Appendix for

Membrane cholesterol depletion as a trigger of Nav1.9-mediated inflammatory pain

Muriel Amsalem[1], Corinne Poilbout[1,2], Géraldine Ferracci[1], Patrick Delmas[1*], Francoise

[1]Aix-Marseille-Université; CNRS; CRN2M UMR 7286, CS80011, Bd Pierre Dramard, 13344 Marseille Cedex 15 France.

[2] Present address: Centre de psychiatrie et de neurosciences, 2 ter rue d'Alésia 75014 Paris.

Table of contents

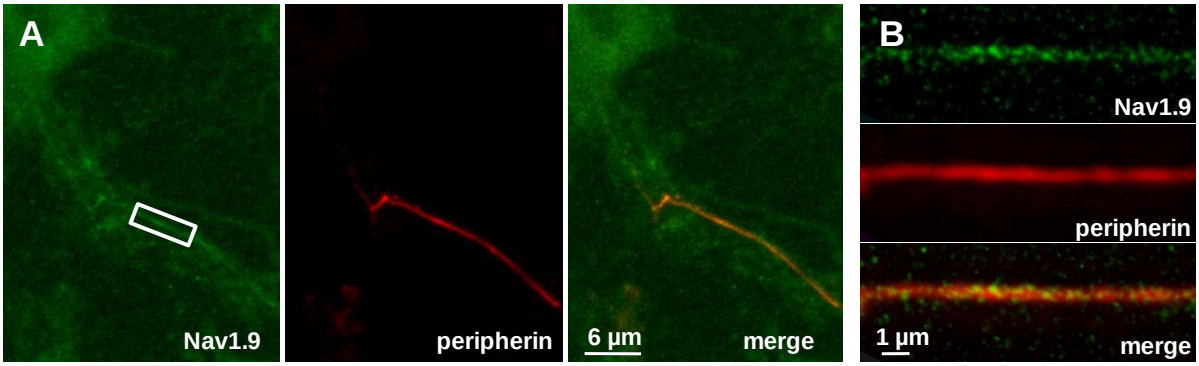
Appendix Figure S1 Immunostaining of Nav1.9 proteins along DRG neuron fibers in skin territory..……..……p3

Appendix Figure S2 Cholesterol oxidation-induced mechanical hypersensitivity is unchanged in Nav1.8 KO mice.……...……..p4

Appendix Figure S3 Time course of Nav1.9 potentiation by MβCD..…….p5

Appendix Figure S4 MβCD modulates Nav1.9 partitioning in DRM-raft like microdomains….p6

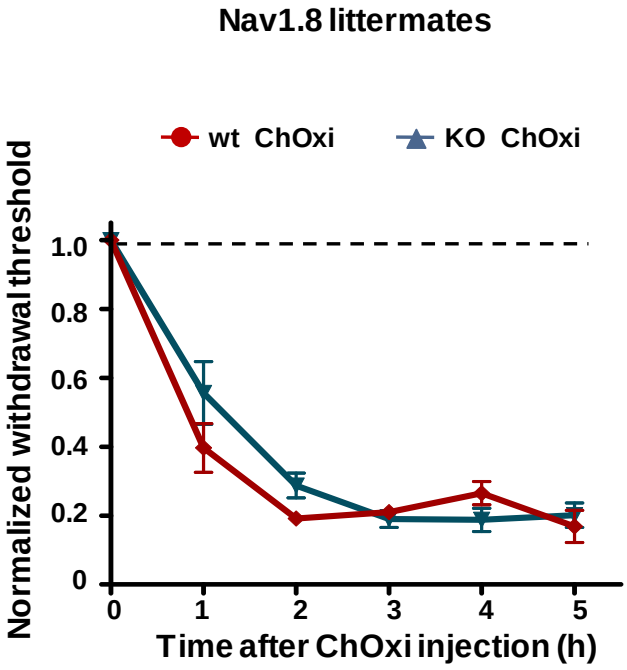
Appendix Figure S5 Identification of cholesterol consensus binding domains on Nav1.9 sequence.……...……..p8



Appendix Figure *S1*.

Immunostaining of Nav1.9 proteins along DRG neuron fibers in skin territory.

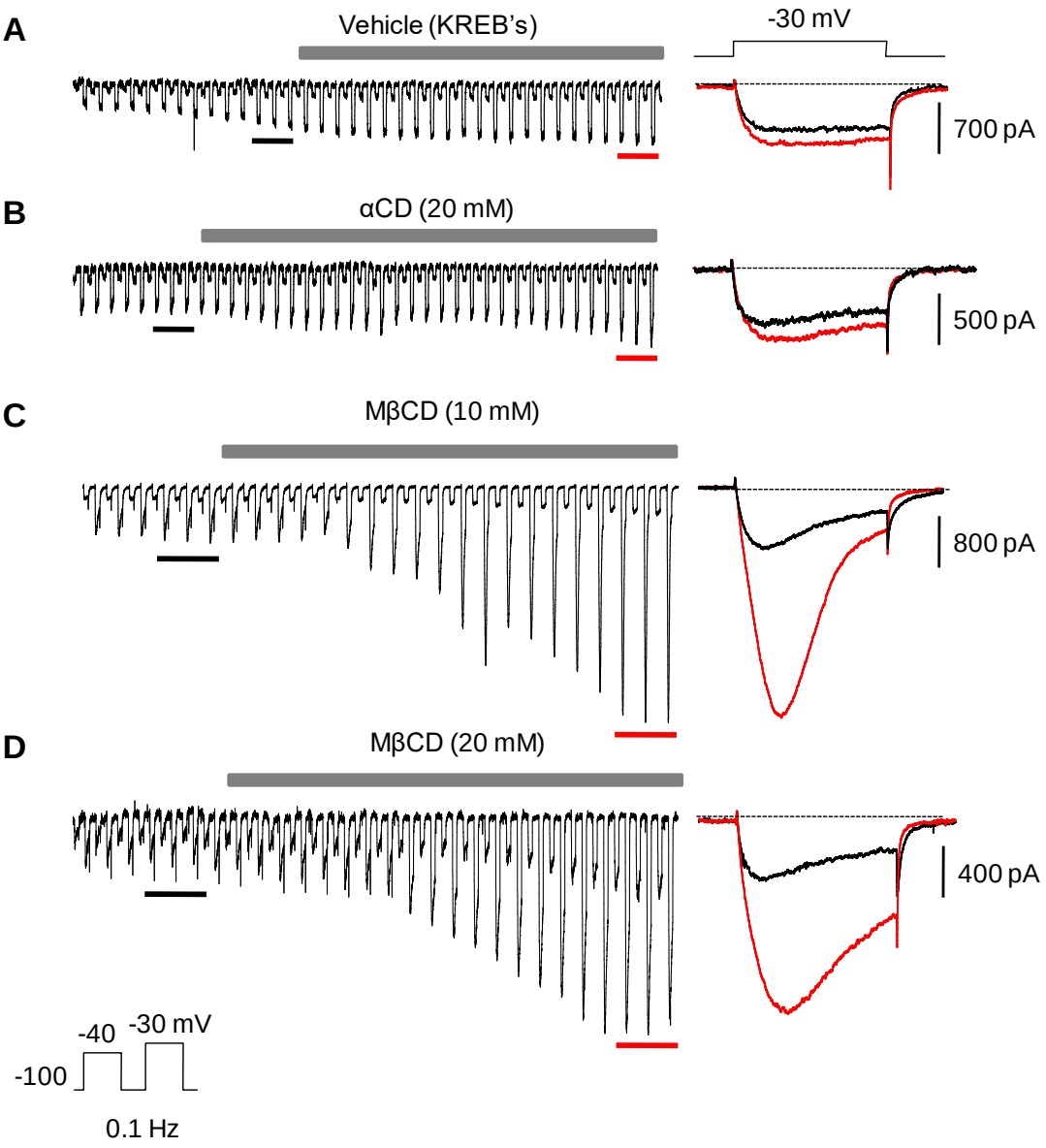
(A) Confocal images of double staining of nerve terminals in frozen sections of mouse skin paw. (B) Magnification of the zone highlighted in A after deconvolution treatment showing a punctate distribution of Nav1.9 channels along a peripherin-positive nerve fiber.



Appendix Figure *S2*.

Cholesterol oxidation-induced mechanical hypersensitivity is unchanged in Nav1.8 KO mice.

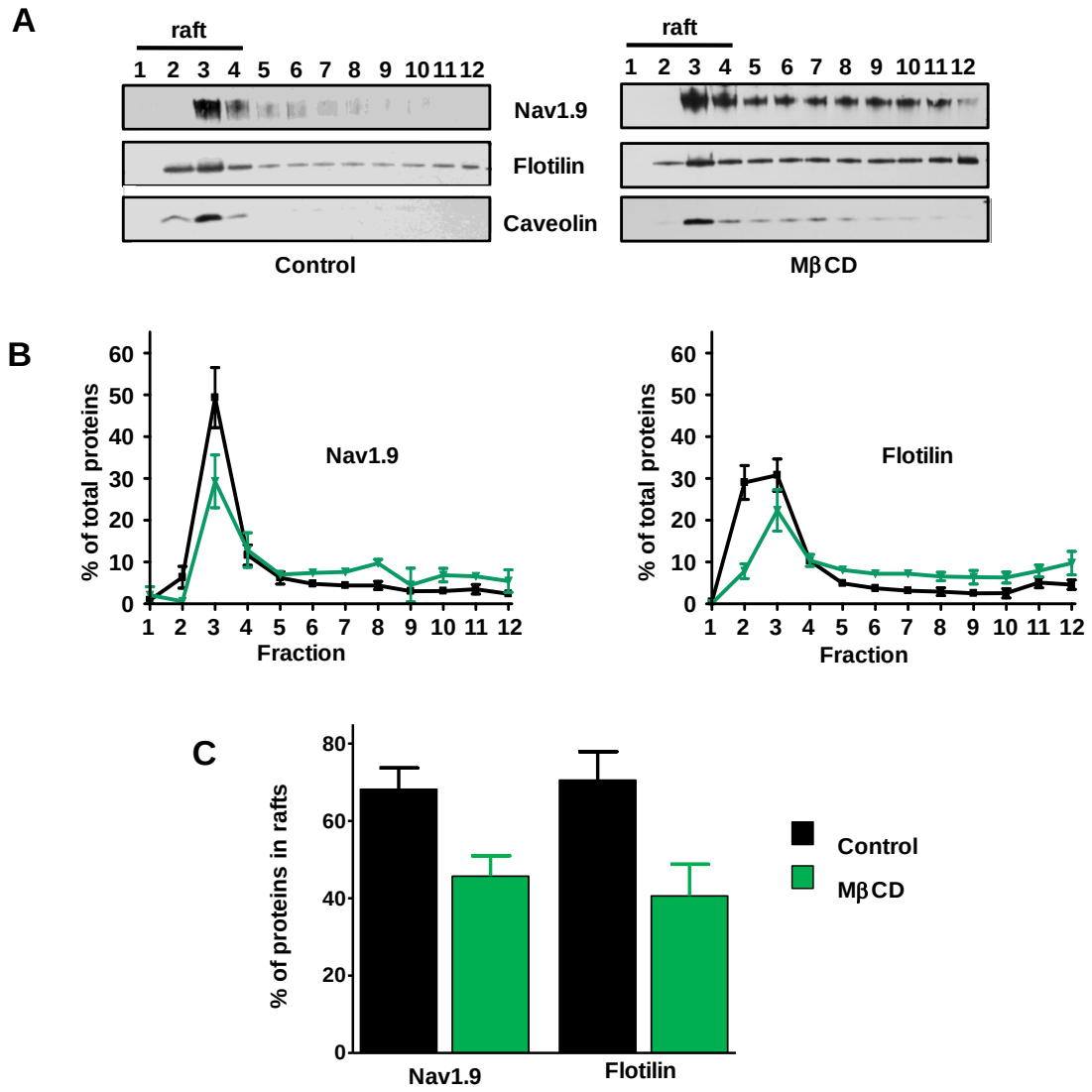
Mechanical hypersensitivity caused by intraplantar injection of ChOxi (4U/ml) is similar in Nav1.8 KO mice (n = 8) compared to wt littermates (n = 6) ($p = 0.39$, two way ANOVA test).



Appendix Figure *S3*.

Time course of Nav1.9 potentiation by MβCD.

Recording of Nav1.9 in DRG neurons superfused with Kreb's (vehicle) (A) or Kreb's supplemented with αCD (20 mM) (B) or MβCD (10 and 20 mM as indicated) (C-D). Nav1.9 currents were evoked by 100 ms-depolarizing double voltage steps to -40 and -30 mV (Vh = -100 mV) every 20 s. Each illustration represents a concatenated sequence of consecutive sweeps. Right inset: superimposed Nav1.9 current traces evoked at -30 mV and collected as indicated by colored lines on concatenated traces (average of 3 consecutive traces).

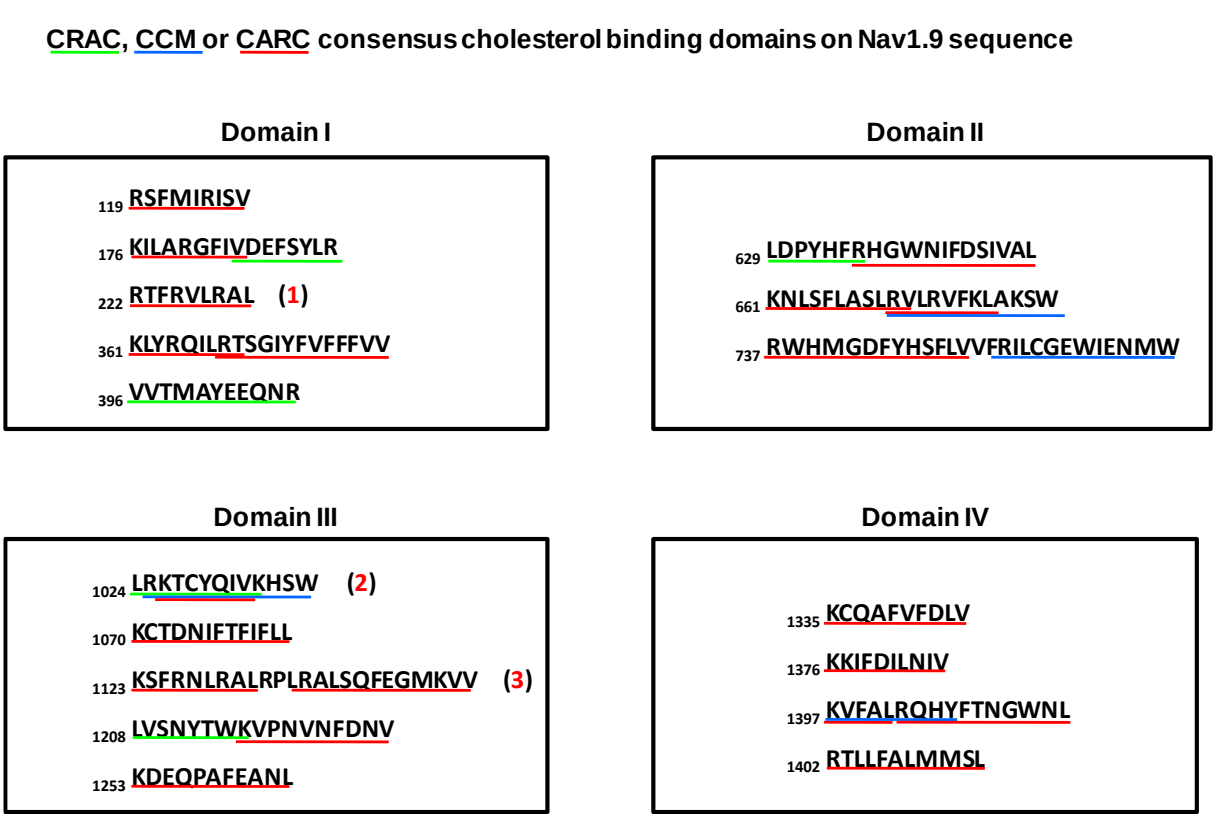


Appendix Figure *S4*.

MβCD modulates Nav1.9 partitioning in DRM-raft like microdomains.

(A) Representative Western blot of DRM fractions of freshly isolated cells from DRGs incubated for 15 min at 37°C with DMEM (left panel, same as Figure 5A) or DMEM + MβCD (40 mM) (right panel). Fraction 1 represents the top of the gradient and fraction 12 the bottom. Lipid rafts-DRM fractions are revealed by the presence of flotilin and caveolin. (B) Quantification of Nav1.9 and flotilin signals obtained for each separated fraction of DMEM-treated control (black line; same as Fig 5) or DMEM + MβCD-treated (green line; n = 2) DRGs. Values of each fraction are expressed as a percentage of the sum of signals from all fractions. (C) Quantification of Nav1.9

and flotilin proteins in raft fractions (sum of fractions 1 to 4) of control and MβCD-treated DRGs (black and green bars, respectively).



Appendix Figure S5.

Identification of cholesterol consensus binding domains on Nav1.9 sequence.

Sequences of CRAC, CCM and CARC domains underlined on transmembrane and juxta membrane domains of Nav1.9 protein sequence, distributed in the 4 structural domains of the channel. Peptide 1, 2 and 3 are indicated in bracket.