

Supplementary information for

Spore-like Propagule: A Special Phenotype in Aconidial *Aspergillus niger* SH2 and Its Mechanism of Formation via CRISPRi

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Figure S4 The construction of CRISPRi on conidia and GlcNAc metabolic pathway genes in the *Aspergillus niger* SH2.

Table S1 The strains and plasmids used in this study.

Strain / Plasmid	Genotype / Marker	Source
<i>E. coli</i> Mach1 TM T1	Host of Molecular cloning	Invitrogen, USA
<i>A. niger</i> SH2	Wild type	A factory, China
<i>A. niger</i> CBS513.88	Wild type	CBS-KNAW, Netherlands
<i>A. niger</i> SH2 $\Delta ku\Delta pyrG$	$\Delta ku\Delta pyrG$	This study
<i>A. niger</i> SH2 $\Delta Dac1$	$\Delta ku\Delta pyrG \Delta Dac1::pyrG$	This study
<i>A. niger</i> SH2 <i>Dac1</i> recovery	$\Delta ku\Delta pyrG$	This study
<i>A. niger</i> SH2 CRISPRi- <i>Dac1</i>	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; <i>Dac1</i> -sgRNA ORF: <i>hygB</i>	This study
<i>A. niger</i> SH2 CRISPRi- <i>Uap1</i>	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; <i>Uap1</i> -sgRNA ORF: <i>hygB</i>	This study
<i>A. niger</i> SH2 CRISPRi- <i>Ngt1</i>	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; <i>Ngt1</i> -sgRNA ORF: <i>hygB</i>	This study
<i>A. niger</i> SH2 CRISPRi- <i>Hxk1</i>	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; <i>Hxk1</i> -sgRNA ORF: <i>hygB</i>	This study
<i>A. niger</i> SH2 CRISPRi- <i>hfbD</i>	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; <i>hfbD</i> -sgRNA ORF: <i>hygB</i>	This study
<i>A. niger</i> SH2 CRISPRi-GC	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; GC-sgRNA ORF: <i>hygB</i>	This study
<i>A. niger</i> SH2 CRISPRi-MAPK1	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; MAPK1-sgRNA ORF: <i>hygB</i>	This study
<i>A. niger</i> SH2 CRISPRi-dCas9-Mxi1	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i>	This study
<i>A. niger</i> SH2 CRISPRi-dCas9	$\Delta ku\Delta pyrG$ dCas9 ORF-AMA1: <i>pyrG</i>	This study
<i>A. niger</i> SH2 CRISPRi-non spacer RNA	$\Delta ku\Delta pyrG$ dCas9-Mxi1 ORF-AMA1: <i>pyrG</i> ; non spacer -sgRNA ORF: <i>hygB</i>	This study

Table S2 The semi-quantitative RT-PCR system.

Green taq mix (Vazyme, China)	10 μ L
cDNA	1 μ L
Primer-F	1 μ L
Primer-R	1 μ L
ddH ₂ O	7 μ L
Total	20 μ L

Cycles: 27

Table S3 The primers used in this study.

Primer name	Primer sequences (5'→3')
Primers used for qPCR	
<i>Dac1</i> -qPCR-F	GACGGGTGTGACTTCATACT
<i>Dac1</i> -qPCR-R	AGTGAGGTTCTCTTTGCCATA
<i>Uap1</i> -qPCR-F	TTTCCAGCAGCACAACTACTT
<i>Uap1</i> -qPCR-R	AGTAGGTGTGAATGTGCTGG
DC-qPCR-F	CACTCATATCCTTGCCGTTT
DC-qPCR-R	ATTCCATTAGTCTCGCAGCAA
GC-qPCR-F	GAAACTTCGCCAACCGCTC
GC-qPCR-R	TCCTCAGGTTTCATCTTCATCA
<i>hxaA</i> -qPCR-F	CCTACCCCGCCACCCAG
<i>hxaA</i> -qPCR-R	GTGTAAGAAGAGGCGATGAG
<i>Ng1</i> -qPCR-F	ACCTGGGTGGCGTGATTGG
<i>Ng1</i> -qPCR-R	GGTGTCGTAGAGACCTTTGA
MAPK1-qPCR-F	ATCCCGACGATAGACAGAGA
MAPK1-qPCR-R	GACATTGCCTCGTCGCTCA

Table S4 The sequences of the *dCas9-MxiI* ORF, common sgRNA cassette and *pyrG* sgRNA expression cassette.

1. The sequences of the <i>dCas9-MxiI</i> ORF
<p>Green: <i>Ptef</i></p> <p>Black: linker</p> <p>Orange: SV40 NLS</p> <p>Blue: <i>dCas9</i></p> <p>Purple: <i>MxiI</i></p> <p>Red: <i>Ttef</i></p> <p>cgagacagcagaatcaccgccaagttaagcctttgtgctgatcatgctctgaacgggccaagttcgggaaaagcaaaggagcgtttagtg aggggcaatttgactcacctcccaggcaacagatgaggggggcaaaaagaaagaaatttctgtagtcaatatggattccgagcatcattttct tgcgtctatcttgctacgtatgttgatcttgacgtgtggatcaagcaacgccactcgtcgtccatcgcaggtggtcgcagacaaattaa aggcgggcaactcgtacagccgcggggtgtccgtgcaaagtacagagtataaaagccgcatcgcaccatcaacgcgttgatgccca gctttttgatccgagaatccaccgtagaggcgatagcaagtaaaagaaagctaaacaaaaaaaaatttctgccctaagccatgaaaacgag atggggtggagcagaaccaaggaagagtcgcgtgggtgctccgtccggaaggtgtttaaaggctcgacgccaaggtgggagcttag gagaagaatttgcacgaggagtgaggcggttaccctccatatccaatgacagatatctaccagccaagggttgagccccccgcttagtc gtcgtcctcgttgccctccataaaaggatttccctccctccacaaaatttcttccctcctccttctgctcgttcagtactatatttcc cttccctcgttctcctccatcttcttccatccatctcgtcctaacttctcgtcagcacctctacgcattactagccgtagtatctgagcacttct cccttttatattccacaaaacataacacacacttcaccggccattaccaagaaaaagcgaagggtgGGGCCCatggacaagaagtatag catcgggctggctatttgaacgaactcgggtggtgggtgtgattacggacgaatacaaggtgccatccaagaagttaaggtcctgggaaa caccgaccgtcactcaatcaagaagaatctcattggagccctgctctcgtatgtgggagaccgccgaagctactcactgaagcgaacgg ctcgcggcggtatatacagcgaagaatcgcatctgctacctccaggagatttccagcaacgaaatggctaaggtgatgactatttctcat cgactcgaagaaagtcttctggtcaggagagataagaagcacgagcgccatccgatcttggtaacattgtggatgaggttgccatcacgaa aagtaccaactatctatctctgtaagaagctggtcgtatgacgagcaggaaggctgatttgcgacttatctacctggcactcgcgcacatgat taagttccgcggccatttctatcgaggggtgacctgaacccgataattctgacgttgataagctcttcatccagttggtccaaacctacaatcag ctgtttgaggaaaacctattaatgcatctggcgtggacgccaaggctatccttccgcgcgctgtctaagtcgcgcgcttggagaaccttat cgcacaactccccggcgaagaaagacggcctcttcggttaatttgattgctgtcacttggtcgtgactcctaactcaagagtaattttgacct ggcagaggatgcgaagctccagttgtctaaggatagctatgatgacgatctcgacaacttgcttgcccaaatcggtgaccagtacgctgatctt ttcctggccgctaagaatctctcagatgcaatcctgctcagtgacattttcggggtcaacaccgagattactaagccccctgtcagctagat gatcaagcggatgatgagcaccatcaggacctcacctgcttaaggccctcgtgcgtcagcaattgcctgagaagtacaaggaaatcttctt gaccaatccaagaacggatacgcagggtatattgatggcggtgcgagccaggaggaattctacaagtttatcaagccgattttggagaagatg gacggcactgaggaactgctcgtcaagctgaatcgcaagatttgctcgttaagcaacgaacgttcgacaacggctccatcccgcaccagat tcacttgggcgagctccacgccatcttcgacgcagggaagatttctaccatttctgaaggacaaccgtgagaagatcgaagattcttaca ttccgaatcccctactatgtgggacctttggccggtgggaattcccatttgcgttgatgaccgaaagagcggaggaaaccatcactccgtgga acttcgaggaagtcgtggacaagggtgcatccgcgagagcttcattgagcggatgaccaatttataagaacctccgaatgaaaagggtcc tgccaaagcattcgtcgtctacgagatttaccgtgtataacgaactgactaagggtcaagtacgtgacggagggaatgcgggaagccagcct tccttcagggggaacaaaagaaggctatcgtgatttgccttttaagaccaatcgtaaaagtactgttaagcagctgaaggaggattatttcaag aagattgaatgttgcactccgtcagatcagcggcggtggaagatcgctttaacgcttccctcggtaacctaccacgacctgctcaagatcattaa ggacaaggatttctcgataacgaggaaaatgaggacatcttgaagatattgtcctcacgttgacacttttgaggaccgcgaaatgatcgag gaacggctcaagacatatgccatttgcgtacgataaggtgatgaagcagctgaagcggcgctgataccggatgggggtcgcttagccg gaagctgatcaacggcattcgagataagcaatctggttaagctatcttgatttcttaagtcggacggcttcgccaaccgcaatttatgacgt tattcacgacgattccctgacgttcaaggaggacatccagaaggcacaagctcaggacaaggggattccctgcacgagcatatcgccaacc</p>

tggtggtatccccggcgatcaagaaggggattctcagaccgtcaaggtgtcgacgagctggtaaggtgatggccgtcataagccaga
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gaagtatggagggttcgatttctctacagtggcatactcgggttcgtgtcggaaggttgagaagggaagtctaagaagctgaagtcggtc
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caccacaacgcgtttatcaacctcaactttcaacaacctcatgccacaaaagcgcgtgtaaaacaaagcgagatttgattgagcaagagggca
ggatccaatgcgcgattcaagacattaaaaatggaaaatttcaaaaaattgcgcccgcagcgcgtgcatacaaaattcatccaatac

2. The sequences of the common sgRNA cassette

Light green: *aamA* upstream homologous arm

Green: *A. fumigatus* U6 promoter

Black: linker

Orange: *Not I* restriction enzyme cutting site

Blue: sgRNA scaffold

Red: *A. oryzae* U6 terminator

Purple: *hygB* cassette

Wathet: *aamA* downstream homologous arm

TGATAGTATCCGAAAGCTGCAAATTGCTTCATCGAGGCTGGCATTTCGATAGAAGAAAG
AATTATAGACAACTAGTCTTGCAATATGACAATTCTCTTTGATTAATAAATGAAAGCACG
CATGTATCAGCCTAATAGCCGAGTGGCGGGCATCTCTGGCGGCCTCCCGAGCAGCGTG
GAATGCGTCCAAGATCCCGTCCGCGGGTCGTCTTCGGTCGGAATGATGACTGGAGCA
GCAGACGATGTCTTGAGCTGAATGCATGTGATATTACATTCCAGGGAGAATTGTCGGC
TATTTAGAACCCTCTCGGCTTAAAAGCCCTATTAGACTATGGGTGCGCTCAAGCCACTA
GCCAGGATATCCCGCTGAACGCTCCATCACCTTGACGCTGAAGTGCAACATGGGACGG

GCTTTAACTTTTCGTAGATATAAGTTTAATTTATCCTCTCCACACCCATAGGGTCGTATGG
TGTC AACCGGTGTAGTCTGCAGGATTTTCATCTCGCTTCGCCAAGCGAGGGCGCCCTAAC
GGGCAGCCTGCAGCTTACCCTGTTAACCCCGGCTCACCACCCCCGAGCAATCCGTCG
CGTCCTCCACGAGTCATAACAAGGTTCTGGGCGTTGTTTCTTACCCCCACTATCAGGCGT
ATTCAGTTAACAGTCAGTAGTCCCGTGTCTGGAGATTTGTTGTTCTGCAACAATTAAAGG
GGACCAGGGTTAAATCCTGGCCCCCGAACTGATCGGAGTTTCGGCCAATGAGAGATGT
TGTATACCCCCGTTTCTGGCAGATGGATTAATTGCCGGCTCCATTTGGCATCCATCAAGC
ATCATACGGGATTAGAAGGGTAGTTCGTGGGTTGATCTGCCGTGCAAGGTGCTCAAGG
CTCTGGAGTCATGCTGAACGCAAATATTTAAGAATCGTCGTCAGGGACAGCGTTTCTCTG
GATAGTCAAGCTGTGCTTGGGACGCTGTTCTGTCTGCTTTGTCAAAACATAATTTCGACG
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TACCCGGGGATCCgctagtggaggtcaacacatcaatgctattttggtttagtcgtccaggcggatcacaaaattgtgtcgttgaca
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gcggttaaatagctgcgccgatggtttctacaaagatcgttatgttatcggcactttgcatcgccgcgctcccgattccgggaagtgttgaca
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GGCTACCAGGAAGCAGCAGGTGTCTACTGCGTCGGCGAAGTCGACAACGGCAACCCT
GCCCTCGACTGCCCATACCAGAAGGTCTTGGACGGCGTCCTCAACTATCCGATGTACAT
CCCCCTATACATTGTTTCATTAGATCTTCGCTAACTCCAACCAGCTACTGGCAACTCCTCT
ACGCCTTCGAATCCTCCAGCGGCAGCATCAGCAACCTCTACAACATGATCAAATCCGTC
GCAAGCGACTGCTCCGATCCGACACTACTCGGCAACTTCATCGAAAACCACGACAATC
CCCGTTTCGCCTCGTATGTCCCACCCCCCTCCCCTCCCTACAATCACACTCACTAATACAT
CTAACAGCTACACCTCCGACTACTCGCAAGCCAAAAACGTCTCAGCTACATCTTCCTC
TCCGACGGCATCCCCATCGTCTACGCCGGCGAAGAACAGCACTACTCCGGCGGCAAGG
TGCCCTACAACCGCGAAGCGACCTGGCTTTTACGGCTACGACACCTCCGCAGAGCTGTA
CACCTGGATAGCCACCACGAACGCGATCCGCAAACTAGCCATCTCAGCTGACTCGGCC
TACATTACCTACGCGGTTTCGTCTTCCCTCCCACCCCTTTACCCCCCACCCTACAAACATC
CCACATACTAACAACATTTCAATAATGAAATAGAATGATGCATTCTACACTGACAGCAA

CACCATCGCAATGCGCAAAGGCACCTCAGGGAGCCAAGTCATCACCGTCCTCTCCAAC
AAAGGCTCCTCAGGAAGCAGCTACACCCTGACCCTCAGCGGAAGCGGCTACACATCC
GGCACGAAGCTGATCGAAGCGTACACATGCACATCCGTGACCGTGGACTCGAGCGGC
GATATCCCCGTGCCGATGGCGTCGGGATTACCGAGAGTTCTTCTGCCCCGCGTCCGTCGT
CGATAGCTCTTCGCTCTGTGGCGGGAGCGGAAGATTATACGTCGAGTAA

3. The sequences of *pyrG* sgRNA expression cassette

Green: *A. fumigatus* U6 promoter

Black: linker

Orange: *pyrG* protospacer

Blue: sgRNA scaffold

Red: *A. oryzae* U6 terminator

gcaggcggttgcaagcgatctatcgtgaatttgtggaggacgtaatggtgaaatgccgaatggcgaagcaggctgggagatcggtggtcg
gacgagcctgatgtagatgggtgcatactttctttctttcacaattcaagatcatgtattagaactacattgctatgttcagcaatatatatgca
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aaaagaggtctgtaagaatgaaagTAAACTCTTCTCCATCGCCGGTTTTAGAGCTAGAAATAGCAAG
TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT
TTTGAGCATTATCAGCTTGATATAGAGGTAGGAATGTATGGAGGTGCAGAATGGCTATT
TTGTTATTGGAGCGGGTTCGAAACGGAGGGCAGGAGACTTTTTCTAAATACGTCACGT
GATATAGAGCTGCT

Table S5 The T7 transcription system of the protospacer RNA.

T7- protospacer- sgRNA scaffold fragment	10 μ L
T7 transcriptase	2 μ L
Buffer	10 μ L
RNase free water	8 μ L
Total	30 μ L

37°C , 16 h.

Table S6 The ethanol precipitation system of the RNA.

sgRNA product	30 μ L
Isopropyl alcohol	300 μ L
RNase free water	120 μ L
Total	450 μ L

-20°C, 2 h

Table S7 The NLS-Cas9 Nuclease cleavage of the target gene system in vitro.

NLS-Cas9 Nuclease	1 μ L
Buffer	2 μ L
Target gene DNA fragment	5 μ L
sgRNA product	2 μ L
RNase free water	10 μ L
Total	20 μ L

37°C, 6 h or overnight

Table S8 The quality control of the RNA samples.

Name of sample	Concentration	OD260/	OD260/	RIN/RQN	28S/18S	Result
	(ng/ μ L)	280	230			
G1	325	2.15	2.46	9.4	2.4	Qualified
G2	452	2.15	2.26	9.5	2.3	Qualified
N1	395	2.14	2.25	9.4	2.1	Qualified
N2	412	2.17	2.13	9.4	2.1	Qualified

Table S9 The expression of conidia-associated genes (CAGs) in the spore-like propagule and normal hypha of *Aspergillus niger* SH2.

Gene ID	Gene name/PFAMs	N FPKM	G FPKM	log ₂ (N/G)	P-value
An12g10720	<i>Cat3</i>	699.69	13.31	5.14	0.0000
An12g10710	Hemerythrin	330.76	16.47	3.75	0.0000
An03g02400	<i>HfbA</i>	17728.19	836.07	4.41	0.0000
An08g09880	<i>HfbD</i>	22884.13	0.00	16.66	0.0000
An15g07300	AAA_22,NB-ARC	606.17	2.69	7.24	0.0000
An17g01885	CHZ	1962.83	31.48	5.38	0.0000
An12g10240	<i>ConJ</i>	883.11	30.08	4.29	0.0000
An15g04670	DUF1295	761.49	9.60	5.73	0.0000
An14g04530	NmrA	398.41	84.82	1.65	0.0000
An03g00920	Epimerase	143.36	0.41	7.79	0.0000
An01g10950	DJ-1_PfpI,ThiJ_like	155.42	18.93	2.46	0.0000
An03g02190	<i>Hxt5</i>	213.22	15.65	3.19	0.0000
An15g04770	Pyridoxal_deC	117.28	10.72	2.87	0.0000
An09g03130	<i>CatA</i>	574.11	14.38	4.74	0.0000
An18g04120	-	579.79	2.91	6.99	0.0000
An11g08160	DUF2235	77.44	2.41	4.41	0.0000
An03g04860	<i>Nce102</i>	349.78	74.09	1.66	0.0000
An15g05990	adh_short_C2	204.54	50.12	1.45	0.0000
An09g06270	-	341.26	5.09	5.48	0.0000
An08g06600	-	178.92	3.42	5.11	0.0000
An01g00280	HAD	107.11	9.28	2.95	0.0000
An02g07350	-	1221.27	159.91	2.35	0.0000
An09g05520	-	228.88	18.76	3.03	0.0000
An11g01750	<i>PilB</i>	101.60	9.01	2.92	0.0000
An02g08740	DUF3292	122.42	5.11	4.00	0.0000
An14g06050	-	683.00	135.41	1.76	0.0000
An08g03030	Lactamase_B_2	53.92	0.75	5.54	0.0000
An08g06620	-	1481.00	46.47	4.41	0.0000
An09g01150	adh_short	292.26	20.23	3.28	0.0000
An16g04420	<i>Ish1</i>	436.62	146.62	1.00	0.0000
An11g06120	<i>GndB</i>	57.25	2.22	4.10	0.0000
An04g04280	DUF1772	260.04	21.23	3.03	0.0000
An04g00100	DUF1761	2478.41	649.49	1.35	0.0000
An18g01860	<i>Fhk1</i>	55.55	9.47	1.97	0.0000
An08g08500	DUF3140,Med22	80.69	2.07	4.65	0.0000
An07g03930	Ferritin_2	512.07	6.67	5.68	0.0000
An04g05790	<i>VosA</i>	79.66	5.85	3.18	0.0000
An14g07380	ADH_zinc_N_2	14.37	0.58	3.98	0.0013
An01g05320	Abhydrolase	229.33	34.01	2.18	0.0000

An11g02200	<i>HppD</i>	217.06	21.50	2.76	0.0000
An12g01460	Bac_rhodopsin	556.33	30.36	3.61	0.0000
An04g09030	- <i>SfcI</i>	187.97	18.01	2.80	0.0000
An16g05030	-	1927.62	162.99	2.99	0.0000
An07g06530	<i>Sur7</i>	711.33	32.00	3.89	0.0000

Table S10 The expression of germination-associated genes (GeAGs) in the spore-like propagule and normal hypha.

Gene ID	Gene name	N FPKM	G FPKM	log ₂ (N/G)	P-value
An16g03330	<i>Bys1</i>	2098.74	802.17	0.81	0.0000
An04g02550	<i>Mef1</i>	70.08	34.82	0.43	0.1667
An04g02000	<i>Ytm1</i>	39.35	15.84	0.74	0.1139
An12g08230	<i>Mrs2</i>	20.86	32.92	-0.66	0.0121
An01g10790	S	15149.26	3.60	12.04	0.0000
An08g03290	<i>Nop52</i>	28.58	12.26	0.64	0.2106
An01g03230	SRP40_C	41.69	30.96	0.43	0.7890
An01g04590	<i>Nat10</i>	30.89	15.94	0.38	0.4410
An16g04970	<i>Cpa2</i>	127.01	94.55	0.43	0.6514
An04g01900	<i>Utp13</i>	33.57	8.40	1.42	0.0118
An15g06390	<i>Rrp5</i>	30.38	14.84	0.46	0.3512
An16g05290	<i>Fcy21</i>	0.60	1.66	-1.48	0.3644
An13g01230	zf-LYAR	40.33	60.69	-0.59	0.0010
An09g06310	<i>Rrp15</i>	24.56	9.38	0.81	0.1537
An04g00680	<i>Nop15</i>	89.00	43.08	0.47	0.1030
An11g10000	<i>Ipi3</i>	16.88	5.13	1.13	0.1695
An07g06640	<i>Grc3</i>	14.51	3.22	1.59	0.1027
An11g06110	<i>Meu1</i>	116.12	64.49	0.27	0.2484
An14g01560	<i>Zuo1</i>	190.47	146.52	0.38	0.4209
An08g09160	<i>Utp10</i>	38.73	17.46	0.57	0.1915
An02g14340	<i>Snu13</i>	347.87	272.73	0.35	0.1931
An02g09200	<i>Rps0</i>	690.43	329.60	0.49	0.0000
An01g14080	<i>Rps22</i>	891.54	686.11	0.38	0.0556
An04g09270	<i>Utp8</i>	42.76	18.71	0.62	0.1706
An02g03860	<i>Rrp12</i>	37.37	19.24	0.38	0.3411
An18g04470	<i>Tbfl</i>	22.59	3.745	1.99	0.1027
An17g01270	<i>Mrpl7</i>	100.17	54.16	0.31	0.2409
An07g06760	<i>Rps20</i>	1230.21	797.74	0.62	0.2337
An15g01160	<i>Ecm16</i>	13.83	4.82	0.94	0.2966
An18g04220	<i>Pet9</i>	2450.64	2298.74	0.09	0.0000
An08g03910	FMO-like,Pyr_redox_3	1434.63	1198.21	0.26	0.0000
An16g07400	<i>Mtd1</i>	109.15	68.72	1.59	0.5989
An02g06530	<i>Chu1</i>	79.01	72.43	0.13	0.1506
An02g07010	<i>Ubi3</i>	1437.33	1151.16	0.32	0.0014
An02g08080	<i>Rpl22</i>	613.64	555.79	0.14	0.0000
An14g06860	<i>Oac1</i>	75.65	51.19	0.56	0.9174
An11g09740	<i>Ipi3</i>	26.22	7.24	1.28	0.0562
An11g11150	<i>Utp22</i>	22.06	6.36	1.22	0.0862
An11g00990	<i>Lag1</i>	86.14	31.09	0.89	0.0076

An11g05510	<i>Fpr3</i>	167.48	115.35	0.54	0.9981
An02g06320	<i>Erg13</i>	237.14	87.53	0.86	0.0000
An01g08850	<i>Cpc2</i>	1023.66	583.26	0.23	0.0009
An09g03120	GPI-anchored	67.62	13.47	5.02	0.0000
An11g06810	<i>Mrpl6</i>	58.94	42.11	0.49	0.9043
An07g07840	<i>Rpl23A</i>	659.48	424.41	0.64	0.3578

Table S11 The expression of genes involved in the production of enzymes involved in cell wall synthesis or processing.

Gene ID	Gene name	N FPKM	G FPKM	log ₂ (N/G)	P-value
An14g05350	<i>AygI</i>	501.47	0.60	9.71	0.0000
An09g05730	<i>FwnA</i>	386.86	6.00	6.01	0.0000
An03g03750	<i>McoC</i>	55.32	36.32	0.61	0.8422
An03g02360	<i>HfbB</i>	16927.17	451.67	5.23	0.0000
An04g08500	Hydrophobin	208.53	11.41	4.19	0.0000
An12g05020	<i>HfbE</i>	63.72	207.81	-1.71	0.0000
An07g03340	<i>HypI</i>	150.61	22.83	2.72	0.0000
An09g06400	<i>CtcA</i>	14.82	13.11	0.18	0.6398
An04g01430	SAP	786.88	1024.45	-0.38	0.0000
An06g01000	CFEM	221.77	937.58	-2.08	0.0000
An07g05570	<i>ChsA</i>	53.69	55.22	-0.04	0.0849
An09g04010	<i>ChsB</i>	158.37	107.73	0.56	0.9117
An12g10380	<i>ChsF</i>	28.14	8.41	1.74	0.0065
An02g02340	<i>ChsL</i>	42.62	140.55	-1.72	0.0000
An02g02360	<i>ChsM</i>	24.41	89.49	-1.87	0.0000
An09g02290	<i>ChsD</i>	25.94	27.15	-0.06	0.2171
An08g10740	DLH	109.61	140.01	-0.35	0.0170
An12g09130	DLH	171.55	86.32	0.99	0.0000
An06g01550	<i>FksA</i>	142.07	106.305	0.42	0.0491
An17g02120	<i>SmiI</i>	84.49	104.34	-0.30	0.5972
An09g03070	<i>AgsE</i>	90.61	39.04	1.21	0.0011
An04g09890	<i>AgsA</i>	7.37	6.34	0.22	0.0001
An11g07660	<i>ExgD</i>	21.16	10.04	1.07	0.6912
An03g05290	<i>BgtB</i>	1027.95	549.34	0.90	0.4602
An07g04650	<i>BgtC</i>	151.93	54.40	1.48	0.0000
An19g00090	LysM,Pectate_lyase_3	1.52	1.39	0.13	0.0003
An16g06800	<i>EglB</i>	74.52	45.39	0.72	0.8607
An03g06220	Glyco_hydro_72,X8	55.86	6.01	3.22	0.5830
An16g02850	Glyco_hydro_16	27.78	13.53	1.04	0.0000
An01g11010	<i>CrhD</i>	120.85	43.99	1.46	0.3482
An07g01160	<i>CrhC</i>	4.14	29.43	-2.83	0.0017
An07g07530	<i>CrhB</i>	38.38	43.09	-0.17	0.0690
An08g03580	<i>BgtI</i>	2301.31	7.81	8.20	0.0000
An10g00400	<i>GelA</i>	532.66	403.66	0.40	0.2242
An16g07040	<i>BtgE</i>	26.48	67.87	-1.36	0.0002

Table S12 The expression and spacer RNA sites of genes involved in the N-acetyl-D-glucosamine metabolic pathway and conidia.

Gene ID	Name	Log ₂ (N/G)	Spacer RNA sequence (5'-3')	Description
An16g09020	<i>Ngf1</i>	2.22	GTATGTGC AGGTGTAA ACAG	N-acetylglucosamine transmembrane transporter activity
An13g00510	<i>Hxk1</i>	3.23	GTGCAAG ACCTTGAC CAATG	Hexokinase
An16g09040	<i>Dac1</i>	1.96	GAAGTGTC TGAACCTAC TAAG	N-acetylglucosamine-6-phosphate deacetylase activity
An12g00480	<i>Uap1</i>	0.12	ACAAGGG TGTGAAAG CGCGG	UDP-N-acetylglucosamine pyrophosphorylase
An08g09880	<i>HfbD</i>	16.66	GGATGATA GAAAACTT CAGG	expressed in dormant conidia
An01g10790	GC	12.03	GGTGACAT TATGACTT CATG	expressed in germinating conidia
An06g02240	H-MAPK	14.58	CCCTGTTC ATTGCTCC AAAA	MAPK signaling pathway

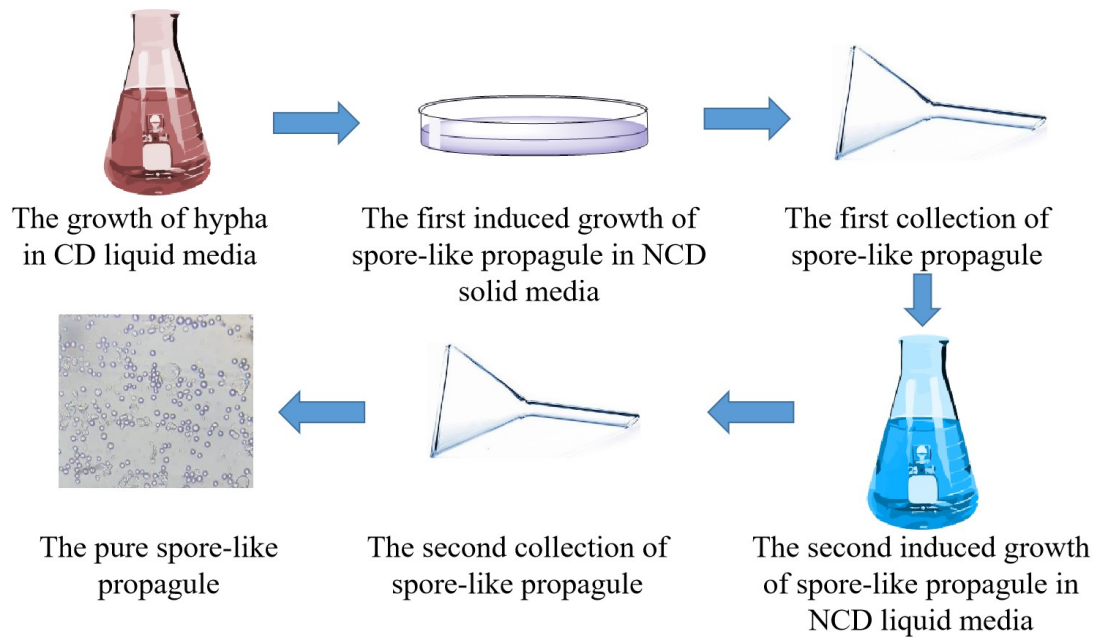


Figure S1 The flowchart of spore-like propagule purification.

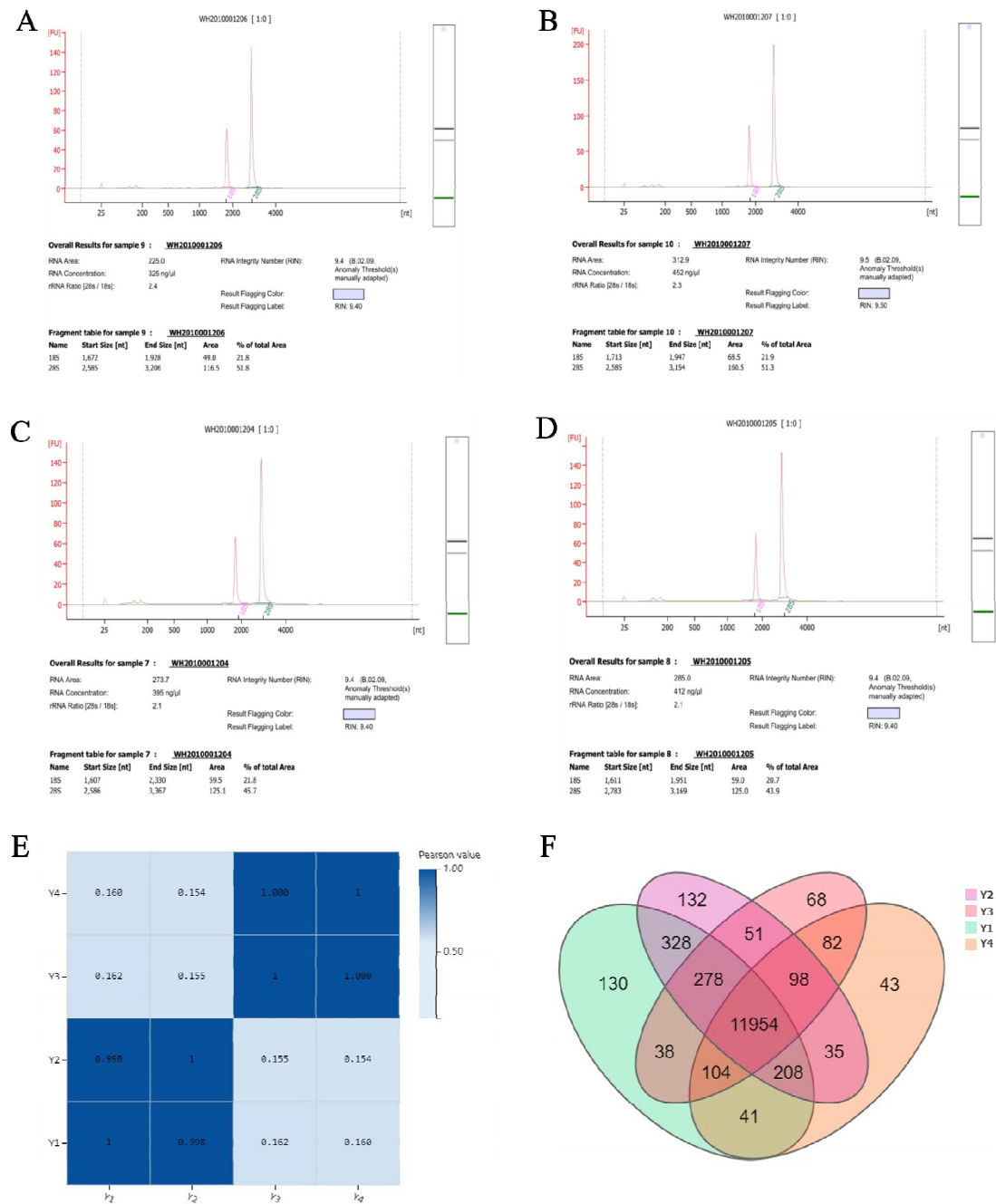


Figure S2 The RNA sequence of spore-like propagule. (A) The result of sample Y-1 (N1) RNA quality was assessed with Agilent 2100. (B) The result of sample Y-2 (N2) RNA quality was assessed with Agilent 2100. (C) The result of sample Y-3 (G1) RNA quality was assessed with Agilent 2100. (D) The result of sample Y-4 (G2) RNA quality was assessed with Agilent 2100. (E) The heat map of correlation between the samples. (F) The venn diagram of expression level between the samples.

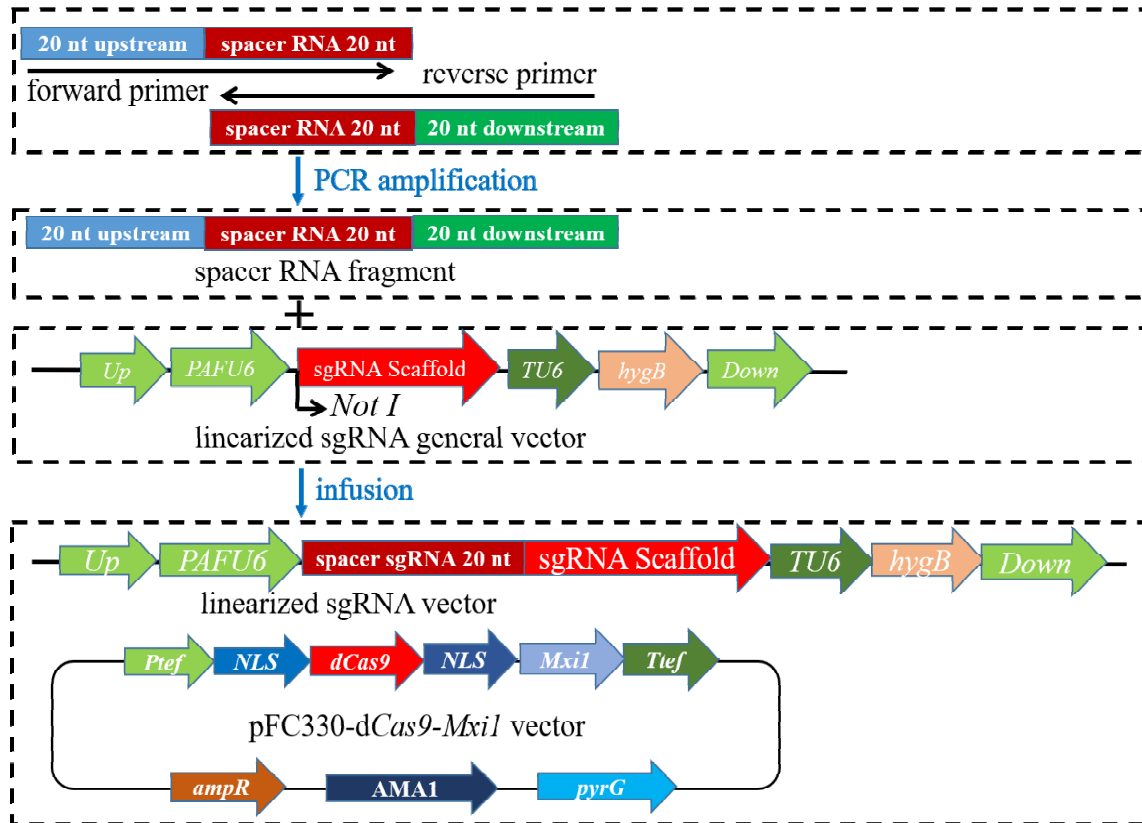


Figure S3 The flow chart of CRISPRi construction on conidia and GlcNAc metabolic pathway genes. The short spacer RNA fragment (total 60 bp) with 20 bp upstream homologous arm, 20 bp spacer and 20 bp upstream homologous arm was obtained by PCR amplification without template DNA. And the short spacer RNA fragment was connected to linearized sgRNA general vector using infusion method. The sgRNA general vector was linearized by *NotI* enzyme.

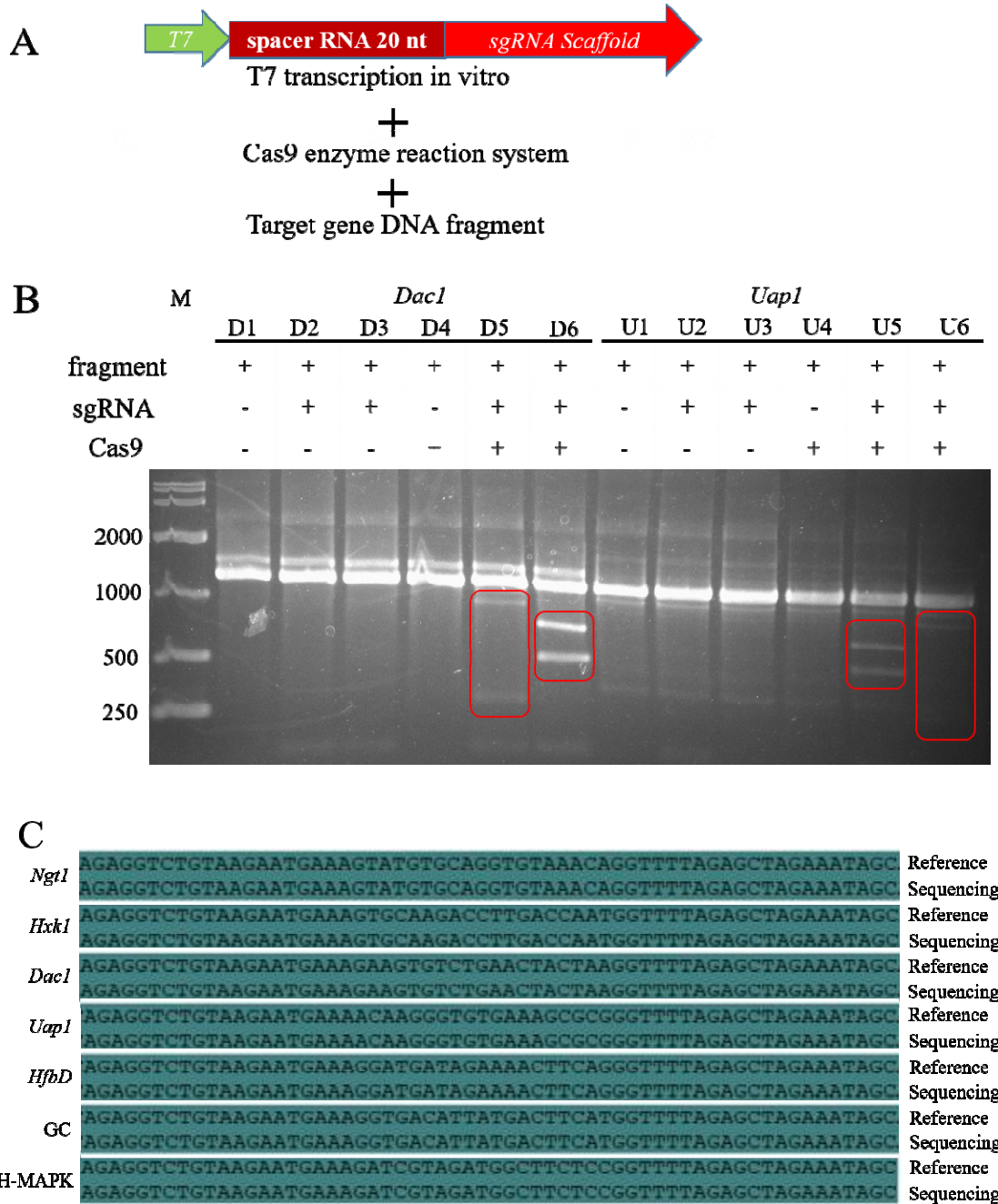


Figure S4 The construction of CRISPRi on conidia and GlcNAc metabolic pathway genes in the *Aspergillus niger* SH2. (A) The T7 transcription of sgRNA in vitro. (B) The Cas9 digestion of a 1200 bp DNA fragment with each sgRNA variant in vitro. (C) The sequencing and alignment of spacer RNA sequence that was connected into linearized sgRNA general vector using infusion method.