Supplementary Information

Genome-scale CRISPR-Cas9 knockout screening in nasopharyngeal carcinoma for radiosensitive and radioresistant genes

Translational Oncology

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Fig. S1 The quality inspection test about CRISPR-PoolTMKOUT-Human-Single vector Vector library sgRNA.

Fig.S2 Determination of the virus library MOI value. (A) CCK8 assay for cell viability under different cell MOI; (B) Light microscope images of C666-1 cells treated with Puro for different times.

Fig. S3 Optical microscopic images of C666-1 cells transfected with lentiviral sgRNA library and treated with irradiation (1Gy, 2Gy, 4Gy) after 2, 4, and 5 days.

Fig. S4 Fluorescence microscopy images of C666-1 cells infected with the CRISPR control or CRISPR/Cas9 (+) virus.

Fig. S5 Fluorescence microscopy images of CNE1 cells infected with the CRISPR control or CRISPR/Cas9 (+) virus.

Fig. S6 Western blot was used to verify CRISPR/Cas9-mediated knockdown effect of 11 key functional genes in CNE1 cells. (A) Western blot of individual gene knockouts in CNE1 cells infected with the CRISPR control or CRISPR /cas9 (+) virus, β-actin was used as loading control. (B) Quantification of individual gene knockout band intensity in western blot.

Fig. S7 RT-qPCR of individual gene knockouts in CNE1 cells infected with the CRISPR control or CRISPR/Cas9 (+) virus.

Fig. S8 CCK8 assay for CNE1 cells with single gene knockout.

Fig. S9 Clony formation was used to verify the successful construction of radiation-resistant 5-8F cells(5-8FR). Reported values were mean ± SD from three independent experiments. **P < 0.01, ***P <0.001; ****P < 0.0001.

Fig. S10 RT-qPCR was used to determine the expression levels of 11 genes in 5-8F cells and 5-8FR to confirm their radioresistant or radiosensitive status.

Table S1. sgRNA sequences of candidate genes for knockdown

Table S2. Primers of candidate genes for RT-qPCR

Table S3. Coverage statistics of library sgRNA

Table S4. Homogeneity analysis of library sgRNA

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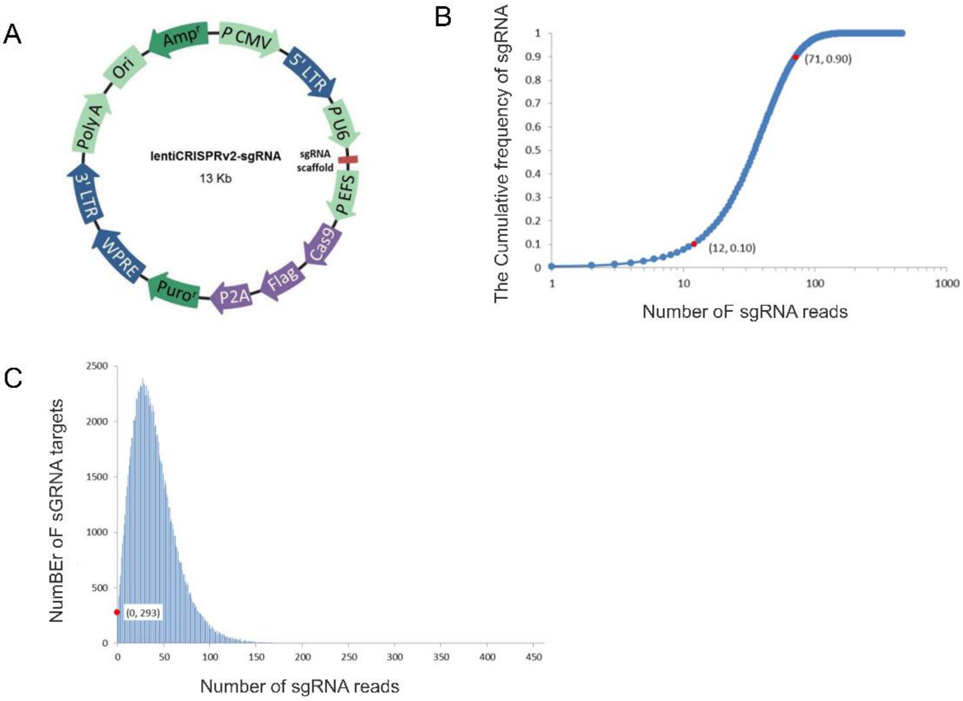
Table S8. Detailed information of GO analysis for the selected nine genes

Table S9. KEGG pathway analysis of candidate genes



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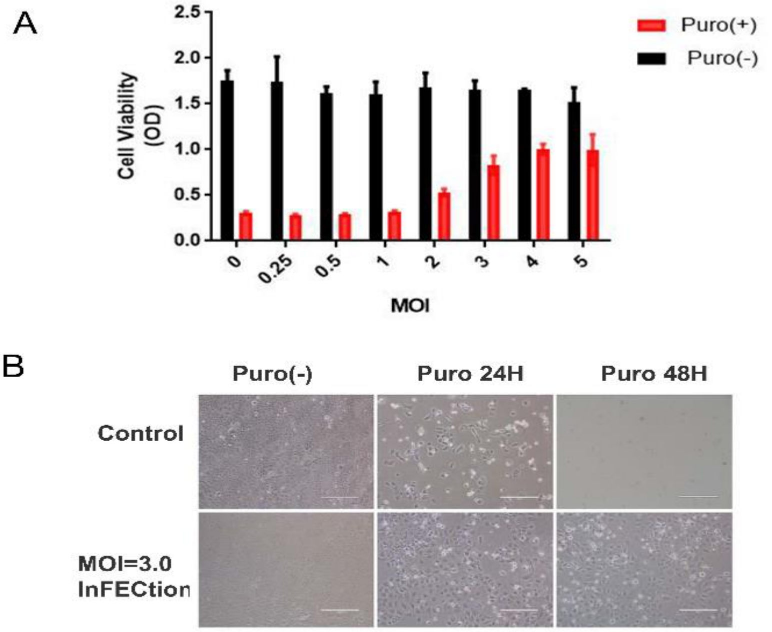

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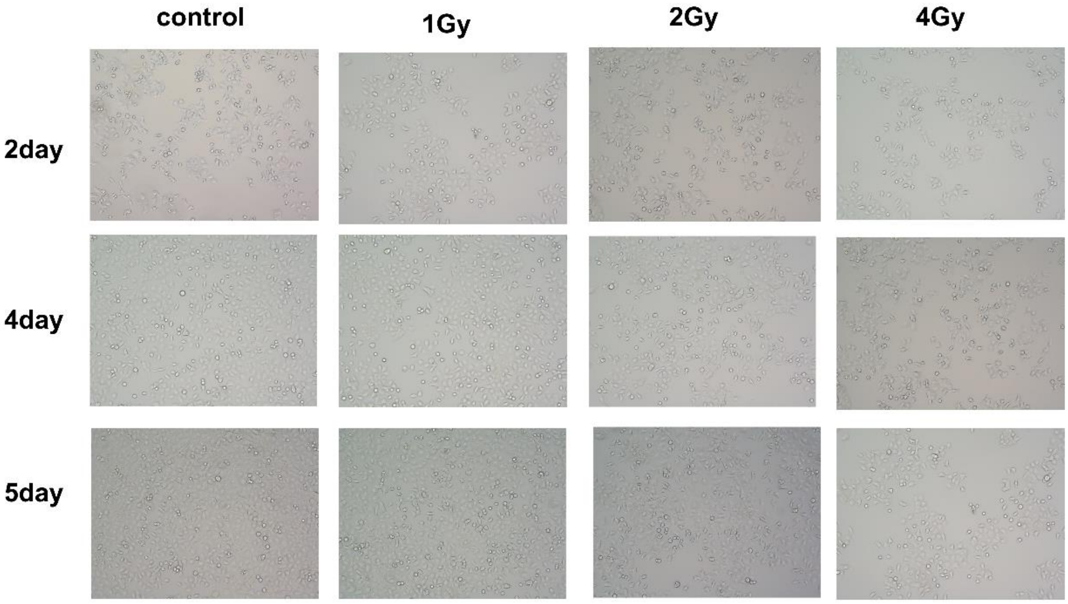

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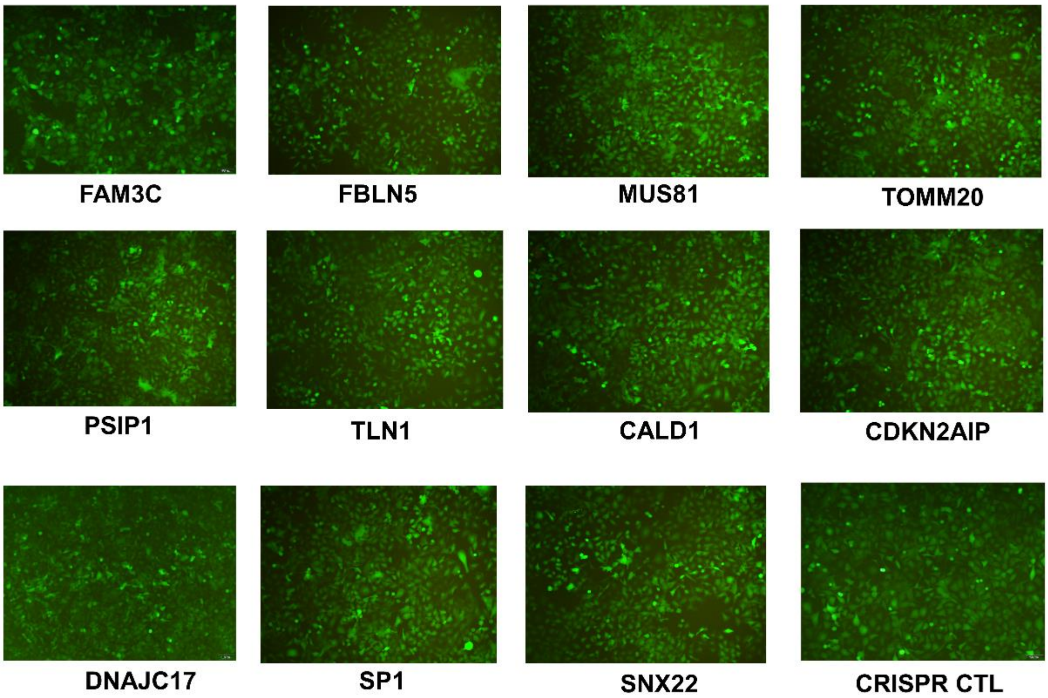

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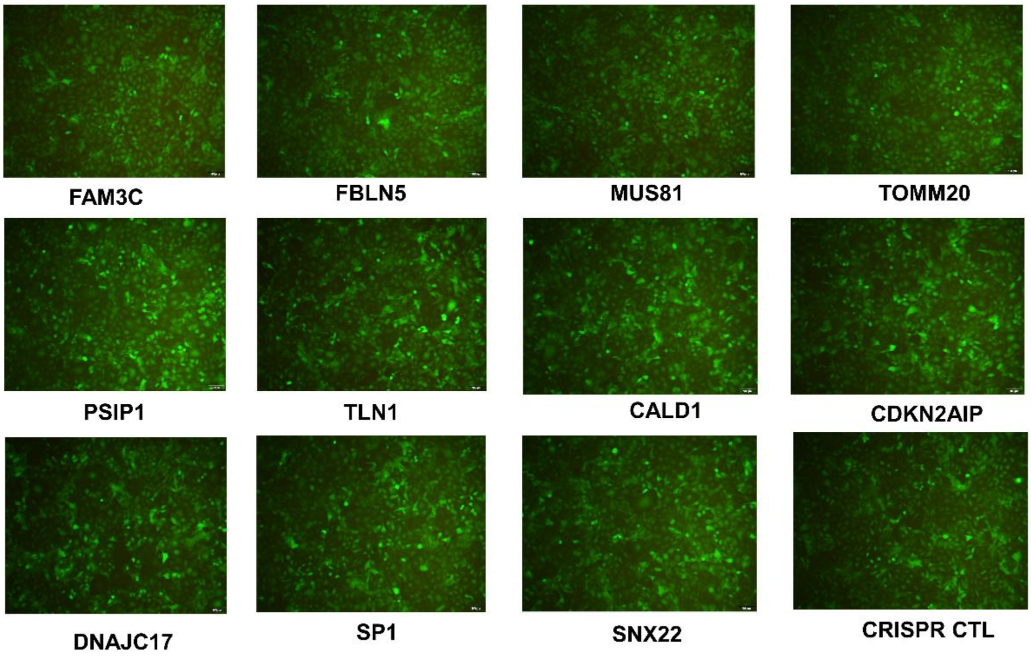
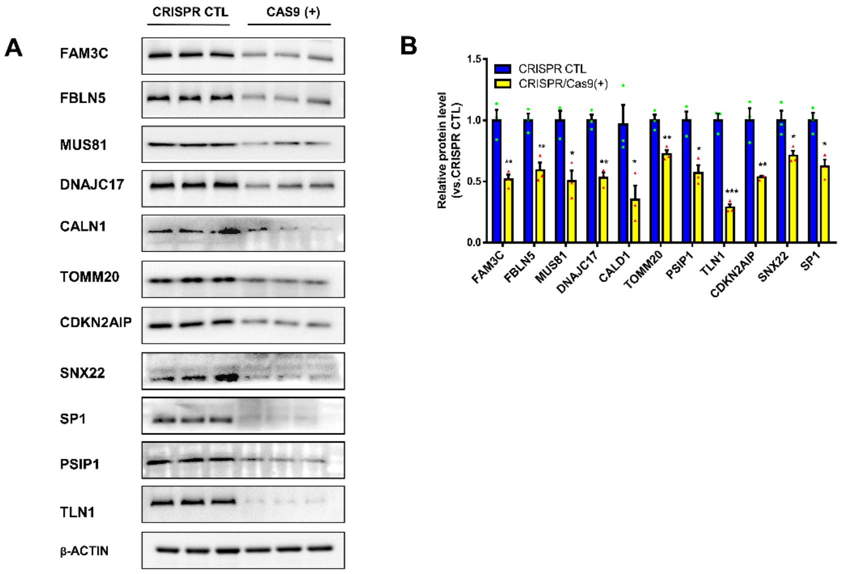


Fig. S5 Fluorescence microscopy images of CNE1 cells infected with the CRISPR control or CRISPR/Cas9 (+) virus.



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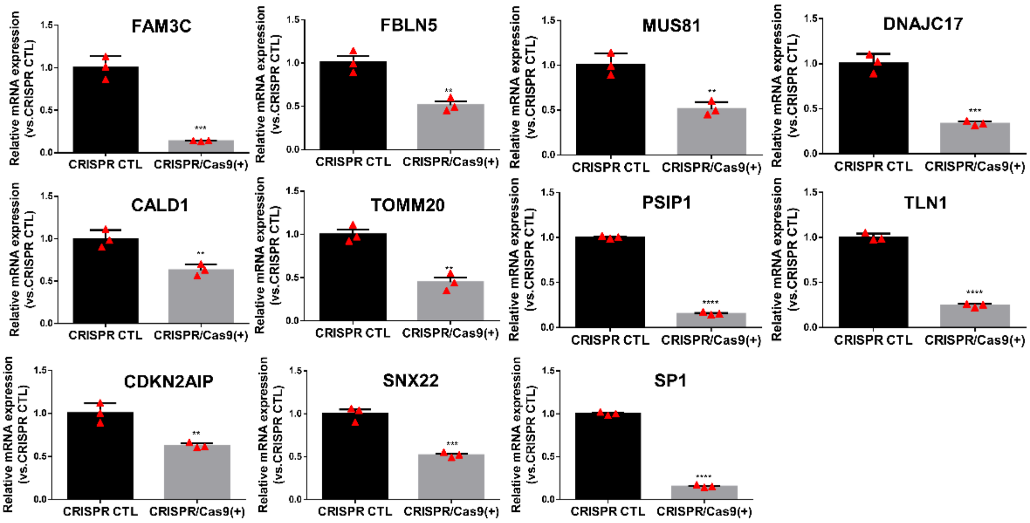

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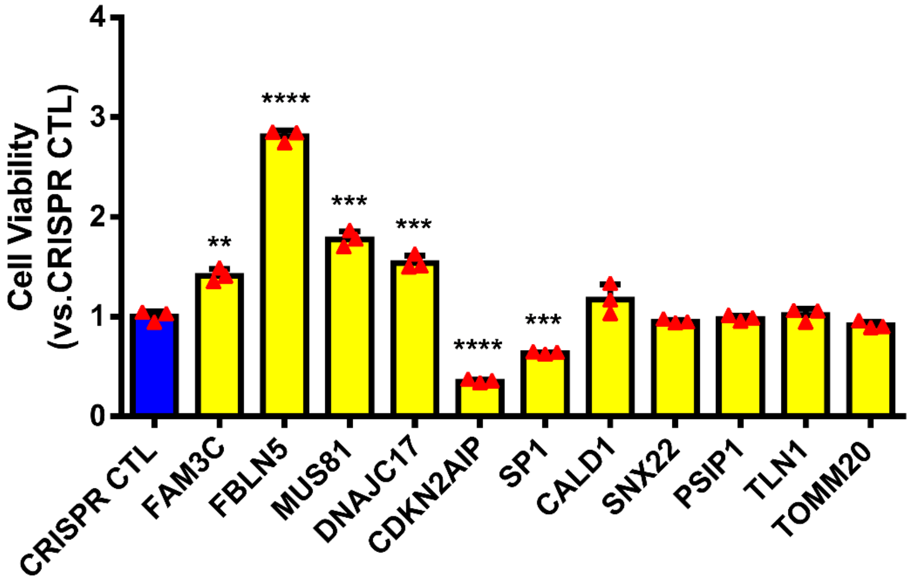

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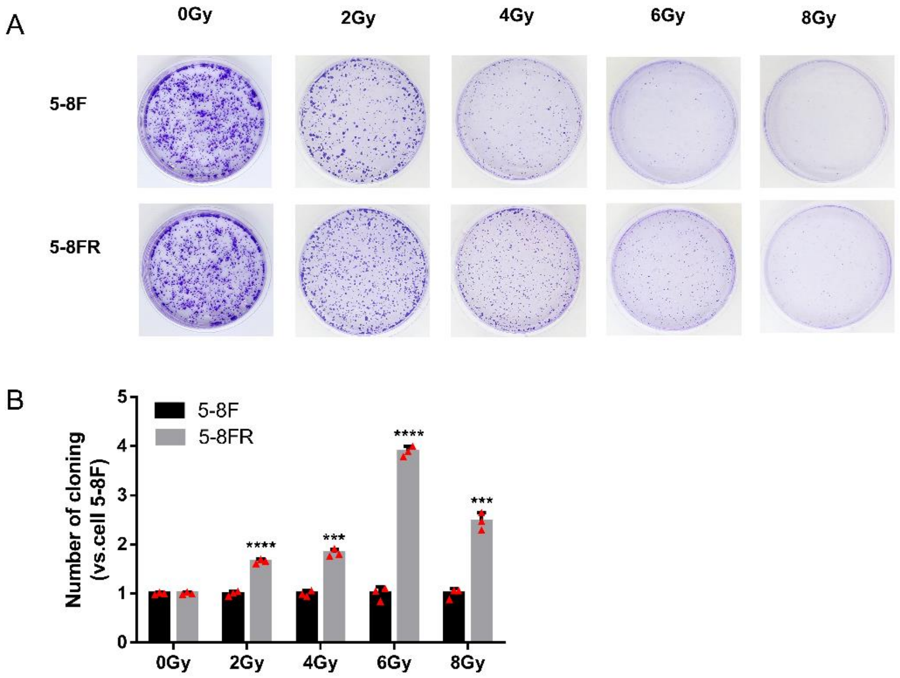

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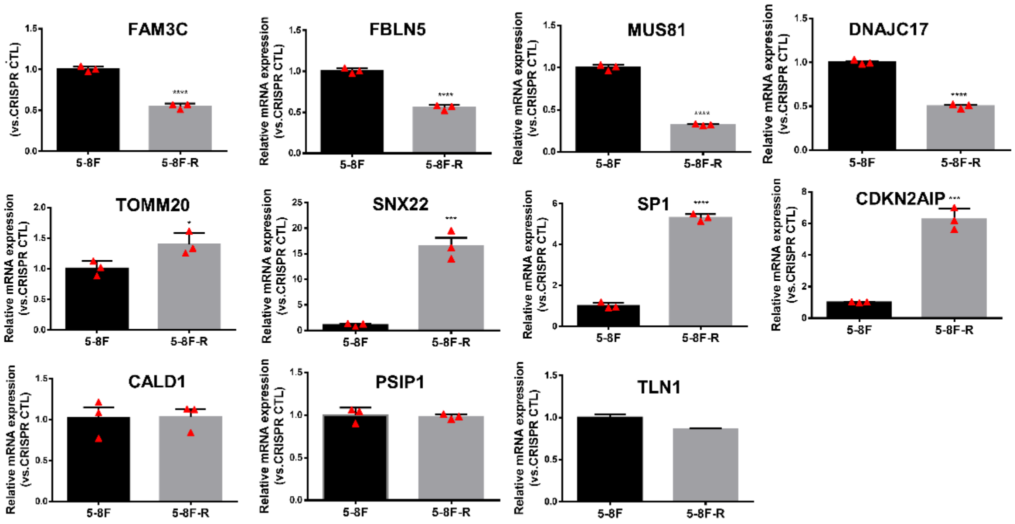

Fig. S10 RT-qPCR was used to determine the expression levels of 11 genes in 5-8F cells and 5-8FR to confirm their radioresistant or radiosensitive status.

Table S1. SgRNA sequences and diversity of candidate genes

Gene symbol	Target Sequences(5'to3')	SgRNA diversity
FAM3C	GAGGAGATGTGGCACCATTTA	3
FBLN5	GCAGCAGACGTGCTACAATTT	5
MUS81	CACGCGCTTCGTATTTCAGAA	5
DNAJC17	CCAGAGCAGCUGAACUCUUTT	5
CALD1	GCAGCAGGCACACCAAAUATT	5
TOMM20	GCTGTTCAGAAGTTCTTCCTT	5
CDKN2AIP	GCUCCUAAAUGCAGCUAUUTT	5
SP1	CCACTCCTTCAGCCCTTATTA	6
SNX22	GCUGCACAAGCGGAUCAAGAATT	5
PSIP1	TTTAGGACCAAAGGATATATT	6
TLN1	GCAGTGAAAGATGTAGCCAAA	5

Table S2. Primers of candidate genes for RT-qPCR

Gene	Sequences
FBLN5	forward: 5′-GCAGCAGACGTGCTACAATTTACAA-3′
	reverse: 5′-CAGGGTTCTCAGCAGGACACATAC-3′
FAM3C	forward: 5′-CTGCAAAGTTGGTGGTAGCTGTG-3′
	reverse: 5′-ACGTGCAGCTGTGTCCAATG-3′
MUS81	forward: 5′-AGTGGAGCCAAGGGAAAAGA-3′
	reverse: 5′-GGGGCACTAACGAGAGGAGA-3′
DNAJC17	forward: 5′-GAGGTTCTCAACCTGGTGCTTTC-3′
	reverse: 5′-GGTTATCCACCAGGCCAACTTC-3′
CALD1	forward: 5′-CCGCATCAATGAATGGCTAACTAA-3′
	reverse: 5′-GTTCCGCTTGCTGGATACGTC-3′
TLN1	forward: 5′-TGTGCCAATGGCTACCTGGA-3′
	reverse: 5′-GAACCAGCCACACGCTTTGA-3′
PSIP1	forward: 5′-ACAGCCCTGTCCTTCAGAGAGTG-3′
	reverse: 5′-CCTCTTCATCCTTCTTAGGCTGCTT-3′
SNX22	forward: 5′-AGTAGTAGCCCTTGCTTCCACAAC-3′
	reverse: 5′-CCAACGCAGGCAAAGACA-3′
CDKN2AIP	forward: 5′-CGCTAGCACGGATGAAGCTG-3′
	reverse: 5′-ATAGGTTGGAGCATCTGTCACTTTG-3′
TOMM20	forward: 5′-CCTGACCTTAAAGATGCTGAAGCTG-3′
	reverse: 5′-ATCTGGAACACTGGTGGTGGAA-3′
SP1	forward: 5′-TCACACGTTCGGATGAGCTACAG-3′

reverse: 5′-ATGAAGCGCTTAGGACACTCAGG-3′

Total number of library sgRNA (N)	123.411
Number of library sgRNA with 0 read times (N0)	293
Library sgRNA coverage rate	99.76%
Reference	>98%

Table S3. Coverage statistics of library sgRNA

Note: Library sgRNA coverage (%) = (N-N0) /N×100% (N= total number of library sgRNA; N0= Number of sgRNA with 0 high-throughput sequencing read times)

Table S4. Homogeneity analysis of library sgRNA

	SgRNA cumulative frequency	SgRNA read times	the difference multiple of sgRNA reading times
The library of virus	0.90	71	5.92
	0.10	12	
Reference			<15

Note: When the cumulative frequency of sgRNA is 0.10 and 0.90, the number of sgRNA reads differs by multiples. Calculation formula of cumulative frequency: The cumulative frequency of sgRNA with reading times of N = the number of all sgRNA with reading times ≤ N/the total number of sgRNA in library

Table S5. Determination of lentivirus titer

The sample	V（ml）	N	Copy number of A gene	Copy number of B gene	C	lentivirus titer（TU/ml）	The average of lentivirus titer（TU/ml）
The library of virus	1.00E-03	4.00E+05	3.81E+04	1.01E+05	0.75	3.02E+08	3.00E+08
	1.00E-03	4.00E+05	3.12E+04	7.66E+04	0.81	3.26E+08	
	1.00E-03	4.00E+05	3.43E+04	9.70E+04	0.71	2.86E+08	

Note: Lentivirus titer (TU/ml) =N ×C/N (N= number of cells infected by lentivirus; C= Number of lentiviruses contained in one cell =2 × Copy number of A gene/copy number of B gene; V= Volume of lentivirus used to infect cells (ml))

Table S6. Library virus purity test results

The sample	Test item	test method	Test result	unit	reference test range
The library of virus	mycoplasma	Nest PCR	negative	——	negative
	endotoxin	LAL	5.9	EU/ml	<50
	chlamydia	Culture method	negative	——	negative
	bacteria	Culture method	negative	——	negative
	fungi	Culture method	negative	——	negative

Table S7. Key genes information in the results of high-throughput sequencing analysis

GeneID	Symbol	Synonyms	description	chromosome	Fold Change
10447	FAM3C	GS3786|ILEI	family with sequence similarity 3 member C	7	2.173134764
10516	FBLN5	ADCL2|ARCL1A|ARMD3|DANCE|EVEC|FIBL5|HNARMD|UP50	fibulin 5	14	2.368614842
80198	MUS81	SLX3	MUS81 structure-specific endonuclease subunit	11	2.142258603
55192	DNAJC17	-	DnaJ heat shock protein family (Hsp40) member C17	15	2.022497019
800	CALD1	CDM|H-CAD|HCAD|L-CAD|LCAD|NAG22	caldesmon 1	7	1.991529948
9804	TOMM20	MAS20|MOM19|TOM20	translocase of outer mitochondrial membrane 20	1	-2.44760528
55602	CDKN2AIP	CARF	CDKN2A interacting protein	4	-2.487205583
6667	SP1	-	Sp1 transcription factor	12	-2.440589176
79856	SNX22	-	sorting nexin 22	15	-2.625790481
11168	PSIP1	DFS70|LEDGF|PAIP|PSIP2|p52|p75	PC4 and SFRS1 interacting protein 1	9	-2.747363999
7094	TLN1	ILWEQ|TLN|talin-1	talin 1	9	-2.77007414

Table S8. Detailed information of GO analysis for the selected nine genes

GO ID	Description	Associated genes	p-value
Biological process			
GO:0100057	regulation of phenotypic switching by transcription from RNA polymerase II promoter	SP1	0.000351
GO:1904828	positive regulation of hydrogen sulfide biosynthetic process	SP1	0.000351
GO:0016031	tRNA import into mitochondrion	TOMM20	0.000701
GO:0070096	mitochondrial outer membrane translocase complex assembly	TOMM20	0.000701
GO:1904826	regulation of hydrogen sulfide biosynthetic process	SP1	0.000701
GO:1905242	response to 3,3',5-triiodo-L-thyronine	TOMM20	0.000701
GO:0051176	positive regulation of sulfur metabolic process	SP1	0.001052
GO:0072429	response to intra-S DNA damage checkpoint signaling	MUS81	0.001402
GO:0000727	double-strand break repair via break-induced replication	MUS81	0.001752
GO:0035927	RNA import into mitochondrion	TOMM20	0.001752
GO:0070814	hydrogen sulfide biosynthetic process	SP1	0.002103
GO:0007008	outer mitochondrial membrane organization	TOMM20	0.002453
GO:0009403	toxin biosynthetic process	SP1	0.002453
GO:1900378	positive regulation of secondary metabolite biosynthetic process	SP1	0.002453
GO:0070813	hydrogen sulfide metabolic process	SP1	0.002803
GO:0072396	response to cell cycle checkpoint signaling	MUS81	0.002803
GO:0072402	response to DNA integrity checkpoint signaling	MUS81	0.002803
GO:0072423	response to DNA damage checkpoint signaling	MUS81	0.002803
GO:0048251	elastic fiber assembly	FBLN5	0.004201
GO:1900376	regulation of secondary metabolite biosynthetic process	SP1	0.004201
Cellular component			
GO:0071953	elastic fiber	FBLN5	0.001317027
GO:0048476	Holliday junction resolvase complex	MUS81	0.001646051
GO:0001652	granular component	CDKN2AIP	0.001974983
GO:0005742	mitochondrial outer membrane translocase complex	TOMM20	0.003618247
GO:0031089	platelet dense granule lumen	FAM3C	0.004603092
GO:0098799	outer mitochondrial membrane protein complex	TOMM20	0.005914921
GO:0031307	integral component of mitochondrial outer membrane	TOMM20	0.006570279
GO:0031306	intrinsic component of mitochondrial outer membrane	TOMM20	0.006897819
GO:0042827	platelet dense granule	FAM3C	0.006897819
GO:0044233	Mitochondria-associated ER Membrane	TOMM20	0.007552622
GO:0044232	organelle membrane contact site	TOMM20	0.009841519
GO:0044420	extracellular matrix component	FBLN5	0.017005762
GO:0032592	integral component of mitochondrial membrane	TOMM20	0.025737478
GO:0098573	intrinsic component of mitochondrial membrane	TOMM20	0.026059594
GO:0017053	transcriptional repressor complex	SP1	0.027668804

GO:0005730	nucleolus	CDKN2AIP; MUS81	0.042342651
GO:0043227	membrane-bounded organelle	DNAJC17; FBLN5; CDKN2AIP; MUS81; TOMM20; SP1; FAM3C	0.046041933
Molecular function			
GO:0048257	3'-flap endonuclease activity	MUS81	0.000693
GO:0008022	protein C-terminus binding	FBLN5; SP1	0.002023
GO:0030943	mitochondrion targeting sequence binding	TOMM20	0.002078
GO:0015450	P-P-bond-hydrolysis-driven protein transmembrane transporter activity	TOMM20	0.00277
GO:0008821	crossover junction endodeoxyribonuclease activity	MUS81	0.003116
GO:0030023	extracellular matrix constituent conferring elasticity	FBLN5	0.003116
GO:0048256	flap endonuclease activity	MUS81	0.003462
GO:0097493	structural molecule activity conferring elasticity	FBLN5	0.003807
GO:0016889	endodeoxyribonuclease activity, producing 3'-phosphomonoesters	MUS81	0.004153
GO:0071837	HMG box domain binding	SP1	0.005534
GO:0008320	protein transmembrane transporter activity	TOMM20	0.007602
GO:0022884	macromolecule transmembrane transporter activity	TOMM20	0.008635
GO:0043425	bHLH transcription factor binding	SP1	0.009667
GO:0035035	histone acetyltransferase binding	SP1	0.010354
GO:0016894	endonuclease activity, active with either ribo- or deoxyribonucleic acids and producing 3'-phosphomonoesters	MUS81	0.010697
GO:0001103	RNA polymerase II repressing transcription factor binding	SP1	0.012071
GO:0004520	endodeoxyribonuclease activity	MUS81	0.014812
GO:0005048	signal sequence binding	TOMM20	0.016522
GO:1904680	peptide transmembrane transporter activity	TOMM20	0.018571
GO:0004536	deoxyribonuclease activity	MUS81	0.020956

GO: Gene Ontology

Table S9. KEGG pathway analysis of candidate genes

Pathway ID	Pathway	Gene name	p-value
ko03440	Homologous recombination	MUS81	0.010076
ko03460	Fanconi anemia pathway	MUS81	0.013112
ko04137	Mitophagy - animal	SP1	0.017076
ko04927	Cortisol synthesis and secretion	SP1	0.017774
ko04350	TGF-beta signaling pathway	SP1	0.021496
ko05231	Choline metabolism in cancer	SP1	0.025442
ko01522	Endocrine resistance	SP1	0.027065
ko04928	Parathyroid hormone synthesis, secretion and action	SP1	0.027297
ko05224	Breast cancer	SP1	0.037695
ko04934	Cushing syndrome	SP1	0.039308
ko04915	Estrogen signaling pathway	SP1	0.039768
ko05202	Transcriptional misregulation in cancers	SP1	0.045285

KEGG: Kyoto Encyclopedia of Genes and Genomes