Kim et al., http://www.jcb.org/cgi/content/full/jcb.201110090/DC1

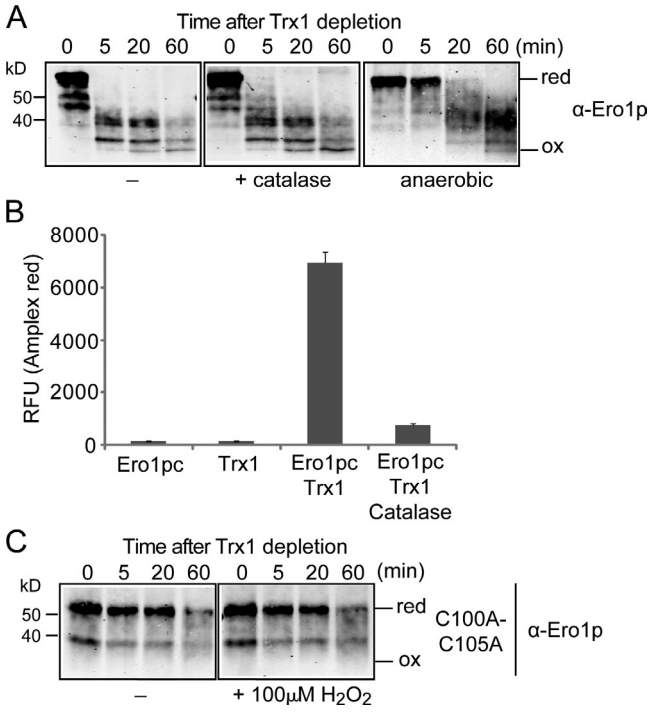

Figure S1. **Autonomous Ero1p inactivation is not mediated by hydrogen peroxide production.** (A) Oxidation (ox) state of Ero1pc after Trx1 depletion in the presence of catalase or under the anaerobic conditions. red, reduced. (B) A control to show catalase effectively removes hydrogen peroxide produced by Ero1p. The amount of hydrogen peroxide was measured by using Amplex UltraRed reagent for 30 min (see Materials and methods). The error bars (SEM) shown represent three independent experiments. RFU, relative fluorescent unit. (C) Exogenous hydrogen peroxide (100 μM) does not enhance oxidation of the regulatory bonds of Ero1pc-C100A-C105A after Trx1 depletion. Reduced and oxidized forms of Ero1p are indicated.

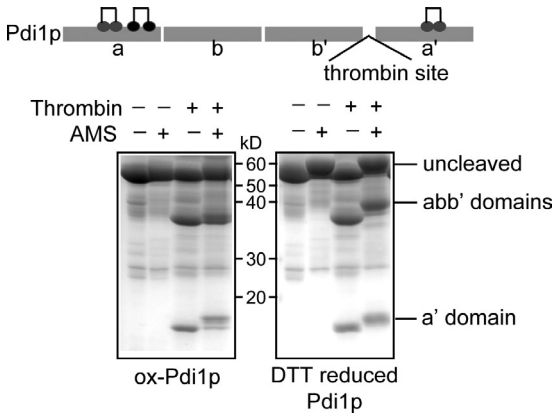

Figure S2. **Air-oxidized Pdi1p can contribute to the oxidation of the Ero1p regulatory bonds.** Recombinant Pdi1p is oxidized during expression and purification such that the a domain is almost fully oxidized, whereas the a′ domain is partially oxidized. A thrombin site was introduced in the X-linker after the b′ domain to monitor the difference in the redox state between the a and a′ domain (shown schematically on the top). Oxidized Pdi1p (ox-Pdi1p; left) was incubated with thrombin for 30 min at 37°C followed by modification of free cysteine thiols with AMS. Samples were analyzed by nonreducing SDS-PAGE. As a control, Pdi1p was pretreated with DTT (right) before thrombin and AMS incubation to monitor the complete reduction of the a and a′ domains.

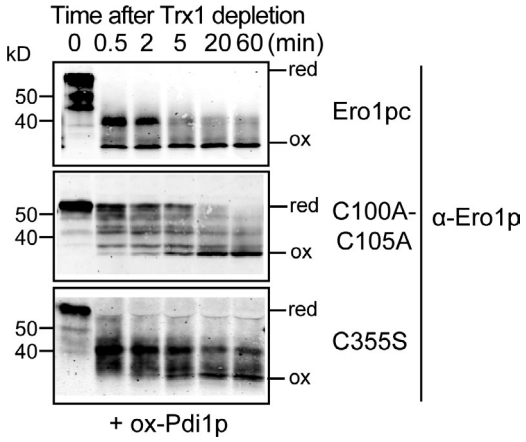

Figure S3. **The endpoint of air oxidation yields a form of recombinant Pdi1p with the a domain almost fully oxidized, and the a′ domain is partially oxidized.** Oxidized Pdi1p (ox-Pdi1p) accelerates oxidation of the Ero1p regulatory bonds in Ero1pc, Ero1pc-C100A-C105A, and Ero1pc-C355S. Ero1p and mutants were reduced (red) by treatment with reduced Trx1 and then mixed with 20 μM oxidized Pdi1p after Trx1 removal.

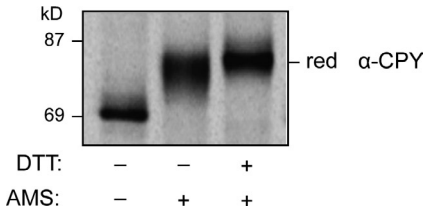

Figure S4. **The oxidation state of CPY in *pdi1Δ* cells.** Pdi1p was depleted by growing *pdi1Δ* cells with glucose-repressible *PDI1* for 15 h in glucose medium. Pulse-labeled and AMS-modified CPY was immunoprecipitated and analyzed. A fully reduced (red) and AMS-modified CPY was prepared as a control.

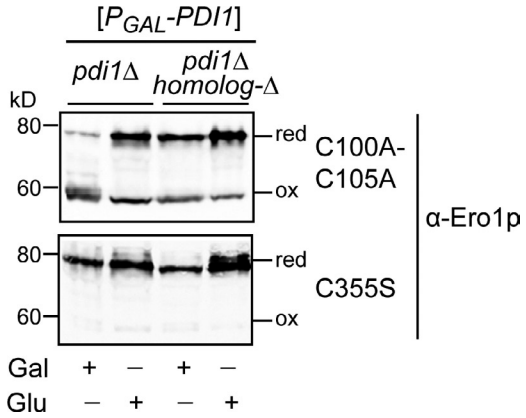

Figure S5. **The oxidation states of Ero1p-C100A-C105A and Ero1p-C355S in the Pdi1p-depleted cells.** Ero1p-C100A-C105A or Ero1p-C355S was ectopically expressed in *pdi1Δ* or *pdi1Δ/PDI homolog-Δ* strain recovered with P_{Gal1}-*PDI1*. Pdi1p was depleted by incubating cells in glucose medium. Reduced (red) and oxidized (ox) forms of Ero1p are indicated.