

Life Sciences Reporting Summary

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

► Experimental design

1. Sample size

Describe how sample size was determined.

No sample size calculation was required for this study.

2. Data exclusions

Describe any data exclusions.

No data exclusion

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

RNA-seq experiments were performed in triplicate. ChIP-seq experiments were performed on independent tissues. All real time quantitative RT-PCR were repeated at least 3 times.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

The study does not involve any randomized experimental group. Phylogenetic groups are based on published data.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

No blinding was required for this study.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- ☒ ☐ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- ☒ ☐ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☒ ☐ A statement indicating how many times each experiment was replicated
- ☒ ☐ The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☒ ☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- ☒ ☐ Test values indicating whether an effect is present
*Provide confidence intervals or give results of significance tests (e.g. *P* values) as exact values whenever appropriate and with effect sizes noted.*
- ☒ ☐ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- ☒ ☐ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

Code for filtering FALCON overlaps can be found at <http://lipm-bioinfo.toulouse.inra.fr/download/til-r/>. This is indicated in the manuscript.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

The data availability statement is included in the Author Information section of the main manuscript

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

ChIP assays were performed using the commercially available antibodies, anti-H3K9ac (Millipore, ref. 07-352) and anti-H3K27me3 (Millipore, ref. 553 07-449), previously validated by other published data and widely used for similar experiments.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No cell lines were used in this study.
The source of plants used in this study is given in Supplementary Table 7 (section 8 of Supplementary Notes).

b. Describe the method of cell line authentication used.

No cell lines were used in this study.

c. Report whether the cell lines were tested for mycoplasma contamination.

No cell lines were used in this study.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No cell lines were used in this study.

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used in this study.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human research was performed in this study.

ChIP-seq Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

► Data deposition

1. For all ChIP-seq data:

- ☒ a. Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- ☒ b. Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

2. Provide all relevant data deposition access links.
The entry may remain private before publication.

ChIPseq raw data have been deposited under SRA numbers SRR6167310, SRR6167311, SRR6167312 and SRR6167313.
Processed data can be accessed through genome browser <https://lipm-browsers.toulouse.inra.fr/pub/RchiOBHm-V2/> (login: reviewer / password: rosaob).

3. Provide a list of all files available in the database submission.

RcHt_17_H3K9ac_mark_Petal.R1.fastq.gz
RcHt_17_H3K9ac_mark_Petal.R2.fastq.gz
RcHt_17_H3K9Ac_Input_Petal.R1.fastq.gz
RcHt_17_H3K9Ac_Input_Petal.R2.fastq.gz
RcHt_17_H3K27me3_mark_Petal.fastq.gz
RcHt_17_H3K27me3_Input_Petal.fastq.gz

4. Provide a link to an anonymized genome browser session (e.g. [UCSC](#)), if available.

The genome and data can be accessible for the reviewer at <https://lipm-browsers.toulouse.inra.fr/pub/RchiOBHm-V2/> (login: reviewer / password: rosaob).

► Methodological details

5. Describe the experimental replicates.

n.a

6. Describe the sequencing depth for each experiment.

Homozygous genome :
H3K9Ac mark, mean coverage : 5.2x; H3K9Ac input, mean coverage : 15x;
H3K27me3 mark, mean coverage : 4.9x; H3K27me3 input : 4.2x
Heterozygous genome :
H3K9Ac mark, mean coverage : 3.6x; H3K9Ac input, mean coverage : 10.2x;
H3K27me3 mark, mean coverage : 3.4x; H3K27me3 input : 2.9x

7. Describe the antibodies used for the ChIP-seq experiments.

H3K9AC : MILLIPORE / 07-352 / n.a / 2325091
H3K27me3 : MILLIPORE / 07-449 / n.a / 2686928

8. Describe the peak calling parameters.

**** H3K9AC mark :**
*** read mapping :**
bowtie2 -x \$INDEX_FILE -m1 \$R1_file -m2 \$R2_file -S \$RESULT_FILE -N1
\$INDEX_FILE = [RchiOBHm-V2.0,RchzRDP12]
- Chip :
\$R1_file = \$DATA_REP/trim_galore/RcHt_17_H3K9ac_mark_Petal.R1.val1.fq.gz
\$R2_file = \$DATA_REP/trim_galore/RcHt_17_H3K9ac_mark_Petal.R2.val2.fq.gz
\$RESULT_FILE = [aln_bowtie2_Rose_K9ac_mark_Flower_vs_RchiOBHm-V2.0.sam,aln_bowtie2_Rose_K9ac_mark_Flower_vs_RchzRDP12.sam]
- Input :
\$R1_file = \$DATA_REP/trim_galore/RcHt_17_H3K9ac_Input_Petal.R1.val1.fq.gz
\$R2_file = \$DATA_REP/trim_galore/RcHt_17_H3K9ac_Input_Petal.R2.val2.fq.gz
\$RESULT_FILE = [aln_bowtie2_Rose_K9ac_Input_Flower_vs_RchiOBHm-V2.0.sam,aln_bowtie2_Rose_K9ac_Input_Flower_vs_RchzRDP12.sam]
*** Peak calling :**
macs2 callpeak -f BAM -t \$mark_file -n \$result_file -g 515588973 -c \$control_file --bdg
- RchiOBHm genome :

9. Describe the methods used to ensure data quality.

```
$mark_file = aln_bowtie2_Rose_K9ac_mark_Flower_vs_RchiOBHm-V2.0.bam
$result_file = aln_bowtie2_Rose_K9ac_Flower_vs_RchiOBHm-V2.0.macs2
$control_file = aln_bowtie2_Rose_K9ac_Input_Flower_vs_RchiOBHm-V2.0.bam
- RchzRDP12 genome :
$mark_file = aln_bowtie2_Rose_K9ac_mark_Flower_vs_RchzRDP12.bam
$result_file = aln_bowtie2_Rose_K9ac_Flower_vs_RchzRDP12.macs2
$control_file = aln_bowtie2_Rose_K9ac_Input_Flower_vs_RchzRDP12.bam
**H3K27me3 :
* read mapping :
bowtie2 -x $INDEX_FILE -U R_file -S $RESULT_FILE -N1
$INDEX_FILE = [RchiOBHm-V2.0,RchzRDP12]
- Chip :
$R_file = RchT_17_H3K27me3_mark_Petal.trimmed.fq.gz
$RESULT_FILE = [aln_bowtie2_Rose_H3K27me3_mark_Flower_vs_RchiOBHm-
V2.0.sam,aln_bowtie2_Rose_H3K27me3_mark_Flower_vs_RchzRDP12.sam]
- Input :
$R_file = RchT_17_H3K27me3_Input_Petal.trimmed.fq.gz
$RESULT_FILE = [aln_bowtie2_Rose_H3K27me3_Input_Flower_vs_RchiOBHm-
V2.0.sam,aln_bowtie2_Rose_H3K27me3_Input_Flower_vs_RchzRDP12.sam]
* Peak calling :
macs2 callpeak -f BAM -t $mark_file -n $result_file -g 515588973 -c $control_file --
bdg
- RchiOBHm genome :
$mark_file = aln_bowtie2_Rose_H3K27me3_mark_Flower_vs_RchiOBHm-
V2.0.bam
$result_file = aln_bowtie2_Rose_H3K27me3_Flower_vs_RchiOBHm-V2.0.macs2
$control_file = aln_bowtie2_Rose_H3K27me3_Input_Flower_vs_RchiOBHm-
V2.0.bam
- RchzRDP12 genome :
$mark_file = aln_bowtie2_Rose_H3K27me3_mark_Flower_vs_RchzRDP12.bam
$result_file = aln_bowtie2_Rose_H3K27me3_Flower_vs_RchzRDP12.macs2
$control_file = aln_bowtie2_Rose_H3K27me3_Input_Flower_vs_RchzRDP12.bam
```

```
** H3K9AC mark :
* RchiOBHm genome :
# peaks with FDR < 5% = 46624
# peaks FC > 5 = 21668
* RchzRDP12 genome :
# peaks with FDR < 5% = 50577
# peaks FC > 5 = 27286
**H3K27me3 mark :
* RchiOBHm genome :
# peaks with FDR < 5% = 13877
# peaks FC > 2 = 6379
* RchzRDP12 genome :
# peaks with FDR < 5% = 20179
# peaks FC > 5 = 11799
```

10. Describe the software used to collect and analyze the ChIP-seq data.

Software used to collect and analysed ChIPseq data are fully described in section 6.2 of Supplementary Notes

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

► Data presentation

For all flow cytometry data, confirm that:

- ☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ 3. All plots are contour plots with outliers or pseudocolor plots.
- ☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

► Methodological details

- | | |
|--|---|
| 5. Describe the sample preparation. | Nuclei were isolated from RcHzRDP12 calli or from young leaves of regenerated plantlets, as previously described ¹⁷ , and stained by adding 1µg/ml DAPI (Sigma) for 1 hour at room temperature. |
| 6. Identify the instrument used for data collection. | FACS data collections were performed using MACSQuant VYB (Miltenyi Biotec) cytometer. |
| 7. Describe the software used to collect and analyze the flow cytometry data. | FACS data analyses were performed using FlowJo software (FlowJo LLC). |
| 8. Describe the abundance of the relevant cell populations within post-sort fractions. | Total nuclei populations were gated using DAPI intensity (Extended Data Fig. 1j, left panels): 16% of 'Old blush' and 13% of RcHzRDP12 nuclei were retained as DAPI+. Next, doubles were eliminated from this population using DAPI-W versus DAPI-H axes (Extended Data Fig. 1j, middle panels): about 97% of DAPI+ nuclei were retained by this gating in both samples. Finally, in DAPI+ singles cells, the proportions of nuclei with different ploidy levels were determined based on their DAPI intensity (Extended Data Fig. 1j, right panels): about 67% and 12% of nuclei were diploids (2N) and tetraploids (4N), respectively in both the "Old Blush" sample and in the RcHzRDP12 sample. |
| 9. Describe the gating strategy used. | Total nuclei populations were gated using DAPI intensity (Extended Data Fig. 1j, left panels). Next, doubles were eliminated from this population using DAPI-W versus DAPI-H axes (Extended Data Fig. 1j, middle panels). Finally, in DAPI+ singles cells, the proportions of nuclei with different ploidy levels were determined based on their DAPI intensity (Extended Data Fig. 1j, right panels). |

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒