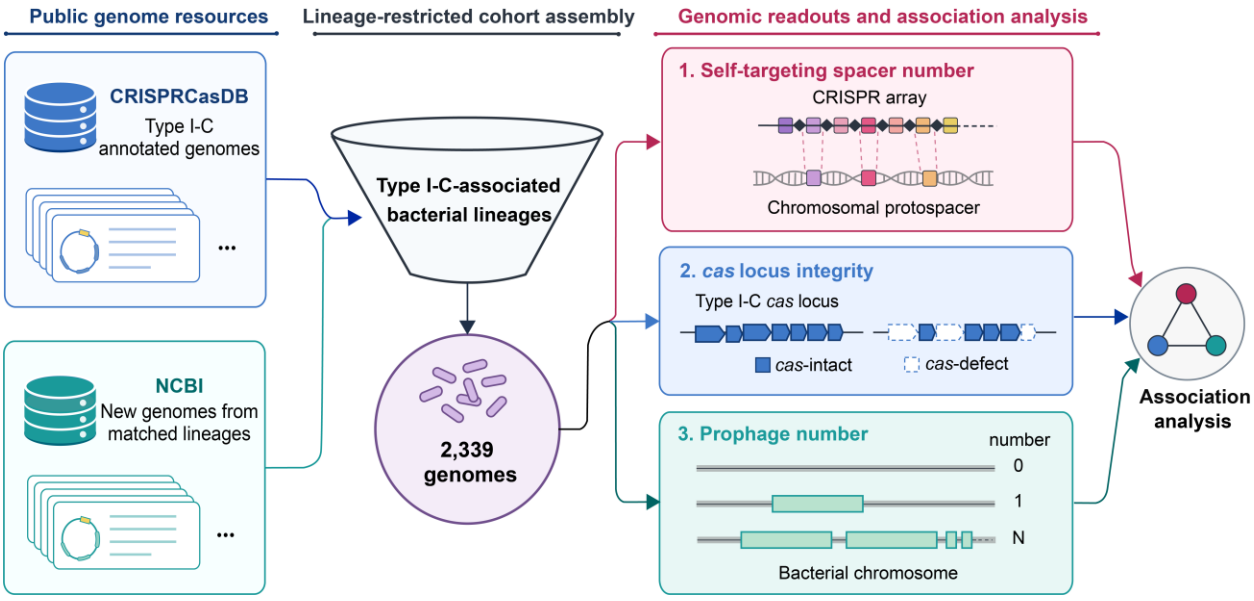
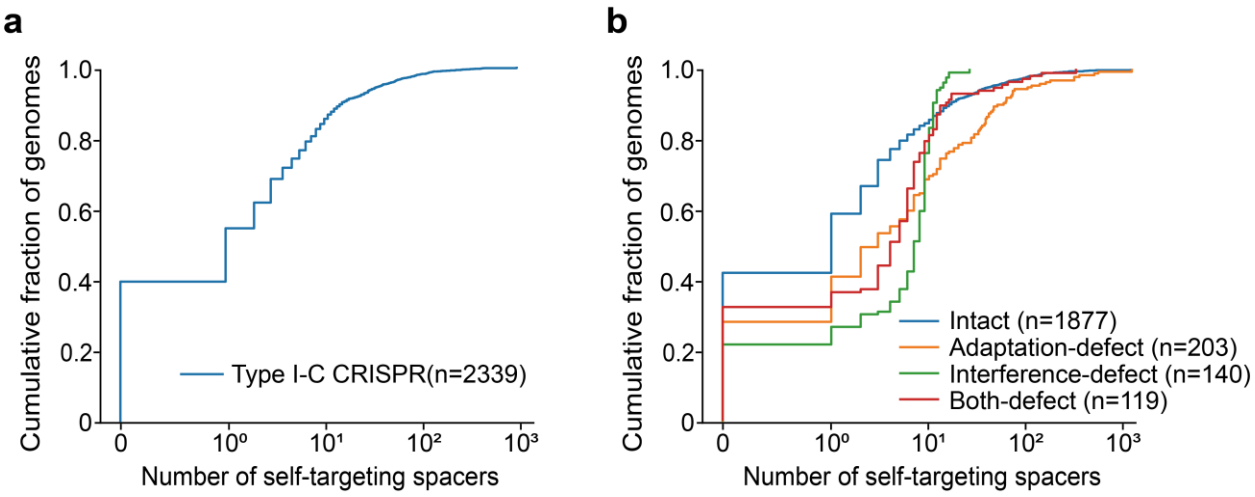
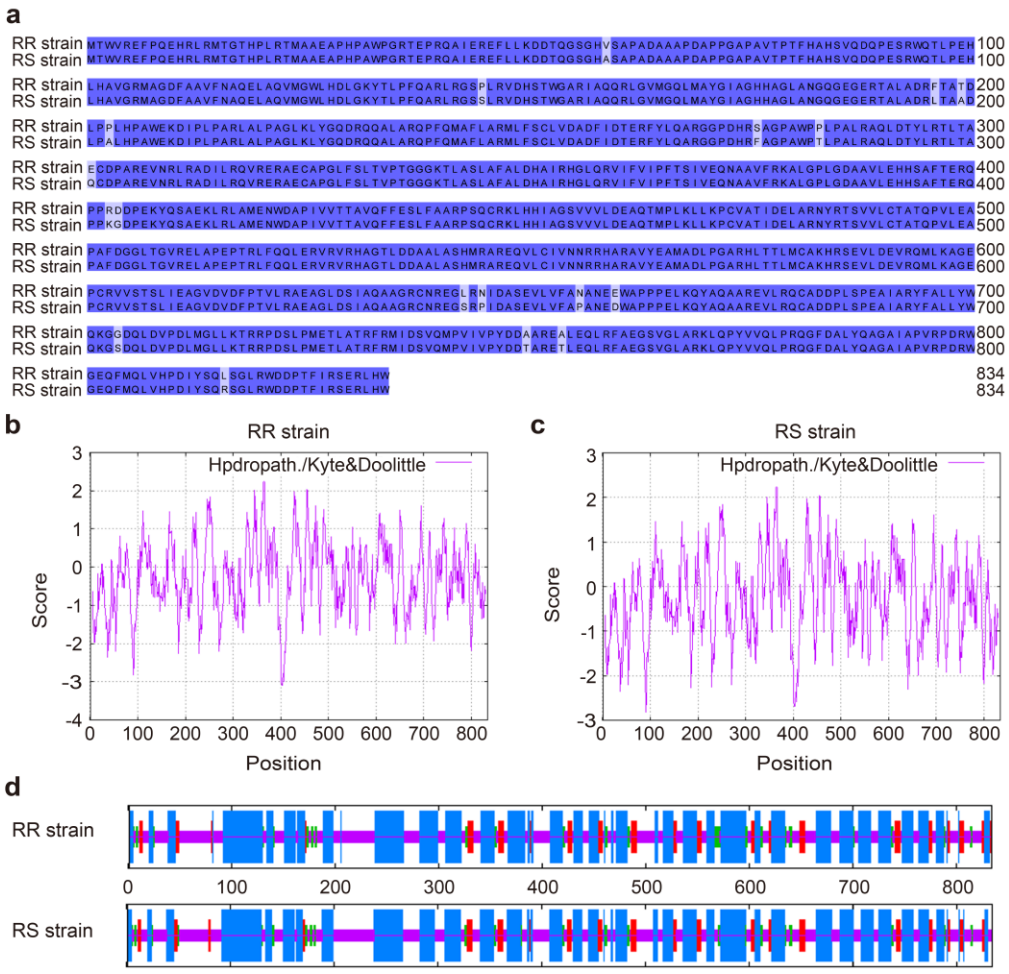


Supplementary Fig. 1 Workflow used to assemble a genome-scale discovery cohort from CRISPRCasdb-annotated type I-C genomes and newly deposited NCBI genomes from type I-C-associated bacterial lineages.



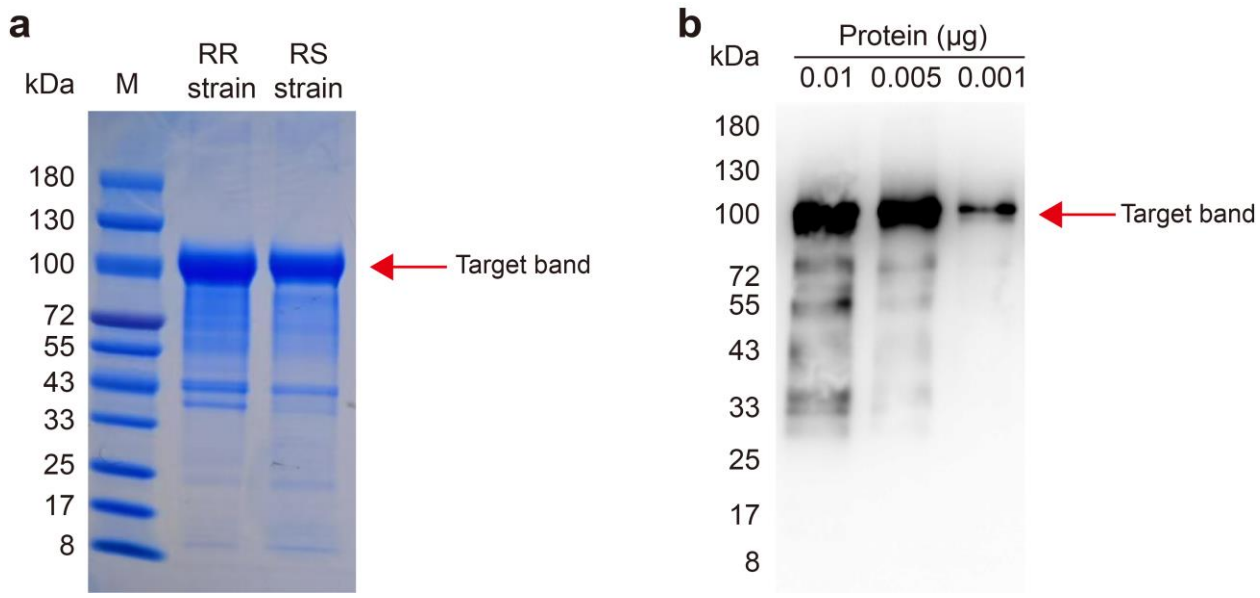
Supplementary Fig. 2 Distributional analyses of genome level self-targeting spacer abundance across genomes from type I-C-associated lineages.

a, Empirical cumulative distribution functions (ECDFs) of self-targeting spacer numbers across the 2,339-genome discovery cohort. **b**, ECDFs of self-targeting spacer numbers stratified by type I-C module integrity. Adaptation-defect genomes carried disruption or absence of *cas1*, *cas2* or *cas4*, interference-defect genomes carried disruption or absence of *cas3*, *cas5*, *cas8* or *cas7*, and both-defect genomes carried defects in both modules.

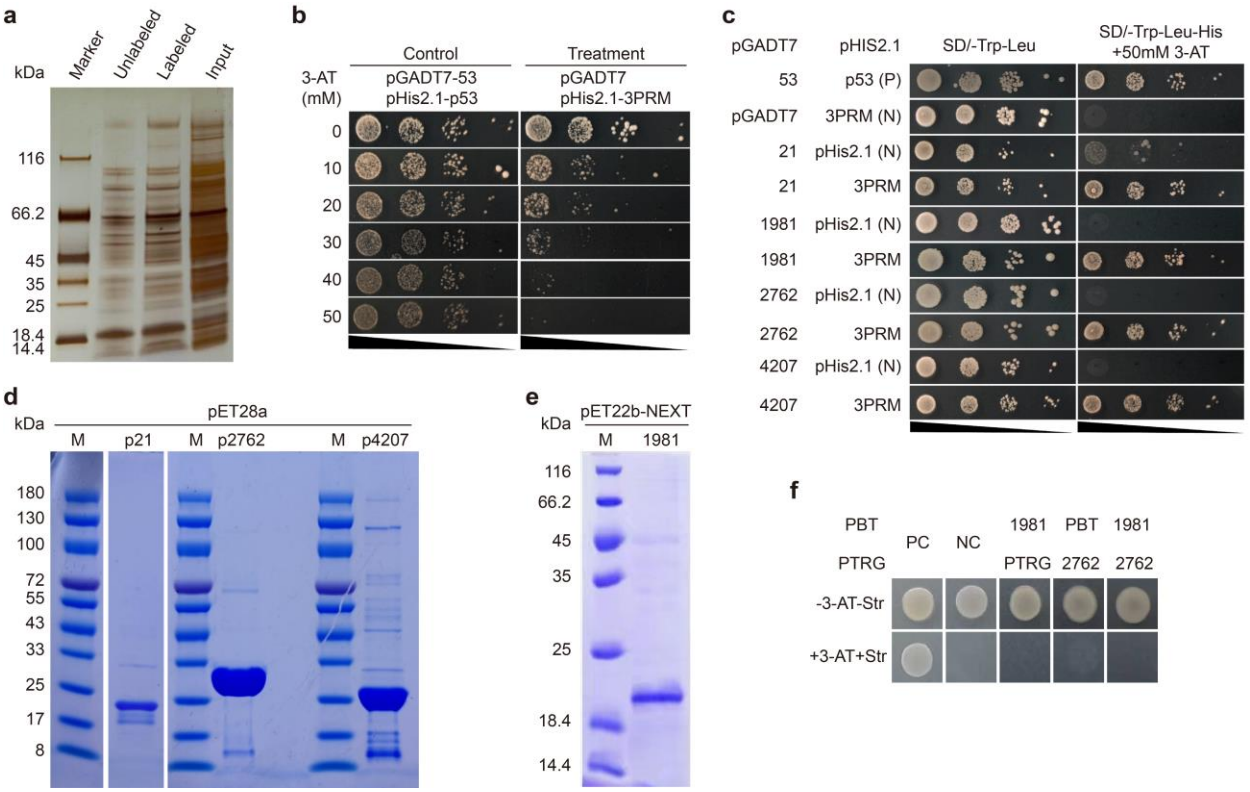


Supplementary Fig. 3 Comparative bioinformatics analysis of Cas3 from both the phage-resistant (PR) and phage-susceptible (PS) strains.

a, Sequence alignment. Cas3 from the two strains shared 97.84% amino acid identity. **b, c**, Kyte&Doolittle hydropathy profiles (ProtScale). ProtParam analysis predicted aliphatic indices of 86.1 and 85.41 and GRAVY scores of −0.262 and −0.263 for PR-strain Cas3 and PS-strain Cas3, respectively (GRAVY, grand average of hydropathicity; range, −2 to 2; positive values, hydrophobic; negative values, hydrophilic). **d**, Secondary-structure prediction using SOPMA. Predicted α-helices accounted for 48.32% and 47.12% of PR-strain Cas3 and PS-strain Cas3, respectively; random coils accounted for 37.65% and 39.21%, extended strands for 8.99% and 8.87%, and β-turns for 5.04% and 4.80%.



Supplementary Fig. 4 Validation of recombinant Cas3 protein preparation and antibody detection. **a**, SDS-PAGE analysis of refolded and concentrated recombinant Cas3 proteins of the PR and PS strains expressed from pET28a. Prominent bands at approximately 100 kDa correspond to the expected Cas3 fusion proteins. M, molecular weight marker. **b**, Western blot validation of the PS strain Cas3-specific polyclonal antibody using recombinant PS-strain Cas3 expressed from pET28a, loaded at 0.01 μg, 0.005 μg and 0.001 μg per lane.



Supplementary Fig. 5 Identification of phage-susceptible strain *cas3* promoter-binding proteins.

a, Silver-stained SDS-PAGE of proteins recovered by DNA pull-down using the PS strain *cas3* promoter probe. The unlabeled lane shows pull-down products obtained with unlabeled DNA probe, beads and PS strain total protein; the labelled lane shows pull-down products obtained with biotin-labelled DNA probe under the same conditions; input indicates total PS strain protein extract. Selected differential bands were excised for mass-spectrometry analysis. Protein concentrations were 1.24 mg ml^{-1} for the unlabeled sample and 1.47 mg ml^{-1} for the labelled sample. Loading volumes were 12 µl for the unlabeled sample, 10 µl for the labelled sample and 10 µl for input. M, molecular weight marker. **b**, Autoactivation control of the 3PRM yeast one-hybrid bait. Three tandem copies of the predicted regulatory motif (PRM) from the PS strain *cas3* promoter were cloned into pHis2.1 and co-transformed with empty pGADT7 into yeast strain Y187. Growth was

assessed on SD/-Leu-Trp-His plates containing 0-50 mM 3-AT. The positive control was p53-pHis2.1 co-transformed with pGADT7-53. **c**, Yeast one-hybrid screening of candidate PS strain *cas3* promoter-binding proteins. P, positive control; N, negative control; pGADT7 and pHis2.1, empty vectors; 3PRM, three tandem PRM bait. **d**, Heterologous expression of p21, p2762 and p4207 using pET28a. **e**, Heterologous expression of p1981 using pET22b-NEXT. M, molecular weight marker. **f**, Interaction between p1981 and p2762 by bacterial two-hybrid analysis. PC indicates the positive control containing pBT-LGF2 and pTRG-Gal11p. NC indicates the negative control containing empty pBT and pTRG vectors. Data represent three independent biological replicates, each with three technical replicates.