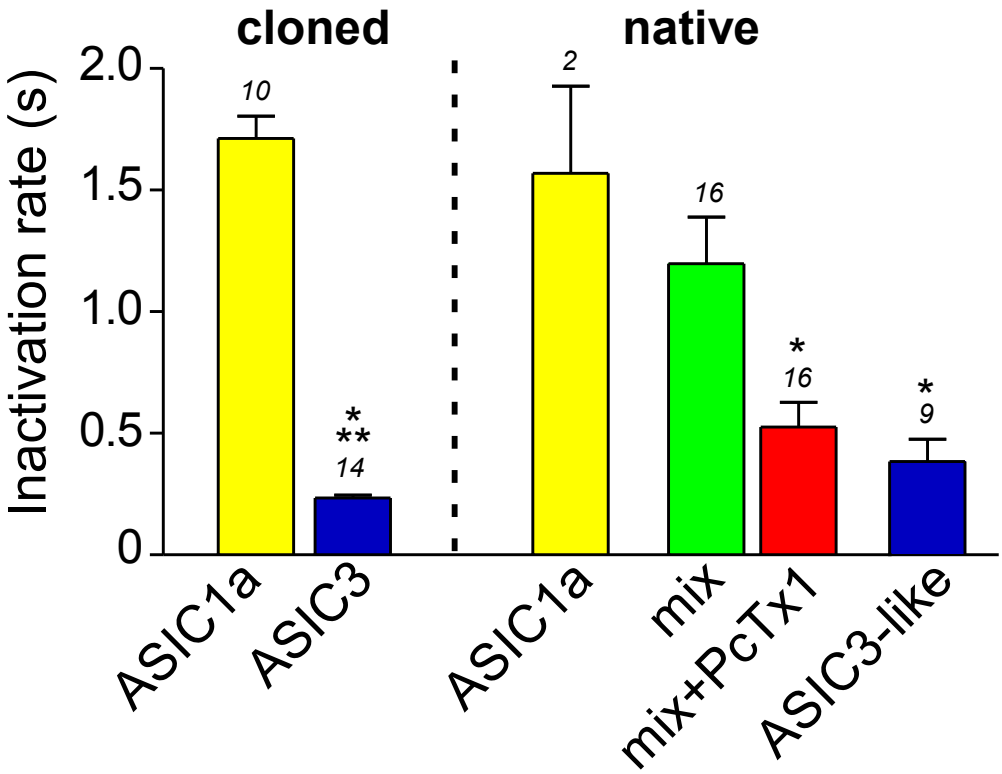
Supplementary information

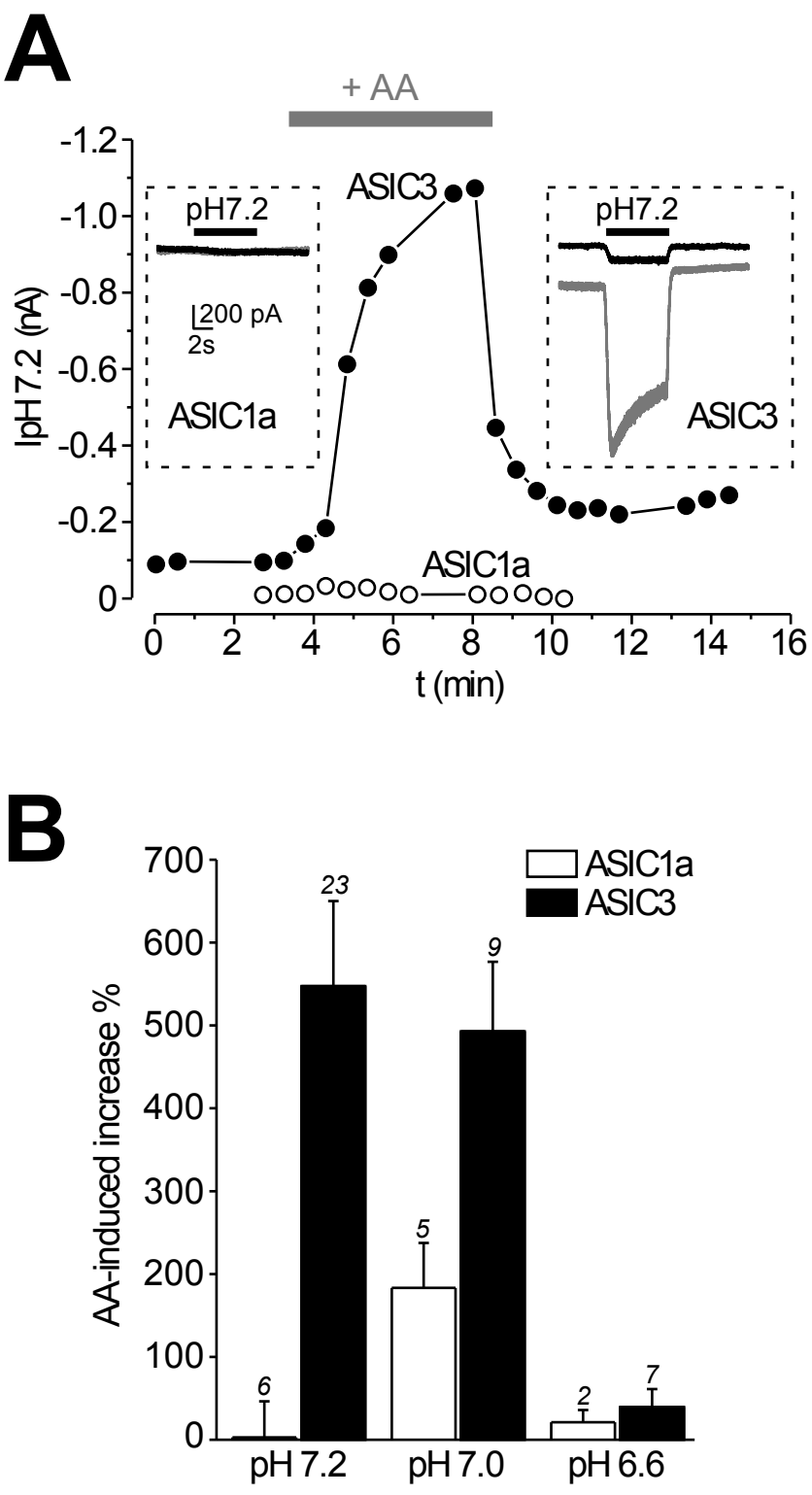
Supplementary Figure 1: Statistical analysis of the kinetics of native and recombinant pH6.6-evoked ASIC currents. The inactivation time constants of the currents were estimated using exponential fits. The number (*n*) of experiment is indicated above each bar (***, P<0.001 and *, P<0.05, significantly different from cloned or native ASIC1a, one-way ANOVA followed by a Tuckey's *post hoc* test). Native ASIC1a and ASIC3-like currents had the same inactivation time constant as compared to that of recombinant ASIC1a and ASIC3 currents expressed in the F-11 DRG cell line ($\tau_{inactivation}$ =1.6 ± 0.4 s, n=2 *vs.* $\tau_{inactivation}$ =1.7 ± 0.09 s, n=10 and $\tau_{inactivation}$ =0.2 ± 0.01 s, n=14 *vs.* $\tau_{inactivation}$ =0.4 ± 0.09 s, n=9 for ASIC1a and ASIC3 recombinant and native currents respectively, P>0.05, one-way ANOVA followed by a Tukey's *post hoc* test). The inactivation time constant of the native mix current ($\tau_{inactivation}$ =1.2 ± 0.2 s, n=16) was significantly reduced by PcTx1 ($\tau_{inactivation}$ =0.5 ± 0.1 s, n=16, P<0.01, paired *t*-test) to a value not significantly different from that of recombinant ASIC3 and native ASIC3-like currents (P>0.05, one-way ANOVA followed by a Tukey's *post hoc* test).

Supplementary Figure 2: Arachidonic acid preferentially potentiates ASIC3 current induced by moderate acidosis. ***A***, The effect of 10 μM arachidonic acid on pH7.2-induced ASIC1a (open circles) and ASIC3 (dark circles) currents is represented as a function of time. Currents were recorded from F-11 transfected cells at -50 mV. Dotted rectangles highlights the maximal effects of the compound obtained on ASIC1a (left) and ASIC3 (right) pH7.2-evoked currents. ***B***, Bar graph showing the AA-induced increase percentage of both ASIC1a (white bars) and ASIC3 (black bars) currents recorded at -80mV from F-11 transfected cells at different pH. The number of experiment (*n*) is indicated in italic above each bar.

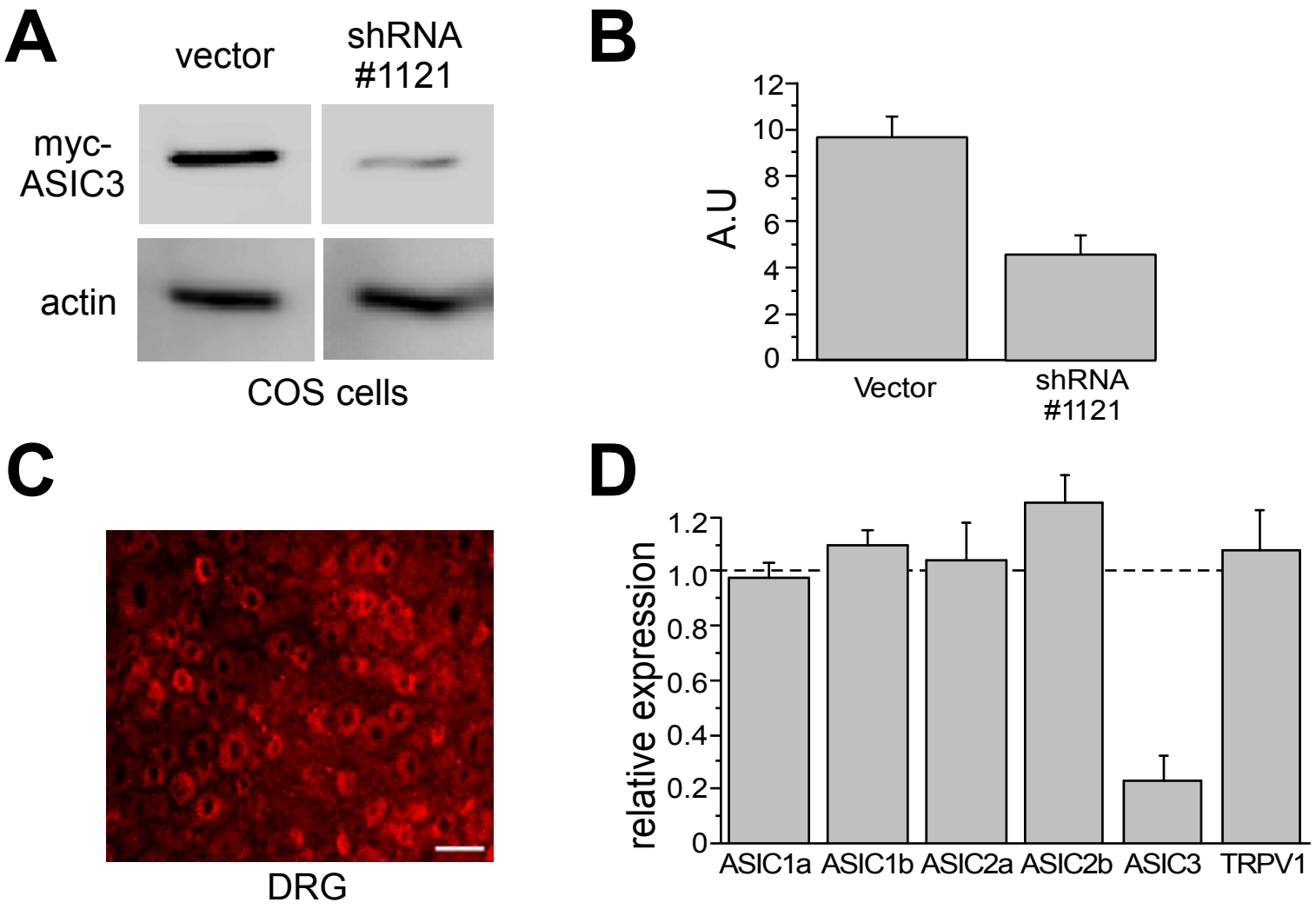
Supplementary Figure 3: *In vitro* evaluation, uptake in lumbar DRG after intrathecal injection and *in vivo* validation of the siRNA targeting ASIC3. The siRNA targeting ASIC3 was inserted into a siRNA expression vector (see methods) and co-transfected in COS cells with a plasmid coding for a myc tagged rat ASIC3 (20:1 ratio). ***A***, Top, the level of ASIC3 protein after co-transfection with the shRNA or with the empty vector as a control was assessed by Western blot with a myc antibody. Bottom, the blot was probed with an antibody against actin to demonstrate equal loading of proteins. ***B***, densitometric quantification of the ASIC3 signal as shown in a (n=6) showing that shRNA #1121 knockdown about 50% of the expression of ASIC3. Note that because of the very robust expression of myc-ASIC3 after transient transfection into COS cells, the knockdown efficiency is probably underestimated in this system. A.U, arbitrary units. ***C***, Cryostat section of lumbar dorsal root ganglia showing uptake of Cy3-labelled siRNA (#1121) into the DRG 24 hours after a single intrathecal injection. Scale bar, 100 µm. ***D***, Relative levels of RNA transcripts encoding ASICs and TRPV1 in lumbar dorsal root ganglia after 3 intrathecal injections at 24-hour intervals of the siRNA targeting ASIC3 or of the vehicle, assessed by RT-qPCR. L5 and L6 ganglia were removed 24 hours after the last injection. Values represent the mean ± s.e.m of 3 different animals, except for TRPV1 where only two animals were used. The siRNA specifically knockdown ASIC3 expression in lumbar dorsal root ganglia.



SUPPLEMENTARY FIGURE 1 (Deval et al)



SUPPLEMENTARY FIGURE 2 (Deval et al)



SUPPLEMENTARY FIGURE 3 (Deval et al)