

Development of synthetic guide RNA libraries for CRISPR-mediated transcriptional activation screening for gain-of-function studies

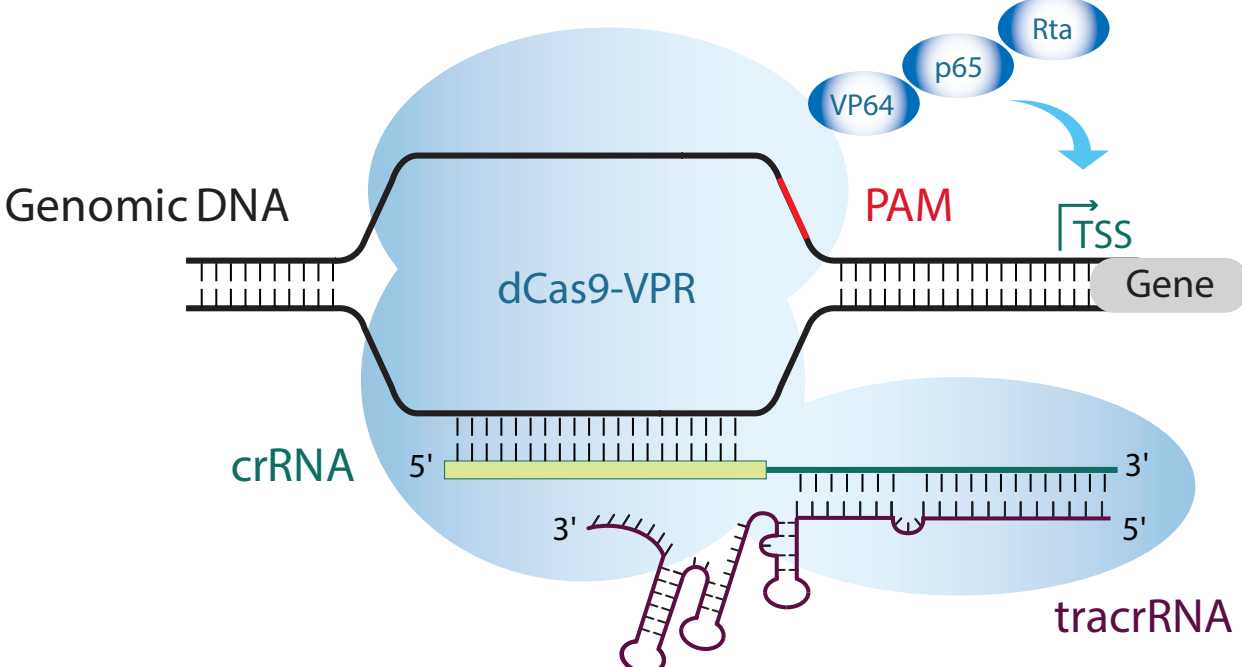
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Abstract

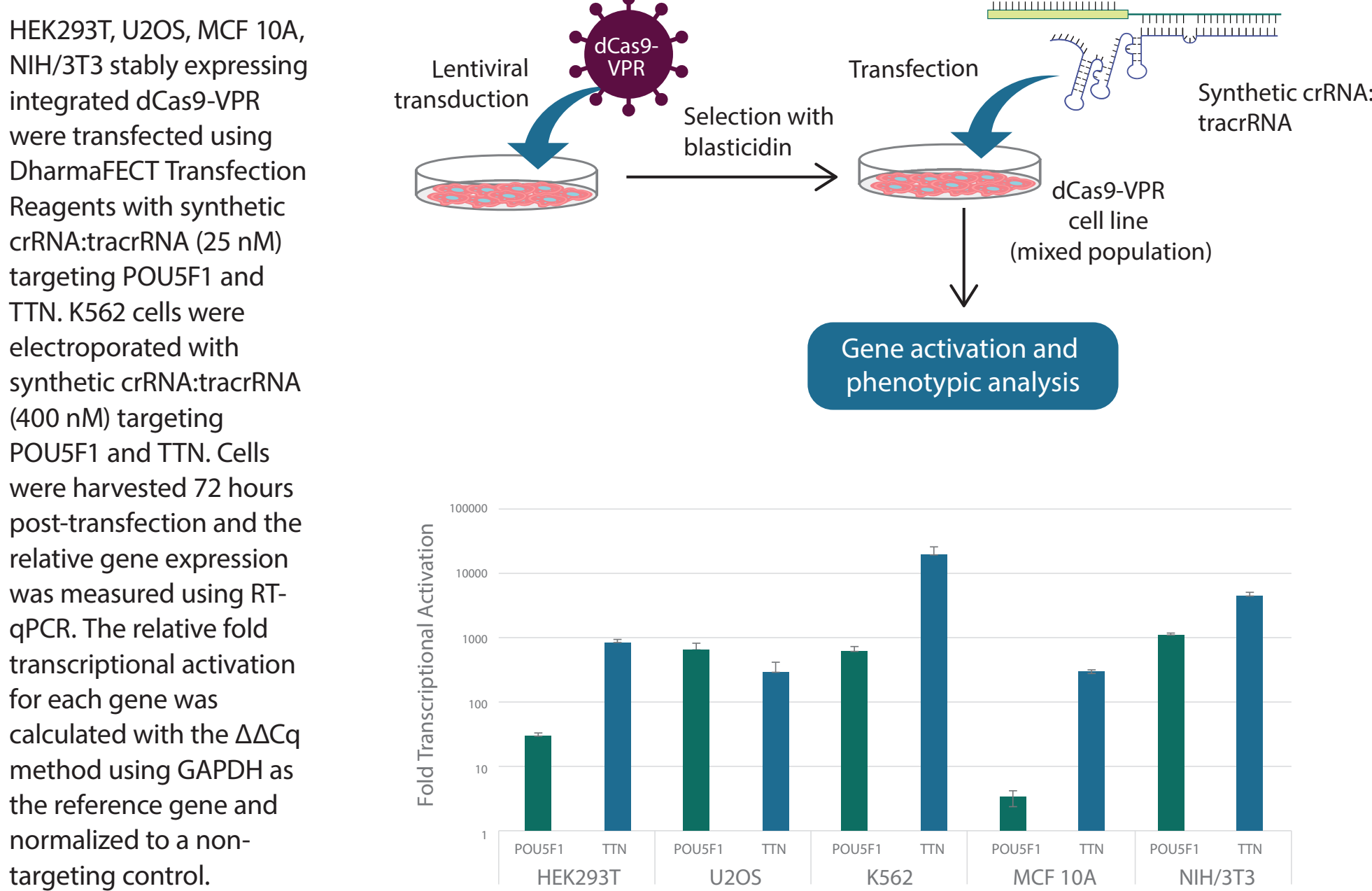
Functional gene analysis studies have been empowered by development of CRISPR-Cas9 gene knockout tools, however the CRISPR-Cas9 system has also been adapted for inhibition or activation of gene transcription. A nuclease-deactivated Cas9 (dCas9) can be fused to various effector domains to produce an RNA-guided transcription factor for either inhibition (CRISPRi) or activation (CRISPRa) of target genes. For overexpression studies, CRISPRa holds significant advantages over traditional vector-based gene expression, because genes are upregulated from their native promoter and endogenous genomic context. The majority of CRISPRa research performed to date has utilized single guide RNA (sgRNA) expressed from a DNA vector, primarily in the context of pooled lentiviral screening approaches. Here we describe the development of CRISPRa synthetic guide RNAs for the use in arrayed screening, so that we combine this next-generation transcriptional activation method with the ability to support more complex assays in a one-gene-per well format. Considerations for arrayed activation screens will be shown. The combination of gain-of-function from CRISPRa with loss-of-function using RNAi or canonical CRISPR-Cas9 for gene knockout allows for robust characterization of gene mechanisms and pathways.

Using synthetic guide RNA with the dCas9-VPR transcriptional activation system

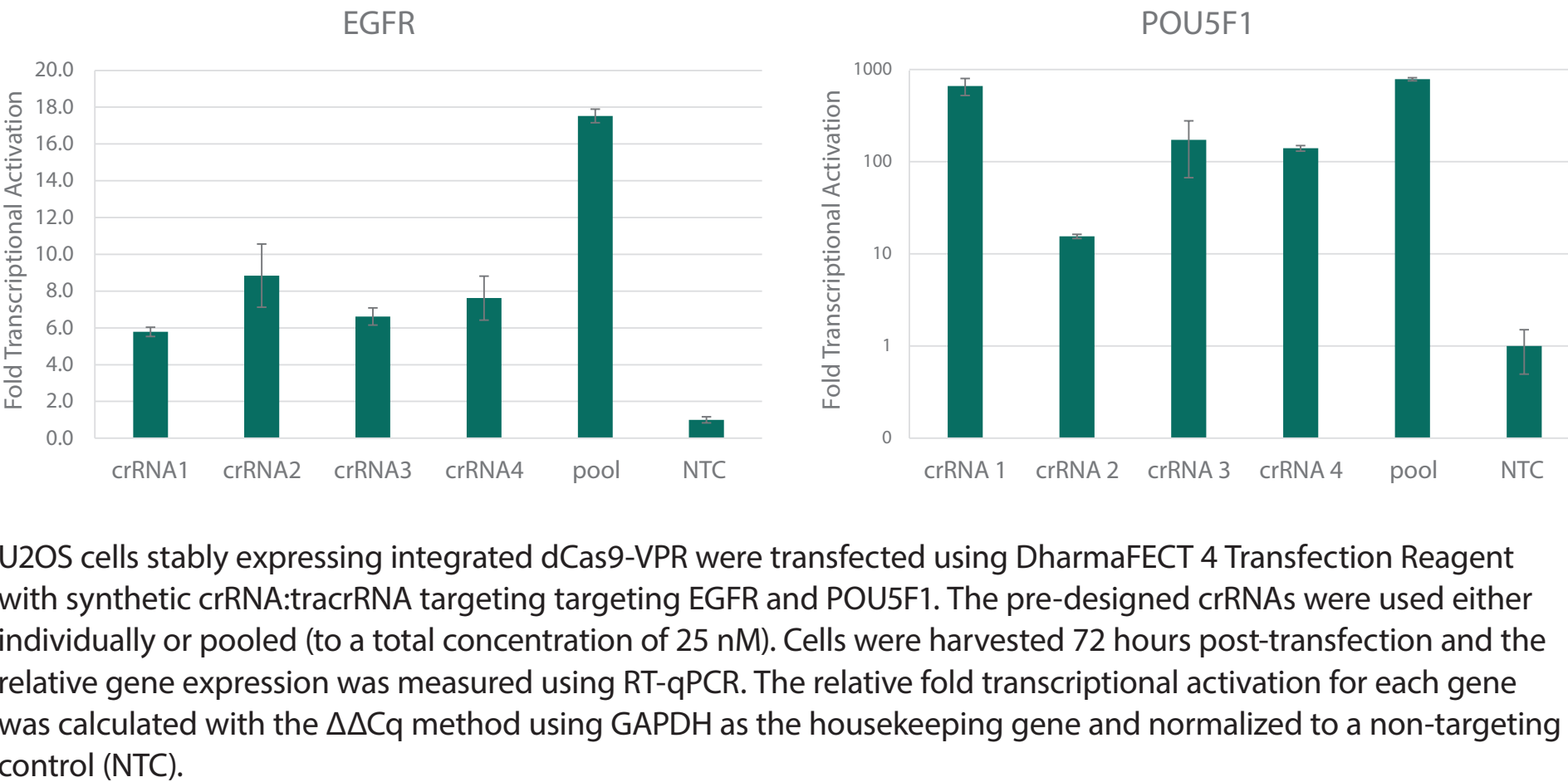


- In the VPR activation system, deactivated Cas9 is fused at the C-terminal end to three transcriptional activators, VP64, p65 and Rta (Chavez *et al.*)
- Edit-RTM CRISPRa synthetic crRNA:tracrRNA mimics the two-part guide RNA found in the native *S. pyogenes* and can be utilized with this system
- The crRNA is complementary to the gene promoter regions and induce transcription
- Designs are based on CRISPRa v2 algorithm based on machine learning (Horlbeck *et al.* 2016)

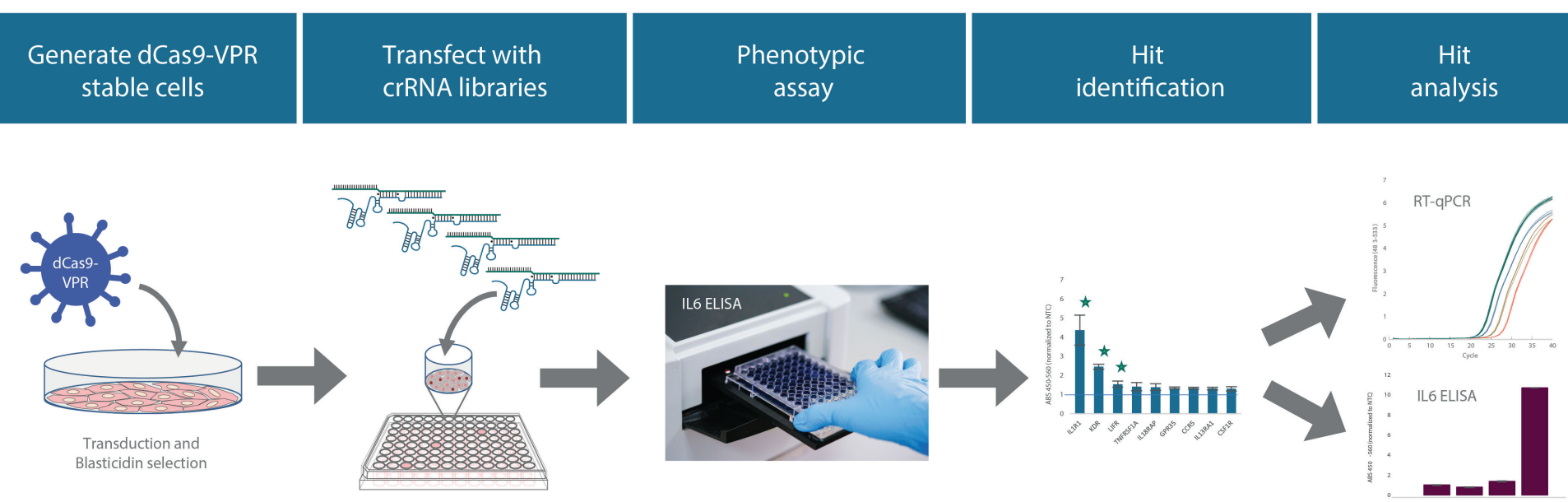
Efficient transcriptional gene activation with synthetic crRNA:tracrRNA in multiple dCas9-VPR stable cells



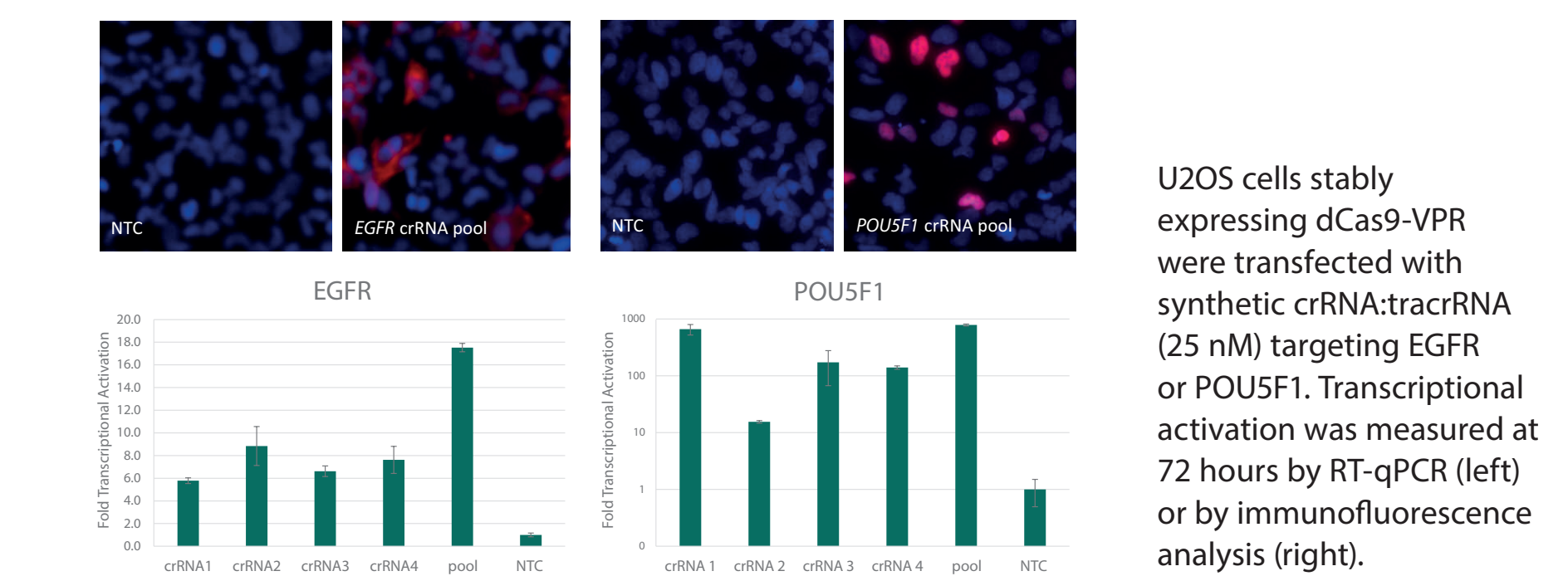
Using multiple synthetic crRNA per gene can enhance activation



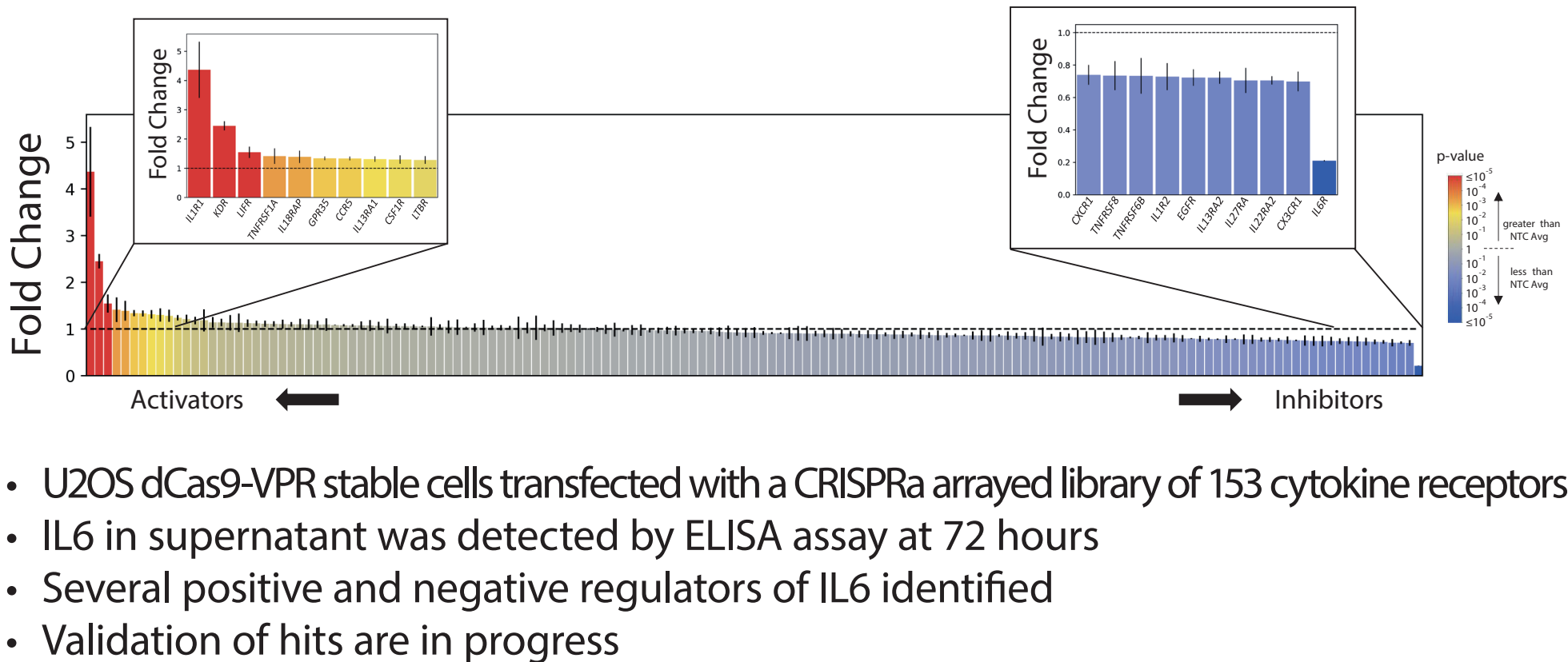
Synthetic guide RNAs are ideal for arrayed CRISPRa screening



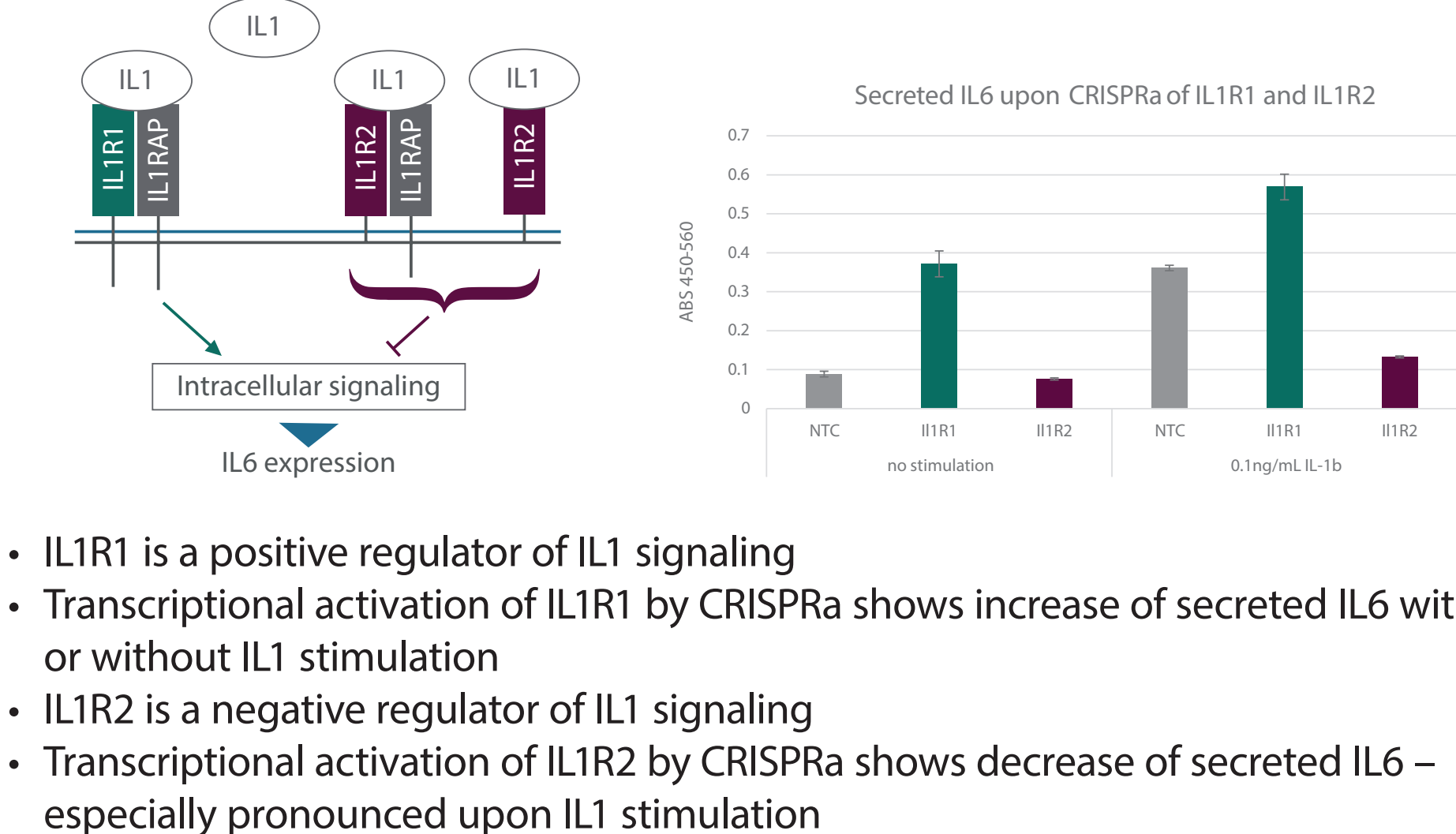
Efficient transcriptional activation on mRNA and protein levels



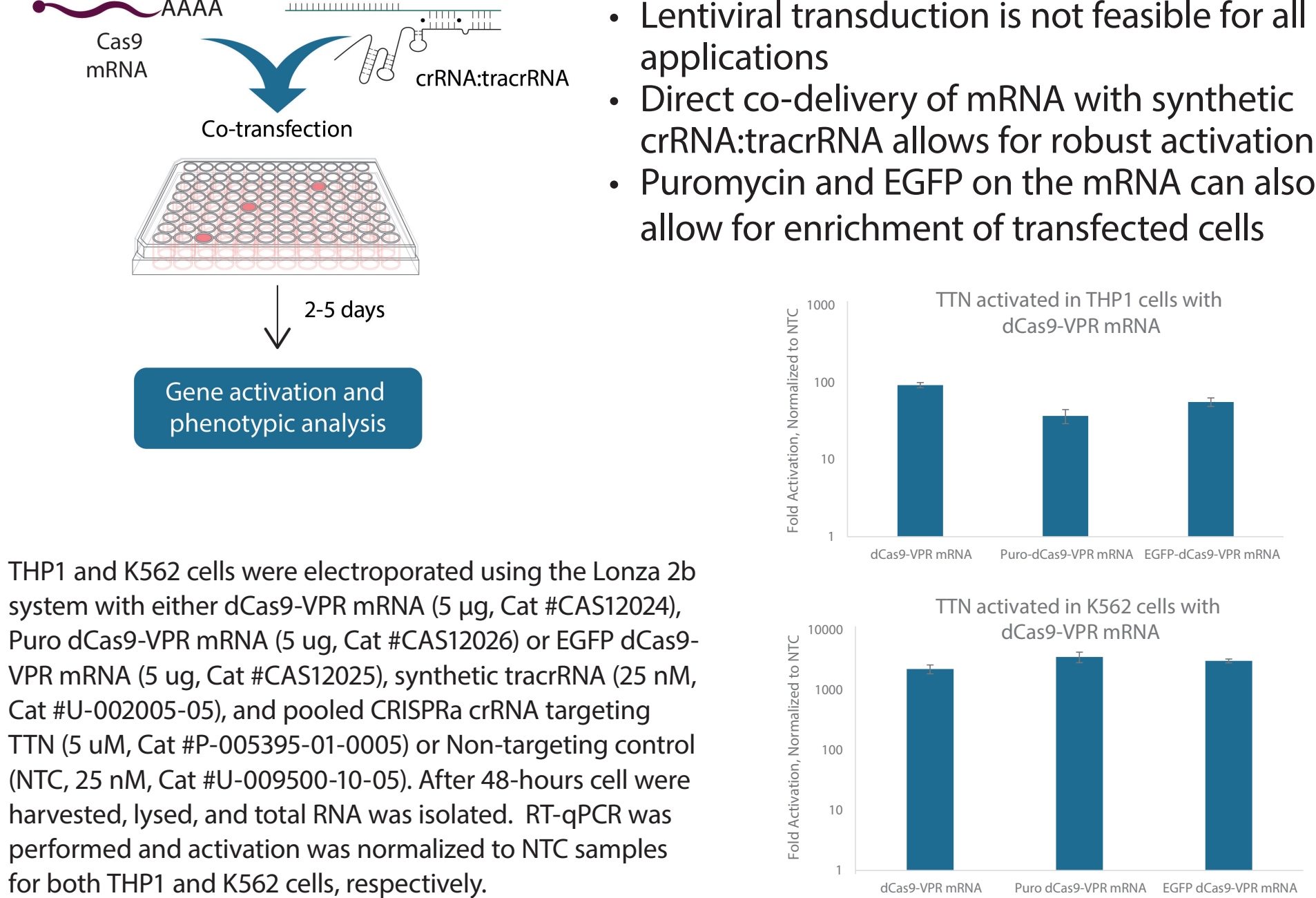
Primary CRISPRa screen identifies IL6 regulators



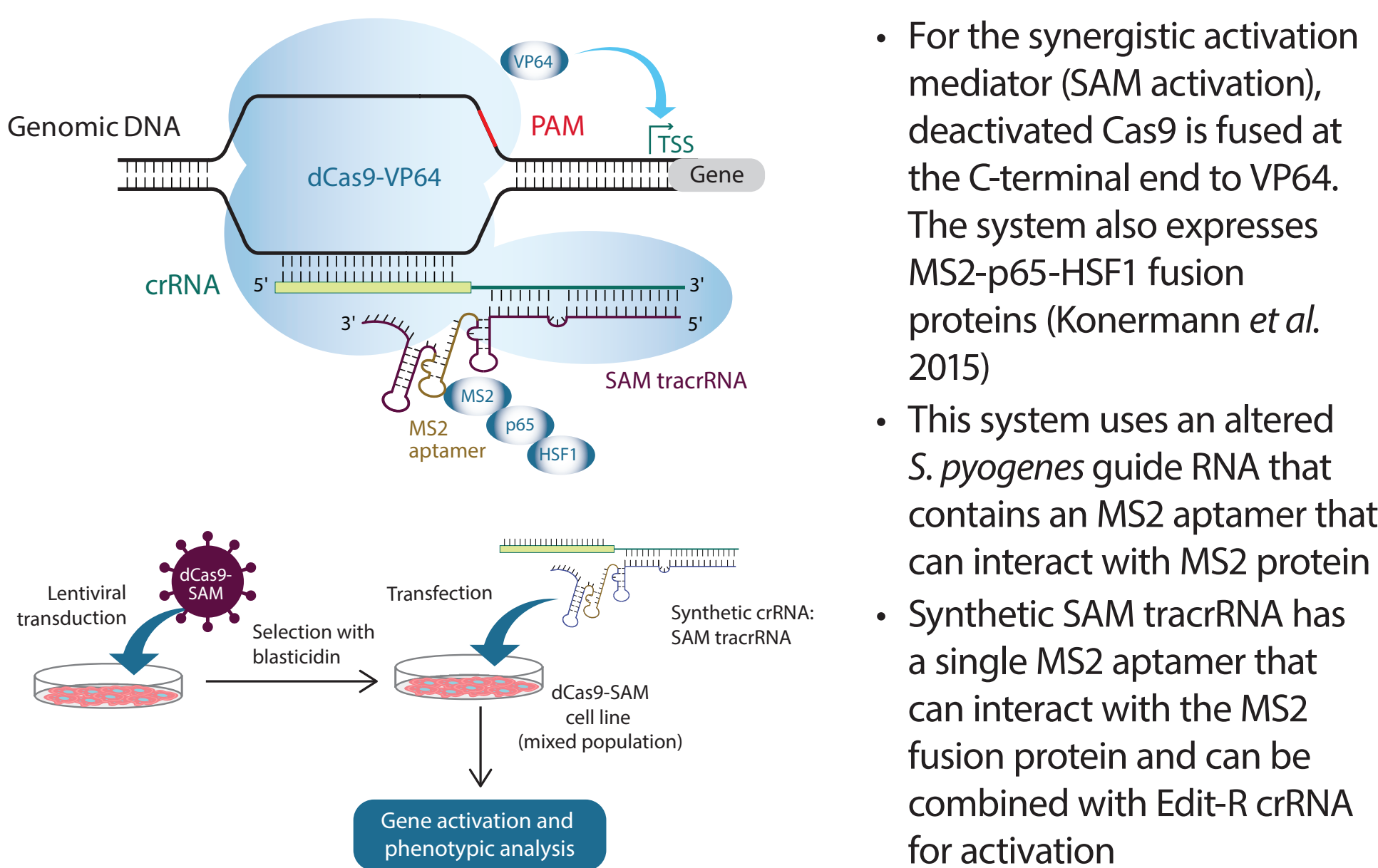
IL1 signaling pathway is one of the major regulators of IL6 expression



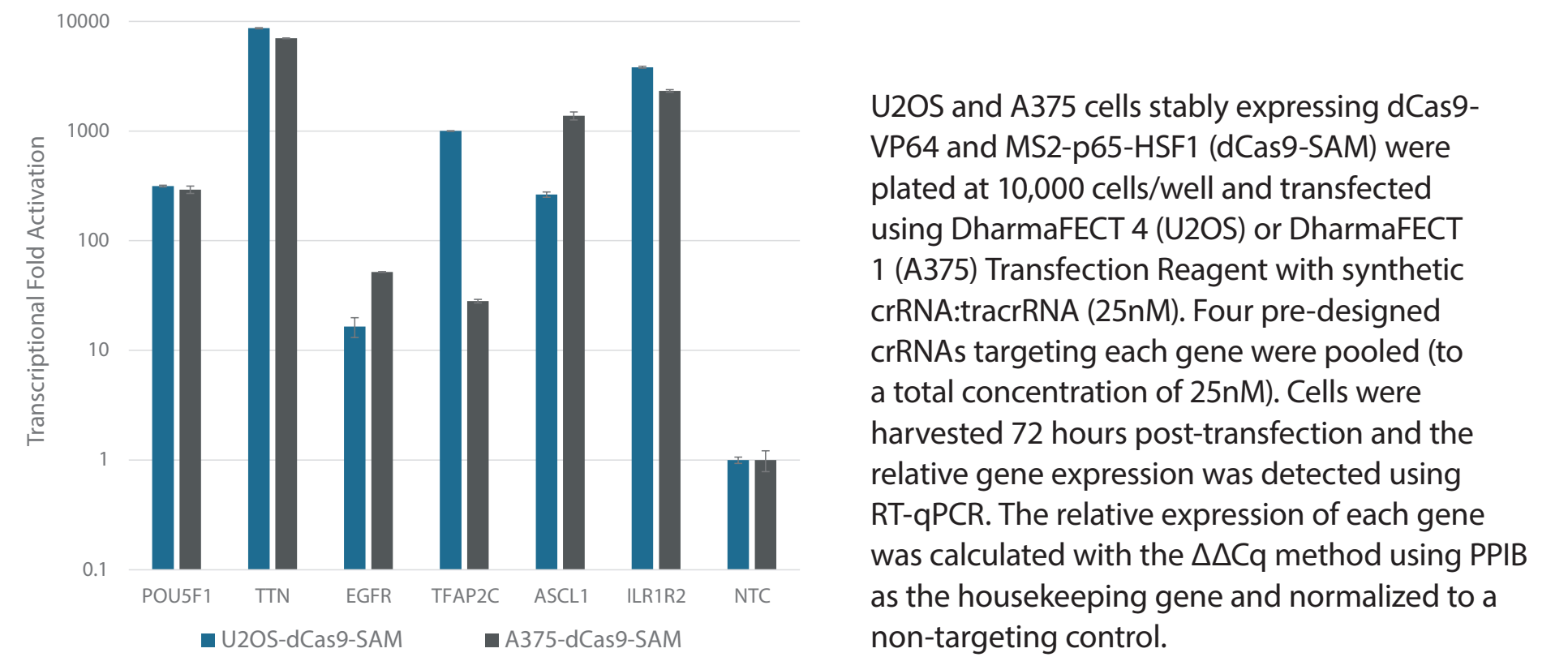
Co-electroporation of dCas9-VPR mRNA and crRNA:tracrRNA results in efficient activation



Synthetic SAM tracrRNA was developed for use with the dCas9-SAM system



Transcriptional activation using dCas9-SAM and synthetic SAM tracrRNA



Conclusions

- Synthetic crRNA:tracrRNA can be used with dCas9-VPR for transcriptional activation
- Pooled crRNAs can enhance the transcriptional gene up-regulation activity
- Synthetic crRNAs can be successfully used for gain-of-function screening in an arrayed format enabling expansion of the phenotypic readouts to high-content and morphology-based assays
- Synthetic guide RNA can be co-delivered with dCas9-VPR mRNA for applications where a stable dCas9-VPR cell line cannot be generated
- Synthetic crRNA can also be used in the dCas9-SAM system when used in conjunction with a synthetic SAM tracrRNA
- Synthetic guide RNAs provide many advantages to expressed guide RNAs in well-by-well screens because they require fewer experimental steps, give a much more uniform delivery concentration and are less prone to off-target effects due to their transient cellular effect