

## ANNEXES

### **VI. CONTRE-INDICATIONS DE L'OHB**

L'OHB est contre-indiquée de manière absolue dans l'épilepsie mal équilibrée, le pneumothorax non drainé, l'emphysème majeur, la cardiopathie ischémique non stabilisée, l'otite aiguë (4).

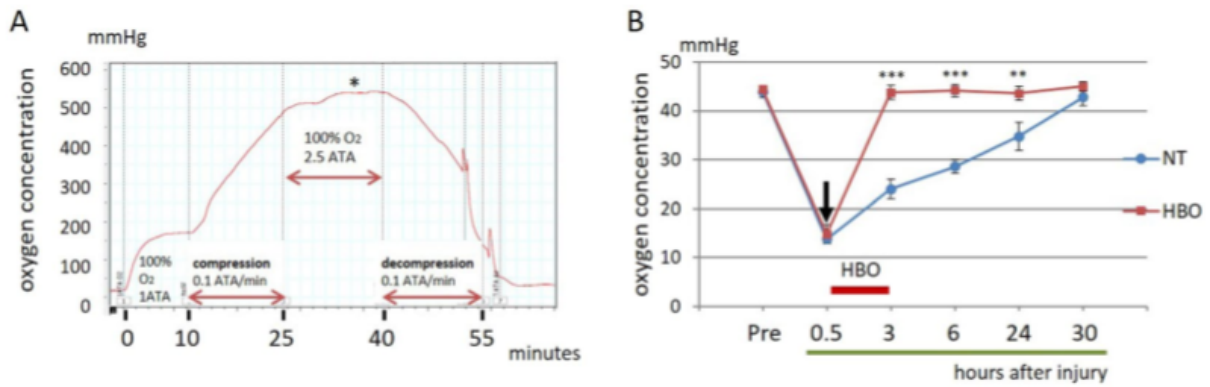
Les contre-indications relatives sont : l'asthme non contrôlé, la grossesse, le kyste osseux, l'emphysème modéré (33).

### **Indications**

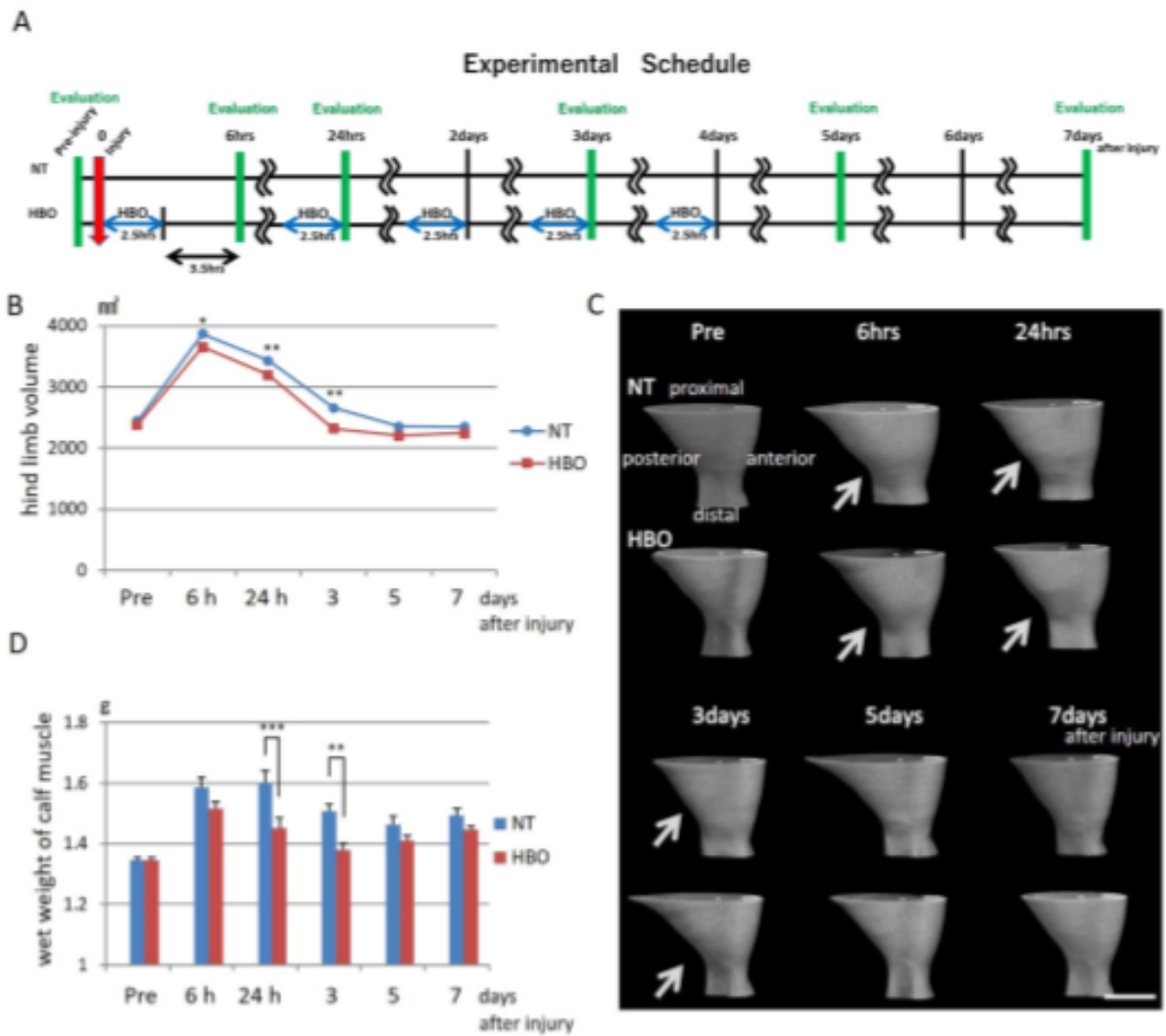
Les indications retrouvées dans la littérature sont l'intoxication au monoxyde de carbone, accident de décompression, embolie gazeuse, infections bactériennes anaérobies ou mixtes des tissus mous, ischémie aiguë des tissus mous, surdité brusque, greffes de peau et lambeaux musculo-cutanés, les abcès intracrâniens, pleuro-pulmonaires, hépatiques, ostéomyélite chronique réfractaire, lésions radio-induites, retards à la cicatrisation, neuroblastome de stade IV.

**Tableau 2** – Indications proposées de l’OHB, urgentes ou pas, d’après l’ECHM et l’UHMS

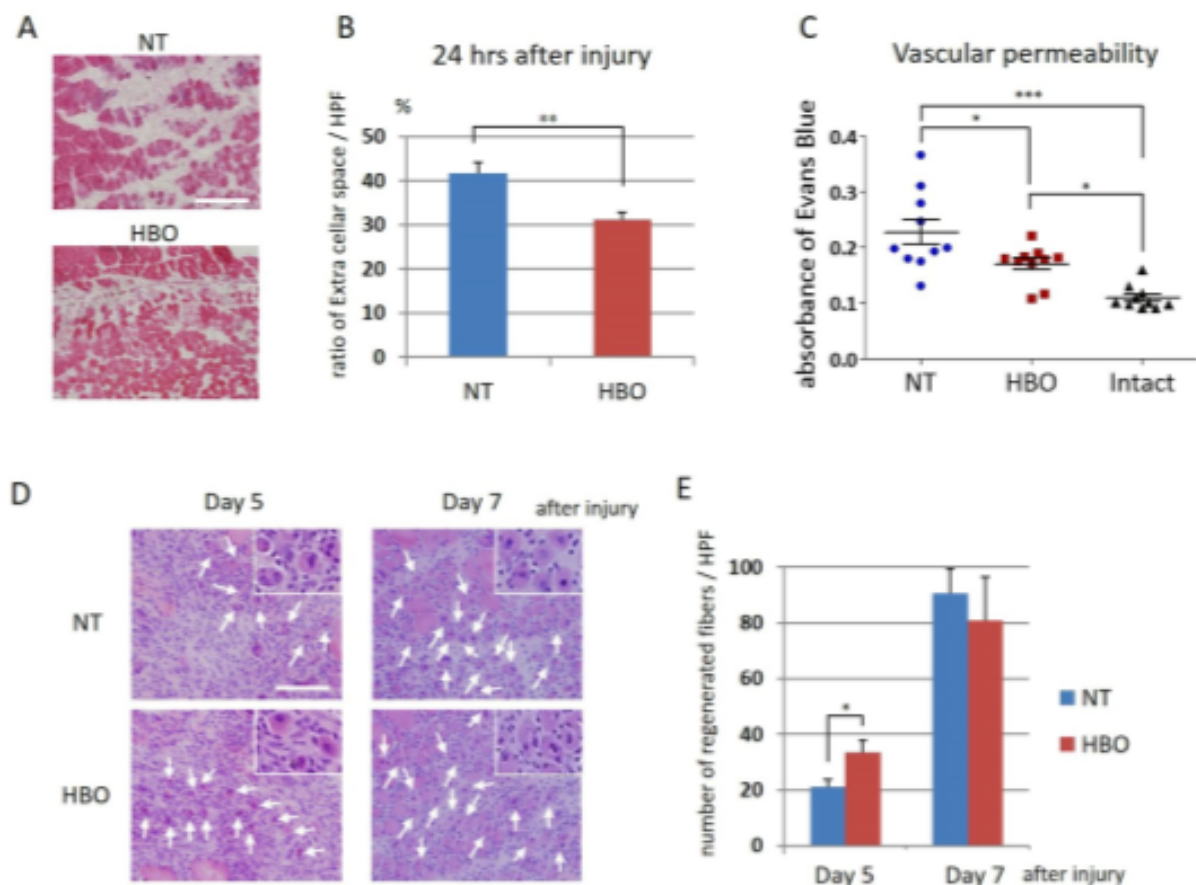
ECHM (Europe) (2)	UHMS (USA) (3)
Embolie gazeuse iatrogène	Embolie gazeuse iatrogène
Intoxication au monoxyde de carbone (CO)	Intoxication au monoxyde de carbone (CO)
Accident de décompression ou désaturation	Intoxication au CO compliquée d’intoxication au cyanure
Infections nécrotiques des tissus mous à germes anaérobies ou mixtes	Myonécrose et gangrène gazeuse
Abcès profonds (cérébral, pleuropulmonaire et hépatique)	Accident de désaturation (plongée sous-marine)
Ischémie de parties molles (lambeaux, greffes, réimplantation de membres, crush syndrome)	Occlusion de l’artère centrale de la rétine
Lésions radio-induites (mandibule, vessie, extraction dentaires, rectum, os longs)	Cicatrisation des plaies difficiles
Cicatrisation difficile (diabète, athérosclérose)	Anémie sévère
Ostéomyélite chronique réfractaire	Abcès intracrânien
Encéphalopathie post-anoxique	Ostéomyélites chronique réfractaires
Brûlures étendues > 20 %	Lésions post-radiques (tissus mous et os)
Surdité brusque	Greffes et lambeaux cutanés
Lésions ischémiques ophtalmologiques	Brûlures thermiques aiguës
Neuroblastome stade IV	
Pneumatose kystique	



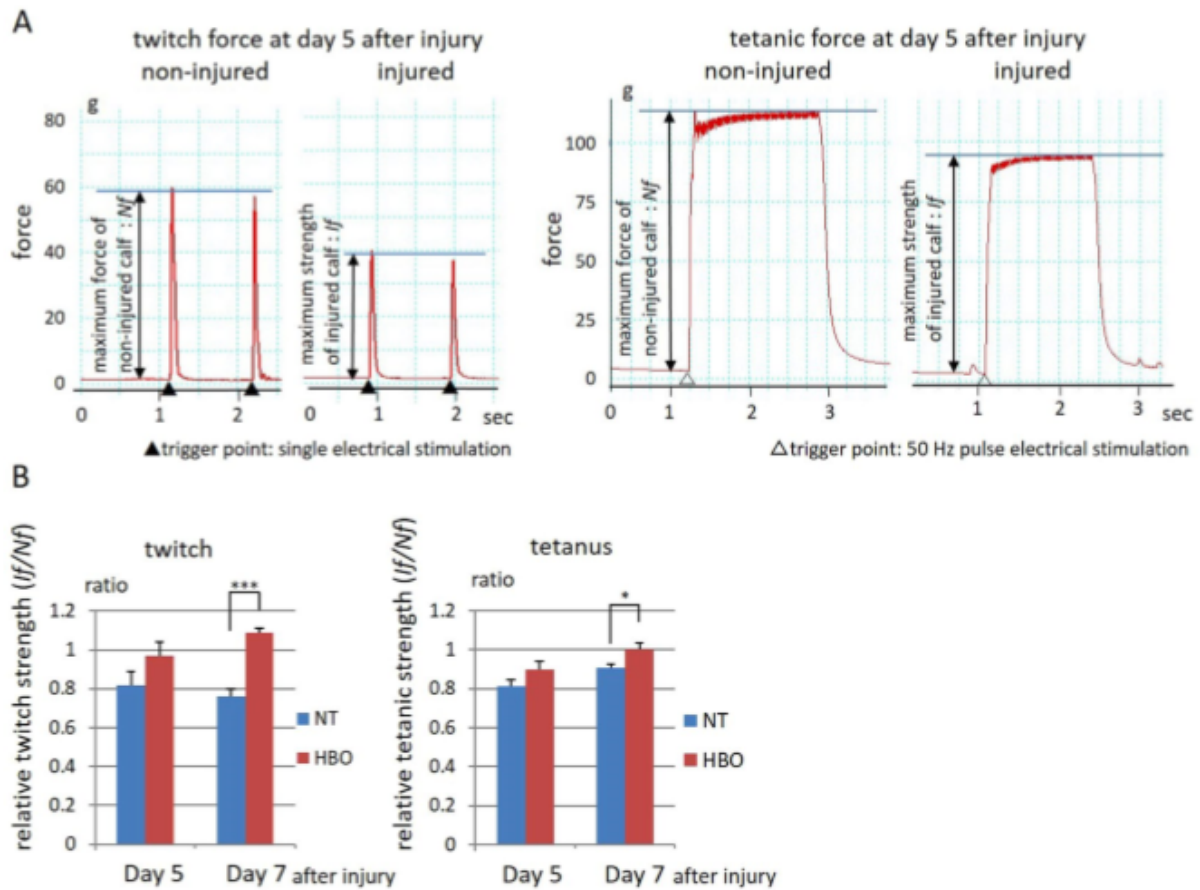
**Figure 1.** HBO reduced the hypoxic environment and maintained high oxygenation in contused muscle. (A) O<sub>2</sub> concentration monitoring using a needle-type probe shows real-time oxygenation in the contused muscle during HBO. The oxygen concentration increased to 540 mmHg (asterisk) during HBO in the experimental protocol (100% oxygen for 15 minutes). Artifacts from body motion were observed during decompression. (B) O<sub>2</sub> concentration in the injured muscle. The tissue oxygen concentration decreased from 45 to 15 mmHg within 30 minutes after injury (arrow). After one session of HBO, hypoxia was reduced and oxygenation was maintained for 30 hours. n = 5. \*\*\*P < 0.001, \*\*P < 0.01 using two-way ANOVA followed by Bonferroni post-tests. Data are the mean ± SEM.



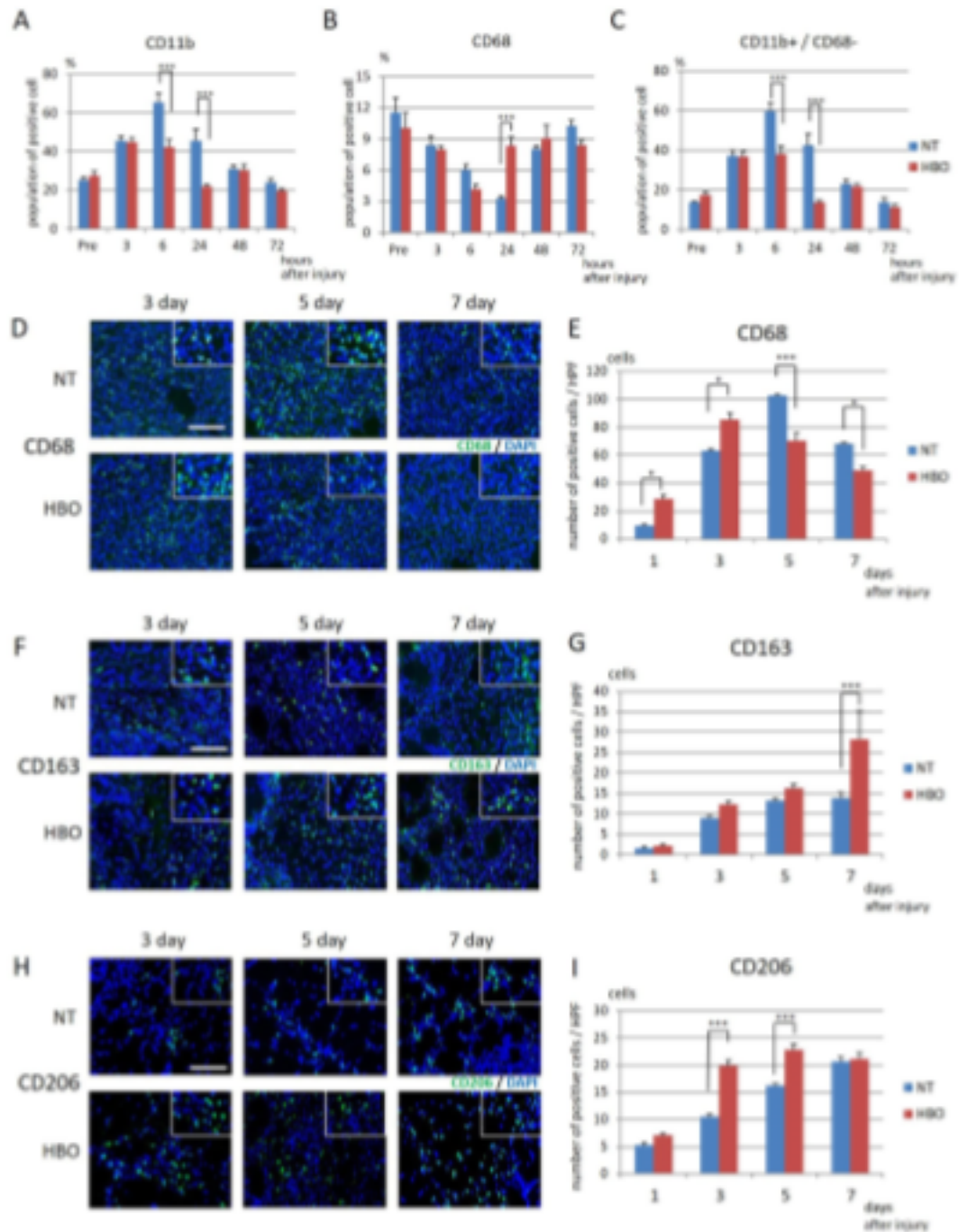
**Figure 2.** HBO reduced the volume of the injured hindlimb and muscle wet weight. (A) Treatment and evaluation schedule of hindlimb volume and calf muscle wet weight. (B)  $\mu$ CT evaluation of hindlimb volume after injury. The volume in the HBO group was lower than that in the NT group at 6 hours, 24 hours, and 3 days after injury.  $n = 13-20$ . \*\* $P < 0.01$ , \* $P < 0.05$  using two-way ANOVA followed by Bonferroni post-tests. (C) 3D images of hindlimbs. Reduced swelling (arrows) was observed in the HBO group. Scale bar: 10 mm. (D) Wet weight of the calf muscles. The wet weight in the HBO group was lower than that in the NT group at 24 hours and 3 days after injury.  $n = 8$ . \*\*\* $P < 0.001$ , \*\* $P < 0.01$  using two-way ANOVA followed by Bonferroni post-tests. Data are the mean  $\pm$  SEM.



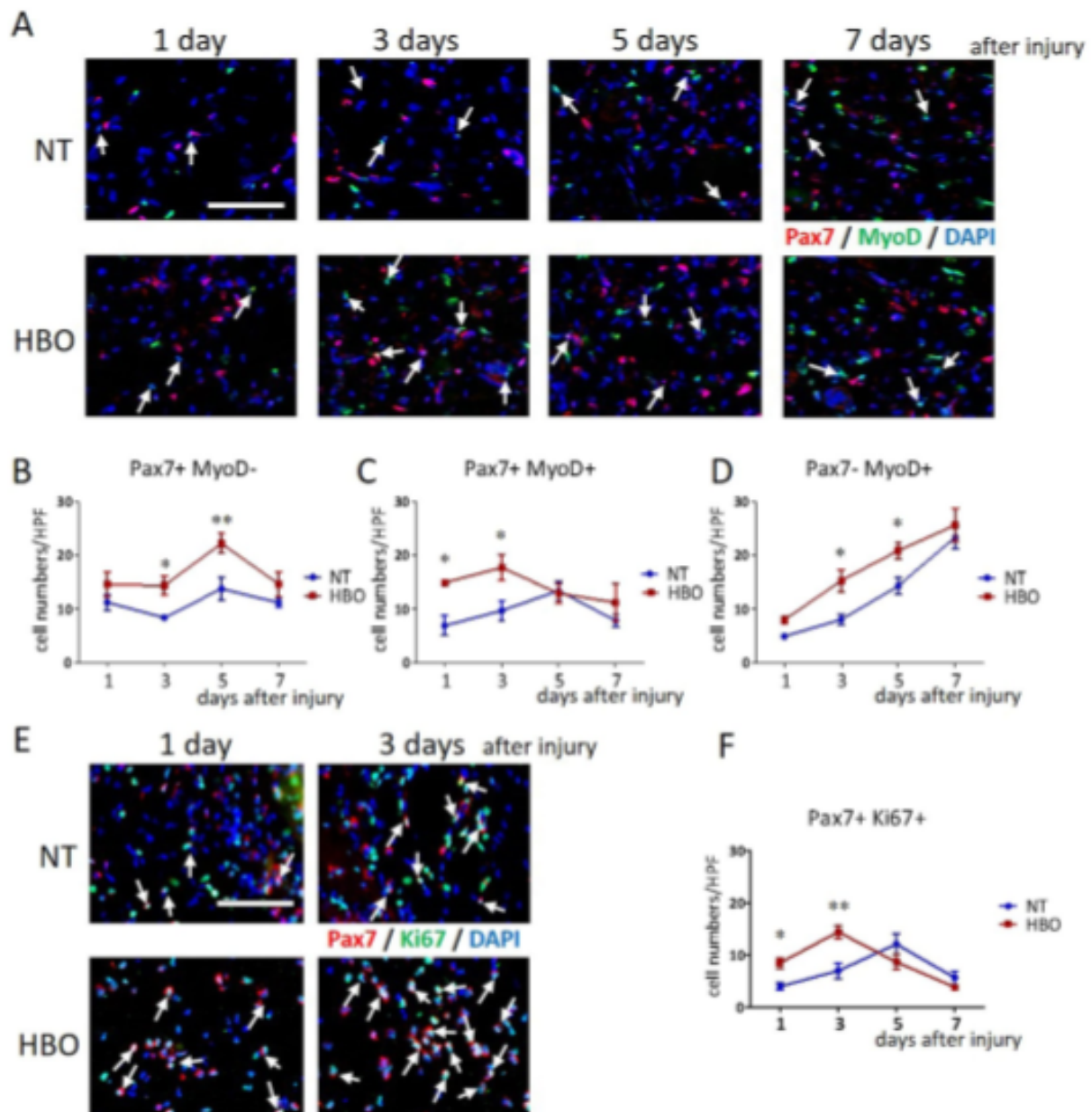
**Figure 3.** HBO improved the early post-injury muscle environment and increased myofiber regeneration. (A) Representative image of the extracellular space (non-stained area) in H&E-stained sections. Scale bar: 100  $\mu$ m (B) The ratio of the extracellular space per HPF was lower in the HBO group.  $n = 5$ .  $**P < 0.01$  using Welch's test. (C) The absorbance of Evans Blue in the supernatant from homogenized muscles was higher in the NT and HBO groups than in the intact group. The absorbance in the HBO group was lower than that in the NT group.  $n = 10$ .  $***P < 0.001$ ,  $*P < 0.05$  using one-way ANOVA followed by Bonferroni post-tests. (D) Nucleus-centered myofibers (insets) were observed from 5 days after injury in the NT group. Regenerated myofibers (arrows) were counted. Scale bars: 100  $\mu$ m (E) The number of regenerating myofibers in the HBO group was higher than that in the NT group at 5 days.  $n = 5$ .  $*P < 0.05$  using Student's t-test. Data are the mean  $\pm$  SEM.



**Figure 4.** HBO increased the isometric tensile strength of the gastrocnemius muscle. (A) Twitch and tetanic forces were recorded and ratios of injured leg ( $I_f$ ) to non-injured leg forces ( $N_f$ ) were calculated. (B) The ratio of twitch muscle strength in the HBO group was higher than that in the NT group at 7 days. The maximum strength of tetanic forces in the HBO group was higher than that in the NT group at 7 days.  $n = 6$ . \*\*\* $P < 0.001$ , \* $P < 0.05$  using Student's  $t$  test. Data are the mean  $\pm$  SEM.



**Figure 5.** HBO decreased the amount of early circulating macrophages and facilitated macrophage infiltration in injured muscles. (A) The proportion of CD11b-positive cells was reduced in the HBO group at 6 hours and 24 hours. (B) The proportion of CD68-positive cells was increased in the NT group at 6 hours and in the HBO group at 24 hours. (C) The proportion of CD11b-positive/CD68-negative cells was lower in the HBO group than in the NT group at 6 hours and 24 hours,  $n = 5$ .  $***P < 0.001$  using two-way ANOVA followed by Bonferroni post-tests. (D) Representative images of CD68-positive cells colocalized with DAPI (insets). (E) The number of CD68-positive cells was increased in the HBO group at 1 day and 3 days after injury. The number of CD68-positive cells was increased in the NT group at 5 days and 7 days after injury,  $n = 5$ .  $***P < 0.001$ ,  $*P < 0.05$  using two-way ANOVA followed by Bonferroni post-tests. (F) Representative image of CD163-positive cells. Scale bar: 100  $\mu\text{m}$ . (G) The number of CD163-positive cells colocalized with DAPI (insets) in the HBO group was increased at 7 days,  $n = 5$ .  $***P < 0.001$  using two-way ANOVA followed by Bonferroni post-tests. (H) Representative image of CD206-positive cells. Scale bar: 100  $\mu\text{m}$ . (I) The number of CD206-positive cells colocalized with DAPI (insets) in the HBO group was increased at 3 days and 5 days,  $n = 5$ .  $***P < 0.001$  using two-way ANOVA followed by Bonferroni post-tests. Data are the mean  $\pm$  SEM.



**Figure 6.** HBO accelerated the proliferation and differentiation of satellite cells in injured muscle. (A) Merged images, cells positive for Pax7 (red), MyoD (green), and DAPI (blue) in injured muscle (arrows). Scale bar: 50  $\mu$ m. (B) The number of Pax7<sup>+</sup>/MyoD<sup>-</sup> cells was significantly increased in the HBO group at 3 days and 5 days. (C) The number of Pax7<sup>+</sup>/MyoD<sup>+</sup> cells was increased in the HBO group at 1 day and 3 days. (D) The number of Pax7<sup>-</sup>/MyoD<sup>+</sup> cells were increased in the HBO group at 3 days and 5 days,  $n = 5$ . \*\* $P < 0.01$ , \* $P < 0.05$  using two-way ANOVA followed by Bonferroni post-tests. (E) Merged images, cells positive for Pax7 (red), Ki67 (green), and DAPI (blue) in injured muscle (arrows). (F) The number of Pax7<sup>+</sup>/Ki67<sup>+</sup> cells was increased in the HBO group at 1 day and 3 days, and peaked at 3 days in the HBO group and at 5 days in the NT group,  $n = 5$ . \* $P < 0.05$  using two-way ANOVA followed by Bonferroni post-tests. Data are the mean  $\pm$  SEM.