Supplemental Figure & Table Descriptions

**Figure S1:** Counts returned for sequenced libraries. (A) Counts distribution for each barcoded lineage X sample (primer pair), omitting reference barcode data. (B) Log-counts distribution for each barcoded lineage X sample (primer pair), omitting reference barcode data. (C) Mean counts for each barcoded lineage across all samples, omitting reference barcode data. (D) Log-mean counts for each barcoded lineage across all samples, omitting reference barcode data.

**Figure S2:** Power to detect fitness differences of different magnitudes as percent change from initial fitness.(A) power to detect fitness change for individual barcodes. (B) power to detect fitness change at the treatment level. N=64, solid line, is power to detect fitness difference between two treatments with 32 barcoded strains in each. N=32, dashed line, is power to detect fitness change for a single treatment with 32 barcoded strains.

**Figure S3:** Joint-Performance in CM, CM + chemical stress. Generalists do not always pay a fitness cost relative to specialist lineages. (A, B, D, E) Black circles are the joint-performance for the specialist lineages in the x-axis, y-axis environments. Orthogonal distance below- and to the left of the dot-dash line connecting the specialist join-performance values indicates a fitness cost. Lineages on the line pay no cost for their generalist history. Lineages orthogonally above- and to the right of the dot-dash line enjoy additional fitness benefits from generalism (i.e., not just no trade-off, but a qualitative advantage). (A) Cost of generalism for a 0%, 40% stress generalist (EH0\_40) relative to 0% (EH0) and 40% stress (EH40) specialists in the NaCl dataset. (D) The same, for the CuSO4 dataset. (B) Cost of generalism for 0%, 80% stress generalist (EH0\_80) versus the 0% (EH0) and 80% stress (EH80) specialists in the NaCl dataset. (E) The same, for the CuSO4 dataset. (C) density plot corresponding to A, B data; distance from dot-dash line. (F) density plot corresponding to D, E data; distance from dot-dash line. 80% power to detect fitness cost of 0.634% for treatments. 80% power to detect fitness cost of 2.163% for individual barcodes.

**Figure S4:** Geometric mean fitness change for hypothetical scenario of 50:50 exposure to 0%, 80% chemical stress. (A) Geometric mean fitness change, NaCl dataset. (B) Geometric mean fitness change, CuSO4 dataset. Lower-triangle insets illustrate geometric mean fitness differences among treatments; significant associations have beta-values, non-significant associations in grey. Asterisks denote treatment differences from 0 (no geometric mean fitness change). Black open circles are treatment median fitness. Black Closed circles are treatment mean fitness with standard error bars depicted. Panels A and B depict data from two, separate, evolution experiments: A depicts data from the “NaCl” experiment; B depicts data from the “CuSO4” experiment. Lineages in A were assayed only in CM and 80% NaCl stress; lineages in B were assayed only in CM and 80% CuSO4 stress.

**Table S1:** Strain construction, yeast strains, and inserted MOBY barcode sequences. (top) Strain construction -Oligo name indicates the name of the forward and reverse primer; oligo names that contain number preceded by “R” are reverse primers. Full Sequence notes the full oligo sequence in the 5’-3’ direction. (bottom) column 1 contains diploid yeast strain ID’s. Column 2 contains the MOBy barcode uptags (in the 5’-3’ direction) that identify the diploid yeast strains in the same row.

**Table S2:** Ion proton sequencing primers. Oligo name indicates the name of the forward or reverse primer; oligo names that contain numbers preceded by an “R” are reverse primers. Full sequence notes the full oligo sequence in the 5’-3’ direction. Barcode sequence shows the unique genetic barcode within each primer, in the 5’-3’ direction, used in multiplexing and demultiplexing libraries.

**Table S3:** Data table of power calculations at the treatment level using error in the fitness assay data; supports Figure S2, B. Data used to generate figure S2, B. Columns, in order, are: degrees of freedom for numerator (u), degrees of freedom for denominator (v), power (power), effect size in % fitness change (f2), significance level (sig.level), and number of entries for which the calculation corresponds (n) – data for n=32 and n=64 included as described in Figure S2 legend.

**Table S4:** Data table of power calculations at the individual barcode level using error in the fitness assay data; supports Figure S2, A. Data used to generate figure S2, A. Columns, in order, are: fitness change (fitchange), psd (population standard deviation), number of replicates (n), and power (power).

**Table S5:** Number (percentage) of lineages that exhibit Positive change in fitness in evolutionary environment (black) and negative fitness change in alternate environment (red) in 0%, 40%, 80% chemical stress for the NaCl and CuSO4 datasets. To quantify cost, the numerator in the evolutionary environment entry becomes the denominator for the other entry in the row such that the alternate (“non-home”) environment reports the number (percentage) of lineages that increased at home and decreased in the non-home environment. Fluctuating treatments that experienced two environments in evolution are split into two rows, one for each “home” environment. 80% power to detect 2.163% fitness change for individual barcodes; barcodes with fitness change < 2.16% and > -2.16% had no significant change in fitness.

**Table S6:** Cost of generalization for treatments EH0\_40, EH20\_60, EH0\_80, EH40\_80 relative to constant environment treatments EH0 and EH80 in 0%, 80% chemical stress for the CuSO4 dataset; linear model

**Table S7:** Cost of generalization for treatments EH0\_40, EH20\_60, EH0\_80, EH40\_80 relative to constant environment treatments EH0 and EH80 in 0%, 80% chemical stress for the NaCl dataset; linear model

**Table S8:** Cost of generalization for individual barcodes from treatments EH0\_40, EH20\_60, EH0\_80, EH40\_80 relative to constant environment treatments EH0 and EH80 in 0%, 80% chemical stress. Barcodes with distances >= 2.16% in the joint-fitness space indicated as “benefit”; those <= -2.16% indicated as “cost”; those <2.16% but > -2.16% indicated as “no cost|benefit”.

**Table S9:** Change in geometric mean fitness under a hypothetical scenario of 50:50 exposure to 0%, 80% chemical stress. Barcodes with geometric mean fitness change <= -2.16% indicated as “Decrease”; those < 2.16% but > -2.16% indicated as “no change”; those >= 2.16% indicated as “Increase”.

**Table S10:** Change in variance fitness and change in arithmetic fitness predict change in geometric mean fitness under a hypothetical scenario of 50:50 exposure to the 0%, 80% stress environments in the NaCl dataset; linear mixed-effects model.

**Table S11:** Change in variance fitness and change in arithmetic fitness predict change in geometric mean fitness under a hypothetical scenario of 50:50 exposure to the 0%, 80% stress environments in the CuSO4 dataset; linear mixed-effects model.