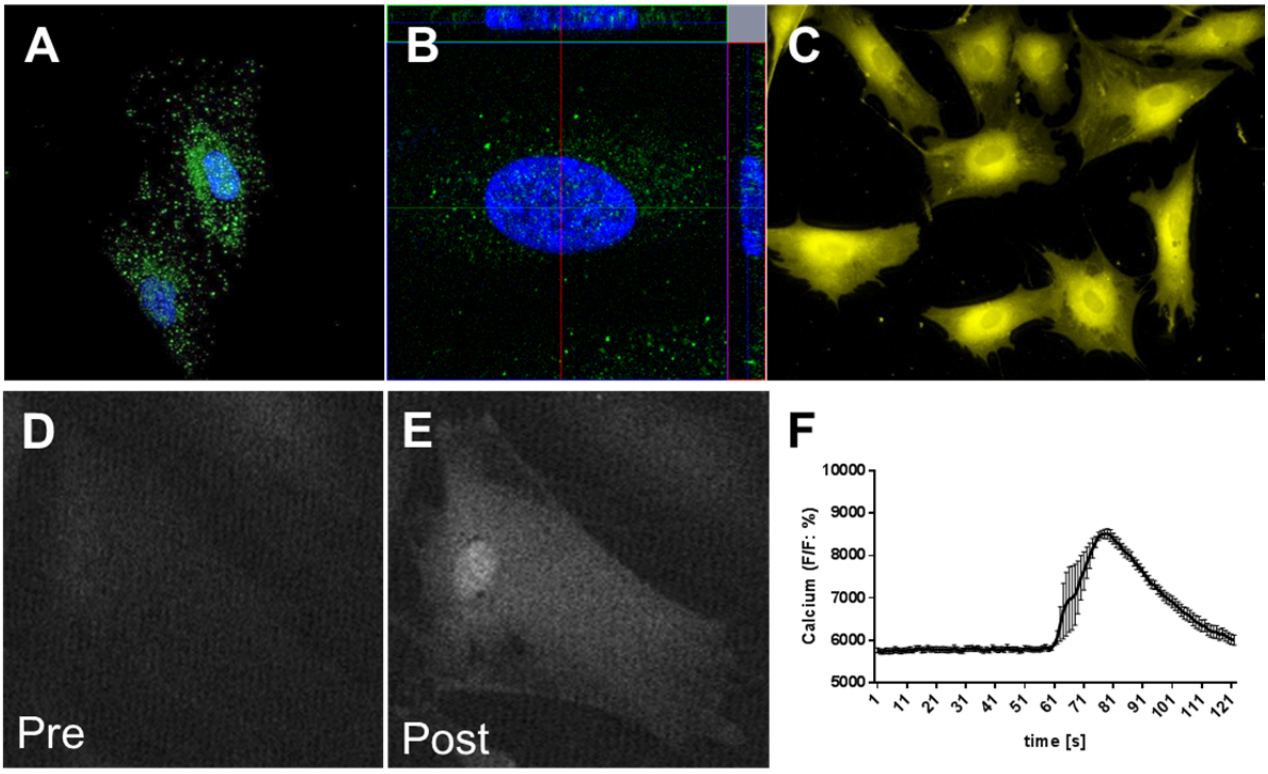
Supplementary Figure 3 Distribution of the neurokinin-1 receptor with substance P application and activation of downstream calcium signaling. Quiescent, uninfected primary human spinal astrocytes (qHA-sps) were treated with 10^{-6} M of subP for 60 minutes, fixed, and analyzed by immunofluorescence using an antibody directed against neurokinin-1 receptor (NK-1R). NK-1R was seen in cytoplasm, predominantly around the nucleus, as expected (*A*, green); a small amount of NK-1R was seen in the nucleus on a z-stack image (*B*, green). Additionally, subP treatment failed to induce lamellipodia; qHA-sps retained their polygonal morphology as visualized by CellTracker Deep Red (*C*, yellow). Calcium imaging of HA-sps before and after application of 10^{-6} M subP demonstrates functional NK-1R downstream signaling, with increased intracellular calcium (*E*, white color) after the addition of subP compared to the absence of intracellular calcium in the same cell pretreatment (*D*). Quantified fluorescence intensity traces showed a characteristic calcium spike after subP application (*F*). Blue coloring indicates cell nuclei. Mag 400X (*A, C, D, E*) and 600X (*B*).


A
B
C
D
Pre
E
Post
F
Calcium (F/F: %)
10000
9000
8000
7000
6000
5000
1
11
21
31
41
51
61
71
81
91
101
111
121
time [s]