Supplementary Material

Table S1. *Aspergillus fumigatus* strains used in this study.

Strain	Progenitor	Description	Reference
Δ*akuB*^{KU80}	CEA17	Δ*ku80*, *pyrG*+	[1]
A1160p+/MFIG001	A1160p-	Δ*ku80*, *pyrG*+	[2]
AFUA_1G10550 KO	A1160p+	Δ*cbfA*, hygro^r	[3]
AFUA_5G10620 KO		Δ*nctC*, hygro^r	
AFUA_7G05440 KO		Δ*fhdA*, hygro^r	
AFUA_2G01260 KO		Δ*srbA*, hygro^r	
AFUA_2G02690 KO		Δ*atrR*, hygro^r	
AFUA_2G14250 KO		Δ*nctA*, hygro^r	
AFUA_5G03920 KO		Δ*hapX*, hygro^r	
AFUA_2G14720 KO		Δ*hapB*, hygro^r	
AFUA_1G14460 KO		ΔAFUA_1G14460, hygro^r	
AFUA_2G10550 KO		Δ*rfeC*, hygro^r	
AFUA_1G09190 KO		Δ*rglT*, hygro^r	
AFUA_3G08520 KO		Δ*rlmA*, hygro^r	
AFUA_1G06900 KO		Δ*crzA*, hygro^r	
AFUA_2G12330 KO		Δ*acuM*, hygro^r	
AFUA_6G09630 KO		Δ*gliZ*, hygro^r	
AFUA_3G12890 KO		Δ*hasA*, hygro^r	
AFUA_3G14750 KO		ΔAFUA_3G14750, hygro^r	
AFUA_3G15290 KO		ΔAFUA_3G15290, hygro^r	
AFUA_4G14540 KO		Δ*tpcE*, hygro^r	
AFUA_5G10130 KO		ΔAFUA_5G10130, hygro^r	
AFUA_5G10040 KO		ΔAFUA_5G10040, hygro^r	
AFUA_7G00130 KO		Δ*fccR*, hygro^r	

Table S1. *Cont.*

Strain	Progenitor	Description	Reference
AFUA_3G03315 KO	A1160p+	*Δ*AFUA_3G03315, hygro^r	[3]
AFUA_6G08550 KO		*Δ*AFUA_6G08550, hygro^r	
AFUA_1G10280 KO		*Δ*AFUA_1G10280, hygro^r	
AFUA_6G03430 KO		*Δ*AFUA_6G03430, hygro^r	
AFUA_2G18040 KO		*ΔfgaPT2*, hygro^r	-
AFUA_6G12050 KO		*ΔpesL*, hygro^r	
AFUA_6G03480 KO	*ΔakuB*^{KU80}	*ΔfmpE*/*ΔfsqF*, PT^r	
AFUA_6G09660 KO		*ΔgliP*, PT^r	
AFUA_8G00540 KO		*ΔpsoA*, PT^r	
AFUA_8G00370 KO		*ΔfmaB*, PT^r	
AFUA_8G00420 KO		*ΔfumR*/*ΔfapR*, PT^r	
AFUA_4G14560 KO		*ΔtynC*, hygro^r	
AFUA_7G00160 KO		*ΔfccA*, PT^r	
AFUA_7G00170 KO		*ΔfccD*, PT^r	
AFUA_5G02330 KO		*Δaspf1*, PT^r	
AFUA_8G00170 KO		*ΔftmA*, PT^r	
AFUA_3G14700 KO		*Δ*AFUA_3G14700, hygro^r	

KO: Knockout (Δ) mutant; hygro^r: Hygromycin resistance; PT^r: Pyrithiamine resistance.

Table S2. Virulence of *A. fumigatus* knockout mutants lacking the same TF-encoding genes in silkworm *vs.* in mouse models.

Knockout gene ID		Generic name	Virulence in silkworm model (compared to WT*)	Data from mouse infection models (with references)			
				Virulence in mouse model (compared to WT)	Fungal WT	Mouse model	Infectious dose (spores)
AFUA_2G01260	AFUB_018340	*srbA*	Attenuated	Strongly attenuated[4]	AfS35	Leukopenic	IT, $3x10^5$
AFUA_2G02690	AFUB_019790	*atrR*	Attenuated	Strongly attenuated[4]	AfS35 Af293		IT, $3x10^5$ IT, $2.5x10^7$
AFUA_5G03920	AFUB_052420	*hapX*	Attenuated	Attenuated[5] Avirulent[5]	ATCC46645	Leukopenic Non-neutropenic	IN, $6x10^4$
AFUA_2G12330	AFUB_027990	*acuM*	Attenuated	Attenuated[6]	Af293	Leukopenic Non-neutropenic	IV, $3x10^3$ germlings IH
AFUA_3G08520	AFUB_040580	*rlmA*	Slightly attenuated	Strongly attenuated[7]	Δaku^{KU80}	Leukopenic	IN, $2.5x10^6$
AFUA_1G06900	AFUB_007280	*crzA*	Slightly attenuated	Avirulent[8] Strongly attenuated[9]	Af293 $\Delta akuB^{KU80}$	Leukopenic	IH IN 2.5-5.0x10^5
AFUA_6G09630	AFUB_075680	*gliZ*	Slightly increased	Unchanged[10] Attenuated[11]	Af293.1 B-5233	Leukopenic Non-neutropenic	IN, $2.5x10^5$ IN, $5x10^6$

*Wild type (WT) strain in this study means A1160p+ (derived from CEA10); AfS35 and Δaku^{KU80} are derived from D141 and CEA10, respectively; IT: Intratracheal infection; IN: Intranasal infection; IV: Intravenous infection; IH: Inhalation.

Table S3. Twenty-four *A. fumigatus* KO mutants tested in the silkworm model (regulatory/backbone genes of secondary metabolism).

Knockout (KO) gene ID		Generic name	SM cluster product	Virulence in silkworm model (compared to WT)	Fungal WT
AFUA_1G10280	AFUB_009690	- (TF)	Unknown	Slightly attenuated	A1160p+
AFUA_3G14750	AFUB_034470	- (TF)	Unknown		
AFUA_6G08550	AFUB_074510	- (TF)	Unknown		
AFUA_7G00130	AFUB_086680	*fccR* (TF)	Fumicycline		
AFUA_4G14560	AFUB_071800	*tynC* (PKS)	Trypacidin		$\Delta akuB^{KU80}$
AFUA_6G03480	AFUB_094810	*fmpE/fsqF* (NRPS-like)	Fumipyrrole/Fumisoquin		
AFUA_3G12890	AFUB_036300	*hasA* (TF)	Hexadehydroastechrome	Unchanged	A1160p+
AFUA_3G15290	AFUB_033930	- (TF)	Unknown		
AFUA_4G14540	AFUB_071780	*tpcE* (TF)	Trypacidin		
AFUA_5G10130	AFUB_057730	- (TF)	Unknown		
AFUA_5G10040	AFUB_057630	- (TF)	Unknown		
AFUA_6G03430	AFUB_094860	*fmpR* (TF)	Fumipyrrole/Fumisoquin		
AFUA_6G12050	AFUB_078040	*pesL* (NRPS)	Unknown		
AFUA_3G03315	AFUB_044930	- (TF)	Siderophore		
AFUA_6G09660	AFUB_075710	*gliP* (NRPS)	Gliotoxin		$\Delta akuB^{KU80}$
AFUA_8G00540	AFUB_086030	*psoA* (PKS-NRPS)	Pseurotin		
AFUA_8G00370	AFUB_086200	*fmaB* (PKS)	Fumagillin		
AFUA_8G00420	AFUB_086150	*fumR/fapR* (TF)	Fumagillin/Pseurotin		
AFUA_7G00160	AFUB_086700	*fccA* (PKS)	Fumicycline		
AFUA_7G00170	AFUB_086710	*fccD* (DMAT)	Fumicycline		
AFUA_5G02330	AFUB_050860	*aspf1*	AspF1		
AFUA_8G00170	AFUB_086360	*ftmA* (NRPS)	Fumitremorgin		
AFUA_2G18040	AFUB_033730	*fgaPT2* (DMAT)	Fumigaclavin	Slightly increased	A1160p+
AFUA_3G14700	AFUB_034520	- (PKS)	Unknown		$\Delta akuB^{KU80}$

SM: secondary metabolite; TF: Transcription factor-encoding gene; PKS: Polyketide synthase-encoding gene; NRPS: Nonribosomal peptide synthetetase-encoding gene; DMAT: Dimethylallyl tryptophan synthase-encoding gene.

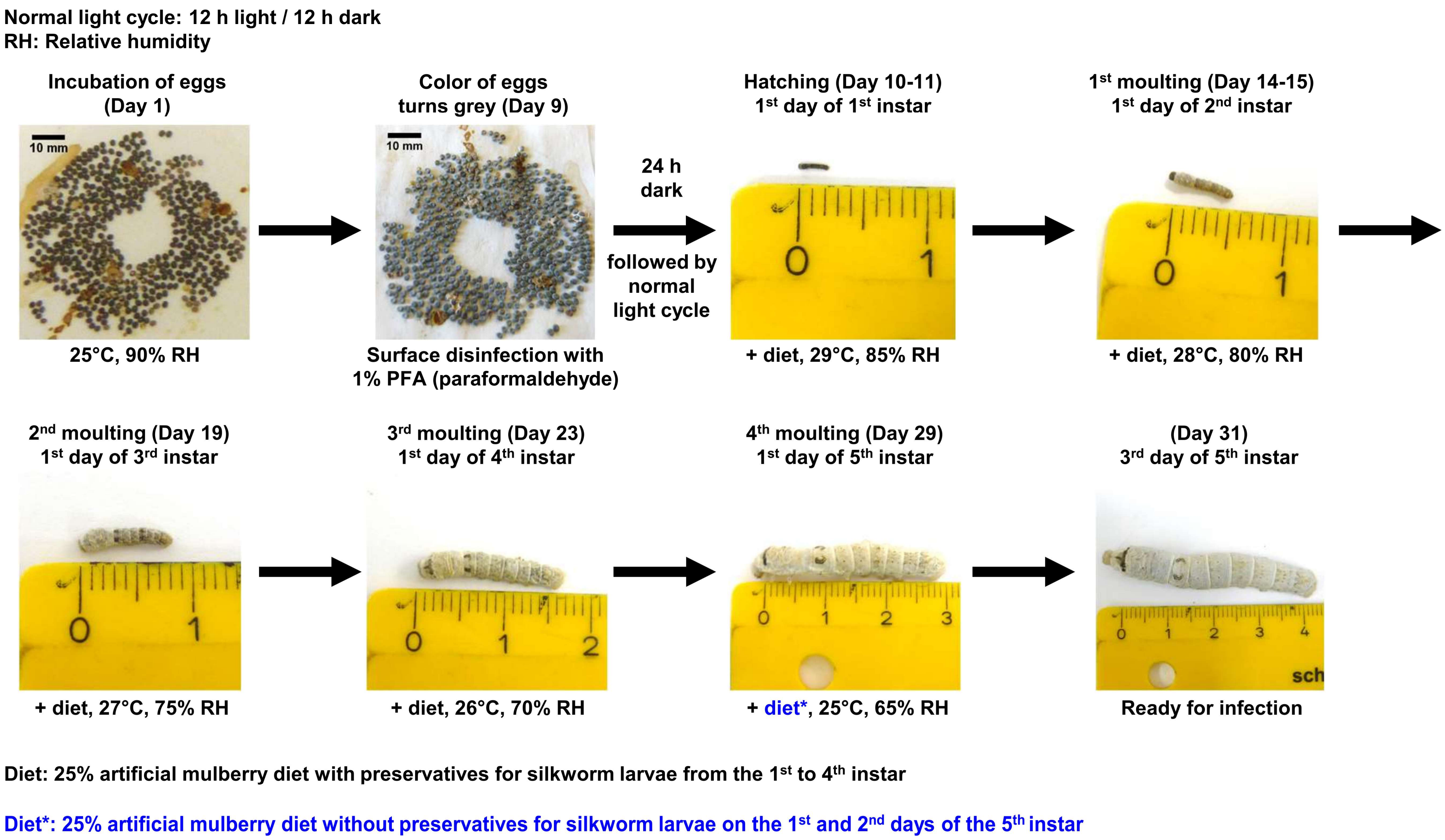

Figure S1. Simplified silkworm rearing protocol (laboratory version).

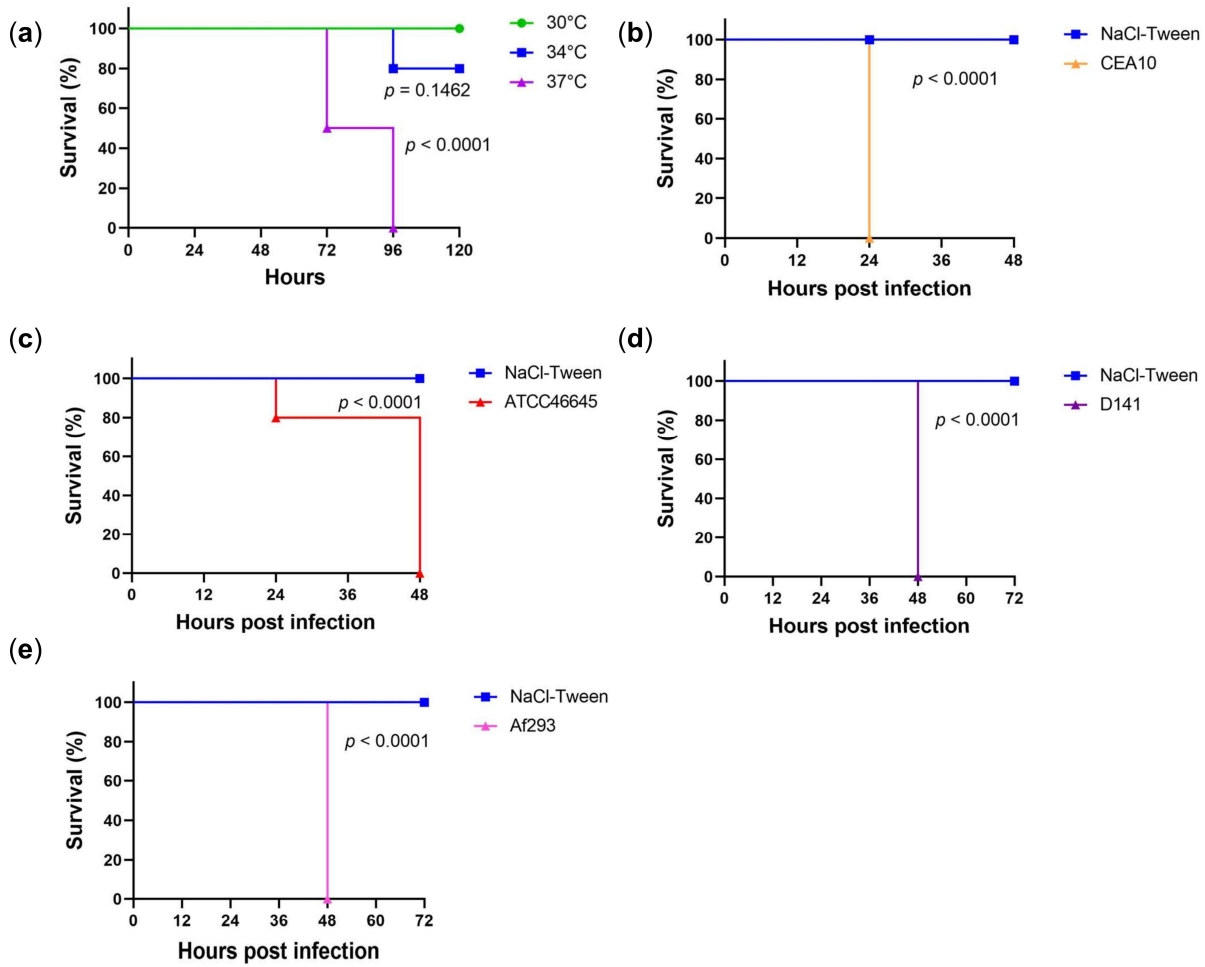

Figure S2. Kaplan-Meier survival curves of silkworms incubated at distinct temperatures without infection or infected with different clinical isolates of *A. fumigatus*. (**a**) Silkworms were fasted during the incubation at indicated temperatures (10 silkworms/group); Kaplan-Meier survival curves were compared using the log-rank test; pairwise comparisons were performed between 30°C and 34°C (p = 0.1462), as well as between 30°C and 37°C ($p < 0.0001$); (**b**)-(**e**) Silkworms were infected with indicated clinical isolates of *A. fumigatus* (10 silkworms/group); silkworms of the control group (n=10) were injected with 50 µl NaCl-Tween; Kaplan-Meier survival curves were compared using the log-rank test; pairwise comparisons were performed between silkworms infected with the indicated fungal isolate and the control group.

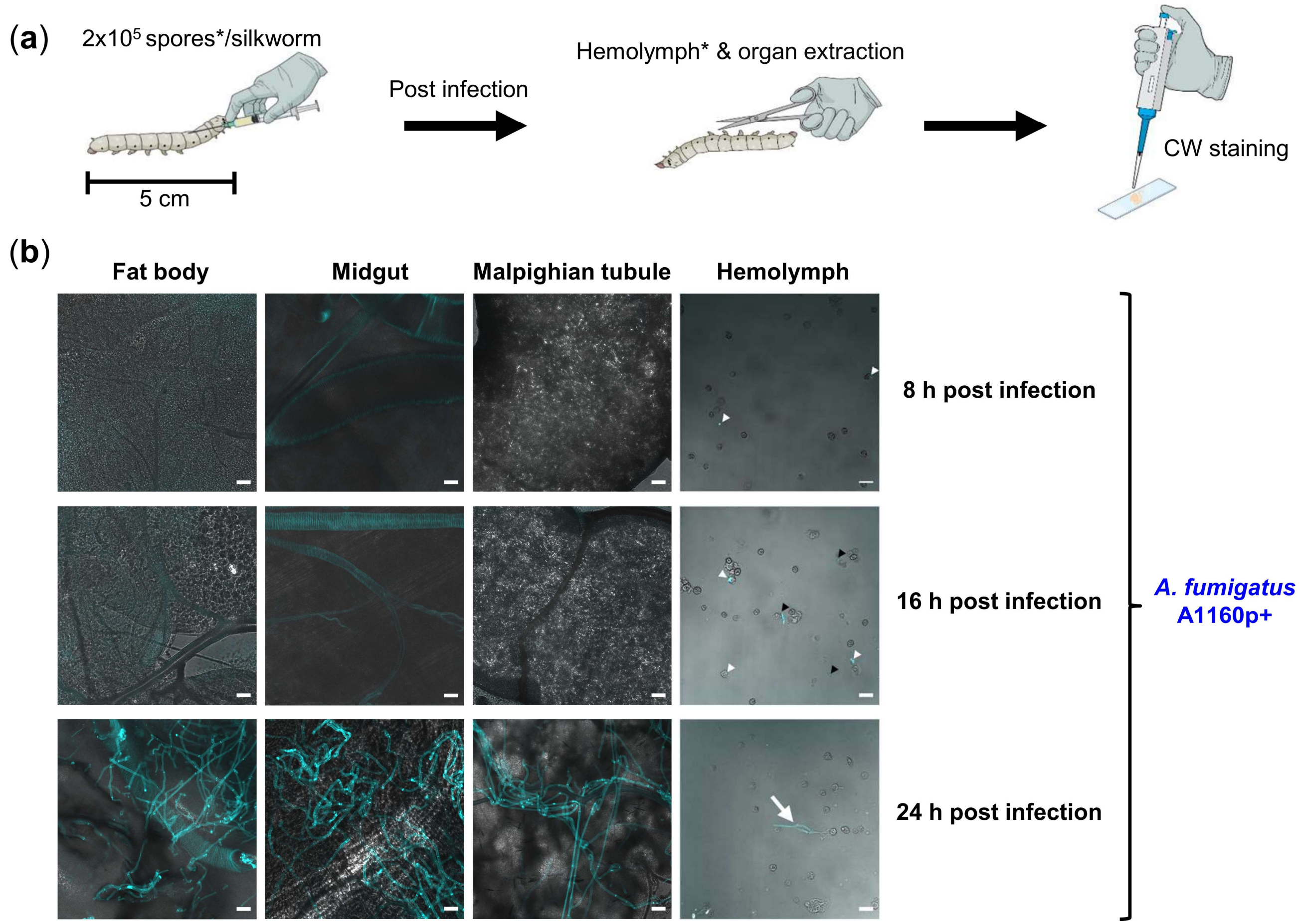

Figure S3. Visualization of fungal burden in hemolymph and organs of *A. fumigatus*-infected silkworms at different time points post infection. (**a**) Illustration of experimental setup; CW: calcofluor white; *for visualization of the fungus in the hemolymph, CW pre-stained spores were used for infection; (**b**) Merged images (calcofluor white in turquoise + brightfield) show fungal growth along the disease progression in the hemolymph: spores (white arrow heads) at 8 h, germ tubes/short hyphae (black arrow heads) at 16 h, and long hyphae (white arrow) at 24 h post infection; hyphae were only detected in the organs at 24 h post infection; scale bars represent 20 μm.

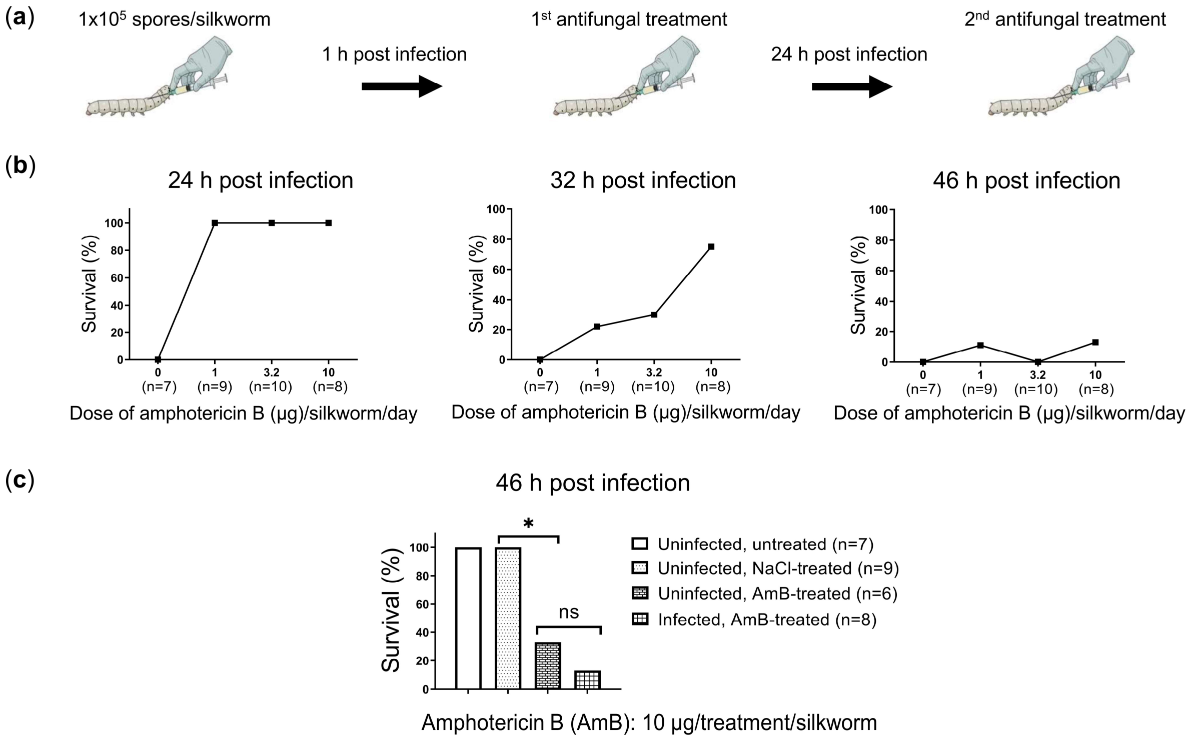

Figure S4. Therapeutic efficacy and safety of amphotericin B in *A. fumigatus*-infected silkworms. (**a**) Illustration of experimental setup, silkworms were reared at 34°C without feeding post infection; (**b**) Dose-dependent therapeutic efficacy of amphotericin B in terms of silkworm survival rate at 24 h, 32 h, and 46 h post infection; n: number of silkworms in each group; (**c**) Severe toxicity of amphotericin B (10 µg/day) in silkworms; uninfected controls were first injected with 50 µl NaCl-Tween; NaCl-treated control was injected with 40 µl 0.9% NaCl at time point 1 h and 24 h following the first injection; statistical analysis of the Kaplan-Meier survival curves of indicated groups (log-rank test; pairwise comparison) was integrated in this diagram, * means p-value < 0.05; ns means not significant (p > 0.05).

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