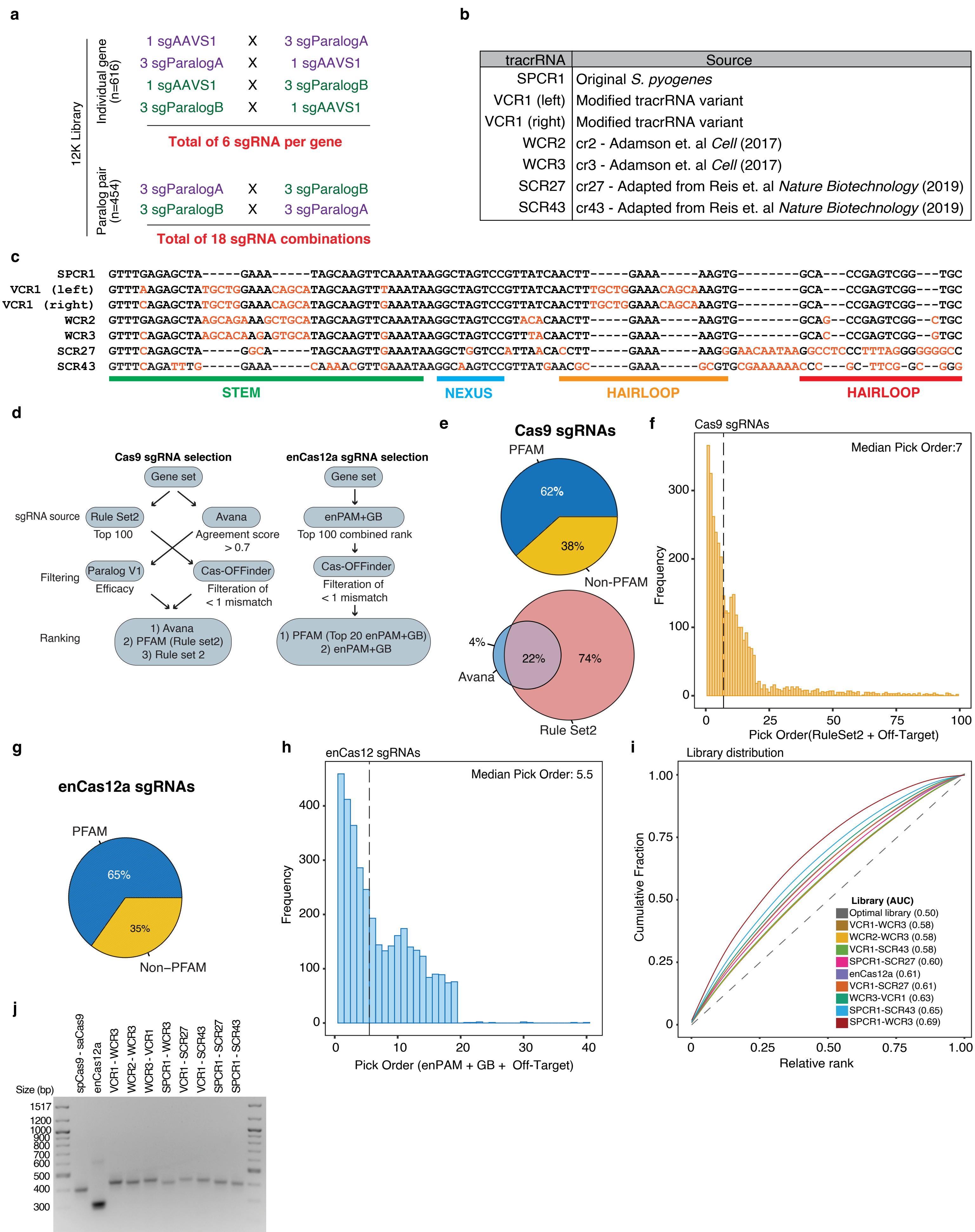
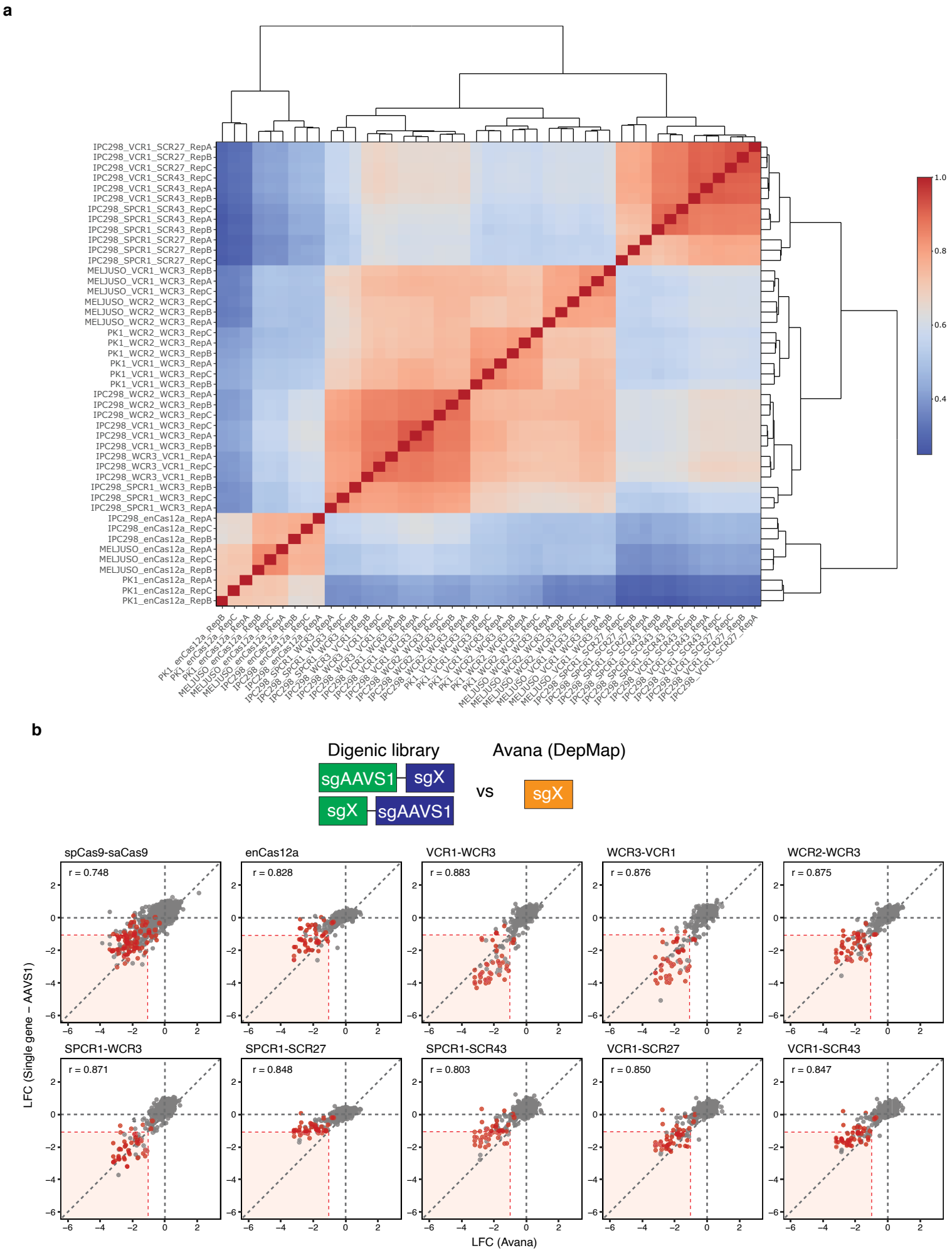
Supplementary Information

Comparative Optimization of Combinatorial CRISPR Screens

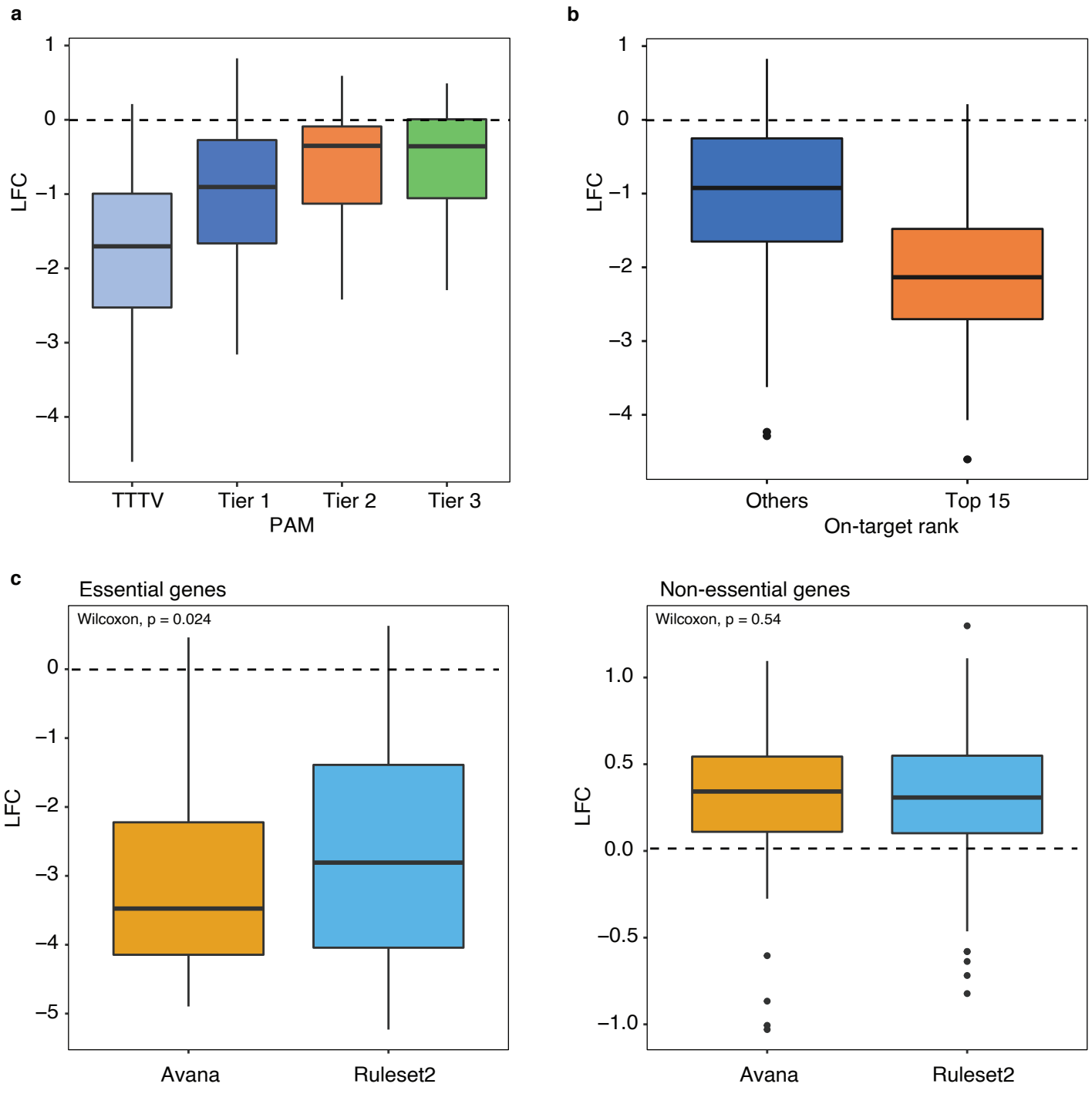
Ruitong Li, Olaf Klingbeil, Davide Monducci, Michael J. Young, Diego J. Rodriguez, Zaid Bayyat, Joshua M. Dempster, Devishi Kesar, Xiaoping Yang, Mahdi Zamanighomi, Christopher R. Vakoc, Takahiro Ito, William R. Sellers


a
12K Library
Individual gene (n=616)
1 sgAAVS1 X 3 sgParalogA
3 sgParalogA X 1 sgAAVS1
1 sgAAVS1 X 3 sgParalogB
3 sgParalogB X 1 sgAAVS1
Total of 6 sgRNA per gene
Paralog pair (n=454)
3 sgParalogA X 3 sgParalogB
3 sgParalogB X 3 sgParalogA
Total of 18 sgRNA combinations
b
tracrRNA | Source
SPCR1 | Original S. pyogenes
VCR1 (left) | Modified tracrRNA variant
VCR1 (right) | Modified tracrRNA variant
WCR2 | cr2 - Adamson et. al Cell (2017)
WCR3 | cr3 - Adamson et. al Cell (2017)
SCR27 | cr27 - Adapted from Reis et. al Nature Biotechnology (2019)
SCR43 | cr43 - Adapted from Reis et. al Nature Biotechnology (2019)
c
SPCR1 GTTTGAGAGCTA-----GAAA-----TAGCAAGTTCAAATAAGGCTAGTCCGTTATCAACTT-----GAAA-----AAGTG--------GCA---CCGAGTCGG---TGC
VCR1 (left) GTTTAAGAGCTATGCTGGAAACAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTTGCTGGAAACAGCAAAGTG--------GCA---CCGAGTCGG---TGC
VCR1 (right) GTTTCAGAGCTATGCTGGAAACAGCATAGCAAGTTGAAATAAGGCTAGTCCGTTATCAACTTTGCTGGAAACAGCAAAGTG--------GCA---CCGAGTCGG---TGC
WCR2 GTTTGAGAGCTAAGCAGAAAGCTGCATAGCAAGTTCAAATAAGGCTAGTCCGTACACAACTT-----GAAA-----AAGTG--------GCAG--CCGAGTCGG--CTGC
WCR3 GTTTCAGAGCTAAGCACAAGAGTGCATAGCAAGTTGAAATAAGGCTAGTCCGTTTACAACTT-----GAAA-----AAGTG--------GCAC--CCGAGTCGG--GTGC
SCR27 GTTTCAGAGCTA-----GGCA-----TAGCAAGTTGAAATAAGGCTGGTCCATTAACACCTT-----GAAA-----AAGGGAACAATAAGGCCTCCCTTTAGGGGGGGC
SCR43 GTTTCAGATTTG-----GAAA-----CAAAACGTTGAAATAAGGCAAGTCCGTTATGAACGC-----GAAA-----GCGTGCGAAAAAACCC---GC-TTCG-GC--GGG
STEM
NEXUS
HAIRLOOP
HAIRLOOP
d
Cas9 sgRNA selection
enCas12a sgRNA selection
Gene set
Gene set
sgRNA source
Rule Set2
Top 100
Avana
Agreement score > 0.7
enPAM+GB
Top 100 combined rank
Filtering
Paralog V1
Efficacy
Cas-OFFinder
Filteration of < 1 mismatch
Cas-OFFinder
Filteration of < 1 mismatch
Ranking
1) Avana
2) PFAM (Rule set2)
3) Rule set 2
1) PFAM (Top 20 enPAM+GB)
2) enPAM+GB
e
Cas9 sgRNAs
PFAM
62%
38%
Non-PFAM
4%
22%
74%
Avana
Rule Set2
f
Cas9 sgRNAs
Median Pick Order:7
Frequency
Pick Order(RuleSet2 + Off-Target)
0
100
200
300
25
50
75
100
g
enCas12a sgRNAs
PFAM
65%
35%
Non–PFAM
h
enCas12 sgRNAs
Median Pick Order: 5.5
Frequency
Pick Order (enPAM + GB + Off-Target)
0
100
200
300
400
10
20
30
40
i
Library distribution
Cumulative Fraction
Relative rank
0
0.25
0.50
0.75
1.00
Library (AUC)
Optimal library (0.50)
VCR1-WCR3 (0.58)
WCR2-WCR3 (0.58)
VCR1-SCR43 (0.58)
SPCR1-SCR27 (0.60)
enCas12a (0.61)
VCR1-SCR27 (0.61)
WCR3-VCR1 (0.63)
SPCR1-SCR43 (0.65)
SPCR1-WCR3 (0.69)
j
spCas9 - saCas9
enCas12a
VCR1 - WCR3
WCR2 - WCR3
WCR3 - VCR1
SPCR1 - WCR3
VCR1 - SCR27
VCR1 - SCR43
SPCR1 - SCR27
SPCR1 - SCR43
Size (bp)
1517
1200
1000
900
800
700
600
500
400
300

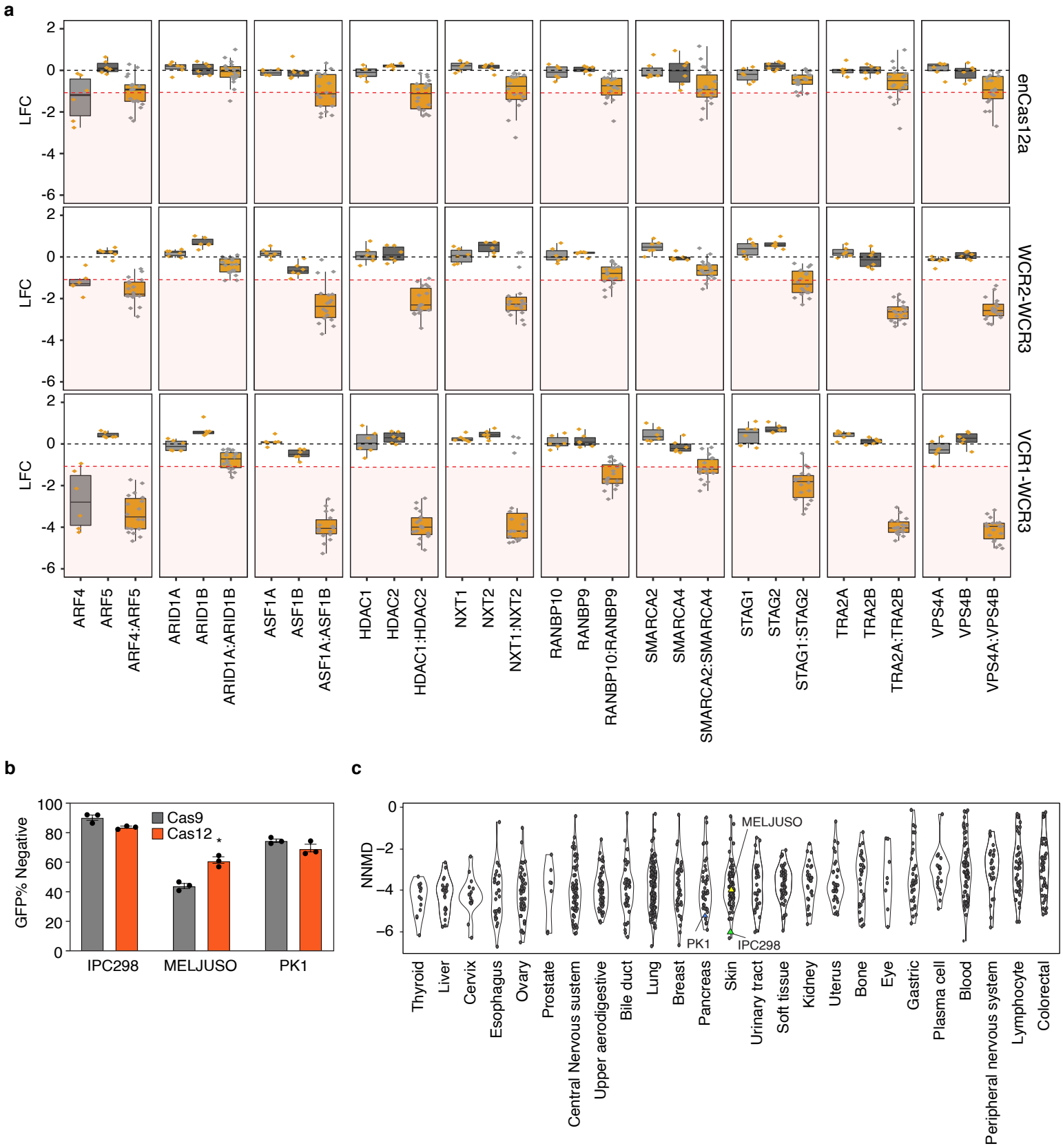
Supplementary Fig. 1: Composition of the combinatorial libraries. (**a**) Schematic of the library design. The nine 12K libraries generated targeting 616 individual genes with 6 sgRNAs coupled with AAVS1 sgRNA cutting control (3 sgRNA in right and left position). Dual knockouts of 454 paralog pairs by 18 sgRNA combinations are utilized. (**b**) Source and nomenclature of alternative tracrRNA sequences. (**c**) Sequence of alternative tracrRNAs. (**d**) Source and prioritization of sgRNAs for Cas9 and enCas12a. (**e**) Pie chart depicting the percentage of Cas9 sgRNAs targeting PFAM and non-PFAM domain (*top*). Venn diagram of sgRNAs from Avana library, Rule Set 2 or both (*bottom*). (**f**) Distribution of Cas9 sgRNAs based on Rule Set 2 Pick Order. Dotted line represents the median Pick Order. (**g**) Pie chart depicting the percentage of enCas12a sgRNAs targeting PFAM and non-PFAM domain. (**h**) Distribution of enCas12a sgRNAs based on Pick Order. Dotted line represents the median Pick Order. (**i**) Area-under-the-curve (AUC) analysis for library distribution from 12K library pDNAs. (**j**) PCR analysis of the pDNA using PCR primers and conditions used to amplify libraries for sequencing analysis. Source data are provided in the Source Data File.


a
IPC298_VCR1_SCR27_RepA
IPC298_VCR1_SCR27_RepB
IPC298_VCR1_SCR27_RepC
IPC298_VCR1_SCR43_RepC
IPC298_VCR1_SCR43_RepA
IPC298_VCR1_SCR43_RepB
IPC298_SPCR1_SCR43_RepC
IPC298_SPCR1_SCR43_RepA
IPC298_SPCR1_SCR43_RepB
IPC298_SPCR1_SCR27_RepA
IPC298_SPCR1_SCR27_RepB
IPC298_SPCR1_SCR27_RepC
MELJUSO_VCR1_WCR3_RepB
MELJUSO_VCR1_WCR3_RepA
MELJUSO_VCR1_WCR3_RepC
MELJUSO_WCR2_WCR3_RepC
MELJUSO_WCR2_WCR3_RepB
MELJUSO_WCR2_WCR3_RepA
PK1_WCR2_WCR3_RepC
PK1_WCR2_WCR3_RepA
PK1_WCR2_WCR3_RepB
PK1_VCR1_WCR3_RepA
PK1_VCR1_WCR3_RepC
PK1_VCR1_WCR3_RepB
IPC298_WCR2_WCR3_RepA
IPC298_WCR2_WCR3_RepB
IPC298_WCR2_WCR3_RepC
IPC298_VCR1_WCR3_RepC
IPC298_VCR1_WCR3_RepA
IPC298_VCR1_WCR3_RepB
IPC298_WCR3_VCR1_RepA
IPC298_WCR3_VCR1_RepC
IPC298_WCR3_VCR1_RepB
IPC298_SPCR1_WCR3_RepB
IPC298_SPCR1_WCR3_RepC
IPC298_SPCR1_WCR3_RepA
IPC298_enCas12a_RepA
IPC298_enCas12a_RepC
IPC298_enCas12a_RepB
MELJUSO_enCas12a_RepA
MELJUSO_enCas12a_RepC
MELJUSO_enCas12a_RepB
PK1_enCas12a_RepA
PK1_enCas12a_RepC
PK1_enCas12a_RepB
1.0
0.8
0.6
0.4
b
Digenic library
sgAAVS1
sgX
sgX
sgAAVS1
vs
Avana (DepMap)
sgX
spCas9-saCas9
r = 0.748
enCas12a
r = 0.828
VCR1-WCR3
r = 0.883
WCR3-VCR1
r = 0.876
WCR2-WCR3
r = 0.875
SPCR1-WCR3
r = 0.871
SPCR1-SCR27
r = 0.848
SPCR1-SCR43
r = 0.803
VCR1-SCR27
r = 0.850
VCR1-SCR43
r = 0.847
LFC (Single gene – AAVS1)
LFC (Avana)

Supplementary Fig. 2: Quality controls for combinatorial systems and the screen performance. (**a**) Pearson correlation heatmap of LFCs between all pairwise combinations of the 12K library screens across three cell lines (IPC298, MELJUSO, PK1). (**b**) Scatterplot of the LFC for each gene against the LFC from Avana DepMap CRISPR dataset in IPC298. Pan-essential genes are annotated in red. Source data are provided in the Source Data File.


a
1
0
−1
−2
−3
−4
LFC
TTTV
Tier 1
Tier 2
Tier 3
PAM
b
1
0
−1
−2
−3
−4
LFC
Others
Top 15
On-target rank
c
Essential genes
Wilcoxon, p = 0.024
0
−1
−2
−3
−4
−5
LFC
Avana
Ruleset2
Non-essential genes
Wilcoxon, p = 0.54
1.0
0.5
0.0
−0.5
−1.0
LFC
Avana
Ruleset2

Supplementary Fig. 3: Evaluation of enCas12a and Cas9 sgRNA design. (**a**) Activity of enCas12a sgRNA targeting essential genes binned by PAM tiers. (**b**) Same as (a) binned by on-target rank. (**c**) Activity of Cas9 sgRNA targeting essential (*left*) or non-essential (*right*) genes grouped by sgRNAs source (DepMap Avana screens or Rule Set 2). Significant difference is determined using Wilcoxon test. For the boxplots, the centerline, lower hinge, and upper hinge correspond to the 50th, 25th, and 75th percentiles, respectively. The upper and lower whiskers extend from the upper and lower hinges to the largest and smallest values no further than 1.5 * IQR (interquartile range). Source data are provided in the Source Data File.


a
enCas12a
WCR2-WCR3
VCR1-WCR3
LFC
ARF4
ARF5
ARF4:ARF5
ARID1A
ARID1B
ARID1A:ARID1B
ASF1A
ASF1B
ASF1A:ASF1B
HDAC1
HDAC2
HDAC1:HDAC2
NXT1
NXT2
NXT1:NXT2
RANBP10
RANBP9
RANBP10:RANBP9
SMARCA2
SMARCA4
SMARCA2:SMARCA4
STAG1
STAG2
STAG1:STAG2
TRA2A
TRA2B
TRA2A:TRA2B
VPS4A
VPS4B
VPS4A:VPS4B
b
GFP% Negative
Cas9
Cas12
IPC298
MELJUSO
PK1
c
NNMD
MELJUSO
PK1
IPC298
Thyroid
Liver
Cervix
Esophagus
Ovary
Prostate
Central Nervous sustem
Upper aerodigestive
Bile duct
Lung
Breast
Pancreas
Skin
Urinary tract
Soft tissue
Kidney
Uterus
Bone
Eye
Gastric
Plasma cell
Blood
Peripheral nervous system
Lymphocyte
Colorectal

Supplementary Fig. 4: Comparison of LFC of pan-essential paralog pairs. (**a**) LFC of individual sgRNA combinations for single-gene and dual knockout of pan-essential paralog pairs in IPC298. The centerline, lower hinge, and upper hinge correspond to the 50th, 25th, and 75th percentiles, respectively. The upper and lower whiskers extend from the upper and lower hinges to the largest and smallest values no further than 1.5 * IQR (interquartile range). n=3 biological replicates; 6 independent sgRNAs per gene and 18 independent sgRNA combination per gene pairs. (b) Cas9 and Cas12 activity assay assessed by percentage of GFP negative cells measured by FACS in Cas9 or enCas12a-stably expressing cells transduced with vector ectopically expressing EGFP and sgEGFP. The data are shown as mean ± SD (n=3 biologically independent replicates). *P-value≤ 0.001, two-way ANOVA. (c) Separation between non- and pan-essential genes computed by NNMD score across CCLE. Source data are provided in the Source Data File.