Supplemental Figure 1. The association of AQP4 with the detergent-resistant membranes (DRM) is dependent on cholesterol in astrocytes. (A) Detergent resistant membranes (DRM) and non-detergent resistant membranes (Non-DRM) were harvested from astrocytes incubated for 8h with 0.5 μg/ml of the cholesterol-sequestering agent, filipin. Immunoblots were probed for the transferrin receptor (TfR), α-tubulin, β-DG, AQP4, flotillin-1 and caveolin-1 and the dot blot labeled for GM1. **(B)** Proteins from astrocytes incubated for 1h with 20 mM of the cholesterol-extracting agent, methyl β-cyclodextrin (MβCD), were harvested in DRM and Non-DRM and immunoblotted for AQP4 and flotillin-1.

Supplemental Figure 2. The morphology of astrocytes remains unchanged after laminin treatment. (A-C) Astrocytes were incubated in the absence or **(D-F)** the presence of 30 nM laminin and labeled for laminin **(A, and D)**. The plasma membrane of these cells was labeled using wheat germ agglutinin conjugated to Alexa Fluor 568 **(**WGA; **B and E)**. Scale bar, 45 μm.

Supplemental Figure 3. Laminin induces the coclustering of GM1-containing lipid rafts with AchR and β-DG in C2C12 myotubes. (A-D) Differentiated C2C12 myotubes were incubated in the absence or **(E-H)** the presence of 30 nM laminin overnight and labeled for GM1 using FITC-conjugated cholera toxin subunit B **(A and E)**, acetylcholine receptors (AchR) using RITC-conjugated α-bungarotoxin **(B and F)** and β-DG **(C and G)**. Scale bar, 8 μm.

Supplemental Figure 4. Laminin but not agrin or fibronectin clusters the the GM1-containing lipid rafts in astrocytes. (A-D) Astrocytes were incubated with 30 nM laminin, **(E-H)** 10 nM C-agrin 4,8 or **(I-L)** 100 nM fibronectin and labeled for GM1 **(A, E and I)**, β-DG **(B, F and J)** and AQP4 **(C, G and K)**. Scale bar, 45 μm.

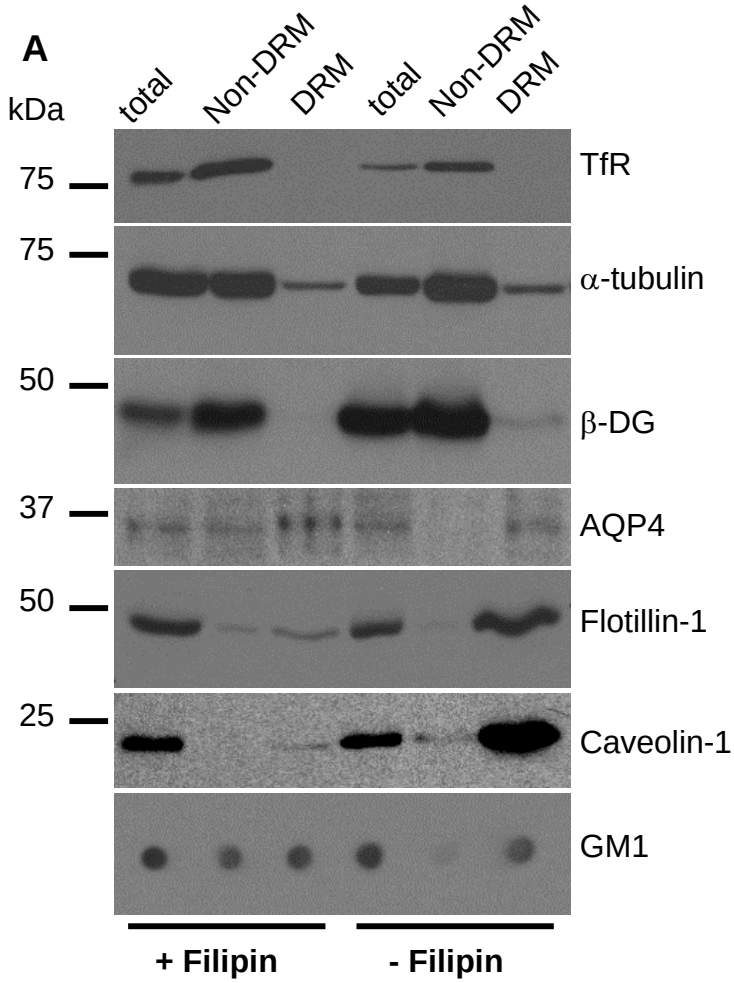
Supplemental Figure 5. The disruption of lipid rafts with the cholesterol-sequestering agent, filipin, inhibits β-DG, AQP4 and laminin clustering. (A-F) Astrocytes incubated with 30 nM laminin or **(G-L)** 30 nM laminin and 0.5 μg/ml of filipin were double immunolabeled for β-DG **(A, G)** and laminin **(B, H)** or β-DG **(D, J)** and AQP4 **(E, K)**. Scale bar, 50 μm.

Supplemental Figure 6. Cholesterol depletion does not alter dystroglycan and AQP4 expression levels in astrocytes. Western blot analysis of α-DG, β-DG and AQP4 total expression levels was performed in astrocytes treated with 10 μM (+Mevastatin) and control astrocytes (+DMSO).

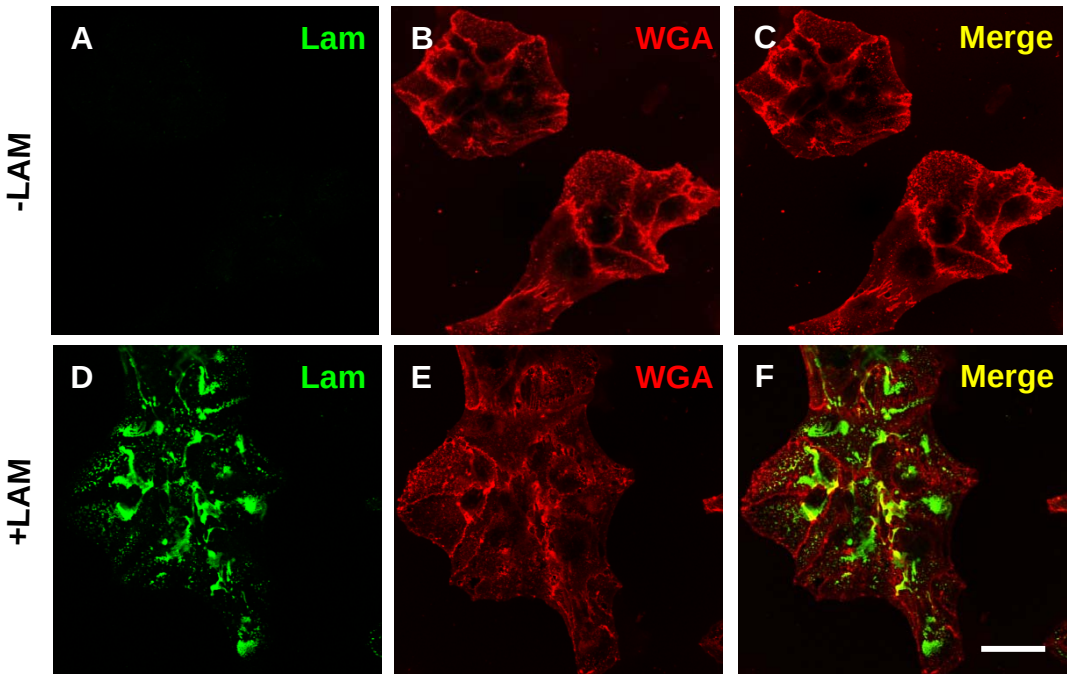
Supplemental Figure 7. The morphology of astrocytes remains unchanged after dystroglycan and AQP4 silencing. (A-C) Astrocytes transfected with siCTL, siDAG1 and siAQP4 were immunolabeled for the glial fibrillary acidic protein (GFAP) and **(D-F)** corresponding differential interference contrast photomicrographs are shown (DIC). Scale bar, 30 μm.

Supplemental Figure 8. β-DG remains associated with non-detergent-resistant membranes in laminin-treated astrocytes. Proteins from astrocytes incubated with (+LAM) or without 30 nM laminin (-LAM) were harvested in detergent resistant membranes (DRM) and non-detergent resistant membranes (Non-DRM) and blotted for AQP4, β-DG and GM1.

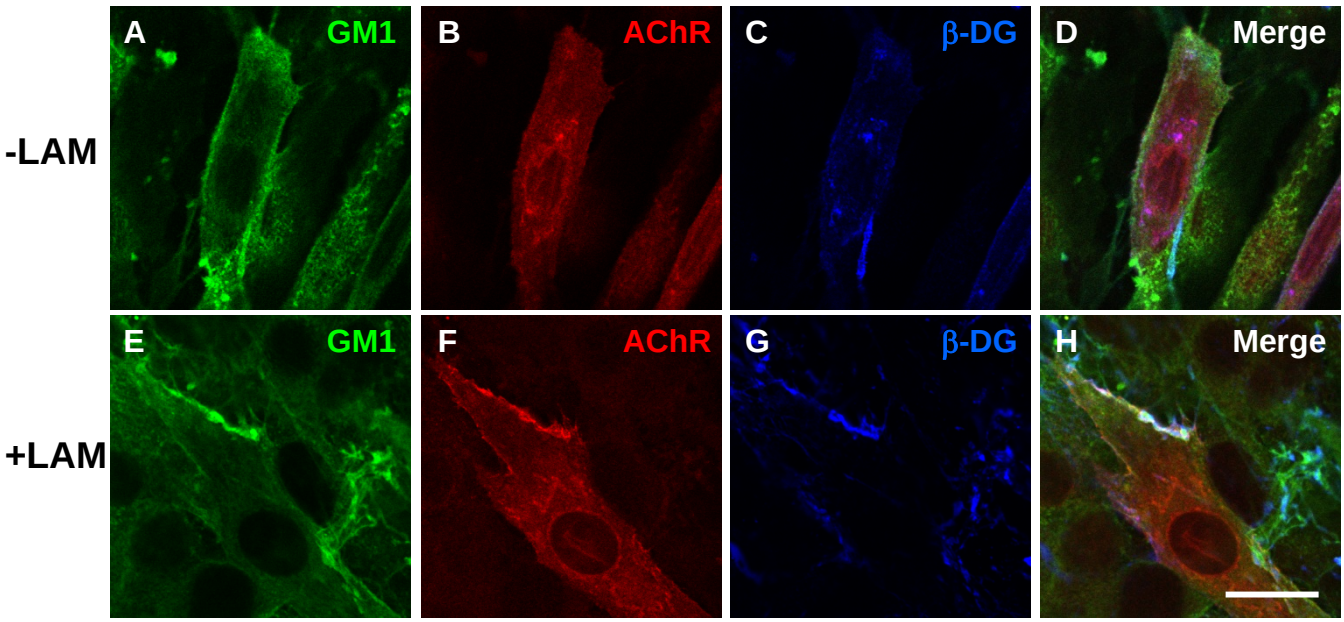
Supplemental Figure. 1



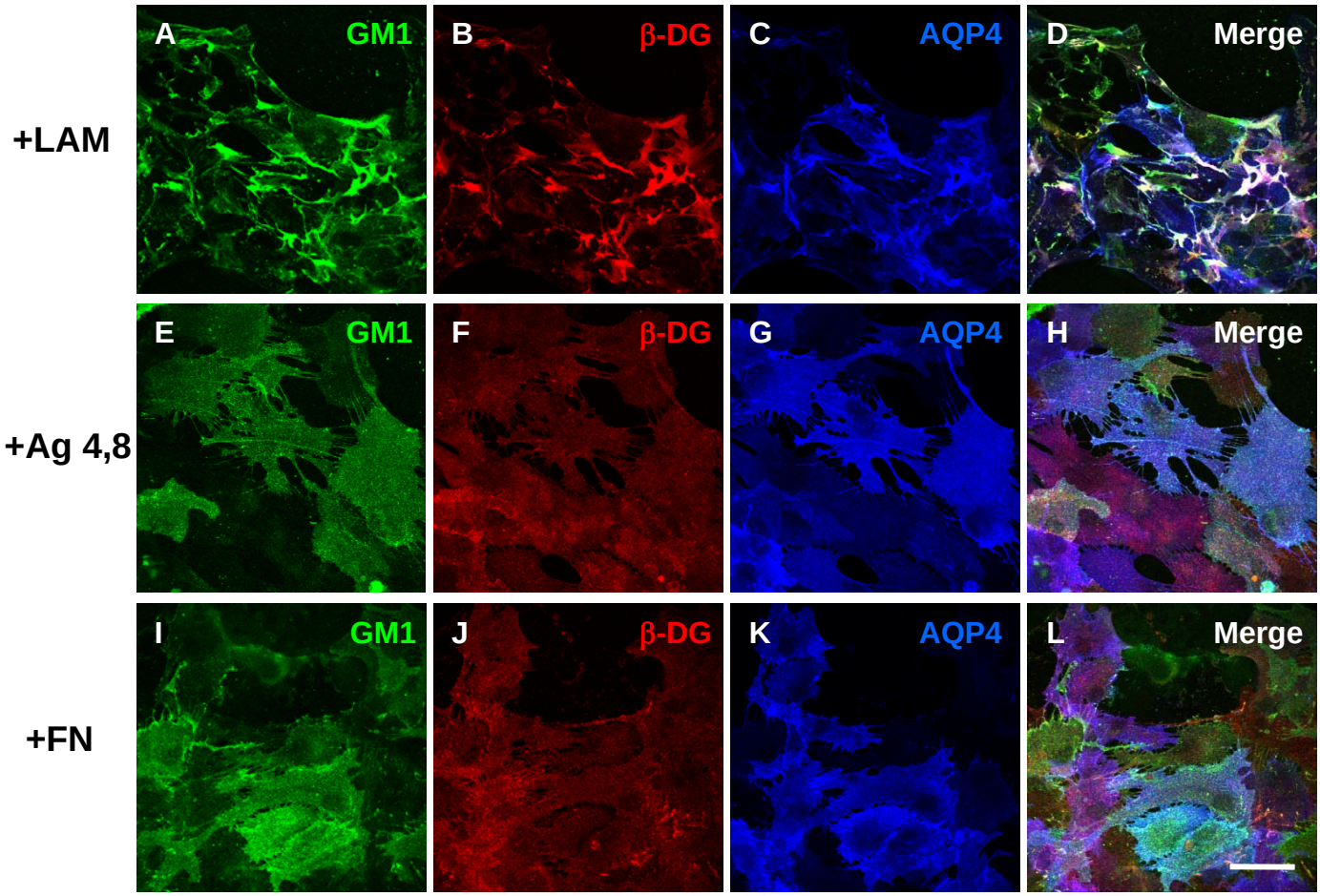
Supplemental Figure. 2



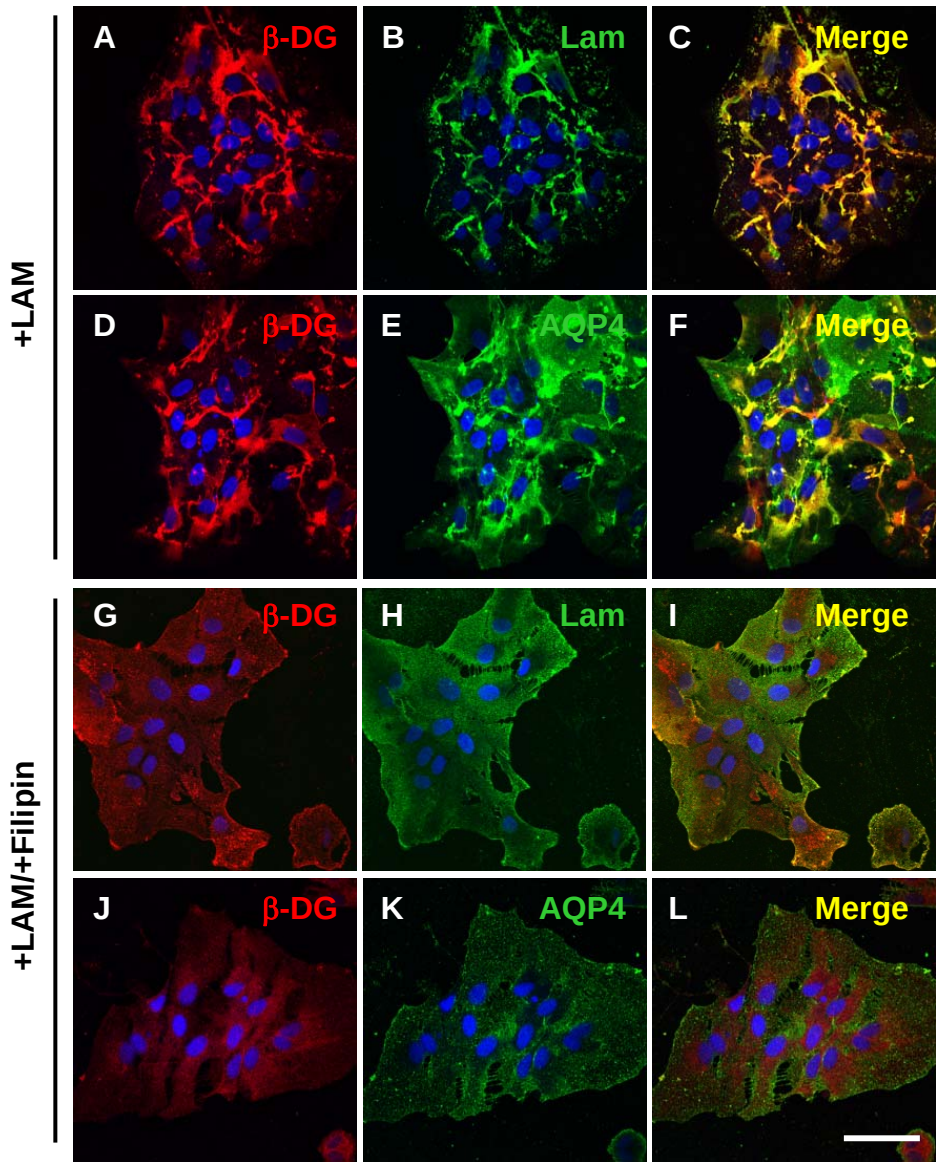
Supplemental Figure. 3



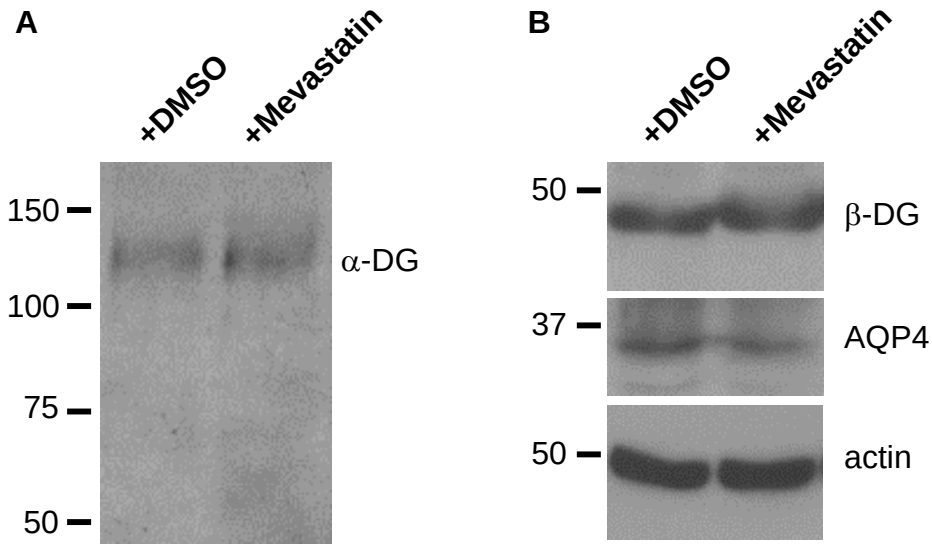
Supplemental Figure. 4



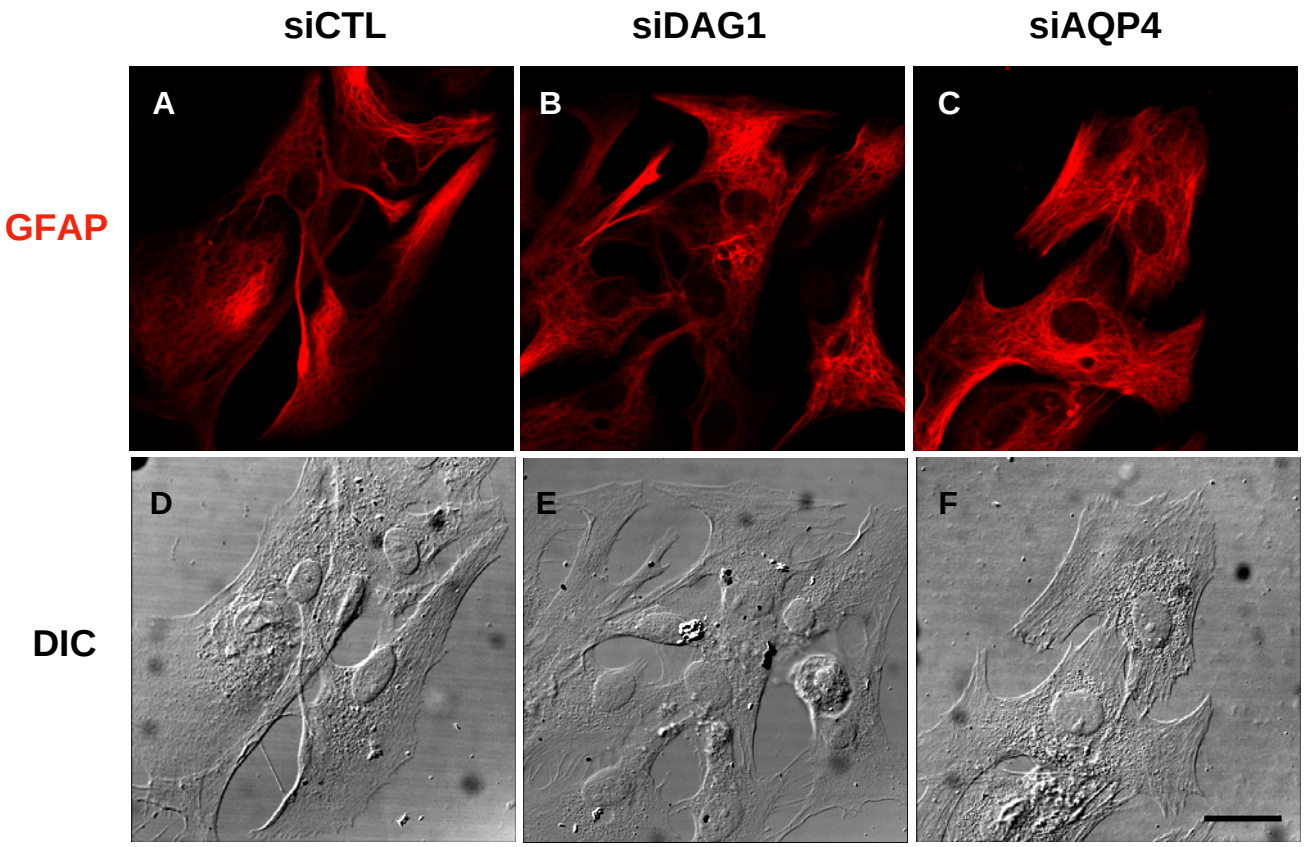
Supplemental Figure. 5



Supplemental Figure. 6



Supplemental Figure. 7



Supplemental Figure. 8