SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Analysis of Band 4.1 protein expression in wild type and 4.1B-/- brains. (A); Detergent-soluble lysates from cerebral cortices of wild type and 4.1B-/- neonatal mice were immunoblotted with anti-4.1B, anti-4.1G, and anti-4.1N rabbit polyclonal antibodies. **(B);** Genomic DNA isolated from wild type, 4.1B+/-, or 4.1B-/- tail snips was analyzed using synthetic PCR-based methods to amplify specific regions of the Protein 4.1B gene.

Supplemental Figure 2. Protein 4.1B is dispensable for astrocyte adhesion to fibronectin. (A, B); Wild type (A) and 4.1B-/- astrocytes (B) were plated on a fibronectin-coated dishes for 30 minutes and then stained with phalloidin-Texas Red to visualize the actin cytoskeleton. Note that comparable morphologies in wild type and 4.1B-/- adherent cells. **(C);** Wild type and 4.1B-/- astrocytes were plated on a fibronectin-coated dishes for 30 minutes and then stained with crystal violet. 4.1B-dependent cell adhesion was quantified by measuring absorbance readings at 562 nm. Note that Protein 4.1B is not essential for astrocyte adhesion to fibronectin. Images are shown at 400x.

Supplemental Figure 3. Proteins 4.1B, 4.1G and 4.1N show diffuse expression patterns in astrocytes during late stages of spreading on fibronectin. (A-C); Antibodies directed against Protein 4.1B (A), 4.1G (B), and 4.1N (C) were used to immunolabel sub-confluent cultures of wild type and 4.1B-/- astrocytes 24 hours after adhesion to fibronectin. Note the diffuse, cytoplasmic patterns of expression in wild type and 4.1B-/- cells. Images are shown at 400x.

Supplemental Figure 4. Proteins 4.1B and 4.1G, but not 4.1N, are enriched at sites of astrocyte cell-cell contact. (A-C); Analysis of sub-cellular localization of Protein 4.1B, 4.1G and 4.1N in confluent monolayers of astrocytes. Antibodies directed against Protein 4.1B (A), 4.1G (B), and 4.1N (C) were used to immunolabel wild type and 4.1B-/- astrocytes 24 hours after adhesion to fibronectin. Note that Proteins 4.1B and 4.1G, but not 4.1N, show sub-cellular localization at sites of direct cell-cell contact in both wild type and 4.1B-/- cells. Images are shown at 400x.

Supplemental Figure 5. Protein 4.1B is dispensable for astrocyte polarity and migration in vitro. (A); Confluent monolayers on mouse brain astrocytes were scratched and 8 hours later cells were immunostained with anti-4.1B, anti-4.1G and anti-4.1N antibodies. Note that the Band 4.1 proteins are not enriched at the leading edges of polarized astrocytes. **(B, C);** Wild type (B) or 4.1B-/- (C) astrocytes were scratched with a pipet tip and migrating cells were labeled with anti-GFAP antibodies at 0, 24 and 48 hours post-wounding. Note the apparently normal patterns of migration in wild type and 4.1B-/- astrocytes.

Supplemental Figure 6. Co-localization of Protein 4.1B and β1 integrins in vivo. (A-C); Horizontal sections through the cerebral cortices of E11.5 mouse embryos were immunofluorescently labeled with anti-4.1B (A) and anti-β1 integrin antibodies (B). Note that Protein 4.1B and β1 integrin co-localize to the plasma membrane throughout the neuroepithelium (C). In addition, β1 integrin but not Protein 4.1B, was also detected in cerebral blood vessels (arrows in B, C). Images are shown at 400x.

Supplemental Figure 7. Protein 4.1B is dispensable for reactive astrogliosis in vivo. (A-D); Paraffin-embedded coronal brain sections from wild type (A, C) or 4.1B-/- adult mice (B, D) before experimental-induced cortical brain injury (A, B) and three days after injury (C, D) were stained with H&E (upper panels) or immunofluorescently labeled with anti-GFAP antibodies (lower panels) to visualize astrocytes. Dashed white lines (C, D) indicate wound boundaries. Note that in the non-injured brain there are no differences in astrocyte morphologies in wild type or 4.1B-/- mice. Also, note that GFAP-expressing reactive astrocytes form glial scars in both control and 4.1B-/- injured brains.

Supplemental Figure 8. Protein 4.1B is not essential for injury-induced reactive astrogliosis in vivo. (A, B); Paraffin-embedded coronal brain sections from wild type (A) or 4.1B-/- adult mice (B) seven days after cortical injury were stained with H&E (upper panels) or immunofluorescently labeled with anti-GFAP antibodies (lower panels) to visualize astrocytes. Dashed white lines indicate wound boundaries. Note that GFAP-expressing reactive astrocytes form an astroglial scar in both control and 4.1B-/- injured brains.

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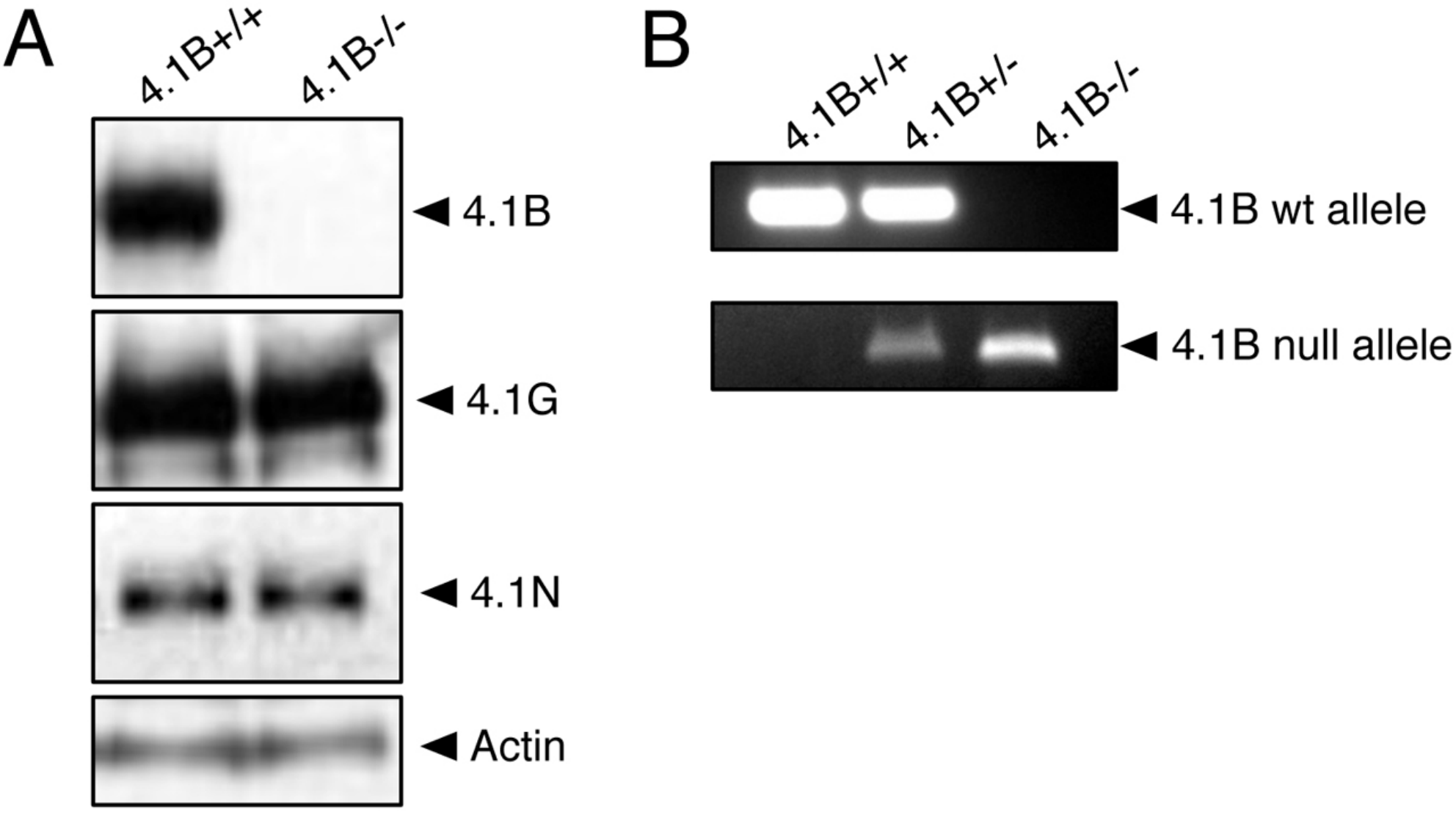
Supplemental Figure 1



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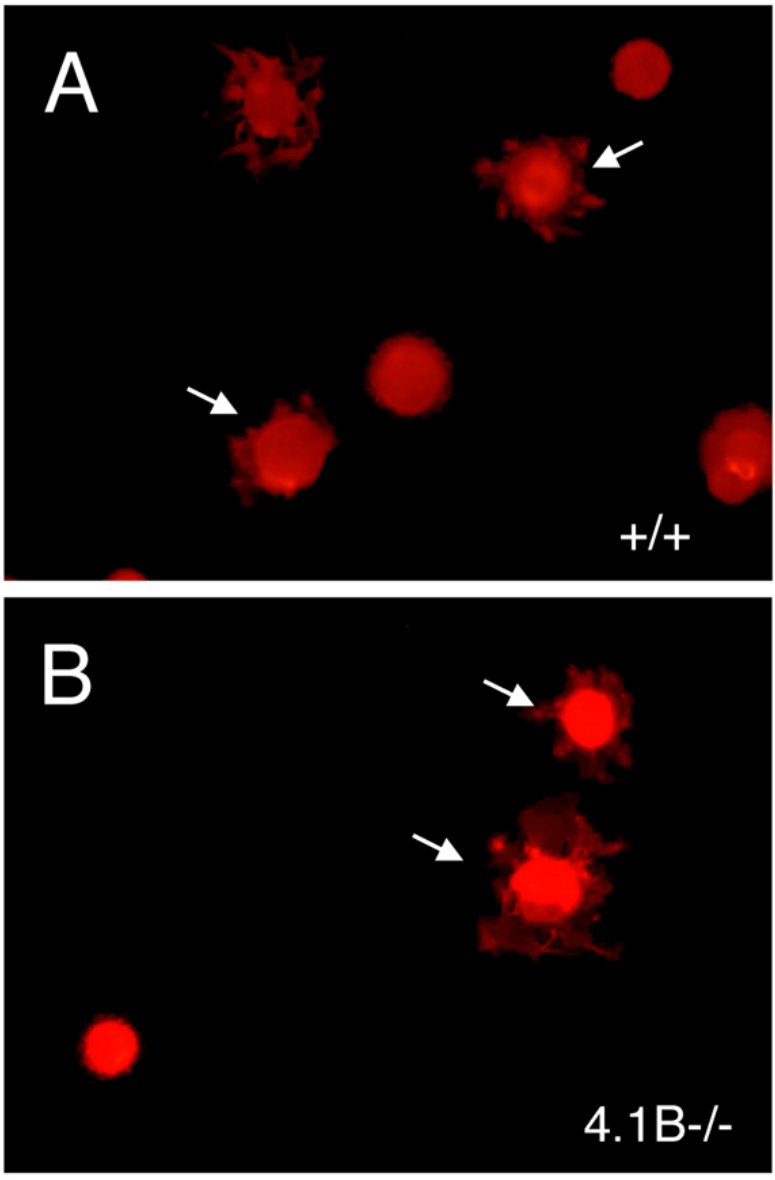
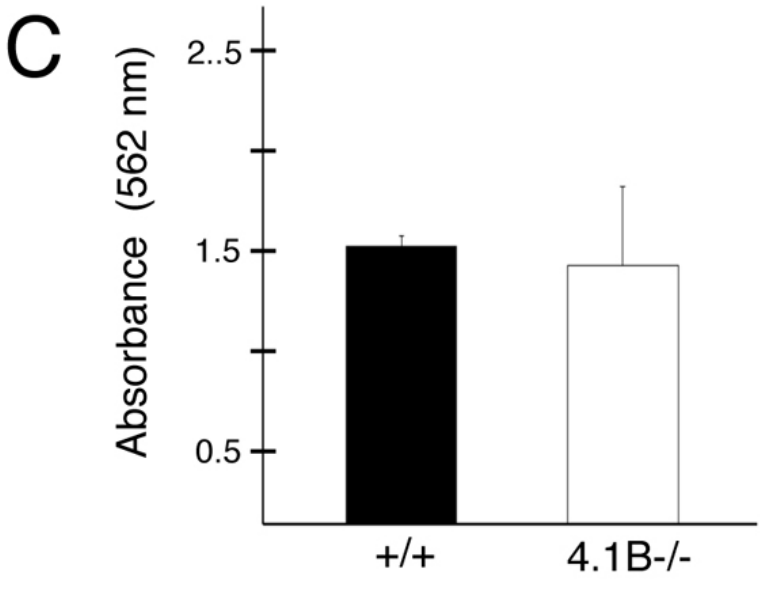
Supplemental Figure 2





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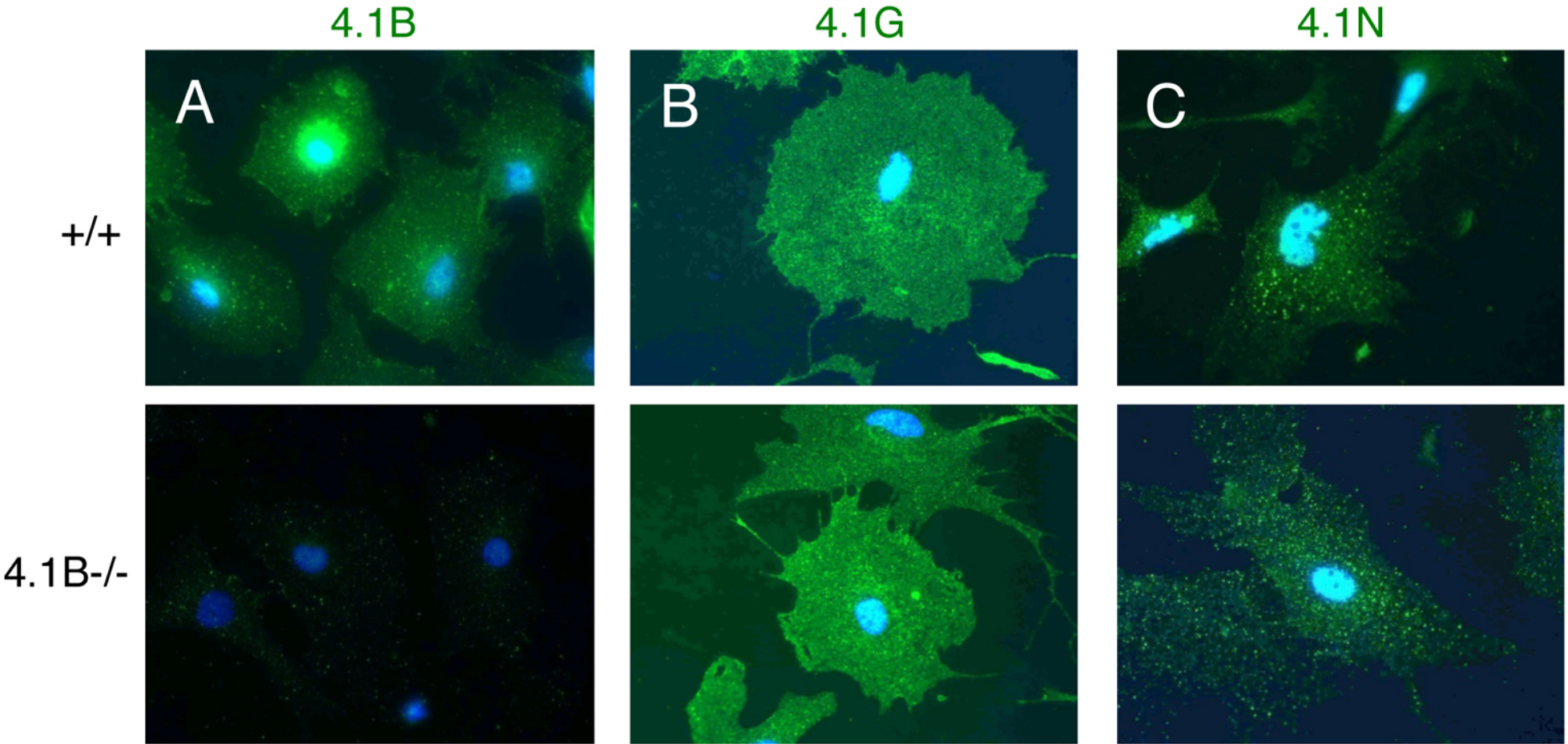
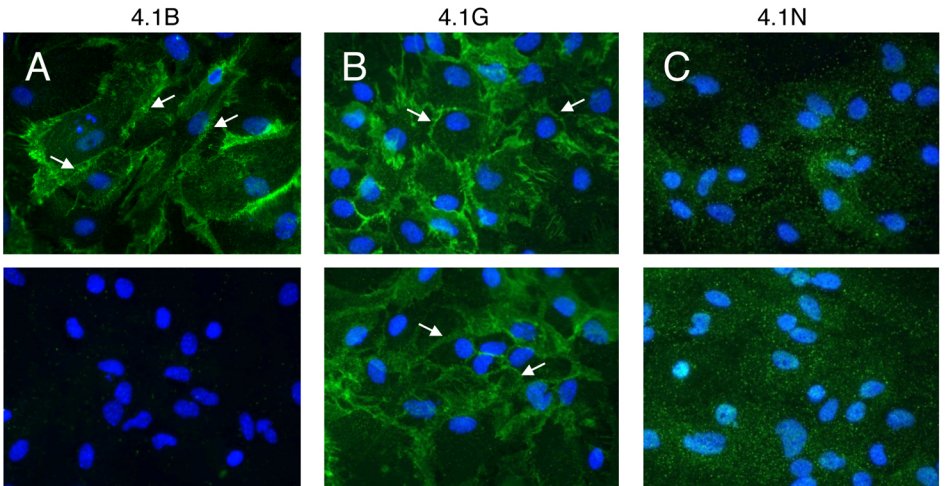
Supplemental Figure 3

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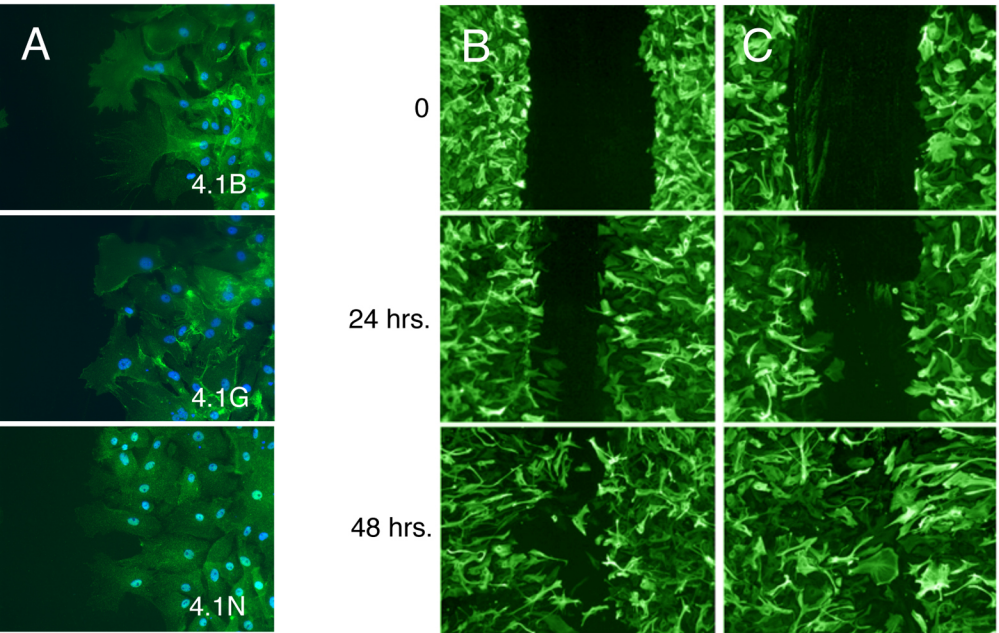
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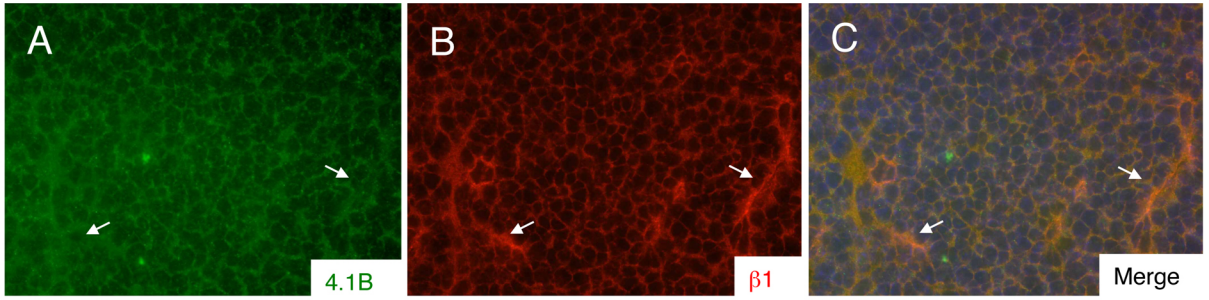
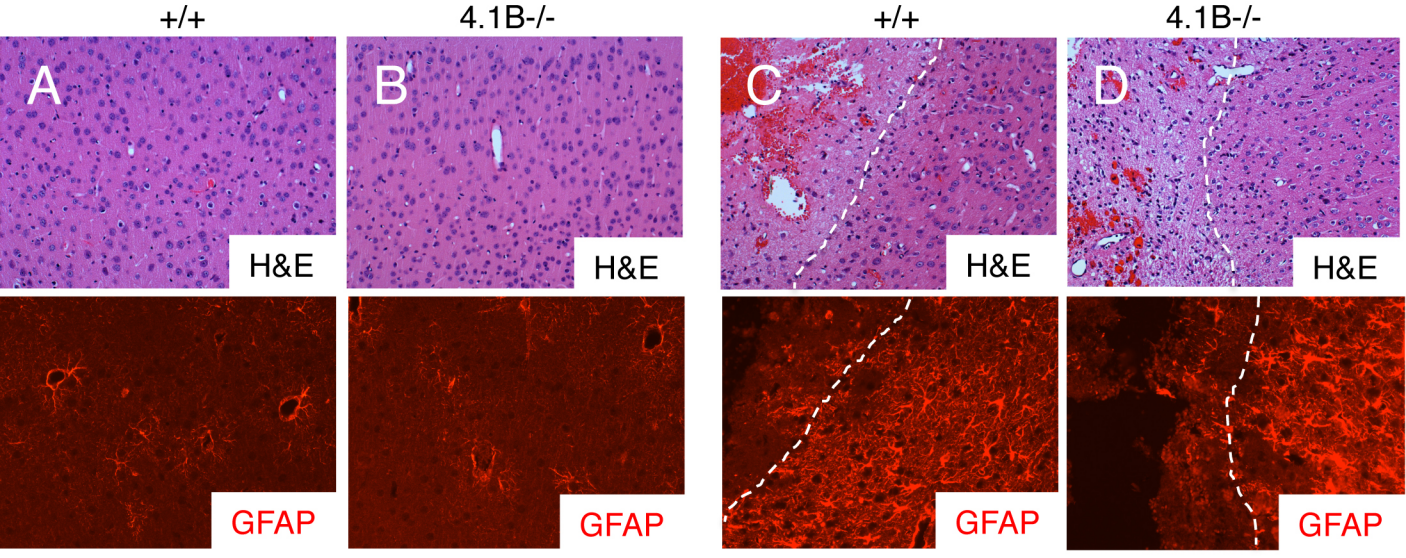
Supplemental Figure 6



Jung and McCarty,
Supplemental Figure 7



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Supplemental Figure 8