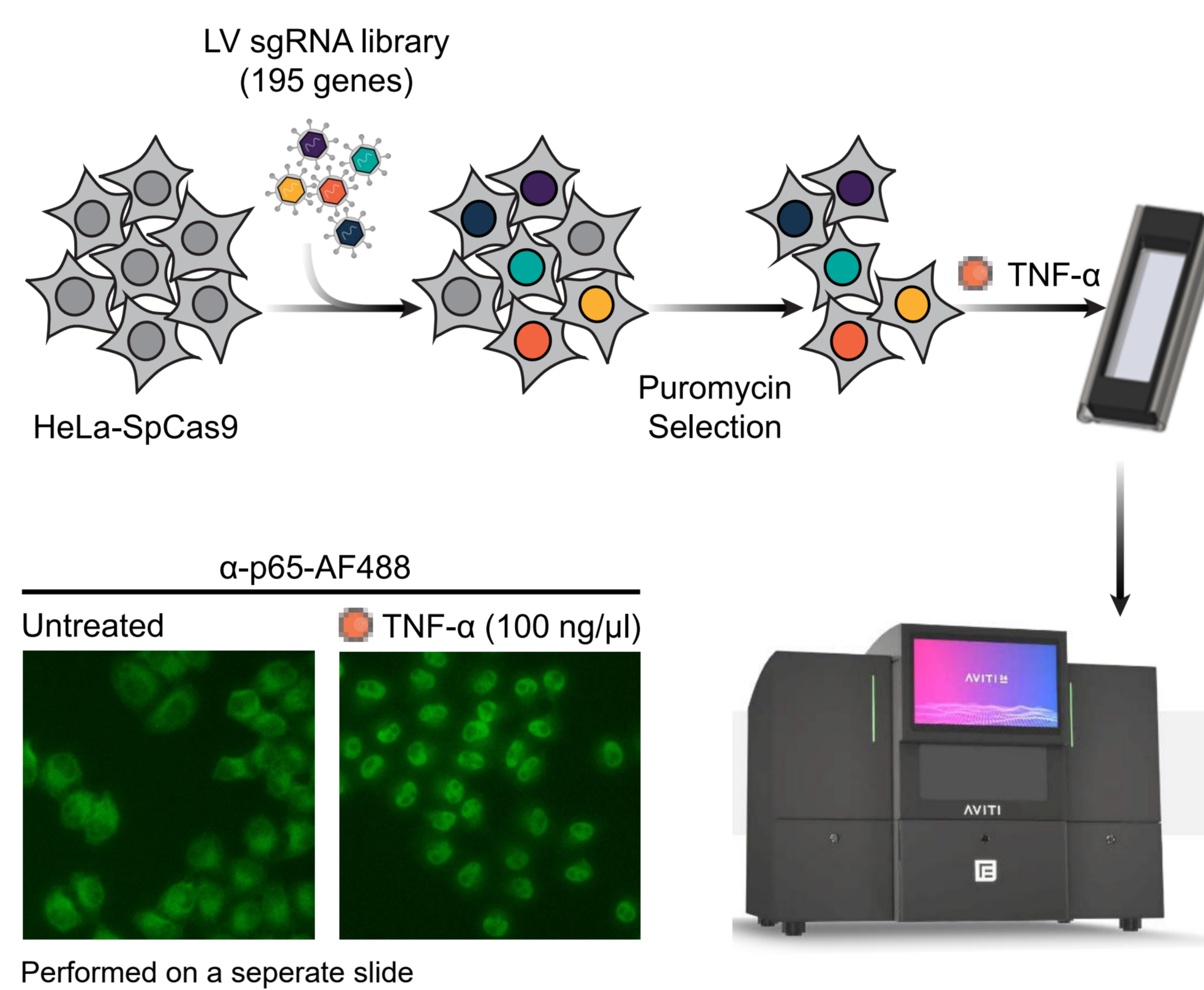


Honigfort et al., 2025

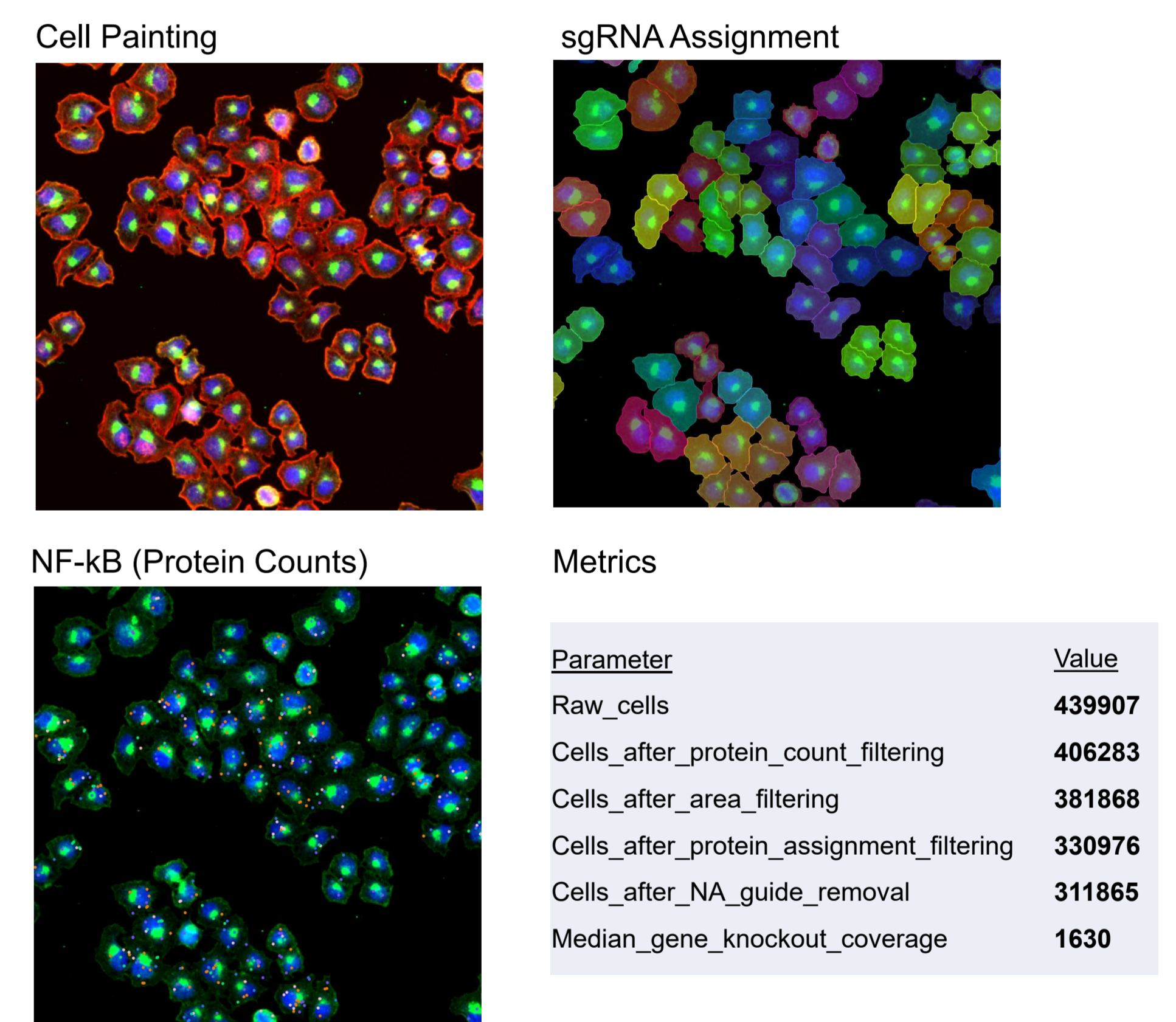
2 Overview of NF- κ B signalling



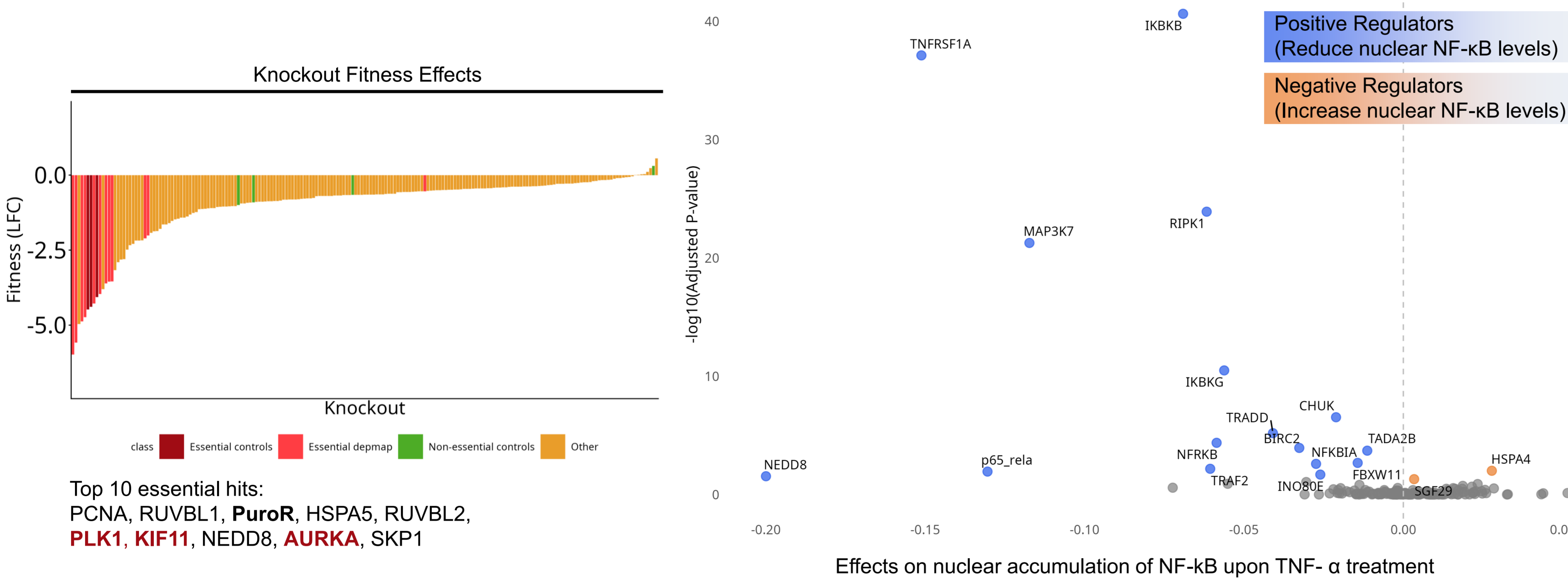
3 Screening Workflow



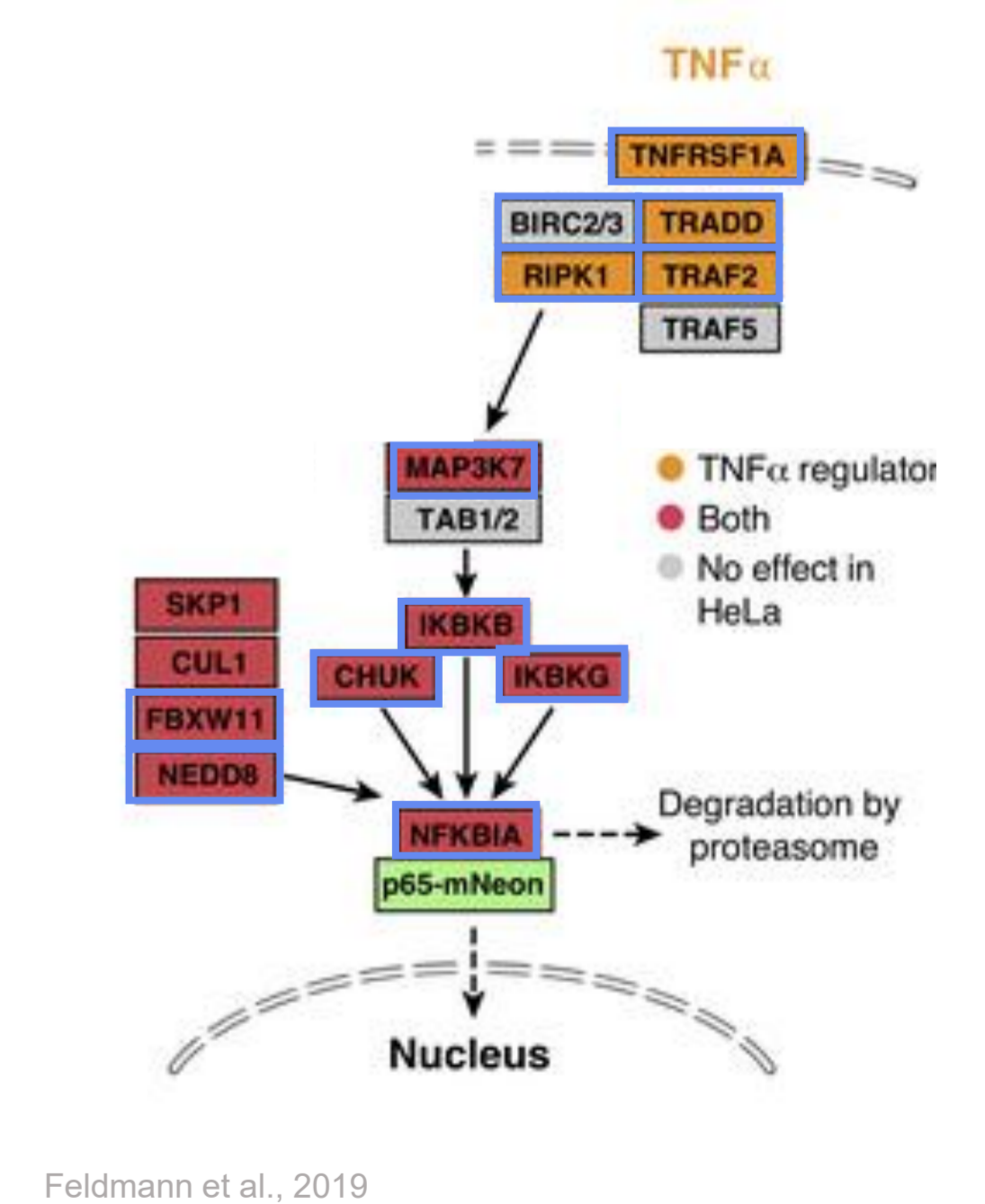
4 Imaging and sgRNA annotation



5 AVITI24 identifies gene perturbations modulating NF- κ B signalling



6 AVITI24 confirms key regulators



Conclusions and Outlook

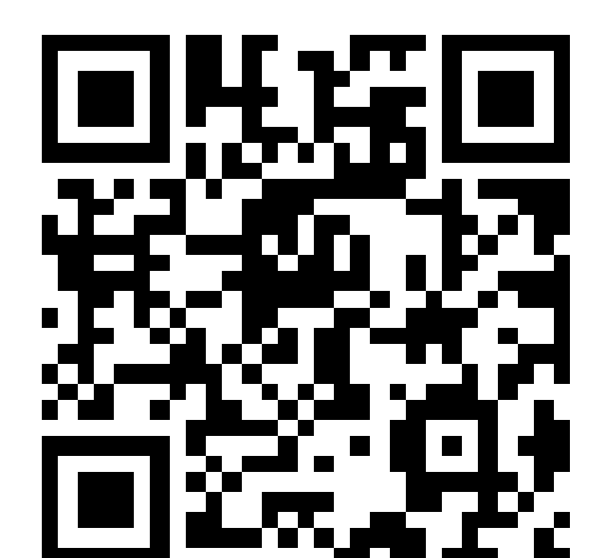
- A total of 15 significant positive and 2 significant negative regulators of NF- κ B signaling were identified
- The AVITI24 platform achieves robust sgRNA detection in approximately 80% of cells
- High-quality cell segmentation enables the study of morphological changes after genetic perturbations
- Strong dropout of sgRNAs targeting essential genes confirms the reliability of the instrument

Next steps:

- Combine the workflow with immunofluorescence to uncover more subtle imaging-based phenotypes

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