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Supplemental information

VGLL4 and MENIN function as TEAD1 corepressors to block pancreatic β cell proliferation

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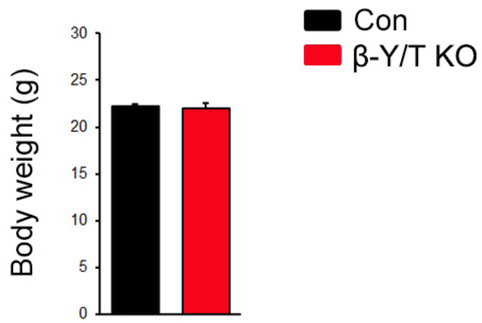
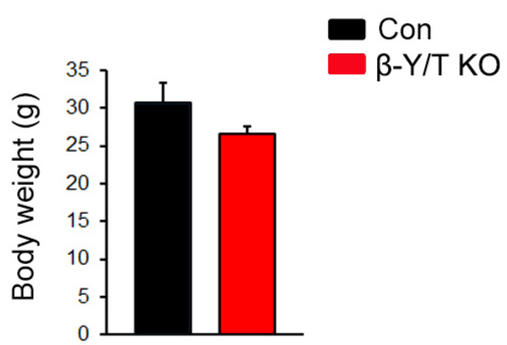
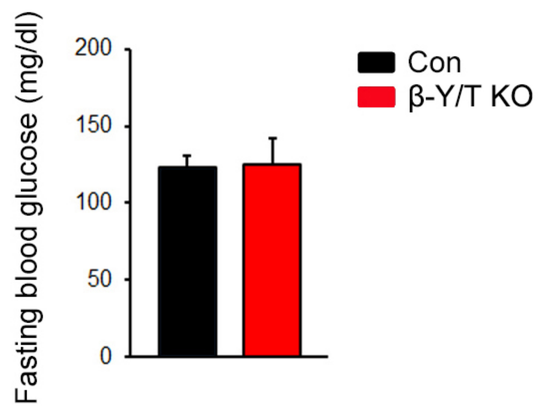
A


Body weight (g)
30
25
20
15
10
5
0
Con
β-Y/T KO

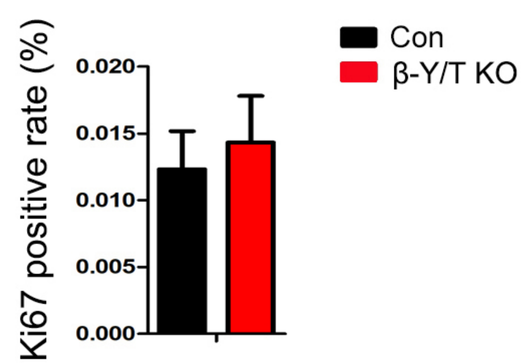
B


Body weight (g)
35
30
25
20
15
10
5
0
Con
β-Y/T KO

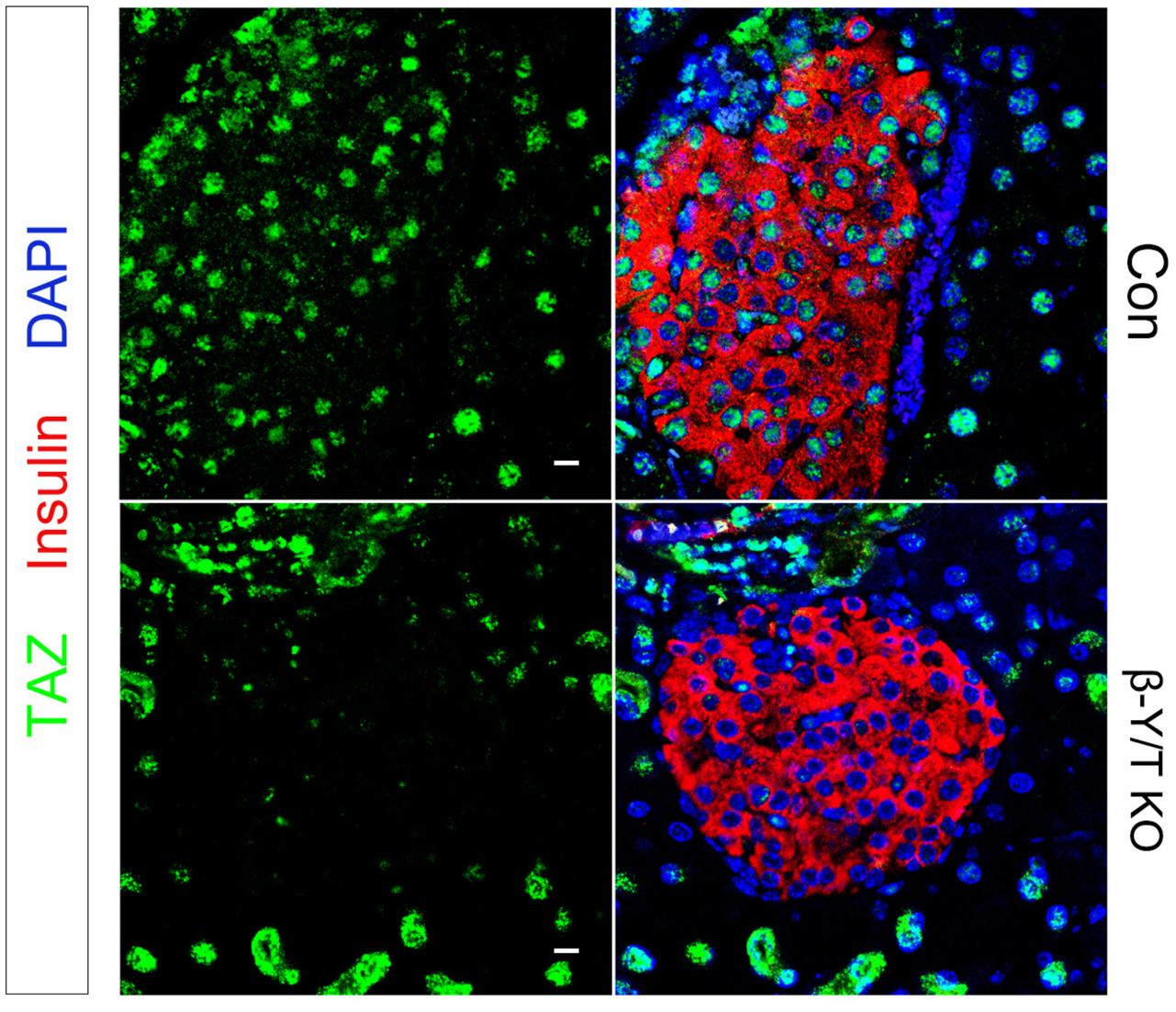
C

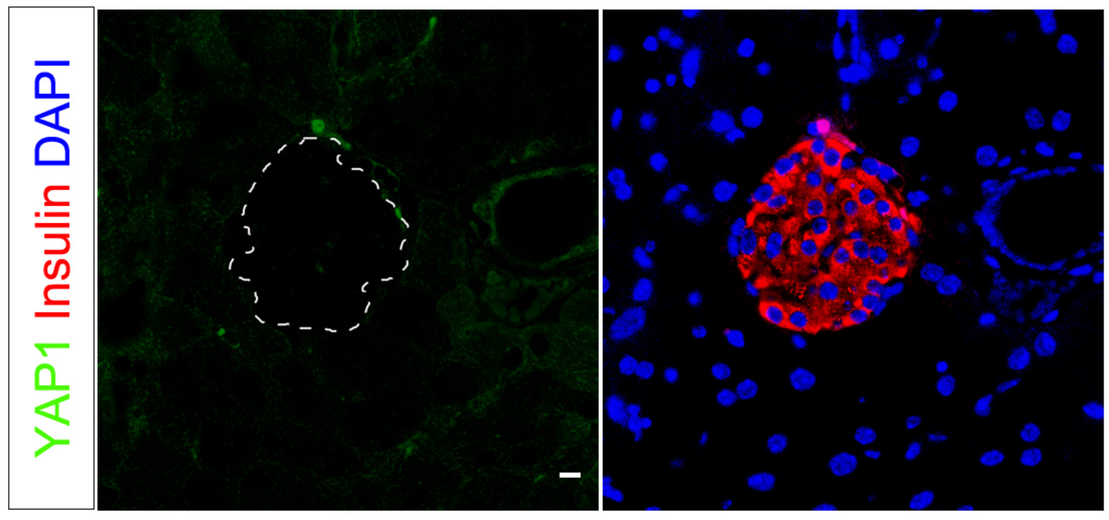

Fasting blood glucose (mg/dl)
200
150
100
50
0
Con
β-Y/T KO

D


Ki67 positive rate (%)
0.020
0.015
0.010
0.005
0.000
Con
β-Y/T KO

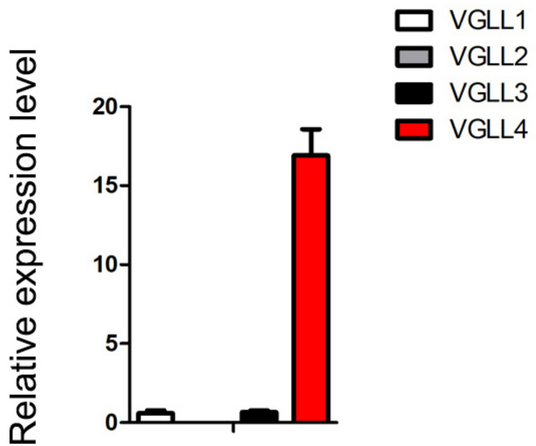
E


TAZ Insulin DAPI
Con
β-Y/T KO

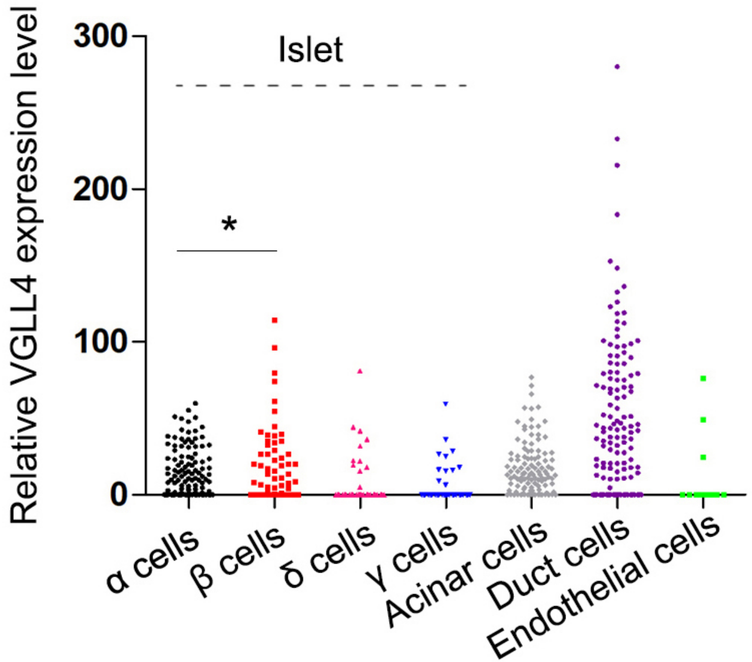

YAP1 Insulin DAPI

Supplementary Figure S1- Phenotype changes of β-Y/T KO, related to Figure 1. (A-B) The bodyweight of β-Y/T KO have no change compared with control at the age of 8 weeks or 8 months (n=5 mice/group). (C) Fasting blood glucose level of β-Y/T KO have no change compared with control (n=6 mice/group). (D) Immunostaining showed that the rate of Ki67-positive β cells has no change between β-Y/T KO and Control (n=3 mice/group). (E) Immunostaining showed that TAZ expression can't detected in β-Y/T KO β cells. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. *p* values were from Student's t test. Error bars represent standard error of the mean (SEM). Scale bar, 10 μm.

A


Relative expression level
20
15
10
5
0
VGLL1
VGLL2
VGLL3
VGLL4

B


Relative VGLL4 expression level
300
200
100
0
Islet
*
α cells
β cells
δ cells
γ cells
Acinar cells
Duct cells
Endothelial cells

Supplementary Figure S2- VGLL4 expression in human islet cells, related to Figure 2. (A) Human islet sequencing showed that in whole islet VGLL4 expression were much higher than the other VGLL family members. (B) Single islet cell sequencing showed that VGLL4 expression were higher in β cells than α cells. *, $p<0.05$. *p* values were from Student's t test. Error bars represent standard error of the mean (SEM).

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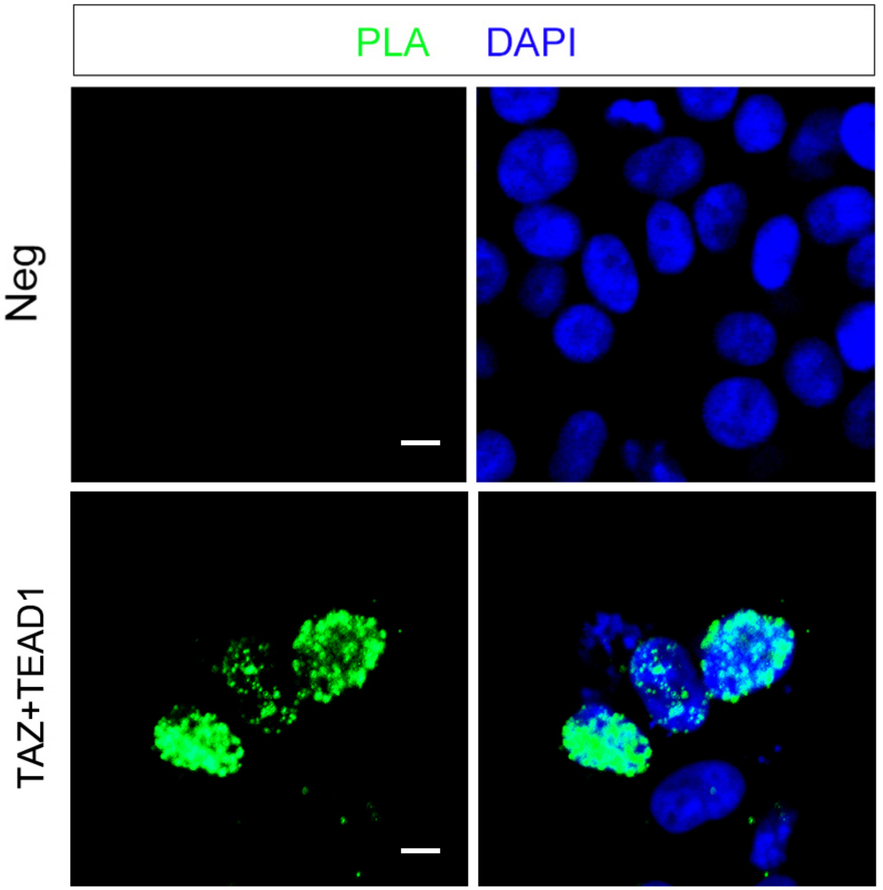
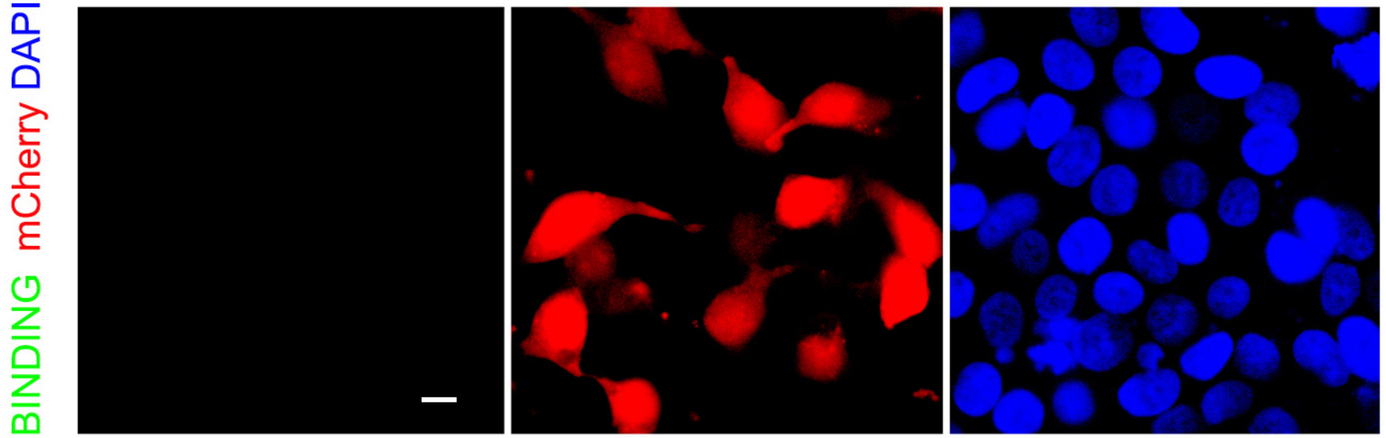
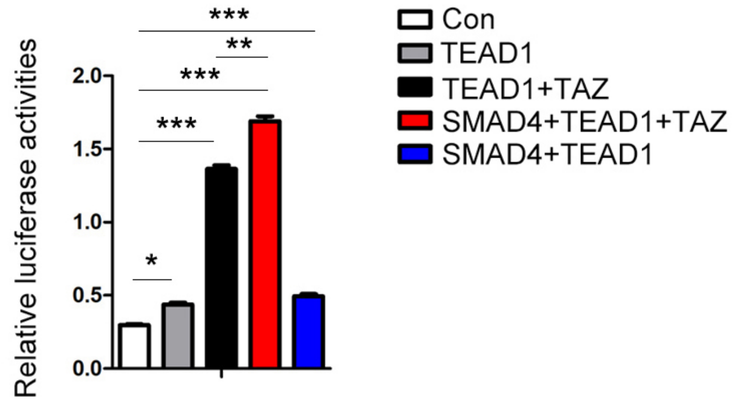
A


PLA
DAPI
Neg
TAZ+TEAD1

B


BINDING
mCherry
DAPI

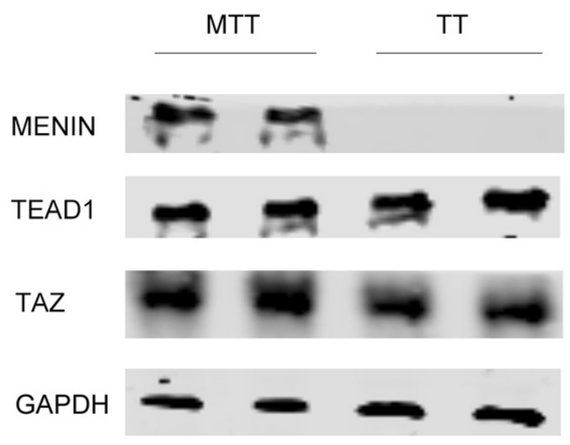
C


Relative luciferase activities
2.0
1.5
1.0
0.5
0.0
***
**
***
***
*
Con
TEAD1
TEAD1+TAZ
SMAD4+TEAD1+TAZ
SMAD4+TEAD1

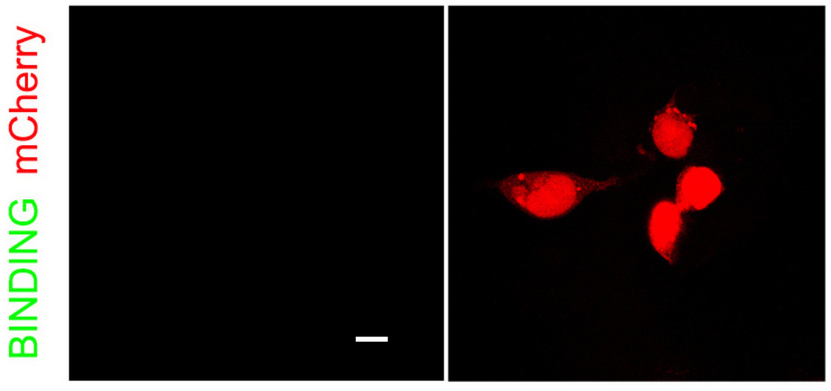
D


Relative luciferase activities
0.8
0.6
0.4
0.2
0.0
***
***
TEAD1+TAZ
MENIN+TEAD1+TAZ
MENIN+JUND+TEAD1+TAZ

E


MTT
TT
MENIN
TEAD1
TAZ
GAPDH

F


BINDING
mCherry

Supplementary Figure S3- MENIN is the functional cofactor of TEAD1, related to Figure 3. (A) As a positive control, proximity ligation assay (PLA) showed that TEAD1 bound TAZ in situ (green dots), while in negative control in which primary antibody were not added, no signal can be observed. (B) Deleting of aa359-371 of TEAD1 (ΔTEAD1) led to a loss of the binding signal between ΔTEAD1 and MENIN in Split-GFP system. (C) Luciferase assay showed that SMAD4 overexpression can't repress TAZ-TEAD1 (TT) pathway activities. On the contrary, it had a weak activation effect on TEAD1 pathway. (D) Luciferase assay showed that TT pathway activities were significantly reduced after MENIN overexpression. However, no change can be observed between MENIN overexpression and MENIN-JUND co-overexpression. (E) Western blot showed that MENIN overexpression can't affect TEAD1 and TAZ expression in Split-GFP system. (F) No GFP binding signal can be observed between MENIN and TAZ in Split-GFP system. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. (A-E) Graphs show mean (±SEM) from at least 3 biological repeat and triplicate replicate assays and show *p* from Student's t test. Scale bar, 10 μm.

#

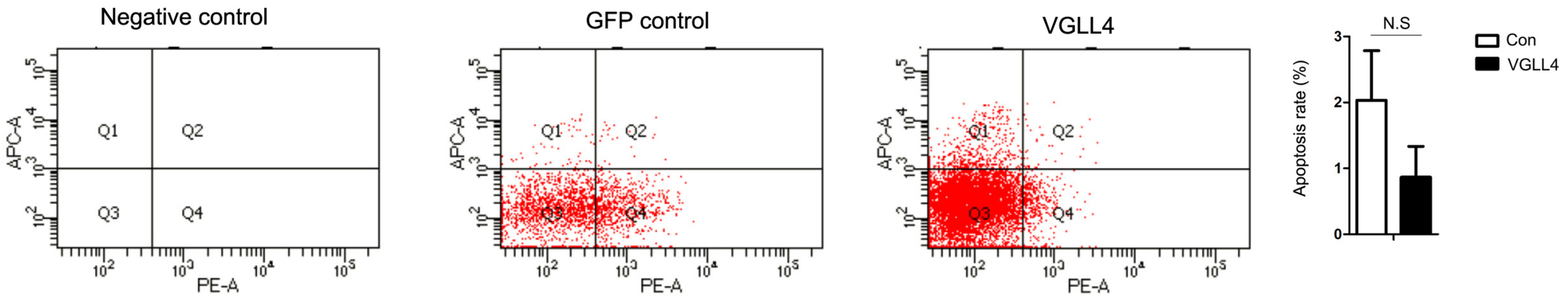
A


CMV
VGLL4
P2A
GFP

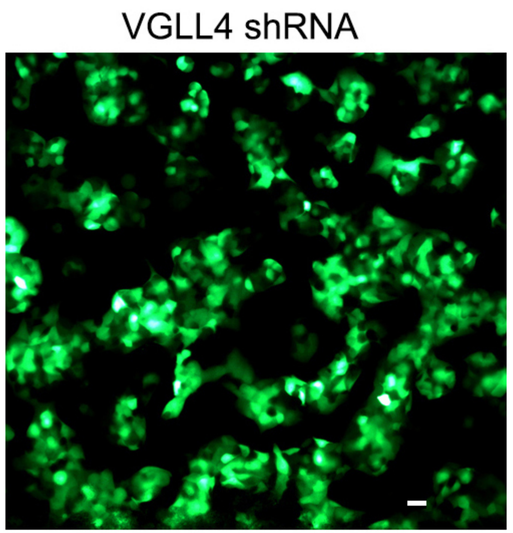
B


Con
VGLL4

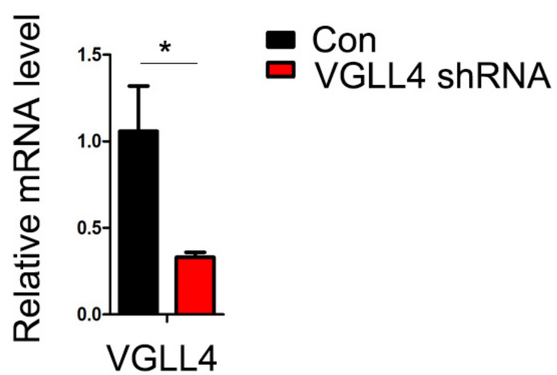
C


Negative control
GFP control
VGLL4
APC-A
PE-A
Q1
Q2
Q3
Q4
N.S
Apoptosis rate (%)
Con
VGLL4

D


VGLL4 shRNA

E


Relative mRNA level
*
Con
VGLL4 shRNA
VGLL4

Supplementary Figure S4- VGLL4 can repress β cell proliferation *in vitro*, related to Figure 4. (A) Schematic representation for VGLL4 lentiviral plasmid. VGLL4 was connected GFP with P2A. (B) Western blot showed that VGLL4 were successfully overexpressed in INS1 cells detected by VGLL4 antibody. (C) Apoptosis rate (Q2) has no change after VGLL4 overexpression compared with GFP overexpression control in INS1 cells, indicated by flow cytometry experiments. Fluorochrome-labeled Annexin V was used to identify apoptotic cells. (D) The transfection rate for VGLL4 shRNA lentivirus is more than 90% in INS2 cells and qPCR showed that VGLL4 knockdown rate is around 70%. *, $p<0.05$. (B-D) Graphs show mean (±SEM) from at least 3 biological repeat and triplicate replicate assays and show *p* from Student's t test.. Scale bar, 10 μm.

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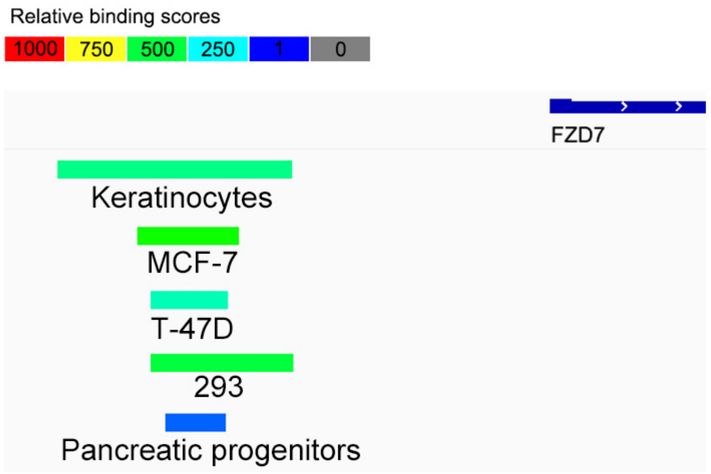
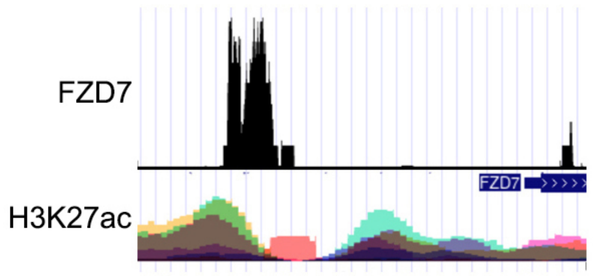
A


Relative binding scores
1000
750
500
250
1
0
FZD7
Keratinocytes
MCF-7
T-47D
293
Pancreatic progenitors

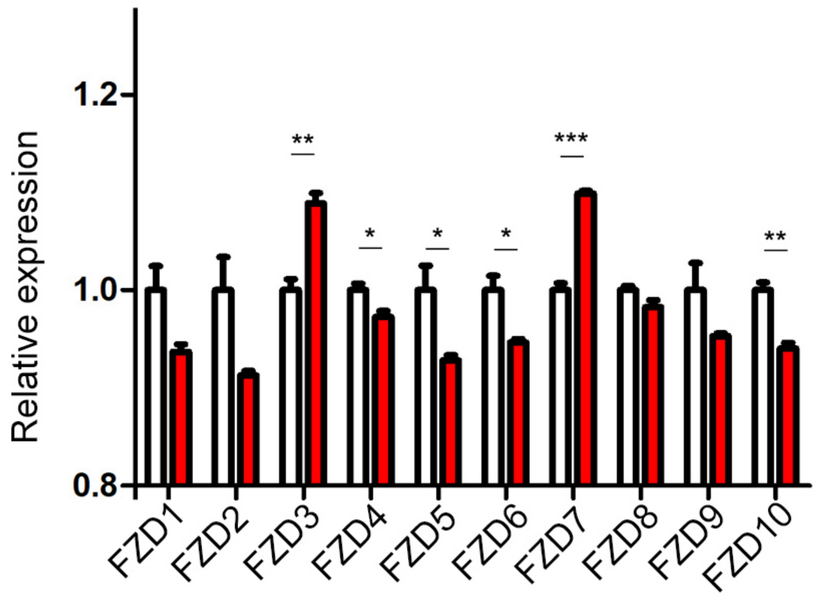
B


FZD7
H3K27ac
FZD7

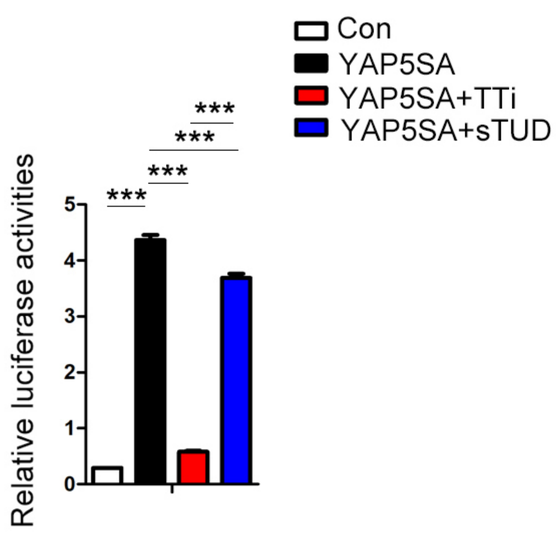
C


-402
CCCTGCTCAGGAATGCGCATCTGAGTGCACCAGG
GTCTTTCCCTTAAACGAAGTTTCTAAACAAGGAGAA
-354

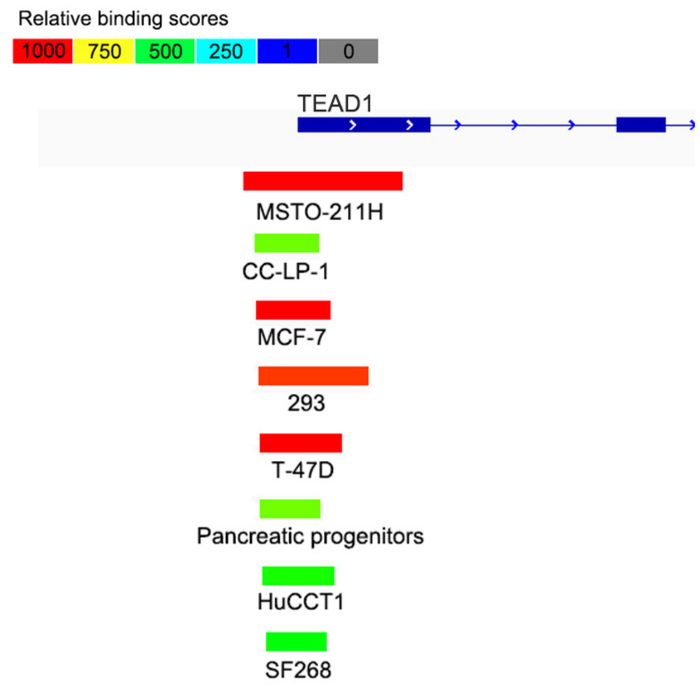
D


Con
MKO
Relative expression
1.2
1.0
0.8
FZD1
FZD2
FZD3
FZD4
FZD5
FZD6
FZD7
FZD8
FZD9
FZD10

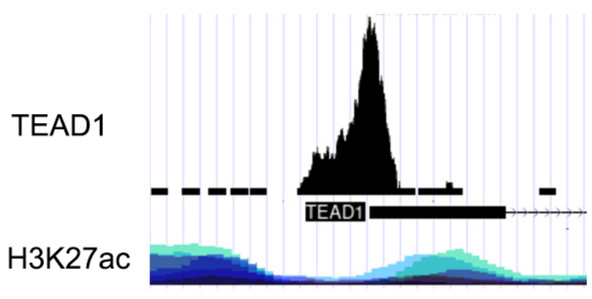
E


Con
YAP5SA
YAP5SA+TTi
YAP5SA+sTUD
Relative luciferase activities
5
4
3
2
1
0

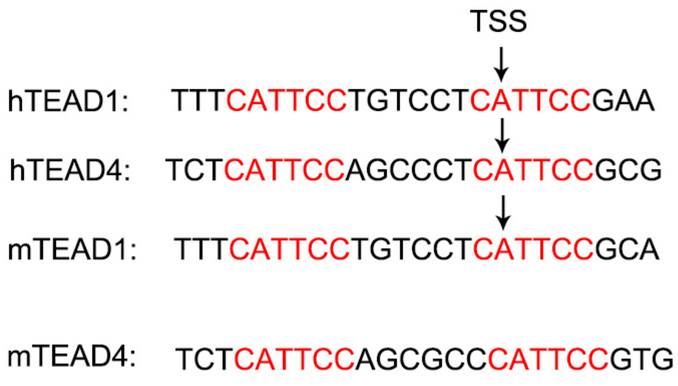
F


Relative binding scores
1000
750
500
250
1
0
TEAD1
MSTO-211H
CC-LP-1
MCF-7
293
T-47D
Pancreatic progenitors
HuCCT1
SF268

G


TEAD1
H3K27ac
TEAD1

H


TSS
hTEAD1: TTTCATTCCTGTCCTCATTCCGAA
hTEAD4: TCTCATTCCAGCCCTCATTCCGCG
mTEAD1: TTTCATTCCTGTCCTCATTCCGCA
mTEAD4: TCTCATTCCAGCGCCCATTCCGTG

I


Con
VGLL4
37KD
Con
MENIN
75KD

Supplementary Figure S5- FZD7 is a target gene of MENIN-TEAD1 signaling pathway, related to Figure 5. (A) Reanalysis of public ChIPseq showed that TEAD1 can bind FZD7 promoter area in different cell types. (B) In pancreatic progenitor cells, a TEAD1 binding peak can be observed in the promoter area of FZD7. (C) There are one classical and one non-classical MCAT can be found in the sequences extracted from the binding peak. (D) Reanalysis of RNAseq showed that FZD3 and FZD7 were significantly increased while FZD4, FZD5, FZD6 and FZD10 significantly reduced after MENIN were conditional knocked out in β cells. (E) Luciferase assay showed that our TEAD1 inhibitor (TTi) had stronger repression effect on YAP5SA pathway than sTDU. The ratio of YAP5SA to TTi or to sTDU is 1 to 5. Graphs show mean (±SEM) from at least 3 biological repeat and triplicate replicate assays. (F) Reanalysis of public ChIPseq showed that TEAD1 can bind TEAD1 promoter area and transcription start site (TSS) in different cell types. (G) In pancreatic progenitor cells, a TEAD1 binding peak can be observed in the promoter area and TSS of TEAD1. (H) A classical MCAT was found just on the TSS and the other one was close to TSS. This tandem MCAT motif was conserved in human TEAD1, 4 and mouse TEAD1. In mouse TEAD4, tandem MACT does not occupy TSS. (I) Western blot showed that VGLL4 were overexpressed in INS1 cells. (J) Western blot showed that MENIN were overexpressed in INS1 cells. (K) Luciferase assay showed that VGLL4 can't inhibit, while MENIN+TEAD1 has a weak repression effect on TEAD1 signaling in the absence of transfected TAZ, indicated by HOPFLASH reporter. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. p were from Student's t test. Error bars represent standard error of the mean (SEM).

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