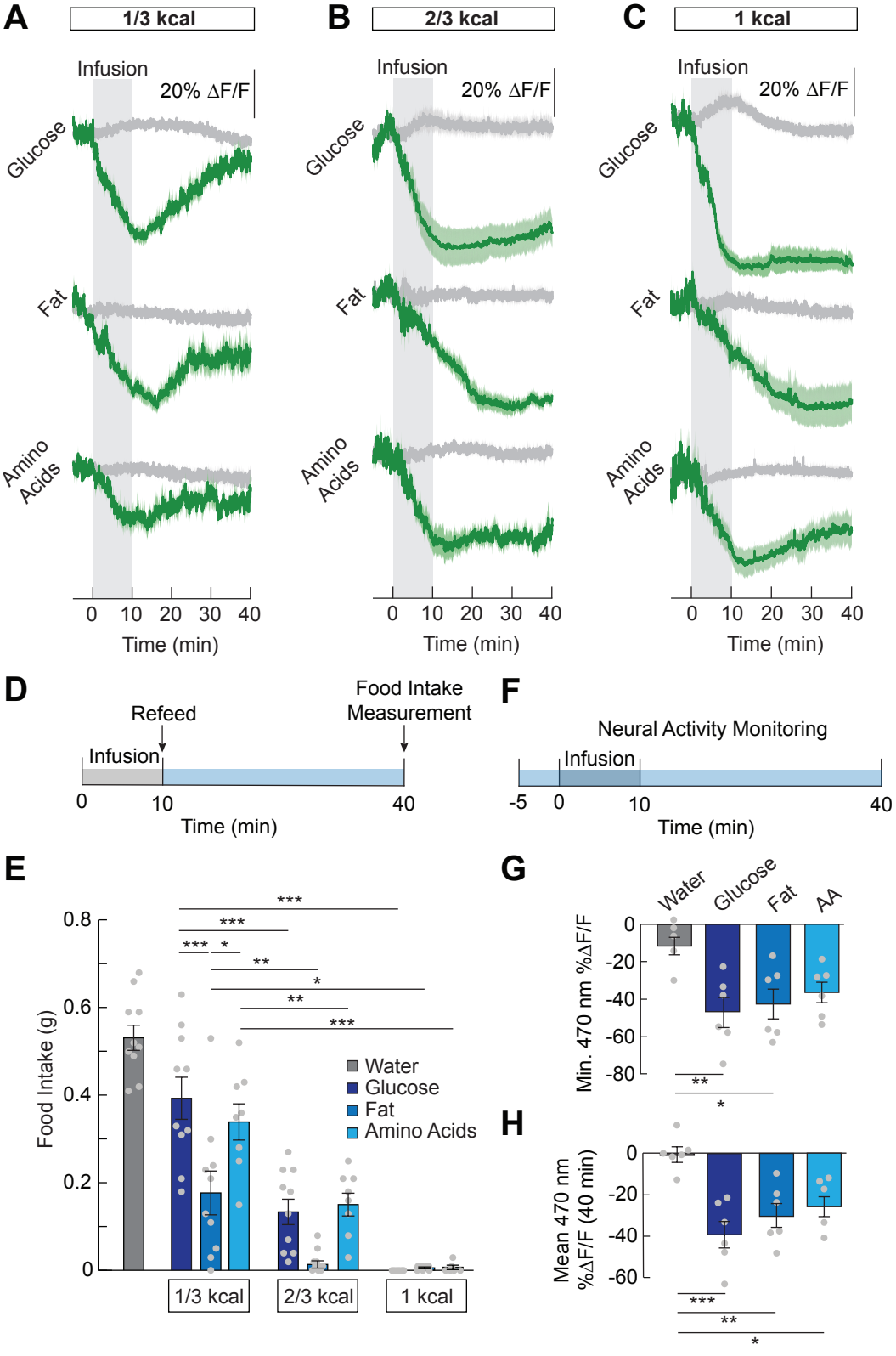

Figure S1, Related to Figure 1

Figure S1, Related to Figure 1. Intra-duodenal macronutrients inhibit AgRP neuron activity and suppress food intake. (**A-C**) AgRP neuron activity changes in response to intra-duodenal infusion of 1/3 (**A**), 2/3 (**B**), or 1 (**C**) kcal of glucose, fat, or amino acids. Signals are aligned to the start of the infusion. Green, 470-nm calcium signal; grey, 405-nm control signal. Dark lines represent means and lighter shaded areas represent SEM. (**D**) Food intake was monitored for 30 min following a 10-min infusion of macronutrients in food-restricted mice. (**E**) Total chow intake after intra-duodenal (ID) infusion of water, glucose, fat, or amino acids (n=6-10/group, one-way ANOVA, $p<0.001$). (**F**) AgRP neuron activity was monitored in response to a 10-min infusion of macronutrients in food-restricted mice. (**G**) Minimum $\Delta F/F$ of the 470-nm signal in AgRP neurons following ID infusion of water, glucose (2/3 kcal), fat (1 kcal), and amino acids (1 kcal, n=6/group, one-way ANOVA, $p<0.01$). (**H**) Mean $\Delta F/F$ of the 470-nm signal in AgRP neurons following ID infusion of water, glucose, fat, and amino acids (n=6/group, one-way ANOVA, $p<0.001$). Data are expressed as mean ± SEM, ns $p>0.05$, t-tests and post-hoc comparisons: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

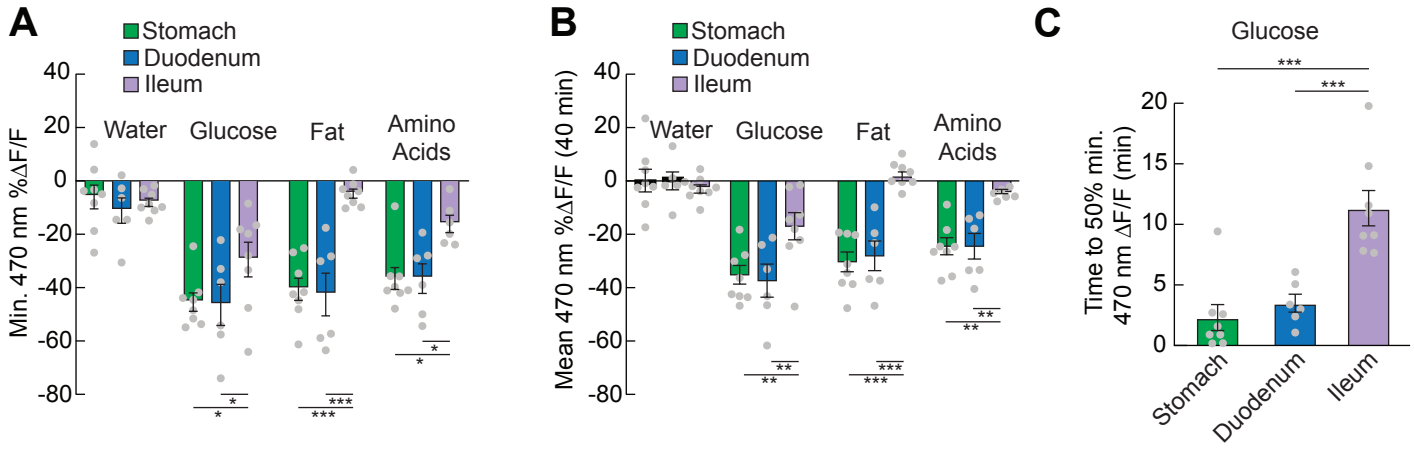

Figure S2, Related to Figure 1

Figure S2, Related to Figure 1. Macronutrient effects on AgRP neuron activity along the gastrointestinal tract. (**A**) Minimum ΔF/F of the 470-nm signal in AgRP neurons with infusions of water, glucose (2/3 kcal), fat (1 kcal) or amino acids (1 kcal) in the stomach (green), duodenum (blue), and ileum (purple, n=6-8/group, two-way ANOVA, $p<0.01$). (**B**) Mean ΔF/F of the 470-nm signal in AgRP neurons with infusions of water, glucose, fat, or amino acids in the stomach, duodenum, and ileum (n=6-8/group, two-way ANOVA, $p<0.001$). (**C**) Latency to 50% maximum reduction in ΔF/F during infusion of 2/3 kcal glucose (n=6-8/group, one-way ANOVA, $p<0.001$). Data are expressed as mean ± SEM, ns $p>0.05$, t-tests and post-hoc comparisons: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

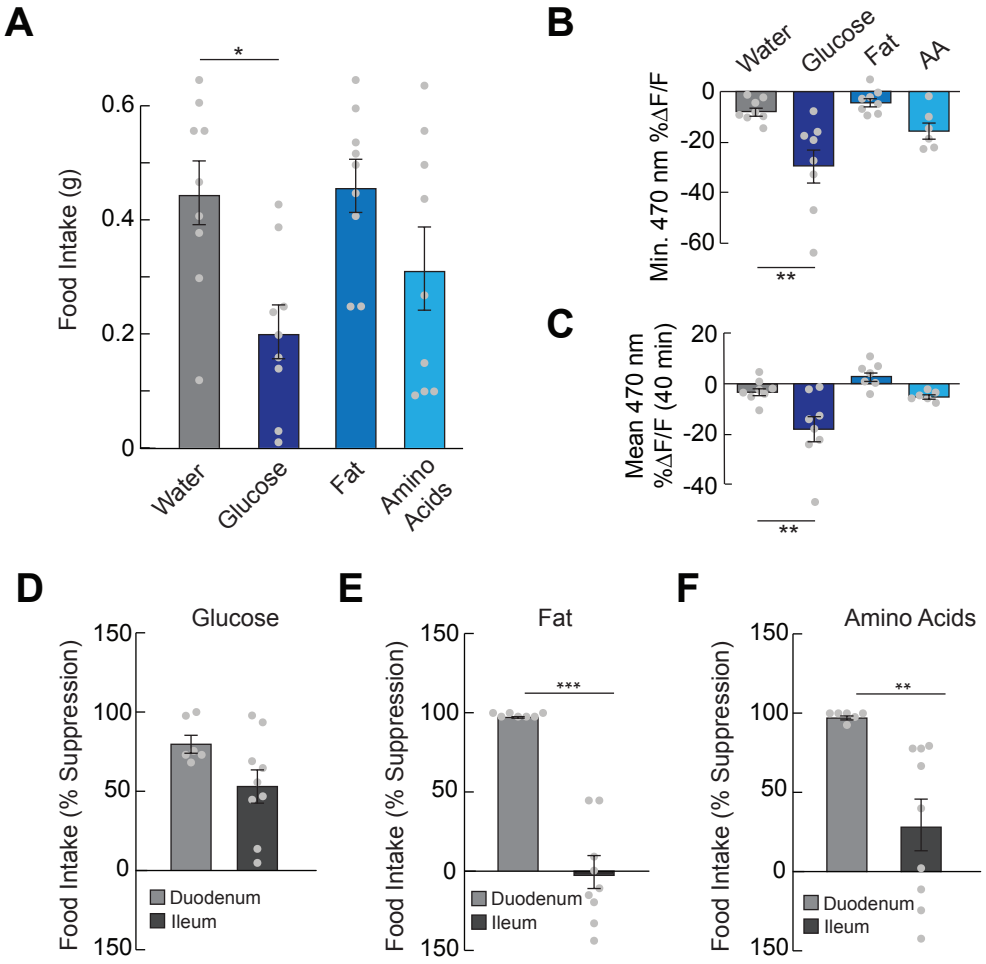

Figure S3, Related to Figure 1

Figure S3, Related to Figure 1. Intra-ileal infusion of glucose, but not fat or protein, inhibits AgRP neuron activity and suppresses food intake. (**A**) Total chow intake 30 mins after intra-ileal (II) infusion of water, glucose (2/3 kcal), fat (1 kcal), or amino acids (1 kcal) (n=9/group, one-way ANOVA, $p<0.01$). (**B**) Minimum $\Delta F/F$ of the 470-nm signal in AgRP neurons following II infusion of water, glucose, fat, or amino acids (n=6-8/group, one-way ANOVA, $p<0.01$). (**C**) Mean $\Delta F/F$ of the 470-nm signal in AgRP neurons following II infusion of water, glucose, fat, or amino acids (n=6-8/group, one-way ANOVA, $p<0.001$). (**D-F**) Food intake suppression following infusions of (**D**) glucose (n=6-9/group, unpaired t-test, $p=0.08$) (**E**) fat (n=7-9/group, unpaired t-test, $p<0.001$) and (**F**) amino acids (n=6-9/group, unpaired t-test, $p<0.01$) in the duodenum (light grey) or ileum (dark grey) normalized to food intake after water infusion. Data are expressed as mean ± SEM, ns $p>0.05$, t-tests and post-hoc comparisons: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

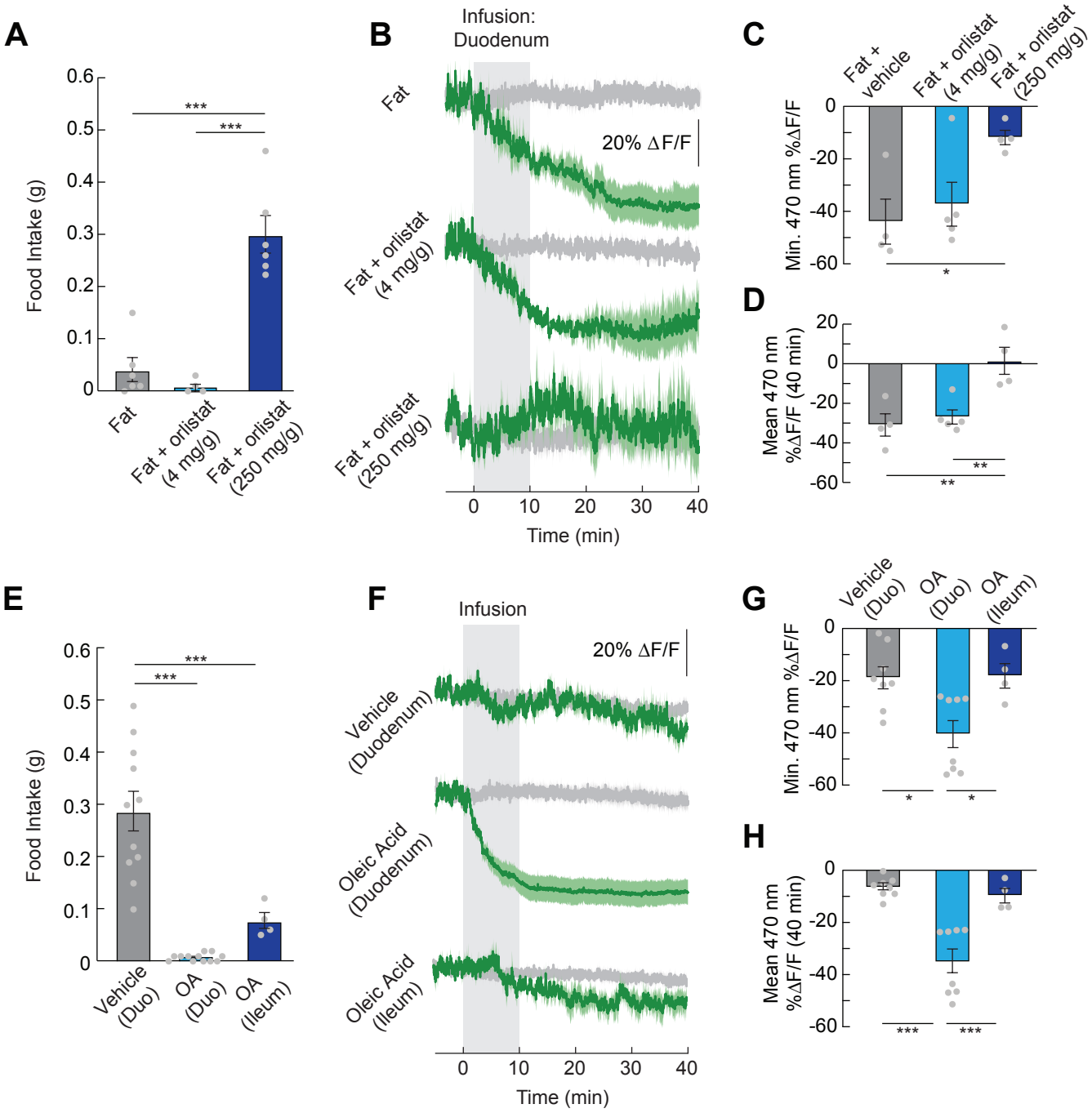

Figure S4, Related to Figure 1

Figure S4, Related to Figure 1. Mechanisms for fat signaling to AgRP neurons. (**A**) Food intake 30 min after intra-duodenal (ID) infusions of fat (1 kcal) or fat with lipase inhibitor (4 or 250 mg orlistat/g fat). (n=4-6/group, one-way ANOVA, $p<0.001$). (**B**) Average $\Delta F/F$ of GCaMP6s signals in AgRP neurons of food-restricted mice during ID infusions of fat solutions in (A). Signals are aligned to the start of the infusion. Green, 470-nm calcium signal; grey, 405-nm control signal. Dark lines represent means and lighter shaded areas represent SEM. (**C**) Minimum $\Delta F/F$ of the 470-nm signal in AgRP neurons during ID infusions of fat solutions in (B) (n=4-5/group, one-way ANOVA, $p<0.05$). (**D**) Mean $\Delta F/F$ of the 470-nm signal from 0 to 40 min in AgRP neurons with ID infusion of fat solutions in (B) (n=4-5/group, one-way ANOVA, $p<0.01$). (**E**) Food intake 30 min after ID or II infusion of vehicle or 12.5% oleic acid (n=4-12/group, one-way ANOVA, $p<0.001$). (**F**) Average $\Delta F/F$ of GCaMP6s signals in AgRP neurons of during intra-duodenal or intra-ileal infusions of vehicle or 12.5% oleic acid. (**G**) Minimum $\Delta F/F$ of the 470-nm signal in AgRP neurons with ID or II infusion of solutions in (F) (n=4-8/group, one-way ANOVA, $p<0.01$). (**H**) Mean $\Delta F/F$ of the 470-nm signal from 0 to 40 min in AgRP neurons with ID or II infusion of solutions in (F) (n=4-8/group, one-way ANOVA, $p<0.001$). Data are expressed as mean ± SEM, ns $p>0.05$, t-tests and post-hoc comparisons: *$p<0.05$, **$p<0.01$, ***$p<0.001$.