Defining Essential Enhancers for Pluripotent Stem Cells Using a Features-Oriented CRISPR-Cas9 Screen

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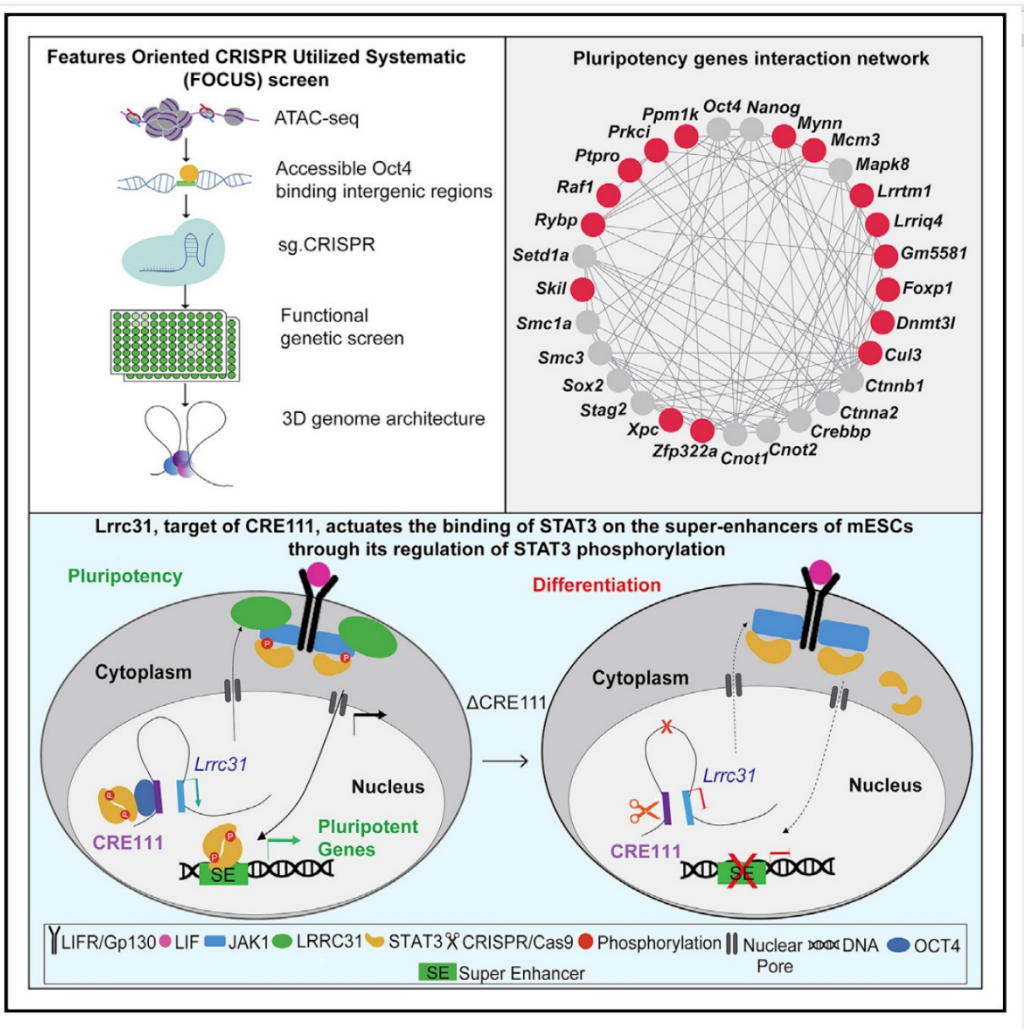
Abstract

cis-regulatory elements (CREs) regulate the expression of genes in their genomic neighborhoods and influence cellular processes such as cell-fate maintenance and differentiation. To date, there remain major gaps in the functional characterization of CREs and the identification of their target genes in the cellular native environment. In this study, we perform a features-oriented CRISPR-utilized systematic (FOCUS) screen of OCT4- bound CREs using CRISPR-Cas9 to identify functional enhancers important for pluripotency maintenance in mESCs. From the initial 235 candidates tested, 16 CREs are identified to be essential stem cell enhancers. Using RNA-seq and genomic 4C-seq, we further uncover a complex network of candidate CREs and their downstream target genes, which supports the growth and self-renewal of mESCs. Notably, an essential enhancer, CRE111, and its target, *Lrrc31*, form the important switch to modulate the LIF-JAK1-STAT3 signaling pathway.

Figure



Highlights

- A focused CRISPR screen identifies essential enhancers for pluripotency maintenance
- Proximal pluripotency genes are identified by 4C-seq
- *Lrrc31* is found to regulate pluripotency through the JAK-STAT signaling pathway
- *Lrrc31* regulates the binding of STAT3 on super-enhancers of mESCs