Characterization of the impact of GMP/GDP synthesis inhibition on replicative lifespan extension in yeast

Ping Liu[1,2,*], Ethan A. Sarnoski[1,2,*], Tolga T. Olmez[1,2], Thomas Z. Young[1,2], and Murat Acar[1,2,3,4,#]

[1] Department of Molecular Cellular and Developmental Biology, Yale University

   219 Prospect Street, New Haven, CT 06511

[2] Systems Biology Institute, Yale University

   850 West Campus Drive, West Haven, CT 06516

[3] Interdepartmental Program in Computational Biology and Bioinformatics, Yale University

   300 George Street, Suite 501, New Haven, CT 06511

[4] Department of Physics, Yale University

   217 Prospect Street, New Haven, CT 06511

[*] These authors contributed equally to this work.

[#] To whom correspondence should be addressed: murat.acar@yale.edu

SUPPLEMENTARY INFORMATION

I. Supplementary Text

II. Supplementary Figures S1-S3

III. Supplementary References

I. SUPPLEMENTARY TEXT

Longevity Placement Test

<u>L</u>ongevity <u>P</u>lacement <u>T</u>est (LPT) is a mutually exclusive, collectively exhaustive method to determine the relationship of a longevity intervention to a known genetic regulator of lifespan. For a given intervention that extends lifespan, any of three possible relationships may exist relative to a known genetic regulator of lifespan: (1) the intervention may act to extend lifespan independently from the known regulator; (2) the intervention may act downstream from the known regulator, converging on a single component of the known regulator's lifespan pathway; (3) the intervention may act upstream from or upon the known regulator, ultimately modulating lifespan through the genetic regulator.

In Step 1 of the LPT, the longevity intervention is applied to a strain in which a genetic regulator of lifespan is deleted. In the event that lifespan extension from the longevity intervention is observed in this background, only possibilities (1) and (2) above remain valid (Fig. S1A). If no lifespan extension is observed, then possibilities (2) and (3) remain valid. Possibility (2) cannot be ruled out in this step, since non-saturating action by the genetic regulator could leave room for lifespan extension by the longevity intervention, while saturating action would preclude it.

In Step 2 of the LPT, an epistatic agent, which prevents lifespan extension from the longevity agent, is applied to a strain in which some upstream member of the genetic regulator's lifespan pathway has been modified to extend lifespan. This step differentiates possibility (2) from the remaining possibility after Step 1. In the event that lifespan extension from the genetic regulator's pathway is suppressed by the epistatic agent, this determines that possibility (2) is correct. In the event that no epistasis is observed, the remaining possibility, (1) or (3), is correct (Fig. S1B).

Preconditions, both physical and experimental, exist for an LPT experiment to conclusively relate a longevity intervention to a given pathway. Two physical factors must exist for an exhaustive LPT test: a longevity agent to test, and an epistatic agent that prevents lifespan extension from the longevity agent. Importantly, the epistatic agent must not directly affect the longevity agent, such as through inactivating it. Ideally, it should exert an opposing effect on some downstream target, ensuring that its suppression will be generalizable to any actor upstream of the longevity agent's target. There also exist constraints on the choice of genetic manipulations for investigation. For Step 1, probe gene deletion should not shorten RLS, as this may mask longevity effects[1]. For Step 2, the intervention which extends RLS must act upon or upstream from the Step 1 probe gene in order to create complete coverage of the pathway. However, the intervention in Step 2 need not always be a gene deletion; for example, deletion of a gene in Step 1 could be complemented by an overexpression in Step 2.
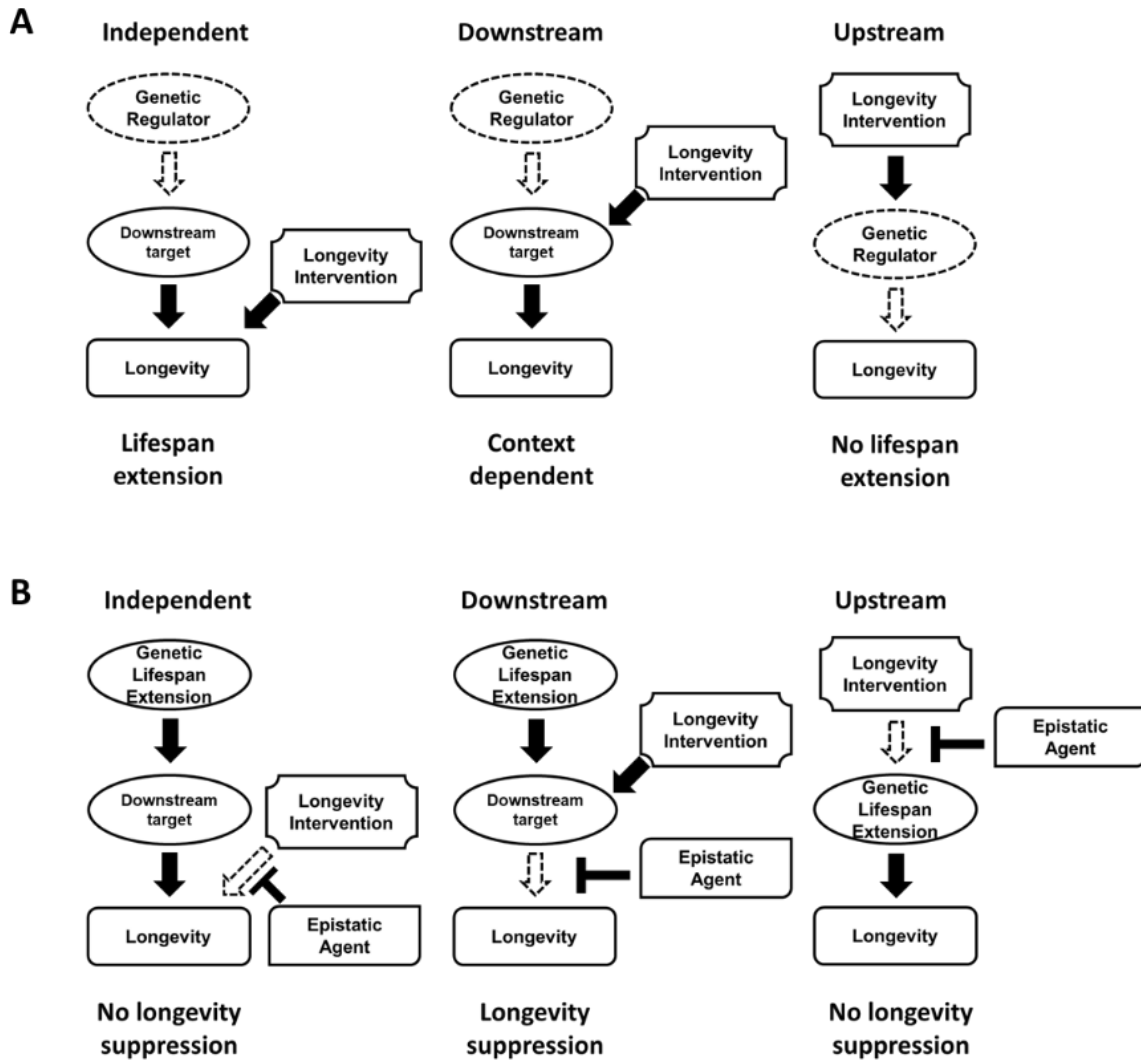
Figure S1. Possible outcomes of the longevity placement test. Possible network architectures, and the outcomes of the LPT in Step 1 (**A**) and Step 2 (**B**). Interactions shown in dashed lines represent those that are prevented, either by the suppression agent, or via deletion of the gene.
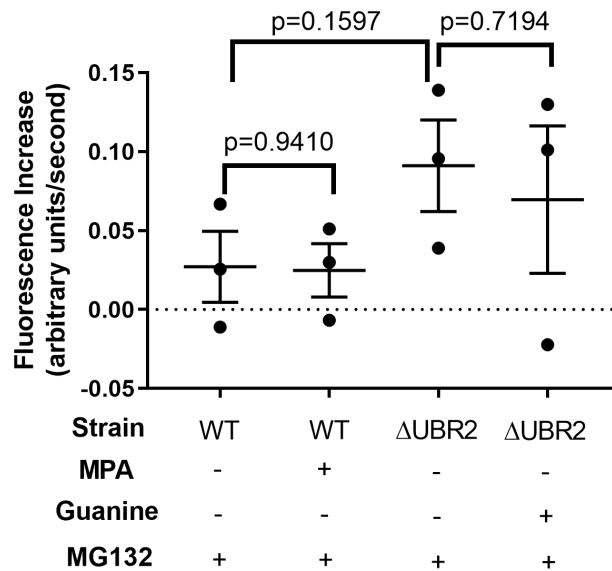
Figure S2. Negative control for proteasome activity measurement. The proteasome activity experiment shown in Fig. 3C is controlled for by adding MG-132, a proteasome inhibitor, to separate wells of the experiment run concurrently. The low rate of fluorescence increase in the presence of MG-132 indicates that our measurements were specific to the proteasome.
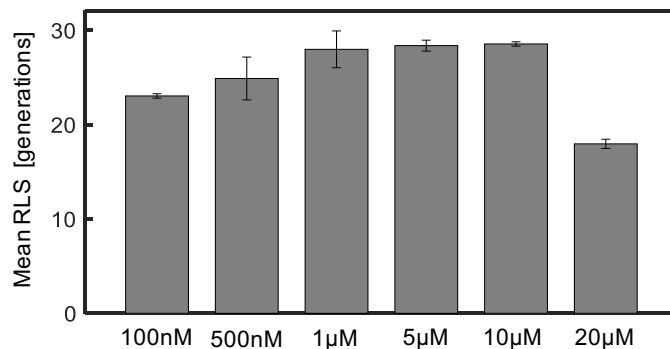

Figure S3. Dose-response characterization of MPA. Mean replicative lifespan across multiple MPA concentrations. Error bars denote S.E.M. (N=2-3). For each MPA concentration, mean RLS from 2 biological replicates was calculated, except for 10µM MPA, for which mean RLS from 3 biological replicates was calculated. Each replicate contributed 100 cells, except for 500nM MPA, for which there were 95 and 105 cells contributed by the two replicates.

III. SUPPLEMENTARY REFERENCES

1. Delaney, J. R. *et al.* Sir2 deletion prevents lifespan extension in 32 long-lived mutants. *Aging Cell* **10,** 1089–1091 (2011).