

# A *Turandot* family gene promotes immunity against sexually transmitted fungal infections in *Drosophila melanogaster*

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## STIs & Immune anticipation

- Mating is fraught with danger of contracting sexually transmitted infections (STIs) [1].
- Pre-emptive activation of immune responses prior to sexual congress could mitigate the costs of STIs [2].
- Anticipatory immune response should also incur fitness costs as otherwise constitutive expression is expected.

## The *Turandot* gene family

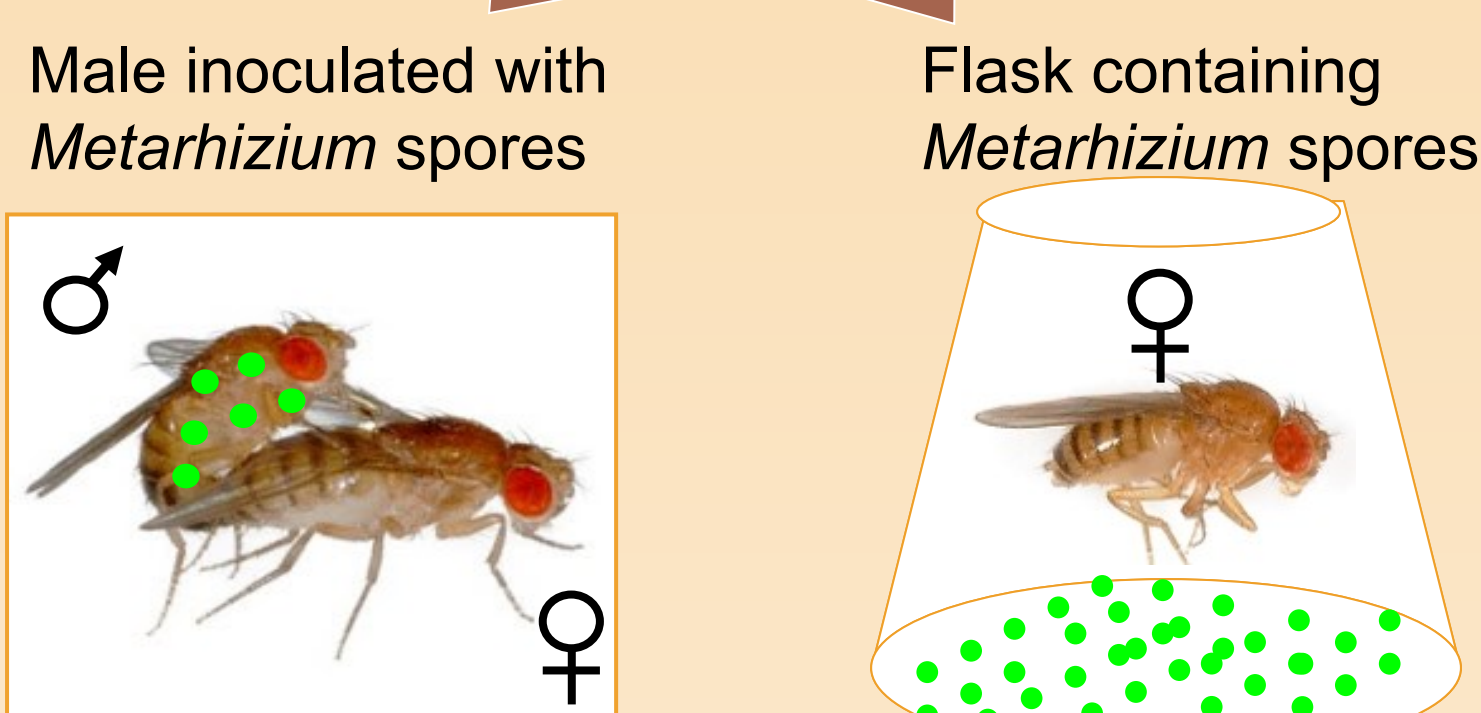
- Female *Drosophila* up-regulate the expression of *Turandot M* and *C* (*TotM* and *TotC*) when they hear the courtship song of the male [3].
- A family of rapidly evolving immune and stress response genes with no known homologs outside *Drosophila* [4].
- *TotM* expression is strongly induced by topical fungal infections [4] and mating [5].

## Hypothesis & Methods

We examined the hypothesis that *TotM* confers protection against fungal STIs using the model entomopathogenic fungus *Metarhizium robertsii*. We used the **Gal4/UAS RNAi** system of targeted gene expression knockdown to assess protection provided by three immune genes: *TotM*, *TotC* and *Dorsal-related immunity factor* (*Dif*).



Four day old adult female

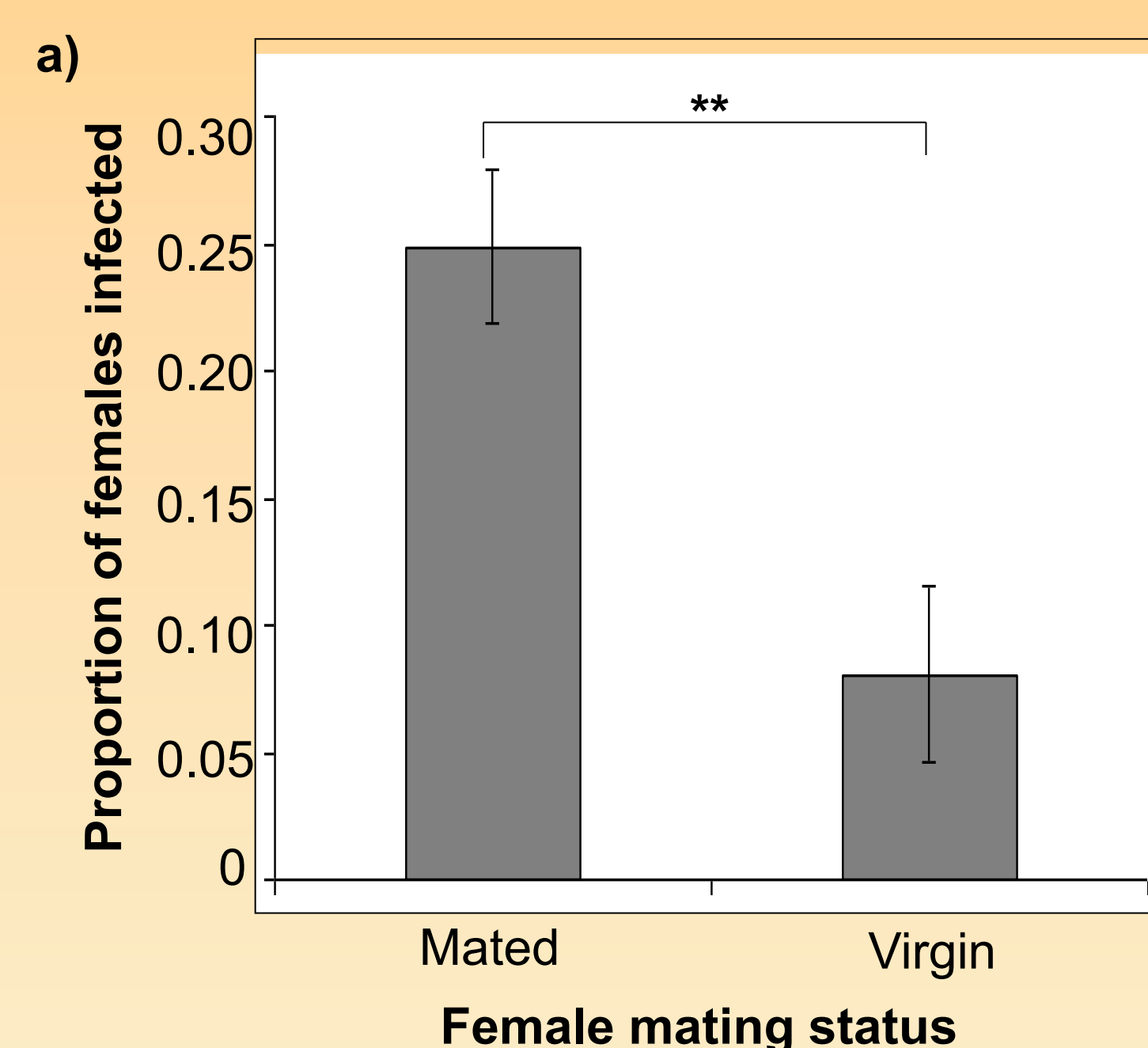


Survival and fecundity assays

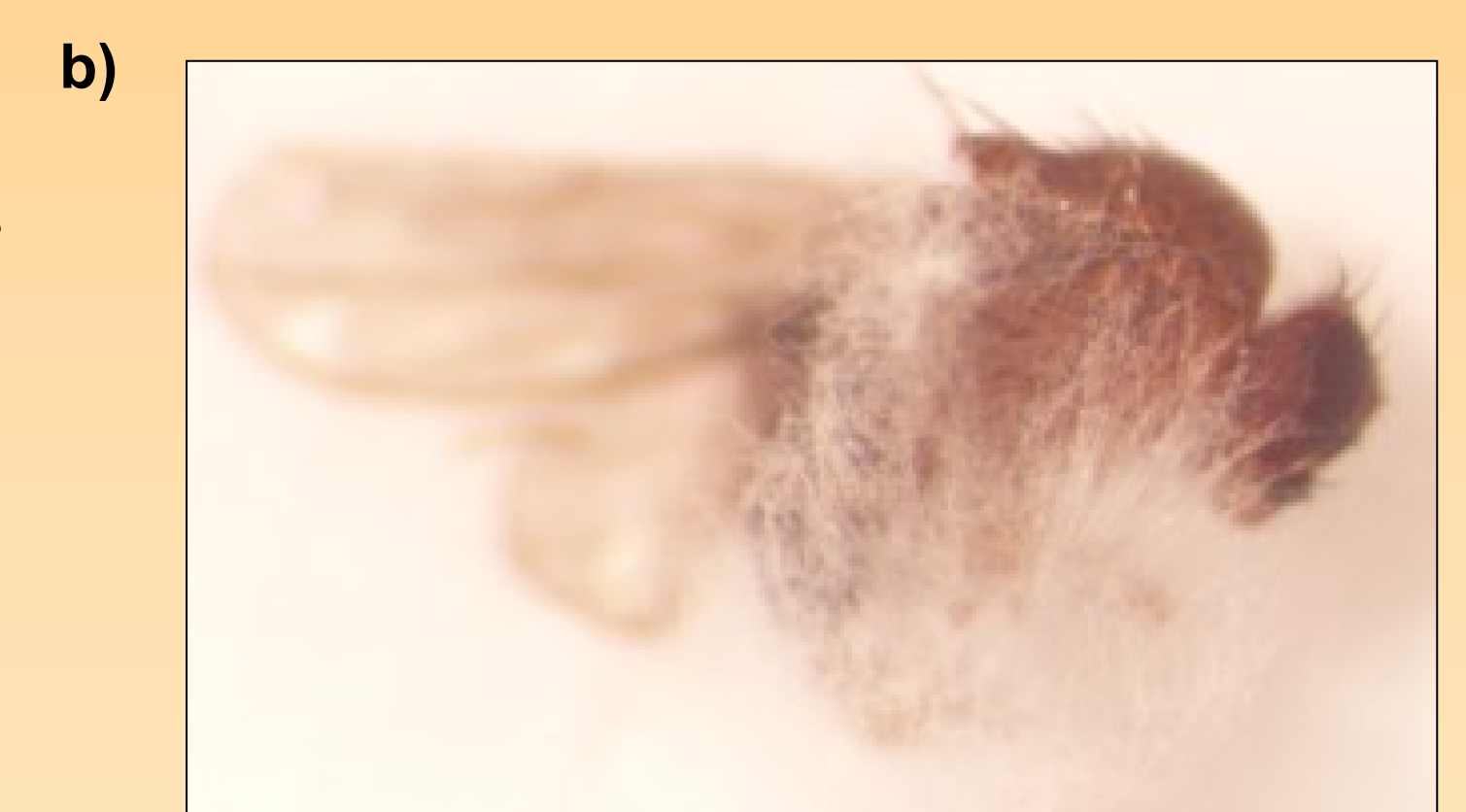
## Summary

1. *Turandot M* provides specific immunity against fungal STIs, but has pleiotropic effects on survival and fecundity in the absence of STIs.
2. Female up-regulation of *TotM* expression in response to male courtship song may represent an example of immune anticipation of mating [2].
3. *Drosophila*-*Metarhizium* is a promising model system for the study of insect STIs, mating and immunity.

## 1. Sexual transmission of *Metarhizium* in *Drosophila*

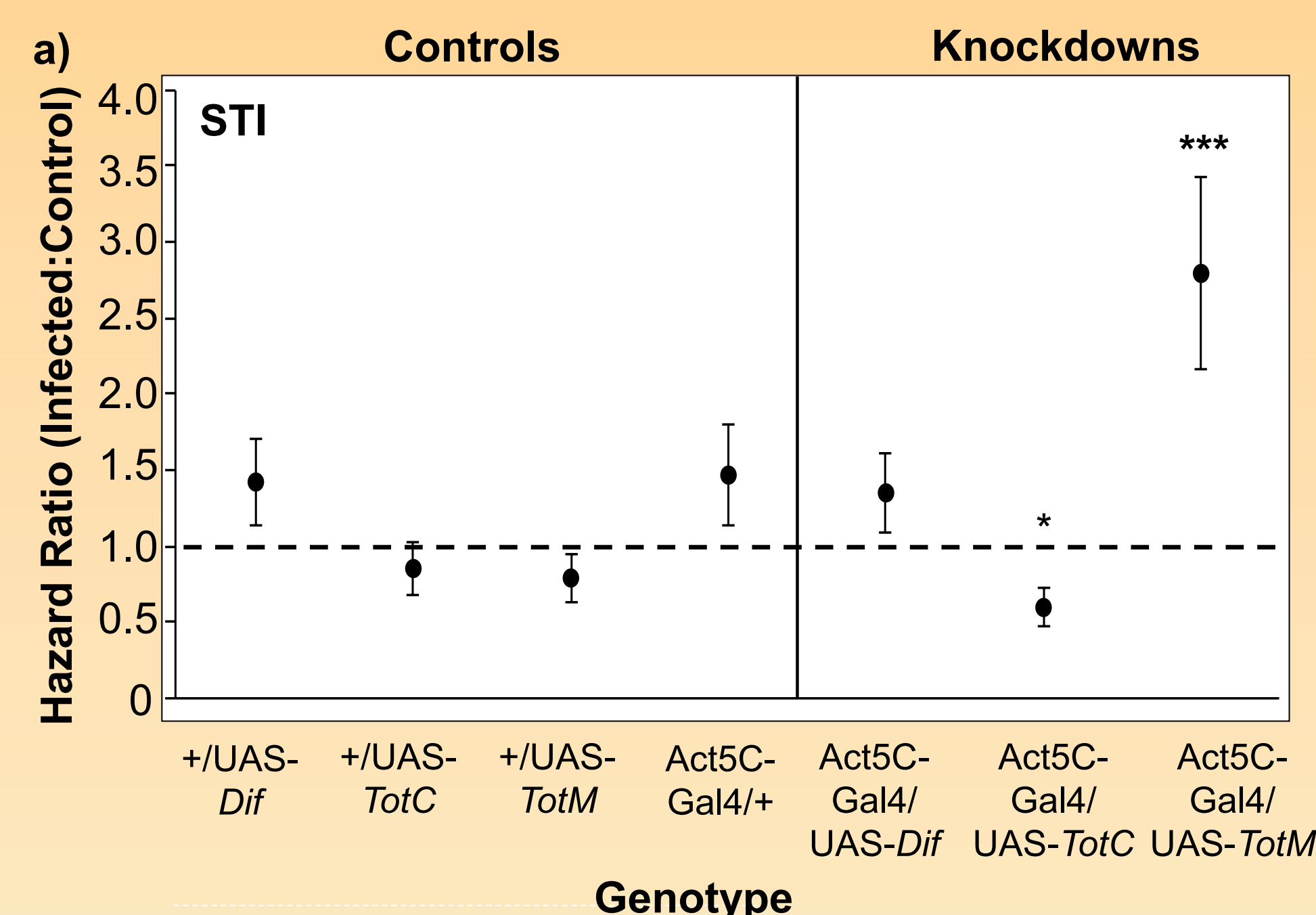


a, when kept with a *Metarhizium*-inoculated male, naïve virgin females that mated were much more likely to acquire infection than those that did not mate ( $\chi^2_1=8.96$ ,  $p=0.0028$ ). Subsequent experiment found that naïve females were eight times more likely to be infected when kept with treated males than when kept with treated females (20.9% vs 2.6%).

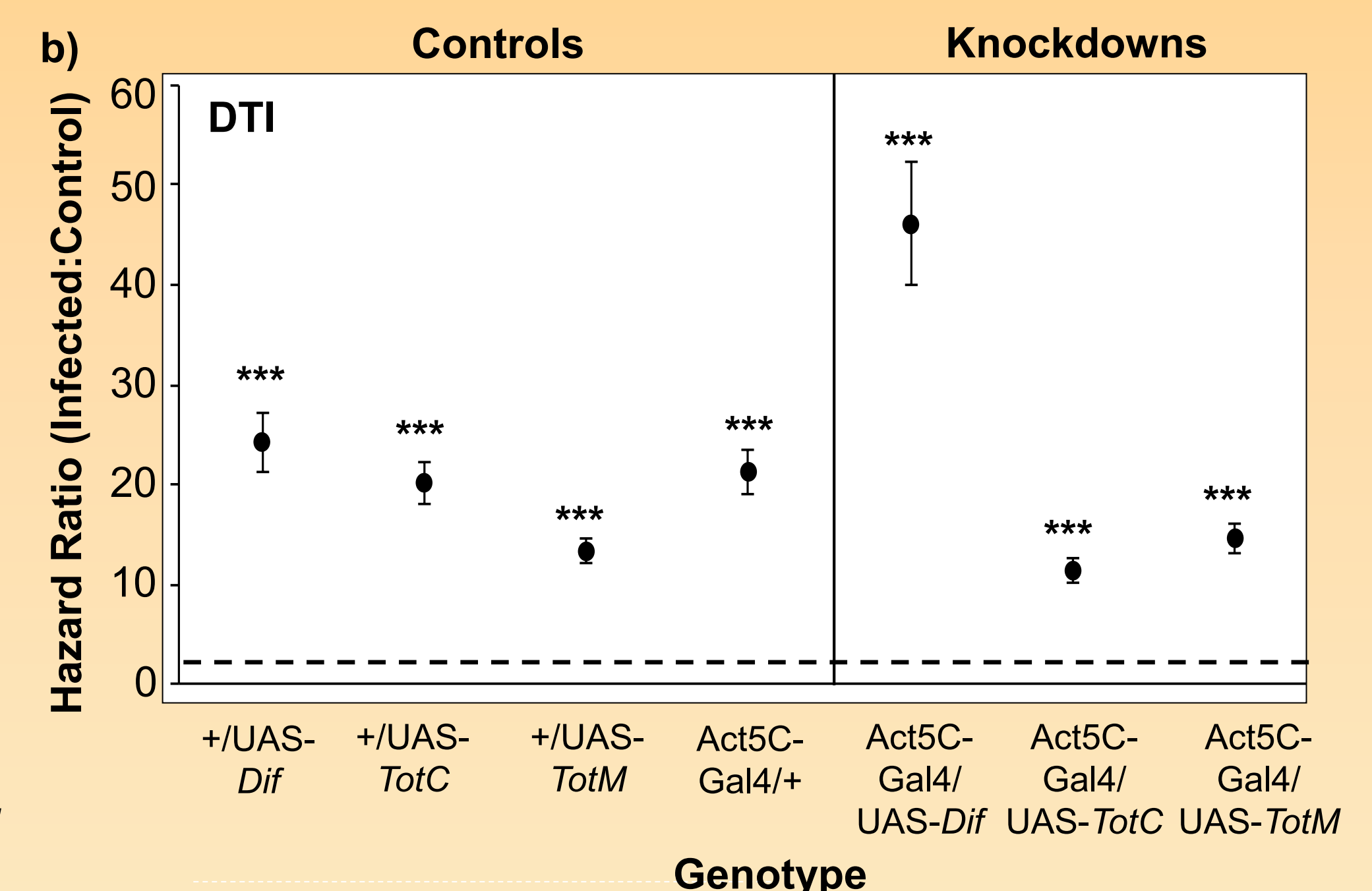


b, *Metarhizium* mycelial growth emerging from an infected fly cadaver. All females were scarified after 24 hours exposure to infected males and incubated at 28°C for 5 days. Cadavers were then examined for signs of *Metarhizium*-like fungal growth

## 2. *Turandot M* enhances survival under sexually transmitted infection (STI) but not direct topical infection (DTI)

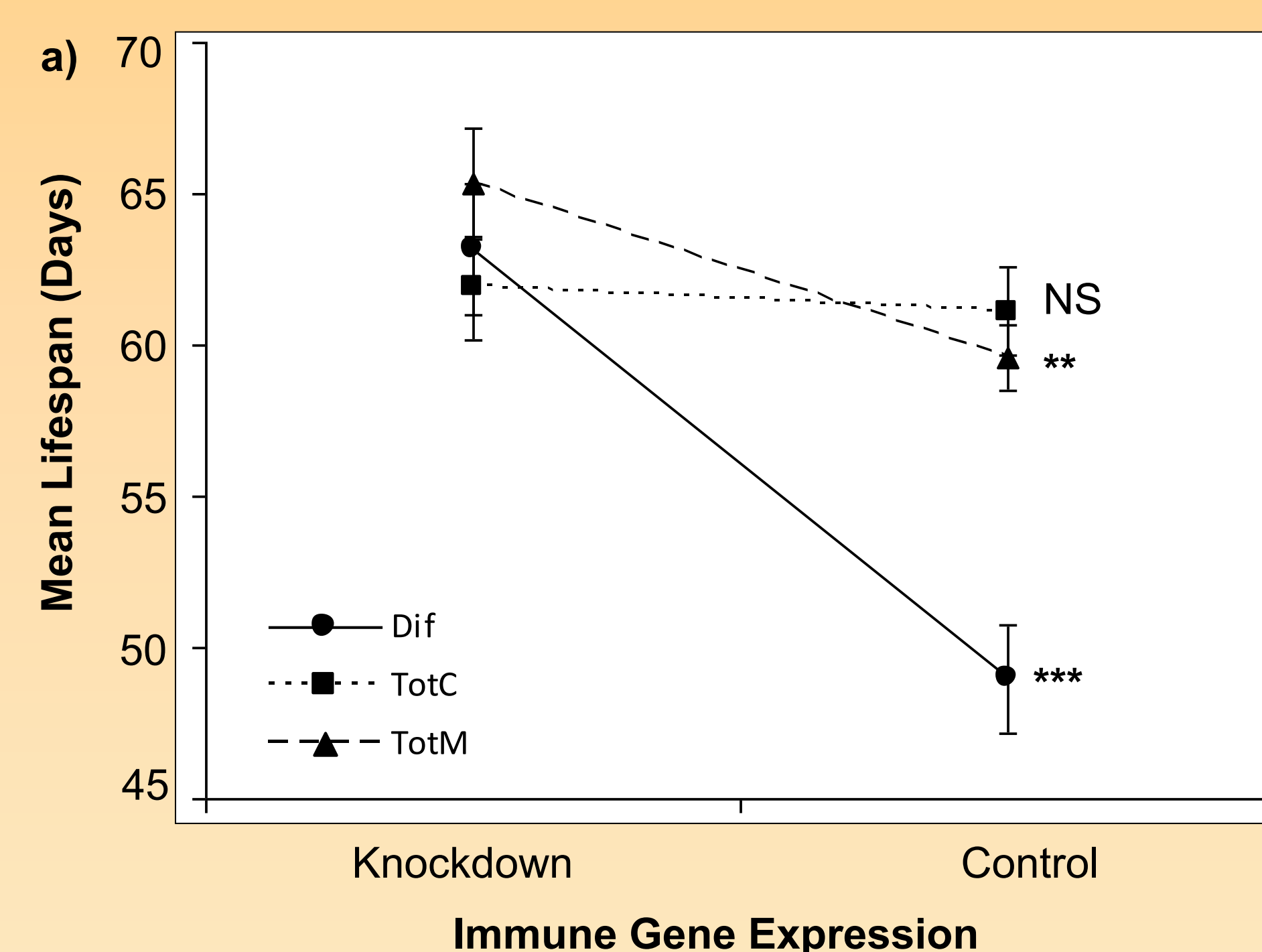


a, Cox proportional hazard ratios of STI relative to uninfected controls with standard errors. Dotted line denotes a hazard ratio of 1, which indicates equal risk of death for infected and control animals. \* indicates statistical significance of  $p < 0.005$  and \*\*\* indicates  $p < 0.001$

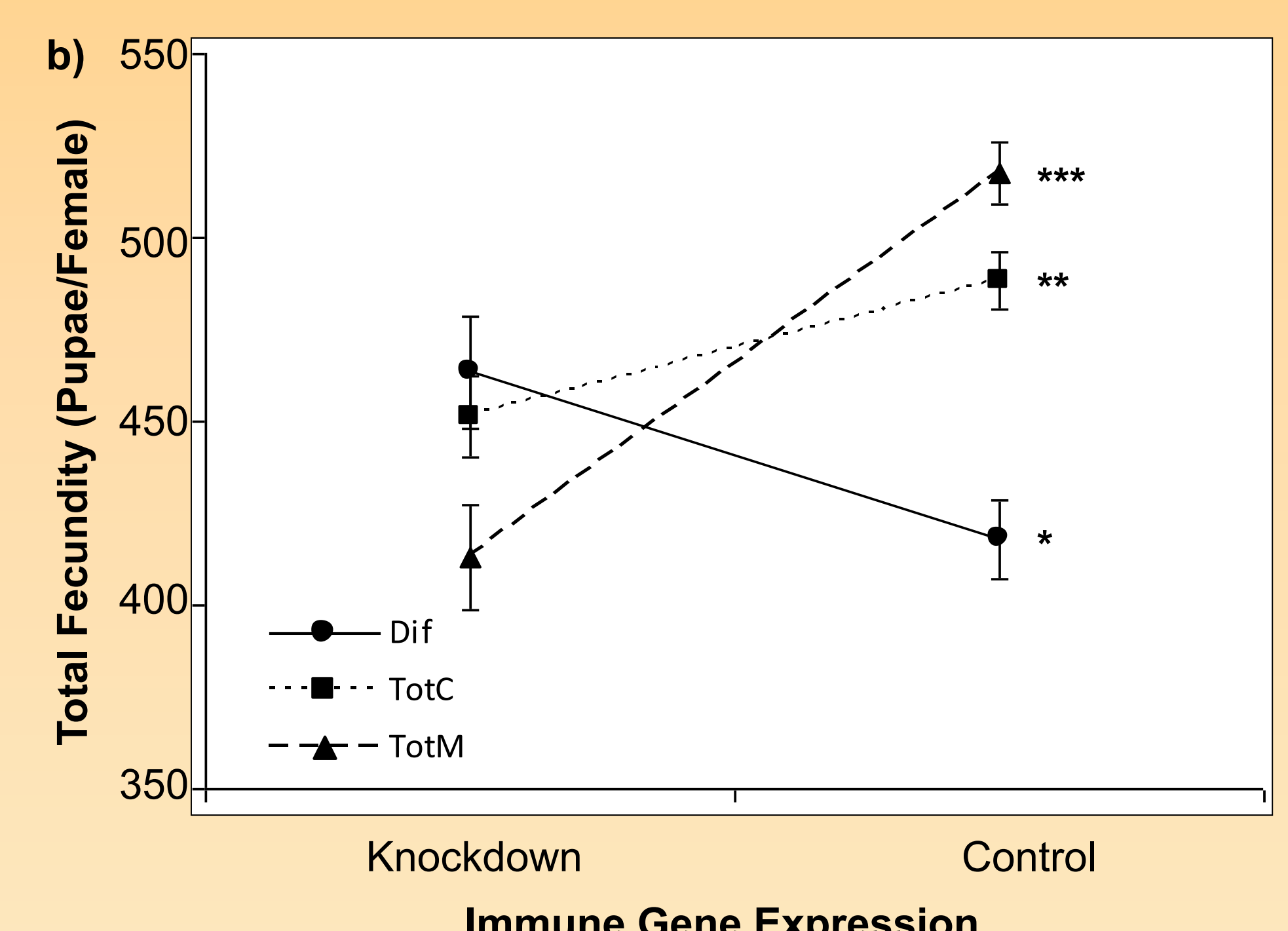


b, Cox proportional hazard ratios of DTI relative to uninfected controls with standard errors. Dotted line denotes a hazard ratio of 1, which indicates equal risk of death for infected and control animals. \*\*\* indicates statistical significance of  $p < 0.001$

## 3. *Turandot M* expression entails survival cost but fecundity benefit



a, the survival cost of immune gene expression. Statistical significance was based on Cox proportional hazard regression of the survival curves of knockdown and its combined control. Statistical significance is indicated by the following: NS,  $p > 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .



b, the fecundity cost of immune gene expression. Statistical significance was based one-way ANOVA of total number of enclosed pupae during the first 9 days post-inoculation. Statistical significances is indicated by the following: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .