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Figure S3.

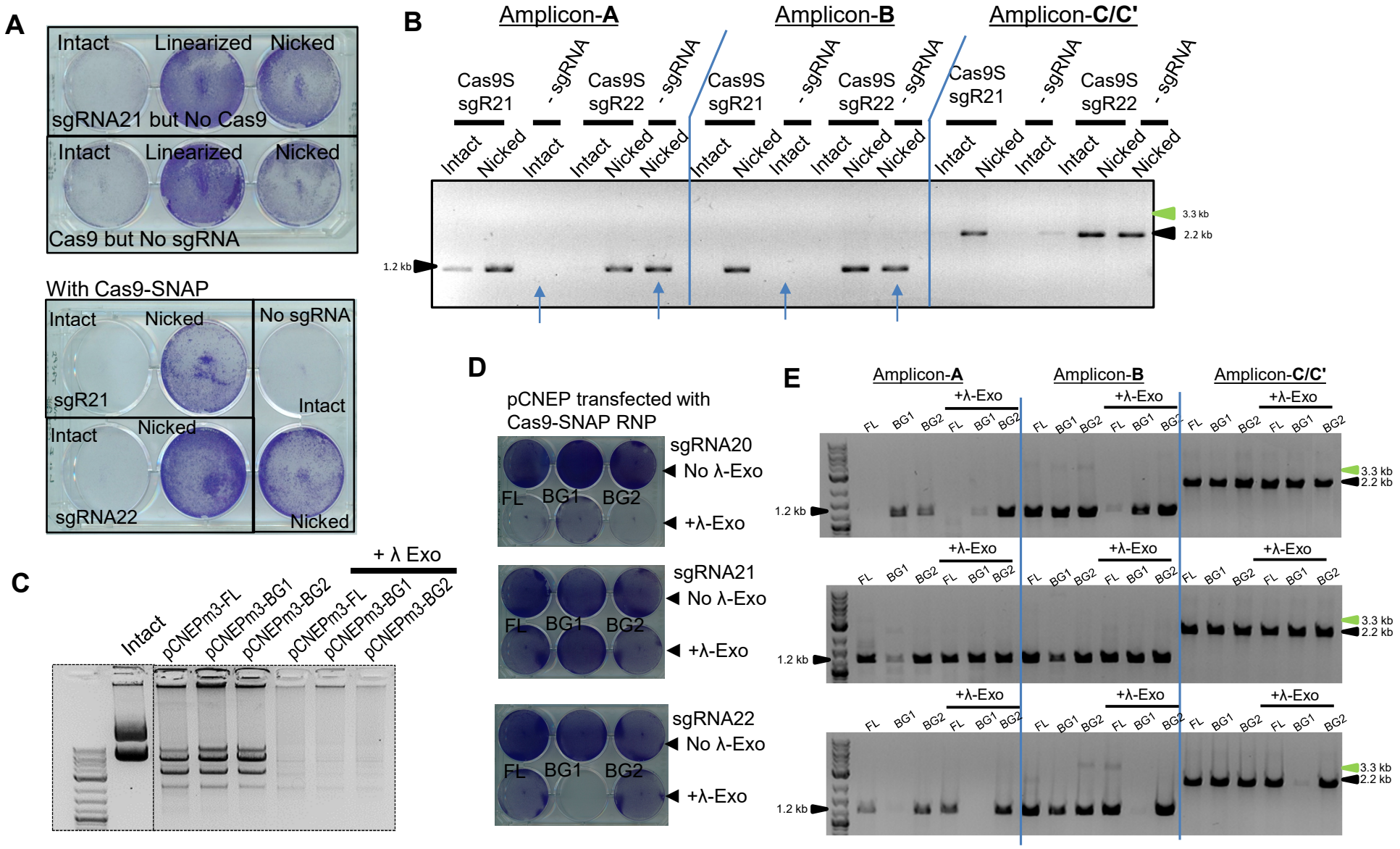

Figure S3. Promiscuous HDR and transgene integration with nicked plasmids in HEK293FT cells.
(A) Crystal violet staining of HEK293FT cells transfected with pCNEPm3 plasmid that is intact versus linearized or nicked with NtBbvCI. Top plate compares having only one component of Cas9 or sgRNA, where the transgene is able to integrate without Cas9-stimulation. Bottom plate further compares complete Cas9-SNAP versus no sgRNA. (B) Genomic PCR assays in the same format as Fig. 3 and 4, detecting the left junction (Amp.-A), right junction (Amp.-B) and transgene-spanning products (Amp.-C). Blue arrows point to specific lanes lacking sgRNA, where the Intact plasmid does not integrate at the EMX1 locus, but the Nicked plasmid is able to integrate in an HDR-like manner without Cas9 stimulation. The black arrowheads point to the 1.2 kb size of Amplicon-A and –B and the 2.2 kb size of Amplicon-C from the endogenous EMX1 locus. A desired HDR event generating a 3.3 kb Amplicon-C' is not observed (green arrowhead). (C) The BG- and FL- labeled plasmids after T4 DNA ligase repair as detailed in Fig. S2, then subjected to digestion with λ-Exonuclease that removes nicked plasmids but leaves behind fully-repaired intact plasmids. (D) Crystal violet staining of puromycin-selected cells after transfected with BG- and FL-labeled pCNEPm3 plasmid with or without λ-Exonuclease treatment. (E) Genomic PCR assays in the same format as (B).