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Figures and figure supplements

Evolution of extreme resistance to ionizing radiation via genetic adaptation of DNA repair

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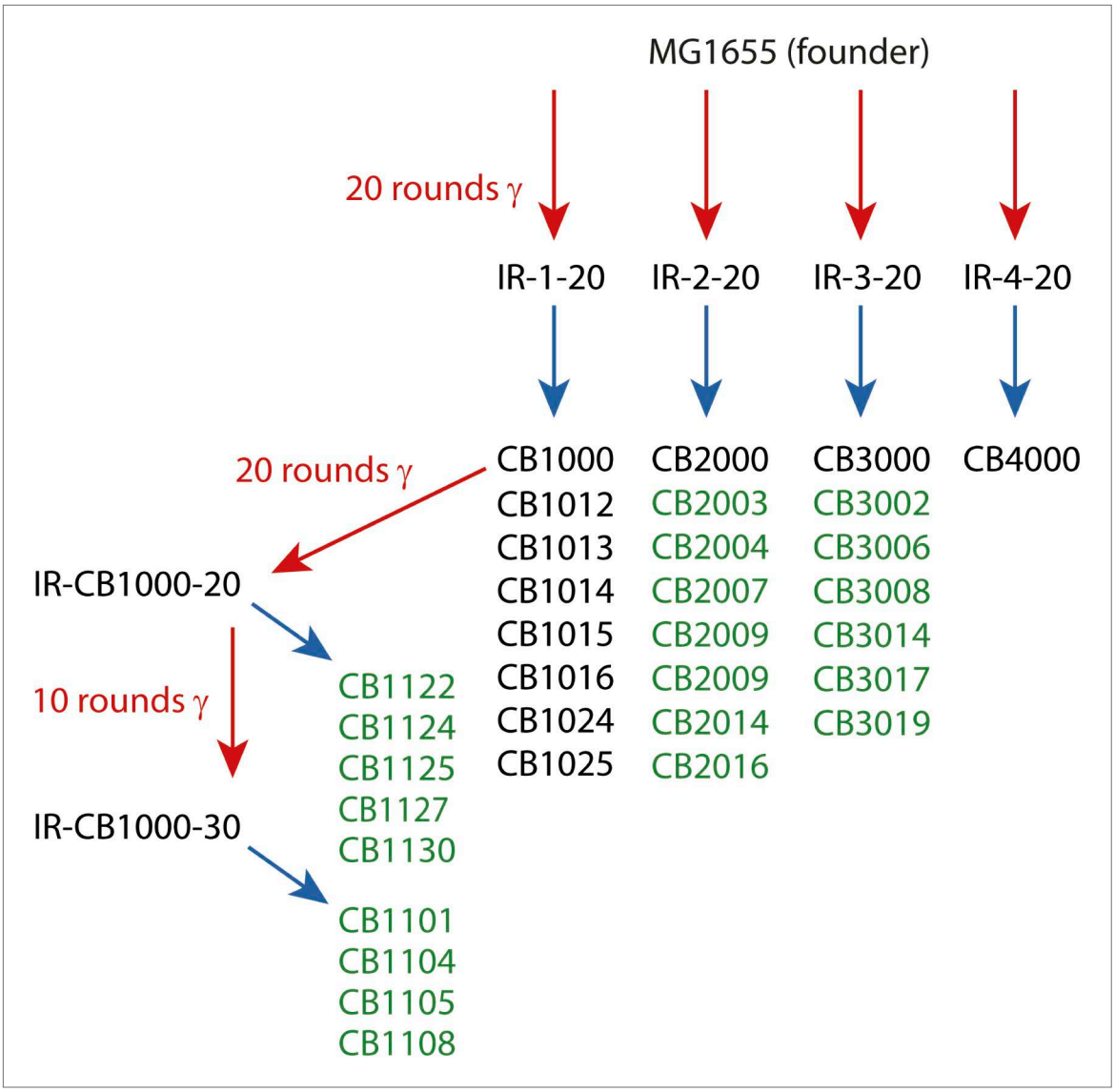

Figure 1. Directed evolution scheme for the evolved isolates described in this paper. Red arrows denote cycles of irradiation and outgrowth. Evolved populations have titles beginning with "IR". Isolates were derived from each listed population, as indicated by blue arrows. Isolates from each population are listed under the respective blue arrows, and each isolate features a name beginning with CB. Isolates listed in green text are described for the first time in this study. The sequences of the remaining isolates were described previously (***Harris et al., 2009***), and are listed here since the genomic data was utilized in the current analysis.

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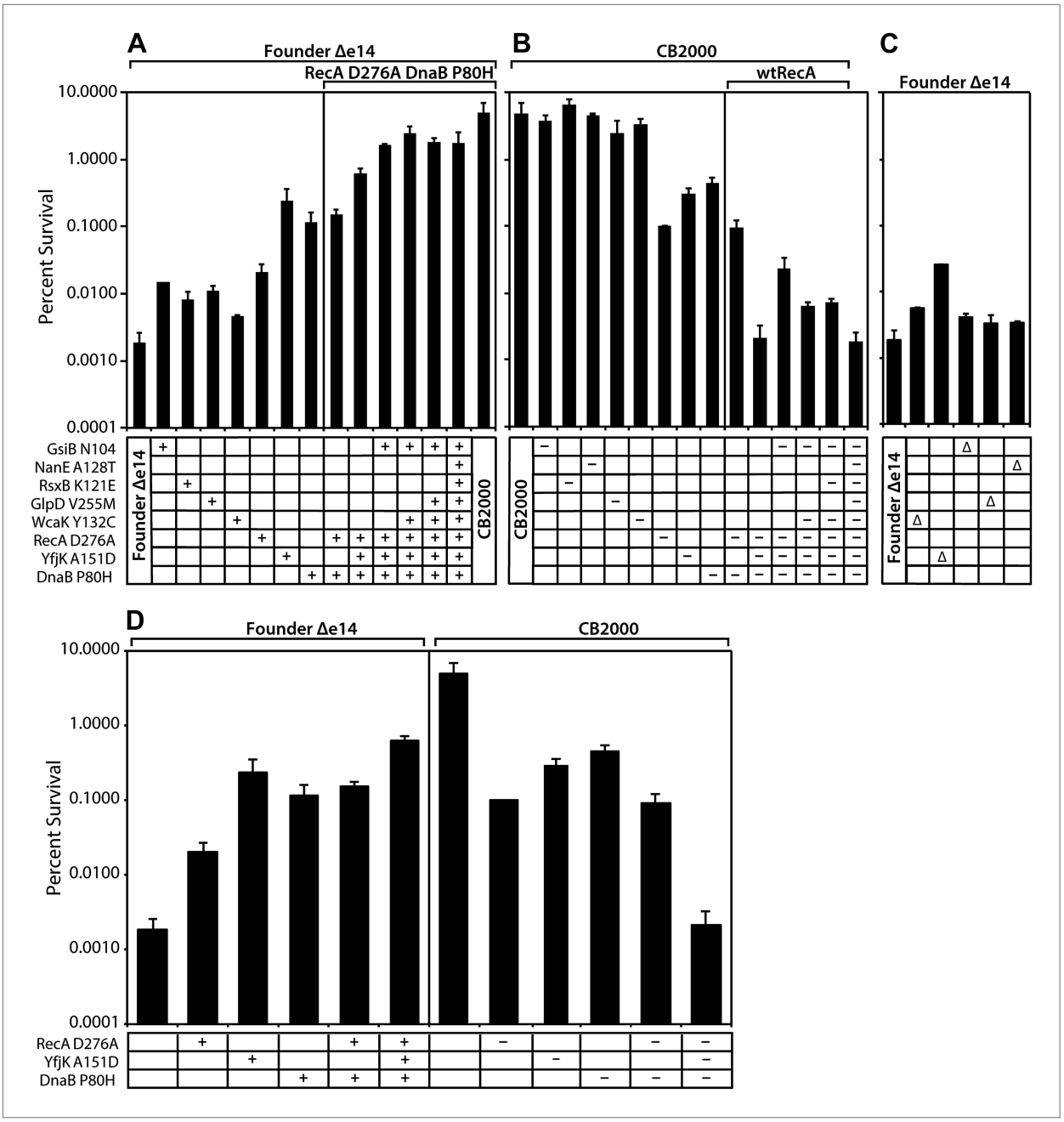

Figure 2. Effects of selected mutations on survival of *E. coli*. Mid-logarithmic phase cultures were irradiated to 3000 Gy and plated to measure survival as described in 'Material and methods'. (**A**) mutations discovered in CB2000 were moved individually and in combination into the Founder Δe14 background. Mutations present in a given strain are indicated by a + symbol. For reasons not understood, it proved impossible to move the *nanE* mutation into this background on its own. CB2000 itself is presented in the final lane. (**B**) the same point mutations (this time including *nanE*) were mutated back to Founder sequence in the CB2000 background. The first lane is CB2000. Mutations converted to the wild type allele in a given strain are indicated by a–symbol. (**C**) non-essential genes assayed in Panel **A** and **B** were deleted in the Founder Δe14 background, with the deleted gene indicated by a Δ symbol. **D**, The effects of mutations in genes *recA*, *dnaB*, and *yfjK* are summarized, with symbols as in panels **A**–**C**. All results were obtained with a ^{137}Cs irradiator (~7 Gy/min).
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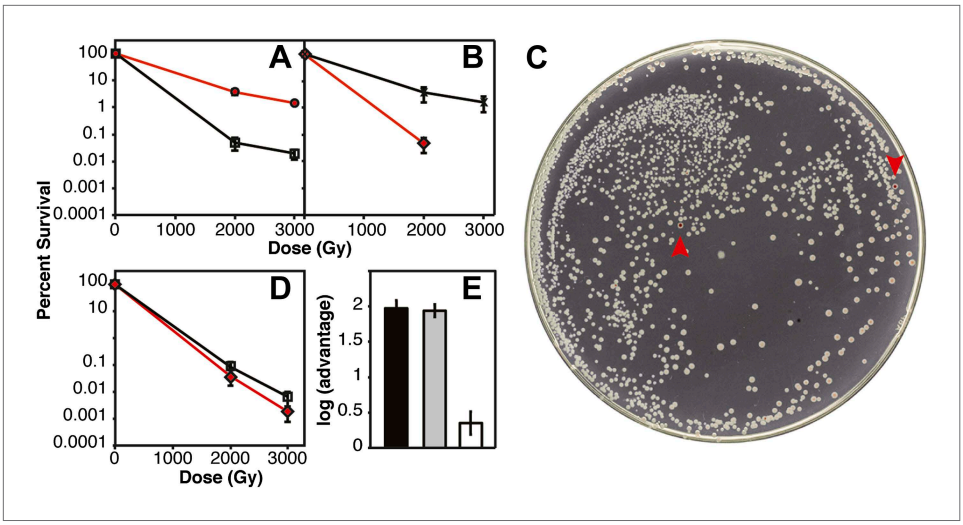

Figure 3. IR survival in direct competition assays. Mid-logarithmic cultures consisting of a 1:1 ratio of the competiting Ara+ and Ara⁻strains were irradiated to 2000 and 3000 Gy and plated on tetrazolium arabinose indicator plates to distinguish the frequency of survival for both strains. (**A**) CB2000 Ara⁻ (red, ^), vs CB2000 wtRecA wtDnaB wtYfjK, □ (**B**) Founder Δe14 Ara⁻, (red, ▵), *vs* Founder Δe14 RecA D276N DnaB P80H YfjK A151D, ◇ (**C**) TA plate of 2000 Gy survival competition of Founder Δe14 Ara⁻vs Founder Δe14 RecA D276N DnaB P80H YfjK A151D. The two red Founder Δe14 Ara⁻colonies are indicated by red arrows. (**D**) Founder Δe14 Ara⁻ (red, ◇), *vs* CB2000 wtRecA wtDnaB wtYfjK, □. (**E**) Log advantage in survival to 2000 Gy of CB2000 Ara⁻over CB2000 wtRecA wtDnaB wt YfjK (in black), Founder Δe14 RecA D276N DnaB P80H YfjK P80H over Founder Δe14 Ara⁻ (in grey), and CB2000 wtRecA wtDnaB wt YfjK over Founder Δe14 Ara⁻ (in white).

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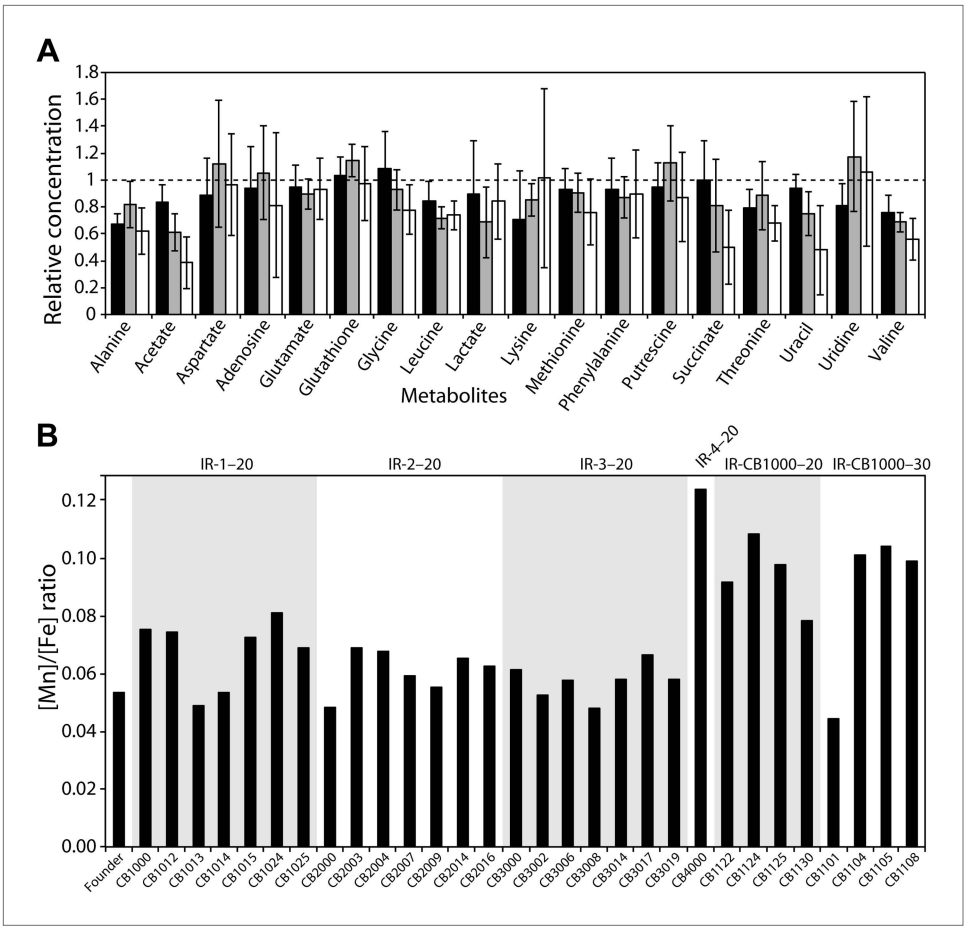

Figure 4. (**A**), Measurements of metabolites from three representative evolved *E. coli* strains as compared to Founder. Metabolites from whole cell pellets collected during logarithmic growth in LB were identified using a two-dimensional ^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC) experiment. Each metabolite is expressed as a ratio of the amount measured in the evolved strain (CB1000, CB1013, or CB2000; black, gray, and white bars, respectively) relative to the Founder. (**B**) Ratios of manganese to iron are plotted for all isolates for which genomic sequences were obtained. The average increase in Mn/Fe ratio in strains derived from the further evolution of CB1000 is 1.4-fold.

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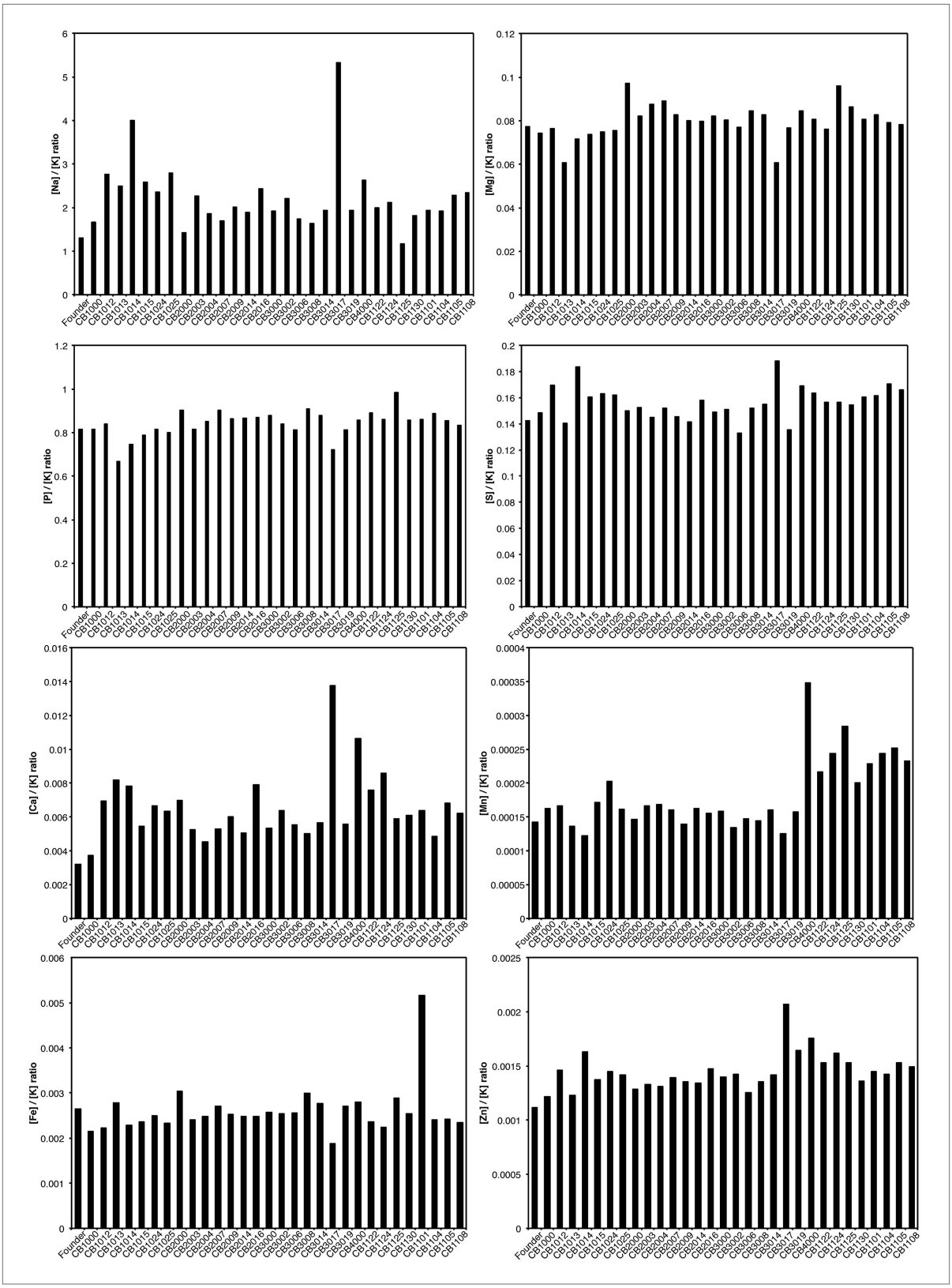

Figure 4—figure supplement 1. Relative levels of metals are unchanged in evolved *E. coli*.

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