

Supplementary Information

An artificial metalloenzyme biosensor can detect ethylene gas in fruits and Arabidopsis leaves

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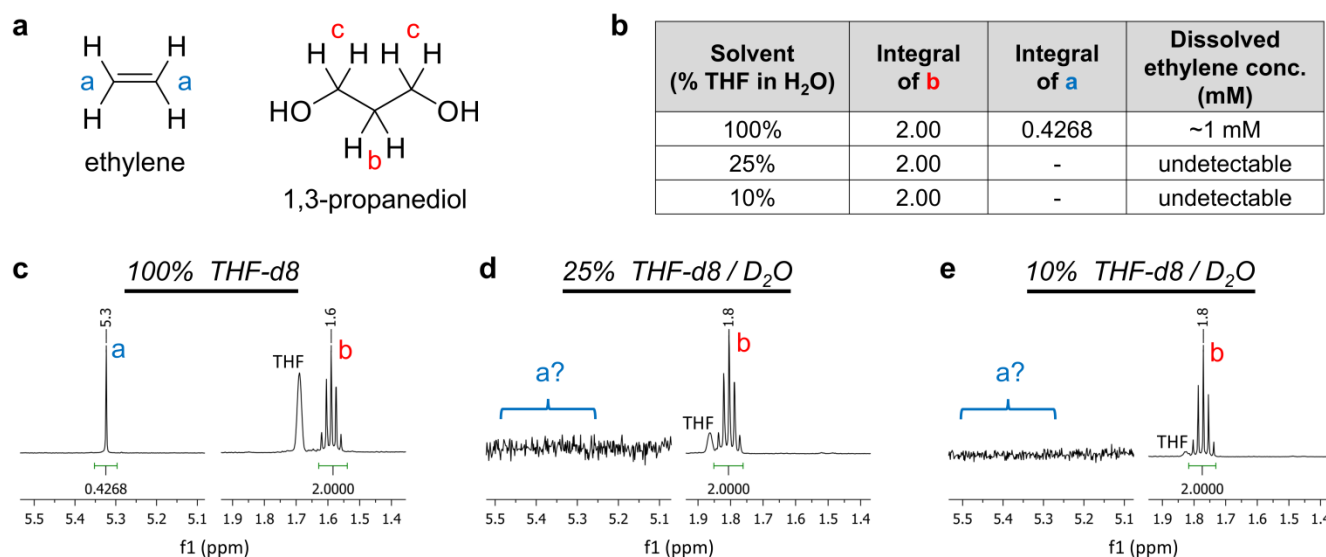
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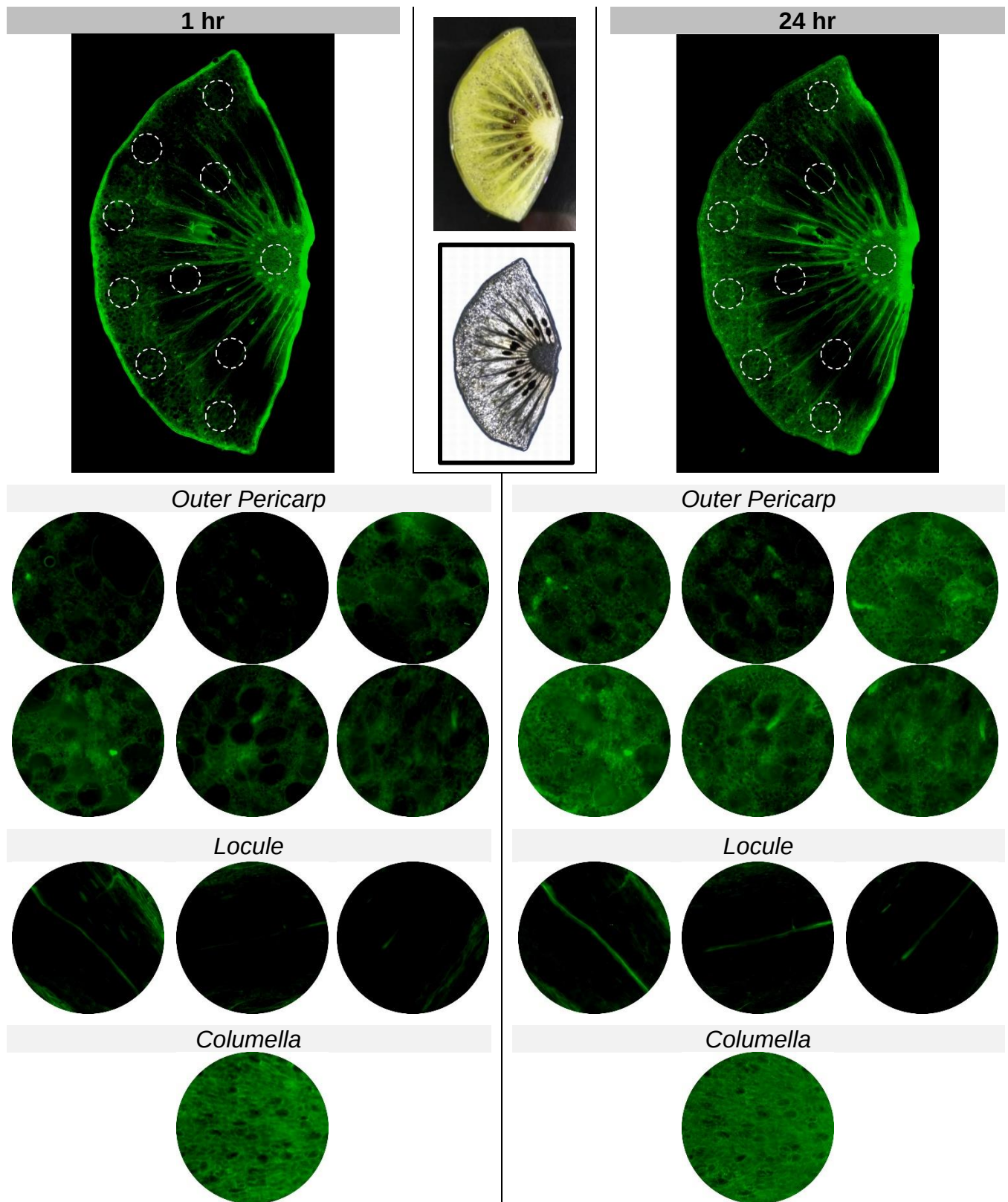
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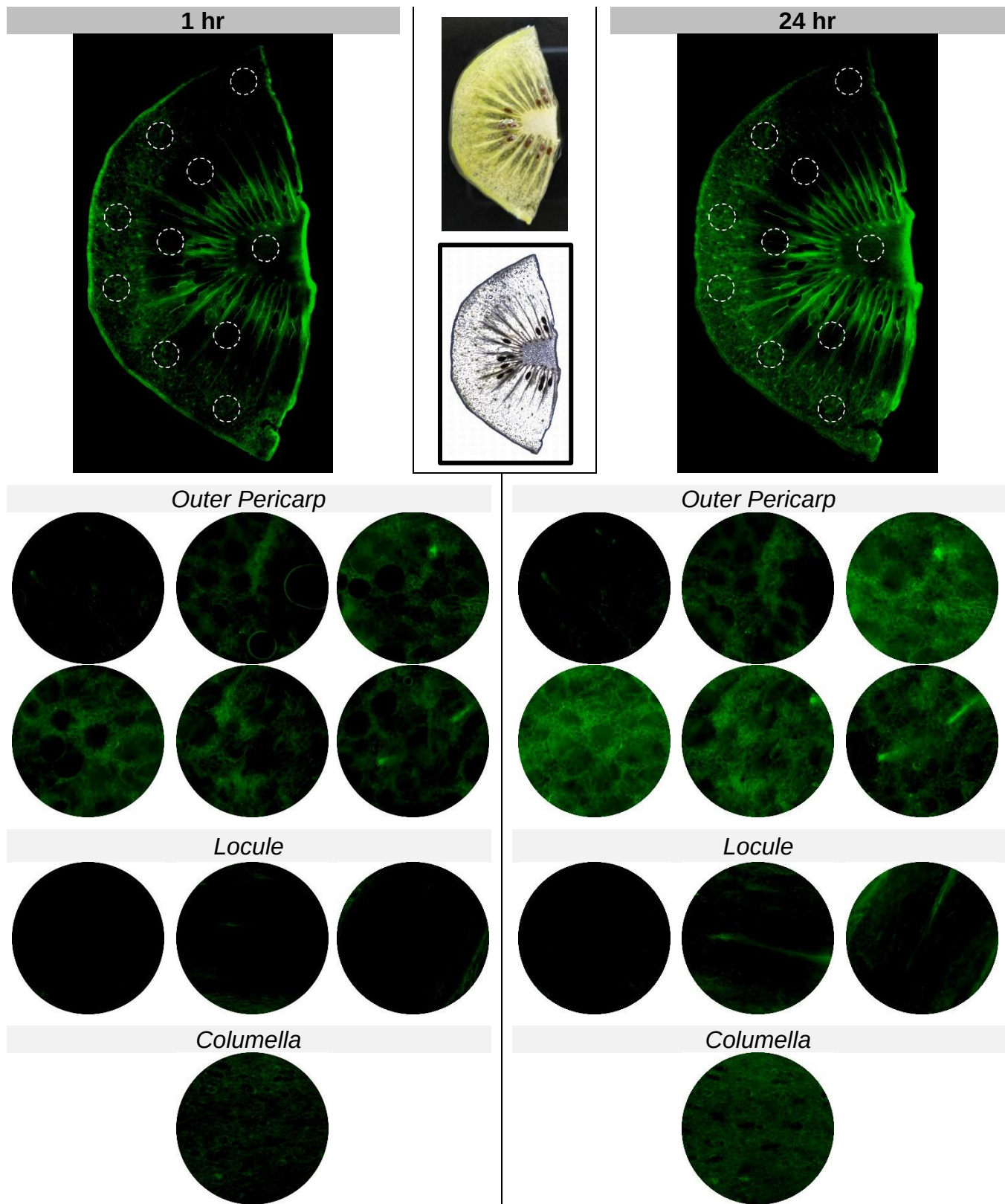
Supplementary Figures



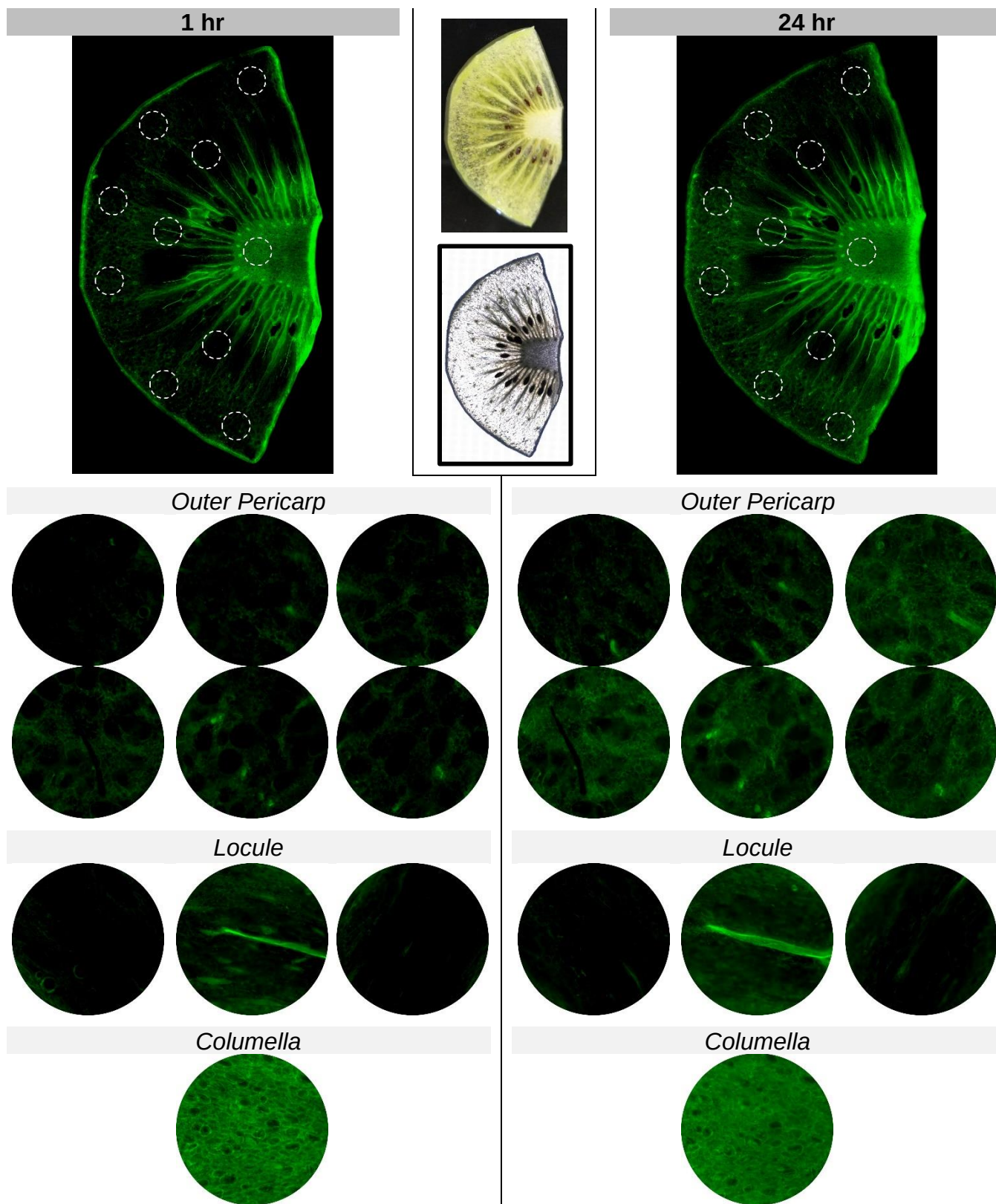
Supplementary Figure 1. NMR-based solubility study of ethylene gas. **(a)** Chemical structures of ethylene and 1,3-propanediol (internal NMR standard). **(b)** Chart comparing the integration ratios of peak a-to-b, thereby allowing the calculation of dissolved ethylene. NMR peaks are shown for the solvents of 100% THF **(c)**, 25% THF in H₂O **(d)**, and 10% THF in H₂O **(e)**. To perform the experiment, 1 ml of ethylene gas was added to airtight sealed NMR tubes containing varying solvent concentrations. Peaks were integrated and then compared to peaks of the internal standard (10 mM of 1,3-propanediol).



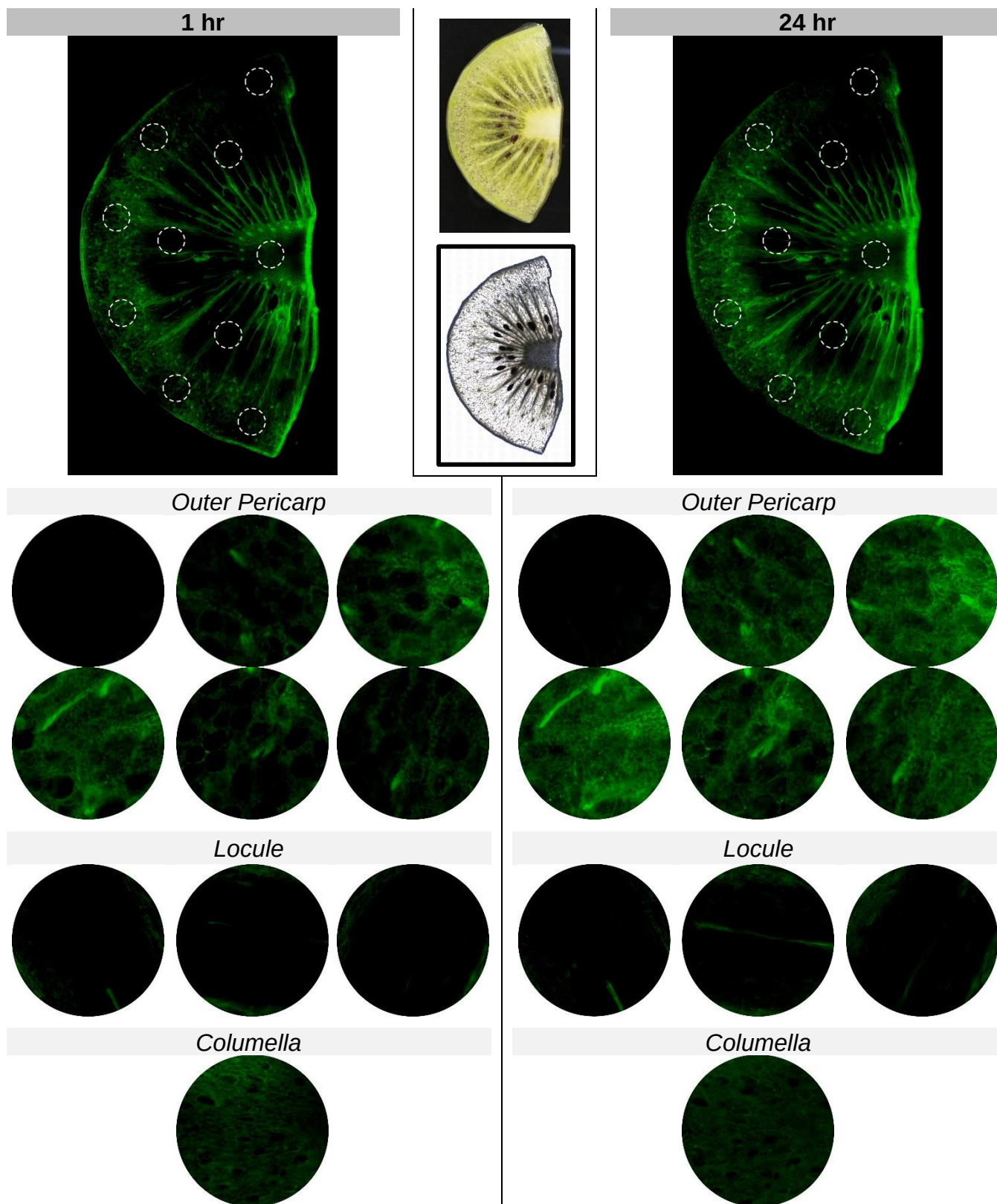
Supplementary Figure 2. Spatial imaging of ripening kiwifruit (sample#1), with emphasis on the outer pericarp, locules, and columella.



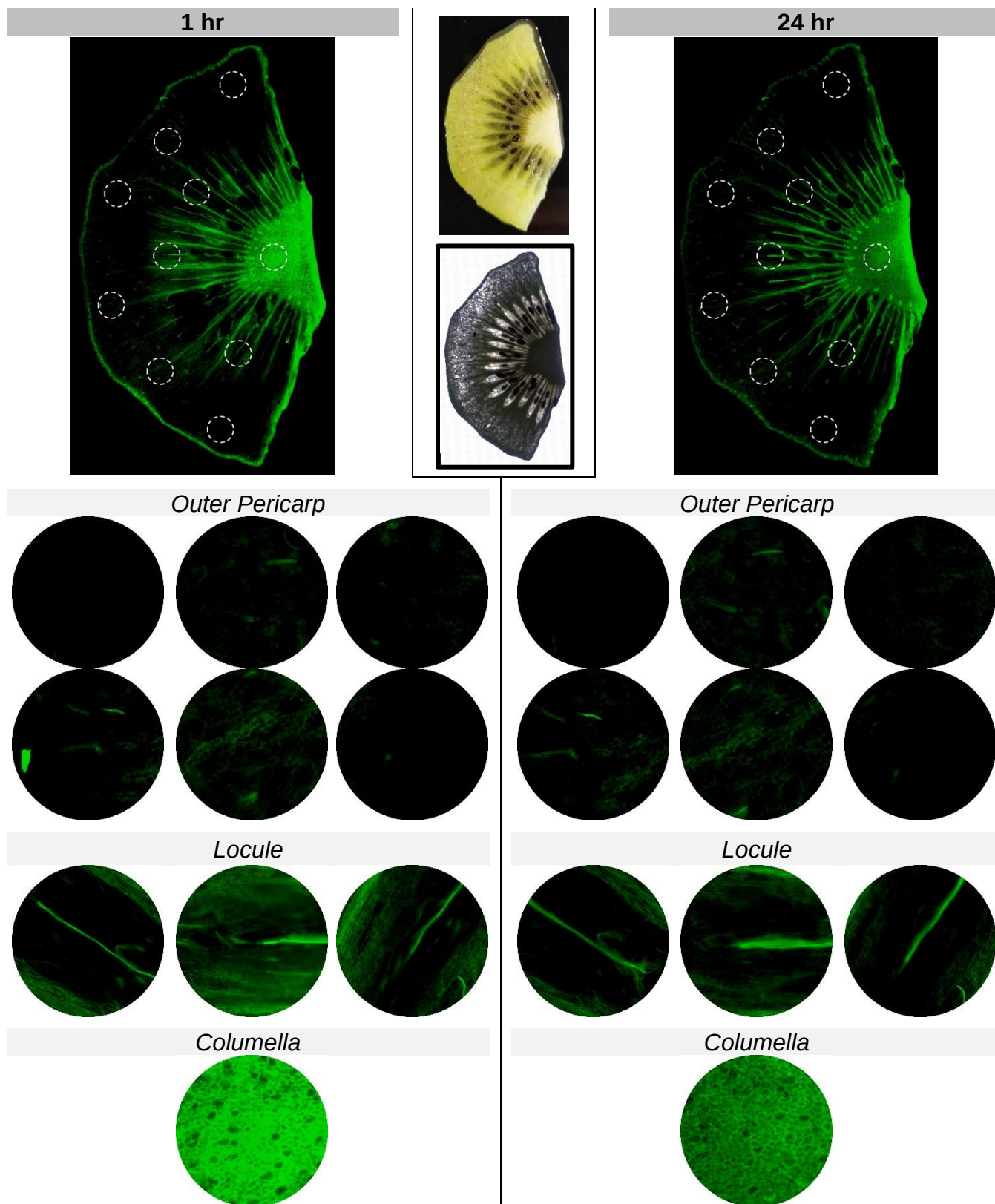
Supplementary Figure 3. Spatial imaging of ripening kiwifruit (sample#2), with emphasis on the outer pericarp, locules, and columella.



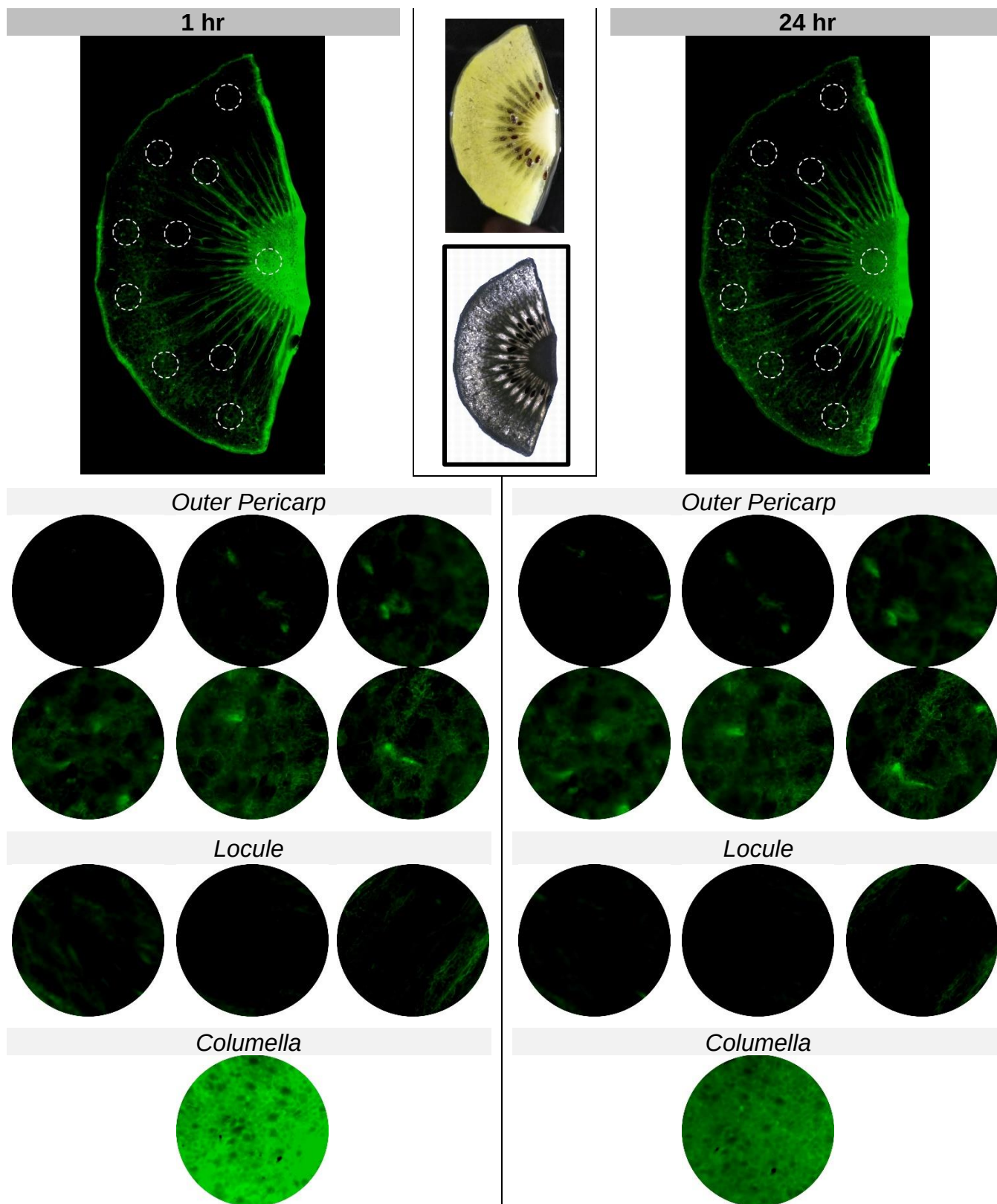
Supplementary Figure 4. Spatial imaging of ripening kiwifruit (sample#3), with emphasis on the outer pericarp, locules, and columella.



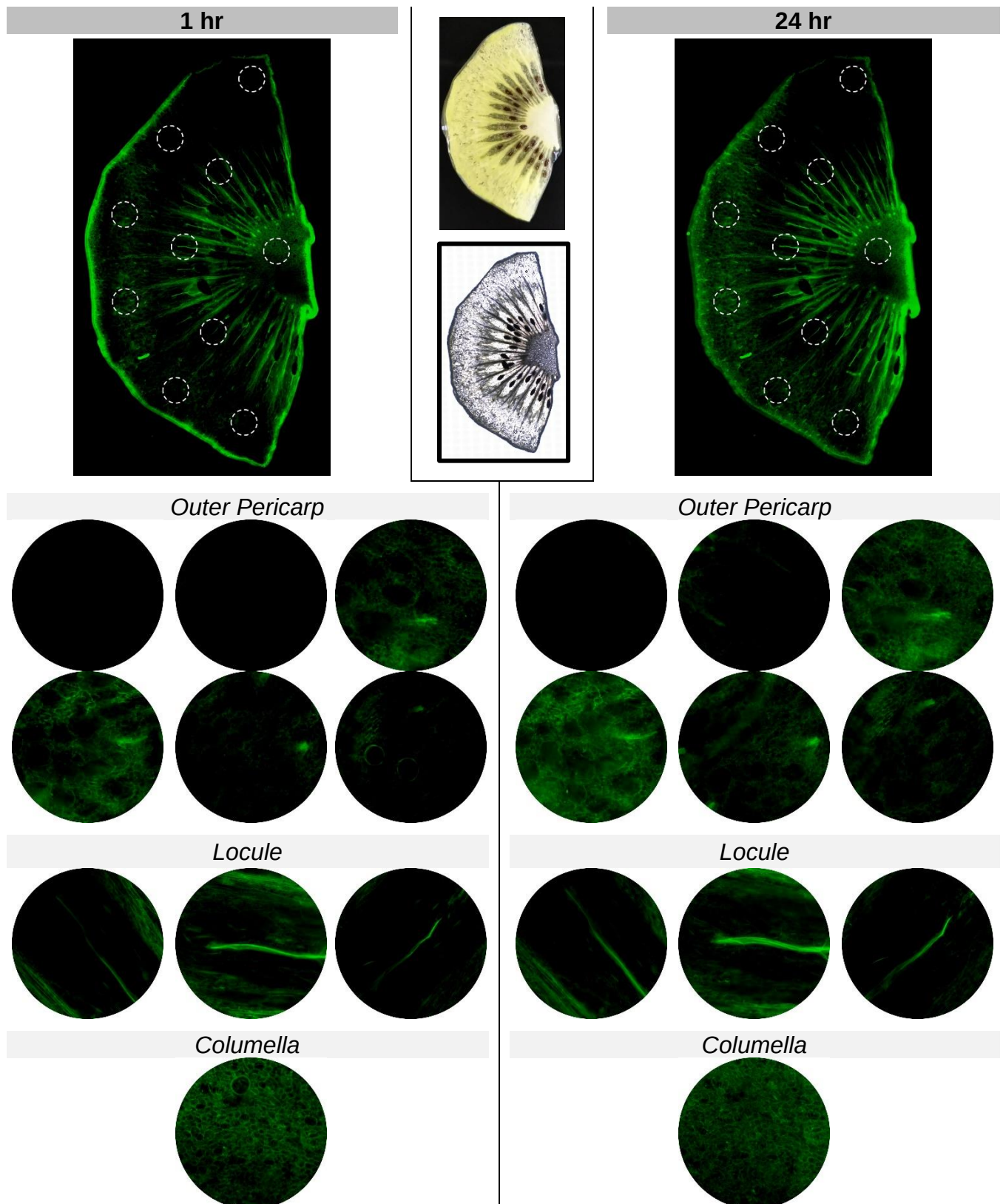
Supplementary Figure 5. Spatial imaging of ripening kiwifruit (sample#4), with emphasis on the outer pericarp, locules, and columella.



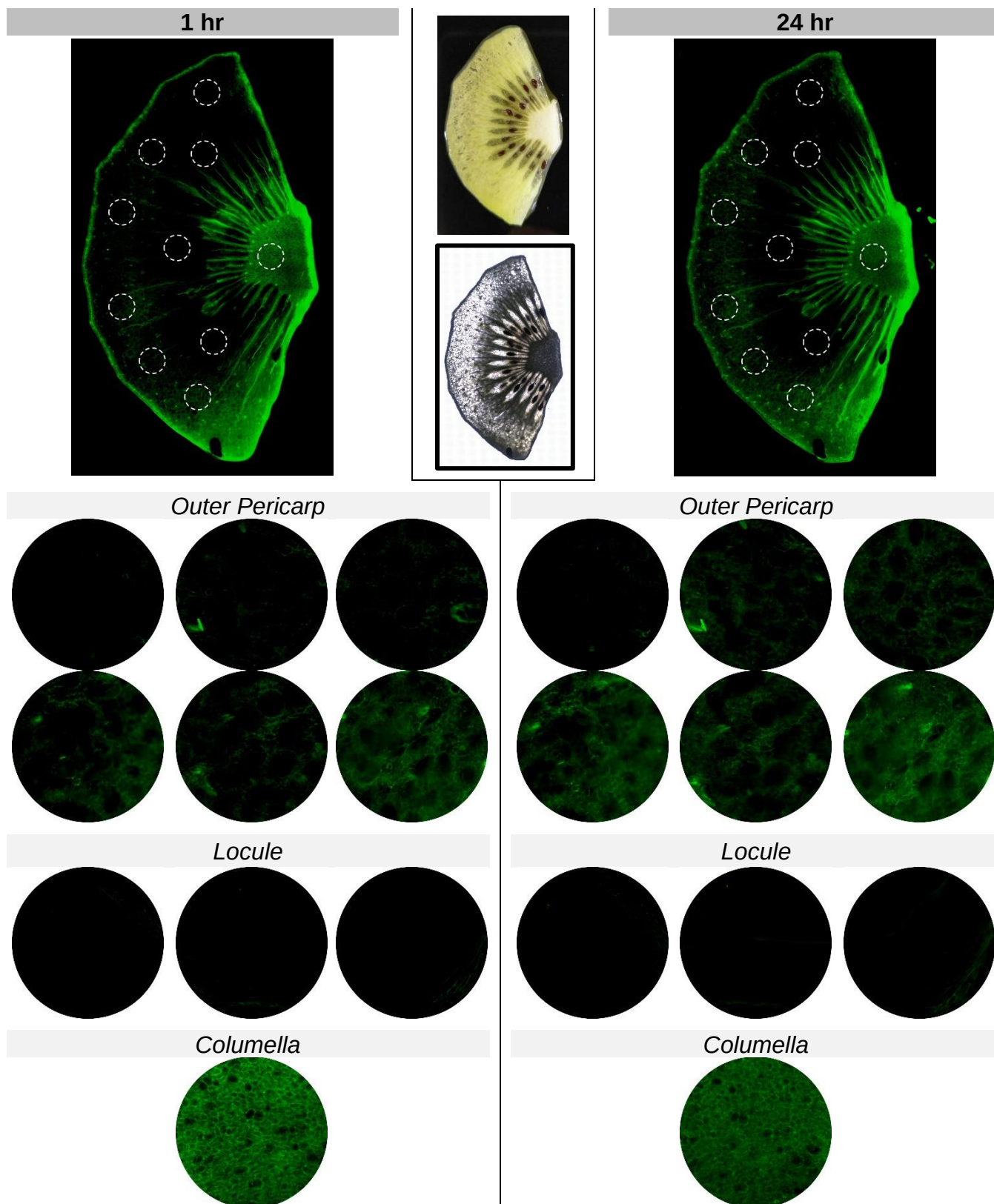
Supplementary Figure 6. Spatial imaging of unripe kiwifruit (sample#1), with emphasis on the outer pericarp, locules, and columella.



Supplementary Figure 7. Spatial imaging of unripe kiwifruit (sample#2), with emphasis on the outer pericarp, locules, and columella.

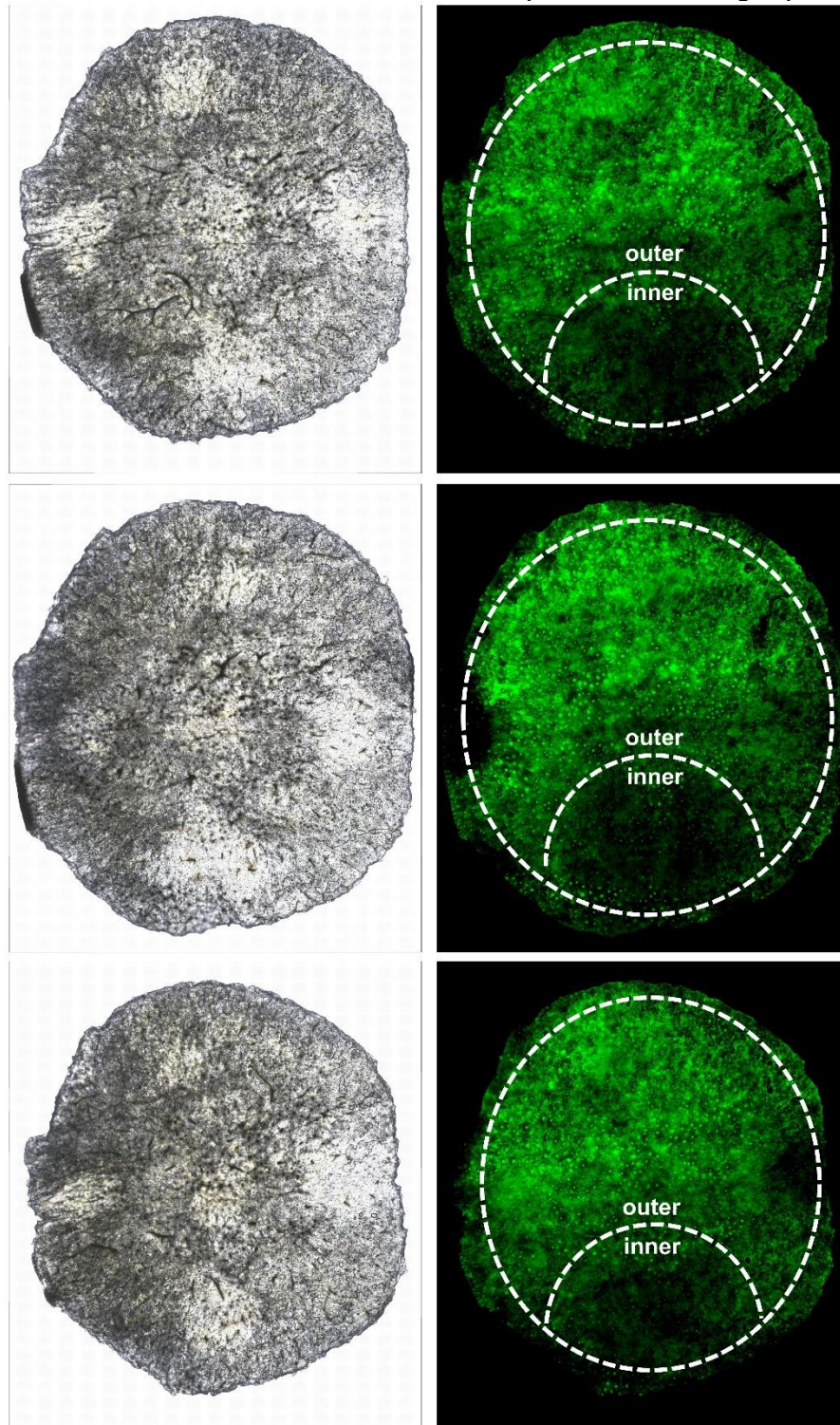


Supplementary Figure 8. Spatial imaging of unripe kiwifruit (sample#3), with emphasis on the outer pericarp, locules, and columella.



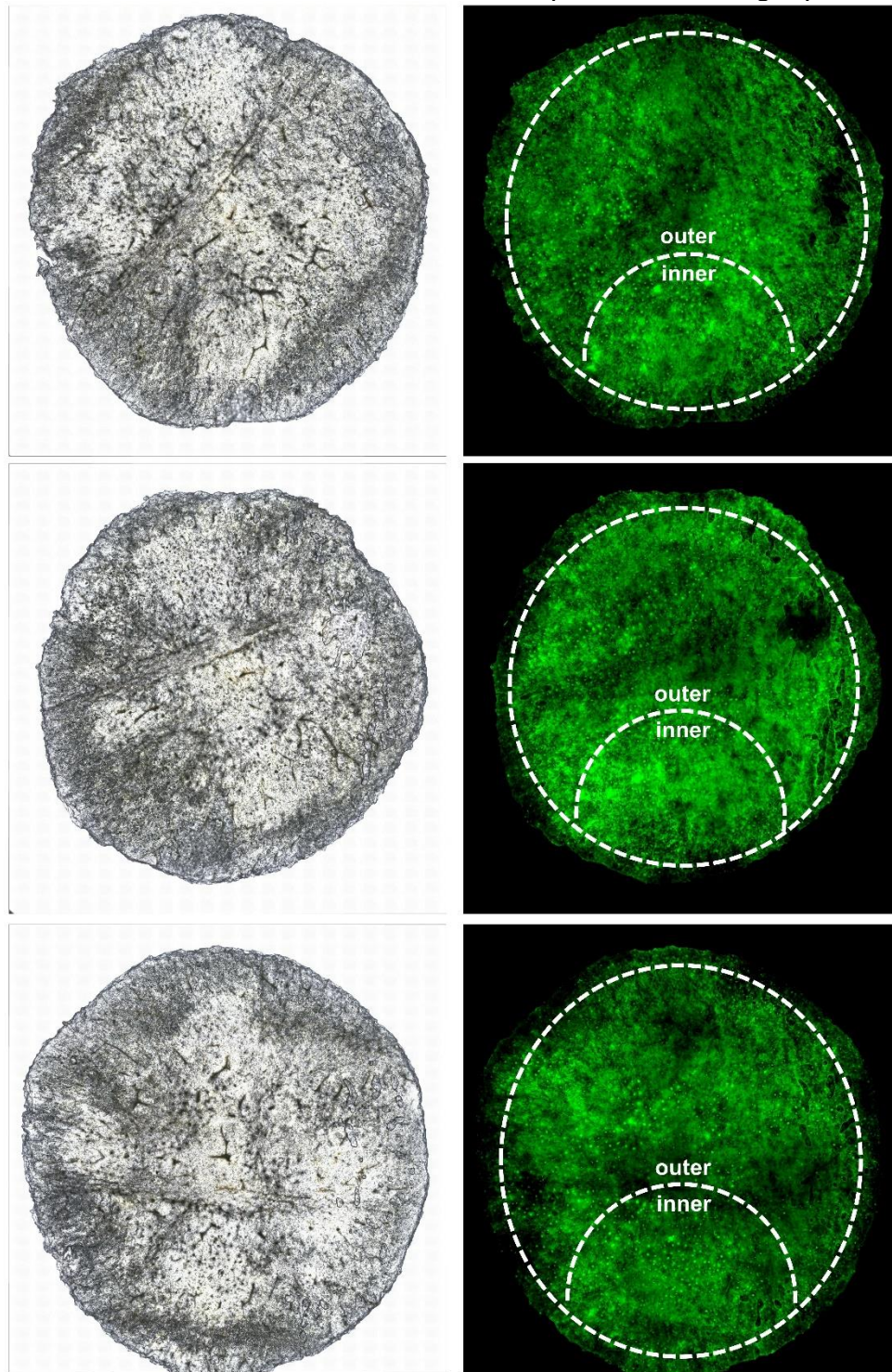
Supplementary Figure 9. Spatial imaging of unripe kiwifruit (sample#4), with emphasis on the outer pericarp, locules, and columella.

fruit: Asian pears
additive: PZA (10 mM) - *bottom position*
water - *top, center, left, right position*



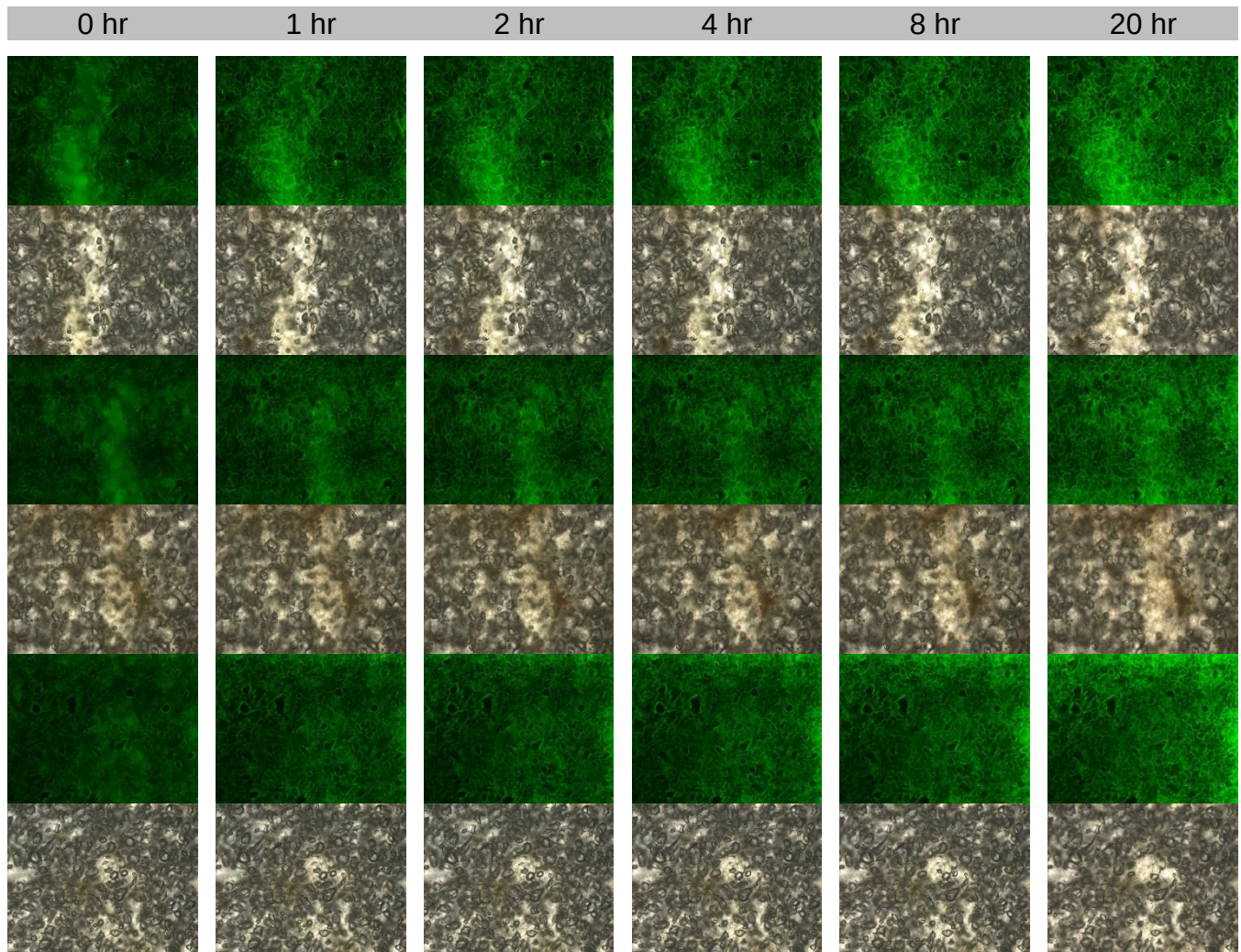
Supplementary Figure 10. Spatial imaging of Asian pear sections supplemented with 10 mM PZA (inner area) and water (outer area).

fruit: Asian pears
additive: ACC (10 mM) - *bottom position*
water - *top, center, left, right position*



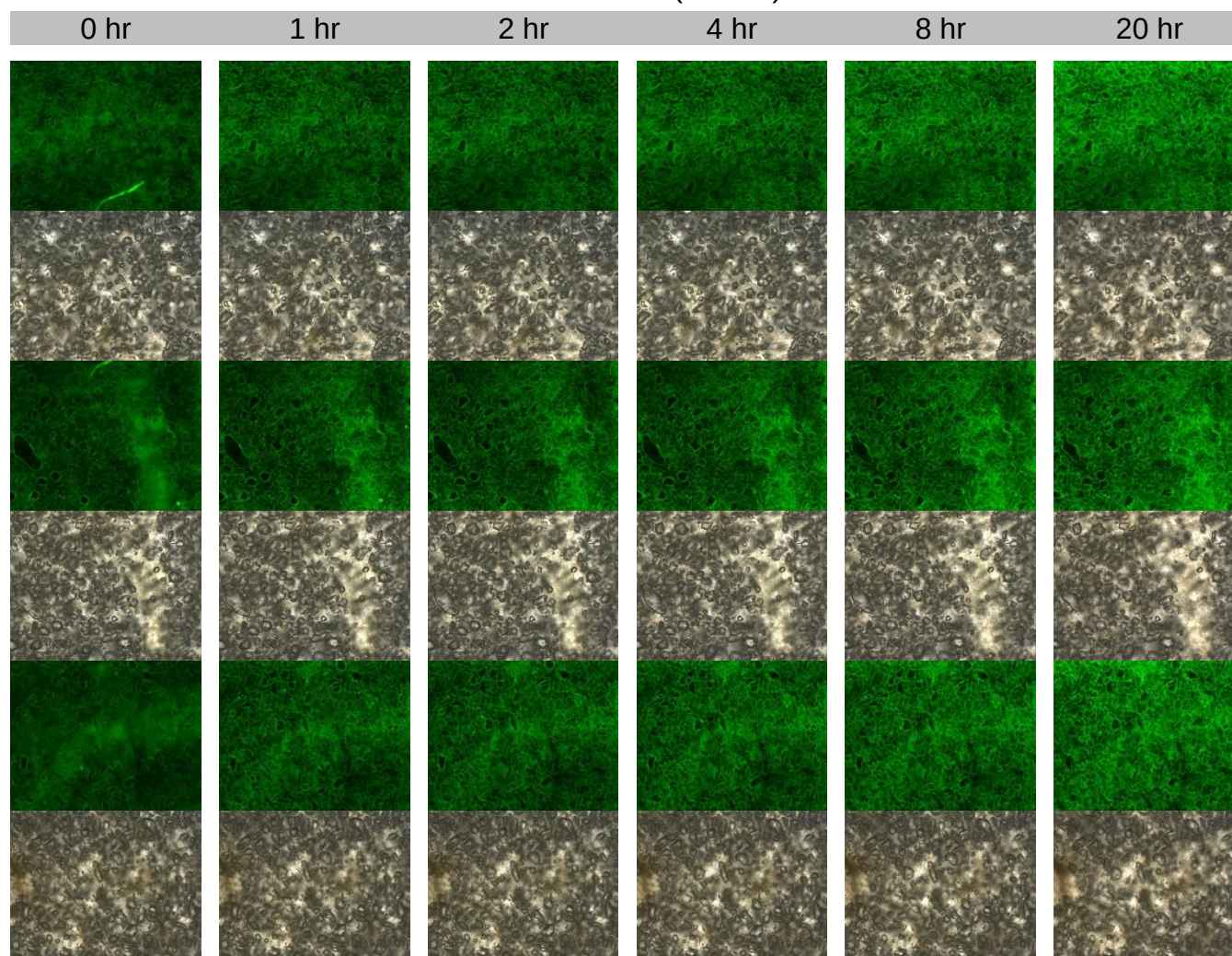
Supplementary Figure 11. Spatial imaging of Asian pear sections supplemented with 10 mM ACC (inner area) and water (outer area).

fruit: Golden Delicious apple
additive: ACC (1 mM)



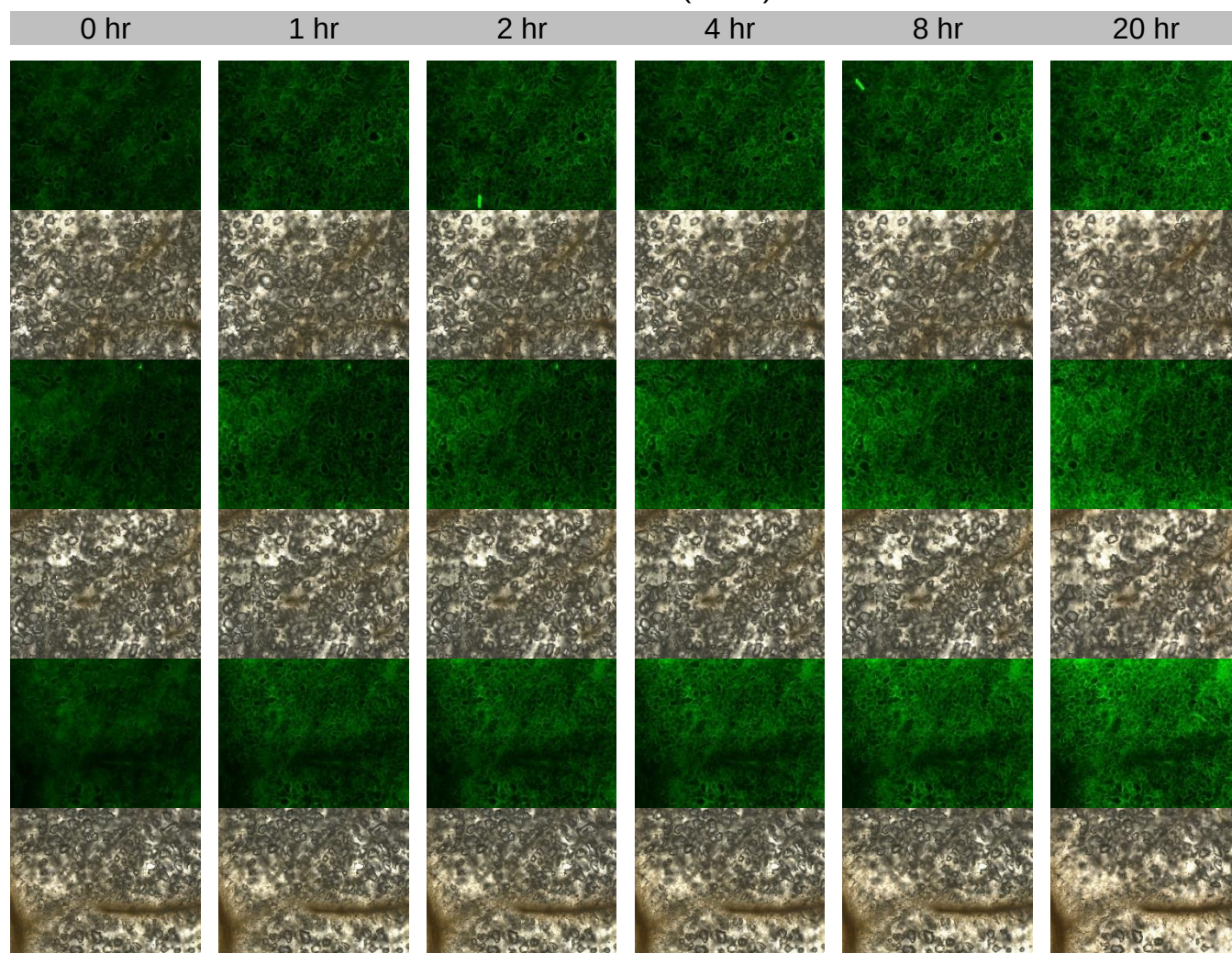
Supplementary Figure 12. Time-based imaging of Golden Delicious apples with 1 mM ACC supplementation.

fruit: Golden Delicious apple
additive: ACC (10 mM)



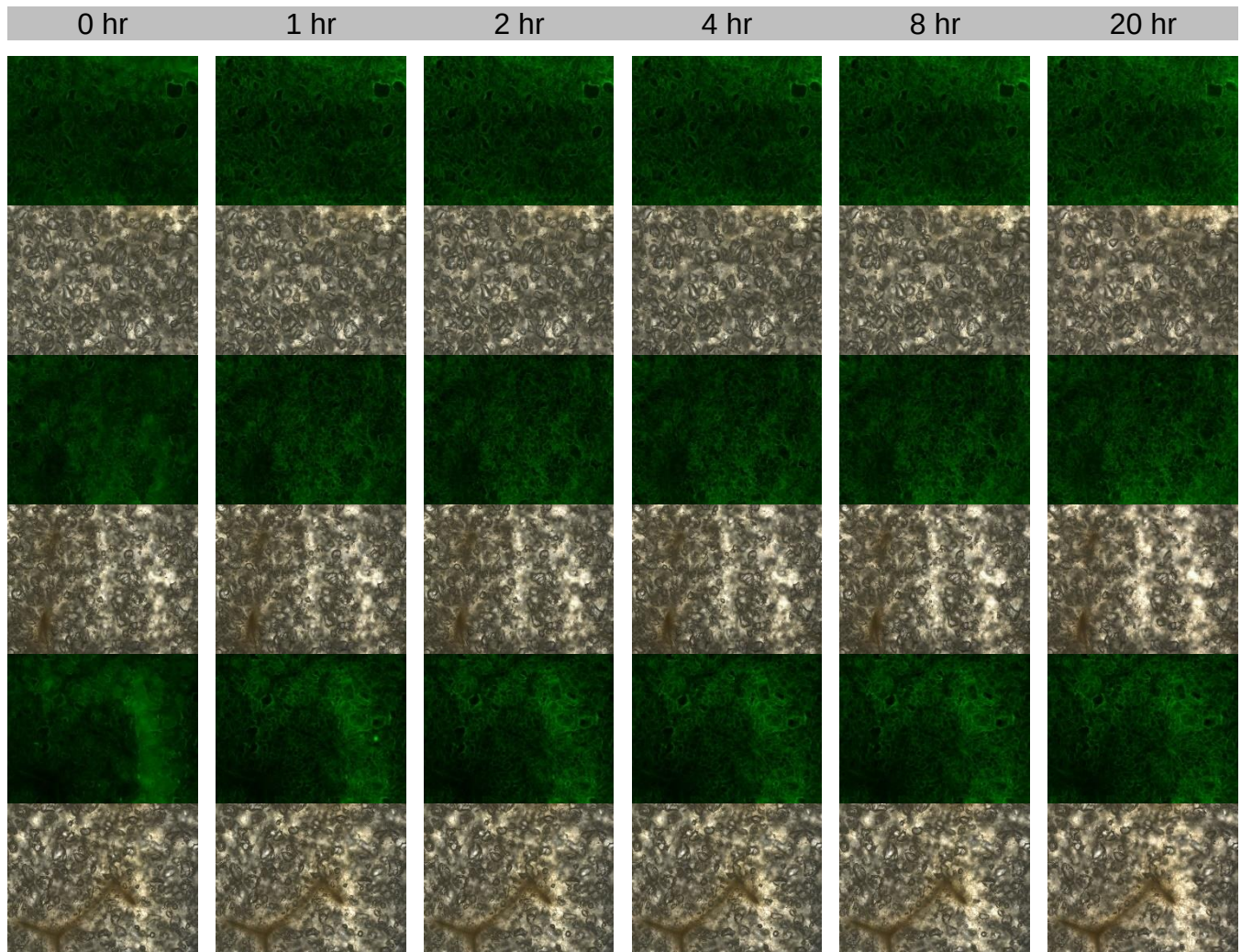
Supplementary Figure 13. Time-based imaging of Golden Delicious apples with 10 mM ACC supplementation.

fruit: Golden Delicious apple
additive: none (water)



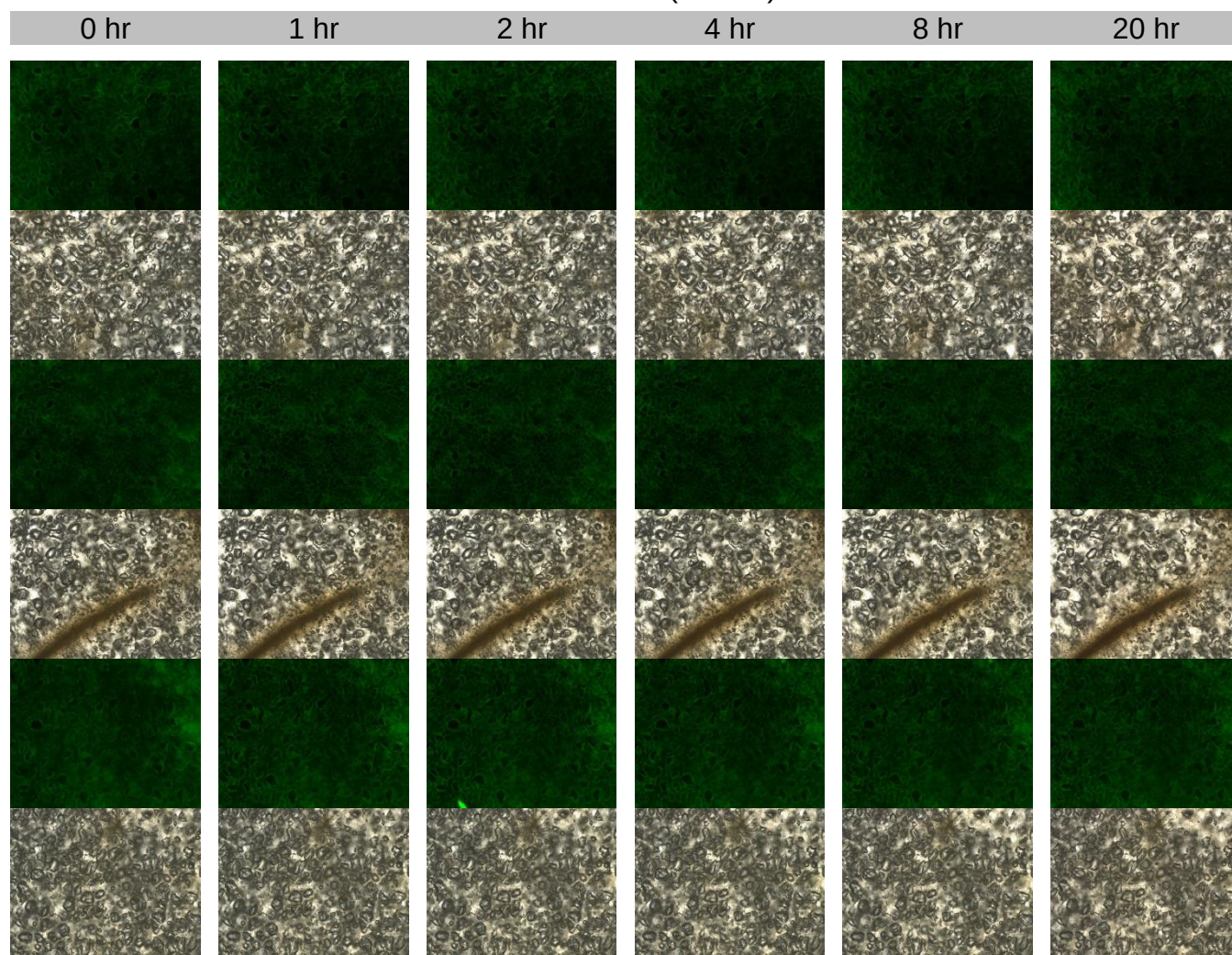
Supplementary Figure 14. Time-based imaging of Golden Delicious apples with no supplementation.

fruit: Golden Delicious apple
additive: PZA (1 mM)

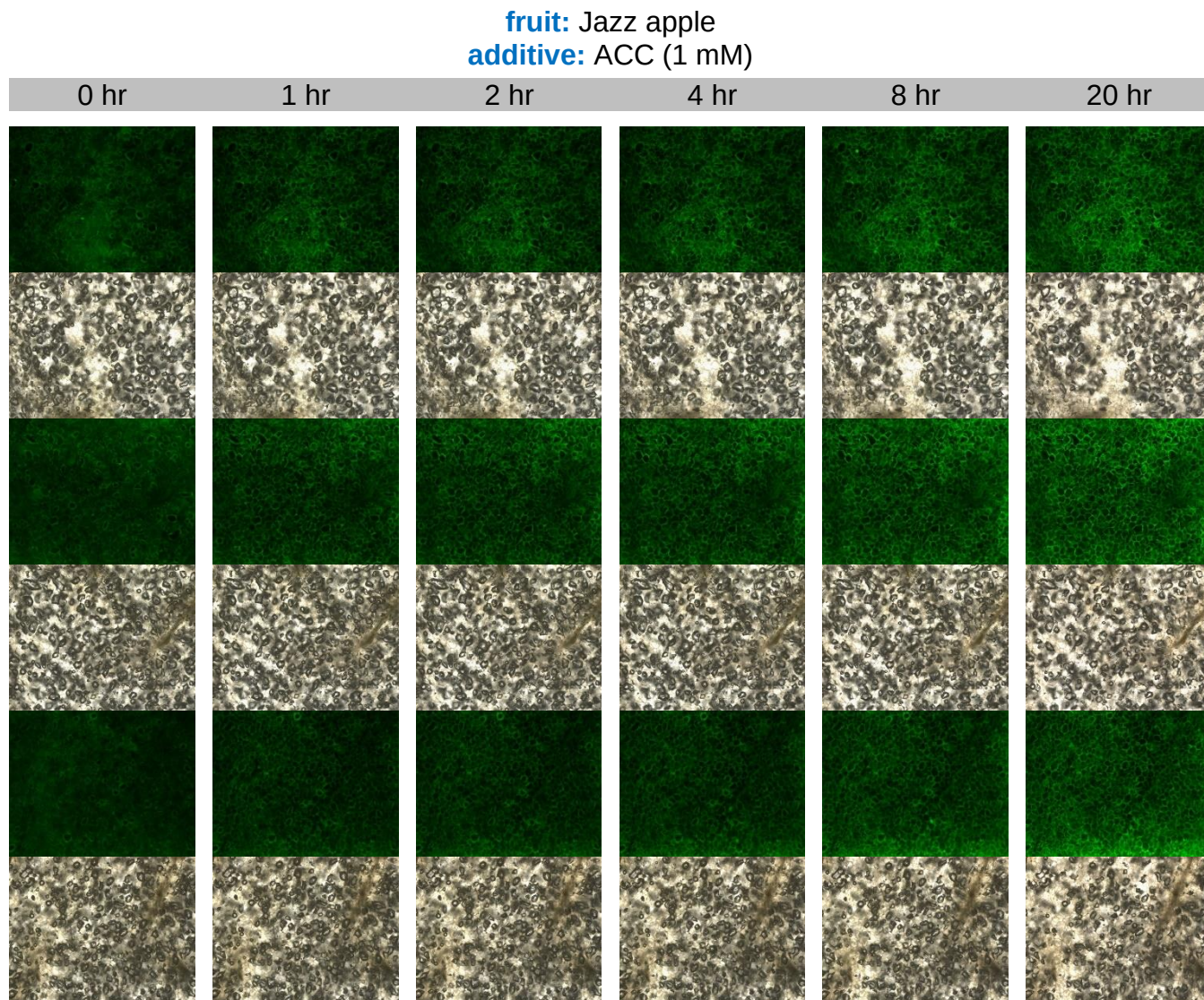


Supplementary Figure 15. Time-based imaging of Golden Delicious apples with 1 mM PZA supplementation.

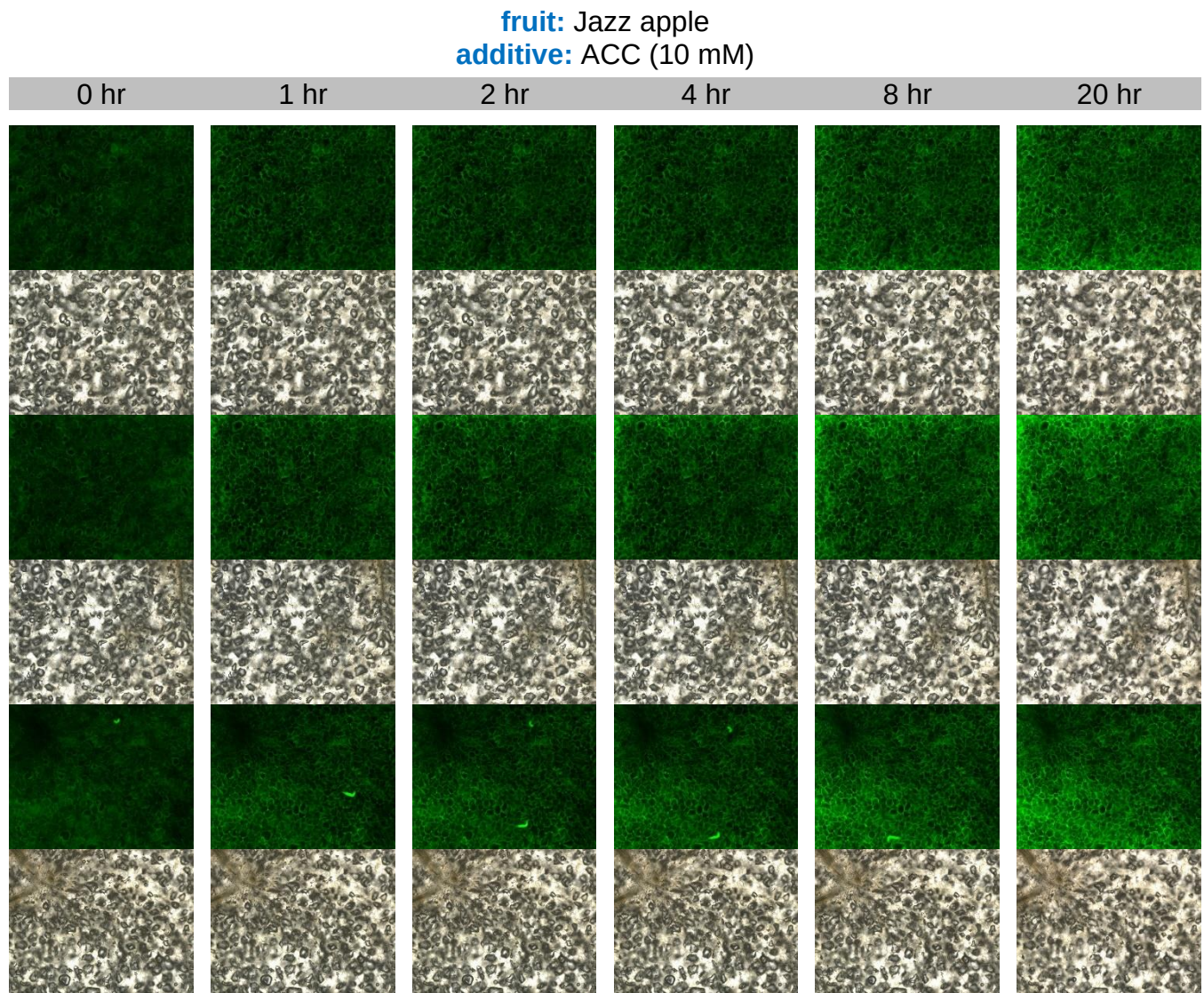
fruit: Golden Delicious apple
additive: PZA (10 mM)



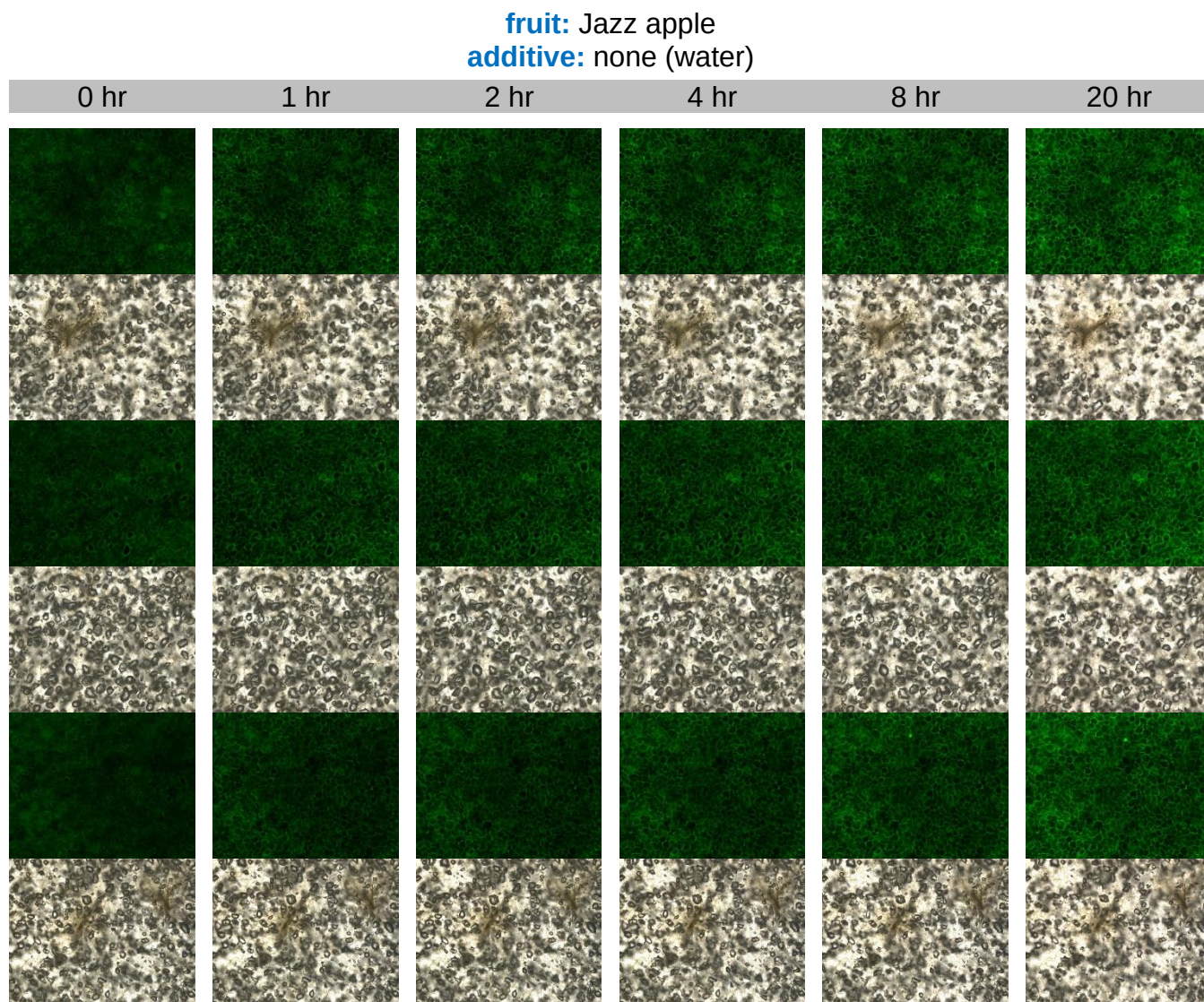
Supplementary Figure 16. Time-based imaging of Golden Delicious apples with 10 mM PZA supplementation.



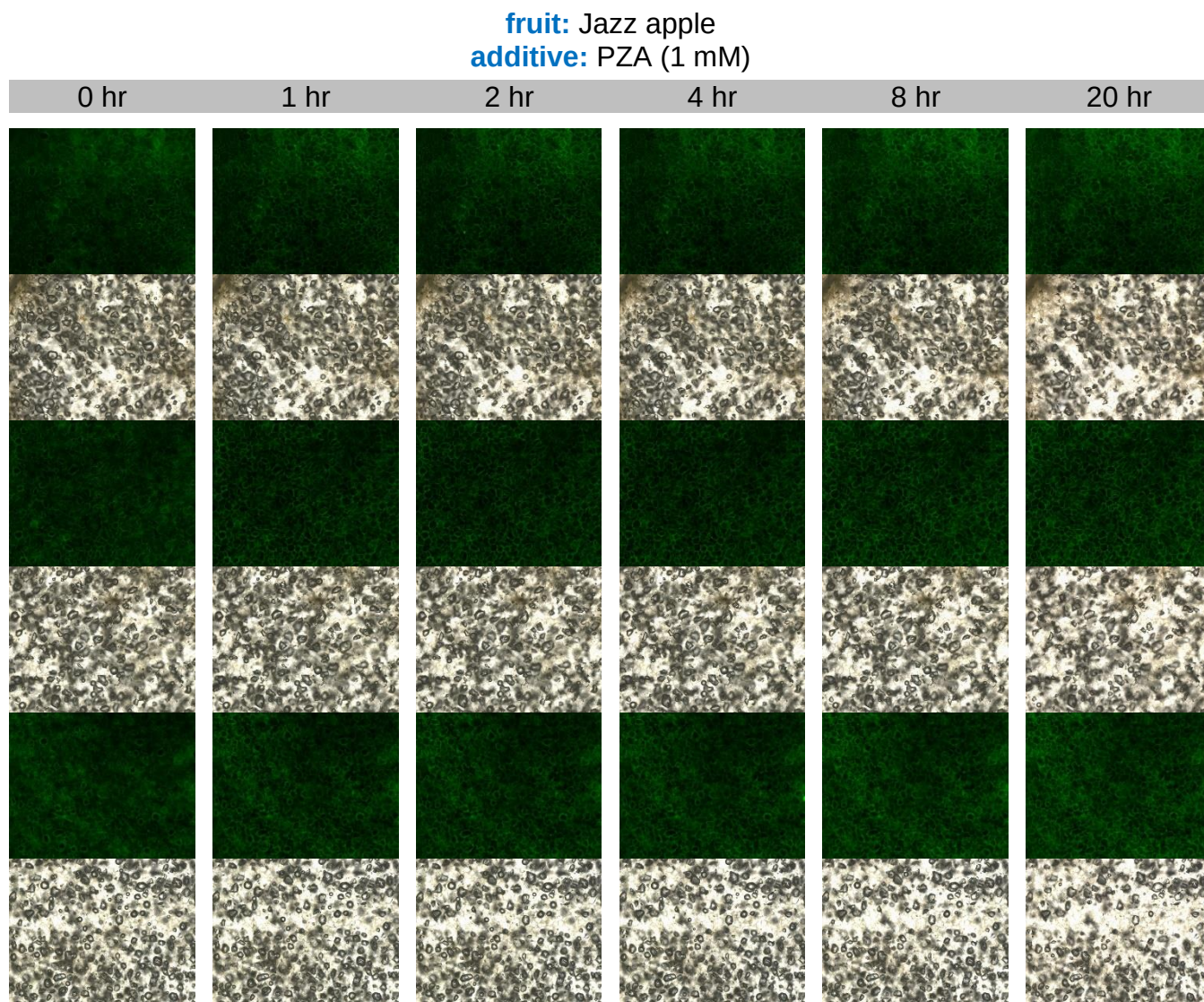
Supplementary Figure 17. Time-based imaging of Jazz apples with 1 mM ACC supplementation.



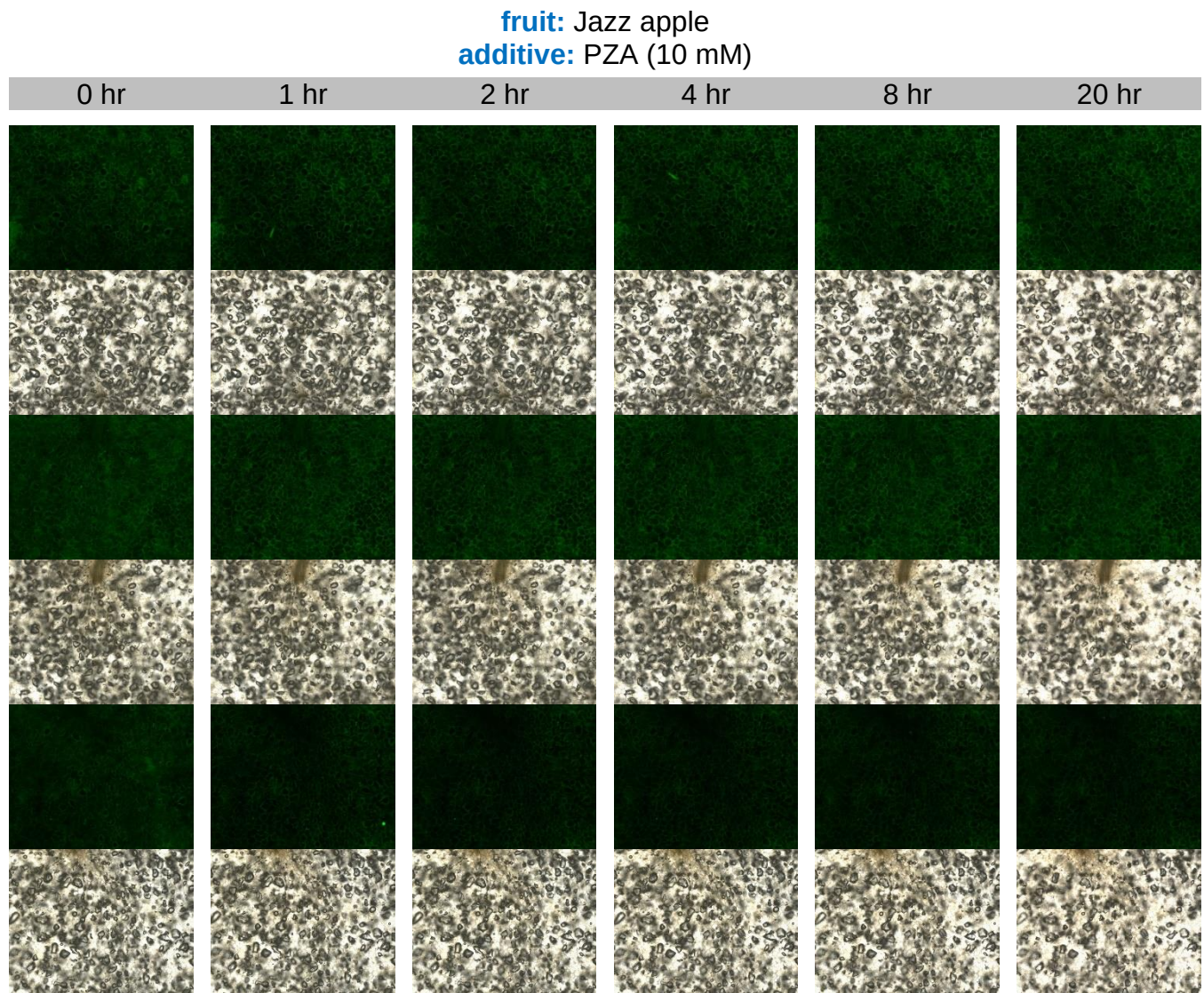
Supplementary Figure 18. Time-based imaging of Jazz apples with 10 mM ACC supplementation.



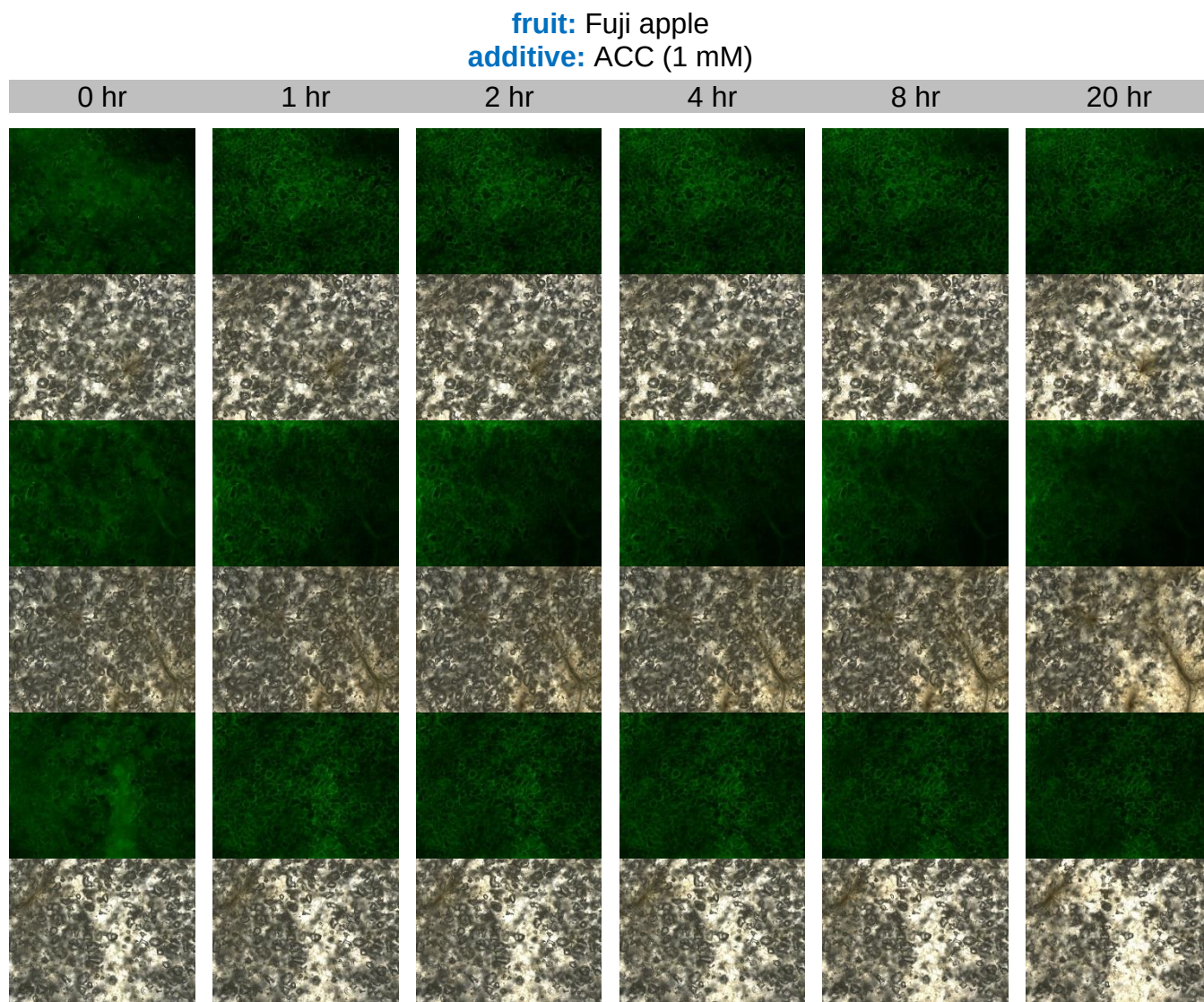
Supplementary Figure 19. Time-based imaging of Jazz apples with no supplementation.



Supplementary Figure 20. Time-based imaging of Jazz apples with 1 mM PZA supplementation.

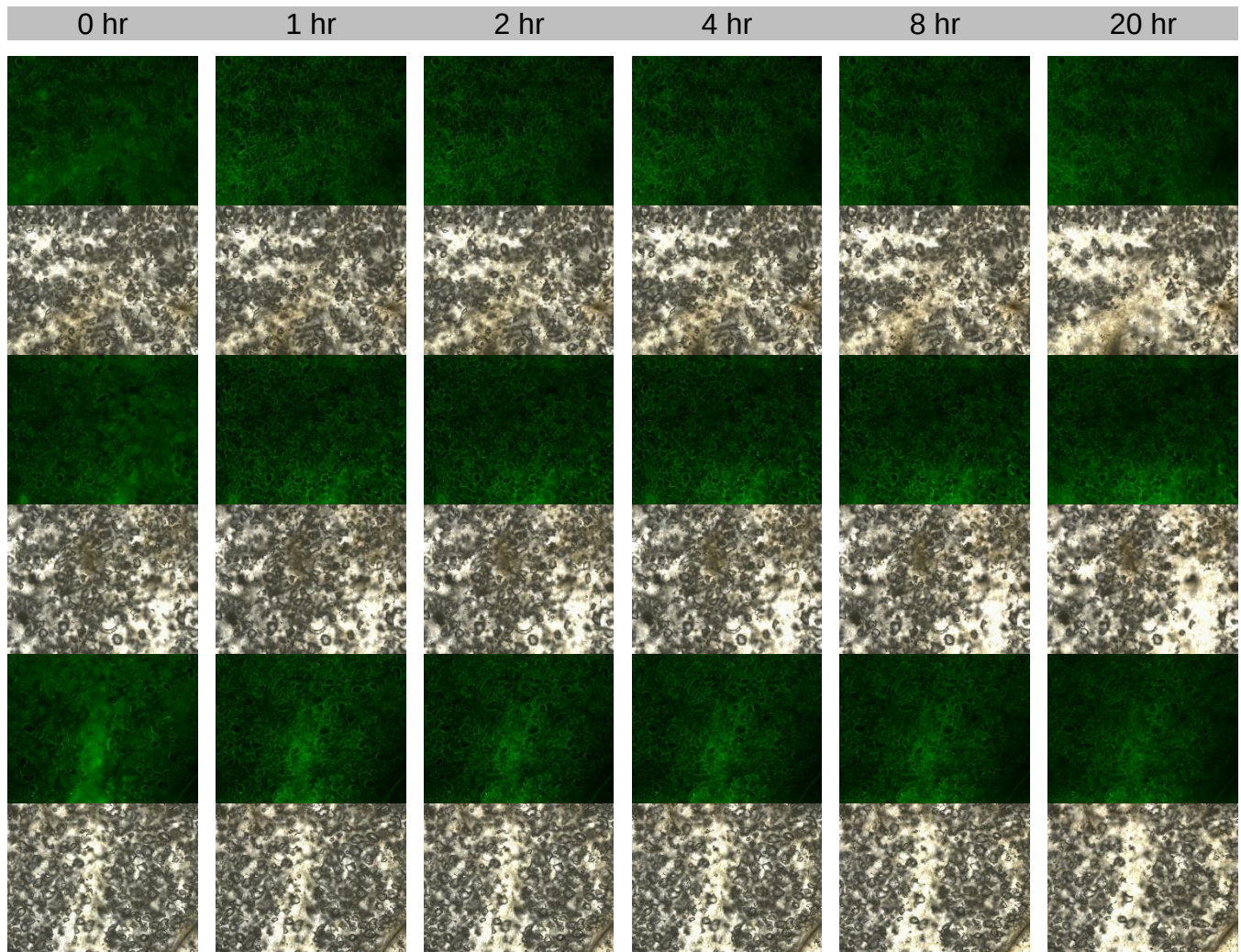


Supplementary Figure 21. Time-based imaging of Jazz apples with 10 mM PZA supplementation.

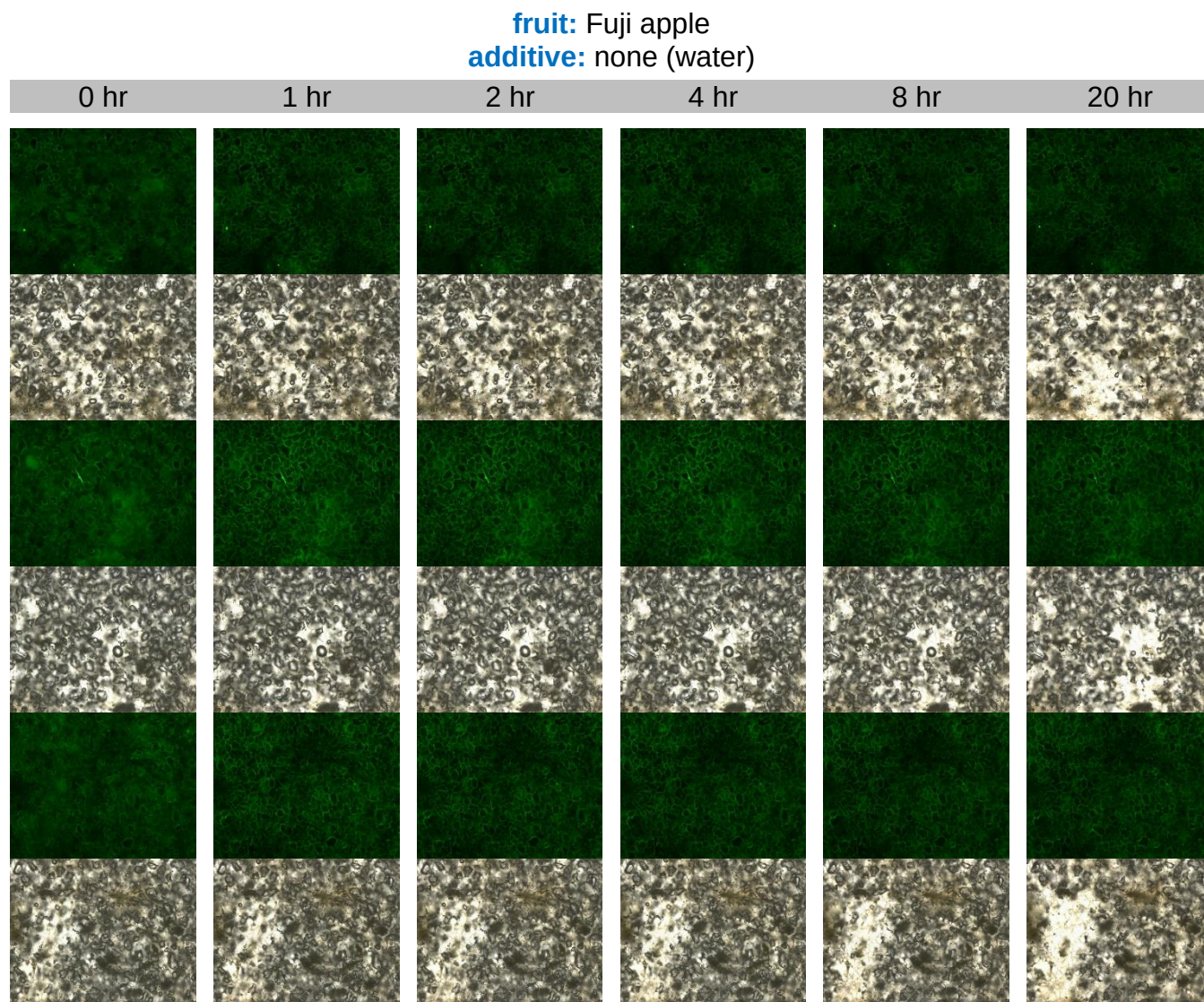


Supplementary Figure 22. Time-based imaging of Fuji apples with 1 mM ACC supplementation.

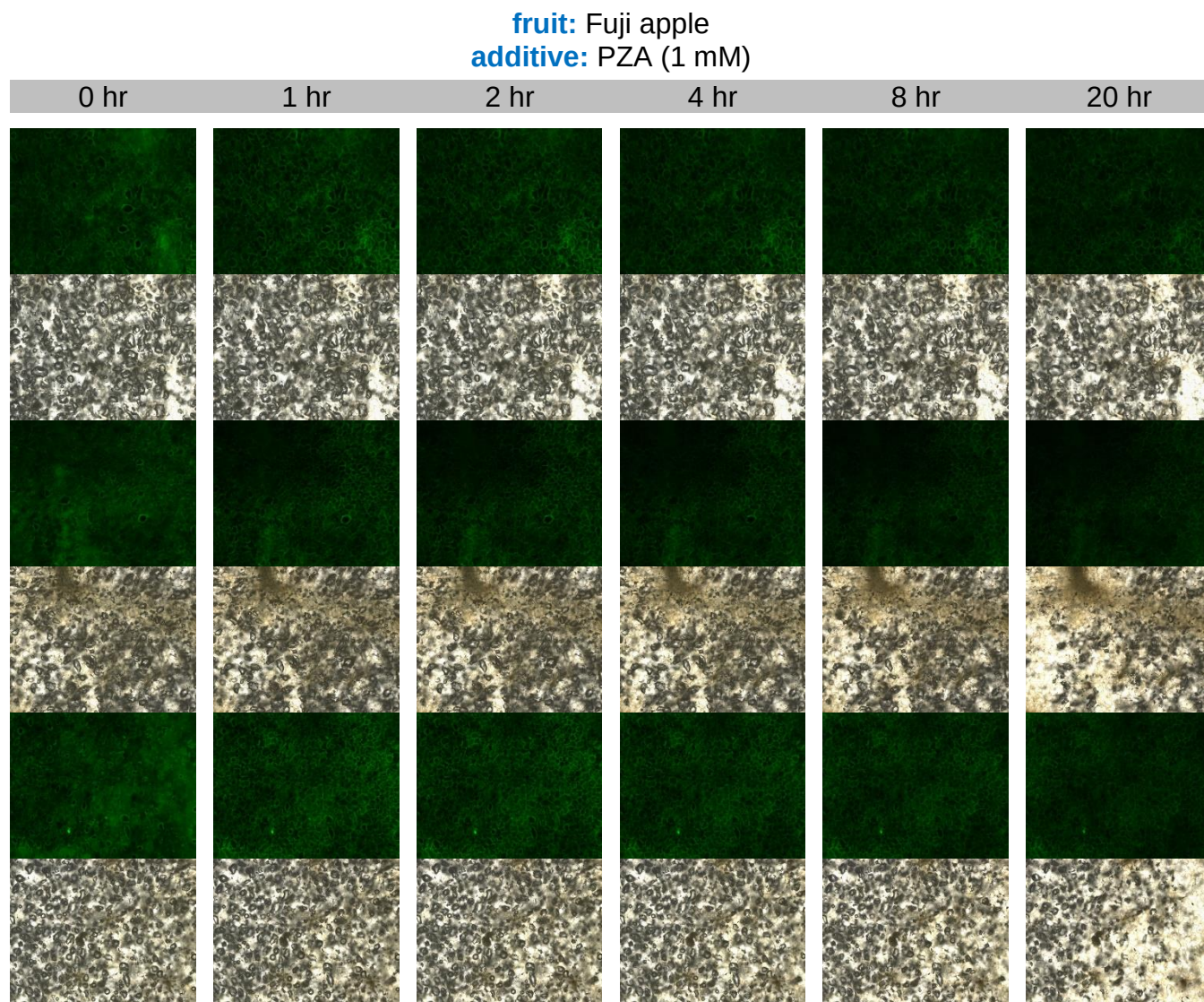
fruit: Fuji apple
additive: ACC (10 mM)



Supplementary Figure 23. Time-based imaging of Fuji apples with 10 mM ACC supplementation.

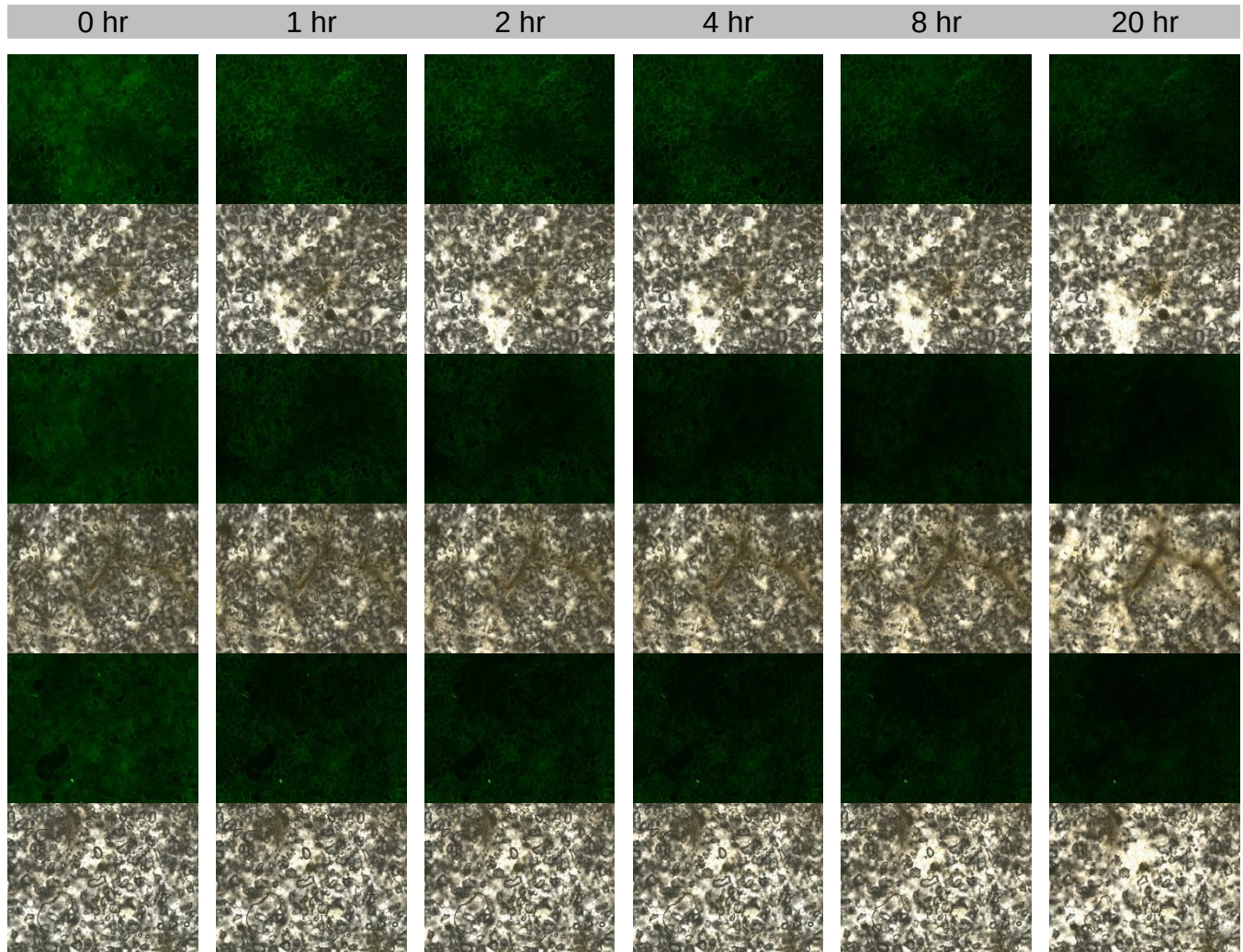


Supplementary Figure 24. Time-based imaging of Fuji apples with no supplementation.

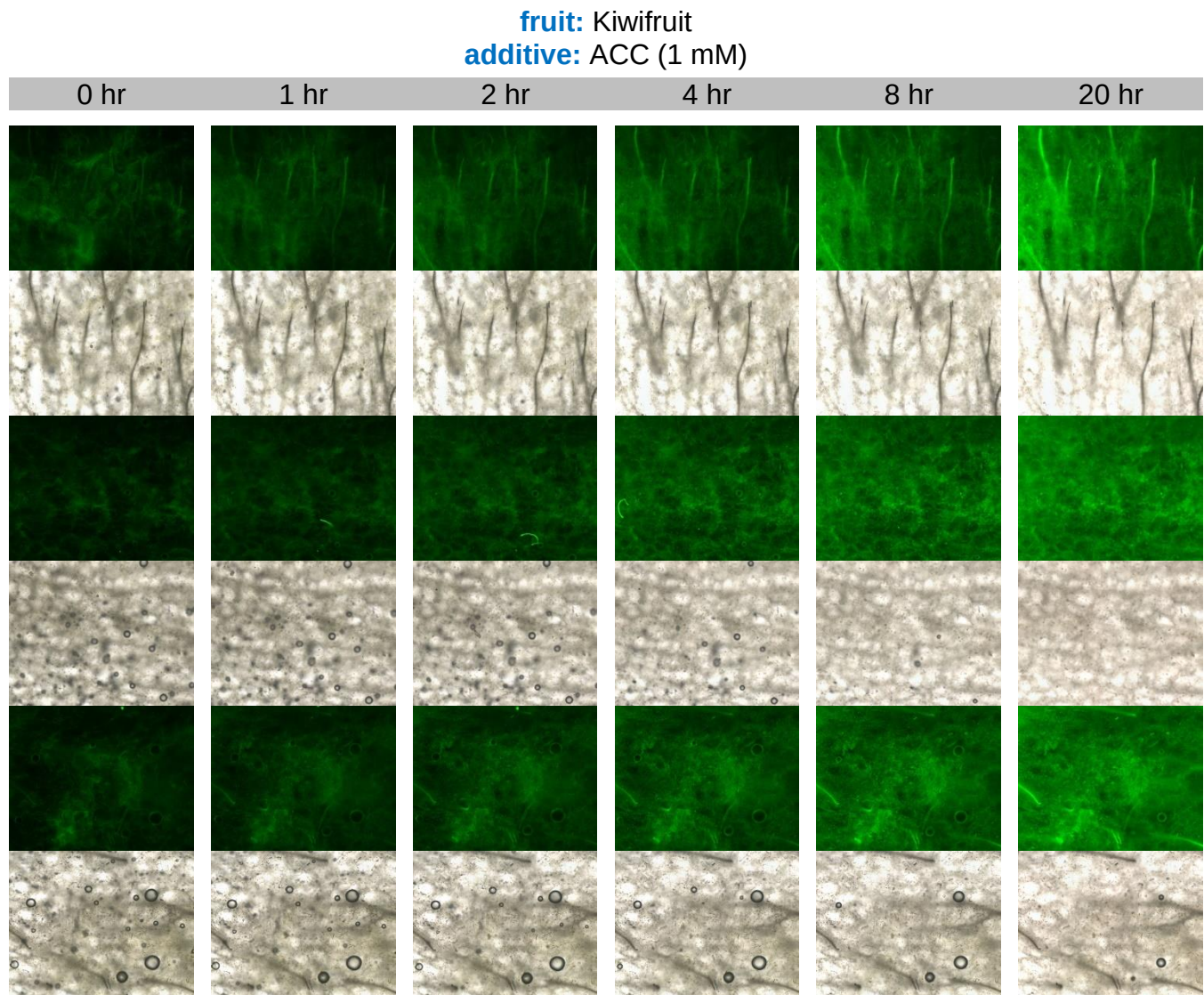


Supplementary Figure 25. Time-based imaging of Fuji apples with 1 mM PZA supplementation.

fruit: Fuji apple
additive: PZA (10 mM)

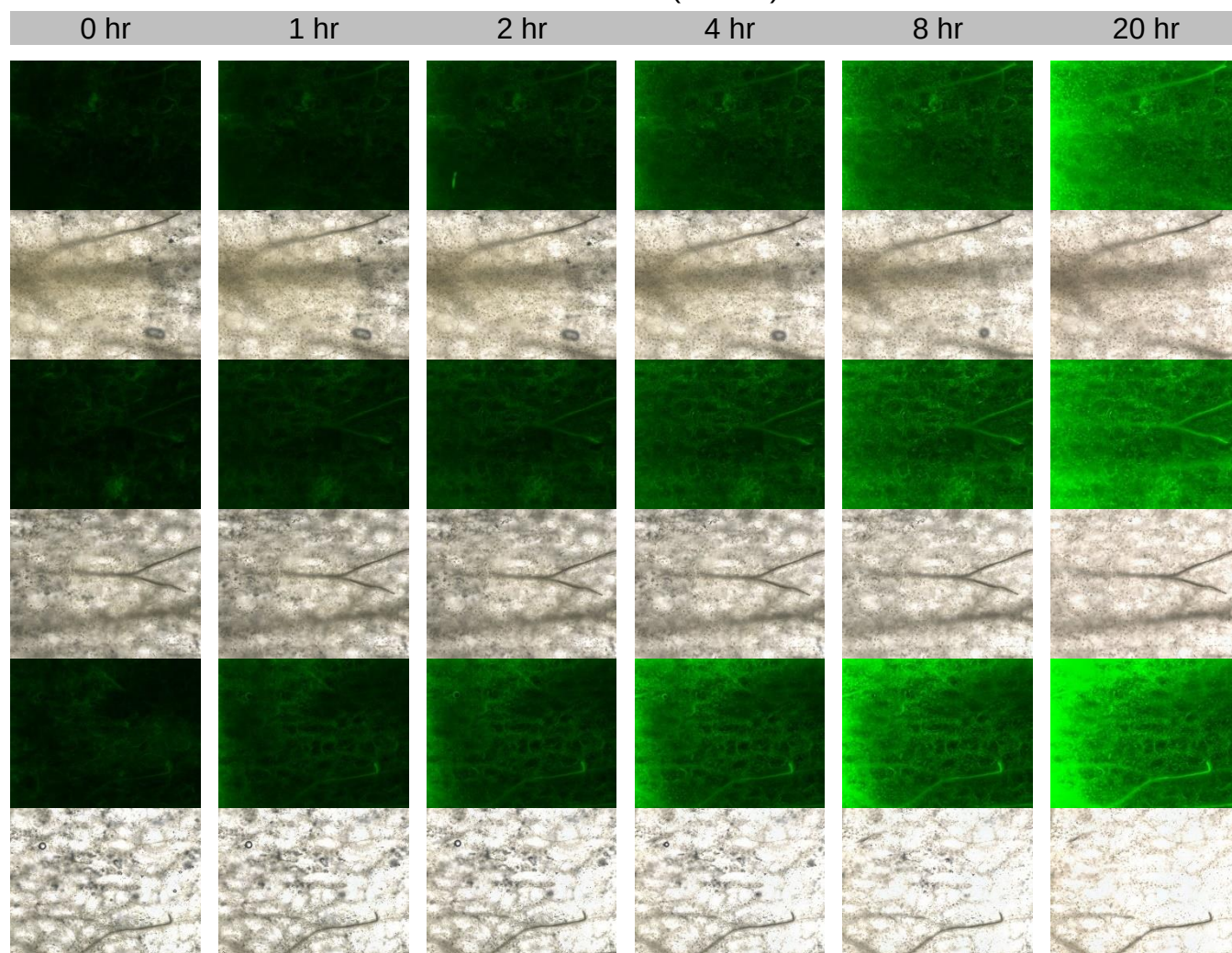


Supplementary Figure 26. Time-based imaging of Fuji apples with 10 mM PZA supplementation.

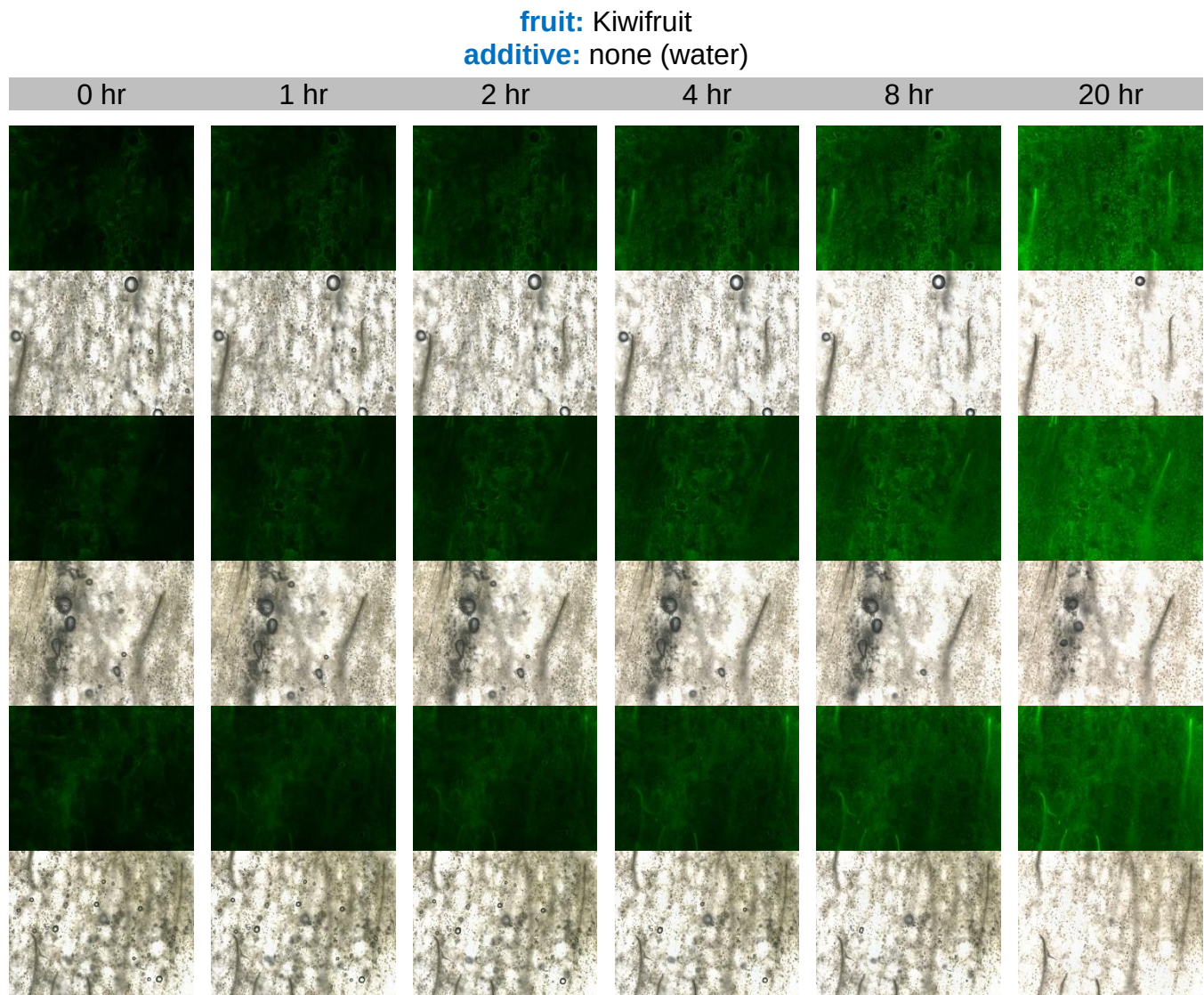


Supplementary Figure 27. Time-based imaging of kiwifruit with 1 mM ACC supplementation.

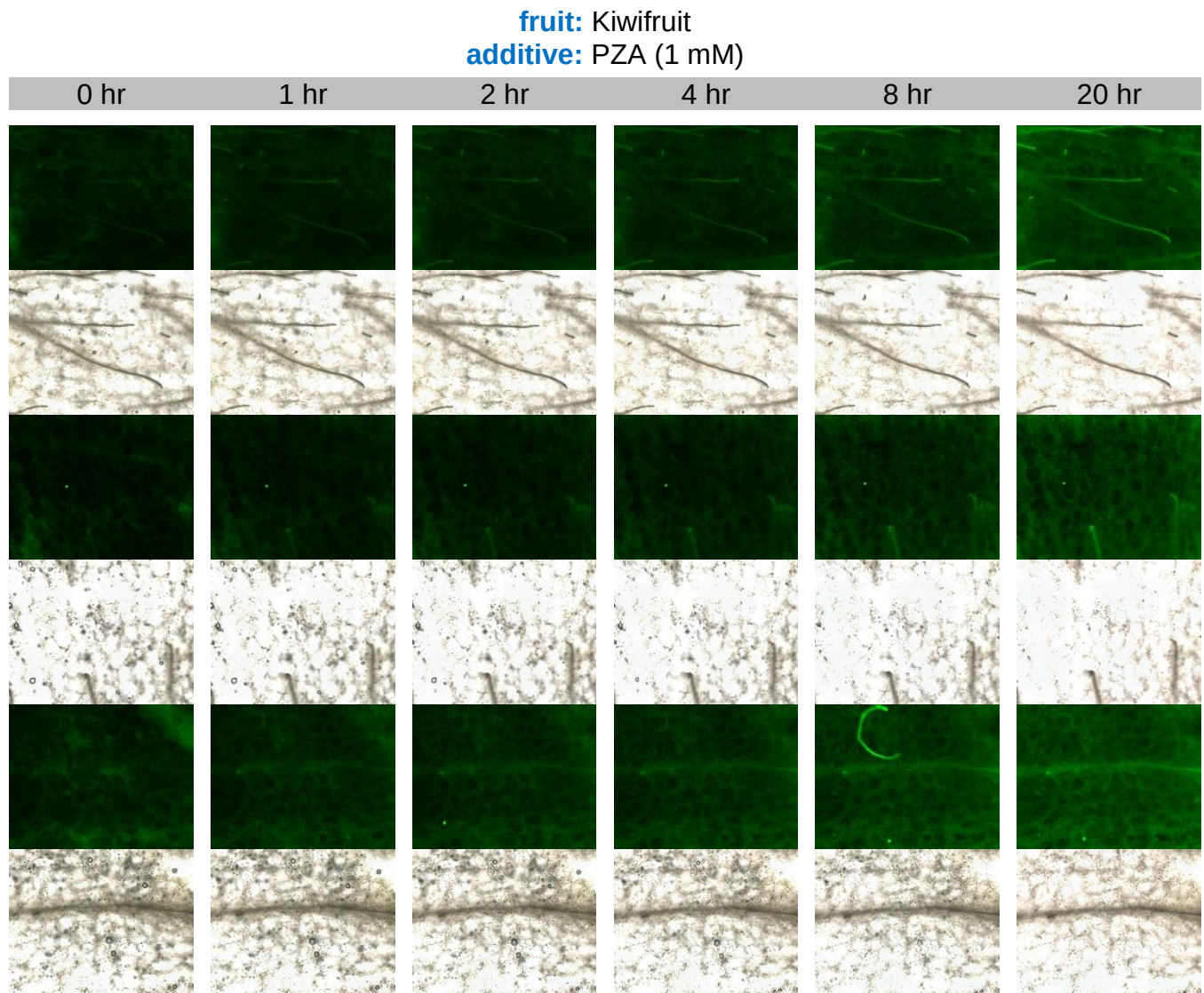
fruit: Kiwifruit
additive: ACC (10 mM)



Supplementary Figure 28. Time-based imaging of kiwifruit with 10 mM ACC supplementation.

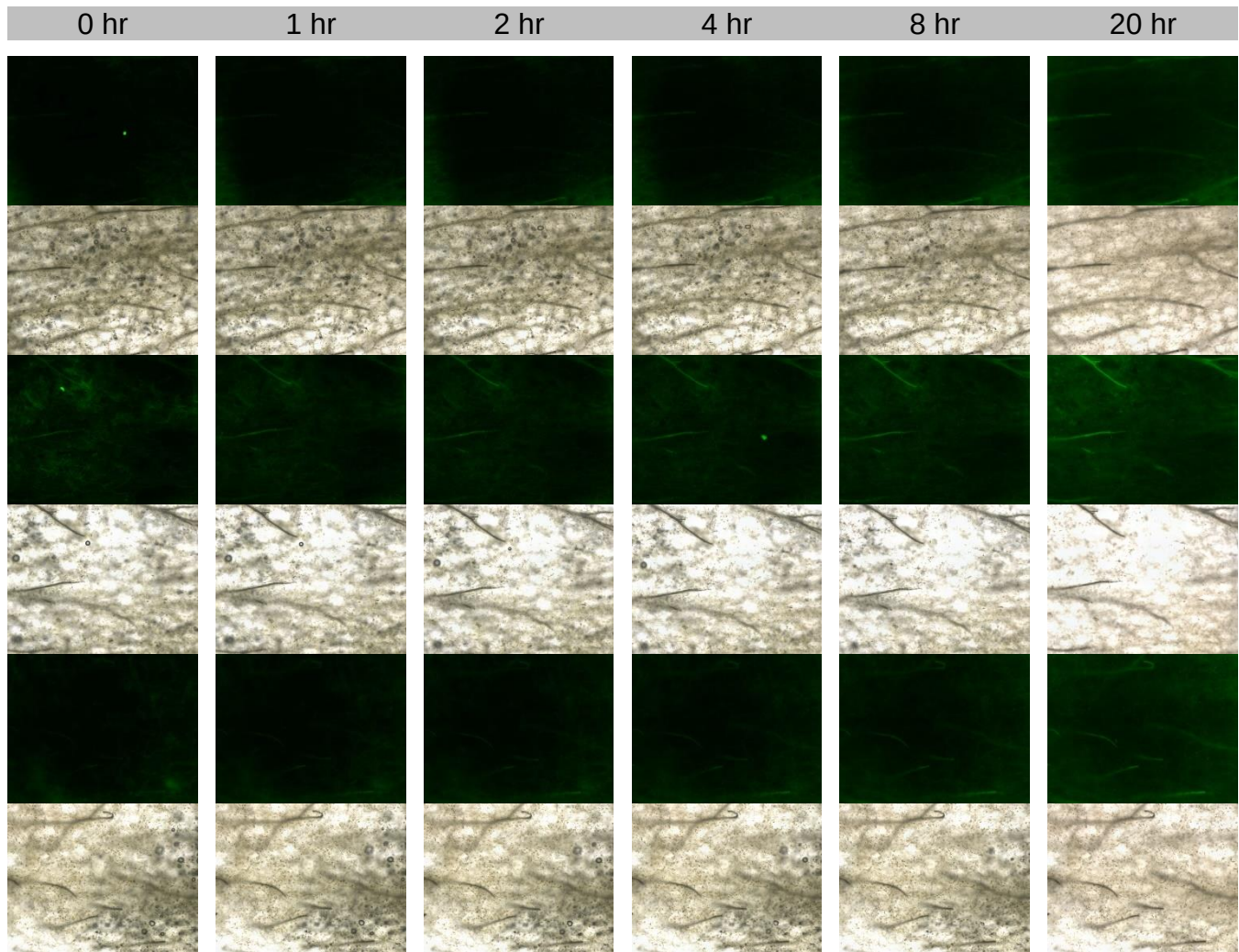


Supplementary Figure 29. Time-based imaging of kiwifruit with no supplementation.

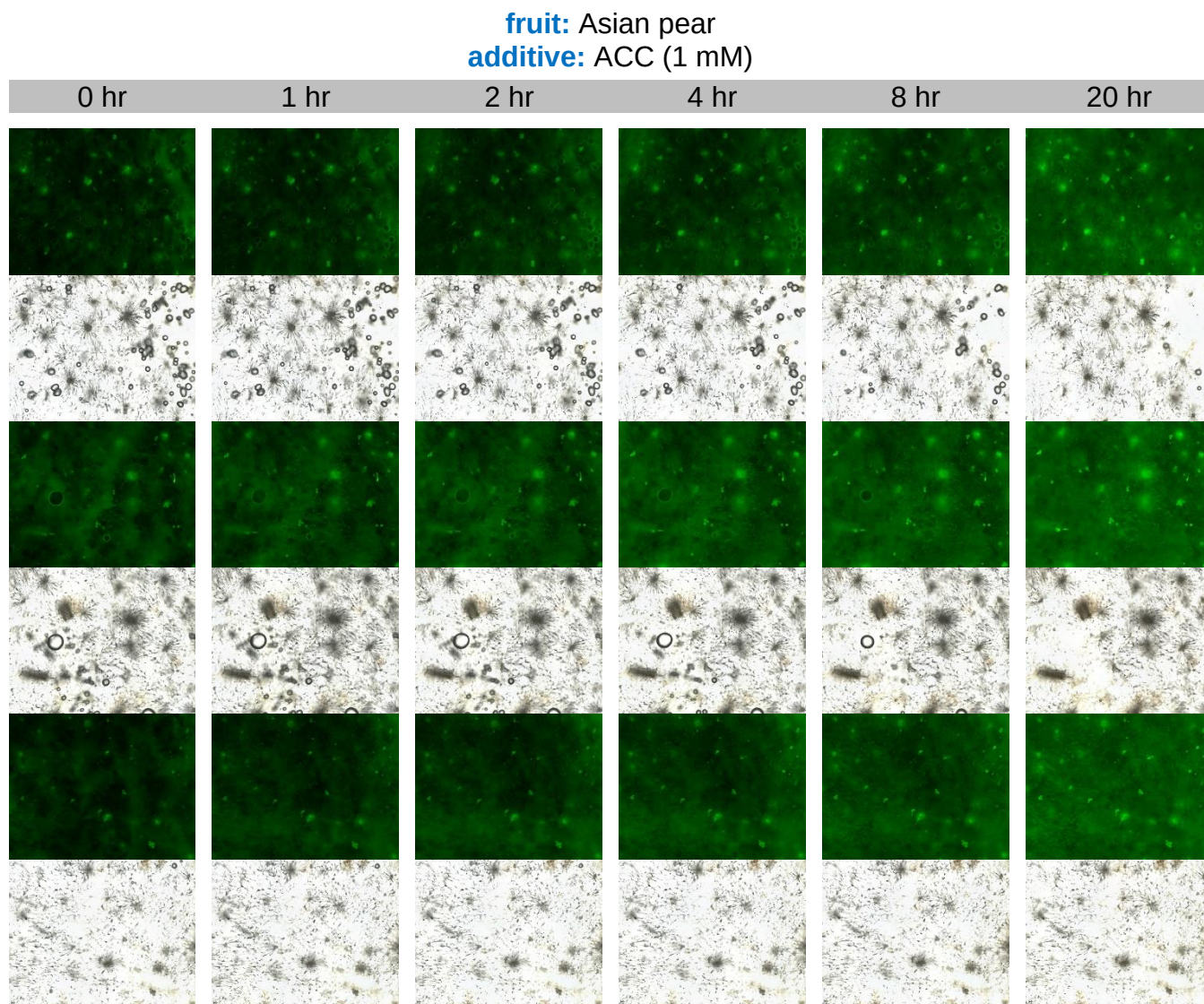


Supplementary Figure 30. Time-based imaging of kiwifruit with 1 mM PZA supplementation.

fruit: Kiwifruit
additive: PZA (10 mM)

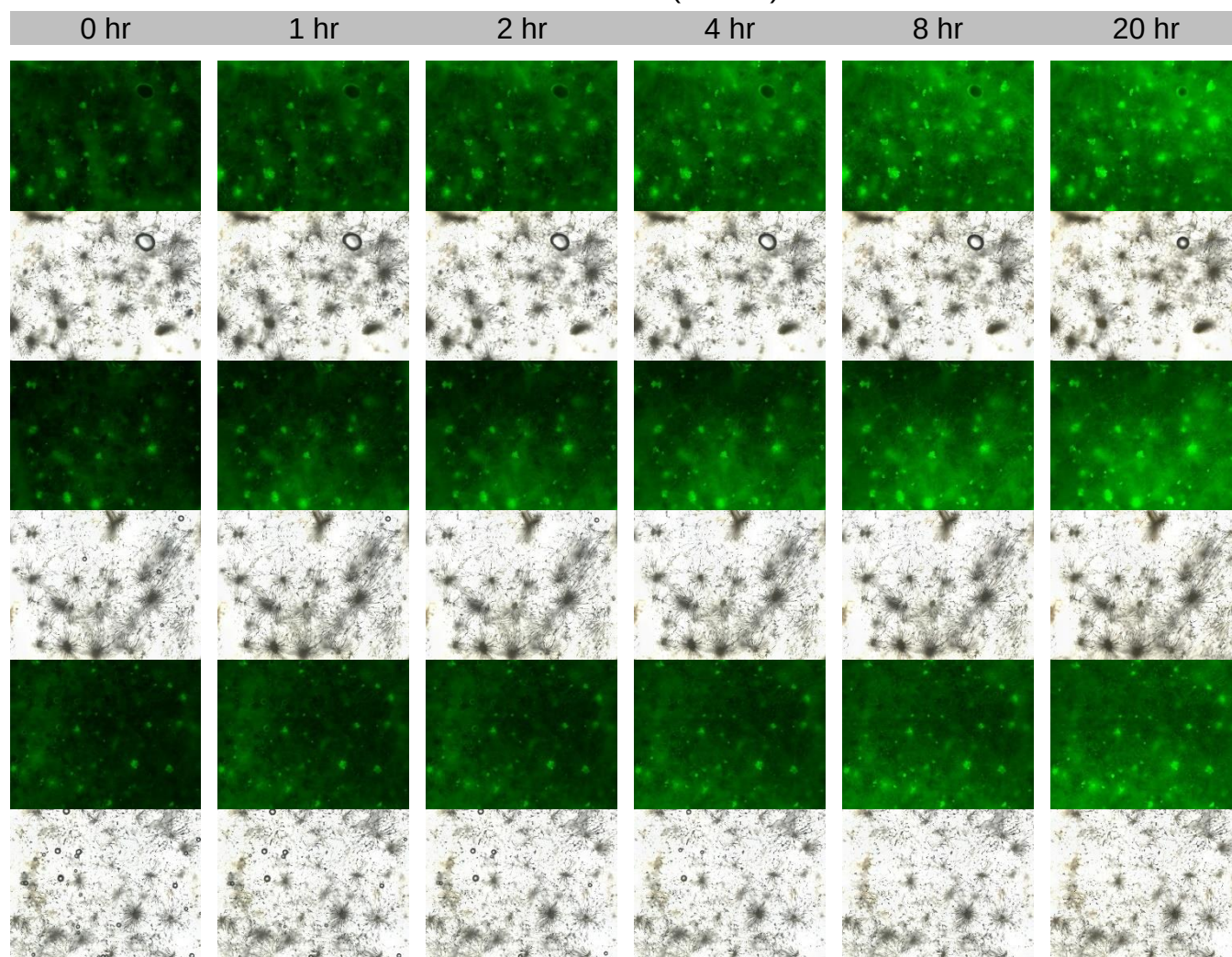


Supplementary Figure 31. Time-based imaging of kiwifruit with 10 mM PZA supplementation.

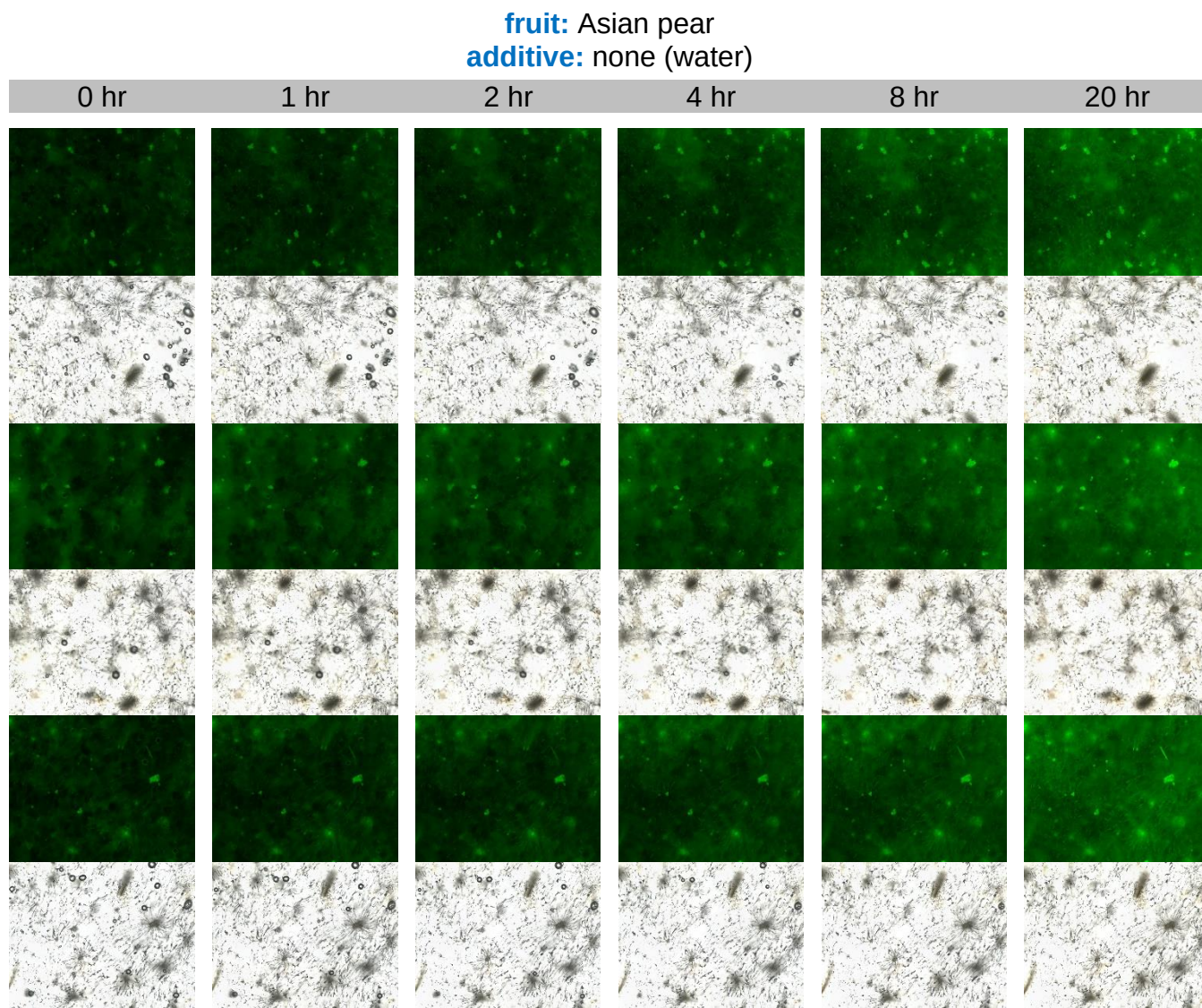


Supplementary Figure 32. Time-based imaging of Asian pear with 1 mM ACC supplementation.

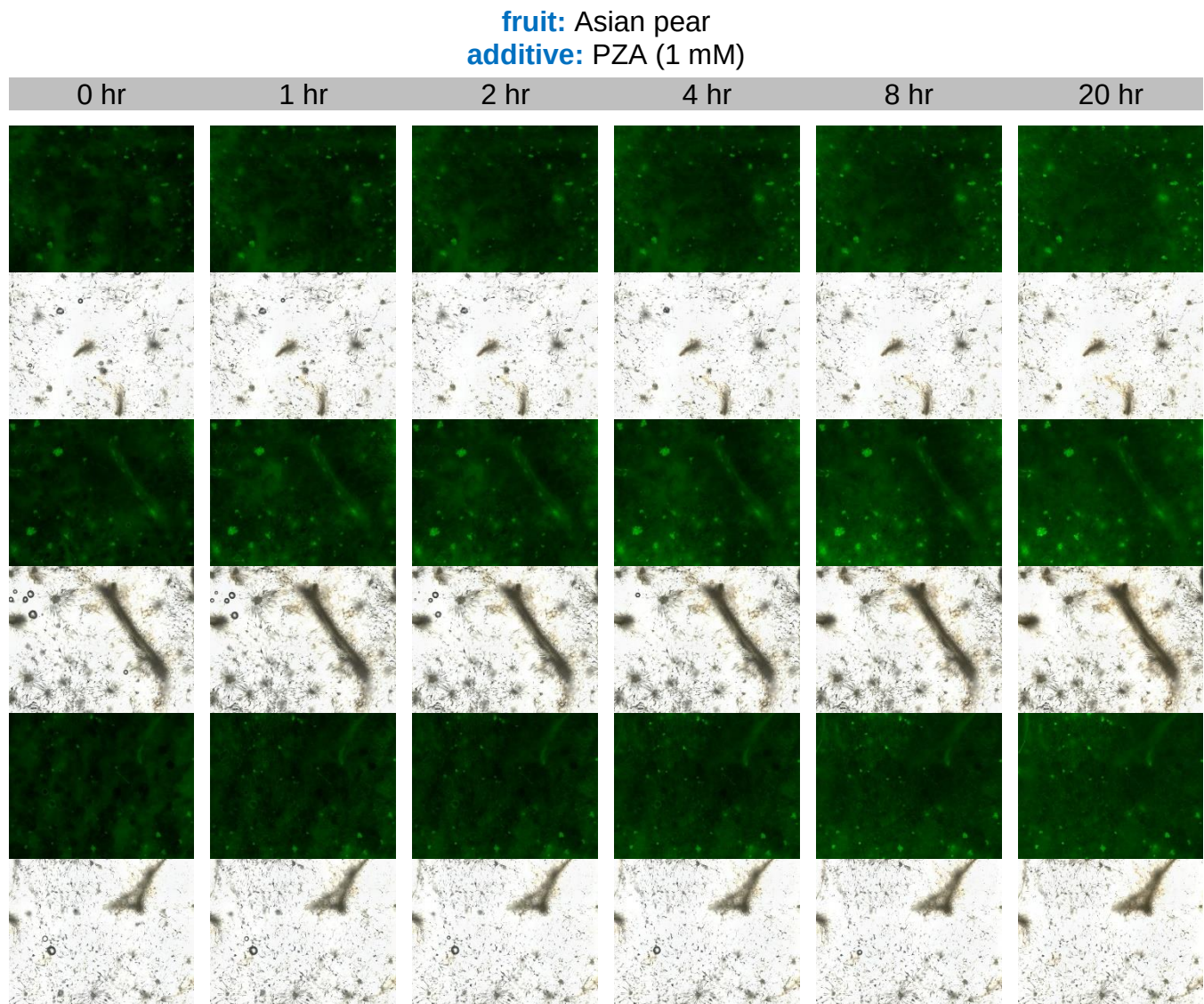
fruit: Asian pear
additive: ACC (10 mM)



Supplementary Figure 33. Time-based imaging of Asian pear with 10 mM ACC supplementation.

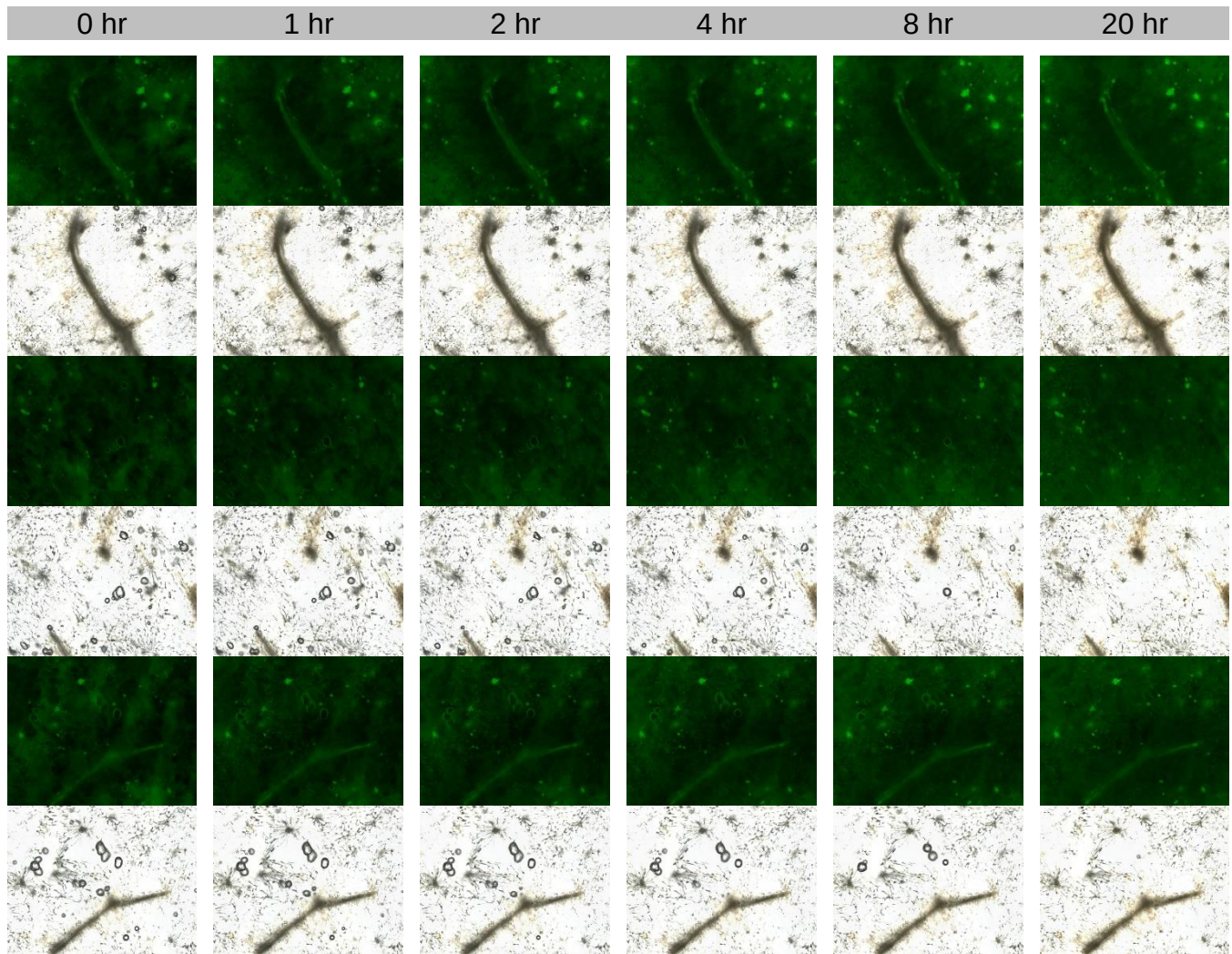


Supplementary Figure 34. Time-based imaging of Asian pear with no supplementation.

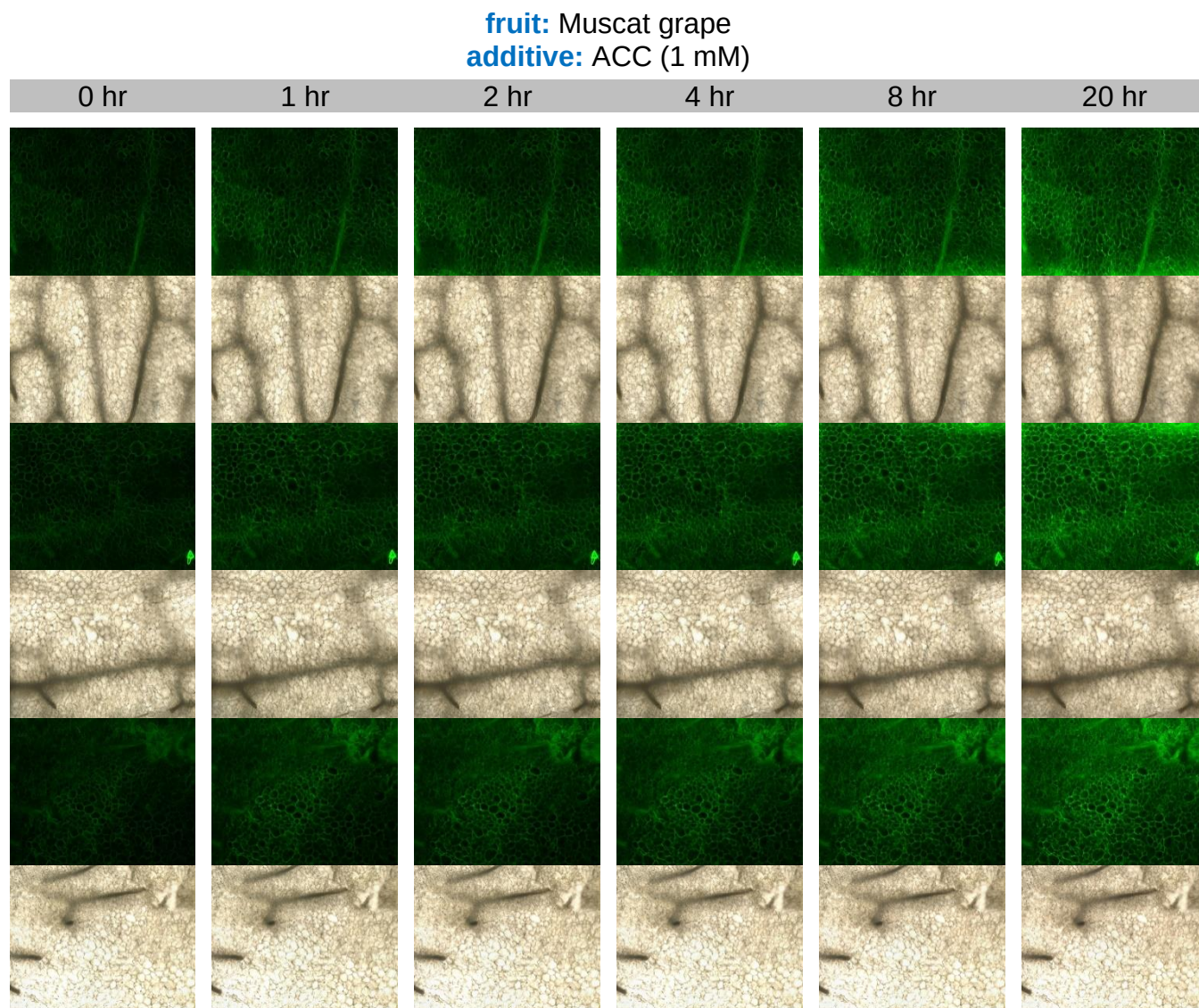


Supplementary Figure 35. Time-based imaging of Asian pear with 1 mM PZA supplementation.

fruit: Asian pear
additive: PZA (10 mM)

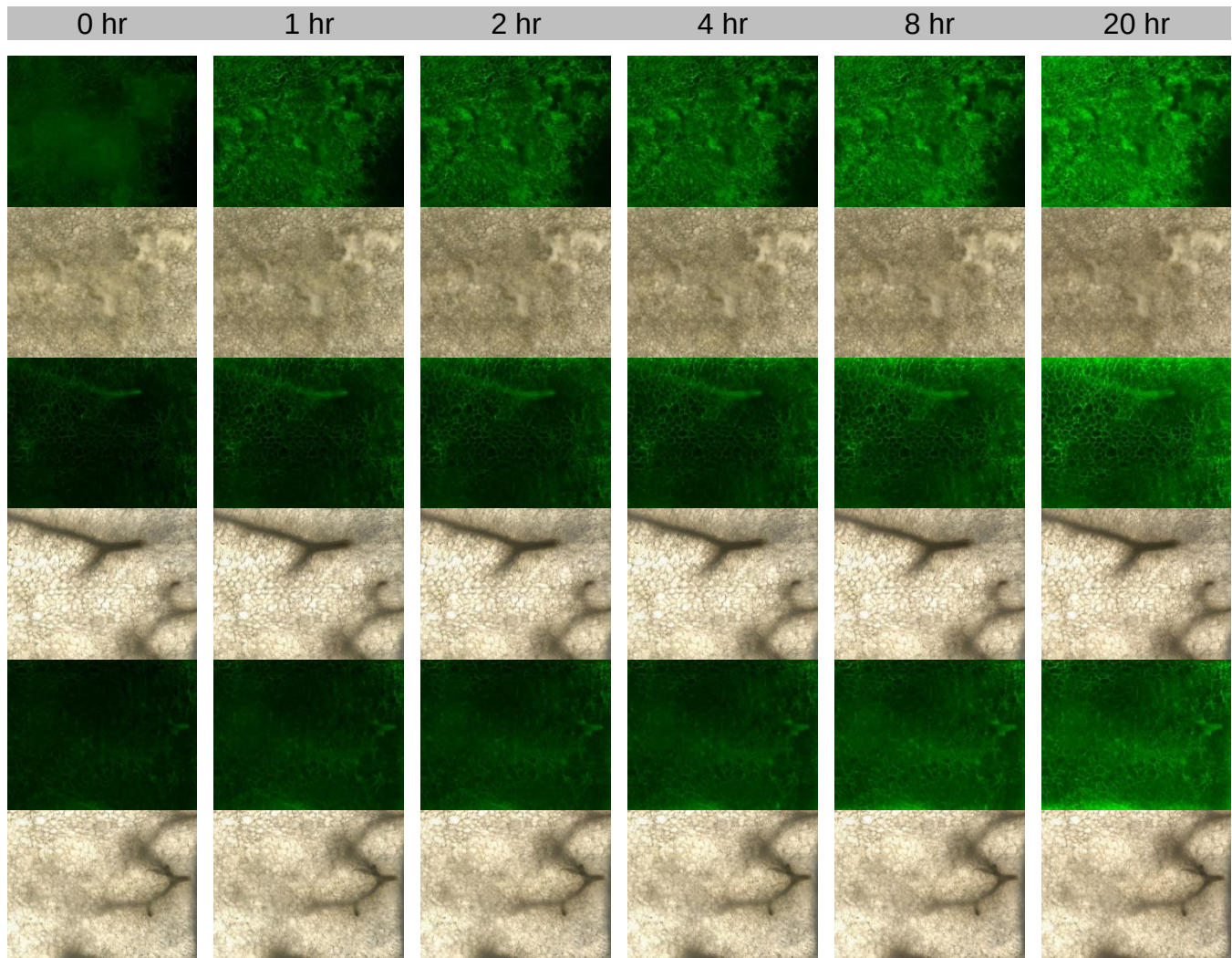


Supplementary Figure 36. Time-based imaging of Asian pear with 10 mM PZA supplementation.



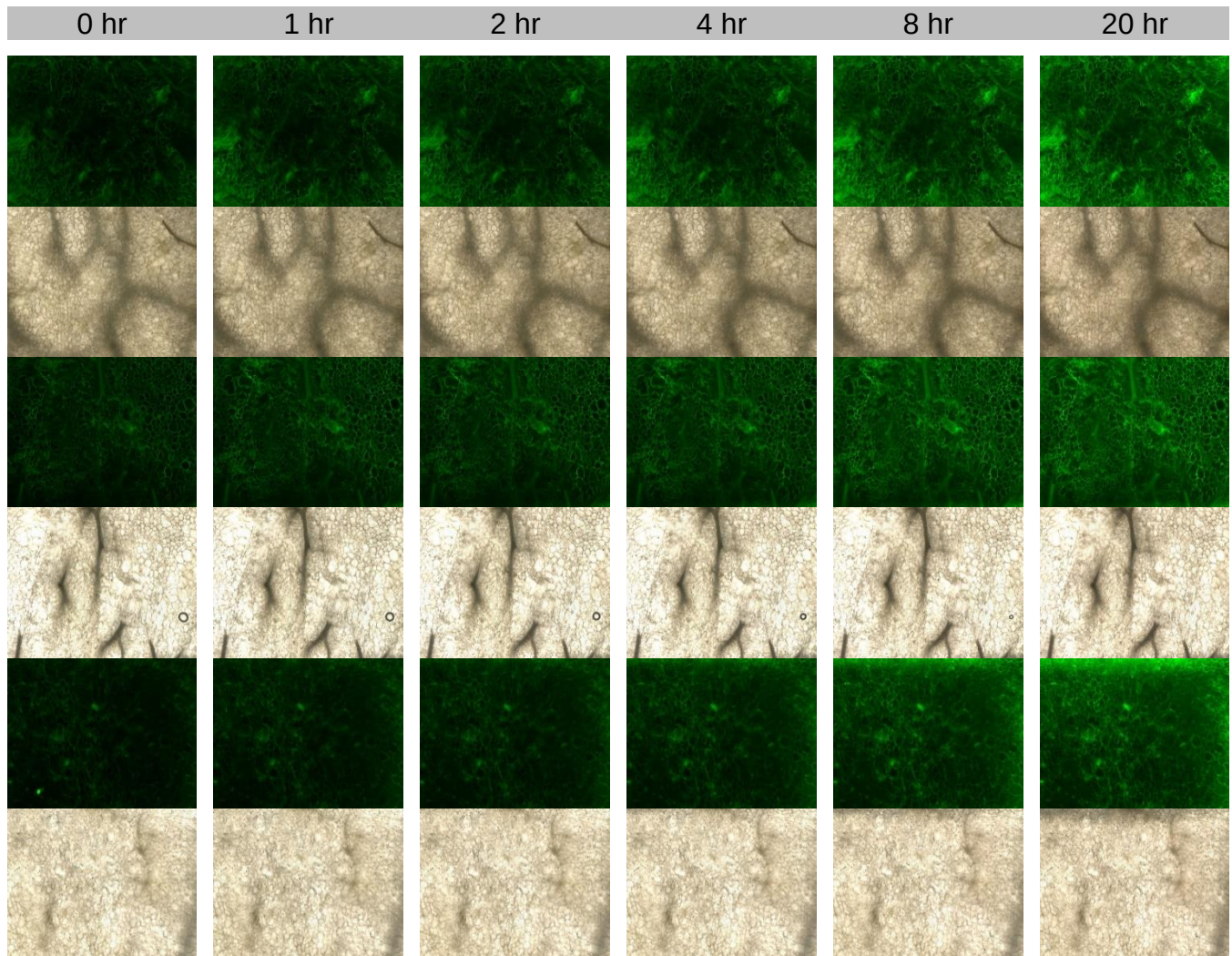
Supplementary Figure 37. Time-based imaging of muscat grape with 1 mM ACC supplementation.

fruit: Muscat grape
additive: ACC (10 mM)

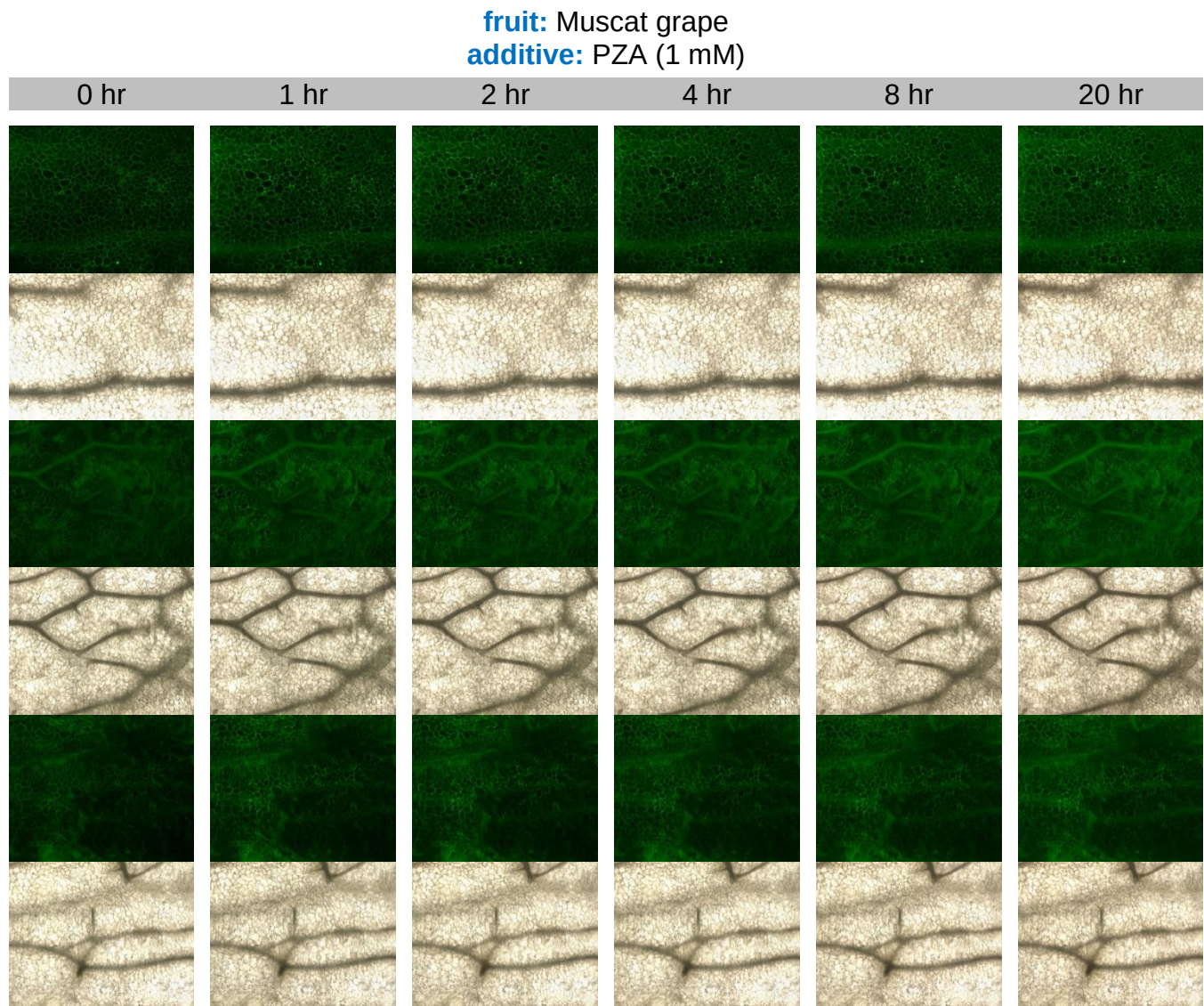


Supplementary Figure 38. Time-based imaging of muscat grape with 10 mM ACC supplementation.

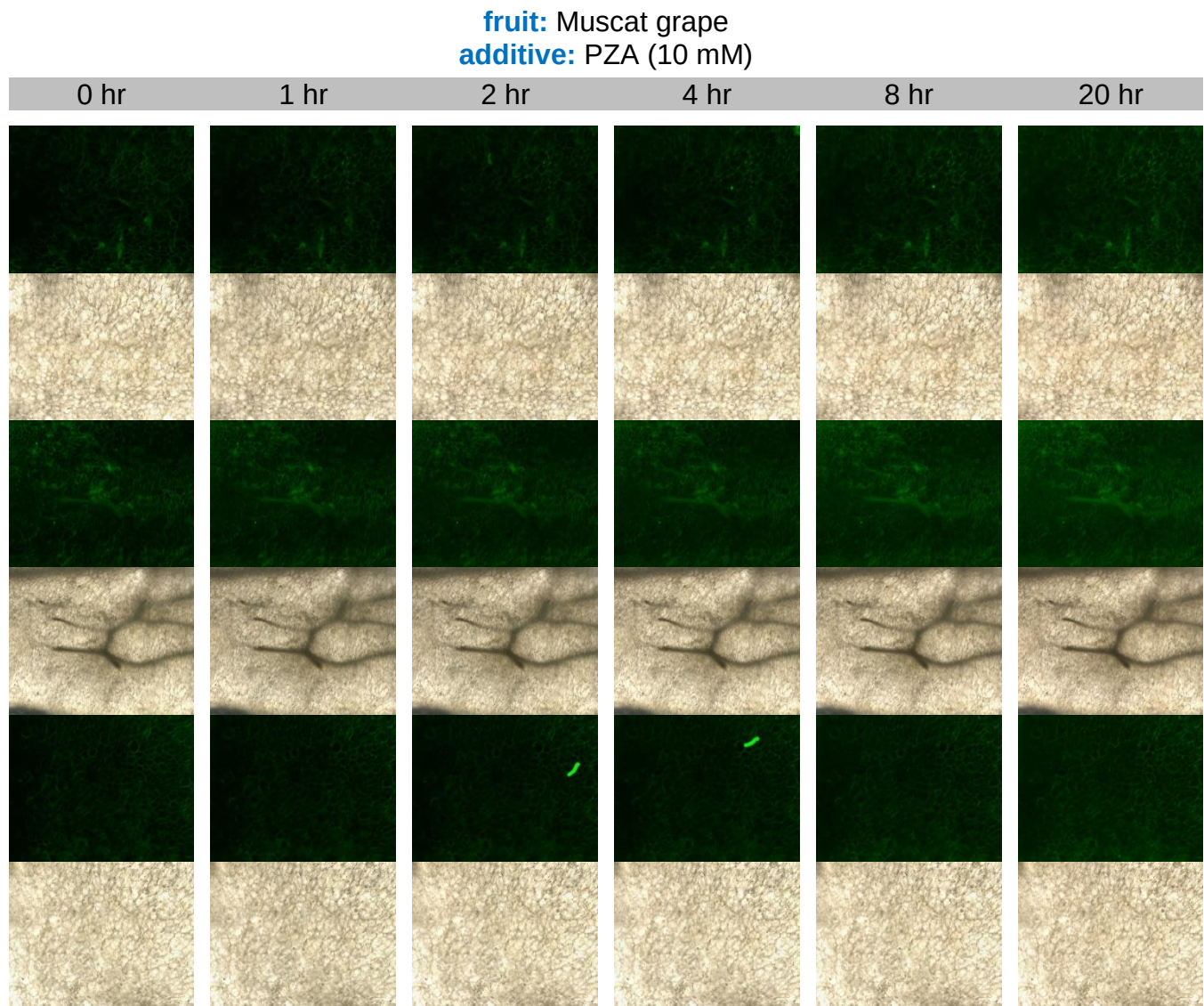
fruit: Muscat grape
additive: none (water)



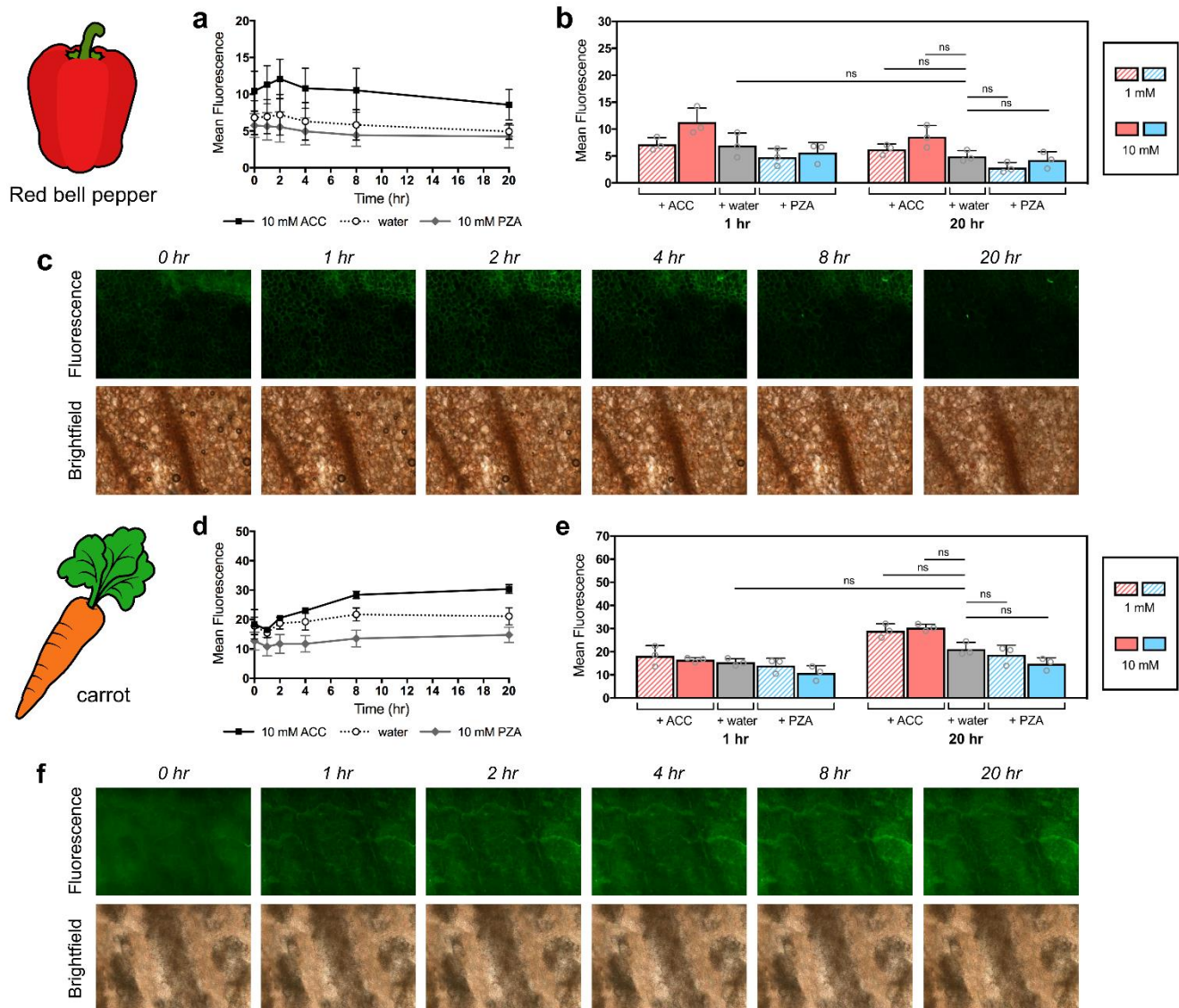
Supplementary Figure 39. Time-based imaging of muscat grape with no supplementation.



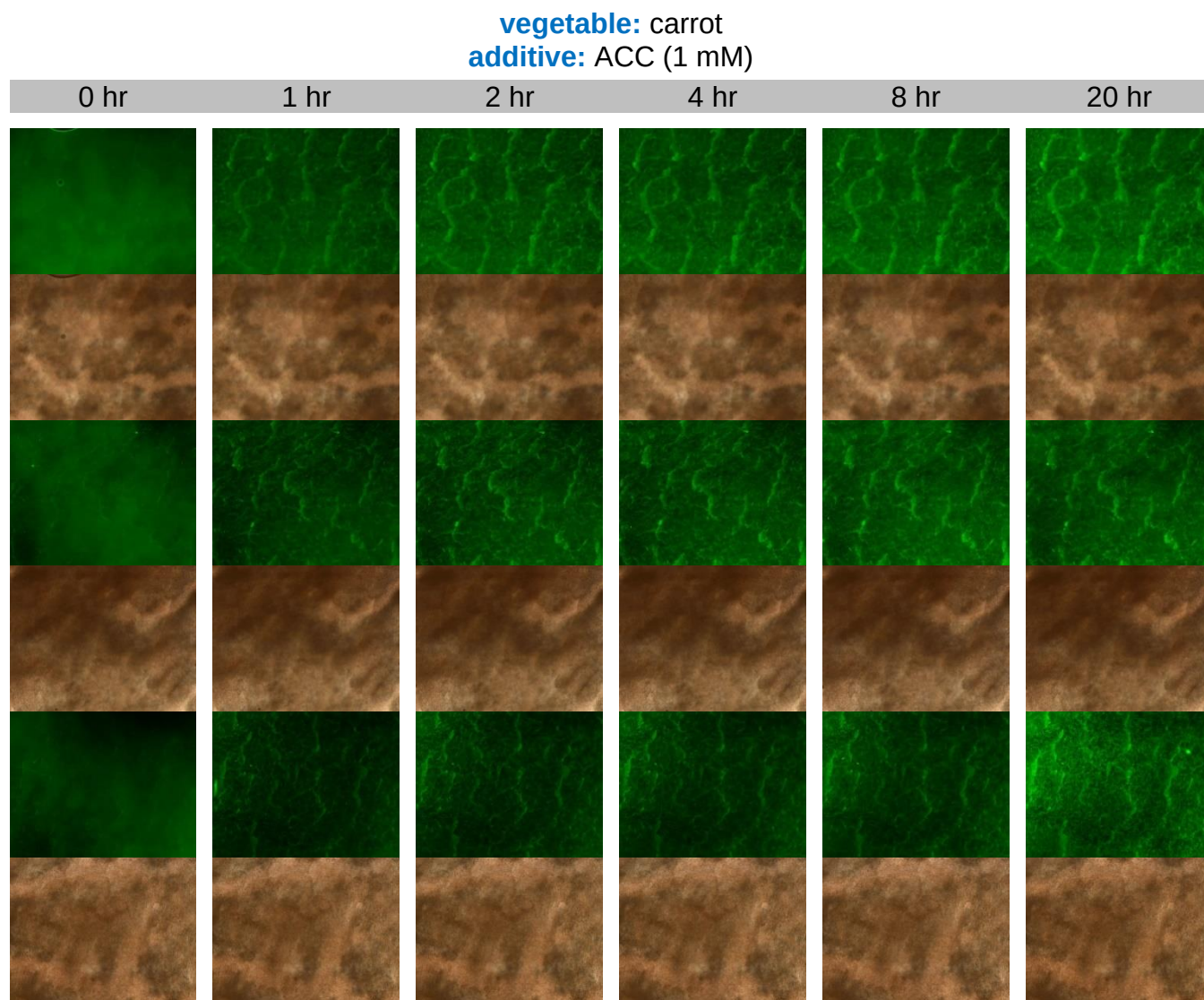
Supplementary Figure 40. Time-based imaging of muscat grape with 1 mM PZA supplementation.



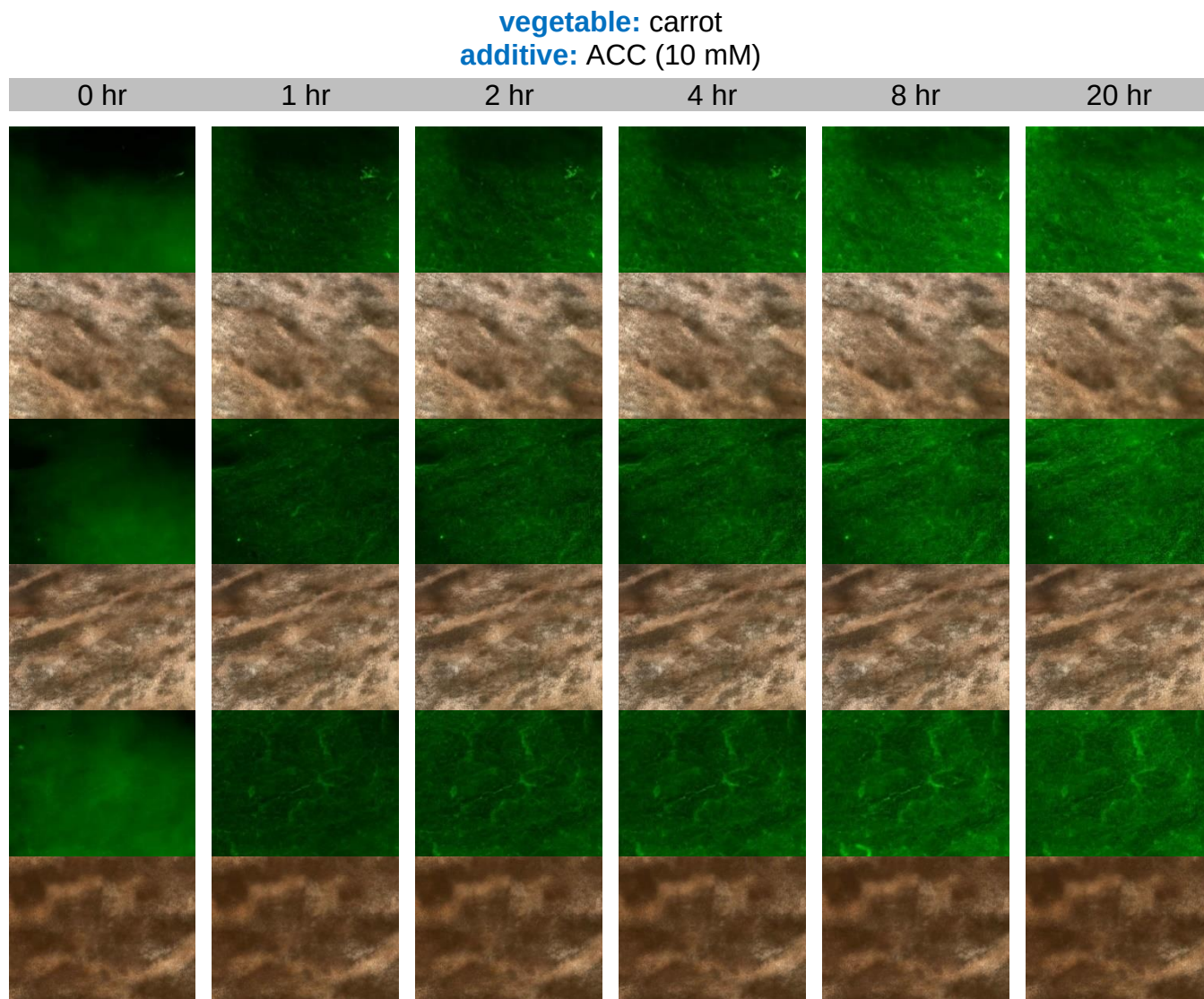
Supplementary Figure 41. Time-based imaging of muscat grape with 10 mM PZA supplementation.



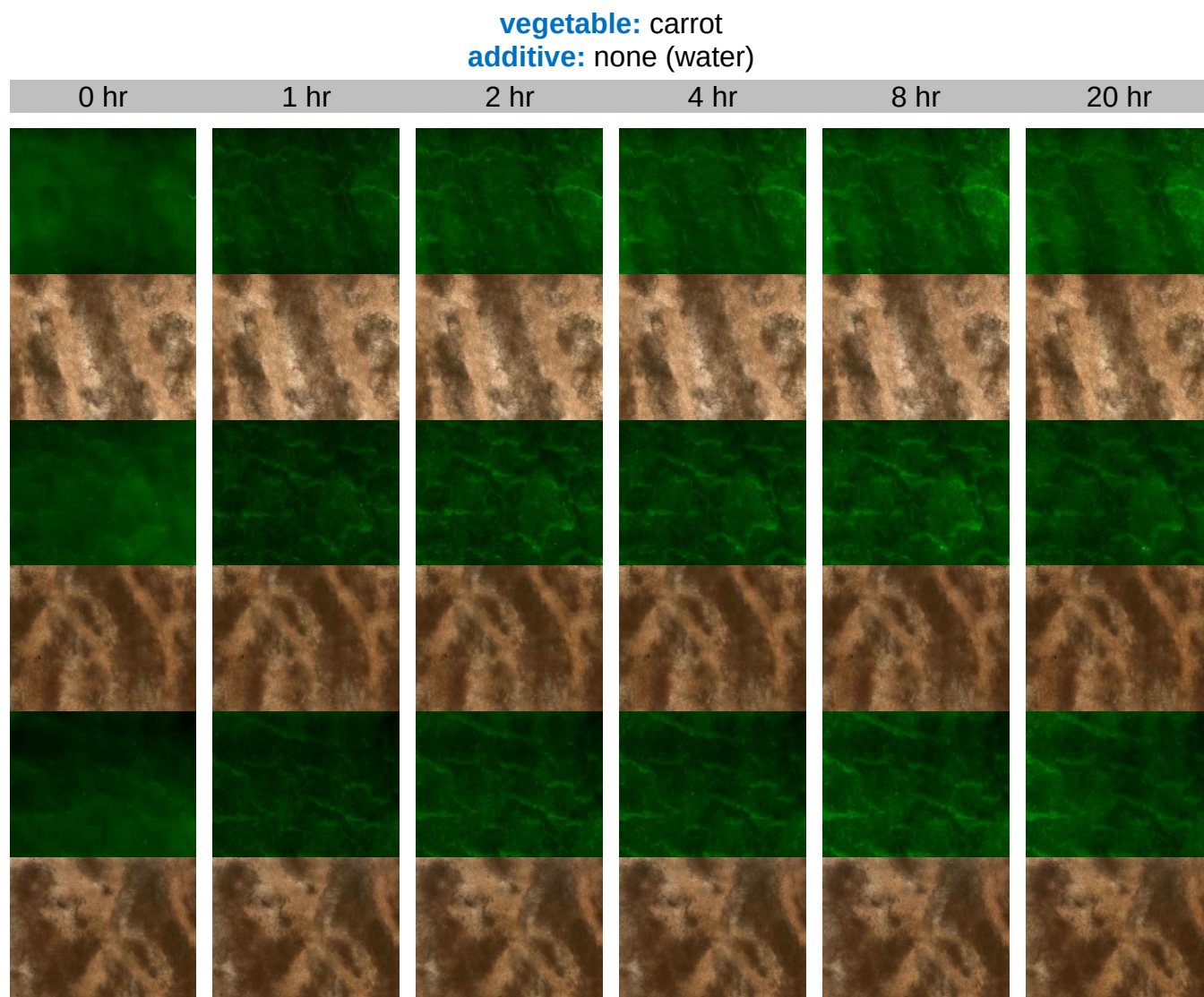
Supplementary Figure 42. Time-dependent detection of ethylene in vegetables. Fluorescence was monitored over time under various conditions (ACC or PZA) for red bell peppers (**a**) and carrots (**d**). A summary and statistical comparison between the 1 hr and 20 hr timepoints for each vegetable are shown (**b,e**), as well as samples images (water-control) over various timepoints (**c,f**). All values were obtained in triplicate. Statistical analysis was performed using a one-way ANOVA with Tukey's multiple comparisons test. * $P < 0.03$, ** $P < 0.002$, *** $P < 0.0002$, **** $P < 0.0001$, ns = not significant. Source data are provided as a Source Data file



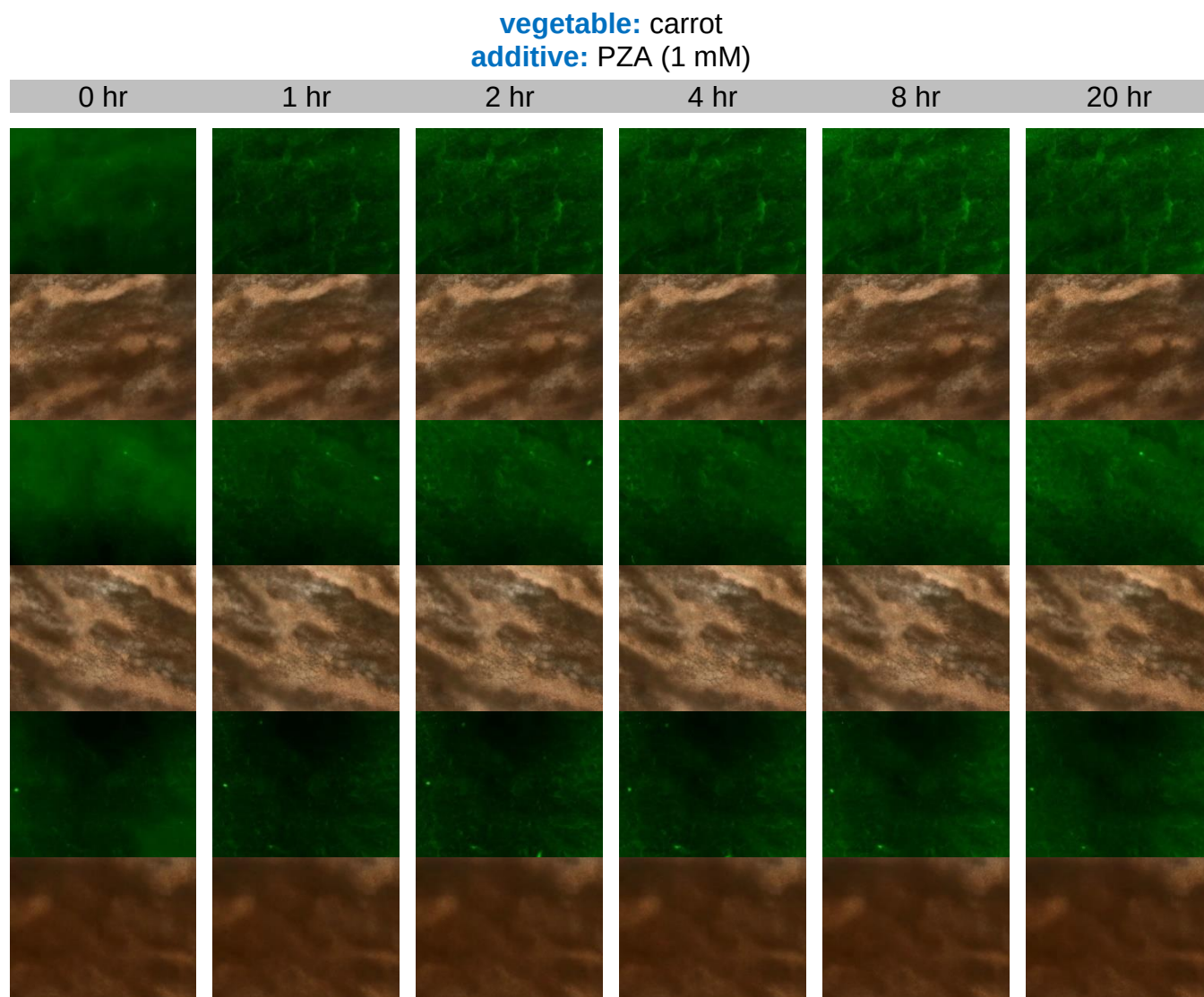
Supplementary Figure 43. Time-based imaging of carrot with 1 mM ACC supplementation.



Supplementary Figure 44. Time-based imaging of carrot with 10 mM ACC supplementation.

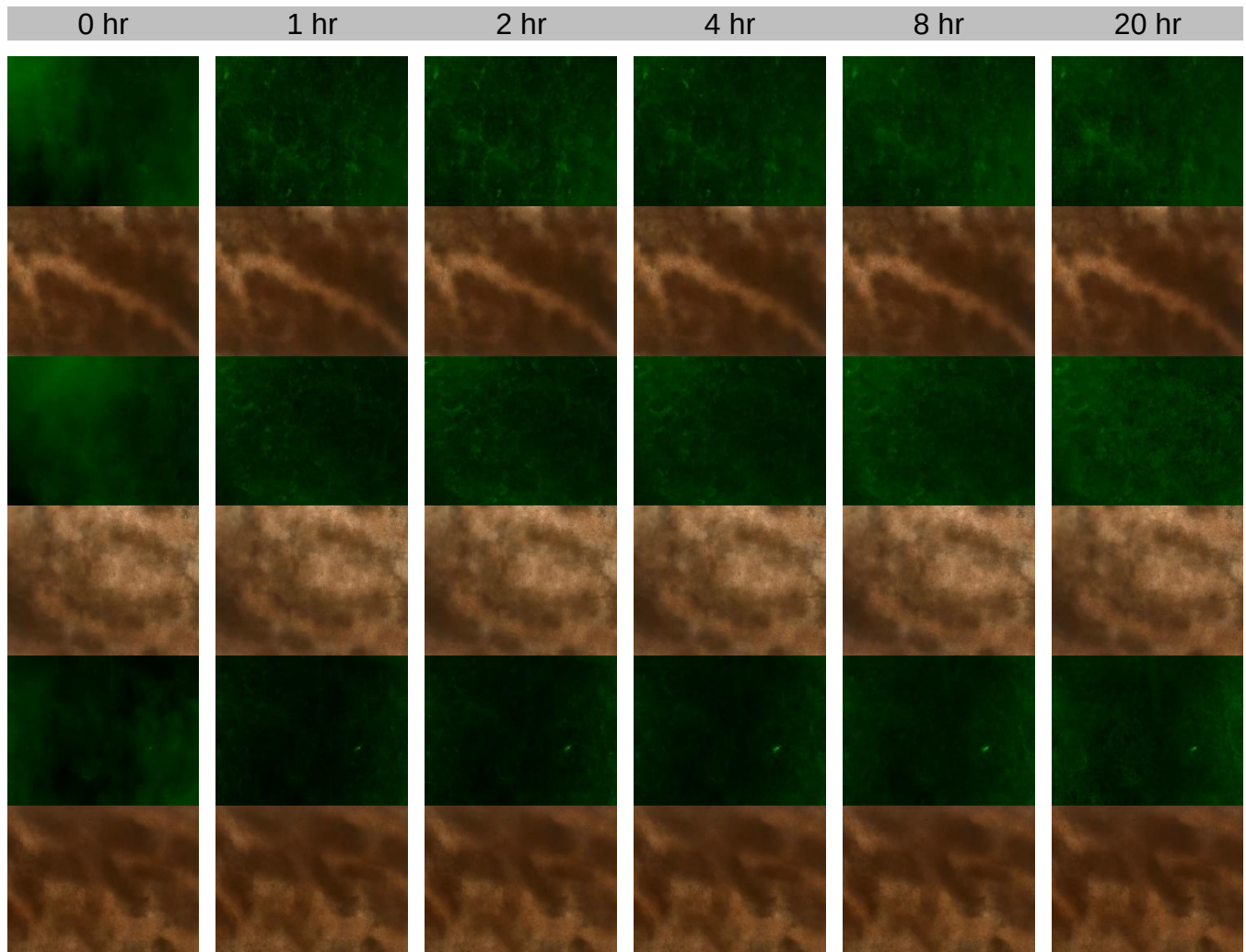


Supplementary Figure 45. Time-based imaging of carrot with no supplementation.



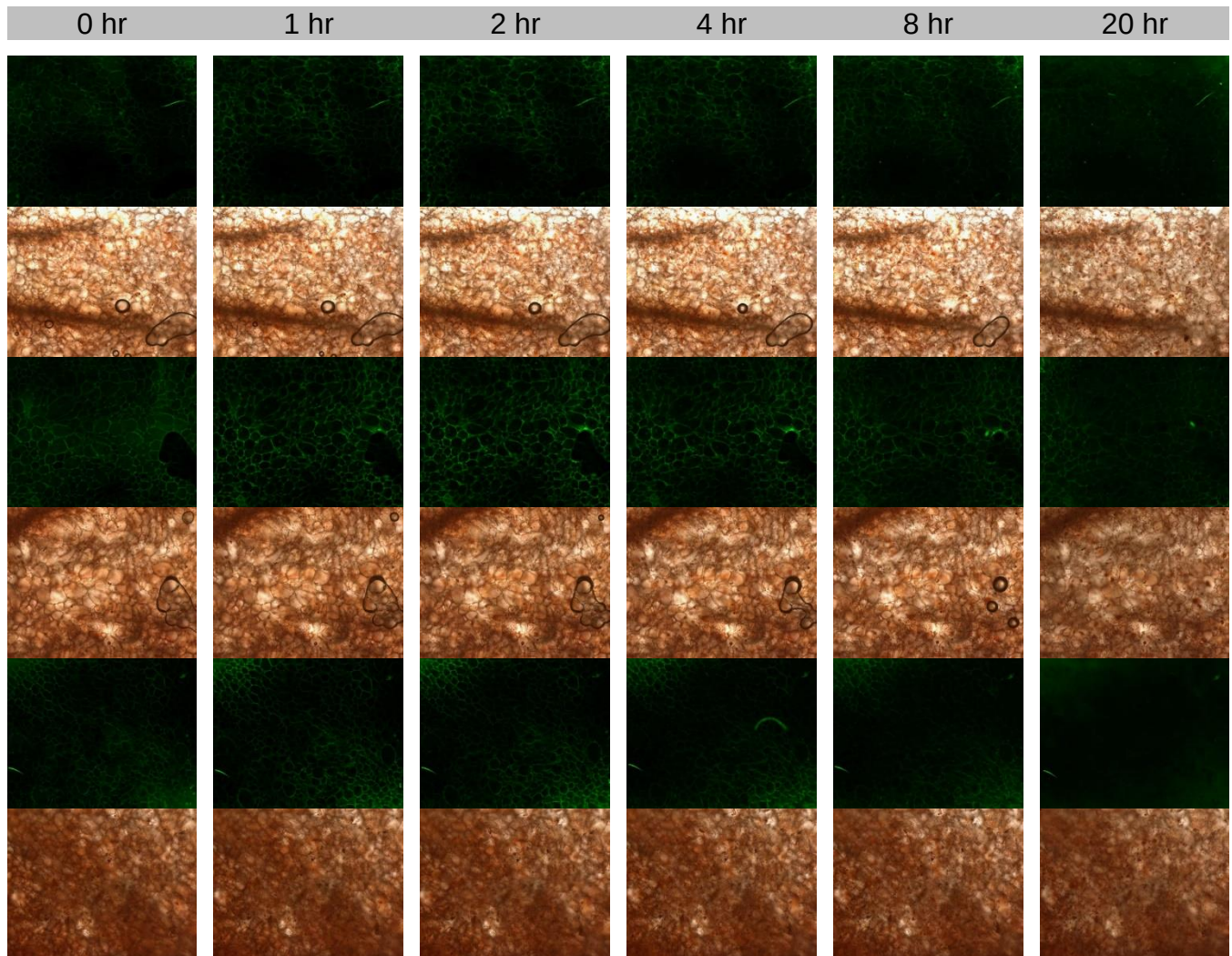
Supplementary Figure 46. Time-based imaging of carrot with 1 mM PZA supplementation.

vegetable: carrot
additive: PZA (10 mM)



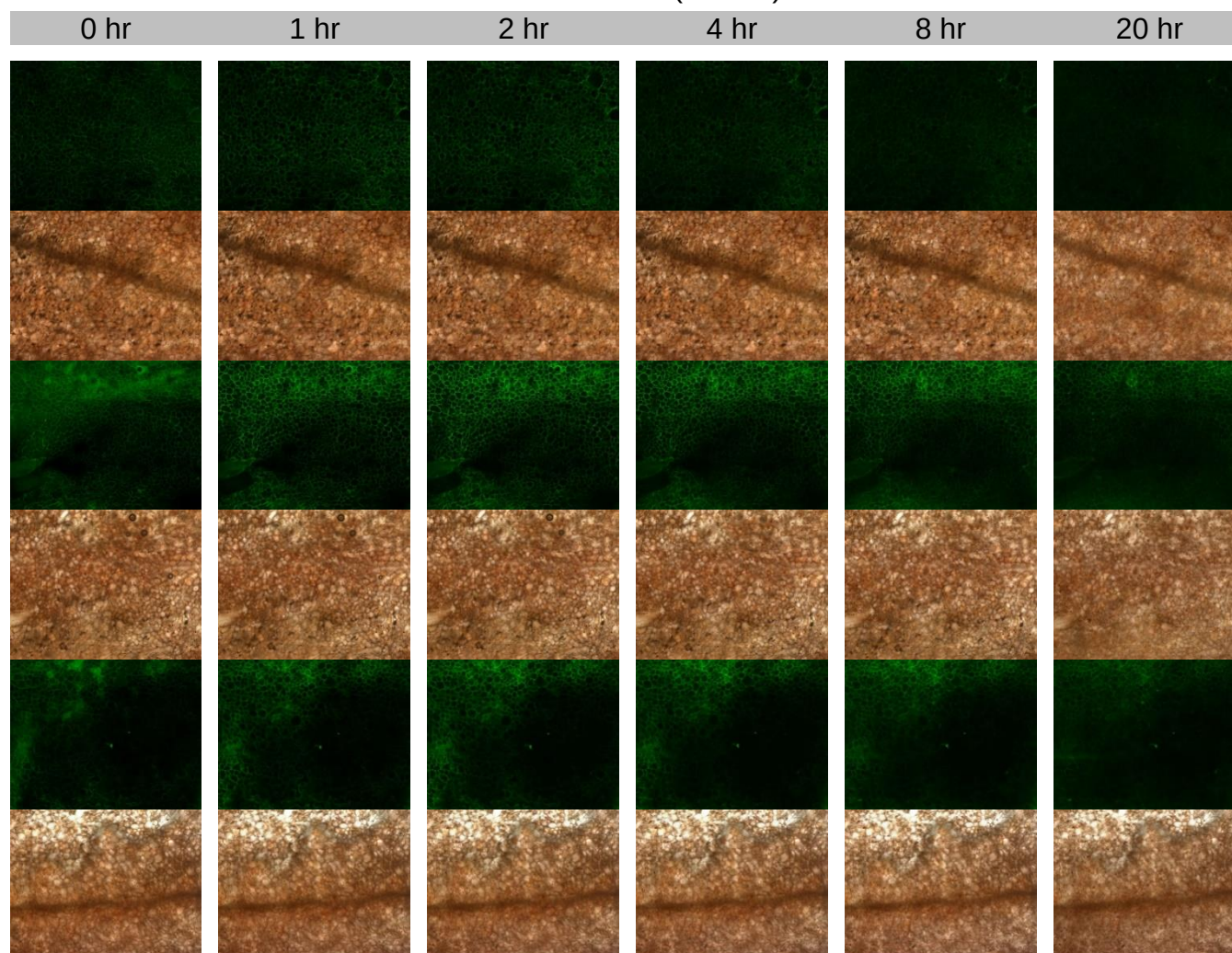
Supplementary Figure 47. Time-based imaging of carrot with 10 mM PZA supplementation.

vegetable: red bell pepper
additive: ACC (1 mM)



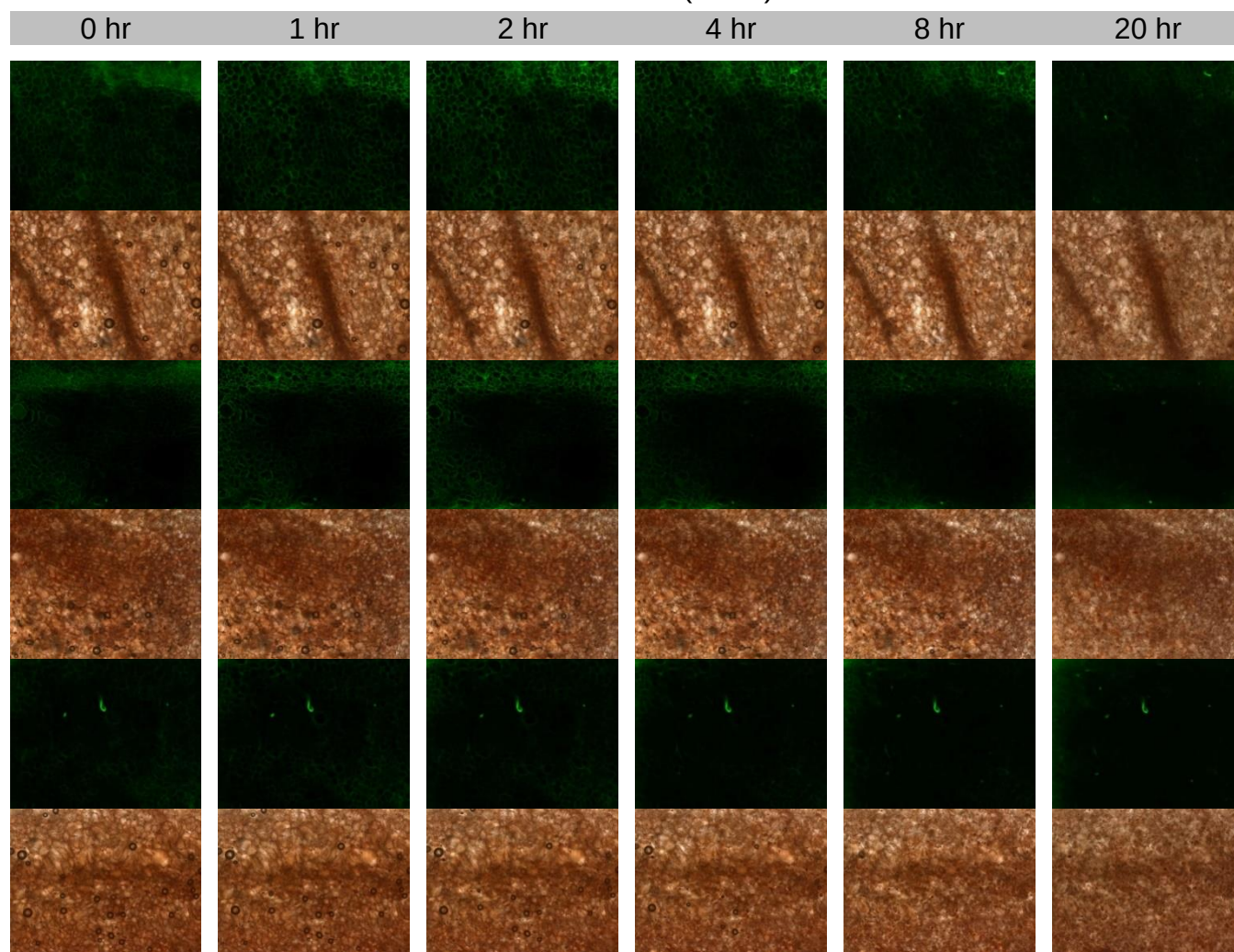
Supplementary Figure 48. Time-based imaging of red bell pepper with 1 mM ACC supplementation.

vegetable: red bell pepper
additive: ACC (10 mM)



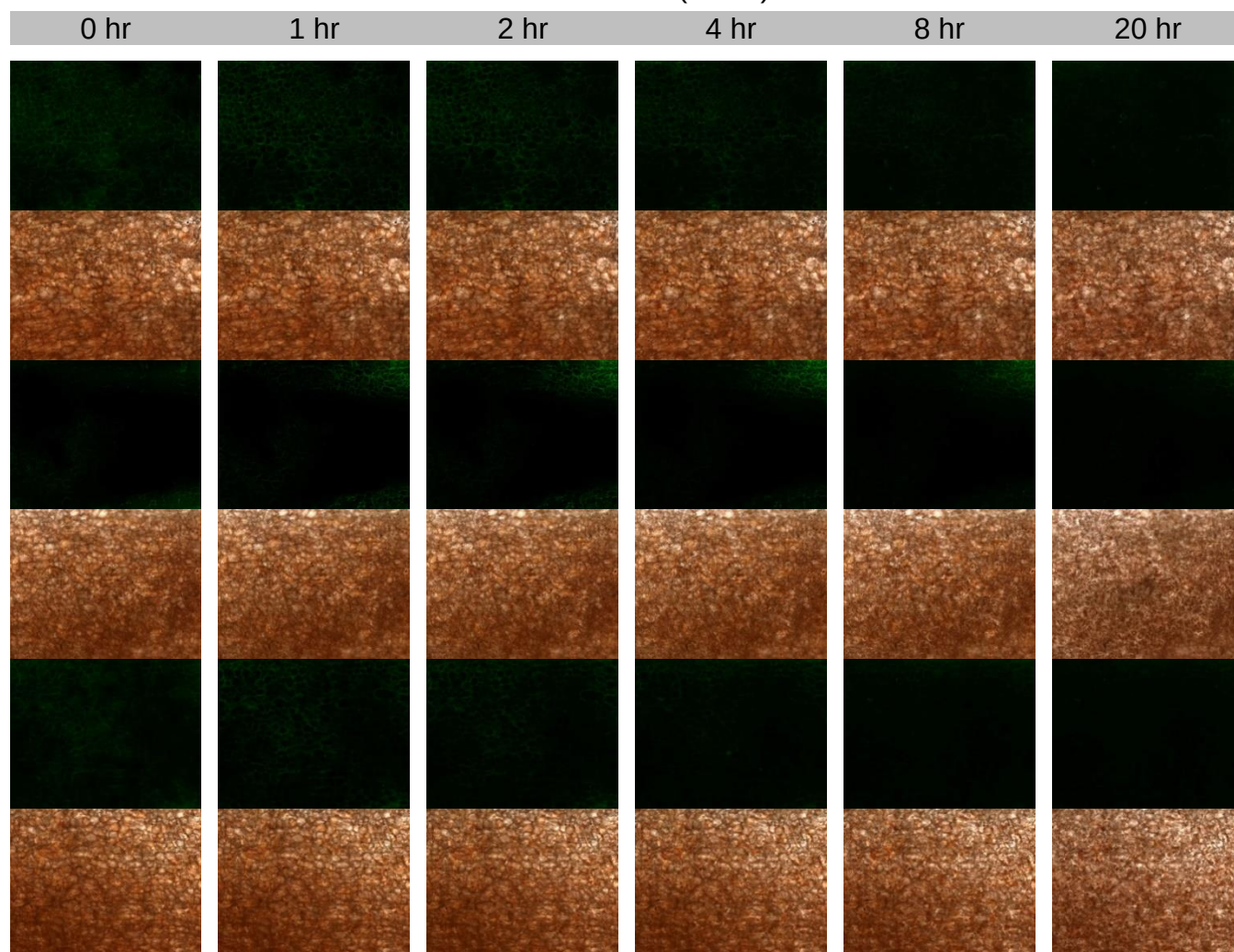
Supplementary Figure 49. Time-based imaging of red bell pepper with 10 mM ACC supplementation.

vegetable: red bell pepper
additive: none (water)



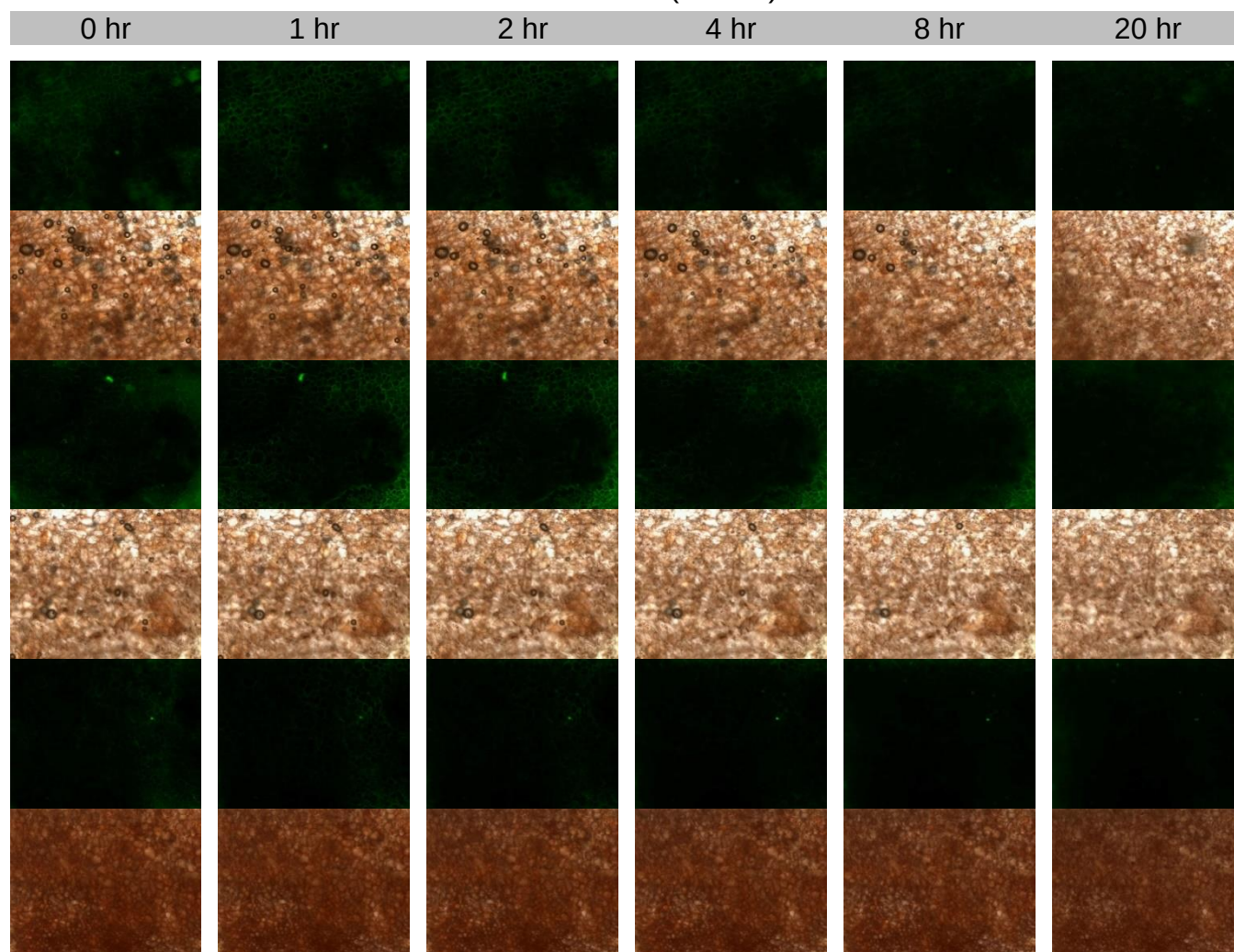
Supplementary Figure 50. Time-based imaging of red bell pepper with no supplementation.

vegetable: red bell pepper
additive: PZA (1 mM)



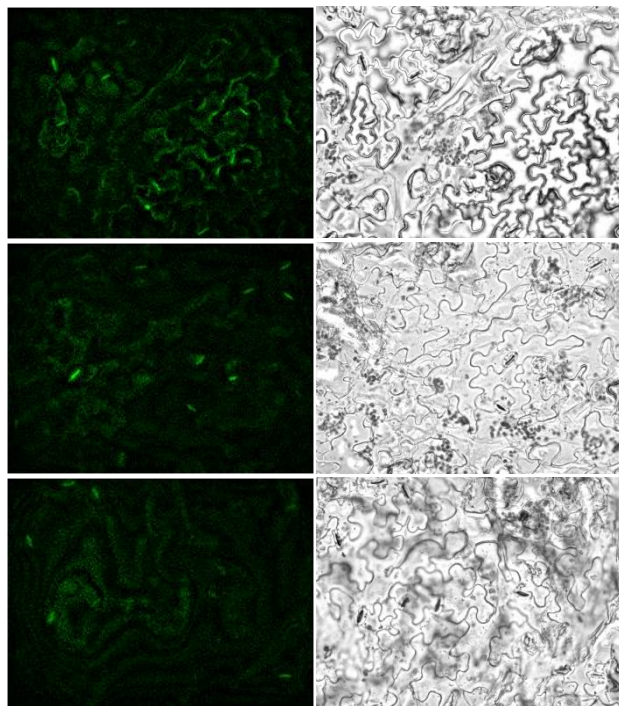
Supplementary Figure 51. Time-based imaging of red bell pepper with 1 mM PZA supplementation.

vegetable: red bell pepper
additive: PZA (10 mM)

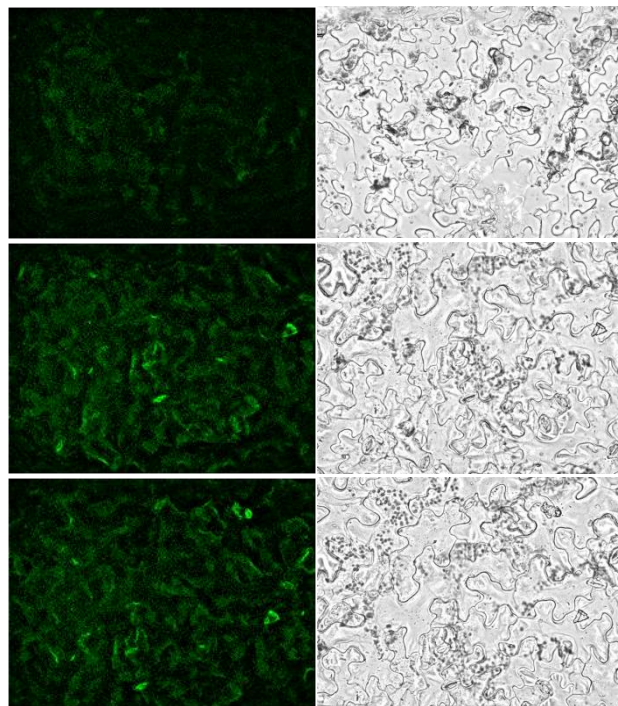


Supplementary Figure 52. Time-based imaging of red bell pepper with 10 mM PZA supplementation.

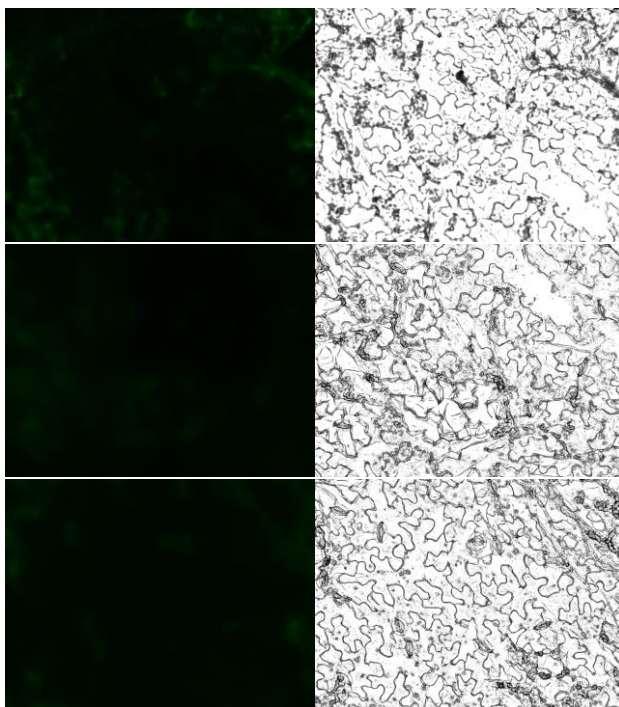
plant: Col-0
additive: none



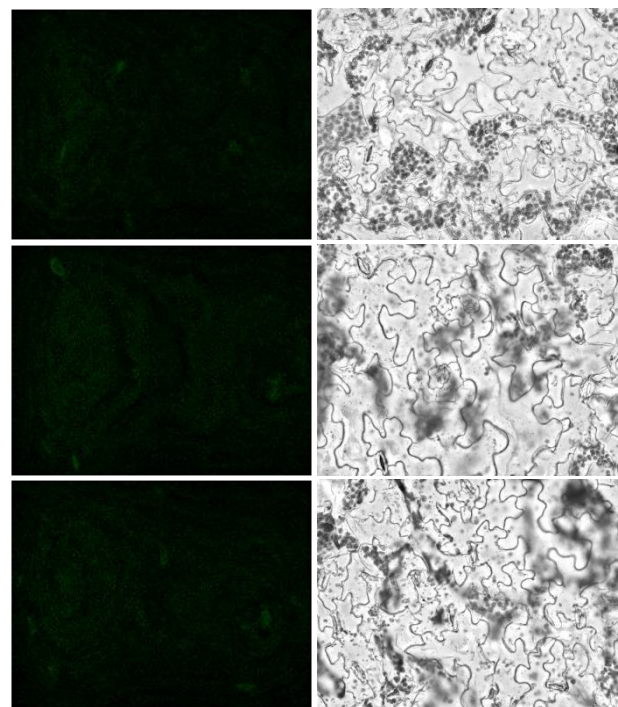
plant: Col-0
additive: ACC (1 mM)



plant: *eto1-1/ckrc1-1*
additive: none

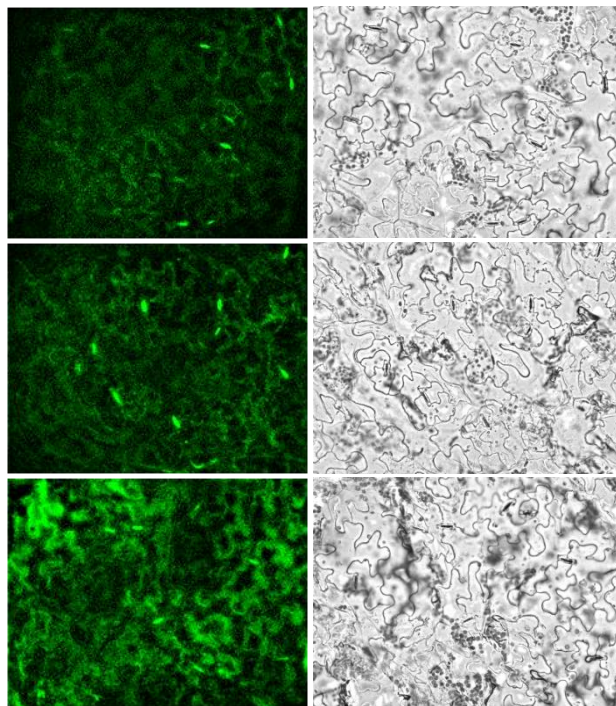


plant: *acs1/2/6/4/5/9/7/11*
additive: none

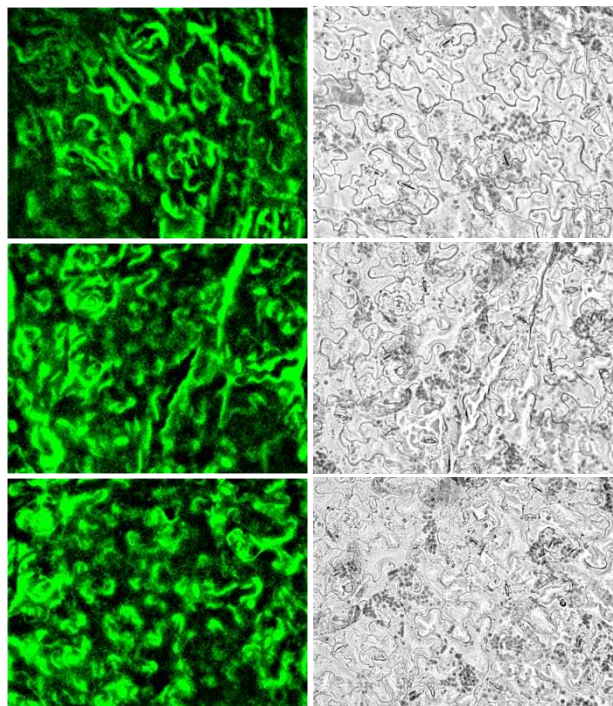


Supplementary Figure 53. Imaging (40× magnification) of epidermal peels treated without **AEP** probe for various strains of *A.thaliana* tissues under various conditions.

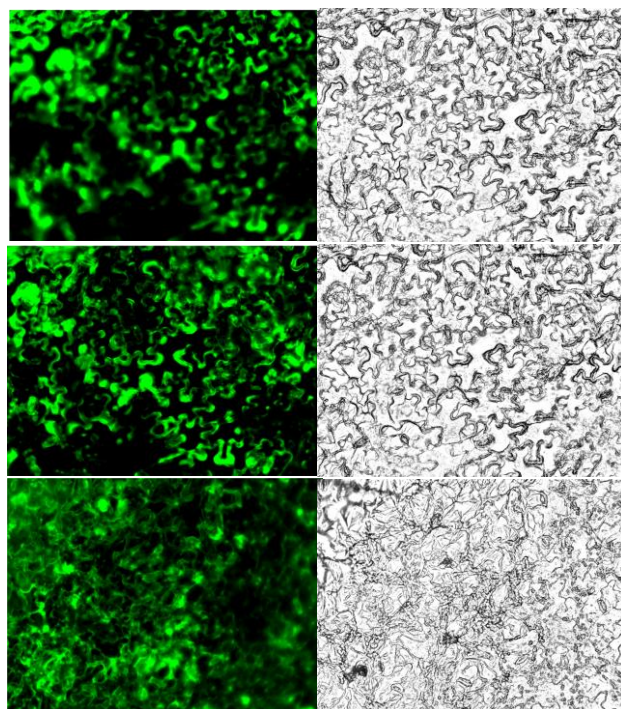
plant: Col-0
additive: none



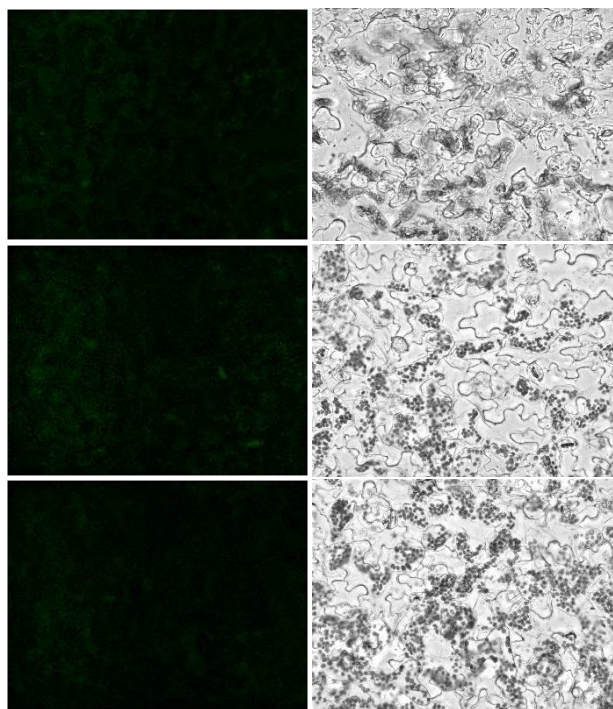
plant: Col-0
additive: ACC (1 mM)



plant: *eto1-1/ckrc1-1*
additive: none

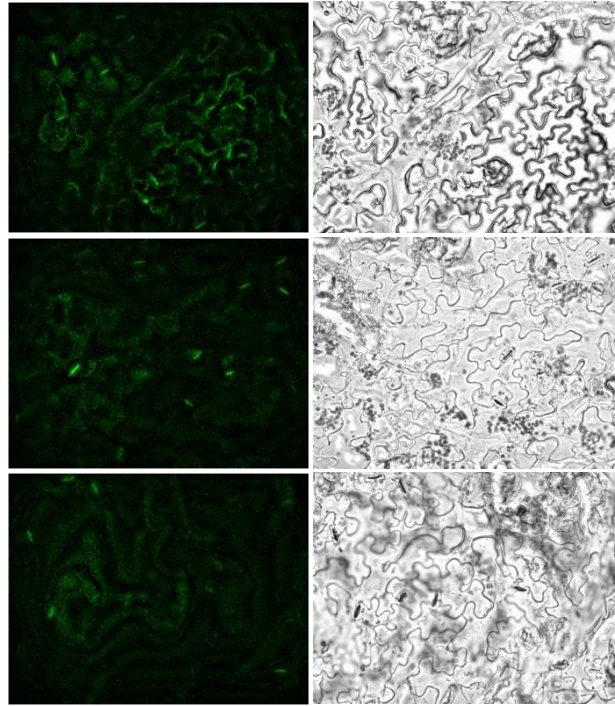


plant: *acs1/2/6/4/5/9/7/11*
additive: none

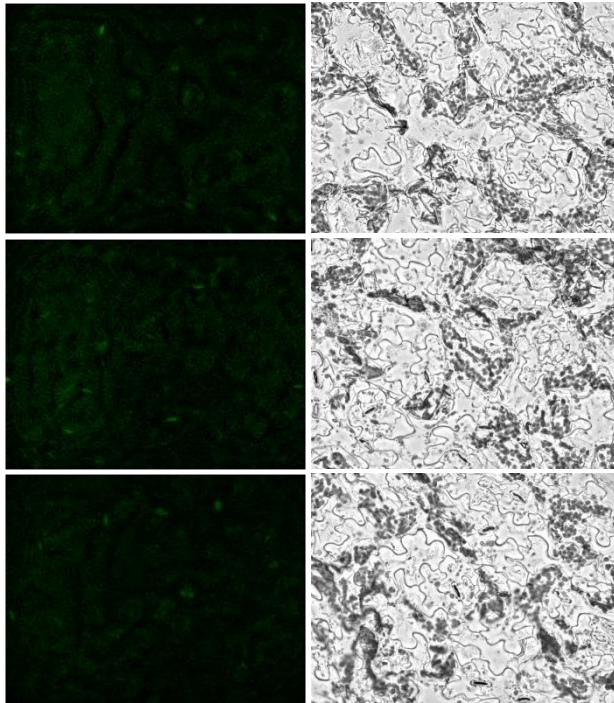


Supplementary Figure 54. Imaging (40× magnification) of epidermal peels treated with **AEP** probe for various strains of *A.thaliana* tissues under various conditions.

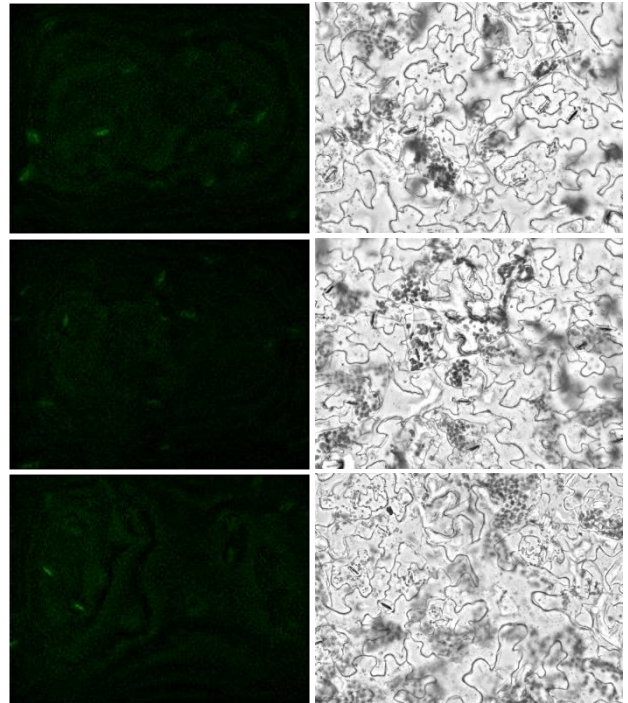
additive: none



additive: flg22 (4.8 μ M)

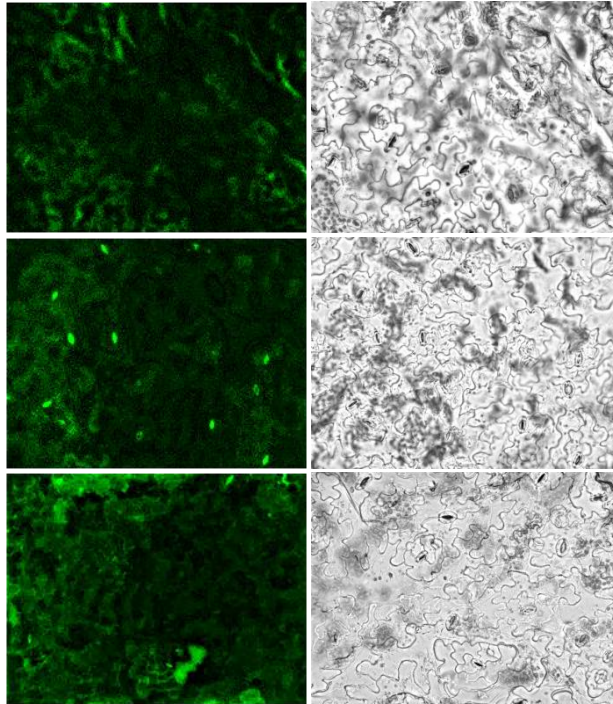


additive: elf18 (4.8 μ M)

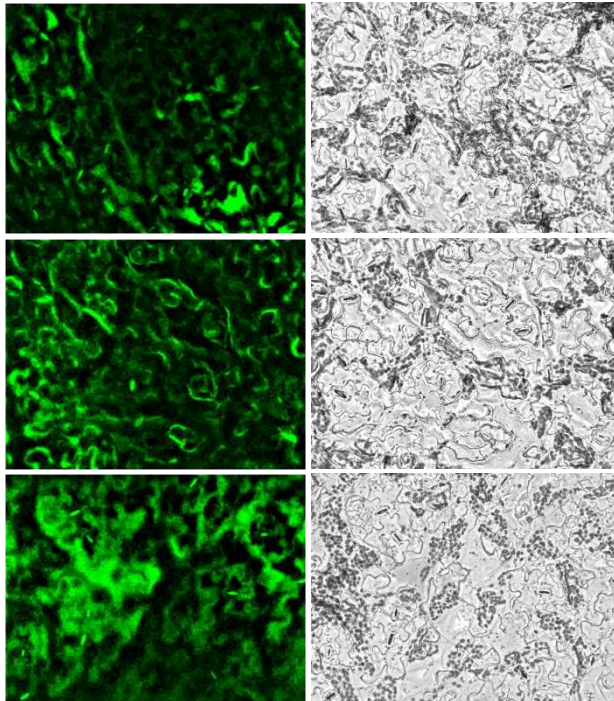


Supplementary Figure 55. Imaging (40 $\times$ magnification) of epidermal peels treated without **AEP** probe for *A.thaliana* Col-0 tissues incubated in the presence and absence of PAMPs.

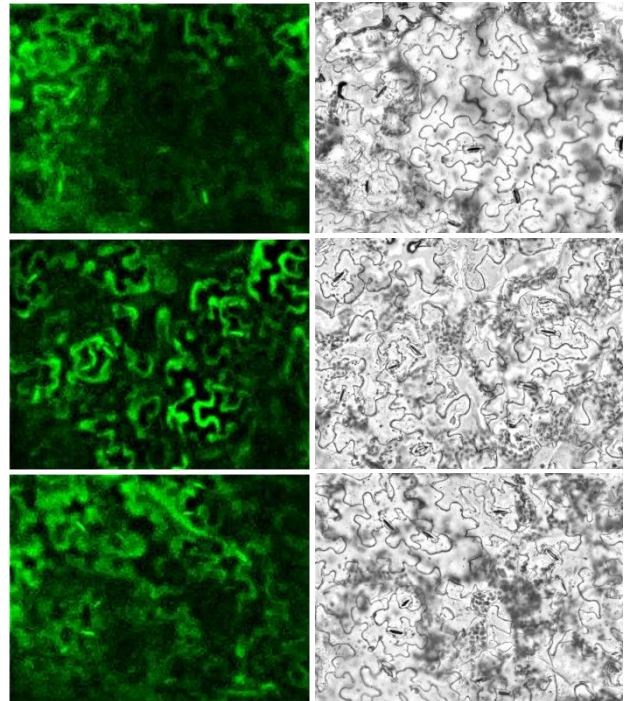
additive: none



additive: flg22 (4.8 μ M)

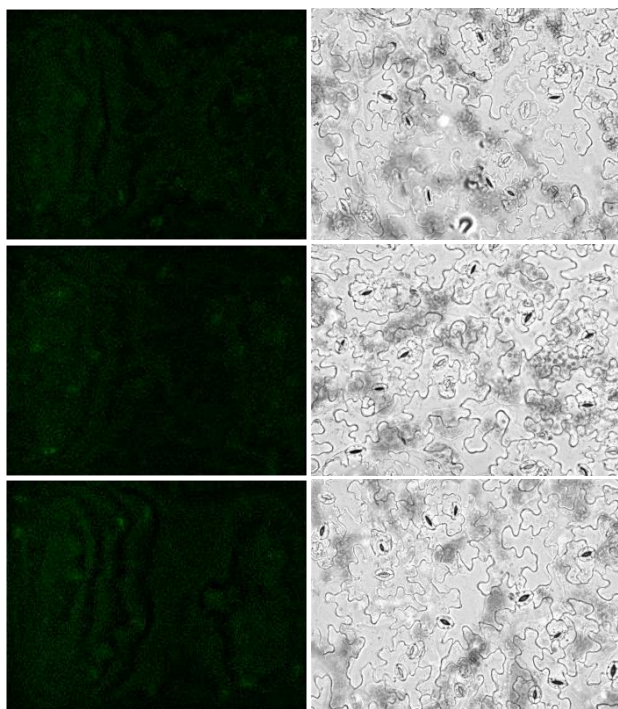


additive: elf18 (4.8 μ M)

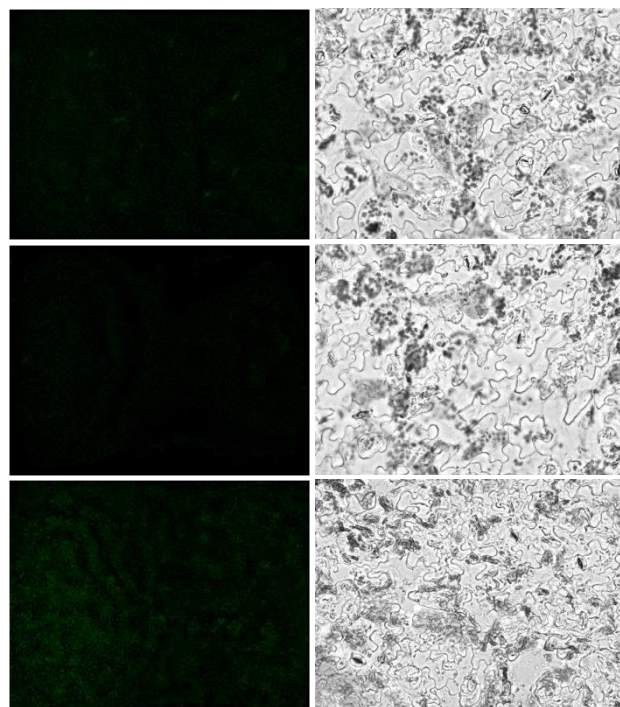


Supplementary Figure 56. Imaging (40 $\times$ magnification) of epidermal peels treated with **AEP** probe for *A.thaliana* Col-0 tissues incubated in the presence and absence of PAMPs.

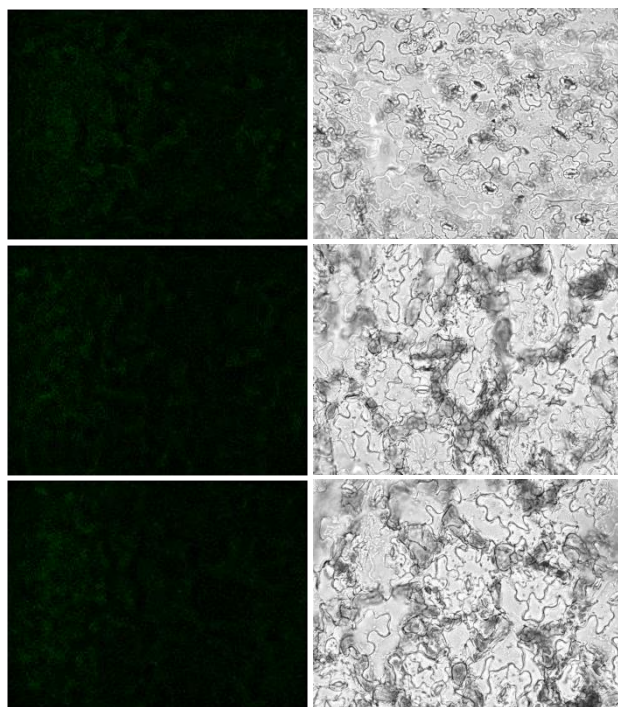
additive: none



additive: flg22 (4.8 μ M)

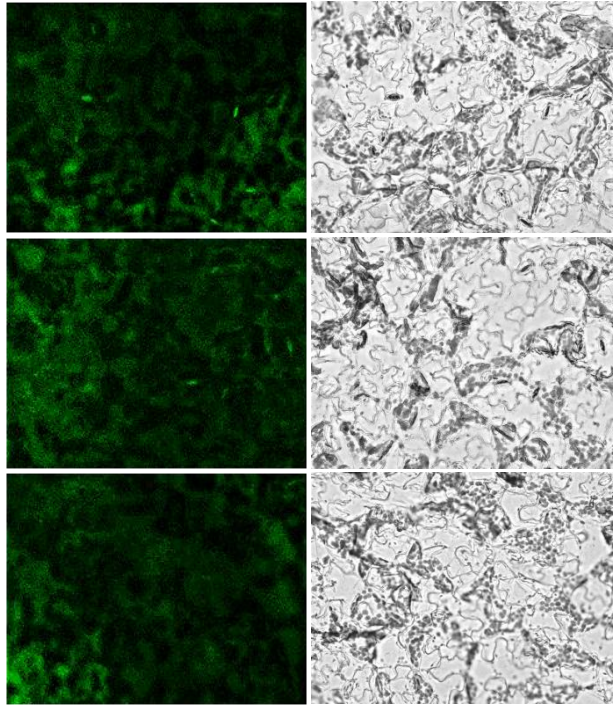


additive: elf18 (4.8 μ M)

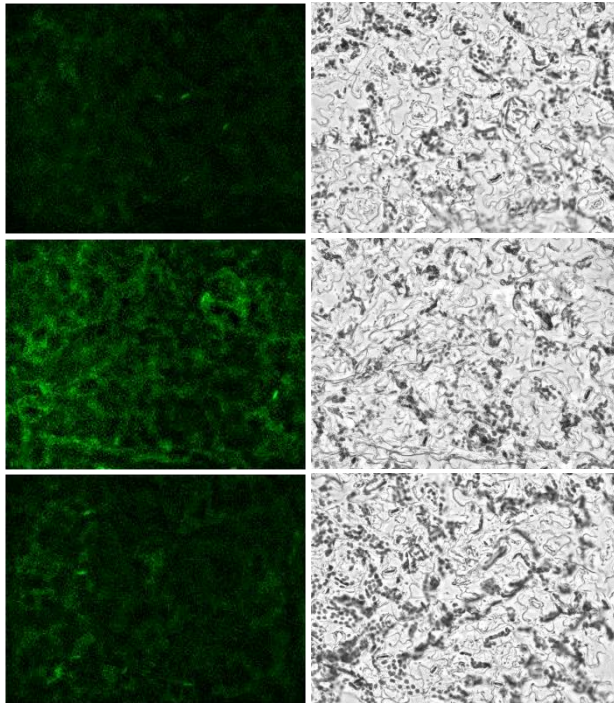


Supplementary Figure 57. Imaging (40 $\times$ magnification) of epidermal peels treated without **AEP** probe for *A.thaliana fls2/efr/cerk1* tissues incubated in the presence and absence of PAMPs.

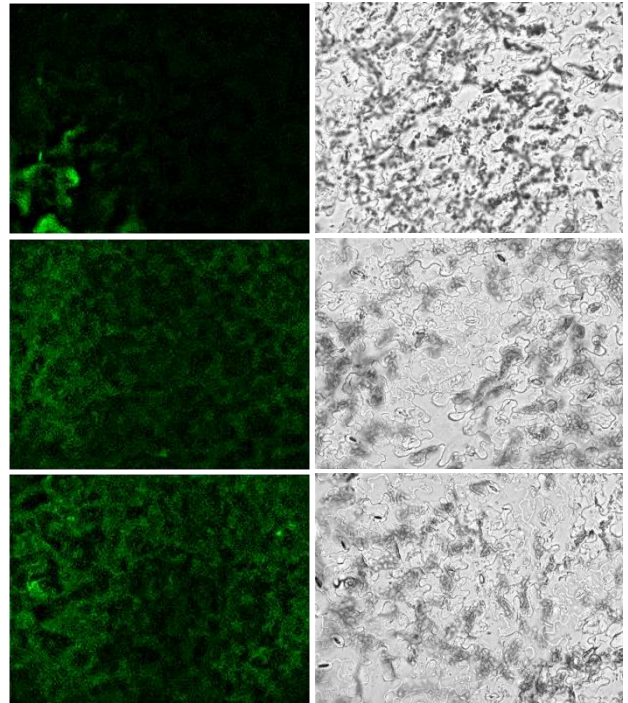
additive: none



additive: flg22 (4.8 μ M)

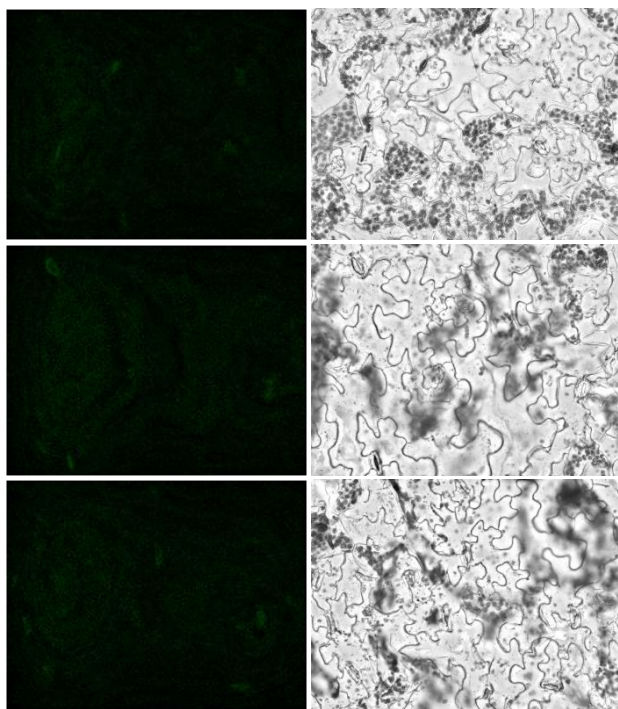


additive: elf18 (4.8 μ M)

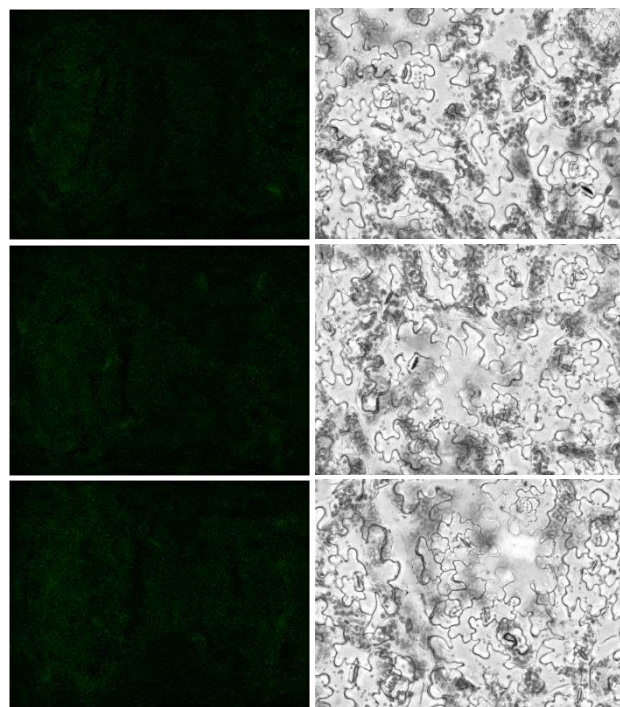


Supplementary Figure 58. Imaging (40 $\times$ magnification) of epidermal peels treated with **AEP** probe for *A.thaliana fls2/efr/cerk1* tissues incubated in the presence and absence of PAMPs.

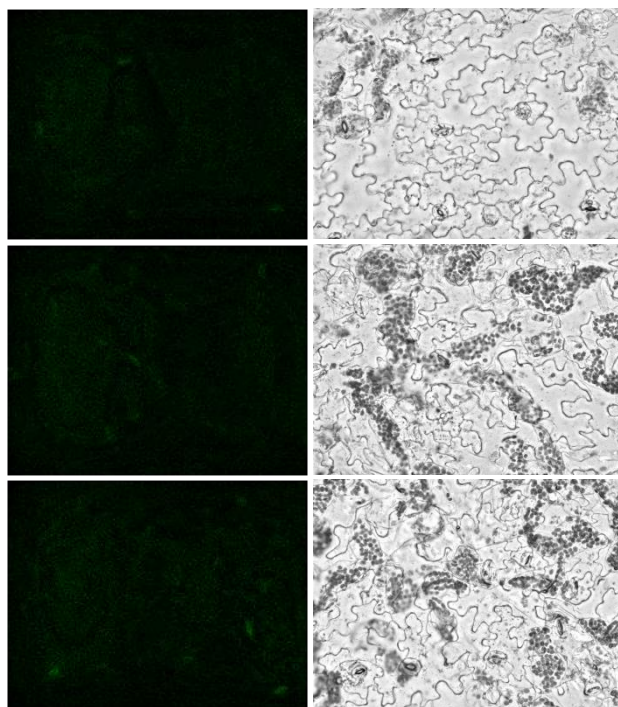
additive: none



additive: flg22 (4.8 μ M)

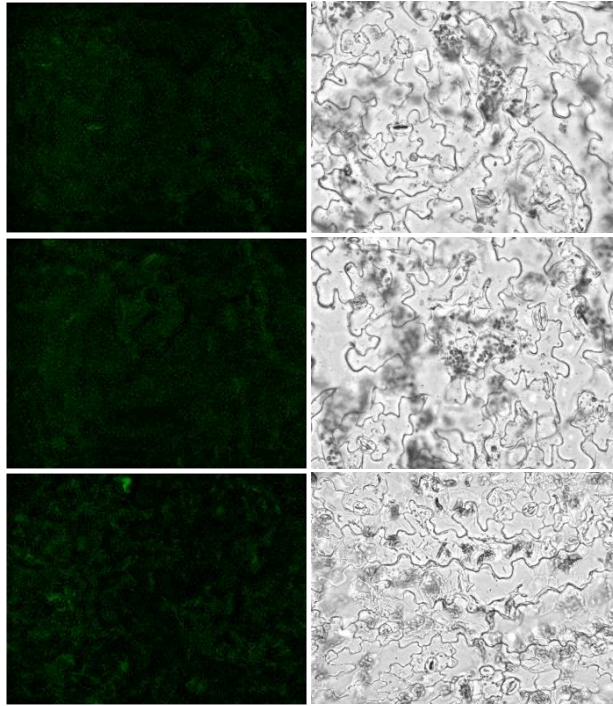


additive: elf18 (4.8 μ M)

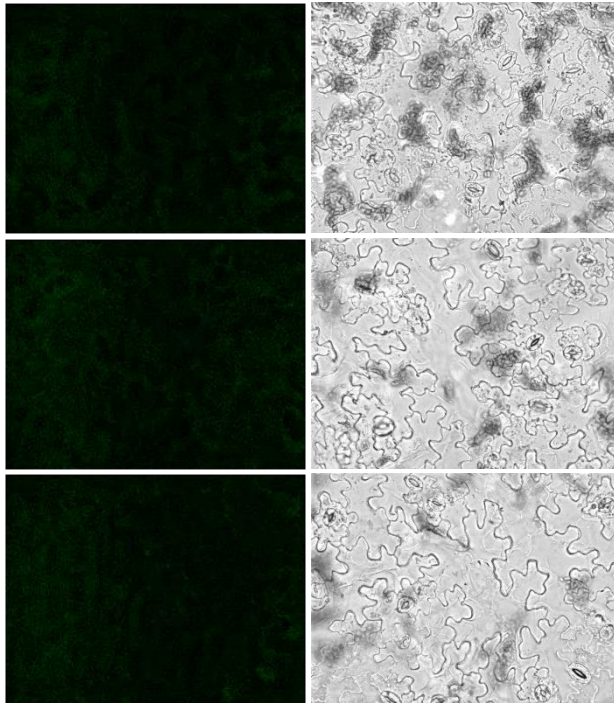


Supplementary Figure 59. Imaging (40 $\times$ magnification) of epidermal peels treated without **AEP** probe for *A.thaliana* *acs1/2/6/4/5/9/7/11* tissues incubated in the presence and absence of PAMPs.

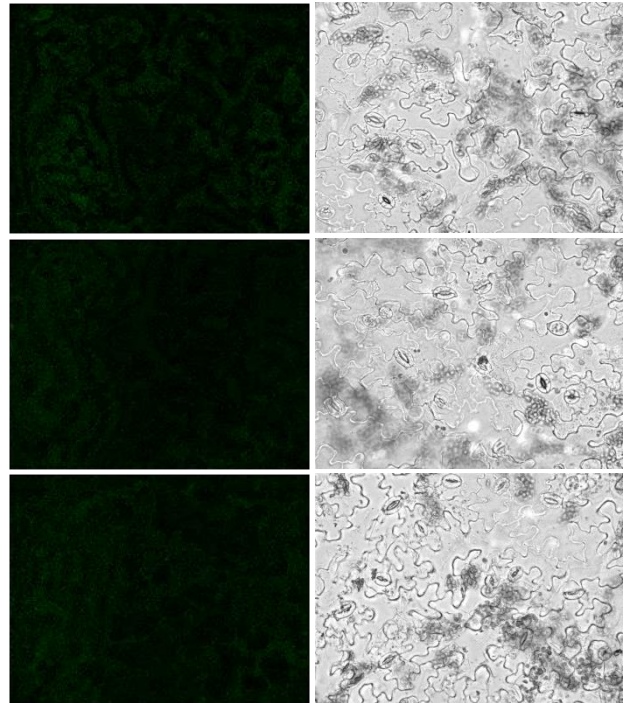
additive: none



additive: flg22 (4.8 μ M)

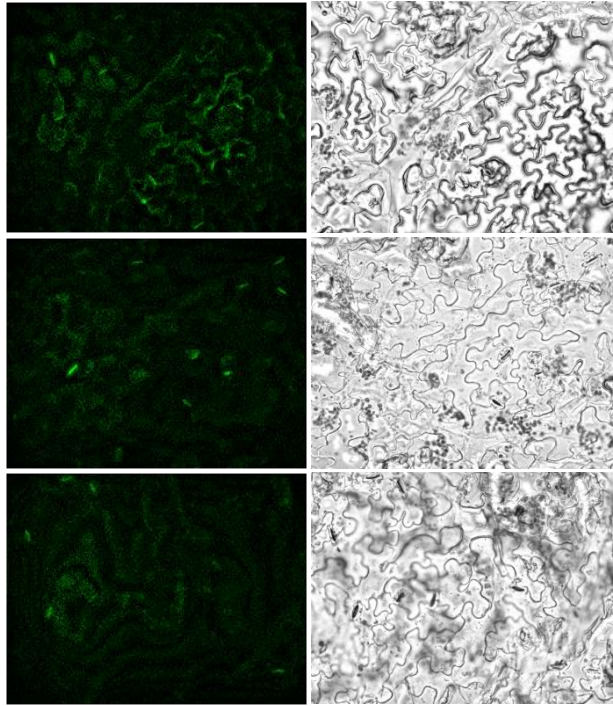


additive: elf18 (4.8 μ M)

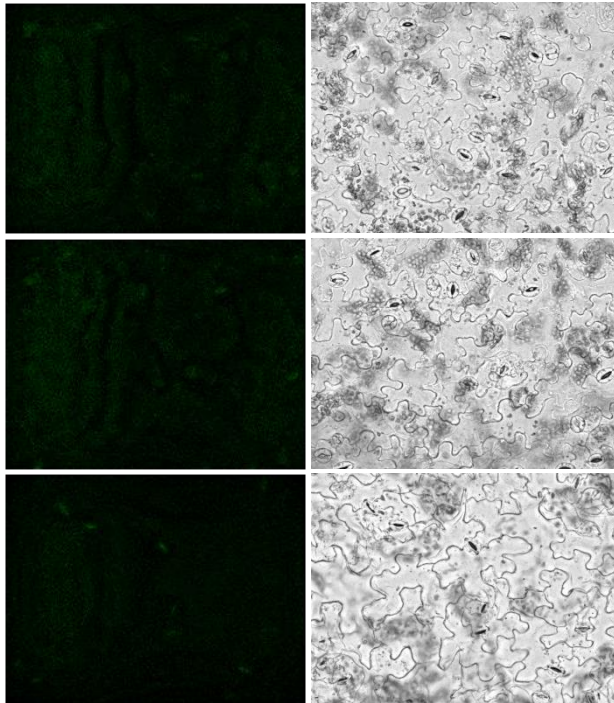


Supplementary Figure 60. Imaging (40 $\times$ magnification) of epidermal peels treated with **AEP** probe for *A.thaliana* *acs1/2/6/4/5/9/7/11* tissues incubated in the presence and absence of PAMPs.

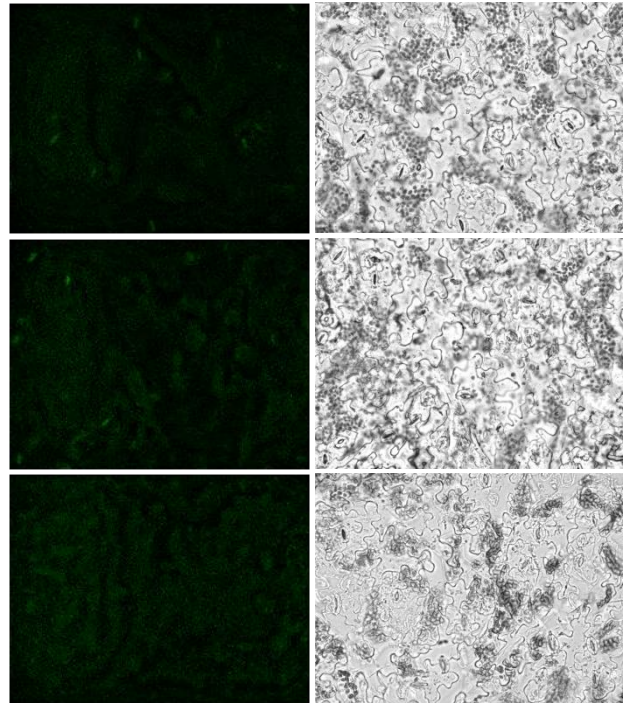
additive: 10 mM MgCl₂



additive: *Pst* AvrRpm1 (OD₆₀₀ = 0.02)

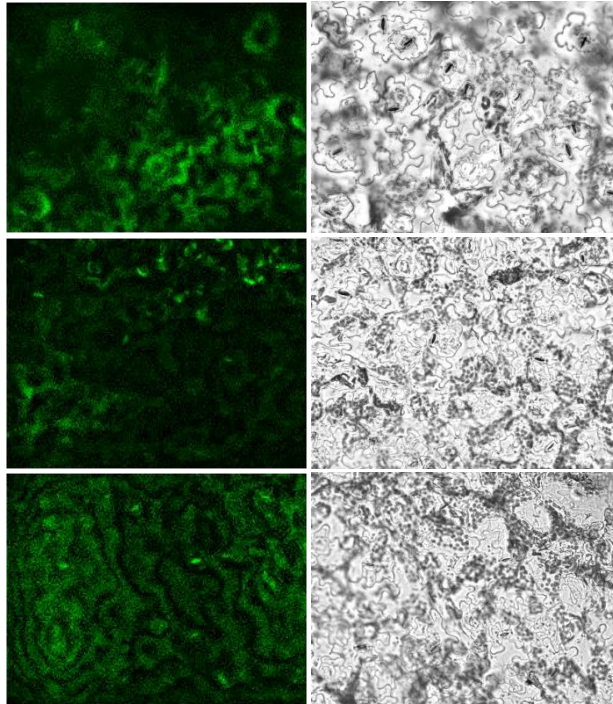


additive: *Pst* AvrRpt2 (OD₆₀₀ = 0.02)

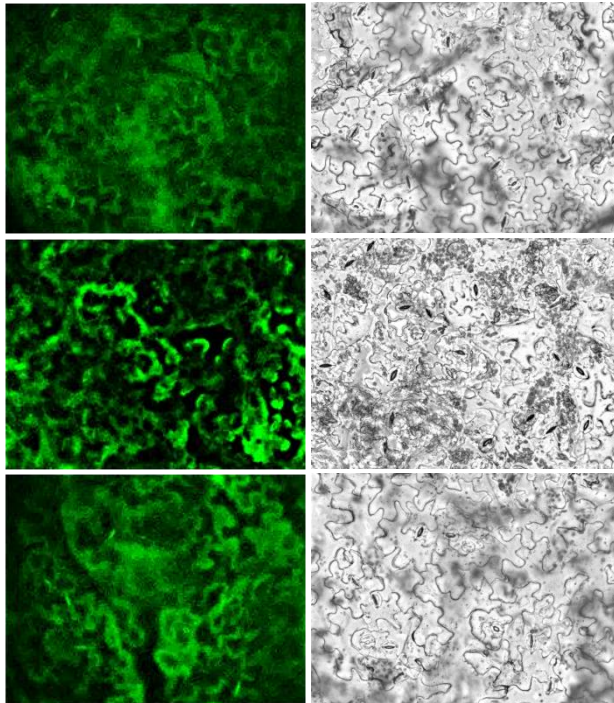


Supplementary Figure 61. Imaging (40× magnification) of epidermal peels treated without **AEP** probe for *A.thaliana* Col-0 tissues incubated in the presence and absence of Avr-producing *Pseudomonas* bacteria.

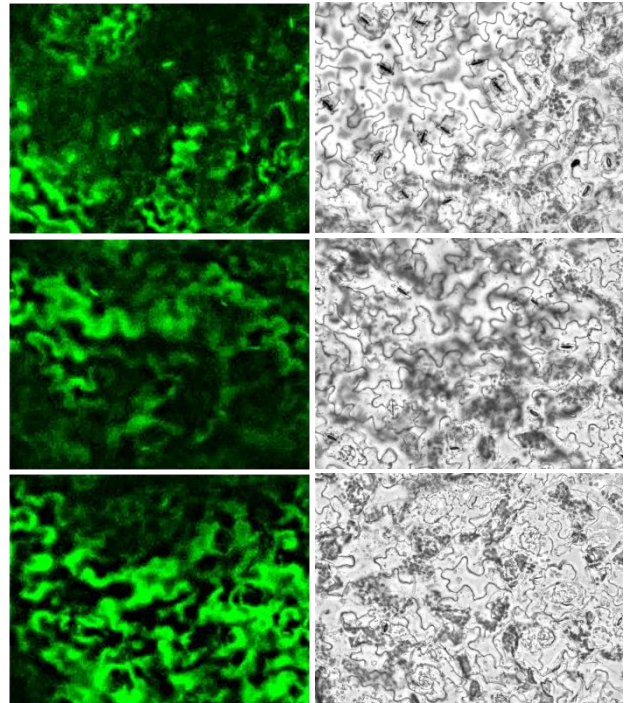
additive: 10 mM MgCl₂



additive: *Pst AvrRpm1* (OD₆₀₀ = 0.02)

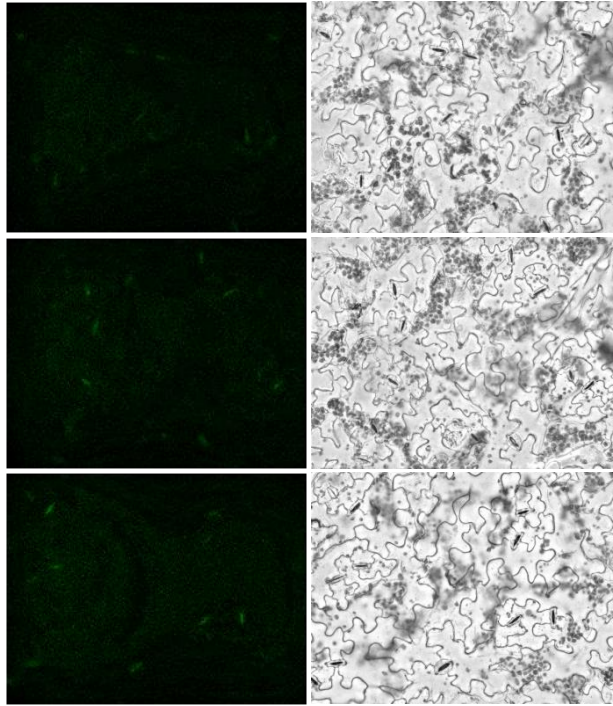


additive: *Pst AvrRpt2* (OD₆₀₀ = 0.02)

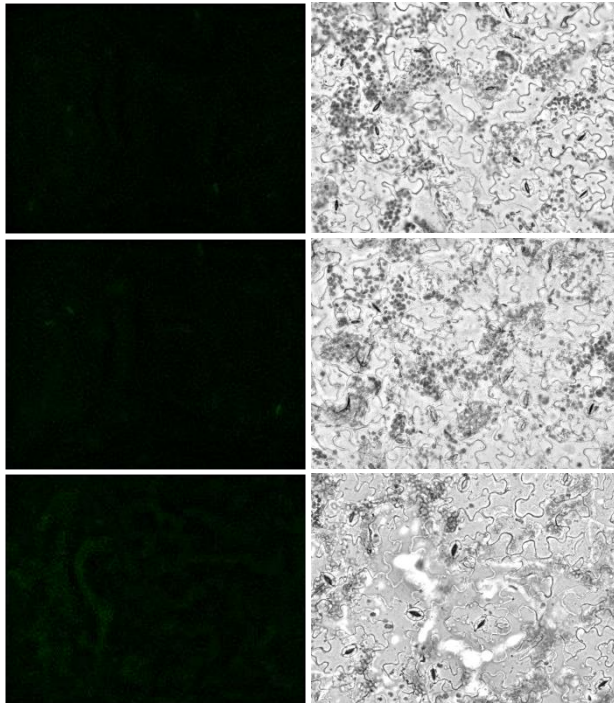


Supplementary Figure 62. Imaging (40× magnification) of epidermal peels treated with **AEP** probe for *A.thaliana* Col-0 tissues incubated in the presence and absence of Avr-producing *Pseudomonas* bacteria.

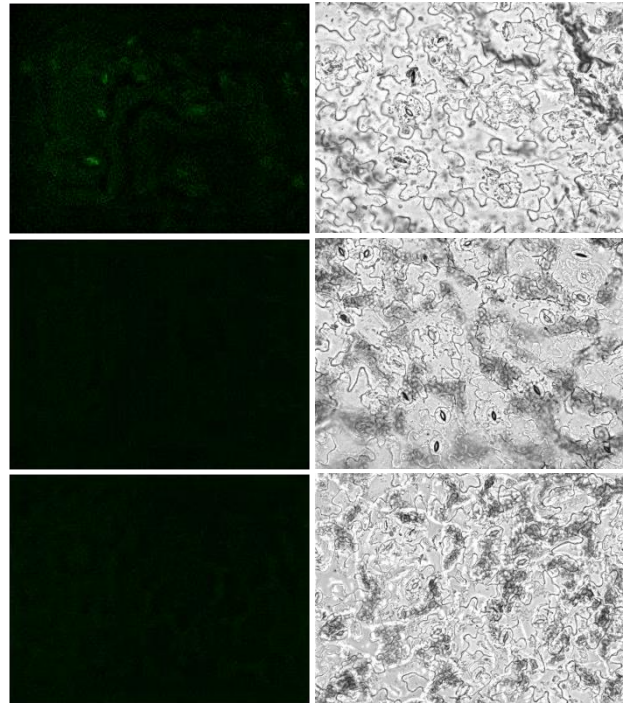
additive: 10 mM MgCl₂



additive: *Pst* AvrRpm1 (OD₆₀₀ = 0.02)

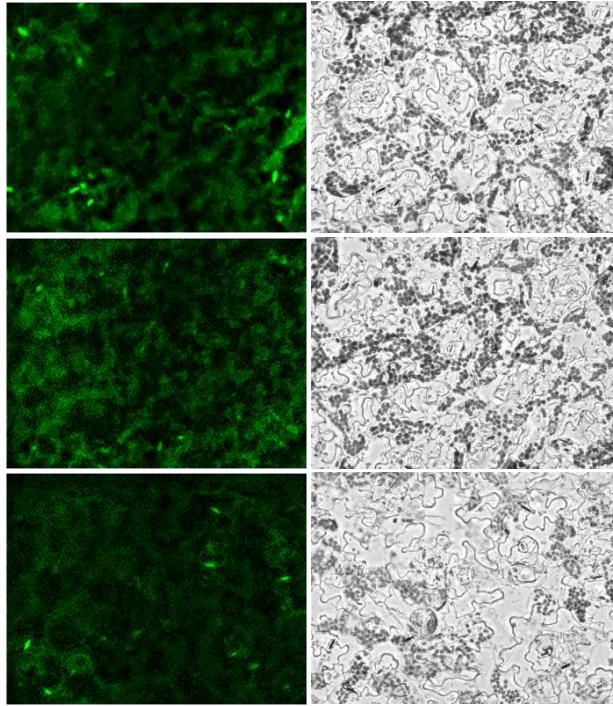


additive: *Pst* AvrRpt2 (OD₆₀₀ = 0.02)

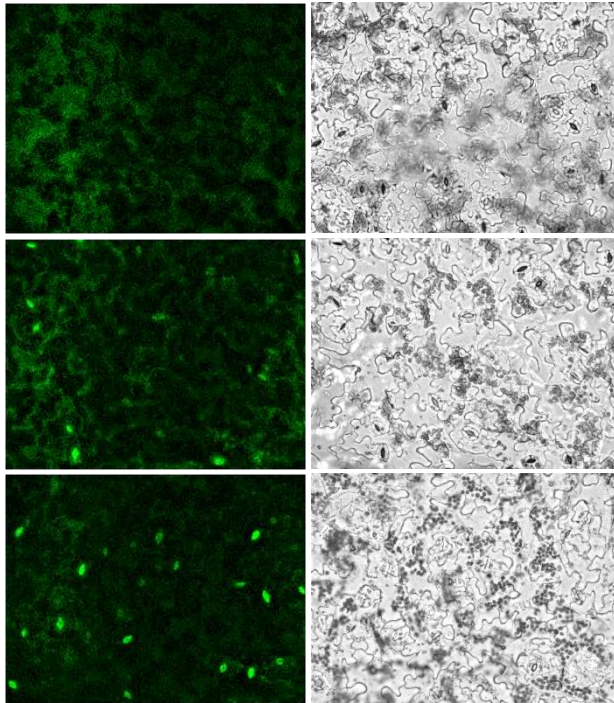


Supplementary Figure 63. Imaging (40× magnification) of epidermal peels treated without **AEP** probe for *A.thaliana rpm1rps2* tissues incubated in the presence and absence of Avr-producing *Pseudomonas* bacteria.

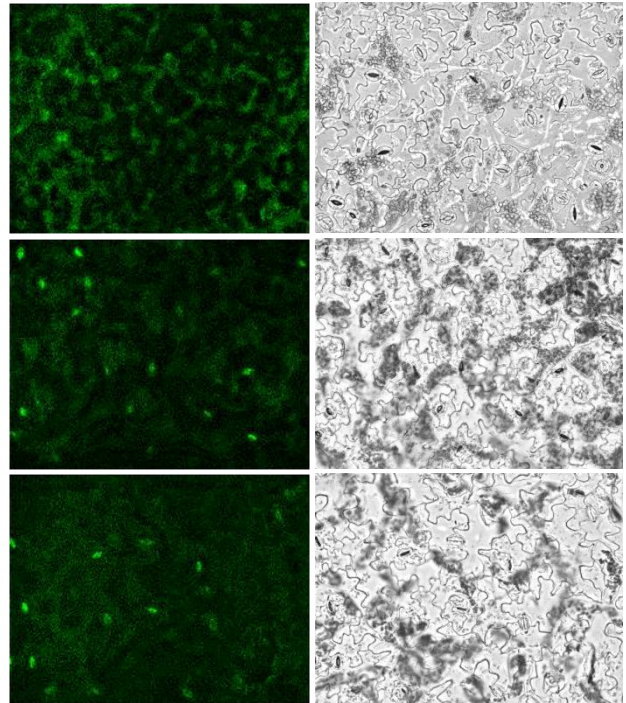
additive: 10 mM MgCl₂



additive: *Pst* AvrRpm1 (OD₆₀₀ = 0.02)

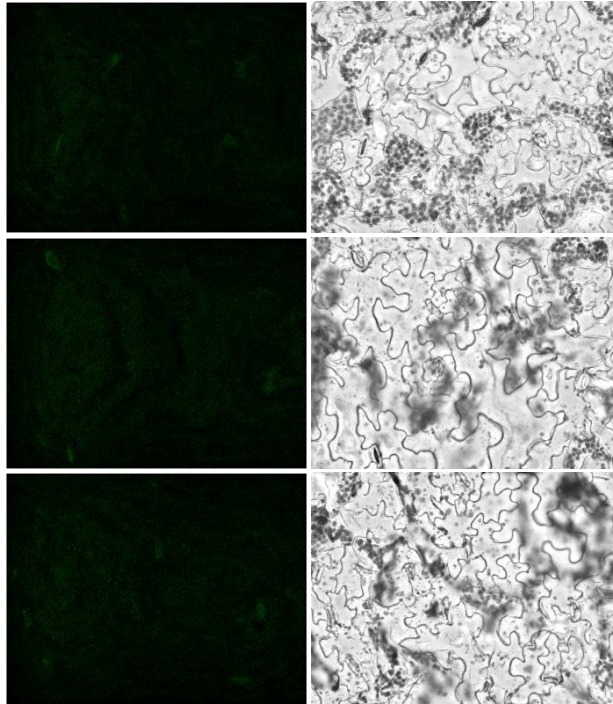


additive: *Pst* AvrRpt2 (OD₆₀₀ = 0.02)

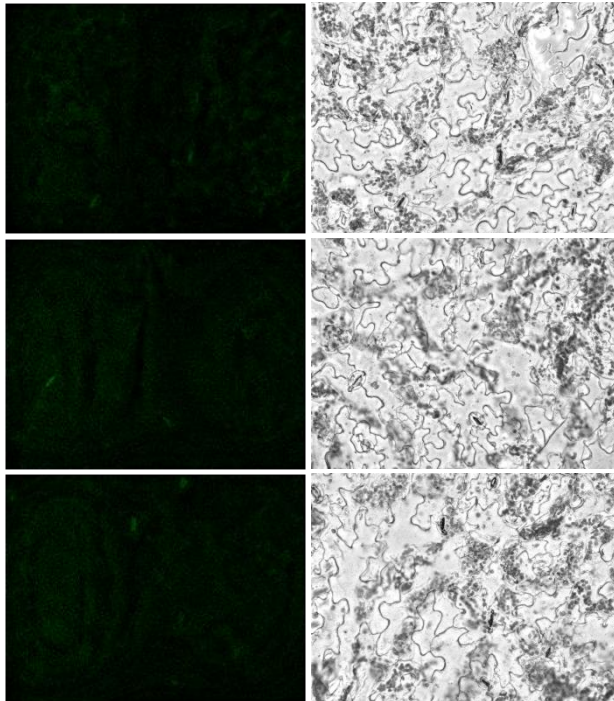


Supplementary Figure 64. Imaging (40× magnification) of epidermal peels treated with **AEP** probe for *A.thaliana rpm1rps2* tissues incubated in the presence and absence of Avr-producing *Pseudomonas* bacteria.

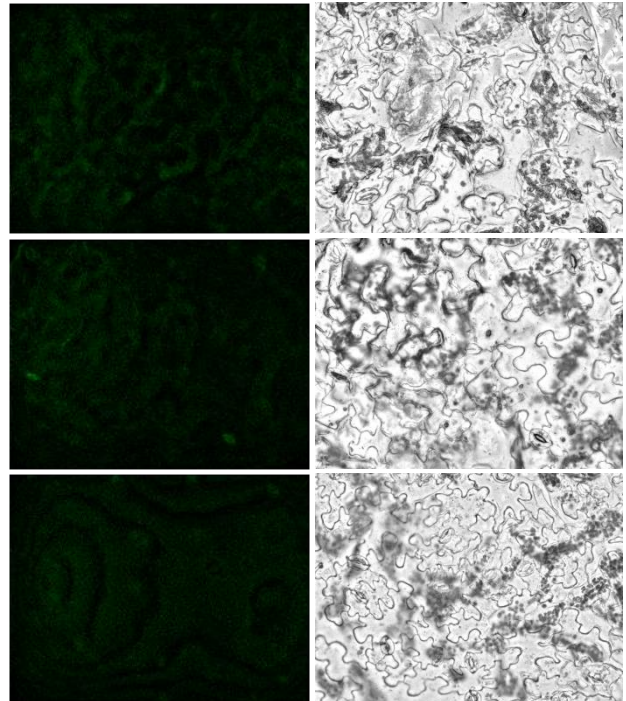
additive: 10 mM MgCl₂



additive: *Pst AvrRpm1* (OD₆₀₀ = 0.02)

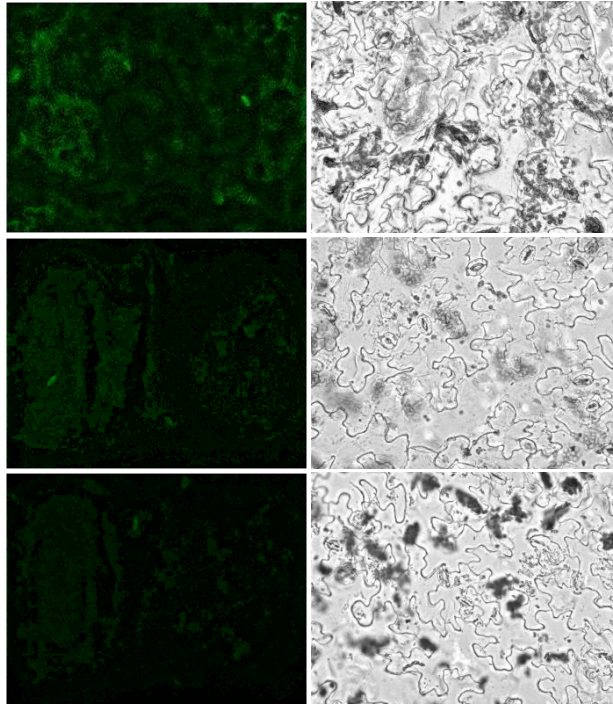


additive: *Pst AvrRpt2* (OD₆₀₀ = 0.02)

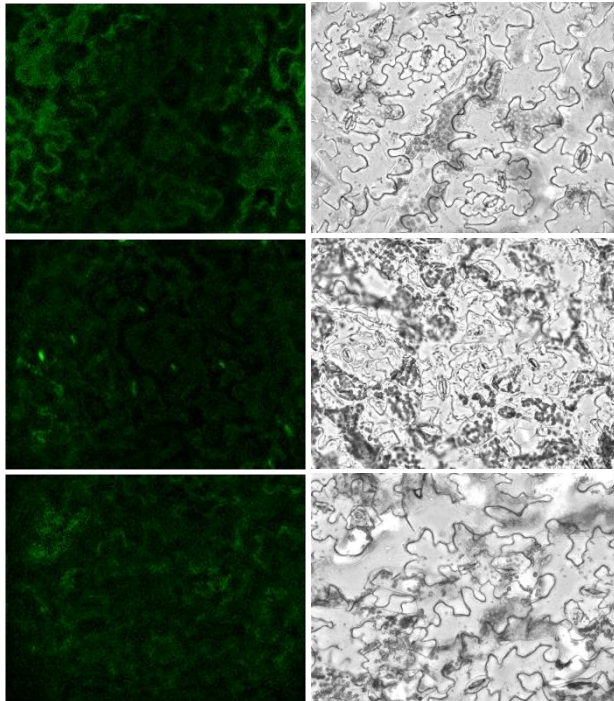


Supplementary Figure 65. Imaging (40× magnification) of epidermal peels treated without **AEP** probe for *A.thaliana acs1/2/6/4/5/9/7/11* tissues incubated in the presence and absence of Avr-producing *Pseudomonas*.

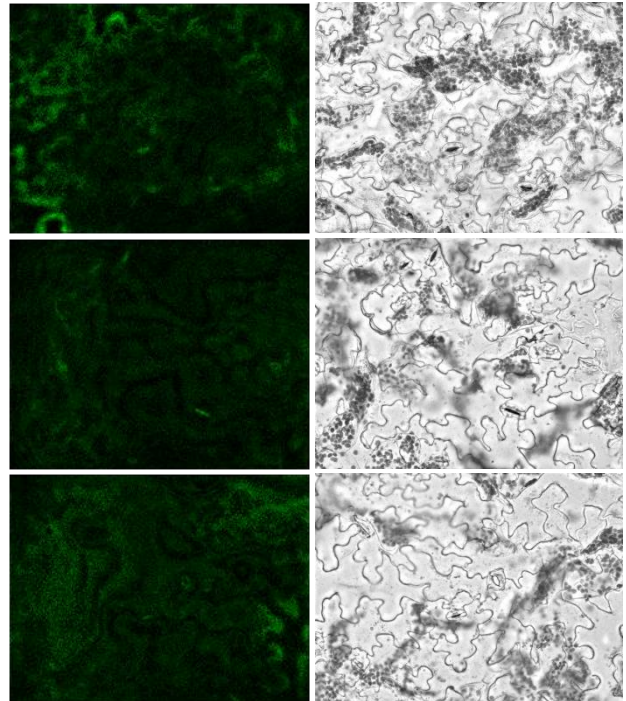
additive: 10 mM MgCl₂



additive: *Pst* AvrRpm1 (OD₆₀₀ = 0.02)



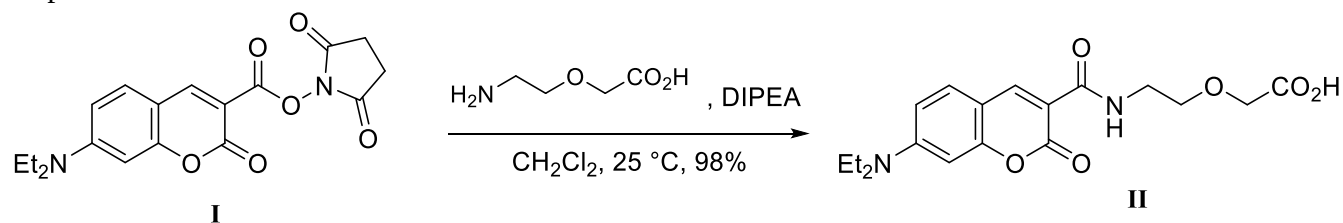
additive: *Pst* AvrRpt2 (OD₆₀₀ = 0.02)



Supplementary Figure 66. Imaging (40× magnification) of epidermal peels treated with **AEP** probe for *A.thaliana* acs1/2/6/4/5/9/7/11 tissues incubated in the presence and absence of Avr-producing *Pseudomonas*.

Supplementary Methods

Preparation of **II**

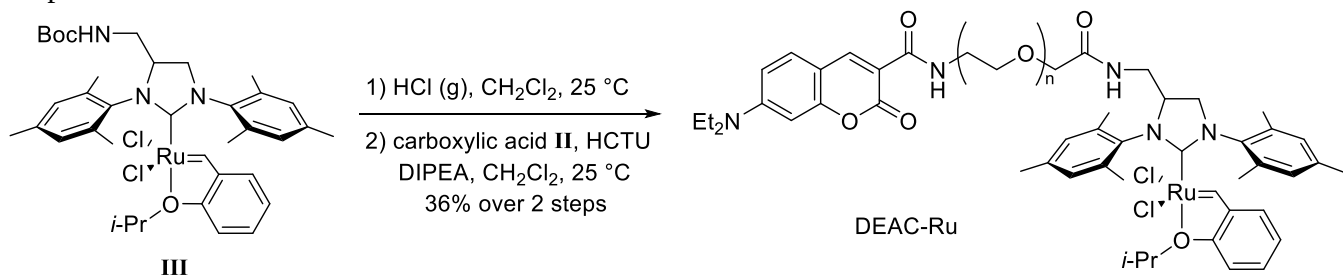


To a solution of *N*-succinimidyl 7-(diethylamino)coumarin-3-carboxylate **I** (100 mg, 0.279 mmol) and 5-amino-3-oxapentanoic acid (36.6 mg, 0.307 mmol) in CH_2Cl_2 (3 mL) was added *N,N*-diisopropylethylamine (110 mg, 0.851 mmol) at 25 °C. After stirring for 16 h, the reaction was stopped by addition of 1 M aq. HCl. The product was then extracted with CH_2Cl_2 ($\times 3$), and the combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was purified by silica-gel flash column chromatography ($\text{CHCl}_3/\text{MeOH} = 9/1 \rightarrow 8/2 \rightarrow \text{CHCl}_3/\text{MeOH}/\text{AcOH} = 79/20/1$) to give the desired product (100 mg, 98.9%) as a yellow solid.

^1H -NMR (500 MHz, CDCl_3 , δ) 1.22 (t, 6H, $J = 6.9$ Hz), 3.41 (q, 4H, $J = 6.9$ Hz), 3.64–3.72 (m, 4H), 4.10 (s, 2H), 6.44 (s, 1H, $J = 2.3$ Hz), 6.61 (d, 1H, $J = 8.8$ Hz), 7.41 (d, 1H, $J = 8.8$ Hz), 8.68 (s, 1H), 9.13 (s, 1H); ^{13}C -NMR (125 MHz, CDCl_3 , δ) 12.4 (2C), 39.6, 45.1 (2C), 69.4, 70.7, 96.5, 108.4, 109.6, 110.0, 131.3, 148.5, 152.6, 157.6, 162.5, 164.1, 173.8;

HRMS (ESI) m/z 363.1555 (363.1551 calcd for $\text{C}_{18}\text{H}_{23}\text{N}_2\text{O}_6$, $[\text{M}+\text{H}]^+$).

Preparation of DEAC-Ru



Prepared according to literature,¹⁻³ a solution of ruthenium complex **III** (80.1 mg, 0.106 mmol) in CH_2Cl_2 (3 mL) was bubbled with HCl gas at 25 °C. The gaseous HCl was generated by dropwise addition of conc. H_2SO_4 to NH_4Cl . After stirring for 45 min, CH_2Cl_2 (1 mL) was added to the reaction with a syringe and was continued to be stirred at the same temperature. After a further 15 min, the reaction was concentrated *in vacuo* to give the intermediate compound, which was used in the next reaction without further purification. HRMS (ESI) m/z 620.1979 (620.1981 calcd for $\text{C}_{31}\text{H}_{41}\text{ClN}_3\text{ORu}$, $[\text{M}-\text{Cl}]^+$).

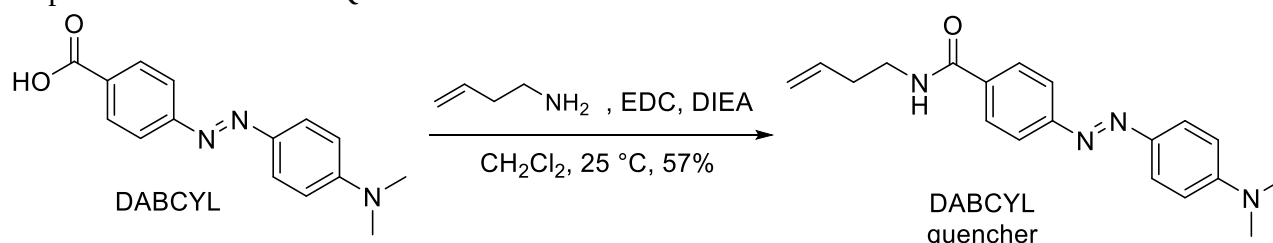
In another flask, a solution of carboxylic acid **II** (42.4 mg, 0.117 mmol) and the coupling agent HCTU (57.6 mg, 0.139 mmol) in CH_2Cl_2 (1 mL) was stirred at 25 °C for 30 min. To this reaction was added the intermediate compound in CH_2Cl_2 (1 mL) followed by *N,N*-diisopropylethylamine (137 mg, 1.06 mmol) at the same temperature. After stirring for 6 h, the reaction was stopped by adding 1 M aq. HCl. The

product was extracted with CH₂Cl₂ (×3), and the combined organic extracts were washed with sat. aq. NaHCO₃ and brine, dried (Na₂SO₄), and concentrated *in vacuo*. The residue was purified by silica-gel flash column chromatography (cyclohexane/EtOAc/CHCl₃/MeOH = 40/40/15/5) to give the desired product (37.7 mg, 35.6%) as a green solid.

¹H-NMR (400 MHz, CDCl₃, δ) 1.24–1.27 (m, 12H), 2.37–2.51 (br m, 18H), 3.47 (q, 4H, *J* = 7.1 Hz), 3.53–3.71 (m, 6H), 3.79 (m, 1H), 3.93 (d, 1H, *J* = 15.3 Hz), 3.99 (d, 1H, *J* = 15.3 Hz), 4.05 (dd, 1H, *J*₁ = *J*₂ = 10.0 Hz), 4.33 (dd, 1H, *J*₁ = *J*₂ = 10.0 Hz), 4.75–4.84 (br m, 1H), 4.89 (sept, 1H, *J* = 6.1 Hz), 6.50 (d, 1H, *J* = 2.3 Hz), 6.66 (dd, 1H, *J*₁ = 2.3 Hz, *J*₂ = 9.1 Hz), 6.78 (d, 1H, *J* = 7.7 Hz), 6.85 (dd, 1H, *J*₁ = *J*₂ = 7.7 Hz), 6.91 (dd, 1H, *J*₁ = 1.7 Hz, *J*₂ = 7.7 Hz), 7.02 (s, overlapped, 2H), 7.04 (s, 1H), 7.07 (s, 1H), 7.42 (d, 1H, *J* = 9.1 Hz), 7.48 (ddd, 1H, *J*₁ = 1.7 Hz, *J*₂ = *J*₃ = 7.7 Hz), 8.67 (s, 1H), 9.09 (s, 1H), 16.50 (s, 1H);

HRMS (ESI) *m/z* 964.3355 (964.3359 calcd for C₅₀H₆₁ClN₅O₆Ru, [M–Cl]⁺).

Preparation of DABCYL Quencher



To a solution of DABCYL Acid (135 mg, 0.50 mmol), 3-buten-1-amine (81 mg, 0.75 mmol), and EDC (117 mg, 0.61 mmol) in anhydrous DCM (10 ml) was added DIEA (436 µl, 2.50 mmol) at 25 °C. After stirring for 16 h, the reaction was stopped by addition of aq. NaHCO₃. The product was then extracted with CH₂Cl₂ (×3), and the combined organic extracts were washed with brine, dried (Na₂SO₄), and concentrated *in vacuo*. The residue was purified by silica-gel flash column chromatography (CHCl₃/MeOH = 95/5) to give the desired product (91.3 mg, 57%) as an orange solid.

¹H-NMR (400 MHz, CDCl₃, δ) 2.41 (dt, 2H, *J*₁ = 7.2 Hz, *J*₂ = 7.2 Hz), 3.11 (s, 6H), 3.56 (dt, 2H, *J*₁ = 5.6 Hz, *J*₂ = 5.6 Hz), 5.13–5.20 (m, 2H), 5.82–5.89 (m, 1H), 6.22 (br s, 1H), 6.74–6.78 (m, 2H), 7.85–7.91 (m, 6H); ¹³C-NMR (100 MHz, CDCl₃, δ) 167.0, 155.0, 152.8, 143.6, 135.3, 134.7, 127.7, 125.4, 122.2, 117.5, 111.5, 40.3, 38.9, 33.8;

HRMS (ESI) *m/z* 323.1858 (323.1866 calcd for C₁₉H₂₃N₄O, [M+H]⁺).

Supplementary References

- Lo, C. et al. Artificial metalloenzymes for olefin metathesis based on the biotin-(strept)avidin technology. *ChemComm* **47**, 12065–12067 (2011).
- Kajetanowicz, A., Chatterjee, A., Reuter, R. & Ward, T. R. Biotinylated Metathesis Catalysts: Synthesis and Performance in Ring Closing Metathesis. *Catal. Lett.* **144**, 373–379 (2014).
- Zhao, J., Kajetanowicz, A. & Ward, T. R. Carbonic anhydrase II as host protein for the creation of a biocompatible artificial metathesase. *Org. Biomol. Chem.* **13**, 5652–5655 (2015).