

Table S1 Strains used in this study

Strains	Characteristic(s)	Source
<i>Aspergillus</i> sp. SCSIO SX7S7	Wild-type strain, depsidones producing	Ref [24]
<i>Spiromastix</i> sp. SCSIO F190	Wild-type strain, spiromarmycin producing	Ref [25]
Δ pyrG-7S7	Δ pyrG::hph	This study
7S7- Δ depD- Δ pyrG	Δ pyrG::hph; Δ depD::afpyrG	This study
7S7- Δ depH- Δ pyrG	Δ pyrG::hph; Δ depH::afpyrG	This study
7S7- Δ depH	Δ pyrG::hph; Δ depH	This study
F190- Δ spm11- Δ amdS	Δ spm11:: Δ amdS	This study
F190- Δ spm11	Δ spm11	This study

Table S2 Plasmids used in this study

Plasmids	Characteristic(s)	Source
pBSKII-toCas9-hph	pBSKII-PtrPC-Flag-toCas9-TtrPC containing the hph cassette	Ref [29]
pFC330	sgRNA expression cassette	Ref [30]
pBluescript II SK plasmid	Plasmid for generating pyrG/amdS expression cassette	Ref [28]
pBSKII-toCas9-hph-sgRNA	Cas9, sgRNA and hph expression vector	This study
pBSKII-toCas9-pyrG-sgRNA	Cas9, sgRNA and pyrG expression vector	This study
pBSKII-toCas9-amdS-sgRNA	Cas9, sgRNA and amdS expression vector	This study

Table S3. Primers used in this study

Name	Sequence (5' to 3')	Experiment
pks-Ptrpc-F	atatcgaattcctgcagcccgcaattaaccctcactaa	Cloning of pyrG/amdS expression cassette
pks-Ptrpc-R	ttcgatgcttggttagaatagg	Cloning of pyrG/amdS expression cassette
amdS-F1	cctattctaccaagcatcgaaatgcctcaatcctggaagaac	Cloning of amdS expression cassette
amdS-R1	gctattaaatcactagaaggcactcctatggagtcaccacatttccc agc	Cloning of amdS expression cassette
pks-Ttrpc-an-F	gagtgccctctagtgatttaatatgctcc	Cloning of pyrG/amdS expression cassette
pks-Ttrpc-an-R	ctagaactagtggatccccggagcattcactaggcaacat	Cloning of pyrG/amdS expression cassette
pyrG-F1	gacttacctattctaccaagcatcgaaatgctgccaagtgcgaat tgac	Cloning of pyrG expression cassette
pyrG-R1	gctattaaatcactagaaggcactctcatgacttgccgcatactctg gcc	Cloning of pyrG expression cassette
psiI-DR-F	ggccgaaatcggaataatccctattataactaatcaagtttttggg gtcgaggtgccg	Cloning of pyrG/amdS expression cassette
DR-R	cctttgccagctggcgtaa	Cloning of pyrG/amdS expression cassette
DR-Ptrpc-F	ttacgccagctggcgaaaggcgcaattaaccctcactaa	Cloning of pyrG/amdS expression cassette
psiI-Ttrpc-R	ccctatctcggtctattcttttgattattataaggagcattcactaggc aacctagg	Cloning of pyrG/amdS expression cassette

7S7- <i>pyrg</i> -flag1-R	gacgagcttactcgtttcgtcctcacggactcatcaggtcggCcg gtgatgtctg	Cloning of sgRNA expression cassette
7S7- <i>pyrg</i> -flag2-F	acgagtaagctcgtcgtcggcggagacggtgacgtgttttagagc tagaaatagca	Cloning of sgRNA expression cassette
7s7- <i>pyrg</i> -test-F	ggctctcgcgcagacgacgc	Amplification of the DNA region surrounding the PAM site of <i>pyrg</i> -7S7
7s7- <i>pyrg</i> -test-R	gtttcatggggatcccacagtcagct	Amplification of the DNA region surrounding the PAM site of <i>pyrg</i> -7S7
7s7- <i>depD</i> -crisper- frag1-R	gacgagcttactcgtttcgtcctcacggactcatcagggaaggcg gtgatgtctgctcaa	Cloning of sgRNA expression cassette
7s7- <i>depD</i> -crisper- frag2-F	acgagtaagctcgtcgtcggagggttctgggacctgtgttttagagct agaaatagca	Cloning of sgRNA expression cassette
7s7- <i>depH</i> -crisper- frag1-R	gacgagcttactcgtttcgtcctcacggactcatcaggcgggcg gtgatgtctgctcaa	Cloning of sgRNA expression cassette
7s7- <i>depH</i> -crisper- frag2-F	acgagtaagctcgtcggcgggatccagggtgctgtgttttagagc tagaaatagc	Cloning of sgRNA expression cassette
7s7- <i>depH</i> -test-F	gctgtcaaacatcctgccca	Amplification of the DNA region surrounding the PAM site of <i>depH</i>
7s7- <i>depH</i> -test-R	aagcgccactccatctcgat	Amplification of the DNA region surrounding the PAM site of <i>depH</i>
7s7- <i>depD</i> -test-F	ctcacggtttatctcgtcga	Amplification of the DNA region surrounding the PAM site of <i>depD</i>
7s7- <i>depD</i> -test-R	aagatgcagcgcacacagac	Amplification of the DNA region surrounding the PAM site of <i>depD</i>
<i>amdS</i> - <i>pyrG</i> -DR- test-F	ccaataggccgaaatcggcaaaatccc	Testing the integrity of <i>pyrG/amdS</i> expression cassette
<i>amdS</i> - <i>pyrG</i> -DR - test-R	gactgggaaaaccctggcgttacccaactta	Testing the integrity of <i>pyrG/amdS</i> expression cassette
f190- <i>spm11</i> - crisper-frag1-R	gacgagcttactcgtttcgtcctcacggactcatcagggttagcggt gatgtctg	Cloning of sgRNA expression cassette
f190- <i>spm11</i> - crisper-frag2-F	acgagtaagctcgtcgttagcatggcgcttgagggttttagagct agaaatagc	Cloning of sgRNA expression cassette
<i>spm11</i> -crisper- test-F	tatgagtcagtgctcacgc	Amplification of the DNA region surrounding the PAM site of <i>spm11</i>
<i>spm11</i> -crisper- test- F1	ggttagcatggcgcttgagg	Amplification of the DNA region surrounding the PAM site of <i>spm11</i>
<i>spm11</i> -crisper- test-R	cattagcaagctcatccggc	Amplification of the DNA region surrounding the PAM site of <i>spm11</i>
ITS1	tccgtaggtgaacctgcgg	Amplification of the ITS region
ITS4	tctccgcttattgatatgc	Amplification of the ITS region

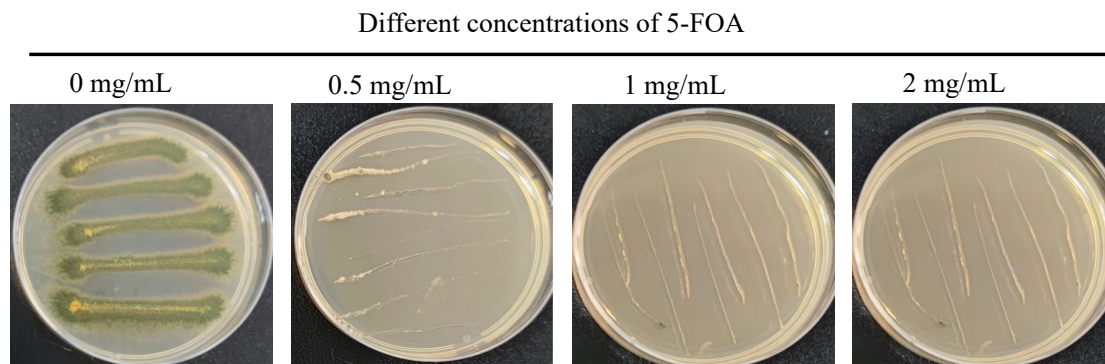


Figure S1. Growth of *Aspergillus* sp. SCSIO SX7S7 on PDA plate with different concentrations of 5-FOA. The spores of *Aspergillus* sp. SCSIO SX7S7 were streaked on the PDA plate with different concentrations of 5-FOA and incubated at 28°C for 7 days.

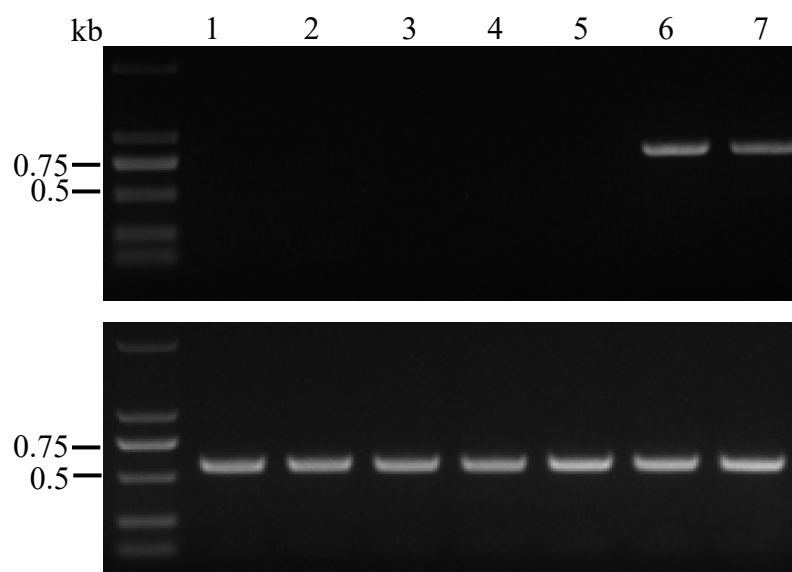


Figure S2. Verification of the $\Delta depH$ mutants by genotyping PCR. Upper panel: DNA amplification of the DNA region surrounding sgRNA binding site of the six clones picked out from the regeneration plate using primers flanking the PAM site in *depH* gene; Lower panel: Checking the DNA template quality of the tested samples using primers flanking the ITS region. PCR amplification with the same primers of DNA from the wild type strain served as positive controls. (Lane 1–6: tested transformants; lane 7: wild-type strain.)

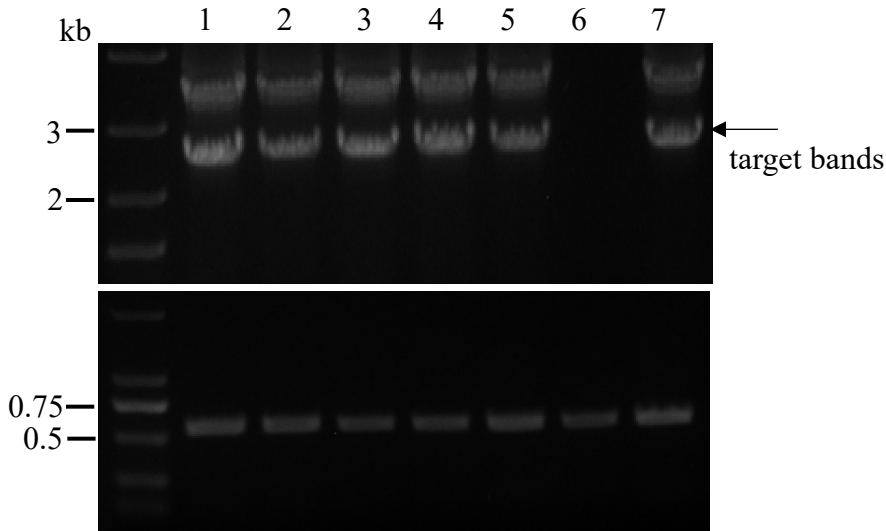


Figure S3. Verification of the integrity of DR flanked *Afp_{pyrG}* expression cassette. Upper panel: PCR was carried out using the primers target the complete DRs flanked *Afp_{pyrG}* marker expression cassette. Lower panel: the DNA template quality of tested samples was validated using primers flanking the ITS region. The wild type strain was set as negative control and plasmid containing DRs flanked *Afp_{pyrG}* marker was set as positive control. (Lane 1-5: five independent $\Delta depH$ mutants; Lane 6: negative control; Lane 7: positive control.)

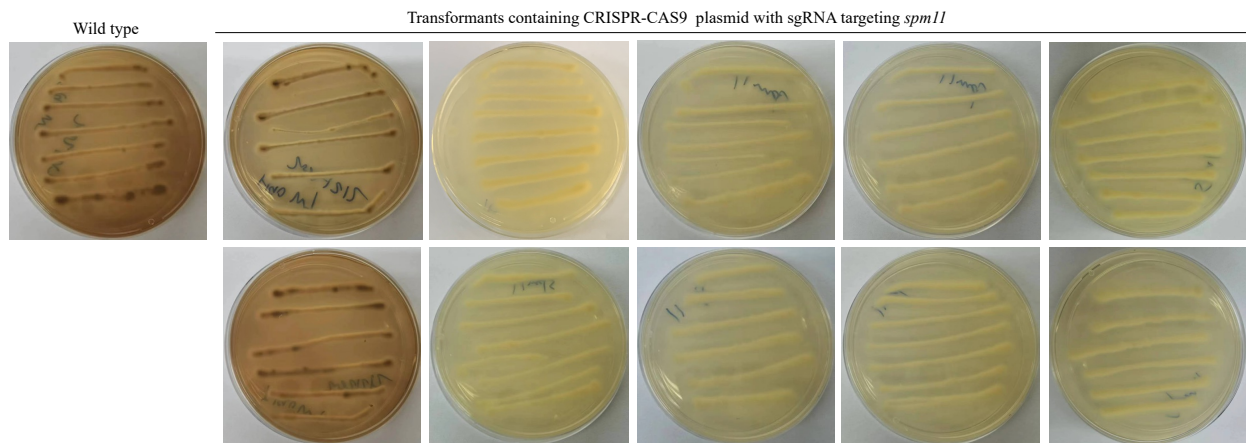


Figure S4. Growth of *Spiromastix* sp. SCSIO F190 strains. The wild type strain and transformants containing CRISPR-CAS9 plasmid with sgRNA targeting *spm11* were grown on PDA for 10 days.

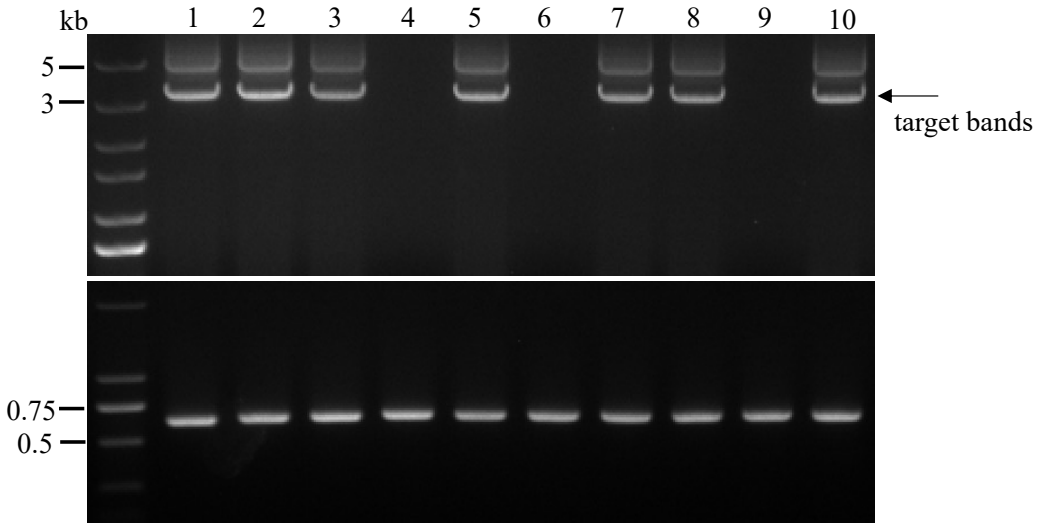


Figure S5. Verification of integrity of DR flanked *AnamdS* expression cassette. Upper panel: PCR was carried out using the primers target the complete DRs franked *AnamdS* marker expression cassette. Lower panel: DNA template quality of tested samples was validated using primers flanking the ITS region. The wild type strain was set as negative control and plasmid containing DRs franked *AnamdS* marker was set as positive control. (Lane 1-8: eight independent $\Delta spm11$ mutants; Lane 9: negative control; Lane 10: positive control.)