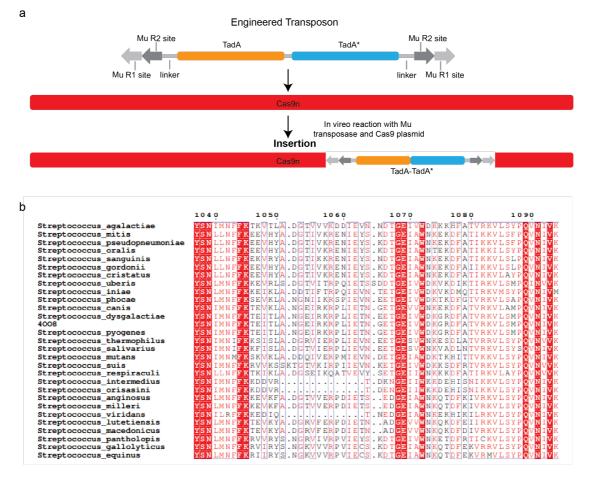
Supplementary Information

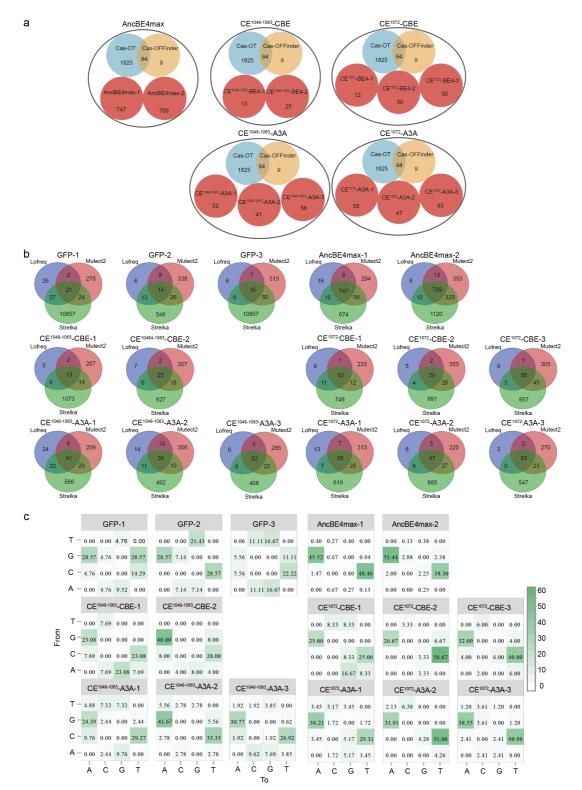
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Supplementary Figure 1: Genetic screen for tolerant nCas9 sites

Supplementary Figure 2: GOTI data in detail



Supplementary Figure 1. Genetic screen for tolerant nCas9 sites. a, The transposon harboring TadA-TadA* and its random insertion into nCas9. **b**, Sequence alignment of SpCas9 (1036 to 1096) among 28 orthologs.



Supplementary Figure 2. GOTI data in detail. a, No overlap between the shared SNVs and the potential gRNA-dependent off-targets predicted by Cas-OT or OFFinder. **b**, SNVs in individual embryos treated with indicated proteins. Three software (Lofreg, Mutect2 and Strelka) were used to identify SNVs, with the shared SNVs detected by all the softwares considered genuine. **c**, Composition of the shared SNVs. The values are the frequencies of the indicated nucleotide alterations.