Supplemental material

Stockstill et al., https://doi.org/10.1084/jem.20170584

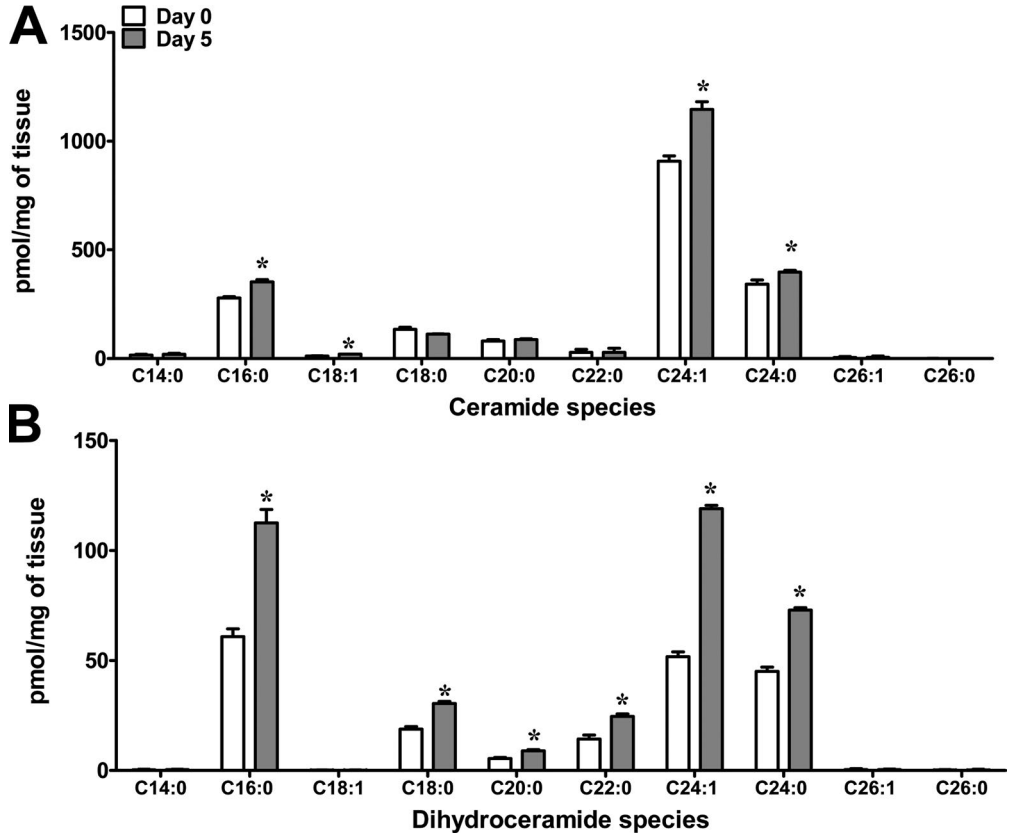

Figure S1. **Bortezomib increases ceramide and dihydroceramide acyl chain species in spinal cord.** Lipids extracted from the DHSC harvested from saline-treated rats (day 0) or bortezomib-treated rats 24 h (day 5) after the last injection of bortezomib were analyzed by LC-ESI-MS/MS (data are representative of two separate experiments). **(A and B)** Spinal levels of individual ceramide (A) and dihydroceramide (B) species are shown. Data are mean ± SD for n = 4 per group; ***,** $P < 0.05$ versus day 0 analyzed by Welch's corrected, unpaired, one-tailed Student's t test. False discovery rate was controlled by Benjamini-Hochberg procedure ($q < 0.05$; $q^* = 0.027$).

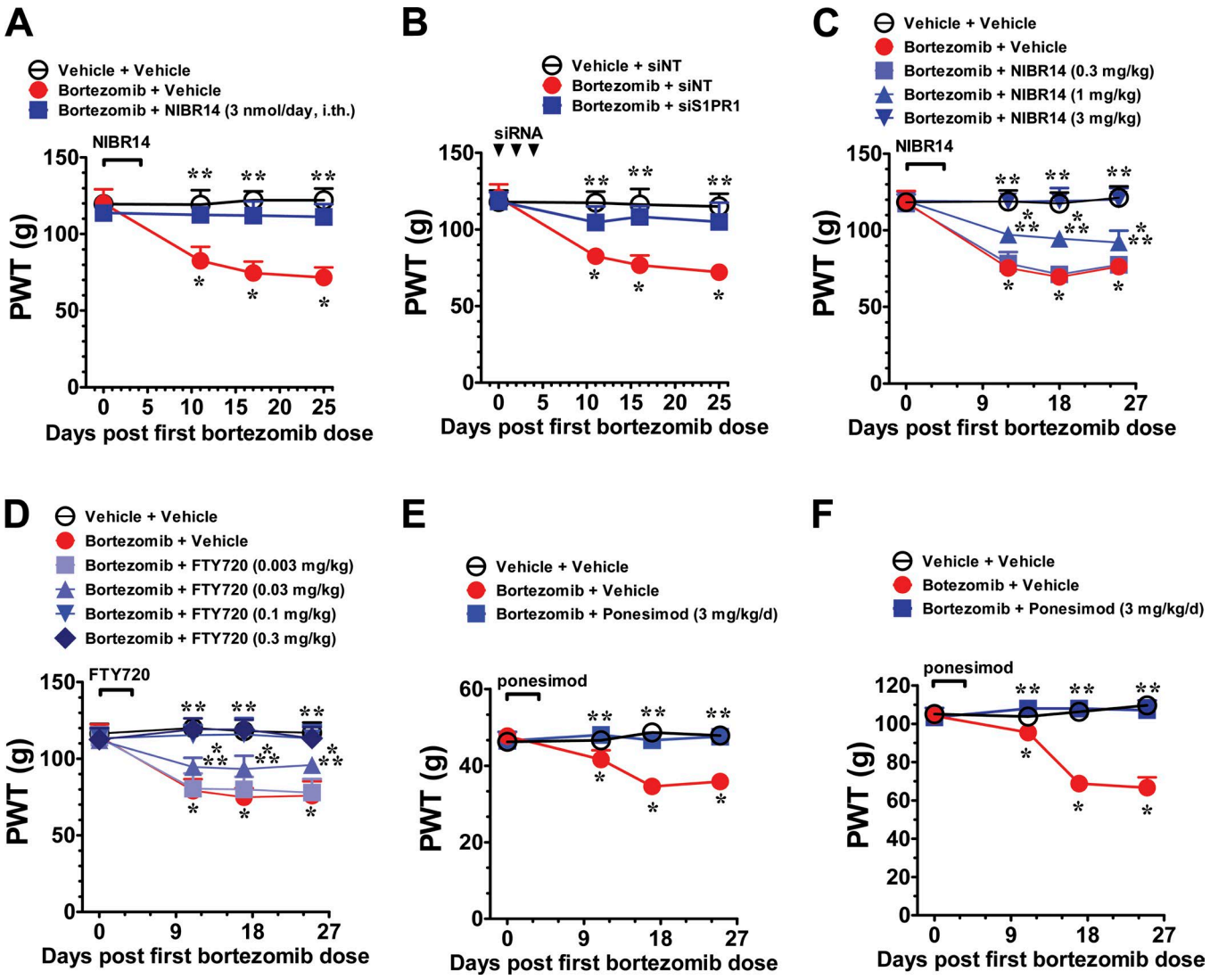

Figure S2. **Targeting S1PR1 prevents bortezomib-induced mechano-hypersensitivity. (A)** Mechano-hyperalgesia was measured in rats treated on days 0–4 with vehicle controls (n = 6) or bortezomib and concurrent i.th. NIBR14 (3 nmol/d, days 0–4; n = 6) or vehicle (10% DMSO; n = 6). **(B)** Mechano-hyperalgesia was measured in rats treated on days 0–4 with bortezomib and concurrent i.th. *S1pr1*-targeting (siS1PR1) or nontargeting (siNT) DsiRNA (2 µg/d on days 0, 2, and 4; n = 6 per group). Control groups were treated with bortezomib vehicle with siNT (n = 6). Pilot studies showed that i.th. NIBR14 or *S1pr1*-targeting DsiRNA had no effect on PWT in vehicle-treated rats (not depicted). **(C and D)** Mechano-hyperalgesia was measured in rats treated with bortezomib (days 0–4) and concurrent oral NIBR14 (0.3, 1, or 3 mg/kg/d; n = 6 per group; C), oral FTY720 (0.003, 0.03, or 0.1 mg/kg/d, n = 6 per group, or 0.3 mg/kg/d, n = 3, D) or their vehicle (2% DMSO in saline; n = 7, C; or n = 11, D). Control groups were treated with bortezomib vehicle and concurrent test agent vehicle (n = 6, C; or n = 9, D). **(E and F)** Mechano-allodynia (E) and mechano-hyperalgesia (F) were measured in rats treated on days 0–4 with bortezomib and concurrent oral administration (days 0–4) of ponesimod (3 mg/kg/d, n = 6) or its vehicle (n = 6). Control groups were treated with bortezomib vehicle and concurrent test agent vehicle (n = 6). Data are mean ± SD for n animals; *****, $P < 0.05$ versus day 0; ******, $P < 0.05$ versus time-matched bortezomib plus vehicle or bortezomib plus siNT by two-way ANOVA with Holm-Sidak.

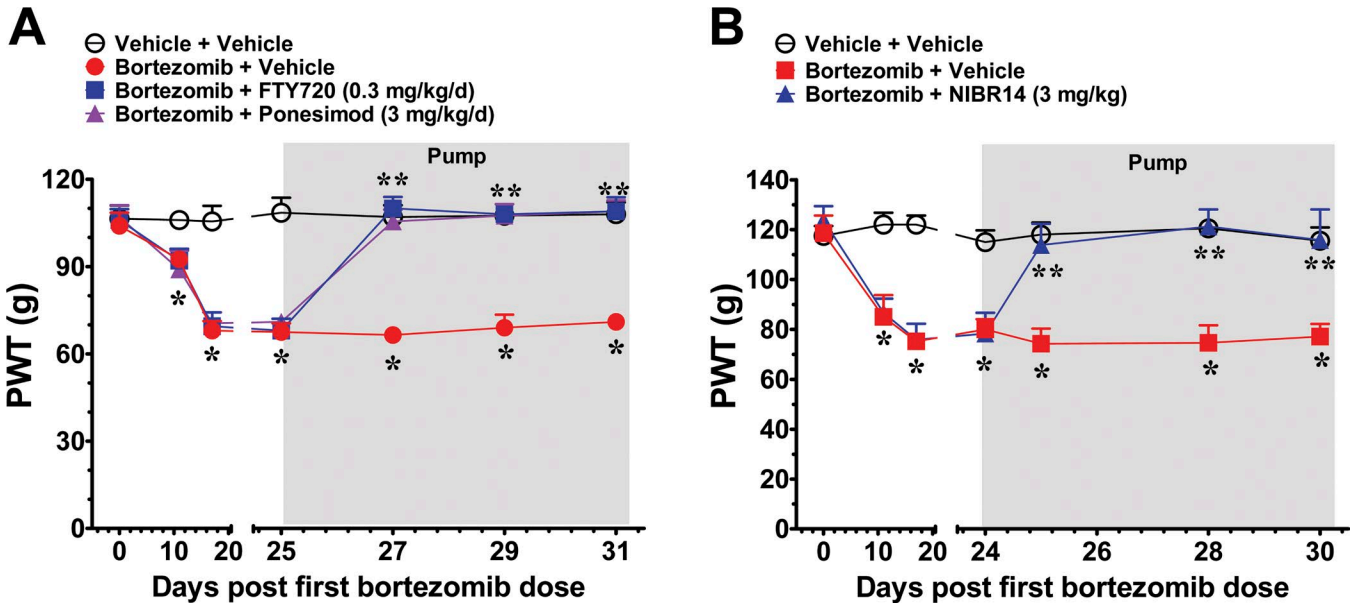

Figure S3. **Targeting S1PR1 reverses bortezomib-induced mechano-hyperalgesia.** On days 24 or 25 after bortezomib, 7-d minipumps were implanted to deliver continuous subcutaneous infusion of vehicle (n = 5, A; or n = 7, B), FTY720 (0.3 mg/kg/d, n = 5, A), ponesimod (3 mg/kg/d, n = 5, B), or NIBR14 (0.3 mg/kg/d, n = 6, B). Control rats were treated with bortezomib and test compound vehicles (3–10% DMSO in saline, n = 5, A and B). Rats were injected immediately after minipump implantation with a respective loading i.p. dose of FTY720 (0.03 mg/kg), ponesimod (3 mg/kg), NIBR14 (3 mg/kg), or vehicle. Data are mean ± SD for n rats; *, $P < 0.05$ versus day 0; **, $P < 0.05$ versus day 24 or 25 by two-way ANOVA with Holm-Sidak.