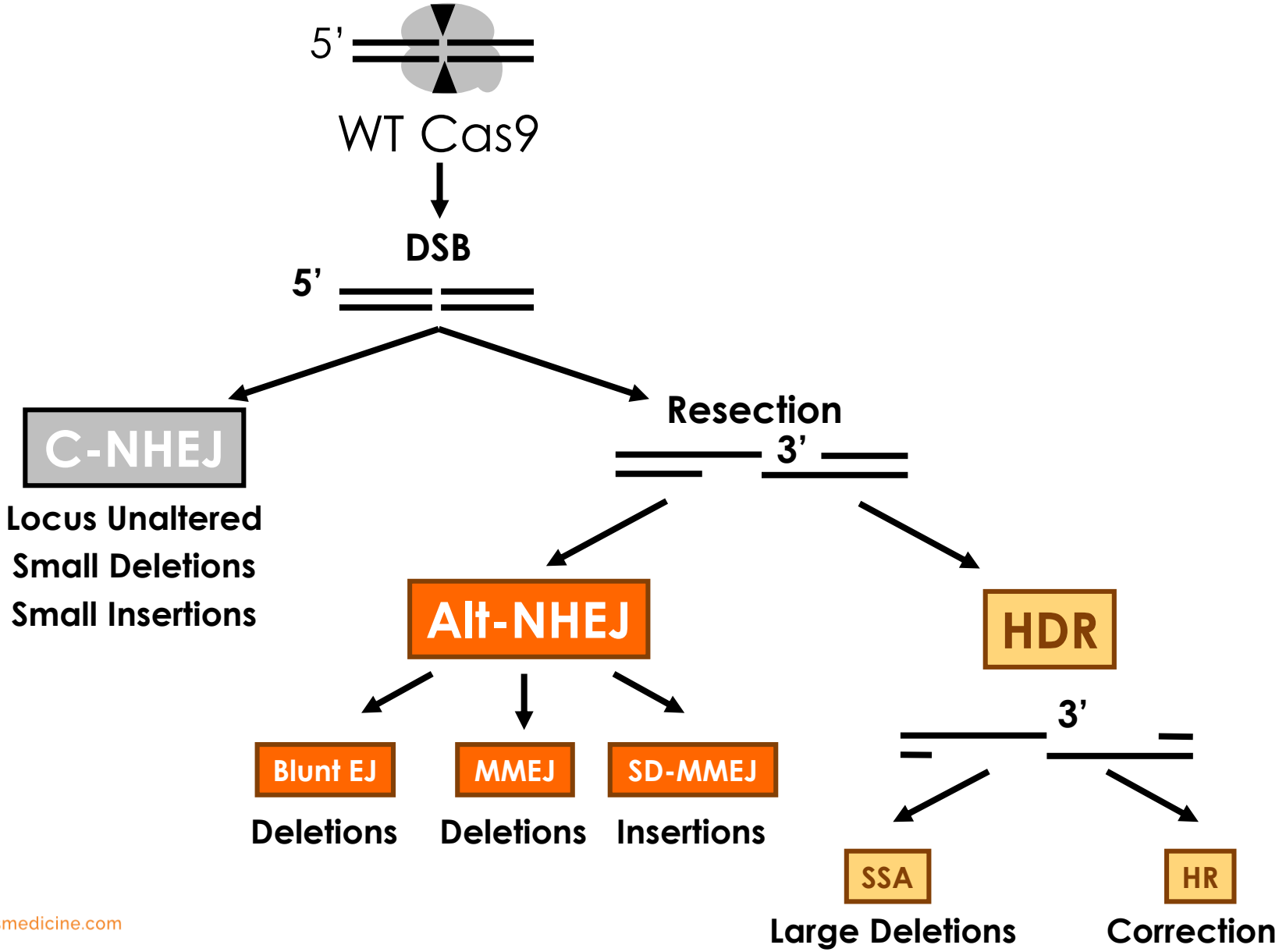
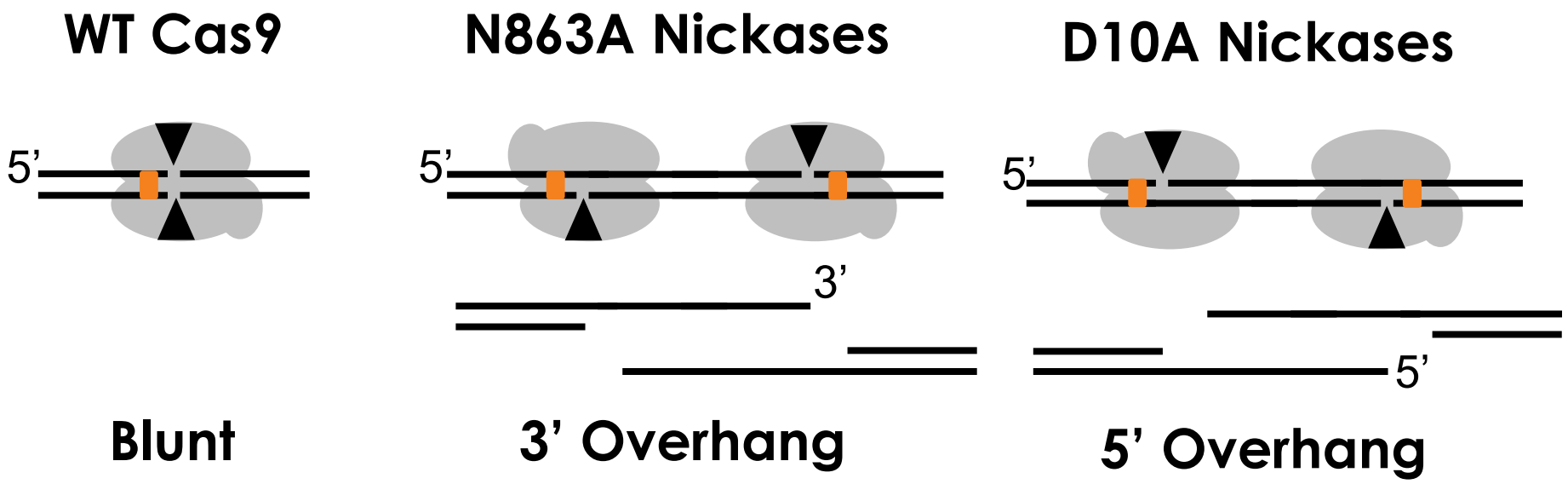
Effect of Different CRISPR/Cas9 Variants on Repair Pathway Choice

Cecilia Cotta-Ramusino

Cas9 Stimulates the Endogenous Repair Pathways

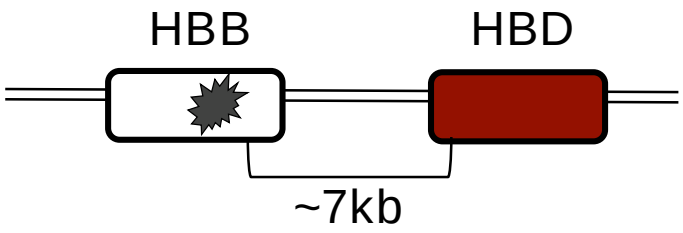


Cas9 is a Flexible Tool

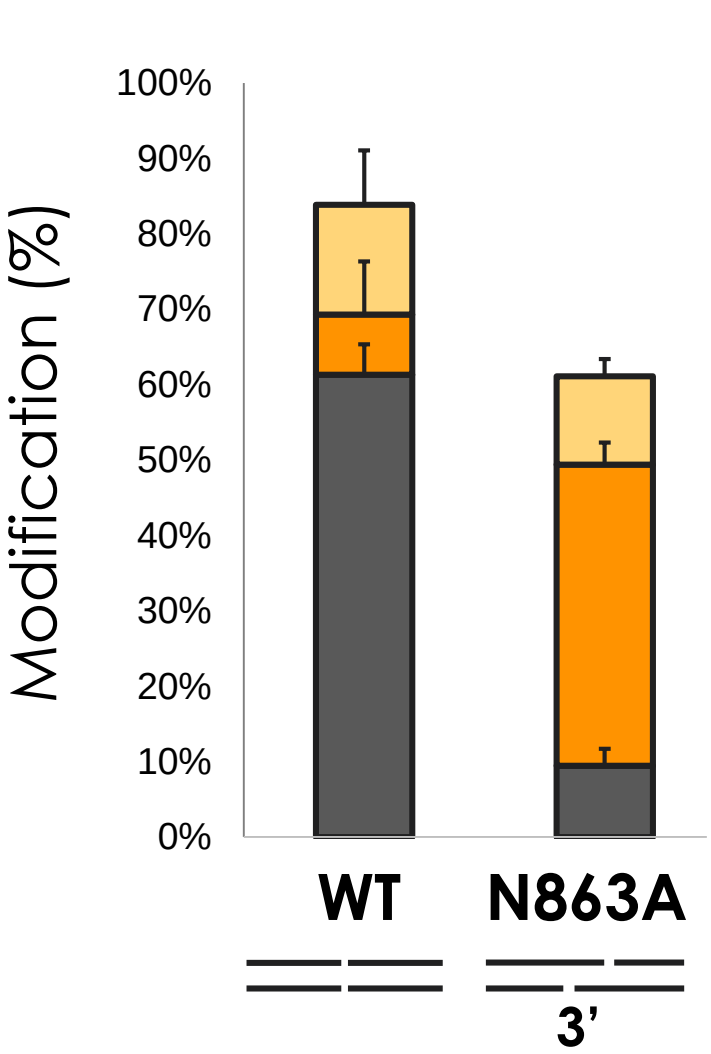


- Could we engage different pathways by using these different variants?
- Could we selectively stimulate HDR?

Sickle Cell Disease: Editing of the HBB locus



DSBs Generated by D10A are Predominantly Repaired by HDR



HDR
Insertions
Deletions

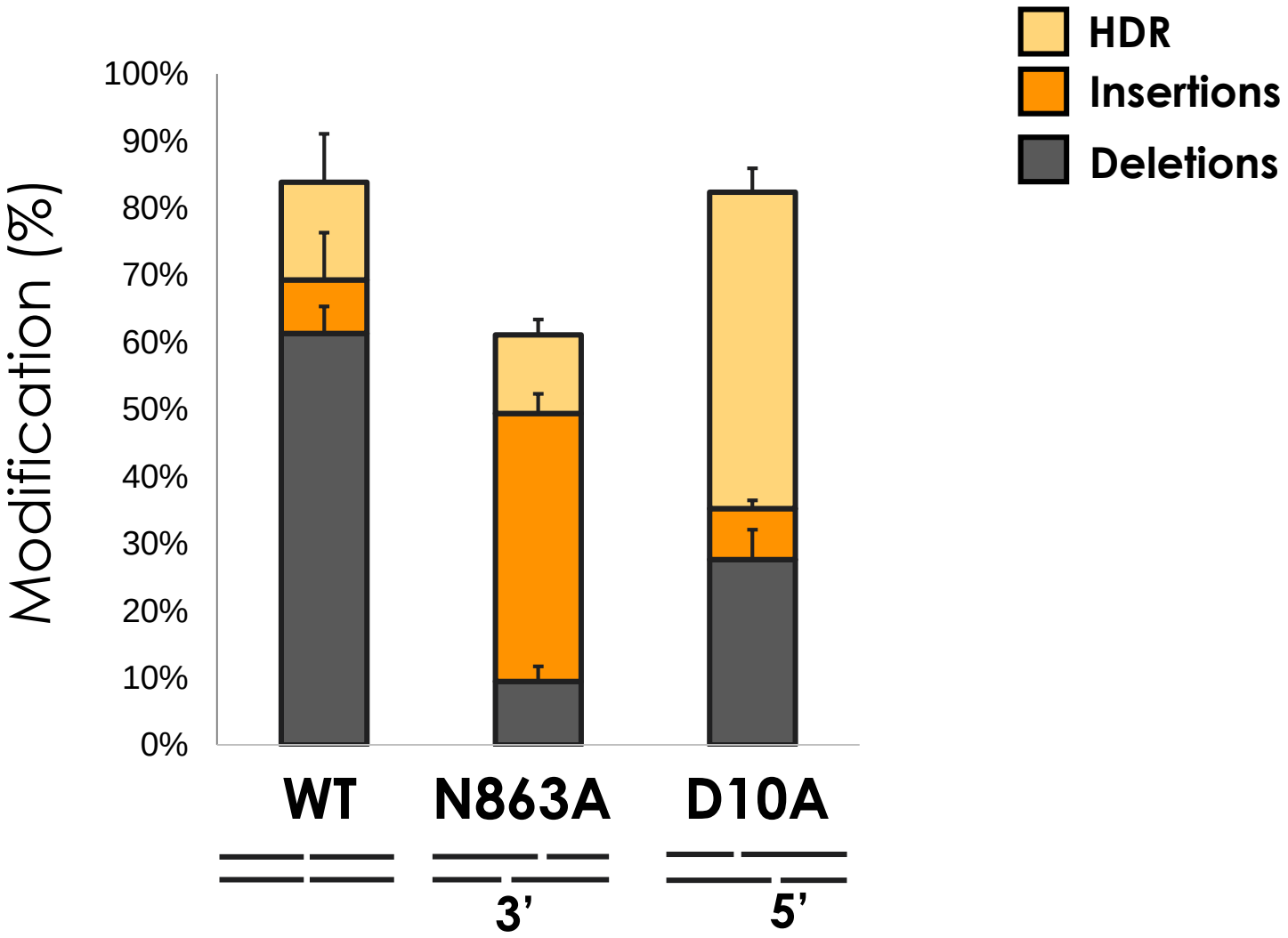
- Long insertions
- Repetitions of the overhang
- Microhomology mediated

Alt-NHEJ

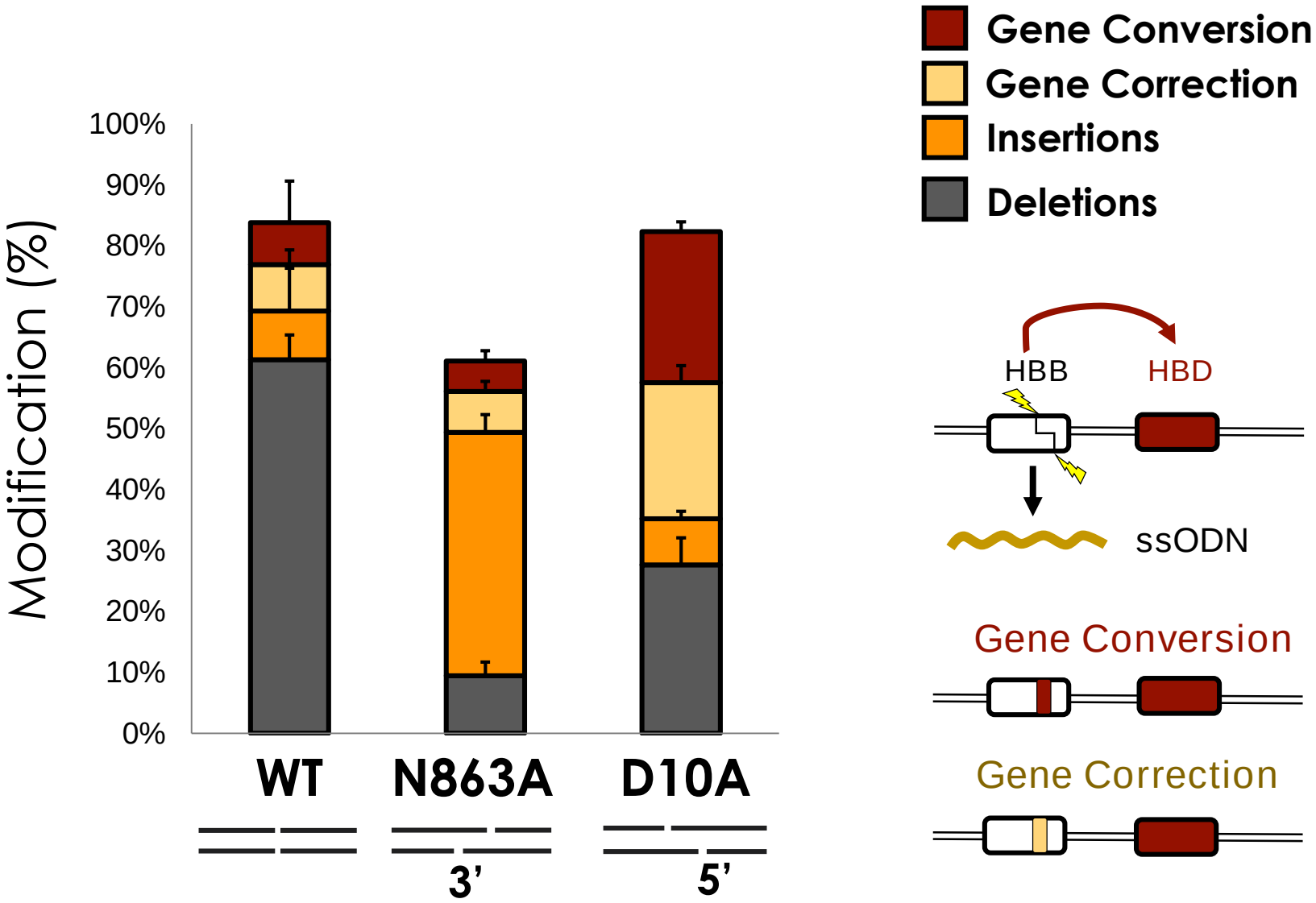
SD-MMEJ

Bothmer et al., Nat Comm 2017

DSBs Generated by D10A are Predominantly Repaired by HDR



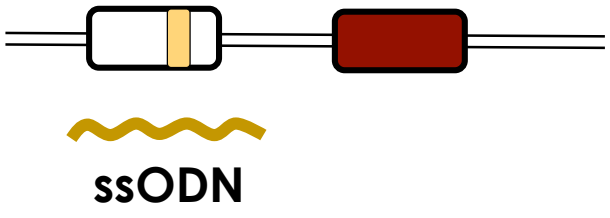
DSBs Generated by D10A are Predominantly Repaired by HDR



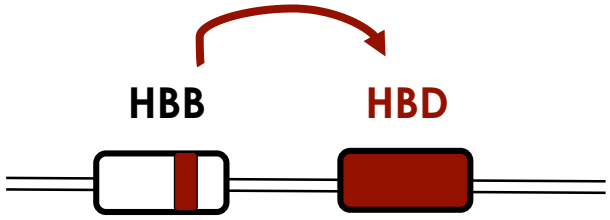
Bothmer et al., Nat Comm 2017

Do Gene Conversion and Gene Correction have the same Genetic Requirement?

Gene Correction

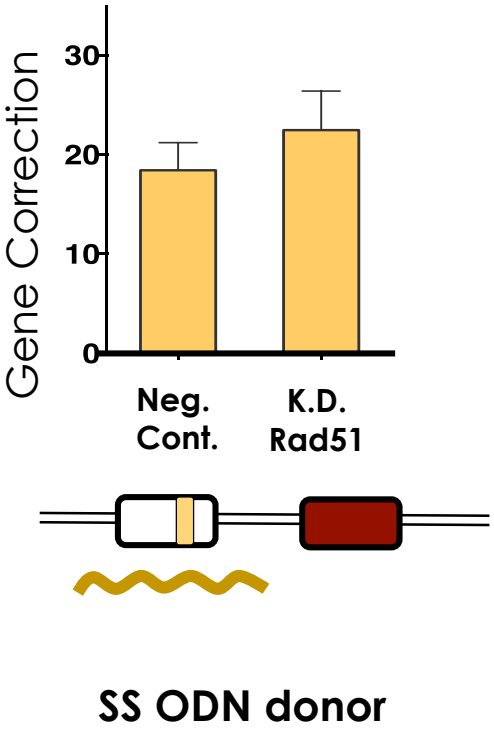


Gene Conversion

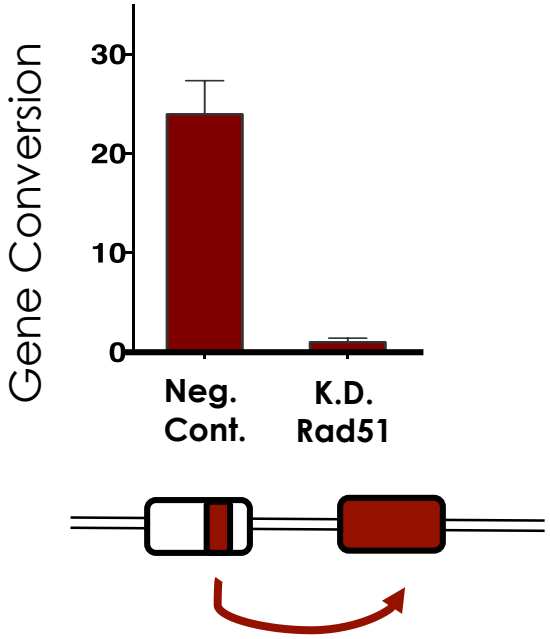


Do they both dependent on the HR pathway?

Gene Conversion and Gene Correction have Different Genetic Requirements

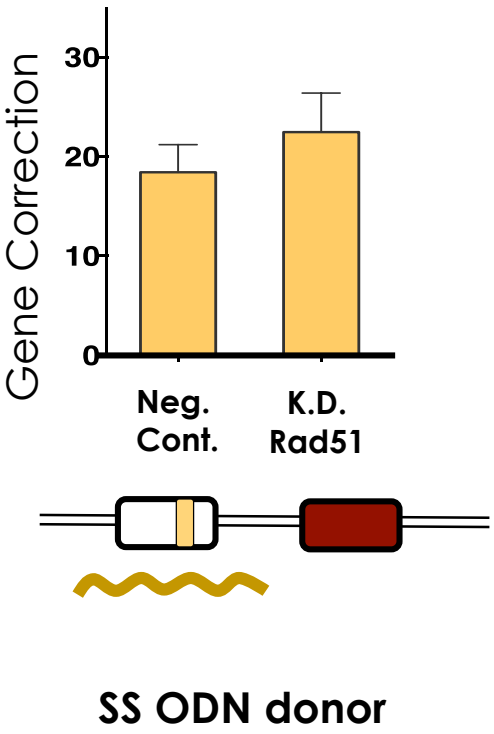


SS ODN donor

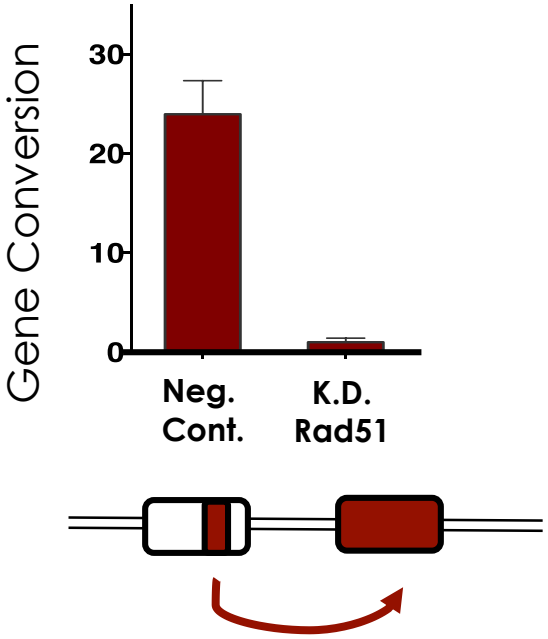


Endogenous HBD

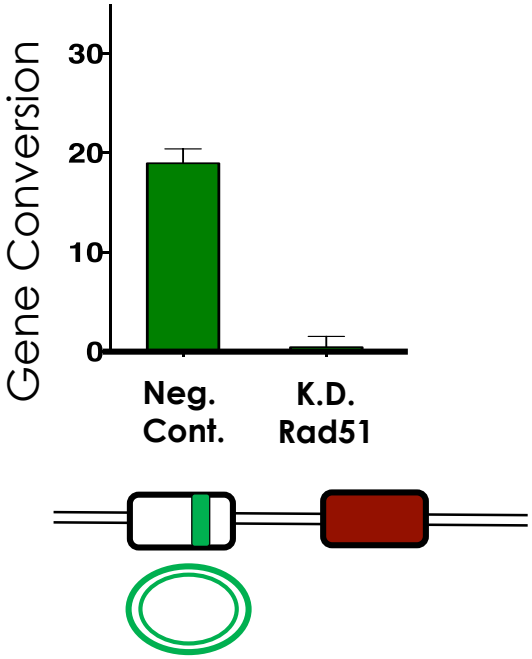
Gene Conversion and Gene Correction have Different Genetic Requirements



SS ODN donor



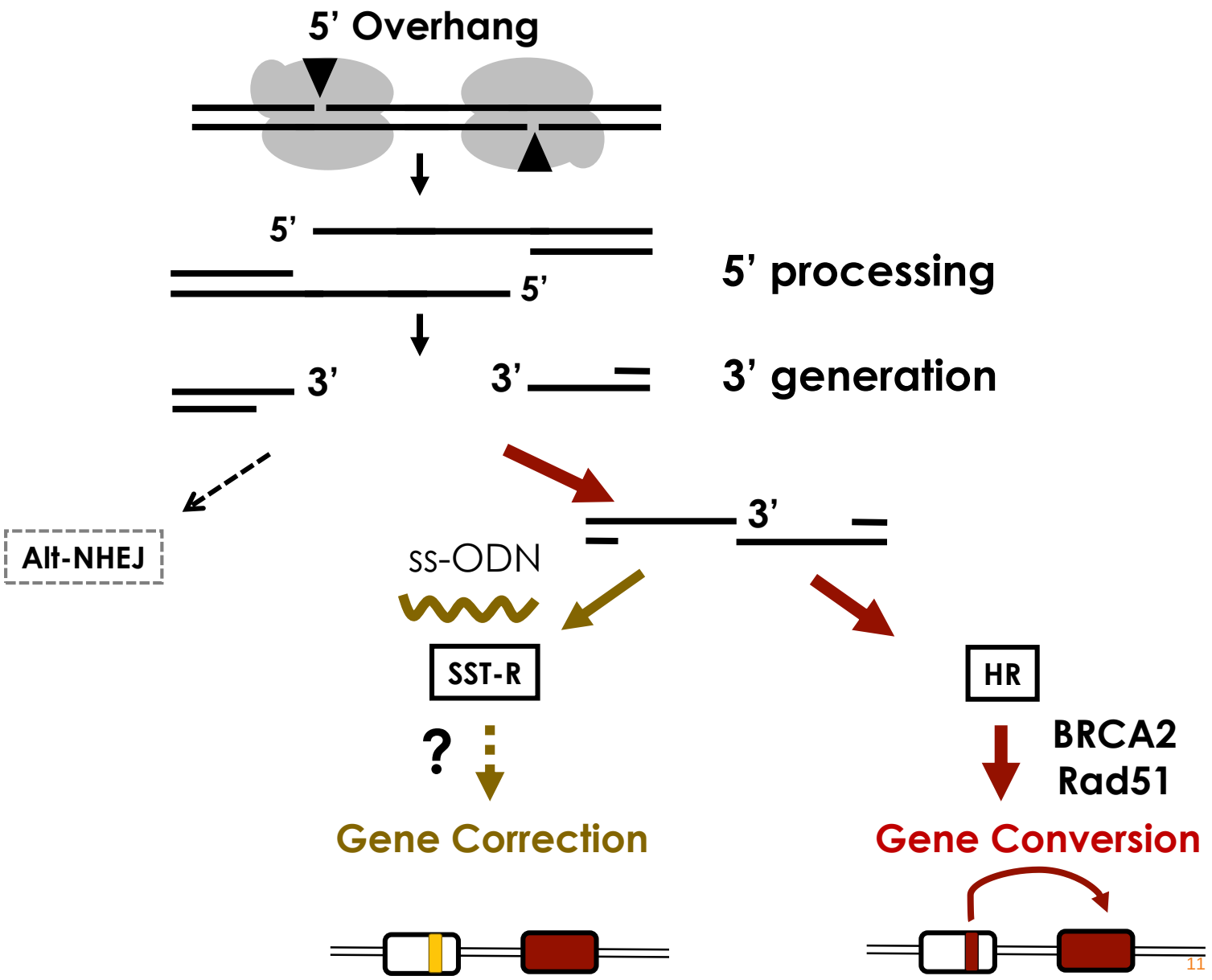
Endogenous HBD



Plasmid donor

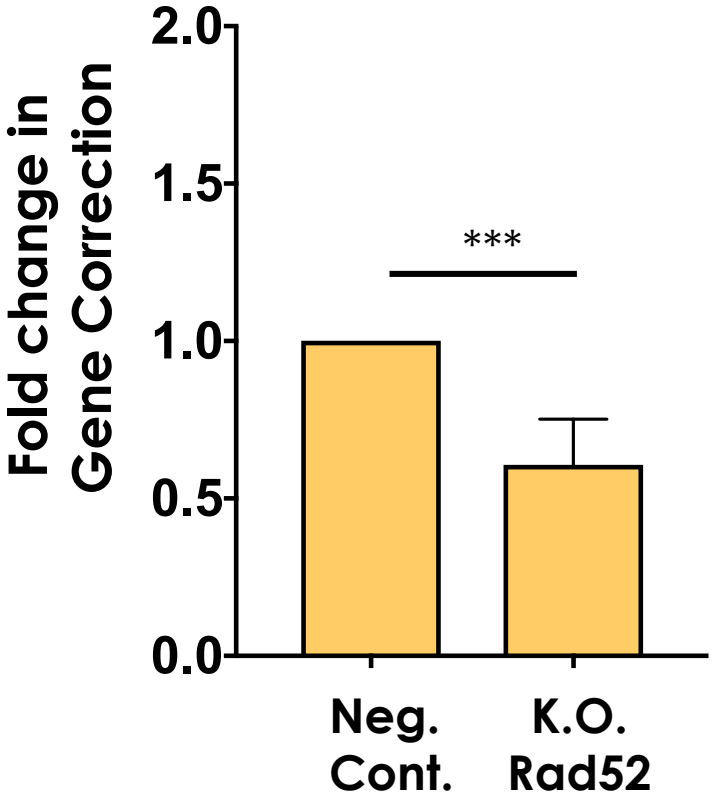
HR is required for repair from double stranded donors (endogenous homology tracks or plasmids) but not single stranded donors

3’ Overhang is Required to Promote Gene Conversion and Gene Correction

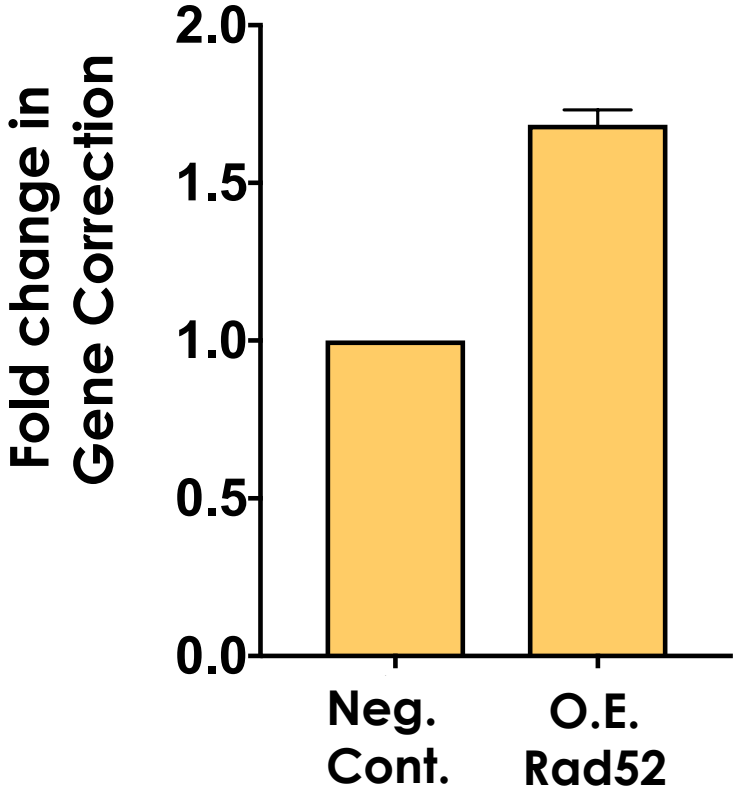


Rad52 has a Role in Promoting SST-R

K.O. of Rad52

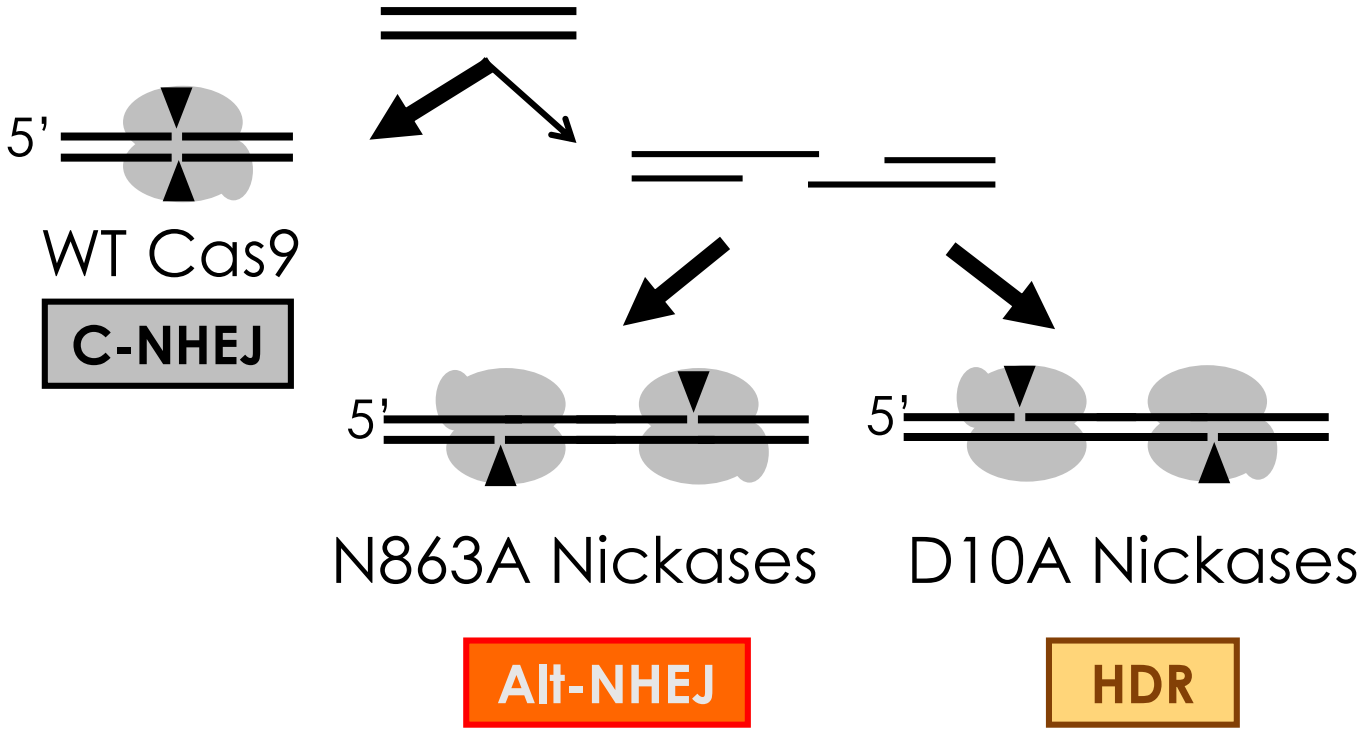


Overexpression of Rad52



Conclusions from the Dual Nick Analysis

- **Different ends activate different DNA repair pathways**

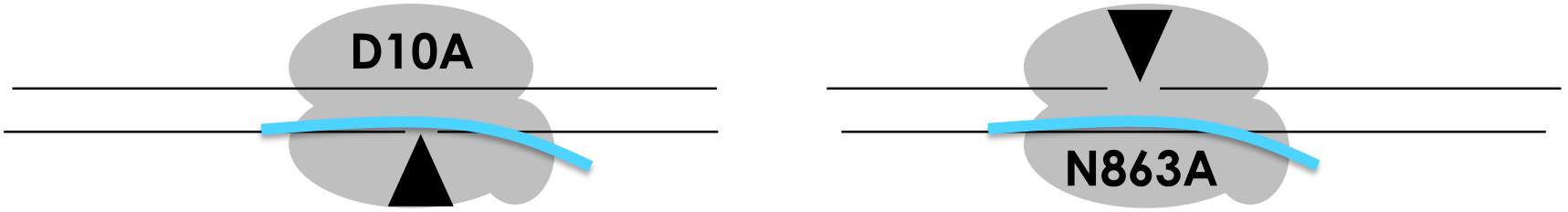


- **Different donors stimulate different pathways**

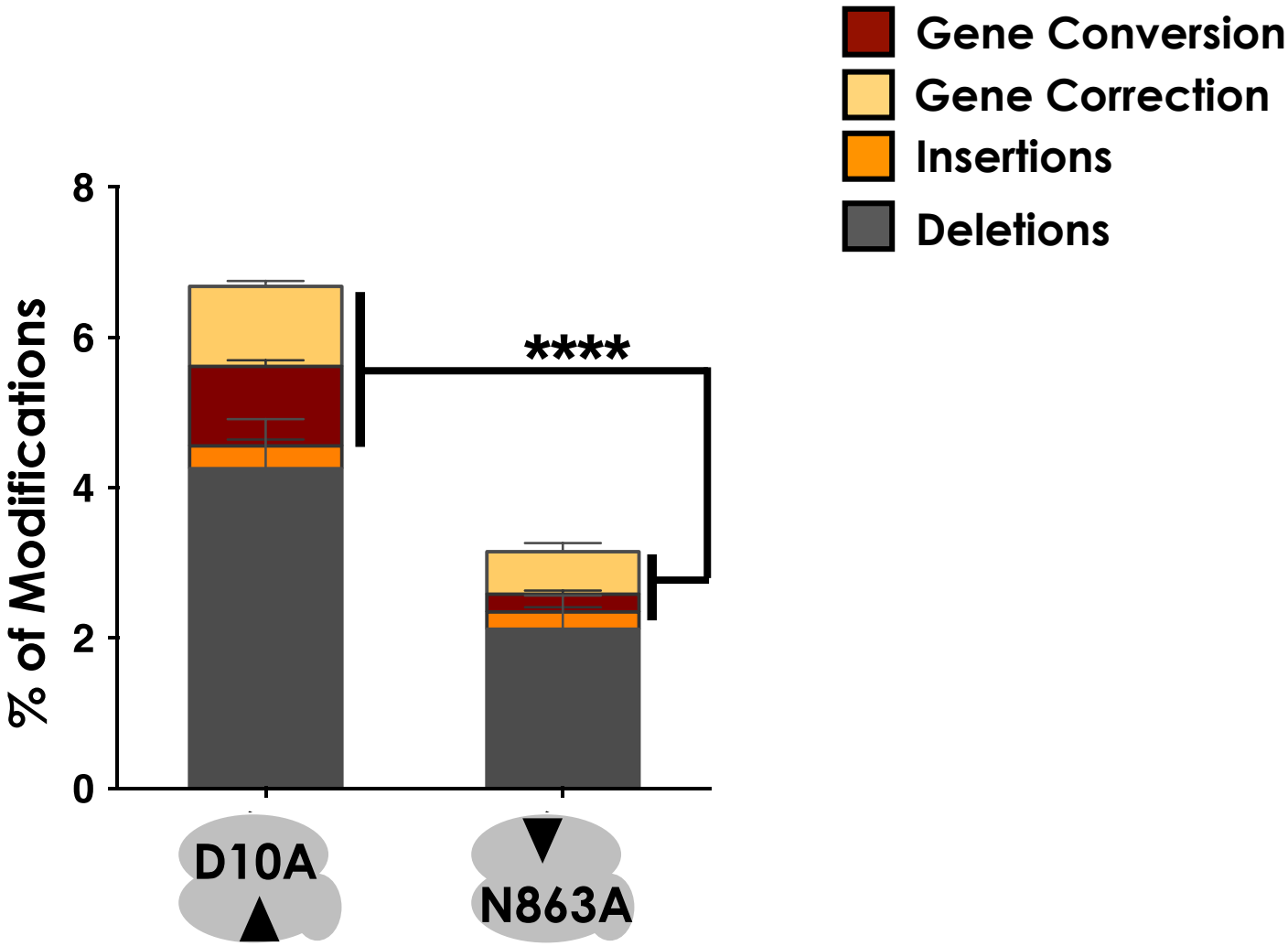
  Gene Correction mediated by ssODN is not HR dependent but partially depends on Rad52

Bothmer et al., Nat Comm 2017

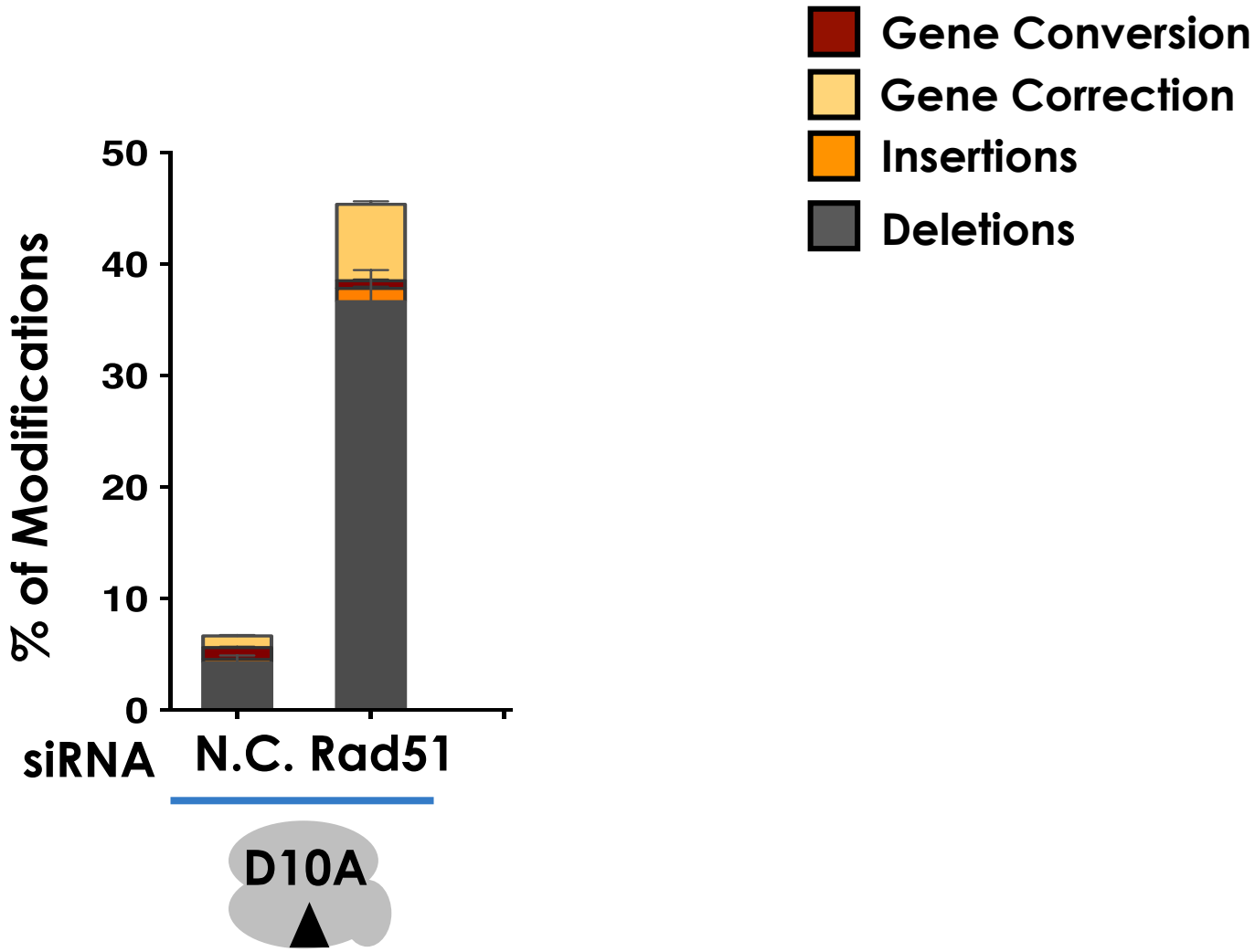
Characterization of the DNA Repair Pathway in Response to Single Nick



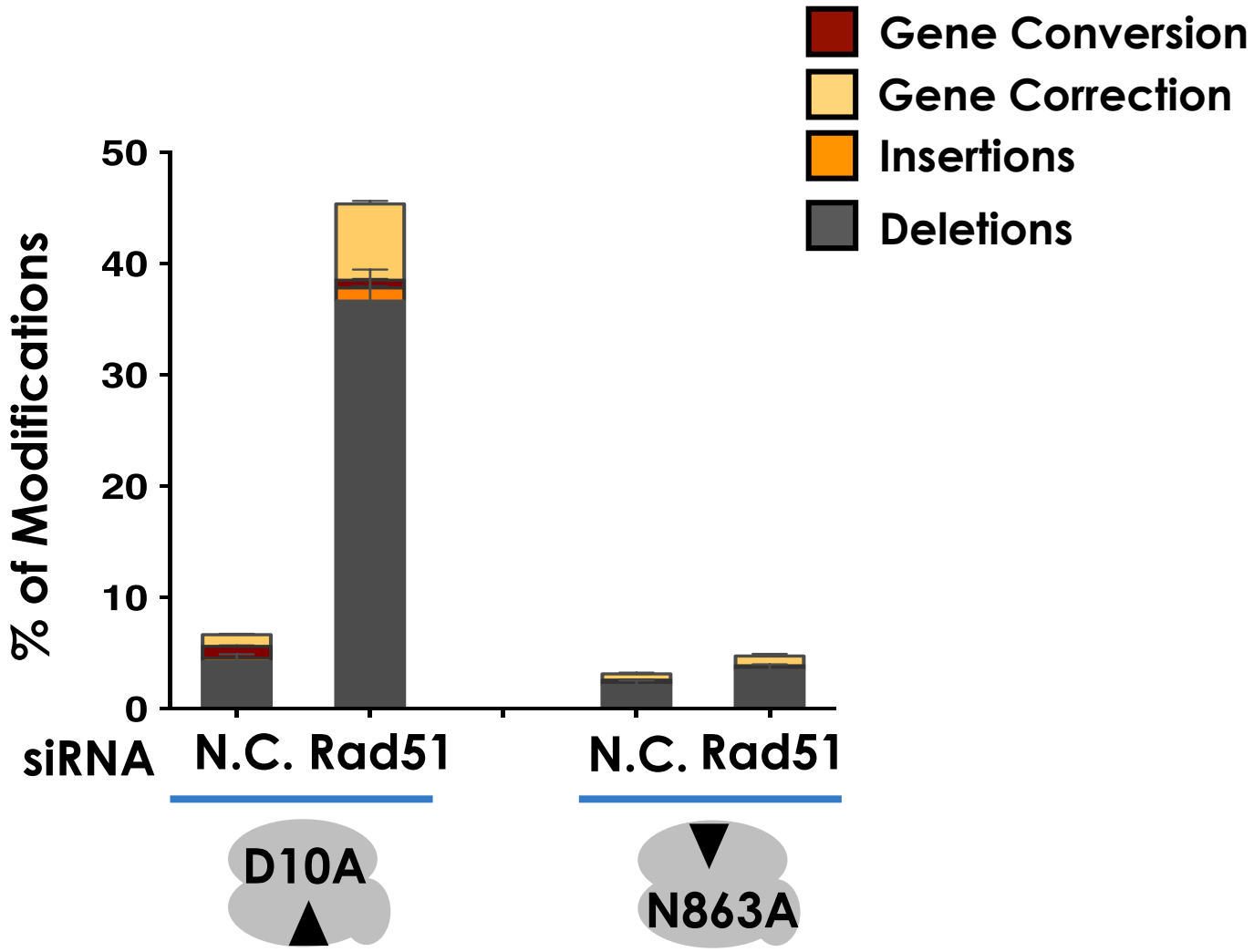
D10A Nick Results in More Frequent HDR Than N863A



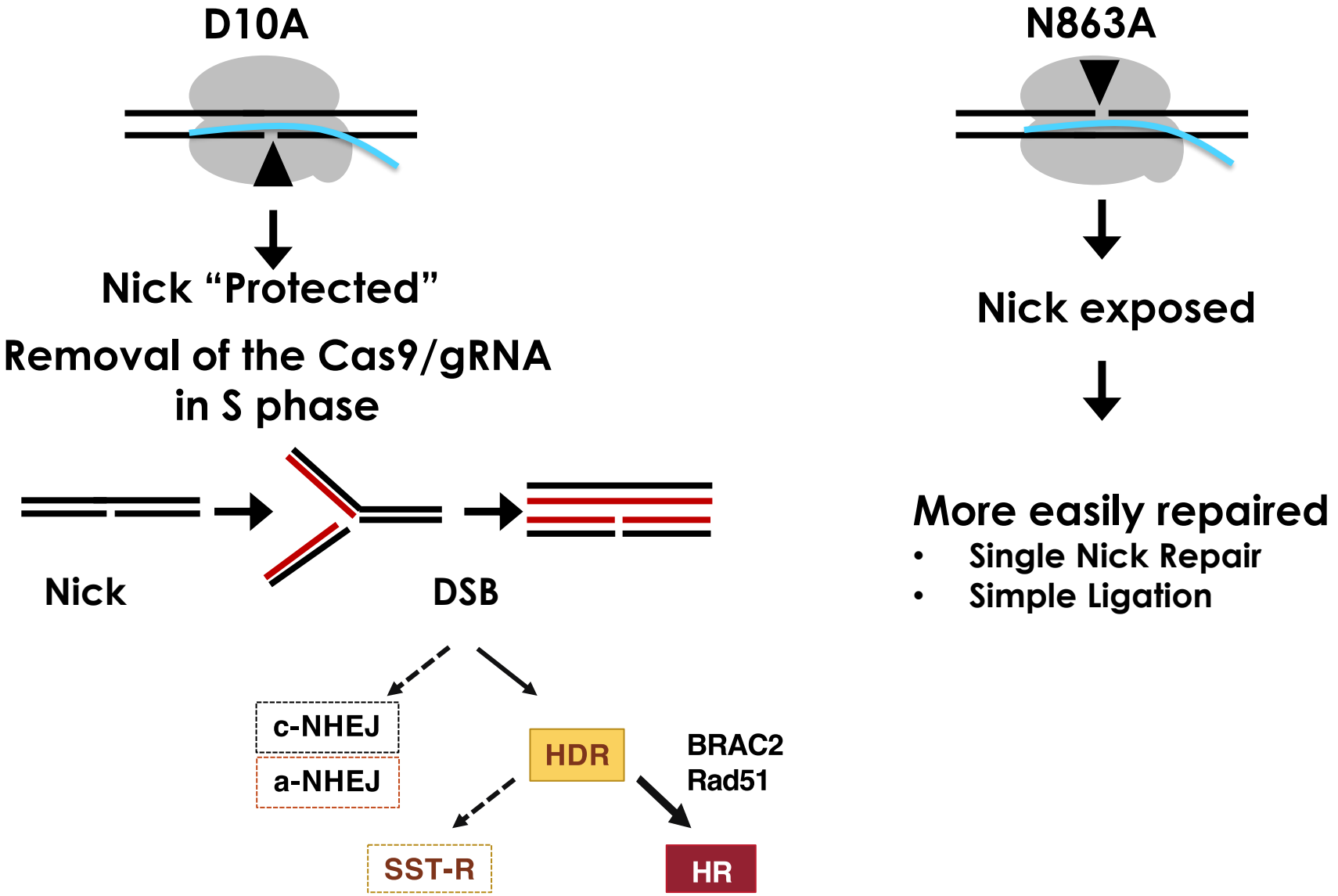
D10A but Not N863A Nick Repair Depends on HR Pathway



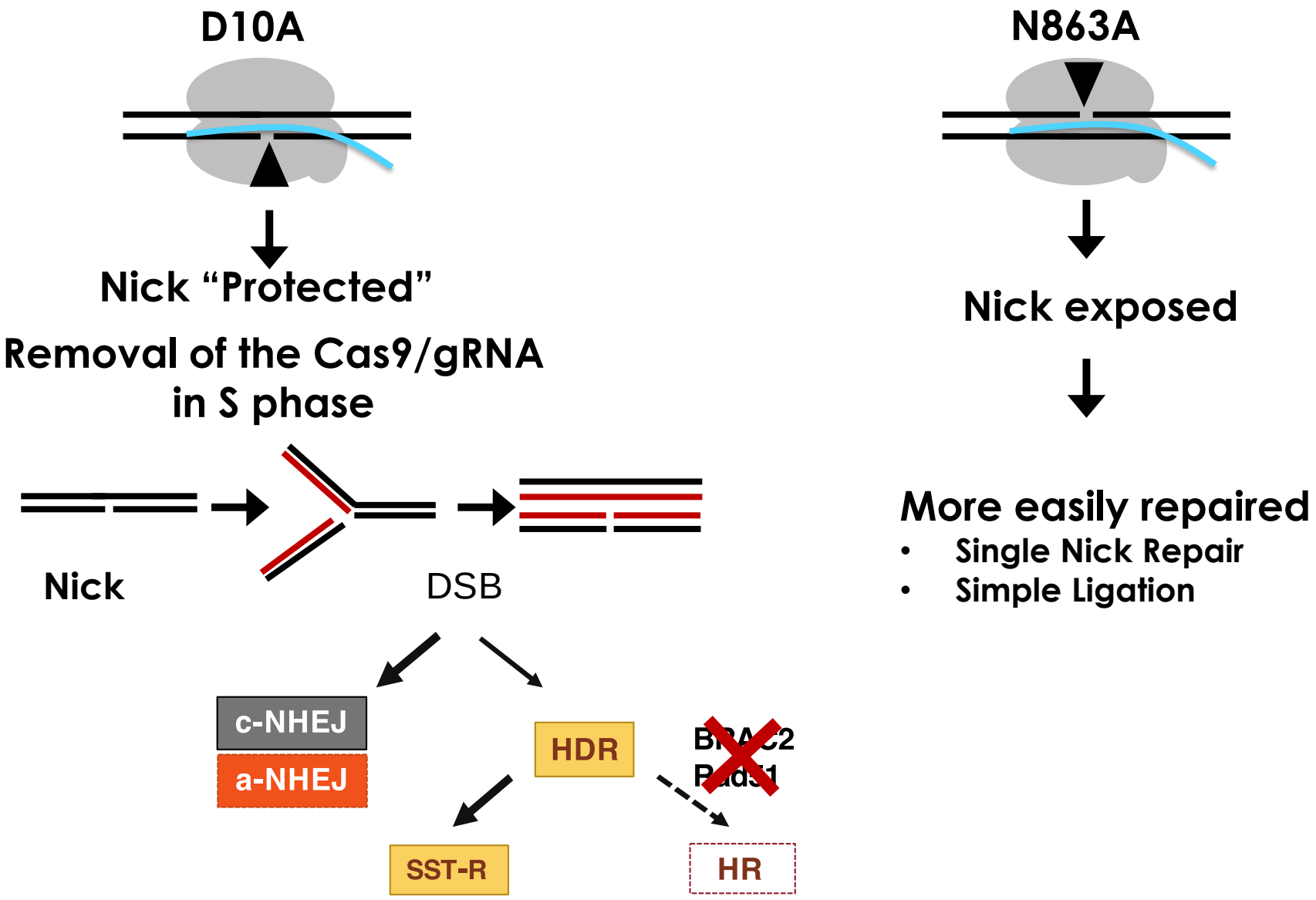
D10A but Not N863A Nick Repair Depends on HR Pathway



Working Model



Working Model



Overall Conclusions

- **Different lesions activate different repair pathways**
- **Different donors activate different repair pathways**
- **Understanding the differential pathway engagement allows for a deterministic approach in designing research and therapeutic genome engineering strategies**

Thank you!