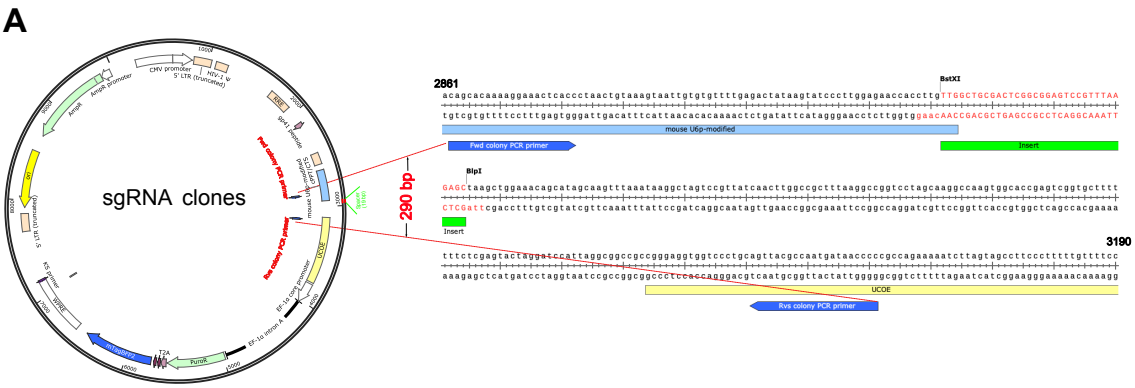
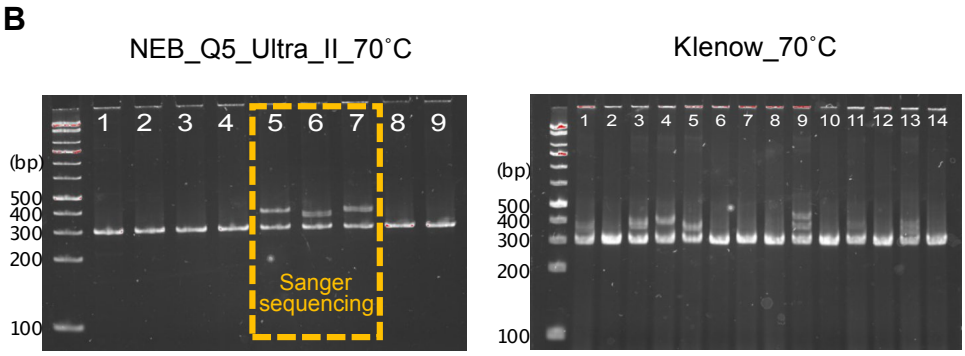
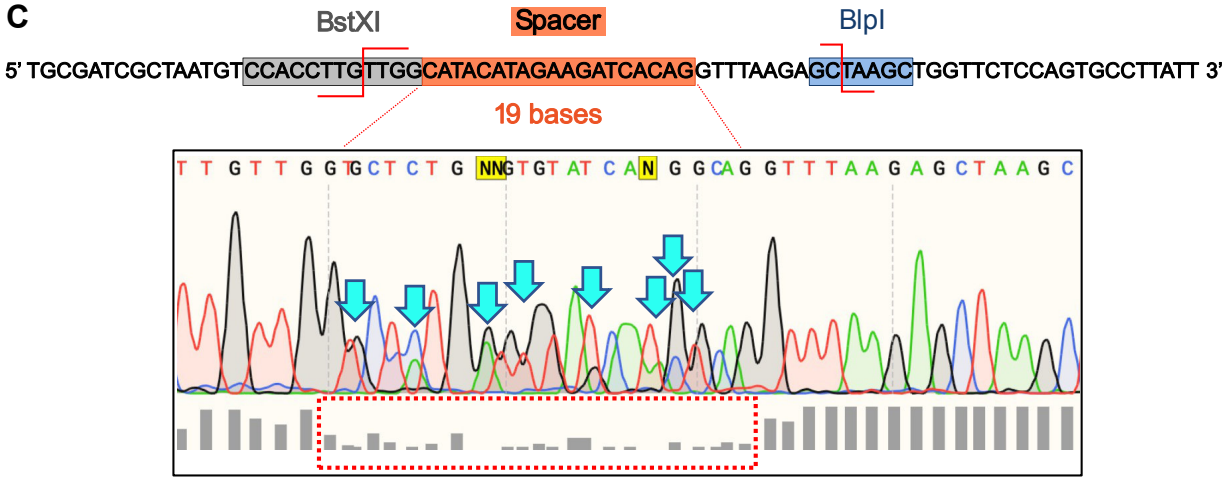
Fig. S1


A
sgRNA clones
290 bp
2861
3190
BstXI
BlpI
mouse U6p-modified
Fwd colony PCR primer
Insert
Rvs colony PCR primer
UCOE


B
NEB_Q5_Ultra_II_70˚C
Klenow_70˚C
(bp)
500
400
300
200
100
Sanger sequencing


C
BstXI
Spacer
BlpI
5' TGCGATCGCTAATGTCCACCTTGTTGGCATACATAGAAGATCACAGGTTTAAGAGCTAAGCTGGTTCTCCAGTGCCTTATT 3'
19 bases


D
Retransformed from two hybrid clones of the NEB_Q5_Ultra_II_70˚C
(bp)
500
400
300
200
100

Fig S1. (A) Diagram of the cloning strategy and colony PCR primer design. (B) Colony PCR of sgRNA clones and gel electrophoresis. (C) Representative Sanger sequencing trace of plasmids from hybrid clones (D) Plasmids from two different hybrid clones were retransformed. Colony PCR was performed, and hybrid clones were not detected.

Fig. S2

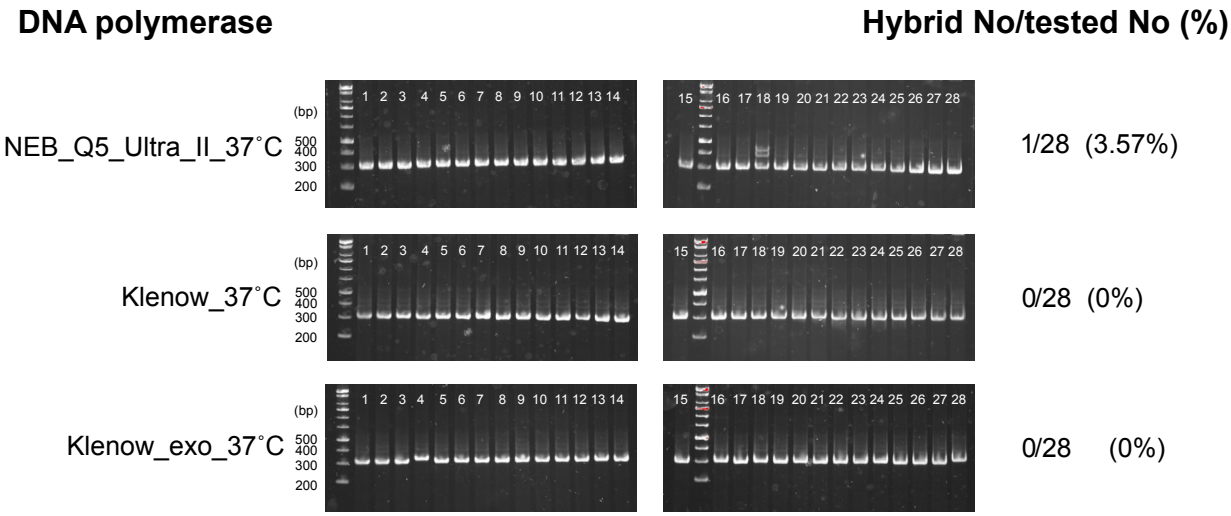


Fig S2. Lowering the elution temperature for the insert gel extraction reduces the formation of hybrid clones, regardless of the insert preparation polymerase used.

Fig. S3

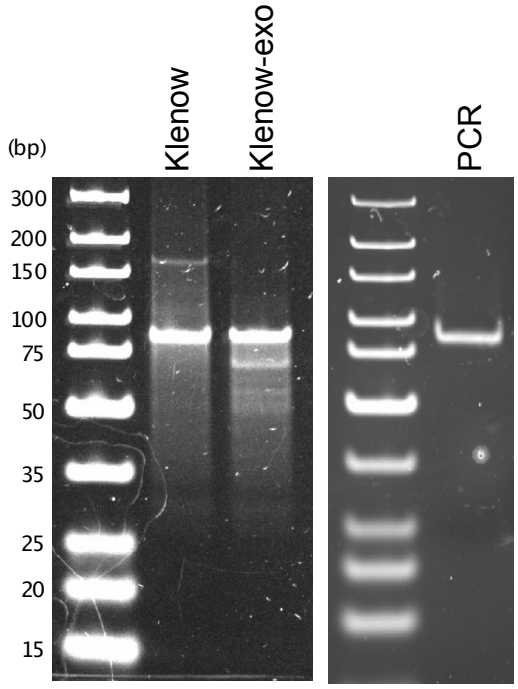


Fig S3. Synthesis of double-stranded sgRNA with the three different DNA polymerases (Klenow, Klenow exo-, and NEB Q5 Ultra II) and gel electrophoresis indicates less by-products with PCR.

Fig. S4

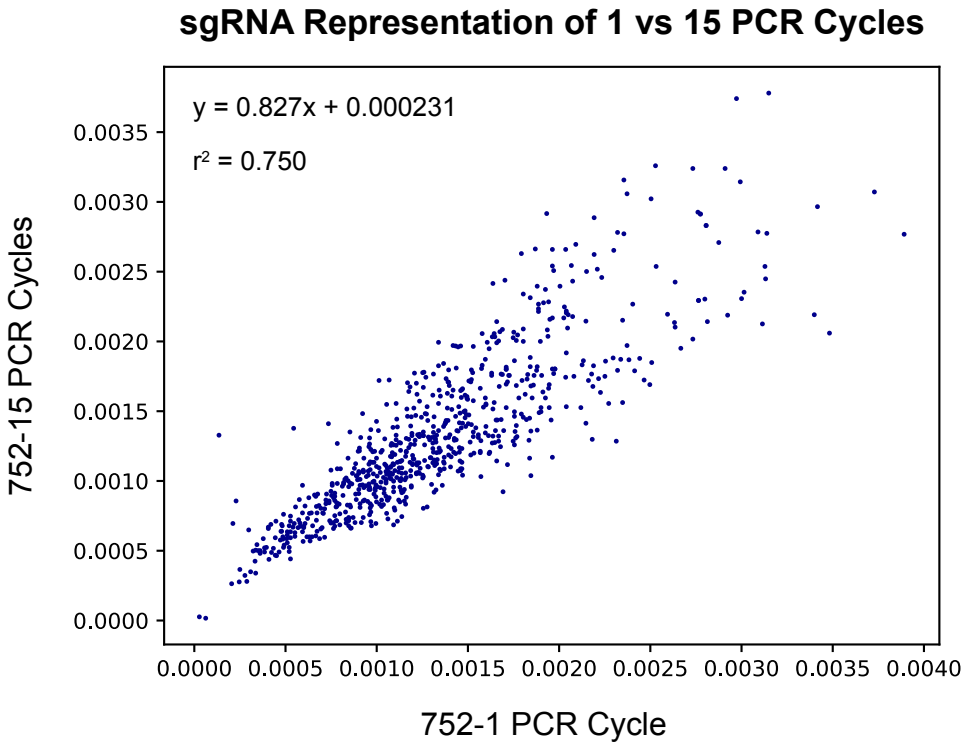
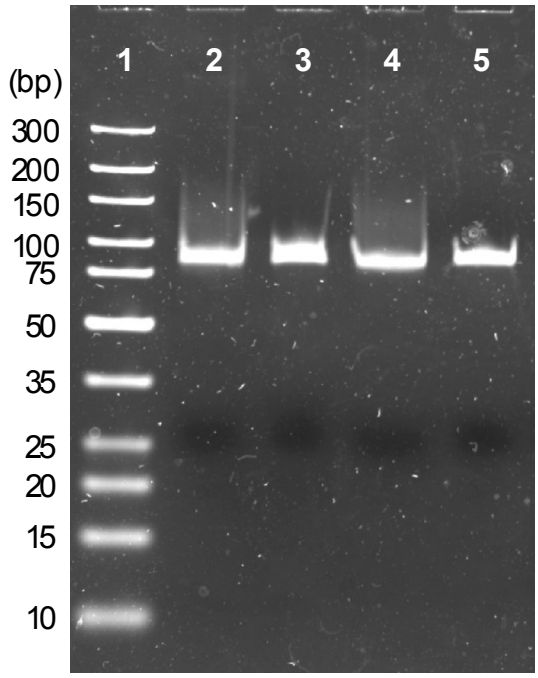


Fig S4. Additional PCR cycles introduce minimal bias in sgRNA libraries. An oligo pool containing 752 guides was amplified by one or 15 cycles of PCR, digested, and cloned into a guide vector. A pairwise comparison of sequence counts of guides in each library is depicted and a linear least-squares regression was performed.

Fig. S5

A


1
2
3
4
5
(bp)
300
200
150
100
75
50
35
25
20
15
10

B

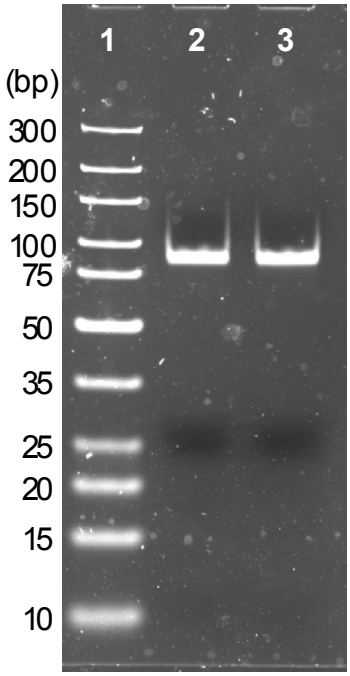

1
2
3
(bp)
300
200
150
100
75
50
35
25
20
15
10

Fig S5. Optimization of PCR cycles and input template concentration reduces overamplification products. (A) Lane 1: DNA ladder, Lanes 2 and 4: 200pM template. Lanes 3 and 5: 10pM template. Lanes 2-5: 13 cycles PCR. (B) Lane 1: DNA ladder. Lane 2: 200pM template, Lane 3: 600pM template. Lanes 2 and 3: 8 cycles PCR.

Table S1

CRISPRi - LGR

		Skew				
	# Guides	**90/10**	**95/5**	**98/2**	**99/1**	**99.5/0.5**
Entire Library	103,074	1.99	2.36	2.86	3.25	3.73
h1	12,930	2.00	2.37	2.86	3.28	3.67
h2	16,197	1.99	2.36	2.84	3.25	3.73
h3	16,699	1.99	2.39	2.89	3.26	3.71
h4	12,161	1.99	2.36	2.88	3.27	3.79
h5	12,509	1.99	2.36	2.87	3.28	3.75
h6	13,090	1.97	2.32	2.80	3.15	3.66
h7	19,488	1.97	2.33	2.81	3.22	3.72

Table S1. Skew ratios of the genome-wide CRISPRi LGR library broken down by sublibraries.

Table S2

CRISPRi - Legacy

		Skew				
	# Guides	**90/10**	**95/5**	**98/2**	**99/1**	**99.5/0.5**
Entire Library	103,074	5.11	10.27	33.29	171.38	inf
h1	12,930	4.97	8.61	19.00	33.77	102.39
h2	16,197	3.27	5.03	9.87	17.60	36.77
h3	16,699	2.91	4.24	6.90	11.04	19.07
h4	12,161	3.24	4.91	8.46	13.88	27.36
h5	12,509	4.51	6.87	11.98	19.05	33.90
h6	13,090	8.78	21.25	130.06	inf	inf
h7	19,488	12.89	44.59	inf	inf	inf

Table S2. Skew ratios of the genome-wide CRISPRi Legacy library broken down by sublibraries.

Table S3

CRISPRa - LGR		Skew				
	# Guides	90/10	95/5	98/2	99/1	99.5/0.5
Entire Library	101,250	1.98	2.46	3.17	3.91	5.10
h1	12,751	2.01	2.51	3.22	3.94	5.47
h2	15,918	2.01	2.51	3.20	3.99	5.26
h3	16,277	1.97	2.42	3.14	3.85	4.95
h4	11,909	1.99	2.49	3.27	4.15	5.61
h5	12,207	1.98	2.45	3.26	4.08	5.78
h6	12,906	1.96	2.41	3.07	3.66	4.58
h7	19,282	1.97	2.41	3.07	3.80	4.54

Table S3. Skew ratios of the genome-wide CRISPRa LGR library broken down by sublibraries.

Table S4

CRISPRa - Legacy		Skew				
	# Guides	90/10	95/5	98/2	99/1	99.5/0.5
Entire Library	101,250	2.80	3.97	6.57	10.99	23.12
h1	12,751	3.04	4.33	7.11	11.42	19.97
h2	15,918	2.76	4.00	6.78	11.66	29.54
h3	16,277	2.74	4.12	7.65	14.83	32.68
h4	11,909	2.55	3.57	6.26	10.42	16.50
h5	12,207	2.90	4.13	6.63	10.61	53.46
h6	12,906	2.08	2.67	3.55	4.52	7.25
h7	19,282	2.77	3.95	6.86	11.65	22.00

Table S4. Skew ratios of the genome-wide CRISPRa Legacy library broken down by sublibraries.

Fig. S6

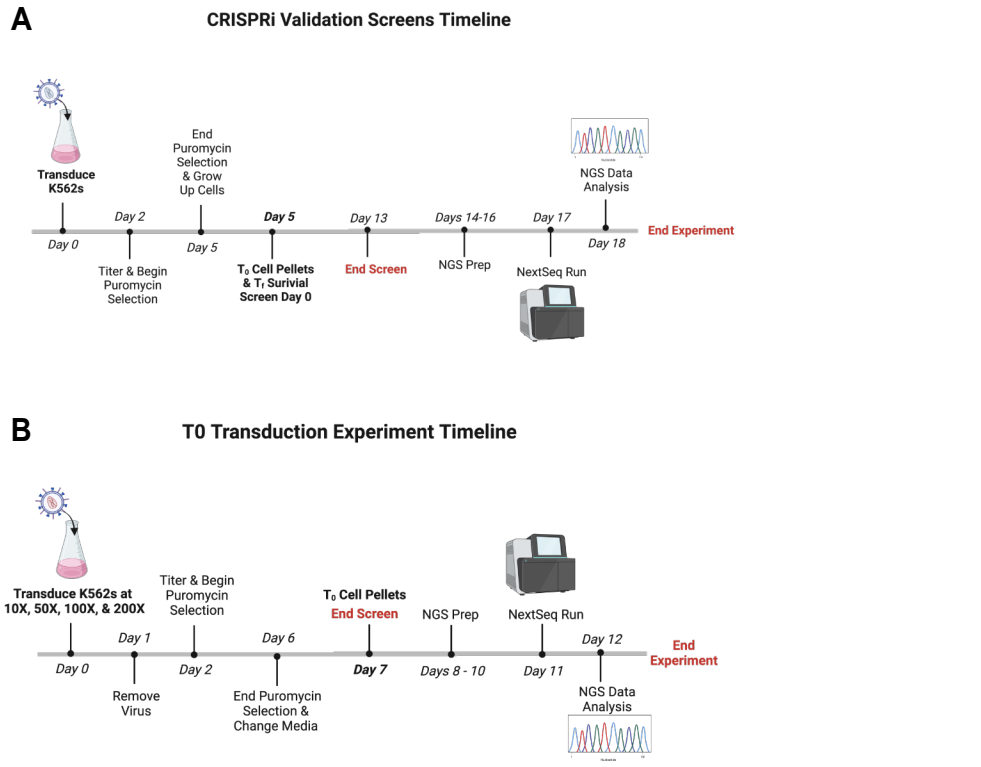
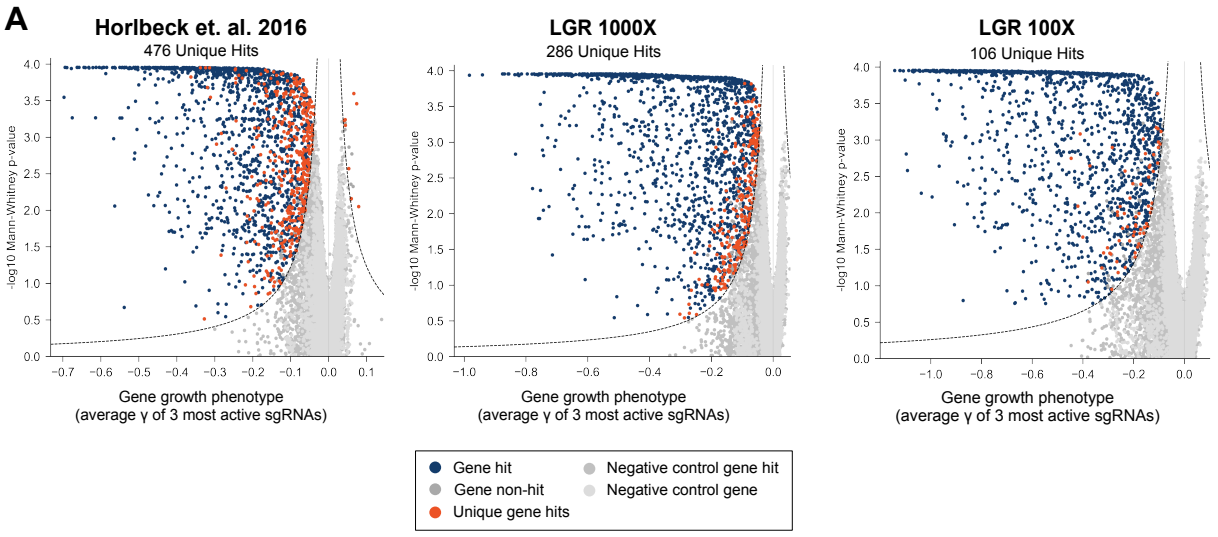


Fig S6. Schematic of the screening timelines for the CRISPRi V2 validation screen (Figure 3), the transduction titration experiment (Figure 4), and the dasatinib survival screen (Figure 5). Timelines include the transduction duration, virus titer determination, puromycin selection length, cell pellet collections, and next-generation sequencing (NGS) sample preparation for each experiment. (A) CRISPRi V2 LGR 1000 and 100-fold cell coverage survival screen timeline. (B) CRISPRi V2 LGR verses legacy transduction titration experiment timeline. (C) LGR verses legacy 100-fold cell coverage dasatinib survival screen timeline.

Fig. S7



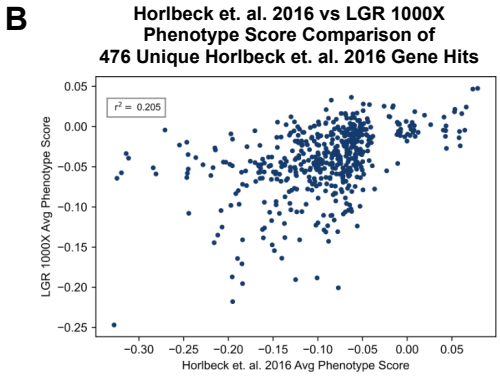


Fig S7. (A) Volcano plots of all three survival screens (Horlbeck et al. 2016, LGR 1000-fold cell coverage, and LGR 100-fold cell coverage). Unique gene hits identified for each library are orange and gene hits that are shared among the libraries are in blue. Unique gene hits for each library are near the cutoff (dotted lines) which are populated by weaker and/or less significant hits. (B) A point comparison of the phenotype score of the 476 unique gene hits found in the Horlbeck et al. 2016 survival screen between the Horlbeck et al. 2016 and LGR 1000-fold screens. The coefficient of determination (r^2) was calculated using a linear least-squares regression.

Table S5

Sample	90/10	95/5	98/2	99/1	99.5/0.5	Number sgRNA Dropouts
LGR_10X_1	5.04	8.7	19	37.06	138.8	765
LGR_10X_2	4.85	8.2	17.07	30.95	62.17	553
Legacy_10X_1	21.88	–	–	–	–	7409
Legacy_10X_2	17.29	610	–	–	–	6141
LGR_50X_1	2.31	2.93	3.84	4.72	5.81	72
LGR_50X_2	2.28	2.86	3.73	4.5	5.51	71
Legacy_50X_1	5.51	11.56	39.8	333.95	–	1092
Legacy_50X_2	5.54	11.42	38.53	392	–	1056
LGR_100X_1	2.29	2.91	3.82	4.67	5.77	69
LGR_100X_2	2.31	2.89	3.78	4.65	5.86	71
Legacy_100X_1	5.42	11	35.5	241.5	–	950
Legacy_100X_2	5.45	10.97	34.69	257.5	–	841
LGR_200X_1	2.22	2.76	3.56	4.33	5.39	71
LGR_200X_2	2.22	2.76	3.6	4.34	5.48	71
Legacy_200X_1	5.32	10.8	34.53	201.29	–	844
Legacy_200X_2	5.35	10.91	35.06	212.67	–	860

Table S5. The majority of dropouts identified in the CRISPRi V2 LGR library (plasmid and K562 samples) was due to the Illumina NextSeq 550 sequencing artifact. The NextSeq 550 is a two-color sequencer that requires signal in the first few cycles to find clusters on the flow cell. Since G bases are dark and lack signal, sgRNAs that begin with a polyG are not detected. Guide library skew ratios and number of sgRNA dropouts in the LGR and legacy samples at 10, 50, 100, and 200-fold cell coverage.

Table S6

Samples	Total sgRNAs Drop Out	% Total Guides	% of Poly G Drop Outs
LGR_10X_1	765	0.742	8.627
LGR_10X_2	553	0.537	11.754
Legacy_10X_1	7409	7.188	1.039
Legacy_10X_2	6141	5.958	1.238
LGR_50X_1	72	0.070	90.278
LGR_50X_2	71	0.069	90.141
Legacy_50X_1	1092	1.059	5.861
Legacy_50X_2	1056	1.025	6.155
LGR_100X_1	69	0.067	94.203
LGR_100X_2	71	0.069	92.958
Legacy_100X_1	950	0.922	6.842
Legacy_100X_2	841	0.816	7.729
LGR_200X_1	71	0.069	88.732
LGR_200X_2	71	0.069	88.732
Legacy_200X_1	844	0.819	7.464
Legacy_200X_2	860	0.834	7.093
Plasmid LGR NextSeq	59	0.057	93.220
Plasmid LGR HiSeq	2	0.002	0.000
Plasmid Legacy NextSeq	770	0.747	14.545

Table S6. In a 103,074 element library, the majority of guides that drop out of the LGR library samples at higher fold cell coverage (50, 100, and 200) are sgRNAs that begin with polyG sequences. Whereas the legacy library samples at the same cell coverages experienced dropouts due to the library quality.

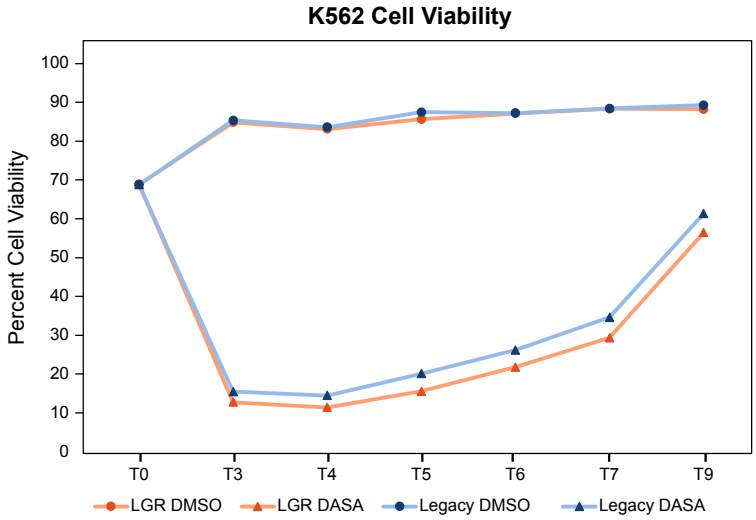
Table S7

LGR CRISPRi Plasmid HiSeq		LGR CRISPRi Plasmid NextSeq	
Skew : 1.92		Skew : 1.99	
Frequency of Drop Out	First 5 Bases of sgRNA	Frequency of Drop Out	First 5 Bases of sgRNA
1	GTCAC	56	GGGGG
1	GCCCG	1	GTCAC
		1	GGGGA
		1	GGCTC

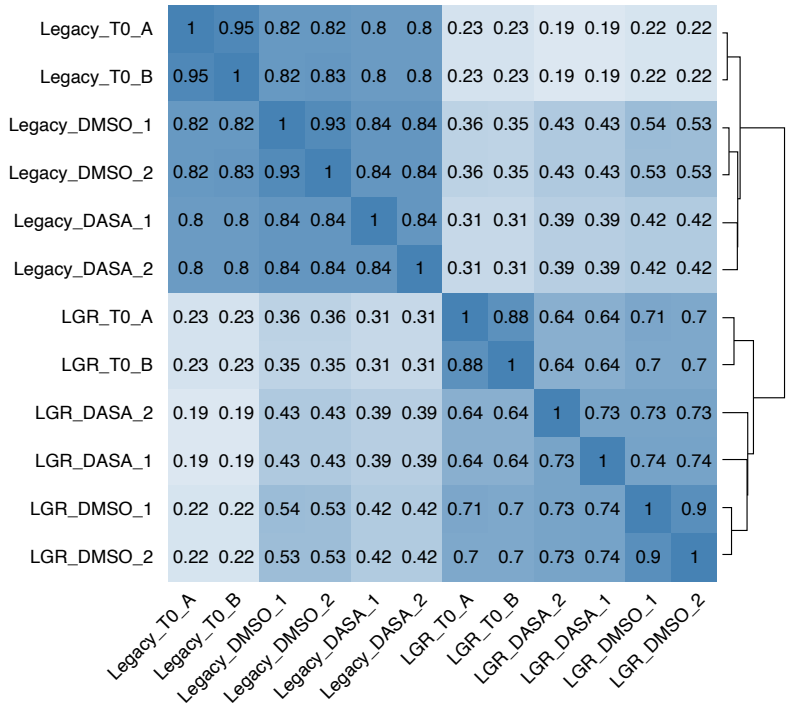
Table S7. Sequence composition analysis of the CRISPRi V2 LGR plasmid library determined that most sgRNAs dropping out began with the polyG sequence in the NextSeq 550 run. When the same library was sequence on the four-color HiSeq 4000, much fewer guides dropped out and a lower 90/10 skew ratio was achieved.

Fig. S8

A


K562 Cell Viability
Percent Cell Viability
100
90
80
70
60
50
40
30
20
10
0
T0
T3
T4
T5
T6
T7
T9
LGR DMSO
LGR DASA
Legacy DMSO
Legacy DASA

B


Legacy_T0_A 1 0.95 0.82 0.82 0.8 0.8 0.23 0.23 0.19 0.19 0.22 0.22
Legacy_T0_B 0.95 1 0.82 0.83 0.8 0.8 0.23 0.23 0.19 0.19 0.22 0.22
Legacy_DMSO_1 0.82 0.82 1 0.93 0.84 0.84 0.36 0.35 0.43 0.43 0.54 0.53
Legacy_DMSO_2 0.82 0.83 0.93 1 0.84 0.84 0.36 0.35 0.43 0.43 0.53 0.53
Legacy_DASA_1 0.8 0.8 0.84 0.84 1 0.84 0.31 0.31 0.39 0.39 0.42 0.42
Legacy_DASA_2 0.8 0.8 0.84 0.84 0.84 1 0.31 0.31 0.39 0.39 0.42 0.42
LGR_T0_A 0.23 0.23 0.36 0.36 0.31 0.31 1 0.88 0.64 0.64 0.71 0.7
LGR_T0_B 0.23 0.23 0.35 0.35 0.31 0.31 0.88 1 0.64 0.64 0.7 0.7
LGR_DASA_2 0.19 0.19 0.43 0.43 0.39 0.39 0.64 0.64 1 0.73 0.73 0.73
LGR_DASA_1 0.19 0.19 0.43 0.43 0.39 0.39 0.64 0.64 0.73 1 0.74 0.74
LGR_DMSO_1 0.22 0.22 0.54 0.53 0.42 0.42 0.71 0.7 0.73 0.74 1 0.9
LGR_DMSO_2 0.22 0.22 0.53 0.53 0.42 0.42 0.7 0.7 0.73 0.74 0.9 1
Legacy_T0_A
Legacy_T0_B
Legacy_DMSO_1
Legacy_DMSO_2
Legacy_DASA_1
Legacy_DASA_2
LGR_T0_A
LGR_T0_B
LGR_DASA_2
LGR_DASA_1
LGR_DMSO_1
LGR_DMSO_2

C

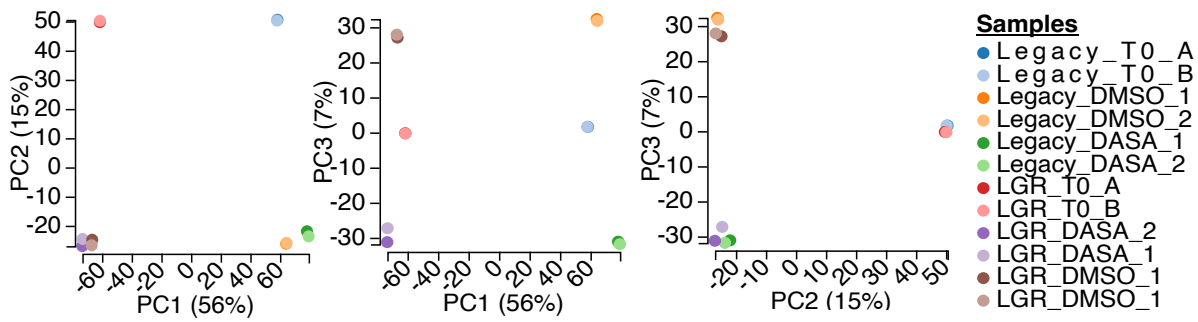

PC2 (15%)
PC1 (56%)
PC3 (7%)
PC1 (56%)
PC3 (7%)
PC2 (15%)
Samples
Legacy_T0_A
Legacy_T0_B
Legacy_DMSO_1
Legacy_DMSO_2
Legacy_DASA_1
Legacy_DASA_2
LGR_T0_A
LGR_T0_B
LGR_DASA_2
LGR_DASA_1
LGR_DMSO_1
LGR_DMSO_1

Fig S8. Dasatinib survival screen quality control measurements for the LGR and legacy library samples. (A) K562 cell viability graph for the average LGR and legacy dasatinib treated (DASA) and control (DMSO) samples over the 10 days of the experiment. Dasatinib treatment was for 72 hours (T0 - T3) and recovery was for 5 days (T3 – T9). (B) Hierarchical clustering of the LGR and legacy library samples using Pearson correlation. (C) Principal Component Analysis (PCA) plot of the LGR and legacy library samples.

Fig. S9

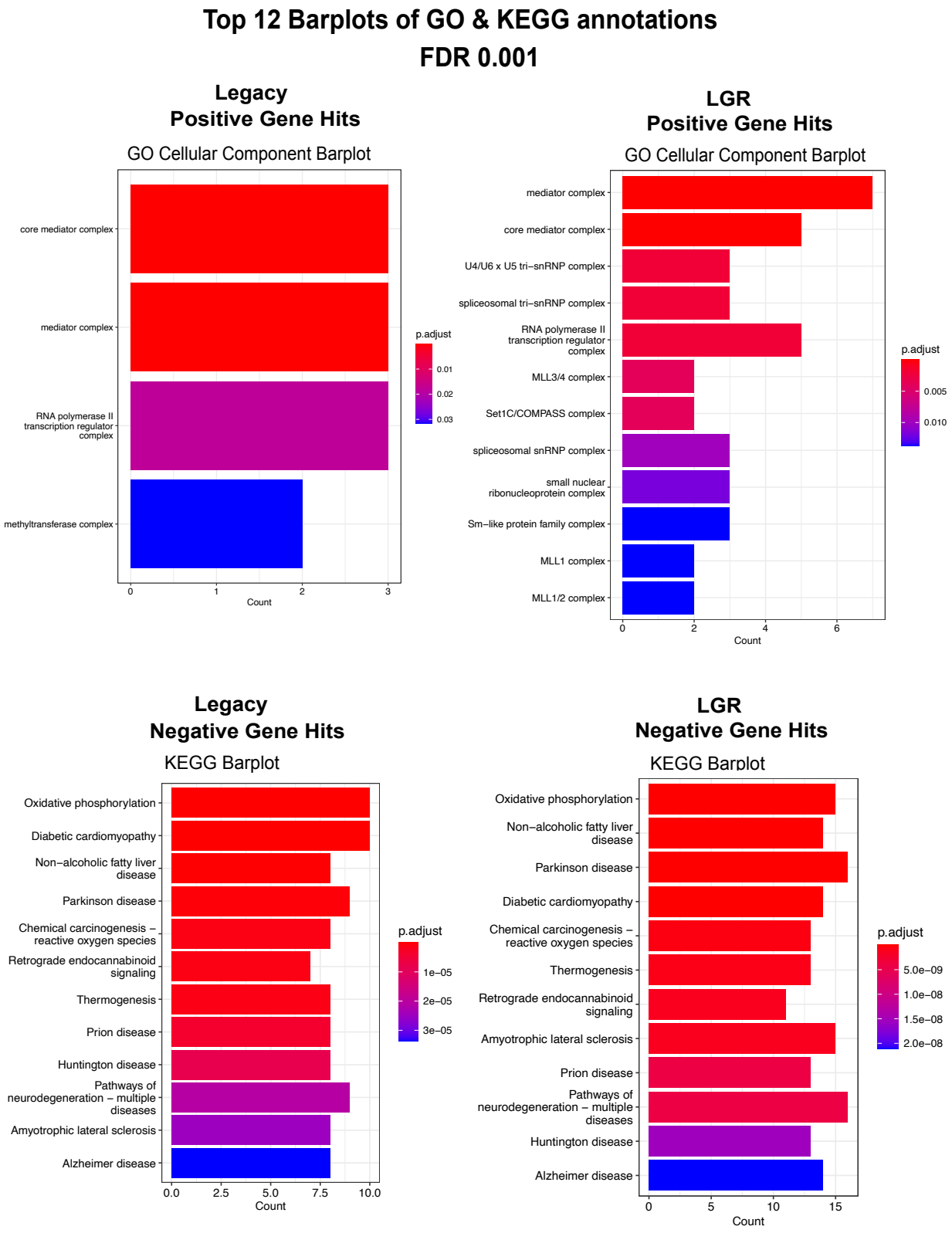

Top 12 Barplots of GO & KEGG annotations
FDR 0.001
Legacy
Positive Gene Hits
GO Cellular Component Barplot
core mediator complex
mediator complex
RNA polymerase II transcription regulator complex
methyltransferase complex
p.adjust
0.01
0.02
0.03
Count
LGR
Positive Gene Hits
GO Cellular Component Barplot
mediator complex
core mediator complex
U4/U6 x U5 tri−snRNP complex
spliceosomal tri−snRNP complex
RNA polymerase II transcription regulator complex
MLL3/4 complex
Set1C/COMPASS complex
spliceosomal snRNP complex
small nuclear ribonucleoprotein complex
Sm−like protein family complex
MLL1 complex
MLL1/2 complex
p.adjust
0.005
0.010
Count
Legacy
Negative Gene Hits
KEGG Barplot
Oxidative phosphorylation
Diabetic cardiomyopathy
Non−alcoholic fatty liver disease
Parkinson disease
Chemical carcinogenesis − reactive oxygen species
Retrograde endocannabinoid signaling
Thermogenesis
Prion disease
Huntington disease
Pathways of neurodegeneration − multiple diseases
Amyotrophic lateral sclerosis
Alzheimer disease
p.adjust
1e−05
2e−05
3e−05
Count
LGR
Negative Gene Hits
KEGG Barplot
Oxidative phosphorylation
Non−alcoholic fatty liver disease
Parkinson disease
Diabetic cardiomyopathy
Chemical carcinogenesis − reactive oxygen species
Thermogenesis
Retrograde endocannabinoid signaling
Amyotrophic lateral sclerosis
Prion disease
Pathways of neurodegeneration − multiple diseases
Huntington disease
Alzheimer disease
p.adjust
5.0e−09
1.0e−08
1.5e−08
2.0e−08
Count

Fig S9. Bar plots ranking the top 12 positive and negative gene hits for the 100-fold cell coverage dasatinib survival screen performed with the legacy (left) and LGR (right) libraries. Gene hits are ranked by p-value assigned by ClusterProfiler (53,56).

Fig. S10

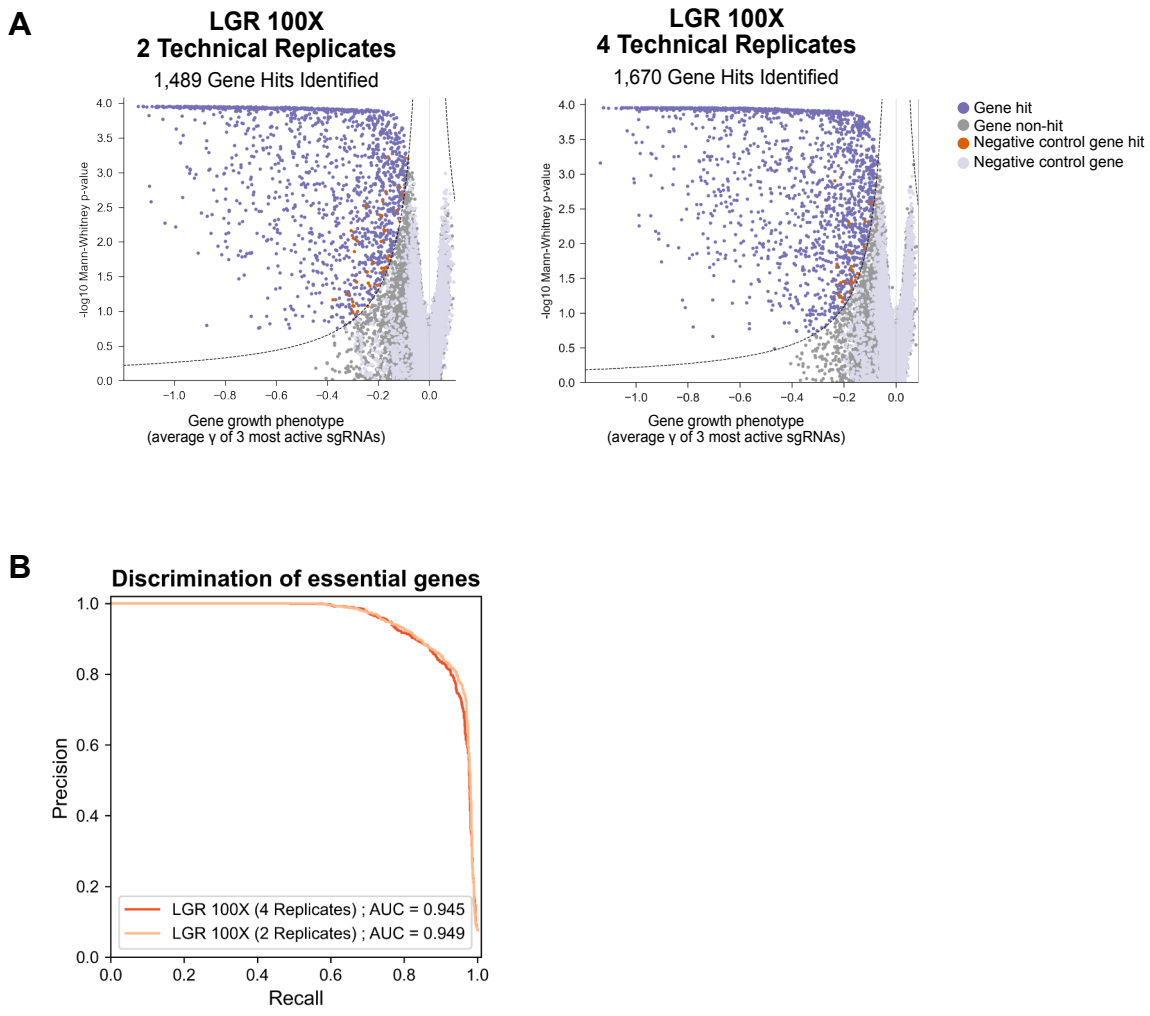


Fig S10. (A) The total number of essential genes identified in the LGR 100-fold cell screen increases as more technical replicates are processed. (B) Precision-recall analysis of the essential genes identified in the LGR 100x screen using either 2 or 4 technical replicates using BAGEL2 (23,24) to measure screening quality. The area under the curve (AUC) for the screen with 2 technical replicates was 0.949 and for 4 technical replicates was 0.945.

Table S8

Screening Coverage	Number of Infected Cells for 100,000 Guide Library	Number of Cells to Transduce with a 25 % Infection	Suspension Cell Line Culture Volume for 1 Sample *(with 3 technical replicates at ~500,000 cells/mL)*	Suspension Cell Line Shaker Flask Size for 1 Sample	Adherent Cell Line Flask Size for 1 Sample
10X	1 million	4 million	6 mL *(30 mL minimum 125mL culture volume)*	125 mL	T25
50X	5 million	20 million	30 mL	125 mL	T75
100X	10 million	40 million	60 mL	125 mL	T175
200X	20 million	80 million	120 mL	250 mL	T175
500X	50 million	200 million	300 mL	500 mL	Two T225
1000X	100 million	400 million	600 mL	1 L	Four T225

Table S8. A table demonstrating the quantity of materials necessary to perform genome-wide screens at increasing coverages (10 to 1000-fold cell coverage). Labware recommendations were acquired from ThermoFisher.