# SUPPLEMENTARY INFORMATION

## High-throughput format for the phenotyping of fungi on solid substrates

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## Supplementary Figures Legends

**Supplementary Fig. 1**. In-well analysis of the distribution of the signal. A grid of 5x5 squares was designed and absorbance values were individually quantified at each of the 25 positions. The signal is uniformly distributed in the center of well and showing lower values at the border of the wells, which correspond with areas shared between the inside of the well and the walls. This experiment was performed in an EnSpire Multimode plate reader (Perkin Elmer).

**Supplementary Fig. 2.** Correlation between inoculum size and lag phase. Serial dilutions of spores were inoculated on minimal solid media containing ammonium as the sole nitrogen source and incubated at 37 °C in the plate reader. Absorbance was recorded for 48 h. The lag phase was plotted against the logarithmic concentration of spores in the inoculum, and the trend line was calculated. The data shows an inverse logarithmic correlation with a coefficient of determination ($R^2$) of 0.994. Data is the average of four independent experiments performed at least in duplicates. Error bars depict the standard error of the sample.

**Supplementary Fig. 3.** Quantitative assessment of the deleterious effects of nitrosative stress on the growth of *A. nidulans*. Using our platform here we have quantified growth of the wild type and the double mutant. The plot displays the growth curves of both strains in ammonium minimal media containing 0, 0.75 or 1.5 mM dNO. Both strains displayed the same growth pattern in the absence of dNO. Addition of dNO to the media increased the length of the lag phase, and this increase was more drastic in the case of the double mutant, *ΔfhbA ΔfhbB*, that hardly grew in the presence of 1.5 mM dNO. The experiment also included both single mutants (not depicted here for clarity). We employed the lag phase as an indicator of the growth of the culture and it was inversely normalized to the lag phase found in the wild type grown under control conditions (ammonium media in the absence of dNO). The experiment was repeated three times, although only one experiment is represented here as an example. Figure 3 contains the calculations of the three independent experiments and errors bars depicting the standard error of each sample.

**Supplementary Fig. 4**. Growth curves corresponding to the samples in Fig. 5b.

**Supplementary Fig. 5**. Growth curves corresponding to the samples in Fig. 6c-d.

**Table S1**

Strains used in this study

| *A. nidulans* strains | Genotype* | Reference |
|---|---|---|
| Cib08 | *biA1 yA2 veA1* | [14] |
| | *argB2 biA1 veA1* | FGSC¶ |
| | *pyroA4 veA1* | FGSC |
| TNO2A21 | *riboB2 pyroA4 nku::argB veA1* | [28] |
| CibA | *ΔfhbA biA1 yA2 veA1* | [14] |
| CibB | *ΔfhbB biA1 yA2 veA1* | [14] |
| CibAB | *ΔfhbA ΔfhbB biA1 yA2 veA1* | [14] |
| Ag07C1#7 | *ΔkdmB::AfriboB ΔhepA::AfpyrG pabaB22 pantoB100 ΔniaD Δnku::argB(?)* | [16] |
| *aflR*(p)::egfp | *aflR(p)::egfp::afriboB::(ΔaflR) riboB2 pyroA4 Δnku::argB veA1* | This study |
| *aflR*(p)::egfp Δ*kdmB* | *aflR(p)::egfp::afriboB::(ΔaflR) kdmBΔ::AfriboB riboB2(?) ΔniaD Δnku::argB(?) veA1* | This study |
| | *gamma-actin::gfp pyroA4 wA3* | From Claudio Scazzocchio |
| | *H1::rfp pabaA1 wA3* | From Claudio Scazzocchio |
| *F. fujikori* strains | | |
| *TRI5*(p)::egfp | *TRI5(p)::egfp, hph, ΔTRI5* | This study |
| *CPS/KS*(p)::egfp | *CPS/KS(p)::egfp, hph, ΔCPS/KS* | This study |

* All strains are *veA1*

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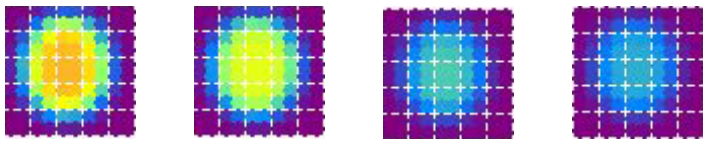
**Table S2**

Primers used in this study.

| Oligo | Sequence |
|---|---|
| YRC_CPS/KS_5F | TAACGCCAGGGTTTTCCCAGTCACGACGCTTCCACCTGTGATTGAGCTCC |
| YRC_CPS/KS-GFP_R | TACTTACCTCACCCTTGGAAACCATCTTGTGTAAGTATTCCAGATCCAC |
| YRC_CPS/KS_3F | TCCTTCAATATCATCTTCTGTCTCCGACAGGGAATACTATGGAGCTGG |
| YRC_CPS/KS_3R | GCGGATAACAATTTCACACAGGAAACAGCGAATCTCAGGTCTGATGGTGG |
| YRC_TRI5_5F | TAACGCCAGGGTTTTCCCAGTCACGACGGACGGCAGACAGCGGCTATACG |
| YRC_TRI5-GFP_R | TACTTACCTCACCCTTGGAAACCATGATGGCAAGGTTGTACTGG |
| YRC_TRI5_3F | TCCTTCAATATCATCTTCTGTCTCCGACGATACTCGTTTGGAGAATGGTGG |
| YRC_TRI5_3R | GCGGATAACAATTTCACACAGGAAACAGCTCGAGCATCGACATCGAGCATCG |
| gfp-F | ATGGTTTCCAAGGGTGAGG |
| hph-F | GTCGGAGACAGAAGATGATATTGAAGGAGC |
| YRC_*aflR*_5F | TAACGCCAGGGTTTTCCCAGTCACGACGAAGCGAAGCACCTCATCAACGG |
| YRC_*aflR*-GFP_R | TACTTACCTCACCCTTGGAAACCATGATATTTGCATATGATACAGG |
| YRC_*aflR*_3F | GGTGTATGCTCGTCACACTCATGACGAGTGTTGGGTGAATGG |
| YRC_alfR_3R | GGATAACAATTTCACACAGGAAACAGCTCTCCGCAATCGCACTTCGCAGG |
| Tgluc-RIBO_R | AGCCAGACTCCTGAACGGCCTCATCTTGTTGGGGGGAAGGGG |
| pRIBO-F | GAGGCCGTTCAGGAGTCTGGCTCG |
| tRIBO-R | CCATTCACCCAACACTCGTCATGAGTGTGACGAGCATACACC |
| hyg_split-mark_F | GGATGCCTCCGCTCGAAGTA |
| hyg_split-mark_R | CGTTGCAAGACCTGCCTGAA |
| GGS2-D-DR2 | TGATCACCGAGGTCTCGTCTTCGG |

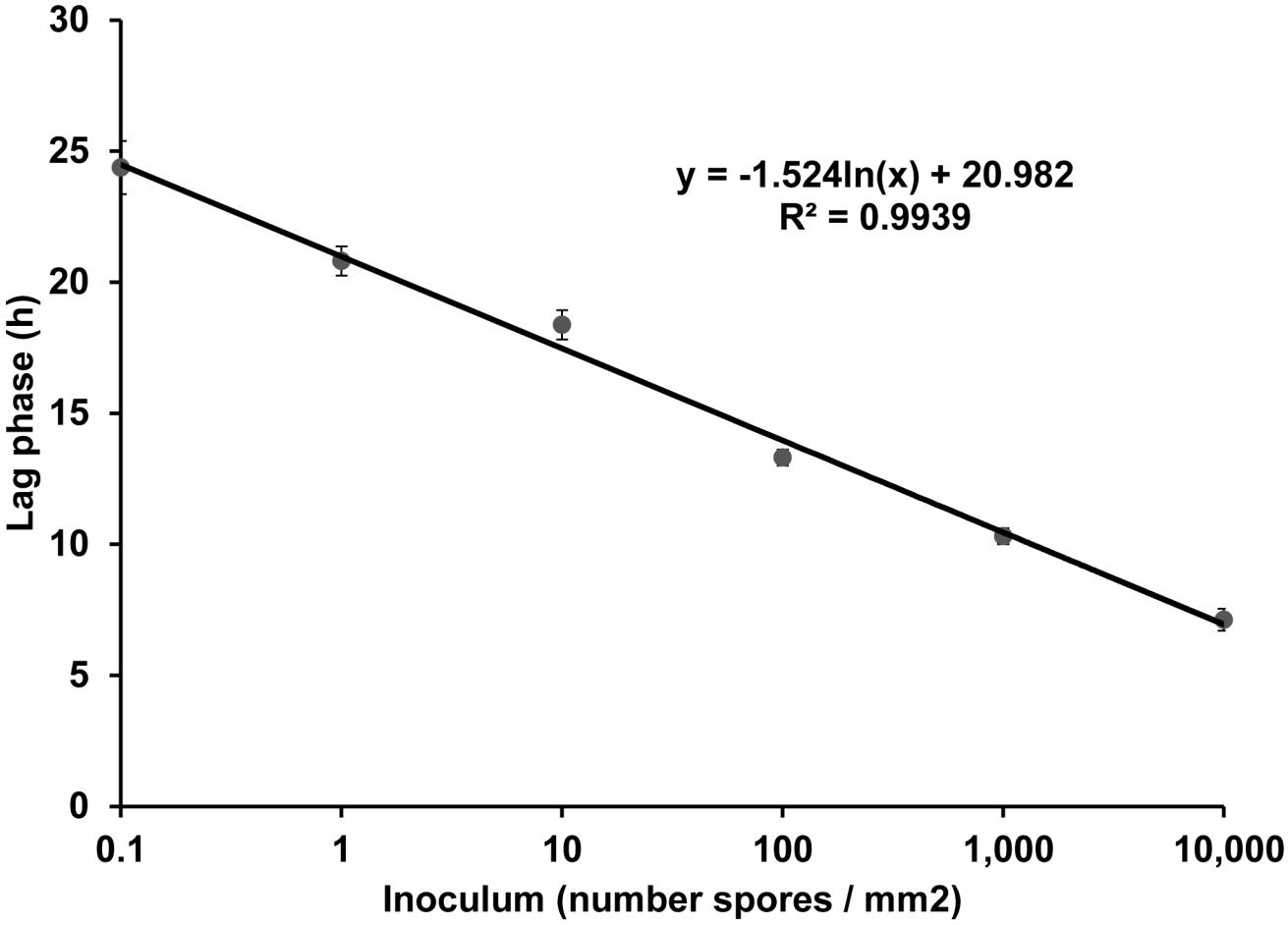
| | |
|---|---|
| ogfp_seqR1 | CGTCTCCCTCACCCTCTCCG |
| pCSN44-trpC-P2 | GTGATCCGCCTGGACGACTAAACC |
| Seq-P450-3R | AGCCTATGGAAGCTTGTAGC |
| pCSN44-trpC-P | CCTCCACTAGCTCCAGCCAAGCCC |
| TRI5-downstream_R | CTCCGACGTATTCGAAGC |
| dPCR_IL_*aflR*up_5F | GCTGACATCCACGATGGTGG |
| dPCR_IL_GFP-out-R | CGACCAAGATTGGAACGACGC |
| dPCR_IL_RIBOout_R | GACTTAGCCAAGTCGACACG |
| dPCR_IL_*aflR*down_3R | GACGGTCTACCTAGTGACCG |

# Supplementary Figure 1



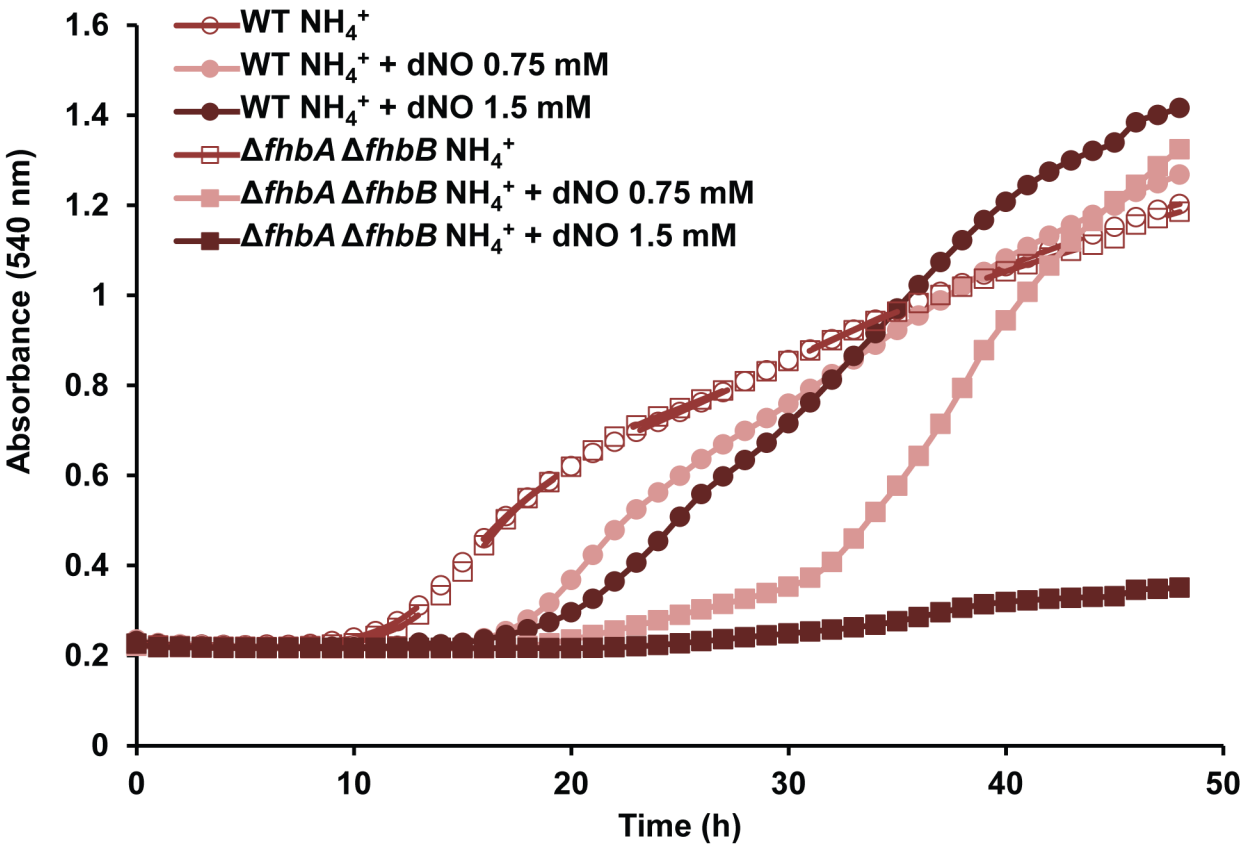
**Supplementary Fig. 1**. In-well analysis of the distribution of the signal. A grid of 5x5 squares was designed and absorbance values were individually quantified at each of the 25 positions. The signal is uniformly distributed in the center of well and showing lower values at the border of the wells, which correspond with areas shared between the inside of the well and the walls. This experiment was performed in an EnSpire Multimode plate reader (Perkin Elmer).

# Supplementary Figure 2




**Supplementary Fig. 2**. Correlation between inoculum size and lag phase. Serial dilutions of spores were inoculated on minimal solid media containing ammonium as the sole nitrogen source and incubated at 37 °C in the plate reader. Absorbance was recorded for 48 h. The lag phase was plotted against the logarithmic concentration of spores in the inoculum, and the trend line was calculated. The data shows an inverse logarithmic correlation with a coefficient of determination ($R^2$) of 0.994. Data is the average of four independent experiments performed at least in duplicates. Error bars depict the standard error of the sample.

# Supplementary Figure 3

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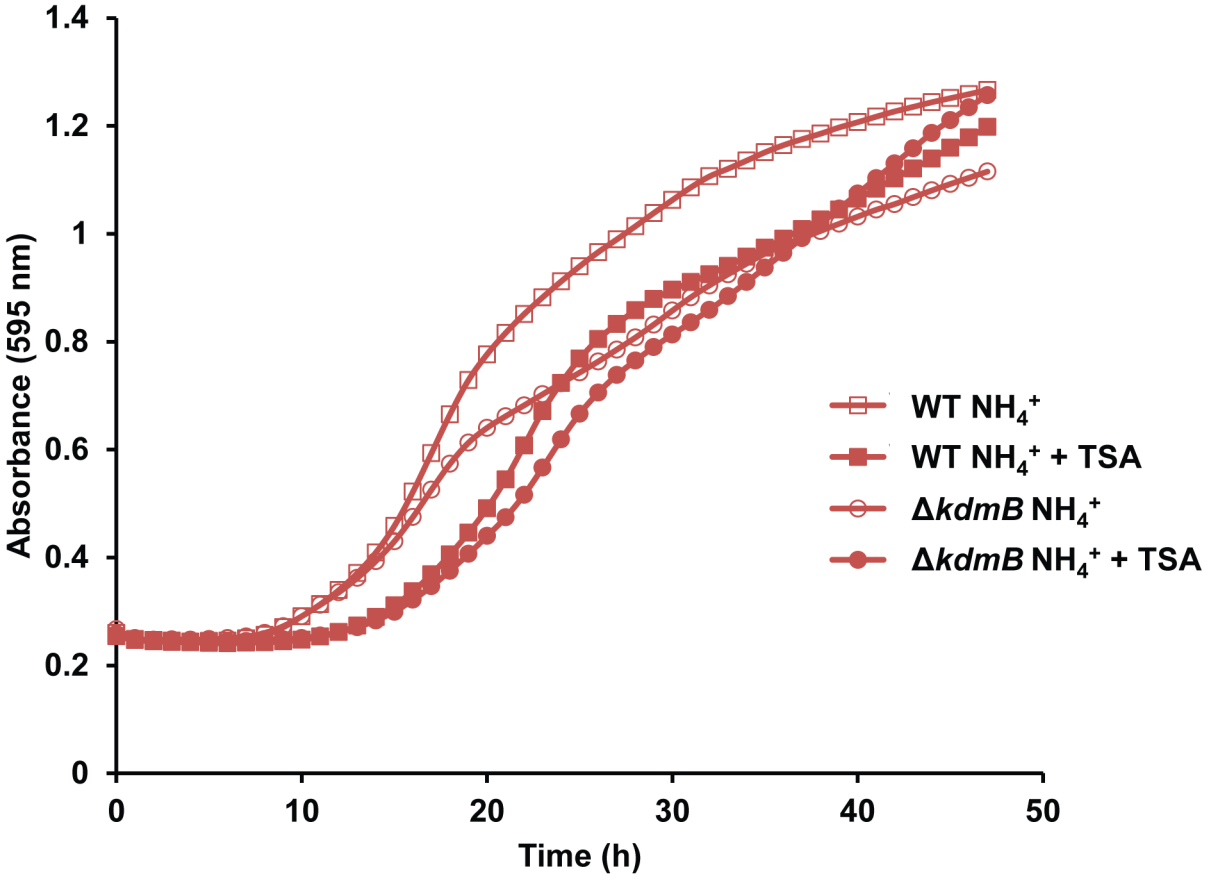
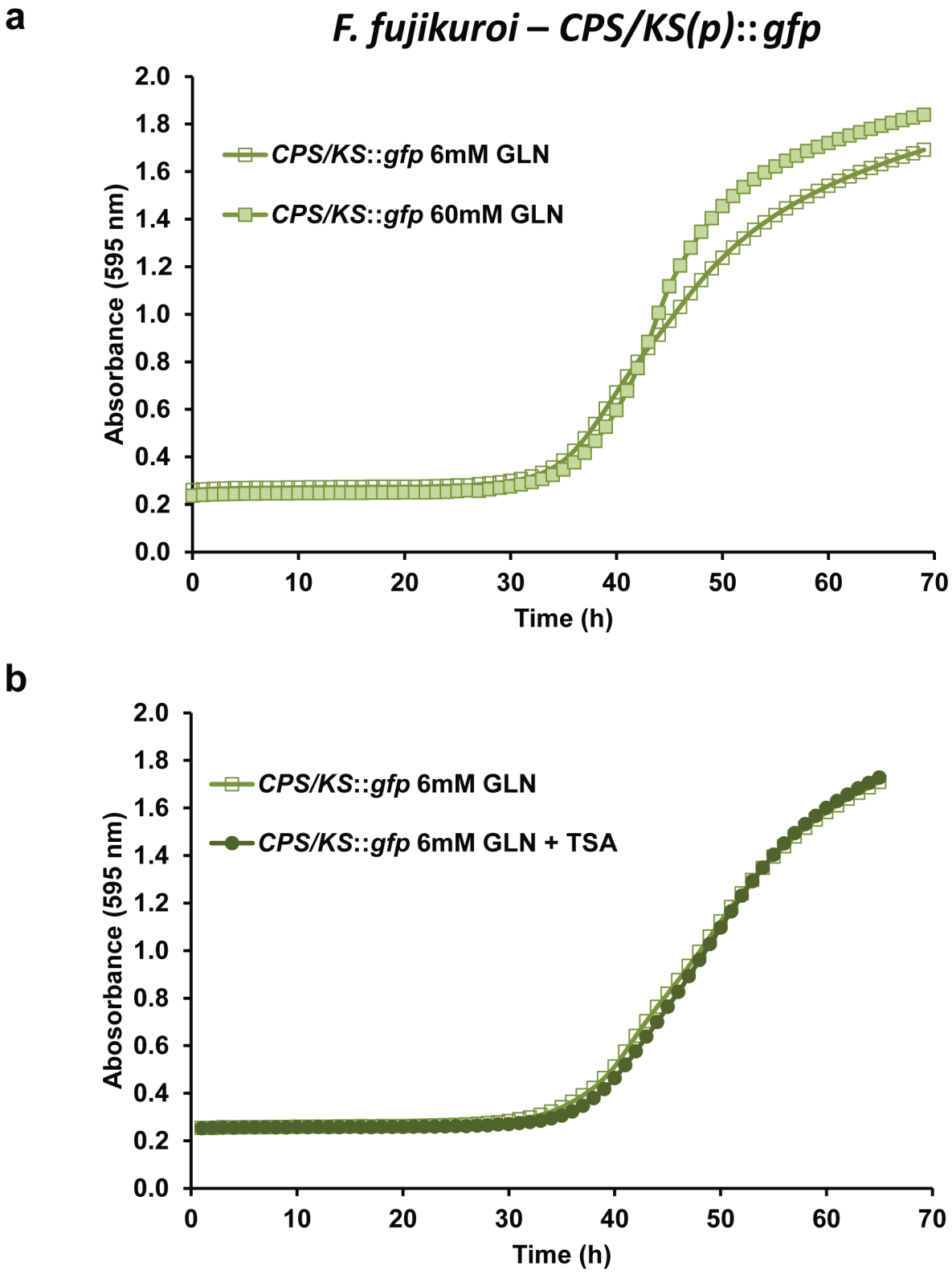
# Supplementary Figure 4




**Supplementary Fig. 4**. Growth curves corresponding to the samples in Fig. 5b.

# Supplementary Figure 5




**Supplementary Fig. 5**. Growth curves corresponding to the samples in Fig. 6c-d.