Supporting Information

Histone lysine-to-methionine mutations reduce histone methylation and cause developmental pleiotropy

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SI Materials and Methods

RNA extraction and RT-PCR. Total RNA was extracted from 0.2 g of plant tissue with Trizol (ThermoFisher). One microgram RNA pretreated with DNase I (M0303L, New England Biolabs) was reverse-transcribed into cDNA using oligoDT primers with superscript III reverse transcriptase according to the manufacturers instructions (ThermoFisher). PCR products were visualized by ethidium bromide staining with *ACTIN 7* as a control.

Gene Ontology analysis. For GO analysis, both upregulated and downregulated genes (Supplemental table 1a,b) from M36 were utilized for a David functional annotation analysis (Huang da et al., 2009). *p*-values are reported as $-\text{Log}_{10}$ *p*-value (Fig. 2B.).

Histone extractions. Two grams of plant tissue were ground into powder and resuspended in 25 mL extraction buffer (10mM HEPES pH 8.0, 1M sucrose, 5mM KCl, 5mM $MgCl_2$, 0.6% triton X-100, 0.4mM PMSF, Pepstatin 1:1000, complete protease inhibitor) with dounce on ice. The extracts were filtered through two layers of miracloth on ice and spin down at 2,627 g for 10 minutes. Nuclei were washed 4 times with extraction buffer and then resuspended into 1mL detergent extraction buffer (10mM Tris-HCl, 10mM NaCl, 3mM $MgCl_2$, 1% triton with pH 7.4) for 5 minutes on ice. The solution was spun at 16,000 g for 5 min at room temperature. The nuclei were resuspended in 0.5mL of 0.4N H_2SO_4 for 1hr at 4°C. After centrifuge (21,000 g for 5 min at 4°C), supernatants were precipitated in 132 µL of TCA (99.9 % tricarboxylic acid + 0.1% sodium deoxycholate), washed two times with acetone, and resuspended in 120 µL deionized water.

Immunofluorescence. Leaves from 21 day-old plants were diced into 1cm pieces and placed into ice-cold fixing buffer (4% paraformaldehyde, 10mM Tris-HCl, 100mM NaCl, 10mM EDTA) for 20 minutes. Fixed plant material was washed two times in fixing buffer without paraformaldehyde for 10 minutes/wash and chopped thoroughly in 500 µL LOB1 buffer (15 mM Tris-HCl, 2 mM EDTA, 0.5 mM spermine, 80mM KCl, 20 mM NaCl, 0.1% Triton X-100). The nuclei were filtered through two layers of miracloth, diluted 1:3 in sorting buffer (100 mM Tris-HCl, 50mM KCl, 2 mM $MgCl_2$, 0.05 % TWEEN 20, 5% sucrose), spotted onto pre-coated poly-lysine slides, and air dried for 4 hours. Slides were fixed in 1x KPBS (128 mM NaCl, 2 mM KCl, 8 mM Na_2HPO_4, 2 mM KH_2P0_4) plus 0.1% Triton-X100 and 4% paraformaldehyde for 5 min at room temperature. Next, slides were blocked in 100 µL 1% BSA in 1x KPBS at 37°C for 30 min, washed three times with 1x KPBS plus 0.1% Triton-X100 for 5 minutes, and incubated with primary αFLAG antibody (F3165, Sigma-Aldrich) at 4°C overnight. After overnight incubation, the slides were washed three times with 1x KPBS for 5 minutes and blocked with secondary antibody (VRDye 490, LI-COR) in a humid chamber for 2 hours. After three washes with 1x KPBS, samples were incubated with 30 µL DAPI in mounting solution and imaged on a Nikon Ti Eclipse microscope.

Mass spectrometry. Acid extracted histones suspended in ddH_2O were initially quantified using the Pierce BCA assay as per manufacturers instructions (www.thermofisher.com). 10 µg of purified histones were prepared in 18 µL ddH_2O. 2 µL of 1M TEAB was added with vortexing. Samples were labeled with 2µL of 1:100 diluted propionic anhydride (Sigma-Aldrich) with brief vortexing and 2 min incubation at room temperature. The reaction was quenched with 1µL 80mM NH_2OH. Samples were then trypsinized overnight at 37°C. Trypsinized peptides were N-

terminal labeled by adding 6 µL of 2% phenylisocyanate in ACN in a fume hood (PIC is a potent inhalation hazard). Samples were incubated for 1 hour at 37°C with light shaking. Peptides were purified using an in-house packed C18 StageTip (3M Empore). Peptides were dried-down and then resuspended in 33 µL of sample diluent (2% ACN, 0.5% Formic Acid).

Histone peptides were injected onto a Dionex Ultimate3000 nanoflow HPLC with a Waters NanoEase C18 column (100 µm × 15 cm, 3 µm) coupled to a Thermo Scientific Q-Exactive mass spectrometer at 700 nL/min. Mobile phases consisted of water + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B). Histone peptides were resolved with a linear gradient of 2−25% (B) over 50 minutes and then to 40% (B) over 10 minutes. The mass spectrometer was operated in DDA mode with dynamic exclusion enabled (exclusion duration = 8 s); MS1 resolution = 70,000; MS1 automatic gain control target = 1×10^6; MS1 maximum fill time = 100 ms; MS2 resolution = 17,500; MS2 automatic gain control target = 2×10^5; MS2 maximum fill time = 500 ms; and MS2 normalized collision energy = 30. For each cycle, one full MS1 scan (300−1100 m/z) was followed by 10 MS2 scans using an isolation window size of 2.0 m/z.

Raw data files were converted to mascot generic files (.mgf) via MSConvert (Chambers et al., 2012). Peptides were identified via Mascot database search engine (version: 2.2.07) (Perkins et al., 1999) using Arab_TAIR database (www.uniprot.org/) with the following settings: ≤1 missed cleavage, +2 and +3 charge state and ≤5 ppm peptide tolerance. Data files were combined to build spectral libraries in Skyline (MacLean et al., 2010). All peptides were quantified via MS1 peak area integration in Skyline. All histone peptide MS1 peaks and their integration bounds were verified using XCalibur Qual Browser (v2.2). The data was normalized

and reported as the percent total of the overall signal intensity for a particular peptide family as previously reported (Krautkramer et al., 2015).

Supplemental References

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