

1 **IKK $\beta$  signaling mediates metabolic changes in the hypothalamus of**

2 **a Huntington's disease mouse model**

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17

18 **Abstract**

19 **Background:** Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat  
20 expansion in the huntingtin (*HTT*) gene. Metabolic changes are associated with HD progression,  
21 and underlying mechanisms are not fully known. As the IKK $\beta$ /NF- $\kappa$ B pathway is an essential  
22 regulator of metabolism, we investigated the involvement of IKK $\beta$ , the upstream activator of NF-  
23  $\kappa$ B in hypothalamus-specific HD metabolic changes.

24 **Methods:** Using viral vectors, we expressed amyloidogenic N-terminal fragments of mutant HTT  
25 (mHTT) fragments in the hypothalamus of mice without IKK $\beta$  in the CNS (IKK $\beta^{-/-}$ ) and control  
26 mice (IKK $\beta^{+/+}$ ). We assessed effects on body weight, metabolic hormones, and hypothalamic  
27 neuropathology.

28 **Results:** Hypothalamic expression of mHTT led to an obese phenotype only in female mice. CNS-  
29 specific inactivation of IKK $\beta$  prohibited weight gain in females, which was independent of  
30 neuroprotection and microglial activation.

31 **Conclusions:** The expression of mHTT in the hypothalamus causes metabolic imbalance in a sex-  
32 specific fashion, and central inhibition of the IKK $\beta$  pathway attenuates the obese phenotype.

33

34 **Keywords:** Huntington's disease, IKK $\beta$  pathway, metabolism, hypothalamus, obesity

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36

## 37 **Introduction**

38 Huntington's disease (HD) is a fatal neurodegenerative disorder caused by a CAG repeat expansion  
39 in the huntingtin (HTT) gene (HDCRG, 1993). Although the clinical diagnosis is based on typical  
40 motor symptoms, affected individuals also suffer from non-motor symptoms such as cognitive  
41 decline, psychiatric symptoms, and metabolic disturbances, which often precede motor symptoms  
42 by several years (Bates et al., 2015; Cheong, Gabery, & Petersen, 2019). Metabolic changes in HD  
43 include weight loss despite adequate or even higher caloric intake (Aziz et al., 2008; Marder et al.,  
44 2009). A higher baseline body mass index (BMI) has been associated with a slower disease  
45 progression (van der Burg et al., 2017). Hence, identifying the underlying mechanisms of  
46 metabolic changes in HD may reveal novel targets for therapeutic interventions to modify disease  
47 progression.

48         The hypothalamus is a master regulator of metabolism (Cakir & Nillni, 2019; Timper &  
49 Bruning, 2017). Imaging studies have identified hypothalamic changes in both prodromal and  
50 symptomatic HD patients (Douaud et al., 2006; Kassubek, Gaus, & Landwehrmeyer, 2004; Politis  
51 et al., 2008; Soneson et al., 2010). Analyses of postmortem hypothalamic tissue from HD cases  
52 and animal models (Cheong et al., 2019) showed loss of neuronal populations expressing orexin  
53 (hypocretin), oxytocin, and vasopressin, as well as altered metabolic pathways in several nuclei  
54 (Baldo et al., 2019; Gabery, Halliday, Kirik, Englund, & Petersen, 2015; Gabery et al., 2010;  
55 Petersen et al., 2005). Inactivation of mutant HTT (mHTT) selectively in the hypothalamus in the  
56 transgenic BACHD mouse model prevented developing a metabolic phenotype with obesity  
57 accompanied by leptin and insulin resistance (Gray et al., 2008; Hult et al., 2011). Similarly, local  
58 expression of mHTT in the hypothalamus using recombinant adeno-associated viral (rAAV)  
59 vectors in mice led to hyperphagic obesity with leptin and insulin resistance (Hult et al., 2011;

60 Soylu-Kucharz, Adlesic, Baldo, Kirik, & Petersen, 2015). Transgene expression was present in  
61 several appetite-regulating hypothalamic cell populations (i.e., AGRP, POMC)(Hult et al., 2011).  
62 As a result, even though these experiments have proved a causal link between hypothalamic  
63 expression of mHTT and the development of metabolic imbalance in mice, the mechanisms of  
64 hypothalamic mHTT induced metabolic imbalance are still unknown.

65         The inhibitor of  $\kappa$ B kinase  $\beta$ / nuclear factor- $\kappa$ B (IKK $\beta$ /NF- $\kappa$ B) signaling pathway plays a  
66 significant role in obesity and overeating and is enriched in the hypothalamus (Meng & Cai, 2011;  
67 X. Zhang et al., 2008). Hyperphagia has been shown to activate IKK $\beta$ /NF- $\kappa$ B in the hypothalamus  
68 through increased endoplasmic reticulum stress, and suppression of IKK $\beta$ /NF- $\kappa$ B results in  
69 reduced food intake and normalized metabolic phenotype in mice (Douglass, Dorfman, Fasnacht,  
70 Shaffer, & Thaler, 2017; X. Zhang et al., 2008; Y. Zhang, Reichel, Han, Zuniga-Hertz, & Cai,  
71 2017). The IKK $\beta$ /NF- $\kappa$ B pathway is also activated by mHTT and has been associated with HD  
72 pathogenesis (Atwal et al., 2011; Becanovic et al., 2015; Khoshnan et al., 2004; Sarkar et al., 2011;  
73 Thompson et al., 2009; Trager et al., 2014). In the R6/1 mouse model of HD, brain-specific  
74 deletion of IKK $\beta$  impaired the behavioral phenotype and led to exacerbated neurodegeneration  
75 with an activated microglial response in the striatum (Ochaba et al., 2019). However, it is not  
76 known whether the IKK $\beta$ /NF- $\kappa$ B pathway is involved in the development of HD metabolic  
77 imbalance. In the present study, our aim was to determine whether inactivation of the IKK $\beta$ /NF-  
78  $\kappa$ B pathway would prevent hypothalamic-induced metabolic changes induced by mHTT. We  
79 therefore performed injections of rAAV vectors expressing mHTT into the hypothalamus of mice  
80 without IKK $\beta$  in the CNS (IKK $\beta$ <sup>-/-</sup>) and compared metabolic effects to control mice with the floxed  
81 allele of IKK $\beta$  (IKK $\beta$ <sup>+/-</sup>).

## 82 **Materials and methods**



## 83 **Animals**

84 The experimental procedures performed on mice were carried out using the approved guidelines  
85 in the ethical permit approved by the Lund University Animal Welfare and Ethics committee in  
86 the Lund-Malmö region (ethical permit numbers M20-11 and M65-13). Generation of  $IKK\beta^{lox/lox}$   
87 (referred to as  $IKK\beta^{+/+}$ ) mice (Li, Omori, Labuda, Karin, & Rickert, 2003) and Nestin-Cre mice  
88 (Betz, Vosshenrich, Rajewsky, & Muller, 1996) was described previously. Nestin/ $IKK\beta^{lox/lox}$   
89 (referred to as  $IKK\beta^{-/-}$ ) mice were generated following several generations of backcrossing. Mice  
90 were obtained through crossing  $IKK\beta^{-/-}$  mice with  $IKK\beta^{+/+}$  mice. The experiments were carried out  
91 on 2-6 months old mice old, both male and female mice, with the genotypes of  $IKK\beta^{-/-}$  mice and  
92 their  $IKK\beta^{+/+}$  WT littermates. Genotyping for  $IKK\beta^{+/+}$  was performed using the following primer  
93 sequence 5'-GTC ATT TCC ACA GCC CTG TGA-3' and 5'-CCT TGT CCT ATA GAA GCA  
94 CAA C-3', as described previously (Chen et al., 2003). The animals were kept at 12 hours night/day  
95 cycle with free access to a standard chow diet and water.

96

## 97 **Adeno-associated viral vectors**

98 To investigate the effect of the IKK pathway on the development of the metabolic phenotype, we  
99 performed stereotactic injections of recombinant adeno-associated viral (rAAV) vectors into the  
100 hypothalamus of  $IKK\beta^{+/+}$  and  $IKK\beta^{-/-}$  mice. The viral vector was a pseudotyped rAAV2/5 vector  
101 (transgene was flanked by two inverted terminal repeats of the AAV2 and packaged in an AAV5  
102 capsid), where the human mHTT gene of 853 amino acids length (853HTT79Q) (Hult et al., 2011).  
103 The human Synapsin-1 promoter drove the mHTT gene expression.

104

## 105 **Viral vector injections**

106 The animals were anesthetized by air mask inhalation of isoflurane (2% isoflurane in O<sub>2</sub>/N<sub>2</sub>O  
107 (3:7)). The mouse head was fixed with a nose clamp and ear bars in the stereotaxic apparatus.  
108 Following the head position's fine-tuning on the stereotaxic frame, the skull was thinned with a  
109 dental drill to make a borehole at the determined anterior-posterior and medial-lateral  
110 hypothalamic coordinates. Subsequently, the final dorsal-ventral coordinates were measured from  
111 the dura mater. The stereotaxic coordinates chosen for hypothalamic injections were: 0.6 mm  
112 posterior to bregma, -0.6 mm lateral to the bregma, and 5.2 mm ventral to the dura mater, selected  
113 according to the mouse brain atlas (Franklin & Paxinos, 2008).

114 The rAAV vector delivery was performed bilaterally in the hypothalamus. The silica glass  
115 capillary (with an outer diameter of ~ 80 µm) attached to a 5 µl Hamilton syringe (Nevada, USA)  
116 was used for the virus delivery. A total volume of 0.5 µl viral vector solution was pulled in a glass  
117 capillary, and the capillary was descended from dura mater to target coordinates slowly. At the  
118 target, the first 0.1 µl of the total volume was injected. After 30 seconds, the viral suspension was  
119 delivered at a rate of 0.05µl/15s until the whole volume was delivered. To allow the brain to absorb  
120 the viral vector solution, the capillary was left at the target for an additional 5 minutes at the end  
121 of the injection. The viral vector concentration used in the study was 2,1E+14 genome copies  
122 (GC)/ml.

123

## 124 **Perfusion and serum collection**

125 To induce deep anesthesia in mice, a terminal dose of pentobarbital (600 mg/kg, Apoteksbolaget)  
126 was injected intraperitoneally. The thoracic cavity was opened to expose the heart, and blood was  
127 collected from the right ventricle with the 16G needle. Subsequently, a small incision was made  
128 to insert a 12-gauge perfusion needle at the tip of the left ventricle. First, the vessels were rinsed  
129 with the saline solution at a rate of 10-12 ml/min for a minute, and then it was switched to freshly  
130 prepared 4% paraformaldehyde (PFA) ~0°C for 8 minutes. Following that, the animals were  
131 decapitated, and the brains were isolated. The brains were placed in 4% PFA for 24 hours at 4°C  
132 for post-fixation. Next, the PFA was replaced with 25% sucrose solution at 4°C for cryoprotection  
133 (~24 hours).

134 Finally, the fixed brains were sectioned coronally on a semi-automated freezing microtome  
135 (Microm HM 450) at 30 µm thick slices and in six series. Until further processing, the brain  
136 sections were stored in antifreeze solution (30% glycerol and 30% ethylene glycol in phosphate  
137 buffer) at -20 °C.

138

### 139 **Metabolic Measurements**

140 Serum insulin and leptin concentrations were assessed in blood collected from the heart left  
141 ventricle prior to perfusion. The blood was kept at room temperature for 30 minutes to clot and  
142 spun for 15 minutes at 2500 g. The serum (supernatant) was stored at -80 C. Serum levels of insulin  
143 (Crystal Chem Inc, Catalog #90080) and leptin (Crystal Chem Inc, Catalog #90030) were assessed  
144 using ELISA according to the manufacturer's instructions.

145

## 146 **Immunohistochemistry**

147 The free-floating brain sections were used for immunohistochemistry (IHC). All the washing steps  
148 (10 minutes/wash) and incubations were performed using gentle agitation on a shaker at room  
149 temperature. The sections were washed three times in Tris-buffered-saline (TBS) in 1% Triton X  
150 (TBS-T) to remove the antifreeze solution. The endogenous peroxidase activity was blocked by  
151 30 minutes of incubation in 10% methanol with 3% H<sub>2</sub>O<sub>2</sub> in TBS. Following that, the sections  
152 were washed three times for 10 minutes in TBS-T. The sections were incubated with 5% serum  
153 and bovine serum albumin (BSA) in TBS-T for 1 hour to reduce nonspecific binding of the primary  
154 and secondary antibodies. Next, the sections were left in the respective primary antibody solutions  
155 in 3% appropriate corresponding serum in TBS-T (anti-huntingtin (sc-8767; 1:500; goat; Santa  
156 Cruz), anti-ubiquitin (1:2000; rabbit; Dako), anti-orexin (1:4000; rabbit; Phoenix  
157 Pharmaceuticals), anti-tyrosine hydroxylase (1:2000; rabbit; Pel-Freez), anti-GnRH (1:3000, anti-  
158 rabbit, Abcam #ab5617), anti-iba-1) and left on shaker for overnight incubation at room  
159 temperature. Next, sections were washed three times for 10 minutes in TBS-T. The secondary  
160 antibody incubation was performed in 3% respective serum or BSA with TBS-T for 1 hour at room  
161 temperature, and the sections were washed three times for 10 minutes in TBS before 3,3'-  
162 diaminobenzidine (DAB) development (Vectastain, ABC kit). Brain sections were mounted on  
163 chromatin-gelatin coated glass slides. The air-dried sections were left in distilled water for 1  
164 minute and dehydrated in increasing ethanol solutions (70%, 95%, 99%). Finally, the samples were  
165 cleared in xylene and covered with glass coverslips using DPX mounting medium (Sigma-  
166 Aldrich).

167

## 168 **Stereological analyses**

169 To estimate the numbers of cells positive for orexin, TH, GnRH (in the anterior hypothalamus;  
170 AHA), and size of the inclusions, we applied unbiased stereological quantification principles by  
171 using the optical dissector method (West, Slomianka, & Gundersen, 1991). Stereological analyses  
172 were performed with a Nikon 80i microscope, which is equipped with an X–Y motorized stage  
173 (Märzhauser, Wetzlar) and a high precision linear encoder (Heidenhain, Traunreut). The position  
174 of the stage and the input from the digital camera were controlled by a computer. The sampling  
175 interval was adjusted to count at least 100 cells for each hypothalamus to minimize the coefficient  
176 of error. The region of interest was delineated under the 4X objective, whereas the counting was  
177 performed using a 60X NA 1.4 Plan-Apo oil objective with a random start systematic sampling  
178 routine (NewCast Module in VIS software; Visiopharm A/S, Horsholm). The border delineation  
179 processes for orexin, TH, GnRH cell populations were defined by the natural contours of cell  
180 populations. The number and size of iba-1 positive microglia/macrophages were quantified in the  
181 anatomical borders of the mediobasal hypothalamus. The number of small, medium and large size  
182 HTT inclusions was quantified with HTT staining (sc-6787). The inclusions ~ 0.04 - 0.1  $\mu\text{m}$  size  
183 considered small, 0.15 - 0.25  $\mu\text{m}$  medium and ~ 0.25-0.5  $\mu\text{m}$  large size inclusions. The same size  
184 of the area was quantified for all genotypes and gender under blinded conditions. The mean number  
185 of assessed inclusions per brain was 755, median 663 with a standard deviation of error 528  
186 inclusions.

187

## 188 **Metabolic tests**

189 For all animals used in the study, body weight was measured bimonthly. Serum insulin and leptin  
190 concentrations were assessed in serum. Blood was collected from the heart left ventricle at 18  
191 weeks post-injection, and they were kept at room temperature for 30 minutes to clot, spun for 15  
192 minutes at 2500 g. Serum (supernatant) was aliquoted and stored at -80 C. Serum levels of leptin  
193 (Crystal Chem Inc, Catalog #90030) and testosterone (Demeditec, Cat.-No.: DEV9911) were  
194 determined with ELISA according to the manufacturer's instructions.

195

## 196 **Statistical analysis**

197 All statistical analyses were performed using Prism 8 software (GraphPad). The data was initially  
198 tested with D'Agostino & Pearson omnibus normality test for normal distribution. Following that,  
199 the data was either subjected to Kruskal–Wallis followed by Dunn's multiple comparison tests or  
200 an unpaired t-test with equal SD. The statistical test results and the type of analysis used for each  
201 experiment are specified in detail in the results section and figure legends. Statistically significant  
202 differences were considered for  $p < 0.05$ .

203

## 204 **Results**

### 205 **Inhibition of the IKK $\beta$ pathway protects from hypothalamic mHTT-induced obesity in** 206 **female mice**

207 To assess the effects of mHTT expression on the development of a metabolic phenotype, we  
208 injected IKK $\beta^{+/+}$  (homozygous for the floxed allele of IKK $\beta$ , control group) and IKK $\beta^{-/-}$

209 (expressing Cre-recombinase under nestin promotor) mice with rAAV vectors expressing mHTT  
210 (AAV5-853HTT79Q vectors are referred to as HD elsewhere) in the hypothalamus of both sexes.  
211 Consistent with our previous findings (Baldo, Soyulu, & Petersen, 2013; Hult et al., 2011; Soyulu-  
212 Kucharz et al., 2015), expression of mHTT in female control mice led to an obese phenotype  
213 (**Figure 1A, C**). The body weight at 18 weeks post-injection was significantly higher in the  
214  $\text{IKK}\beta^{+/+}\text{HD}$  mice (n=16 and mean= 55.6g, SD=10.2) compared to  $\text{IKK}\beta^{-/-}\text{HD}$  mice (n=20 and  
215 mean=40.6g, SD=9.3,  $p<0.0001$ ) and uninjected mice of the two genotypes (uninjected  $\text{IKK}\beta^{+/+}$ :  
216 n=15 and mean=43.7g, SD=7.4,  $p=0.0026$ ; uninjected  $\text{IKK}\beta^{-/-}$ : n=13 and mean=37.1g, SD=9.8,  
217  $p<0.0001$ ). Hence, inactivation of the  $\text{IKK}\beta$  pathway in Nestin-expressing cells protected female  
218 mice from hypothalamic mHTT induced obesity (**Figure 1A**). The brain-specific deletion of  $\text{IKK}\beta$   
219 did not affect the circulating insulin and leptin levels in mice (Meng & Cai, 2011). Therefore, in  
220 this study, we assessed the serum levels of insulin and leptin levels only in mice expressing mHTT  
221 in the hypothalamus. In line with the prevention of body weight gain,  $\text{IKK}\beta^{-/-}\text{HD}$  mice displayed  
222 significantly lower serum levels of insulin and leptin than  $\text{IKK}\beta^{+/+}\text{HD}$  mice (**Figure 1D**).

223  $\text{IKK}\beta$  did not affect the body weight in male mice with or without injections of AAV5-  
224 853HTT79Q as assessed up to 18 weeks post-injection (**Figure 1B, F**). However, analyses of  
225 serum levels of insulin and leptin showed that even though there was no effect on body weight,  
226 levels of insulin and leptin were significantly elevated in  $\text{IKK}\beta^{-/-}\text{HD}$  mice compared to  $\text{IKK}\beta^{+/+}\text{HD}$   
227 mice (**Figure 1G and 1H**).

228

229  **$\text{IKK}\beta$  is not involved in the mHTT-mediated loss of orexin and TH positive cell populations**  
230 **in HD mice**

231 The development of the metabolic phenotype in HD has been associated with expression of mHTT  
232 in the hypothalamus (Hult et al., 2011; Soylyu-Kucharz et al., 2015). Here we tested whether the  
233 rescue of the metabolic phenotype observed in  $\text{IKK}\beta^{-/-}$ HD mice was due to the preservation of  
234 metabolism-regulating neuronal populations known to be affected in HD (Cheong et al., 2019). In  
235 female mice, there was no benefit of  $\text{IKK}\beta$  silencing as the number of orexin positive cells was  
236 comparable in  $\text{IKK}\beta^{+/+}$ HD (cell loss ~64%) and  $\text{IKK}\beta^{-/-}$ HD groups (cell loss ~82%), and both  
237 groups had a significantly lower number of orexin cells compared to uninjected groups of female  
238 mice (**Figure 2A and 2B**). Male  $\text{IKK}\beta^{-/-}$ HD mice had significantly lower orexin and A13 TH  
239 positive cells than  $\text{IKK}\beta^{+/+}$ HD mice in the hypothalamus (**Supplementary Figure 1A and 1B**).

240 The loss of A13 TH positive cells was ~80% in  $\text{IKK}\beta^{-/-}$ HD and ~65% in  $\text{IKK}\beta^{+/+}$ HD when  
241 compared to WT and  $\text{IKK}\beta^{-/-}$  uninjected female mice (**Figure 2C and 2D**).

242 Reduced testosterone levels are associated with increased circulating levels of leptin and  
243 insulin, even in the absence of increased BMI (Luukkaa et al., 1998; Pitteloud et al., 2005). The  
244 hypothalamic-pituitary-gonadal (HPG) axis, which regulates testosterone production, is altered in  
245 HD (Bird, Chiappa, & Fink, 1976; Kalliolia et al., 2015; Markianos, Panas, Kalfakis, &  
246 Vassilopoulos, 2005; Papalexi et al., 2005; Petersen & Bjorkqvist, 2006; Saleh et al., 2009; Soylyu-  
247 Kucharz, Baldo, & Petersen, 2016; Van Raamsdonk et al., 2007). The number of GnRH positive  
248 cells was comparable (**Figure 2F and Supplementary Figure 1C**); however, the total circulating  
249 level of testosterone was diminished in  $\text{IKK}\beta^{-/-}$ HD male mice by 60% compared to  $\text{IKK}\beta^{+/+}$ HD  
250 male mice expressing mHTT in the hypothalamus (**Supplementary Figure 1D**).

251

252 **The number or size of Iba-1 positive microglial cells in the mediobasal hypothalamus.**



253 Given that IKK $\beta$  is one of the mediators of microglial activation and energy balance (Karin, 1999;  
254 Liu, Zhang, Joo, & Sun, 2017), we investigated whether protection from hypothalamic mHTT  
255 induced obesity in IKK $\beta^{-/-}$  female mice was due to alteration in microglial activation. Nonetheless,  
256 the number and size of Iba1 positive cells in the mediobasal hypothalamus of both IKK $\beta^{+/+}$ HD and  
257 IKK $\beta^{-/-}$ HD female mice were comparable (**Figure 3A-C**).

### 258 **IKK $\beta^{-/-}$ HD female mice display an increased number of small-sized inclusions of mHTT**

259 Reduction of IKK $\beta$  activity decreases the cleavage of both WT and mHTT and prevents the  
260 accumulation of mHTT inclusions (Khoshnan, Ko, Tescu, Brundin, & Patterson, 2009; Thompson  
261 et al., 2009). IKK $\beta$  silencing studies also showed impaired clearance of mHTT and worsening HD  
262 pathological phenotypes in vivo and vitro (Khoshnan et al., 2009; Thompson et al., 2009). In our  
263 model, inclusions were increased in IKK $\beta^{-/-}$ HD compared to IKK $\beta^{+/+}$ HD in both female and male  
264 mice (**Figure 4A-C**). The small size inclusions were responsible for the increase, as the number  
265 of medium or large size inclusions were similar between IKK $\beta^{+/+}$ HD and IKK $\beta^{-/-}$ HD groups  
266 (**Figure 4D-F**). Altogether, these results show the increase in inclusion formation correlates with  
267 previous reports on IKK $\beta$  silencing in HD (Criollo et al., 2010; Khoshnan et al., 2009; Ochaba et  
268 al., 2019).

269

## 270 **Discussion**

271 Alterations in energy metabolism may affect disease progression in HD as weight loss is part of  
272 the clinical phenotype, and a higher BMI has been associated with the slower clinical decline (van  
273 der Burg et al., 2017). Understanding the underlying biological cause of metabolic disturbances in

274 HD may unravel novel therapeutic targets for this fatal neurodegenerative disorder. The IKK $\beta$ /NF-  
275  $\kappa$ B pathway has been implicated in HD pathogenesis (Khoshnan & Patterson, 2011), but it has not  
276 been investigated in the context of HD metabolic and hypothalamic alterations. Here, we expressed  
277 mHTT in the hypothalamus of control mice and compared effects to mice with IKK $\beta$  inactivated  
278 in the CNS. We found that hypothalamic mHTT expression induces obese phenotype selectively  
279 in the female mice and inactivation of the IKK $\beta$  prevents it. Gender differences play a role in HD  
280 as the severity and rate of the motor symptoms progression has been suggested to be faster in  
281 women than men with HD (Zielonka et al., 2013; Zielonka et al., 2018; Zielonka & Stawinska-  
282 Witoszynska, 2020). Previous studies indicated that sex also affects the HD metabolic and  
283 behavioral manifestation in animal models (Dorner, Miller, Barton, Brock, & Rebec, 2007;  
284 Sjogren et al., 2019; Soylu-Kucharz et al., 2016). Even though the expression of mHTT in the  
285 hypothalamus of male mice did not affect body weight, male IKK $\beta$ <sup>-/-</sup>HD displayed high serum  
286 leptin and insulin levels. As testosterone deficiency is associated with metabolic syndrome  
287 exemplified by increased circulating leptin levels and insulin resistance, it is possible that the  
288 increase in serum leptin and insulin levels could be due to reduced circulating testosterone levels  
289 in IKK $\beta$ <sup>-/-</sup>HD mice.

290 Obese phenotype can occur due to increased caloric intake, decreased activity, metabolic  
291 rate, or combination of these factors. Previously, we demonstrated that the obese phenotype caused  
292 by hypothalamic mHTT expression was due to hyperphagia as general motor activity and basal  
293 metabolic rate were unaltered in these mice (Hult et al., 2011). The mice in this study were housed  
294 in a separate animal unit that lacks behavior testing platforms and limiting the number of in-house  
295 cages. Therefore, we were not able to re-test basic parameters such as food intake and locomotor  
296 activity. However, as we have previously shown that obese phenotype was caused by increased

297 food intake, we speculate that the silencing of the IKK $\beta$  expression prohibited hyperphagia-  
298 induced obesity in HD mice. The orexin and A13 TH neuronal populations are involved in  
299 metabolism regulation (Adeghate et al., 2020; Shi et al., 2013) and they are affected in HD (Gabery  
300 et al., 2010; Hult et al., 2011; Petersen et al., 2005; Soylu-Kucharz et al., 2015). As the inactivation  
301 of the IKK $\beta$  signaling did not affect the preservation of these cells, we can speculate that orexin  
302 and A13 TH neuropathology are not the central cell populations responsible for the development  
303 of HD bodyweight alterations.

304 In conclusion, our study shows that hypothalamic expression of mHTT leads to a metabolic  
305 imbalance in a sex-specific fashion. The weight gain phenotype induced by the mHTT in female  
306 mice is prevented by IKK $\beta$  inactivation and is independent of orexin TH neuroprotection, and  
307 microglial activation.

308

### 309 **Author contributions statements**

310 RS, ÅP, and AK conceived and designed the experiments. RSK performed the experiments. RSK  
311 and ÅP analyzed the data. RSK and ÅP wrote the first draft of the manuscript. All authors were  
312 involved in editing the manuscript and approved the final version.

313

### 314 **Conflict of interest**

315 The authors declare no conflict of interest.

316

### 317 **Figure legends**

318 **Figure 1: Inactivation of the IKK $\beta$  pathway inhibits the development of obesity-induced by**  
319 **mHTT expression in the hypothalamus of female mice**

320 IKK $\beta^{+/+}$  and IKK $\beta^{-/-}$  mice were injected bilaterally into the hypothalamus with AAV-HTT853-  
321 79Q vectors and assessed using metabolic analyses. **(A)** Female IKK $\beta^{+/+}$  mice develop increased  
322 body weight after hypothalamic injections of AAV5-HTT853-79Q vectors which is prevented in  
323 IKK $\beta^{-/-}$  female mice (two-way repeated measures ANOVA, effect of time F (2, 62) = 8.920,  
324  $p < 0.0001$ ; effect of genotype F (1, 31) = 20.83,  $p < 0.0001$ ; effect of genotype x time F (2, 62) =  
325 8.920,  $p = 0.0004$ ; followed by a Sidak's multiple comparisons test:  $p = 0.0026$  at 8 weeks and  $p <$   
326  $0.0001$  at 18 weeks). **(B)** Male IKK $\beta^{+/+}$  and IKK $\beta^{-/-}$  mice do not develop obesity after AAV5-  
327 HTT853-79Q vector injections (two-way repeated measures ANOVA, effect of time F (2, 72) =  
328 190.4,  $P < 0.0001$ ; effect of genotype F (1, 36) = 2.359,  $P = 0.1333$ ; effect of genotype x time F  
329 (2, 72) = 1.446,  $P = 0.2422$ , followed by a Sidak's multiple comparisons test). **(C)** Body weight  
330 changes at 18 weeks post-injection in females (One-way ANOVA, effect of treatment F (3, 60) =  
331 11.69,  $P < 0.0001$  followed by Sidak's multiple comparisons test). Serum **(D)** insulin (two-tailed,  
332 unpaired t-test,  $n = 16/19$ ,  $p = 0.0082$ ) and **(E)** leptin (two-tailed, Mann Whitney test,  $n = 16/19$ ,  
333  $p = 0.0003$ ) concentrations measured by ELISA in females at 18 weeks post injection. **(F)** Body  
334 weight changes at 18 weeks post-injection in males (One-way ANOVA, effect of treatment F (3,  
335 55) = 0.7312,  $P = 0.5378$  followed by Sidak's multiple comparisons test). Serum **(G)** insulin (two-  
336 tailed, Mann Whitney test,  $n = 15/21$ ,  $p = 0.0448$ ) and **(H)** leptin (two-tailed, unpaired t-test,  
337  $n = 15/21$ ,  $p = 0.0023$ ) assessments at 18 weeks. Data are represented as box and whisker plots (25–  
338 75 percentile (boxes), min to max (whiskers), median (horizontal line), mean (+)).

339

340 **Figure 2: Quantitative analysis of the neuronal populations expressing orexin, TH in the A13**  
341 **area, and GnRH in female mice at 18-week post-injection of AAV-HTT853-79Q vectors. (A)**  
342 **Representative** immunohistochemically stained sections show orexin immunopositive cells in the  
343 hypothalamus. **(B)** Stereological analysis of orexin immunopositive cells in female mice at the 18  
344 weeks time point (Kruskal-Wallis test followed by Dunn's multiple comparisons test  $p=0.0002$ ;  
345  $n=4-8$ /group). **(C)** Representative photomicrographs show the A13 TH immunopositive cell  
346 population in the hypothalamus. **(D)** Numbers of A13 TH positive cells in the hypothalamus's zona  
347 incerta area (Kruskal-Wallis test followed by Dunn's multiple comparisons test  $p=0.0001$ ;  $n=4-$   
348  $9$ /group). **(E)** Representative photomicrographs illustrate the GnRH positive cells in the anterior  
349 hypothalamic area of the hypothalamus. **(F)** Stereological quantification of GnRH positive cells in  
350 the anterior hypothalamic area (Kruskal-Wallis test followed by Dunn's multiple comparisons  
351  $p=0.0802$ ;  $n=4-8$ /group). Points on scatter graphs represent total cell count for individual mice, the  
352 lines are means, and the whiskers indicate  $\pm$  SEM. Scale bars represent 200  $\mu\text{m}$ .

353

354 **Figure 3: No effect of the IKK $\beta$  pathway on the degree of iba-1 positive cell activation at 18**  
355 **weeks post-injection in females.** Stereological assessment of **(A)** the total number of iba-1  
356 positive cells (two-tailed, unpaired t-test,  $n=7/8$ ,  $p=0.477$ ) and **(B)** the size of Iba-1 positive cells  
357 (two-tailed, Mann-Whitney test,  $n=7/8$  animals,  $n=783/911$  cells/genotype,  $p=0.9601$ ) in the  
358 mediobasal hypothalamus (MBH) 18 weeks after injections of AAV5-HTT853-79Q vectors in  
359 IKK $\beta^{+/+}$  and IKK $\beta^{-/-}$  mice. In (A), data are represented as scatter dot plots, and bars represent mean  
360  $\pm$  SEM, and in (B), data are represented as scatter dot plots, and lines represent median.

361

362 **Figure 4: Inactivation of the IKK $\beta$  pathway leads to increased numbers of huntingtin**  
363 **inclusions in the hypothalamus.** Representative photomicrographs of sections processed for  
364 immunohistochemistry for **(A)** huntingtin (using the sc-8767 antibody) and **(B)** ubiquitin  
365 demonstrating the formation of inclusions in the hypothalamus after injections of AAV-HTT853-  
366 79Q vectors in IKK $\beta^{+/+}$  and IKK $\beta^{-/-}$  mice. Stereological quantification of sections processed with  
367 the huntingtin antibody show **(C)** the total number of inclusions (Females: two-tailed, Mann-  
368 Whitney test, n=5/group, p=0.0008; Males: two-tailed, Mann-Whitney test, n=5/group, p<0.0001),  
369 **(D)** large-sized inclusions (Females: two-tailed, Mann-Whitney test, n=5/group, p=0.3095; Males:  
370 two-tailed, unpaired t-test, n=5/group, p=0.8992), **(E)** medium-sized inclusions (Females: two-  
371 tailed, unpaired t-test, n=5/group, p=0.0635); Males: two-tailed, unpaired t-test, n=5/group,  
372 p=0.0911) and **(F)** small-sized inclusions (Females: two-tailed, Mann-Whitney test, n=5/group,  
373 p=0.0079; Males: two-tailed, unpaired t-test, n=5/group, p<0.0001) inclusions in female and male  
374 mice at 18 weeks post-injection. Points on scatter graphs represent total inclusion count for  
375 individual mice, the lines are means, and the whiskers indicate  $\pm$  SEM. Scale bars represent 200  
376  $\mu$ m and 25  $\mu$ m on lower and higher magnifications, respectively.

377

378 **Supplementary figure 1: Effects on the orexin, GnRH, and A13 TH cell populations in the**  
379 **hypothalamus in male mice after injections of AAV-HTT853-79Q vectors.** Stereological  
380 quantification of **(A)** orexin (two-tailed, unpaired t-test, n=8/group, p=0.001), **(B)** TH (two-tailed,  
381 unpaired t-test, n=8/group, p=0.0471) and **(C)** GnRH (two-tailed, unpaired t-test, n=7/group,  
382 p=0.3648) positive cells in IKK $\beta^{+/+}$  and IKK $\beta^{-/-}$  males expressing mHTT in the hypothalamus at  
383 18 weeks post-injection. **(D)** Changes in serum testosterone levels in male IKK $\beta^{+/+}$  and IKK $\beta^{-/-}$   
384 mice at 18 weeks post-injection of AAV-853HTT-79Q in the hypothalamus (two-tailed, Mann-

385 Whitney test,  $n=8/14$ ,  $p=0.0159$ ). Data are represented as scatter dot plots, the lines are means, and  
386 the whiskers indicate  $\pm$  SEM.

387

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Figure 1

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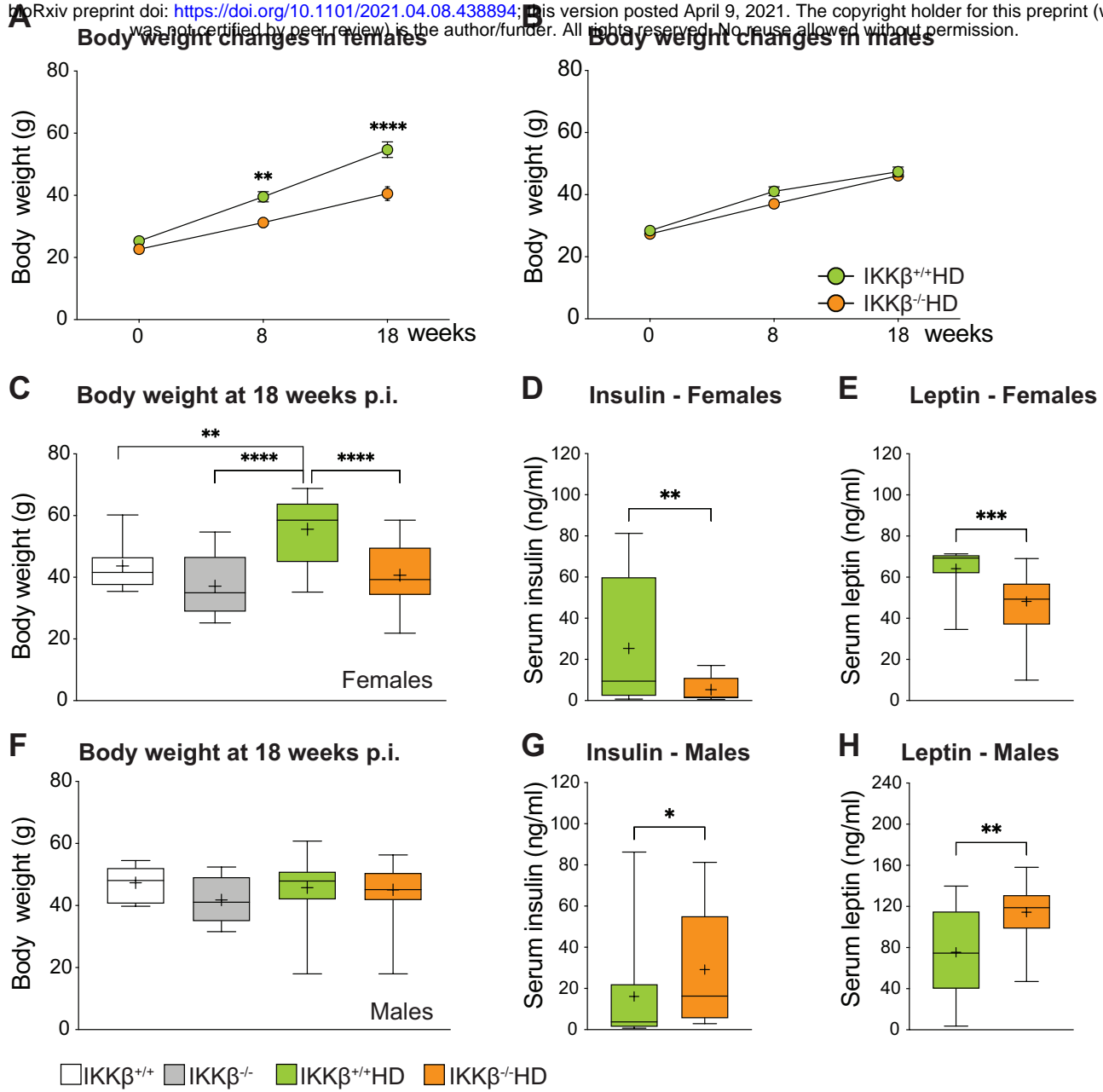
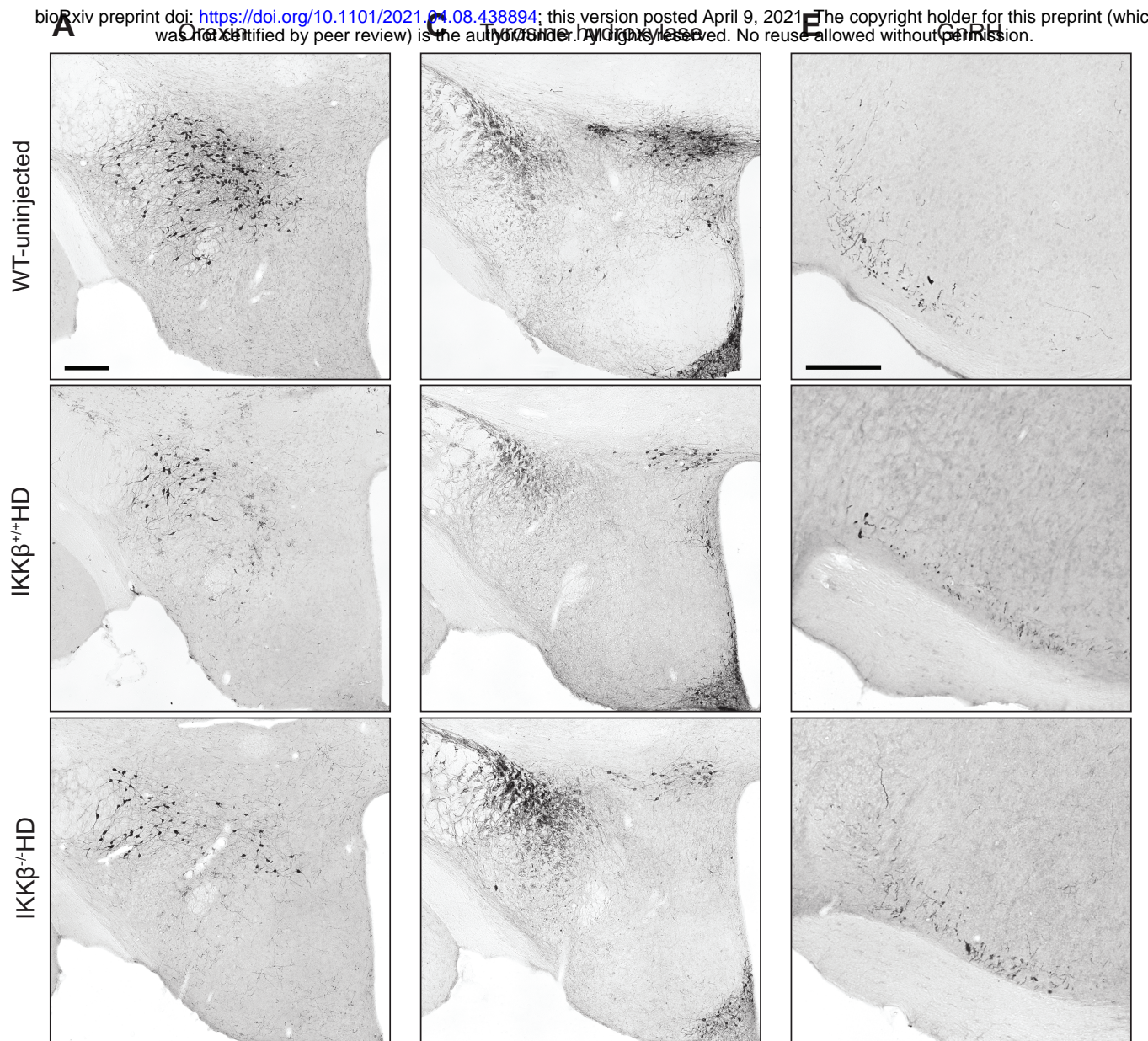


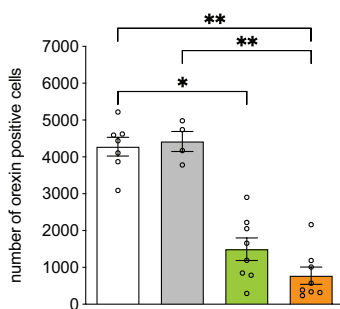


Figure 2

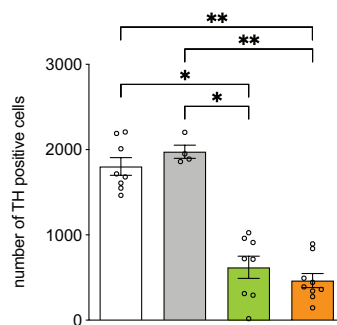
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**B** Orexin+ cells in females



**D** A13 TH+ cells in females



**F** GnRH+ cells in females

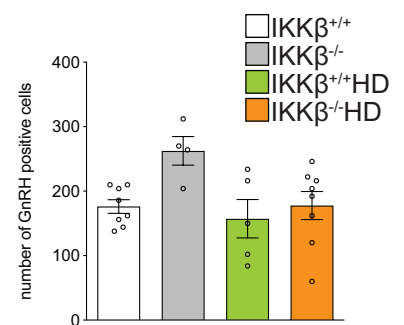


Figure 3

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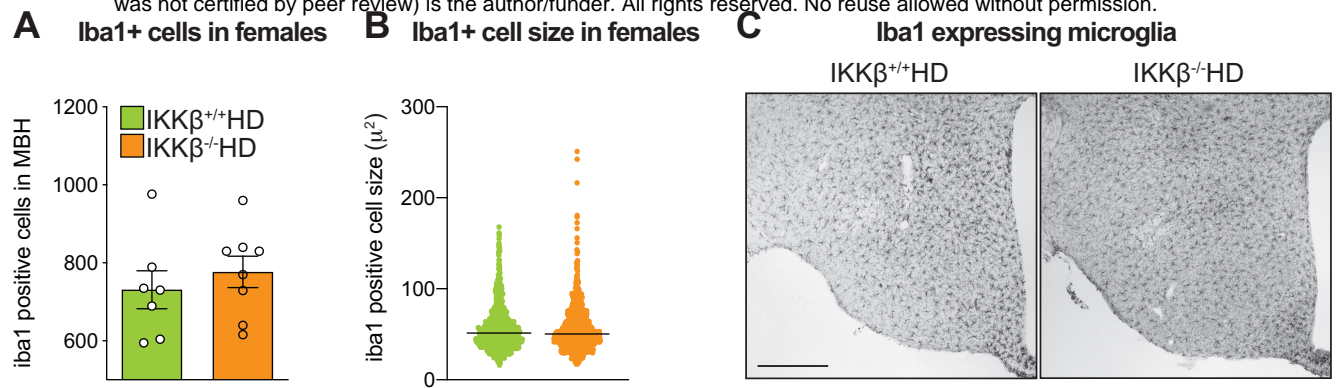
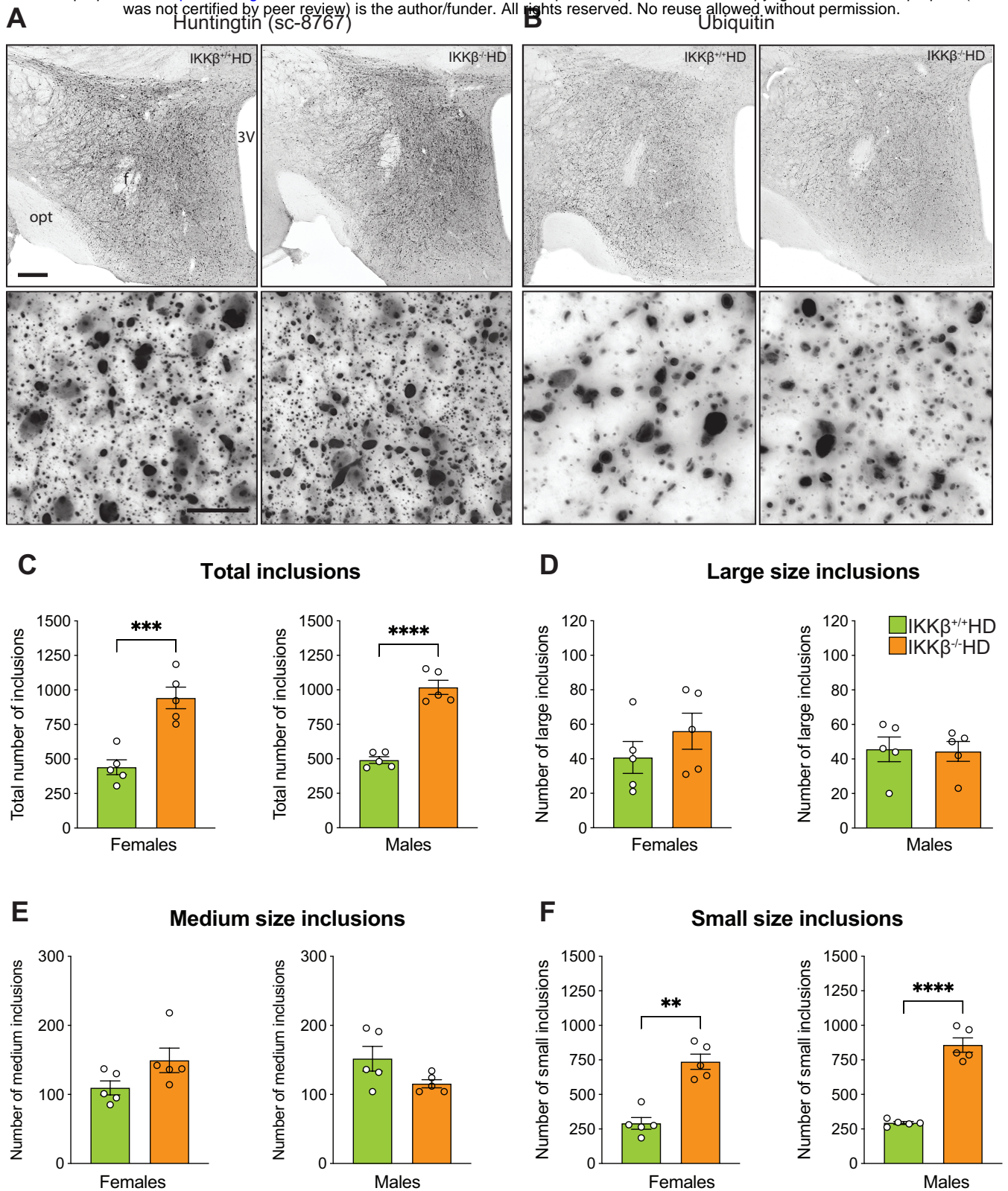




Figure 4

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## Supplementary figure 1

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