**In vivo competitive fitness profiling reveals protein kinases required for adaptation of Aspergillus fumigatus to the murine host environment**

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**Introduction**

Annual mortality due to fungal infections exceeds that caused by malaria. More than 10 million people worldwide suffer from lung diseases caused by the environmental mould Aspergillus fumigatus [1]. Only a few drugs are available to treat fungal infections, and resistance against these drugs is rising. There is an unmet medical need for new therapeutics to treat drug-resistant infections [1].

Our understanding of the factors that drive pathogenicity in A. fumigatus are limited. Functional genomic approaches have been used to great effect to establish an in depth understanding of factors that govern pathogenicity in several bacterial species. As part of the A. fumigatus genome-wide knockout program [2], we have defined a complement of 154 protein kinase encoding genes and generated a library of 120 genetically barcoded protein kinase null mutants.

We have employed Bar-seq to characterise the fitness profiles of the protein kinase null mutant collection when exposed to a series of nutritional and environmental stressors. Clustering of these fitness profiles reveals groups of kinases that phenocopy each other. Only some of these groups form previously defined signalling pathways. We have also performed Bar-seq in a murine infection model and identify that the Yak1 kinase plays an important role in virulence and adaptation to iron depleted environments.

**Protein kinase Yak1 is required for virulence and this is linked to its role in adaptation to metal ion depletion**

**Key insights**

1. Competitive fitness profiling can provide a robust dataset for uncovering factors involved in pathogenicity in A. fumigatus and help define the mechanistic basis for virulence.
2. Competitive fitness profiles can link protein kinases within signaling cascades and provide a useful sensor to assess how fungi perceive their environment.
3. The Yak1 null mutant exhibits a number of significant phenotypic defects especially under low iron, high temperature and itraconazole induced stress.
4. The Yak1 null mutant exhibits virulence defects in larvae that are linked with its inability to adapt to iron depletion.
5. Fitness defects in vitro are not absolute indicators of pathogenicity, but provide valuable insights.

**REFERENCES**