



Figure S2. Gene expression analysis of different HA-FIT Ox lines used in the selection of lines HA-FIT 8 and HA-FIT 9

Reverse transcription-qPCR analysis of A, *FIT*, B, *FRO2* and C, *IRT1*; in Col-0, two independent HA₇-FIT lines (#7: HA-FIT 7, #8: HA-FIT 8) and three independent HA₃-FIT lines (#4: HA-FIT 4, #6: HA-FIT 6, #9: HA-FIT 9), showing that ectopic over-expression of *FIT* took place in all transgenic lines, while *IRT1* and *FRO2* were induced only at - Fe; plants were grown in the 14-day growth system and exposed to + Fe (+) or - Fe (-). Roots (R) and leaves (L) were harvested separately and processed for experiments. The horizontal line (fictive line for visualization) in the *FIT* diagram (in A) represents the threshold of *FIT* expression level, which was needed to obtain ectopic expression of *FRO2* (in B) and *IRT1* (in C) in leaves. D, Reverse transcription-qPCR analysis of *FIT*, *FRO2* and *IRT1* in roots of Col-0, and the selected lines, HA-FIT 9 and HA-FIT 8. Plants were grown in the 14-day agar

growth system at + or – Fe; n = 2. E, as in D using leaves. *FRO2* and *IRT1* were ectopically expressed in leaves of HA-FIT8, but not of HA-FIT9 plants. Note that in leaves of the HA-FIT8 line, but not of the HA-FIT9 line, *FIT* over-expression levels were beyond the level indicated by the fictive line in A.