Pro-inflammatory mediators modulate the heat-activated ion channel TRPV1 via the scaffolding protein AKAP79/150

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Summary

The ability of vertebrates to detect and avoid damaging extremes of temperature depends on activation of ion channels belonging to the thermo-TRP family. Injury or inflammation lowers the threshold for detection of painful levels of heat, a process known as heat hyperalgesia. A wide range of inflammatory mediators is able to promote heat hyperalgesia, and these activate at least three distinct intracellular signalling pathways which lower the threshold of the heat-activated ion channel TRPV1. Here we show that modulation of the sensitivity of TRPV1 by the protein kinases PKA and PKC, and by the phosphatase calcineurin, depends on the formation of a signalling complex between the scaffolding protein AKAP79/150 and TRPV1. We identify a critical region in the TRPV1 C-terminal which mediates binding of AKAP79/150. If binding is prevented then sensitisation by both bradykinin and PGE_2 is abrogated. AKAP79/150 is therefore a final common element in heat hyperalgesia, on which the effects of multiple pro-inflammatory mediators converge.

Introduction

Animals detect thermal stimuli via the activation of primary sensory nerve terminals responding to a range of temperatures from noxious cold, through the non-noxious range, and extending to damaging extremes of heat (Darian-Smith, 1984; Perl, 1984; Lumpkin and Caterina, 2007). Thermal stimuli activate inward membrane currents in temperature-sensitive neurons (Cesare and McNaughton, 1996; Reid and Flonta, 2001), and they do so by the direct activation of temperature-sensitive ion channels belonging to the thermo-TRP family (Jordt et al., 2003; Venkatachalam and Montell, 2007; Nilius et al., 2007). The best-studied thermo-TRPs are as follows: TRPV1, which is activated by noxious temperatures above 43°C (Caterina et al., 1997); TRPV2, activated by higher temperatures, above 52°C (Caterina et al., 1999); TRPV3 and TRPV4, activated in the range of non-noxious warmth, c. 35- 37°C (Smith et al., 2002; Xu et al., 2002; Guler et al., 2002; Watanabe et al., 2002); TRPM8, activated by cooling below c. 30°C (McKemy et al., 2002); and TRPA1, which may be activated by more extreme cooling (Story et al., 2003), though other recent studies have failed to confirm the activation of this ion channel by cold (Bautista et al., 2006).

The temperature thresholds of thermally activated ion channels are modulated by a range of extracellular factors (Huang et al., 2006; Venkatachalam and Montell, 2007). The temperature threshold of TRPV1 is lowered by a wide range of pro-inflammatory mediators such as bradykinin, prostaglandin E_2 (PGE_2) and nerve growth factor (NGF). The lowering of the TRPV1 threshold is critical for heat hyperalgesia, the process by which the threshold for heat pain is lowered following inflammation or injury, because heat hyperalgesia is ablated when TRPV1 is genetically deleted (Davis et al., 2000; Caterina et al., 2000).

Three pathways are known to lead to sensitization of TRPV1. The activation of the G_q-coupled B1 and B2 receptors by bradykinin lowers the temperature threshold of TRPV1 by promoting phosphorylation by PKCε (Cesare and McNaughton, 1996; Cesare et al., 1999; Numazaki et al., 2002; Bhave et al., 2003). An alternative possibility, that metabolism of PIP_2

by PLCβ may release TRPV1 from PIP_2-mediated inhibition (Chuang et al., 2001), is not supported by more recent evidence showing that PIP_2 in fact activates rather than inhibiting TRPV1 (Stein et al., 2006; Lishko et al., 2007). In sensory neurons PGE_2 activates the EP_4 receptor, which is G_s–coupled and causes sensitization by promoting PKA-dependent phosphorylation of TRPV1 (Bhave et al., 2002; Distler et al., 2003). In a third pathway leading to sensitization of TRPV1, NGF potentiates TRPV1 by activating PI3 kinase and the tyrosine kinase Src, leading to phosphorylation of TRPV1 at Y200 and to promotion of trafficking of TRPV1 to the neuronal cell membrane (Zhang et al., 2005; Stein et al., 2006). A proposal that NGF sensitizes TRPV1 by activating PLCγ and thus reducing levels of PIP_2 (Chuang et al., 2001; Prescott and Julius, 2003) is not supported by more recent evidence (Zhang et al., 2005).

The speed and specificity of the action of kinases is in many cases enhanced by the use of scaffolding proteins which pre-assemble the kinases into signalling complexes with their substrates. The AKAP (A Kinase Anchoring Protein) family of scaffolding proteins were originally named for their ability to target PKA to appropriate substrates, but are now known to assemble a wide range of kinases and phosphatases into signalling complexes with appropriate targets (Leiser et al., 1986; Bregman et al., 1989; reviewed by Smith et al., 2006). AKAP79/150 targets PKA, PKC and the Ca-dependent phosphatase calcineurin to appropriate substrates (Coghlan et al., 1995; Klauck et al., 1996). AKAP79 is the human homolog, while AKAP150, the mouse homolog, seems to be similar in function to AKAP79 but contains an additional large central domain.

A number of ion channels are subject to modulation by AKAPs, including glutamate receptors, calcium channels and M-type potassium channels (Gao et al., 1997; Colledge et al., 2000; Altier et al., 2002; Hoshi et al., 2003; Hoshi et al., 2005; Sandoz et al., 2006; Oliveria et al., 2007; Lu et al., 2007; Chai et al., 2007; Navedo et al., 2008). An AKAP may be involved in the modulation of TRPV1 by PKA (Rathee et al., 2002; Distler et al., 2003) but neither the AKAP family member involved nor its binding site on TRPV1 have been

identified, and the possibility that other kinases such as PKC and Src may also be assembled into a functional signalling complex important in the regulation of TRPV1 has not been explored. These questions are addressed in the present paper.

Results

Co-expression of AKAP79/150 and TRPV1 in sensory neurons

Several AKAPs are expressed in sensory neurons, amongst them AKAP79/150 (Rathee et al., 2002). Fig. 1A shows that the vast majority of rat DRG neurons which express TRPV1 (left panel) also express AKAP150 (centre panel and merged image in right panel). We found that 91.1 ± 1.7 % of neurons expressing TRPV1 also expressed AKAP150 (n=352). Evidence for a physical association between the two in sensory neurons was obtained in co-immunoprecipitation experiments in which pull-down of TRPV1, using an antibody raised to the TRPV1 N-terminal domain, also brought down AKAP150 (Fig. 1B).

In later experiments in this study it was important to be able to manipulate endogenous AKAP79/150 levels in both DRG neurons and in a HEK293 expression system. We constructed an siRNA against AKAP150 and AKAP79, as used by Hoshi et al (2005) but in a vector which co-expresses GFP to enable identification of successfully transfected cells. The siRNA against AKAP150 reduced expression levels in transfected rat DRG neurons to 12.1 ± 1.9% of control (Fig. 1C, n=6), and the siRNA against AKAP79 reduced expression in HEK293 cells to 15.9 ± 2.6% of control (Fig. 1D, n =22). A similar but lesser downregulation was seen in Western blots of HEK293 cell protein (Fig. 1E, 30.4 ± 2.5% of control, n=3), a difference which is expected because not all cells are transfected. In all cases a scrambled siRNA with the same base composition was completely ineffective (Fig. 1C-E).

Binding of AKAP79 to thermo-TRPs

The experiments in Fig. 2 show that AKAP79 expressed in HEK293 cells binds to several members of the thermo-TRP family of ion channels. AKAP79 expressed in HEK293 cells co-immunoprecipitates with several thermo-TRPs (Fig. 2A), particularly with TRPV1 and TRPV4, and to a lesser extent TRPM8 and TRPA1. Endogenous AKAP79 can also co-immunoprecipitate lesser amounts of TRPV1 (Fig. 1B, top left panel). Fig. 2C shows that the

binding between TRPV1 and AKAP79 does not depend on the use of the V5 tag attached to the C-terminal as it is also observed when an antibody raised to the N-terminal TRPV1 domain is used.

We next localised the TRPV1 binding site for AKAP79 by constructing GST-coupled fragments of the four intracellular domains of TRPV1, expressed in bacteria, and exploring binding to AKAP79 *in vitro*. The major binding domain is in the C-terminal portion of TRPV1 (Fig. 2D), with only weak binding observed to the large N-terminal domain and no detectable binding to either of the intracellular loops. When the GST-coupled C-terminal domain was progressively truncated there was an increase in binding on removal of the region between amino acids 759 and 777, probably because of removal of an inhibitory region, but more significantly there was a substantial loss of binding on removal of amino acids 736 to 749 (Fig. 2E, left panel; note that all residue numbering is for hTRPV1). Deletion of just this region caused a major reduction in binding, while other nearby deletions were much less effective (Fig. 2E, right panel). We repeated this deletion in intact TRPV1, expressed in HEK293 cells, and found that removal of this region also caused a substantial reduction in the binding of AKAP79 to intact TRPV1 (Supplementary Fig. 1A). We next constructed a 14aa peptide, identical to this binding domain, which should be able to compete for the site on AKAP79 to which TRPV1 binds. At a concentration of 200μM this peptide largely prevented binding of AKAP79 to the GST-coupled C-terminal fragment of TRPV1, while a scrambled peptide of the same composition was ineffective (Fig. 2F). The peptide was also highly effective in displacing AKAP79 from binding to intact TRPV1 (Fig. 2G).

AKAP79/150 promotes activation of TRPV1

We found that expression of AKAP79 enhanced the sensitivity of TRPV1 to capsaicin even in the absence of overt activation of PKC or PKA by pro-inflammatory mediators (Fig. 3A, compare red points and curve with black points and curve). Conversely, expression of

AKAP79 with the PKC binding site deleted (Hoshi et al., 2005) reduced the sensitivity of TRPV1 (green points and curve). The fact that AKAP79 can enhance sensitivity, while deletion of the PKC binding site on AKAP79 has a dominant negative effect, shows the phosphorylation by PKC is important in modulating sensitivity of TRPV1 even in the resting cell. The results also show that endogenous AKAP79 does not saturate its binding sites on TRPV1, because overexpression of AKAP79 has an effect greater than that of endogenous AKAP79.

Deletion of the PKA binding site had a smaller effect than deletion of the PKC site (grey squares), showing that phosphorylation by constitutively active PKA is less important than PKC in the resting cell. The effect of expression of an AKAP79 with the calcineurin (PP2B) binding site deleted was indistinguishable from that of wild-type AKAP79 (blue points), suggesting that calcineurin bound to AKAP79 makes little contribution to dephosphorylating TRPV1 at resting levels of calcium.

TRPV1 ion channels can be opened even in the absence of agonist by strong membrane depolarisation (Voets et al., 2004). Activation of PKC potentiates this process by enabling opening of TRPV1 at lower membrane potentials (Matta and Ahern, 2007), and our demonstration that AKAP79 is involved in potentiation of TRPV1 by PKC suggests that it will have a similar effect. Fig. 3B shows that activating PKC or overexpressing AKAP79 both have similar effects in potentiating the opening of TRPV1 by membrane depolarisation, and moreover that the actions are to some extent additive. Fig. 3C and D demonstrate that overexpression of AKAP79 shifts the voltage activation curve to less depolarised potentials by 54mV, and that subsequent full activation of PKC causes an additional 32mV negative shift. In summary, the experiments in Fig. 3 show that expression of AKAP79 promotes activation of TRPV1 by both capsaicin and membrane potential, and moreover that this action depends mainly on the binding of PKC to AKAP79.

AKAP79/150 is essential for sensitization of TRPV1via PKC and PKA pathways

We next investigated whether AKAP79/150 mediates the sensitization of TRPV1 caused by pro-inflammatory mediators. For these experiments we used a low dose of capsaicin as a convenient activator of TRPV1, because the sensitivity of TRPV1 to heat, capsaicin, low pH and a range of other activators is known to be enhanced in a similar way by pro-inflammatory mediators (Vellani et al., 2001).

In rat sensory neurons bradykinin caused a substantial enhancement of the inward current mediated by TRPV1 (Fig. 4A), a process which has been shown to be due to phosphorylation of serines 502 and 801 by PKCε (Cesare and McNaughton, 1996; Cesare et al., 1999; Numazaki et al., 2002; Bhave et al., 2003). Incorporation of the 14aa peptide which prevents binding of AKAP150 to TRPV1 completely inhibited sensitization by Bk, while the scrambled peptide was ineffective (Fig. 4A, B). An alternative way of demonstrating an involvement of AKAP150 is to downregulate endogenous expression using siRNA. Fig 4A and B show that removal of AKAP150 in this way almost completely inhibited the action of Bk in sensory neurons, while a scrambled siRNA was ineffective. Finally, simultaneously transfecting with AKAP79, which is insensitive to the siRNA against AKAP150 (Hoshi et al., 2005), rescued the sensitization by BK, and in fact the overexpression of AKAP79 causes an enhanced level of sensitization (final bar, Fig. 4B).

Bradykinin also caused a substantial sensitization of TRPV1 in HEK293 cells transfected with the G_q-coupled B2 receptor (Fig. 4C). Sensitization was abolished by deletion of the PKC binding site on AKAP79, by downregulation of endogenous AKAP79 with siRNA, or by mutation of the S502 and S801 sites on TRPV1, which are known targets for phosphorylation by PKC (Numazaki et al., 2002; Bhave et al., 2003); see Fig. 4D. Deletion of the PKA binding site, however, had a lesser effect than deletion of the PKC site (Fig. 2C), as expected because PKC is the main kinase activated by bradykinin. The experiments in Fig 4 show that simple activation of PKC by a pro-inflammatory mediator

such as bradykinin is insufficient to cause sensitization of TRPV1. Formation of a scaffolding complex between TRPV1, AKAP79 and PKC is an essential pre-requisite for PKC to sensitize TRPV1.

A second important pro-inflammatory mediator is the prostaglandin PGE_2, which sensitises TRPV1 in sensory neurons via both the G_q-coupled EP_1 receptor and the G_s-coupled EP_4 receptor (Lopshire and Nicol, 1998; Southall and Vasko, 2001; Bhave et al., 2002; Moriyama et al., 2005; Lin et al., 2006). We found that application of PGE_2 to isolated rat sensory neurons caused a substantial sensitization, and as was the case with Bk, sensitisation by PGE_2 was completely ablated by inclusion of the 14aa peptide which blocks the AKAP79/150 – TRPV1 interaction (Fig. 5A,B). Downregulating endogenous AKAP150 by the use of siRNA had a similar inhibitory effect, and transfection of AKAP79 rescued the sensitization by PGE_2. Interestingly, overexpression of AKAP79 did not produce a level of sensitization above that in untransfected neurons, in contrast to the enhanced sensitization seen with Bk. This difference is discussed further below.

In HEK293 cells co-expressing TRPV1 and the EP_1 receptor, application of PGE_2 caused significant sensitization (Fig. 5C, and see example traces in Supplementary Fig. 2A). Over-expression of AKAP79 enhanced sensitization (Fig. 5C, bar 3), which we attribute to a greater availability of the PKC binding site, an explanation which is reinforced by the similar enhancement observed when AKAP79 with the PKA site deleted was expressed (Fig. 5C, bar 4). Sensitization was abolished, however, by expression of AKAP79 with the PKC site deleted, or by downregulation of endogenous AKAP79 by the use of siRNA (Fig. 5C, bars 5, 6). These results show that sensitization of TRPV1 via the EP_1 receptor is completely dependent on the presence of AKAP79 containing a PKC binding site.

The results above show that PKC is able to phosphorylate TRPV1 only when AKAP79/150 is available to position the kinase appropriately. We also demonstrated this directly by the use of a phospho-serine antibody (Supplementary Fig. 1B). Serine

phosphorylation was enhanced when PKC was activated with PMA, but when endogenous AKAP79 was eliminated by transfection of siRNA the effect of PKC activation was abolished.

In HEK293 cells transfected with the EP_4 receptor we found that sensitization was abolished by deletion of the PKA binding site on AKAP79, or by down-regulation of endogenous AKAP79 by the use of siRNA, while deletion of the PKC site was without significant effect (Fig. 5 D, and see example traces in Supplementary Fig. 2B). These results show that sensitization via the EP_4 receptor depends on the presence of AKAP79 containing a functional PKA binding site.

An interesting difference between Fig. 5C and D, which is also mirrored in the experiments on DRG neurons in Figs. 4B and 5B, is that sensitization via the PKC pathway is enhanced when AKAP79/150 is overexpressed, but sensitization via the PKA pathway is already maximal with endogenous levels of AKAP79/150. Thus endogenous levels of AKAP79/150 are not adequate to ensure full phosphorylation of TRPV1 when PKC is activated, and phosphorylation is boosted by transfection of further AKAP79/150, while endogenous levels of AKAP79/150 are adequate to ensure maximal phosphorylation of TRPV1 following activation of PKA.

NGF sensitizes TRPV1 mainly by causing increased trafficking to the surface membrane via a pathway involving the tyrosine kinase Src (Zhang et al., 2005). Does this pathway also depend on AKAP79/150? Fig. 6A shows that NGF enhanced the membrane current activated by a near-saturating concentration of capsaicin, in agreement with previous studies (Zhang et al., 2005; Stein et al., 2006). This effect is not, however, reduced by inclusion of the AKAP-TRPV1 blocking peptide in the pipette. We conclude that Src potentiates membrane trafficking of TRPV1 via an AKAP79/150-independent mechanism.

Involvement of AKAP79/150 in desensitization

AKAP79/150 contains a binding site for the calcium-dependent phosphatase calcineurin (PP2B) (Coghlan et al., 1995; Klauck et al., 1996), suggesting that AKAP79/150 could play a role in desensitization of TRPV1, which is partly mediated by calcineurin (Docherty et al., 1996; Koplas et al., 1997; Vellani et al., 2001). In Fig. 6C we investigated desensitization by applying a near-saturating conditioning dose of capsaicin, which evoked a large inward current and caused strong desensitization of the current evoked by a second capsaicin exposure. Note that in these experiments the magnitude of desensitization depends strongly on the preceding calcium influx (Koplas et al., 1997) and therefore it is important to select cells in which the calcium influxes in response to the conditioning pulse are similar, particularly in view of the fact (see Fig. 7C and discussion below) that treatments such as upregulating AKAP79 by transfection, or downregulating it by the use of siRNA, modulate the maximum current amplitude. See legend to Fig. 6D for further details.

Desensitization was largely abolished by removal of external Ca (Fig. 6D, bar 2). Over-expression of AKAP79 strongly enhanced desensitization, while downregulation of endogenous AKAP79 by the use of siRNA substantially reduced it (Fig. 6C, D bars 3,4). AKAP79 with either the PKA site or the PKC site deleted behaved similarly to WT AKAP79 (Fig. 6D, bars 5,6) showing that these kinases are not involved in desensitization when not activated by exogenous stimuli. Deletion of the calcineurin binding site, on the other hand, substantially reduced desensitization compared to that observed with expression of WT AKAP79 (Fig. 6D, last bar). In similar experiments on sensory neurons, inclusion of the 14aa blocking peptide also reduced desensitisation (Fig. 6E, F).

These results implicate the calcineurin binding site of AKAP79 in the process of calcium-dependent desensitization of TRPV1. We note, however, that while removal of extracellular calcium almost completely abolished desensitization, removing the involvement of AKAP79, by downregulating AKAP79 with siRNA, by deleting the calcineurin binding

site, or (in neurons) by preventing binding of AKAP79 to TRPV1 with the inhibitory peptide, reduced but did not abolish desensitization (see Fig. 6C-F). A likely explanation is that another Ca-dependent mechanism of desensitisation is also present, in addition to the calcineurin/AKAP79 pathway. One possibility is that calmodulin may mediate calcium-dependent desensitization of TRPV1, either by activating CaM kinase (Jung et al., 2004; Novakova-Tousova et al., 2007) or by direct binding to a site in the intracellular N-terminal domain (Lishko et al., 2007).

AKAP79/150 mediates translocation of TRPV1 to the membrane

In Fig 7 we investigated a possible effect of AKAP79/150 on trafficking of TRPV1 to the membrane. We first measured membrane expression of TRPV1 using biotinylation of surface membrane proteins (Fig. 7A, B) or by expressing a functional TRPV1 in which an HA tag had been engineered into the third extracellular loop (Fig. 7H). We also obtained an independent measure of surface membrane TRPV1 from the maximal membrane current elicited by a saturating dose of capsaicin (Fig. 7C, D). With all three methods over-expression of AKAP79 caused a robust increase in surface membrane TRPV1 expression. Downregulation of AKAP79 using siRNA reduced but did not eliminate membrane expression (Fig. 7C, D). Deletion of either the PKC or the PKA binding site on AKAP79 largely negates the effect of AKAP79 in promoting translocation of TRPV1 to the membrane, while deletion of the PP2B binding site is without effect (Fig 7A-D). The PKA inhibitor H89 negates the effect of AKAP79 on trafficking of TRPV1 to the membrane (Fig. 7G, last lane). Thus AKAP79-mediated phosphorylation of TRPV1 by both PKC and PKA is important in translocation of TRPV1 to the membrane.

We next sought to identify the residues on TRPV1 whose phosphorylation by PKC or PKA promotes trafficking to the membrane. Mutating S502 abolished the enhanced membrane expression caused by over-expression of AKAP79 (Fig. 7E) or activation of PKA by forskolin (Fig. 7F). By contrast, mutation of the S117 residue, which has been implicated

in sensitization of TRPV1 by PKA (Bhave et al., 2002), does not inhibit AKAP79-dependent membrane trafficking (Fig. 7G). These experiments implicate phosphorylation of the S502 site as the main mechanism by which AKAP79 mediates translocation of TRPV1 to the surface membrane.

Discussion

The experiments reported here show that inflammatory mediators acting via PKA or PKC depend completely on AKAP79/150 to mediate their effects on TRPV1 (see summary diagram in Fig. 8). Two main mechanisms of sensitization have been identified in previous studies: an enhancement of gating of TRPV1 ion channels already in the membrane, and an increase in trafficking of TRPV1 to the membrane.

In the first and best-studied mechanism, mediators such as PGE_2 or bradykinin activate either PKA or PKCε, which phosphorylate TRPV1 and increase the probability of gating of the channel by a wide range of activators. In the present study we find that sensitization of channel gating depends on the ability of the relevant kinase to bind to AKAP79/150, and in turn on the ability of AKAP79/150 to bind to TRPV1. The observation that sensitization is abolished by disrupting either of these binding mechanisms, or by removal of AKAP79/150 using siRNA, implies that these two kinases must be pre-assembled in a signalling complex with TRPV1 in order to have any effect, and thus that free PKA or PKCε are unable to phosphorylate TRPV1 to any significant extent. The fact that inhibiting binding or removing AKAP79/150 completely inhibits sensitization adds further evidence to show that other proposed mechanism of sensitization, such as depletion of membrane PIP_2 following activation of PLC (Chuang et al., 2001; Prescott and Julius, 2003), do not make any significant contribution to sensitization of TRPV1.

AKAP79/150 is also involved in trafficking of TRPV1 to the surface membrane. Previous studies have shown that trafficking can be promoted by phosphorylation of TRPV1 either by Src (Zhang et al., 2005) or by PKC (Morenilla-Palao et al., 2004). The first mechanism is insensitive to disruption of binding between AKAP79/150 and TRPV1, showing that Src does not depend on AKAP79/150 to assemble it into a signalling complex with TRPV1. The ability of PKC and PKA to enhance trafficking does, however, depend absolutely on the presence of functional AKAP79/150. We have shown that the main target

of both kinases in promoting trafficking is S502,a residue which is also involved in the potentiation of channel gating (Numazaki et al., 2002; Bhave et al., 2003).

We have concentrated mainly on the functional consequences of binding of AKAP79/150 to TRPV1, but we have also obtained biochemical evidence for interactions of AKAP79 with other members of the thermo-TRP family, in particular TRPV4, TRPM8 and TRPA1 (Fig. 2). The functional consequences of AKAP79 binding for the activation of these channels remains to be explored.

We have identified a 14aa domain in the C-terminal domain of TRPV1 which mediates binding of AKAP79. Previous studies have found that AKAP79 binds diffusely to other proteins (Colledge et al., 2000), so the identification of a small binding domain is something of a surprise. We did not find any obviously homologous binding domains on other thermo-TRPs, apart from an area of weak homology between residues 705 and 724 of the C-terminal domain of TRPA1, which suggests that AKAP79/150 binds to other thermo-TRP proteins at non-homologous domains. A second possibility is that different intermediary proteins are involved in the binding to other thermo-TRPs, as was found for the binding of AKAP79/150 to the NMDA and AMPA receptors (Colledge et al., 2000).

The dependence of sensitization of TRPV1 on AKAP79/150 raises the possibility that disrupting binding may reverse heat hyperalgesia *in vivo*. The binding site of AKAP79/150 to TRPV1 may therefore prove to be an attractive target for the development of novel analgesics.

Experimental Procedures

Methods were as described previously (Zhang et al., 2005) with the following exceptions. Binding site deletions in AKAP79 for PKC, PKA and PP2B were performed using overlapping PCR to remove regions identified by Hoshi et al (2005). AKAP79 and binding site deletion mutants were GFP-coupled so that successfully transfected cells could be identified for whole cell patch clamp experiments. AKAP79 and AKAP150 were downregulated by using siRNA constructs (Hoshi et al., 2005) but cloned into the GFP-expressing vector GFP-pGSH1 (Gelantis Inc) so that transfected cells could be identified from their GFP fluorescence for patch-clamp studies. DRG neurons were transfected using the Amaxa Nucleofector according to the manufacturer's instructions. A peptide blocking the binding site between AKAP79 and TRPV1, and scrambled control peptide (see Fig. 2), were obtained from Sigma purified to 75% purity.

Whole-cell patch clamp recordings were carried out at a holding potential of -60mV and at room temperature, as described previously (Zhang et al., 2005), except for experiments in which activation of TRPV1 was explored as a function of membrane voltage (Fig 3), when the holding potential was 0mV. External solution for DRG neuron experiments contained 140mM NaCl, 4mM KCl, 10mM HEPES, 1.8mM $CaCl_2$, 1mM $MgCl_2$ and 5mM Glucose, pH 7.4, while for experiments with HEK293 cells (with the exception of those shown in Fig. 6) $CaCl_2$ was replaced with 5mM EGTA to inhibit desensitization. For recording maximal current density (Fig. 7C), calcium free solution was used and $[Na]_o$ was reduced to30mM (substituted with choline chloride) in order to reduce the maximum membrane current amplitude, as described previously (Zhang et al., 2005). For experiments using inhibitory peptide DRG neurons were dialysed with peptide-containing intracellular solution for 10 minutes before starting recording.

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Experimental procedures are described in more detail in Supplementary Material.

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Figure legends

Fig.1. AKAP150 is co-expressed with TRPV1 in sensory neurons

A. TRPV1 (left) and AKAP150 (centre) co-express in small (<25μm dia) rat DRG neurons (merged image at right). The majority of TRPV1 is present in subcellular vesicles (Zhang et al., 2005). 91.1 ± 1.7% of TRPV1-positive neurons express AKAP150 (n=352). Scale bar 10μm.

B. Immunoprecipitating TRPV1 from rat DRG neurons co-immunoprecipitates AKAP150 (upper blot, right lane). A control experiment using rat IgG does not immunoprecipitate either TRPV1 or AKAP150.

C. AKAP150 can be downregulated in rat DRG neurons by the use of an siRNA against AKAP150. The vector chosen for the siRNA expression also co-expressed GFP so that successfully transfected neurons could be identified (left column). A scrambled siRNA had no effect on AKAP150 levels in successfully transfected neurons (top) but the siRNA against AKAP150 downregulated expression (middle; mean 12.1 ± 1.9% of untransfected neurons in same images, n=6). Lower pair of images compares the effect of siRNA in adjacent transfected and untransfected neurons. Scale bar 20μm.

D. Similar experiment in which AKAP79 in HEK293 cells was downregulated to a mean value of 15.9 ± 2.6% of control (n=22) by the use of an siRNA against AKAP79. AKAP79 and siRNA against AKAP79 were simultaneously co-expressed. Scale bar 10μm.

E. Western blot showing downregulation of AKAP79 in HEK293 cells by siRNA and lack of effect of scrambled siRNA. Mean downregulation was to 30.4 ± 2.5% of control (n=3), but note that this includes some untransfected cells (c. 20% of total). Bottom image shows same blot stripped and reprobed for β tubulin to check similar loading in each lane.

Fig. 2. AKAP79 binds to a region in the TRPV1 C terminal domain.

A. Members of the thermo-TRP family of ion channels co-immunoprecipitate AKAP79. TRP proteins were immuno-precipitated with an antibody to a V5 tag fused to the

TRP C-termini and association of co-transfected AKAP79 was probed as shown in top blot. Expression levels of TRP proteins were similar in all cases (middle blot, which is top blot stripped and reprobed), apart from the first lane in which no TRP was transfected. Note the double principal bands for most TRPs, due to the presence of glycosylated (mature) and non-glycosylated (immature) protein (Jahnel et al., 2001). The V5 antibody does not cross-react with any endogenous HEK293 cell proteins (see left lane, in which no TRP was transfected), and fainter bands of lower M.Wt. are due the presence of truncated versions of the V5-tagged TRPs. Lower blot of total cell lysate (TCL) shows that AKAP79 expression was similar in all lanes.

B. Converse experiment to that shown in A; AKAP79 was immunoprecipitated and association with the TRP proteins shown at top was probed with the V5 antibody. Association of TRP proteins with AKAP79 in both A and B was in the order TRPV1≈TRPV4>TRPA1≈TRPM8 >TRPV2≈TRV3. Note that endogenous AKAP79 expressed in HEK293 cells (left lane, middle panel) precipitates TRPV1, though to a lesser extent, even in the absence of transfected AKAP79 (see top panel, weak band in left lane).

C. Co-ip of TRPV1 and AKAP79 demonstrated without use of the V5 antibody. Immunoprecipitation with a TRPV1 antibody raised to the N-terminal (right lane) co-ips AKAP79.

D. GST-coupled fragments of the hTRPV1 N-terminal (residues 1-433), intracellular loop 1 (498-513), intracellular loop 2 (557-579) and C-terminal (681-839) were expressed in E.coli, purified and tested for their ability to co-ip AKAP79. Note that all residue numbering is for the hTRPV1 used in these experiments. Bottom image, from an independent run, shows Coomassie stain of similar gel to demonstrate that expression levels of GST-coupled constructs were similar in all lanes. Principal band

in each lane is the GST-coupled construct; other bands are bacterial proteins incompletely removed during purification.

E. GST-coupled fragments of the hTRPV1 C-terminal domain (681-839) were truncated as shown (left) or internal regions were deleted (right) and were examined for ability to co-immunoprecipitate AKAP79. Bottom image at left shows GST-coupled fragments stained with Coomassie in an independent run; bottom image at right shows blot above stripped and reprobed for GST to demonstrate similar loading in all lanes.

F. A 14aa peptide of sequence KDDYRWCFRVDEVN, corresponding to residues 736-749 of hTRPV1, displaces binding between AKAP79 and TRPV1 GST-coupled C-terminal. A peptide with the same composition but with scrambled sequence VCEYDVFDRNDKWR is ineffective.

G. Inhibitory peptide (see F) displaces binding between intact TRPV1 and AKAP79, while scrambled peptide is ineffective. Peptide added at stated final concentrations directly to lysate of HEK293 cells transfected with TRPV1 and AKAP79.

Fig. 3. AKAP79 promotes activation of TRPV1

A. Peak inward current as a function of capsaicin concentration (5s pulse) in HEK293 cells transfected with hTRPV1. Control dose-response relation (black points and curve, $K_{½}$ = 244nM, Hill coefficient 1.82) is sensitized, i.e. displaced to lower capsaicin concentrations with no change in Hill coefficient, by co-transfection with AKAP79 (red points and curve, $K_{½}$ = 92nM), and desensitized by co-transfection with AKAP79 with the PKC binding site deleted (green points and curve, $K_{½}$ = 634nM). Deletion of PKA binding site of AKAP79 (squares, $K_{½}$ = 180nM, curve not shown) reduced sensitivity less than deletion of the PKC binding site, while deletion of the

calcineurin binding site was indistinguishable from WT AKAP79 (blue points, $K_{½}$ = 112nM, curve not shown).

B. Whole-cell voltage-clamp experiments on HEK293 cells. Voltage-clamp pulse protocol shown at left. Other traces show currents in cells transfected with hTRPV1 and with transfected AKAP79 and/or exposed to PMA (1μM) as indicated.

C. Activation of TRPV1 by membrane voltage. Points derived from traces shown in B, as detailed in Methods. Curves are Boltzmann functions best-fitted to experimental points (see Methods).

D. Average values of $V_{½}$ obtained in experiments such as those shown in B and C. Numbers of experiments for each bar are (from left) 14, 13, 43 and 20. Error bars show ± SEM. Significance levels are **, $p<0.01$ and ***, $p<0.001$ with respect to TRPV1 alone; ###, $p<0.001$ with respect to TRPV1+AKAP79.

Fig. 4. AKAP79 mediates sensitization of TRPV1 by bradykinin

A. Whole-cell voltage clamp experiments on small (<25μm dia) rat DRG neurons. From top: bradykinin causes potent sensitisation of inward current activated by a subsaturating pulse of capsaicin (100nM, 5s); inclusion of blocking peptide (200μM) in the patch clamp pipette abolished sensitisation; downregulation of AKAP150 by the use of siRNA largely abolished sensitization; sensitization was rescued by co-transfection of AKAP79, which is insensitive to the siRNA against AKAP150. These experiments were conducted in the presence of 1.8 mM external [Ca^{2+}], so substantial desensitization is observed in response to successive applications of capsaicin following application of Bk. Note that almost all TRPV1-positive neurons also expressed B2 receptors for Bk, as shown from the observation that >90% of naïve neurons responding to capsaicin showed an enhancement of response following

exposure to Bk. The suppression of sensitization by the inhibitory peptide or siRNA cannot therefore be attributed to chance selection of neurons not expressing receptors for Bk.

B. Collected results of experiments similar to those in A. Bars show maximum amplitude of first response following application of Bk relative to last response before application. Bradykinin caused sensitization (bar 2; *, $p<0.05$ compared to control, n=19). Inclusion of peptide abolished sensitization (bar 3; ###, $p<0.001$ compared to bar 2, n=23). Scrambled peptide was ineffective in reversing sensitisation by bradykinin (bar 4; *, $p<0.05$ compared to control, n=13). SiRNA against AKAP150 largely abolished sensitization (bar 5; #, $p<0.05$ compared to bar 2, n=15) but this effect was not seen with scrambled siRNA (bar 6; *, $p<0.05$ compared to control, n=20). Abolition of sensitization following transfection with siRNA could be rescued by co-transfection of AKAP79, which is insensitive to the siRNA against AKAP150 (bar 7; ##, $p<0.01$ compared to bar 2, n=22).

C. Whole-cell voltage clamp experiments on HEK293 cells transfected with TRPV1 and bradykinin B2 receptor. Bradykinin (1μM) caused potent sensitisation of the inward current activated by a subsaturating pulse of capsaicin (20nM, 5s). Sensitization was abolished by co-transfection of AKAP79 with the PKC binding site deleted, which has a dominant negative effect, or by down-regulation of endogenous AKAP79 expression with siRNA. Note that these experiments were conducted in the absence of external Ca^{2+}, which explains why desensitization is absent.

D. Collected results of experiments similar to those in C. Bradykinin caused potent sensitization (bar 2; ***, $p<0.001$ compared to control, n=25). Mutation of either or both target phosphorylation sites S502 and S801 to alanine largely abolished sensitization (bars 3-5, #, $p<0.05$ and ##, $p<0.01$ with respect to bar 2; n=5, 8 and 12).

Overexpressing AKAP79 causes enhanced sensitization (bar 6; #, $p<0.05$ compared to bar 2, n=16). Deleting the PKC binding site on AKAP79 largely abolishes sensitization (bar 7; ##, $p<0.01$ compared to bar 2, n=23). Deleting the PKA binding site is less effective (bar 8; not significant compared to bar 2, n=10). Sensitization is abolished by downregulating expression of AKAP79 by the use of siRNA (bar 9; ###, $p<0.001$ compared to bar 2, n=17).

Fig. 5. Sensitization by PGE_2 is mediated by AKAP79/150

A. PGE_2 causes potent sensitisation of inward current activated by a sub-saturating pulse of capsaicin (100nm, 5s) in small DRG neurons. From top: control neurons; blocking peptide (200μM) included in the patch clamp pipette abolished sensitisation; downregulation of AKAP150 with siRNA largely abolished sensitization; sensitization was rescued by co-transfection of AKAP79. Break in trace is 10min period during which PGE_2 was constantly perfused without application of capsaicin.

B. Collected results of experiments similar to those in A. PGE_2 caused sensitization (bar 2; *, $p<0.05$ compared to control, n= 9). Inclusion of peptide abolished sensitization (bar 3; ##, $p<0.01$ compared to bar 2, n=13). SiRNA against AKAP150 largely abolished sensitization (bar 4; ##, $p<0.01$ compared to bar 2, n=19). Abolition of sensitization following transfection with siRNA could be rescued by co-transfection of AKAP79 (bar 5; **, $p<0.01$ compared to control, n=11).

C. Collected results of whole-cell voltage clamp experiments on HEK293 cells transfected with TRPV1 and G_q-coupled prostaglandin EP1 receptor (see example traces in Supplementary Fig. 2A). PGE_2 (1μM) caused potent sensitization (bar 2; **, $p<0.01$ compared to control, n=27). Overexpressing AKAP79 causes enhanced sensitization (bar 3; ##, $p<0.01$ compared to bar 2, n=5). Deleting the PKA binding

site on AKAP79 has similar effect to WT AKAP79 (bar 4; ##, $p<0.01$ compared to bar 2, n=15). Deleting the PKC binding site abolishes sensitization (bar 5; #, $p<0.05$ compared to bar 2, n=20). Sensitization is abolished by downregulating expression of AKAP79 by the use of siRNA (bar 6; #, $p<0.05$ compared to bar 2, n=13).

D. Similar experiments in HEK293 cells transfected with the G_s-coupled EP_4 receptor (see example traces in Supplementary Fig. 2B). PGE_2 (1μM) caused potent sensitization (bar 2; **, $p<0.01$ compared to control, n=13). Over-expressing AKAP79 does not further enhance sensitization (bar 3; ***, $p<0.001$ compared to control, n=6). Deleting the PKA binding site on AKAP79 abolishes sensitization (bar 4; #, $p<0.05$ compared to bar 2, n=8). Deleting the PKC binding site has no effect on sensitization (bar 5; *, $p<0.05$ compared to control, n=10). Sensitization is abolished by down-regulating expression of AKAP79 by the use of siRNA (bar 6; ##, $p<0.01$ compared to bar 2, n=7).

Fig. 6. AKAP79 partly mediates Ca-dependent desensitization of TRPV1

A. AKAP79 does not mediate the sensitizing effects of NGF. From top: DRG neurons were exposed to near-saturating pulses of capsaicin (5s, 1μM) separated by a 10min interval; application of NGF between pulses (100ng/ml, 10min) enhanced the inward current activated by capsaicin; the enhancement was not affected by inclusion of blocking peptide (200μM) in the patch pipette.

B. Collected results of experiments similar to those in A. *, $p<0.05$; **, $p<0.01$ compared to control (n=12,15,13).

C. Activation of TRPV1 by a near-saturating dose of capsaicin (1μM, 5s) causes strong desensitization to a subsequent capsaicin application (top trace). Desensitization is enhanced by transfection of AKAP79 (middle trace), and is reduced but not abolished

by downregulation of AKAP79 with siRNA (lower trace). Cells selected to have similar currents in response to conditioning application of capsaicin (see D).

D. Collected results of experiments similar to those in C. The magnitude of desensitisation depends on the amplitude of the preceding inward current through TRPV1 (Koplas et al., 1997), so to control for this potential source of variability data were only included from HEK293 cells with initial inward currents between 3.5 and 5.5nA (i.e. current density between 100-200pA/pF). Desensitization is almost entirely Ca-dependent (bar 2; ***, $p<0.001$ compared to bar 1, n=12). Desensitization is enhanced by transfection of AKAP79 (bar 3; *, $p<0.05$ compared to bar 1, n=9) and is reduced but not abolished by downregulating AKAP79 with siRNA (bar 4; *, $p<0.05$ compared to bar 1, n=8). Desenstization with PKA or PKC binding sites deleted is similar to WT AKAP (bars 5, 6; *, $p<0.05$, **, $p<0.01$ compared to bar 1, n=7,5), but desensitization is reduced by deletion of PP2 binding site (bar 7; not significant compared to bar 1, n=5).

E. Similar experiments in DRG neurons. Desensitization is reduced by inclusion of peptide in patch pipette.

F. Collected results of experiments on DRG neurons. Neurons selected with initial currents between 1.0 and 2.0nA. Significance level **, $p<0.01$ compared to first bar, n=19.

Fig. 7. AKAP79/150 promotes trafficking of TRPV1 to the cell surface membrane

A. Surface membrane expression of TRPV1 in HEK293 cells, measured by biotinylation of surface membrane TRPV1 in intact cells (Zhang et al., 2005), is promoted by transfection with AKAP79. The effect of AKAP79 transfection is inhibited by deletion of the PKA or PKC binding sites on AKAP79, but is not affected by deletion

of the PP2B (calcineurin) binding site. Lower image shows parallel experiment in which total TRPV1 was probed in total cell lysate to demonstrate constant loading of all lanes.

B. Mean band density obtained in 3 independent experiments. ***, $p<0.001$ compared to bar 1; ###, $p<0.001$ compared to bar 2.

C. Functional surface membrane expression of TRPV1 in HEK293 cells, measured in whole-cell voltage clamp experiments in HEK293 cells from maximal current elicited by a saturating dose of capsaicin (10μM applied for 1s). Note that in these experiments the very large currents can cause loss of voltage clamp, and experiments were therefore carried out in $[Na]_o$ = 30mM (see Methods). Maximum current amplitude is increased by transfection of AKAP79 and is inhibited by downregulation of AKAP79 using siRNA.

D. Mean results from experiments similar to C. Maximum current density is reduced by downregulation of endogenous AKAP79 using siRNA (bar 2; *, $p<0.05$ compared to bar 1; n=20). AKAP79 transfection enhances maximum current density (bar 3; **, $p<0.01$ compared to bar 1; n=42) but the effect is negated by PKA or PKC site deletions, but not by PP2B site deletion. Bar 4, #, $p<0.05$ compared to bar 3, n= 28; bar 5, #, $p<0.05$ compared to bar 3; bar 4, not significantly different from bar 3, n= 24.

E. Enhancement of TRPV1 trafficking to the membrane by AKAP79 depends on the TRPV1 S502 site. Blots in E-G show surface membrane, obtained by biotinylation of intact HEK293 cells, and total TRPV1 as in A. The enhanced TRPV1 surface expression caused by co-transfection with AKAP79 is abolished by the S502A mutation. Mean band densities from left, relative to major band at top left: 1.0, 1.44, 1.0, 1.03, n=3.

F. Enhancement of TRPV1 trafficking to the membrane by PKA depends on the S502 site. Forskolin (100μM, 30 min) applied as shown to enhance PKA activity. Mean band densities from left, relative to major band at top left: 1.0, 2.11, 1.0, 0.86, n=3.

G. Mutation of the S117 site, which has also been shown to be a target for PKA (Bhave et al., 2002), has no effect on enhancement of translocation of TRPV1 to the membrane caused by overexpression of AKAP79. Final lane shows that translocation caused by overexpression of AKA79 is partially PKA-dependent as it is inhibited by the PKA inhibitor H89 (1μM for 30 minutes). Mean band densities from left, relative to major band at top left: 1.0, 1.66, 1.85, 1.01, n=2.

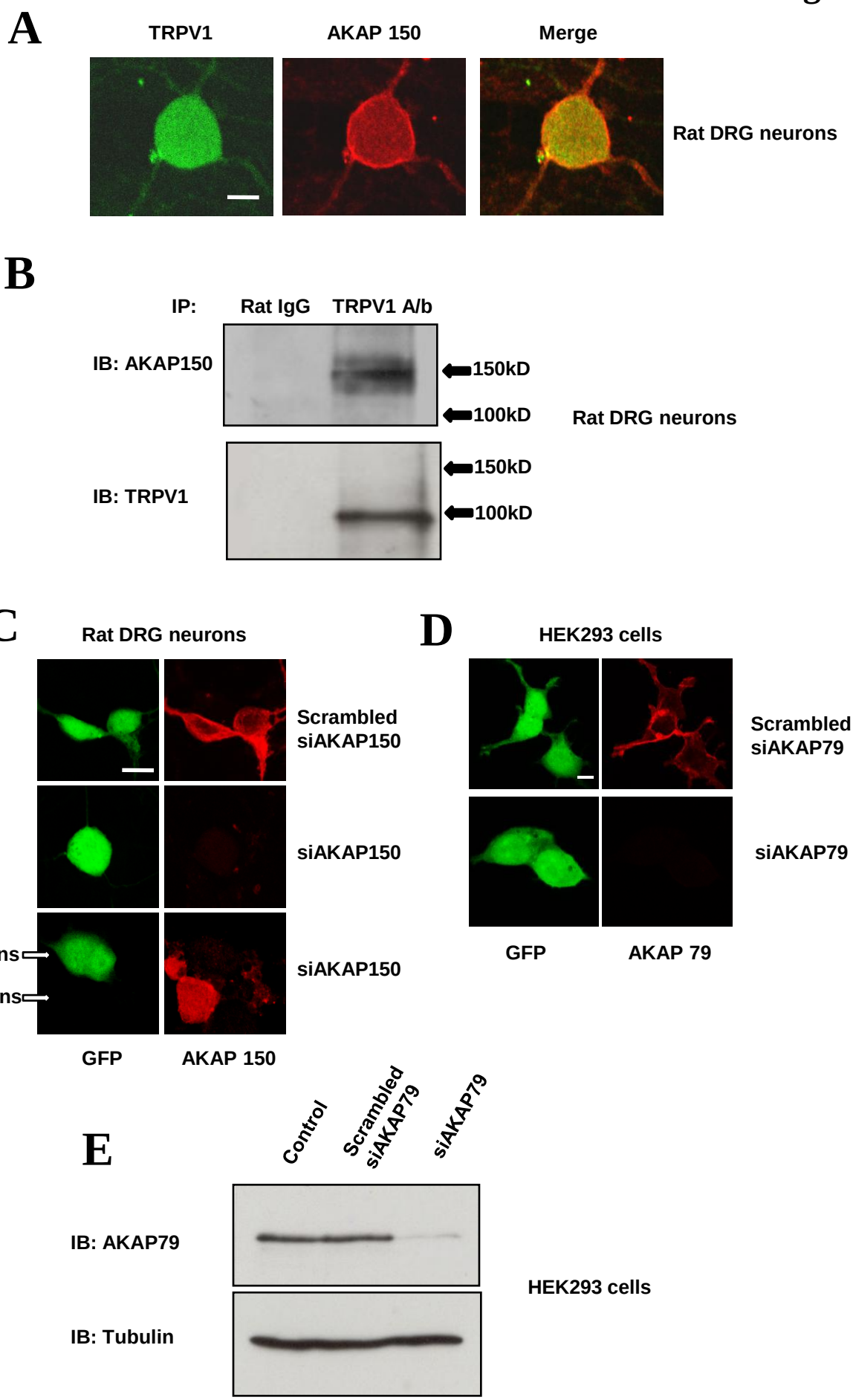
H. Surface membrane expression of TRPV1 in HEK293 cells, measured by immunoprecipitation of HA-tagged surface membrane TRPV1 in intact cells (see Methods), is promoted by overexpression of AKAP79. Mean band densities from left, relative to major band at top left: 1.0, 1.4, n=1.

Fig. 8. AKAP79/150 forms a signalling complex with TRPV1

The scaffolding protein AKAP79/150 binds to a 14aa region in the C-terminal domain of the heat-activated ion channel TRPV1 (orange cylinder). The formation of this scaffolding complex aligns PKCε, the phosphatase calcineurin (PP2B) and PKA so as to control phosphorylation of key sites on TRPV1. The principal site involved in control of trafficking of TRPV1 to the membrane is S502. The tyrosine kinase Src can also promote trafficking to the membrane by phosphorylating Y200 (Zhang et al., 2005) but does so independently of AKAP79/150. TRPV1 has six ankyrin repeats in its N-terminal domain (Lishko et al., 2007).

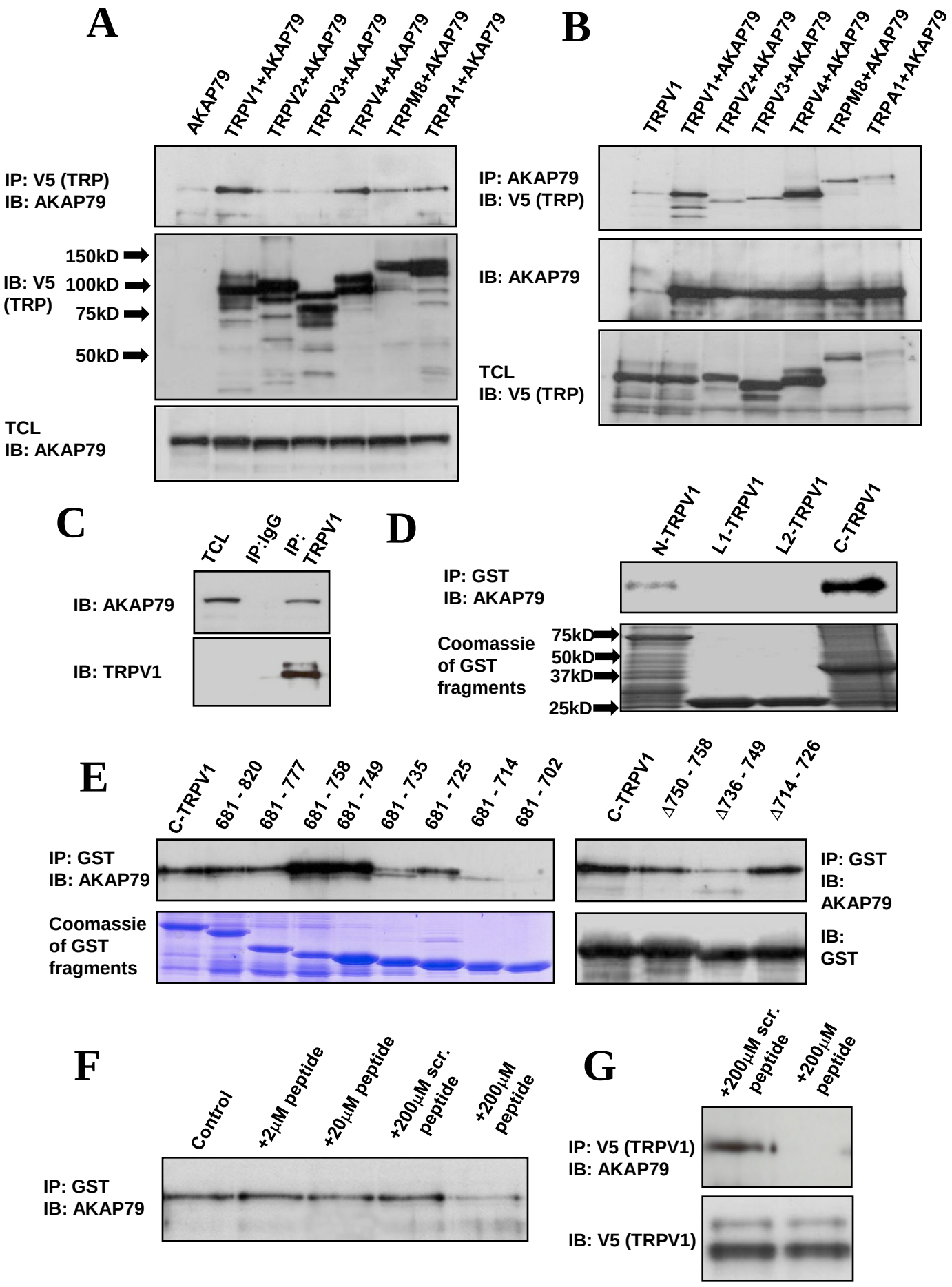
Figure

Fig. 1


A
TRPV1
AKAP 150
Merge
Rat DRG neurons
B
IP:
Rat IgG
TRPV1 A/b
IB: AKAP150
150kD
100kD
Rat DRG neurons
IB: TRPV1
150kD
100kD
C
Rat DRG neurons
Scrambled siAKAP150
siAKAP150
trans
untrans
siAKAP150
GFP
AKAP 150
D
HEK293 cells
Scrambled siAKAP79
siAKAP79
GFP
AKAP 79
E
Control
Scrambled siAKAP79
siAKAP79
IB: AKAP79
IB: Tubulin
HEK293 cells

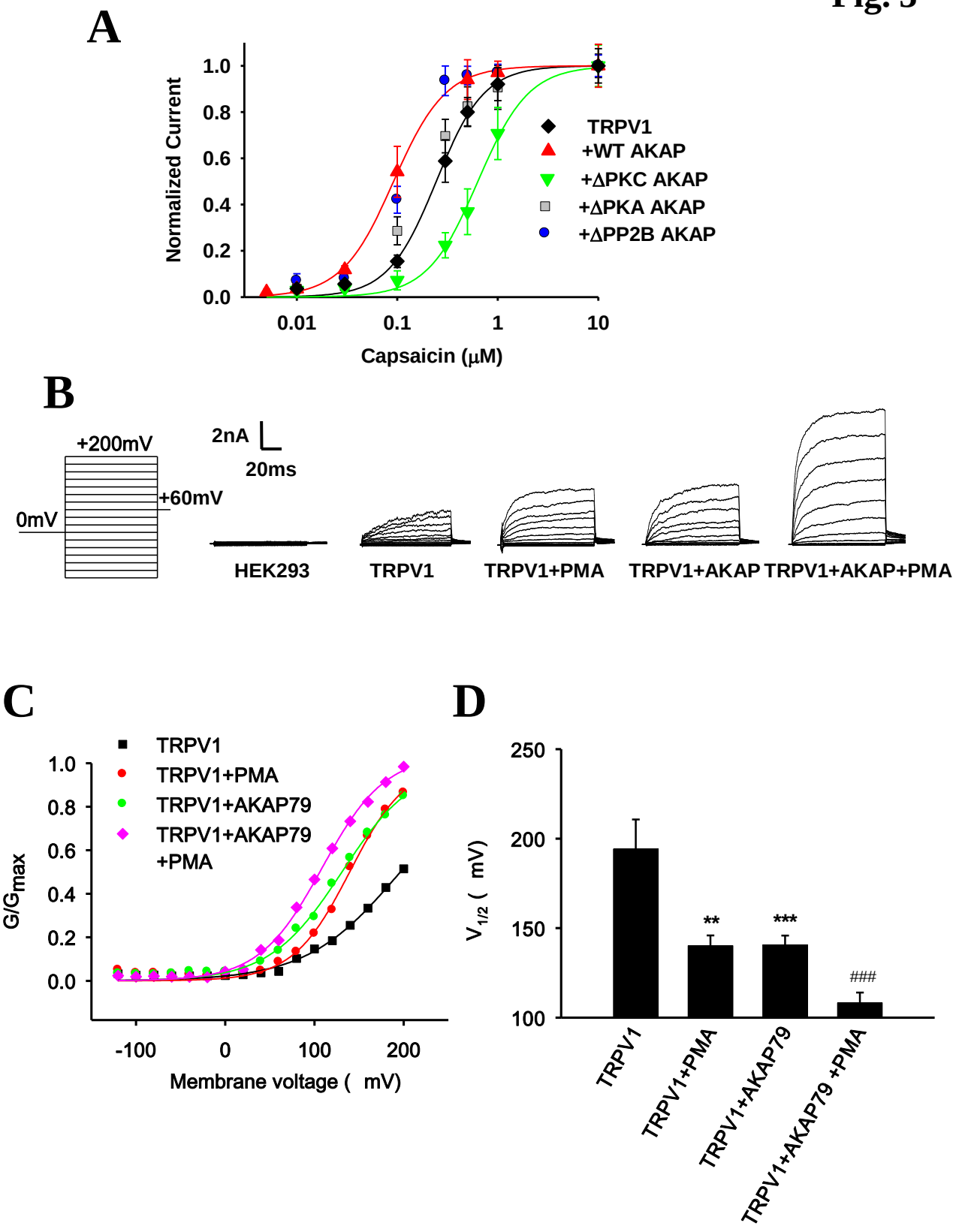
Figure

Fig. 2


A
AKAP79
TRPV1+AKAP79
TRPV2+AKAP79
TRPV3+AKAP79
TRPV4+AKAP79
TRPM8+AKAP79
TRPA1+AKAP79
IP: V5 (TRP)
IB: AKAP79
IB: V5 (TRP)
150kD
100kD
75kD
50kD
TCL
IB: AKAP79
B
TRPV1
TRPV1+AKAP79
TRPV2+AKAP79
TRPV3+AKAP79
TRPV4+AKAP79
TRPM8+AKAP79
TRPA1+AKAP79
IP: AKAP79
IB: V5 (TRP)
IB: AKAP79
TCL
IB: V5 (TRP)
C
TCL
IP:IgG
IP: TRPV1
IB: AKAP79
IB: TRPV1
D
N-TRPV1
L1-TRPV1
L2-TRPV1
C-TRPV1
IP: GST
IB: AKAP79
Coomassie of GST fragments
75kD
50kD
37kD
25kD
E
C-TRPV1
681 - 820
681 - 777
681 - 758
681 - 749
681 - 735
681 - 725
681 - 714
681 - 702
IP: GST
IB: AKAP79
Coomassie of GST fragments
C-TRPV1
Δ750 - 758
Δ736 - 749
Δ714 - 726
IP: GST
IB: AKAP79
IB: GST
F
Control
+2μM peptide
+20μM peptide
+200μM scr. peptide
+200μM peptide
IP: GST
IB: AKAP79
G
+200μM scr. peptide
+200μM peptide
IP: V5 (TRPV1)
IB: AKAP79
IB: V5 (TRPV1)

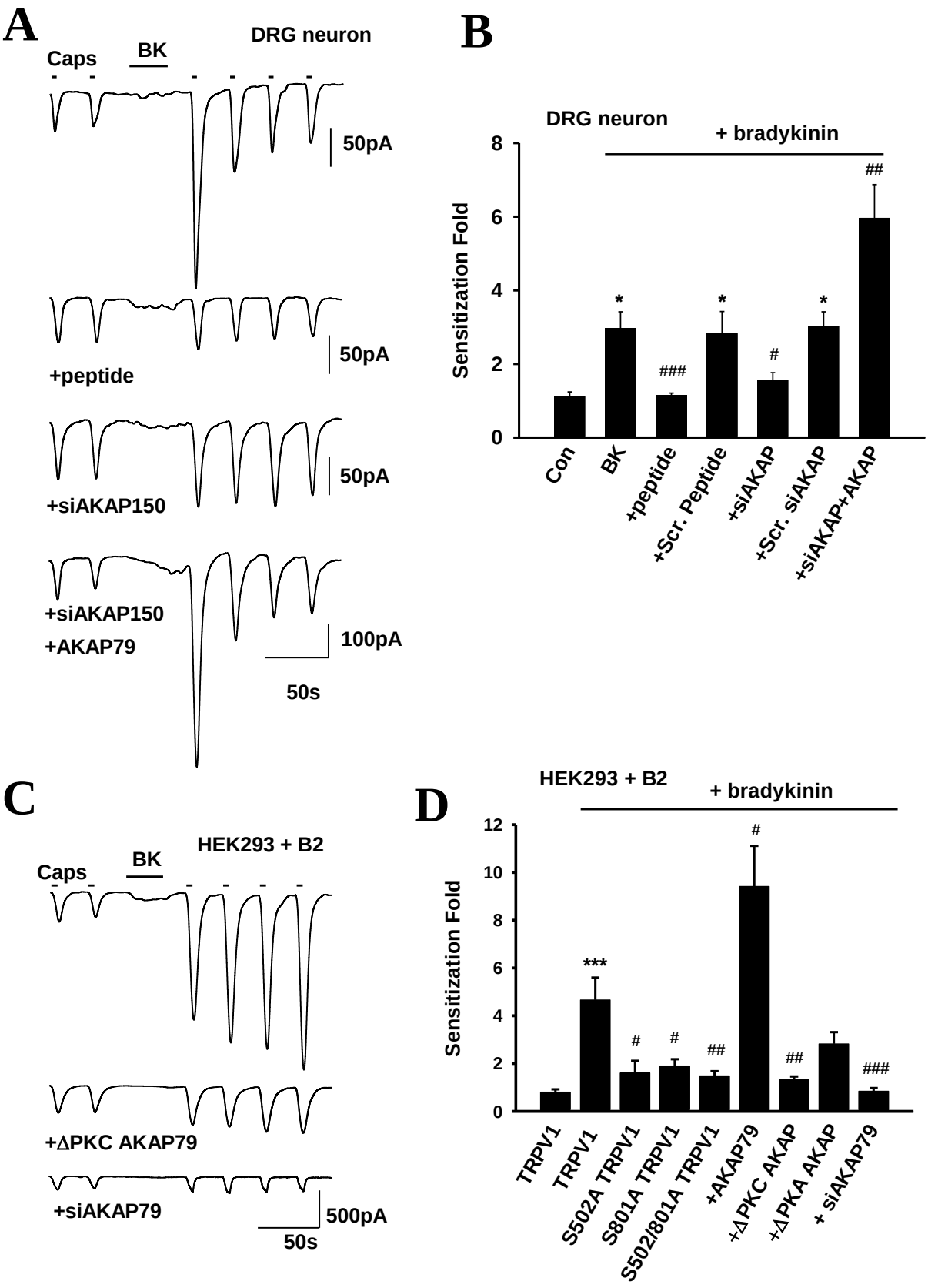
Figure

Fig. 3


A
Normalized Current
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
Capsaicin (μM)
TRPV1
+WT AKAP
+ΔPKC AKAP
+ΔPKA AKAP
+ΔPP2B AKAP
B
+200mV
0mV
+60mV
2nA
20ms
HEK293
TRPV1
TRPV1+PMA
TRPV1+AKAP
TRPV1+AKAP+PMA
C
G/Gmax
TRPV1
TRPV1+PMA
TRPV1+AKAP79
TRPV1+AKAP79 +PMA
-100
0
100
200
Membrane voltage (mV)
D
V1/2 (mV)
250
200
150
100
**
***
###
TRPV1
TRPV1+PMA
TRPV1+AKAP79
TRPV1+AKAP79 +PMA

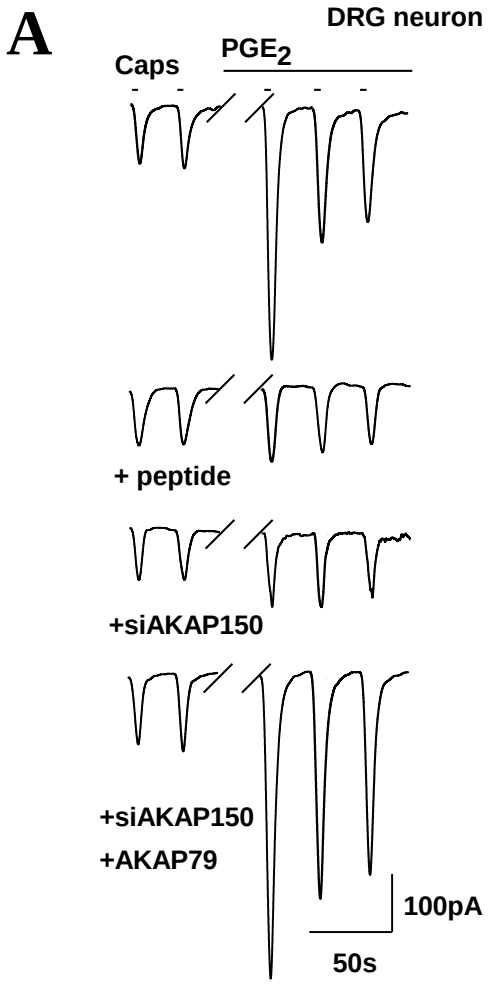
Figure

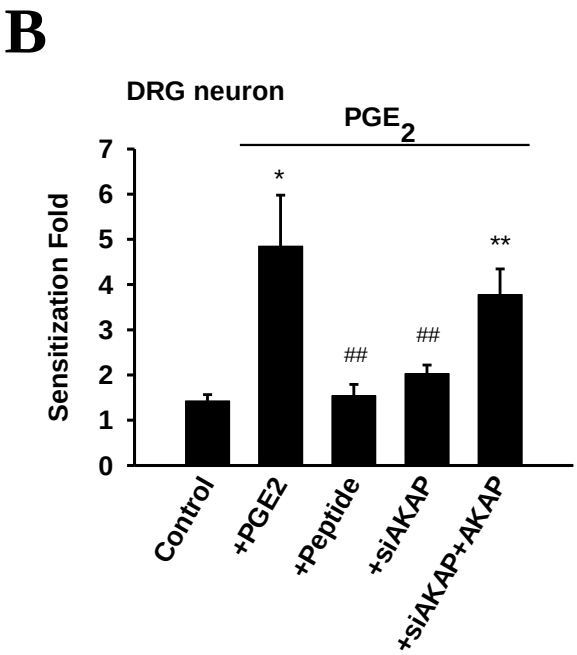
Fig. 4

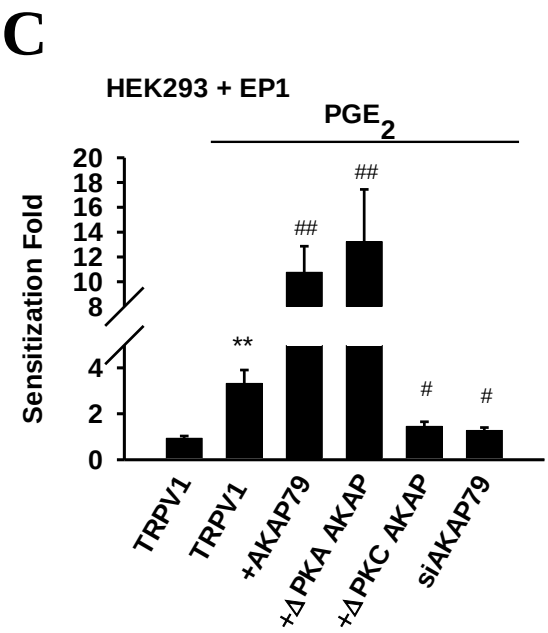

A
DRG neuron
Caps
BK
50pA
50pA
+peptide
50pA
+siAKAP150
+siAKAP150
+AKAP79
100pA
50s
B
DRG neuron
+ bradykinin
Sensitization Fold
Con
BK
+peptide
+Scr. Peptide
+siAKAP
+Scr. siAKAP
+siAKAP+AKAP
C
HEK293 + B2
Caps
BK
+ΔPKC AKAP79
+siAKAP79
500pA
50s
D
HEK293 + B2
+ bradykinin
Sensitization Fold
TRPV1
TRPV1
S502A TRPV1
S801A TRPV1
S502I801A TRPV1
+AKAP79
+ΔPKC AKAP
+ΔPKA AKAP
+ siAKAP79

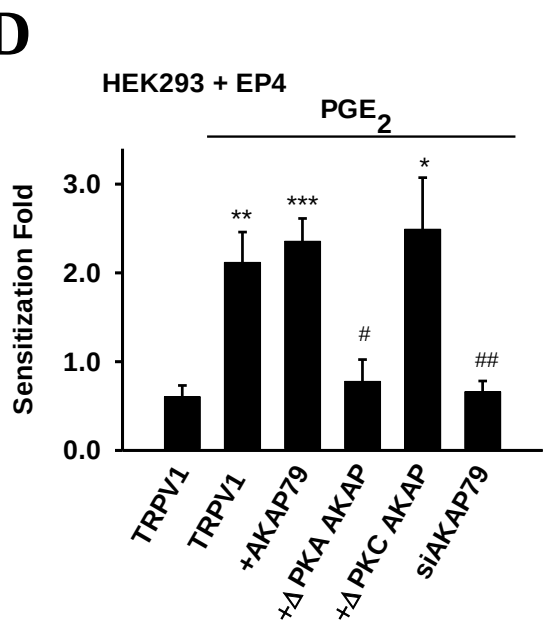
Figure

Fig. 5


A
DRG neuron
Caps
PGE2
+ peptide
+siAKAP150
+siAKAP150
+AKAP79
100pA
50s

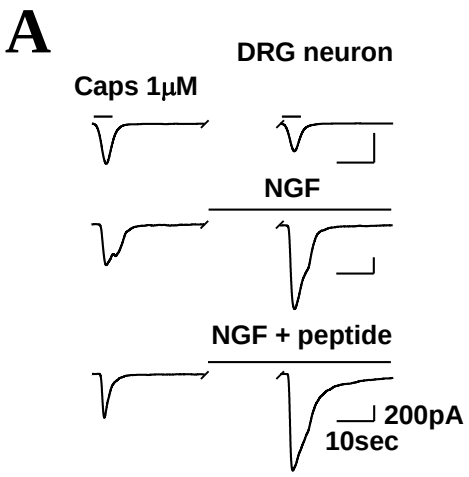

B
DRG neuron
PGE2
Sensitization Fold
0
1
2
3
4
5
6
7
*
**
##
##
Control
+PGE2
+Peptide
+siAKAP
+siAKAP+AKAP

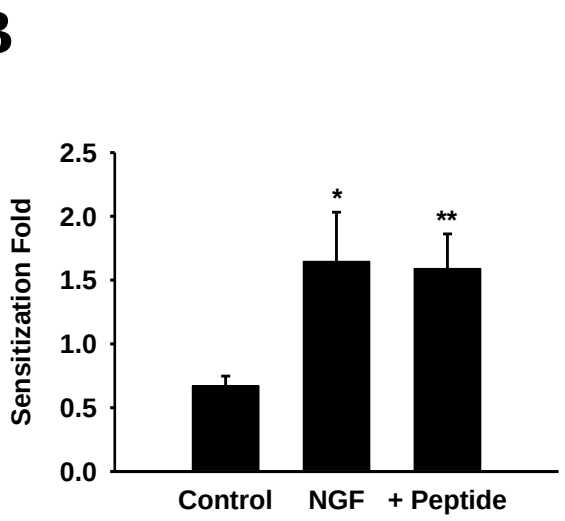

C
HEK293 + EP1
PGE2
Sensitization Fold
0
2
4
8
10
12
14
16
18
20
**
##
##
#
#
TRPV1
TRPV1
+AKAP79
+Δ PKA AKAP
+Δ PKC AKAP
siAKAP79

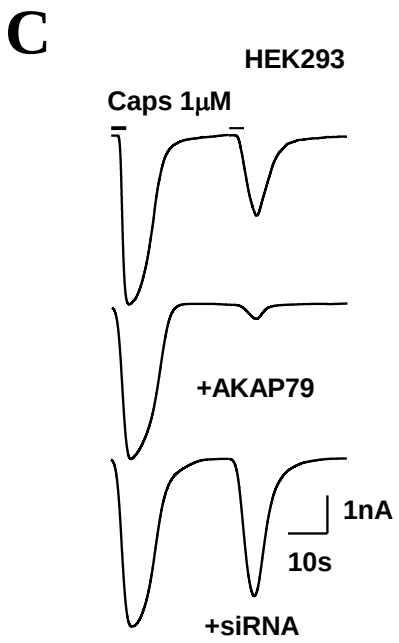

D
HEK293 + EP4
PGE2
Sensitization Fold
0.0
1.0
2.0
3.0
**
***
#
*
##
TRPV1
TRPV1
+AKAP79
+Δ PKA AKAP
+Δ PKC AKAP
siAKAP79

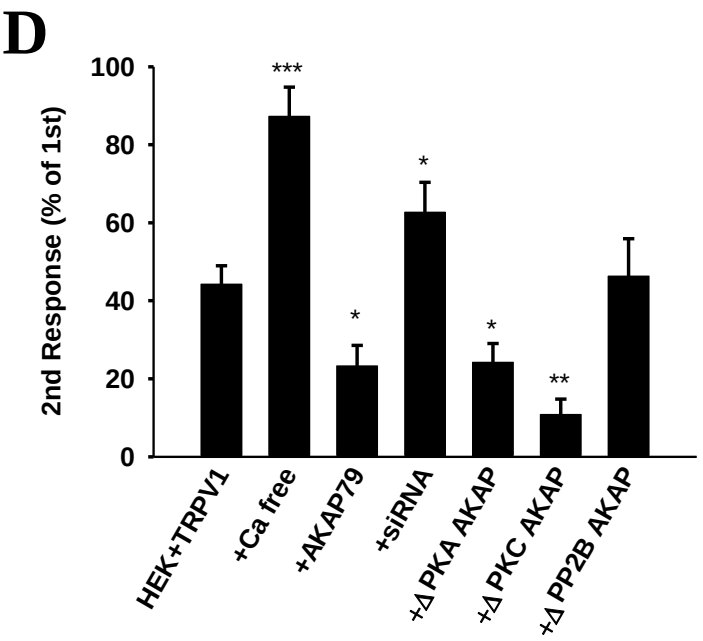
Figure

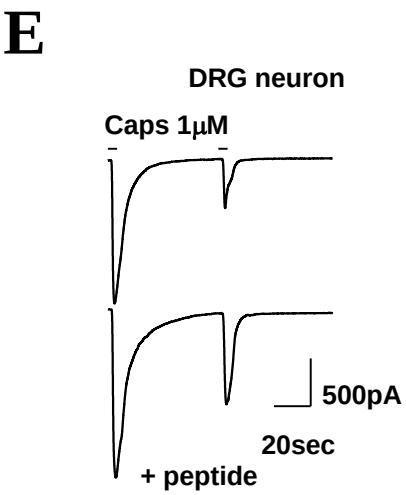
Fig. 6


A
DRG neuron
Caps 1μM
NGF
NGF + peptide
200pA
10sec


B
Sensitization Fold
2.5
2.0
1.5
1.0
0.5
0.0
*
**
Control
NGF
+ Peptide


C
HEK293
Caps 1μM
+AKAP79
1nA
10s
+siRNA


D
2nd Response (% of 1st)
100
80
60
40
20
0
***
*
*
*
**
HEK+TRPV1
+Ca free
+AKAP79
+siRNA
+Δ PKA AKAP
+Δ PKC AKAP
+Δ PP2B AKAP


E
DRG neuron
Caps 1μM
500pA
20sec
+ peptide

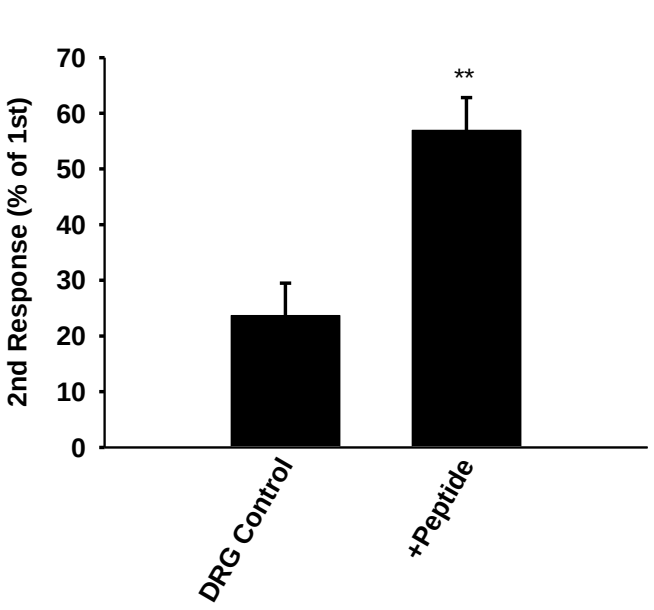
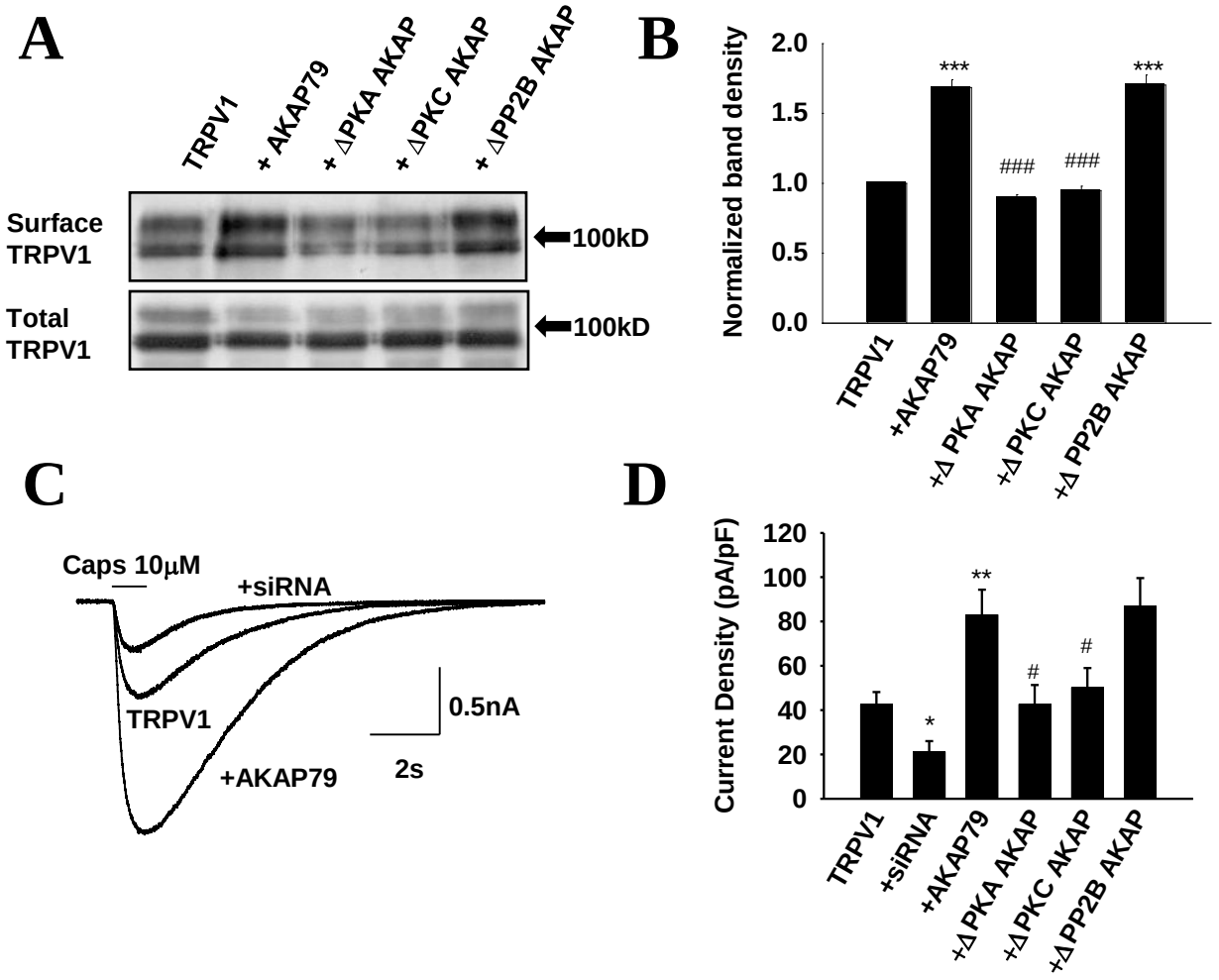

F
2nd Response (% of 1st)
70
60
50
40
30
20
10
0
**
DRG Control
+Peptide

Fig. 7


A
TRPV1
+ AKAP79
+ ΔPKA AKAP
+ ΔPKC AKAP
+ ΔPP2B AKAP
Surface TRPV1
Total TRPV1
100kD
100kD
B
Normalized band density
2.0
1.5
1.0
0.5
0.0
***
***
###
###
TRPV1
+AKAP79
+Δ PKA AKAP
+Δ PKC AKAP
+Δ PP2B AKAP
C
Caps 10μM
+siRNA
TRPV1
+AKAP79
0.5nA
2s
D
Current Density (pA/pF)
120
100
80
60
40
20
0
**
*
#
#
TRPV1
+siRNA
+AKAP79
+Δ PKA AKAP
+Δ PKC AKAP
+Δ PP2B AKAP
E
WT TRPV1
S502A TRPV1
AKAP79 – + – +
Surface TRPV1
Total TRPV1
F
WT TRPV1
S502A TRPV1
Forskolin – + – +
Surface TRPV1
Total TRPV1
G
TRPV1
+ AKAP79
S117A + AKAP79
H89 + AKAP79
Surface TRPV1
Total TRPV1
H
TRPV1
+AKAP79
HA tag: Surface TRPV1
Total TRPV1

Figure

Fig.8