Supplemental information, Figure S4

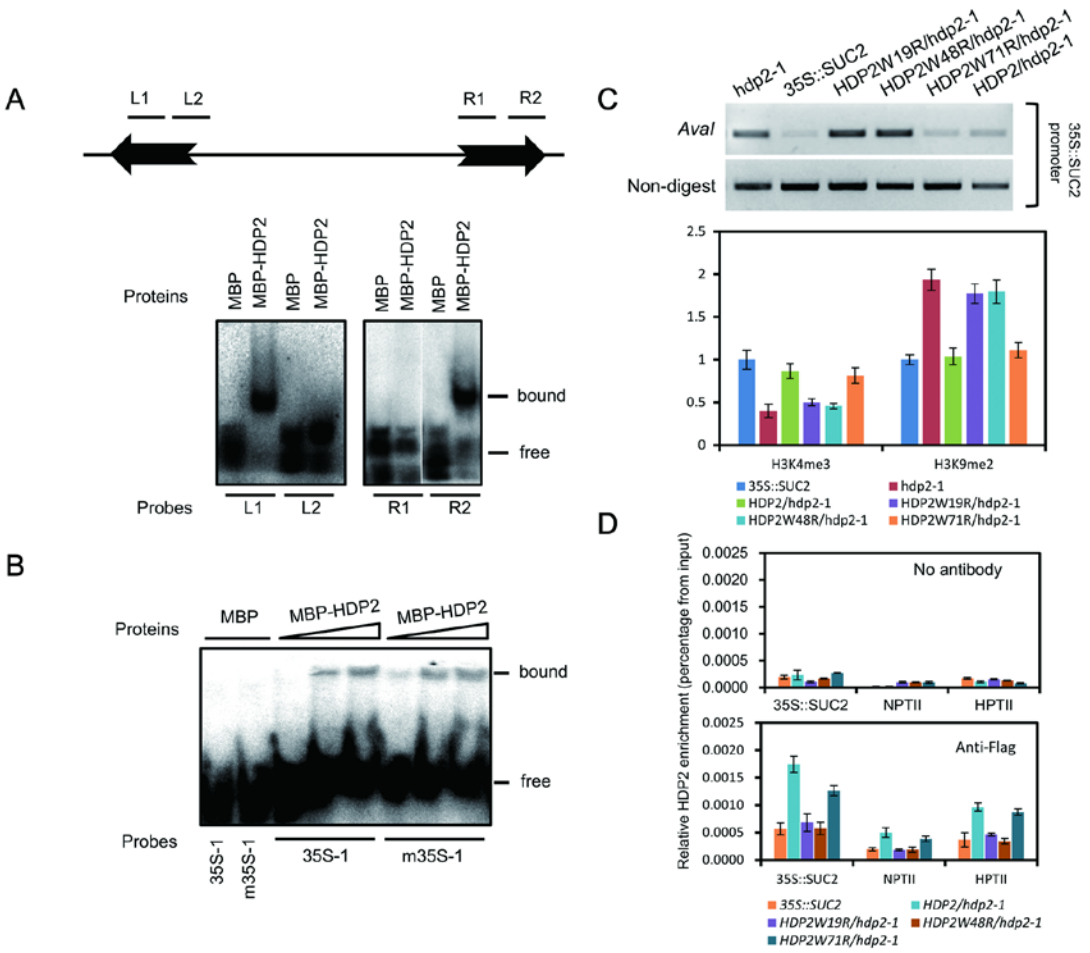

Figure S4 DNA-binding activity of HDP2 and its role in anti-silencing and DNA demethylation. Related to **Figure 4**. **(A)** EMSA assay showing the DNA-binding activity of HDP2. MBP-fused HDP2 recombinant protein was incubated with four double-stranded DNA probes from the TIRs sequence of zebrafish Harbinger3N_DR transposon [11]. Mobility shifted bands were observed when MBP-HDP2 was incubated with L1 and R2 probes, suggesting that HDP2 bears DNA-binding activity. MBP served as a negative control. Bound and free probes are labeled with black arrows. See also Supplementary information, Table S3 for the sequence of DNA probes. **(B)** HDP2 showing binding activity to both unmethylated and methylated

DNA probes. The 35S-1 region (See also Figure 2A) from *35S::SUC2* promoter region was selected to synthesize methylated and unmethylated double-stranded DNA probes via PCR amplification. Equal amounts of methylated and unmethylated DNA probes were incubated with increasing amounts of MBP-HDP2 proteins. MBP-HDP2 displayed similar DNA-binding patterns to these two probes. “35S-1” and “m35S-1” represent unmethylated and methylated DNA probes, respectively. **(C)** HDP2 DNA-binding mutants cannot rescue the DNA hypermethylation and reduced transcriptional activity of *35S::SUC2* promoter caused by *hdp2-1* mutation. Upper panel: chop-PCR showing HDP2 W19R and W48R DNA-binding mutants cannot rescue the hypermethylation of *hdp2-1*. Chop-PCR was performed in wild-type HDP2 and HDP2 DNA-binding mutant transgenic plants to test the effects of HDP2 DNA-binding activity on DNA demethylation. Lower panel: ChIP-qPCR showing the effects of HDP2 DNA-binding mutants on the accumulations of histone H3K4me3 and H3K9me2 modifications at *35S::SUC2* transgene promoter region. **(D)** ChIP assay showing HDP2 W19R and W48R mutations abolished its enrichment at transgene promoter regions. Wild-type and DNA-binding mutant HDP2 transgenic plants were subjected to ChIP assay using anti-Flag antibody to test the effects of HDP2 DNA-binding activity on its enrichment at transgene promoter regions. No antibody was used as negative control.