

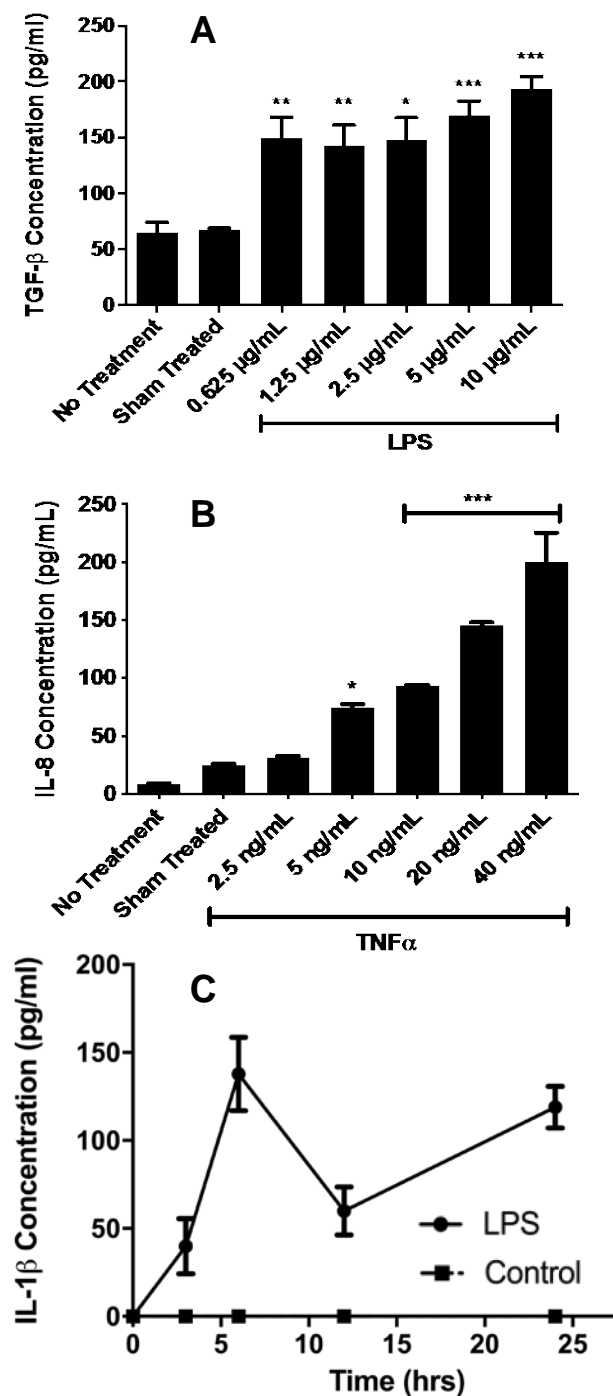
The VR23 anti-tumor compound also shows strong anti-inflammatory effects on a human rheumatoid arthritis cell model and acute lung inflammation in mice

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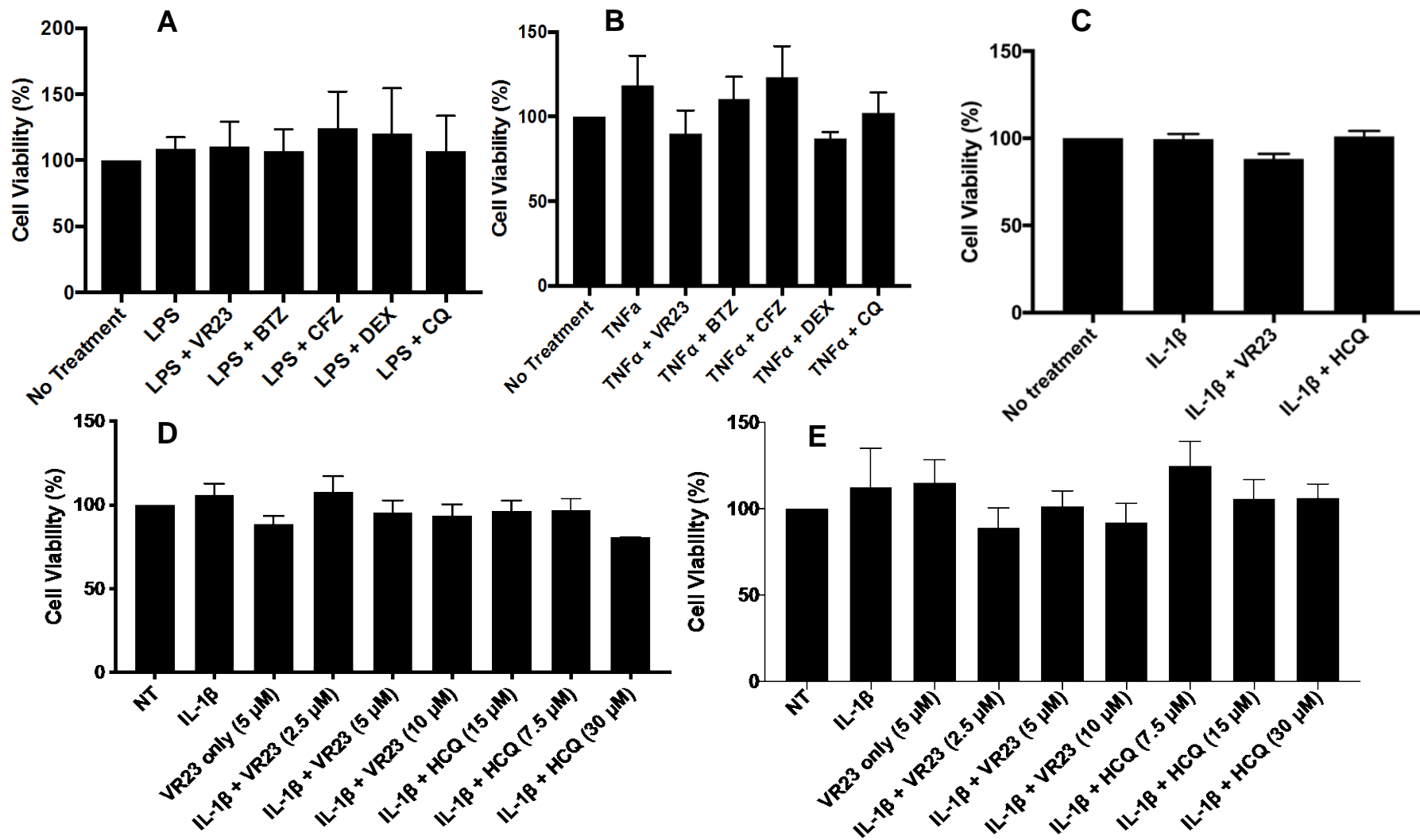
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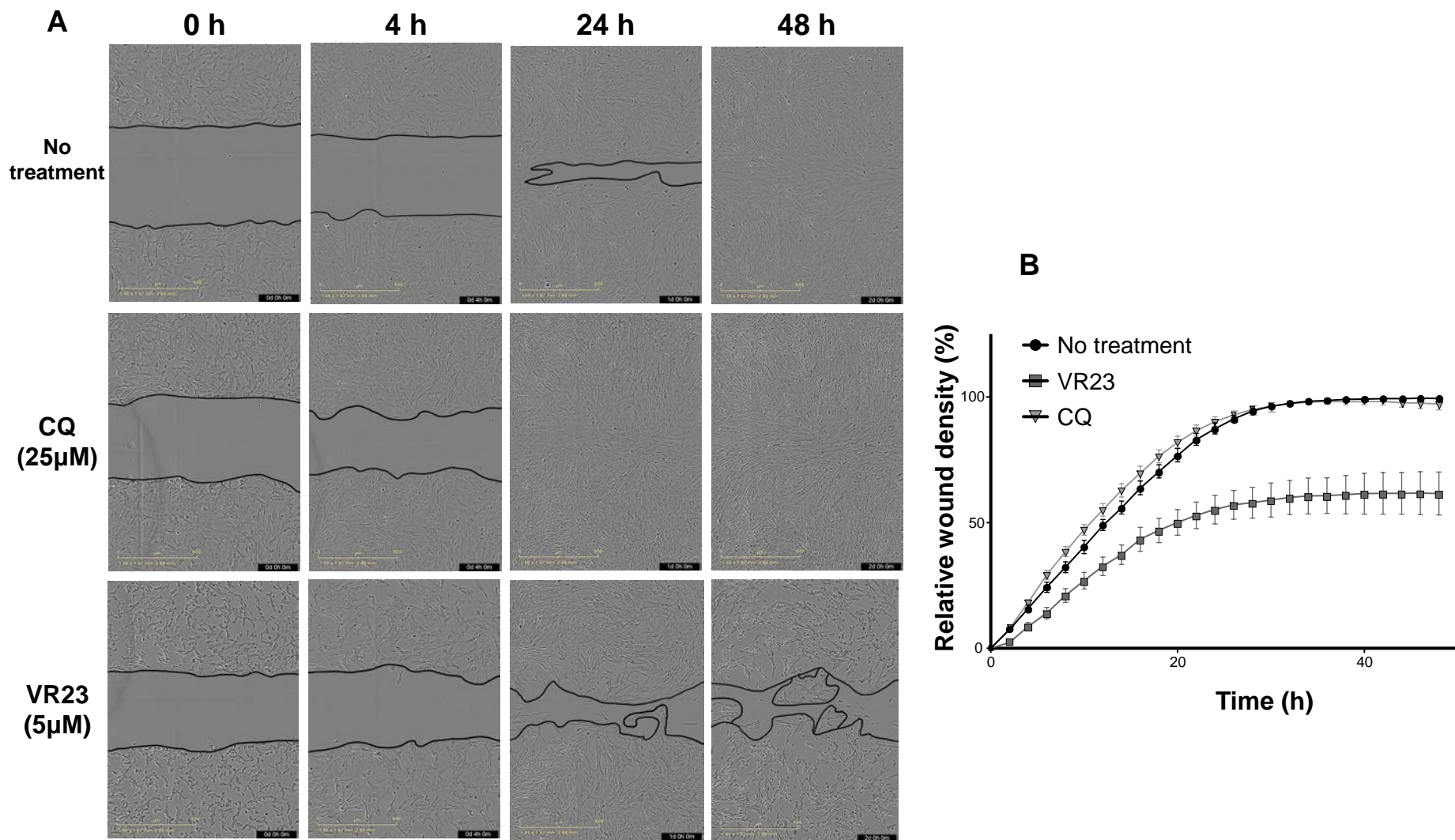
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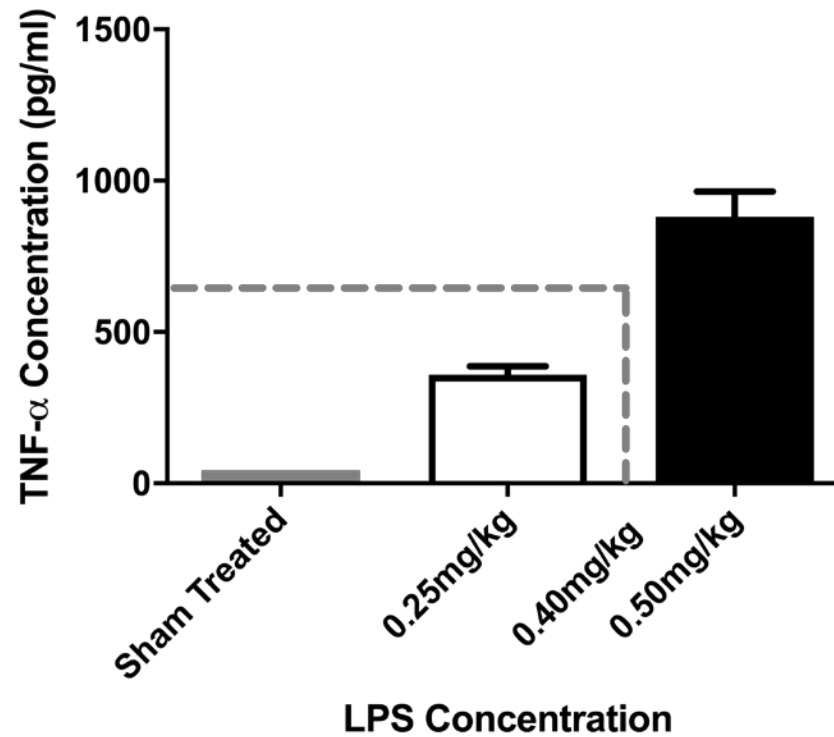
Supplemental Figure S1. The levels of inflammatory cytokines secreted in the presence of LPS or TNF- α . (A) Levels of TGF- β secreted in the supernatant of THP-1 cells stimulated with varying concentrations of LPS. (B) Levels of IL-8 in the supernatant of THP-1 cells stimulated with varying concentrations of TNF- α . (C-F) Levels of IL-1 β (C), TNF- α (D), IL-6 (E) and IL-8 (F) measured in the supernatant of THP-1 cells stimulated with LPS (5 μ g/mL) as the function of time. (G) Levels of IL-8 in the supernatant of THP-1 cells stimulated with TNF- α (10 ng/mL) for different durations. The comparison between groups was made using one-way ANOVA. *, **, and *** are $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, which denote significant differences from the no treatment group, determined by a Dunnett's test. The values presented are mean \pm SEM (n=3).



Supplemental Figure S2. Cell growth and viability are largely unaffected under the experimental conditions employed in this study. (A-D) The viability rates are shown for THP-1 (A,B) or SW982 (C) cells after they were treated with LPS (5 μ g/mL), TNF- α (10 ng/mL), or IL-1 β (1 ng/mL) in the absence or presence of different compounds for 6 h. Results of the viability tests with HFLS-N (D) and HFLS-RA (E) cells (5,000 cells/well) measured at 6-h post-stimulation in the absence (control) or presence of varying concentrations of VR23 or HCQ. For abbreviation, see text. Figures A-C treated with compounds at their relative IC₅₀ values. The comparison between groups was made using one-way ANOVA. * is $p < 0.05$, which denotes a significant difference from the LPS, TNF- α , or IL-1 β only group, determined by a Dunnett's test. The values presented are mean \pm SEM (n=3).



Supplemental Figure S3. The results of the scratch wound assay of SW982 cells. (A) Images were taken using Incucyte S3 Live Cell Analysis System (10 \times) at every 2 h intervals (although shown only four time points). Scratch borders are outlined in black on the images. (B) Relative wound density calculated over 48-h time period.



Supplemental Figure S4. The optimal LPS concentration for the induction of TNF- α in the mouse lung was determined to be 0.4 mg/kg body weight..