An enhanced CRISPR repressor for targeted mammalian gene regulation

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Supplementary Table 1 Sequences of dCas9-Krab and dCas9-KRAB-MeCP2

>dCas9-KRAB

Labels: dCas9; SV40 nucleus localization signal (NLS); KRAB; glycine serine-rich linker (bold); stop codon (italic)

ATGGACAAGAAGTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGCTGGGCCGTCA
TTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCA
CAGCATAAAGAAGAACCTCATTGGCGCCCTCCTGTTCGACTCCGGGGAGACGGCCGAAGC
CACGCGGCTCAAAAGAACAGCACGGCGCAGATATACCCGCAGAAAGAATCGGATCTGCTAC
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GTGGACGAGGTGGCGTACCATGAAAAGTACCCAACCATATATCATCTGAGGAAGAAGCTTG
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CTCGGGCTGACCCCCAACTTTAAATCTAACTTCGACCTGGCCGAAGATGCCAAGCTTCAACT
GAGCAAAGACACCTACGATGATGATCTCGACAATCTGCTGGCCCAGATCGGCGACCAGTAC
GCAGACCTTTTTTTGGCGGCAAAGAACCTGTCAGACGCCATTCTGCTGAGTGATATTCTGCG
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CACCACCAAGACTTGACTTTGCTGAAGGCCCTTGTCAGACAGCAACTGCCTGAGAAGTACAA
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GGTACACCTCTACAAAGGAGGTCCTGGACGCCACACTGATTCATCAGTCAATTACGGGGCT
CTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAG
AAGAGGAAGGTG**AGTGGTGGAGGAAGTGGCGGGTCAGGGTCG**ATGGACGCGAAATCACTT
ACGGCATGGTCGAGAACACTGGTTACGTTCAAGGACGTGTTTGTGGACTTTACACGTGAGG
AGTGGAAATTGCTGGATACTGCGCAACAAATTGTGTATCGAAATGTCATGCTTGAGAATTAC
AAGAACCTCGTCAGTCTCGGATACCAGTTGACGAAACCGGATGTGATCCTTAGGCTCGAAAA
GGGGGAAGAACCTTGGCTGGTA*TAG*

>dCas9-KRAB-MeCP2

Labels: dCas9; SV40 nucleus localization signal (NLS); KRAB; TRD domain of MeCP2; glycine serine-rich linker (bold); stop codon (italic)

ATGGACAAGAAGTACTCCATTGGGCTCGCTATCGGCACAAACAGCGTCGGCTGGGCCGTCA
TTACGGACGAGTACAAGGTGCCGAGCAAAAAATTCAAAGTTCTGGGCAATACCGATCGCCA
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CACGCGGCTCAAAAGAACAGCACGGCGCAGATATACCCGCAGAAAGAATCGGATCTGCTAC
CTGCAGGAGATCTTTAGTAATGAGATGGCTAAGGTGGATGACTCTTTCTTCCATAGGCTGGA
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AGAAATACGGCGGATTCGATTCTCCTACAGTCGCTTACAGTGTACTGGTTGTGGCCAAAGTG
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AGCGATCAAGCTTCGAAAAAAACCCCATCGACTTTCTCGAGGCGAAAGGATATAAAGAGGTC
AAAAAAGACCTCATCATTAAGCTTCCCAAGTACTCTCTCTTTGAGCTTGAAAACGGCCGGAA
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GAGCAGAAGCAGCTGTTCGTGGAACAACACAAACACTACCTTGATGAGATCATCGAGCAAAT
AAGCGAATTCTCCAAAAGAGTGATCCTCGCCGACGCTAACCTCGATAAGGTGCTTTCTGCTT
ACAATAAGCACAGGGATAAGCCCATCAGGGAGCAGGCAGAAAACATTATCCACTTGTTTACT
CTGACCAACTTGGGCGCGCCTGCAGCCTTCAAGTACTTCGACACCACCATAGACAGAAAGC
GGTACACCTCTACAAAGGAGGTCCTGGACGCCACACTGATTCATCAGTCAATTACGGGGCT
CTATGAAACAAGAATCGACCTCTCTCAGCTCGGTGGAGACAGCAGGGCTGACCCCAAGAAG
AAGAGGAAGGTG**AGTGGTGGAGGAAGTGGCGGGTCAGGGTCG**ATGGACGCGAAATCACTT
ACGGCATGGTCGAGAACACTGGTTACGTTCAAGGACGTGTTTGTGGACTTTACACGTGAGG
AGTGGAAATTGCTGGATACTGCGCAACAAATTGTGTATCGAAATGTCATGCTTGAGAATTAC
AAGAACCTCGTCAGTCTCGGATACCAGTTGACGAAACCGGATGTGATCCTTAGGCTCGAAAA
GGGGGAAGAACCTTGGCTGGTA**TCGGGAGGTGGTTCGGGTGGCTCTGGATCA**AGCCCAAA
GAAGAAACGGAAGGTGGAAGCCTCAGTGCAGGTGAAAAGGGTGCTGGAAAAATCCCCCGG
CAAACTCCTCGTGAAGATGCCCTTCCAGGCTTCCCCTGGCGGAAAAGGTGAAGGGGGTGG
CGCAACCACATCTGCCCAGGTCATGGTCATCAAGCGACCTGGAAGGAAAAGAAAGGCCGAG
GCTGACCCTCAGGCCATTCCAAAGAAACGGGGACGCAAGCCAGGGTCCGTGGTCGCAGCT
GCAGCAGCTGAGGCTAAGAAAAAGGCAGTGAAGGAAAGCTCCATCCGCAGTGTGCAGGAG
ACTGTCCTGCCCATCAAGAAGAGGAAGACTAGGGAGACCGTGTCCATCGAGGTCAAAGAAG
TGGTCAAGCCCCTGCTCGTGTCCACCCTGGGCGAAAAATCTGGAAAGGGGCTCAAAACATG

CAAGTCACCTGGACGGAAAAGCAAGGAGTCTAGTCCAAAGGGGCGCTCAAGCTCCGCTTCT
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GCCACTGCTCCCACCACCTCCACCACCTGAGCCACAGTCAAGCGAAGACCCCATCAGCCCA
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GCCTGGAGAGTGATGGCTGTCCAAAAGAACCCGCCAAGACCCAGCCTATGGTGGCAGCCG
CTGCAACTACCACCACAACCACAACTACCACAGTGGCCGAAAAATACAAGCATCGCGGCGA
GGGCGAACGAAAGGACATTGTGTCAAGCTCCATGCCCAGACCTAACCGGGAGGAACCAGT
CGATAGTAGGACACCCGTGACTGAGAGAGTCTCA*TAG*

Supplementary Table 2 A list of differentially expressed (DE) genes with log_2 FC above 1.5 threshold in RNA-seq experiment. (Note that all of these genes have positive log_2 FC, which corresponds to increased transcriptional activity).

dCas9				
	Log_2FC	Log_2CPM	P-Value	FDR
HSPA6[1]	6.57	8.3400	2.00E-25	2.74E-21
DNAJB1[1]	2.24	8.2800	6.09E-23	4.16E-19
HSPA7[1]	7.28	2.1290	9.68E-16	4.41E-12
HSPA1A[1]	4.69	2.7660	4.75E-13	1.62E-09
CRYAB[1]	6.55	2.0320	4.09E-12	1.12E-08
ANKRD1[2]	2.54	2.3950	4.02E-11	9.16E-08
ZFAND2A[1]	1.65	4.2370	3.74E-10	7.29E-07
ATF3[2]	1.11	6.0300	2.30E-07	3.93E-04
DNAH17[1]	1.74	3.5590	2.92E-07	4.44E-04
SCG2	4.11	0.1960	1.35E-05	1.75E-02
VGF[1]	2.33	1.9290	1.41E-05	1.75E-02
FOS[2]	1.41	2.9560	1.65E-05	1.88E-02
GDF15[1]	2.43	1.7580	2.68E-05	2.82E-02
dCas9-KRAB				
	Log_2FC	Log_2CPM	P-Value	FDR
HSPA6[1]	7.19	8.3398	2.26E-26	2.65E-22
DNAJB1[1]	2.62	8.2801	3.88E-26	2.65E-22
HSPA1A[1]	6.36	2.7657	8.82E-21	4.02E-17
HMOX1	2.07	5.5632	1.31E-20	4.48E-17
CRYAB[1]	8.14	2.0315	2.86E-18	7.82E-15
HSPA7[1]	7.82	2.1293	5.79E-18	1.32E-14
VGF[1]	4.6	1.9288	4.29E-16	8.37E-13
ZFAND2A[1]	2	4.2367	6.13E-14	1.05E-10
PPP1R15A[3]	1.47	5.3442	2.88E-12	4.36E-09
GDF15[1]	4.08	1.7576	6.69E-12	9.13E-09
DNAH17[1]	2.34	3.5595	2.04E-11	2.53E-08
CLU	1.49	4.588	6.38E-11	7.26E-08
SERPINH1	1.24	6.3749	1.08E-10	1.14E-07
FOXJ1[3]	6.5	0.4447	1.47E-10	1.43E-07
ANXA1	2.55	1.9991	1.02E-09	9.33E-07
FOS[2]	1.86	2.9564	3.62E-09	3.09E-06
NCRNA00306	3.6	0.6114	1.87E-08	1.50E-05
CSRNP1	1.32	4.5789	2.82E-08	2.14E-05
ANKRD1[2]	2	2.3946	1.28E-07	9.22E-05

ACHE	1.89	2.0935	2.28E-06	1.56E-03
SCG2	4.28	0.1963	4.42E-06	2.75E-03
ATF3[2]	1.02	6.0304	4.42E-06	2.75E-03
HLA-G	3.54	-0.0433	5.02E-06	2.98E-03
INPP5D[3]	2.93	0.983	5.69E-06	3.24E-03
RELB	1.56	2.6216	6.66E-06	3.64E-03
PNLDC1	3.2	0.0906	8.19E-06	4.15E-03
CYP4F3	7.12	-0.4274	8.21E-06	4.15E-03
MMP12	4.58	-0.6846	9.52E-06	4.64E-03
TUBB3	1.15	3.9455	1.39E-05	6.53E-03
PLK2	1.23	3.6057	1.52E-05	6.92E-03
IRX4	2.55	0.425	2.43E-05	1.07E-02
DUSP8	1.22	3.6476	3.69E-05	1.57E-02
FERMT3	2.22	0.6882	5.66E-05	2.34E-02
ACRC	1.63	1.6191	7.17E-05	2.88E-02
IL11	1.92	0.9971	9.89E-05	3.86E-02
dCas9-KRAB-MeCP2				
	Log$_2$FC	**Log$_2$CPM**	**P-Value**	**FDR**
HSPA6[1]	6.7	8.34	1.27E-25	1.73E-21
DNAJB1[1]	2.24	8.28	6.26E-23	4.28E-19
HSPA1A[1]	5.8	2.766	4.26E-18	1.94E-14
HSPA7[1]	6.99	2.129	2.26E-14	7.71E-11
CRYAB[1]	6.59	2.032	4.73E-12	1.29E-08
DNAH17[1]	2.43	3.559	8.96E-12	2.04E-08
INPP5D[3]	3.96	0.983	1.65E-09	3.23E-06
GDF15[1]	3.29	1.758	2.00E-08	3.41E-05
CYP4F3	7.81	-0.427	3.40E-07	5.16E-04
FOXJ1[3]	5.41	0.445	4.53E-07	6.19E-04
ZFAND2A[1]	1.33	4.237	1.24E-06	1.54E-03
PPP1R15A[3]	1.09	5.344	1.46E-06	1.66E-03
VGF[1]	2.55	1.929	2.43E-06	2.56E-03
NCRNA00306	2.75	0.611	2.56E-05	2.50E-02
DUSP8[3]	1.23	3.648	3.32E-05	2.92E-02
PLA2G4C	1.89	1.495	3.55E-05	2.92E-02
ANXA1	1.76	1.999	3.64E-05	2.92E-02

Note: [1] indicates genes common to all three repressor groups, [2] indicates common genes between dCas9 and dCas9-KRAB, and [3] indicates common genes between dCas9-KRAB and dCas9-KRAB-MeCP2. FC = fold-change, CPM = counts per million, FDR = false discovery rate. n=2 biologically independent samples. For DE analyses, see **Supplementary Note 2**.

Supplementary Table 3. A list of down-regulated genes identified in RNA-seq experiment. Note that all of these genes have log_2 FC below 1.5 threshold.

dCas9-KRAB				
	Log_2FC	**Log_2CPM**	**P-Value**	**FDR**
EIF5B[1]	-0.59	5.4743	6.88E-07	2.54E-04
TMEM44	-1.329	2.2374	5.03E-06	1.37E-03
HNRNPA2B1[1]	-0.463	8.6101	1.72E-05	3.86E-03
HNRNPD	-0.451	5.9149	2.69E-05	5.74E-03
HIST1H2AE	-0.822	3.4547	4.20E-05	8.44E-03
TAF15	-0.497	4.9187	1.88E-04	3.02E-02
NFXL1	-0.444	5.0202	2.40E-04	3.62E-02
ODZ3	-0.452	4.8766	2.44E-04	3.62E-02
dCas9-KRAB-MeCP2				
	Log_2FC	**Log_2CPM**	**P-Value**	**FDR**
EIF5B[1]	-0.63	5.474	2.87E-07	1.87E-04
HNRNPA2B1[1]	-0.427	8.61	5.75E-05	1.96E-02
LRRFIP1	-0.398	5.767	1.54E-04	4.34E-02
PRPF19	-0.359	6.983	1.55E-04	4.34E-02
RNU6ATAC	-0.662	3.595	1.67E-04	4.36E-02

Note: [1] indicates genes down-regulated in both dCas9-KRAB and dCas9-KRAB-MeCP2 groups. None of the down-regulated genes showed a near sequence match to the *CXCR4* targeting sgRNA. No downregulated genes were observed in the dCas9 group. FC = fold-change, CPM = counts per million, FDR = false discovery rate. n=2 biologically independent samples. For DE analyses, see **Supplementary Note 2**.

Supplementary Table 4 Mean fold changes of all essential gene-targeting sgRNAs and *p*-values for statistical comparison between essential and non-essential guides in HAP1 lethality screen.

Experiment	Mean essential guides $\log_2$ OR	P-value[#]
dCas9_day0	-0.008	0.1329
dCas9_day7	0.001	0.669
dCas9_day14	-0.001	0.6289
dCas9-KRAB_day0	-0.015	0.005708
dCas9-KRAB _day7	-0.056	1.83E-07
dCas9-KRAB _day14	-0.081	5.41E-19
dCas9-KRAB-MeCP2_day0	0.031	0.9996
dCas9-KRAB-MeCP2_day7*	-2.358	4.87E-78
dCas9-KRAB-MeCP2_day14**	-2.586	3.52E-80

[#] p-value is derived from one-tailed Welch T-test comparing the $\log_2$ OR of the essential guides versus the $\log_2$ OR of the non-essential guides. See **Supplementary Note 3** for detailed analysis and statistical test.
* Log_2 Odds-ratio ($\log_2$ OR) that was -Infinity, i.e. complete depletion, were replaced by the least finite OR for day 7, i.e. -7.607176
** $\log_2$ Odds-ratio ($\log_2$ OR) that were -Infinity, i.e. complete depletion, were replaced by the least finite OR for day 14, i.e. -8.867043

Supplementary Table 5 Mean fold changes of all essential gene-targeting sgRNAs and *p*-values for statistical comparison between essential and non-essential guides in SH-SY5Y lethality screen.

Experiment	mean essential guides log2 OR	P-value#
dCas9_day0	-0.0517	0.0009321
dCas9_day7	-0.10179	9.92E-16
dCas9_day14	-0.12011	3.76E-20
dCas9_day22	-0.149066	1.31E-23
dCas9-KRAB_day0	-0.1341297	3.79E-22
dCas9-KRAB _day7	-0.3476919	4.72E-37
dCas9-KRAB _day14	-0.3733395	2.16E-44
dCas9-KRAB _day22	-0.386887	2.24E-52
dCas9-KRAB-MeCP2_day0	-0.1937839	5.27E-26
dCas9-KRAB-MeCP2_day7	-0.4740388	2.55E-55
dCas9-KRAB-MeCP2_day14	-0.3774654	1.19E-63
dCas9-KRAB-MeCP2_day22	-0.525382	3.08E-70

p-value is derived from one-tailed Welch T-test comparing the log_2 OR of the essential guides versus the log_2 OR of the non-essential guides. See **Supplementary Note 3** for detailed analysis and statistical test.

Supplementary Table 6 Mean fold changes of all essential gene-targeting sgRNAs and *p*-values for statistical comparison between essential and non-essential guides in HEK293T lethality screen.

Experiment	mean essential guides log2 OR	P-value[#]
dCas9_day0	0.003201572	0.12
dCas9_day7	-0.03381157	1.18E-06
dCas9_day14	-0.05741197	2.43E-08
dCas9-KRAB_day0	-0.07741853	0.02
dCas9-KRAB _day7	-0.06196394	1.53E-14
dCas9-KRAB _day14	-0.007220968	4.15E-15
dCas9-KRAB-MeCP2_day0	-0.03050618	2.34E-06
dCas9-KRAB-MeCP2_day7	-0.120637	7.67E-38
dCas9-KRAB-MeCP2_day14	-0.1226212	4.63E-34

[#] p-value is derived from one-tailed Welch T-test comparing the $\log_2$ OR of the essential guides versus the $\log_2$ OR of the non-essential guides. See **Supplementary Note 3** for detailed analysis and statistical test.

Supplementary Table 7. Shown is the total number of sgRNAs showing depletion within or outside of the optimal targeting window previously defined for repression. Data was based on our pooled essentiality screens where 370 essential gene-targeting sgRNAs were tested.

a. Summary of depleted sgRNAs in HAP1 cells at day 14

Targeting window	Total number of essential sgRNAs	Number of significantly depleted sgRNAs with dCas9	Number of significantly depleted sgRNAs with dCas9-KRAB	Number of significantly depleted sgRNAs with dCas9-KRAB-MeCP2
Within -50 to +200bp from TSS	213	35	94	181
Outside -50 to +200bp from TSS	144	19	47	113

b. Summary of depleted sgRNAs in SH-SY5Y cells at day 22

Targeting window	Total number of essential sgRNAs	Number of significantly depleted sgRNAs with dCas9	Number of significantly depleted sgRNAs with dCas9-KRAB	Number of significantly depleted sgRNAs with dCas9-KRAB-MeCP2
Within -50 to +200bp from TSS	213	75	117	149
Outside -50 to +200bp from TSS	144	27	58	77

c. Summary of depleted sgRNAs in 293T cells at day 14

Targeting window	Total number of essential sgRNAs	Number of significantly depleted sgRNAs with dCas9	Number of significantly depleted sgRNAs with dCas9-KRAB	Number of significantly depleted sgRNAs with dCas9-KRAB-MeCP2
Within -50 to +200bp from TSS	213	50	64	84
Outside -50 to +200bp from TSS	144	26	29	49

Supplementary Table 8 Sequence of oligos used to construct dual guide RNA library

Target genes	Sequence of oligos used for cloning	Note
BLM	[TTTTCGTCTCTCACCG] AGGAAACGGAAGAACCCGAG [gttttagagctatgctgaaaagca]	first spacer BLM_1
WRN	[TTTTCGTCTCTCACCG] CCGGCTTGTACTCGGCAGCG [gttttagagctatgctgaaaagca]	first spacer WRN_1
RECQL1	[TTTTCGTCTCTCACCG] GCTGAACGGACCGACCCGGA [gttttagagctatgctgaaaagca]	first spacer RecQL1_1
RECQL4	[TTTTCGTCTCTCACCG] TCGCTGGACGATCGCAAGCG [gttttagagctatgctgaaaagca]	first spacer RecQL4_1
RECQL5	[TTTTCGTCTCTCACCG] CGACGGATATAAGATTGCGT [gttttagagctatgctgaaaagca]	first spacer RecQL5_1
BLM	[TTTTCGTCTCTCACCG] CCTCGCACGCAGACTCCTAG [gttttagagctatgctgaaaagca]	first spacer BLM_2
WRN	[TTTTCGTCTCTCACCG] CTAGCACTATAGATACCCCG [gttttagagctatgctgaaaagca]	first spacer WRN_2
RECQL1	[TTTTCGTCTCTCACCG] GAGATCGGAGAGTCGGACAC [gttttagagctatgctgaaaagca]	first spacer RecQL1_2
RECQL4	[TTTTCGTCTCTCACCG] TGGAGCGGCTGCGGGACGTG [gttttagagctatgctgaaaagca]	first spacer RecQL4_2
RECQL5	[TTTTCGTCTCTCACCG] TGAGTTGGGGTTGTGTATAG [gttttagagctatgctgaaaagca]	first spacer RecQL5_2
BLM	[TTTTCGTCTCTCACCG] CCGCTAGGAGTCTGCGTGCG [gttttagagctatgctgaaaagca]	first spacer BLM_3
WRN	[TTTTCGTCTCTCACCG] GATGTGTACTGTGTGCGCCG [gttttagagctatgctgaaaagca]	first spacer WRN_3
RECQL1	[TTTTCGTCTCTCACCG] AAGATTTTACTCCCGAGTAG [gttttagagctatgctgaaaagca]	first spacer RecQL1_3
RECQL4	[TTTTCGTCTCTCACCG] CTGGACGATCGCAAGCGCGG [gttttagagctatgctgaaaagca]	first spacer RecQL4_3
RECQL5	[TTTTCGTCTCTCACCG] TTTAATTCTTGGGCGGACCA [gttttagagctatgctgaaaagca]	first spacer RecQL5_3
GFP	[TTTTCGTCTCTCACCG] CAAGTTCAGCGTGTCCGGCG [gttttagagctatgctgaaaagca]	first spacer GFP_1
LACZ	[TTTTCGTCTCTCACCG] AGGTAGCAGAGCGGGTAAAC	first spacer LACZ_1

	[gttttagagctatgctgaaaagca]	
LUC	[TTTTCGTCTCTCACCG] AACGCCTTGATTGACAAGGA [gttttagagctatgctgaaaagca]	first spacer LUC_1
RPL34 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] TGGTGAGCTGTGGCTACTCA [gttttagagctatgctgaaaagca]	first spacer RPL34_1
RPL11 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] TCTCTTCCTGCTCTCCATCA [gttttagagctatgctgaaaagca]	first spacer RPL11_1
RPS24 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] CCATCATGGTGAGTCTCCCT [gttttagagctatgctgaaaagca]	first spacer RPS24_1
GFP	[TTTTCGTCTCTCACCG] CAGCTCGATGCGGTTCACCA [gttttagagctatgctgaaaagca]	first spacer GFP_2
LACZ	[TTTTCGTCTCTCACCG] TTTGTGGACGAAGTACCGAA [gttttagagctatgctgaaaagca]	first spacer LACZ_2
LUC	[TTTTCGTCTCTCACCG] ACAACTTTACCGACCGCGCC [gttttagagctatgctgaaaagca]	first spacer LUC_2
RPL34 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] CTCCTCGGATGGCAGCCGAT [gttttagagctatgctgaaaagca]	first spacer RPL34_2
RPL11 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] CCAGCTACTCACCGCCATGA [gttttagagctatgctgaaaagca]	first spacer RPL11_2
RPS24 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] TCCGTGCGCGTTGATATGAT [gttttagagctatgctgaaaagca]	first spacer RPS24_2
GFP	[TTTTCGTCTCTCACCG] CATGCCGAGAGTGATCCCGG [gttttagagctatgctgaaaagca]	first spacer GFP_3
LACZ	[TTTTCGTCTCTCACCG] AGGGCGGCTTCGTCTGGGAC [gttttagagctatgctgaaaagca]	first spacer LACZ_3
LUC	[TTTTCGTCTCTCACCG] AGCTATTCTGATTACACCCG [gttttagagctatgctgaaaagca]	first spacer LUC_3
RPL34 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] GAATGCAGCAAAGTCCCGGG [gttttagagctatgctgaaaagca]	first spacer RPL34_3
RPL11 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] CGGCCTGCCATGGATGGCGA [gttttagagctatgctgaaaagca]	first spacer RPL11_3
RPS24 (ESSENTIA L GENE)	[TTTTCGTCTCTCACCG] CCGCGTATCCGAGCCATCCG [gttttagagctatgctgaaaagca]	first spacer RPS24_3
BLM	[TTTTCGTCTCTAAAC] CTCGGGTTCTTCCGTTTCCT[cggtgACCCAGGCGG CGCACAAG]	second spacer BLM_1
WRN	[TTTTCGTCTCTAAAC] CGCTGCCGAGTACAAGCCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer WRN_1

RECQL1	[TTTTCGTCTCTAAAC] TCCGGGTCGGTCCGTTCAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer RecQL1_1
RECQL4	[TTTTCGTCTCTAAAC] CGCTTGCGATCGTCCAGCGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RecQL4_1
RECQL5	[TTTTCGTCTCTAAAC] ACGCAATCTTATATCCGTCG[cggtgACCCAGGCGG CGCACAAG]	second spacer RecQL5_1
CHEK1	[TTTTCGTCTCTAAAC] CCTGGTACCATTCCTCCACC[cggtgACCCAGGCGG CGCACAAG]	second spacer CHEK1_1
CHEK2	[TTTTCGTCTCTAAAC] CCTGGAGCCGCACACTCTCC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHEK2_1
SLX4	[TTTTCGTCTCTAAAC] CCCGGGTGCCGACTCCCAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX4_1
DNA2	[TTTTCGTCTCTAAAC] CCGGTTCCGCTGTCTTTTCT[cggtgACCCAGGCGG CGCACAAG]	second spacer DNA2_1
EME1	[TTTTCGTCTCTAAAC] CTATCAGGAGATCTACTTCC[cggtgACCCAGGCGG CGCACAAG]	second spacer EME1_1
GEN1	[TTTTCGTCTCTAAAC] CTCGGCTTTCCCTTGCCGGC[cggtgACCCAGGCG GCGCACAAG]	second spacer GEN1_1
RNF4	[TTTTCGTCTCTAAAC] ACTTCCGCTTCGGAGGCCTC[cggtgACCCAGGCG GCGCACAAG]	second spacer RNF4_1
SLX1	[TTTTCGTCTCTAAAC] GTCGGCGAGCGCGTACCATT[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX1_1
TOP3A	[TTTTCGTCTCTAAAC] CCTCAGCACCGAATCCAGTA[cggtgACCCAGGCG GCGCACAAG]	Second spacer TOP3A_1
TOP3B	[TTTTCGTCTCTAAAC] CTATTTCCGGGTCCAGCCGC[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3B_1
WDHD1	[TTTTCGTCTCTAAAC] CAGTGGCGGAGGCTCGGTCA[cggtgACCCAGGCG GCGCACAAG]	second spacer WDHD1_1
CHTF8	[TTTTCGTCTCTAAAC] CGCGGCCAACGGGCGACAAC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHTF8_1
SOD1	[TTTTCGTCTCTAAAC] CGTCTCCGCGACTACTTTAT[cggtgACCCAGGCGG CGCACAAG]	second spacer SOD1_1
GFP	[TTTTCGTCTCTAAAC] CGCCGGACACGCTGAACTTG[cggtgACCCAGGCG GCGCACAAG]	second spacer GFP_1
LACZ	[TTTTCGTCTCTAAAC] GTTTACCCGCTCTGCTACCT[cggtgACCCAGGCGG CGCACAAG]	second spacer LACZ_1

LUC	[TTTTCGTCTCTAAAC] TCCTTGTCAATCAAGGCGTT[cggtgACCCAGGCGG CGCACAAG]	second spacer LUC_1
RPL34 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] TGAGTAGCCACAGCTCACCA[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL34_1
RPL11 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] TGATGGAGAGCAGGAAGAGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL11_1
RPS24 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] AGGGAGACTCACCATGATGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPS24_1
BLM	[TTTTCGTCTCTAAAC] CTAGGAGTCTGCGTGCGAGG[cggtgACCCAGGCG GCGCACAAG]	second spacer BLM_2
WRN	[TTTTCGTCTCTAAAC] CGGGGTATCTATAGTGCTAG[cggtgACCCAGGCG GCGCACAAG]	second spacer WRN_2
RECQL1	[TTTTCGTCTCTAAAC] GTGTCCGACTCTCCGATCTC[cggtgACCCAGGCGG CGCACAAG]	second spacer RecQL1_2
RECQL4	[TTTTCGTCTCTAAAC] CACGTCCCGCAGCCGCTCCA[cggtgACCCAGGCG GCGCACAAG]	second spacer RecQL4_2
RECQL5	[TTTTCGTCTCTAAAC] CTATACACAACCCCAACTCA[cggtgACCCAGGCGG CGCACAAG]	second spacer RecQL5_2
CHEK1	[TTTTCGTCTCTAAAC] TCCCTCACTAATCTAGACCC[cggtgACCCAGGCGG CGCACAAG]	second spacer CHEK1_2
CHEK2	[TTTTCGTCTCTAAAC] CTCTGCTGGCTGAGGCTGCG[cggtgACCCAGGCG GCGCACAAG]	second spacer CHEK2_2
SLX4	[TTTTCGTCTCTAAAC] CGGAGCCAGCGAGGGAGACG[cggtgACCCAGGC GGCGCACAAG]	second spacer SLX4_2
DNA2	[TTTTCGTCTCTAAAC] CTCCGCTCACAGCTCCGCCG[cggtgACCCAGGCG GCGCACAAG]	second spacer DNA2_2
EME1	[TTTTCGTCTCTAAAC] CCTGAACACCGCTCTGCAGA[cggtgACCCAGGCG GCGCACAAG]	second spacer EME1_2
GEN1	[TTTTCGTCTCTAAAC] CCCGTGCCTACCAGCTTCCC[cggtgACCCAGGCG GCGCACAAG]	second spacer GEN1_2
RNF4	[TTTTCGTCTCTAAAC] CGAGAAAGATGCCGCCGCCT[cggtgACCCAGGCG GCGCACAAG]	second spacer RNF4_2
SLX1	[TTTTCGTCTCTAAAC] CGGTACCGGGGCCGCGTCTA[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX1_2
TOP3A	[TTTTCGTCTCTAAAC] CGCTTCGGTCACGTCCCCAC[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3A_2

TOP3B	[TTTTCGTCTCTAAAC] GAGCTGGATCCGCGGTGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3B_2
WDHD1	[TTTTCGTCTCTAAAC] CTAGGGCCCGTTCTCCGCAG[cggtgACCCAGGCG GCGCACAAG]	second spacer WDHD1_2
CHTF8	[TTTTCGTCTCTAAAC] CTCGGCTCGCCATTCTTCTC[cggtgACCCAGGCGG CGCACAAG]	second spacer CHTF8_2
SOD1	[TTTTCGTCTCTAAAC] TCGTCGCCATAACTCGCTAG[cggtgACCCAGGCGG CGCACAAG]	second spacer SOD1_2
GFP	[TTTTCGTCTCTAAAC] TGGTGAACCGCATCGAGCTG[cggtgACCCAGGCG GCGCACAAG]	second spacer GFP_2
LACZ	[TTTTCGTCTCTAAAC] TTCGGTACTTCGTCCACAAA[cggtgACCCAGGCGG CGCACAAG]	second spacer LACZ_2
LUC	[TTTTCGTCTCTAAAC] GGCGCGGTCGGTAAAGTTGT[cggtgACCCAGGCG GCGCACAAG]	second spacer LUC_2
RPL34 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] ATCGGCTGCCATCCGAGGAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL34_2
RPL11 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] TCATGGCGGTGAGTAGCTGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL11_2
RPS24 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] ATCATATCAACGCGCACGGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RPS24_2
BLM	[TTTTCGTCTCTAAAC] CGCACGCAGACTCCTAGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer BLM_3
WRN	[TTTTCGTCTCTAAAC] CGGCGCACACAGTACACATC[cggtgACCCAGGCG GCGCACAAG]	second spacer WRN_3
RECQL1	[TTTTCGTCTCTAAAC] CTACTCGGGAGTAAAATCTT[cggtgACCCAGGCGG CGCACAAG]	second spacer RecQL1_3
RECQL4	[TTTTCGTCTCTAAAC] CCGCGCTTGCGATCGTCCAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RecQL4_3
RECQL5	[TTTTCGTCTCTAAAC] TGGTCCGCCCAAGAATTAAA[cggtgACCCAGGCGG CGCACAAG]	second spacer RecQL5_3
CHEK1	[TTTTCGTCTCTAAAC] CTCTGAATGTCGGCGGCTCC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHEK1_3
CHEK2	[TTTTCGTCTCTAAAC] CATATGACTCACCGCGTGAG[cggtgACCCAGGCG GCGCACAAG]	second spacer CHEK2_3
SLX4	[TTTTCGTCTCTAAAC] CCGCGGAGCATTGCCTGCGC[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX4_3

DNA2	[TTTTCGTCTCTAAAC] CGCGTCCAGGATGGAGCAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer DNA2_3
EME1	[TTTTCGTCTCTAAAC] CAGGCCTGCGACCGGGGACG[cggtgACCCAGGCG GCGCACAAG]	second spacer EME1_3
GEN1	[TTTTCGTCTCTAAAC] CCGAGTCCGGTCACTGCGGA[cggtgACCCAGGCG GCGCACAAG]	second spacer GEN1_3
RNF4	[TTTTCGTCTCTAAAC] CGCAGCGCGGCTCCCCCAAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RNF4_3
SLX1	[TTTTCGTCTCTAAAC] TACTAAGGCGTACGTCAACG[cggtgACCCAGGCG GCGCACAAG]	second spacer SLX1_3
TOP3A	[TTTTCGTCTCTAAAC] CACAGCGACCTGGAACTACA[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3A_3
TOP3B	[TTTTCGTCTCTAAAC] CCCCGGGAACAAGGACCGGA[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP3B_3
WDHD1	[TTTTCGTCTCTAAAC] GAGTGGGGACTCACCCGGGT[cggtgACCCAGGCG GCGCACAAG]	second spacer WDHD1_3
CHTF8	[TTTTCGTCTCTAAAC] CCAATCCCGGCTCGGCCCTC[cggtgACCCAGGCG GCGCACAAG]	second spacer CHTF8_3
SOD1	[TTTTCGTCTCTAAAC] TTCAGCACGCACACGGCCTT[cggtgACCCAGGCG GCGCACAAG]	second spacer SOD1_3
GFP	[TTTTCGTCTCTAAAC] CCGGGATCACTCTCGGCATG[cggtgACCCAGGCG GCGCACAAG]	second spacer GFP_3
LACZ	[TTTTCGTCTCTAAAC] GTCCCAGACGAAGCCGCCCT[cggtgACCCAGGCG GCGCACAAG]	second spacer LACZ_3
LUC	[TTTTCGTCTCTAAAC] CGGGTGTAATCAGAATAGCT[cggtgACCCAGGCGG CGCACAAG]	second spacer LUC_3
RPL34 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] CCCGGGACTTTGCTGCATTC[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL34_3
RPL11 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] TCGCCATCCATGGCAGGCCG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPL11_3
RPS24 (ESSENTIA L GENE)	[TTTTCGTCTCTAAAC] CGGATGGCTCGGATACGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RPS24_3
TOP1	[TTTTCGTCTCTAAAC] AAGTTCGCATTTGGGCTCAC[cggtgACCCAGGCGG CGCACAAG]	second spacer TOP1_1
FEN1	[TTTTCGTCTCTAAAC] CCGGGAGCGACGGGGTCCGC[cggtgACCCAGGC GGCGCACAAG]	second spacer FEN1_1

EXO1	[TTTTCGTCTCTAAAC] TTCGCGCGCTGTGTAGGCAA[cggtgACCCAGGCG GCGCACAAG]	second spacer EXO1_1
RNASEH1	[TTTTCGTCTCTAAAC] CGCCGGTGACGGAAGTGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH1_1
LIG4	[TTTTCGTCTCTAAAC] CCGGTCTGTTGCCCCACAGA[cggtgACCCAGGCG GCGCACAAG]	second spacer LIG4_1
BRCA1	[TTTTCGTCTCTAAAC] TCTGTCAGCTTCGGAAATCC[cggtgACCCAGGCGG CGCACAAG]	second spacer BRCA1_1
MRE11	[TTTTCGTCTCTAAAC] CGGGAGAGAACGGCGTCCGT[cggtgACCCAGGCG GCGCACAAG]	second spacer MRE11_1
CTIP	[TTTTCGTCTCTAAAC] CCGAGATTGCCTCGGGATTC[cggtgACCCAGGCG GCGCACAAG]	second spacer CTIP_1
RNASEH2A	[TTTTCGTCTCTAAAC] CATCGACGCCCAGGACGCAA[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH2A_1
RAD51B	[TTTTCGTCTCTAAAC] CCTTAAGACTCGGGATCGTC[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51B_1
XRCC3	[TTTTCGTCTCTAAAC] CCCGCGGGTTCCGCACTCCT[cggtgACCCAGGCG GCGCACAAG]	second spacer XRCC3_1
RAD51C	[TTTTCGTCTCTAAAC] CCGAGCTTAGCAAAGGTAAC[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51C_1
BRCA2	[TTTTCGTCTCTAAAC] CCTAGTTTCAGAAGCTCGCG[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA2_1
RAD52	[TTTTCGTCTCTAAAC] TCTTGTTACTCCCTAGCAGT[cggtgACCCAGGCGG CGCACAAG]	second spacer RAD52_1
RTEL	[TTTTCGTCTCTAAAC] CGGCGAACCTTCCAGAACCG[cggtgACCCAGGCG GCGCACAAG]	second spacer Rtel_1
FBH1	[TTTTCGTCTCTAAAC] CGTCTGCGGCCTCACGCACT[cggtgACCCAGGCG GCGCACAAG]	second spacer Fbh1_1
FANCM	[TTTTCGTCTCTAAAC] CTACGGTTCCGATCCCCATC[cggtgACCCAGGCGG CGCACAAG]	second spacer FANCM_1
TOP1	[TTTTCGTCTCTAAAC] CCGCTTACCTGCGCCTCCTC[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP1_2
FEN1	[TTTTCGTCTCTAAAC] CCCGCCGCTAAGCTGAGAAG[cggtgACCCAGGCG GCGCACAAG]	second spacer FEN1_2
EXO1	[TTTTCGTCTCTAAAC] GTGTTCTGCGTTGCCGGCCG[cggtgACCCAGGCG GCGCACAAG]	second spacer EXO1_2

RNASEH1	[TTTTCGTCTCTAAAC] CCGGCGCTCAACACCGCACT[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH1_2
LIG4	[TTTTCGTCTCTAAAC] GCGTGCTTGAGCCCGGTGAC[cggtgACCCAGGCG GCGCACAAG]	second spacer LIG4_2
BRCA1	[TTTTCGTCTCTAAAC] TCCAGGAAGTCTCAGCGAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA1_2
MRE11	[TTTTCGTCTCTAAAC] TGGGTCGCGATTGTGGGGCT[cggtgACCCAGGCG GCGCACAAG]	second spacer MRE11_2
CTIP	[TTTTCGTCTCTAAAC] CCGAGTGTAGCCCGGGCCCG[cggtgACCCAGGCG GCGCACAAG]	second spacer CTIP_2
RNASEH2A	[TTTTCGTCTCTAAAC] CGGGCACAGGCGAACTCAGG[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH2A_2
RAD51B	[TTTTCGTCTCTAAAC] CCAATATCGAAACCCACGAG[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51B_2
XRCC3	[TTTTCGTCTCTAAAC] CGGGTTCCGCACTCCTCTTC[cggtgACCCAGGCG GCGCACAAG]	second spacer XRCC3_2
RAD51C	[TTTTCGTCTCTAAAC] CGCTGGGGCGTGCGGCGTGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51C_2
BRCA2	[TTTTCGTCTCTAAAC] CGGGTGTCTTTTGCGGCGGT[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA2_2
RAD52	[TTTTCGTCTCTAAAC] TTCATTTCTTGGACATCCGG[cggtgACCCAGGCGG CGCACAAG]	second spacer RAD52_2
RTEL	[TTTTCGTCTCTAAAC] TTGCTTTGTGCTCCCGGCGG[cggtgACCCAGGCG GCGCACAAG]	second spacer Rtel_2
FBH1	[TTTTCGTCTCTAAAC] CCGTGTGGAAAACTTAACCT[cggtgACCCAGGCGG CGCACAAG]	second spacer Fbh1_2
FANCM	[TTTTCGTCTCTAAAC] TCGGTGGTTGTCGGCCTAAT[cggtgACCCAGGCG GCGCACAAG]	second spacer FANCM_2
TOP1	[TTTTCGTCTCTAAAC] CACAGGCCGGTTCGCCGTCT[cggtgACCCAGGCG GCGCACAAG]	second spacer TOP1_3
FEN1	[TTTTCGTCTCTAAAC] CGAACCAAGCTTTAGCCGCC[cggtgACCCAGGCG GCGCACAAG]	second spacer FEN1_3
EXO1	[TTTTCGTCTCTAAAC] CTGGGCGGGGCCGCAAGGAA[cggtgACCCAGGCG GCGCACAAG]	second spacer EXO1_3
RNASEH1	[TTTTCGTCTCTAAAC] ACAGAGTCGCCTTGGCCGCC[cggtgACCCAGGCG GCGCACAAG]	second spacer RNASEH1_3

LIG4	[TTTTCGTCTCTAAAC] CACAGACTTCTCGCCGCCTG[cggtgACCCAGGCG GCGCACAAG]	second spacer LIG4_3
BRCA1	[TTTTCGTCTCTAAAC] CCCGTCAAAGAATACCCATC[cggtgACCCAGGCGG CGCACAAG]	second spacer BRCA1_3
MRE11	[TTTTCGTCTCTAAAC] CGGAATTCAGGTTTACGGCC[cggtgACCCAGGCG GCGCACAAG]	second spacer MRE11_3
CTIP	[TTTTCGTCTCTAAAC] CGGTGGGAAAGCCGACCCCT[cggtgACCCAGGCG GCGCACAAG]	second spacer CTIP_3
RNASEH2A	[TTTTCGTCTCTAAAC] AGACCCGCTCCTGCAGTATT[cggtgACCCAGGCGG CGCACAAG]	second spacer RNASEH2A_3
RAD51B	[TTTTCGTCTCTAAAC] CACAACGGCACCCCACATGA[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51B_3
XRCC3	[TTTTCGTCTCTAAAC] CGGGTCTCCATTGCCCGAGC[cggtgACCCAGGCG GCGCACAAG]	second spacer XRCC3_3
RAD51C	[TTTTCGTCTCTAAAC] CTGCATTTCAAAGCGGAACG[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD51C_3
BRCA2	[TTTTCGTCTCTAAAC] CGCCGGTCACAAATCTGTCC[cggtgACCCAGGCG GCGCACAAG]	second spacer BRCA2_3
RAD52	[TTTTCGTCTCTAAAC] CCGGGGTGGTTCTAGCCGTG[cggtgACCCAGGCG GCGCACAAG]	second spacer RAD52_3
RTEL	[TTTTCGTCTCTAAAC] TCGACTGGAGTCGGTTGAGT[cggtgACCCAGGCG GCGCACAAG]	second spacer Rtel_3
FBH1	[TTTTCGTCTCTAAAC] CAGTGCGTGAGGCCGCAGAC[cggtgACCCAGGCG GCGCACAAG]	second spacer Fbh1_3
FANCM	[TTTTCGTCTCTAAAC] CACGTCTGAAAAAGCGTTCT[cggtgACCCAGGCGG CGCACAAG]	second spacer FANCM_3

Note: Sequences in bracket '[ ]' are homologous to guide expression vector. First spacer represents oligo sequence cloned into the first guide position, while second spacer represents oligo sequence cloned into the second guide position in the vector.

Supplementary Table 9 Sequence of sgRNAs used in the studies

Target gene	sgRNA sequence
EYFP reporter[1]	TACCTCATCAGGAACATGT
NEAT1 sgRNA1	GCGACAGGGAGGGATGCGCGCC
NEAT1 sgRNA2	GCGCGCCTGGGTGTAGTTGT
NEAT1 sgRNA3	GAAGTGGCTAGCTCAGGGCTTC
CXCR4	CAGGTAGCAAAGTGACGCCGA
SEL1L sgRNA1	GCAGGAAGAGCAGCGGCGAGG
SEL1L sgRNA2	GGGGGCGGATACTGACCCG
SEL1L sgRNA3[2]	GATACTGACCCGAGGACGCCG
ARPC2 sgRNA1[2]	TGTCGGTGAAGCGGCAGTGG
ARPC2 sgRNA2	CAGGCGGGTTCAGGCTTCGG
ERK1 sgRNA1[2]	GGGAGCCCCGTAGAACCGAG
ERK1 sgRNA2	CACCGCCCTCCTCCCCACGG
BRCA1 sgRNA1	GGATTTCCGAAGCTGACAGA
BRCA1 sgRNA2[2]	GCTCGCTGAGACTTCCTGGA
BLM sgRNA1	AGGAAACGGAAGAACCCGAG
BLM sgRNA2	CCTCGCACGCAGACTCCTAG
MET1 sgRNA1	TGAGCAGATGCGGAGCCGAG
MET1 sgRNA2	ACTGGTTCCTGGGCACCGAA
RHOA sgRNA1	AGTTCCCGTGATGCCCCACG
RHOA sgRNA2	GCGCGCCTCCGAGTGCCCAG
CHK2 sgRNA1	GGAGAGTGTGCGGCTCCAGG
CHK2 sgRNA2	CGCAGCCTCAGCCAGCAGAG
CHK1 sgRNA1	GGTGGAGGAATGGTACCAGG
CHK1 sgRNA2[2]	GGGTCTAGATTAGTGAGGGA
CANX_-700*	TGAAGTGAGATTAGGTGTCA
CANX_-335*	GTTGGGTTGGAACGCCCCGA
CANX_-505*	GGTTCTGCTCACGCCCGTAG
CANX_-22*	GCTCGCTCGCGCGGCAGCGG
CANX_-1[2]*	GGCCGAGGCCTCTTGGTTCTG
CANX_47*	GCGCCGCAGTAAAGAGAGAGG
CANX_155*	TCGGGCCTGTGAGGACCTCG
CANX_263*	CGACGCGCCCGCCGTGAGCG
CANX_472*	GAGTAACTGGGTAAAAGTAT
CANX_642*	ACCAGAAGGAGAACACGCAG
SYVN1_-1032[3,4]*	GGAAAACGCAAGGCACAAAG
SYVN1_-734[3,4]*	AACGTTCCCGGAGGCCAGCC
SYVN1_-601[3,4]*	ACCTTTGCTGGCCTATAGAA
SYVN1_-555[3,4,6]*	AACTTATCGCAACCAATCAG
SYVN1_-339[3,4,6]*	CAGGTGGTACAGCCCGCAAG

SYVN1_-194[3,4]*	ATTACCTTCCGACCACCTCT
SYVN1_-116[3,4,6]*	CCTACGTGGGCCCATAGCAA
SYVN1_-43[3,4]*	ACACCTCACTTCCGGCGGCG
SYVN1_19[3,5]*	CCGCTCAATCCGCGCGACTG
SYVN1_45[3,5]*	GGCGCTGGGTTCCTGGTGAGT
SYVN1_183[3,5]*	GCACCGGCGTCTGAGGTCTC
SYVN1_228[2,3,5,6]*	GTTGCGGGCGTCGCAGGCA
SYVN1_292[3,5]*	GAGAGCAGCAGCGGGACGGG
SYVN1_480[3,5]*	TGAGAGCAGCCAAGGCACAG
SYVN1_702[3,5]*	TAAGTGATCACACTGACGCA
SYVN1_844[3,5]*	TCGTGCTGTGCAAAATAGCC

1: Guide RNA targeting EYFP reporter in the reporter screen assay
2: Guide RNA used in single gene targeting experiments
3: Guide RNA used in ‘mixed gRNAs 1-16’ group shown in Figure 2b and Supplementary Figure 8
4: Guide RNA used in ‘mixed gRNAs 1-8’ group shown in Figure 2b and Supplementary Figure 8
5: Guide RNA used in ‘mixed gRNAs 8-16’ group shown in Figure 2b and Supplementary Figure 8
6: Guide RNA used in ‘mixed best 4 gRNAs’ group shown in Figure 2b and Supplementary Figure 8
*: The numerical number indicates the position of spacer relative to transcription start site of the target gene.

Supplementary Table 10 Sequences of all qPCR primers in the studies.

Target gene	**Forward qPCR primer sequence**	**Reverse qPCR primer sequence**
NEAT1	GTGGCTGTTGGAGTCGGTAT	ATTCACTCCCCACCCTCTCT
CXCR4	ACTACACCGAGGAAATGGGCT	CCCACAATGCCAGTTAAGAAGA
SEL1L	GAGGGGGAAAGTGTCACAGA	GGTCAAAGCTGGTTTCCGTA
SYVN	ACCAGCATCCCTAGCTCAGA	TCCTCAGGCATCTCCTCTGT
ARPC2	CTGGAGGTGAACAACCGCAT	GACCCCATCGAAATCTGCAAA
ERK1	ATGTCATCGGCATCCGAGAC	GGATCTGGTAGAGGAAGTAGCA
BRCA1	CTCAAGGAACCAGGGATGAA	GCTGTAATGAGCTGGCATGA
BLM	CAGACTCCGAAGGAAGTTGTAT G	TTTGGGGTGGTGTAACAAATGA T
MET1	AGCAATGGGGAGTGTAAAGAGG	CCCAGTCTTGTACTCAGCAAC
RHOA	GGAAAGCAGGTAGAGTTGGCT	GGCTGTCGATGGAAAAACACAT
CHK1	ATATGAAGCGTGCCGTAGACT	TGCCTATGTCTGGCTCTATTCTG
CHK2	GCGCCTGAAGTTCTTGTTTC	CGTAAAACGTGCCTTTGGAT
CANX	GATCCAGACGCAGAGAAACC	CATCCAGGAGCTGACTCACA
TERC	CCCTAACTGAGAAGGGCGTA	GCTCTAGAATGAACGGTGGAA
XIST	AGGTCAGGCAGAGGAAGTCA	CTGCCTCCCGATACAACAAT
ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
CAS9	GAGTTGACGCCAAAGCAATC	TACCAAACAGGCCGTTCTTC

Supplementary Table 11 Sequence of sgRNA2-7SK template

sgRNA1 tail; 7SK promoter
gttttagagctatgctgaaaagcatagcaagttaaaataaggcagtgatttttaatccagtccgtacacaacttgaaaaagtgcgcaccg attcggtgcttttttttCCTACGCACCTCGTCGAACCCTCACTGCAGTATTTAGCATGCCCCACCCATCT GCAAGGCATTCTGGATAGTGTCAAAACAGCCGGAAATCAAGTCCGTTTATCTCAAACTTTAG CATTTTGGGAATAAATGATATTTGCTATGCTGGTTAAATTAGATTTTAGTTAAATTTCCTGCTG AAGCTCTAGTACGATAAGTAACTTGACCTAAGTGTAAAGTTGAGATTTCCTTCAGGTTTATAT AGCTTGTGCGCCGCCTGGGTcaccg

Supplementary Table 12 Sequence of PCR primers for next generation sequencing

PCR 1 primers	Sequence
Forward primer (lethality screen)	**CTTTCCCTACACGACGCTCTTCCGATCT** NNNNNNCCCTTGGAGAAAAGCCTTGTTT
Reverse primer (lethality screen)	**GGAGTTCAGACGTGTGCTCTTCCGATCT**TGTACAAGAAAGCTGGGTCTAG
Forward primer (epistasis screen)	**CTTTCCCTACACGACGCTCTTCCGATCT** NNNNNNCTTGTGGAAAGGACGAAACACC
Reverse primer (epistasis screen)	**GGAGTTCAGACGTGTGCTCTTCCGATCT**CATTTGTCTCGAGGTCGAGAATTC

Sequences in bold are adaptor sequence for next generation sequencing.

Supplementary Table 13 PCR cycling conditions to amplify libraries for next generation sequencing

PCR 1

Step	Temperature	Duration
Step 1	95 °C	8 min
Step 2	95 °C	30 sec
Step 3	60 °C	30 sec
Step 4	72 °C	30 sec# / 1 min*
Repeat step 2-4 for a total a 25-30 cycles		
Step 5	72 °C	2 min

extension time for lethality screen library; * extension time for epistasis screen library

PCR 2

Step	Temperature	Duration
Step 1	95	3 min
Step 2	95	10 sec
Step 3	55	20 sec
Step 4	72	30 sec
Repeat step 2-4 for a total a 5-10 cycles		

Supplementary Note 1 Interpretation of pi scores. A positive pi-score suggests that the fitness effect of the gene pair knockdown is less than expected from individual knockdowns (e.g., loss of two proteins in a pathway or complex), while a negative pi-score means that the fitness effect was more pronounced than expected based on the individual fitness effects from single-gene knockdowns (e.g., synthetic lethal effects).

Supplementary Note 2 This section describes the strategy used to identify and analyze genes with differential expression (DE) in our RNA-seq experiments. Related results are **Supplementary Figure 10-11** and **Supplementary Table 2**.

To analyze raw reads from RNA-sequencing experiments and profile whole transcriptome activity induced by dCas9, dCas9-KRAB and dCAS9-KRAB-MeCP2 repressors, we implemented edgeR quasi-likelihood (edgeR-quasi) pipeline for DE. EdgeR-quasi uses negative binomial generalized linear model[1] with F-tests[2], and holds advantages over other methods as it provides speed and reliable error rate control. For the DE analyses, we utilize edgeR-quasi and limma-voom pipelines for two independent biological replicates of dCas9, dCas9-KRAB and dCAS9-KRAB-MeCP2 repressors. The sample size of n=2 in each repressor group is reasonable due to the low biological variability characteristic of cell culture experiments. Our analysis involves importing of raw counts, filtering of lowly expressed counts, normalization due to library size bias, DE, and clustering testing.

First, we tested DE between each repressor group relative to a control group (delivered sgRNA only) using the edgeR QL functions set to *robust=TRUE* in *glmQLFit* to reduce the number of false positives from genes with extreme dispersions (very low and very high). In **Supplementary Figure 10a-c**, we plot these results on two axes - $\log_2$ fold change (FC) versus averaged $\log_2$ counts per million (CPM), where positive $\log_2$ FC indicates upregulated genes while negative $\log_2$ FC represent downregulated genes relative to the negative control. DE genes at FDR of 5% and corrected using Benjamini-Hochberg method are shown in grey, whereas genes with no significant fold change are shown in black. *glmQLFTest* function identifies all DE only based on statistical significance including genes with small fold changes. To remove such bias, we apply the TREAT method[3] which leverages a negative binomial framework using the edgeR's *glmTrea*t function, and simultaneously tests for significance and differential fold change at a cutoff of $\log_2$ FC > 1.5. This method is more stringent as it requires larger p-values for calling genes and leads to fewer detected genes. It therefore provides better specificity in recognizing genes with true biological function. The resulting genes are plotted in red on **Supplementary Figure 10a-c** and summarized in the table on **Supplementary Figure 10d**. The identity of these genes are listed in **Supplementary Table 2**. The application of log fold change cutoff of 1.5 results in no downregulated genes, and significantly reduces the number of upregulated genes.

We display expression patterns of transcriptional changes by plotting the top 35 genes with DE in the control, dCas9, dCas9-KRAB and dCas9-KRAB-MeCP2 groups. Clustering of genes with correlated expression provides insights into the biological effects of repressor's activity. To display relative changes in genes across the four groups, we performed scaling such that each gene has a mean of zero and standard deviation of 1. The displayed gene clusters are based on Euclidean distance, $(1-R)^2/2$ between each gene pair where R is the Pearson's correlation of the two genes. A scale bar key of normalized Log_2 CPM represents large negative (colored blue) and positive (colored red) correlations. Genes with large positive correlations correspond to small Euclidean distances and cluster together (**Supplementary Figure 11a**).

Lastly, we examine activated and repressed genes across all three repressor groups (all normalized to the negative control) by applying the limma-voom workflow. We performed linear modelling in limma, and used *lmFit* and *contrasts.fit* functions followed by empirical Bayes model, *eBayes*. This workflow removes variance-associated dependencies on the mean. In **Supplementary Figure 11b-c**, we plot genes on Venn diagrams for downregulation and upregulation where we define significance at 5% p-value with no log fold change cutoff as a less stringent method for examining transcriptome-wide transcriptional offsets for the different repressors. Based on these results, dCas9-KRAB repressor shows the largest clusters of activated genes followed by dCas9-KRAB-MeCP2, and dCas9.

Supplemental Note 3 This section describes the methods and bioinformatics analyses we used to interpret the repressor screens in HAP1, SH-SY5Y and 293T cell lines. Related results are **Supplementary Table 4-6** and **Supplementary Data 2-4**.

Alignment of sequencing reads to reference contig

The sequencing reads were aligned to a reference contig sequence (NNNNNNcccttggagaaaagccttgtttNNNNNNNNNNNNNNNNNNNNgtttaagagctaga aatagcaagtttaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttctagaccca gctttcttgtacaaaaaaaagaattcctgcagccccgataaaataaaag) using the alignment software BWA-MEM (version 0.7.8)[4]. We used samtools (version 1.2) to extract aligned sequences that have a mapping quality score >= 30[5]. The 20-nucleotide variable sequence was then match to the 20-nucleotide sgRNA library with 683 guide sequences. Sequences that do not match any of the 683 guide sequences were discarded from the analysis.

Analysis of the repressor sgRNA library

We first sequenced the sgRNA library to determine the distribution of the guide sequences. A total 129,362 sequences map to a guide sequence within the library of 683 guides. However, 53 of the guides were severely underrepresented, i.e. less than 50 reads mapped to the guide (< 0.04%) and

they were removed from all further analysis.

Comparing the guide sequences for all the conditions

For all the conditions, we compared the mapped guides against the corresponding control experiment. The control experiment for all the conditions is the experiment performed without any repressor, i.e. without dCas9, dCas9-KRAB or dCas9-KRAB-MeCP2. For each guide, we compared its frequency for the condition against its frequency for the control. This is done by calculating the odds-ratio (OR) for each guide using the formula,

$$OR_i = \frac{\frac{test_i}{testTotal}}{\frac{control_i}{controlTotal}}$$

where $test_i$ is the number of reads that map to guide *i* for the test condition and $control_i$ is the number of reads that map to guide *i* for the control while *testTotal* and *controlTotal* are the total number of reads for the test condition and control, respectively. If the guide is enriched in the test condition, the OR would be > 1 while if the guide is depleted, the OR would be < 1 (**Supplementary Data 2-4** list the OR and p-value of each tested guide).

Determining if the essential guides are depleted

Out of the 630 guides from the library, 370 target essential genes (essential guides) while the remaining 260 target non-essential genes. To determine if the essential guides were significantly depleted, we performed a 1-tailed Welch T-test between the $\log_2$ OR of the essential guides versus the $\log_2$ OR of the non-essential guides (**Supplementary Table 4-6**). Also, for the dCas9-KRAB-MeCP2 for HAP1 day 7 and day 14, there were a number of guides that were completely depleted, ie. they had 0 reads for the test condition. This presents a problem as the $\log_2$ OR for those guides would be $-\infty$.To allow for the T-test, we replaced the log2 OR for those guides to the minimal finite $\log_2$ OR for that condition, which is -7.61 for day 7 and -8.87 for day 14.

Supplementary Note 4 This section describes detailed methods used to analyze the repressor-dual gRNA screen. The analysis was performed using an adapted version of a published workflow for computing genetic interactions using a combinatorial CRISPR-Cas9 knockouts[6] as follows.

Read alignment

Pair-end reads were first aligned to the sequence immediately upstream and downstream of the 20 bp protospacers, thus allowing us to extract protospacer sequences from each read. The protospacer sequences were then aligned to expected sequences, allowing for 3 mismatches (3< ½ * min(hamming(g_i, g_j) for all i, j). To ensure the robustness of the mapping, constructs with fewer than 5

reads mapped will be excluded from downstream analysis. Only when both reads in a pair were matched with a designed construct sequence was the pair considered for downstream scoring.

Quantification of fitness and gRNA fitness and gRNA–gRNA interactions

We modeled the cell population change over the 14 day duration of the screen using an assumption of exponential growth[6]. For each synthesized construct, we estimated the relative abundance (x_c) of the sub-population of cells harboring the construct. We did this by using the count of the reads that aligned to the designed construct.

$$x_c(t) = a_c + f_c t - log_2 \sum_c 2^{a_c + f_c t}$$

In which a_c denotes the initial abundance of the construct at day 0, f_c is the fitness of the cells with the construct. Since each gene is targeted by at least 3 gRNAs, gene-gene fitness $f_{g_i g_j}$ are calculated by collapsing f_c from all dual gRNA constructs targeting the same gene pairs.

We quantified genetic interactions as the difference between summation of single gene fitnesses and the double gene fitness, as follows:

$$f_{g_i g_j} = f_{g_i} + f_{g_j} + \pi_{g_i g_j}$$

Single gene fitnesses f_{g_i}, f_{g_j} were obtained by fitting of above equations to the screen data. The residual of the fit, $\pi_{g_i g_j}$, denotes the genetic interaction score (i.e., pi-score) for a gene pair $g_i\, g_j$. Note that theoretically f_{g_i}, f_{g_j} can be more easily obtained by measuring the fitness of the constructs that contain a negative control gene (lacZ, GFP and luc). We noticed that the negative controls lacZ and luc seemed neutral to cell fitness when a regression model was used to estimate the single gene fitnesses f_{g_i} from the fitness of all constructs $f_{g_i g_j}$ in which f_{g_i} participates, as previously done[6]. Interestingly, GFP gRNAs, expected to serve as a third negative control, showed positive fitness, suggesting that the used GFP targeting guide may be hitting an off-target sequence within the mammalian genome that when mutated causes a fitness benefit. **Supplementary Data 7** lists the genetic interactions uncovered in our screen.

Validation of genetic interaction screens by examining the topology of protein complex network and genetic interactions

We first filtered out gene-pairs that show zero interaction ($\{g_i g_j \; \forall \; |\pi_{ij}| > 0\}$), and the shortest path was computed for the remaining gene-pairs. The shortest paths between gene pairs were computed based on a network of experimentally characterized protein complexes[7] The number of intermediate genes that connect the gene pair as determined by the protein complex network is treated as the distance between the two genes ($d_{g_i g_j}$). $d_{g_i g_j}$ values are subsequently multiplied by the genetic interaction score π_{ij} derived from the 3 repression screens for gene-pair $g_i g_j$ to get the enrichment score $ES_{ij} = d_{g_i g_j} \cdot \pi_{ij}$. The overall enrichment score can then be calculated by the summing all enrichment

scores ($ES_{\mathrm{overall}} = \sum_{\mathrm{i,j}} \hat{}ES_{\mathrm{ij}}$). Permutation tests are performed by shuffling the genetic interaction scores and repeating the above steps for a total of 10000 permutations. As valid screen should have a smaller $ES_{overall}$ compared to a non-valid one, left-tailed p values are reported.

Supplementary Note 5 This section describes detailed materials and methods used to perform circuit experiments.

Cell culture

Transfections were performed on HEK293ft cells using polyethylenemine (PEI). Cells were cultured in Debulcco's Modified Eagle Medium (DMEM), with 10% Fetal Bovine Serum (FBS), non-essential amino acids (NEAA), glutamine, sodium pyruvate, and penicillin/streptomycin. The day prior to transfection, cells were passaged and split into 24-well plates, then allowed to grow to 70-90% confluence. Mixes of DNA were used with a 2:1 PEI:DNA ratio to transfect the 90% confluent cells using standard transfection protocols. All conditions were transfected in quadruplicate. For inducible circuits, doxycycline was added to cultures to a concentration of 2000ng/uL. Media and inducers were changed daily post-transfection until flow cytometry was performed 72 hours later. In wells designated for control experiments, corresponding plasmid DNA under study was replaced by equal amount of empty DNA plasmid. 72 hours post-transfection, cells were collec-ted for flow cytometry assay.

Flow Cytometry and Data analysis

72 hours post-transfection, cells were trypsinized, washed with Hank's Balanced Salt Solution (HBSS) with 2% FBS, then resuspended in 200uL HBSS+FBS. Then flow cytometry was performed using a BD FACSCelesta flow cytometer. 200,000 events were collected, measuring forward scatter (FSC), side scatter (SSC), EBFP expression (BV421), and EYFP expression (BB515). Data were analyzed using FlowJo (FlowJo, LLC). For analysis, all data were compensated using single color and non-transfected controls. Cells were then gated by FSC and SSC to separate healthy, living cells from dead cells and debris. Living cells were further gated by EBFP; laser voltage was set in such a way as to make non-fluorescing cells express at 10^2 or lower, so the BV421 gate was set at 10^2 .

Cells with above 10^2 a.u of EBFP expression were considered transfected and were further analyzed by taking the geometric mean of the population's EYFP (BB515) expression. The geometric means of all samples were exported and further analyzed in Excel (Microsoft) or Prism (GraphPad Software). For single repression circuits, fold repression was calculated by dividing the average EYFP expression of the unrepressed samples by the EYFP of each of the four replicates. These four measures were then used to find average fold repression and standard error of the mean (s.e.m.).

For layered transcriptional repression circuits, fraction of maximum expression was calculated by dividing the average EYFP expression of the

unrepressed samples by the EYFP of each of the four replicates. These four measures were then used to find average fraction of maximum and standard deviation. For inducible layered transcriptional repression circuits, fold derepression was calculated by dividing the EYFP expression of each sample in the +Dox condition by the average EYFP expression of their No Dox counterpart. These four measures were then used to find the average fold derepression and s.e.m.

For transfections involving CXCR4 as the output, after supernatant was removed, cells in each well were labeled with 5 ul of CD184 (CXCR4) monoclonal antibody (eBioscienceTM) conjugated to PE and diluted (1:40) in HBSS without calcium and magnesium supplemented with 2% FBS and incubated at $4^{o}C$ for 30 minutes. Cells were then centrifuged and supernatant was removed. They were then resuspended in 7-AAD solution to exclude dead cells and subjected to FACS. Flow cytometry measurements were performed using BD FACSCelesta with the following settings: EBFP measured with 405 nm laser and a 450/40 filter, EYFP measured with 488 nm laser and a 530/30 filter, 7-AAD measured with a 488 nm laser and a 695/40 filter and CXCR4 measured with 561 nm laser and a 586/15 filter. At least 300,000 events were gathered from each well. Appropriate compensation controls were dedicated for each experiment. Untransfected and unstained cells were used as negative control. Similarly, untransfected cells stained only with CD184-PE antibody were used for CXCR4 control, cells transfected only with EBFP were used as BFP controls. Finally, a mixture of live and dead cells from untransfected wells were used as 7-AAD controls. Data from flow cytometry was analyzed using FlowJo software. Briefly, live cells (7-AAD negative population) were selected and then gated for EBFP expression $>10^3$ A.U. Geometric mean of PE fluorescence level was then calculated in this population.

Statistical analysis

Statistical comparison was performed using one-tailed Student's T-test with a *p*-value < 0.05 as the threshold for significance. In all synthetic reporter gene circuits, sample size (n) of 4 biologically independent samples (cell cultures) was used for statistical test. In the endogenous gene circuit, n of 3 biologically independent samples (cell cultures) was used for statistical test.

Plasmids

The plasmids used in each circuit, along with a brief description of their function, are as follows:

Circuit		1	2	3	4	5
Background Plasmids and dCas9	ND220	20	20	20	20	25
	114	20	20	20	20	20
	Csy4			50	50	
	dCas9 Variant	70	70	70	70	70
gRNA repression devices	3475	100	200			500
	LR2002			100	200	
	U6/Tal4-sgRNA-CXCR4					50
TALE repressor	473		50		50	50
Reporter	1341	20		20		
	ND252		20		20	
	Midi129					

- dCas9 Variants: CMV promoter driving dCas9, dCas9-KRAB, or dCas9-KRAB-MeCP2 expression.
- Background plasmids:
    1. ND220: CAG promoter driving EBFP expression. EBFP expression was used to gate transfected cells.
    2. 114: Hef1a promoter driving Gal4-VP16 and rtTA expression; the protein products are separated by a T2A amino acid sequence. The Gal4-VP16 activator binds upstream of the CRP promoter, driving its expression in the absence of a repressor. rtTA, when combined with Dox, binds upstream of the TRE promoter, driving its expression.
    3. Csy4: PGK promoter driving Csy4 expression. The Csy4 protein was used to cleave gRNAs in an mRNA transcript into functional gRNAs.
- Regulators in single repression circuits:
    1. 3475: U6 promoter driving gRNA expression.
    2. LR2002: TRE promoter driving gRNA expression 3' to iRFP flanked by Csy4 target sites.
    3. U6/Tal14_CXCR4 gRNA: gRNA targeting endogenous CXCR4 locus under U6 promoter (repressed by TALER).
- Regulators in layered transcriptional repression circuits:
    1. 473: CRP promoter driving TALER expression.
- Reporters:
    1. 1341: CRP promoter driving EYFP expression.
    2. Midi129:CRP promoter driving EYFP expression, containing two gRNA target sites flanking the mini CMV.

ND252: pTal promoter driving EYFP expression.

Supplementary References:

1. McCarthy, D. J., Chen, Y, and Smyth, G. K. Differential Expression Analysis of Multifactor RNA-Seq Experiments with Respect to Biological Variation. *Nucleic Acids Research* **40**, 4288–97 (2012).

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Supplementary Data 1 A list of differentially expressed genes considered significant at FDR < 0.05 in the RNA-seq experiment.
Supplementary Data 2 A list of all sgRNA sequences in single guide RNA library and their log2 odd ratios in HAP1 lethality screen.
Supplementary Data 3 A list of all sgRNA sequences in single guide RNA library and their log2 odd ratios in SH-SY5Y lethality screen.
Supplementary Data 4 A list of all sgRNA sequences in single guide RNA library and their log2 odd ratios in 293T lethality screen.
Supplementary Data 5 A list of non-essential gene-targeting sgRNAs that showed depletion in lethality screens.
Supplementary Data 6 Summary of rank-ordered genes identified from sgRNA enrichment analysis performed using MAGeCK software.
Supplementary Data 7 Genetic interactions captured through repressor screens.
Supplementary Data 8 DNA sequences and species origins of all protein domains used to construct the different repressors in this study.