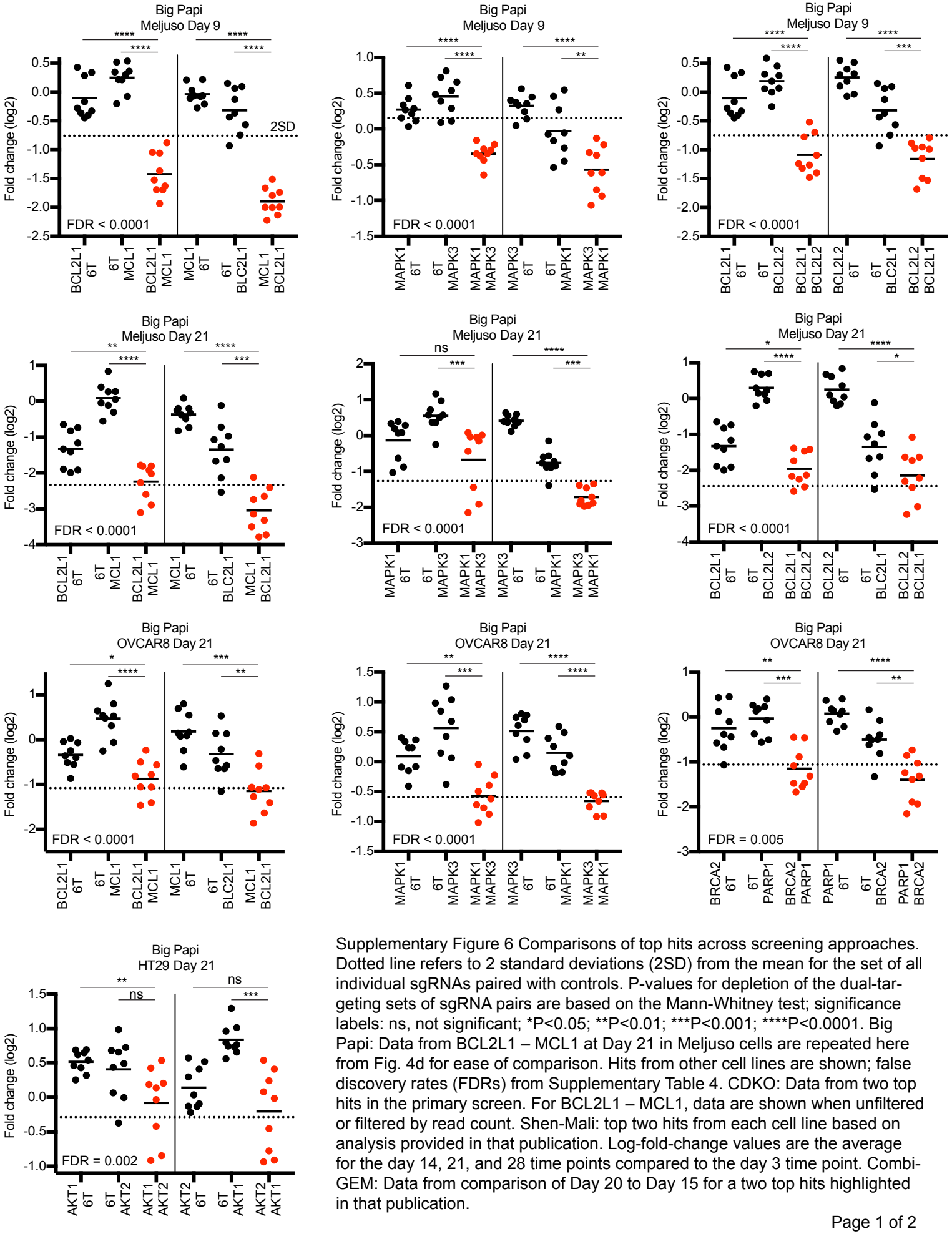
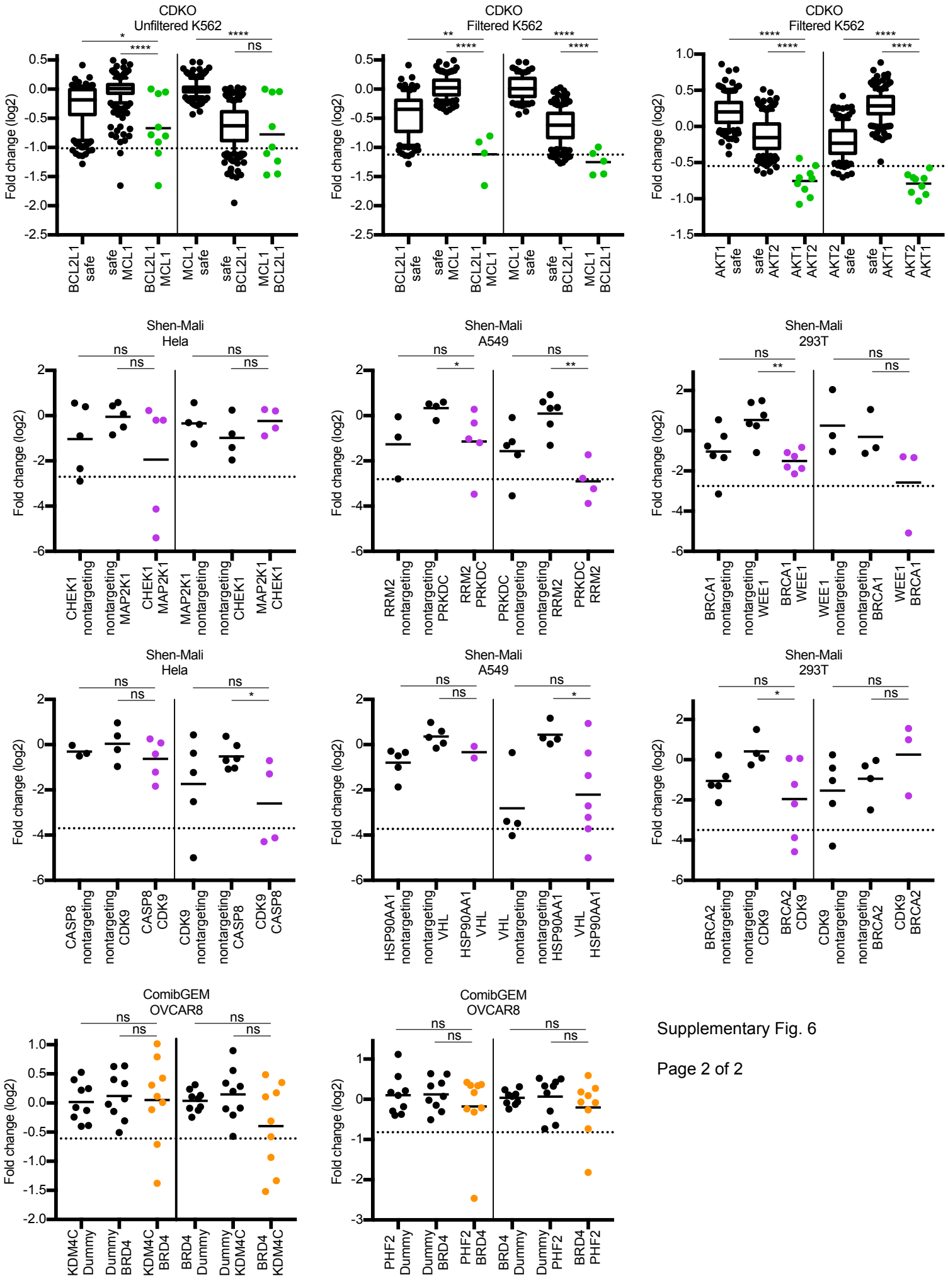


Supplementary Figure 6 Comparisons of top hits across screening approaches. Dotted line refers to 2 standard deviations (2SD) from the mean for the set of all individual sgRNAs paired with controls. P-values for depletion of the dual-targeting sets of sgRNA pairs are based on the Mann-Whitney test; significance labels: ns, not significant; *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Big Papi: Data from BCL2L1 – MCL1 at Day 21 in Meljuso cells are repeated here from Fig. 4d for ease of comparison. Hits from other cell lines are shown; false discovery rates (FDRs) from Supplementary Table 4. CDKO: Data from two top hits in the primary screen. For BCL2L1 – MCL1, data are shown when unfiltered or filtered by read count. Shen-Mali: top two hits from each cell line based on analysis provided in that publication. Log-fold-change values are the average for the day 14, 21, and 28 time points compared to the day 3 time point. Combi-GEM: Data from comparison of Day 20 to Day 15 for a two top hits highlighted in that publication.



Supplementary Fig. 6

Supplementary Note 1 Estimating false positive and false negative rates.

To estimate the specificity of our screening system and analytical approach, we assumed that true positive synthetic lethal interactions occur only within our pre-defined gene groups, whereas interactions across pre-defined groups are false positives. Likewise, for buffering interactions, we assumed that all true-positive interactions occur in the special case where both Cas9s are targeted to the same gene, which is expected from our model of independent gene action and has been observed previously in combinatorial screens[16,20]. Both of these assumptions are conservative, in that true (but currently uncharacterized) synthetic lethal interactions across our pre-defined groups or buffering interactions between genes will be counted as false positives. We calculated the true positive rate at different FDR thresholds for data from both individual cell lines as well as all leave-one-out iterations (**Fig. 4e**). We see similar estimates for the true positive rate for both synthetic lethal and buffering interactions, suggesting the independent assumptions made for each were reasonable. At an FDR threshold of 0.1, the empirically-determined true positive rate ranged from 72 – 85%, not far from the theoretical value of 90% (i.e. 10% false discoveries), suggesting that our analysis approach is well-calibrated.

The false negative rate of a genetic screen is notoriously difficult to determine empirically, because for the majority of screens, there are not well-validated sets of true positive genes. For synthetic lethal interactions, there is no reference set of interactions validated to occur in all cell lines. False negatives arise when the reagents targeting the gene are ineffective; for genetic screens that target single genes, there is no data-driven

way to determine which genes failed to score because of ineffective targeting purely on the basis of screening results. In these data, however, we can use buffering interactions where both Cas9s are targeted to the same gene to validate the effectiveness of the sgRNAs. Buffering in this special case indicates that both the SaCas9 and SpCas9 sgRNAs must have effectively targeted the gene. Failure to detect a buffering interaction for an individual gene is evidence of failure to effectively target the gene with either or both Cas9s, and thus we can empirically determine a false negative rate. This is a conservative assumption, as it assumes that a gene has a measurable viability effect in a cell, which will not always be true. Buffering interactions were detected with approximately equal prevalence across all cell lines, including 786O cells, which were bereft of strong synthetic lethal interactions (**Supplementary Fig. 7a**). We observed a lower false negative rate when information from multiple cell lines was combined (**Fig. 4f**). For example, at an FDR of 0.1, we determine a false negative rate of 57% when using individual cell lines, whereas combining information from 5 lines gives a false negative rate of 33%. The empirically determined true positive and false negative rates of the Big Papi screening system suggest that this is an efficient screening approach, especially when assayed across multiple cell lines

SUPPLEMENTARY NOTE 2

pPapi, U6 – H1 region:

GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGA
GAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACGTGA
CGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAATGG
ACTATCATATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATATCTT
GTGGAAAGGACGAAACACCG**G**
GGGCGAGGAGCTGTTCACCG
GTTTGAGAGCTAGAAATAGCAAGTTCAAATAAGGCTAGTCCGTTATCAACTTGAAAA
AGTGGCACCGAGTCGGTGCTTTTTT
GAACCG
ACGGATGATCTCGTGCAC
CGGTTC
AAAAAATCTCGCCAACAAGTTGACGAGATAAACACGGCATTTTGCCTTGTTTAAGTA
GATTCTGTTTCCAGAGTACTTAAAC
CTACATAGAAGGTGTTGGGCG
CGGGAAAGAGTGGTCTCATACAGAACTTATAAGATTCCCAAATCCAAAGACATTTC
ACGTTTATGGTGATTTCCCAGAACACATAGCGACATGCAAATATTGCAGGGCGCCA
CTCCCCTGTCCCTCACAGCCATCTTCCTGCCAGGGCGCACGCGCGCTGGGTGTTC
CCGCCTAGTGACACTGGGCCCGCGATTCCTTGGAGCGGGTTGATGACGTCAGCGT
TCGAATTCGCTAGCTAGGTCTTGAAAGGAGTGGGAATTGG

U6 promoter (forward) Fwd PCR Primer **+1G of transcription**
S. pyogenes guide
S. pyogenes tracrRNA
Barcode 1
Overlap Extension
Barcode 2
S. aureus tracrRNA
S. aureus guide
+1G of transcription H1 promoter (reverse) Rev PCR Primer
Anchors for sequencing deconvolution

PCR PRIMERS FOR SEQUENCING DECONVOLUTION:

Forward (P5)
5'AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT[s]TTGTGGAAAGGACGAAACACCG

Reverse (P7)
5'CAAGCAGAAGACGGCATACGAGATNNNNNNNNGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAATTCCCACTCCTTTCAAGACC

P5/P7 flowcell attachment sequence
Illumina sequencing primer
[Stagger region] / Barcode region
Vector primer binding sequence

Staggers	
Length	Sequence
0	
1	C
2	GC
3	AGC
4	CAAC
6	TGCACC
7	ACGCAAC
8	GAAGACCC

Barcodes					
TTGAACCG	AATCCACG	AACTCACG	TTGAGTTA	TTGACACG	CCAGTGCG
AATCCAGC	TTCTACTA	AATCTGAT	GGCTCACG	AAGAACGC	GGTCTGTA
CCGAGTTA	AATCGTGC	AATCACTA	CCTCTGAT	GGCTTGGC	GGTCACGC
AACTGTTA	GGCTACCG	CCGAACCG	CCTCCATA	GGAGGTGC	CCGACATA
TTGAGTAT	AAGAACTA	GGTCCATA	TTGAACGC	CCAGGTGC	AAGAGTCG
TTCTCAGC	AACTTGTA	AACTTGGC	TTCTTGCG	GGTCTGGC	CCGAACGC
CCTCCAAT	CCAGTGAT	GGTCTGCG	GGCTGTCG	GGCTTGCG	TTAGGTAT
TTAGACTA	TTGATGCG	GGAGGTCG	CCGAGTCG	TTGATGTA	AATCACCG
GGTCACCG	GGTCGTGC	CCAGTGGC	TTCTTGTA	GGCTACGC	GGTCGTTA
CCTCTGTA	GGAGTGTA	AACTACGC	AATCTGCG	TTCTGTCG	GGCTGTAT
TTGACAAT	TTAGACCG	AACTTGCG	GGAGCAAT	CCAGACTA	GGTCGTCG
AAGACATA	CCGAACAT	AACTGTCG	CCGAGTGC	AACTTGAT	TTAGTGAT
AATCCAAT	GGTCCACG	AAGATGCG	AAGAACAT	AACTACTA	CCAGGTCG
CCGAGTAT	GGCTCAAT	TTGAACAT	GGAGACAT	AAGAGTAT	TTGACATA
TTCTCATA	CCGAACTA	TTCTACGC	TTGAGTGC	AACTACCG	TTAGGTTA
AACTGTGC	CCTCACCG	GGCTACAT	GGCTACTA	CCTCGTAT	AAGATGGC

sgRNA SEQUENCES

Cas9	Target Gene	Guide #	Guide sequence (5' → 3')	Note
Flow cytometry (Figure 1)				
S. aureus	EGFP	1	GCCGGTGGTGCAGATGAACTT	
S. aureus	CD81	1	CGCCCAACACCTTCTATGTAG	Not used in this study
S. aureus	CD81	2	GATACAGGAGGTTGGTGGTCT	
S. aureus	CD81	3	AGGCTGTGGTGAAGACCTTCC	
S. pyogenes	EGFP	1	GGGCGAGGAGCTGTTCACCG	
S. pyogenes	CD81	1	TGATGACGCCAACAACGCCA	
S. pyogenes	CD81	2	GACAAAGCCCCAGATGCCGG	Not used in this study
S. pyogenes	CD81	3	CTCCCAGCTCCAGATACAGG	