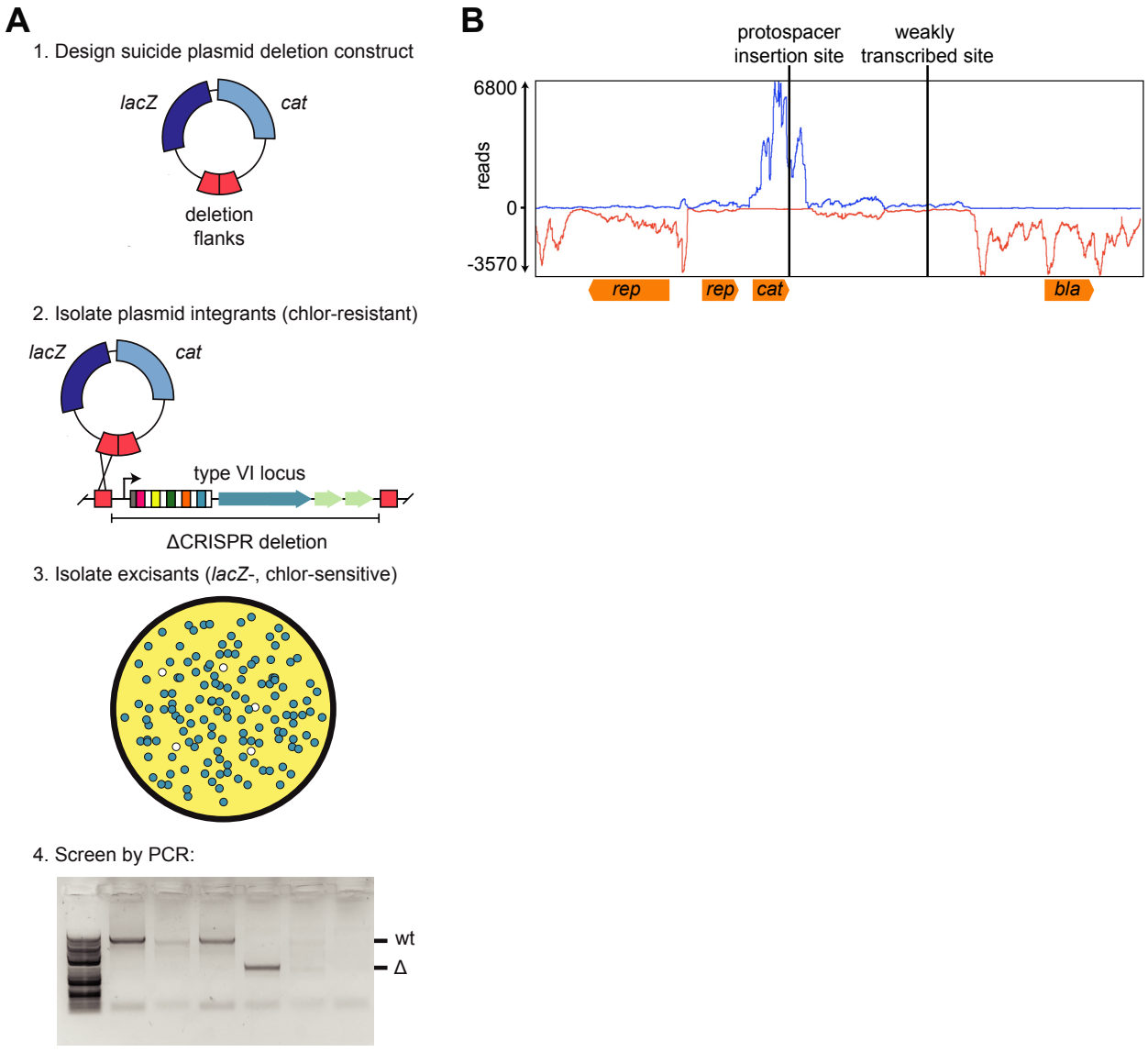

Figure S1: Listeria seeligeri strain construction and pTarget plasmids, related to Fig. 1. **A**. Four step protocol for generating knockouts and knockins in *Listeria seeligeri.* 1. The mutant allele is cloned into a suicide vector encoding a chloramphenicol resistance cassette for selection in *L. seeligeri*, as well as a *lacZ* gene as a plasmid reporter (generates blue color on X-gal plates). Once cloned, the allelic exchange construct is transformed into an SM10 *E. coli* strain for conjugative mating into *L. seeligeri.* 2. The suicide plasmid is introduced into *L. seeligeri* via conjugative mating, selecting on media containing chloramphenicol (15 µg/mL) and nalidixic acid (50 µg/mL) to select for plasmid integration and eliminate *E. coli* donor, respectively. Integrants are isolated by streaking on the same media. 3. Isolates are passaged twice in liquid culture without selection to allow the plasmid to be excised. Cultures are diluted and plated onto media containing X-gal, and after two days blue colonies are apparent. White colonies (excisants) are restreaked and confirmed to be chloramphenicol sensitive. 4. Excisants are screened by PCR and Sanger sequencing to check for the mutant allele. **B**. Stranded RNAseq coverage plot of *L. seeligeri* pTarget plasmid, showing protospacer insertion sites in 3' UTR of cat gene and weakly transcribed downstream site. Reads from top strand are in blue, bottom strand in red.

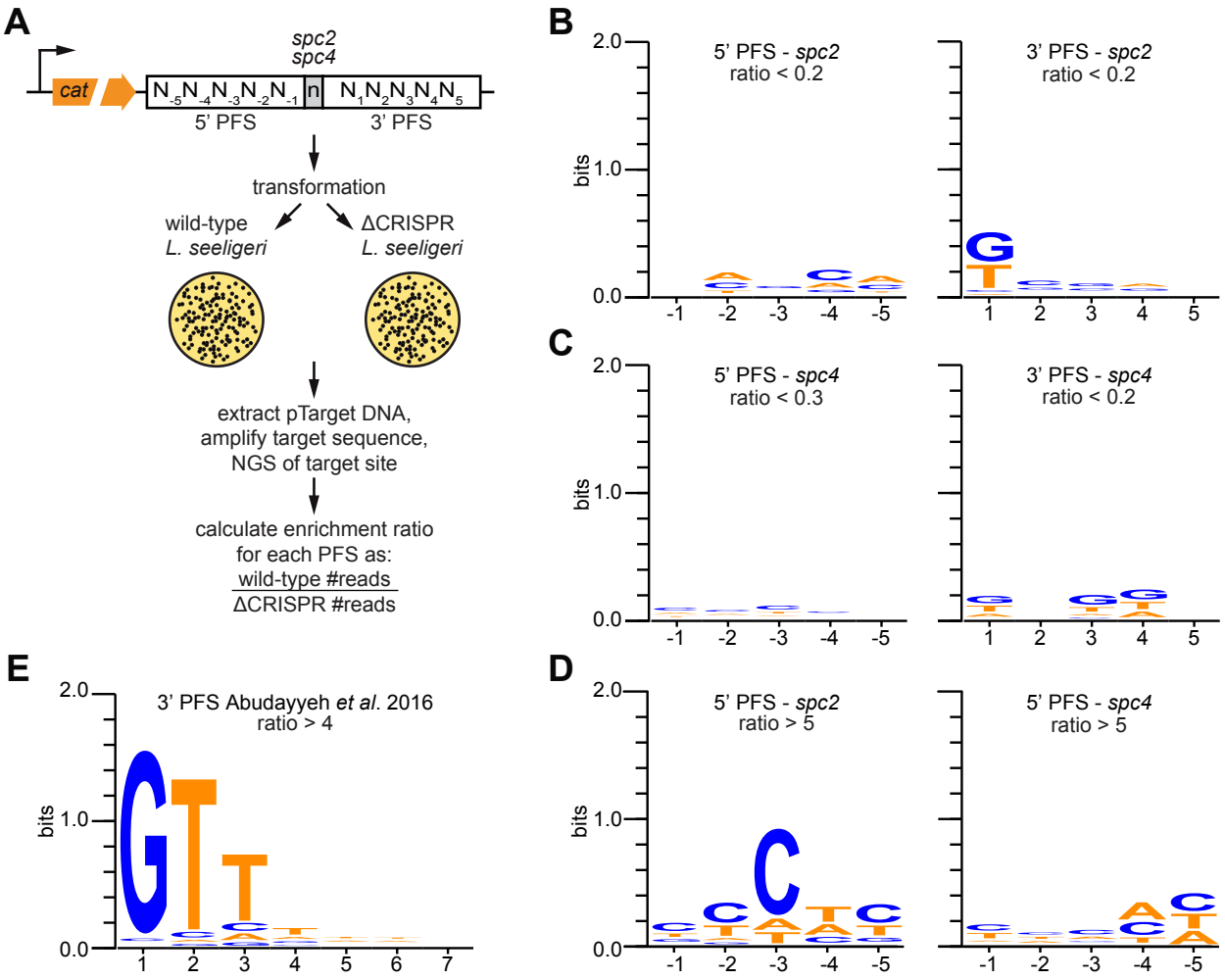

Figure S2: PFS screening strategy, targeted and enriched flanking sequences, related to Fig. 3. **A.** Screening strategy to identify protospacer flanking sequence (PFS) requirements of the *L. seeligeri* type VI CRISPR system. Five randomized nucleotides were introduced at the 5' or 3' end of the *spc2* or *spc4* target sequence and these libraries were transformed into wild-type and ΔCRISPR *L. seeligeri* by conjugation. Transconjugants were pooled, target plasmids were isolated and the target flanking sites were deep sequenced. Flanking sequence representation was normalized to total reads and the WT:ΔCRISPR ratio was calculated for each 5nt sequence. **B.** Weighted sequence logos for 3' target flanking sequences depleted more than five fold in the wild type strain. Logos representing the 5' and 3' PFS from the *spc2* (**B**) and *spc4* (**C**) libraries are both shown. **D.** Weighted sequence logos for 5' target flanking sequences enriched more than five-fold in the CRISPR+ strain for each library. **E.** Sequence logos for 3' target flanking sequences enriched more than four-fold in the LshCas13 beta lactamase PFS screen from Abudayyeh et al. 2016.

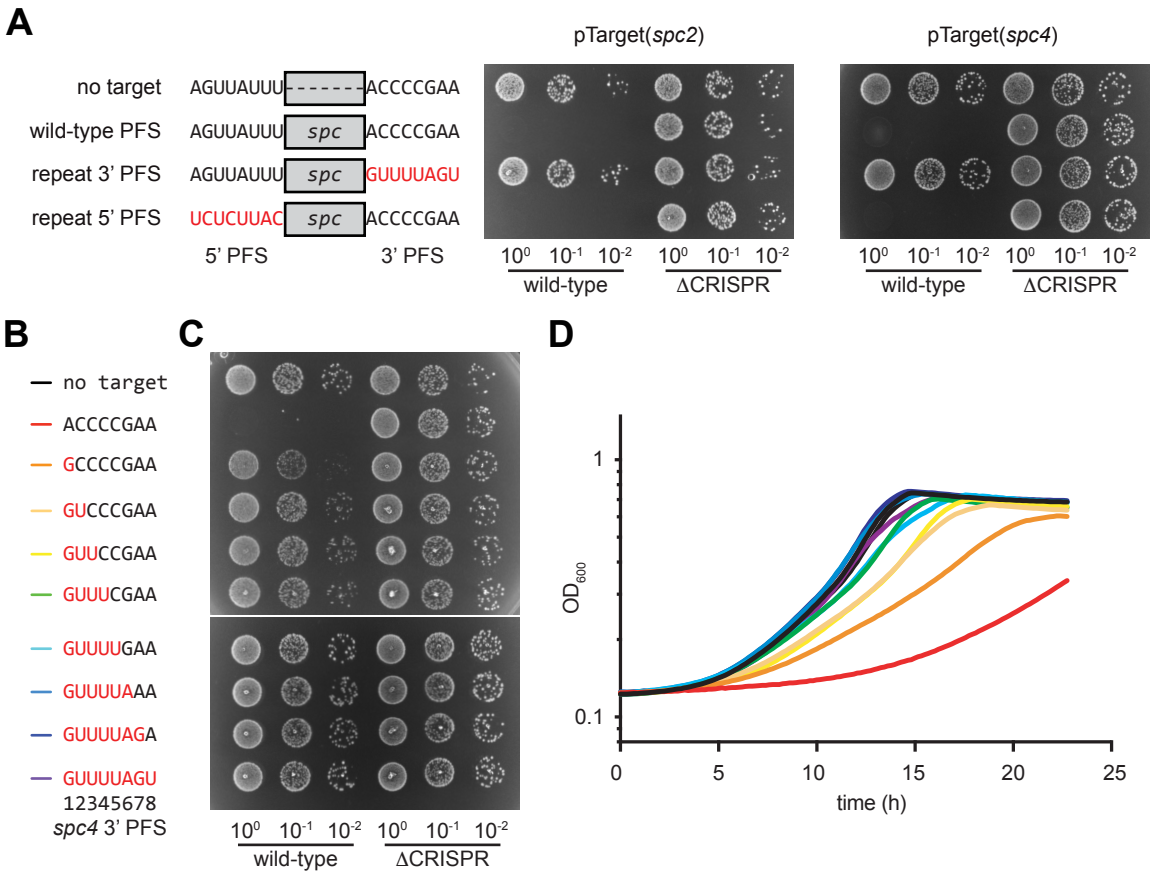

Figure S3: Repeat-like sequences on 3' end of the protospacer confer protection from type VI CRISPR targeting, related to Fig 4. A. Conjugation assay showing 8nt of repeat-like sequence on the 3' end (but not the 5' end) of the protospacer (*spc2* or *spc4*) confers protection from targeting. **B.** Series of *spc4* pTarget plasmids containing repeat-derived sequences (shown in red) were tested for type VI CRISPR interference using conjugation assay and (D) liquid growth assay. Traces represent the mean from four biological replicates.

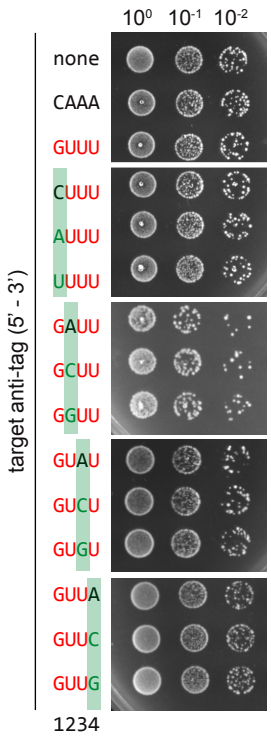

Figure S4: Target plasmid stability in ΔCRISPR strain, related to Fig. 5. Conjugation of pTarget plasmids from Fig 5 into ΔCRISPR strain. Each plasmid contains a mutation in the anti-tag region highlighted in green.

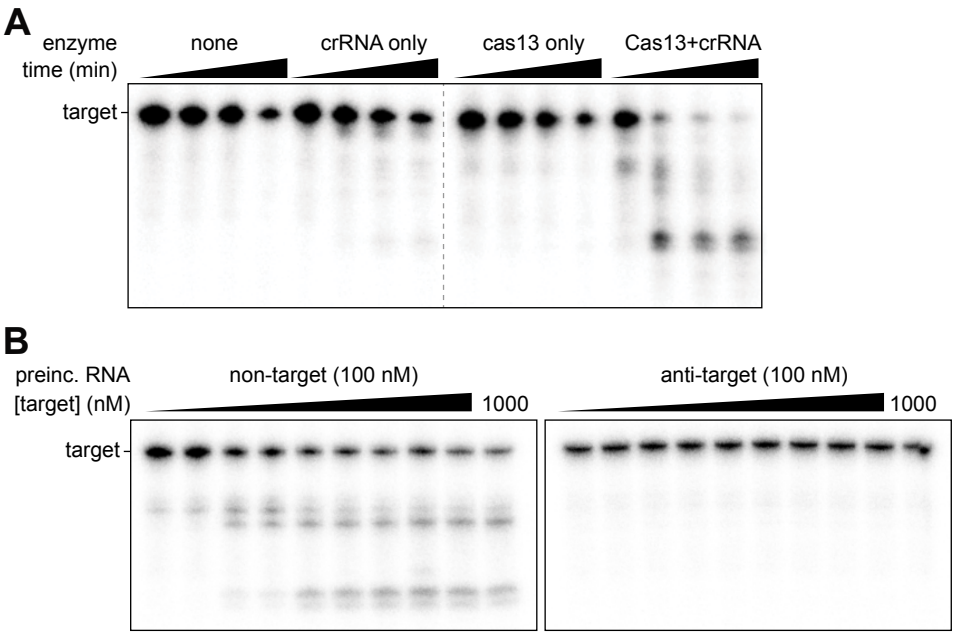

Figure S5: In vitro RNA cleavage assays with LbuCas13 and cleavage inhibition by anti-target RNA, related to Fig. 6. A. *In vitro* RNA cleavage time course with purified LbuCas13 and labeled RNA targets. The indicated RNA substrates were incubated with the Cas13 and / or crRNA and cleavage products analyzed by SDS-PAGE. **B.** Inhibition of *trans*-RNA cleavage by anti-target RNA. 10 nM LbuCas13, 10 nM crRNA, and 10 nM labeled nontarget RNA were incubated with 100 nM nontarget RNA or anti-target RNA. Reactions were initiated with target RNA at 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, or 1000 nM and cleavage products analyzed after 60 minutes.

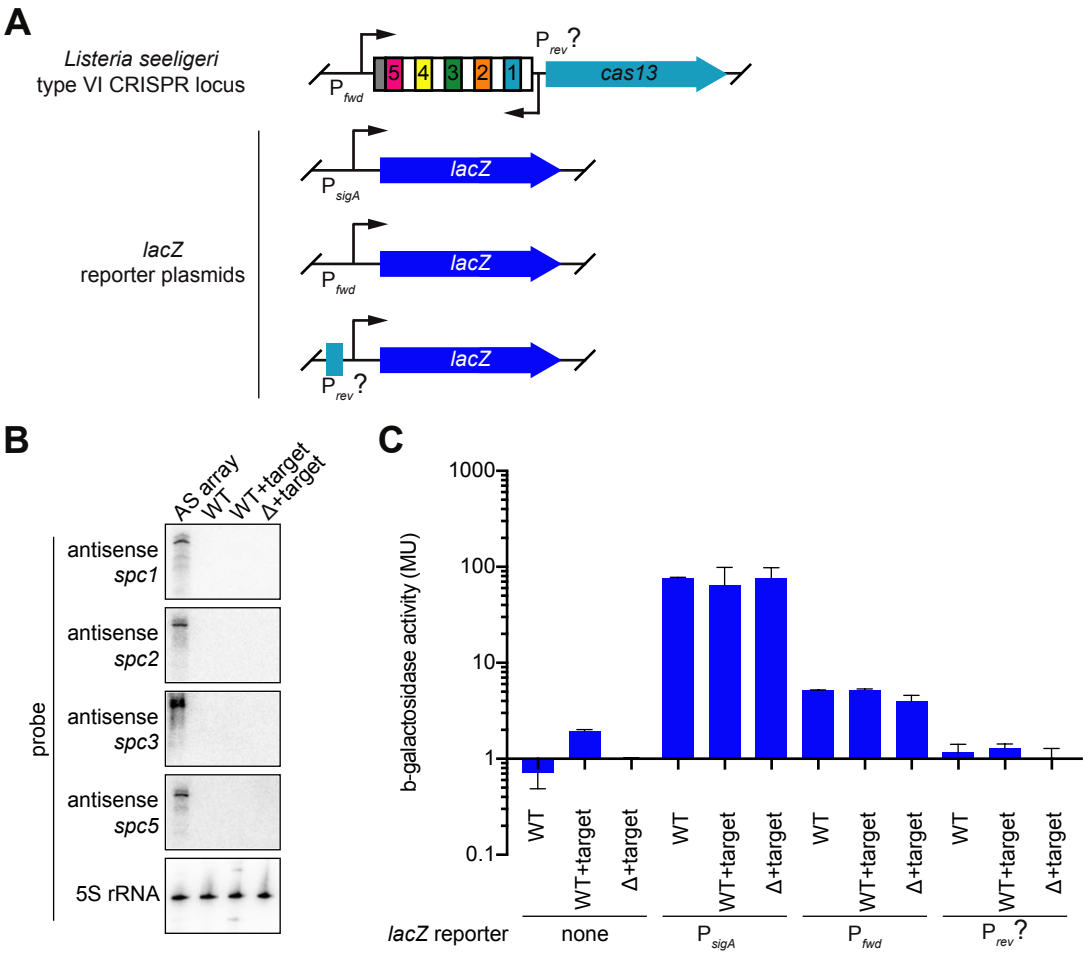

Figure S6: Antisense CRISPR array transcripts are not produced in vivo, related to Figure 7. A. LacZ reporter constructs used to assess antisense transcription from putative promoters downstream of the CRISPR array. A constitutive sigma 70-dependent promoter (PsigA) was fused to *lacZ* as a positive control. Promoters preceding (fwd) and following (rev) the array were tested. **B.** Northern blot analysis using probes against antisense spacer sequences or 5S rRNA loading control in the indicated strains. A strain overexpressing the antisense CRISPR array was analyzed as a positive control **C.** β-galactosidase assay of the reporters in **A.** Expression from the PsigA, Pfwd, and Prev promoters was measured in wild type with and without *spc4* target induction, as well as in the ΔCRISPR strain. Error bars represent standard error of the mean from two biological replicates.