

## Efficient Targeted Knock-in in Non-Dividing Cells Using Engineered Nucleases

Recent advances in targeted genome editing have transformed biomedical research. With the advent of the CRISPR/Cas9 system, genome editing has become faster, cheaper and more accurate, and is now an invaluable tool for researchers in the biological sciences. Although this technology has been revolutionary for the research community and holds great potential for gene therapy, it is not an efficient tool for targeted integration of transgenes in non-dividing cells. Currently, site specific transgene integration using CRISPR/Cas9 exploits the homology-directed repair (HDR) pathway, which is inefficient in primary cell types and only functions in actively dividing cells. This limits the utility of CRISPR/Cas9 and other engineered nucleases in non-dividing cells, which are the major constituents of adult tissues. Investigators at Salk have developed a homology-independent targeted integration strategy which overcomes these limitations. This new strategy allows for efficient targeted knock-in in both dividing and non-dividing cells *in vitro*, and more importantly, *in vivo*. This innovative approach will not only help to advance basic biological research, but has the potential to make targeted gene therapy safer and more efficient.

### APPLICATIONS:

- *In vivo* targeted gene-replacement therapy
- Tissue and animal disease models
- Cellular engineering (stem cells, CAR-T cells)
- Live tracking of post-mitotic cells

### ADVANTAGES:

- Works in both dividing and non-dividing cells
- Applicable to *in vitro* and *in vivo* systems
- Only targeted gene insertion method applicable to non-dividing cells
- Increased efficiencies compared to traditional methods

**STAGE OF DEVELOPMENT:** Proof of concept in rat model of retinitis pigmentosa

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**PATENT STATUS:** US Provisional Application

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<http://www.salk.edu/news-release/new-gene-editing-technology-partially-restores-vision-blind-animals/>

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