How to review a paper

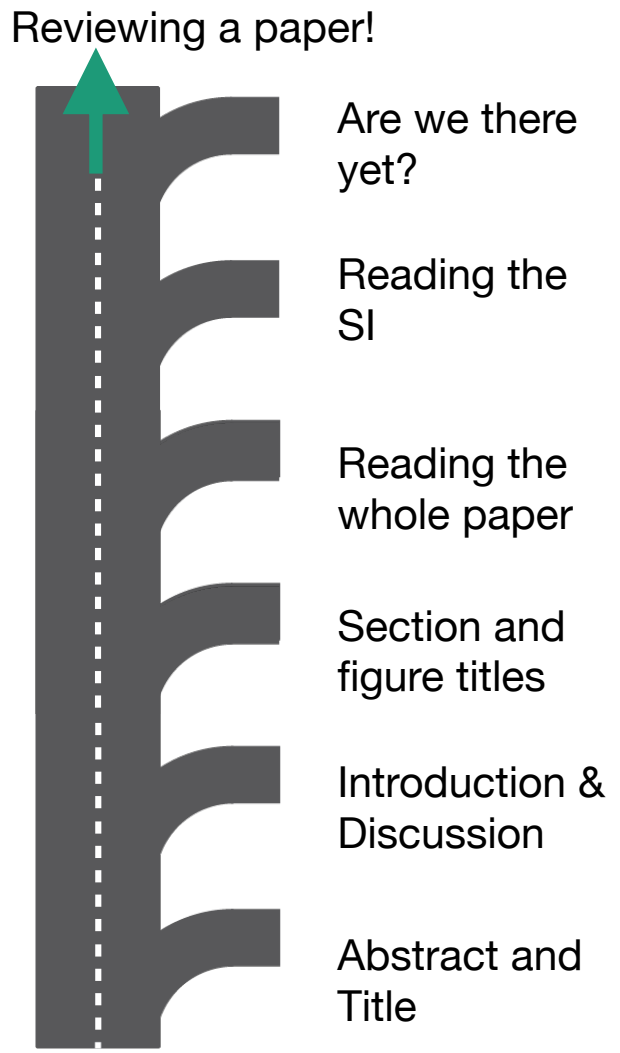
UROP Training

Kasey Love

Adapted from Christopher Johnstone

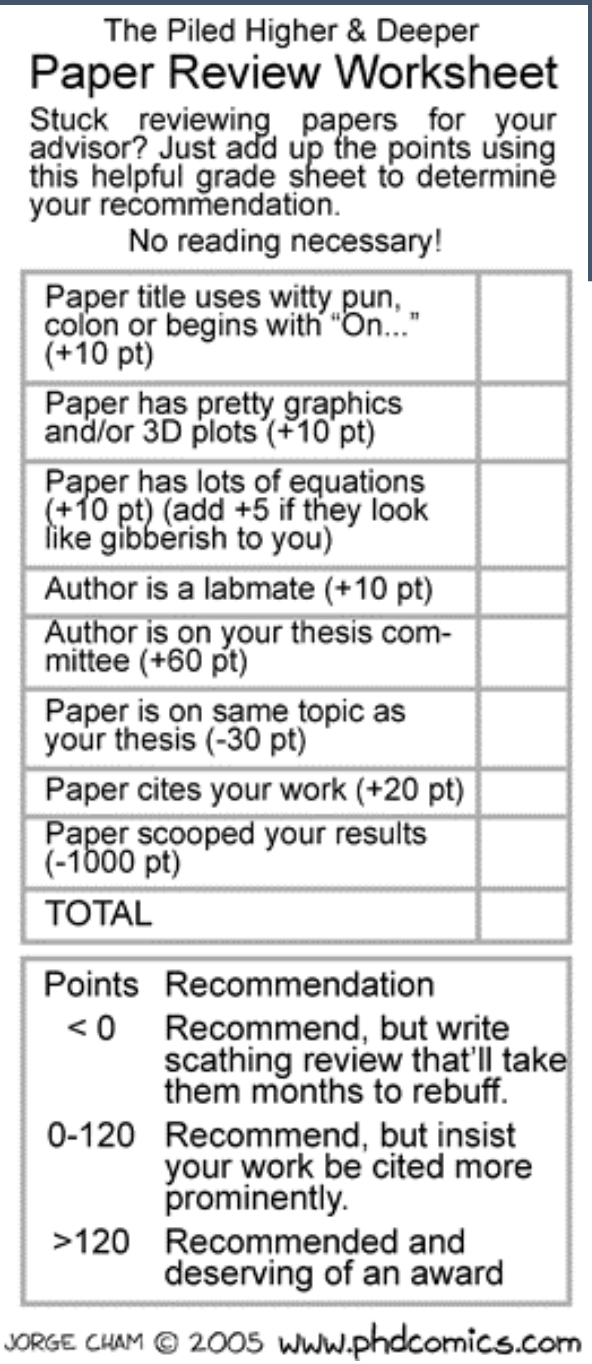
What do you mean, review a paper?

- Step one: identify a paper worth reviewing by reading!
- “Reviewing” is a more involved engagement with a paper
- It typically involves:
  - **Situating** the paper in the broader field
  - Contextualizing for **your audience**
  - **Criticizing and evaluating** the work
  - Concisely telling **their story**
- Specifics depend on the type of review you are doing



Some types of review

	“News and Views”	**Journal club**	**Paper peer review**
Added context	+++	++	+, as needed
Context audience	General journal readership	Research group	Specific field
Evaluation	+	++	++++



The Piled Higher & Deeper

Paper Review Worksheet

Stuck reviewing papers for your advisor? Just add up the points using this helpful grade sheet to determine your recommendation.

No reading necessary!

Paper title uses witty pun, colon or begins with “On...” (+10 pt)	
Paper has pretty graphics and/or 3D plots (+10 pt)	
Paper has lots of equations (+10 pt) (add +5 if they look like gibberish to you)	
Author is a labmate (+10 pt)	
Author is on your thesis committee (+60 pt)	
Paper is on same topic as your thesis (-30 pt)	
Paper cites your work (+20 pt)	
Paper scooped your results (-1000 pt)	
TOTAL	

Points	Recommendation
< 0	Recommend, but write scathing review that’ll take them months to rebuff.
0-120	Recommend, but insist your work be cited more prominently.
>120	Recommended and deserving of an award

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A suggested recipe for journal club

1. **Read** the paper, including looking at the SI
2. Write a **summary**, including highlights/limitations/etc.
3. From the discussion and intro sections, identify needed **extra context** to understand the problem/solution (engineering) or observation/hypothesis (science)
4. Decide how to **group figures** together to tell the story
5. While thinking through the story, **identify limitations** and alternative hypotheses

An example

ACS SyntheticBiology

pubs.acs.org/synthbio

Research Article

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*

Cite This: *ACS Synth. Biol.* 2020, 9, 2316–2323

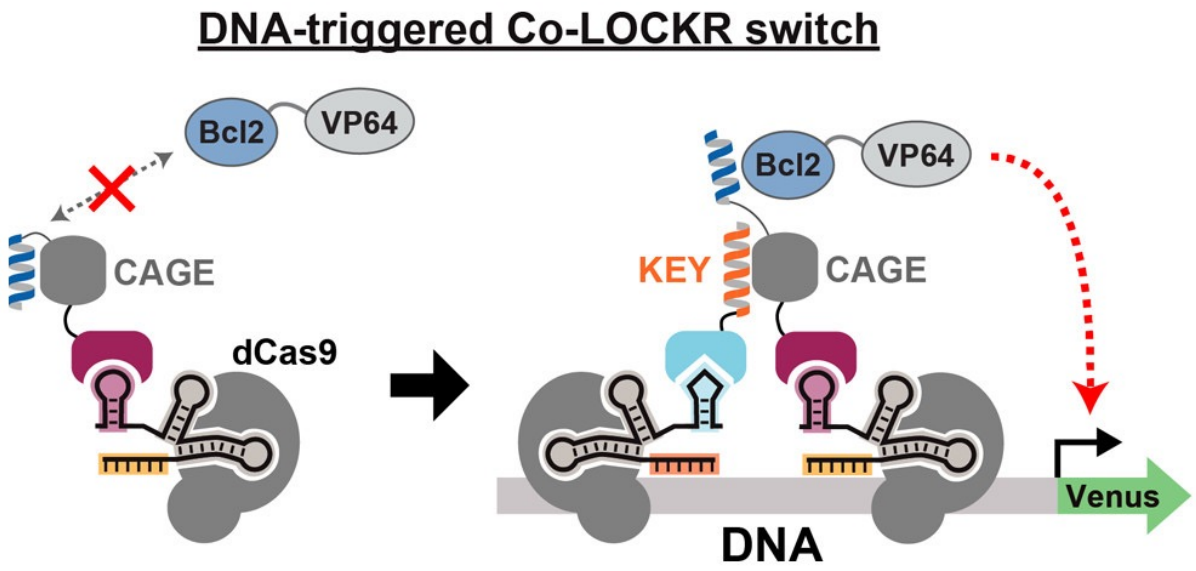
Read Online

Start with the summary

- Orient the audience to key points: What did the paper do, and why should we care?
- Can use various frameworks, such as:
  - Highlights - Limitations – Relevance
  - Summary – Weaknesses – Open Questions
- Can organize as an outline for the presentation

CRISPR-Cas + Co-LOCKR induces gene activation with reduced background

- Highlights: Colocalization of two CRISPR-Cas complexes opens the Co-LOCKR switch and allows for binding of an activation domain, triggering expression of a reporter gene. This system decreases off-target effects of effectors activated by DNA binding.
- Limitations: Low fold-change activation, requires two DNA target sites spaced appropriately apart, and contains many components



- Relevance: Could be used to decrease background for epigenetic modifiers, improve formation of long-range DNA loops, or implement AND logic

Provide relevant context

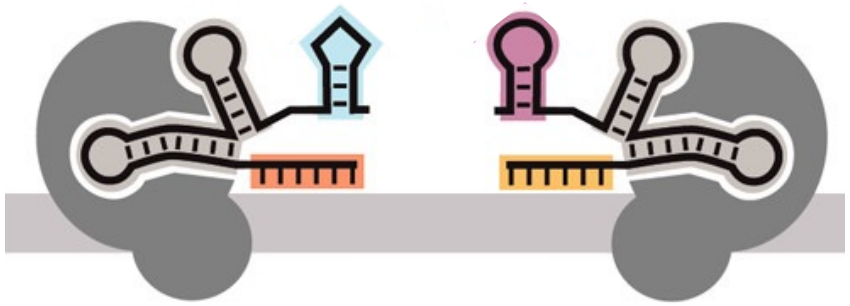
- Motivation: What problem are the authors trying to solve? Or, what question are they trying to answer?
  - Beginning the presentation with background on the engineering challenge or scientific question can help convey to the audience **why** the technology/findings are interesting
- Previous work: What prior research does the paper use or build on?
  - Citations in the paper can provide sources for relevant info and graphics (e.g., overview of a system, details on key molecules/pathways)
- Similar/competing work: Have others employed alternative approaches to the same problem/question? How is this work different?
- Can also include explanations of important techniques/technologies, depending on audience background

Off-target effects can occur when unbound effector proteins are functional

- Effector proteins that act when bound to DNA can have nonspecific effects
  - e.g., off-target epigenetic modification, high background imaging signal
- Goal: engineer a **DNA-triggered** effector protein with **reduced off-target effects**
- Solution: effector activity is dependent on co-localization of two **CRISPR-Cas** complexes via **Co-LOCKR** switch
  - Engineer Co-LOCKR affinity such that colocalization is required to open the switch
  - Off-target colocalization is very rare, so nonspecific effector activation should be minimal

CRISPR-Cas complexes provide DNA specificity

- dCas9: catalytically inactive CRISPR effector protein, binds to DNA location specified by guide RNA
- scRNA: scaffold guide RNA, CRISPR guide RNA with 3’ hairpins that recruit RNA binding proteins

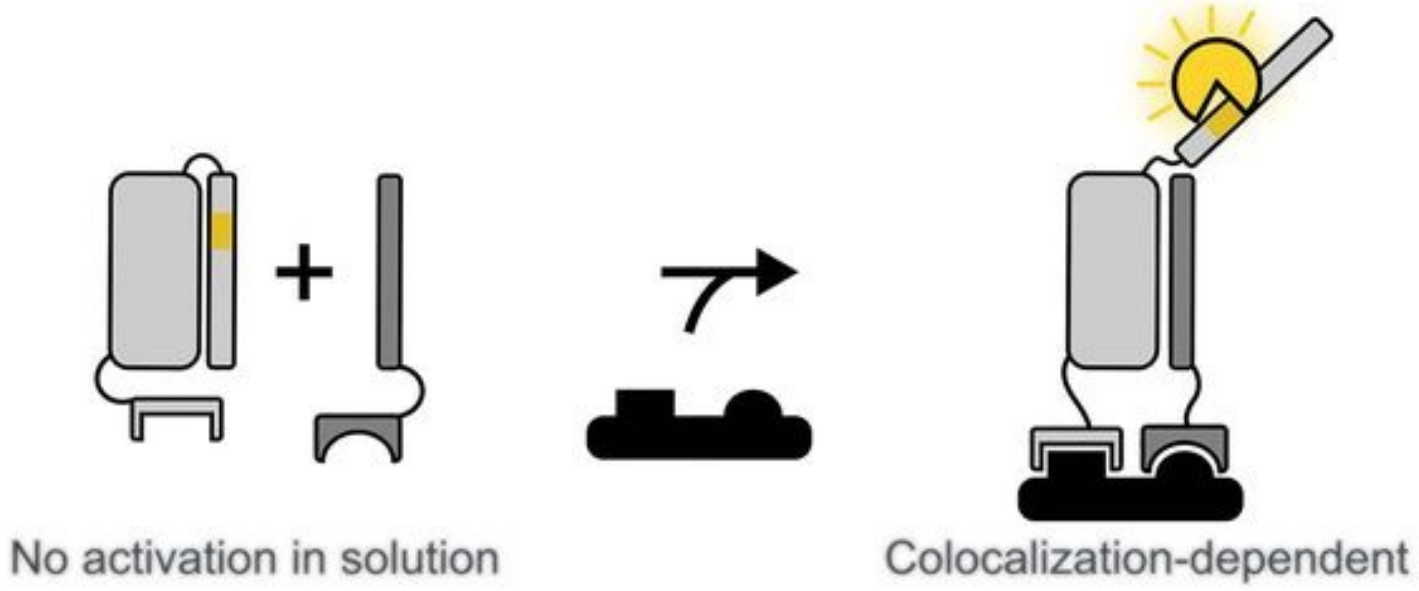


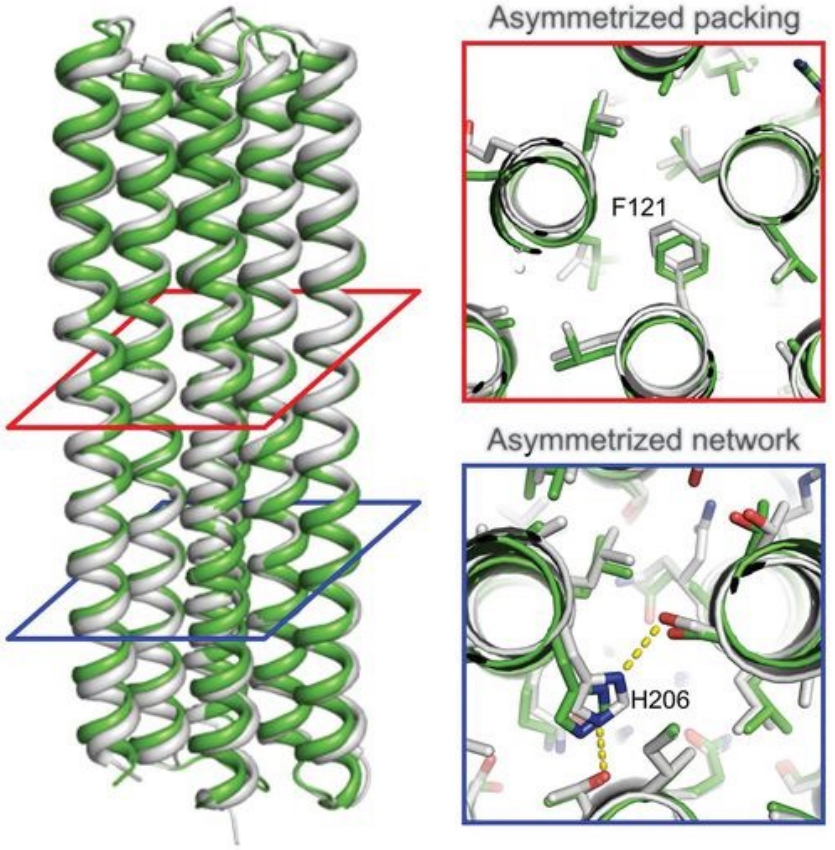
DNA
dCas9
scRNA
- spacer sequences
- RNA binding protein hairpins

Co-LOCKR switch allows activator binding upon colocalization

Co-LOCKR: colocalization-dependent latching orthogonal cage-key

- *de novo* designed alpha-helical protein switch



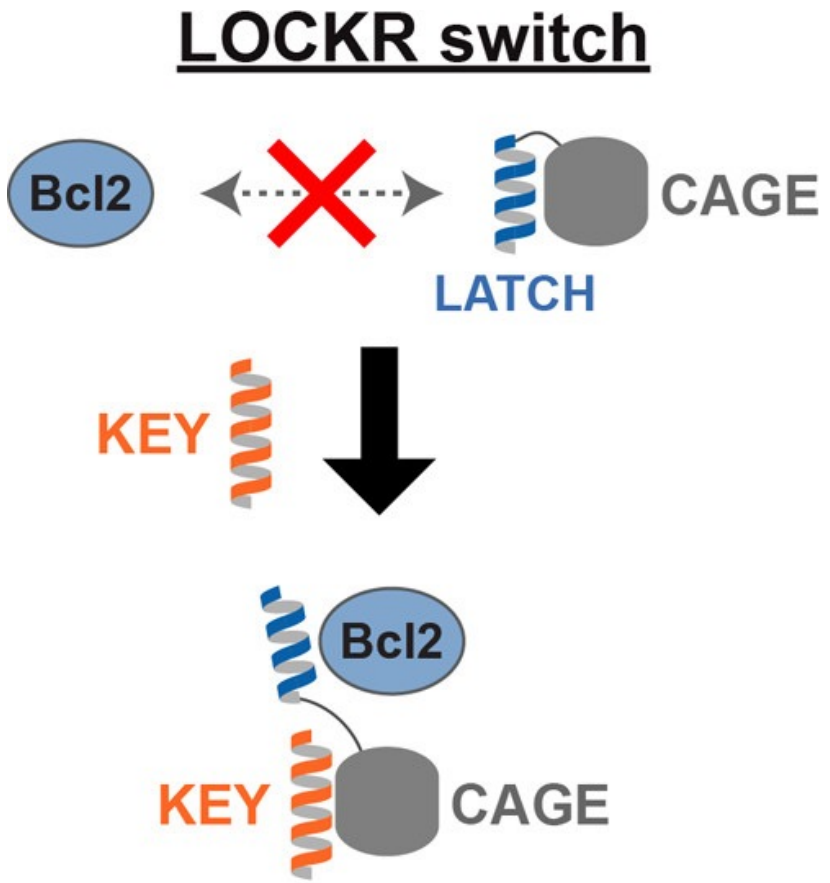


Lajoie MJ*, Boyken SE*, Salter AI*, et al. *Science* **369** (2020).

Co-LOCKR switch allows activator binding upon colocalization

Co-LOCKR: colocalization-dependent latching orthogonal cage-key

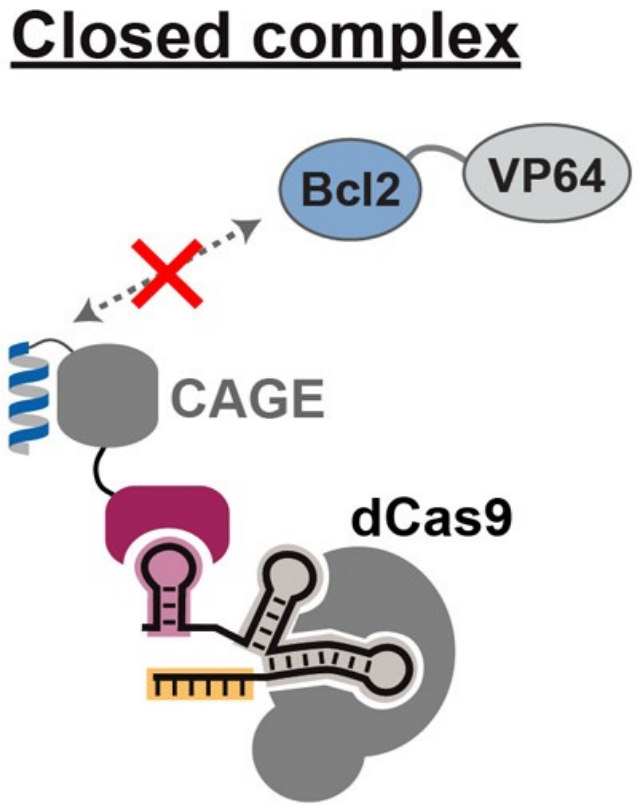
- KEY binds CAGE and displaces LATCH, permitting Bcl2 to bind LATCH



Co-LOCKR switch allows activator binding upon colocalization

Co-LOCKR: colocalization-dependent latching orthogonal cage-key

- KEY binds CAGE and displaces LATCH, permitting Bcl2 to bind LATCH
- CAGE and KEY are fused to different RNA binding proteins that bind to scRNA, exposing LATCH only upon CRISPR colocalization

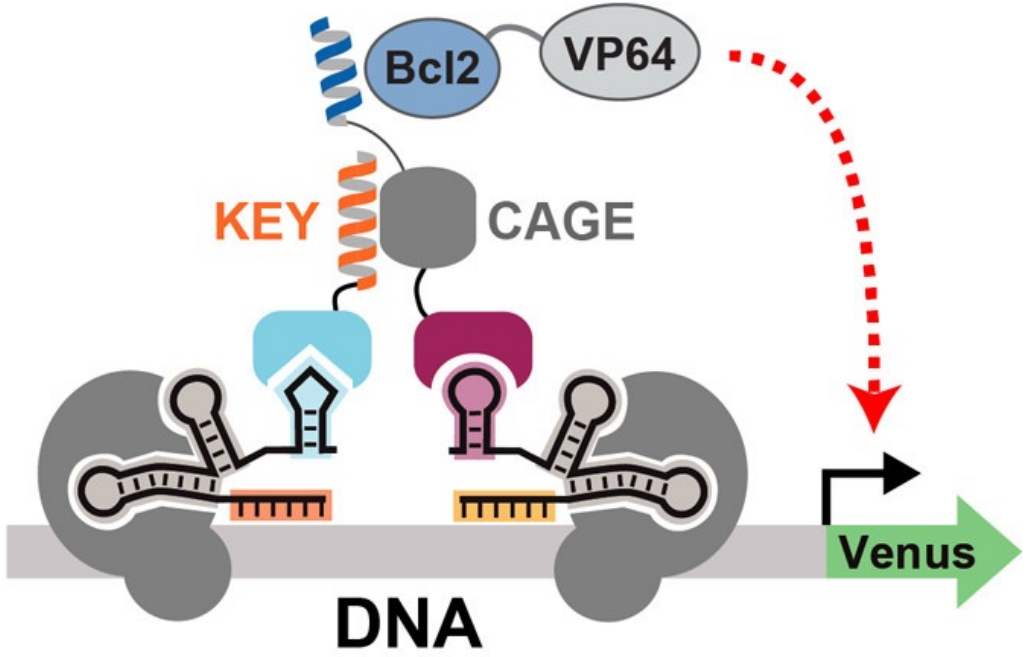


Co-LOCKR switch allows activator binding upon colocalization

Co-LOCKR: colocalization-dependent latching orthogonal cage-key

- KEY binds CAGE and displaces LATCH, permitting Bcl2 to bind LATCH
- CAGE and KEY are fused to different RNA binding proteins that bind to scRNA, exposing LATCH only upon CRISPR colocalization
- Bcl2 is fused to VP64 (transcriptional activator) and binds to LATCH, activating target gene expression

Colocalized open complex



Tell the story

- Think about how to group the figures into logical parts
  - Often, subsection headings and major claims are a good place to start
  - Usually in the order presented in the paper, but sometimes not
- Decide what info/figures from the SI are important (if any)
  - Can always make extra slides in case questions come up
- Determine whether any new graphics are necessary
  - e.g., redraw or reorganize confusing diagrams, add animations or annotations to existing figures

Claims: Section headings

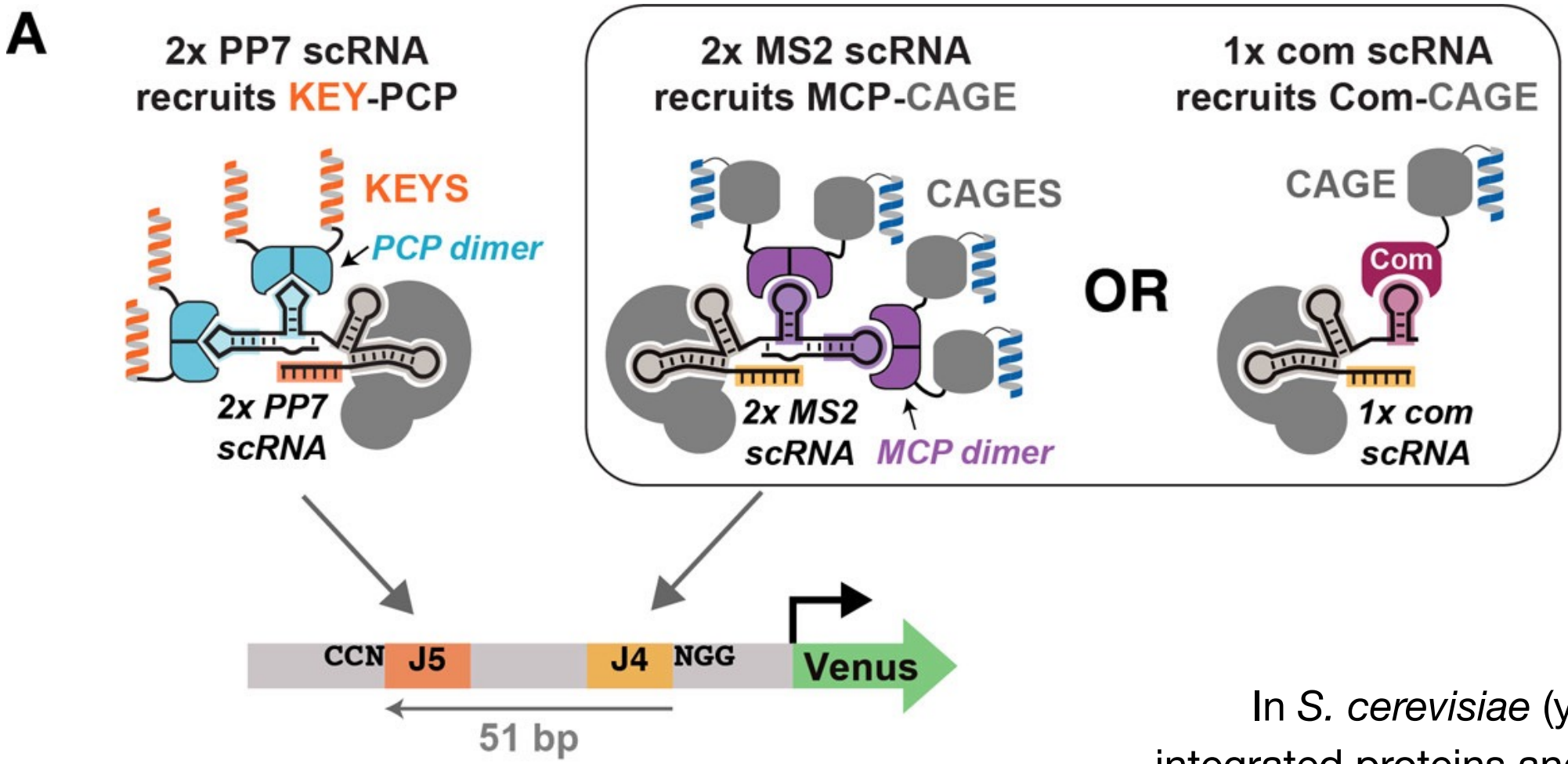
- Colocalization on genomic DNA can activate a Co-LOCKR switch (Fig. 2, S1, S2)

- Direct protein fusions to orthogonal CRISPR-Cas complexes can activate a Co-LOCKR switch (Fig. S3)
- Switch activation is sensitive to the distance between the CRISPR-Cas complexes (Fig. 3, S4)
- Optimization of the Com-cage RNA-mediated Co-LOCKR switch (Fig. 4, 5; T. S2)

Main demonstration of function + background calculation

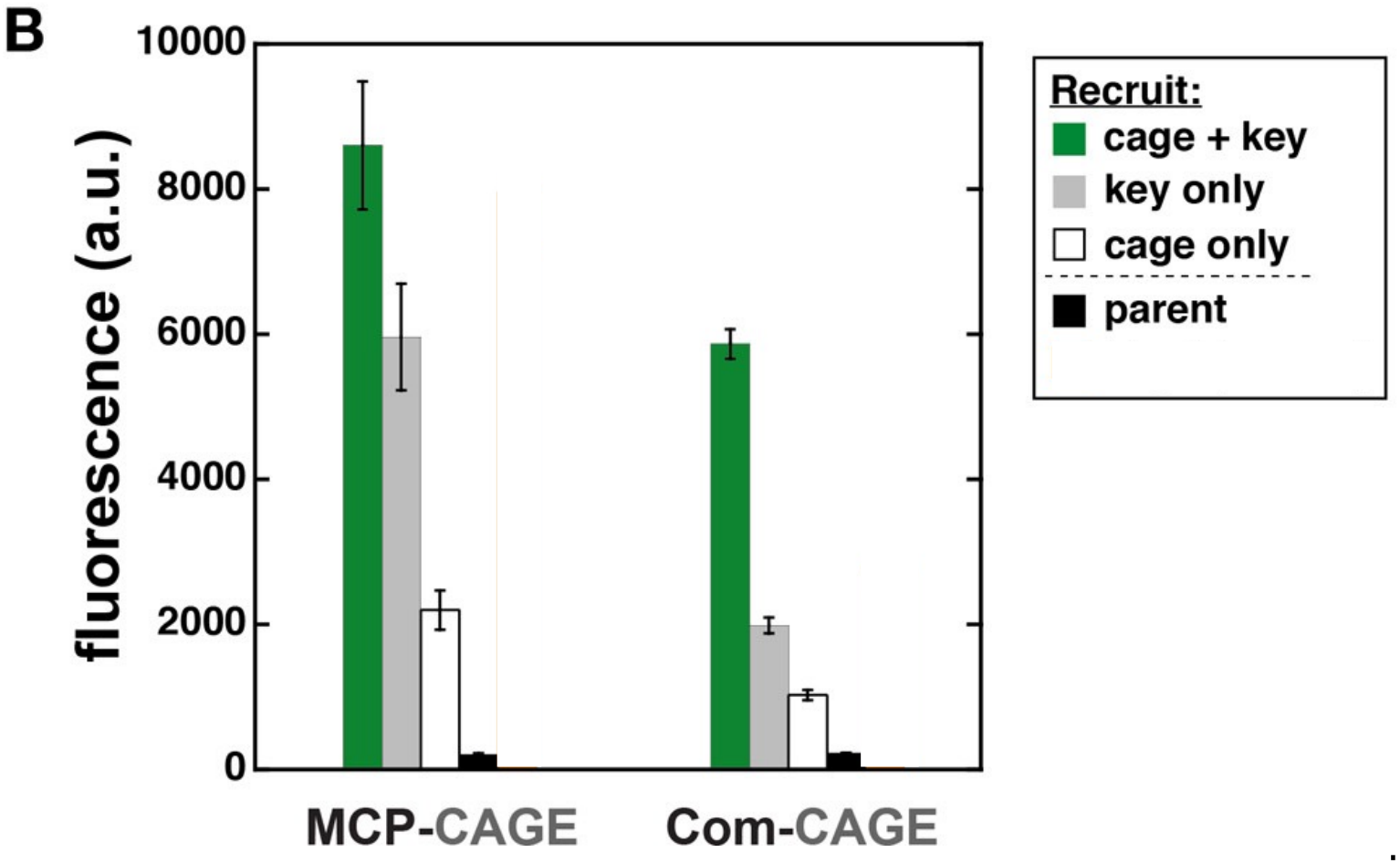
Module optimization

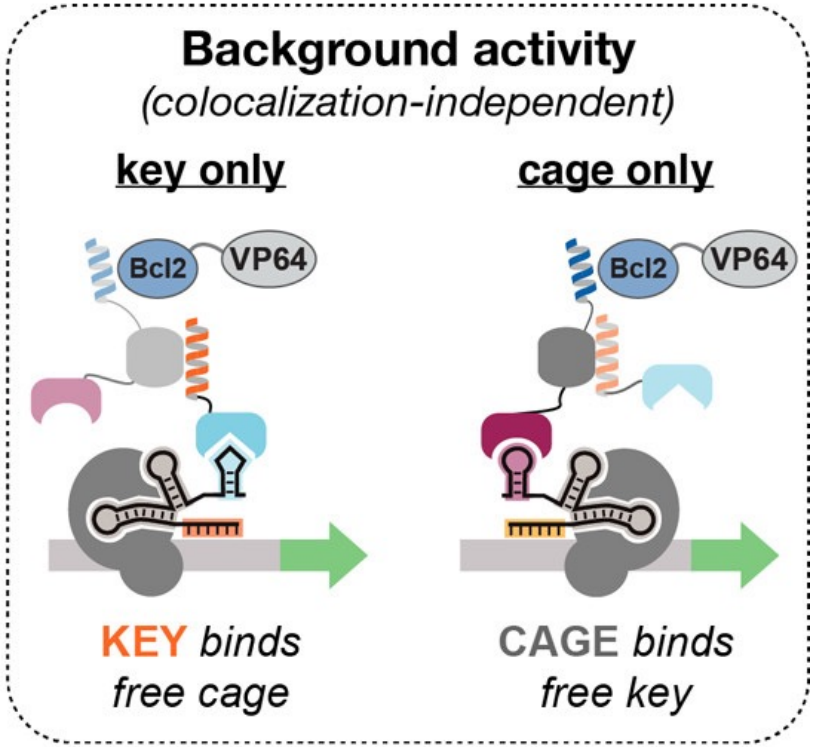
Colocalization on genomic DNA can activate a Co-LOCKR switch



In *S. cerevisiae* (yeast) strains with integrated proteins and plasmid scRNA

Colocalization on genomic DNA can activate a Co-LOCKR switch

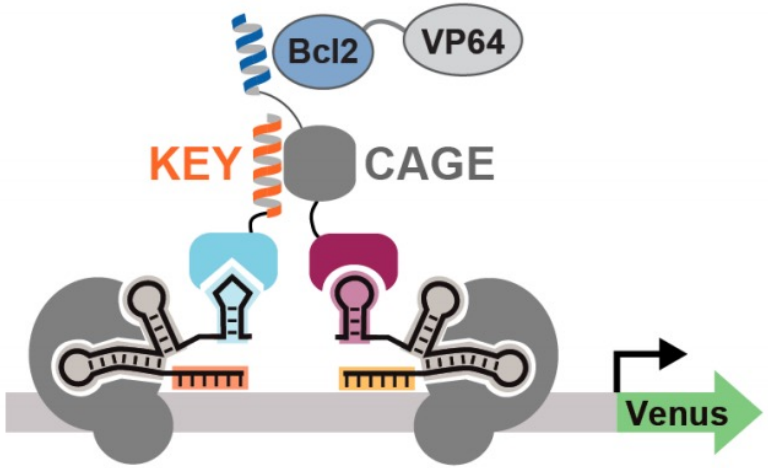




In *S. cerevisiae* (yeast) strains with integrated proteins and plasmid scRNA

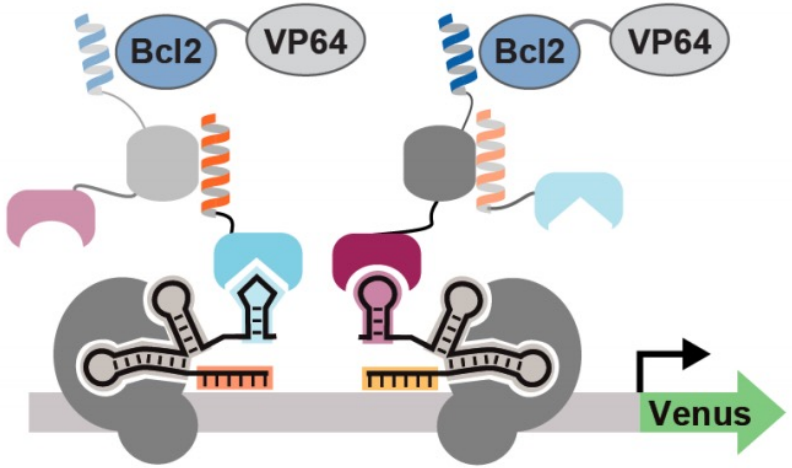
Assessment of background fluorescence reveals colocalization-dependence

Colocalization-dependent activation



vs.

Background activity
(colocalization-independent cage opening)

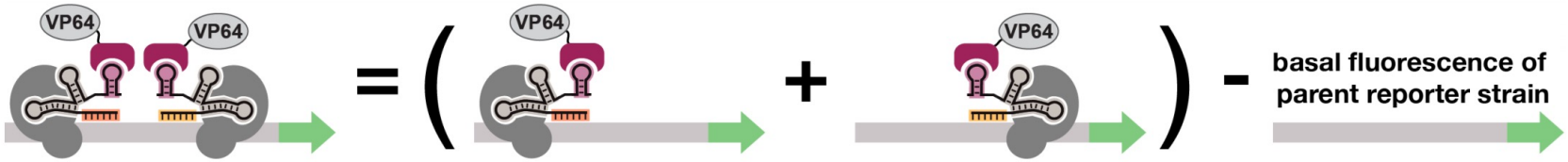


KEY *binds free cage*

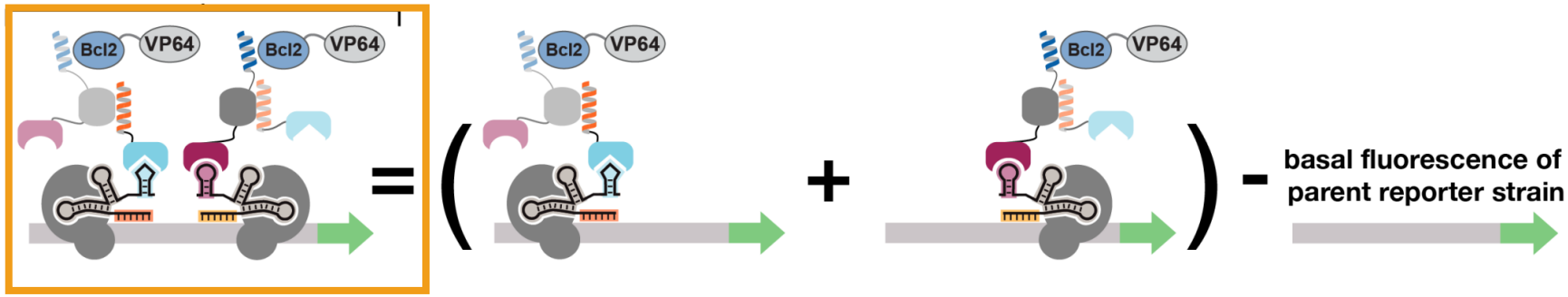
CAGE *binds free key*

Assessment of background fluorescence reveals colocalization-dependence

IF transcriptional activation from adjacent gRNA target sites is additive:



THEN we can assess colocalization-dependent activation using:



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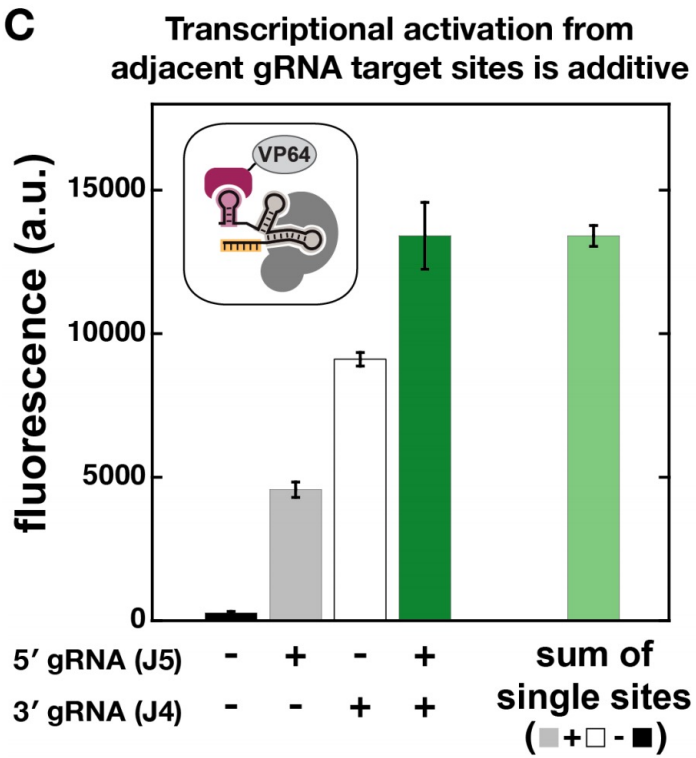
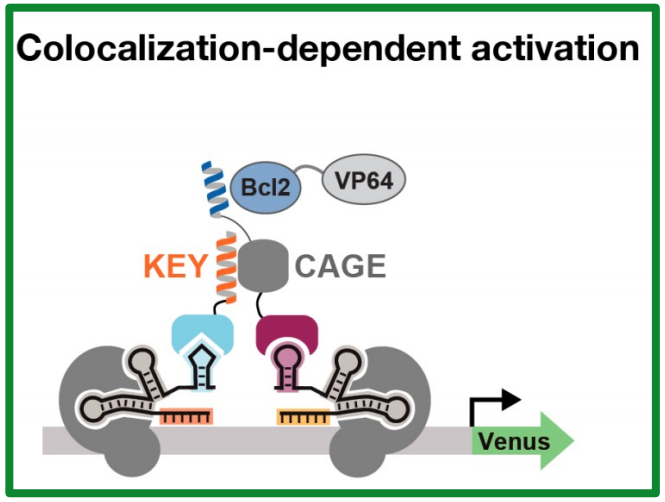
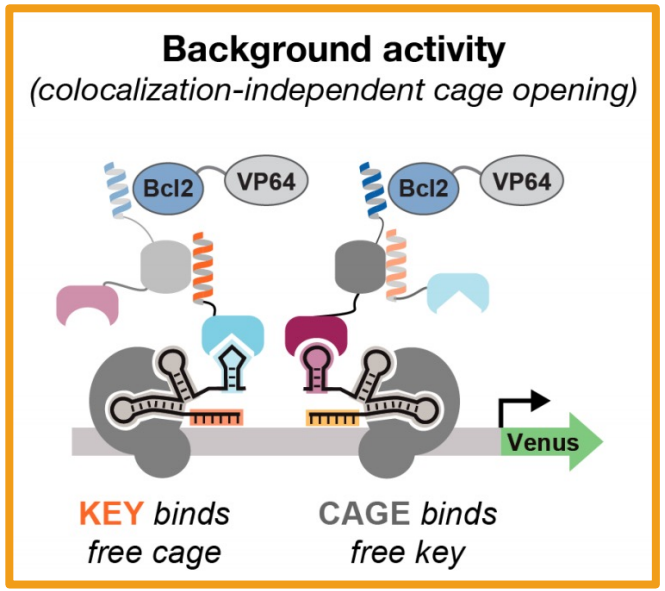
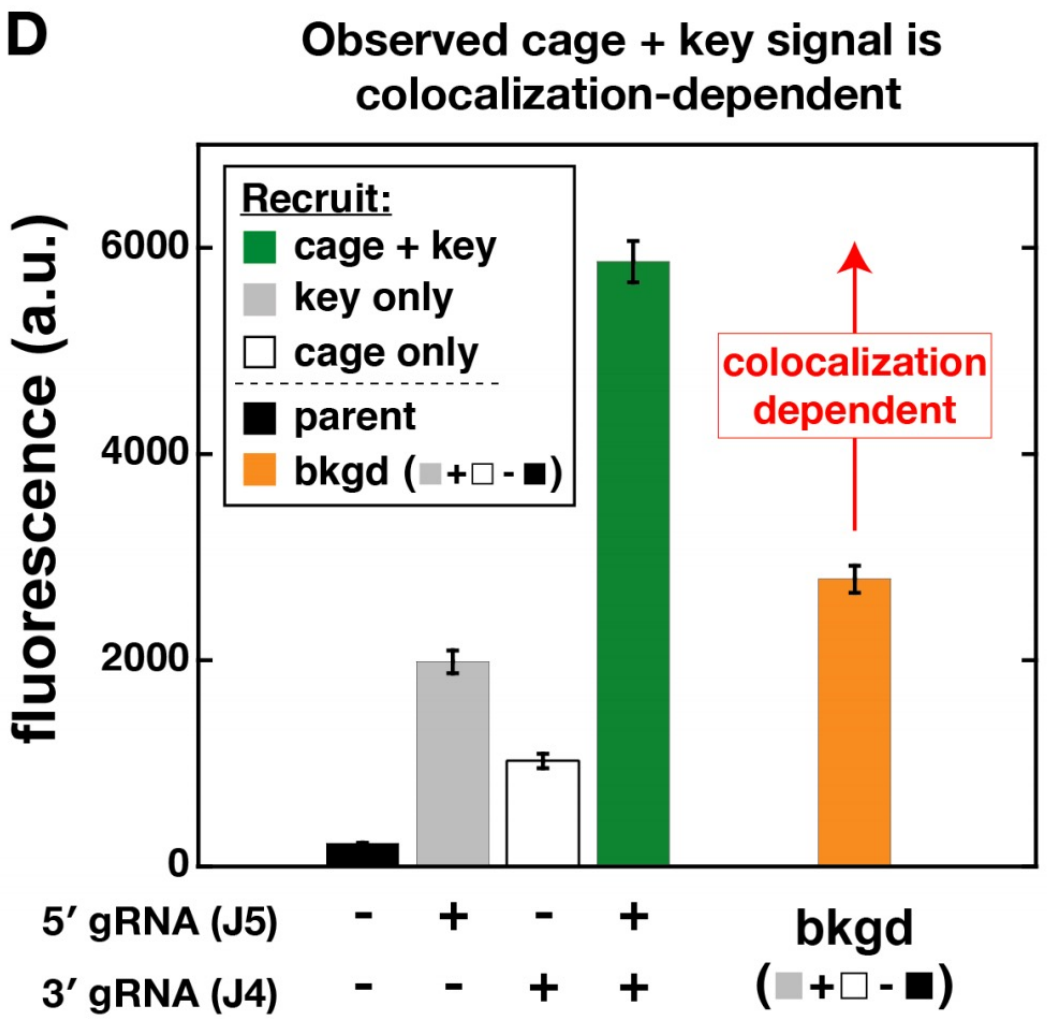


Colocalization-dependent activation = **Observed fluorescence signal** - **Background activity** *(not colocalization-dependent)*

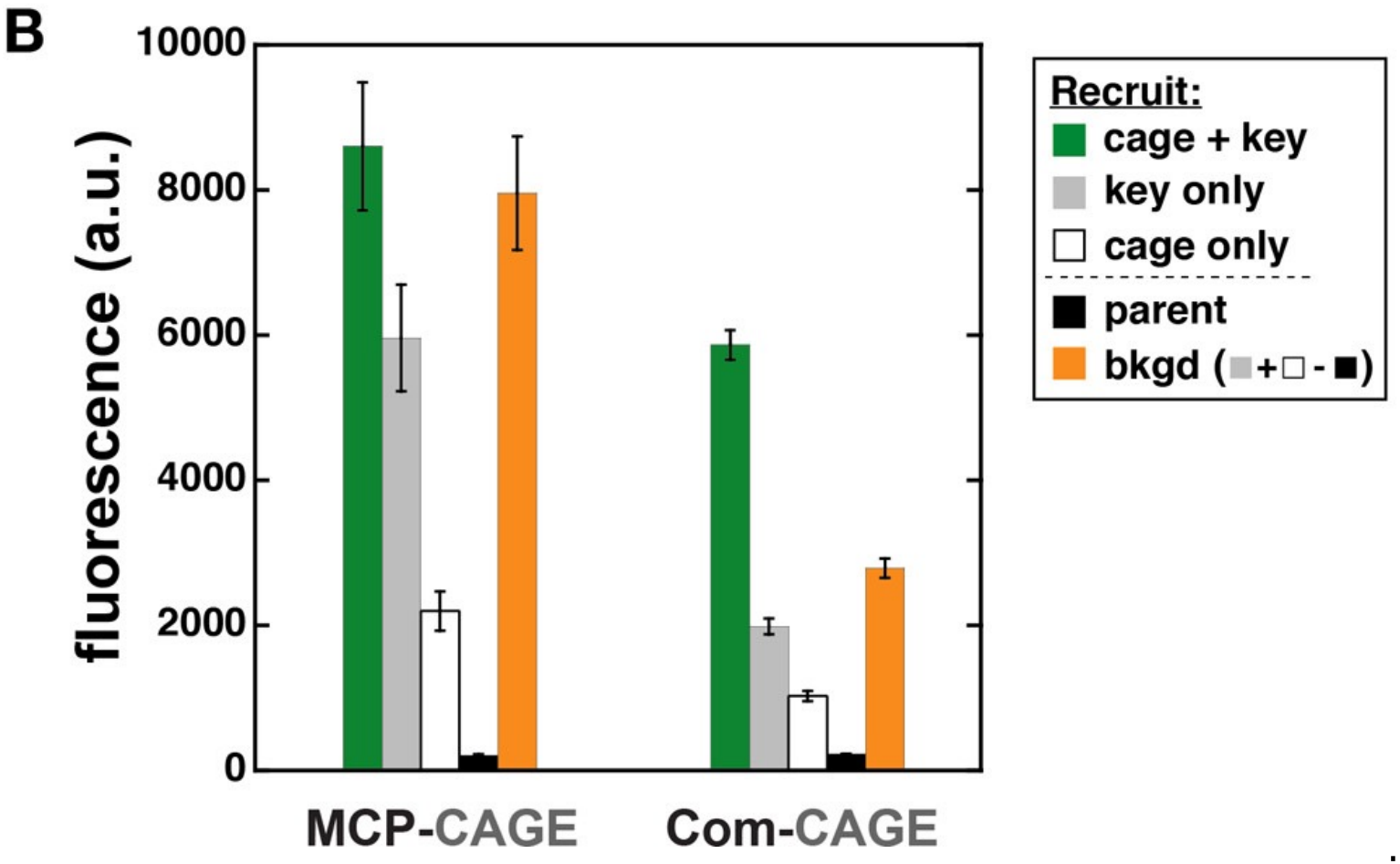
Assessment of background fluorescence reveals colocalization-dependence

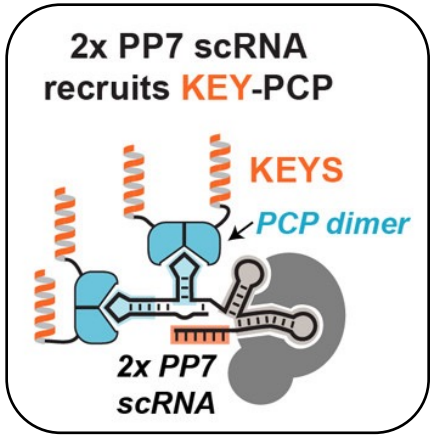


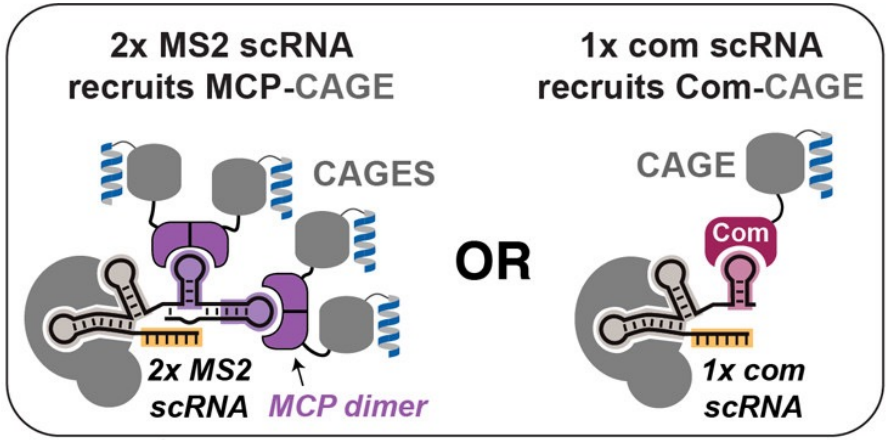




Colocalization on genomic DNA can activate a Co-LOCKR switch







In *S. cerevisiae* (yeast) strains with integrated proteins and plasmid scRNA

Module optimization

- RNA recruitment
  - RNA hairpin-RNA binding protein (RBP) pairs
  - Number of RNA hairpins on scRNA
  - Direct fusion versus RNA recruitment of key and cage
  - Linker length between RBP and key – *no effect*
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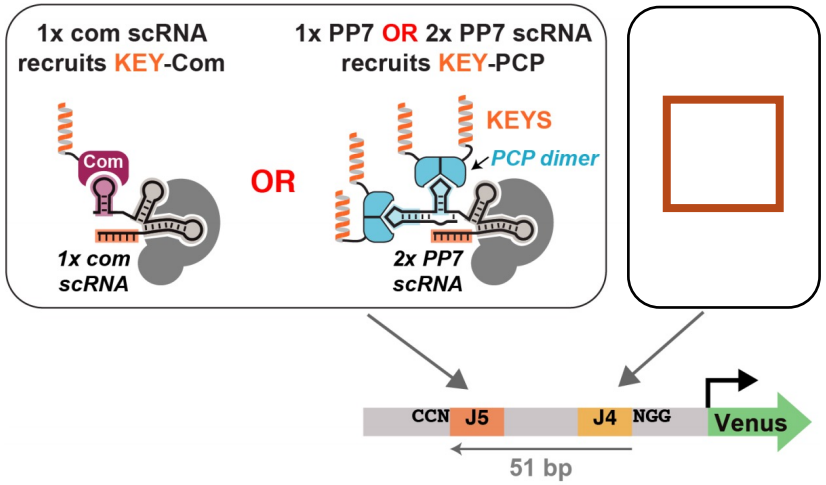
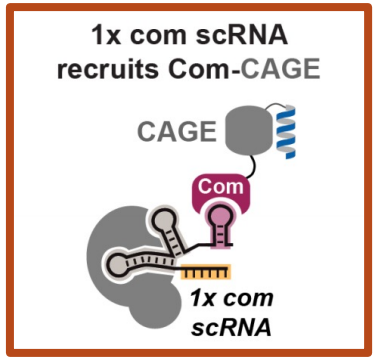
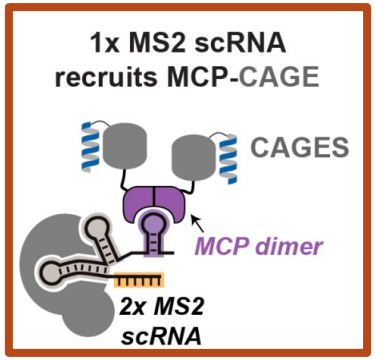
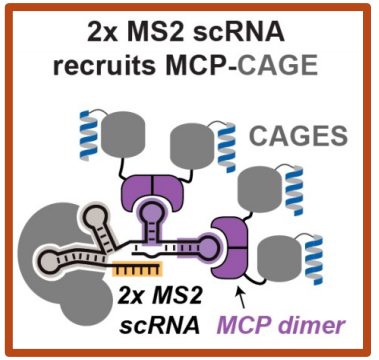
- Expression level of RBP-key and Bcl2-VP64 proteins
- Cage-key interaction strength

Alternative topologies reveal best combination of RNA hairpins and RBPs





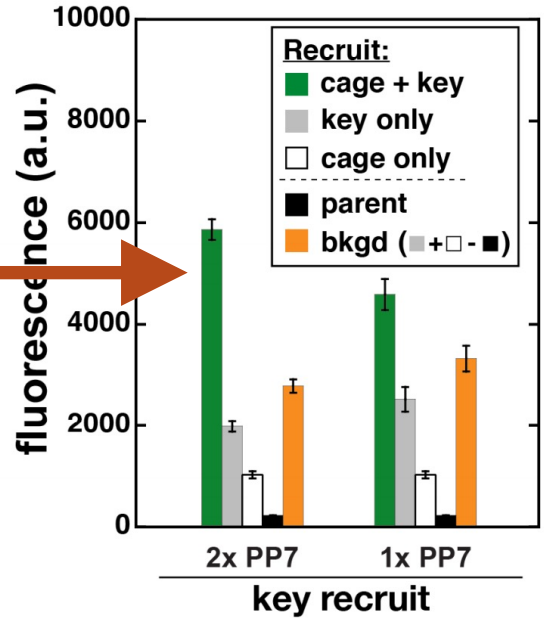


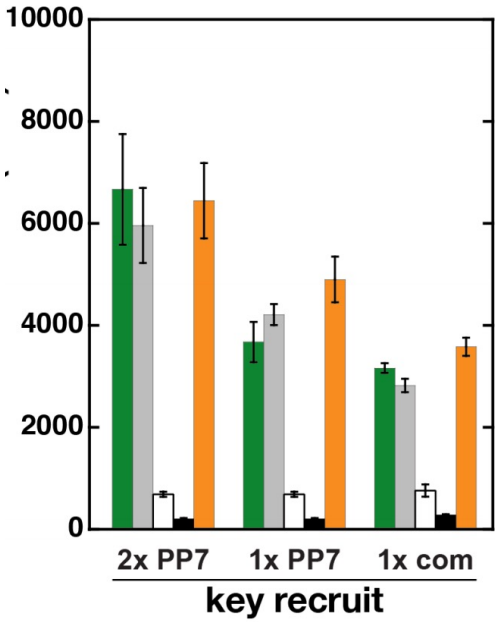


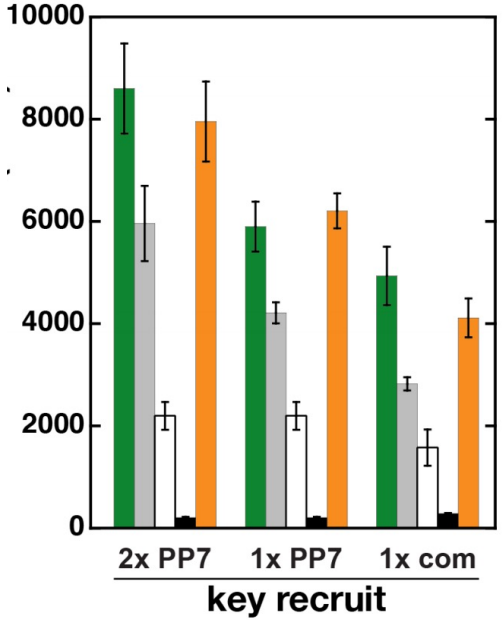
Best Design

1x com + Com-CAGE

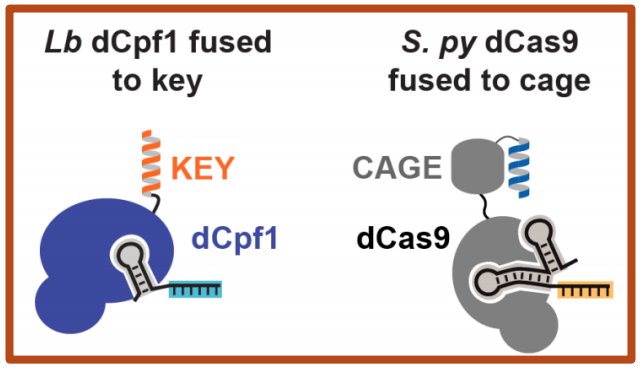
2x PP7 + KEY-PCP

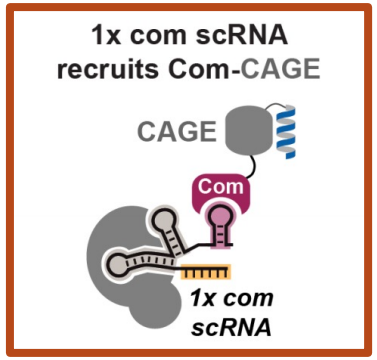


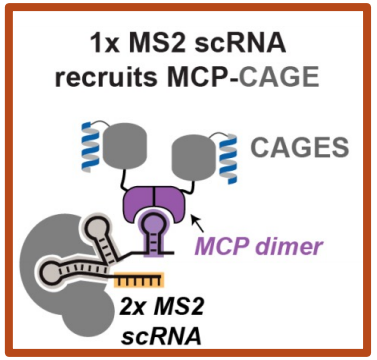


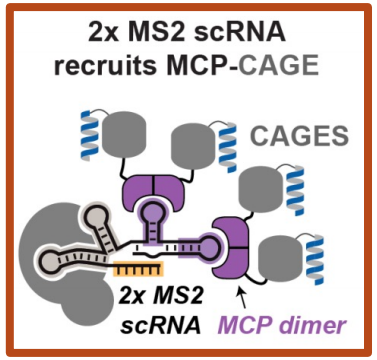


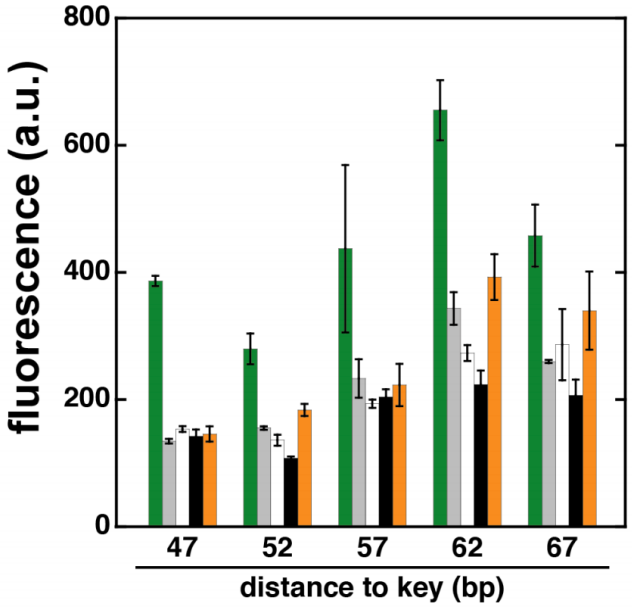
Alternative topologies reveal best combination of RNA hairpins and RBPs

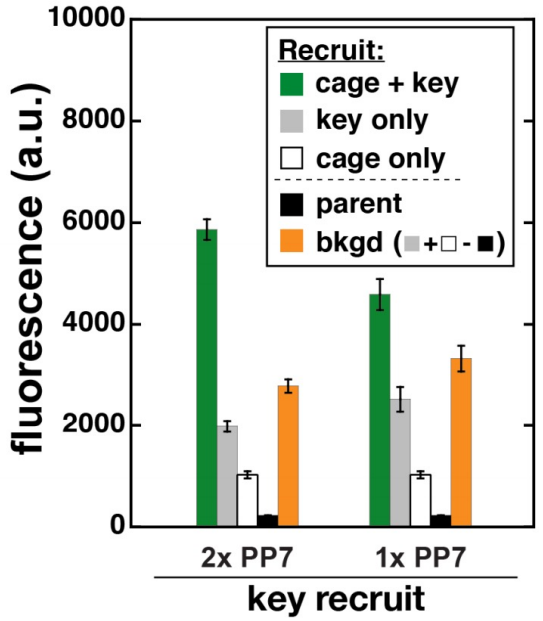


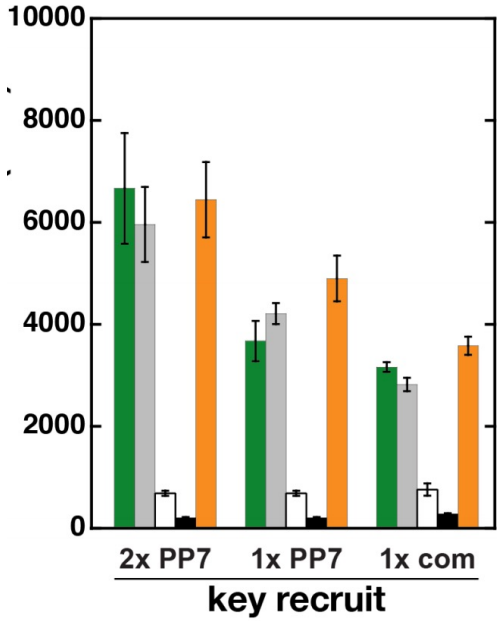


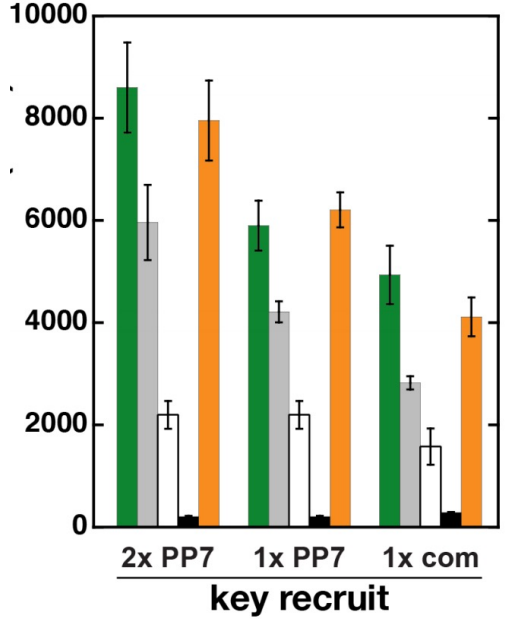












Module optimization

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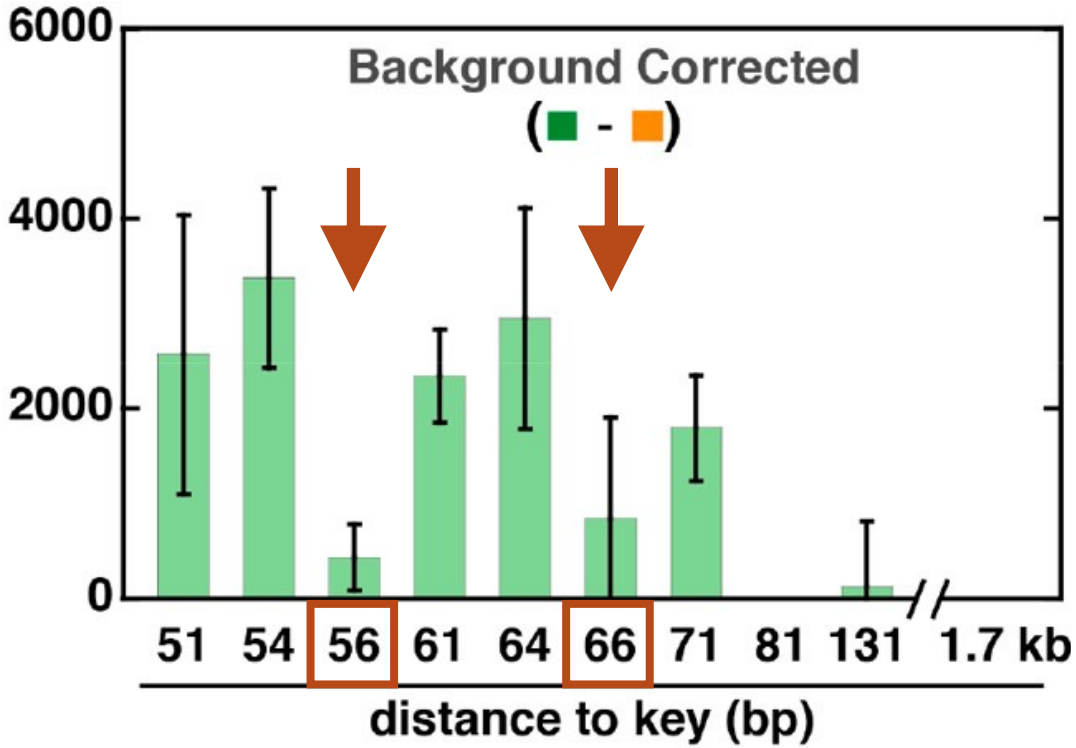
Switch activation is sensitive to target site spacing



Half-turn difference leads to loss of function

→ Periodicity is important

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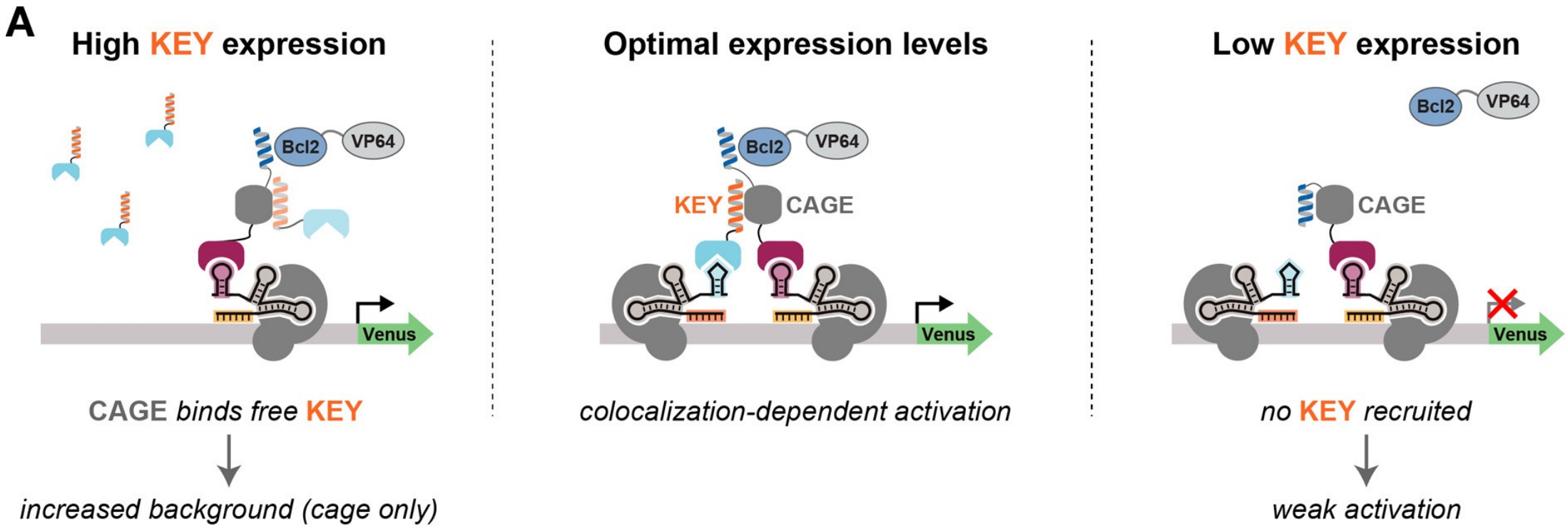
Expression level of KEY and activator affects module function



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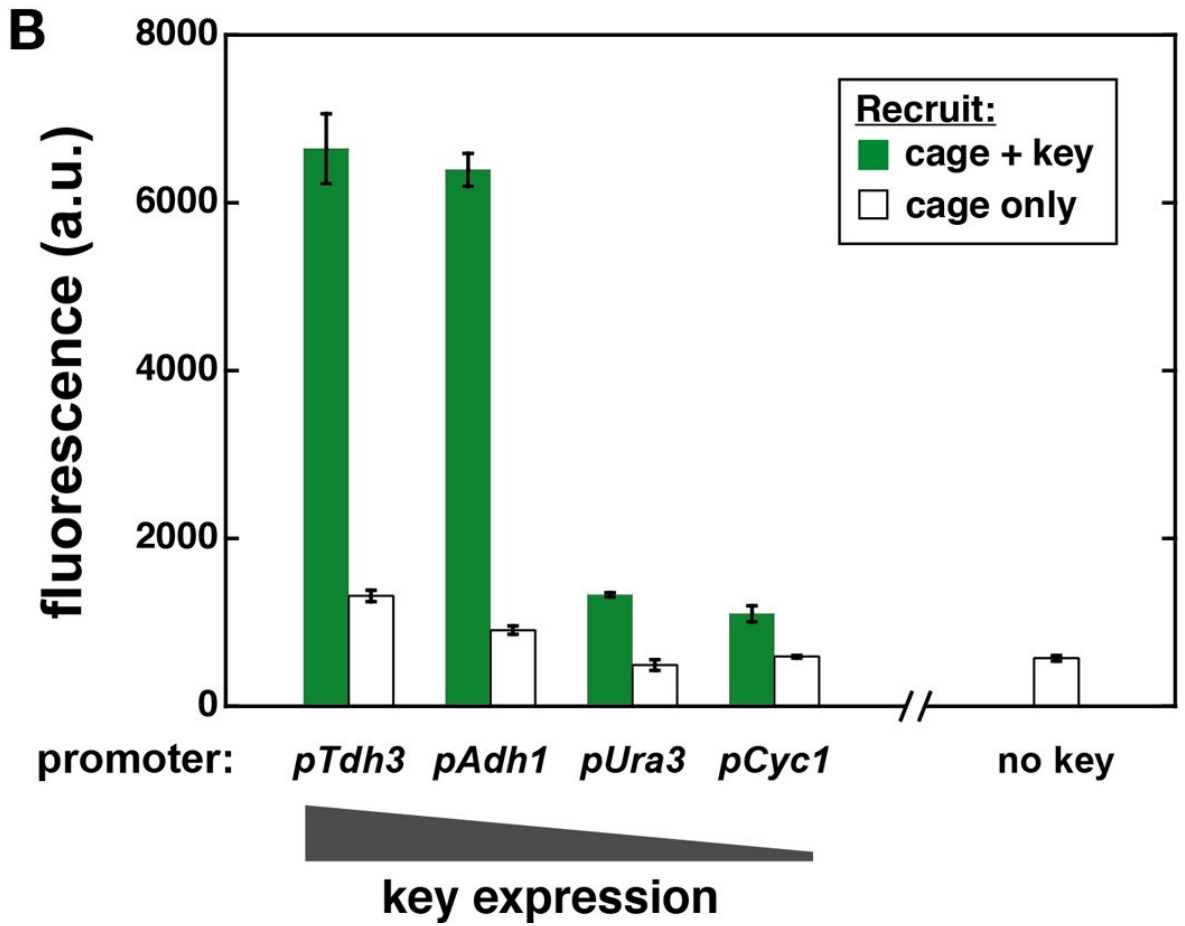

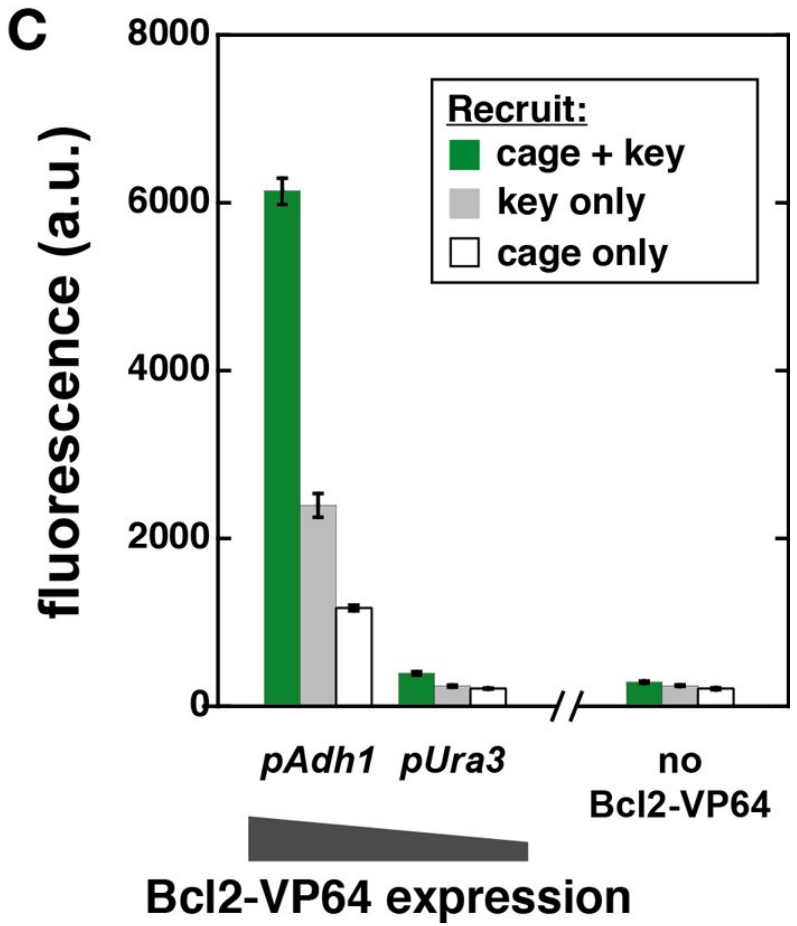

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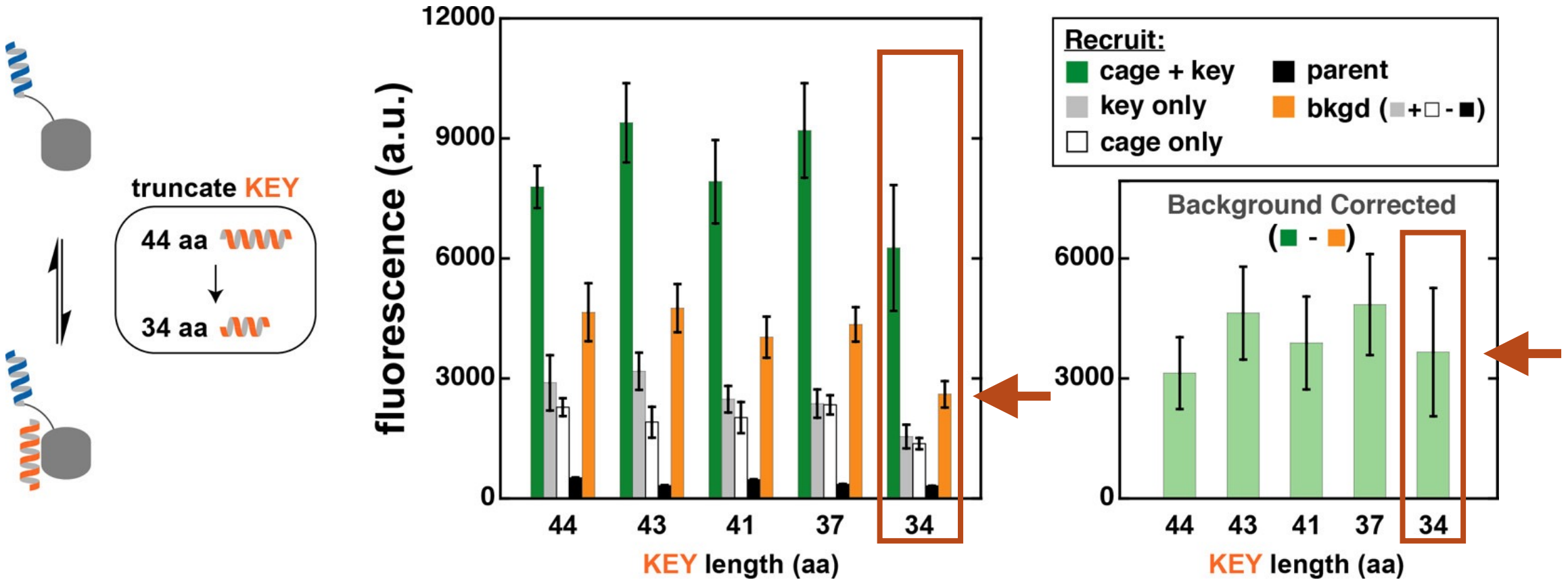
- Cage-key interaction strength

Tuning the length of the KEY peptide reduces background activation



Finish the story

- Evaluate the work
  - Are there experiments that are missing?
  - Do the strength of the claims match the strength of the data?
  - Are there alternative interpretations/explanations of the results?
- Consider limitations
  - Where does the solution/explanation fall short?
- Mention potential next steps / future work
- Finally, connect this work to your (lab's) research, if relevant
  - What implications do these findings have for your work?
  - Can these systems/technologies be used by the lab?

Advantages, limitations, and future work

- Colocalization-dependence means that proteins can be **moderately expressed** while maintaining **low background** activation
- Limitations:
  - Relatively low fold-change activation (~2x)
  - Requires two DNA target sites spaced appropriately apart
  - Contains many components (dCas9, two scRNAs, cage, key, and activator)
- Further optimization: tune cage-latch affinity, tune protein expression levels

Advantages, limitations, and future work

- Implementation in mammalian systems?
- System could be adapted to use other DNA-binding domains (e.g., Co-LOCKR + zinc fingers)
- Potential applications:
  - Split protein epigenetic modifiers
  - Engineering long-range DNA loops
  - AND-gate logic

Tips and tricks

- Can download high-quality images/figures online (rather than screenshotting)
- Be sure to cite any graphics/info you use from papers other than the one you're reviewing
  - Common format: First author(s), et al. *Journal* **Issue#**, (Year).
- Graphics, figures, added annotations, and animations are very helpful!