Supplementary Figure 1

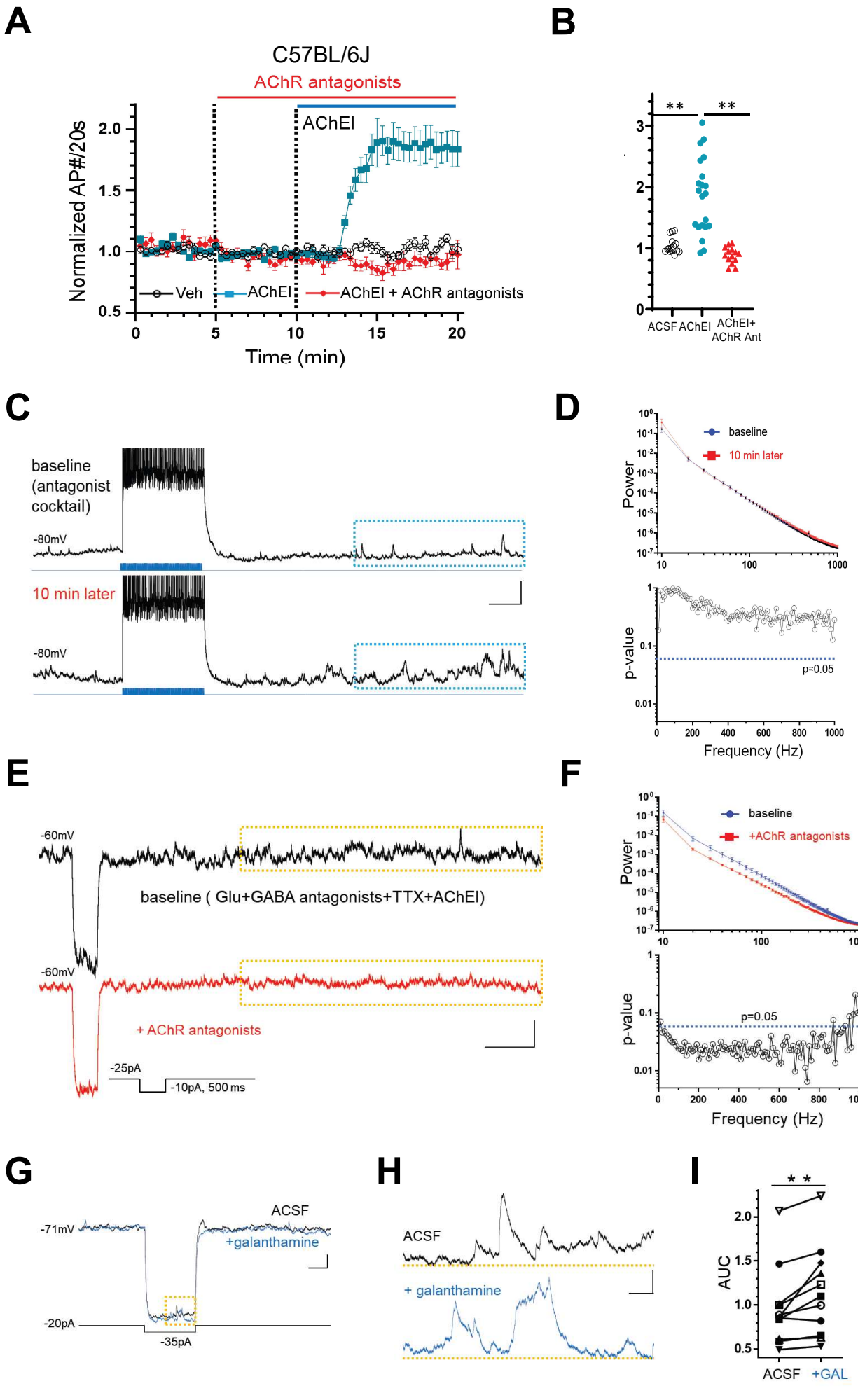

Figure S1. ACh-mediated PSPs in MHb cholinergic neurons. A. Left, average number of APs in response to bath application of vehicle (ACSF), AChEI (denoted by the blue bar), or of AChEIs in the presence of AChR antagonists (application in time denoted by the blue bar) in C57BL/6J. Each data point represents the normalized average AP during 20 s intervals. **B.** Scatter plot of AP frequency change at the end of 10 min application of ACSF, AChEI, or AChEIs in the presence AChR antagonists (One-way ANOVA $F(2,47)=27.83$, $p<0.0001$; $^{**}p<0.01$ Tukey's multiple comparison test). **C.** Representative whole-cell current-clamp recording of MHb cholinergic neurons from ChAT::ChR2 after 10 min of stimulation and after another 10 min of stimulation without Mec. Scale bar: 2 sec, 10 mV. **D.** Normalized power spectrum (Top, Mean ± SEM) from traces in panel (C) (n=6; repeated 2-way ANOVA, interaction frequency x power, $F(99,495)=2.320$, $p<0.0001$). (Bottom) Plot of p-value vs. frequency. Dotted line represents $p=0.05$ (paired t-test at each interval, n=6). **E.** Representative whole-cell current-clamp recording of MHb cholinergic neurons from ChAT::GFP mice. PSPs were evident and recorded in the presence of iGluR and GABA receptor antagonists (NBQX, CPP, SR95531, CGP52432), TTX and AChEI (Top trace) and after addition of AChR antagonists (Mecamylamine & atropine) which reduced PSP amplitude (Bottom trace, n=5). Downward deflection was elicited by a -10 pA (500 ms) test pulse. Scale bar: 2 sec, 5 mV. **F.** Normalized power spectrum (Top, Mean ± SEM) from traces in panel (E) (n=5; repeated 2-way ANOVA, interaction frequency x power, $F(99,396)=5.984$, $p<0.0001$). (Bottom) Plot of p-value vs. frequency. Dotted line represents $p=0.05$ (paired t-test at each interval, n=5). **G.** Analysis of PSPs as in panel (E) in response to the AChEI, galantamine (20 μM) from C57BL/6J mice. Scale bar: 0.4 sec, 4 mV. **H.** Amplification of PSPs during hyperpolarization shown in panel (H). **I.** AUC of PSPs in the absence and presence of galantamine (n=11; ACSF, 0.97 ± 0.14; GAL, 1.25 ± 0.15; paired t-test, $t=3.227$, $df=10$, $p=0.0091$). Scale bar: 60 ms, 1 mV.

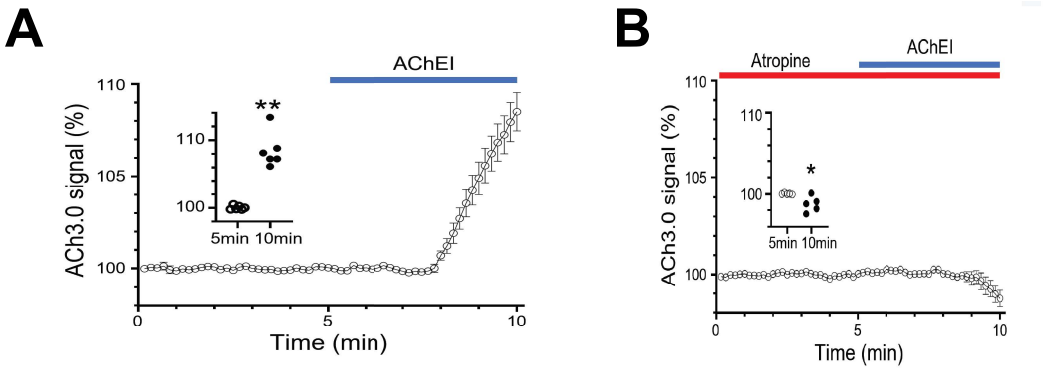

Figure S2. ACh3.0 signal after AChEI, ACh, ACSF. A. Percent change in ACh3.0 signal before and during bath application of AChEI in the IPN. ACh3.0 (AAV9-hSyn-ACh3.0) signal (10 sec bin) increased over time from 210 sec after AChEI application. Inset, summed average signal change at baseline and during AChEI application (n=6; 5 min, 100.0 ± 0.1%; 10 min, 116.2 ± 1.4%; paired t-test, t=11.29, df=5, p<0.0001). **B.** Percent change in ACh3.0 signal as in panel (A) but in the presence of atropine (n=5; 5 min, 100.1 ± 0.0%; 10 min, 98.8 ± 0.4%; paired t-test, t=3.111, df=4, p=0.0359).

Supplementary Figure 3

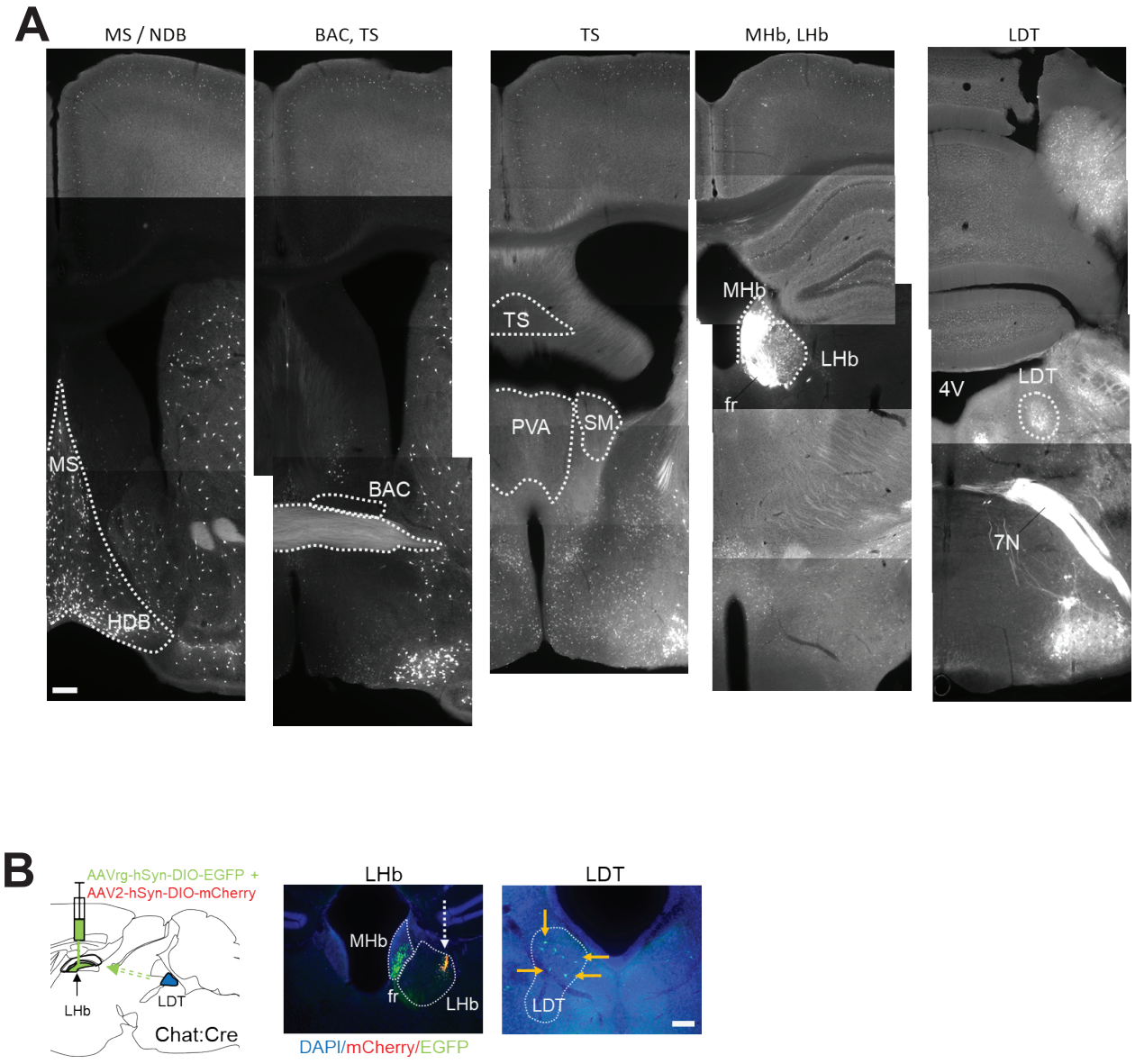

Figure S3. Chat::GFP mouse shows overall cholinergic neuron distribution. A. Chat::GFP gives a guide to candidate areas that could project to MHb. Note: TS is virtually devoid of Chat neuron. Scale bar: 200 μm. **B.** Left, experimental strategy for retrograde labeling of cholinergic inputs to the LHb. AAVrg-hSyn-DIO-eGFP was co-injected into the LHb of ChAT::Cre mice with AAV2-hSyn-DIO-mCherry as an anterograde injection marker. Middle, photomicrograph of the injection site depicting eGFP and mCherry expression in LHb. Right, eGFP expression in LDT of ChAT::Cre mice after retrograde virus injection in LHb. Scale bar: 200 μm.