Online Supplements

Iron chelation inhibits the development of pulmonary vascular remodeling

Chi-Ming Wong,[1] Ioana R. Preston,[2] Nicholas S. Hill,[2] and Yuichiro J. Suzuki[1]*

[1]Department of Pharmacology and Physiology,
Georgetown University Medical Center, Washington, DC 20057 USA

and

[2]Pulmonary, Critical Care and Sleep Division,
Tufts Medical Center, Tupper Research Institute, Boston, MA 02111 USA

*To whom correspondence should be addressed:

Dr. Yuichiro J. Suzuki
Department of Pharmacology and Physiology
Georgetown University Medical Center
NW403 Medical-Dental Building
3900 Reservoir Road NW
Washington, DC 20057 USA

TEL: (202) 687-8090
FAX: (202) 687-8825
e-mail: ys82@georgetown.edu

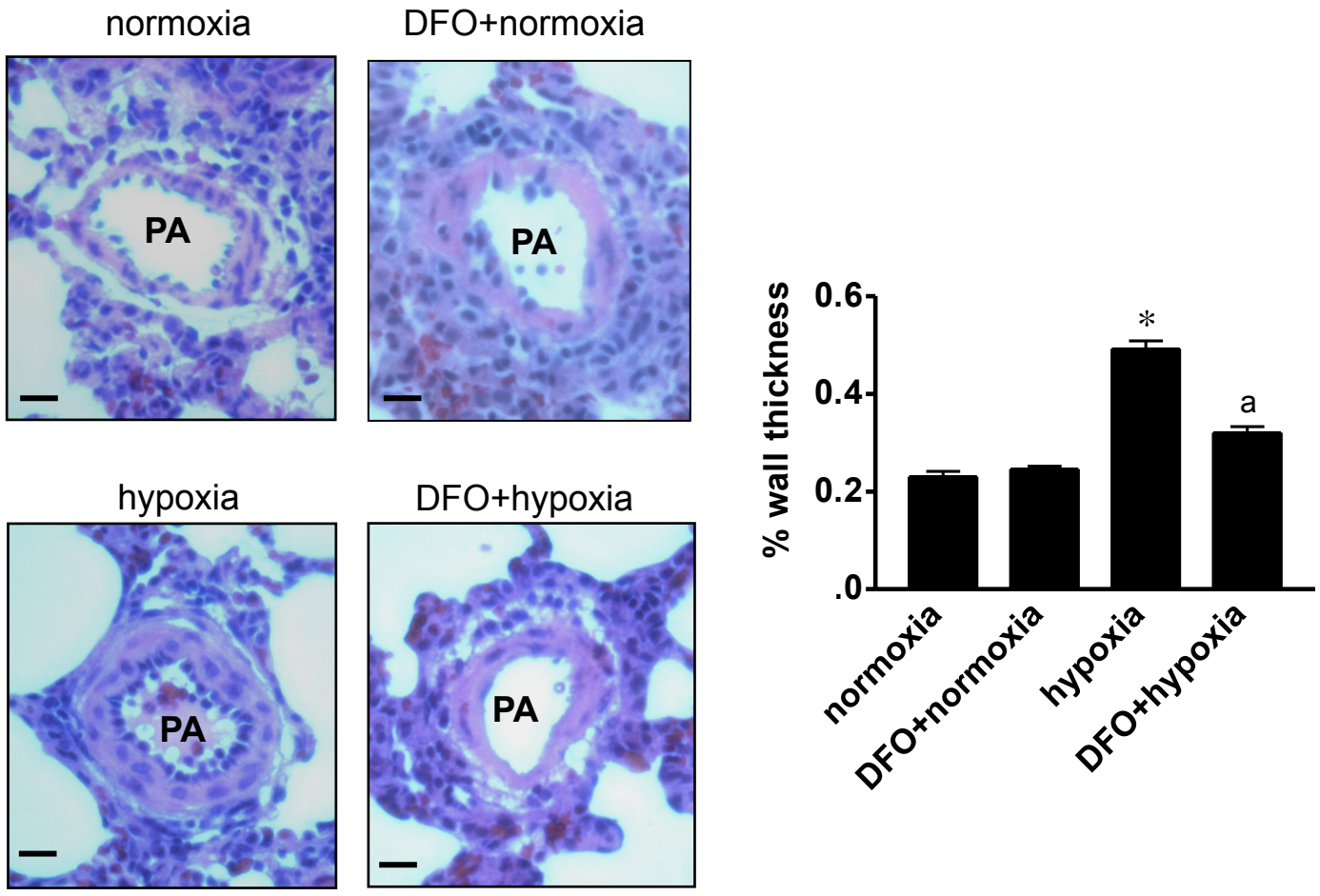


Fig. S1 Effects of deferoxamine (DFO) on chronic hypoxia-induced pulmonary hypertension in intact rats. Rats were intraperitoneally injected daily with saline (vehicle control) or 20 mg/kg body weight of DFO during 2-week exposure to normoxia or hypoxia (10% O_2). H & E staining showing large pulmonary arteries (PA) with the diameter ranging 116 - 363 μm. Scale bars, 50 μm. The bar graph represents means ± SEM of % wall thickness (n = 3 - 6). *, $P<0.05$ vs. normoxia; a, $P<0.05$ vs. hypoxia.

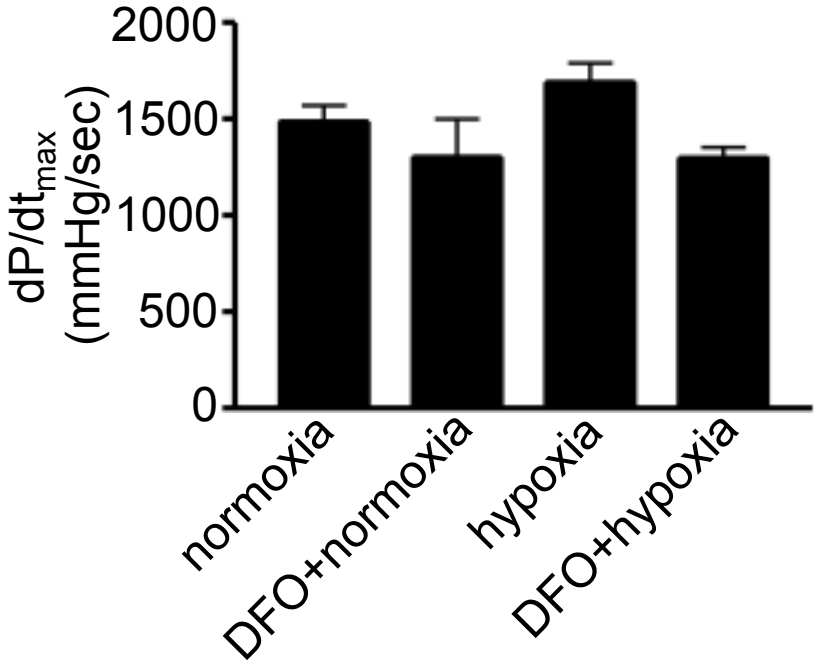


Fig. S2 Effects of deferoxamine (DFO) on right ventricular contractility. Rats were intraperitoneally injected daily with saline (vehicle control) or 20 mg/kg body weight of DFO during 2-week exposure to normoxia or hypoxia (10% O_2). Right ventricular dP/dt_{max} (as an indication of contractility) was measured using a Millar catheter (n = 3). No significant differences were detected.

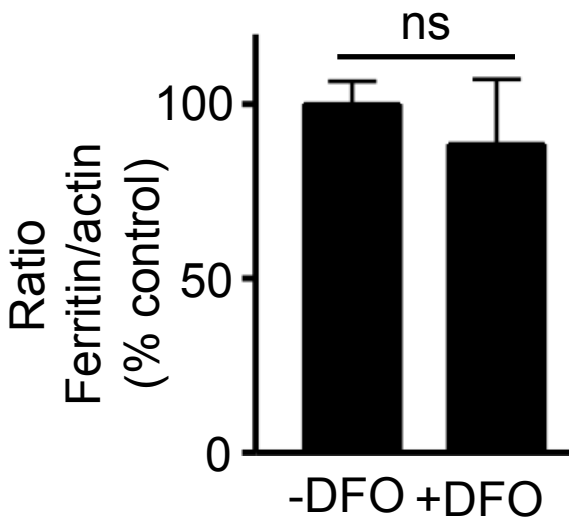


Fig. S3 Effects of chronic hypoxia and deferoxamine (DFO) on pulmonary arterial ferritin levels in rats. Rats were intraperitoneally injected daily with saline (vehicle control) or 20 mg/kg body weight of DFO. Western blotting was performed on pulmonary arterial homogenates to monitor ferritin and b-actin expression. Bar graphs represent means ± SEM (n = 3). ns, not significantly different.

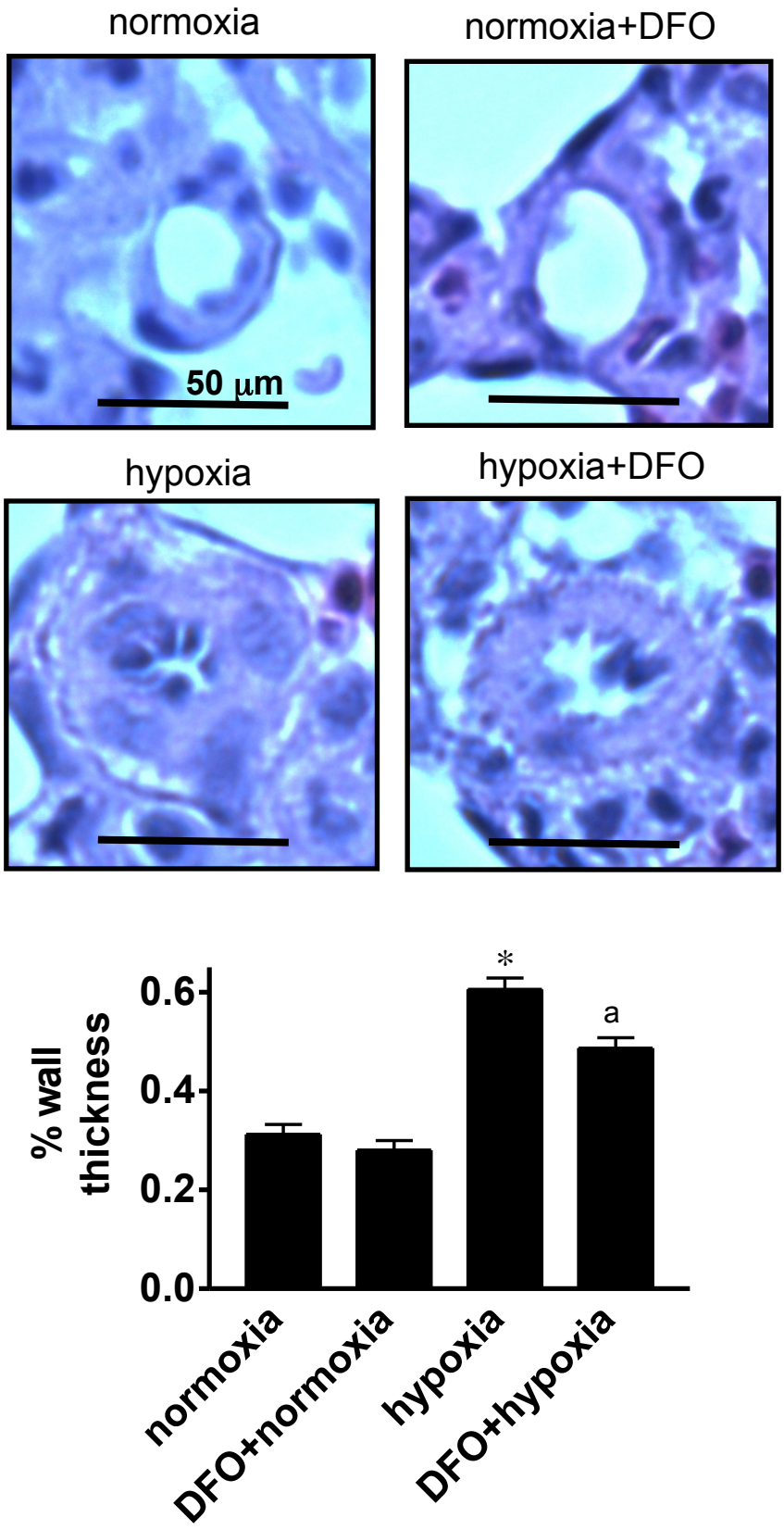
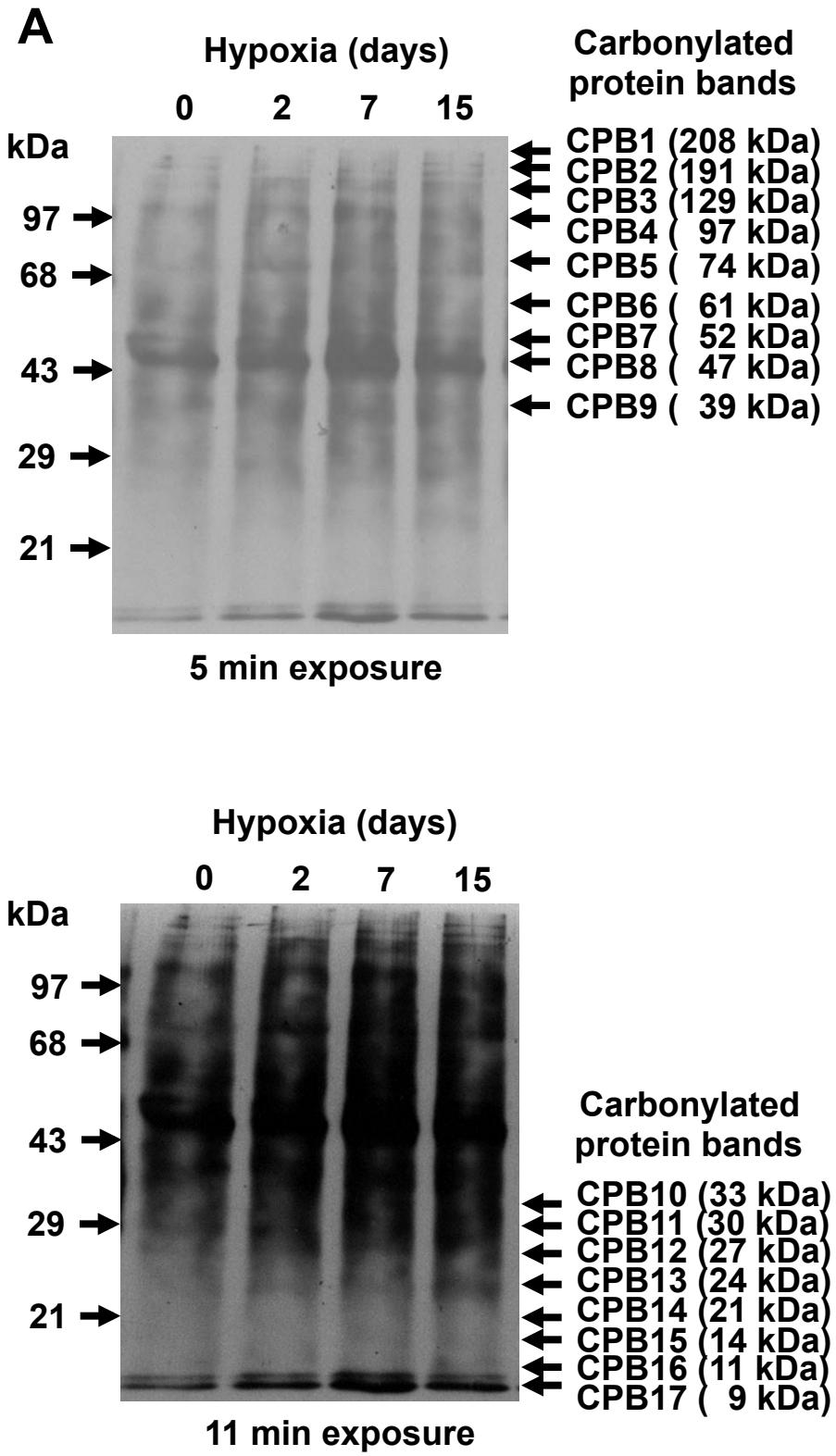


Fig. S4 Effects of deferoxamine (DFO) on the already developed pulmonary vascular remodeling in intact rats. Rats were treated with normoxia or hypoxia (10% O_2) for 2 weeks, then intraperitoneally injected with saline or 20 mg/kg body weight of DFO. Rats were then placed back in normoxia or hypoxia for 3 days. The images show representative data on histology (H & E staining) of pulmonary arteries (PA). Scale bars, 50 μm. The bar graph represents means ± SEM of % wall thickness (n = 4). *, P<0.05 vs. normoxia; a, P<0.05 vs. hypoxia.

Fig. S5 Wong et al.



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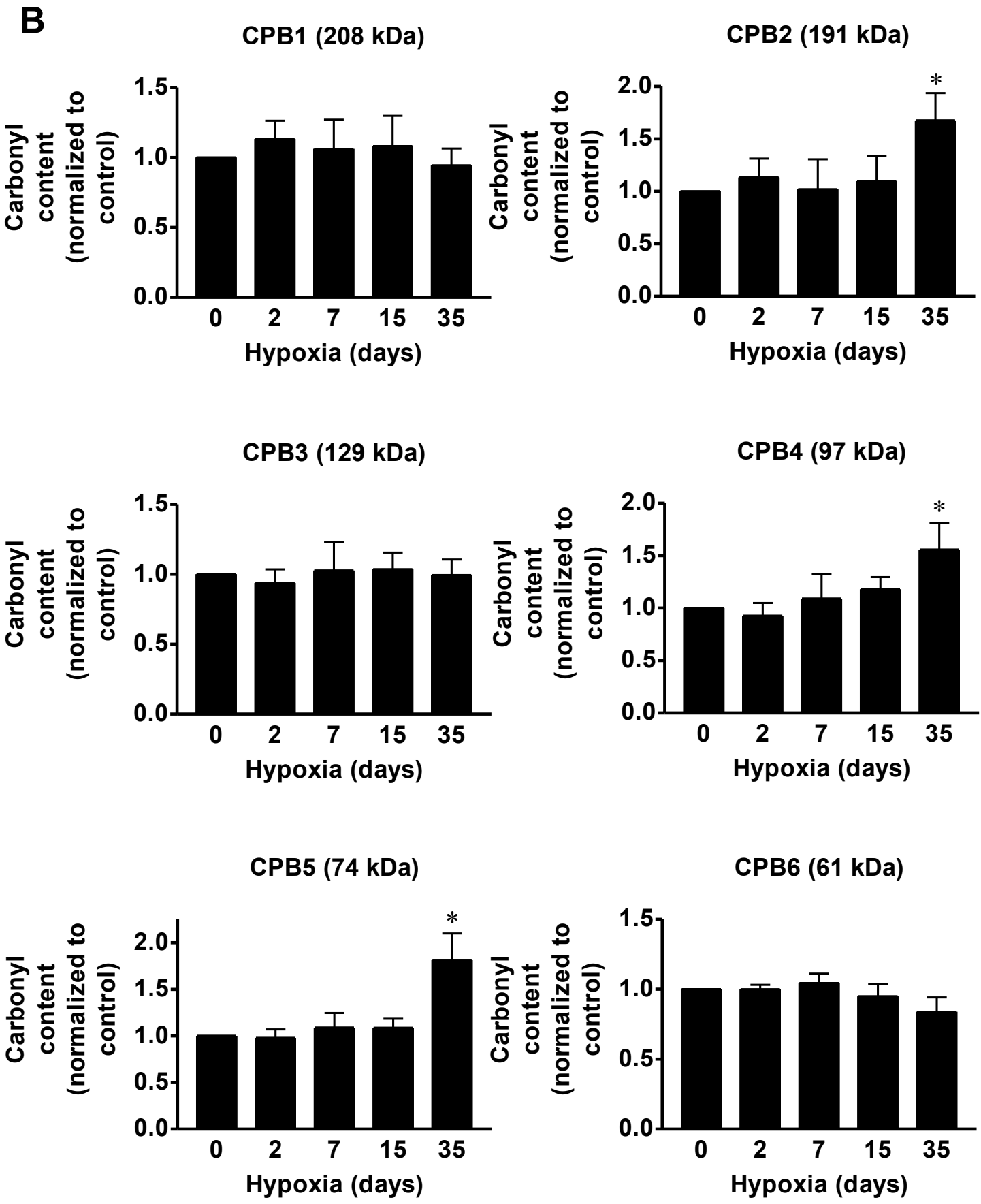

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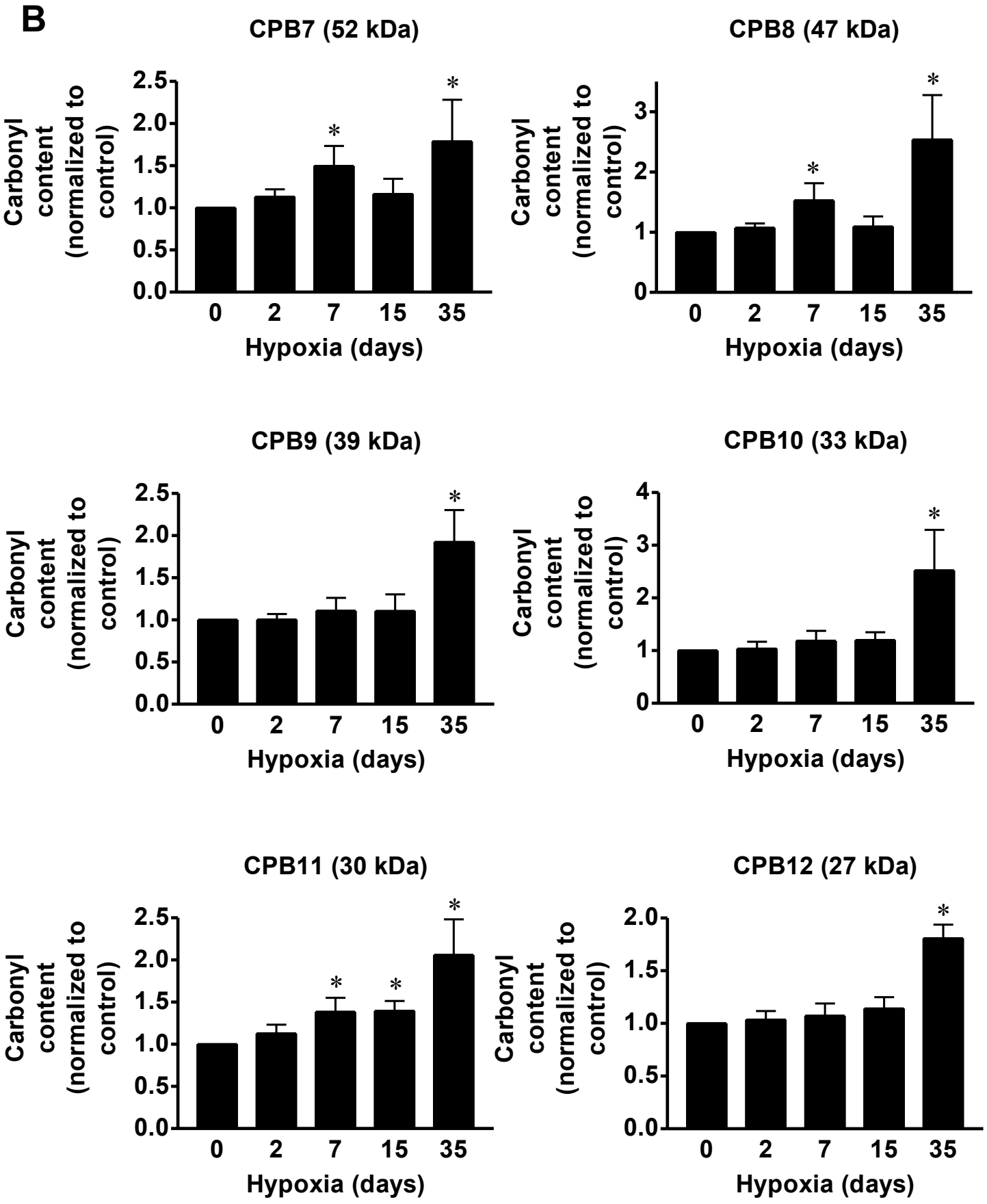

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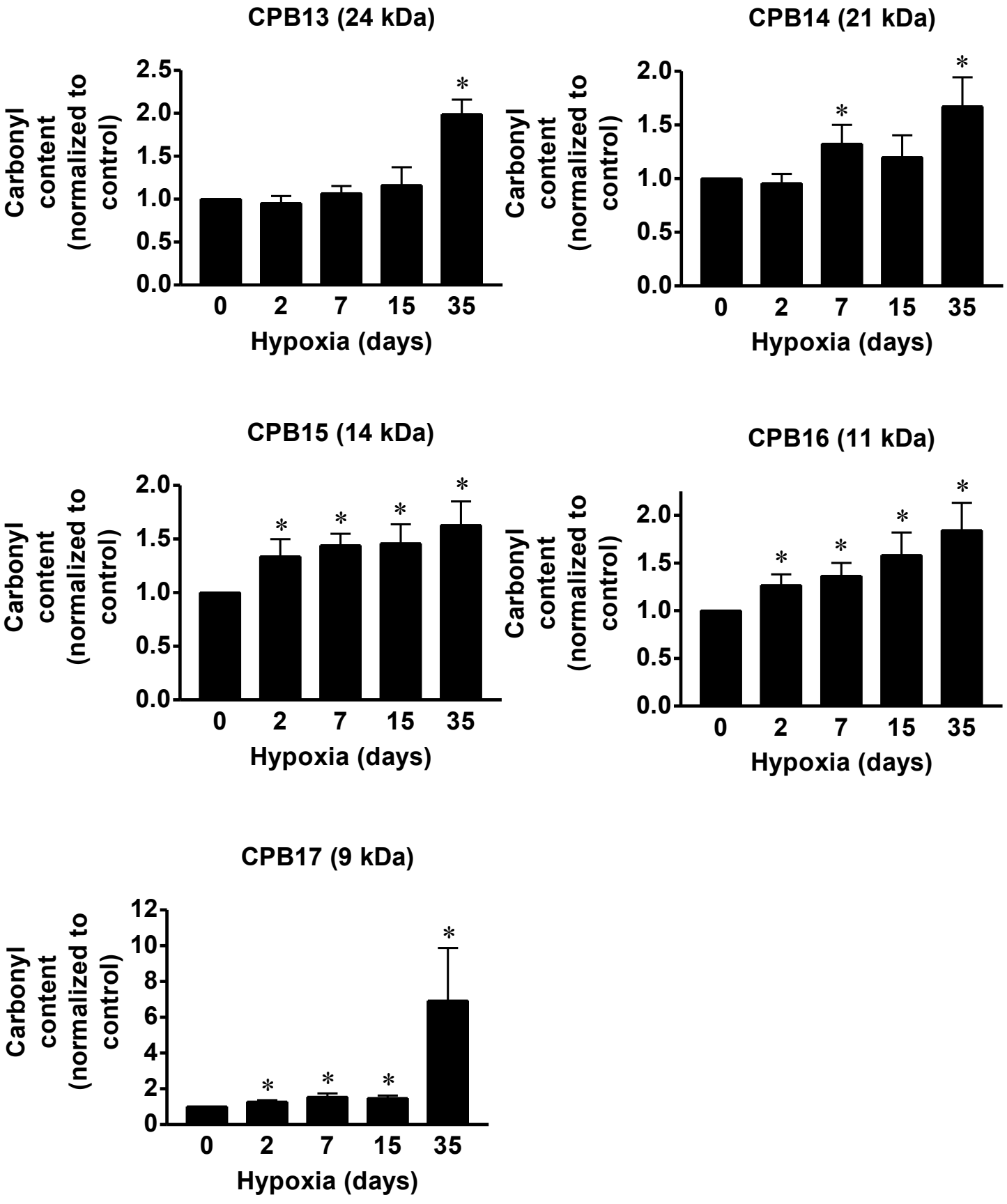

Fig. S5 Effects of chronic hypoxia on protein carbonylation in an *in vivo* model of pulmonary hypertension. Rats were subjected to chronic hypoxia (10% O_2) for durations indicated. After treatment, pulmonary arteries were isolated and homogenized. Proteins were derivatized with DNPH and subjected to Western blotting with rabbit polyclonal IgG for DNP to monitor total carbonylated proteins. (A) Numbers were assigned for each of carbonylated protein (CPB) bands, CPB1 - CPB17. The molecular weights for carbonylated proteins were calculated using the DNP-conjugated standard proteins. 5 and 11 min exposures of the film are shown. (B) The bar graph represents means ± SEM (n = 4). *, $P<0.05$ vs. normoxia (0 day hypoxia).

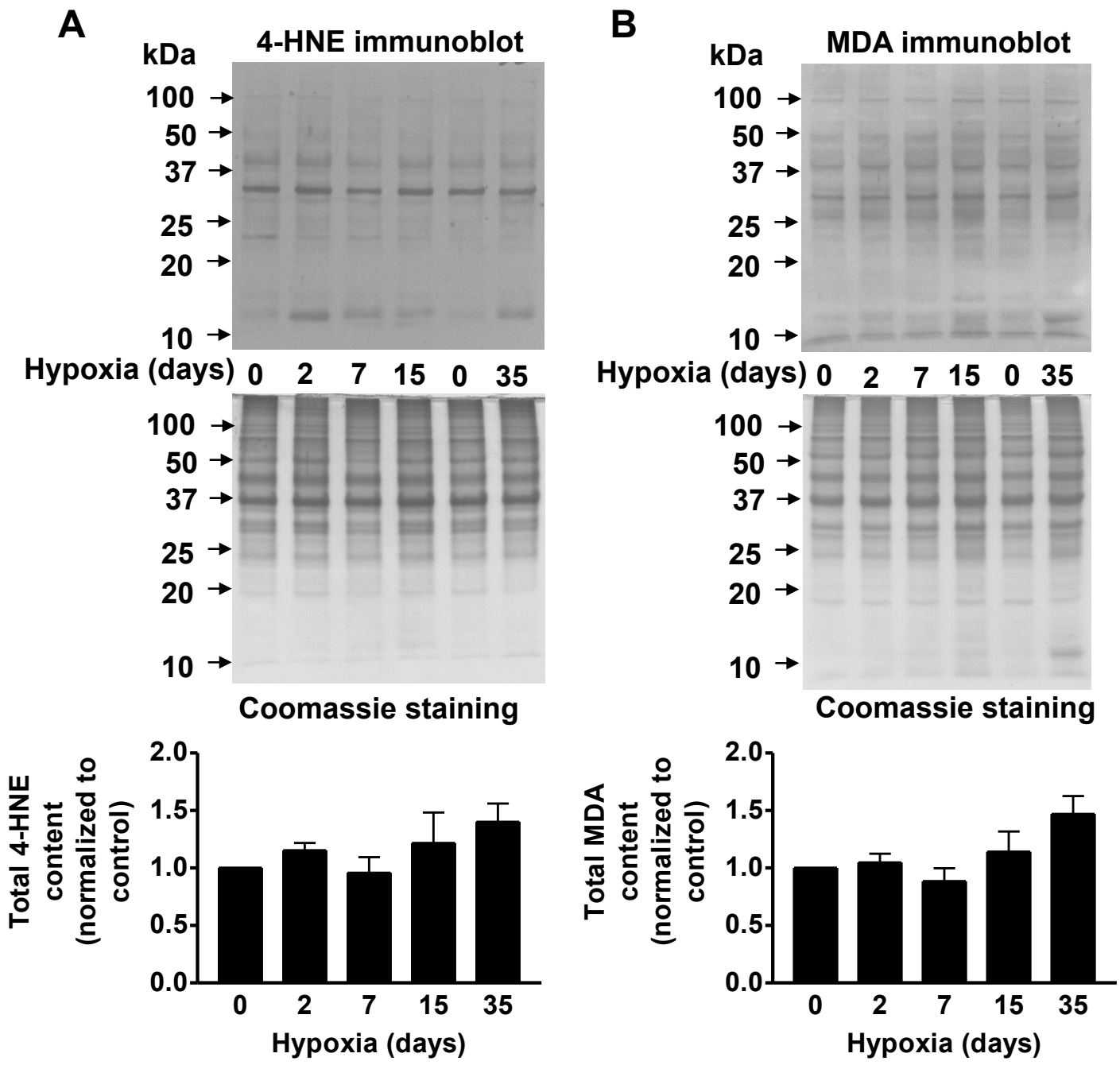

Fig. S6 Effects of chronic hypoxia on secondary protein carbonylation in an *in vivo* model of pulmonary hypertension. Rats were subjected to chronic hypoxia (10% O_2) for indicated durations. After treatment, pulmonary arteries were isolated and homogenized. Samples were subjected to Western blotting with (A) 4-HNE and (B) MDA antibodies. Total protein levels were monitored by Coomassie Blue staining. The bar graph represents means ± SEM (n = 4). No significant differences were detected.

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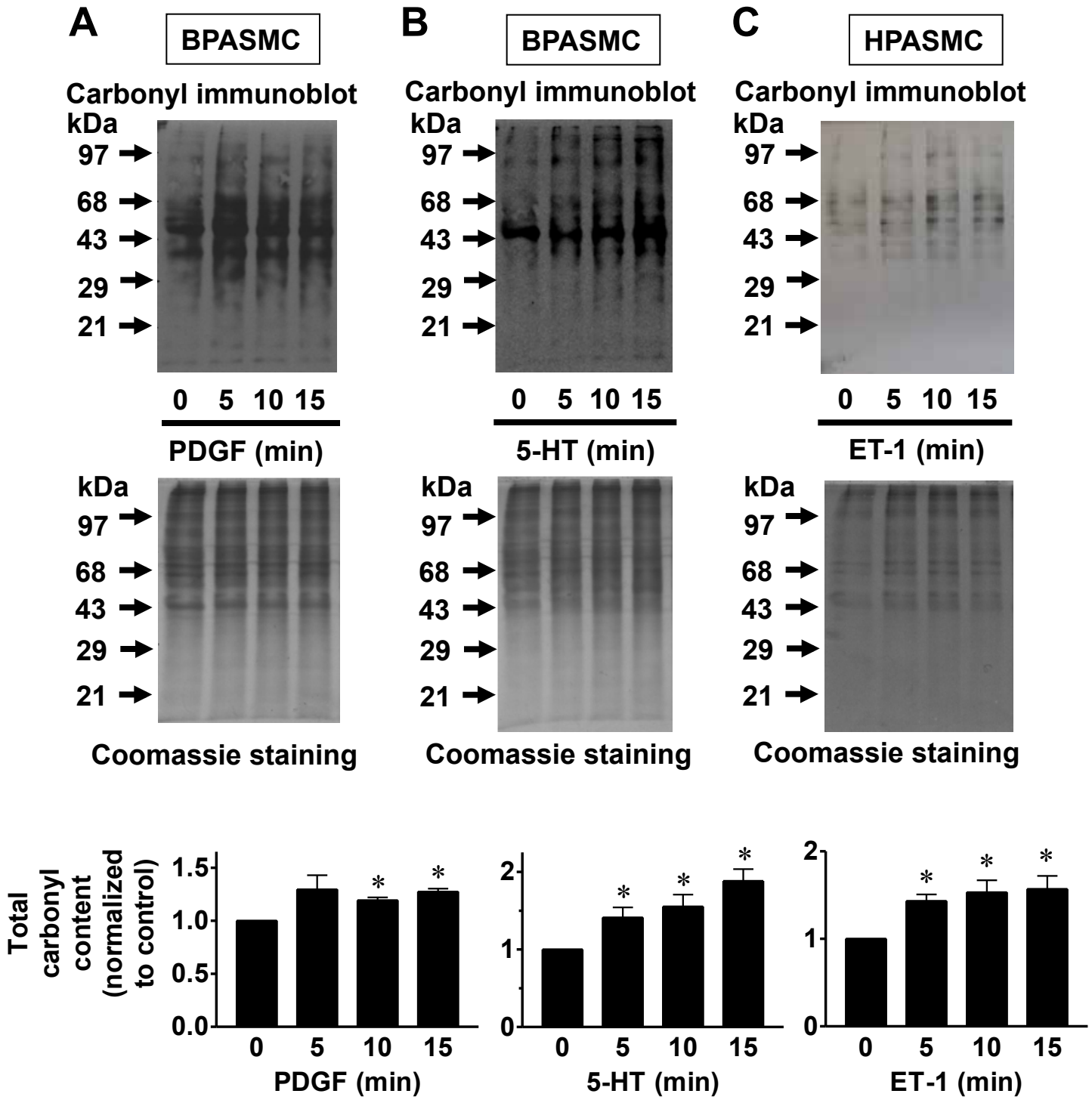

Fig. S7 Effects of PDGF and 5-HT on total protein carbonylation in pulmonary artery SMCs. Growth-arrested bovine pulmonary artery SMCs were treated with (A) PDGF (10 ng/mL) or (B) 5-HT (1 μmol/L) for indicated durations. (C) Human pulmonary artery SMCs were treated with ET-1 (30 nmol/L). Cell lysates were prepared, derivatized with DNPH, and subjected to Western blotting with rabbit polyclonal IgG for DNP to detect changes in the level of protein carbonyls. A negative control without the addition of DNPH is also shown in the far right lane. Protein expression levels were monitored by Coomassie Blue staining. Bar graphs represent means ± SEM (n = 4 – 8). *, $P<0.05$ vs. untreated (0 control).

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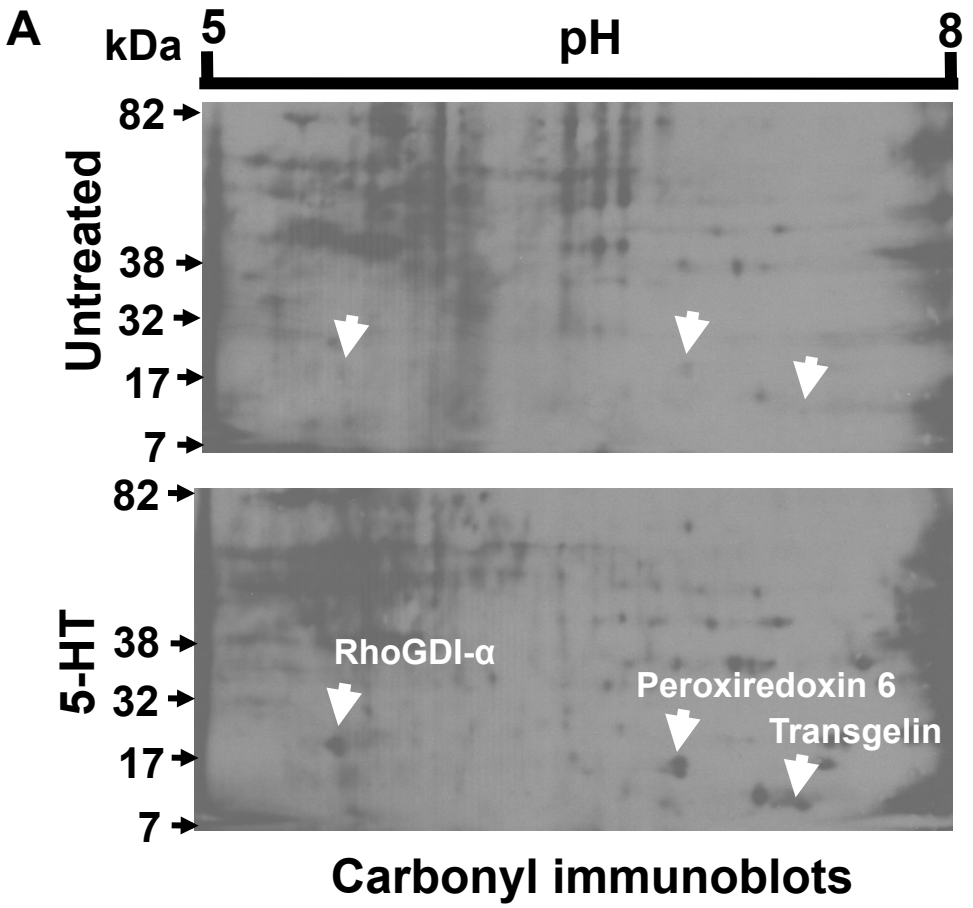

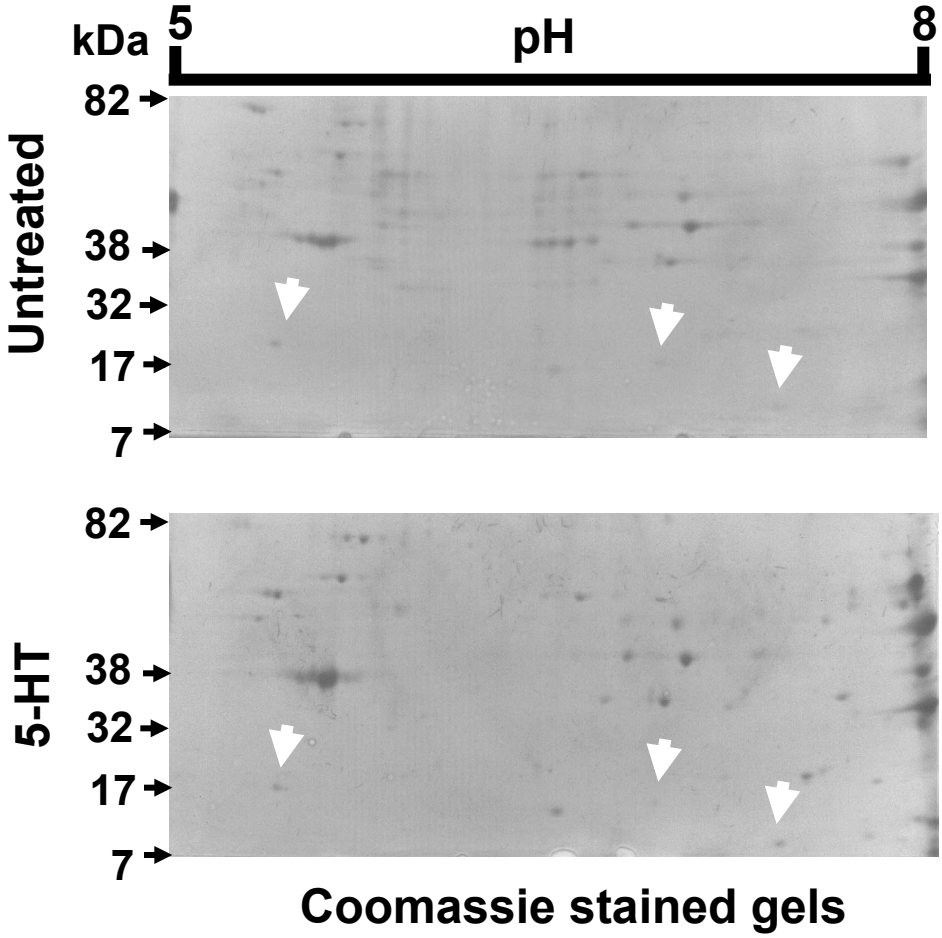

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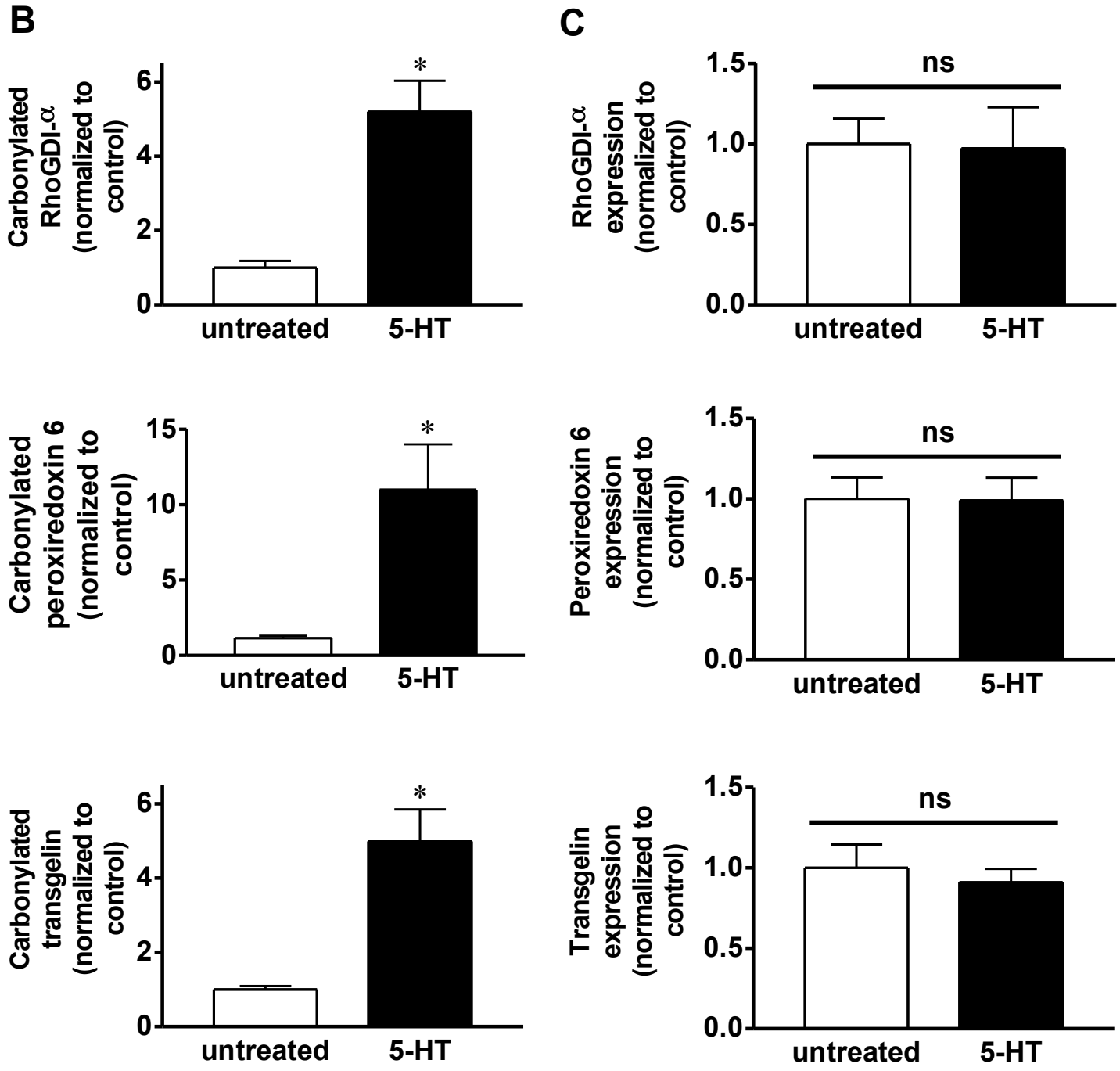

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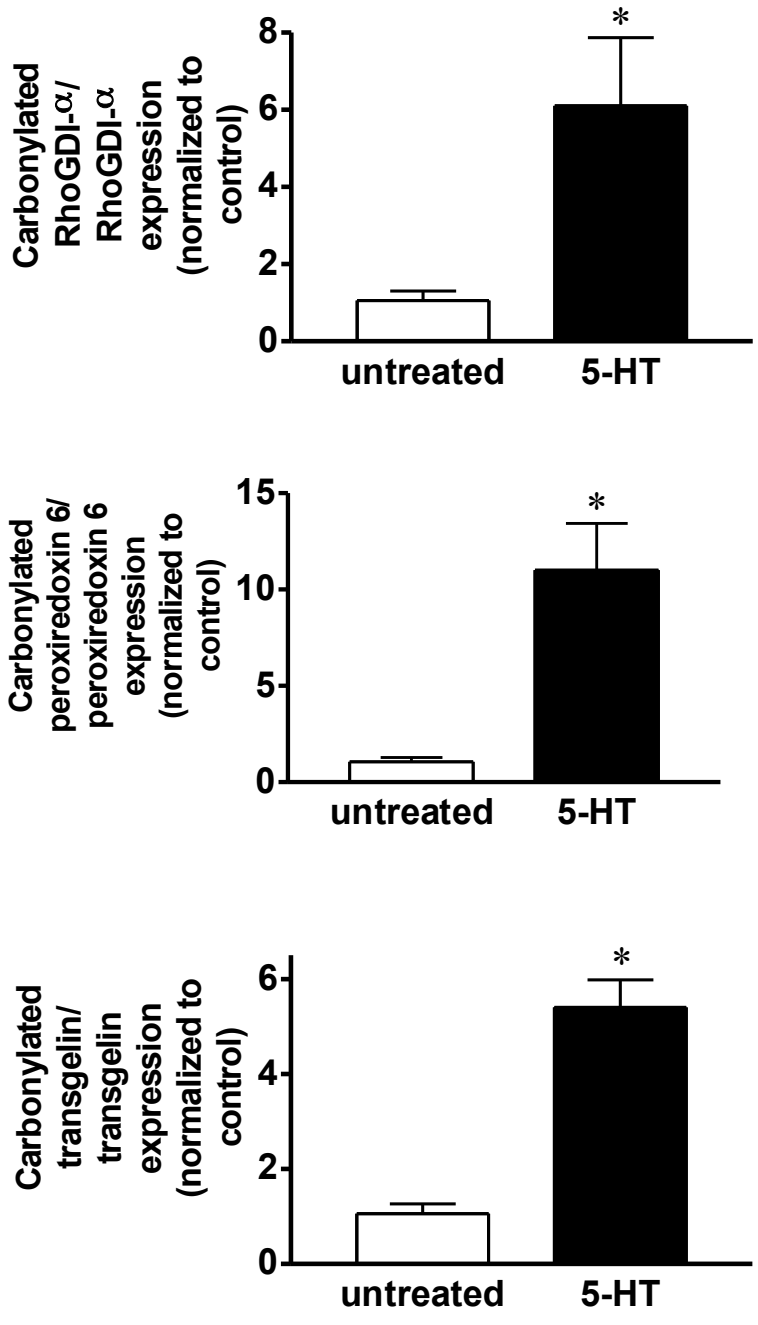

Fig. S8 Proteomic analysis of carbonylated proteins in response to 5-HT in pulmonary artery SMCs. Growth-arrested bovine pulmonary artery SMCs were treated with 5-HT (1 μmol/L) for 15 min. Cell lysates were subjected to 2-dimensional gel electrophoresis and immunoblotted with DNP antibody. Gels that were run in parallel with the immunoblotting were stained with Coomassie Blue. (A) Spots that were indicated by the arrow exhibited a significant increase in carbonyl content. Corresponding spots from the Coomassie Blue-stained gel were analyzed by mass spectrometry. Bar graphs represent means ± SEM of (B) carbonylated proteins, (C) protein expression, and (D) the ratio (n = 3). *, $P<0.05$. ns, not significantly different.

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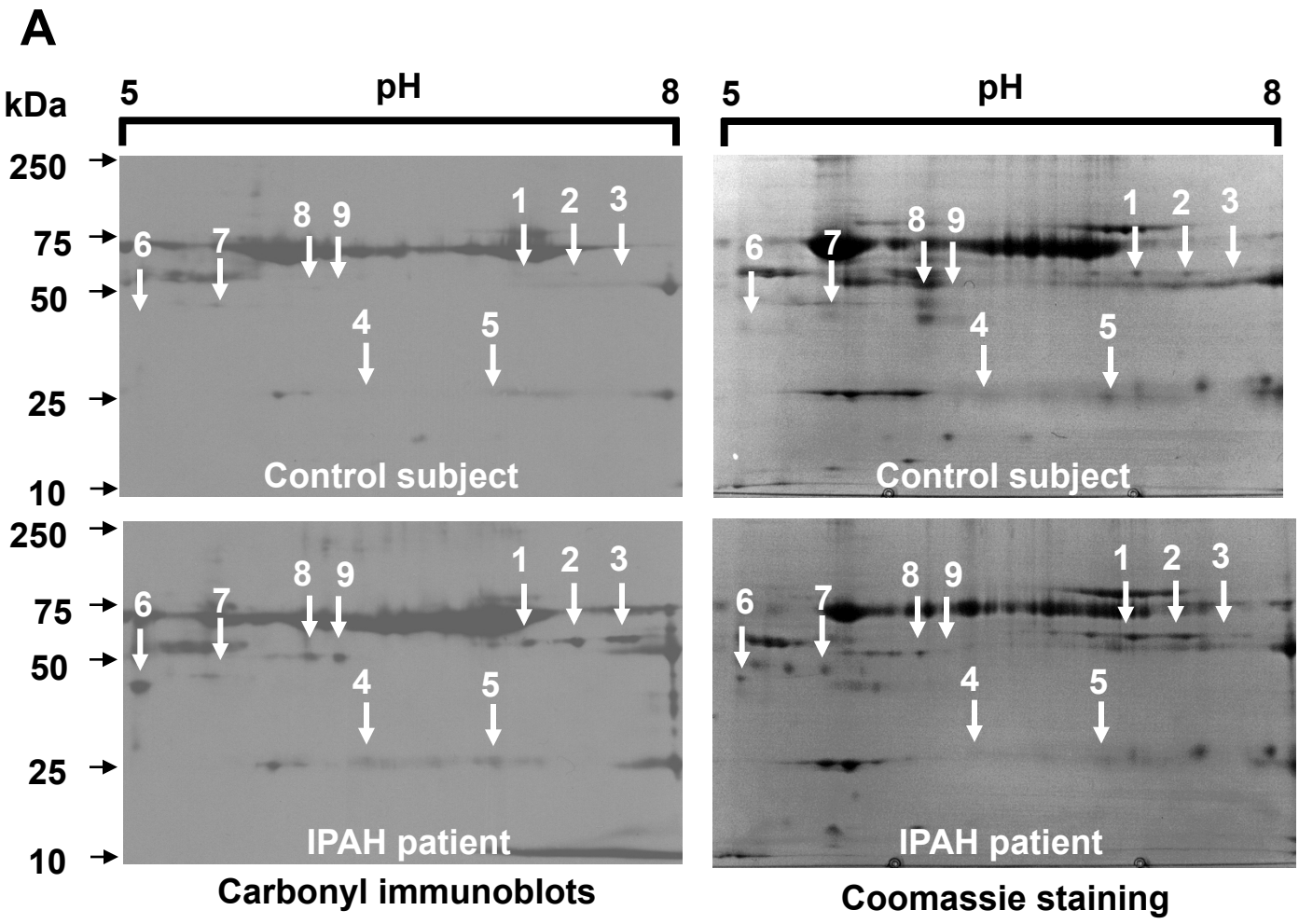

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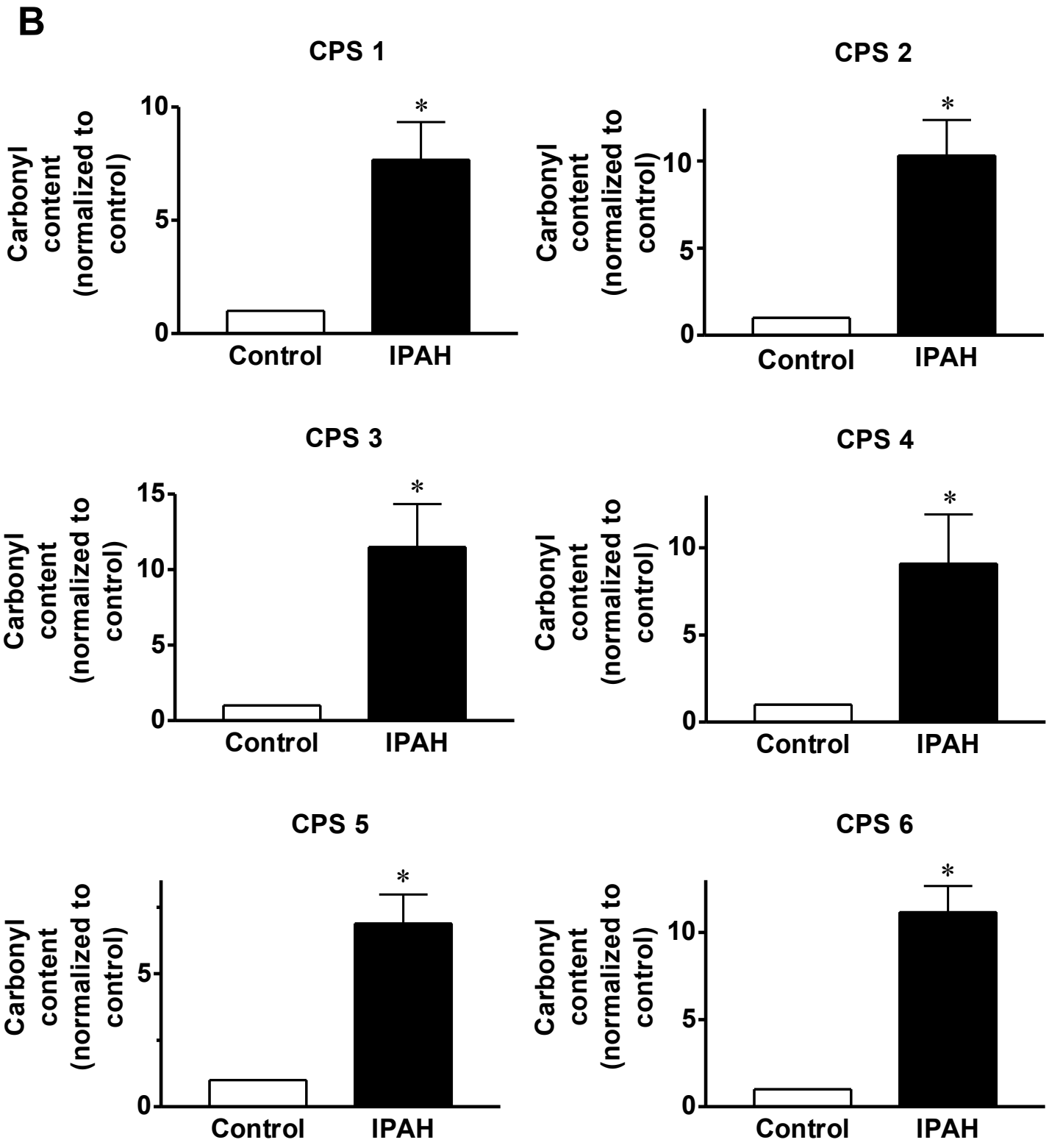

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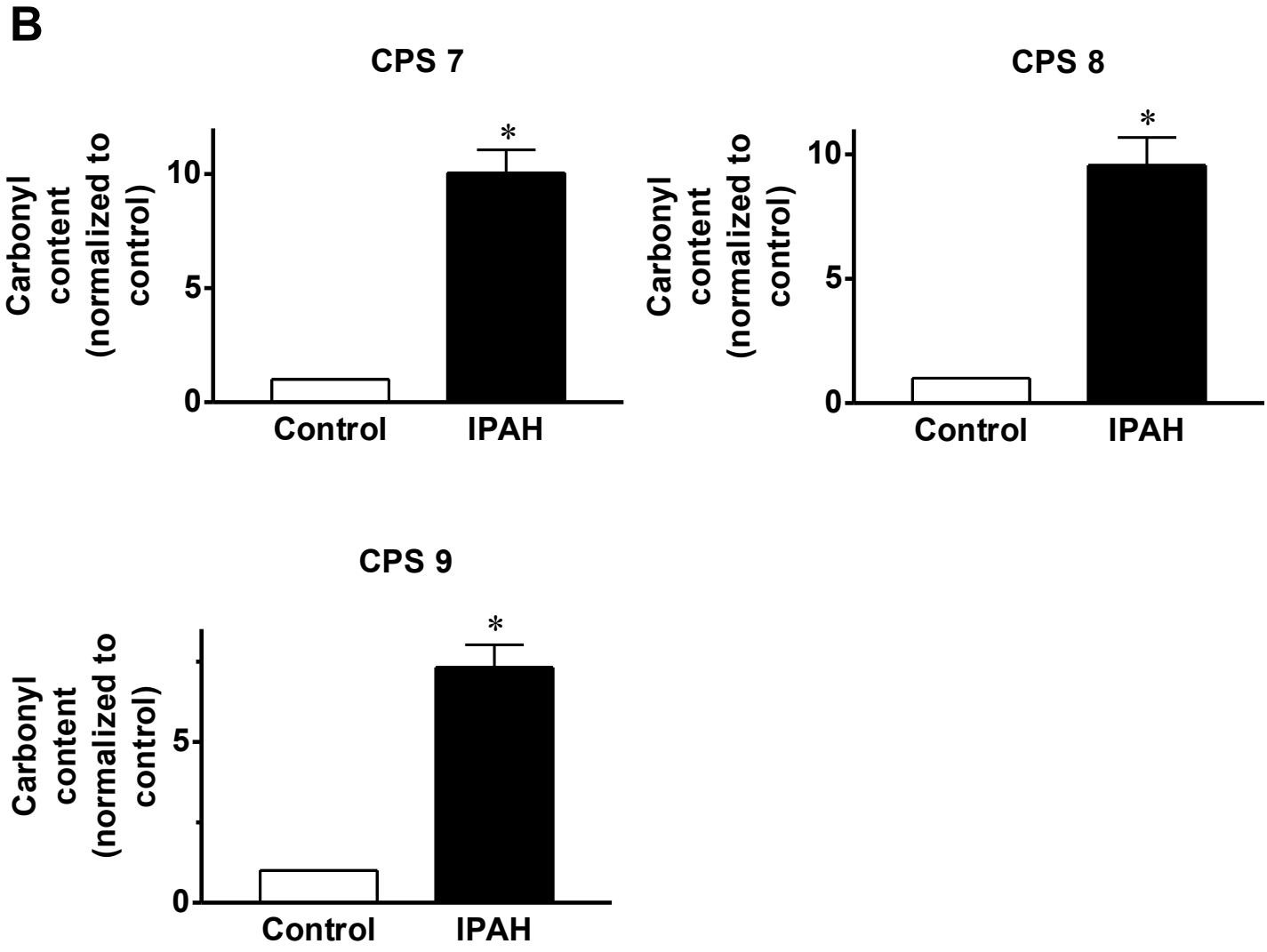

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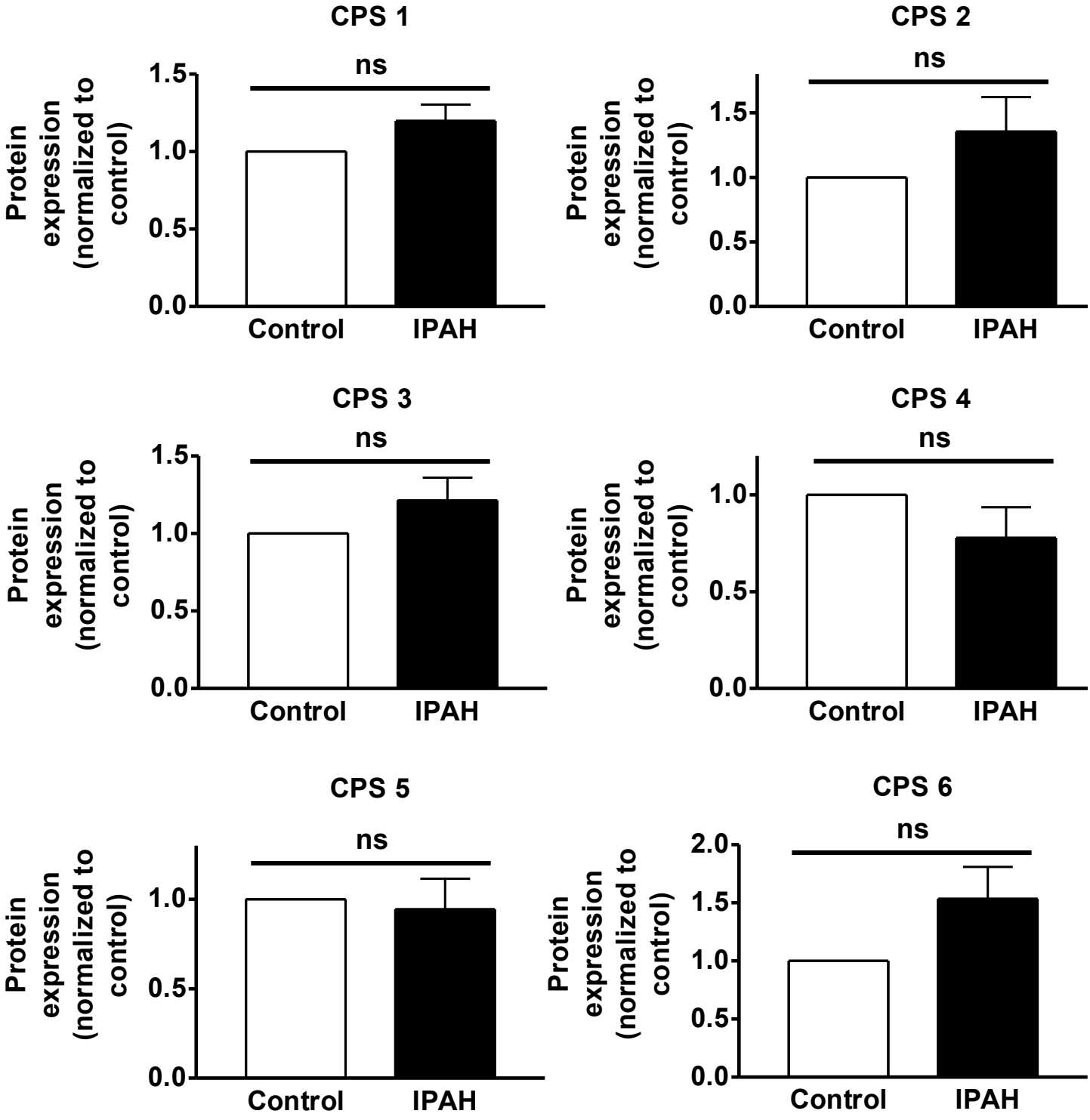

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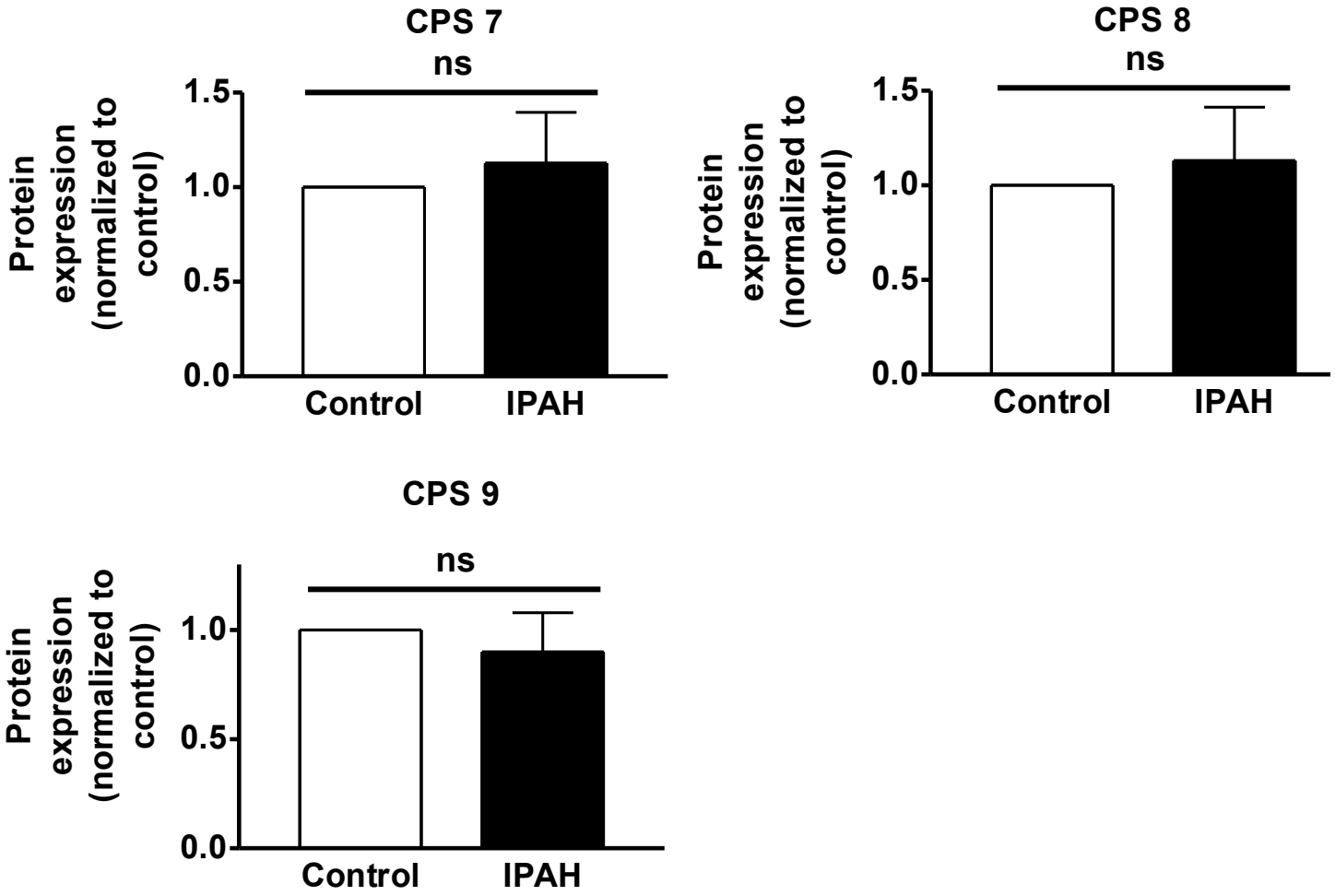

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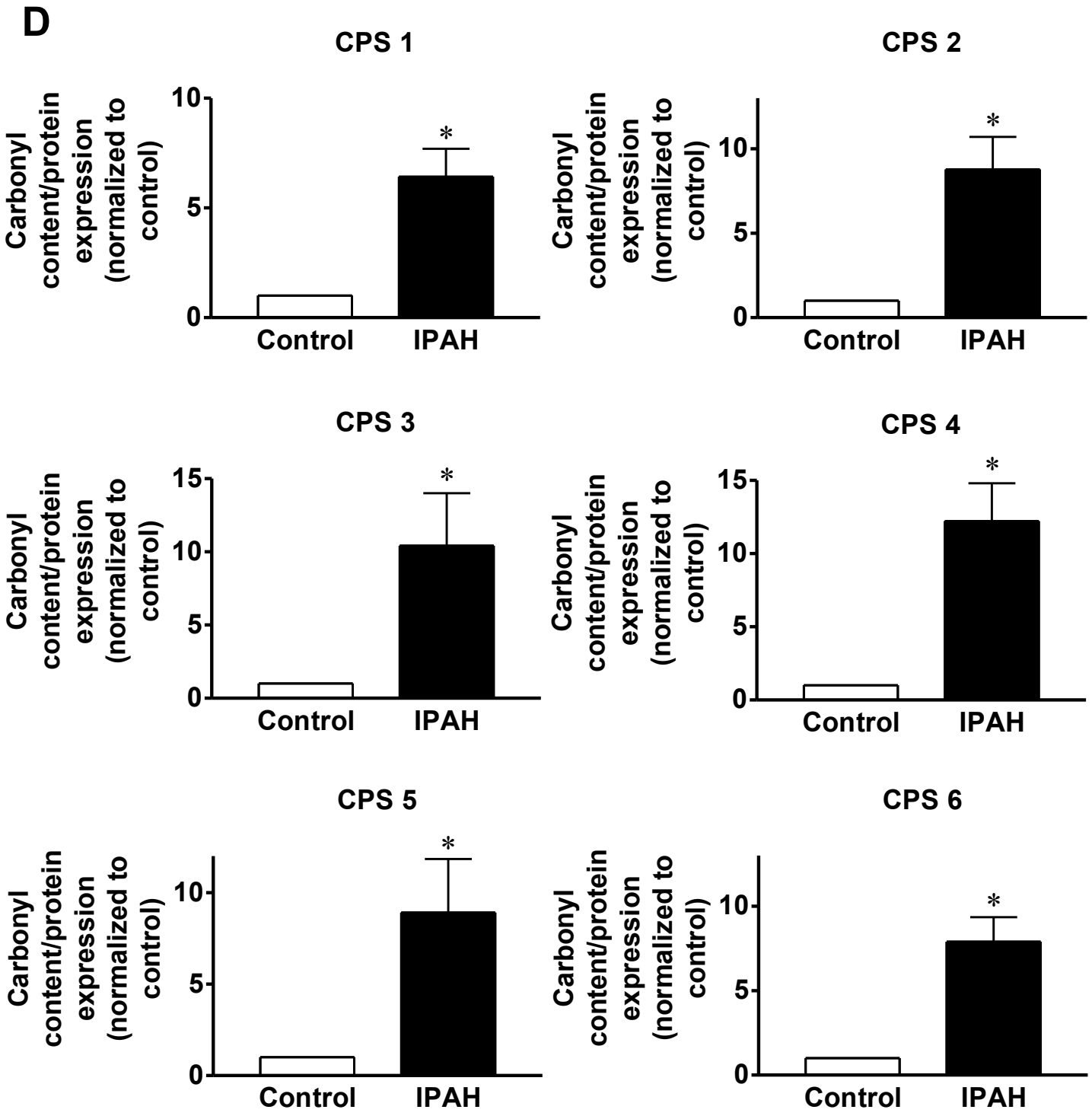

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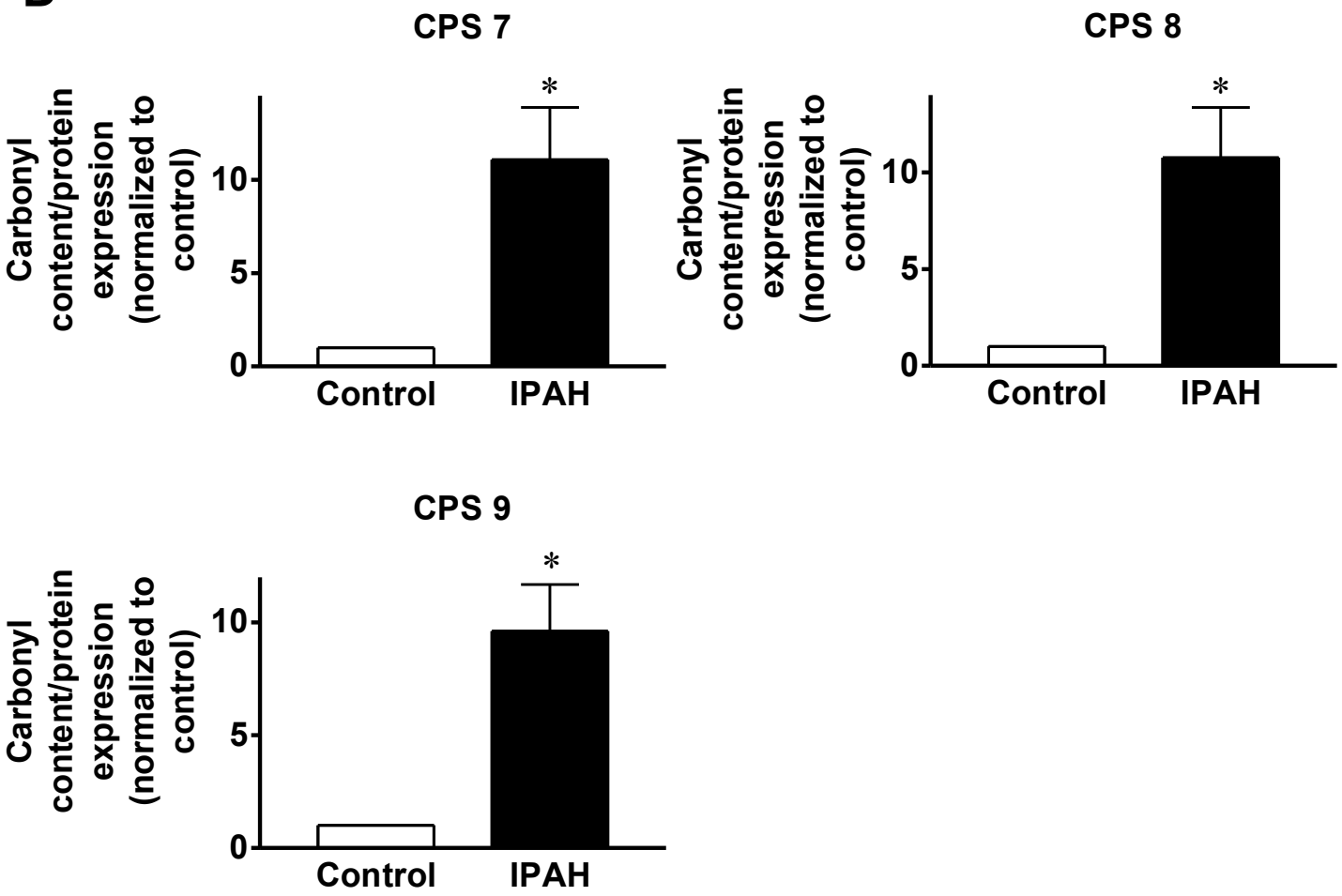

Fig. S9 Proteomic analysis of carbonylated proteins in IPAH. (A) Representative 2-dimensional gels with 9 carbonylated protein spots (CPS) that were identified to have significantly higher carbonyl content in IPAH samples compared to controls. (B,C,D) Bar graphs represent means ± SEM (n = 5). *, $P<0.05$. ns, not significantly different.

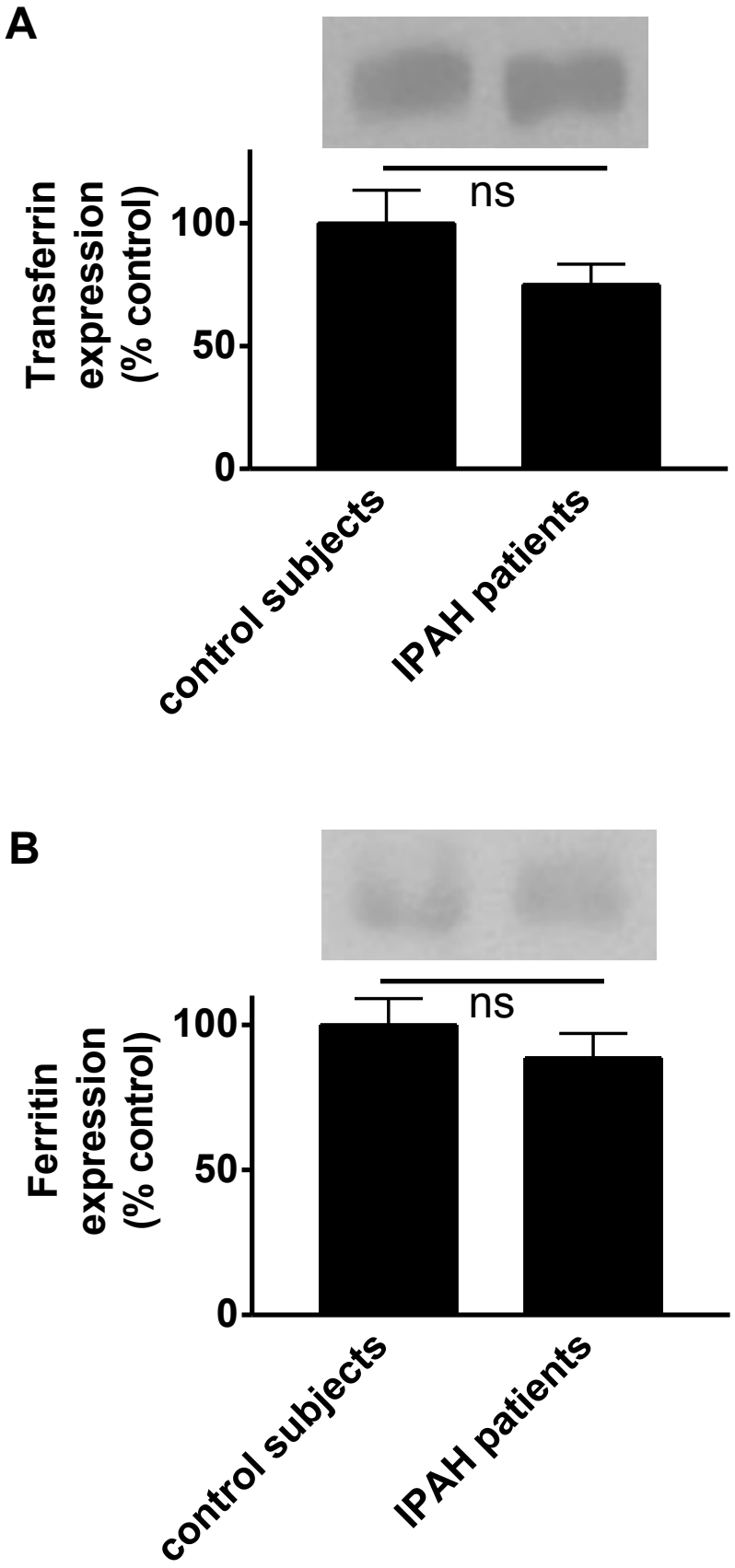

Fig. S10 Transferrin and ferritin levels in IPAH patients and control subjects. Plasma samples from IPAH patients and age- and gender-matched control subjects were subjected to Western blotting with (A) transferrin and (B) ferritin antibodies. Bar graphs represent means ± SEM (n = 6 – 7). ns, not significantly different.