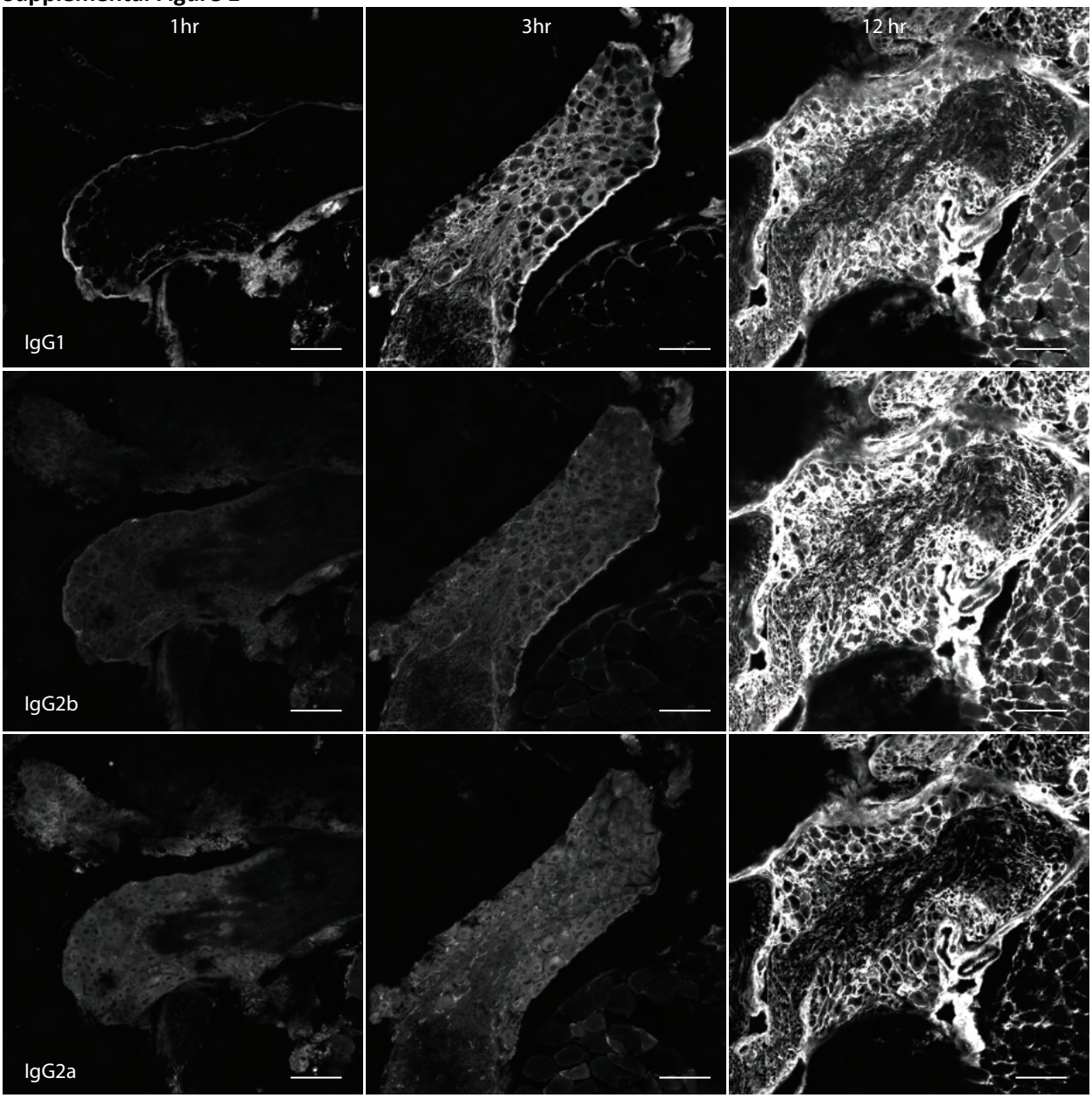
SUPPLEMENTAL FIGURES

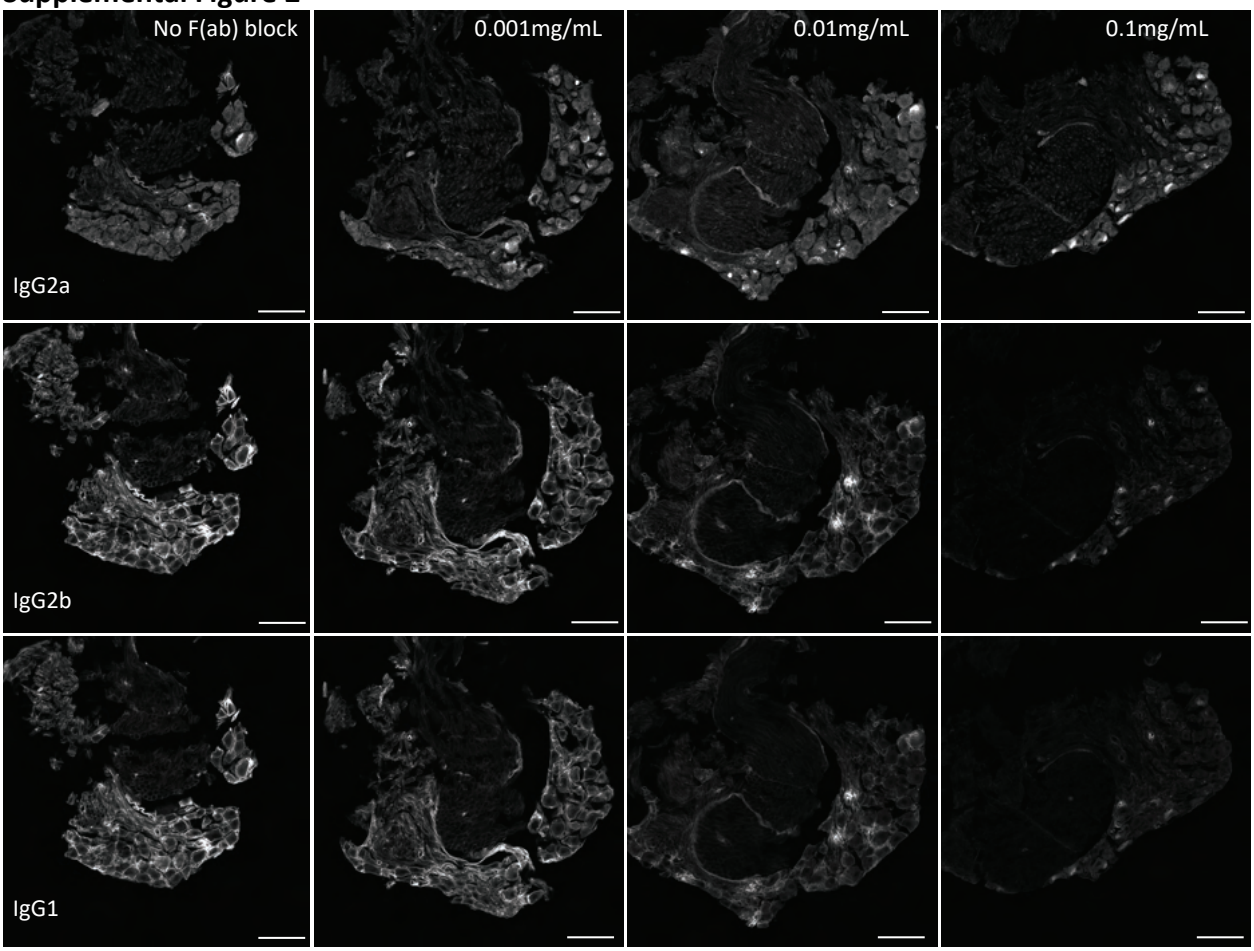
Supplemental Figure 1



Supplemental Figure 1 legend

Off-target mouse anti-IgG1, IgG2b, and IgG2a immunofluorescence increases with fixation time. Images of DRG sections labeled with fluorescently tagged antibodies which target IgG1 (top), IgG2b (middle) and IgG2a (bottom) after DRG were fixed in ice cold 4% PFA for 1, 3 and 12 hours. DRG sections are from the same mouse. Scale bars are 100 µm.

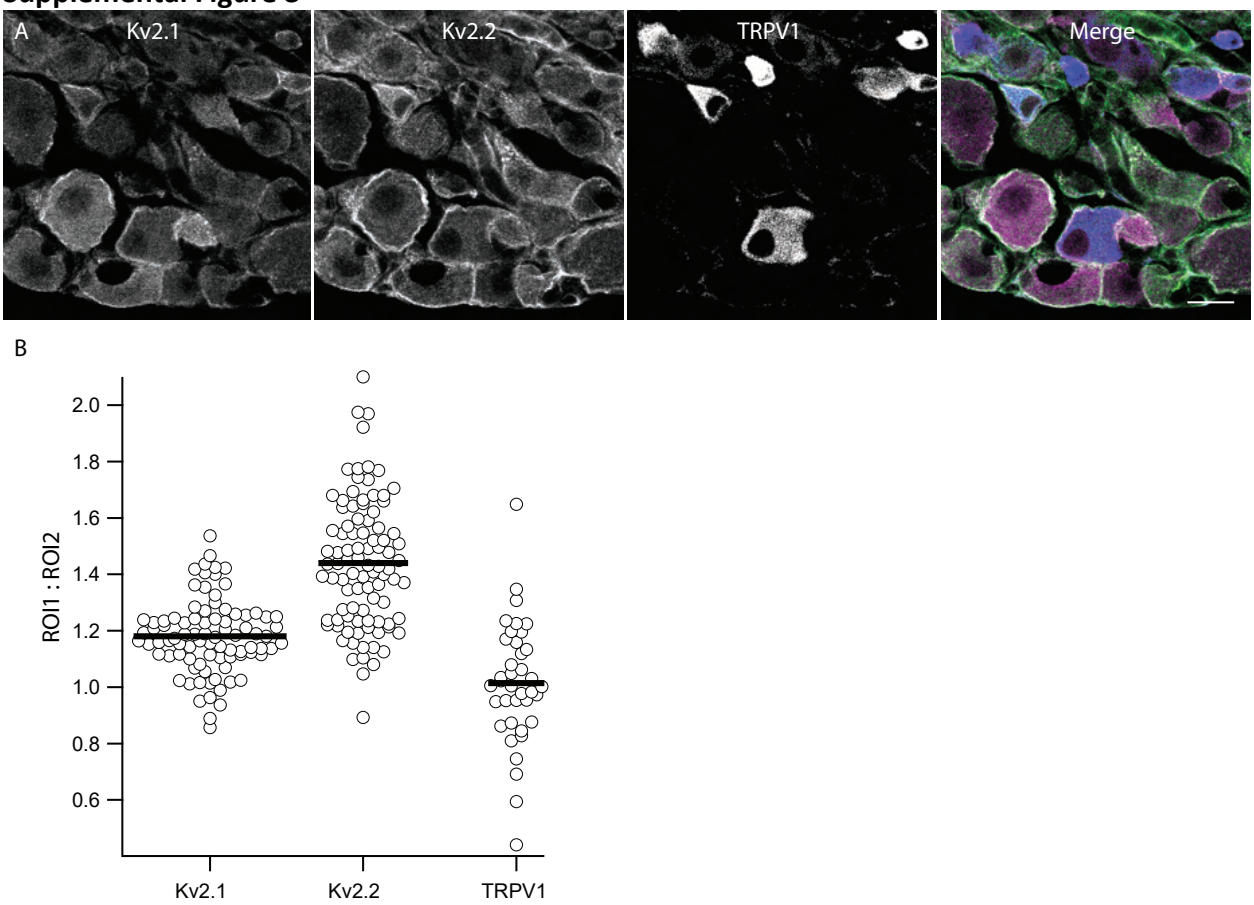
Supplemental Figure 2



Supplemental Figure 2 legend

Pre-incubation of mouse DRG sections in IgG H+L Fab fragments reduces off target secondary antibody labeling. Representative images of DRG sections from the same DRG treated with increasing concentrations (left to right) of IgG H+L Fab fragment and the same concentration of secondary antibody used in experiments throughout this study. Images were taken with identical imaging settings and are set to the same brightness and contrast. Scale bars are 100 µm.

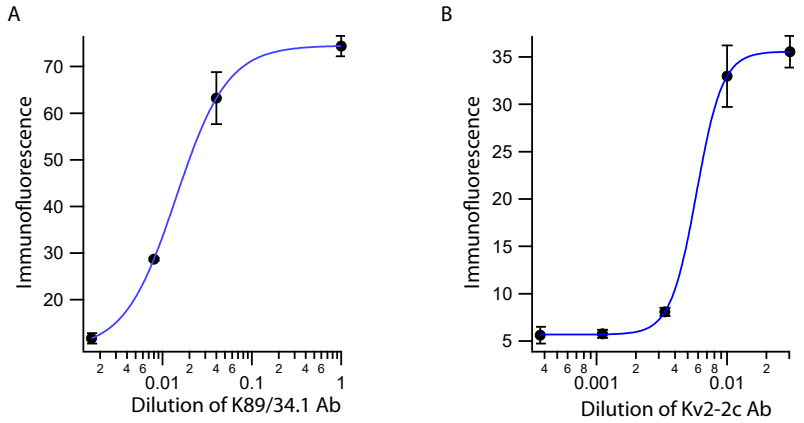
Supplemental Figure 3



Supplemental Figure 3 legend

Kv2.1 and Kv2.2 protein are enriched at the outer edge of DRG neuron somas relative to TRPV1. ***A***, Anti-Kv2.1, anti-Kv2.2 and anti-TRPV1 immunofluorescence from lumbar DRG neurons. Prominent cytoplasmic anti-TRPV1 immunofluorescence was observed in a subset of small diameter neurons. In merge image anti-Kv2.1, anti-Kv2.2 and anti-TRPV1 immunofluorescence are magenta, green and blue respectively. Scale bar is 20 µm. ***B***, Ratio of average anti-Kv2.1, anti-Kv2.2 or anti-TRPV1 immunofluorescence from outer and inner ROIs for individual neurons.

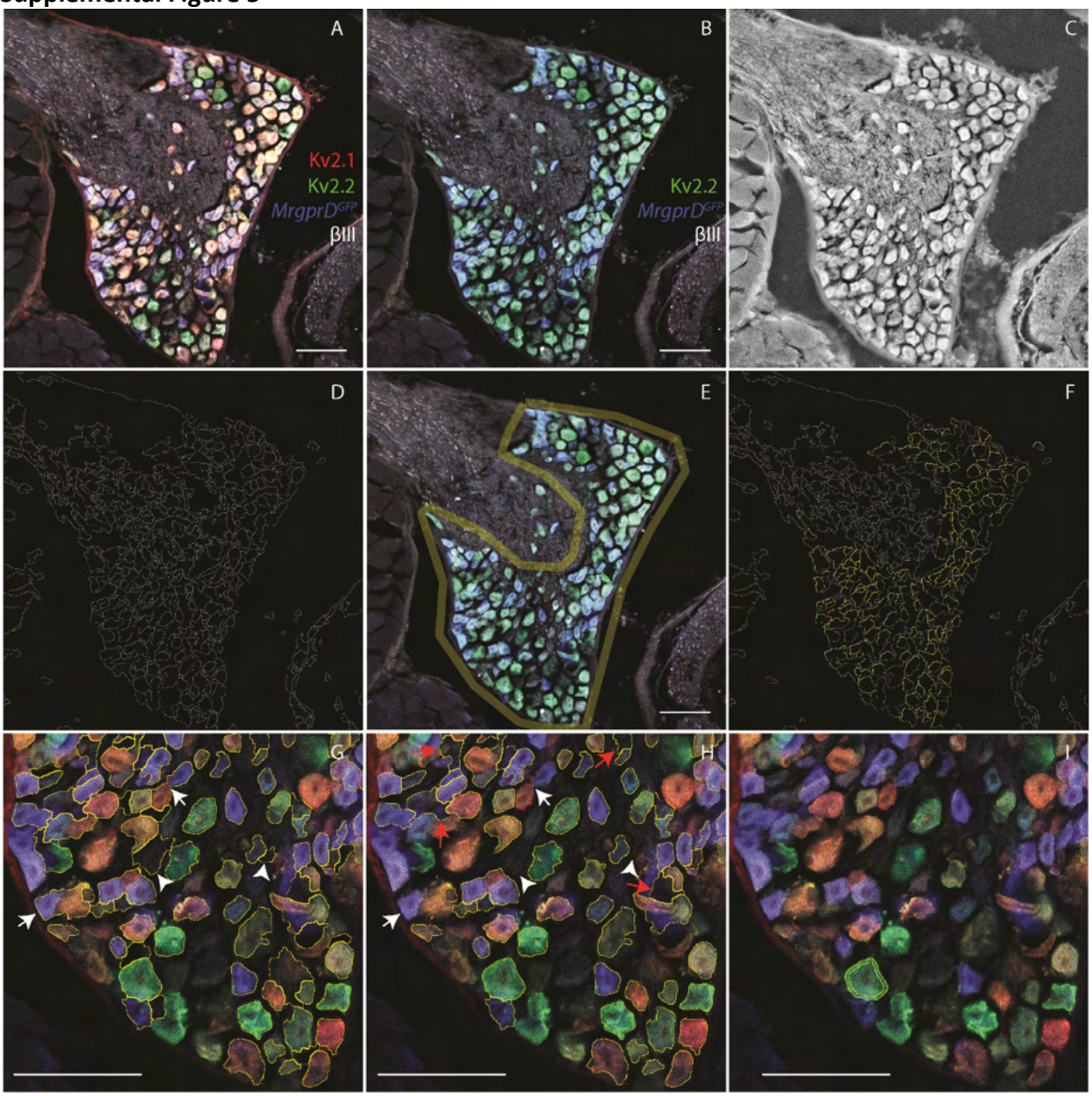
Supplemental Figure 4



Supplemental Figure 4 legend

Kv2 antibodies used in knockout experiments are at saturating concentrations. ***A***, Concentration response of immunofluorescence from sections labeled with anti-Kv2.1 antibody used in Figure 2. Blue line is a Hill fit of the data. 1:1 = 2 sections, 1:25 = 3 sections, 1:125 = 2 sections, 1:625 = 3 sections ***B***, Concentration response of immunofluorescence from sections labeled with anti-Kv2.2 antibody used in Figure 3. Blue line is a Hill fit of the data. 1:33 = 2 sections, 1:100 = 10 sections, 1:300 = 4 sections, 1:900 = 4 sections, 1:2700 = 3 sections

Supplemental Figure 5

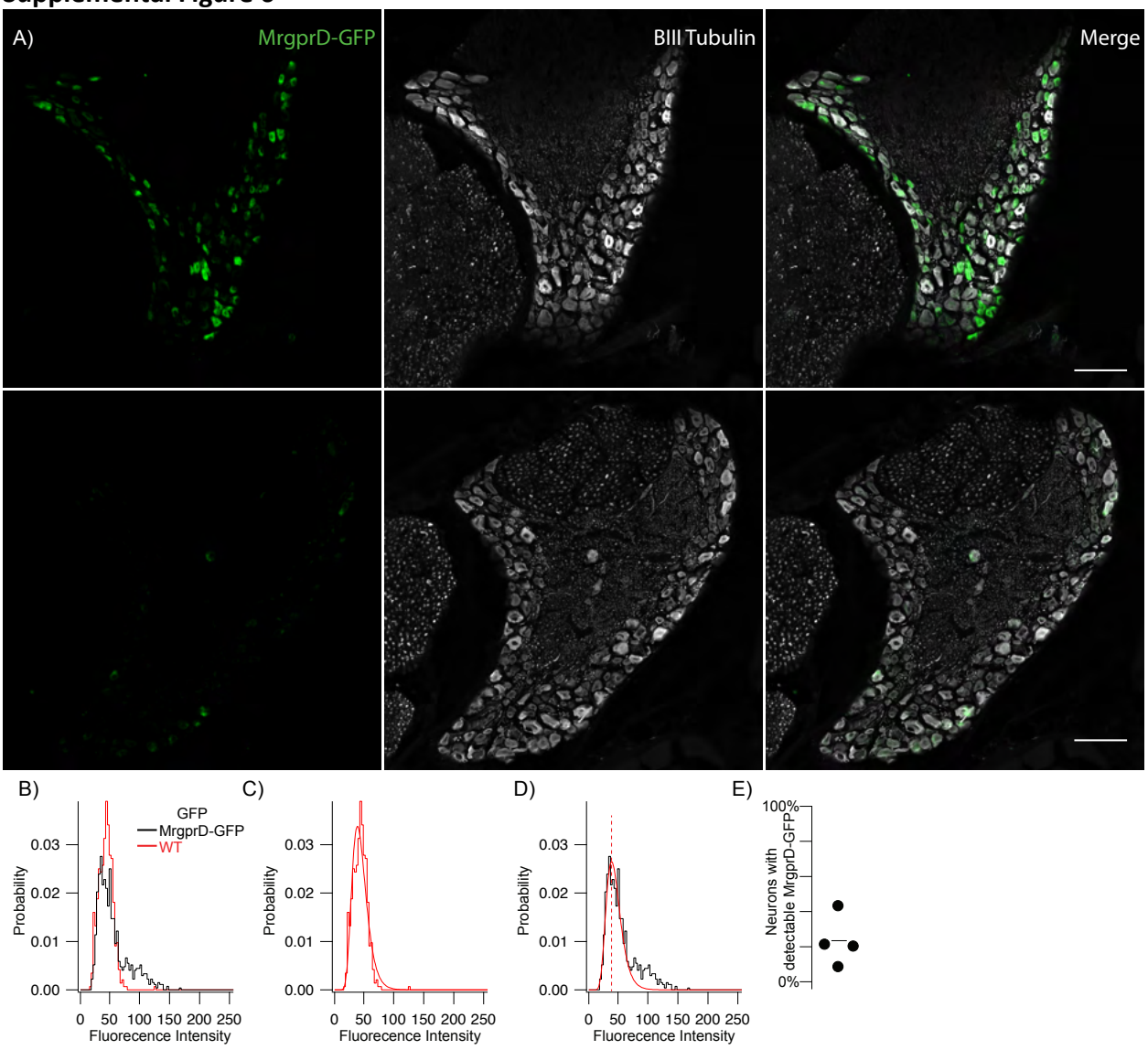


Supplemental Figure 5 legend

A method to sample neurons in DRG imaging data using watershed segmentation identifies the outer region of neurons. ***A***, Image of anti-Kv2.1, anti-Kv2.2, anti-βIII tubulin immunofluorescence and MrgprD-GFP fluorescence. ***B***, Same image as A with anti-Kv2.1 immunofluorescence channel removed as this is an example of processing data for Kv2.1 KO analysis. ***C***, Grayscale image of average fluorescence from all three channels shown in B. Gaussian and median filters were applied to image to improve watershed segmentation. ***D***, Watershed segmentation of image in C using the MorphoLibJ Morphological Segmentation Plugin in Fiji. ***E***, Example of manually drawn boundary that encompasses the neuron somas in the DRG so that only these watershed lines are selected. ***F***, Selected ROIs from watershed segmentation (yellow). ROIs were excluded based on roundness and size using the Analyze

Particles tool in Fiji. ***G***, ROIs in F overlaid on DRG image showing that some ROIs are selecting regions that do not contain neurons (arrow heads) or are selecting multiple neurons (arrows). ***H***, ROIs after processing using an in-house R script which removes ROIs that do not contain neurons (arrow heads) and ROIs that contain two neurons (arrows). This script did not remove all ROIs that do not contain neurons (red arrows). Each experiment performed was done alongside controls where the primary antibodies were omitted and fluorescence from these control sections was used by the in-house R script to identify and remove ROIs that do not contain neurons. ***I***, Example of automatically generated annulus that encompasses the outer edge of the soma. Scale bars are 100 µm.

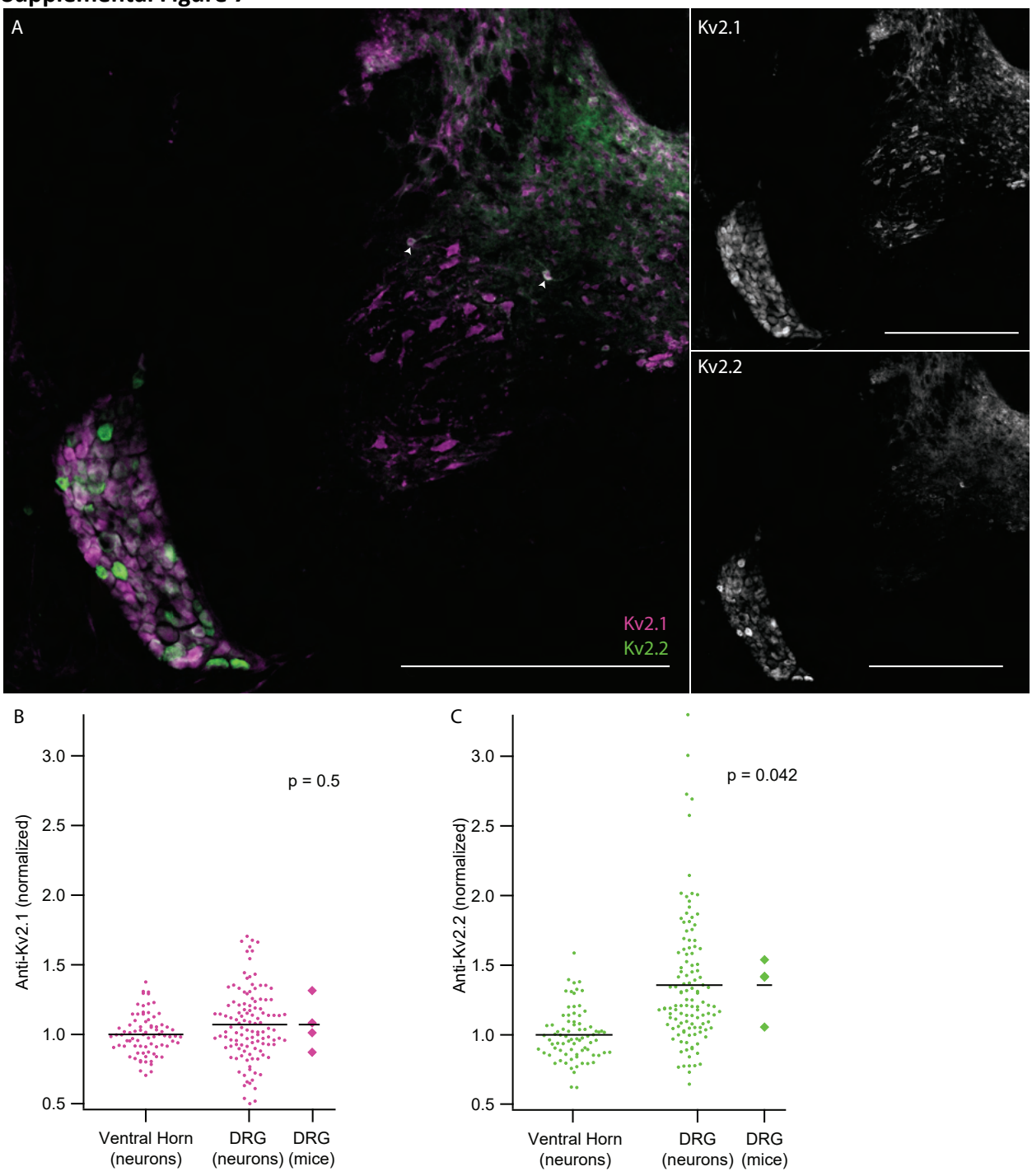
Supplemental Figure 6



Supplemental Figure 6 legend

Method used to estimate percent of neurons expressing Kv2.1 and Kv2.2 reliably predicts the percentage of neurons that express GFP in MrgprD-GFP mice. ***A***, MrgprD-GFP (top) and WT (bottom) DRG sections immunolabeled for BIII tubulin (white). Images were taken with identical imaging settings and are set to the same brightness and contrast. Scale bars are 100 µm. ***B***, Distribution of fluorescence intensity from MrgprD-GFP (black) and WT (red) neurons. Data represents the fluorescence intensity of 905 MrgprD-GFP neurons from 9 DRG sections from 1 mouse or 477 WT neurons from 5 DRG sections from 1 mouse. DRG sections were taken from 7 week old female mice and are from the 1st lumbar DRG. ***C***, WT data shown in B fit with a log normal distribution (red fit). ***D***, MrgprD-GFP data shown in B fit with the WT distribution (red fit) where width and mean were constrained to the WT distribution and amplitude was unconstrained (equation 1). Only MrpgrD-GFP data to the left of the mean intensity of WT neurons (red dotted line) was used for the fit. ***E***, Percent of neurons with detectable GFP protein of 4 mice (3 females 1 male). All DRG sections were taken from the 1st lumbar DRG.

1146 **Supplemental Figure 7**



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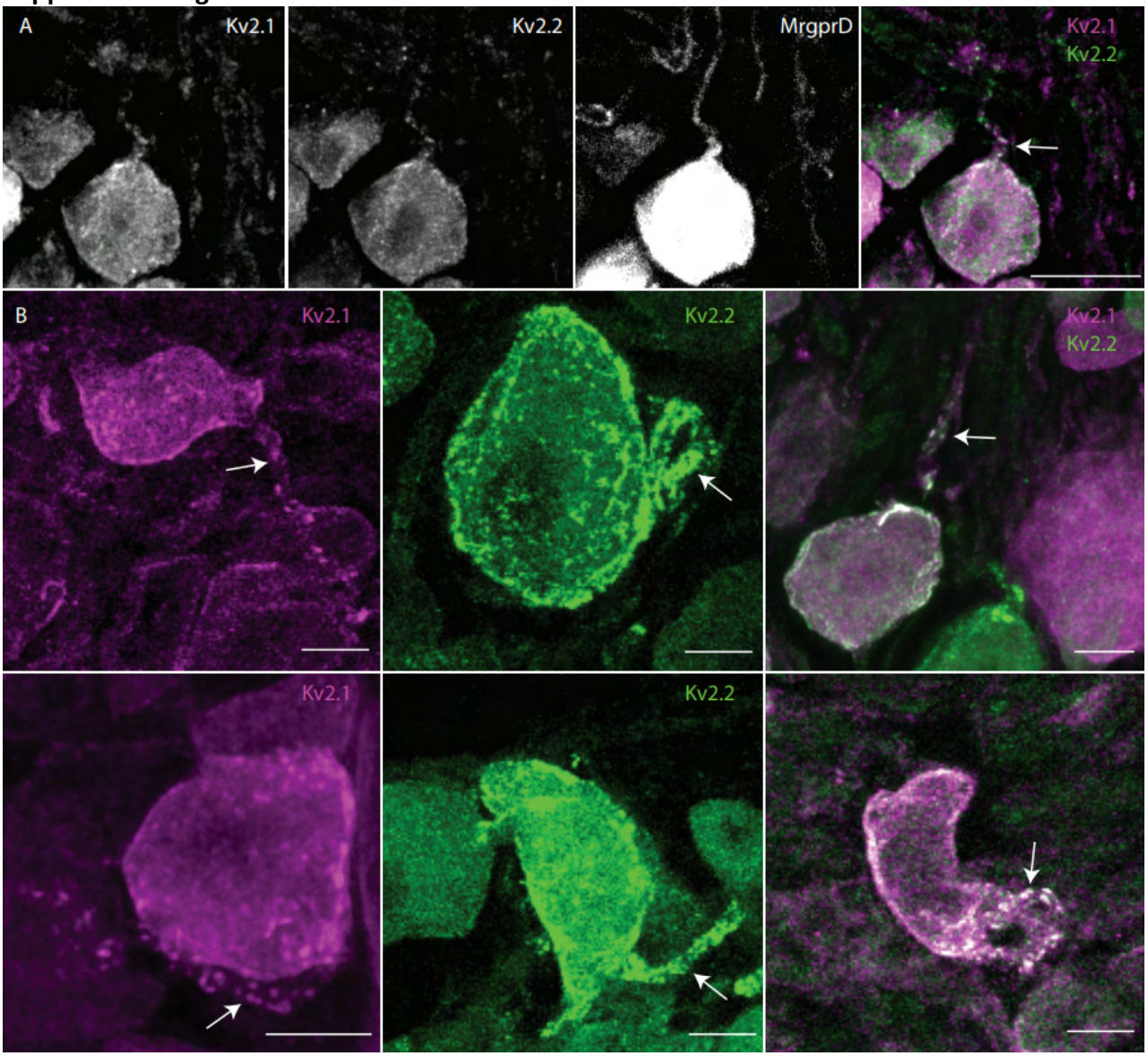
1148 **Supplemental Figure 7 legend**

1149 DRG neurons have enriched Kv2.2 protein compared to neurons in the ventral horn. ***A***, Anti-
1150 Kv2.1 (magenta) and anti-Kv2.2 (green) immunofluorescence in a spinal cord section from the
1151 13th thoracic vertebra (left). Anti-Kv2.1 immunofluorescence (right top) and anti-Kv2.2
1152 immunofluorescence (right bottom). Arrow heads show neurons in the spinal cord with anti-
1153 Kv2.2 immunofluorescence. Scale bars are 500 µm. ***B***, Anti-Kv2.1 immunofluorescence from

individual neurons (circles) in the DRG and ventral horn normalized to the average fluorescence intensity of neurons in the ventral horn. Diamonds to the right of data represent the average intensity in the DRG of individual mice. Significant differences from 1 were calculated for individual mice using Students t-test. N = 4 mice n = 116 in DRG and n = 77 in ventral horn. ***C,*** Identical analysis shown in B with anti-Kv2.2 immunofluorescence.

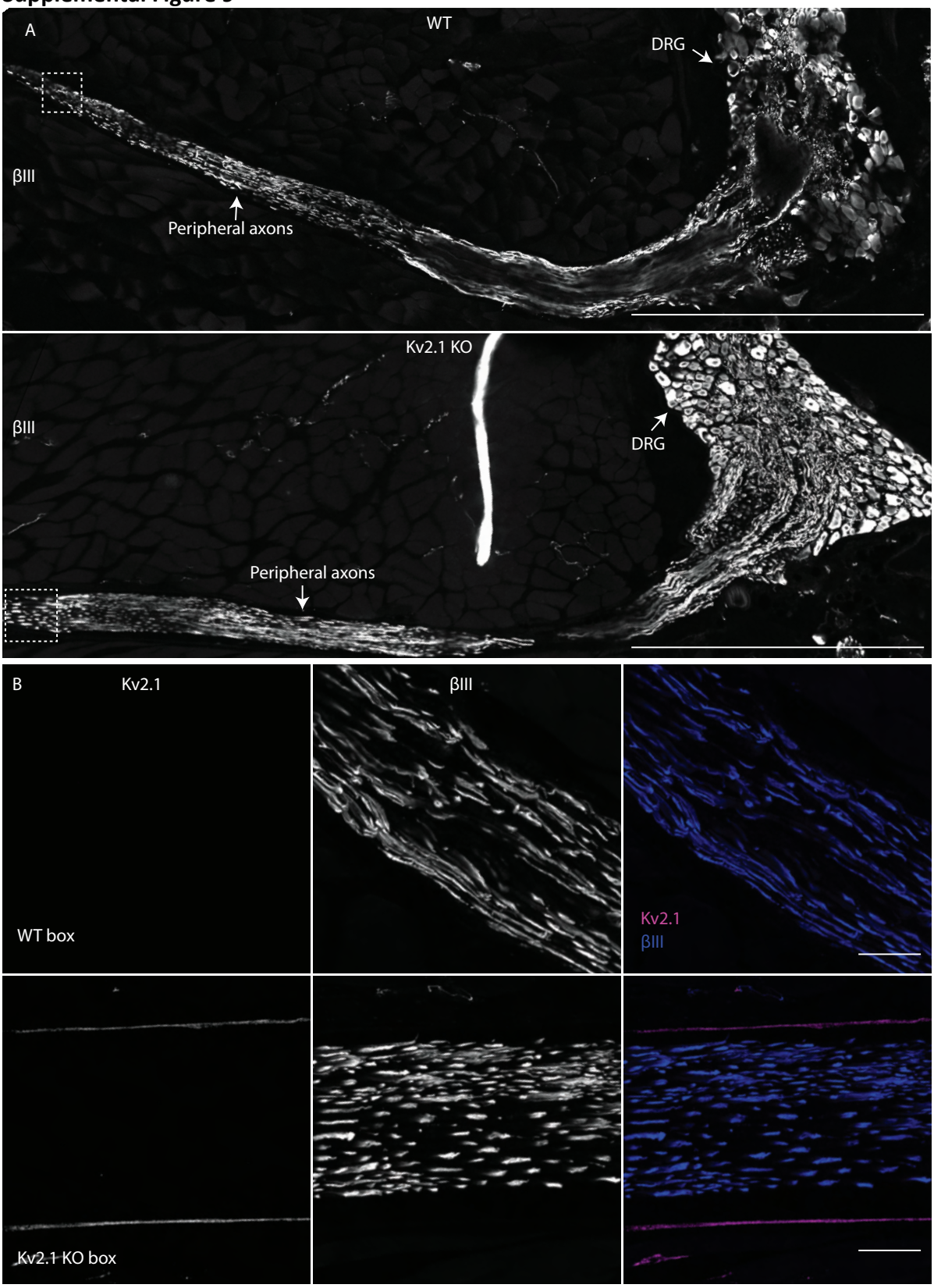
Supplemental Figure 8



Supplemental Figure 8 legend

Kv2 channels are expressed on the stem axon of mouse DRG neurons. ***A***, Z-projection with anti-Kv2.1 and anti-Kv2.2 immunofluorescence on the stem axon of a neuron in the DRG of a MrgprD-GFP mouse. ***B***, Gallery of z-projected images of DRG neurons with anti-Kv2.1 and/or anti-Kv2.2 immunofluorescence on stem axons. Arrows indicate stem axons. Scale bars are 10 µm

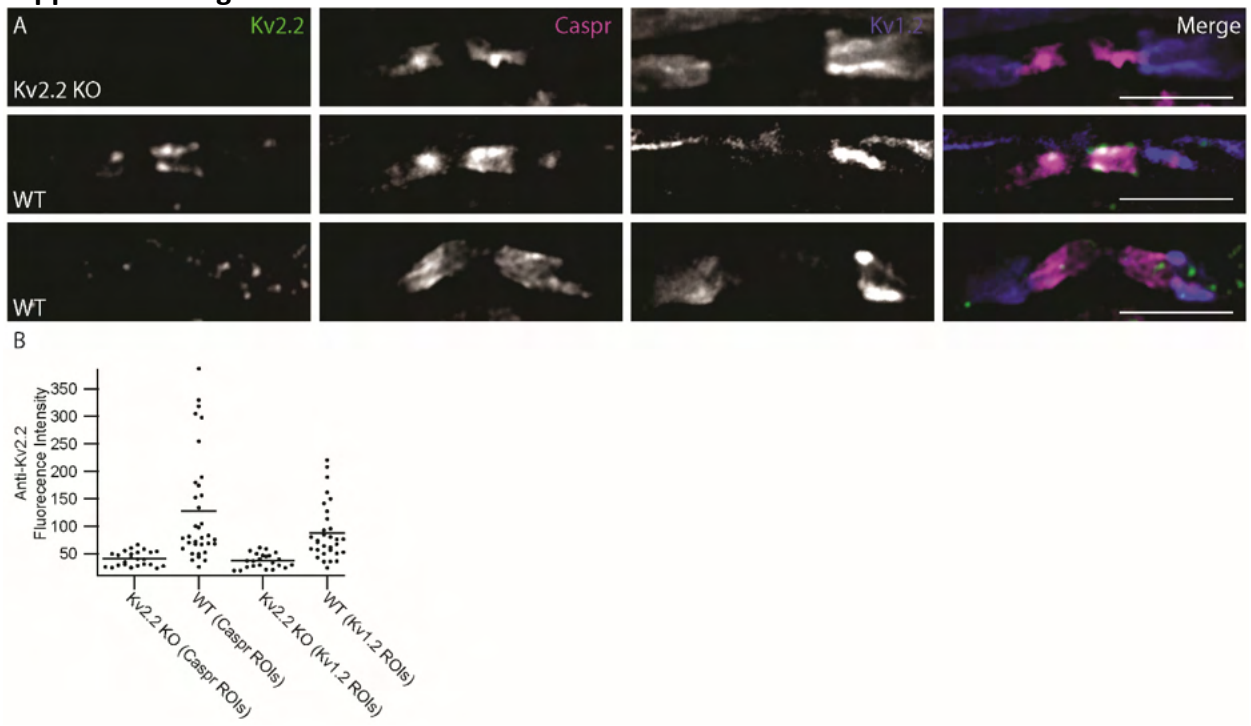
Supplemental Figure 9



Supplemental Figure 9 legend

Kv2.1 channels were not detected in peripheral axons of DRG neurons. ***A***, WT (top) and Kv2.1 KO (bottom) sections containing the DRG and peripheral axons from the 12th thoracic DRG in age and sex matched 7 week old mice immunolabeled for βIII tubulin (white). Scale bar is 500 µm. ***B***, High magnification z-projection of anti-Kv2.1 and anti-βIII immunofluorescence from box in A of WT and Kv2.1 KO mice. Scale bars are 20 µm.

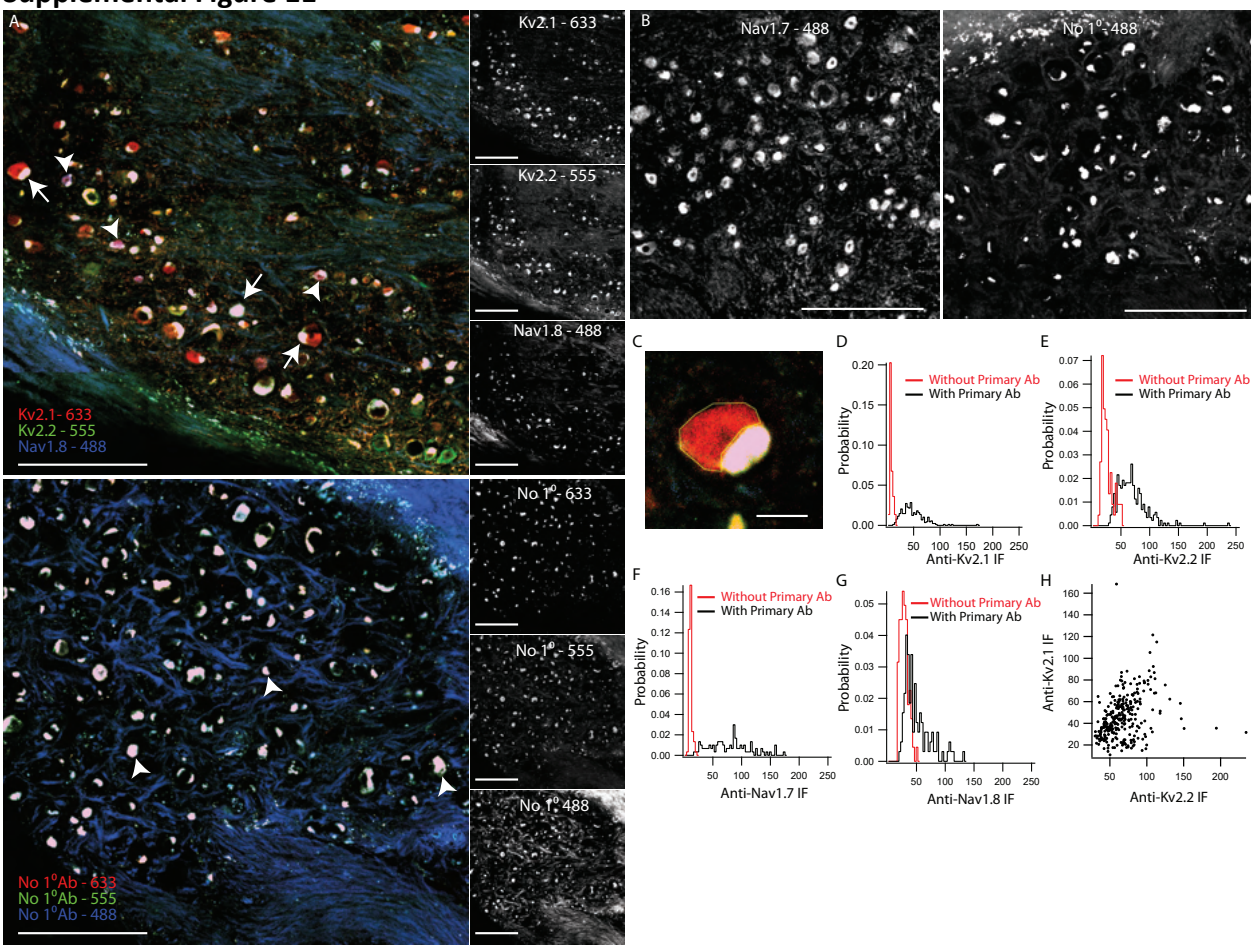
Supplemental Figure 10



Supplemental Figure 10 legend

Kv2.2 is expressed in myelinated fibers of DRG neuron axons. ***A***, Kv2.2 KO (top) and WT (middle and bottom) sections containing the peripheral axons from the 12th thoracic DRG in 28 week old mice immunolabeled for Kv2.2, Caspr and Kv1.2. Middle panels are an exemplar of prominent Kv2.2 immunofluorescence in CASPR labeled axons and bottom panels are an exemplar of prominent Kv2.2 clusters in the Kv1.2 labeled axons. Scale bars are 5 µm. ***B***, Analysis of anti-Kv2.2 immunofluorescence intensity in CASPR and Kv1.2 labeled regions of age and sex matched WT and Kv2.2 KO mice. Individual points represent single ROIs drawn around anti-CASPR or anti-Kv1.2 immunofluorescence.

Supplemental Figure 11

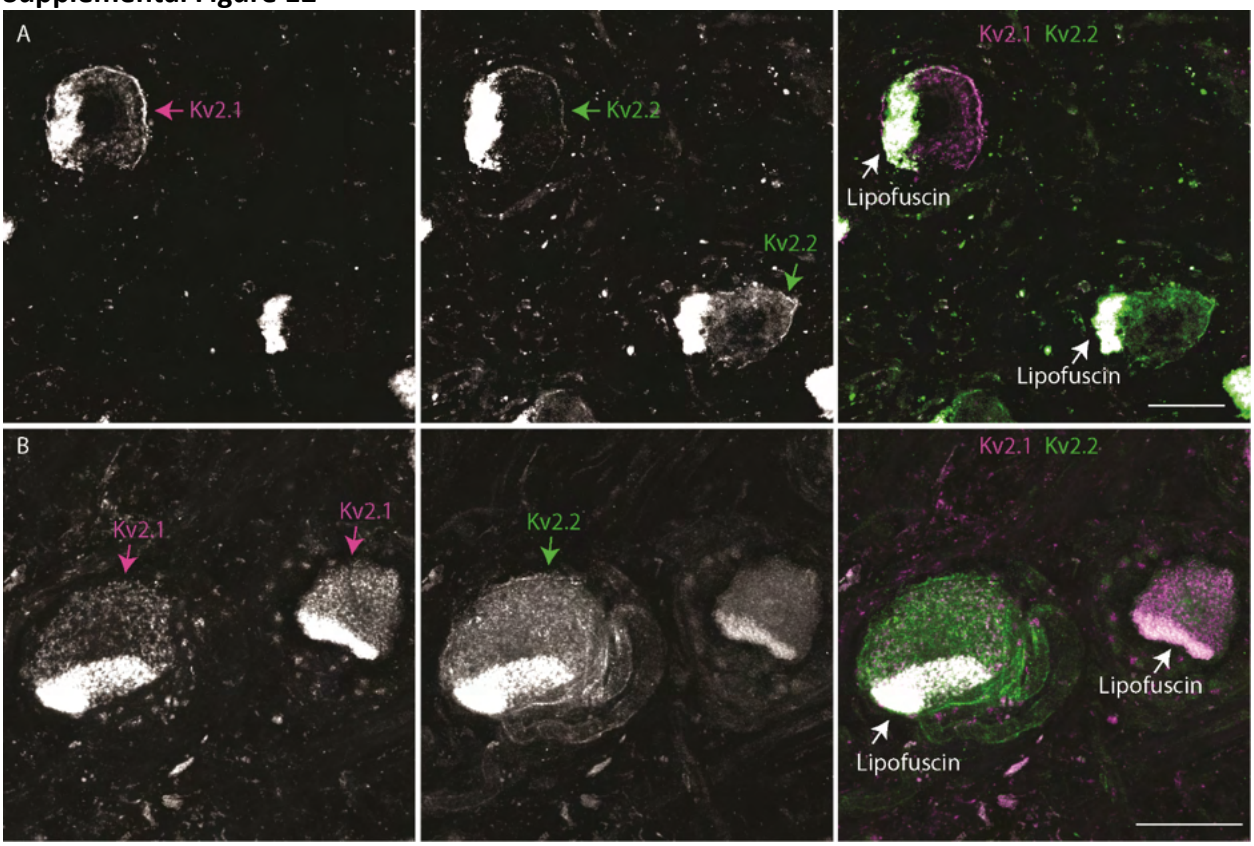


Supplemental Figure 11 legend

Fluorescence from human DRG neurons labeled with ion channel targeting antibodies is distinct from human DRG neurons where ion channel targeting antibodies were omitted. ***A***, Top: Immunofluorescence from human DRG section labeled with anti-Kv2.1, anti-Kv2.2 and anti-Nav1.8 antibodies. Bottom: Fluorescence from human DRG section where the primary antibodies are omitted. Arrows in top and bottom images indicate examples of autofluorescence from apparent intracellular lipofuscin. Arrow heads in top image identify anti-Nav1.8 immunofluorescence. Images on the right are fluorescence from each fluorescence channel of the top and bottom images. Number next to target protein label represents excitation wavelength. DRG sections from top and bottom images are from the same DRG. Scale bars are 500 µm. ***B***, Left: Immunofluorescence from human DRG section labeled with anti-Nav1.7 antibody. Right: Fluorescence from human DRG section where the primary antibody has been omitted. Number next to target protein label represents excitation wavelength. DRG sections in left and right images are from the same DRG. Scale bars are 500 µm. ***C***, Exemplar manually drawn ROI to analyze fluorescence intensity in human DRG neurons that omits apparent lipofuscin autofluorescence. Scale bar is 50 µm. ***D***, Distribution of fluorescence intensity of human DRG neurons labeled with an anti-Kv2.1 antibody (black) or when the anti-Kv2.1 antibody was omitted (red). Data represents the fluorescence intensity of 293 neurons labeled with anti-Kv2.1 antibody or 73 neurons where the anti-Kv2.1 antibody was omitted. ***E***,

Distribution of fluorescence intensity of human DRG neurons labeled with anti-Kv2.2 antibody (black) or when the anti-Kv2.2 antibody was omitted (red). Data represents the fluorescence intensity of 293 neurons labeled with anti-Kv2.2 antibody or 73 neurons where the anti-Kv2.2 antibody was omitted. ***F***, Distribution of fluorescence intensity of human DRG neurons labeled with anti-Nav1.7 antibody (black) or when the anti-Nav1.7 antibody was omitted (red). Data represents the fluorescence intensity of 99 neurons labeled with anti-Nav1.7 antibody or 99 neurons where the anti-Nav1.7 antibody was omitted. ***G***, Distribution of fluorescence intensity of human DRG neurons labeled with anti-Nav1.8 antibody (black) or when the anti-Nav1.8 antibody was omitted (red). Data represents the fluorescence intensity of 293 neurons labeled with anti-Nav1.8 antibody or 73 neurons where the anti-Nav1.8 antibody was omitted. ***H***, Fluorescence intensity of human neurons labeled with both anti-Kv2.1 and anti-Kv2.2 antibodies. Individual points represent individual neurons. All images are from donor #2. Detailed information on each donor can be found in the *Human Tissue Collection* section of the methods.

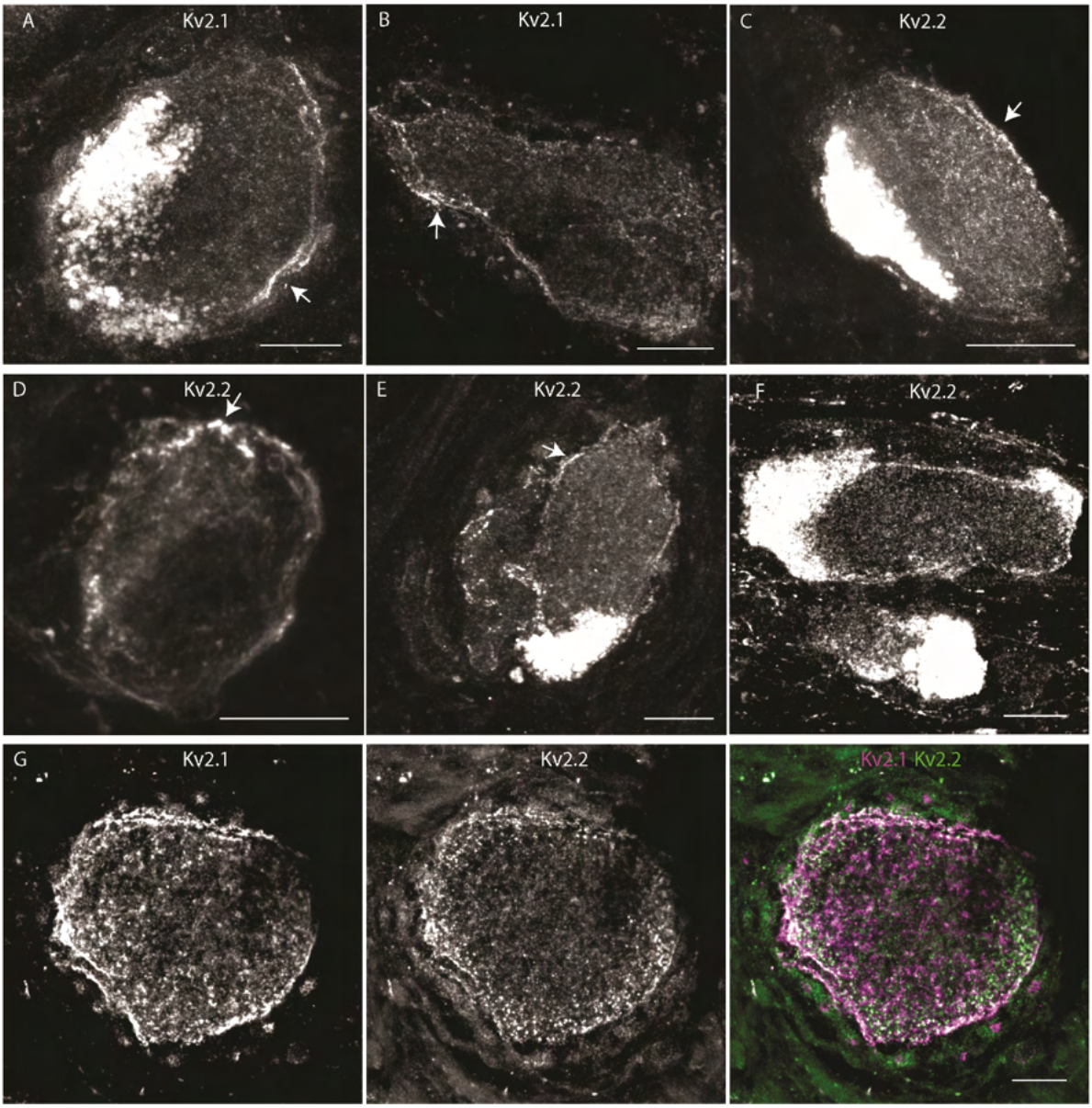
Supplemental Figure 12



Supplemental Figure 12 legend

Immunofluorescence from human DRG neurons from donor #2 ***A*** and donor #3 ***B*** labeled with anti-Kv2.1 and anti-Kv2.2 antibodies. Autofluorescence attributed to lipofuscin is labeled in right panels while apparent Kv2.1 and Kv2.2 protein are labeled in left and middle panels respectively. Scale bars are 50 µm. Detailed information on each donor can be found in the *Human Tissue Collection* section of the methods.

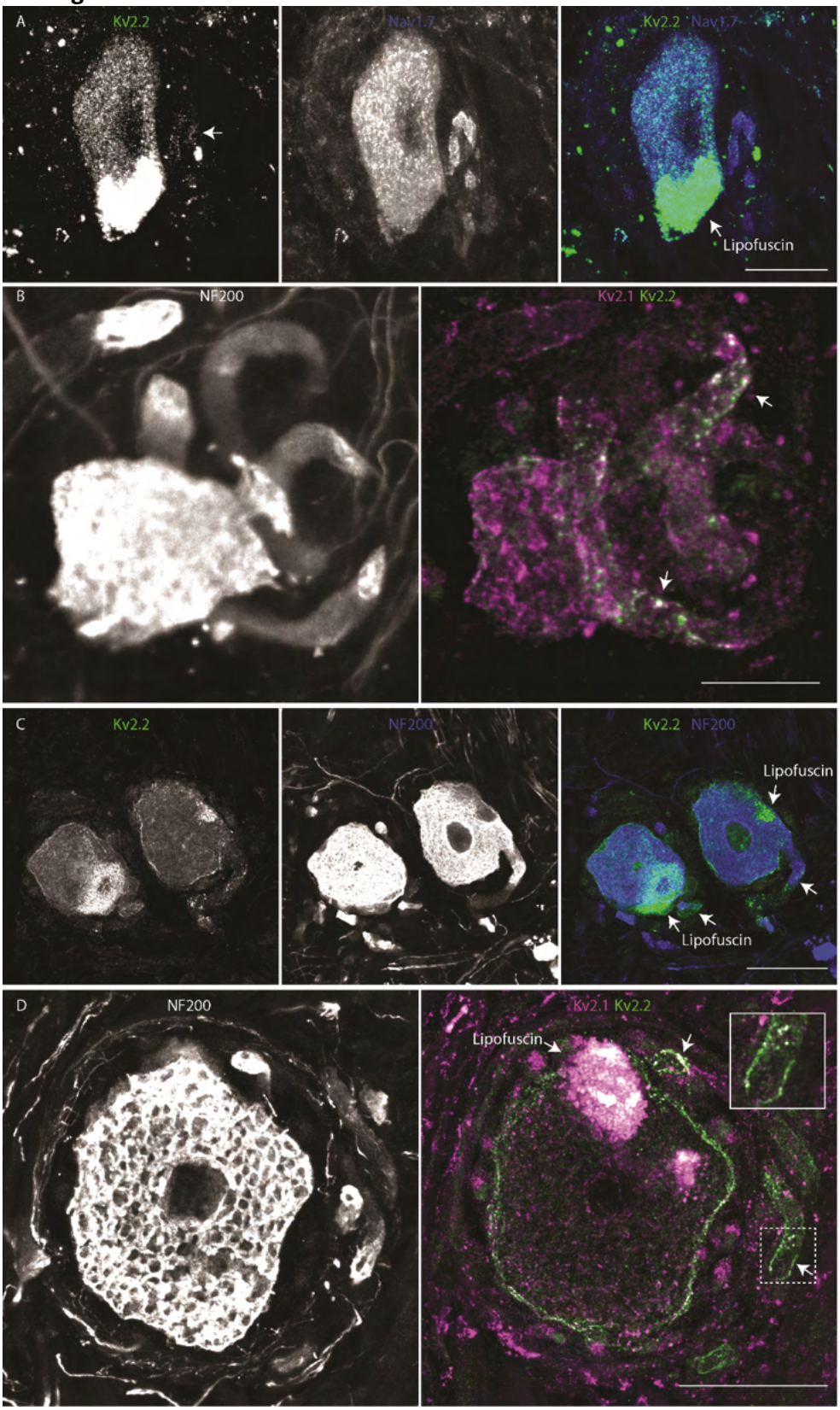
Supplemental Figure 13



Supplemental Figure 13 legend

Kv2 channels are enriched at the outer edge of human DRG neurons. ***A-B***, Exemplar z-projections of anti-Kv2.1 immunofluorescence enriched at the outer surface of human DRG neurons. Arrows indicate asymmetric clusters. Images are from donor #2 Scale bars are 20 µm. ***C-F***, Exemplar z-projections of anti-Kv2.2 immunofluorescence enriched at the outer surface of a human DRG neurons. Arrows indicate asymmetric clusters. Image in ***E*** is from donor #3 while all other images are from donor #2. Scale bars are 20 µm. ***G***, Exemplar z-projection of anti-Kv2.1 and anti-Kv2.2 immunofluorescence both enriched at the outer surface of a human DRG neuron soma. Image is from donor #2. Scale bar is 20 µm. Detailed information on each donor can be found in the *Human Tissue Collection* section of the methods.

1249 **Supplemental Figure 14**



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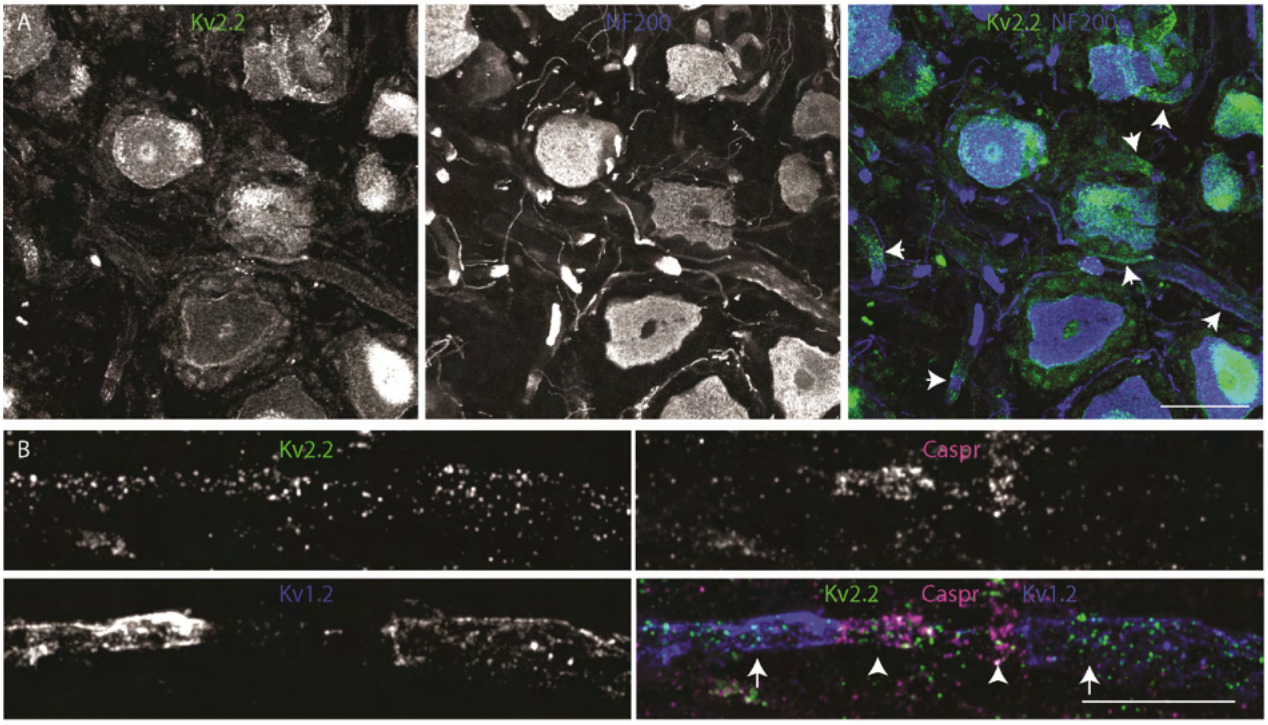
Supplemental Figure 14 legend

A, Z-projection of anti-Kv2.2 and anti-Nav1.7 immunofluorescence in a human DRG neuron soma and stem axon. Arrow in merge indicates the stem axon of the DRG neuron. Apparent lipofuscin autofluorescence is labeled in merge. Image is from donor #2. Scale bar is 20 µm. ***B,*** Z-projection of anti-Kv2.1 (magenta), anti-Kv2.2 (green) (right) and anti-NF200 (left) immunofluorescence in a human DRG neuron soma and stem axon. Arrows in merge indicate the stem axon of the DRG neuron. Image is from donor #1. Scale bar is 20 µm. ***C,*** Z-projection of anti-Kv2.2 (left) and anti-NF200 (middle) immunofluorescence in a human DRG neuron soma and stem axon. Arrow in merge indicates the stem axon of the DRG neuron. Apparent lipofuscin autofluorescence is labeled in merge. Image is from donor #1. Scale bar is 50 µm. ***D,*** Z-projection of anti-Kv2.1 (magenta), anti-Kv2.2 (green) (right) and anti-NF200 (left) immunofluorescence in a human DRG neuron soma and stem axon. Arrows in merge indicate the stem axon of the DRG neuron. Apparent lipofuscin autofluorescence is labeled in merge. Image is from donor #3. Scale bar is 50 µm. Detailed information on each donor can be found in the *Human Tissue Collection* section of the methods.

Supplemental Figure 15



Supplemental Figure 15 legend

A, Z-projection of anti-Kv2.2 (left) and anti-NF200 (middle) immunofluorescence of human DRG. Arrows in merge represent exemplar axons which have clear anti-Kv2.2 immunofluorescence. Image is from donor #1. Scale bar is 50 µm. ***B***, Z-projection of anti-Kv2.2 (upper left), anti-CASPR (upper right) and anti-Kv1.2 (bottom left) immunofluorescence of human DRG axon. Image is from donor #2. Scale bar is 10 µm. Detailed information on each donor can be found in the *Human Tissue Collection* section of the methods.