Cao et al., http://www.jgp.org/cgi/content/full/jgp.201311025/DC1

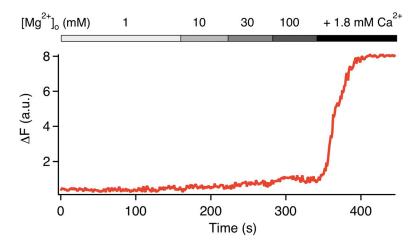
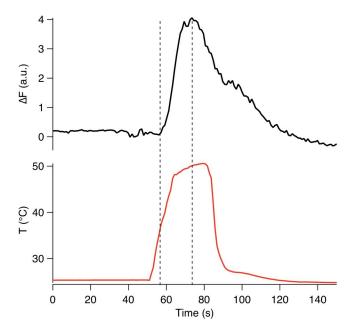


Figure S1. Fluorescence intensity increase upon channel opening was mainly induced by  $Ca^{2+}$ , while  $Mg^{2+}$  could also induce a small fluorescence signal. A TRPV1-expressing cell was loaded with Fluo-4; channel activation was induced by increasing concentration of  $Mg^{2+}$  in the absence of  $Ca^{2+}$ . At the end of the recording, the cell was exposed to a solution containing  $100 \text{ mM } Mg^{2+}$  supplemented with  $1.8 \text{ mM } Ca^{2+}$ . The large increase in fluorescence intensity indicates that the fluorescence increase observed in this study was predominantly from binding of  $Ca^{2+}$  instead of  $Mg^{2+}$  to Fluo-4.



**Figure S2.** Representative time course of the temperature-dependent fluorescence intensity change (top) upon heating (bottom) in a TRPV1-expressing cell. Broken lines indicate the times when the activation threshold temperature (first) and the peak current (second) were reached. Decline of fluorescence intensity started while the temperature remained high.