1 **Bromodomain inhibition reveals FGF15/19 as a target of epigenetic regulation**

2 **and metabolic control**

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24 **Summary**

25 Epigenetic regulation is an important factor in glucose metabolism, but underlying
 26 mechanisms remain largely unknown. Here we demonstrated that
 27 bromodomain-containing proteins (Brds), transcriptional regulators binding to acetylated
 28 histone, are potent modulators of glucose metabolism via the gut-liver farnesoid X
 29 receptor (FXR)-fibroblast growth factor 15/19 (FGF15/19) pathway. In vivo inhibition of
 30 Brd4 by the inhibitor JQ1 in mice strongly inhibited ileal expression of FGF15, resulting
 31 in decreased FGFR4-related signaling, increased glucose production in the liver and
 32 hyperglycemia. Adverse metabolic effects of BRD4 inhibition were reversed by
 33 overexpression of FGF19, with improvement in hyperglycemia. At a cellular level, we
 34 demonstrate that BRD4 binds to the promoter region of FGF19 in human intestinal cells;
 35 BRD inhibition by JQ1 reduces binding to the FGF19 promoter and downregulates
 36 FGF19 expression. Thus, we identify Brd4 as a novel transcriptional regulator of
 37 intestinal FGF15/19 in ileum, and a contributor to hepatic and systemic glucose
 38 metabolism.

39

40 **Keywords**

41 Epigenetics, bromodomain inhibition, FGF15/19, glucose metabolism

42 **Introduction**

43 Type 2 diabetes (T2D) is a complex disorder influenced by interactions between
44 multiple genetic loci and environmental factors (Pinney and Simmons, 2010).
45 Environmental factors contributing to metabolic disease, such as intrauterine
46 environment, diet and physical activity, may mediate risk via epigenetic mechanisms,
47 such as DNA methylation, histone modification, and noncoding RNAs. Given that
48 epigenetic mediators are influenced by metabolic signals and in turn modulate
49 transcriptional and/or developmental responses, understanding mechanisms by which
50 epigenetic signals influence metabolic disease risk is a key scientific challenge.

51 One key epigenetic mark is histone acetylation, which mediates chromatin accessibility
52 to transcriptional factors and coactivators (Verdin and Ott, 2015). In turn, histone
53 acetylation is mediated by histone acetyltransferase (HAT) and removed by histone
54 deacetylase (HDAC). Modulation of histone acetylation can alter systemic metabolism.
55 For example, mice with heterozygous deficiency of the HAT CREB-binding protein
56 (CBP), remain insulin sensitive, despite lipodystrophy (Yamauchi et al., 2002).
57 Conversely, deletion of HDAC in skeletal muscle increases lipid oxidation, energy
58 expenditure and insulin resistance (Gaur et al., 2016; Hong et al., 2017). Together,
59 these data demonstrate that histone acetylation plays an important role in energy

60 expenditure and glucose metabolism.

61 The Bromodomain and Extra-Terminal Domain (BET) family of proteins has two
62 tandem bromodomains that recognize acetylated lysine of histones or non-histone
63 targets. The mammalian BET family comprises bromodomain-containing proteins 2
64 (Brd2), Brd3, Brd4 and BrdT in mammals, which are “readers” that bind to acetylated
65 histones and recruit transcription factors (Marmorstein and Zhou, 2014). While Brds are
66 recognized for their potential as a target in cancer therapeutics, Brds also influence
67 metabolism. For example, mice with genetic disruption of Brd2 have severe obesity, but
68 normal glucose metabolism, potentially via increased peroxisome-proliferator-activated
69 receptor (PPAR)- γ activity (Wang et al., 2009). Brd4 binds to enhancers regulating
70 adipogenesis and myogenesis (Lee et al., 2017). Moreover, Brd4 is a coactivator of
71 nuclear factor- κ B, (Huang et al., 2009; Mauro et al., 2011), and inhibition of Brd4 can
72 modulate OXPHOS capacity (Barrow et al., 2016). However, the role of BET proteins in
73 systemic metabolism remains ill-defined.

74 Mouse fibroblast growth factor (FGF) 15 and its human ortholog FGF19 share about
75 50% amino acid identity and have similar physiological functions to regulate
76 intestine-to-liver crosstalk via a complex feedback loop (Markan and Potthoff, 2016).
77 Bile acids are synthesized from cholesterol in the liver and enter the enterohepatic

78 circulation. After entry into the intestinal lumen, bile acids are transported into
79 enterocytes by apical sodium dependent bile acid transporter (ASBT) and activate the
80 farnesoid X receptor (FXR) to promote FGF15/19 gene transcription and secretion of
81 FGF15/19 into the circulation. In the liver, FGF15/19 binds to and activates the FGF
82 receptor 4 (FGFR4)/ β -klotho receptor complex, leading to suppression of bile acid
83 synthesis via repression of the rate-limiting enzyme cytochrome P450 7A1 (CYP7A1).
84 FGF15/19 also exerts potent effects on glucose metabolism, reducing blood glucose via
85 increased activity of Agouti-related peptide/Neuropeptide Y neurons in hypothalamus
86 (Liu et al., 2018; Morton et al., 2013) and modulation of hepatic metabolism, including
87 inhibition of gluconeogenesis and lipogenesis and increased glycogen synthesis
88 (Owen et al., 2015). We now demonstrate that the FGF15/19 signaling pathway is a
89 target of epigenetic modification by the bromodomain inhibitor, JQ-1.

90

91 **Results**

92 ***Brd4 inhibition by JQ-1 decreases body weight and induces hyperglycemia***

93 JQ-1, a Brd inhibitor, shows high selectivity for Brd4 (Filippakopoulos et al., 2010). To
94 determine the effect of Brd4 inhibition on body weight and glucose metabolism, we
95 treated CD1 mice with JQ-1 (25 mg/kg intraperitoneally) for 11 days (**Figure 1A**). This

dose was previously shown to be without toxic effects during long term treatment (Filippakopoulos et al., 2010). JQ-1 decreased body weight modestly (Veh: 41.4 ± 0.9 g; JQ-1: 36.8 ± 0.5 g, $p < 0.01$) (**Figure 1B**). Modest effects on food intake were observed in one cohort (Veh: 5.4 ± 0.2 g/day; JQ-1: 3.7 ± 1.1 g/day), but not in subsequent cohorts of mice treated with the same dose of JQ-1. Blood glucose was significantly increased in JQ-1 treated mice vs. vehicle, both after a 4-hour fast (Veh: 186 ± 17 mg/dl; JQ-1: 264 ± 14 mg/dl, $p < 0.01$, **Figure 1C**) and during intraperitoneal glucose tolerance testing (peak glucose: Veh: 384 ± 18 mg/dl; JQ-1: 592 ± 8 mg/dl, $p < 0.01$) (**Figure 1D-E**).

We next analyzed potential contributors to hyperglycemia in JQ-1-treated mice. While fasting plasma insulin did not differ, insulin levels were significantly decreased in JQ-1-treated mice after glucose injection (50% reduction at 15 minutes, $p < 0.05$) (**Figure 1F**). Glucose similarly reduced glucagon levels in both vehicle and JQ-1-treated mice (**Figure 1G**). JQ-1 had no effect on pancreatic islet size (Veh; $15,427 \pm 3,503 \mu\text{m}^2$, JQ; $14,763 \pm 1,928 \mu\text{m}^2$) (**Figure 1H, I**). There was no difference in insulin sensitivity as determined by ITT (**Figure 1J**). Glycerol tolerance testing revealed greater increase in blood glucose in JQ-1-treated mice vs. vehicle-treated mice (**Figure 1K, L**).

Fasting hyperglycemia in JQ-1-treated mice without change in fasting insulin and increased glycemic response to glycerol suggested increased gluconeogenesis. We

114 therefore asked whether this was related to impaired insulin action or cell autonomous
115 insulin resistance. Hepatic insulin action *in vivo*, as measured by Akt phosphorylation
116 (**Figure S1A-C**) did not differ. Moreover, basal glucose production in primary
117 hepatocytes did not differ with JQ-1, and the effect of insulin to suppress glucose
118 production was only modestly reduced (vehicle vs. JQ-1, $p = 0.05$, **Figure S1D**).
119 Together, these data suggested that reduction in systemic insulin sensitivity or cell
120 autonomous hepatic insulin action was not likely the dominant mediator of *in vivo*
121 hyperglycemia.

122

123 ***JQ-1 alters hepatic gene expression and sterol metabolism***

124 To identify potential molecular mediators of fasting hyperglycemia *in vivo*, we analyzed
125 gene expression in the liver using microarray. 1471 genes were upregulated and 1036
126 genes were downregulated by JQ-1 (FDR<0.25). Pathway analysis using Gene Set
127 Enrichment Analysis (GSEA) and REACTOME pathways (**Table S1**) revealed
128 upregulation of multiple pathways related to glucose production and lipid metabolism
129 (biosynthesis of triglycerides, fatty acids, and cholesterol; β -oxidation) in JQ-1-treated
130 mice (**Figure S1E, F**). Analysis of individual genes revealed increased expression of
131 genes related to gluconeogenesis (fold change vs. vehicle: *Pepck*: 1.6 ± 0.2 , $p < 0.01$,

132 *G6pase*: 2.0 ± 0.6 , **Figure S1G, H**). Effects on lipid metabolism were more striking,
133 with significant increases in expression of genes regulating FA synthesis (*Acc*: 1.9 ± 0.2 ,
134 $p < 0.01$; *Fasn* 2.2 ± 0.3 , $p < 0.01$), β -oxidation (*Ppara*: 1.6 ± 0.1 , $p < 0.01$; *Cpt1a*: $1.2 \pm$
135 0.0 , $p < 0.01$), and cholesterol synthesis (e.g. *Hmgcr*, *Hmgcs1*, and *Srebp2*) (**Figure**
136 **S1I-O**).

137 Given these prominent patterns of lipid-related gene expression in JQ-1 treated mice,
138 we analyzed plasma and hepatic lipids. There were no differences in either plasma or
139 hepatic triglycerides (**Figure S2A, B**). By contrast, cholesterol levels were reduced by
140 52% in plasma ($p < 0.05$), with 28% reduction in liver content in JQ-1-treated mice
141 (plasma: veh; $165 \pm \text{mg/dl}$, JQ-1; $79 \pm 29 \text{ mg/dl}$, $p < 0.05$; liver: veh; $0.94 \pm 0.1 \text{ mg/g}$
142 tissue, JQ-1; $0.68 \pm 0.1 \text{ mg/g tissue}$, $p < 0.05$) (**Figure S2C, D**).

143 Reductions in both plasma and tissue cholesterol levels, despite significantly increased
144 expression of cholesterol synthetic genes, suggested that cholesterol catabolic
145 pathways, such as bile acid metabolism, may be upregulated. Indeed the major
146 enzymes of both classic and alternative bile acid synthesis pathways were upregulated
147 by JQ-1, with a 1.4-fold increase in the rate-limiting enzyme *Cyp7a1* ($p = 0.08$) and
148 1.2-fold increase in *Cyp27a1* ($p < 0.01$) (**Figure S1P, Q and Figure S2E**). However,
149 there was no net difference in hepatic and plasma bile acid composition between

150 vehicle and JQ-1-treated mice (**Figure S2F, G**), potentially linked to downregulation of
 151 the downstream enzymes Cyp7b1 and Cyp8b1 (reduced by 56 and 46% respectively,
 152 **Figure S2E**).

153 The complex pattern of lipid, sterol, and bile acid gene expression and metabolism in
 154 JQ-1-treated liver raised the possibility that FGF receptor-dependent signaling might be
 155 reduced by JQ-1. Indeed, 10 of 14 top-ranking pathways *downregulated* in JQ-1-treated
 156 mice were related to FGFR signaling (FDR <0.25, **Figure 2A**). Expression of Fgfr4 as
 157 determined by PCR was decreased by 27% ($p < 0.01$) with a similar trend for its
 158 coreceptor beta-Klotho (17% lower, $p = 0.07$). Moreover, Fgfr signaling was significantly
 159 reduced with a 46% reduction in phosphorylation of FGF receptor substrate 2 (FRS2) in
 160 liver protein extracts of JQ-1 treated mice ($p < 0.01$, **Figure S1A, B**).

161

162 ***Brd4 inhibition by JQ-1 reduces ileal expression of Fgf15***

163 Upregulation of bile acid synthetic enzymes and downregulation of FGFR-mediated
 164 signaling suggest that JQ1 modulated systemic and hepatic lipid and bile acid
 165 metabolism via the complex regulatory loop involving the bile acid-responsive hormone
 166 FGF15/19. FGF15 (FGF19 in humans) is produced in intestinal enterocytes in response
 167 to bile acids, secreted into the bloodstream, and binds FGF receptors in the liver to

168 inhibit both bile acid synthesis and cholesterol metabolism (Kim et al., 2015; Kliewer
169 and Mangelsdorf, 2015).

170 Luminal bile acids are transported into enterocytes by ASBT (Slc10a2), where they
171 can bind to FXR and activate its transcriptional activity to increase expression of its
172 targets including *Fgf15*, *Shp*, *Slc15a* and *Slc51b* (Wong et al., 2011). While there was
173 no change in no change in Slc10a2, Tgr5, Fxr, or RXRa expression in ileum of
174 JQ-1-treated mice (not shown), we observed profound alterations in expression of FXR
175 target genes. Expression of *Fgf15* and *Shp* was reduced by 90% and 95% ($p < 0.01$ for
176 all), with similar trend for *Slc51a* and *Slc51b* (**Figure 2E-H**). In agreement, plasma
177 FGF15 was decreased by 29% in JQ-1-treated mice (veh; 2.1 ± 0.1 ng/ml, JQ-1; $1.4 \pm$
178 0.1 ng/ml, $p < 0.05$) (**Figure 2I**).

179 We next evaluated potential mechanisms mediating JQ-1 decreases in *Fgf15*
180 expression. Previous studies have shown that RNAi-mediated silencing of *Brd4* reduces
181 intestinal cellular diversity (Bolden et al., 2014), potentially via reduced intestinal stem
182 cell differentiation (Nakagawa et al., 2016); thus, alterations in number or function of
183 specific intestinal cell subtypes could potentially contribute to JQ-1-mediated changes in
184 intestinal FGF15 levels. Histologic analysis revealed no change in the overall villus
185 structure in JQ-1-treated mice (**Figure 2J**). However, JQ-1 was associated with reduced

186 numbers of Paneth cells, as previously reported (Bolden et al., 2014) (**Figure S3A**). In
187 parallel, mRNA levels of Reg4, a Paneth cell marker, were decreased by JQ-1, but
188 expression of the microbicidal functional marker α -defensin cryptdin-4 (Crp4) did not
189 differ (**Figure S3G, K**) (Ouellette, 2011). Moreover, there was no change in expression
190 of other cell type markers for enterocytes (keratin 20 (Krt20)), enteroendocrine cells
191 (hepatocyte nuclear factor (HNF) 1 α), neuroendocrine cells (chromogranin A (ChgA)),
192 goblet cells (mucin 2 (Muc2)), stem cells (leucine-rich orphan G-protein-coupled
193 receptor 5 (Lgr5)), or intestinal neuroendocrine peptides (*Gcg* or *Pyy*), suggesting
194 JQ-1-mediated reduction in Fgf15 expression was not related to global alterations in cell
195 type distribution (**Figure S3B-J**).

196

197 ***JQ-1 inhibits Brd4 binding to the Fgf15 promoter in HT-29 cells***

198 Marked downregulation of ileal expression of Fgf15 in JQ-1 treated mice suggested
199 that the bile acid-Fgf15 feedback loop might play a prominent role in systemic metabolic
200 effects of JQ-1. To test this hypothesis at a cellular level, we utilized the human intestinal
201 cell line HT-29, which constitutively expresses FGF19 at a high level (Vergnes et al.,
202 2013). HT-29 cells were treated with 500 nM JQ-1 for 24 hours. While there was no
203 change in *BRD4* expression (**Figure 3A**), expression of the Brd4 target gene *MYC* was

204 reduced by 83% with JQ-1 ($p < 0.01$), as predicted (**Figure 3B**). In parallel, expression
205 of *FGF19* was reduced by 96% ($p < 0.01$) with similar dramatic reduction in *SHP* (89%
206 reduction, $p < 0.01$) (**Figure 3C, D**). There was no change in *FXR* or *SLC51B*
207 expression (data not shown), consistent with prior evidence indicating BRD4 does not
208 bind to FXR or SLC51B (GSE73319 (McClelland et al., 2016) and ENCSR514EOE
209 (Consortium, 2012). Moreover, secretion of FGF19 into culture medium was completely
210 abolished by JQ-1, with >98% decrease in the conditioned medium ($p < 0.01$, **Figure 3E**).
211 To determine whether JQ-1 mediated reduction in expression of FGF19 and SHP was
212 mediated by modulation of BRD4 binding to promoter regions of these genes, we
213 performed CHIP-PCR analysis using an anti-BRD4 antibody (**Figure 3F**) (Rathert et al.,
214 2015). As expected, BRD4 bound to its target Myc (control); Brd4 also bound robustly to
215 promoter sequences of both FGF19 and SHP (**Figure 3G, H**). This was markedly
216 inhibited by JQ-1, with a >90% reduction in binding (**Figure 3G, H**).

217

218 ***Overexpression of FGF19 reverses glucose intolerance induced by JQ-1***

219 To determine the requirement for FGF15/19 signaling in mediating the metabolic
220 effects of JQ-1, we treated mice with AAV-FGF19 or GFP control vectors, and then
221 treated mice with JQ-1 (25 mg/kg, ip) for 10 days (**Figure 4A**). Plasma FGF19 was not

222 detectable in AAV-GFP controls, but was readily detected in plasma of AAV-FGF19 mice
 223 **(Figure 4B)**. Consistent with prior studies (Lan et al., 2017; Morton et al., 2013),
 224 AAV-FGF19-treated mice had lower body weight and blood glucose levels at baseline
 225 **(Figure 4C)** but weight did not change during JQ-1 treatment in either GFP or FGF19
 226 groups **(Figure 4C)**. As with prior cohorts, JQ-1 increased blood glucose in AAV-GFP
 227 control mice as early as 4 days after the onset of treatment, but this effect was not
 228 observed in AAV- FGF19 mice. Glucose tolerance testing at day 10 of treatment again
 229 revealed significant impairment in JQ-1 treated mice (35% increase in glucose AUC, $p <$
 230 0.01) **(Figure 4D)**. By contrast, the ability of JQ-1 to impair glucose tolerance was fully
 231 reversed in mice overexpressing FGF19 **(Figure 4D, E)**. Moreover, overexpression of
 232 FGF19 reduced both basal and glucose-stimulated insulin levels **(Figure 4F)**, indicating
 233 FGF19-mediated reversal of glucose tolerance in JQ-1 treated mice is
 234 insulin-independent.
 235

236 **Discussion**

237 Our studies identify the intestinal Fgf15/19 hormonal axis as a target of epigenetic
238 and transcriptional regulation by the bromodomain protein Brd4 and its inhibitor JQ-1.
239 We demonstrate that pharmacological inhibition of Brd4 induces hyperglycemia and
240 impaired glucose tolerance in mice, via inhibition of intestinal FGF15 expression and
241 reduced Fgfr4-mediated signaling in liver. ChIP-PCR analysis revealed that FGF19 and
242 SHP are novel targets of Brd4 in intestine, and that JQ-1 reduced Brd4 binding to the
243 promoter of these targets, reduced their expression, and reduced secretion of FGF19.
244 Experimental overexpression of FGF19 normalized JQ-1-induced hyperglycemia. Thus,
245 Brd4-dependent transcription is a potent regulator of intestinal endocrine function and
246 systemic glucose metabolism via FGF15/19-dependent mechanisms.

247 Our data provide new evidence supporting epigenetic regulation of intestinal function
248 and metabolism and identification of new gene targets of Brd4 regulation. Prior studies
249 have demonstrated that genetic or pharmacologic inhibition of Brd4-dependent
250 signaling (Bolden et al., 2014) (Nakagawa et al., 2016) can modulate intestinal cell
251 populations. While we also find that JQ-1 decreased the number of Reg4+ Paneth cells,
252 there was no change in the Paneth cell functional marker Crp4 (Ouellette, 2011)
253 (**Figure S3**) nor in ileal expression of cytokines (data not shown). Moreover, JQ-1 did

not alter markers of additional intestinal cell populations. Thus, effects of JQ-1 in our experimental conditions do not appear to require alterations in Paneth cell-linked host defense systems or cell type distribution. Rather, our data point to new targets of Brd4 action in the intestine – including regulation of enterocyte Fgf15/19 expression – with secondary effects on hepatic and systemic glucose and sterol lipid metabolism.

Fgf15/19 expression and secretion in the intestine can be regulated by bile acid binding to the luminal bile acid receptor Tgr5 or the nuclear receptor Fxr. We observed no change in expression of Tgr5, the apical bile acid transporter Slc10a2, Fxr, or Rxr, and plasma levels of bile acids did not differ significantly in JQ-1-treated mice. We cannot exclude the possibility that luminal signaling via bile acids or other metabolites could contribute to reduced intestinal Fgf15 expression and secretion in response to JQ-1 in vivo. However, our data in cultured cells suggest that JQ-1 exerts a direct, cell autonomous effect on transcription and secretion of Fgf15/19 via inhibition of Brd4.

Our data suggest that inhibition of Fgf15/19 is also the dominant mediator of in vivo metabolic effects of JQ-1. Fgf15/19 is increasingly recognized as a potent regulator of systemic glucose metabolism. Direct administration or transgenic overexpression of FGF19 in mice fed either chow or high fat diet improves glucose tolerance, despite lower insulin levels (Fu et al.; Tomlinson et al., 2002), and reverses obesity,

272 hyperglycemia, and insulin resistance in mice with genetic obesity (Fu et al., 2004;
273 Morton et al., 2013). Such improvements in systemic glucose metabolism may result
274 in part from FGF-19 effects to increase insulin-independent glucose uptake (Morton et
275 al.) and reduce adiposity (Tomlinson et al., 2002). Interestingly, increased plasma
276 levels of Fgf19 have been implicated as a mediator of the potent impact of bariatric and
277 intestinal surgery to reduce blood glucose levels, with sustained remission of T2D
278 (Bozadjieva et al., 2018) and development of post-bariatric hypoglycemia in some
279 patients (Mulla et al., 2019).

280 A key mediator of systemic effects of FGF15/19 in normal physiology is altered liver
281 metabolism, with reduced gluconeogenesis and lipogenesis and increased glycogen
282 synthesis (Nies et al., 2015). Consistent with reduction in hepatic signaling by Fgf15/19
283 in JQ-1-treated mice, we observed (1) downregulation of Fgfr4 and β -Klotho (Klb)
284 pathways and reduced signaling, as indicated by reduced Frs2 phosphorylation (**Figure**
285 **2A-F**), (2) increased hepatic glucose production, as indicated by increased glucose
286 after glycerol injection (**Figure 1K**) and increased gluconeogenic gene expression
287 (**Supplemental Figure 1**), (3) reduced plasma and hepatic cholesterol, and (4) global
288 changes in the hepatic transcriptome within both glucose and lipid metabolic pathways.
289 Given that the effects of JQ-1 in primary hepatocytes were small in magnitude, cell

290 autonomous effects in the liver do not appear to be dominant. Rather, JQ-1 mediated
291 repression of intestinal FGF15 expression, secretion, and signaling drives in vivo
292 systemic and hepatic glucose metabolism, as demonstrated by reversal of
293 hyperglycemia with restoration of systemic Fgf19 levels.

294 We acknowledge that JQ-1 may have additional Fgf15/19-independent effects.
295 Neither systemic insulin tolerance nor hepatic insulin signaling were affected by
296 short-term treatment with JQ-1, indicating insulin resistance was not a major contributor
297 to hyperglycemia despite prior reports of Brd4 effects on adipogenesis and myogenesis
298 (Lee et al., 2017). While there were no differences in pancreatic islet size or insulin
299 staining, glucose-stimulated insulin levels were lower in JQ-1-treated mice. While
300 BRD4 can modulate senescence-associated genes in islets in the setting of
301 autoimmunity in mice (Thompson et al., 2019), our data indicate that effects of JQ-1 on
302 insulin secretion are not dominant in our model, as FGF19 overexpression reduced
303 insulin levels but still reversed JQ-1-mediated hyperglycemia. Additional systemic or
304 extrahepatic metabolic effects of inhibition of Brd4-mediated transcription (Lee et al.,
305 2017; Thompson et al., 2019) could also contribute to hyperglycemia induced by JQ-1.

306 In summary, inhibition of the bromodomain protein Brd4 by JQ-1 inhibits FGF15
307 and SHP expression in ileum, resulting in reduced FGFR signaling in liver, altered sterol

308 metabolism, increased gluconeogenesis, and hyperglycemia. Effects of JQ-1 on
309 glucose metabolism were FGF15/19 dependent, as overexpression of FGF19 reversed
310 hyperglycemia induced by JQ-1. Thus, our studies identify FGF15/19 as a hormonal
311 target of epigenetic regulation potentially contributing to systemic metabolic control.
312

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318

319 **METHODS**

320 ***Animal Care and Studies***

321 Male CD-1 mice obtained from Envigo (South Easton, MA, USA) were housed (3-5
322 per cage) in at 24 °C under a 12-hour light/dark cycle, with free access to food and
323 water. All animal experiments were approved by the IACUC at Joslin Diabetes Center
324 and conducted in accordance with the NIH Guide for the Care and Use of Laboratory
325 Animals.

326 ***Metabolic parameters***

327 Glucose tolerance was assessed after intraperitoneal injection of glucose (1 or 2 g/kg
328 body weight after a 16 hour fast. Blood glucose was measured via tail vein sampling at
329 the indicated time points. Insulin tolerance was assessed after intraperitoneal injection
330 of human insulin (0.5 units/kg body weight, Lilly) after a 4-hour fast. Plasma insulin and
331 glucagon were measured using an enzyme-linked immunosorbent assay (ELISA) kit
332 (Crystal Chem and R&D, respectively). Plasma triglyceride (TG) and total cholesterol
333 were measured using colorimetric assays (Cayman Chemical and Cell Biolabs).

334 ***In vivo treatment with the bromodomain inhibitor JQ-1***

335 The Brd4 inhibitor JQ1 was synthesized and purified in the laboratory of Dr. Jun Qi
336 (DFCI). For in vivo experiments, a stock solution (50 mg/mL in DMSO) was diluted to a

337 working concentration of 5 mg/mL in 10% hydroxypropyl β -cyclodextrin (Sigma). Mice
338 were injected at a dose of 25 or 50 mg/kg given intraperitoneally. Vehicle controls were
339 given an equal amount of DMSO in 10% hydroxypropyl β -cyclodextrin. For in vitro
340 experiments, JQ1 was dissolved in DMSO and added to cells at indicated
341 concentrations, with equal volume of DMSO as control.

342 ***Adenoviral overexpression of FGF19***

343 Mice received a single tail vein injection of 3×10^{11} vector genome adeno-associated
344 virus (AAV)-FGF19 or a control virus encoding green fluorescent protein (GFP;
345 AAV-GFP). After 16 days of treatment, tissues were collected.

346 ***Quantitative real-time PCR***

347 Total RNA was extracted using Trizol reagent (Thermo Fisher Scientific, Inc., Waltham,
348 MA, USA), and cDNA was synthesized using a High-Capacity cDNA Reverse
349 Transcription Kit (Thermo Fisher Scientific) according to manufacturer's instructions.
350 Quantitative real-time PCR was performed using SYBR Green (Bio-Rad, Hercules, CA,
351 USA). Expression was normalized by *Rn18s* (18S rRNA), *Rpl13a* or *36B4*. Primer
352 sequences are provided in **Table S2**.

353 ***Microarray analysis***

354 Total RNA was isolated from liver tissue from 4 representative mice per group. RNA
355 quality was assessed using Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto,
356 CA), and samples were processed for microarray analysis (Affymetrix Mouse Gene 2.0
357 ST, Molecular Phenotyping Core, Joslin).

358 ***Bioinformatic analysis***

359 For liver transcriptomics and metabolomics datasets, principal component analysis
360 (PCA) revealed that the first principal component was an extraneous source of variation,
361 so it was accounted for as a covariate in linear modeling (Leek et al., 2010).
362 Metabolomics/lipidomics sample weights were unbiasedly estimated (Ritchie et al.,
363 2006) and used in linear modelling. Linear modeling differential analysis was done with
364 the R package limma (Ritchie et al., 2015). Nominal p-values were corrected for multiple
365 testing using the false discovery rate (FDR). Transcriptomic pathway and transcription
366 factor prediction analysis was done using the Roast method (Wu et al., 2010) with
367 pathways defined by Reactome (Fabregat et al., 2018).

368 ***Immunohistochemistry (IHC)***

369 Pancreata were dissected and fixed in 4% paraformaldehyde, embedded in paraffin,
370 and sectioned. Sections were stained using H&E or immunostained for lysozyme
371 (1:1000; ab108508, Abcam) and insulin (1:100; #4590, Cell Signaling technology). Islet

372 area was calculated from analysis of more than 100 islets per mouse (Adobe
373 Photoshop).

374 ***Cell culture***

375 The human intestinal cell line (HT-29) cells (American Type Cell Collection, Manassas,
376 VA, USA) were maintained in McCoy's 5A medium (ThermoFisher Scientific)
377 supplemented with 10% FBS, 1% PenStrep (ThermoFisher Scientific) in a humidified
378 atmosphere with 5% CO₂ at 37 °C.

379 ***Primary hepatocyte isolation and measurement of glucose production***

380 Primary hepatocytes were isolated from C57BL/6 mice after liver perfusion with
381 collagenase and seeded 1 x 10⁵ cells/ml into collagen coated plates containing DMEM
382 (ThermoFisher Scientific). The media were changed after 4 hours. Primary hepatocyte
383 experiments were performed the day after isolation. Glucose production in primary
384 hepatocytes was measured as preciously described (Matsumoto and Sakai, 2012).
385 Briefly, after 6 h serum starvation with or without JQ-1 and FGF19, cells were cultured in
386 glucose and phenol red-free DMEM with or without insulin (10, 100 nM), JQ-1 (250 nM)
387 and FGF19 (100 ng/ml) for 5 h. The medium was used to determine glucose
388 concentrations with the Glucose (HK) Assay Kit (Sigma).

389 ***Chromatin immunoprecipitation (ChIP)-PCR***

390 ChIP was performed on HT-29 cells cultured in the presence or absence of
391 JQ1 (250 nM, 24 hr). Chromatin pooled from approximately 1×10^6 HT-29 cells was
392 used for each immunoprecipitation. HT-29 cells were fixed directly on the dish with 1%
393 formaldehyde for 10 minutes followed by quenching with 0.125M glycine for 5 minutes.
394 Chromatin was extracted, followed by shearing on a Tekmar Sonic Disruptor (Cincinnati,
395 OH, USA) (3 cycles, 80% Amp and 6 sec pulse, 5 min on/off). The sonicated chromatin
396 was immunoprecipitated with 5 µg of antibody (anti-BRD4, Bethyl #A301-985A). bound
397 to Dynabeads (Invitrogen) as previously described (Anand et al., 2013). Cross-linking
398 was reversed in immunoprecipitate and input chromatin samples prior to purification of
399 genomic DNA. Target and non-target regions of genomic DNA were amplified by PCR or
400 qRT-PCR in both the immunoprecipitates and input samples using SYBR Green
401 chemistry. Enrichment was calculated as a percentage of input DNA for each sample.
402 ChIP-PCR primer sequences are shown in **Table S2**. ChIP-PCR primers for MYC were
403 previously described (Rathert et al., 2015).

404 ***Metabolomics***

405 Tissues harvested after 11 days of treatment were used for analysis of plasma and liver
406 metabolites, using liquid chromatography-mass spectrometry (LC/MS) to determine
407 metabolites (Roberts et al., 2012). Missing data were imputed with half of the minimum

408 intensity of the metabolite, and the imputed data were quantile normalized and
409 $\log_2$ -transformed.

410 ***Statistical analysis***

411 Data are expressed as mean $\pm$ SEM. One-way ANOVA and repeated-measures
412 ANOVA followed by multiple comparison tests (Bonferroni/Dunn method) were used
413 where applicable. Student's *t*-test was used to analyze the differences between two
414 groups. Differences were considered significant at $P < 0.05$.

415

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540

541 **Figure Legends**

542 **Figure 1. JQ-1 induces hyperglycemia *in vivo*.**

543 (A) Experimental protocol. (B) Body weight changes during JQ-1 treatment ($n = 5-6$).
 544 (C) Fasting blood glucose levels (4-h fasting, day 10, $n = 5-6$). (D, E) Blood glucose
 545 levels and area under the curve (AUC) during ipGTT (day 6). (F, G) Plasma insulin and
 546 glucagon levels during ipGTT. (H) H&E and insulin staining of pancreatic sections. Scale
 547 bar, 200 and 100 μm . (I) Islet size ($n = 5-6$; >100 islets/mouse). (J, K) Intraperitoneal
 548 insulin tolerance test (day10) and glycerol tolerance test (day 4). $*P < 0.05$, $**P < 0.01$,
 549 vs. vehicle-treated mice (Veh). Data are expressed as means $\pm$ SEM.

550 **Figure 2. JQ-1 modulates hepatic transcription related to Fgfr signaling, reduces**
 551 **Fgfr signaling, and reduces ileal Fgf15 and Shp expression *in vivo*.**

552 (A-C) Microarray analysis in the liver was performed in JQ-1 vs. vehicle-treated mice (n
 553 $= 3$ per group). Minus $\log_{10}$ (p-value) (A) and upregulated or downregulated gene
 554 numbers (B) in pathways related to FGFR signaling. (C) Heatmap demonstrate $\log_2$ fold
 555 change (JQ-1 vs. vehicle) in gene expression in pathways related to FGFR ligand
 556 binding and activation. (D) Schematic of bile acid signaling in ileal enterocytes. (E-H)
 557 Expression levels of genes related to bile acid signaling in ileum (*Fgf15* (E), *Nr0b2*
 558 (SHP) (F), *Slc51a* (OST α) (G) and *Slc51b* (OST β) (H)) ($n = 5-6$). Expression levels were

559 normalized by those of *Rpl13*. (I) Plasma FGF15 level in JQ-1 vs. vehicle-treated mice
560 ($n = 5-6$). $*P < 0.05$, $**P < 0.01$, vs. vehicle-treated mice (Veh). Data are expressed as
561 means $\pm$ SEM. (J) H&E staining of intestinal sections. Scale bar, 100 μ m.

562 **Figure 3. BRD4 binds promoter regions of FGF19 and SHP to modulate**
563 **expression in HT-29 cells.**

564 HT-29 cells were treated with 250 or 500 nM JQ-1 or DMSO (Veh) for 12-24 h. (A-D)
565 Gene expression levels of *BRD4* (A), *MYC* (B), *FGF19* (C), and *NR0B2* (SHP) (D) ($n =$
566 4). The levels were normalized by those of *RN18S*. (E) FGF19 level in conditioned
567 media ($n = 4$). (F) Primer design for ChIP assays for *FGF19* and *NR0B2* (SHP)
568 promoter regions. (G-H) Cells were incubated with JQ-1 or DMSO (Veh) for 24 h and
569 then harvested for the ChIP assay. The precipitated DNA was analyzed by PCR (G) and
570 real-time PCR (H) ($n = 4$). $*P < 0.05$, $**P < 0.01$, vs. vehicle-treated mice (Veh). Data are
571 expressed as means $\pm$ SEM.

572 **Figure 4. Overexpression of FGF19 reverses hyperglycemia induced by JQ-1.**

573 (A) Experimental protocol. (B) Plasma FGF19 levels in AAV-GFP- and
574 AAV-FGF19-treated mice. (C) Body weight in AAV-GFP- and AAV-FGF19-treated mice
575 during JQ-1 treatment. (D-E) Blood glucose levels (D) and area under the curve (AUC)
576 (E) during ipGTT (day10). (F) Plasma insulin levels during ipGTT. $*P < 0.05$, $**P < 0.01$,

577 vs. AAV-GFP-treated mice (GFP). Data are expressed as means $\pm$ SEM.

A **B** **C** **D** **E** **F** **G** **H** **I** **J** **K** **L**

A**Pathways related to FGFR signaling** **B** **C** **D** **I** **J** **Ileum****E** **F** **G** **H**

F

G

H

A **B** **C** **D** **E** **F**