

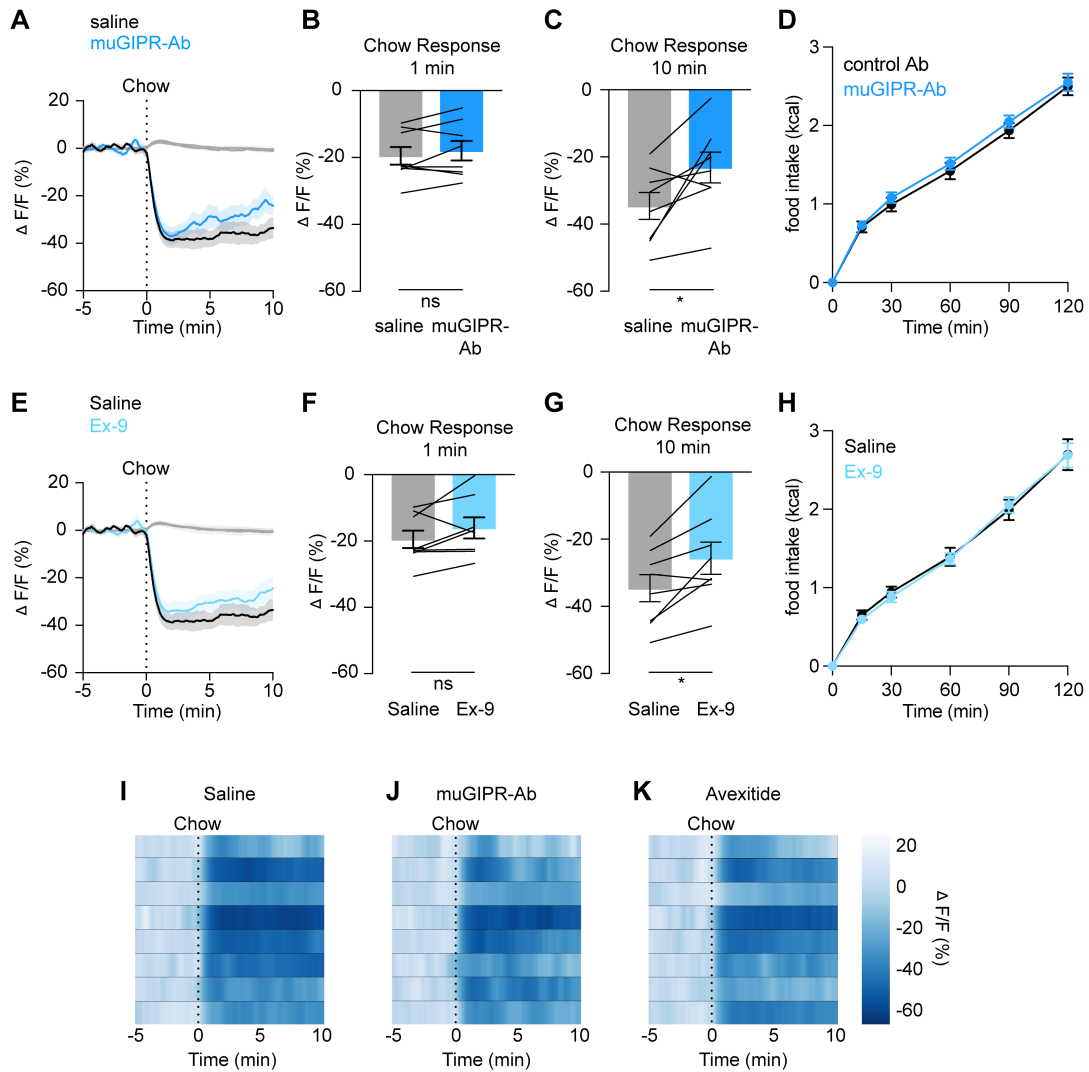
Supplemental Figure 1. Nutrient-mediated AgRP neuron inhibition is stable over time in untreated mice

(A,E,I) Calcium signal in AgRP neurons from fasted mice during infusion of glucose **(A)**, intralipid **(E)**, and Ensure **(I)** at baseline and 7-10 days later as indicated. $n = 9-10$ mice per group.

(B,F,J) Average $\Delta F/F$ in mice from **(A,E,I)** at the end of nutrient infusion. **(B)** paired t-test, $p=0.3471$; **(F)** paired t-test, $p=0.1781$; **(J)** paired t-test, $p=0.2725$.

(C,D,G,H,K,L) Heat maps showing $\Delta F/F$ in individual mice from **(A,E,I)** during nutrient infusion.

(A,E,I) Isosbestic traces for all recordings are shown in gray. **(C,D,G,H,K,L)** Vertical dashed lines indicate the start and end of nutrient infusions. **(B,F,J)** Lines represent individual mice. Error bars indicate mean \pm SEM.



Supplemental Figure 2. GIPR and GLP-1R blockade cause delayed attenuation of the AgRP neural response to chow

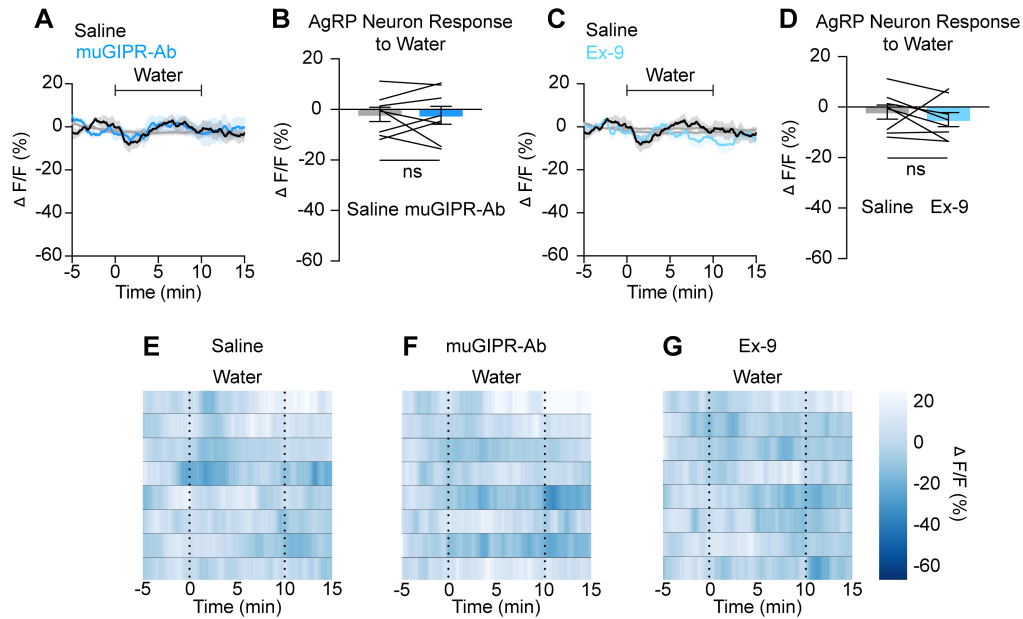
(A,E) Calcium signal in AgRP neurons from fasted mice presented with chow after pre-treatment with muGIPR-Ab **(A)**, Ex-9 **(E)**, or saline as indicated. $n = 8$ mice per group.

(B,C,F,G) Average $\Delta F/F$ in mice from **(A,E)** 1 minute **(B,F)** and 10 minutes **(C,G)** after chow presentation. **(B)** paired t-test, $p=0.2629$; **(C)** paired t-test, $p=0.0318$; **(F)** paired t-test, $p=0.1270$; **(G)** paired t-test, $p=0.0111$).

(D,H) Two-hour chow intake following an overnight fast and pre-treatment with muGIPR-Ab versus control Ab at the time of fasting **(D)** or Ex-9 versus saline immediately before re-feeding **(H)** as indicated in C57BL/6 mice. $n = 16$ mice per group. **(D)** two-way ANOVA treatment \times time interaction, $p=0.8420$; **(H)** two-way ANOVA treatment \times time interaction, $p=0.7569$).

(I,J,K) Heat maps showing $\Delta F/F$ in individual mice from **(A,E)** after chow presentation.

(A,E) Isosbestic traces for all recordings are shown in gray. **(A,E,I,J,K)** Vertical dashed lines indicate chow presentation. **(B,C,F,G)** Lines represent individual mice. Error bars indicate mean \pm SEM. T-tests: $*p < 0.05$.



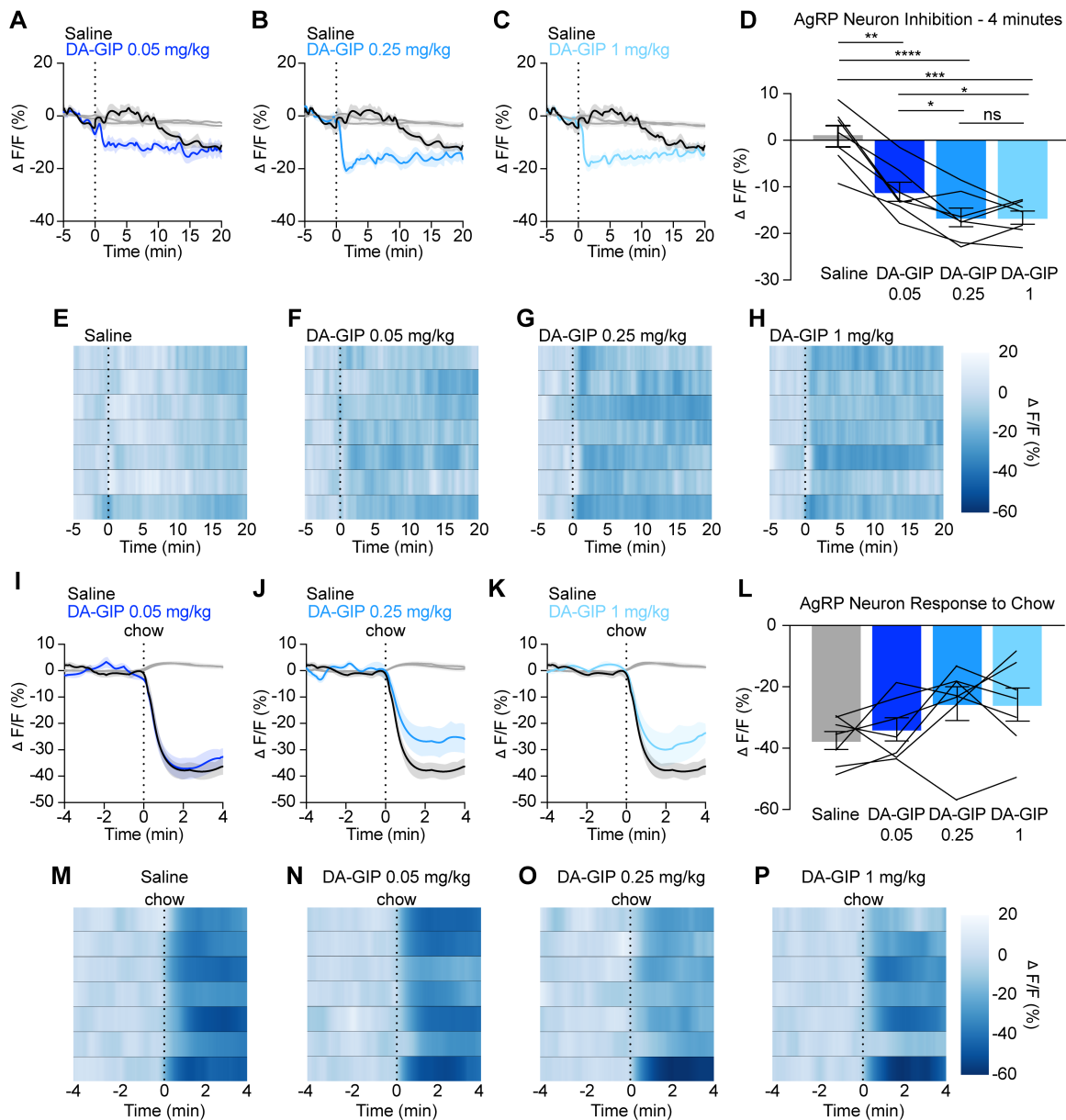
Supplemental Figure 3. GIPR and GLP-1R blockade do not alter AgRP neural response to water infusion

(A,C) Calcium signal in AgRP neurons from fasted mice during water infusion after pre-treatment with muGIPR-Ab **(A)**, Ex-9 **(C)** or saline as indicated. $n = 8$ mice per group.

(B,D) Average $\Delta F/F$ in mice from **(A,C)** at the end of water infusion. **(B)** paired t-test, $p=0.9004$; **(D)** paired t-test, $p=0.3644$.

(E,F,G) Heat maps showing $\Delta F/F$ in individual mice from **(A,C)** during water infusion.

(A,C) Isosbestic traces for all recordings are shown in gray. **(E,F,G)** Vertical dashed lines indicate start and end of water infusions. **(B,D)** Lines represent individual mice. Error bars indicate mean \pm SEM.



Supplemental Figure 4. AgRP neuron responses to GIPR agonists are dose-dependent

(A-C) Calcium signal in AgRP neurons from fasted mice injected with DA-GIP at 0.05 mg/kg (A), 0.25 mg/kg (B), or 1 mg/kg (C) compared to saline as indicated. $n = 7$ mice per group.

(D) Average $\Delta F/F$ in mice from (A-C) 4 minutes after injection. (one-way ANOVA, $p < 0.0001$).

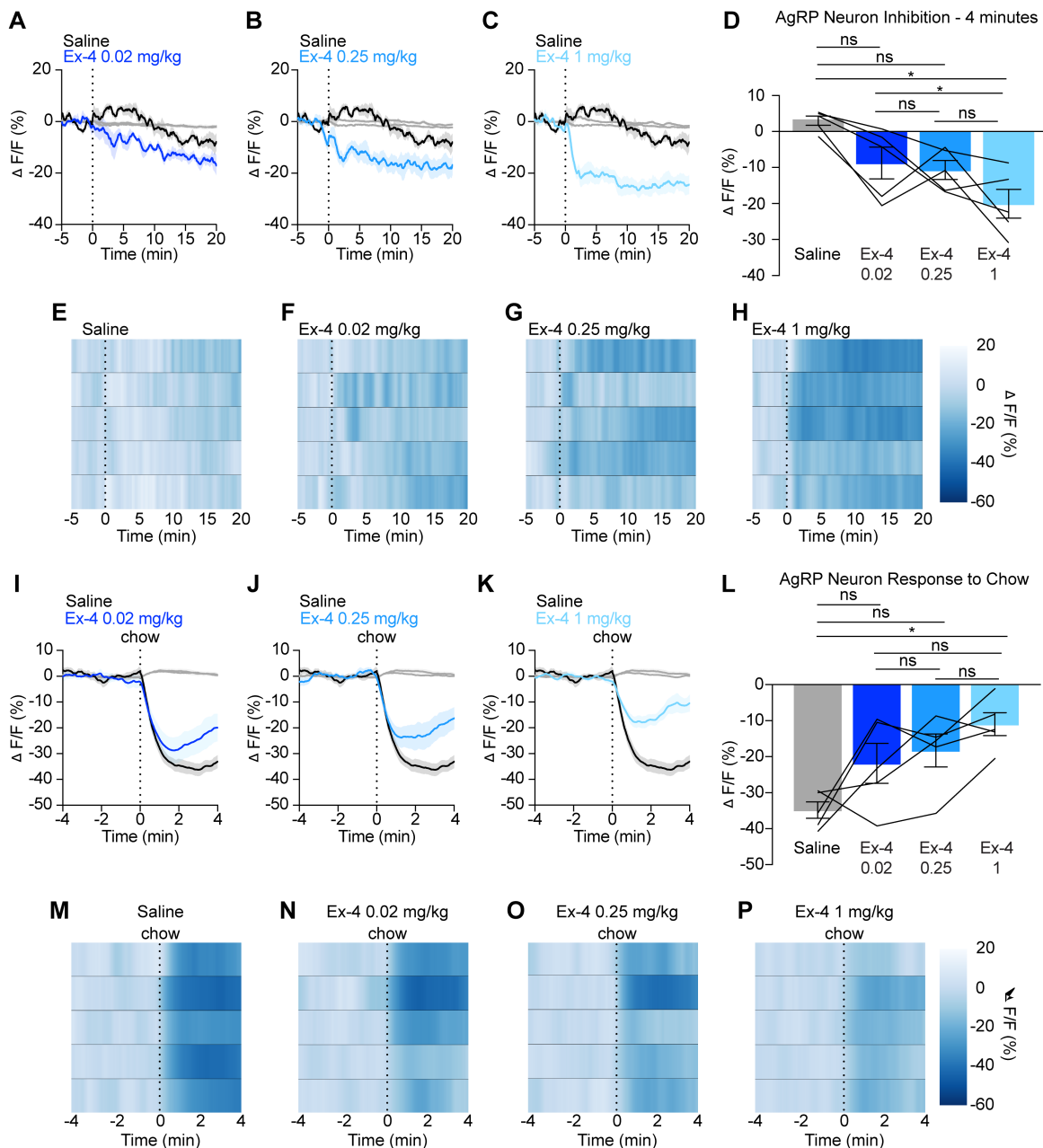
(E-H) Heat maps showing $\Delta F/F$ in individual mice from (A-C) injected with saline (E), DA-GIP at 0.05 mg/kg (F), 0.25 mg/kg (G), or 1 mg/kg (H) as indicated.

(I-K) Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after pre-treatment with saline or DA-GIP at 0.05 mg/kg (I), 0.25 mg/kg (J), or 1 mg/kg (K) as indicated. $n = 7$ mice per group.

(L) Average $\Delta F/F$ in mice from (I-K) 4 minutes after chow presentation. (one-way ANOVA, $p = 0.0738$).

(M-P) Heat maps showing $\Delta F/F$ in individual mice from (I-K) after chow presentation.

(A-C, I-K) Isosbestic traces for all recordings are shown in gray. (A-C, E-H, I-K, M-P) Vertical dashed lines indicate the time of injection or chow presentation. (D, L) Lines represent individual mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



Supplemental Figure 5. AgRP neuron responses to GLP-1R agonists are dose-dependent

(A-C) Calcium signal in AgRP neurons from fasted mice injected with Ex-4 at 0.02 mg/kg **(A)**, 0.25 mg/kg **(B)**, or 1 mg/kg **(C)** compared to saline as indicated. $n = 5$ mice per group.

(D) Average $\Delta F/F$ in mice from **(A-C)** 4 minutes after injection. (one-way ANOVA, $p=0.0119$).

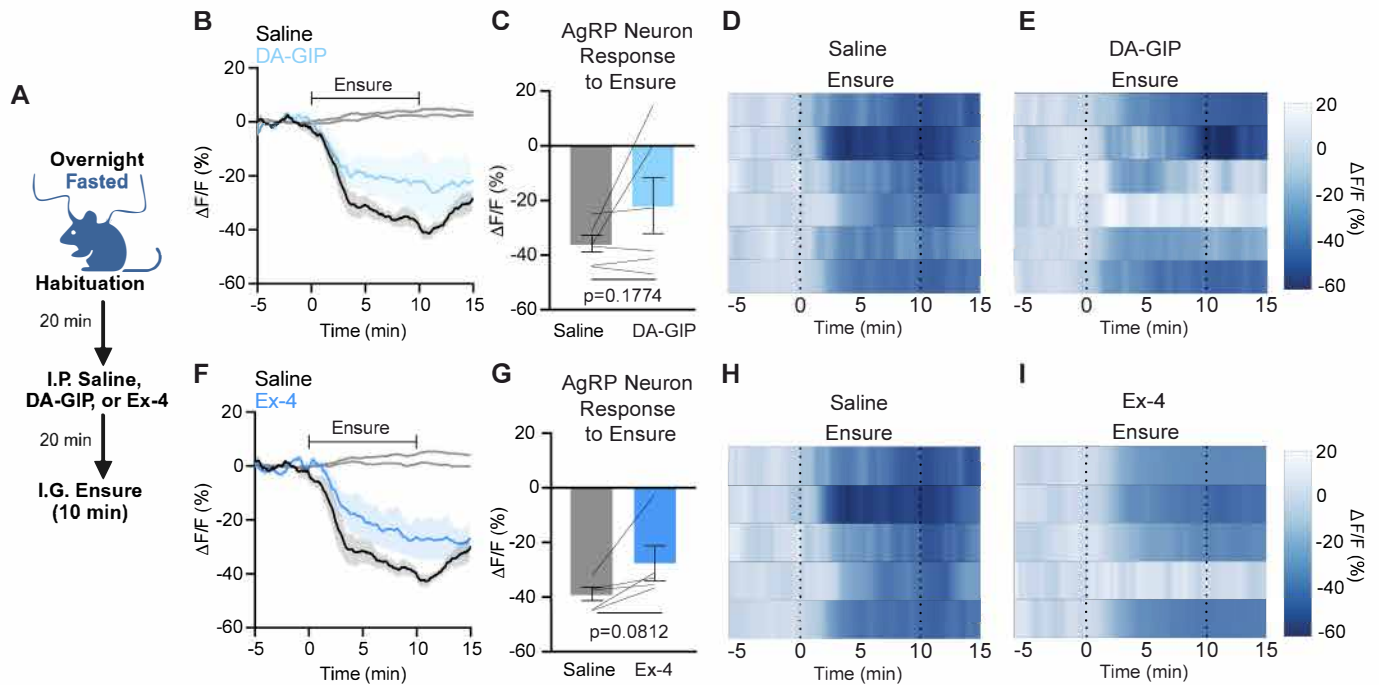
(E-H) Heat maps showing $\Delta F/F$ in individual mice from **(A-C)** injected with saline **(E)**, Ex-4 at 0.02 mg/kg **(F)**, 0.25 mg/kg **(G)**, or 1 mg/kg **(H)** as indicated.

(I-K) Calcium signal in AgRP neurons from fasted mice presented with chow 20 minutes after pre-treatment with saline or Ex-4 at 0.02 mg/kg **(I)**, 0.25 mg/kg **(J)**, or 1 mg/kg **(K)** as indicated. $n = 5$ mice per group.

(L) Average $\Delta F/F$ in mice from **(I-K)** 4 minutes after chow presentation. (one-way ANOVA, $p=0.0219$).

(M-P) Heat maps showing $\Delta F/F$ in individual mice from **(I-K)** after chow presentation.

(A-C, I-K) Isosbestic traces for all recordings are shown in gray. **(A-C, E-H, I-K, M-P)** Vertical dashed lines indicate the time of injection or chow presentation. **(D, L)** Lines represent individual mice. Error bars indicate mean \pm SEM. Post-hoc comparisons: $*p<0.05$.



Supplemental Figure 6. GIPR and GLP-1R agonists do not attenuate intragastric nutrient-mediated AgRP neuron inhibition

(A) Experimental schematic.

(B,F) Calcium signal in AgRP neurons from fasted mice during intragastric infusion of Ensure 20 minutes after pre-treatment with saline, DA-GIP (1 mg/kg; **B**), or Ex-4 (0.5 mg/kg; **F**) as indicated. $n = 5-6$ mice per group.

(C,G) Average $\Delta F/F$ in mice from **(B, F)** at the end of Ensure infusion. (**C**) paired t-test, $p = 0.1774$; (**G**) paired t-test, $p = 0.0812$.

(D,E,H,I) Heatmaps showing $\Delta F/F$ in individual mice from **(B, F)** during Ensure infusion after pre-treatment with saline (**D, H**), DA-GIP (**E**), or Ex-4 (**I**).

(B,F) Isosbestic traces for all recordings are shown in gray. **(C,G)** Lines represent individual mice. **(D,E,H,I)** Vertical dashed lines indicate the start and end of Ensure infusion. Error bars indicate mean \pm SEM.