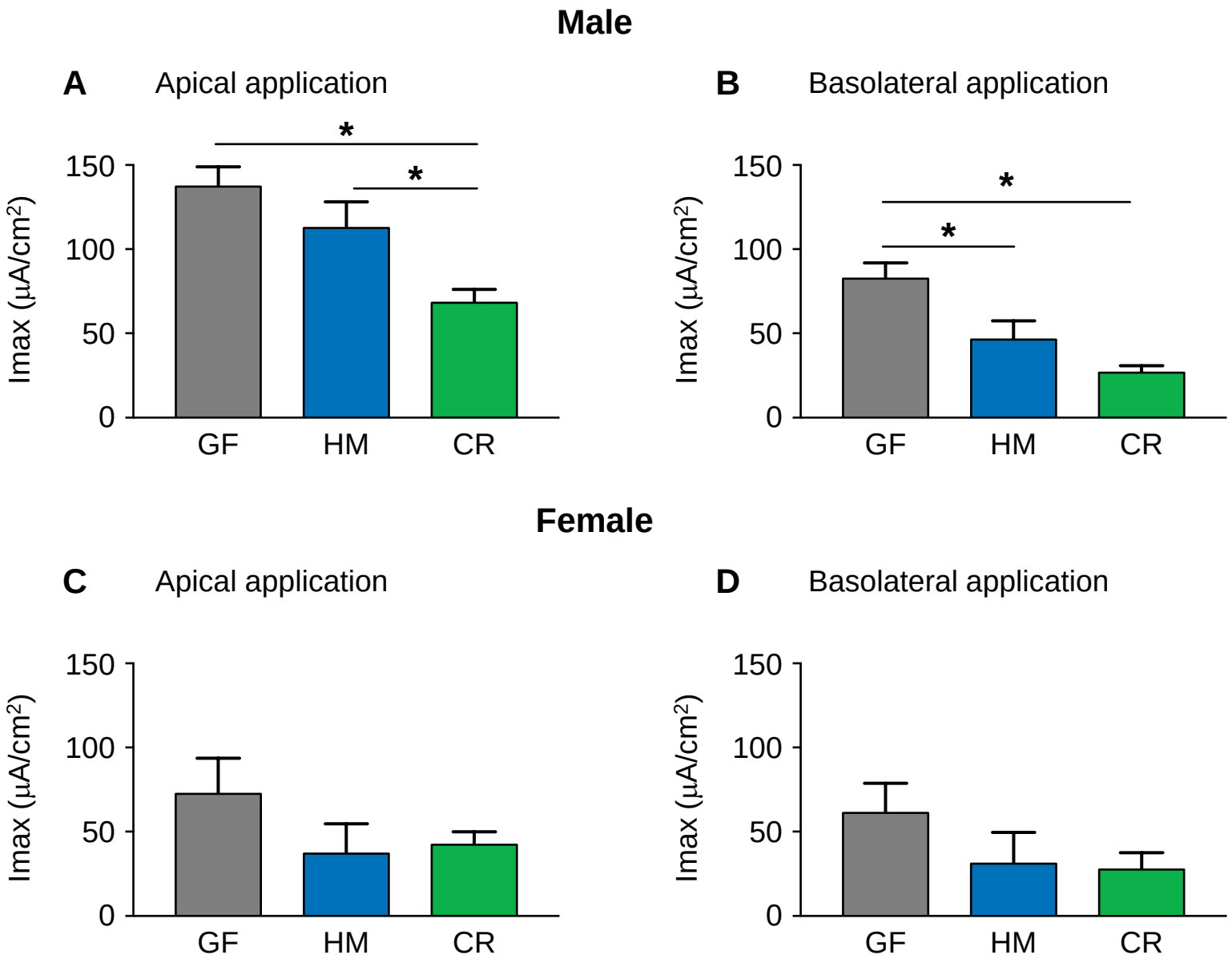

Figure S1 (related to Figure 1). Tryptamine evoked maximal increase in *Isc* (Imax) response is higher in GF males than HM or CR male mice but not in female mice. *Imax* in response to tryptamine when applied apically (A) and basolaterally (B) in male and female (C, D) GF, HM and CR mice. n=4-6, one-way *ANOVA,* $^{*}P<0.05$.

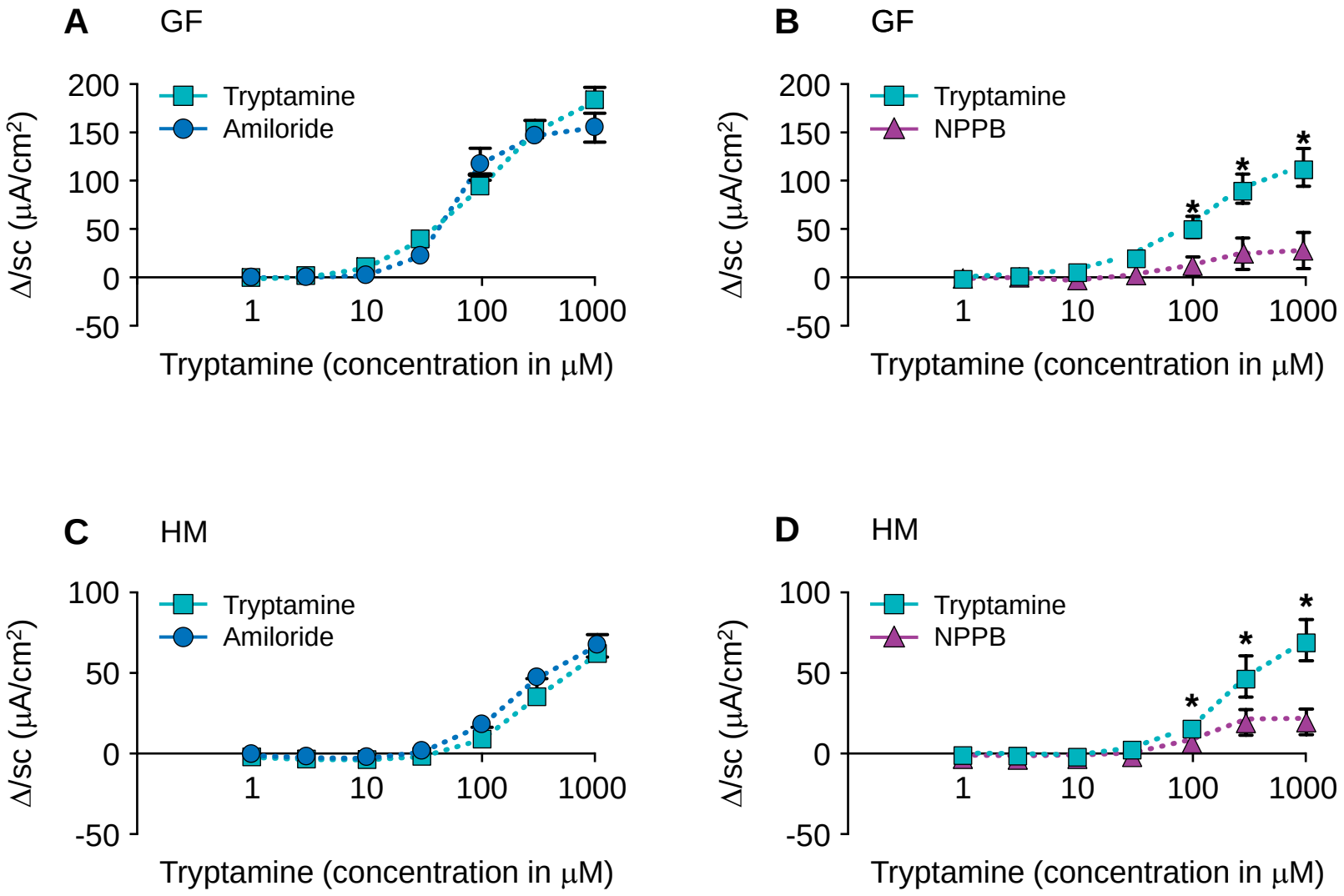

Figure S2 (related to Figure 1). Tryptamine evoked increase in *Isc* is mediated by anionic transport in both GF and HM mice. Δ*Isc* response following basolateral application of cumulative concentrations of tryptamine either alone or in presence of amiloride (5μM) in GF (A) and HM mice (C). Δ*Isc* response following basolateral application of cumulative concentrations of tryptamine either alone or in presence of 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB; 100μM) in GF (B) or HM mice (D). n=4 male mice, *two-way ANOVA*, $^{*}P<0.05$.

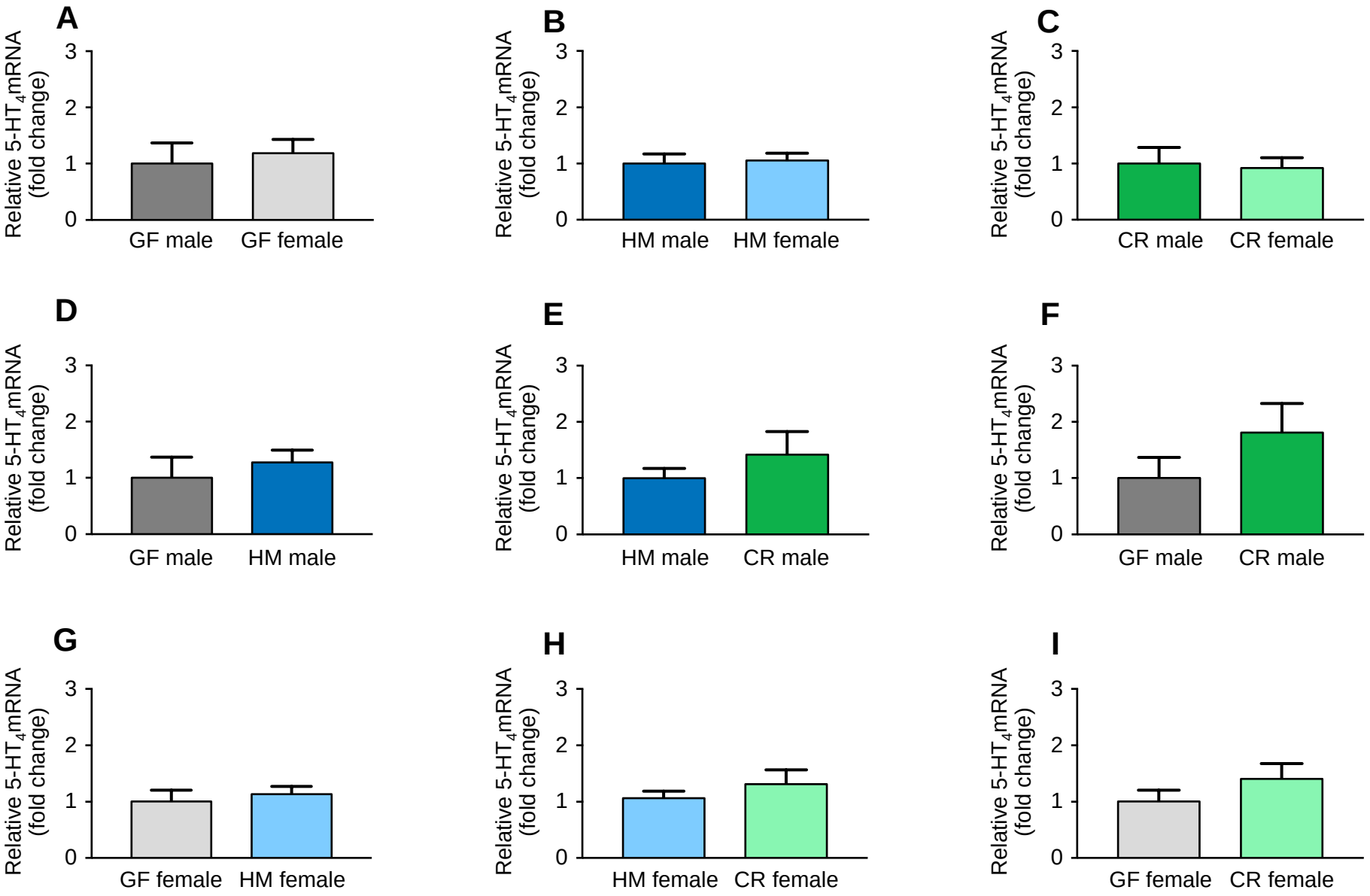

Figure S3 (related to Figure 2). Sex and colonization status does not alter 5-HT_4R mRNA expression. No change in 5-HT_4R mRNA expression is observed between sex in GF (A), HM (B) and CR (C) mice. Colonization status does not affect 5-HT_4R mRNA expression in male (D-F) and female (G-I) mice. n=5-6, un-paired t-test, *P*>0.05.

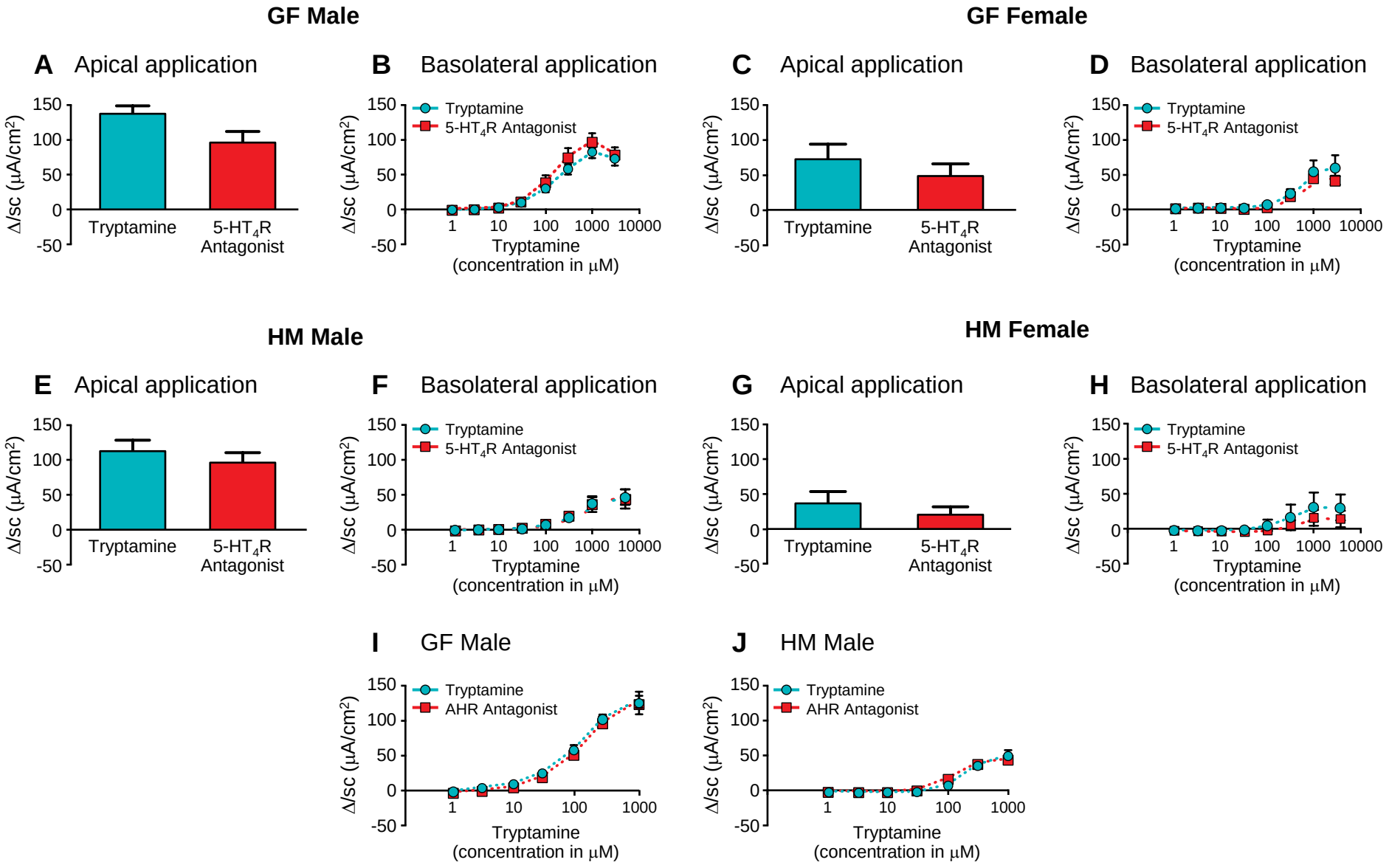

Figure S4 (related to Figure 2). Tryptamine evoked Δ*Isc* is not inhibited by 5-HT$_3$R antagonist and aryl hyrdocarbon receptor antagonist. Δ*Isc* in response to tryptamine applied apically (3mM, paired *t-test*) and cumulative concentrations of tryptamine applied basolaterally (*two-way ANOVA*) respectively, either alone or in the presence of ondansetron (100nM) in GF male, (A,B) GF female (C,D), HM male (E,F), HM female (G,H). Δ*Isc* response following basolateral application of cumulative concentrations of tryptamine either alone or in presence of aryl hydrocarbon receptor antagonist CH-223191 (10μM) in GF (I) and HM (J) male mice. n=4-6, **P*<0.05.

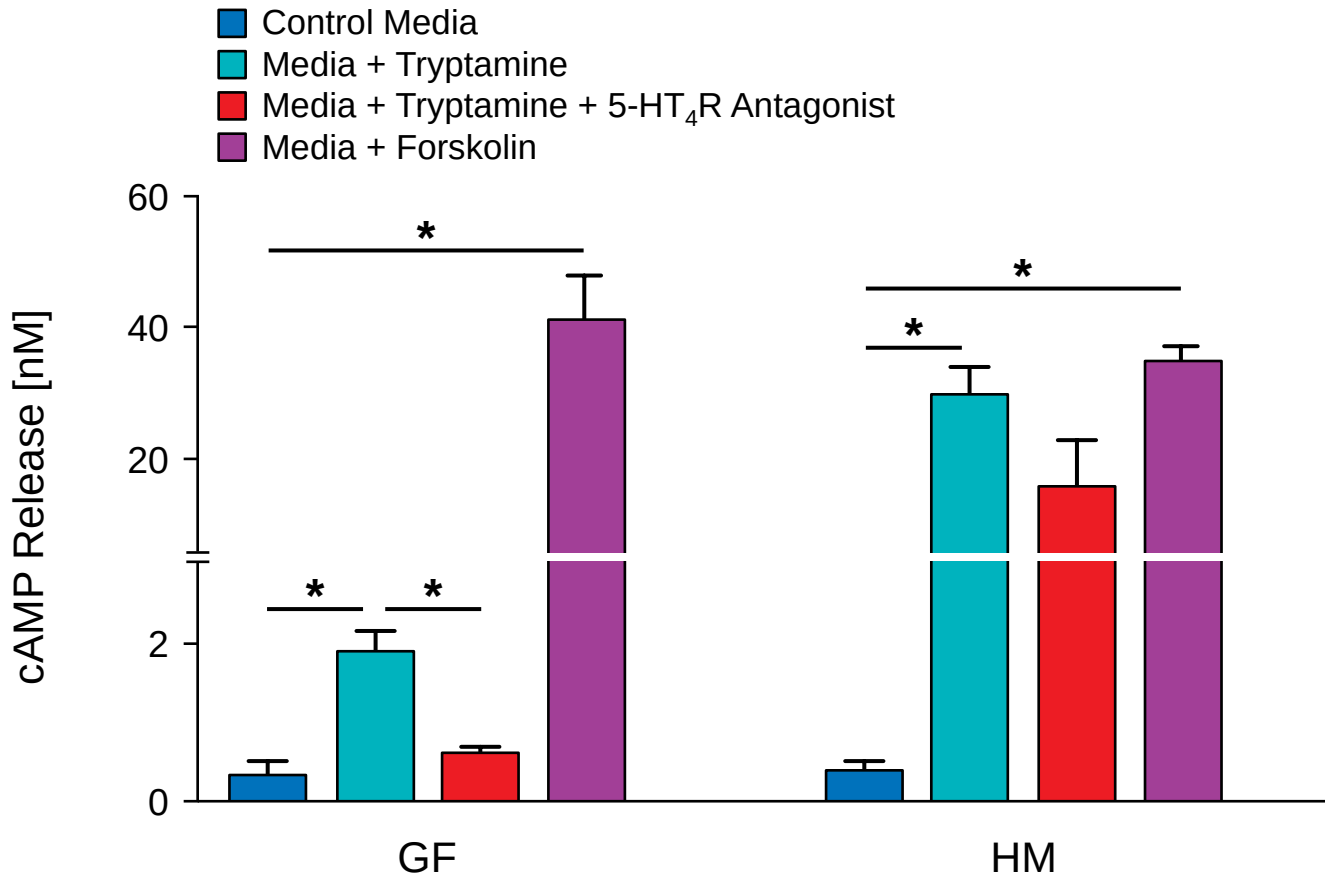

Figure S5 (related to Figure 6). Tryptamine increases cAMP in colonoids irrespective of microbial colonization. Total cAMP in colonoids from GF and HM male mice after incubation for an hour with either control media alone, media with tryptamine (1mM), media with tryptamine (1mM) 30 minutes after pre-treatment with GR-113808 (100 nM) and media with forskolin. n=3, *P<0.05, one-way *ANOVA*.

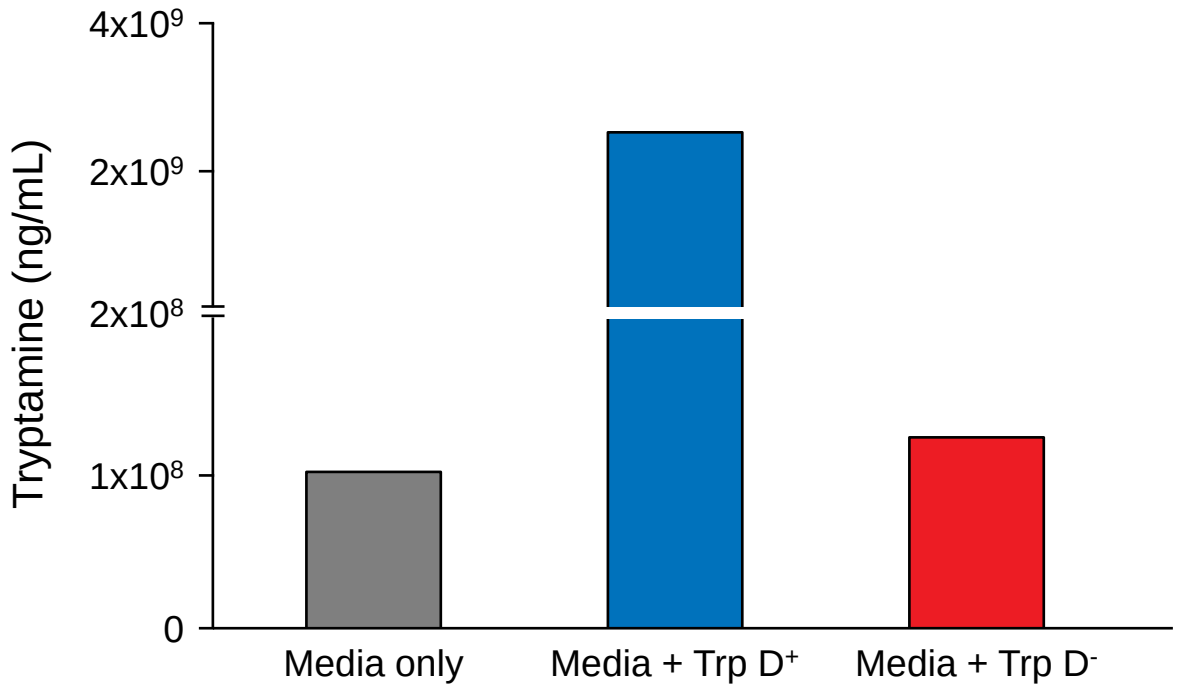

Figure S6 (related to Figure 7). Genetically engineered *B. thetaiotaomicron* with tryptophan decarboxylase gene produces tryptamine *in vitro*. Tryptamine concentrations measured after overnight inoculation of TYG media with either engineered *B. thetaiotaomicron* Trp D^+ or control *B. thetaiotaomicron* Trp D^- relative to media only control.

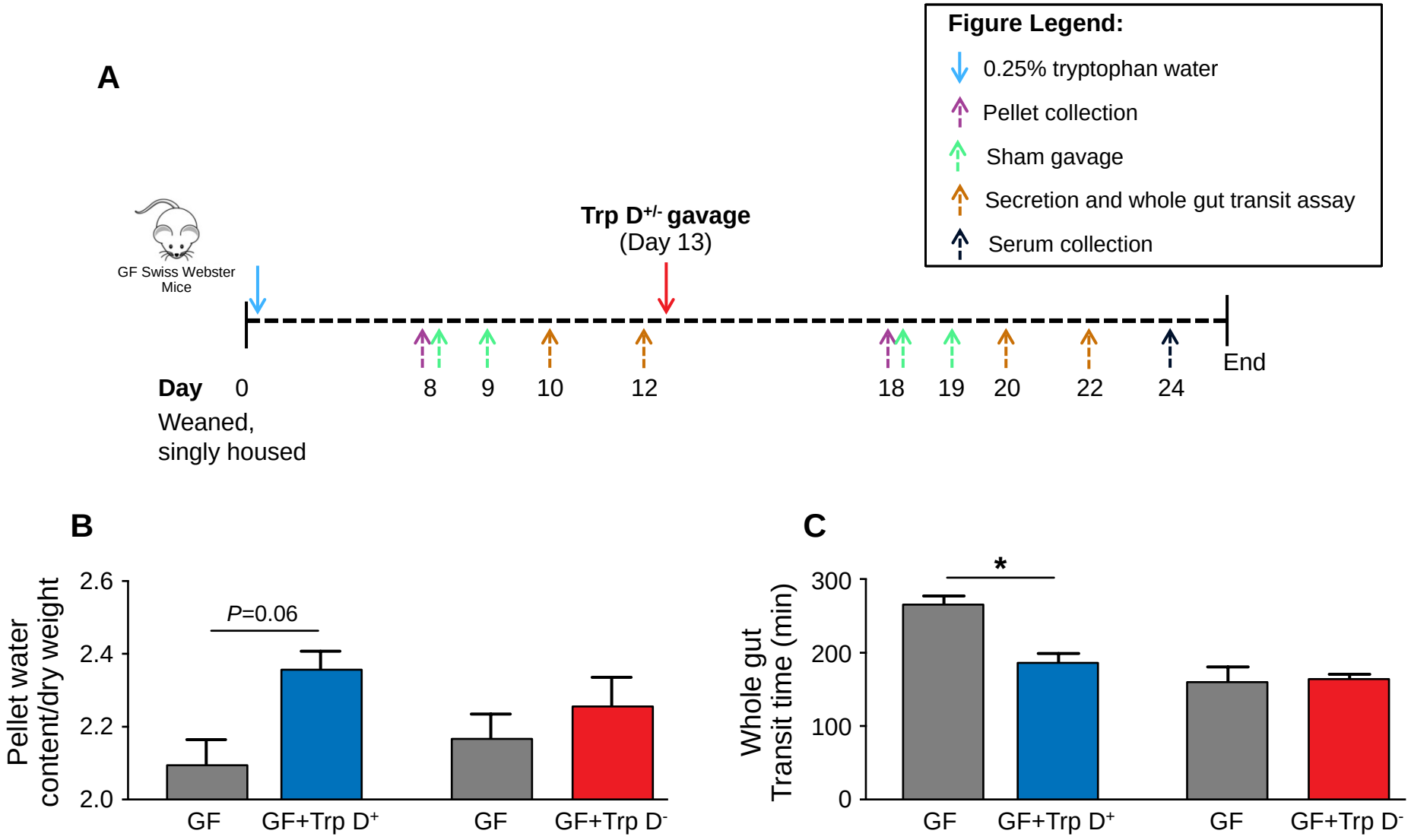

Figure S7 (related to Figure 7G, H). Experimental design for assessing the effect of bacterially produced tryptamine on colonic secretion and whole gut transit time in GF mice colonized with either engineered *B. thetaiotaomicron* Trp D^+ or control *B. thetaiotaomicron* Trp D^-. Experimental design and timeline for monocolonization experiments (A). Change in pellet water content per dry weight (B) and absolute whole gut transit time (C) in GF mice colonized with *B. thetaiotaomicron* Trp D^+ and control *B. thetaiotaomicron* Trp D^-. Mean ± SEM, n=5, one-way *ANOVA*, **P*<0.05.