Original Article

Peptidylarginine deiminase inhibition prevents diabetes development in NOD mice.

Running title: PAD inhibition protects NOD mice from diabetes

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Abstract

Protein citrullination plays a role in several autoimmune diseases. Its involvement in murine and human type 1 diabetes has recently been recognized through the discovery of antibodies and T-cell reactivity against citrullinated peptides. In the current study, we demonstrate that systemic inhibition of peptidylarginine deiminases (PADs), the enzymes mediating citrullination, through BB-CI-amidine treatment, prevents diabetes development in NOD mice. This prevention was associated with reduced levels of citrullination in the pancreas, decreased circulating autoantibody titers against citrullinated GRP78 and reduced spontaneous NETosis of bone marrow-derived neutrophils. Moreover, BB-CI-amidine treatment induced a shift from Th1 to Th2 cytokines in the serum and an increase in the frequency of regulatory T cells in the blood and spleen. In the pancreas, BB-CI-amidine treatment preserved insulin production and was associated with a less destructive immune infiltrate, characterized by reduced frequencies of effector memory CD4⁺ T cells and a modest reduction in the frequency of IFN γ -producing CD4⁺ and CD8⁺ T cells. Our results point to a role of citrullination in the pathogenesis of autoimmune diabetes, with PAD inhibition leading to disease prevention through modulation of immune pathways. These findings provide insight in the potential of PAD inhibition for treating autoimmune diseases like type 1 diabetes.

Introduction

Emerging evidence demonstrates a role for post-translational modifications (PTMs) in the pathogenesis of type 1 diabetes (1–10). One of these PTMs is citrullination, the conversion of arginine into citrulline, mediated by peptidylarginine deiminases (PADs), of which 5 isozymes have been described (11). The loss of a positively charged arginine in peptides enhances their binding affinity to type 1 diabetes predisposing HLA-DR4 molecules (12,13) and may thereby elicit autoreactive T-cell responses. Indeed, it has been shown that autoreactive CD4⁺ T cells of patients with type 1 diabetes recognize citrullinated GAD65 (4). Moreover, our group showed that citrullinated glucose regulated protein 78 (GRP78) is an autoantigen in NOD mice and in human type 1 diabetes (5,6,10). We also provided direct evidence for cytokine-induced citrullination of GRP78 in INS-1E beta-cells (5) and human islets (10), indicating that GRP78 can be citrullinated in beta-cells in the absence of immune cells. In addition, we showed that inflammatory cytokines induce translocation of GRP78 from the endoplasmic reticulum to the beta-cell membrane and its subsequent secretion (5,14), providing the ideal environment to become citrullinated in the extracellular space (15). Although it remains unknown which cells are responsible for protein citrullination in the pancreas, PAD2 is highly expressed in NOD islets relative to C57Bl/6 islets (5,16). Also increased levels of PAD4 in neutrophils of type 1 diabetes individuals have been reported (17,18). Interestingly, PAD4 is crucial for the formation of neutrophil extracellular traps (NETs) (19), a process associated with the pathogenesis of type 1 diabetes (20,21). Neutrophils are the first immune cells to infiltrate the islets of NOD mice (20) and have been found in the human pancreas prior to disease onset (21), with a substantial fraction forming NETs (21). Moreover, different studies targeting neutrophil activity or NETs showed a marked protection against diabetes development in NOD mice (20,22–24).

Citrullination does not occur exclusively in type 1 diabetes. In other autoimmune diseases, citrullinated proteins are present in inflamed target tissues and are associated with the break in immune tolerance (25). In rheumatoid arthritis, for example, citrullinated autoantigens are known to be causative (25). Several studies have reported improved disease outcome without any signs of *in vivo* toxicity (26–30) using pan-PAD inhibitors, such as Cl-amidine or BB-Cl-amidine, in animal models for rheumatoid arthritis (26,31,32), multiple sclerosis (33), systemic lupus erythematosus (30,34) and ulcerative colitis (27–29). Disease improvement, through direct inhibition of citrullination, was shown to be linked to decreased NET formation (NETosis) (34–37), modulation of dendritic cell function (37) and a shift in Th1/Th2 profiles (31). Based on these results, PAD inhibition is gaining interest as a strategy to treat or prevent autoimmune diseases that are associated with abnormal PAD activity.

The mechanism by which BB-Cl-amidine and its mother compound Cl-amidine inactivates PAD has been well described (38). Both compounds irreversibly inactivate PAD enzymes through covalent modification of a conserved cysteine in the active site of the PAD enzymes, thereby having an effect on all PAD enzymes. When PAD enzymes become activated they undergo a calcium-dependent conformational change that moves a nucleophilic cysteine residue into the active site and, only then, this cysteine is available for the PAD inhibitor (38). Compared to Cl-amidine, BB-Cl-amidine has a longer *in vivo* half-life (1.75 h vs. ~15 min), a higher cellular potency (EC₅₀ of 8.8±0.6 µM vs >200 µM for Cl-amidine) and a comparable selectivity for the different PAD enzymes (34). With no reports on the potential efficacy of PAD inhibition in type 1 diabetes models, we here evaluated the effect of BB-Cl-amidine in NOD mice. We observed full protection against diabetes development, associated with a decrease in citrullination and in autoantibody titers against citrullinated GRP78. Effects on innate and adaptive immune responses were also observed, with decreased NETosis of bone marrow-derived neutrophils, increased serum Th2 cytokines and regulatory T cells (T_{reg}) in

peripheral tissues, and a decrease in effector memory CD4⁺ T cells (T_{EM}) and IFN γ -producing CD4⁺ and CD8⁺ T cells in the pancreas. These findings point to a role for dysregulated PAD activity and citrullination in autoimmune diabetes and provide initial insight into PAD inhibition as a potential therapeutic strategy.

Research Design and Methods

Mice and treatment regimen

Non-obese diabetic (NOD) mice were inbred and housed under semi-barrier conditions in our animal facility. Eight-week-old female NOD mice were used. Treatments involved subcutaneous injections with BB-Cl-amidine (1µg/g body weight) or vehicle (25% DMSO in PBS) six times per week until 25 weeks of age for diabetes incidence, or until 13 weeks of age for mechanistic studies (29,31,34,39). Experiments were approved by the Institutional Animal Ethics Committee of KULeuven.

Dispersion of murine islets and sorting

Islets were isolated from 10-week-old NOD mice using collagenase (40). After overnight resting, the islets were dispersed into single-cell suspensions by incubation for 90sec at 37°C in 0.025% trypsin-EDTA (Thermo Fisher). Cells were surface stained for cell sorting by flow cytometry as described (40). A detailed protocol is provided in Supplementary data.

RNA isolation, cDNA synthesis and qPCR

RNA isolation and cDNA generation was performed as previously described (40). SYBR green qPCR was performed on a QuantStudio 3 (Thermo Fisher) per manufacturer's instructions. Gene expression levels of *insulin 1 (Ins1)*, *glucagon (Glc)*, *CD45*, *Padi2* and *Padi4* were normalized to the geometrical mean of 3 housekeeping genes (*actin*, *RPL27* and *HPRT*) and analyzed by relative quantification using the $2^{-\Delta\Delta C_t}$ method.

Immunohistochemistry

Five- μm sections of paraffin embedded pancreata were collected at 50 μm intervals. Heat-mediated antigen retrieval was performed on deparaffinized sections using citrate buffer (pH6.1; 20min, Dako). To detect citrullination, endogenous peroxidase activity was blocked with 0.5% H_2O_2 in methanol for 20min. Sections were incubated with rabbit anti-citrulline (1:50, Abcam) for 1h and with anti-rabbit HRP Envision (Dako) for 30min. Sections were incubated with DAB (Dako), counterstained with haematoxylin (2min), dehydrated and mounted with DEPEX. For insulin detection, sections were blocked with 10% normal goat serum (Dako) for 20min and incubated with guinea pig anti-insulin (1:150, Dako) for 1h and Alexa Fluor®633-conjugated goat anti-guinea pig (1:500, Life) for 30min. Sections were counterstained with Hoechst (1:2000, Thermo Fisher) and mounted with Mowiol (Polysciences). All incubations were done at room temperature (RT). Imaging was performed using an Olympus BX41 or Zeiss LSM 780 confocal NLO microscope.

Diabetes incidence

Mice were tested for clinical signs of diabetes by evaluating glucose levels in the urine with Diastix Reagent Strips (Bayer). As soon as glucosuria was measured, further follow-up was done by measuring blood glucose levels. Mice were considered diabetic when glucosuria was present and blood glucose levels were higher than 200mg/dL for two consecutive days.

Insulinitis

At least 25 islets per mouse were scored for lymphocytic infiltration in a blinded manner using hematoxylin-eosin stained sections.

Insulin content

Pancreata were homogenized in acidic ethanol (91% ethanol, 9% 1M H₃PO₄) at 4°C overnight and sonicated. Insulin content was determined in the supernatant by ELISA (Mercodia) and normalized to the weight of the pancreas.

SDS-PAGE and Western blotting

Protein samples (50µg) were separated on 4-12% Bis-Tris gels (Invitrogen) and blotted onto PVDF membranes (Hybond-ECL, GE Healthcare). To detect citrullination, the anti-modified citrulline Kit (Millipore) was used. Membranes were incubated with SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Fisher) and revealed using the ImageQuant LAS 500 system (GE Healthcare). Blots were normalized by re-probing for GAPDH (Thermo Fisher).

Proteomic analysis making use of biotin-phenylglyoxal labeling

DMSO (n=4) and BB-Cl-amidine treated (n=4) mice pancreata were pooled in buffer (50mM HEPES pH 7.6, 0.5% NP40 and 1mM PMSF) and homogenized using a tissue homogenizer (maximum speed, 3x20sec at 10min intervals). The lysed tissue samples were centrifuged (12,000 rpm, 30min) at 4°C. The supernatants were isolated, and the protein concentration was determined using the DC™ (detergent compatible) assay. Equal protein concentrations from DMSO and BB-Cl-amidine treated samples were labeled with biotin-phenylglyoxal (biotin-PG) in quadruplicate as previously described (41). A detailed protocol is provided in Supplementary data.

TMT labeling and LC-MS/MS analysis

A total of eight peptide digests were obtained from quadruplicate biotin-PG labeling of the DMSO and BB-Cl-amidine treated samples. The samples were labeled using a TMT labeling

system and prepared for LC-MS/MS analysis on a NanoAcquity UPLC (Waters Corporation) coupled to an Orbitrap Fusion Lumos Tribrid (Thermo Fisher) mass spectrometer. The raw data were processed using Proteome Discoverer (Thermo Fisher, version 2.1.1.21) and searched against SwissProt murine (downloaded 07/2019) database using Mascot (Matrix Science, version 2.6.2). A detailed protocol is provided in Supplementary data.

Autoantibody assays

Mouse serum autoantibody levels against citrullinated GRP78 were determined by an in-house developed MesoScale Discovery (MSD) ELISA. *In vitro* citrullinated GRP78 (IVC GRP78) was generated as described (5). Multi-array 96 plates (MSD) were coated (overnight, 4°C) with 5µg/mL IVC GRP78 in PBS (pH 7) or with 3% skimmed milk in PBS (uncoated control). Blocking was performed with 3% skimmed milk in PBS with 0.5% Tween 20 (2h at RT). Mouse serum was diluted 1:5 in blocking solution and incubated for 2h at RT. Wells were incubated for 2h at RT with SULFO-TAG labeled goat anti-mouse IgG. Read buffer was added and detection was done on an MSD reader. To exclude nonspecific signals, each sample had its background signal (serum binding in uncoated well) subtracted from the reading.

NETosis

Bone marrow-derived cells were harvested from the humeri, tibiae and femurs and neutrophils were isolated using the Neutrophil Isolation Kit (Miltenyi). Neutrophils were cultured on coverslips, pre-coated with 0.01% Poly L-lysine (Sigma), for 3h at 37°C in 5% CO₂. Paraformaldehyde-fixed neutrophils were stained with Hoechst (Thermo Fisher) and NETosis was quantified blindly using a Nikon Eclipse TI microscope. Percentage of NET releasing cells was calculated by normalizing to the total amount of cells. Images were taken on a Zeiss LSM 880 confocal Airyscan microscope.

Cytokine measurement in serum

Serum levels of IFN γ , IL-4 and IL-10 were determined using a V-plex assay from MSD, by a single measurement. All concentrations were above the lower limit of detection (LLOD).

Immune cell phenotyping

Blood was incubated 3 times with NH₄Cl to lyse red blood cells. Single cell suspensions from spleen were obtained by mechanical disruption, while the pancreas was enzymatically digested for 15min at 37°C in RPMI (72400, Thermo Fisher) supplemented with 5% bovine serum, 0.05mM beta-mercaptoethanol, 100U/mL penicillin, 100U/mL streptomycin, 1mg/mL collagenase VII and 0.02mg/mL DNase I. For phenotypic characterization of T_{reg}, T_{EM}, central memory (T_{CM}) and naïve (T_{naïve}) T-cell subsets, cell suspensions were stained with Zombie Yellow Fixable Viability dye (Biolegend) and with antibodies against CD4 (BD), CD3, CD8, CD44, CD62L, CD25 and Foxp3 (eBioscience). Intracellular staining was performed with Foxp3/Transcriptional Factor Staining Buffer Set (eBioscience). To evaluate the effect of BB-Cl-amidine treatment on cytokine production, 5 x 10⁵ cells from the spleen and pancreas were stimulated *in vitro* with 0.025 μ g/mL phorbol-12-myristate-13-acetate (PMA) (Sigma) and 0.25 μ g/mL ionomycin (Sigma) in the presence of GolgiStop (1:500, BD) for 4h at 37°C in 5% CO₂. Next, cells were incubated with antibodies against CD4 and CD8, treated with Cytofix/Cytoperm (BD), and stained with anti-IFN γ antibody (eBioscience). Samples were acquired on a BD FACS Canto II and analyzed with FlowJo software (TreeStar). The percentage of cytokine-producing cells was determined and the expression level of the cytokine was assessed using geometric mean fluorescence intensity (MFI). For each

experiment, the geometric MFI of each sample was normalized to the average geometric MFI of the DMSO group (MFI fold change).

Statistics

Data were analyzed using GraphPad Prism 8 or Scaffold software. Diabetes incidence was evaluated by Kaplan-Meier survival analysis with Mantel-Cox log-rank test. Data were expressed as mean $\pm$ SEM and, after exclusion of outliers, data were analyzed by one-way ANOVA, unpaired t-test with Welch's correction, or Mann-Whitney U test, as indicated. $P < 0.05$ was considered statistically significant.

Data and Resource Availability

All datasets generated during the current study are available from the corresponding author upon request.

Results

Pancreatic islets express *Padi2* both in endocrine and in infiltrating immune cells

Although we have previously shown that islets of 3- and 10-week-old NOD mice have elevated PAD activity levels and enhanced *Padi2* mRNA expression compared to C57Bl/6 islets (5), it remains to be elucidated which cell population within the islets is responsible for this high PAD activity. To investigate this, we performed flow cytometry sorting on 10-week-old NOD islet cells making use of an antibody panel consisting of CD71, CD24, CD49f and CD45 to enable isolation of highly purified alpha- and beta-cells (40), as well as immune cells. The purity of immune cell fraction was confirmed by marginal expression of *insulin 1* (*ins1*) and *glucagon* (*glc*). Purity of alpha- and beta-cell fractions was confirmed by complete absence of leukocyte marker *CD45* in both fractions, and marginal expression levels of *glc* (in beta-cells) and *ins1* (in alpha-cells) (Fig. 1A-C). In immune, as well as in alpha- and beta-cells, *Padi2* was clearly expressed (Fig. 1D), with a trend to higher levels in immune and beta-cells, as compared to alpha-cells. Of note, *Padi4* mRNA levels were below the detection limit (data not shown). In line with this, immunohistochemical analysis of citrullination in pancreas sections of NOD mice demonstrated intense staining in infiltrated immune cells, while endocrine islet cells stained weakly positive. Parallel staining in age-matched C57Bl/6, also showed citrullination in the endocrine islet cells (Fig. 1E and Suppl. Fig. 1). These results indicate that citrullination occurs in immune cells infiltrated in the islets and, also in endocrine alpha- and beta-cells even in non-pathological conditions.

BB-Cl-amidine treatment prevents diabetes development in NOD mice and preserves insulin production

To examine the role of citrullination on diabetes development, NOD mice were treated with BB-Cl-amidine. Treatment was started at 8 weeks of age, a time point at which insulinitis is already ongoing in NOD mice. BB-Cl-amidine treatment fully prevented diabetes development, with all mice free of diabetes until 25 weeks of age, against 44% diabetes-free in DMSO treated mice ($P < 0.001$; Fig. 2A). Therapy was well tolerated as confirmed by normal weight curves (Suppl. Fig. 2). Evaluation of pancreatic insulin content at 13 weeks of age, before the onset of diabetes, showed a 4-fold increase of insulin in the pancreas of BB-Cl-amidine treated mice compared to the control ($P < 0.01$; Fig. 2B and C), pointing to preserved insulin production in BB-Cl-amidine treated mice.

BB-Cl-amidine treatment reduces citrullination in the pancreas and autoantibody formation against citrullinated GRP78

To evaluate the direct effect of BB-Cl-amidine on inhibition of citrullination, we performed immunohistochemical staining of citrullinated protein residues on pancreatic sections, showing a reduced staining intensity in BB-Cl-amidine treated compared to DMSO treated mice (Fig. 3A and Suppl. Fig. 3). Quantification of the overall level of citrullinated proteins in the pancreas of BB-Cl-amidine and vehicle-treated NOD mice by Western blotting demonstrated that PAD inhibition reduced the total level of protein citrullination by about 50% ($P < 0.01$; Fig. 3B and C). This finding was further confirmed and extended by more quantitative LC-MS/MS analysis. A total of 1,003 citrullinated proteins were identified in pancreata from BB-Cl-amidine and vehicle treated mice, of which 765 were significantly decreased in abundance by

the treatment regimen (Fig. 3D and Suppl. Table 1 and 2). Interestingly, citrullinated GRP78, reported by our group as an autoantigen in murine and human type 1 diabetes (5,10), was the most significantly reduced citrullinated protein, with a decrease of 2.66-fold ($P<0.0001$). We have previously reported the generation of autoantibodies against citrullinated GRP78 in NOD mice (5). In line with this, we observed here that BB-Cl-amidine treatment decreased autoantibody titers against citrullinated GRP78, both at 13 ($P<0.05$; Fig. 3E) and 25 weeks of age ($P<0.05$; Fig. 3F).

BB-Cl-amidine treatment decreases spontaneous NET formation in bone marrow-derived neutrophils

Neutrophils have been suggested to play an important role in the pathogenesis of autoimmune diabetes, as they are the first immune cells to infiltrate the islets of NOD mice (20). Also in type 1 diabetes patients, neutrophil infiltration is observed in the pancreas, even prior to disease onset, with some degree of NETosis (21). Since citrullination is a prerequisite for the initiation of NETosis, we questioned here if BB-Cl-amidine treatment could have an impact on the propensity of neutrophils to form NETs. To this end, spontaneous NETosis was assessed in bone marrow-derived neutrophils isolated from BB-Cl-amidine or vehicle-treated mice. NETosis was significantly reduced in neutrophils from mice treated with BB-Cl-amidine ($P<0.05$; Fig. 4). This finding shows that systemic administration of BB-Cl-amidine has an effect on the capacity of neutrophils to form NETs.

BB-Cl-amidine alters the phenotype and cytokine production of immune infiltrate in the islets

The finding of preserved insulin production by BB-Cl-amidine tempted us to investigate the degree and phenotype of immune infiltration. To this end, immunohistochemical evaluation of islet immune infiltration at 13 weeks of age showed only a moderate overall decrease of insulinitis in the BB-Cl-amidine group (Fig. 5A), suggesting a less destructive form of insulinitis. To evaluate this further, we measured *in vitro* cytokine production of the immune cells isolated from the pancreas. Although the overall frequency of IFN γ -producing T cells was low in both groups, mice treated with BB-Cl-amidine presented a significantly lower percentage of IFN γ -producing CD4⁺ and CD8⁺ T cells ($P < 0.01$ and $P < 0.05$ respectively; Fig. 5B and C). Evaluating the phenotype of immune cells in the pancreas (representative gating strategy in Suppl. Fig. 4A), revealed a significant decrease in the percentage of CD4⁺ T_{EM} cells ($P < 0.05$; Fig. 5E) in BB-Cl-amidine treated mice, whereas no changes were observed in the frequency of T_{reg} (Fig. 5D). No differences were observed in CD4⁺ and CD8⁺ T_{naïve} and T_{CM} subsets (Suppl. Fig. 5). Collectively, these data indicate that, although only a minor change in the overall level of insulinitis, BB-Cl-amidine alters the phenotype of infiltrating immune cells, as demonstrated by the decrease in Th1 cytokine production and reduction of T_{EM} cells locally in the pancreas.

BB-Cl-amidine treatment is associated with a Th1 to Th2 serum cytokine shift, and of T_{EM} to Treg in the periphery

Next, we sought to investigate the effect of BB-Cl-amidine treatment on Th1 and Th2 profiles and immune cell phenotype in the periphery, as BB-Cl-amidine has been shown to act through this pathway in other disease models (31). First, we measured serum levels of Th1 and Th2 cytokines in BB-Cl-amidine or vehicle-treated NOD mice. Whereas no significant changes were measured in circulating IFN γ titers (Fig. 6A), IL-4 and IL-10 titers were significantly increased in the BB-Cl-amidine treated group ($P < 0.01$ for both; Fig. 6B and C). This resulted in an overall reduction in the IFN γ /IL-10 ratio ($P < 0.05$; Fig. 6D) (42). In the spleen of BB-Cl-

amidine treated mice, there was a decrease in IFN γ production indicated by the reduced frequency of IFN γ -producing CD4 $^{+}$ T cells ($P<0.01$; Fig. 6E), as well as the lower expression of IFN γ in IFN γ -producing CD4 $^{+}$ and CD8 $^{+}$ T-cell populations ($P<0.0001$ for both; Fig. 6F and H). To specifically investigate the effect on the cellular phenotype of immune cell subsets, we used flow cytometry to study T-cell subsets in the blood and spleen (representative gating strategy in Suppl. Fig. 4B and C). This revealed that BB-Cl-amidine induced a significant increase in the percentage of T $_{reg}$ in blood ($P<0.05$; Fig. 7A) and spleen ($P<0.01$; Fig. 7D). Regarding T $_{EM}$ (CD4 $^{+}$ and CD8 $^{+}$) in the periphery, there was a significant decrease in the percentage of CD4 $^{+}$ T $_{EM}$ cells in the blood and of splenic CD8 $^{+}$ T $_{EM}$ cells ($P<0.05$ and $P<0.001$, respectively; Fig. 7B and F). No changes were observed in CD4 $^{+}$ and CD8 $^{+}$ T $_{CM}$ populations in the blood and spleen, whereas CD4 $^{+}$ and CD8 $^{+}$ T $_{naïve}$ in blood (both $P<0.05$; Suppl. Fig. 6A and C) and CD8 $^{+}$ T $_{naïve}$ in spleen ($P<0.01$; Suppl. Fig. 6G) were increased in BB-Cl-amidine treated mice. These findings suggest that in the periphery, BB-Cl-amidine induces a shift towards Th2 cytokine production, a decrease in T $_{EM}$ cells and an induction of T $_{reg}$ cells.

Discussion

This study describes the effect of the pan-PAD inhibitor BB-CI-amidine, showing a complete prevention of diabetes development in NOD mice. This finding highlights the involvement of inflammation-induced citrullination in diabetes development and adds type 1 diabetes to the list of autoimmune diseases in which citrullination participates in disease pathogenesis, a list that includes rheumatoid arthritis (26,31,32), multiple sclerosis (33), lupus (30,34) and ulcerative colitis (27–29). This observation may have significant implications for the development of new therapeutic strategies to mitigate the progression and/or development of type 1 diabetes.

We here provide evidence that the previously reported high expression of *Padi2* in islets of NOD mice (5,16) originates from both the endocrine cells, including alpha- and beta-cells, as well as from the infiltrating immune cells, thereby conclusively demonstrating that beta-cells themselves have the capacity to citrullinate. This finding formed the basis for evaluating the effect of PAD inhibition in NOD mice. In the current treatment regimen, we started treatment at 8 weeks of age, a time point at which insulinitis is already ongoing. The observation that inhibition of PAD activity, and thus citrullination, protected NOD mice against diabetes development suggests that citrullination may play a role in the amplification of the disease rather than being an initial trigger in breaking of immune tolerance. This strengthens our earlier findings of minor autoreactivity against citrullinated GRP78 in prediabetic NOD mice (5) and the increased occurrence of autoantibodies and autoreactive T-cell responses in long-standing T1D patients versus new onset patients (10). This view also fits well with the knowledge that citrullination is mostly associated with inflammation, thereby playing a role in several autoimmune diseases, where citrullination takes place in the inflamed target tissues (26–34). The low levels of citrullination in islets of C57Bl/6 mice further demonstrate that citrullination

is present already under physiological conditions, however, the degree of citrullination is markedly increased in NOD islets associated with immune infiltration.

BB-Cl-amidine treatment is effective in decreasing overall levels of citrullination in the pancreas, as shown by Western blotting and quantitative LC-MS/MS. Of interest, the most significant reduction was measured for citrullinated GRP78. In line with this, the level of autoantibodies against citrullinated GRP78 is decreased in the circulation of BB-Cl-amidine treated NOD mice. Although autoantibodies are not thought to be pathogenic in type 1 diabetes, this finding underlines the important effect of BB-Cl-amidine on reducing the antigenicity of beta-cell proteins in the inflamed islet environment.

Pan-PAD inhibitors are known to alter NET release (34–37). Neutrophils and NETosis are thought to play an important role in the initiation and perpetuation of type 1 diabetes (20,21,43). Here, we show that BB-Cl-amidine reduces spontaneous NET release *ex vivo* from bone marrow-derived neutrophils of NOD mice, a finding that may be linked to a decrease in citrullinated histone H3 levels. Indeed, citrullination of histone H3, mediated by activation of PADs in neutrophils, is an initiating factor for the formation of NETs (44), and has been shown to be reduced by PAD inhibition (36). Our results are in line with studies in lupus (30,34) and collagen-induced arthritis (37), demonstrating that reduced NETosis in response to PAD inhibition is associated with disease amelioration.

Looking at the islet level, BB-Cl-amidine treatment resulted in only a marginal reduction of insulitis, whereas pancreatic insulin levels were preserved. However, we did observe a significant reduction in the percentage of CD4⁺ T_{EM} cells and in the frequency of IFN γ -producing CD4⁺ and CD8⁺ T cells in the pancreas of BB-Cl-amidine treated mice. The observed reduction in IFN γ production may reflect an impaired effector function of the infiltrating immune cells (45). These results suggest that the immune cell infiltrate in BB-Cl-amidine treated mice is less aggressive (46).

Our results on increased frequency of T_{reg} in the blood and spleen demonstrate a peripheral effect of BB-Cl-amidine. Additionally, splenic $IFN\gamma$ production of BB-Cl-amidine treated mice was significantly reduced. These results suggest a more regulatory environment that favors immune tolerance and prevents intensification of the immune assault. Also, with Th1/Th2 polarization thought to play a role in T1D pathogenesis, the significant increase in IL-4 and decrease in the $IFN\gamma/IL-10$ ratio in the circulation of BB-Cl-amidine treated mice supports this hypothesis. Of note, whether Th1/Th2 polarization is a cause rather than a consequence of disease development, is not fully established. In line with these findings, an induction of Th2 cells by BB-Cl-amidine has been shown in a mouse model of rheumatoid arthritis (31) and a direct effect of BB-Cl-amidine on enhancing the differentiation of Th2 subsets through inhibition of GATA3 citrullination was demonstrated mechanistically by Sun *et al.* (47).

For the first time, a pan-PAD inhibitor was used in a very long treatment regimen (17 weeks), with no detrimental side effects observed. This, together with reported disease amelioration with the use of PAD inhibitors in other autoimmune models, opens the road to potential clinical therapeutic applications. However, since PADs also participate in physiological processes (11), such as gene regulation and nerve myelination, such therapeutic treatment regimens will need careful consideration. The recent development of specific PAD2 (48) and PAD4 (49) inhibitors, as well as reversible PAD inhibitors (50), may be a solution, but their efficacy to treat autoimmune diseases remains to be confirmed. Results from ongoing studies may further invite to consider PAD inhibition as a therapeutic strategy, not only for type 1 diabetes but also for other autoimmune diseases.

In conclusion, treatment of NOD mice with BB-Cl-amidine resulted in complete diabetes prevention, associated with decreased protein citrullination, anti-citrulline autoantibody formation, NETosis, and *in situ* $IFN\gamma$ responses. We postulate that these effects, combined with

the direct effect of PAD inhibition on T cells, might lead to a less aggressive inflammatory environment in the islets and in the periphery. Our findings align with previous reports that citrullination plays a pathogenic role in type 1 diabetes and demonstrate that PAD inhibition is an attractive target for clinical therapeutic applications in autoimmunity.

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Author Contributions. FMCS performed design, conduction, analysis and interpretation of the data and wrote and edited the manuscript. SB helped with the design of the NETosis data, besides conducting and analyzing them. YB contributed by injecting mice and performing experiments. RT and PRT conducted and analyzed the proteomic analysis of the biotin-PG labeled samples and the LC-MS/MS. DPC helped with mice treatment and contributed to the design and analysis of the FACS data. CB and RS performed and analyzed the islets isolation, sorting and qPCR. AC helped with conduction of experiments. SY and RM contributed to the design and interpretation of islet experiments. SM and PRT provided the BB-Cl-amidine. CM, LO and MB designed research, interpreted data and wrote and edited the manuscript. MB also conducted and analyzed experiments. All authors edited the manuscript and gave their final approval of the version to be published. CM and LO are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Figures legends

Figure 1 - *Padi2* is expressed in immune, alpha- and beta-cells from NOD islets. A-C: mRNA expression level in pancreatic immune cells (Imm), alpha-cells and beta-cells for the leukocyte marker *CD45* (A); alpha-cell marker glucagon (*Glc*) (B); beta-cell marker insulin 1 (*Ins1*) (C); and *Padi2* (D) (n = 3; 3 independent experiments were performed with one mouse per experiment). E: Representative immunohistochemical staining against citrulline in pancreas sections of C57Bl/6 and NOD mice (scale bar = 50µm). All sections were mounted on one slide to enable good comparison. Data in A–D are mean ± SEM and were analyzed by one-way ANOVA. **P<0.01, ***P<0.001 and ****P<0.0001.

Figure 2 - BB-Cl-amidine treatment prevents diabetes development in NOD mice and preserves pancreatic insulin production. A: Percentage of diabetes free NOD mice treated with DMSO or BB-Cl-amidine. Eight-week-old female littermate NOD mice were injected subcutaneously with vehicle (25% DMSO in PBS) or BB-Cl-amidine (1µg/g body weight) six times per week until 25 weeks of age and followed-up for diabetes development (n = 17-18 mice per group; 2 independent cohorts were performed with at least eight mice per group per experiment). B: Pancreatic insulin content of 13-week-old NOD mice treated with DMSO or BB-Cl-amidine from 8-13 weeks of age (n = 8-10; two independent experiments were performed with 4-5 mice per group per experiment). C: Illustrative images of insulin staining in the pancreas of 13-week-old NOD mice treated from 8-13 weeks of age with DMSO or BB-Cl-amidine (scale bar = 50µm). Data in A were evaluated by Kaplan-Meier survival analysis with Mantel-Cox log-rank test. Data in B are mean ± SEM and were analyzed by unpaired t-test with Welch's correction. **P<0.01 and ***P<0.001.

Figure 3 - BB-Cl-amidine treatment reduces citrullination in the pancreas and the formation of circulating autoantibodies. A: Representative immunohistochemical staining against citrulline in pancreas sections of 13-week-old DMSO or BB-Cl-amidine treated NOD mice (scale bar = 50µm). All sections were mounted on one slide to enable good comparison. B: Representative Western blot with anti-modified citrulline antibody in pancreatic lysates of 13-week-old DMSO or BB-Cl-amidine treated NOD mice. C: Optical density ratio (sum of citrullinated bands/GAPDH band) of pancreas samples from DMSO or BB-Cl-amidine treated mice (n = 4 mice per group). D: Volcano plot showing significant fold changes in citrullinated proteins in pancreas of NOD mice treated with BB-Cl-amidine, from 8 until 13 weeks of age, compared to DMSO treated NOD mice. The x axis represents log₂ expression fold changes (BB-Cl-amidine/DMSO), and the y axis represents the adjusted p-values (as -log₁₀). E-F: Comparison between the serum levels of anti-citrullinated GRP78 antibodies in NOD mice, treated with DMSO or BB-Cl-amidine, at 13 weeks of age (E) and at 25 weeks of age (F) (n = 9-17 samples per group; two independent experiments were performed, with at least two mice per group per experiment). Data in C, E and F are mean ± SEM and were analyzed by unpaired t-test with Welch's correction. Data in D were analyzed by Mann-Whitney U test. *P<0.05 and **P<0.01.

Figure 4 - BB-Cl-amidine treatment reduces NET release. A-B: Bone marrow-derived neutrophils were isolated from 13-week-old NOD mice treated with DMSO or BB-Cl-amidine and cultured for 3h. Spontaneous NET formation was blindly evaluated by fluorescence microscopy. A: Representative images of spontaneous NETosis of bone marrow-derived neutrophils from DMSO or BB-Cl-amidine treated mice (scale bar = 10µm). White arrows point to NET forming cells. B: Percentage of NET formation was quantified blindly by fluorescence microscopy, after Hoechst staining (n = 10-15 mice per group; two independent

experiments were performed, with at least four mice per group per experiment). Each dot indicates the value for a single mouse. Data in B are mean $\pm$ SEM and were analyzed by unpaired t-test with Welch's correction. * $P < 0.05$.

Figure 5 – BB-Cl-amidine alters the phenotype of immune infiltrate in the islets. A: Insulinitis score of 13-week-old NOD mice treated with vehicle (DMSO) or BB-Cl-amidine from 8-13 weeks of age ($n = 6$ mice per group; two independent experiments were performed, with three mice per group per experiment). Score 0 (□): intact islets; 1 (▤): peri-insulinitis; 2 (■): $< 50\%$ infiltration; 3 (▨): $> 50\%$ infiltration; and 4 (■): complete destruction. B-C: Immune cells isolated from the pancreas of 13-week-old DMSO or BB-Cl-amidine treated NOD mice were stimulated *in vitro* with PMA and ionomycin in the presence of GolgiStop. Representative flow cytometry plots and percentage of IFN γ^+ cells (%IFN γ^+) in CD4 $^+$ (B) and CD8 $^+$ (C) T cells isolated from pancreas ($n = 9-12$ mice per group; two independent experiments were performed with at least three mice per group per experiment). D-F: Representative flow cytometry plots and percentage of T $_{reg}$ (CD4 $^+$ CD25 $^+$ Foxp3 $^+$) (D), CD4 $^+$ T $_{EM}$ (CD4 $^+$ Foxp3 $^-$ CD44 HIGH CD62L $^-$) (E) and CD8 $^+$ T $_{EM}$ (CD8 $^+$ Foxp3 $^-$ CD44 HIGH CD62L $^-$) (F) in pancreas of 13-week-old NOD mice treated with DMSO or BB-Cl-amidine ($n = 15-16$ mice per group; two independent experiments were performed with at least six mice per group per experiment). Data in B-F are mean $\pm$ SEM and were analyzed by unpaired t-test with Welch's correction. * $P < 0.05$ and ** $P < 0.01$. T $_{reg}$, regulatory T cells; T $_{EM}$, effector memory T cells.

Figure 6 - BB-Cl-amidine treatment is associated with a Th1 to Th2 shift in the periphery. A-D: Serum cytokine levels of IFN γ (A), IL-4 (B), IL-10 (C) and ratio IFN γ /IL-10 (D) in 13-week-old NOD mice treated from 8-13 weeks with DMSO or BB-Cl-amidine ($n = 28-35$ mice per group; four independent experiments were performed with at least seven mice per group per experiment). E-H: Splenocytes isolated from the pancreas of 13-week-old DMSO or BB-Cl-amidine treated NOD mice were stimulated *in vitro* with PMA and ionomycin in the presence of GolgiStop ($n = 18-19$ mice per group; two independent experiments were performed with at least eight mice per group per experiment). Cytokine production was measured by flow cytometry in CD4 $^+$ and CD8 $^+$ T cell populations considering the 2 following parameters: 1) percentage of IFN γ^+ cells (%IFN γ^+) and 2) fold change of the MFI of IFN γ in IFN γ^+ cells (MFI IFN γ^+). Per experiment, the geometric MFI of each sample was normalized to the average geometric MFI of the DMSO group. Representative flow cytometry plots and histograms depicting the geometric MFI of IFN γ between the two groups are shown above the pooled data. E-F: IFN γ^+ (E) and MFI IFN γ^+ (F) in splenic CD4 $^+$ T cells. G-H: %IFN γ^+ (G) and MFI IFN γ^+ (H) in splenic CD8 $^+$ T cells. Data in A-H are mean $\pm$ SEM and were analyzed by unpaired t-test with Welch's correction. * $P < 0.05$, ** $P < 0.01$ and **** $P < 0.0001$.

Figure 7 - BB-Cl-amidine increases the frequency of T $_{reg}$ cells in the periphery. Representative flow cytometry plots and percentage of T $_{reg}$ (CD4 $^+$ CD25 $^+$ Foxp3 $^+$), CD4 $^+$ T $_{EM}$ (CD4 $^+$ Foxp3 $^-$ CD44 HIGH CD62L $^-$) and CD8 $^+$ T $_{EM}$ (CD8 $^+$ Foxp3 $^-$ CD44 HIGH CD62L $^-$) in peripheral blood (A-C) and spleen (D-F) from 13-week-old NOD mice treated with DMSO or BB-Cl-amidine ($n = 15-19$ mice per group; two independent experiments were performed with at least six mice per group per experiment). Data in A-F are mean $\pm$ SEM and were analyzed by unpaired t-test with Welch's correction. * $P < 0.05$ and ** $P < 0.01$. T $_{reg}$, regulatory T cell; T $_{EM}$, effector memory T cell.

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121x104mm (600 x 600 DPI)

Figure 2 - BB-Cl-amidine treatment prevents diabetes development in NOD mice and preserves pancreatic insulin production.

A: Percentage of diabetes free NOD mice treated with DMSO or BB-Cl-amidine. Eight-week-old female littermate NOD mice were injected subcutaneously with vehicle (25% DMSO in PBS) or BB-Cl-amidine (1μg/g body weight) six times per week until 25 weeks of age and followed-up for diabetes development (n = 17-18 mice per group; 2 independent cohorts were performed with at least eight mice per group per experiment). B: Pancreatic insulin content of 13-week-old NOD mice treated with DMSO or BB-Cl-amidine from 8-13 weeks of age (n = 8-10; two independent experiments were performed with 4-5 mice per group per experiment). C: Illustrative images of insulin staining in the pancreas of 13-week-old NOD mice treated from 8-13 weeks of age with DMSO or BB-Cl-amidine (scale bar = 50μm). Data in A were evaluated by Kaplan-Meier survival analysis with Mantel-Cox log-rank test. Data in B are mean ± SEM and were analyzed by unpaired t-test with Welch’s correction. **P<0.01 and ***P<0.001.

172x54mm (600 x 600 DPI)

Figure 3 - BB-Cl-amidine treatment reduces citrullination in the pancreas and the formation of circulating autoantibodies. A: Representative immunohistochemical staining against citrulline in pancreas sections of 13-week-old DMSO or BB-Cl-amidine treated NOD mice (scale bar = 50µm). All sections were mounted on one slide to enable good comparison. B: Representative Western blot with anti-modified citrulline antibody in pancreatic lysates of 13-week-old DMSO or BB-Cl-amidine treated NOD mice. C: Optical density ratio (sum of citrullinated bands/GAPDH band) of pancreas samples from DMSO or BB-Cl-amidine treated mice (n = 4 mice per group). D: Volcano plot showing significant fold changes in citrullinated proteins in pancreas of NOD mice treated with BB-Cl-amidine, from 8 until 13 weeks of age, compared to DMSO treated NOD mice. The x axis represents log₂ expression fold changes (BB-Cl-amidine/DMSO), and the y axis represents the adjusted p-values (as -log₁₀). E-F: Comparison between the serum levels of anti-citrullinated GRP78 antibodies in NOD mice, treated with DMSO or BB-Cl-amidine, at 13 weeks of age (E) and at 25 weeks of age (F) (n = 9-17 samples per group; two independent experiments were performed, with at least two mice per group per experiment). Data in C, E and F are mean ± SEM and were analyzed by

unpaired t-test with Welch's correction. Data in D were analyzed by Mann-Whitney U test. * $P < 0.05$ and ** $P < 0.01$.

129x182mm (600 x 600 DPI)

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172x59mm (600 x 600 DPI)

Figure 5 – BB-Cl-amidine alters the phenotype of immune infiltrate in the islets. A: Insulinitis score of 13-week-old NOD mice treated with vehicle (DMSO) or BB-Cl-amidine from 8-13 weeks of age ($n = 6$ mice per group; two independent experiments were performed, with three mice per group per experiment). Score 0 (white): intact islets; 1 (vertical lines): peri-insulinitis; 2 (grey): <50% infiltration; 3 (horizontal lines): >50% infiltration; and 4 (black): complete destruction. B-C: Immune cells isolated from the pancreas of 13-week-old DMSO or BB-Cl-amidine treated NOD mice were stimulated *in vitro* with PMA and ionomycin in the presence of GolgiStop. Representative flow cytometry plots and percentage of IFN γ ⁺ cells (%IFN γ ⁺) in CD4⁺ (B) and CD8⁺ (C) T cells isolated from pancreas ($n = 9-12$ mice per group; two independent experiments were performed with at least three mice per group per experiment). D-F: Representative flow cytometry plots and percentage of T_{reg} (CD4⁺ CD25⁺ Foxp3⁺) (D), CD4⁺ T_{EM} (CD4⁺ Foxp3⁻ CD44^{HIGH} CD62L⁻) (E) and CD8⁺ T_{EM} (CD8⁺ Foxp3⁻ CD44^{HIGH} CD62L⁻) (F) in pancreas of 13-week-old NOD mice treated with DMSO or BB-Cl-amidine ($n = 15-16$ mice per group; two independent experiments were performed with at least six mice per group per experiment). Data in B-F are mean $\pm$ SEM and were analyzed by unpaired t-test with Welch's correction. * $P < 0.05$ and ** $P < 0.01$. T_{reg}, regulatory T cells; T_{EM}, effector memory T cells.

147x170mm (600 x 600 DPI)

Figure 6 - BB-CI-amidine treatment is associated with a Th1 to Th2 shift in the periphery. A-D: Serum cytokine levels of IFN γ (A), IL-4 (B), IL-10 (C) and ratio IFN γ /IL-10 (D) in 13-week-old NOD mice treated from 8-13 weeks with DMSO or BB-CI-amidine (n = 28-35 mice per group; four independent experiments were performed with at least seven mice per group per experiment). E-H: Splenocytes isolated from the pancreas of 13-week-old DMSO or BB-CI-amidine treated NOD mice were stimulated in vitro with PMA and ionomycin in the presence of GolgiStop (n = 18-19 mice per group; two independent experiments were performed with at least eight mice per group per experiment). Cytokine production was measured by flow cytometry in CD4⁺ and CD8⁺ T cell populations considering the 2 following parameters: 1) percentage of IFN γ ⁺ cells (%IFN γ ⁺) and 2) fold change of the MFI of IFN γ in IFN γ ⁺ cells (MFI IFN γ ⁺). Per experiment, the geometric MFI of each sample was normalized to the average geometric MFI of the DMSO group. Representative flow cytometry plots and histograms depicting the geometric MFI of IFN γ between the two groups are shown above the pooled data. E-F: IFN γ ⁺ (E) and MFI IFN γ ⁺ (F) in splenic CD4⁺ T cells. G-H: %IFN γ ⁺ (G) and MFI IFN γ ⁺ (H) in splenic CD8⁺ T cells. Data in A-H are mean $\pm$ SEM and were analyzed by unpaired t-test with Welch's correction. *P<0.05, **P<0.01 and ****P<0.0001.

172x144mm (600 x 600 DPI)

Figure 7 - BB-CI-amidine increases the frequency of T_{reg} cells in the periphery. Representative flow cytometry plots and percentage of T_{reg} (CD4⁺ CD25⁺ Foxp3⁺), CD4⁺ T_{EM} (CD4⁺ Foxp3⁻ CD44^{HIGH} CD62L⁻) and CD8⁺ T_{EM} (CD8⁺ Foxp3⁻ CD44^{HIGH} CD62L⁻) in peripheral blood (A-C) and spleen (D-F) from 13-week-old NOD mice treated with DMSO or BB-CI-amidine (n = 15-19 mice per group; two independent experiments were performed with at least six mice per group per experiment). Data in A-F are mean ± SEM and were analyzed by unpaired t-test with Welch's correction. *P<0.05 and **P<0.01. T_{reg}, regulatory T cell; T_{EM}, effector memory T cell.

147x162mm (600 x 600 DPI)

SUPPLEMENTARY DATA

Supplementary Figure 1 – Negative staining controls for immunohistochemistry with anti-citrulline antibody. Representative immunohistochemistry images of the negative control staining. The negative control was performed by omitting the primary anti-citrulline antibody in pancreas sections of C57Bl/6 and NOD mice (shown in Figure 1 of the manuscript). All sections were mounted on one slide to enable good comparison (scale bar = 50µm).

SUPPLEMENTARY DATA

Supplementary Figure 2 – BB-Cl-amidine treatment does not alter body weight in NOD mice. Eight-week-old female littermate NOD mice were injected subcutaneously with vehicle (25% DMSO in PBS) or BB-Cl-amidine (1 μ g/g body weight) six times per week until 25 weeks of age. Body weight of NOD mice treated with DMSO or BB-Cl-amidine (n = 17-18 mice per group; 2 independent cohorts were performed with at least eight mice per group per experiment).

SUPPLEMENTARY DATA

Supplementary Figure 3 – Negative staining controls for immunohistochemistry with anti-citrulline antibody. Representative immunohistochemistry images of the negative control staining. The negative control was performed by omitting the primary anti-citrulline antibody in pancreas sections of 13-week-old NOD mice treated with DMSO or BB-Cl-amidine from 8-13 weeks of age (shown in Figure 3 of the manuscript). All sections were mounted on one slide to enable good comparison (scale bar = 50µm).

SUPPLEMENTARY DATA

Supplementary Figure 4 – Gating strategy for T cell phenotyping. Gating strategy for T cell phenotyping in the pancreas (A), blood (B) and spleen (C). Left panels show the gating strategy for obtaining live CD3⁺ T cells. Top right panel shows gating strategy for obtaining T_{reg} (CD4⁺ CD25⁺ Foxp3⁺) cells. Bottom right panels show gating strategy for obtaining CD4⁺ T_{EM} (CD4⁺ Foxp3⁻ CD44^{HIGH} CD62L⁻), CD4⁺ T_{CM} (CD4⁺ Foxp3⁻ CD44^{HIGH} CD62L⁺) and CD4⁺ T_{naïve} (CD4⁺ Foxp3⁻ CD44^{LOW} CD62L⁺). CD8⁺ T_{EM} (CD8⁺ Foxp3⁻ CD44^{HIGH} CD62L⁻), CD8⁺ T_{CM} (CD8⁺ Foxp3⁻ CD44^{HIGH} CD62L⁺) and CD8⁺ T_{naïve} (CD8⁺ Foxp3⁻ CD44^{LOW} CD62L⁺) are obtained in the same way as CD4⁺, but in the CD8⁺ gate (not shown). Plots are representative of 2 independent experiments (n = 15-19), with at least six mice per group per experiment. ZY, Zombie Yellow; T_{reg}, regulatory T cells; T_{EM}, effector memory T cells; T_{CM}, central memory T cells.

A

Pancreas

SUPPLEMENTARY DATA

B

Blood

SUPPLEMENTARY DATA

C

Spleen

SUPPLEMENTARY DATA

Supplementary Figure 5 – Phenotype of pancreatic T cells. A-D: Percentage of CD4⁺ T_{naïve} (CD4⁺ Foxp3⁻ CD44^{LOW} CD62L⁺) (A), CD4⁺ T_{CM} (CD4⁺ Foxp3⁻ CD44^{HIGH} CD62L⁺) (B), CD8⁺ T_{naïve} (CD8⁺ Foxp3⁻ CD44^{LOW} CD62L⁺) (C) and CD8⁺ T_{CM} (CD8⁺ Foxp3⁻ CD44^{HIGH} CD62L⁺) (D) in the pancreas of 13-week-old NOD mice treated with DMSO or BB-Cl-amidine (n = 15-16 mice per group; two independent experiments were performed with at least six mice per group per experiment). T_{CM}, central memory T cell.

SUPPLEMENTARY DATA

Supplementary Figure 6 – Phenotype of peripheral T cells. Percentage of CD4⁺ T_{naïve} (CD4⁺ Foxp3⁻ CD44^{LOW} CD62L⁺), CD4⁺ T_{CM} (CD4⁺ Foxp3⁻ CD44^{HIGH} CD62L⁺), CD8⁺ T_{naïve} (CD8⁺ Foxp3⁻ CD44^{LOW} CD62L⁺) and CD8⁺ T_{CM} (CD8⁺ Foxp3⁻ CD44^{HIGH} CD62L⁺) in different organs of 13-week-old NOD mice treated with DMSO or BB-CI-amidine (n = 15-19 mice per group; two independent experiments were performed with at least six mice per group per experiment). A-D: Frequency of CD4⁺ T_{naïve} (A), CD4⁺ T_{CM} (B), CD8⁺ T_{naïve} (C) and CD8⁺ T_{CM} (D) in peripheral blood. E-H: Frequency of CD4⁺ T_{naïve} (E), CD4⁺ T_{CM} (F), CD8⁺ T_{naïve} (G) and CD8⁺ T_{CM} (H) in spleen. *P<0.05 and **P<0.01. T_{CM}, central memory T cell.

SUPPLEMENTARY DATA

Isolation of pancreatic islets

For isolation of NOD adult islets 0.5mg/ml collagenase P (Sigma) diluted in HBSS (Thermo Fisher) was injected in the main pancreatic duct while the ampulla of Vater was clamped. Next, the pancreas was collected and incubated in 0.5mg/ml collagenase P at 37°C for 11min. Islets were handpicked and cultured overnight in RPMI 1640 (Thermo Fisher) supplemented with 10% (vol/vol) Fetal Bovine Serum (FBS) (Biowest).

Flow cytometry sorting

Islets were dispersed into single-cell suspensions by incubation for 90sec at 37°C in 0.025% trypsin-EDTA (Thermo Fisher). Similarly as previously described (1), for cell surface staining, cells were incubated with antibodies at 4°C for 15min in the dark in FACS medium (HBSS + 2% FBS), rinsed in FACS medium and resuspended in FACS medium with propidium iodide (1/4000, Sigma) before FACS acquisition and sorting. The following antibodies were used: EpCam-BV605 (G8.8, Biolegend BLE118227), CD31-PerCPy5.5 (MEC13.3, Biolegend BLE102522), CD45-PerCPy5.5 (30-F11, Biolegend BLE103132), CD24-APC-Fire 750 (MA/69, Biolegend BLE101840), CD49f-PE (GoH3, Biolegend BLE313612), CD71-FITC (RI7217, Biolegend BLE113806), TER-119-PerCPy5.5 (TER-119, Biolegend BLE116228), TCRb-PECy7 (H57-597, Biolegend BLE109222), CD4-APC (GK1.5, Biolegend BLE100412), CD44-BV421 (IM7, BD Biosciences # 563970). For each antibody, optimal dilution was determined by titration. Cell sorting was carried out using a FACSARIA III (BD). Data were analyzed using FlowJo 9.9.4 (RRID: SCR_008520). Dead cells were excluded from analyses using propidium iodide (fluorescence in both PE-A and PerCPy5.5-A channels, exclusion of the diagonal) and non-pancreatic lineages were excluded using the pan-hematopoietic marker CD45, the erythroid marker TER119, and the endothelial marker CD31. Pancreatic alpha- and beta-enriched fractions were isolated as described previously. As we determined that more than 95% of Lin⁺ cells are CD45⁺, sorted Lin⁺ were considered as CD45⁺ cells. For qPCR cells were collected directly in Buffer RLT for qPCR (Qiagen).

Quantitative RT-PCR

RNA was isolated with the RNeasy Micro Kit (Qiagen) and RNA sample was reverse-transcribed using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher) and a Biometra T3 Thermocycler (Analytik Jena). All kits were used per the manufacturer's instructions. SYBR green qPCR was performed on a QuantStudio 3 (Thermo Fisher) per the manufacturer's instructions.

Proteomic analysis making use of biotin-phenylglyoxal labeling

DMSO (n=4) and BB-Cl-amidine treated (n=4) mice pancreata were pooled in buffer (50mM HEPES pH 7.6, 0.5% NP40 and 1mM PMSF) and homogenized using a tissue homogenizer (20sec with maximum speed with 10min time interval three times). The lysed tissue samples were centrifuged (12,000rpm, 30min) at 4°C. The supernatants were isolated, and the protein concentration was determined using the DC™ (detergent compatible) assay. Equal protein

SUPPLEMENTARY DATA

concentrations from DMSO and BB-Cl-amidine treated samples were labeled with biotin-phenylglyoxal (biotin-PG) in quadruplicate as previously described (2). Briefly, supernatants from DMSO and BB-Cl-amidine treated samples (300µg) were diluted in buffer (100mM HEPES pH 7.6) to a final concentration of 1µg/µL in a reaction volume of 300µL and incubated with 20% trichloroacetic acid (60µL of 100% TCA) and 0.5mM biotin-PG (5µL of 5mM stock) for 30min at 37°C. After a 30min incubation, the reaction was quenched with 60µL of 0.1M citrulline dissolved in 50mM HEPES pH 7.6. Proteins were precipitated by placing the reaction mixtures on ice for 30min followed by centrifugation (13,500rpm, 15min) at 4°C. The supernatants were discarded, and the protein pellet was washed twice with cold acetone and dried.

Protein pellets were then resuspended in 1.2% SDS in PBS, boiled for 10min and sonicated for 12-15min. Once dissolved, samples were diluted in PBS buffer (0.2% final SDS concentration) and incubated with streptavidin agarose slurry (170µL) overnight at 4°C, and a further 3h at 25°C. Streptavidin beads were then washed with PBS containing 0.2% SDS (1X), with PBS alone (3X), and water (3X) in order to remove any unbound proteins. Streptavidin beads were heated in a buffer containing 500µL of 6M urea in PBS and 10mM DTT (65°C, 20min) followed by iodoacetamide (20mM) addition and further incubation for 30min at 37°C. The beads were pelleted by centrifugation (1,400 x g for 3min), resuspended in a premixed solution of 2M urea, 1mM CaCl₂ and 2µg trypsin in PBS and incubated overnight at 37°C. The beads were then pelleted by centrifugation and the supernatant, containing the peptide digests, was collected. The pellet was washed twice with water (50µL) and the collected supernatant fractions were combined. Formic acid (15µL) was added to the peptide mixture.

TMT labeling of tryptic digest

A total of eight peptide digests were obtained from quadruplicate labeling of the DMSO and BB-Cl-amidine treated samples. These samples were subjected to desalting using commercially available Pierce C18 Spin columns (Thermo Fisher) to remove buffer components that may interfere with TMT labeling. The desalting procedure followed the manufacturer's protocol. Peptide concentration was determined before as well as after desalting using the commercially available Pierce Quantitative Colorimetric Peptide Assay kit (Thermo Fisher) to ensure that the peptides were not lost during the desalting procedure. The samples were then evaporated to dryness in a Speedvac. The eight peptide digests were then labeled using a commercially available TMT labeling system (Thermo Fisher). Briefly, the samples were resuspended in 100mM HEPES pH 8.6 (100µL) in eight different Eppendorf tubes and treated with 8µL of TMT10plex labeling reagent. The samples were incubated for 1h at RT. The reaction was further quenched using 2.5µL of 5% hydroxylamine and samples were further incubated for 15min at RT. After quenching excess TMT reagent, the TMT-labeled samples (90µL) from each tube were mixed together in a separate Eppendorf tube. The combined sample were evaporated to dryness in a Speedvac and then stored at -80°C until mass spectrometry (MS) analysis.

LC-MS/MS and Data Processing

Liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed on a NanoAcquity UPLC (Waters Corporation) coupled to an Orbitrap Fusion Lumos Tribrid (Thermo Fisher) mass spectrometer, operating in the positive ion mode (1.4kV, applied by liquid junction). Mobile phases were A (0.1% (v/v) formic acid in water) and B (0.1% (v/v)

SUPPLEMENTARY DATA

formic acid in acetonitrile). The peptides were reconstituted in 35 μ L of 5% (v/v) acetonitrile, 0.1% (v/v) formic acid in water, and 3.8 μ L was injected in 5% B at 4 μ L/min for 4min to a 100 μ m ID fused-silica pre-column (Kasil frit) packed with 2cm of ProntoSIL C18 AQ (Bischoff Chromatography, 200 $\text{\AA}$, 5 μ m) particles. Peptides were then separated and eluted at 300nL/min by an *in-house* packed fused silica analytical column (gravity-pulled tip) packed with 25cm Magic C18AQ (Bruker-Michrom, 100 $\text{\AA}$, 3 μ m) particles. In brief, the gradient was 10-35% B (0-100min); 35-65% B (100-120min); 95% B (121-126min), 5% B (127-140min). Mass spectra were acquired in the Orbitrap mass analyzer using a resolution of 60,000 (m/z 200), an ion target population of $7e^5$, and maximum ion injection time of 30msec. Tandem mass spectra were acquired using data dependent acquisition using the following parameters: cycle time, 2sec; intensity threshold, $2e^4$; isolation width, 0.8Da; HCD collision energy, 38%; resolution, 30,000; ion population, $1e^5$; maximum ion injection time, 110 msec.

The raw data were processed using Proteome Discoverer (Thermo Fisher, version 2.1.1.21) and searched against SwissProt murine (downloaded 07/2019) database using Mascot (Matrix Science, version 2.6.2). The mass tolerance was 10ppm for precursor and 0.05Da for the fragment ions. Full tryptic cleavages were considered along with static modifications of carbamidomethylation (cysteine), TMT labelling (peptide N-terminal and lysine) and dynamic modifications of acetylation (protein N-terminal), oxidation (methionine) and glutamine to pyroglutamate conversion (peptide N-terminal). Peptides were assembled to proteins using Scaffold (Proteome Software, version 4.11.0) and were filtered to a 1% FDR using Peptide Prophet (3). The protein threshold was set to 99% probability using Protein Prophet (4) and only proteins with 2 or more filtered peptides were considered. The data was further processed by Scaffold Q+S (4.11.0) for quantitative analysis.

References:

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SUPPLEMENTARY DATA

Supplementary Table 1. Differentially regulated citrullinated proteins in pancreas of BB-CI-amidine treated versus DMSO treated NOD mice

* P<0.05 (Log₁₀p-value = 1.122)

	Log ₂ Ratio	Log ₁₀ p-value (*)	Accession
1	-1.606163	1.3050913	sp Q8BU14 SEC62_MOUSE
2	-1.5756054	2.4036503	sp Q8CAQ8-2 MIC60_MOUSE
3	-1.5686817	2.758175	sp P47915 RL29_MOUSE
4	-1.5622921	3.109283	sp P35564 CALX_MOUSE
5	-1.5391521	1.3050913	sp Q9ERG0-2 LIMA1_MOUSE
6	-1.5389996	3.4577034	sp Q9D0F3 LMAN1_MOUSE
7	-1.5316582	15.25562	sp P08113 ENPL_MOUSE
8	-1.5246878	4.14842	sp Q60997-2 DMBT1_MOUSE
9	-1.5179377	11.206782	sp Q64285 CEL_MOUSE
10	-1.5131435	1.6794106	sp Q9Z0Y2 PA21B_MOUSE
11	-1.5123882	1.6794106	sp Q6ZQ58 LARP1_MOUSE
12	-1.5034275	3.109283	sp P47963 RL13_MOUSE
13	-1.4920053	5.8520784	sp P27659 RL3_MOUSE
14	-1.4888325	2.4036503	sp Q8BKC5 IPO5_MOUSE
15	-1.4820528	1.3050913	sp Q8C0D5 EFL1_MOUSE
16	-1.4796295	1.3050913	sp Q3U0V1 FUBP2_MOUSE
17	-1.4736061	1.6794106	sp P13020-2 GELS_MOUSE
18	-1.4711285	14.724141	sp Q78PY7 SND1_MOUSE
19	-1.4627047	1.3050913	sp P26043 RADI_MOUSE
20	-1.460556	2.044628	sp Q8BI84-3 TGO1_MOUSE
21	-1.45401	11.539065	sp Q9JKR6 HYOU1_MOUSE
22	-1.4527073	9.2091	sp Q9D8E6 RL4_MOUSE
23	-1.4449883	4.8330717	sp Q8BM55 TM214_MOUSE
24	-1.4447136	5.17366	sp Q9Z1Z0 USO1_MOUSE
25	-1.4443893	3.8039558	sp Q8VEK3-2 HNRPU_MOUSE
26	-1.4433832	1.6794106	sp Q9CQX2 CYB5B_MOUSE
27	-1.4416866	14.778499	sp Q8VDJ3 VIGLN_MOUSE
28	-1.4405212	10.541792	sp P08003 PDIA4_MOUSE
29	-1.4388752	1.3050913	sp P62911 RL32_MOUSE
30	-1.4383736	5.8520784	sp P62754 RS6_MOUSE
31	-1.4367504	3.8039558	sp Q8R1B4 EIF3C_MOUSE
32	-1.4338951	6.527502	sp Q99PL5 RRBP1_MOUSE
33	-1.4318275	7.5362897	sp Q8VDD5 MYH9_MOUSE
34	-1.4302464	3.109283	sp P84099 RL19_MOUSE
35	-1.4291286	2.4036503	sp Q7TPR4 ACTN1_MOUSE
36	-1.4274426	4.4913836	sp P47911 RL6_MOUSE
37	-1.4266815	6.527502	sp Q7TPZ8 CBPA1_MOUSE

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38	-1.4237385	2.758175	sp Q91ZV0-3 MIA2_MOUSE
39	-1.4216404	5.5132895	sp P12970 RL7A_MOUSE
40	-1.4201832	1.3050913	sp Q4PJX1 ODR4_MOUSE
41	-1.420105	1.6794106	sp Q99LM2 CK5P3_MOUSE
42	-1.4167576	3.8039558	sp Q8VIJ6 SFPQ_MOUSE
43	-1.416626	3.109283	sp P41105 RL28_MOUSE
44	-1.4158173	1.6794106	sp Q9Z1D1 EIF3G_MOUSE
45	-1.4133005	10.541792	sp P23116 EIF3A_MOUSE
46	-1.4126663	2.758175	sp Q9D733 GP2_MOUSE
47	-1.4123726	1.3050913	sp P61514 RL37A_MOUSE
48	-1.4122839	13.544657	sp D3Z6P0-2 PDIA2_MOUSE
49	-1.4114666	>20	sp P20029 BIP_MOUSE
50	-1.4096727	1.6794106	sp Q8VE88-2 F1142_MOUSE
51	-1.4092903	1.3050913	sp Q9JHU9 INO1_MOUSE
52	-1.4076557	6.527502	sp Q99MR8 MCCA_MOUSE
53	-1.407486	9.2091	sp P14211 CALR_MOUSE
54	-1.4055767	1.3050913	sp Q64514-2 TPP2_MOUSE
55	-1.4040947	2.758175	sp Q9DB77 QCR2_MOUSE
56	-1.4026165	1.6794106	sp Q3U1J4 DDB1_MOUSE
57	-1.4025726	4.4913836	sp Q8BMA6 SRP68_MOUSE
58	-1.4007816	3.109283	sp Q6ZQ38 CAND1_MOUSE
59	-1.4003506	4.14842	sp Q61035 SYHC_MOUSE
60	-1.39781	1.3050913	sp O08553 DPYL2_MOUSE
61	-1.3975468	2.4036503	sp P70168 IMB1_MOUSE
62	-1.3956604	1.3050913	sp P97449 AMPN_MOUSE
63	-1.3956413	1.6794106	sp P97379-2 G3BP2_MOUSE
64	-1.3956404	2.044628	sp Q8VCT4 CES1D_MOUSE
65	-1.3933754	2.044628	sp Q3TXS7 PSMD1_MOUSE
66	-1.3922386	3.4577034	sp Q9CZM2 RL15_MOUSE
67	-1.3906231	1.3050913	sp Q9CYA0 CREL2_MOUSE
68	-1.3901138	2.044628	sp O08795-2 GLU2B_MOUSE
69	-1.3888931	3.4577034	sp Q99L45 IF2B_MOUSE
70	-1.387639	1.3050913	sp Q8VDM4 PSMD2_MOUSE
71	-1.3873711	3.4577034	sp P62918 RL8_MOUSE
72	-1.3868294	3.4577034	sp Q8K2B3 SDHA_MOUSE
73	-1.3841171	4.8330717	sp Q60864 STIP1_MOUSE
74	-1.3825274	11.870765	sp Q8K009 AL1L2_MOUSE
75	-1.3819504	1.3050913	sp P19783 COX41_MOUSE
76	-1.3817673	5.8520784	sp O55029 COPB2_MOUSE
77	-1.3795776	4.8330717	sp Q61941 NNTM_MOUSE
78	-1.3793259	11.870765	sp Q02053 UBA1_MOUSE
79	-1.3792286	5.8520784	sp Q6P5E4 UGGG1_MOUSE
80	-1.3786354	6.864285	sp Q91YW3 DNJC3_MOUSE
81	-1.3778496	>20	sp P58252 EF2_MOUSE
82	-1.3770294	3.4577034	sp Q60597-2 ODO1_MOUSE

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83	-1.3759804	4.8330717	sp Q8VDN2 AT1A1_MOUSE
84	-1.3738422	1.6794106	sp Q69ZS7-2 HBS1L_MOUSE
85	-1.3731365	1.6794106	sp Q9CY27 TECR_MOUSE
86	-1.3721466	7.2005315	sp P62242 RS8_MOUSE
87	-1.3701077	4.14842	sp P57780 ACTN4_MOUSE
88	-1.3683395	2.044628	sp Q61937 NPM_MOUSE
89	-1.3667011	4.4913836	sp Q504N0 CBPA2_MOUSE
90	-1.3636742	2.044628	sp Q8R146-2 APEH_MOUSE
91	-1.3631992	1.3050913	sp P45700 MA1A1_MOUSE
92	-1.3621225	4.4913836	sp Q61316 HSP74_MOUSE
93	-1.3612518	2.044628	sp Q8BWW4 LARP4_MOUSE
94	-1.3611755	2.758175	sp Q6P1B1 XPP1_MOUSE
95	-1.360982	1.6794106	sp P46935 NEDD4_MOUSE
96	-1.3584862	4.14842	sp P42932 TCPQ_MOUSE
97	-1.3582964	2.758175	sp Q9WVE8 PACN2_MOUSE
98	-1.3576298	7.2005315	sp O55143 AT2A2_MOUSE
99	-1.3571434	1.6794106	sp P34022 RANG_MOUSE
100	-1.3570337	1.3050913	sp Q8R010 AIMP2_MOUSE
101	-1.3560905	9.2091	sp P29341 PABP1_MOUSE
102	-1.3555489	8.20651	sp Q922D8 C1TC_MOUSE
103	-1.3548622	3.4577034	sp Q99K01-2 PDXD1_MOUSE
104	-1.3539143	5.17366	sp Q9ER72-2 SYCC_MOUSE
105	-1.3538799	5.8520784	sp Q9Z1Q9 SYVC_MOUSE
106	-1.3524313	3.8039558	sp Q921G7 ETFD_MOUSE
107	-1.350872	2.4036503	sp Q9WUM5 SUCA_MOUSE
108	-1.3487844	5.17366	sp Q9DBG7 SRPRA_MOUSE
109	-1.3481827	1.3050913	sp P10630-2 IF4A2_MOUSE
110	-1.3480272	1.3050913	sp O08529 CAN2_MOUSE
111	-1.3476143	5.17366	sp P07901 HS90A_MOUSE
112	-1.3463306	1.3050913	sp P62900 RL31_MOUSE
113	-1.3462315	2.758175	sp Q9Z2I9 SUCB1_MOUSE
114	-1.3461132	9.875973	sp P63017 HSP7C_MOUSE
115	-1.3459053	4.14842	sp P19253 RL13A_MOUSE
116	-1.3458977	5.5132895	sp Q99K67 AASS_MOUSE
117	-1.3458471	1.3050913	sp P62960 YBOX1_MOUSE
118	-1.3456907	2.044628	sp P19096 FAS_MOUSE
119	-1.3452873	3.109283	sp Q80X90 FLNB_MOUSE
120	-1.3451614	2.4036503	sp P46460 NSF_MOUSE
121	-1.3440971	5.17366	sp P50580 PA2G4_MOUSE
122	-1.3432941	2.758175	sp Q60854 SPB6_MOUSE
123	-1.3426933	2.758175	sp Q93092 TALDO_MOUSE
124	-1.3426638	1.3050913	sp Q6NZC7 S23IP_MOUSE
125	-1.3417797	5.5132895	sp Q08642 PADI2_MOUSE
126	-1.3408585	9.2091	sp P63038 CH60_MOUSE
127	-1.3408566	1.3050913	sp Q99KB8-2 GLO2_MOUSE

SUPPLEMENTARY DATA

128	-1.3404121	1.3050913	sp P99024 TBB5_MOUSE
129	-1.3401604	2.044628	sp P09405 NUCL_MOUSE
130	-1.3398399	2.044628	sp Q8BJ64 CHDH_MOUSE
131	-1.3393288	4.8330717	sp Q91VD9 NDUS1_MOUSE
132	-1.3380337	3.109283	sp Q922Q1 MARC2_MOUSE
133	-1.3370905	1.3050913	sp Q99J99 THTM_MOUSE
134	-1.3368435	7.2005315	sp Q99MN1 SYK_MOUSE
135	-1.3365211	7.5362897	sp P25444 RS2_MOUSE
136	-1.3362446	8.541041	sp Q8BH04 PCKGM_MOUSE
137	-1.3361301	4.14842	sp P62717 RL18A_MOUSE
138	-1.3357277	4.14842	sp Q76MZ3 2AAA_MOUSE
139	-1.3349895	6.1901226	sp P11499 HS90B_MOUSE
140	-1.3349838	6.1901226	sp Q8C1A5 THOP1_MOUSE
141	-1.3348017	1.3050913	sp Q8BH24 TM9S4_MOUSE
142	-1.3339005	2.044628	sp Q8BHN3-2 GANAB_MOUSE
143	-1.3320112	7.2005315	sp Q921I1 TRFE_MOUSE
144	-1.3313932	1.3050913	sp Q9CXI5 MANF_MOUSE
145	-1.3310795	1.3050913	sp Q6IRU5-2 CLCB_MOUSE
146	-1.3307247	4.4913836	sp Q8JZQ9 EIF3B_MOUSE
147	-1.329732	5.17366	sp Q9CZ13 QCR1_MOUSE
148	-1.3291674	2.044628	sp Q8BJW6 EIF2A_MOUSE
149	-1.3288612	4.14842	sp Q9DBG6 RPN2_MOUSE
150	-1.3288498	1.3050913	sp P47757-2 CAPZB_MOUSE
151	-1.3287697	4.8330717	sp P61222 ABCE1_MOUSE
152	-1.3275337	2.044628	sp Q91WD5 NDUS2_MOUSE
153	-1.3272305	1.6794106	sp Q3V3R1 C1TM_MOUSE
154	-1.3262081	11.206782	sp P27773 PDIA3_MOUSE
155	-1.3258724	3.109283	sp Q9JKF1 IQGA1_MOUSE
156	-1.325594	3.4577034	sp P62267 RS23_MOUSE
157	-1.3255043	5.5132895	sp Q9Z110-2 P5CS_MOUSE
158	-1.3244228	2.4036503	sp Q9R111 GUAD_MOUSE
159	-1.3243294	5.17366	sp P16331 PH4H_MOUSE
160	-1.323288	10.209015	sp Q05920 PYC_MOUSE
161	-1.3188515	3.8039558	sp E9PVA8 GCN1_MOUSE
162	-1.3182621	3.109283	sp P16332 MUTA_MOUSE
163	-1.318121	4.14842	sp Q61768 KINH_MOUSE
164	-1.3170776	3.109283	sp Q91VR5 DDX1_MOUSE
165	-1.3169403	4.14842	sp Q8VE47 UBA5_MOUSE
166	-1.3169346	1.3050913	sp P13439 UMPS_MOUSE
167	-1.3159828	1.3050913	sp Q8R4N0 CLYBL_MOUSE
168	-1.3152256	2.044628	sp Q8CGK3 LONM_MOUSE
169	-1.3147907	1.3050913	sp O35295 PURB_MOUSE
170	-1.314601	1.3050913	sp Q3THS6 METK2_MOUSE
171	-1.3144493	3.4577034	sp Q8BP67 RL24_MOUSE
172	-1.3129902	5.5132895	sp Q8BU30 SYIC_MOUSE

SUPPLEMENTARY DATA

173	-1.3128166	1.3050913	sp Q8R081 HNRPL_MOUSE
174	-1.312418	9.875973	sp P40142 TKT_MOUSE
175	-1.3121223	5.8520784	sp Q9QZE5 COPG1_MOUSE
176	-1.312088	1.3050913	sp Q5FWK3 RHG01_MOUSE
177	-1.3116188	5.17366	sp P17892 LIPR2_MOUSE
178	-1.3114376	1.3050913	sp Q60931 VDAC3_MOUSE
179	-1.3113327	3.109283	sp Q64727 VINC_MOUSE
180	-1.311204	2.758175	sp P56399 UBP5_MOUSE
181	-1.310852	3.8039558	sp Q8BGD9 IF4B_MOUSE
182	-1.3106852	3.109283	sp Q9D8U3 ERP27_MOUSE
183	-1.3085461	5.8520784	sp Q8R2E9 ERO1B_MOUSE
184	-1.3082428	2.758175	sp Q9R0N0 GALK1_MOUSE
185	-1.3080387	13.879043	sp Q8CGC7 SYEP_MOUSE
186	-1.3077297	8.87523	sp Q99KI0 ACON_MOUSE
187	-1.3073292	1.3050913	sp Q9EQH3 VPS35_MOUSE
188	-1.3064976	1.6794106	sp Q99K23 UFSP2_MOUSE
189	-1.3062477	2.4036503	sp O08807 PRDX4_MOUSE
190	-1.3061066	5.17366	sp Q6ZWX6 IF2A_MOUSE
191	-1.3054523	3.8039558	sp Q9CPR4 RL17_MOUSE
192	-1.3040752	8.20651	sp Q8BGQ7 SYAC_MOUSE
193	-1.3038893	1.3050913	sp A2ALS5-3 RPGP1_MOUSE
194	-1.3038769	1.3050913	sp Q61712 DNJC1_MOUSE
195	-1.3026886	1.6794106	sp Q62433 NDRG1_MOUSE
196	-1.301486	5.8520784	sp Q8BMJ2 SYLC_MOUSE
197	-1.3014832	1.6794106	sp Q9EPJ9-2 ARFG1_MOUSE
198	-1.3013763	5.5132895	sp P10852 4F2_MOUSE
199	-1.3013611	4.14842	sp Q9WU78-3 PDC6I_MOUSE
200	-1.300457	3.109283	sp P31230 AIMP1_MOUSE
201	-1.3002872	3.109283	sp P50136 ODBA_MOUSE
202	-1.3000164	3.4577034	sp Q9Z0X1 AIFM1_MOUSE
203	-1.2996407	1.6794106	sp P45952 ACADM_MOUSE
204	-1.2996159	3.109283	sp P46978 STT3A_MOUSE
205	-1.2995377	4.8330717	sp P80313 TCPH_MOUSE
206	-1.2984924	>20	sp P09103 PDIA1_MOUSE
207	-1.2976894	2.044628	sp Q99JY9 ARP3_MOUSE
208	-1.2975521	6.527502	sp P80318 TCPG_MOUSE
209	-1.2969227	1.3050913	sp Q64324 STXB2_MOUSE
210	-1.2958546	2.4036503	sp Q9CR57 RL14_MOUSE
211	-1.2954254	5.5132895	sp Q6NZJ6 IF4G1_MOUSE
212	-1.2949848	3.8039558	sp Q7TSV4 PGM2_MOUSE
213	-1.2946405	5.17366	sp Q7TMK9-2 HNRPQ_MOUSE
214	-1.2941017	7.2005315	sp Q8R0Y6 AL1L1_MOUSE
215	-1.2937813	2.4036503	sp Q8VCT3 AMPB_MOUSE
216	-1.2937784	4.14842	sp P80316 TCPE_MOUSE
217	-1.2931099	15.4774685	sp Q01853 TERA_MOUSE

SUPPLEMENTARY DATA

218	-1.2930288	1.3050913	sp Q9CXR1 DHRS7_MOUSE
219	-1.291956	5.17366	sp Q91WT9-2 CBS_MOUSE
220	-1.291832	4.4913836	sp Q9JK88 SPI2_MOUSE
221	-1.2915516	9.875973	sp Q6P8U6 LIPP_MOUSE
222	-1.2909584	3.4577034	sp Q5SUR0 PUR4_MOUSE
223	-1.2907934	4.4913836	sp P28271 ACOC_MOUSE
224	-1.2906647	4.8330717	sp Q9CWJ9 PUR9_MOUSE
225	-1.2903461	1.6794106	sp Q9DB25 ALG5_MOUSE
226	-1.2898369	4.8330717	sp O70194 EIF3D_MOUSE
227	-1.2889051	5.17366	sp Q91V92 ACLY_MOUSE
228	-1.2882395	5.5132895	sp P80314 TCPB_MOUSE
229	-1.2875557	3.8039558	sp Q8CHT0 AL4A1_MOUSE
230	-1.28619	5.17366	sp Q9JHU4 DYHC1_MOUSE
231	-1.2860632	9.875973	sp Q03265 ATPA_MOUSE
232	-1.2860603	4.8330717	sp Q9D8S3 ARFG3_MOUSE
233	-1.2857866	2.4036503	sp Q9DCX2 ATP5H_MOUSE
234	-1.2849884	1.6794106	sp O88487 DC1I2_MOUSE
235	-1.2849503	1.6794106	sp P68372 TBB4B_MOUSE
236	-1.2847481	6.527502	sp Q5XJY5 COPD_MOUSE
237	-1.2844143	2.044628	sp Q9Z1Z2 STRAP_MOUSE
238	-1.2842455	7.5362897	sp P26638 SYSC_MOUSE
239	-1.2839375	1.3050913	sp Q8VBV7 CSN8_MOUSE
240	-1.2835426	6.864285	sp P38647 GRP75_MOUSE
241	-1.2834921	2.044628	sp Q9CY58-2 PAIRB_MOUSE
242	-1.2819977	5.17366	sp Q68FD5 CLH1_MOUSE
243	-1.2819004	5.5132895	sp Q9JIF7 COPB_MOUSE
244	-1.2816324	3.8039558	sp Q8CCJ3 UFL1_MOUSE
245	-1.2815514	8.87523	sp P26443 DHE3_MOUSE
246	-1.2805405	3.4577034	sp P26039 TLN1_MOUSE
247	-1.2801208	1.3050913	sp Q9CPX6 ATG3_MOUSE
248	-1.2797565	2.4036503	sp Q00PI9 HNRL2_MOUSE
249	-1.2796917	3.109283	sp Q8BH59 CMC1_MOUSE
250	-1.2796316	1.6794106	sp Q9WUM3 COR1B_MOUSE
251	-1.2790489	9.2091	sp Q3UPL0-2 SC31A_MOUSE
252	-1.2786016	2.758175	sp Q8VCA8 SCRN2_MOUSE
253	-1.2774887	3.109283	sp Q9CQA3 SDHB_MOUSE
254	-1.27707	6.864285	sp Q99MN9 PCCB_MOUSE
255	-1.2770615	1.3050913	sp Q9WVJ2 PSD13_MOUSE
256	-1.2764082	8.541041	sp Q91YQ5 RPN1_MOUSE
257	-1.2756062	3.109283	sp Q9Z2I8-2 SUCB2_MOUSE
258	-1.2742653	1.6794106	sp P29758 OAT_MOUSE
259	-1.2741928	2.044628	sp P81117 NUCB2_MOUSE
260	-1.2736826	1.3050913	sp P20357 MTAP2_MOUSE
261	-1.2730732	1.6794106	sp Q3UM45 PP1R7_MOUSE
262	-1.2729797	1.3050913	sp Q80UU9 PGRC2_MOUSE

SUPPLEMENTARY DATA

263	-1.2727833	1.3050913	sp Q61792 LASP1_MOUSE
264	-1.2726507	5.8520784	sp Q8BMS1 ECHA_MOUSE
265	-1.2719345	1.3050913	sp O70503-2 DHB12_MOUSE
266	-1.2717094	2.044628	sp O54724 CAVN1_MOUSE
267	-1.2707272	2.758175	sp P24527 LKHA4_MOUSE
268	-1.2707233	2.4036503	sp Q9DBC7 KAP0_MOUSE
269	-1.2700119	4.4913836	sp P80317 TCPZ_MOUSE
270	-1.268858	6.864285	sp P20152 VIME_MOUSE
271	-1.2685776	1.3050913	sp P97855 G3BP1_MOUSE
272	-1.268301	3.109283	sp Q8VCR2-2 DHB13_MOUSE
273	-1.2682877	4.4913836	sp O08749 DLDH_MOUSE
274	-1.2681694	3.4577034	sp Q99L88 SNTB1_MOUSE
275	-1.266922	1.6794106	sp P61982 1433G_MOUSE
276	-1.2664375	8.20651	sp P14148 RL7_MOUSE
277	-1.266159	2.758175	sp Q8K0C9 GMDS_MOUSE
278	-1.2659197	1.3050913	sp Q9D8S4 ORN_MOUSE
279	-1.2655277	3.8039558	sp P68368 TBA4A_MOUSE
280	-1.2654095	13.544657	sp Q61753 SERA_MOUSE
281	-1.2650509	4.14842	sp Q99LF4 RTCB_MOUSE
282	-1.2646408	5.5132895	sp Q91WQ3 SYYC_MOUSE
283	-1.263113	7.5362897	sp Q922R8 PDIA6_MOUSE
284	-1.2624445	1.3050913	sp Q921F4 HNRL_MOUSE
285	-1.2621841	12.202081	sp Q91VI7 RINI_MOUSE
286	-1.261591	5.8520784	sp Q9CZD3 GARS_MOUSE
287	-1.2613306	4.8330717	sp P11983 TCPA_MOUSE
288	-1.261198	2.758175	sp O09167 RL21_MOUSE
289	-1.2602272	1.3050913	sp O35129 PHB2_MOUSE
290	-1.2596807	12.202081	sp Q8CIE6 COPA_MOUSE
291	-1.2593307	2.758175	sp Q60932-2 VDAC1_MOUSE
292	-1.2590218	6.527502	sp P47962 RL5_MOUSE
293	-1.2587128	2.044628	sp Q9D7S7-2 RL22L_MOUSE
294	-1.2579155	2.758175	sp Q8C0C7 SYFA_MOUSE
295	-1.2578335	2.044628	sp Q8VCK7 SYCN_MOUSE
296	-1.2575798	1.3050913	sp O70310 NMT1_MOUSE
297	-1.2570591	2.758175	sp Q8BG32 PSD11_MOUSE
298	-1.256978	1.3050913	sp Q99JX3-2 GORS2_MOUSE
299	-1.2556381	1.3050913	sp Q68FL4 SAHH3_MOUSE
300	-1.2545471	4.14842	sp Q9JMH6-2 TRXR1_MOUSE
301	-1.2544708	5.5132895	sp Q91YI0 ARLY_MOUSE
302	-1.254446	2.044628	sp P62264 RS14_MOUSE
303	-1.2543488	6.864285	sp Q9CR35 CTRB1_MOUSE
304	-1.2530174	12.533645	sp Q91ZA3 PCCA_MOUSE
305	-1.252924	2.044628	sp Q9CXF4 TBC15_MOUSE
306	-1.2526445	1.3050913	sp Q8K1M6-2 DNM1L_MOUSE
307	-1.2522888	2.4036503	sp Q91W90 TXND5_MOUSE

SUPPLEMENTARY DATA

308	-1.2520561	2.044628	sp P30999-2 CTND1_MOUSE
309	-1.2511292	1.3050913	sp Q9QXK3-4 COPG2_MOUSE
310	-1.2510643	2.758175	sp P14115 RL27A_MOUSE
311	-1.2509689	1.3050913	sp Q61749-2 EI2BD_MOUSE
312	-1.2500534	7.871603	sp Q9CPY7-2 AMPL_MOUSE
313	-1.2498074	2.044628	sp Q9D1R9 RL34_MOUSE
314	-1.2494869	9.542674	sp P00688 AMYP_MOUSE
315	-1.2493353	2.044628	sp Q921F2 TADBP_MOUSE
316	-1.2482071	4.4913836	sp P97429 ANXA4_MOUSE
317	-1.2477598	1.3050913	sp Q3UW53 NIBAN_MOUSE
318	-1.2468872	7.5362897	sp P14206 RSSA_MOUSE
319	-1.245183	4.8330717	sp Q9D662 SC23B_MOUSE
320	-1.2451668	4.8330717	sp Q8QZT1 THIL_MOUSE
321	-1.2449217	4.14842	sp P05201 AATC_MOUSE
322	-1.2442493	2.044628	sp O55234 PSB5_MOUSE
323	-1.2440205	1.3050913	sp Q91W50 CSDE1_MOUSE
324	-1.2438259	1.6794106	sp Q9D1M7 FKB11_MOUSE
325	-1.2428455	1.6794106	sp P50544 ACADV_MOUSE
326	-1.2422352	4.14842	sp P17563 SBP1_MOUSE
327	-1.2422047	6.527502	sp Q9D0R2 SYTC_MOUSE
328	-1.2420607	2.4036503	sp P40124 CAP1_MOUSE
329	-1.242033	3.4577034	sp Q9EP69 SAC1_MOUSE
330	-1.2414513	1.3050913	sp P68037 UB2L3_MOUSE
331	-1.2410183	2.4036503	sp Q9JMD3 STA10_MOUSE
332	-1.2408857	13.879043	sp P07724 ALBU_MOUSE
333	-1.2403259	1.6794106	sp P61620 S61A1_MOUSE
334	-1.2402878	7.871603	sp Q5BKQ4 LIPR1_MOUSE
335	-1.2398195	2.4036503	sp Q501J6 DDX17_MOUSE
336	-1.2398071	2.4036503	sp P43137 LIT1_MOUSE
337	-1.2398014	2.4036503	sp Q80UM7 MOGS_MOUSE
338	-1.2397232	2.4036503	sp Q9DBT5 AMPD2_MOUSE
339	-1.2394905	1.3050913	sp P63085 MK01_MOUSE
340	-1.2394314	1.6794106	sp Q61655 DD19A_MOUSE
341	-1.2394104	3.109283	sp P07759 SPA3K_MOUSE
342	-1.2393808	2.044628	sp P26040 EZRI_MOUSE
343	-1.2392197	1.3050913	sp Q9D8W5 PSD12_MOUSE
344	-1.2390957	2.4036503	sp Q9D1D4 TMEDA_MOUSE
345	-1.2390442	3.109283	sp Q8R050-2 ERF3A_MOUSE
346	-1.2390137	8.541041	sp Q9EQ20 MMSA_MOUSE
347	-1.2386875	2.044628	sp P16546-2 SPTN1_MOUSE
348	-1.2385035	2.4036503	sp P50516 VATA_MOUSE
349	-1.2384434	4.4913836	sp Q3ULD5 MCCB_MOUSE
350	-1.2383823	1.6794106	sp Q9Z2M7 PMM2_MOUSE
351	-1.2382393	3.109283	sp Q9D0E1-2 HNRPM_MOUSE
352	-1.2381363	3.8039558	sp Q9CQ52 CEL3B_MOUSE

SUPPLEMENTARY DATA

353	-1.2380524	5.17366	sp Q61656 DDX5_MOUSE
354	-1.2374115	2.044628	sp Q64737 PUR2_MOUSE
355	-1.2358093	2.4036503	sp P62821 RAB1A_MOUSE
356	-1.2353916	1.6794106	sp Q8BWF0 SSDH_MOUSE
357	-1.2348747	4.4913836	sp Q922Q8 LRC59_MOUSE
358	-1.2344933	7.2005315	sp Q9D8N0 EF1G_MOUSE
359	-1.2338982	5.5132895	sp P32921-2 SYWC_MOUSE
360	-1.233263	4.4913836	sp P53395 ODB2_MOUSE
361	-1.2329149	1.6794106	sp Q62318 TIF1B_MOUSE
362	-1.2329006	1.3050913	sp O70378 EMC8_MOUSE
363	-1.2326508	2.758175	sp P46471 PRS7_MOUSE
364	-1.232357	6.864285	sp P14152 MDHC_MOUSE
365	-1.2322598	2.4036503	sp Q9WTP7 KAD3_MOUSE
366	-1.2322226	6.864285	sp P51881 ADT2_MOUSE
367	-1.2318592	3.8039558	sp P60229 EIF3E_MOUSE
368	-1.2318182	2.044628	sp Q64105 SPRE_MOUSE
369	-1.2316704	2.758175	sp Q9D6R2 IDH3A_MOUSE
370	-1.2314072	2.044628	sp Q8CC86 PNCB_MOUSE
371	-1.2311535	3.8039558	sp Q8BWT1 THIM_MOUSE
372	-1.230875	2.4036503	sp Q9D1M0 SEC13_MOUSE
373	-1.2305536	2.044628	sp P51150 RAB7A_MOUSE
374	-1.2305307	1.6794106	sp Q00612 G6PD1_MOUSE
375	-1.2305117	4.4913836	sp Q8BFR5 EFTU_MOUSE
376	-1.2304802	3.109283	sp O08547 SC22B_MOUSE
377	-1.2302799	2.4036503	sp O09131 GSTO1_MOUSE
378	-1.230217	2.4036503	sp P07146 TRY2_MOUSE
379	-1.2294235	5.5132895	sp P62702 RS4X_MOUSE
380	-1.2292967	8.87523	sp Q9DBF1-2 AL7A1_MOUSE
381	-1.2286272	3.109283	sp Q9DBE8 ALG2_MOUSE
382	-1.228198	1.3050913	sp Q8K268 ABCF3_MOUSE
383	-1.2280216	2.4036503	sp Q9EPU0-2 RENT1_MOUSE
384	-1.2277203	3.4577034	sp P35980 RL18_MOUSE
385	-1.2275372	4.8330717	sp Q9DCS3 MECR_MOUSE
386	-1.2258224	2.044628	sp P67984 RL22_MOUSE
387	-1.2256184	2.044628	sp P46638 RB11B_MOUSE
388	-1.2253809	1.6794106	sp Q61699-2 HS105_MOUSE
389	-1.2244892	1.6794106	sp Q99KJ8 DCTN2_MOUSE
390	-1.224266	3.109283	sp Q9WUQ2 PREB_MOUSE
391	-1.2236204	1.3050913	sp P63330 PP2AA_MOUSE
392	-1.2234249	1.6794106	sp Q9DBE0 CSAD_MOUSE
393	-1.2232704	1.3050913	sp O35639 ANXA3_MOUSE
394	-1.2221279	2.044628	sp Q9DBJ1 PGAM1_MOUSE
395	-1.2198334	4.14842	sp Q91X79 CELA1_MOUSE
396	-1.2187748	3.4577034	sp Q9CZN7-2 GLYM_MOUSE
397	-1.2182426	2.044628	sp P47955 RLA1_MOUSE

SUPPLEMENTARY DATA

398	-1.2181587	1.3050913	sp Q8VDL4-3 ADPGK_MOUSE
399	-1.21809	1.3050913	sp P22892 AP1G1_MOUSE
400	-1.2178001	5.5132895	sp Q9JHI5 IVD_MOUSE
401	-1.2174044	1.3050913	sp Q8BTY8-2 SCFD2_MOUSE
402	-1.2172241	3.109283	sp P16460 ASSY_MOUSE
403	-1.2171345	1.6794106	sp P62897 CYC_MOUSE
404	-1.2166939	1.6794106	sp Q9WV32 ARC1B_MOUSE
405	-1.2147427	3.109283	sp O08663 MAP2_MOUSE
406	-1.2141209	2.044628	sp P60766 CDC42_MOUSE
407	-1.2137928	1.3050913	sp Q62087 PON3_MOUSE
408	-1.2136002	5.5132895	sp P06745 G6PI_MOUSE
409	-1.2135181	7.2005315	sp Q8BML9 SYQ_MOUSE
410	-1.2135086	2.044628	sp P10639 THIO_MOUSE
411	-1.2134705	2.758175	sp O35945 AL1A7_MOUSE
412	-1.213131	7.5362897	sp Q9D154 ILEUA_MOUSE
413	-1.2126694	7.5362897	sp P97351 RS3A_MOUSE
414	-1.2123632	3.4577034	sp P67778 PHB_MOUSE
415	-1.2122631	4.8330717	sp Q8QZS1 HIBCH_MOUSE
416	-1.2121181	6.864285	sp P14869 RLA0_MOUSE
417	-1.2116718	6.1901226	sp Q61598 GDIB_MOUSE
418	-1.2113695	3.109283	sp O88685 PRS6A_MOUSE
419	-1.2113047	1.3050913	sp Q9DBG3-2 AP2B1_MOUSE
420	-1.2110729	2.4036503	sp Q9DB29 IAH1_MOUSE
421	-1.2109756	4.8330717	sp Q922B2 SYDC_MOUSE
422	-1.2109451	2.044628	sp Q6PDI5-2 ECM29_MOUSE
423	-1.2104816	2.044628	sp P62814 VATB2_MOUSE
424	-1.2103176	1.3050913	sp Q00623 APOA1_MOUSE
425	-1.2098598	1.6794106	sp P52480 KPYM_MOUSE
426	-1.2095823	2.044628	sp Q60605-2 MYL6_MOUSE
427	-1.2094078	4.4913836	sp Q68FL6 SYMC_MOUSE
428	-1.2086239	1.3050913	sp P17225 PTBP1_MOUSE
429	-1.2081375	3.109283	sp P59325 IF5_MOUSE
430	-1.2079468	2.758175	sp Q61425 HCDH_MOUSE
431	-1.2077026	4.14842	sp P63101 1433Z_MOUSE
432	-1.2072868	2.044628	sp P16254 SRP14_MOUSE
433	-1.2065659	2.4036503	sp Q9DBL1 ACDSB_MOUSE
434	-1.20607	2.4036503	sp Q60928 GGT1_MOUSE
435	-1.2052937	5.17366	sp Q99LB7 SARDH_MOUSE
436	-1.2052326	1.6794106	sp P22599 A1AT2_MOUSE
437	-1.2049294	3.109283	sp P62830 RL23_MOUSE
438	-1.2048836	2.044628	sp Q9DB20 ATPO_MOUSE
439	-1.20401	2.044628	sp Q9QUR6 PPCE_MOUSE
440	-1.2036858	2.758175	sp P15947 KLK1_MOUSE
441	-1.2035866	3.4577034	sp P19001 K1C19_MOUSE
442	-1.2031517	1.3050913	sp Q6PB66 LPPRC_MOUSE

SUPPLEMENTARY DATA

443	-1.202982	1.3050913	sp P50096 IMDH1_MOUSE
444	-1.2026825	3.8039558	sp Q9CYR6 AGM1_MOUSE
445	-1.2026491	2.044628	sp Q9CZ30 OLA1_MOUSE
446	-1.2025928	2.4036503	sp P62245 RS15A_MOUSE
447	-1.2021599	2.758175	sp Q8K0C5 ZG16_MOUSE
448	-1.2017212	2.758175	sp Q91X83 METK1_MOUSE
449	-1.2005901	1.6794106	sp Q922W5 P5CR1_MOUSE
450	-1.2005043	7.2005315	sp P47856-2 GFPT1_MOUSE
451	-1.200057	2.4036503	sp Q9DCL9 PUR6_MOUSE
452	-1.1999035	12.202081	sp P05784 K1C18_MOUSE
453	-1.1997299	3.8039558	sp Q99JR1 SFXN1_MOUSE
454	-1.199727	3.8039558	sp P24549 AL1A1_MOUSE
455	-1.1993027	8.87523	sp P14824 ANXA6_MOUSE
456	-1.199131	7.871603	sp Q8BP47 SYNC_MOUSE
457	-1.1989794	3.8039558	sp Q9D1A2 CNDP2_MOUSE
458	-1.1985054	1.6794106	sp Q9CQM9 GLRX3_MOUSE
459	-1.1983871	1.6794106	sp Q9JMA1 UBP14_MOUSE
460	-1.1983643	2.044628	sp P47740 AL3A2_MOUSE
461	-1.1980343	2.4036503	sp P60867 RS20_MOUSE
462	-1.1972475	4.14842	sp P97807-2 FUMH_MOUSE
463	-1.1962757	1.3050913	sp P62874 GBB1_MOUSE
464	-1.1960812	2.4036503	sp P00687 AMY1_MOUSE
465	-1.1949425	2.758175	sp O54734 OST48_MOUSE
466	-1.1939955	2.4036503	sp P54775 PRS6B_MOUSE
467	-1.1938419	5.8520784	sp O88844 IDHC_MOUSE
468	-1.1938324	5.5132895	sp Q64442 DHSO_MOUSE
469	-1.1936865	1.3050913	sp A3KMP2 TTC38_MOUSE
470	-1.1935654	1.3050913	sp P62855 RS26_MOUSE
471	-1.1931896	6.1901226	sp P56480 ATPB_MOUSE
472	-1.1927719	9.2091	sp Q9D0I9 SYRC_MOUSE
473	-1.1926117	3.4577034	sp Q9JII6 AK1A1_MOUSE
474	-1.19244	1.6794106	sp P97364 SPS2_MOUSE
475	-1.1923447	1.3050913	sp O88342 WDR1_MOUSE
476	-1.1920319	1.6794106	sp P70349 HINT1_MOUSE
477	-1.1919155	1.6794106	sp Q9QXB9 DRG2_MOUSE
478	-1.191803	2.4036503	sp P12787 COX5A_MOUSE
479	-1.1913805	5.17366	sp P57776-2 EF1D_MOUSE
480	-1.1909008	2.758175	sp Q3UGC7 EI3JA_MOUSE
481	-1.1903687	2.4036503	sp Q9DCD0 6PGD_MOUSE
482	-1.1902828	1.3050913	sp Q9ESP1 SDF2L_MOUSE
483	-1.1898289	1.6794106	sp Q9DCC4 P5CR3_MOUSE
484	-1.189579	1.6794106	sp Q505F5 LRC47_MOUSE
485	-1.1889296	1.3050913	sp P31786 ACBP_MOUSE
486	-1.1883411	4.14842	sp P45376 ALDR_MOUSE
487	-1.1882343	4.8330717	sp Q6ZWW3 RL10_MOUSE

SUPPLEMENTARY DATA

488	-1.1880875	2.4036503	sp Q9CQ60 6PGL_MOUSE
489	-1.1876583	1.6794106	sp Q9JHH9 COPZ2_MOUSE
490	-1.1874218	1.3050913	sp P70404 IDHG1_MOUSE
491	-1.1870861	4.14842	sp Q9WUA2 SYFB_MOUSE
492	-1.1869183	1.3050913	sp P70372 ELAV1_MOUSE
493	-1.1869135	1.6794106	sp Q99KV1 DJB11_MOUSE
494	-1.1862221	1.3050913	sp P97315 CSRP1_MOUSE
495	-1.1851444	3.4577034	sp Q8BRF7 SCFD1_MOUSE
496	-1.1847076	1.6794106	sp P10107 ANXA1_MOUSE
497	-1.1841087	4.8330717	sp Q8VCN5 CGL_MOUSE
498	-1.1839962	3.109283	sp Q91Z53 GRHPR_MOUSE
499	-1.1836319	1.3050913	sp Q8BJY1 PSMD5_MOUSE
500	-1.1835632	3.8039558	sp P35979 RL12_MOUSE
501	-1.1831312	6.1901226	sp P17751 TPIS_MOUSE
502	-1.1825371	1.6794106	sp Q9EPE9 AT131_MOUSE
503	-1.1824512	5.8520784	sp P05208 CEL2A_MOUSE
504	-1.182064	1.3050913	sp Q99LD9 EI2BB_MOUSE
505	-1.1816406	1.3050913	sp P63073 IF4E_MOUSE
506	-1.1809025	3.109283	sp P41216 ACSL1_MOUSE
507	-1.1803513	1.3050913	sp Q9CQC6 BZW1_MOUSE
508	-1.180027	2.044628	sp Q02248 CTNB1_MOUSE
509	-1.1800194	4.8330717	sp Q9JLJ2 AL9A1_MOUSE
510	-1.1799641	3.109283	sp P47758 SRPRB_MOUSE
511	-1.1795483	2.4036503	sp Q99KK7 DPP3_MOUSE
512	-1.1788673	6.864285	sp P47738 ALDH2_MOUSE
513	-1.1785793	1.6794106	sp P63005-2 LIS1_MOUSE
514	-1.1780281	1.6794106	sp Q99KR7 PPIF_MOUSE
515	-1.1776447	3.4577034	sp Q3TW96 UAP1L_MOUSE
516	-1.1773138	2.044628	sp Q99020 ROAA_MOUSE
517	-1.1772804	2.758175	sp Q3ULJ0-2 GPD1L_MOUSE
518	-1.1771526	5.8520784	sp P09411 PGK1_MOUSE
519	-1.176693	1.3050913	sp Q9WUP7-2 UCHL5_MOUSE
520	-1.1761904	5.8520784	sp P06151 LDHA_MOUSE
521	-1.1761398	4.4913836	sp P62281 RS11_MOUSE
522	-1.1751757	3.8039558	sp Q99L04 DHRS1_MOUSE
523	-1.1737366	2.4036503	sp Q99PT1 GDIR1_MOUSE
524	-1.1736565	2.758175	sp Q3UPH1 PRRC1_MOUSE
525	-1.1735573	6.1901226	sp P05202 AATM_MOUSE
526	-1.1734276	1.6794106	sp P61089 UBE2N_MOUSE
527	-1.1726398	2.4036503	sp Q9QUM9 PSA6_MOUSE
528	-1.1719694	3.109283	sp P60843 IF4A1_MOUSE
529	-1.1717148	1.3050913	sp Q60692 PSB6_MOUSE
530	-1.1711302	3.109283	sp P35505 FAAA_MOUSE
531	-1.1709566	5.8520784	sp O88986 KBL_MOUSE
532	-1.170826	3.4577034	sp P14576 SRP54_MOUSE

SUPPLEMENTARY DATA

533	-1.1706753	2.4036503	sp Q9CQR2 RS21_MOUSE
534	-1.17066	6.1901226	sp Q6ZWN5 RS9_MOUSE
535	-1.1703405	11.539065	sp Q61024 ASNS_MOUSE
536	-1.1703348	2.044628	sp Q8BWW3 ERF1_MOUSE
537	-1.1702461	5.5132895	sp P51410 RL9_MOUSE
538	-1.16922	1.6794106	sp Q9Z1N5 DX39B_MOUSE
539	-1.1691589	1.6794106	sp P62751 RL23A_MOUSE
540	-1.166542	1.3050913	sp P35293 RAB18_MOUSE
541	-1.1659575	2.044628	sp Q8K183 PDXK_MOUSE
542	-1.1644859	1.3050913	sp B2RSH2 GNAI1_MOUSE
543	-1.1639977	2.044628	sp Q99L47 F10A1_MOUSE
544	-1.1637392	2.758175	sp Q9Z204-2 HNRPC_MOUSE
545	-1.1633167	3.4577034	sp P35486 ODPA_MOUSE
546	-1.16325	9.2091	sp P17182 ENOA_MOUSE
547	-1.1630039	2.4036503	sp P68254-2 1433T_MOUSE
548	-1.1629868	1.6794106	sp P42669 PURA_MOUSE
549	-1.1629524	1.3050913	sp Q99JT9 MTND_MOUSE
550	-1.1629381	1.6794106	sp Q8BFY9-2 TNPO1_MOUSE
551	-1.1622543	2.044628	sp Q8BVI4 DHPR_MOUSE
552	-1.1620283	1.3050913	sp Q8R059 GALE_MOUSE
553	-1.1615868	1.3050913	sp P61924 COPZ1_MOUSE
554	-1.160984	4.14842	sp Q9Z0N1 IF2G_MOUSE
555	-1.1597481	2.4036503	sp Q9QZD9 EIF3I_MOUSE
556	-1.1596756	2.044628	sp Q62465 VAT1_MOUSE
557	-1.1595402	2.044628	sp Q99JY0 ECHB_MOUSE
558	-1.1595078	1.3050913	sp Q9QYJ0 DNJA2_MOUSE
559	-1.1592178	1.3050913	sp B2RQC6-2 PYR1_MOUSE
560	-1.1588058	3.8039558	sp P62082 RS7_MOUSE
561	-1.1584663	3.109283	sp Q91YT0 NDUV1_MOUSE
562	-1.1580219	2.758175	sp Q9CZU6 CISY_MOUSE
563	-1.1574211	2.758175	sp Q8VEM8 MPCP_MOUSE
564	-1.1571522	2.044628	sp Q9CQ62 DECR_MOUSE
565	-1.1565971	1.6794106	sp Q9Z2U0 PSA7_MOUSE
566	-1.156208	1.3050913	sp Q63932 MP2K2_MOUSE
567	-1.1557693	2.4036503	sp P07356 ANXA2_MOUSE
568	-1.1547451	4.4913836	sp Q9CZX8 RS19_MOUSE
569	-1.1545506	1.3050913	sp Q9Z0L8-2 GGH_MOUSE
570	-1.154439	8.87523	sp Q9D964 GATM_MOUSE
571	-1.1544151	1.3050913	sp Q9CPQ8 ATP5L_MOUSE
572	-1.1543827	2.4036503	sp Q9D2G2-2 ODO2_MOUSE
573	-1.1542625	2.758175	sp Q9CPV4 GLOD4_MOUSE
574	-1.1527195	2.044628	sp P49312-2 ROA1_MOUSE
575	-1.1526899	1.6794106	sp Q9WUM4 COR1C_MOUSE
576	-1.1514435	1.3050913	sp Q9D358-2 PPAC_MOUSE
577	-1.1514091	2.044628	sp P13707 GPDA_MOUSE

SUPPLEMENTARY DATA

578	-1.1512756	1.6794106	sp Q99L13 3HIDH_MOUSE
579	-1.1506748	1.3050913	sp P54822 PUR8_MOUSE
580	-1.1505117	2.044628	sp Q91V41 RAB14_MOUSE
581	-1.1504059	4.14842	sp O70251 EF1B_MOUSE
582	-1.1497898	4.4913836	sp P62270 RS18_MOUSE
583	-1.1495504	3.109283	sp Q9WUK2-2 IF4H_MOUSE
584	-1.1489086	1.6794106	sp Q9EQP2 EHD4_MOUSE
585	-1.1481438	2.758175	sp Q8BTZ7 GMPPB_MOUSE
586	-1.1479921	1.3050913	sp P12367 KAP2_MOUSE
587	-1.1472387	1.3050913	sp Q9Z1Q5 CLIC1_MOUSE
588	-1.146718	1.6794106	sp P35276 RAB3D_MOUSE
589	-1.146718	4.14842	sp Q9R0P3 ESTD_MOUSE
590	-1.1466923	1.6794106	sp Q62186 SSRD_MOUSE
591	-1.145338	2.758175	sp Q8CG76 ARK72_MOUSE
592	-1.1451931	7.5362897	sp P10126 EF1A1_MOUSE
593	-1.1447754	1.6794106	sp O08605 MKNK1_MOUSE
594	-1.1446972	1.3050913	sp Q8K3J1 NDUS8_MOUSE
595	-1.1445351	6.527502	sp P54071 IDHP_MOUSE
596	-1.1444817	1.6794106	sp Q9DB05 SNAA_MOUSE
597	-1.1439724	5.8520784	sp Q71RI9-2 KAT3_MOUSE
598	-1.1431608	3.4577034	sp O89079 COPE_MOUSE
599	-1.142704	1.6794106	sp Q60759 GCDH_MOUSE
600	-1.1425323	1.6794106	sp P03336 GAG_MLVAV
601	-1.1424065	3.109283	sp P63325 RS10_MOUSE
602	-1.1409607	3.109283	sp P63242 IF5A1_MOUSE
603	-1.1404762	5.8520784	sp P16858 G3P_MOUSE
604	-1.1403866	13.879043	sp P11679 K2C8_MOUSE
605	-1.1400976	1.3050913	sp Q3THK7 GUAA_MOUSE
606	-1.1398773	4.14842	sp P62259 1433E_MOUSE
607	-1.1394539	3.8039558	sp P55264-2 ADK_MOUSE
608	-1.1384506	2.758175	sp Q07417 ACADS_MOUSE
609	-1.1378603	3.4577034	sp P97461 RS5_MOUSE
610	-1.1370087	1.3050913	sp Q9JHR7 IDE_MOUSE
611	-1.1349316	1.3050913	sp Q8VCW8 ACSF2_MOUSE
612	-1.1329117	1.6794106	sp O55022 PGRC1_MOUSE
613	-1.1324539	9.875973	sp Q62167 DDX3X_MOUSE
614	-1.1315155	2.4036503	sp P60335 PCBP1_MOUSE
615	-1.1312065	1.6794106	sp P16045 LEG1_MOUSE
616	-1.1303768	5.5132895	sp P05064 ALDOA_MOUSE
617	-1.1301899	2.758175	sp P52825 CPT2_MOUSE
618	-1.1299324	2.044628	sp Q8QZY1 EIF3L_MOUSE
619	-1.1297264	1.6794106	sp Q9DBZ5 EIF3K_MOUSE
620	-1.1294155	2.044628	sp P10518 HEM2_MOUSE
621	-1.1289959	4.14842	sp Q9Z2W0 DNPEP_MOUSE
622	-1.1285181	1.6794106	sp P62880 GBB2_MOUSE

SUPPLEMENTARY DATA

623	-1.1283092	2.4036503	sp Q8BMF4 ODP2_MOUSE
624	-1.1281643	1.6794106	sp P99027 RLA2_MOUSE
625	-1.1278677	5.5132895	sp Q99K85 SERC_MOUSE
626	-1.1272163	1.6794106	sp Q64471 GSTT1_MOUSE
627	-1.1268978	1.3050913	sp Q61510 TRI25_MOUSE
628	-1.1265507	2.4036503	sp Q9D172 GAL3A_MOUSE
629	-1.1265383	3.109283	sp Q04447 KCRB_MOUSE
630	-1.1263762	3.109283	sp P80315 TCPD_MOUSE
631	-1.126358	3.109283	sp Q35969-2 GAMT_MOUSE
632	-1.1262836	2.044628	sp Q9R1P4 PSA1_MOUSE
633	-1.1249123	8.20651	sp P50247 SAHH_MOUSE
634	-1.1245079	4.4913836	sp P24369 PIIB_MOUSE
635	-1.1243048	3.109283	sp Q9R0P5 DEST_MOUSE
636	-1.1225996	1.6794106	sp Q62393-2 TPD52_MOUSE
637	-1.1221457	1.6794106	sp Q8BP48 MAP11_MOUSE
638	-1.1221275	3.4577034	sp O08709 PRDX6_MOUSE
639	-1.1220894	2.044628	sp Q9JJ18 RL38_MOUSE
640	-1.1220264	3.8039558	sp Q35855 BCAT2_MOUSE
641	-1.1214447	1.6794106	sp Q99KC8 VMA5A_MOUSE
642	-1.120224	4.4913836	sp Q9D051 ODPB_MOUSE
643	-1.1194305	1.3050913	sp P47791-2 GSHR_MOUSE
644	-1.1193085	1.6794106	sp Q9JLZ3 AUHM_MOUSE
645	-1.1181297	1.3050913	sp Q8QZR5 ALAT1_MOUSE
646	-1.1180878	1.3050913	sp Q9DCH4 EIF3F_MOUSE
647	-1.1177883	2.044628	sp P49722 PSA2_MOUSE
648	-1.1174593	2.044628	sp Q9CQ65 MTAP_MOUSE
649	-1.1165123	1.3050913	sp Q9QUI0 RHOA_MOUSE
650	-1.1159191	2.758175	sp Q9WTP6-2 KAD2_MOUSE
651	-1.115036	2.758175	sp Q9DCS2 MTL26_MOUSE
652	-1.1111603	2.044628	sp Q9DCN2-2 NB5R3_MOUSE
653	-1.110589	1.3050913	sp Q91V76 CK054_MOUSE
654	-1.1104412	2.044628	sp Q9Z0S1 BPNT1_MOUSE
655	-1.1097298	2.044628	sp P57759 ERP29_MOUSE
656	-1.1096573	1.6794106	sp Q8CHP8 PGP_MOUSE
657	-1.1084394	1.6794106	sp Q9CPP6 NDUA5_MOUSE
658	-1.1083565	2.044628	sp Q6P3A8-2 ODBB_MOUSE
659	-1.1078281	2.4036503	sp P23591 FCL_MOUSE
660	-1.1077929	2.044628	sp Q99JW2 ACY1_MOUSE
661	-1.1074533	2.4036503	sp Q8CDN6 TXNL1_MOUSE
662	-1.1061687	1.3050913	sp P62334 PRS10_MOUSE
663	-1.1043339	2.4036503	sp O08756 HCD2_MOUSE
664	-1.1033707	5.8520784	sp Q99LC5 ETFA_MOUSE
665	-1.1031055	1.6794106	sp P70296 PEBP1_MOUSE
666	-1.1027069	1.6794106	sp Q9CQC2 COL_MOUSE
667	-1.1026287	1.3050913	sp Q9CYN2 SPCS2_MOUSE

SUPPLEMENTARY DATA

668	-1.1007032	1.3050913	sp P61082 UBC12_MOUSE
669	-1.0978203	5.5132895	sp P48758 CBR1_MOUSE
670	-1.0973434	2.758175	sp Q99J77 SIAS_MOUSE
671	-1.0968475	3.8039558	sp P62301 RS13_MOUSE
672	-1.0965519	3.8039558	sp Q64674 SPEE_MOUSE
673	-1.0955677	1.6794106	sp Q05816 FABP5_MOUSE
674	-1.0951557	2.758175	sp Q9D7B6 ACAD8_MOUSE
675	-1.09441	2.758175	sp P35700 PRDX1_MOUSE
676	-1.0940285	2.044628	sp P08228 SODC_MOUSE
677	-1.0935612	1.3050913	sp Q99JX4 EIF3M_MOUSE
678	-1.0930023	1.6794106	sp Q922H4 GMPPA_MOUSE
679	-1.0929871	1.3050913	sp Q60668-2 HNRPD_MOUSE
680	-1.0912857	3.8039558	sp P63276 RS17_MOUSE
681	-1.0904655	3.109283	sp P28474 ADHX_MOUSE
682	-1.0902042	1.6794106	sp P62835 RAP1A_MOUSE
683	-1.0896196	1.3050913	sp P62849-2 RS24_MOUSE
684	-1.0894089	1.3050913	sp P56391 CX6B1_MOUSE
685	-1.0887585	1.3050913	sp Q9R062 GLYG_MOUSE
686	-1.0879536	2.044628	sp Q9JK38 GNA1_MOUSE
687	-1.0871983	4.4913836	sp P18760 COF1_MOUSE
688	-1.086916	1.6794106	sp O70400 PDLI1_MOUSE
689	-1.0858173	2.758175	sp Q61171 PRDX2_MOUSE
690	-1.0835028	1.3050913	sp Q61490 CD166_MOUSE
691	-1.0825024	1.3050913	sp O08691 ARGI2_MOUSE
692	-1.0819969	1.3050913	sp P52760 RIDA_MOUSE
693	-1.0818291	3.109283	sp Q60930 VDAC2_MOUSE
694	-1.0809746	4.8330717	sp Q9DCW4 ETFB_MOUSE
695	-1.0768194	2.044628	sp Q8VC28 AK1CD_MOUSE
696	-1.0767021	1.3050913	sp P18242 CATD_MOUSE
697	-1.0762653	8.87523	sp P08249 MDHM_MOUSE
698	-1.0760937	7.5362897	sp P68040 RACK1_MOUSE
699	-1.0760603	2.044628	sp P53994 RAB2A_MOUSE
700	-1.075716	2.044628	sp Q9CPU0 LGUL_MOUSE
701	-1.0754766	1.6794106	sp Q91ZJ5-2 UGPA_MOUSE
702	-1.0735226	1.6794106	sp P62827 RAN_MOUSE
703	-1.0692596	1.6794106	sp Q61990-2 PCBP2_MOUSE
704	-1.0691986	1.3050913	sp Q9Z2U1 PSA5_MOUSE
705	-1.0689297	3.109283	sp Q9D819 IPYR_MOUSE
706	-1.0689259	2.044628	sp O35215 DOPD_MOUSE
707	-1.0682068	1.3050913	sp P30115 GSTA3_MOUSE
708	-1.0657978	2.044628	sp Q3SYP2 CTRC_MOUSE
709	-1.0642433	2.758175	sp P52196 THTR_MOUSE
710	-1.0620289	2.758175	sp Q99L20 GSTT3_MOUSE
711	-1.0597897	2.044628	sp P62196 PRS8_MOUSE
712	-1.0595093	4.8330717	sp Q8BH95 ECHM_MOUSE

SUPPLEMENTARY DATA

713	-1.0560722	2.758175	sp Q9WVA4 TAGL2_MOUSE
714	-1.0556202	4.14842	sp P63323 RS12_MOUSE
715	-1.0548668	4.8330717	sp P10649 GSTM1_MOUSE
716	-1.0540771	10.209015	sp P62908 RS3_MOUSE
717	-1.0534363	2.758175	sp P68369 TBA1A_MOUSE
718	-1.0515995	1.6794106	sp Q91V64 ISOC1_MOUSE
719	-1.0514774	2.044628	sp Q8R164 BPHL_MOUSE
720	-1.0509663	2.4036503	sp P61458 PHS_MOUSE
721	-1.0502453	1.6794106	sp P85094 ISC2A_MOUSE
722	-1.0480881	1.6794106	sp P59999 ARPC4_MOUSE
723	-1.0436516	1.3050913	sp Q9CQE8 RTRAF_MOUSE
724	-1.0411072	1.3050913	sp Q9D6J6 NDUV2_MOUSE
725	-1.040082	3.109283	sp Q9DBP5 KCY_MOUSE
726	-1.0398941	1.3050913	sp P62852 RS25_MOUSE
727	-1.038868	2.758175	sp P42125 ECI1_MOUSE
728	-1.0380573	3.109283	sp P53026 RL10A_MOUSE
729	-1.0365582	3.8039558	sp P48036 ANXA5_MOUSE
730	-1.0364647	1.3050913	sp O54984 ASNA_MOUSE
731	-1.0322914	1.3050913	sp Q91VR2 ATPG_MOUSE
732	-1.0300941	1.3050913	sp P56959 FUS_MOUSE
733	-1.0268536	1.3050913	sp P38060 HMGCL_MOUSE
734	-1.0248852	4.4913836	sp P17742 PPIA_MOUSE
735	-1.024353	2.044628	sp O55142 RL35A_MOUSE
736	-1.0221062	1.3634305	sp P30416 FKBP4_MOUSE
737	-1.0180988	1.6794106	sp P63028 TCTP_MOUSE
738	-1.0178442	3.109283	sp P62962 PROF1_MOUSE
739	-1.0130997	2.4036503	sp Q9CXW4 RL11_MOUSE
740	-1.0070839	4.4913836	sp O88569-2 ROA2_MOUSE
741	-1.0063515	1.6794106	sp P62858 RS28_MOUSE
742	-1.0013771	5.17366	sp P19157 GSTP1_MOUSE
743	-0.9943714	2.758175	sp P99029-2 PRDX5_MOUSE
744	-0.9920082	3.4577034	sp Q9QXF8 GNMT_MOUSE
745	-0.9902792	1.3050913	sp O35658 C1QBP_MOUSE
746	-0.98579407	3.109283	sp P14131 RS16_MOUSE
747	-0.9848137	1.3050913	sp Q07076 ANXA7_MOUSE
748	-0.98314095	2.758175	sp P62889 RL30_MOUSE
749	-0.97930527	2.044628	sp Q9CQM5 TXD17_MOUSE
750	-0.9738865	3.8039558	sp P15626 GSTM2_MOUSE
751	-0.96040344	1.3050913	sp P00329 ADH1_MOUSE
752	-0.95006275	1.6794106	sp Q91X72 HEMO_MOUSE
753	-0.94696236	1.3050913	sp Q9WVL0 MAAI_MOUSE
754	-0.9423332	1.6794106	sp P02535-2 K1C10_MOUSE
755	-0.9328289	1.3050913	sp Q9R1P1 PSB3_MOUSE
756	-0.9205513	1.6794106	sp Q01768 NDKB_MOUSE
757	-0.918993	1.3050913	sp P16125 LDHB_MOUSE

SUPPLEMENTARY DATA

758	-0.91344833	3.109283	sp P01942 HBA_MOUSE
759	-0.8999491	1.3050913	sp Q64010-2 CRK_MOUSE
760	-0.8672409	1.6794106	sp P04117 FABP4_MOUSE
761	-0.85577774	1.3050913	sp Q9JKB1 UCHL3_MOUSE
762	-0.80261326	1.3050913	sp P15532 NDKA_MOUSE
763	-0.76784515	2.4036503	sp P02088 HBB1_MOUSE
764	-0.6644497	1.3050913	sp P02798 MT2_MOUSE
765	-0.57154083	1.3050913	sp P02802 MT1_MOUSE

Supplementary Table 2. Citrullinated proteins in pancreas of BB-Cl-amidine treated versus DMSO treated NOD mice not significantly altered by the treatment

** P≥0.05 (Log₁₀p-value = 1.122)

	Log ₂ Ratio	Log ₁₀ p-value (**)	Accession
1	-1.7894802	0.49851555	sp P23953 EST1C_MOUSE
2	-1.6683903	0.91601306	sp O08786 CCKAR_MOUSE
3	-1.6681643	0.49851555	sp P53996 CNBP_MOUSE
4	-1.6120167	0.91601306	sp P83882 RL36A_MOUSE
5	-1.580823	0.91601306	sp Q9JKB3-2 YBOX3_MOUSE
6	-1.5767965	0.49851555	sp P48774 GSTM5_MOUSE
7	-1.5470638	0.49851555	sp O35643 AP1B1_MOUSE
8	-1.5351009	0.91601306	sp Q8CHW4 EI2BE_MOUSE
9	-1.5293589	0.91601306	sp Q9CQJ6 DENR_MOUSE
10	-1.5259628	0.91601306	sp P70441 NHRF1_MOUSE
11	-1.519249	0.91601306	sp P63328-2 PP2BA_MOUSE
12	-1.4998703	0.91601306	sp Q6GQT9 NOMO1_MOUSE
13	-1.4967995	0.91601306	sp Q99LE6 ABCF2_MOUSE
14	-1.4886131	0.91601306	sp O54692 ZW10_MOUSE
15	-1.4832001	0.91601306	sp Q9ERK4 XPO2_MOUSE
16	-1.4829292	0.91601306	sp Q791V5 MTCH2_MOUSE
17	-1.481122	0.49851555	sp Q8BFZ3 ACTBL_MOUSE
18	-1.4794674	0.91601306	sp Q9D281 NXP20_MOUSE
19	-1.4741497	0.91601306	sp Q11136 PEPD_MOUSE
20	-1.4740906	0.91601306	sp Q60598 SRC8_MOUSE
21	-1.472992	0.91601306	sp Q91XE8 TM205_MOUSE
22	-1.4608564	0.91601306	sp P14685 PSMD3_MOUSE
23	-1.4468403	0.91601306	sp A2ALW5 DJC25_MOUSE
24	-1.4434948	0.91601306	sp Q6PB93-2 GALT2_MOUSE
25	-1.4420519	0.49851555	sp P48722-2 HS74L_MOUSE
26	-1.4326811	0.91601306	sp O88543 CSN3_MOUSE

SUPPLEMENTARY DATA

27	-1.4253664	0.91601306	sp Q9QXX4 CMC2_MOUSE
28	-1.4231462	0.91601306	sp Q9Z1W9 STK39_MOUSE
29	-1.4202251	0.49851555	sp Q9D2M8-2 UB2V2_MOUSE
30	-1.414794	0.91601306	sp P35235-2 PTN11_MOUSE
31	-1.4048777	0.91601306	sp P17918 PCNA_MOUSE
32	-1.4022331	0.49851555	sp P84084 ARF5_MOUSE
33	-1.3996744	0.91601306	sp Q62448-2 IF4G2_MOUSE
34	-1.3991852	0.49851555	sp P61205 ARF3_MOUSE
35	-1.3975315	0.49851555	sp Q8R5C5 ACTY_MOUSE
36	-1.3934231	0.91601306	sp P08752 GNAI2_MOUSE
37	-1.3886375	0.49851555	sp P63094-2 GNAS2_MOUSE
38	-1.3862152	0.91601306	sp Q9D379 HYEP_MOUSE
39	-1.3848953	0.91601306	sp Q8BMP6 GCP60_MOUSE
40	-1.3750544	0.91601306	sp P70195 PSB7_MOUSE
41	-1.3742962	0.91601306	sp P47964 RL36_MOUSE
42	-1.3739471	0.91601306	sp Q9CR62 M2OM_MOUSE
43	-1.3707561	0.49851555	sp P21107 TPM3_MOUSE
44	-1.368391	0.91601306	sp P32067 LA_MOUSE
45	-1.3655033	0.91601306	sp Q9R099 TBL2_MOUSE
46	-1.3651772	0.91601306	sp Q8BW75 AOFB_MOUSE
47	-1.3639383	0.91601306	sp P36536 SAR1A_MOUSE
48	-1.3636379	0.91601306	sp Q9DC51 GNAI3_MOUSE
49	-1.3608398	0.91601306	sp Q62446 FKBP3_MOUSE
50	-1.3594933	0.91601306	sp Q9D8V0-3 HM13_MOUSE
51	-1.3579845	0.91601306	sp Q9D2R4 TMD11_MOUSE
52	-1.3569355	0.91601306	sp Q8R1F6-2 HID1_MOUSE
53	-1.3559589	0.91601306	sp O08788-2 DCTN1_MOUSE
54	-1.3549404	0.91601306	sp Q9CR68 UCRI_MOUSE
55	-1.3546295	0.91601306	sp Q8BVE3 VATH_MOUSE
56	-1.3509512	0.91601306	sp Q9CWK8 SNX2_MOUSE
57	-1.3440714	0.91601306	sp Q9D5V6 SYAP1_MOUSE
58	-1.3432989	0.49851555	sp P53996-2 CNBP_MOUSE
59	-1.3417873	0.91601306	sp Q9JIZ0 CMLO1_MOUSE
60	-1.3416767	0.49851555	sp P26041 MOES_MOUSE
61	-1.3410187	0.91601306	sp Q8BX90 FND3A_MOUSE
62	-1.3388853	0.91601306	sp P47226-2 TES_MOUSE
63	-1.3375111	0.91601306	sp Q8BLF1 NCEH1_MOUSE
64	-1.3332996	0.91601306	sp Q9CQF9 PCYOX_MOUSE
65	-1.3274622	0.91601306	sp Q920E5 FPPS_MOUSE
66	-1.3102036	0.91601306	sp P19536 COX5B_MOUSE
67	-1.3058357	0.91601306	sp P60670-2 NPL4_MOUSE
68	-1.3058205	0.91601306	sp P10853 H2B1F_MOUSE
69	-1.3057585	0.91601306	sp Q99K28-2 ARFG2_MOUSE
70	-1.3037853	0.91601306	sp O88544 CSN4_MOUSE
71	-1.3005562	0.91601306	sp Q9JII5-2 DAZP1_MOUSE

SUPPLEMENTARY DATA

72	-1.2993164	0.91601306	sp Q6ZPJ3 UBE2O_MOUSE
73	-1.2982349	0.91601306	sp O70435 PSA3_MOUSE
74	-1.2982273	0.91601306	sp P28656 NP1L1_MOUSE
75	-1.2957315	0.91601306	sp Q8K4F5 ABHDB_MOUSE
76	-1.2951851	0.49851555	sp Q8BG05-2 ROA3_MOUSE
77	-1.2950277	0.91601306	sp P61079 UB2D3_MOUSE
78	-1.2935734	0.49851555	sp Q9DCG6 PBLD1_MOUSE
79	-1.2907848	0.91601306	sp P97328 KHK_MOUSE
80	-1.2887697	0.91601306	sp Q8BWG8-2 ARRB1_MOUSE
81	-1.2876129	0.91601306	sp Q60872 IF1A_MOUSE
82	-1.2811394	0.49851555	sp Q6IFX2 K1C42_MOUSE
83	-1.2802505	0.91601306	sp Q9CXT8 MPPB_MOUSE
84	-1.2752724	0.91601306	sp Q8VDM6-2 HNRL1_MOUSE
85	-1.2748566	0.91601306	sp Q4VAA2-2 CDV3_MOUSE
86	-1.2747765	0.91601306	sp Q9WVP1-2 AP1M2_MOUSE
87	-1.2743025	0.91601306	sp P62737 ACTA_MOUSE
88	-1.273632	0.91601306	sp P61161 ARP2_MOUSE
89	-1.272152	0.91601306	sp Q922Q4 P5CR2_MOUSE
90	-1.2720823	0.91601306	sp Q8R1Q8 DC1L1_MOUSE
91	-1.2703238	0.91601306	sp Q9JJK5 HERP1_MOUSE
92	-1.2654572	0.91601306	sp Q9CZY3 UB2V1_MOUSE
93	-1.2645807	0.91601306	sp Q6ZPE2-2 MTMR5_MOUSE
94	-1.2635231	0.91601306	sp Q60710-2 SAMH1_MOUSE
95	-1.262888	0.91601306	sp P50431 GLYC_MOUSE
96	-1.2597857	0.91601306	sp Q3TZM9-2 ALG11_MOUSE
97	-1.2529278	0.91601306	sp Q9CXN7 PBLD2_MOUSE
98	-1.2518997	0.49851555	sp P62137 PP1A_MOUSE
99	-1.2499361	0.91601306	sp Q8VI75 IPO4_MOUSE
100	-1.2495613	0.91601306	sp Q99LB2 DHRS4_MOUSE
101	-1.2485752	0.91601306	sp P22907-2 HEM3_MOUSE
102	-1.2483006	0.91601306	sp P56395 CYB5_MOUSE
103	-1.2480507	0.49851555	sp P21107-2 TPM3_MOUSE
104	-1.2457695	0.91601306	sp Q9DCJ5 NDUA8_MOUSE
105	-1.243103	0.91601306	sp Q9D0F9 PGM1_MOUSE
106	-1.2426987	0.91601306	sp Q91X52 DCXR_MOUSE
107	-1.2419586	0.49851555	sp Q8R180 ERO1A_MOUSE
108	-1.238163	0.91601306	sp P27046 MA2A1_MOUSE
109	-1.2355175	0.91601306	sp P00405 COX2_MOUSE
110	-1.2326698	0.91601306	sp Q922B1 MACD1_MOUSE
111	-1.2316475	0.49851555	sp Q9D6F9 TBB4A_MOUSE
112	-1.2304764	0.91601306	sp Q61081 CDC37_MOUSE
113	-1.2287579	0.91601306	sp P58281-2 OPA1_MOUSE
114	-1.2281294	0.91601306	sp Q3B7Z2 OSBP1_MOUSE
115	-1.2280521	0.91601306	sp Q8R1F1 NIBL1_MOUSE
116	-1.2269764	0.91601306	sp Q6Y7W8 GGYF2_MOUSE

SUPPLEMENTARY DATA

117	-1.2266569	0.91601306	sp Q9Z0U1 ZO2_MOUSE
118	-1.2253742	0.91601306	sp Q6PA06-2 ATLA2_MOUSE
119	-1.2230349	0.91601306	sp Q3UMF0-2 COBL1_MOUSE
120	-1.2195797	0.91601306	sp P49442 INPP_MOUSE
121	-1.219389	0.91601306	sp Q9DCF9-2 SSRG_MOUSE
122	-1.2190657	0.49851555	sp P61164 ACTZ_MOUSE
123	-1.2168465	0.91601306	sp Q9DC16 ERGI1_MOUSE
124	-1.2116909	0.91601306	sp O55028 BCKD_MOUSE
125	-1.2110748	0.49851555	sp P53810 PIPNA_MOUSE
126	-1.2081051	0.91601306	sp P53811 PIPNB_MOUSE
127	-1.2073975	0.91601306	sp Q91WK2 EIF3H_MOUSE
128	-1.2065105	0.49851555	sp Q80SW1 SAHH2_MOUSE
129	-1.2050934	0.91601306	sp Q9WUL7 ARL3_MOUSE
130	-1.2028103	0.91601306	sp Q8C181-2 MBNL2_MOUSE
131	-1.2007256	0.91601306	sp Q9D1Q6 ERP44_MOUSE
132	-1.2003794	0.91601306	sp P47857-2 PFKAM_MOUSE
133	-1.1973591	0.91601306	sp Q8CIM3 D2HDH_MOUSE
134	-1.1967716	0.91601306	sp P45878 FKBP2_MOUSE
135	-1.1963501	0.91601306	sp Q8VCM8 NCLN_MOUSE
136	-1.1960773	0.91601306	sp Q9DCS9 NDUBA_MOUSE
137	-1.1942635	0.91601306	sp Q9D8C2 TSN13_MOUSE
138	-1.1915073	0.91601306	sp Q05144 RAC2_MOUSE
139	-1.1868706	0.91601306	sp Q6ZWQ7 SPCS3_MOUSE
140	-1.184555	0.91601306	sp P62983 RS27A_MOUSE
141	-1.1835709	0.91601306	sp Q64310 SURF4_MOUSE
142	-1.1805344	0.91601306	sp Q9R112 SQOR_MOUSE
143	-1.1790028	0.91601306	sp Q9DCT2 NDUS3_MOUSE
144	-1.1763353	0.49851555	sp P70333 HNRH2_MOUSE
145	-1.1745644	0.91601306	sp Q9CQZ5 NDUA6_MOUSE
146	-1.171978	0.91601306	sp P99026 PSB4_MOUSE
147	-1.1718845	0.91601306	sp Q9DBH5 LMAN2_MOUSE
148	-1.171689	0.49851555	sp Q61133 GSTT2_MOUSE
149	-1.1700516	0.91601306	sp P68510 1433F_MOUSE
150	-1.1697206	0.91601306	sp Q9CZ44-3 NSF1C_MOUSE
151	-1.1675911	0.91601306	sp Q9JM14 NT5C_MOUSE
152	-1.1669292	0.91601306	sp P24547 IMDH2_MOUSE
153	-1.165226	0.91601306	sp Q921X9 PDIA5_MOUSE
154	-1.16292	0.91601306	sp Q80UM3 NAA15_MOUSE
155	-1.162157	0.91601306	sp Q8JZU2 TXTP_MOUSE
156	-1.1541367	0.91601306	sp Q9CYZ2 TPD54_MOUSE
157	-1.151825	0.91601306	sp Q924M7 MPI_MOUSE
158	-1.1503906	0.49851555	sp O35737 HNRH1_MOUSE
159	-1.1486397	0.91601306	sp Q5YD48-2 A1CF_MOUSE
160	-1.1478958	0.91601306	sp P60824 CIRBP_MOUSE
161	-1.1461182	0.49851555	sp Q3UV17 K22O_MOUSE

SUPPLEMENTARY DATA

162	-1.1445503	0.91601306	sp Q9D892 ITPA_MOUSE
163	-1.1439457	0.91601306	sp Q3TJZ6 FA98A_MOUSE
164	-1.1354609	0.91601306	sp Q9D0K2 SCOT1_MOUSE
165	-1.1338282	0.91601306	sp P62141 PP1B_MOUSE
166	-1.1316261	0.91601306	sp Q99J39-2 DCMC_MOUSE
167	-1.1280613	0.91601306	sp P32233 DRG1_MOUSE
168	-1.1258507	0.91601306	sp Q8R0X7 SGPL1_MOUSE
169	-1.1228218	0.91601306	sp P62274 RS29_MOUSE
170	-1.1226807	0.91601306	sp Q9JM76 ARPC3_MOUSE
171	-1.1201134	0.91601306	sp Q9R1P0 PSA4_MOUSE
172	-1.1139317	0.91601306	sp P47754 CAZA2_MOUSE
173	-1.1125727	0.91601306	sp P28658 ATX10_MOUSE
174	-1.1081991	0.91601306	sp Q01405 SC23A_MOUSE
175	-1.1076202	0.91601306	sp P48193-2 41_MOUSE
176	-1.1073151	0.91601306	sp Q9DC69 NDUA9_MOUSE
177	-1.1060591	0.49851555	sp Q78ZA7 NP1L4_MOUSE
178	-1.0976181	0.91601306	sp P61750 ARF4_MOUSE
179	-1.0975285	0.91601306	sp Q9CQC9 SAR1B_MOUSE
180	-1.0948553	0.91601306	sp Q9Z2X1-2 HNRPF_MOUSE
181	-1.0947847	0.91601306	sp Q9JHW2 NIT2_MOUSE
182	-1.0917206	0.91601306	sp Q9QYJ3 DNJB1_MOUSE
183	-1.0882874	0.91601306	sp P51174 ACADL_MOUSE
184	-1.083662	0.91601306	sp Q7TQI3 OTUB1_MOUSE
185	-1.0804901	0.91601306	sp P70695 F16P2_MOUSE
186	-1.0790787	0.91601306	sp Q6ZWU9 RS27_MOUSE
187	-1.0770531	0.91601306	sp Q99LX0 PARK7_MOUSE
188	-1.076642	0.91601306	sp Q8K1E0-2 STX5_MOUSE
189	-1.0762386	0.91601306	sp Q99LC8 EI2BA_MOUSE
190	-1.0753365	0.91601306	sp Q99KR3 LACB2_MOUSE
191	-1.072854	0.91601306	sp Q9D1G1 RAB1B_MOUSE
192	-1.071846	0.91601306	sp P61202-2 CSN2_MOUSE
193	-1.0705929	0.49851555	sp Q9WV02-2 RBMX_MOUSE
194	-1.0703392	0.91601306	sp P29391 FRIL1_MOUSE
195	-1.067997	0.49851555	sp P55258 RAB8A_MOUSE
196	-1.0677948	0.91601306	sp Q8K310 MATR3_MOUSE
197	-1.0526981	0.91601306	sp Q9CR16 PPID_MOUSE
198	-1.047514	0.91601306	sp Q91VF2 HNMT_MOUSE
199	-1.044919	0.91601306	sp Q8VC30 TKFC_MOUSE
200	-1.0421238	0.91601306	sp O88696 CLPP_MOUSE
201	-1.0341625	0.91601306	sp P56380 AP4A_MOUSE
202	-1.0339527	0.91601306	sp P61979-2 HNRPK_MOUSE
203	-1.0335789	0.91601306	sp Q99LB6-2 MAT2B_MOUSE
204	-1.0331154	0.91601306	sp Q9CPT4 MYDGF_MOUSE
205	-1.0248833	0.91601306	sp Q9CQS8 SC61B_MOUSE
206	-1.0220718	0.49851555	sp Q6IFZ6 K2C1B_MOUSE

SUPPLEMENTARY DATA

207	-1.0210285	0.91601306	sp Q9DCT8 CRIP2_MOUSE
208	-1.0209751	0.91601306	sp Q8VCR7 ABHEB_MOUSE
209	-1.0161228	0.91601306	sp P63260 ACTG_MOUSE
210	-1.0129232	0.91601306	sp Q6P8X1 SNX6_MOUSE
211	-1.0076065	0.91601306	sp P61358 RL27_MOUSE
212	-0.9983959	0.91601306	sp P27048 RSMB_MOUSE
213	-0.9941635	0.91601306	sp P07758 A1AT1_MOUSE
214	-0.9922199	0.91601306	sp Q9D7A6 SRP19_MOUSE
215	-0.98636055	0.91601306	sp Q9QXT0 CNPY2_MOUSE
216	-0.98517704	0.91601306	sp Q8BH64 EHD2_MOUSE
217	-0.98493385	0.91601306	sp P97823-2 LYPA1_MOUSE
218	-0.98397064	0.49851555	sp Q9CZ42-3 NNRD_MOUSE
219	-0.9828243	0.91601306	sp Q64133 AOFA_MOUSE
220	-0.98198223	0.91601306	sp Q6ZWR4-2 2ABB_MOUSE
221	-0.9791479	0.49851555	sp Q9DCV7 K2C7_MOUSE
222	-0.9758949	0.49851555	sp Q91VM5 RMXL1_MOUSE
223	-0.9753418	0.91601306	sp Q9R1P3 PSB2_MOUSE
224	-0.96315384	0.91601306	sp P34884 MIF_MOUSE
225	-0.9358063	0.49851555	sp P17426-2 AP2A1_MOUSE
226	-0.9327469	0.91601306	sp Q3UGR5 HDHD2_MOUSE
227	-0.93006516	0.91601306	sp P60710 ACTB_MOUSE
228	-0.923213	0.49851555	sp P17427 AP2A2_MOUSE
229	-0.8823776	0.91601306	sp P23492 PNPH_MOUSE
230	-0.8738289	0.91601306	sp P50543 S10AB_MOUSE
231	-0.872838	0.91601306	sp P20108 PRDX3_MOUSE
232	-0.8728094	0.49851555	sp Q922U2 K2C5_MOUSE
233	-0.8593445	0.91601306	sp Q78XF5 OSTC_MOUSE
234	-0.83586407	0.49851555	sp P61027 RAB10_MOUSE
235	-0.8286371	0.91601306	sp P52480-2 KPYM_MOUSE
236	-0.7517929	0.91601306	sp P00920 CAH2_MOUSE
237	-0.6766529	0.49851555	sp Q8VED5 K2C79_MOUSE
238	-0.1389122	0.39426914	sp P16015 CAH3_MOUSE