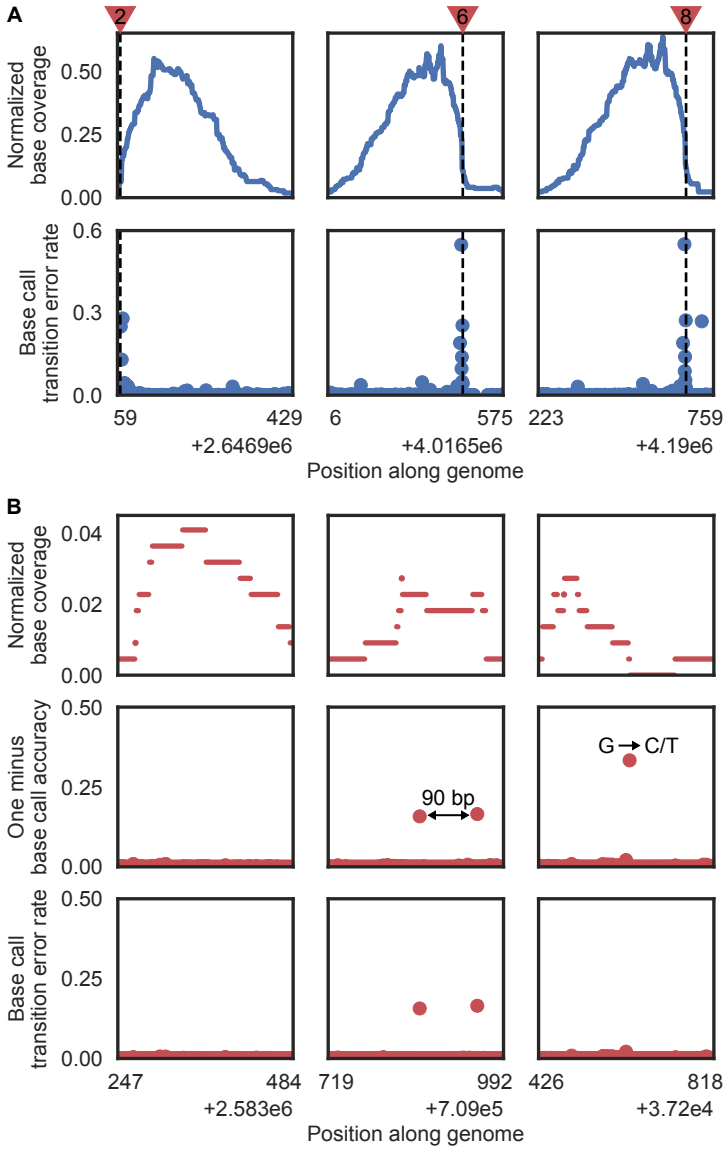

Figure S9: Representative sequencing peaks in the E. coli genome called by MACS2 that did and did not contain nicks. A) In total, nine nicks were expected to be incorporated into the E. coli genome as a result of D10A spCas9 nickase and the two guide RNAs used. One guide RNA had a single target site (Fig. 5D), while the other had eight, five of which are shown in Fig. 5C and the rest here. **B)** Nicks with low penetrance in a sample cannot be identified from sequencing coverage and peak calling alone as background peaks may result from non-specific binding of DNA to the streptavidin beads used for purification. The mutational signal that is unique to DENT-seq can be used to filter out peaks that do not contain nicks, allowing for detection of such low penetrance nicks. Other metrics such as peak height or quality and p-values cannot recapitulate this level of performance (Fig. 5B). Sequencing peaks called by MACS2 may have very little mutational signal (left). Alternatively, signal may be present but not occur at consecutive loci as would be expected from incorporation of consecutive P and K residues (middle). Finally, mutational signal may be detected that does not represent a transition mutation (C↔T or A↔G) and therefore could not have resulted from P and K residues being incorporated (right). The latter two scenarios can occur from real variants in the sample DNA relative to the reference, mis-incorporations during PCR, or sequencing errors.