

The histone methyltransferase SUV420H2 regulates brown and beige adipocyte thermogenesis

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Activation of brown adipose tissue (BAT) thermogenesis increases energy expenditure and alleviates obesity. Here we discover that histone methyltransferase suppressor of variegation 4-20 homolog 2 (Suv420h2) expression parallels that of Ucp1 in brown and beige adipocytes and that Suv420h2 knockdown significantly reduces, whereas Suv420h2 overexpression significantly increases Ucp1 levels in brown adipocytes. Suv420h2 knockout (H2KO) mice exhibit impaired cold-induced thermogenesis and are prone to diet-induced obesity. In contrast, mice with specific overexpression of Suv420h2 in adipocytes display enhanced cold-induced thermogenesis and are resistant to diet-induced obesity. Further study shows that Suv420h2 catalyzes H4K20 trimethylation at eukaryotic translation initiation factor 4E-binding protein 1 (4e-bp1) promoter, leading to down-regulated expression of 4e-bp1, a negative regulator of the translation initiation complex. This in turn up-regulates PGC1 α protein levels, which is associated with increased expression of thermogenic program. We conclude that Suv420h2 is a key regulator of brown/beige adipocyte development and thermogenesis.

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1 The Histone Methyltransferase SUV420H2 Regulates Brown and Beige Adipocyte Thermogenesis

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34 **Abstract**

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36 Activation of brown adipose tissue (BAT) thermogenesis increases energy expenditure and alleviates obesity.
37 Here we discover that histone methyltransferase suppressor of variegation 4-20 homolog 2 (*Suv420h2*)
38 expression parallels that of *Ucp1* in brown and beige adipocytes and that *Suv420h2* knockdown significantly
39 reduces, whereas *Suv420h2* overexpression significantly increases *Ucp1* levels in brown adipocytes. *Suv420h2*
40 knockout (H2KO) mice exhibit impaired cold-induced thermogenesis and are prone to diet-induced obesity. In
41 contrast, mice with specific overexpression of *Suv420h2* in adipocytes display enhanced cold-induced
42 thermogenesis and are resistant to diet-induced obesity. Further study shows that *Suv420h2* catalyzes H4K20
43 trimethylation at eukaryotic translation initiation factor 4E-binding protein 1 (*4e-bp1*) promoter, leading to down-
44 regulated expression of *4e-bp1*, a negative regulator of the translation initiation complex. This in turn up-
45 regulates PGC1 α protein levels, which is associated with increased expression of thermogenic program. We
46 conclude that *Suv420h2* is a key regulator of brown/beige adipocyte development and thermogenesis.

47
48 Key words. SUV420H2, Histone methyltransferase, PGC1 α , mitochondria, brown adipocytes, beige adipocytes,
49 obesity.

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62 Introduction

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64 Obesity is a risk factor for a panel of metabolic disorders, including insulin resistance/type 2 diabetes,
65 hypertension, fatty liver diseases, dyslipidemia, cardiovascular diseases and certain types of cancer. Persistent
66 energy imbalance due to excess energy intake over energy expenditure results in obesity. The total energy
67 expenditure can be divided into basic metabolic rate, physical activity and adaptive thermogenesis (1). Brown
68 fat is a major player in adaptive thermogenesis (2, 3) due to the unique presence of UCP1 in mitochondrial inner
69 membrane, which uncouples oxidative phosphorylation from ATP synthesis, thereby dissipating energy as heat
70 (2, 3). Recent studies also point to several UCP1-independent mechanisms in thermogenesis (4, 5). Rodents
71 have two types of brown adipocytes: classic brown adipose tissue (BAT) is mainly confined to interscapular area
72 and newly discovered beige adipocytes or beige fat (BeAT) is sporadically dispersed in white adipose tissue
73 (WAT) and can be induced by β -adrenergic activation (6-8).

74 Activation of brown/beige adipocyte thermogenesis increases energy expenditure and ameliorates
75 obesity (9, 10). Given the recent discovery of thermogenic brown fat in humans (11-13), it is conceivable that
76 brown/beige adipocyte thermogenesis is a promising target for therapeutic treatment of obesity.

77 Epigenetic mechanisms, including histone modifications have emerged as key links between
78 environmental factors (e.g., diets) and complex diseases (e.g., obesity). However, how epigenetic mechanisms
79 regulate brown/beige adipocyte function have been less explored. To identify functional epigenetic markers that
80 regulate brown/beige adipocyte development, we surveyed the expression of most epigenetic enzymes,
81 including histone methyltransferases, demethylases, and histone deacetylases, that catalyze histone
82 methylation and acetylation during the early postnatal development of mouse beige adipocytes, and found that
83 the expression pattern of suppressor of variegation 4-20 homolog 2 (*Drosophila*) (*Suv420h2*) mirrors that of
84 *Ucp1*. Using genetic mouse models with loss- or gain- functions of *Suv420h2*, we determined the role of
85 *Suv420h2* in cold-induced thermogenesis, energy metabolism and diet-induced obesity.

87 Results

88 *Suv420h2 is important in regulating Ucp1 expression*

89 Xue et al previously reported that beige adipocytes in WAT can be transiently induced in mice during
90 early postnatal development, which peaked at 20 days of age and gradually disappeared thereafter (14).
91 Although the mechanism underlying the transient induction of the developmental beige adipocytes remains

92 unclear, the expression pattern of *Ucp1* in WAT during this period offers a unique framework for identifying
93 factors that regulate brown/beige cell development. Thus, we surveyed the expression patterns of most
94 epigenetic enzymes, including histone methyltransferases, demethylases, and deacetylases, in mouse inguinal
95 WAT (iWAT) during postnatal development from postnatal day 5 (P5) to day 120 (P120), and compared them to
96 that of *Ucp1*. For the preliminary screening, we pooled 4 RNA samples from each time point. (14) We found that
97 *Ucp1* expression in iWAT during postnatal development followed similar patterns to that observed in
98 retroperitoneal WAT (rWAT) (14), peaked at P20 and gradually disappeared thereafter (**Supplemental Figure**
99 **1A**). Among the four genes encoding histone methyltransferases (*Suv420h1*, *Suv420h2*, and SET domain
100 containing protein 8 (*Setd8*)), and demethylase PHD finger protein 8 (*Phf8*) that are responsible for histone H4
101 lysine 20 (H4K20) methylation, we discovered a unique expression pattern of *Suv420h2* (**Supplemental Figure**
102 **1B-E**), which mimicked that of *Ucp1*. We then further confirmed our results on *Ucp1* and *Suv420h2* expression
103 using four individual RNA samples (**Figure 1A-B**). In adult rodents, *Suv420h2* expression was much higher in
104 interscapular BAT (iBAT) than in other fat depots, including iWAT, epididymal WAT (eWAT) and rWAT (**Figure**
105 **1C**). We also found that *Suv420h2* expression was much higher than that of *Suv420h1* in adipose tissues
106 (**Supplemental Figure 1F**). As expected, a 7-day cold exposure at 5°C in 2-3-month-old male mice stimulated
107 *Ucp1* expression in iWAT (**Figure 1D**). Interestingly, *Suv420h2* expression parallels that of *Ucp1* in iWAT during
108 cold exposure (**Figure 1E**).

109 In addition, differentiation of mouse immortalized brown adipocyte cell BAT1 (15, 16) is characterized by
110 significant up-regulation of *Ucp1* expression (**Figure 1F**), which is paralleled by a gradual increase of *Suv420h2*
111 expression (**Figure 1G**).

112 H4K20 can be mono-, di- and trimethylated (H4K20me1, H4K20me2 and H4K20me3, respectively) (17,
113 18). SETD8 is the only known monomethyltransferase; whereas SUV420H1 and SUV420H2 are responsible for
114 di- and trimethylation of H4K20 (17, 18). We therefore further studied the roles of *Suv420h1*, *Suv420h2* and in
115 general H4K20 methylation in regulating brown/beige adipocyte function. We found that knocking down
116 *Suv420h2* with siRNA in BAT1 cells (**Supplemental Figure 2**) significantly decreased H4K20me3 levels around
117 50% without affecting H4K20me1 and H4K20me2 levels (**Figure 1H**); whereas overexpressing *Suv420h2*
118 significantly increased H4K20me3 levels in BAT1 cells without changing H4K20me1 or H4K20me2 (**Figure 1I**).

119 Interestingly, knocking down *Suv420h2* in BAT1 cells significantly suppressed, whereas overexpressing
120 *Suv420h2* significantly enhanced NE-stimulated *Ucp1* expression (**Figure 1J-K**).

121 Since knocking down *Suv420h2* resulted in around 50% reduction of H4K20me3 and both SUV420H1
122 and SUV420H2 catalyze H4K20 trimethylation, we also explored possible physiological function of *Suv420h1* in
123 regulating brown adipocyte thermogenesis. Interestingly, overexpressing *Suv420h1* (**Supplemental Figure 3**)
124 resulted in a significantly decreased NE-stimulated *Ucp1* expression (**Figure 1L**), indicating that *Suv420h1* and
125 *Suv420h2* may have opposite effects on brown adipocyte thermogenic function. To further explore this
126 possibility, we knocked down *Suv420h2* in BAT1 brown adipocytes and further treated cells with SUV420H1/H2
127 inhibitor A196 that has been shown to achieve an 80% reduction of H4K20me3 levels in treated cells (19).
128 *Suv420h2* knockdown significantly reduced *Suv420h2* expression without changing *Suv420h1* levels in BAT1
129 cells; whereas combined *Suv420h2* knockdown and A196 treatment did not change *Suv420h1* expression nor
130 did it further change *Suv420h2* expression (**Supplemental Figure 4A-B**). As expected, knocking down
131 *Suv420h2* significantly suppressed NE-stimulated expression of genes important for brown adipocyte
132 thermogenesis, including *ucp1* (**Figure 1M**), type 2 deiodinase (*Dio2*) (**Figure 1N**) and acyl-CoA thioesterase 2
133 (*Acot2*) (**Figure 1O**), a gene shown to facilitate mitochondrial fatty acid oxidation (20). Interestingly, combined
134 treatment of BAT1 cells with *Suv420h2* knockdown and A196 reversed the inhibitory effects of *Suv420h2*
135 knockdown on these gene expression levels, and restored them to that of control group (**Figure 1M-O**). These
136 data collectively demonstrate that *Suv420h1* and *Suv420h2* regulates brown adipocyte thermogenesis, with
137 *Suv420h2* serving as a potential positive regulator; whereas *suv420h1* may negatively regulate brown adipocyte
138 thermogenesis.

139

140 *Suv420h2* regulates the development of brown and beige fat

141 Recent data suggest that mice lacking both *Suv420h1* and *Suv420h2* exhibited increased mitochondria
142 respiration in brown adipocytes, improved glucose tolerance, and were resistant to diet-induced obesity (21).
143 However, since our *in vitro* data suggest that *Suv420h1* and *Suv420h2* may exert opposite effects on brown
144 adipocyte function, it is important to delineate the functions of *Suv420h1* and *Suv420h2* separately in mouse
145 models. Our gene expression data suggest that *Suv420h2* mirrors *Ucp1* expression during the postnatal
146 development of beige adipocytes, we thus interrogated the role of *Suv420h2* in the development of brown and

147 beige adipocytes *in vivo*. We first examined brown and beige adipose tissue development in mice with whole body
148 *Suv420h2* knockout (H2KO) (22) at postnatal day 20 (P20), when the developmental beige adipocytes appear at
149 peak while brown fat development also ascends to mature (14). As expected, *Suv420h2* mRNA was not detectable
150 in fat depots in H2KO mice, including iBAT, iWAT, eWAT and rWAT; in addition, there was also no difference in
151 adipose tissue *Suv420h1* expression between WT and H2KO mice (**Supplemental Figure 5A-B**). Interestingly,
152 iBAT from H2KO mice had significantly decreased UCP1 protein expression and less UCP1 staining compared to
153 that of WT controls (**Figure 2A-B, Supplemental Figure 6A**). This was associated with enlarged adipocyte size
154 (**Figure 2C**), as shown by a shift of significantly decreased smaller adipocyte and reciprocally increased larger
155 adipocyte numbers in iBAT of 20-day-old H2KO mice compared to that of WT mice (**Figure 2D**). Likewise, iWAT
156 from 20-day-old H2KO mice also had significantly lower UCP1 protein expression (**Figure 2E**) and less multilocular
157 beige adipocytes with UCP1 staining (**Figure 2F, Supplemental Figure 6B-C**), suggesting a less appearance of
158 the developmental beige adipocytes in iWAT of H2KO mice. In consistence, iWAT from 20-day-old H2KO mice
159 had enlarged adipocyte size (**Figure 2G**), with a shift of significantly decreased smaller adipocyte and a tendency
160 of reciprocally increased larger adipocyte numbers (**Figure 2H**).

161 We also generated transgenic mice (AH2Tg mice) overexpressing *Suv420h2* specifically in adipocytes
162 under the control of adiponectin promoter (**Supplemental Figure 7A**). AH2Tg mice exhibited a significant increase
163 of *Suv420h2* mRNA in all fat depots including iBAT, iWAT, eWAT and rWAT without affecting *Suv420h1* levels
164 (**Supplemental Figure 7B-C**). iBAT from 20-day-old AH2Tg mice exhibited enhanced UCP1 protein levels and
165 more UCP1 staining (**Figure 2I-J, Supplemental Figure 8A**). In addition, overexpression of *Suv420h2* in
166 adipocytes resulted in reduced adipocyte size in iBAT during postnatal development at P20 (**Figure 2K**), as shown
167 by a shift of significantly increased smaller adipocyte and a reciprocally decreased larger adipocyte number (**Figure**
168 **2L**). In consistence, AH2Tg mice exhibited higher UCP1 protein levels and more UCP1-positive multilocular beige
169 adipocytes in iWAT (**Figure 2M-N, Supplemental Figure 8B-C**). iWAT from AH2Tg mice also exhibited reduced
170 adipocyte size (**Figure 2O**), as shown by a shift of significantly increased smaller adipocyte and reciprocally
171 decreased larger adipocyte number (**Figure 2P**). In sum, these data suggest that *Suv420h2* promotes brown and
172 beige adipocytes formation during the postnatal development.

175 In adult mice, beige adipocytes can be induced by chronic cold exposure. To determine the role of
176 *Suv420h2* in cold-induced brown and beige adipocyte thermogenesis, we subjected 3-month-old male H2KO,
177 AH2Tg and their respective WT littermates to a chronic 7-day cold challenge. During the cold exposure, H2KO
178 mice displayed significantly lower body temperature compared to their littermate controls (**Figure 3A**),
179 suggesting that *Suv420h2* deficiency causes cold intolerance. Moreover, H2KO mice had higher fat mass in
180 iWAT, eWAT and rWAT after the cold challenge (**Figure 3B**), suggesting a less efficiency in utilizing stored
181 energy in fat depots. This was consistent with larger adipocytes observed in both iBAT and iWAT of H2KO mice
182 (**Figure 3C**), with a shift of reduced smaller adipocyte and a reciprocally increased larger adipocyte numbers in
183 both iBAT and iWAT of cold-challenged H2KO mice, although the increase of larger adipocyte numbers in iWAT
184 did not reach statistical significance (**Figure 3D**). In addition, cold-challenged H2KO mice exhibited decreased
185 expression of *Ucp1* in both iBAT and iWAT (**Figure 3E-F**), along with reduced expression of other cold-induced
186 thermogenic genes, including peroxisome proliferator activated receptor γ (*Ppar γ*), cell death-inducing DNA
187 fragmentation factor, alpha subunit-like effector A (*Cidea*), muscle type carnitine palmitoyltransferase 1b (*Cpt1b*),
188 epithelial V-like antigen 1 (*Eva1*), palmitoyl acyl-Coenzyme A oxidase 1 (*Acox1*) and cytochrome c oxidase subunit
189 I (*Cox1*) in iBAT, and *Ppara α* , PR domain containing 16 (*Prdm16*), *Cidea* and *Cpt1b* in iWAT (**Figure 3E-F**). As
190 expected, *Suv420h2* deficiency resulted in decreased H4K20me3 levels in both iBAT and iWAT, along with
191 decreased UCP1 protein levels (**Figure 3G-H**). Consistent with these findings, IHC analysis revealed less UCP1
192 staining in iBAT and less UCP1-positive beige adipocytes in iWAT of cold-challenged H2KO mice (**Figure 3I**,
193 **Supplemental Figure 9A-C**). Seahorse analysis of primarily isolated brown adipocytes revealed reduced basal
194 and maximal oxygen consumption rate (OCR) in H2KO mice relative to WT controls (**Figure 3J**), suggesting that
195 *Suv420h2* deletion compromised mitochondrial function via a cell-autonomous manner.

196 In contrast, AH2Tg mice with adipocyte *Suv420h2* overexpression exhibited an opposite phenotype.
197 Specifically, AH2Tg mice displayed higher body temperature compared to their littermate controls during the cold
198 challenge (**Figure 4A**), suggesting an increased cold tolerance. Cold-challenged AH2Tg mice also had
199 decreased fat mass in iWAT, eWAT and rWAT (**Figure 4B**). In consistence, iBAT and iWAT from cold-challenged
200 AH2Tg mice had smaller adipocytes (**Figure 4C**), as shown by a shift of significantly increased smaller adipocyte
201 and a tendency of reciprocally decreased larger adipocyte numbers (**Figure 4D**). In addition, iBAT and iWAT
202 from cold-challenged AH2Tg mice exhibited enhanced expression of *Ucp1* and other thermogenic genes, such

203 as *Ppara*, *Pparγ*, *Cox1*, Otopetrin 1 (*Otop1*), *Eva1* and elongation of very long chain fatty acids (FEN1/Elo2,
204 SUR4/Elo3, yeast)-like 3 (*Elovl3*) in iBAT, and *Ppara*, *Cidea*, *Cpt1b*, *Otop1* and *Elovl3* in iWAT (**Figure 4E-F**).
205 Moreover, *Suv420h2* overexpression in adipocytes led to a significant increase in H4K20me3 as well as UCP1
206 levels in both iBAT and iWAT, (**Figure 4G-H**). In consistent with these findings, IHC analysis revealed a stronger
207 UCP1 staining in iBAT and higher UCP1-positive beige adipocyte induction in iWAT (**Figure 4I, Supplemental**
208 **Figure 10A-C**). In addition, Seahorse analysis revealed enhanced maximal oxygen consumption rate (OCR) in
209 primary adipocytes isolated from AH2Tg mice (**Figure 4J**), suggesting that *Suv420h2* overexpression increases
210 mitochondrial function in a cell-autonomous manner.

211 *Suv420h2* regulates mitochondrial bioenergetic program

212 To gain further insight into how *Suv420h2* regulates brown/beige fat thermogenesis, we performed RNAseq
213 analysis in iWAT of 7-day-cold-challenged H2KO and AH2Tg mice. Analysis of differentially expressed genes with
214 online software (<https://github.com/PerocchiLab/ProFAT>) (23) predicted an overall reduced browning probability
215 in *Suv420h2*-deficient iWAT, with a reciprocal increase in gene expression profile resembling that of WAT (**Figure**
216 **5A**). This was consistent with a down-regulation of BAT-specific, and an up-regulation of WAT-specific gene
217 expression in *Suv420h2*-deficient iWAT (**Figure 5A**). In contrast, analysis of differentially expressed genes in iWAT
218 between WT and AH2Tg mice revealed an overall enhanced browning probability, evidenced by enhanced BAT-
219 specific, and reduced WAT-specific gene expression (**Figure 5B**). Interestingly, we found that groups of BAT-
220 specific genes were reciprocally regulated in iWAT between H2KO and AH2Tg mice, including *Ucp1*, *Ucp3*, *Cpt1b*,
221 *Otop1*, *Kcnk3*, *S100b* (**Figure 5A-C**), highlighting the importance of *Suv420h2* in beige fat thermogenesis. More
222 strikingly, genes involved in mitochondrial bioenergetic pathways, including electron transport chain, fatty acid β -
223 oxidation and TCA cycle stood out as converged pathways that were down- or up-regulated in H2KO and AH2Tg
224 mice, respectively (**Figure 5D** and **Supplemental Figure 11A-B**).

225 To further investigate how SUV420H2 regulates pathways in mitochondria function and thermogenesis, we
226 performed assay for transposase-accessible chromatin sequencing (ATAC-seq) analysis in iBAT of 7-day-cold-
227 challenged WT and AH2Tg mice. We compared genome-wide alterations in chromatin accessibility landscape
228 assessed by ATAC-seq with that of gene expression patterns assessed by RNA-seq and discovered a strong
229 correlation between chromatin accessibility status and gene expression changes. As illustrated in **Figure 5E**,
230

231 the decreases in read densities of genes of two selective clusters (Clusters 1 and 2, **Figure 5E**) based on variable
232 degree of peaks in AH2Tg iBAT, which indicates less chromatin accessibility, were highly associated with the
233 down-regulations of the corresponding gene expression, including several genes known to negatively regulate
234 brown/beige adipocyte thermogenesis and energy metabolism, such as nicotinamide N-methyltransferase
235 (*Nnmt*)(24), natriuretic peptide receptor 3 (*Npr3*)(25), twist basic helix-loop-helix transcription factor 1
236 (*Twist1*)(26) and zinc finger protein 423 (*Zfp423*)(27). In addition, we also identified two clusters of genes that
237 showed more chromatin accessibility and were associated with increased gene expression (Clusters 3 and 4,
238 **Figure 5E**), including several genes encoding mitochondrial electron transporting chain proteins, such as
239 complex I component *Ndufa10*, complex III component ubiquinol-cytochrome c reductase, Rieske iron-sulfur
240 polypeptide 1 (*Uqcrcfs1*) and complex IV component heme A:farnesyltransferase cytochrome c oxidase assembly
241 factor 10 (*Cox10*).

242 Our data suggest that *Suv420h2* regulates pathways involved in mitochondrial bioenergetics. Indeed,
243 immunoblotting analysis of mitochondrial respiratory chain proteins revealed down regulation of complex I NADH
244 dehydrogenase 1 β subcomplex 8 (CI-NDUFB8), complex II succinate dehydrogenase complex, subunit B (CII-
245 SDHB) and complex III cytochrome b-c1 complex subunit 2 (CIII-UQCRC2) in both iBAT and iWAT of H2KO
246 mice (**Figure 6A-B**), while up-regulation of CI-NDUFB8, CII-SDHB and complex IV mitochondrially encoded
247 cytochrome c oxidase I (CIV-MTCO1) in iBAT and iWAT of AH2Tg mice cold exposure (**Figure 6C-D**).

248 *H4K20me3 is elevated at the promoter of 4E-BP1*

249 Since genes responsible for mitochondrial function appears to be the most significant feature of
250 *Suv420h2* regulated pathways, we next explored whether *Pgc1 α* , the master regulator of mitochondrial
251 biogenesis (28), is involved in this process. We first studied whether *Pgc1 α* mRNA and protein levels were
252 regulated during postnatal development and cold exposure. While both *Pgc1 α* mRNA and protein levels were
253 significantly higher in iWAT of 20-day-old mice compared to that of 3-month-old mice (**Figure 7A-B**), H4K20me3
254 level at *Pgc1 α* promoter was not significantly different in iWAT across the developmental course (**Supplemental**
255 **Figure 12**). Further, while *Pgc1 α* mRNA expression was only transiently up-regulated in iWAT one day after cold
256 exposure, cold-induced increase in PGC1 α protein levels was observed at seven days after cold exposure
257 (**Figure 7C-D**). These data suggest that *Pgc1 α* expression may not depend on promoter H4K20 trimethylation,
258

259 and PGC1 α protein level may be regulated independent of mRNA expression, at least during chronic cold
260 exposure.

261 Similarly, although our ATACseq and RNAseq data suggested that overexpressing *Suv420h2* in
262 adipocytes resulted in a more open chromatin structure at *Pgc1 α* locus, along with increased *Pgc1 α* expression
263 peaks (**Supplemental Figure 13A**), quantitative RT-PCR analysis showed that *Pgc1 α* expression was not
264 significantly changed in iBAT and iWAT of H2KO (**Figure 3E-F**) or AH2Tg mice (**Figure 4E-F**) after cold
265 exposure. We also did not observe any changes in *Pgc1 α* expression in BAT1 brown adipocytes with *Suv420h2*
266 knockdown and with combined *Suv420h2* knockdown and A196 treatment (**Supplemental Figure 13B**).
267 Interestingly, *Suv420h2* deletion in H2KO mice decreased, while *Suv420h2* overexpressing in AH2Tg mice
268 increased PGC1 α protein content in both iBAT and iWAT (**Figure 7E-F, 7G-H**). Thus, our data suggest that
269 PGC1 α protein level may be regulated independently of its mRNA expression, and *Suv420h2* may be involved
270 in the regulation of PGC1 α protein levels.

271 PGC1 α is a short-lived protein and therefore its protein level is tightly regulated by either protein synthesis
272 or degradation. PGC1 α protein levels can be regulated by protein degradation (29, 30) or synthesis (31). The E3
273 ligases F-box and WD-40 domain protein 7 (FBXW7) and ring finger protein 34 (RNF34) have been previously
274 shown to promote PGC1 α protein ubiquitination and degradation (29, 30), whereas PGC1 α protein translation
275 can be regulated by the eukaryotic translation initiation eIF4F complex, as the negative regulator of the eIF4F
276 complex, the eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1), has been shown to negatively
277 regulate PGC1 α protein synthesis (31). There was no change in the expression of *Fbxw7* and *Rnf34* between
278 WT and H2KO, and between WT and H2Tg mice (**Supplemental Figure 14**). (31) Interestingly, our ATACseq
279 and RNAseq data suggest that overexpressing *Suv420h2* in adipocytes resulted in a more closed chromatin
280 structure at *4e-bp1* locus, which was associated with reduced *4e-bp1* expression (**Figure 8A**). Indeed, *4e-bp1*
281 expression was significantly up-regulated in iBAT and iWAT of cold-challenged H2KO mice, but tended to
282 decrease in iBAT and was significantly decreased in iWAT and eWAT of cold-challenged AH2Tg mice (**Figure**
283 **8B-C**). In consistence, 4E-BP1 protein levels in iBAT and iWAT were increased in cold-challenged H2KO mice,
284 but decreased in cold-challenged AH2Tg mice (**Figure 8D-E, 8F-G**).

285 We also measured 4E-BP1 protein levels in iWAT of C57BL/6J mice during postnatal development and
286 cold challenge. Interestingly, 4E-BP1 protein levels were significantly increased in iWAT of 3-month-old mice as

287 compared to that of 20-day-old mice (**Figure 8H**). Since 4E-BP1 negatively regulates PGC1 α protein levels (31),
288 this may explain the decreased PGC1 α protein levels in iWAT of 3-month-old mice (**Figure 7-B**). On the other
289 hand, cold exposure significantly reduced 4E-BP1 protein levels (**Figure 8H**), which may contribute to the
290 increased PGC1 α protein levels in iWAT of cold-challenged mice (**Figure 7-D**).

291 Mechanistically, ChIP assay revealed that H4K20me3 levels at *4e-bp1* promoter (**Supplemental Figure**
292 **15**) (32-34) was significantly decreased in both iBAT and iWAT of H2KO mice (**Figure 9A-B**). Thus, *Suv420h2*
293 deletion may decrease histone repressive mark H4K20me3 at *4e-bp1* locus, resulting in increased *4e-bp1*
294 expression, which could lead to decreased PGC1 α protein levels seen in H2KO mice. In contrast, *Suv420h2*
295 overexpression in AH2Tg mice increased *4e-bp1* promoter H4K20me3 levels in iBAT and iWAT (**Figure 9C-D**),
296 which may lead to decreased expression of *4e-bp1*, potentially contributing to increased PGC1 α protein levels
297 observed in AH2Tg mice.

298 To further confirm that SUV420H2 regulates PGC1 α protein levels via regulation of *4e-bp1* expression,
299 we knocked down both *Suv420h2* and *4e-bp1* in BAT1 brown adipocytes. As shown in **Figure 9E**, knocking
300 down *Suv420h2* significantly increased 4E-BP1 levels in BAT1 brown adipocytes, similarly to those observed in
301 H2KO mice (**Figure 8D-E**); whereas combined knockdown of both *Suv420h2* and *4e-bp1* significantly reduced
302 4E-BP1 levels (**Figure 9E**). Interestingly, knocking down of *Suv420h2* tended to reduce basal PGC1 α protein
303 levels, and significantly reduced NE-stimulated PGC1 α protein levels in BAT1 brown adipocytes. Further
304 knocking down of *4e-bp1* blocked this effect, and restored PGC1 α protein level to that of control group (**Figure**
305 **9F**). These data suggest that 4E-BP1 mediates SUV420H2's effect on regulating PGC1 α protein levels.

306 We also explored other possible SUV420H2 downstream targets that could potentially mediate
307 SUV420H2's function in regulating brown/beige adipocyte function. Pedrotti et al (21) reported that deletion of
308 both *Suv420h1* and *Suv420h2* resulted in enhanced mitochondria respiration in brown adipocytes, possibly via up-
309 regulation of the expression of *Ppar γ* , a master regulator of brown and white adipocyte lipid and glucose
310 metabolism, and thermogenic function (35, 36). However, we observed no difference in chromatin accessibility and
311 RNA expression peaks at *Ppar γ* locus in our ATACseq and RNAseq data from cold-challenged WT and AH2Tg
312 mice (**Supplemental Figure 16A**). In addition, there were no consistent changes in cold-induced *Ppar γ* mRNA
313 (**Figures 3E-F and 4E-F**) and protein (**Supplemental Figure 16B-C**) levels in iBAT and iWAT between WT and
314 H2KO mice and between WT and AH2Tg mice.

315 *Prdm16* is emerged as an important regulator of brown adipocyte development (16, 37). However, we did
316 not observe any differences in chromatin accessibility and RNA expression at *Prdm16* locus in our ATACseq and
317 RNAseq data (**Supplemental Figure 17A**). In addition, there were no consistent changes in cold-induced *Prdm16*
318 mRNA (**Figure 3E-F, Figure 4E-F**) and protein (**Supplemental Figure 17B-C**) levels in iBAT and iWAT between
319 WT and H2KO mice and between WT and AH2Tg mice.

320 *Twist1* and *Zfp423* negatively regulate brown/beige adipocyte thermogenesis and energy homeostasis (26,
321 27). *Twist1* interacts with PGC1 α on PGC1 α -target genes to suppress mitochondrial metabolism and uncoupling
322 (26); whereas *Zfp423* suppresses adipocyte thermogenic capacity by interfering with several important factors for
323 brown adipocyte function, such as early B cell factor 2 (*Ebf2*) and *Prdm16* (27, 38). Our ATACseq and RNAseq
324 data indicated that chromatin accessibility and RNA expression peaks at *Twist1* and *Zfp423* loci (**Supplemental**
325 **Figure 18A and 19A**) were decreased in WT and AH2Tg mice after cold exposure. In addition, the expression of
326 *Twist 1* (**Supplemental Figure 18B-C**) and *Zfp423* (**Supplemental Figure 19B-C**) was increased in iWAT of
327 H2KO mice but reciprocally decreased in iWAT of AH2Tg mice after cold exposure. However, ChIP assay
328 demonstrated that H4K20me3 levels at *Twist1* (**Supplemental Figure 18D-E**) or *Zfp423* (**Supplemental Figure**
329 **19D-E**) promoter was not different in iBAT and iWAT between cold-challenged WT and H2KO mice and between
330 cold-challenged WT and AH2Tg mice. (Thus, while changes in *Twist1* and *Zfp423* expression might potentially
331 contribute to altered brown/beige adipocyte function observed in our H2KO and AH2Tg mice, they are not likely
332 mediated via *Suv420h2*-regulated H4K20 methylation.

333 Estrogen-related receptor gamma (*Esrrg*) is emerged as a positive regulator of mitochondrial oxidative
334 metabolism and thermogenesis via both *Pgc1 α* -dependent and independent mechanisms (39, 40). Our ATACseq
335 and RNAseq data indicated that chromatin accessibility and RNA expression peaks at *Esrrg* locus were increased
336 in cold-challenged WT and AH2Tg mice (**Supplemental Figure 20A**). In addition, *Esrrg* mRNA and protein levels
337 were decreased in iWAT of H2KO mice (**Supplemental Figure 20B-C**) but reciprocally increased in iWAT of
338 AH2Tg mice (**Supplemental Figure 20D-E**) after cold exposure. However, H4K20me3 level at *Esrrg* promoter was
339 not different in iBAT and iWAT between H2KO and WT (**Supplemental Figure 20F-G**) and between AH2Tg and
340 WT mice (**Supplemental Figure 20H-I**), suggesting the altered *Esrrg* expression in H2KO and AH2Tg mice was
341 not dependent on *Suv420h2*.

342 We further investigated whether ESRRG protein level could be regulated by 4E-BP1. As shown in
343 **Supplemental Figure 20J**, *Suv420h2* knockdown significantly reduced NE-stimulated ESRRG protein levels in
344 BAT1 cells; however, further knockdown of *4e-bp1* blocked this effect and restored ESRRG protein levels to that
345 of control group. Thus, *Esrrg* may be another potential target besides *Pgc1α* mediating *Suv420h2*'s effect on
346 brown/beige adipocyte thermogenesis. However, similarly to that of *Pgc1α*, *Esrrg* may not be a direct target of
347 *Suv420h2*, as *Esrrg* promoter H4K20me3 levels in iBAT and iWAT were not different in cold-challenged H2KO and
348 AH2Tg mice compared to their respective WT controls. Instead, ESRRG protein levels may be regulated by 4E-
349 BP1-mediated translational regulation, similarly to that of PGC1α.

350 We further explored whether other brown/beige adipocyte-related genes could be potential direct targets
351 for *Suv420h2* by comparing H4K20me3 level at the promoters of several genes in iWAT during postnatal
352 development. However, we did not observe differences in H4K20me3 levels at the promoters of *Ucp1*
353 (**Supplemental Figure 21A**), RB transcriptional corepressor 1 (*Rb1*) (**Supplemental Figure 21B**), a negative
354 regulator of brown adipocyte thermogenesis (41), and Kruppel-like transcription factor 2 (*Klf2*) (**Supplemental**
355 **Figure 21C**), a negative regulator of adipogenesis (42), in iWAT along the postnatal developmental course..

356 *Suv420h2 is important in regulation of diet-induced obesity*

357 To determine the role of *Suv420h2* in diet-induced obesity, we challenged H2KO, AH2Tg, and their
358 respective WT littermates with a high fat diet (HFD). When housed at ambient room temperature (20-22°C), H2KO
359 mice had increased fat mass in iWAT and eWAT despite no change in body weight (**Supplemental Figure 22A-**
360 **B**). This was associated with decreased energy expenditure in H2KO mice evident by reduced oxygen
361 consumption and heat production (**Supplemental Figure 22C-D**) without changes in locomotor activity
362 (**Supplemental Figure 22E**) and food intake (**Supplemental Figure 22F**).

363 Similarly, while there was no change in body weight (**Supplemental Figure 23A**), HFD-challenged AH2Tg
364 mice housed at ambient room temperature had decreased fat mass in eWAT without changes in other fat pads
365 (**Supplemental Figure 23B**). Ah2Tg mice also exhibited increased energy expenditure as shown by increased
366 oxygen consumption and heat production (**Supplemental Figure 23C-D**) without changes locomotor activity
367 (**Supplemental Figure 23E**) and food intake (**Supplemental Figure 23F**).

368 We previously reported that mild cold stress under ambient room temperature (20-22°C) may trigger non-
369 shivering thermogenesis (43). Thus, we also conducted HFD feeding experiments under thermoneutrality (30°C).

370 When housed under thermoneutrality, H2KO mice gained more weight starting after 4 weeks of HFD feeding
371 (**Figure 10A**) with increased fat mass in iBAT, iWAT and rWAT depots (**Figure 10B**), . and exhibited glucose
372 intolerance and insulin resistance assessed by GTT and ITT, respectively (**Figure 10C-D**). In contrast, HFD-
373 challenged AH2Tg mice gained less weight under thermoneutrality with lower fat mass in iBAT, iWAT and rWAT
374 (**Figure 10E-F**), and exhibited improved glucose tolerance and insulin sensitivity as shown by GTT and ITT (**Figure**
375 **10G-H**). Thus, our data indicate that *Suv420h2* is important in regulating diet-induced obesity.

377 Discussion

378 Xue et al previously discovered developmentally induced beige adipocytes (14). To identify functional
379 epigenetic marks that regulate brown/beige adipocyte development, we surveyed the expression of epigenetic
380 enzymes responsible for histone modifications during the postnatal development of beige adipocytes and
381 discovered a unique expression pattern of the histone methyltransferase *Suv420h2*, which mirrors that of *Ucp1*.
382 Using genetic models with gain or loss of functions of *Suv420h2*, we demonstrate that *Suv420h2* promotes the
383 development of brown and beige adipocytes postnatally, enhances cold-induced thermogenesis and prevents
384 diet-induced obesity.

385 Methylation of H4K20 was one of the first histone modifications to be discovered, and is evolutionarily
386 conserved from yeast to humans (17, 18). H4K20 can be mono-, di- and trimethylated (17, 18). SET8/PR-SET7
387 is the only known monomethyltransferase; whereas SUV420H1 and SUV420H2 are responsible for the di- and
388 trimethylation of H4K20 (17, 18). The methylation states of H4K20 exert different biological function. Whereas
389 H4K20me1 and H4K20me2 are involved in DNA replication and DNA damage repair, respectively, H4K20me3
390 is a hallmark of silenced heterochromatic regions and is also enriched in chromatin regions that contain silenced
391 genes (17, 18, 44). H4K20me3 plays an important role in dynamic biological functions, including development,
392 cellular differentiation, aging and cancer development (45-49). Here we demonstrate that H4K20me3, catalyzed
393 by SUV420H2, may also be involved in the regulation of brown/beige fat thermogenesis and energy metabolism
394 through 4E-BP1-PGC1 α axis.

395 The enrichment of genes involved in mitochondrial functions revealed by our RNA-seq analysis drew our
396 attention to *Pgc1 α* , a master regulator of mitochondrial biogenesis and thermogenesis (28). (29, 30, 50)It has
397 been demonstrated that PGC1 α protein translation can be regulated by the eukaryotic translation initiation

398 complex (31). The eukaryotic translation initiation factor 4F (eIF4F) complex is composed of eIF4E (mRNA
399 m7GTP 5' cap-binding protein), eIF4G (a scaffolding protein), and eIF4A (an ATP-dependent RNA helicase)
400 (51). Recognition of the mRNA 5' cap structure by eIF4E is a rate-limiting step in translational initiation, and is
401 hence tightly regulated (52). The activity of eIF4E is regulated through interaction with the three inhibitory 4E-
402 BPs, 4E-BP1, 2 and 3. The 4E-BPs compete with eIF4G for a shared binding site on eIF4E (53), thereby
403 negatively regulating eIF4F complex formation and translation initiation. Cold exposure down-regulates 4E-BP1
404 expression in BAT, which is mediated through β 3-adrenergic agonist stimulated signaling pathways (54).
405 Importantly, deletion of 4E-BP1 in mice results in greater reduction of adiposity, increased energy expenditure,
406 up-regulated *Ucp1* expression and beige adipocyte induction in WAT; this is primarily due to increased eIF4F
407 complex formation, leading to increased PGC1 α protein translation (31). Indeed, we discovered that *4e-bp1*
408 promoter H4K20me3 level is increased in *Suv420h2* overexpressing adipocytes, leading to down-regulated *4e-*
409 *bp1* expression and corresponding up-regulated PGC1 α protein levels. The enhanced PGC1 α protein levels may
410 drive the mitochondrial biogenesis in *Suv420h2* overexpressing adipocytes, resulting in increased brown fat
411 thermogenesis.

412 SUV420H2 catalyzes the deposition of trimethylation to histone H4k20, which in turn represses gene
413 transcription (17, 18). In the current study, we observed that overexpressing *Suv420h2* increased, whereas
414 *Suv420h2* knockout decreased thermogenic gene expression in brown adipocytes. Thus, we could reasonably
415 predict that SUV420h2 may repress a putative negative regulator of thermogenesis, which in turn promotes
416 thermogenesis. Indeed, we have measured H4K20me3 levels at the promoters of several positive regulators of
417 thermogenesis, including *Pgc1 α* , *Ppar γ* , *Prdm16* and *Esrrg*, none of them showed any differences in promoter
418 H4K20me3 level between H2KO and AH2Tg mice, suggesting they are not direct targets for *Suv420h2*. We
419 have also measured H4K20me3 levels at the promoters of several negative regulators of thermogenesis in
420 adipose tissues, including *4e-bp1*, *Twist1*, *Zfp423* and *Rb1*. Only *4e-bp1* fit our criteria with decreased promoter
421 H4K20me3 mark in H2KO mice and reciprocally increased promoter H4K20me3 levels in AH2Tg mice. Future
422 studies with ChIP-seq using *Suv420h2* or H4K20me3 antibodies is warranted to identify *Suv420h2*- or
423 H4K20me3- target genes.

424 In the current study, we also identified PGC1 α as one of the targets whose protein synthesis could be
425 regulated by 4E-BP1-dependent regulation of the eukaryotic translation initiation eIF4F complex activity. In

426 addition, whereas *Esrrg* mRNA transcription may not be directly regulated by SUV420H2, our data suggest that
427 ESRRG protein levels may be regulated by 4E-BP1-mediated regulation of the eukaryotic translation initiation
428 eIF4F complex activity, similarly to that of PGC1 α . Although 4E-BP1 may regulate the whole translational
429 machinery, the specificity may be regulated in part by specific transcriptional factor complexes on each target
430 genes. Thus, future experiments with ribosome profiling or Ribo-seq technologies (55, 56) could be performed
431 to identify potential protein candidates that are dependent on SUV420H2/H4K20me3/4E-BP1-regulated cap-
432 dependent protein translation.

433 Along the course of our study, there were two papers published studying the roles of SUV420H1/H2
434 proteins in brown/beige adipocyte thermogenesis. Pedrotti et al reported that deletion of both *Suv420h1* and
435 *Suv420h2* in brown adipocytes increased brown fat thermogenesis and ameliorated obesity via activating *Ppar γ*
436 regulated gene networks (21). The results were opposite to what we observed in our genetic models. The exact
437 reason for this discrepancy is not clear. However, different genetic models were used in these two studies. For
438 our purpose to distinguish the functions of *Suv420h2* from that of *Suv420h1*, we used animal models with
439 *Suv420h2* deletion without affecting the expression of *Suv420h1*, whereas Pedrotti et al used animal models
440 with *Suv420h1/Suv420h2* double deletion. Interestingly, we observed that either *Suv420h2* deletion or *Suv420h1*
441 overexpression suppressed brown adipocyte thermogenic gene expression, suggesting that whereas *Suv420h2*
442 may positively regulate brown adipocyte thermogenesis, *Suv420h1* may serve as a potential negative regulator.
443 Thus, one possible reason accounting for the differences between our mouse models and those published by
444 Pedrotti et al (21) is that deletion of *Suv420h1* in the *Suv420h1* and *Suv420h2* double knockout mouse model
445 may dominate the phenotypes, thus resulting in increased mitochondrial function and thermogenesis in brown
446 adipocytes; whereas in our animal model of *Suv420h2* deletion, reduced *Suv420h2* function along with normal
447 or possibly enhanced *Suv420h1* function could collectively lead to impaired brown/beige adipocyte
448 thermogenesis. Future studies using genetic models with gain- and loss-functions of individual *Suv420h* proteins
449 are warranted to carefully dissect the effect of *Suv420hs* on adaptive thermogenesis.

450 Zhao et al (57) reported that mice with adipocyte-specific *Suv420h2*/lysine methyltransferase 5C (*Kmt5c*)
451 deletion exerted decreased thermogenic gene expression in WAT and BAT, and were prone to diet-induced
452 obesity and associated metabolic disorders, which was similar to the phenotypes observed in our H2KO models.
453 Mechanistically, the authors showed that enhanced expression of a negative regulator of brown fat

454 thermogenesis, transformation related protein 53 (*Trp53*) in *Suv420h2/Kmt5c* knockout (KO) mice, due to
455 decreased H4K20me3 on its proximal promoter, was responsible for the metabolic phenotypes (57). In our
456 current study, we have identified a new mechanism, in which *Suv420h2* suppresses the expression of a negative
457 regulator of PGC1 α protein translation, *4e-bp1*, by increasing repressive mark H4K20me3 on its promoter, thus
458 promoting brown/beige adipocyte mitochondrial oxidative metabolism and thermogenesis. These
459 complementary studies could significantly enhance our understandings of how *Suv420h1/h2* regulates
460 brown/beige adipocyte thermogenesis and whole body metabolic homeostasis.

461 In our current study, we observed significant differences in the metabolic phenotypes in our animal
462 models during a cold challenge, whereas the differences diminished in animals challenged with an obesogenic
463 HFD at ambient temperature. It is possible that diet-induced thermogenesis and cold-induced thermogenesis
464 may be triggered by different stimulations. In the context of increased energy needs (cold environment), the
465 purpose of BAT activation is to increase heat production and maintain temperature stability. This is in contrast
466 to a positive energy balance in diet-induced obesity, in which increased heat is not necessary, but energy
467 expenditure increases owing to diet-induced thermogenesis, a phenomenon in which excess caloric
468 consumption increases metabolic rate and stimulates BAT thermogenesis (2). Thermogenesis might be
469 stimulated via different mechanisms, depending on whether it is triggered through cold or other factors (58).
470 Additionally, cold and diet can lead to differential gene expression patterns in brown and white adipose tissue
471 (59). Our previous data also showed that BAT responded differently in response to a HFD or a cold challenge
472 (60). Thus, it is possible that there are differences in metabolic phenotypes in our animal model during a cold
473 challenge vs a HFD challenge.

474 We also observed that metabolic differences during a HFD challenge was more evident in animals
475 housed under thermoneutrality compared to the ambient temperature. Mice housed at ambient room temperature
476 have a metabolic rate and food intake around 1.5 times higher than mice housed at thermoneutrality (3). While
477 diet-induced thermogenesis might be primarily dependent on UCP1-dependent brown fat thermogenesis,
478 metabolic rate in response to a cold environment could be influenced by factors other than brown fat adaptive
479 thermogenesis, for example, shivering, skin/fur insulation, and most importantly, adipose tissue response to
480 sympathetic activation (3, 61). These factors could mask the true intrinsic energetic demands in response to a
481 high fat diet if mice are housed at ambient temperature that presents mild cold stress condition, which may be

partly responsible for the differences in metabolic phenotypes observed in our animal models housed at different environmental temperatures. The thermogenic adaptation to diet-induced obesity in an animal model may be partially dependent on the difference in environment temperature.

In summary, we discovered a unique expression pattern of the histone methyltransferase *Suv420h2*, which mirrors the appearance of developmental beige adipocytes. Using genetic models with loss- or gain-of-functions of *Suv420h2*, we demonstrate that *Suv420h2* promotes the development of brown and beige adipocytes postnatally, enhances cold-induced thermogenesis and prevents diet-induced obesity, possibly through 4E-BP1-PGC1 α axis. We conclude that *Suv420h2* is a key regulator of brown/beige fat thermogenesis, energy metabolism and diet-induced obesity.

Materials and Methods

Sex as a biological variant. Our study examined both male and female mice. However, we found there was a sex-dimorphic effects and the phenotypes were more profound in males. Thus, results from male mice were reported.

Mice. Mice with whole body *Suv420h2* knockout (H2KO) were kindly provided by Dr. Gunnar Schotta (Ludwig Maximilian University, Munich, Germany)(22). To generate transgenic mice with adipocyte-specific *Suv420h2* overexpression (AH2Tg), a bacterial artificial chromosome (BAC) containing the mouse adiponectin gene was used, and full-length coding sequence of the mouse *Suv420h2* gene was PCR-amplified and inserted into the ATG position at exon 2 of the adiponectin gene in the BAC using homologous recombination. The adiponectin BAC carrying *Suv420h2* was linearized and microinjected into pronuclei of fertilized embryos of C57BL/6J mice at Georgia State University transgenic core facility.

Metabolic analysis. Mice were housed in a temperature- and humidity-controlled environment with a 12/12-hour light–dark cycle and had *ad libitum* access to water and food. H2KO, AH2Tg mice and their respective littermate controls were fed a regular chow diet (LabDiet 5001, LabDiet, St. Louis, MO, 13.5% calories from fat) or a HFD (Research Diets D12492, 60% calorie from fat) diet for up to 24 weeks. Various metabolic measurements were characterized. Body weight was monitored weekly. Body composition including fat and lean mass was analyzed using a Minispec NMR body composition analyzer (Bruker BioSpin Corporation; Billerica, MA). Food intake was measured in single-housed animals over seven consecutive days. Energy expenditure

510 and locomotor activity were assessed using PhenoMaster metabolic cage systems (TSE Systems, Chesterfield,
511 MO). Insulin sensitivity was assessed by GTT and ITT, respectively(62, 63). Blood glucose was measured by
512 OneTouch Ultra Glucose meter (LifeScan, Milpitas, CA). At the end of experiments, tissues including BAT and
513 WAT were dissected, weighed, and frozen in liquid nitrogen for further analysis.

514 Cold exposure. H2KO, AH2Tg mice and their respective littermate controls were subjected to a cold
515 challenge (5-6°C) for 7 days. To measure body temperature, some animals were surgically implanted with a
516 temperature transponder (BioMedic Data Systems, Seaford, DE) in the peritoneal cavity (62, 63). At the end of
517 experiment, WAT and BAT were dissected, weighed and frozen for further analysis.

518 Cell culture and SiRNA knockdown. Immortalized BAT1 brown adipocyte cells (15, 16) were kindly
519 provided by Dr. Patrick Seale (University of Pennsylvania, Philadelphia, PA). BAT1 brown adipocytes were
520 grown and differentiated as we described (63). *Suv420h2*, *4e-bp1* targeting siRNA, and non-targeting scramble
521 siRNA were purchased from Dharmacon (Lafayette, CO) (**Supplemental Table 1**). Plasmids containing
522 *Suv420h1* and *Suv420h2* cDNAs were purchased from Open Biosystems (Huntsville, AL) (**Supplemental Table**
523 **1**). *Suv420h2*, *4e-bp1* siRNAs, or *Suv420h1* or *Suv420h2* overexpression plasmids were electroporated into
524 BAT1 brown adipocytes using Amaxa Nucleofector II Electroporator (Lonza) with an Amaxa cell line nucleofector
525 kit L (Lonza) (63). In some experiments, after *Suv420h2* siRNAs knockdown, BAT1 cells were further treated
526 with the SUV420H1/H2 inhibitor A196 (5µM) (Sigma-Aldrich, St. Louis, MO, catalog #SML1565) (19) for an
527 additional 24 hours. In other experiments, BAT1 brown adipocytes were treated with both *Suv420h2* and *4e-bp1*
528 siRNAs to knockdown both *Suv420h2* and *4e-bp1*. Some cells were also treated with either vehicle (PBS) or the
529 adrenergic agonist norepinephrine (1µM) for 4 hours.

530 Quantitative RT-PCR analysis of gene expression. Total RNA was extracted from fat tissues using Tri
531 Reagent kit (Molecular Research Center, Cincinnati, OH) (62, 63). The expression of target genes was measured
532 by quantitative RT-PCR analysis with a TaqMan Universal PCR Master Mix kit (ThermoFisher Scientific,
533 Waltham, MA) using an Applied Biosystems QuantStudio 3 real-time PCR system (ThermoFisher Scientific) (62,
534 63). The TaqMan primers/probe pairs for the gene expression measurements were either purchased from
535 Applied Biosystems (ThermoFisher Scientific) (**Supplemental Table 2**) or commercially synthesized (Applied
536 Biosystems, the sequences were listed in **Supplemental Table 3**).

537 Immunoblotting. Protein levels of target genes were measured by immunoblotting as we described (62,
538 63). Briefly, fat tissues were disrupted with a homogenizer in a modified radioimmunoprecipitation assay (RIPA)
539 lysis buffer supplemented with protease and phosphatase inhibitor mixtures (Sigma-Aldrich, St. Louis, MO). After
540 centrifugation, supernatants were resolved by SDS-PAGE, and transferred to nitrocellulose membranes (Bio-
541 Rad, Hercules, CA), followed by incubating with various primary antibodies and Alexa Fluor 680-conjugated
542 secondary antibodies (Life Science Technologies). The blots were developed with a Li-COR Imager System
543 (Li-COR Biosciences, Lincoln, NE). The antibody information was listed in **Supplemental Table 4**.

544 Immunohistochemistry (IHC). IHC was conducted as we described(62, 63). Briefly, fat tissues were fixed
545 in neutral formalin, embedded in paraffin and sliced into 5 μ m sections. The tissue slides were used for
546 hematoxylin and eosin (H&E) staining, or immunochemical-staining with primary and secondary antibodies,
547 which were further developed with an alkaline phosphatase substrate using Vector Red Substrate kit (Vector
548 Laboratories, SK-5100). Histological images were captured using an Olympus DP73 photomicroscope and
549 CellSens software (Olympus, Waltham, MA). The adipocyte size was measured using ImageJ software with
550 Adiposoft plug-in (64). The primary and secondary antibodies are listed in **Supplemental Table 4**.

551 Chromatin immunoprecipitation (ChIP) assays. ChIP assays were performed with a ChIP assay kit
552 (Upstate BioTechnology, Lake Placid, NY) as we described (63). Briefly, fat tissues were fixed and dounce-
553 homogenized for nuclei isolation. The nuclei were used for sonication to shear DNA, followed by
554 immunoprecipitation and elution. The immunoprecipitated DNA was quantitated by real-time PCR using SYBR
555 green. The information for primer sequences was shown in **Supplemental Table 5**.

556 Oxygen consumption rate (OCR) measurement. Brown adipocyte OCR was measured using a XF 96
557 Extracellular Flux Analyzer (Agilent, Santa Clara, CA) as we described (63). OCR measurement began with a
558 basal respiration recording, followed by addition of other reagents including oligomycin for inhibition of the
559 coupled respiration and FCCP for maximal respiration.

560 RNAseq analysis. Total RNA was isolated from iWAT of cold-challenged WT, H2KO and AH2Tg mice.
561 Equal amount of RNA from 6 animals/group was pooled and sent to Beijing Genomics Institute (BGI, Shenzhen,
562 China) for RNAseq analysis.. Clean reads were aligned to the mouse reference genome (University of California
563 Santa Cruz Mouse Genome Browser mm9 Assembly) using SOAPaligner/SOAP2 (65). Differential expression
564 analysis was performed using DESeq2 (66). Differentially expressed genes between groups were defined as

565 Log2 fold change cutoff threshold of 0.5 and false discovery rate (FDR)<0.05. Pathway analysis was performed
566 using the clusterProfiler (67). The RNAseq data was also used to predict adipose tissue browning capacity with
567 an online bioinformatic software (<https://github.com/PerocchiLab/ProFAT>) (23).

568 Assay for transposase-accessible chromatin sequencing (ATAC-seq) analysis. ATAC-seq was
569 conducted according to the Omni-ATAC-seq protocol as described (68). Briefly, BAT tissues were dounce-
570 homogenized, filtered and centrifuged in iodixanol solution to obtain nuclei. The nuclei were treated with
571 Nextera Tn5 transposase (Illumina, San Diego, CA) for the transposition reaction, followed by DNA purification
572 and PCR amplification with NEBNext 2X MasterMix (New England BioLabs, Ipswich, MA) and Nextera Index
573 primers (Illumina). The ATAC libraries were further size-purified and sent to Novogene (Durham, NC) for
574 sequencing. The ATAC-seq analysis was performed on the Galaxy server as described(68). Briefly, the
575 adaptor-trimmed sequencing reads were mapped to the mm10 mouse reference genome using Bowtie2 (69).
576 After removing PCR duplicates and reads mapped to ENCODE blacklist regions, the ATAC-seq peaks were
577 called using MACS2 (70). Finally, differential ATAC-seq peaks between groups were identified using DiffBind.
578 The integration of RNA-seq and ATAC-seq data was carried out in R, and heatmaps were generated using the
579 ComplexHeatmap package (71).

580 Statistical analysis. Data were expressed as mean \pm SEM. Statistical tests were performed using SPSS
581 software (version 16.0, SPSS Inc, Chicago, IL, USA). Differences between groups were analyzed for statistical
582 significance by Student's t-test, one-way or two-way ANOVA, or two-way ANOVA with repeated measures as
583 appropriate. Statistical significance was accepted at $p < 0.05$.

584 Study Approval. All animal procedures conducted in the study were approved by the Institutional Animal
585 Care and Use Committee (IACUC) at Georgia State University (GSU).

586 Data availability. The RNA-seq and ATAC-seq data have been deposited to Gene Expression Omnibus
587 (GEO) database with the accession code GSE244457 and GSE245509, respectively. Values for graphs in the
588 Figures and Supplemental Figures are provided in the Supporting Data Values file.

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594 **Author Contributions.** XC performed most of the experiments and analyzed the data; QC, FL, JJ, ZL, and XY
595 assisted with various experiments; HDS performed bioinformatic analysis of RNAseq and ATACseq data; GS
596 and LY contributed to conceptual and technical inputs and review/edits on manuscript; HS and BZ conceived
597 and designed the study and wrote the manuscript.

598 **Declaration of Interest.** The authors declare no conflict of interests.

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754

755

756 **Figure Legends.**

757 **Figure 1.** *Suv420h2* is important in regulating *Ucp1* expression.

758 (A)-(B) The expression of *Ucp1* (A) and *Suv420h2* (B) in iWAT of male C57BL/6J mice during postnatal development, n=4
759 for postnatal days 10 and 60, and n=5 for postnatal days 15, 20 and 30. *p<0.05 by one-way ANOVA followed by Tukey's
760 multiple comparisons test.

761 (C) The expression of *Suv420h2* in brown and white adipose tissues in 2-3 months old male C57BL/6J mice, n=4/group.
762 *p<0.05 by one-way ANOVA followed by Tukey's multiple comparisons test.

763 (D)-(E) The expression of *Ucp1* (D) and *Suv420h2* (E) in iWAT of 2-3 months old male C57BL/6J mice during a 5°C cold
764 exposure challenge, n=10 in (D) and n=4 in (E). *p<0.05 by one-way ANOVA followed by Tukey's multiple comparisons
765 test.

766 (F)-(G) The expression of *Ucp1* (F) and *Suv420h2* (G) during BAT1 brown adipocyte differentiation, n=4-6/group. *p<0.05
767 by one-way ANOVA followed by Tukey's multiple comparisons test.

768 (H)-(I) H4K20 mono-, di- and tri-methylation levels in BAT1 brown adipocytes with scramble siRNA (Control) and
769 *Suv420h2* (H2KD) siRNA knockdown (H) or with empty vector (Control) and *Suv420h2* (H2OE) overexpression (I),
770 n=3/group. *p<0.05 by unpaired two-tailed Student's t-test. Blots were run in parallel at the same time.

771 (J)-(K) The expression of *Ucp1* in BAT1 brown adipocytes with *Suv420h2* knockdown (J) or *Suv420h2* overexpression (K),
772 n=4-6/group. *p<0.05 by two-way ANOVA followed by Tukey's multiple comparisons test.

773 (L) The expression of *Ucp1* in BAT1 brown adipocytes with overexpression of either empty vector (Control) or *Suv420h1*
774 (H1 OE), n=4-5/group. *p<0.05 by two-way ANOVA followed by Tukey's multiple comparisons test.

775 (M-O) The expression of *Ucp1* (M), *Dio2* (N) and *Acot2* (O) in BAT1 brown adipocytes with *Suv420h2* knockdown and
776 further treated with either vehicle (dimethyl sulfoxide (DMSO)) or the SUV420H1/H2 inhibitor A196. Four-day
777 differentiated BAT1 cells were treated with either scramble or *Suv420h2* siRNA via electroporation. On day 6 of
778 differentiation, cells were further treated with either DMSO or the SUV420H1/H2 inhibitor A196 (5μM) for 24 hours.
779 Before harvesting, cells were further treated with either PBS or NE (1μM) for 4 hours, n=3-4/group. Bars with a different
780 letter indicate statistical significance at p<0.05 as analyzed by two-way ANOVA followed by Tukey's multiple
781 comparisons test. Control: Scramble siRNA+DMSO; H2KD: *Suv420h2* siRNA+DMSO; H2KD+A196: *Suv420h2* siRNA+A196.

782 All data are expressed as mean ± SEM.

783

784 **Figure 2.** *Suv420h2* regulates the development of brown and beige fat.

785 (A) –(D) UCP1 protein levels (A), UCP1 immunostaining (B), H&E staining (C), and quantification of adipocyte size (D) in
786 iBAT of 20-day-old H2KO and WT mice housed at room temperature. In (A), n=4-5/group. *p<0.05 vs. WT by
787 unpaired two-tailed Student's t-test. In (B), images are representatives from 3 replicate animals/group. Images from
788 additional animals can be found in Supplemental Figure 6A. Scale bar=70μm in (B) and (C). In (D), n=3/group.
789 *p<0.05 as analyzed by two-way ANOVA followed by Tukey's multiple comparisons test.

790 (E) –(H) UCP1 protein levels (E), UCP1 immunostaining (F), H&E staining (G) and Quantification of adipocyte size (H) in
791 iWAT of 20-day-old H2KO and WT mice housed at room temperature. In (E), n=3/group. *p<0.05 vs. WT by unpaired
792 two-tailed Student's t-test. In (F), images are representatives from 3 replicate animals/group. Images from additional

793 animals can be found in Supplemental Figure 6B-C. Scale bar=140 μ m in (F) and (G). In (H), n=4/group. *p<0.05 as
794 analyzed by two-way ANOVA followed by Tukey's multiple comparisons test.

795 (I) –(L) UCP1 protein levels (I), UCP1-immunostaining (J), H&E staining (K) and quantification of adipocyte size (L) in
796 iBAT of 20-day-old AH2Tg and WT mice housed at room temperature. In (I), n=4-5/group. *p<0.05 vs. WT by
797 unpaired two-tailed Student's t-test. In (J), images are representatives from 3 replicate animals/group). Images from
798 additional animals can be found in Supplemental Figure 8A Scale bar=70 μ m in (J) and (K). In (L), n=3/group. *p<0.05
799 as analyzed by two-way ANOVA followed by Tukey's multiple comparisons test.

800 (M) –(P) UCP1 protein levels (M), UCP1-immunostaining (N), H&E staining (O) and Quantitation of adipocyte size (P) in
801 iWAT of 20-day-old AH2Tg and WT mice housed at room temperature. In (M), n=3/group. *p<0.05 vs. WT by
802 unpaired two-tailed Student's t-test. In (N), images are representatives from 3 replicate animals/group. Images from
803 additional animals can be found in Supplemental Figure 8B-C. Scale bar=140 μ m in (N) and (O). In (P), n=3/group.
804 *p<0.05 as analyzed by two-way ANOVA followed by Tukey's multiple comparisons test.

805 All data are expressed as mean \pm SEM. UCP1-positive multilocular brown/beige adipocytes were shown in dark purplish
806 red color, and were indicated with black arrows; and UCP1-negative unilocular white adipocytes were shown in light
807 color, and were indicated with red arrows.

808 **Figure 3.** H2KO mice have impaired brown/beige adipocyte thermogenesis during a 7-day cold challenge. Three-month-
809 old male H2KO and their WT littermates were challenged with a chronic 7-day cold at 5°C.

810 (A) –(B) Core body temperature (A) and fat pad weight (B) in WT and H2KO mice during the cold challenge. In (A), n=7
811 for WT and n=5 for H2KO. *p<0.05 by two-way ANOVA with repeated measures followed by Tukey's multiple
812 comparisons test. In (B), n=5/group. *p<0.05 by unpaired two-tailed Student's t-test.

813 (C) –(D) H&E staining (C) and quantification of adipocyte size (D) in iBAT and iWAT of WT and H2KO mice after the 7-day
814 cold exposure. In (C), scale bar=70 μ m for iBAT and 140 μ m for iWAT. In (D), n=3/group. *p<0.05 by two-way ANOVA
815 followed by Tukey's multiple comparisons test.

816 (E)-(F) Gene expression analysis in iBAT (E) and iWAT (F) of WT and H2KO mice after the 7-day cold exposure, n=6/group
817 in (E) and n=5/group in (F). *p<0.05 by unpaired two-tailed Student's t-test.

818 (G)-(H) UCP1 protein and H4K20me3 levels in iBAT (G) and iWAT (H) of WT and H2KO mice after the 7-day cold exposure,
819 n=5 (WT), n=3 (H2KO) in (G) and n=4/group in (H). *p<0.05 by unpaired two-tailed Student's t-test.

820 (I) UCP1 immunostaining in iBAT and iWAT of WT and H2KO mice after the 7-day cold exposure (Representative images
821 from 3 replicate animals/group). Images from additional animals can be found in Supplemental Figure 9A-C. UCP1-
822 positive multilocular brown/beige adipocytes were shown in dark purplish red color, and were indicated with black
823 arrows; and UCP1-negative unilocular white adipocytes were shown in light color, and were indicated with red arrows.
824 Scale bar=70 μ m for iBAT and 140 μ m for iWAT.

825 (J) Oxygen consumption rate (OCR) in primary brown adipocytes isolated from iBAT of male WT and H2KO mice
826 measured by a Seahorse XF 96 Extracellular Flux Analyzer, n=9 (WT) and 8 (H2KO). *p<0.05 by two-way ANOVA with
827 repeated measures followed by Tukey's multiple comparisons test or by unpaired two-tailed Student's t-test.

828 All data are expressed as mean \pm SEM.

830 **Figure 4.** AH2Tg mice have enhanced brown/beige adipocyte thermogenesis during a 7-day cold challenge. Three-
831 month-old male AH2Tg and their WT littermates were challenged with a chronic 7-day cold at 5°C.

832 (A) –(B) Core body temperature (A) and fat pad weight (B) in WT and AH2Tg mice during the cold challenge. In (A), n=7
833 for WT and n=6 for H2KO. *p<0.05 by two-way ANOVA with repeated measures followed by Tukey’s multiple
834 comparisons test. In (B), n=7/group. *p<0.05 by unpaired two-tailed Student’s t-test.

835 (C) –(D) H&E staining (C) and quantification of adipocyte size (D) in iBAT and iWAT of WT and AH2Tg mice after the 7-day
836 cold exposure. In (C), scale bar=70µm for iBAT and 140µm for iWAT. In (D), n=3/group in iBAT and n=4/group in iWAT.
837 *p<0.05 by two-way ANOVA followed by Tukey’s multiple comparisons test.

838 (E)-(F) Gene expression analysis in iBAT (E) and iWAT (F) of WT and AH2Tg mice after the 7-day cold exposure,
839 n=7/group in (E) and n=8/group in (F). *p<0.05 by unpaired two-tailed Student’s t-test.

840 (G)-(H) UCP1 protein and H4K20me3 levels in iBAT (G) and iWAT (H) of WT and AH2Tg mice after the 7-day cold
841 exposure, n=3/group. *p<0.05 by unpaired two-tailed Student’s t-test.

842 (I) UCP1 immunostaining in iBAT and iWAT of WT and AH2Tg mice after the 7-day cold exposure (Representative images
843 from 3 replicate animals/group). Images from additional animals can be found in Supplemental Figure 10A-C. UCP1-
844 positive multilocular brown/beige adipocytes were shown in dark purplish red color, and were indicated with black
845 arrows; and UCP1-negative unilocular white adipocytes were shown in light color, and were indicated with red arrows.
846 Scale bar=70µm for iBAT and 140µm for iWAT.

847 (J) Oxygen consumption rate (OCR) in primary brown adipocytes isolated from iBAT of male WT and AH2Tg mice
848 measured by a Seahorse XF 96 Extracellular Flux Analyzer, n=8 (WT) and 9 (H2KO). *p<0.05 by two-way ANOVA with
849 repeated measures followed by Tukey’s multiple comparisons test or by unpaired two-tailed Student’s t-test.

850 All data are expressed as mean ± SEM.

851

852 **Figure 5.** SUV420H2 regulates mitochondrial bioenergetic program.

853 (A)-(B) RNAseq analysis of BAT-specific gene expression in iWAT of male H2KO mice (A) and male AH2Tg mice (B) after
854 the 7-day cold exposure using an online software (<https://github.com/PerocchiLab/ProFAT>). The WAT reference
855 aggregate and BAT reference aggregate were derived from the online software.

856 (C) Heatmaps of genes that are reciprocally regulated in iWAT of H2KO and AH2Tg mice after cold exposure.

857 (D) Analysis of pathways that are reciprocally regulated in iWAT of H2KO and AH2Tg mice after cold exposure.

858 (E) Comparison of genome-wide alterations in chromatin accessibility landscape assessed by ATAC-seq with the
859 corresponding gene expression assessed by RNA-seq of AH2Tg and WT mice after the 7-day cold exposure.

860

861 **Figure 6.** SUV420H2 regulates mitochondrial respiratory chain complex protein levels.

862 (A)-(B) Immunoblotting of mitochondrial respiratory chain complex proteins in iBAT (A) and iWAT (B) of H2KO and WT
863 mice after the 7-day cold exposure, n=5-7/group in (A) and n=3/group in (B). *p<0.05 by unpaired two-tailed Student’s t-
864 test.

865 (C)-(D) Immunoblotting of mitochondrial respiratory chain complex proteins in iBAT (C) and iWAT (D) of AH2Tg and WT
866 mice after the 7-day cold exposure, n=3/group. *p<0.05 by unpaired two-tailed Student’s t-test.

867 All data are expressed as mean \pm SEM.

868 **Figure 7.** SUV420H2 regulates brown/beige adipocyte thermogenesis through post-transcriptional regulation of PGC1 α
869 protein levels.

870 (A)-(B) PGC1 α mRNA (A) and Protein (B) levels in iWAT of C57B6/6J mice during postnatal development, n=5/group in (A)
871 and n=3/group in (B). *p<0.05 by unpaired two-tailed Student's t-test.

872 (C)-(D) PGC1 α mRNA (C) and Protein (D) levels in iWAT of C57B6/6J mice during cold exposure, n=4/group. In (C), bars
873 with a different letter indicate statistical significance at p<0.05 as analyzed by one-way ANOVA followed by Tukey's
874 multiple comparisons test; in (D), *p<0.05 by unpaired two-tailed Student's t-test.

875 (E)-(F) PGC1 α protein levels in iBAT (E) and iWAT (F) of H2KO and WT mice after the 7-day cold exposure, n=6/group.
876 *p<0.05 by unpaired two-tailed Student's t-test.

877 (G)-(H) PGC1 α protein levels in iBAT (G) and iWAT (H) of AH2Tg and WT mice after the 7-day cold exposure, n=3/group.
878 *p<0.05 by unpaired two-tailed Student's t-test.

879 All data are expressed as mean \pm SEM.

880

881 **Figure 8.** 4E-BP1 mRNA and protein levels are reciprocally regulated in H2KO and AH2Tg animals after cold exposure.

882 (A) ATAC-seq analysis of chromatin accessibility and RNAseq peak data at *4e-bp1* gene locus in AH2Tg and WT mice after
883 a 7-day cold exposure.

884 (B)-(C) Expression of *4e-bp1* in various adipose tissues of H2KO (B) and AH2Tg (C) mice after cold exposure, n=5/group in
885 (B), and n=7 (WT) and 6 (AH2Tg) in (C). *p<0.05 by unpaired two-tailed Student's t-test.

886 (D)-(E) 4E-BP1 protein levels in iBAT (D) and iWAT (E) of H2KO and WT mice after the 7-day cold exposure, n=7 (WT) and
887 5 (H2KO). *p<0.05 by unpaired two-tailed Student's t-test. Blots were run in parallel at the same time.

888 (F)-(G) 4E-BP1 protein levels in iBAT (F) and iWAT (G) of AH2Tg and WT mice after the 7-day cold exposure, n=8 (WT) and
889 7 (AH2Tg) in (F), and n=6 (WT) and 9 (AH2Tg) in (G). *p<0.05 by unpaired two-tailed Student's t-test.

890 (H) 4E-BP1 protein levels in iWAT of C57BL/6J mice during postnatal development and after a cold challenge, n=3/group.
891 Bars with a different letter indicate statistical significance at p<0.05 as analyzed by one-way ANOVA followed by Tukey's
892 multiple comparisons test. Blots were run in parallel at the same time.

893 All data are expressed as mean \pm SEM.

894

895 **Figure 9.** SUV420H2 regulates PGC1 α protein levels through increasing H4K20me3 at *4e-bp1* promoter.

896 (A)-(B) H4K20me3 levels at the promoter regions of *4e-bp1* as assessed by ChIP assay in iBAT (A) and iWAT (B) of H2KO
897 and WT mice after a 7-day cold exposure, n=3/group. *p<0.05 by two-way ANOVA followed by Tukey's multiple
898 comparisons test.

899 (C)-(D) H4K20me3 levels at the promoter regions of *4e-bp1* as assessed by ChIP assay in iBAT (C) and iWAT (D) of AH2Tg
900 and WT mice after a 7-day cold exposure, n=3/group. *p<0.05 by two-way ANOVA followed by Tukey's multiple
901 comparisons test.

902 (E)-(F) Basal and NE-induced 4E-BP1 (E), and PGC1 α (F) protein levels in BAT1 brown adipocytes treated with either
903 *Suv420h2* knockdown or combined *Suv420h2/4e-bp1* knockdown, n=4-6/group. In (E), Bars with a different letter
904 indicate statistical significance at p<0.05 as analyzed by two-way ANOVA followed by Tukey's multiple comparisons test.
905 In (F), *p<0.05 by two-way ANOVA followed by Tukey's multiple comparisons test.

906 All data are expressed as mean \pm SEM.

907

908 **Figure 10.** SUV420H2 regulates diet-induced obesity.

909 (A)-(D) Body weight (A), fat pad mass (B), glucose tolerance test (GTT) (C) and insulin tolerance test (ITT) (D) in H2KO and
910 WT mice fed a HFD when housed at thermoneutrality,. In (A) and (B), n=7/group. *p<0.05 by two-way ANOVA with
911 repeated measures followed by Tukey's multiple comparisons test in (A) and unpaired two-tailed Student's t-test in (B).
912 In (C) and (D), n=6-7/group. *p<0.05 by two-way ANOVA with repeated measures followed by Tukey's multiple
913 comparisons test.

914 (E)-(H) Body weight (E), fat pad mass (F), glucose tolerance test (GTT) (G) and insulin tolerance test (ITT) (H) in AH2Tg
915 and WT mice fed a HFD when housed at thermoneutrality. n=6-7/group. *p<0.05 by two-way ANOVA with repeated
916 measures followed by Tukey's multiple comparisons test in (E), (G) and (H), and unpaired two-tailed Student's t-test in
917 (F). In (G) and (H), n=6-7/group. *p<0.05 by two-way ANOVA with repeated measures followed by Tukey's multiple
918 comparisons test.

919 All data are expressed as mean \pm SEM.

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Figure 1

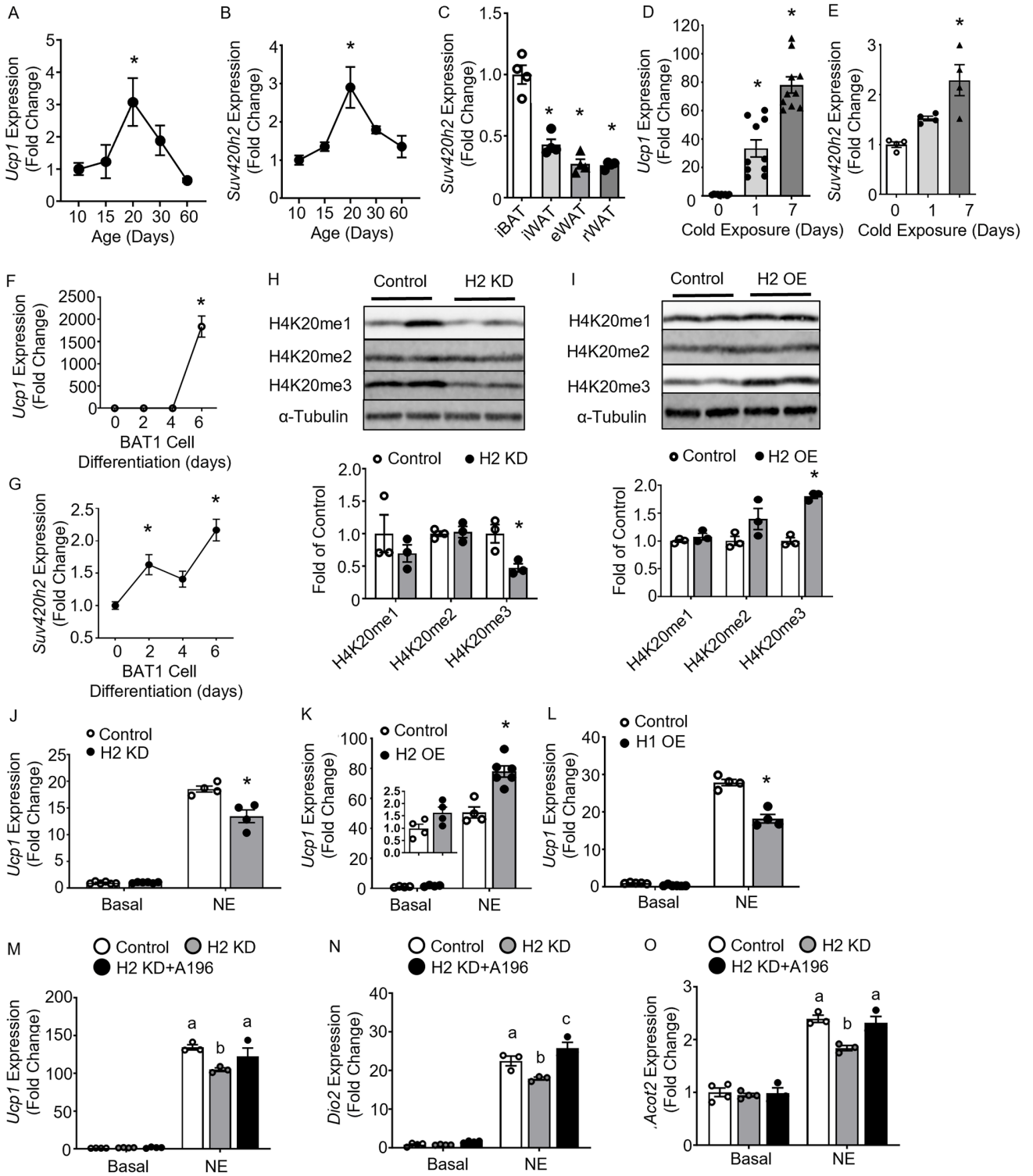


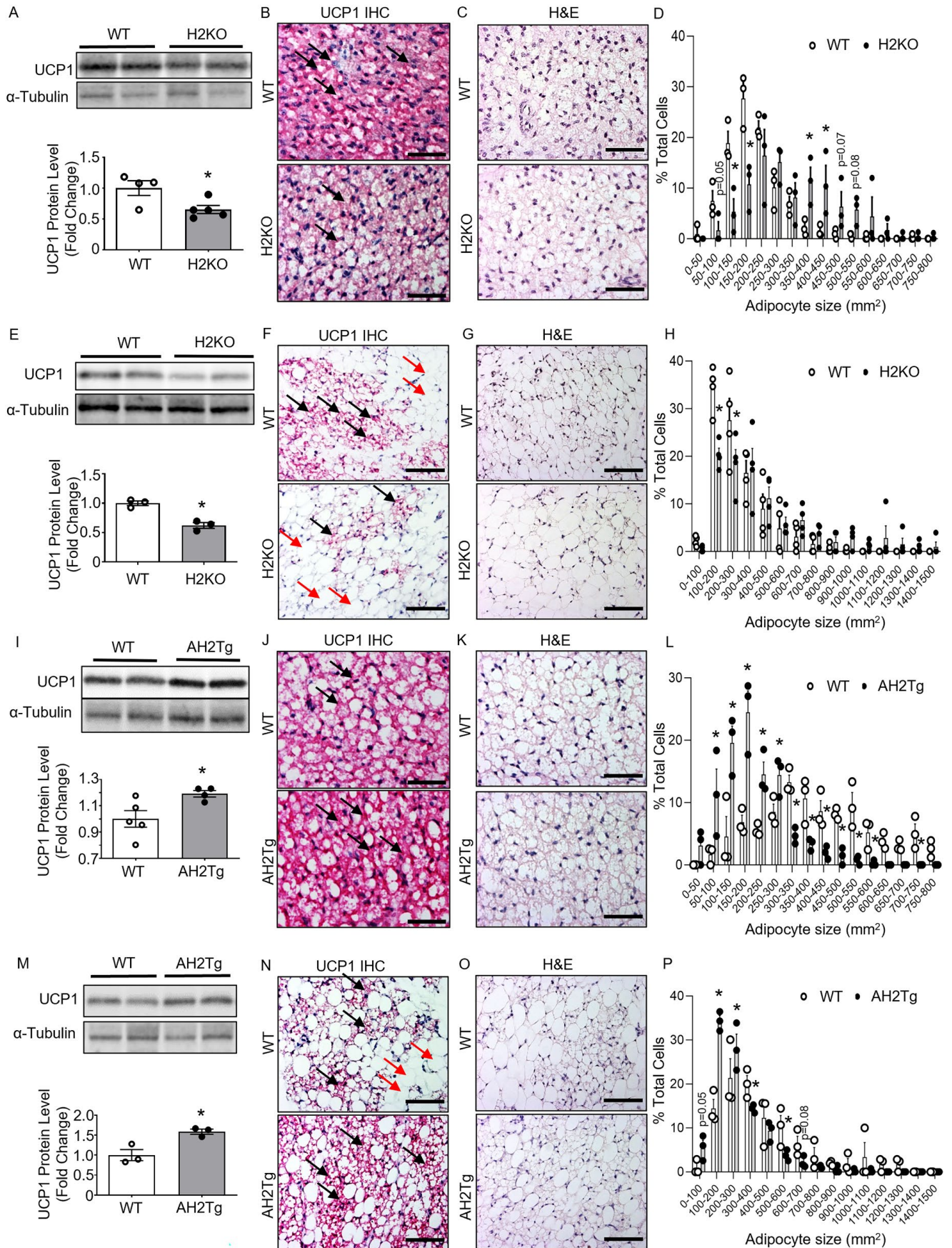
Figure 2

Figure 3

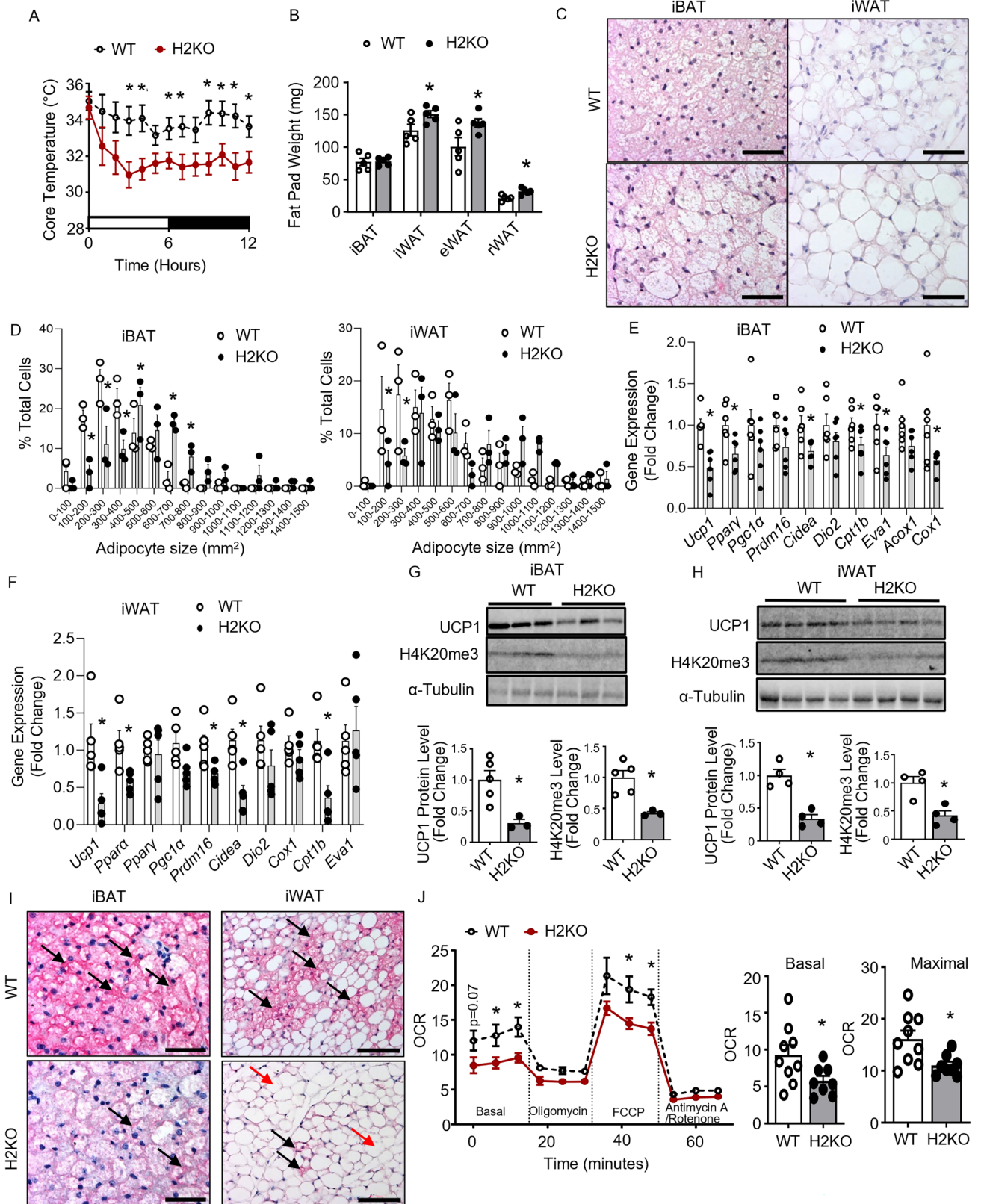


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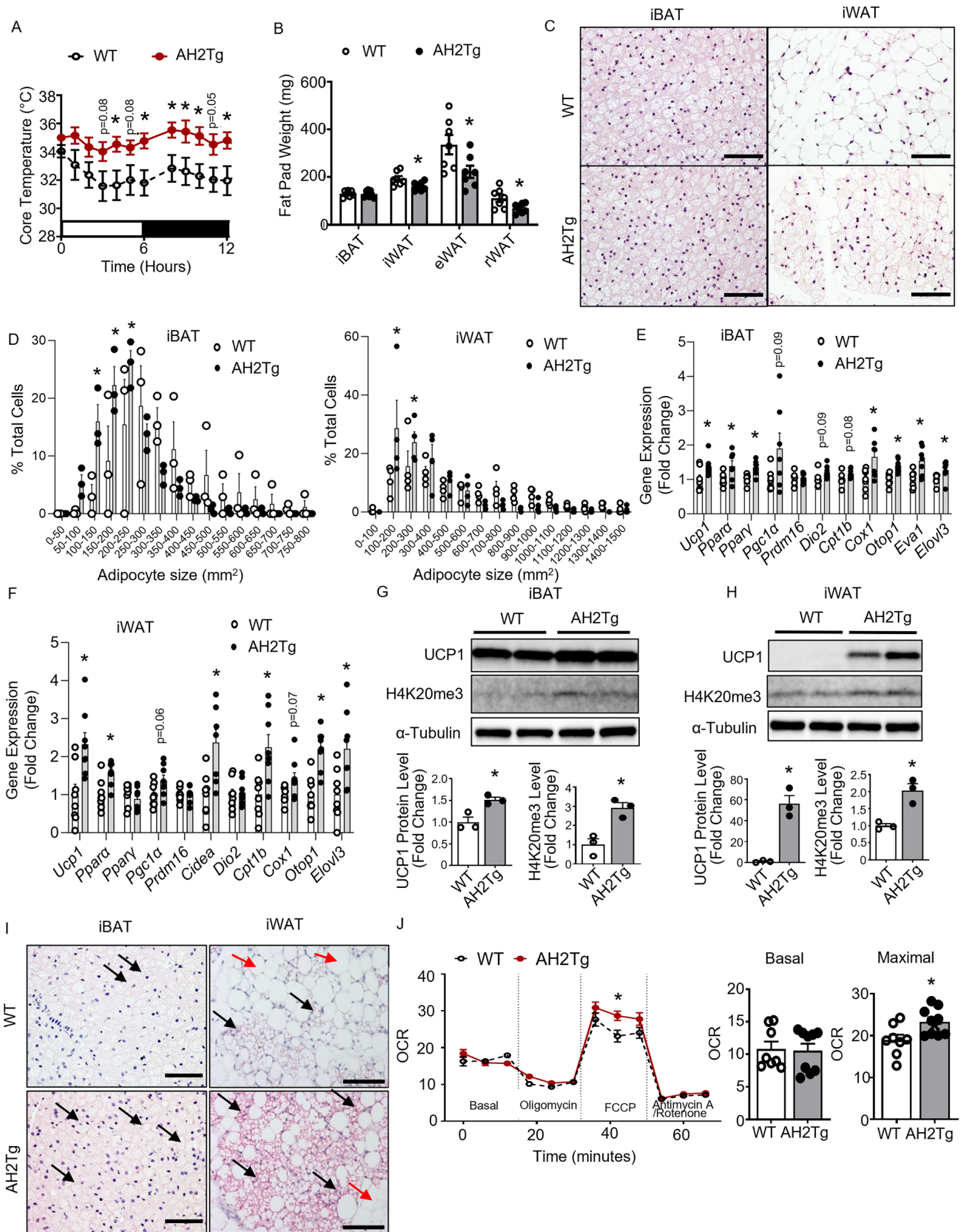


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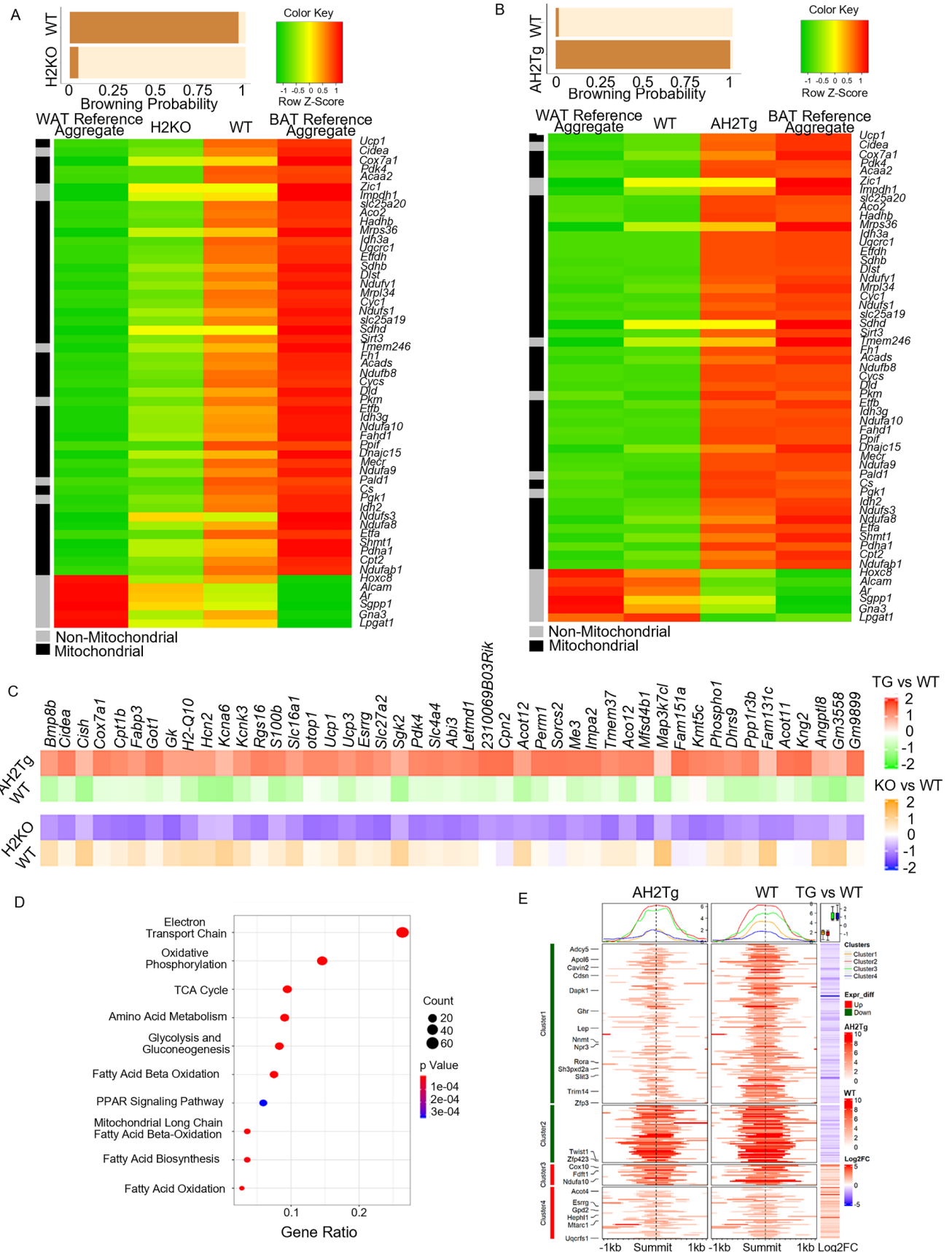


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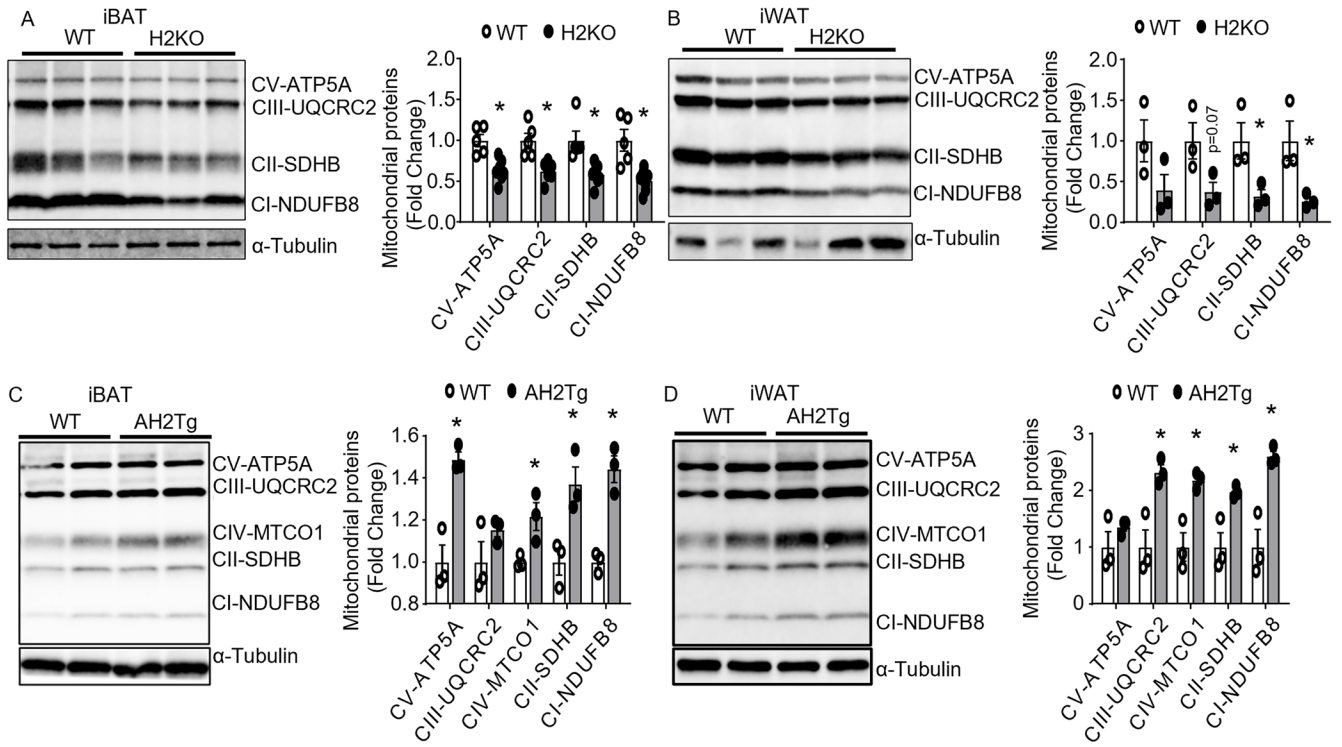


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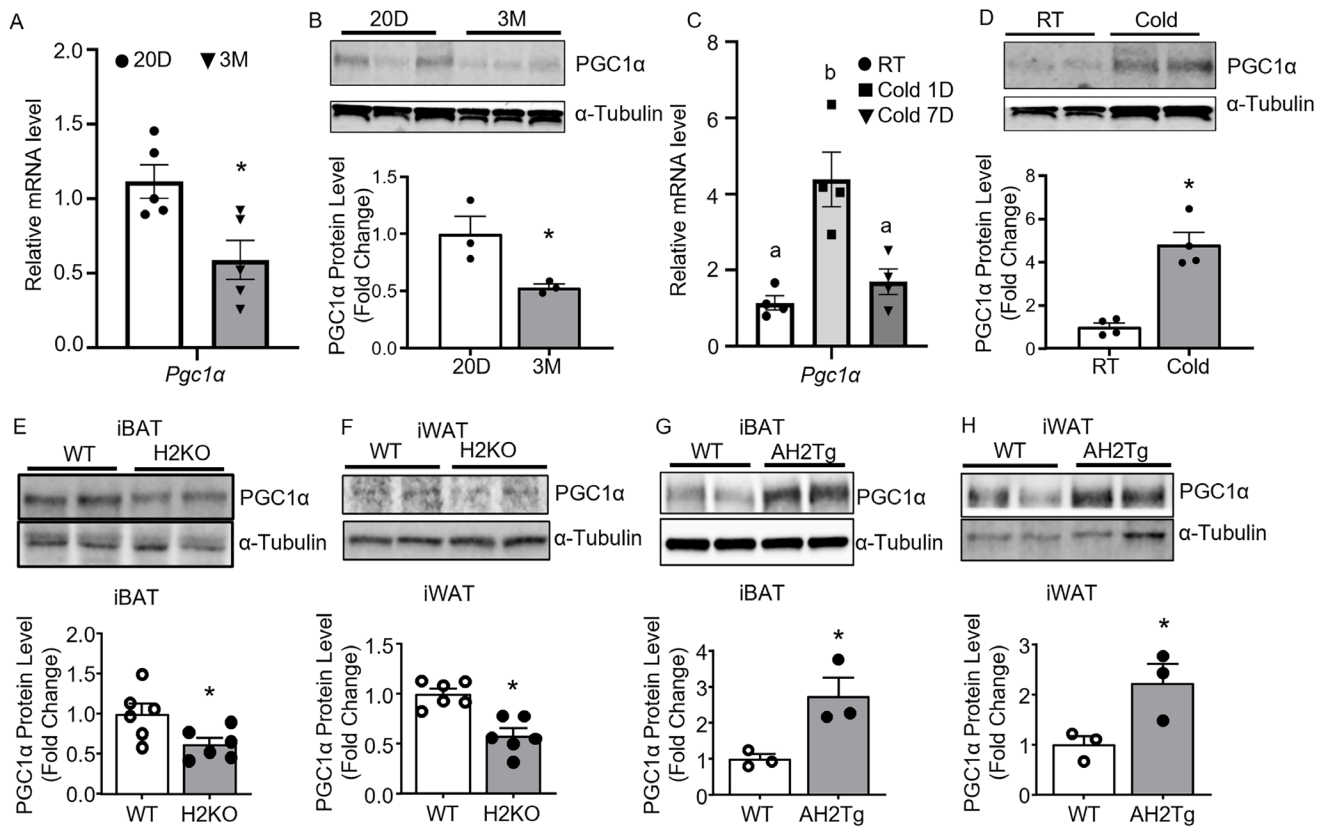


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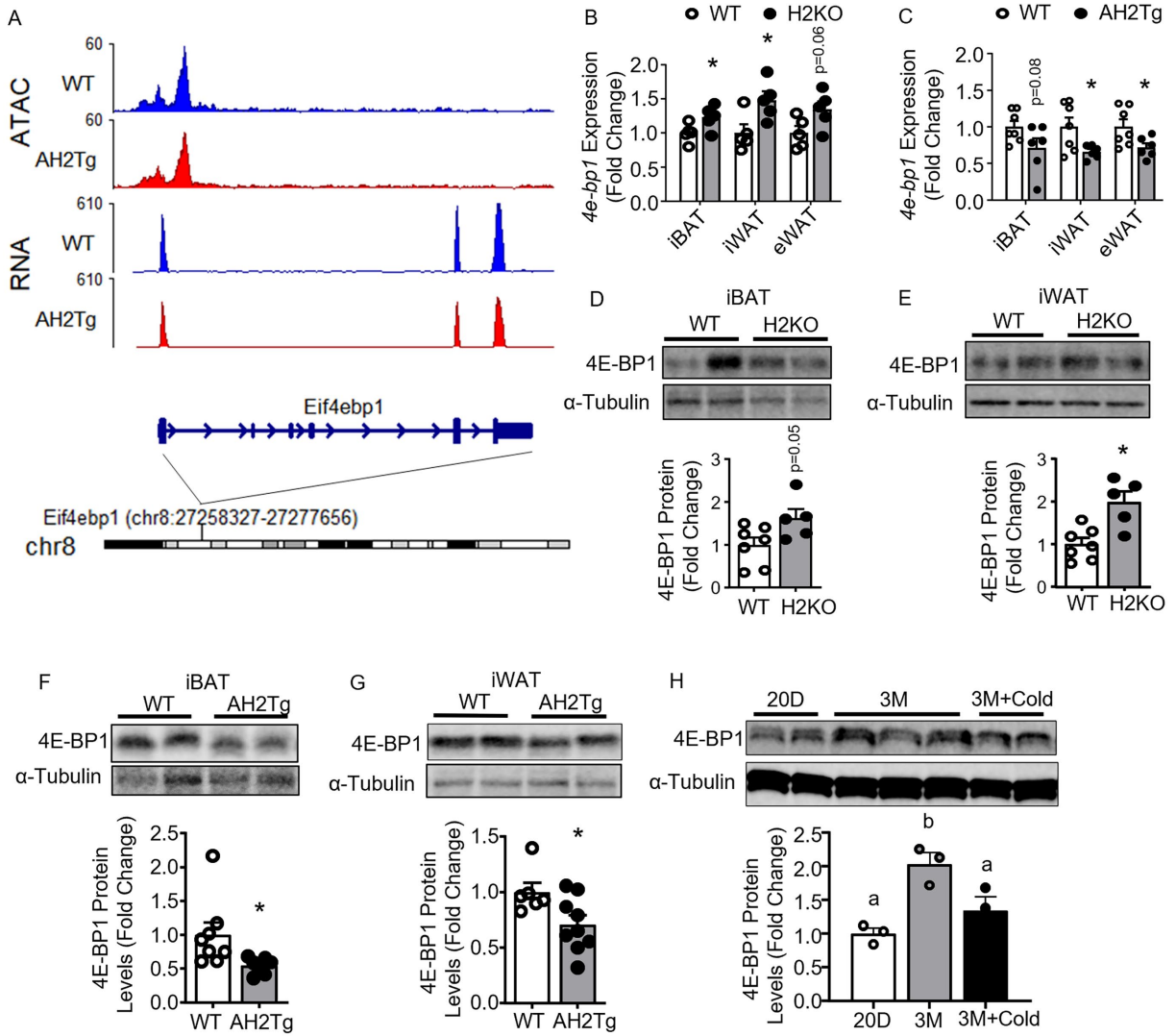


Figure 9

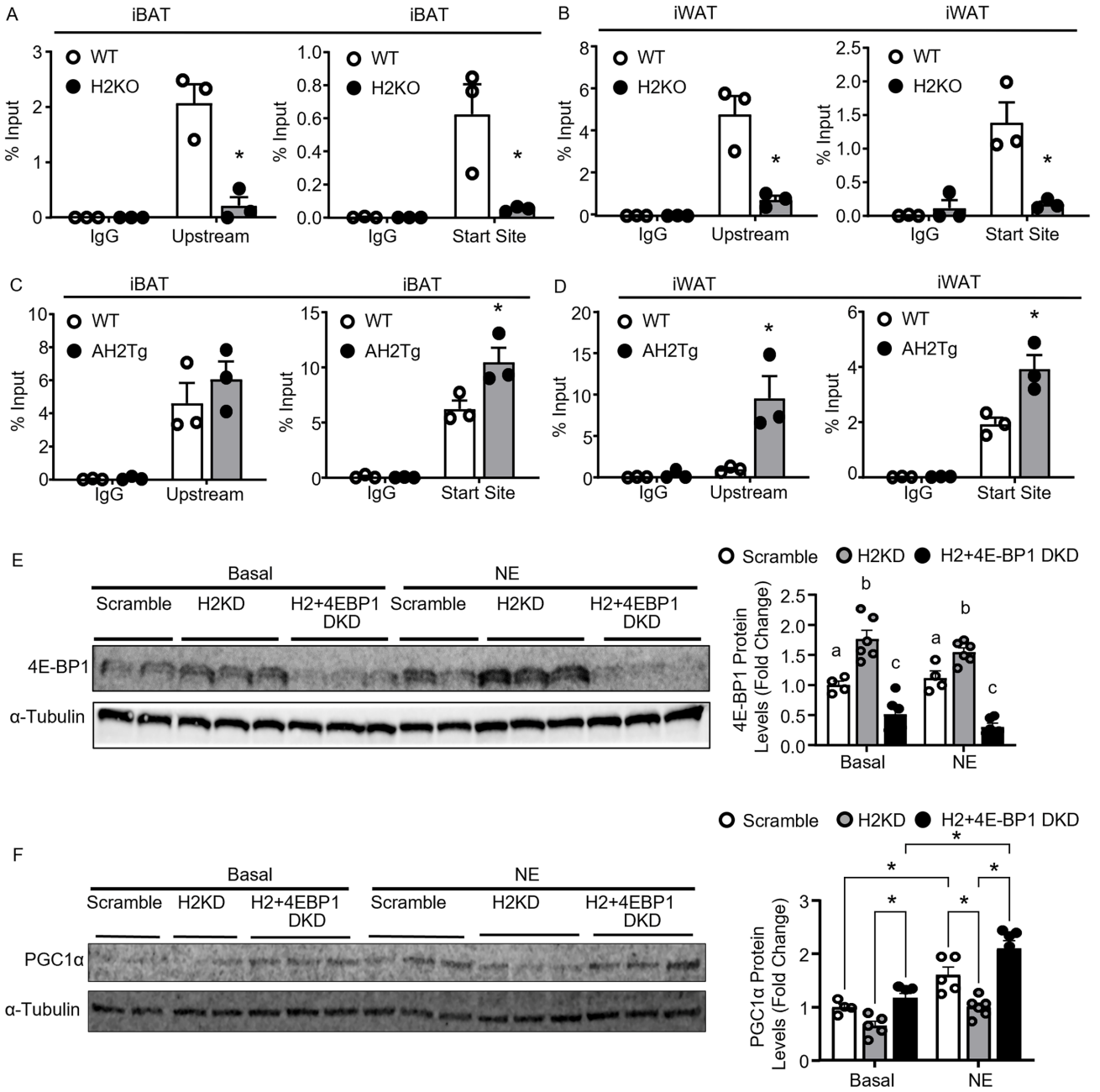


Figure 10

