



SUPPLEMENTARY FIG. S2. Effects of hemin on deep tissue hyperalgesia and BW in HbSS-BERK, HbAA-BERK, TLR4-KO HbSS-BERK, and TLR4-KO HbAA-BERK mice. Animals were treated as described in Supplementary Figure S5A. (A, B) Deep tissue/musculoskeletal hyperalgesia as measured by grip force in HbSS- and HbAA-BERK mice. (C, D) Deep tissue/musculoskeletal hyperalgesia as measured by grip force in TLR4-KO mice. (E, F) BW. Age in months \pm SEM. HbAA-BERK: 4.81 ± 0.06 (Veh, $n=5$); 4.77 ± 0.03 (hemin, $n=6$) and HbSS-BERK: 4.79 ± 0.04 (Veh, $n=5$); 4.75 ± 0.05 (hemin, $n=6$). SS-TLR4-KO (Veh, 5.73 ± 1.04 , $n=5$); SS-TLR4-KO (hemin, 5.86 ± 1.04 , $n=5$); AA-TLR4-KO (Veh, 6.04 ± 0.26 , $n=5$); AA-TLR4-KO (hemin, 5.77 ± 0.26 , $n=5$). Statistical analysis of hemin versus corresponding vehicle at each time-point (two-way ANOVA, Bonferroni) and each time-point versus BL in hemin groups (one-way ANOVA, Bonferroni) showed no significant differences. All data are mean \pm SEM. AA-TLR4-KO: TLR4 knockout HbAA-BERK; SS-TLR4-KO: TLR4 knockout HbSS-BERK. ANOVA, analysis of variance; BL, baseline; BW, body weight; TLR4-KO, TLR4 knockout (genetic deletion); Tx, treatment.