

1 **Enhanced Ghrelin Levels and Hypothalamic Orexigenic AgRP and NPY Neuropeptide**
2 **Expression in Models of Jejuno-Colonic Short Bowel Syndrome**

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36 **Abstract**

37 Short bowel syndrome (SBS) patients developing hyperphagia have a better outcome.
38 Gastrointestinal endocrine adaptations help to improve intestinal functions and food
39 behaviour. We investigated neuroendocrine adaptations in SBS patients and rat models with
40 jejunio-ileal (IR-JI) or jejunio-colonic (IR-JC) anastomosis with and without parenteral
41 nutrition. Circulating levels of ghrelin, PYY, GLP-1, and GLP-2 were determined in SBS rat
42 models and patients. Levels of mRNA for proglucagon, PYY and for hypothalamic
43 neuropeptides were quantified by qRT-PCR in SBS rat models. Histology and
44 immunostaining for Ki67, GLP-1 and PYY were performed in SBS rats. IR-JC rats, but not
45 IR-JI, exhibited significantly higher crypt depths and number of Ki67-positive cells than
46 sham. Fasting and/or postprandial plasma ghrelin and PYY concentrations were higher, or
47 tend to be higher, in IR-JC rats and SBS-JC patients than in controls. *Proglucagon* and *Pyy*
48 mRNA levels were significantly enhanced in IR-JC rats. Levels of mRNA coding
49 hypothalamic orexigenic NPY and AgRP peptides were significantly higher in IR-JC than in
50 sham rats. We demonstrate an increase of plasma ghrelin concentrations, major changes in
51 hypothalamic neuropeptides levels and greater induction of PYY in SBS-JC rats and patients
52 suggesting that jejunio-colonic continuity creates a peculiar environment promoting further
53 gut-brain adaptations.

54

55 INTRODUCTION

56 Short bowel syndrome (SBS) results from extensive resection of the small intestine. It is the
57 leading cause of chronic intestinal failure and is treated principally by parenteral nutrition
58 (PN). The degree of malabsorption depends on both the extent of the resection and the
59 anatomy of the remnant bowel. Adaptive phenomena occur during the two to three years
60 following the surgery and are characterized by improvements in intestinal absorption^{1,2},
61 increase of hormonal secretion^{3,4}, development of an hyperphagia^{2,5} and dysbiosis of the gut
62 microbiota^{6,7,8}. These changes, which are highly variable and unique to each patient, may
63 facilitate weaning off of PN. Oral intake plays a major role in these adaptations⁹ as does the
64 presence of the terminal ileum and/or colon.

65 Gut hormones are key factors in this spontaneous intestinal adaptation¹⁰. Increased secretions
66 of glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2) have been reported
67 in preclinical models^{11,12} and in SBS patients with the colon in continuity³, but not in SBS
68 patients with jejunostomy (i.e. with no colon and no ileum)^{4,13}. These hormones are secreted,
69 together with Peptide YY (PYY), by the enteroendocrine L cells of the ileum and colon, in
70 response to nutrient stimulation¹⁴. They play key roles in orchestrating gastrointestinal
71 functions, such as intestinal trophicity¹², expression of intestinal nutrient transporter^{10,15,16} and
72 gastro-intestinal motility^{4,17}. The role of GLP-2 in these intestinal functions has been largely
73 documented¹⁸ in SBS patients and its therapeutic use (in the recombinant form of
74 Teduglutide) has now been approved in adults with SBS dependent on PN. At last, PYY and
75 GLP-1 are considered to be molecular mediators of the ileal brake¹⁹. They inhibit gastric
76 emptying and intestinal transit, thereby increasing the time for the nutrients to be absorbed by
77 the intestinal mucosa.

78 The development of hyperphagia, which increases over time in SBS patients after
79 reestablishment of colonic continuity², makes it possible to decrease dependence on PN
80 by increasing net nutrient absorption². It is associated with a good prognosis for the

81 weaning off of PN^{2,20}. The molecular and physiological mechanisms underlying the
82 development of this compensatory overeating in SBS patients remain unknown. In
83 addition to their gastro-intestinal effects, gut hormones (such as CCK, PYY, GLP-1 and
84 ghrelin) participate in the regulation of appetite and food intake¹⁴. Ghrelin, is the unique
85 orexigenic gut hormone^{21,22}. Plasma ghrelin levels increase before meal, stimulating
86 hunger and meal initiation, and then decrease after the meal²¹. These peripheral
87 gastrointestinal signals are integrated into the hypothalamus, which contains two
88 populations of neurons: orexigenic neurons co-expressing neuropeptide Y (NPY) and
89 agouti-related peptide (AgRP) and anorexigenic neurons co-expressing pro-
90 opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript
91 (CART)^{14,23} Ghrelin increases food intake through the activation of hypothalamic
92 orexigenic neurons²⁴. Little, if anything, is known about the expression of these
93 neuropeptides in preclinical models of intestinal resection. Only one study has reported data
94 for the fasting concentrations of ghrelin and PYY in SBS patients with adaptive hyperphagia
95 but their postprandial levels were not evaluated²⁵. Finally, the measure of circulating gut
96 hormones is recognized of great interest for the follow-up of patients with gastrointestinal
97 pathology and/or surgery. However their baseline and postprandial concentrations are rarely
98 evaluated in combined panels in SBS patients with or without ileum nor compared with
99 control subjects and little is known about what the colon or the ileum specifically brings to
100 intestinal endocrine adaptation in SBS.

101 To address the colonic cellular and molecular mechanisms of adaptive hormonal
102 secretions, we set up two rat models of extensive intestinal resection with colon in continuity
103 and with or without a resected ileum. We use these models to analyze food intake,
104 hypothalamic neuropeptides expression and to evaluate gastro-intestinal production and
105 secretion of gut hormones (ghrelin, PYY, GLP-1 and GLP-2) in comparison with sham-

106 operated animals. We further analyzed the clinical relevance of hormonal adaptation in
107 relation to food intake (fasting and post-prandial gut hormone secretion) in jejuno-colonic and
108 jejuno-ileal SBS patients.

109

110 **RESULTS**

111 **Preclinical models**

112 *Characteristics of rat models:*

113 All rats were on oral nutrition with free access to food, but a group of IR-JC rats received also
114 PN to partially compensate for their malabsorption. Seven days after surgery, sham rats had
115 gained 6% of their pre-operative weight, whereas IR jejuno-ileal (IR-JI) and jejuno-colonic
116 (IR-JC) rats had lost 14% and 29% of their pre-operative weight, respectively (Figure 1B). IR
117 jejuno-colonic rats under enteral and parenteral nutrition (IR-JC PN) had lost 17% of their
118 pre-operative weight without increasing their food intake suggesting that PN (12 kcal/day)
119 was beneficial for the nutritional status of the IR-JC rats. Total calorie intake (oral intake and
120 parenteral nutrition) (Figure 1C) increased in all groups during the experiment although it
121 remained lower in IR-JC rats, in particular IR-JC rats. Consistent with their weight loss,
122 plasma leptin and albumin levels were significantly lower in IR-JC and IR-JI groups than in
123 the sham group ($P<0.05$) (Figure 1D and 1E), and tended to be lower for leptin in IR-JC PN
124 group.

125 Histological examination of the colon mucosa (Figure 2A) showed that IR-JC rats had
126 significantly deeper colonic crypts compared to sham rats ($P<0.001$ IR JC vs sham and
127 $P<0.05$ IR JC-PN vs sham) (Figure 2B), reflecting morphological adaptation. In addition they
128 displayed higher number of Ki67-positive cells per crypt (Figure 2A and C) ($P<0.05$) than
129 sham rats. On the contrary IR-JI rats were not significantly different from sham rats. The
130 proliferative index was not significantly different between groups suggesting that the
131 epithelial homeostasis was recovered at that post-operative time (Figure 2D)

132 PAS mucin staining and Muc2 immunostaining of the residual colon mucosa of IR-JC rat,
133 revealed an increase of mucus production compared to sham rat (Figure 2E and F), as
134 previously described for other preclinical models of large intestinal resection²⁶. This greater
135 number of goblet cells is probably a consequence of the increase of the crypt depth observed
136 in IR-JC rats. The density of these cells was not modified in IR-JC compared to sham (figure
137 2F).

138 ***Higher plasma concentrations of gut hormones in rat models of SBS:*** Fasting plasma
139 ghrelin concentration was significantly higher in IR-JC rats with and without PN than in sham
140 rats ($P<0.01$) (Figure 3A), whereas no such difference was observed in IR-JI rats. Fasting
141 plasma GLP-1 and GLP-2 concentrations (Figure 3B and C) were also higher in IR-JC rats
142 with and without PN ($P<0.05$ and <0.01 , respectively versus sham, for GLP-2). Fasting
143 plasma PYY concentration, which was barely detectable in sham rats, was increased in IR-JI
144 rats and was further enhanced in IR-JC rats ($P<0.05$) (Figure 3D). Unfortunately we were not
145 able to determine plasma PYY in IR-JC PN rats and plasma GLP-1 and GLP-2 in IR-JI rats
146 due to technical problems. However the higher plasma GLP-1, GLP-2 and PYY levels
147 observed in overnight fasted rats were also found in non-fasted IR-JC rats ($P<0.01$ or $P<0.05$
148 vs sham) (Supplemental Figure S1).

149 ***No change in the densities of PYY and GLP-1 positive cells but higher levels of mRNA***
150 ***coding for proglucagon and PYY:*** We investigated whether the elevation of these hormone
151 levels was due to an increase in the number of enteroendocrine cells producing them and/or
152 an increase in the hormone production by these cells. The density of GLP-1 positive cells was
153 similar in the 3 groups of IR rats and in sham rats (Figure 4A and C). The density of PYY-
154 positive cells was unchanged in IR-JC rats without PN, and slightly lower in the colon
155 mucosa of IR-JC rats with PN (Figure 4B and D). Nevertheless, since the crypt depth is
156 enhanced by 50% (Figure 2A and B), this means that the total number of these cells will be
157 increased similarly. The levels of mRNA coding for Gcg and PYY were 4 and 3-fold higher,

158 respectively, in the colon mucosa of IR-JC rats than sham rats ($P<0.001$) (Figure 4E and F).
159 IR-JC rats with PN had mRNA levels coding for Gcg and PYY similar to those observed in
160 sham and IR-JI rats, but lower than those observed in IR-JC rats ($P<0.05$).

161 ***Higher levels of mRNA coding for hypothalamic orexigenic neuropeptides (AgRP, NPY)***
162 ***and lower levels of mRNA coding for anorexigenic neuropeptides (POMC, CART) in rat***

163 ***models of SBS:*** We investigated changes in the levels of hypothalamic neuropeptides after
164 intestinal resection by analyzing mRNA levels for orexigenic (AgRP and NPY) and
165 anorexigenic (CART and POMC) neuropeptides within the hypothalamus (Figure 5).

166 IR-JC rats had significantly higher mRNA levels of AgRP and NPY than the sham rats
167 ($P<0.001$) (Figure 5A and B). These levels were also significantly higher in IR-JC rats with
168 PN than in sham rats ($P<0.05$). Actually, mRNA levels of hypothalamic neuropeptides were
169 not statistically different in IR-JC rats with PN or without PN.

170 Conversely, IR-JC rats (with or without PN) had significantly lower mRNA levels of POMC
171 than the animals of the sham group ($P<0.001$ and $P<0.05$ respectively) (Figure 5C and D).

172 The decrease of mRNA levels coding CART was more subtle ($P<0.05$). These results show
173 that jejunocolonic continuity induces substantial changes in the expression of hypothalamic
174 neuropeptides controlling food intake and PN only slightly compensates this effect.

175 **Clinical study**

176 ***Characteristics of SBS patients and control subjects:*** We studied SBS patients (clinical
177 characteristics and median are in Table 1) with jejunocolonic (SBS-JI, $N=5$) or jejunocolonic
178 (SBS-JC, $N=4$) anastomosis. All SBS patients were considered to be in a steady state and had
179 a remnant small bowel of less than 80 cm in length. All had normal albumin and prealbumin
180 levels. Their concentration of plasma leptin was normal or low (Table 1). The average of total
181 oral intake was 2271 (± 288) kcal/day equivalent to 1.8 (± 0.73) times their resting energy
182 expenditure (REE). Six SBS patients were considered to have a high level of oral intake or
183 hyperphagia², i.e. with oral intake >1.5 times their REE (Table 1). Six of the SBS patients

184 were dependent on PN (average of 1181 ± 787 kcal/perfusion). Patient's gut hormone
185 concentrations were compared with those of healthy subjects (C, $N=5$) that were disease-free
186 with normal eating behaviour.

187 ***Higher plasma concentrations of active ghrelin, GLP-1, GLP2 and PYY in SBS patients:***

188 As expected, active ghrelin levels decreased 30 minutes after eating the calibrated meal in
189 control subjects, remaining low for at least 90 minutes (Supplemental Figure S2A). Fasting
190 active ghrelin levels tended to be higher in SBS patients than in healthy controls
191 (Supplemental Figure S2A), were significantly higher at 30 minutes in SBS patients ($P<0.05$
192 vs controls) and decreased only at 90 minutes. The calculated total area under the curve
193 (AUC), reflecting the absolute levels of ghrelin and the post-prandial response, was
194 significantly greater (by a factor of two, $P<0.05$ vs controls) in SBS patients (Supplemental
195 Figure S2B). Distinguishing jejuno-colonic from jejuno-ileal SBS patients revealed that
196 fasting active ghrelin elevation tended to be higher for jejuno-colonic SBS patients than for
197 jejuno-ileal SBS patients and controls (Figure 6A). Jejuno-colonic SBS patients exhibited a
198 delayed meal-induced inhibition of plasma ghrelin concentration whereas no meal-induced
199 inhibition of ghrelin concentration was observed in jejuno-ileal SBS patients (Figure 6A).
200 Fasting plasma GLP-1 (3.286 ± 0.706 for SBS patients vs 1.553 ± 0.063 for controls, $P < 0.05$
201 in Mann-Whitney test) and GLP-2 (3.664 ± 0.716 for SBS patients vs 1.394 ± 0.252 for
202 controls $P < 0.05$ in Mann-Whitney test) concentrations were both higher in SBS patients than
203 in controls subjects (see also Supplemental Figure S2C and E). These increases were similar
204 in jejuno-colonic and jejuno-ileal SBS patients (Figure 6C and E). After the calibrated meal,
205 the average plasma levels of these two hormones remained higher in SBS patients than in
206 control subjects (Figure 6C and E and supplemental Figure S2C and E), but the calculated
207 incremental AUC (iAUC), reflecting the postprandial secretion response, did not differ
208 significantly between patients and controls (Figure 6D and F and Supplemental Figure S2D
209 and F).

210 Fasting plasma PYY concentration was three times higher in SBS patients than in controls
211 ($P<0.01$ in Mann-Whitney tests) (Supplemental Figure S2G). This concentration increased
212 significantly after a calibrated meal remaining higher in SBS patients than in controls.
213 Accordingly, the calculated iAUC was significantly larger than that for controls ($P<0.01$)
214 (Supplemental Figure S2H). The difference in PYY levels (Figure 6G) and PYY iAUC
215 ($P<0.01$) (Figure 6H) with respect to controls was significantly greater in jejuno-colonic SBS
216 patients than in those with jejuno-ileal anastomosis. Consistent with these observations, PYY
217 levels were inversely correlated with the length of the remaining small bowel in SBS patients
218 (Spearman coefficient: -0.76 ; $P<0.05$). However, no correlations were found between
219 circulating PYY, GLP-1 or ghrelin levels and daily oral intake or hyperphagic status in SBS
220 patients.

221

222 **DISCUSSION**

223 This study is the first to demonstrate an increase of plasma ghrelin levels, major
224 changes in the mRNA levels of hypothalamic orexigenic AgRP and NPY neuropeptides, and
225 the elevation of PYY, together with GLP-2 and GLP-1, in rats with large intestinal resection
226 including ileum, that are preclinical models of SBS. We also found that SBS patients had
227 higher fasting and postprandial circulating ghrelin and PYY levels than controls, in particular
228 patients with jejuno-colonic anastomosis, further emphasizing the relevance of our preclinical
229 model.

230 Since morphological and hormonal secretion adaptations have been reported to be maximal in
231 different rat models of resection between day 4 and day 12 after intestinal resection^{11,27,28}, the
232 experimental period for the rat model was set up at 7 days. The colonic morphological
233 adaptation observed in our IR-JC model are in agreement with previous reports for patient
234 with jejuno-colonic anastomosis, i.e increase of crypt depth and of proliferating cell number
235 with a controlled hyperplasia²⁹ and increased GLP-2 secretions³.

236 Ghrelin is a well-known physiological hunger signal^{30,31} playing a major role in meal
237 initiation²¹. Ghrelin-induced hunger involves the activation of central orexigenic pathways³²
238 and stimulation of the expression of AgRP and NPY within the hypothalamus^{24,33,34}. Higher
239 levels of fasting ghrelin and of mRNA coding for orexigenic neuropeptides (AgRP and NPY)
240 suggests a role for ghrelin in the activation of central orexigenic signals in IR jejuno-colonic
241 rats. The lower levels of circulating leptin (an anorexigenic hormone) may also have
242 contributed to the higher expression of the orexigenic neuropeptide²³ in these IR rats. Of note,
243 in a previous report³⁶, and in our study, leptin concentrations in SBS patients was low but not
244 different from controls. Furthermore, Molina et al. demonstrated that leptin concentrations did
245 not correlate with hyperphagia in short bowel-patients³⁶.

246 The central and peripheral variations of mRNA or peptide levels may also reflect the
247 nutritional status of the rats. Ghrelin plays a role in long-term energy balance regulation,
248 protecting against prolonged energy deficiency³². Indeed, prolonged starvation increases
249 plasma ghrelin concentration in mice³⁷ and may also influence NPY and AgRP mRNA
250 levels³⁸. We observed no hyperphagia in the IR rats during these 7 days of adaptation and all
251 IR rats lost weight. Furthermore, the IR jejuno-colonic rats without PN, which are the leanest
252 rats, had the highest levels of orexigenic signals suggesting that this may be an under-
253 nutrition response to promote food intake. IR jejuno-colonic rats with PN were heavier than
254 jejuno-colonic rats without PN. Parenteral nutrition may ensure better hydration and improve
255 energy balance by partially compensating for malabsorption, despite providing a moderate
256 amount of calories. But, IR-JC rats with PN also exhibited significant increase in their
257 orexigenic signals compared to IR jejuno-ileal rats even if these two groups had similar body
258 weight. Overall, these observations suggest that the specific environment created by
259 connecting the jejunum to the colon (including modifications of the lumen contents of
260 nutrients, biliopancreatic secretions and microbiota metabolites) may trigger a specific

261 activation of peripheral and central orexigenic cues beyond the known effect of
262 undernutrition/starvation.
263 Evidence for the clinical relevance of these findings was provided by the study in human SBS
264 patients. Indeed, these patients, who were not undernourished, had similar levels of leptin
265 than healthy subjects and had high plasma concentrations of active ghrelin, with fasting and
266 postprandial active ghrelin concentrations higher in jejuno-colonic SBS patients than in
267 control individuals. In SBS patients, the postprandial inhibition of ghrelin levels was delayed,
268 suggesting that this hunger signal persisted after the initiation of the meal in these patients. It
269 remains unclear whether these persistently high ghrelin levels could induce hyperphagia in the
270 long-term. Despite these differences, no relationships were found between ghrelin
271 concentrations and the amount of ingested calories or the presence of hyperphagia, possibly
272 because of the small number of SBS patients in this study. Further evaluation in a larger
273 cohort of SBS patients would be required to unveil such relations.

274 The average concentrations of GLP-2, GLP-1 and PYY were systematically increased
275 in IR rats, consistent with data reported for SBS patients (this study and³). Signals of hunger
276 and satiety would be expected to operate in opposite ways. However, this does not appear to
277 be the case here, given the concomitantly high concentrations of anorexigenic¹⁴ PYY and
278 GLP-1 and orexigenic ghrelin found in IR rats and SBS patients. As suggested by others for
279 GLP-1^{39,40}, the high circulating concentrations of PYY, GLP-1 and GLP-2 may affect
280 principally gastrointestinal tract functions rather than the central nervous system. In
281 particular, these hormones decrease intestinal motility and transit time¹⁸, increasing the time
282 for which nutrients are in contact with the remaining epithelium and thereby optimizing their
283 distribution and absorption along the gastrointestinal tract. GLP-2 and PYY can also improve
284 water and electrolyte absorption within the colon^{41,42}. More specifically GLP-2 is also well
285 known for its intestinotrophic effect^{12,18}, and its increase could support, at least partially, the
286 elevation of the colonic surface area we observed. Thus, this enhanced secretion of gut

287 hormone reflects adaptive intestinal response, to improve overall digestion, absorption of
288 water and nutrients and the epithelial surface available for this absorption.

289 The increase of fasting and postprandial secretion of PYY in SBS patients (this study and ⁴)
290 was larger in jejunocolonic than in jejunoleal SBS patients and rats compared to control.
291 No such differences were observed for GLP-1 and GLP-2 levels. Enteroendocrine cells are
292 distributed from the duodenum to the colon but cells producing PYY are mostly restricted to
293 the ileum and colon, and recently, it has been shown in rat that the highest concentrations of
294 PYY along the gastrointestinal tract are found in the distal ileum and proximal and distal
295 colon⁴³. The higher PYY levels could result from an increase in the number of cells producing
296 PYY due to morphological adaptation of the residual colon mucosa. The densities of PYY
297 (and GLP-1) producing cells in colon were not affected in IR rats, suggesting that the
298 homeostasis of epithelial cell differentiation was maintained in the colon of resected rats. But,
299 the remaining colon mucosa in jejunocolonic IR rats was hyperplastic with an enlarged
300 number of proliferating cells and such changes were not observed in the colon mucosa when
301 part of the ileum was preserved. A direct consequence of this colonic overgrowth would be a
302 local increase in the number of enteroendocrine cells that in turn may contribute to the higher
303 plasma concentrations of gut hormones. Alternatively, the colonic hormones-producing cells,
304 and especially the PYY producing cells, may also have become highly responsive to the new
305 environment created by the direct connection of the jejunum to the residual colon. The lumen
306 of the residual colon typically contains large amounts of undigested nutrients⁵ and a dysbiotic
307 microbiota^{6,8,44}, and is thought to display changes in the fermentative activity generating
308 short-chain fatty acids (SCFAs). SCFAs can modulate the function of enteroendocrine cells
309 secreting GLP-1/GLP-2/PYY *in vitro*^{45,46} and possibly *in vivo*^{46,47}. Bile acids could also
310 participate to the increased colonic production of GLPs and PYY^{48,49}, as well as to the
311 increase number of proliferating cells⁵⁰, observed in jejunocolonic individuals. Whether the

312 bile acids are present in different amount and composition in the colonic lumen content of the
313 jejunum-ileal, jejunum-colonic and sham operated rat remain to be evaluated.

314 In conclusion, we demonstrate that SBS is associated with an increase in fasting plasma
315 ghrelin concentration and an induction of orexigenic signals in the hypothalamus, in a rat
316 model with jejunum-colonic anastomosis and that post-prandial ghrelin inhibition is impaired in
317 SBS patients. The induction of peripheral gut hormones, such as PYY, is stronger in the
318 absence of the ileum. Intestinal resection with jejunum-colonic anastomosis creates a specific
319 environment promoting increase hunger signals and improving gastrointestinal functions
320 associated with nutrient absorption. Further studies are required to determine whether these
321 changes are correlated with the development of hyperphagia, a good prognosis for weaning
322 off of PN.

323

324 **MATERIALS AND METHODS**

325 This study followed accepted ethical, scientific and medical standards and was conducted in
326 accordance with recognized international standards, including the principles of the
327 Declaration of Helsinki.

328 **Animal studies**

329 All experimental procedures were performed in accordance with the European Community
330 guidelines and were approved by and the Paris Nord local ethics committee and the Ministry
331 of Education and Research (N°02285.01).

332 6-week-old male Wistar rats (n=28) weighing 250-300 g (Elevage Janvier, Le Genest-St-Isle,
333 France) were used. The animals were housed in cage with free access to standard rat chow
334 diet (C1314 Altromin, Genestil, Royaucourt, France) and water, and maintained under
335 standardized temperature (23 °C ± 1°C) and 12h light-dark cycles (lights on at 7:00 a.m.).

336 Animals were allowed to acclimatize to their environment for 7 days before experimentation.

337 ***Surgical and experimental procedure:*** On the day of surgery, rats were randomly assigned to
338 undergo resection of 70% of the small bowel with jejunio-ileal anastomosis (IR-JI), 80% of
339 small bowel including the ileum, ileocecal valve and 20% of colon with jejunio-colonic
340 anastomosis (for IR-JC), or a jejunio-jejunal transection with re-anastomosis (Sham) (figure
341 1A). Twelve hours before surgery, animals were fasted but allowed *ad libitum* access to
342 water. They were anesthetized by intraperitoneal injection of pentobarbital sodium (Ceva,
343 Libourne, France). Standard aseptic procedures were used throughout the surgery. Intestinal
344 length was measured in a standardized method. The bowel was resected 15 cm distal to the
345 ligament of Treitz to either 3 cm distal to the cecum for IR-JC rat groups or to 10 cm
346 proximal to the cecum for IR JI rat groups and followed respectively by jejunio-colonic or
347 jejunio-ileal anastomosis with 6-0 Prolene running sutures. Sham-operated rats underwent a
348 jejunio-jejunal transection at 15 cm below the ligament of Treitz and re-anastomosed. Animals
349 received subcutaneously Xylocaïne 1% (100 µl / 100 g) (Astra, France) and Penicillin G
350 (20,000 units/kg) (Panpharma, Luitre, France) to prevent postoperative pain and infections.
351 Subcutaneous injection of 10 ml Bionolyte G5 (Sodium chloride [0.4%], Glucose [5.5%],
352 Potassium Chloride [0.2%]) (Baxter, Maurepas, France) was given to prevent postoperative
353 dehydration. Liquid diet (Nutrison, Nutricia, France) was available *ad libitum* 48h after
354 surgery. The IR jejunio-colonic rats were under oral nutrition without or with PN through a
355 catheter implanted in the jugular vein. PN diet, prepared aseptically, was perfused 48h after
356 surgery via a syringe pump (Precidor-Infors AG Basel) at 1.5 ml/h during 8h daily, providing
357 12 kcal/day. Free access to food and PN administration were restored 48 hours after surgery
358 and total calorie intake was calculated daily.

359 Previous studies have reported maximal intestinal morphological changes between day 4 and
360 12 after the surgery^{11,27}. Thus, we set 7 days as the experimental period for this study. Body
361 weight was monitored daily and food intake was determined from day 2 to 7 post-surgery. At
362 day 7, overnight fasted rats were anesthetized; blood was collected in chilled tubes containing

363 heparin or heparin and dipeptidyl peptidase IV (DPP-IV) inhibitor (Roche Applied Science).
364 Plasma was aliquoted and stored in -80 °C until analyses. Then the rats were sacrificed,
365 hypothalamus and intestinal mucosa scrappings were collected and rapidly frozen until
366 extraction of total ARN for RTq-PCR analyses. Other intestinal segments were formalin-fixed
367 overnight and embedded in paraffin for classical histology and immunohistochemistry studies
368 (*see Supplementary Material*).

369 ***Quantification of gene expression in colon mucosa and hypothalamus:*** Total RNA was
370 extracted from unfrozen colon mucosa scrappings and from hypothalamus tissue with Trizol
371 reagent (Invitrogen, Saint Aubin, France). After reverse transcription with 8µg total RNA,
372 RTq-PCR real-time qPCR was performed in duplicate for each cDNA, using specific primers
373 and the ABI PRISM 7000 sequence detection system and TaqMan universal PCR technology
374 as described in details in *Supplementary Material* and compared to mean target gene
375 expression of sham operated rats as calibrator samples. Fold induction was calculated using
376 the comparative $2^{-\Delta\text{ct}}$ Method.

377 **Clinical study**

378 ***Human subjects.*** Nine adult patients with SBS were recruited from our tertiary care center for
379 intestinal failure (Beaujon Hospital). They gave informed consent for participation to the
380 study. The protocol was approved (N°15-046) by the Institutional Review Board-IRB-
381 00006477- of HUPNVS, Paris 7 University, APHP

382 The patients were in a steady state, with their last surgery realized more than 6 months before
383 the blood test. They all were on a non-restricted oral diet and 6 of them had PN dependence.
384 Criteria for inclusion were extensive small bowel resection, with a remnant small bowel
385 length <150 cm, with some colon in continuity (jeuno-colonic anastomosis) or with ileum in
386 continuity (jeuno-ileal anastomosis). Criteria for exclusion were upper gastrointestinal tract
387 surgery, small bowel lesions in the remnant gut, organ failure other than digestive, evolutive
388 neoplasia, intestinal fistulae and treatment by growth hormone, GLP-2, steroids or

389 immunosuppressive therapy in the 4 months preceding the study. The patients had no signs of
390 sepsis and no antibiotherapy.

391 Five adult healthy subjects with normal BMI (between 18.5-24.9 kg/m²) who gave their
392 informed consent for the study were also recruited (ANSM authorization B131095-81).

393 Procedures followed were in accordance with the ethical standards of the Helsinki Declaration
394 of 1975, as revised in 1983.

395 **Study design.** The blood samples were collected in SBS and control subjects during their
396 routine monitoring after an overnight fast (SBS on PN receiving saline solution to avoid
397 dehydration), before (T0), 30 and 90 min after consumption of a standardized breakfast of 725
398 kcal (100 g of bread, 20 g of butter, 20 g of jam, 30 g of French emmental, 20 g of powder
399 skimmed milk, 10 g of sugar, 250 ml of tea or coffee and 500 ml of Vichy St-Yorre water).

400 Blood samples were drawn in EDTA tube Vacutainer (Becton Dickinson, France) containing
401 DPP-IV inhibitor (DPP-IV-010, Millipore) and serine protease inhibitor, 4-(Aminoethyl)-
402 benzenesulfonyl fluoride hydrochloride or Pefabloc (Roche Applied Science). Samples were
403 centrifuged between 863 to 2000g for 10 min. Aliquoted plasma was stored in -80 °C until
404 analyses.

405 **Plasma hormone analyses**

406 Human and rat plasma concentrations of PYY, GLP-1, GLP-2 and ghrelin were quantified as
407 described in details (*Supplementary Material*). The hormone assessment results were
408 expressed as absolute value and incremental area under the curve (iAUC) calculated by
409 subtracting baseline levels from all subsequent readings or total area under the curve (tAUC)
410 for ghrelin.

411 **Statistical analyses**

412 All values are expressed as the mean \pm S.E.M. Non-parametric tests were used: Mann-
413 Whitney test to compare two groups and Kruskal-Wallis test followed by Dunn's adjusted
414 multiple comparisons to compare more than two groups were performed using GraphPad

415 Prism version 6.0 for Windows (GraphPad Software, San Diego, CA, USA). A value of
416 $P < 0.05$ was considered statistically significant.

417

418 **SUPPLEMENTARY MATERIAL and FIGURES** are presented in “Supplementary
419 Information”

420

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543

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555 analyzed and interpreted the data and wrote the manuscript; FJ and IP recruited the humans
556 subjects for the clinical study; LG, LB, ACJ, BSI, FC, JBC, CM, MLG, AB, JLB, IP
557 contributed to the acquisition of data; BSI and LRP performed the surgeries for the preclinical
558 model; MLG, JBC, JNF, MT, JML have critically read the manuscript. All authors approved
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561 financial interests

562

563 **FIGURE LEGENDS**

564 **Figure 1: Body weight loss, plasma leptin levels and histological adaptation of the**
565 **remaining colon mucosa after intestinal resection in rats.** (A) Schematic representation of
566 the surgeries and colonic tissue collections at day 7 post-surgery. Length of resected or
567 remnant part of intestinal segments are not proportional. (B) Comparative time-dependent
568 body weight loss represented as percent of the preoperative weight and (C) total calorie intake
569 (oral intake and parenteral nutrition) in IR jejunum-ileal (IR-JI), IR jejunum-colonic (IR-JC), IR
570 jejunum-colonic PN (IR-JC PN) and sham-operated (Sham) rats during 7 days after surgery.
571 Black box corresponds to the period of the postoperative care. (D-E) Plasma levels of leptin
572 (C) and albumin (E) in the same groups. Data are represented as mean \pm SEM of n=6 for
573 sham, n=4 to 6 for IR jejunum-ileal, n= 10 for IR jejunum-colonic, n=5 to 6 for IR jejunum-colonic
574 with PN. *P<0.05, **P<0.01, vs sham-operated rats based on non-parametric Kruskal-Wallis
575 test followed by Dunn's adjusted multiple comparisons.

576 **Figure 2: histological adaptation of the remaining colon mucosa after intestinal resection**
577 **in rats.** (A) Representative photomicrographs of colon mucosa of Sham, IR jejunum-ileal (JI),
578 IR jejunum-colonic (JC) and IR jejunum-colonic with PN (JC-PN) rats stained with HPS and
579 immunostained with an anti-Ki67 antibody. (B) Measurement of colon mucosa crypt depth in
580 μm (at least 5 crypts analyzed by rat). (C) Quantification of Ki67 positive cells per crypt (at
581 least 5 crypts analyzed by rat), (D) density of Ki67 positive cells expressed as number of Ki67
582 positive cells per 100 μm of crypt. (E-F) Representative photomicrographs of colon mucosa of
583 sham and IR-JC rats stained with Periodic Acid Schiff (PAS) (E) and Representative
584 photomicrographs of colon mucosa of sham and IR-JC rats immunostained with anti-Muc2
585 antibody (F) with quantification of Muc2 positive cells per area in mm^2 . Data are represented
586 as mean \pm SEM of n=6 for sham, n=4 to 6 for IR jejunum-ileal, n= 10 for IR jejunum-colonic,
587 n=5 to 6 for for IR jejunum-colonic with PN. *P<0.05, **P<0.01, vs sham-operated rats based

588 on non-parametric Kruskal-Wallis test followed by Dunn's adjusted multiple comparisons.

589 Scale bar: 50 μ m

590 **Figure 3: Increased levels of plasma gut hormones after intestinal resection in rats.**

591 Fasting plasma levels of ghrelin (A), GLP-1 (B) GLP-2 (C) and PYY (D) in IR jejuno-ileal
592 (JI), IR jejuno-colonic (JC), IR jejuno-colonic with PN (JC-PN) and sham-operated (Sham)
593 overnight fasted rats 7 days after surgery. Data are represented as mean \pm SEM of n=6 for
594 sham, n=4 or not available (NA) for IR-JI, n=4 to 10 for IR JC, n=4 to 6 or not available (NA)
595 for IR-JC PN. *P<0.05, **P<0.01, vs sham-operated rats based on non-parametric Kruskal-
596 Wallis test followed by Dunn's adjusted multiple comparisons.

597 **Figure 4: Unchanged number of PYY and GLP-1 positive cells but enhanced levels of**

598 *proglucagon and Pyy mRNA after intestinal resection in rats.* (A-B) Representative

599 photomicrographs of GLP-1 (A) and PYY (B) immunostaining on colon mucosa sections

600 from sham-operated and IR-JC rats (cytoplasm of GLP-1 or PYY cells are stained in brown),

601 scale bar:50 μ m. (C-D) Quantification of GLP-1 (C) and PYY (D) positive cells per area in

602 mm². Data are represented as mean \pm SEM of n=4 for sham, n=4 or not available (NA) for IR

603 jejuno-ileal (JI), n= 5 for IR jejuno-colonic (JC), n= 6 for for IR jejuno-colonic with PN (JC-

604 PN). (E-F) Colonic mucosa mRNA levels of proglucagon (*Gcg*) (E) and *Pyy* (F) normalized

605 to *L19*, 7 days after surgery. Data are represented as mean \pm SEM of n= 6 for sham, n= 5 for

606 IR-JI, n= 9 for IR-JC, n= 6 for IR-JC with PN. *P<0.05, **P<0.01, ***P<0.001 vs sham-

607 operated rats and # P<0.05 ## P<0.01 vs IR-JC based on non-parametric Kruskal-Wallis test

608 followed by Dunn's adjusted multiple comparisons.

609 **Figure 5: Expression of mRNA coding for hypothalamic orexigenic (AgRP, NPY) and**

610 **anorexigenic (POMC, CART) neuropeptides after intestinal resection in rats.** Expression

611 of (A) *Agrp*, (B) *Npy*, (C) *Pomc* and (D) *Cart* mRNA normalized to *Hprt* mRNA in

612 hypothalamus in IR jejuno-ileal (JI), IR jejuno-colonic (JC), IR jejuno-colonic with PN (JC-

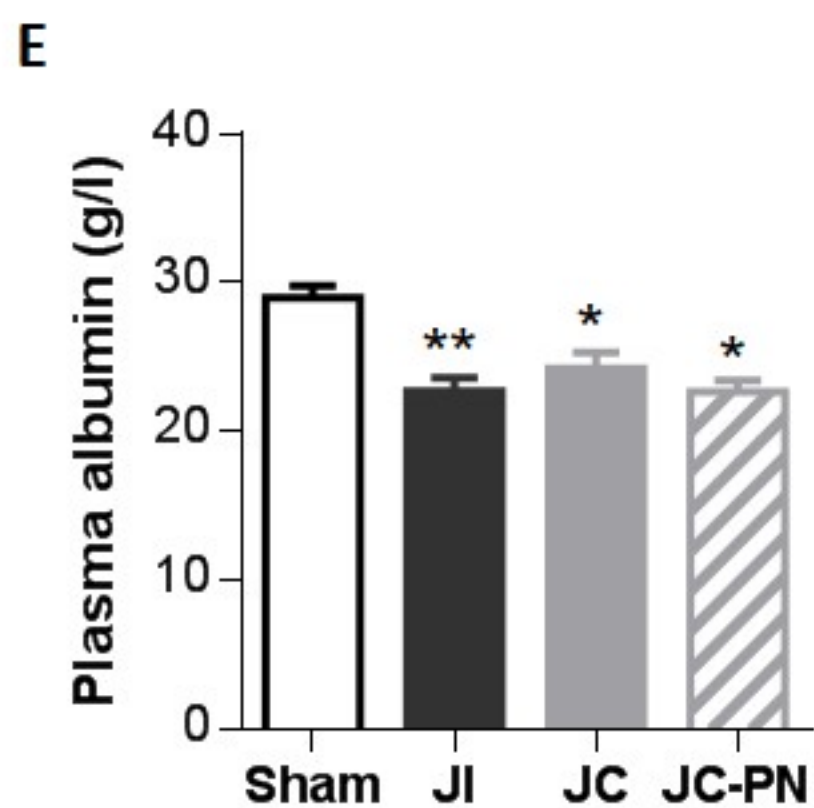
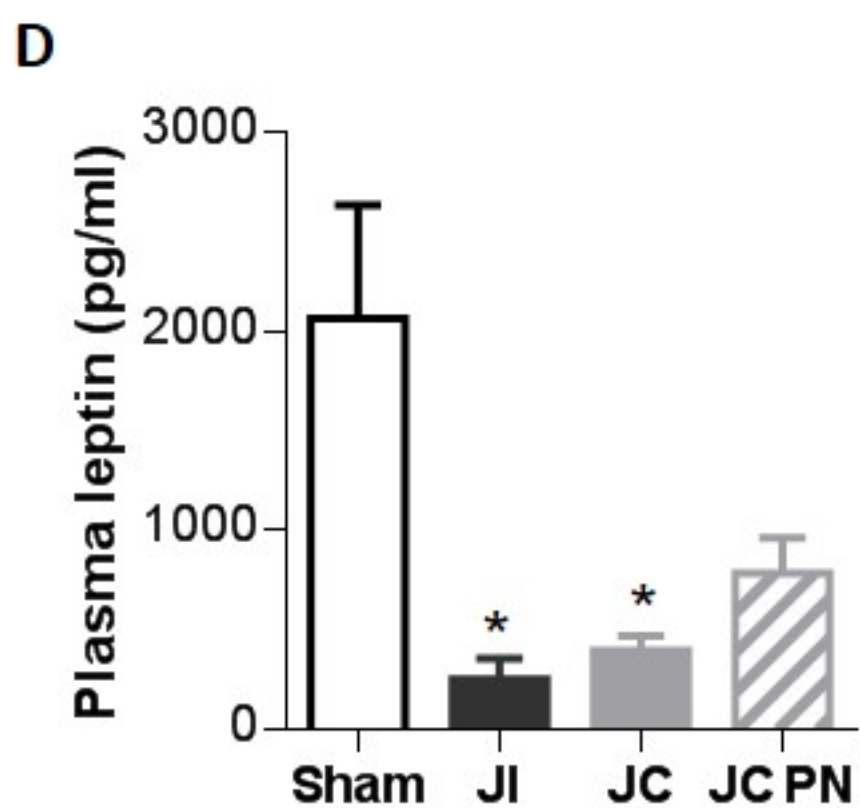
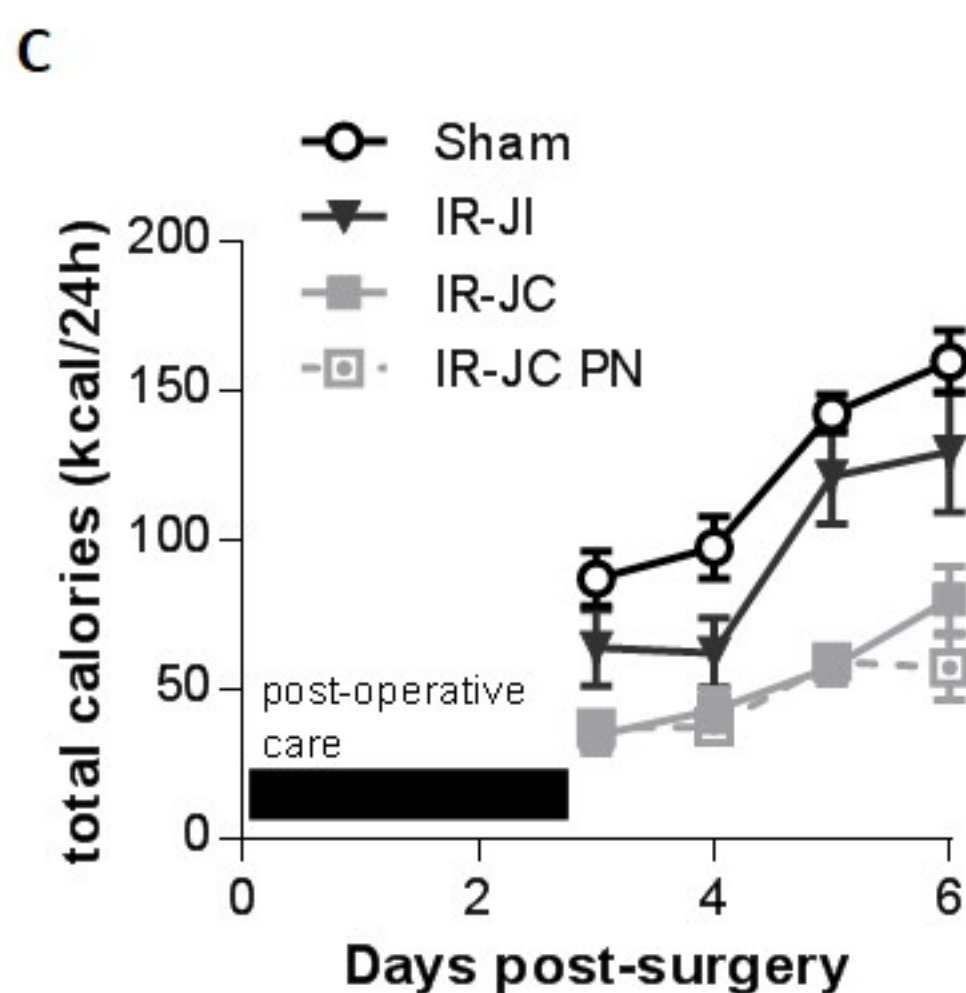
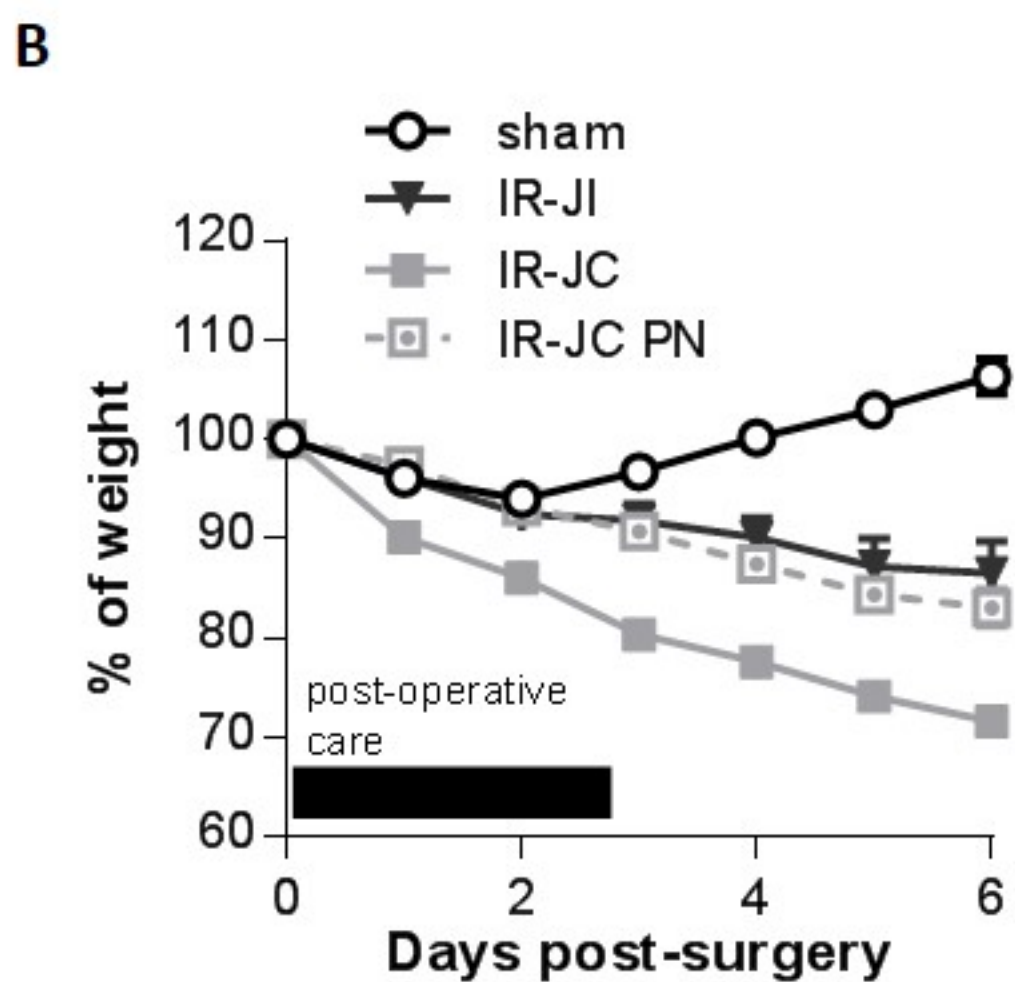
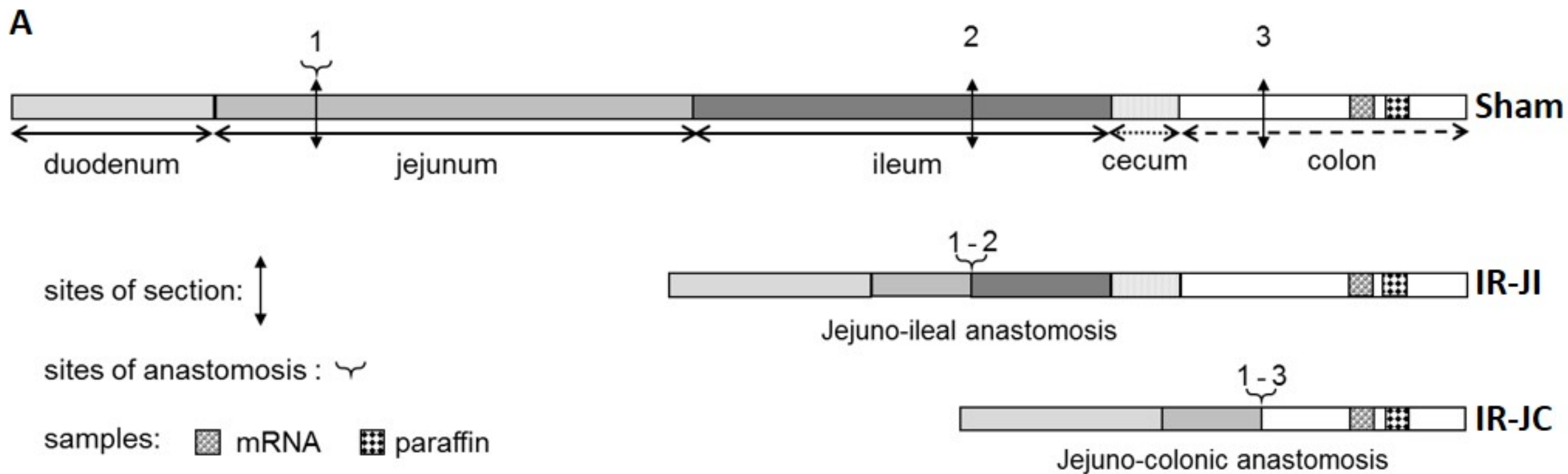
613 PN) and sham-operated (Sham) rats 7 days after surgery. Data are represented as mean \pm
614 SEM of n=6 for sham, n=5 for IR-JI, n=10 for IR-JC, n=6 for for IR-JC with PN. *P<0.05,
615 ***P<0.001 vs sham-operated rats based on non-parametric Kruskal-Wallis test followed by
616 Dunn's adjusted multiple comparisons.

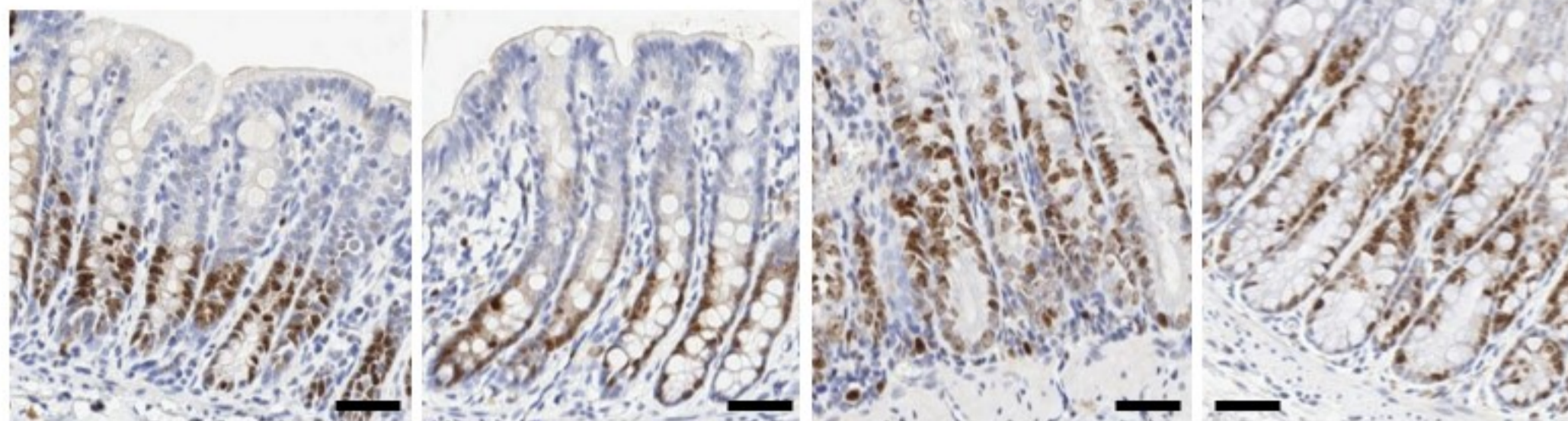
617 **Figure 6: Increased fasting and post-prandial levels of plasma active Ghrelin, GLP-1,**
618 **GLP2 and PYY in SBS patients.** Plasma concentrations and secretory response to a
619 calibrated meal (750 kcal) in SBS jejuno-ileal (JI) (blue) and SBS jejuno-colonic (JC)
620 (orange) patients compared to healthy subjects (C) (black) of: **(A-B)** active ghrelin (pg/mL)
621 before (T0) and after (T30, T90) the meal **(A)** and ghrelin secretory response expressed as the
622 total area under the curve (AUC) **(B)** ; **(C-D)** GLP-1 (pg/mL) before (T0) and after (T30, T90)
623 the meal **(C)** and GLP-1 secretory response expressed as the incremental area under the curve
624 (iAUC) **(D)**; **(E-F)** GLP-2 (pg/mL) before (T0) and after (T30, T90) the meal **(E)** and
625 secretory response expressed as the iAUC **(F)**; **(G-H)** PYY (pg/mL) before (T0) and after
626 (T30, T90) the meal **(G)**, and secretory response expressed as the iAUC **(H)**. All values are
627 expressed as the mean \pm S.E.M of n=5 for healthy subjects, n= 5 for jejuno-ileal SBS patients
628 and n=4 for jejuno-colonic SBS patients. *P<0.05, **P<0.01, ***P<0.001 vs healthy subjects
629 and # P<0.05, ## P<0.01 and ### P<0.001 vs jejuno-ileal SBS patients based for A, C, E, G,
630 on Bonferroni's multiple comparisons test and for B, D, F, H on non-parametric Kruskal-
631 Wallis test followed by Dunn's adjusted multiple comparisons.

632

SBS patients (sex)	age	BMI	remnant jejunum/ileum length (cm)	Remnant colon (%)	Delay since continuity reestablishment (months)	total oral intake (kcal/day)	PN/Week (n)	PN support (kcal/perfusion)	REE (kcal/day)	oral intake /REE	Leptin (ng/ml)	Prealbumin (g/l)
P1 (M)	50	22	10 / 40 (SBS-JI)	100	12	3000	2	1612	1442	2,1	2,2	0,305
P2 (F)	59	16	20 / 0 (SBS-JC)	75	132	2200	4	2450	1070	2,1	2,2	0,194
P3 (F)	52	20	20 / 50 (SBS-JI)	100	24	2200	0	0	1188	1,9	1,99	0,163
P4 (F)	49	19	12 / 10 (SBS-JI)	100	96	3600	3	1498	1192	3,0	3,09	0,221
P5 (F)	39	23	70 / 5 (SBS-JI)	100	60	1500	0	0	1419	1,1	14,5	0,187
P6 (F)	32	19	0 / 0 (SBS-JC)	50	12	840	7	1910	1348	0,6	2,71	0,313
P7 (M)	73	20	10 / 40 (SBS-JI)	100	96	3000	0	0	1214	2,5	1,99	0,263
P8 (M)	57	19	15 / 0 (SBS-JC)	50	120	1600	5	1662	1195	1,3	4,56	0,289
P9 (F)	63	25	80 / 0 (SBS-JC)	50	12	2500	2	1498	1307	1,9	43,18	0,288
Median	52	20	15 [10-45]/	100	60	2200	2	1498	1214	1,9	2,71	0,276
[25-75]	[49-59]	[19-22]	5 [0-40]	[50-100]	[12-96]	[1600-3000]	[0-4]	[0-1662]	[1192-1348]	[1,3-2,1]	[2,2-4.56]	[0,194-0,289]

Table 1: Clinical and nutritional characteristics of SBS patients. BMI: body mass index (Kg/m²). SBS-JI = SBS patients with jejunum-ileal anastomosis, SBS-JC = SBS patients with jejunum-colonic anastomosis. Remnant jejunum and ileum length and colon in continuity were expressed in cm and in % of common colon length, respectively. Delay was in months from the last surgery establishing an anastomosis between the jejunum and the remaining colon. Total oral was expressed in Kcal/day. Parenteral nutrition (PN) support was expressed in number (n) of perfusion per week (PN/week) and kcal/perfusion. Resting energy expenditure (REE) was expressed in kcal/day. Hyperphagia is defined as oral intake/REE >1.5. Plasma levels of leptin are in ng/mL and prealbumin are in g/L. Values are median and 25th and 75th percentile. Etiology of SBS were arterial mesenteric infraction (n=5), volvulus (n=1), intestinal occlusion (n=1) and surgical complications (n=3) with no residual lesions of the remnant intestine



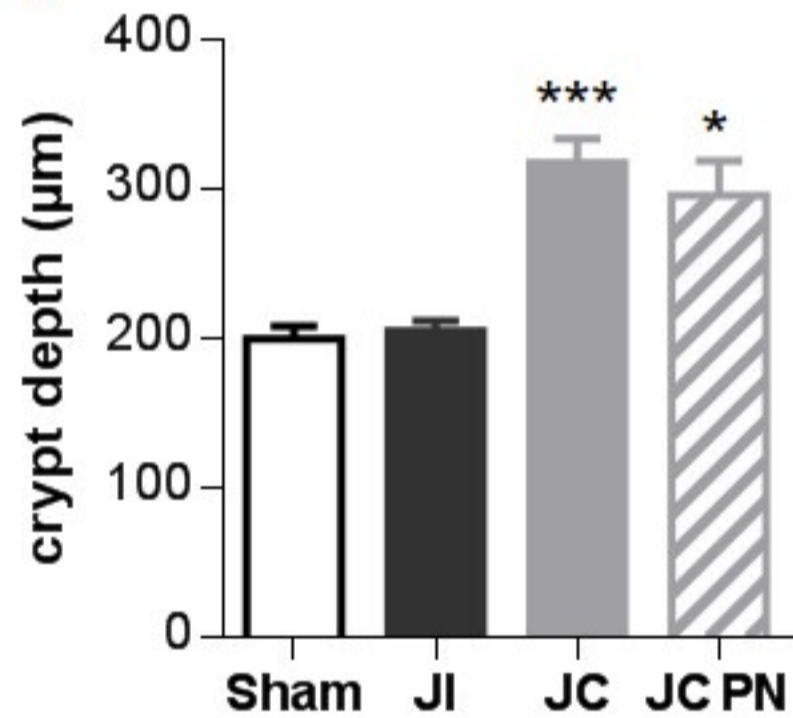
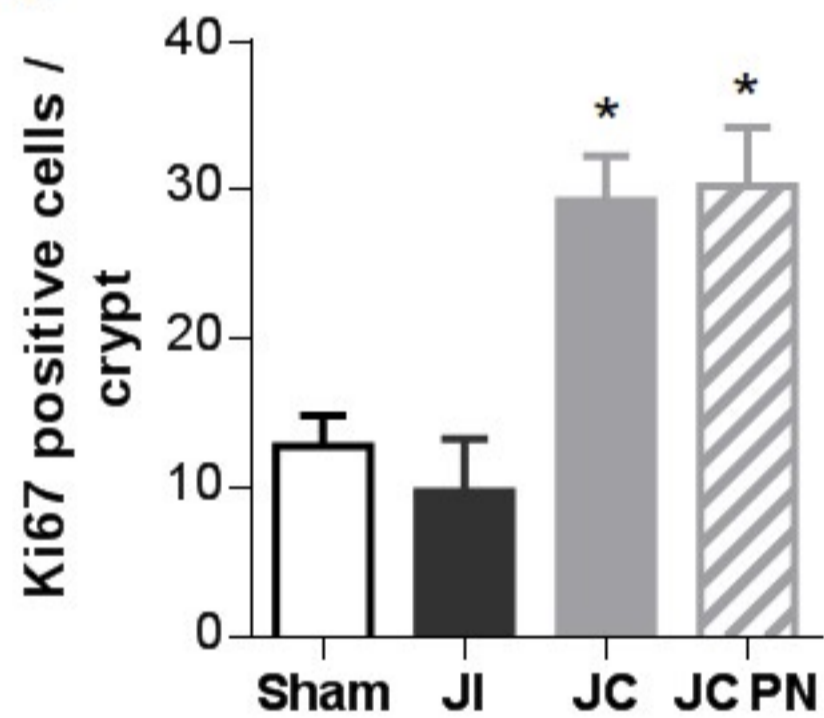
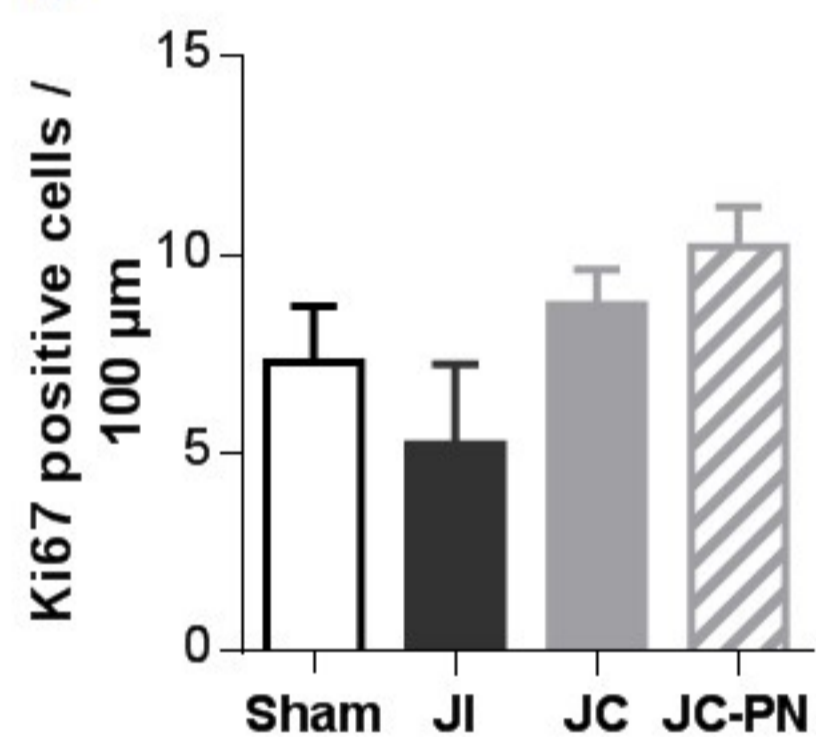
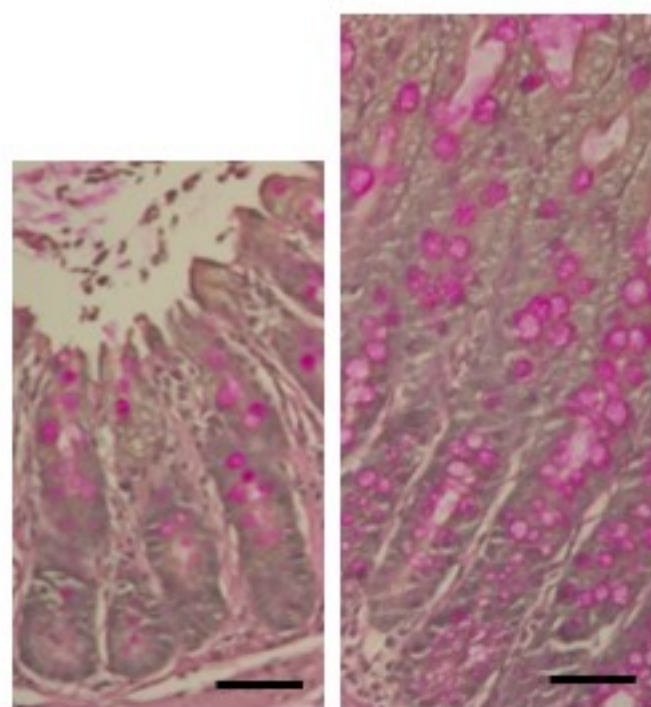
A**KI67⁺**

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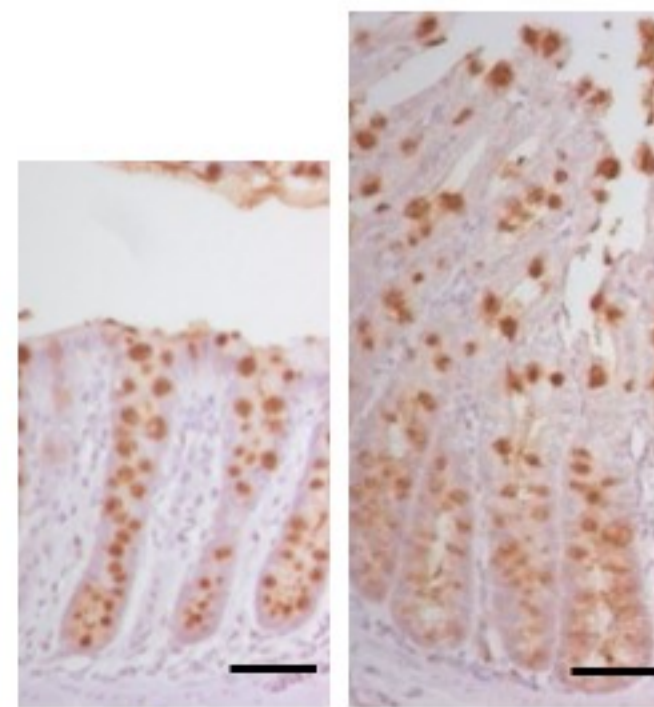
JC

JC-PN

B**C****D****E****PAS**

Sham

JC

F**Muc2⁺**

Sham

JC

