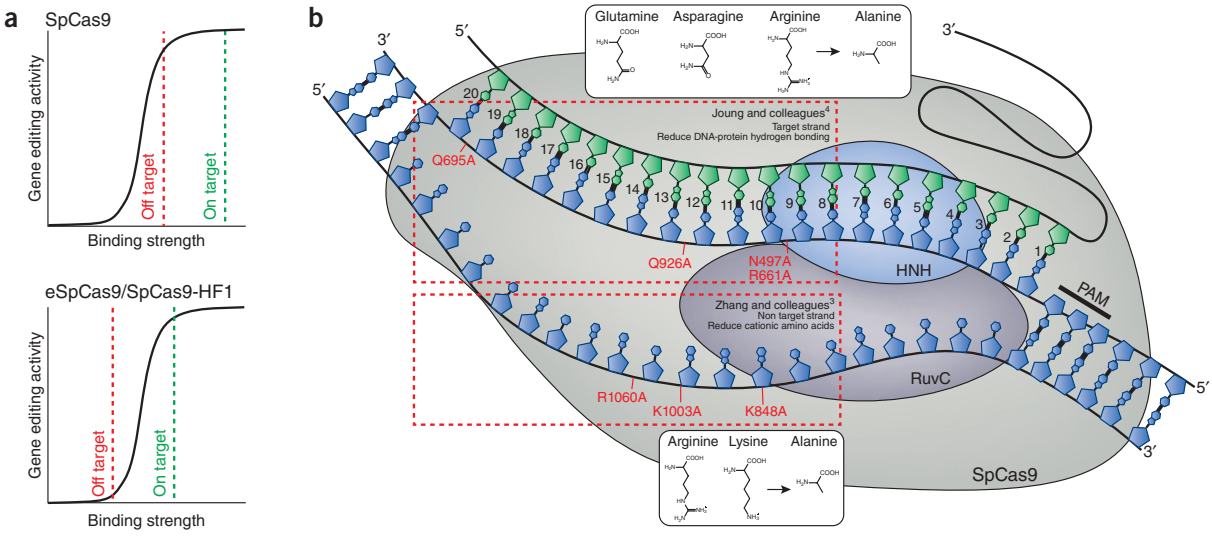

Figure 1 Engineering increased specificity into the Cas9 nuclease. (**a**) Wild-type Cas9 binds both on-target and off-target sites with sufficient energy for efficient gene editing, but decreasing overall binding strength can preferentially reduce activity at off-target sites. (**b**) Different regions of the Cas9 protein (HNH and RuvC) are altered to decrease nonspecific interactions with the DNA backbone. PAM, protospacer-adjacent motif.

may be sufficient[5]. However, additional rigor is warranted for therapeutic applications in which eliminating the potential for off-target modification of tumor suppressor genes or oncogenes is essential[2]. Regardless, the beauty of this work is the simplicity of using eSpCas9 or SpCas9-HF1 in place of wild-type SpCas9. There is no barrier to using these variants in any application. Future work will undoubtedly include expanding this approach to Cas9 variants from other species and to epigenome editing tools that use catalytically inactive Cas9 (ref. 8). This work was already begun by Zhang and colleagues[3] for the *Staphylococcus aureus* Cas9 (ref. 9), although a complete characterization of these variants remains to be performed.

The new SpCas9 variants are particularly remarkable in that no off-target effects could be found for several sgRNAs using unbiased genome-wide assays. However, an important caveat is the limitation of these methods for detecting low levels of off-target activity[5]. All of these assays depend on next-generation sequencing technologies, which have an inherent error rate that varies by target sequence but can be as high as 0.1% in many cases. Although off-target activity below this level is likely acceptable for many research uses, it could be a concern for therapeutic applications in which hundreds of millions of patient cells are being treated. Similarly, reducing the off-target activity below this level will be particularly valuable for *in vivo* genome editing in which available delivery methods lead to prolonged Cas9 expression[10]. Moreover, any differences between eSpCas9 or SpCas9-HF1, or any further improvements to their specificity, such as combining the amino acid substitutions in each variant, are unmeasurable by current techniques. Thus, increasing the sensitivity of methods for detecting off-target effects is an important goal for the field.

Diminishing off-target effects to below the detection limits of the most sensitive assays available was unimaginable even recently. These new advances promise even further progress toward clinical applications of Cas9 that require the utmost specificity.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.

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