Supplemental data

Figure legends

Figure 1. Specificity of Kv4.3 antibody. (A) In the rat spinal cord (only the right side is shown), the mouse anti-Kv4.3 (mKv4.3, NeuroMab, 1:200) and the rabbit anti-Kv4.3 (rKv4.3, Alomone, the AN01-04 batches, AN04 as a representative, 1: 200) show the same immunostaining patterns in lamina II. The non-specific rabbit anti-Kv4.3 (rKv4.3, Alomone, the AN05-07 batches, AN05 as a representative, 1:500) also labels many cell bodies in the ventral horn. (B) In the confocal images of rat DRGs, the NeuroMab mKv4.3 (1:150) and the Alomone rKv4.3 (AN04, 1:150) antibodies label the same subset of DRG neurons. 94% of mKv4.3(+) neurons (226/241 of total cell counts) show rKv4.3 IR and 97% of rKv4.3(+) neurons (218/225 of total cell counts) show mKv4.3 IR. Scale bar in B: 510 μm (A, left), 470 μm (A, middle), 540 μm (A, right); 73 μm (B).

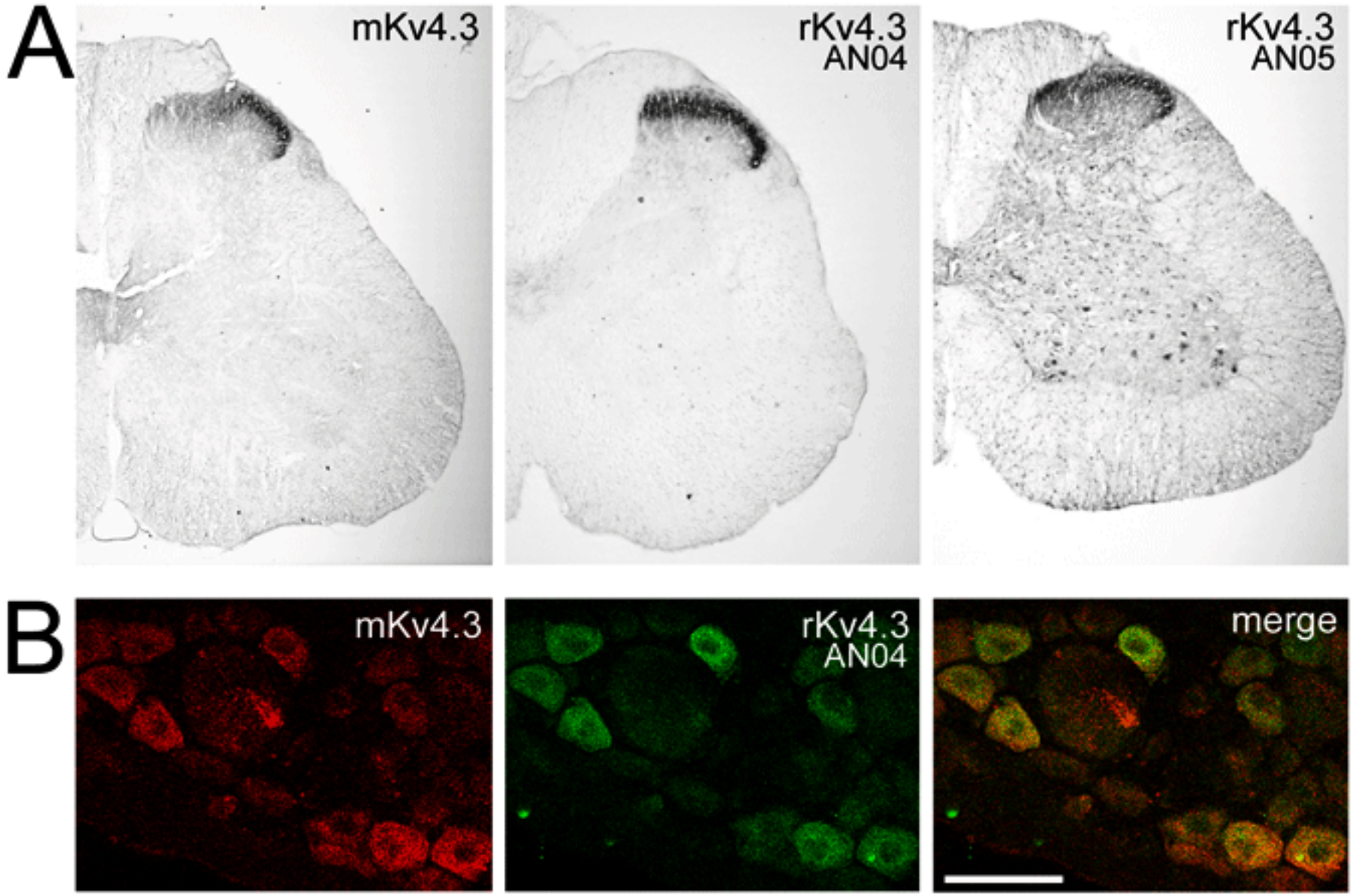
Figure 2. Quantification of IR in DRG neurons. A representative image (Kv3.4 IR in the L6 DRG of non-ligated side) is used for illustration. Cells with obviously positive IR (arrowheads) were randomly selected, circled along the somatic surfaces, and the IR within each circled area was measured. The average of 20 positive cells was assigned as the sample value. Cells without significant IR in the same image (arrows) were measured and the average of 5 negative cells was assigned as the background

value. Scale bar, 25 μm.

Figure 3. Apoptosis of DRG neurons 7 days after spinal nerve ligation. Sections of L5 DRGs were processed with terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) followed by sheep anti-fluorescein antibody conjugated with horse-radish peroxidase (Roche, Mannheim, Germany). After visualization by diaminobenzidine reaction in the presence of ammonium nickel sulfate, apoptotic nuclei (black color in the middle of cells, arrows) were detected in both large- (upper panel) and small-sized (lower panel) neurons. For the labeling of normal nuclei (light brown color in the middle of cells, arrowheads), DRG sections were further immunostained with anti-NeuN and processed for diaminobenzidine reaction. Scale bar: 38 μm (upper panel), 33 μm (lower panel).

Figure 4. Fluorescence intensity in the L5 DRG neurons after intrathecal injection with a single dose of fluorescence-tagged ODNs. Except the image A under bright field, the images B-G were taken under dark field with 1 s of exposure time by a 20X objective lens in Nikon Eclipse E800 microscope connected to a fluorescent light source. Rats injected with vehicle (A and B, as background) or non-fluorescence-tagged Kv3.4 antisense ODNs (C, as control) were sacrificed 5 h later. (D-G) Rats injected with fluorescence-tagged antisense Kv3.4 ODNs were sacrificed 3 h (D), 5 h (E), 8 h (F), or 12 h (G) later. (H) Quantitative data. The intensity of fluorescence was measured by the ImageJ 1.36b

software (National Institutes of Health, Bethesda, MD, USA). N = 3, ** $p < 0.01$, *** $p < 0.001$, comparing each time point with the control by Student's *t* test. Scale bar in G: 95 μm (A-G)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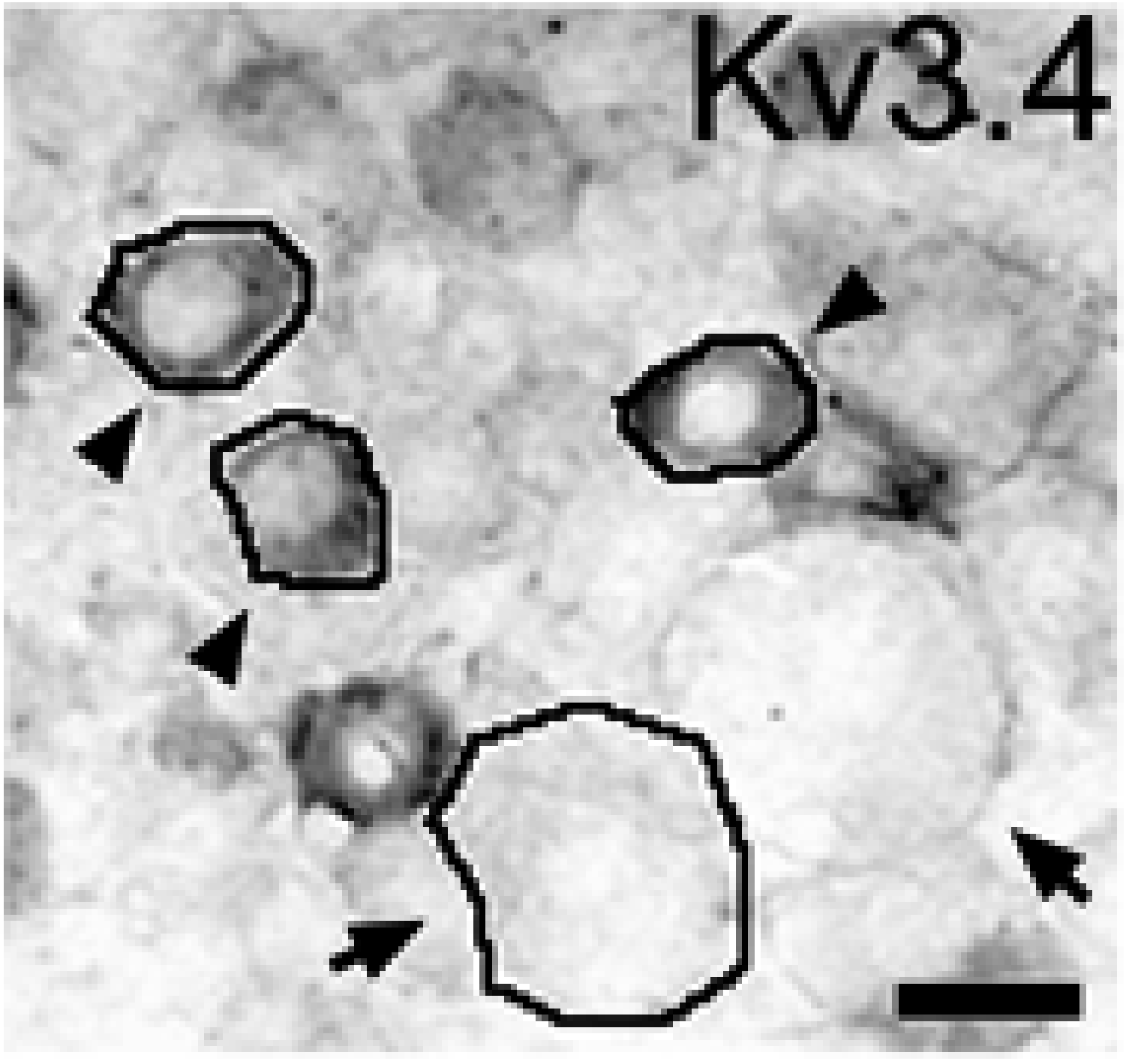
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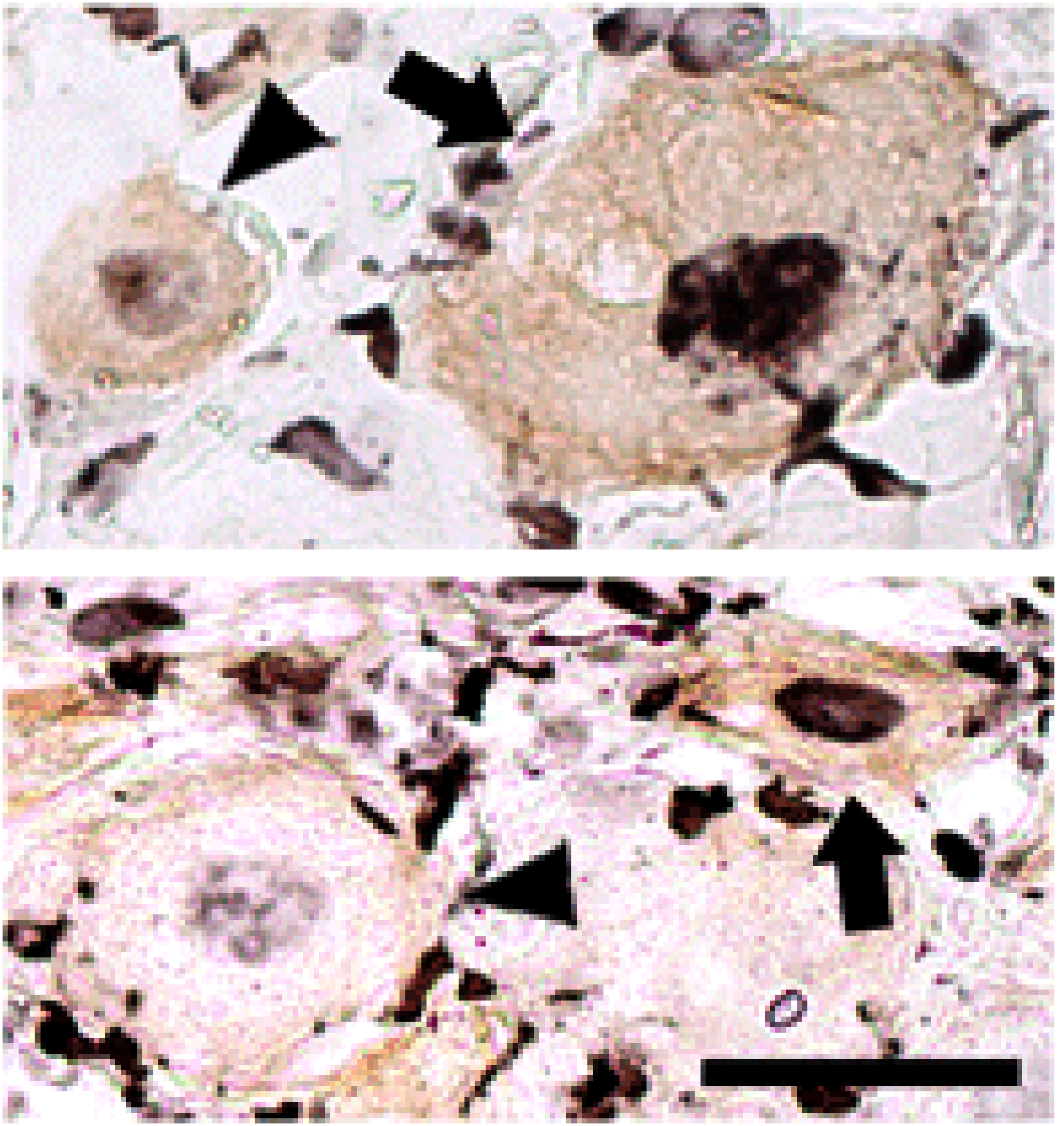

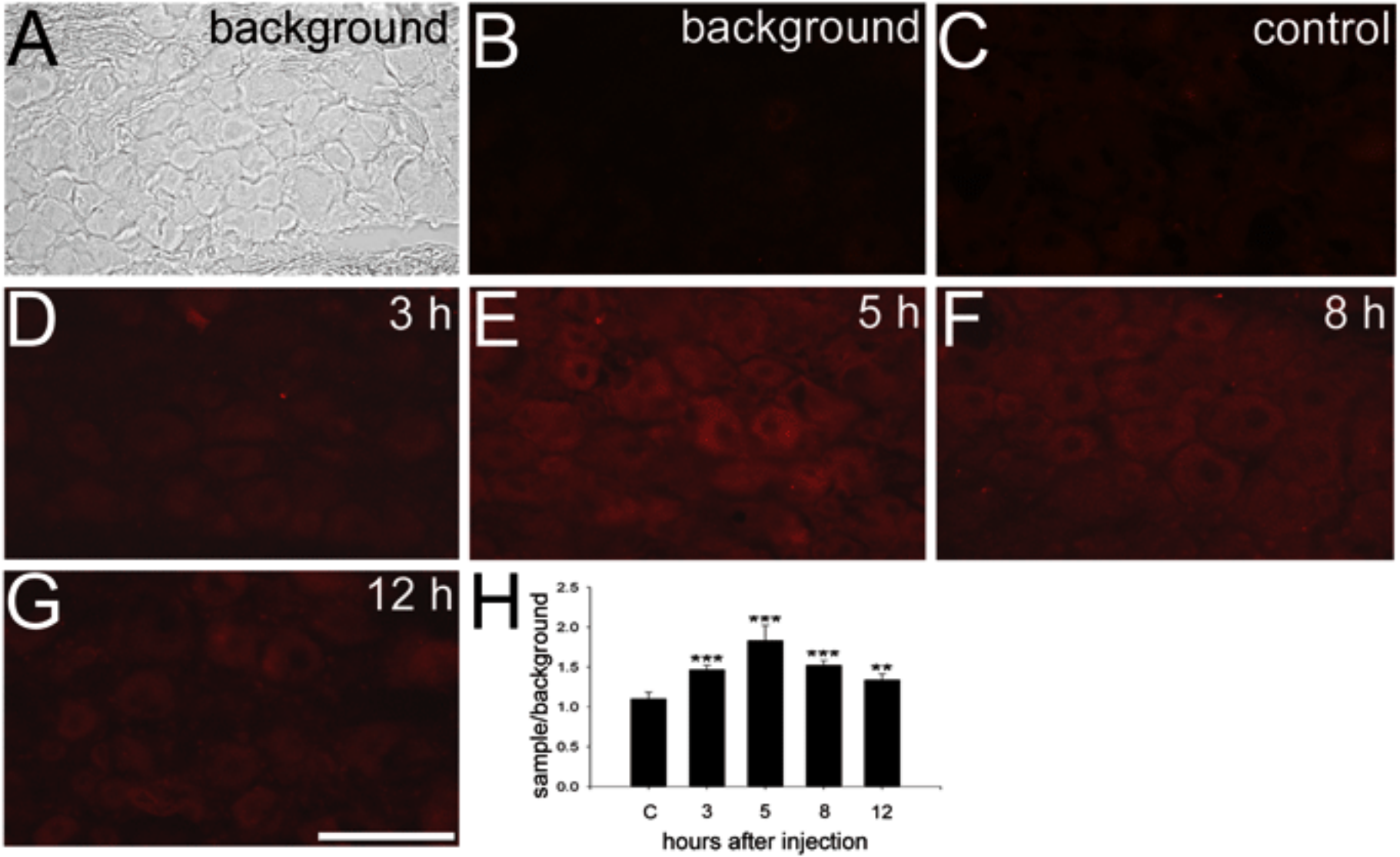
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background
C
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D
3 h
E
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G
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sample/background
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1.0
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C
3
5
8
12
hours after injection