Legends of supplementary material

NOTE:

A part from the controls described in the manuscript, we have also added CDK6 presence as positive control in human pancreas and negative control in mice pancreas, as CDK6 presence in healthy human pancreas has been reported previously [1] and CDK6 absence in healthy murine pancreas has been also reported [2].

[1] Tomita T (2004) Cyclin-dependent kinase (cdk6) and p16 in pancreatic endocrine neoplasms. Pathology 36: 566-570

[2] Martin J, Hunt SL, Dubus P, et al. (2003) Genetic rescue of Cdk4 null mice restores pancreatic beta-cell proliferation but not homeostatic cell number. Oncogene 22: 5261-5269

Sup Fig 1. Immunohistochemical controls showed no cross-positivity for the different channels. The columns from left to right are the same sections observed in the different channels (red, green and blue respectively). A: Incubation of the samples with only the secondary antibodies showed no staining. B and C: Staining for CDK4 (C-22 antibody) (B) or CDK6 (C-21 antibody) (C) and both secondary antibodies showed no staining in the green and blue channels. D: Staining for insulin and both secondary antibodies show no staining in the red and blue channels. E: Nuclear staining using Hoechst did not show any staining in red and green channels. Images are at 40x magnification using an epifluorescence microscope.

Sup Fig 2. Immunostaining for Cdk4 and Cdk6 in the pancreas of C57B/6 as a negative control for Cdk6. In the upper row, the images from left to right correspond to pancreas immunostained for either Cdk4 (C-22 antibody), Cdk6 (C-21 antibody) or without a primary antibody. Notice that Cdk6 staining in the pancreas of C57B/6 exhibits an unspecific diffuse, non-nuclear staining consistent with the absence of the protein. The next rows are the same sections stained for insulin and Hoechst. The lowest row is the merge of the above images. Images are at 40x magnification using an epifluorescence microscope.

Sup Fig 3. Immunostaining for Cdk4 in the pancreas of the Cdk4 KO mice as a negative control for Cdk4 staining (C-22 antibody). Image is at 40x magnification using an epifluorescence microscope.

Sup Fig 4. Competition peptide study shows no staining and signal specificity in human pancreas sections. The rows from top to bottom are CDK4 (C-22 antibody) with (left panel) or without (right panel) competition peptide, insulin, nuclear staining and the merge image of the same section stained with these antibodies. Notice that CDK4 specific nuclear staining disappears when the antibody is incubated with the competition peptide. Images are at 20x magnification using an epifluorescence microscope.

Sup Fig 5. Western Blot controls. A: Representative Western Blot of CDK4 (C-22 antibody), CDK6 (C-21 antibody) and beta-actin for HeLa cells (positive control), human islets and mice islets. B: Representative Western Blot of CDK4 (H-22 antibody), CDK4 (DCS-31 antibody), CDK4 (DCS-156 antibody) and beta-actin for WT Cdk4 mouse pancreas (positive control), knockout-Cdk4 mouse pancreas (negative control) and human islets. C: Representative Western Blot of CDK4 (DCS-156 antibody) for WT Cdk4 mouse pancreas (positive control), knockout-Cdk4 mouse pancreas (negative control) and 4 different human pancreas.

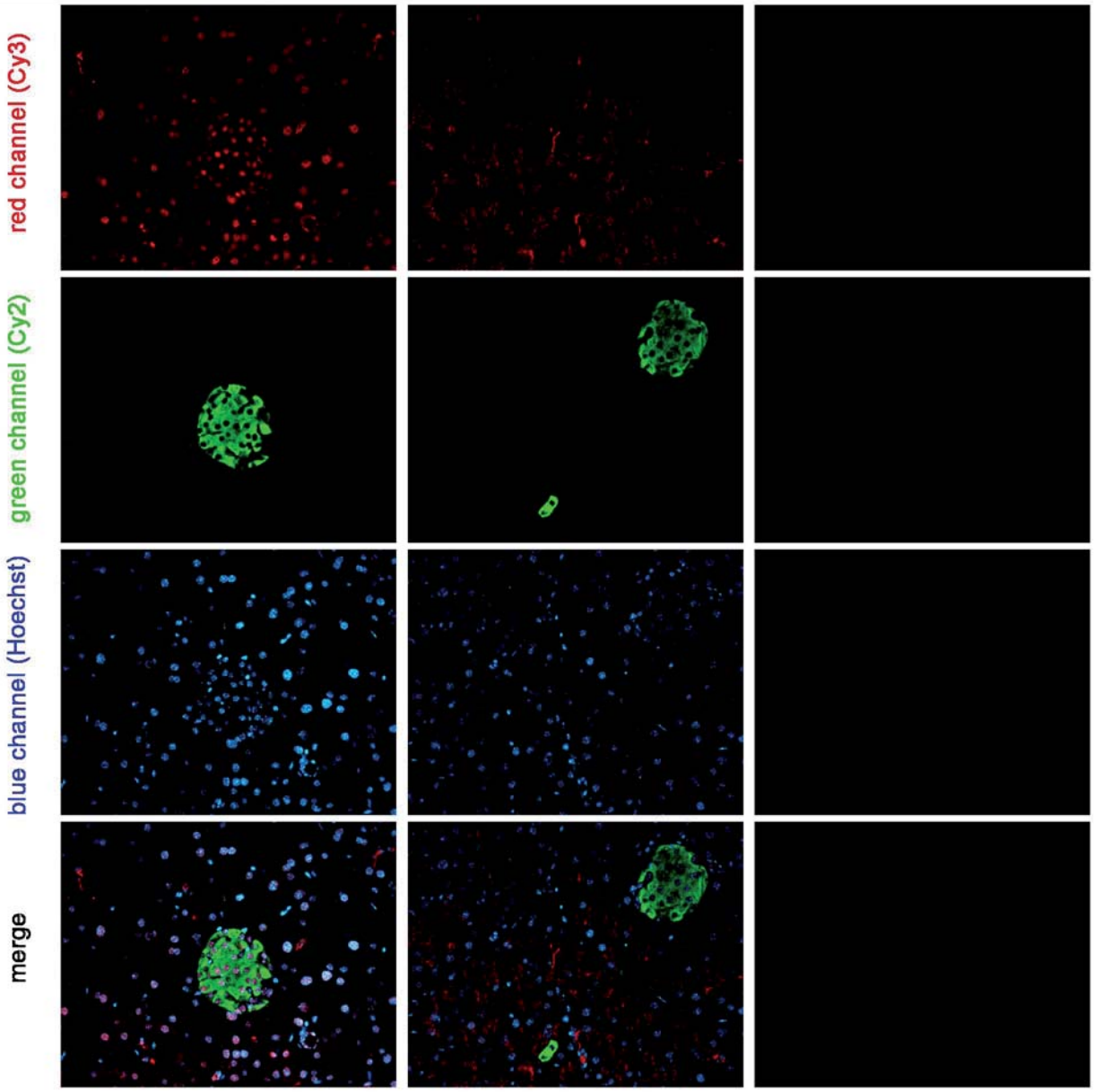
Sup Fig 6. Competition peptide study shows no staining and signal specificity. Representative Western Blot of CDK4 (C-22 antibody) expression in WT Cdk4 mouse pancreas, knockout-Cdk4 mouse pancreas and HeLa cells. (positive, human control sample). Upper panel: incubated with anti Cdk4 C-22 antibody; lower panel: Cdk4-specific competition peptide (sc-260-P) was added to the C-22 antibody solution in order to prevent the binding of C-22 to CDK4 in protein samples on the Western Blot membrane.

Sup Fig 1

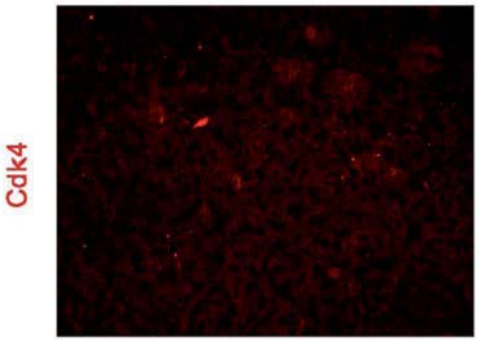
red channel (Cy3)
green channel (Cy2)
blue channel (Hoechst)
Primary
Secondary
Nuclear staining
A
-
anti-rabbit-Cy3
anti-guinea pig-Cy2
-
B
CDK4
anti-rabbit-Cy3
anti-guinea pig-Cy2
-
C
CDK6
anti-rabbit-Cy3
anti-guinea pig-Cy2
-
D
insulin
anti-rabbit-Cy3
anti-guinea pig-Cy2
-
E
-
anti-rabbit-Cy3
anti-guinea pig-Cy2
+ (Hoechst)

Sup Fig 2

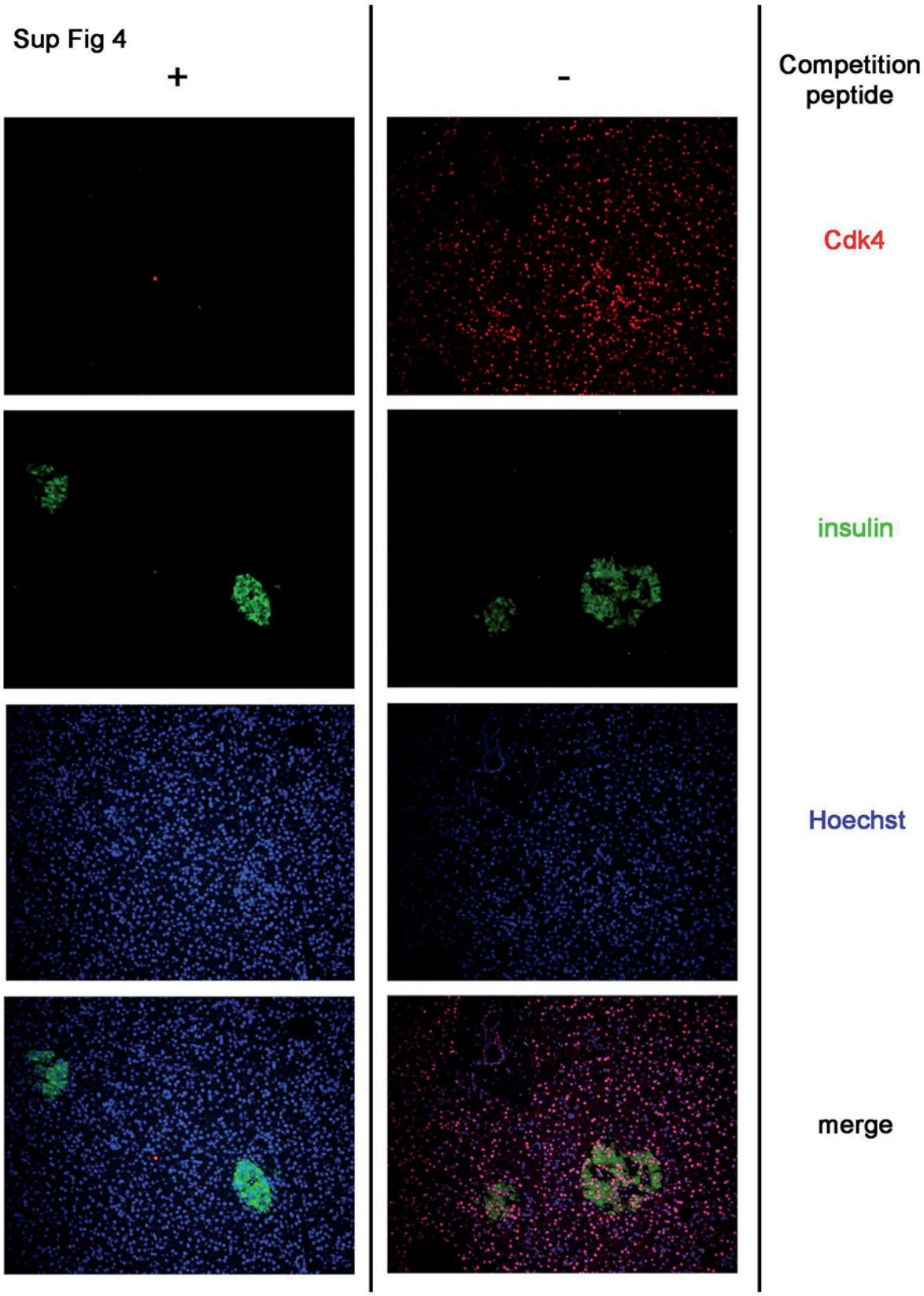
Primary	Cdk4 insulin	Cdk6 insulin	-
Secondary	anti-rabbit-Cy3 anti-guinea pig-Cy2	anti-rabbit-Cy3 anti-guinea pig-Cy2	anti-rabbit-Cy3 anti-guinea pig-Cy2
Nuclear staining	+ (Hoechst)	+ (Hoechst)	-



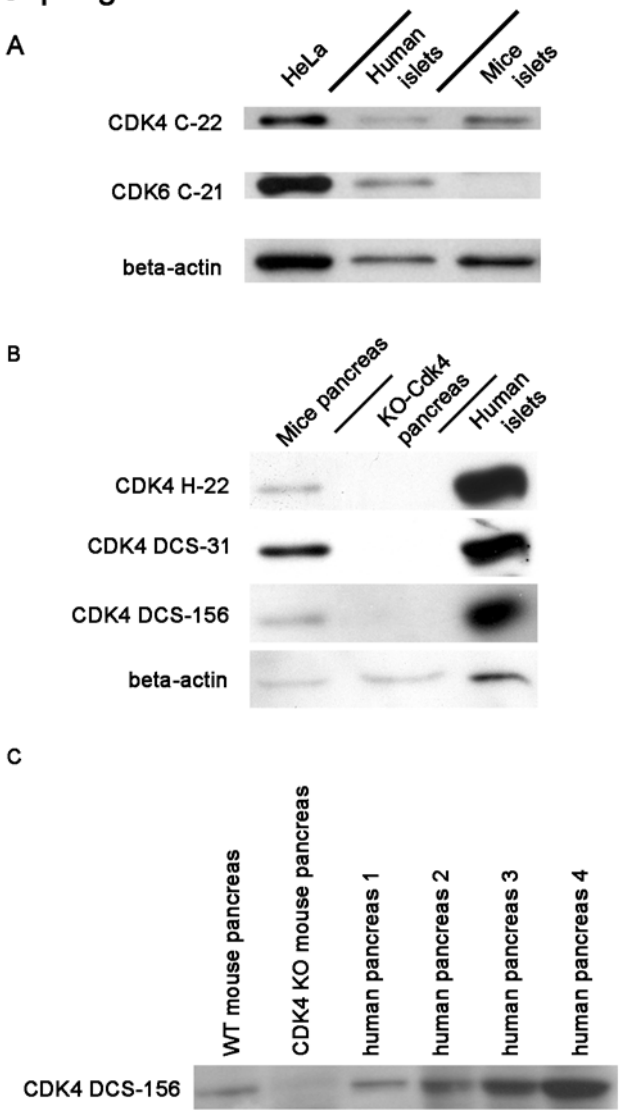
Sup Fig 3



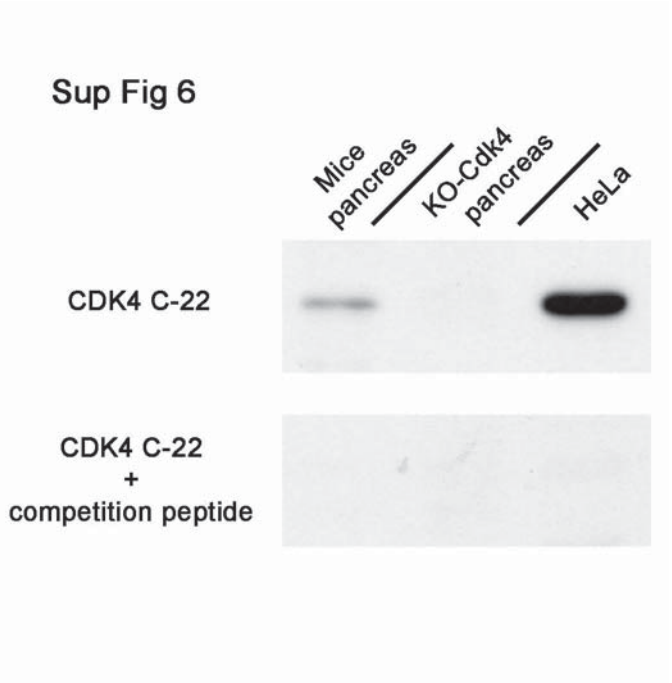
Sup Fig 4



Sup Fig 5


A
HeLa
Human islets
Mice islets
CDK4 C-22
CDK6 C-21
beta-actin
B
Mice pancreas
KO-Cdk4 pancreas
Human islets
CDK4 H-22
CDK4 DCS-31
CDK4 DCS-156
beta-actin
C
WT mouse pancreas
CDK4 KO mouse pancreas
human pancreas 1
human pancreas 2
human pancreas 3
human pancreas 4
CDK4 DCS-156

Sup Fig 6


Mice pancreas
KO-Cdk4 pancreas
HeLa
CDK4 C-22
CDK4 C-22
+
competition peptide