

## Supplemental Information

# Hydrogen Attenuates Inflammation by Inducing Early M2 Macrophage Polarization in Skin Wound Healing

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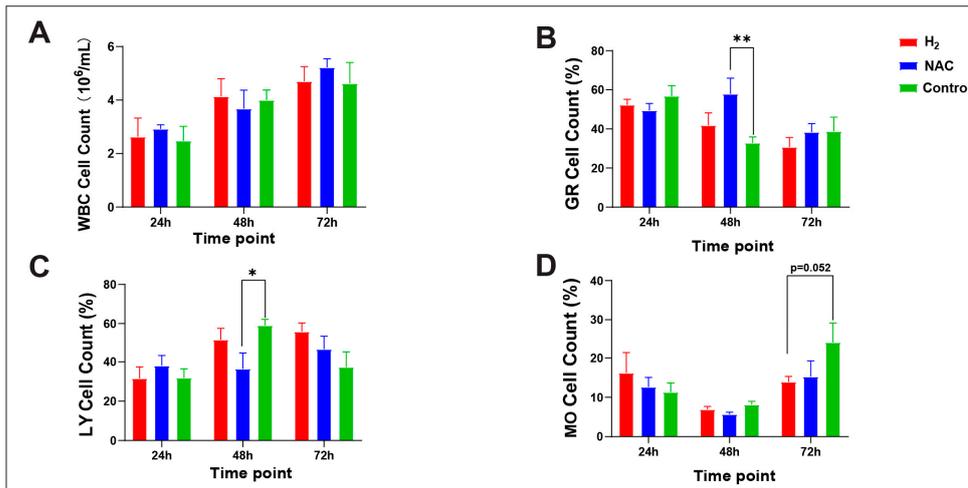
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## Results

### *H<sub>2</sub> inhibited the systemic inflammatory response*

Skin defects may cause systemic inflammatory responses, so we performed routine blood tests to determine the number of leukocytes and the percentages of neutrophils, lymphocytes, and monocytes in the blood of each group on the first three days (Fig. 4). The number of leukocytes in the blood corresponds to the state of systemic inflammation of the organism. From day 1 to day 3, no significant difference was observed in Leukocyte counts in all groups (Fig. 4A). Neutrophils remove cellular debris and release cytokines to create conditions for subsequent repair. The proportion of neutrophils in the H<sub>2</sub> group gradually decreased from 24 h to 72 h; at 48 h, the proportion of neutrophils was higher in the H<sub>2</sub> and NAC groups than in the control group ( $P < 0.01$  between the NAC group and the control group), while at 72 h, the proportion of neutrophils in the H<sub>2</sub> group was lower than in the control group (Fig. 4B). The proportion of lymphocytes in the NAC group was significantly lower than in the control group ( $P < 0.01$ ) and no significant difference was observed compared with the H<sub>2</sub> group at 48 h; at 72 h, the proportion of lymphocytes was higher in the H<sub>2</sub> group than in the other two groups (Fig. 3C), and no significant differences observed. At 72 h, monocyte counts in the H<sub>2</sub> group were lower than those in the control groups ( $P < 0.052$ ). Therefore, taking insight into the blood cell count results, systemic inflammatory responses in the H<sub>2</sub> group have no obvious changes at the first 72 h, while the monocytes number in H<sub>2</sub> group was slightly decreased on the 72 h.



**Figure S1.** Routine blood tests during the inflammatory stages of wound healing. Routine blood tests of the three groups. **(A)** White blood cell (WBC) count. **(B)** Granulocytes (GR). **(C)** Lymphocytes (LY). **(D)** Monocytes (MO). Percentages in whole blood at 24, 48, and 72 h after wounding. Data were analyzed by two-way ANOVA and plotted as mean±SEM. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .