CELL BIOLOGY

Functional recovery of islet β cells in human type 2 diabetes: Transcriptome signatures unveil therapeutic approaches

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Remission of type 2 diabetes (T2D) can occur after hypocaloric diet, bariatric surgery, or pharmacological treatments and associates with improved β cell function. Here, we studied islets from nondiabetic ($n = 15$) and T2D ($n = 21$) donors. We examined whether T2D β cell dysfunction can be rescued, charted the underlying molecular mechanisms by RNA sequencing, and mined transcriptomes for drug targets. Glucose responsiveness of T2D β cells improved in 60% of preparations after 3-day culture in euglycemic conditions. This was accompanied by changes in expression of >400 genes involved in functional or inflammatory pathways. Drug repurposing and target identification analyses predicted chemical and genetic hits, including JAK inhibitors, which were validated in a β cell line, human islets, and db/db mice. Therefore, defective β cell glucose responsiveness in T2D can recover, demonstrating β cell functional plasticity. The recovery associates with transcriptomic traits, pointing to targetable defects to induce T2D remission.

INTRODUCTION

Type 2 diabetes (T2D) is generally assumed to be a relentlessly progressive disease due to combined genetic and environmental factors that cause pancreatic β cell dysfunction and damage (*1*–*4*). However, remission of T2D has been observed in variable proportions of patients after therapeutic interventions (*5*–*11*). Following carbohydrate restriction and very low-calorie diets, diabetes remission can rapidly occur. It is associated with increased hepatic insulin sensitivity, partial recovery of first-phase insulin secretion, and reduction of hepatic and pancreatic triacylglycerol content (*7*). Bariatric surgery can promote early remission of T2D before major weight loss (*9*, *10*), with partial restoration of first-phase insulin release in response to intravenous glucose administration observed already by 1 to 4 weeks after surgery (*10*). Increased glucagon-like peptide-1GLP-1(GLP-1) release and potentiated incretin effects may play a role, but the ultimate mechanisms of the recovery of β cell function after bariatric surgery remain to be defined (*11*). Glucose-lowering drugs such as metformin, pioglitazone, and incretin-related drugs improve β cell function in patients with newly diagnosed or established T2D and also in people at high T2D risk (*12*–*14*). However, these effects are usually lost when the medication is withdrawn (*12*–*15*). Of interest, islets isolated from T2D donors and cultured for 24 to 48 hours in the presence of metformin showed improved β cell glucose responsiveness, accompanied by better ultrastructural features, changes in expression of genes associated with cellular redox balance, and reduced induction of the adenosine 5′-triphosphate (ATP)–conducting mitochondrial outer membrane voltage-dependent anion channel-1 (VDAC1) (*16*, *17*). It remains unclear whether and to which extent β cells from T2D subjects can regain function once removed from the in vivo "diabetic" microenvironment, what molecular mechanisms are involved, and whether the defects can be targeted pharmacologically. We presently studied a large number of human islet preparations from nondiabetic (ND; $n = 15$) and T2D donors ($n = 21$). We observed that the defective β cell glucose responsiveness in T2D can be recovered by culture in a "ND" milieu, demonstrating unexpected functional plasticity of T2D β cells. Notably, this functional improvement was associated

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with specific transcriptomic traits, unveiling potential mechanisms to be therapeutically targeted for T2D remission.

RESULTS

Partial functional recovery of T2D β cells in a ND milieu

As shown in Fig. 1, we studied islets from 36 organ donors [tables S1 (15 ND) and S2 (21 T2D)]. T2D donors had higher blood glucose levels and body mass index (BMI) (both $P = 0.01$). After 2 days of recovery from isolation, glucose-stimulated insulin secretion (GSIS) was assessed for ND and T2D islets ("basal" condition). Following an additional 3-day culture at 5.5 or, in selected T2D cases, 11.1 mM glucose, GSIS was again measured ("cultured" condition). As illustrated in Fig. 2A, the insulin stimulation index (ISI; i.e., the ratio of insulin release at 16.7 mM glucose over release at 3.3 mM glucose) was lower for T2D than for ND islets (confirming previous results) (*18*, *19*), both basally and after culture. This was essentially due to increased insulin secretion at 3.3 mM glucose and a trend for lower release at 16.7 mM glucose in T2D (Fig. 2B). In ND islets, the increased release at 3.3 mM glucose after culture was paralleled by grater secretion at 16.7 mM glucose (Fig. 2B), leading to a slight reduction of ISI (Fig. 2A). In T2D islets, we observed that ISI improved in 13 of the 21 cultured preparations ("improvers", ISI fold change relative to basal above 1), while it was unchanged or decreased in the remaining 8 ("nonimprovers," ISI fold change relative to basal 1 or below) (Fig. 2C). In this series of experiments, ISI values in ND islets showed similar trajectory as that of T2D nonimprovers (Fig. 2, C and D). This indicates that the differences between improvers and nonimprovers were mainly due to ameliorated glucose sensitivity in the improvers and not worsened function of the nonimprovers. The increased ISI of improvers after culture (Fig. 2E) was due to stable insulin release at 3.3 mM glucose and higher secretion in response to 16.7 mM glucose (Fig. 2F). In a subgroup of five T2D preparations, islets were cultured in parallel at 5.5 or 11.1 mM glucose. In two cases (20/54 and 21/26), there was a slight deterioration of insulin release after culture at either condition (fig. S1). In three preparations (20/41, 21/40 and 21/91), ISI increased when the islets were maintained at 5.5 mM glucose (basal: 1.5 ± 0.2, cultured: 2.4 ± 0.3, $P < 0.05$). However, after culture at 11.1 mM glucose, ISI did not improve in these three preparations (1.7 ± 0.1; fig. S1).

Although the number of cases was relatively low, there was no statistically significant difference in clinical donor characteristics between improvers and nonimprovers (table S3, A and B). Antidiabetic pharmacological therapy was disparate and not different between the two groups, with 23% of improvers and 25% of nonimprovers being on insulin treatment (alone or in combination). Information on the chronic use of medications beyond their antidiabetic therapy was not available. Islet morphometry studies, performed before islet isolation on the pancreas of 10 improvers and 7 nonimprovers, showed similar islet density and area and superimposable proportions of insulin-, glucagon-, and somatostatin-containing cells in the two groups (fig. S2, A to F). β cell apoptosis in pancreatic sections was comparable and infrequent in improver and nonimprover islets (0.03 ± 0.02 and 0.02 ± 0.02%, respectively). In keeping with these morphometric analyses, transcriptome-based deconvolution analysis of islet cell proportions showed similar percentages of β cells in improver and nonimprover islets, with no significant effect of culture (fig. S2G). Accordingly, when an apoptosis score (calculated as the average expression of apoptosis-related genes from islet transcriptomes) (*20*), was used, we found no difference for nonimprover islets before and after culture, suggesting that increased apoptosis was not a cause of their functional decline (fig. S2H). Cultured improver islets showed a decrease in the score, pointing to activation of protective responses (fig. S2H).

Improver and nonimprover islets have distinct transcriptome signature changes

The transcriptomes of five improver and five nonimprover islet preparations were compared. Several genes tended to be differentially expressed, but without reaching the significance threshold [false discovery rate (FDR) <0.05] either before or after culture (table S4). Although a possible effect of these genes on β cell function cannot be excluded, these modest differences were not further analyzed. However, several differentially expressed genes (DEGs; FDR <0.05) were found by comparing the transcriptomes of cultured improver and nonimprover islets versus their respective basal condition. DEGs were 438 for the improver (233 up-regulated and 205 down-regulated) and 1512 for the nonimprover islets (588 up-regulated and 924 down-regulated) (Fig. 3, A and B, and table S5). The 20 most significantly up- and down-regulated genes for the improver islets following culture at 5.5 mM glucose are shown in table S6. Among the up-regulated DEGs were: *PPARGC1A*, a transcriptional coactivator that regulates genes involved in energy metabolism via cAMP response element binding protein (CREB) and nuclear respiratory factors (*21*); *BAAT*, involved in bile acid metabolism (*22*); *SLC17A4*, *ATP13A4*, and *SMIM24*, implicated in ion transport; *CTSD* and *CTSK*, proteolytic enzymes involved in the activation of hormones and growth factors and modulation of apoptosis (*23*); and a few genes pertaining to inflammatory/immune processes, such as *LYZ*, *CIITA*, *PIGR*, and *CXCL9*. The increase in *ADRA2A* was unexpected, as higher ADRA2A expression in islets from *ADRA2A* risk allele carriers has previously been associated with reduced GSIS (*24*). Among the down-regulated genes, several are involved in inflammatory/immune reactions, including *IL13RA2*, *FGF2*, *CSF3*, and *SOCS3*. As shown in Fig. 3 (C to F) and detailed in table S7, there were 171 DEGs in common between improver and nonimprover islet transcriptomes, of which only 2 oppositely regulated, showing that most of the changes were specific to the two functional trajectories. Among the DEGs in improver islets, 17 harbor T2D GWAS variants (table S8A) (https://www.ebi.ac.uk/gwas/) (*25*). The down-regulation of *C2CD4B* expression in improver islets is consistent in direction of effect with a *C2CD4B* GWAS variant that reduces T2D risk and that colocalizes with an eQTL, decreasing human islet *C2CD4B* expression (*2*). We noted that 46 and 48 DEGs of improver islets are associated with recovery of human β cell function after washout of in vitro lipotoxicity (table S8B) or glucotoxicity (table S8C) (*26*), with the same direction of effect. We performed hypergeometric tests to test whether the observed overlap exceeds random expectation. The overlap was significant (fig. S3), indicating that the shared gene expression changes may reflect meaningful biological associations.

By Gene Set Enrichment Analysis (GSEA), Gene Ontology biological processes with significant (FDR <0.05) enrichment were identified (Fig. 4, A and B, and table S9). Positively enriched processes were 65 and 149 in the improver and nonimprover islets, respectively. Among them, 35 terms were unique in improvers and 119 in nonimprovers (table S9). Negatively enriched terms were 362 in the improver and 318 in the nonimprover preparations, of which 174 unique in improvers and 130 in nonimprovers (table S9). On the whole,

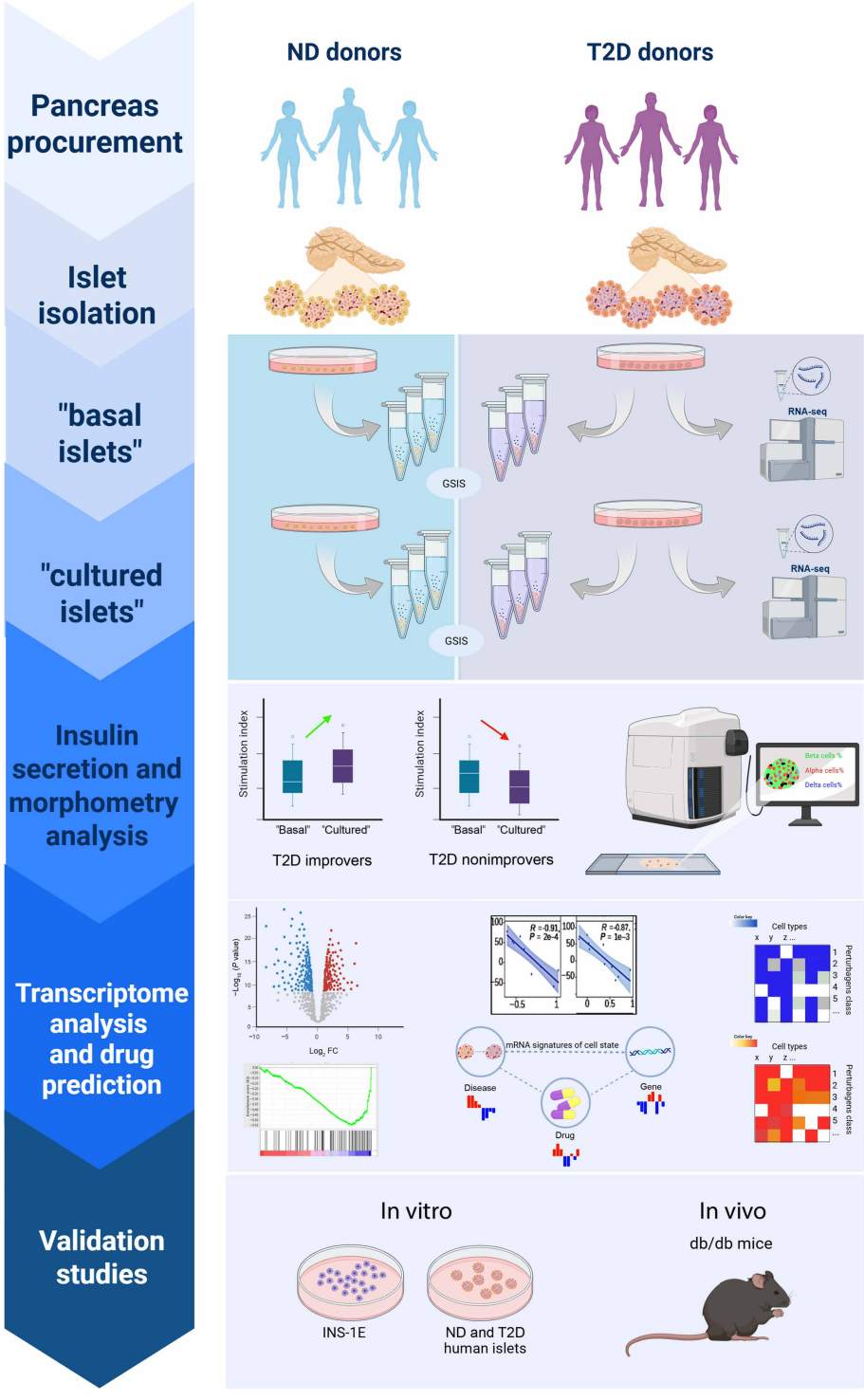

Fig. 1. Experimental design. Islets were isolated from the pancreas of ND and T2D donors and then cultured for 2 days in plain culture medium (containing 5.5 mM glucose) to allow recovery from the isolation stress. Islets were then evaluated in terms of GSIS and (for T2D islets) transcriptome features (basal). Afterward, ND and T2D islets were cultured for ~3 days at 5.5 (nrmoglycemia) and (for a subgroup of T2D islets) also at 11.1 (moderate hyperglycemia") mM glucose, followed by GSIS and transcriptome analysis (T2D islets only). Based on changes in GSIS between cultured versus basal assessments, T2D islets were classified as improvers or nonimprovers. Transcriptomes of improver and nonimprover T2D islets were compared, differential signatures used to identify potential therapeutic targets, and validation studies performed by in vitro and in vivo experiments. Refer to text for further details. Created in BioRender [Suleiman, M. (2025); https://BioRender.com/32jzgsb.

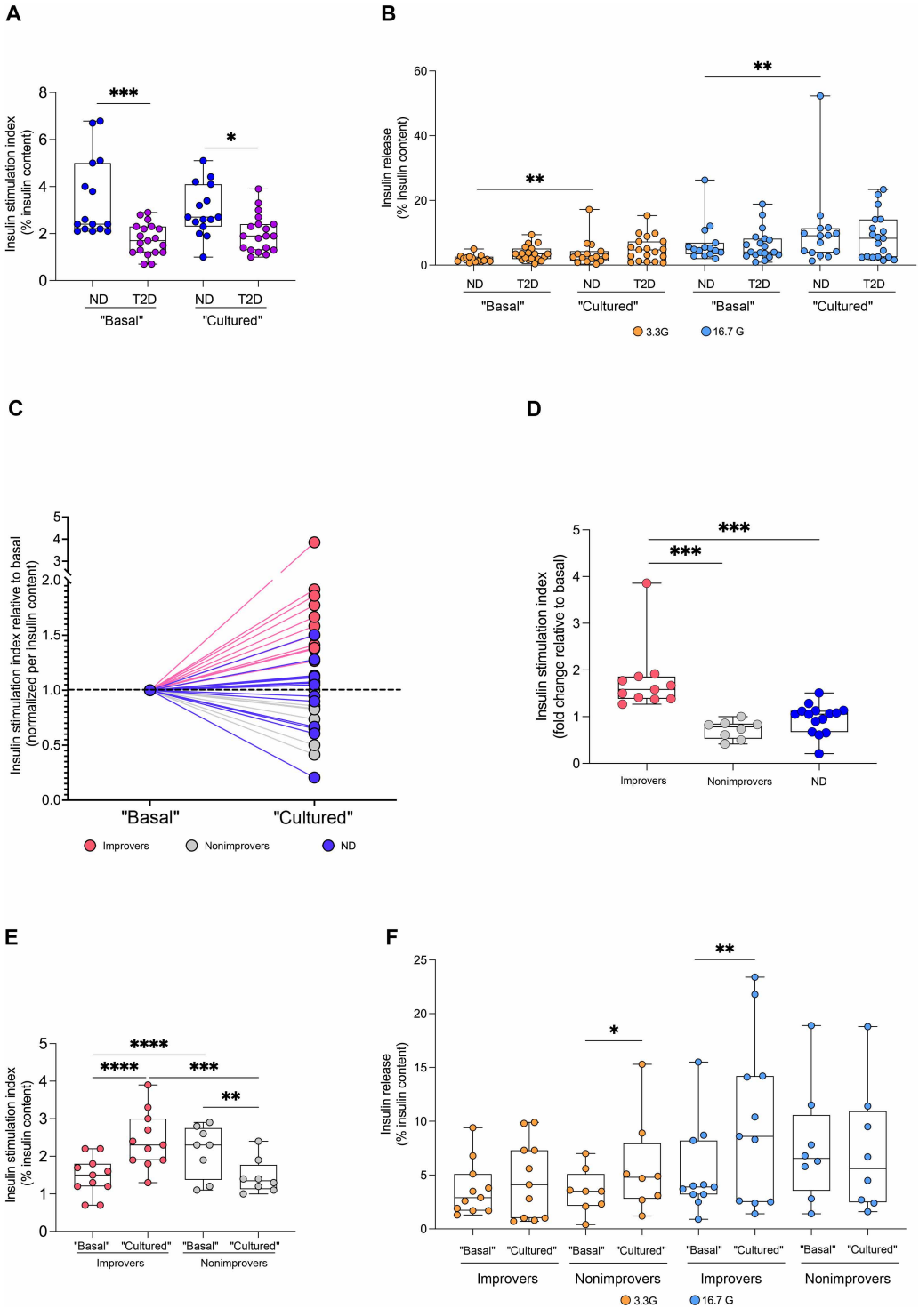

Fig. 2. Insulin secretion in response to glucose from ND and T2D islets. (**A**) ISIs from ND and T2D islets before (basal) and after (cultured) culture at 5.5 mM glucose. (**B**) Insulin release in response to 3.3 and 16.7 mM glucose from ND and T2D islets before (basal) and after (cultured) 3-day culture at 5.5 mM glucose. (**C**) ISI of cultured islets relative to basal values; the ratio was used to define 13 improver (magenta) and 8 nonimprover (gray) T2D islet preparations; ND islets are indicated in blue. (**D**) ISI fold changes relative to basal in the three groups (T2D improvers, T2D nonimprovers, and ND), confirming that improvers had better β cell glucose responsiveness after culture, with no difference between nonimprovers and ND. (**E**) ISI of improver and nonimprover islet preparations (basal and cultured). (**F**) Insulin release in response to 3.3 and 16.7 mM glucose from improver and nonimprover T2D islets before (basal) and after 3-day culture at 5.5 mM glucose. Statistical analysis was performed by two-way analysis of variance (ANOVA). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

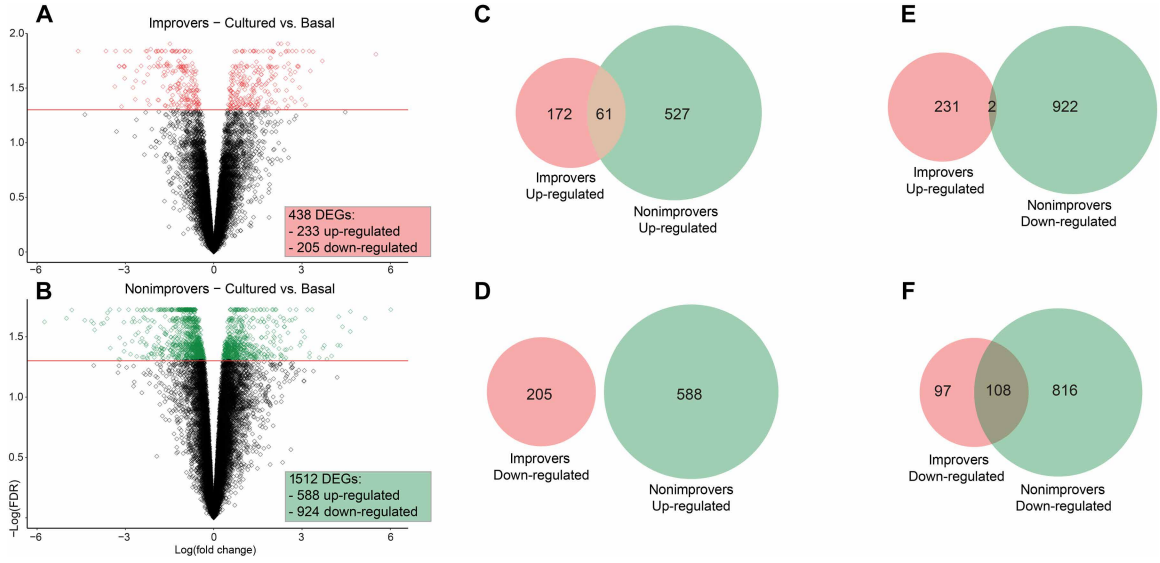

Fig. 3. Transcriptomes of improver and nonimprover T2D islets. (**A** and **B**) DEGs in volcano plots showing the difference of fold change and significance between cultured and basal islets for (A) improvers and (B) nonimprovers. The red line marks the 0.05 FDR significance threshold. (**C** to **F**) Overlapping DEGs between improvers and nonimprovers. The Venn diagrams show the number of DEGs either shared or unique to improvers or nonimprovers. The comparisons include genes regulated in the same (C and F) or opposite directions (D and E).

many terms were associated with carbohydrate/lipid metabolism and inflammatory/immune processes. Of interest, among the positively changed processes uniquely enriched in the improver islet transcriptomes were "positive regulation of peptide hormone secretion," "positive regulation of insulin secretion involved in cellular response to glucose stimulus," and "positive regulation of insulin secretion," with genes relevant to β cell function and identity (*PFKFB2*, *UNC3*, *CRH*, *PDX1*, *TCF7L2*, *NKX6.1*, and *CLTRN*) (table S9).

Correlations between β cell function and gene expression

To identify genes that could play a direct role in β cell recovery, we correlated changes in gene expression and those in insulin secretion (culture versus basal conditions), in combined improvers and nonimprovers. Among the DEGs after culture (table S5), 213 displayed a significant correlation with insulin release, 55 positively and 158 negatively (table S10). Of them, 14 have been previously linked to T2D, either by genetic association (*C2CD4B*, *H19*) (https://www.ebi.ac.uk/gwas/) or by β cell functional recovery after washout of lipo- [*DLGAP1-AS1*, interleukin-24 (IL-24), *PKNOX2*, *EMP1*, *LAMP3*, *GAP43*, and *C2CD4B*] or glucotoxicity (*MMP1*, *LAMC3*, *PAPPA*, *NT5DC2*, *PTPRE*, *GAP43*) (*26*); for 12 genes, the direction of effect was the same as presently observed (down/down). The top significant associations for positively or negatively correlated DEGs are illustrated in Fig. 4 (C and D). Among the genes positively correlated with improved β cell function, SYT8 favors insulin gene transcription and is involved in exocytosis (*27*), the long noncoding RNA MIR210HG potentiates hypoxia-inducible factor–1a (HIF-1a) translation, which in turn up-regulates the expression of glycolysis enzymes (*28*), and RASD2 has been proposed as a putative regulator of insulin secretion (*29*). Of those inversely correlated with GSIS, we found PAPP-A, a metalloproteinase which cleaves insulin-like growth factor binding proteins (*30*); ARHGAP44, an inhibitor of guanosine triphosphatase activity (*31*); CA8, a negative regulator of GLP-1 secretion abundantly expressed in the cerebellum and also in islets (*32*); and SBNO2, involved in inflammatory response (*33*). Overall, the transcriptomic study shows that variations in β cell function correlate with changes in the expression of numerous genes, with plausible roles for several of these in improving insulin secretion in T2D.

Mining transcriptomes identifies potential therapeutic targets

To identify potential therapeutic targets, we compared the top 150 up- and down-regulated genes from the improver islet preparations against human transcriptomes induced by chemical or genetic perturbagens in the Connectivity Map database (Fig. 5) (*34*). The Connectivity Map contains transcriptome changes induced in human cell lines by chemical (libraries of ~3000 compounds) or genetic (silencing or overexpression of ~2000 genes) perturbations. By comparing differential islet transcriptomes with Connectivity Map signatures, chemical or genetic perturbagens may be identified in silico that reproduce gene signatures of β cell functional restoration. We identified 22 positively correlated chemical perturbagens for the up-regulated signatures and 9 for the down-regulated signatures in improver islets (Fig. 5, A and B). These include mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitors for both and, among the more significant ones, Janus kinase (JAK), phosphatidylinositol 3-kinase (PI3K), and histone deacetylase (HDAC) inhibitors for the up-regulated genes. Among the genetic perturbagens of the Connectivity Map, the knockdown of 687 genes was positively correlated with the top 150 up-regulated genes of the improver islets, while 3799 genes were positively correlated with the top 150 down-regulated genes (Fig. 5, C and D, and table S11, A and B). We also submitted DEGs specific to improvers to the iLINCs database (http://ilincs.org) to predict potential drugs and genetic perturbations (*35*). In this platform, 1468 chemicals and knockdown of 632 genes had transcriptomes that correlated positively with the improver islet gene signature (table S11, C and D). In line with the differential transcriptome analyses, JAK inhibitors and other drugs

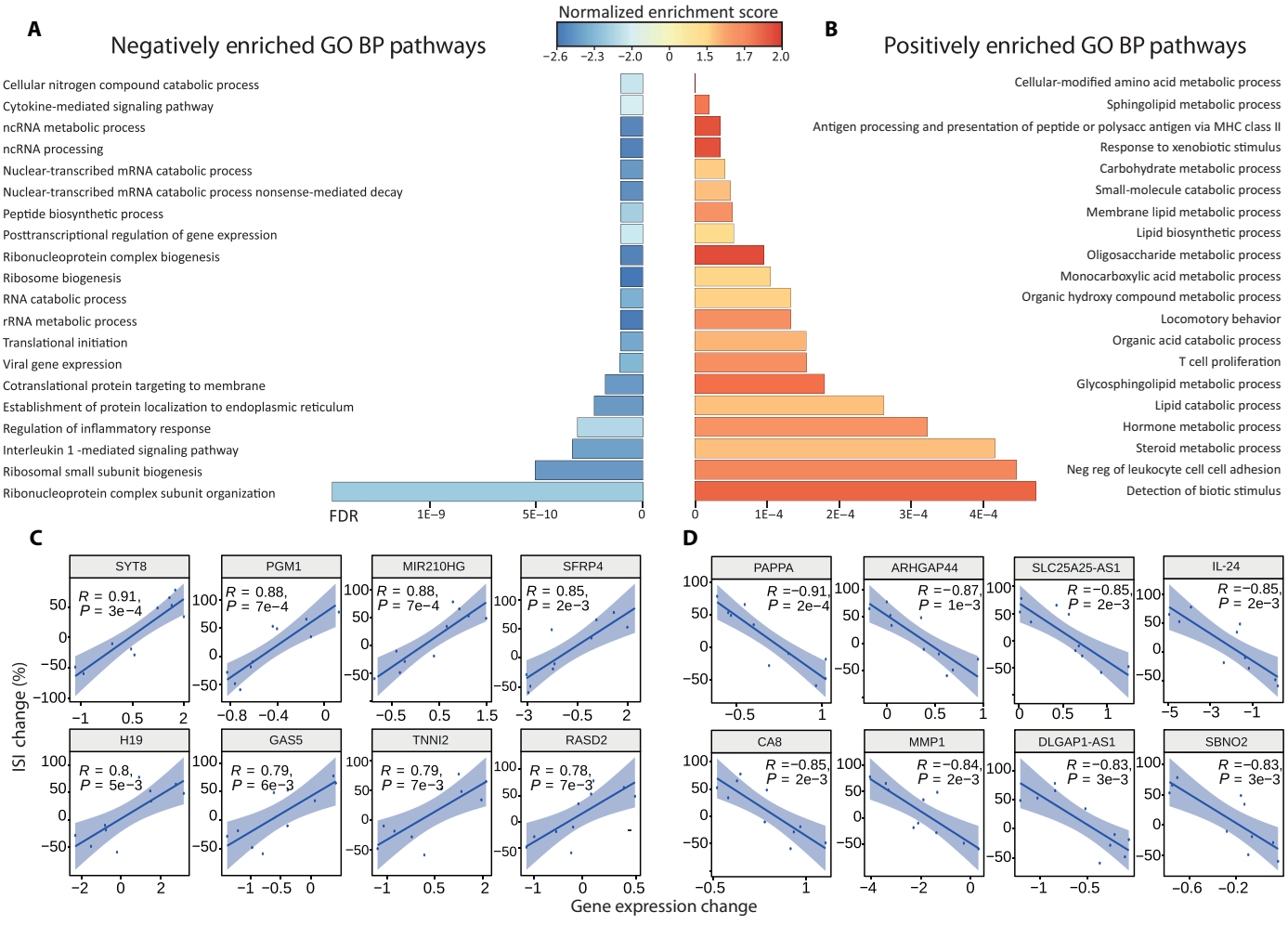

Fig. 4. Enriched pathways and gene expression changes in cultured T2D islets. (**A** and **B**) Most significantly enriched functional pathways in the improver cultured versus basal islets. The bar plots show significance and normalized enrichment scores of the 20 most significantly enriched Gene Ontology terms [Biological Pathway (BP)] in negative (A) or positive (B) direction. (**C** and **D**) Most significant correlations between gene expression and insulin secretion changes (cultured versus basal) shown as scatterplots. The eight genes with the most significant positive (C) and negative (D) correlations are shown.

with potential anti-inflammatory effects were shared between the two databases (MEK inhibitors, targeting MAP2K1/2). Crossmatching genetic perturbagens with genes that were negatively correlated with GSIS showed several common genes (Fig. 5, C and D), including *PIM1* (encoding a serine/threonine protein kinase involved in regulating inflammatory signaling pathways and immune cell activation) (*36*) and *CPD* (encoding carboxypeptidase D that is up-regulated by elevated glucose in mouse and human islets) (*37*).

The JAK inhibitor baricitinib improves insulin secretion in a clonal β cell line and T2D human islets

Baricitinib is a JAK1 and JAK2 inhibitor used to treat inflammatory and autoimmune diseases, including type 1 diabetes (*38*–*40*). To test this predicted chemical perturbagen for its potential to restore insulin secretion, we first used the clonal rat INS-1E β cell model. The exposure of INS-1E cells to high glucose, palmitate, or proinflammatory cytokines reduced ISI values (fig. S4, A, D, and G). Baricitinib partially prevented these deleterious effects. Unexpectedly, baricitinib also potentiated insulin release from control, nonstressed cells (fig. S4, A, B, D, E, and H). In addition, the drug protected β cells from cytokine-induced apoptosis (fig. S4I), in line with previous findings in human β cells (*41*). To further characterize these observations, we studied the effects of baricitinib on INS-1E cells exposed to palmitate using optical microscopy approaches. By fluorescence lifetime imaging microscopy, baricitinib was shown to enter INS-1E cells (fig. S5). Super-resolution microscopy demonstrated that palmitate disrupts the highly interconnected and dense tubular network of mitochondria, which appeared fragmented into numerous small punctate particles (fig. S6). Baricitinib restored, at least in part, mitochondrial morphology, with normalization of mitochondria area, perimeter, and circularity (fig. S6).

We next assessed the potential of baricitinib to restore β cell function in the "gold standard" model of human islets from T2D donors. In static incubation experiments, glucose-stimulated insulin release of ND islets ($n = 6$ preparations) was not affected by the drug (Fig. 6, A and B), at odds with the potentiation of GSIS in clonal rat β cells under nonstressed conditions (fig. S4). T2D islets ($n = 6$ preparations) showed lower GSIS, and this improved with

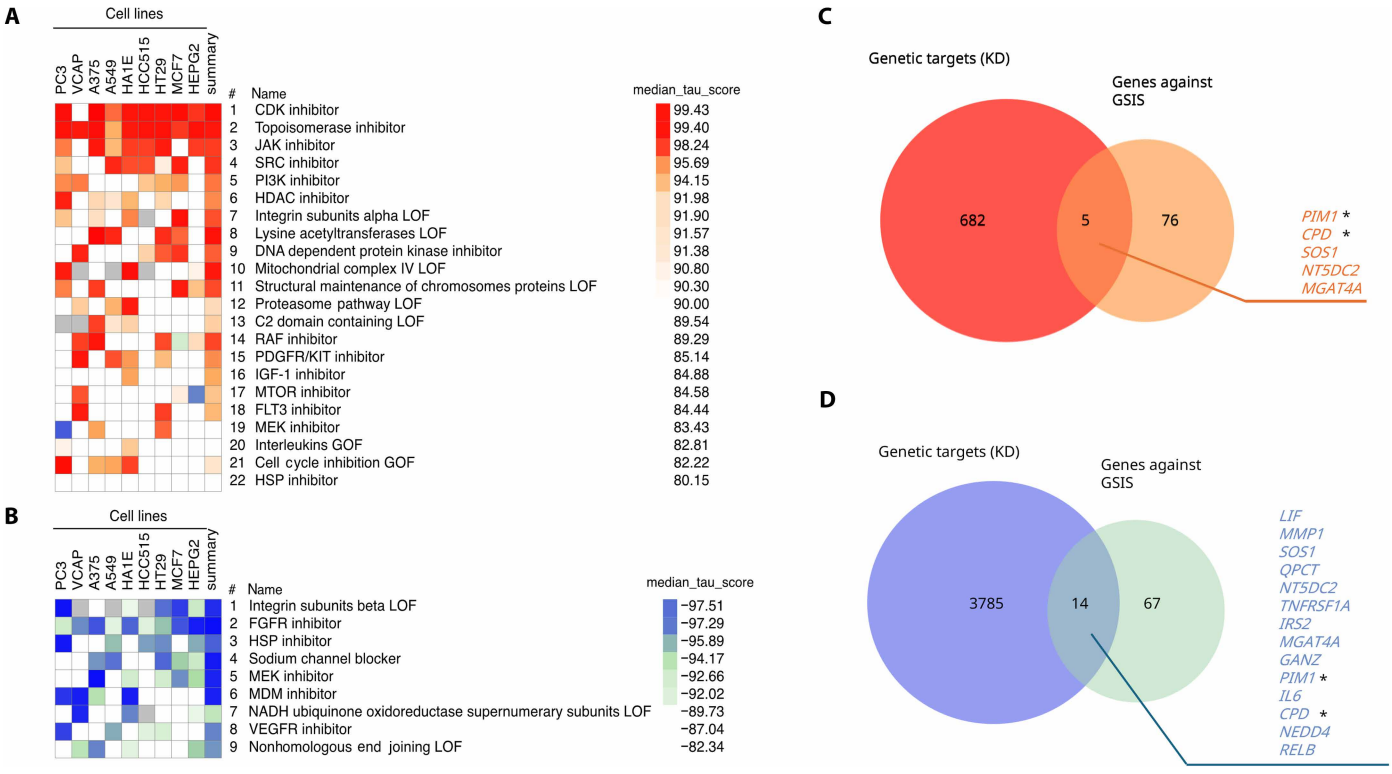

Fig. 5. Identification of potential therapeutic targets through mining of differential signatures. The top 150 up-regulated (**A** and **C**) and top 150 down-regulated (**B** and **D**) genes were analyzed in the Connectivity Map to identify chemical (A and B) and genetic perturbagens (C and D). Genetic targets identified in Connectivity Map were crossmatched with genes negatively correlated with GSIS. Genes modulated in nonimprovers were excluded from these analyses. Signatures generated by knocking down genes are positively correlated with the improvers and were considered as potential targets. Only classes with a |median tau score| >80 were considered as potential chemical perturbations. The genetic targets also identified in the iLINCs database are marked with an asterisk. KD, knockdown.

72-hour treatment with baricitinib, resulting in an increased ISI by ~30% (Fig. 6, A and B). For seven ND and one T2D islet preparations, we also studied dynamic insulin secretion using a perifusion system (fig. S7). The results confirmed that baricitinib does not affect insulin release from ND β cells (fig. S7A). Although limited to one only preparation, the perifusion results of T2D islets (fig. S7B) were in line with the static incubation data, showing better insulin response to glucose and exendin-4 (a GLP-1 receptor agonist) for islets exposed to baricitinib. In these functional experiments, islet cell viability and insulin content were not changed by baricitinib (fig. S8, A to D).

To evaluate whether baricitinib induced changes in expression of 16 key genes associated with GSIS (Fig. 4, C and D), we explored transcriptomes of baricitinib-treated T2D islets ($n = 4$). For two genes, namely, *TNNI2* and *IL24*, the expression was significantly modified by baricitinib (Fig. 6C), with a direction of effect consistent with the changes in Fig. 4 (C and D). *TNNI2* encodes troponin I, the inhibitory subunit of the troponin complex in fast skeletal muscle. By chromatin interaction studies, it has been shown that the *INS* gene physically interacts with the *TNNI2* and neighboring *SYT8* gene locus in human islets, with the *INS* promoter regulating their expression in a glucose-dependent manner (*42*). *IL24* encodes IL-24, a member of the IL-10 family of cytokines. IL-24 mRNA expression has been shown to be up-regulated in T2D versus ND islets (*26*, *43*) and in ND islets exposed to palmitate, alone or in combination with high glucose (*26*)). The transcriptome of baricitinib-treated T2D islets suggests that these genes might be involved in the improvement of insulin secretion.

JAK inhibitors block interferon (IFN) signaling (*41*), and they have been successfully tested in type 1 diabetes (*39*, *40*). We examined whether IFN signaling is present in T2D β cells using previously published single-cell RNA sequencing (RNA-seq) data from the Human Pancreas Analysis Program (HPAP) (see Materials and Methods). We used a β cell IFN-stimulated gene signature score based on ~50 genes induced by IFNs in human β cells that has been shown to be threefold higher in β cells from individuals with type 1 diabetes as compared to β cells from ND individuals (see also Fig. 6D) (*44*). T2D β cells had a 30% higher IFN-stimulated gene score than ND β cells (Fig. 6D). Accordingly, "type 1 IFN production" signaling is significantly and uniquely down-regulated in the improver islets (see table S9).

We examined two additional chemical perturbagens predicted to reproduce the transcriptome signature of improver islets, namely, the HDAC inhibitor PCI-34051 and the PI3K inhibitor wortmannin (fig. S9). In two T2D islet preparations, these drugs did not induce functional recovery (fig. S9). Together, these results show that baricitinib may confer β cell functional restoration by attenuating mild IFN signaling present in T2D islets or by other mechanisms that remain to be characterized.

Baricitinib partially preserves insulin secretion in vivo in a mouse model of T2D

To test if baricitinib exerts beneficial effects on diabetic β cell function in vivo, db/db mice (a model of severe T2D) (*45*) were treated

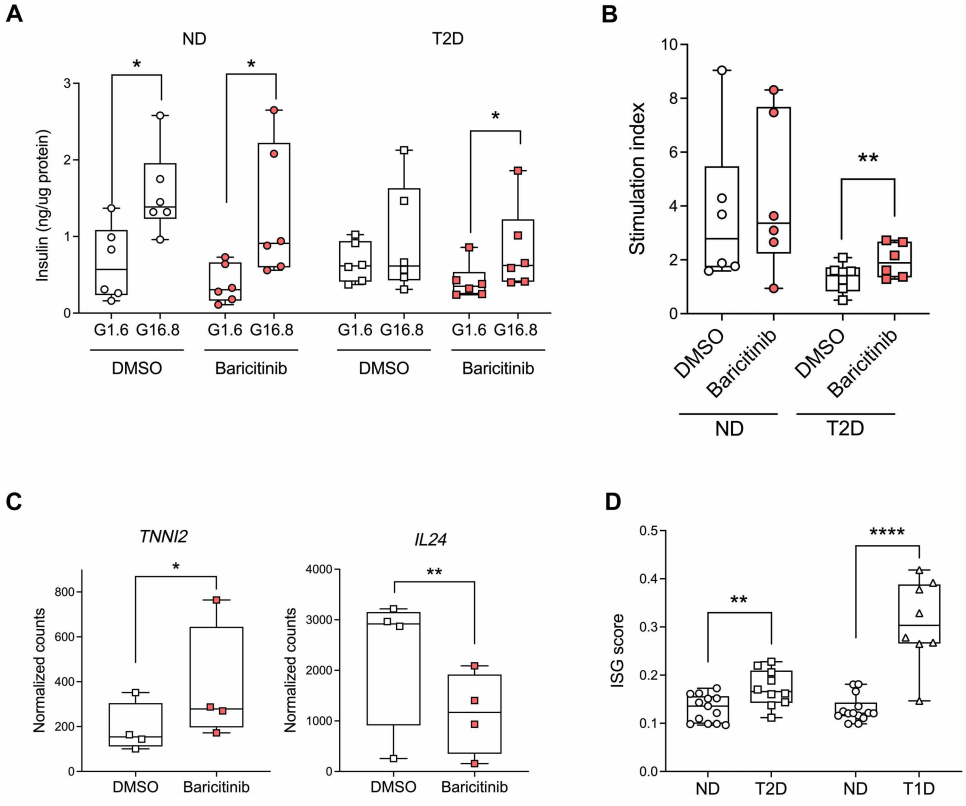

Fig. 6. Baricitinib improves β cell function in T2D but not in ND islets. (**A**) ND (circle, six donors) or T2D (square, six donors) islets were treated with 4 μM baricitinib (red) or DMSO (white), and insulin secretion was assessed at 1.6 and 16.8 mM glucose. (**B**) Data from (A) presented as ISI. (**C**) Expression levels of *TNNI2* and *IL24* by RNA-seq of T2D islets (four donors) treated or not with baricitinib. Counts normalized by DESeq2 median of ratios method. (**D**) β cell IFN-stimulated gene (ISG) score was computed on the basis of single-cell RNA-seq data from 8 T2D and 13 matched ND donors or 10 T1D and 14 matched ND donors from the HPAP (see text for details). Boxes depict the 25th and 75th percentiles, and the line represents the median. Statistical significance was determined using paired or unpaired two-tailed *t* test. $*P < 0.05$, $**P < 0.01$, and $****P < 0.0001$.

daily with baricitinib (20 mg/kg) or vehicle for a period of 4 weeks. Baricitinib-treated mice exhibited better weight maintenance over time (Fig. 7A) and slightly lower food and water intake (fig. S10, A to C). HbA1c levels increased similarly in baricitinib- and vehicle-treated animals (from 4.7 ± 0.1 to 10.6 ± 0.4% for baricitinib and from 4.7 ± 0.2 to 10.9 ± 0.4% for vehicle) (fig. S10D). Insulin tolerance tests or clamp studies were not done to evaluate the effect of baricitinib on insulin sensitivity, but proxy measures showed a trend toward improved insulin action after the 4-week treatment (fig. S11). Oral glucose tolerance tests (OGTTs) showed no major difference in blood glucose levels between the two groups, with similar increases over the 4-week period to severe hyperglycemia (Fig. 7, B and C). As previously observed in db/db mice (*46*, *47*), insulin levels during the OGTT were not glucose responsive (Fig. 7D). In vehicle and baricitinib treatment groups, insulin levels and insulin area under the curve were comparable at the start, but after 4 weeks, the baricitinib-treated mice had significantly higher insulin levels (Fig. 7, D and E), as a result of a less pronounced decline in insulin secretion (Fig. 7F).

DISCUSSION

Defective insulin secretion in T2D is due to β cell functional alterations and, to a lesser extent, to loss of β cell mass (*48*–*51*). In the present study, the recovery of human T2D β cell glucose responsiveness occurred in almost two-thirds of islet preparations after 3 days of culture in medium with normal glucose concentration (5.5 mM) and devoid of other putative metabolic and inflammatory stresses of the in vivo T2D milieu. Glucose-stimulated ISI, a robust marker of β cell glucose sensitivity (*18*, *19*), increased by around 60% in 13 of 21 T2D islet preparations (Fig. 2). This trajectory is reminiscent of that of islets from ND subjects upon removal of in vitro lipo- or glucotoxic culture conditions (*26*), demonstrating remarkable β cell functional plasticity and resilience. T2D diabetes remission after low-calory diets (*6*) or bariatric surgery (*8*, *9*) may occur in proportions similar to that observed for T2D β cell recovery in the present study, i.e., around 50 to 60%; it is more rare for pharmacological treatment (*15*). Although there is agreement that information on human islet function generated ex vivo reasonably reproduces the in vivo situation (*19*), the present ex vivo functional recovery model may not exactly reproduce β cell functional recovery as it occurs in vivo in diet- or bariatric surgery-treated T2D individuals.

Albeit improved, insulin secretion after T2D islet culture remains lower than that of ND islets (*18*, *19*); in one-third of cases, β cell function did not improve. T2D remission after caloric restriction or bariatric surgery is less likely in subjects with longer duration of disease (*5*, *6*, *8*, *52*). This does not seem to be the case in our ex vivo study, since improver and nonimprover islet preparations came from

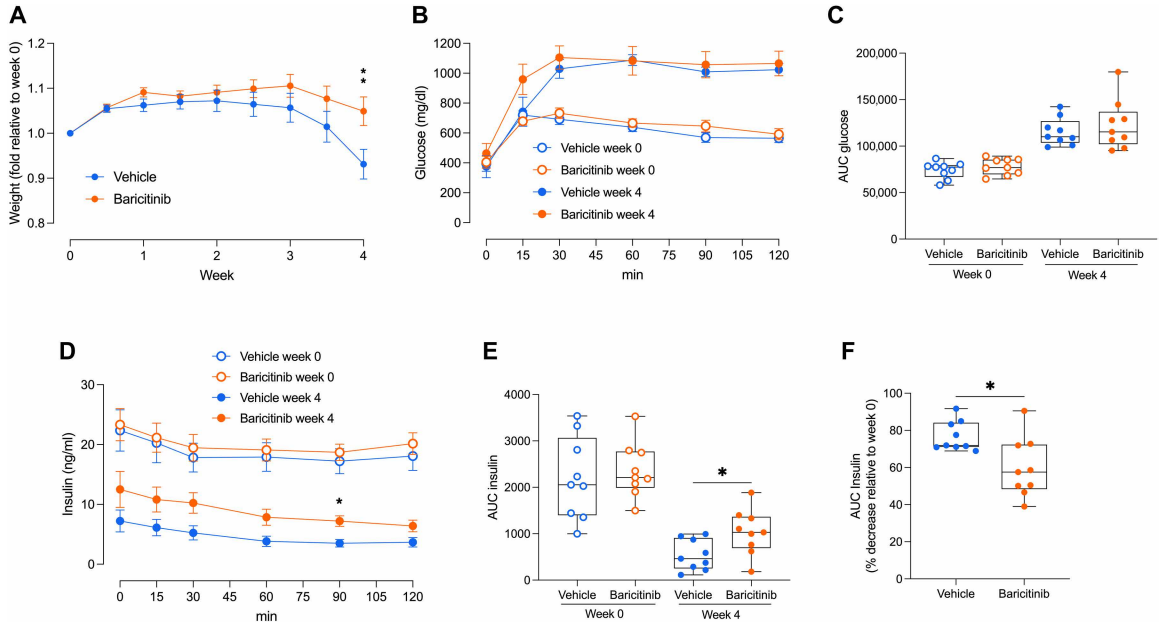

Fig. 7. Baricitinib partially preserves insulin secretion in db/db mice. (**A**) Weight, expressed as fold change relative to baseline (week 0) in baricitinib (orange) treated db/db mice as compared to vehicle (blue). (**B**) Glucose levels during OGTT basally and after 4 weeks of treatment with baricitinib or vehicle. (**C**) Glucose area under the curve (AUC) during OGTT before (week 0) and after 4 weeks (week 4) of treatment with baricitinib or vehicle. (**D**) Insulin levels during OGTT. (**E**) OGTT insulin AUC before and after 4-week treatment with baricitinib or vehicle. (**F**) Decline in insulin AUC over 4 weeks, expressed as percent decreased value relative to week 0. Statistical analysis between baricitinib- and vehicle-treated groups at the same time point was performed by unpaired two-tailed *t* test or two-way ANOVA with Sidak correction for multiple comparisons. $^{*}P < 0.05$ and $^{**}P < 0.01$.

donors with similar clinical characteristics and duration of T2D (average 11 and 9 years, respectively; table S3). We did not observe major differences in islet and β cell morphometry between improver and nonimprover samples. Rates of islet loss or islet cell death during culture were not measured in these experiments. However, indirect assessments of islet mass and composition basally and after culture (deconvolution of islet RNA-seq data and apoptosis gene expression signature) (fig. S2) argue against major changes.

Whether longer culture, pharmacological compounds and/or interference with genetic targets (see below) may lead to greater T2D β cell functional rescue remains to be assessed. No β cell functional improvement was seen in islets cultured at 11.1 mM glucose, suggesting that glucose levels in people with T2D should be kept as close as possible to the physiological range to preserve/restore β cell function (*53*).

Transcriptomic traits of T2D islets have been reported by a few groups, showing numerous DEGs in comparison with ND islets (*18*, *54*, *55*). A relevant finding of the present study is the observation that T2D β cell functional improvement is associated with specific transcriptome changes. In particular, we found up-regulation of genes implicated in β cell function and structure and down-regulation of genes implicated in inflammatory responses (tables S5 to S7). Of the genes that have been identified in T2D GWAS (https://www.ebi.ac.uk/gwas/) (*25*), 17 changed in expression during β cell functional recovery in ND islets following washout of palmitate or high glucose exposure (table S8) (*26*). Many gene expression changes were correlated with improved insulin release (Fig. 4 and table S10), suggesting their direct impact on β cell function. For some of these, experimental evidence already exists. SYT8 knockdown in islets from two donors attenuated glucose- and arginine-induced insulin secretion by more than 50% (*42*). In INS-1 cells, the overexpression of GAS5 increased insulin content and potentiated insulin release (*56*), while NR3C2 was associated with reduced insulin secretion (*57*). The up-regulation of PAX4 and down-regulation of FGF2 and MYC point to restoration of a more differentiated β cell phenotype (*58*, *59*). Thus, the present study provides a comprehensive gene signature of β cell functional plasticity and resilience in T2D islets, including many targets to potentially restore β cell function.

To explore this further, the islet differential gene expression signature was mined in the Connectivity Map and integrative library of integrated network-based cellular signatures (iLINCS) repositories of human cell transcriptomes induced by chemical and genetic perturbagens (Fig. 5). Searching for chemical perturbagens may lead to drug repurposing, an attractive approach that lowers drug development costs and shortens development timeframes (*59*). Unexpectedly, among the top hits predicted to potentially improve T2D β cell function are JAK inhibitors that act downstream of type I and II IFN receptors. We tested baricitinib, a JAK1 and JAK2 inhibitor used to treat inflammatory diseases, such as atopic dermatitis, rheumatoid arthritis, psoriasis, alopecia areata, ulcerative colitis, and autoimmune diabetes (*38*, *39*). Baricitinib has been recently shown to preserve β cell function in people with recent-onset type 1 diabetes (*40*). Our validation experiments demonstrate that baricitinib can indeed protect clonal rat β cells from metabolic and proinflammatory insults. It improved insulin secretion of islets isolated from T2D subjects. The drug was also tested in db/db mice, a model of severe obesity and T2D. The mice were severely hyperglycemic (blood glucose levels around 400 mg/dl), indicating advanced β cell dysfunction. Yet, 4-week baricinitib administration partially preserved insulin secretion, showing that baricitinib delayed the progression

of β cell failure (Fig. 7). The drug preserved body weight as compared with vehicle-receiving mice. The weight loss in the latter group was similar to other reports (*60*). We did not perform insulin tolerance or clamp tests to evaluate insulin sensitivity, but proxy indices of insulin action showed a trend toward better insulin action after 4-week baricitinib treatment (fig. S11).

With less aggressive models of hyperglycemia the results obtained with baricitinib are more evident. In a previous study (*61*), using high-fat– and high-sugar–fed C57BL/6 mice, baricitinib (10 mg/kg, 16-week oral administration) improved glucose tolerance during OGTTs and lowered nonfasting glucose levels compared to untreated high-fat– and high-sugar–fed mice. In another study (*62*), Wistar rats, rendered diabetic by administration of 10% fructose and streptozotocin, were treated with tofacitinib (10 or 20 mg/kg orally). Tofacitinib improved glucose tolerance during OGTTs at 4 and 8 weeks of treatment, along with an increase in HOMA-B, a measure of β cell function (albeit imprecise). The results in these rodent models suggest that baricitinib may be more effective as an intervention in early and/or less severe stages of diabetes.

Anecdotal evidence from clinical studies further supports our discovery. In 13 patients with rheumatoid arthritis who also had T2D, baricitinib—but not tofacitinib, a JAK1 and JAK3 inhibitor—improved HbA1c levels after 6 months of treatment (*63*). In two of the seven baricitinib-treated patients, the dose of concomitant oral antihyperglycemic drugs was reduced. Tofacitinib improved HOMA2-B after 6 months in 32 rheumatoid arthritis patients with T2D (*64*). In a phase 2, double-blind, randomized clinical trial involving individuals with T2D and diabetic kidney disease (*65*), the efficacy of four different doses of baricitinib in reducing albuminuria was evaluated. Although glucose control was not among the primary or secondary end points of the study, a 0.5% decrease in HbA1c was observed at 24 weeks in the group of patients receiving the highest baricitinib dose (4 mg/day). Collectively, these findings support a potential beneficial effect of baricitinib on glucose regulation, although available studies were not designed to determine whether the improvements were driven by enhanced β cell function, improved insulin sensitivity, or both. Larger prospective studies with more accurate measurements of β cell function are warranted to assess potential benefits of baricitinib in T2D.

It is of interest that baricitinib treatment of T2D islets up-regulates *TNNI2* and down-regulates *IL24*, in keeping with gene expression changes that are correlated with changes in GSIS (Fig. 4, C and D). The expression of *TNNI2* and its neighbor *SYT8* is induced by high glucose in human islets; SYT8 has been shown to promote human islet insulin secretion, but the role of TNNI2 has not been examined (*42*). *IL24* expression is induced in T2D islets and under glucolipotoxic conditions. *IL 24* expression is also increased in db/db mouse islets; it elicits mouse islet oxidative and endoplasmic reticulum stress and impairs GSIS (*66*). IL-24–neutralizing antibodies improved glucose tolerance of high-fat diet–fed mice (*66*). Baricitinib might exert its beneficial effects on GSIS in T2D islets through these gene expression changes.

T2D islets show that histological and molecular signs of inflammation [(*67*–*70*) and present study] and other anti-inflammatory approaches, such as interleukin-1-receptor antagonism, have been tested to improve insulin secretion in T2D patients and subjects with impaired glucose tolerance, although with scarce success (*71*, *72*). It is unclear whether this anti-inflammatory therapy reduces inflammation in islets or in other metabolically relevant tissues (*72*). The anti-inflammatory cytokines IL-4 and IL-10 are human β cell protective ex vivo against the pro-inflammatory cytokines IL-1β, tumor necrosis factor–α (TNF-α), and IFN-γ (*73*).

As an additional example of chemical perturbagens, we identified Src family tyrosine kinases that tonically inhibit Ca^{2+}-dependent insulin secretion. Src inhibitors enhance rat INS-1 cell and islet insulin secretion (*74*) and recover β cell function following palmitate exposure of islets from obese diabetic and hyperlipidemic KK-A^{Y} mice (*75*) or diabetic Goto-Kakizaki rats (*76*). Not all predictions of chemical perturbagens were validated, e.g., the HDAC and PI3K inhibitors that we tested were ineffective.

The in silico predictions using Connectivity Map and iLINCs also identified putative genetic targets, and *PIM1* and *CPD* were identified in both databases. Their expression also changes inversely with GSIS during T2D islet culture (table S10). The PIM1 kinase promotes cell proliferation and survival; PIM1 inhibition has shown promise in hematological, prostate, and triple-negative breast cancers (*77*–*79*). PIM1 also regulates inflammation and immune cells and has emerged as a therapeutic target in immunoinflammatory diseases, such as autoimmune uveitis, inflammatory bowel disease, asthma, and rheumatoid arthritis (*36*). While in clonal rat β cells its expression is positively correlated with insulin release (*80*, *81*), its role in human islets remains largely unexplored. CPD protein (but not mRNA) levels are up-regulated by elevated glucose and insulin in rodent and human islets (*82*). CPD is structurally similar to carboxypeptidase E, but its specific function is not known. CPD is localized in the plasma membrane and trans-Golgi network, while carboxypeptidase E is found in secretory granules where it mediates the final steps of insulin processing (*82*). The role of CPD in β cell function remains to be elucidated.

In conclusion, the present study shows that glucose responsiveness, typically impaired in T2D β cells, was rescued in 60% of the cases by exposure to a ND milieu, demonstrating relevant functional plasticity of T2D β cells and their resilience to long-term metabolic stress. β cell functional recovery is associated with transcriptomic traits, revealing several chemical and genetic targets to induce T2D remission. We validated the in silico prediction that baricitinib may improve T2D β cell function, pointing to a targeted therapeutic approach for the disease.

MATERIALS AND METHODS

Study design

For the functional and molecular recovery experiments, organ donor pancreata were handled in Pisa before 30 November 2021 and processed as previously described (*26*, *83*, *84*), with permission by the Ethics Committee of the University of Pisa (21 November 2013, #2615), upon written consent of donors' next-of-kin. As shown in Fig. 1, islets were isolated by collagenase digestion and density gradient purification. Islets prepared from ND and T2D donors were cultured for 2 days in control medium to allow recovery from isolation stress and were then evaluated in terms of GSIS (ND and T2D islets) and whole transcriptome features (a subgroup of T2D islets) (basal assessment). Subsequently, islets were cultured for ~3 days at 5.5 mM glucose ("normoglycemia" condition) and, for five T2D islet preparations, also at 11.1 mM glucose ("moderate hyperglycemia" condition), followed again by GSIS and transcriptome analysis (this is denoted as cultured assessment). At this stage, islets were classified as improvers or nonimprovers based on the change in GSIS compared to basal assessment. In subgroups of improvers and

nonimprovers, islet morphometry was performed on pancreatic tissue, banked before islet isolation. Correlations between changes of β cell glucose responsiveness and transcriptome traits were assessed. Drug repurposing and target identification analyses for β cell functional recovery were performed to predict chemical and/or genetic hits. Last, validation studies were conducted in a β cell line, human islets [provided by University of Lille, University of Alberta (Edmonton) and University of Barcelona], and in db/db mice.

Pancreatic islet isolation and culture

Isolated islets were prepared by enzymatic digestion and density gradient centrifugation, as previously detailed (*26*, *83*–*85*). Then, they were allowed to recover from the isolation procedure by 2 days of culture in a 5% CO_2 incubator at 37°C. As reported previously (*26*, *83*–*85*), culture medium was M199 medium containing 5.5 mM glucose and supplemented with 10% (v/v) adult bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (50 μg/ml), and amphotericin B (750 ng/ml). Afterward, ND and T2D islets were cultured respectively for 3.3 ± 0.4 and 3.3 ± 0.2 days in medium containing 5.5 mM glucose. Five T2D islet preparations were cultured in parallel also at 11.1 mM glucose. Insulin secretion in response to glucose (3.3 and 16.7 mM) was assessed with handpicked islets (1 to 3 replicates for each experimental point: 1 replicate, 55 points; 2 replicates, 53 points; 3 replicates: 28 points; total replicates, 245) of similar size (approximate diameter of 100 to 150 μm), as previously described (*26*, *83*, *84*). Glucose-stimulated insulin concentrations were measured by a radioimmunometric assay (DIAsource ImmunoAssays, Nivelles, Belgium), islet insulin content was quantified after overnight acid-alcohol extraction, and the ISI was calculated as the ratio of insulin release at 16.7 mM glucose over release at 3.3 mM glucose.

Morphometry studies

Islet morphometry was performed as described (*83*, *86*) on tissue samples obtained before islet isolation. Paraldehyde-fixed, paraffin-embedded tissue sections (7-μm thickness) were analyzed by immunofluorescence for insulin, glucagon, and somatostatin, and β cell apoptosis was also quantified (see Supplementary Material for details). In addition, transcriptome data (see below) were used for deconvolution analysis (*87*, *88*) of T2D islets to assess the effect of culture on the β cell proportion.

Transcriptome studies

The transcriptome of T2D islets before and after culture was studied. RNA was extracted and analyzed as previously reported (see also the Supplementary Materials) (*18*, *26*, *89*–*91*). The 20 samples analyzed were as follows: 5 improvers, basal and cultured, and 5 nonimprovers, basal and cultured. Identification codes were: improvers, 19/71, 21/40, 21/91, 24/25, and 24/53; nonimprovers, 19/64, 21/26, 24/34, 24/49, and 24/65 (table S3). Genes were filtered for a minimal average expression of 1 cpm. Normalization of reads was accomplished for library size using the edgeR Bioconductor package. To assess differences in gene expression, the limma function with voom approach from the limma Bioconductor package was used (*92*). Transcriptomes of cultured versus basal islets were compared, with a significance threshold of 0.05 (Benjamini-Hochberg corrected *P* value, FDR). GSEA was performed with fast GSEA (v. 1.16) on the Biological Process sets from Gene Ontology (version 7.2), with parameters minSize = 15 and maxSize = 500. Input gene lists were ranked according to signed significance ($-\log_{10}$ *P* value × fold change sign) of the tested contrasts. The association between changes in gene expression and ISI values was tested by linear regression analysis for each gene using relative gene expression (independent variable) and ISI (dependent variable) variations between cultured and basal conditions, according to the formula $\left(\frac{\text{Cultured}-\text{Basal}}{\text{Basal}}\right) \cdot 100$.

Connectivity Map and iLINCS analyses

The top 150 genes that were significantly (FDR < 0.05) up- and down-regulated after culture in improver islets were analyzed using the cloud-based CLUE software (https://clue.io) and compared with the Connectivity Map database (*34*). Because the differential signatures identified in nonimprover islets are unrelated to β cell functional recovery, we excluded these differential genes from the analysis. Differential signatures of improver islets were matched with those in the Connectivity Map, that include nine human cell lines exposed to chemical perturbagens used in experimental and clinical settings, and also to genetic perturbagens. Perturbagens that were positively correlated (|median tau score| >80) were considered as potential therapeutic hits since they produce gene expression programs similar to those of improver islets. We also submitted DEGs specific to improvers to the iLINCs database (http://ilincs.org) to mine for potential drugs and genetic perturbations (*35*). Perturbagens with a positive correlation (concordance score > 0.6) were considered potential targets for both drugs and genes.

IFN-stimulated gene signature and apoptosis score

We previously established a β cell IFN-stimulated gene signature score by selecting mRNAs that exhibited a >3-fold up-regulation in EndoC-βH1 cells exposed to IFN-α or IFN-γ but not induced by IL-1β or TNF-α (*44*). The 53 IFN-stimulated genes included are reported in Supplementary Materials and Methods. The gene score was computed in single-cell RNA-seq data from the HPAP (https://hpap.pmacs.upenn.edu) (*93*) and reanalyzed as described (*44*). Samples were 10 from individuals with T2D and 13 from age-matched ND donors, and 8 from subjects with type 1 diabetes and 14 from age-matched ND donors. Of the 53 IFN-stimulated genes, 48 were detected in the single-cell RNA-seq data from HPAP. Their average expression level was calculated as the IFN-stimulated gene signature score for each β cell, and the score of all β cells from a donor was averaged. The apoptosis score was defined as the average expression of 140 apoptosis-related genes based on the Reactome gene sets, as previously described (*20*).

Validation of chemical perturbagen predictions in clonal rat β cells

INS-1E cells (catalog no. C00118009, AddexBio, San Diego, USA), tested negative for mycoplasma, were cultured as previously described (*18*) and then exposed for 24 hours to glucotoxic, lipotoxic, or proinflammatory cytokine stresses (see the Supplementary Materials for details). Insulin release in response to glucose (2.5 and 16.7 mM) and insulin content were assessed as previously reported. Fluorescence lifetime imaging microscopy and super-resolution microscopy experiments were performed as previously described (see also the Supplementary Materials) (*94*, *95*).

Validation of chemical perturbagen predictions in T2D islets

Human islets from 13 ND (age 49.4 ± 3.8 years, 7 male/6 female, BMI 27.1 ± 1.2 kg/m^2) and 6 T2D donors (age 64.0 ± 3.6 years, 3 males/3 females, BMI 32.7 ± 1.8 kg/m^2; table S12) were isolated at University

of Lille, University of Alberta (Edmonton), Université Catholique de Louvain, and University of Barcelona with approval of the local Ethics Boards (P2019/498 and A2024/211) and informed consent by donors' families for the use of pancreatic tissue in research and cultured in Brussels as described (*96*). The β cell purity of the islets, determined by insulin immunofluorescence (*97*), was 49.0 ± 3.7% (ND) and 33.8 ± 4.0% (T2D) (table S12). Human islets were treated for 72 hours with 4 μM baricitinib or dimethyl sulfoxide (DMSO) (vehicle). Islet cell viability was detected by fluorescence microscopy after staining with the DNA binding dyes Hoechst 33342 (5 μg/ml) and propidium iodide (5 μg/ml). A total of 20 to 30 islets were handpicked and washed with glucose-free Krebs buffer (Univercell Biosolutions, Toulouse, France), preincubated in 1.6 mM glucose Krebs for 30 min, and incubated with 1.6 and 16.8 mM glucose for 1 hour, and supernatant was collected for human insulin enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden). Dynamic insulin secretion was carried out on 30 to 50 handpicked human islets using the PERI5LM perifusion instrument (Biorep Technologies) with a flow rate of 100 μl/min and sampling every 4 min. Islets were perfused with Krebs buffer (Univercell Biosolutions), and after 90 min of equilibration in 2.8 mM glucose, samples were collected for human insulin ELISA (Mercodia). After the secretion studies, islets were collected for insulin and protein content analysis. Cellular insulin was extracted using acid ethanol (95% ethanol, and 5% 12-N hydrochloric acid). Insulin secretion and content were normalized to total protein content, measured by protein assay dye (Bio-Rad). RNA of ~120 handpicked T2D islets (Lille H1151, H1183A, and H1194 and Barcelona 192/23; table S12) treated or not with baricitinib was prepared using the RNeasy Plus Marco Kit + QIAshredder (QIAGEN, Hilden, Germany) by cell lysing and homogenizing steps. Total RNA concentration and RNA integrity number (RIN) values were assessed using Agilent Bioanalyzer 2100 (Agilent Technologies, Wokingham, UK) and Agilent RNA Nano Chips (Agilent Technologies). RNA RIN values were 9.0 ± 0.24 for DMSO-treated T2D islets and 8.8 ± 0.19 for baricitinib-treated T2D islets, demonstrating suitability for sequencing. Raw reads were quality-filtered using fastp (v0.20.1) (*98*). Filtered reads ranged from 150 to 1000 million per sample. Salmon (v1.4.0) (*99*) was used for gene expression quantification, correcting for guanine-cytosine biases. GENCODE version 38 (GRCh38.p13) was selected as the human reference genome (*100*). Differential analysis was assessed with DESeq2 (1.38.30) (*101*), considering a Benjamini-Hochberg FDR < 0.05 as significant.

In vivo animal studies

All procedures were conducted in accordance with the European Community Council Directive 86/609/EEC and the European Directive 2010/63/EU for animal experiments and authorized by the Italian Ministry of Health (authorization 1024/2023-PR, dated 06 December 2023). All efforts were made to minimize the number of animals and reduce their suffering. The study complies with the ARRIVE guidelines (*102*). No exclusion criteria were pre-established. No data were omitted from the analysis. Eighteen 5-week-old male db/db mice [BKS.Cg-Dock7m+/+LeprdbJ, Charles River, 607BKS(DB/DB) Wilmington, MA, USA] were housed under controlled environmental conditions (22°C, 50% humidity, 12-hour light/12-hour dark cycle) with ad libitum access to food and water. After a week of acclimatization, 12-hour fasting glycemia and glycated hemoglobin (HbA1c) were measured on tail vein blood using Glucocard G meter (Menarini Diagnostics, Florence, Italy) and Cobas b101 instrument (Roche Diagnostics, Indianapolis, IN, USA). Mice (mean body weight: 35.5 ± 0.4 g) were randomly assigned to baricitinib (20 mg/kg, suspended in 0.5% methylcellulose, $n = 9$) or vehicle (0.5% methylcellulose, $n = 9$). At treatment initiation, mean glycemia was 405 ± 40 mg/dl in the baricitinib group and 396 ± 52 mg/dl in the vehicle group. HbA1c levels were 4.7 ± 0.1 and 4.7 ± 0.2%, respectively. Treatments were administered once daily by oral gavage for 4 weeks. Body weight, food intake, and water consumption were monitored twice weekly. An OGTT was conducted before and after the 4-week treatment in 12-hour fasted mice given a glucose challenge (2 g/kg; glucose Carlo Erba Reagent, Milan, Italy) via oral gavage. Blood samples were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min using Microvette collection tubes (SARSTEDT, Nümbrecht, Germany). Plasma insulin was measured using an Ultra-Sensitive Mouse Insulin ELISA (Crystal Chem, #90080, Elk Grove Village, IL, USA), following the manufacturer's instructions.

Statistical analysis

Clinical and isolated islet data are presented as means ± SE, unless otherwise specified (see Results). Differences between groups were assessed by the two-tailed paired or unpaired Student's *t* test, the Mann-Whitney test, or the one-way repeated measure analysis of variance (ANOVA) test, as appropriate (see Results). The two-way ANOVA test was used to evaluate the effects of culture duration and functional response. A P value < 0.05 was considered statistically significant. The analyses of gene expression data were performed as reported in the "Transcriptome studies" section.

Supplementary Materials

The PDF file includes:

Supplementary Text
Figs. S1 to S11
Tables S1 to S3, S6, and S12
Legends for tables S4, S5, S7 to S11

Other Supplementary Material for this manuscript includes the following:

Tables S4, S5, S7 to S11

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