Systematic Evaluation of Retroviral LTRs as *cis*-regulatory Elements in Mouse Embryos

Jian Yang[1,2], and Lauryn Cook[1,2], and Zhiyuan Chen[1,2*]

[1]Reproductive Sciences Center, Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, 45229, USA;

[2]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH 45267, USA;

[*]To whom correspondence should be addressed: zhiyuan.chen@cchmc.org

Abstract

In mammals, many retrotransposons are de-repressed during zygotic genome activation (ZGA). However, their functions in early development remain elusive largely due to the challenge to simultaneously manipulate thousands of retrotransposon insertions in embryos. Here, we applied CRISPR interference (CRISPRi) to perturb the long terminal repeats (LTR) MT2_Mm, a well-known ZGA and totipotency marker that exists in ~2667 insertions throughout the mouse genome. CRISPRi robustly perturbed 2485 (~93%) MT2_Mm insertions and 1090 (~55%) insertions of the closely related MT2C_Mm in 2-cell embryos. Remarkably, such perturbation caused down-regulation of hundreds of ZGA genes and embryonic arrest mostly at the morula stage. Mechanistically, MT2 are globally enriched for open chromatin and H3K27ac and function as promoters/enhancers downstream of OBOX/DUX proteins. Thus, we not only provide direct evidence to support the functional importance of MT2 activation in development, but also systematically define *cis*-function of MT2 in embryos by integrating functional perturbation and multi-omic analyses.