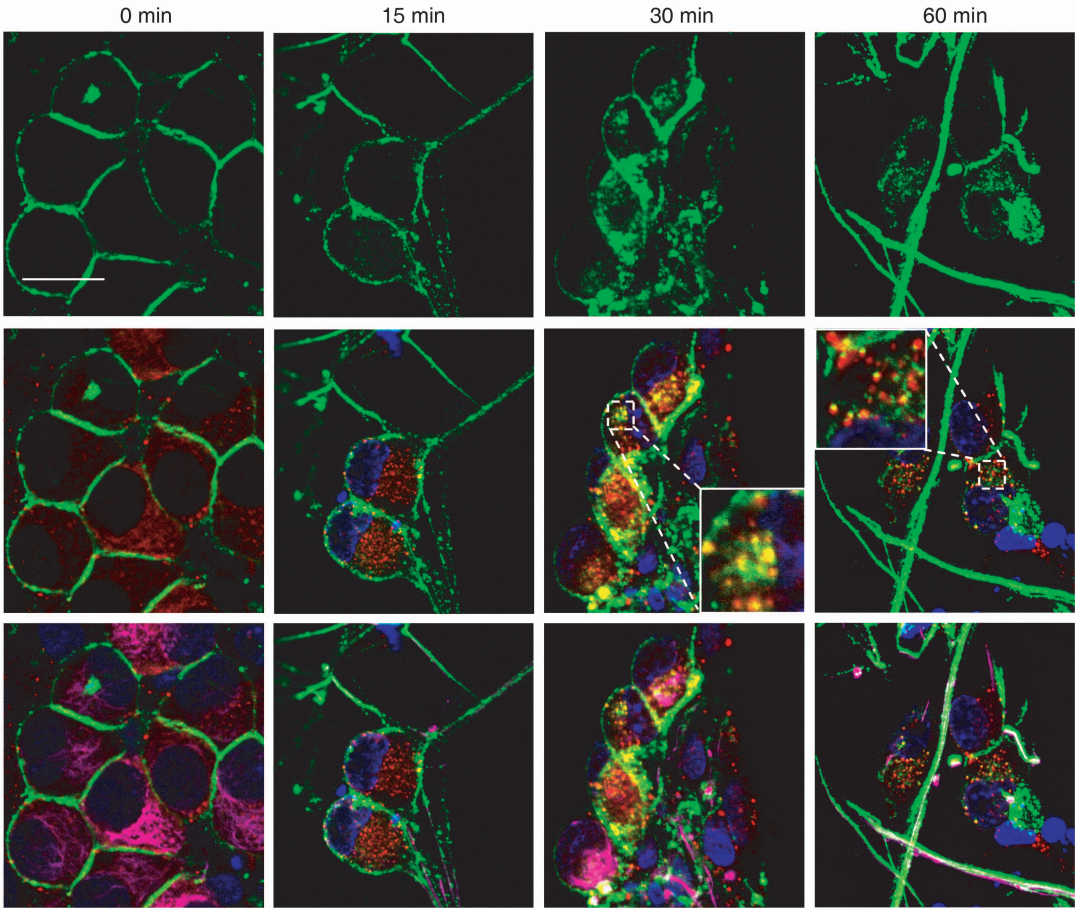
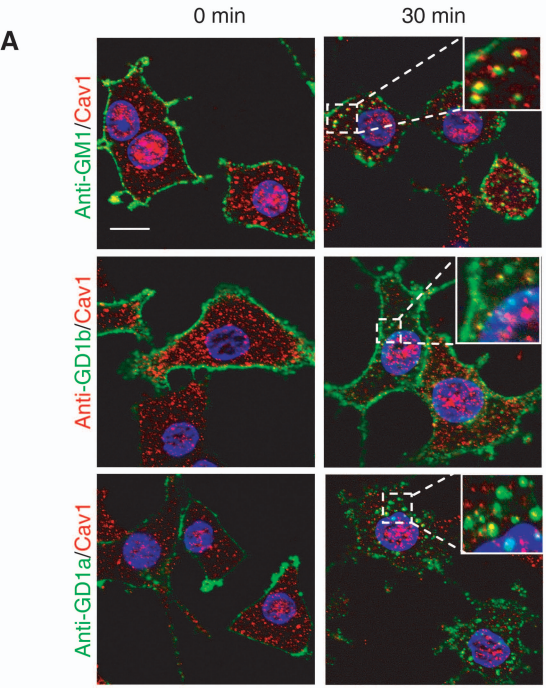
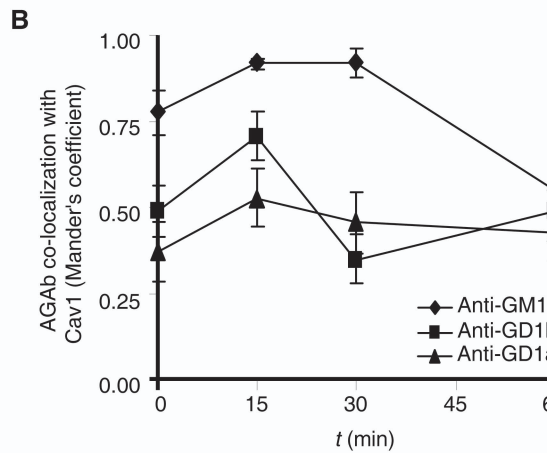
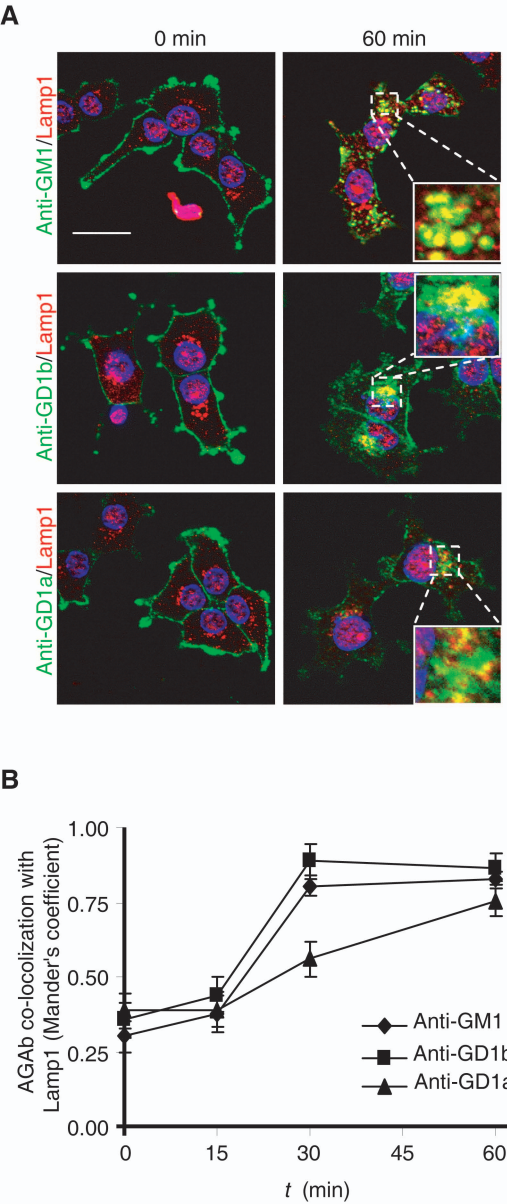
Supplemental Figure 1



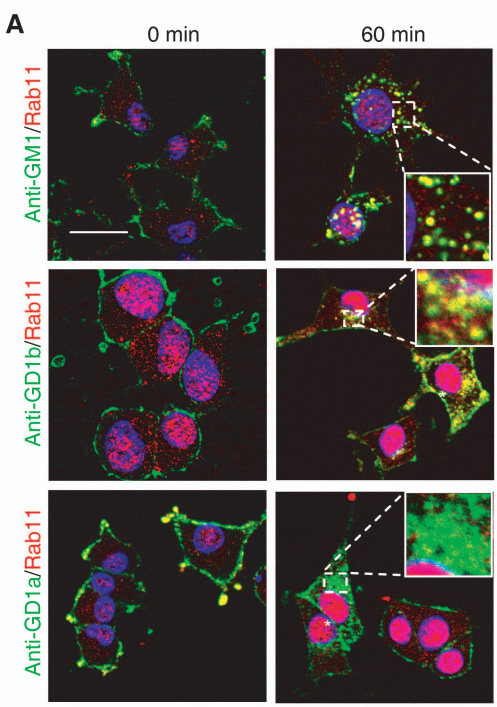

A
0 min
30 min
Anti-GM1/Cav1
Anti-GD1b/Cav1
Anti-GD1a/Cav1

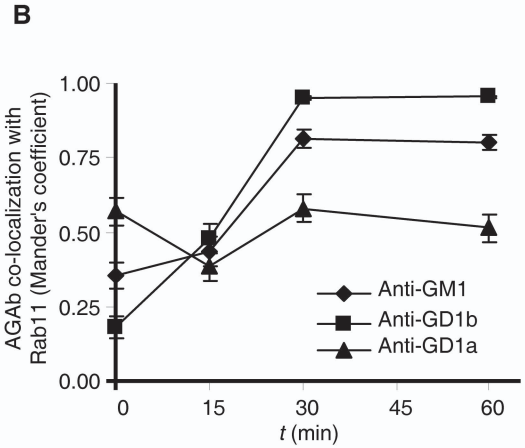

B
AGAb co-localization with Cav1 (Mander's coefficient)
1.00
0.75
0.50
0.25
0.00
0
15
30
45
60
t (min)
Anti-GM1
Anti-GD1b
Anti-GD1a

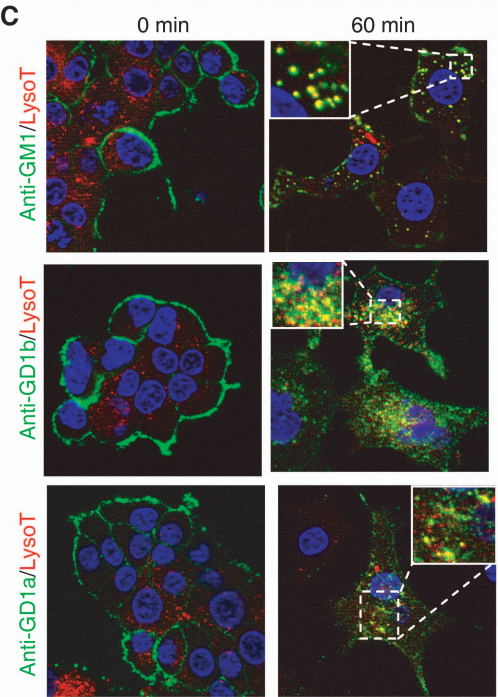
Supplemental Figure 3

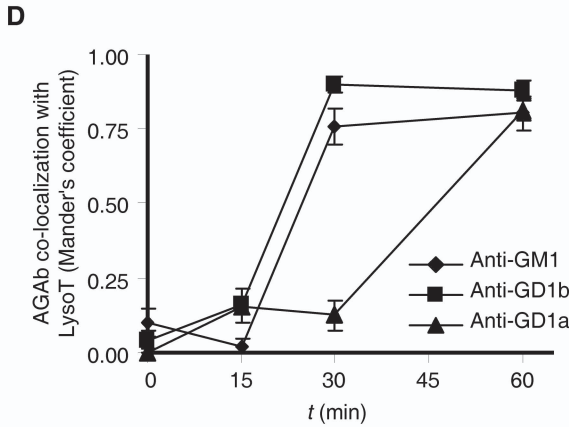


Supplemental Figure 4

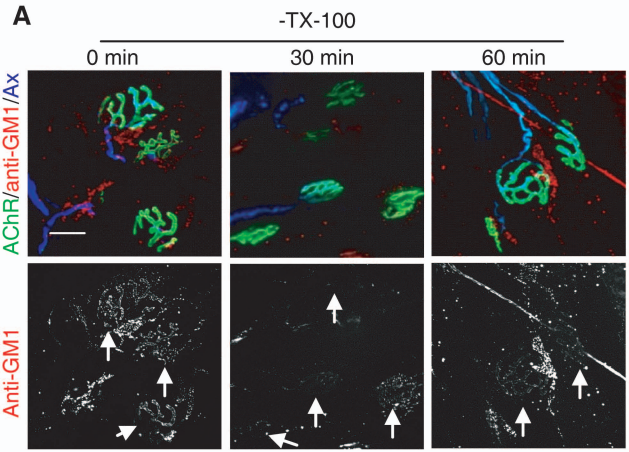


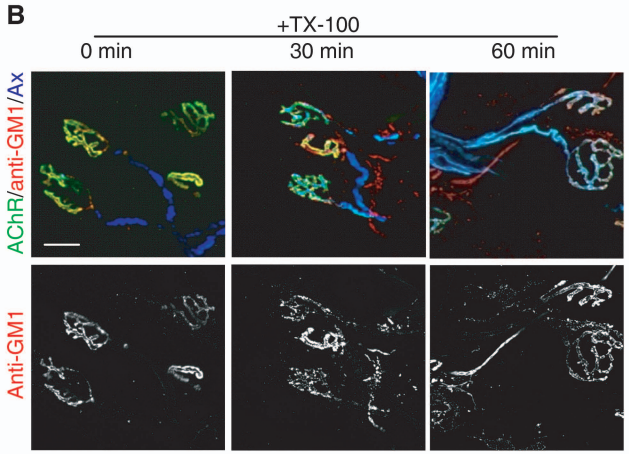


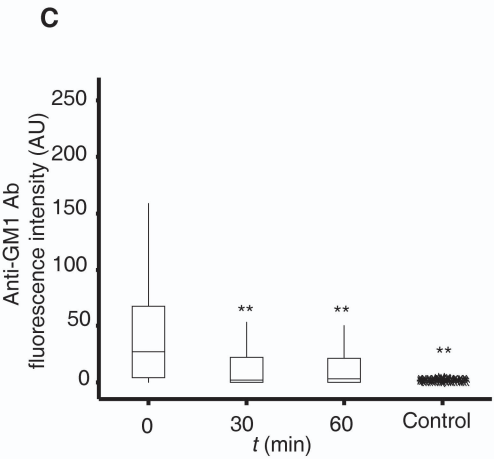


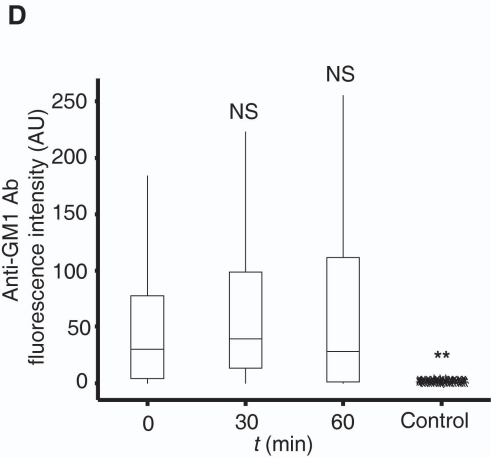


Supplemental Figure 5

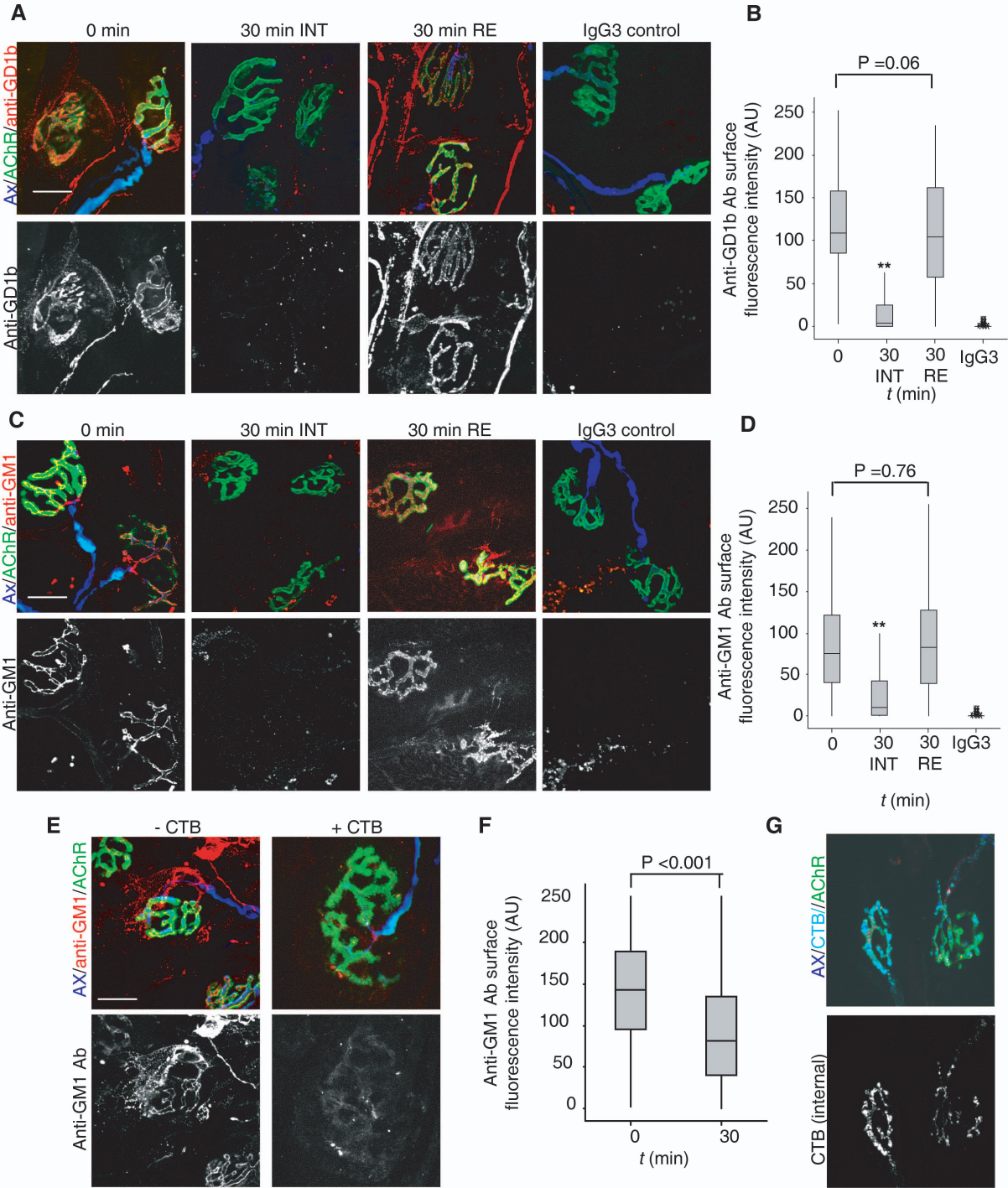




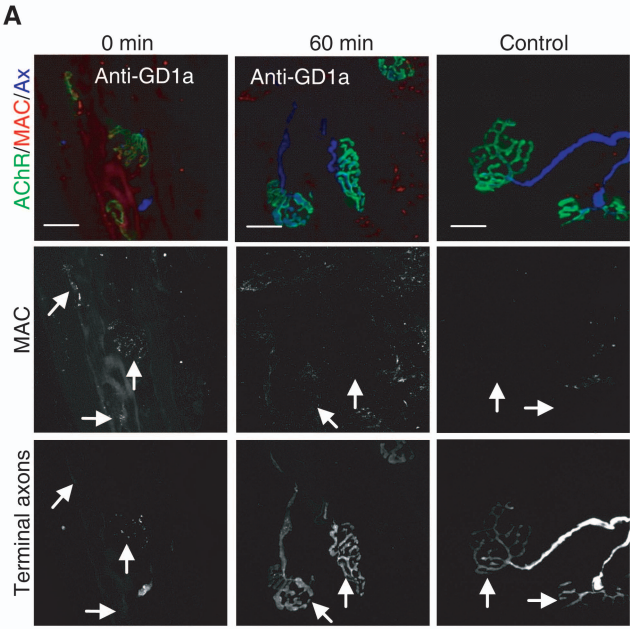


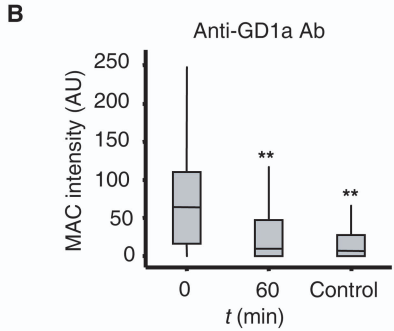


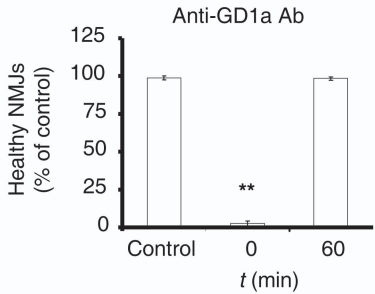
Supplemental Figure 6



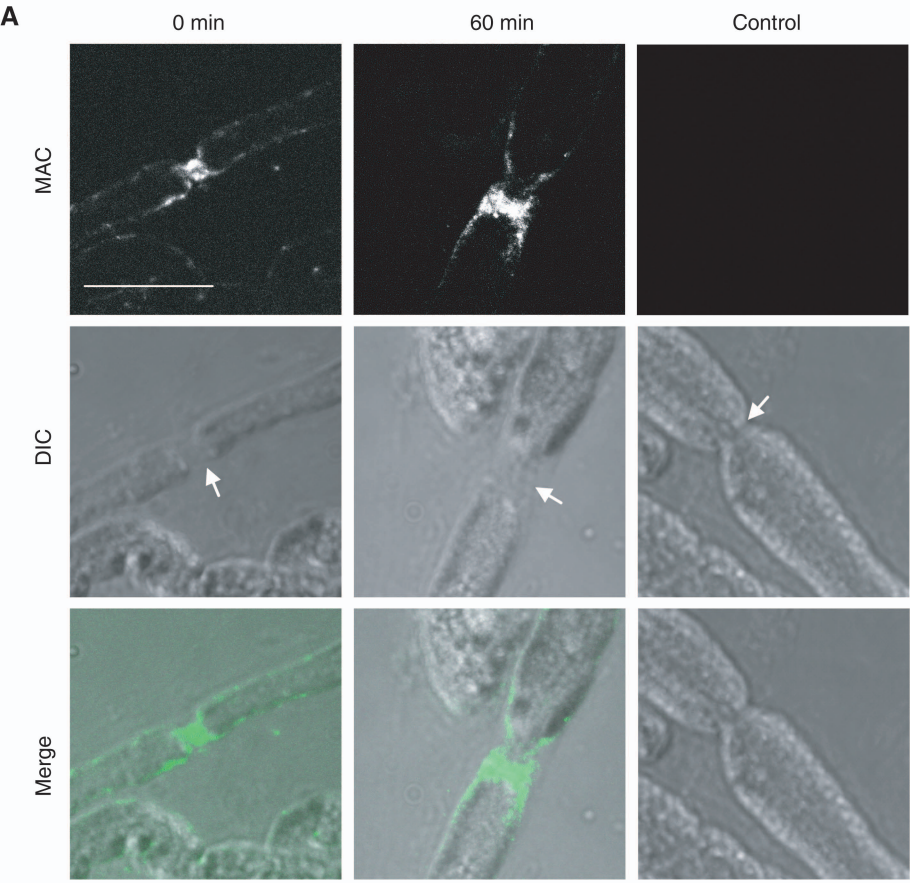
Supplemental Figure 7

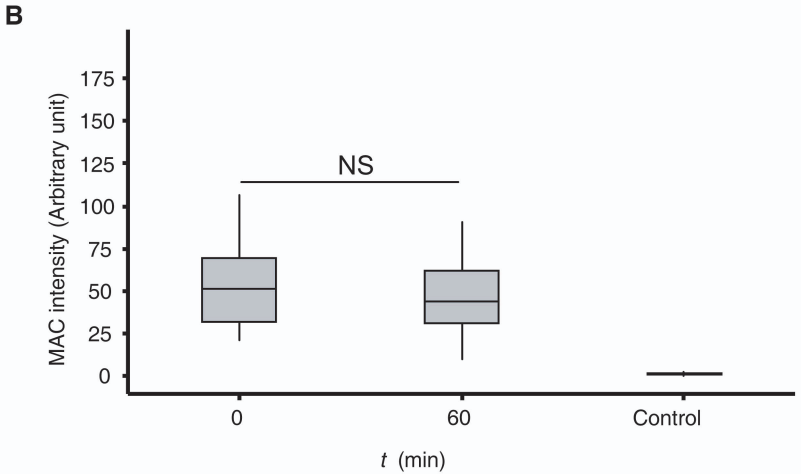






Supplemental Figure 8





Supplemental Figure legends

Supplemental Figure 1

Anti-GD1b Ab uptake by primary dorsal root ganglion (DRG) neurons. Neurons isolated from rat dorsal root ganglion (DRG) were labeled on ice with anti-GD1b Ab (green) and further incubated at 37ºC for 15, 30, or 60 minutes. Neurons were identified using anti-NFM Ab (pink, lower panels). The cells were permeabilized and further stain for the early endosome marker EEA1 (red). Anti-GD1b, NFM and EEA1 were visualized using the appropriate secondary Ab and analyzed by fluorescence microscopy. Scale bar 50 μm.

Supplemental Figure 2

AGAb colocalize with the detergent-resistant membrane (DRM) compartment. (**A**) PC12 cells labeled with AGAbs at 4ºC were incubated 37ºC for 15, 30, and 60 minutes to allow endocytosis. The cells were fixed, permeabilized and further labeled with the rabbit anti-caveolin-1 (Cav1). Internalized and surface AGAbs were detected using the appropriate FITC-labeled secondary Ab and the Cav1-positive vesicles revealed using the TRITC-labeled secondary Ab, and analyzed by fluorescence microscopy. Boxed areas show the degree of overlap (yellow, yellow-green or orange) between AGAbs and Cav1-prositive vesicles. Scale bar 20 μm. (**B**) The colocalization between AGAbs and Cav1-positive vesicle was estimated using the AxioVision software. Five to 10 dots per cells were quantified and at least 25 cells per condition were analyzed and the average of colocalization is plotted. AGAbs fully or partially overlap with the Cav1-positive vesicles. Shown are mean of ± SEM (n=3).

Supplemental Figure 3

AGAbs associate with the late endosomal compartment. (**A**) PC12 cells labeled with AGAbs (anti-GD1a, anti-GD1b, and anti-GM1) at 4ºC were incubated 37ºC to allow endocytosis for 15, 30, and 60 minutes. The cells were fixed, permeabilized and further incubated with the rabbit anti-Lamp1. Internalized and surface AGAbs was detected using the appropriate FITC-labeled secondary Ab and the late endosome detected with the TRITC-labeled secondary Ab. Note the colocalization (yellow; boxed area) of AGAbs with the late endosome. Scale bar 20μM. (**B**) AGAbs overlap with the late endosome was investigated using the AxioVision software. Five to 10 dots per cells was investigated and at least 25 cells per condition were analyzed and the average Mander's coefficient calculated. Plotted are mean of ± SEM (n=3).

Supplemental Figure 4

AGAbs localize to the recycling and lysosomal compartments. (**A**) PC12 cells labeled with AGAbs (anti-GD1a, anti-GD1b, and anti-GM1) at 4ºC were incubated 37ºC to allow endocytosis for 15, 30, and 60 minutes. The cells were fixed, permeabilized and further labeled with the Rab11 Ab. Internalized and surface AGAbs and Rab11 were detected using the appropriate secondary antibodies and analyzed by fluorescence microscopy. Non-specific nuclear staining seen with the anti-Rab11 Ab (white asterisk) was excluded from analysis (**C**) PC12 cells were preincubated at 37ºC for 1 hour with LysoTracker®-containing DMEM to label acidic vesicles. The cells were then

further treated with AGAbs and analyzed as described above. Note the colocalization (yellow, boxed area) of all 3 antibodies with the acidic vesicle. (**B** and **D**) The colocalization of AGAbs with the recycling and lysosomal compatment was assessed using the colocalization module of the AxioVision software. Five to 10 dots per cells were quantified and at least 25 cells per condition were analyzed and the average Mander's coefficient calculated. Shown are mean of ± SEM (n=3). Scale bar 20 μm.

Supplemental Figure 5

Internalization of Aanti-GM1 Ab from presynaptic membranes at NMJs occurs rapidly at physiological temperature (37ºC). (**A**) Triangularis sterni (TS) muscle was labeled with anti-GM1 Ab on ice and subsequently incubated at 37ºC for 30 and 60 minutes to allow endocytosis. Surface anti-GM1 Ab was detected with secondary Ab and analyzed by fluorescence microscopy. Arrows indicate the expected site of surface anti-GM1 Ab signal overlying the AChR signal at the NMJ upon incubation at 37ºC. (**B**) Following microscopic analysis, the same TS preparations were permeabilized (+TX-100) and the total internalized and surface anti-GM1 Ab imaged using secondary Ab. (**C-D**) Fluorescence images for anti-GM1 Ab were quantitated using the imageJ software. (**C**) Anti-GM1 Ab was rapidly depleted from the cell surface over time. (**D**) Following permeabilization, no significant difference in total anti-GM1 Ab signal overlying the NMJ was observed, compared with surface Ab levels at t=0. At least 150 NMJs was analyzed per time point and results are from 3 independent experiments. N=3, $^{**}p<0.001$. Scale bars 20 μM. Control tissues

were labeled with the fluorescent secondary Ab and showed no significant binding or uptake.

Supplemental Figure 6

AGAb uptake does not deplete the density of ganglioside at the presynaptic nerve terminal membrane. TS preparations were exposed anti-GD1b (**A**) or anti-GM1 (**B**) Abs for 2hrs at 4ºC, then imaged directly to determine baseline staining levels prior to any AGAb internalization (0 min of internalization time point). Separate TS preparations were incubated with unlabeled anti-GD1b (**A**) and anti-GM1 antibodies (**C**) for 2hrs at 4ºC, then allowed to internalize antibodies for 30 min at 37ºC, and then reverted to 4ºC. Preparations were then stained with secondary Ab to determine the residual amount of surface AGAb following the 30 min uptake period (all panels, 30 min time points). As can be seen the vast majority of Ab is internalised by 30 mins. In these TS preparations, surface Ab binding was comparable to levels obtained at baseline staining (0 mins). Control tissues stained with anti-TNP Ab (IgG3 control) followed by fluorescent-labeled secondary Ab showed no binding. (**E**) TS preparations were incubated with or without CTB for 2hrs at 4ºC, then allowed to internalize CTB for 30 min at 37ºC (shown in **G**). Preparations were reverted to 4ºC and stained with anti-GM1 Ab (**E, F**). A 43% reduction in surface GM1 was observed. NMJs were detected by staining the AChRs with α-BTx. Scale bar 20 µm. AGAb signal is determined using ImageJ software. At least 150 NMJs were investigated per condition and two independent experiments were performed.

Supplemental Figure 7

Anti-GD1a Ab uptake attenuates complement activation ex vivo, and protects nerve terminals from complement-mediated injury. (**A**) TS from wild-type mice were labeled at 4ºC with anti-GD1a Ab respectively, then further incubated at 37ºC for 60 minutes (or retained at 4ºC) before exposure to NHS. Control tissues were exposed to H-I NHS. Membrane attack complex (MAC) deposits at the endplates were detected with anti-C5-b9 Ab. MAC deposits at NMJs of TS incubated at 37ºC for 60 minutes were greatly reduced compared to incubation maintained at 4ºC. Arrowheads indicate expected positions of NMJs. Scale bar 20µm. (**B**) Quantitation of MAC was conducted using ImageJ software. At least 150 NMJs were investigated per condition. n=3, ***p*<0.001. (**C**) The NMJ was assessed for the loss of intra-terminal cytosolic CFP overlying the acetylcholine receptor staining and the percentage of NMJs that retained CFP (healthy NMJs) was calculated. An increase in the number of healthy NMJs in tissue incubated for 60 mins at 37ºC was observed. Data are the mean from 3 independent experiments and at least 150 NMJs were investigated per condition. ***p*=0.001.

Supplemental Figure 8

Complement activation at the node of Ranvier is unaffected by incubation at physiological temperature. Teased sciatic nerve fibres from GD3s$^{-/-}$ mice were labeled with anti-GM1 Ab, then incubated at 37ºC for 60 mins or maintained at 4^{o}C (0 mins), prior to exposure to 40% NHS. (**A**) Illustrative images of MAC deposit profile at the node of Ranvier in tissue incubated at 37ºC (60 min) or maintained at 4^{o}C (0 min). Control tissue was treated with anti-GM1 Ab, then

exposed to HI-NHS. The node of Ranvier appear elongated in tissue incubated at both 4ºC and 37ºC (arrow points, DIC panel), indicating injury caused by MAC deposits. (**B**) Nodes of Ranvier were indentified and the level of MAC deposit estimated using ImageJ software. 25 NoR were investigated per condition in 3 independent experiments. NS, not significant. Scale bar 20 µM.