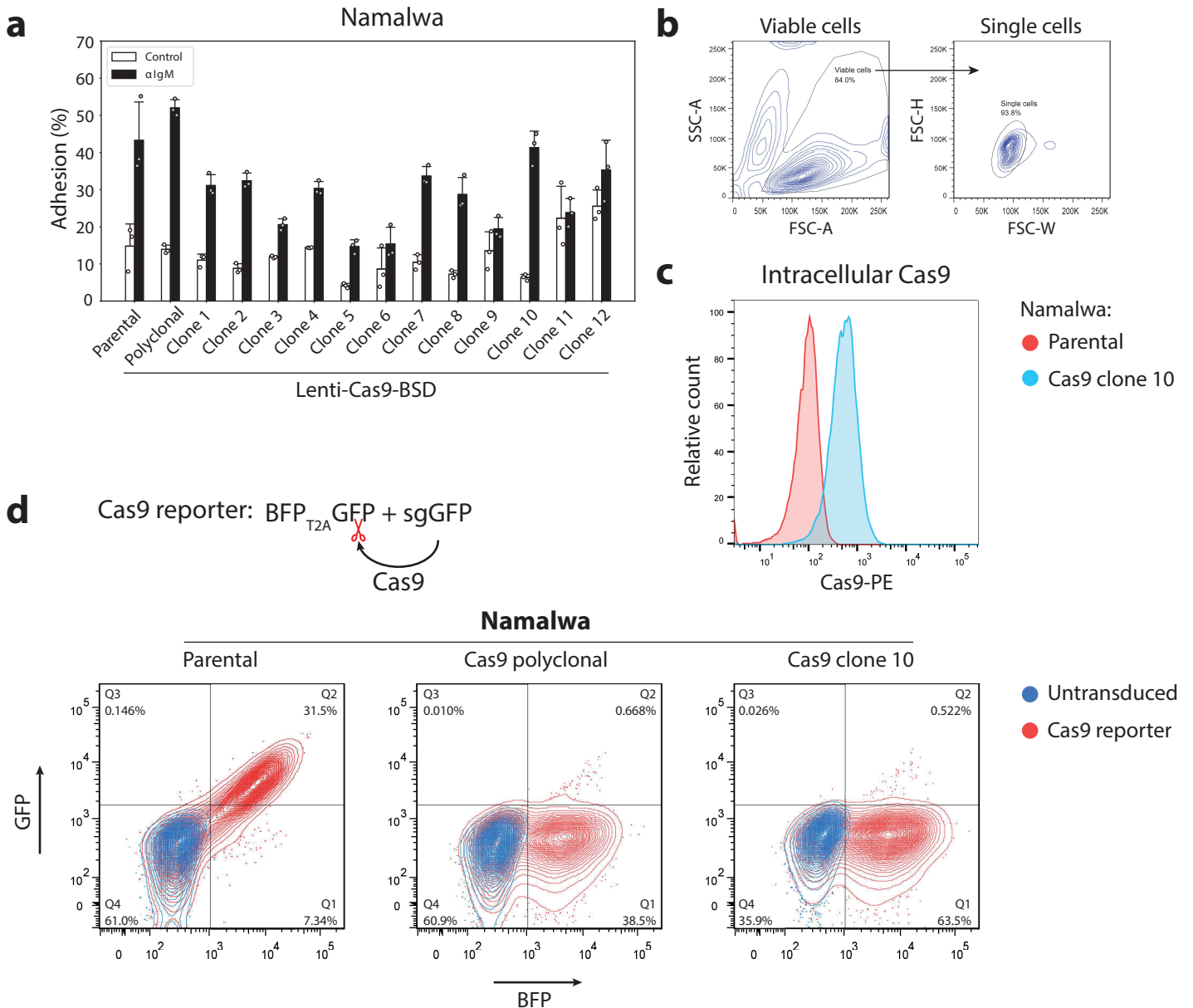


SUPPLEMENTARY TABLE AND FIGURES

GuideID	BrunelloID	Guide sequence	Gibson cloning oligo for LentiGuide x BsmBI
sgNEG	BRDT-2	ATGGCCCTTTCAACGTCCTG	TATCTTGTGGAAGGACGAAACACCGATGGCCCTTTCAACGTCCTGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgNT	NT-1	AAAAAGCTTCCGCCTGATGG	TATCTTGTGGAAGGACGAAACACCGAAAAAGCTTCCGCCTGATGGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgBTK-A	BTK-2	CTGTGTTTGCTAAATCCACA	TATCTTGTGGAAGGACGAAACACCGCTGTGTTTGCTAAATCCACAGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgBTK-B	BTK-3	GATGGTAGTTAATGAGCTCA	TATCTTGTGGAAGGACGAAACACCGGATGGTAGTTAATGAGCTCAGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgPAK2-A	PAK2-5	CTAGGAACATCGTGAGCAT	TATCTTGTGGAAGGACGAAACACCGCTAGGAACATCGTGAGCATGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgPAK2-B	PAK2-8	TGACGTTGCACTGGGACAGG	TATCTTGTGGAAGGACGAAACACCGTGACGTTGCACTGGGACAGGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgPKM-A	PKM-6	GCTGTGGCTCTAGACACTAA	TATCTTGTGGAAGGACGAAACACCGGCTGTGGCTCTAGACACTAAGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgPKM-B	PKM-7	TCACCTGCCTTCAGCCCGAGT	TATCTTGTGGAAGGACGAAACACCGTCACCTGCCTTCAGCCCGAGTGTGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgCRKL-A	CRKL-5	ACCGCTCCGCTCGGTATATG	TATCTTGTGGAAGGACGAAACACCGACCGCTCCGCTCGGTATATGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgCRKL-B	CRKL-6	CCTGGGCGCAGGCTCGATGA	TATCTTGTGGAAGGACGAAACACCGCCTGGGCGCAGGCTCGATGAGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgPTK2B-A	PTK2B-6	CTGGGGACATCCATTTGATG	TATCTTGTGGAAGGACGAAACACCGCTGGGGACATCCATTTGATGTTTTAGAGCTAGAAATAGCAAGTTAAAA
sgPTK2B-B	PTK2B-7	GCGGAACAAGAACTCCCTGA	TATCTTGTGGAAGGACGAAACACCGCGCGAACAAGAACTCCCTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAA

Supplementary table 1. CRISPRguide sequences.



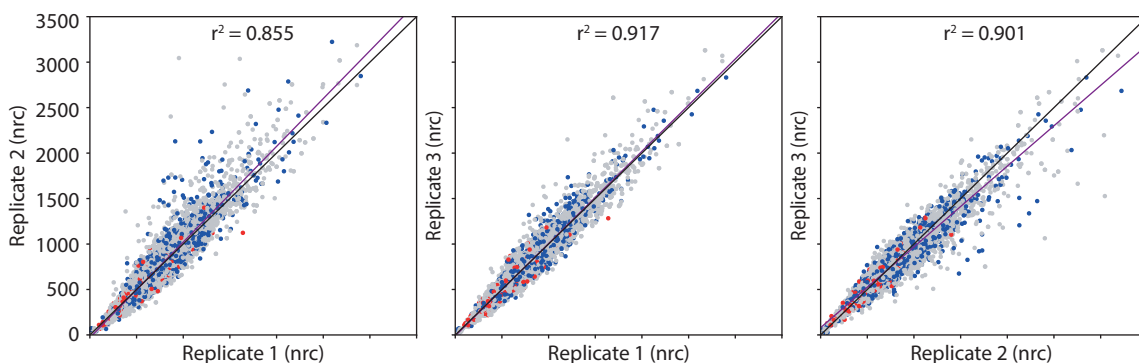
Supplementary figure 1. Generation of Cas9 clones. **a** Namalwa cells were transduced with Lenti-Cas9-BSD, blasticidin-selected, and sorted 1 cell/well. Growing clones were first tested for algM-induced adhesion to fibronectin. The clone with the best stimulated/unstimulated ratio was selected for screening (clone 10). Graph is presented as mean + SD of triplicates. The clone selection was performed once. Source data are provided as a Source Data file. **b** Gating strategy to select viable single cells for FACS analysis. **c** Clone 10 was tested for Cas9 expression by intracellular FACS. Cas9 expression was analyzed once. **d** Clone 10 was tested for Cas9 activity with the Cas9-reporter. Cas9 activity results in loss of GFP, but not BFP expression. Cas9 activity was regularly tested, and we observed no loss of Cas9 activity, even after prolonged culturing in the absence of blasticidin.

a

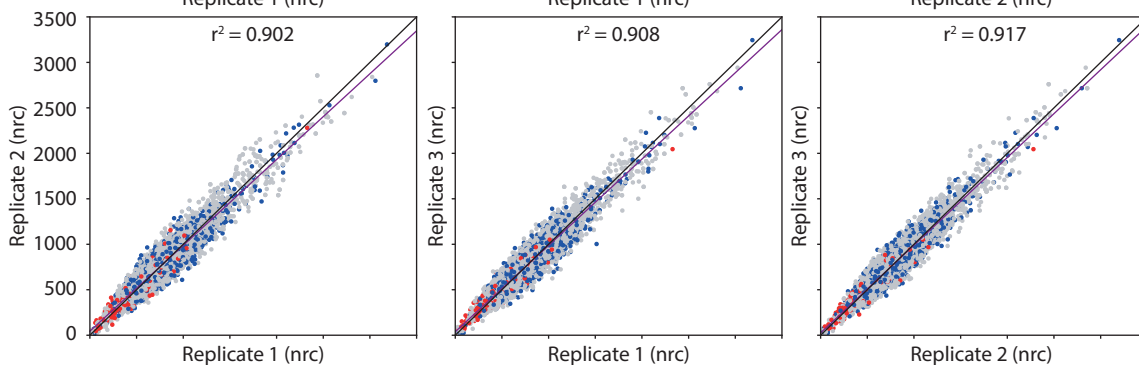
Correlation plots of replicates

Guides against: ● Viability genes ● Non-expressed genes ● Other genes

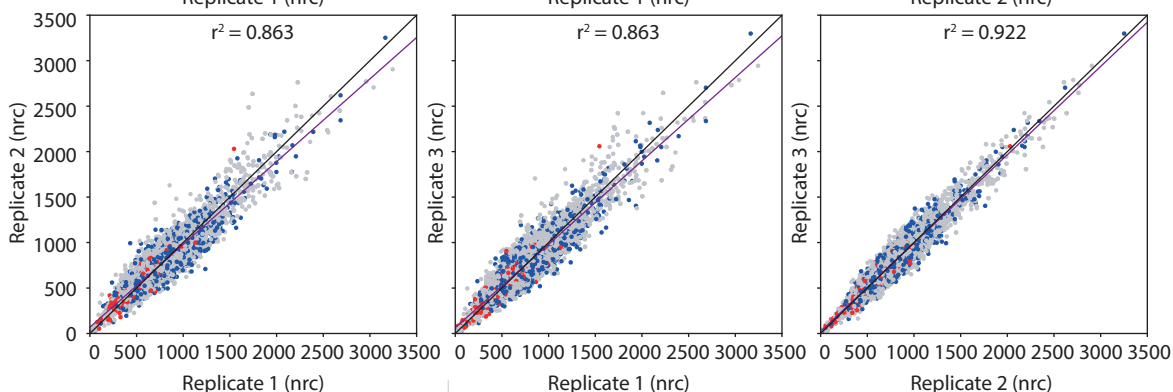
Pre-adhesion



algM-adhesion

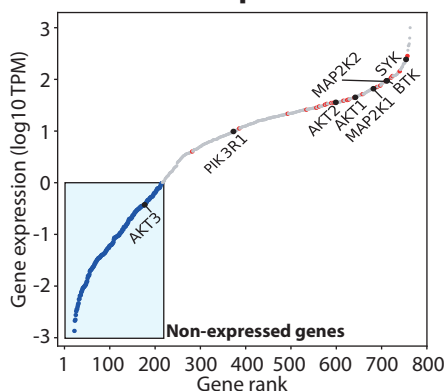


PMA-adhesion

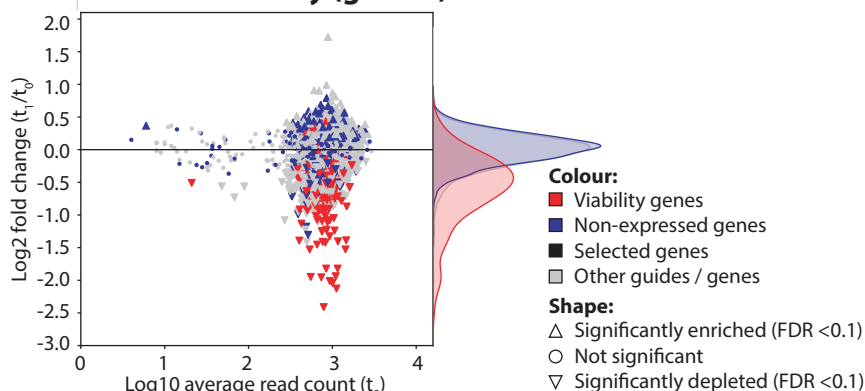


b

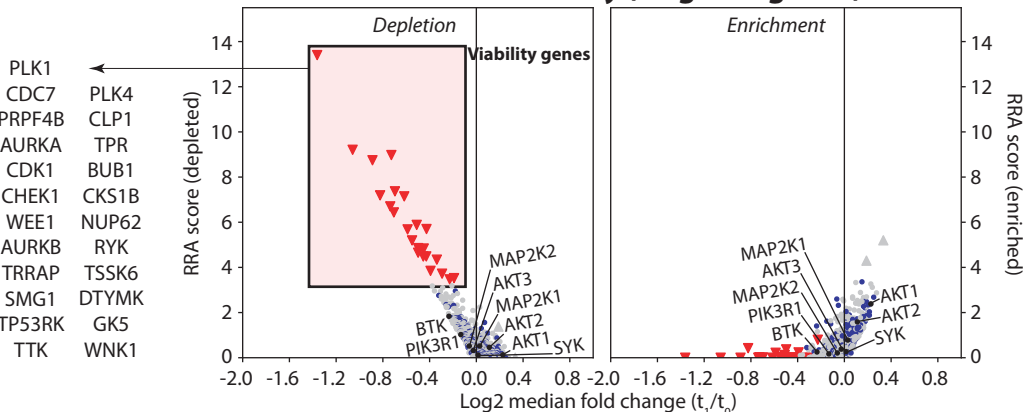
Gene expression



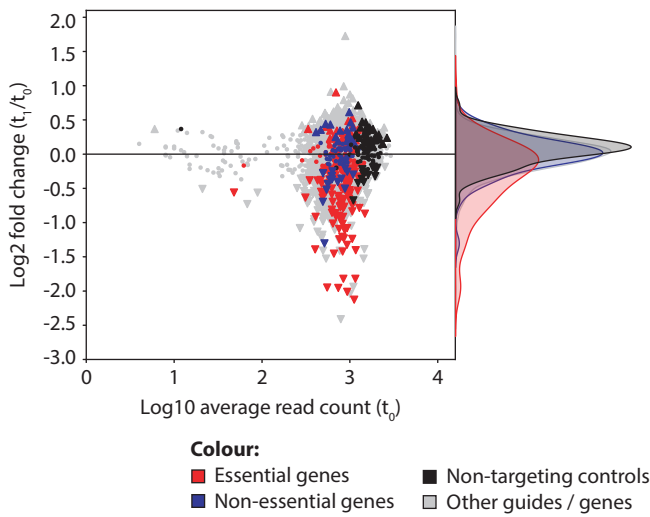
c Short-term lethality (guides)



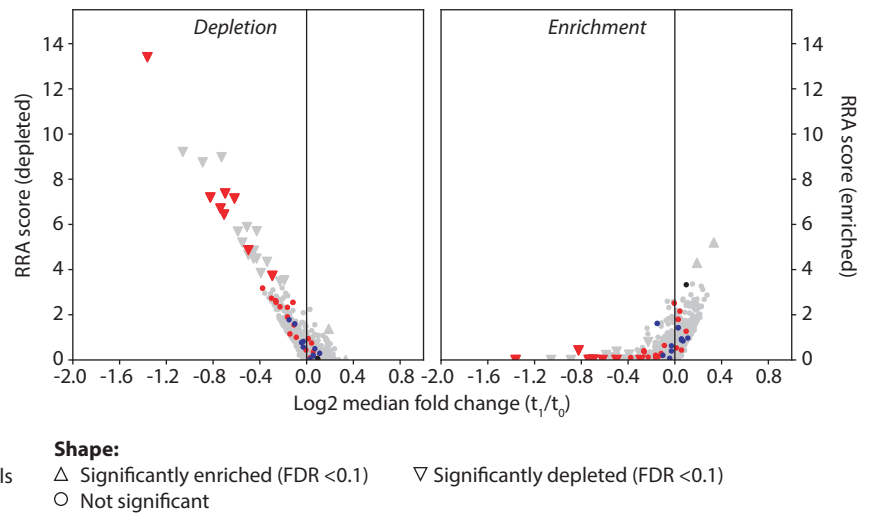
Short-term lethality (targeted genes)



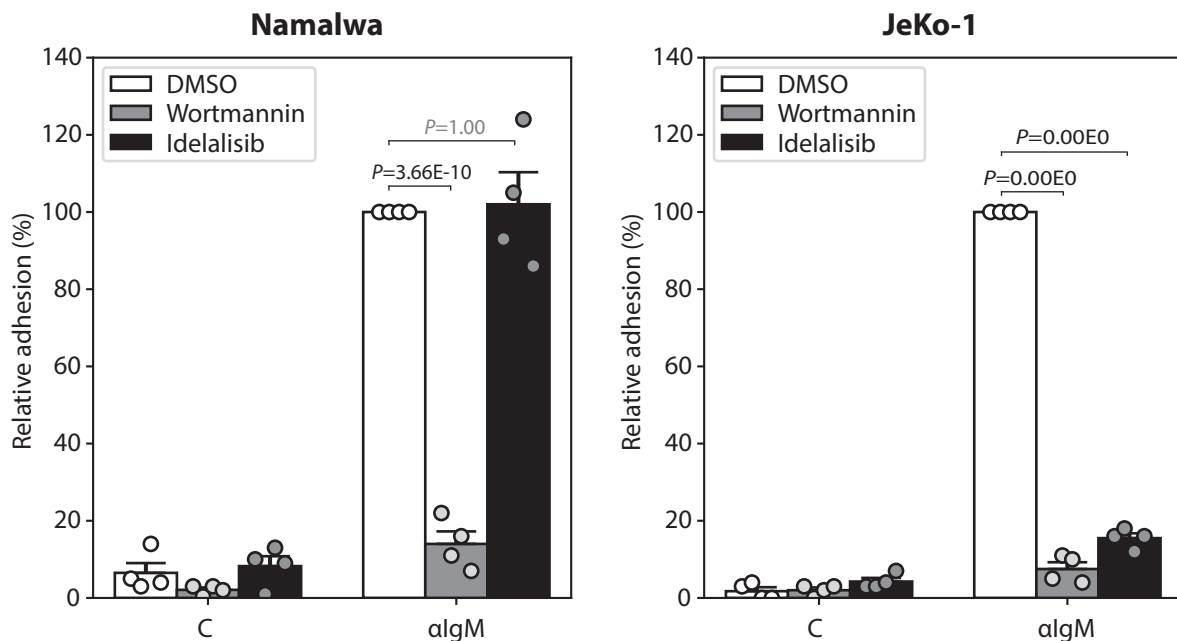
d Short-term lethality (guides)



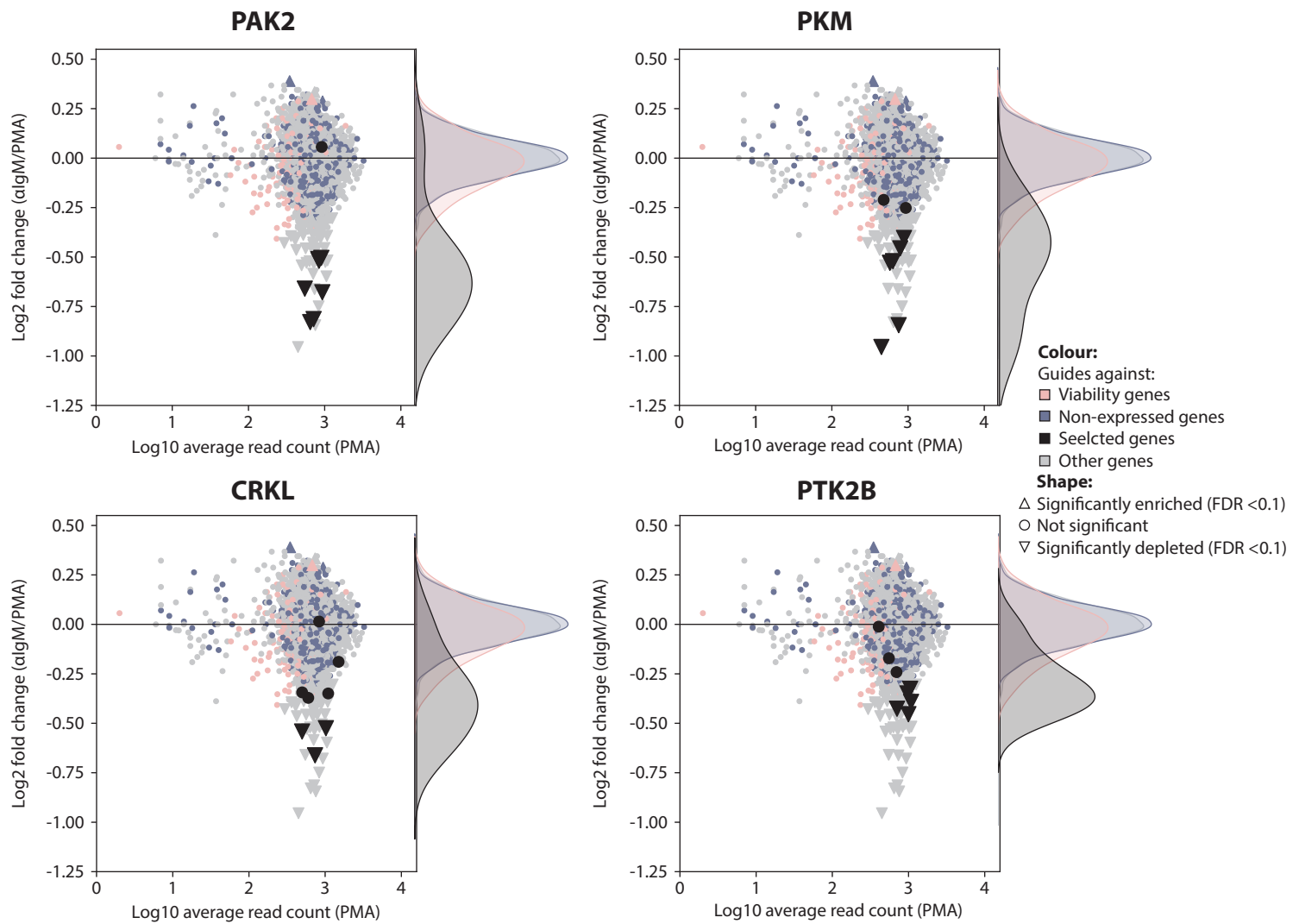
Short-term lethality (targeted genes)



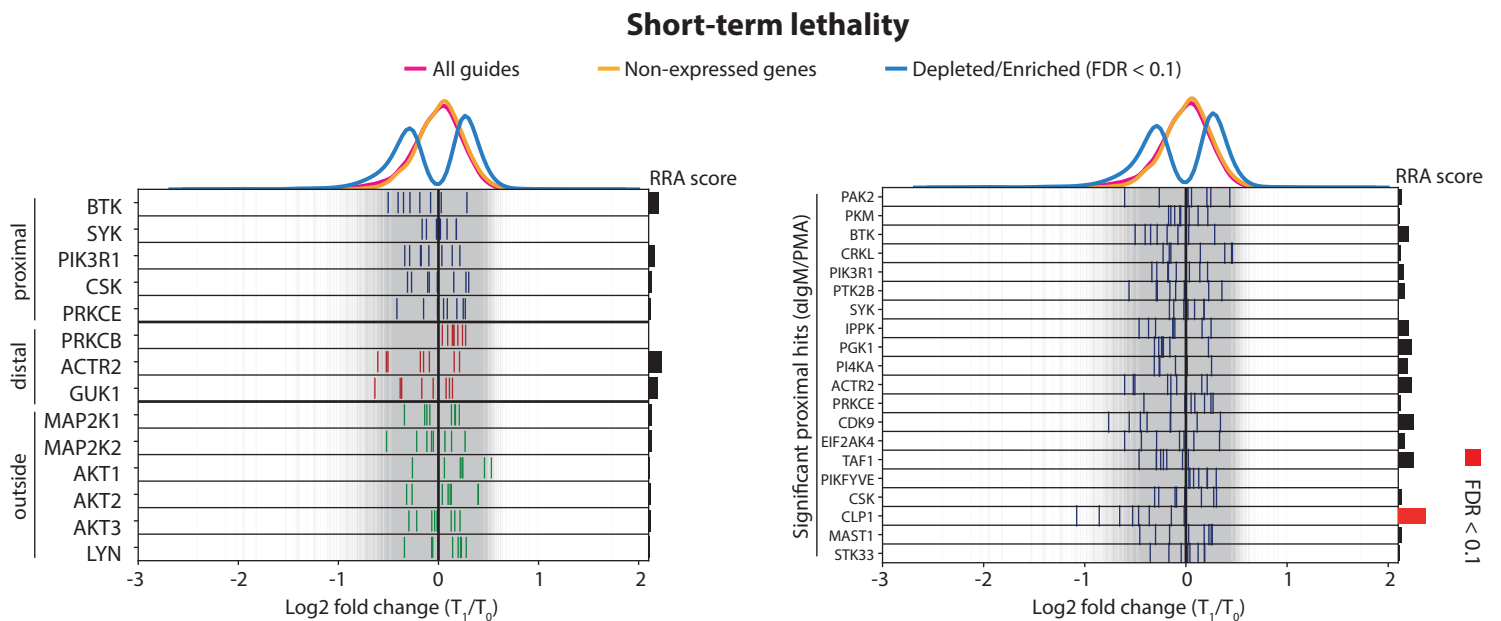
Supplementary figure 2. Replicate plots of the adhesion screen and definition of non-expressed and viability genes. **a** Replicate plots of guide distribution in pre-adhesion, algM-induced adhesion, and PMA-induced adhesion samples. nrc: normalized read counts, black line $y=x$, purple line: linear fit. **b** Genes represented in the Brunello kinome-centered library were selected from public Namalwa RNAseq data, and ranked by gene expression. Genes which express less than 1 transcript per million (TPM) were defined as non-expressed genes in Namalwa. Of note, the non-essential/non-expressed gene BRDT had an expression level of 0.0 TPM (not shown). **c** MA and volcano plots of pre-adhesion samples ($t_1 = 8$ days upon library transduction) compared to the library distribution (t_0). The genes in which the corresponding guides dropped out significantly (FDR < 0.1, DESeq2 followed by α RRA_{depletion}) were defined as (strong) viability genes. The screen was performed with 3 independent replicates. **d** The same MA and volcano plots as in c, but here the guides against essential and non-essential genes (described by Hart *et al*³¹) and non-targeting control guides are depicted. Source data are provided as a Source Data file.



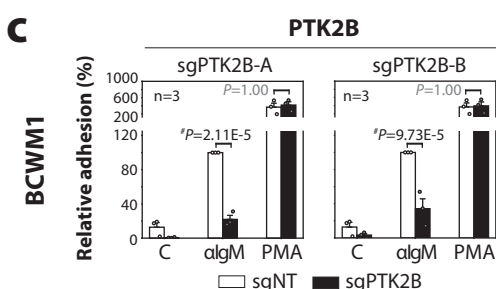
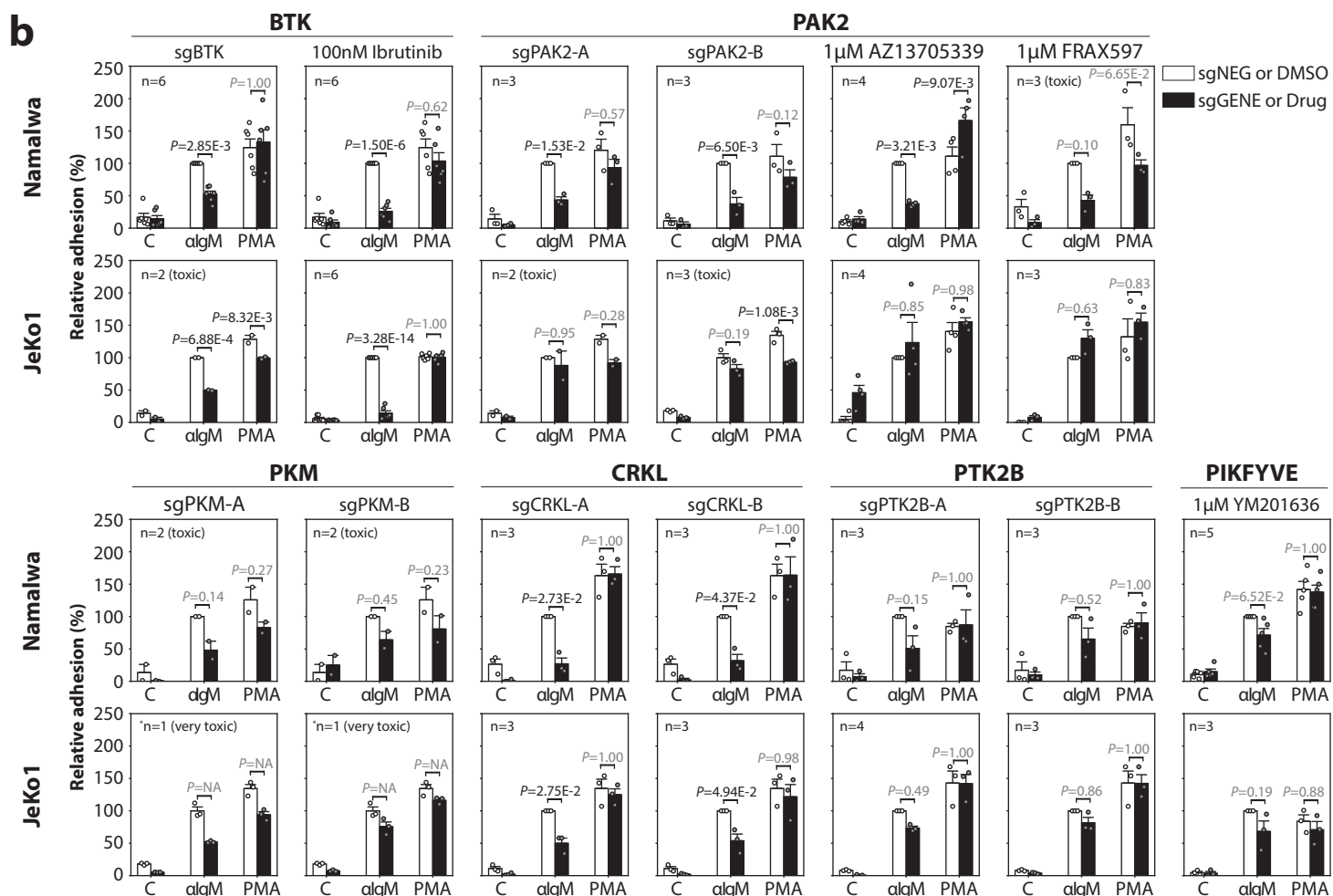
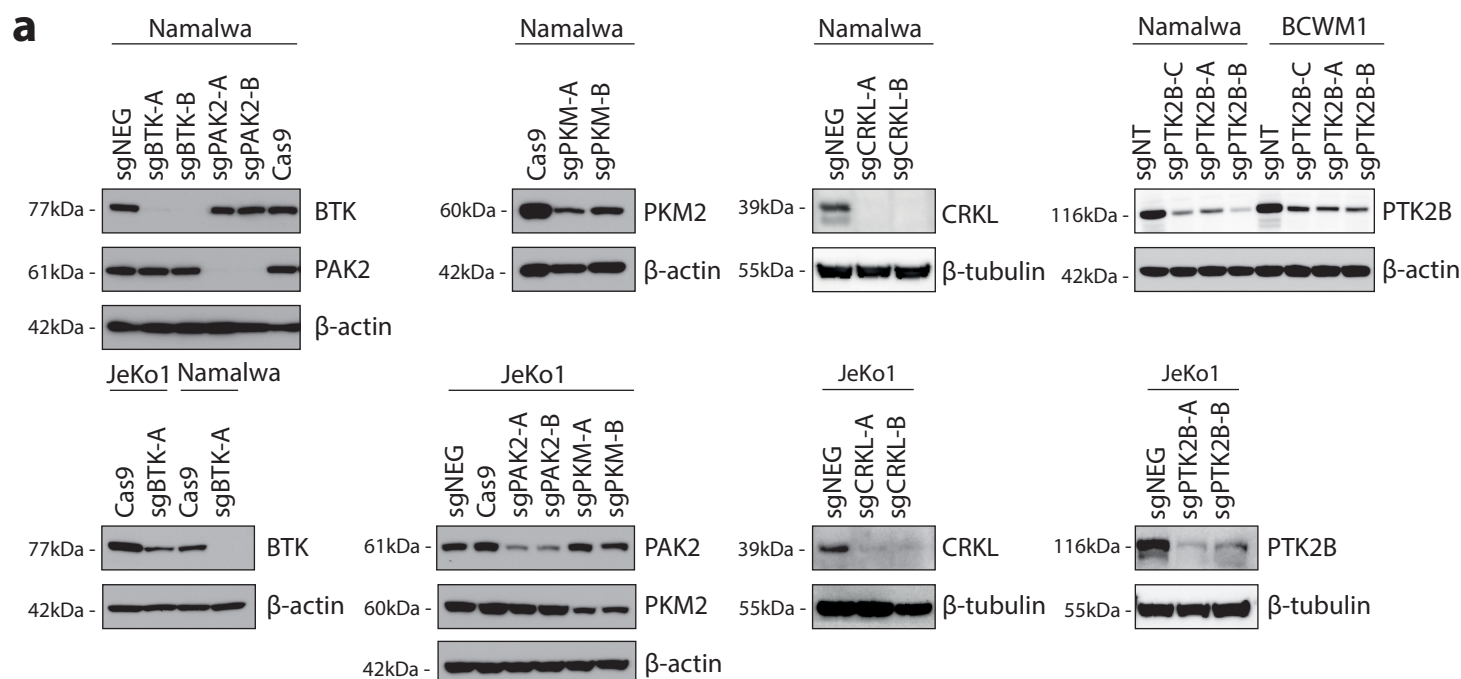
Supplementary figure 3. PI3K δ is not essential for BCR-controlled adhesion of Namalwa cells. Namalwa and JeKo-1 cells pretreated with 100nM wortmannin (pan-PI3K inhibitor), 1 μ M idelalisib (PI3K δ inhibitor), or DMSO control, were stimulated with algM, and allowed to adhere to fibronectin-coated surfaces. Graphs are presented as normalized means + SEM (100% = untreated algM-stimulated cells). P -values are adjusted P -values from two-way ANOVA followed by Tukey HSD post hoc test (two-tailed and paired design), of 4 independent experiments each assayed in triplicate. Source data are provided as a Source Data file.



Supplementary figure 4. MA plots of algM-induced adhesion relative to PMA-induced adhesion of the novel regulators of proximal BCR-integrin signaling. Statistics was performed with DESeq2. The screen was performed with 3 independent replicates. Source data are provided as a Source Data file.



Supplementary figure 5. Analysis of the short-term lethality of the guides targeting genes involved in the antigen/BCR-integrin axis. Extension of figures 3 (left) and 4a (right), showing the effect on short-term lethality of the guides against BCR signaling components. Statistics was performed with DESeq2 followed by α RRA. The RRA scores are α RRA_{depletion} scores. The screen was performed with 3 independent replicates. Source data are provided as a Source Data file.



Supplementary figure 6. Validation of the novel proximal BCR-signaling hits. **a** CRISPR knock outs of BTK, PAK2, PKM, CRKL, and PTK2B in Namalwa and JeKo1 cells, and PTK2B in BCWM1 cells were verified by western blot. A guide against the nonessential/nonexpressed gene BRDT (sgNEG) or a nontargeting guide (sgNT) were used as control guides. Cas9 represents the parental cells. Knockout upon each transduction was at least two times verified by western blot. **b-c** Namalwa, JeKo1, and BCWM1 cells with indicated knock out or 1h pretreated with indicated drug, were stimulated with algM or PMA, and allowed to adhere to fibronectin-coated surfaces. Graphs are presented as normalized means + SEM (100% = untreated algM-stimulated cells). P-values are adjusted P-values from two-way ANOVA followed by Tukey HSD post hoc test (two-tailed and paired design), of n independent experiments each assayed in triplicate. *Technical replicates are shown. *PMA values were excluded in the statistics, because they were in another range than the algM values. Source data are provided as a Source Data file.