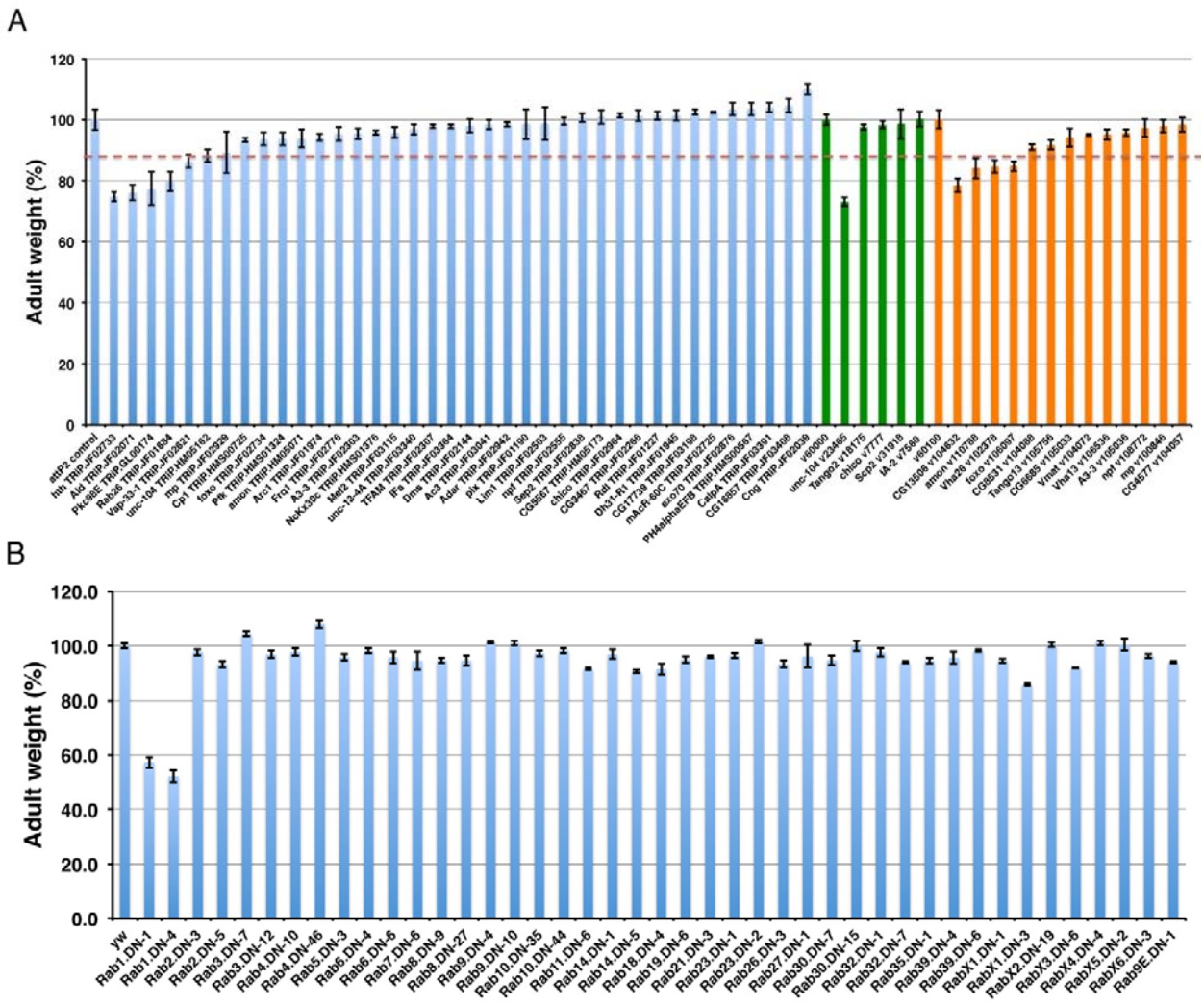


**Figure S1 RNAi screen of IPC-enriched genes and Rab-DNs for growth phenotypes. (A)** 56 *UAS-RNAi* lines covering 50 genes were crossed to *Ilp2-Gal4* and the progeny male adults were examined for their weights. These genes represent a wide range of molecular functions. They encode signaling molecules, transcription factors, neuropeptide receptors, motor proteins, sugar metabolic enzymes, synapse organizers, and etc. RNAi lines from different RNAi libraries are color-coded. Blue: Harvard TRiP lines; Green: VDRC GD lines; Orange: VDRC KK lines. The red-dashed line indicates the 90% adult weight cutoff. Error bars represent S.E.M. **(B)** 43 *UAS-Rab-DNs* lines covering 29 Rabs were crossed to *Ilp2-Gal4* and the progeny male adults were examined for their weight. Error bars represent S.E.M.

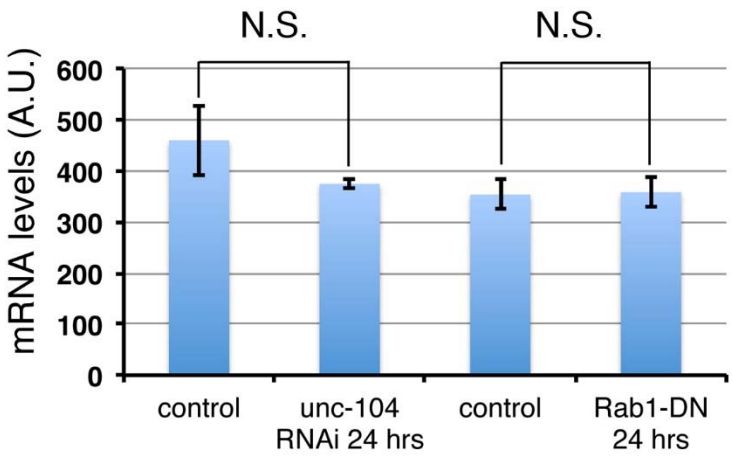


**Figure S2 Measurement of larval brain *Ilp2* transcript levels when *unc-104* mRNA was depleted or Rab1 protein function was inhibited.** *Ilp2* transcript levels were compared by quantitative RT-PCR between control and *unc-104* knockdown in IPCs for 24 hrs (*tubGal80$^{ts}$*, *Ilp2>unc-104 RNAi*), or Rab1 inhibition in IPCs for 24 hrs (*tubGal80$^{ts}$*, *Ilp2>Rab1-DN*). *Rpl32* was used as an internal control. N.S.: non significant. Error bars represent S.E.M.

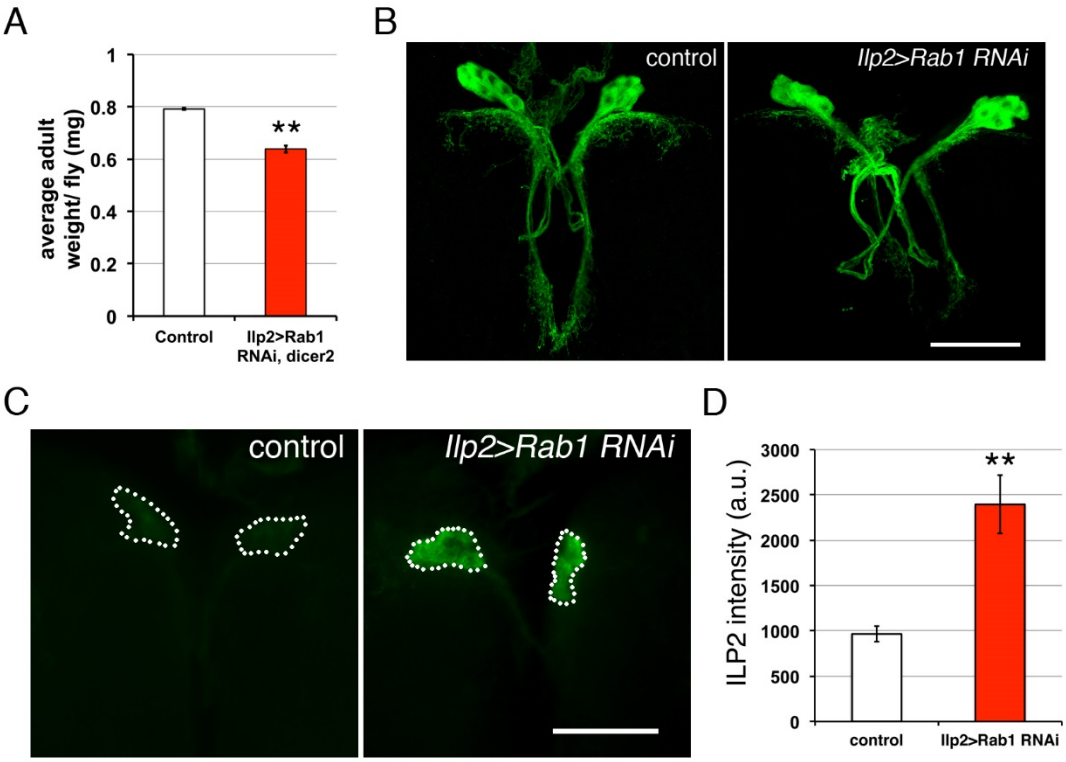


**Figure S3 Expressing Rab1 RNAi in IPCs results in accumulation of ILP2 in IPCs. (A)** Adult weights were compared between control flies (*Ilp2-Gal4>attP2 control*, n = 63 flies) and flies expressing *Rab1 RNAi* in IPCs (*Ilp2>Rab1 RNAi*, *dicer2*; n = 60 flies). **(B)** IPC neurite structure was labeled with *Ilp2>mCD8-GFP* in *Ilp2>Rab1 RNAi*, *dicer2* and control (*Ilp2>dicer2*, *attP2 control*) brains. **(C-D)** ILP2 fluorescence intensities in the brain IPC cell bodies were compared between control (n = 8 brains) and *Rab1 RNAi* expressed in IPCs (n = 8 brains). Scale bars: 50µm. Error bar: S.E.M. **p<0.01.

**Files S1-S4**

Available for download as .mov files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.160663/-/DC1

**File S1 Movie of IPCs at different developmental stages.** Green: *Ilp2>mCD8-GFP*; Red: *Ilp2>nuclear-RFP*; blue: DAPI.

**File S2 Rotating view of larval IPCs at 93 hrs after embryo deposition.** Green: *Ilp2>mCD8-GFP*; Red: *Ilp2>nuclear-RFP*.

**File S3 Rotating view of pupal IPCs at 146 hrs after embryo deposition.** Green: *Ilp2>mCD8-GFP*; Red: *Ilp2>nuclear-RFP*.

**File S4 Rotating view of adult IPCs at 239 hrs after embryo deposition.** Green: *Ilp2>mCD8-GFP*; Red: *Ilp2>nuclear-RFP.*

**File S5**

**Supplemental Materials and Methods**

**Detailed protocol for sequencing library construction**

1) 400ng of amplified mRNA was fragmented to 10-200nt using 10x RNA fragmentation buffer (Ambion) and was purified using regular ethanol precipitation method with 0.35µl of GlycoBlue (Ambion). 2) 3' end the RNA samples were dephosphorylated using 10x Antarctic Phosphatase Buffer and 0.5 µl Antarctic Phosphatase (NEB) at 37 °C for 20 minutes. The reaction was heat inactivated at 75°C for 10 minutes. 3) 5' end of RNA samples was phosphorylated using 10x T4 DNA ligase buffer (it has 1mM ATP final) and T4 PNK (NEB) at 37 °C for 30 minutes. The RNAs in the reactions were purified using ammonium acetate and ethanol precipitation with 2µl of GlycoBlue (Ambion). 4) The RNA samples are then ligated to 3' linker (5'-/5rApp/CTG TAG GCA CCA TCA AT/3ddC/-3') (synthesized by IDT) using T4 RNA ligase 1 (NEB), 5X ATP-free T4 RNA ligase buffer (16.5 mM DTT, 41.5% glycerol, 250 mM HEPES-KOH, pH8.3, 50 mM $MgCl_2$, 50 µg/ml acetylated BSA), and 10% DMSO at 37 °C for one hour. The RNAs in the reactions were purified using ammonium acetate and ethanol precipitation with 2µl of GlycoBlue (Ambion). The RNA samples were then run on 6% TBE-Urea PAGE Gel (Invitrogen). The 100-200nt bands were cut and elute overnight with 400µl stop solution (1M ammonium acetate and 10mM EDTA) at 4°C overnight. The RNAs in the supernatant was purified using regular ethanol precipitation method with 2µl of GlycoBlue (Ambion). 5) The RNA samples are ligated to 5' linker (with bar code) using T4 RNA ligase 1 (NEB), 10x T4 RNA ligase 1 buffer (NEB), and 10% DMSO at 37°C for 1 hour. The RNAs was purified by ammonium acetate and ethanol precipitation and gel purification as described in step 4. The 5' barcoded linkers are synthesized by IDT. IPC1: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rCrUrGrG-3', IPC2: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rCrGrUrC-3', Control 1: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rArCrUrU-3', Control 2: 5'-/5AmMC6/ ACG CTC TTC CGA TCT rCrCrCrU-3'. 6) cDNA of the RNA samples were reverse transcribed using SuperScript III (Invitrogen) following manufacture's protocol. The primer sequence used for reverse transcription is 5'-ATT GAT GGT GCC TAC AG-3'. 7) The cDNA samples were amplified using Taq (NEB) following manufacture's protocol. Forward primer: 5'-GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC T-3'. Reverse primer: 5'-CAA GCA GAA GAC GGC ATA CGA GCT CTT CCG ATC TAT TGA TGG TGC CTA CAG-3'. The PCR products (200-300nt) were purified using Qiagen PCR purification kit. The purified PCR samples were diluted to 10nM and were sequenced using Illumina GA II sequencing system.

**Table S1 Number of aligned mRNA reads to the *D. melanogaster* Refseq mRNA.**

| Samples | Barcode | Total # of post-filter reads | Mapped to dm3 genome and transcriptome |
|---|---|---|---|
| Control | ACTT | 1,077,110 | 239,757 |
| | TCGC | 3,175,833 | 398,659 |
| IPC | CTGG | 3,118,626 | 592,440 |
| | CGTC | 3,833,784 | 417,340 |

**Tables S2-S5**

Available for download as Excel files at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.160663/-/DC1

**Table S2** List of all genes with aligned reads in laser-captured IPC and control samples.

**Table S3** List of 193 IPC-enriched genes with their annotated molecular functions.

**Table S4** Full list of biological functional clusters annotated by DAVID with IPC-enriched transcripts.

**Table S5** List of 109 IPC-enriched genes and their mouse orthologs. The mouse orthologs that have higher expression levels in beta cells compared to other non-beta cell tissues (Ku et al.) are indicated.

**Table S6 *Drosophila* stocks used in this study.**

| | | |
|---|---|---|
| *yw* | *UAS YFP.Rab2 DN-5* | *UAS YFP.Rab23 DN-2* |
| $w^{1118}$ | *UAS YFP.Rab3 DN-7* | *UAS YFP.Rab26 DN-3* |
| *Ilp2-Gal4/Cy* (Eric Rulifson) | *UAS YFP.Rab3 DN-12* | *UAS YFP.Rab27 DN-1* |
| *UAS-GFP-myc-2xFYVE* (Bloomington) | *UAS YFP.Rab4 DN-10* | *UAS YFP.Rab30 DN-7* |
| *UAS-Grasp65-GFP* (Bloomington) | *UAS YFP.Rab 4 DN-46* | *UAS YFP.Rab30 DN-15* |
| *UAS-mCD8-GFP* (Liqun Luo) | *UAS YFP.Rab5 DN-3* | *UAS YFP.Rab32 DN-1* |
| *UAS-mtdTomato* (Liqun Luo) | *UAS YFP.Rab6 DN-4* | *UAS YFP.Rab32 DN-7* |
| *UAS- nuclear-RFP* (Liqun Luo) | *UAS YFP.Rab6 DN-6* | *UAS YFP.Rab35 DN-1* |
| *UAS-Tau-LacZ* (Bloomington) | *UAS YFP.Rab7 DN-6* | *UAS YFP.Rab39 DN-4* |
| *UAS-Khc::nod-LacZ* (Bloomington) | *UAS YFP.Rab8 DN-9* | *UAS YFP.Rab39 DN-6* |
| *UAS-dicer2* (VDRC) | *UAS YFP.Rab8 DN-27* | *UAS YFP.RabX1 DN-1* |
| *yv; attP2, y+* (control for TRiP RNAi lines, Bloomington) | *UAS YFP.Rab9 DN-4* | *UAS YFP.RabX1 DN-3* |
| *UAS-lamin-GFP* (Bloomington) | *UAS YFP.Rab9 DN-10* | *UAS YFP.RabX2 DN-19* |
| *Rab1-Gal4* (Robin Hiesinger) | *UAS YFP.Rab10 DN-35* | *UAS YFP.RabX3 DN-6* |
| *Ilp2-Gal4* (Ping Shen) | *UAS YFP.Rab10 DN-44* | *UAS YFP.RabX4 DN-4* |
| *UAS-unc-104-GFP* (Bill Saxton) | *UAS YFP.Rab11 DN-6* | *UAS YFP.RabX5 DN-2* |
| *UAS-unc-104-mCherry-HA* (Tom Schwarz) | *UAS YFP.Rab14 DN-1* | *UAS YFP.RabX6 DN-3* |
| *UAS-ANF-GFP* (Bill Saxton) | *UAS YFP.Rab14 DN-5* | *UAS YFP.Rab9E DN-1* |
| *Elav-Gal4* (Bloomington) | *UAS YFP.Rab18 DN-4* | *UAS YFP.Rab1 WT-1* |
| *UAS YFP.Rab1 DN-1* | *UAS YFP.Rab19 DN-6* | *VDRC RNAi* lines |
| *UAS YFP.Rab1 DN-4* | *UAS YFP.Rab21 DN-3* | *TRiP RNAi* lines |
| *UAS YFP.Rab2 DN-3* | *UAS YFP.Rab23 DN-1* | $tub\text{-}Gal80^{ts}$ (Bloomington) |