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1 **The HDAC inhibitor trichostatin A impairs pancreatic β -cell**
2 **function through an epigenome-wide reprogramming**

3 Frédéric Oger^{1, *}, Maeva Moreno¹, Mehdi Derhourhi¹, Bryan Thiroux¹, Lionel
4 Berberian¹, Cyril Bourouh¹, Emmanuelle Durand¹, Souhila Amanzougarene¹, Alaa
5 Badreddine¹, Etienne Blanc¹, Olivier Molendi-Coste², Laurent Pineau², Gianni
6 Pasquetti³, Laure Rolland^{1, 4}, Charlène Carney¹, Florine Bornaque^{1, 4}, Emilie Courty^{1,}
7 ⁴, Céline Gheeraert², Jérôme Eeckhoute², David Dombrowicz², Julie Kerr-Conte³,
8 François Pattou³, Bart Staels², Philippe Froguel^{1, 5}, Amélie Bonnefond^{1, 5, †} and Jean-
9 Sébastien Annicotte^{1, 4, †, *}

10 ¹ Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, CNRS, U1283 - UMR 8199 -
11 EGID, F-59000 Lille, France.

12 ² Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011 - EGID, F-59000, Lille,
13 France.

14 ³ Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1190 - EGID, F-59000, Lille,
15 France.

16 ⁴ Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1167 – RID-AGE-Facteurs
17 de risque et déterminants moléculaires des maladies liées au vieillissement, F-59000,
18 Lille, France.

19 ⁵ Department of Metabolism, Digestion and Reproduction, Imperial College London;
20 London, United Kingdom

21 **Footnotes**

22 † Co-senior authors

23 * Corresponding authors:

24 frederik.oger@univ-lille.fr

25 jean-sebastien.annicotte@inserm.fr

26 Running title: HDAC and β -cell function

27 **Abstract**

28 **Objective:** The pancreatic islets of Langerhans contain distinct cell subtypes including
29 insulin-producing β cells. Although their cell-specific gene expression pattern defines
30 their identity, the underlying molecular network driving this transcriptional specificity is
31 not fully understood. Among the numerous transcriptional regulators, histone
32 deacetylases (HDAC) enzymes are potent chromatin modifiers which directly regulate
33 gene expression through deacetylation of lysine residues within specific histone
34 proteins. The precise molecular mechanisms underlying HDAC effects on cellular
35 plasticity and β -cell identity are currently unknown.

36 **Methods:** The pharmacological inhibition of HDAC activity by trichostatin A (TSA) was
37 studied in the mouse Min6 and human EndocBH1 cell lines, as well as primary mouse
38 sorted β cells and human pancreatic islets. The molecular and functional effects of
39 treating these complementary β -cell models with TSA was explored at the epigenomic
40 and transcriptomic level through next-generation sequencing of chromatin
41 immunoprecipitation (ChIP) assays (ChIP-seq) and RNA sequencing (RNA-seq)
42 experiments, respectively.

43 **Results:** We showed that TSA alters insulin secretion associated with β -cell specific
44 transcriptome programming in both mouse and human β -cell lines, as well as on
45 human pancreatic islets. We also demonstrated that this alternative β -cell
46 transcriptional program in response to HDAC inhibition is related to an epigenome-
47 wide remodeling at both promoters and enhancers.

48 **Conclusions:** Taken together, our data indicate that full HDAC activity is required to
49 safeguard the epigenome, to protect against loss of β -cell identity with unsuitable
50 expression of genes associated with alternative cell fates.

51

52 **Keywords:** epigenome, HDAC, insulin secretion, pancreatic beta cell.

53 1. Introduction

54 The endocrine pancreas is composed of distinct cell subtypes including α
55 (producing and secreting glucagon), β (producing and secreting insulin), δ (producing
56 and secreting somatostatin), ϵ (producing and secreting ghrelin) and PP (producing
57 and secreting pancreatic polypeptide) cells that play a crucial role in the regulation of
58 glucose homeostasis [1]. A specific gene expression pattern defines each endocrine
59 cell identity but the underlying molecular network that controls this transcriptional
60 specificity remains elusive. The roles of some tissue-specific transcription factors, such
61 as PDX1 or MAFA, in the maintenance of expression of genes controlling β -cell
62 phenotype is well-known [2], but the contribution of chromatin modifiers in the
63 maintenance of β -cell identity is less documented. Among these putative epigenomic
64 regulators, there are histone acetyl transferases (HAT) and histone deacetylases
65 (HDAC) that directly regulate gene expression through acetylation/deacetylation of
66 lysine residues within specific histone and non-histone proteins [3].

67 HDACs are zinc metallo-enzymes divided into three main classes on the basis
68 of their protein sequence homologies with yeast deacetylase enzymes [4]. Briefly,
69 class I HDACs, composed of HDAC1, HDAC2, HDAC3 and HDAC8, are closely related
70 to yeast Rpd3 (reduced potassium dependency 3) transcriptional regulator. Class II
71 HDACs, including HDAC4, HDAC5, HDAC7, HDAC9 (class IIa) and HDAC6, HDAC10
72 (class IIb), share domains with similarity to yeast Hda1 (histone deacetylase I), whereas
73 HDAC11 belongs to the class IV. All these HDACs have a conserved catalytic domain
74 and are therefore considered as ancestral enzymes that play a crucial role in the
75 regulation of gene expression. While these enzymes have been considered as
76 transcriptional inhibitors due to the resulting compaction of chromatin structure upon
77 histone deacetylation [5], studies have also demonstrated that HDAC enzymes can
78 actively contribute to cell-specific gene expression [6], suggesting that HDACs could
79 play a dual active and inhibiting role in the regulation of gene expression to maintain
80 cell identity.

81 The pharmacological inhibition of HDAC has gained a strong interest following
82 the demonstration that HDAC inhibitors (HDACi) harbor anticancer properties [7].
83 HDACi suberoylanilide hydroxamic acid (SAHA, vorinostat) has been approved by the
84 Food and Drug Administration for cancer therapy. HDACi may have potential as

85 treatments for type 2 diabetes (T2D). Indeed, HDAC inhibition prevents cytokine-
86 induced toxicity in β cells [8-10], and improves β -cell proliferation [11]. *Hdac3* β -cell
87 specific knock down and pharmacological HDAC inhibition improve glucose tolerance
88 [12; 13] and insulin sensitivity [14] in mice.

89 However, the treatment of the rodent β -cell line β -TC3 with HDACi also induces
90 a loss of cell identity through a decrease of β -cell markers, correlated to an increase
91 of α -cell marker expression within β cells [15], but the impact of HDACi on chromatin
92 remodeling and the subsequent modulation of gene expression within β cells has not
93 yet been interrogated.

94 Here, we explored the molecular and functional effects of treating β -cell models
95 with the HDACi Trichostatin A (TSA) at the epigenomic and transcriptomic level. We
96 show that TSA treatment leads to an epigenome-wide redistribution of histone marks
97 that are enriched in active promoters and enhancers.

98 **2. Materials and methods**

99 **2.1. Cell culture and chemicals**

100 Chemicals, unless stated otherwise, were purchased from Sigma-Aldrich. Min6 cells
101 (Addexbio) were maintained in DMEM high Glucose glutaMAX medium (Gibco, 31966-
102 021) supplemented with 15 % SVF, 0.1 % β -mercaptoethanol and antibiotics
103 (penicillin/streptomycin). Trichostatin A (Sigma, T1952-200UL) was used at different
104 time and concentrations, as indicated. EndoC- β H1 cells were purchased from Human
105 Cell DesignTM and cultured according supplier recommendations.

106 Human pancreatic tissue was harvested from human, non-diabetic, adult donors.
107 Isolation and pancreatic islet culture were performed as previously described [16].

108 **2.2. Dot blot**

109 Min6 cells were cultured in 24-well plates ($2 \cdot 10^5$ cells/well) and were resuspended in
110 100 μ L of RIPA buffer (Thermo Scientific, 89901), then incubated at 4 °C during 30 min
111 under agitation. After centrifugation (15000 g, 20 min, 4 °C), supernatant was
112 harvested and protein concentration was measured using BCA assay (Pierce, 23227).
113 Samples were denatured (95 °C, 5 min) and 1 μ g of total protein were dotted on
114 nitrocellulose membrane using a dot blotter (Cleaver Scientific Ltd, CSL-D96). After
115 drying (15 min, RT), membranes were saturated in saturation buffer (5 % free fatty
116 acids milk in TBS 1X, 2h, room temperature), then incubated with primary antibody
117 diluted in saturation buffer (16 h, 4 °C). After incubation with HRP-coupled secondary
118 antibody diluted in saturation buffer (1 h, room temperature), membranes were
119 revealed using ECL kit (Pierce, 34076) on Chemidoc XRS+ imager (Biorad). Images
120 were processed and analyzed using ImageJ software. The list of antibodies used in
121 this study is available in the [Supplementary Table S1](#).

122 **2.3. Animal procedures**

123 Mice were maintained according to European Union guidelines for the use of laboratory
124 animals. *In vivo* experiments were performed in compliance with the French ethical
125 guidelines for studies on experimental animals (animal house agreement no. A59-
126 35015, Authorization for Animal Experimentation, project approval by our local ethical
127 committee no. APAFIS#2915-201511300923025v4). C57bl6J (Charles River
128 Laboratories) and Rip-Cre::Lox-STOP-Lox-Tomato (obtained after breeding of Rip-Cre

129 mice [17] with LSL-Td-Tomato (Jax, #stock number 007905)) mice were maintained
130 under 12 hours light/dark cycle and were fed *ad libitum*.

131 **2.4. Mouse pancreatic islet isolation and cell sorting**

132 Mouse pancreatic islets were prepared as described previously [18]. Briefly, pancreata
133 were digested using type V collagenase (Sigma, ref C9263-1G) for 10 min at 37 °C.
134 After digestion, pancreatic islets were separated in a density gradient medium
135 (Histopaque) and purified by handpicking under a microscope. Pancreatic islets were
136 cultured for 24 hours before further processing. For cell sorting experiments, mouse
137 pancreatic islets isolated from RipCre-tdTomato mice were trypsinized for 7 minutes
138 with 1 mM of trypsin (Gibco). Dissociated pancreatic cells were directly run into an
139 Influx sorter (Becton Dickinson®) equipped with a 86 µm nozzle and tuned at a
140 pressure of 24.7 psi and a frequency of 48.25 kHz. Sample fluid pressure was adjusted
141 to reach an event rate of 10 000 events/second. β cells and other pancreatic cells
142 were selected as Tomato + (Tom+, β cells) and Tomato – (Tom-, non-β cells) and
143 sorted in purity mode (phase mask 16/16) directly in RNeasy kit Lysis buffer for further
144 processing.

145 **2.5. Glucose stimulated insulin secretion (GSIS)**

146 Min6 cells cultured in 96-well plates ($2 \cdot 10^4$ cells/well) were glucose-starved in 200 µL
147 of starvation buffer (Krebs Ringer buffer (KRB) supplemented with BSA 0.5 %, 1 h, 37
148 °C, 5 % CO₂). After incubation, the starvation buffer was discarded and cells were
149 incubated in 200 µL of 2.8 mM glucose-concentrated starvation buffer (1 h, 37 °C, 5 %
150 CO₂). After 2.8 mM glucose samples recovering, cells were incubated in 200 µL of 20
151 mM glucose-concentrated starvation buffer (1 h, 37 °C, 5 % CO₂). After 20 mM glucose
152 samples recovering, the intracellular insulin content was recovered in 100 µL of lysis
153 buffer (Ethanol 75 %, HCl 1.5 %). Insulin concentration was measured using mouse or
154 human Insulin ELISA kits according to the manufacturer's instructions (Merckodia).

155 **2.6. RNA extraction and RT-qPCR**

156 Total RNA was extracted from Min6 cells using RNeasy Plus mini kit and from
157 pancreatic islets using RNeasy Plus micro kit (Qiagen, ref 74034) following the
158 manufacturer's instruction. Total RNA sample concentrations were determined using

159 a Nanodrop spectrophotometer (Implen). 100 ng of RNA was used for reverse
160 transcription (RT) using Transcriptor universal cDNA master mix (Roche) for Min6 cells
161 or superscript III enzyme (Invitrogen) for mouse pancreatic islets according to the
162 manufacturer's instructions. Gene expression was measured through quantitative real-
163 time PCR (qPCR) using FastStart SYBR Green master mix (Roche) according to the
164 manufacturer's recommendations in a LightCycler 480 II device (Roche). Mouse RT-
165 qPCR results were normalized to endogenous cyclophilin reference mRNA levels. The
166 results are expressed as the relative mRNA level of a specific gene expression using
167 the formula $2^{-\Delta\Delta C_t}$. The oligonucleotides sequences used for various experiments are
168 listed in [Supplementary Table S2](#).

169 **2.7. RNA sequencing (RNA-seq)**

170 RNA quality was verified using RNA 6000 nanochips (Agilent, #5067-1511) on the
171 Agilent 2100 bioanalyzer (Agilent, #G2939A). 500 ng of purified RNA with RNA integrity
172 number (RIN) ≥ 8 was subsequently used for library preparation with the TruSeq
173 Stranded mRNA library Prep (Illumina, #20020594) and sequenced on the Illumina
174 NextSeq500 system using a paired-end 2 \times 75 bp protocol. Raw sequencing data are
175 available upon request.

176 **2.8. Chromatin immunoprecipitation sequencing (ChIP-seq)**

177 $20 \cdot 10^6$ Min6 cells were treated with formaldehyde at a final concentration of 1% to
178 cross link DNA and protein complexes during 10 min. The reaction was stopped by the
179 addition of Glycine 0.125 M during 5 min. Cells were lysed and DNA-protein complexes
180 were sheared using the Bioruptor Pico (Diagenode, #B01060010) for 8 minutes. The
181 sheared chromatin was followed by immunoprecipitation with either the non-specific
182 antibody IgG (Santa Cruz, #sc2025), H3K4me1 (Active motif, #39297), H3K4me3
183 (Active motif, #61379), H3K27me3 (Active motif, #61017), H3K27ac (Active motif,
184 #39685) or H3K9ac (Abcam, #ab47915). 1 ng of eluted and purified DNA was used to
185 prepare libraries with the Nextflex rapid DNA seq kit 2.0 (Perkin Elmer, #NOVA-5188-
186 01) on the Illumina NextSeq500 system using a single read 100 bp protocol. Raw
187 sequencing data are available upon request.

188 **2.9. Bioinformatic analysis**

189 *2.9.1. RNA-seq*

190 The demultiplexing of sequence data (from BCL files generated by Illumina sequencing
191 systems to standard FASTQ file formats) was performed using bcl2fastq Conversion
192 Software (Illumina; version 2.19.1). Trimming of residuals adapters and low-quality
193 reads was performed using Cutadapt software (version 1.7.1). Subsequently,
194 sequence reads from FASTQ files were mapped to the mouse genome (mm10) using
195 STAR Aligner (version 2.5.2b). On average, 38M reads were generated per sample,
196 and 93.5 % +/- 0.9% of them were accurately mapped. The counting of the different
197 genes and isoforms was performed using RSEM (version 1.3). Finally, differential
198 expression was performed using DESeq2 package.

199 *2.9.2. ChIP-seq*

200 The demultiplexing of sequence data (from BCL files generated by Illumina sequencing
201 systems to standard FASTQ file formats) was performed using bcl2fastq Conversion
202 Software (Illumina; version 2.20). Trimming of residuals adapters and low-quality reads
203 was performed using TrimGalore (version 0.4.5). Subsequently, sequence reads from
204 FASTQ files were mapped to the mouse genome (mm10) using Bowtie2 Aligner
205 (version 2.3.5.1). Finally peak-calling was performed using MACS2 software (version
206 2.2.7.1).

207 *2.9.3. Pathway analysis*

208 RNA-seq data and integrated ChIP-seq/RNA-seq data were uploaded to Metascape
209 website [19] or Ingenuity Pathway Analysis software (Qiagen) as specified. For RNA-
210 seq data, an adjusted p-value < 0.05, LogFC>1 and LogFC<-1 were set as thresholds
211 as indicated and pathway analyses were performed using the core analysis function,
212 including canonical pathways, upstream regulators, diseases, biological functions and
213 molecular networks.

214 **2.10. Statistical analysis**

215 Data are presented as mean ± s.e.m. Statistical analyses were performed using a two-
216 tailed unpaired Student's t-test, one-way analysis of variance (ANOVA) followed by
217 Dunnett's post hoc test or two-way ANOVA with Tukey's post hoc tests comparing all
218 groups to each other, using GraphPad Prism 9.0 software. Differences were
219 considered statistically significant at p < 0.05 (*p < 0.05, ** p < 0.01, *** p < 0.001 and
220 **** p < 0.0001).

221 3. Results

222 3.1. TSA-mediated HDAC inhibition increases global histone acetylation 223 associated to impaired insulin secretion and decreased expression of β - 224 cell identity genes.

225 We first characterized the role of HDAC inhibition in Min6 cells through a
226 pharmacological approach using the pan-HDAC inhibitor TSA. A time-course TSA
227 treatment (0.5 μ M) of Min6 cells was performed from 15 min to 24 h and acetylation of
228 lysine 27 (H3K27ac), lysine 9 (H3K9ac) and global acetylation (PanH3ac) levels of
229 histone H3 were monitored by dot blot to validate global HDAC inhibition (Figure 1A).
230 TSA induced a time-dependent increase of H3K27ac (Figures 1A and B), H3K9ac
231 (Figures 1A and C) and PanH3ac (Figures 1A and D), indicating that Min6 cells were
232 sensitive to HDAC inhibitor treatment. Given that the sub-maximal histone H3K27 and
233 H3K9 acetylation signal was observed 16 h after TSA treatment (Figures 1A, B and C),
234 subsequent experiments were performed at this time point. To ensure that TSA did not
235 induced cytotoxicity, the viability of Min6 cells upon 16 h of TSA treatment was
236 assessed by FACS through annexin V and propidium iodide labeling to identify both
237 apoptosis events and cell viability, respectively. Neither annexin V (Supplementary
238 Figure S1A) nor propidium iodide (Supplementary Figure S1B) positive cells were
239 significantly overrepresented upon TSA treatment compared to vehicle (DMSO 0.1%)
240 treatment, indicating that TSA did not affect Min6 cell viability. Then, to determine the
241 effects of HDAC inhibition on specific acetylation and methylation sites of histone H3
242 involved in transcriptional regulation, the acetylation level of lysine 9 and 27 of histone
243 3 (H3K9ac and H3K27ac, respectively) and the trimethylation level of lysine 27
244 (H3K27me₃) were specifically monitored by dot blot in Min6 cells after 16h of TSA
245 treatment. Compared to vehicle-treated cells, TSA induced a significant increase of
246 both H3K9ac (Figures 1E and F) and H3K27ac (Figure 1E and G) indicating that the
247 effects of HDAC inhibition in Min6 cells could be partly related to the increased
248 acetylation of these histone marks. Trimethylation level of lysine 27 of H3 (H3K27me₃)
249 was significantly decreased upon TSA treatment (Figure 1E and H). Then, to evaluate
250 the impact of TSA on functional properties of Min6 cells, insulin secretion was
251 measured through glucose-stimulated insulin secretion (GSIS) assays. TSA treatment
252 significantly increased insulin secretion in low glucose conditions (2.8 mM) and
253 significantly decreased insulin secretion in high glucose conditions (20 mM, Figure 1I).

254 These functional effects were associated with a decreased expression of genes
255 involved in β -cell functions, such as *Pdx1* or *Mafa* (Figure 1J). These results suggest
256 that HDACs maintain functional properties of β cells through mechanisms that imply
257 the modulation of histone acetylation and gene expression.

258 **3.2. Epigenome-wide histone mark and transcriptome profiling identifies** 259 **distinct functional genomic regions in Min6 cells.**

260 With the aim to restrict our analysis to the effects of HDAC inhibition on
261 functional genomic regions, we first performed profiling of H3K4me3, H3K4me1,
262 H3K27ac and H3K27me3 through ChIP-seq experiments in untreated, control Min6
263 cells to delineate these specific chromatin features in basal conditions. As expected,
264 H3K4me3 and H3K27ac were mostly enriched within gene promoter (74% and 58%,
265 respectively, Supplementary Figures 2A and 2B) whereas H3K4me1 and H3K27me3
266 were mostly enriched both within gene body (46% and 40%, respectively) and
267 intergenic regions (25% and 44%, respectively, Supplementary Figures 2C and 2D).
268 Interestingly, this systematic histone mark profiling at the epigenome-wide level
269 allowed us to precisely circumscribe 6 distinct functional genomic regions based on
270 combinatorial histone mark enrichment within both promoter and distal intergenic
271 regions. Indeed, at the promoter level, we defined active (*i.e.*, both H3K4me3 and
272 H3K27ac enriched; Figure 2A), bivalent (*i.e.*, both H3K4me3 and H3K27me3 enriched;
273 Figure 2B) and inactive (*i.e.*, no histone marks enrichment; Figure 2C) promoters.
274 Within distal intergenic regions, we were able to define active enhancers (*i.e.*, both
275 H3K27ac and H3K4me1 enriched; Figure 2D), poised enhancers (*i.e.*, both H3K4me1
276 and H3K27me3 enriched; Figure 2E) and heterochromatin (*i.e.*, only H3K27me3
277 enriched; Figure 2F).

278 To go further in the characterization of these distal functional intergenic regions, their
279 associated genes were defined using Genomic Regions Enrichment of Annotations
280 Tool (GREAT) software [20].

281 Secondly, with the aim to link these chromatin features to the associated-gene
282 expression level, transcriptomic analysis through RNA-seq was performed in untreated
283 Min6 cells. The genes associated with active regions such as active promoter as well
284 as active enhancers were significantly found to be the most expressed compared to
285 genes associated with other chromatin features (*i.e.*, bivalent and inactive promoter,

286 poised enhancers and heterochromatin, [Figure 3A](#)). The expression level of genes
287 associated with poised enhancers was not significantly different to the expression level
288 of genes associated with heterochromatin ([Figure 3A](#)). Then, we analyzed the gene
289 ontology of genes associated with these functional genomic regions. This analysis
290 showed that biological processes related to genes associated with functionally active
291 genomic regions were enriched either in pathways involved in general biological
292 functions such as mitotic cell cycle process ($-\text{Log}_{10}(q)=22$, active promoter, [Figure 3B](#))
293 or in β -cell specific functions such as the regulation of secretion ($-\text{Log}_{10}(q)=34$, active
294 enhancers, [Figure 3C](#)). Conversely, genes associated with inactive bivalent, poised
295 genomic regions were rather enriched in pathways unrelated to β -cell functions such
296 as embryonic morphogenesis ($-\text{Log}_{10}(q)=35$, bivalent promoters, [Figure 3D](#); $-\text{Log}_{10}(q)=325$,
297 heterochromatin, [Figure 3F](#)), dorsal spinal cord development ($-\text{Log}_{10}(q)=19$, poised enhancers,
298 [Figure 3E](#)) or cell fate commitment ($-\text{Log}_{10}(q)=29$, bivalent promoters, [Figure 3D](#);
299 $-\text{Log}_{10}(q)=325$, heterochromatin, [Figure 3F](#)). Due to the large number of genes associated
300 with inactive promoters in Min6 cells, no specific pathway was identified for these associated
301 genes (data not shown). Altogether, these results indicate that, based on the combinatorial
302 enrichment of a selection of histone marks involved in transcriptional regulation in Min6 cells,
303 these chromatin features were functional and directly related to gene expression level and the
304 regulation of biological process involved in β -cell function.

306 3.3. The functional genomic regions identified in Min6 cells overlap with 307 functional chromatin segments found in mouse islets.

308 To go further in the characterization of these functional genomic regions in Min6
309 cells, we next assessed whether they overlapped with functional genomic regions
310 recently identified in mouse islets [21]. Therefore, we first sought to determine the
311 overlapping level between the genomic regions (peaks) independently enriched in a
312 selection of histone marks in Min6 cells and the functional genomic segments resulting
313 from functional chromatin segmentation (categorized from A to X) defined in mouse
314 islets of Langerhans [21]. This analysis showed that most of the regions enriched in
315 H3K4me3 in Min6 cells were mostly found in active (segments A to D) or bivalent
316 (segment K to M) chromatin segments, suggesting that the active promoter regions of
317 mouse β cells are conserved in Min6 cells ([Figure 4A](#), H3K4me3). This result was
318 corroborated by data obtained with the genomic regions enriched in H3K27ac in Min6

319 cells showing that these also mostly overlapped with the segments of active chromatin
320 (segments A to D, [Figure 4A](#), H3K27ac). Furthermore, the regions enriched with
321 H3K4me1 in Min6 cells were also conserved since they predominantly overlapped with
322 the active chromatin segments (segments from C to E) and to a lesser extent with the
323 bivalent regions (segment K, [Figure 4A](#), H3K4me1). Regarding the regions enriched
324 in H3K27me3 in Min6 cells, these were mainly found in the segments of silent
325 chromatin (segments N to P, [Figure 4A](#), H3K27me3). Taken together, this analysis
326 demonstrated that the regions enriched in histone marks involved in transcriptional
327 regulation are, at least partially, shared between Min6 cells and mouse pancreatic
328 islets.

329 This comparative analysis led us to consider the conservation level of the
330 functional genomic regions characterized in Min6 cells ([Figure 2](#)) with the functional
331 chromatin segments defined in mouse islets [21]. The functional genomic regions of
332 Min6 cells were also conserved compared to functional chromatin segments defined
333 in mouse islets ([Figure 4B](#)). Indeed, we showed that the most active promoters in Min6
334 cells were related to active promoter segments (segments A and B, [Figure 4B](#), active
335 promoter) while bivalent promoters were related to bivalent segments (segments L and
336 M, [Figure 4B](#), bivalent promoter) and inactive promoters were mostly related to silent
337 segments (segments N to Q segments, [Figure 4B](#), inactive promoter). Regarding the
338 distal intergenic functional regions, the active enhancers mainly overlapped with the
339 distal active segments (segments C to E, [Figure 4B](#), active enhancers) whereas the
340 poised enhancers were mainly related to the bivalent segments (K and M segments,
341 [Figure 4B](#), poised enhancers). Regarding the heterochromatin regions, these were
342 mostly related to the inactive segments (mainly segment N, [Figure 4B](#),
343 heterochromatin). Each conserved functional genomic region was exemplified through
344 a series of selected chromatin features (gene promoter or intergenic region, [Figures](#)
345 [4C and 4D](#)), such as the β -cell marker *Pdx1*, the α -cell marker somatostatin receptor
346 1 (*Sstr1*) or the ameloblast specific gene, Amelotin (*Amtn*). Finally, we selected the
347 functional chromatin regions that were conserved between Min6 cells and mouse
348 islets. To reach this aim, we selected only the functional chromatin regions of Min6
349 cells with more than 30% overlap with each related functional chromatin segment
350 within the mouse islets. Subsequently, the expression level of the genes associated
351 with these conserved genomic functional regions was analyzed ([Figure 4E](#)). The

352 results notably showed that the genes associated with the conserved active chromatin
353 regions (active promoter and active enhancers) are most expressed compared to the
354 genes associated with the inactive regions in line with our data generated without
355 applying the conservation filter (Figure 4E). Altogether, these results showed that the
356 functional chromatin regions are mostly shared and conserved between Min6 cells and
357 mouse islets, indicating that Min6 cells represent a pertinent β -cell model to explore
358 the effect of HDAC inhibition at the genome-wide level.

359 **3.4. HDAC inhibition differentially alters acetylation level of conserved** 360 **functional genomic regions in Min6 cells.**

361 The correlation between the alteration of β -cell function and the increase of
362 histone acetylation in response to HDAC inhibition (Figure 1) prompted us to
363 interrogate the effects of TSA treatment on histone acetylation within previously
364 characterized conserved functional genomic regions in Min6 cells. To reach this aim,
365 Min6 cells were treated either with vehicle (DMSO 0.1%) or TSA (0.5 μ M) during 16
366 hours and H3K9ac, H3K27ac and H3K27me3 were profiled through ChIP-seq
367 experiments (Figure 5A). Compared to the vehicle condition, TSA treatment led to the
368 detection of a higher number of peaks for both H3K9ac (27438 for TSA *versus* 13771
369 for DMSO) and H3K27ac (66347 for TSA *versus* 13451 for DMSO) (Figure 5B).
370 Concomitantly, TSA treatment drastically reduced the number of peaks for H3K27me3
371 (17367 for DMSO *versus* 1253 for TSA) (Figure 5B). Consistent with the results from
372 dot blot experiments (Figures 1A to D), these data however indicated that the increase
373 of H3K9ac and H3K27ac signal intensity in response to TSA treatment may not be only
374 related to an increase of signal intensity within constitutively acetylated regions but
375 also to an increase in the number of *de novo* acetylated genomic sites. This was
376 corroborated by the analysis of the genomic distribution of H3K9ac and H3K27ac in
377 response to TSA treatment showing a significant redistribution of H3K9ac and
378 H3K27ac enriched regions especially towards gene bodies and distal genomic regions
379 for H3K9ac (Supplementary Figures 3A and B, H3K9ac) and gene bodies for H3K27ac
380 (Supplementary Figures 3C and D, H3K27ac). The genomic distribution of H3K27me3
381 was less modified suggesting that it was rather the loss of signal than the genomic
382 redistribution affecting H3K27me3 in response to TSA treatment (Supplementary
383 Figures 3E and F, H3K27me3).

384 Considering that a genomic redistribution of H3K9ac and H3K27ac occurred in
385 response to TSA treatment, we then hypothesized that the levels of H3K9ac and
386 H3K27ac acetylation could be altered within the conserved functional genomic regions
387 described above (Figure 2 and Supplementary Figure 2). In addition, the H3K27me3
388 signal was also monitored in these regions. First, a promoter-focused analysis
389 centered on the transcription start site (TSS) +/- 2.5 kb was performed. Surprisingly,
390 this analysis revealed that H3K9ac signal was completely blunted after treating Min6
391 cells with TSA within shared active promoters while the H3K27ac signal was weakly
392 affected or even slightly increased in the close vicinity of the promoters whereas
393 H3K27me3 signal was not modulated (Figure 5C). Conversely, an increase of H3K9ac
394 and, to a lesser extent, H3K27ac signal, associated with a decrease of H3K27me3,
395 was detected within shared bivalent promoters upon TSA condition (Figure 5D).
396 Considering shared inactive promoters, neither H3K9ac, H3K27ac nor H3K27me3
397 signal was modulated by TSA treatment (Supplementary Figure 4A). This promoter-
398 based analysis at genome-wide level showed that TSA treatment led to distinct effects
399 on histone acetylation depending on the basal activation level of the promoters.
400 Acetylation level in response to TSA treatment was next interrogated within conserved
401 functional distal intergenic regions at genome-wide level focusing on the center of the
402 region +/- 2.5 kb. Within shared poised enhancers, both H3K9ac and H3K27ac signal
403 was increased whereas H3K27me3 signal was decreased (Figure 5E) while shared
404 active enhancers were weakly enriched with H3K9ac and H3K27ac without modulation
405 of H3K27me3 signal upon TSA treatment (Figure 5F). Finally, shared heterochromatin-
406 focused analysis showed that H3K27me3 was decreased within these regions
407 whereas H3K9ac and H3K27ac signals were weakly affected (Supplementary Figure
408 4B). Taken together, these genome-wide analyses suggest that TSA treatment in Min6
409 cells differentially impacts acetylation profile depending on the type of functional
410 genomic regions. This implied that gene expression level could be directly affected by
411 these changes after genomic reprogramming of acetylation.

412 **3.5. Genomic redistribution of histone acetylation upon HDAC inhibition** 413 **directly reprograms the transcriptome in Min6 cells.**

414 We interrogated the functional outcomes of the modulation of histone
415 acetylation profile within conserved functional genomic regions through transcriptome-
416 wide analysis in Min6 cells. To reach this aim, RNA-seq was then performed on 16 h

417 vehicle- and TSA-treated Min6 cells. By applying an adjusted p-value cut-off threshold
418 at 0.05 ($P_{adj} < 0.05$), 5636 genes were significantly downregulated and 6571 genes
419 were significantly upregulated (Supplementary Table S3). Given this high number of
420 deregulated genes, we applied a second cut-off threshold based on Log2 fold change
421 (Log2FC) to select only the most deregulated genes. Using these two cut-off
422 thresholds, 2626 genes were downregulated ($P_{adj} < 0.05$, $\text{Log}_2\text{FC} < -1$) and 3877 genes
423 were upregulated ($P_{adj} < 0.05$, $\text{Log}_2\text{FC} > 1$) upon TSA treatment (Figure 6A,
424 Supplementary Table S3). Ingenuity pathway analysis (IPA) revealed that
425 downregulated genes were associated with canonical pathways involved in
426 kinetochore metaphase signaling ($-\text{Log}_{10}(P)=16$), mitotic roles of Polo-like kinase ($-\text{Log}_{10}(P)=9$), but most interestingly, with insulin secretion ($-\text{Log}_{10}(P)=9$), Maturity
428 Onset of Diabetes of Young (MODY, $-\text{Log}_{10}(P)=7$) and GPCR-mediated nutrient
429 sensing ($-\text{Log}_{10}(P)=5$, Figure 6B, Supplementary Table S4). In line with this, several
430 key β -cell genes were negatively affected by TSA treatment, such as *Slc2a2*, *Nkx2-2*,
431 *G6pc2*, *Kcnj11*, *Mafa*, *Pdx1* or *P2ry1* (Supplementary Figure 5A and Supplementary
432 Table S3). Conversely, upregulated genes were associated to pathways unrelated to
433 β -cell functions, such as axonal guidance, pulmonary fibrosis or cardiac hypertrophy
434 (Figure 6C, Supplementary Table S5). Based on these results, the deregulated genes
435 were classified according to the conserved functional genomic region with which they
436 were associated and the proportion of each group was plotted in a pie chart. Among
437 the downregulated genes, this analysis showed that more than 25% were genes with
438 an active promoter (Figure 6D) and 10% of the downregulated genes were associated
439 with heterochromatin regions. Genes associated with other functional genomic regions
440 were marginally represented (less than 15%). In addition, this analysis showed that
441 48% were not associated with any previously characterized functional genomic region,
442 likely due to a lower analytical power related to the restricted number of histone marks
443 profiled in this study. These results were directly correlated to the functional genomic
444 data showing that the active promoters harbored a drastic decrease in the H3K9ac
445 signal potentially responsible to gene expression downregulation (Figure 5B).

446 Regarding upregulated genes, 46% were associated with silent functional
447 genomic regions (bivalent promoter, 18%, heterochromatin, 23% and poised
448 enhancers, 5%) while genes associated with active functional genomic regions were
449 less represented (active promoter, 1% and active enhancers, 3%) (Figure 6E). Again,

450 48% of the downregulated genes were not associated with previously defined
451 functional genomic regions. This analysis however highlighted the direct link between
452 altered epigenomic profile and increased gene expression in response to TSA
453 treatment since previous data demonstrated that silent regions showed either an
454 enrichment in H3K9ac/H3K27ac associated with a depletion in H3K27me3 (*i.e.*,
455 bivalent promoter and poised enhancers; [Figures 5D and 5E](#), respectively) or only
456 depletion in H3K27me3 (*i.e.*, heterochromatin; [Supplementary Figure 4B](#)). These
457 observations were exemplified through a series of selected β -cell (*Pdx1* and *Mafa*) and
458 α -cell markers (*Arx* and *Mafb*) demonstrating that β -cell genes were depleted in
459 H3K9ac/H3K27ac marks, whereas α -cell genes were slightly increased in
460 H3K9ac/H3K27ac marks but depleted in H3K27me3 mark ([Supplementary Figure 5C](#)).

461 Consistent with the analysis of the effects of TSA on H3K9ac, H3K27ac and
462 H3K27me3 levels within the conserved functional genomic regions in Min6 cells, these
463 results showed a direct functional repercussion of the epigenomic alteration of these
464 regions on the regulation of gene expression. This was all the more likely since the
465 genes associated with inactive promoters whose H3K9ac, H3K27ac and H3K27me3
466 profile were marginally altered in response to TSA treatment were not enriched neither
467 in downregulated genes (2.17%, [Figure 6D](#)) nor in upregulated genes (2.09%, [Figure](#)
468 [6E](#)). Gene ontology analysis using Metascape revealed that most of the downregulated
469 genes upon TSA treatment were associated to chromatin organization, RNA splicing
470 and insulin secretion ([Figure 6F](#)). Conversely, upregulated genes were enriched in
471 pathways that are unrelated to β -cell function, such as fat cell differentiation or lipid
472 biosynthetic process ([Figure 6G](#)). Altogether, these data suggest that TSA treatment
473 functionally affects cell fate through an epigenome-wide remodeling associated to
474 transcriptome changes that alter β -cell identity and function.

475 **3.6. The transcriptome of FACS-sorted TSA-treated mouse β cells identifies** 476 **partially conserved genomic mode of action of TSA-mediated HDAC** 477 **inhibition.**

478 Having demonstrated a direct link between the epigenome alteration and
479 modulation of gene expression in Min6 cells in response to HDAC inhibition, we next
480 sought to determine whether the transcriptome as well as the associated-chromatin
481 features could be conserved in mouse β cells. Pancreatic islets of Langerhans isolated

482 from RIPCre-tdTomato mice expressing the red fluorescent protein td-Tomato under
483 the control of the β -cell specific Cre recombinase were treated by either vehicle (DMSO
484 0.1%) or TSA (0.5 μ M, 16 h) and then subjected to FACS analysis to sort Tomato
485 positive cells, corresponding to a β -cell enriched population. RNA-seq experiment was
486 performed on this β -cell enriched population (Figure 7A). In order to compare these
487 data with those obtained in Min6 cells, an adjusted p-value and Log2FC cut-off
488 thresholds ($P_{adj} < 0.05$ and $-1 > \text{Log}_2\text{FC} > 1$) were applied, respectively. In these
489 conditions, 1182 genes were significantly downregulated and 1331 genes were
490 significantly upregulated upon TSA treatment (Figure 7B, Supplementary Table S6).
491 IPA further confirmed that downregulated genes from TSA-treated sorted β -cells were
492 mostly associated to insulin secretion and MODY signaling pathways (Figure 7C,
493 Supplementary Table S7), whereas upregulated genes were found to be involved in
494 the regulation of axonal guidance signaling, molecular mechanisms of cancer or WNT/
495 β -catenin signaling (Figure 7D, Supplementary Table S8).

496 In order to focus our analysis on conserved transcriptomic pattern between
497 FACS-sorted β cells and Min6 cells, we next intersected the related dataset in a Venn
498 diagram (Figure 7E). Among upregulated genes defined in FACS-sorted TSA-treated
499 β -cells, 53% (694 genes) and 2% (26 genes) were shared with upregulated and
500 downregulated genes detected in Min6 cells, respectively. Of note, 46% of upregulated
501 genes were specific to FACS-sorted TSA-treated β -cell. Downregulated genes in TSA-
502 treated β -cell sorted were also mostly conserved compared to downregulated genes
503 in TSA-treated Min6 cells since 53% were shared whereas only 5.6% were upregulated
504 in Min6 cells. TSA treatment also induced a β -cell sorted specific transcriptomic
505 signature since 41% of genes were not overlapping neither with downregulated nor
506 upregulated genes in Min6 cells. This analysis suggested that HDAC inhibition through
507 TSA treatment induced a transcriptomic signature resulting at least partly from
508 conserved molecular mechanisms between *ex vivo* TSA-treated β -cell and the Min6 β -
509 cell model.

510 To go further in the functional characterization of these conserved deregulated
511 genes upon TSA treatment, a gene ontology analysis using IPA was conducted on
512 both conserved down- and upregulated genes. Conserved downregulated genes were
513 associated with biological processes mostly related to β -cell function (e.g., regulation
514 of hormone levels and endocrine pancreas development, Figure 7F) whereas

515 conserved upregulated genes were rather associated with biological processes
516 unrelated to β -cell function (e.g., axon development and fat cell differentiation, [Figure](#)
517 [7G](#)). Altogether, this analysis suggested that the alteration of β -cell function upon
518 HDAC inhibition could be conserved and related to selective downregulation of β -cell
519 specific genes. To ascertain this hypothesis, we conducted a Gene Set Enrichment
520 Analysis (GSEA) to monitored β -cell specific gene set enrichment within conserved
521 downregulated genes ([Supplementary Figure 5B](#)). As expected, β -cell specific genes
522 were mostly enriched in vehicle- compared to TSA-treated β -cell-sorted, thus
523 supporting our gene ontology analysis.

524 With the aim to connect our transcriptomic data with functional genomic data
525 from mouse β cells, we next sought to determine the functional genomic regions to
526 which conserved deregulated genes were associated. By using the previously defined
527 conserved functional genomic regions ([Figure 2B](#)), we were able to demonstrate that
528 most of the conserved downregulated genes were associated to active regions (active
529 promoter, 42% and active enhancers, 13%) ([Figure 7H](#)) whereas upregulated genes
530 were rather associated to silent regions (bivalent promoter, 18%, poised enhancers,
531 6% and heterochromatin, 19%) ([Figure 7I](#)). Of note, these results were close to results
532 obtained in Min6 cells ([Figures 6D and 6E](#)) suggesting that a part of genomic regions
533 functionally affected upon TSA treatment were conserved between β -cell-sorted and
534 Min6 cells.

535 **3.7. Transcriptome-wide analysis of EndoC- β H1 and human islets upon HDAC** 536 **inhibition identifies conserved TSA-sensitive genes related to β -cell** 537 **identity.**

538 Given that HDAC inhibition led to an important redistribution of histone
539 acetylation at genome-wide level leading to a drastic modulation of gene expression at
540 transcriptome-wide level both in Min6 and mouse β -cell sorted cells, we next asked
541 whether TSA treatment could also reprogram the transcriptome of human β cells.
542 Consequently, EndoC- β H1 cells and human pancreatic islets were independently
543 treated either with vehicle (DMSO 0.1%) or TSA (0.5 μ M) during 16 hours and the
544 resulting transcriptome was recorded through RNA-seq experiments as depicted in
545 [Figures 8A and 8B](#), respectively.

546 In EndoC- β H1 cells, by applying an adjusted p-value cut-off threshold at 0.05
547 ($P_{adj}<0.05$), 4717 genes were significantly downregulated and 5443 genes were
548 significantly upregulated ([Supplementary Table S9](#)). As in Min6 cells, we applied a
549 second cut-off threshold based on Log2 fold change (Log2FC) to select only the most
550 deregulated genes. Using these two cut-off thresholds, 2793 genes were
551 downregulated ($P_{adj}<0.05$, $\text{Log}_2\text{FC}<-1$), 3243 genes were upregulated ($P_{adj}<0.05$,
552 $\text{Log}_2\text{FC}>1$) upon TSA treatment ([Figure 8C and Supplementary Table S9](#)). These
553 results showed that the transcriptome of EndoC- β H1 cell line was highly sensitive to
554 and profoundly affected by TSA treatment, as observed in the Min6 cell line and FACS-
555 sorted β cells. Pathway analysis of downregulated genes suggested that TSA affects
556 pathways controlling cell cycle and cell proliferation, but also insulin secretion
557 ($\text{Log}(p\text{Val})= 1.8$; $Z\text{-score}=-5.38$; [Figure 8D, Supplementary Tables S9 and S10](#)).
558 Amongst genes that are downregulated and identified as β -cell identity genes [22],
559 *ABCC8*, *MAFA*, *GLP1R* and *PDX1* were found to be negatively affected. Conversely,
560 TSA-mediated upregulated genes were associated to canonical pathways that were
561 unrelated to β -cell functions, such as fibrosis, axonal guidance or osteoarthritis
562 pathways ([Figure 8E, Supplementary Tables S9 and S11](#)). In non-diabetic human
563 islets, 1099 genes were significantly downregulated and 854 genes were significantly
564 upregulated, after applying cut-off thresholds ($P_{adj}<0.05$, $-1>\text{Log}_2\text{FC}>1$, [Figure 8F,](#)
565 [Supplementary Tables S12](#)). Among the canonical pathways that were affected in
566 downregulated genes, phagosome formation, G-protein coupled receptor signaling
567 and CREB signaling in neurons were the most represented ([Figure 8G and](#)
568 [Supplementary Table S13](#)). Again, insulin secretion signaling pathway was found to
569 be affected by TSA treatment ($\text{Log}(p\text{Val})= 1.64$; $Z\text{-score}=-3.15$; [Supplementary Table](#)
570 [S13](#)). For upregulated genes, IPA revealed that genes involved in pathways unrelated
571 to pancreatic islet function were enriched following TSA treatment, such as axonal
572 guidance signaling, synaptogenesis or semaphorin signaling pathways ([Figure 8H and](#)
573 [Supplementary Table S14](#)). Although we did not measure the GSIS of EndoC- β H1 and
574 human islets in response to TSA treatment, these results suggest that TSA induces a
575 profound transcriptomic reprogramming in human β -cell line.

576 **4. Discussion**

577 Here we show that blocking HDAC activity with TSA profoundly impairs insulin
578 secretion. This functional effect is associated to an epigenomic reprogramming,
579 associated to loss of β -cell identity genes, in mouse as well as in human β cells or
580 islets. Although the contributions of HDACs to endocrine pancreas development are
581 well circumscribed [23; 24], our results demonstrate that they also contribute to the
582 maintenance of mature β -cell identity, plasticity and function.

583 It has been recently suggested that enhancement of ROS (reactive oxygen species)
584 production upon TSA treatment in Min6 cells could have negative effects of HDACi on
585 β -cell function [25]. An increase of IRS2 (Insulin Receptor Substrate) expression in
586 response to TSA treatment in Min6 cells has been reported to be causal of the
587 impairment of β -cell function upon HDAC inhibition [26]. Our transcriptomic analyses
588 reveal that TSA treatment decreases rather than increases IRS2 expression
589 ([Supplementary Table S3](#)) and mostly affects the expression of a large set of genes
590 involved in various biological process, suggesting that the effect of HDACi on β -cell
591 function is probably more complex than anticipated. Indeed, among deregulated genes
592 in response to TSA treatment, most of β -cell specific genes are downregulated
593 whereas a series of α -cell specific genes as well as genes unrelated to endocrine
594 functions are upregulated. In accordance with previous results from Kubicek and
595 colleagues observed in TSA-treated murine β -TC6 cells [15], these results suggest that
596 global HDAC inhibition leads to an alternative transcriptional program leading to a
597 switch from β -cell identity towards an undefined alternative identity partly overlapping
598 with α -cell identity. Also in line with the analysis restricted to some β -cell markers from
599 Yamoto E [25], our data confirm that β -cell identity is effectively directly impaired by
600 HDAC inhibition.

601 In this context, we demonstrate here that this alternative transcriptional program
602 is likely consecutive to a drastic alteration of epigenome upon HDAC inhibition in β -
603 cell, especially through modification of histone acetylation profile within promoters.
604 Whereas the definition of H3K9ac-enriched promoters at genome-wide level is
605 consistent with global increase of histone acetylation level, it is much more
606 counterintuitive to identify H3K9ac-depleted promoters upon HDAC inhibition. Indeed,

607 we found most of the β -cell specific genes promoters surprisingly H3K9ac-depleted
608 in response to HDAC inhibition and correlated to a decrease of gene expression. As
609 TSA is an unspecific class I and class II HDAC inhibitor, this experimental evidence
610 suggests that one or several class I and/or class II HDAC enzymes could play a direct
611 active role in the regulation of expression of genes involved in the β -cell identity. In
612 accordance with this hypothesis, class I (*i.e.*, HDAC1, 2 and 3) and class IIb (*i.e.*,
613 HDAC6) HDAC enzymes were shown to be bound within promoter of transcriptionally
614 active gene promoters in human CD4+ T cells [27]. However, we did not demonstrate
615 any involvement of neither HDAC1 nor HDAC6 in the direct regulation of
616 transcriptionally active genes in β cell (data not shown) suggesting that (*i*) conversely
617 to observations from CD4+ T cells, HDAC1 and HDAC6 are not directly involved in the
618 regulation of β -cell specific genes, (*ii*) other HDAC could be implicated in this process
619 such as HDAC2 and/or HDAC3 [13; 27]. Alternatively, depletion of acetylation in
620 promoter of transcriptionally active genes upon HDAC inhibition in β cell could be
621 consecutive to the loss of a histone acetyl transferase enzyme binding within promoter
622 of these genes which has been recently proposed for CBP/p300 as an alternative
623 mechanistic model explaining promoter histone deacetylation in response to HDAC
624 inhibition in endothelial cells [28].

625 We also provided evidences that HDAC inhibition also leads to enhancer
626 remodeling. Enhancers are cell specific non-coding elements of the genome involved
627 in long-distance cell-specific regulation of gene expression directly related to cell
628 identity, especially β -cell identity [29]. It has been recently reported that the HDAC
629 inhibition using largazole induces a H3K27ac-depletion at enhancers in HCT116 cells
630 [30] suggesting a role of HDAC in enhancer activity. Although it remains to
631 experimentally demonstrate that HDACs directly bind to β -cell enhancers, our results
632 emphasize a crucial role of HDAC enzymes in enhancer activity to maintain an
633 appropriate transcriptional pattern. At a mechanistic level, the level of enhancer activity
634 is especially directly related to the amount of local production of enhancers RNA
635 (eRNA) [31] playing a direct role in the regulation of enhancer target genes expression
636 [32] In addition, as eRNA are transcribed from active enhancers, they exhibit tissue
637 and lineage specificity, and serve as markers of cell state and function [33].
638 Consequently, we hypothesize that remodeling of β -cell enhancers upon HDAC
639 inhibition could lead to an alteration of the β -cell specific eRNA expression profile, thus

640 partly contributing to affect specific gene expression profile as recently pointed out in
641 human BT474 cells [34]. To date, eRNAs are not well characterized in β cells and the
642 link between HDAC and eRNA needs to be further investigated to better define β -cell
643 identity as well as improve molecular knowledge on β -cell function. It would be
644 interesting to investigate the role of distinct HDAC using selective or specific HDACi
645 [35].

646 Although this study connects the negative effects of HDACi on β -cell function to
647 the global remodeling of epigenome (*i.e.*, histone acetylation) at genome-wide level,
648 we cannot rule out that alteration of acetylome at the proteome-wide level upon HDAC
649 inhibition is related to the observed phenotype. Indeed, acetylation is a largely
650 widespread conserved post-translational modification also affecting non-histone
651 proteins/enzymes to regulate their functions [36]. As acetylome recently defined in rat
652 islet is enriched in metabolic pathways of β cells related to nutrient sensing [37], we
653 propose that HDAC inhibition using hydroxamic acid such as TSA leads to a global
654 protein deacetylation involved in these pathways impairing protein/enzyme functions
655 concomitantly to epigenome remodeling inducing in turn β -cell failure. Nevertheless,
656 given the specificity of action of TSA on class I and II HDAC, this implies that these
657 two classes of enzymes play a role on both nuclear and cytoplasmic non-histone
658 protein deacetylation process, which has not been yet reported in β cell whereas Sirt3
659 belonging to class III HDAC takes part to this process in rat Ins1 cells [37].

660 In summary, based on a pharmacological approach using the pan-HDAC
661 inhibitor TSA, we provide new information indicating that HDAC inhibition negatively
662 acts on β -cell transcriptional program through epigenome-wide remodeling, leading to
663 an alteration of β -cell functional properties. These results also indicate that β -cell
664 identity is likely under the control of HDAC activity acting both on gene expression as
665 well as enhancer activity. Other investigations are currently in progress to better define
666 the role of each HDAC in this process.

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684 **Authors contributions**

685 Conceptualization, F.O., and J.S.A.; Methodology, F.O., M.M., Am.B. and J.S.A.;

686 Investigation, F.O., M.M., M.D., B.T., L.B., C.B., E.D., S.A., Al.B., E.B., O.M.C., L.P.,

687 G.P., L.R., C.C., F.B. and E.C.; Resources, C.G., J.E., D.D., J.K.C., F.P., B.S., P.F.,

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692

693 **Conflict of interest**

694 The authors declare that there is no conflict of interests regarding the publication of
695 this article.

696

697 **Figure legends**

698 **Figure 1. The increase of histone H3 acetylation level upon TSA treatment is**
699 **correlated to alteration of functional properties of Min6 cells. (A)** Dot blot kinetic
700 analysis of H3K9ac, H3K27ac and pan-acetylated histone H3 (Pan H3ac) levels upon
701 TSA treatment (0.5 μ M) in Min6 cells at the indicated time. Vehicle (DMSO 0.1%) was
702 used as negative control. **(B-D)** Densitometry analysis of A was performed by
703 comparing the mean of signal at each time point to mean signal in vehicle (n=3). **(E)**
704 Dot blot analysis of H3K9ac, H3K27ac and H3K27me3 levels upon TSA treatment (0.5
705 μ M, 16h) in Min6 cells. Vehicle (DMSO 0.1%) was used as negative control. **(F-H)**
706 Densitometry analysis of E is expressed as fold of H3K9ac (F), H3K27ac (G) and
707 H3K27me3 (H) signals in TSA-treated cells compared to signals in vehicle-treated cells
708 (n=3). **(I)** Glucose-stimulated insulin secretion of TSA-treated cells (0.5 μ M, 16h, n=7).
709 Vehicle (DMSO 0.1%) was used as a control. Results are presented as fold insulin
710 secretion in response to 20 mM glucose compared to 2.8 mM glucose +/- SEM. **(J)**
711 Quantitative RT-PCR analysis of key β -cell identity genes in vehicle and TSA-treated
712 Min6 cells (n=3). Results in B, C, D, F, G, H, I, J are displayed as means +/- SEM. *
713 $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

714 **Figure 2. Histone marks profiling by ChIP-seq defines distinct functional**
715 **genomic regions in Min6 cells. (A)** Characterization of active promoters in Min6 cells.
716 Heatmap displayed signal centered on transcription start site (TSS) +/- 2.5kb for
717 H3K4me3, H3K27ac and H3K27me3 within each active promoter (n=8303 regions,
718 2549 genes). Mean signal centered on TSS +/- 2.5kb for H3K4me3, H3K27ac and
719 H3K27me3 within active promoters is displayed. **(B)** Characterization of bivalent
720 promoters in Min6 cells. Heatmap displayed signal centered on TSS +/- 2.5kb for
721 H3K4me3 and H3K27me3 within each bivalent promoter (n=3685 regions, 1323
722 genes). Mean signal centered on TSS +/- 2.5kb for H3K4me3 and H3K27me3 within
723 bivalent promoters is displayed. **(C)** Characterization of inactive promoters in Min6
724 cells. Heatmap displayed signal centered on TSS +/- 2.5kb for H3K4me3, H3K27ac
725 and H3K27me3 within each inactive promoter (n=7632 genes). Mean signal centered
726 on TSS +/- 2.5kb for H3K4me3, H3K27ac and H3K27me3 within inactive promoters is
727 displayed. **(D)** Characterization of active enhancers in Min6 cells. Heatmap displayed
728 signal centered on the center of the regions +/- 2.5kb for H3K4me1 and H3K27ac
729 within each active enhancer (n=1597 regions). Mean signal centered on the center of

730 the regions +/- 2.5kb for H3K4me1 and H3K27ac within active enhancers is displayed.
731 **(E)** Characterization of poised enhancers in Min6 cells. Heatmap displayed signal
732 centered on the center of the regions +/- 2.5kb for H3K4me1, H3K27ac and H3K27me3
733 within each poised enhancer (n=683 regions). Mean signal centered on centered on
734 the center of the regions +/- 2.5kb for H3K4me1, H3K27ac and H3K27me3 within
735 poised enhancers is displayed. **(F)** Characterization of heterochromatin in Min6 cells.
736 Heatmap displayed signal centered on the center of the regions +/- 2.5kb for
737 H3K4me1, H3K27ac and H3K27me3 within heterochromatin (n=21292 regions). Mean
738 signal centered on centered on the center of the regions +/- 2.5kb for H3K4me1,
739 H3K27ac and H3K27me3 within heterochromatin is displayed.

740 **Figure 3. Functional genomic regions in Min6 cells are directly associated with**
741 **gene expression level and distinct biological processes. (A)** RNA-seq based
742 expression level of genes associated with functional genomic regions in Min6 cells.
743 Results are displayed as mean of $\text{Log}_2(\text{TPM}+1)$ for each functional genomic regions
744 associated-genes. The violin plot displays median and quartile values for each group
745 of genes. **(B)** Gene ontology (GO) analysis of active promoter associated-genes was
746 performed using Metascape by filtering output only on GO Biological process. The 5
747 more enriched GO Biological processes are displayed as $-\text{Log}_{10}(q)$ (*i.e.*, $-\text{Log}_{10}(\text{Padj})$).
748 **(C)** GO was performed using Genomic Regions Enrichment of
749 Annotations Tool (GREAT) following the two nearest genes association rule (cut-
750 off<1000kb). The 5 more enriched GO biological processes are displayed as $-\text{Log}_{10}(q)$
751 (*i.e.*, $-\text{Log}_{10}(\text{Padj})$). **(D)** GO analysis was performed using Metascape by filtering
752 output only on GO Biological process. The 5 more enriched GO Biological processes
753 are displayed as $-\text{Log}_{10}(q)$ (*i.e.*, $-\text{Log}_{10}(\text{Padj})$). **(E)** GO analysis was performed using
754 GREAT following the two nearest genes association rule (cut-off<1000kb). The 5 more
755 enriched GO biological processes are displayed as $-\text{Log}_{10}(q)$ (*i.e.*, $-\text{Log}_{10}(\text{Padj})$). **(F)**
756 GO analysis was performed using GREAT following the two nearest genes association
757 rule (cut-off<1000kb). The 5 more enriched GO biological processes are displayed as
758 $-\text{Log}_{10}(q)$ (*i.e.*, $-\text{Log}_{10}(\text{Padj})$).

759 **Figure 4. Functional genomic regions in Min6 cells are partly conserved with**
760 **functional genomic segments in mouse islets of Langerhans. (A)** Percentage
761 overlap of H3K4me3, H3K27ac, H3K4me1 and H3K27me3 peaks in Min6 cells with
762 the distinct genomic segments (A to J, active segments; K to U, silent segments; V to

763 X, others segments) from mouse pancreatic islets chromatin segmentation [21]. Peaks
764 for each histone mark were intersected with each mouse islets chromatin segment to
765 define the overlap associated-percentage. Results are displayed as heatmap scaled
766 on this percentage of overlap. **(B)** Percentage overlap of functional genomic regions in
767 Min6 cells with the distinct genomic segments from mouse islets of Langerhans
768 chromatin segmentation [21]. Each specific functional genomic region was intersected
769 with each mouse pancreatic islets chromatin segment to define the overlap associated-
770 percentage. Results are displayed as heatmap scaled on this percentage of overlap.
771 **(C-D)** Examples of conserved functional genomic regions. Figures are adapted from
772 the IGB genome browser screenshots and genomic coordinates are indicated. **(E)**
773 RNA-seq based expression level of genes associated with conserved functional
774 genomic regions in Min6 cells (active promoters: 2370 genes, bivalent promoters: 1191
775 genes, inactive promoters: 3055 genes, active enhancers: 837 genes, poised
776 enhancers: 648 genes, heterochromatin: 3308 genes). Results are displayed as mean
777 of $\text{Log}_2(\text{TPM}+1)$ for each conserved functional genomic regions associated-genes.
778 The violin plot displays median and quartile values for each group of genes.

779 **Figure 5. TSA treatment leads to a genomic redistribution of H3K9ac, H3K27ac**
780 **and H3K27me3. (A)** Scheme representing the strategy used in Min6 cells. **(B)** Number
781 of H3K9ac, H3K27ac and H3K27me3 peaks in vehicle- and TSA-treated Min6 cells.
782 **(C-F)** H3K9ac, H3K27ac and H3K27me3 signal in conserved active promoters (C),
783 bivalent promoters (D), poised enhancers (E) and active enhancers (F) in vehicle- and
784 TSA-treated Min6 cells. Heatmaps and mean signals centered on TSS +/- 2.5kb are
785 represented.

786 **Figure 6. Redistribution of histone acetylation at genome-wide level upon TSA**
787 **treatment in Min6 cells is associated with differential gene expression at**
788 **transcriptome-wide level. (A)** Volcano plot displaying downregulated (red circles),
789 upregulated (green circles) and not deregulated genes (black circles) in TSA-treated
790 cells compared to vehicle-treated cells according to two cut-off thresholds based on
791 adjusted p-value (P_{adj}) and Log_2 fold change (Log_2FC). **(B-C)** Ingenuity Pathway
792 analysis (IPA) of downregulated (B) and upregulated (C) genes in Min6 cells upon TSA
793 treatment. **(D-E)** Pie chart displaying proportion of TSA-dependent downregulated (D)
794 and upregulated (E) genes in Min6 cells associated with a specific functional genomic

795 region. **(F-G)** Metascape analysis of genes with an active promoter that are
796 downregulated (F) and upregulated (G) upon TSA treatment.

797 **Figure 7. Transcriptome of FACS-sorted β -cell from *ex vivo* TSA-treated mouse**
798 **Langerhans islets is partly shared with Min6 cells. (A)** Schematic representation of
799 β -cell preparation from mouse Langerhans islets. **(B)** Volcano plot displaying
800 downregulated (red circles), upregulated (green circles) and not deregulated genes
801 (black circles) in β -cell from TSA-treated mouse Langerhans islets compared to β -cell
802 from vehicle-treated mouse Langerhans islets according to two cut-off thresholds
803 based on adjusted p-value (P_{adj}) and Log2 fold change (Log2FC). **(C-D)** Top 5
804 canonical pathways identified by IPA of downregulated (C) and upregulated (D) genes
805 in TSA-treated mouse sorted- β cells. **(E)** Venn diagram identifying common TSA-
806 dependent up- and downregulated genes between Min6 cells and β -cell from mouse
807 Langerhans islets. **(F-G)** GO analysis of common TSA-dependent downregulated (F)
808 and upregulated (G) genes. Gene ontology analysis was performed using Metascape
809 by filtering output only on GO Biological process. The 5 more enriched GO Biological
810 processes are displayed as $-\text{Log}_{10}(q)$ (*i.e.*, $-\text{Log}_{10}(P_{adj})$). **(H-I)** Pie chart displaying
811 proportion of TSA-dependent downregulated (H) and upregulated (I) genes in β -cell
812 from mouse Langerhans islets associated with a specific functional genomic region.

813 **Figure 8. (A-B)** Schematic representation of EndoC- β H1 and human islets treatment
814 by TSA and subsequent RNA-seq analysis. **(C)** Volcano plot displaying downregulated
815 (red circles), upregulated (green circles) and not deregulated genes (black circles) in
816 EndoC- β H1 control and TSA-treated cells. **(D-E)** Top 5 canonical pathways identified
817 by IPA of downregulated (D) and upregulated (E) genes in TSA-treated EndoC- β H1
818 cells. **(F)** Volcano plot displaying downregulated (red circles), upregulated (green
819 circles) and not deregulated genes (black circles) in TSA-treated human islet
820 compared to untreated controls. **(G-H)** Top 5 canonical pathways identified by IPA of
821 downregulated (D) and upregulated (E) genes in TSA-treated human pancreatic islets.

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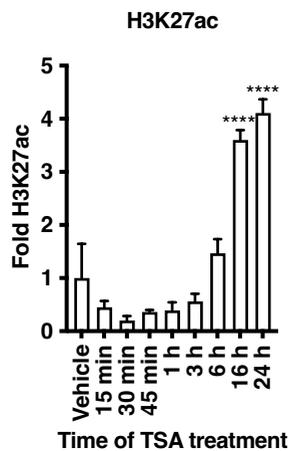
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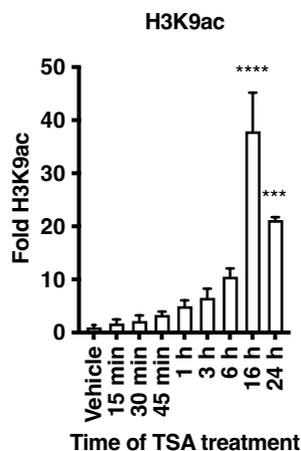
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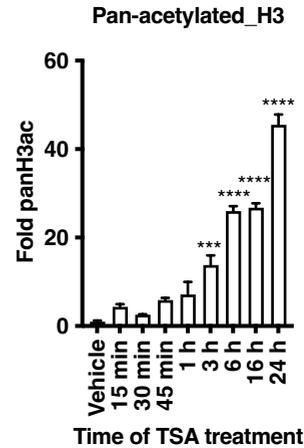
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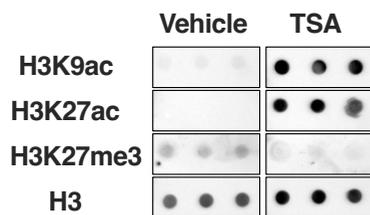
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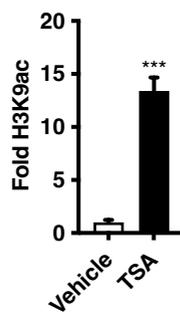
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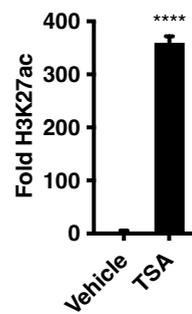
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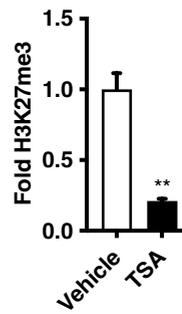
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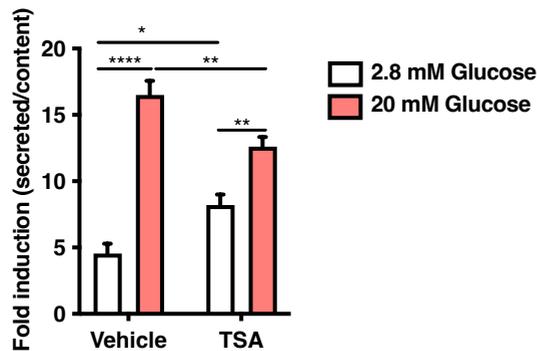
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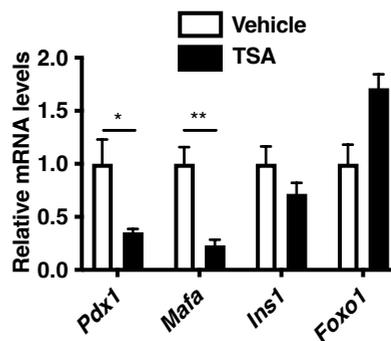
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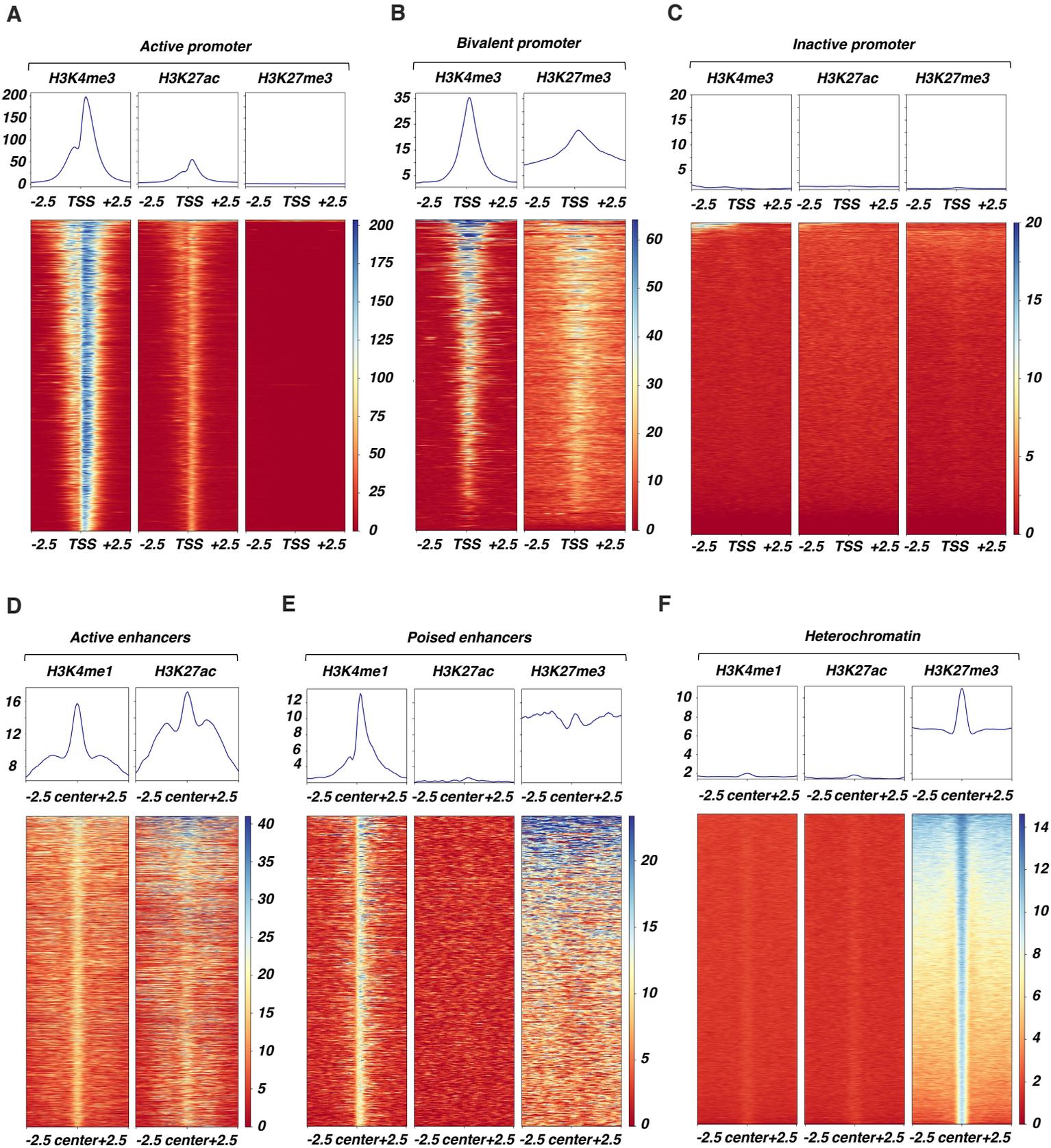


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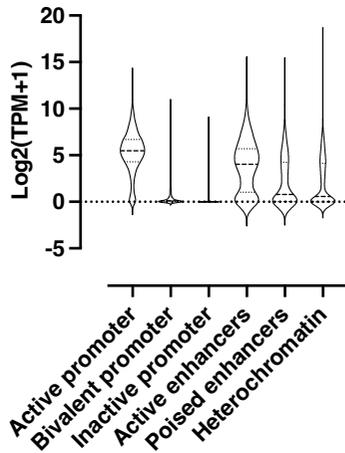


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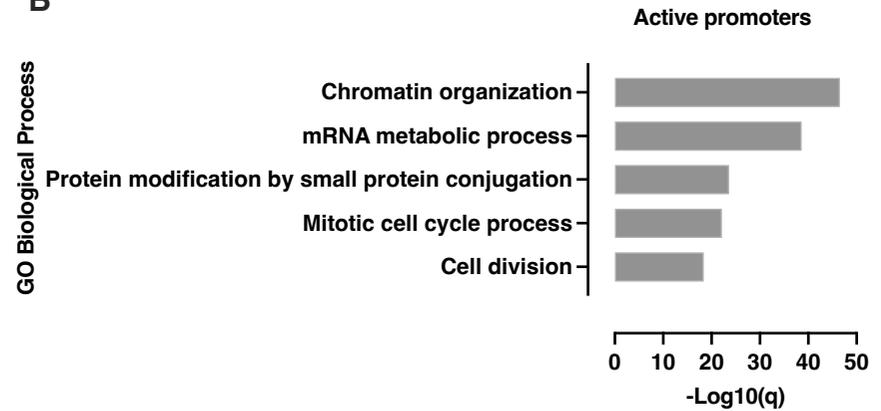




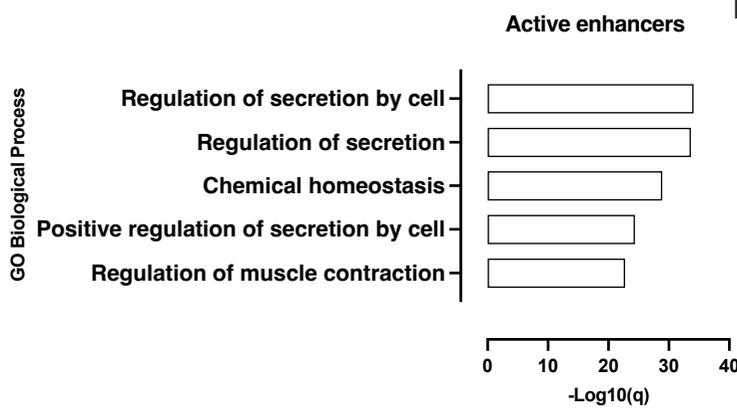
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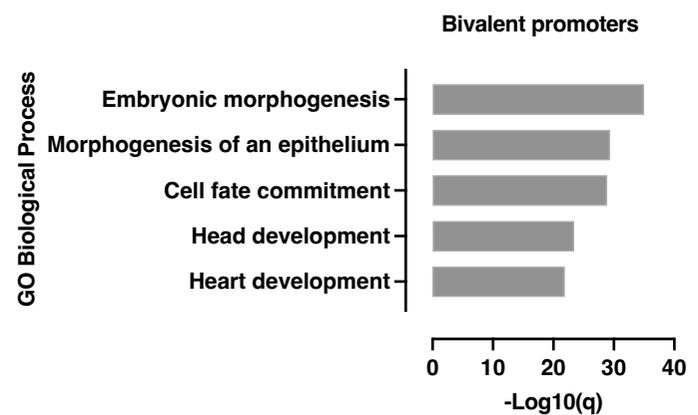
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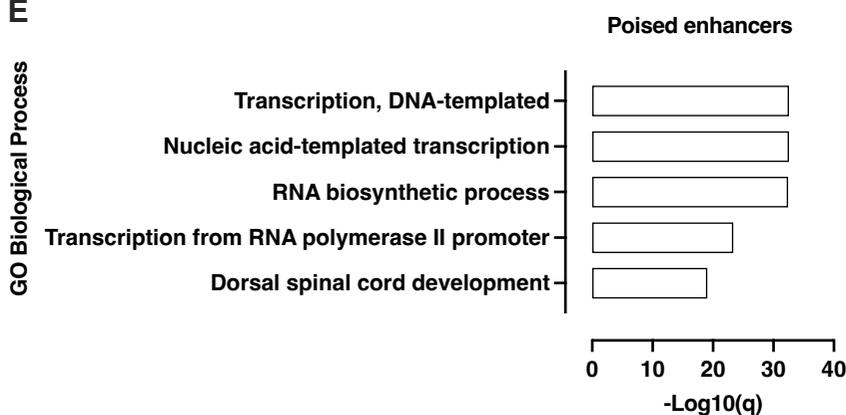
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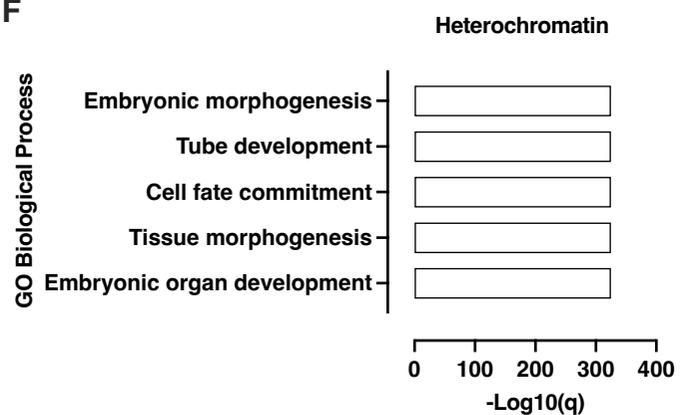
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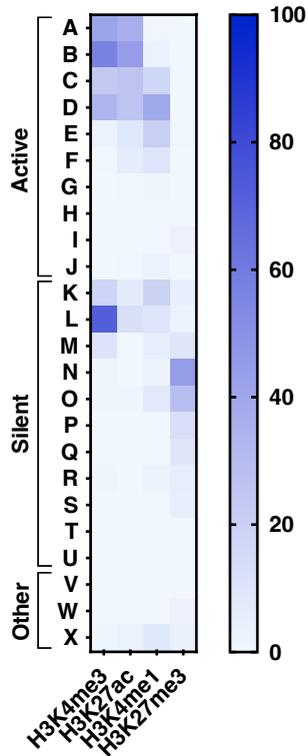
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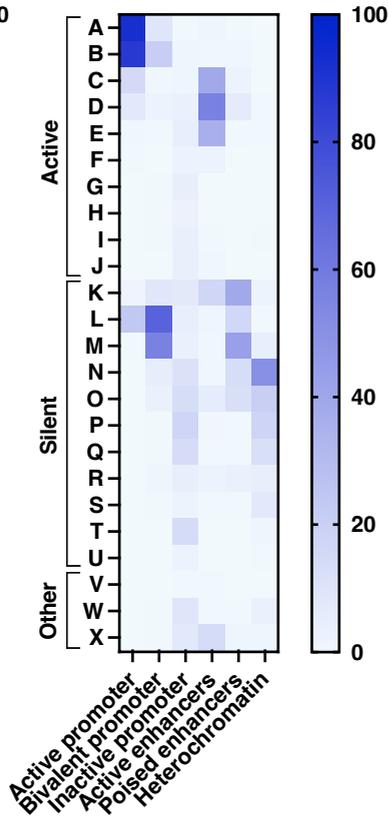
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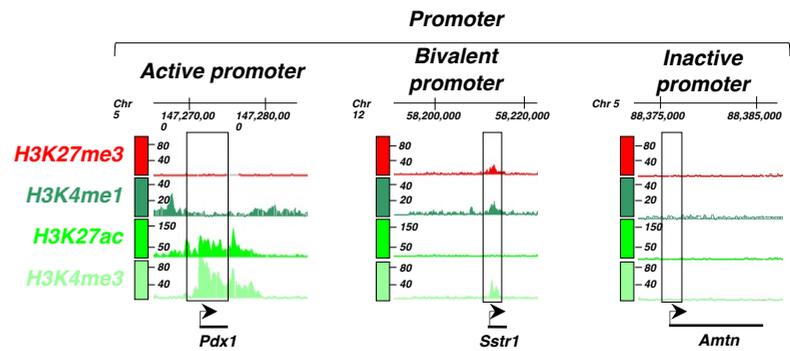
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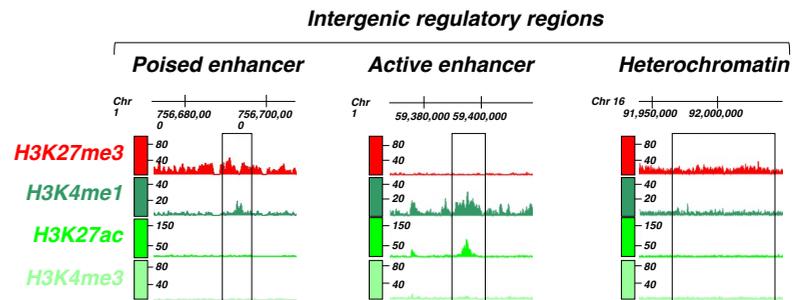
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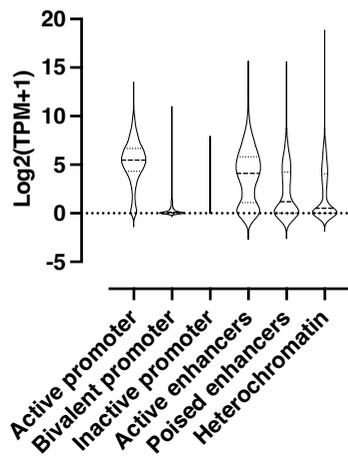
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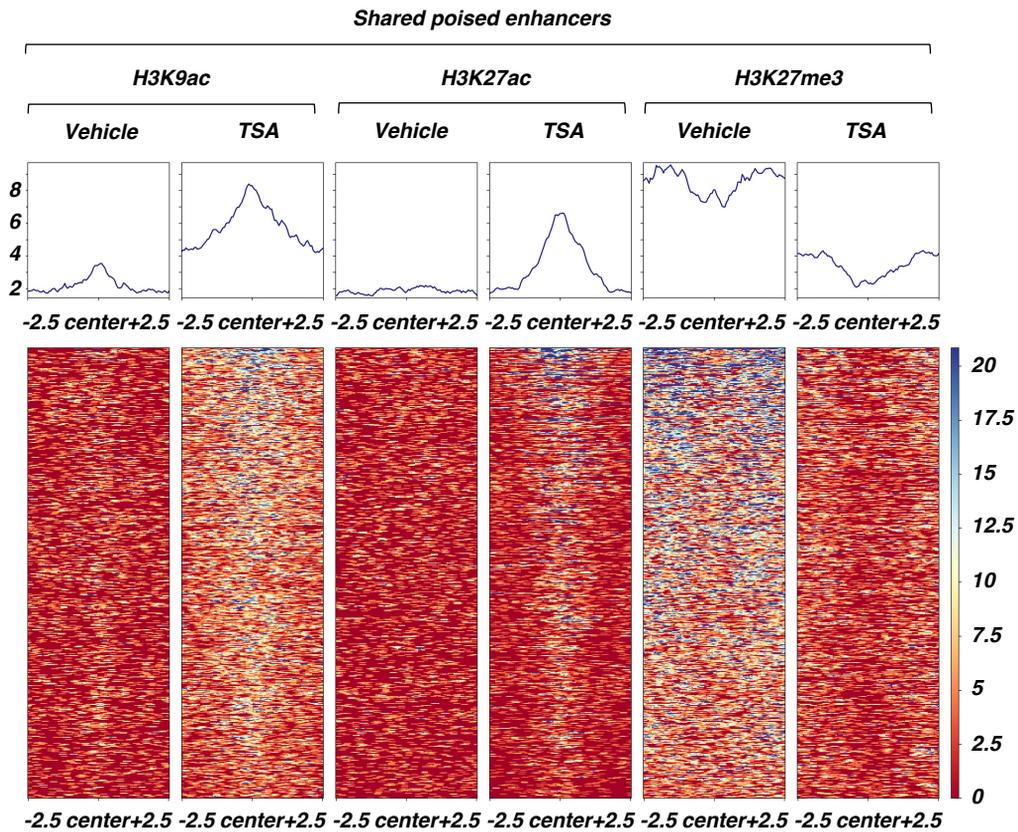
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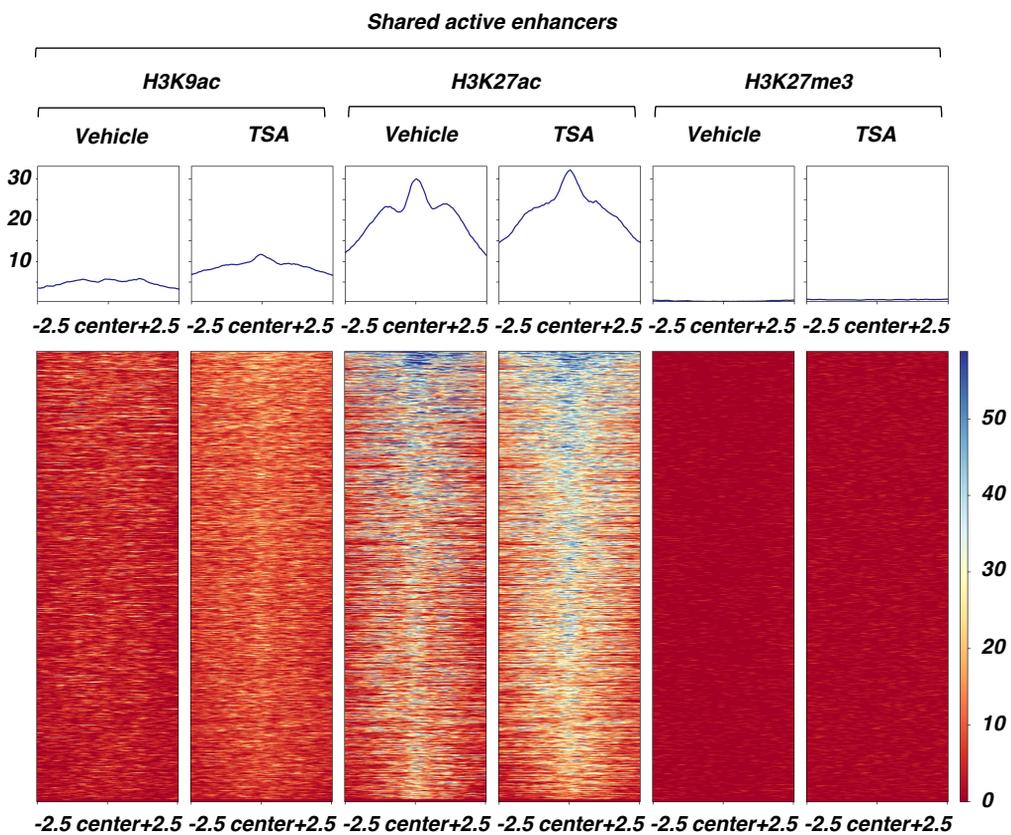
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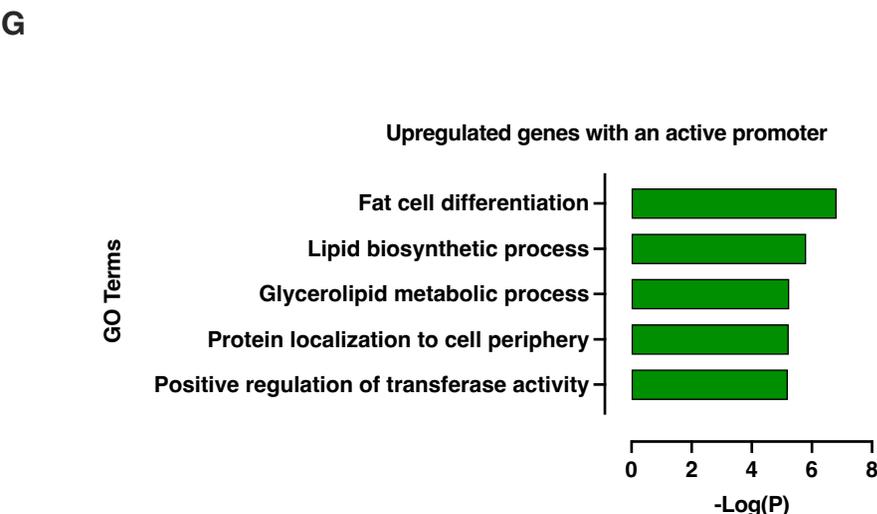
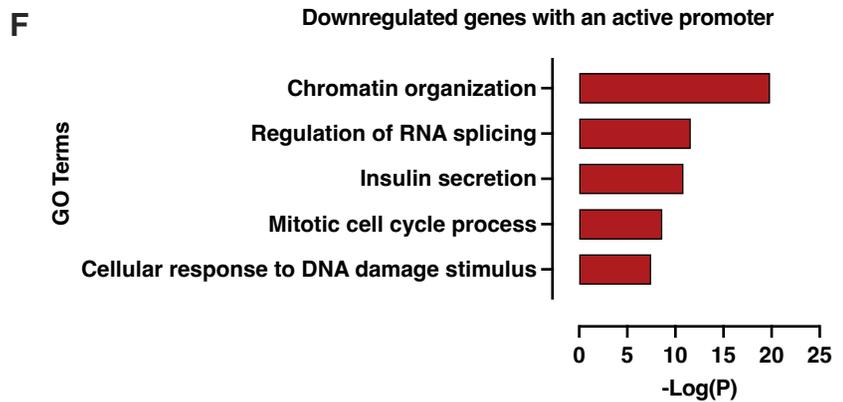
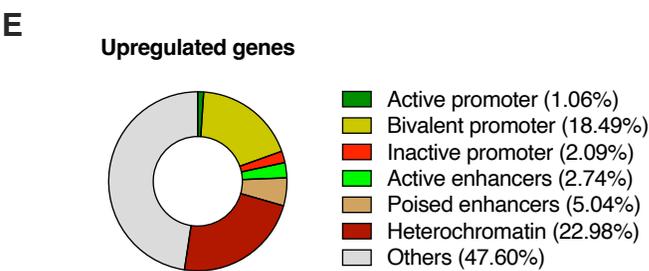
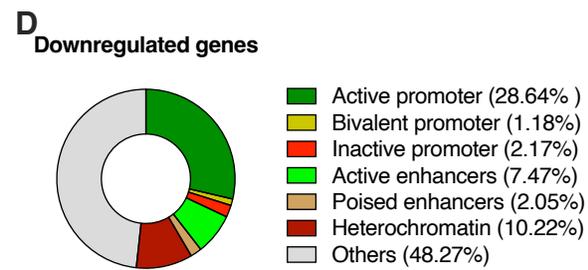
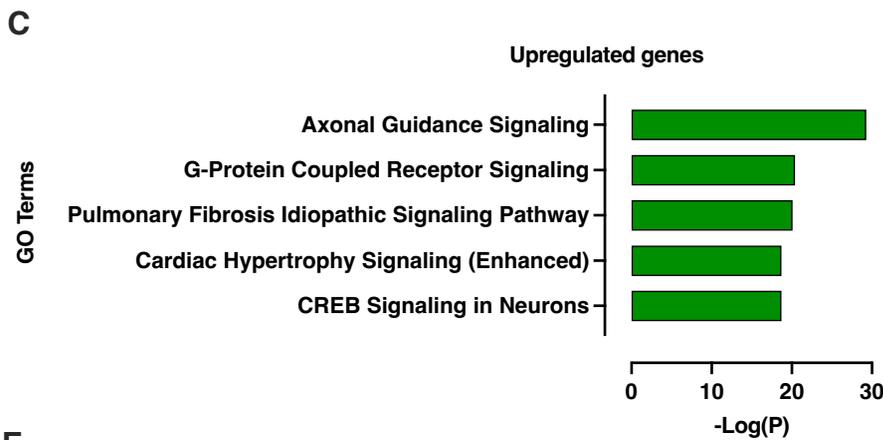
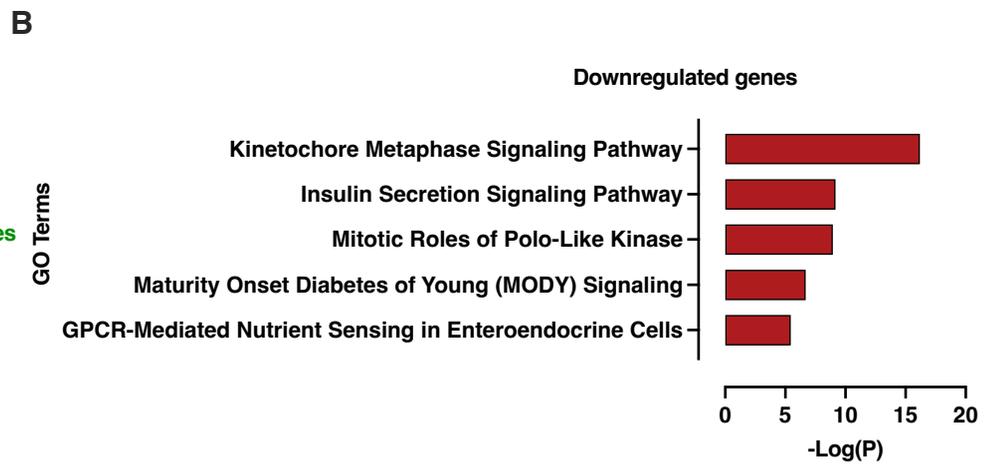
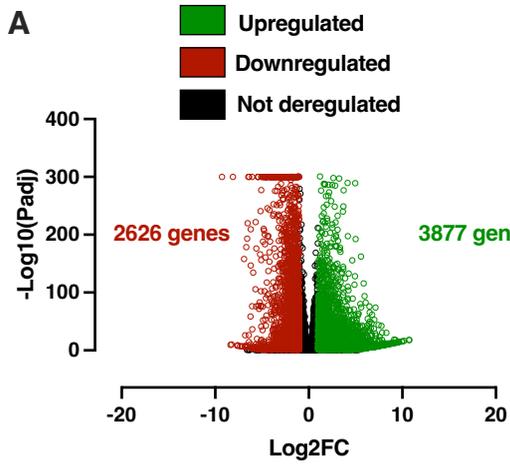


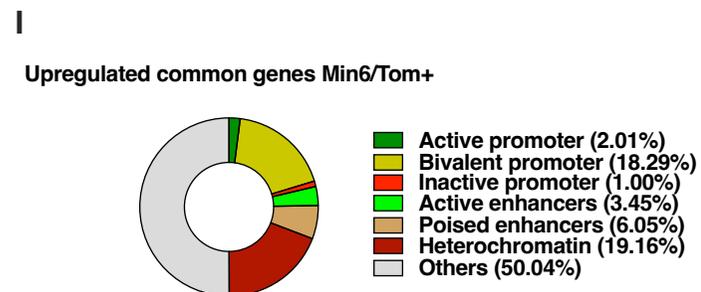
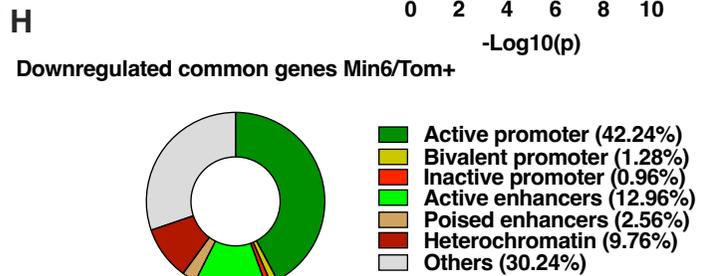
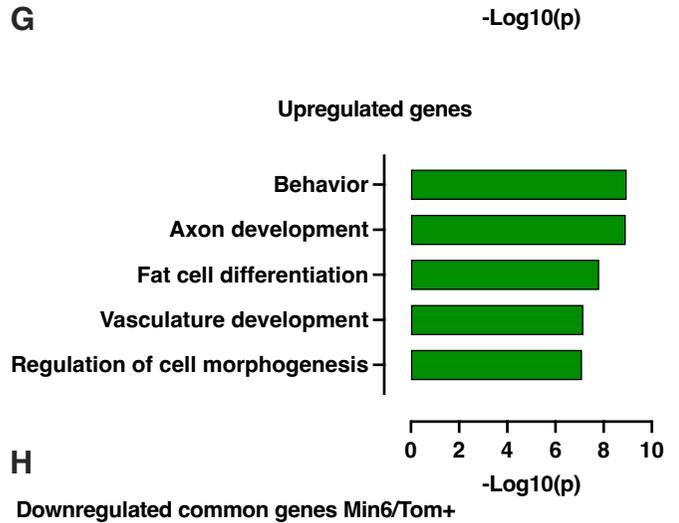
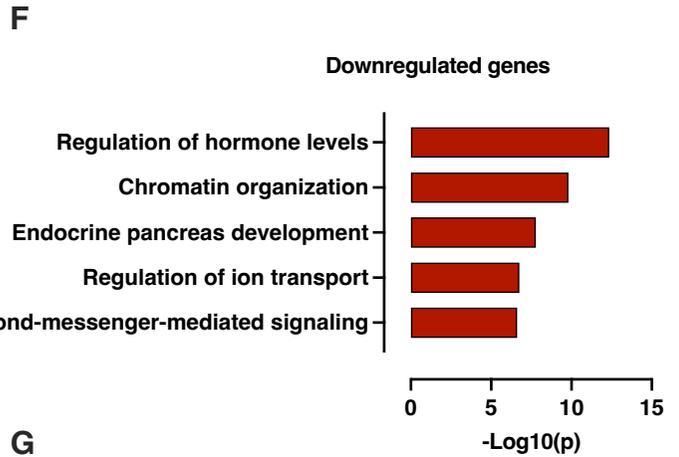
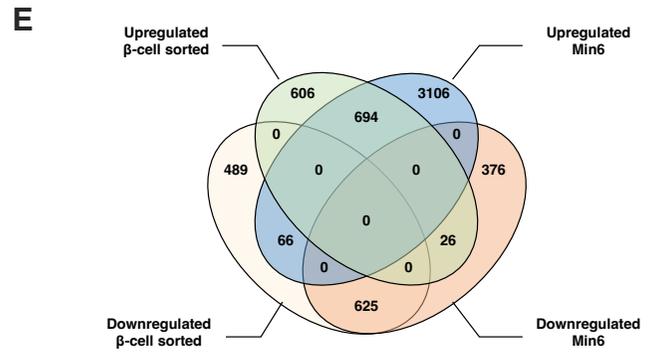
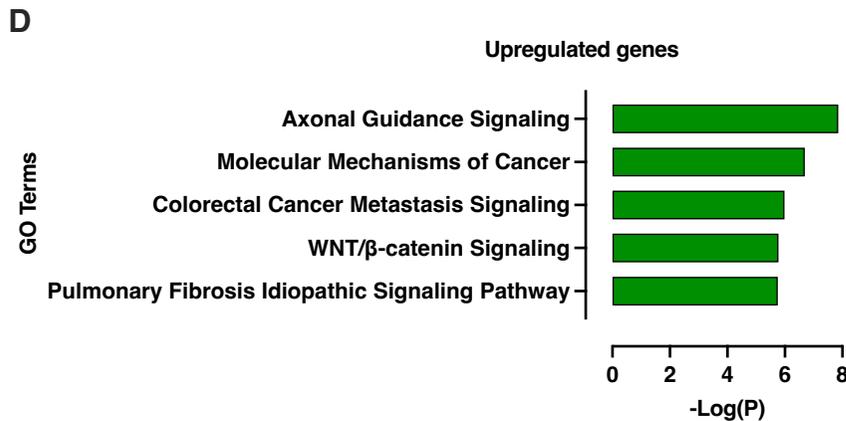
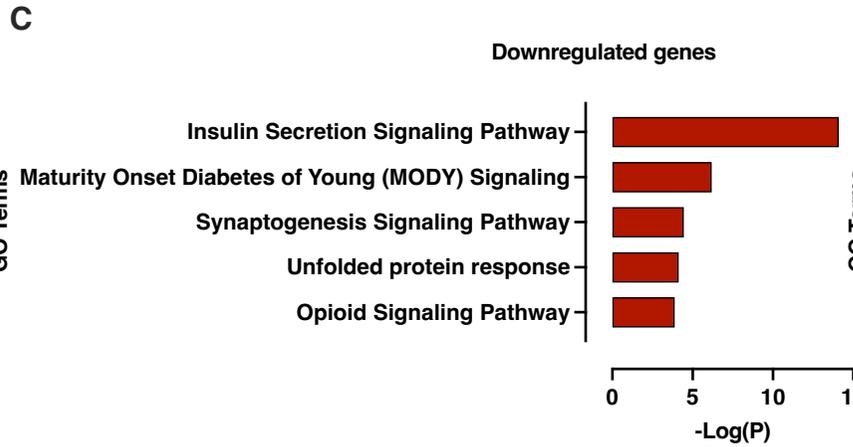
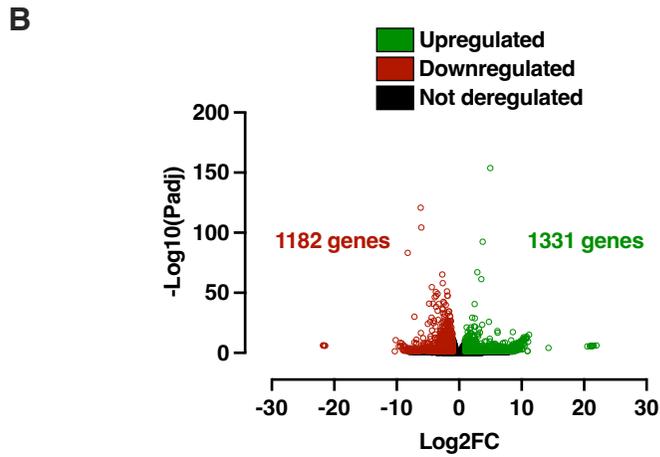
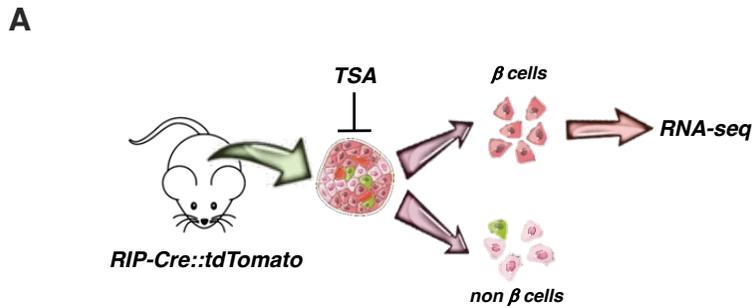
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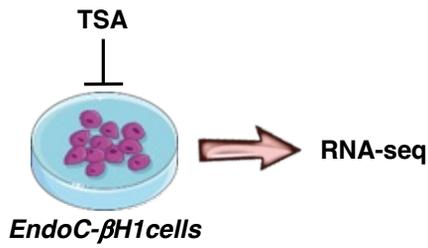
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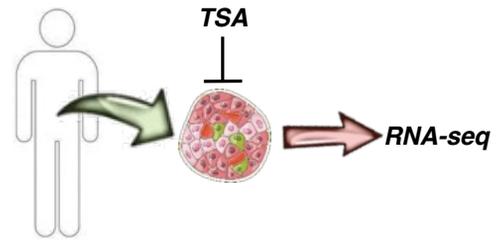




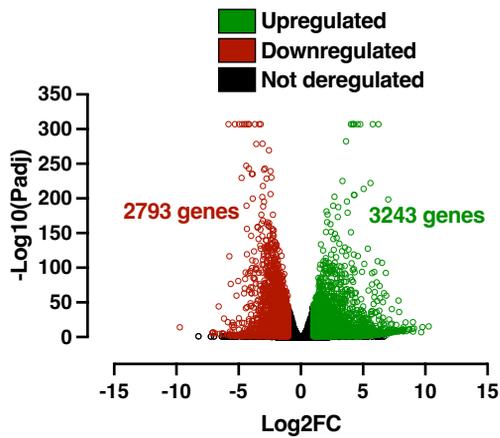
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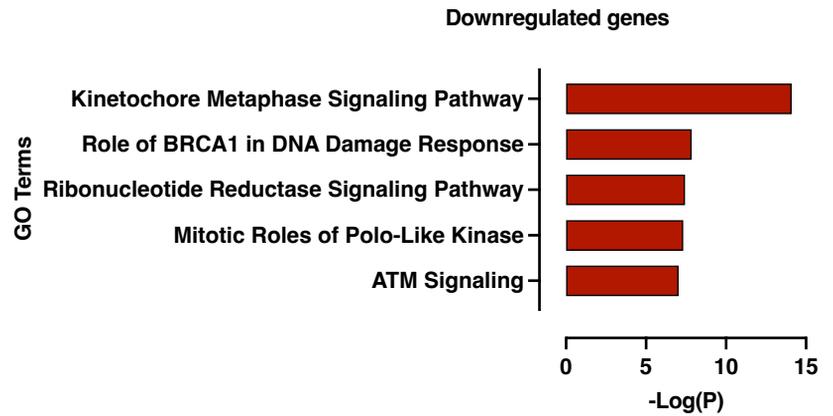
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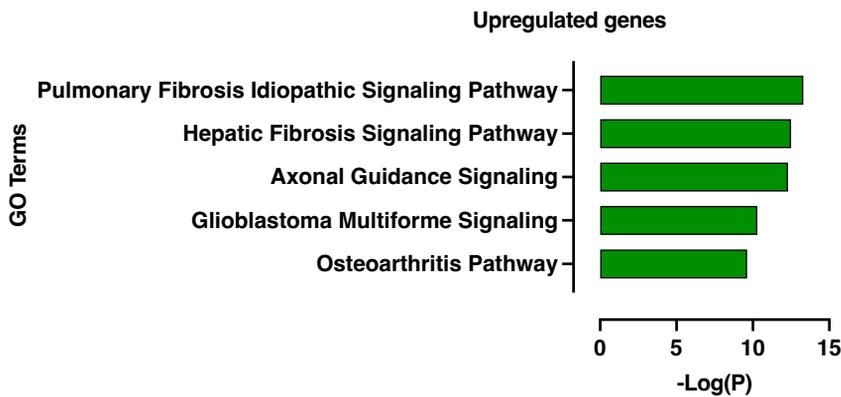
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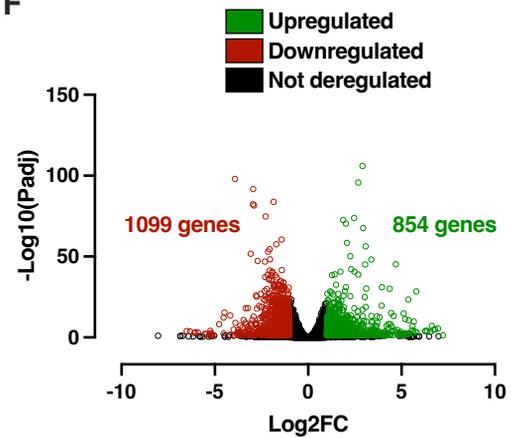
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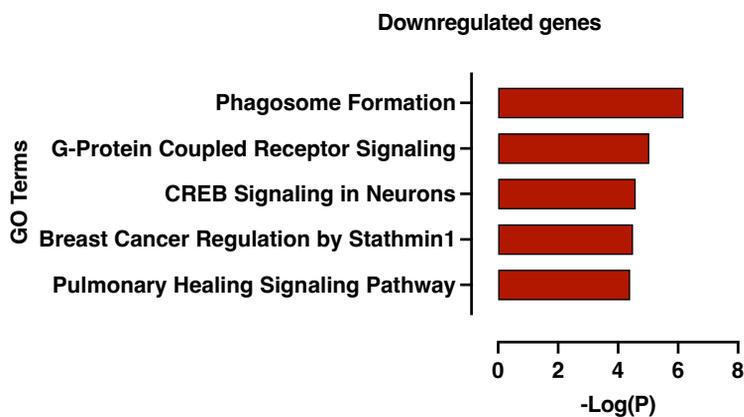
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