

Supporting Information to

Disruption of the MICOS complex leads to an aberrant cristae structure and an unexpected, pronounced lifespan extension in *Podospora anserina*

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Figure S1

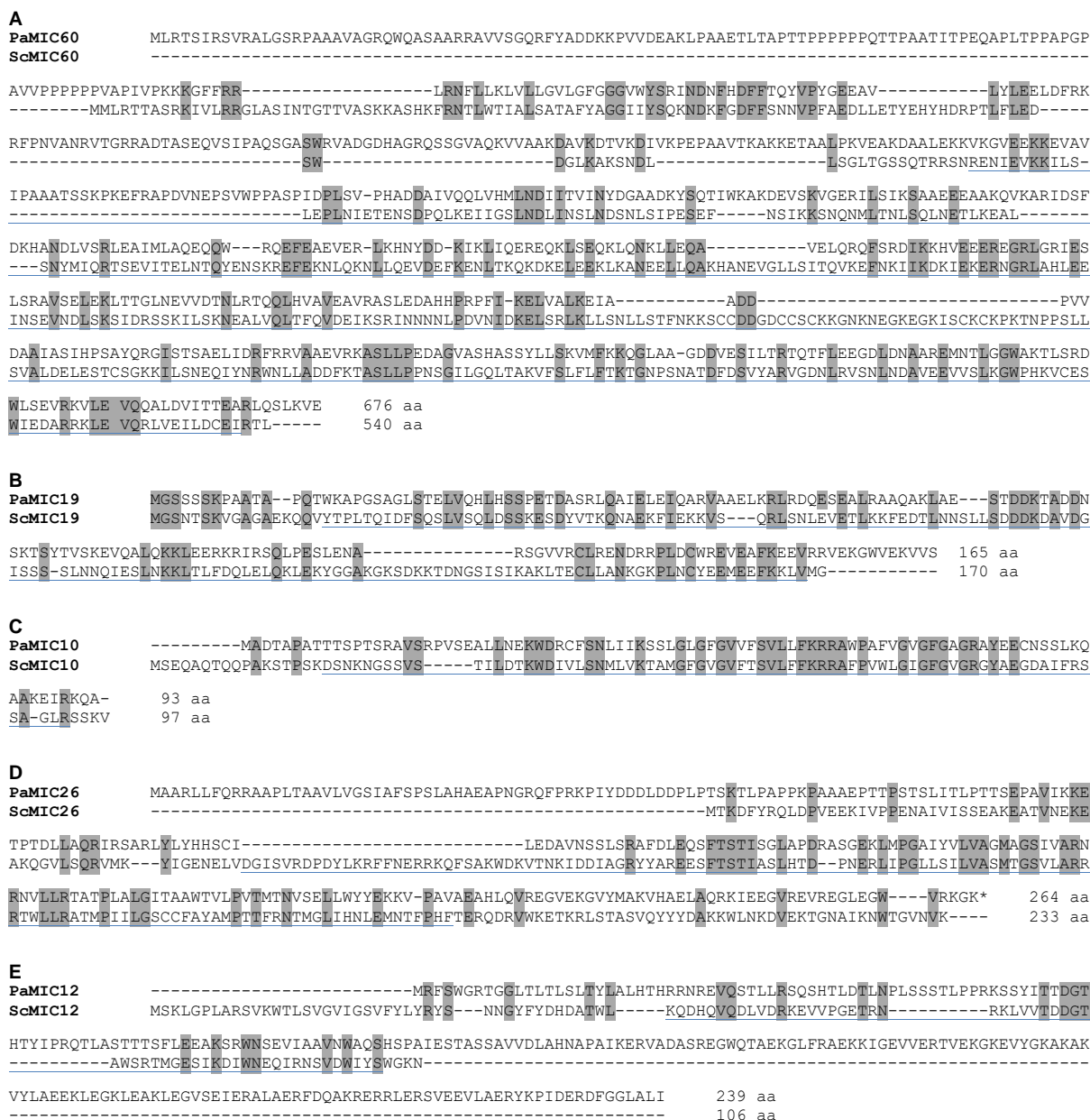


Figure S1: Amino acid sequence alignments of *P. anserina* MICOS proteins with their yeast homologs. Amino acid sequence alignment of (A) *P. anserina* MIC60 (PaMIC60; UniProt: B2A9R4) and *S. cerevisiae* MIC60 (ScMIC60; UniProt: P36112) (B) *P. anserina* MIC19 (PaMIC19; UniProt: B2AUM5) and *S. cerevisiae* MIC19 (ScMIC19; UniProt: P43594), (C) of *P. anserina* MIC10 (PaMIC10; UniProt: B2AWQ3) and *S. cerevisiae* MIC10 (ScMIC10; UniProt: Q96VH5), (D) of *P. anserina* MIC26 (PaMIC26; UniProt: B2AYB9) and *S. cerevisiae* MIC26 (ScMIC26; UniProt: P50087) and (E) of *P. anserina* MIC12 (PaMIC12; UniProt: B2B4I6) and *S. cerevisiae* MIC12 (ScMIC12; UniProt: P38341) using EMBOSS Needle (http://www.ebi.ac.uk/Tools/psa/emboss_needle/). Identical amino acids are marked by grey boxes. Conserved domains are marked by blue lines in the *S. cerevisiae* sequences.

Figure S2

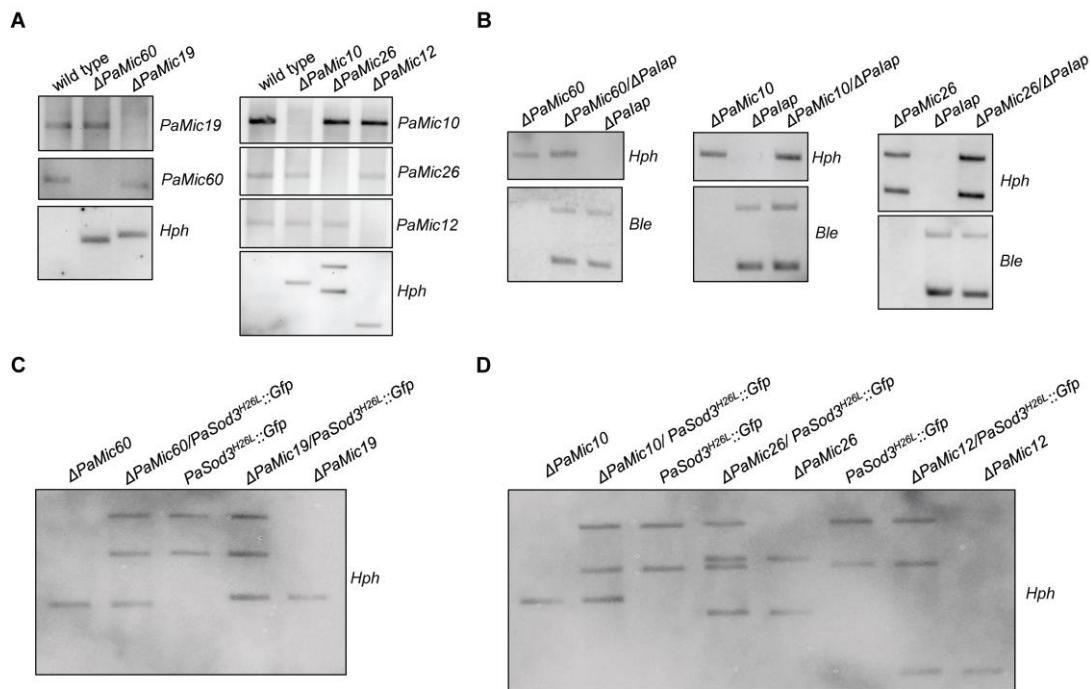


Figure S2: Southern blot verification of MICOS mutants. (A) Southern blot analyses verifying the replacement of *PaMic60*, *PaMic19*, *PaMic10*, *PaMic26* and *PaMic12* by a hygromycin resistance gene (*Hph*). *PaMic60*, *PaMic19*, *PaMic10*, *PaMic26*, *PaMic12* and *Hph* probes were used as indicated. (B) Southern blot analyses verifying the newly generated double mutants $\Delta PaMic60/\Delta Palap$, $\Delta PaMic10/\Delta Palap$ and $\Delta PaMic26/\Delta Palap$. *Ble* (phleomycin resistance), *Hph* (hygromycin resistance) probes were used as indicated. (C+D) Southern blot analysis validating the genetic constitution of different *PaSod3^{H26L}::Gfp* mutants and their respective control strains. *Hph* (hygromycin resistance) probe was used.

Figure S3

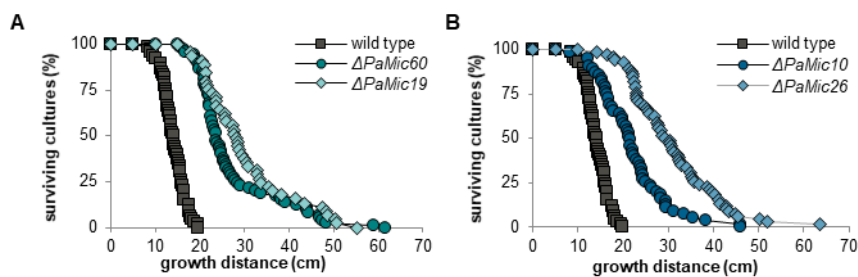


Figure S3: Ablation of MICOS leads to unexpected longevity. (A) Survival curves of *P. anserina* wild type (n = 66); $\Delta PaMic60$ (n = 76) and $\Delta PaMic19$ (n = 45) grown on standard M2 medium. (B) Survival curves of *P. anserina* wild type (n = 66); $\Delta PaMic10$ (n = 48) and $\Delta PaMic26$ (n = 66) grown on standard M2 medium.

Figure S4

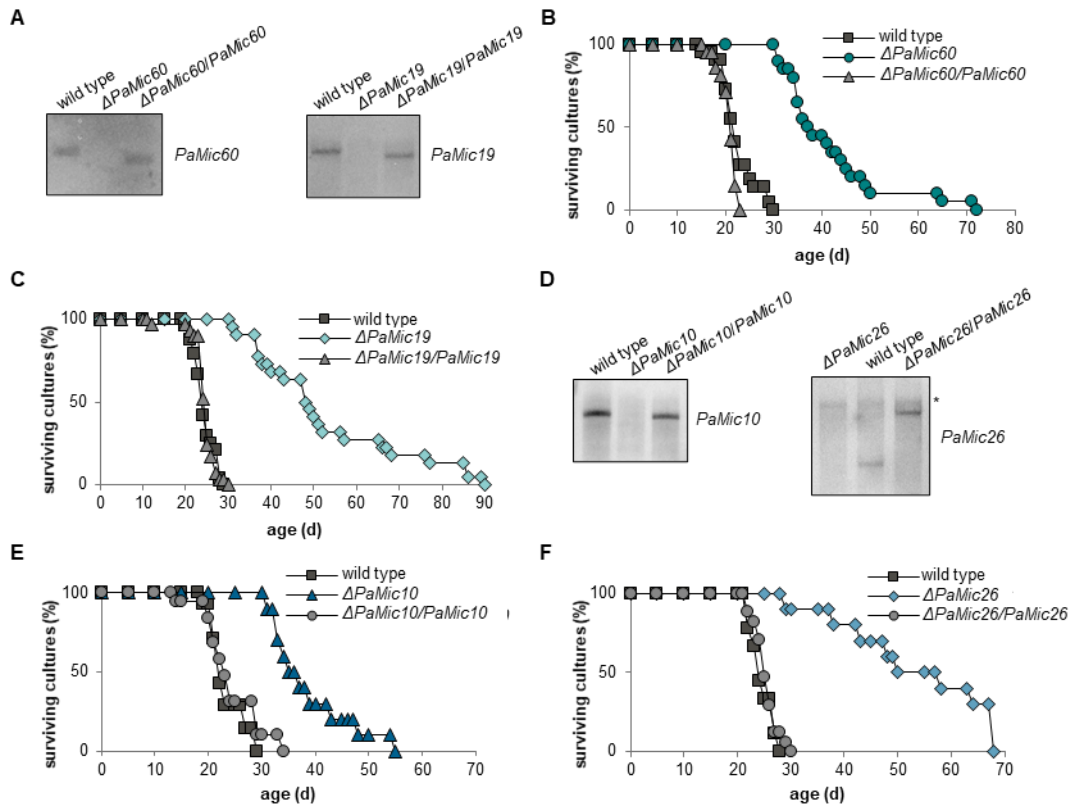


Figure S4: Southern blot verification and lifespan analysis of MICOS complementation strains. (A) Southern blot analysis validates the genotype of different complementation strains and their respective control strains. *PaMic60* and *PaMic19* specific probes were used to detect the corresponding genes. (B) Survival curves of *P. anserina* wild type (n = 22); $\Delta PaMic60$ (n = 20) and $\Delta PaMic60/PaMic60$ (n = 21) grown on standard M2 medium. (C) Survival curves of *P. anserina* wild type (n = 24); $\Delta PaMic19$ (n = 22) and $\Delta PaMic19/PaMic19$ (n = 29) grown on standard M2 medium. (D) Southern blot analysis validates the genotype of different complementation strains and their respective control strains. *PaMic10* and *PaMic26* specific probes were used to detect the corresponding genes. An asterisk (*) marks an unspecific signal. (E) Survival curves of *P. anserina* wild type (n = 14); $\Delta PaMic10$ (n = 10) and $\Delta PaMic10/PaMic10$ (n = 19) grown on standard M2 medium. (F) Survival curves of *P. anserina* wild type (n = 9); $\Delta PaMic26$ (n = 10) and $\Delta PaMic26/PaMic26$ (n = 17) grown on standard M2 medium.

Figure S5

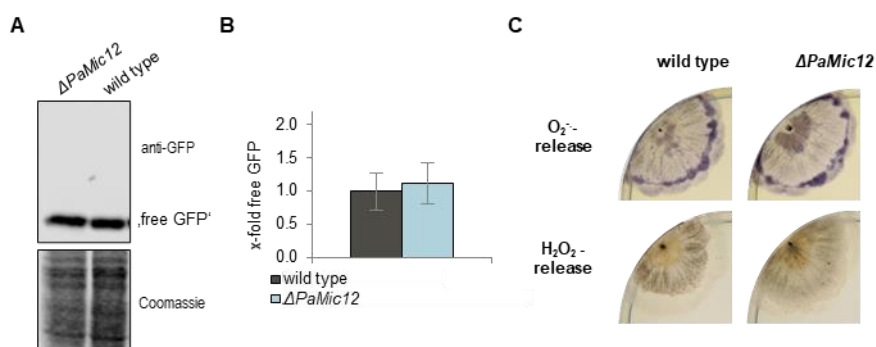


Figure S5: Mitophagy and ROS release in the *PaMic12* deletion mutant. (A) Monitoring mitophagy by western blot analysis of total protein extracts from *PaSod3^{H26L}::Gfp* (here wild type) and $\Delta PaMic12/PaSod3^{H26L}::Gfp$ ($\Delta PaMic12$) cultures with a GFP antibody (each 4 biological replicates). (B) Quantification of 'free GFP' protein level normalized to the Coomassie stained gel. Protein level in *PaSod3^{H26L}::Gfp* cultures was set to 1. Data represent mean \pm SD. (C) Qualitative determination of superoxide anion and hydrogen peroxide release, respectively, by histochemical NBT- and DAB-staining of *P. anserina* wild-type and $\Delta PaMic12$ strains.

Figure S6

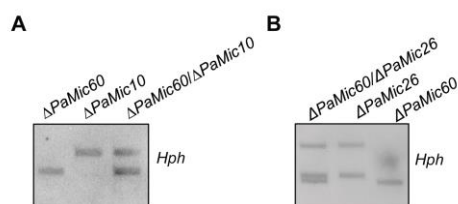


Figure S6: Southern blot analysis of MICOS double deletion mutants. Southern blot analysis verifies the genotype of (A) $\Delta PaMic60/\Delta PaMic10$ and of (B) $\Delta PaMic60/\Delta PaMic26$ and their respective control strains using an *Hph*-specific gene probe.

Table S1

Table S1: Oligonucleotides used for amplification of flanking regions for deletion of specific genes. The restriction sites are underlined in the sequences. All oligonucleotides were purchased from Biomers, Ulm, Germany.

Gene	oligonucleotide		restriction site
	name	sequence (5'-3')	
<i>PaMic10</i> (<i>Pa_7_7950</i>)	PaMic10_KO5	GCTCTGCAGATCACCAAATCAAATTCACGC	PstI
	PaMic10_KO6	GAGGATCCTTGCTGTCTTCTTCTACC	BamHI
	Mic10OEx1	CAGGTACCGCTAGGGAGAAGAGAGG	KpnI
	Mic10OEx2	GCGGAAGCTTGTGATTGTGTGGGAGTG	HindIII
<i>PaMic12</i> (<i>Pa_2_1415</i>)	PaMic12-KO1	GGATCGATGACCGCCTTGTCCCTTGAG	ClaI
	PaMic12-KO2	GCCGAAGCTTGTGTTTCAGATAACCGTTG	HindIII
	PaMic12-KO3	GCACTGCAGGGGAGGTTGATAAGGGGAG	PstI
	PaMic12-KO4	GAGGATCCGATTGGGCCGTTGATTGCG	BamHI
<i>PaMic26</i> (<i>Pa_1_10590</i>)	PaMic26-KO1	GCATCGATACCGCTGGGCTACCTAGAGC	ClaI
	PaMic26-KO2	GCCGAAGCTTGGTCTCGACCCAATTGG	HindIII
	PaMic26-KO3	GCTCTGCAGGGGAATGTTGTGTG	PstI
	PaMic26-KO4	GAGGATCCCTACCGCCAAACCACTCTC	BamHI
<i>PaMic60</i> (<i>Pa_1_1530</i>)	PaMic60-KO1	GAGGATCCAGGACTCAGGCGTATGAC	BamHI
	PaMic60-KO2	GACTGCAGGAGGGAGATGGAGTGGCC	PstI
	PaMic60-KO3	GCGATATCAAGGGACCAACGGGACGTC	EcoRV
	PaMic60-KO4	GCAAGCTTTGCCCTTGCCTTTTGTCC	HindIII
<i>PaMic19</i> (<i>Pa_1_19620</i>)	PaMic19-KO1	GCGATCGATGTTGTACGAGCATCAAG	ClaI
	PaMic19-KO2	GGAAGCTTGTGAGGGATTGTCTGGTTG	HindIII
	PaMic19-KO3	GGCTGCAGGGTGGTAACGAAGTTGG	PstI
	PaMic19-KO4	GCGGATCCATGATCCCATCTCTTC	BamHI

Table S2

Table S2: Oligonucleotides used for amplification of genes for complementation. The restriction sites are underlined in the sequences. All oligonucleotides were purchased from Biomers, Ulm, Germany.

Gene	oligonucleotide		restriction site
	name	sequence (5'-3')	
<i>PaMic10</i> (<i>Pa_7_7950</i>)	Mic10OEx1	CAGGT <u>ACCGCTAGGG</u> GAGAAGAGAGG	KpnI
	Mic10-Kom1	GTGT <u>CGACGTGGATTTAGGCCG</u> GAGGATG	Sall
<i>PaMic26</i> (<i>Pa_1_10590</i>)	Mic26-3	GAGT <u>CGACGCCACCTATGTCGATAAC</u>	Sall
	Mic26-4	<u>CGATCGATGAGGG</u> GAGATTGTAGCG	Clal
<i>PaMic60</i> (<i>Pa_1_1530</i>)	PaMic60_1	GTCTCGAGTATGACCGGCAGTGGATG	XhoI
	PaMic60_2	GAAGAGCCGCAGTCTCCTTC	5'Phosphat
	PaMic60_3	CCAAGGTCGAAGCCAAGGATG	5'Phosphat
	PaMic60_4	CAGGT <u>ACCGGACCGGAGTAAGACTG</u>	KpnI
<i>PaMic19</i> (<i>Pa_1_19620</i>)	PaMic19_KO1	GCGATCGATGTTGTACGAGCATCAAG	Clal
	PaMic19-5	GAGGT <u>ACCGGAGGTTAGGGAGG</u> CACTG	KpnI

Table S3

Table S3: Oligonucleotides used for the construction of specific gene probes. All oligonucleotides were purchased from Biomers, Ulm, Germany.

Gene	oligonucleotide		base pairs (bp)
	name	sequence (5'-3')	
<i>PaMic10</i> (<i>Pa_7_7950</i>)	Pa_7_7950-1	TCAGCGAAGCCCTCCTCAAC	311
	Pa_7_7950-2	TAGGCTTGCTTCTGATCTC	
<i>PaMic12</i> (<i>Pa_2_1415</i>)	PaMic12-1	CTAACCTACCTAGCCCTCC	289
	PaMic12-2	GCGGTTGATTCGATAGCG	
<i>PaMic26</i> (<i>Pa_1_10590</i>)	PaMic26-1	GGTCGTCAGTTTGTACGTC	297
	PaMic26-2	GATGCAGGAGTGGTGGTAG	
<i>PaMic60</i> (<i>Pa_1_1530</i>)	Pa_1_1530-1	GCGTACATCGATACGATCAG	319
	Pa_1_1530-2	GTGCTTGCTCGGGAGTAATC	
<i>PaMic19</i> (<i>Pa_1_19620</i>)	PaMic19-1	CCAGCACCTCCATTCTC	306
	PaMic19-2	CTTCTTCTGGAGAGCTTGC	

Table S4

Table S4: Oligonucleotides used for the quantitative real-time PCR (qPCR). All oligonucleotides were purchased from Biomers, Ulm, Germany.

Gene	oligonucleotide	
	name	sequence (5'-3')
<i>PaMic10</i> (<i>Pa_7_7950</i>)	Pa_7_7950-1	TCAGCGAAGCCCTCCTCAAC
	Pa_7_7950-2	TAGGCTTGCTTCTGATCTC
<i>PaMic60</i> (<i>Pa_1_1530</i>)	Pa_1_1530-3	GCCTCTAGCTACCTCTTG
	Pa_1_1530-4	TTGCAGTCTGGCTCGGTTG
<i>PaMic19</i> (<i>Pa_1_19620</i>)	PaMic19-1	CCAGCACCTCCATTCTC
	PaMic19-2	CTTCTTCTGGAGAGCTTGC
<i>PaPorin</i> (<i>Pa_2_9780</i>)	Porin-RT-for	TCTCCTCCGGCAGCCTTG
	Porin-RT-rev	CGGAGGCGGACTTGTGAC

Table S5

Table S5: Overview of lifespan and growth rate of *P. anserina* wild type and MICOS deletion mutants. The *p*-values were determined with SPSS (IBM; SPSS Statistics, New York, USA) with three different tests. Indicated are the *p*-values of the lifespan curves in comparison to the wild type. “*p*-value 1” = Log Rank (Mantel-Cox); “*p*-value 2” = Breslow (Generalized Wilcoxon); “*p*-value 3” = Tarone-Ware.

	wild type	$\Delta PaMic60$	$\Delta PaMic19$	$\Delta PaMic10$	$\Delta PaMic12$	$\Delta PaMic26$
mean lifespan (d) \pm SD	25 \pm 3.97	47 \pm 16.37	50 \pm 16.05		23 \pm 3.75	52 \pm 14.96
Maximum lifespan (d)	32	105	90		33	100
<i>p</i> -value 1	/	3.29E-37	1.84E-25	2.21E-18	0.13	7.87E-33
<i>p</i> -value 2	/	9.39E-32	1.93E-20	3.97E-15	0.06	1.28E-27
<i>p</i> -value 3	/	1.76E-34	6.28E-23	9.27E-17	0.07	3.33E-30
growth rate (cm/d) \pm SD	0.67 \pm 0.05	0.62 \pm 0.04	0.62 \pm 0.03	0.62 \pm 0.04	0.65 \pm 0.09	0.65 \pm 0.03
growth distance (cm) \pm SD	14.2 \pm 2.6	27.5 \pm 9.7	30.3 \pm 9.8		13.9 \pm 2.1	30.7 \pm 9.5
biological replicates	66	74	45	48	54	66

Table S6

Table S6: Overview of lifespan and growth rate of *P. anserina* wild type and Mic60-subcomplex deletion mutants with and without paraquat. The *p*-values were determined with SPSS (IBM; SPSS Statistics, New York, USA) with three different tests. Indicated are the *p*-values of the lifespan curves in comparison to the wild type without paraquat. “*p*-value 1” = Log Rank (Mantel-Cox); “*p*-value 2” = Breslow (Generalized Wilcoxon); “*p*-value 3” = Tarone-Ware. Additionally, *p*-values of the lifespan curves compared to the corresponding deletion mutant without paraquat are given. “*p*-value 4” = Log Rank (Mantel-Cox); “*p*-value 5” = Breslow (Generalized Wilcoxon); “*p*-value 6” = Tarone-Ware.

	wild type	$\Delta PaMic60$	$\Delta PaMic19$	wild type	$\Delta PaMic60$	$\Delta PaMic19$
	0 μ M paraquat			80 μ M paraquat		
mean lifespan (d) \pm SD	23 \pm 2.4	48 \pm 17.8	50 \pm 13.8	44 \pm 16.8	72 \pm 27.5	76 \pm 24.3
Maximum lifespan (d)	28	107	86	99	129	113
<i>p</i> -value 1	/	7.96E-15	1.03E-09	3.67E-11	7.96E-15	1.03E-09
<i>p</i> -value 2	/	1.18E-12	8.09E-08	4.24E-09	1.18E-12	8.09E-08
<i>p</i> -value 3	/	1.00E-13	9.56E-09	4.13E-10	1.00E-13	9.56E-09
<i>p</i> -value 4	/	/	/	/	0.0003	0.001
<i>p</i> -value 5	/	/	/	/	0.001	0.003
<i>p</i> -value 6	/	/	/	/	0.0004	0.002
growth rate (cm/d) \pm SD	0.62 \pm 0.04	0.60 \pm 0.04	0.57 \pm 0.02	0.59 \pm 0.04	0.55 \pm 0.03	0.57 \pm 0.02
growth distance (cm) \pm SD	13.2 \pm 1.8	27.4 \pm 10.9	29.7 \pm 7.5	21.4 \pm 8	32.5 \pm 11.1	36.1 \pm 12.2
biological replicates	26	27	15	26	27	15
	0 mM ascorbic acid			1 mM ascorbic acid		
mean lifespan (d) \pm SD	23 \pm 2.7	54 \pm 9.5	57 \pm 11.3	27 \pm 7.1	49 \pm 11.1	49 \pm 12.3
Maximum lifespan (d)	30			46	71	
<i>p</i> -value 1	/	3.98E-11	2.56E-10	0.048	3.98E-11	3.86E-10
<i>p</i> -value 2	/	2.56E-09	1.54E-08	0.121	2.56E-09	1.79E-08
<i>p</i> -value 3	/	3.47E-10	2.17E-09	0.080	3.47E-10	2.79E-09
<i>p</i> -value 4	/	/	/	/	0.052	0.039
<i>p</i> -value 5	/	/	/	/	0.039	0.030
<i>p</i> -value 6	/	/	/	/	0.043	0.031
growth rate (cm/d) \pm SD	0.67 \pm 0.02	0.62 \pm 0.01	0.63 \pm 0.02	0.62 \pm 0.03	0.61 \pm 0.02	0.59 \pm 0.01
growth distance (cm) \pm SD	13.7 \pm 1.7			14.4 \pm 3.9	27.5 \pm 6.6	26.8 \pm 7.8
biological replicates	21	19	17	21	19	17

Table S7

Table S7: Overview of lifespan and growth rate of *P. anserina* wild type and Mic10-subcomplex deletion mutants with and without paraquat or ascorbic acid. The *p*-values were determined with SPSS (IBM; SPSS Statistics, New York, USA) with three different tests. Indicated are the *p*-values of the lifespan curves in comparison to the wild type without paraquat or ascorbic acid. “*p*-value 1” = Log Rank (Mantel-Cox); “*p*-value 2” = Breslow (Generalized Wilcoxon); “*p*-value 3” = Tarone-Ware. Additionally, *p*-values of the lifespan curves compared to the corresponding deletion mutant without paraquat or ascorbic acid are given. “*p*-value 4” = Log Rank (Mantel-Cox); “*p*-value 5” = Breslow (Generalized Wilcoxon); “*p*-value 6” = Tarone-Ware.

	wild type	$\Delta PaMic10$	$\Delta PaMic26$	wild type	$\Delta PaMic10$	$\Delta PaMic26$
	0 μ M Paraquat			80 μ M Paraquat		
mean lifespan (d) \pm SD	26 \pm 4.1	40 \pm 12.4	53 \pm 12.6	55 \pm 22.6	30 \pm 11.6	25 \pm 6.1
Maximum lifespan (d)	32	75	89	107	53	32
<i>p</i> -value 1	/	5.65E-09	7.24E-10	9.91E-10	0.02	0.75
<i>p</i> -value 2	/	2.61E-07	7.90E-08	8.53E-08	0.31	0.76
<i>p</i> -value 3	/	4.11E-08	7.86E-09	1.02E-08	0.10	0.72
<i>p</i> -value 4	/	/	/	/	0.01	1.87E-08
<i>p</i> -value 5	/	/	/	/	0.004	1.99E-07
<i>p</i> -value 6	/	/	/	/	0.007	5.96E-08
growth rate (cm/d) \pm SD	0.68 \pm 0.03	0.61 \pm 0.04	0.66 \pm 0.01	0.59 \pm 0.1	0.54 \pm 0.03	0.51 \pm 0.02
growth distance (cm) \pm SD	14.8 \pm 2.6	20.4 \pm 5.8	30.8 \pm 7.7	25.6 \pm 11.2	14.2 \pm 7.3	10.6 \pm 1.8
biological replicates	27	28	15	27	28	15
	0 mM ascorbic acid			1 mM ascorbic acid		
mean lifespan (d) \pm SD	24 \pm 2.2	39 \pm 12.9	58 \pm 18.5	29 \pm 10.8	27 \pm 9.1	37 \pm 5.3
Maximum lifespan (d)	29		100	55	41	46
<i>p</i> -value 1	/	2.01E-05	1.12E-09	0.322	0.173	7.26E-06
<i>p</i> -value 2	/	2.67E-04	9.48E-09	0.982	0.691	1.22E-04
<i>p</i> -value 3	/	7.55E-05	3.22E-09	0.661	0.402	2.98E-05
<i>p</i> -value 4	/	/	/	/	0.01	3.39E-07
<i>p</i> -value 5	/	/	/	/	0.02	4.37E-06
<i>p</i> -value 6	/	/	/	/	0.01	1.23E-06
growth rate (cm/d) \pm SD	0.65 \pm 0.03	0.67 \pm 0.02	0.64 \pm 0.01	0.61 \pm 0.01	0.60 \pm 0.04	0.60 \pm 0.01
growth distance (cm) \pm SD	14.1 \pm 1.4		35.3 \pm 12.4	16.1 \pm 6.8	15.7 \pm 5.4	20.1 \pm 2.9
biological replicates	15	11	21	15	11	21

Table S8

Table S8: Overview of lifespan and growth rate of *P. anserina* wild type and MICOS deletion mutants and double deletion mutants. The *p*-values were determined with SPSS (IBM; SPSS Statistics, New York, USA) with three different tests. Indicated are the *p*-values of the lifespan curves in comparison to the wild type. “*p*-value 1” = Log Rank (Mantel-Cox); “*p*-value 2” = Breslow (Generalized Wilcoxon); “*p*-value 3” = Tarone-Ware. Moreover, *p*-values of the lifespan curves compared to $\Delta PaMic10$ or $PaMic26$. “*p*-value 4” = Log Rank (Mantel-Cox); “*p*-value 5” = Breslow (Generalized Wilcoxon); “*p*-value 6” = Tarone-Ware. And, *p*-values of the lifespan curves compared to $\Delta PaMic60$. “*p*-value 7” = Log Rank (Mantel-Cox); “*p*-value 8” = Breslow (Generalized Wilcoxon); “*p*-value 9” = Tarone-Ware.

	wild type	$\Delta PaMic60$	$\Delta PaMic10$	$\Delta PaMic26$	$\Delta PaMic60/\Delta PaMic10$	$\Delta PaMic60/\Delta PaMic26$
mean lifespan (d) \pm SD	26 ± 3.5	50 ± 16.1	39 ± 12.5	52 ± 12.6	101 ± 35.8	121 ± 45.9
Maximum lifespan (d)	32	100	63	86	176	233
<i>p</i> -value 1	/	2.82E-16	2.23E-07	1.70E-09	1.17E-12	3.09E-12
<i>p</i> -value 2	/	2.52E-14	4.26E-06	9.63E-08	1.22E-10	3.09E-10
<i>p</i> -value 3	/	2.72E-15	1.04E-06	1.33E-08	1.26E-11	3.27E-11
<i>p</i> -value 4	/	0.02 / 0.59	/	/	8.88E-11	2.64E-08
<i>p</i> -value 5	/	0.01 / 0.24	/	/	4.19E-09	2.61E-07
<i>p</i> -value 6	/	0.02 / 0.35	/	/	5.88E-10	8.07E-08
<i>p</i> -value 7	/	/	0.02	0.59	4.64E-09	4.58E-10
<i>p</i> -value 8	/	/	0.01	0.24	9.68E-08	2.29E-08
<i>p</i> -value 9	/	/	0.02	0.35	1.86E-08	2.97E-09
growth rate (cm/d) \pm SD	0.68 $\pm 0,03$	0.63 $\pm 0,04$	0.70 $\pm 0,04$	0.66 $\pm 0,01$	0.66 $\pm 0,02$	0.64 $\pm 0,02$
growth distance (cm) \pm SD	15.4 ± 1.7	30.6 ± 10.2	24.3 ± 9.1	30.8 ± 7.7	57.9 ± 20.5	67.6 ± 26.6
biological replicates	25	34	26	15	22	21