Pain Reports

Supplemental materials

Transmembrane protein 100 is expressed in neurons and glia of dorsal root ganglia and is reduced following painful nerve injury (five figures)

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Supplemental Figure 1

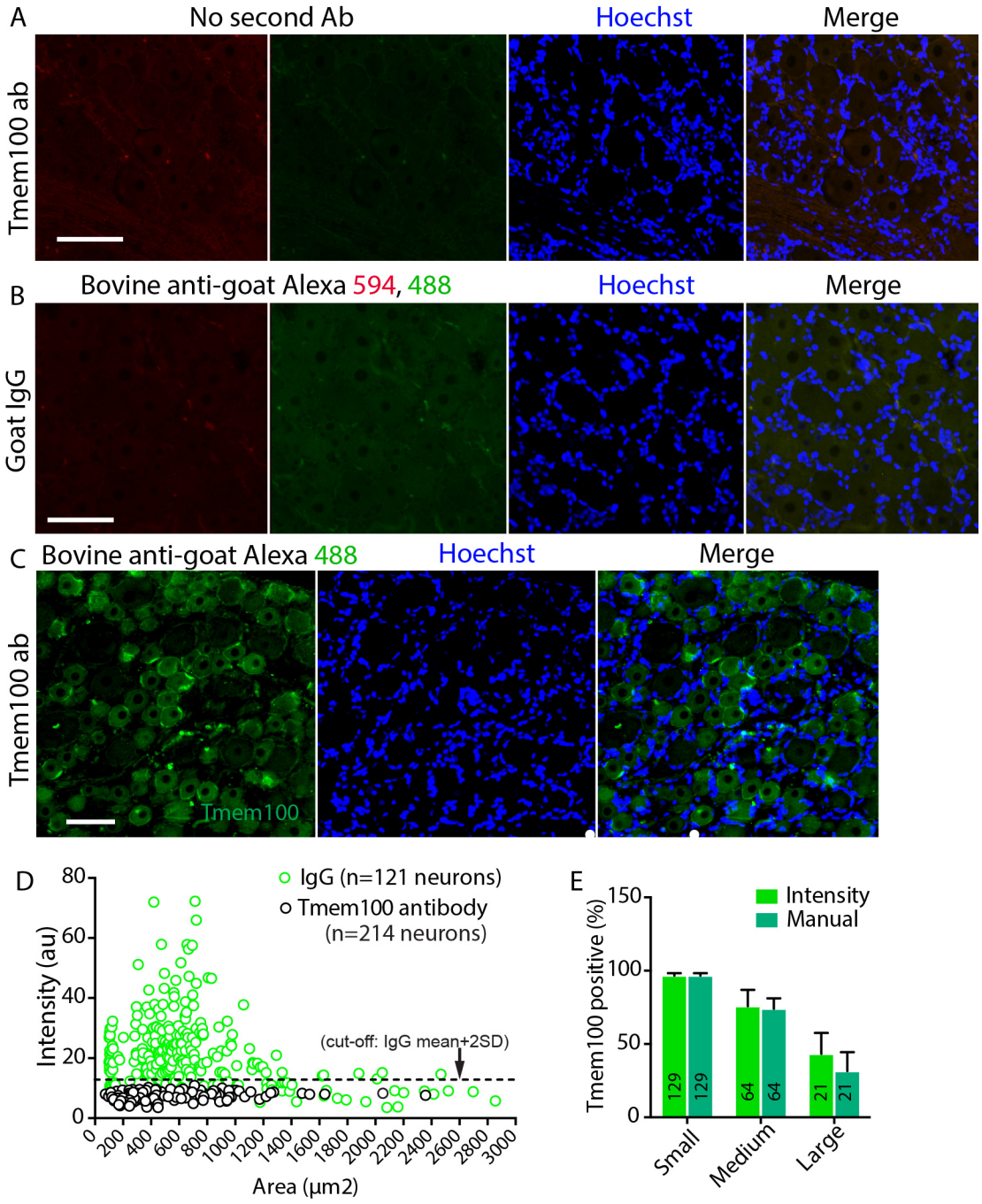

Figure S1. Controls for Tmem100 IHC and positive standardization.

Red and green color images are captured after goat Tmem100 (1:100) antibody staining (no second antibody staining) of DRG section from naïve rat, with Hoechst nuclear counterstain and merged images in the panels as indicated (**A**). Second antibody negative controls for double-labeling performed using goat IgG (**B**) followed by Alexa 594- or 488-conjugated bovine anti-goat second antibodies. IHC delineates clear Tmem100 immunopositivity (**C**) compared to controls (A and B). A scatter plots show size-dependent immunostaining intensity of Tmem100 in DRG neurons, with a dashed line indicating cutoff level of negative controls (**D**). The unassisted observer-based method (Manual) for tmem100 immunopositivity is highly comparable to the results of photoshop software assisted digital intensity analysis (Intensity, **E**). The number in each bar is the number of analyzed by Intensity and Manual immunopositive annotation, respectively, in small-, medium, and large-sized neurons. Scale bar: 100μm for all panels.

Supplemental Figure 2

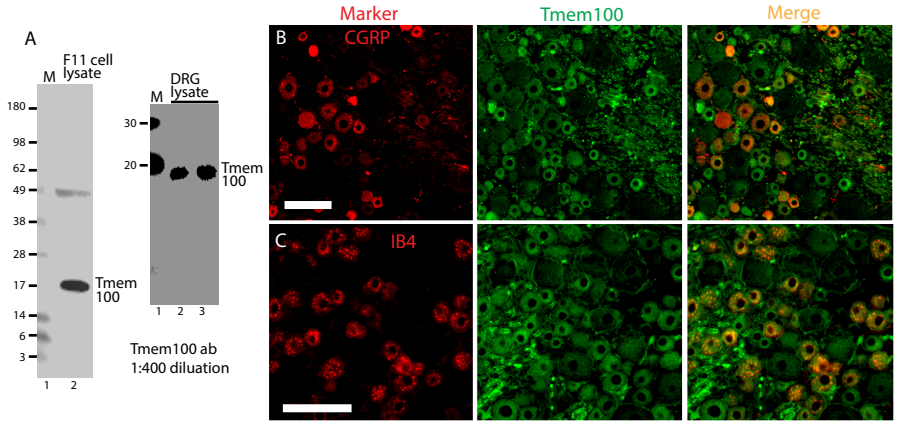

Figure S2. Characterization of Tmem100 using a rabbit anti-Tmem100 antibody. Immunoblots shows Tmem100 expression on the lysates from F11 cell (rat DRG neurons) (**A, left panel**) and DRG tissue (**A, right panel**) using a rabbit anti-Tmem100 antibody. Representative IHC montage images show Tmem100 colocalization with CGRP (**B**) and IB4 (**C**) staining in naïve DRG sections. Scale bar: 100μm for all panels.

Supplemental Figure 3

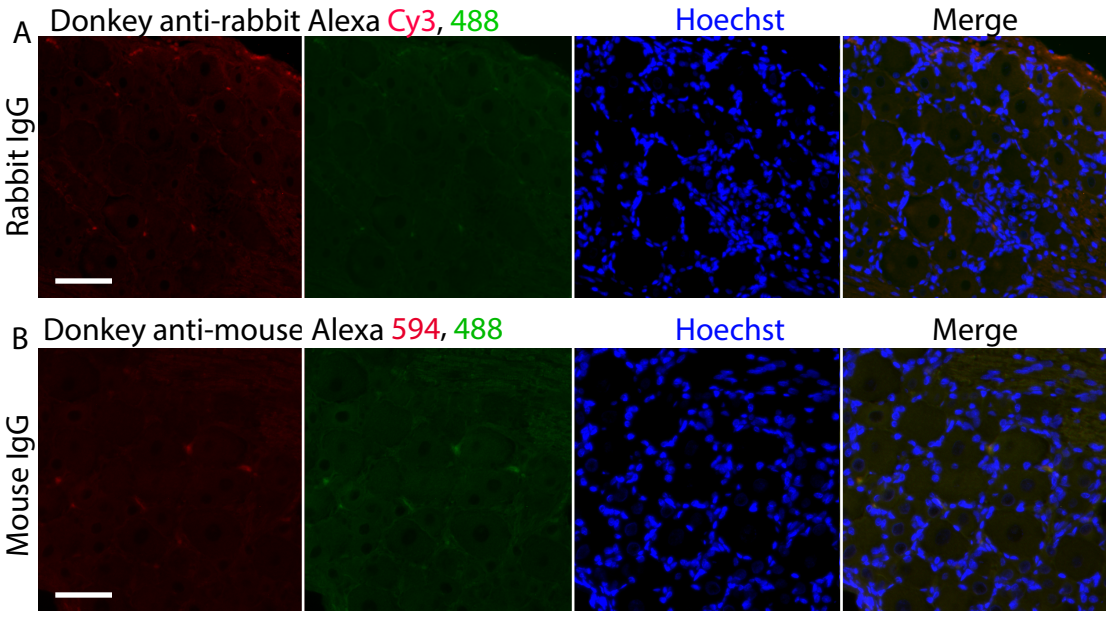

Figure S3. Negative controls for IHC using rabbit and mouse antibodies.

Representative montage images show staining using rabbit IgG (**A**) or mouse IgG (**B**) followed by donkey anti-rabbit alexa cy3-conjugated (red) or alexa 488-conjugated (green) second antibodies on DRG sections from naïve rat, with Hoechst nuclear counterstain and merged images in the panels as indicated. Scale bar: 100μm for all panels.

Supplemental Figure 4

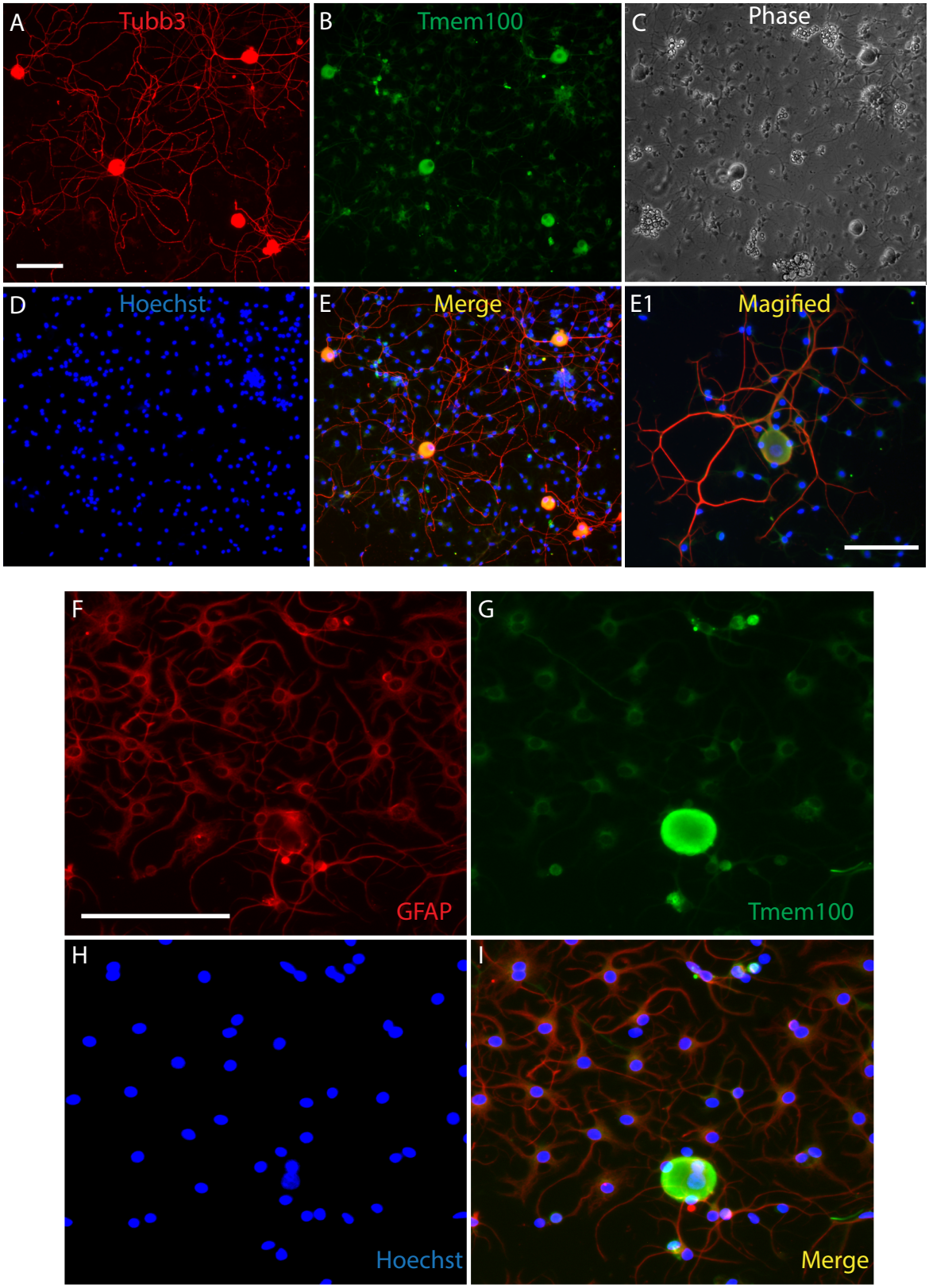

Figure S4. ICC of Tmem100 expression in DRG dissociated cultures.

The panels **A-E1** show the representative images of immunostaining and phase images of pan-neuronal PM marker Tubb3 (**A**, red), Tmem100 (**B**, green), Phase (**C**), Hoechst cell nuclear (**D**, blue), showing Tmem100 and Tubb3 colabeling in the merged image (**E**), with a magnified image from a different field (**E1**). The panels **F-I** show the representative images of immunostaining of GFAP (**F**, red), Tmem100 (**G**, green), Hoechst (**H**, blue), showing Tmem100 and GFAP colabeling in the merged image (**I**). Scale bar: 100μm for all panels.

Supplemental Figure 5

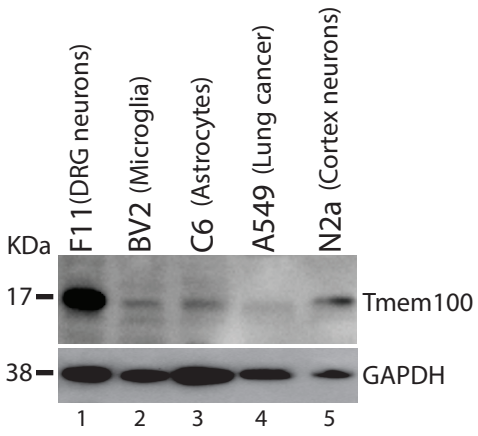

Figure S5. Immunoblot analysis of Tmem100 expression in different cell lines. Cell lysates were prepared from the various neural cell lines including F11 (rat DRG neurons), BV2 (mouse microglia), C6 (rat astrocytes), and N2A (mouse brain cortex neurons), as well as A549 (human non-small-cell lung cancer, NSCLC), as indicated. 40μg of each cell lysate protein was loaded on the gel and analyzed by immunoblotting using a rabbit anti-Tmem100 antibody (top panel). GAPDH immunoblotting (bottom panel) was used as the loading control.