

Restoration of proteostasis in the endoplasmic reticulum reverses an inflammation-like response to cytoplasmic DNA in *Caenorhabditis elegans*

Ashley B. Williams*, Felix Heider*, Jan-Erik Messling*, Matthias Rieckher*, Wilhelm Bloch †, and Björn Schumacher*

*Institute for Genome Stability in Ageing and Disease, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany

*Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), Center for Molecular Medicine Cologne (CMMC) and Systems Biology of Ageing Cologne, University of Cologne, Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany

†Department of Molecular and Cellular Sports Medicine, German Sports University, 50933 Cologne, Germany

Running title: *C. elegans* response to DNA

Keywords: innate immunity, DNA sensing, inflammatory responses, *Caenorhabditis elegans*, DNase II

Corresponding author:

Dr. Björn Schumacher

University of Cologne
Institute for Genome Stability in Aging and Disease
Joseph-Stelzmann-Str. 26
Cologne 50931
GERMANY

bjoern.schumacher@uni-koeln.de

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Controls for the cytoplasmic DNA imaging in Figure 2 and explanation of pharynx size measurement in Figure 3. **a.** DAPI staining of *nuc-1* worms mock-infected with OP50. **b.** Diagram of the method used to calculate the surface area of the pharyngeal bulbs (see the Materials and Methods).

Figure S2. Supplemental data for the *fshr-1(RNAi)* experiment shown in Figure 6. **a** and **b.** Plots showing that *fshr-1* RNAi does not affect the basal pumping rates of wildtype (left) or *nuc-1* (right) worms when fed OP50. **b** and **c.** Confirmation of the RNAi efficacy via *P. aeruginosa* (PA14) sensitivity assays. Shown are Kaplan-Meier survival plots of PA14-infected wildtype (left) and *nuc-1* (right) worms. The statistical significance was calculated via the log-rank test; *** $P < 0.001$.

Figure S3. Supplemental data showing that *pmk-1* is likely not involved in the ODN-dependent tissue declines. **a.** Plot showing that the *pmk-1* RNAi does not affect the basal pumping rate in *nuc-1* worms fed OP50. **b.** Plot showing that *pmk-1(RNAi)* induces sensitivity to PA14 as indicated by sharp drops in the pharyngeal pumping rates at 12 and 24 h post-infection. For a and b, the data are given as the mean \pm S.D, and the statistical significance was assessed via unpaired, two-tailed Student's *t* tests; *** $P < 0.001$. **c.** A Kaplan-Meier survival plot of PA14-infected *nuc-1* worms with and without *pmk-1* RNAi. The statistical significance was calculated via the log-rank test; *** $P < 0.001$. **d.** Plot showing the effect of *pmk-1* RNAi on the pumping rate of ODN-infected *nuc-1* worms. The statistical significance was calculated via two-way ANOVA; n.s., not significant.

Figure S4. Controls for the proteostasis experiments. **a.** Plot showing the relative expression levels of the XBP-1 target gene *hsp-4* in UPEC-infected *nuc-1* worms with and without tunicamycin treatment 48 h post-infection. The statistical significance was assessed via an unpaired, two-tailed Student's *t* test; * $0.01 < P < 0.05$. **b.** Experimental timeline for the results shown in c. L4 *nuc-1* larvae were infected with UPEC with and without tunicamycin and 4 μ 8c. Their pharyngeal pumping rates were then scored 48 h post-infection. **c.** Plots of the pharyngeal pumping rates of UPEC-infected *nuc-1* worms treated as described in b. The pumping rate of each individual animal is shown and the data are summarized as the mean \pm S.D. **d.** Schematic of UPR^{ER} activation via *sams-1* depletion. **e.** Plots of the pharyngeal pumping rates of UPEC-infected wildtype and *nuc-1* worms with control RNAi (*gfp*) or RNAi against *sams-1*. **f.** Plots of the pharyngeal pumping rates of OP50 mock-infected *nuc-1* worms. For c, e and f, the statistical significance was assessed via unpaired, two-tailed Student's *t* tests; n.s. $P > 0.05$, ** $0.01 > P > 0.001$, *** $P < 0.001$, **** $P < 0.0001$.