

# Evolutionary capacitance driven by HSP90 during the *de novo* evolution of multicellularity

Dung Lac<sup>1</sup>, Sayantan Datta<sup>1</sup>, Kai Tong<sup>1</sup>, Kristopher Montrose<sup>2</sup>, Anthony Burnett<sup>1</sup>, Gonensin

Ozan Bozdag<sup>1</sup>, Juha Saarikangas<sup>2</sup>, and William Ratcliff<sup>1</sup>

<sup>1</sup>Georgia Institute of Technology, Atlanta, Georgia, United States

<sup>2</sup>University of Helsinki, Helsinki, Finland



RATCLIFF LAB

<https://ratclifflab.biosci.gatech.edu/>

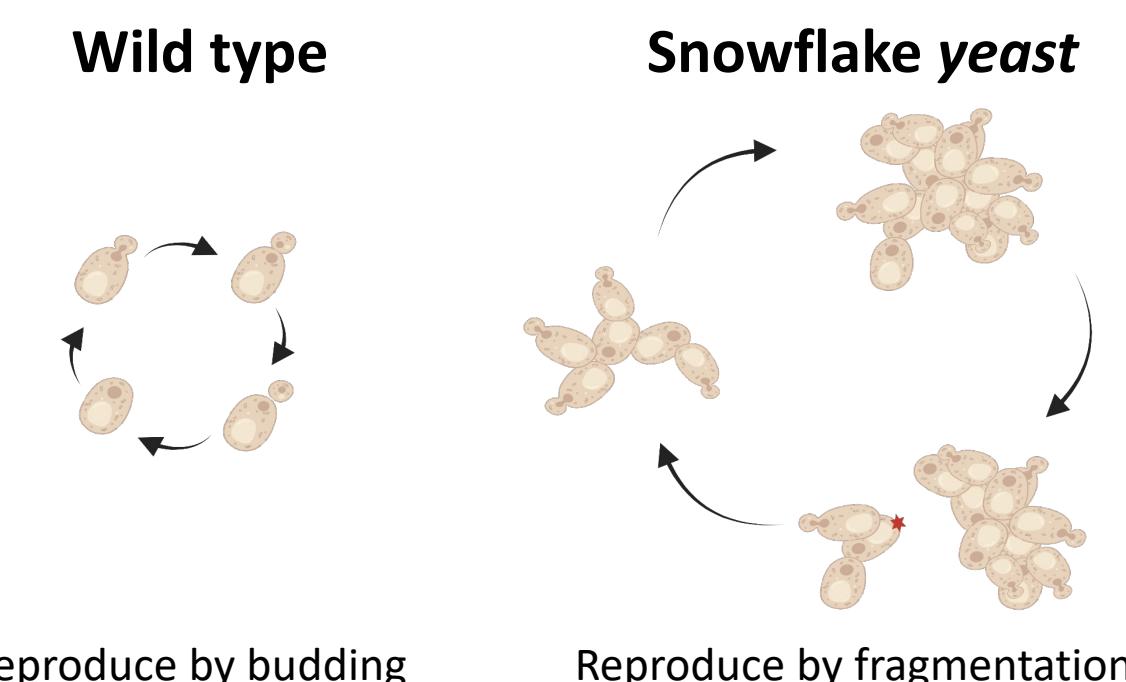
dlac3@gatech.edu

## Snowflake yeast

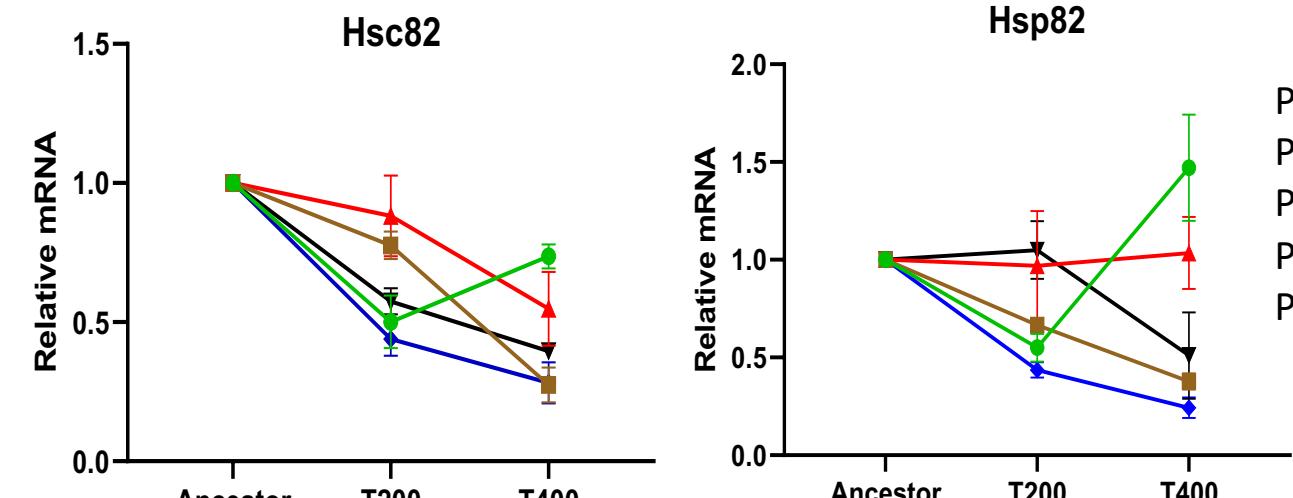
Model system of nascent multicellularity which is capable of *in vitro* evolution [1, 2].

**Snowflake yeast**  
*Saccharomyces cerevisiae*  
ace2::KANMX4/ace2::KANMX4 knockout  
Undifferentiated multicellular cluster

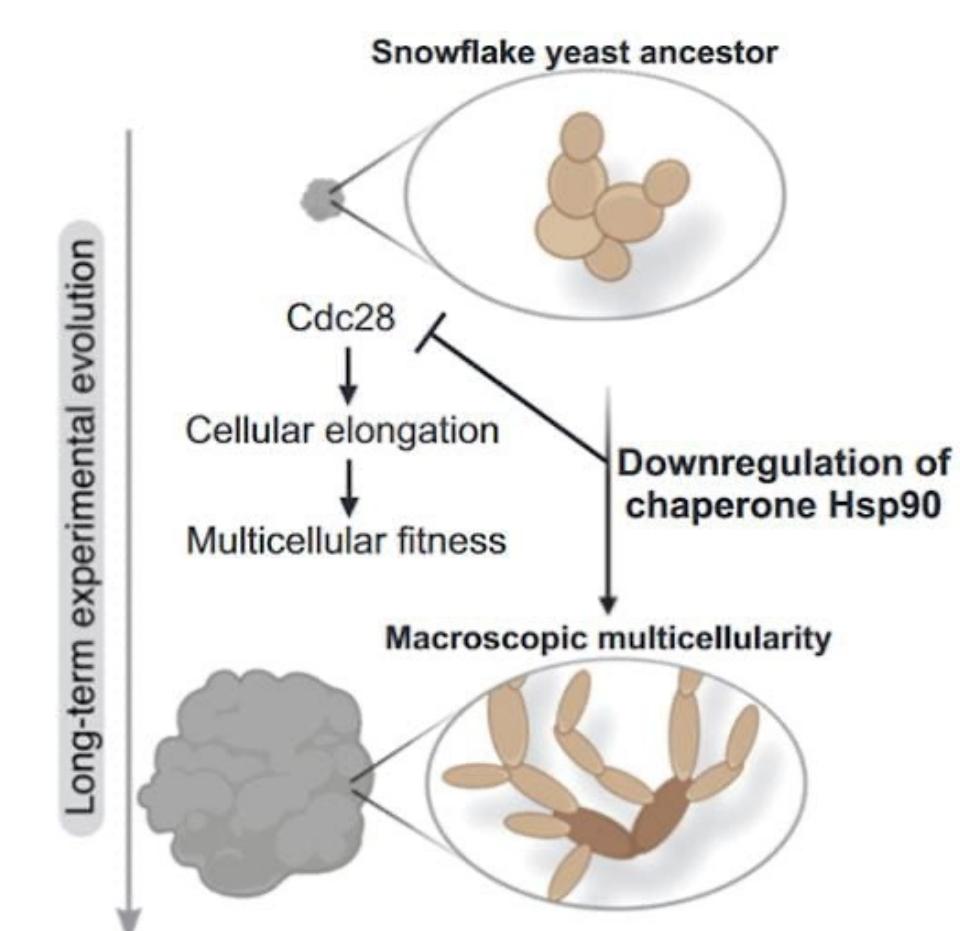
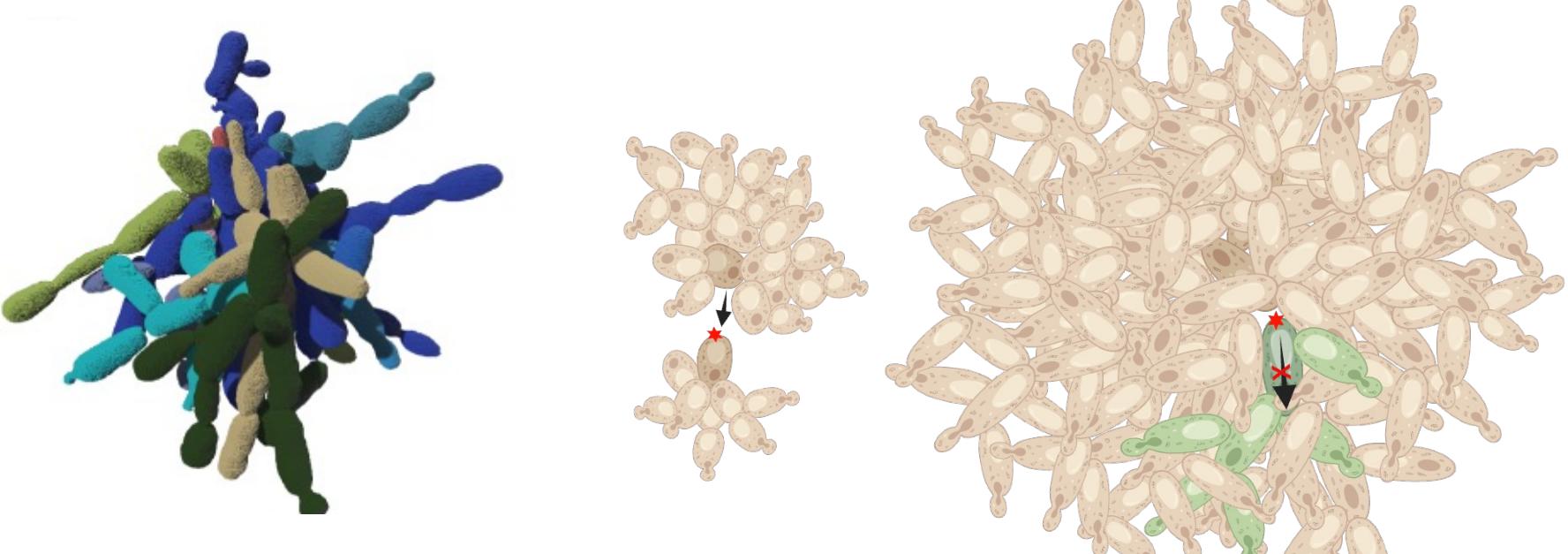
Snowflake yeast has evolved into macroscopic size in a long-term evolution experiment of multicellularity (MuLTEE) [4].



## Reduced HSP90 evolves as a mechanism of increasing group size in the MuLTEE



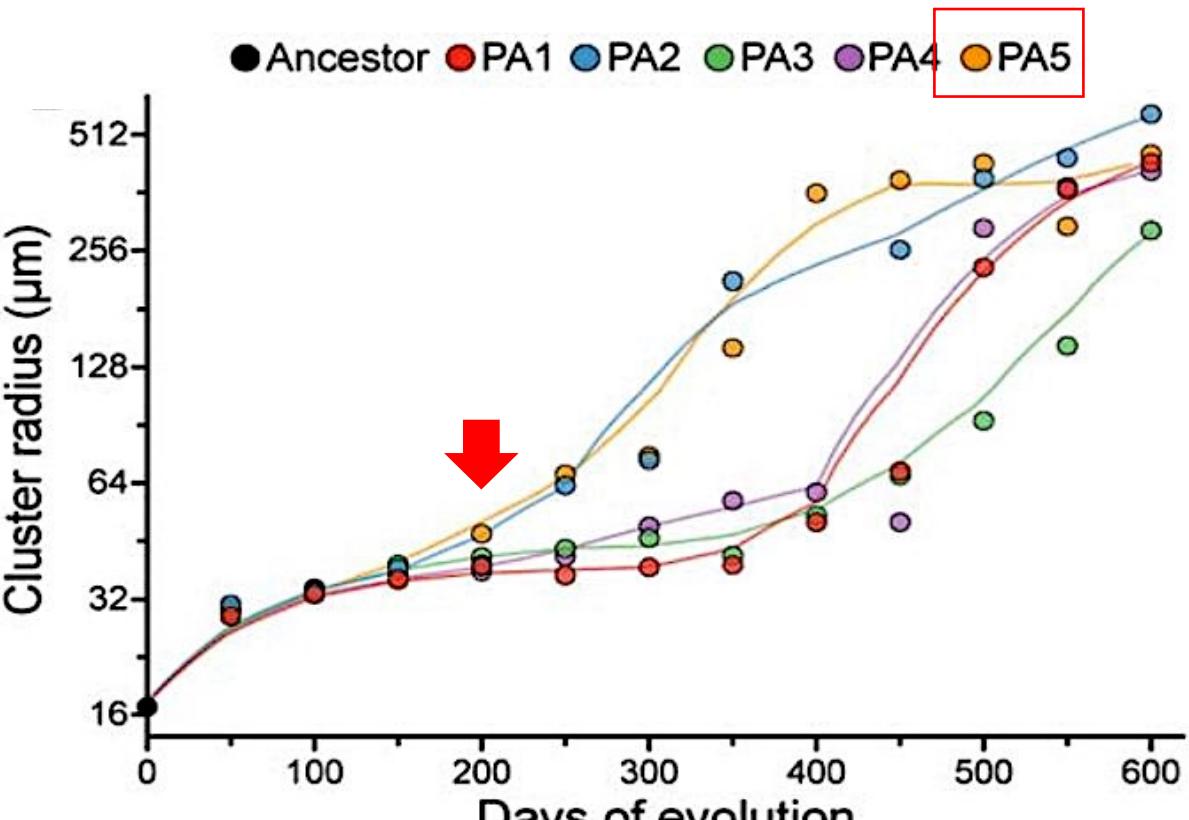
- Hsc82 and Hsp82 are two isoforms of heat shock protein 90 (HSP90). Hsc82 is the constitutively-expressed version important for day to day function.
- HSP90 expression declines rapidly during evolution, with lineages evolving larger group size losing HSP90 expression more rapidly.



Elongated cellular morphology and side budding behavior provide the biophysical properties to form larger and more resilient multicellular groups [7]. These traits are affected by HSP90.

HSP90 drives cellular elongation by modulating the stability and activity of the central cell cycle kinase Cdc28 [8].

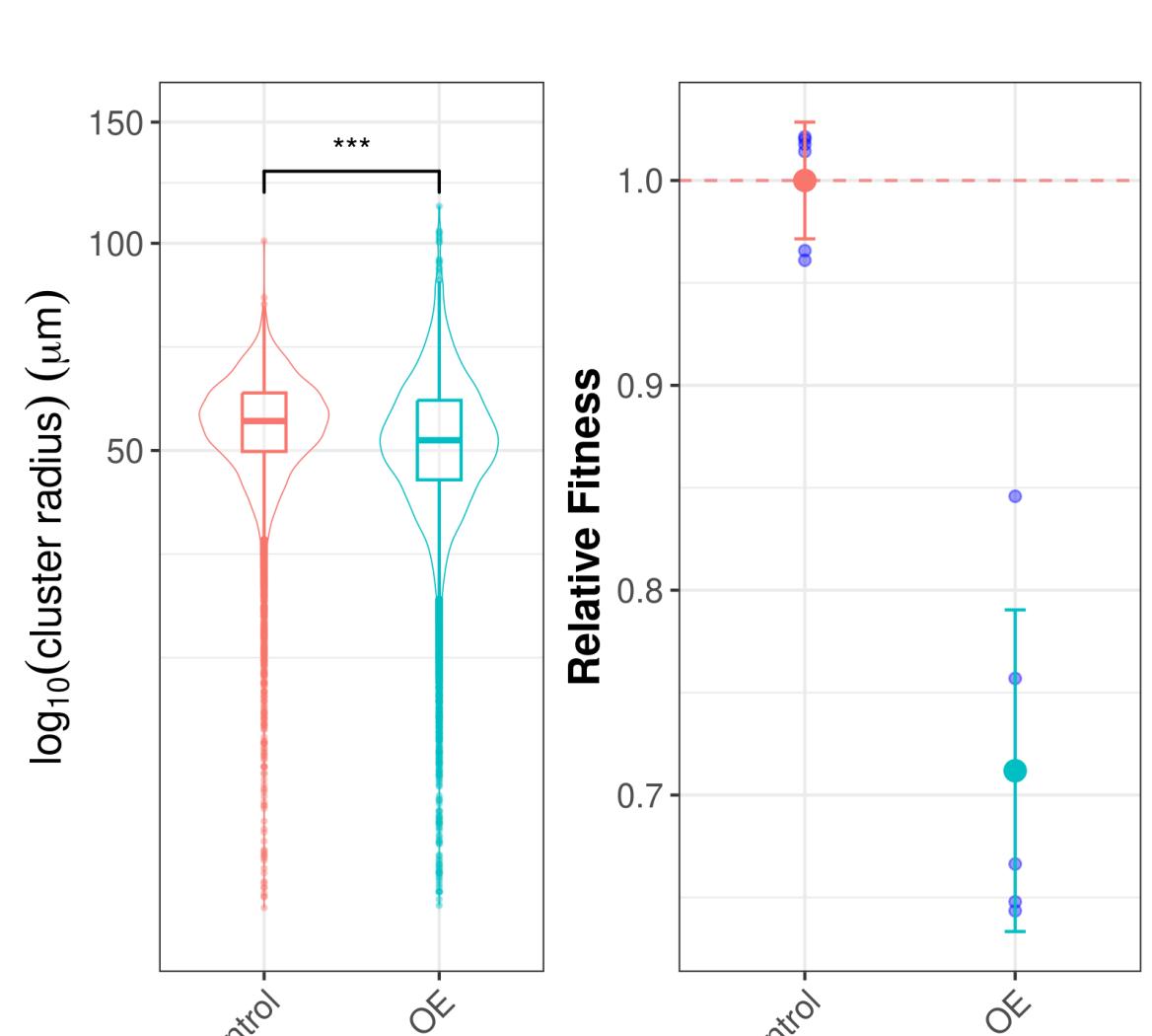
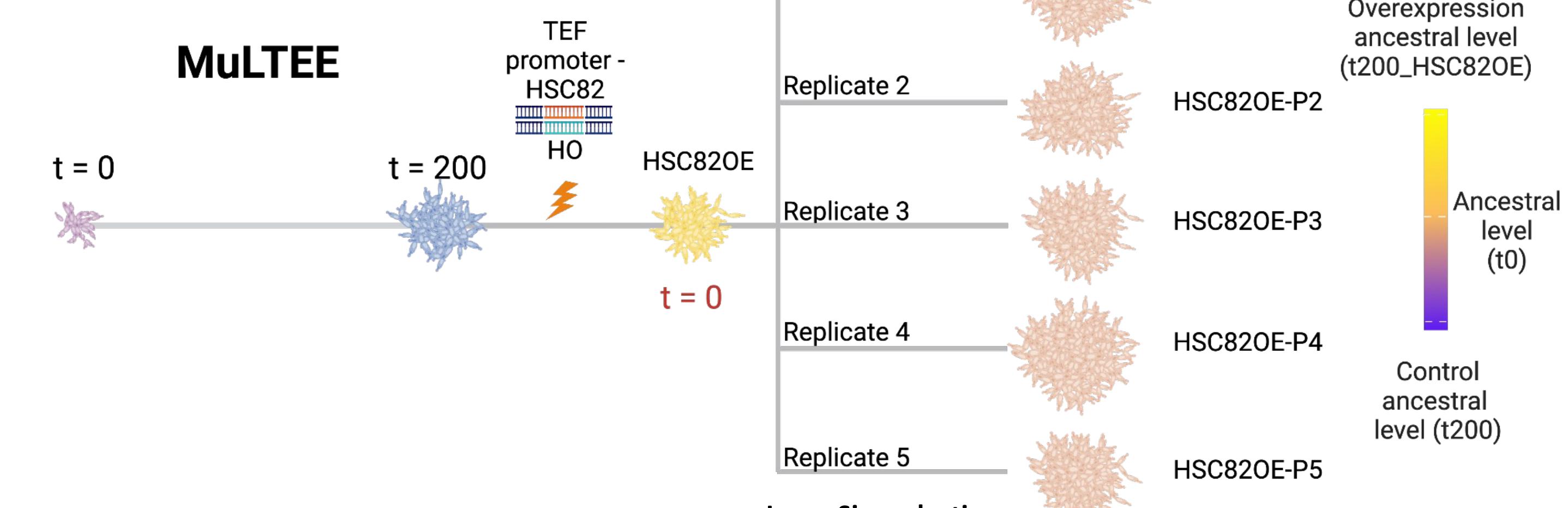
## Will over-expressing HSP90 suppress macroscopic evolution? We conducted an evolutionary replay experiment.



We went back to before the evolution of macroscopic size (t200) in the lineage to show the most rapid decline in HSP90 expression (PA5) and overexpressed the more important isoform of HSP90 (Hsc82).

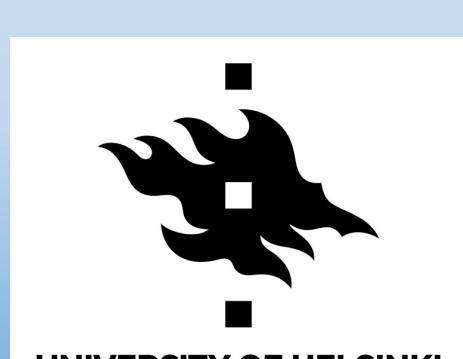
We ran the experiment for 100 daily rounds of selection, examining the evolution of novel traits and fitness.

## Evolutionary replay experiment



Overexpressing Hsc82 modestly reduced the size and fitness of our t200 strain under our selection conditions (daily transfers including settling selection).

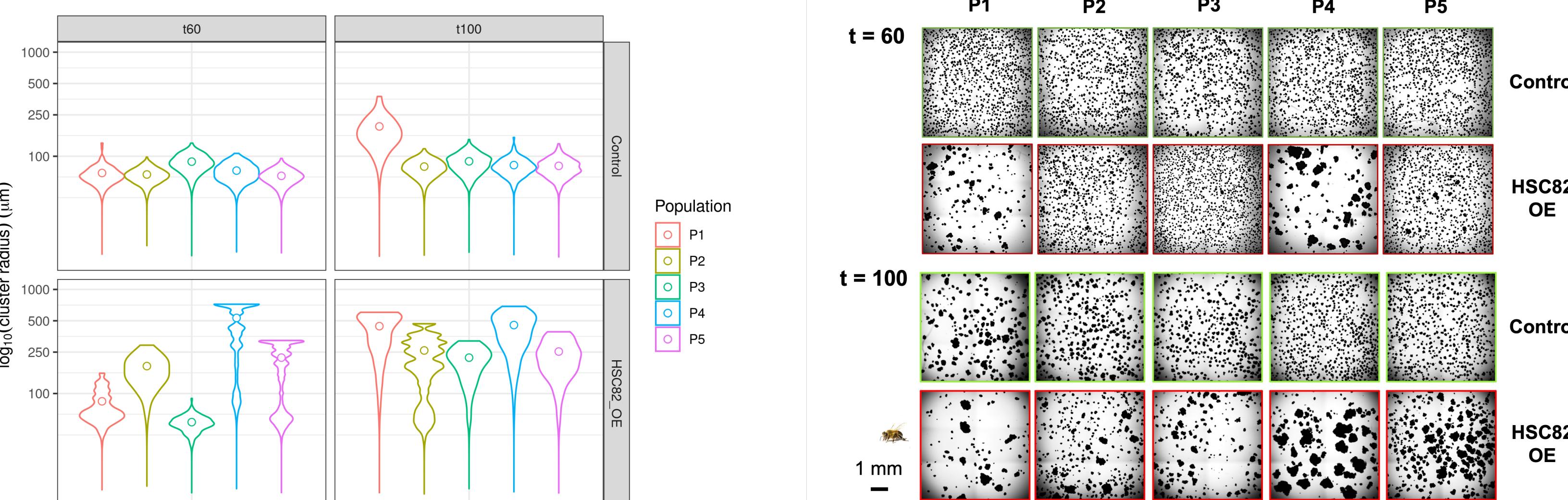
## Acknowledgments



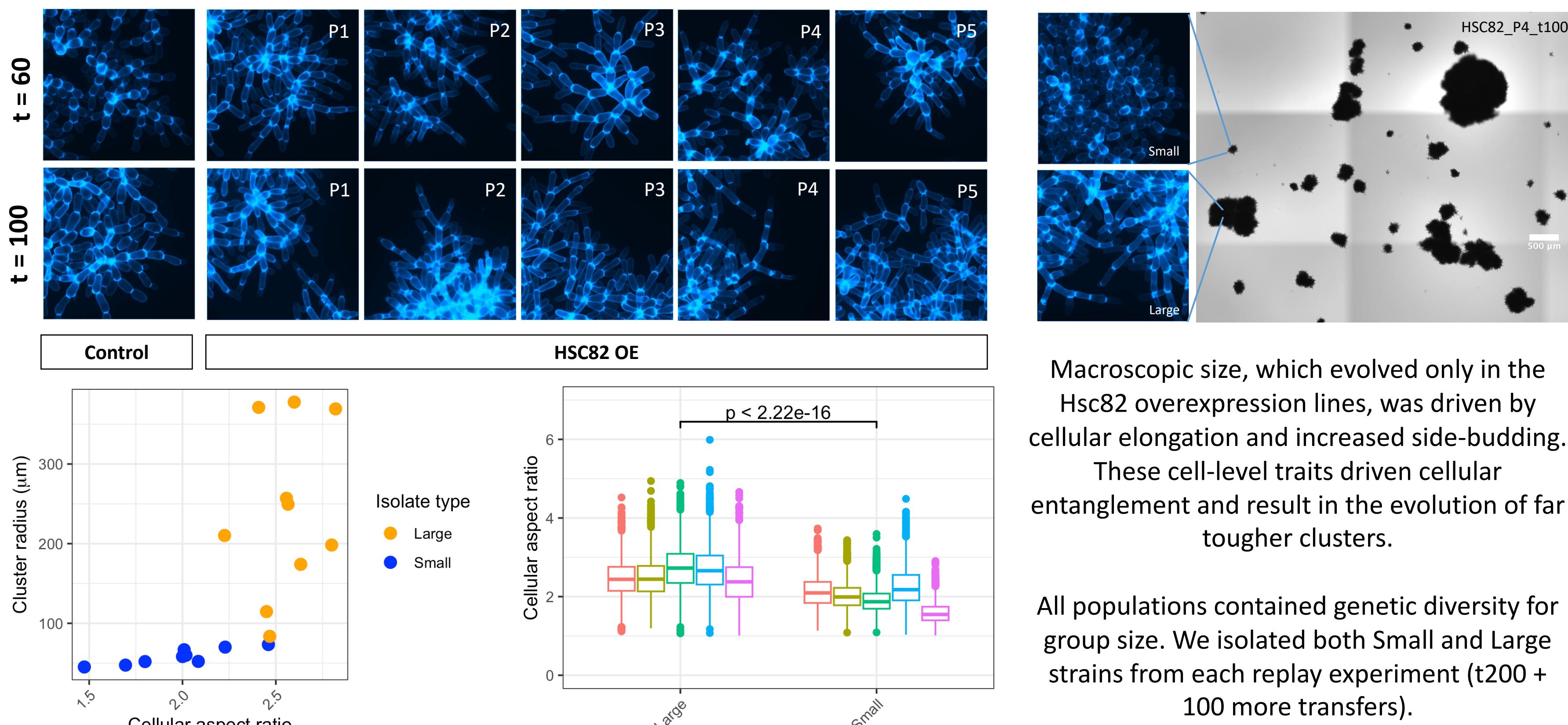
## References:

- Ratcliff, W. C., Denison, R. F., Borrello, M., & Travisano, M. Experimental evolution of multicellularity. *Proc. Natl. Acad. Sci. USA* **109**, 1595–1600. (2012).
- Ratcliff, W. C. et al. Origins of multicellular evolvability in snowflake yeast. *Nat. Commun.* **6**:6102 doi: 10.1038/ncomms7102. (2015).
- Aleza C Gerstein and Judith Berman, Current Opinion in Microbiology. 2015; 26:130–136.
- Bozdag, G., Ozan, et al. Oxygen suppression of macroscopic multicellularity. *Nat. Commun.* **12**, 1–10. (2021).
- Rutherford, Suzanne L., and Susan Lindquist. Hsp90 as a capacitor for morphological evolution. *Nature*. **396**:6709, 336–342. (1998)
- Girstmair, Hannah, et al. The Hsp90 isoforms from *S. cerevisiae* differ in structure, function and client range. *Nat Commun* **10**, 1–15 (2019).
- Bozdag, G., Ozan, et al. De novo evolution of macroscopic multicellularity. *Nature*. (2023).
- Kristopher, Montrose, et al. Proteostatic tuning underpins the evolution of novel multicellular traits. *bioRxiv*. (2023).

## Surprising result: rather than constraining it, overexpressing HSP90 accelerated multicellular adaptation

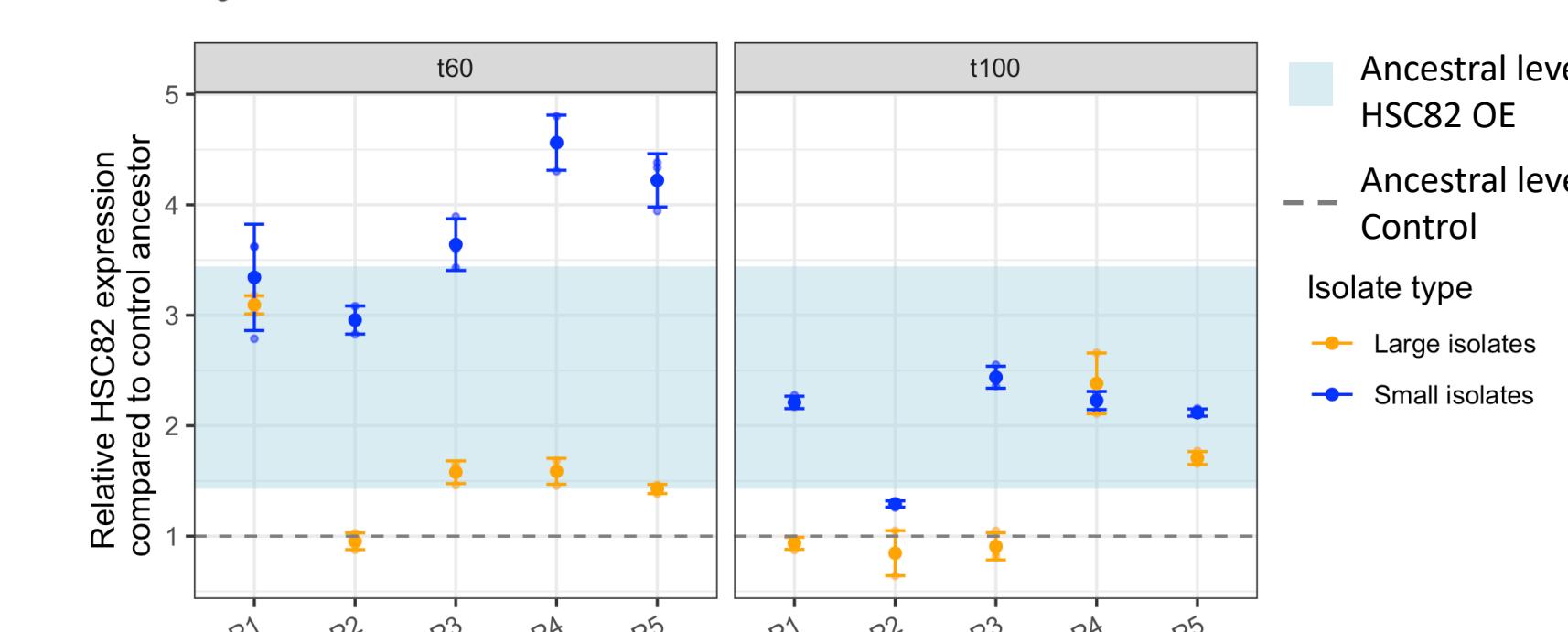


Within 100 days of additional evolution, all five HSP90 (yeast gene Hsc82) overexpression lineages evolved macroscopic size. Even the smallest population (HSC82 OE P1) was twice as large as the largest parallel-evolving control (Control P1).



Macroscopic size, which evolved only in the Hsc82 overexpression lines, was driven by cellular elongation and increased side-budding. These cell-level traits driven cellular entanglement and result in the evolution of far tougher clusters.

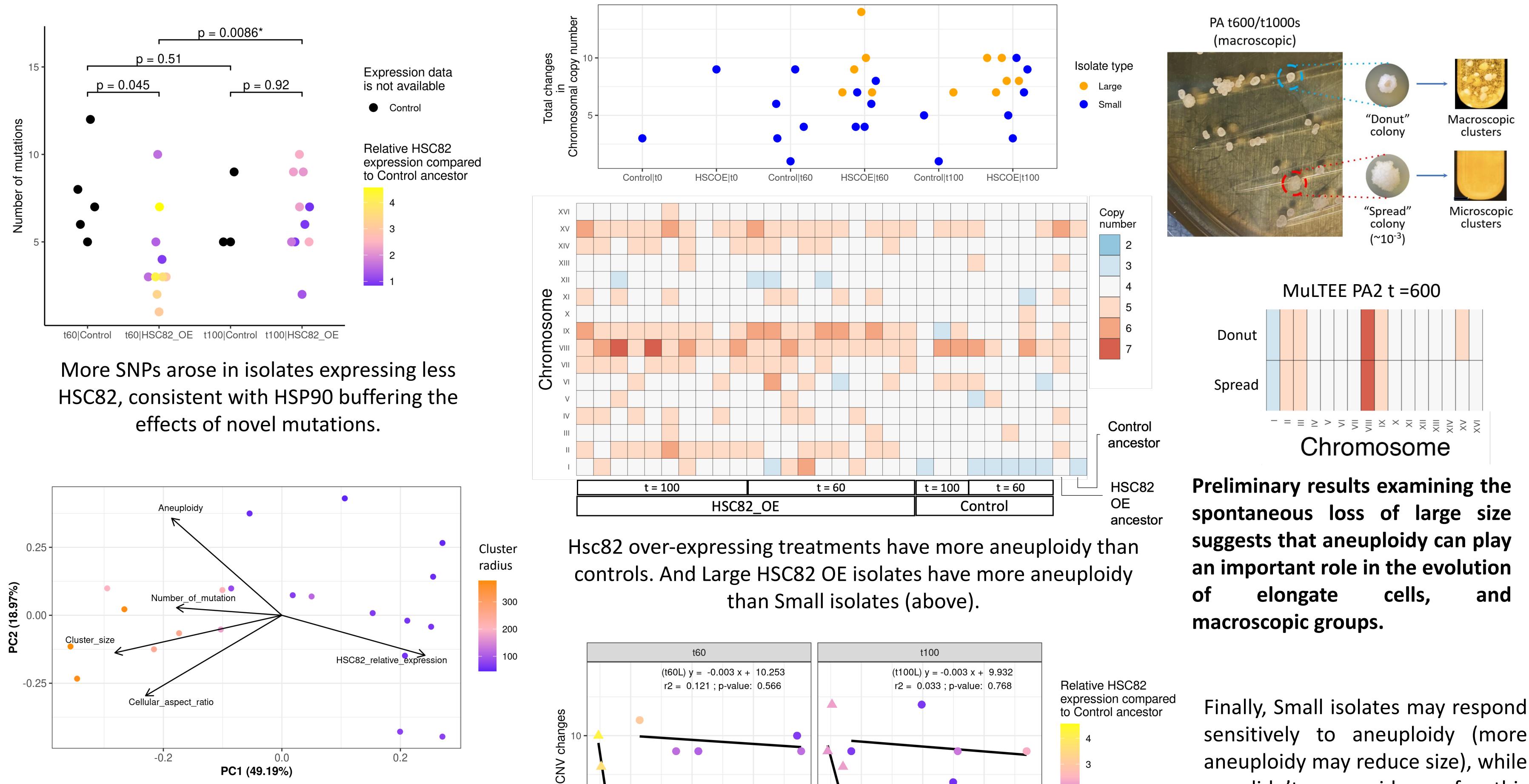
All populations contained genetic diversity for group size. We isolated both Small and Large strains from each replay experiment (t200 + 100 more transfers).



Consistent with what we saw in the MuLTEE, HSP90 expression levels rapidly decline during the replay experiment.

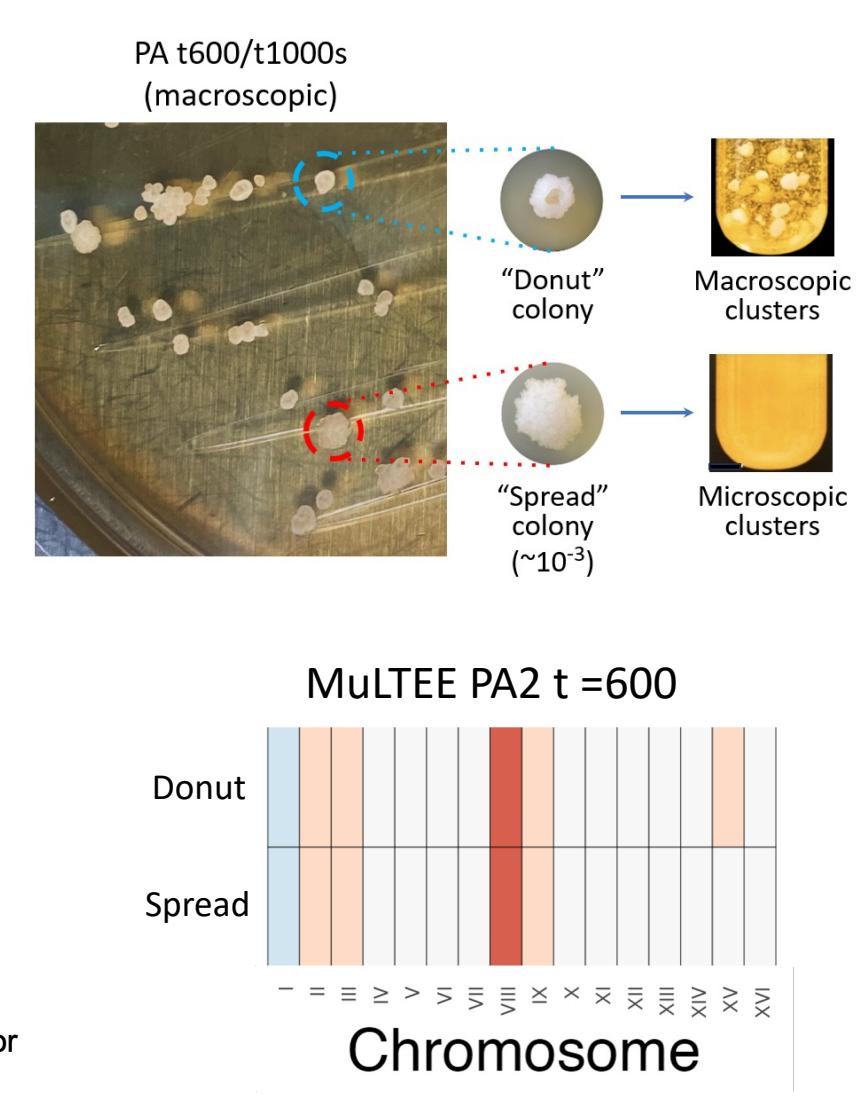
And, just like before, within each population, genotypes forming large groups lost HSP90 expression more rapidly than those remaining small.

## How does transient expression of HSP90 facilitate multicellular evolution?



More SNPs arose in isolates expressing less HSC82, consistent with HSP90 buffering the effects of novel mutations.

HSC82 over-expressing treatments have more aneuploidy than controls. And Large HSC82 OE isolates have more aneuploidy than Small isolates (above).



Preliminary results examining the spontaneous loss of large size suggests that aneuploidy can play an important role in the evolution of elongate cells, and macroscopic groups.

Finally, Small isolates may respond sensitively to aneuploidy (more aneuploidy may reduce size), while we didn't see evidence for this with our Large isolates, which were all highly aneuploid. Not sure if this is important- curious about your thoughts!

## Conclusions

- One of the most convergently evolving traits in the MuLTEE is reduced HSP90 (yeast gene HSC82) expression. This initially increases cluster size by downregulating its client protein the cyclin CDC28, resulting in more polarized growth and increased group size.
- We performed an evolutionary replay experiment going back to before the evolution of macroscopic multicellularity, and over-expressing HSP90. Rather than slowing the rate of multicellular evolution like we expected, this greatly accelerated it.
- As with the MuLTEE, HSP90 expression rapidly declined, with strains evolving large size losing HSP90 expression the fastest.
- This work (which is still in progress!) suggests that *losing* HSP90 expression may have an impact independent of persistently low expression. This could be explained by transient HSP90 expression facilitating evolutionary valley crossing, temporarily buffering the consequences of novel mutations. Alternatively, rapid changes in HSP90 expression may increase aneuploidy, impacting cell shape and group size.

Let me know if you have any questions or want to discuss more!

