G Protein–Coupled Receptor Kinase 6 Acts as a Critical Regulator of Cytokine-Induced Hyperalgesia by Promoting Phosphatidylinositol 3-Kinase and Inhibiting p38 Signaling

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Supplemental Data

Supplementary Figure 1. IL-1β-induced inflammatory response in WT and GRK6−/− mice. WT and GRK6−/− mice (n = 4 per group) received an intraplantar injection of IL-1β (1000 pg/5 μl). 24 h after injection (A) COX2, (B) TNF-α and IL-6 mRNA expression in the injected paw was determined. IL-6 mRNA expression was below detection limit. Expression was corrected for GAPDH expression. All data are expressed as mean ± SEM.

Supplementary Figure 2. 6 GRK2, β-arrestin1, and β-arrestin2 mRNA levels in DRGs during chronic neuropathic and inflammatory pain. (A-C) GRK2 (A), β-arrestin1 (B), or β-arrestin2 (C) mRNA expression levels in DRGs innervating the contralateral (-C) or ipsilateral(-I) side of sham-operated (n=4) and L5 SNL (n=5) mice 4 wks after surgery. (D-F) GRK2 (D), β-arrestin1 (E), or β-arrestin2 (F) mRNA expression levels in DRGs of mice 6 d after intraplantar injection of vehicle or carrageenan (n=5). All data are expressed as mean ± SEM. * p<0.05.
Supplementary Figure 3. NGF-induced hyperalgesia in GRK6−/− mice. WT and GRK6−/− mice (n = 4 per group) received an intraplantar injection of NGF (A: 1.25 μg/5 μl; B: 200 ng/5 μl) and the decrease in heat withdrawal latency was determined over time. All data are expressed as mean ± SEM.

Supplementary Figure 4. No role for COX2 in enhanced IL-1β-induced hyperalgesia in GRK6−/− mice. The COX2 inhibitor indomethacin (5 mg/kg) was injected intraperitoneally (i.p.) 30 min prior and 12 h after IL-1β injection (1). WT and GRK6−/− mice received a single intrathecal injection of IL-1β (1 ng) and heat withdrawal latencies were determined over time. n=6 per group.


Supplementary Figure 5. Role of TRPV1 in ongoing IL-1β-induced hyperalgesia in GRK6−/− mice. The TRPV1 antagonist capsazepine was injected intraplantarly 30 min. prior to measurement of heat withdrawal latency 24 h after IL-1β (1000 pg/paw) injection in WT and GRK6−/− mice. Thermal hyperalgesia is depicted as mean percentage decrease ± SEM in heat withdrawal latency (n=4-8). **p < 0.01.