
Figures and figure supplements

Delivery of circulating lipoproteins to specific neurons in the *Drosophila* brain
regulates systemic insulin signaling

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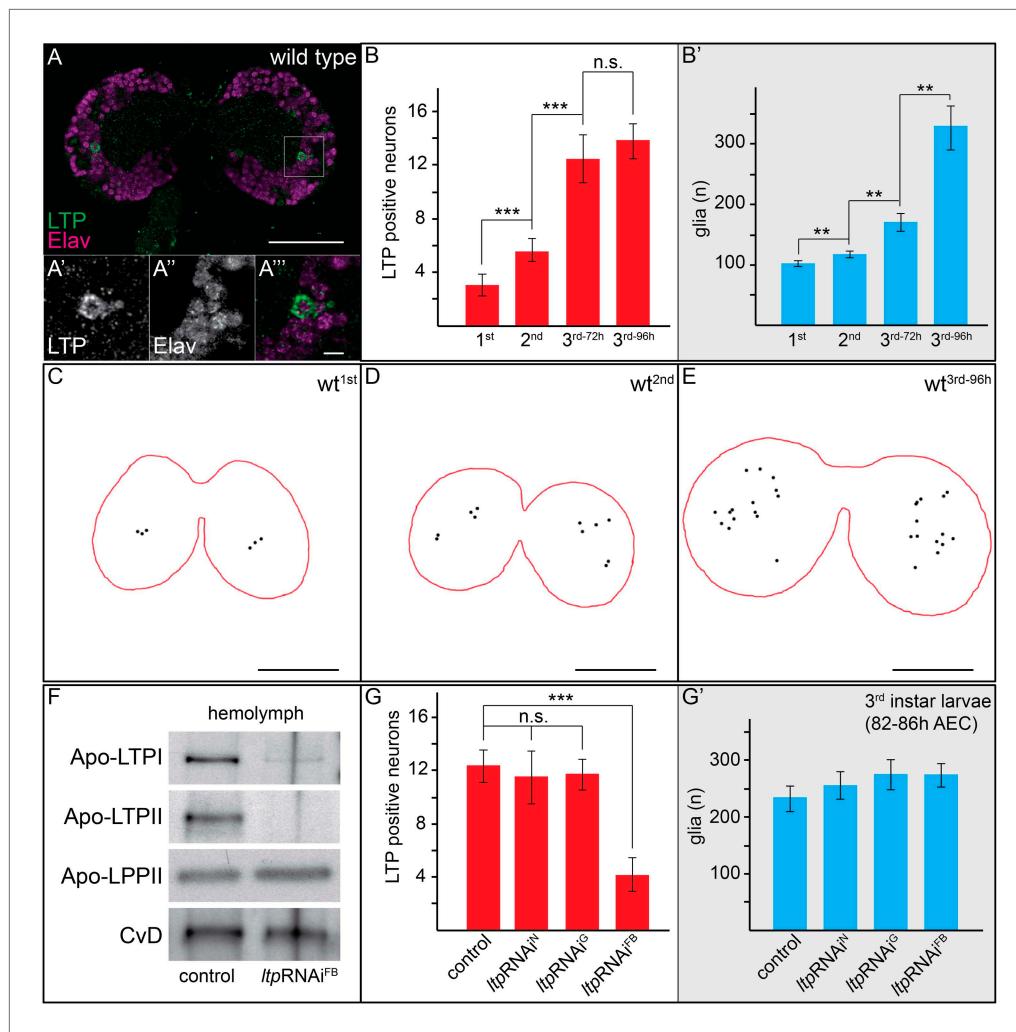


Figure 1. Circulating LTP crosses the BBB and accumulates on neurons. **(A)** Confocal section of the CNS from a wt larva reared on YF (wt^{YF}), at the level of the big commissure, stained for LTP (green) and Elav (magenta). **(A'-A'')** Magnified boxed region in **(A)** **(B-B')** Total numbers of LTP-positive neurons/brain lobe **(B)** or Repo-positive glia/brain **(B')** of wt^{YF} larvae of different ages, quantified from 50–60 confocal sections per brain. Numbers indicate larval instar; superscripts indicate age in hours after egg collection. **(C-E)** Cartoons depict positions of all LTP-positive neurons (black dots) identified in confocal stack of three wt^{YF} brains of different ages, stained for LTP and Elav. **(F)** Western blot showing equal volumes of hemolymph from control^{YF} larvae, and larvae with FB-specific knock-down of *ltp* (*ltpRNAi^{FB}*). Blots are probed for Apo-LTPI, Apo-LTPII, Apo-LPPII and Cv-D. **(G and G')** Total numbers of LTP-positive neurons/brain lobe **(G)** and Repo-positive glia/brain **(G')** quantified from control^{YF} larvae, and larvae where *ltp* has been knocked-down in neurons (*ltpRNAi^N*), glia (*ltpRNAi^G*), or FB (*ltpRNAi^{FB}*). Error bars indicate standard deviation. *** = $p < 0.001$, ** = $p < 0.01$, n.s. = not significant (Student's *t* test). Scale bars indicate 50 μ m **(A and C-E)** or 5 μ m **(A'-A'')**.

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| Panel 1 | | 1 st | 2 nd | 3 rd -72h | 3 rd -96h |
|---|--|-------------------------|-------------------------|--------------------------|--------------------------|
| larval developmental stage | | 1 st | 2 nd | 3 rd -72h | 3 rd -96h |
| LTP positive neurons brain hemisphere (n=10) | | 3,0 ^{+/−0,8} | 5,5 ^{+/−0,8} | 12,4 ^{+/−1,8} | 13,8 ^{+/−1,3} |
| p value (neurons) | | [0,0002] | [7,29E-06] | [0,0607] | |
| Repo positive glia brain lobes (n=5) | | 101,2 ^{+/−5,2} | 116,4 ^{+/−5,9} | 169,6 ^{+/−15,5} | 328,8 ^{+/−36,3} |
| p value (glia) | | [0,0018] | [0,0020] | [0,0012] | |

Figure 1—figure supplement 1. Numbers of LTP positive neurons rise over larval development. Panel shows numbers (see also **Figure 1B,B'**) of LTP positive neurons/brain hemisphere (red) and Repo positive glia/brain (blue) from staged First, second, early and mid third instar (3rd-72h = 72 hr and 3rd-96h = 96 hr after egg collection) wt larvae.

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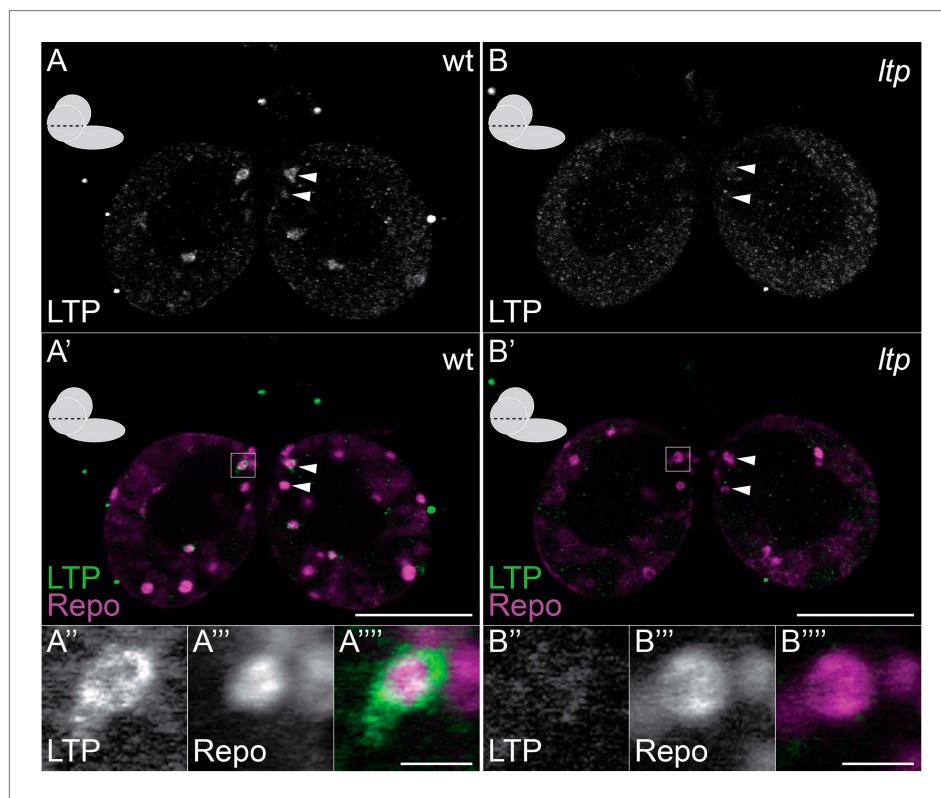


Figure 1—figure supplement 2. Small glial subsets enrich LTP positive. (A–B'') Confocal section at the big commissure from wt (A–A'') or *ltp* (B–B'') staged larval brains probed for Repo (magenta) and LTP (green). Boxed regions (A' and B') enlarged in (A''–A''') and (B''–B''') respectively. Scale bars indicate 50 μ m (A–B') and 5 μ m (A''' and B''').

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| Panel 2 | control | <i>ltpRNAi^N</i> | <i>ltpRNAi^G</i> | <i>ltpRNAi^{FB}</i> |
|---|--------------------------|----------------------------|----------------------------|-----------------------------|
| LTP positive neurons brain hemisphere (n=10) | 12,3 ^{+/−1,2} | 11,5 ^{+/−2,0} | 11,7 ^{+/−1,2} | 4,1 ^{+/−1,3} |
| p value (neurons) | 0,059 | 0,055 | — | 1,00E-05 |
| Repo positive glia brain lobes (n=5) | 233,3 ^{+/−22,9} | 254,7 ^{+/−24,2} | 274,3 ^{+/−26,0} | 273,7 ^{+/−21,0} |

Figure 1—figure supplement 3. Circulating LTP crosses the BBB and enriches on neurons. Panel shows numbers (see also **Figure 1G,G'**) of LTP positive neurons/brain hemisphere (red) and Repo positive glia/brain (blue) from *ltpRNAi/+* (controls) and larvae upon LTP knock-down in neurons (*ltpRNAi^N*), glia (*ltpRNAi^G*) and fat bodies (*ltpRNAi^{FB}*). Larvae reared on yeast food, superscript numbers indicate standard deviation values.

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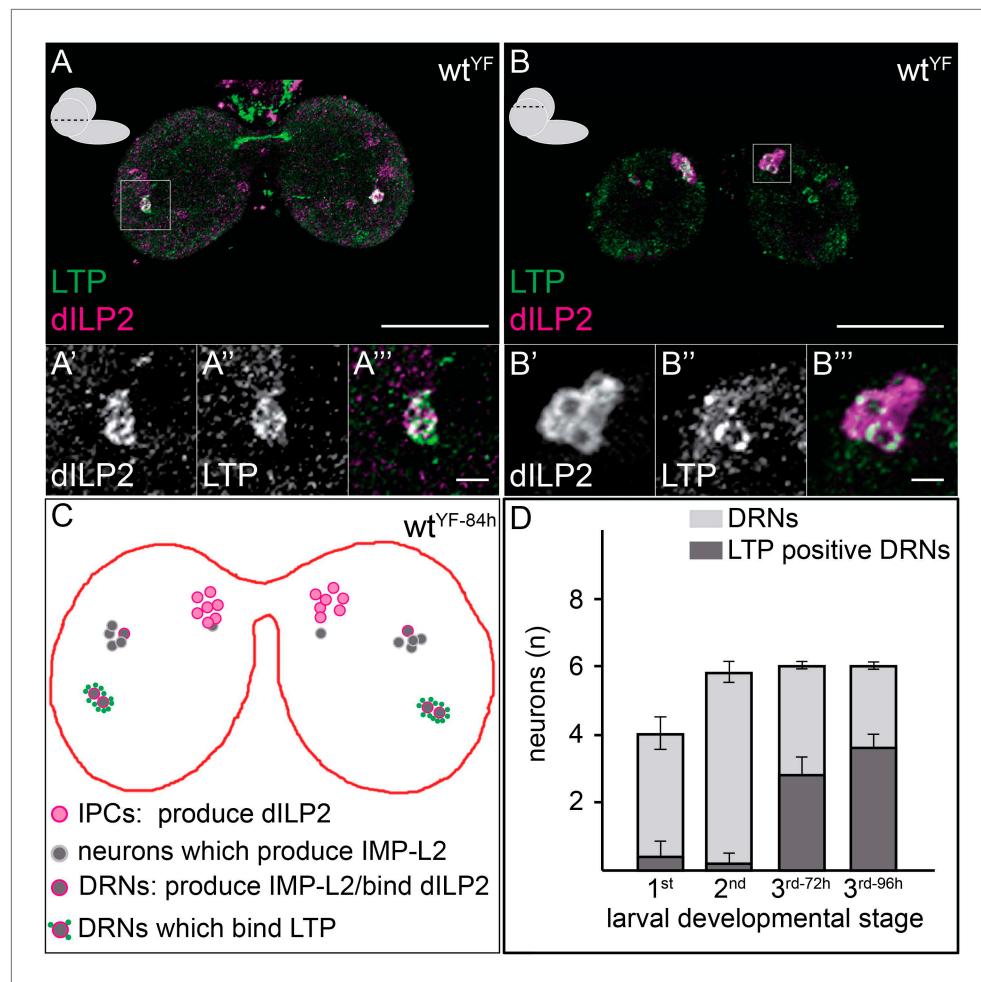


Figure 2. LTP accumulates on neurons positive for Dilp2 and IMPL2. Confocal sections at the level of (A–A'') or dorsal to (B–B'') the big commissure from third instar wt^{YF} larval brains stained for Dilp2 (magenta) and LTP (green). Boxed regions show LTP/Dilp2 double-positive neurons. Scale bar = 50 μ m (A and B) or 5 μ m (A'' and B''). (C) Cartoon drawn from a single sectioned brain showing positions of IPCs (magenta), IMPL2-producing cells that recruit Dilp2 (DRNs) (grey with magenta rim), and IMPL2-producing cells negative for Dilp2 (grey). LTP (green dots) is found on a subset of DRNs. (D) shows average number of neurons double-positive for Dilp2/IMPL2 (DRNs, grey) and the number of these that are LTP-positive (black) in larval brains of different stages. Error bars = standard deviation.

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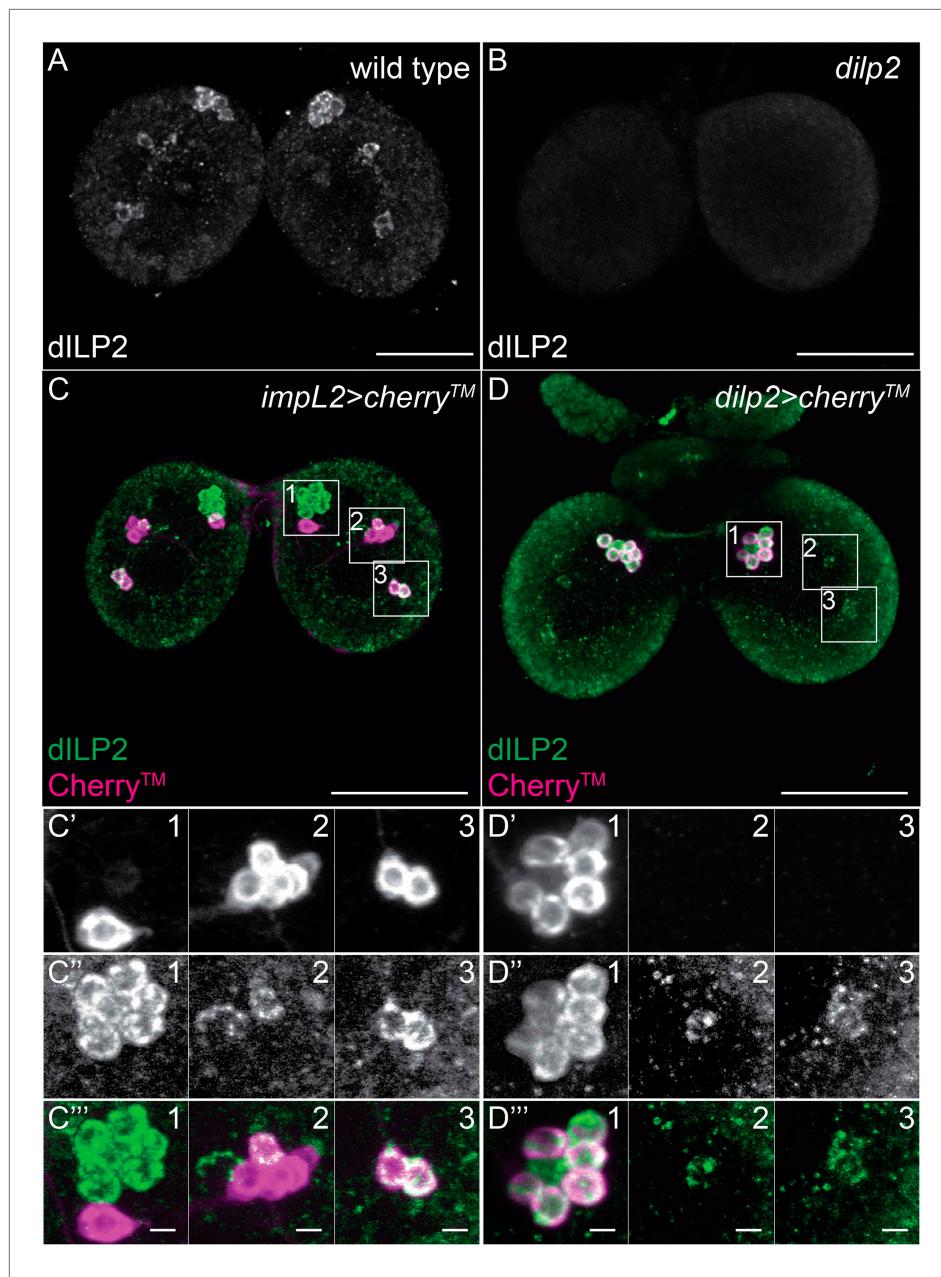


Figure 2—figure supplement 1. A small subset of Dilp2 positive neurons expresses IMPL2. (A-D'') 40 μ m confocal stack projections from wt (A), *dilp2* (B), *impL2>cherryTM* (C-C'') and *dilp2>cherryTM* (D-D'') larval brains probed for Dilp2 (green) and CherryTM (magenta). Boxed sections (C,D) magnified in C'-C'' or D'-D'' respectively. CherryTM shown in C',D' and Dilp2 in C'',D''. Scale bars = 50 μ m (A-D) or 5 μ m (C'',D'').

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| Panel 3 | | | | |
|--|-----------------------|-----------------------|-----------------------|-----------------------|
| larval developmental stage | 1 st | 2 nd | 3 rd -72h | 3 rd -96h |
| DRNs brain lobes (n=5) | 4,0 ^{+/−0.5} | 5,8 ^{+/−0.3} | 6,0 ^{+/−0} | 6 ^{+/−0} |
| LTP positive DRNs brain lobes (n=5) | 0,4 ^{+/−0.4} | 0,2 ^{+/−0.3} | 2,8 ^{+/−0.5} | 3,6 ^{+/−0.4} |

Figure 2—figure supplement 2. In the last larval developmental stage DRNs enrich LTP. Panel shows numbers (see also **Figure 2D**) of DRNs (grey) and LTP positive DRNs (dark grey) from 1st, 2nd or early and mid 3rd instar (3rd-72h = 72hr and 3rd-96h = 96 hr after egg collection) larvae reared on yeast food.

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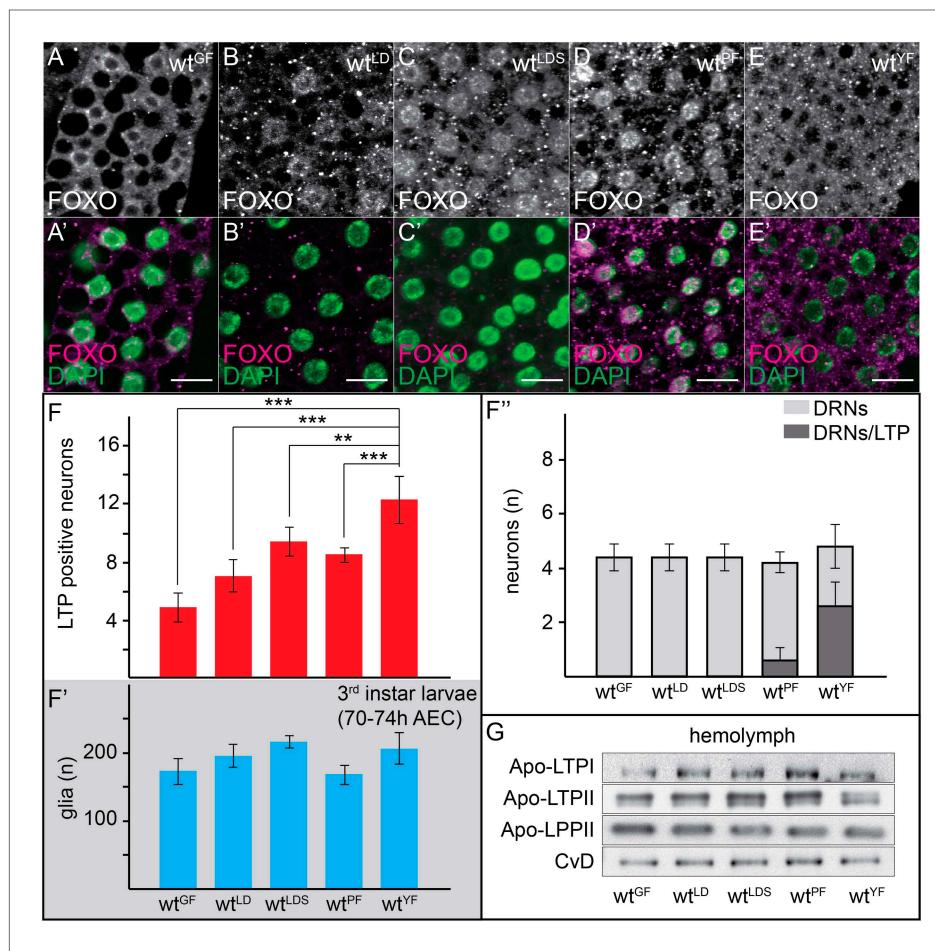


Figure 3. Neuronal LTP accumulation is diet-dependent. (A–E) Confocal stack projections of fat bodies from early third instar larvae raised on GF (A and A'), LDF (B and B'), LDSF (C and C'), PF (D and D') and YF (E and E') probed for FOXO (A–E and A–E' magenta) and DAPI (A–E', green). Scale bars = 20 μ m. (F–F'') average number of LTP-positive neurons/brain lobe (F), glial cells/brain (F') and LTP-positive DRNs (F'') in brains of larvae transferred from YF to indicated diets in the late second instar. Error bars indicate standard deviation. T-test significance: **p < 0.01, ***p < 0.001. (G) Equal hemolymph volumes from wt larvae raised on indicated food sources Western blotted and probed for the indicated Apolipoproteins.

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Panel 4

| food type | GF | LD | LDS | PF | YF |
|---|-------------------------|------------------------|-------------------------|-------------------------|-------------------------|
| LTP positive neurons brain hemisphere (n=10) | 4,8 ^{+-1,0} | 6,9 ^{+-1,1} | 9,3 ^{+-1,0} | 8,4 ^{+-0,5} | 12,1 ^{+-1,6} |
| p value (neurons) | | | | | |
| | 1,60E-04 | 3,23E-04 | 0,0035 | 5,37E-04 | |
| Repo positive glia brain lobes (n=5) | 171,8 ^{+-18,8} | 193,8 ^{+-9,7} | 214,5 ^{+-17,0} | 167,0 ^{+-14,7} | 204,3 ^{+-22,9} |
| DRNs brain lobes (n=5) | 4,4 ^{+-0,5} | 4,4 ^{+-0,5} | 4,4 ^{+-0,5} | 4,2 ^{+-0,4} | 4,8 ^{+-0,8} |
| LTP positive DRNs brain lobes (n=5) | 0 ⁺⁻⁰ | 0 ⁺⁻⁰ | 0 ⁺⁻⁰ | 0,6 ^{+-0,5} | 2,6 ^{+-0,9} |

Figure 3—figure supplement 1. Only yeast food promotes LTP enrichments on DRNs. Panel shows numbers (see also **Figure 3F–F'**) of LTP positive neurons/brain hemisphere (red) and Repo positive glia/brain (blue), and DRNs/brain (light grey) or LTP positive DRNs/brain (dark grey) from wt larvae reared on YF till late 2nd instar and transferred for 16hr on GF, LD, LDS, PF or YF. p-values (Students t' test) are indicated, superscript numbers = standard deviation.

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Panel 5

| food type | YF | PF | YF>PF | PF>YF |
|---|------------------------|------------------------|------------------------|-----------------------|
| LTP positive neurons brain hemisphere (n=10) | 10,1 ^{+-0,8} | 8,4 ^{+-1,3} | 7,8 ^{+-1,3} | 10,1 ^{+-1,0} |
| p value (neurons) | | | | |
| | 0,013 | 0,002 | n.s. | |
| Repo positive glia brain lobes (n=5) | 154,3 ^{+-3,6} | 155,3 ^{+-8,6} | 157,5 ^{+-9,9} | 137 ^{+-3,7} |
| DRNs brain lobes (n=5) | 5,4 ^{+-0,9} | 4,4 ^{+-0,5} | 5,6 ^{+-0,9} | 5,6 ^{+-0,5} |
| LTP positive DRNs brain lobes (n=5) | 3,4 ^{+-0,5} | 1,2 ^{+-0,8} | 1,0 ^{+-1,2} | 3,2 ^{+-0,8} |

Figure 3—figure supplement 2. LTP enrichments on DRNs are reversible. Panel shows numbers (see also **Figure 1—figure supplement 3A,A',C**) of LTP positive neurons/brain hemisphere (red) and Repo positive glia/brain (blue), and DRNs/brain (light grey) or LTP positive DRNs/brain (dark grey) from larvae reared on YF, PF, larva reared on PF till late 2nd instar and transferred on YF and larva reared till late 2nd instar on YF and transferred on PF. p-values (Students t' test) are indicated, superscript numbers = standard deviation.

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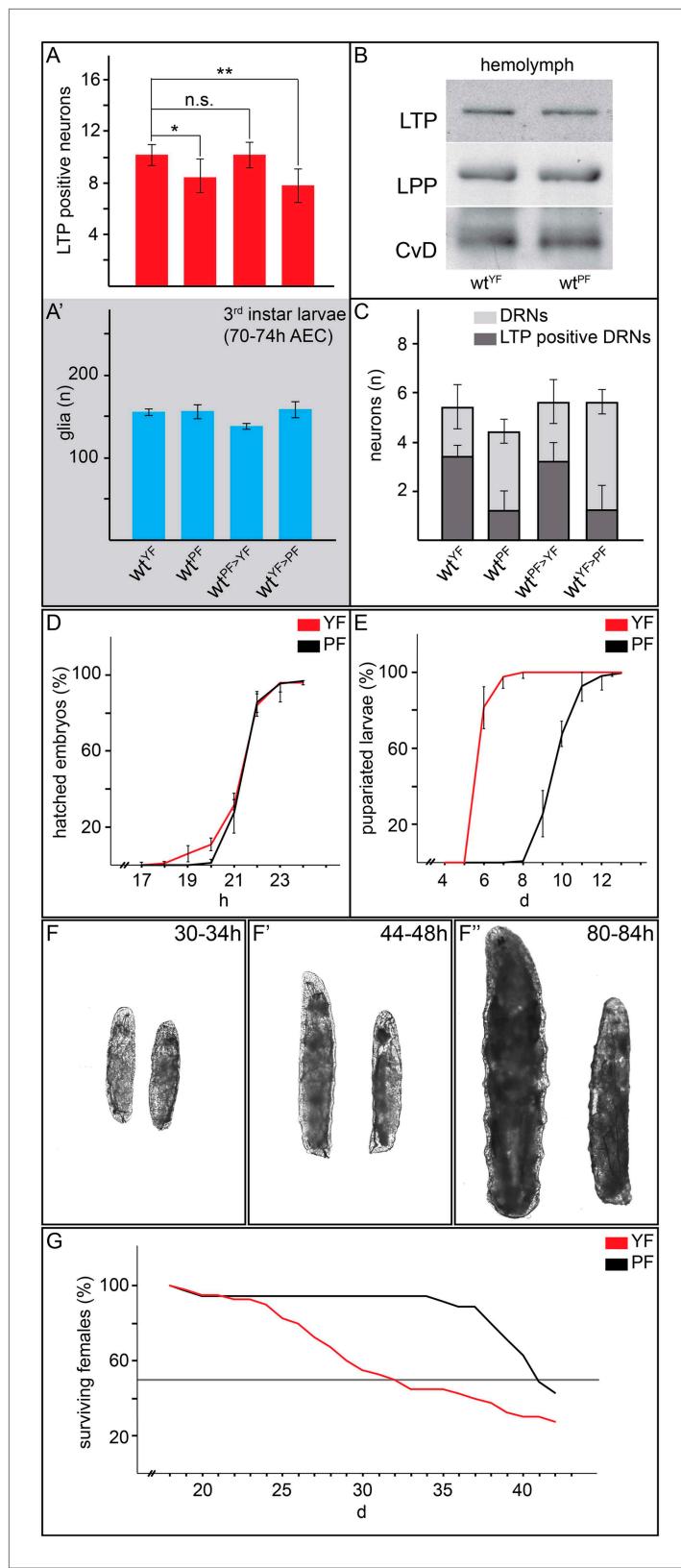


Figure 4. Yeast food promotes fast larval development but reduces average life span. (A, A', C) Charts depict LTP positive neurons/brain hemisphere (A) and Repo positive glia/brain (A'), and DRNs or LTP positive

Figure 4. Continued on next page

Figure 4. Continued

DRNs/brain (**C**) from larvae reared on YF, PF, raised on PF till late second instar and transferred for 16hr on YF, and raised on YF till late second instar and transferred for 16 hr on PF. p-values (Student's ttest) are indicated, * = $p < 0.05$, ** = $p < 0.01$, n.s. = not significant, error bars = standard deviation. (**B**) Equal volumes hemolymph from early third instar wt larvae reared on YF or PF probed for Apo-LTP, Apo-LPP and Cvd. (**D, E, G**) Plotted are percentages (Y-axis) of hatched embryos (**D**; $n^{YF} = 198$, $n^{PF} = 198$; parental flies kept for three generations on respective food types before embryo collection), pupariated larvae (**E**; $n^{YF} = 163$ and $n^{PF} = 133$; time of pupal development was unchanged) and of living mated females (**G**; $n^{YF} = 40$ and $n^{PF} = 35$) over time (X-axis). Please note, LD_{50} indicated with grey line (**G**). (**F-F'**) Exemplary photographs of staged wt larvae bred on YF (left) or PF (right). Hours after egg collection (AEC) are indicated in top right corner.

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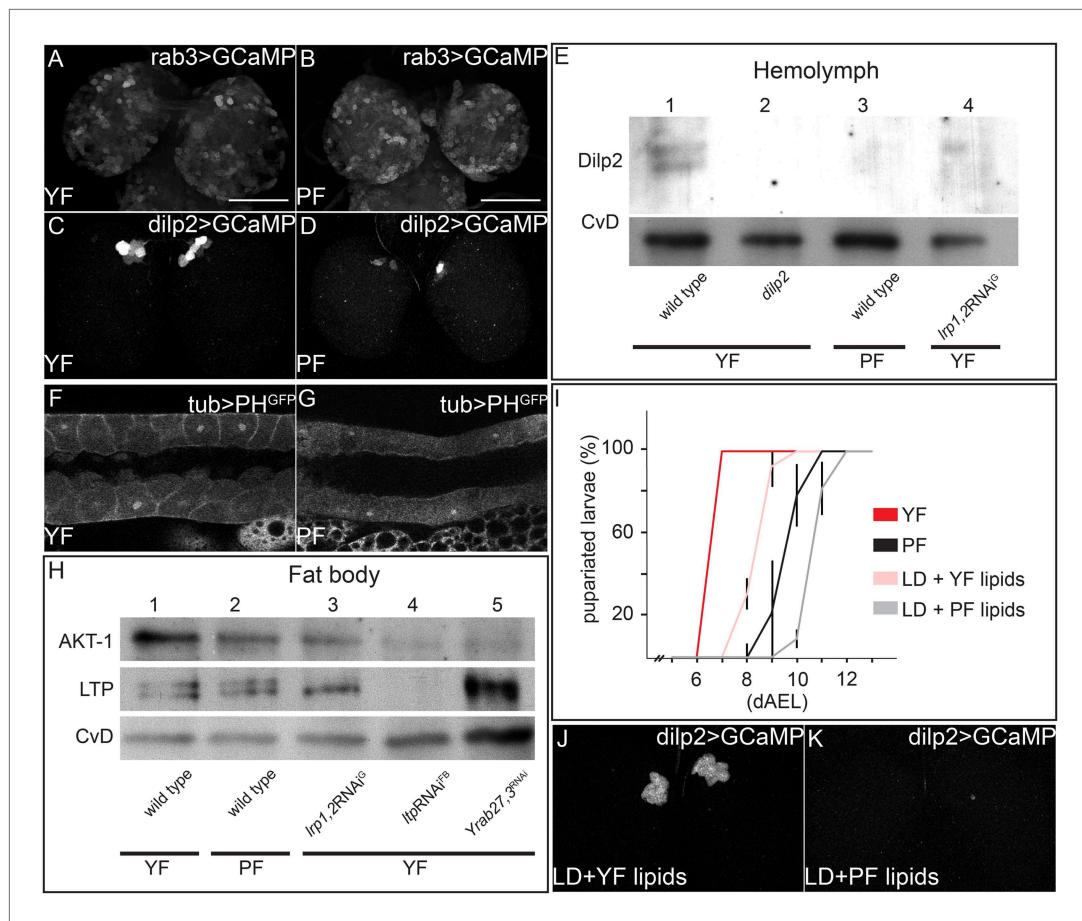


Figure 5. Yeast food components activate IPCs to release Dilp2 and induce systemic Insulin signaling. **(A–D)** show GFP fluorescence from collapsed confocal stacks of whole brains expressing GCaMP under the control of *rab3*-GAL4 (**A** and **B**) or *dilp2*-GAL4 (**C** and **D**) from larvae reared on YF (**A** and **C**) or PF (**B** and **D**). **(E)** Hemolymph blots probed for Dilp2 and CvD from wild type (lanes 1 and 3), *dilp2* mutant (lane 2) and *repo*-GAL4>*lrp1,2RNAi* (lane 4) larvae reared on yeast food (YF) or plant food (PF) as indicated. **(F and G)** show single confocal sections of salivary glands expressing the PIP₃ reporter PH^{GFP} under the direct control of the tubulin promoter. Larvae were reared on YF (**F**) or PF (**G**). **(H)** Western blots from fat body lysates probed for phospho-AKT, LTP and CvD as indicated. Lysates are from wild type (lanes 1, 2), *repo*-GAL4>*lrp1,2RNAi* (lane 3), *lrp1,2RNAi* (lane 4) and *YFP-rab27;YFP-rab3;impl2-GAL4>gfpRNAi* (lane 5). Larvae were reared on yeast food (YF) or plant food (PF) as indicated. **(I)** shows percentages of larvae that have pupariated at different days after egg laying (dAEL). Larvae were reared on yeast food (red, n = 49), on plant food (black, n = 34), or on lipid-depleted food supplemented with either yeast lipids (light red, n = 23) or plant lipids (grey, n = 24). **(J and K)** show GFP fluorescence from collapsed confocal stacks of whole brains expressing GCaMP under the control of *dilp2*-GAL4 from larvae fed on lipid-depleted food supplemented with yeast lipids (**J**) or lipid-depleted food supplemented with plant lipids (**K**). See also **Videos 7 and 8**.

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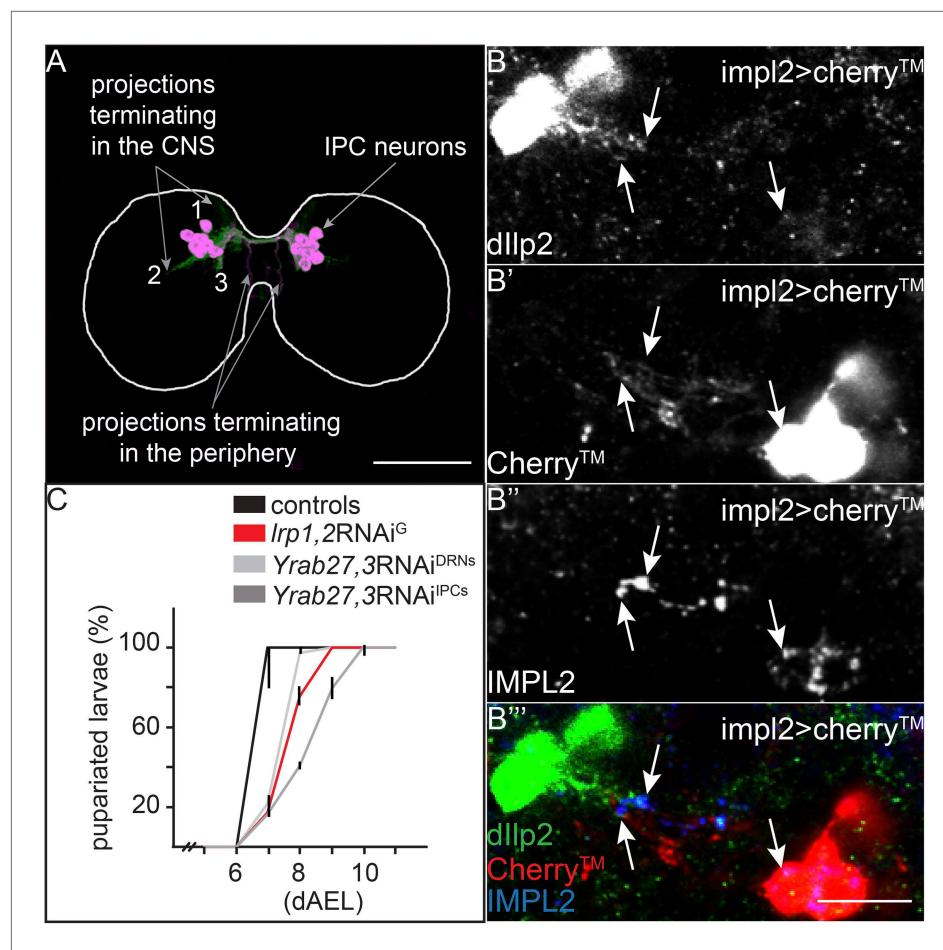


Figure 6. IPC and IMPL2-positive neurons are in direct contact. **(A)** Projected confocal stacks of an early third instar brain expressing transmembrane Cherry under the control of *dilp2-GAL4*, stained for Cherry (green) and Dilp2 (magenta). Most Dilp2 is detected in projections that terminate in the periphery (as determined from examining individual sections). However, Cherry staining reveals other projections that enter the central neuropil ([Linneweber et al., 2014](#)) or remain in other regions of the CNS ([Levin et al., 2011](#); [Bjordal et al., 2014](#)). **(B)** Shows a single confocal section from *impl2-GAL4>cherry*TM larval brains probed for Dilp2 (**B**, green in **B''**), Cherry (**B'**, red in **B''**) and IMPL2 (**B''**, blue in **B''**). White arrows point to colocalization between Dilp2 and IMPL2. Scale bars indicate 50 μ m (**A**) or 10 μ m (**B-B''**). **(C)** Shows percentages of yeast food-reared larvae of different genotypes that have pupariated at different days after egg laying (dAEL). Black indicates pooled results from two control genotypes: UAS:*lrp1,2RNAi*/+ and UAS:*gfpRNAi*/+ ($n = 81$). Red indicates *repo-GAL4>lrp1,2RNAi* ($n = 109$). Light grey indicates *YFP-rab27;YFP-rab3;impl2-GAL4>gfpRNAi* ($n = 76$). Dark grey indicates *YFP-rab27;YFP-rab3;dilp2-GAL4>gfpRNAi* ($n = 120$).

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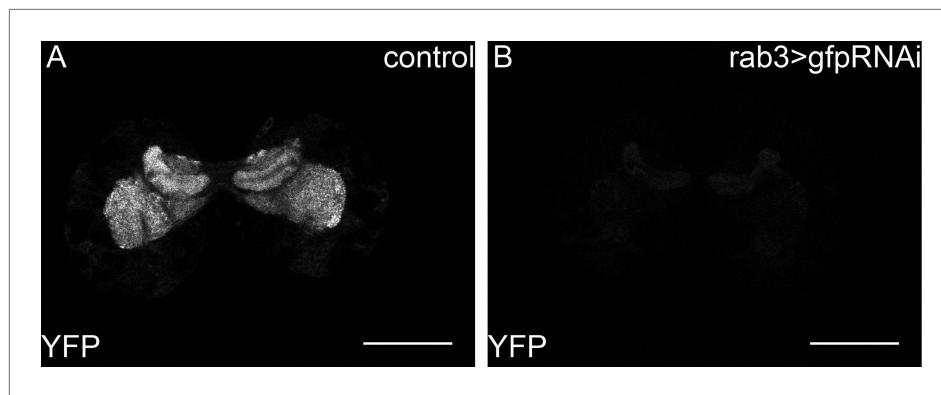


Figure 6—figure supplement 1. *Y-rab3* and *Yrab27* are reduced by *gfpRNAi*. (A,B) show confocal sections at the level of the big brain commissure from control *YFPPrab27;YFPPrab3;UAS:gfpRNAi* (A) and *YFPPrab27;YFPPrab3;rab3Gal4> UAS:gfpRNAi* (B) larval brains. Scale bars indicate 50 μ m.

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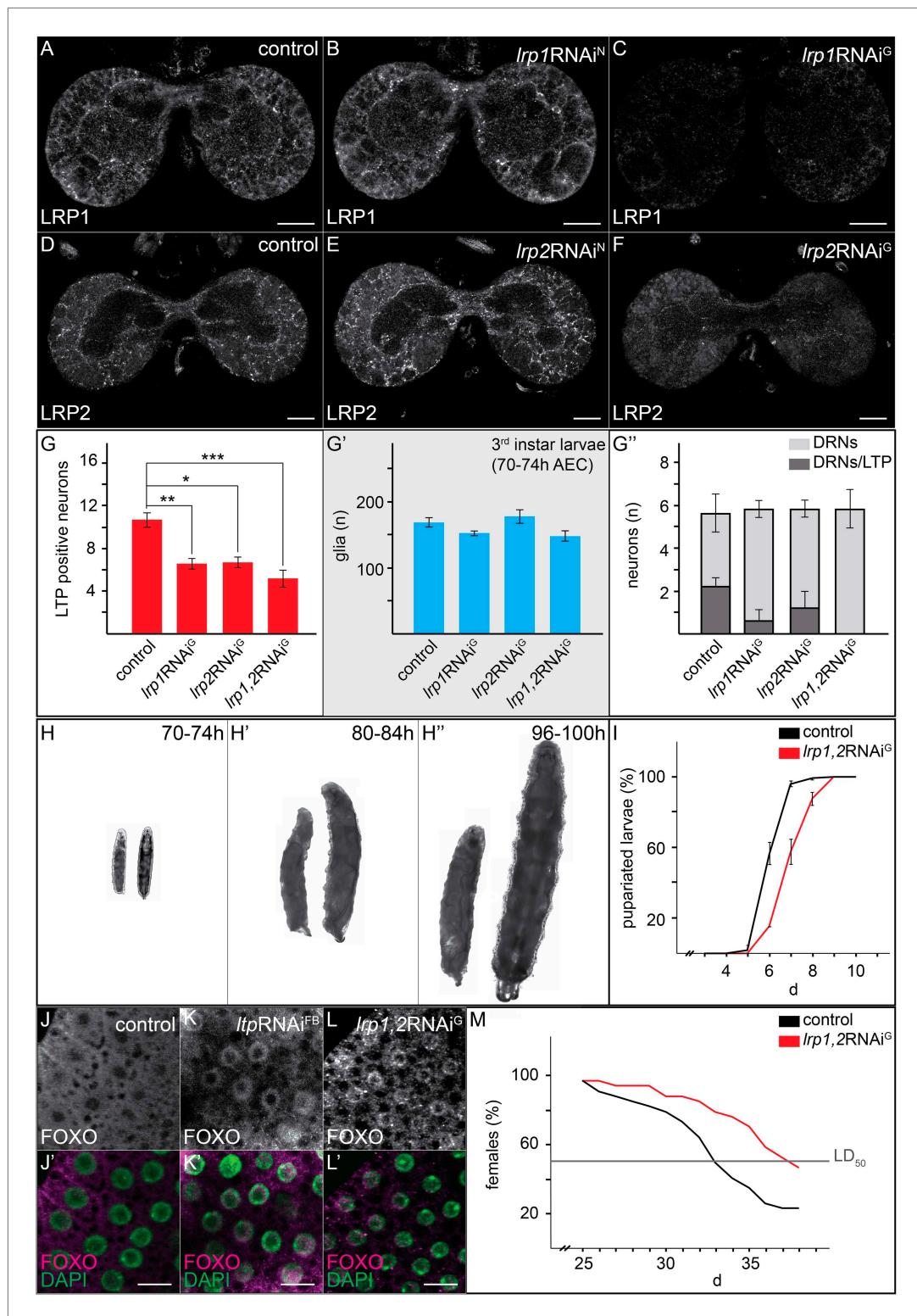


Figure 7. Glial LRP1 and LRP2 receptors move LTP across the BBB. (A–F) Confocal brain sections at the level of the big commissure stained for LRP1 (A–C) or LRP2 (D–F) from control^{YF} larvae (A and D) or larvae with neuronal (B and E) or glial (C and F) knock-down of each receptor. Glial knock down reduces LRP1 and LRP2. (G–G'') Average number of LTP-positive neurons/brain lobe (G) Repo-positive glia/brain (G') or fraction of LTP-positive DRNs (G'') in larval brains with glial knock-down of LRP1 and LRP2 singly or in combination, as indicated. Error bars indicate

Figure 7. Continued

standard deviation. T-test significance: * $p = 0.052$, ** $p > 0.01$, *** $p < 0.001$. (H–H'') control larvae (right) and larvae with glial knock-down of both LRP1/LRP2 (left) photographed at indicated times after egg collection. (I) Percent of control ($n = 216$, black) or double LRP1/LRP2 knock down ($n = 183$, red) larvae pupariating over time. (J–L') Confocal stack projections of fat bodies from control (J and J'), fat body (K and K') or glial (L and L') LRP1,2 knock down larvae stained for FOXO (magenta) and DAPI (green). Scale bars = 20 μ m. (M) Percent survival of control ($n = 34$, black) or glial LRP1/2 double knock-down ($n = 33$, red) flies fed with YF; grey line indicates 50% survival.

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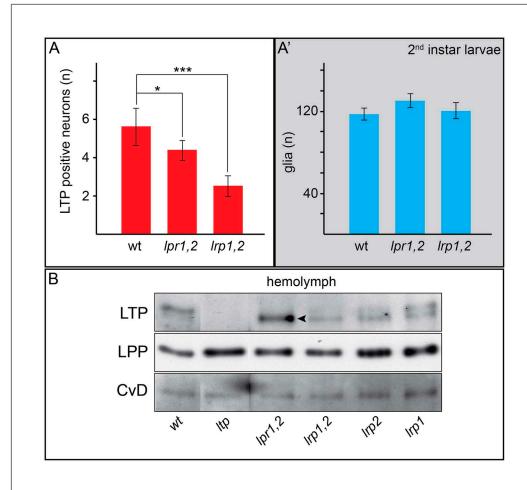


Figure 7—figure supplement 1. Neuronal LTP enrichments are reduced in *lpr1,2*. (A,A') Charts show LTP positive neurons/brain hemisphere (A) and Repo positive glia/brain (A') from wt, *lpr1,2* and *lpr1,2* larva reared on YF. * = $p < 0.05$, *** = $p < 0.001$ (Students t' test), error bars = standard deviation. (B) Equal volumes hemolymph probed for Apo-LTP, Apo-LPP and CvD. Please note increased LTP levels present in *lpr1,2* hemolymph (arrowhead).

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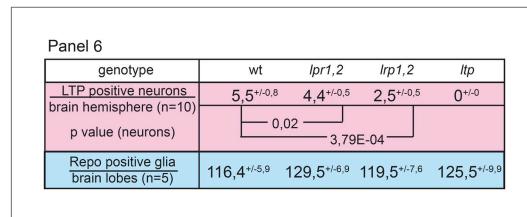


Figure 7—figure supplement 2. Neuronal LTP enrichments are only mildly affected in *lpr1,2*. Panel shows numbers of LTP positive neurons/brain hemisphere (red) and Repo positive glia/brain (blue) from staged wt, *lpr1,2* and *lpr1,2* larvae reared on YF. Superscript numbers = standard deviation, p-values (Students t' test) are indicated.

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| knock-down | control | <i>lrp1</i> RNAi ^G | <i>lrp2</i> RNAi ^G | <i>lrp1,2</i> RNAi ^G |
|---|-------------------------|-------------------------------|-------------------------------|---------------------------------|
| LTP positive neurons brain hemisphere (n=10) | 10,6 ^{+/−0,7} | 6,5 ^{+/−0,5} | 6,6 ^{+/−0,5} | 5,1 ^{+/−0,8} |
| p value (neurons) | 0,003 | 0,052 | | 1,61E-06 |
| Repo positive glia brain lobes (n=5) | 167,0 ^{+/−7,3} | 151 ^{+/−3,9} | 176,3 ^{+/−7,8} | 146,3 ^{+/−7,8} |
| DRNs brain lobes (n=5) | 5,6 ^{+/−0,9} | 5,8 ^{+/−0,4} | 5,8 ^{+/−0,4} | 5,5 ^{+/−0,4} |
| LTP positive DRNs brain lobes (n=5) | 2,2 ^{+/−0,4} | 0,6 ^{+/−0,5} | 1,2 ^{+/−0,8} | 0 ^{+/−0} |

Figure 7—figure supplement 3. Both receptors, LRP1 and LRP2, promote LTP transport in glia. Panel shows numbers (see also **Figure 4G–G'**) of LTP positive neurons/brain hemisphere (red) and Repo positive glia/brain (blue), and DRNs/brain (light grey) or LTP positive DRNs/brain (dark grey) from *lrp1,2*RNAi/+ (controls) and larvae upon glial knock-down of LRP1 (*lrp1*RNAi^G), LRP2 (*lrp2*RNAi^G), and LRP1,2 (*lrp1,2*RNAi^G) protein. Larvae reared on YF, p-values (Students t' test) are indicated, superscript numbers = standard deviation.

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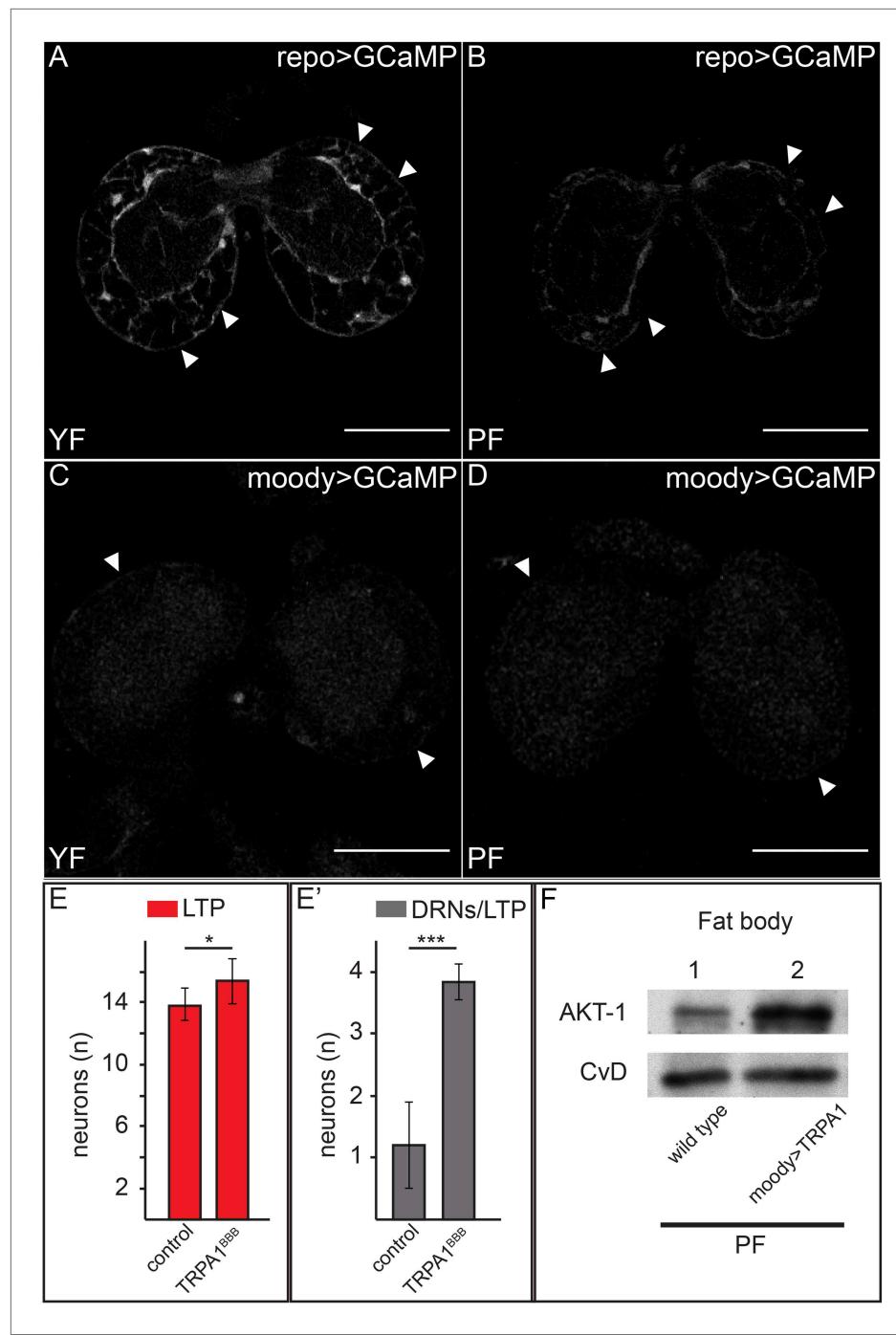


Figure 8. Increased free Ca^{++} levels promote systemic Insulin signaling. **(A–D)** Show GFP fluorescence of the GCaMP reporter construct in confocal sections of larval brains at the level of the big brain commissure. GCaMP was expressed either in all glia under the control of *repo*-GAL4 **(A and B)** or in BBB glia under the control of *moody*-GAL4 **(C and D)**. Larvae were reared on yeast food **(A and C)** or plant food **(B and D)**. White arrowheads point to blood brain barrier and scale bars indicate 50 μm . **(E)** shows the average number of LTP-positive neurons per brain lobe (**E**, red, $n = 10$) or the average number neurons co-staining for LTP and Dilp2 (DRNs) (**E'**, grey, $n = 5$) from either *UAS:trpa1* (control) larvae or larvae expressing *TRPA1* under the control of *moody*-GAL4 ($\text{TRPA1}^{\text{BBB}}$). All larvae were reared on plant food at 29C. Error bars show standard deviations. * indicates $p < 0.02$, *** indicates $p < 0.0002$. **(F)** Western blot of fat body lysates from control (*UAS:trpa1*+/+) larvae (lane 1) or larvae expressing *TRPA1* in BBB glia under the control of *moody*-GAL4 (lane 2), probed for phospho-AKT1 and CvD. Larvae were all reared on plant food (PF).

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