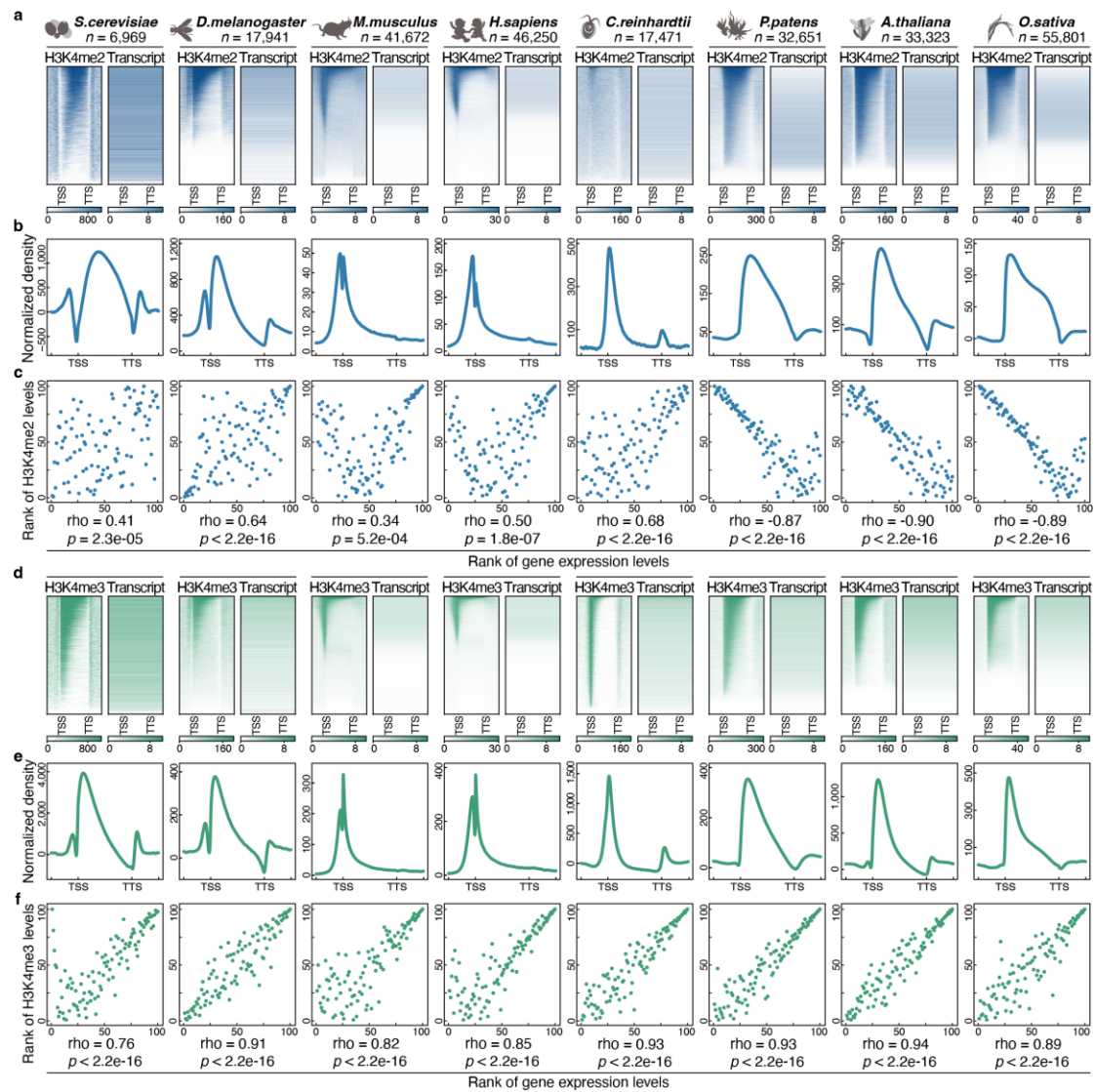
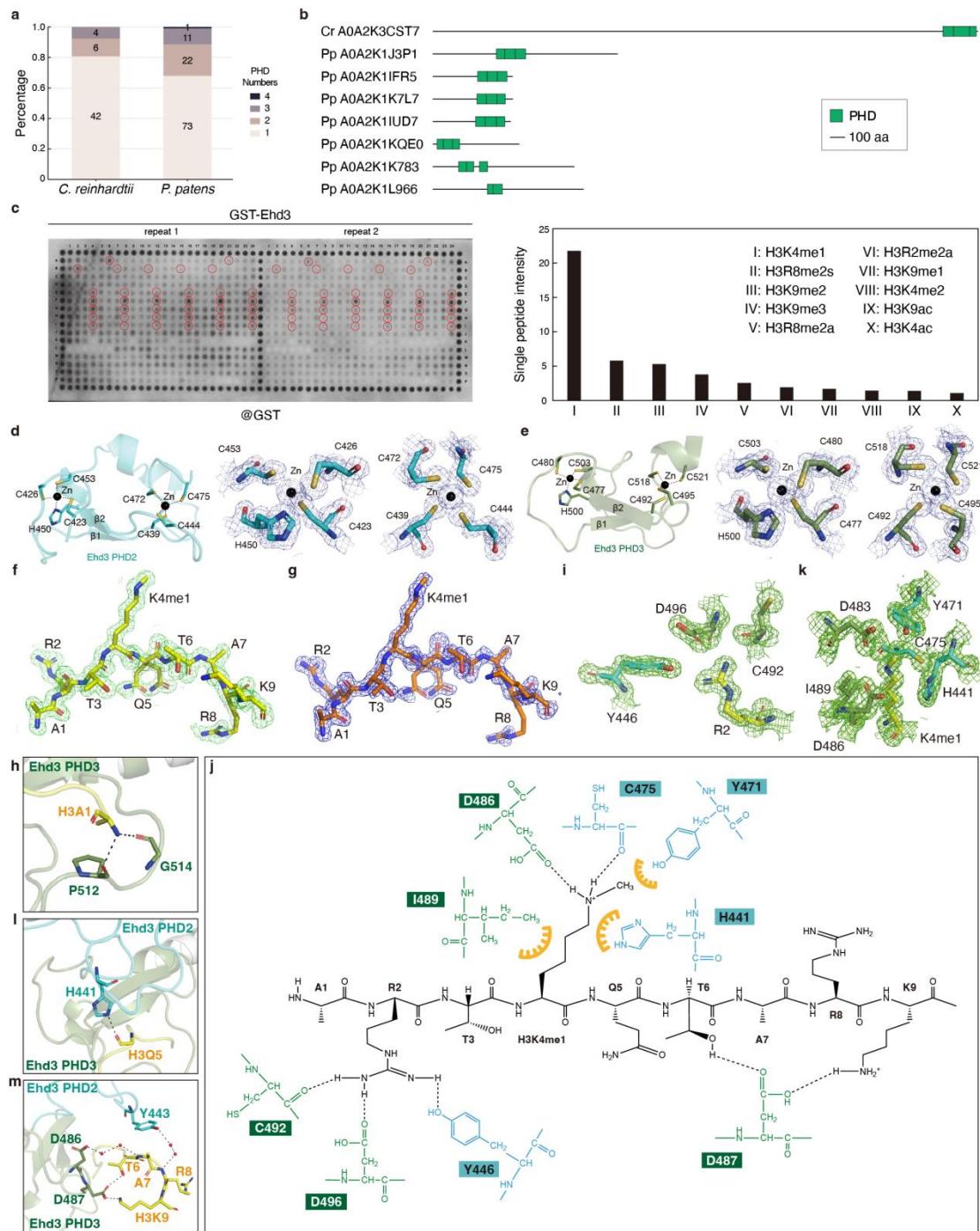




Supplementary Fig. 1: Characterization of the genome-wide distributions of H3K4me2 and H3K4me3 across diverse species.

a,d, Heatmaps showing the individual gene distribution of H3K4me2 (a) and H3K4me3 (d), together with their associated transcription level (log₂-transformed) in eight species. All genes are sorted based on the enrichment of H3K4me2 (a) or H3K4me3 (d). Gradient colors indicate levels of histone modification enrichment or transcription. **b,e**, Integrative genomic distributions of H3K4me2 (b) and H3K4me3 (e) in various species. **c,f**, Scatter plots presenting the correlations of H3K4me2 (c) and H3K4me3 (f) levels with gene transcription levels in eight species. The species from left to right are *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Mus musculus*, *Homo sapiens*, *Chlamydomonas reinhardtii*, *Physcomitrium patens*, *Arabidopsis thaliana*, and *Oryza sativa*, respectively. The transcribed genes enriched with H3K4me2 or H3K4me3

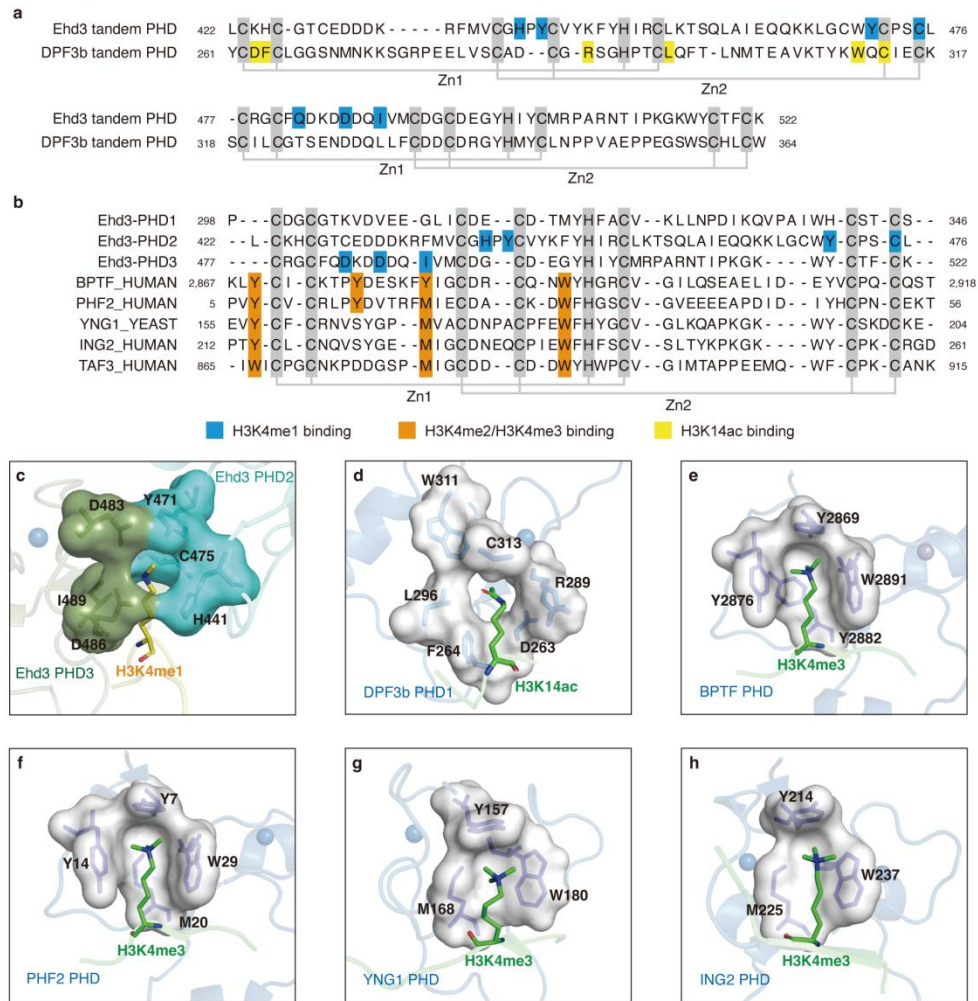
modifications were divided into 100 groups based on the levels of their transcription and modification. The average values of each group of modifications and transcription were calculated for analysis. The heatmaps and plots present the region from 3 kb upstream of the transcription start site (TSS) to 3 kb downstream of the transcription termination site (TTS). For scatter plots, Spearman's rank correlation coefficient indicates the correlation between the methylation level and gene transcription level. The p -values were determined based on two-sided Spearman's rank correlation test. Public data were downloaded and analyzed for *Saccharomyces cerevisiae* (ref. ¹⁷), *Drosophila melanogaster* (GSE55555, GSE47285, and SRR1197331), *Mus musculus* (ref. ¹⁸), *Homo sapiens* (refs. ^{19,20}), and *Arabidopsis thaliana* (ref. ²¹).



Supplementary Fig. 2: Ehd3 recognizes H3K4me1 peptide.

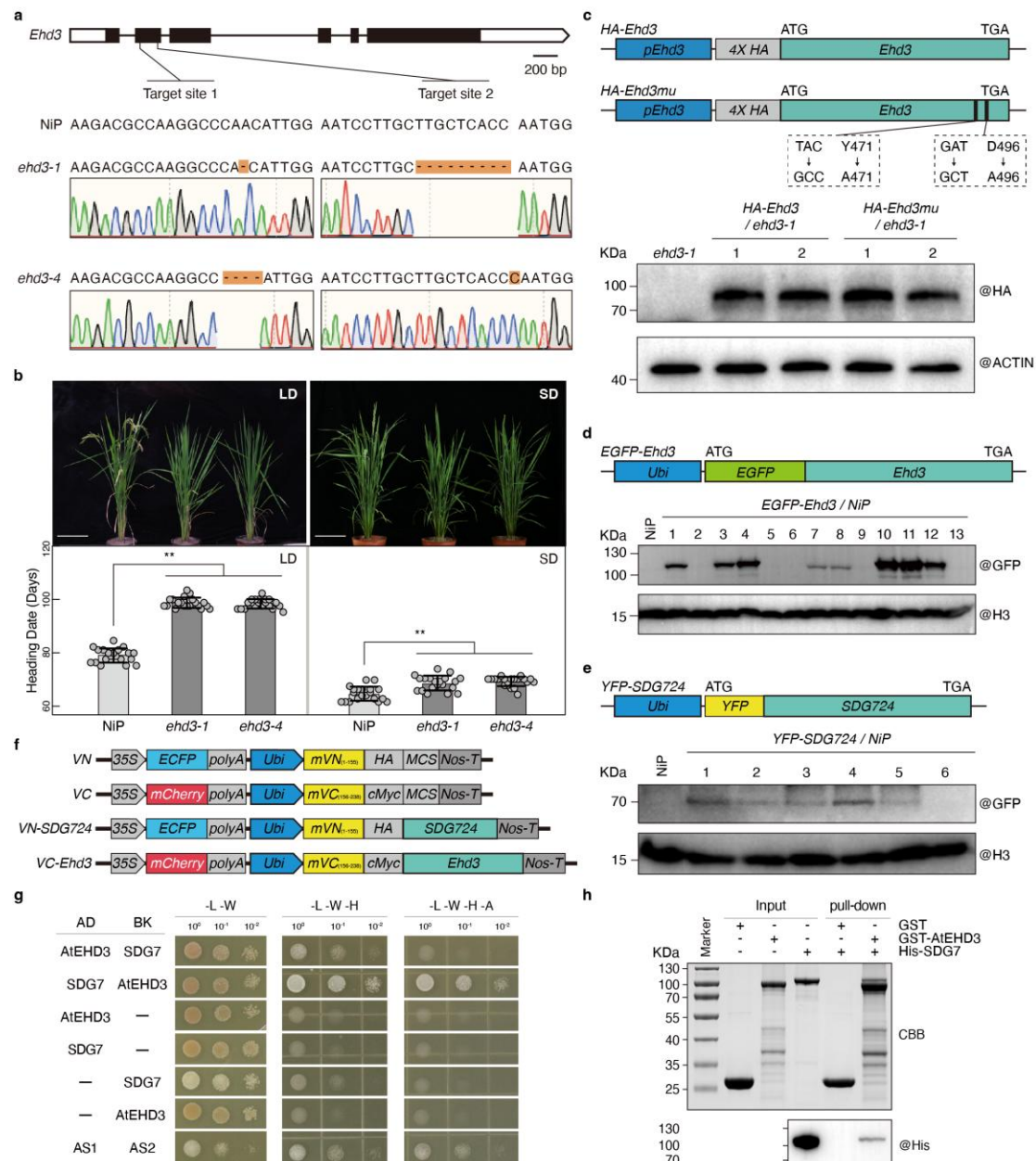
a, Intersection comparison of the percentage of plant homeodomain (PHD) domain-containing proteins with varying domain numbers (1–4) in *Chlamydomonas reinhardtii* and *Physcomitrium patens*. Percentage represents each number of proteins with 1–4 PHD domains relative to the total number of PHD domain-containing proteins. The number of proteins with 1–4 PHD domains is labeled in the graph. **b**, Protein domain architecture of tandem PHD domain-containing proteins in *Chlamydomonas reinhardtii*

and *Physcomitrium patens*. Cr, *Chlamydomonas reinhardtii*; Pp, *Physcomitrium patens*. UniProt (<https://www.uniprot.org>) entries of the proteins are marked behind the species abbreviations. **c**, Representative array images showing histone binding preferences of glutathione-S-transferase (GST)–Ehd3 on a histone peptide microarray. Red circles indicate the locations of H3K4me1-modified peptides. Histogram showing the relative binding intensity of GST–Ehd3 to the indicated modified histone peptide. **d,e**, Critical residues of Ehd3 plant homeodomain 2 (PHD2) (d) and PHD3 finger (e) involved in zinc ion binding are shown as sticks. Zinc ions are depicted as spheres in black. The $2F_o-F_c$ electron density maps are contoured at 1.5 sigma levels. **f**, Overall conformation of the bound H4K3me1 peptide. The $2F_o-F_c$ electron density map of the peptide is contoured at 1.0 sigma levels. **g**, Unbiased omit map of the bound H4K3me1 peptide. The F_o-F_c electron density map of the peptide is contoured at 3.0 sigma levels. **h**, Detailed interaction of Ehd3 with H3A1. **i**, Residues responsible for H3R2 recognition. The $2F_o-F_c$ electron density maps of the peptide are contoured at 1.5 sigma levels. **j**, Schematic representation showing the intermolecular interactions between Ehd3 and the side chains of H3K4me1 peptide residues. The hydrogen bonds are highlighted by dashed lines. **k**, Residues responsible for H3K4me1 recognition. The $2F_o-F_c$ electron density maps of the peptide are contoured at 1.5 sigma levels. **l,m**, Detailed interaction of Ehd3 with H3Q5 (l) and with H3T6, H3A7, H3R8, and H3K9 (m). Source data are provided as a Source Data file.



Supplementary Fig. 3: Structure-based characterization of the representative PHD finger.

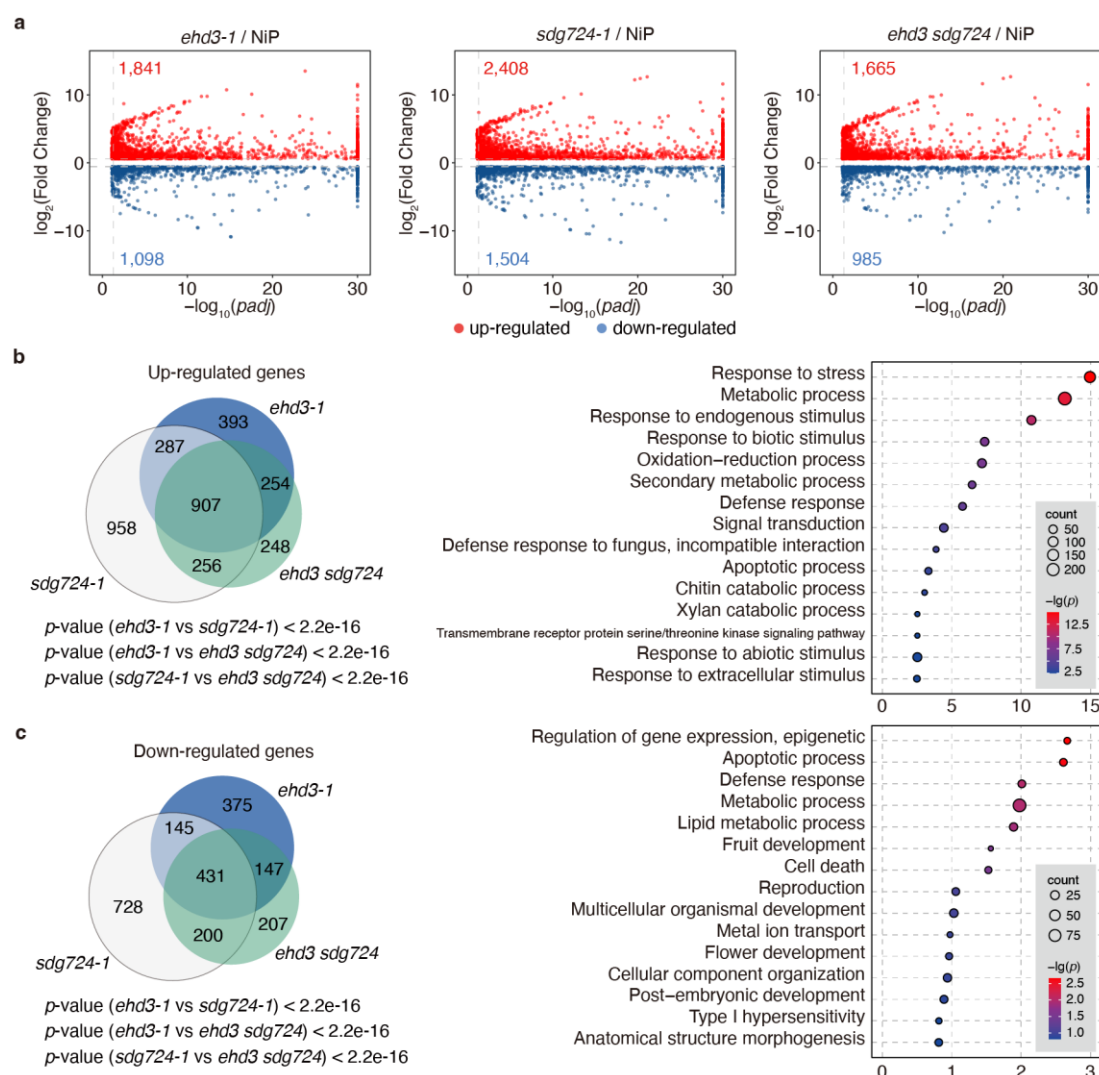
a,b, Sequence alignment of the tandem PHD of Ehd3 with that of DPF3b (a), and PHD fingers in Ehd3 with those in other PHD-containing proteins (b). The critical residues of PHD fingers are classified into three groups, distinguished by different colors as indicated. Zinc-coordinating residues are shaded in light grey. **c-h**, Representative structure of the PHD domain including Ehd3 (c), DPF3b (d), BPTF (e), PHF2 (f), YNG1 (g), and ING2 (h), in complex with various H3 modifications. The published PDB structures include: 5szb (DPF3b, ref. ²⁵), 2f6j (BPTF, ref. ²⁶), 3kqi (PHF2, ref. ²⁷), 2jmg (YNG1, ref. ²⁸), and 2g6q (ING2, ref. ²⁹).



Supplementary Fig. 4: Generation of CRISPR/Cas9 mutants and transgenic plants related to *Ehd3* and *SDG724*, as well as analysis of the interaction between *AtEHD3* and *SDG7* in Arabidopsis.

a, Identification of the *ehd3* mutants generated by CRISPR/Cas9 editing. The untranslated regions, exons, and introns of *Ehd3* are indicated by blank rectangles, black rectangles, and black lines, respectively. The altered sequences in the *ehd3-1* and *ehd3-4* mutants are highlighted in orange. **b**, Representative images showing wild-type rice ‘Nipponbare’ (NIP), *ehd3-1*, and *ehd3-4* plants grown under long-day (LD) and short-day (SD) conditions, respectively. Scale bar, 20 cm. Histograms show days to

heading in NiP, *ehd3-1*, and *ehd3-4* plants under LD and SD conditions. Values are shown as means $\pm$ standard deviation of 20 individual plants. Statistical significances were determined with one-sided Ordinary one-way ANOVA test followed two-sided Tukey's Honestly Significant Difference test: ** adjusted *p*-value < 0.01. **c**, Schematic representation showing the *pEhd3::4 $\times$ HA-Ehd3* (HA-Ehd3) and *pEhd3::4 $\times$ HA-Ehd3mu* (HA-Ehd3mu) constructs. The western blot shows comparable levels of HA-Ehd3 and HA-Ehd3mu protein in the transgenic lines. **d,e**, Schematic representation showing the *EGFP-Ehd3* (D) and *YFP-SDG724* (E) constructs. The western blot shows the EGFP-Ehd3 (D) and YFP-SDG724 (E) protein in the different transgenic lines. **f**, Schematic representation of vectors for bimolecular fluorescence complementation assays. The mVN (1–155 amino acids (aa)) and mVC (156–238 aa) are the N- and C-terminals of mVenus, respectively. To evaluate plasmid transfection and gene expression efficiency, the marker genes *ECFP* and *mCherry* are incorporated into the vectors. **g**, Yeast two-hybrid assay showing the interaction between Arabidopsis AtEHD3 and SDG7 in yeast cells. AS1 and AS2 were used as the positive control. **h**, *In vitro* GST-pulldown assay showing the interaction between Arabidopsis AtEHD3 and SDG7. His-SDG7 was pulled down by GST-AtEHD3 but not GST. The upper panel shows the SDS-PAGE stained with Coomassie Brilliant Blue (CBB) and the lower panel shows the western blot using the anti-His antibody. Source data are provided as a Source Data file.

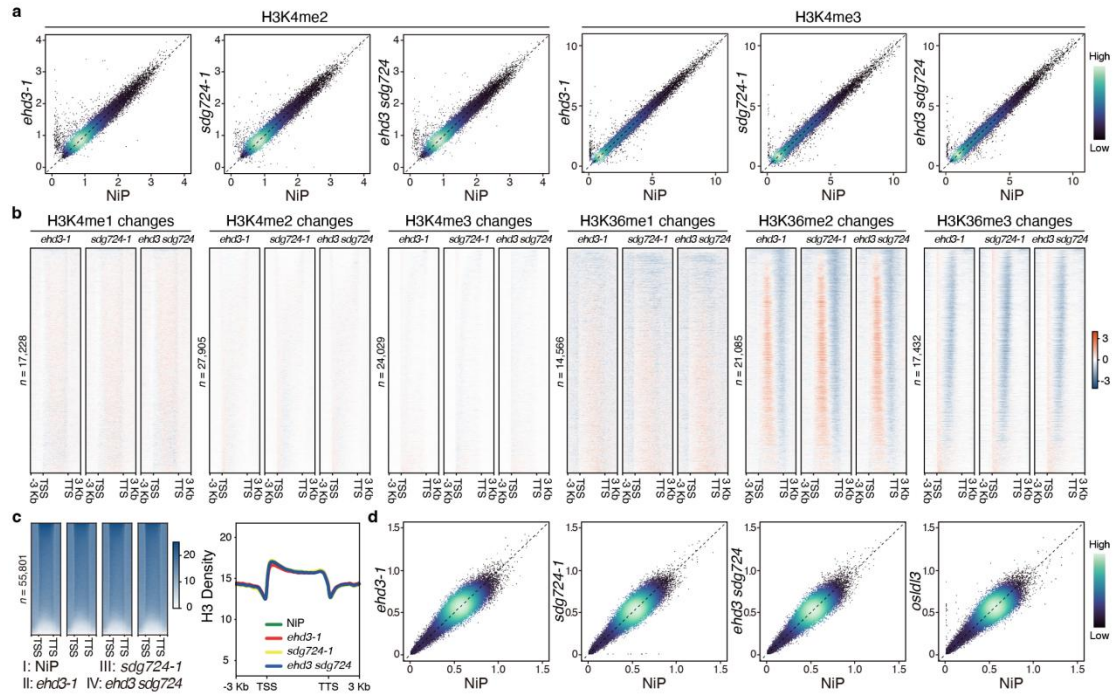


Supplementary Fig. 5: Ehd3 and SDG724 coregulate expression of a set of genes.

a, Volcano plots showing differentially expressed genes identified by RNA-seq analysis in the *ehd3-1*, *sdg724-1*, and *ehd3 sdg724* mutants compared with the wild-type NiP. The x-axis indicates the adjusted p -value, and the y-axis indicates the fold change in transcription. Up- and down-regulated genes (red and blue dots, respectively) were identified based on changes and adjusted p -value satisfying thresholds ($p\text{-value} \leq 0.05$ and $|\text{fold change}| \geq 1.5$, shown as dashed lines). The p -values were calculated using two-sided Wald test and adjusted using the Benjamini–Hochberg correction. **b,c**, Venn diagrams showing the overlap of up-regulated (**b**, left) or down-regulated (**c**, left) genes in the *ehd3-1*, *sdg724-1*, and *ehd3 sdg724* mutant plants. The p -values were determined by one-sided Fisher's exact test. Dotplots enrichment map showing biological processes associated with up-regulated genes (**b**, right) or down-regulated genes (**c**,

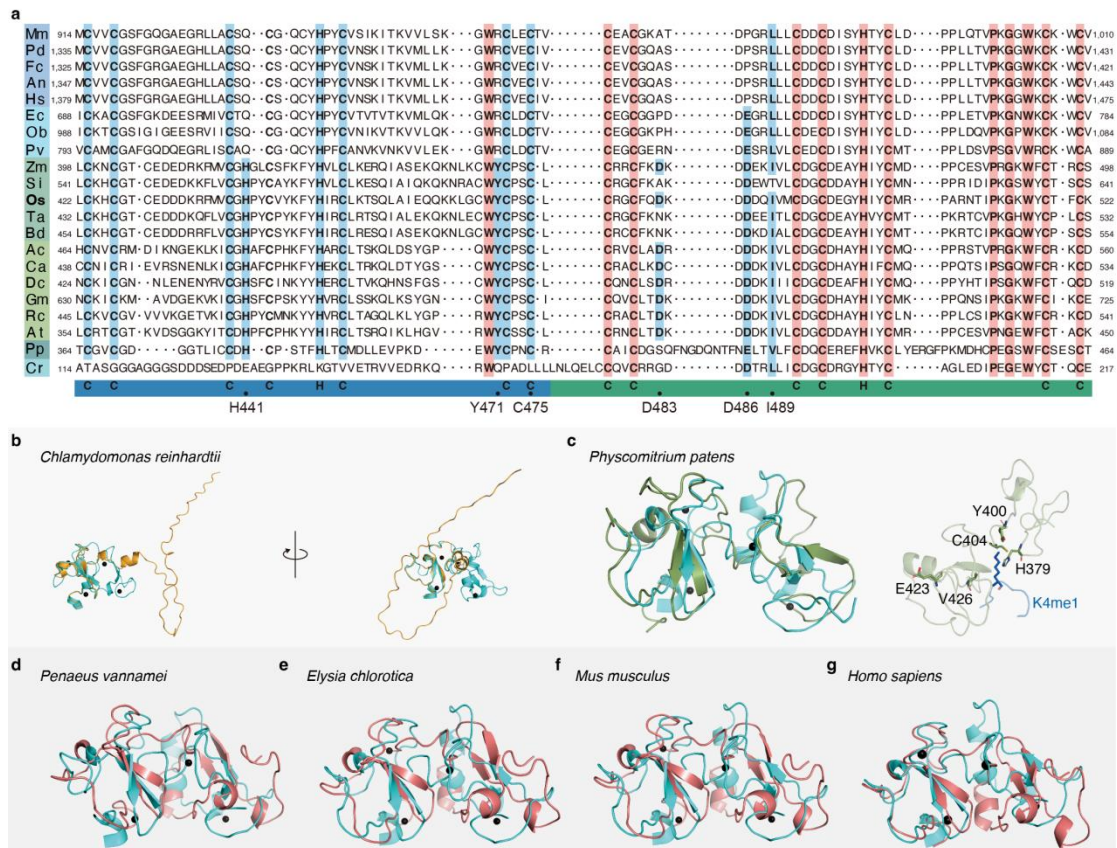
110 right). The top 15 catalogs selected by p -value (a modified one-sided Fisher's exact test)
111 are presented. The color of the dot depends on the p -value, and the size is determined
112 by the respective gene number.

113



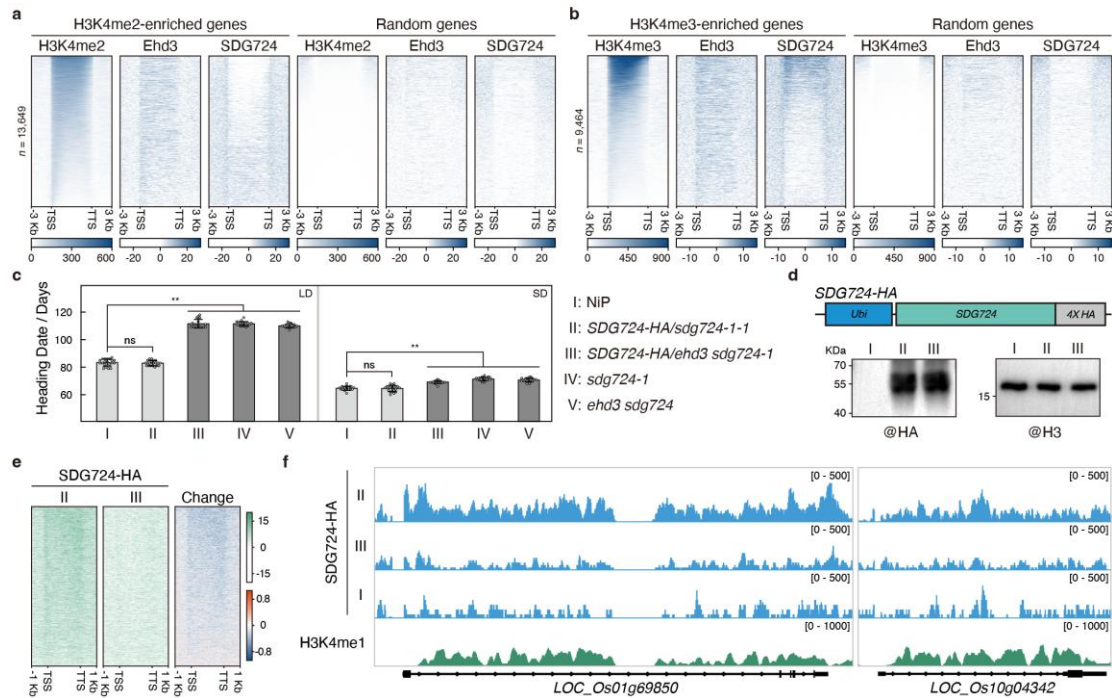
Supplementary Fig. 6: Genome-wide changes of H3K4/H3K36 methylations and H3 levels in the mutant plants.

a, Scatter plots comparing H3K4me2 and H3K4me3 between the wild-type NiP and the *ehd3-1*, *sdg724-1*, and *ehd3 sdg724* mutants. Each dot represents the histone methylation level of one peak corrected with MAnorm. The transition from deep blue to cyan indicates the increase of dot density. **b**, Heatmaps showing the changes of H3K4me1/me2/me3 and H3K36me1/me2/me3 levels in the *ehd3-1*, *sdg724-1*, and *ehd3 sdg724* mutants, respectively, compared with NiP, from 3 kb upstream of the TSS to 3 kb downstream of the TTS. All genes are sorted based on the enrichment of the corresponding histone methylation identified in NiP. Numbers of genes are shown on the left. **c**, Heatmaps and metaplots showing the H3 distribution from 3 kb upstream of the TSS to 3 kb downstream of the TTS in NiP, *ehd3-1*, *sdg724-1*, and *ehd3 sdg724*. All genes in heatmaps are sorted based on enrichment of H3. Numbers of genes are shown on the left. **d**, Scatter plots comparing H3 between NiP and the *ehd3-1*, *sdg724-1*, *ehd3 sdg724*, *osl3* mutants. Each dot represents the H3 level of one gene corrected with MAnorm. The transition from deep blue to cyan indicates the increase of dot density. Gradient colors indicate log₂-transformed fold changes (b) and H3 levels (c).



Supplementary Fig. 7: Sequence alignment and structure superimposition of the tandem PHD.

a, Tandem PHD sequence alignment for the corresponding species in Fig. 5a. Identical residues are highlighted in red. The dots indicate the critical residues responsible for interaction with H3K4me1 in the co-crystal structure of the Ehd3 tandem PHD–H3K4me1 complex. **b–g**, Structure superimposition of the tandem PHD in A0A2K3D188 (b), A0A2K1L966 (c), A0A3R7Q333 (d), A0A433TT05 (e), Q8BRH4 (KMT2C) (f), and O14686 (KMT2D) (g) with Ehd3. The structure of Ehd3 is colored in cyan. The protein codes represent the UniProt entries (<https://www.uniprot.org>).

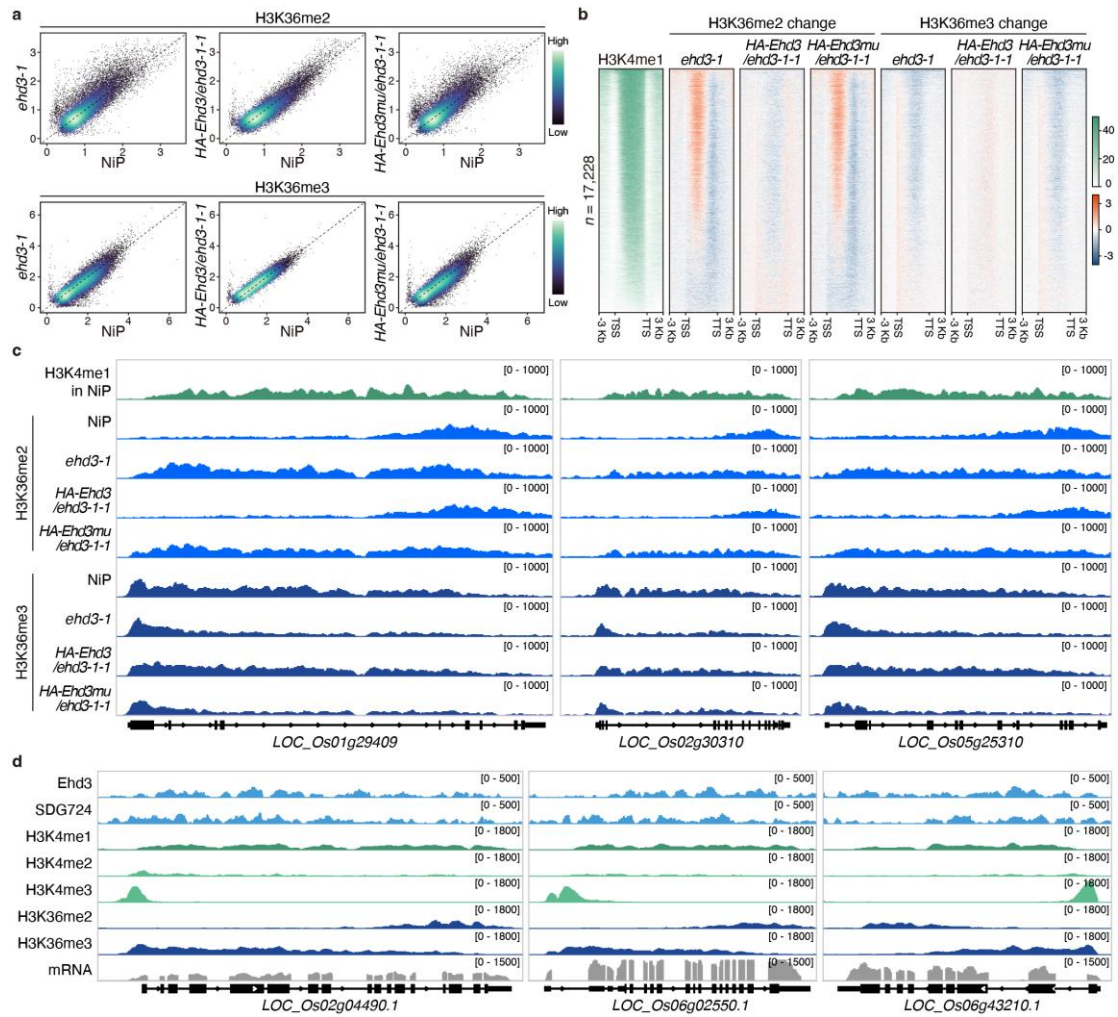


Supplementary Fig. 8: Enrichment of Ehd3/SDG724 within H3K4me2- and H3K4me3-occupied genes, as well as the changed distribution of SDG724 in the absence of Ehd3.

a–b, Heatmaps showing the enrichment of Ehd3 or SDG724 within H3K4me2 without H3K4me1-occupied genes (a) and H3K4me3 without H3K4me1-occupied genes (b) from 3 kb upstream of the TSS to 3 kb downstream of the TTS. An equal number of genes lacking H3K4me1/H3K4me2 and H3K4me1/H3K4me3 enrichment were randomly selected as a control. All genes are sorted based on the enrichment of H3K4me2 or H3K4me3 identified in the wild-type NiP. Numbers of genes are shown on the left. **c**, Histograms showing days to heading in NiP (I), *SDG724-HA/sdg724-1-1* (II), *SDG724-HA/ehd3 sdg724-1* (III), *sdg724-1* (IV) and *ehd3 sdg724* (V) plants under LD and SD conditions. Values are shown as means $\pm$ standard deviation of 20 individual plants. Statistical significances were determined by one-sided Ordinary one-way ANOVA test followed two-sided Tukey's Honestly Significant Difference test: ** adjusted *p*-value < 0.01 ; ns (not significant). **d**, Schematic representation showing the *Ubi::SDG724-HA* construct. The western blot shows comparable levels of SDG724-HA protein in the *SDG724-HA/sdg724-1-1* (II) and *SDG724-HA/ehd3 sdg724-1* (III) plants. **e**, Heatmaps showing SDG724 levels within H3K4me1-occupied genes from 1

163 kb upstream of the TSS to 1 kb downstream of the TTS in the *SDG724-HA/sdg724-1-*
164 *1* (II) and *SDG724-HA/ehd3 sdg724-1* (III) plants. All genes are sorted based on
165 enrichment of H3K4me1 identified in NiP. **f**, Integrative Genomics Viewer images
166 showing CUT&Tag signals in NiP (I), *SDG724-HA/sdg724-1-1* (II), and *SDG724-*
167 *HA/ehd3 sdg724-1* (III) plants, as well as H3K4me1 in NiP. Gradient colors indicate
168 enrichment levels of ChIP-seq data (a, b), levels of SDG724 CUT&Tag signal (e), or
169 log₂-transformed fold changes. Source data are provided as a Source Data file.

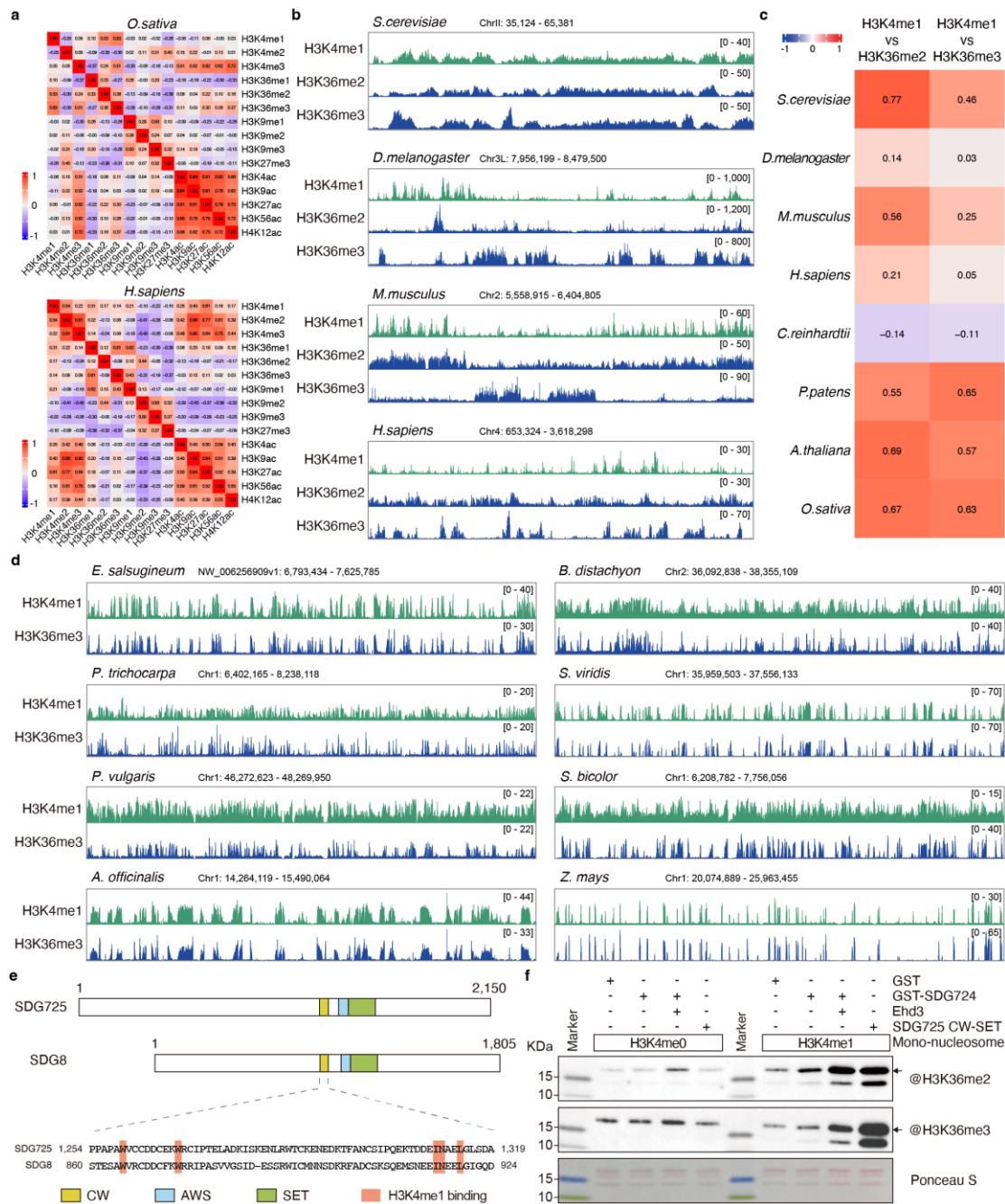
170



Supplementary Fig. 9: Changes of H3K36me2 and H3K36me3 profiles in the *ehdl-1*, *HA-Ehdl3/ehdl-1-1* and *HA-Ehdl3mu/ehdl-1-1* plants, as well as the distributions of Ehdl3, SDG724, H3K4me1/me2/me3 and H3K36me2/me3 along the representative genes in NiP.

a, Scatter plots comparing H3K36me2 and H3K36me3 levels between the wild-type NiP and the *ehdl-1*, *HA-Ehdl3/ehdl-1-1*, and *HA-Ehdl3mu/ehdl-1-1* plants. The transition from deep blue to cyan indicates the increase of dot density. **b**, Heatmaps showing changes in H3K36me2 and H3K36me3 levels within H3K4me1-enriched genes, compared with the wild-type NiP, from 3 kb upstream of the TSS to 3 kb downstream of the TTS in the *ehdl-1*, *HA-Ehdl3/ehdl-1-1*, and *HA-Ehdl3mu/ehdl-1-1* plants. All genes are sorted based on enrichment of H3K4me1 identified in the wild-type NiP. Numbers of genes are shown on the left. Gradient colors indicate histone modification enrichment levels or log₂-transformed fold changes. **c**, Integrative

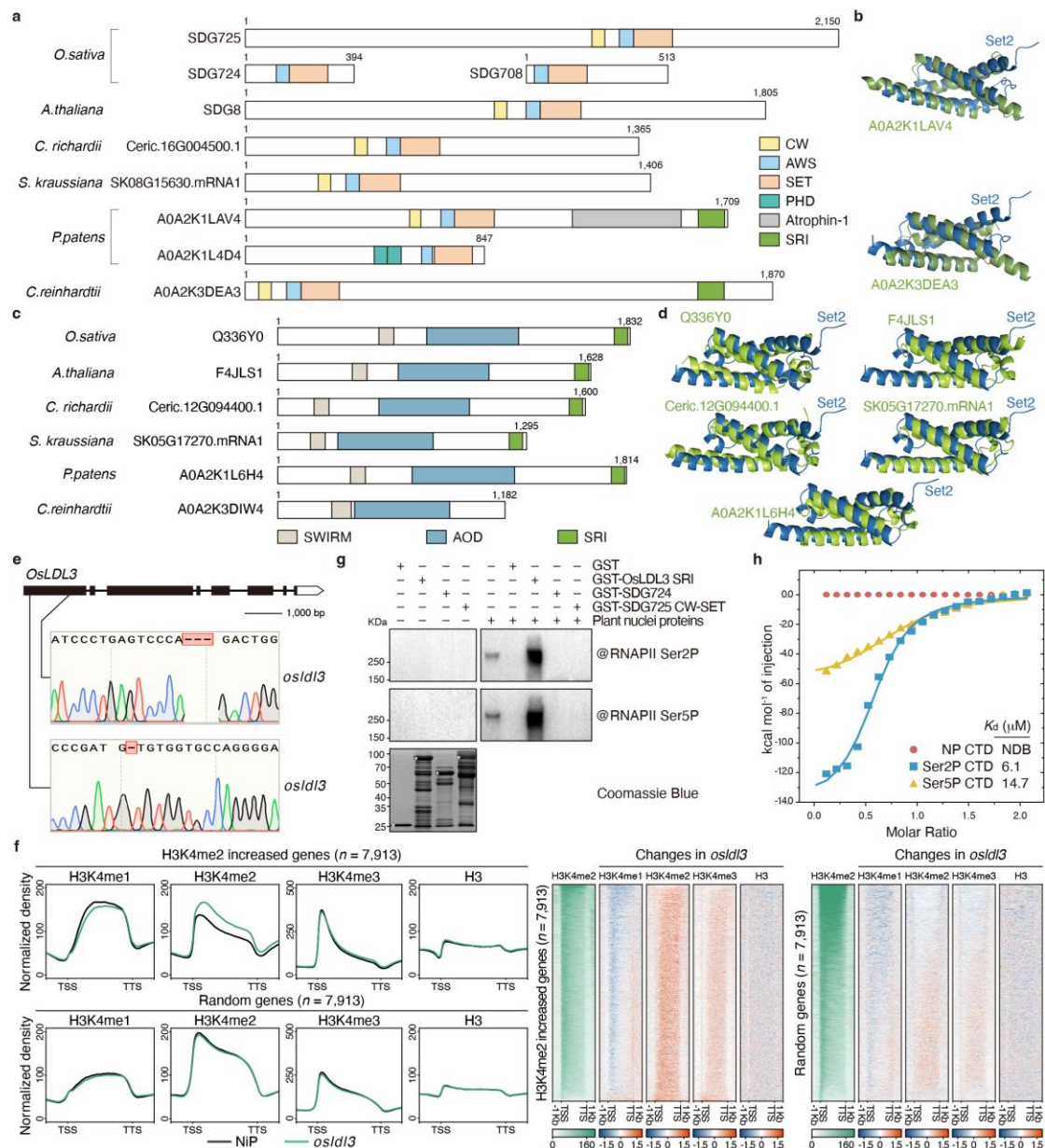
Genomics Viewer images of ChIP-seq data showing H3K4me1 level in the wild-type NiP, as well as H3K36me2 and H3K36me3 levels in NiP, *ehd3-1*, *HA-Ehd3/ehd3-1-1*, and *HA-Ehd3mu/ehd3-1-1* plants. **d**, Integrative Genomics Viewer images of ChIP-seq and RNA-seq data showing the distributions of Ehd3, SDG724, H3K4me1, H3K4me2, H3K4me3, H3K36me2, H3K36me3, and mRNA along the representative genes in the wild-type NiP.



Supplementary Fig. 10: H3K4me1 directs H3K36me2 and H3K36me3 deposition in plants.

a, Pearson correlation heatmap of histone modifications in *Oryza sativa* and *Homo sapiens*. For genes enriched of H3K4me1 (identified by Macs2 with q -value ≤ 0.05), each gene was first scaled to a fixed length using the computeMatrix tool in deepTools. Histone modification levels across each gene body were then quantified and subjected to Z-score normalization. The normalized data from all genes were used to calculate Pearson correlation coefficients. One replicate of ChIP-seq data from each sample was

selected for analysis. The transition from blue to red indicates the change in the correlation coefficient from -1 to 1. **b**, Integrative Genomics Viewer images of ChIP-seq data showing the H3K4me1, H3K36me2, and H3K36me3 profile plots of the representative region in *Saccharomyces cerevisiae*, *Drosophila melanogaster*, *Mus musculus*, and *Homo sapiens*. **c**, Correlation analysis was performed to quantify the colocalization of H3K4me1 with H3K36me2/me3 across species. Pearson pairwise correlation coefficients were calculated from genome-wide coverage tracks (binned at 500-bp resolution) using multiBigwigSummary and plotCorrelation in deepTools. The transition from blue to red indicates the change in the correlation coefficient from -1 to 1. **a-c**, Public data were downloaded and analyzed for *Saccharomyces cerevisiae* (ref. ⁴²), *Drosophila melanogaster* (ref. ³⁹), *Mus musculus* (ref. ³⁹), *Homo sapiens* (refs. ^{35-38,40}), *Arabidopsis thaliana* (refs. ^{21,41}), and *Oryza sativa* (refs. ^{33,34} and GSE126436). **d**, Integrative Genomics Viewer images of published ChIP-seq data showing the H3K4me1 and H3K36me3 profile plots of the representative region in *Eutrema salsugineum*, *Populus trichocarpa*, *Phaseolus vulgaris*, *Asparagus officinalis*, *Brachypodium distachyon*, *Setaria viridis*, *Sorghum bicolor*, and *Zea mays* (ref. ²²). **e**, Schematic representation showing similar protein domain architecture of rice SDG725 and Arabidopsis SDG8. Sequence alignment of the CW domain showing the conserved residues responsible for H3K4me1 binding, highlighted in orange rectangles. **f**, A replicate for histone methyltransferase analysis showing that Ehd3 stimulates the H3K36 methyltransferase activity of SDG724. H3K4me0 and H3K4me1 mononucleosomes were used as the substrates. GST and truncated SDG725 (containing the CW and SET domains) were used as negative and positive controls, respectively. Source data are provided as a Source Data file.



Supplementary Fig. 11: Plant H3K4 demethylase LDL3 interacts with phosphorylated RNA polymerase II (RNAPII) via the Set2–Rpb1 interacting (SRI) domain, facilitating demethylation of H3K4me2 to H3K4me1.

a, Schematic representation of H3K36 methyltransferases in *Chlamydomonas reinhardtii*, *Physcomitrium patens*, *Selaginella kraussiana*, *Ceratopteris richardii*, *Arabidopsis thaliana*, and *Oryza sativa*. **b**, Structure superimposition of SRI domain in *Chlamydomonas reinhardtii* A0A2K3DEA3 and *Physcomitrium patens* A0A2K1LAV4 with *Saccharomyces cerevisiae* Set2 (PDB: 2a7o). **c**, Schematic representation of LDL3 homologous proteins in *Chlamydomonas reinhardtii*, *Physcomitrium patens*, *Selaginella kraussiana*, *Ceratopteris richardii*, *Arabidopsis*

thaliana, and *Oryza sativa*. **d**, Structure superimposition of SRI domain in *Physcomitrium patens* A0A2K1L6H4, *Selaginella kraussiana* SK05G17270.mRNA1, *Ceratopteris richardii* Ceric.12G094400.1, *Arabidopsis thaliana* F4JLS1, and *Oryza sativa* Q336Y0 with *Saccharomyces cerevisiae* Set2 (PDB: 2a7o). The protein codes represent the UniProt entries, except for *Selaginella kraussiana* and *Ceratopteris richardii* (<https://sk.ccg.gun/bblast>). **e**, Identification of *osldl3* mutants. Untranslated regions, exons, and introns are indicated by blank rectangles, black rectangles, and black lines, respectively. **f**, Integrative genomic distributions and heatmaps showing occupancies and changes of H3K4me1/me2/me3 and H3 in NiP and *osldl3*. In both NiP and *osldl3*, H3K4me2-enriched genes (identified by Macs3, q -value < 0.05) were further screened for those showing increased H3K4me2, as determined by MANorm (fold change ≥ 1.2 , Bayesian model based p -value ≤ 0.01). An equal number of random genes from the H3K4me2-enriched set that did not display increased H3K4me2 were used as the negative control. The plots and heatmaps present the region from 1 kb upstream of the TSS to 1 kb downstream of the TTS. Gradient colors indicate histone modification enrichment levels or log₂-transformed fold changes. **g**, Semi-*in vivo* pulldown showing the interaction between OsLDL3 and Ser-2- or Ser-5-phosphorylated RNA polymerase II (RNAPII). Total nuclear proteins extracted from NiP seedlings were precipitated using GST–OsLDL3 SRI, GST–SDG724, and GST–SDG725 CW-SET. GST served as a negative control. The products were analyzed by Coomassie Brilliant Blue staining and western blot using antibodies against Ser-2- and Ser-5-phosphorylated RNAPII. The asterisk represents the corresponding recombinant protein. **h**, Isothermal titration calorimetry results showing the binding affinities of the SRI domain of OsLDL3 with differently modified RNAPII C-terminal domain (CTD). NP, non-phosphorylated CTD peptide; Ser2P, Ser-2-phosphorylated CTD peptide; Ser5P, Ser-5-phosphorylated CTD peptide; NDB, no detectable binding. Source data are provided as a Source Data file.