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Supplemental information

Modular vector assembly enables rapid assessment of emerging CRISPR technologies

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SUPPLEMENTARY INFORMATION

Supplementary Figures

Supplementary Figure 1. Modular vector overview, related to Figure 1.

Supplementary Figure 2. Tiling screens for identification of positive control guides, related to Figure 2.

Supplementary Figure 3. Applications for viral delivery, related to Figure 3.

Supplementary Figure 4. Pooled Golden Gate assembly, related to Figure 4.

Supplementary Figure 5. Overview of the Fragmid design process, related to STAR Methods.

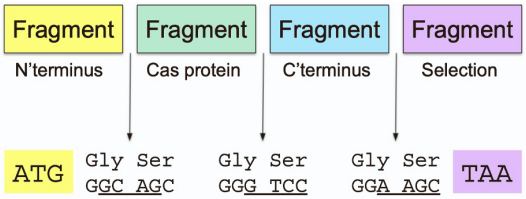
Supplementary Figure 6. Applications for *Drosophila* cell-based and *in vivo* expression, related to STAR Methods.

A

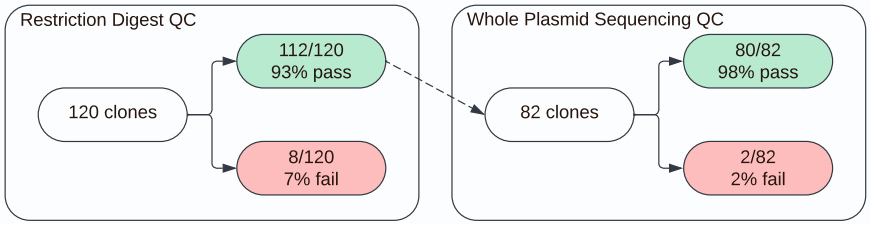
Module	Overhang (5' -> 3')
0	CAGA
1	ACAC
2	AGGA
3	GCAG
4	GTCC
5	AAGC
6	ATTC
9	CGTC
10	TTCG

Modules	Name	Description
0-1	Guide cassette	For expression of guide RNAs
1-2	Pol 2 promoter	For expression of protein coding components
2-3	N' terminus	For N' terminal domains
3-4	Cas protein	For Cas enzymes
4-5	C' terminus	For C' terminal domains
2-5	Selection	For antibiotic or fluorescent selection markers
5-6	2A selection	For antibiotic or fluorescent selection markers, via 2A sites
5-9	Guide cassette	For expression of guide RNAs, compatible with the AAV destination vector
6-9	CROP-seq	For expression of guide RNAs in the 3'LTR of a lentivirus
9-10	Barcode	For inserting a barcode for pooled assemblies

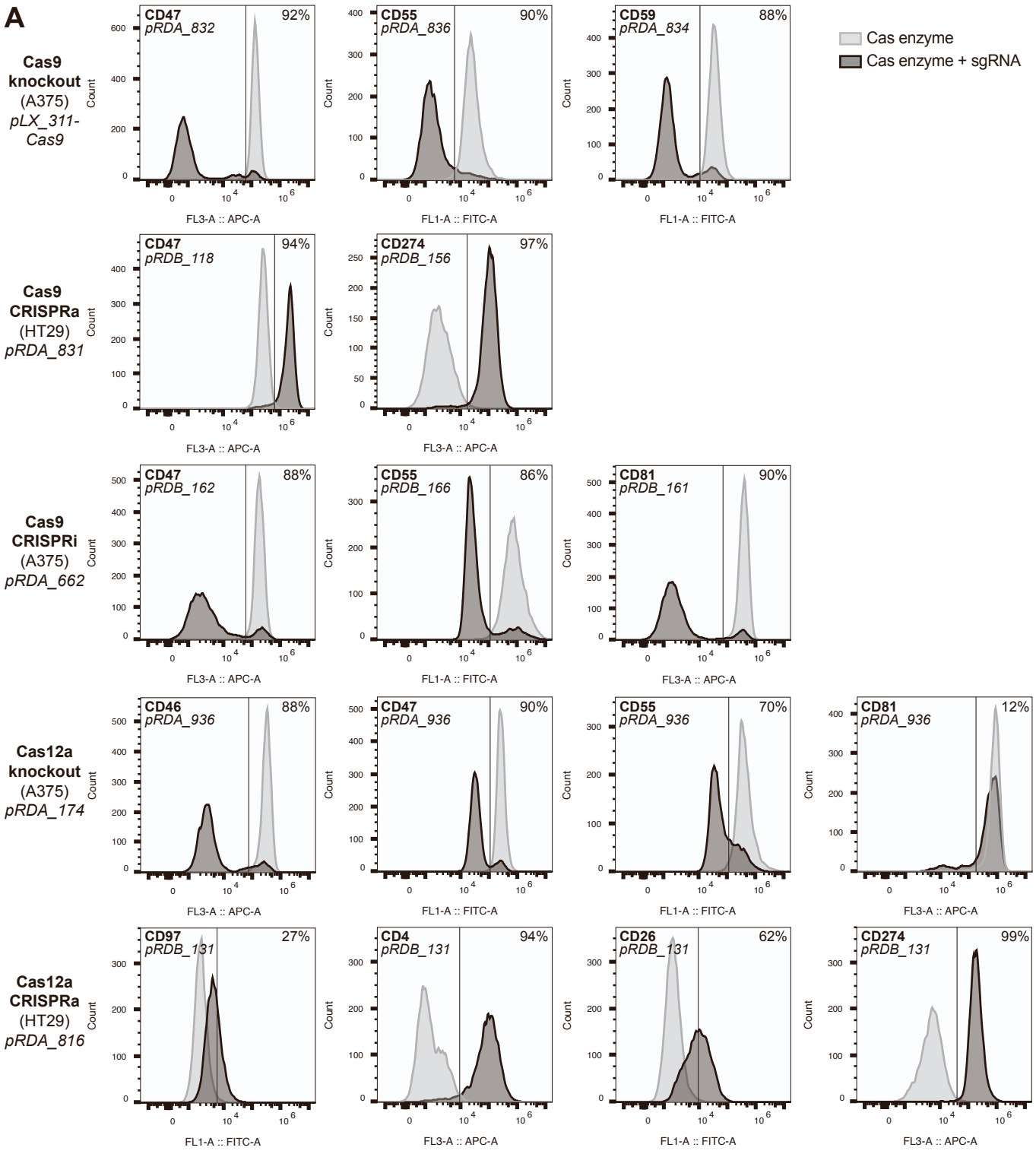
B



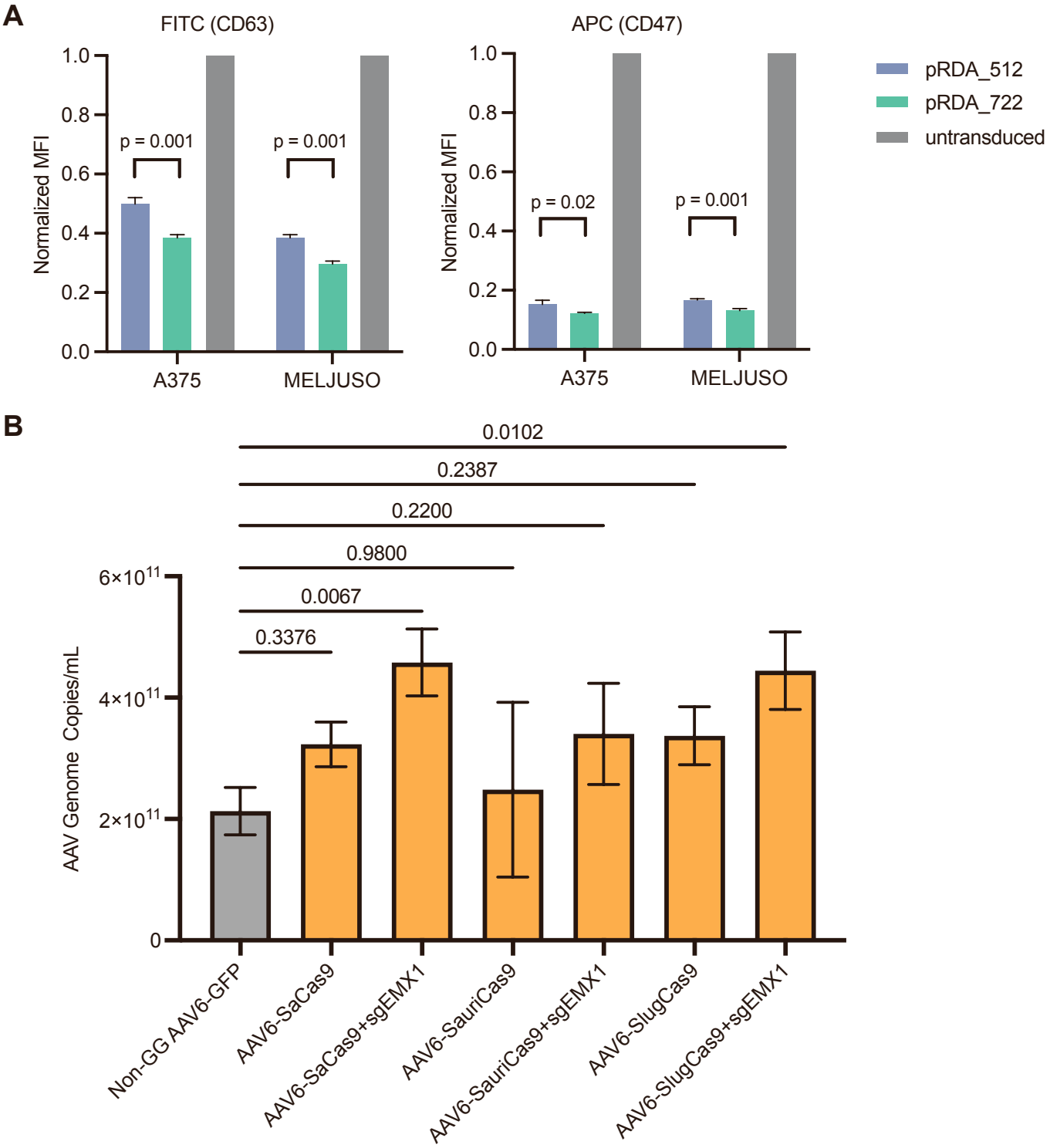
C



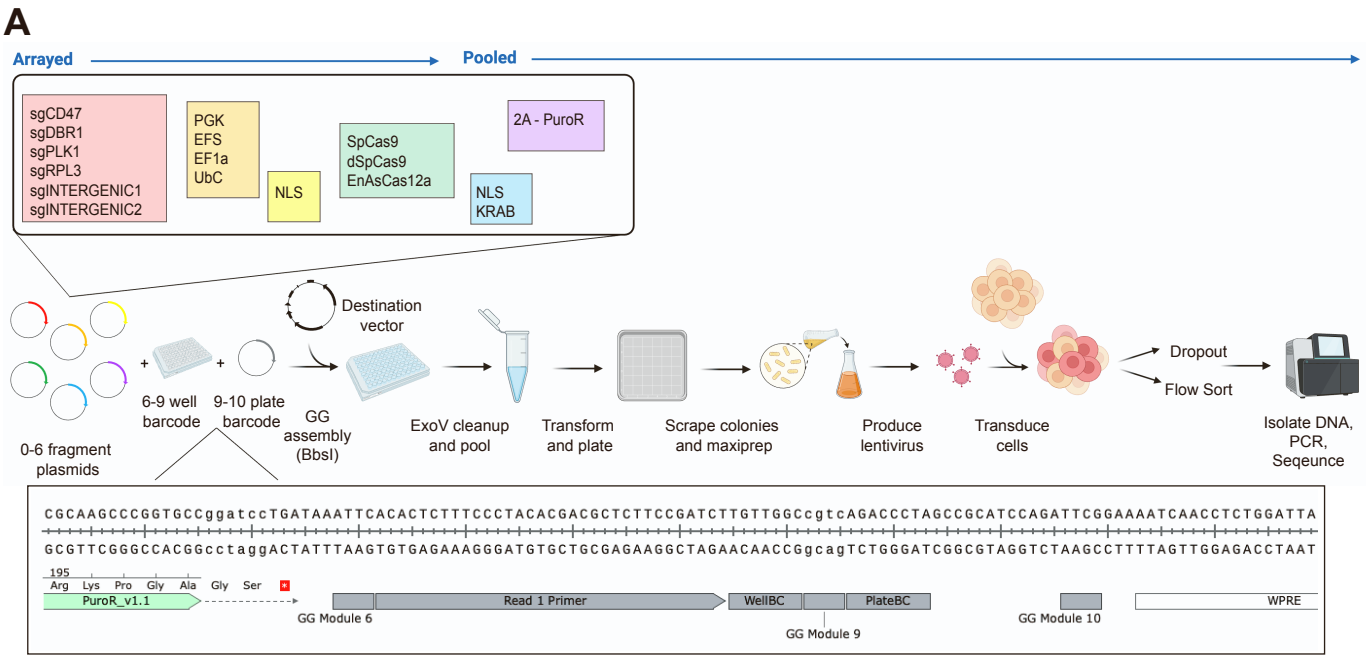
Supplementary Figure 1. Modular vector overview, related to Figure `1. A) Table of BbsI overhang sequences and associated module numbers (left). Table of fragment types, descriptions, and module numbers (right). B) Schematic depicting glycine-serine linkers encoded by BbsI overhang sequences for coding fragment types. C) Schematic depicting Golden Gate assembly fidelity at multiple Quality Control (QC) checkpoints.



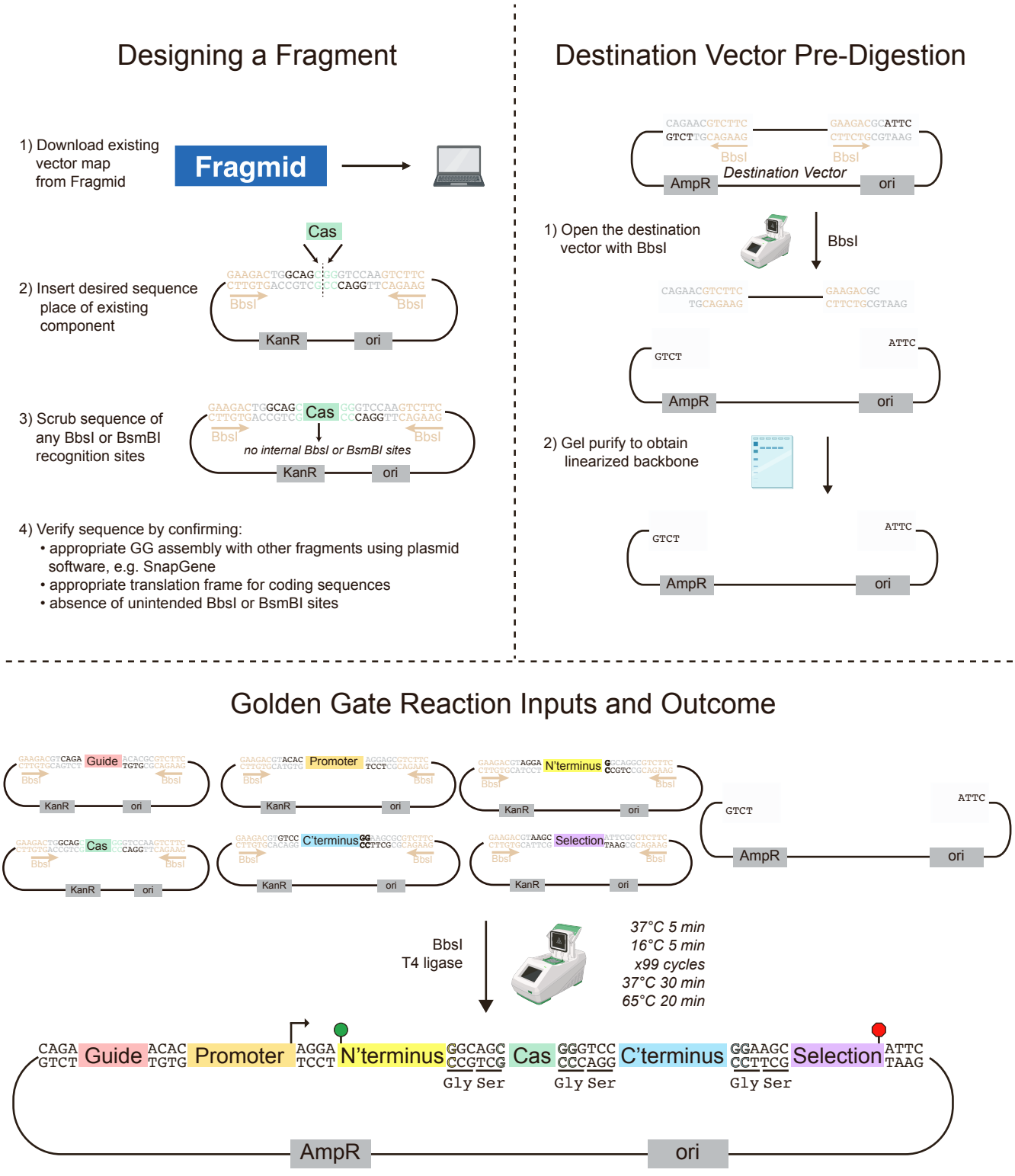
Supplementary Figure 2. Validation of positive control guides, related to Figure 2. A) Histograms showing cell surface marker expression levels (APC or FITC channel) when targeted in validation experiments for each enzyme-technology combination. Cell line information is indicated in parentheses; vector information is indicated in italics. For each cell surface marker, the percentage of cells successfully up- or down-regulated relative to controls is indicated in the top right of the plot. For each EnAsCas12a technology, data are shown from one array that expresses four guides, one targeting each gene. Data from one representative replicate shown for each experiment.



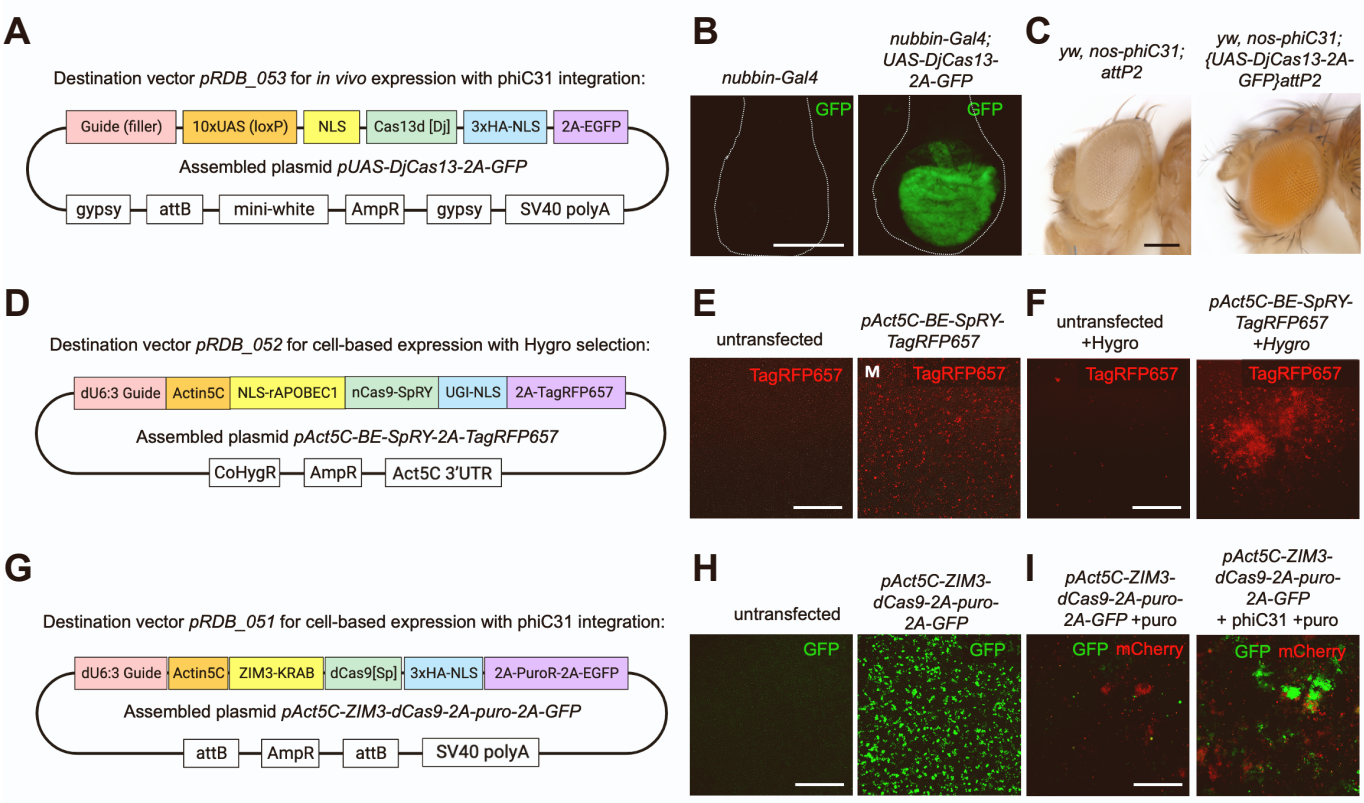
Supplementary Figure 3. Applications for viral delivery. related to Figure 3. A) Comparison of CD47 and CD63 knockout activity in A375 and MelJuSo cells expressing an all-in-one EnAsCas12a construct assembled in the pRDA_512 and pRDA_722 destination vectors. Barplots show normalized MFI values for CD47 (left) and CD63 (right) knockout assessed in triplicate, with the box indicating the mean and the whiskers the standard deviation. Stained parental cells are depicted in gray. Two-tailed t-test used to calculate p-values. B) Barplot depicting AAV viral titer (genome copies/mL) quantified by ddPCR for SaCas9, SauriCas9, and SlugCas9 constructs, with and without the 21 nt EMX1 guide cloned into the pRDA_ 889 AAV destination vector. GFP control construct in a non-GG vector is depicted in gray. Bar indicates mean, whiskers indicate standard deviation for n = 3 replicates. One-way ANOVA with multiple hypothesis correction used to calculate p-values.



Supplementary Figure 4. Pooled Golden Gate assembly, related to Figure 4. A) Schematic of partially-pooled GG assembly, including fragments used and dual barcoding strategy. An example assembled vector is shown at the bottom, indicating the position of the Plate and Well Barcodes, as well as the Read 1 Primer for Illumina sequencing and the modules (overhangs) used for Golden Gate cloning. See also Supplementary Data 3. B) Cumulative distribution of barcodes in pooled GG library pDNA. C) Replicate correlations (Pearson's r) for the pooled GG library screened in MelJuSo cells in duplicate for the CD47-sort screen (left) and viability screen (right).



Supplementary Figure 5. Overview of the Fragmid design process, related to STAR Methods. Design of new fragments (top left) should ensure that BbsI overhang identity is maintained, along with the appropriate reading frame for translated elements. Pre-digestion of Destination Vector (top right) is recommended to reduce cloning background but may not be required. In the final assembled vector, RNA pol II transcription is indicated by the arrow, while translation start and stop are indicated by the green circle and red hexagon.



Supplementary Figure 6. Applications for Drosophila cell-based and in vivo expression, related to STAR Methods. A) Schematic of pUAS-DjCas13-2A-GFP, assembled in destination vector pRDB_053, for in vivo expression. B) Wing imaginal discs from nubbin-Gal4; UAS-DjCas13-2A-GFP, but not nubbin-Gal4 controls, express GFP in the wing pouch. C) Injection of pUAS-DjCas13-2A-GFP into yw, nos-phiC31; attP2 flies produces transformants with orange eyes. D) Schematic of pAct5C-BE-SpRY-2A-TagRFP657 assembled in destination vector pRDB_052, for cell-based expression with Hygromycin selection. E) S2R+ cells transfected with pAct5C-BE-SpRY-2A-TagRFP657 express TagRFP657 (red). F) Hygromycin selects for S2R+ cells expressing pAct5C-BE-SpRY-2A-TagRFP657 (red). G) Schematic of pAct5C-ZIM3-dCas9-2A-puro-2A-GFP assembled in destination vector pRDB_051, for cell-based expression with phiC31 integration. H) S2R+ cells transfected with pAct5C-ZIM3-dCas9-2A-puro-2A-GFP express GFP (green). I) S2R+ PT5 cells transfected with pAct5C-ZIM3-dCas9-2A-puro-2A-GFP + phiC31 integrate the plasmid into the attP sites (marked by increased GFP, and reduced mCherry fluorescence). Scale bars, 100 μm (B, C); 300 μm (E, F, H, I).