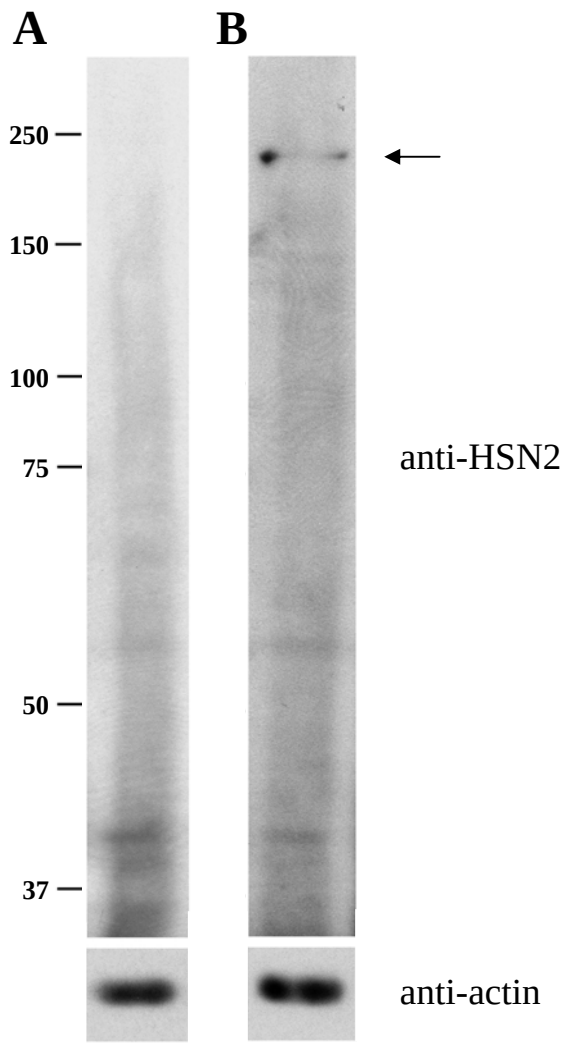
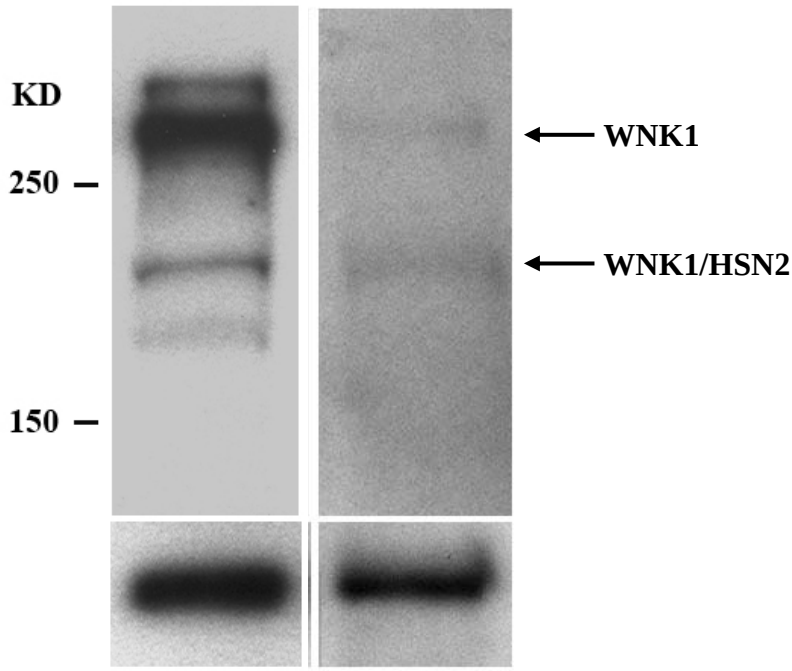
Shekarabi_Suppl_Fig1

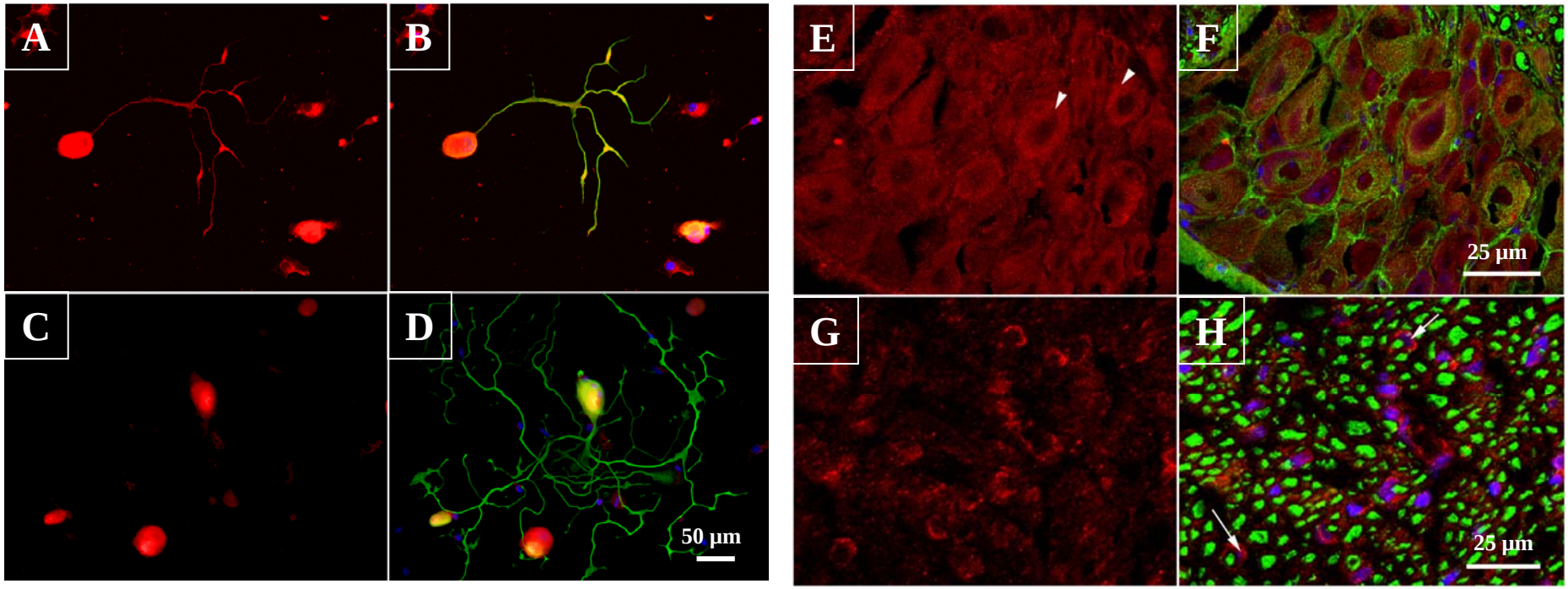


Supplemental Figure 1: Peptide competition experiments on adult mouse DRG protein and tissue confirm the specificity of the anti-HSN2 antibody. Whole protein lysates from adult mouse DRG were analyzed by SDS-PAGE. **(A)** One blot was detected with anti-HSN2 following its overnight (4^{o}C) pre-incubation with the antigenic peptide that was used to produce it. **(B)** An identical blot to the one in A detected with the anti-HSN2 antibody without any pre-incubation with its antigenic peptide. Equal loading was confirmed using an anti-actin antibody.



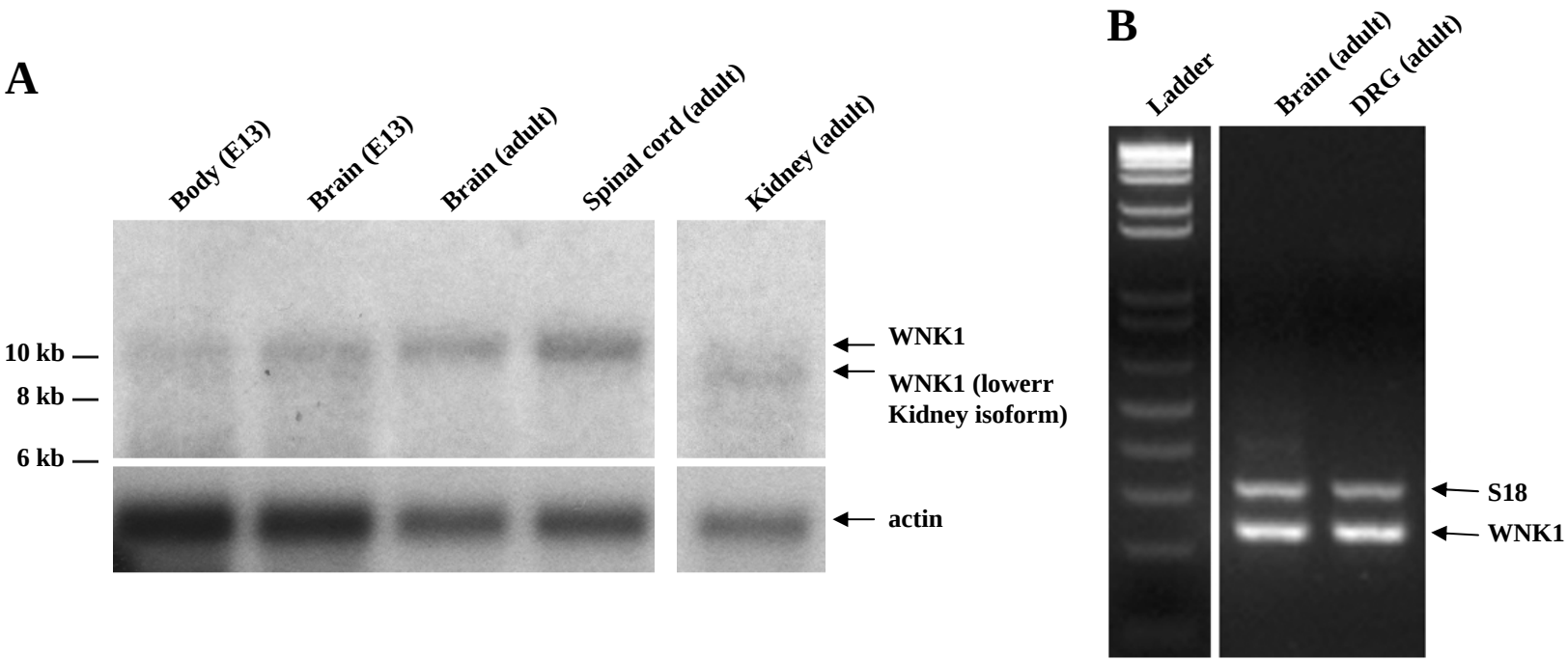
Supplemental Figure 2: Comparison of alternative anti-WNK1 antibodies. In addition to the rabbit polyclonal anti-WNK1 (Alpha Diagnostic, cat #WNK11-A) used in the report, we tested two other anti-WNK1 antibodies. A rabbit polyclonal antibody from R&D systems (cat #AF2849) which recognizes a portion of WNK1 located between 816-1173 a.a. (beginning of exon 12 to the first quarter of exon 17) was tested (left lane). A sheep polyclonal antibody from Kinasource (cat #AB-170) which recognizes a portion of WNK1 located between 60-661 a.a. (exon 1 to the middle of exon 2) was tested (right lane). Overall it can be concluded that the antibody from Alpha Diagnostic only detects WNK1 when its translation is initiated from the first ATG. Equal amount of proteins (50 µg) were loaded on the two lanes. The membranes (which came from the same SDS-PAGE) were stripped of the primary antibodies signal and redetected with an antibody specific to actin (Chemicon).

Shekarabi_Suppl_Fig3



Supplemental Figure 3: (A) The immunofluorescence detection of WNK1/HSN2 in primary culture of adult mouse sensory neurons with the anti-HSN2 antiserum showed its expression along the axons (red). The WNK1/HSN2 protein was also detected in the soma. **(B)** Overlaid image of the WNK1/HSN2 signal from (A) and of a SMI-31/-32 detection (neuronal markers mix, green) confirms the expression of WNK1/HSN2 in the axons (yellow). **(C)** Immunofluorescence detection of WNK1 (Alpha diagnostic, red) was examined in a parallel set of primary cultures. No WNK1 signal was visible in the axons and the signal appears mostly limited to the cell body. **(D)** Overlaid image of the WNK1 signal from (C) and of a neuronal marker (SMI-31/-32 mix, green) show the absence of WNK1 in the axons projections. WNK1 immunoreactivity (Alpha Diagnostics antibody) was examined in adult mouse DRG **(E)** and sciatic nerve **(G)** cross sections**.** Observations from (E) to (H) were made using a confocal microscope and not a regular fluoresent microscope like those from (A) to (D). Sections from **(E)** and **(G)** were detected with the anti-WNK1 antibody (red) and a very low expression of WNK1 was observed in the sensory neurons (arrow heads in E). Overlaid images of the WNK1 signal (red) from (E) and (G), of the SMI-31/-32 mix (green) and of a nuclear stain by TOTO- 3 iodide (blue) are shown in **(F)** and **(H).** These overlaid images of the WNK1 expression in the DRG and sciatic nerve confirm that there is virtually no expression of it in the axons fibers. Schwann cells of the sciatic nerve express WNK1 as they appeared to express WNK1/HSN2 (Figure 5 D). The blue is is pseudocolored and shows nuclei staining.

Shekarabi_Suppl_Fig4



Supplemental Figure 4: RNA expression analysis of the *WNK1* messenger without the *HSN2* exon. (A) A Northern analyses of *WNK1* expression in the mouse. The gel was prepared from different adult and E13 embryo mouse tissues. The membrane was hybridized with a 850 bp probe located outside the *HSN2* region (the probe covers the end of *WNK1* exon 1 to exon 6). Following the autoradiography detection of the signal the WNK1 messenger was observed at the previously reported positions (~10.5 kb for every tissues and ~9.0 kb for the kidney). **(B**) RT-PCR amplifications with primers recognizing sequences in *WNK1* exon 8 and 10 were made (using regular taq polymerase and not the long range PCR mix) with DRG RNA. These amplifications generated a fragment fitting with the size (250 bp) of *WNK1* when the *HSN2* exon is not used in the DRG (and also in the brain which was used as a control). The RT-PCR mixes also contained primers to amplify S18 RNA which generated the expected 324 bp.

Supplemental Table 1

Primer	Sequence
1	GTTTCTAGTATCCAAGCACAATCT
2	TTGTGTTCCCATCTTTCTGCT
3a	GAAACTGGACGTTCATGAATAG
3b	GGAAGGAGGTGCTCTTAC
4	CAAGGAACCACATCTCAGCAGGTCT
5	TGACTCACTGGCTGTGCAGTAGT
7	ATGTCTGACGGTGCCGCCGAG
8	ATGGACAAGGACAGTCGTGGG
9	CCCAGCCTCGTGGGGAGCAAA
10	TGACATCGAAATCGGCAGAGGCT
11	GTCATTGTTCACCATAATTGTTGCTATCTC
5’UTRP1F	AAAGCGGAGAGTTTTCCCGGGCG
Exon 6R	CTTCTGCTAATTCTACCCGTACCC

Supplemental Table 1: List of the different primers illustrated in Figure 2B used to performed the RTPCR reactions