Supplementary Figure Legends

Figure S1 Clinical diagnosis, insulin and glucagon positivity of PanNET samples used in the study.
The numbers of insulin- or glucagon-positive tumors (including partially positive tumors) with or without *PHLDA3* LOH were counted, and analyzed by Fisher's exact test (B, C).
NF; non-functional PanNET. ND; no data.

Figure S2 Analysis of the genomic sequence of the *PHLDA3* gene within the ORF region.
We used 4 primers (3-seq: gcc cgc atc aag gcc gtg, 4-seq: ggg tag cat gaa gga aag atg, 5-seq: gcc gca gcc tgg agc ttt c, 6-seq: ctt cgg tca cca gcg tga ag) to determine the genomic sequence of the ORF region of the *PHLDA3* gene. Genomic DNAs from PanNET patients were PCR amplified using primers 3-seq and 4-seq, or 5-seq and 6-seq. Subsequently, the PCR products were directly sequenced.

Figure S3 5-aza-C treatment of MIN6 cells, a mouse PanNET cell line.
(A) PHLDA3 protein levels were analyzed by Western blotting.
(B) Total RNAs were isolated, and *PHLDA3* mRNA levels were analyzed as in Fig. 8A.

Figure S4 Methylation status of *PHLDA3* promoter in PanNETs (samples without LOH at the *PHLDA3* locus was analyzed). Genomic DNAs were prepared and analyzed as described in Fig. 4G.

Figure S5 Effect of PHLDA3 expression on Akt activity and phosphorylation of Akt downstream signaling molecules in *PHLDA3* -/- MEFs.
PHLDA3 -/- MEFs were transduced with Ad-LacZ or Ad-PHLDA3 at a moi of 60. Cells were harvested 36 hrs post-infection. Akt activation, phosphorylation of Akt downstream signaling molecules were analyzed by Western blotting and quantified by normalization to total Akt levels (P-Akt) or by β-actin levels (P-p70 S6K, P-Mdm2). PHLDA3 expression was detected by anti-HA anitibody.

Figure S6 PHLDA3 protein levels in isolated rat islets.
PHLDA3 protein levels were determined by Western blotting, using isolated rat islets subjected to γ-ray irradiation (20 Gy) or left untreated.

Figure S7 Calculation of islet sizes of 3-month-old (12- to 13-week-old) mice.
Indicated numbers of mice were analyzed. Size distributions of islets (A) and average islet sizes (B) are shown.

Figure S8 Immunohistochemical staining of pancreas sections from 10-month-old *PHLDA3* +/+ and -/- mice. Sections were stained with antibody against Ki67 and counter-stained with hematoxylin. Representative images are shown.

Figure S9 Blood glucose and plasma insulin levels in *PHLDA3*-deficient mice.
(A) Body weights, plasma insulin levels and blood glucose levels were analyzed using the indicated numbers (n) of 6-month-old *PHLDA3* +/+ and -/- mice. Plasma insulin levels and blood glucose levels were determined in blood taken from tail vein samples. Mice were fed ad libitum. Data are presented as mean ± SEM. (**, $p<0.01$)
(B) Indicated numbers (n) of overnight-fasted 10-month-old mice were subjected to the same analysis. Plasma insulin levels were determined in blood from the inferior vena cava. Blood glucose levels were determined in blood from tail vein samples. (*, $p<0.05$)

Figure S10 Chromogranin A levels in islets of *PHLDA3* +/+ and -/- mice.
Pancreas sections from 10-month-old *PHLDA3* +/+ and -/- mice were stained with antibody against Chromogranin A. Representative images are shown (A). Chromogranin A expression levels were analyzed quantitatively in 5 or 10 semi-hyperplasic islets and hyperplastic islets (larger than 0.05 mm^2 islet area) using TissueFAXS (B). Data are presented as mean ± SEM.

Figure S11 Glut2 expression and localization in islets of *PHLDA3* +/+ and -/- mice.
Pancreas sections from 10-month-old *PHLDA3* +/+ and -/- mice were stained with antibody against Glut2. Representative images are shown (A; *PHLDA3* +/+ islet, B; -/- islet , C; -/- hyperplastic islet). Glut2 expression levels in 3 representative islet cells

were quantitated from line-profile fluorescence intensities in images obtained with a Keyence BZ-9000 fluorescence microscope.

Figure S12 Detection of IAPP (islet amyloid polypeptide) deposits in islets of *PHLDA3* +/+ and -/- mice.

Pancreas sections from 10-month-old *PHLDA3* +/+ and -/- mice were stained with Congo red and counter-stained with HE. Representative images are shown. A small section of the intestine of an amyloidosis patient is shown as a positive control for amyloid deposits. Deposited amyloid exhibits a typical green birefringence in polarized light, as is seen in the amyloidosis patient.

A

	tumor type	insulin	glucaon	PHLDA3 LOH
PanNET1	NF	−	−	+
PanNET2	NF	+ (partially)	+ (partially)	+
PanNET3	NF	−	−	+
PanNET4	NF	ND	ND	+
PanNET5	NF	+ (partially)	−	+
PanNET6	NF	ND	ND	+
PanNET7	NF	+ (partially)	+ (partially)	+
PanNET8	NF	−	−	+
PanNET9	NF	ND	ND	+
PanNET10	NF	+ (partially)	+ (partially)	+
PanNET11	NF	−	−	+
PanNET12	NF	−	−	+
PanNET13	NF	−	−	+
PanNET14	NF	−	+ (partially)	+
PanNET15	NF	+ (partially)	+ (partially)	+
PanNET16	NF	−	−	−
PanNET17	NF	+ (partially)	−	−
PanNET18	NF	+ (partially)	+ (partially)	−
PanNET19	NF	ND	ND	+
PanNET20	NF	+	−	+
PanNET21	NF	+ (partially)	+ (partially)	+
PanNET22	NF	ND	ND	+
PanNET23	insulinoma	+	−	+
PanNET24	NF	ND	ND	+
PanNET25	NF	−	−	+
PanNET26	NF	ND	ND	+
PanNET27	NF	+ (partially)	−	+
PanNET28	NF	+ (partially)	−	+
PanNET29	NF	ND	ND	+
PanNET30	NF	ND	ND	+
PanNET31	NF	−	+ (partially)	+
PanNET32	NF	−	+ (partially)	+
PanNET33	NF	−	−	+
PanNET34	NF	−	−	+
PanNET35	NF	−	+ (partially)	+
PanNET36	NF	−	+ (partially)	+
PanNET37	NF	−	−	+
PanNET38	NF	+ (partially)	+ (partially)	+
PanNET39	NF	−	−	+
PanNET40	NF	−	−	−
PanNET41	NF	+ (partially)	+ (partially)	−
PanNET42	NF	+ (partially)	−	−
PanNET43	NF	−	+ (partially)	−
PanNET44	NF	−	+ (partially)	−
PanNET45	NF	−	+ (partially)	−
PanNET46	NF	−	−	−
PanNET47	NF	−	+ (partially)	−
PanNET48	NF	−	−	−
PanNET49	NF	−	+	−
PanNET50	insulinoma	+	−	−
PanNET51	NF	ND	ND	ND
PanNET52	NF	−	−	ND
PanNET53	NF	−	+	ND
PanNET54	insulinoma, gastrinoma	+	+	ND

B

	ins+	ins−
LOH+	11 (41 %)	16 (59 %)
LOH−	5 (36 %)	9 (64 %)

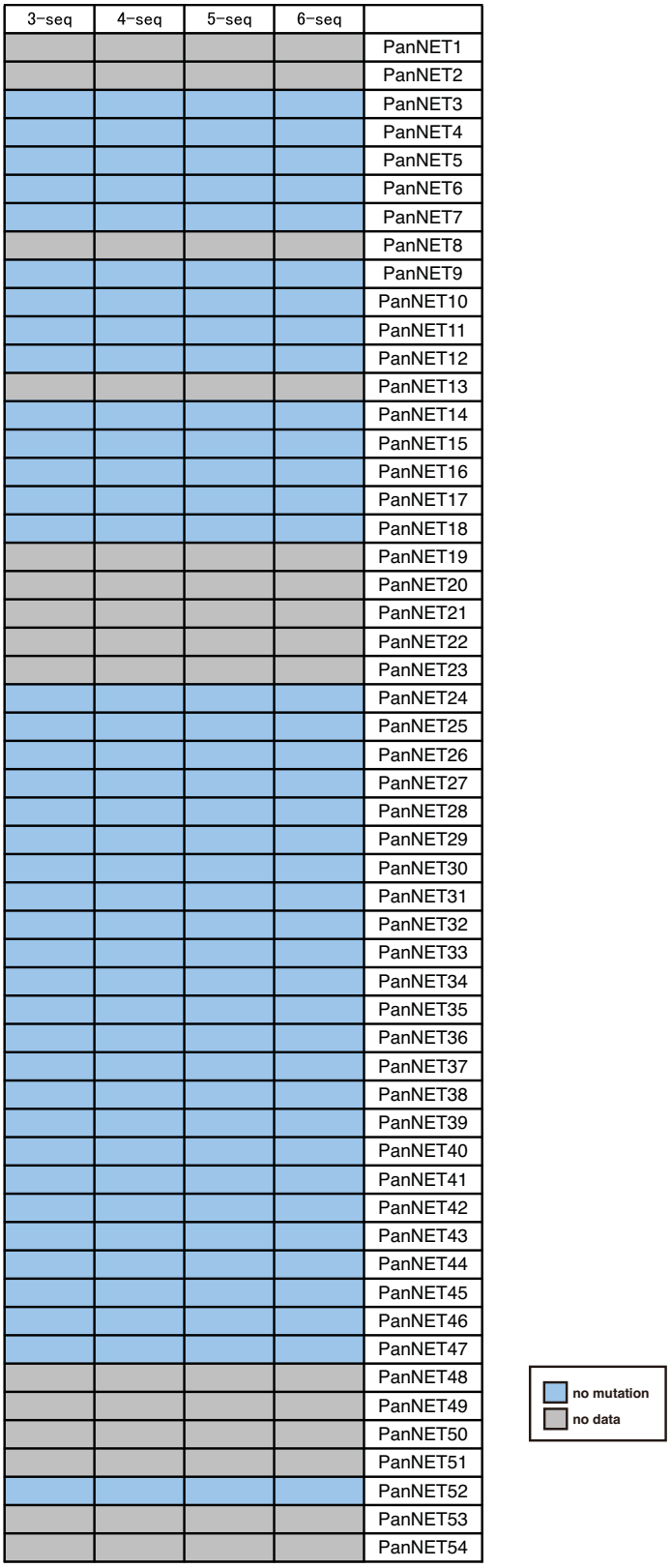
(Fisher's exact test, p=1)

C

	glu+	glu−
LOH+	11 (41 %)	16 (59 %)
LOH−	7 (50 %)	7 (50 %)

(Fisher's exact test, p=0.74)

Ohki_Fig.S2


3-seq
4-seq
5-seq
6-seq
PanNET1
PanNET2
PanNET3
PanNET4
PanNET5
PanNET6
PanNET7
PanNET8
PanNET9
PanNET10
PanNET11
PanNET12
PanNET13
PanNET14
PanNET15
PanNET16
PanNET17
PanNET18
PanNET19
PanNET20
PanNET21
PanNET22
PanNET23
PanNET24
PanNET25
PanNET26
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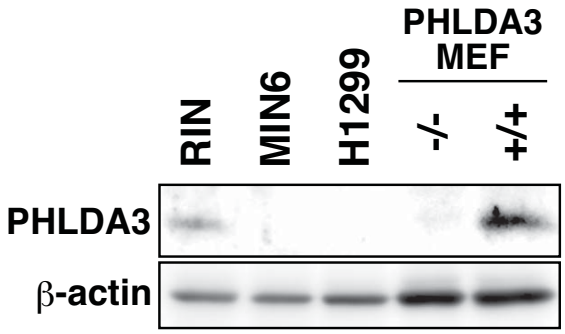
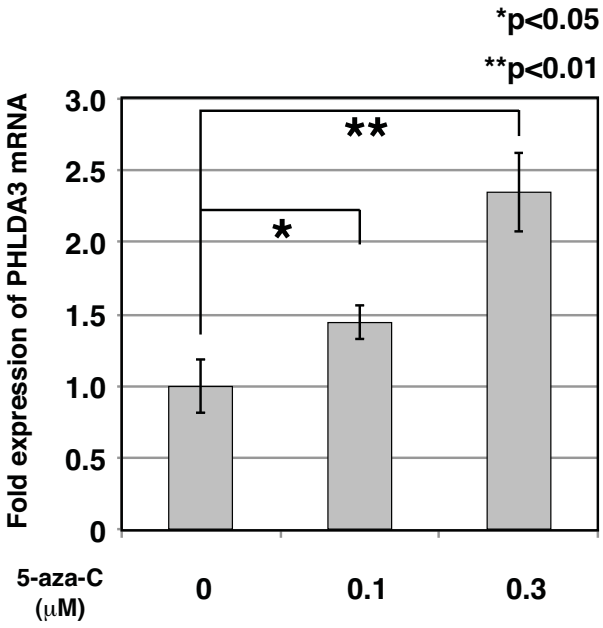
PanNET28
PanNET29
PanNET30
PanNET31
PanNET32
PanNET33
PanNET34
PanNET35
PanNET36
PanNET37
PanNET38
PanNET39
PanNET40
PanNET41
PanNET42
PanNET43
PanNET44
PanNET45
PanNET46
PanNET47
PanNET48
PanNET49
PanNET50
PanNET51
PanNET52
PanNET53
PanNET54
no mutation
no data

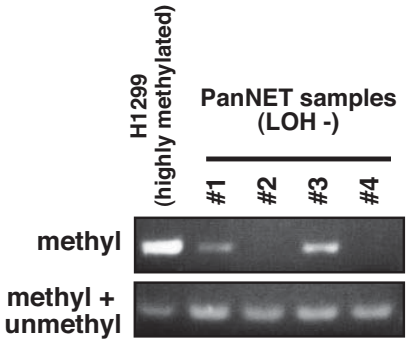
A

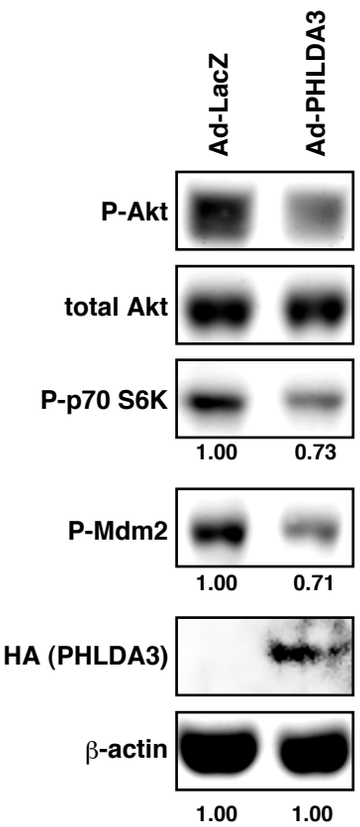

PHLDA3
MEF
RIN
MIN6
H1299
-/-
+/+
PHLDA3
β-actin

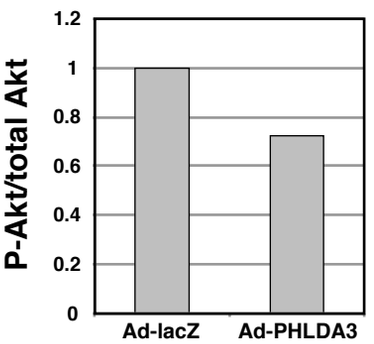
B


*p<0.05
**p<0.01
Fold expression of PHLDA3 mRNA
3.0
2.5
2.0
1.5
1.0
0.5
0
**
*
5-aza-C
(μM)
0
0.1
0.3

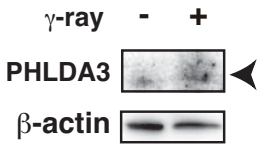
Ohki_Fig.S4


H1299
(highly methylated)
PanNET samples
(LOH -)
#1
#2
#3
#4
methyl
methyl +
unmethyl


Ad-LacZ
Ad-PHLDA3
P-Akt
total Akt
P-p70 S6K
1.00
0.73
P-Mdm2
1.00
0.71
HA (PHLDA3)
β-actin
1.00
1.00

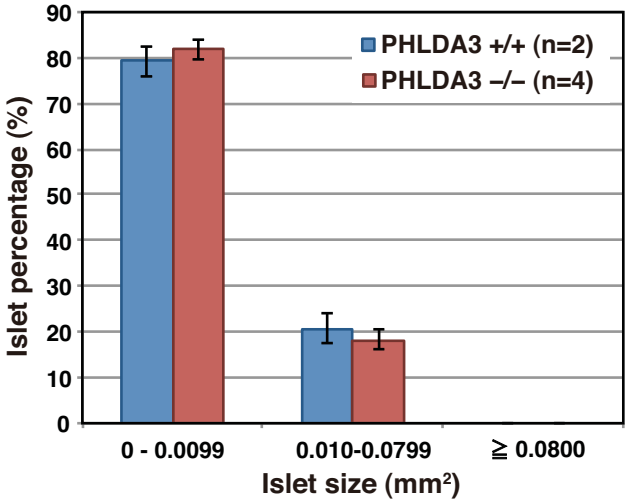

P-Akt/total Akt
1.2
1
0.8
0.6
0.4
0.2
0
Ad-lacZ
Ad-PHLDA3

Ohki_Fig.S6

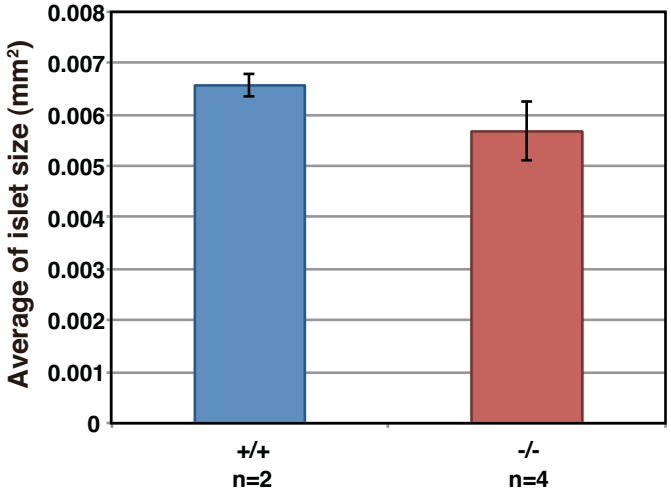


Ohki_Fig.S7

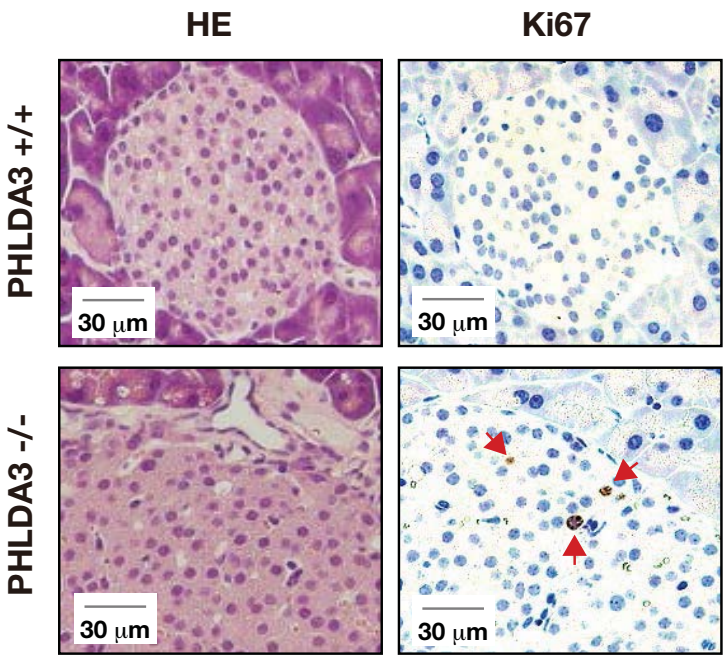
A



B

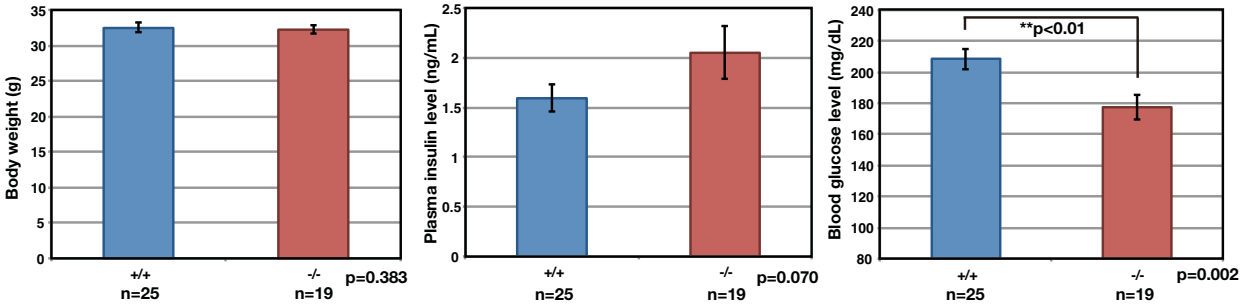


Ohki_Fig.S8

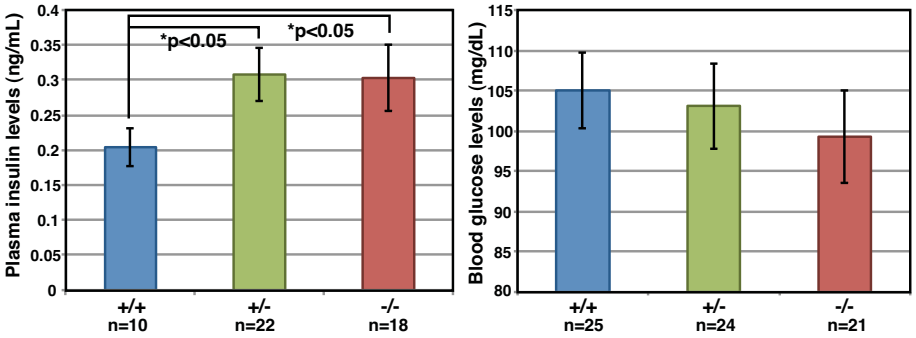


Ohki_Fig.S9

A



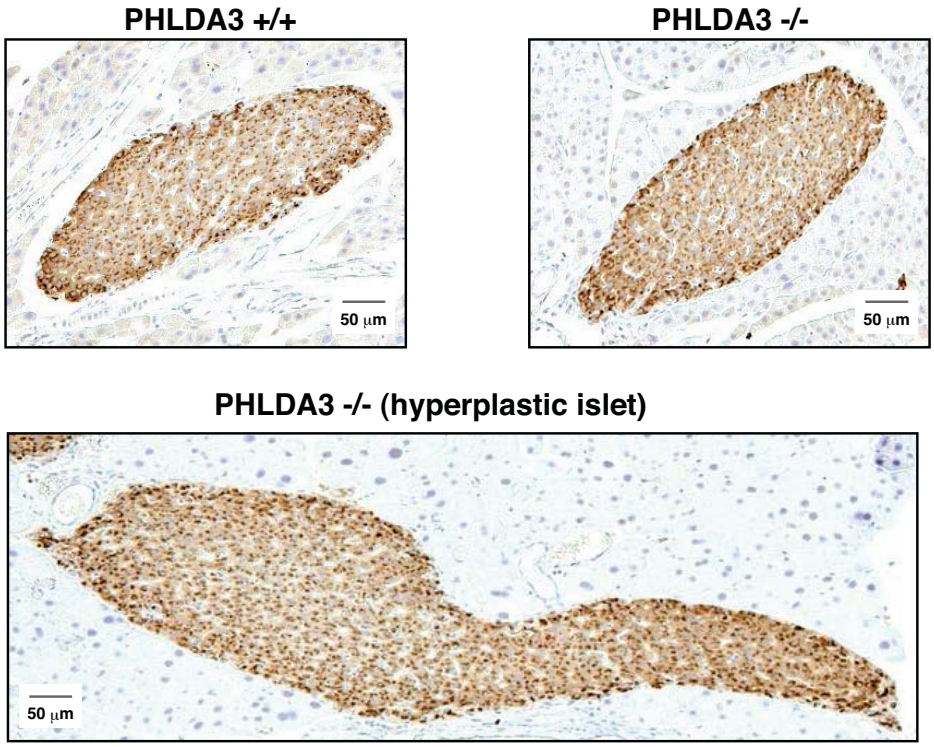
B



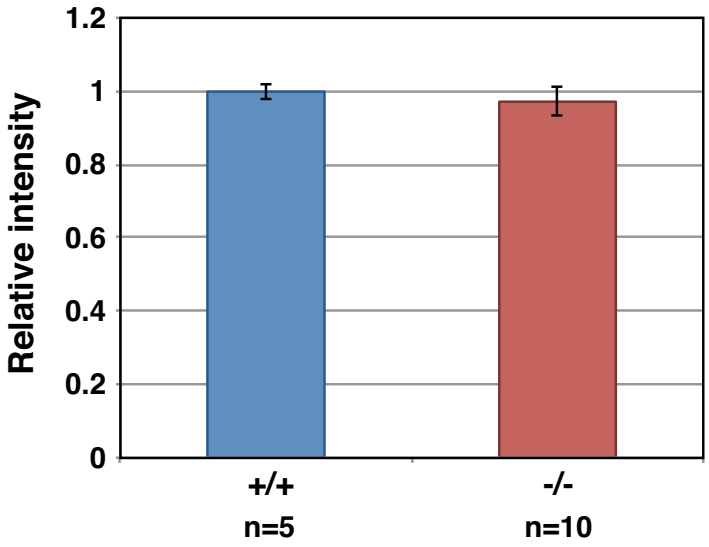
	p value
+/+ vs +/–	0.017
+/+ vs –/–	0.043
+/– vs –/–	0.467

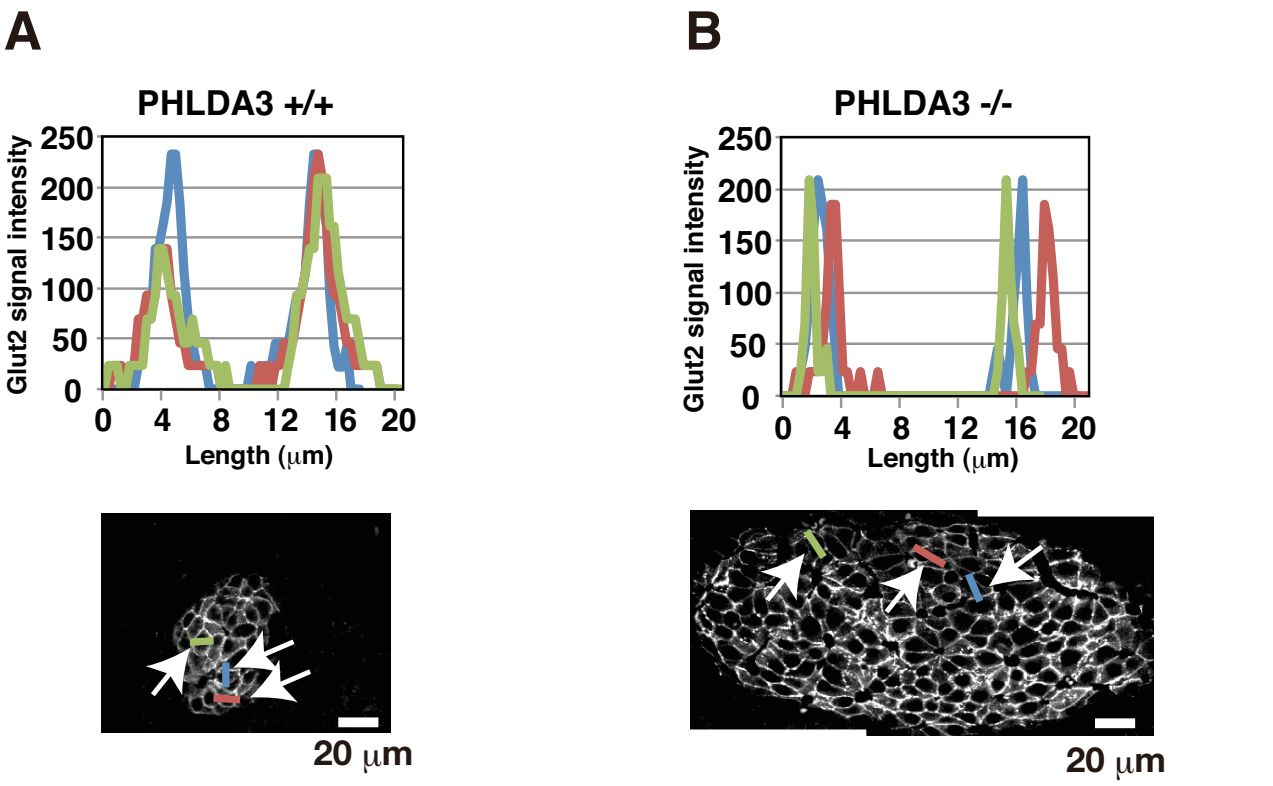
	p value
+/+ vs +/–	0.396
+/+ vs –/–	0.220
+/– vs –/–	0.314

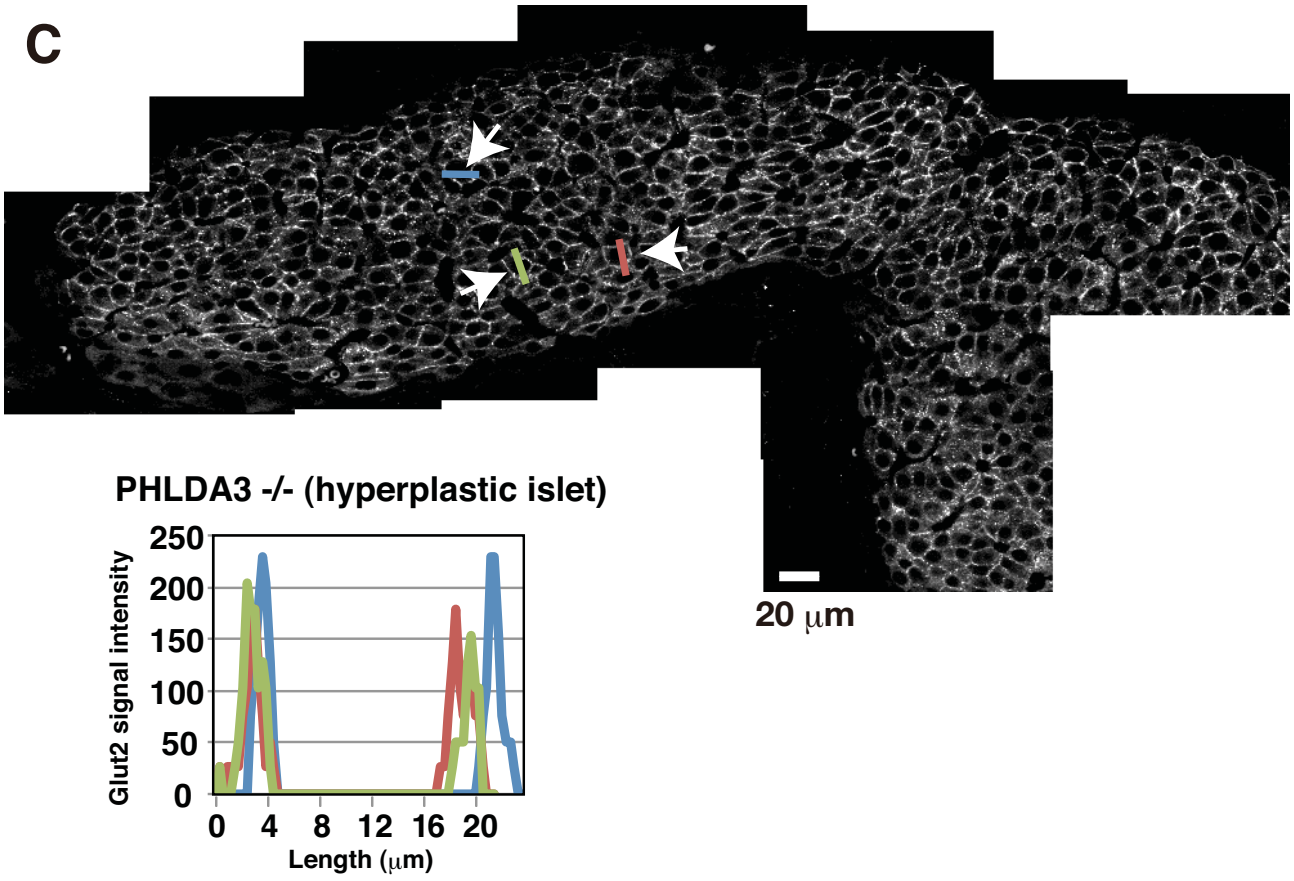
A

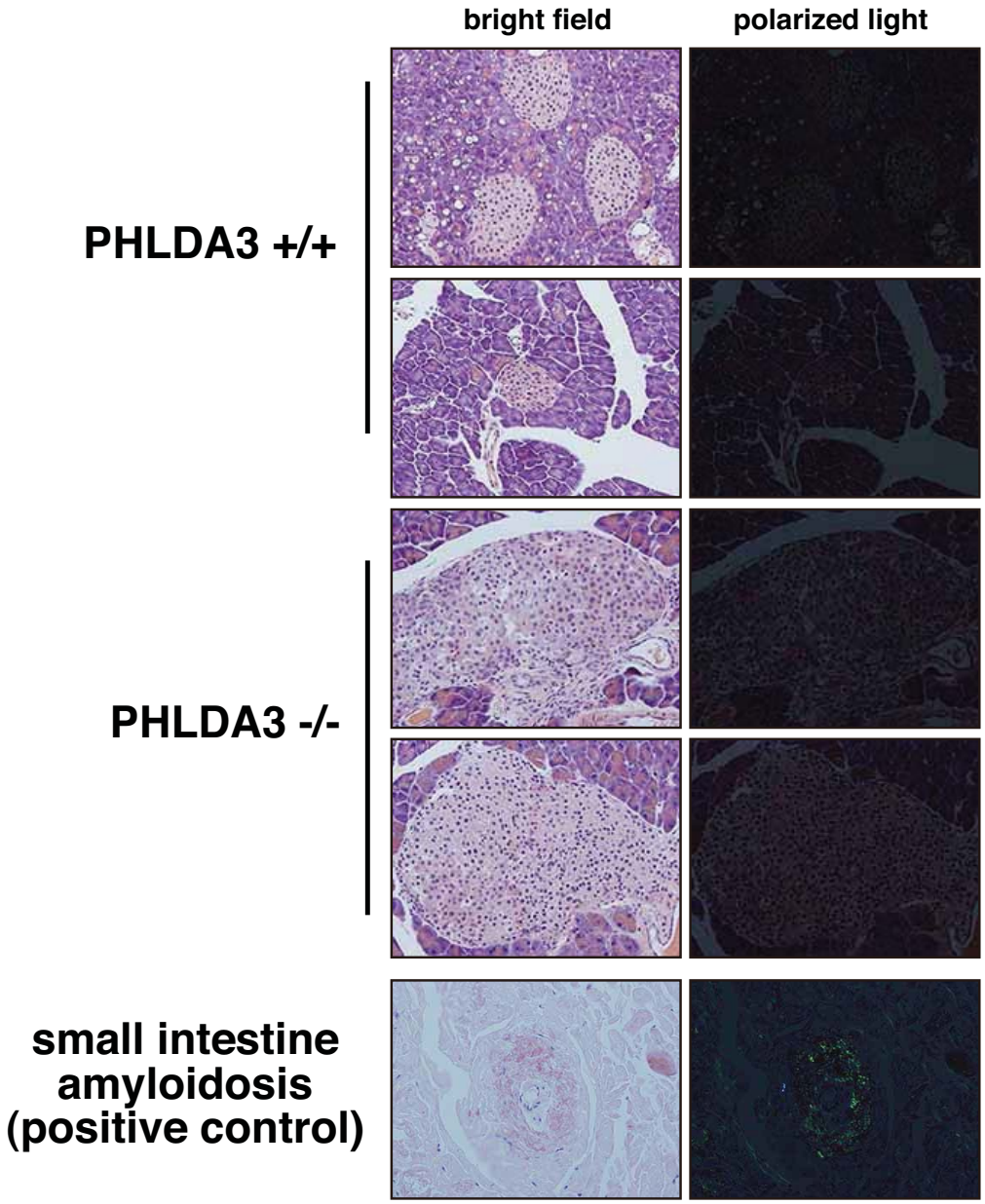

PHLDA3 +/+
PHLDA3 -/-
50 μm
50 μm
PHLDA3 -/- (hyperplastic islet)
50 μm

B


Relative intensity
1.2
1
0.8
0.6
0.4
0.2
0
+/+
n=5
-/-
n=10


A
PHLDA3 +/+
Glut2 signal intensity
250
200
150
100
50
0
0 4 8 12 16 20
Length (μm)
20 μm
B
PHLDA3 -/-
Glut2 signal intensity
250
200
150
100
50
0
0 4 8 12 16 20
Length (μm)
20 μm


C
20 μm
PHLDA3 -/- (hyperplastic islet)
Glut2 signal intensity
250
200
150
100
50
0
0 4 8 12 16 20
Length (μm)


bright field
polarized light
PHLDA3 +/+
PHLDA3 -/-
small intestine amyloidosis (positive control)