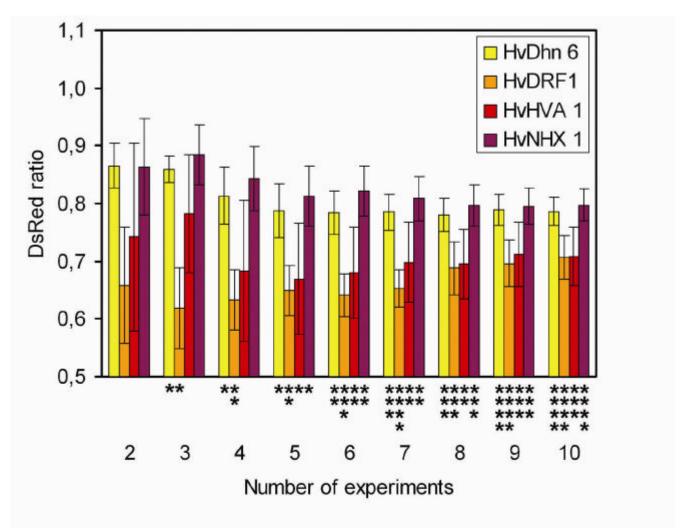
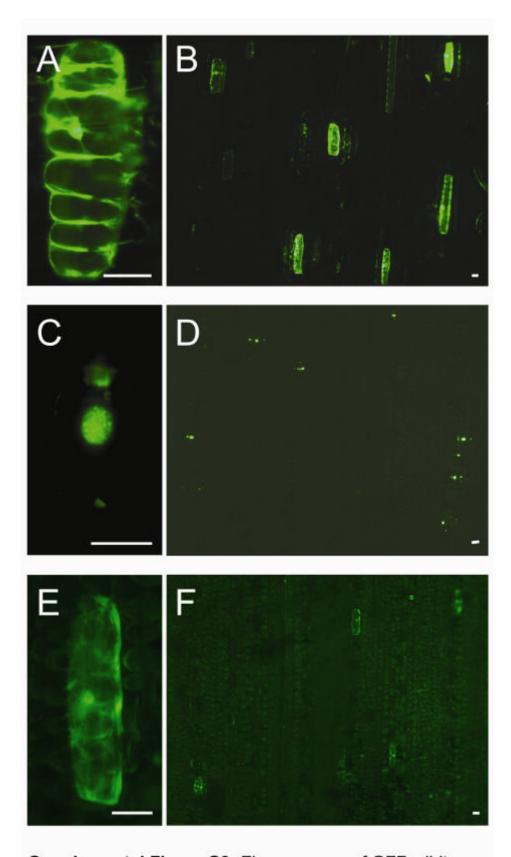


**Supplemental Figure S1:** Schematic diagram of Constructs used for transient expression of GFP-fusion proteins and for transient-induced gene silencing (GATEWAY destination vector pIPKTA30N). Amino acids 459-686 were deleted from fusion protein encoded by pIPKTA40\_DRF1 in order to stabilize it. T, terminator; att R1 and attR2, attachment sites for LR clonase; CmR, chloramphenicol resistance gene; ccdB, negativ selection marker; I, intron from *RGA2* gene of wheat.



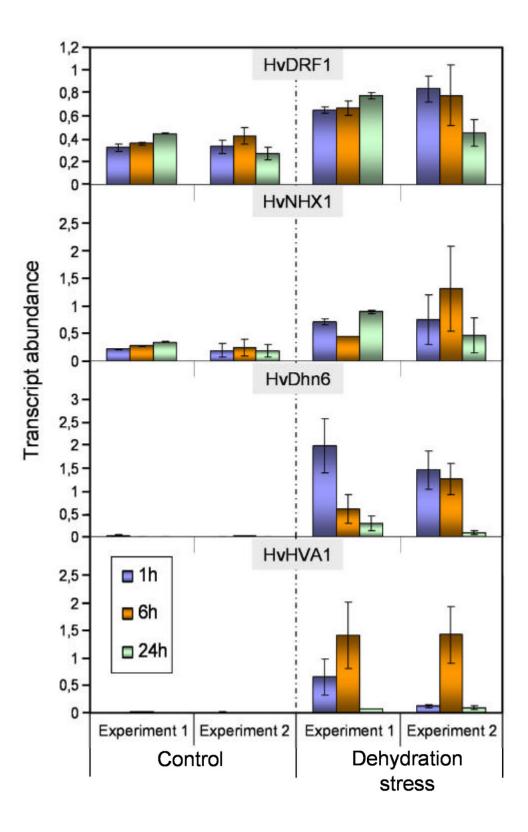
**Supplemental Figure S2:** Minimum number of independent TIGS experiments required for statistically significant effects of four candidate genes.

The ratio of normalized numbers of DsRed-fluorescing cells (RNAi test construct versus pIPKTA30N empty vector) in dehydration-stressed leaves is shown. Mean values + SEM from the indicate dnumber of independent experiments. Increasing numbers of stars below each column indicate increasing statistical significance of TIGS effect. \*, p<0.05; \*\*,p<0.01; \*\*\*,p<0.001; \*\*\*\*,p<0.0001.



**Supplemental Figure S3:** Fluorescence of GFP wildtype and DREB:GFP fusion proteins upon transient expression in barley epidermal cells.

(A, B) Wildtype GFP; (C,D) HvDREB1:GFP fusion protein; (E,F) GFP:HvDRF1:GFP fusion protein. (A, C, E) A typical cell expressing GFP shown at larger magnification. Scale bar, 20  $\mu$ m.



**Supplemental Figure S4:** Reproducibility of gene-regulation in the detached dehydration-stress system of barley. Leaf segments were bombarded with pGFP plus pUbi-DsRed-nos, 24 h prior to the dehydration stress treatment. RNA was extracted in two independent dehydration experiments at the times indicated. Mean ± range of two independent qPCR runs using the same cDNA samples.