HvNramp5 mediates uptake of cadmium and manganese, but not iron in barley

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Supplemental Data

Supplemental Figure S1. Sequence, gene and protein structure, and phylogenetic analysis of *HvNramp5.*

Supplemental Figure S2. Gene expression level in the roots of *HvNramp5* RNAi lines and WTs.

Supplemental Figure S3. Concentration of Cu and Zn in the *HvNramp5* RNAi lines at different Mn concentrations.

Supplemental Figure S4. Phenotypic analysis of *HvNramp5* RNAi lines at different Cd concentrations.

Supplemental Figure S5. Phenotypic analysis of *HvNramp5* RNAi lines at different Fe concentrations.

A

The full-length cDNA (1638 bp):

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Amino acids (545aa):

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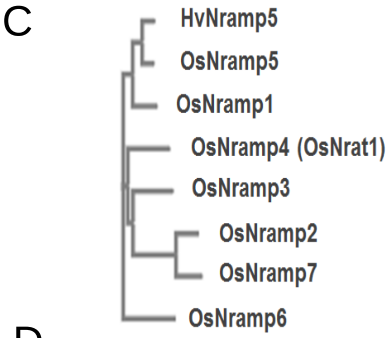

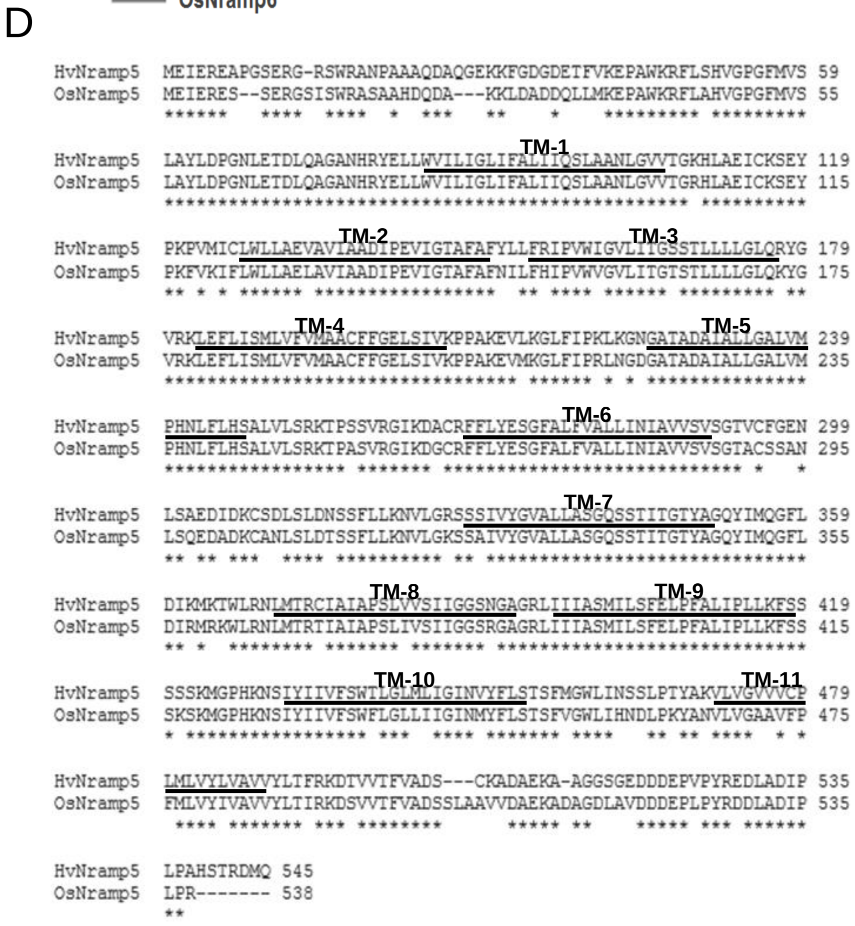

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HvNramp5  MEIEREAPGSERG-RSWRANPAAAQDAQGEKKFGDGDETFVKEPAWKRFLSHVGPGFMVS 59
OsNramp5  MEIERES--SERGSISWRASAAHDQDA---KKLDADDQLLMKEPAWKRFLAHVGPGFMVS 55
          ******   ****  ****  *  ***   **    *    ********* *********

                                           TM-1
HvNramp5  LAYLDPGNLETDLQAGANHRYELLWVILIGLIFALIIQSLAANLGVVTGKHLAEICKSEY 119
OsNramp5  LAYLDPGNLETDLQAGANHRYELLWVILIGLIFALIIQSLAANLGVVTGRHLAEICKSEY 115
          ************************************************* **********

                          TM-2                       TM-3
HvNramp5  PKPVMICLWLLAEVAVIAADIPEVIGTAFAFYLLFRIPVWIGVLITGSSTLLLLGLQRYG 179
OsNramp5  PKFVKIFLWLLAELAVIAADIPEVIGTAFAFNILFHIPVWVGVLITGTSTLLLLGLQKYG 175
          ** * * ****** *****************  ** **** ****** ********* **

                      TM-4                                  TM-5
HvNramp5  VRKLEFLISMLVFVMAACFFGELSIVKPPAKEVLKGLFIPKLKGNGATADAIALLGALVM 239
OsNramp5  VRKLEFLISMLVFVMAACFFGELSIVKPPAKEVMKGLFIPRLNGDGATADAIALLGALVM 235
          ********************************* ****** * * ***************

                                               TM-6
HvNramp5  PHNLFLHSALVLSRKTPSSVRGIKDACRFFLYESGFALFVALLINIAVVSVSGTVCFGEN 299
OsNramp5  PHNLFLHSALVLSRKTPASVRGIKDGCRFFLYESGFALFVALLINIAVVSVSGTACSSAN 295
          ***************** ******* **************************** *   *

                                               TM-7
HvNramp5  LSAEDIDKCSDLSLDNSSFLLKNVLGRSSSIVYGVALLASGQSSTITGTYAGQYIMQGFL 359
OsNramp5  LSQEDADKCANLSLDTSSFLLKNVLGKSSAIVYGVALLASGQSSTITGTYAGQYIMQGFL 355
          ** ** ***  **** ********** ** ******************************

                             TM-8                       TM-9
HvNramp5  DIKMKTWLRNLMTRCIAIAPSLVVSIIGGSNGAGRLIIIASMILSFELPFALIPLLKFSS 419
OsNramp5  DIRMRKWLRNLMTRTIAIAPSLIVSIIGGSRGAGRLIIIASMILSFELPFALIPLLKFSS 415
          ** *  ******** ******* ******* *****************************

                              TM-10                            TM-11
HvNramp5  SSSKMGPHKNSIYIIVFSWTLGLMLIGINVYFLSTSFMGWLINSSLPTYAKVLVGVVVCP 479
OsNramp5  SKSKMGPHKNSIYIIVFSWFLGLLIIGINMYFLSTSFVGWLIHNDLPKYANVLVGAAVFP 475
          * ***************** ***  **** ******* ****   ** ** ****  * *

HvNramp5  LMLVYLVAVVYLTFRKDTVVTFVADS---CKADAEKA-AGGSGEDDDEPVPYREDLADIP 535
OsNramp5  FMLVYIVAVVYLTIRKDSVVTFVADSSLAAVVDAEKADAGDLAVDDDEPLPYRDDLADIP 535
           **** ******* *** ********      ***** **    ***** *** ******

HvNramp5  LPAHSTRDMQ 545
OsNramp5  LPR------- 538
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Fig. S1 Sequence, gene and protein structure, and phylogenetic analysis of *HvNramp5*

A, Full-length cDNA sequence of *HvNramp5* and deduced amino acid sequences from barley cultivar Golden Promise. B, Schematic gene structure of *HvNramp5*. C, Phylogenetic tree of HvNramp5 and seven Nramp proteins in rice. D, Alignment of amino acid sequence between HvNramp5 and OsNramp5. Predicted transmembrane domains in HvNramp5 are marked with black lines.

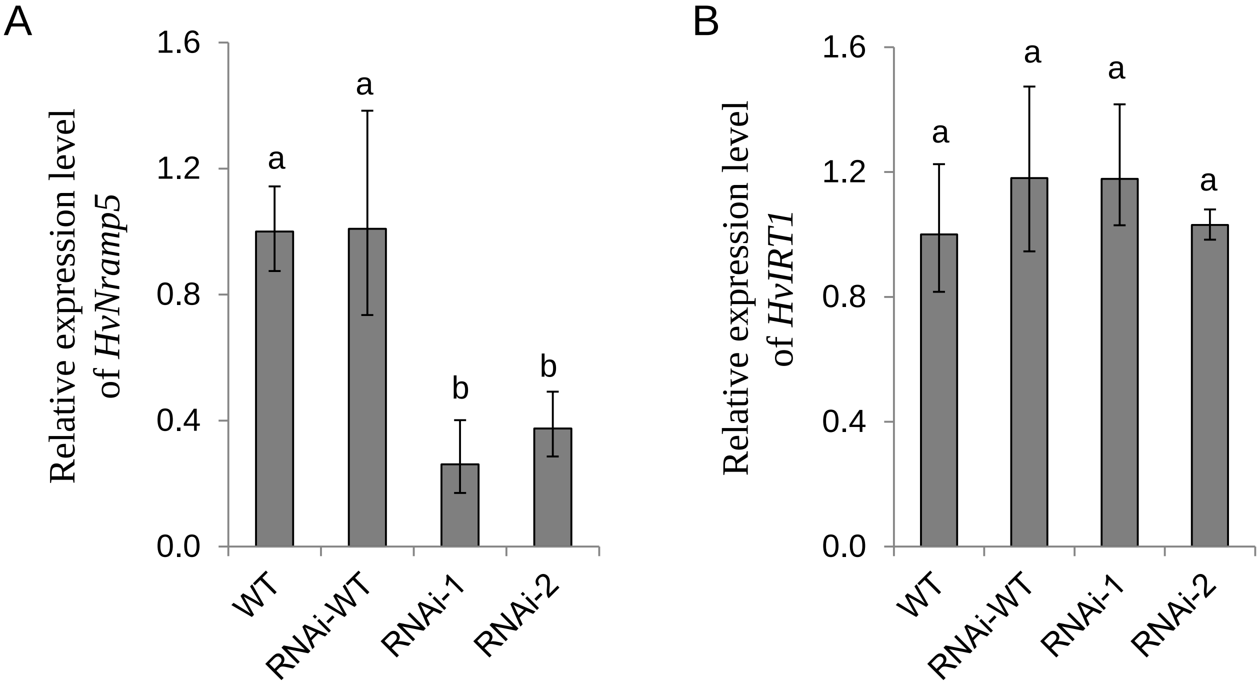

Fig. S2 Gene expression level in the roots of *HvNramp5* RNAi lines and WTs.

A-B, Relative expression level of *HvNramp5* (A) and *HvIRT1* (B) in the roots. Roots of two HvNramp5 RNAi lines, the homozygous wild type from the RNAi population (RNAi-WT) and wild-type barley (WT, cv. Golden Promise) were sampled for RNA extraction and expression analysis by quantitative RT-PCR. Expression relative to WT is shown. Data are means ± SD of three biological replicates and different small letter indicates significant difference at $p < 0.05$ by Tukey’s test.

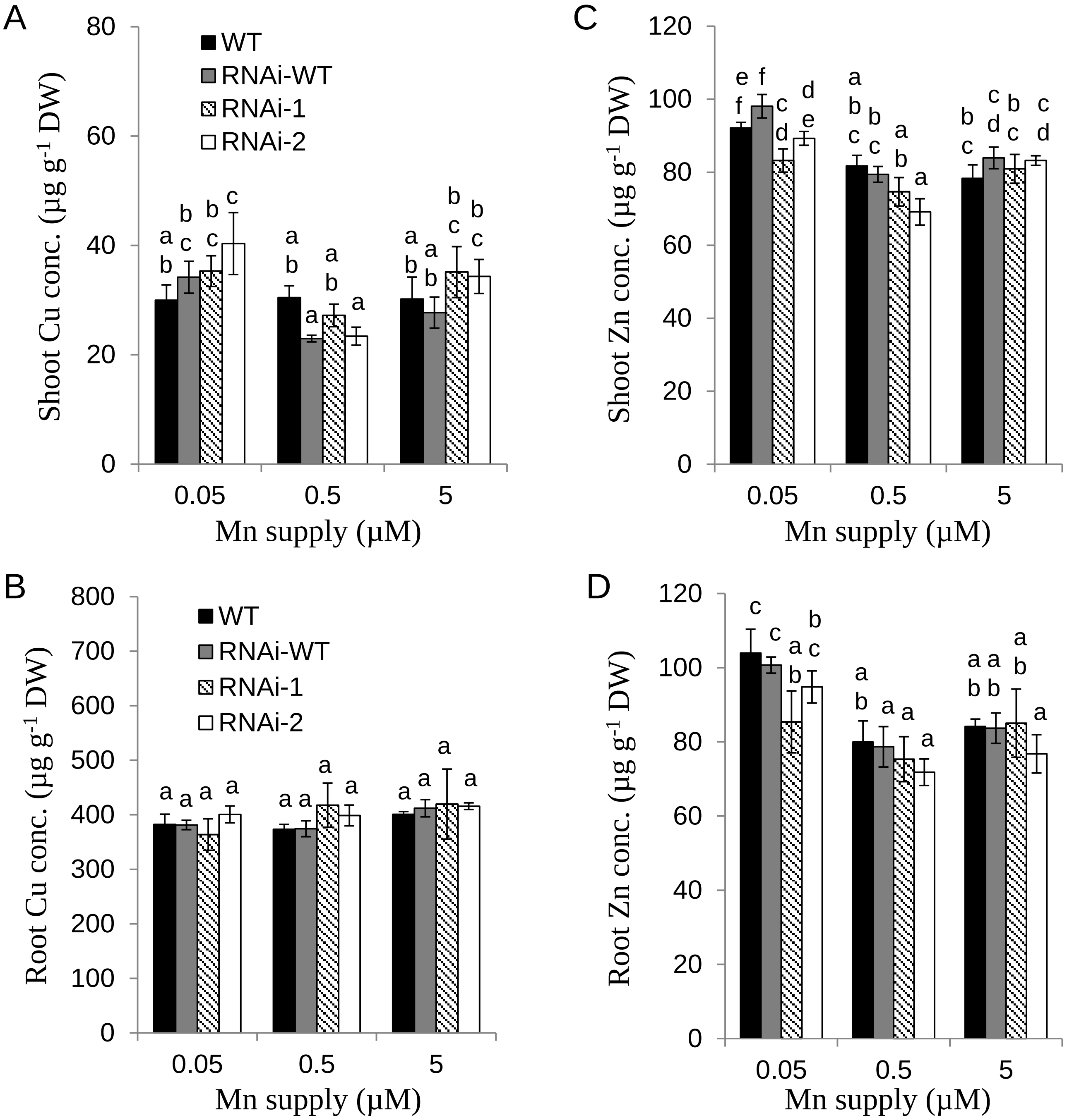

Fig. S3 Concentration of Cu and Zn in the *HvNramp5* RNAi lines at different Mn concentrations

Wild-type (WT) barley (cv. Golden Promise), the homozygous wild type (RNAi-WT) and two homozygous transgenic RNAi lines (RNAi-1, RNAi-2) derived from two independent events were cultivated in a nutrient solution containing 0.1, 0.5 or 1 µM Cd for 14 d. The concentration of Cu (A, B) and Zn (C, D) in the shoots (A, C) and roots (B, D) was determined by ICP-MS. Data are means ± SD of three biological replicates and different small letter indicates significant difference at $p < 0.05$ by Tukey's test.

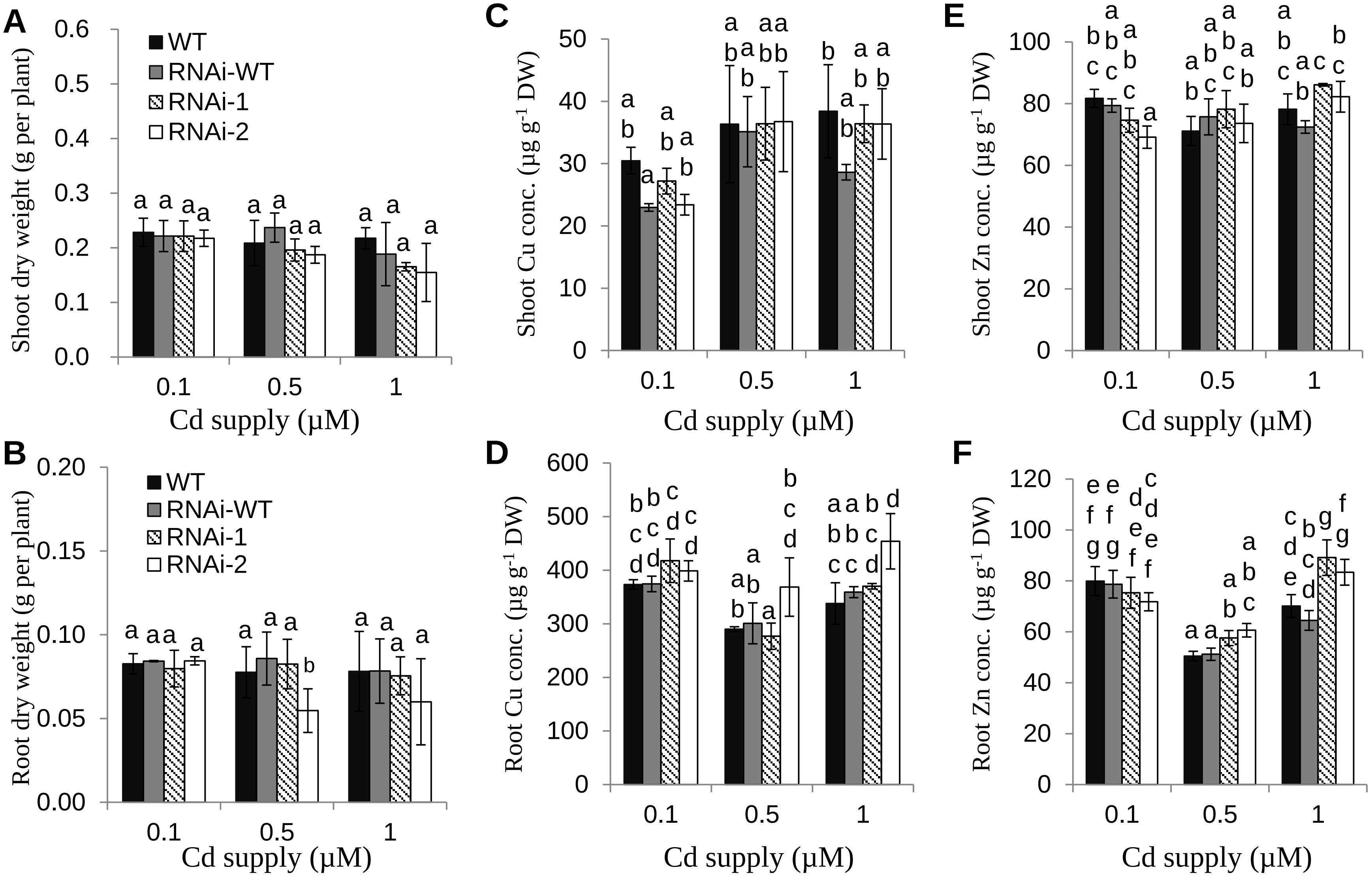

Fig. S4 Phenotypic analysis of *HvNramp5* RNAi lines at different Cd concentrations

Wild-type (WT) barley (cv. Golden Promise), the homozygous wild type (RNAi-WT) and two homozygous transgenic RNAi lines (RNAi-1, RNAi-2) derived from two independent events were cultivated in a nutrient solution containing 0.1, 0.5 or 1 µM Cd for 14 d. Dry weight of the roots (A) and shoots (B) was recorded. Concentration of Cu (C, D) and Zn (E, F) in the shoots (C, E) and roots (D, F) was determined by ICP-MS. Data are means ± SD of three biological replicates and different small letter indicates significant difference at $p < 0.05$ by Tukey's test.

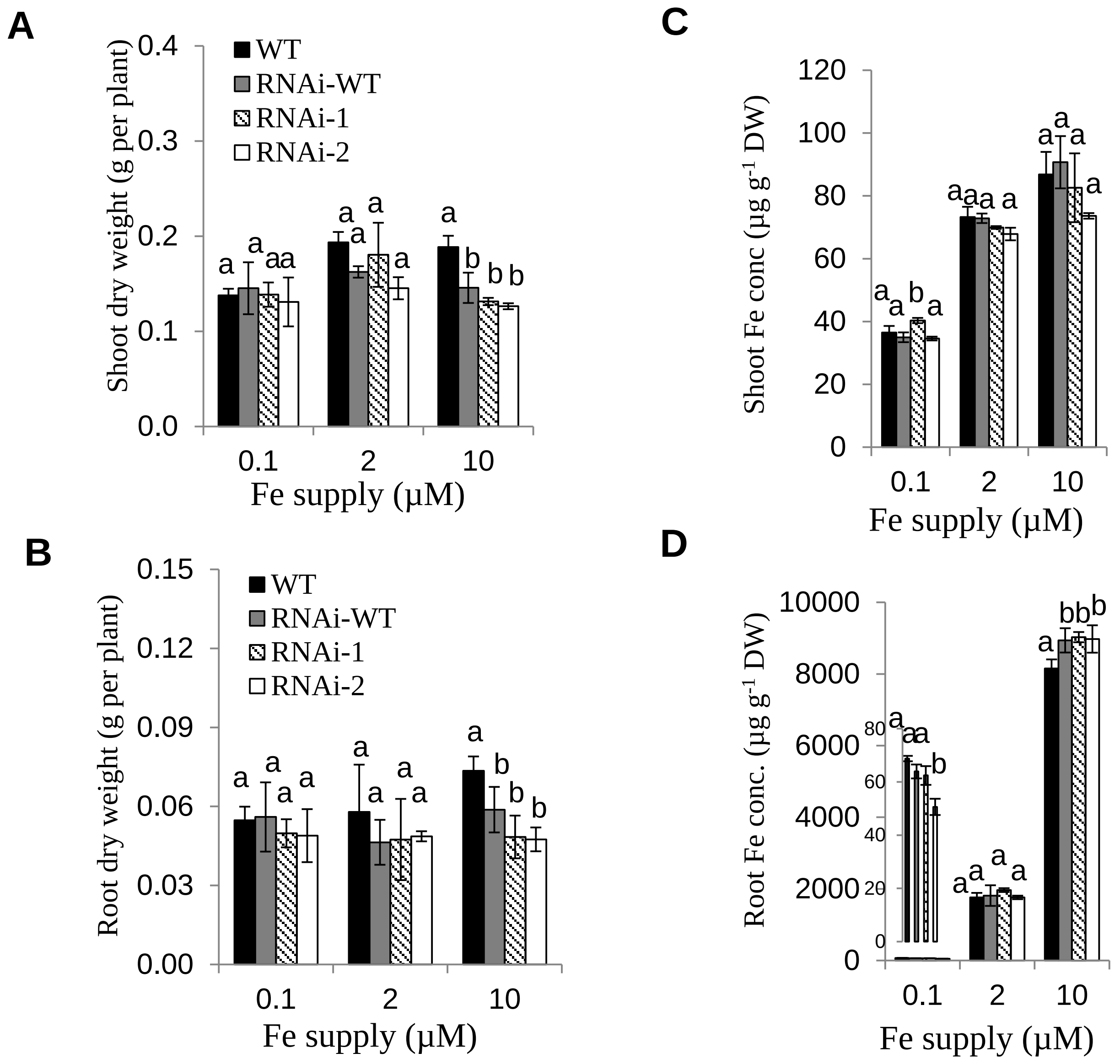

Fig. S5 Phenotypic analysis of *HvNramp5* RNAi lines at different Fe concentrations

Wild-type (WT) barley (cv. Golden Promise), the homozygous wild type (RNAi-WT) and two homozygous transgenic RNAi lines (RNAi-1, RNAi-2) derived from two independent events were cultivated in a nutrient solution containing 0.1, 2 or 10 µM $FeSO_4$ for 14 d. Dry weight of the roots (A) and shoots (B) was recorded. Concentration of Fe in the shoots (C) and roots (D) was determined by ICP-MS. Data are means ± SD of three biological replicates and different small letter indicates significant difference at $p < 0.05$ by Tukey’s test.