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β1 integrin–extracellular matrix interactions are essential for maintaining exocrine pancreas architecture and function

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Integrin receptors are responsible for integrating extracellular matrix signals inside the cell. The most prominent integrin receptor, β1 integrin, has a role in cell function, survival and differentiation. Recently, we demonstrated a profound *in vivo* role of β1 integrin expression in the pancreas on glucose homeostasis and islet function. Here, we extend these results by examining the role of β1 integrin in exocrine pancreatic structure and function. Adult C57Bl/6 mice hemizygous for a collagen type Iα2 (*Col1a2*) promoter-controlled tamoxifen-inducible Cre recombinase gene and homozygous for loxP-β1 integrin were injected with tamoxifen or corn oil to generate mice deleted or not for β1 integrin. Pancreata derived from these male mice were analyzed by quantitative reverse transcriptase-polymerase chain reaction, western blot and immunofluorescence. Our results showed that β1 integrin-deficient mice displayed a significant decrease in pancreas weight with a significant reduction of amylase, regenerating islet-derived protein II and carboxypeptidase-A expression ($P<0.05$–0.01). Compared with control pancreata, β1 integrin-deficient pancreata showed reduced mRNA expression of extracellular matrix (collagen type Iα2, fibronectin and laminin) genes ($P<0.05$), detached acini clusters and lost focal adhesion structure. Moreover, β1 integrin-deficient pancreatic acinar cells displayed decreased proliferation ($P<0.05$) and increased apoptosis ($P<0.001$). Apoptosis was reduced to that of controls when isolated exocrine clusters were cultured in media supplemented with extracellular matrix proteins. Taken together, these results implicate β1 integrin as an essential component for maintaining exocrine pancreatic structure and function.

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Integrin receptors have a significant role in cell–cell and cell–extracellular matrix (ECM) contacts in many different tissue types. There are 18 α and 8 β receptors capable of forming 24 heterodimeric interactions; yet, half these interactions are made up by β1 integrin receptors.[1] This β1 integrin receptor group is largely responsible for attachment to the ECM.[2] Upon stimulation, β1 integrin has been shown to mediate cell motility, survival, proliferation and differentiation.[2–5]

Pancreatic acini are enclosed round structures that produce digestive enzymes.[6] Basal lamina covers the acini at the basal surface, stimulating acini integrin receptors.[6] Pancreatic stellate cells (PSCs) are identified as periacinar fibroblast-like cells of the pancreas that express glial fibrillary acidic protein (GFAP) and produce ECM proteins in support of surrounding tissue.[7] PSCs are also, in part, responsible for the fibrosis observed in chronic pancreatitis.[8,9] Previous studies have shown that α3β1 integrin is essential for proper apical/basolateral cell surface receptor organization and basement membrane formation in the submandibular gland.[10] As well, β1 integrin deficiency has shown to interfere with laminin-1 expression and basement membrane synthesis and assembly in embryoid bodies[11] and teratoma,[12] as well as collagen IV expression in the lens fiber of mice.[13] This is also true for proper fibronectin assembly, which requires α5β1 integrin.[14] β1 integrin has been studied in many tissues, yet the role of β1 integrin in the postnatal exocrine pancreas is almost wholly unknown. One recent study examined the effect of loss of β1 integrin expression in acinar cells during development; ~6-week-old β1 integrin-deficient mice were susceptible to pancreatitis and displayed aberrant acinar cell

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polarity and necrosis.[15] More recently, we have begun to probe the role of β1 integrin expression in the postnatal, developed pancreas, using a tamoxifen-dependent cre recombinase expressed under the control of a collagen I-specific promoter/enhancer. We demonstrated that adult mice deficient in β1 integrin showed impaired glucose tolerance with a significant reduction in pancreatic β-cell function, consistent with the onset of diabetes. Furthermore, these β1 integrin-deficient mice displayed a significant decrease in pancreatic focal adhesion kinase and extracellular signal-regulated kinase 1/2 activation, along with increased caspase-3 cleavage and decreased cyclin D1 expression.[16] However, the effects of β1 integrin deficiency on pancreatic exocrine morphology and function in conditional β1 integrin-deficient mice have still to be determined.

Here, we used mice homozygous for a loxP-β1 integrin allele and hemizygous for tamoxifen-dependent cre recombinase expressed under the control of a collagen type I promoter to analyze pancreatic exocrine morphology and function *in vivo*.[16] We found that mice with β1 integrin deficiency controlled by the collagen I promoter had a primary defect in PSCs and islets in the pancreas, which led to a significant decrease in ECM products in the exocrine compartment. β1 integrin-deficient mice displayed significantly decreased food intake with a loss of body weight, which was associated with reduced pancreatic amylase, carboxypeptidase A and regenerating islet-derived protein II expression. These β1 integrin-deficient mice also demonstrated decreased exocrine cell proliferation and increased apoptosis. Interestingly, cultured acinar cell clusters isolated from β1 integrin-deficient mouse pancreata in the presence of ECM proteins showed an improved acinar cell apoptosis. This study indicates that sufficient ECM and β1 integrin interactions are essential for maintaining exocrine pancreatic integrity and function.

MATERIALS AND METHODS

Conditional β1 Integrin-Deficient Mice

To generate conditional β1 integrin-deficient mice (β1KO) in collagen I-producing cells, floxed β1 integrin mice were crossed with C57BL/6 mice containing CRE-ER^T (tamoxifen-inducible cre recombinase) gene downstream of the collagen type Iα2 (*Col1a2*) promoter, as described previously.[17] Progeny mice with positive genotype, as analyzed by polymerase chain reaction (PCR),[17] were induced by intraperitoneal injection of 1 mg tamoxifen (4-hydroxytamoxifen; Sigma, St Louis, MO, USA) per mouse per day for 5 days at 3 weeks of age.[16] Corn oil-injected Cre-positive and tamoxifen-injected Cre-negative mouse groups were merged as controls (Ctrl) and experiments were carried out on male mice at 4 and 7 weeks post-injection. Deletion of β1 integrin was confirmed by quantitative RT-PCR, western blot and immunofluorescence as described previously.[16] A Rosa26loxP-STOP-lacZ mouse (Jackson Laboratories), which does not express the β-galactosidase reporter gene unless cre recombinase is expressed in the nucleus, was crossed with *Col1a2*-Cre-ER^T to identify the cells within the pancreas that expressed cre under control of the *Col1a2* promoter.[17] All protocols were approved by the Animal Use Subcommittee at the University of Western Ontario in accordance with the guidelines of the Canadian Council of Animal Care.

Body and Pancreas Weight and Food Intake Studies

The body and pancreas weight of β1 integrin-deficient and control mice were measured at 4 and 7 weeks post-injection. At 4 weeks post-injection, mice from both β1 integrin-deficient and control groups were separated individually and had their food weighed daily at 0900 hours and monitored their food intake for a week. Data were expressed as average food intake per mouse per day.

Acinar Cell Culture Experiments

To isolate acinar cell clusters, both β1 integrin-deficient and control mouse pancreata at 3 weeks post-injection were dissected and digested with collagenase XI (1 mg/ml; Sigma). Acinar cell clusters were cultured in modified RPMI 1640 media[18] with either 1% BSA (Sigma) or 10% FBS (Invitrogen, Burlington, ON, Canada), reported to be enriched for ECM proteins,[19,20] for 24 h. Cell clusters from four experimental groups (β1KO-BSA, Ctrl-BSA, β1KO-FBS, and Ctrl-FBS) were harvested and processed for immunofluorescence with at least four mouse pancreata per experimental per group used.

RNA Extraction, Real-Time RT-PCR

Total RNA was extracted from pancreata of β1 integrin-deficient and control mice at 1 week post-injection using the miRNeasy kit (Qiagen, Germantown, MD, USA).[16] For each reverse transcription reaction, 2 μg of total RNA from whole pancreatic tissue were used with oligo(dT) and random primers, as well as Superscript reverse transcriptase (Invitrogen). Sequences of PCR primers used for RT-PCR with expected size of product are listed in Table 1. Real-time RT-PCR analyses were performed as described previously.[16] Data were normalized to levels of 18S rRNA subunit and relative gene expression was calculated based on the $2^{\Delta\Delta}$CT method as PCR signals from β1 integrin-deficient pancreata relative to control pancreata.[16]

Protein Extraction and Western Blot Analysis

β1 integrin-deficient and control pancreata, as well as acinar cell clusters, were sonicated in Nonidet-P40 lysis buffer to extract protein. Equal amounts (2 μg) of lysate pancreatic proteins from each experimental group were separated by 10 or 12% sodium dodecyl sulfate-polyacrylamide gel electrophesis and transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were incubated with appropriate diluted primary antibodies as listed: mouse anti-amylase (Santa Cruz Biotechnology, Santa Cruz, CA, USA); mouse anti-carboxypeptidase A (AbD serotec; Raleigh, NC, USA); mouse anti-regenerating islet-derived protein II and goat anti-β1 integrin (R&D System, Minneapolis, MN,

Table 1 Sequences of primers used in real-time PCR

Primer name	Accession number	Primer pair sequence 5′-3′ (sense/antisense)	Fragment size (bp)
Amy1	NM_007446.2	CAAGTGGAGGTATCGAGAAC	149
		CCCTGCTACGCCAATGTCAA	
Col1a1	NM_007742.3	CCTCCCAGTGGCGGTTATGAC	383
		GGGTTCGGGCTGATGTACCAG	
Col1a2	NM_007743.2	AGGGTGAAACTGGTCTCCGA	374
		CCCTCCATCACCACGACTTC	
Fn1	NM_010233.1	AACTACGATGCCGATCAGAA	263
		CCCTCCTCGTGACGCTTGTG	
Lama1	NM_008480.2	GGCGGTATAACAACGGAACC	180
		GCAATCCACCCACGTAAATC	
Lamb1	NM_010721.2	CGAGAGTATGAGGCGGCACT	285
		CACTTCCACCAAGCGGGTCT	
Lamc1	NM_010683.2	TAGCCAAATTAGCCGACTGC	122
		GCTCCCTGGAGGCGATCTCA	
18S	NR_003278.1	GTAACCCGTTGAACCCCATTC	151
		CCATCCAATCGGTAGTAGCG	

USA). Proteins were detected using ECL™-Plus Western blot detection reagents (Perkin-Elmer, Wellesley, MA, USA) and exposed using the Versadoc Imaging System (Bio-Rad Laboratories). Densitometric quantification of bands at subsaturation levels was performed using the Quantity One software (Bio-Rad Laboratories) and normalized to appropriate loading controls. Data are expressed as relative expression level of protein to the loading control.[16]

Serum Assays for Amylase and Pancreatic Lipase

Serum was collected from 7 weeks post-injected β1 integrin-deficient and control mice. To measure serum amylase levels, a Phadebas Test tablet (Magle Life Sciences, Lund, Sweden) was added to diluted serum samples, based on the manufacturer's instructions. Amylase activity was measured with a spectrophotometer and determined by comparing absorbance values to a standard curve.[21] To examine the serum level of pancreatic lipase, serum samples were mixed with lipase enzyme substrate and enzyme activator as per the manufacturer's instructions. Pancreatic lipase activity was measured using a spectrophotometer and a formula offered by Genzyme Diagnostics (Charlottetown, PEI, Canada).[22]

Extracellular Matrix Protein Analysis

Pancreata from β1 integrin-deficient and control mice at 7 weeks post-injection were dissected and fixed in 4% paraformaldehyde. In all, 4-μm-thick pancreatic tissue sections were prepared from the entire length of the pancreas and stained with hematoxylin and eosin, Masson's trichrome (for total collagen analysis) and picrosirius red (for birefringent collagen I staining), as described previously.[16] To evaluate quantitatively fibronectin concentration in the pancreas of β1 integrin-deficient and control mice, a fibronectin mouse sandwich ELISA kit was used (Abcam, Cambridge, MA, USA).

Immunofluorescence and TUNEL Assay

Sets of pancreatic tissue sections (4 μm thick) were immunofluorescently stained with appropriate diluted primary antibodies as listed: rabbit anti-β1 integrin (Millipore, Temecula, CA, USA); rabbit anti-*Escherichia coli* β-galactosidase (Abcam, Cambridge, MA, USA); mouse anti-GFAP (Pharmingen, Mississauga, ON, Canada); guinea-pig anti-insulin (Zymed, San Francisco, CA, USA) and mouse anti-laminin (Developmental Studies Hybridoma Bank, Iowa City, IA, USA). Fluorescent secondary antibodies were obtained from Jackson Immunoresearch Laboratories (West Grove, PA, USA) and 4′-6-diamidino-2-phenylindole (Sigma) was used for nuclear counterstaining.[16]

To assess the structural organization of focal adhesion contacts in pancreatic acinar cells, an Actin Cytoskeleton and Focal Adhesion Staining kit (Chemicon, Temecula, CA, USA) containing mouse anti-vinculin monoclonal antibody and TRITC-conjugated phalloidin was used. Cell proliferation was examined using Ki67 labeling (Abcam).

To examine the cells undergoing apoptosis, the terminal deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay was performed.[23] Briefly, pancreatic sections were pretreated with 0.1% trypsin, and then incubated with the TUNEL reaction mixture conjugated with fluorescein-dUTP (Roche, Montreal, QC, Canada). Percent cell proliferation and apoptosis were calculated by counting Ki67- or TUNEL-labeled cells in exocrine tissue from at least 12 randomly selected fields of view per pancreatic section.[16]

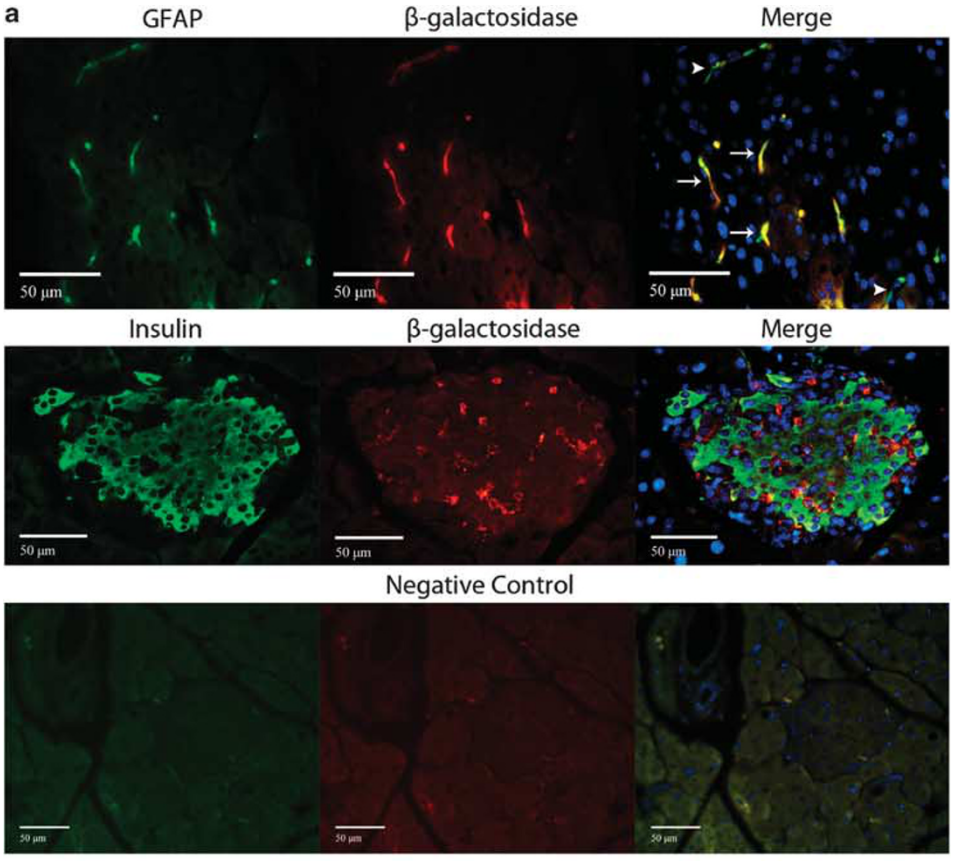
Statistical Analysis

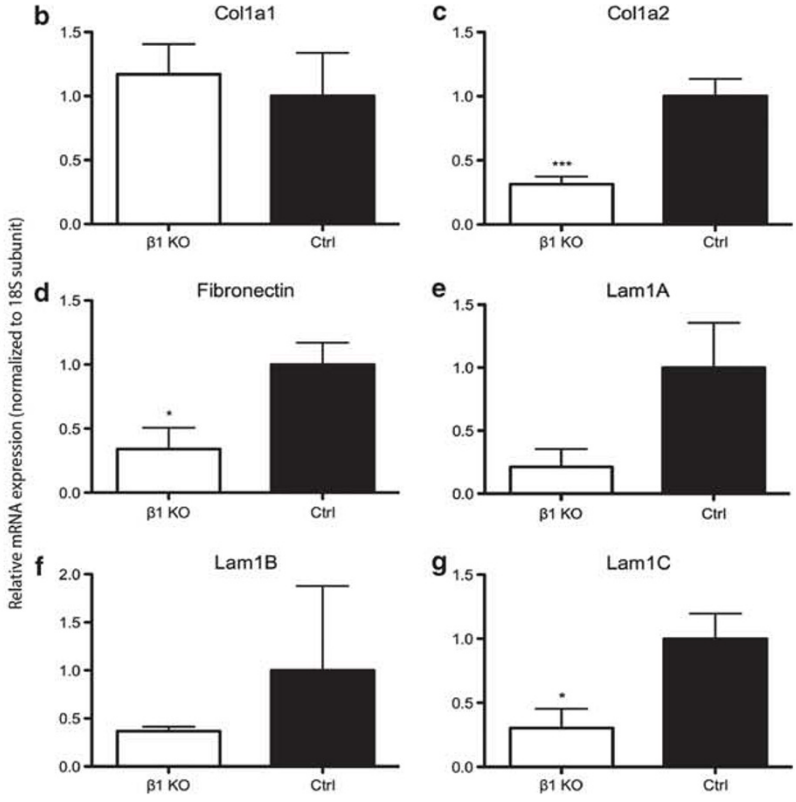
Data are expressed as means ± s.e.m. Statistical significance was determined using the unpaired Student's *t*-test. Differences were considered to be statistically significant when $P<0.05$.

RESULTS

Loss of β1 Integrin Observed in PSCs and Islets with a Significant Reduction in ECM Expression in the Pancreas of β1 Integrin-Deficient Mice

To determine the pancreatic cell types that would be directly affected by the knockout of β1 integrin, transgenic Rosa26loxP-STOP-lacZ mice were crossed with Col1a2-CRE(ER)^T mice.[17] The resultant mice were injected with tamoxifen to allow expression of β-galactosidase in cells specifically expressing cre recombinase under control of the *Col1a2* promoter. Cells within the pancreas positive for β-galactosidase were detected with an anti-β-galactosidase antibody (Figure 1a). Double labeling using an antibody directed against GFAP, a marker of PSCs,[24] revealed that the *Col1a2* promoter was active in PSCs (Figure 1a). Moreover, β-galactosidase expression was also found in islets of the pancreas (Figure 1a). These results


a
GFAP
β-galactosidase
Merge
50 μm
Insulin
β-galactosidase
Merge
50 μm
Negative Control
50 μm


b
Col1a1
c
Col1a2
d
Fibronectin
e
Lam1A
f
Lam1B
g
Lam1C
Relative mRNA expression (normalized to 18S subunit)
β1 KO
Ctrl
***
*

indicate that these two cell populations express cre recombinase under the control of the *Col1a2* promoter.

To examine the effects of β1 integrin loss in PSCs and islets on pancreas function, mice hemizygous for Col1a2-CRE(ER)^T and homozygous for loxP-β1 integrin were generated. The resultant mice were injected with either tamoxifen or corn oil to generate mice deleted or not for β1 integrin in PSCs and islets. To assess whether β1 integrin-deficient mice pancreas showed altered ECM expression, quantitative RT-PCR analysis of collagen type Iα1 (*Col1a1*), *Col1a2*, fibronectin 1 (*Fn1*), laminin α1 (*Lama1*), laminin β1 (*Lamb1*) and laminin γ1 (*Lamc1*) mRNA was performed at 1 week post-injection. We found a significant decrease in the expression of *Col1a2* ($P<0.001$), *Fn1* ($P<0.05$) and *Lamc1* ($P<0.05$) mRNA in β1 integrin-deficient mice pancreata when compared with controls (Figure 1b–g). These results indicate that β1 integrin expression on PSCs and islets is critical in mediating ECM gene expression in the pancreas.

Disturbed Acinar Cell–Cell Contacts and Focal Contact Complex Observed in the β1 Integrin-Deficient Mouse Pancreas

Loss of β1 integrin in pancreatic PSCs and islets resulted in decreased ECM expression; therefore, we set to investigate any alterations in pancreatic morphology. Based on hematoxylin and eosin stain, we found that pancreatic acini similarly organized between β1 integrin-deficient and control mice. However, the detachment of acini clusters from one another was observed along with very basophilic stain in the periphery of the acinus in β1 integrin-deficient pancreata (Figure 2a). Masson's trichrome (Figure 2b, upper panel) and picrosirius red (Figure 2b, lower panel) staining revealed a reduction in collagen fibers and connective tissue between the acini clusters. Furthermore, analysis of pancreatic fibronectin protein levels in β1 integrin-deficient mice showed a significant decrease compared with controls ($^*P<0.05$; Figure 2c). Actin cytoskeleton and focal adhesion staining showed more robust cytoskeletal fibers along with more pronounced and continuous vinculin stain in control, but not β1 integrin-deficient, mouse pancreas at 7 weeks post-tamoxifen injection (Figure 2d). Likewise, there was less colocalization of F-actin and vinculin, suggesting fewer focal adhesions in 7 weeks post-tamoxifen-injected β1 integrin-deficient mice when compared with controls (Figure 2d). Finally, anti-laminin immunofluorescence showed a dramatic loss of laminin in 7 weeks post-tamoxifen-injected β1 integrin-deficient mouse pancreas, whereas controls show clear laminin at cell–cell contacts (Figure 2e).

Reduced β1 Integrin in Acinar Cells, along with Significantly Reduced Pancreas Weight and Food Intake Observed in β1 Integrin-Deficient Mice

Although the β-galactosidase stain showed a direct effect on PSCs and islets, but not acinar cells in the β1 integrin-deficient mouse pancreas, reduced β1 integrin expression stain in the acinar cell population was observed in 1 week post-tamoxifen-injected β1 integrin-deficient mice when compared with controls, but not at 3 days (Figure 3a and b). Protein expression analyses confirm these results, indicating a significant reduction in β1 integrin in β1 integrin-deficient mice at 7 days, but not 3 days, when compared with controls ($^*P<0.05$; Figure 3c and d). This suggests that loss of β1 integrin expression in the acinar cells may be secondary to the loss of ECM proteins. Analysis of body and pancreas weight in 4 and 7 weeks post-tamoxifen-injected β1 integrin-deficient mice showed a significantly reduced ratio of pancreas to body weight at 7 weeks post-injection when compared with control mice ($^*P<0.05$; Figure 3e). As well, a significant decrease in food intake was observed in 4 weeks post-tamoxifen-injected β1 integrin-deficient mice compared with controls ($^{***}P<0.001$; Figure 3f).

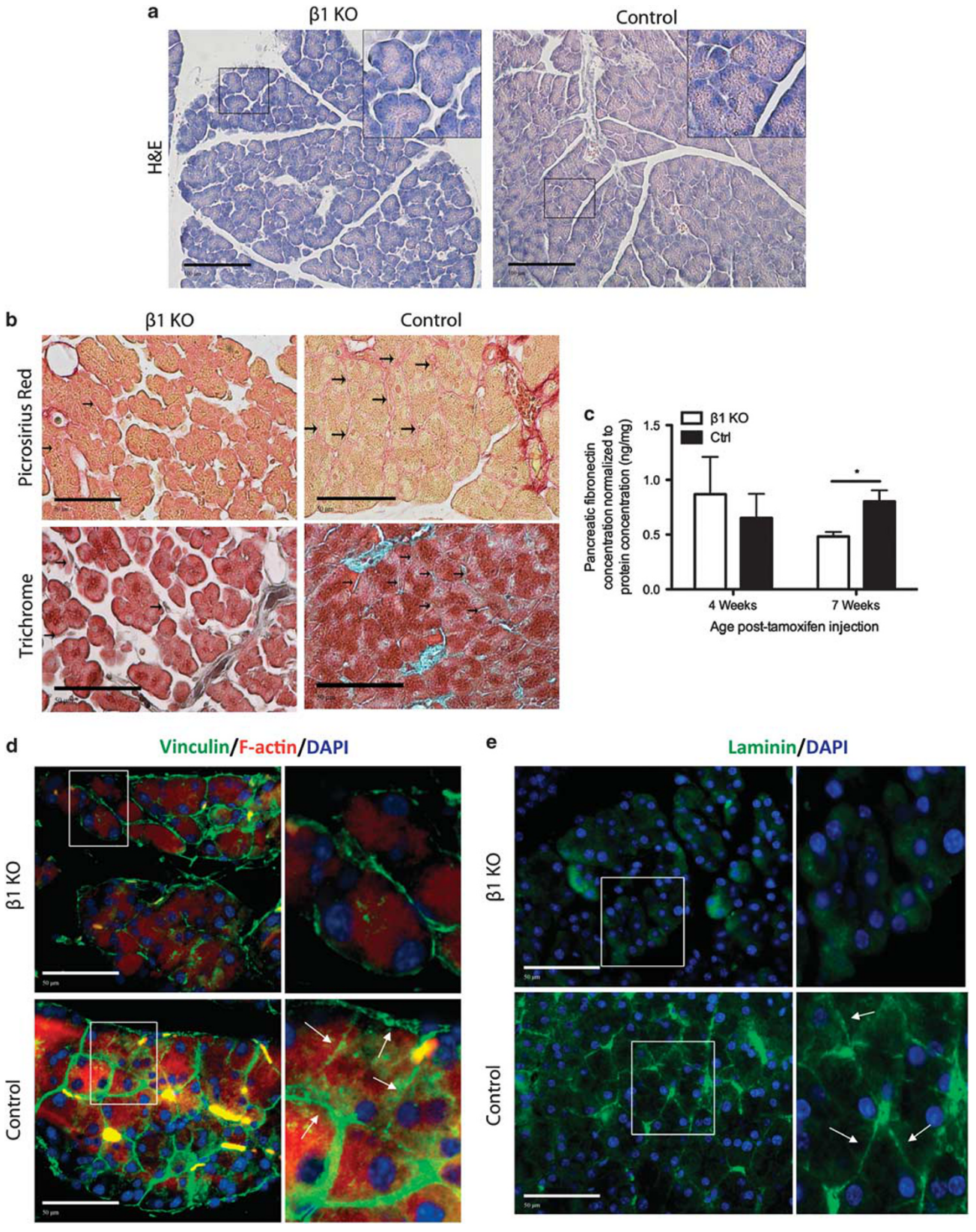
Significantly Reduced Amylase Expression Observed in β1 Integrin-Deficient Mice

To assess the defect of β1 integrin deficiency on exocrine pancreatic products, the expression of amylase mRNA and protein, as well as serum amylase levels, was examined. We found that β1 integrin-deficient mice compared with controls had significant decreases in amylase mRNA expression ($^*P<0.05$; Figure 4a), with a 50% reduction in serum amylase level ($^{***}P<0.01$; Figure 4b) and total amylase protein expression in the pancreas ($^*P<0.05$; Figure 4d). Furthermore, serum pancreatic lipase activity was significantly reduced in β1 integrin-deficient mice, but not controls ($^*P<0.05$; Figure 4c). Protein expression level of carboxypeptidase A and regenerating islet-derived protein II was also significantly decreased in β1 integrin-deficient pancreata when compared with controls at 7 weeks post-injection ($^*P<0.05$–$^{***}P<0.01$; Figure 4e and f), confirming reduced pancreatic exocrine cell function in β1 integrin-deficient mice.

Reduced β1 Integrin in Acinar Cells Led to Significantly Increased Cell Apoptosis, which was Partially Rescued by Culturing Acinar Cell Clusters with ECM Proteins

To analyze the proliferative and apoptotic status of β1 integrin-deficient mouse pancreas, Ki67 immunofluorescence staining and TUNEL was conducted. The percent of Ki67-

Figure 1 (**a**) Representative immunofluorescence images of the pancreas of a transgenic Rosa26loxP-STOP-lacZ mouse crossed with a Col1a2-CRE(ER)^T mouse, whereby the β-galactosidase reporter gene is expressed only if cre recombinase is present using anti-glial fibrillary acidic protein (GFAP), anti-β-galactosidase and anti-insulin antibodies (arrows indicate colocalization of GFAP and β-galactosidase, while arrowheads show no colocalization). (**b**) Relative mRNA expression (normalized to 18S subunit) of collagen type Iα1 (*Col1a1*), *Col1a2*, fibronectin 1 (*Fn1*), laminin α1 (*Lama1*), laminin β1 (*Lamb1*) and laminin γ1 (*Lamc1*) of β1 integrin-deficient and control mouse pancreas at 1 week post-tamoxifen injection ($n=3$–5). Data are expressed as means ± s.e.m. $^*P<0.05$, $^{***}P<0.001$ *vs* controls.


a
β1 KO
Control
H&E
b
β1 KO
Control
Picrosirius Red
Trichrome
c
β1 KO
Ctrl
Pancreatic fibronectin concentration normalized to protein concentration (ng/mg)
1.5
1.0
0.5
0.0
*
4 Weeks
7 Weeks
Age post-tamoxifen injection
d
Vinculin/F-actin/DAPI
β1 KO
Control
e
Laminin/DAPI
β1 KO
Control

positive proliferating acinar cells was significantly decreased in β1 integrin-deficient mice at 7 weeks post-tamoxifen injection when compared with control mice (*$P<0.05$; Figure 5a). Furthermore, a significant increase in acinar cells undergoing apoptosis was observed in β1 integrin-deficient mice (***$P<0.001$; Figure 5b).

Since the primary β1 integrin defect was in PSCs and islets, we hypothesized that the pancreatic exocrine dysfunction is due to problematic ECM stimulation of acini. To investigate this, we isolated acinar cell clusters from control and β1 integrin-deficient pancreata and cultured them with or without FBS that contains rich ECM proteins.[19,20] β1 integrin-deficient acinar cell clusters cultured with BSA showed significantly increased cell apoptosis when compared with the control cell clusters (*$P<0.05$; Figure 5c). However, the number of TUNEL-positive cells in β1 integrin-deficient acinar cell clusters was reduced and reached control levels when cultured with FBS medium (*$P<0.05$; Figure 5c).

DISCUSSION

This study analyzed the effects of β1 integrin deficiency on pancreatic exocrine tissue. Our results demonstrate that β1 integrin deficiency under control of the collagen I promoter directly affected PSCs and islets, and this led to significantly reduced ECM protein production in the pancreas. PSCs support parenchyma by secreting ECM components that integrins use as ligands. Indeed, PSCs in human pancreatic acini stain positive for collagens I, III and IV, laminin, fibronectin and other ECM proteins.[7] Furthermore, there is a necessity of α5β1 integrin in connective tissue growth factor stimulation of PSC collagen I synthesis.[25] It has also been reported that PSC secretion of growth factors and ECM components are, in part, responsible for fibrosis in chronic pancreatitis.[8,9] Our results support previous research, in that β1 integrin is required to maintain PSC expression of certain ECM proteins, including *Col1a2*, *Fn1* and *Lamc1*.

Our observation of reduced pancreatic ECM mRNA was concomitant with disrupted cell–cell contacts between acini along with reduced connective tissue and collagen fibers, laminin immunoreactivity and acinar cell expression of β1 integrin. It is unclear precisely which process preceded the other, but studies have demonstrated that β1 integrin loss led to improper basement membrane assembly and laminin expression in teratoma[12] and embryoid bodies,[11] implicating β1 integrin as an essential part of ECM maintenance. It has also been demonstrated that ECM proteins can regulate integrin expression in human fibroblasts.[26] Ablation of β1 integrin by inactivating monoclonal antibody treatment disrupted cell–cell contacts of keratinocytes.[27] Likewise, mouse mammary gland alveoli deficient of β1 integrin could not attach to the basement membrane laminin substratum.[28] This study also showed decreased focal adhesion contacts in β1 integrin-deficient mammary epithelium when compared with controls.[27] Taken together, the findings of this study demonstrate that reduced β1 integrin in PSCs had a direct effect on ECM expression, which in turn effected acinar cell β1 integrin expression, cell–cell interactions, and subsequently cell proliferation and death.

β1 integrin has been well-established as upstream of the mitogen-activated protein kinase pathway, which has profound effects on cell survival and proliferation.[16,23] Our previous analysis of β1 integrin-deficient mice demonstrated a significant reduction in focal adhesion kinase and extracellular signal-regulated kinase 1/2 phosphorylation, indicating reduced mitogen-activated protein kinase signaling.[16] Our results corroborate these studies by demonstrating a reduction in pancreatic β1 integrin, along with significantly decreased proliferation and increased apoptosis. To elucidate if the observed effects of pancreatic exocrine dysfunction were due to β1 integrin or ECM protein deficiency, isolated exocrine cell clusters from β1 integrin-deficient and control mouse pancreas were cultured in ECM-enriched medium. Our data indicate that apoptosis was significantly reduced in β1 integrin-deficient cell clusters when cultured in media with FBS, suggesting that supplementing ECM via FBS to β1 integrin-deficient cell clusters can improve their survival status. This strengthens our working model, whereby pancreatic exocrine defects associated with β1 integrin deficiency may be largely due to insufficient ECM proteins.

It was noted that food intake was significantly reduced in β1 integrin-deficient mice at 4 weeks post-tamoxifen injection along with a significant decrease in body weight.[16] Many studies have established a positive correlation between food consumption and weight gain.[29,30] Food intake is controlled by a brain–gut axis associated with multiple factors including pancreatic enzyme secretion.[31–33] Our data demonstrated a significant reduction in pancreatic lipase, amylase and carboxypeptidase A expression, major enzymes produced by the exocrine pancreas, in β1 integrin-deficient mice when compared with controls, suggesting that the pancreatic insufficiency occurred because of a loss of ECM proteins

Figure 2 (**a**) Hematoxylin and eosin, (**b**) picrosirius red (upper panels) and trichrome (lower panels) staining of β1 integrin-deficient and control mouse pancreas at 7 weeks post-injection. Black arrows indicate positive collagen and connective tissue stain in controls with fewer in β1 integrin-deficient mice. (**c**) Relative fibronectin concentration between β1 integrin-deficient and control (Ctrl) mice at 4 and 7 weeks post-injection ($n=3$–4). Data are expressed as means ± s.e.m. *$P<0.05$ *vs* control. Immunofluorescence was used to investigate focal adhesions (**d**) and laminin expression (**e**). Focal contacts are defined by vinculin (green) and actin filaments by tetramethyl rhodamine isothiocyanate (TRITC)-conjugated phalloidin (red). Nuclei are counterstained by 4′-6-diamidino-2-phenylindole (DAPI) (blue). Boxed regions display zoom, while arrows display cell–cell contacts in focal adhesion and laminin immunofluorescence in control, but not β1 integrin-deficient mouse pancreas. Scale bar = 100 μm (**a**) and 50 μm (**b**, **d** and **e**).
KO, knockout.

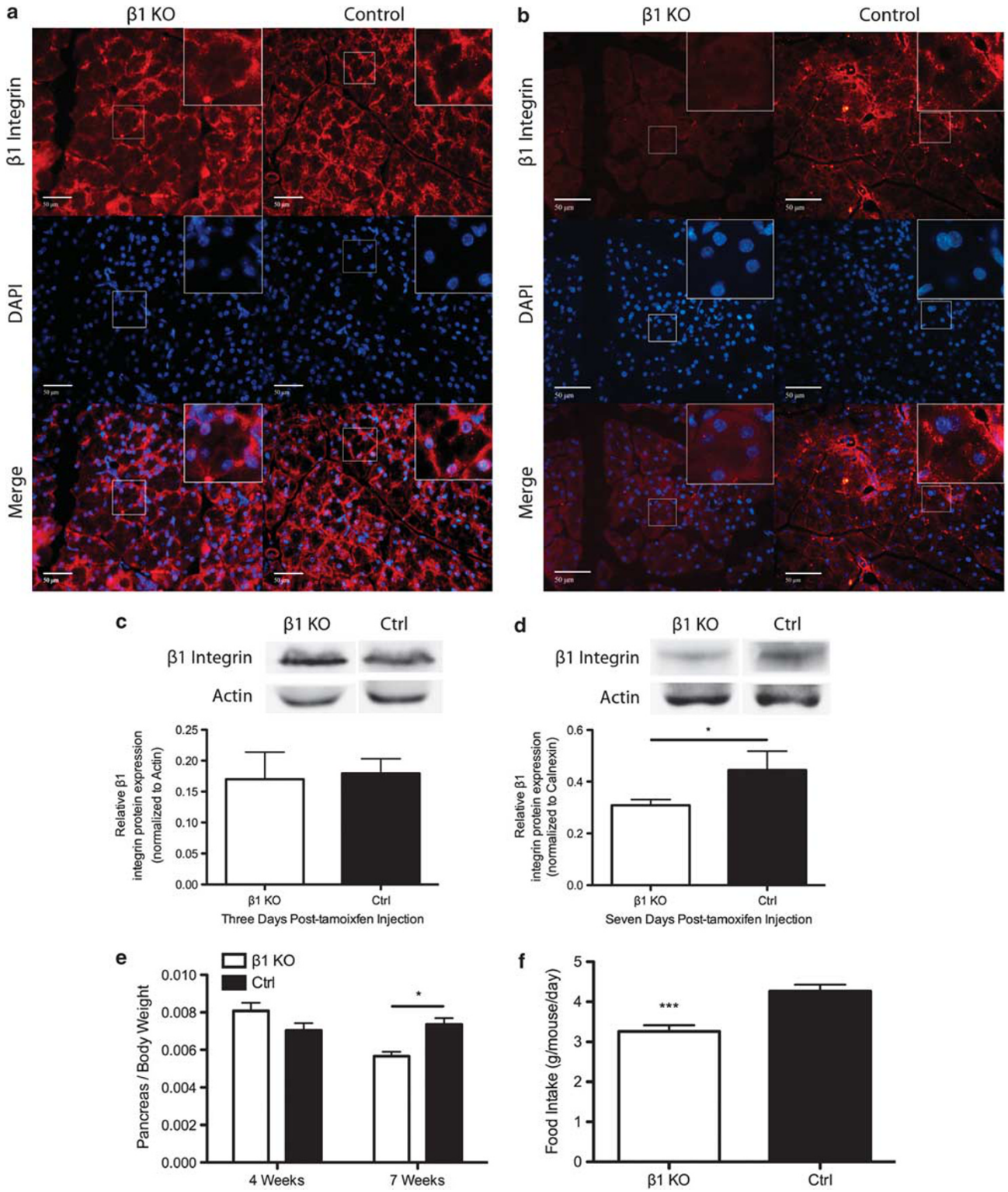


Figure 3 Representative immunofluorescence images of β1 integrin-deficient and control mouse pancreas at 3 (**a**) and 7 days (**b**) post-tamoxifen injection using an anti-β1 integrin antibody. Western blot analyses of β1 integrin-deficient and control mouse β1 integrin expression at 3 (**c**) and 7 days (**d**) post-tamoxifen injection ($n = 4$). (**e**) Pancreas to body weight ratio in β1 integrin-deficient and control mice at 4 and 7 weeks post-injection ($n = 7$–17). (**f**) Food intake data from 4 weeks post-tamoxifen-injected β1 integrin-deficient mice with respective controls ($n = 7$–8). Data are expressed as means ± s.e.m. $^*P < 0.05$; $^{***}P < 0.001$ *vs* controls. Scale bar = 50 μm. Ctrl, control; KO, knockout.

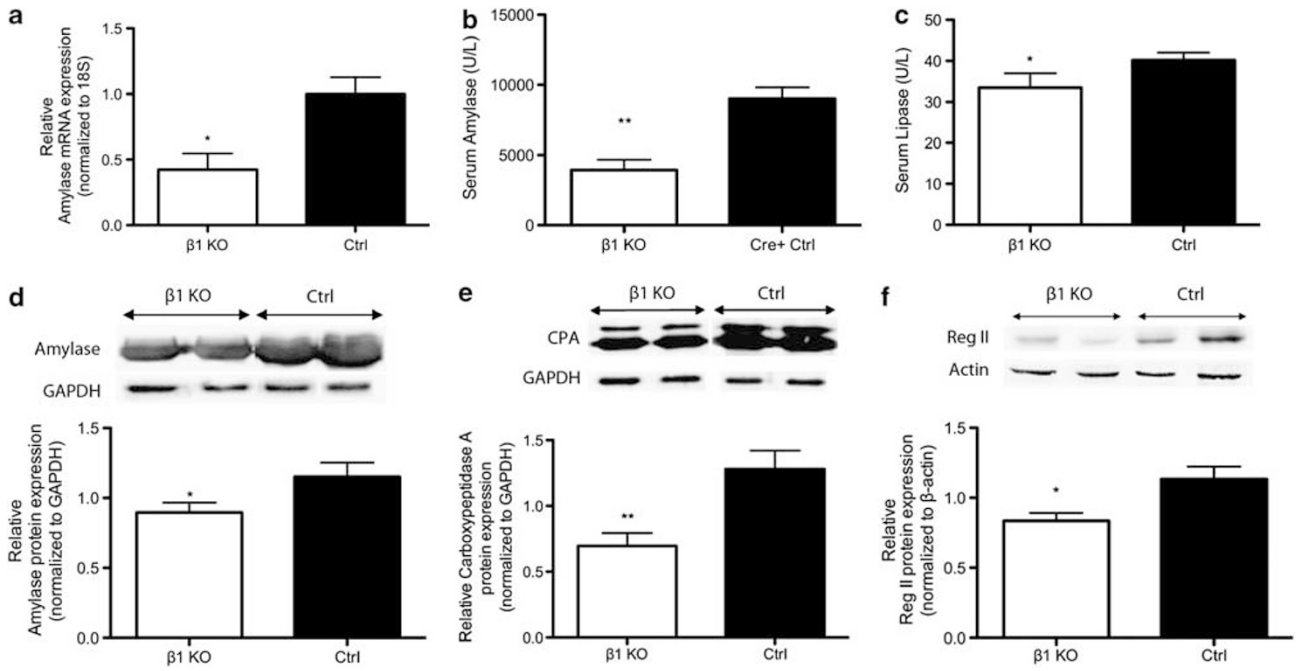


Figure 4 (**a**) Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis of amylase mRNA expression in 1 week post-injected β1 integrin-deficient and control mouse pancreas ($n = 3$). Analysis of serum (**b**) and pancreatic protein (**d**) amylase and serum pancreatic lipase (**c**) in 7 weeks post-injected β1 integrin-deficient and control mice ($n = 3$–10). Relative carboxypeptidase-A (CPA) (**d**) and regenerating islet-derived protein II (Reg-II) (**e**) protein expression in the pancreas of 7 weeks post-injected β1 integrin-deficient and control mice pancreas ($n = 4$). Data are expressed as means ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$ *vs* controls. Ctrl, control; KO, knockout.

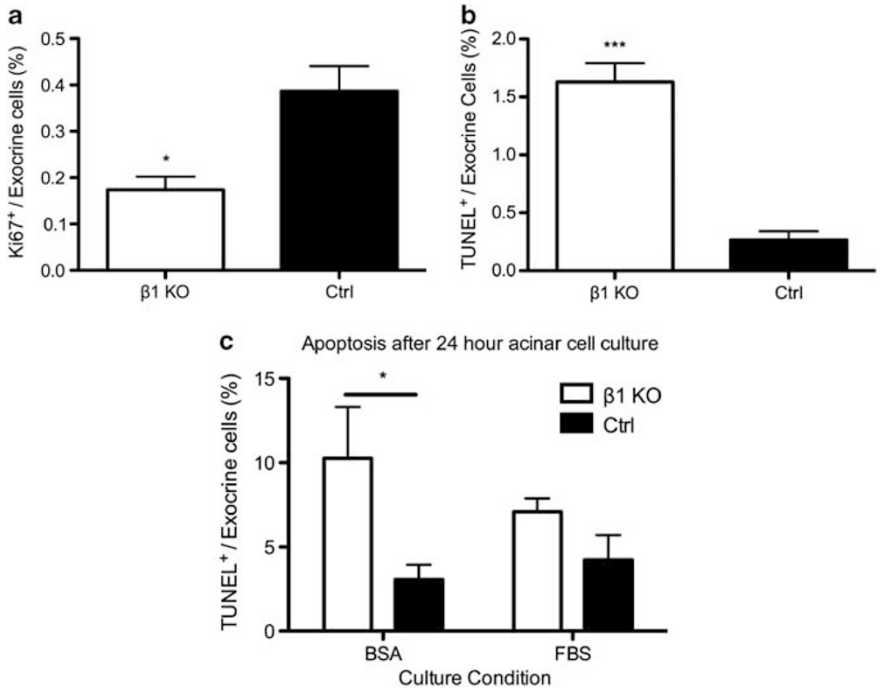


Figure 5 Quantitative analysis of cell proliferation (**a**) and apoptosis (**b**) in the exocrine pancreas of β1 integrin-deficient and control mice at 7 weeks post-injection. (**c**) Apoptosis in exocrine cell clusters cultured in either bovine serum albumin (BSA) or fetal bovine serum (FBS) for 24 h. The percentage of Ki67- or terminal deoxynucleotidyl transferase mediated nick-end labeling (TUNEL)-positive cells over total number of cells counted was calculated and data are expressed as means ± s.e.m. ($n = 4$). $^*P < 0.05$, $^{***}P < 0.001$ *vs* controls. Ctrl, control; KO, knockout.

and β1 integrin in the pancreas. The pancreas of β1 integrin-deficient mice became significantly underweight at 7 weeks post-tamoxifen injection,[16] which was more drastic than the drop in body weight as shown by the ratio of pancreas to body weight. This ratio was significantly decreased in β1 integrin-deficient mice when compared with controls, further indicating a severe pancreatic defect. Meanwhile, other research has shown that reduction in food intake decreases serum[32] and pancreatic[33] amylase, which may be related to lower insulin levels. These studies are in line with our research, whereby β1 integrin-deficient mice display reduced pancreatic insulin along with reduced serum amylase activity and pancreatic amylase protein and mRNA expression.[16] Therefore, the lack of pancreatic ECM proteins or β1 integrin affected pancreatic enzyme levels, which may have negatively affected food intake, and thus body weight.

In summary, β1 integrin-deficient mice showed significant pancreatic exocrine dysfunction compared with controls. The decrease in pancreas to body weight ratio, essential pancreatic digestive enzymes and ECM matrix expression all suggest that β1 integrin and ECM proteins have an important role in maintaining pancreatic function and differentiation. Furthermore, we observed increased cell death and reduced cell proliferation, indicating disturbed cell survival/apoptosis homeostasis. A recovery in apoptosis was observed after β1 integrin-deficient cell clusters were provided ECM proteins. Taken together, these results implicate β1 integrin as an essential component to maintain ECM expression along with exocrine pancreatic structure and function.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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