Supporting Information

Conditional recruitment to a DNA-bound CRISPR-Cas complex using a colocalization-dependent protein switch

Robin L. Kirkpatrick, Kieran Lewis, Robert A. Langan, Marc J. Lajoie, Scott E. Boyken, Madeleine Eakman, David Baker, and Jesse G. Zalatan

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Figure S1: Reporter activation is colocalization-dependent

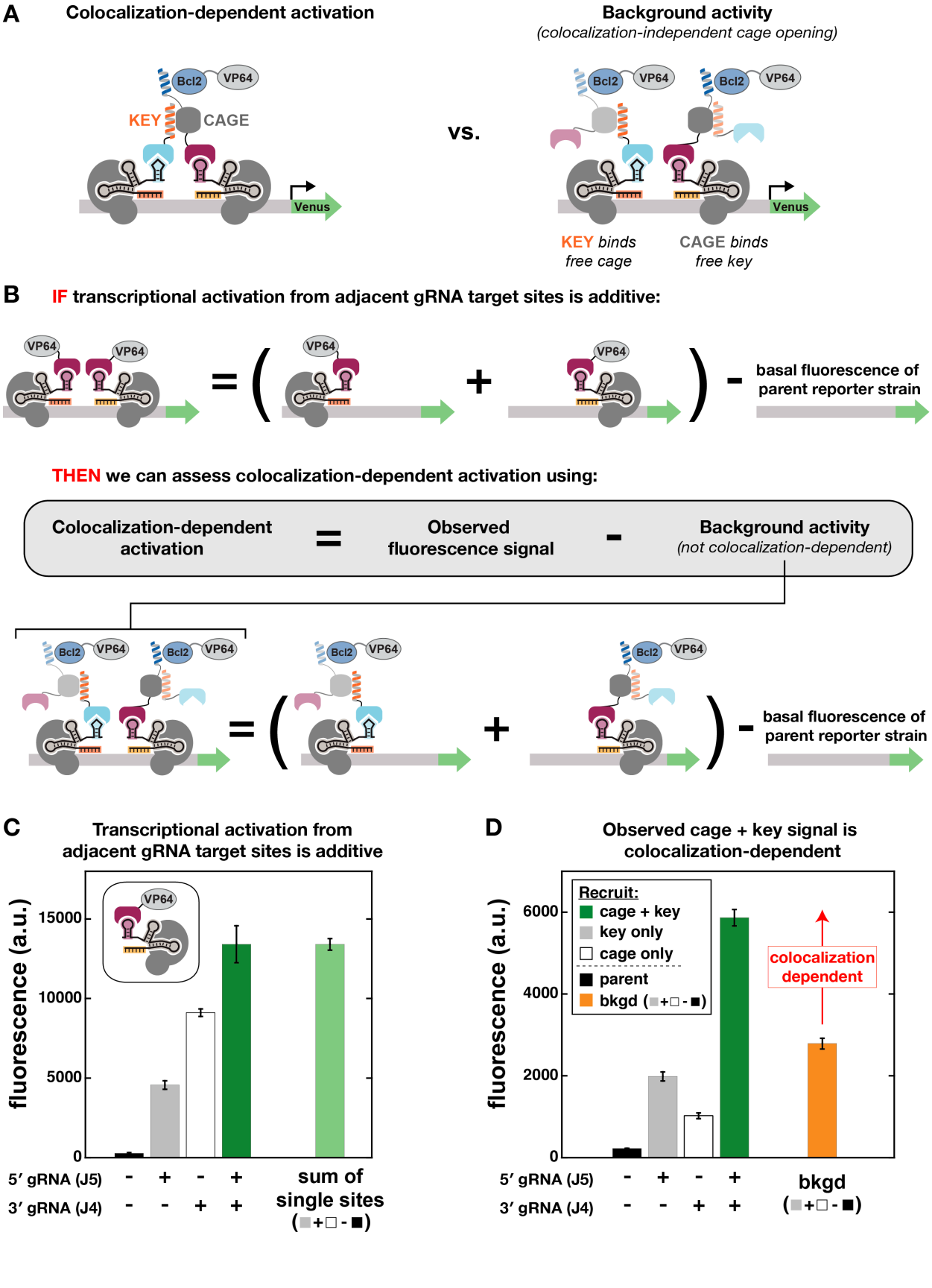

Figure S1. Reporter gene activation with the CRISPR-Cas Co-LOCKR switch is colocalization-dependent. (A) Observed reporter gene activation could arise from colocalization-dependent switch opening, which allows the Bim peptide to recruit the Bcl-VP64 activator. Alternatively, reporter gene activation could arise from recruitment of Bcl-VP64 to each CRISPR-Cas complex. Although the co-LOCKR is designed to open only in when cage and key are colocalized, high concentrations of cage or key could drive the equilibrium towards the open state. (B) We can separately measure reporter gene activation from a recruited cage in the presence of co-expressed key, and recruited key in the presence of co-expressed cage. To predict the contribution from colocalization-independent cage opening when both cage and key are recruited, we need to assess whether VP64 recruitment to adjacent gRNA sites is additive. We can measure reporter gene activation when VP64 is recruited to both sites or each site individually. We can calculate an additive sum from each single target site for comparison to the observed activation when VP64 is recruited to both sites. The sum must be corrected for basal fluorescence from the parent reporter strain, which will be included twice if observed when the values for each single target site are added. This correction can be explicitly derived as:

$$A_{\text{both sites}} = A_{\text{site1}} + A_{\text{site2}}$$

$$(\text{Obs} - \text{basal})_{\text{both sites}} = (\text{Obs} - \text{basal})_{\text{site1}} + (\text{Obs} - \text{basal})_{\text{site2}}$$

$$\text{Obs}_{\text{both sites}} = \text{Obs}_{\text{site1}} + \text{Obs}_{\text{site2}} - \text{basal}$$

In practice, the correction for basal parental strain fluorescence is negligible. (C) Reporter gene activation from directly recruiting VP64 to both target sites is indistinguishable from the additive sum of VP64 recruitment to each individual target site. Data without either guide present was obtained by transforming the parental yRK266 strain with empty vector (pRS316). (D) Reporter gene activation from the co-LOCKR switch is larger than the background activation predicted from the sum of measured background activity from independent recruitment of cage and key. This co-LOCKR switch uses a 2x PP7 scRNA to recruit key-PCP and a 1x com scRNA to recruit Com-cage (Figure 2). Data for parent was obtained with the unmodified parent strain yKL014. Fluorescence values in (C) and (D) are mean ± SD for at least three biological replicates. The errors are propagated by adding in quadrature.

Figure S2: Alternate RNA recruitment modules affect switch activation

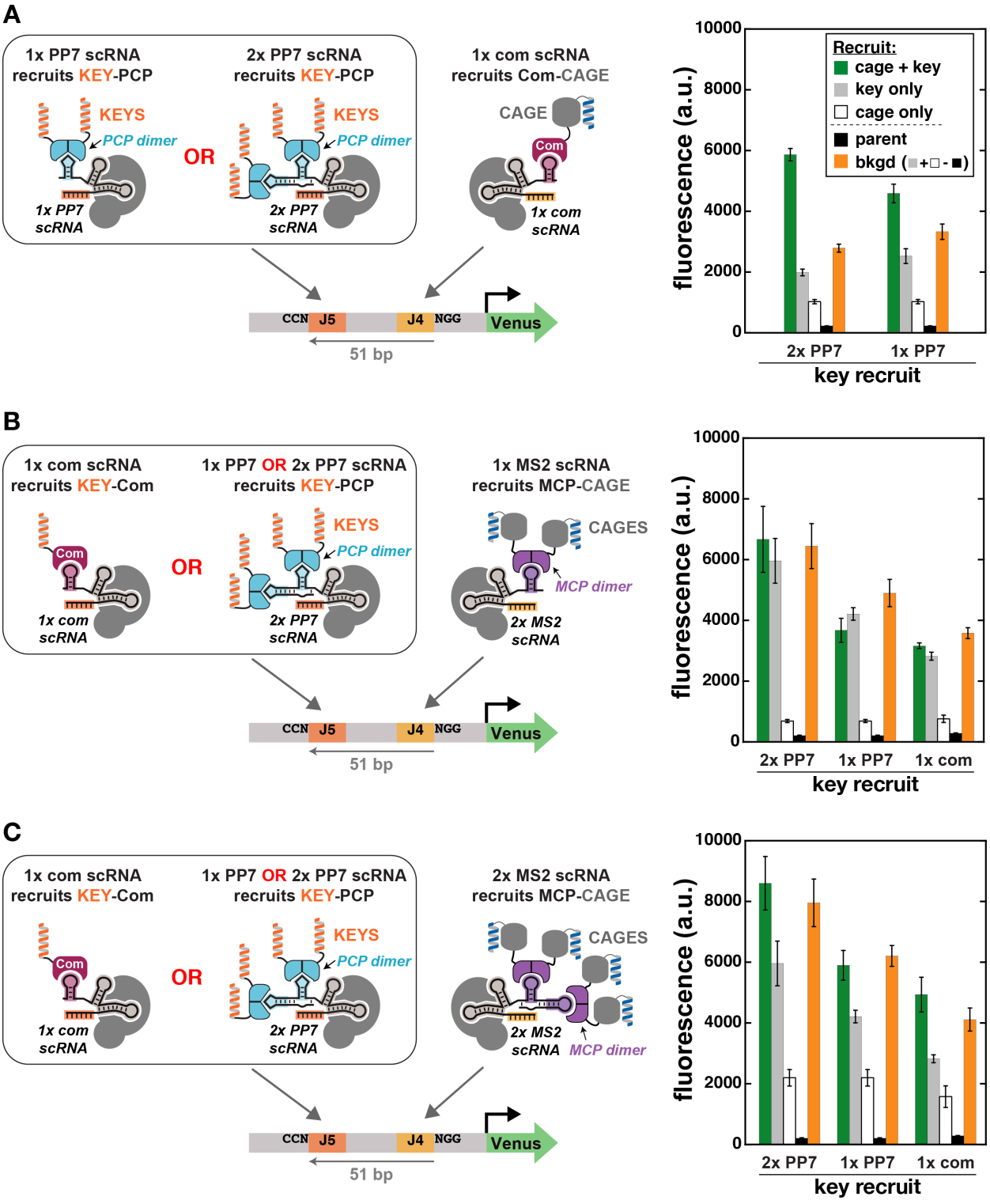

Figure S2. Alternative RNA recruitment modules affect switch activation. (A) Either a 2x PP7 or a 1x PP7 scRNA targets the upstream site (J5) and recruits either four or two key-PCP fusion proteins. A 1x com scRNA recruits one Com-cage fusion protein to the downstream site (J4). Both

key recruitment strategies produce switch activation above background. The 2x PP7 switch produces more activation than 1x PP7. (B) Either a 2x PP7, 1x PP7, or 1x Com scRNA recruits key fusion proteins to the upstream site (J5). A 1x MS2 scRNA recruits two MCP-cage fusion proteins to the downstream site (J4). In each case, there is no significant switch activation above background. (C) Either a 2x PP7, 1x PP7, or 1x Com scRNA recruits key fusion proteins to the upstream site (J5). A 2x MS2 scRNA recruits four MCP-cage fusion proteins to the downstream site (J4). In each case, there is no significant switch activation above background. For A-C, fluorescence values are mean ± SD for at least three biological replicates. Data for the parent strains were obtained from the unmodified yKL014, yKL016, or yRK456 strains. Background values are the additive sums of the "key only" and "cage only" samples (Figure S1). We did not test every possible pairwise combination of MS2, PP7, and com scRNA recruitment strategies because the MCP and PCP proteins are structurally homologous and behave similarly in CRISPR gene activation assays.[1]

Figure S3: RNA-mediated recruitment is more effective than direct dCas9 fusions

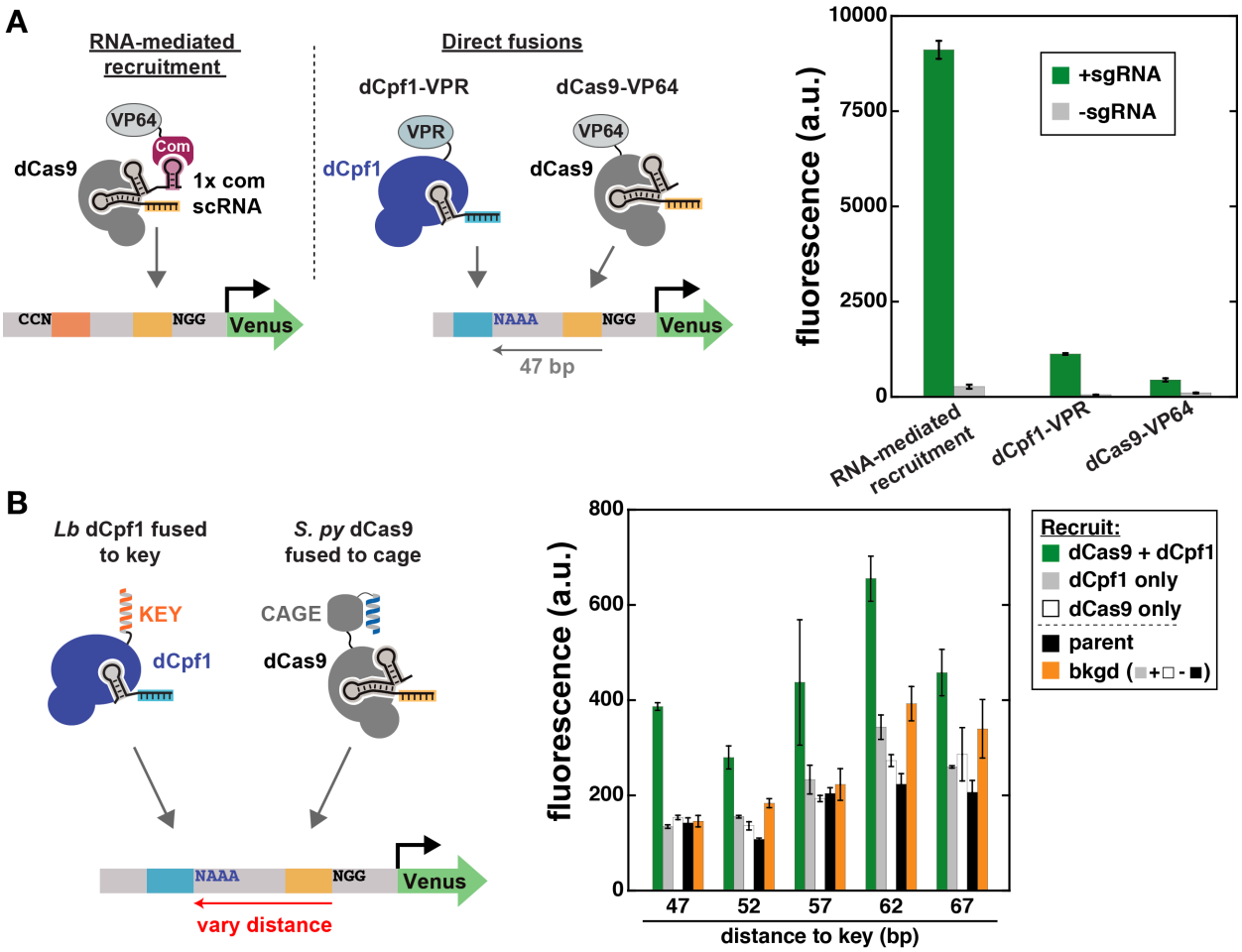

Figure S3. RNA recruitment is more effective than direct dCas9 fusions for co-LOCKR-mediated gene activation. (A) RNA recruitment is more effective than direct dCas9 fusions for reporter gene activation in yeast. Reporter gene activation with a 1x com scRNA and the Com-VP64 fusion protein was measured with the same reporter construct used for co-LOCKR-mediated activation. Reporter gene activation for dLbCpf1-VPR[2] and dCas9-VP64 was measured using a modified reporter gene construct where the upstream dCas9 PAM sites have been replaced with dCpf1-compatible PAM sites. Both dCpf1-VPR and dCas9-VP64 give significant reporter gene activation, although the values are substantially smaller than that observed with RNA-mediated recruitment of Com-VP64. This observation is consistent with prior results in yeast; in mammalian cells, dCas9 fusion proteins and RNA-mediated recruitment produce comparable reporter gene activation.[1] dCpf1-VPR is more effective than dCas9-VP64, likely because dCpf1 is fused to the tripartite VPR (VP64-p65-Rta) activator, which is more effective than VP64.[3] (B) Colocalization of dCpf1-Key and dCas9-CAGE results in significant gene activation, well above the background predicted from the sum of colocalization-independent cage opening (Fig S1). The observed reporter gene activation is substantially smaller than that observed with RNA-mediated recruitment (Fig 2). Varying the distance between dCpf1 and dCas9 has modest effects. Data for parents were obtained by cotransforming the parental reporter strains yKL069, yKL073, yKL074, yKL075, and yKL076 with the off-target guide RNA plasmids pRK020 and pKL053. Fluorescence values are mean ± SD for at least three biological replicates.

Figure S4: Varing the key-PCP linker length has no significant effect

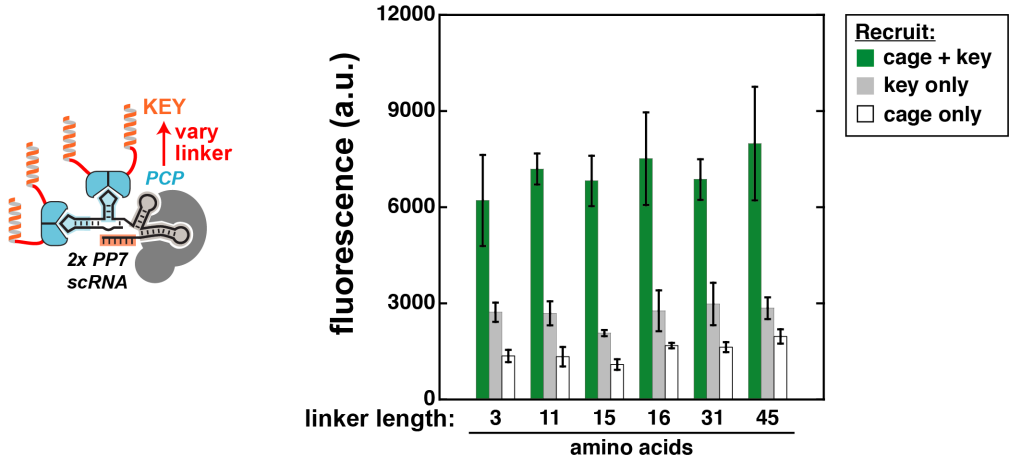

Figure S4. Varying the key-PCP linker length has no significant effect on reporter gene activation. The linker was varied between 3 and 45 amino acids (complete sequences appended below), but we observe no change in colocalization-dependent activation (cage + key) or background activation (key only, cage only). The 3 amino acid linker was used for all other experiments. Fluorescence values are mean ± SD for at least three biological replicates.

Table S1. Yeast strains

Strain[a]	Genotype	Figure
SO992	W303 *MAT**a** ura3 leu2 trp1 his3 can1R ade*	
yKL014	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	2, 3, 4, 5 S1D, S2, S4
yKL016	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-MCP-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	2, S2
yRK266	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pAdh1-Com-VP64*	S1C, S3A
yKL006	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage mfa2::Kan^R pAdh1-BCL2-VP64*	3, 4
yRK244	SO992 *trp1::pR4-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage mfa2::Kan^R pAdh1-BCL2-VP64 HO::HygB^R pAdh1-CLkey$_{44}$-PCP*	3
yRK245	SO992 *trp1::pR5-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage mfa2::Kan^R pAdh1-BCL2-VP64 HO::HygB^R pAdh1-CLkey$_{44}$-PCP*	3
yKL029	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{43}$-PCPmfa2::Kan^R pAdh1-BCL2-VP64*	5
yKL030	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{41}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	5
yKL031	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{37}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	5
yKL032	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{34}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	5
yRK456	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-MCP-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-Com mfa2::Kan^R pAdh1-BCL2-VP64*	S2
yRK273	SO992 *trp1::R6-Venus leu2::pTdh3-dCas9-VP64*	S3A
yKL079	SO992 *trp1::R6-Venus leu2::pTdh3-dLbCpf1-VPR*	S3A
yKL069	SO992 *trp1::pR6-Venus leu2::pTdh3-CLkey$_{44}$-dLbCpf1 HO::HygB^R pUra3-dCas9-2xNLS-CLcage mfa2::Kan^R pAdh1-BCL2-VP64*	S3B
pKL073	SO992 *trp1::pR6+5-Venus leu2::pTdh3-CLkey$_{44}$-dLbCpf1 HO::HygB^R pUra3-dCas9-2xNLS-CLcage mfa2::Kan^R pAdh1-BCL2-VP64*	S3B
yKL074	SO992 *trp1::pR6+10-Venus leu2::pTdh3-CLkey$_{44}$-dLbCpf1 HO::HygB^R pUra3-dCas9-2xNLS-CLcage mfa2::Kan^R pAdh1-BCL2-VP64*	S3B
yKL075	SO992 *trp1::pR6+15-Venus leu2::pTdh3-CLkey$_{44}$-dLbCpf1 HO::HygB^R pUra3-dCas9-2xNLS-CLcage mfa2::Kan^R pAdh1-BCL2-VP64*	S3B
yKL076	SO992 *trp1::pR6+20-Venus leu2::pTdh3-CLkey$_{44}$-dLbCpf1 HO::HygB^R pUra3-dCas9-2xNLS-CLcage mfa2::Kan^R pAdh1-BCL2-VP64*	S3B
yKL021	*trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pCyc1-CLkey$_{44}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	4
yKL022	*trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pUra3-CLkey$_{44}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	4
yKL023	*trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pTdh3-CLkey$_{44}$-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	4
yRK325	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-PCP mfa2::Kan^R pUra3-BCL2-VP64*	4
yRK454	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-PCP*	4
yME007	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-(11aa)-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	S4
yME002	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-(15aa)-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	S4
yME008	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-(16aa)-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	S4
yME009	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-(31aa)-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	S4

yME003	SO992 *trp1::pJ1-Venus leu2::pTdh3-dCas9 his3::pTef1-Com-CLcage HO::HygB^R pAdh1-CLkey$_{44}$-(45aa)-PCP mfa2::Kan^R pAdh1-BCL2-VP64*	S4

[a] The SO992 parental strain has been described previously.[1] The Venus fluorescent reporter gene is a derivative of GFP.[4]

Table S2. Yeast protein expression plasmids

Plasmid[a]	**Parent Vector**[b]	**Marker**	**Promoter**[c]	**Gene**	**Used for yeast strain**
pJZC518	pNH605	*leu2*	*pTdh3*	dCas9-3xNLS	yKL006, yKL014, yKL016 yKL021, yKL022, yKL023, yKL029, yKL030, yKL031, yKL032, yRK244, yRK245, yRK266, yRK325, yRK454, yRK456
pRK201	pJW609	*kanMX*	*pAdh1*	Bcl2-VP64	yKL006, yKL014, yKL016, yKL021, yKL022, yKL023, yKL029, yKL030, yKL031, yKL032, yKL069, yKL073, yKL074, yKL075, yKL076, yRK244, yRK245, yRK456
pKL054	pJW609	*kanMX*	*pUra3*	Bcl2-VP64	yRK325
pKL001	pNH603	*his3*	*pTef1*	Com-CLcage	yKL006, yKL014, yKL021, yKL022, yKL023, yKL029, yKL030, yKL031, yKL032, yRK244, yRK245, yRK325, yRK454
pKL003	pNH603	*his3*	*pTef1*	MCP-CLcage	yKL016, yRK456
pRK442	pJW607	*hphMX*	*pAdh1*	CLkey$_{44}$-Com	yRK456
pKL006	pJW607	*hphMX*	*pAdh1*	CLkey$_{44}$-PCP	yKL014, yKL016, yRK244, yRK245, yRK325, yRK454
pKL024	pJW607	*hphMX*	*pCyc1*	CLkey$_{44}$-PCP	yKL021
pKL025	pJW607	*hphMX*	*pUra3*	CLkey$_{44}$-PCP	yKL022
pKL026	pJW607	*hphMX*	*pTdh3*	CLkey$_{44}$-PCP	yKL023
pKL028	pJW607	*hphMX*	*pAdh1*	CLkey$_{43}$-PCP	yKL029
pKL029	pJW607	*hphMX*	*pAdh1*	CLkey$_{41}$-PCP	yKL030
pKL030	pJW607	*hphMX*	*pAdh1*	CLkey$_{37}$-PCP	yKL031
pKL031	pJW607	*hphMX*	*pAdh1*	CLkey$_{34}$-PCP	yKL032
pME007	pJW607	*hphMX*	*pAdh1*	CLkey$_{44}$-(11aa)-PCP	yME007
pME002	pJW607	*hphMX*	*pAdh1*	CLkey$_{44}$-(15aa)-PCP	yME002
pME008	pJW607	*hphMX*	*pAdh1*	CLkey$_{44}$-(16aa)-PCP	yME008
pME009	pJW607	*hphMX*	*pAdh1*	CLkey$_{44}$-(31aa)-PCP	yME009

pME003	pJW607	*hphMX*	*pAdh1*	CLkey$_{44}$-(45aa)-PCP	yME003
pRK271	pNH604	*trp1*	*pJ1*	Venus	yKL006, yKL014, yKL016, yKL029, yKL030, yKL031, yKL032, yKL021, yKL022, yKL023, yRK266, yRK325, yRK454, yRK456
pRK339	pNH604	*trp1*	*pR4*	Venus	yRK244
pRK340	pNH604	*trp1*	*pR5*	Venus	yRK245
pRK369	pNH604	*trp1*	*pR6*	Venus	yKL069, yKL079, yRK273
pKL065	pNH604	*trp1*	*pR6+5*	Venus	yKL073
pKL066	pNH604	*trp1*	*pR6+10*	Venus	yKL074
pKL067	pNH604	*trp1*	*pR6+15*	Venus	yKL075
pKL068	pNH604	*trp1*	*pR6+20*	Venus	yKL076
pKL055	pJW607	*hphMX*	*pUra3*	dCas9-2xNLS-CLcage	yKL069, yKL073, yKL074, yKL075, yKL076
pKL058	pNH605	*leu2*	*pTdh3*	CLkey$_{44}$-dLbCpf1	yKL069, yKL073, yKL074, yKL075, yKL076
pJZC506	pNH603	*his3*	*pAdh1*	Com-VP64	yRK266
pJZC527	pNH605	*leu2*	*pTdh3*	dCas9-VP64	yRK273
pRK364	pNH605	*leu2*	*pTdh3*	dLbCpf1-VPR	yKL079

[a] pJZC518 and pJZC506 have been described previously.[1] pKL058 and pRK364 were cloned from addgene #104567;[5] dLbCpf1-VPR expression in yeast has been previously described.[2]

[b] The parent vectors used in this work are designed to integrate in single copy in the yeast genome. The pNH600 and pJW600 series of vectors have been described previously.[6,7] Vectors containing auxotrophic markers (*leu2*, *his3, trp1*) integrate at the corresponding endogenous locus. The pJW609 parent vector (kanMX marker) integrates at the *mfa2* locus and confers G418 resistance (*Kan*^{R}). The pJW607 parent vector (hphMX marker) integrates at the HO locus and confers hygromycin B resistance (*HygB*^{R}). Each of these vectors uses the same *C. alb. Adh1* terminator.

[c] *S. cerevisiae* promoter pTdh3 is among the strongest yeast promoters. pAdh1 and pTef1 produce similar expression levels and are relatively strong, but somewhat weaker than pTdh3. pUra3 is a relatively weak promoter.[8,9] The minimal 247 bp pCyc1 promoter is the weakest promoter used here.[10] Based on microarray data, the endogenous pTdh3 and pUra3 promoters vary in expression level by >100-fold.[9] Complete reporter gene promoter sequences for pJ1, pR4, pR5, and pR6 derivatives are appended below.

Table S3. Guide RNA expression plasmids[a]

Plasmid	Parent Vector	sgRNA Target Site[b]	sgRNA/scRNA Design[c]	Figure
pKL011	pRS316	1) J4 2) J5	1) 2x MS2 scRNA 2) 2x PP7 scRNA	2, S2
pKL013	pRS316	1) J4 2) J5	1) 1x com scRNA 2) 2x PP7 scRNA	2, 3, 4, 5, S1D, S2, S4
pKL014	pRS316	1) J4 2) J6	1) 1x com scRNA 2) 2x PP7 scRNA	3
pRK323	pRS316	1) J4 2) J7	1) 1x com scRNA 2) 2x PP7 scRNA	3
pRK324	pRS316	1) J4 2) J8	1) 1x com scRNA 2) 2x PP7 scRNA	3
pRK325	pRS316	1) J4 2) J9	1) 1x com scRNA 2) 2x PP7 scRNA	3
pRK326	pRS316	1) J4 2) J10	1) 1x com scRNA 2) 2x PP7 scRNA	3
pKL040	pRS316	1) J4 2) J5	1) 1x com scRNA 2) 1x PP7 scRNA	S2
pRK433	pRS316	1) J4 2) J5	1) 1x MS2 scRNA 2) 2x PP7 scRNA	S2
pRK440	pRS316	1) J4 2) J5	1) 2x MS2 scRNA 2) 1x PP7 scRNA	S2
pKL047	pRS316	1) J4 2) J5	1) 1x MS2 scRNA 2) 1x PP7 scRNA	S2
pRK441	pRS316	1) J4 2) J5	1) 1x MS2 scRNA 2) 1x com scRNA	S2
pRK290	pRS316	1) J4 2) J5	1) 2x MS2 scRNA 2) 1x com scRNA	S2
pRK275	pRS316	J4	2x MS2 scRNA	2, S2
pKL038	pRS316	J4	1x MS2 scRNA	S2
pKL016	pRS316	J4	1x com scRNA	2, 3, 4, 5, S1D, S2, S3, S4
pKL048	pRS316	J5	1x com scRNA	S1C, S2
pKL036	pRS316	1) J4 2) J5	1) 1x com scRNA 2) 1x com scRNA	S1C
pKL018	pRS316	J5	2x PP7 scRNA	2, 3, 4, 5, S1D, S2, S4
pKL019	pRS316	J6	2x PP7 scRNA	3
pME010	pRS316	J7	2x PP7 scRNA	3
pME011	pRS316	J8	2x PP7 scRNA	3
pME012	pRS316	J9	2x PP7 scRNA	3
pME013	pRS316	J10	2x PP7 scRNA	3
pKL039	pRS316	J5	1x PP7 scRNA	S2
pRK373	pRS313	TET	sgRNA	S3
pRK020	pRS313	W17 (off target control)	sgRNA	S3
pRK375	pRS316	R6	sgRNA (LbCpf1)	S3
pKL053	pRS316	TET_2 (off target control)	sgRNA (LbCpf1)	S3

[a] Guide RNA constructs were expressed from the pRS316 (*ura3* marker) or pRS313 (*his3* marker) CEN/ARS plasmid backbones with the SNR52 promoter and a SUP4 terminator.[1,11,12] sgRNA/scRNA designs and corresponding sequences have been described previously.[1,2,13] Guide RNA target sequences are provided in Table S4. All sgRNA and scRNA constructs (sgRNAs with 3' RNA recruitment domains)[1] are *S. py* Cas9 guide sequences unless otherwise noted.

Table S4. Guide RNA target sites

sgRNA	DNA Sequence	CRISPR system	Reporter[a]
J4	CGGTGTCCTGCGGTTACCAA	*S. py dCas9*	pJ1, pR4, pR5
J5	AGGTCGCCCGTGGTGGCCCA	*S. py dCas9*	pJ1, pR4, pR5
J6	TGGTGGCCCATGGTCACCAT	*S. py dCas9*	pJ1, pR4, pR5
J7	TGGTCACCATAGGTCACCCT	*S. py dCas9*	pJ1
J8	AGGTCACCCTTGGCAACCAA	*S. py dCas9*	pJ1
J9	AGGTCTCCGGTGGATACCGT	*S. py dCas9*	pJ1
J10	CCGGATCAAGATTGTACGTA	*S. py dCas9*	pJ1
W17	GAAGTCAGTTGACAGAGTCG	*S. py dCas9*	*off target control*
TET	ACTTTTCTCTATCACTGATA	*S. py* dCas9	pR6-Venus
R6	CGGTGCAAAGCAAAGTAAAG	*Lb dCpf1*	pR6-Venus
TET_2	CCACTCCCTATCAGTGATAGAGA	*Lb dCpf1*	*off target control*

[a] J4 is used to recruit the cage to the reporter. J5-10 are upstream target sites separated from J4 by 51, 61, 71, 81, 131, and 1671 bases respectively in the pJ1 reporter. The pR4 promoter has a 3 base insertion to access separation distances 54 and 64 bases (with J5 and J6). The pR5 promoter has a 5 base insertion to access separation distances 56 and 66 bases (with J5 and J6). Complete reporter gene promoter sequences for pJ1, pR4, pR5, and pR6 are appended below.

The target site separation distances are defined as the distance between PAM sites:

51 base separation for dCas9 target sites in pJ1:

```
CCN(N20)nnnnnnnnnn(N20)NGG
        ___________________
            (51 bases)
```

47 base separation for dCpf1 and dCas9 target sites in pR6:

```
(N20)TAAAnnnnnnnnnnnnnnnnnnnnnn(N20)NGG
          ___________________________
                (47 bases)
```

Reporter Gene Promoters

All reporter gene promoters contain an array of gRNA target sites, a pCyc1 promoter fragment (bases -147 to -4, lowercase letters), an ATG start codon, and a Venus fluorescent reporter gene (annotated in green, only first 18 bases shown).

Promoters to vary distance between dCas9 target sites (Figure 2):

J4 (CGGTGTCCTGCGGTTACCAA), J5 (TGGGCCACCACGGGCGACCT), and J6 (**ATGGTGACCATGGGCCACCA**) sequences are annotated.

>pJ1

GCCTACGGTATCCACCGGAGACCTATGGCAGCCTCCGGCCGCCATAGGACACCTTTGGTTGCCAAGGGTGACCT**ATG**
GTGACCATGGGCCACCACGGGCGACCTCAGGTATCCTGCGGTGTCCTGCGGTTACCAAAGGCGTCCTTTGGGTTCCA
CCGGATACCTCCGGAAAGTGAAAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaactctt
gttttcttctttctctaaatattctttccttatacattaggtcctttgtagcataaattactatacttctatagac
acgcaaacacaaatacacacactaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA…

>pR4

GCCTACGGTATCCACCGGAGACCTATGGCAGCCTCCGGCCGCCATAGGACACCTTTGGTTGCCAAGGGTGACCT**ATG**
GTGACCATGGGCCACCACGGGCGACCTGACCAGGTATCCTGCGGTGTCCTGCGGTTACCAAAGGCGTCCTTTGGGTT
CCACCGGATACCTCCGGAAAGTGAAAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaact
cttgttttcttctttctctaaatattctttccttatacattaggtcctttgtagcataaattactatacttctata
gacacgcaaacacaaatacacacactaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA…

>pR5

GCCTACGGTATCCACCGGAGACCTATGGCAGCCTCCGGCCGCCATAGGACACCTTTGGTTGCCAAGGGTGACCT**ATG**
GTGACCATGGGCCACCACGGGCGACCTCTGACCAGGTATCCTGCGGTGTCCTGCGGTTACCAAAGGCGTCCTTTGGG
TTCCACCGGATACCTCCGGAAAGTGAAAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaa
ctcttgttttcttctttctctaaatattctttccttatacattaggtcctttgtagcataaattactatacttcta
tagacacgcaaacacaaatacacacactaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA
…

Promoters to vary distance between dCpf1 and dCas9 target sites (Figure S3)

R6 (CTTTACTTTGCTTTGCACCG) and TET (ACTTTTCTCTATCACTGATA) gRNA target sequences are annotated.

>pR6

CTTTACTTTGCTTTGCACCGTAAACGTAAAGGAAACGCACTCAGGATACTTTTCTCTATCACTGATACGGAAAGTGA
AAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaactcttgttttcttctttttctctaaat
attctttccttatacattaggtcctttgtagcataaattactatacttctatagacacgcaaacacaaatacacaca
ctaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA…

> pR6+5

CTTTACTTTGCTTTGCACCGTAAACGTAAAGGAAACGATTCGCACTCAGGATACTTTTCTCTATCACTGATACGGAA
AGTGAAAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaactcttgttttcttctttttctc
taaatattctttccttatacattaggtcctttgtagcataaattactatacttctatagacacgcaaacacaaatac
acacactaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA…

> pR6+10

CTTTACTTTGCTTTGCACCGTAAACGTAAAGGAAACGATTGCTACCGCACTCAGGATACTTTTCTCTATCACTGATA
CGGAAAGTGAAAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaactcttgttttcttctt
ttctctaaatattctttccttatacattaggtcctttgtagcataaattactatacttctatagacacgcaaacaca
aatacacacactaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA…

> pR6+15

CTTTACTTTGCTTTGCACCGTAAACGTAAAGGAAACGATTGCTACTCCAGCGCACTCAGGATACTTTTCTCTATCAC
TGATACGGAAAGTGAAAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaactcttgttttc
ttcttttctctaaatattctttccttatacattaggtcctttgtagcataaattactatacttctatagacacgcaa
acacaaatacacacactaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA…

> pR6+20

CTTTACTTTGCTTTGCACCGTAAACGTAAAGGAAACGATTGCTACTCCAGCCAAGCGCACTCAGGATACTTTTCTCT
ATCACTGATACGGAAAGTGAAAGTCGAGCTCGGTACCCtatggcatgcatgtgctctgtatgtatataaaactcttg
ttttcttctttttctctaaatattctttccttatacattaggtcctttgtagcataaattactatacttctatagaca
cgcaaacacaaatacacacactaaattaCCCGGATCAATTCGGGATGCTCGAGTCTAAAGGTGAAGAATTA…

Protein Sequences

Transcriptional activator

> NLS-Bcl2-linker-VP64

MPKKKRKVMAHAGRTGYDNREIVMKYIHYKLSQRGYEWDAGDVGAAPPGAAPAPGIFSSQPGHTPHPAASRDPVART
SPLQTPAAPGAAAGPALSPVPPVVHLTLRQAGDDFSRRYRRDFAEMSSQLHLTPFTARGRFATVVEELFRDGVNWGR
IVAFFEFGGVMCVESVNREMSPLVDNIALWMTEYLNRHLHTWIQDNGGWDAFVELYGPSMRGSGRADALDDFDLDML
GSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLIN

Cage constructs fused to RNA binding proteins

>NLS-linker-MCP-linker-CLcage

MPKKKRKVGSMASNFTQFVLVDNGGTGDVTVAPSNFANGIAEWISSNSRSQAYKVTCSVRQSSAQNRKYTIKVEVPK
GAWRSYLNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANSGIYGSGSGSELARKLLEASTKLQRLNIRLA
EALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDAAKESEKILEEAREAISGSGSEL
AKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIREALEHAKRRSKEIIDEAERAIRAAKRE
SERIIEEARRLIEKGSGSGSELARELLRAHAQLQRLNLELLRELLRALAQLQELNLDLLRLASELTDEIWIAQELRR
IGDEFNAYYADAERLIREAAAASEKISREAERLIR

>NLS-linker-Com-linker-CLcage

MPKKKRKVGSMKSIRCKNCNKLLFKADSFDHIEIRCPRCKRHIIMLNACEHPTEKHCGKREKITHSDETVRYGSGSG
SELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRRAEKEIDDA
AKESEKILEEAREAISGSGSELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPATIREALEH
AKRRSKEIIDEAERAIRAAKRESERIIEEARRLIEKGSGSGSELARELLRAHAQLQRLNLELLRELLRALAQLQELN
LDLLRLASELTDEIWIAQELRRIGDEFNAYYADAERLIREAAAASEKISREAERLIR

Key constructs fused to the PCP RNA binding protein

Key truncations are shown in Figure 4 and extended linkers are shown in Figure S4. The 15 and 45 amino acid linkers are derived from the disordered tether from the regulatory domain of PKA, which is well-characterized structurally and biochemically.[14] The 11, 16, and 31 amino acid linkers are derived from the XTEN linker, a flexible, extended polypeptide.[15,16]

Vary key length:

>NLS-linker-CLkey$_{44}$-linker-PCP *(44 aa key)*

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIRGSGMSKTIVLSVGEATRTLTE
IQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHDVTIVANSTEASRK
SLYDLTKSLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{43}$-linker-PCP *(43 aa key)*

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLKGSGMSKTIVLSVGEATRTLTEI
QSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHDVTIVANSTEASRKS
LYDLTKSLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{41}$-linker-PCP *(41 aa key)*

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERGSGMSKTIVLSVGEATRTLTEIQS
TADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHDVTIVANSTEASRKSLY
DLTKSLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{37}$-linker-PCP

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISRGSGMSKTIVLSVGEATRTLTEIQSTADR
QIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHDVTIVANSTEASRKSLYDLTK
SLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{34}$-linker-PCP

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKGSGMSKTIVLSVGEATRTLTEIQSTADRQIF
EEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHDVTIVANSTEASRKSLYDLTKSLV
ATSQVEDLVVNLVPLGR

Vary key-PCP linker length:

> NLS-linker-CLkey$_{44}$-11aalinker-PCP

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIRGSETPGTSESAMSKTIVLSVG
EATRTLTEIQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHDVTIVA
NSTEASRKSLYDLTKSLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{44}$-15aalinker-PCP

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIRRQESDTFIVSPTTFHMSKTIV
LSVGEATRTLTEIQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHDV
TIVANSTEASRKSLYDLTKSLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{44}$-16aalinker-PCP

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIRSGSETPGTSESATPESMSKTI
VLSVGEATRTLTEIQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVDSGLPKVRYTQVWSHD
VTIVANSTEASRKSLYDLTKSLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{44}$-31aalinker-PCP

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIRSGSETPGTSESATPESGSETP
GTSESATPESMSKTIVLSVGEATRTLTEIQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAYRVNLKLDQADVVD
SGLPKVRYTQVWSHDVTIVANSTEASRKSLYDLTKSLVATSQVEDLVVNLVPLGR

> NLS-linker-CLkey$_{44}$-45aalinker-PCP

MPKKKRKVGSGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIRRQESDTFIVSPTTFHTQESSA
VPVIEEDGESDSDSEDADLEVPVPMSKTIVLSVGEATRTLTEIQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTA
YRVNLKLDQADVVDSGLPKVRYTQVWSHDVTIVANSTEASRKSLYDLTKSLVATSQVEDLVVNLVPLGR

dCas9 fused to cage

> dCas9-2xNLS-linker-CLcage

MLEDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRR
KNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLR
LIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIAQL
PGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDIL
RVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM
DGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNS
RFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKP
AFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENED
ILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANR
NFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQ
TTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDAIVPQS
FLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLV
ETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIK
KYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDK
GRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGK
SKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPS
KYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAE
NIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDEGADPKKKRKVDPKKK
RKVGSGSGSELARKLLEASTKLQRLNIRLAEALLEAIARLQELNLELVYLAVELTDPKRIRDEIKEVKDKSKEIIRR
AEKEIDDAAKESEKILEEAREAISGSGSELAKLLLKAIAETQDLNLRAAKAFLEAAAKLQELNIRAVELLVKLTDPA
TIREALEHAKRRSKEIIDEAERAIRAAKRESERIIEEARRLIEKGSGSGSELARELLRAHAQLQRLNLELLRELLRA
LAQLQELNLDLLRLASELTDEIWIAQELRRIGDEFNAYYADAERLIREAAAAASEKISREAERLIR

dCpf1 fused to key

> NLS-linker-CLkey$_{44}$-dLbCpf1-NLS

LEPKKKRKVGSGPGSDEARKAIARVKRESKRIVEDAERLIREAAAASEKISREAERLIRGSGSGSKLEKFTNCYSLS
KTLRFKAIPVGKTQENIDNKRLLVEDEKRAEDYKGVKKLLDRYYLSFINDVLHSIKLKNLNNYISLFRKKTRTEKEN
KELENLEINLRKEIAKAFKGNEGYKSLFKKDIIETILPEFLDDKDEIALVNSFNGFTTAFTGFFDNRENMFSEEAKS
TSIAFRCINENLTRYISNMDIFEKVDAIFDKHEVQEIKEKILNSDYDVEDFFEGEFFNFVLTQEGIDVYNAIIGGFV
TESGEKIKGLNEYINLYNQKTKQKLPKFKPLYKQVLSDRESLSFYGEGYTSDEEVLEVFRNTLNKNSEIFSSIKKLE
KLFKNFDEYSSAGIFVKNGPAISTISKDIFGEWNVIRDKWNAEYDDIHLKKKAVVTEKYEDDRRKSFKKIGSFSLEQ
LQEYADADLSVVEKLKEIIIQKVDEIYKVYGSSEKLFDADFVLEKSLKKNDAVVAIMKDLLDSVKSFENYIKAFFGE
GKETNRDESFYGDFVLAYDILLKVDHIYDAIRNYVTQKPYSKDKFKLYFQNPQFMGGWDKDKETDYRATILRYGSKY
YLAIMDKKYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFSKKWMAYYNPSEDIQKIYKNGTFKKGDMFNLN
DCHKLIDFFKDSISRYPKWSNAYDFNFSETEKYKDIAGFYREVEEQGYKVSFESASKKEVDKLVEEGKLYMFQIYNK
DFSDKSHGTPNLHTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVVHPANSPIANKNPDNPKKTTTLSYDVY
KDKRFSEDQYELHIPIAINKCPKNIFKINTEVRVLLKHDDNPYVIGIARGERNLLYIVVVDGKGNIVEQYSLNEIIN
NFNGIRIKTDYHSLLDKKEKERFEARQNWTSIENIKELKAGYISQVVHKICELVEKYDAVIALEDLNSGFKNSRVKV
EKQVYQKFEKMLIDKLNYMVDKKSNPCATGGALKGYQITNKFESFKSMSTQNGFIFYIPAWLTSKIDPSTGFVNLLK
TKYTSIADSKKFISSFDRIMYVPEEDLFEFALDYKNFSRTDADYIKKWKLYSYGNRIRIFRNPKKNNVFDWEEVCLT
SAYKELFNKYGINYQQGDIRALLCEQSDKAFYSSFMALMSLMLQMRNSITGRTDVDFLISPVKNSDGIFYDSRNYEA
QENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKVKIAISNKEWLEYAQTSVKHASKRPAATKKAGQAKKK

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