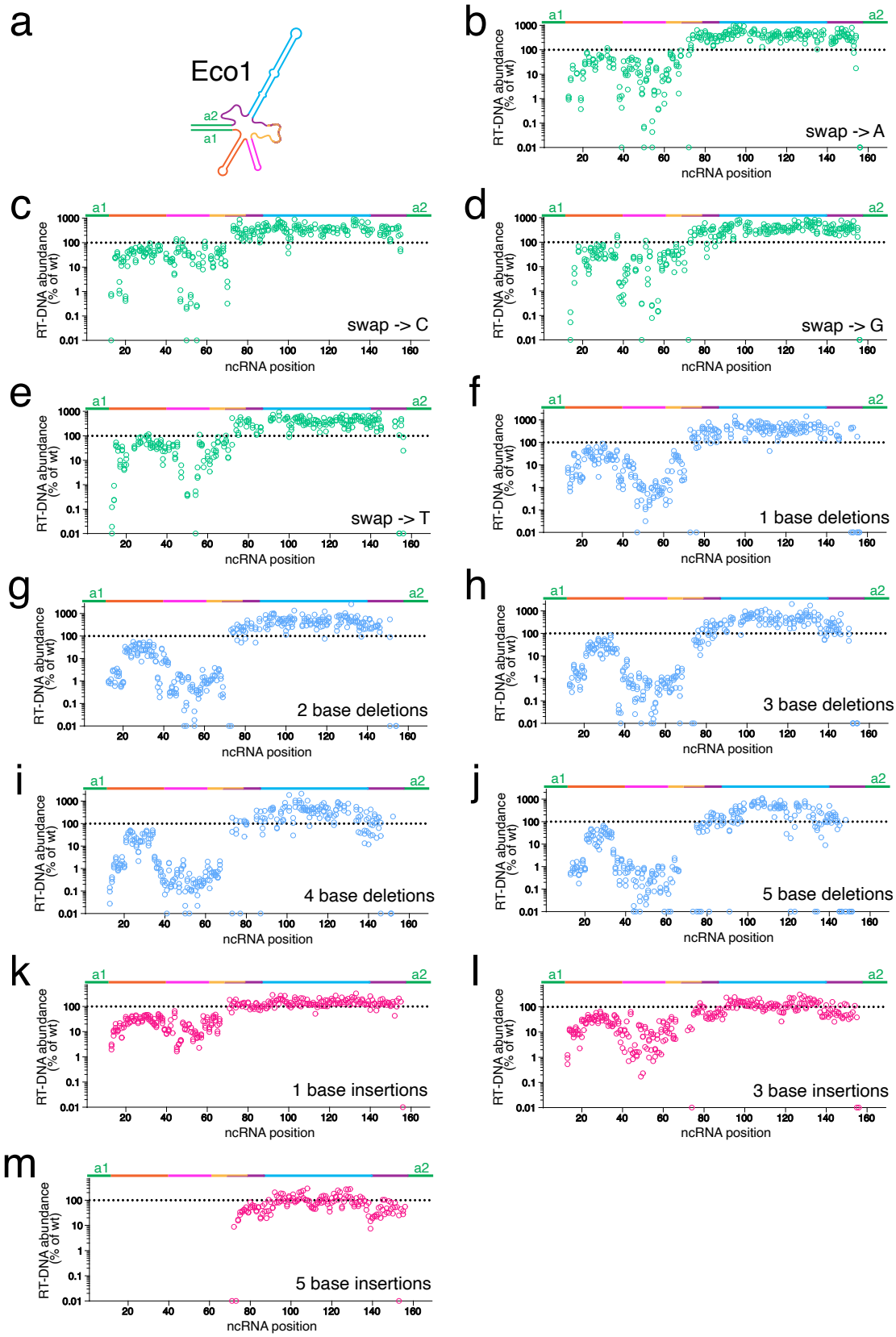
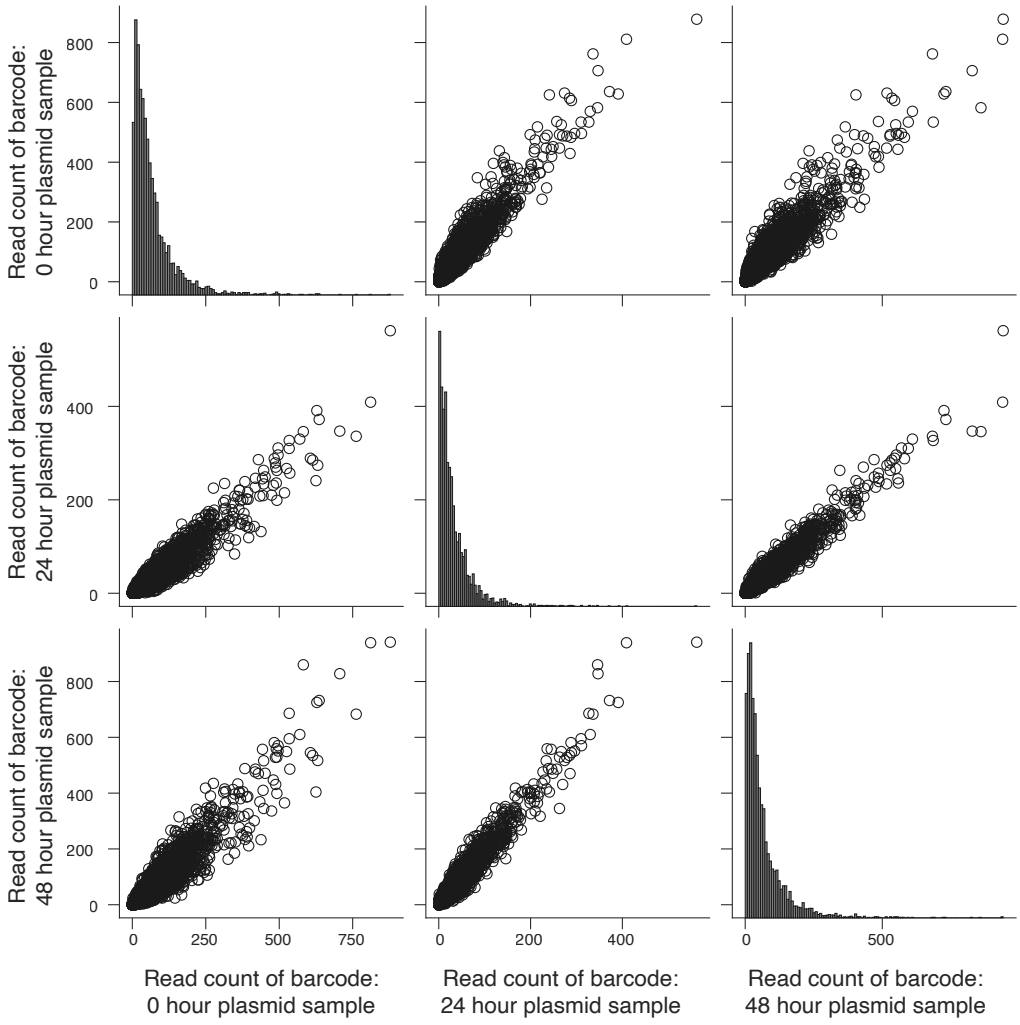
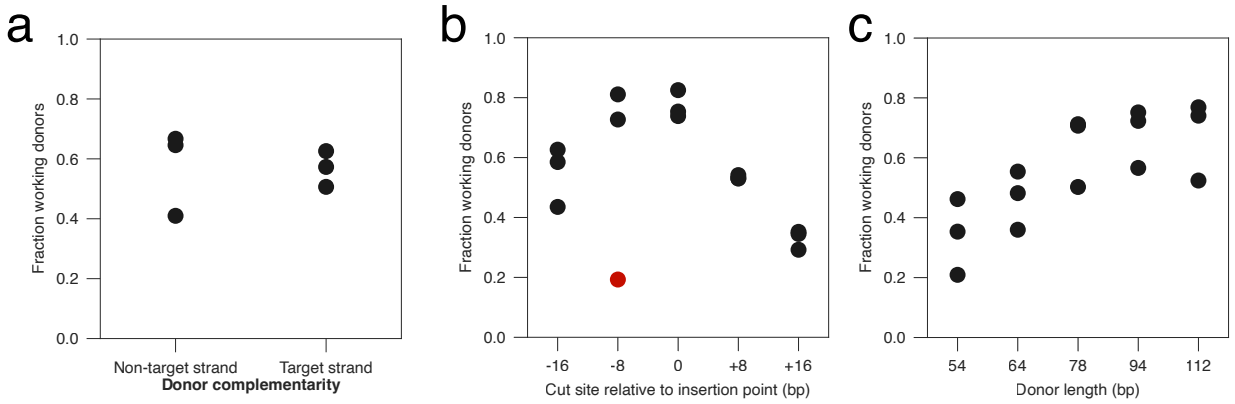
Supplementary Figures



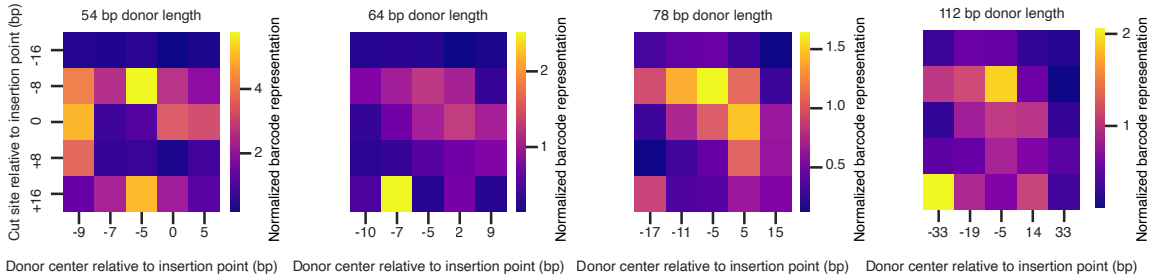
Supplementary Figure 1. Substitution, deletion, and insertion sub-library RT-DNA production in *E. coli*. **a.** Retron-Eco1 ncRNA structure. **b.** RT-DNA production of N→A nucleotide swap, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **c.** RT-DNA production of N→C nucleotide swap, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **d.** RT-DNA production of N→G nucleotide swap, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **e.** RT-DNA production of N→T nucleotide swap, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **f.** RT-DNA production of single-base deletions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **g.** RT-DNA production of two-base deletions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **h.** RT-DNA production of 3-base deletions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **i.** RT-DNA production of 4-base deletions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **j.** RT-DNA production of 5-base deletions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **k.** RT-DNA production of single-base insertions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **l.** RT-DNA production of 3-base insertions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate. **m.** RT-DNA production of 5-base insertions, starting at a specified ncRNA position relative to wild-type RT-DNA. Each open circle represents an individual biological replicate.



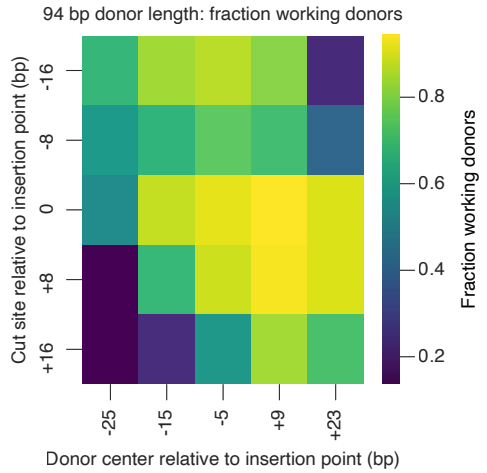
Supplementary Figure 2. Correlation in plasmid read counts over an example 48-hr editing window in *S. cerevisiae*. Correlation between individual plasmid barcode read counts at 0 hr, 24 hr, and 48 hr of editing for the first biological replicate of the site 1 library. Each open circle represents an individual barcode read count.



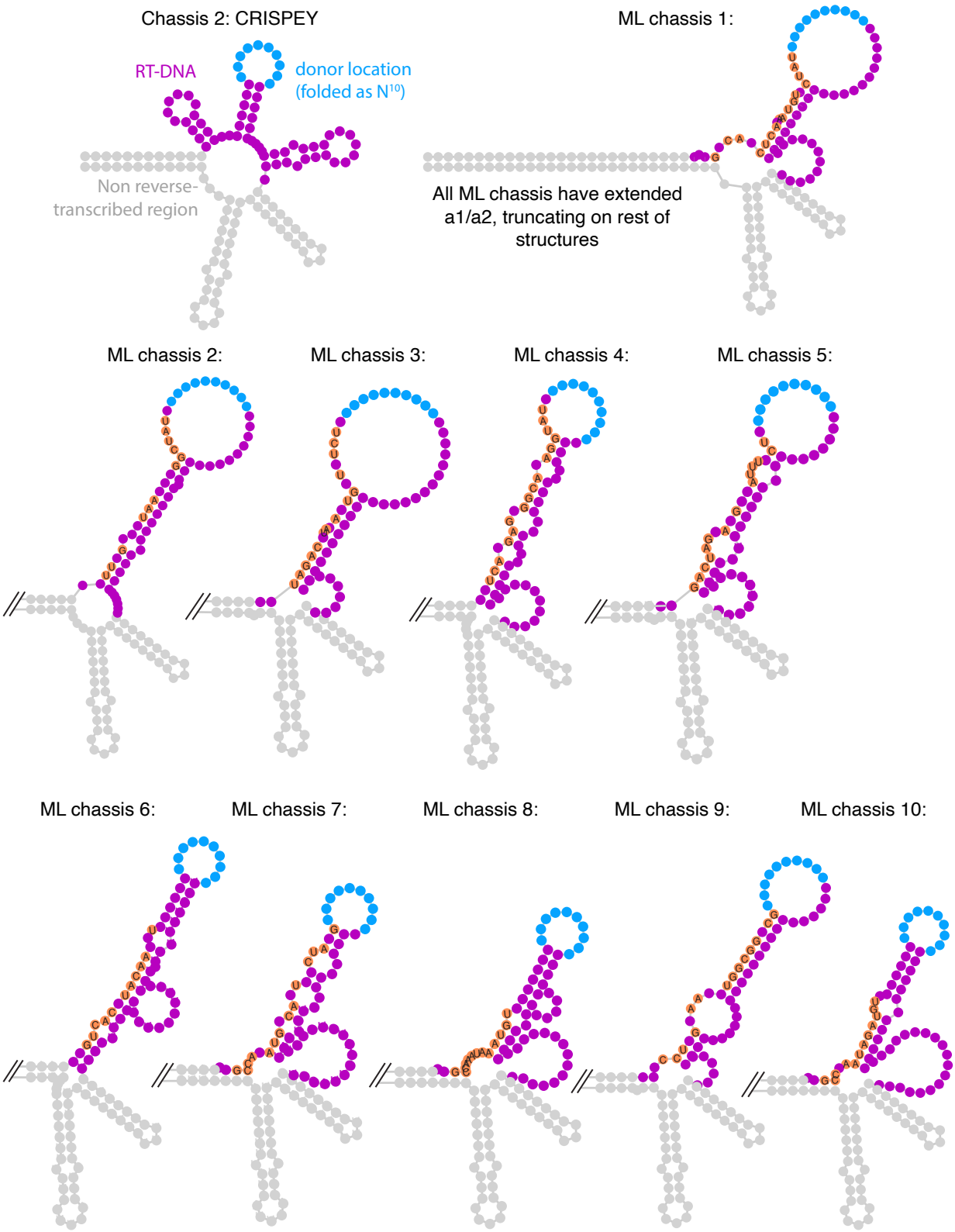
Supplementary Figure 3. Fraction working editors for different editing variables. a. Fraction working donors that are complementary to the non-target strand vs. target strand when reverse-transcribed into donor DNA. Each closed circle represents the mean of the three biological replicates for that site. **b.** Fraction working donors across all tested PAMs. Each closed circle represents the mean of the three biological replicates for that site. The red closed circle represents the PAM excluded from the analysis, as the fraction working donors is below 20%. **c.** Fraction working donors across all tested donor lengths. Each closed circle represents the mean of the three biological replicates for that site.



Supplementary Figure 4. Normalized barcode representation of donors of varying cut sites vs. donor centers in *S. cerevisiae*. Heat map of normalized barcode representation of cut site vs. donor center (54, 64, 78, and 112 nucleotide donor length), normalized to the cut site at the barcode insertion site, and donor center of -5 bp from the barcode insertion site. Each square represents the mean of all biological replicates across all sites.

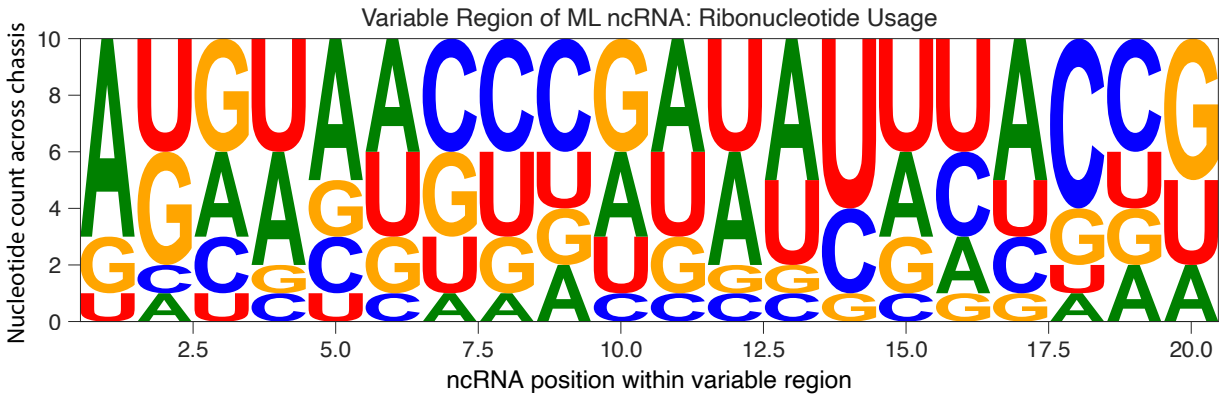


Supplementary Figure 5. Standard deviation of normalized barcode representation of donors of varying cut sites vs. donor centers in *S. cerevisiae*. Heat map of the standard deviation of normalized barcode representation of cut site vs. donor center (94 nucleotide donor length), normalized to the cut site at the barcode insertion site, and donor center of -5 bp from the barcode insertion site. Each square represents the standard deviation of all biological replicates across all sites.

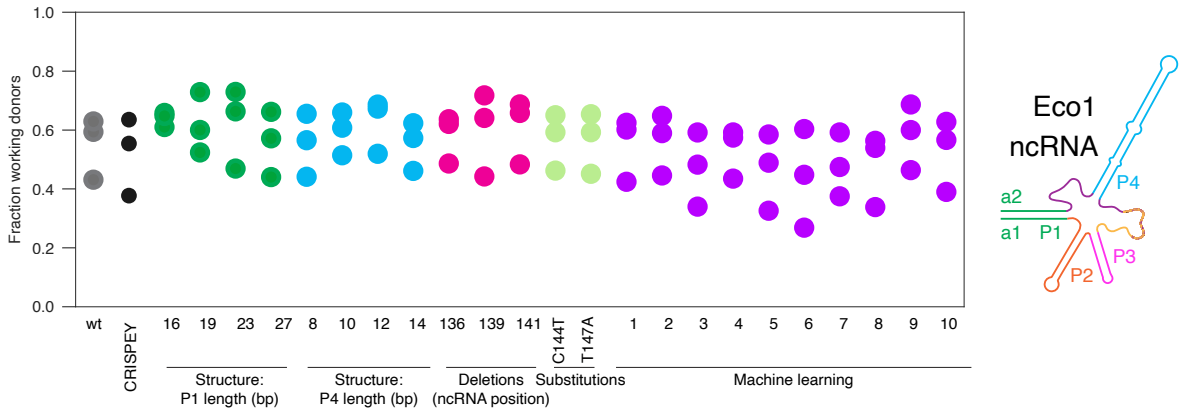


Supplementary Figure 6. ncRNA structure and sequence of top machine learning ncRNA chassis. CRISPEY and ML chassis were folded using RNAfold (Institute for Theoretical Chemistry, University of Vienna webtool) using N_{10} to stand in for the variable donor region (light blue). *msr* annotated in grey and

RT-DNA annotated in purple. Nucleotides with changes from the CRISPEY reference are highlight in red with the nucleotide identity annotated in black.



Supplementary Figure 7. Usage of ribonucleotides in ML ncRNA chassis across variable region. Ribonucleotide height scaled with usage, created by the Python logomaker package.



Supplementary Figure 8. Fraction working editors across ncRNA chassis. Fraction of working donors across all ncRNA chassis. Each closed circle represents the mean of the three biological replicates for that site.