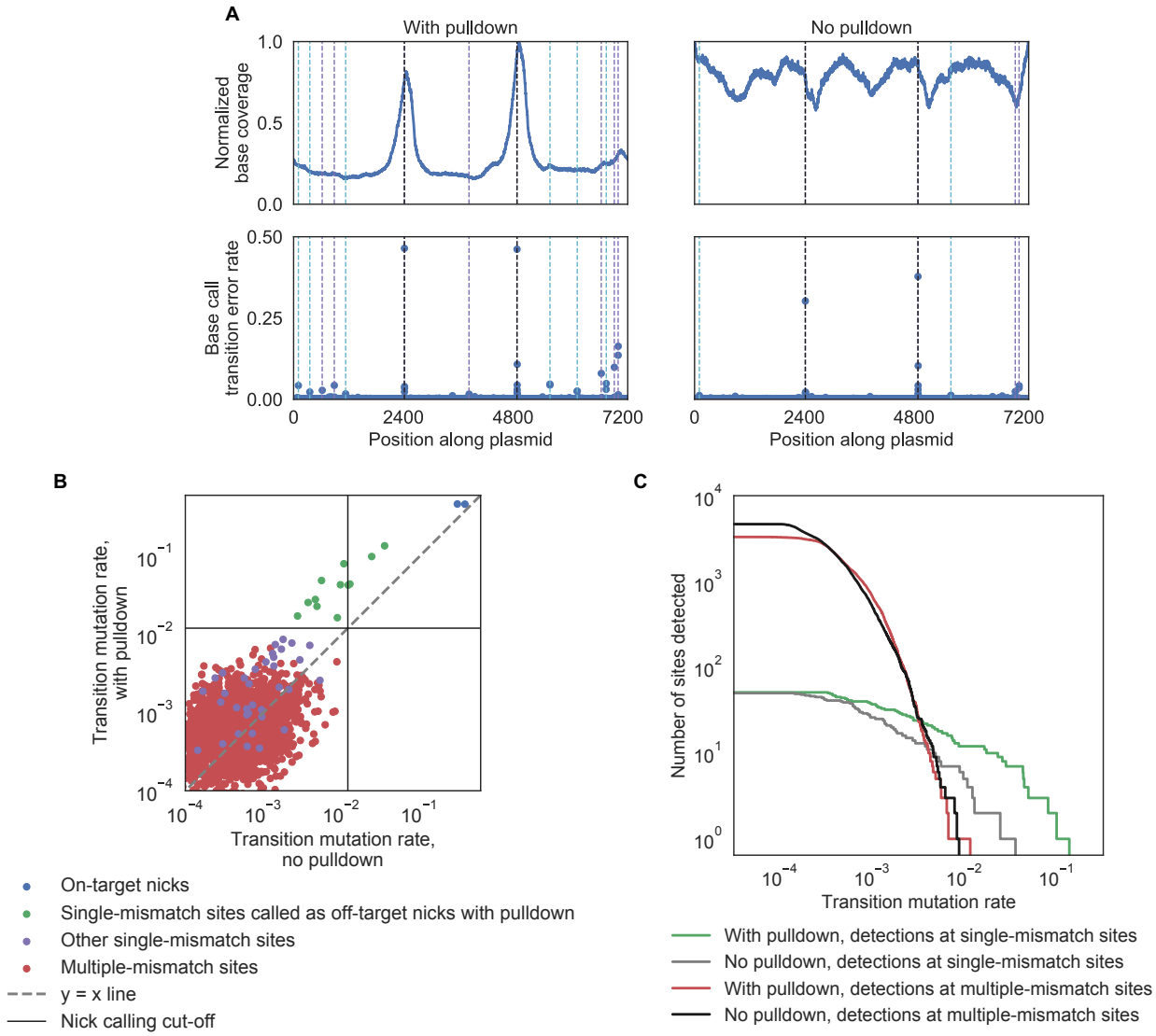

Figure S8: Comparison of Nb.BsmI off-target identification with and without enrichment on streptavidin. A) Normalized read coverage and transition mutation rate across the whole plasmid when DENT-seq is performed with (left) and without (right) the streptavidin pulldown. The left panel displays the same data shown in Fig. 4B and is recreated here for the comparison. Only 4 Nb.BsmI off-targets are identifiable without the pulldown as compared to 12 with. In addition, identified off-targets show less transition mutational signal without the pulldown. **B)** Comparison of mutational signal across the plasmid with and without the pulldown. Identifiable off-targets all have significantly higher signal when the pulldown is performed compared to when it is not performed. Signal at single-mismatch sites not identified as Nb.BsmI off-targets and at multiple-mismatch sites does not appear to differ between the two scenarios. Site mismatch categorization is performed by comparing the plasmid reference sequence to the Nb.BsmI on-target sequence. **C)** Number of sites identified as the transition mutation cutoff is changed, sorted by level of homology to the Nb.BsmI on-target sequence. Sites with a single mismatch to the Nb.BsmI on-target sequence have higher signal when the pulldown is performed (green vs. gray lines) while the signal at sites with more mismatches does not change (red vs. black lines). Data in green here (all single-mismatch sites) represent both data in green (single-mismatch sites identified as Nb.BsmI off-targets) and purple (other single-mismatch sites) in **B**. It is important to note that while single-mismatch sites are more likely to represent true off-target cutting by Nb.BsmI than multiple mismatch sites, DENT-seq mutational signal at mismatch sites can in principle arise from either off-target cutting or noise from the DENT-seq assay.