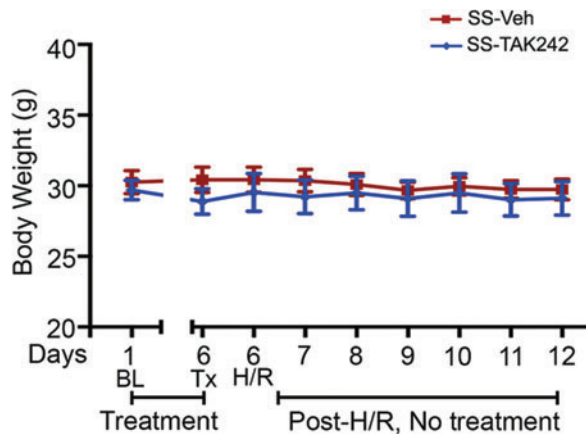


**SUPPLEMENTARY FIG. S3. TLR4 inhibition with TAK242 or knockout of TLR4 reduced hyperalgesia in HbSS-BERK mice.** Animals were treated as described in Supplementary Figure S5B. (A) BW. Mean age of each group of mice in months  $\pm$  SEM: HbAA-BERK (AA-WT,  $5.92 \pm 0.72$ ,  $n=8$ ); HbSS-BERK (SS-WT,  $6.00 \pm 0.88$ ,  $n=8$ ); AA-TLR4-KO ( $5.88 \pm 0.96$ ,  $n=8$ ); SS-TLR4-KO ( $6.02 \pm 0.89$ ,  $n=5$ ). In (B),  $6.04 \pm 0.18$  (HbAA, Veh,  $n=6$ ),  $5.92 \pm 0.33$  (HbAA, TAK242,  $n=6$ ),  $6.07 \pm 0.05$  (HbSS, Veh,  $n=8$ ) and  $6.00 \pm 0.33$  (HbSS, TAK242,  $n=8$ ). (B) Mechanical hyperalgesia assessed by paw withdrawal threshold to a series of mechanical stimuli of increasing force. All data are mean  $\pm$  SEM.  $**p < 0.001$  (Student's unpaired  $t$  test). Statistical analysis of BW for treatment versus corresponding Veh at each time-point (two-way ANOVA, Bonferroni) or each time-point versus BL in each treatment group (one-way ANOVA, Bonferroni) showed no significant differences. AA: HbAA-BERK; AA-TLR4-KO: TLR4 knockout HbAA-BERK; SS: HbSS-BERK; SS-TLR4-KO: TLR4 knockout HbSS-BERK. WT, wild type.



**SUPPLEMENTARY FIG. S4. Pretreatment with TAK242 decreased acute pain in sickle mice.** Animals were treated as described in Supplementary Figure S5C. BW. Mean age of each group is given as months  $\pm$  SEM:  $7.51 \pm 0.04$  ( $n=5$ ) for SS-Veh and  $7.39 \pm 0.07$  ( $n=5$ ) for SS-TAK242. Each value is the mean  $\pm$  SEM. TAK242 versus corresponding vehicle at each time-point (two-way ANOVA, Bonferroni), or each time-point versus BL of corresponding group (one-way ANOVA, Bonferroni), versus Tx pre-H/R of corresponding group (one-way ANOVA, Bonferroni), and versus H/R of corresponding group showed no significant differences. H/R, hypoxia/reoxygenation.