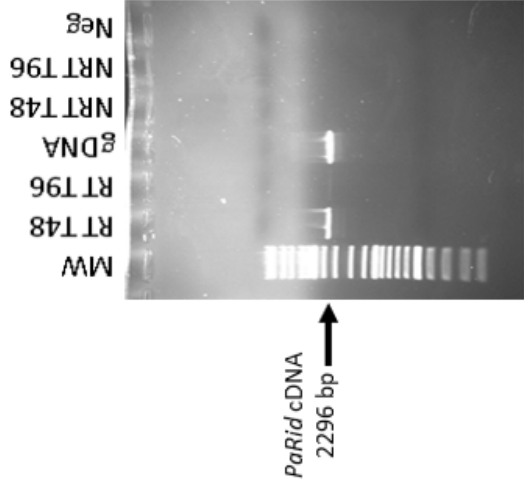
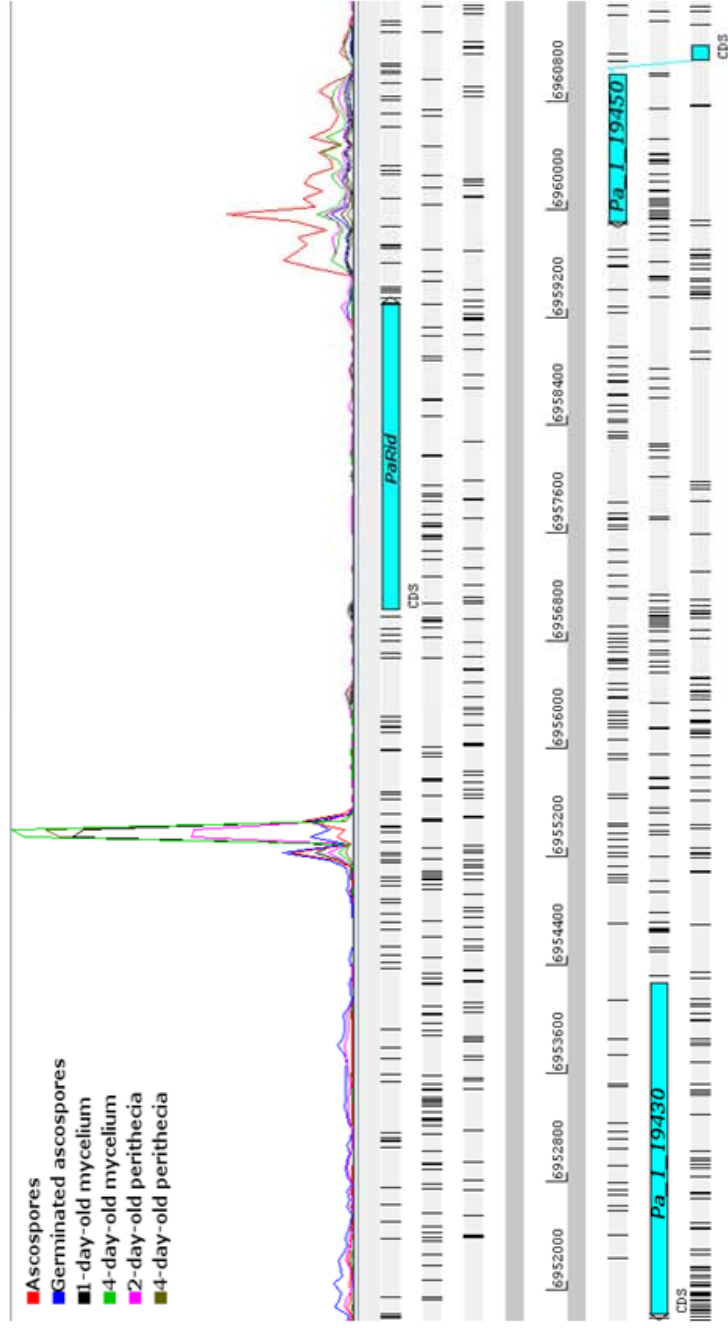
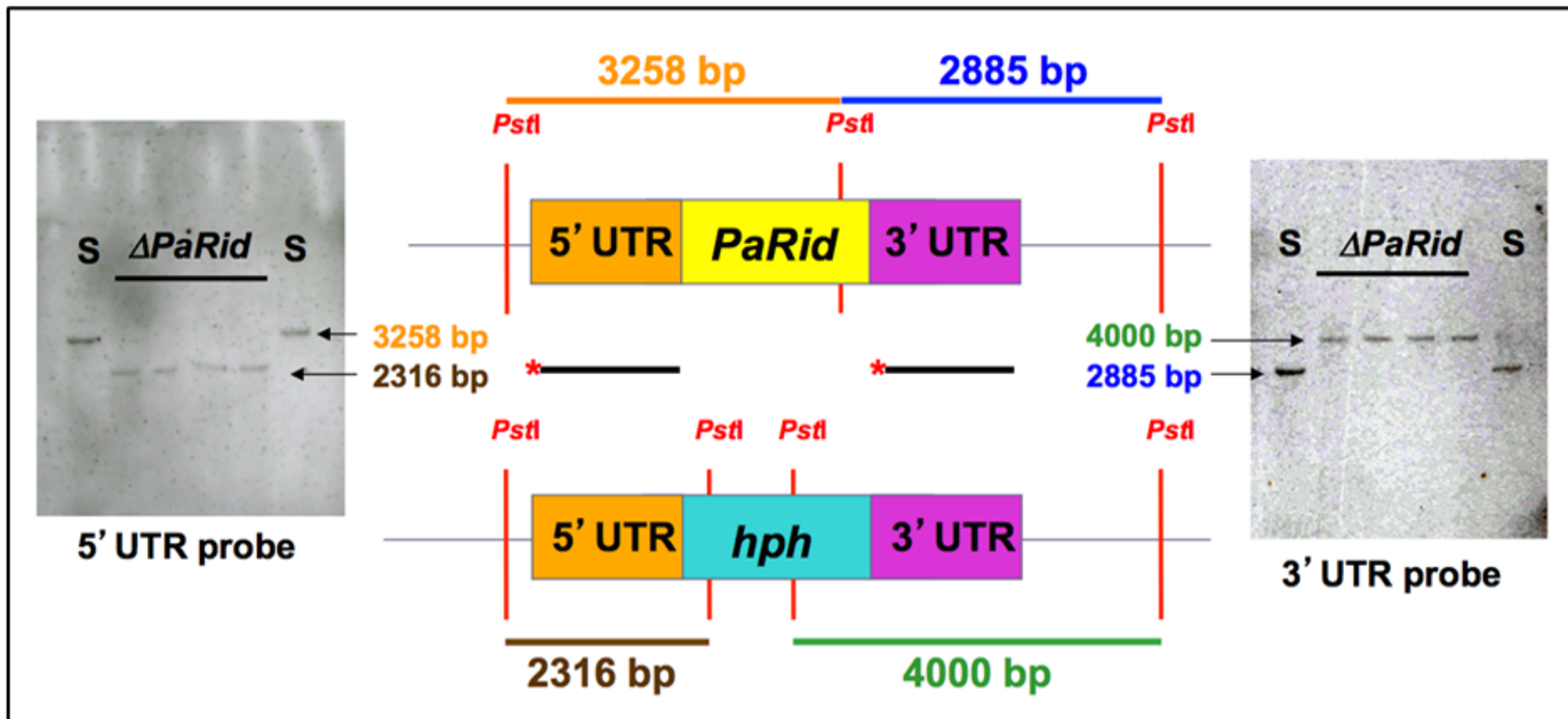


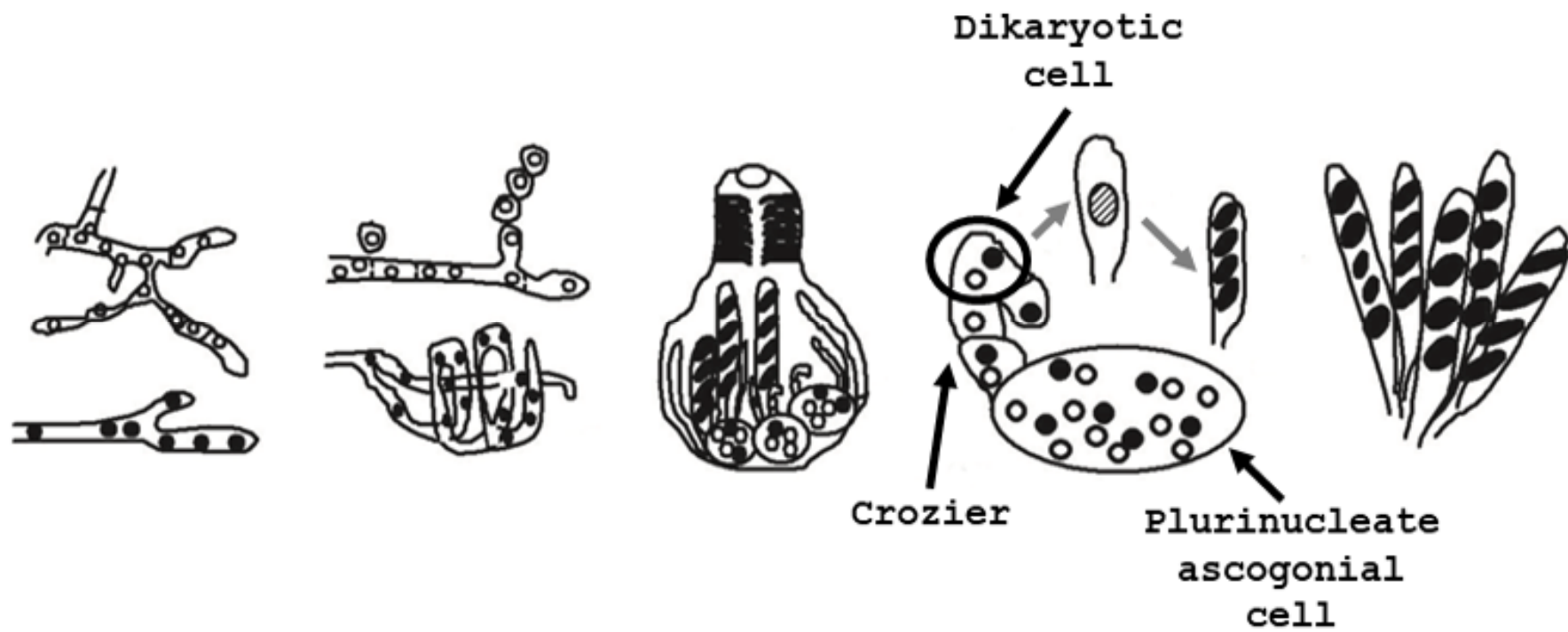
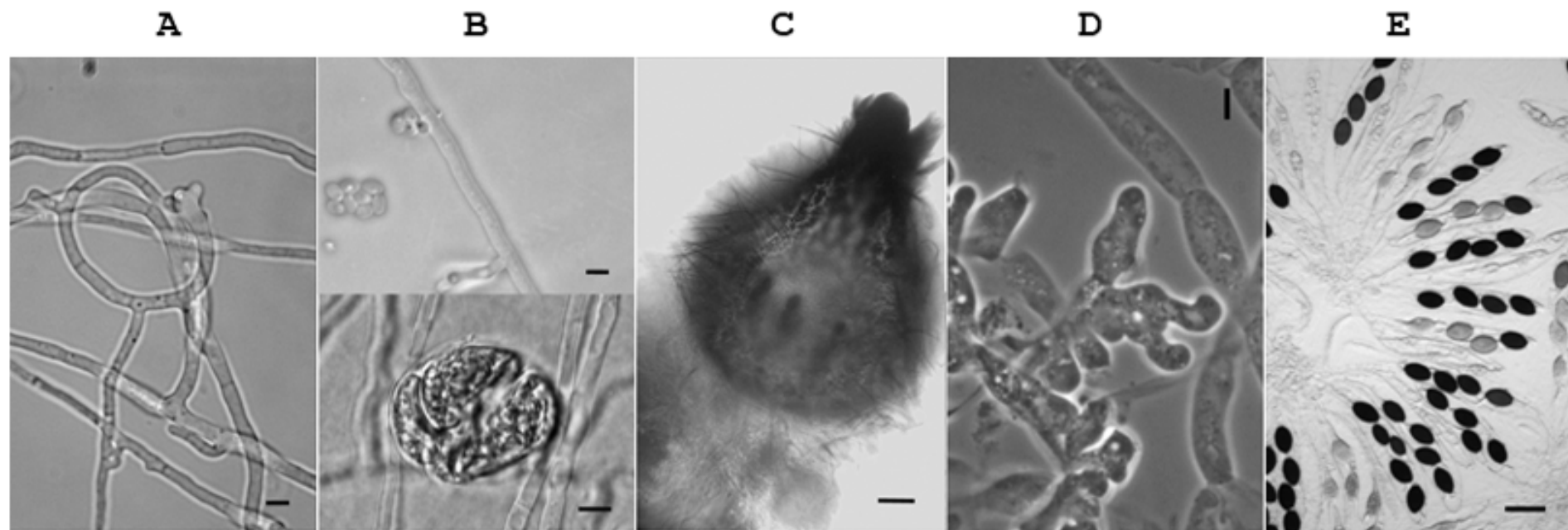
S1B



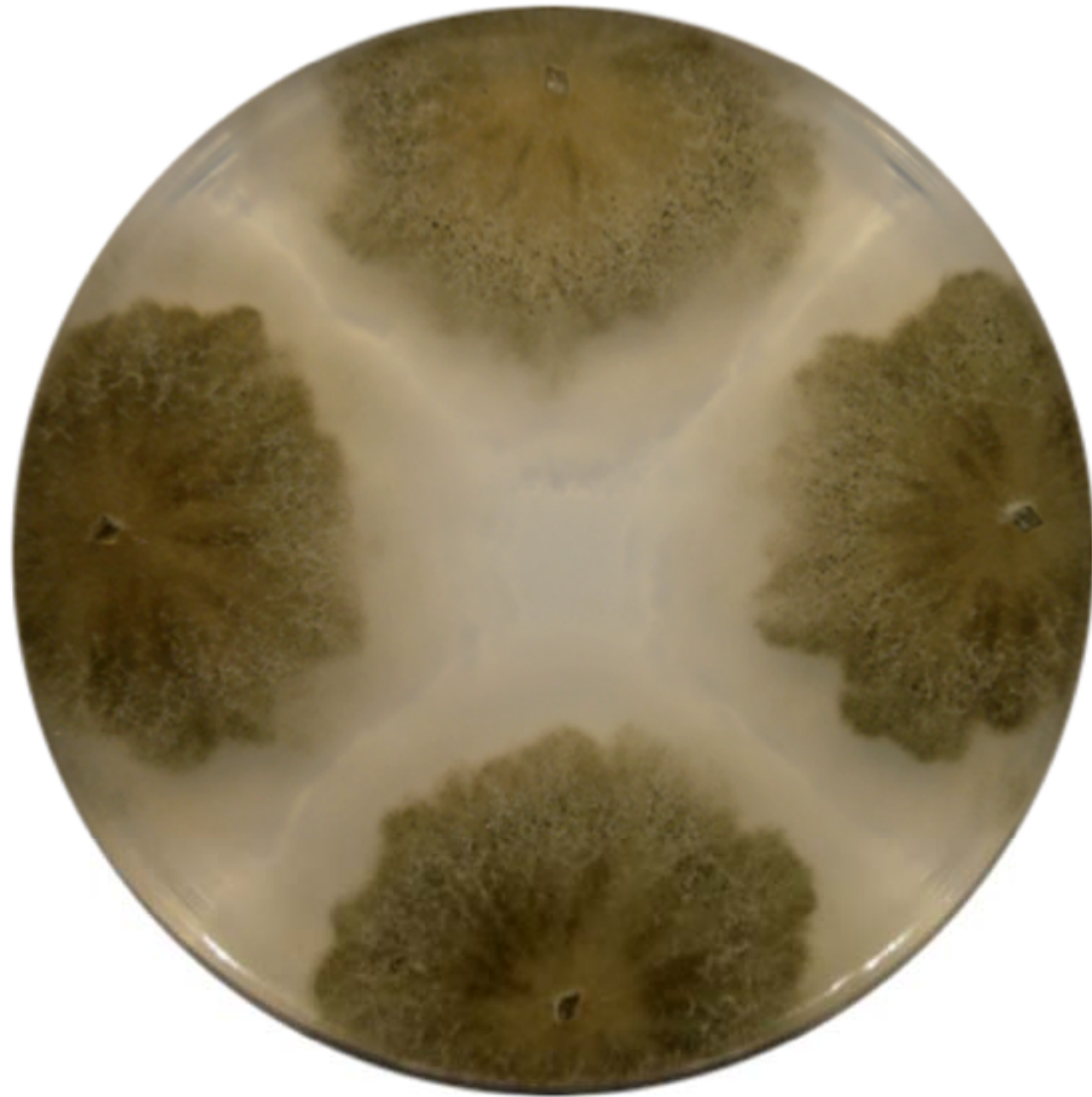
S1C







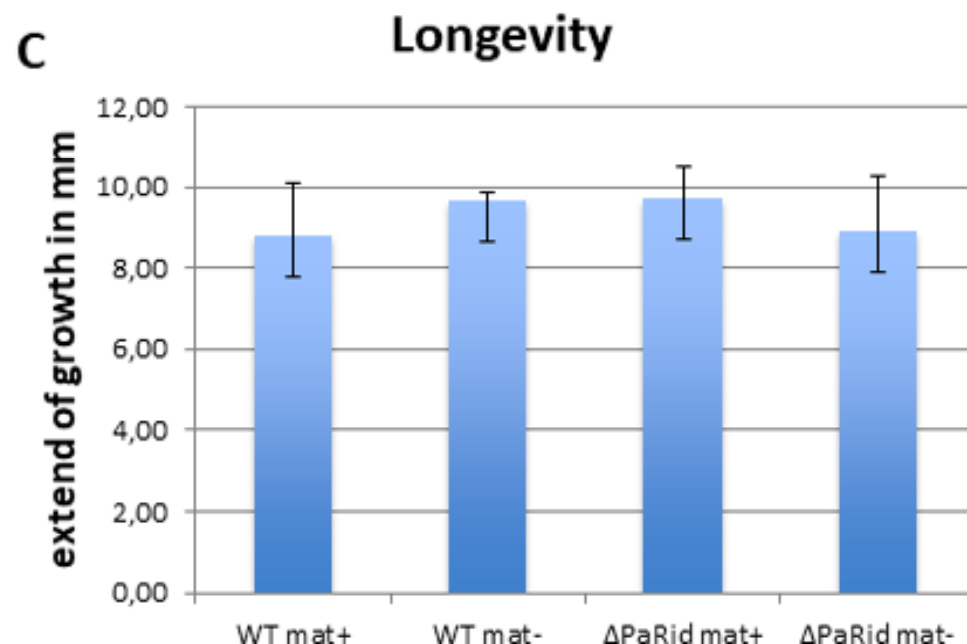
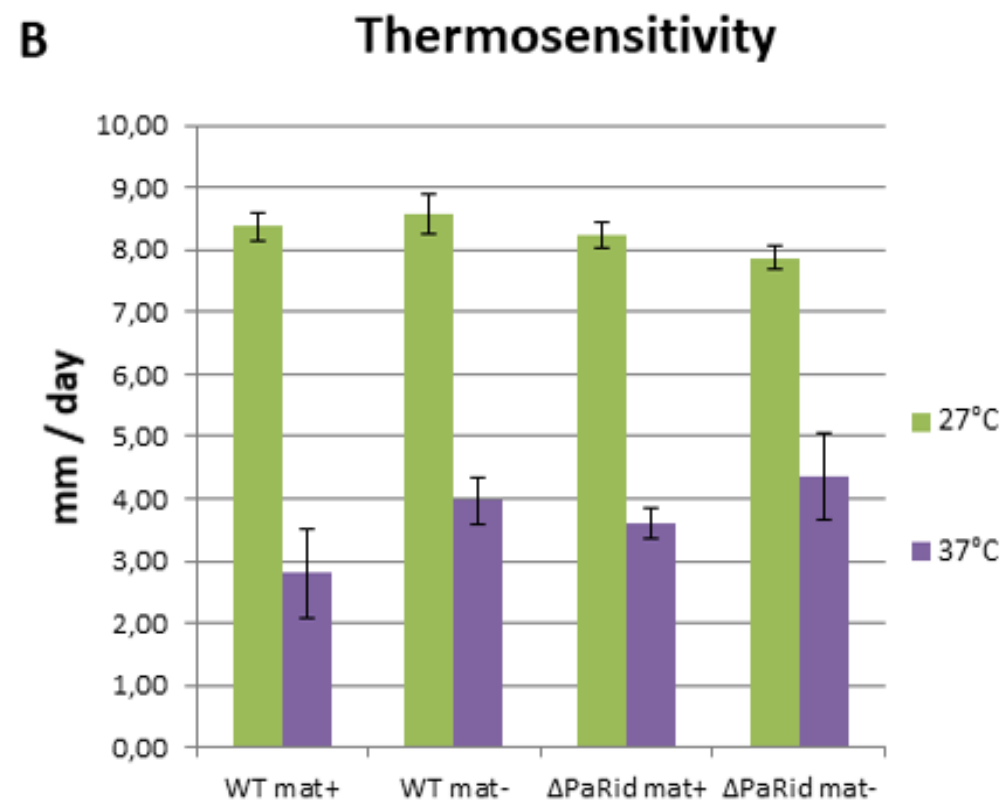
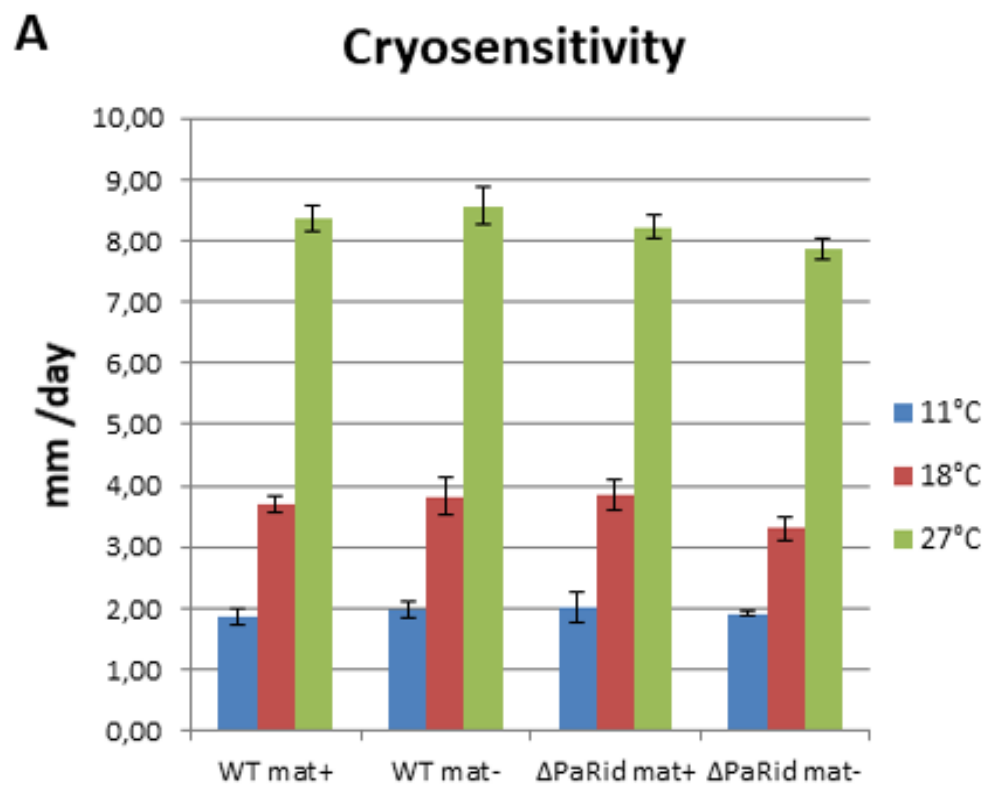
*PaRid*<sup>+</sup>



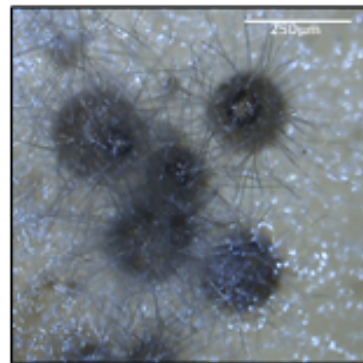
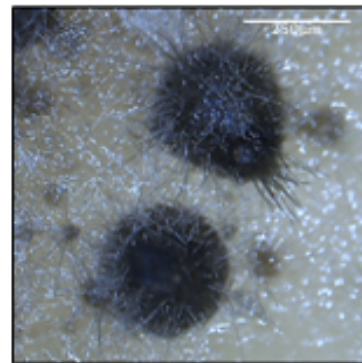
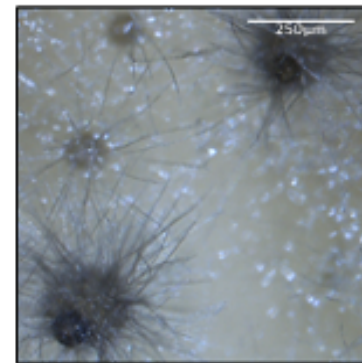
$\Delta PaRid$   
*AS4:PaRid:HA*

$\Delta PaRid$   
*AS4:PaRid:GFP*

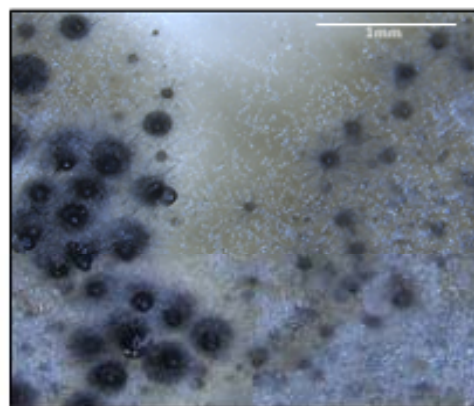
$\Delta PaRid$



6A

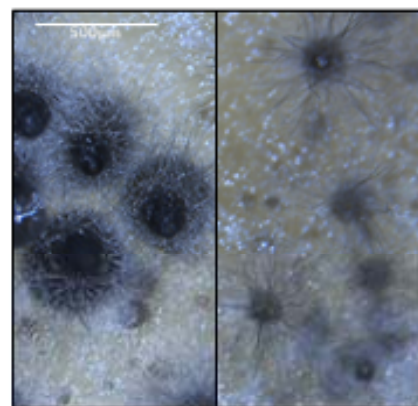
 $\Delta PaRid$  $PaRid^+$  $\Delta Smr1$ 

6B



♀  $PaRid^+$     ♀  $\Delta PaRid$   
 X                    X

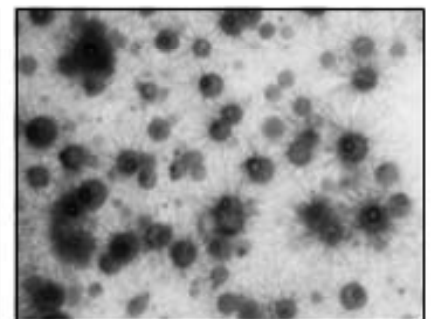
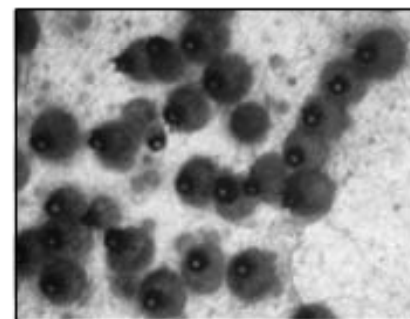
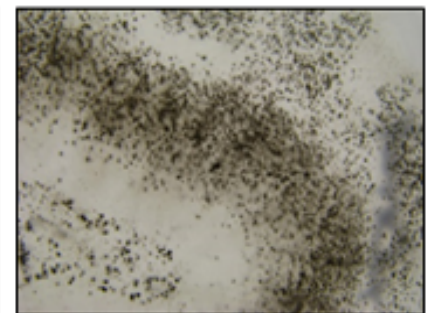
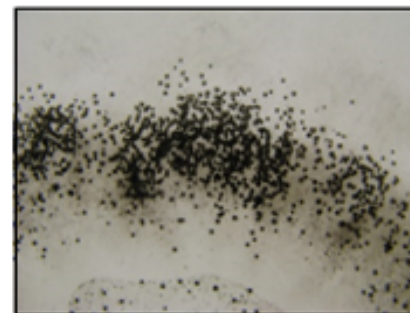
♂  $\Delta PaRid$     ♂  $PaRid^+$



♀  $PaRid^+$     ♀  $\Delta PaRid$   
 X                    X

♂  $\Delta PaRid$     ♂  $PaRid^+$

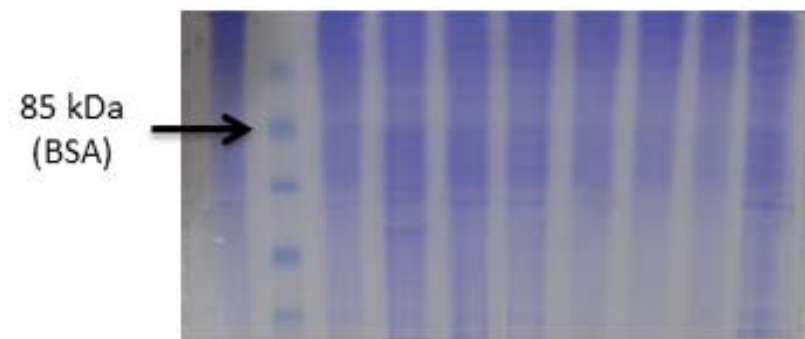
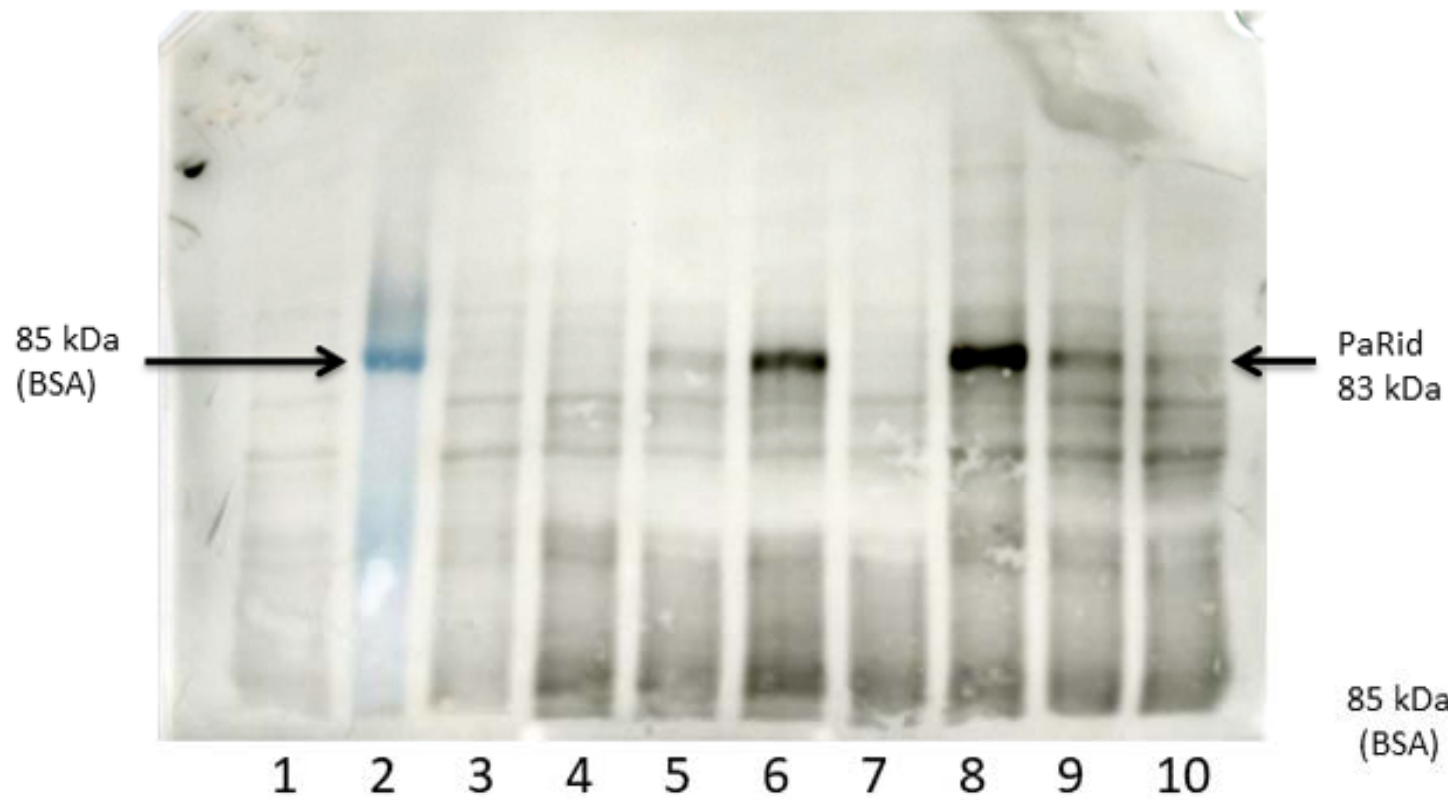
6C

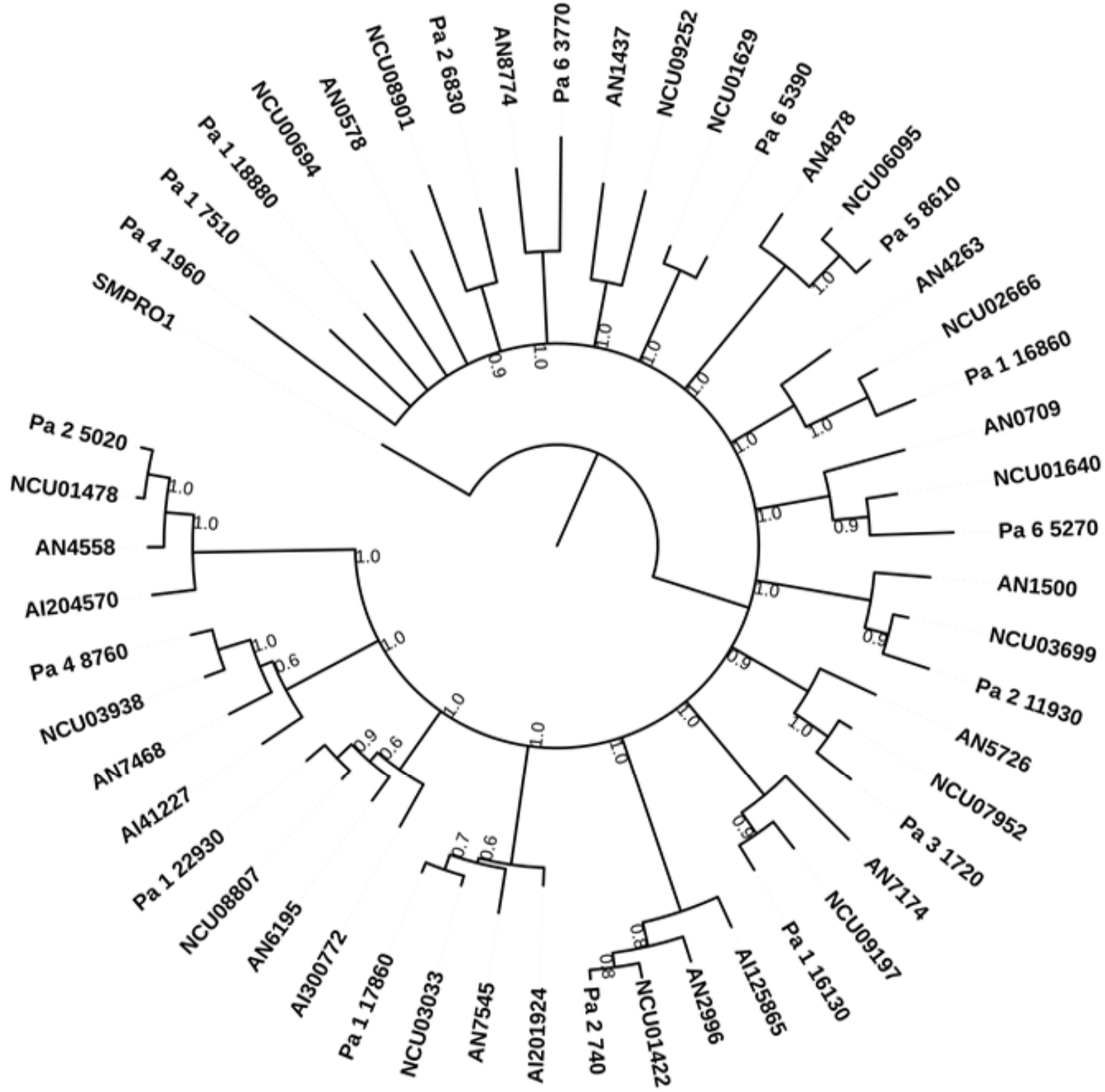


$PaRid^+$   $mat^+$   
 $PaRid^+$   $mat^-$   
 $\Delta mat$

$\Delta PaRid$   $mat^+$   
 $\Delta PaRid$   $mat^-$   
 $\Delta mat$

*PaRid*<sup>+</sup>  
MW  
*PaRid*<sup>+</sup>  
 $\Delta$ *PaRid*  
 $\Delta$ *PaRid* + *PaRid*<sup>+</sup>  
 $\Delta$ *PaRid* + AS4*PaRid*<sup>+</sup>  
 $\Delta$ *PaRid* + *PaRid*<sup>+</sup>-GFP  
 $\Delta$ *PaRid* + AS4-*PaRid*<sup>C403S</sup>  
 $\Delta$ *PaRid* + *PaRid*<sup>C403S</sup>  
 $\Delta$ *PaRid* + *PaRid*<sup>C403S</sup>







**S1 Table. Primers used in this study.**

Primer Names	Primer sequences
ΔRID2F	CATTGGTCTTCCCAGTGACGTAACCAAC
ΔRID2R	cttaccgctgttgagatccagttcgatGAGAAGGAGCAAGAAGGATAAGGGCAACC
ΔRID3F	GGTTGCCCTTATCCTTCTTGCTCCTTCTCatcgaactggatctcaacagcggttaag
ΔRID3R	GCTTCATAAGATCGCCAATAACATGCCACggttcagggcagggctcgtaaataag
ΔRID4F	ctatttaacgaccctgccctgaaccGTGGCATGTTATTGGCGATCTTATGAAGC
ΔRID4R	GCCAAATTCTGAACAGGGGTAGCTTTG
RIDmut1	TCCCCTCCGTCTCAGTTCTGGTCCCCGGC
RIDmut2	CAGAACTGAGACGGAGGGGAGAGGTGGAGG
PaRIDHAFXbal	<u>ATCTAGATCAA</u> AATCACTGACCACTCAGC
PaRIDHAREcoRI	<u>AGAATTCC</u> GTCTCCCAATCAATAACC
AS4PaRIDmutF	gtgatcgcaaaaccgtcaggaattACAAAAAATGGCCGAAATTTACAACCCG
PaRIDmutAS4R	cgggttgtaaattcggccattttttgtAATTCCTGACGGTTTTGCGATCAC
PaRIDAS4Xbal	<u>ATCTAGACGTCTCCCAATCAATAACC</u>
RIDGFPEcoRI	<u>AGAATTC</u> ATGGTGAGCAAGGGCGAGGAGC
RIDGFPRClal	<u>AATCGATGGCGCTT</u> GACTTGACAGCTCGTCC
NcRIDHAFNotI	<u>AGCGGCCGCTGGAGGTATCCATTGACTTTCC</u>
NcRIDHARBamHI	<u>AGGATCCTAGTCTTGAGTCGTCGAAAAGCTCC</u>
dmtAFXbal	<u>ATCTAGAATGAGACAGTCAGCCTATATTTACCTAAC</u>
dmtARBamHI	<u>AGGATCCTTGGTTTTCTGTGCAATTCAC</u>
AiRIDAS4Xbal	<u>ATCTAGAACACCATGT</u> CGGAAAGAAGATACG
AiRIDHAREcoRI	<u>AGAATTCCTCCATCCTCTCGAAATCCTTCTCC</u>
PaRIDF1	CGGTTGCCGACTTCTGTT
PaRIDF2	GGACATTCTCCAATACTGGACA
PaRIDF3	TTCACTGATCTGGAATATGCTG
PaRID1R	TCGGGTGTTTTGGAGTGG
NcRIDF2	AACATCTTGATGACATTCAG
NcRIDF3	ATCGATATTACAGAAGACG
NcRIDF4	ATGGTCATTTATTAGAGTGG
NcRIDF4bis	TGTCTGAACAAACGTTCCGGC
NcRIDF5	TCGAATGCTTAGATAAACGGG
NcRIDF6	ACAAGGACGGGTTCAAAGGCC
NcRIDF7	TCTTGAAGGAGTTGGGCGG
AiRIDF3	TGTCTGAAGCGCTTCGTCTACGGG
5s-PaRid	GTTGTTACTCACACAAAAAATGG
3s-PaRid	CCACTCTCCTGCCAAAAGTCAATC

**S2 Table. Vegetative phenotypes analyses.**

	<i>PaRid</i> <sup>+</sup>	$\Delta PaRid$
Growth rate on M2		
Germination	100 %	100 %
Senescence		
Cell fusion	Efficient	Efficient
Vegetative Incompatibility S vs s	Cell death	Cell death
Neutral osmo-sensitivity (saccharose 200g/l)	100 %	100 %
Neutral osmo-sensitivity (sorbitol 200g/l)	100 %	100 %
Ionic sensitivity (KCl 0.5M)	100 %	100 %
Ionic sensitivity (NaCl 0.5M)	100 %	100 %
10 <sup>-4</sup> M TBX sensitivity	100 %	100 %
5. 10 <sup>-5</sup> M Menadione sensitivity	100 %	100 %
50 µg/ml calcofluor sensitivity	100 %	100 %
5mM caffeine sensitivity	100 %	100 %

For each condition, 3 independent experiments were performed.

**S3 Table. Sexual reproduction phenotypic analyses.**

	<i>PaRid<sup>+</sup> mat<sup>+</sup> X PaRid<sup>+</sup> mat<sup>-</sup></i>	<i>ΔPaRid mat<sup>+</sup> X ΔPaRid mat<sup>-</sup></i>
Spermatia	$1.55 \cdot 10^6 \pm 3.77 \cdot 10^5$	$1.4 \cdot 10^6 \pm 3.28 \cdot 10^5$
Perithecia	[WT] 4477 ± 456	[micro] 4333 ± 368
Ascospores production (96H post-fertilization)	WT production	<b>None</b>

For each condition, 5 independent experiments were performed.

**S4 Table. Grafting experiments**

Recipient strain	<i>PaRid</i> <sup>+</sup>		$\Delta$ <i>PaRid</i>	
	<i>PaRid</i> <sup>+</sup>	$\Delta$ <i>PaRid</i>	<i>PaRid</i> <sup>+</sup>	$\Delta$ <i>PaRid</i>
Grafted perithecia				
Perithecium phenotype	[WT]	[ <b>micro</b> ]	[WT]	[ <b>micro</b> ]
Ascospore production	[WT]	<b>None</b>	[WT]	<b>None</b>

Grafted perithecia per experiment N=9, 3 independent experiments

**S7 Table. Strains used in this study**

Alleles	Promoter	Expressed protein	Number of independent tested transformants
<i>PaRid mat+</i>	Native PaRid	Wild-type PaRid	2
<i>PaRid mat-</i>	Native PaRid	Wild-type PaRid	2
$\Delta$ <i>PaRid mat+</i>	None	None	2
$\Delta$ <i>PaRid mat-</i>	None	None	2
$\Delta$ <i>mat</i>	Native PaRid	Wild-type PaRid	1
<i>PaRid-HA</i>	Native PaRid	Wild-type PaRid HA tagged	109
<i>PaRid-GFP-HA</i>	Native PaRid	Wild-type PaRid HA and GFP tagged	63
<i>AS4-PaRid-HA</i>	Highly and constitutively active <i>AS4</i>	Wild-type PaRid HA tagged	78
<i>AS4-PaRid-GFP-HA</i>	Highly and constitutively active <i>AS4</i>	Wild-type PaRid HA and GFP tagged	95
<i>PaRid<sup>C403S</sup>-HA</i>	Native PaRid	Point-mutated catalytically dead HA tagged	89
<i>AS4-PaRid<sup>C403S</sup>-HA</i>	Highly and constitutively active <i>AS4</i>	Point-mutated catalytically dead HA tagged	55