Expanded View Figures

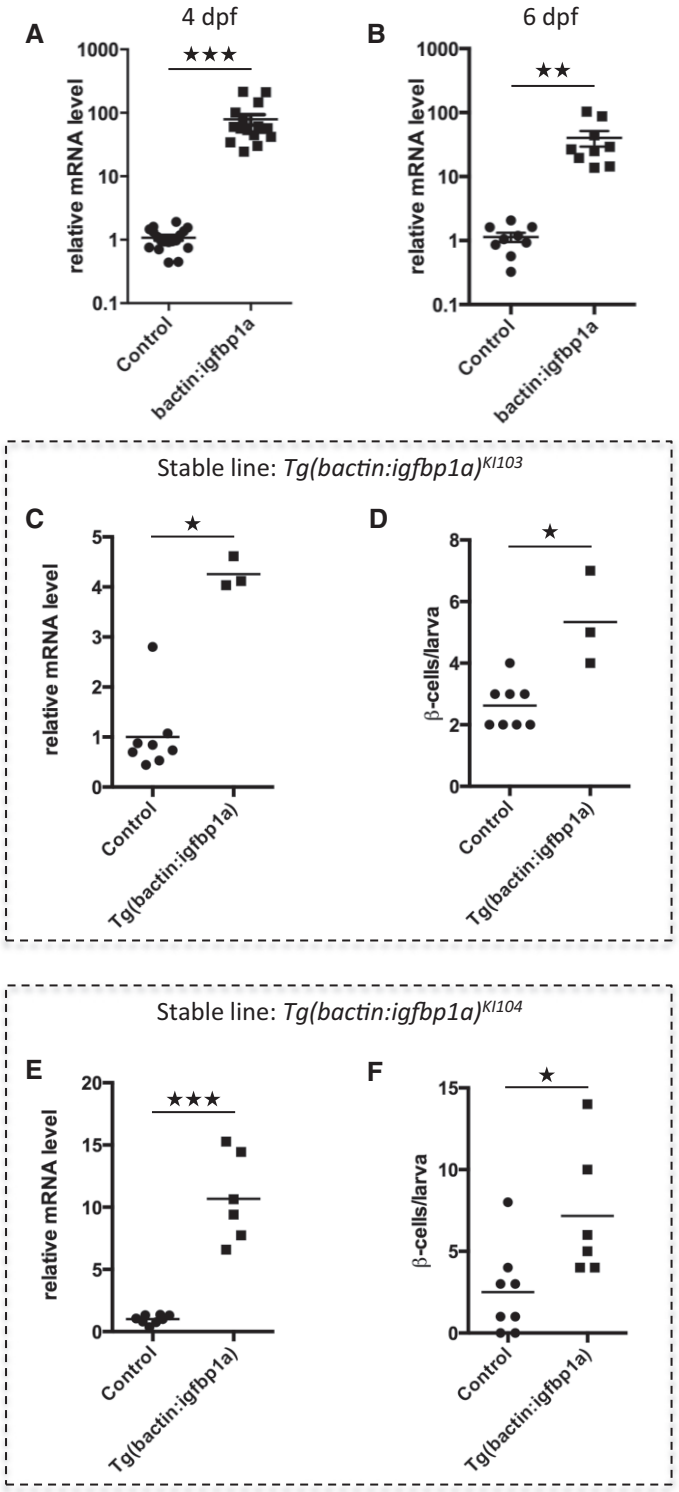

Figure EV1. Expression level of *igfbp1a* in mosaic over-expressing larvae and stable transgenic lines.

A, B Relative *igfbp1a* mRNA levels in mosaic *bactin:igfbp1a* transgenic larvae. Control or *bactin:igfbp1a*-injected embryos were collected for RNA purification and qPCR analysis at 4 dpf (A) or 6 dpf (B). *Igfbp1a* expression was approximately 80 times higher in *bactin:igfbp1a* overexpressing larvae than in control larvae. ***$P < 0.0001$ and **$P = 0.0024$. Each data point represents one larva; thus, it can be seen that substantial *Igfbp1a* overexpression was attained in all *bactin:igfbp1a*-injected embryos that generated mosaically overexpressing larvae.

C–F Relative *igfbp1a* mRNA levels (C, E) in two independent stable *Tg*(*bactin:igfbp1a*) lines, which have the line designations KI103 and KI104. Control and *Tg*(*bactin:igfbp1a*) larvae were processed for qPCR analysis at 6 dpf. Both transgenic lines (data for each line are shown separately, in panels C and E) have moderately, but significantly, higher expression of *igfbp1a* than sibling controls; *$P = 0.0121$ and ***$P = 0.0007$, respectively. (D, F) Like the larvae mosaically overexpressing *igfbp1a*, the stable *Tg*(*bactin:igfbp1a*) lines also have an increase in β-cell regeneration. We crossed the *Tg*(*bactin:igfbp1a*) lines into the double transgenic *Tg*(*ins:H2B-GFP*);*Tg*(*ins:Flag-NTR*) to allow visualization of β-cell regeneration. We treated the resulting offspring with metronidazole (MTZ) from 3 to 4 dpf to ablate the β cells and then allowed them to regenerate until 6 dpf. Both *Tg*(*bactin:igfbp1a*)^{KI103} and *Tg*(*bactin:igfbp1a*)^{KI104} stable lines had a significant increase in β-cell regeneration (*$P = 0.0121$ and *$P = 0.0107$, respectively).

Data information: Results are presented as mean and individual values and analyzed with *t*-tests (A, B) or Mann-Whitney tests (C–F). Related to Fig 1.

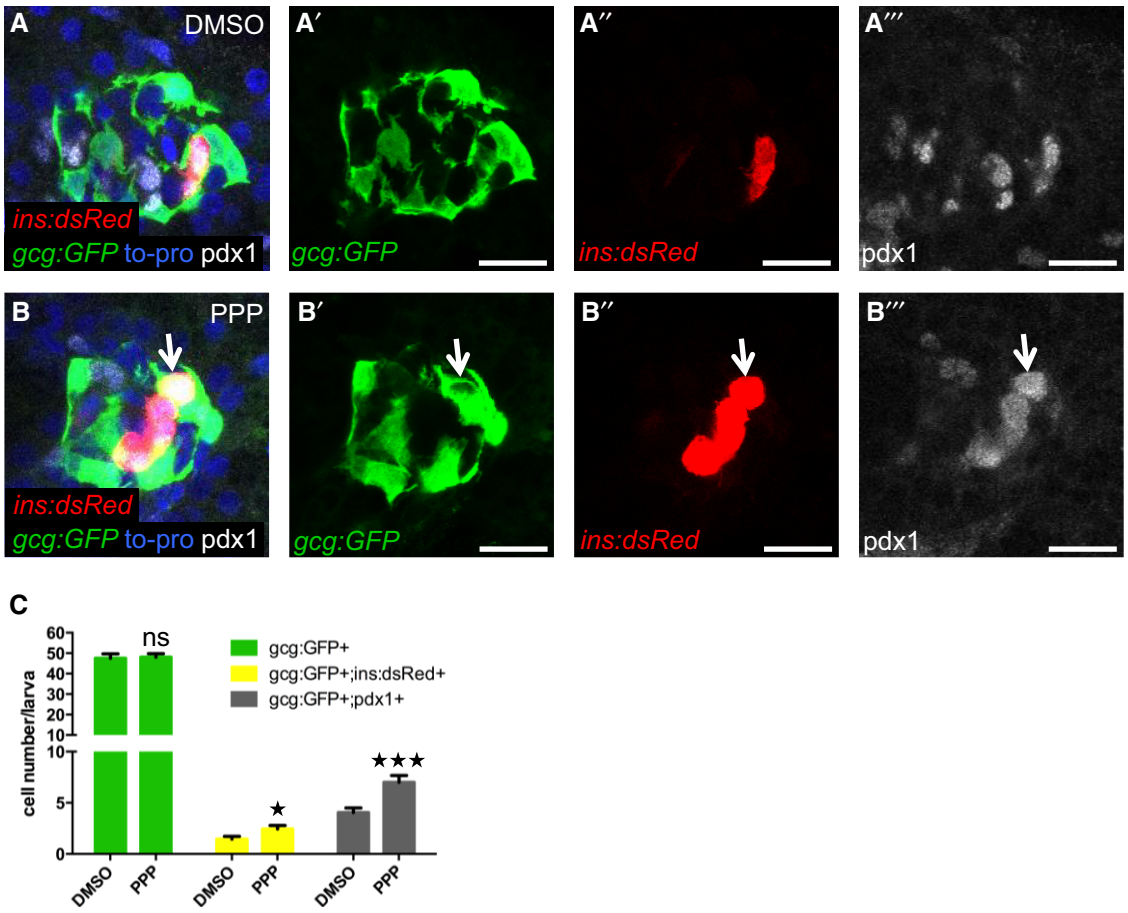

Figure EV2. The IGF1R inhibitor PPP increases β-cell regeneration and co-expression of insulin and pdx1 with glucagon.

A–C Pdx1 expression in 6-dpf larvae during regeneration. *Tg*(*ins:dsRed*);*Tg*(*gcg:GFP*);*Tg*(*ins:Flag-NTR*) larvae were treated with MTZ from 3 to 4 dpf to ablate the β cells and subsequently treated with DMSO or the IGF1R inhibitor PPP from 4 to 6 dpf. Representative confocal images (A, B) at 6 dpf of DMSO- or PPP-treated larvae. A $gcg^+ins^+pdx1^+$ cell is indicated by an arrow. Scale bars: 15 μm. (C) Quantification of gcg^+, gcg^+ins^+, and $pdx1^+gcg^+$ cells (P = 0.8409, $^*P$ = 0.0232, and $^{***}P$ = 0.0007, respectively) in DMSO- and PPP-treated groups. n = 20 for each group. Results are presented as mean values ± SEM and analyzed with ANOVA. Related to Fig 4.

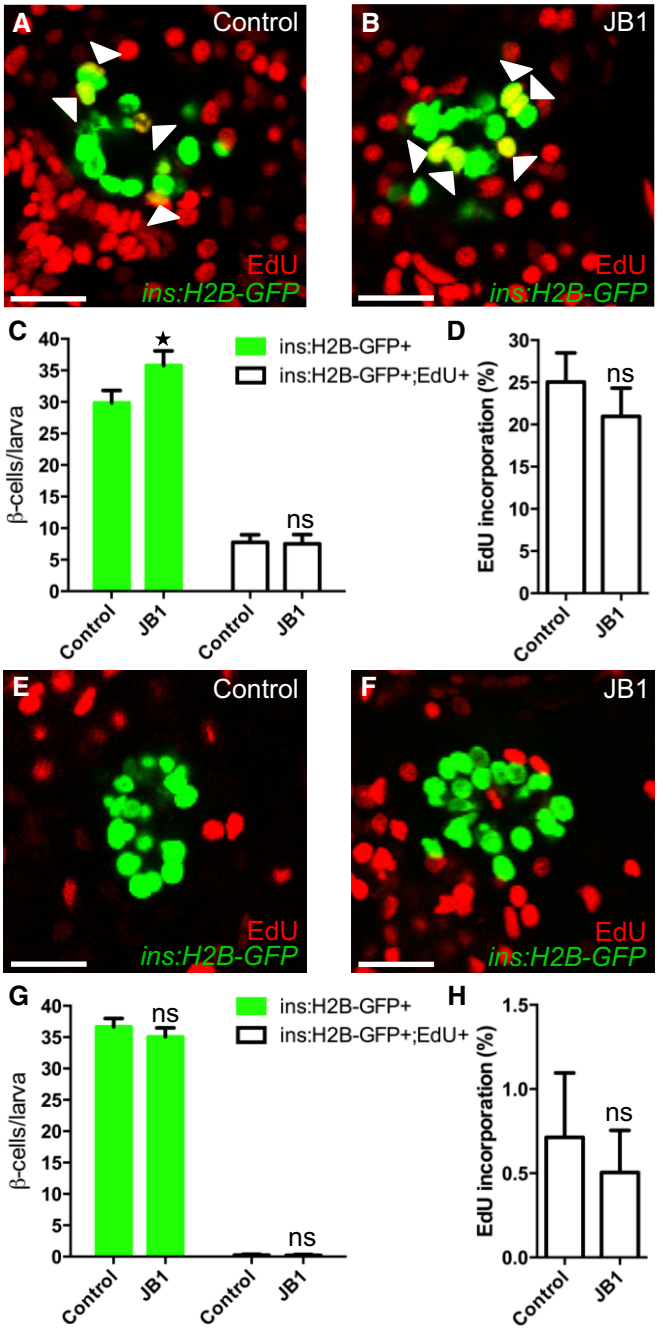

Figure EV3. The IGF1R inhibitor JB1 promotes β-cell regeneration.

A–D *Tg(ins:H2B-GFP);Tg(ins:Flag-NTR)* larvae were treated with MTZ from 3 to 4 dpf to ablate the β cells. DMSO or JB1 was then injected into the larval pericardial cavity at 4 dpf, and the larvae were treated with EdU from 4 to 6 dpf. Representative confocal images (A, B) at 6 dpf of DMSO- and JB1-injected larvae, showing β cells in green and the β cells that incorporated EdU as yellow (green and red overlap; see arrowheads). Scale bars: 20 μm. (C) Quantification of the number of all β cells (green bars) and of β cells that incorporated EdU (white bars) per larva at 6 dpf. **P* = 0.0422; ns=non-significant (*P* = 0.9944). (D) Rate of β-cell proliferation, shown as the percentage of β cells that incorporated EdU. ns=non-significant (*P* = 0.5885). *n* = 12 larvae in the DMSO-injected group; *n* = 19 larvae in the JB1-injected group.

E–H To determine whether inhibition of Igf signaling affects β-cell proliferation during regular development, we treated DMSO- or JB1-injected *Tg(ins:H2B-GFP)* larvae with EdU in the absence of β-cell ablation. DMSO or JB1 was injected into pericardial cavity of *Tg(ins:H2B-GFP)* larvae at 4 dpf, and the larvae were then treated with EdU from 4 to 6 dpf. Representative confocal images (E, F) at 6 dpf of DMSO- and JB1-injected larvae, showing β cells in green and the cells that incorporated EdU in red. Scale bars: 20 μm. (G) Quantification of the number of all β cells (green bars) and of β cells that incorporated EdU (white bars) from 4 to 6 dpf. ns=non-significant (*P* = 0.4088 and 0.9997, respectively). (H) Rate of β-cell proliferation, shown as the percentage of β cells that incorporated EdU. *P* = 0.8504. *n* = 20 larvae in the DMSO-injected group, *n* = 27 larvae in the JB1-injected group.

Data information: Results are presented as mean values ± SEM and analyzed with two-way ANOVA (C, D, G and H). Related to Fig 5.

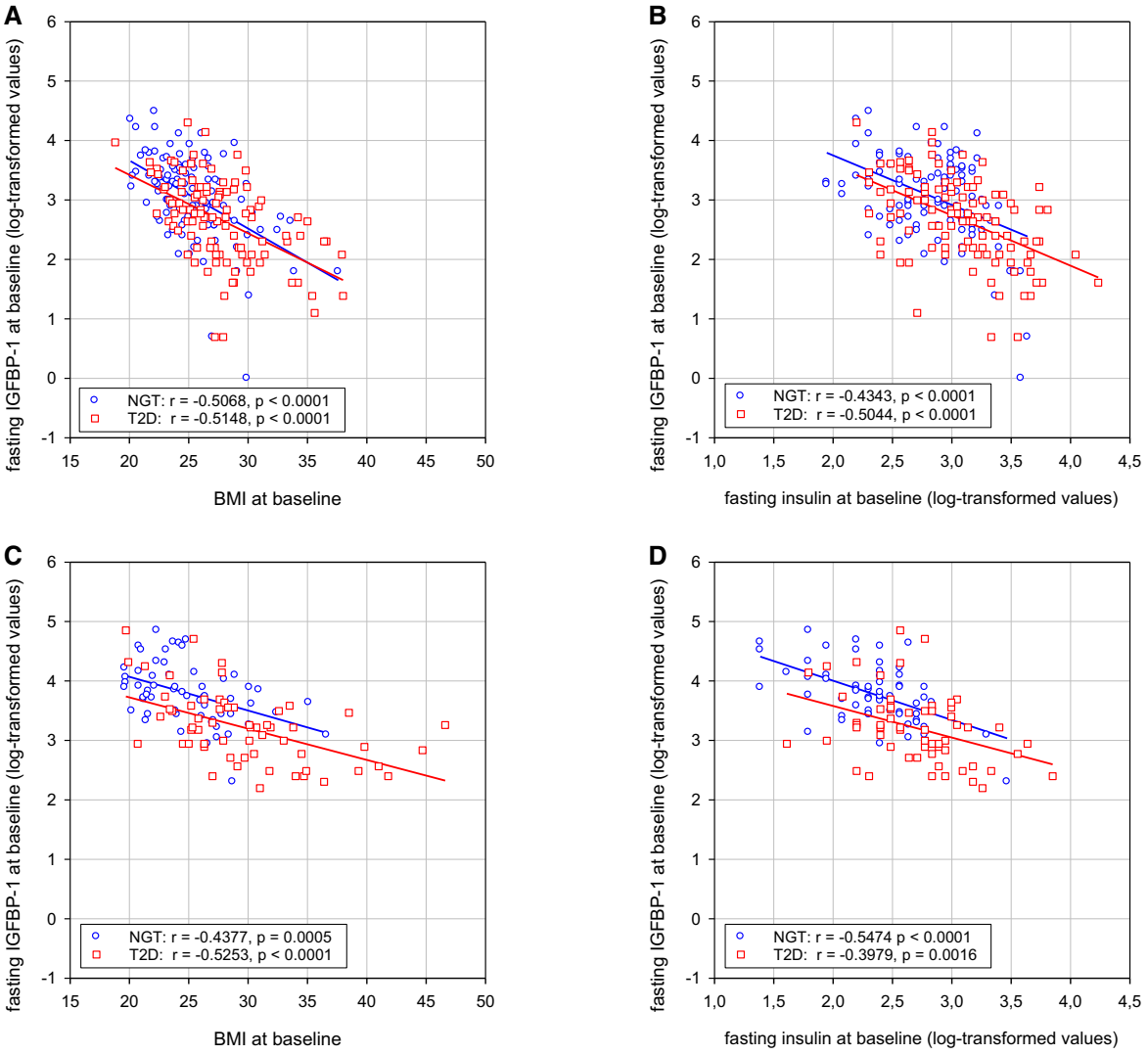

Figure EV4. Correlations of IGFBP1 levels with BMI and insulin levels.

A–D Correlations between baseline levels of fasting IGFBP1 levels and BMI (A, C) or fasting insulin levels (B, D) in men (A, B) and women (C, D). The correlations between these baseline values are significant both in subjects who developed T2D (red squares) and in controls with a normal glucose tolerance (NGT) (blue circles) at follow-up after 8–10 years. Results are presented as individual values with linear regression. Related to Table 1.