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1 H3K27me3 natural variation selectively marks genes predicted to be important for
2 differentiation in unicellular algae

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28 **Abstract**

29 In multicellular organisms H3K27me3 has been shown to be deposited by Polycomb Repressive
30 Complex 2 (PRC2) to establish and maintain gene silencing, critical for cell fate and
31 developmentally regulated processes. PRC2 complex is absent in both yeasts *Saccharomyces*
32 *cerevisiae* and *Schizosaccharomyces pombe*, which initially suggested that PRC2 arose with
33 the emergence of multicellularity. However, its discovery in several unicellular species
34 including microalgae questions its role in unicellular eukaryotes. Here, we show in the model
35 diatom *Phaeodactylum tricornutum* (Pt), using mutants in the homologue of the catalytic
36 subunit of PRC2, *enhancer of zeste E(z)*, that Pt *E(z)* is responsible for di and tri-methylation
37 of lysine 27 of histone H3. H3K27me3 depletion abolishes cell morphology in Pt providing
38 evidence for a role of H3K27me3 in cell differentiation in unicellular species. Genome wide
39 profiling of H3K27me3 in fusiform and triradiate cells further revealed genes that may specify
40 cell identity. These results suggest a role for PRC2 and its associated histone mark in cell
41 differentiation in unicellular species and highlights their ancestral function in a broader
42 evolutionary context than is currently appreciated.

43 **Introduction**

44 Tri-methylation of lysine 27 of histone H3 (H3K27me3) is a mark deposited by Polycomb
45 Repressive Complex 2 (PRC2), which mediates silencing of gene expression during
46 differentiation and development in both animals and plants¹⁻³. PRC2 is comprised of four core
47 proteins, highly conserved among multicellular organisms: the histone methyltransferase
48 (HMTase) enhancer of zeste *E(z)*, the WD40 domain containing polypeptide Extra Sex Comb
49 *Esc*, the C2H2 type zinc finger protein Suppressor of zeste 12 *Su(z)12* and the Nucleosome
50 remodeling factor 55 kDa subunit *Nurf-55*^{4,5}. The absence of PRC2 in the unicellular yeast
51 models *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* initially led to suggestions
52 that it arose to regulate cell differentiation in multicellular organisms⁶. This hypothesis has
53 recently been questioned because components of PRC2 and the associated mark H3K27me3
54 are found in several unicellular species that belong to different lineages including, but not only,
55 Stramenopiles, Alveolates and Rhizaria (SAR)⁷⁻⁹ (Supplementary Fig. 1a-d), thus questioning
56 the function of such a widespread complex in single celled organisms.

57 Attempts to understand the role of H3K27me3 in the unicellular green alga *Chlamydomonas*
58 *reinhardtii* were not conclusive because tri-methylation of lysine 27 could not be assessed
59 reliably due to its nominal mass which was found similar to acetylation of lysine 27 of the same
60 histone⁷. However, we have previously identified H3K27me3 by mass spectrometry and
61 mapped its localization genome-wide in the pennate diatom *Phaeodactylum tricornutum*⁹ (Pt),
62 which belongs to the stramenopile group of eukaryotes, only distantly related to the animal
63 (Opisthokonta) and plant (Archaeplastida) eukaryotic crown groups. Pt has different
64 morphotypes, fusiform (FM hereafter), which is the most prevailing morphology among the
65 sampled accessions known so far, triradiate (TM hereafter), oval (OM hereafter) and cruciform
66 (CM hereafter)¹⁰⁻¹³ (Fig. 1a). Each morphotype can switch reversibly into a different
67 morphology in response to several growth and environmental cues¹⁰. FM is the most stable
68 morphotype while switching is more prominent in TM, CM and OM, which tend to convert to
69 FM in the growth conditions used in this study^{10,11}.

70 Interestingly, western blot analysis using a monoclonal antibody against the mark in FM, TM,
71 CM and OM cells revealed a strong correlation between the complexity of the morphology
72 (branching of the cell) and the absolute quantity of H3K27me3, which is higher in both CM
73 and TM cells compared to FM and OM cells (Supplementary Fig. 1e), suggesting that PRC2
74 activity controls cell differentiation in *P. tricornutum*. Cell differentiation is often orchestrated
75 by H3K27me3-mediated silencing that underlies the establishment and maintenance of cellular

76 identity in multicellular model species¹⁴. These results prompted us to investigate the putative
77 role of H3K27me3 in cell differentiation in a unicellular model. In silico annotation of
78 polycomb complex members and identification of H3K27me3 by mass spectrometry¹⁵ in a well-
79 developed experimental model such as *P. tricornutum* (Supplementary Fig. 1a-c) might give
80 new insights for the unsolved questions in the study of PRC2 complex in multicellular species,
81 especially the availability of different morphotypes which is unique to *P. tricornutum* among
82 stramenopiles, present an opportunity to decipher its role in single celled organisms with respect
83 to its potential contribution to establish morphotype switches as well as its function in an
84 evolutionary context.

85 To gain insights into the function of $E(z)$ and its associated H3K27me3 mark in Pt, we generated
86 two CRISPR/cas9 knockouts of the gene in each of the three morphotypes (FM, TM and CM)
87 leading to putative loss of function mutations including premature stop codon and frameshifts
88 (Fig. 1b). Light microscopy analysis of $E(z)$ knockouts shows a change in cell morphology
89 which becomes shorter in the FM background. Both triradiate and cruciform morphologies were
90 abolished in TM and CM, respectively while transgenic lines carrying cas9 control vectors in
91 each morphotype remain unchanged (Fig. 1c). Whereas *P. tricornutum* transgenic lines with
92 the Cas9 control vector show similar H3K27me3 enrichment than the wild type (Supplementary
93 Fig. 2a). knockout of $E(z)$ led to an overall depletion of H3K27me3 shown by western blot
94 using a monoclonal antibody against the mark (Fig. 1d), suggesting that the loss or diminution
95 of $E(z)$ activity, and hence H3K27me3, causes the observed changes in cell morphology (Fig.
96 1c; Supplementary Fig. 2a; Table S1). Overall, this suggests that $E(z)$ and its associated mark
97 are required for morphotype switch/to establish specific cell identity.

98 Mass spectrometry analysis of histones extracted from both wild type and $E(z)$ knockout
99 confirmed the loss of H3K27me3 and revealed a depletion of di-methylation of H3K27,
100 corroborating the role of $E(z)$ in di and tri-methylation of lysine 27 of histone H3
101 (Supplementary Fig. 2b,c,d). This is similar to fungi¹⁶ and mammals¹⁷ but different from *A.*
102 *thaliana* where PRC2 loss of function leads to specific depletion of H3K27me3¹⁸, although in
103 vitro assays with reconstituted *A. thaliana* PRC2 components showed mono, di and tri-
104 methylation of lysine 27 of histone H3^{19,20}. Western blot analysis of $E(z)$ knockout mutants
105 using a monoclonal antibody against H3K27me2 (Supplementary Fig. 2e) confirmed the
106 depletion of the mark from the mutants, supporting further the mass spectrometry analysis and
107 the role of $E(z)$ in di-methylation of lysine 27 of histone H3 in *P. tricornutum*.

108 Similar to animals, plants and *Neurospora crassa*, H3K27me3 appears not to be essential for
109 cell survival in *P. tricornutum*, as indicated by the overall growth of *E(z)* knockout lines, which
110 are only slightly retarded compared to wild type lines (Supplementary Fig. 2f).

111 To further investigate the role of H3K27me3 and its targets in different morphotypes, we carried
112 out a genomic approach and performed Chromatin Immuno-Precipitation (ChIP) on two
113 biological replicates of TM morphotypes using an antibody against H3K27me3 followed by
114 DNA sequencing (ChIP-Seq) to generate a map of H3K27me3 distribution, which we compared
115 to the one previously generated in FM²¹. ChIP-Seq data analysis revealed a similar H3K27me3
116 enrichment profile between TM and FM that localizes principally on transposable elements
117 (TEs), with 58% and 60% of the reads overlapping with TE annotations for FM²¹ and TM,
118 respectively (Fig. 2a). The mark was found to occupy, on average, ~11.6% of the genome in
119 FM cells, targeting approximately 15% of genes (consistent with¹⁵) and ~13.2% of the genome
120 within TM, targeting 19% of genes in agreement with the absolute amount of H3K27me3 to be
121 elevated in TM compared to FM as detected by western blot (Supplementary Fig. 1e). Indeed,
122 more genes are marked by H3K27me3 in TM than in FM (Fig. 2a, Supplementary Fig. 3a),
123 although most of the PRC2 targets are shared between both morphotypes (Fig. 2a) and exhibit
124 globally broad coverage over the annotation (Supplementary Fig. 3b). Among the PRC2 target
125 genes, 635 and 297 genes are found to be specifically marked by H3K27me3 in TM and FM,
126 respectively (Fig. 2a). We used ChIP followed by quantitative PCR (ChIP-qPCR) to validate
127 H3K27me3 enrichment over specifically marked loci in both backgrounds, which corroborated
128 the genome wide data for most of the tested genes (Fig. 2b, c). Common marked loci and
129 unmarked loci were tested as internal controls (Supplementary Fig. 3c). To test for the loss of
130 H3K27me3 in *E(z)* knockouts, we performed ChIP-qPCR in FM and TM as well as the
131 respective mutants, which confirmed the depletion of H3K27me3 in these mutants (Fig. 1e,f).

132 To gain insights into the functional categories enriched in H3K27me3 target genes that are
133 shared between the two morphotype or specific, we applied to the updated Phatr3 annotation a
134 gene ontology analysis using DAMA²² and CLADE²³ which is a machine learning methodology
135 that uses pHMMs, positive score matrix and support vector machines to infer the corresponding
136 most probable GO category to genes. DAMA and Clade allow a more sensitive remote
137 homology that permits to assign to genes with no or poor domain conservation the
138 corresponding GO categories that would have been missed by other methods. Are considered
139 statistically significant, only GO classes that are represented by at least 3 standard deviations
140 above the average of observed entries.

141 Out of 1,640 H3K27me3 marked genes, 753 could not be assigned to a more specific GO
142 category and are therefore marked as unknown. The genes that are marked in both morphotypes
143 show top enrichment in RNA related biological processes such as RNA-dependent DNA
144 biosynthetic process, RNA phosphodiester bond hydrolysis and RNA-DNA hybrid
145 ribonuclease activity. Genes marked by H3K27me3 specifically in TM displayed top
146 enrichment exclusively in (1) glycoprotein biosynthetic processes involved in the transfer of
147 sugar moieties that might determine different sugar composition of the cell wall which is known
148 to be sugar rich in *P. tricornutum*²⁴, (2) Peptidyl tyrosine dephosphorylation processes with
149 Ankyrin repeats proteins known to act as scaffold for connecting molecular interactions, likely
150 important for development of the numerous signaling pathways associated generally to more
151 complex multicellular organisms²⁵ (Fig. 2d; Supplementary Table S2). Genes that are
152 specifically marked in FM cells exhibit enrichment in categories such as peptidyl-tyrosine
153 phosphorylation containing genes with central roles as modulators of cell differentiation and
154 cell fate decisions²⁶ (Fig. 2d; Supplementary Table S2). Interestingly, additional genes
155 specifically marked in each FM or TM share categories with predicted functions in positive
156 regulation of (1) GTPase activity with a role in cell morphology changes, and neurite outgrowth
157 and guidance²⁷ as well as the differentiation of many cell types, including neurons, T
158 lymphocytes and myocytes²⁸; (2) protein ubiquitination shown to play a role in the complex
159 regulation of the levels and function of many proteins and signaling pathways involved in
160 determining cell fate²⁹. Overall, the genes that are specifically marked in the TM or FM
161 morphotypes reflect processes related to cell growth, proliferation and differentiation.

162 We have reported previously that H3K27me3 marked genes in FM are characterized by low
163 expression¹⁵, consistent with the role of H3K27me3 as a repressive mark. Interestingly, when
164 genes are marked by H3K27me3 in both FM and TM, their expression is lower compared to
165 the genes that are uniquely marked in FM and to a lesser extent in TM (Fig. 3a,b). This suggests
166 that specifically marked genes are kept under less stringent and tight repression which might be
167 due to their putative role in morphotype switch, which is known to be a dynamic process¹⁰.

168 Considering the conserved role of H3K27me3 in repression, we tested the effect of $E(z)$
169 knockout on gene expression. Therefore, RNA sequencing (RNA-seq) of two biological
170 replicates of the $E(z)$ mutant (Del6) was carried out and compared to previously generated
171 RNA-seq in the wild type (FM). Around 1/4 of all genes are (23%, 2795 out of 12152
172 annotations) differentially expressed in the $E(z)$ mutant (P-value. < 0.05), (Supplementary Fig.
173 3d; Supplementary Table S2), indicating an essential role in gene regulation by PRC2 in Pt.

174 We further monitored by RT-qPCR the expression of 27 specifically marked genes in the $E(z)$
175 knockout of the TM and found that 18 genes out of 27 showed a gain of expression in the mutant
176 compared to the TM background, demonstrating further that depletion of H3K27me3 likely
177 releases the repression of target genes and correlates with the loss of the triradiate morphology
178 (Fig. 3c). Although the remaining genes showed no change or even a gain in expression, these
179 genes can be targets of other repressive or active marks as shown previously with co-occurrence
180 of several repressive marks over genic regions⁹. The analysis of the R value³⁰ which reflects
181 the entropy, and therefore the variability in expression of genes between FM, TM and loci
182 marked in both morphotypes, showed a higher value in specifically marked genes compared to
183 commonly marked ones (Fig. 3d). This supports further the finding that specific enrichment in
184 each of the morphotypes are less silenced and potentially more dynamic compared to genes
185 marked by H3K27me3 in both TM and FM cells whereas commonly H3K27me3 marked genes
186 are globally silenced.

187 To substantiate the assumption that phenotypic plasticity and morphotype switch are regulated
188 by PRC2 in *P. tricornutum*, we took advantage of the lack of stability of the TM phenotype and
189 its tendency to switch to FM. Specifically, we used clonal cell samples with FM and TM
190 morphologies from the same genetic background (TM), which switches habitually to fusiform
191 and therefore contains a mixture of FM and TM cells. We reasoned that the activity of $E(z)$
192 should correlate with H3K27me3 levels in the following way: (1) a pure triradiate population
193 isolated from TM-N (named here TM-T1): highest level of H3K27me3), (2) a population of
194 cells from TM after N generations (N is 60 ± 5) of cell division containing a mixture of triradiate
195 and fusiform morphotypes (TM-N): medium level of H3K27me3) and (3) fusiform cells
196 isolated from the triradiate background TM (TM-Fusi): lowest level of H3K27me3 (Fig. 4a).
197 $E(z)$ transcript levels show a clear decrease in TM-N and TM-Fusi compared to TM-T1 (Fig.
198 4b) which correlates with the switch from TM to FM, reflecting a lower activity of $E(z)$ and
199 H3K27me3 levels. We then asked whether specifically H3K27me3-marked loci in TM lose the
200 mark upon cell switching to FM after multiple generations of sub-culturing leading to TM-N
201 and in transformed fusiform cells (TM-Fusi). As expected, ChIP-qPCR showed clearly a loss
202 of the mark in a population containing a mixture of fusiform and triradiate cells (TM-N) as well
203 as in TM-Fusi compared to TM-T1 (Fig. 4c,d), which contains only triradiate cells, thus
204 correlating the morphology with the level of enrichment in H3K27me3 over specific genes.

205 In summary, we have demonstrated in this study the role in *P. tricornutum* of $E(z)$ as a histone
206 methyltransferase responsible for di and tri-methylation of lysine 27 of histone H3. Knockout

207 of $E(z)$ causes H3K27me3 depletion and loss of triradiate cell shape maintenance, providing
208 evidence for the involvement of $E(z)$ and its associated mark in establishing and/or maintaining
209 cell morphology in unicellular species. We showed the dynamic nature of the mark, depending
210 on the specific morphology between and within *P. tricornutum* accessions that correlate with
211 the level of H3K27me3 enrichment. We showed differential marking in two different
212 accessions of *P. tricornutum*, FM versus TM, which identified genes related to cell fate
213 decisions compared to commonly marked genes. This is the first evidence of the involvement
214 of H3K27me3 in cell differentiation in unicellular eukaryotes only distantly related to animals
215 and plants. Our study points to the emerging function of PRC2 and its H3K27me3 associated
216 mark as a determinant of the establishment and maintenance of cell morphology in single celled
217 species such as *P. tricornutum* that shows signs of differentiation of the cell into diverse
218 morphologies. This same function likely diversified with the emergence of multicellularity with
219 PRC2 orchestrating development in plants and animals.

220

221 **Methods**

222 **Strains and growth conditions**

223 *Phaeodactylum tricornutum* Bohlin Clone Pt1 8.6 (CCMP2561) (referred as FM) and Clone
224 Pt8Tc (referred as TM) cells were grown as described previously ³¹.

225 **Isolation and immunoprecipitation of chromatin**

226 Chromatin isolation and immunoprecipitation were performed as described previously ³². The
227 following antibodies were used for immunoprecipitation: H3K27me3 (07-449) from Millipore
228 and H3K27me3 from cell signaling technology. qPCR on recovered DNA was performed as
229 described previously ³²

230 **CRISPR/Cas9 plasmid construction**

231 hCAS9n (Cas9 from *Streptococcus pyogenes*, adapted to human codon usage, fused to SV40
232 nuclear localization sequence, and containing a D10A mutation) was amplified from
233 pcDNA3.3-TOPO-hCAS9n (kindly received from Dr. Yonatan B. Tzur, Hebrew University of
234 Jerusalem), using the primers 5'-CAC CAT GGA CAA GAA GTA CTC-3' and 5'- TCA CAC
235 CTT CCT CTT CTT CTT-3'. The PCR product was first cloned into pENTR using pENTR/D-
236 TOPO cloning kit (ThermoFisher Scientific), and then sub-cloned into a PT pDest, containing

237 an N-terminal HA-tag³¹, following the manufacturer's protocol, which was named pDest-HA-
238 hCAS9n.

239 For the sgRNA vector we first cloned the snRNA U6 promoter³³ from *P. tricornutum* genomic
240 DNA using the primers 5'- AAA CGA CGG CCA GTG AAT TCT CGT TTC TGC TGT CAT
241 CAC C-3' and 5'- TCT TTA ATT TCA GAA AAT TCC GAC TTT GAA GGT GTT TTT TG-
242 3'. PU6::unc-119_sgRNA (kindly received from Dr. Yonatan B. Tzur) backbone was amplified
243 using the primers 5'-CAA AAA ACA CCT TCA AAG TCG GAA TTT TCT GAA ATT AAA
244 GA-3' and 5'-GGT GAT GAC AGC AGA AAC GAG AAT TCA CTG GCC GTC GTT T-3'.
245 The two PCR products were used as template for a second round fusion PCR reaction as
246 described in³⁴. We further transformed the resulting product into *E. coli*, and extracted the
247 ligated plasmid. The terminator sequence of the *P. tricornutum* U6 was amplified using the
248 primers 5'-CATTCTAGAAGAACCGCTCACCCATGC-3' and 5'-
249 GTTAAGCTTGAAAAGTTCGTCGAGACCATG-3', digested by XbaI/HindIII and ligated
250 into XbaI/HindIII digested pU6::unc-119. The resulting vector, ptU6::unc-119-sgRNA, was
251 used as template to replace the target sequence to *E(Z)* target by PCR using primers
252 32817TS12fwd GTG TCG GAG CCC GCC ATA CCG TTT TAG AGC TAG AAA TAG C
253 and 32817TS12rev GGT ATG GCG GGC TCC GAC ACC GAC TTT GAA GGT GTT TTT
254 TG. Target sequences were picked using PhytoCRISP-Ex³⁵.

255 **Transformation of *P. tricornutum* cells and screening for mutants**

256 Wild type cells of the reference strain FM and the TM were transformed with three plasmids
257 (pPhat1, Cas9 and guide RNA with the target sequence) as described previously³⁶. Positive
258 transformants were validated by triple PCR screen for pPhaT1 shble primers (ACT GCG
259 TGCACTTCGTGGC/TCGGTCAGTCCTGCTCCTC), sgRNA
260 (GAGCTGGAAATTGGTTGTC/GACTCGGTGCCACTTTTTCAAGTT) and CAS9n
261 (GGGAGCAGGCAGAAAACATT/TCACACCTTCCTCTTCTTCTT). For each colony, a
262 rapid DNA preparation was performed as described previously and fragment of 400 bp was
263 amplified with primers flanking the target sequence in the *E(z)* gene. The forward primer used
264 is 5'-TAAGATGGAGTATGCCGAAATTC-3' and reverse primer is 5'-
265 AGGCATTTATTCGTGTCTGTTCG-3' PCR product was run in 1% agarose gel and a single
266 band was extracted using Machery Nagle kit and according to the manual manufacturer. PCR
267 product was sequenced using the primer 5'-AGCCACCCTGCGTAACTGAAAAT-3'.

268 To make sure that the fusiform cells originating from the switch of TM are not contaminants
269 from the FM, each of the TM-T1, TM-Fusi and TM-N were checked for their genetic
270 background whether it is FM or TM using a molecular marker designed around a 400 bp
271 insertion in the FM background (Supplementary Fig. 4g), identified from genome sequencing
272 of FM and TM strains of *P. tricornutum*³⁷. The PCR check confirmed that all the cell samples
273 described above are in the TM genetic background.

274

275 **Validation of enrichment and expression of target genes**

276 **qPCR:** Total RNA was extracted from TM and FM cells as described previously³¹ and cDNA
277 was synthesized with cDNA high yield synthesis kit according to the manufacturer user manual.
278 Quantitative PCR was performed as described previously³¹ using the primer list in
279 Supplementary Table S3

280 **Proteomics and PRM Measurements**

281 Three independent histone purifications recovered from FM wild type cells as well as *E(z)*
282 knockout mutant Del6 in FM genetic background were simultaneously separated by SDS-
283 PAGE and stained with colloidal blue (LabSafe Gel Blue GBiosciences). Three gel slices were
284 excised for each purification and in-gel digested by using trypsin/LysC (Promega). Peptide
285 extracted from each set were pooled and analyzed by nanoLC-MS/MS using an Ultimate 3000
286 system (Thermo Scientific) coupled to a TripleTOFTM 6600 mass spectrometer (AB Sciex).
287 Peptides were first trapped onto a C18 column (75 μ m inner diameter \times 2 cm; nanoViper
288 Acclaim PepMapTM 100, Thermo Scientific) with buffer A (2/98 MeCN/H₂O in 0.1% formic
289 acid) at a flow rate of 2.5 μ L/min over 4 min. Separation was performed on a 50 cm x 75 μ m
290 C18 column (nanoViper C18, 3 μ m, 100 \AA , Acclaim PepMapTM RSLC, Thermo Scientific)
291 regulated to 50°C and with a linear gradient from 1% to 30% buffer B (100 MeCN in 0.085%
292 formic acid) at a flow rate of 400 nL/min over 90 min. The mass spectrometer was operated in
293 PRM top30 high sensitivity mode with 100 ms acquisition time for MS1 and MS2 scans
294 respectively with included precursor mass list for 600 sec (see Supplementary Table S4)

295 **PRM Data Analysis**

296 The PRM data were analyzed using Skyline version 3.7.0.11317 MacCoss Lab Software,
297 Seattle, WA; <https://skyline.ms/project/home/software/Skyline/begin.view>, fragment ions for
298 each targeted mass were extracted and peak areas were integrated. The peptide areas were log₂

299 transformed and the mean log₂- area was normalized by the mean area of peptide STDLLIR
300 using software R version 3.1.0. On each peptide a linear model was used to estimate the mean
301 fold change between the conditions, its 97.5% confidence interval and the p-value of the two
302 sided associated t-test. The p-values were adjusted with the Benjamini-Hochberg procedure³⁸.
303 The mass spectrometry proteomics data have been deposited to the ProteomeXchange
304 Consortium via the PRIDE [1] partner repository with the dataset identifier PXD012347.

305

306 **Western blot analysis:** Chromatin was extracted from wild type as well as mutants of both TM
307 and FM cells and western blot performed as described previously³².

308

309 **Sequencing and computational data analysis**

310 **ChIP-Seq:** Chromatin Immunoprecipitation (ChIP) was done with monoclonal cell cultures
311 grown using single triradiate cell from Pt8 population (referred as TM). CHIP-Seq was
312 performed as described previously^{15,39}. Two replicates were performed and showed a good
313 Pearson correlation (Supplementary Fig. 5). Raw reads were filtered and low quality read-pairs
314 were discarded using FASTQC with a read quality (Phred score) cutoff of 30. Using the genome
315 assembly published in 2008 as reference (Pt1 8.6), we performed reference-assisted mapping
316 of filtered reads using BOWTIE. We then performed the processing and filtering of the
317 alignments using SAMTOOLS and BEDTOOLS. SICER⁴⁰ was then used to identify
318 significant enriched H3K27me3 peaks by comparing it with the INPUT. Differential
319 H3K27me3 peak enrichment analysis between FM and TM backgrounds was also done using
320 SICER-df plugin. Peaks with Padj < 0.05 differential enrichment or depletion were considered
321 significant. Functional inferences were obtained by overlapping the differentially enriched
322 peaks over structural annotations from Phatr3 genome annotation⁴¹.

323 **RNA sequencing (RNA-Seq):** Total RNA was extracted from FM, TM, and FM Ez-KO (Del6)
324 cell lines. RNA expression and differential gene expression analysis was performed using
325 Eoulsan version 1.2.2 with default parameters⁴². Genes having at least 2 folds expression
326 change with P-value < 0.05 were considered as significant different expressed genes (DEGs).

327 **Whole genome sequencing (WGS):** Whole genome sequencing was performed using DNA
328 extracted from monoclonal cell cultures grown using single triradiate cell taken from Pt8 and
329 Pt1 accession, referred to as Pt8tc and Pt1 8.6, respectively. At least 6 µg of genomic DNA
330 from each accession was used to construct a sequencing library following the manufacturer's

331 instructions (Illumina Inc.). Paired-end sequencing libraries with a read size of 100 bp and an
332 insert size of approximately 400 bp were sequenced on an Illumina HiSeq 2000 sequencer at
333 Berry Genomics Company (China) and Fasteris for Pt1 8.6 and Pt8tc, respectively. Low quality
334 read-pairs were discarded using FASTQC with a read quality (Phred score) cutoff of 30. Using
335 the genome assembly published previously⁴³, we performed reference-assisted assembly of all
336 the accessions. We used BOWTIE (-n 2 -X 400) for mapping the high quality NGS reads to
337 the reference genome followed by the processing and filtering of the alignments using
338 SAMTOOLS and BEDTOOLS. For estimating the genetic diversity between Pt1 8.6 and Pt8Tc
339 genome, GATK⁴⁴ configured for diploid genomes, was used for variant calling, which included
340 single nucleotide polymorphisms (SNPs), small insertions and deletions ranging between 1 and
341 300 base pairs (bp). The genotyping mode was kept default (genotyping mode =
342 DISCOVERY), Emission confidence threshold (-stand_emit_conf) was kept 10 and calling
343 confidence threshold (-stand_call_conf) was kept at 30. The minimum number of reads per base
344 to be called as a high quality SNP was kept at 4 (i.e., read-depth $\geq 4x$). SNPEFF was used to
345 annotate the functional nature of the polymorphisms.

346 **GO enrichment analysis:** GO categories were grouped by 3 different levels of expression
347 according to a simple density clustering algorithm (also confirmed by iterative k-means
348 clustering).

349 **Data Availability:**

350 All data are available through NCBI Sequence Read Archive with accession number
351 PRJNA565539.

352

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474

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484 **Competing interests**

485 The authors declare no competing interests.

486

487 **Figure legends**

488 **Figure 1. *Phaeodactylum tricornutum* morphotypes and enhancer of zeste knockout**
489 **mutants. (a)** *P. tricornutum* morphotypes (top left fusiform, scale bar = 3 μ m, top right oval,
490 bottom left triradiate, scale bar = 2 μ m, bottom right cruciform). Scale bars correspond to 1 μ m
491 in OM and CM. **(b)** Sequence chromatograms of PCR product from WT cells and CRISPR cas9
492 mutants of enhancer of zeste showing the different indels in FM, TM and CM. **(c)** Light
493 microscopy images of WT, E(z) KO and Cas9 control cells. Empty vector controls containing
494 Cas9 and Shble antibiotic resistance gene show no loss of H3K27me3 and retain the wild type
495 morphology suggesting that morphology distortion is not due to the transformation but to the
496 absence of H3K27me3. **(d)** Western blots of WT and two E(z)KOs from each morphotype
497 using a monoclonal antibody against H3K27me3. Histone H4 was used as a loading control.
498 ChIP-QPCR enrichment levels of H3K27me3 on genes in WT and E(z) KO in FM **(e)** and TM
499 **(f)** backgrounds.

500

501 **Figure 2. Genomic features of H3K27me3 targets in FM and TM cells. (a)** Venn diagrams
502 showing the number of common and specific genomic features [Genes, Transposable elements
503 (TEs), and Intergenic Regions (IGRs)] targeted by H3K27me3 in TM (orange circles) and FM
504 (blue circles). ChIP-QPCR validation of H3K27me3 specifically marked genes in **(b)** TM and
505 **(c)** FM morphotypes. **(d)** Distribution of the most frequent GO terms on genes marked with
506 H3K27me3. The distribution was sub-divided into different categories, where TM and FM
507 represent the GOs observed exclusively on triradiate and fusiform genes, respectively. A third
508 category (Both) also presents a GO distribution for genes observed on both morphotypes.

509

510

511 **Figure 3. Gene expression profiles of 3HK27me3 specific and common targets in FM and**
512 **TM. (a)** The box plot represents mean enrichment of H3K27me3 (Y-axis), with log₁₀ scaling,
513 over genes marked specifically in FM (blue), TM (orange), and also on genes marked in both
514 (commonly marked) morphotypes. The enrichment profile is generated using number of genes
515 marked by H3K27me3 specifically in each morphotype and also in both. The significant
516 H3K27me3 enrichment difference between specifically marked and commonly marked is
517 estimated using two-tailed t-test with P value < 0.0001, denoted by “*****”. **(b)** Expression of

518 genes marked specifically in FM (blue), TM (orange), and in both phenotypic backgrounds with
519 same principle aesthetics and categorical genes, used in 2f. The significance/non-significance
520 of the variability of expression between specifically and commonly marked genes is estimated
521 using two-tailed t-test with P value = 0.0433, as denoted by “*”. “ns” denote non-significant.
522 (c) Relative expression level of H3K27me3 targets genes in the TM and enhancer of zeste knock
523 out M2-11. In the plot, fold change $\log_2(\text{PtM2-11}/\text{Pt8Tc})$ values are shown (d) Boxplot
524 showing the entropy value distribution of H3K27me3 marked genes. Entropy values measures
525 the differential expression of genes under different experimental conditions. Entropy values are
526 derived from expression data (fragments per kilobase of exon per million fragments mapped)
527 under five different experimental conditions. Genes marked specifically by H3K27me3 in TM
528 (Triradiate) shows higher variation in expression followed by FM (Fusiform) specific
529 H3K27me3 marked genes. Horizontal lines represent the median entropy values. The
530 significant of FM/TM-specific H3K27me3 marked genes and genes marked on both conditions
531 are estimated using two-tailed t-test with P value < 0.0001.

532

533

534 **Figure 4. H3K27me3 enrichment levels and morphotype switch.** (a) Schematic diagram
535 showing how TM-N, TM-T1 and TM-Fusi were generated. After generations of culture in lab
536 growth condition (ASW media, 19°C, 12h/12h light dark period), some triradiate cells switch
537 to fusiform forming a mixture of FM and TM cells, named TM-N. A single fusiform cell from
538 TM-N was picked and propagated clonally giving rise to TM-Fusi. Similarly, single triradiate
539 cell was isolated from TM-N and its clonal propagation gave a population of pure triradiate
540 cells named TM-T1. (b) Relative expression level of Enhancer of Zest in FM, TM-N, TM-T1
541 and TM-Fusi respectively, two pairs of primer were designed at N-terminal of Enhancer of
542 Zeste gene, and at the CXC domain respectively. (c) ChIP-QPCR enrichment levels of
543 H3K27me3 in TM-T1 and TM-Fusi. (d) ChIP-QPCR showing enrichment levels of H3K27me3
544 in TM-T1 and TM-N.

545

546 **Supplementary Table 1.** Cell counts of different morphologies and cell size measurements in
547 each wild type morphotype and knock out of E(z)

548 **Supplementary Table 2.** List of genes marked by H3K27me3 with their annotation and GOs.

549 **Supplementary Table 3.** List of the primers used in this study

550 **Supplementary Table 4.** Mass spectrometry quantification of Di- and Tri-methylation of
551 H3K27me3

552 **Supplementary Figure 1.** Phylogeny (a-c). (d) Western blots using a monoclonal antibody
553 against H3K27me3 on protein or chromatin extracts of different species representative of the
554 super SAR lineage (1: *Bigelowiella_natans*, 2: *Gymnophora dimorpha*, 3: *Skeletonema*
555 *marinoi*, 4: *Thalassiosira pseudonana*, 5: *Raphoneis sp*, 6: *Synedra sp*, 7:
556 *Asterionellopsisglacialis*, 8: *Thalassiosira rotula*, 9: *Phaeodactylum tricornutum*, 10:
557 *Isocrhysis lutea*, 11: *Amhedinium klebselii*, 12: *Amhedinium carteri*). (e) Western blot on
558 chromatin extracts from OM, FM, TM and CM with a monoclonal antibody against H3K27me3
559 showing a significant difference in enrichment levels of H3K27me3 in TM and CM which are
560 higher compared to FM and OM.

561

562 **Supplementary Figure 2.** (a) Western blot of chromatin extracts of Cas9 control lines from
563 each of FM, TM and CM. H4 histone antibody was used as a loading control. (b, c) Mass
564 spectrometry quantification of di and tri-methylation of lysine 27 of histone H3 in both wild
565 type and enhancer of zeste knockout mutant. MS/MS spectrum of the $[M + 2H]^{2+}$ precursor ion
566 of histone H3 (27–36 residue peptide) tri-methylated or di-methylated on K27. Broken bonds
567 above and below sequence denote b and y ions, respectively, that were annotated from the
568 spectrum. (d) Abundance of H3 K27 di- and tri-methylated KSAPATGGVK peptide. Y axis
569 shows normalized \log_2 (WT/Mutant) of the di-methylated and tri-methylated peptides. All
570 measurements have been performed in triplicate, and error bars indicated the 97.5% confidence
571 interval (see supplementary Table 1). (e) Western blot of chromatin extracts from wild type FM
572 and TM as well as E(z) knockouts in both backgrounds with a monoclonal antibody against
573 H3K27me2. H4 histone antibody was used as loading control. (f) Growth curves of wild type,
574 enhancer of zeste mutants and cas9 control line in each of FM, TM and CM. (g) Gel picture of
575 a molecular marker distinguishing FM and TM and amplifying an insertion in one allele present
576 in FM but absent in TM.

577

578 **Supplementary Figure 3.**

579 (a) Total genome coverage of H3K27me3 within TM (orange) and FM (blue) showing a higher
580 mapping of H3K27me3 in TM compared to FM. (b) mean distribution of H3K27me3 over 500
581 bp upstream, gene body, and 500 bp downstream region of all the gene targets in TM (orange
582 line) and FM (blue line). (c) Unmarked genes (J01910, J31617), commonly marked genes
583 (J34600, J44413) were chosen as internal controls. FM (EG00164) and TM specifically marked
584 (J49062) genes were used as controls for the reproducibility of independent ChIP-QPCR
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586 TM compared to the wild-type (WT). The significant expression difference between Ez(KO)
587 and WT is estimated using two-tailed t-test with P value < 0.0001

588

589 **Supplementary Figure 4.** Scatter plots with Pearson correlation coefficient displaying the
590 relationship between TM and E(z) knock out RNA Seq replicates.

591 **Supplementary Figure 5.** Scatter plots with Pearson correlation coefficient displaying the
592 relationship between TM ChIP-Seq replicates

593

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605 *Asterionellopsis glacialis*, 8: *Thalassiosira rotula*, 9: *Phaeodactylum tricornutum*, 10:
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637

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640 **Supplementary Figure 5.** Scatter plots with Pearson correlation coefficient displaying the
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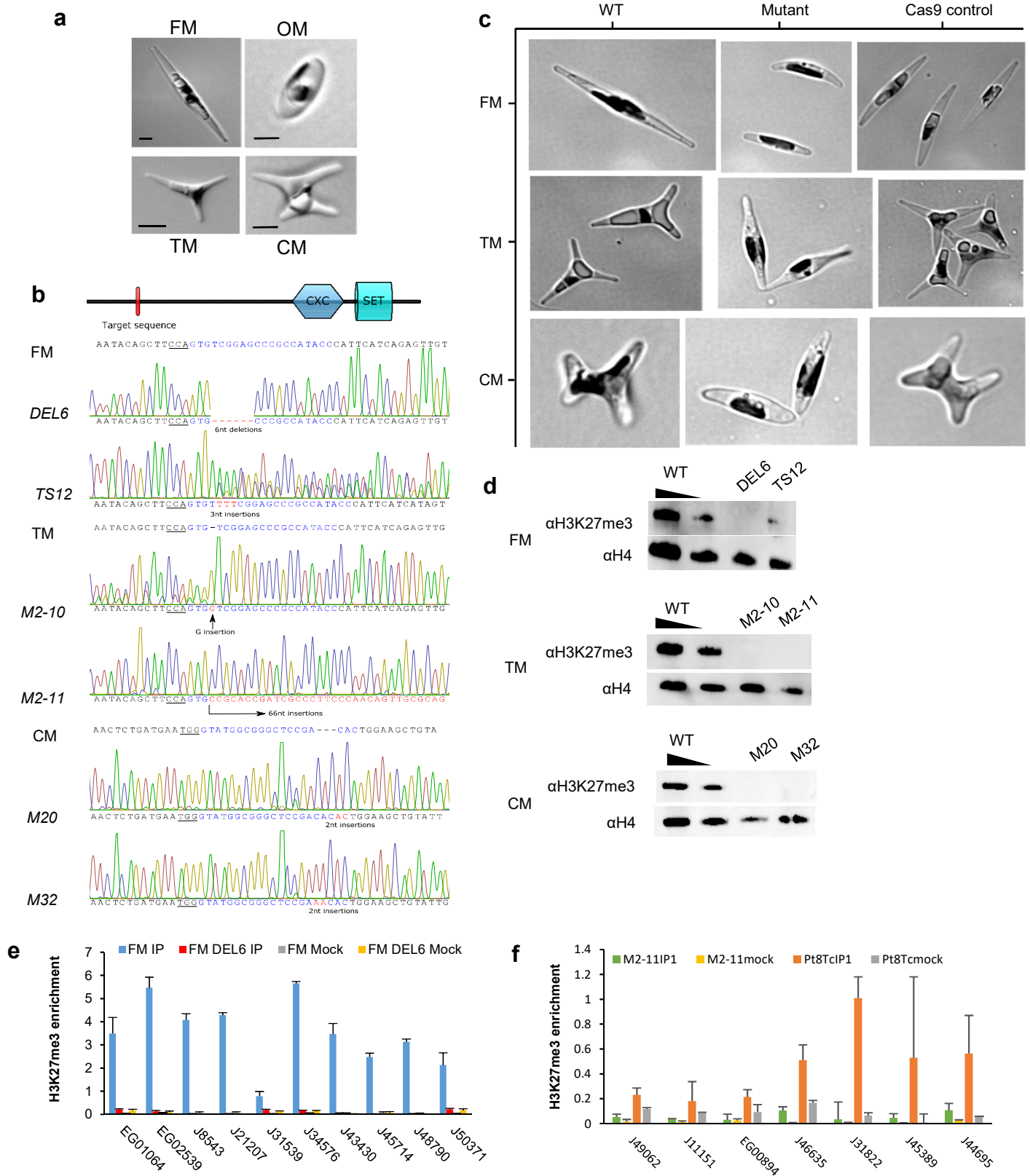


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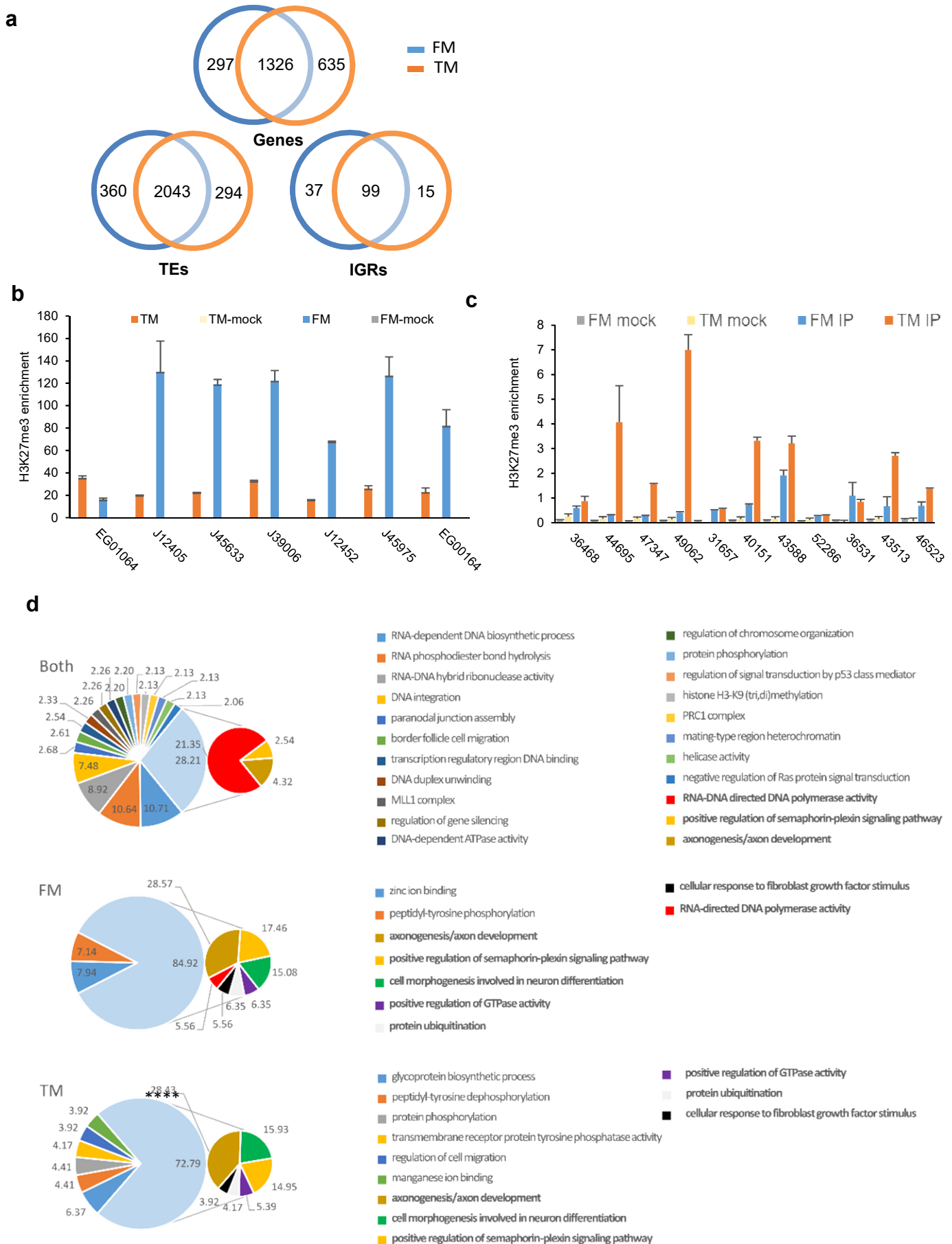


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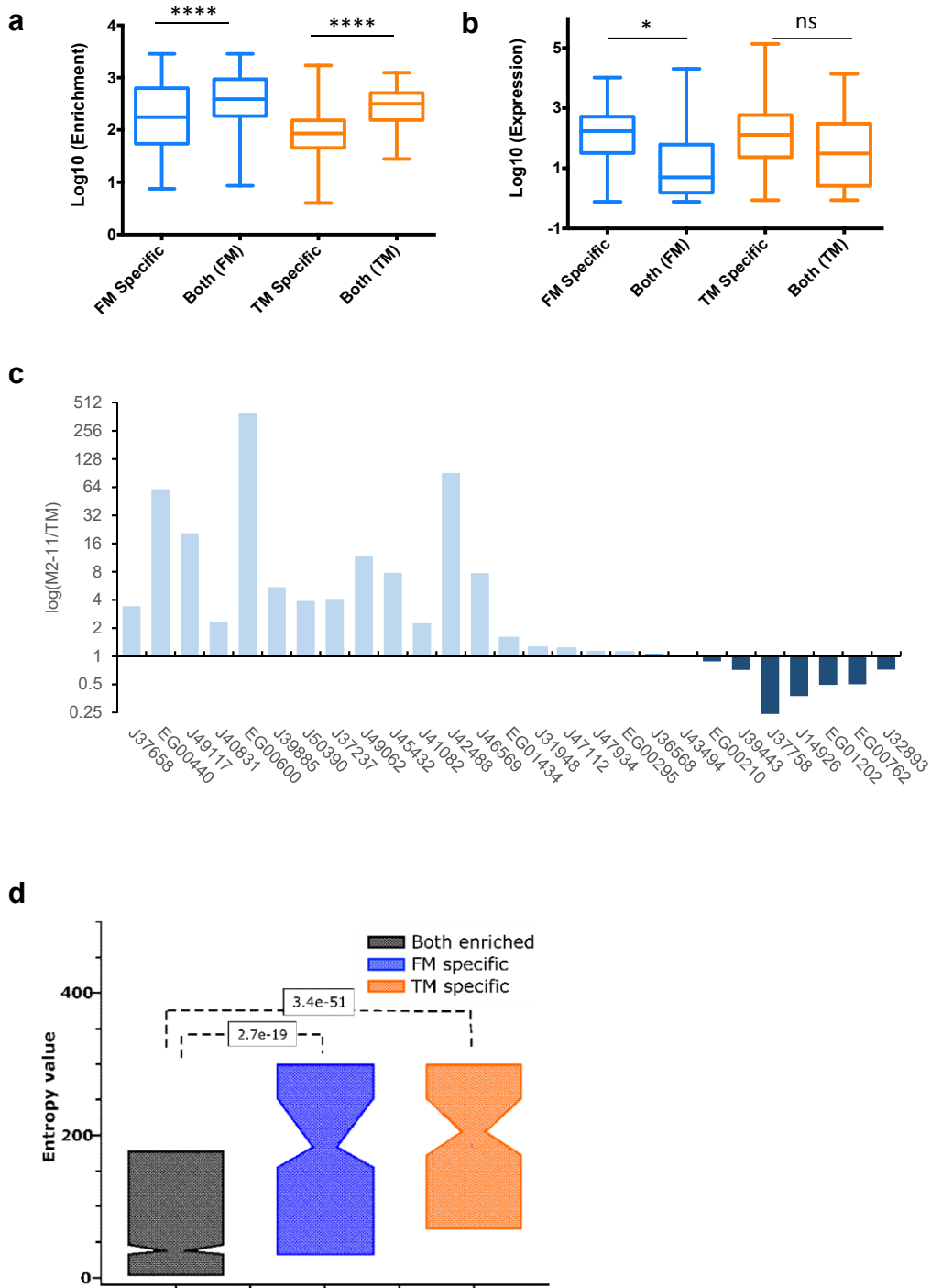


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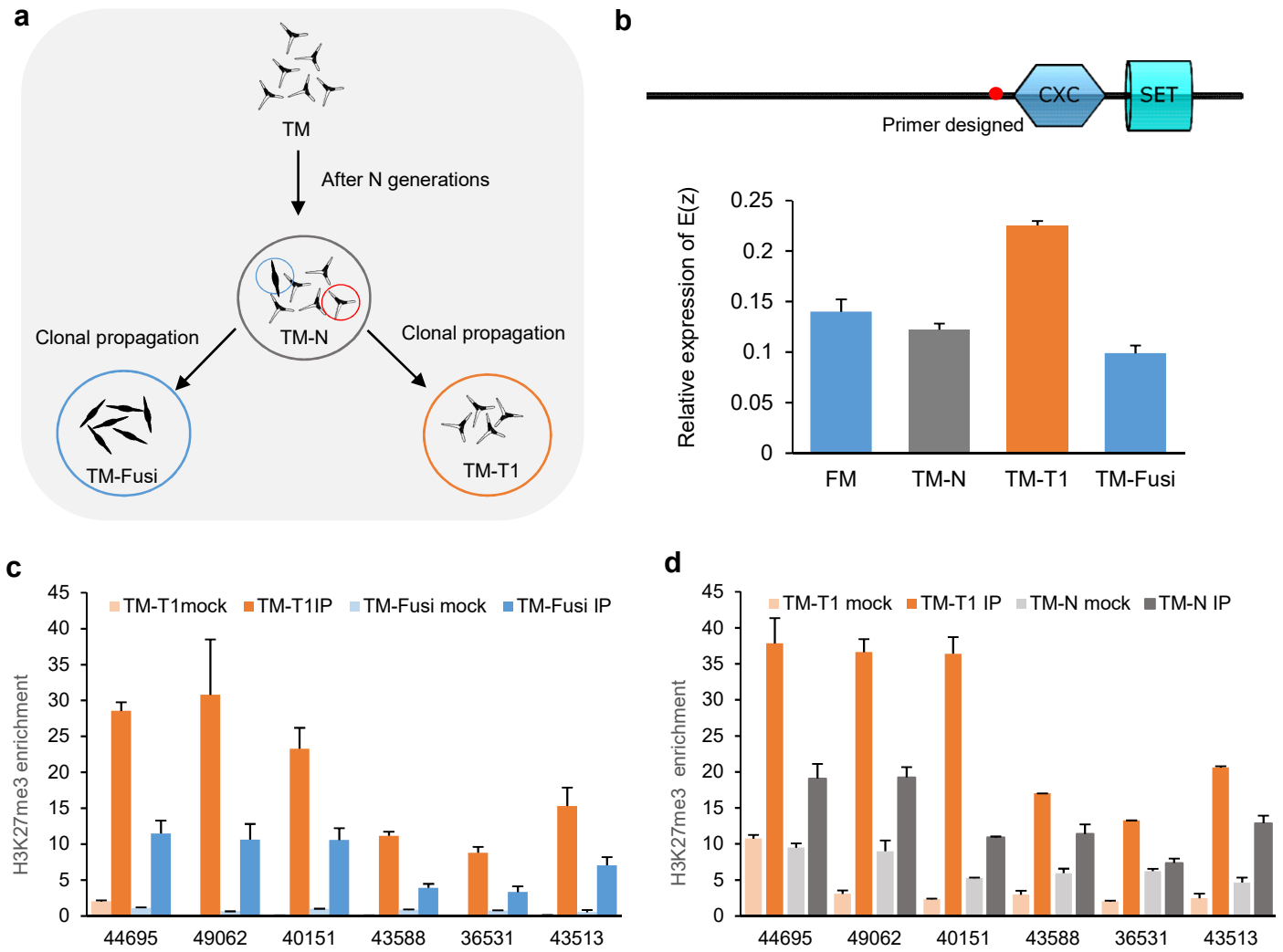


Figure 4

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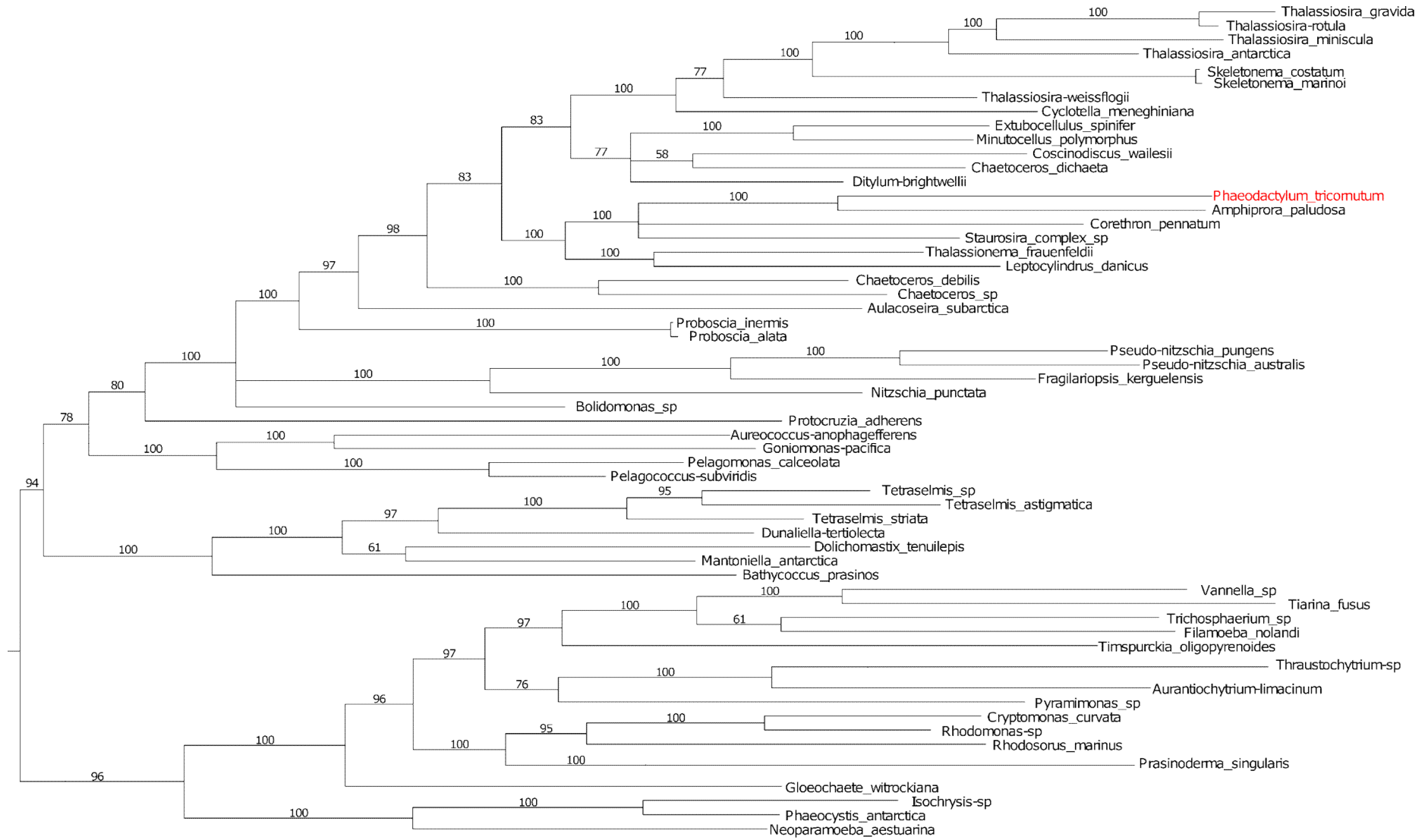


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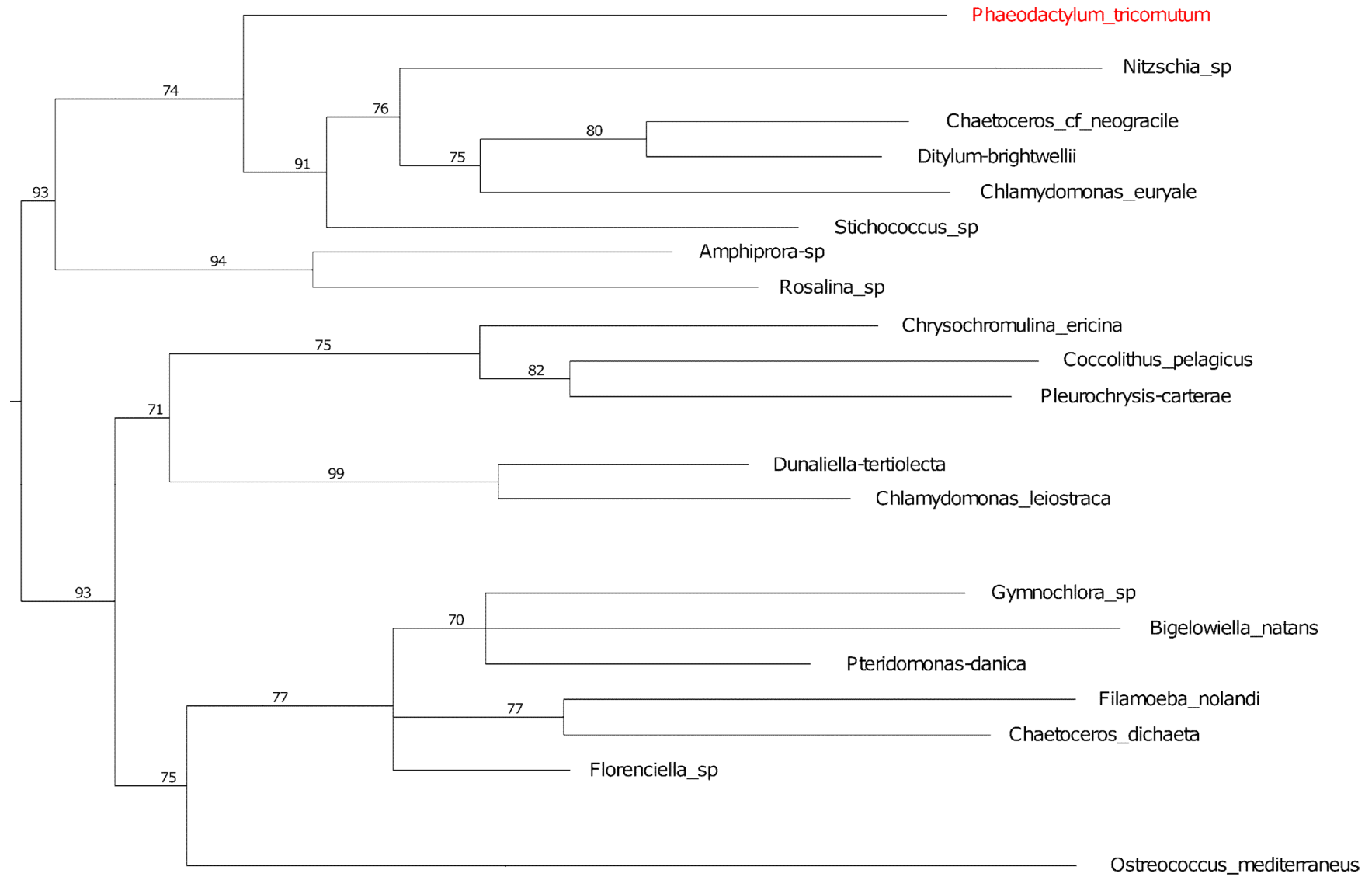


Figure S1

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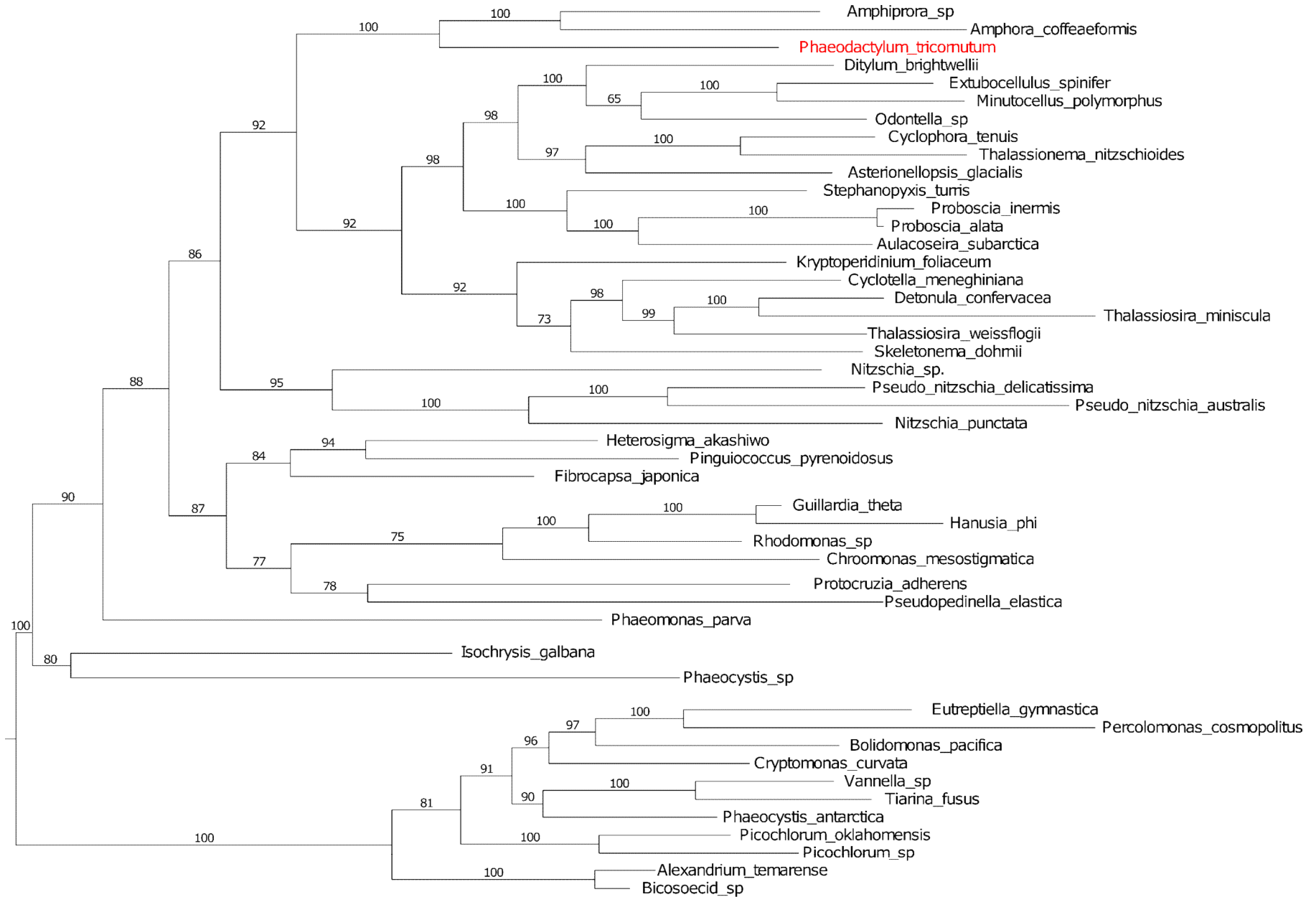
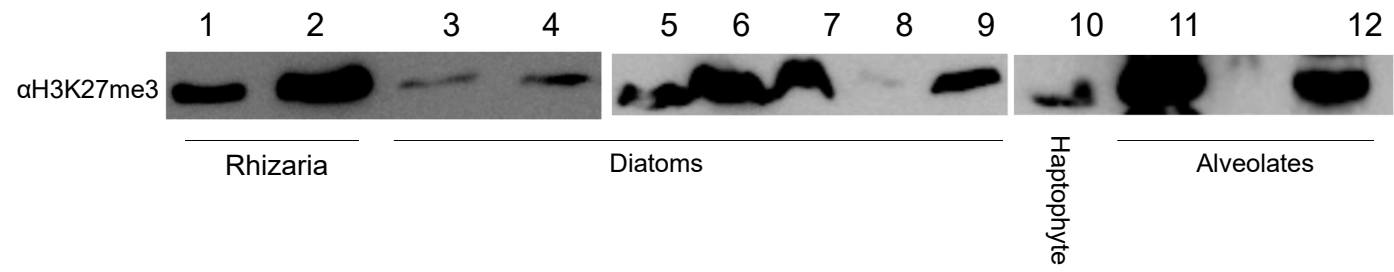


Figure S1

d



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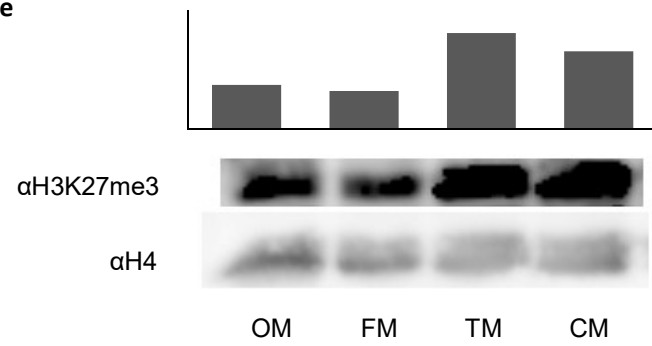


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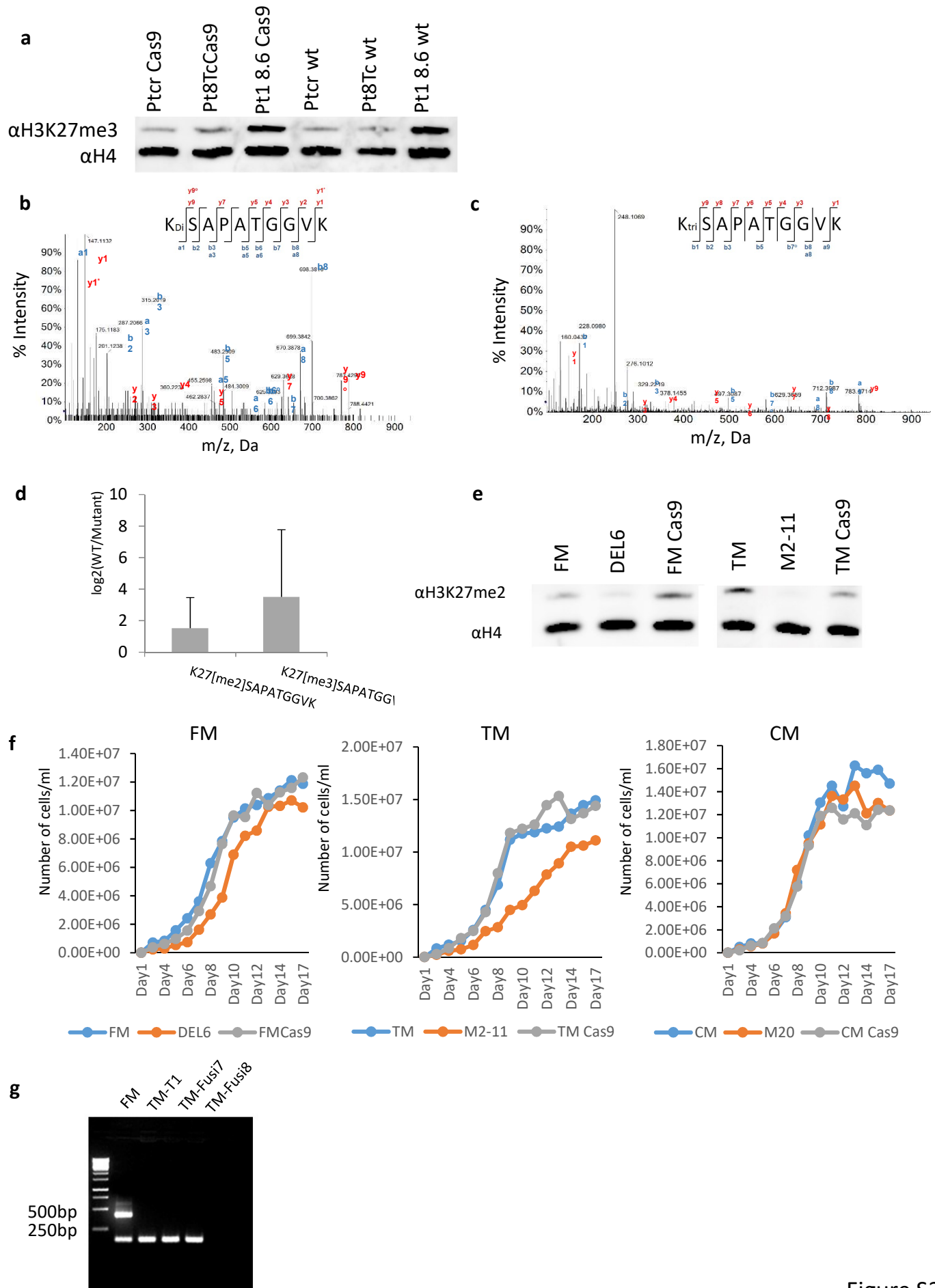


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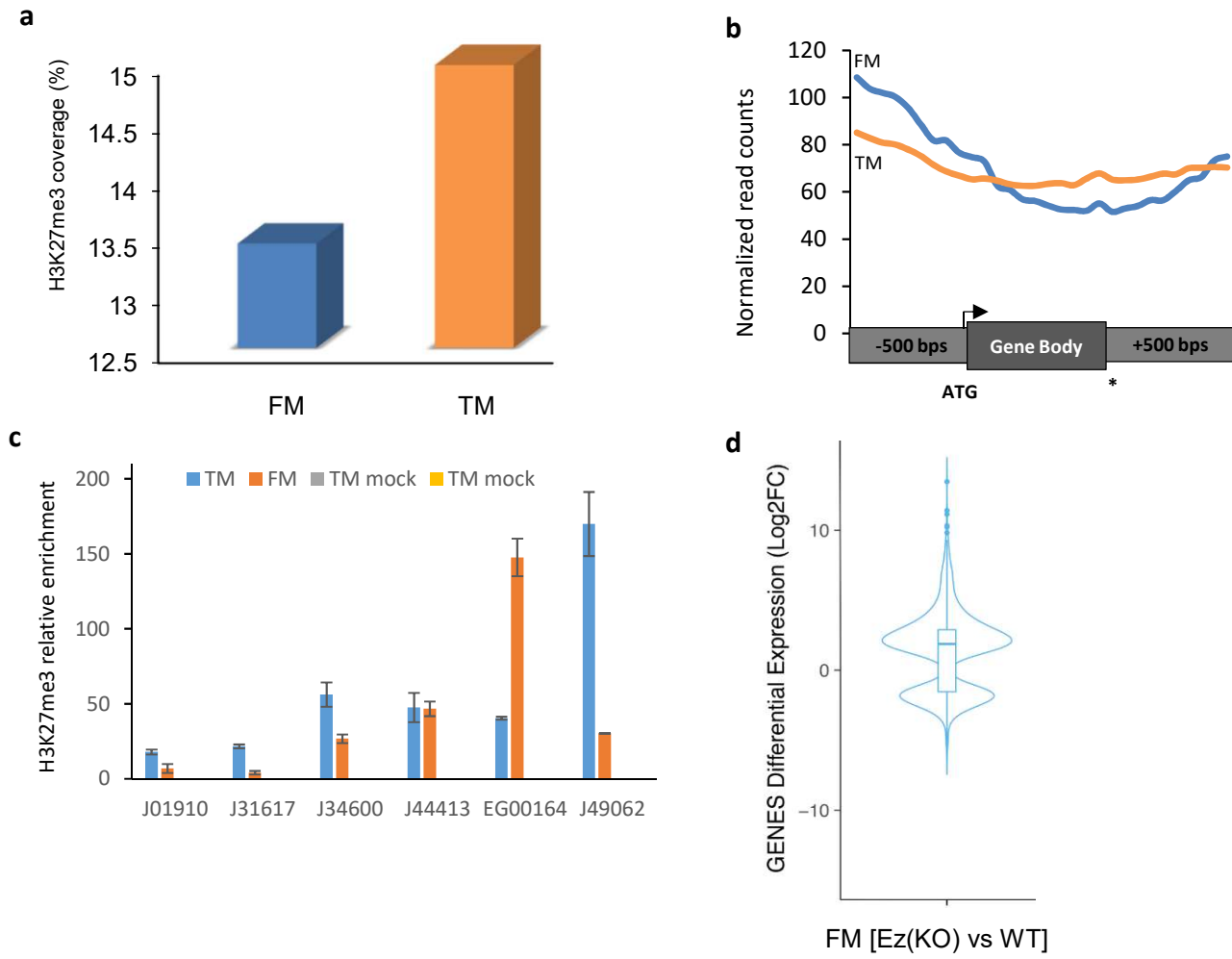


Figure S3

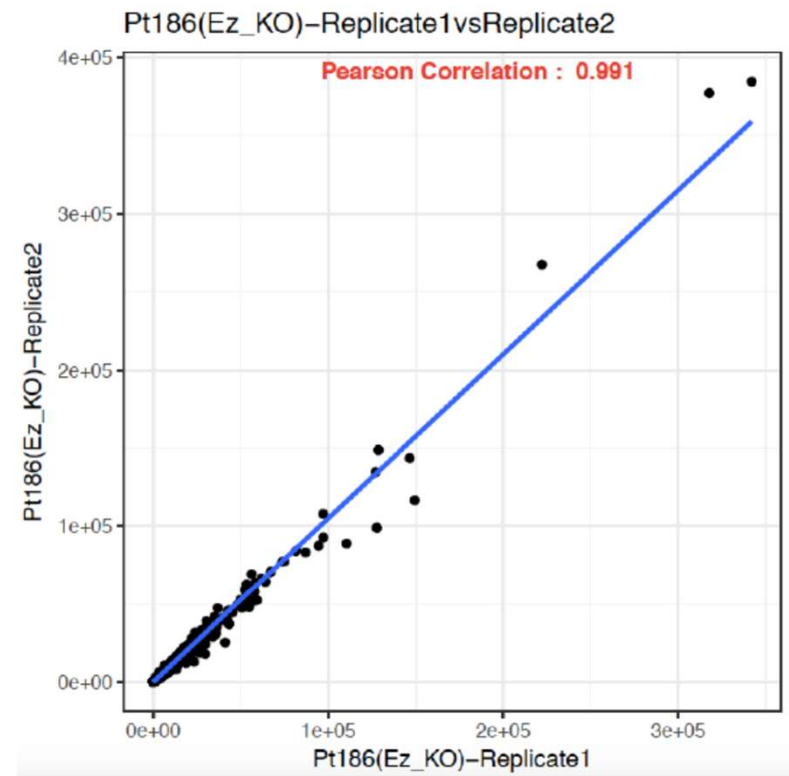
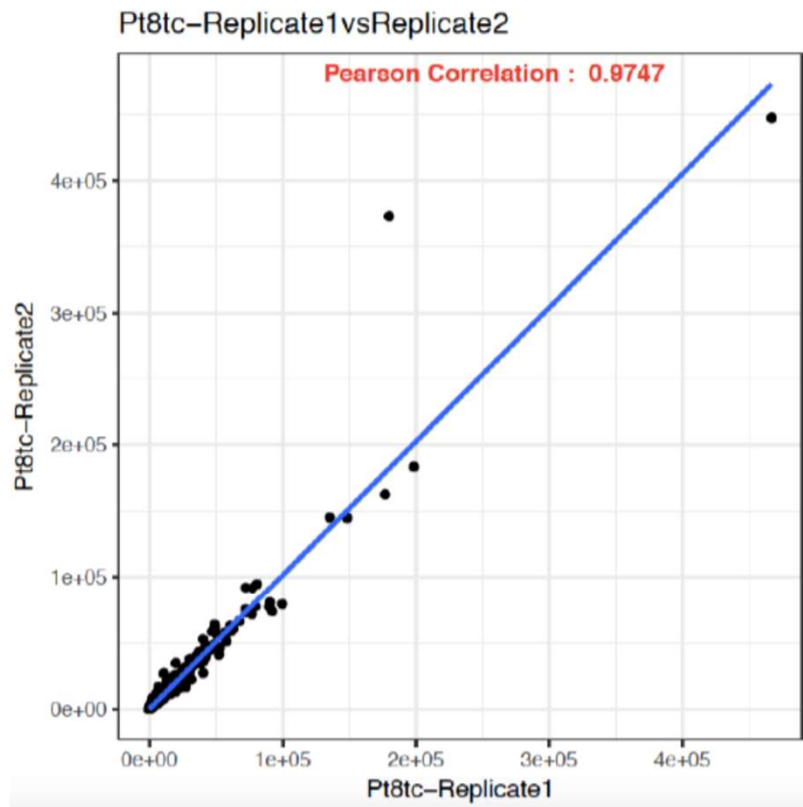


Figure S4

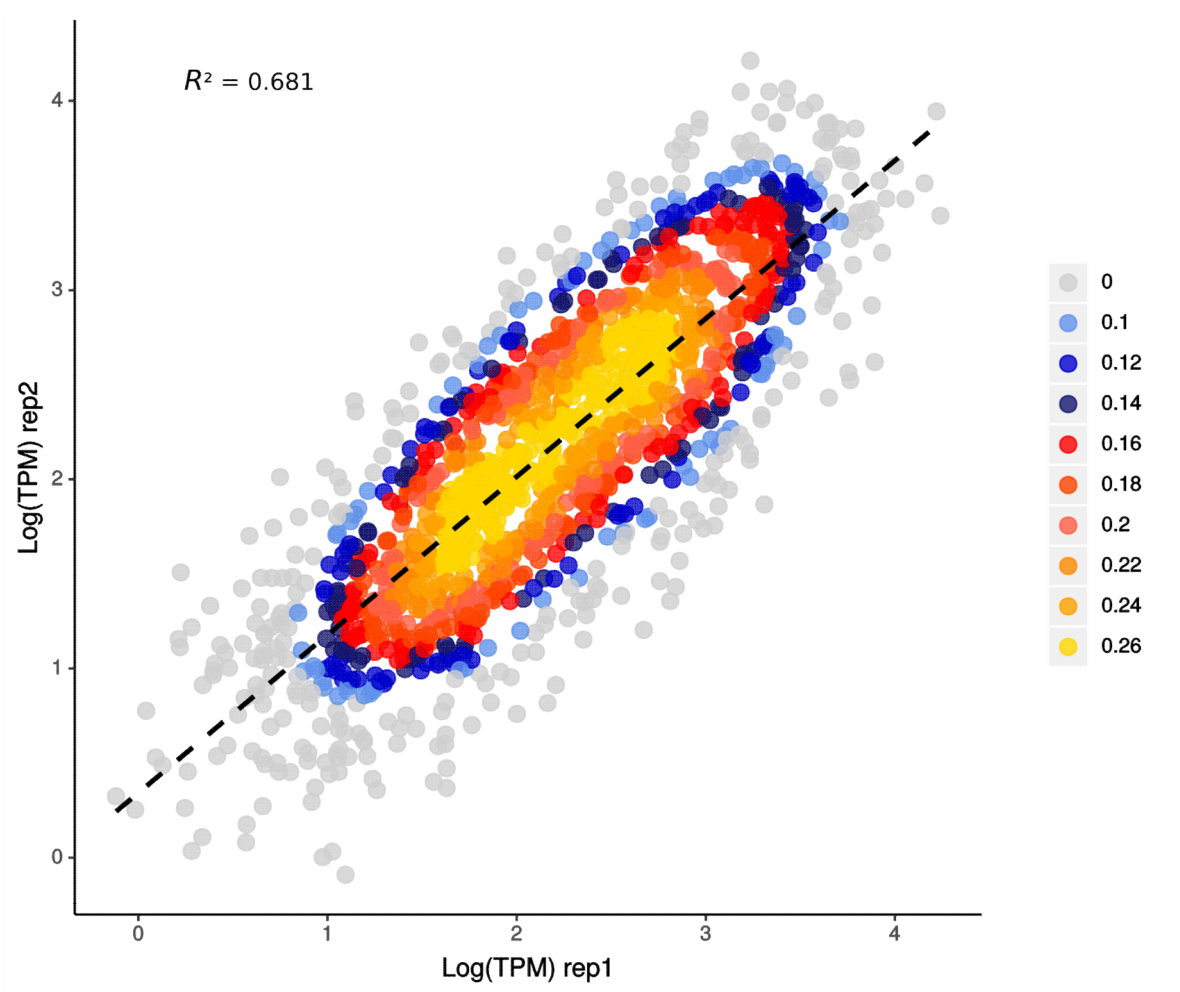


Figure S5

Table 1. Cell counts of different morphologies and cell size measurements in each wild type morphotype and knock out of *E(z)*

	FM	TM	CM	OM	Majority	Percentage	Size
FM	107	0	0	0	FM	100%	26.58 μ m
TM	0	100	0	0	TM	100%	L:13.59 μ m; W:11.48 μ m
CM	4	31	66	0	CM	67.34%	-
OM	0	0	0	100	OM	100%	-
TM-N	63	55	0	0	FM	53.38%	-
T1	0	219	0	0	TM	100%	-
DEL6	74	0	0	0	FM	100%	19.91 μ m
TS12	41	0	0	0	FM	100%	-
M2-10	65	0	0	0	FM	100%	-
M2-11	74	0	0	0	FM	100%	21.04 μ m
M20	32	0	0	0	FM	100%	23.59 μ m

Table 2. Mass spectrometry quantification of Di- and Tri-methylation of H3K27me3

peptide sequence	Protein Modification (without Nterm Met)	peptide used for normalization	log ₂ (WT/Mutant)	CI 2.5%	CI 97.5%	adjusted p value
KSAPATGGVK	Dimethyl (K:27)	STDLLIR	1.52038639	1.09023167	1.95054112	2.10E-07
KSAPATGGVK	Trimethyl (K:27)	STDLLIR	3.5091078	2.7625983	4.25561731	7.82E-10