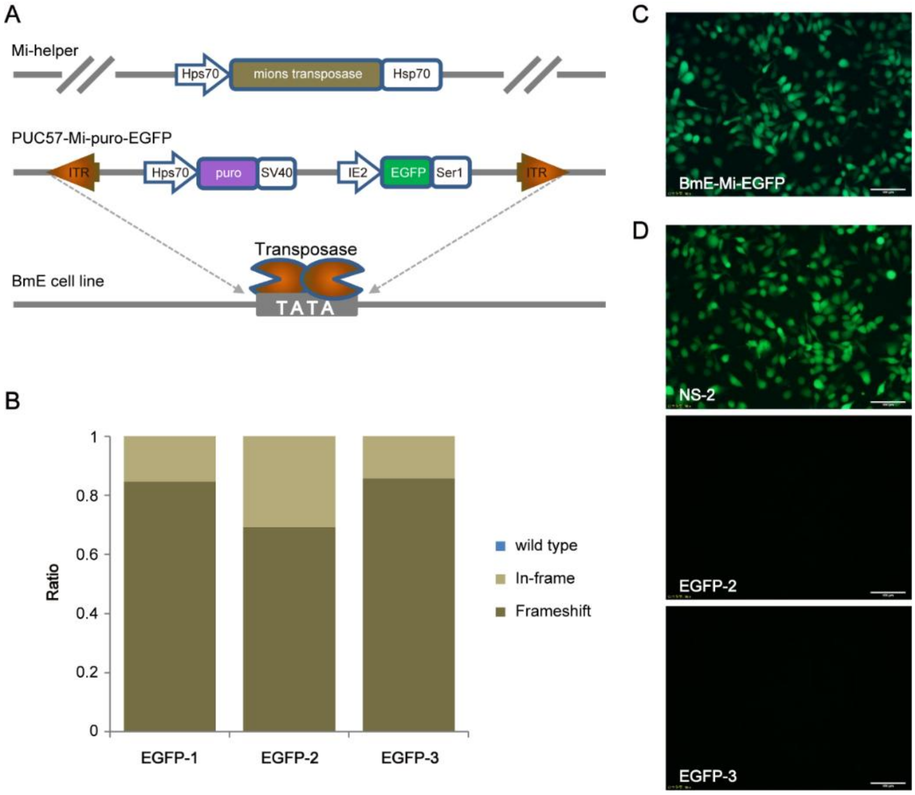
Supplemental Figures

Supplemental Fig S1



Supplemental Fig S1. The *Minos* delivery method and *piggy*Bac-CRISPR system in *B. mori* cells.

(A) Schematics of *Minos* delivery vectors PUC57-Mi-puro-EGFP and Mi-helper. **(B)** Sanger sequencing analysis of the ratio of in-frame/frameshift for EGFP-1 (left), EGFP- 2 (middle), and EGFP-3 (right) target regions of BmE-Mi-EGFP cells. **(C)** Fluorescent images of BmE-Mi-EGFP cells. **(D)** Fluorescent images of BmE-Mi-EGFP knockout experiments.

Supplemental Fig S2

sgRNA 1

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT

sgRNA 2 **sgRNA 3**

CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGG

GCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACC

ACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTAC

GGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTT

CTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTT

CAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGC

GACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGA

CGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACG

TCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGA

TCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG

CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTA

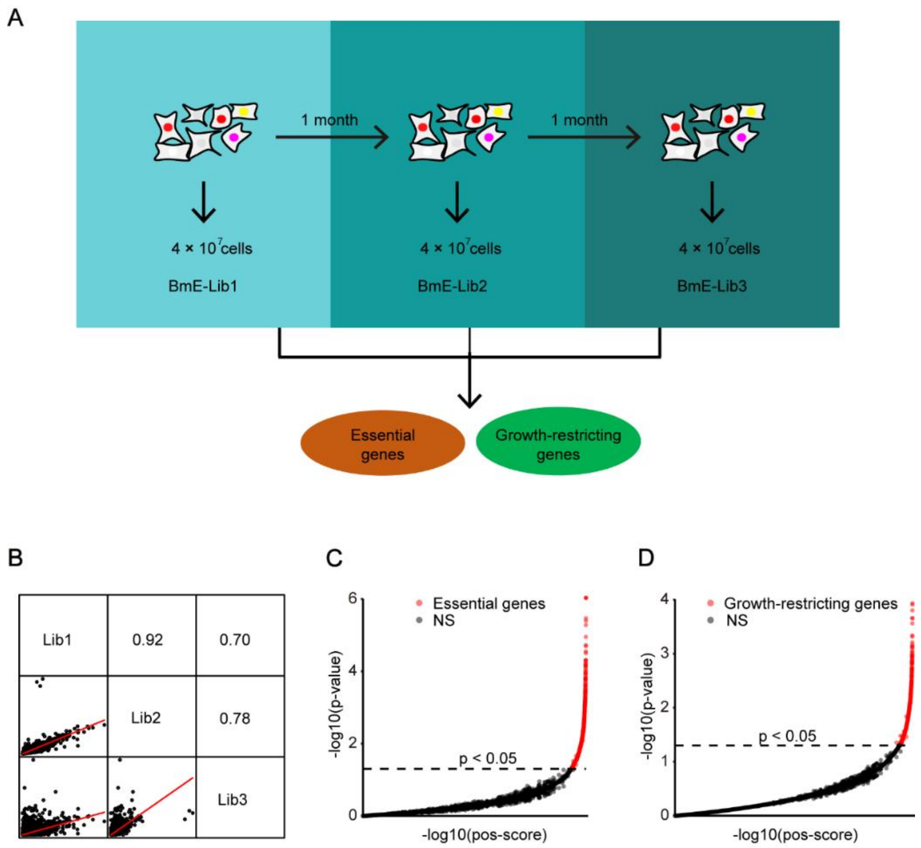
CCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATC

ACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGG

ACGAGCTGTACAAGTAA

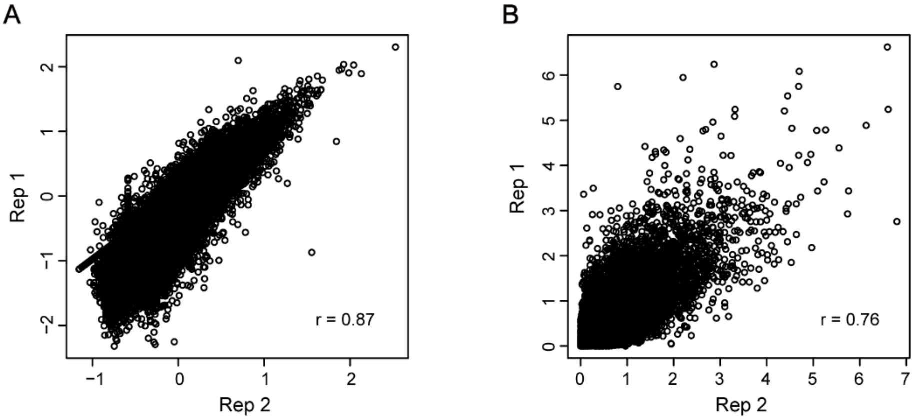
Supplemental Fig S2. Design of three sgRNAs in the coding region of EGFP.

Supplemental Fig S3



Supplemental Fig S3. Screening of essential and growth-restricting genes in the BmE cell line.

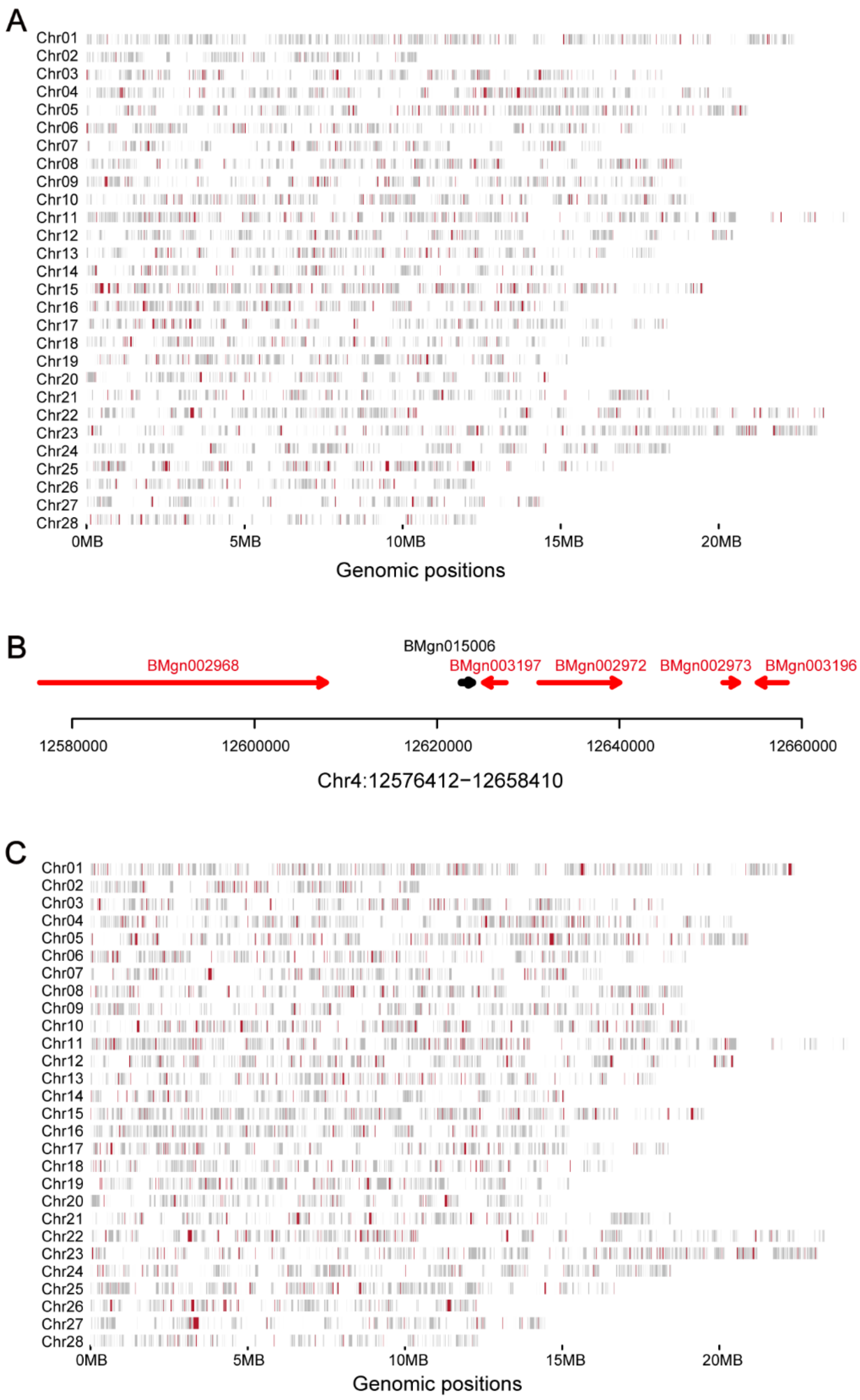
(A) Flowchart for screening essential and growth-restricting genes in BmE cells. **(B)** Correlation analysis of BmE-Libs among three time points. **(C)** Screening of essential genes based on the MAGeCK method (p-value < 0.05). **(D)** Screening of growth-restricting genes based on the MAGeCK method (p-value < 0.05).



Supplemental Fig S4. The correlation analysis of two biological replicates for fitness screen.

(A) Plot documenting log10(fold-changes) of all sgRNAs included in two independent biological replicates for fitness screen. **(B)** Plot documenting log10(negative scores) for all genes included in two independent biological replicates for fitness screen.

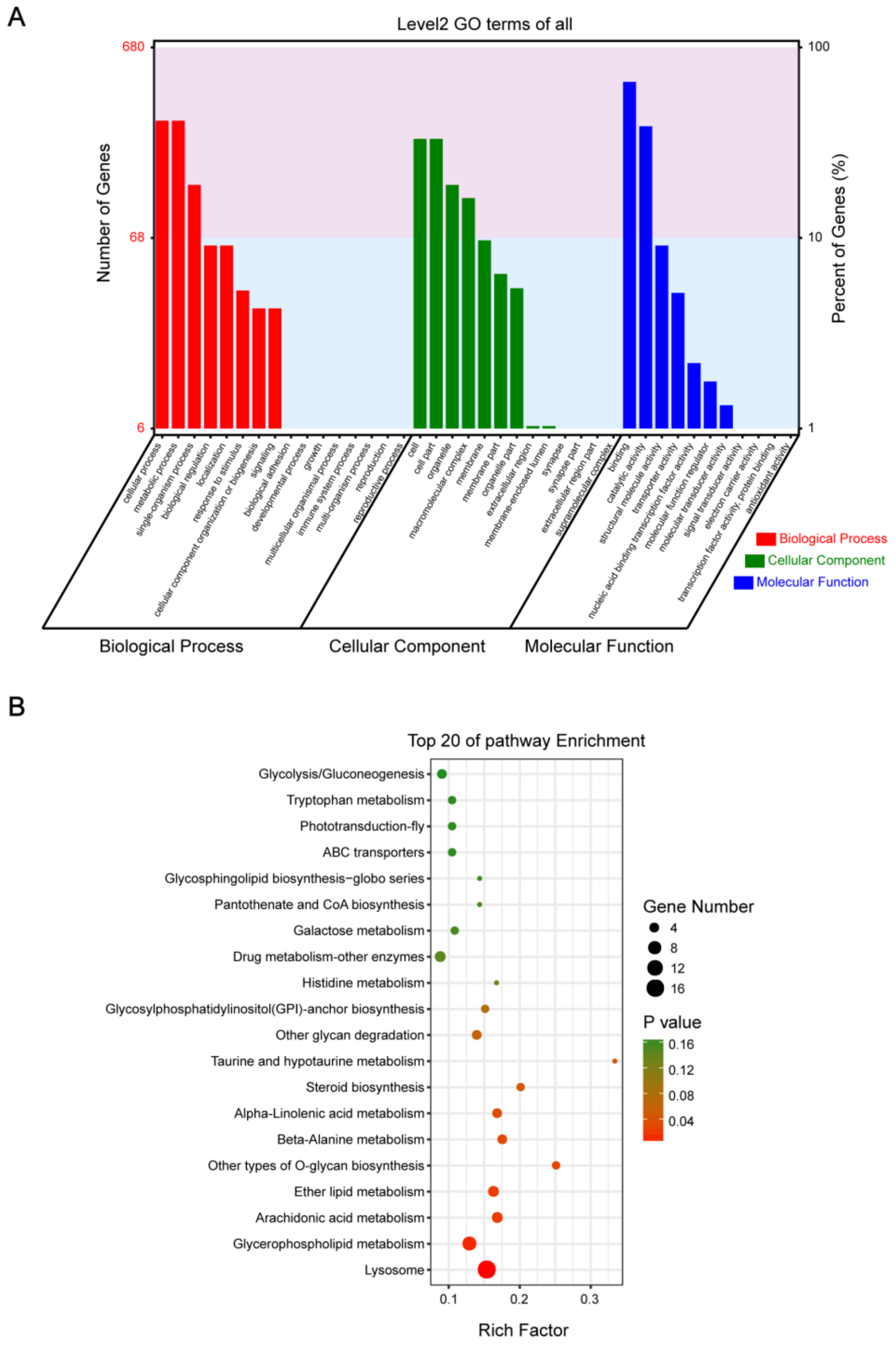
Supplemental Fig S5



Supplemental Fig S5. Chromosome distribution of essential and growth-restricting genes.

Essential genes **(A)** and growth-restricting genes **(C)** distributed evenly on the chromosomes. red lines: essential or growth-restricting genes, grey lines: all genes. **(B)** The enriched region of essential genes in chromosome 4 (12576412–12658410) that contained a cluster of six genes.

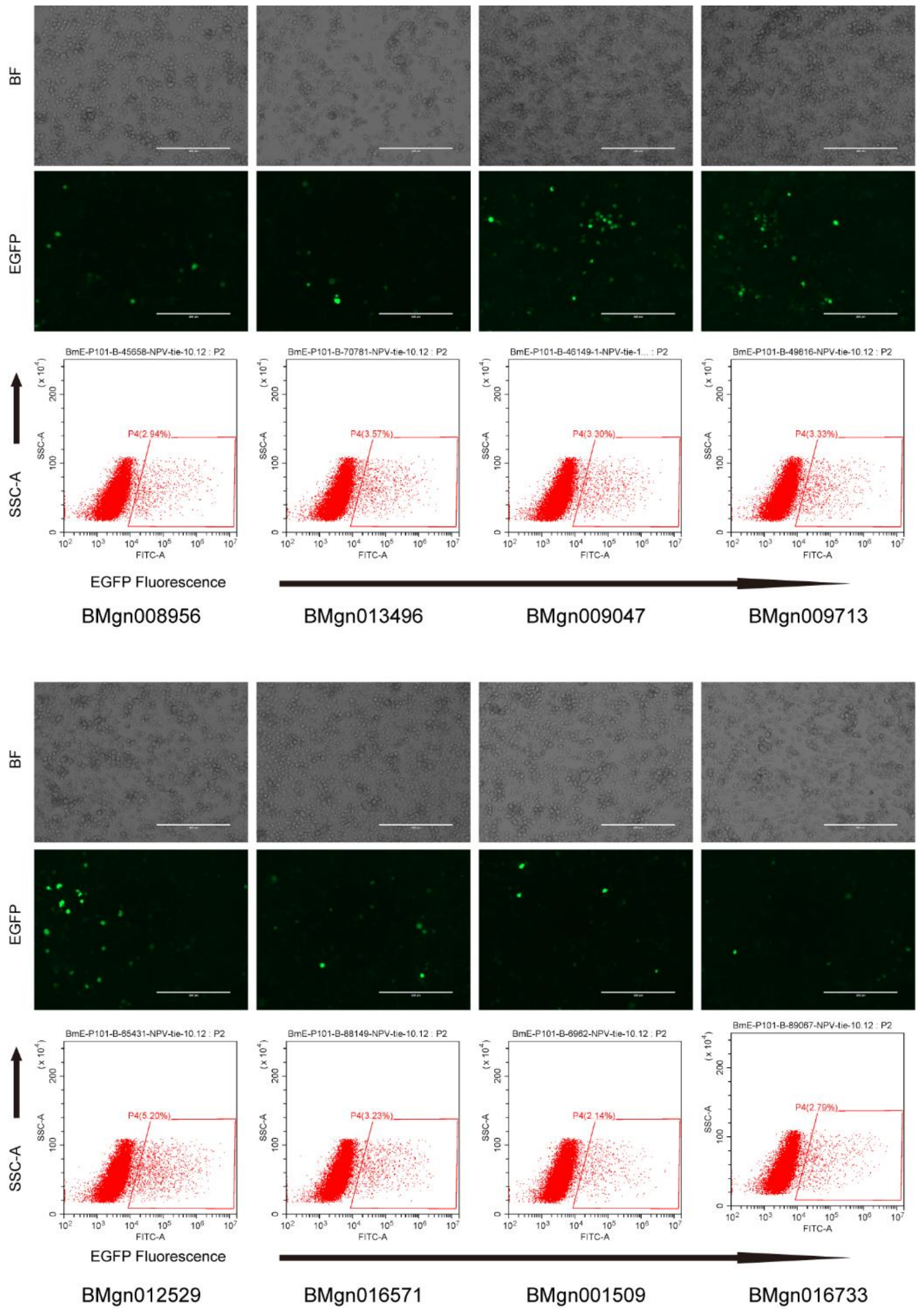
Supplemental Fig S6



Supplemental Fig S6. Functional Analysis of essential and growth-restricting genes in BmE cells.

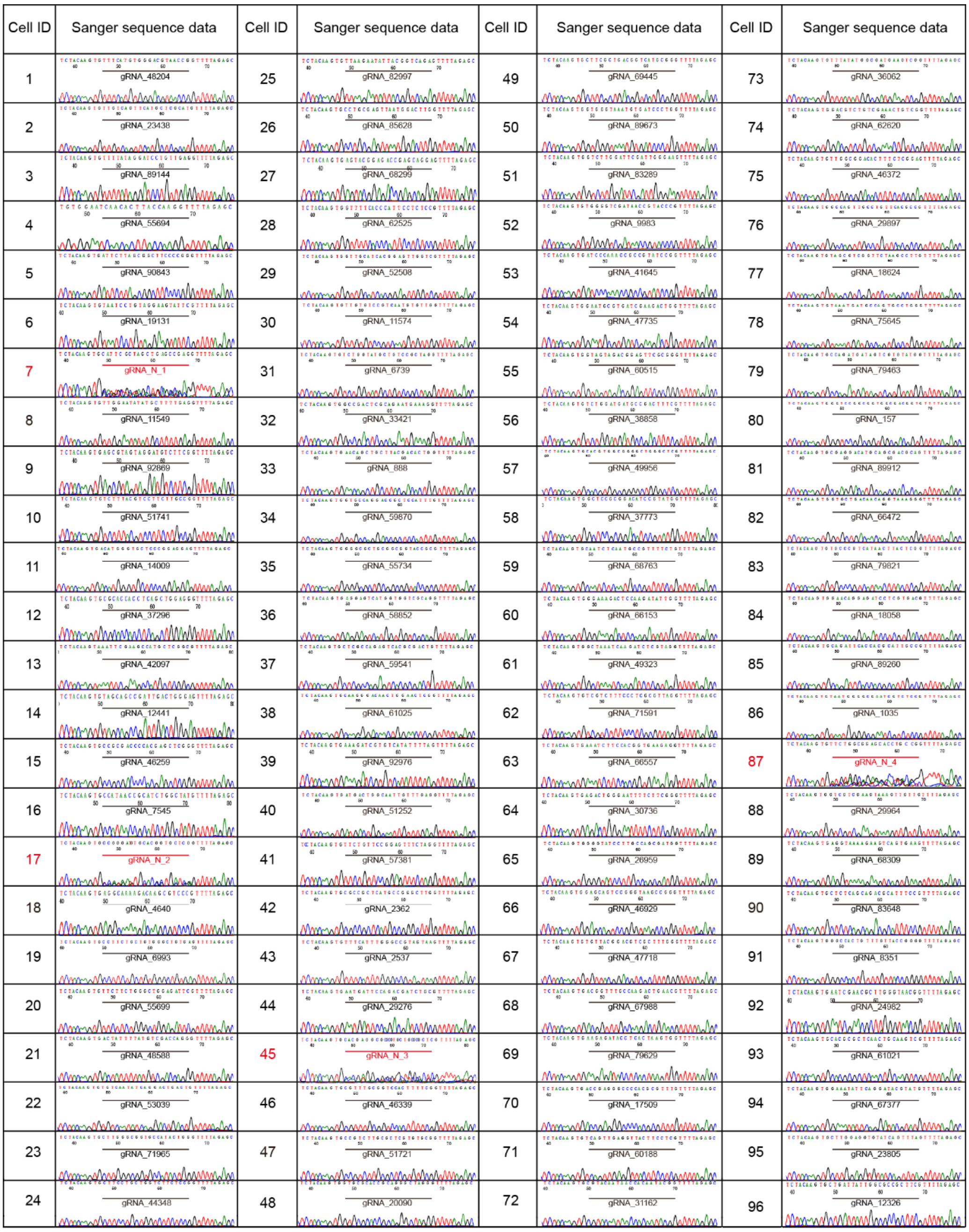
(A) GO classification of essential genes. **(B)** Top 20 KEGG pathway enrichment of growth-restricting genes.

Supplemental Fig S7



Supplemental Fig S7. Functional analysis of genes involved in *B. mori*-BmNPV interactions. Fluorescent images analysis (upper) and flow cytometry analysis (lower) of knockout cells infected with BmNPV at an MOI of 1. The gene numbers were indicated at the bottom.

Supplemental Fig S8



Cell ID	Sanger sequence data	Cell ID	Sanger sequence data	Cell ID	Sanger sequence data	Cell ID	Sanger sequence data
1	gRNA_48204	25	gRNA_82997	49	gRNA_69445	73	gRNA_36062
2	gRNA_23438	26	gRNA_85628	50	gRNA_89673	74	gRNA_62620
3	gRNA_89144	27	gRNA_68299	51	gRNA_83289	75	gRNA_46372
4	gRNA_55694	28	gRNA_62525	52	gRNA_9983	76	gRNA_29897
5	gRNA_90843	29	gRNA_52508	53	gRNA_41645	77	gRNA_18624
6	gRNA_19131	30	gRNA_11574	54	gRNA_47735	78	gRNA_75645
7	gRNA_N_1	31	gRNA_6739	55	gRNA_60515	79	gRNA_79463
8	gRNA_11549	32	gRNA_33421	56	gRNA_38858	80	gRNA_157
9	gRNA_92869	33	gRNA_888	57	gRNA_49956	81	gRNA_89912
10	gRNA_51741	34	gRNA_59870	58	gRNA_37773	82	gRNA_66472
11	gRNA_14009	35	gRNA_55734	59	gRNA_68763	83	gRNA_79821
12	gRNA_37296	36	gRNA_58852	60	gRNA_66153	84	gRNA_18058
13	gRNA_42097	37	gRNA_59541	61	gRNA_49323	85	gRNA_89260
14	gRNA_12441	38	gRNA_61025	62	gRNA_71591	86	gRNA_1035
15	gRNA_46259	39	gRNA_92976	63	gRNA_66557	87	gRNA_N_4
16	gRNA_7545	40	gRNA_51252	64	gRNA_30736	88	gRNA_29964
17	gRNA_N_2	41	gRNA_57381	65	gRNA_26959	89	gRNA_68309
18	gRNA_4640	42	gRNA_2362	66	gRNA_46929	90	gRNA_83648
19	gRNA_6993	43	gRNA_2537	67	gRNA_47718	91	gRNA_8351
20	gRNA_55699	44	gRNA_29276	68	gRNA_67988	92	gRNA_24982
21	gRNA_48588	45	gRNA_N_3	69	gRNA_79629	93	gRNA_61021
22	gRNA_53039	46	gRNA_46339	70	gRNA_17509	94	gRNA_67377
23	gRNA_71965	47	gRNA_51721	71	gRNA_60188	95	gRNA_23805
24	gRNA_44348	48	gRNA_20090	72	gRNA_31162	96	gRNA_12326

Supplemental Fig S8. A summary of Sanger sequencing results for single cells.

The four-color chromatogram of DNA sequencing results for each single cell is placed right to the cell ID. The chromatograms with single peaks and matched to the sgRNA sequences in the library are labeled with black line and sgRNA number in the library. The cell ID in red indicates that the single cell harbors more than one integrated sgRNAs.