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Supplementary information

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# Protein-responsive gut hormone tachykinin directs food choice and impacts lifespan

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**Supplementary Table S1: Fly stocks**

| Short name | Full genotype | Source | Reference |
|---|---|---|---|
| *Akh-GAL4* | *Akh-GAL4* on 3 | BDSC #25684 | Lee and Park[1] |
| *Akh*$^{null}$ | *Akh*$^{SK1}$, a CRISPR-induced frame-shift | Gift of Shu Kondo (Tokyo University of Science) | Hentze *et al.*[2] |
| *Akh*$^{null}$ *Tk*$^{Gut}$*-GAL4* | *R57C10-GAL80- WPRE[su(Hw)attP8]; +; Akh*$^{null}$*, Tk::2A::GAL4* | Prepared for this work | *Tk>* (Deng *et al.*[3]), BDSC #84693. *R57C10-GAL80*, Harris *et al.*, 2015 [4], gift of Ryusuke Niwa (Univeristy of Tsukuba). *Akh*$^{null}$ (Hentze *et al.*[2]), gift of Shu Kondo (Tokyo University of Science). |
| *CaLexA* system | *LexAop-CD8::GFP::2A::CD8::GFP; UAS-mLexA::VP16::NFAT, LexAop-CD2::GFP/TM6B, Tb* | BDSC #66542 | Masuyama *et al.*[5] |
| *CaLexA-Luciferase* | *LexAop-Luciferase; UAS-mLexA::VP16::NFAT/TM6B, Tb* | Gift of Michael Rosbash (Brandeis) | Guo, Chen, and Rosbash[6] |
| *CaLexA-Luciferase, Tk::2A::GAL4* | *LexAop-Luciferase; Tk::2A::GAL4, UAS-mLexA::VP16::NFAT/TM6B, Tb* | Prepared for this work | *Tk>* (Deng *et al.*[3]), BDSC #84693. *LexAop-Luciferase, UAS-mLexA::VP16::NFAT* [6], gift of Michael Rosbash (Brandeis). |
| *Cg*$^{TS}$*-GAL4* | *Cg-GAL4 Tub-GAL80*$^{TS}$ on 2 | Prepared for this work | *Tub-GAL80TS* [7], BDSC #7108. *Cg-GAL4*, BDSC #7011. |
| *ChAT-GAL4* | *ChAT-GAL4*$^{19B}$ *UAS-GFP*$^{S65T}$ on 2 | BDSC #6793 | Salvaterra and Kitamoto[8] |
| *dimm-GAL4* | *P{w[+mW.hs]=GawB}crc[c929]* | BDSC #25373 | |
| *Ilp2-GAL4, UAS-GFP* | *Ilp2-GAL4.R 2/CyO* | BDSC #37516 | Rulifson[9] |
| *R34G02-GAL4* | *R34G02-GAL4[attP2]* | BDSC #49797 | Jenett *et al.*[10]; Jourjine *et al.*[11] |
| *R57C10-GAL4* | *R57G02-GAL4[attP2]* | BDSC #39171 | Jenett *et al.*[10] |
| *R57C10-GAL80* | *R57C10-GAL80-WPRE[su(Hw)attP8]* on X | Gift of R. Niwa, University of Tsukuba | Harris *et al.*[4] |
| *R57C10*$^{TS}$*-GAL4* | *Tub-GAL80TS; R57C10-GAL4[attP2]* | BDSC #39171 + #7108 | |
| *Tdc2-GAL4* | *Tdc2-GAL4* on 2 | BDSC #9313 | |
| *TH-GAL4* | *pale-GAL4/CyO* | BDSC #95269 | Friggi-Grelin *et al.*[12] |
| *tim-GAL4* | *timeless-GAL4 on 2* | BDSC #7126 | Emery *et al.*[13] |
| *Tk::2A::GAL4* | *TI{2A-GAL4}Tk[2A-GAL4]* on 3 | BDSC #84693 | Deng *et al.*[3] |
| *Tk*$^{Gut}$*-GAL4 (Tk*$^{Gut}$*>)* | *R57C10-GAL80-WPRE[su(Hw)attP8]; +; Tk::2A::GAL4* | Prepared for this work | *Tk>* (Deng *et al.*[3]), BDSC #84693. *R57C10-GAL80* (Harris *et al.*[4]), gift of Ryusuke Niwa (Tsukuba). |
| *TkR99D::2A::GAL4* | *Mi{Trojan-GAL4.2}TkR99D[MI10336-TG4.2]* | BDSC #76208 | Lee *et al.*[14] |
| *UAS-AKH-RNAi* | *UAS-Akh-RNAi*$^{v105063}$ on 2 | VDRC #105063 | Dietzl *et al.*[15] |
| *UAS-AkhR-RNAi*$^{KK}$ | *UAS-AkhR-RNAi*$^{v109300}$ on 2 | VDRC #109300 | Dietzl *et al.*[15] |
| *UAS-AkhR-RNAi*$^{TRiP}$ | *UAS-AkhR-RNAi*$^{TRiP.JF03256}$*[attP2]* | BDSC #29577 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-CG16700-RNAi* | *UAS-CG16700-RNA*$^{v45188}$ on 3 | VDRC #45188 | Dietzl *et al.*[15] |
| *UAS-CG2930-RNAi* | *UAS-CG2930-RNAi*$^{v7031}$ on 3 | VDRC #7031 | Dietzl *et al.*[15] |
| *UAS-CG31248-RNAi* | *UAS-CG31248-RNAi*$^{TRiP.JF03189}$*[attP2]* | BDSC #28761 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-Eaat1-RNAi* | *UAS-Eaat1-RNAi*$^{TRiP.HMS02659}$*[attP40]/CyO* | BDSC #43287 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-gb-RNAi* | *UAS-gb-RNAi*$^{v1262}$ on 2 | VDRC #1262 | Dietzl *et al.*[15] |
| *UAS-GCaMP6s* | *20xUAS-IVS-GCaMP6s[VK00005]* on 3 | BDSC #42749 | Chen *et al.*[18] |
| *UAS-Gcn2-RNAi* | *UAS-Gcn2-RNAi*$^{TRiP.GL00267}$*[attP2]* | BDSC #35355 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-mCD8::GFP* | *UAS-mCD8::GFP.L[LL5], UAS-mCD8::GFP.L[2]* on 2 | BDSC #5137 | Lee and Luo, 1999[19] |
| *UAS-mnd-RNAi* | *UAS-mnd-RNAi*$^{v42485}$ on 3 | VDRC #42485 | Dietzl *et al.*[15] |
| *UAS-Rheb* | *UAS-Rheb*$^{EP50.084,w-}$*/TM6B,Tb* | Gift of Ernst Hafen and Hugo Stocker (ETH Zürich). | Stocker *et al.*[20] |
| *UAS-sbm-RNAi* | *UAS-sbm-RNAi*$^{TRiP.HMC03347}$*[attP2]* | BDSC #51791 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-slif-RNAi*$^{GD}$ | *UAS-slif-RNAi*$^{v45590}$ on 3 | VDRC #45590 | Dietzl *et al.*[15] |
| *UAS-slif-RNAi*$^{KK}$ | *UAS-slif-RNAi*$^{v110425}$*/CyO* | VDRC #110425 | Dietzl *et al.*[15] |
| *UAS-tdTomato* | *10xUAS-IVS-myr::tdTomato[su(Hw)attP8])* on X | BDSC #32223 | Pfeiffer *et al.*[21,22] |
| *UAS-tdTomato; CaLexA* | *10xUAS-IVS-myr::tdTomato[su(Hw)attP8]; LexAop-CD8::GFP::2A::CD8::GFP; UAS-mLexA-VP16-NFAT, lexAop-rCD2::GFP/TM6B, Tb* | BDSC #32223 combined with BDSC #66542 | Malita et al.[23]; *tdTomato* (Pfeiffer *et al.*[21,22]), BDSC #32223. *CaLexA* system (Masuyama *et al.*[5]), BDSC #66542. |

| | | | |
|---|---|---|---|
| *UAS-tdTomato; CaLexA, Tk::2A::GAL4* | *10xUAS-IVS-myr::tdTomato[su(Hw)attP8]; LexAop-CD8::GFP::2A::CD8::GFP; Tk::2A::GAL4, UAS-mLexA-VP16-NFAT/TM6B, Tb* | Prepared for this work | *tdTomato* (Pfeiffer *et al.*[21,22]) BDSC #32223. *CaLexA* system (Masuyama *et al.*[5]), BDSC #66542. *Tk>* [3], BDSC #84693. |
| *UAS-TkR99D-RNAi$^{TRiP1}$* | *UAS-TkR99D-RNAi$^{TRiP.JF02663}$[attP2]* | BDSC #27513 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-TkR99D-RNAi$^{TRiP2}$* | *UAS-TkR99D-RNAi$^{TRiP.HMC03749}$[attP40]* | BDSC #55732 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-Tk-RNAi$^{KK}$* | *UAS-Tk-RNAi$^{v103662}$ on 2* | VDRC #103662 | Dietzl *et al.*[15] |
| *UAS-Tk-RNAi$^{sh}$* | *UAS-Tk-RNAi$^{v330743}$[attP40]* | VDRC #330743 | Dietzl *et al.*[15] |
| *UAS-Tk-RNAi$^{sh}$; Akh$^{null}$* | *UAS-Tk-RNAi$^{v330743}$[attP40]; Akh$^{null}$* | Prepared for this work | *Tk-RNAi$^{sh}$*, VDRC #330743. *Akh$^{null}$* (Hentze[2]), gift of Shu Kondo. |
| *UAS-Tk-RNAi$^{sh}$; UAS-TrpA1* | *UAS-Tk-RNAi$^{v330743}$[attP40]; UAS-TrpA1/TM6B, Tb* | Prepared for this work | *Tk-RNAi$^{sh}$*, VDRC #330743. *UAS-TrpA1* on 3, gift of Christian Wegener (Würzburg). |
| *UAS-TOR-RNAi* | *UAS-TOR-RNAi$^{TRiP.HMS01114}$[attP2]* | BDSC #34639 | Ni et al.[16]; Perkins et al.[17] |
| *UAS-TrpA1 (II)* | *UAS-TrpA1* | BDSC #26263 | Hamada et al.[24] |
| *UAS-TrpA1 (III)* | *UAS-TrpA1[attP2]/TM6B, Tb* | Gift of Christian Wegener (Würzburg) | |
| *UAS-TrpA1; UAS-TOR-RNAi* | *UAS-TrpA1(B).K[attP16]; UAS-TOR-RNAi$^{TRiP.HMS01114}$[attP2]* | Prepared for this work | *TrpA1* on 2 (Hamada et al.[24]), BDSC #26263. *TOR-RNAi*, BDSC #34639. |
| *UAS-TrpA1; UAS-Tsc1/2* | *UAS-TrpA1(B).K[attP16]; UAS-Tsc1, UAS-Tsc2* | Prepared for this work | TrpA1 on 2 (Hamada et al.[24]), BDSC #26263. *UAS-Tsc1, UAS-Tsc2* (Tapon *et al.*[25]), gift of Ernst Hafen and Hugo Stocker, also available as BDSC #80576. |
| *UAS-TrpA1-RNAi* | *UAS-TrpA1-RNAi$^{TRiP.HMS05348}$[attP2]* | *BDSC #66905* | Ni et al.[16]; Perkins et al.[17] |
| *TrpA1::GFP* | *TrpA1::GFP* on 3 | Gift of Yang Xiang | Gong et al.[26] |
| *UAS-Tsc1/2* | *UAS-Tsc1, UAS-Tsc2* on 3 | BDSC #80576 | *UAS-Tsc1, UAS-Tsc2* (Tapon *et al.*[25]) is also available as BDSC #80576. |
| *VGlut1-GAL4* | *VGlut1-GAL4$^{OK371}$* on 2 | BDSC #26160 | Mahr and Aberle[27] |
| *w$^{1118}$* | *w$^{1118}$; +; +* | VDRC #60000 | |
| *w$^{1118}$* | *w$^{1118}$; +; +* | Gift of Christen Mirth (Monash University) | |

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## Supplementary Table S2: Oligo sequences used for qPCR

| **Oligos for *Drosophila* genes** | **Forward** | **Reverse** |
|---|---|---|
| *Rp49* | AGTATCTGATGCCCAACATCG | CAATCTCCTTGCGCTTCTTG |
| *AKH* | TCCCAAGAGCGAAGTCCTCA | CCAGAAAGAGCTGTGCCTGA |
| *slif* | CTGACGGCTTTGGGAGTGG | AATCAGGAAGGAAATGGTCACC |
| *Tk* | CAATTCCTTTGTGGGGATGCG | CTGCTGTTTTCCTCTCAAGTCAT |
| **Oligos for mouse genes** | **Forward** | **Reverse** |
| *RPLP0* | AGTATCTGATGCCCAACATCG | CAATCTCCTTGCGCTTCTTG |
| *TAC1* | ATTCCTTTGTTGGACTAATGGGC | ACGTCTTCTTTCGTAGTTCTGC |

## Supplementary Table S3: Reagents used in intestinal organoid culture

| Compound | Company | Stock | Usage Dilution |
|---|---|---|---|
| 2 mM EDTA in PBS | Sigma Aldrich #03692 | | |
| DMEM | Sigma Aldrich #D6546 | | Foundation of “2-D medium” |
| Advanced DMEM/F12 | Gibco #12634010 | | Foundation for ”basal medium” for 3-D organoids |
| GlutaMAX | Gibco #31966021 | 100x | 1:100 to make basal medium |
| HEPES | | 1M | 10 mM in basal medium |
| Penicillin/Streptomycin (Pen/Strep) | | 100x | 1:100 to make basal medium (0.1 U/mL pen, 0.1 mg/mL strep final) |
| B27 supplement (minus Vit. A) | Invitrogen #12587-010 | 50x | 1:50 to make ENR |
| EGF (mouse) | Gibco #PMG8045 | 50 µg/mL in 0.1% BSA | 1:1000 to make ENR (50 ng/mL final) |
| N2 supplement | Gibco #17502048 | 100x | 1:100 to make ENR |
| N-Acetyl-L-Cysteine (NAC) | Sigma Aldrich #A96165-5G | 500 mM, 500x | 1:500 to make ENR (1 mM final) |
| Nicotinamide | Sigma Aldrich #N0636/N33776 | 1 M, 100x | 1:100 to make ENR (10 mM final) |
| Noggin (mouse) | PeproTech #250-38 | 100 µg/mL in 0.1% BSA | 1:1000 to make ENR (100 ng/mL final) |
| ROCK inhibitor Y27632 | Tocris #1254 | 10 mM | 1:1000, added just before use (10 µM final) |