

Figure S1. Variation in the number of lysosomes during phagolysosome development. Number and locations of lysosomes in (a) Uninfected, (b) Phagosome-containing conidia, (c) Germling-containing phagosome, (d) Conidia-containing phagolysosome and (e) Germling-containing phagolysosome of A549 alveolar epithelial cells. Red = conidia, green = lysosome, magenta = plasma membrane. Scale Bar = 5 μ m. Magenta = Cell Mask Deep Red, Green = GFP-LAMP1, Red = MFIGRag29 (Table S1).

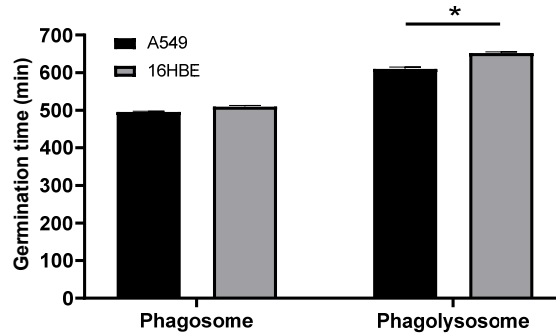


Figure S1. Germination of *A. fumigatus* spores within phagolysosomes is delayed compared to germination time within phagosomes. Data shown as mean \pm standard deviation of three biological and technical replicates. * $P < 0.05$. Asterisks represent differences between cell lines.

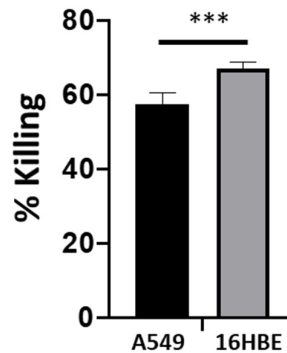


Figure S3: Proportion of killed spores within acidic phagosomes of 16HBE and A549 epithelial cell. Data shown as mean \pm standard deviation of three biological and technical replicates. Asterisks represent differences between cell lines (** $P < 0.001$).

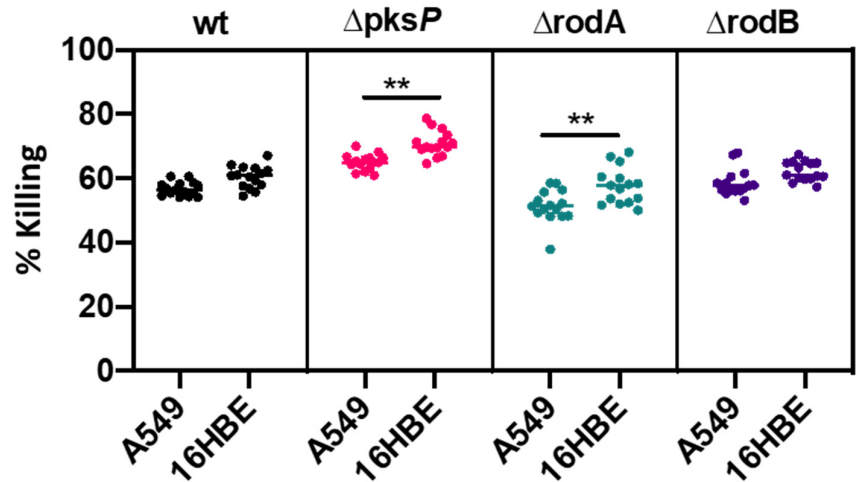


Figure S4: Percentage of *A. fumigatus* spore killed within epithelial cells. Data represents Mean and standard deviation of three biological and technical replicates. Asterisks represents differences between cell lines. (** $P < 0.001$).

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

Strain	Function	Gene name	Parental strain
A1160	-	-	CEA10 (CBS1144.89)
MFIGGFP4	Cytoplasmic GFP	-	A1163 (FGSC A1163) (<i>pyrG::βtub-GFP,pyrG⁺</i>)
MFIGRag29	Cytoplasmic RFP	-	A1163 (FGSC A1163) (<i>pyrG::βtub-TurboRFP635,pyrG⁺</i>)
A1160_ΔrodA	Rodlet layer protein A	AFUB_057130	A1160, ΔKu80 <i>pyrG⁺</i>
A1160_ΔrodB	Rodlet layer protein B	AFUB_016640	A1160, ΔKu80 <i>pyrG⁺</i>
A1160_ΔpKsP	Polyketide synthase required for DHN-melanin	AFUB_033290	A1160, ΔKu80 <i>pyrG⁺</i>

Table S2. Comparison of automated and manual spore calling results within phagolysosomes (PL) in five independent images. Cohen's K represents Kappa test.

	Manual			Automated			Cohen's K % Agreement	
	Total	Within fused PL	% Fusion	Total	Within fused PL	% Fusion		
1	25	12	48	28	15	53	0.84	92.68
2	27	6	22	23	10	43	0.65	85.18
3	44	17	38	42	19	45	0.82	91.12
4	20	4	20	35	10	28	0.42	76.92
5	39	16	41	31	14	45	0.74	91.30
Total	155	55		159	68		0.7	87.5%
% Fusion		35%			42%			

Table S3. Comparison of automated and manual spore calling results within acidified phagosomes (AF) in five independent images. Cohen's K represents Kappa test.

	Manual			Automated			Cohen's K	% Agreement
	Total	Within AP	%	Total	Within AP	%		
1	22	5	23	27	7	26	0.79	93.10
2	27	8	30	23	5	21	0.57	84.73
3	35	10	28	37	6	16	0.64	89.13
4	23	2	8	19	2	10	0.43	84
5	38	6	16	34	7	20	0.90	97.56
Total	145	31		140	27		0.66	89.7
% Acidified		21%			19%			