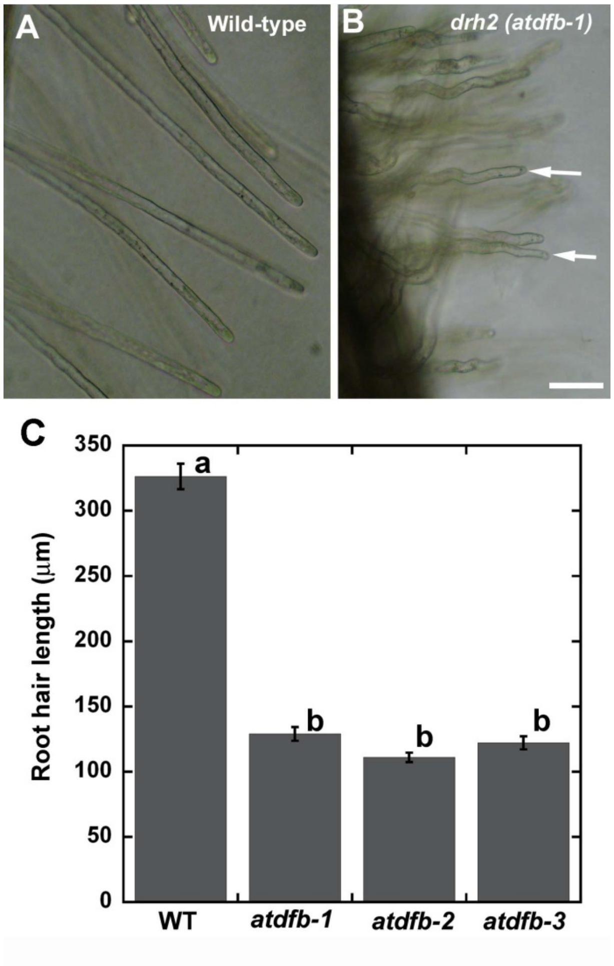
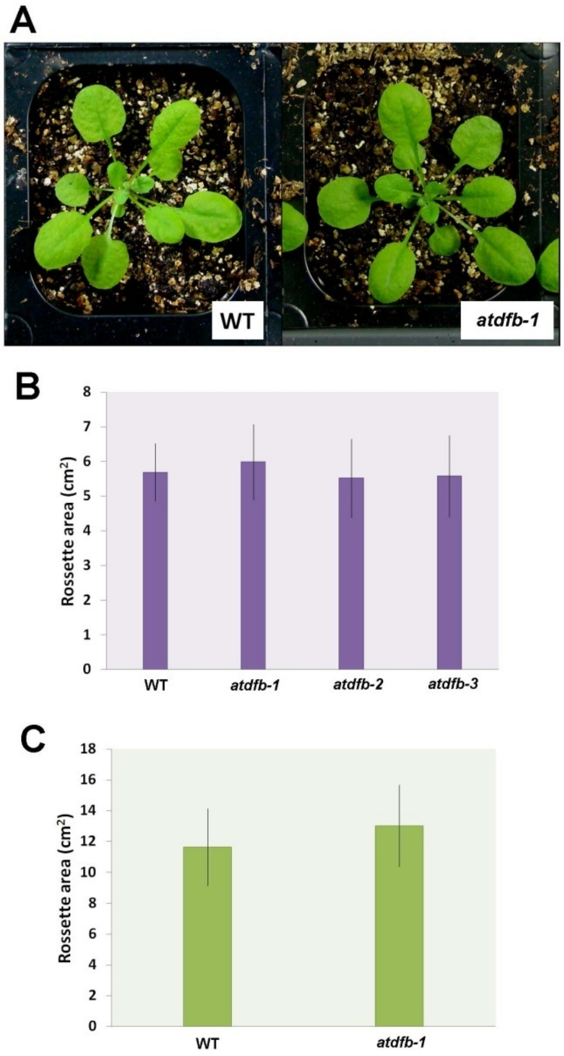
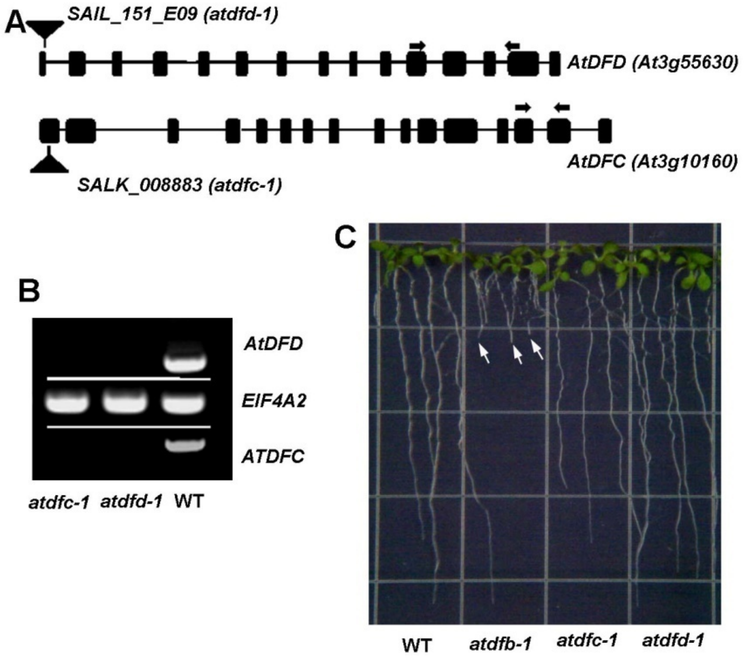
Supplemental Figures



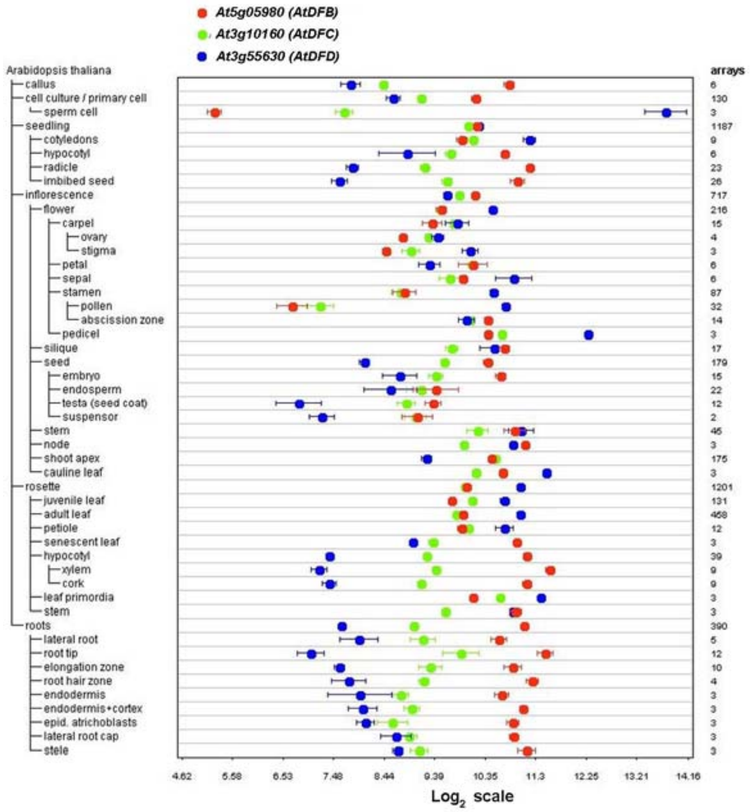
Supplemental Figure S1. *drh2 (atdfb-1)* has short and wavy root hairs. Wild type (A) and mutant (B) root hairs from 4 day old seedlings. Note that the mutant has root hairs that are about 3-fold shorter compared to wild-type (arrows). (C) Quantification of root hair length in *atdfb-1*, *atdfb-2*, and *atdfb-3*. Values are means ± SE of 100 root hairs from 20 seedlings. Means with different letters are statistically significant (Tukey's test; p<0.05).



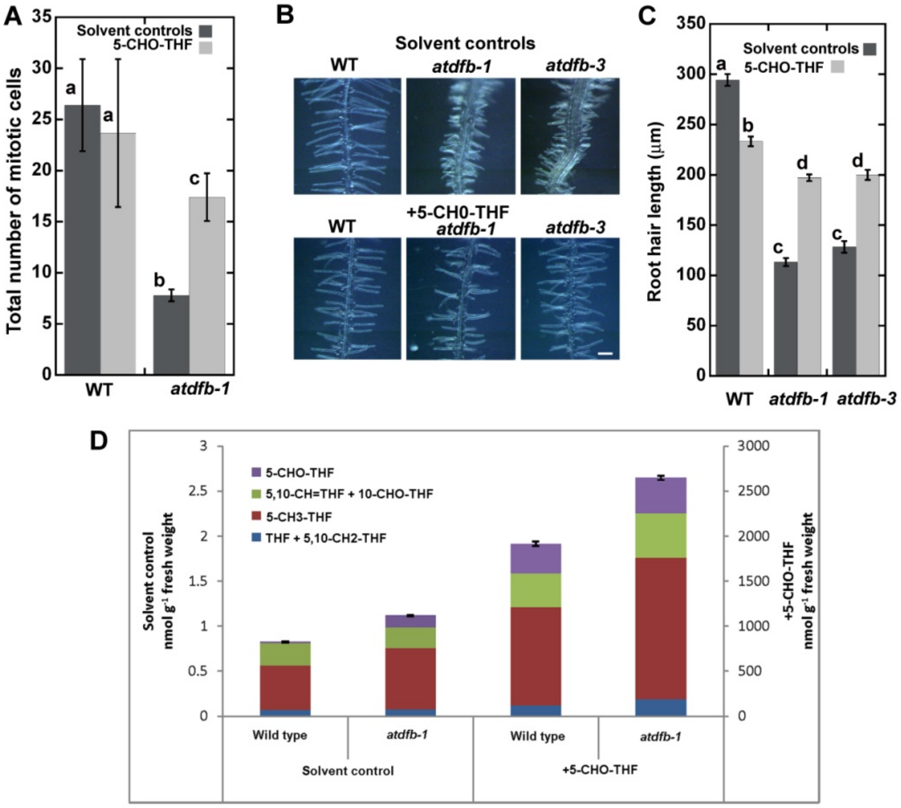
Supplemental Figure S2. *AtDFB* knockouts have no obvious defects in shoot development when compared to wild type. A, Representative image of the above ground organs of 17 day old wild type and *atdfb-1* plants from seed directly germinated in soil. Rosette area of 10 day (B) and 17 day (C) old wild type and *atdfb* seedlings. Values are means ± SD of 18 plants. Rosette area of *atdfb* showed no significant difference compared to wild type.



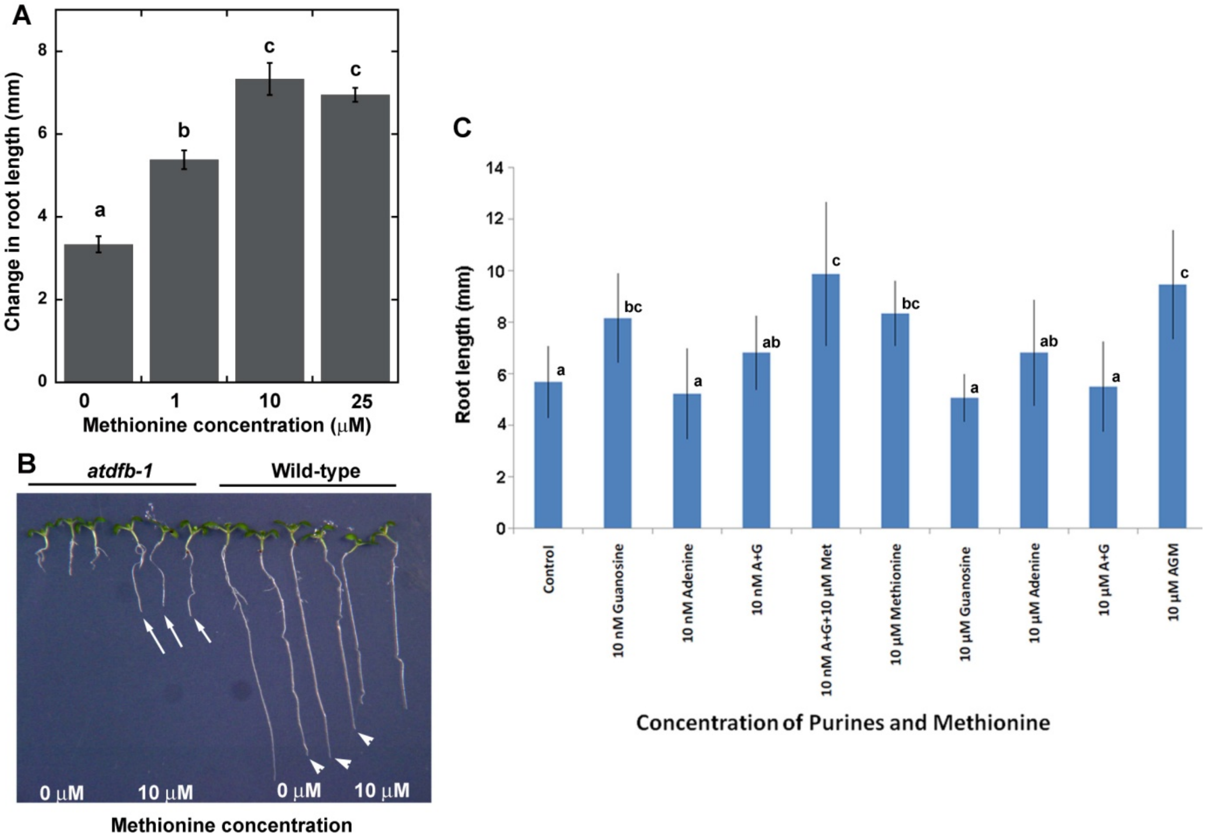
Supplemental Figure S3. Identification of mutants to *AtDFC* and *AtDFD*. A, Schematic diagram of the genome organization of *AtDFC and AtDFD* showing T-DNA insertion sites for SAIL_151_EO9 (*atdfd-1*) and SALK_008883 (*atdfc-1*). B, Semi quantitative RT-PCR of *AtDFC and AtDFD* in wild type (WT), *atdfc-1* and *atdfd-1*. C, 12 day old seedlings of wild type (WT), *atdfb-1*, *atdfc-1* and *atdfd-1*. Only *atdfb-1* (arrows) has reduced primary root growth.



Supplemental Figure S4. *AtDFB* is more strongly expressed in roots compared to *AtDFC* and *AtDFD*. *In silico* expression profiling of *AtDFB*, *AtDFC* and *AtDFD* based on publicly available microarray data sets from Genevestigator (Zimmermann et al., 2004). The X axis is in the log$_2$ scale.



Supplemental Figure S5. 5-formyl tetrahydrofolate (5-CHO-THF) rescues the cell division and root hair phenotypes of *atdfb*. A, Number of mitotic cells in the apical 100 µm of *atdfb-1* increases when exposed to exogenous 5-CHO-THF. Data are means of 5 roots ± SE. Means with different letters are statistically significant (Tukey's test; $p<0.05$). Root hair length of *atdfb* is enhanced by 5-CHO-THF (B, C). Data are means from 50 to 80 root hairs ± SE from 10-15 independent seedlings. Means with different letters are significantly different (Tukey's test; $p < 0.05$). D, Folate hyper-accumulation in roots of 5-CHO-THF complemented seedlings. Analyses were conducted on roots that were rinsed with distilled water and dried before analysis to avoid 5-CHO-THF transfer from the media. Y-axis values to the left correspond to solvent control data whereas Y-axis values to the right correspond to seedlings treated with 5-CHO-THF. Data are means ± SE from three independent replicates. Bar = 100 µm (B).



Supplemental Figure. S6. Exogenous methionine and guanosine promotes root growth in *atdfb* seedlings. A, 10 μM methionine triggered the strongest induction of root growth in *atdfb-1* with a more than 2-fold increase in root length compared to non-treated mutant roots. Means with different letters are statistically significant (Tukey's test; $p < 0.05$). B, Representative images of *atdfb-1* and wild type seedlings with and without exogenous methionine. Although *atdfb-1* roots exposed to methionine were longer than untreated *atdfb-1* roots (arrows), their length was still significantly less than wild type roots (arrowheads). C, Exogenous guanosine (G) but not adenine (A) partially promotes root growth of *atdfb-1*. Data are means from 15 to 25 roots ± SE. Means with different letters are statistically significant (Tukey's test; $p < 0.05$).