Tumour-derived Dilp8/INSL3 induces cancer anorexia by regulating feeding neuropeptides via Lgr3/8 in the brain

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In patients with advanced-stage cancer, cancer-associated anorexia affects treatment success and patient survival. However, the underlying mechanism is poorly understood. Here, we show that Dilp8, a *Drosophila* homologue of mammalian insulin-like 3 peptide (INSL3), is secreted from tumour tissues and induces anorexia through the Lgr3 receptor in the brain. Activated Dilp8-Lgr3 signalling upregulated anorexigenic nucleobinding 1 (*NUCB1*) and downregulated orexigenic short neuropeptide F (*sNPF*) and *NPF* expression in the brain. In the cancer condition, the protein expression of Lgr3 and *NUCB1* was significantly upregulated in neurons expressing *sNPF* and *NPF*. *INSL3* levels were increased in tumour-implanted mice and *INSL3*-treated mouse hypothalamic cells showed *Nucb2* upregulation and *Npy* downregulation. Food consumption was significantly reduced in intracerebrospinal *INSL3*-injected mice. In patients with pancreatic cancer, higher serum *INSL3* levels increased anorexia. These results indicate that tumour-derived Dilp8/*INSL3* induces cancer anorexia by regulating feeding hormones through the Lgr3/Lgr8 receptor in *Drosophila* and mammals.

Anorexia, which is common in patients with advanced-stage cancer, leads to progressive weight loss and contributes to cancer-related mortality. It is closely linked to cancer-associated cachexia^{1–3}, which severely affects the quality of life of patients with cancer^{6,7}. In a case study of 66 cancer patients near the end of life, more than 60% of patients showed anorexia despite not receiving chemotherapy^{8,9}. This suggests that the development of anorexia in patients with cancer is independent from the treatment. However, the molecular mechanism underlying cancer anorexia is largely unknown.

In the cancer anorexia-cachexia syndrome, secreted tumour-derived humoral cytokines play an essential role in inter-organ/tissue communication^{10–13}. Systemic coordination and integration among organs and tissues by inter-organ communication is important in normal conditions and in pathologies such as cancer and metabolic syndrome¹⁴. Numerous evidence suggest that tumours elicit inflammatory responses^{15,16} and metabolic dysfunction in muscle and fat through the activation of pro-inflammatory cytokines^{17–20}. Systemic inflammatory cytokines such as tumour necrosis factor- α , interleukin-1 α (IL-1 α), IL-1 β , IL-6, transforming growth factor- β and parathyroid-hormone-related protein (PTHrP) function as tumour-derived hormones that mediate muscle wasting in mouse cancer models and human cancer patients^{21–23}. Recently, growth differentiation factor (GDF15) has also been implicated in cancer cachexia due to the correlation of GDF15 with food intake and body weight loss in numerous chronic human diseases^{24–26}.

Recent studies have shown that ImpL2, a *Drosophila* homologue of mammalian insulin-like growth factor binding protein (IGFBP), is secreted from tumour tissue and impairs muscle function and tissue wasting by inhibiting IGF-like signalling in *Drosophila*^{27,28}. These *Drosophila* cancer models reveal how tumour-secreted cytokines cause cachexia-like tissue wasting, demonstrating that *Drosophila* is a good model system to elucidate the mechanisms of the tumour–host interaction.

In this Article, we establish a *Drosophila* cancer anorexia model by overexpressing the known oncogene *yorkie* and demonstrate that tumour-derived Dilp8 systemically regulates the expression of feeding neuropeptides through its receptor Lgr3 in the brain. We also show that this cancer anorexia pathway is conserved in mammals. Tumour-derived insulin-like 3 peptide (INSL3), a mammalian homologue of Dilp8, regulates anorexigenic *Nucb2* and orexigenic *Npy* through its receptor Lgr8 in mouse hypothalamic cells and pancreatic-tumour-cell-implanted mice. In addition, we show that the level of INSL3 is substantially increased in the serum of patients with pancreatic cancer.

Results

Cancer anorexia precedes organ wasting in the *Drosophila* eye tumour model. To investigate the molecular mechanism of cancer-induced anorexia in *Drosophila*, we established a *Drosophila* cancer model by overexpressing *yorkie* (*yki*), a transcriptional coactivator of the Hippo pathway^{29–31}, in adult eyes using the *GMR-Gal4*

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Fig. 1 | Cancer anorexia precedes organ wasting in the *Drosophila* eye tumour model. **a**, The life span of the constitutively active form of *yki* flies (*GMR>yki^{SA}*) was shorter than that of control flies (*GMR-Gal4*). **b**, The 10-day-old *yki* overexpressing flies (*GMR>yki^{WT}* and *GMR>yki^{SA}*) showed decreased food intake compared with that of the control in the blue dye feeding assay. $n = 5$ biologically independent experiments; *GMR>yki^{WT}* $*P = 0.013$, *GMR>yki^{SA}* $**P = 0.006$. **c**, The ovaries of *GMR>yki^{SA}* flies showed a severe wasting phenotype after 15 days. Scale bar, 200 μ m. **d**, The triglyceride levels of *GMR>yki^{SA}* flies showed reduced lipid levels after 15 days. $n = 3$ biologically independent experiments; day 15 $*P = 0.044$, day 20 $*P = 0.019$. **e**, Reduced food intake was observed in five-day-old *GMR>yki^{SA}* flies. $n = 3$ biologically independent experiments; day 5 $*P = 0.041$, day 10 $*P = 0.015$, day 15 $*P = 0.016$, day 20 $*P = 0.039$. Data are presented as mean $\pm$ s.e.m. Statistical significance was determined with a two-tailed Student's *t*-test; $*P < 0.05$, $**P < 0.01$.

driver. Mutations in the Hippo pathway lead to malignant tumour formation in *Drosophila* and mammals, and are often detected in human cancers^{32–34}. Overexpression of wild-type *yki* in the eyes (*GMR>yki^{WT}*) caused a mild rough eye phenotype, whereas the constitutively active form *GMR>yki^{S168A}* (hereafter referred to as *GMR>yki^{SA}*) resulted in a dramatic protrusion of the eyes caused by massive cell proliferation (Extended Data Fig. 1a and Supplementary Fig. 1a). We examined whether the survival rate was lower in the fly tumour model than in normal flies, similar to the high mortality of patients with cancer. The results showed that *GMR>yki^{SA}* flies had a significantly shorter life span (Fig. 1a). Next, cancer anorexia in *GMR>yki^{SA}* flies was examined by measuring the relative food intake using blue dye³⁵ and capillary feeder (CAFE) assays^{36,37}. Food intake was $\sim 30\%$ lower in 10-day-old *GMR>yki^{SA}* adult flies than in the controls of both assays (Fig. 1b and Extended Data Fig. 1b,c), suggesting that the fly cancer model mimics human cancer anorexia.

Impl2 induces cachexia-like wasting in intestinal malignant tumours and in a tumour allograft model^{27,28}. We avoided tumour

formation in tissues involved in the brain–gut axis to minimize the effects on feeding behaviour. In our fly eye cancer model (*GMR>yki^{SA}*), a mild wasting phenotype in the ovary and reduced triglyceride levels in the fat tissues were observed up to 10 days, whereas a severe wasting phenotype developed after 15–20 days (Fig. 1c,d and Extended Data Fig. 1d,e). The thoracic muscle of 15-day-old *GMR>yki^{SA}* adult flies also showed an abnormal mitochondrial structure with irregular packing of mitochondria between the muscle fibres, which indicates the muscle wasting phenotype (Extended Data Fig. 1f and Supplementary Fig. 1b). *GMR>yki^{SA}* flies also showed reduced food intake after five days (Fig. 1e). These results indicated that cancer anorexia seems to precede cachexia-like organ wasting in *GMR>yki^{SA}* flies.

Tumour-derived Dilp8 suppresses food intake through its receptor Lgr3. Recent studies show that Dilp8, Impl2 and Upd2 are tumour-secreted factors^{27,28}. RNA-sequencing (RNA-seq) analysis confirmed that the mRNA levels of these three genes were highly

increased in the heads of *GMR>yki^{SA}* flies (Extended Data Fig. 2a). *Dilp8* and *Upd2* were highly expressed in five-day-old flies, whereas *ImpL2* mRNA expression was peaked in the 15-day-old fly cancer model (Fig. 2a–c). Because cancer anorexia precedes organ wasting, *Dilp8* or *Upd2* may be involved in cancer anorexia rather than organ wasting. Next, relative food intake was measured after inhibiting *Dilp8*, *ImpL2* and *Upd2* in the *GMR>yki^{SA}* cancer condition. *Dilp8* or *ImpL2* inhibition (*Dilp8-RNAi* or *ImpL2-RNAi*) significantly rescued the reduced food intake in the *GMR>yki^{SA}* cancer model compared with the effect of inhibition of *Upd2* (Fig. 2d). To further test our findings in the *GMR>yki^{SA}* tumour model, we generated a second fly tumour model in which *Ras^{v12}* was overexpressed in the eye using the *sevenless-Gal4* driver. We confirmed that *sev>Ras^{v12}* flies also display anorexia and that this phenotype was effectively rescued by *Dilp8-RNAi* (Extended Data Fig. 2b). These findings indicate that *Dilp8* is probably a key secreted factor for inducing cancer anorexia. Consistently, *Dilp8* mRNA level was increased ~2.5-fold in tumour tissue (eye disc) but not in non-tumour tissue (brain) (Fig. 2e). To determine whether *Dilp8* is secreted from *GMR>yki^{SA}* tumour tissues, we generated an antibody against *Dilp8* and confirmed the specificity in the *Dilp8^{M100727}* hypomorphic mutant^{38,39} (Extended Data Fig. 2c). Western blot analysis detected *Dilp8* in the adult haemolymph of *GMR>yki^{SA}* flies (Fig. 2f), suggesting that tumour-derived *Dilp8* is released into the circulating haemolymph to relay the signal to the target tissues. The elevated *Dilp8* protein level in *GMR>yki^{SA}* fly heads was rescued in *GMR>yki^{SA}, Dilp8-RNAi* fly heads (Extended Data Fig. 2d,e).

Recent studies show that the *Lgr3* relaxin protein is a *Dilp8* receptor, which functions in the homeostatic control of organ size during development^{38,40,41}. To determine whether *Dilp8* and *Lgr3* have any function in the regulation of feeding behaviour, we examined the mRNA expression of these molecules in response to food deprivation and performed a feeding assay with *Dilp8* and *Lgr3* mutants. *Dilp8* and *Lgr3* expression levels gradually decreased in response to starvation and rapidly increased after refeeding (Fig. 2g,h). To test food intake in *Dilp8* and *Lgr3* mutants, the *Lgr3^{Δ50}* deletion mutant was generated by CRISPR-Cas9-mediated mutagenesis. *Lgr3^{Δ50}* was a strong hypomorphic mutant (Extended Data Fig. 2f,g). *Dilp8^{M100727}* and *Lgr3^{Δ50}* mutant flies showed significantly increased food intake in blue dye and CAFE assays (Fig. 2i,j). *GMR>yki^{SA}, Dilp8^{M100727}* and *GMR>yki^{SA}, Lgr3^{Δ50}* flies reversed the cancer anorexia phenotype observed in *GMR>yki^{SA}* flies (Extended Data Fig. 2h). Conversely, *Dilp8* overexpression flies (*GMR>Dilp8*) showed reduced food intake (Extended Data Fig. 2i). These results reveal a metabolic function of *Dilp8-Lgr3* signalling in the regulation of food intake.

Lgr3 is predominantly expressed in the adult head, and its expression in the head is dramatically increased in the cancer condition (Extended Data Fig. 2j), indicating that *Lgr3* can receive the ligand signal in the adult head. We found that the *Dilp8* and *Lgr3* mutations could not reverse the tumour phenotype caused by *yki* overexpression (Extended Data Fig. 2k and Supplementary Fig. 2a), implying that the *Dilp8-Lgr3* axis is not involved in autonomous cancer growth.

***Dilp8-Lgr3* signalling regulates anorexigenic *NUCB1* and orexigenic *sNPF* and *NPF* expression in the *Drosophila* brain.** To identify the feeding regulators that suppress food intake in response to *Dilp8* secretion, we examined the expression levels of *Drosophila* neuropeptides involved in feeding behaviour by RNA-seq analysis in *GMR>yki^{SA}* flies (Supplementary Table 1). The results showed alterations in the expression of *NUCB1*, a homologue of the mammalian anorexigenic factor *NUCB2*, and the orexigenic neuropeptides *sNPF* and *NPF* in cancer. Consistent with RNA-seq data, the mRNA level of *NUCB1* was increased and *sNPF* and *NPF* levels were decreased in *GMR>yki^{SA}* flies (Fig. 3a–c).

To determine whether *Dilp8-Lgr3* signalling regulates the mRNA levels of *NUCB1*, *sNPF* and *NPF*, the mRNA expression of *NUCB1*, *sNPF* and *NPF* was examined in the heads of *Dilp8* and *Lgr3* mutant and overexpressing flies. *NUCB1* mRNA levels were decreased in the *Dilp8* mutant (*Dilp8^{M100727}*) and increased when *Dilp8* was overexpressed. By contrast, *sNPF* and *NPF* mRNA levels were increased in the *Dilp8* mutant and reduced in response to *Dilp8* overexpression (Fig. 3d,e). Consistent with these results, *NUCB1*, *sNPF* and *NPF* mRNA expression levels showed the same expression pattern in *Lgr3^{Δ50}* mutant and *Lgr3*-overexpressing flies (Fig. 3d,f). To show *Lgr3* can regulate *sNPF* and *NPF* mRNA expressions cell-autonomously because *Lgr3-Gal4* expression is overlapped with a small subset of *sNPF* neurons (Extended Data Fig. 2l and Supplementary Fig. 2b), we crossed *UAS-sNPF RNAi* and *UAS-NPF RNAi* flies with the *Lgr3-Gal4* line. Both *Lgr3>sNPF RNAi* and *Lgr3>NPF RNAi* showed significant knockdown of mRNA levels (Extended Data Fig. 2m,n). Taken together, these results indicate that the anorexigenic peptide *NUCB1* and the orexigenic neuropeptides *sNPF* and *NPF* are inversely regulated by tumour-derived *Dilp8* and its receptor *Lgr3* to induce cancer anorexia.

Drosophila *NUCB1* is a homologue of mammalian *NUCB2*, which is described as a satiety molecule⁴². However, the biological function of *Drosophila* *NUCB1* has not been studied. *NUCB2* and *Drosophila* *NUCB1* show 62% amino-acid similarity. The signal peptide and dibasic cleavage sites are highly conserved (Extended Data Fig. 3), suggesting that nesfatin-1, the 82-amino-acid anorexigenic peptide processed from *NUCB2*, can also be produced via *NUCB1* processing. We tested whether the role of *NUCB1* is also involved in feeding suppression. Consistent with mammalian *NUCB2*⁴², *Drosophila* *NUCB1* mRNA expression in the adult head was decreased under the starvation condition and increased by refeeding (Fig. 3g). Nesfatin-1 (a processed form of *NUCB1*) overexpression in neurons (*MJ94>Nesfatin1*) inhibited food intake; conversely, *NUCB1* inhibition in neurons (*MJ94>NUCB1-RNAi*) increased food intake (Fig. 3h). These results demonstrate that *NUCB1* has an evolutionarily conserved role as an anorexigenic factor both in *Drosophila* and mammals.

***NUCB1*- and *Lgr3*-expressing neurons are markedly increased and overlapped in the cancer condition.** To determine whether secreted *Dilp8* and its receptor *Lgr3* systemically regulate *NUCB1* in the target organ, the expression pattern of *NUCB1* in neurons was examined in the adult brain of *GMR>yki^{SA}*. A *Drosophila* *NUCB1* antibody was generated for immunostaining and its specificity was assessed by western blot (Extended Data Fig. 4a). In addition, the co-localization of *NUCB1* and *Lgr3* was assessed to determine whether *NUCB1*-expressing neurons directly receive tumour-derived *Dilp8* signals through the *Lgr3* receptor. In the normal non-tumour condition, endogenous *NUCB1* was expressed at a low level in few neuronal cells (Fig. 4a,b, arrowheads, Extended Data Fig. 4b,c and Supplementary Fig. 3a). *Lgr3* expression in the adult brain was also weak, and the neuronal cells were difficult to distinguish at the endogenous level (Fig. 4c, arrowheads, and Extended Data Fig. 4c). The signal intensity of both *NUCB1* and *Lgr3* neurons was significantly increased in the cancer condition (*GMR>yki^{SA}*) and the signals showed perfect overlap (Fig. 4e–h, boxes, 4i–k, arrows, Extended Data Fig. 4d and Supplementary Fig. 3b), suggesting that *Lgr3* and *NUCB1* double-positive neurons are anorexigenic neurons in the cancer condition. These findings were supported by the almost undetectable expression of *Lgr3* and low level of *NUCB1* in the *Lgr3* mutant (*Lgr3^{Δ50}*) in the cancer condition, which were similar to the levels in the control (Fig. 4l–o, arrowheads, Extended Data Fig. 4e and Supplementary Fig. 3c). Additionally, *NUCB1*-expressing neurons in the cancer condition overlapped with a subset of *sNPF* and *NPF* neurons (Extended Data Fig. 4f,g, arrows, and Supplementary Fig. 4a,b). These results

Fig. 2 | Tumour-derived Dilp8 suppresses food intake through its receptor Lgr3. a–c, *Dilp8* mRNA expression levels peaked in 5-day-old *GMR>yki^{SA}* flies and gradually decreased (**a**), while *Upd2* (**b**) and *ImpL2* (**c**) mRNA expression levels peaked in 5-day-old and 15-day-old *GMR>yki^{SA}* flies, respectively. $n=4$ biologically independent experiments: day 5 $^{**}P=0.003$, day 10 $^{*}P=0.025$, day 15 $^{**}P=0.001$, day 20 $^{**}P=0.002$ (**a**); day 5 $^{**}P=0.002$, day 10 $^{***}P=0.0004$, day 15 $^{***}P=0.0002$, day 20 $^{**}P=0.003$ (**b**); day 5 $^{**}P=0.0076$, day 10 $^{***}P=0.0006$, day 15 $^{***}P=0.00058$, day 20 $^{***}P=0.0009$ (**c**). **d**, Among the tumour-derived cytokines *Dilp8*, *ImpL2* and *Upd2*, *Dilp8*-RNAi significantly rescued the reduced food intake by *GMR>yki^{SA}* in the feeding assay. $n=3$ biologically independent experiments; *GMR>yki^{SA}* $^{*}P=0.043$, *GMR>yki^{SA}Dilp8-RNAi* $^{*}P=0.018$, *GMR>yki^{SA}ImpL2-RNAi* $^{*}P=0.041$, *GMR>yki^{SA}Upd2-RNAi* NS=0.792. **e**, *Dilp8* mRNA level was increased ~2.5-fold only in tumour tissues (eye disc). $n=3$ biologically independent experiments; brain NS=0.103, eye disc $^{*}P=0.047$. **f**, Circulating Dilp8 was detected in the adult haemolymph of *yki^{SA}* overexpressing flies. An asterisk indicates a non-specific band. $n=3$ biologically independent experiments. **g,h**, The expression levels of *Dilp8* (**g**) and its receptor *Lgr3* (**h**) gradually decreased in response to starvation and rapidly increased after refeeding. $n=3$ biologically independent experiments: 12 h $^{*}P=0.056$, 24 h $^{*}P=0.001$, refeed $^{**}P=0.005$ (**g**); 12 h $^{*}P=0.029$, 24 h $^{*}P=0.039$, refeed $^{**}P=0.003$ (**h**). **i,j**, *Dilp8^{M100727}* and *Lgr3^{Δ50}* mutants showed increased food intake compared to the *w⁻* control flies in the blue dye feeding assay (**i**) and CAFE assay (**j**): $n=6$ biologically independent experiments, *Dilp8^{M100727}* $^{**}P=0.0067$, *Lgr3^{Δ50}* $^{**}P=0.007$ (**i**); $n=3$ biologically independent experiments (**j**). Data are presented as mean $\pm$ s.e.m. Statistical significance was determined with a two-tailed Student's *t*-test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Fig. 3 | Dilp8-Lgr3 signalling regulates anorexigenic *NUCB1* and orexigenic *sNPF* and *NPF* expression in the *Drosophila* brain. **a–c, The relative mRNA level of *NUCB1* (**a**) was increased (** $P=0.0075$), and *sNPF* (**b**) (** $P=0.0097$) and *NPF* (**c**) mRNA levels (* $P=0.010$) were decreased in *GMR>yki^{SA}*. $n=3$ biologically independent experiments. **d**, *NUCB1* mRNA levels were decreased, whereas *sNPF* and *NPF* mRNA levels were increased in *Dilp8^{M100727}* and *Lgr3^{Δ50}* mutants. $n=3$ biologically independent experiments; *NUCB1*, *Dilp8^{M100727}* * $P=0.012$, *Lgr3^{Δ50}* * $P=0.015$; *sNPF*, *Dilp8^{M100727}* * $P=0.016$ and *Lgr3^{Δ50}* ** $P=0.005$; *NPF*, *Dilp8^{M100727}* ** $P=0.004$, *Lgr3^{Δ50}* * $P=0.023$. **e,f**, *NUCB1* mRNA levels were increased, whereas *sNPF* and *NPF* mRNA levels were decreased, in *Dilp8*-overexpressing (*GMR>Dilp8*) (**e**) and *Lgr3*-overexpressing (*Lgr3>Lgr3*) (**f**) flies. $n=3$ biologically independent experiments: *NUCB1* ** $P=0.0026$, *sNPF* * $P=0.0138$, *NPF* * $P=0.014$ (**e**); *NUCB1* ** $P=0.006$, *sNPF* * $P=0.017$, *NPF* * $P=0.011$ (**f**). **g**, *Drosophila* *NUCB1* mRNA expression was decreased under the starvation condition and was increased by refeeding. $n=3$ biologically independent experiments; 6 h * $P=0.002$, 12 h * $P=0.007$, 24 h * $P=0.007$, refeed * $P=0.011$. **h**, The relative food intake was reduced when nesfatin-1 (a processed form of *NUCB1*) was overexpressed (*MJ94>Nesfatin1*) and increased when *NUCB1* was inhibited in neurons (*MJ94>NUCB1-RNAi*). $n=4$ biologically independent experiments; *MJ94>Nesfatin1* * $P=0.035$, *MJ94>NUCB1-RNAi* * $P=0.022$. Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined with two-tailed Student's *t*-test; * $P<0.05$, ** $P<0.01$.**

demonstrated that tumour-derived Dilp8 effectively regulates *NUCB1*, *sNPF* and *NPF* expression through the Lgr3 receptor in *NUCB1*- and Lgr3-expressing neurons.

We further confirmed *NUCB1*, *sNPF* and *NPF* expression in the cancer condition by quantitative polymerase chain reaction with reverse transcription (qRT-PCR). The increased *NUCB1* expression

in the *GMR>yki^{SA}* cancer model was significantly suppressed in the *Lgr3* mutant (Fig. 4p). Consistent with the qRT-PCR results, western blot analysis showed that the upregulation of the NUCB1 protein in the cancer condition was restored to the control level in the *Lgr3* mutant (Extended Data Fig. 5a,b). Conversely, the downregulation of *sNPF* and *NPF* mRNA in the cancer condition was restored by the *Lgr3* mutation (Fig. 4q,r).

Because tumour-derived Dilp8 systemically controls the expression of *NUCB1*, *sNPF* and *NPF* in the brain, we investigated whether these neuropeptides are regulated by Dilp8. An ex vivo culture assay was performed by adding synthetic Dilp8 peptide to the culture medium of dissected fly adult brains (Extended Data Fig. 5c). *NUCB1* mRNA expression was upregulated at 0.5 h and the levels were maintained for >2 h (Fig. 4s). *sNPF* and *NPF* were downregulated in response to the Dilp8 peptide (Fig. 4t,u). Notably, *Lgr3* expression was upregulated at early time points (Extended Data Fig. 5d), consistent with the immunostaining results showing that *Lgr3* neurons were upregulated in the cancer condition compared with the levels in the non-cancer normal condition. Taken together, these data support our previous results that *NUCB1*, *sNPF* and *NPF* are regulated by Dilp8-*Lgr3* signalling to induce cancer anorexia.

INSL3-LGR8 signalling regulates anorexigenic *Nucb2* and orexi-genic *Npy* expression in mouse hypothalamic cells. To identify a Dilp8 homologue in mammals, we searched for a mammalian *Lgr3* homologue by phylogenetic comparative analysis. Mammalian *Lgr7/8* (RXFP2, relaxin/insulin-like family peptide receptor 2) was identified as a homologue of *Drosophila* *Lgr3* (Extended Data Fig. 6a). *Lgr8* is a receptor for INSL3 and *Lgr7* is a receptor for H2 relaxin⁴³. A separate phylogenetic comparative analysis showed that Dilp8 and INSL3 are closely related peptides (Extended Data Fig. 6b). Comparison of the mature structures of INSL3 and Dilp8 showed that Dilp8 and INSL3 have six conserved invariant cysteine residues for disulfide bond formation (Fig. 5a). These findings indicate that *Drosophila* Dilp8-*Lgr3* signalling may be conserved in the mammalian INSL3-*Lgr8* pathway.

The hypothalamus is a feeding centre in mammals and it expresses numerous neuropeptides involved in the regulation of feeding behaviour^{44,45}. *Lgr8* protein and mRNA were predominantly expressed in the hypothalamus and hippocampus of the mouse brain (Extended Data Fig. 6c,d and Supplementary Fig. 5). To test whether INSL3 regulates *Nucb2* and *Npy* expression through the *Lgr8* receptor, mouse hypothalamic mHypoE-N3 cells, which express *Lgr8* (Extended Data Fig. 6e), were treated with INSL3. Consistent with the fly data, INSL3-treated N3 cells showed increased *NUCB2* and reduced *NPY* expression (Fig. 5b). However, in mHypoE-N39 cells, which express low levels of *Lgr8* (Extended Data Fig. 6e), INSL3 treatment had no significant effect on *Nucb2* and *Npy* expression

(Fig. 5c). In the *Lgr8* knockdown N3 cells with *Lgr8* siRNA, INSL3 treatment had no significant effect on *Nucb2* and *Npy* expression (Fig. 5d,e). Conversely, in *Lgr8*-overexpressed N39 cells obtained by the transfection of *Lgr8*, INSL3 treatment showed increased *Nucb2* and reduced *Npy* expression (Fig. 5f,g). These results indicate that *Nucb2* and *Npy* are regulated by INSL3-*Lgr8* signalling in mammalian cells.

INSL3 regulates *Nucb2* and *Npy*, and induces anorexia in the mouse tumour model. To test cancer cachexia-anorexia in the mouse model, we generated tumour-implanted mouse models that were subcutaneously (s.c.) implanted with two different cancer cell lines, C26 (colon carcinoma cells) and LLC (Lewis lung carcinoma cells), and monitored for food intake and body weight changes. After two weeks of tumour inoculation, C26 implanted mice showed body weight loss (Extended Data Fig. 6f), but not LLC implanted mice (Extended Data Fig. 6g), even though the tumour weights of both tumours were similar (Extended Data Fig. 6h). Daily food intake was significantly reduced in C26 implanted mice (Fig. 6a), but not in LLC implanted mice (Fig. 6b). Interestingly, the level of secreted INSL3 in the conditioned media of C26 cells was higher than that of the LLC cells (Fig. 6c). In the C26 implanted mice model, daily food intake and body weight were measured. From day 11, food intake was significantly decreased but body weight was unchanged (Fig. 6d,e). When we analysed the tissue wasting, muscle wasting and its molecular markers (*MuRF-1*, *MAFbx*, *Fbxo31* and *Musl1*) were unaffected at day 12, but showed an increase at day 21 (Fig. 6f,i and Extended Data Fig. 6i,j). However, lipolysis in the fat tissue and its molecular markers (*Atgl* and *Hsl*) were increased at day 12 and day 21 (Fig. 6f,j and Extended Data Fig. 6i,j). In the hypothalamic tissues of C26 tumour implanted mice, *Nucb2* mRNA levels were increased at day 12 and 21 (Fig. 6g,j), but *Npy* expression was decreased only at day 21 (Fig. 6h,k). However, no such changes were observed in the hypothalamic sample of LLC tumour implanted mice (Extended Data Fig. 6k,l). These results indicate that cancer anorexia precedes the appearance of muscle wasting in the C26 tumour implanted mice model.

To address whether INSL3 regulates food intake, we then measured the food intake of mice after intracerebrospinal (i.c.v.) injection of artificial CSF (aCSF) or INSL3. The food intake was significantly reduced in INSL3-injected mice compared with aCSF-injected mice (Fig. 6l), indicating that INSL3 can reduce food intake by functioning in the brain. We next tested whether peripheral INSL3 regulates food intake by delivering INSL3 peptide intraperitoneally (i.p.) and measuring the food intake. However, i.p. INSL3 did not induce significant changes in food intake or gene expression of *Nucb2* and *Npy* in the hypothalamus (Extended Data Fig. 6m–o). We hypothesized that systemic changes induced by the

Fig. 4 | *NUCB1*- and *Lgr3*-expressing neurons are markedly increased and overlapped in the cancer condition. **a–d**, Immunostaining with antibodies against *NUCB1* and *Lgr3* showed that a small number of neuronal cells expressed low levels of endogenous *NUCB1* and *Lgr3* in the adult brain in normal non-tumour condition (arrowheads): nc82 (**a**); *NUCB1* (**b**); *Lgr3* (**c**); *NUCB1* and *Lgr3* (**d**). **e–h**, In the cancer condition caused by *GMR>yki^{SA}*, *NUCB1* and *Lgr3* immunostaining was significantly increased in the same neuronal cells (arrows): nc82 (**e**); *NUCB1* (**f**); *Lgr3* (**g**); *NUCB1* and *Lgr3* (**h**). **i–k**, Enlarged image of the white dashed boxes in **f–h**, showing increased intensity and overlapped *NUCB1*- and *Lgr3*-expressing neurons (arrows). **l–o**, The *Lgr3* mutant in the *yki* overexpression condition (*GMR>yki^{SA}*, *Lgr3^{Δ50}*) showed almost undetectable expression of *Lgr3* and a low level of *NUCB1* (arrowheads): nc82 (**l**); *NUCB1* (**m**); *Lgr3* (**n**); *NUCB1* and *Lgr3* (**o**). Scale bars, 100 μm. **p**, Quantitative PCR of adult heads showed that *NUCB1* mRNA levels were increased by approximately twofold in *GMR>yki^{SA}* (**P* = 0.021), reduced in the *Lgr3^{Δ50}* mutant (**P* = 0.019) and restored to the control level in *GMR>yki^{SA}*, *Lgr3^{Δ50}* (**P* = 0.049). *n* = 3 biologically independent experiments. **q,r**, Quantitative PCR of adult heads showed that *sNPF* (**q**) and *NPF* (**r**) mRNA levels were reduced to less than half of the control (*GMR-Gal4*) in *GMR>yki^{SA}* (***P* = 0.001 (**q**), ***P* = 0.004 (**r**)), increased in the *Lgr3^{Δ50}* mutant (**P* = 0.006 (**q**); **P* = 0.045 (**r**)) and restored in *GMR>yki^{SA}*, *Lgr3^{Δ50}* (***P* = 0.028 (**q**); **P* = 0.027 (**r**)). *n* = 3 biologically independent experiments. **s–u**, Dilp8 treatment increased *NUCB1* mRNA levels (**s**) and decreased *sNPF* (**t**) and *NPF* (**u**) mRNA levels in the ex vivo culture. *n* = 3 biologically independent experiments: 0.5 h ***P* = 0.002, 1 h ***P* = 0.0097, 2 h ***P* = 0.012 (**s**); 0.5 h ***P* = 0.007, 1 h ****P* = 0.0009, 2 h ***P* = 0.003 (**t**); 5 h ***P* = 0.0088, 1 h ***P* = 0.0023, 2 h ****P* = 0.0004 (**u**). Data are presented as the mean ± s.e.m. Statistical significance was determined with a two-tailed Student's *t*-test; **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

tumour may be required for CNS actions of peripherally delivered INSL3. To test this notion, we performed i.p. injection of INSL3 to LLC tumour-bearing mice that did not show anorexia. These experiments resulted in significantly reduced food intake and increased

Nucb2 mRNA expression in the hypothalamus (Fig. 6m–o). Taken together, these results suggest that cancer-derived INSL3 causes anorexia in mammals, similar to the effect of tumour-derived Dilp8 in *Drosophila*.

Fig. 5 | INSL3-LGR8 signalling regulates anorexigenic *Nucb2* and orexigenic *Npy* expression in mouse hypothalamic cells. **a**, The structural homology of human INSL3 and fly DILP8. **b**, Treatment in the INSL3 peptide increased the *Nucb2* mRNA level (1 h $^{**}P=0.0037$, 2 h $^{**}P=0.0036$) and decreased the *Npy* mRNA level (1 h $^{*}P=0.029$, 2 h $^{*}P=0.018$) in mHypoE-N3 cells. $n=3$ biologically independent experiments. **c**, No significant changes in *Nucb2* and *Npy* mRNA levels were detected after INSL3 treatment in mHypoE-N39 cells. $n=3$ biologically independent experiments. **d,e**, Knockdown of *Lgr8* in N3 cells showed no significant changes in *Nucb2* (**d**) (control $^{**}P=0.0019$, siLgr8 NS=0.698) and *Npy* (**e**) mRNA levels (control $^{**}P=0.0035$, siLgr8 NS=0.431) by INSL3 treatment. $n=3$ biologically independent experiments. **f,g**, *Lgr8* overexpression in N39 cells showed increased *Nucb2* (**f**) ($^{*}P=0.0195$) and decreased *Npy* mRNA level (**g**) ($^{*}P=0.034$) with INSL3 treatment. $n=3$ biologically independent experiments. Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined with two-tailed Student's *t*-test; $^{*}P<0.05$, $^{**}P<0.01$.

Serum INSL3 levels correlate with anorexia severity in patients with pancreatic cancer. Approximately 80% of pancreatic ductal adenocarcinoma (PDAC) patients present the cancer anorexia-cachexia syndrome^{46,47}. To test cancer anorexia in a mouse pancreatic cancer model, NSG mice were s.c. implanted with two different human-derived pancreatic cancer cell lines, Capan1 or Panc1, and food intake and anorexia-associated body mass were analysed. Tumour volume was gradually increased in both tumour-implanted mice (Extended Data Fig. 7a). Reduced food intake was observed at five weeks in the Capan1-implanted group compared with that in the non-tumour-implanted control or Panc1-implanted group (Fig. 7a). The wasting phenotype was observed in both tumour-implanted mice starting at five weeks after implantation (Extended Data Fig. 7b). The secreted INSL3 level was dramatically higher in Capan1 cancer cell culture media than in HPDE controls and Panc1 cells (Fig. 7b). In Capan1 cells, the INSL3 expression level was significantly high among relaxin-INSL family genes (Extended Data Fig. 7c). These results confirm that INSL3 is an evolutionarily conserved tumour-derived factor for inducing anorexia in cancer.

We compared INSL3 levels in the blood of patients with pancreatic cancer ($n=19$), patients with pancreatic benign disease ($n=5$) and patients with non-pancreatic benign disease ($n=7$) (Supplementary Table 2). This revealed that INSL3 serum level was significantly increased in patients with pancreatic cancer compared to other benign disease groups (Fig. 7c). Increased INSL3 expression was also found in pancreatic tumour tissue compared to normal tissue (Fig. 7d and Supplementary Fig. 6). In addition, we checked the correlation of INSL3 serum levels and feeding problems in a human PDAC patient group ($n=19$) and a non-PDAC patient group ($n=8$) (Supplementary Table 3). There was a negative correlation between serum INSL3 level and calorie intake in PDAC patients ($R^2=0.206$), but a significant positive correlation in non-PDAC patients ($R^2=0.0991$) (Fig. 7e,f). Also, INSL3 serum level was increased significantly in PDAC patients with various degrees of anorexia compared with PDAC patients without anorexia (Fig. 7g). These results indicate that tumour-derived INSL3 is associated with the induction of cancer anorexia in patients with pancreatic cancer.

Discussion

In this study, we have examined the cancer anorexia mechanism induced by the secretion of Dilp8/INSL3 from tumour tissues, which systemically controls the expression of feeding peptide hormones through the Lgr3/Lgr8 receptor in the brain (Fig. 7h). Dilp8 secretion in our *yki* tumour model was strongly supported by a recent study showing that Yorkie (*yki*) and its binding partner Scalloped directly regulate *Dilp8* expression⁴⁸. The present data demonstrate that Dilp8 and its mammalian orthologue INSL3 are critical factors involved in cancer anorexia in *Drosophila* and mammals.

Two recent studies using fly cancer models reported that the inhibition of insulin signalling induces systemic organ wasting, a characteristic of cancer cachexia^{27,28}. Both papers reported that Impl2, a mammalian IGFBP that is secreted from hyper-proliferating tissues and tumours, causes systemic organ wasting independently of food consumption. Consistent with previous data, our fly cancer model showed the organ wasting phenotype, and the severity of the wasting phenotype increased time-dependently (Fig. 1c,d). Importantly, the anorexia phenotype was observed at early stages, consistent with the fact that most cancer patients show loss of appetite at the pre-cachexia stage⁴. Although the inhibition of *Dilp8* does not restore the cancer-induced organ-wasting phenotype^{27,28}, *Dilp8* inhibition rescued the cancer anorexia phenotype (Fig. 2d), suggesting that Dilp8 and Impl2 play critical roles in regulating cancer anorexia and cachexia, respectively.

Another *Drosophila* tumour-secreted factor, Upd2, does not cause organ wasting^{27,28} or anorexia in flies (Fig. 2d), whereas mouse IL-6 (equivalent to Upd2) induces white adipose tissue browning in the cachexia condition⁴⁹. A recent report shows that tumour-derived IL-6/Upd induces non-cell-autonomous autophagy around tumour tissues⁵⁰. This paper suggests that tumour cells can acquire nutrition by eating adjacent cells for growth and proliferation.

We discovered a fly anorexigenic hormone, *Drosophila* NUCB1, a homologue of mammalian NUCB2. Nesfatin-1, a processed form of NUCB2, was initially discovered as a potential anorexigenic modulator of food intake and body weight independent of leptin signalling^{42,51–53}. The NUCB2 precursor protein is expressed in the hypothalamic region, including the supraoptic nucleus, lateral hypothalamic area, arcuate nucleus, paraventricular nucleus⁴² and parabrachial nucleus (PBN)⁵⁴. Recent findings indicate that calcitonin gene-related peptide (CGRP)-positive neurons in the PBN region contribute to cancer-induced appetite suppression in mice implanted with LLC cells⁵⁵. The *Drosophila* NUCB1/nesfatin-1 described in this study is identified as an evolutionarily conserved anorexigenic molecule expressed in the fly brain. We propose that NUCB1-expressing neurons in the brain are important for anorexigenic regulation in normal and cancer conditions. Despite substantial evidence supporting that nesfatin-1 binding to G protein-coupled receptors increases the cAMP response^{56–58}, a receptor specific to NUCB1/nesfatin-1 remains to be identified.

In this Article, we have demonstrated that tumour-secreted Dilp8 induces cancer anorexia through the Lgr3 receptor, and INSL3 and Lgr8 are predicted to be mammalian orthologues of Dilp8 and Lgr3, respectively. We have also showed that the regulation of INSL3 in mice is conserved in cancer anorexia. The secreted INSL3 in cell culture medium and INSL3 serum level were increased in the mouse cancer model and in human cancer patients, respectively. Consistently, food intake was reduced when INSL3 was injected

Fig. 6 | INSL3 regulates *Nucb2* and *Npy*, and induces anorexia in the mouse tumour model. **a,b**, Food intake was reduced in C26 tumour mice (**a**) ($***P=2.49 \times 10^{-8}$) but not in LLC tumour mice (**b**) (NS = 0.295). $n=7$ mice. **c**, A higher secreted level of INSL3 was seen in C26 culture medium than in LLC medium. $n=3$ biologically independent experiments, $**P=0.026$. **d**, Food intake was significantly reduced from day 11 in C26 tumour implanted mice. Control $n=6$ mice, C26 $n=8$ mice; day 11 $*P=0.013$, day 12 NS = 0.078, day 13 $***P=0.0009$. **e**, Body weight was unchanged in C26 tumour mice until day 13. Control $n=6$ mice, C26 $n=8$ mice. **f**, Muscle wasting (*MuRF-1*, *MAFbx*, *Fbxo31* and *Musa1*) and fat lipolysis (*Atgl* and *Hsl*) molecular markers of C26 tumour mice at day 12 in gastrocnemius (GCM) and epididymal white adipose tissue (eWAT). Control $n=5$ mice, C26 $n=6$ mice; *Atgl* $**P=0.0017$; *Hsl* $***P=0.0003$. **g,h**, Significantly increased *Nucb2* (**g**) ($*P=0.017$) and unchanged *Npy* (**h**) (NS = 0.164) mRNA levels in C26 tumour mice hypothalamus at day 12. Control $n=5$ mice, C26 $n=5$ mice. **i**, Increased muscle wasting and fat lipolysis molecular markers of C26 tumour mice at day 21. Control $n=5$ mice, C26 $n=6$ mice; *MuRF-1* $*P=0.033$, *MAFbx* $*P=0.042$, *Fbxo31* NS = 0.082, *Musa1* NS = 0.368, *Atgl* $*P=0.022$, *Hsl* $**P=0.005$. **j,k**, Significantly increased *Nucb2* (**j**) ($*P=0.035$) and decreased *Npy* (**k**) ($**P=0.008$) mRNA levels in C26 tumour mice hypothalamus at day 21. Control $n=5$ mice, C26 $n=5$ mice. **l**, The synthetic INSL3 peptide i.c.v. injection reduced food intake at both $5 \mu\text{g} \mu\text{l}^{-1}$ ($*P=0.011$) and $1 \mu\text{g} \mu\text{l}^{-1}$ ($***P=0.009$). $n=9$ mice. **m-o**, The synthetic INSL3 peptide i.p. injected LLC tumour-bearing mice showed reduced food intake (**m**) (PBS $n=13$ mice, INSL3 $n=13$ mice; $***P=0.0007$) and increased *Nucb2* mRNA level (**n**) ($**P=0.002$), but the *Npy* mRNA level was unchanged (**o**) (PBS $n=3$ mice, INSL3 $n=4$ mice). Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined with two-tailed Student's *t*-test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

into the brain of mice. Taken together, the fly and mammalian data support our cancer anorexia model (Fig. 7h). These findings also suggest that Dilp8/INSL3-Lgr3 signalling is a potential therapeutic target in cancer anorexia.

Online content

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Fig. 7 | Serum INSL3 levels correlate with anorexia severity in patients with pancreatic cancer. **a**, The food intake of Capan1-implanted mice at five weeks was decreased compared with that in control or Panc1-implanted mice. $n=4$ mice for each group, $**P=0.009$. **b**, The secreted level of INSL3 in Capan1 culture media was remarkably increased compared with those in the control. $n=4$ biologically independent experiments, $***P=0.0001$. **c**, A highly increased INSL3 level was detected in the serum of patients with pancreatic cancer. One-way analysis of variance (ANOVA) $*P=0.040$. **d**, The protein level of INSL3 was increased in pancreatic tumour tissue. Scale bars, 100 μm. **e, f**, Unlike the non-PDAC group (**e**), patients in the PDAC group showed a decrease in food intake with increasing INSL3 levels (**f**). **g**, The INSL3 serum level was increased significantly in PDAC patients with various degrees of anorexia than in PDAC patients without anorexia. $n=6$ anorexia-absent patients, $n=13$ anorexia-present patients, $*P=0.029$. **h**, Our model of the cancer anorexia mechanism. Data are presented as the mean $\pm$ s.e.m. Statistical significance in **a**, **b** and **g** was determined with a two-tailed Student's t -test, and in **c**, **e** and **f** with one-way ANOVA; $*P<0.05$, $**P<0.01$, $***P<0.001$.

author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41556-020-00628-z>.

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Methods

Drosophila culture and stocks. *Drosophila melanogaster* were maintained at 25 °C on standard media. *GMR-Gal4*, *UAS-yki^{WT}*, *UAS-yki^{S168A}*, *NUCB1-RNAi*, *Dilp8^{M100727}* were obtained from the Bloomington Stock Center and *Dilp8-RNAi*, *ImpL2-RNAi* and *Upd2-RNAi* were obtained from Vienna Drosophila RNAi Center (VDRC). *UAS-Dilp8* and *UAS-Lgr3* were gifts from A.M. Gontijo (NOVA Medical School) and *Lgr3-Gal4* was a gift from P. Leopold (Institute of Biology Valrose). The *pUAS-Nesfatin1* transgenic fly was generated by the p-element-mediated germline transformation method⁵⁹ containing the processed form of *NUCB1 cDNA*.

CRISPR/Cas9-mediated mutagenesis. The *Lgr3* mutant was generated by CRISPR/Cas9-mediated mutagenesis⁶⁰. The single-guide RNA target sequence was designed and generated by Toolgen (toolgen.com). Mutant screening by T7E1 assay and direct sequencing followed the protocol described in ref. ⁶¹. The *Lgr3* mutant consists of a 50-base-pair deletion upstream of the target sequences. Capital letters indicate the second exon of the *Lgr3* genomic region, bold capital letters indicate target sequences and underlined lower-case letters indicate deleted sequences:

ctaggaattataaaaggtacaaacaaagatattaaatgtttcaagacacaaatgtttgcttgatgctaacag-
ctctaggaattatattttggttaaaatcatcagatgtttgtttgattccccaaaatctgtgagaaactagttgaact-
cacatgcaaatgctccgctgctctgagtgataattattgggatcgtccctttccagGACCCCTGCCCGCGC
GCCAGCTTCGCCTGCGACAAACGGCACGCTGTGCGTTCCAAGGCGCCAG
ATGTGCGACAGTCGGAACGATTGCGCCGACAGCAGCGACGAGAATCCG

RNA preparation and quantitative real-time PCR. Adult heads from 20 flies were collected for RNA preparation. Total RNA was extracted using the easy-BLUE reagent (iNTRON Biotechnology). All RNA samples were treated with RNase-free DNase (Promega) and cDNA was synthesized using a SuperScript III First-Strand synthesis system (Invitrogen). For qRT-PCR analysis, an ABI Prism 7900 sequence detection system (Applied Biosystems) and SyberGreen PCR Core reagents (Applied Biosystems) were used. mRNA levels were expressed as the relative fold change against the normalized *rp49* mRNA. The comparative cycle threshold (Ct) method (User Bulletin 2, Applied Biosystems) was used to analyse the data.

RNA-sequencing analysis. To extract total RNAs for RNA-seq experiments, 30 adult heads were dissected and isolated with easy-BLUE reagent (iNTRON Biotechnology). RNA quality control and data analyses were performed by ebiogen (ebiogen.com).

Generation of the NUCB1 and Dilp8 antibody. NUCB1 antibody was generated by the immunization of rabbits with the synthetic peptide (TQNKKDHKEAAESSTPATAD) and the immunization of guinea pigs with the synthetic peptide (EEKHKDEKHLHPGNGKAQ) of the NUCB1 protein (Abfrontier). Dilp8 antibody was generated by the immunization of rats with the same peptide sequence as described in ref. ⁶².

Immunostaining. For immunostaining, adult brains were dissected in phosphate buffered saline (PBS), fixed in 4% paraformaldehyde (PFA) and blocked in 5% bovine serum albumin (BSA). Primary antibodies were incubated overnight at 4 °C and secondary antibodies were incubated for 2 h at room temperature. The tissues were mounted with Vectashield mounting medium (Vectashield) and fluorescence images were acquired by a FluoView confocal microscope (Olympus). sNPF (1:200), NUCB1 (1:100), *Lgr3* (1:200, a gift from M. Dominguez), NPF (1:2,000, a gift from P. Shen), brp (nc82, 1:100, DSHB) primary antibodies and anti-mouse immunoglobulin-G (IgG) Alexa 488, anti-guinea pig IgG Alexa 594 and anti-rabbit Alexa 633 secondary antibodies (1:200, Molecular Probes) were used.

Western blot analysis. Total proteins from adult heads were lysed by PRO-PREP protein extraction buffer (iNTRON biotechnology). Western blot analyses were performed as described previously⁶³. β -actin (1:3,000, DSHB), *Lgr8* (1:1,000, Novus), NUCB1 (1:1,000) and *Dilp8* (1:1,000) primary antibodies and anti-rabbit IgG (1:5,000, Santa Cruz), anti-rat IgG (1:5,000, Invitrogen) and anti-mouse IgG (1:5,000, Invitrogen) secondary antibodies were used.

Measurement of Drosophila life span. Flies used in life span experiments were collected upon eclosion over a 48-h period, with a total of 20 vials ($n \approx 200$) for each genotype. Flies were transferred to fresh food every other day, and dead flies were scored and counted. Lifespan analyses were performed and Log rank test statistics calculated using the online application OASIS2⁶⁴.

Drosophila food intake assay. *Drosophila* food intake was measured by two different assays. The CAFE assay³⁷ was performed with three-day-old adult male flies. Four flies were placed in the CAFE device³⁷ containing a 5% sucrose solution in calibrated glass micropipettes (VWR) and adapted for 12 h before the assay. At time zero, the micropipettes with 5% sucrose solution were replaced, and the amount of liquid consumed was measured every 12 h. For colorimetric food intake assay, flies were starved in PBS-containing vials for 2 h and fed for 15 min in vials containing 0.05% FD&C Blue dye, 7% sucrose and 5% yeast. The flies were frozen, homogenized in PBS, and centrifuged twice for 25 min each. The supernatant was measured at 625 nm. Each experiment consisted of 20 flies and the assay was repeated three times.

Measurement of total body triglyceride level. Ten adult flies were ground in PBS solution and centrifuged. The supernatant was used for analysis. Total glycerol and triglyceride levels were measured using a serum triglyceride determination kit (Sigma). The protein levels were measured to normalize the triglyceride level.

Drosophila starvation. Twenty *w⁺* adult flies were starved overnight and fed for 2 h to achieve physiological synchronization. Then, starvations for the experiment were started until each time point. The heads from starved flies were collected for qPCR analysis.

Ex vivo culture assay. The adult brains were dissected and cultured in 100 μ l of Schneider's medium (Sigma) with or without *Drosophila* Dilp8 peptide (Phoenix) at room temperature and collected for RNA preparation.

Cell culture and mouse models. The embryonic mouse hypothalamic cell lines mHypoE-N3 and mHypoE-N39, purchased from Cellutions Biosystems, were maintained at 37 °C, 5% CO₂ in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. Cells were treated with 100 nM mouse INSL3 peptide (Phoenix Pharmaceuticals) after 1 h of starvation. mHypoE-N3 cells were transfected with siRNA targeting mouse *Lgr8* (Bioneer, 140498-1, 140498-2, 140498-3) using Lipofectamine RNAiMAX (Invitrogen, 13778150) for 48 h according to the manufacturer's protocol and then treated with vehicle or INSL3 for 2 h.

C26 and LLC were obtained from the American Type Culture Collection (ATCC). C26 cells were maintained in RPMI-1640 (Hyclone) containing 10% FBS (Hyclone) and 1% antibiotic-antimycotic solution (AA; Gibco/Thermo Fisher Scientific). LLCs were maintained in DMEM (Hyclone) containing 10% FBS and 1% AA.

Seven-week-old female Balb/c mice or C57BL/6 mice (Narabio Inc.) were housed in a specific pathogen-free animal facility (KAIST, Daejeon, Korea) in a temperature-controlled environment at 23 °C under a 12-h:12-h light-dark cycle. For tumour-implanted mouse models, 5×10^5 C26 cells (into Balb/c) or LLC cells (into C57BL/6) were administrated a s.c. injection in 100 μ l of sterilized PBS (tumour-implanted group) or 100 μ l of sterilized PBS without cells (control group). To identify the effects of C26 or LLC tumour growth on body weight and food intake, body weight was measured every other day and food intake was weighed daily after two weeks of tumour inoculation when C26 mice displayed symptoms of body weight loss. Mice were euthanized at 21 days after tumour cell injection. Organs were collected and used for further analysis. In each animal experiment, mice were randomly selected to each group. All mouse experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Korea Advanced Institute of Science and Technology.

Human pancreatic adenocarcinoma Capan1 and Panc1 cell lines were obtained from the ATCC, maintained in the ATCC recommended growth media, and incubated at 37 °C, 5% CO₂. Briefly, Capan1 and Panc1 cells were grown in Iscove's modified Dulbecco's medium with 10% FBS and antibiotics. For tumour-implanted mouse models, NOD.Cg-Prkdc^{cid} Il2rg^{tm1Wjl}/SzJ (NSG) mice (The Jackson Laboratory) were housed in a pathogen-free animal facility at KRIBB and used for tumour implantation studies. Six-week-old male NSG mice were implanted with 5.0×10^5 Capan1 or Panc1 cells s.c. in their abdomens. Weight and food intake were measured weekly. Tumour measurements were performed every week with a caliper, and volumes were calculated using the following formula: $V = 1/2(\text{length (mm)} \times \text{width (mm)})^2$. This study is compliant with all relevant ethical regulations regarding animal research.

RNA fluorescence in situ hybridization. RNA fluorescence in situ hybridization (FISH) was performed using an RNAscope multiplex fluorescence kit (ACDbio, 323133). In brief, mice were anaesthetized with Zoletil (Virbac Korea, Zoletil 50) and transcardially perfused with ice-cold PBS followed by 1% PFA. Brains were extracted, post-fixed overnight in 4% PFA at 4 °C, then 40- μ m-thick coronal brain sections were obtained on a vibratome (Leica, VT1200S) and collected in six equal series. Slices were stored at -20 °C in hand-made anti-freezing solution (poly and glycerol mixture) until RNA FISH was performed. The sections were removed from anti-freezing solution and washed six times in Tris-buffered saline, for 10 min. After washing, sections were mounted directly onto Histobond (Marienfeld, 0810001) slides and the sections were dried at 60 °C overnight. The following day, slides were dehydrated with 100% ethanol and dipped in boiling target retrieval solution for 20 min. A hydrophobic barrier was created and slides were treated with protease III for 30 min at 40 °C. The probe against *Rxfp2* mRNA (ACDbio, 589261-C3) was added into slides. After 3 h of incubation at 40 °C, hybridize-Amp probes #1-4 were applied on slides sequentially to amplify the signal. For every RNA FISH run, the *Escherichia coli* gene *dapB* was used as a negative control, and *Mm-Polr2a*, *Mm-PP1B* and *Mm-UBC* were used as housekeeping genes. All sections were counterstained with DAPI (Invitrogen, 62248). Fluorescent images were captured using a Zeiss LSM 880 confocal scanning microscope (Carl Zeiss, LSM 880). Contrast and brightness were corrected using ZEN blue software.

Enzyme-linked immunosorbent assay. Cultured media for C26, LLC, Capan1, Panc1 and HPDE (human pancreatic duct epithelial) cell lines and patients' blood

were used for INSL3 detection. The process was performed as described in the INSL3 enzyme-linked immunosorbent assay kit datasheet from MyBioSource Inc. All samples were measured at individual levels and the results were from more than three independent experiments.

Stereotaxic surgery and intracerebrospinal injection. A guide cannula (26 G; Plastics One, C315GS-5/SPC) was inserted into the lateral ventricle (1.0 mm lateral; −0.7 mm posterior to bregma; 1.3 mm below the skull surface) of mice using a stereotaxic apparatus (KOPF, model 900LS). Mice were anaesthetized with Zoletil (Virbac Korea, Zoletil 50) during stereotaxic surgery and were allowed to recover after surgery for at least seven days. Food intake and body weight were recorded during the recovery period and mice that did not regain normal weight were excluded from studies. After the recovery period, proper insertion of the cannula was assessed by measuring the drinking response to i.c.v. injection of angiotensin II ($2 \mu\text{g}\mu\text{l}^{-1}$; Sigma, A9525). For i.c.v. injections to the lateral ventricle, aCSF (Tocris, 3525) or INSL3 peptide (Anygen) dissolved in aCSF at a concentration of $5 \mu\text{g}\mu\text{l}^{-1}$ or $1 \mu\text{g}\mu\text{l}^{-1}$ was injected over 60 s in a final volume of 1 μl using a 33-G injector cannula (Plastics One, C315I/SPC) extending 0.5 mm beyond the tip of the i.c.v. cannula.

Data collection from patients with pancreatic cancer. The study complied with the Declaration of Helsinki, and the protocol was reviewed and approved by the Institutional Review Board of Asan Medical Center (IRB no. 2017-0305). Written informed consent was obtained from all participants. Patient-, surgery- and oncology-related data were obtained from medical records. Peripheral blood was drawn from patients with pancreatic cancer and other diseases who were diagnosed by pathological examination at Asan Medical Center in South Korea. The plasma was separated and stored at -70°C until analysis. Anorexia grade was measured by the Common Terminology Criteria for Adverse Events (CTCAE) method version 4.0 (<https://www.upToDate.com/contents/common-terminology-criteria-for-adverse-events>) and classified as absence (Grade 0) and presence (Grade 1, 2, 3). Calorie intake was measured by the 24-hour dietary recall method^{65,66}. This study is compliant with all relevant ethical regulations regarding research involving human participants.

Statistics and reproducibility. All experiments were performed more than three times with similar results. Statistical analyses were conducted using GraphPad Prism version 8.1 (GraphPad Software) and Microsoft Excel-based application (Microsoft Office Professional Plus 2016) for the Student *t*-test statistical analysis. To compare two normally distributed groups, unpaired two-tailed *t*-tests were used. For multiple comparisons between normally distributed groups (Supplementary Tables 1 and 2), one-way ANOVA and Mann–Whitney U-test were used. Lack of an asterisk means non-significant ($P > 0.05$). Values in this paper are presented as mean $\pm$ s.e.m., *P* values are indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this Article.

Data availability

The RNA-seq data have been deposited in the GEO database under accession code [GSE154404](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154404). Data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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Author contributions

E.Y., K.-S.L. and K.Y. designed the research. S.C.K. provided key reagents. E.Y., H.S., W.Y., E.J., S.H.H., D.-W.K. and T.H.R. performed experiments. E.Y., S.K., J.M.S., S.C.K., K.-S.L. and K.Y. analysed the data. E.Y., K.-S.L. and K.Y. wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Extended Data Fig. 1 | Generation of the *Drosophila* cancer anorexia model. **a, *Drosophila* cancer model was established by overexpressing a wild-type (*yki^{WT}*) and a constitutively active form of *yki* (*yki^{S168A}*) using *GMR-Gal4*. Expression of the wild-type of *yki* (*GMR>yki^{WT}*) caused the mild rough eye phenotype, whereas the active form (*GMR>yki^{SA}*) showed dramatic protrusion of the eye. Scale bars are 200 μm. **b**, The 10 day-old *yki* overexpression flies showed decreased food intake compared with that of the control in the blue dye feeding assay. *n* = 3 biologically independent experiments; 1.5 days **P* = 0.022, 2 days ***P* = 0.003. **c**, *UAS-yki^{WT}* and *UAS-yki^{SA}* control flies showed normal feeding. *n* = 3 biologically independent experiments. **d**, The triglyceride levels of *GMR>yki^{SA}* was analyzed with female flies. *n* = 3 biologically independent experiments; day 10 **P* = 0.038, day 15 **P* = 0.035, day 20 **P* = 0.012. **e**, Quantification of ovary size in Fig. 1c. *n* = 3 biologically independent experiments; day 10 ****P* = 0.0006, day 15 ***P* = 0.0014, day 20 ****P* < 0.001. **f**, The thoracic muscle of 15-day-old *GMR>yki^{SA}* showed abnormal mitochondrial structure and irregular packing of mitochondria between muscle fibers. *n* = 3 biologically independent experiments. Scale bar is 10 μm. Data are presented as the mean ± s.e.m. Statistical significance was determined with two-tailed Student's *t*-test; **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Statistical source data.**

Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | RNA-seq analysis, anorexia in *sev* > *Rasv12* flies, *Lgr3* Δ 50 generation, *Lgr3* expression in adult tissues, and eye phenotypes in *GMR* > *yki*^{SA} and the mutants of *Dilp8* and *Lgr3*. **a**, RNA-seq analysis showed the increased levels of three tumor-secreted factors (*Dilp8*, *ImpL2*, and *Upd2*) in *GMR* > *yki*^{SA} flies. **b**, *sev* > *Ras*^{v12} flies showed anorexia and this phenotype was effectively rescued by *Dilp8*-RNAi. *n* = 3 biologically independent experiments; *sev* > *Ras*^{v12} **P* = 0.012, *sev* > *Ras*^{v12} *Dilp8*-RNAi **P* = 0.013, *sev* > *Ras*^{v12} *ImpL2*-RNAi **P* = 0.019. **c**, *Dilp8* antibody test was performed by western blot and showed almost no expression in the *Dilp8*^{M100727} mutant. **d**, *Dilp8* protein was increased in *GMR* > *yki*^{SA} fly heads and reduced in *GMR* > *yki*^{SA}, *Dilp8*-RNAi fly heads by western blot. **e**, The quantification of d using ImageJ program. *n* = 3 biologically independent experiments; *GMR* > *yki*^{SA} **P* = 0.047, *GMR* > *yki*^{SA} *Dilp8*-RNAi **P* = 0.045. **f**, The *Lgr3* Δ 50 mutant was obtained using the CRISPR/Cas9-mediated mutagenesis. It consists of 50 base pair deletion in the intron region upstream of target sequences. **g**, *Lgr3* mRNA expression in the *Lgr3* Δ 50 mutant showed that the *Lgr3* Δ 50 mutant is a strong hypomorphic allele. *n* = 3 biologically independent experiments, ***P* = 0.007. **h**, *GMR* > *yki*^{SA}, *Dilp8*^{M100727} and *GMR* > *yki*^{SA}, *Lgr3* Δ 50 flies respectively blunted or reversed the cancer anorexia phenotype observed in *GMR* > *yki*^{SA} flies. *n* = 4 biologically independent experiments; *GMR* > *yki*^{SA} **P* = 0.010, *GMR* > *yki*^{SA} *Dilp8*^{M100727} **P* = 0.012, *GMR* > *yki*^{SA} *Lgr3* Δ 50 **P* = 0.027. **i**, *Dilp8* overexpression flies (*GMR* > *Dilp8*) showed reduced food intake. *n* = 6 biologically independent experiments, **P* = 0.034. **j**, In *GMR* > *yki*^{SA} and *GMR*-*Gal4* control flies, the *Lgr3* mRNA expression level in the head is very high relative to other tissues. *n* = 3 biologically independent experiments; head **P* = 0.017; thorax ***P* = 0.006, gut **P* = 0.035. **k**, The eye phenotype in *GMR* > *yki*^{SA} is similar with those of *GMR* > *yki*^{SA}, *Dilp8*^{M100727} and *GMR* > *yki*^{SA}, *Lgr3* Δ 50. Scale bar is 200 μ m. **l**, *Lgr3*-*Gal4* expression marked by GFP. *Lgr3* expression was not detectable in sNPF neurons. Scale bar is 100 μ m. **m-n**, sNPF (m) (**P* = 0.018) and NPF (n) (**P* = 0.048) mRNA levels were reduced by RNAi lines with *Lgr3*-*Gal4*. *n* = 3 biologically independent experiments. Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined with two-tailed Student's *t*-test; **P* < 0.05, ***P* < 0.01. Statistical source data and unprocessed western blots.

cleavage site for Nesfatin1

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Extended Data Fig. 4 | Intensity quantification of NUCB1 and Lgr3 antibodies immunostaining. **a**, NUCB1 antibody specificity was tested by the NUCB1 inhibition using *heat-shock Gal4* (*Hs>NUCB1-RNAi*). **b**, Staining intensity of neurons was measured with LSM 5 software program by drawing a dotted line as shown. **c-e**, The staining intensity of neuronal cells in Fig. 4 was quantified using LSM 5 software program and illustrated as intensity graphs. Control ($n=5$), *GMR>yki^{SA}* ($n=10$), *GMR>yki^{SA}, Lgr3^{Δ50}* ($n=5$). **f-g**, A subset of sNPF (**f**) and NPF (**g**) expressing neurons in the cancer condition (*GMR>yki^{SA}*) were overlapped with NUCB1 expressing neurons. Scale bars are 100 μ m. Statistical source data and unprocessed western blots.

Extended Data Fig. 5 | NUCB1 protein expression in the cancer condition, ex vivo analysis, and *Lgr3* expression by the Dilp8 treatment. **a**, In western blot of adult heads with the NUCB1 antibody, NUCB1 protein level is increased in *GMR>yki^{SA}*, reduced in the *Lgr3^{Δ50}* mutant, and restored to the control level in *GMR>yki^{SA}, Lgr3^{Δ50}*. **b**, The western blot bands of (a) were quantified using ImageJ program. $n=3$ biologically independent experiments; *GMR>yki^{SA}* $**P=0.003$, *Lgr3^{Δ50}* $*P=0.038$, *GMR>yki^{SA}, Lgr3^{Δ50}* $**P=0.002$. **c**, The schematic presentation of ex vivo culture assay in which the synthetic Dilp8 peptide was treated to the dissected adult brains in the culture media. **d**, *Lgr3* mRNA level was increased after Dilp8 peptide treatment in the ex vivo culture. $n=3$ biologically independent experiments; 0.5 hr $*P=0.020$, 1 hr $*P=0.021$. Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined with two-tailed Student's *t*-test; $*P<0.05$, $**P<0.01$. Statistical source data and unprocessed western blots.

Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Conservation of INSL3/Lgr8 in mammals, and cachexia-anorexia phenotype of C26 or LLC implanted mice. **a**, The phylogenetic comparative analysis of *Drosophila* Lgr3 and mammalian Lgr family shows that Lgr7/8 is a homologue of Lgr3. **b**, The phylogenetic comparative analysis of fly Dilp8 and mammalian INSL3 shows that they are classified as close peptides. **c**, Lgr8 protein is strongly expressed in the hypothalamic region and hippocampus of the mouse brain. **d**, In the RNA FISH, *Lgr8* mRNA is strongly expressed in the hypothalamic region and hippocampus of the mouse brain. **e**, *Lgr8* is expressed in the mouse hypothalamic N3 cells, but not in N39 cells. **f-g**, Body weight was changed in C26 tumor implanted mice (f) not in LLC tumor implanted mice (g). $n = 7$ mice for both groups. f, 2w + 2day $*P = 0.027$, 2w + 4day $***P = 0.0004$, 2w + 6day $**P = 0.0034$. **h**, Tumor volume and weight changes were measured in C26 and LLC implanted mice. $n = 6$ mice for both groups. **i-j**, Muscle and fat weight of C26 tumor implanted mice at day 12 (i) and day 21 (j). Tibialis anterior (TA), gastrocnemius (GCM), inguinal white adipose tissue (iWAT), and epididymal white adipose tissue (eWAT). Control $n = 3$ mice, C26 day 12 $n = 5$ mice, C26 day 21 $n = 4$ mice. i, iWAT $*P = 0.024$, eWAT $*P = 0.094$. j, GCM $*P = 0.038$, iWAT $**P = 0.001$, eWAT $*P = 0.013$. **k-l**, *Nucb2* (k) and *Npy* (l) mRNA levels were not changed in LLC implanted mice. $n = 5$ mice for both groups. **m**, INSL3 i.p. injected wild-type mice didn't show reduction of food intake. $n = 7$ mice for both groups. **n-o**, *Nucb2* (n) and *Npy* (o) mRNA levels were not changed in INSL3 i.p. injected wild-type mice hypothalamus. $n = 3$ mice for both groups. Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined with two-tailed Student's *t*-test; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Statistical source data and unprocessed western blots.

Extended Data Fig. 7 | Anorexia-associated body mass changes of human pancreatic cancer implanted mice. **a**, Tumor volumes were increased in Panc1 and Capan1 implanted mice. $n = 4$ mice for three groups; 3 week $*P = 0.049$, 4 week $*P = 0.049$, 5 week $*P = 0.033$. **b**, Body mass were reduced in Panc1 and Capan1 implanted mice compared to the control mice. $n = 4$ mice for each group; 3 week Panc1 $**P = 0.0014$ and Capan1 $*P = 0.049$, 5 week Panc1 $***P < 0.0001$ and Capan1 $***P < 0.0001$. 6 week Panc1 $***P < 0.0001$ and Capan1 $***P < 0.0001$. **c**, Relaxin-INSL family mRNA expression levels in Capan1 cells. $n = 3$ biologically independent experiments; Rxn vs INSL3 $**P = 0.003$. Data are presented as the mean $\pm$ s.e.m. Statistical significance was determined with two-tailed Student's t -test; $*P < 0.05$. Statistical source data.

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Data collection

Data collection was done using Excel program (Microsoft Office Professional Plus 2016) .

Data analysis

Statistical analyses were conducted using GraphPad Prism version 8.1 (GraphPad Software, CA, US) and Microsoft Excel-based application (Microsoft Office Professional Plus 2016). Anorexia grade was measured by Common Terminology Criteria for Adverse Events (CTCAE) method version 4.0. Lifespan analyses were performed with Log rank test statistics calculated using the on-line application OASIS2. In quantitative RT-PCR, the comparative cycle threshold (Ct) method (User Bulletin 2, Applied Biosystems) was used. Contrast and brightness for mouse fluorescent images were corrected using ZEN blue software. Staining intensity of Drosophila neuronal cells was quantified using LSM 5 software program.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data generated or analyzed in this study are included in this manuscript. All RNAseq data have been deposited in GEO database and the accession code is GSE154404.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample sizes of fly and mouse experiments were empirically determined. Progressively increasing numbers of mice from pilot experiments to reach statistical significance between different groups. Clinical sample sizes were determined in accordance with clinical availability that can assure the statistical significance.
Data exclusions	There are no data exclusions of fly and mouse experiments in this manuscript. Human patients who were not available of clinical information or not fitted in diagnostic criteria were excluded.
Replication	All fly experiments were repeated at least three times and results were reproducible. All mouse experiments were replicated at least twice and results were reproducible. The difference between the experimental group and control group were statistically significant in the repeated experiments.
Randomization	Experimental and control animals bred in identical conditions were randomized in each experiment. All animals were randomly assigned into the tested group after manipulation or treatment. Cells were cultured under identical conditions until random assignment to control and experimental groups. Scheme of data generation and analyses of specimens from randomized groups were designed to avoid batch effects.
Blinding	No blinding was done in fly and mouse experiments. All experiments performed with multiple steps including the material preparation, treatment, biochemical and molecular analysis, as well as behavioral assay. Fly and mouse experiments were not amenable to blinding as subjects from control and test group conditions become evident due to obvious differences in data obtained from downstream analyses. Furthermore, all experimental data for fly and mouse studies were generated by quantitative methods of analyses that are not affected by user bias. For prospective study with human patients, data were given a code to identify their groups and data analysis or histological scoring were blinded to experimental groups.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	In the immunostaining of fly samples, anti-sNPF (our lab generated, 1:200, Lee et al., JBC, 2004), anti-NUCB1 (1:100 in this study), anti-Lgr3 (1:200, a gift from M.Dominguez, not commercial), anti-NPF (1:2000, a gift from P. Shen, not commercial), anti-brp (1:100, DSHB, nc82), anti- β -actin (DSHB, JLA20), anti-rabbit IgG (Santa Cruz, sc2357), anti-rat IgG (Invitrogen, 31470), anti-mouse IgG (Invitrogen, 31430) were used. In mammalian study, anti-Lgr8 (1:1000, Novus, NBP1-01001) was used. In human patient study, anti-INS3 (1:100, Abcam, ab227974) was used. Conjugated fluorescent secondary antibodies were obtained from Molecular Probe.
Validation	We generated two antibodies (anti-Dilp8 and anti-NUCB1) for fly experiments and purified by the affinity chromatography. Specificity of new generated antibodies was validated using knockdown or knockout flies. All commercial antibody lots are routinely tested by the manufacturers. anti-INS3 is validated by abcam and suitable for WB, IHC-C, IP. anti-Lgr8 is validated by Novus and suitable for WB, IHC.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	C26 and LLC was obtained from the American Type Culture Collection (ATCC). Embryonic Mouse Hypothalamic Cell Lines, mHypoE-N3 and mHypoE-N39 cell lines purchased from Cellutions Biosystems. Human pancreatic adenocarcinoma Capan1 and Panc1 cell lines were obtained from the American Type Culture Collection (ATCC).
Authentication	Morphological shape of cell lines was monitored with the microscopic examination.
Mycoplasma contamination	All cell lines were mycoplasma negative confirmed by the standard PCR method.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines are used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Wild type and mutant strains of <i>Drosophila melanogaster</i> were used in this study, which obtained from Bloomington <i>Drosophila</i> Resource Center, Vienna Stock Center, and other <i>Drosophila</i> researchers. Both male and female flies were used in this study. 5-days old flies were used except for aging experiments. <i>Mus musculus</i> strain C57BL6/J strain males of between 8-10 wk of age and Balb/c strain females of between 7-12 wk of age were used for mouse experiments. For tumor implanted mouse models, 6 wk-old male NSG mice (Jackson Laboratory) were used.
Wild animals	No wild animals were used in the study.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All mouse experiments were approved by the Institutional Animal Care and Use Committee of Korea Institute of Advanced Science and Technology.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Patients who have diagnosed with pancreatic cancer or benign pancreatic disease or other benign disease other than pancreas were included. Patients' information were described in the supplemental tables 1-2 of the manuscript.
Recruitment	Patients, who were admitted to the Division of Hepato-Biliary Pancreatic Surgery in Asan Medical Center for the diagnosis and treatment of diseases, have recruited with full informed consent.
Ethics oversight	Protocol was reviewed and approved by the Institutional Review Board of Asan Medical Center (IRB No2017-0305)

Note that full information on the approval of the study protocol must also be provided in the manuscript.