Smad3 deficiency improves islet-based therapy for diabetes and diabetic kidney injury by promoting β cell proliferation *via* the E2F3-dependent mechanism

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Supplementary Figures and Tables

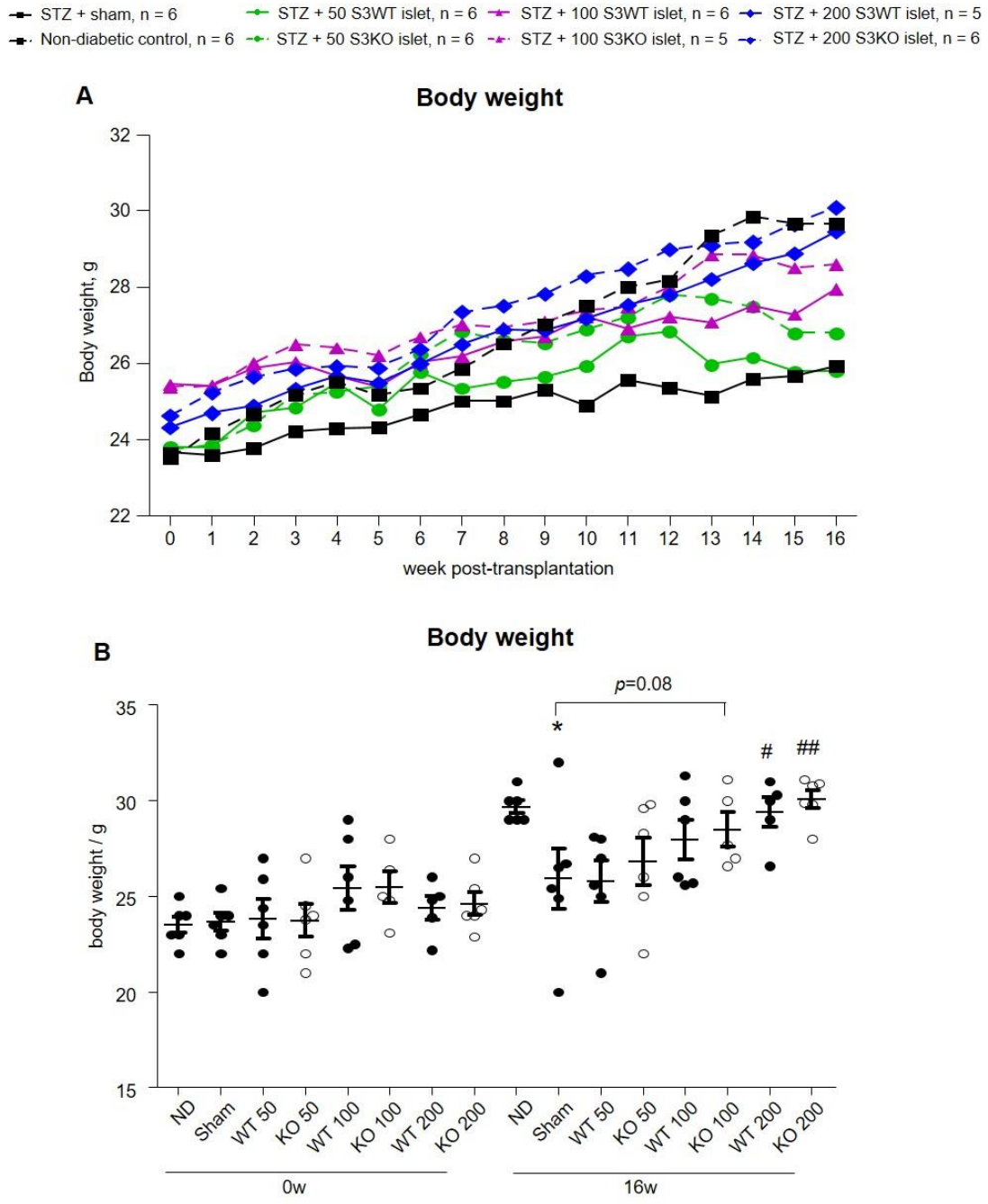

Figure S1. Effect of Smad3KO or WT islet transplantation on body weight in STZ-induced diabetic mice. (**A**) The mean value of weekly body weight over 16 weeks. **(B)** Body weight of mice before (0 week) and after islet therapy at week 16. Each dot represents one animal. ND, non-diabetic normal control. *$p < 0.05$ versus the age-matched normal control (ND). $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus the age-matched Sham group.

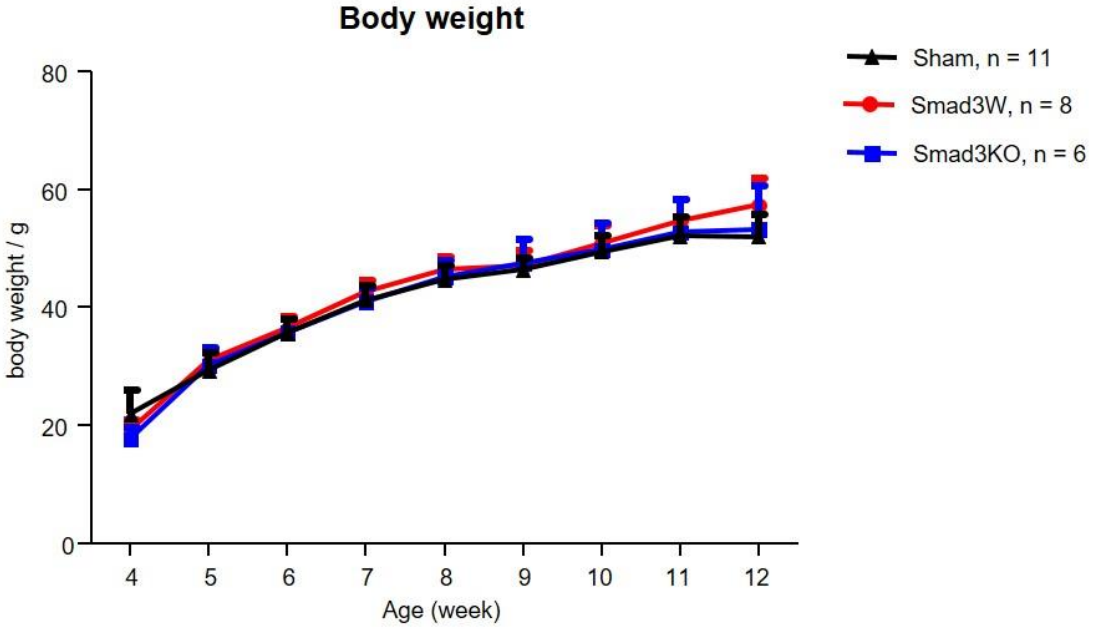

Figure S2. Transplantation of Smad3KO or Smad3WT islets doesn't influence the gain of body weight of recipient db/db mice.

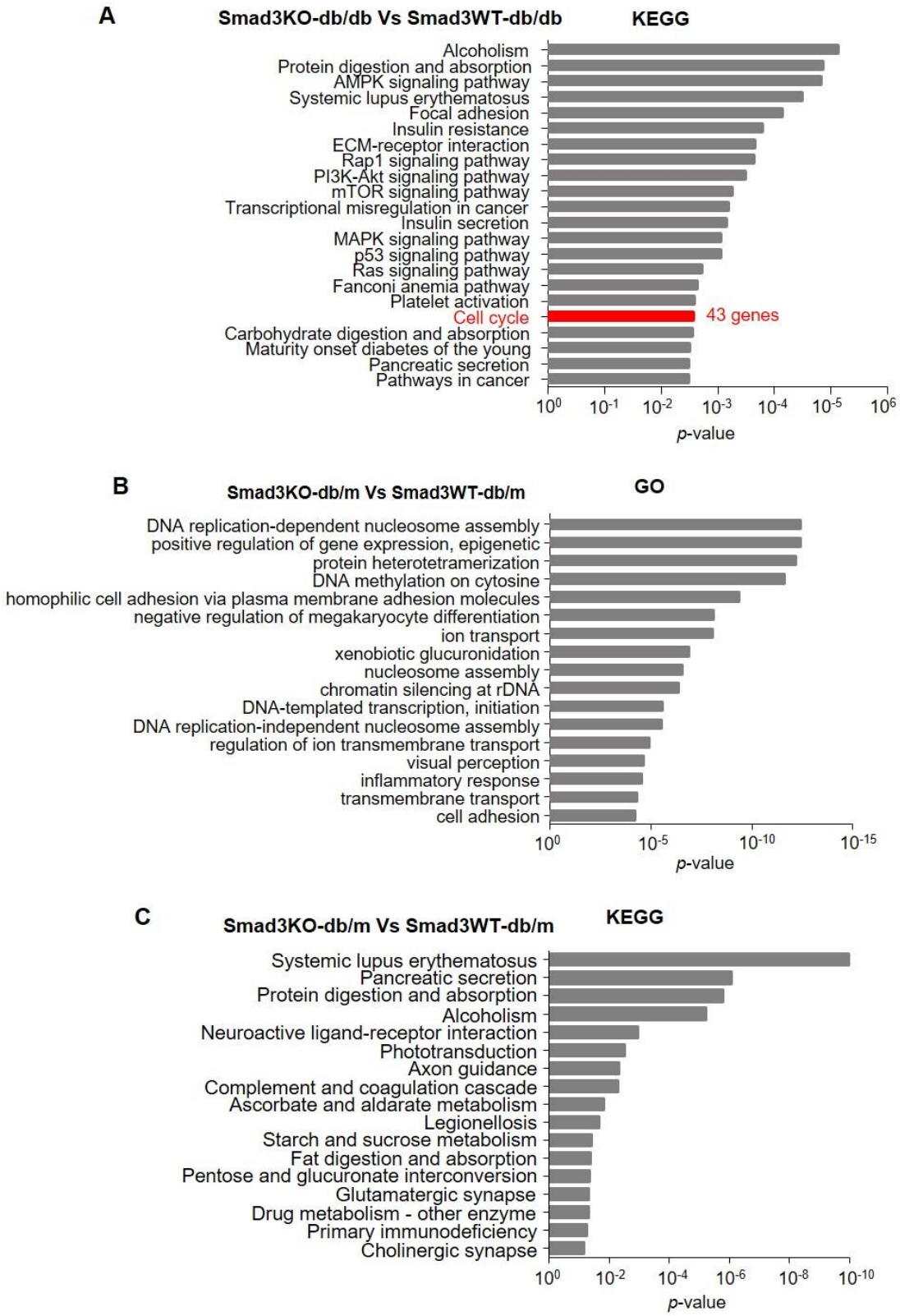

Figure S3. Bioinformatic analysis of DEGs in islets by RNA-seq. (A) KEGG pathway analysis of islet DEGs between Smad3WT-db/db and Smad3KO-db/db. (**B** and **C)** GO (GOTERM_BP_DIRECT subcategory) and KEGG analysis of DEGs in islets between Smad3WT-db/m and Smad3KO-db/m.

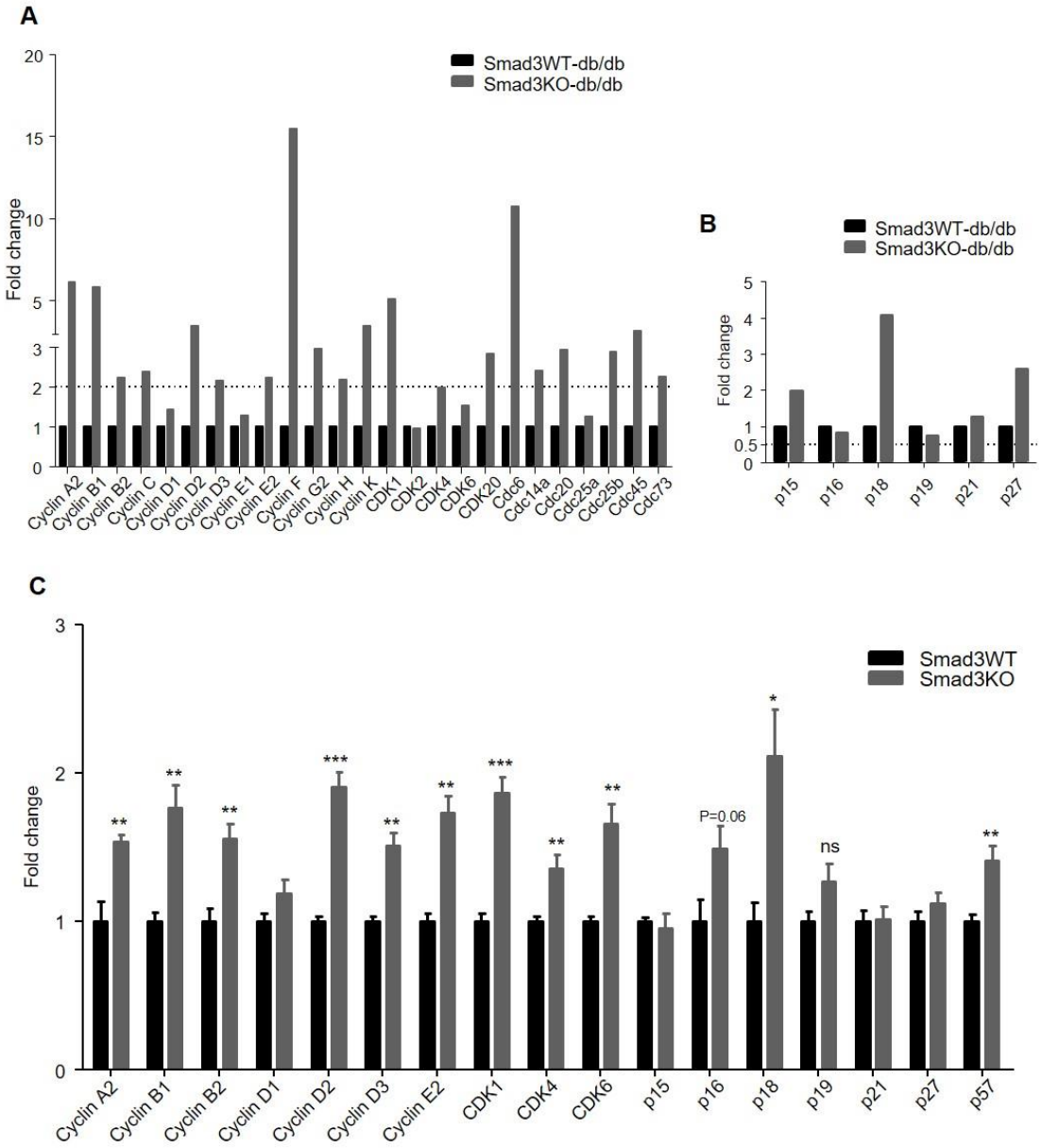

Figure S4. Expression of cell cycle-related genes in the islets isolated from Smad3WT-db/db and Smad3KO-db/db mice, and in cultured Smad3WT and Smad3KO islet cells. (A) Relative expression of genes associated with cyclin, CDK and CDC family revealed by RNA-seq. **(B)** Relative expression of genes from CDKI family revealed by RNA-seq. **(C)** RT-PCR detects the expression of selected cell cycle-related genes in 48 h-cultured islet cells. n = 4 culture replicates for each group. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ versus Smad3WT. ns, no significance.

Table S1. List of primers used in this study.

Primer name	Sequence (forward/reverse)	Application
E2f3.ChIP RT-PCR	CATGAAGCTGCCAGGAATGA TCCAACCAATGACGTGAGAG	ChIP RT-PCR
E2f3.ChIP PCR	CAGAGAGGGTGCTTGTTAGA GTGTCAAAGGCAAGTTGGAC	ChIP PCR
RT.β-actin	AGAGGGAAATCGTGCGTGAC CAATAGTGATGACC TGGCCGT	RT-PCR
RT.E2F3	AAGTCCTGAGATGCAGGTTC TGAGCGGACTGTATCGACTT	RT-PCR
RT.Cyclin A2	CTTACCCAGTACTTCCTGCA CTCTGTCCTGTGACTGTGTA	RT-PCR
RT.Cyclin B1	AGGCCGTGACAAAGGCATAA GCCTAAACTCAGAAGCAACAACA	RT-PCR
RT.Cyclin B2	TTCTCTGATGCTCTGCTCTG ACAGAGTTTCCTGCAGAAGC	RT-PCR
RT.Cyclin D1	ATCTGCATCACCCTGAGAGT GCTGTGGCTTTTCAGCAAAG	RT-PCR
RT.Cyclin D2	GTTCCTAAGAACCAGGCAGC GGTCACTTACCCTGCTAGCA	RT-PCR
RT.Cyclin D3	CTGCTCTTAGAGGGAACAGCC GCACCCTTAAGACCCCACAA	RT-PCR
RT.Cyclin E2	GCTTTGGCTTCTTCTACTCG GTTGACACAGTCCTCATCTC	RT-PCR
RT.CDK1	CAACAGAACCATCGCACTGA AGCAGGCTGTTTAGAGGCTA	RT-PCR
RT.CDK4	CTTTGCAGAGATGTTCCGTC GGTCAGCATTTCCAGTAGCA	RT-PCR
RT.CDK6	AGCTTCTAGAGGCTTCAGCA GGTAAGATGCAGGAAGATGC	RT-PCR
RT.p15	GAAGACTGCAAGCACGAAGA AGCTGCAGAAAATGCGTAGG	RT-PCR
RT.p16	GACATCAAGACATCGTGCGA GGATTTAGCTCTGCTCTTGG	RT-PCR
RT.p18	CCTTATGAAGCACACAGCCT CACATTCACTGCAGGCTTGT	RT-PCR
RT.p19	CTAGAGTTCTGATCCAGCTC CCAGAAGCATAGTGGATACC	RT-PCR
RT.p21	AGGCATATCTAGGCACTTGC CCACACACCATAGAATGCTC	RT-PCR
RT.p27	TCTCAGGCAAACTCTGAGGA AGAATCTTCTGCAGCAGGTC	RT-PCR
RT.p57	GCAAACGTCTGAGATGAGTT TCCTTGCACATGGTACAGAG	RT-PCR

Table S2. List of antibodies used in this study.

Antibody	Company	Catalog No.	Application
Primary antibody			
Rabbit anti-Smad3 ChIP grade	Abcam, USA	ab28379	ChIP, IP
Rabbit anti-PCNA	Santa Cruz	Sc-7907	WB, F-IHC
Rabbit anti-E2F3	Abcam	ab50917	WB, IHC
Mouse anti-BrdU	Dako	M0744	F-IHC
Alexa Fluor 488-conjugated insulin monoclonal antibody	eBioscience	53-9769-82	F-IHC, F-ICC
Rabbit anti-insulin	Abcam	ab181547	F-IHC, F-ICC
Rabbit anti-Col1a1	Cell Signaling,	72026	IHC
Secondary antibody			
DyLight™ 800 conjugated goat anti-rabbit IgG (H&L)	RockLand, USA	611-145-002	WB
DyLight™ 680 conjugated sheep anti-mouse IgG (H&L)	RockLand, USA	610-644-002	WB
DyLight™ 800 conjugated rabbit anti-mouse IgG (H&L)	RockLand, USA	610-445-002	WB
Rhodamine-conjugated Goat Anti-Mouse IgG Antibody	Merck Minipore	AP124R	F-IHC, F-ICC
Donkey anti-Rabbit IgG Secondary Antibody, Alexa Fluor 488	Thermo Scientific	A21206	F-IHC, F-ICC
Mouse anti-Rabbit IgG HRP (Light-chain specific)	Abmart	M21006	WB (after IP)

Note: ChIP, chromatin immunoprecipitation. WB, Western blot. IHC, immunohistochemistry. F-IHC, fluorescent immunohistochemistry. F-ICC, fluorescent immunocytochemistry. IP, immunoprecipitation.