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Figures and figure supplements

Epigenetic modulation of type-1 diabetes via a dual effect on pancreatic macrophages and β cells

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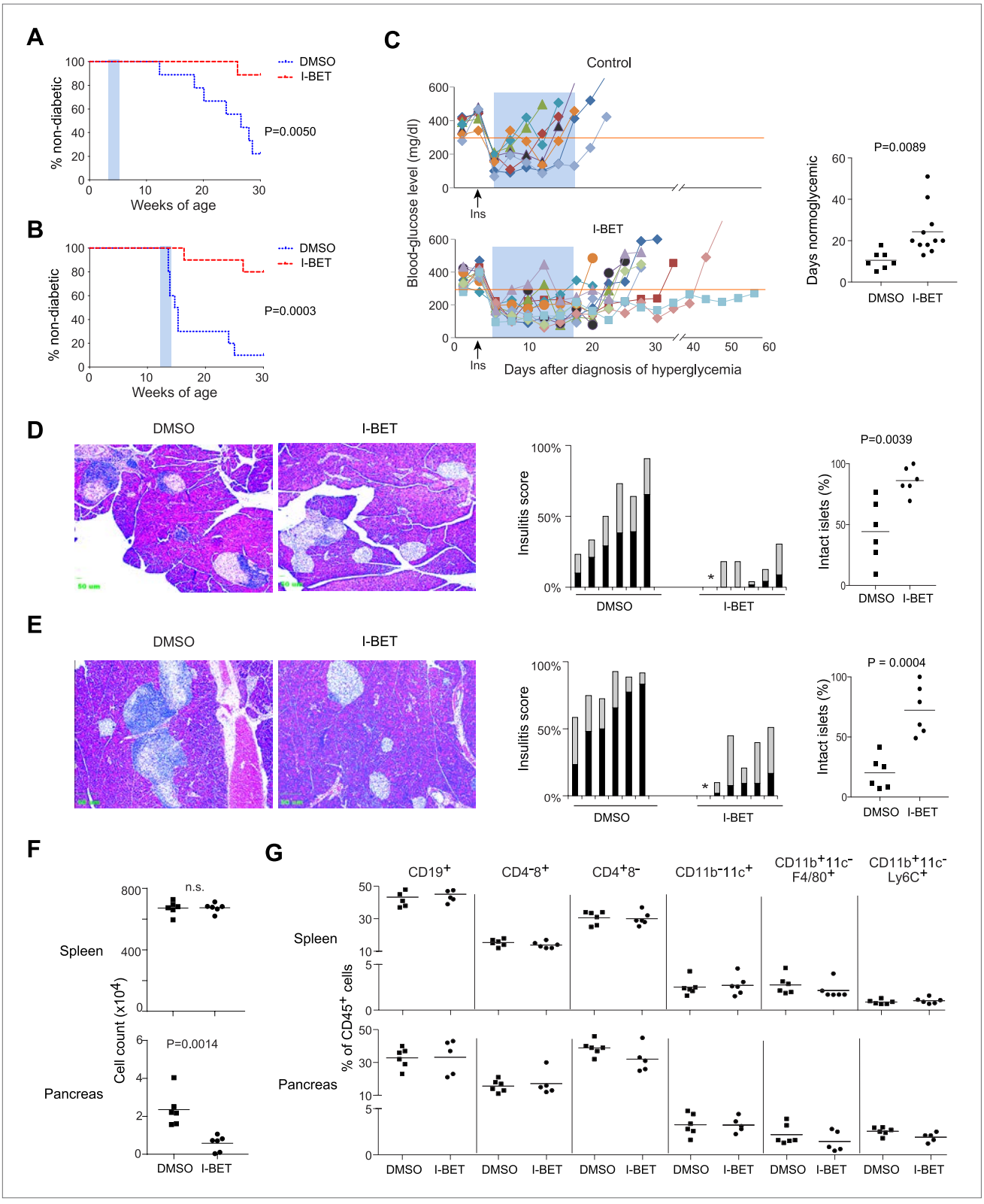

Figure 1. I-BET151 inhibits diabetes and insulitis in NOD mice. Female NOD mice were treated with I-BET151 in DMSO (10 mg/kg daily) or just DMSO from 3–5 weeks (**A** and **D**) or 12–14 weeks (**B** and **E**) of age. (**A** and **B**) Pre-diabetic mice. Hyperglycemia was monitored until 30 weeks of age. n = 10 per group. Blue shading = treatment window. (**C**) Recent-onset diabetic mice. Left: Individual blood-glucose curves. An insulin pellet was implanted subcutaneously within 1 day of diabetes diagnosis (arrow). 2 days later, I-BET151 (10 mg/kg) or DMSO was administered daily for 2 weeks (shaded blue). Right: duration of

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normoglycemia. n = 7 or 11. (**D** and **E**) Insulitis was visualized by H&E staining of paraffin sections. Left: representative histology. Middle: insulitis scores for individual mice. Grey = peri-insulitis; black = insulitis. The asterisk indicates no insulitis in any of the sections examined. Right: summary of the proportions of intact islets for individual mice. (**F**) Left: Total $CD45^+$ cells from the spleen (upper panel) or pancreatic islets (lower panel) from mice treated with I-BET151 or DMSO as per ***Figure 1B***, and analyzed at 14 weeks. n = 6. (**G**) Summary data on the major immune-cell subsets as a fraction of $CD45^+$ cells, from the spleen (upper panels) or pancreas (lower panels). n = 5 or 6. p values in **A** and **B** are from Gehan-Breslow-Wilcoxon tests and in **C**–**G** are from Student's *t* tests.
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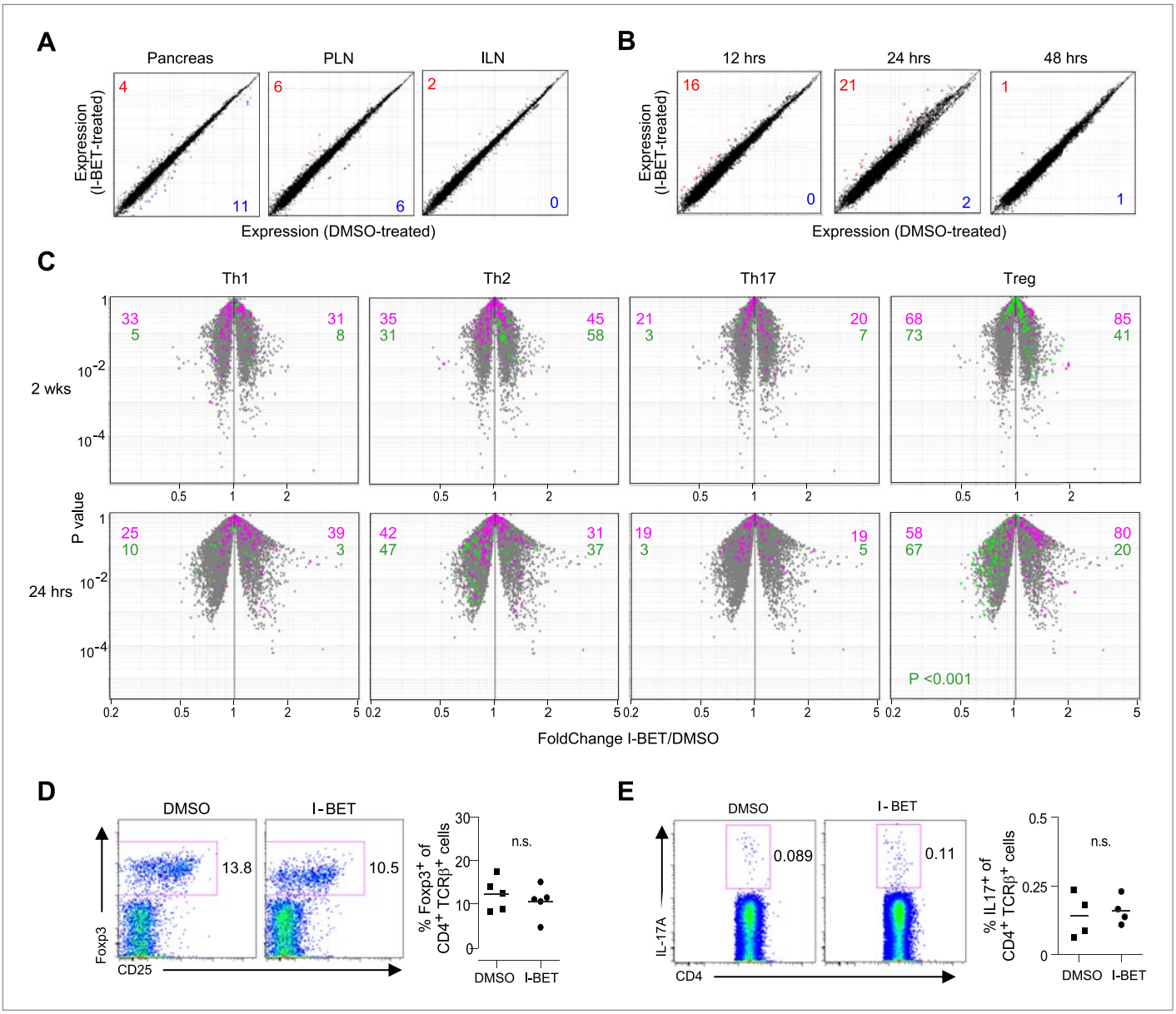

Figure 2. Little impact of BET-protein inhibition on CD4+ T cells in NOD mice. (**A**) Microarray-based transcriptional profiling of TCR+CD4+ cells sorted from pancreata, pancreatic lymph nodes (PLNs) and inguinal lymph nodes (ILNs). Comparison plot of I-BET151- and DMSO-treated mice as per ***Figure 1B*** and analyzed at 14 weeks of age. Red, transcripts increased >twofold by I-BET151; blue, transcripts >twofold decreased. (**B**) Analogous plots of TCR+CD4+ cells sorted from the pancreas of mice given a single I-BET151 (10 mg/kg) or DMSO injection, and analyzed 12, 24 or 48 hr later. (**C**) Th1, Th2, Th17 or Treg signatures (see 'Materials and methods') were superimposed on volcano plots comparing the transcriptomes of TCR+CD4+ cells from the pancreas of mice treated with I-BET151 or DMSO either as per ***Figure 1B*** and analyzed at 14 weeks of age (upper panels) or with a single injection and analyzed 24 hr later (lower panels). Purple: over-represented signature transcripts; Green: under-represented signature transcripts. (**D** and **E**) Proportions of Treg (**D**) or Th17 (**E**) cells within the TCR+CD4+ population in the pancreas of I-BET151- or DMSO-treated mice. Left, representative cytofluorometric dot plots; right, summary data. n = 4–5. p values in panel **C** are from the Chi-squared test (the single significant value is shown; all others were not significant) and in **D**–**E** from Student's *t* tests.

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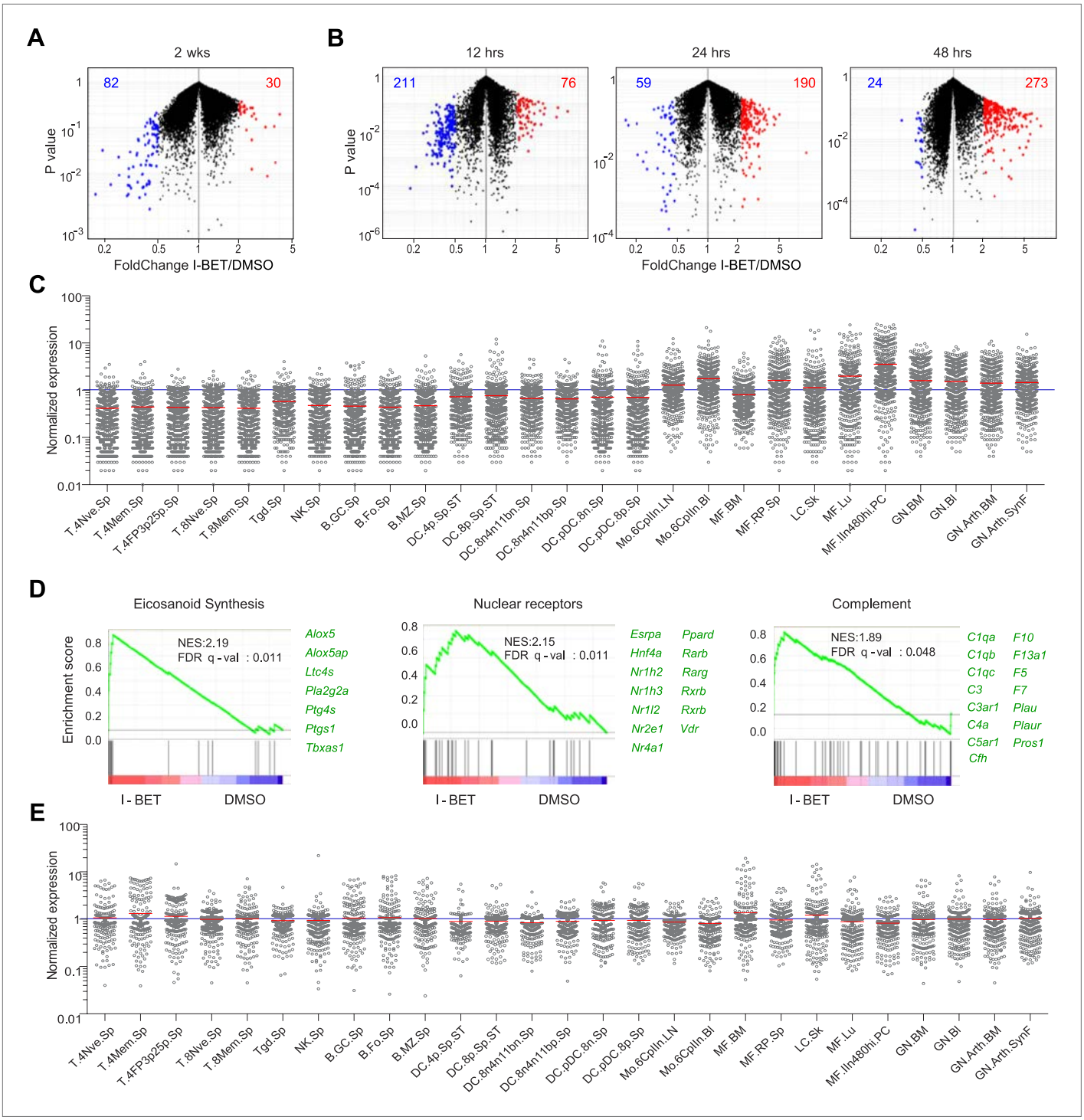

Figure 3. I-BET151 treatment promotes an MF-like, anti-inflammatory transcriptional program in pancreatic CD45⁺ cells. (**A** and **B**) A volcano plot comparing the transcriptomes of pancreatic CD45⁺ cells from mice treated with I-BET151 or DMSO as per ***Figure 1B***. Red: transcripts increased >twofold; blue: transcripts decreased >twofold; numbers of modulated transcripts are indicated in the corresponding color. (**B**) Analogous plots for mice given a single injection of I-BET151 (10 mg/kg) or DMSO only, and analyzed 12, 24 or 48 hr later. (**C**) Cell-type distribution of the totality of transcripts whose expression was increased >twofold in panels **A** and **B** (red). Expression data for and definition of the various cell-types came from ImmGen (www.Immgen.org). Langerhans cells of the skin (LC.SK) have been re-positioned as per recent data (***Gautier et al., 2012***). Expression values were row-normalized. (**D**) GSEA of the totality of transcripts increased in pancreatic CD45⁺ cells of mice treated with I-BET151 (red dots in panels **A** and **B**). NES, normalized enrichment score. Representative genes showing increased expression on the right. (**E**) A plot analogous to that in panel **C** for the totality of transcripts >twofold under-represented in drug-treated mice (blue dots in panels **A** and **B**).

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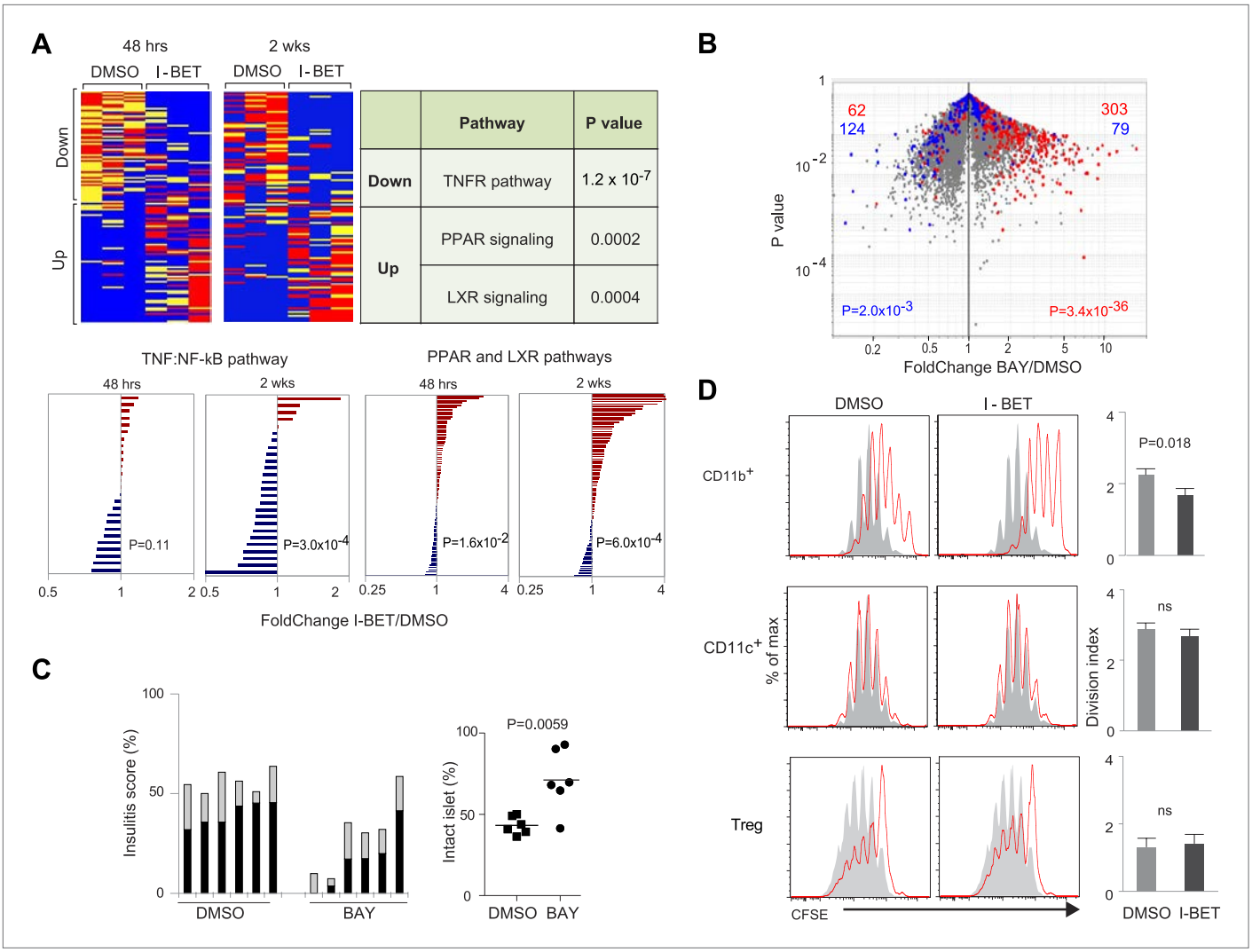

Figure 4. The NF-κB signaling pathway is a major focus of I-BET151's influence on NOD leukocytes. (**A**) Upper panels: The inhibitor's effect on NF-κB-regulated genes—defined as per http://www.bu.edu/nf-kb/gene-resources/target-genes. Left, relevant transcripts from pancreatic CD45$^+$ cells of NOD mice treated long- or short-term with I-BET151 or DMSO. Red: over-represented; blue: under-represented. 2 wk: long-term, treatment as per ***Figure 1B***; 48 hr: short-term, treatment with a single 10 mg/kg dose and analyzed 48 hr later. Right, signaling pathways represented by the enriched or impoverished transcripts in the data to the left, via Ingenuity pathway analysis (www.ingenuity.com). Lower panels: Gene sets corresponding to the TNFα-induced canonical NF-κB pathway (***Schaefer et al., 2009***) or the PPAR and LXR pathways (http://www.genome.jp/kegg/pathway/hsa/hsa03320.html) were retrieved from the Broad Institute's Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb), and their expression levels in CD45$^+$ cells from pancreas of I-BET151- or vehicle-treated mice plotted. (**B**) 12-week-old NOD mice were injected once ip with BAY 11–7082 (10 mg/kg), sacrificed 24 hr later, and CD45$^+$ cells from the pancreas isolated and transcriptionally profiled. A volcano plot comparing treatment with BAY 11–7082 and DMSO, with genes >twofold increased (in red) or decreased (in blue) by I-BET151 treatment (pooled from all time-points of ***Figure 3A,B***) superimposed. (**C**) Effect of a single dose of 10 mg/kg BAY 11–7082 on insulitis in 12-week-old NOD mice, analyzed 24 hr after injection. Left: insulitis scores. Right: summary data for the fraction of islets with no infiltrate. Grey, peri-insulitis; Black, insulitis. (**D**) Suppression of in vitro T cell proliferation by cell populations isolated from the pancreas of I-BET151- or DMSO- treated mice (as per ***Figure 1B***). The CD11b$^+$CD11c$^-$ (top), CD11b$^-$CD11c$^+$ (middle) and TCRβ$^+$CD4$^+$CD25$^+$ (bottom) fractions of CD45$^+$ cells were sorted. To the left are representative plots of CFSE dilution; to the right are summary data quantifying division indices (see 'Materials and methods' for details). p values in **A** and **B** are from the chi-squared test, and in **C** and **D** are from the Student's *t* test.

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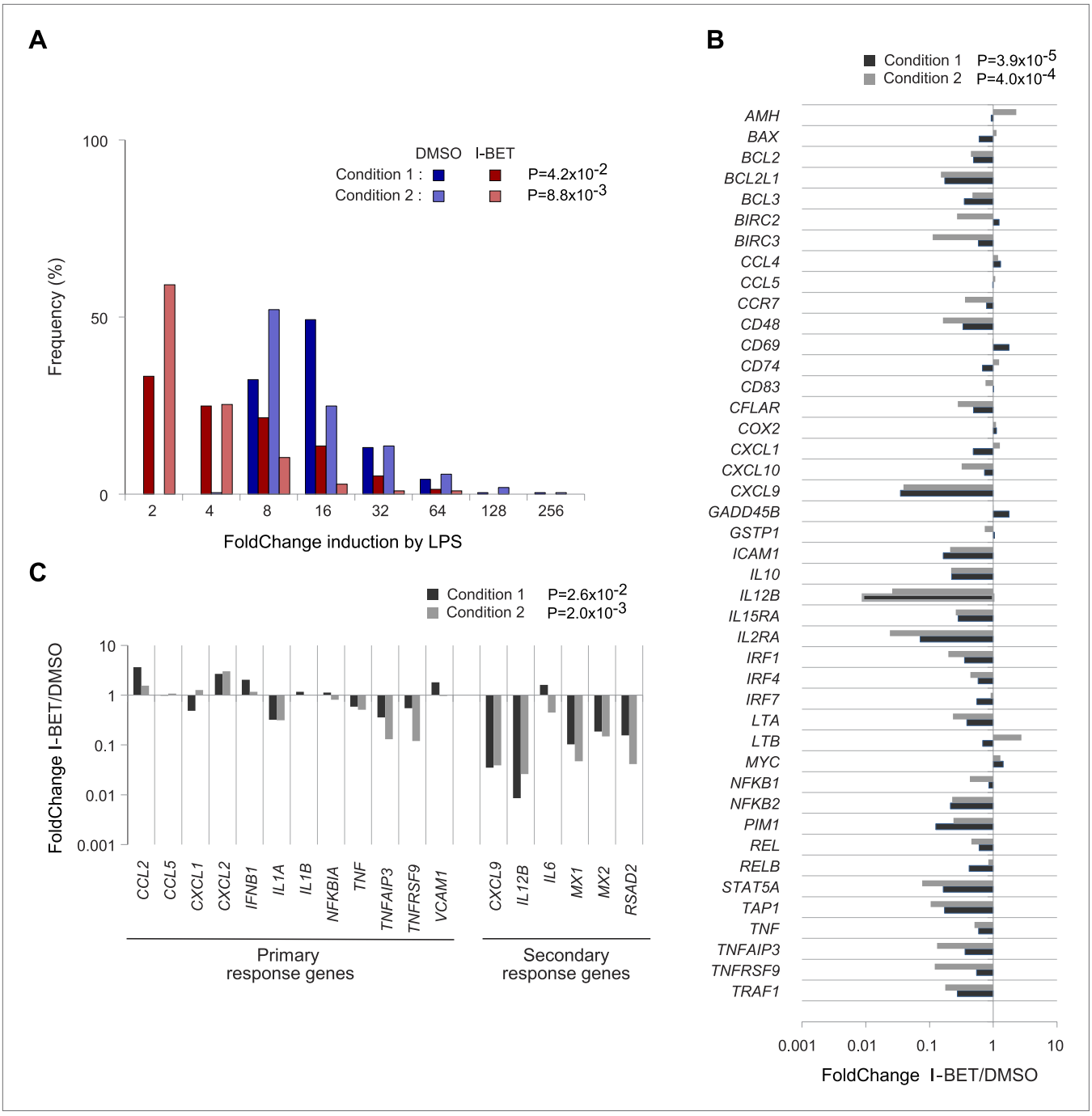

Figure 5. Effects of I-BET151 on human monocyte-derived MFs. Cultures of human MFs were differentiated from peripheral-blood-derived CD14⁺ cells, pre-cultured for 30 min with I-BET151 (red bars) or just DMSO (blue bars), and stimulated for another 4 hr after the addition of LPS (100 ng/ml) or vehicle only. Microarray data from two conditions, as indicated and detailed in 'Materials and methods'. (**A**) Distribution of FCs of I-BET151/DMSO for LPS-induced genes (according to the data on human monocyte-derived MFs treated with LPS or just vehicle). (**B**) FCs of I-BET151/DMSO of the human orthologues of the set of murine NF-κB-regulated genes illustrated in ***Figure 4A***. (**C**) Effects of I-BET151 on primary and secondary LPS-response genes (defined as per [***Hargreaves et al., 2009***; ***Ramirez-Carrozzi et al., 2009***]).

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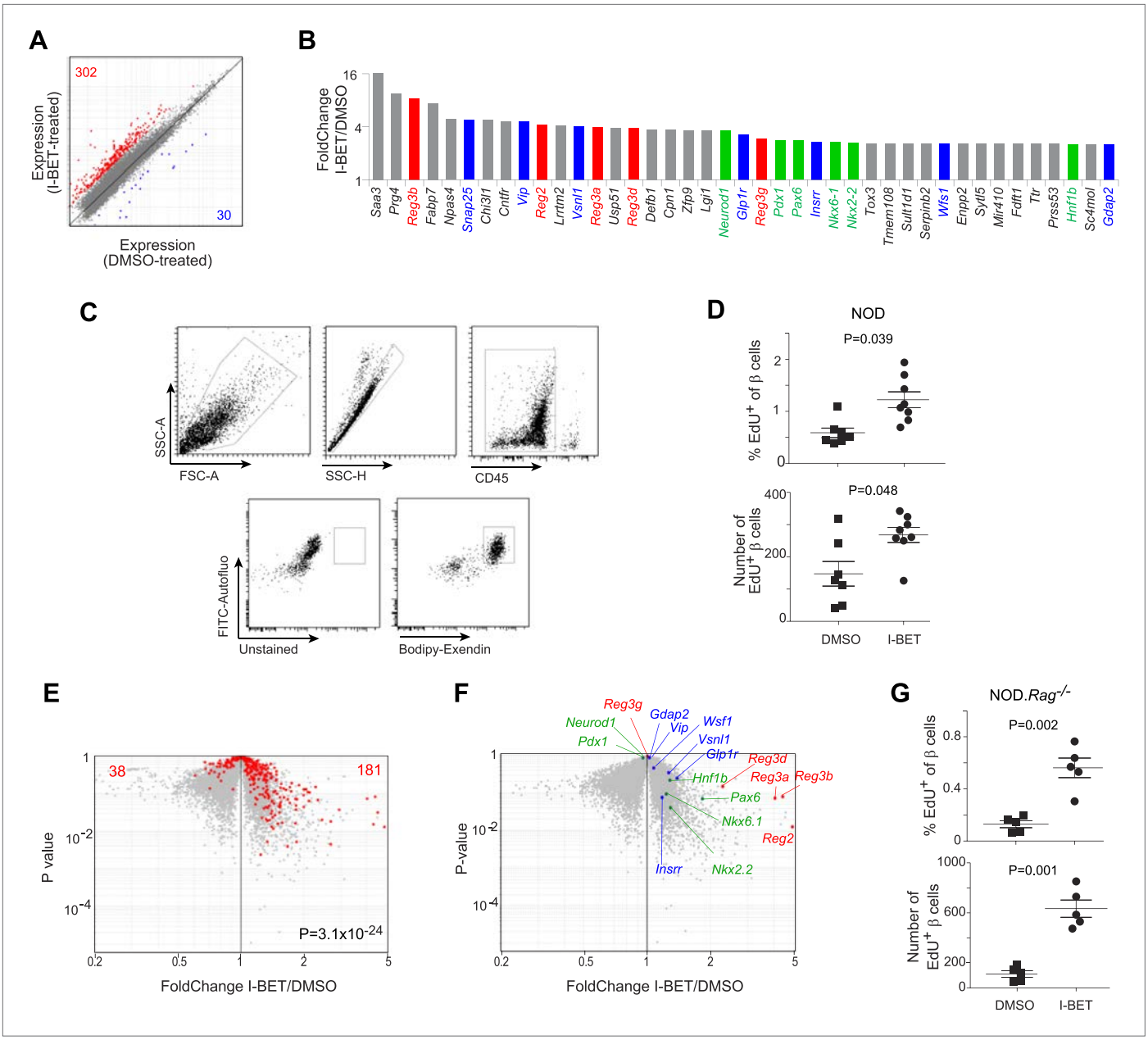

Figure 6. BET-protein inhibition promotes regeneration of islet β cells. (**A**) Pancreatic β cells were cytofluorometrically sorted from mice treated with I-BET151 or DMSO as per ***Figure 1B***, and microarray-based transcriptional profiling performed. Red: transcripts increased >twofold by I-BET151; blue, transcripts >twofold decreased. (**B**) NOD β-cell transcripts increased by I-BET151 ranked by FC vis-à-vis DMSO treatment. Red, regenerating islet-derived (Reg) transcripts; green, transcripts encoding transcription factors important for β-cell differentiation and function; blue, transcripts encoding proteins that enhance insulin production. (**C**, **D**, **G**) Cytofluorometric quantification of EdU^{+} β cells from NOD (**D**) or NOD.Rag$^{-/-}$ (**G**) mice treated with I-BET151 or DMSO as in ***Figure 1B*** and injected with EdU during the last 24 hr n = 5–8. Panel **C** shows the sorting strategy. (**E**) The set of red transcripts from panel **A** was superimposed on volcano plots comparing gene expression by β cells from NOD.Rag$^{-/-}$ mice treated as in ***Figure 1B*** with I-BET151 vs DMSO. (**F**) Relevant I-BET151-induced transcripts highlighted in panel **B** are situated on the volcano plot of panel **E**. (**G**) p values: *<0.05; **<0.01; *<0.001—from the Student's *t* test for panels **D** and **G**, and from the chi-squared test for panel **E**.

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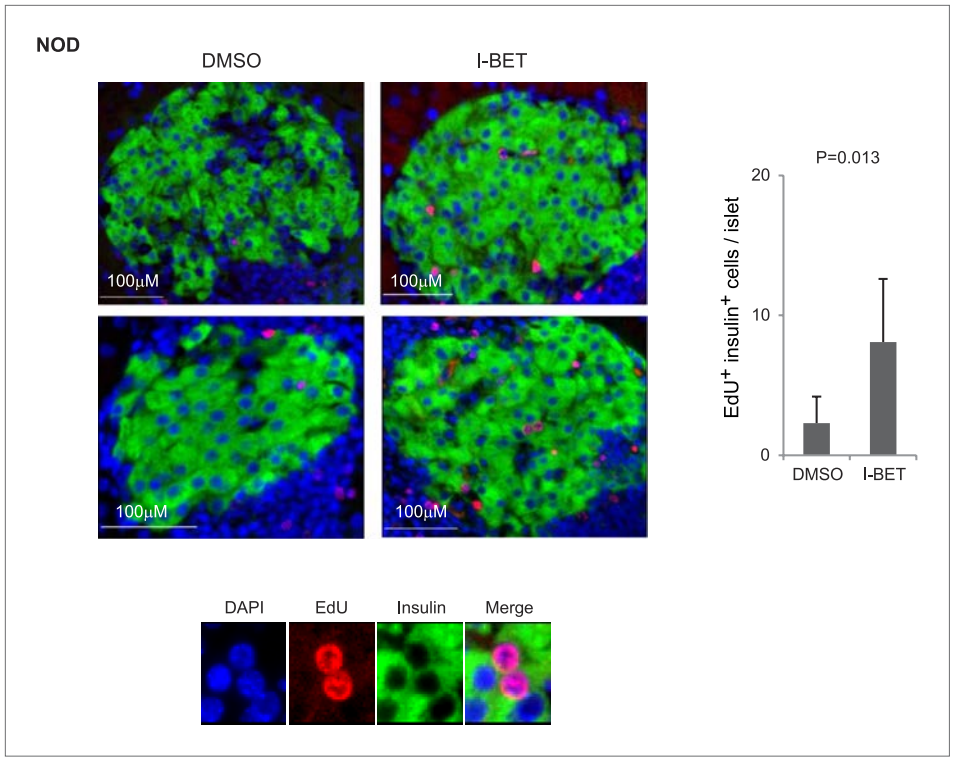

Figure 6—figure supplement 1. Histologic analysis of β-cell proliferation in response to I-BET151 in NOD mice. NOD mice were treated with I-BET151 or DMSO as in ***Figure 1B***. EdU was administrated during the last 24 hr. Frozen sections of pancreas were stained for insulin and EdU. Left, representative islet images; two for each condition; right, summary quantification. Small images in color are legends for insulin, EdU and DAPI, respectively. Red, EdU; green, insulin; blue, DAPI.

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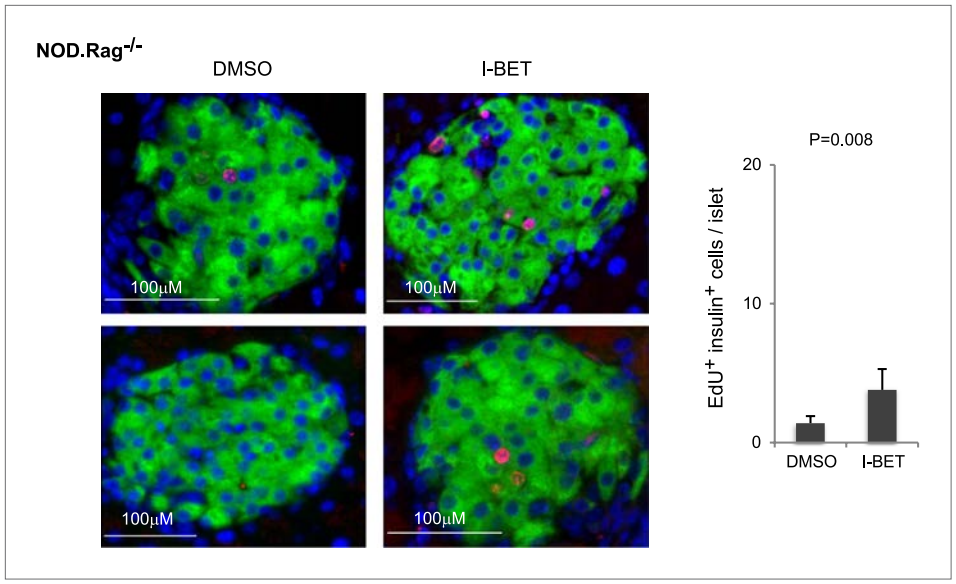

Figure 6—figure supplement 2. Histologic analysis of β-cell proliferation in response to I-BET151 in NOD.Rag$^{-/-}$ mice. NOD.Rag$^{-/-}$ mice were treated with I-BET151 or DMSO as in ***Figure 1B***. EdU was administrated during the last 24 hr. Frozen sections of pancreas were stained for insulin and EdU. Left, representative islet images, two for each condition; right, summary quantification.

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