Supplemental data figure legends

FIG. S1. Pak1 expression profiles in selected tissues of C57BL/6 and CD1 mice. Protein lysates were prepared from indicated tissues of 8-weeks-old male C57BL/6 or CD1 mouse. Equal amount of proteins (40 µg) were loaded for Western blotting with Pak1 and γ-tubulin antibodies.

FIG. S2. Wnt ligand Wnt3A stimulates gcg promoter and mRNA expression in GLUTag cell line. A: GLUTag cells were transfected with 1 µg of 2.4kb-gcg-LUC overnight, serum-starved for 16 h, and treated with indicated concentration of Wnt3A for 2 h. Cells are subsequently harvested for LUC analysis. B: GLUTag cells were treated with 2.5 nM Wnt3A or Wnt11 (a non-canonical Wnt ligand as control) for 2h, followed by RNA extraction and qRT-PCR.

FIG. S3. IPA3 pre-treatment has no effect on gcg expression in the pancreatic α cell line InR1-G9. InR1-G9 cells were serum-starved for 16 h, pre-treated with or without IPA3 (10 µM, 1h) followed by treatment with or without insulin (100 nM, 4h). RNA was then extracted for Northern blotting. DMSO is the vehicle for IPA3.

FIG. S4. Body weight monitoring of Pak1−/− mice in C57BL/6-129 mixed genetic background. Measurements performed with n=5 adult male mice per group.

FIG. S5. Pak1−/− mice exhibit comparable pancreatic islet architecture. Pancreas sections of a Pak1−/− mouse and an age-matched wild-type controls (age 9 or 10wks) were prepared for co-
immunostaining of insulin (brown) and glucagon (red). Arrow points to the islet shown in the
insets, bar indicates A and C: 1 mm, B and D: 100 μm.

FIG. S6. Pak1−/− mice exhibited reduced weight of distal ileum. Male mice were sacrificed for
organ extraction and organ weight measurement at the age of 16 wks (A) (n≥6) or 8 wks (B)
(n≥6). Pak1−/− mice showed significant reduction in distal ileum weight (measurement performed
using 5 cm segments), but no difference was observed for pancreas, liver, epididymal fat weight
(data not shown).
Fig. S1

Control  Wnt3A
0  (0.25 nM)
1  Wnt3A
2  (2.5 nM)

Luciferase (fold change)

A

B

Basal
DMSO
IPA3

Wnt3A
Wnt11

Basal
Insulin

N.S.

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Fig. S2

Fig. S3

C57BL/6

CD1

Pak1
γ-tubulin

Pak1
γ-tubulin

DMSO
IPA3

Basal
Insulin

gcg

tubulin

Basal
Insulin
Fig. S6