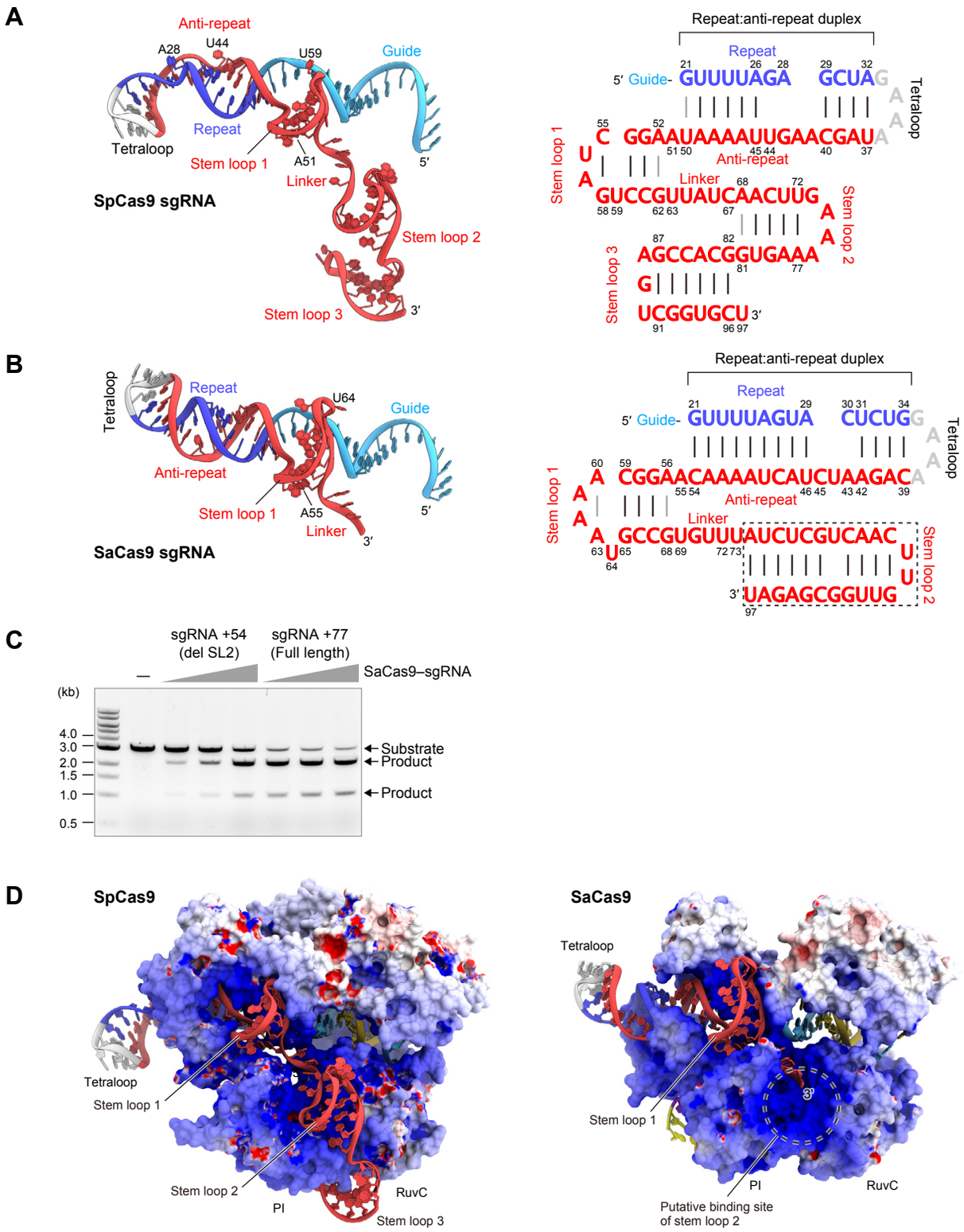
A
Anti-repeat
A28
U44
U59
Guide
Repeat
Tetraloop
Stem loop 1
A51
Linker
5′
SpCas9 sgRNA
Stem loop 2
Stem loop 3
3′
Repeat:anti-repeat duplex
Repeat
5′ Guide- GUUUUAGA GCUAG
Tetraloop
Stem loop 1
C GGAAUAAAAUUGAACGAU
U
A
Anti-repeat
Linker
GUCCGUUAUCAACUUG
Stem loop 2
AGCCACGGUGAAA
Stem loop 3
G
UCGGUGCU 3′
B
Tetraloop
Repeat
U64
Guide
Anti-repeat
Stem loop 1
A55
Linker
5′
3′
SaCas9 sgRNA
Repeat:anti-repeat duplex
Repeat
5′ Guide- GUUUUAGUA CUCUGG
Tetraloop
Stem loop 1
A CGGAACAAAAUCAUCUAAGAC
A
A
Anti-repeat
Linker
A GCCGUGUUUAUCUCGUCAAC
Stem loop 2
U
3′ UAGAGCGGUUG
C
sgRNA +54
(del SL2)
sgRNA +77
(Full length)
SaCas9–sgRNA
(kb)
4.0
3.0
2.0
1.5
1.0
0.5
Substrate
Product
Product
D
SpCas9
Tetraloop
Stem loop 1
Stem loop 2
PI
RuvC
Stem loop 3
SaCas9
Tetraloop
Stem loop 1
PI
RuvC
Putative binding site
of stem loop 2

Figure S4