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A degron-based CRISPRi method to finely tune gene expression at the single-cell level

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Since not only the presence or absence of gene products dictate the cell state, but also their dosage, gene expression levels must be precisely tuned through quantitative modulation of transcriptional regulators. One example is the up-regulation of Xist, the master regulator of X-chromosome inactivation, which is induced only in female cells via a double dose of X chromosomal genes. Interrogation of dosage-sensitive gene regulation requires experimental tools to precisely tune activity of transcriptional regulators. However, most of the currently employed approaches to manipulate gene expression, rely on the strong up- or down-regulation of a target gene. We are thus developing a tunable CRISPR interference (CRISPRi) technique to quantitatively control gene expression. We employ catalytically dead Cas9 (dCas9) fused with a repressor domainand a degron-domain, in order to control the level of repression by varying ligand concentration. We first systematically compared several degron-tags fused to dCas9 and selected the one with the largest dynamic range. Next, we fused different repressor domains to the degron-dCas9 and are now testing the ability of our synthetic repressors to titrate an endogenous gene fused with a fluorescent reporter in mouse embryonic stem cells. Through this approach we will assess the tunability at the single cell level. We will then use the system to quantify the dose-response relationship between Xist regulators and Xist expression with single-cell resolution.