Supplemental Materials for:

Simultaneous enhancement of iron deficiency tolerance and iron accumulation in rice by combining the knockdown of *OsHRZ* ubiquitin ligases with the introduction of engineered ferric-chelate reductase

Takanori Kobayashi*, Keisuke Maeda, Yutaro Suzuki, and Naoko K. Nishizawa

Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi, Ishikawa 921-8836, Japan

*Corresponding author; Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, 1-308 Suematsu, Nonoichi, Ishikawa 921-8836, Japan; Tel/Fax: +81 76 227 7505; E-mail: abkoba@ishikawa-pu.ac.jp

Figure S1

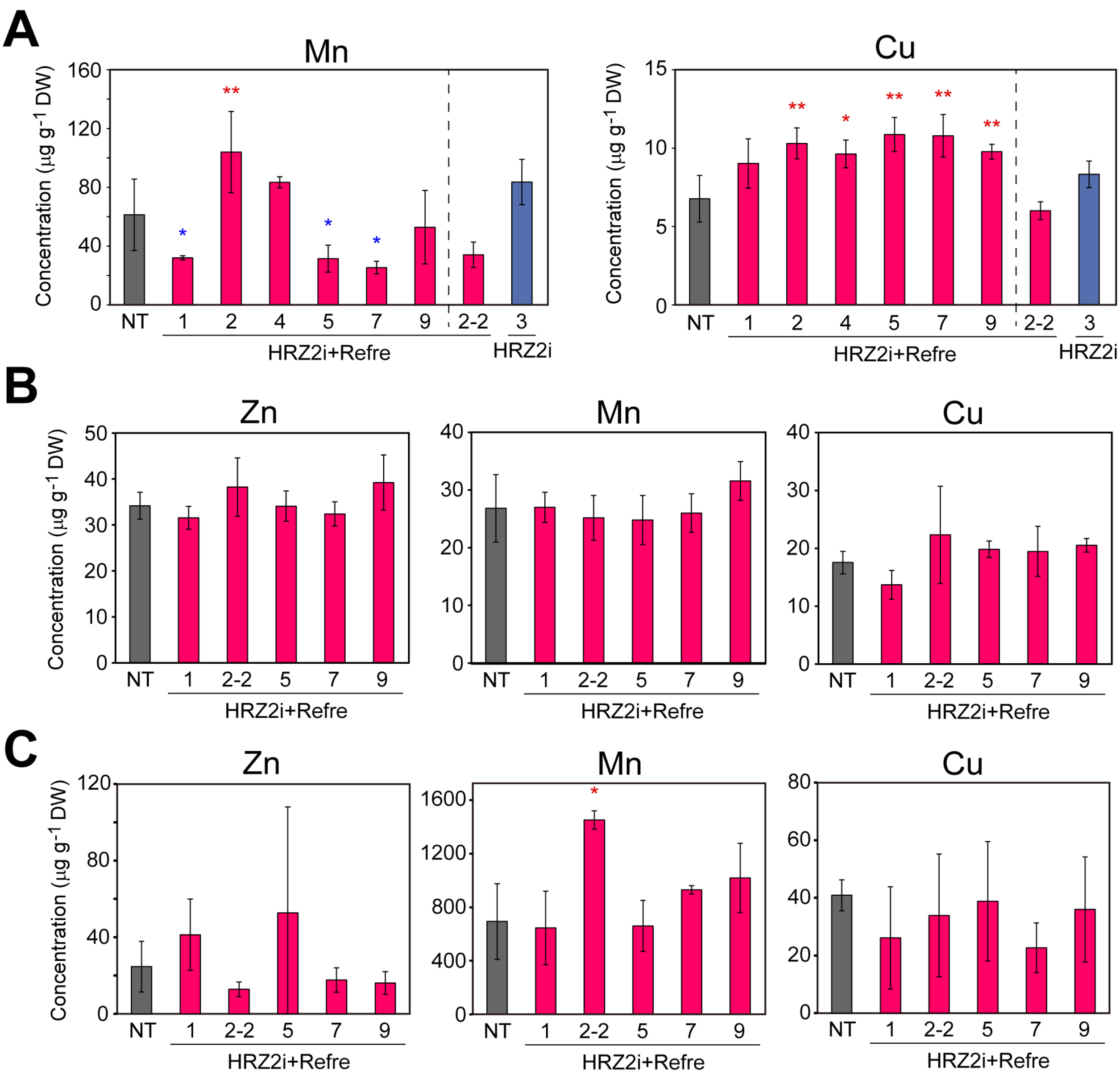

Figure S1. Additional metal concentrations in the HRZ2i+Refre lines. **A** Mn and Cu concentrations in the brown seeds after pot culture in Fe-sufficient soil in Figure 1B. The following seeds were analyzed: NT, non-transformants; HRZ2i+Refre, T_1 seeds of lines 1, 2, 4, 5, 7 and 9, and T_2 seeds of line 2-2; HRZ2i, T_2 seeds of line 3 (Kobayashi et al., 2013). Means ± SD are shown (n = 7 for NT, n = 6 for HRZ2i+Refre lines 2 and 5; n = 5 for HRZ2i line 3; n = 4 for HRZ2i+Refre lines 7 and 9; and n = 3 for HRZ2i+Refre lines 1, 4 and 2-2). **B, C** Zn, Mn, and Cu concentrations in the brown seeds (**B**) or straw (**C**) after pot cultivation and seed maturation in calcareous soil with non-submerged conditions in Figure 2. The following lines were cultivated: NT, non-transformants; HRZ2i+Refre, T_1 lines of 1, 5, 7, and 9, and T_2 line of 2-2. The means ± SD are shown (n = 4 for HRZ2i+Refre lines 2-2, 5, 7, and 9 in **B**; n = 2 for NT in **B**; n = 3 for HRZ2i+Refre line 1 in **B** and all lines in **C**). DW: dry weight. Asterisks indicate significant differences compared to the NT (two-sample Student's *t*-test; $*P < 0.05$; $**P < 0.01$).

Figure S2

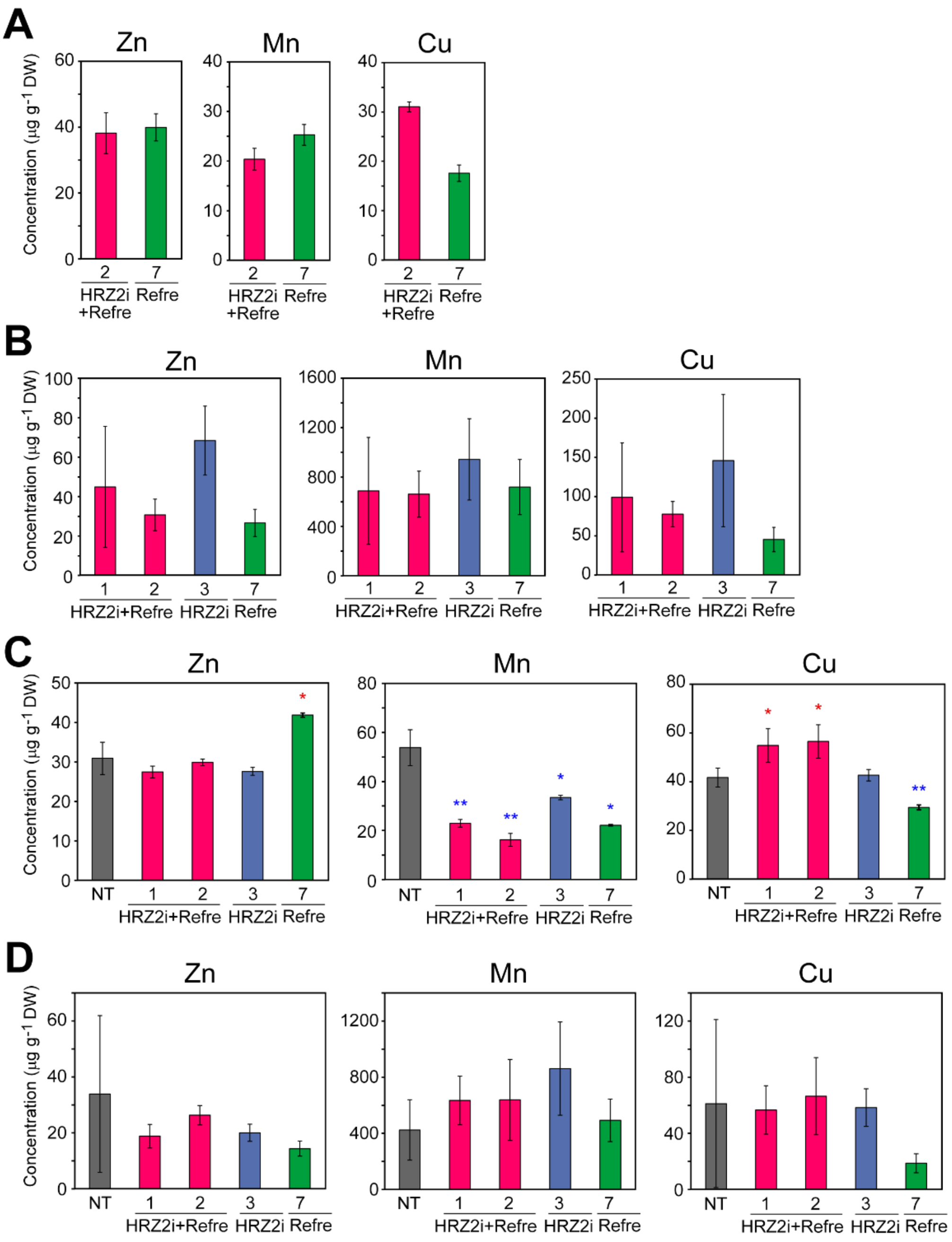

Figure S2. Additional metal concentrations in the HRZ2i+Refre lines compared with single transformation lines after pot cultivation in calcareous soil. **A, B** Zn, Mn, and Cu concentrations in brown seeds (**A**) or straw (**B**) in the non-submerged conditions in Figure 3. **C, D** Zn, Mn, and Cu concentrations in brown seeds (**C**) or straw (**D**) in the submerged conditions in Figure 4. The following lines were cultivated: NT, non-transformants; HRZ2i+Refre, T_1 lines of 1 and 2; HRZ2i, T_2 line 3; Refre, T_3 line 7. The means ± SD are shown. **A, B**: n = 6 for HRZ2i+Refre line 2 for seed; n = 3 for Refre line 7 for seed; n = 7 for HRZ2i+Refre line 2 for straw; n = 4 for other lines for straw. **C, D**: n = 3 for all lines for seed and NT for straw; n = 8 for HRZ2i+Refre line 2 for straw; n = 4 for other lines for straw. DW: dry weight. Asterisks indicate significant differences compared to the NT (two-sample Student's *t*-test; $*P < 0.05$; $**P < 0.01$).

Figure S3

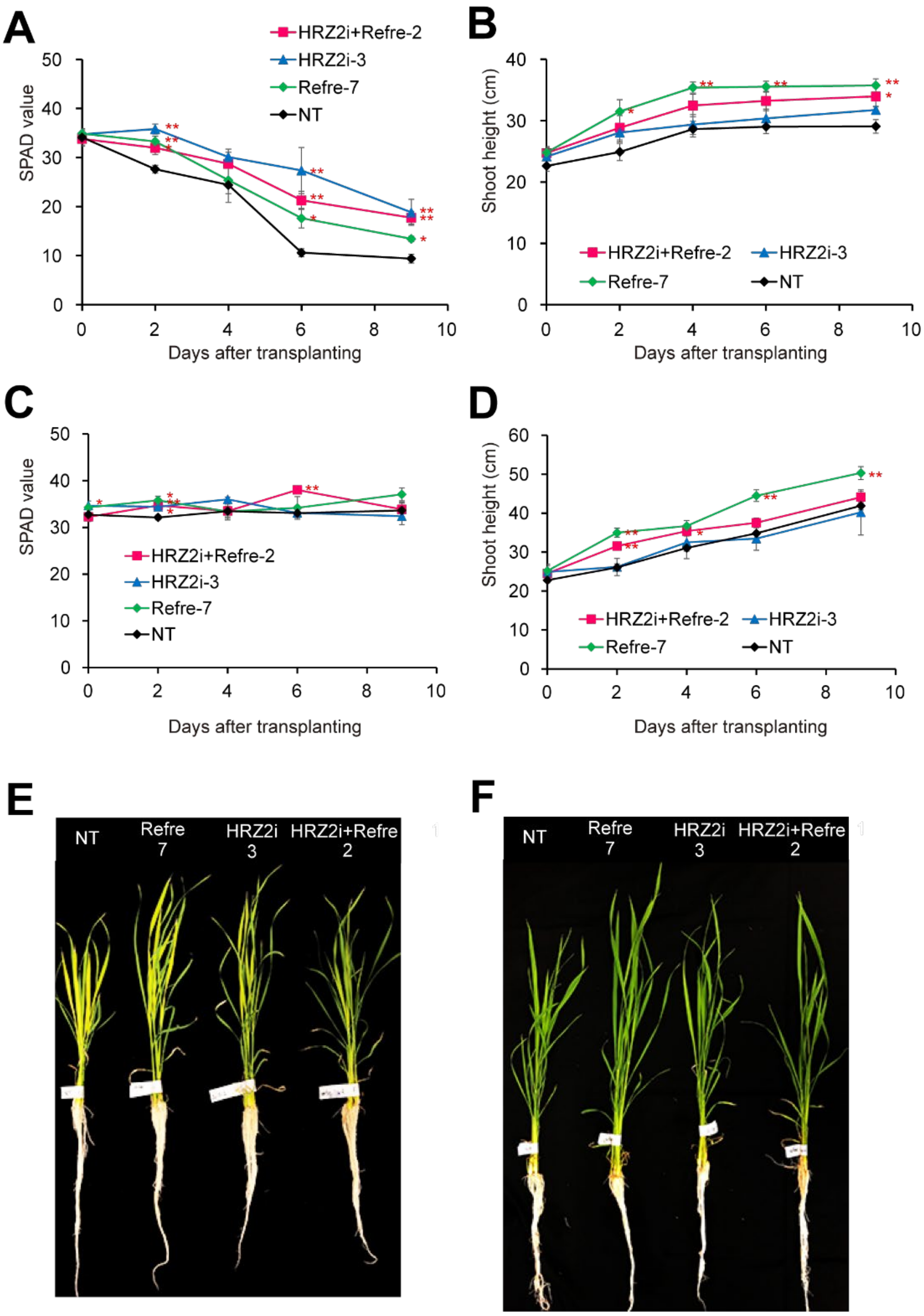

Figure S3. Features of the HRZ2i+Refre lines compared with single transformation lines under hydroponic culture. **A** SPAD value of the newest leaves during Fe-deficient culture. **B** Shoot height during Fe-deficient culture. **C** SPAD value of the newest leaves grown in Fe-sufficient conditions. **D** Shooting height in Fe-sufficient culture. **E, F** Representative plants after 9 d of Fe-deficient (**E**) or Fe-sufficient (**F**) culture. Three plants were bundled. For **A-D**, the means ± SD are shown are shown (n = 3 HRZ2i line 3 in **C** and **D**; n = 4 for others). Asterisks indicate significant differences compared to the NT (two-sample Student's *t*-test; $*P < 0.05$; $**P < 0.01$).