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. 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:** Generalists pay a fitness cost relative to specialist lineages. Orthogonal distance below- and to the left of the dot-dash line 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.

**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).

**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 using error in the fitness assay data; supports Figure S2. Data used to generate figure S2. 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:** Number (percentage) of lineages that exhibit Positive change in fitness in 0%, 40%, 80% chemical stress in the NaCl and CuSO4 datasets. “Home” column is the number of lineages from that treatment that exhibited positive fitness changes “at home”. To quantify cost, the numerator in this entry becomes the denominator for the other entries in the row such that the “non-home” environments report the number (percentage) of lineages that exhibited fitness increases at home *and* in the non-home environment.

**Table S5:** 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.

**Table S6:** 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.