



**SUPPLEMENTARY FIG. S6.**  $P_{cut}$  measurement informs biophysical explanations of cleavage outcomes. **(A)** Probability density functions of cleavage efficiency of Cas-bound and whole reaction controls differ. The histogram of Cas-bound cleavage efficiency is shown in blue, while the whole population is shown in orange. **(B)** Box plot of  $P_{cut}$  of single mismatches along the VEGFA target. Reactions were run at 10:1 RNP:target library for 30 min.  $P_{cut}$  demonstrates position dependence. **(C)** Measuring the efficiency of cutting in bound and unbound states informs limitations on Cas9 cleavage. Interpretation of Figure 5C.