Developmental Cell, Volume *48*

Supplemental Information

Tumor-Derived Ligands Trigger Tumor Growth and Host Wasting via Differential MEK Activation

Wei Song, Serkan Kir, Shangyu Hong, Yanhui Hu, Xiaohui Wang, Richard Binari, Hong-Wen Tang, Verena Chung, Alexander S. Banks, Bruce Spiegelman, and Norbert Perrimon

Supplemental Figure and Table Legends

Supplemental Figure 1. Host wasting in yki^{3SA}-tumor-bearing flies, related to Figure 1 and 2. (**A-D**) TAG (**A**), glucose (**B**), and trehalose levels (**C**) (n=3, 30 flies/group), and climbing speeds (**D**, n=60) in yki^{3SA}-tumor-bearing flies. Thorax muscle degenerative phenotypes (**E**) protein amounts (**F**, n=3, 30 flies/group), muscle protein synthesis (**G**, left, n=3, 30 flies/group) and degradation rates (**G,** right, n=3, 30 flies/group), and of yki^{3SA}-tumor-bearing flies at day 8. (**H**) TAG levels of indicated flies after 8 days at 29°C (n=3, 30 flies/group). (**I**) Lipolysis rates of fat bodies, isolated from indicated flies at 29°C at day 6, with or without Tram treatment (n=3, 30 abdomens/group). (**J-K**) TAG levels (**J,** n=3, 30 flies/group) and climbing speeds (**K,** n=60) of flies bearing yki^{3SA}-gut tumors with dERK-RNAi (*esg-GAL4, UAS-GFP, tub-GAL80^{TS} /+; UAS-yki^{3SA}/UAS-dERK-RNAi*). (**L-N**) Appearance (**L**, up), midgut ISCs (**L**, down), TAG levels (**M,** n=3, 30 flies/group) and climbing speeds (**N,** n=60) of flies with dERK^{SEM} overexpression in ISCs (*esg-GAL4, UAS-GFP, tub-GAL80^{TS} /UAS-dERK^{SEM}*). Data are presented as means ± SEM. * $p < 0.05$.

Supplemental Figure 2. Pharmaceutical MEK inhibition in host tissues improves yki^{3SA} tumor-induced systemic wasting, related to Figure 2. (**A-B**) Protocol used for feeding yki^{3SA}-tumor-bearing flies with Tram after tumor induction (**A**) and schematic MEK suppression by Tram (**B**). (**C-M**) Phenotypes of systemic wasting in yki^{3SA}-tumor-bearing flies (*esg-GAL4, UAS-GFP, tub-GAL80^{TS} /+; UAS-yki^{3SA}/+*) at day 8: bloating phenotypes (**C,** up), gut tumor phenotypes (**C,** middle), muscle degenerative phenotypes (**C,** down), MEK activation in the fat body and muscle (**D**), bloating rates (**E**,

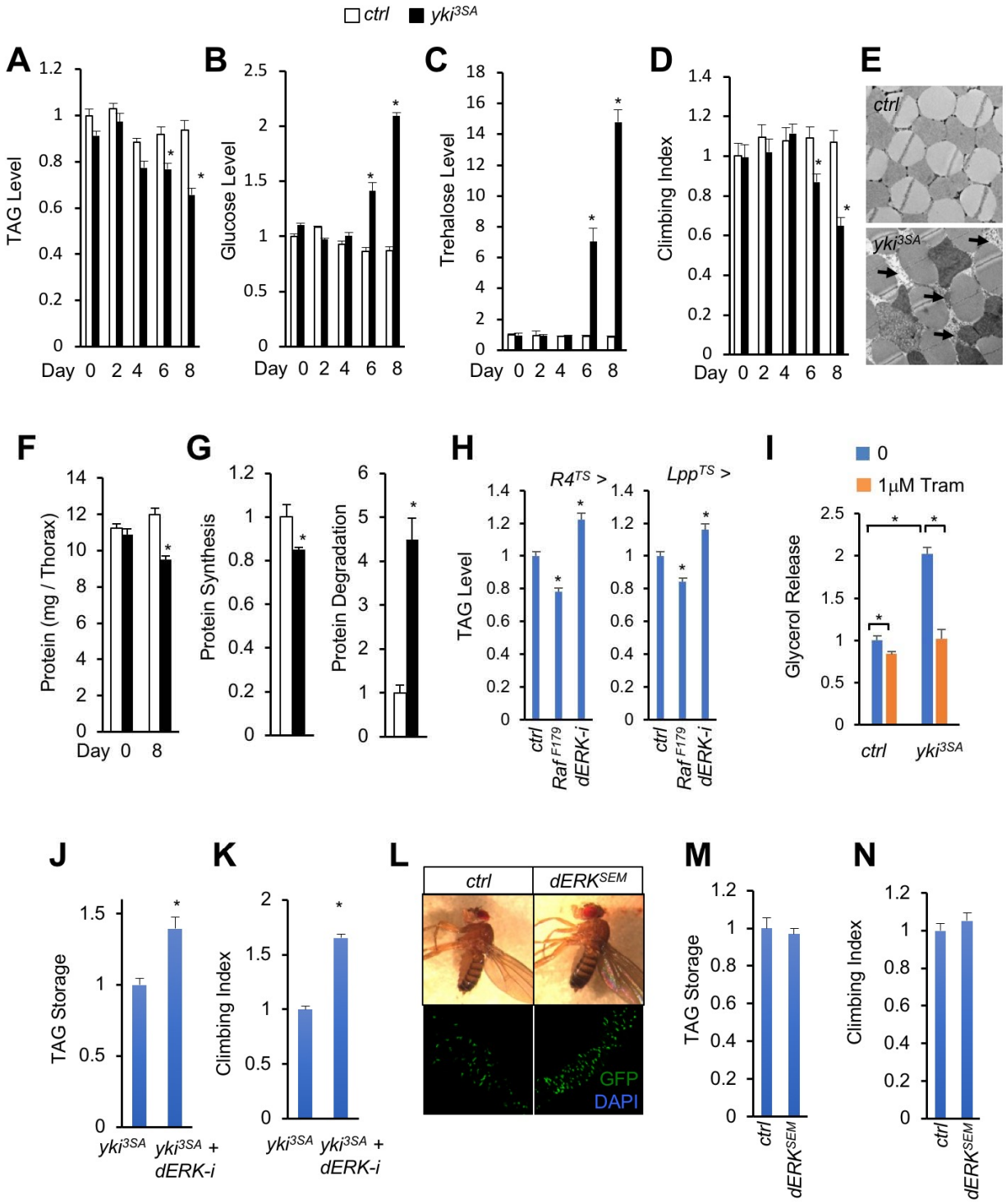
n=3, 60 flies/group), climbing speeds (**F,** n=60), lipid storages (**G,** n=3, 30 flies/group), trehalose and glucose levels in the hemolymph (**H, J,** n=3, 60 flies/group) and whole fly (**I, K,** n=3, 30 flies/group), and lifespan (**L** and **M,** n=120). (**N**) Midgut phenotypes of yki^{3SA}-tumor-bearing flies, which were treated with Tram from day 4, at day 30. Data are presented as means ± SEM. * $p < 0.05$.

Supplemental Figure 3. Pharmaceutical MEK inhibition in host tissues only improves yki^{3SA}+dERK^{SEM} tumor-induced systemic wasting, related to Figure 2 and 3. (**A-B**) Appearances (**A**, up), midgut morphology (**A**, down), lipid and trehalose storage (**B,** n=3, 30 flies/group), and climbing speed (**B,** n=60) of control flies treated with Tram from day 0. (**C-E**) Bloating (**C**, up) and gut tumor (**C,** down), ERK activation in the fat body and muscles (**D**), TAG storage and trehalose levels (**E**, n=3, 30 flies/group), and climbing speed (**E**, n=60) of yki^{3SA}+dERK^{SEM}-tumor-bearing flies at day 8 that were treated with PD from simultaneously with tumor induction. (**F**) Heat map indicating differentially expressed genes that encode trypsins (up) and gut hormones (down) in *yki^{3SA}* midguts. (**G-I**) Midgut phenotypes (Pros^{+} cells) and appearances (**G**), wasting phenotypes including TAG and trehalose (**H**, n=3, 30 flies/group) levels and climbing speed (**H**, n=60), and survival rates (**I,** n=120) of indicated flies. Genotype for *N^{intra}* is *esg-GAL4, UAS-GFP, tub-GAL80^{TS}/UAS-N^{intra}.* (**J**) Midgut *Pvf1* mRNA levels of flies bearing yki^{3SA} or yki^{3SA}+dERK^{SEM} tumors at day 8. (**K-M**) Midgut (**K**, left, n=3, 30 guts/group) and head (**K**, right, n=3, 30 heads/group) mRNA levels, Ilp2 levels in IPCs (**L**), and food intake (**M**, n=4, 80 flies/group) of yki^{3SA}+dERK^{SEM} tumor-bearing flies (*esg-GAL4, UAS-GFP, tub-GAL80^{TS}/UAS-dERK^{SEM}; UAS-yki^{3SA}*/+) at day 8 that were fed 10

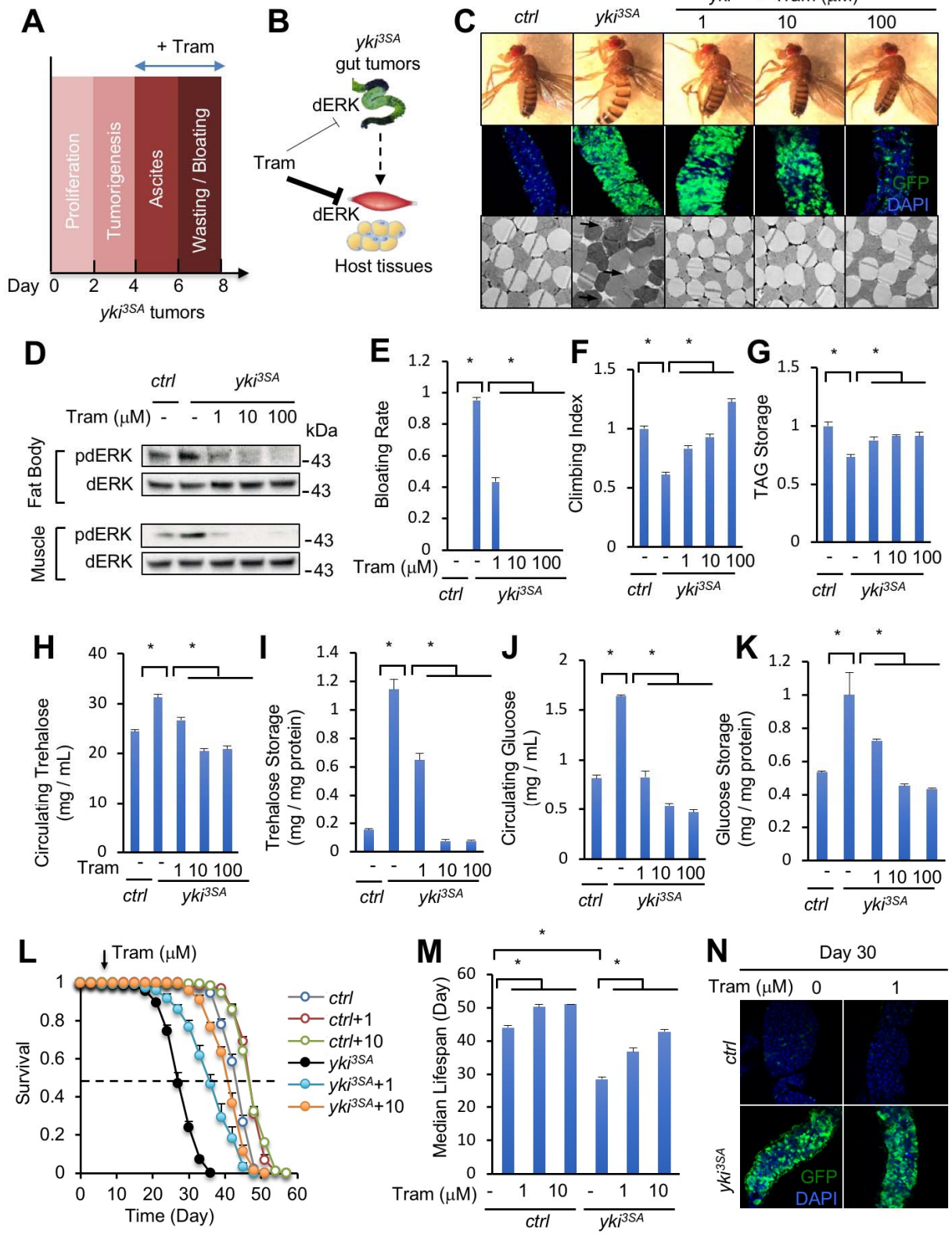
μM Tram simultaneously with tumor induction. (**N**) Midgut *ImpL2* mRNA levels in flies bearing yki^{3SA} tumors with RNAi at day 10 (n=3, 30 guts/group). Data are presented as means ± SEM. * $p < 0.05$.

Supplemental Figure 4. MEK inhibition alleviates mammalian myotube atrophy and lipolysis, related to Figure 2 and 3. (**A**) MEK/ERK activation in differentiated C2C12 myotubes (day 5) treated with LLC conditioned differentiation medium (3:1 diluted with fresh differentiation medium) at different time points (up) or with conditioned differentiation medium plus Tram for 1h (down). (**B-E**) Gene expression (**B,** n=3), protein degradation rates (**C,** n=3), myotube diameters (**D**, n=30, quantification of myotube images), and myotube morphologies (**E**) in C2C12 myotubes (day 5) treated with LLC conditioned differentiation medium with or without 0.1 μM trametinb for 24h. (**F**) MEK/ERK activation in differentiated 3T3-L1 adipocytes (day 10) treated with LLC conditioned growth medium (3:1 diluted with fresh growth medium) at different time points (up) or with conditioned growth medium plus Tram for 1h (down). (**G**) Lipolysis rates in 3T3-L1 adipocytes (day 10) treated with control or LLC-conditioned growth medium for 24h and then treated with 0.1 μM Tram for 1h (n=3). (**H-I**) Lipid storages (**H,** n=3) and lipid staining (**I**) in 3T3-L1 adipocytes (day 10) treated with LLC-conditioned growth medium with or without 0.1 μM Tram for 24h. Data are presented as means ± SEM. * $p < 0.05$.

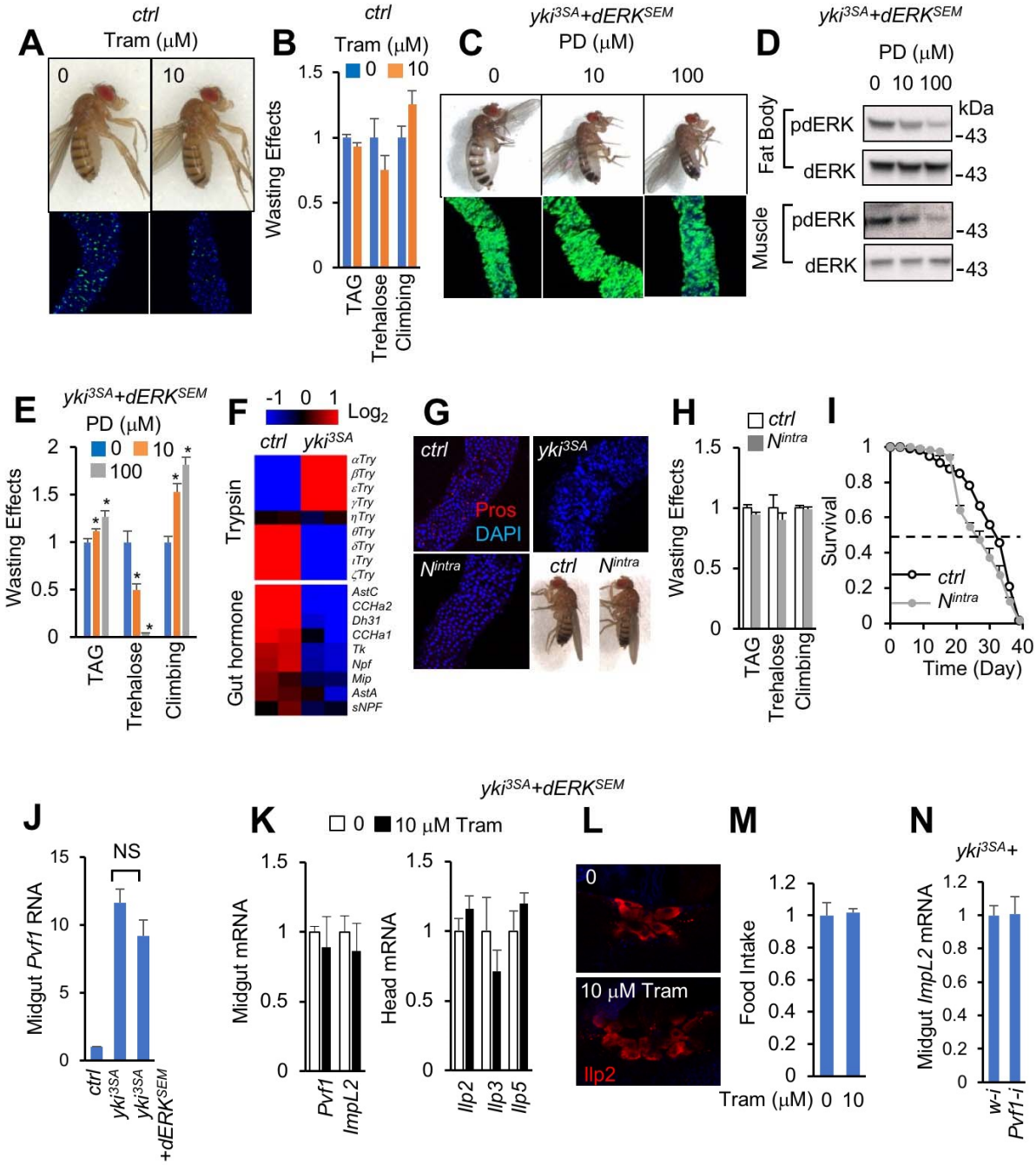
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4