Supplementary information

Live imaging of H3K9 acetylation in plant cells

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Supplementary Figure S1. H3K9ac-mintbody-GFP expressed in plant cells can bind to anti-mouse IgG.

Whole cell extracts from H3K9ac-mintbody-GFP or GFP expressing tobacco BY-2 cells were subjected to immunoprecipitation with anti-mouse IgG antibody on magnetic beads. After the immunoprecipitation, H3K9ac-mintbody-GFP and GFP were detected by immunoblotting. Only H3K9ac-mintbody-GFP was immunoprecipitated with the anti-mouse IgG antibody. For the full-length western blots see Supplementary Fig. S5.



Supplementary Figure S2. The amount of nuclear H3K9ac-mintbody-GFP was correlated with the amount of H3K9ac in tobacco BY-2 cells.

(a) Immunoblotting analysis of H3K9ac, H3, and H3K9ac-mintbody-GFP in the nuclear fractions of tobacco BY-2 cells treated with 1 μ M TSA and 10 μ M C646. The H3 level was used as a loading control. For the full-length western blots see Supplementary Fig. S6a.

(b, c) Quantitative analysis of the levels of nuclear H3K9ac (b) and H3K9ac-mintbody-GFP (c). The intensity of each band shown in (a) was analysed with ImageJ. Each protein level was normalized to the H3 level.

(d) Immunoblotting analysis of H3K9ac-mintbody-GFP in whole cell fractions of tobacco BY-2 cells treated with 1 μ M TSA and 10 μ M C646. The loaded proteins were stained with colloidal gold. For the full-length western blots see Supplementary Fig. S6b.

(e) Quantitative analysis of the levels of whole cell H3K9ac-mintbody-GFP. The intensity of each band shown in (d) was analysed with ImageJ. Each protein level was normalized to the level of the proteins stained with colloidal gold.



Supplementary Figure S3. H3K9ac-mintbody-GFP is unlikely to be expressed in the cytoplasm under the control of the 35S or RPS5a promoter.

Root cells of *A. thaliana* expressing GFP and H3K9ac-mintbody-GFP under the control of the 35S or RPS5a promoter were observed by confocal laser microscopy. T_2 plants were used for the H3K9ac-mintbody-GFP observations. Compared with GFP alone, H3K9ac-mintbody-GFP was not visible in the cytoplasm. Bars, 10 μ m.

	R	composition	Mw	in vitro IC50 inhibitory activity (±S.D.)		
				HDAC1	HDAC4	HDAC6
Ky-2	CONHOH	$C_{26}H_{37}O_6N_5$	515.60	$0.00873 \ \mu M \pm 0.00056$	$0.01997 \mu M \pm 0.00217$	$0.16345 \mu\text{M} \pm 0.01243$
Ky-14	COCH ₂ N(CH ₃) ₃	C29H32O5N5	541.68	19.98 µM ± 3.16	17.86 μM ± 1.93	>100 µM

Supplementary Table S1. Characteristics of Ky-2 and Ky-14.



Supplementary Figure S4. Full-length Western blots from Fig.1.

Full-length western blot shown in Figure 1a (a), in Figure 1b (b), and in Figure 1c (c). Lanes used are outline with a black rectangle.



Supplementary Figure S5. Full-length Western blots from Supplementary Fig.S1. Lanes used are outline with a black rectangle.



Supplementary Figure S6. Full-length Western blots from Supplementary Fig.S2.

Full-length western blot shown in Supplementary Figure S2a (a), and in Supplementary Figure S2d (b). Lanes used are outline with a black rectangle.