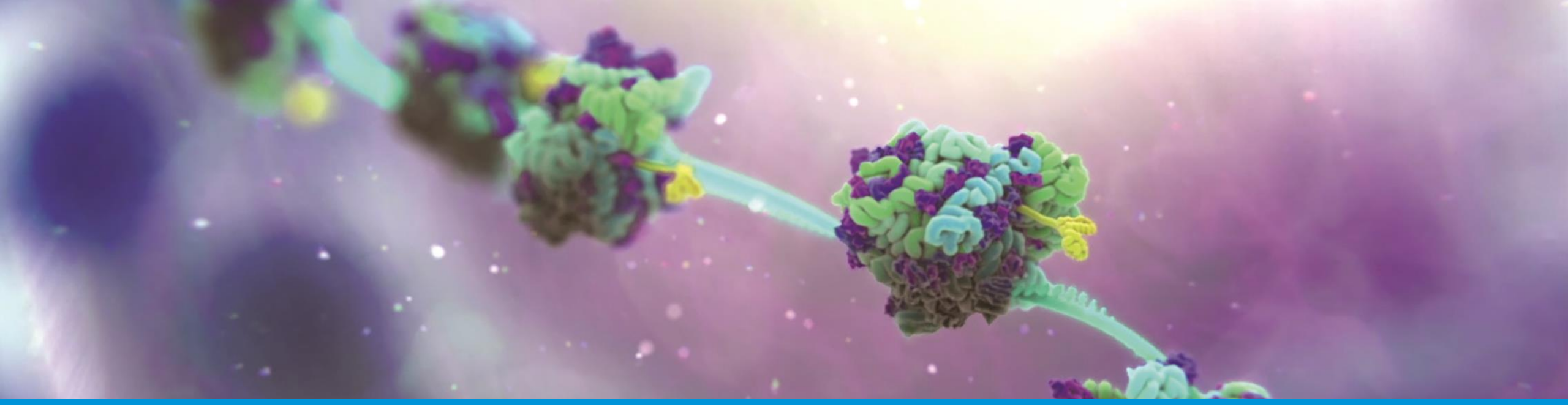

DNA-binding domains containing novel repeat sequences enable temperature-tunable gene editing in primary human cells

Yemi Osayame[1], Franklin Kostas[1], Mitchell Kopacz[1], Mackenzie Parmenter[1], Christopher B. Rohde[1], Matthew Angel[1]

[1]Factor Bioscience Inc., Cambridge, MA 02141

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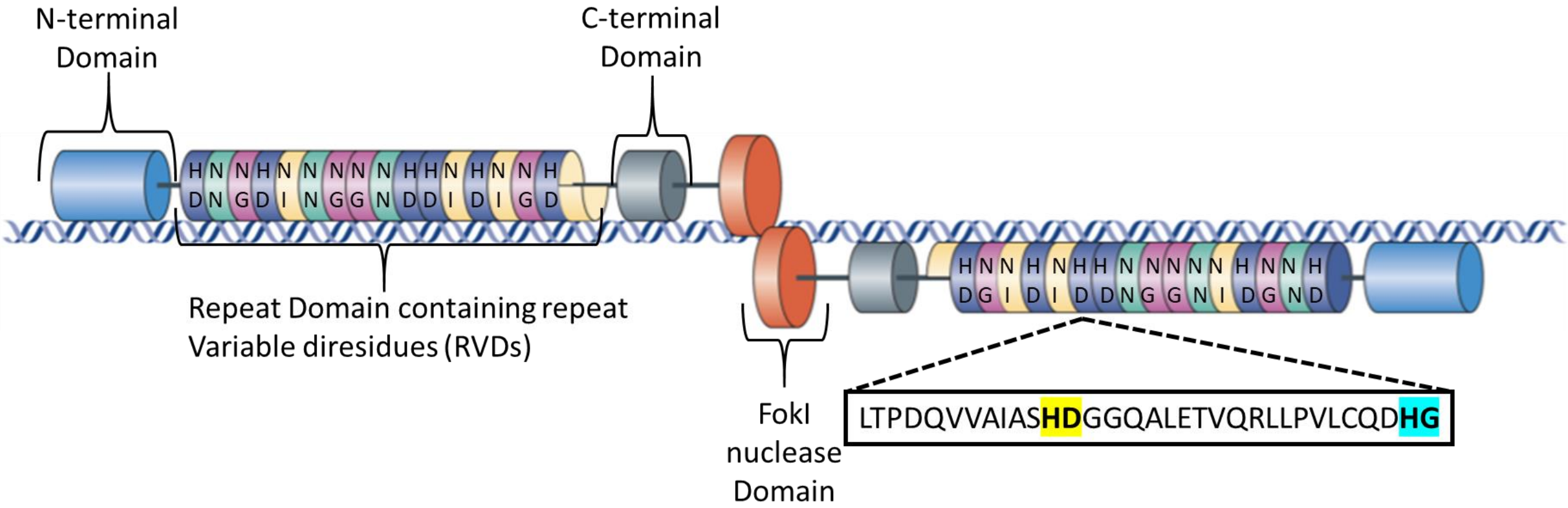
ASGCT 2021

05.11.21

Dimerizing gene editing proteins



Modified, Image taken from Joung, J. and Sander J. Nat Rev Mol Cell Biol, Vol 14 49-55, 2013.

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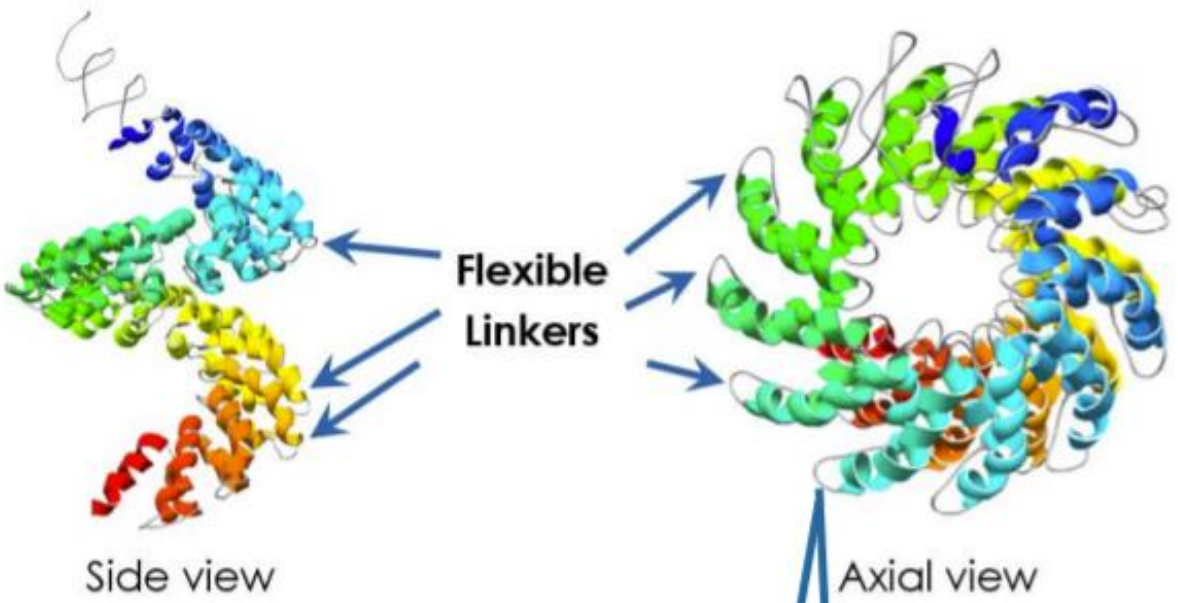
Methods

- Site directed mutagenesis
- Golden Gate Assembly
- mRNA Synthesis
- Cell Transfection
- Editing Efficiency via T7E1



Kopacz, M., et al. Mol Ther, Vol 28 No 4S1, 2020.

Linkers Tested
PHGP
GTHG *UltraSlice
GGGG
GAHD
THGG
GSGS
PHGG
GPHD
GKGG
GRGG
PHDG
AHDG
NHGG
GGHG
IVHG

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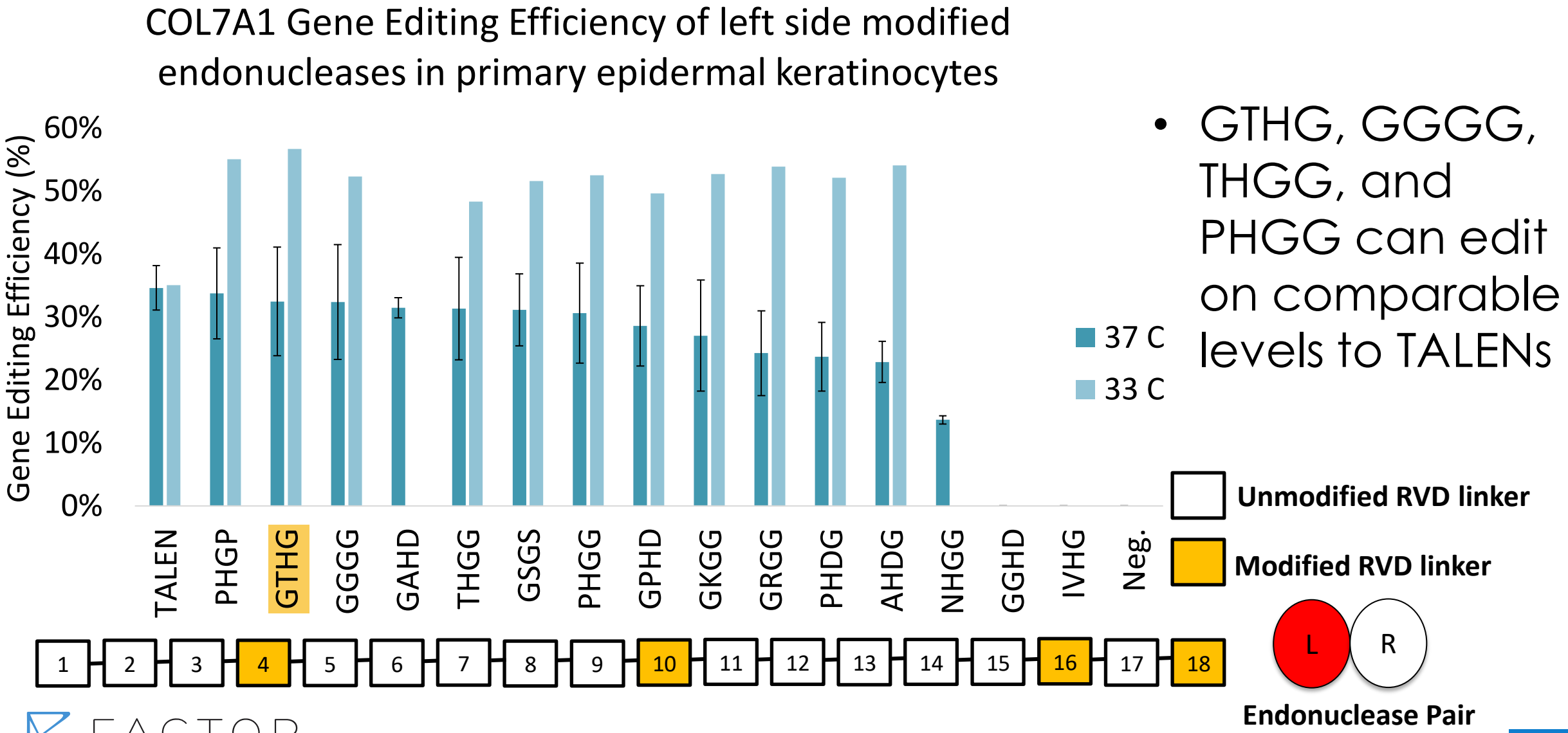
Novel link sequences yield temperature-dependent gene editing



- GTHG, GGGG, THGG, and PHGG can edit on comparable levels to TALENs

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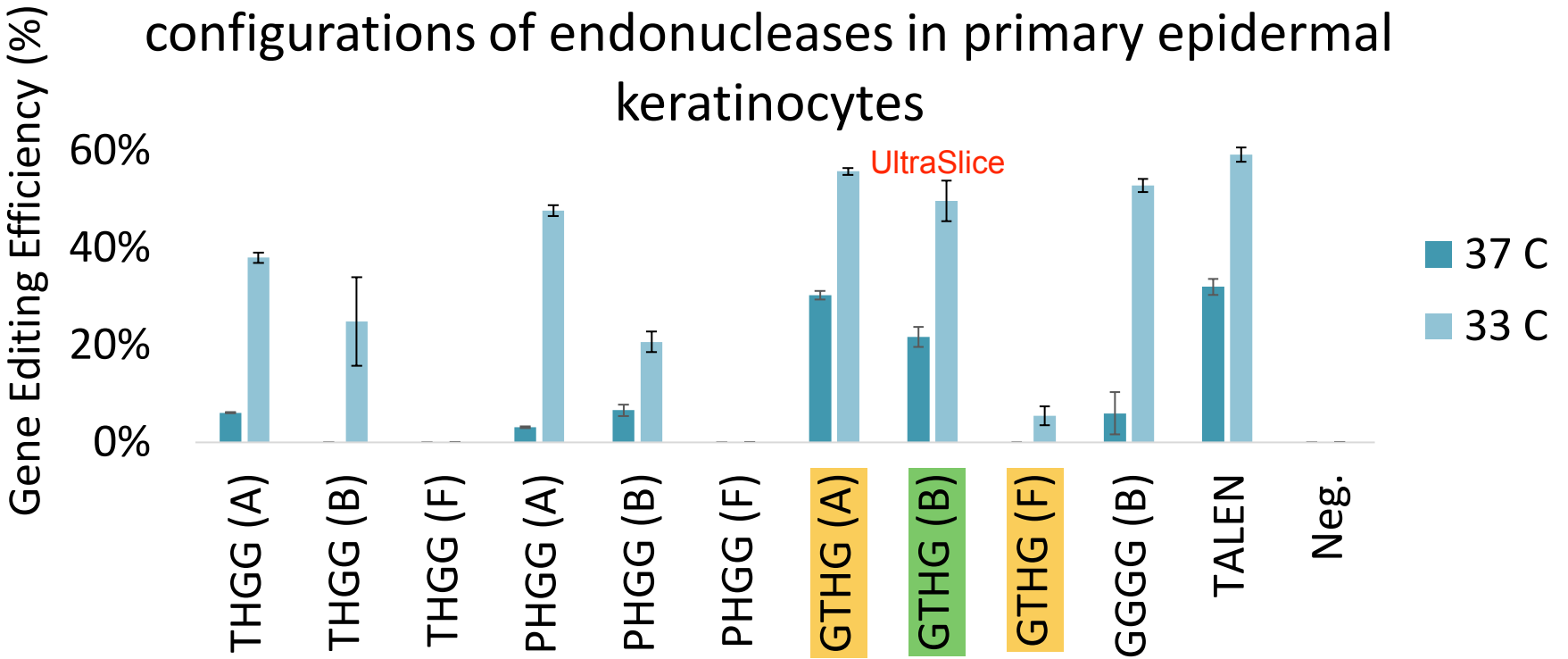
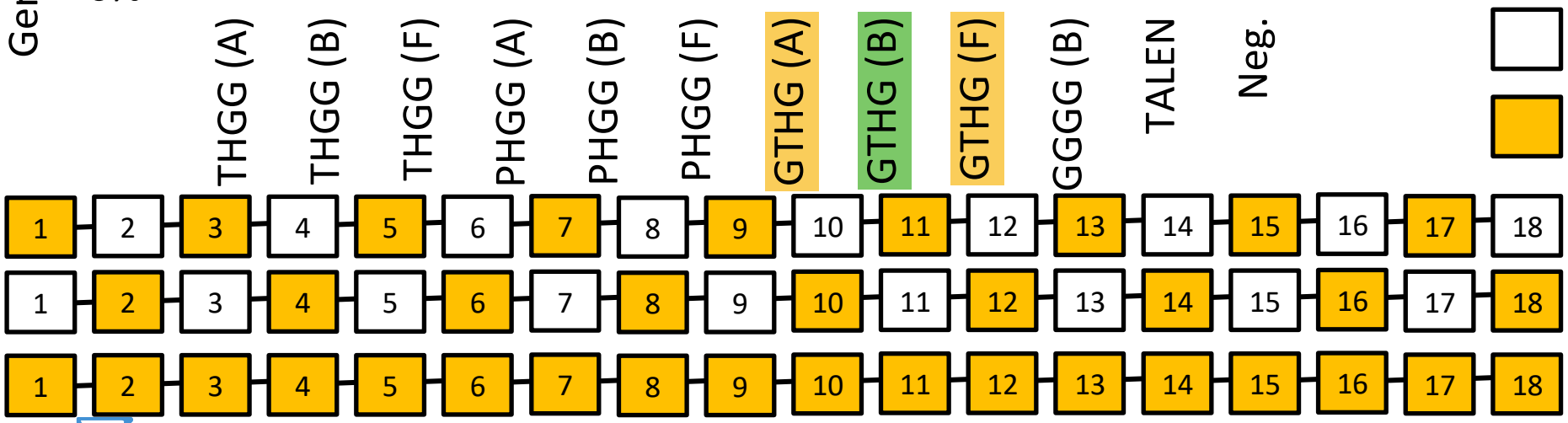
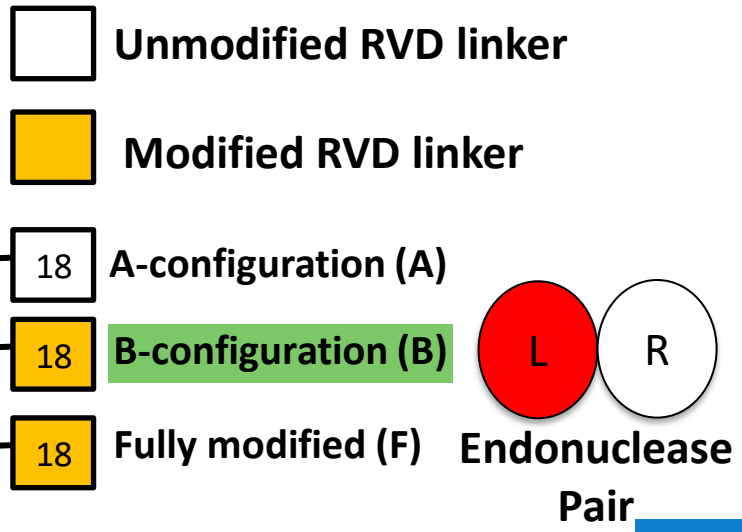
Novel link sequences with alternate configurations yield temperature-dependent gene editing





- Linker sequence and configuration impacts gene editing



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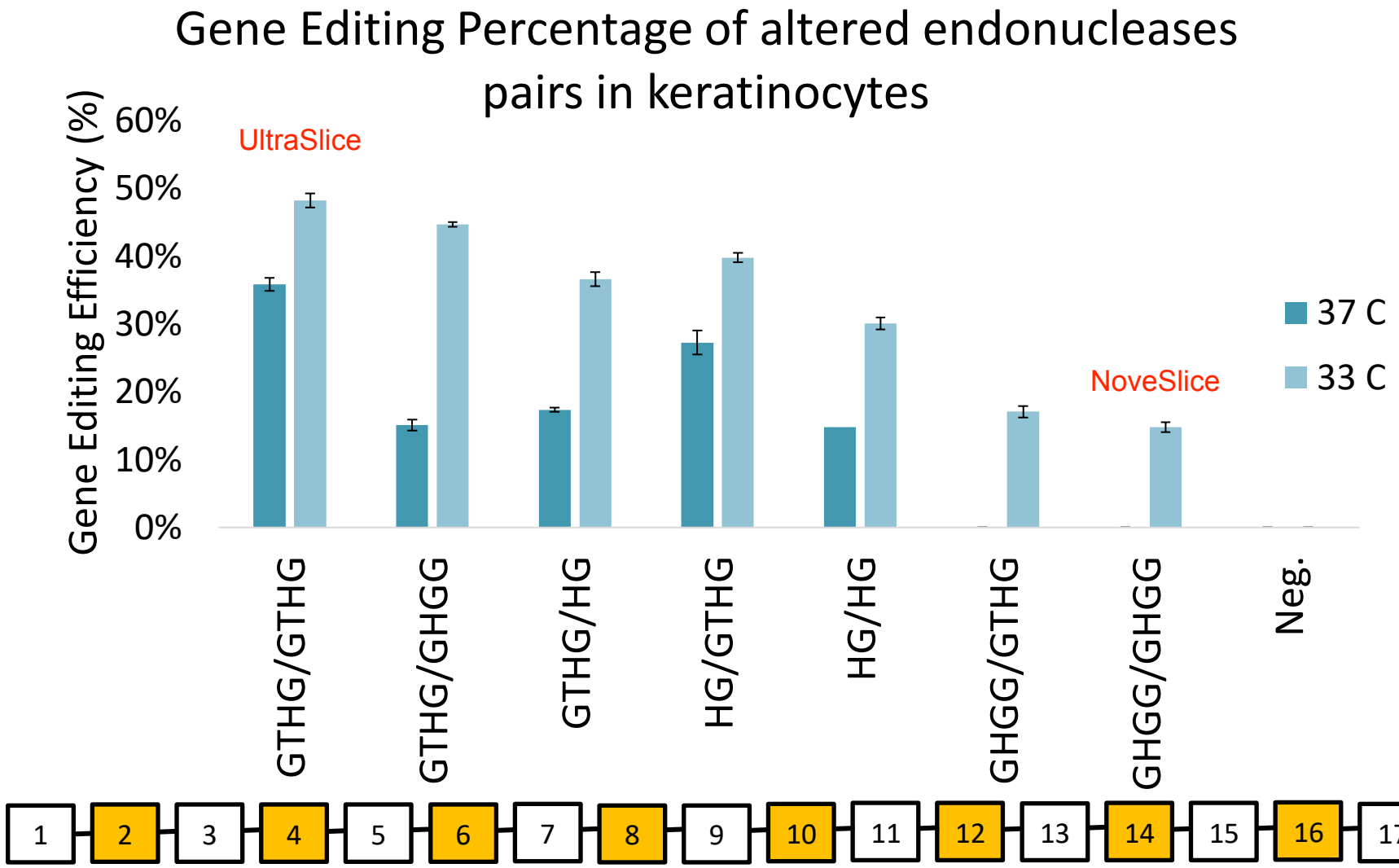
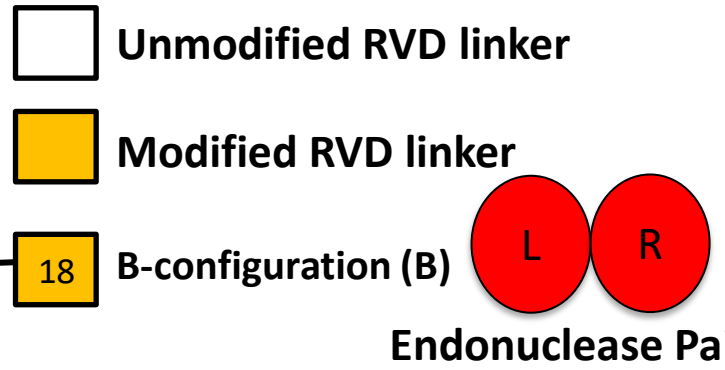
Modified linker pairs can enhance gene-editing efficiency



- Altering one side of an endonuclease pair can result in temperature selective gene editing



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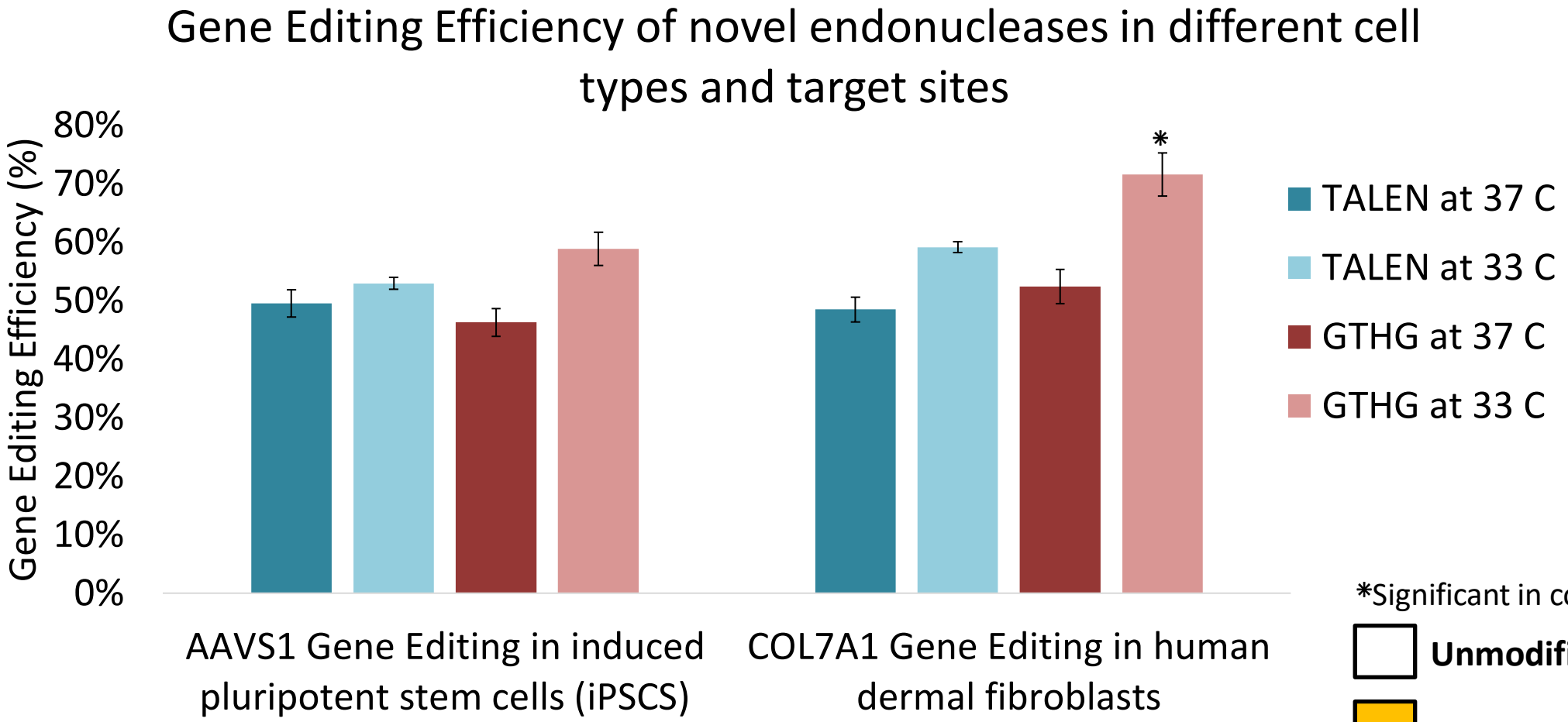
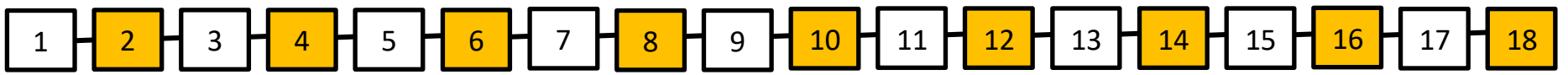
GTHG linked nucleases enable genome editing in several cell types and at multiple target sites



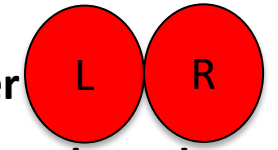
*Significant in comparison to TALEN control

Unmodified RVD linker

Modified RVD linker



B-configuration (B)



Endonuclease Pair

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Conclusions

- Library of different linkers can result in variable amounts of gene editing at different temperatures – "temperature tunability"
- GTHG linked repeats result in comparable or increased editing
- Linker modified endonucleases could lead to temperature specific therapies
  - Ex. Dystrophic Epidermolysis Bullosa (DEB)

Acknowledgements

- I would like to thank everyone at Factor Bioscience Inc, for their help and support.

*CR, MA, and FK are authors on several gene editing patents, include the repeat sequences disclosed here

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