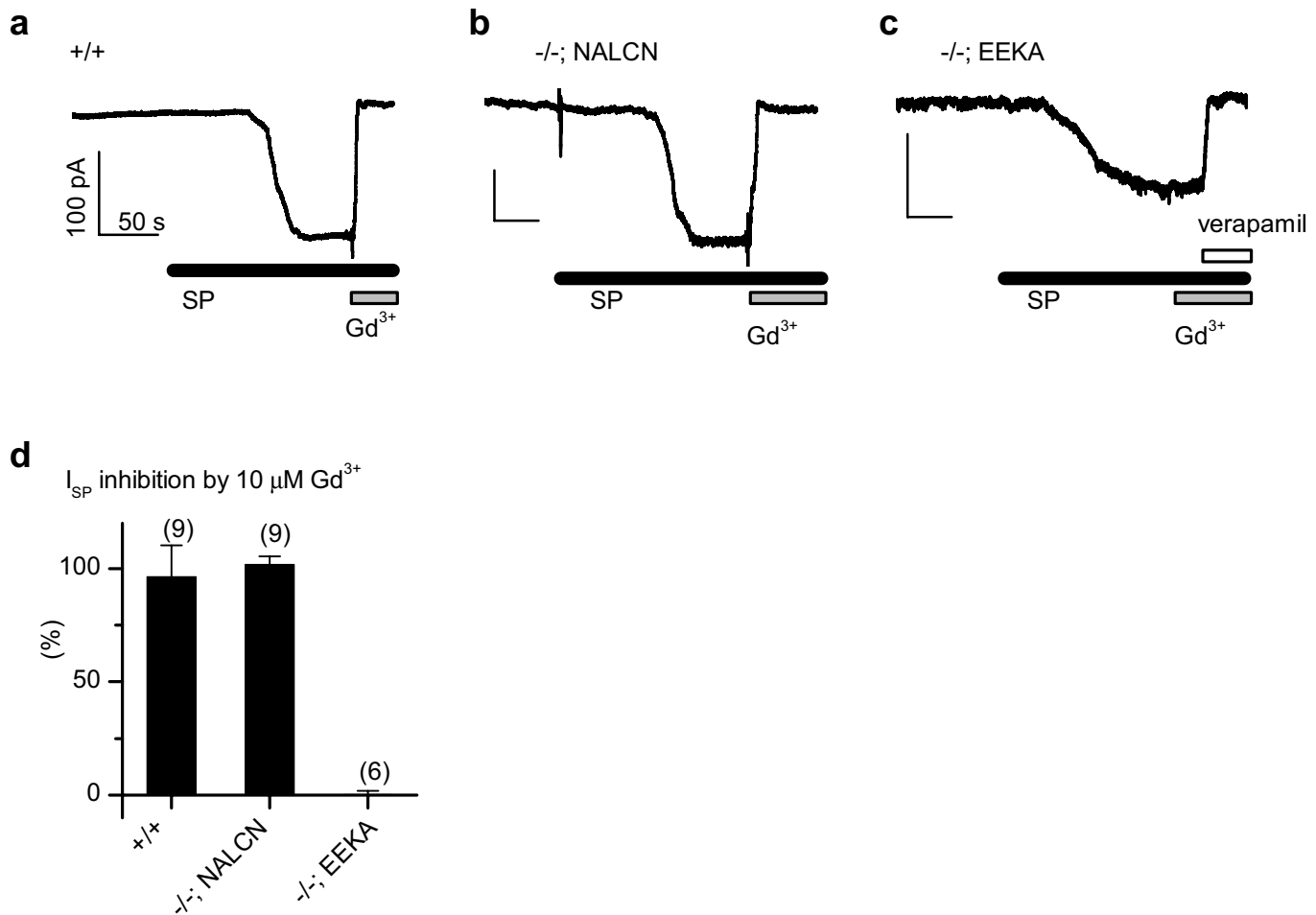
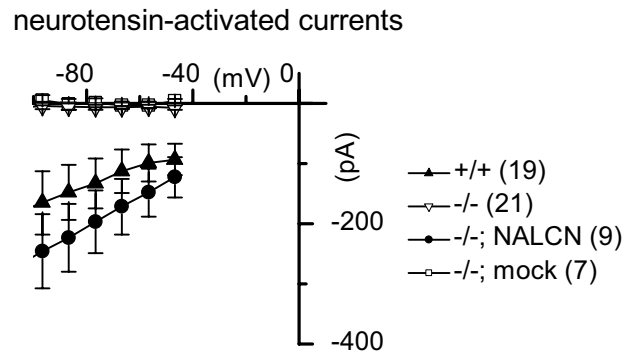


**Supplementary Figure 1: A  $\text{Gd}^{3+}$ -resistant NALCN pore mutant (EEKA).** **a**, A schematic showing a NALCN mutant with the EEKE motif sequence in the putative channel pore filter mutated to EEKA. **b**, **c**, Representative blockade by 10  $\mu\text{M}$   $\text{Gd}^{3+}$  of the wild-type NALCN (EEKE) (**b**) and the pore mutant (EEKA) (**c**) overexpressed in HEK293T cells. Step protocols (300 ms,  $V_h = 0$  mV, from -80 to +80 mV in step of 20 mV) were used. Both the wild-type and the EEKA mutant could be blocked by 1 mM verapamil<sup>10</sup>.

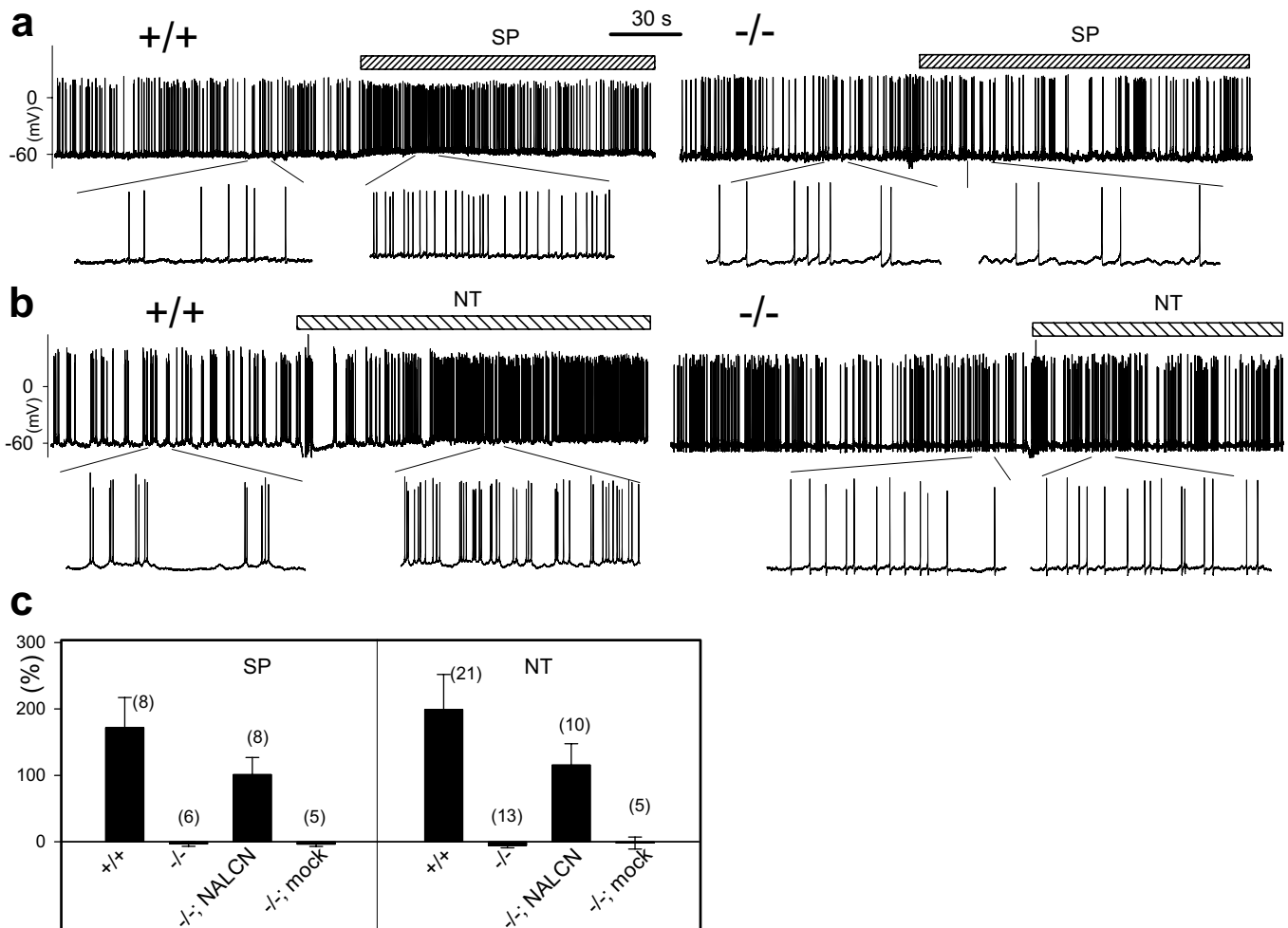


**Supplementary Figure 2. Alteration of  $I_{SP}$  pharmacology by a pore mutation in NALCN.** **a-c**, Representative hippocampal neuron  $I_{SP}$  currents induced by bath application of  $1 \mu\text{M SP}$  (indicated by bars) from a wild-type (**a**),  $Nalcn^{-/-}$  transfected with NALCN (**b**) or with the pore mutant (EEKA, **c**). Currents were recorded in a Tyrode's solution containing  $10 \text{ mM}$  extracellular  $\text{K}^+$  at holding potential of  $E_{\text{K}}$  ( $\text{K}^+$  Nernst potential,  $-67 \text{ mV}$ ) to minimize contribution from  $\text{K}^+$  current. Pipette solution was the same as the K.Asip pipette used in current clamp. The current in (**c**) was insensitive to  $10 \mu\text{M Gd}^{3+}$  but was blocked by  $1 \text{ mM}$  verapamil. **d**, Summary of percentages of inhibition by  $10 \mu\text{M Gd}^{3+}$ . Some neurons had  $>100\%$  inhibition of  $I_{SP}$  (amplitude calculated as the difference between inward current sizes before and after SP application) presumably because  $\text{Gd}^{3+}$  also blocked the basal leak current that existed before SP application<sup>10</sup>. Error bars, mean  $\pm$  s.e.m.



**Supplementary Figure 3: Requirement of NALCN in the neurotensin -activated cation current ( $I_{NT}$ ) in ventral tegmental area neurons.**  $I_{NT}$  amplitudes were obtained at various voltages in the wild-type (+/+), mutant (-/-) and mutant neurons transfected with NALCN (-/-; NALCN) or with an empty vector (-/-; mock). Data were obtained by subtracting currents recorded before from those recorded after NT application. The lines for mutant (-/-) and mutant transfected with empty vector (-/-; mock) overlap and are not distinguished. Currents at potentials positive to -40 mV were not studied because of complications by voltage-activated currents in these neurons. Error bars, mean  $\pm$  s.e.m.

## Supplementary Figure 4

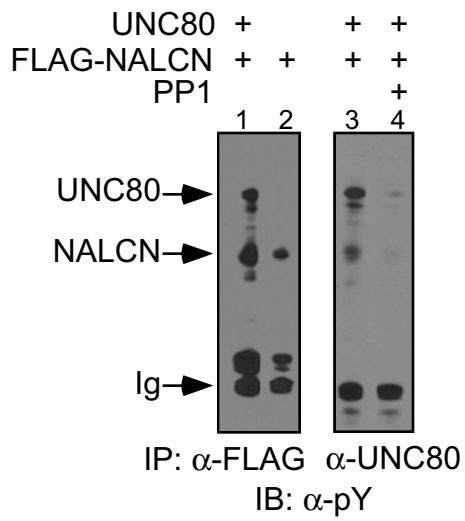


**Supplementary Figure 4: Requirement of NALCN in the potentiation of neuronal firing frequency by SP and NT.** **a, b**, Bath application of SP (1  $\mu$ M, **a**) or NT (1  $\mu$ M, **b**) increased the firing frequency of VTA neurons from wild-type (*left*) but not the *Nalcn*<sup>-/-</sup> mutant (*right*). Recordings of 10 s are expanded below each panel. **c**, Average increases of firing frequency (in %) by SP and NT in wild-type (+/+), mutant (-/-), mutant neurons transfected with NALCN (-/-; NALCN) or with an empty vector (-/-; mock). Error bars, mean  $\pm$  s.e.m.

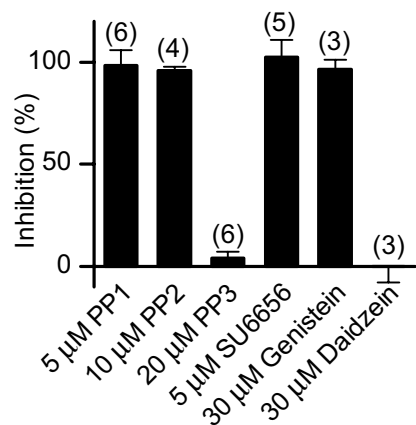
## Supplementary Figure 5

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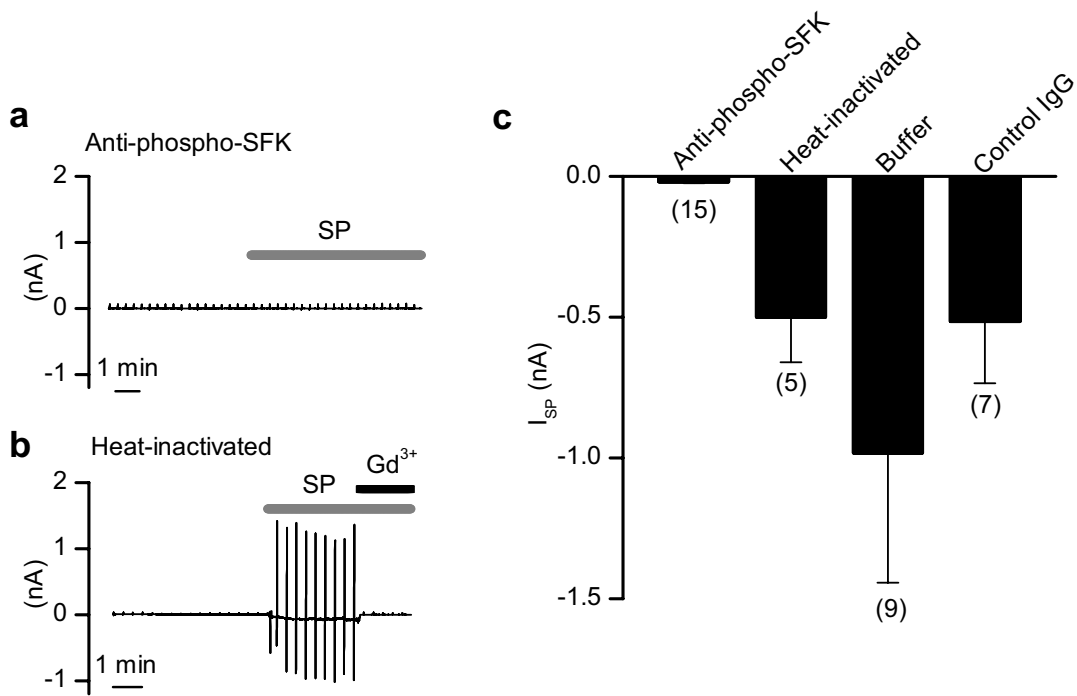
**Supplementary Figure 5: Deduced amino acid sequence of mUNC80.** The peptide sequence used for antibody generation is underlined. A putative PDZ domain-binding motif in the carboxyl-terminus is in bold.



**Supplementary Figure 6: Tyrosine phosphorylation of mUNC80 and NALCN.** Lysates from HEK293T cells co-transfected with NALCN (FLAG-tagged), Src, together with or without (lane 2) mUNC80 were immunoprecipitated using anti-FLAG (lanes 1, 2) or anti-UNC80 (lanes 3, 4) antibodies, and probed with anti-phosphorylated tyrosine antibody. Cells used in lane 4 were treated with SFK inhibitor PP1 (10  $\mu$ M for 2h) before being lysed. Lanes 1, 2 and lanes 3, 4 are from two separate gels.

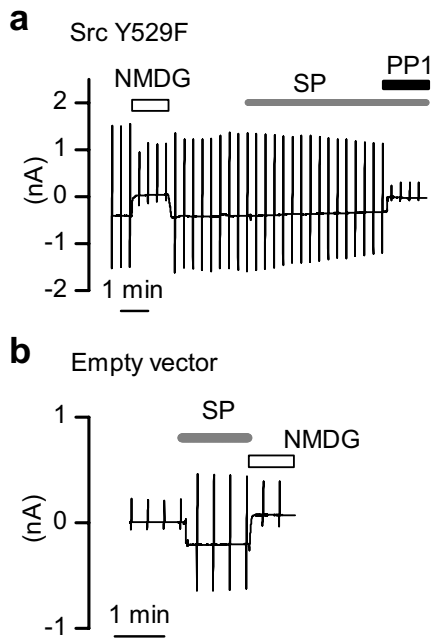


**Supplementary Figure 7: Inhibition of  $I_{SP}$  by SFK inhibitors.** Recordings were done with HEK293T cells transfected with NK1R, NALCN and mUNC80. Phosphotyrosine kinase inhibitor genistein (with daidzein as a control) and SFK inhibitors PP1, PP2 (with an inactive analog, PP3, as a control), and SU6656 were bath-applied at concentrations as indicated.  $I_{SP}$  amplitudes at -100 mV were used for analysis. Error bars, mean  $\pm$  s.e.m.

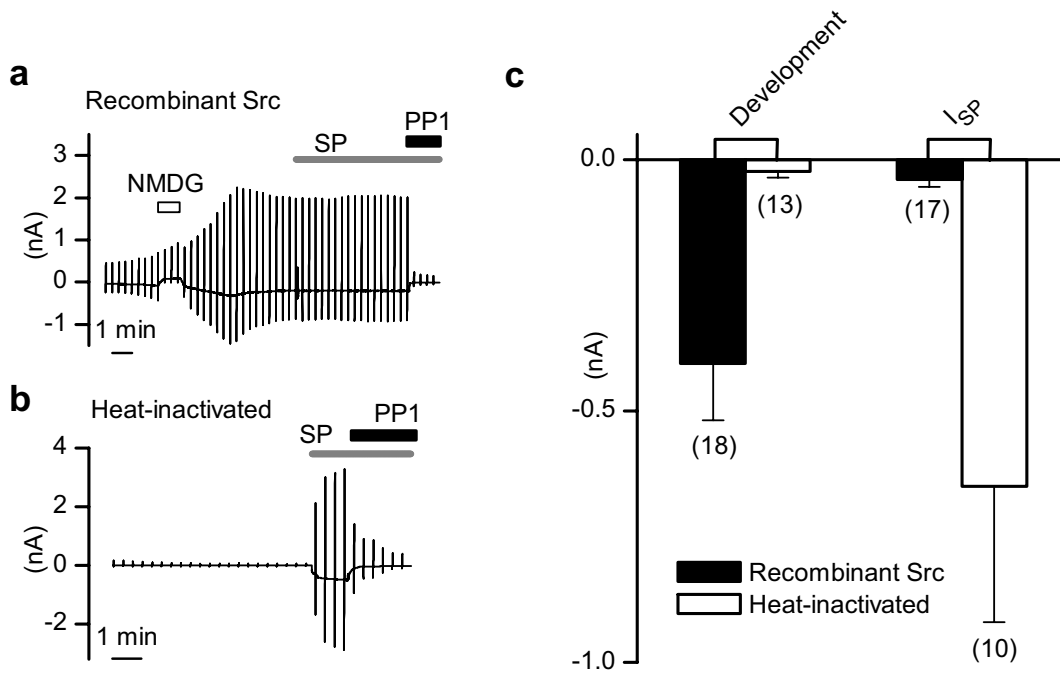


**Supplementary Figure 8: Inhibition of  $I_{SP}$  by an anti-phospho-SFK antibody.** **a, b,** Representative recordings of  $I_{SP}$  in HEK293T cells transfected with NK1R, mUNC80, and NALCN with pipette solutions containing anti-phospho-SFK antibody (**a**, 1  $\mu\text{g}/\text{ml}$ , 1:1000 dilution) or heat-inactivated antibody (**b**). **c,** Summary of  $I_{SP}$  sizes (at -100 mV) recorded with pipette solutions containing anti-phospho-SFK antibody, heat-inactivated anti-phospho-SFK antibody, antibody storage buffer, or control IgG. Recordings were done using ramp protocols ( $V_h = -20$  mV; -100 to +100 mV in 1 s, every 20 s). Error bars, mean  $\pm$  s.e.m.





**Supplementary Figure 9:** Representative currents recorded from HEK293T cells transfected with NK1R, mUNC80, NALCN, and a constitutively active Src (Y529F) (**a**) or an empty vector (**b**). PP1, 20  $\mu$ M. Open bars indicate perfusion with bath containing NMDG to replace  $\text{Na}^+$  and  $\text{K}^+$ .



**Supplementary Figure 10: Activation of NALCN by a recombinant active Src protein.** **a**, **b**, Representative recordings from HEK293T cells transfected with NK1R, mUNC80, and NALCN with pipette solution containing a recombinant active Src (**a**, ~1.6 units/ml) or heat-inactivated protein (**b**). An inward current developed upon intracellular dialysis with pipette solution containing the recombinant active protein (**a**). After the current reached a steady state, application of SP (1  $\mu$ M) did not induce an additional current ( $I_{SP}$  for **c**). **c**, Summary of current development (at -100 mV, as recorded in **a**) by cell dialysis with recombinant protein and additional currents activated by SP bath application after the cellular dialysis. PP1, 20  $\mu$ M. Open bars indicate perfusion with bath containing NMDG to replace  $\text{Na}^+$  and  $\text{K}^+$ . Recordings were done using ramp protocols ( $V_h = -20$  mV; -100 to +100 mV in 1 s, every 20 s). Error bars, mean  $\pm$  s.e.m.

## **List of Key Genes (Proteins) Appearing in the Manuscript**

*Nalcn* (NALCN)

*unc-79* (UNC-79)

*unc-80* (UNC-80)

*tac1r* (NK1R)