

Supplemental Figure 1. YFP-HMR is localized mainly to the cytoplasm and fails to rescue the nuclear defect of the hmr mutant. (A) Fluorescent microscopy images of the representative YFP-HMR localization patterns in YFP-HMR/hmr-5 line #1 (YFP-HMR #1). YFP-HMR localization was observed using 4-d-old seedlings grown in 10 µmol m⁻² s⁻¹ continuous red light for 96 hours. Seedlings were stained with DAPI and imaged by a Zeiss AXIO Observer Z1 fluorescence microscope with a DAPI filter set (excitation, < 395 nm; emission, BP 445/50 nm) and a YFP filter set (excitation, BP 500/25 nm; emission BP 535/30 nm). Images were deconvolved using Huygens Essential. DAPI is shown in red and YFP in green. White arrowheads indicate the position of nuclei. Strong YFP signals were observed in the cytoplasm of all cells. In most cells, YFP was not observable in the nuclues (top panels). In some cells, weak or strong YFP signals could be observed in the nucleus (bottow two panels). Scale bars equal 20 µm. (B) Quantification of YFP-HMR localization. The percentage of cells with YFP signal in the cytoplasm or nucleus were calculated (n=379). Error bars represent the standard deviation of eight seedlings. These results show that YFP-HMR is mainly localized to the cytoplasm. (C) Hypocotyl length measurements of 4-d-old Col-0, hmr-5, and two independent YFP-HMR/hmr-5 (YFP-HMR) lines, #1 and #4, grown under 10 µmol m⁻² s⁻¹ continuous red light for 96 hours. Error bars represent standard errors of the measurements from at least 20 seedlings. Samples with different letters exhibit statistically significant differences in hypocotyl length (ANOVA, Tukey's HSD, P < 0.01, n > 20). (D) qRT-PCR analysis of four HMR-dependent and PIF-induced direct target genes in 4-d-old Col-0, hmr-5, and the YFP-HMR lines. Seedlings were grown under 10 µmol m⁻² s⁻¹ continuous red light for 96 hours. Transcript levels were calculated relative to those of PP2A. Error bars represent the standard error of three replicates.