

Supplemental Material

Research Articles | Cellular/Molecular

Abnormal neuronal excitability and reduced parvalbumin expression in Shank3-deficient parvalbumin neurons of the thalamic reticular nucleus

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Supplemental information:

Fig. S1. Data analysis of AP firing by Clampfit (A) and the relationship between KB and peak calcium concentration (B).

Fig S2. AP firing by WT TRN PV and SOM neurons in response to 100 pA current injection.

- A.** Membrane potential traces of PV and SOM neurons in response to 100 pA current injection. AP firing by PV neurons exhibits two distinct modes, burst mode and tonic mode. In contrast, SOM neurons only show tonic mode. In addition, in tonic firing mode, PV neurons exhibit little adaptation whereas SOM neurons show strong frequency adaption, which is presumably calcium-dependent.
- B.** Compared to SOM neurons, PV neurons exhibit increased AP numbers, frequency, and number of burst firings.
- C.** Statistics. Animal numbers: 6 (PV), 5 (SOM). Neuron numbers: 10 (PV), 10 (SOM). P-value: <0.0001 (AP number), 0.0001 (Frequency), <0.0001 (burst number).

Fig. S3. AP firing by WT, Shank3^{-/-}, and HCN2^{-/-} TRN PV neurons.

- A.** AP firing elicited by +400 pA current injection.
- B.** Left, time interval of tonic firing for the last 8 APs. Right, half-width.
- C.** Half-amplitude of all APs (including Na spikes in burst firing and APs in tonic firing).
- D.** Total number of APs (Na spikes in burst firing and APs in tonic firing). Animals used: 6 (WT), 4 (Shank3^{-/-}), 4 (HCN2^{-/-}). Neuron numbers: 10 (WT), 10 (Shank3^{-/-}), 10 (HCN2^{-/-}). P-value: WT-Shank3^{-/-}, WT-HCN2^{-/-}, (tonic interval) 0.011 0.0002; (half-width) 0.822 0.739, (half-amplitude 400pA) 0.013 0.627, (AP) <0.0001 <0.0001.
- E.** AP firing by WT and HCN2^{-/-} TRN PV neurons recorded at the RMP around -60 mV.
- F.** Statistics of total AP numbers. Animals used: 5 (WT), 6 (HCN2^{-/-}). Neuron numbers: 10 (WT), 10 (HCN2^{-/-}). P-value (400 pA): 60 mV, 0.0001; 70 mV, 0.001.
- G.** Statistics of AP frequency. P-value (400 pA): 60 mV, 0.0001; 70 mV, 0.0008.

Fig. S4. Single or two Burst firings by WT and Shank3^{-/-} TRN neurons elicited by minimal current injections.

For comparison purposes, the RMP of recorded neurons was held around -70 mV through adjusting the holding current. The amplitude of depolarizing current injections was gradually increased by 1-2 pA each time, from the level of 25 pA, to approach the goal of either one or two burst firings.

- A.** Top, single burst firing by WT, Shank3^{-/-}, and HCN2^{-/-} neurons. Bottom, statistics. Animals used: 3 (WT), 2 (Shank3^{-/-}), 3 (HCN2^{-/-}). Neuron numbers: 17 (WT), 13 (Shank3^{-/-}), 18 (HCN2^{-/-}). P-value: peak to AHP, (WT Shank3^{-/-}) 0.016, (WT HCN2^{-/-}) 0.039; AHP, (WT Shank3^{-/-}) 0.063, (WT HCN2^{-/-}) 0.216, Latency (WT Shank3^{-/-}) 0.747, (WT HCN2^{-/-}) 0.94310.
- B.** Top, two burst firing by WT, Shank3^{-/-}, and HCN2^{-/-} neurons. Bottom, statistics. Animals used: 5 (WT), 2 (Shank3^{-/-}), 3 (HCN2^{-/-}). Neuron numbers: 13 (WT), 13 (Shank3^{-/-}), 15 (HCN2^{-/-}). P-value: peak to peak, (WT Shank3^{-/-}) 0.047, (WT HCN2^{-/-}) 0.024.

Fig. S5. Faster decay of calcium transients in dendrites of Shank3^{-/-} TRN PV neurons.

- A.** IR-DIC image of the TRN region adjacent to the VB area in the thalamus (top). Two-photon fluorescence of an TRN GABA neuron filled with AF594 (red channel) and Fluo-5F.
- B.** Current injection (top, 500 pA x 10 msec, 3 stimulations at 1 Hz), membrane potential (middle), and calcium transients recording from the soma of a WT (left) and a Shank3^{-/-} TRN GABA neurons.
- C.** Overlay of calcium transient traces recorded from WT and Shank3^{-/-} TRN neurons at the soma and four dendritic positions (25, 50, 75, 100 μ m from the soma).
- D.** Statistics of the time constant of calcium transients. Animals used: 5 (WT), 5 (Shank3^{-/-}). Brain slice numbers: 6 (WT), 5 (Shank3^{-/-}). P-value: (soma-dendrites) 1.0 0.001 0.369 0.066 0.033.
- E.** Zoomed view over the stimulation shown in B (WT).
- F.** Zoomed view over the stimulation shown in B 9 (Shank3^{-/-}).

Fig. S6. Immunostaining of PV protein in WT, Shank3^{-/-}, and HCN2^{-/-} thalamus.

- A.** Representative fluorescence images showing PV expression in WT, Shank3^{-/-}, and HCN2^{-/-} in TRN and ventral basal (VB) area of the thalamus.

- B.** Statistical results of averaged neuron number in each brain slice (left) and mean fluorescence intensity corresponding to PV expression (right). Animals used: 3 (WT), 3 (Shank3^{-/-}), and 3 (HCN2^{-/-}). Brain slices imaged: 12 (WT), 12 (Shank3^{-/-}), and 12 (HCN2^{-/-}). P-value: (neuron number) WT-Shank3^{-/-} 0.564, WT-HCN2^{-/-} 0.106; PV fluorescence, WT-Shank3^{-/-} 0.046, WT-HCN2^{-/-}, 0.0002.
- C.** Staining results of brain slices with anti-PV antibody omitted. Fluorescence intensity was digitally enhanced to illustrate the TRN region.
- D.** Fluorescence images of WT and HCN2^{-/-} TRN stained by anti-PV antibody. Preparation of brain slice, procedure of immunostaining, and collection of confocal images were all identical for WT and HCN2^{-/-} samples.
- E.** Statistics of fluorescence intensity and cell density. Animals used: 6 (WT), 6 (HCN2^{-/-}). Brain slices imaged: 17 (WT), 19 (HCN2^{-/-}). P-value: (neuron number) 0.735; PV fluorescence, <0.0001.
- F.** In-situ hybridization (ISH) data of PV (left) and the corresponding reference mouse brain atlas (right), obtained from websites of Allen Institute. A zoomed view of the TRN region is shown below.

Fig. S7. Ratiometric calcium imaging of TRN PV neurons.

- A.** Zoomed view over the stimulation shown in Fig. 5 (WT).
- B.** Zoomed view over the stimulation shown in Fig. 5 (Shank3^{-/-}).
- C.** Replotting Fig. 5F (left) and 5G (right) to illustrate the intercepts between the regression line and the X-axis and the derivation of K_E .
- D.** Parameters used in Fura-8 ratiometric imaging including ΔF values.
- E.** Results of a representative Shank3^{-/-} TRN neuron.
- F.** Calcium transients after perfusion of dyes into cytosol reaching an equilibrium.

Fig. S8. Adding additional EGTA in intracellular solution partially reversed the abnormal AP firing exhibited by Shank3^{-/-} TRN neurons.

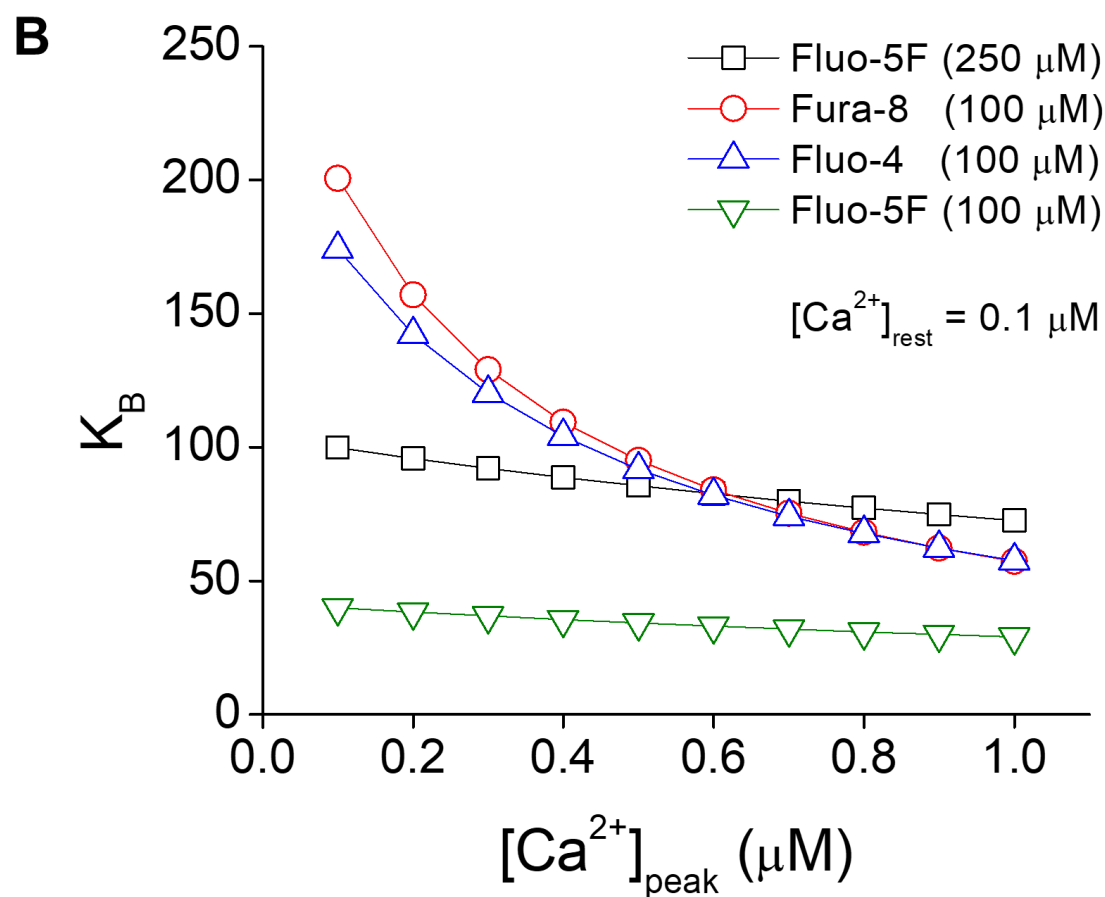
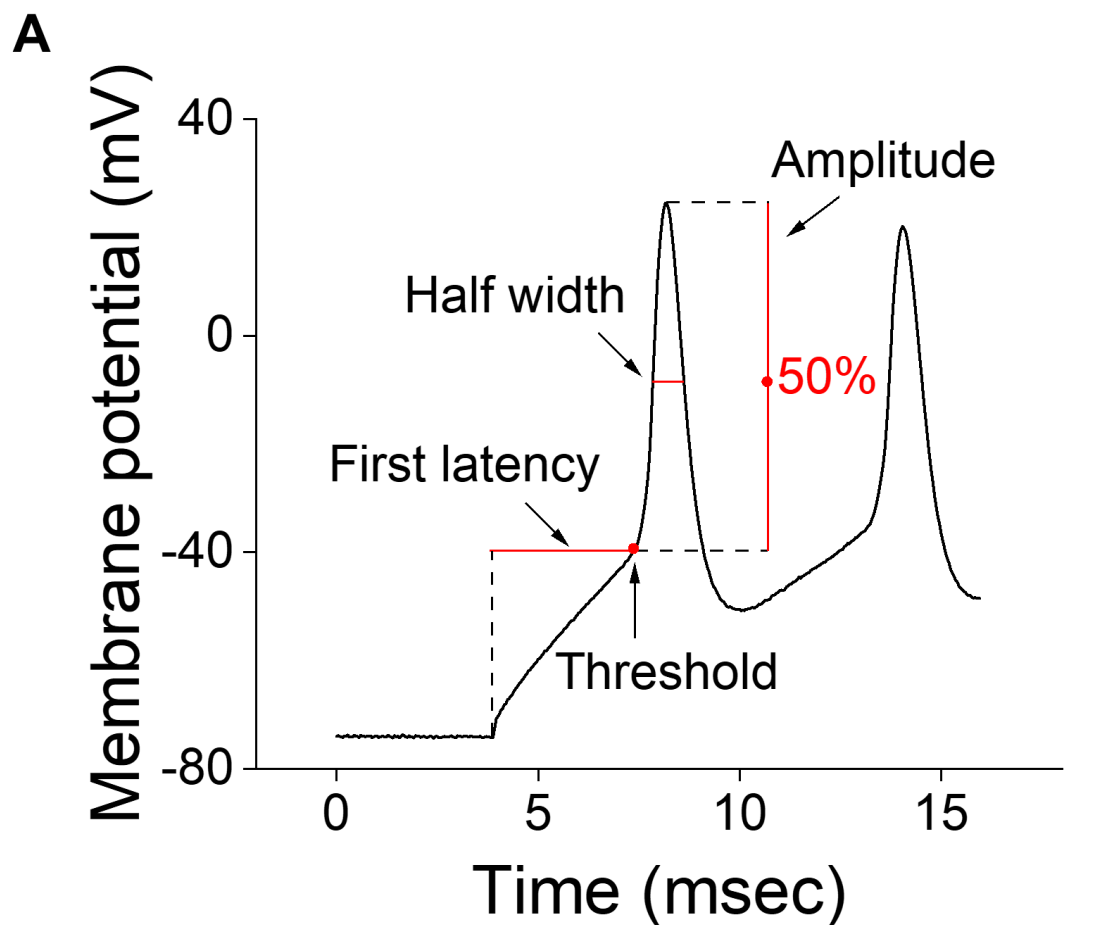
- A.** Membrane potential recordings in response to 50, 100, 150 pA current injections. Adding 2 mM EGTA to the pipette solution decreased the episodes of burst firing and increased the frequency of tonic firing.
- B.** Statistics. Top, burst firing episodes (left) and tonic firing frequency (right). Bottom, half-amplitude of APs (left) and AP number (right). Statistics of half-width (left) and time interval of the last 8 AP in tonic firing (right). Animals used: 7 (WT), 6 (Shank3^{-/-}), 4 (Shank3^{-/-} EGTA). Neuron recorded: 15 (WT), 15 (Shank3^{-/-}), 15 (Shank3^{-/-} EGTA). P-value: burst number, WT Shank3^{-/-} 0.0026, Shank3^{-/-} - Shank3^{-/-} EGTA 0.0099, WT -Shank3^{-/-} EGTA, 0.363; tonic frequency, WT - Shank3^{-/-} <0.0001, WT - Shank3^{-/-} EGTA, <0.0001; AP number, WT - Shank3^{-/-} <0.0001, WT - Shank3^{-/-} EGTA, <0.0001; half width, WT - Shank3^{-/-}, 0.192, WT – Shank3^{-/-} EGTA 0.075.
- C.** Statistics of the latency of the 1st Na spike. P-value: first latency, WT - Shank3^{-/-} 0.741, Shank3^{-/-} - Shank3^{-/-} EGTA 0.0022, WT - Shank3^{-/-} EGTA, 0.022; tonic interval, WT - Shank3^{-/-} <0.0001, WT - Shank3^{-/-} EGTA, <0.0001.
- D.** Two burst firings elicited by minimal current injections.
- E.** Statistics of time interval between the first Na spike of two burst firings. Animals used: 5 (WT), 5 (Shank3^{-/-}), 5 (Shank3^{-/-} EGTA). Neuron recorded: 13 (WT), 13 (Shank3^{-/-}), 10 (Shank3^{-/-} EGTA). P-value: WT - Shank3^{-/-} 0.047, Shank3^{-/-} - Shank3^{-/-} EGTA 0.248, WT - Shank3^{-/-} EGTA, 0.243.

Fig. S9. Impacts of adding additional EGTA in intracellular solution on rebound burst and tonic firings in Shank3^{-/-} TRN neurons.

- A.** Membrane potential recordings in response to hyperpolarizing current injections.
- B.** Statistics. Animals used: 2 (WT), 2 (Shank3^{-/-}), 2 (HCN2^{-/-}). Neuron recorded: 10 (WT), 10 (Shank3^{-/-}), 10 (HCN2^{-/-}). P-value: burst Na number, WT - Shank3^{-/-} 0.026, Shank3^{-/-} - Shank3^{-/-} EGTA 0.382, WT-Shank3^{-/-} EGTA, 0.11702; burst Na frequency, WT-Shank3^{-/-} 0.006, Shank3^{-/-} - Shank3^{-/-} EGTA, 0.016, WT - Shank3^{-/-} EGTA, 0.754; latency, WT - Shank3^{-/-} 0.032, Shank3^{-/-} - Shank3^{-/-} EGTA, 0.355, WT - Shank3^{-/-} EGTA 0.154.

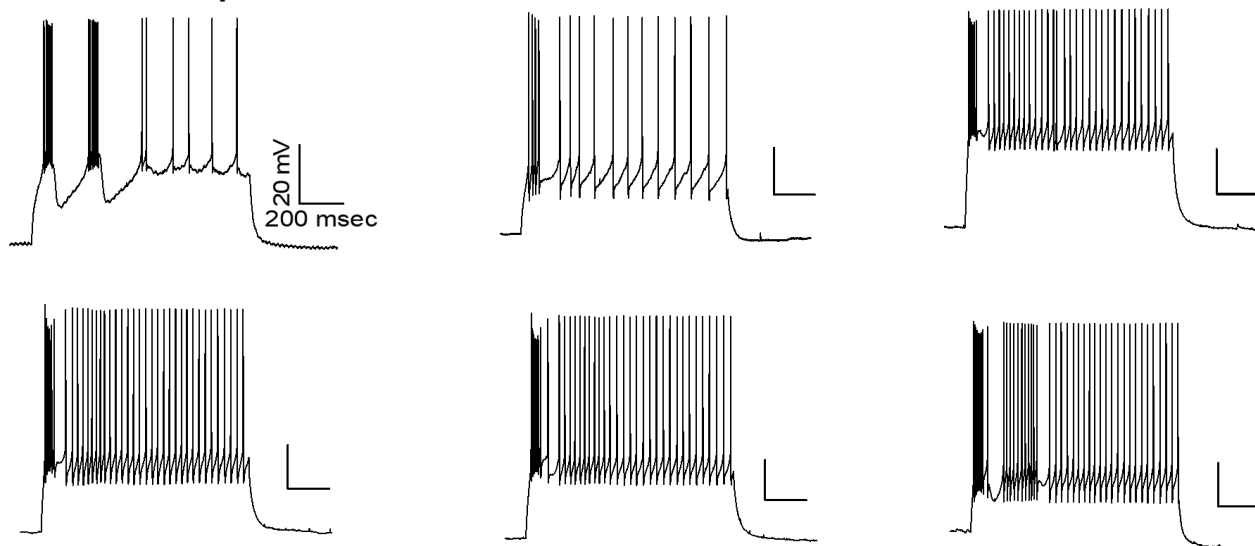
Fig. S10. AP firing of WT and Shank3^{-/-} TRN PV neurons recorded in the absence of EGTA in intracellular pipette solution.

- A.** Statistics of burst firing number, tonic frequency, AP number, and first latency. Animal used: 3 (WT), 3 (Shank3^{-/-}). Neurons recorded, 18 (WT), 18 (Shank3^{-/-}). P-value: Burst number (100, 150, 200, 250 pA) 0.007 0.005 0.002 0.0005; AP number, 0.495; 1st latency, 0.883.
- B.** Statistics of RMP and Rin. P-value: RMP, 0.758; Rin, 0.033.
- C.** Statistics of Na spike number, time interval of 1st Na spike to AHP, time interval 1st Na spikes of two burst firings. P-value: Na spike number, 0.914; 1st Na spike to AHP, 0.308; burst to burst, 0.126.
- Figure S11. Both somatic and dendritic calcium transients of HCN2^{-/-} TRN neurons exhibit faster decay.**
- A.** Top, two-photon fluorescence image of a RTN GABA neuron filled with AF594 (red channel) and Fluo-5F.
- B.** Overlay of normalized calcium transients recorded from the soma and four dendritic locations of TRN GABA neurons from WT and HCN2^{-/-} mice. Notice the stimulation protocols were different between WT (500 pA x 10 msec, 3 or 4 stimulations at 1 Hz) and HCN2^{-/-} (500 pA x 10 msec, 4 stimulations at 2 Hz) neurons. Animals used, 5 (WT), 4 (HCN2^{-/-}). Neurons recorded, 6 (WT), 5 (HCN2^{-/-}).
- C.** Decay time constant of the calcium transients recorded from soma and four dendritic locations of WT and HCN2^{-/-} TRN PV neurons (25, 50, 75, 100 μ m from soma). P-value: soma-dendrites 0.090, 0.002, 0.153, 0.038, 0.141.
- D.** A working model for downstream events caused by Shank3 deficiency: Shank3 deficiency -> HCN channelopathy -> reduced PV expression and calcium-buffering capacity.
- E.** Statistics of time constants measured at the soma. Animals used: 5 (WT), 5 (Shank3^{-/-}), 4 (HCN2^{-/-}). Neurons recorded, 6 (WT), 5 (Shank3^{-/-}), 7 (HCN2^{-/-}). P-values: WT – Shank3^{-/-}, 0.999; WT – HCN2^{-/-}, 0.359.
- F.** Statistics of time constants measured in dendrites. Total data points: 17 (WT), 15 (Shank3^{-/-}), 17 (HCN2^{-/-}). P-values: WT – Shank3^{-/-}, <0.0001; WT – HCN2^{-/-}, <0.0001.

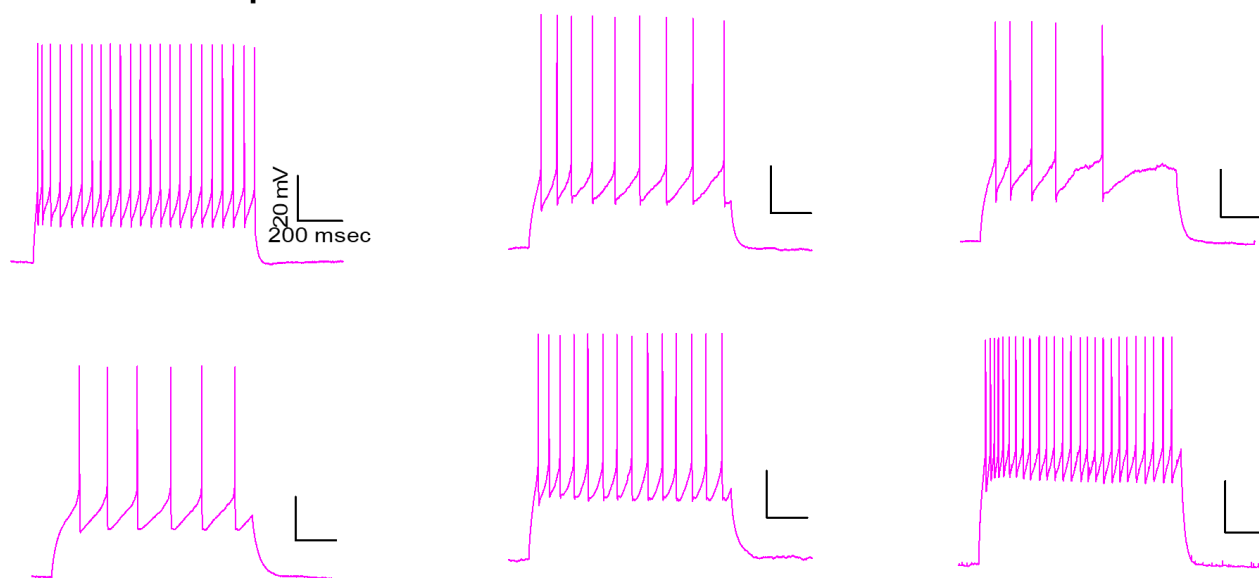
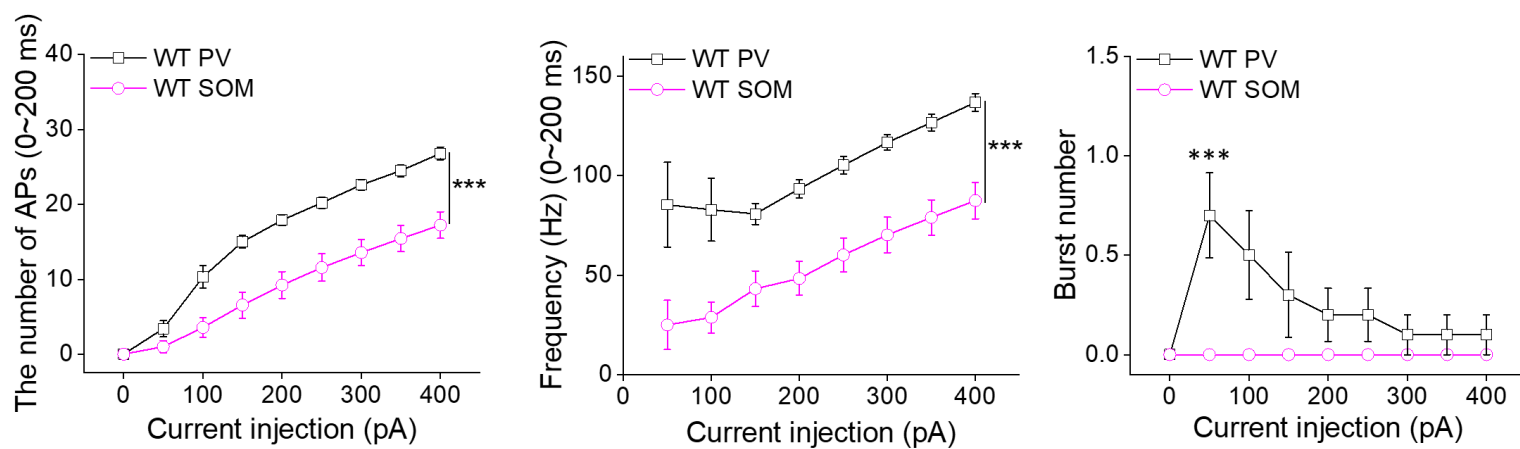


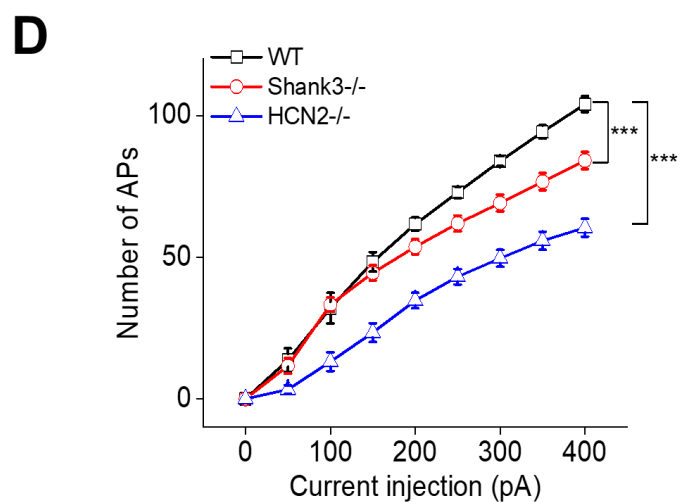
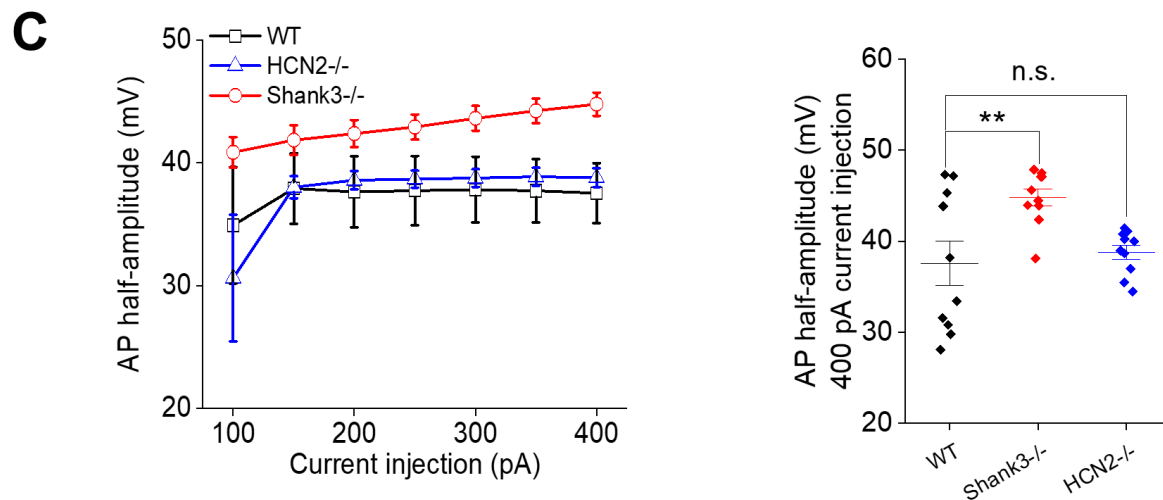
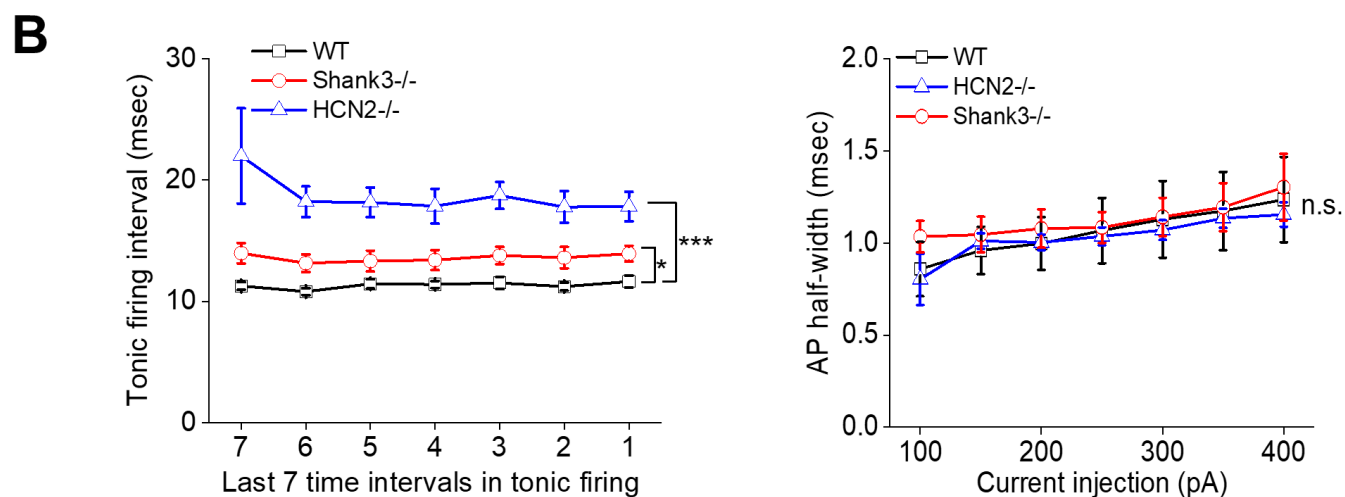
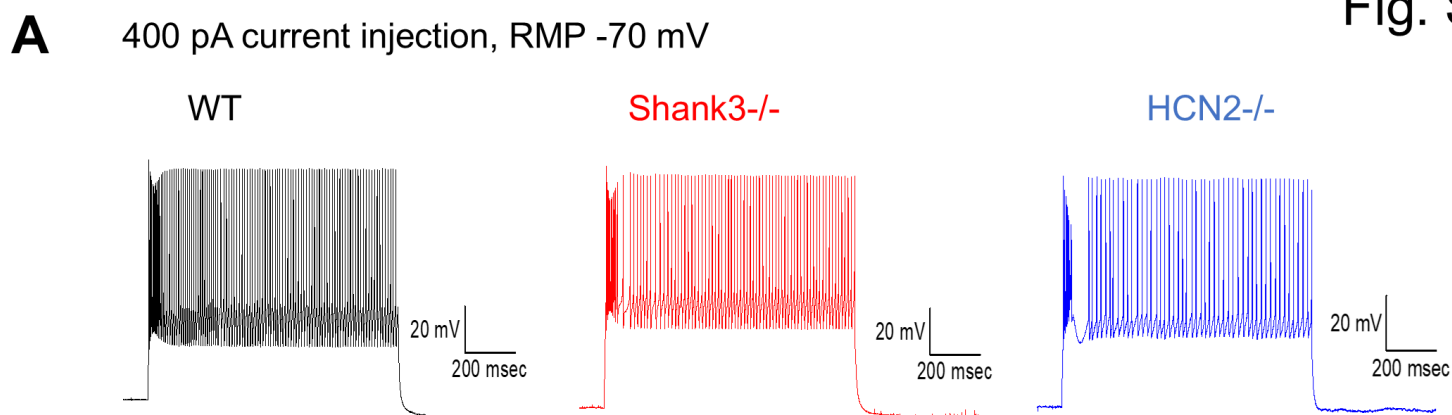
A

WT PV 100 pA

**B**

WT SOM 100 pA

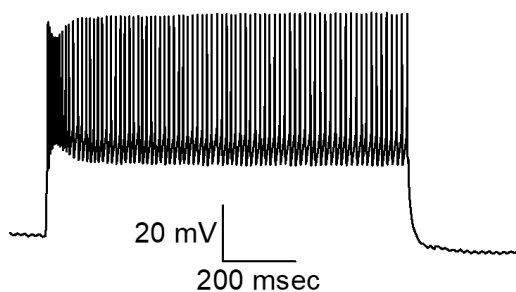
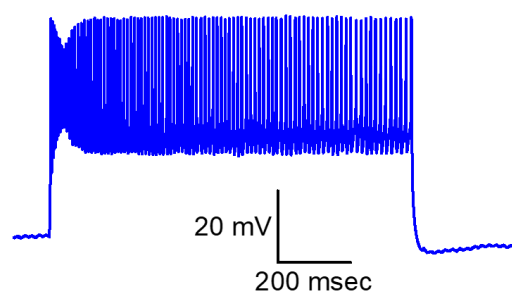
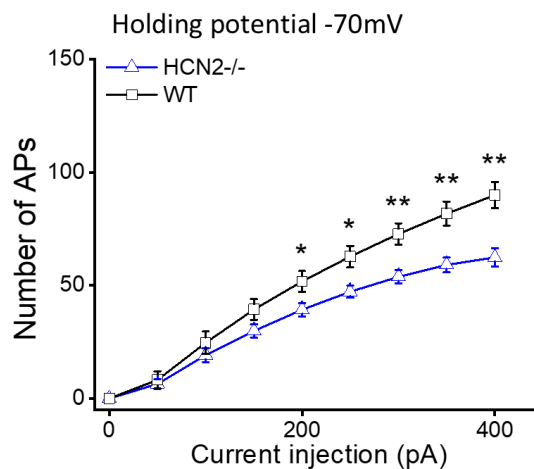
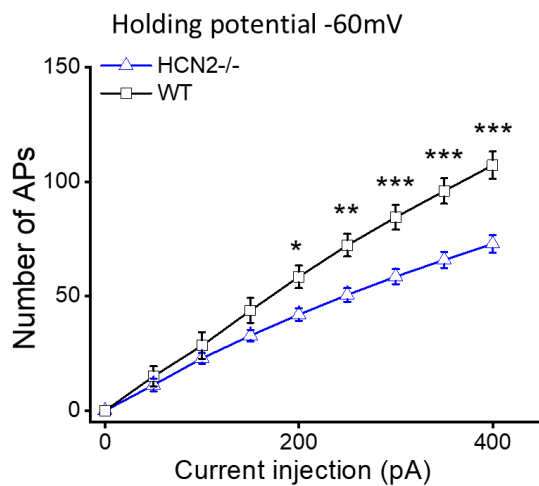
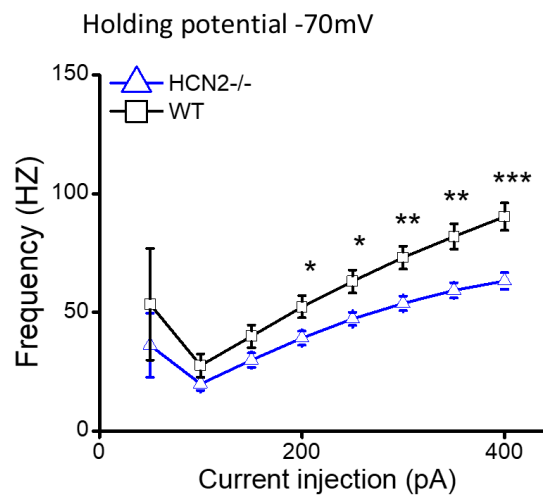
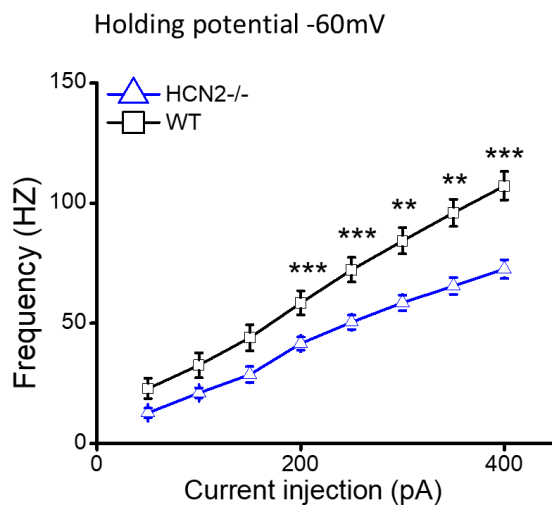
**C**

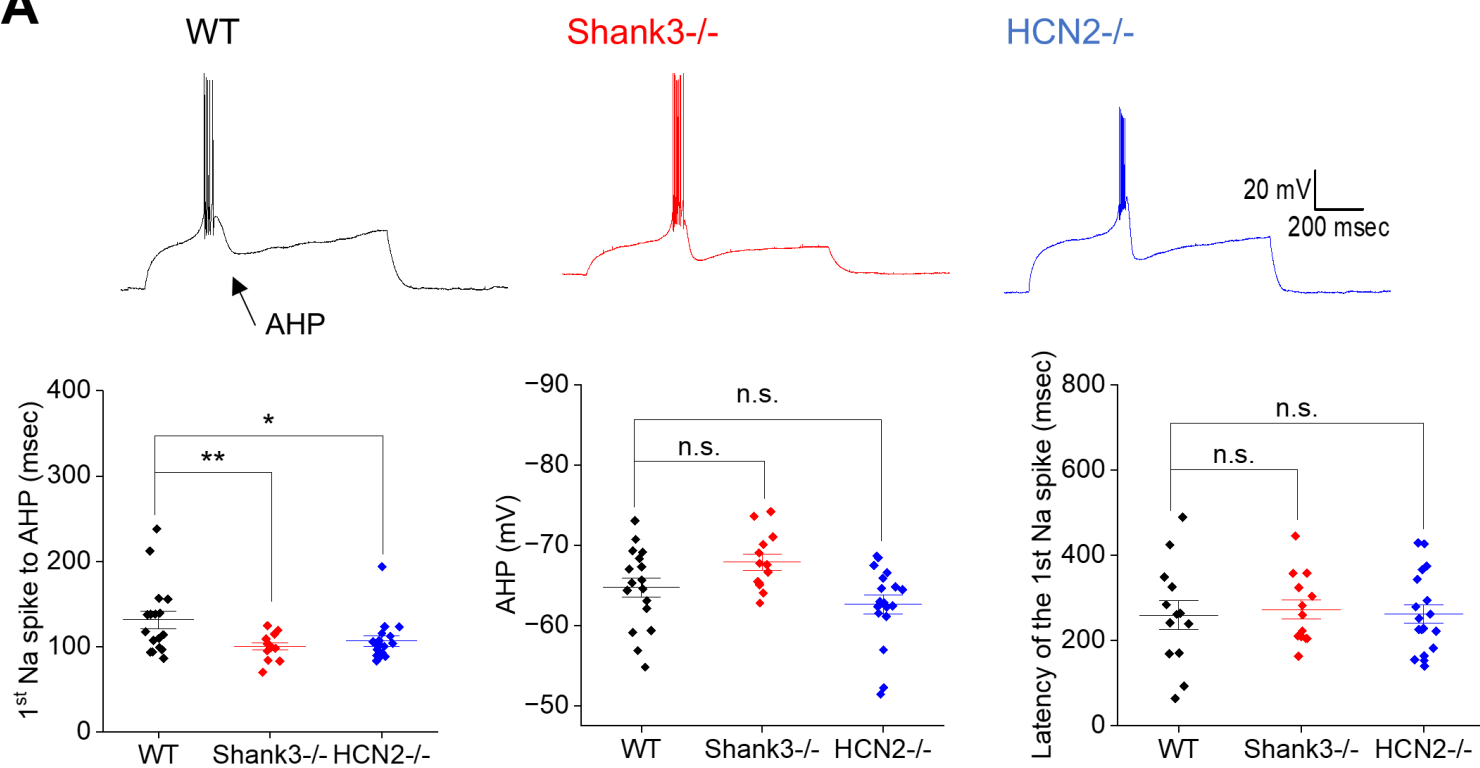
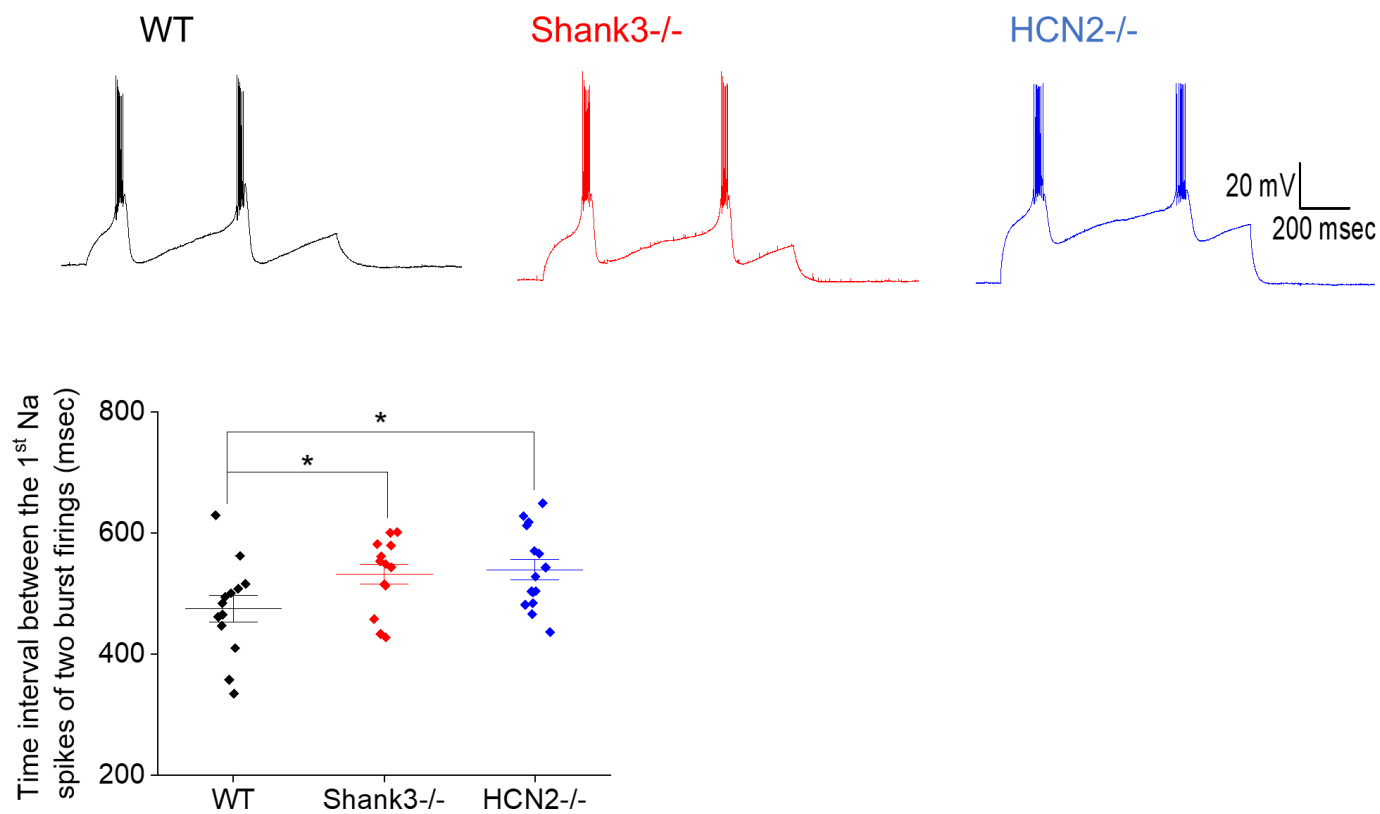


E

TRN PV neuron (-60 mV, 400 pA)

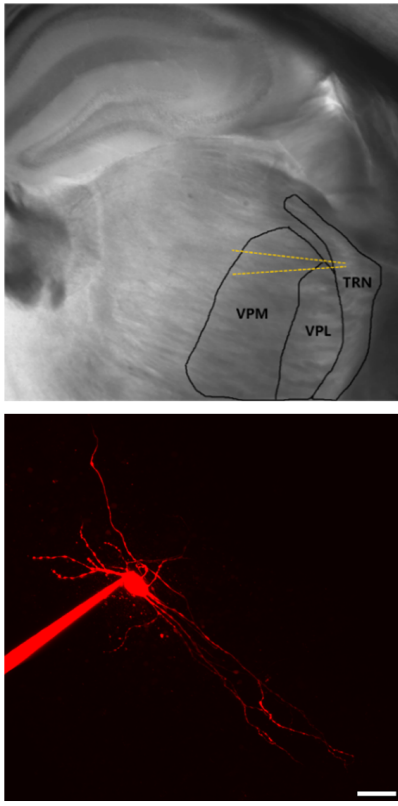
WT

HCN2^{-/-}**F****G**

A**B**

A

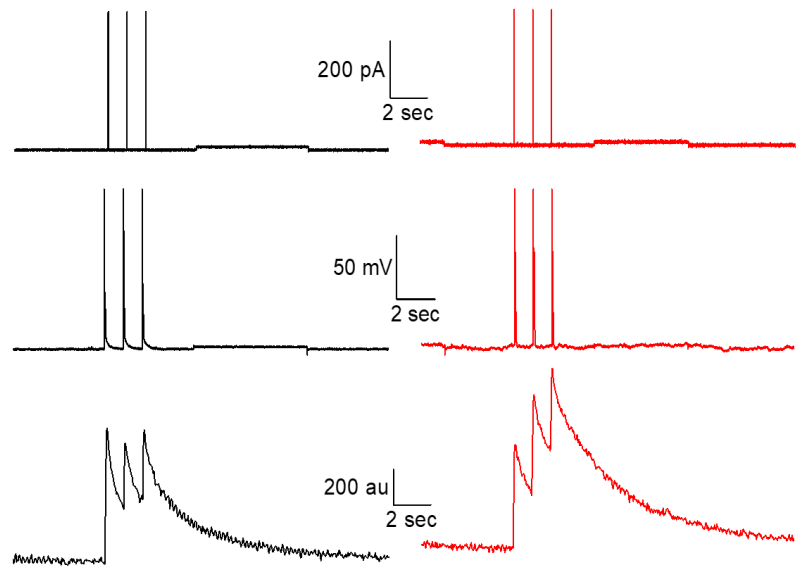
TRN PV neuron



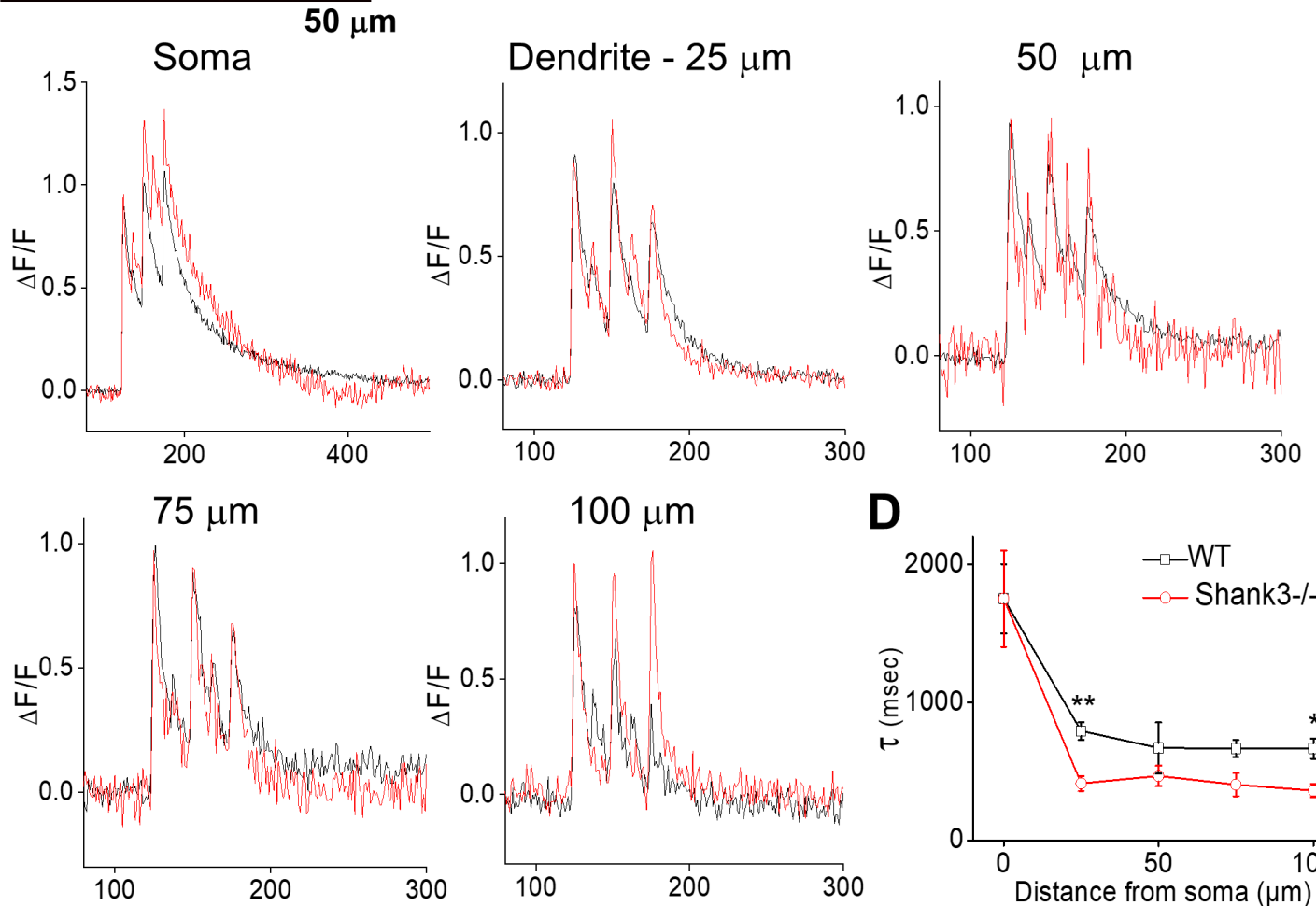
B

WT
(Fluo-5F, 250 μ M)

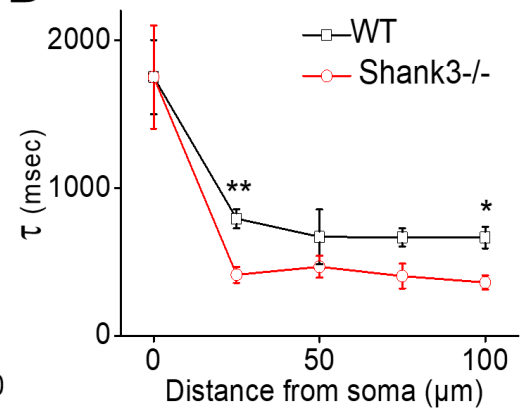
Shank3^{-/-}
(Fluo-5F, 250 μ M)

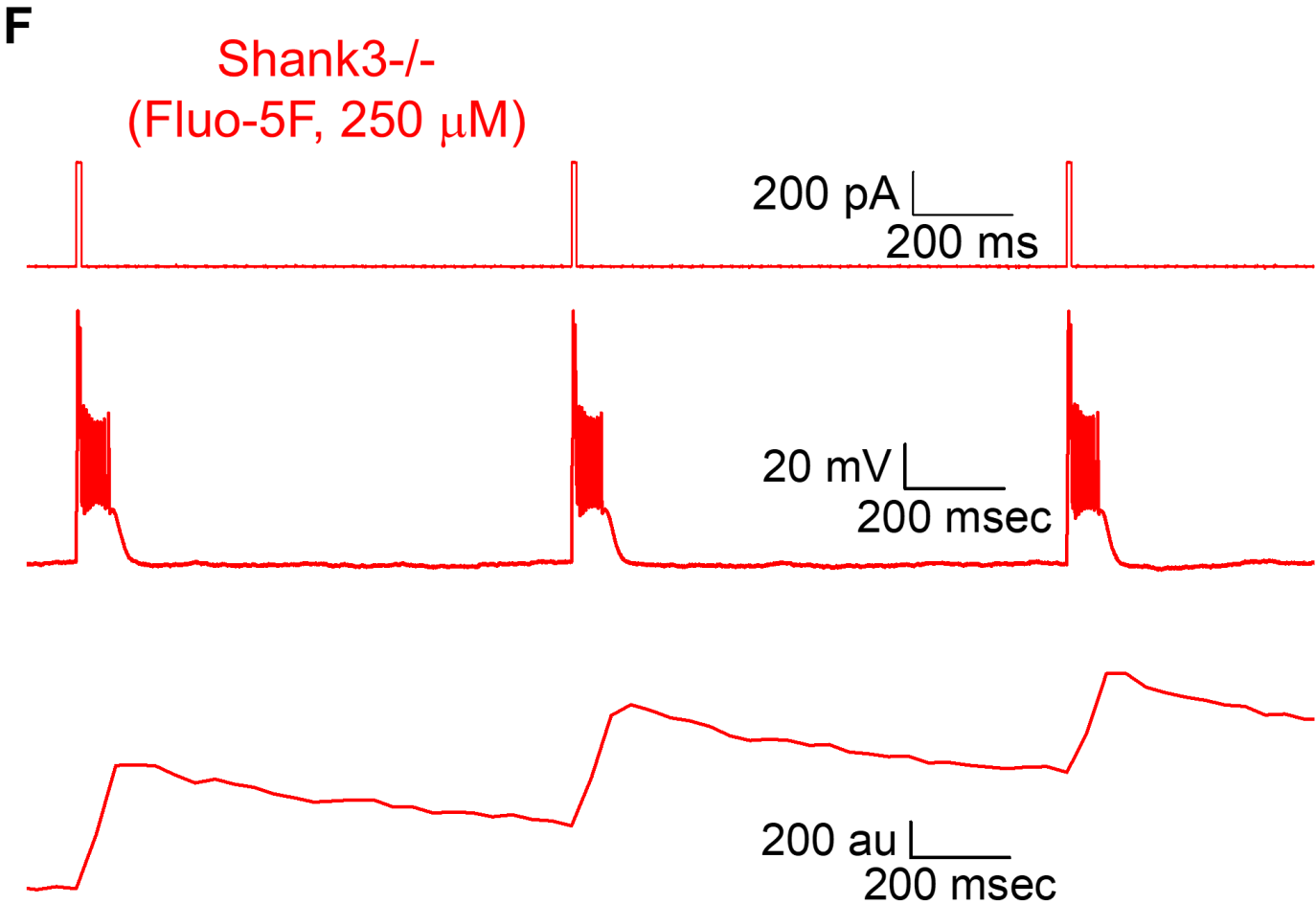
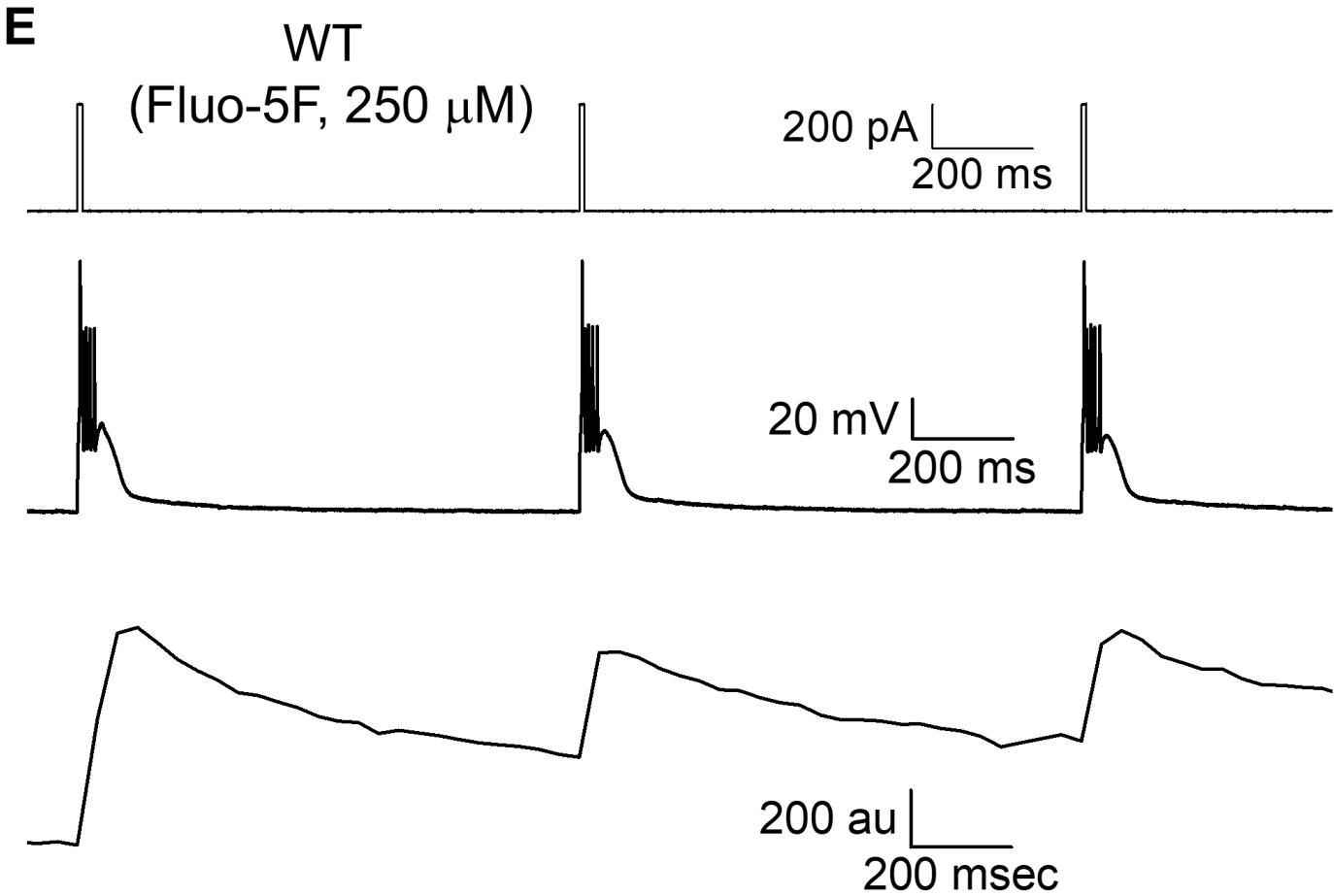


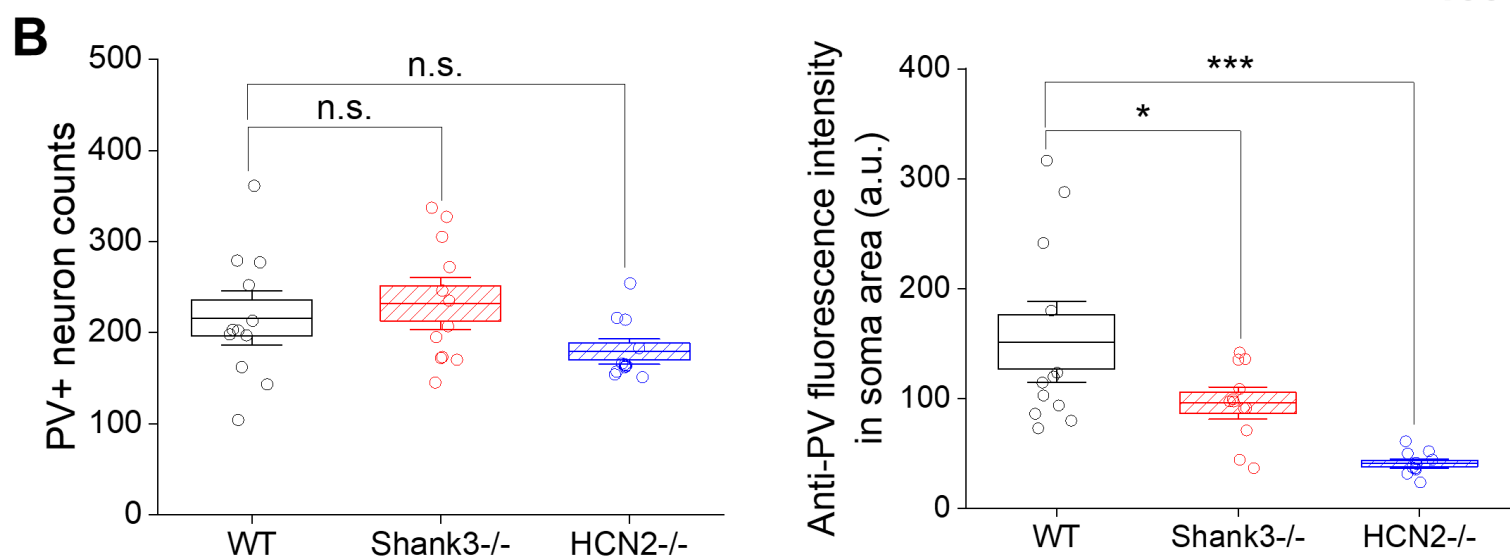
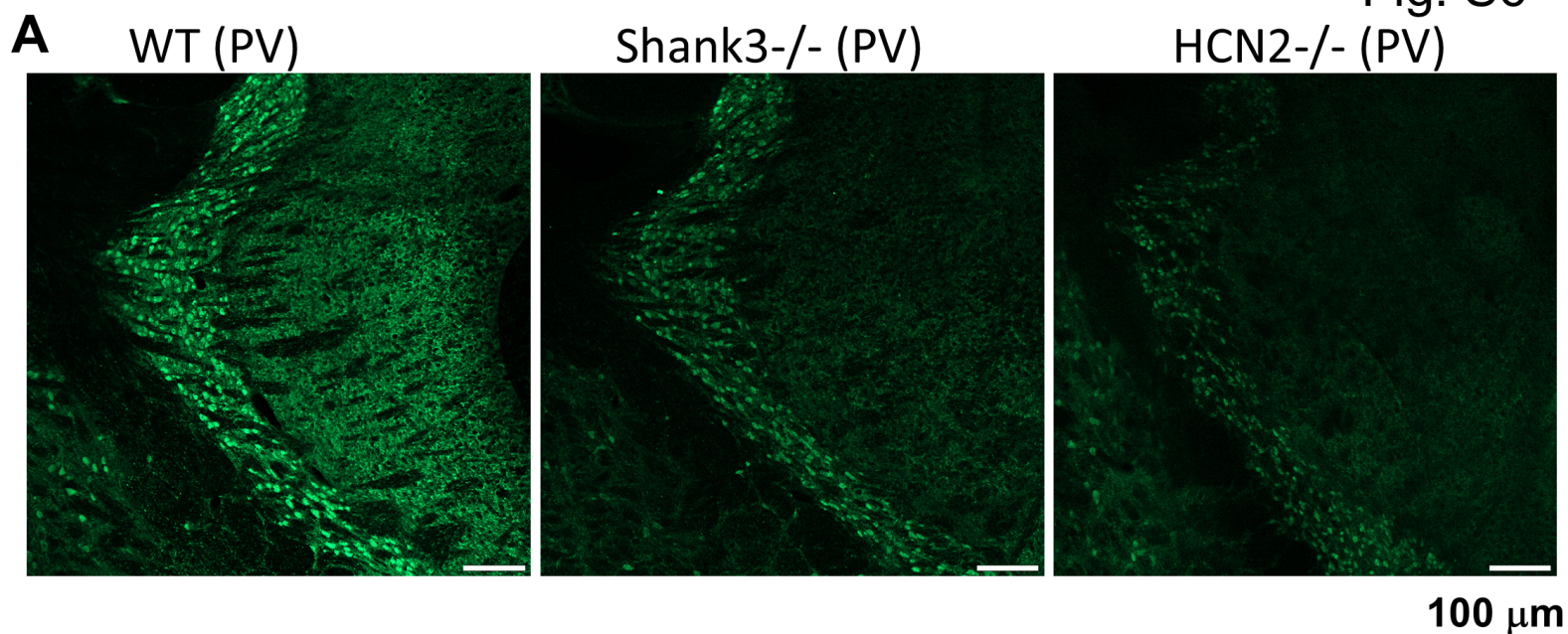
C



D







C Negative controls (omit primary antibody; intensity enhanced to show the TRN region)

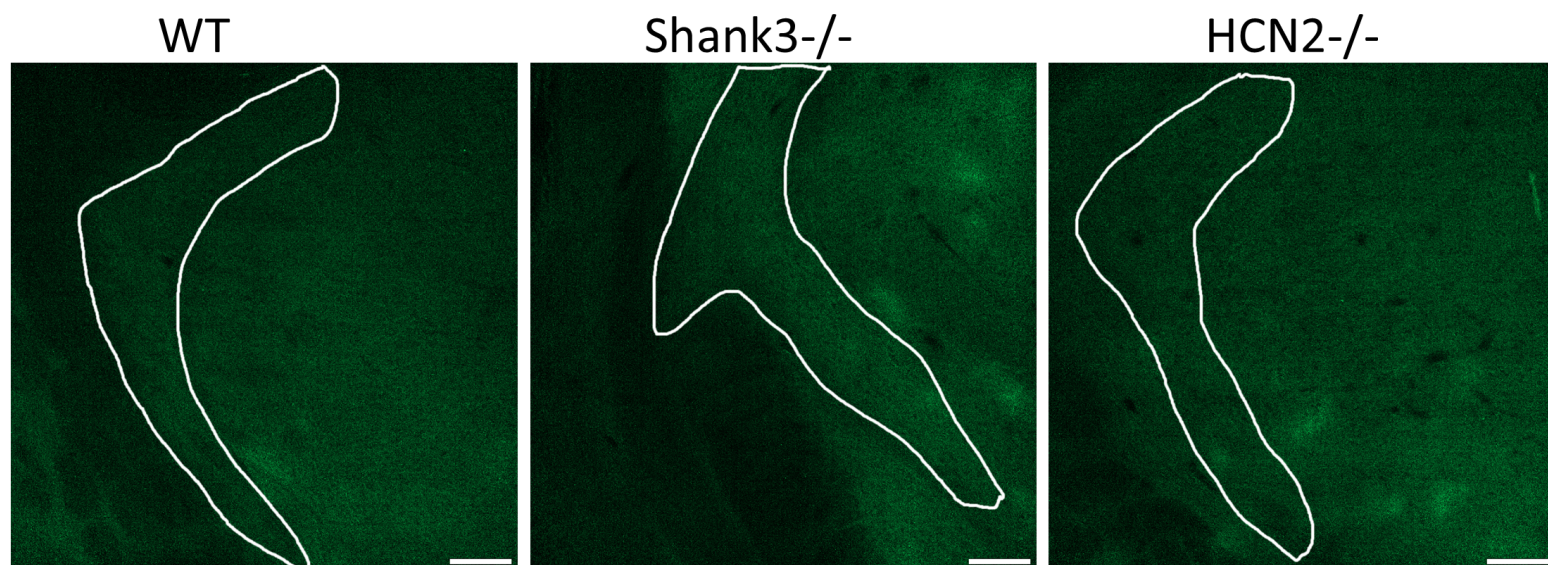
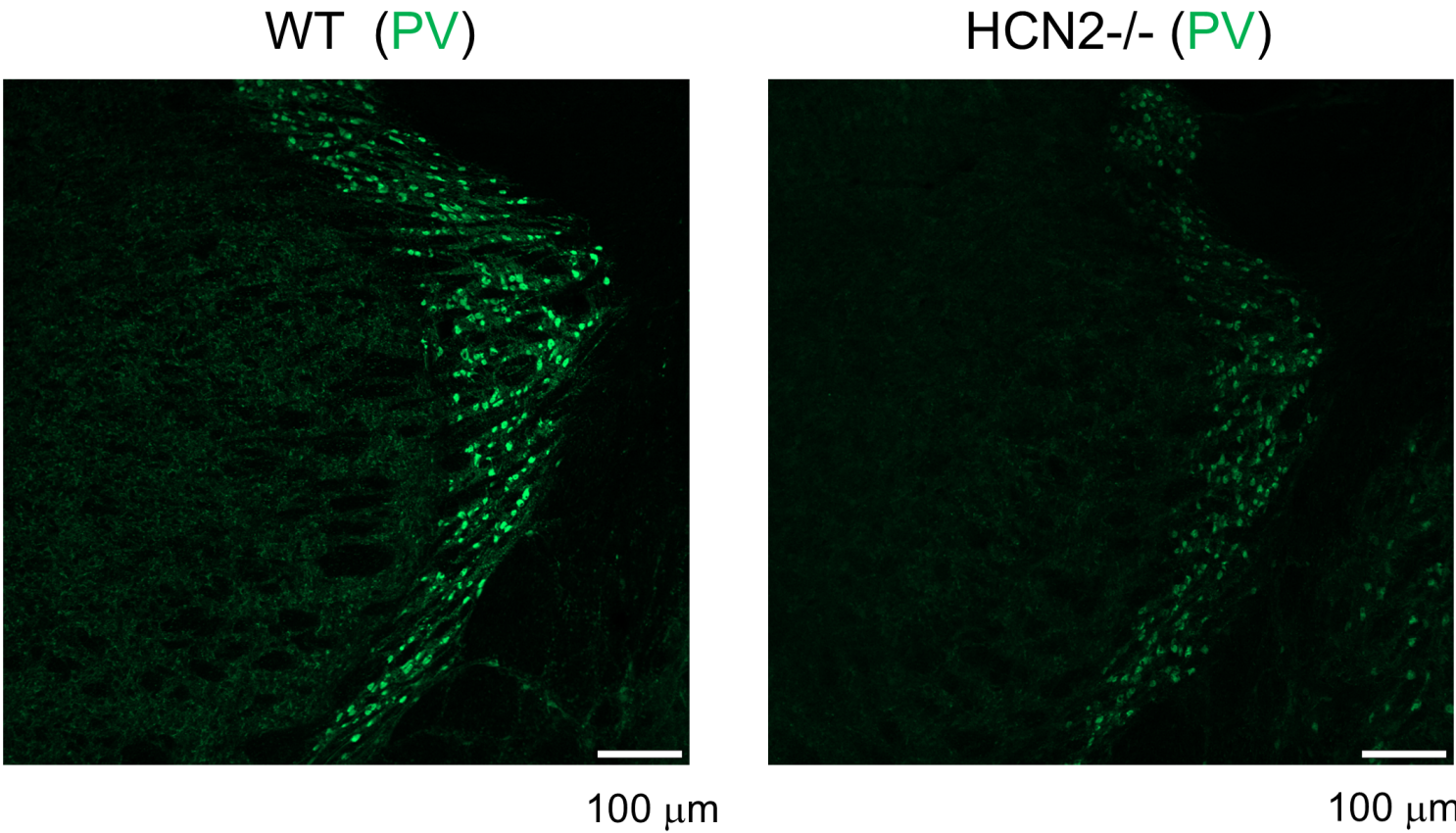
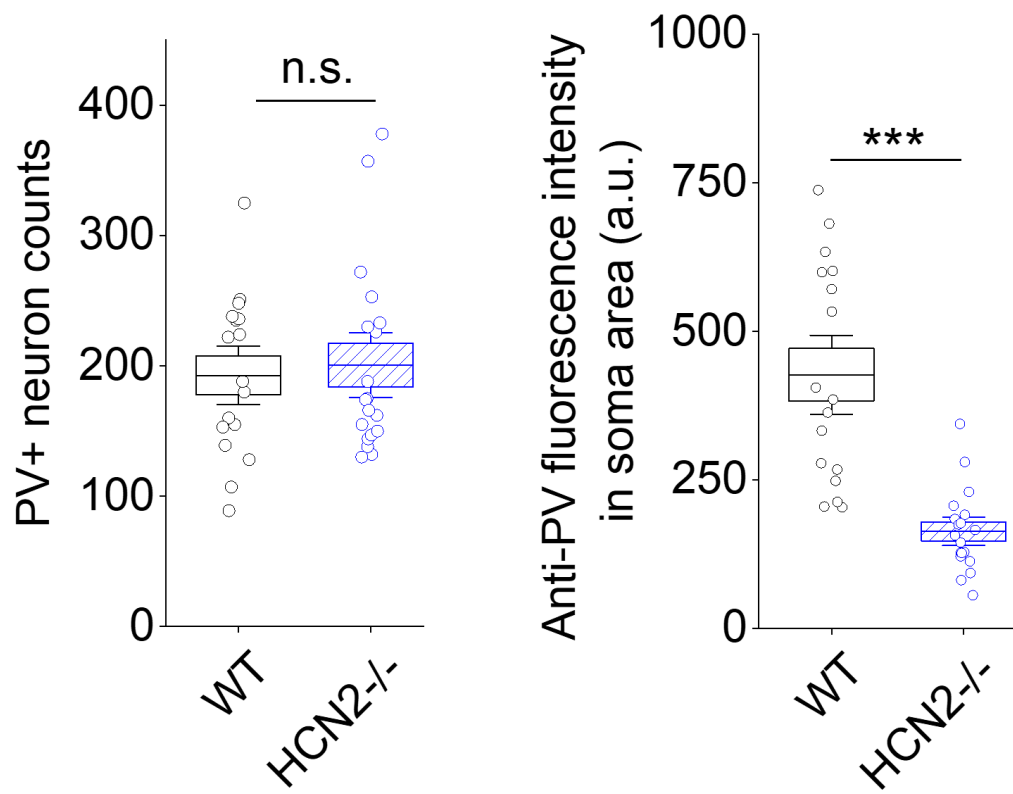


Fig. S6

D

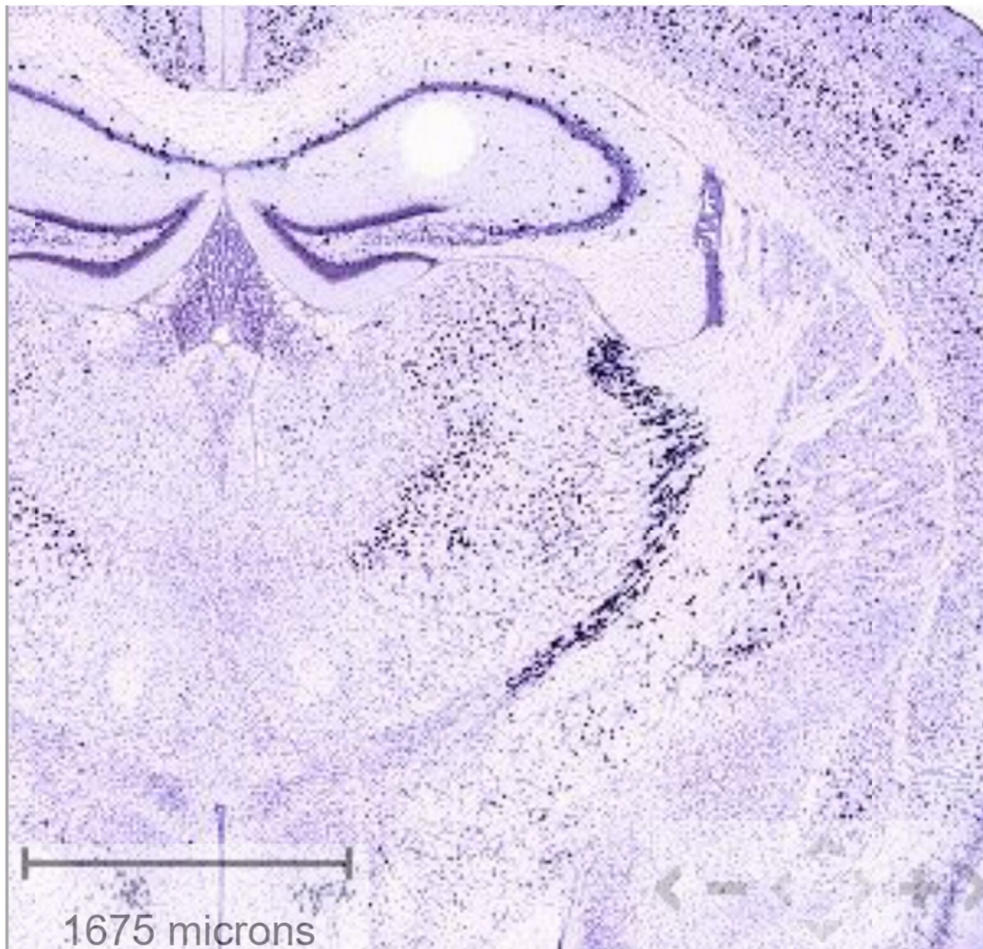
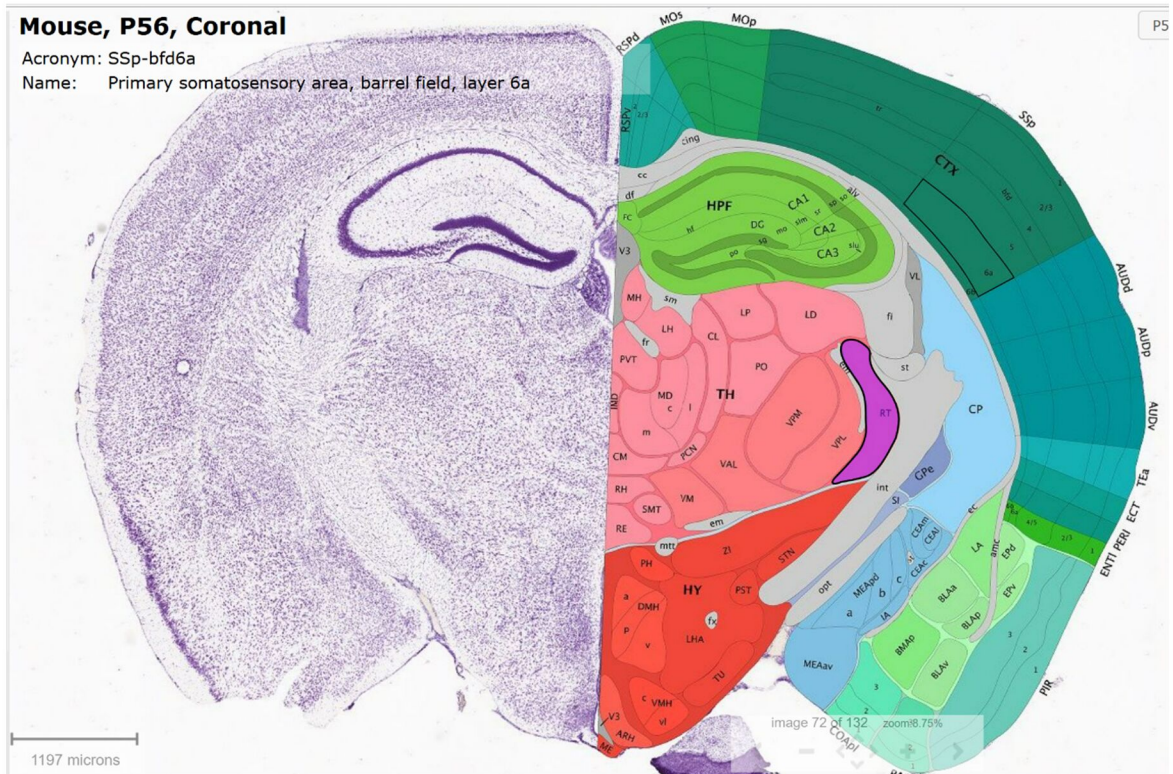


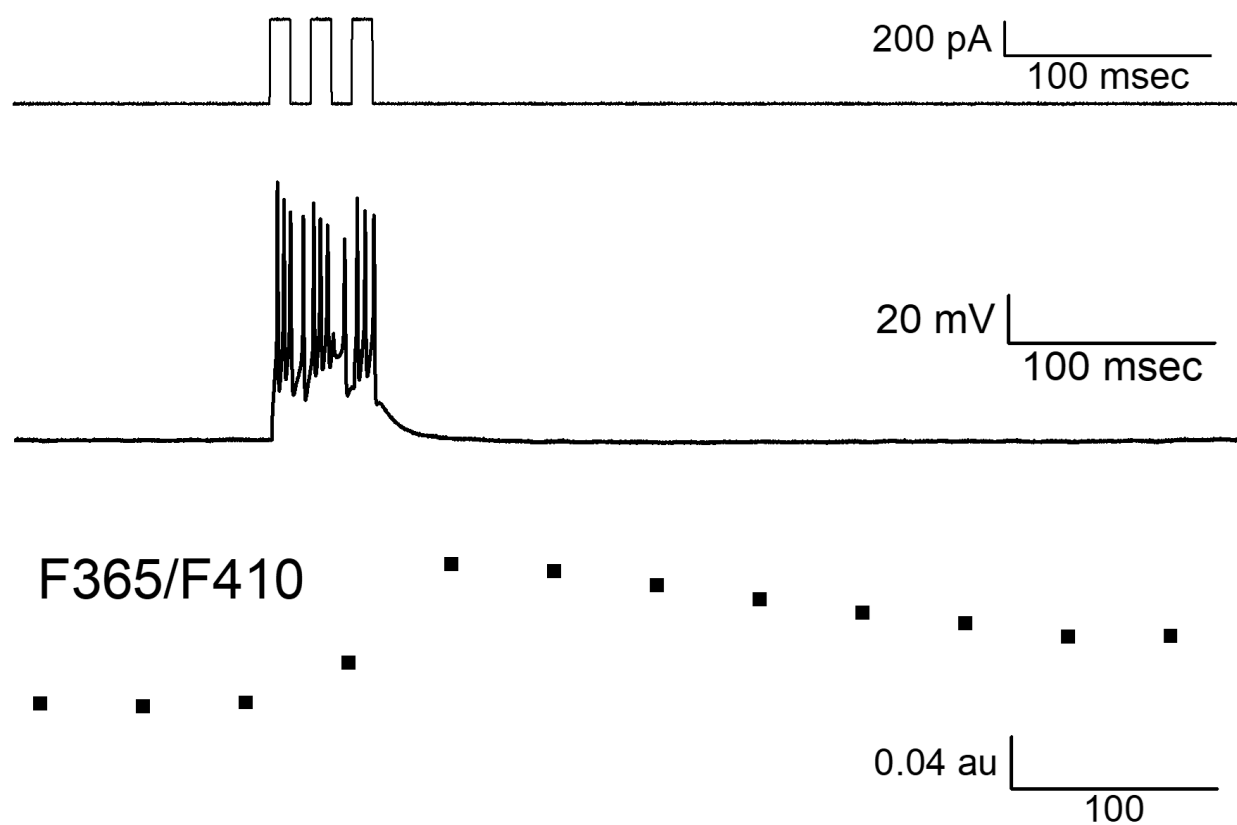
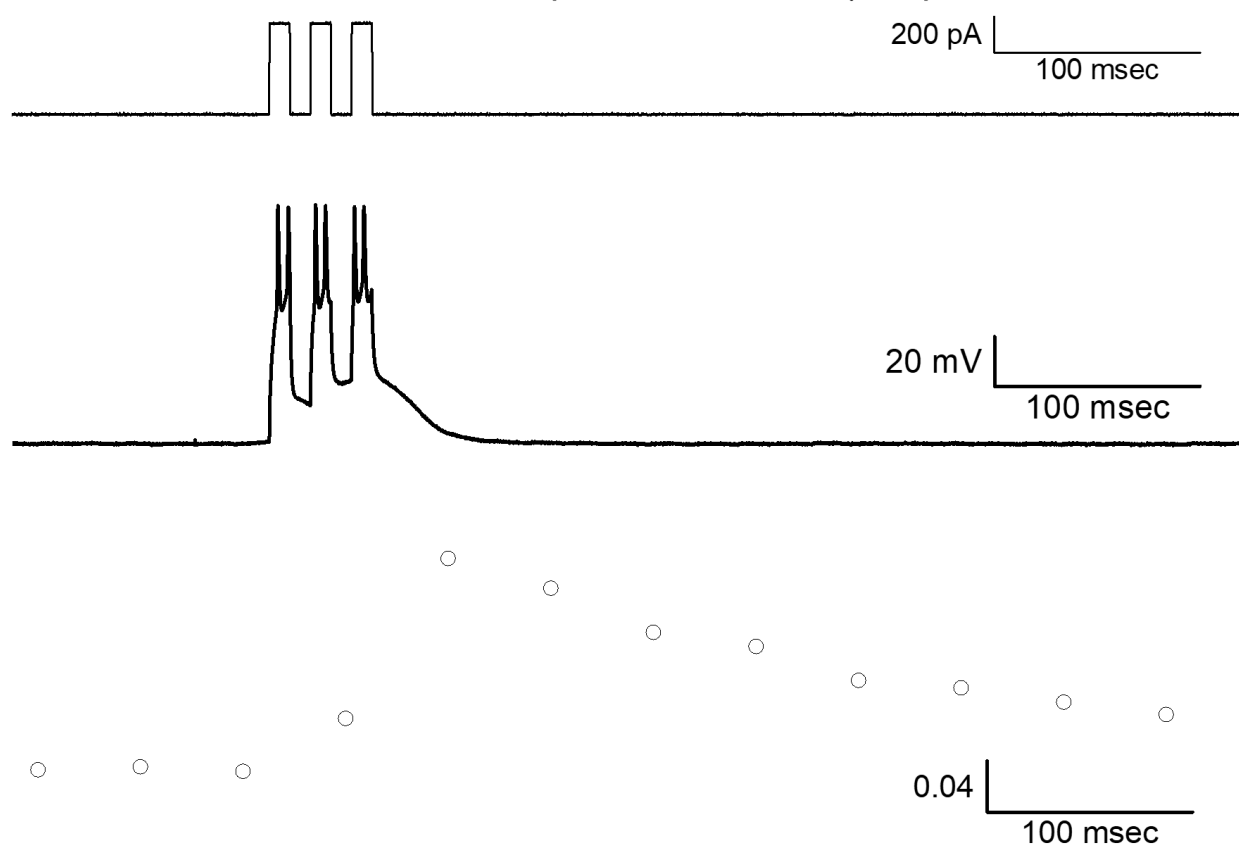
E



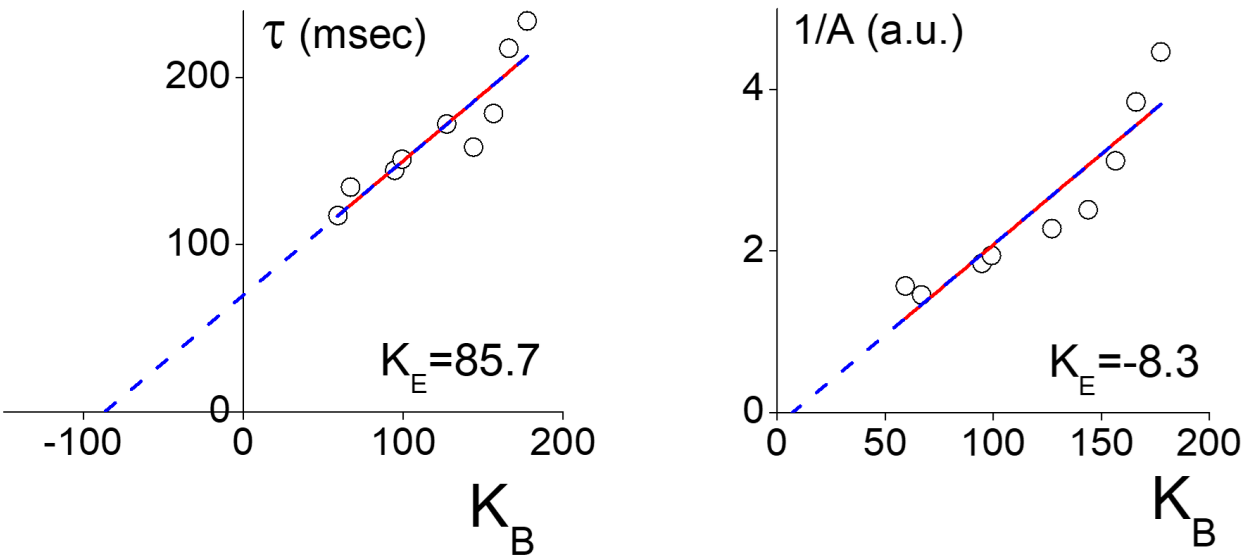
F Parvalbumin *in situ* hybridization image data (Allen Institute)

Fig. S6



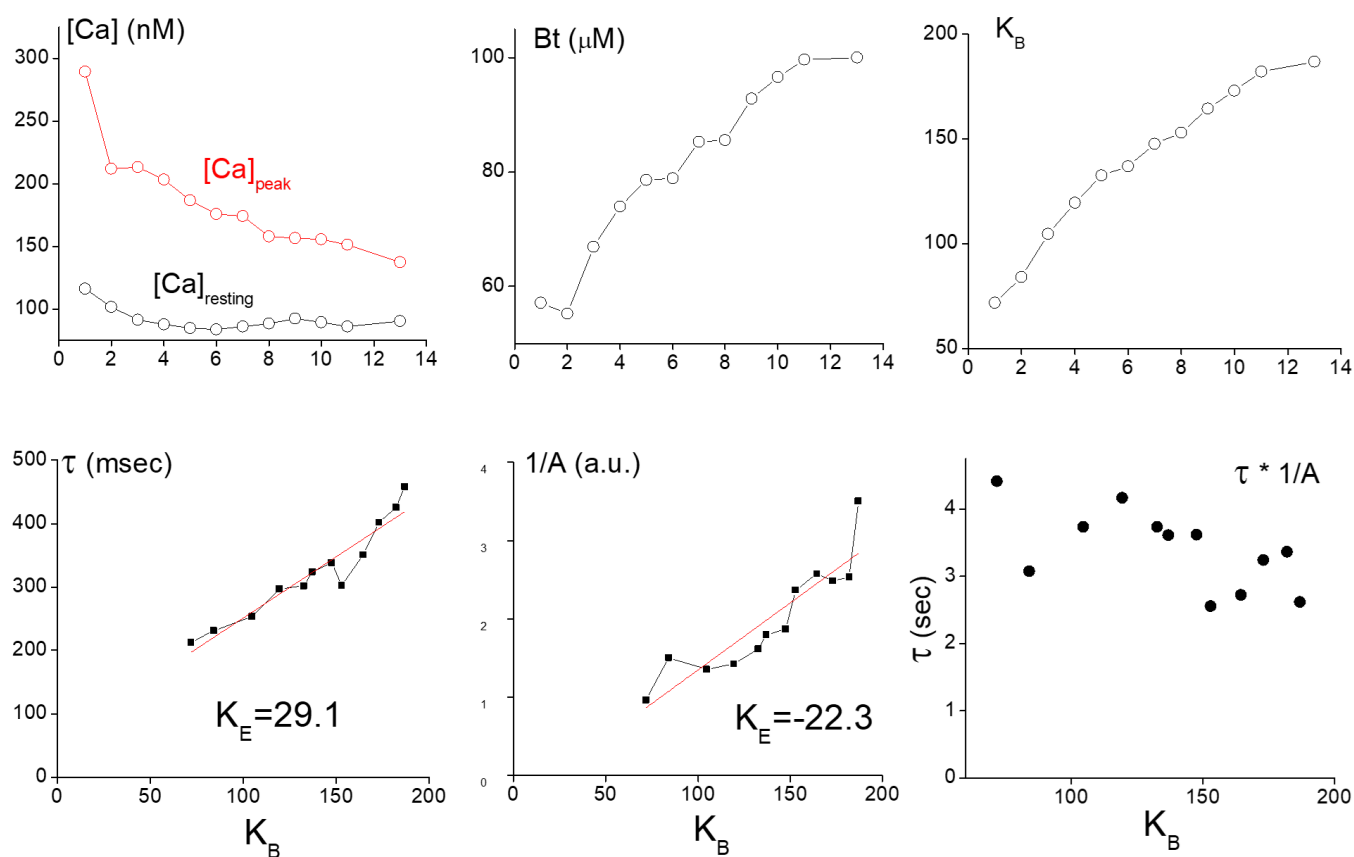
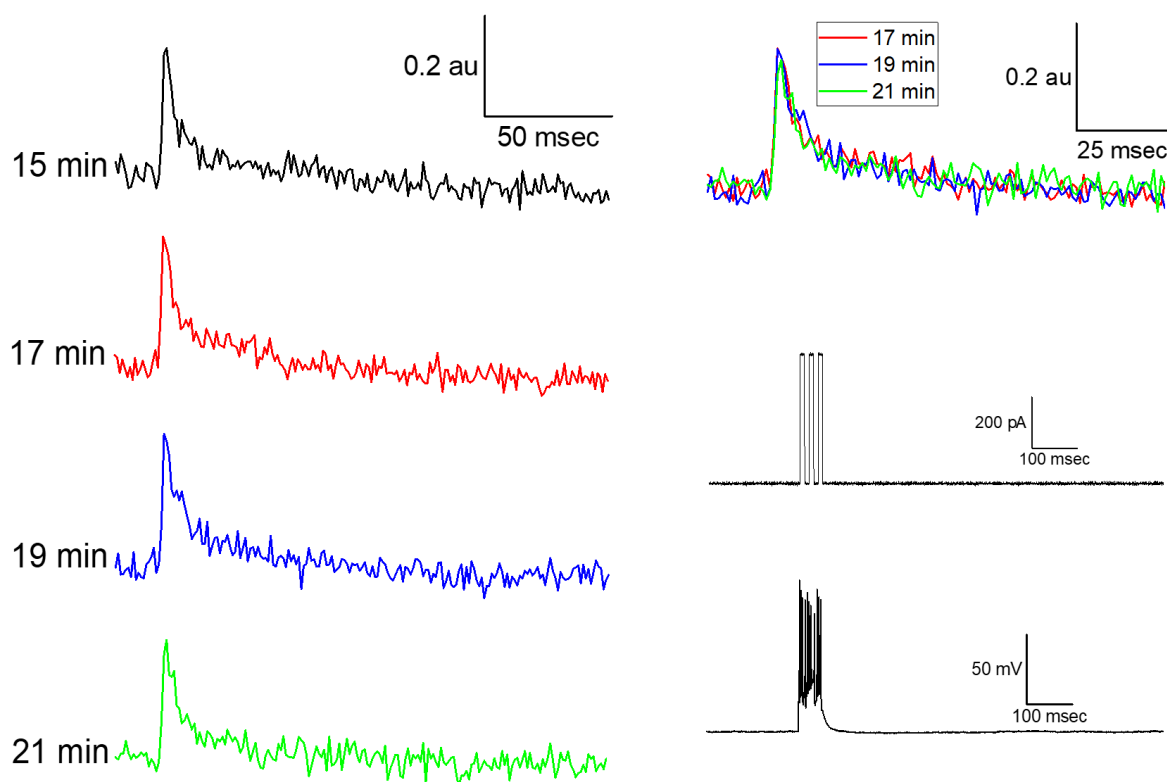
A WT TRN neuron (Fura-8, 100 μ M)**B** Shank3^{-/-} TRN neuron (Fura-8, 100 μ M)

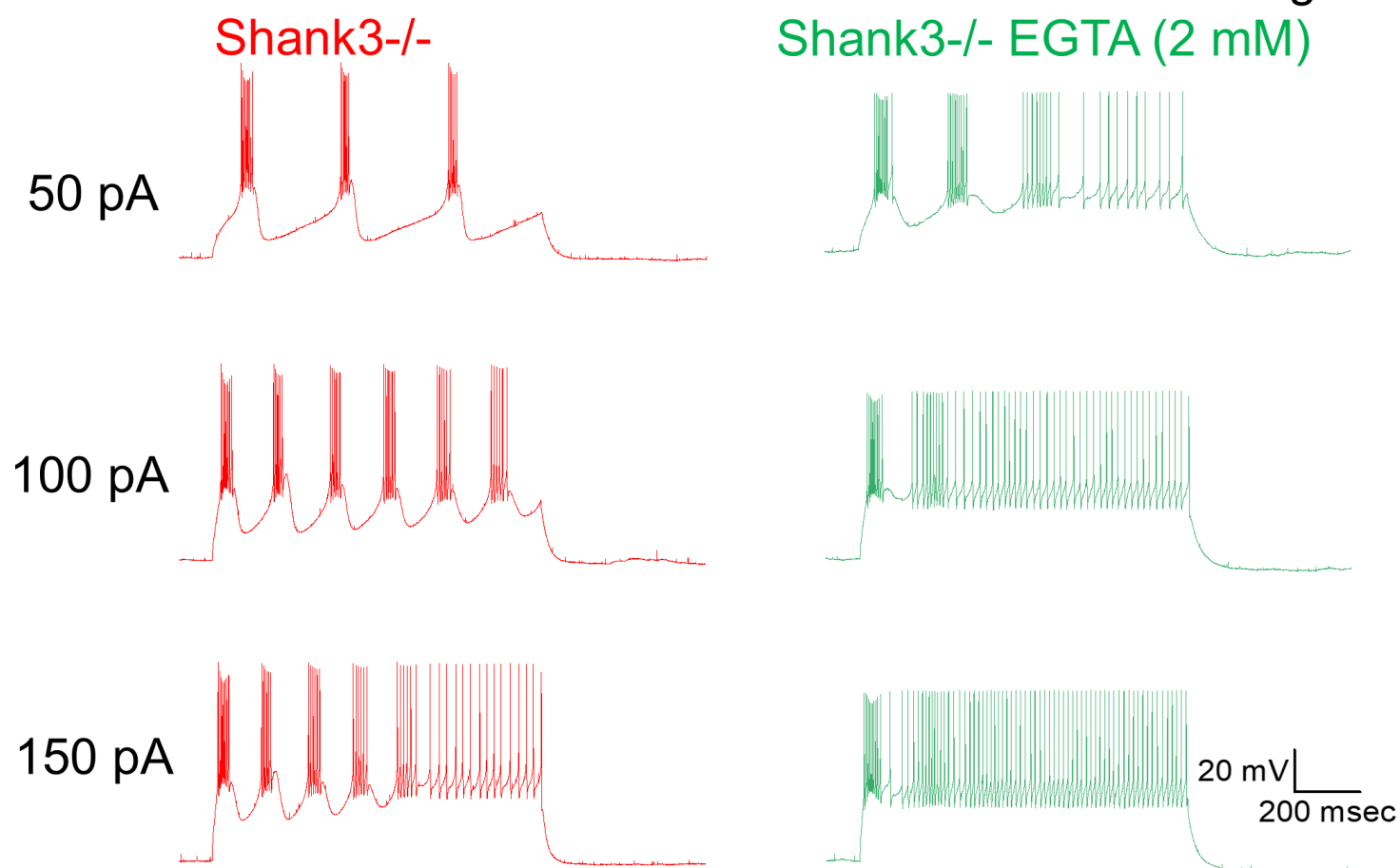
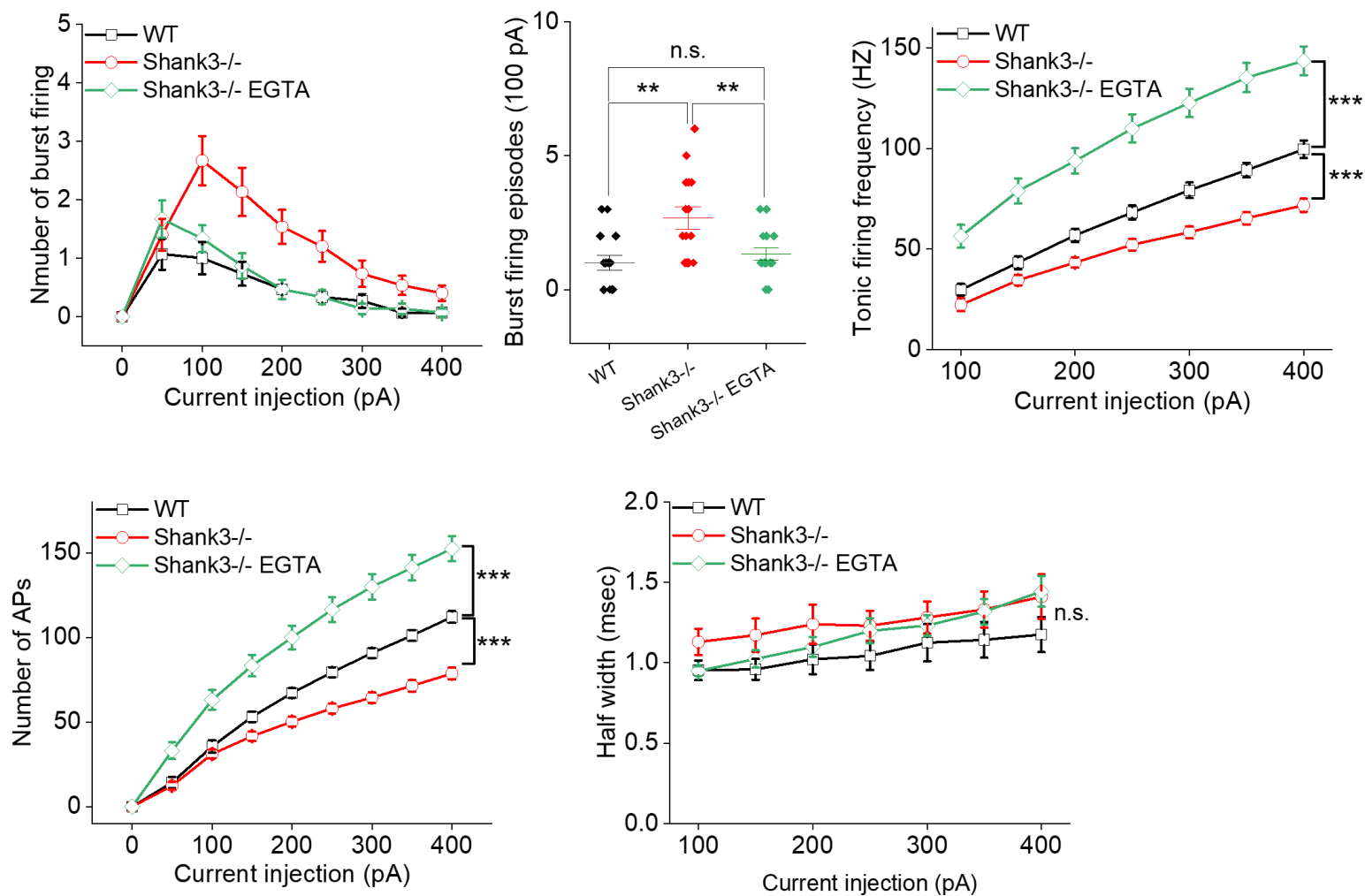
C WT TRN neuron

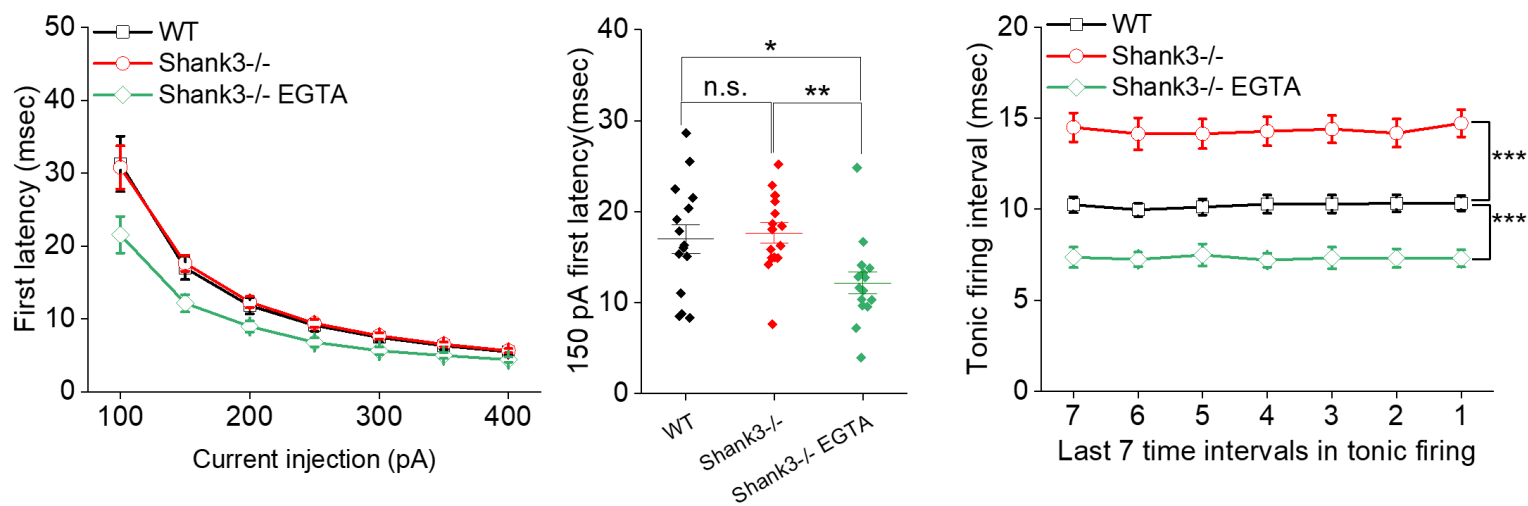
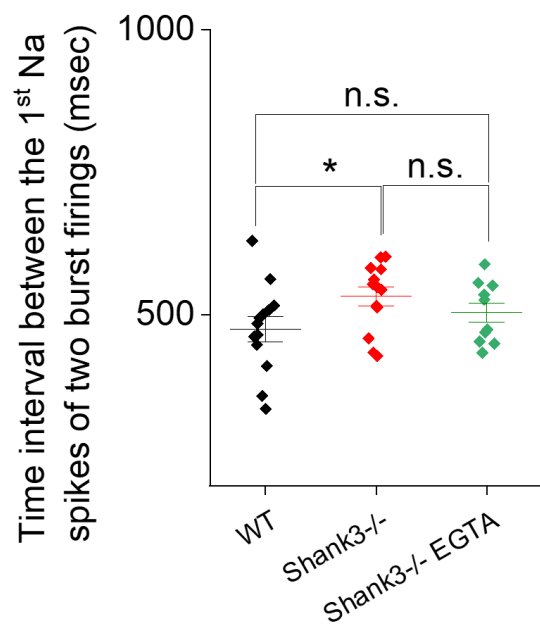


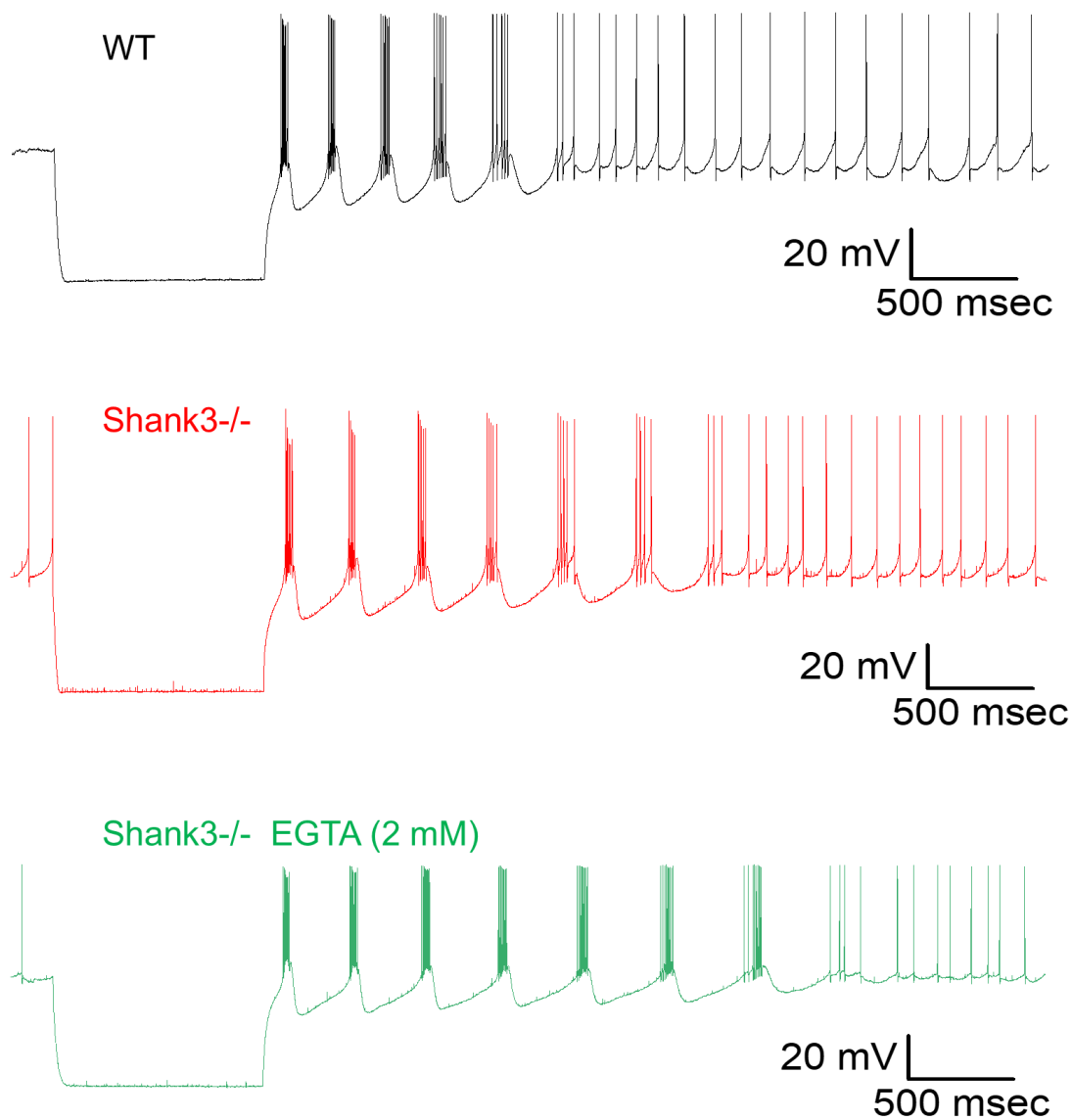
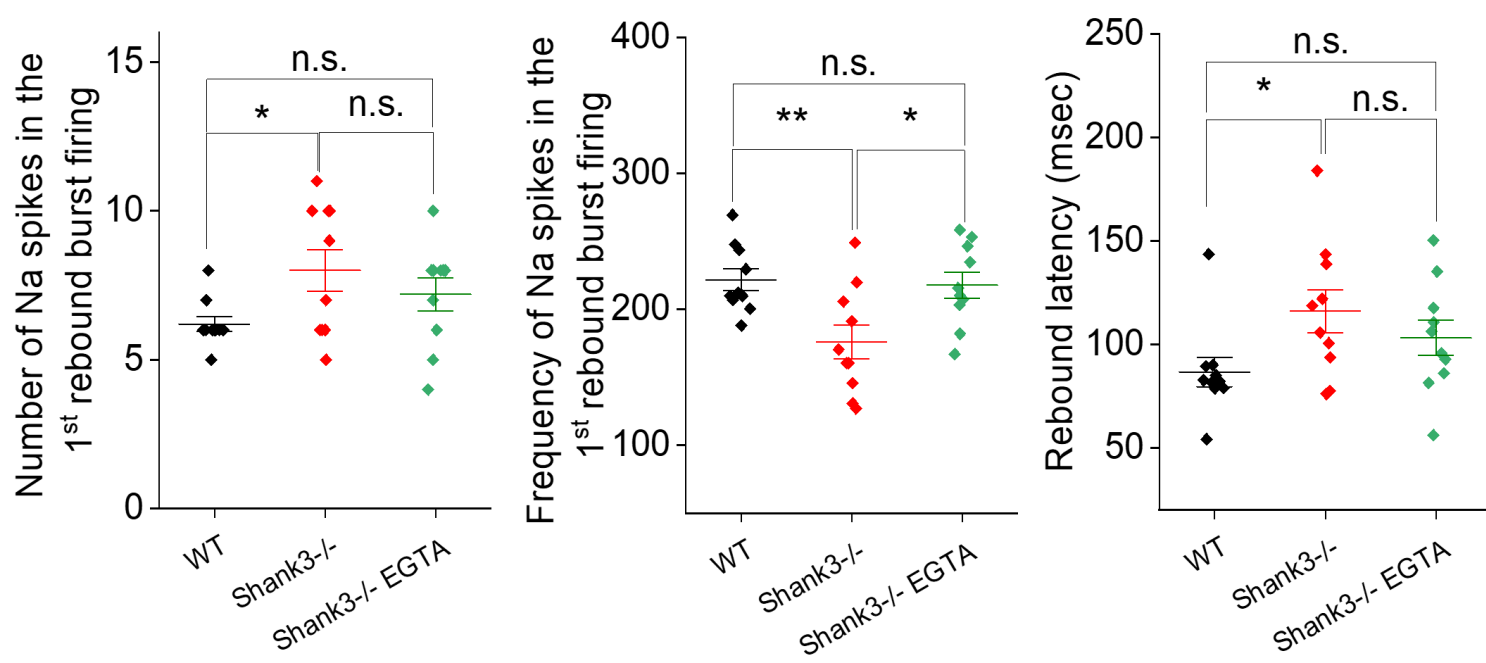
D

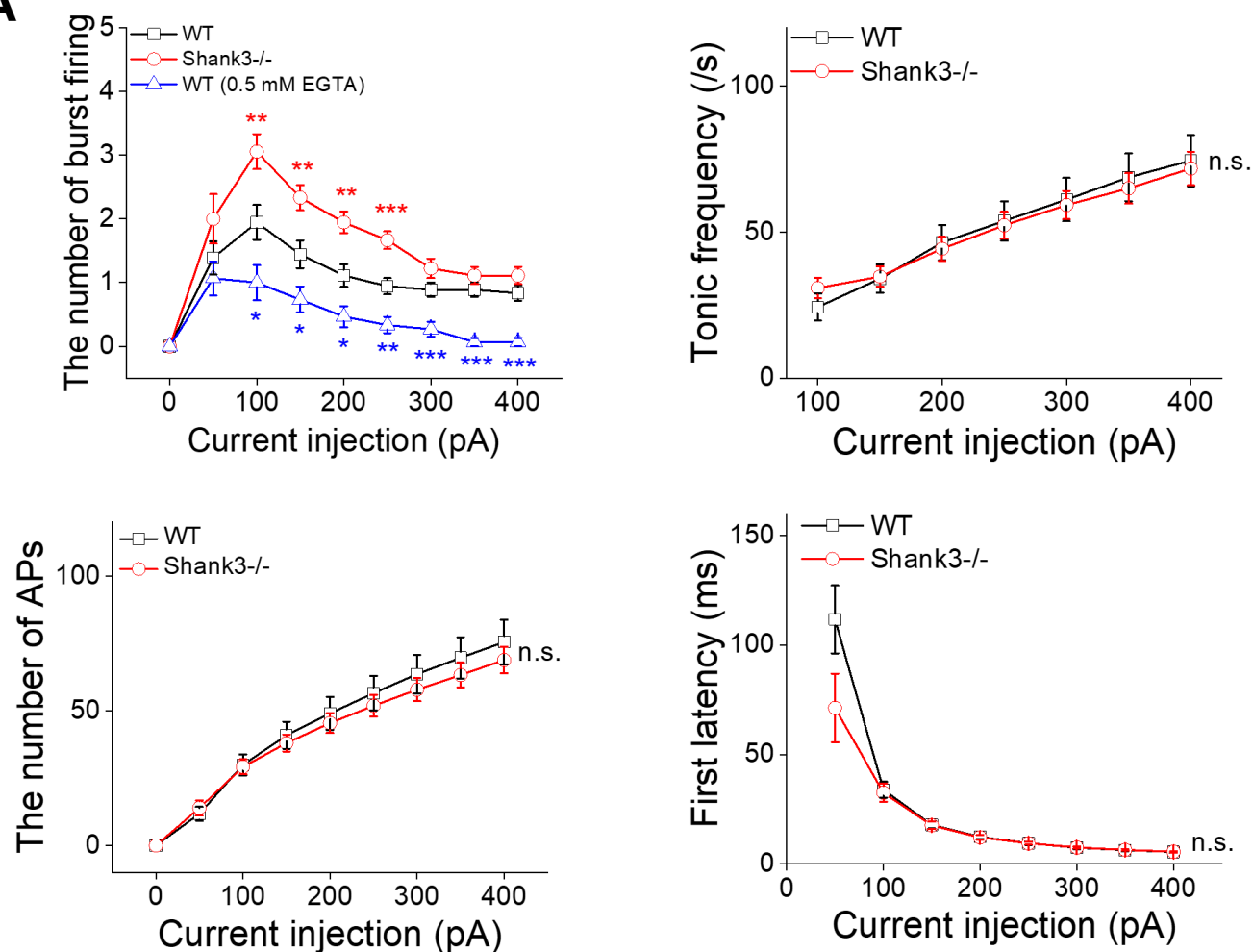
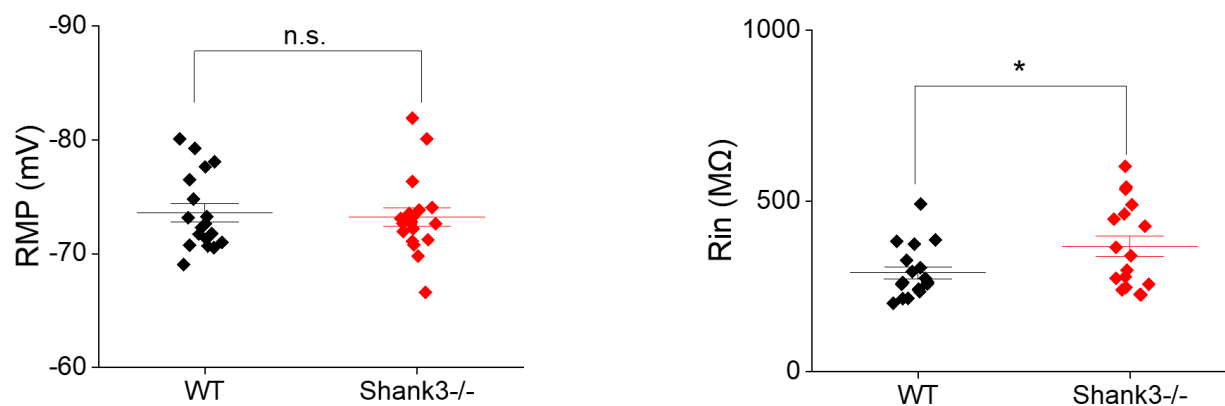
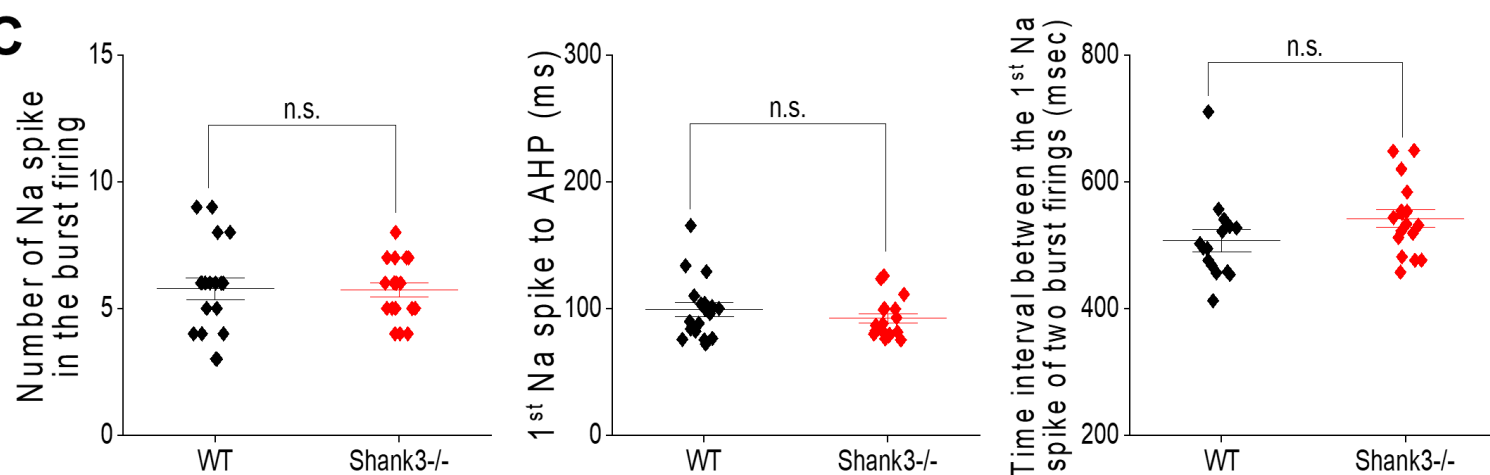
Fura-8	Kd	260 nM	[B]p	100 μ M
10 mM CaCl ₂	365 nm	2995.7	Rmax	76.8
	410 nm	39.0		
10 mM EGTA	365 nm	616.0	Rmin	0.889
	410 nm	692.8		
	alpha	0.755	Keff	12263 μ M

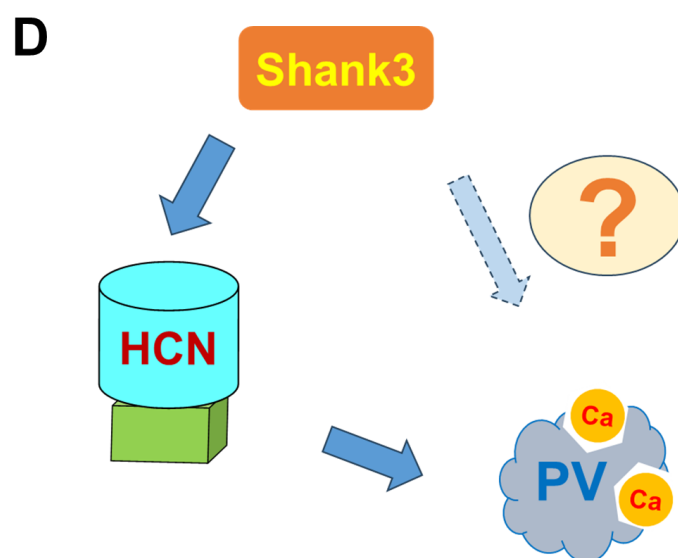
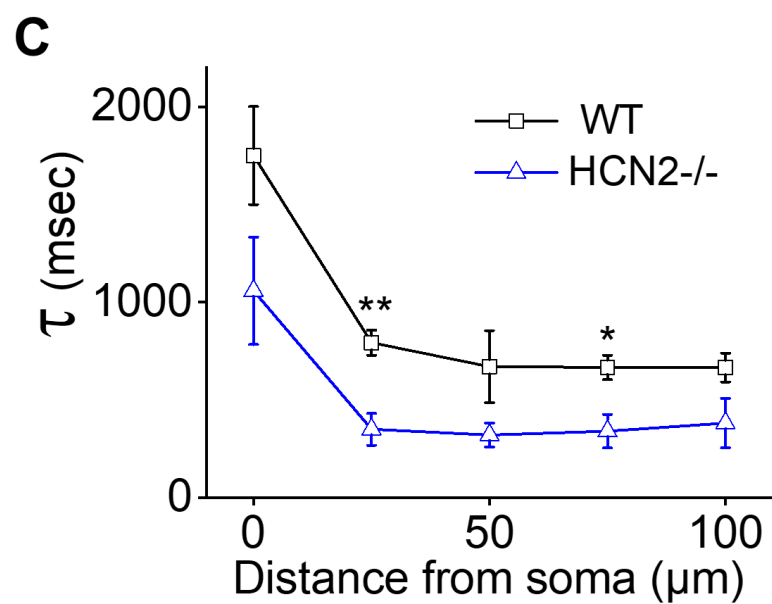
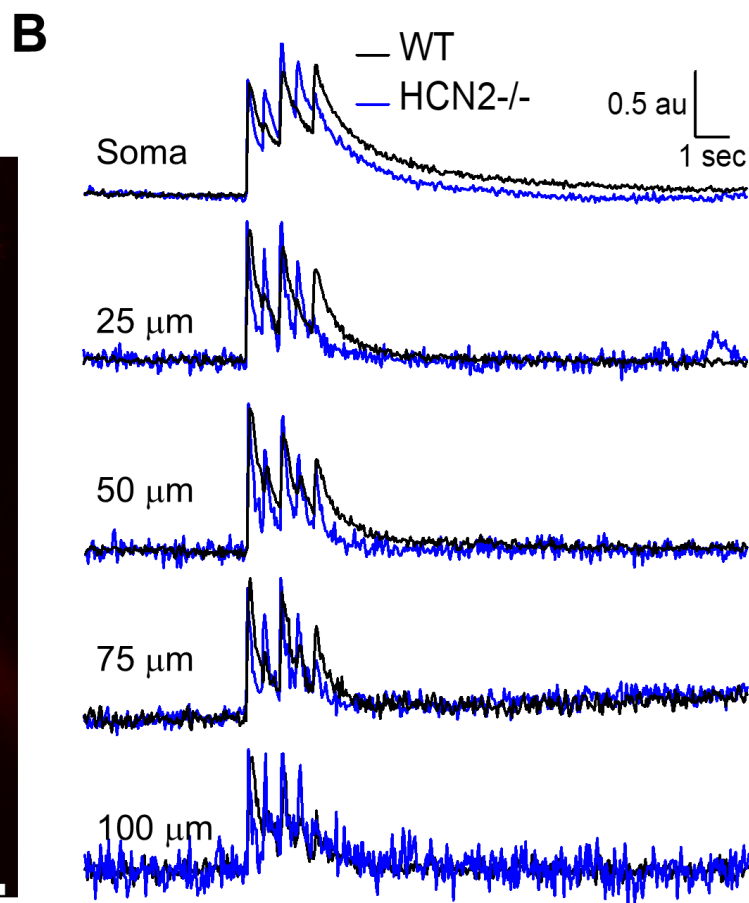
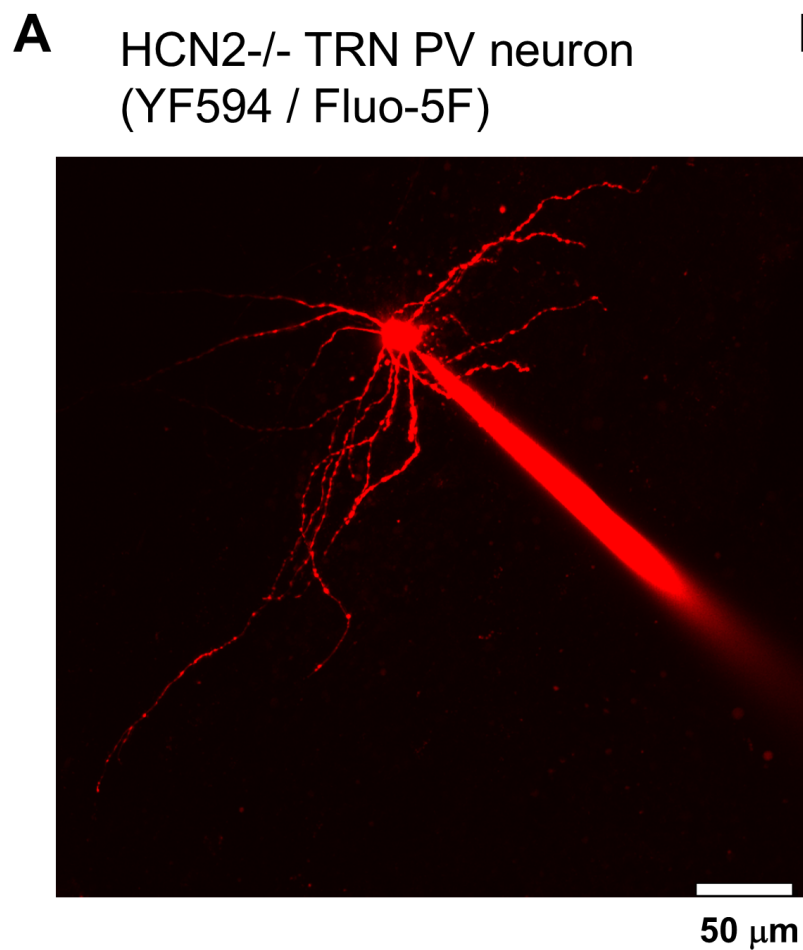
E Shank3^{-/-} TRN neuron**F**

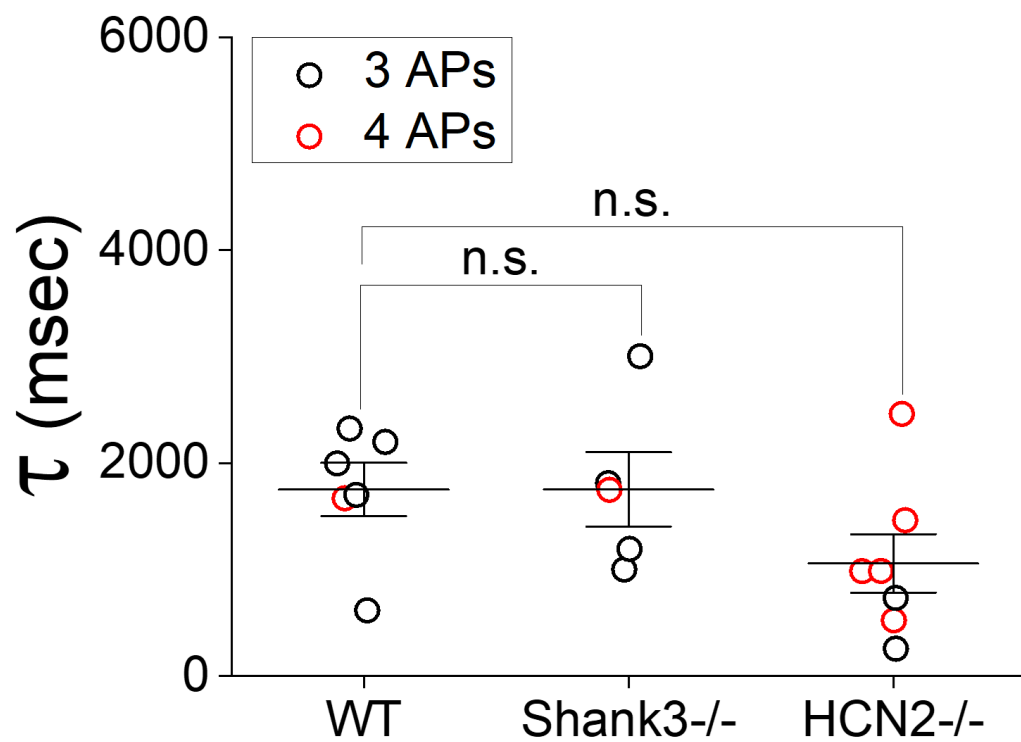
A**B**

C**D****E**

A**B**

A**B****C**



E**Soma****F****Dendrite**