SUPPLEMENTARY INFORMATION

Low costs of adaptation to dietary restriction

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Table S1: YPD medium recipe used for experimental evolution and reproductive fitness assays.

Ingredient	**Concentration (g/L)**
D-glucose	20 (NR) or 5 (DR)
Yeast extract	10
Peptone	20
Adenine	0.1
Tryptophan	0.1

Table S2: SDC medium recipe used for assaying survivorship.

Ingredient	**Concentration (g/L)**
D-glucose	20 (NR) or 5 (DR)
Yeast nitrogen base	6.7
myo-inositol	0.08
Uracil	0.08
PABA	0.04
Adenine	0.18
Tryptophan	0.18
Leucine	0.16
Alanine	0.08
Arginine	0.08
Asparagine	0.08
Aspartic acid	0.08
Cysteine	0.08
Glutamine	0.08
Glutamic acid	0.08
Glycine	0.08
Histidine	0.08
Isoleucine	0.08
Lysine	0.08
Methionine	0.08
Phenylalanine	0.08
Proline	0.08
Serine	0.08
Threonine	0.08
Tyrosine	0.08
Valine	0.08

Table S3. Life expectancy ANOVA table.

Factor	*df*	SS	MS	*F*	*P*	Significance
Evolution	1	12.7	12.7	0.699	0.415333	
Assay	1	388.5	388.5	21.358	0.000283	***
Evolution × Assay	1	5.2	5.2	0.284	0.601630	
Residuals	16	291.0	18.2			

Significance codes: ***: $P < 0.001$

Table S4. Reproductive fitness ANOVA table.

Factor	*df*	SS	MS	*F*	*P*	Significance
Evolution	1	0.0010	0.0010	0.095	0.76195	
Assay	1	0.3449	0.3449	33.159	2.94×10^{-5}	***
Evolution × Assay	1	0.1429	0.1429	13.738	0.00192	**
Residuals	16	0.1664	0.0104			

Significance codes: ***: $P < 0.001$; **: $P < 0.01$

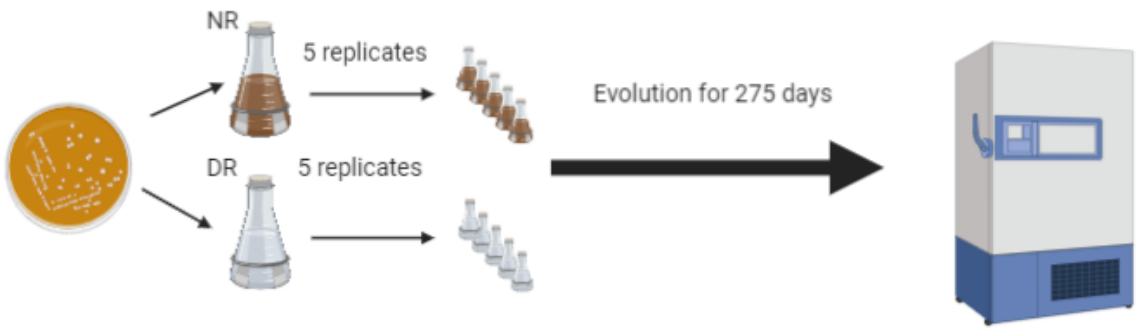

Figure S1. Details on experimental evolution methods. The ancestor W303 yeast strain was grown on a YPD-medium Petri plate. A clonal colony of was taken to inoculate the experiment. For each diet, 5 replicate populations were inoculated per treatment. Each day for 275 days, the 5 × 2 = 10 populations were 100-fold diluted into fresh NR or DR medium in a new flask each day. After 275 days, all populations were cryopreserved to be used for phenotypic assays.

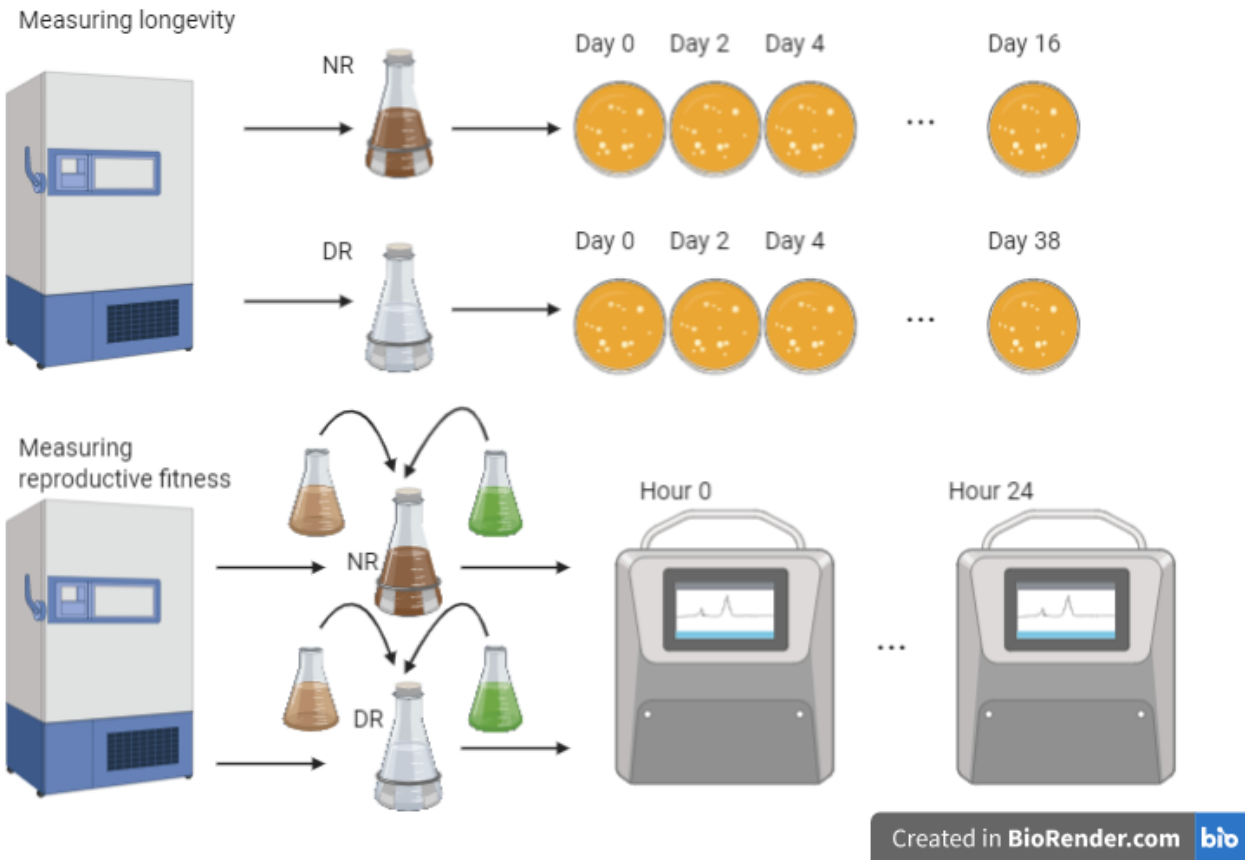

Figure S2. We sampled the longevity and the reproductive fitness of the cryopreserved populations. *Longevity:* For each cryopreserved population, cells were grown to stationary phase (in duplicate or triplicate) for 3 days on either NR or DR diet. We then sampled each population every 2 days. We assayed for survivorship at each time point by plating a dilution of the sample and counting colony-forming units (CFUs). We continued sampling until survivorship fell below 1% (16 days on NR; 38 days on DR). Survivorship values were used to calculate life expectancy. *Reproductive fitness*: For each cryopreserved population, we cultured the cells and then inoculated a mixed culture containing both the strain of interest and a GFP-labeled competitor strain. Each mixed culture was tested on each diet in at least triplicate. Mixed cultures were sampling via flow cytometry immediately to assay the initial non-GFP:GFP cell ratio. After 24 hr, mixed cultures were sampled again and non-GFP:GFP cell ratio was again assayed. The change in non-GFP:GFP cell ratio over 24 hr of growth and reproduction was used to calculate reproductive fitness.

Calculating number of generations: The number of generations is an important parameter to consider for experimental evolution because it measures the amount of evolutionary time that has passed. One possible concern for our experiment might be that populations could grow more slowly on DR due to nutrient limitation. Therefore it was important to verify that the number of generations exposed to experimental evolution were not fewer for the DR treatment.

In pilot experiments, we observed that yeast cultures serially inoculated with 1% v/v transfers (100-fold dilution) had sufficiently short generation time that the original optical density at 600 nm (OD) and population size were restored within 24 hr, in both NR and DR conditions. That is to say, we knew that ≥

$\log_2(100) \cong 6.64$ population doublings (generations) occurred within 24 hr [1], and the doubling time must < 24 ÷ 6.64 = 3.6 hr. This is reasonable given that *S. cerevisiae* commonly exhibit average doubling times ≅ 2 hr in the literature (e.g. [2]). We also used OD measurements to generate growth curves over 24 hours. Growth curves confirmed that maximum OD was reached within 24 hr, at which point population growth ceased (stationary phase).

Therefore, we knew that at least 6.64 generations/day * 275 days = 1827 generations of evolution had occurred for both treatments. We note that this is a conservative estimate because during stationary phase, populations can be dynamic with some cell turnover despite the lack of population growth. One possible concern is that growth rate could be slower on DR due to the nutrient limitation, leading to fewer cryptic generations for the DR treatment. To verify that cryptic generations were not fewer for the DR treatment, we measured the performance of the ancestor strain in OD-based growth curves (**figure S3**). We observed maximum growth rates of 0.275 hr^{-1} for NR, and 0.287 hr^{-1} for DR, which was not significantly lower than the NR growth rate (t = 2.27, P = 0.967).

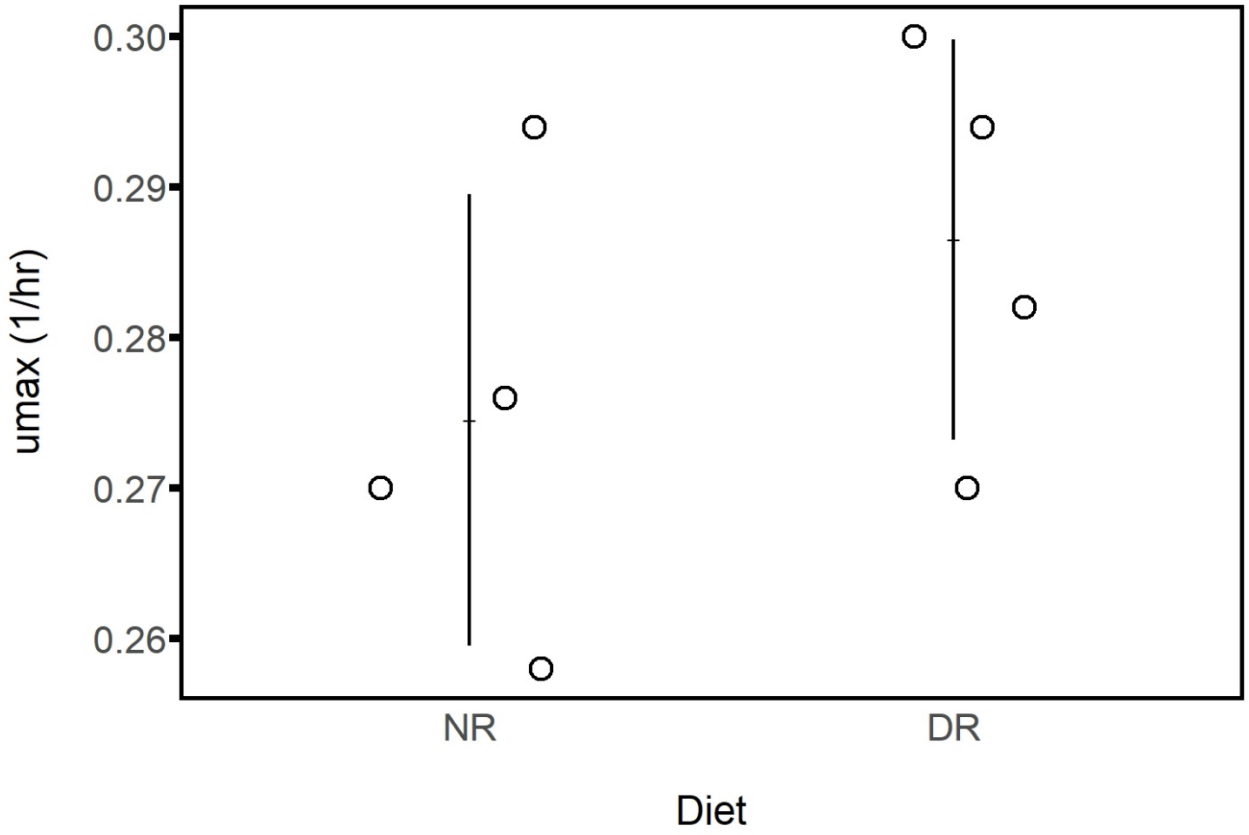

Figure S3. Growth rate via OD-based growth curves. Growth rate of the ancestor strain was not lower on DR diet compared to NR diet. Error bars represent ± 2 standard errors of the mean (SEM).

P**opulation size**:
Another important parameter for experimental evolution is the population size. Population size influences the dynamics of evolution by modulating the relative force of natural selection vs. genetic drift, as well as the number of new mutations generated and exposed to natural selection each generation. One possible concern is that population sizes might be smaller on DR due to the nutrient limitation. We

therefore tested whether cell densities (i.e., population sizes) were smaller on the DR diet compared to the NR diet using both flow cytometry and CFU counts. Measured via flow cytometry, population size was not significantly smaller on DR compared to NR (t = 0.90, P = 0.764; **figure S4**). Measured via CFU counts (10^5-fold dilution, 100 µL spread-plated) as well, population size was not significantly smaller on DR compared to NR (t = 1.20, P = 0.862; **figure S5**). The similar cell densities might be attributable to a shift from primarily fermentation-based metabolism toward efficient, respiration-based metabolism that occurs in diet restricted yeast [3], enabling larger population sizes on less carbon.

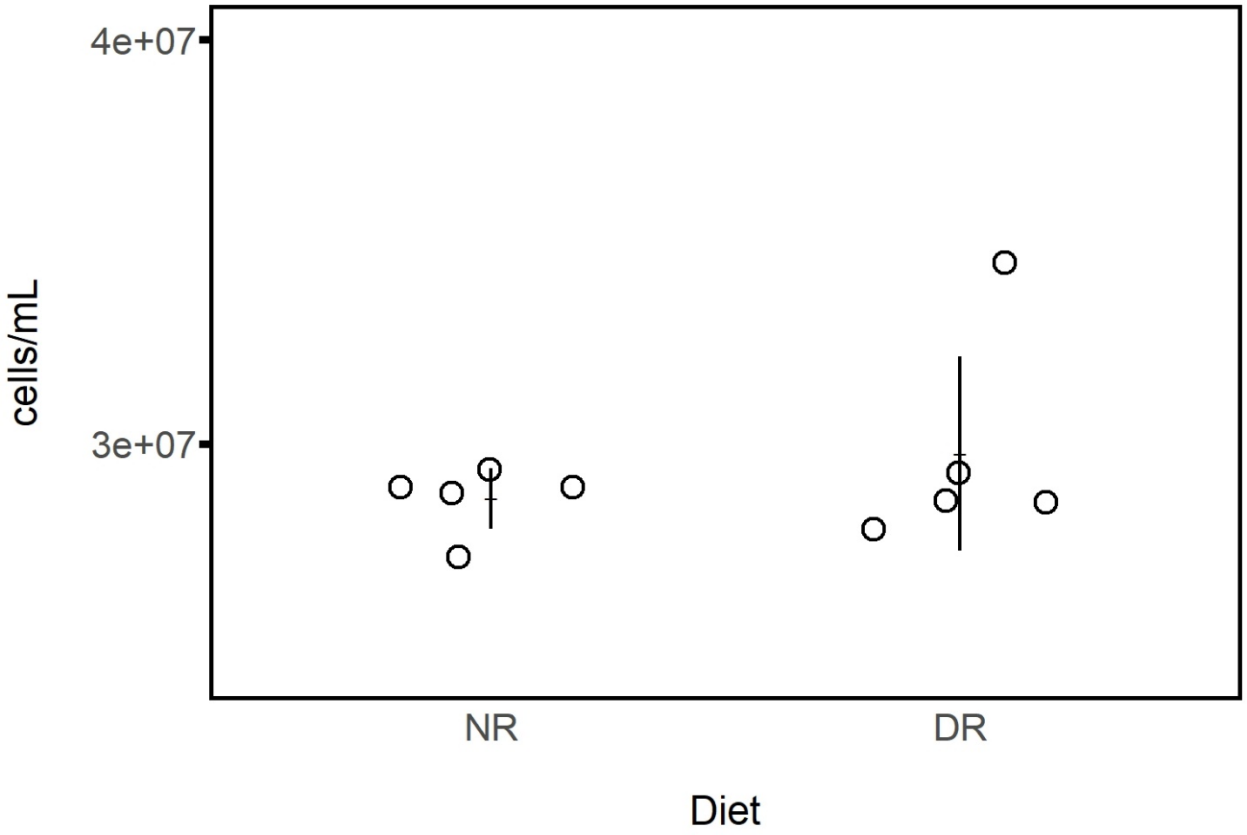

Figure S4. Population sizes were calculated via flow cytometry. We measured cell densities of the ancestor strain on both NR and DR diets. Cell densities were not lower on DR, suggesting that population sizes were similar for the two evolution treatments during experimental evolution. Error bars represent ± 2 SEM.

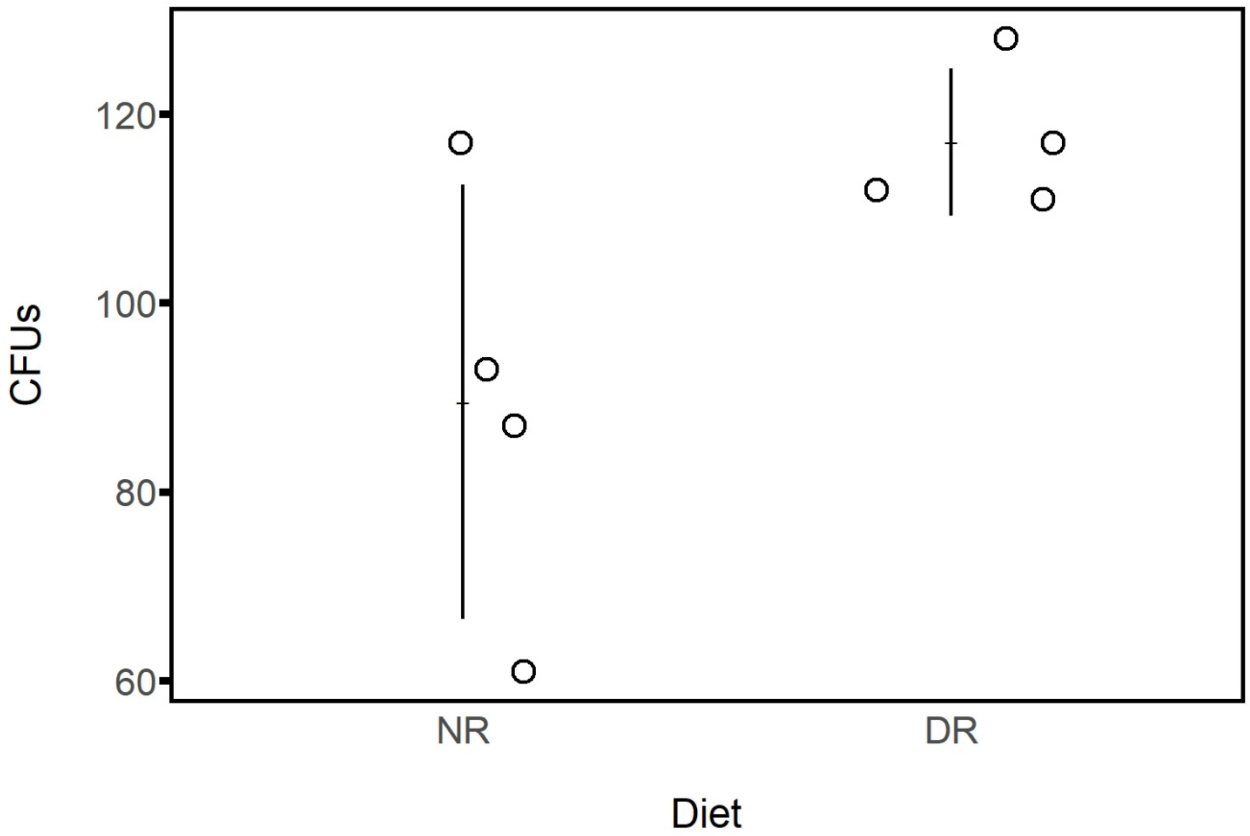

Figure S5. Populations sizes were also calculated via CFU count assays. We measured cell densities of the ancestor strain on both NR and DR diets. Again, cell densities were not lower on DR, suggesting that population sizes were similar for the two evolution treatments during experimental evolution. Error bars represent ± 2 SEM.

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