

Figure 1

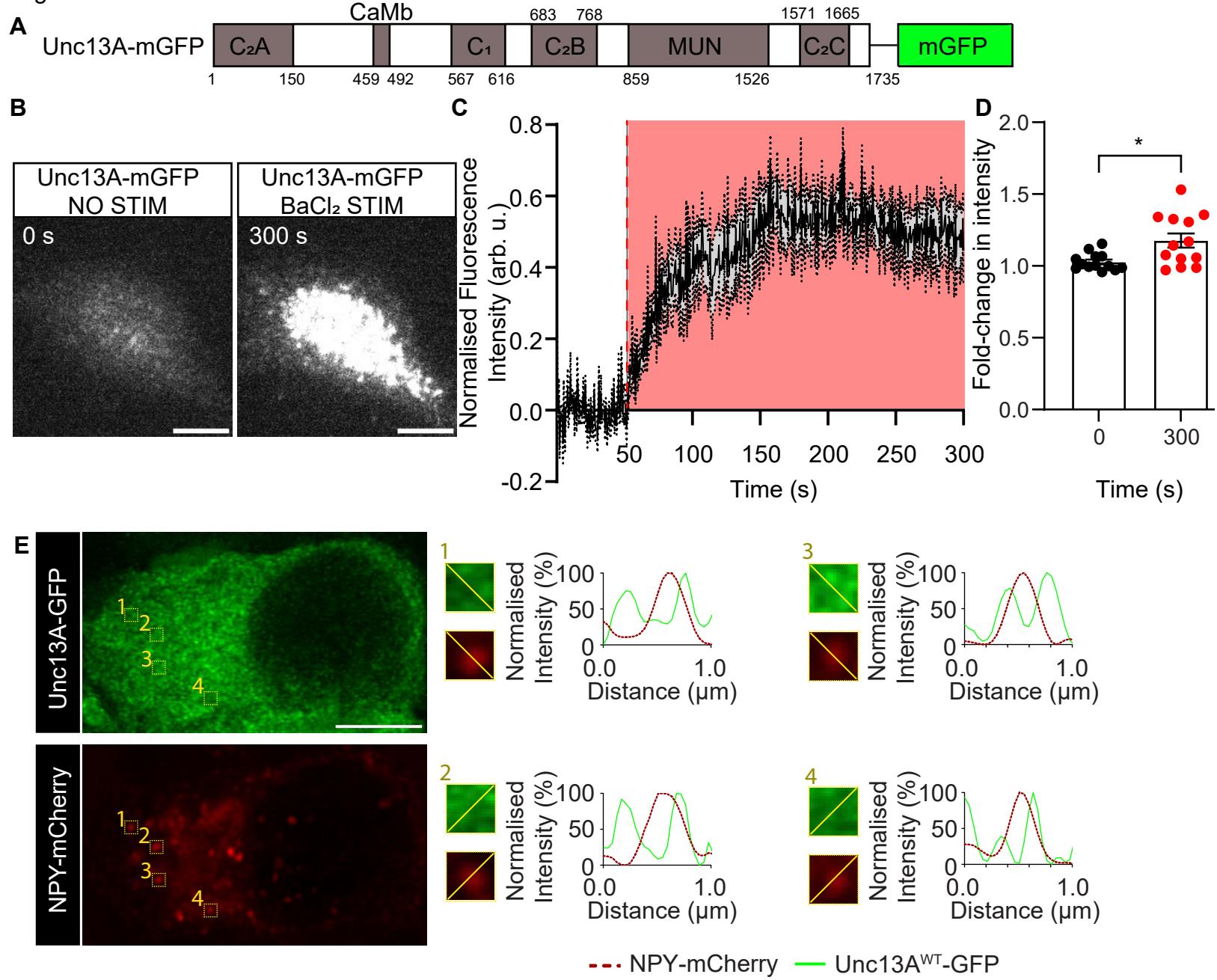
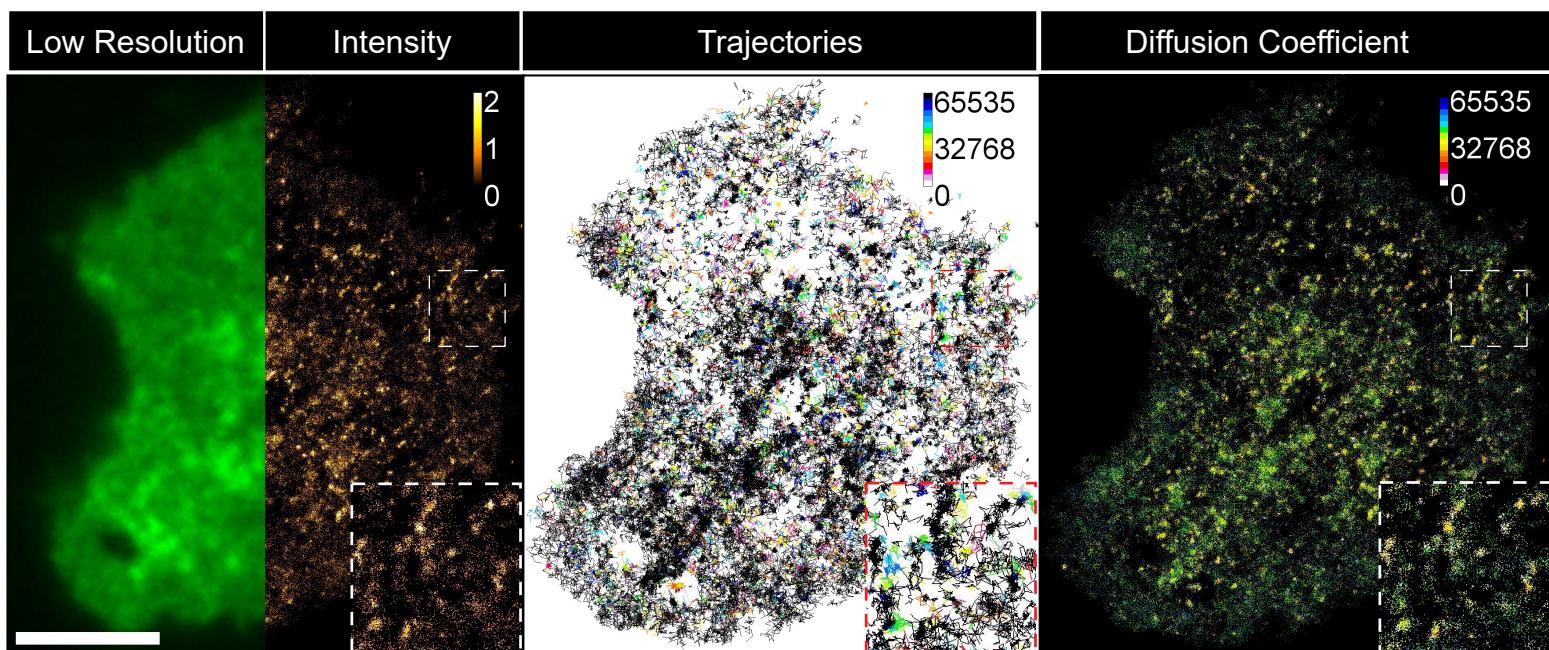
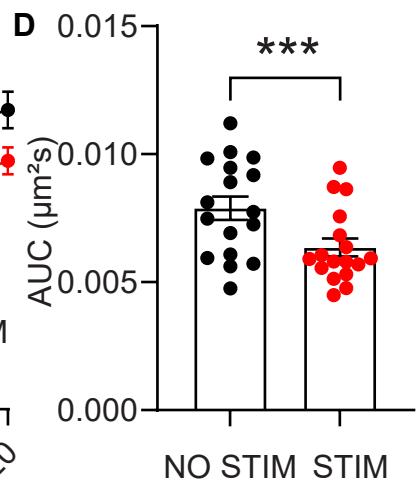
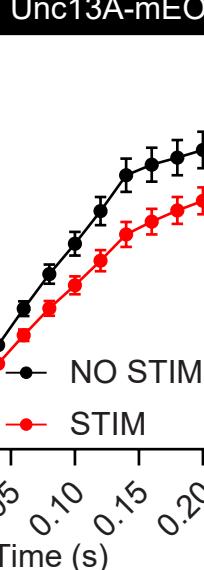
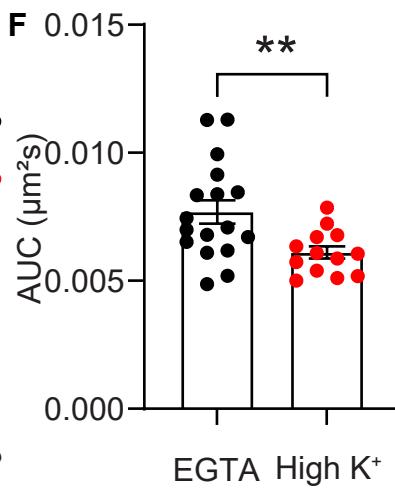
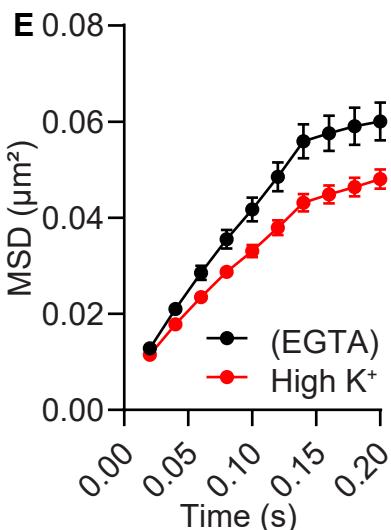
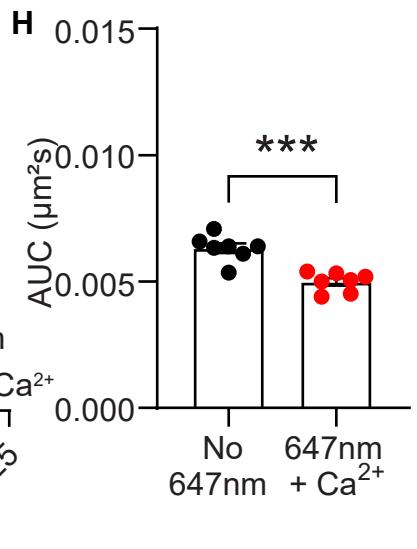
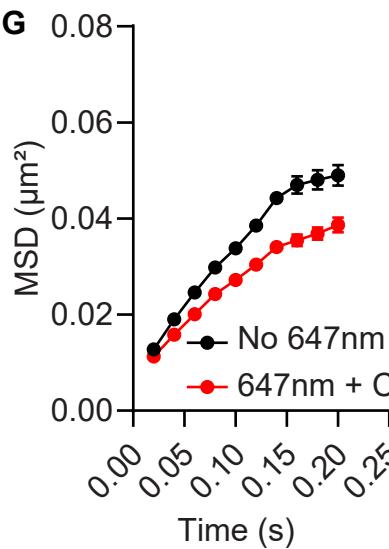


Figure 2

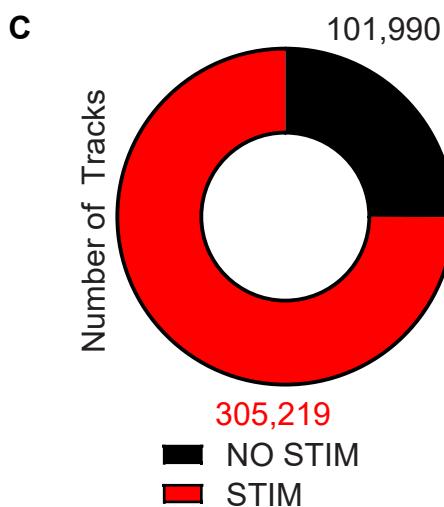
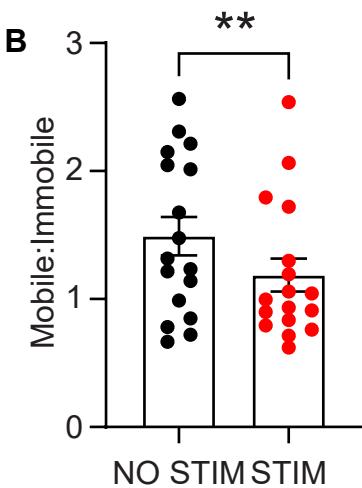
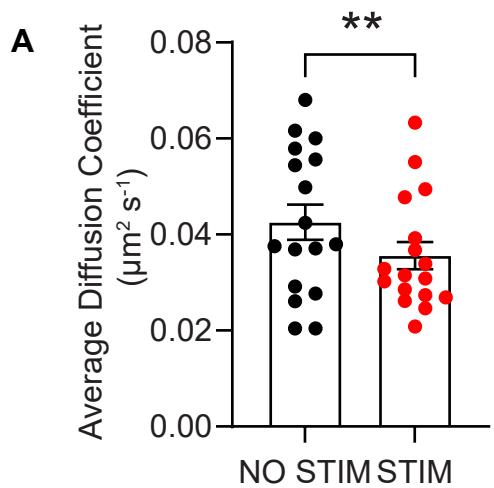
Unc13A-mEOS2

A**B**Unc13A-mEOS2 (+ BaCl₂)Unc13A-mEOS2 (+ High K⁺)Unc13A-mEOS2 (+ FCK-ChrimsonR^{GFP})

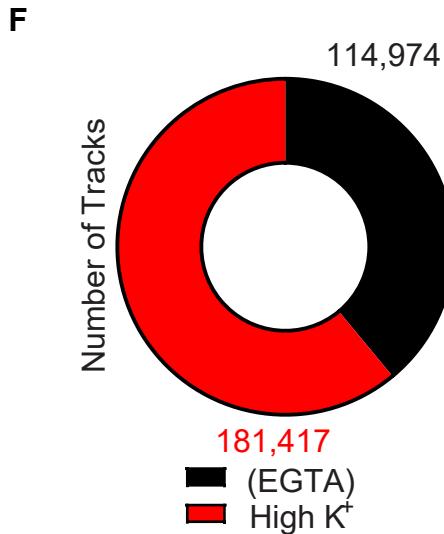
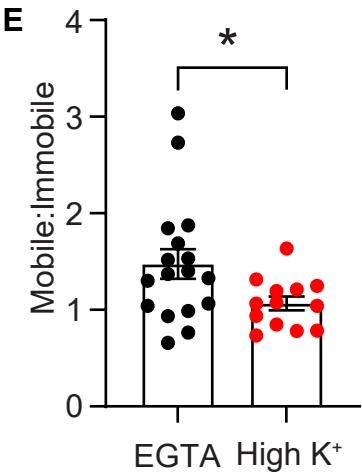
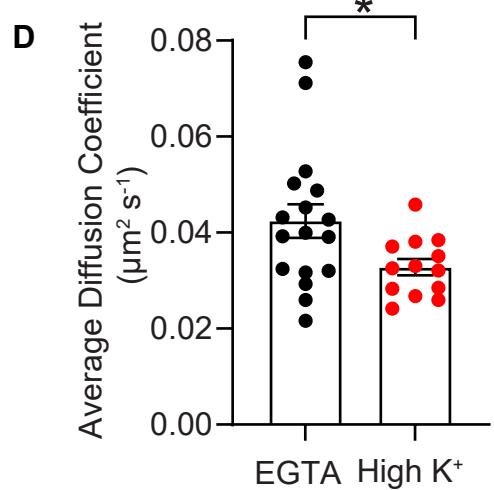
Endogenous Unc13A-mEOS2

Supplementary Figure 1

Unc13A-mEOS2 (+ BaCl₂)



Unc13A-mEOS2 (+ High K⁺)



Unc13A-mEOS2 (+ FCK-ChrimsonR^{GFP})

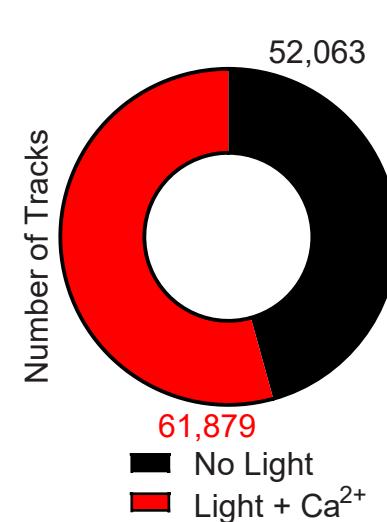
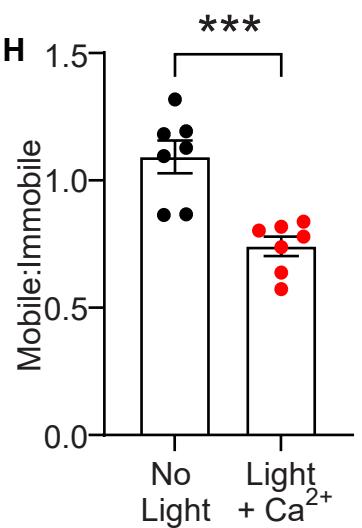
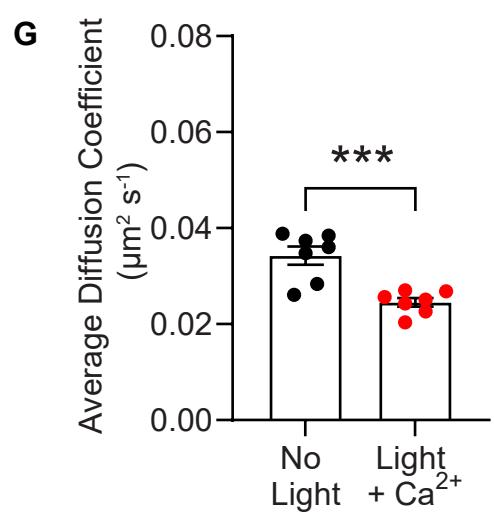
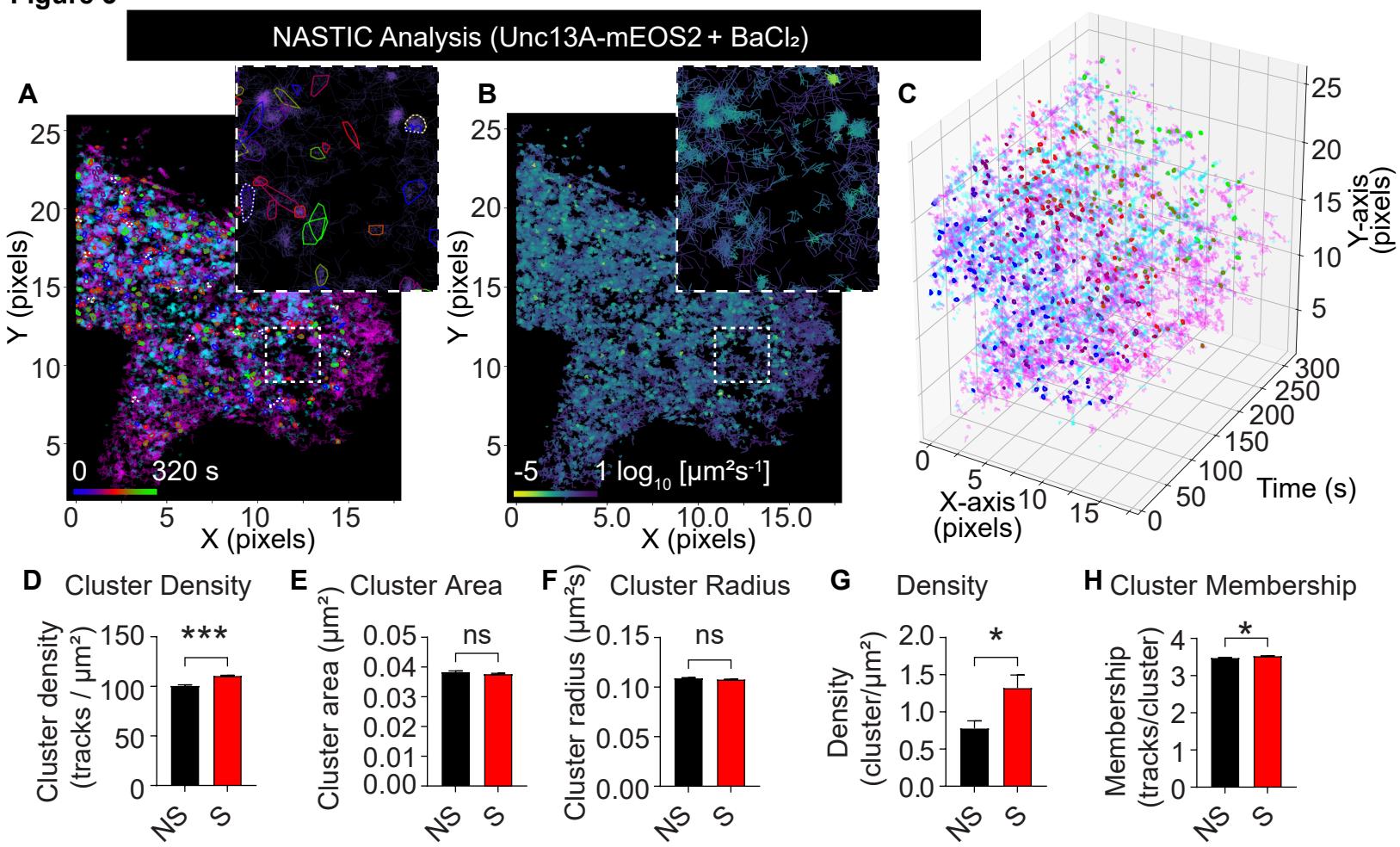
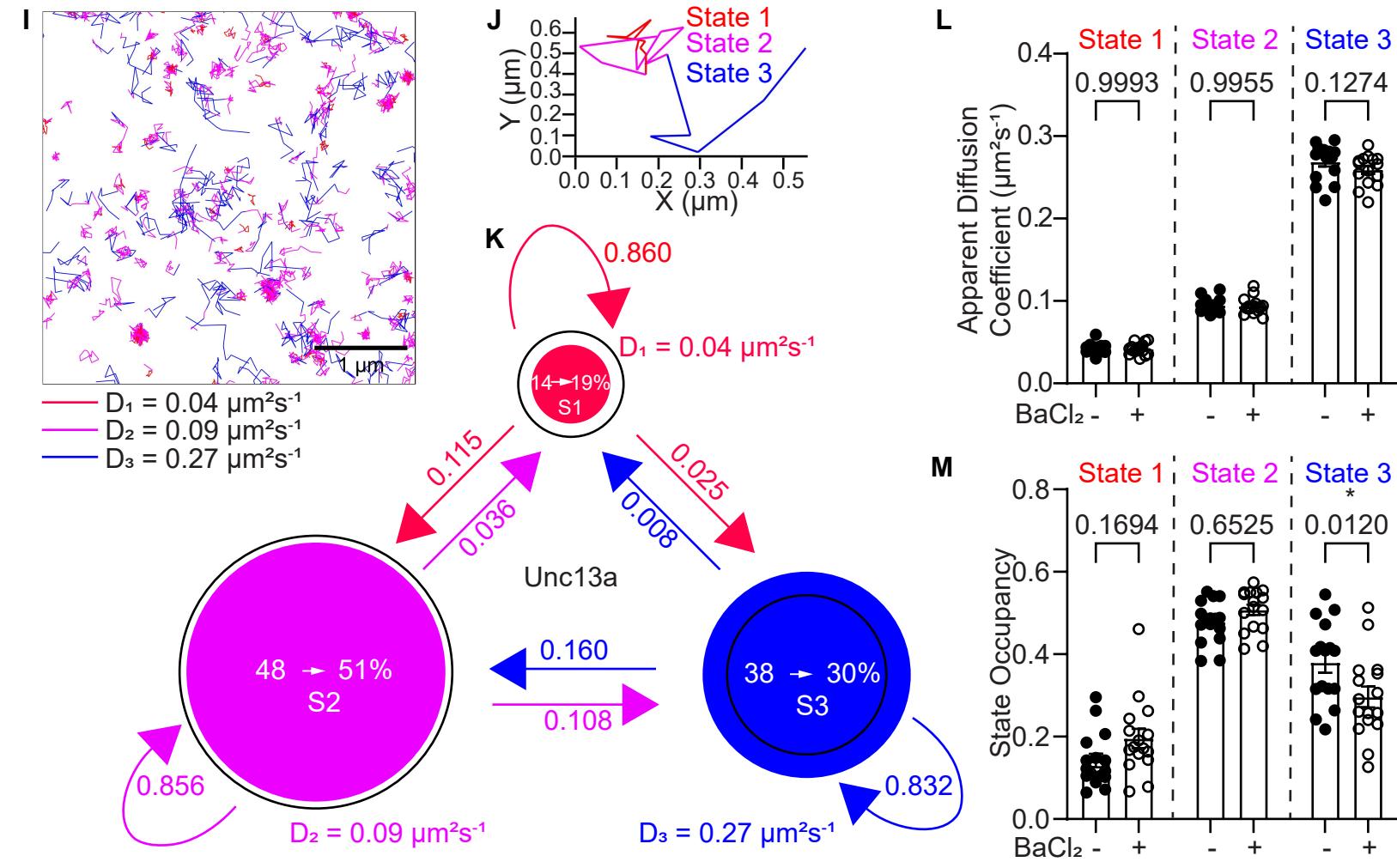


Figure 3**HMM Analysis (Unc13A-mEOS2 + BaCl₂)**

Supplementary Figure 2

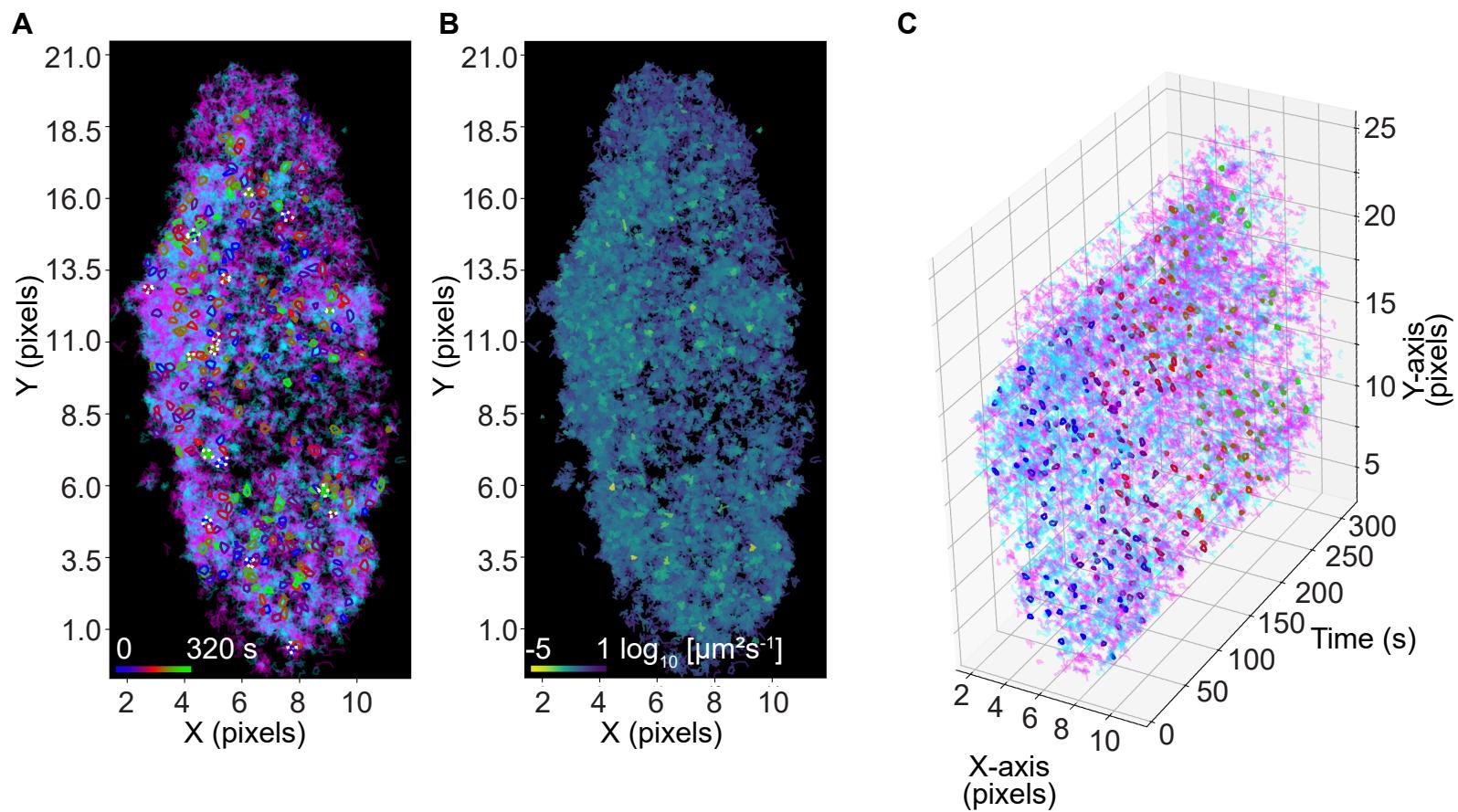
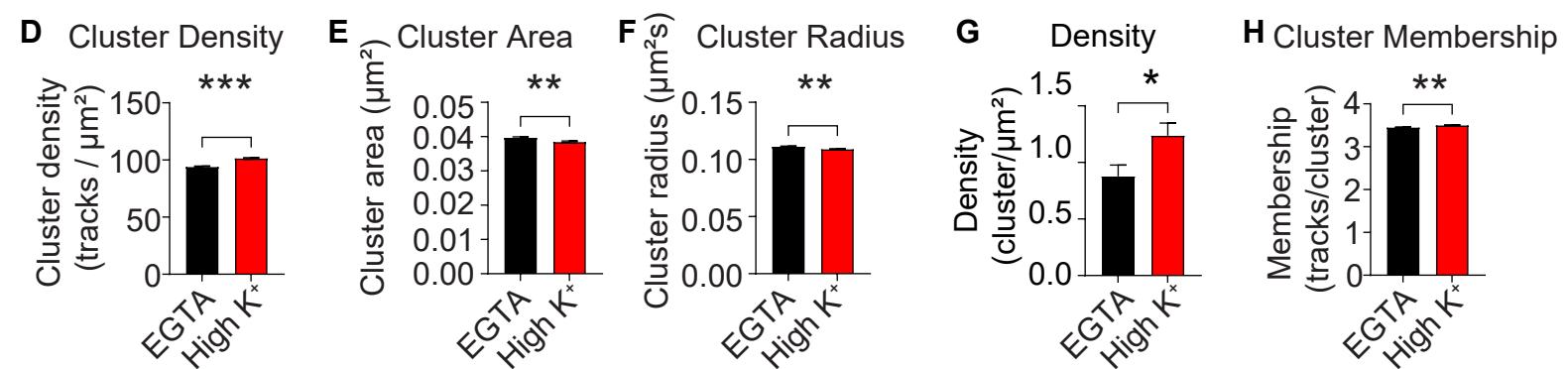
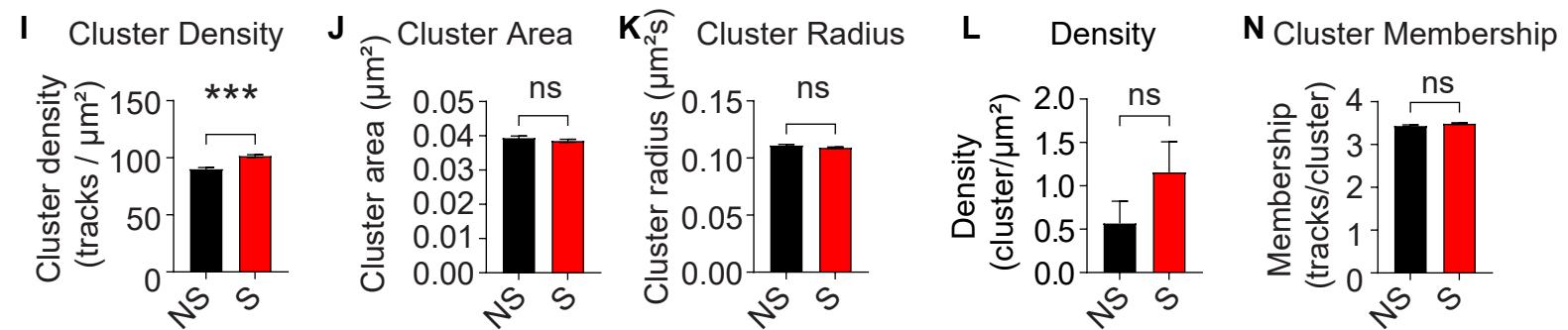
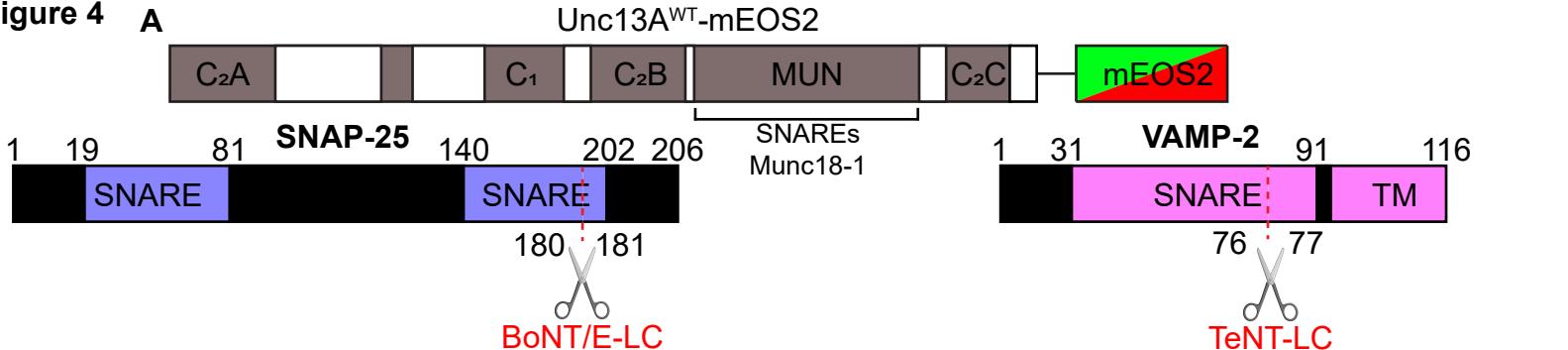
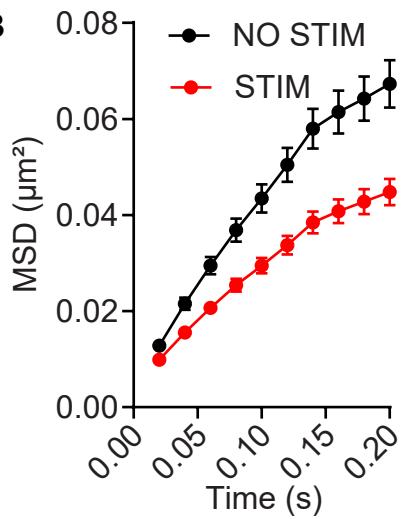
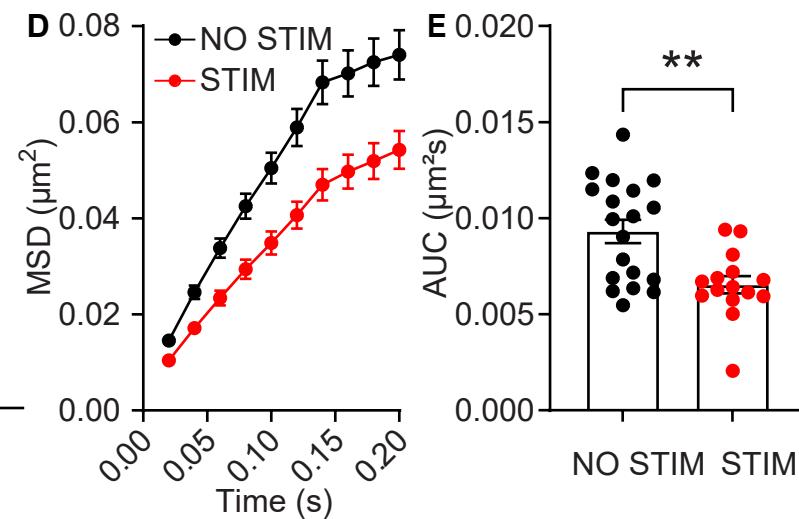
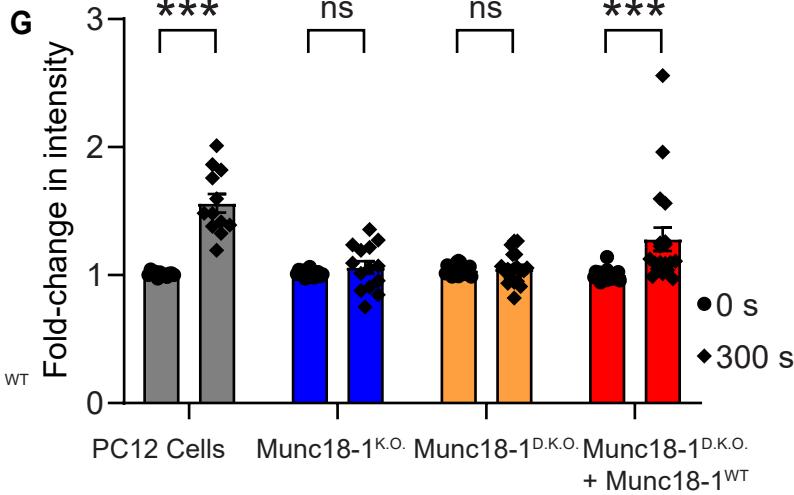
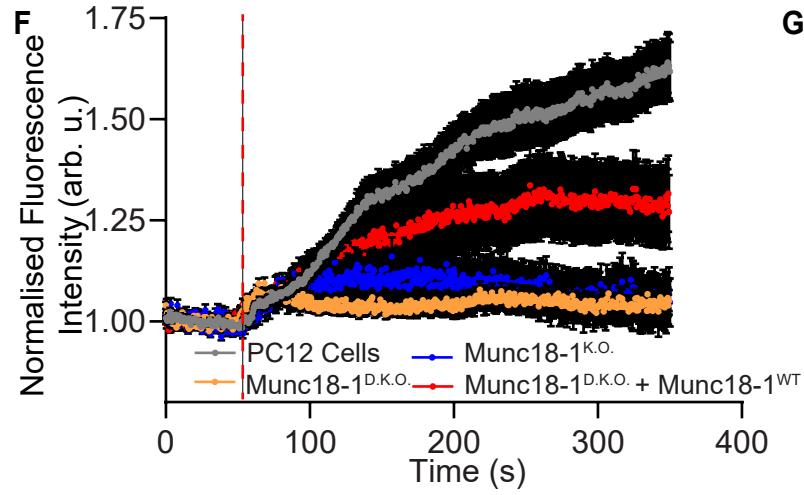
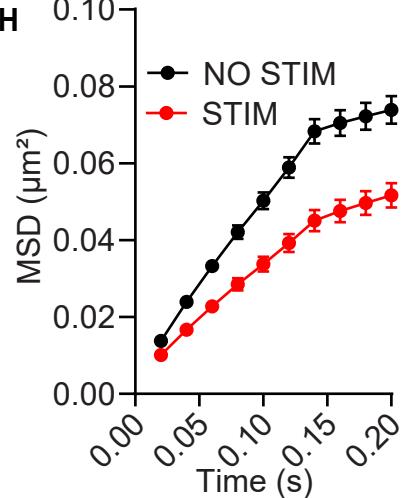
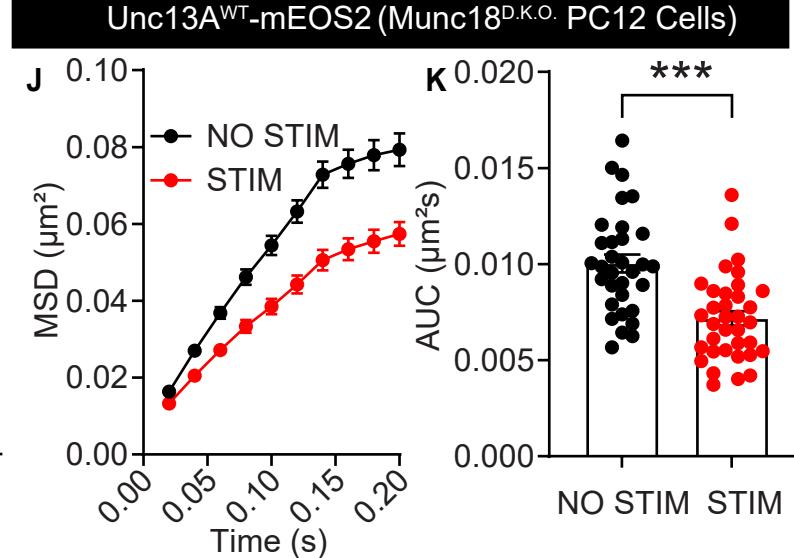
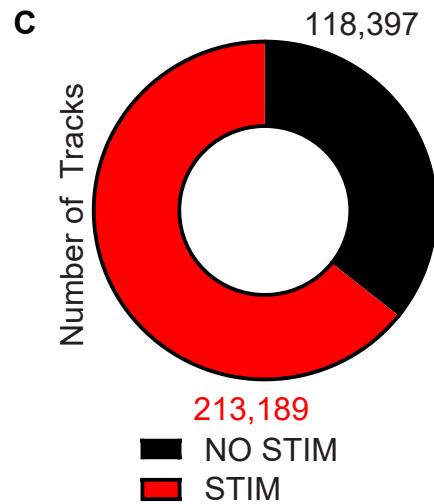
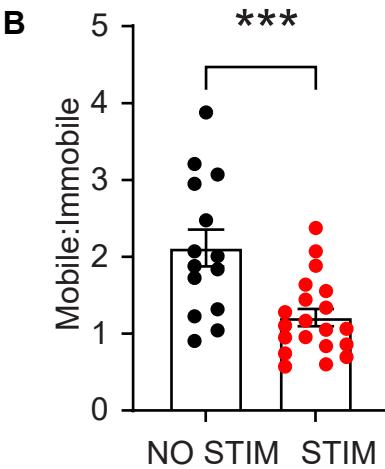
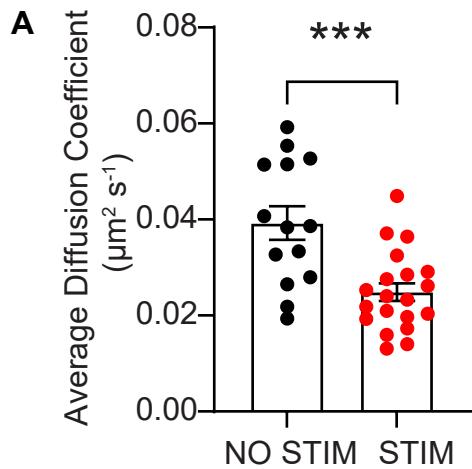
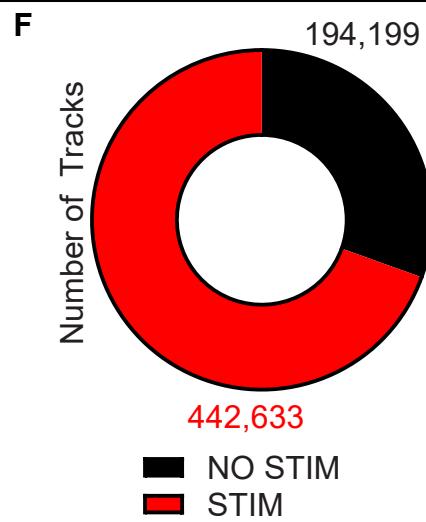
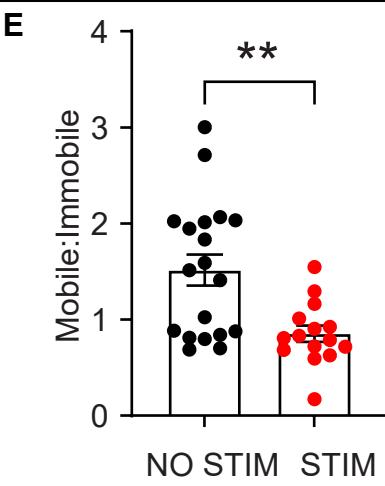
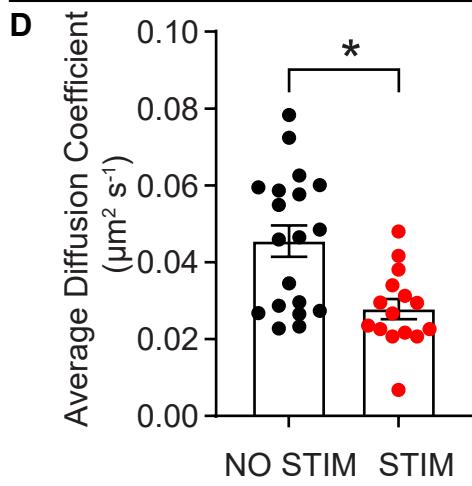
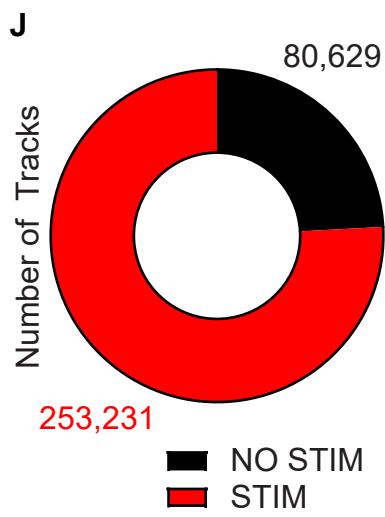
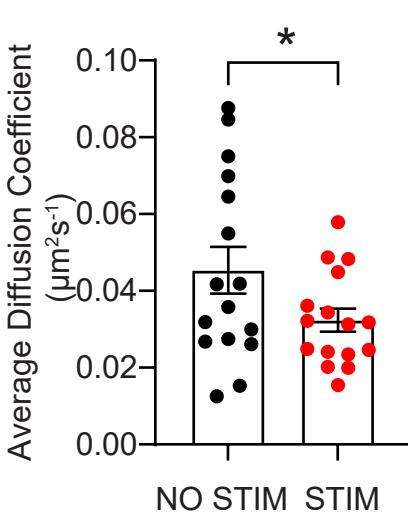
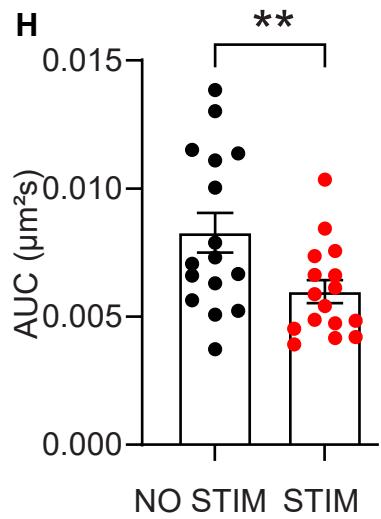
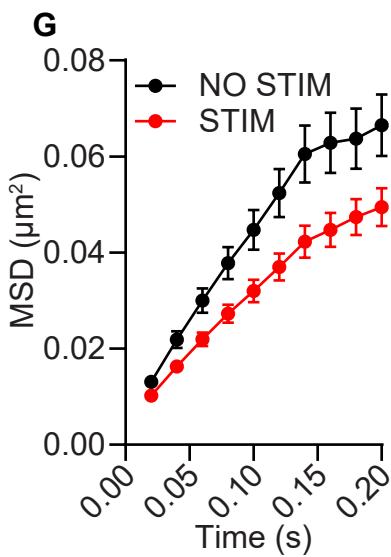
NASTIC Analysis (Unc13A-mEOS2 + High K⁺)NASTIC Analysis (Unc13A-mEOS2 + ChrimsonR^{GFP})

Figure 4 A**Unc13A^{WT}-mEOS2 + BoNT/E-LC****Unc13A^{WT}-mEOS2 + TeNT-LC****Unc13A^{WT}-mEOS2 + TeNT-LC****VAMP2-pHluorin (Native, Munc18-1^{K.O.}, Munc18^{D.K.O.} PC12 Cells)****Unc13A^{WT}-mEOS2 (Munc18^{K.O.} PC12 Cells)****Unc13A^{WT}-mEOS2 (Munc18^{D.K.O.} PC12 Cells)**

Supplementary Figure 3

Unc13A^{WT}-mEOS2 + BoNT/E-LCUnc13A^{WT}-mEOS2 + TeNT-LCUnc13A^{WT}-mEOS2 + Munc18-1^{WT::GFP} (Munc18^{K.O.} PC12 Cells)

Supplementary Figure 4

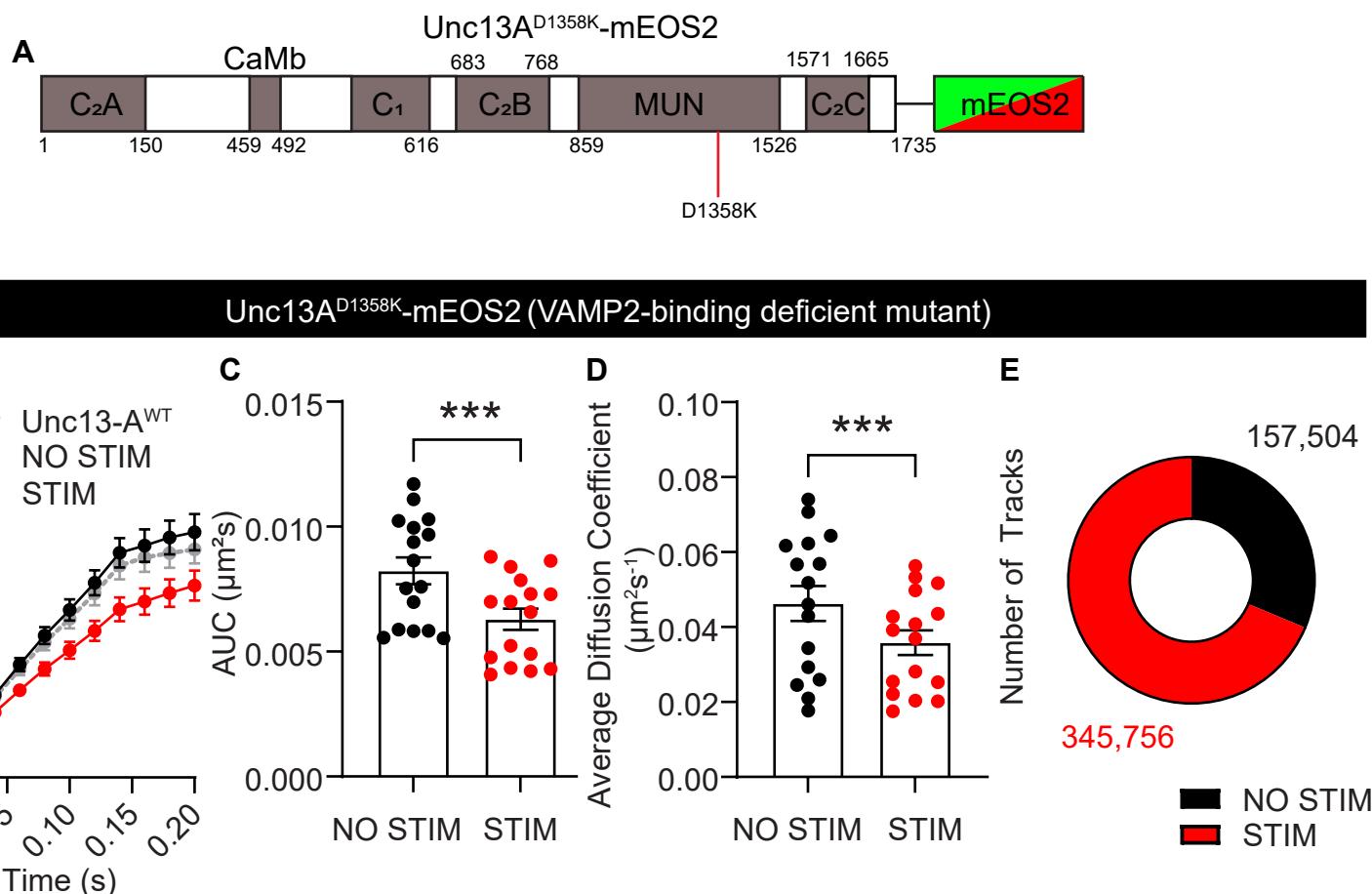
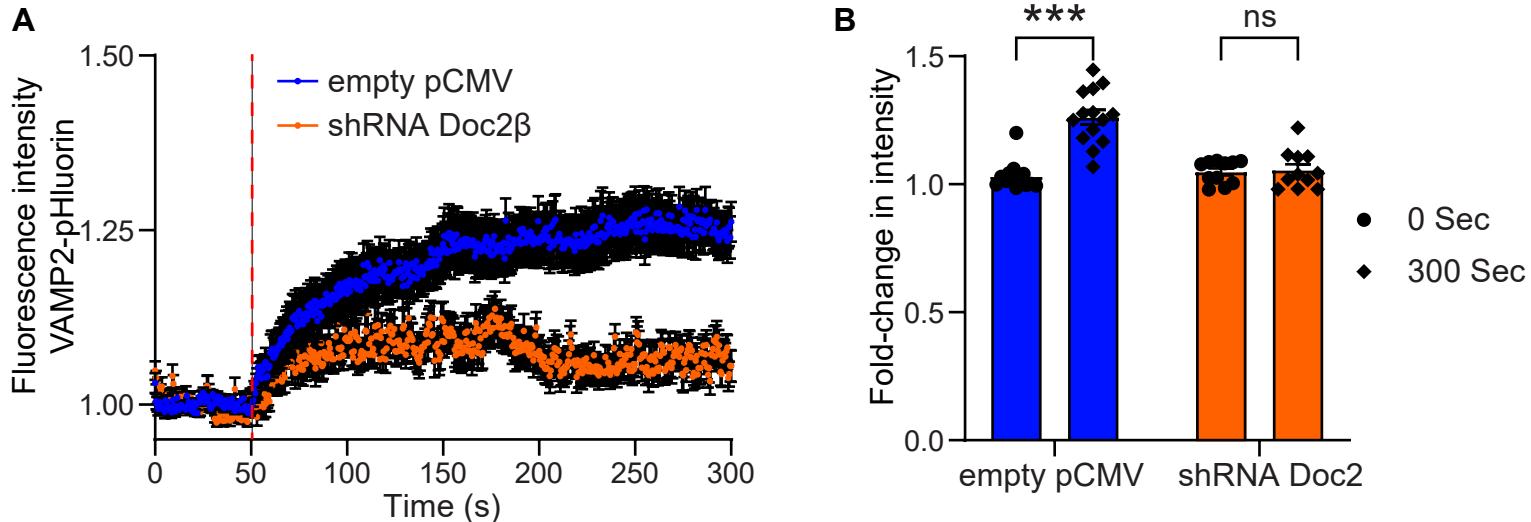
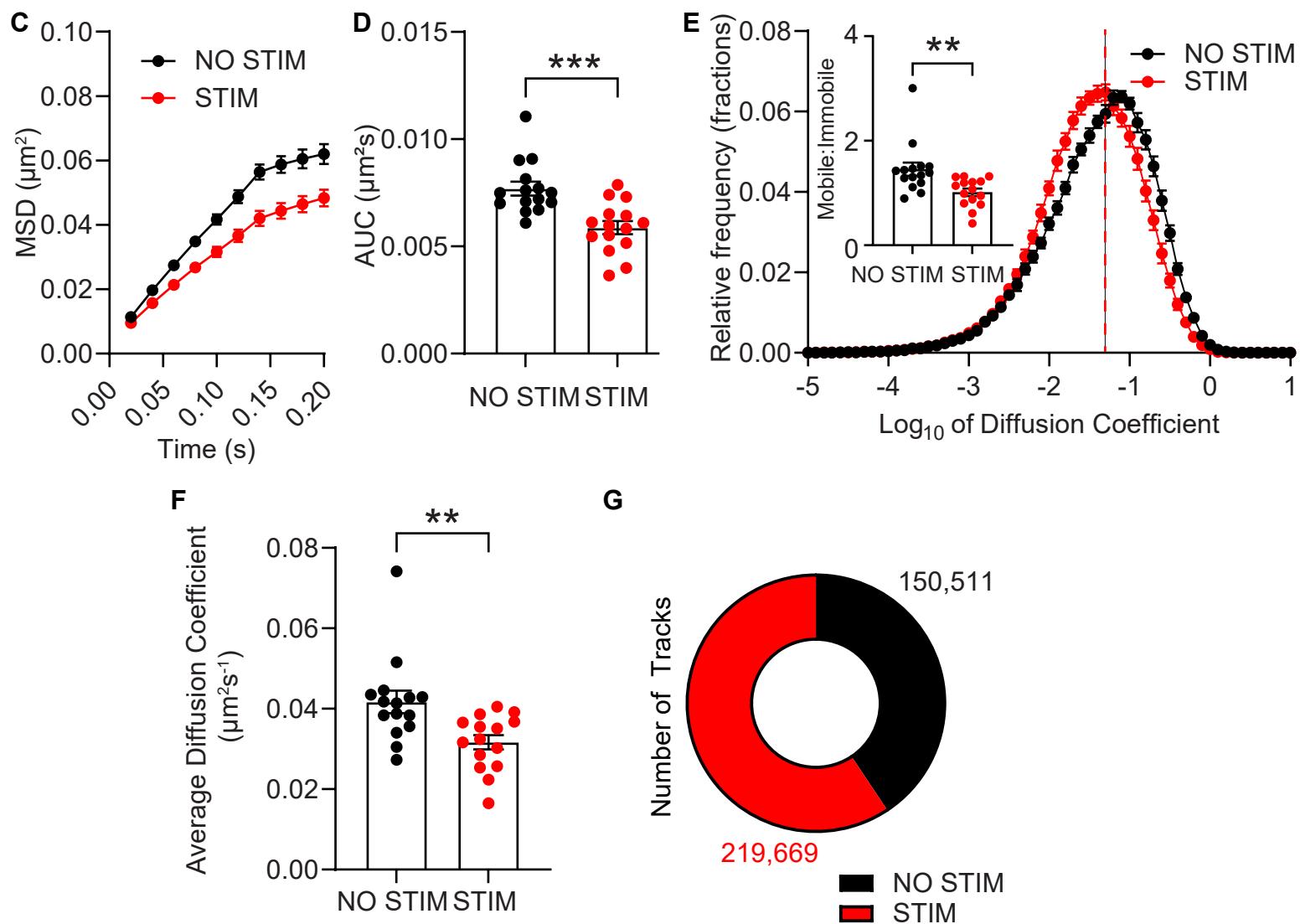
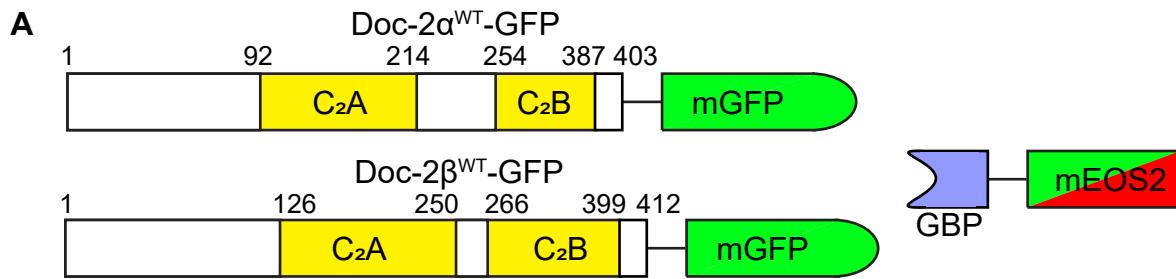
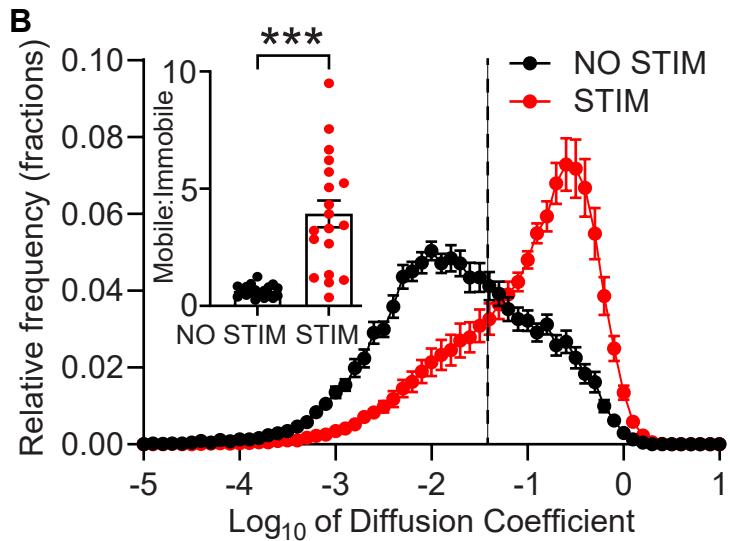
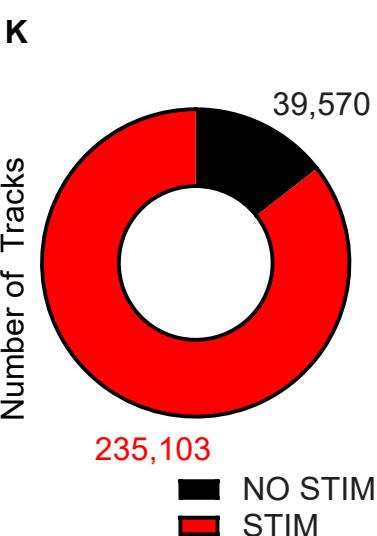
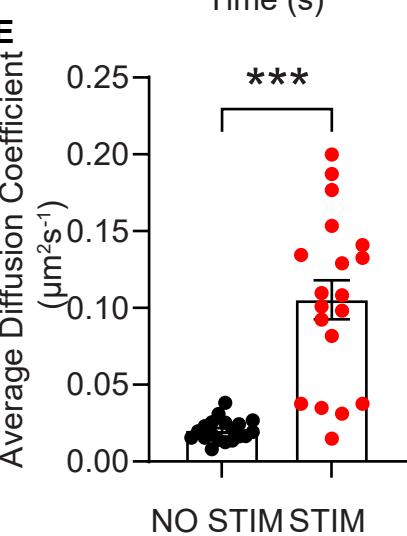
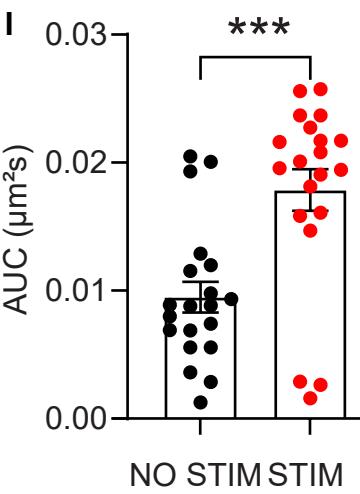
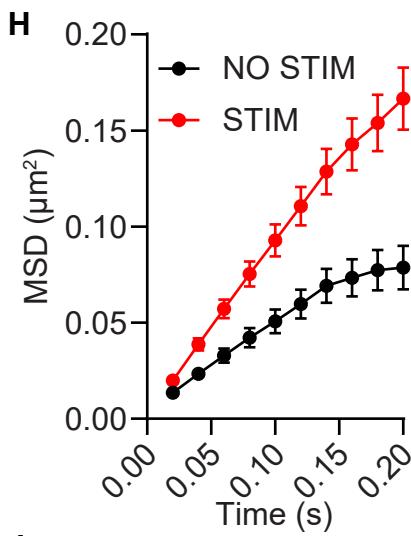
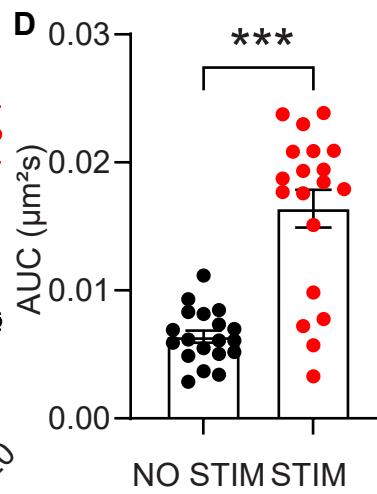
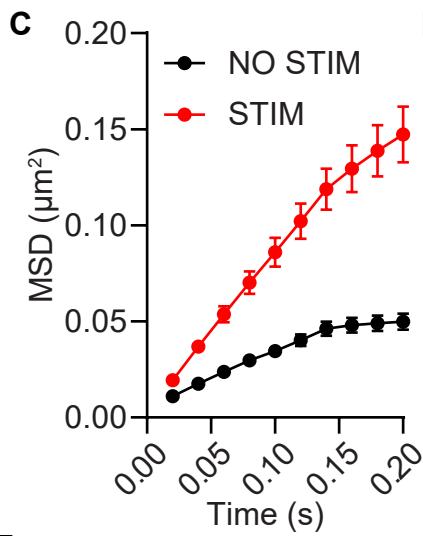
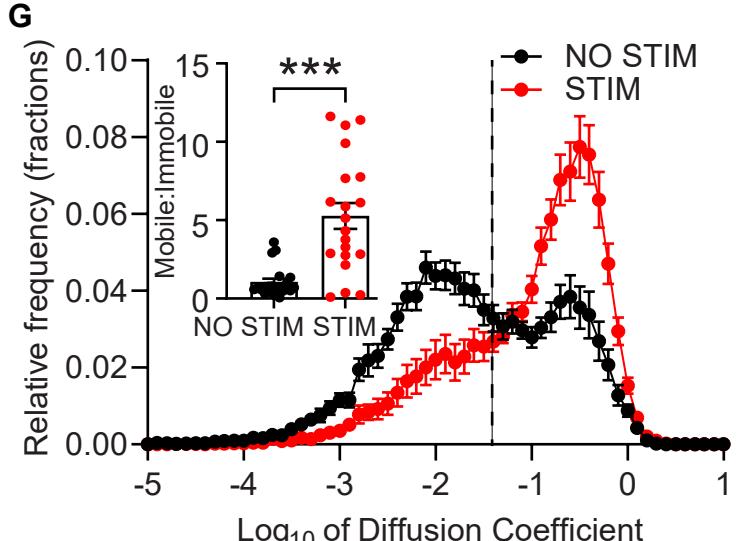
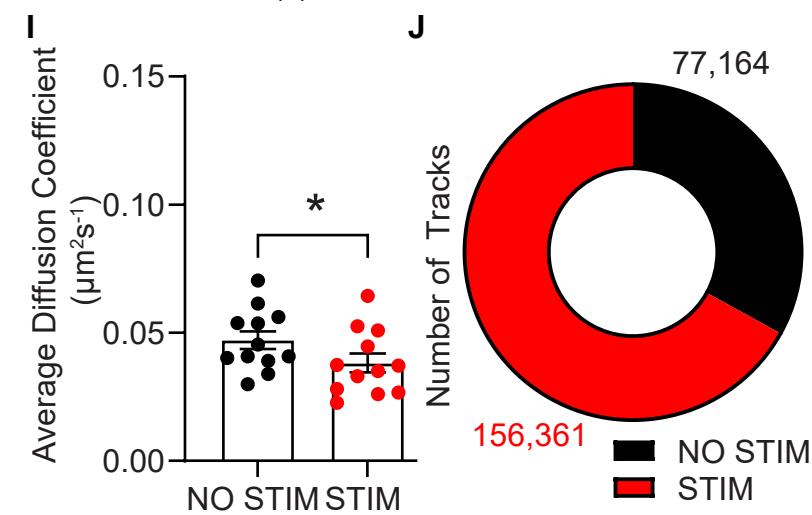
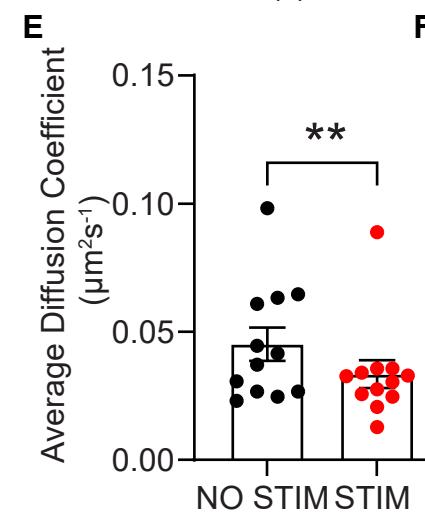
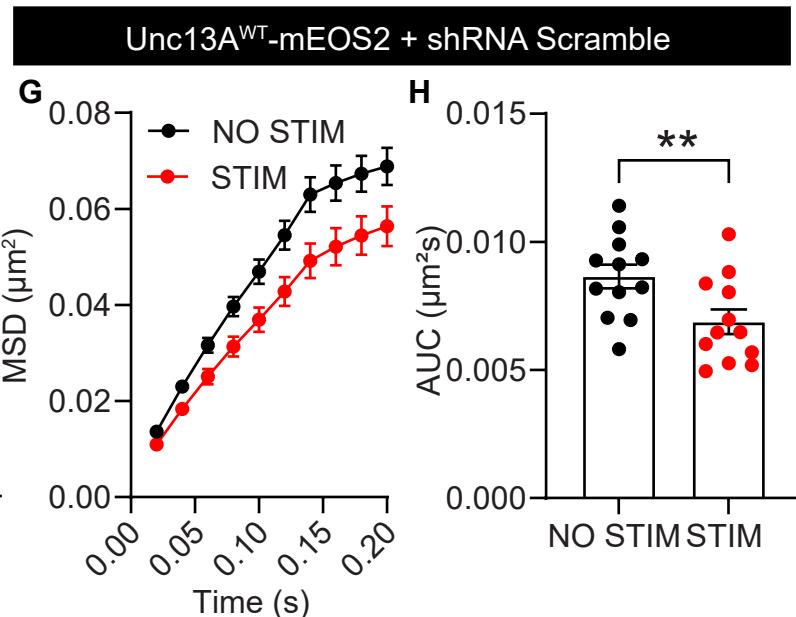
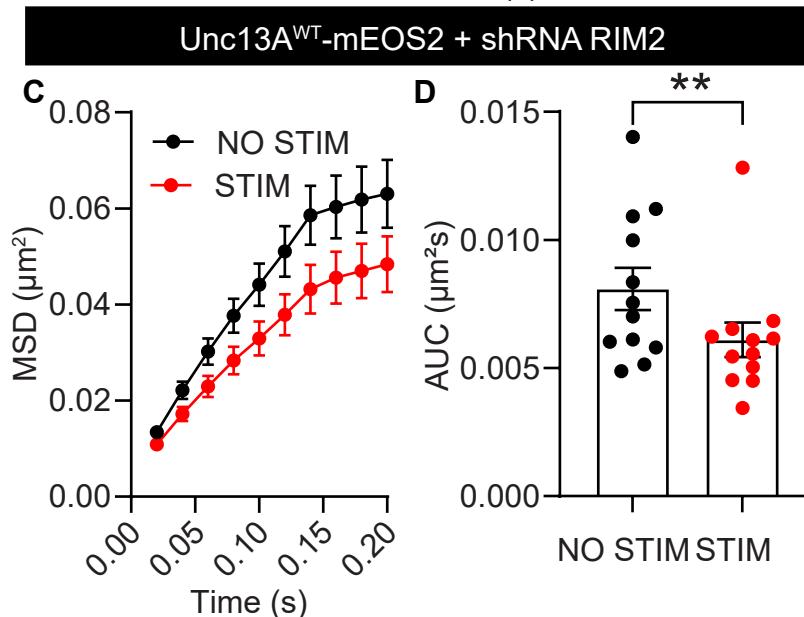
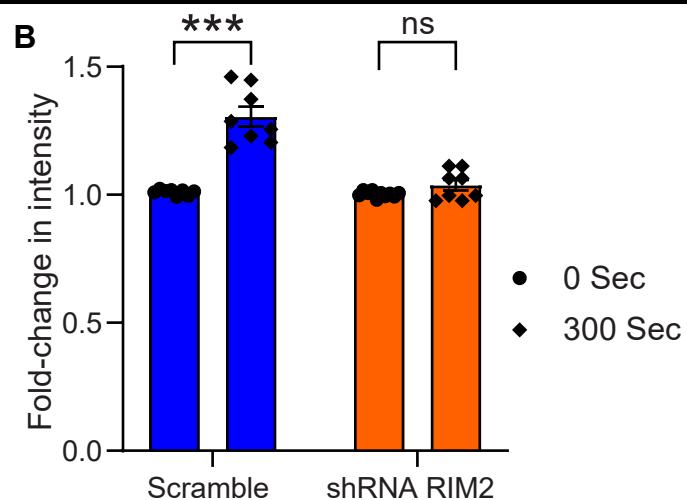
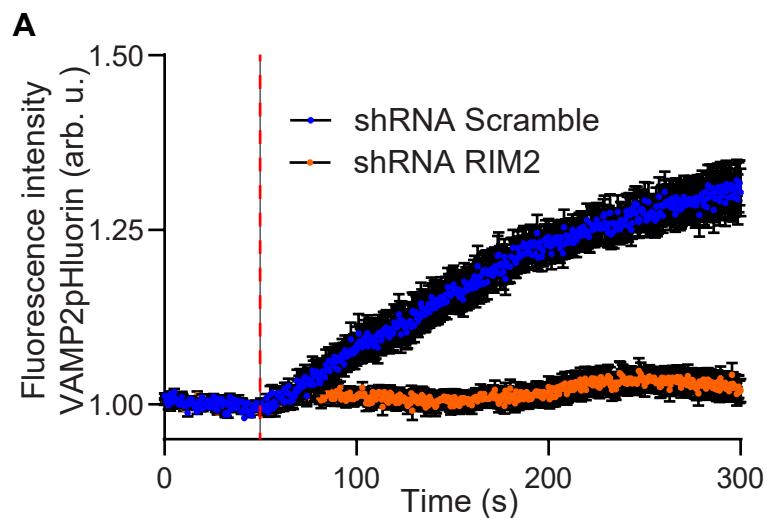


Figure 5VAMP2-pHluorin + shRNA Doc2 β Munc13-1^{WT}-mEOS2 + shRNA Doc2 β 

Supplementary Figure 5

Doc2 α ^{WT}-GFP + GBP-mEOS2Doc2 β ^{WT}-GFP + GBP-mEOS2

VAMP2-pHluorin + shRNA RIM2



Supplementary Figure 6

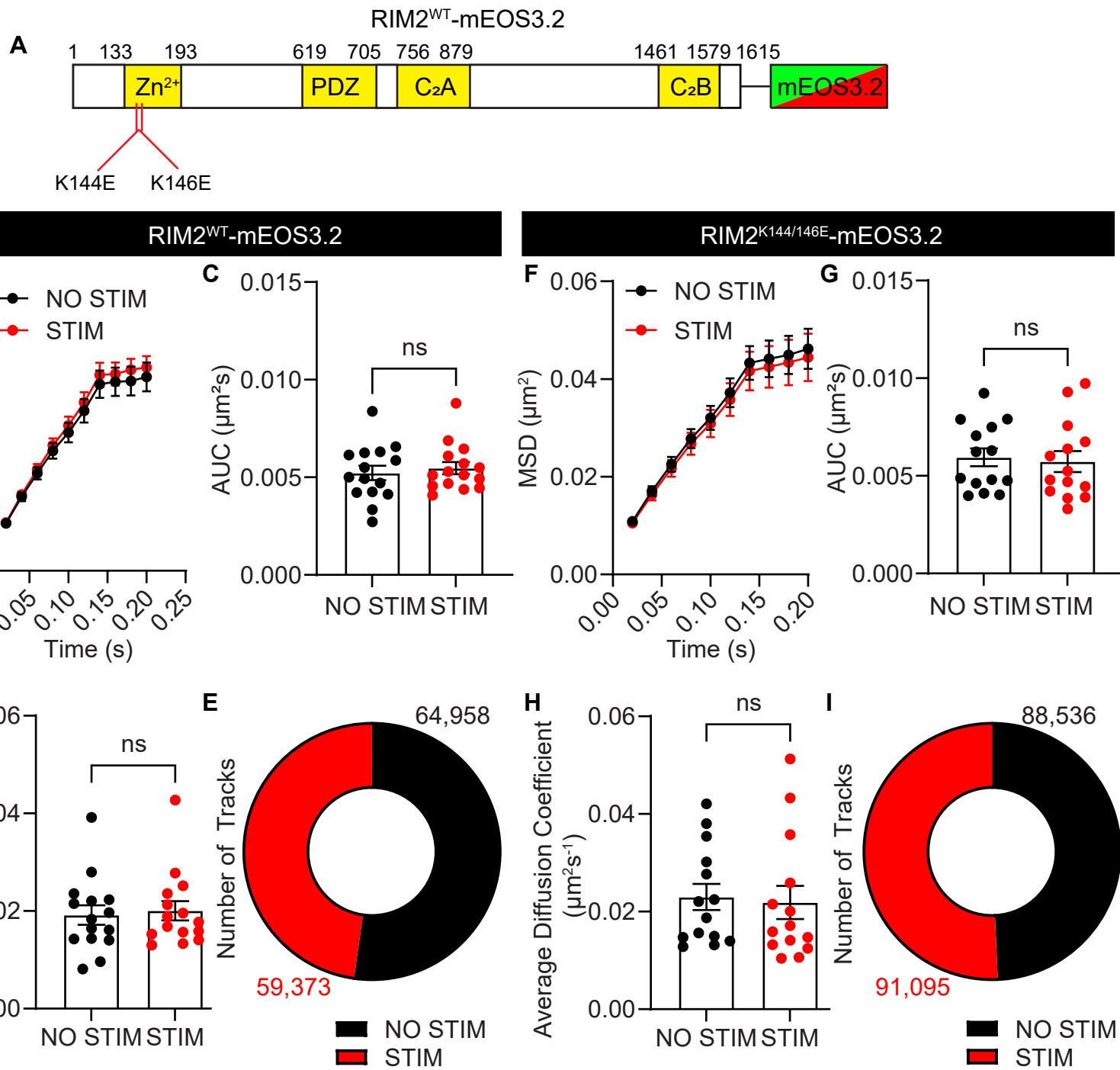
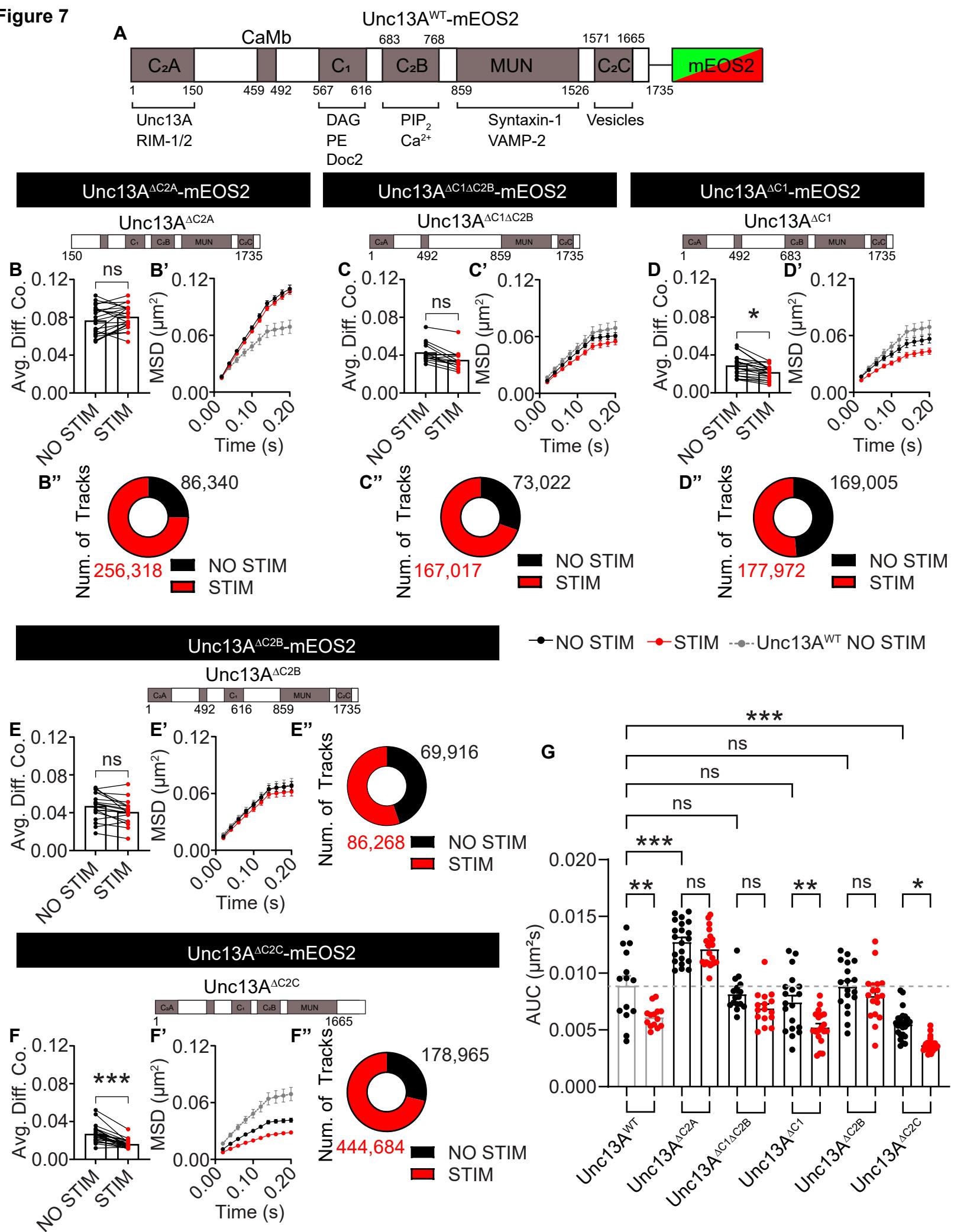
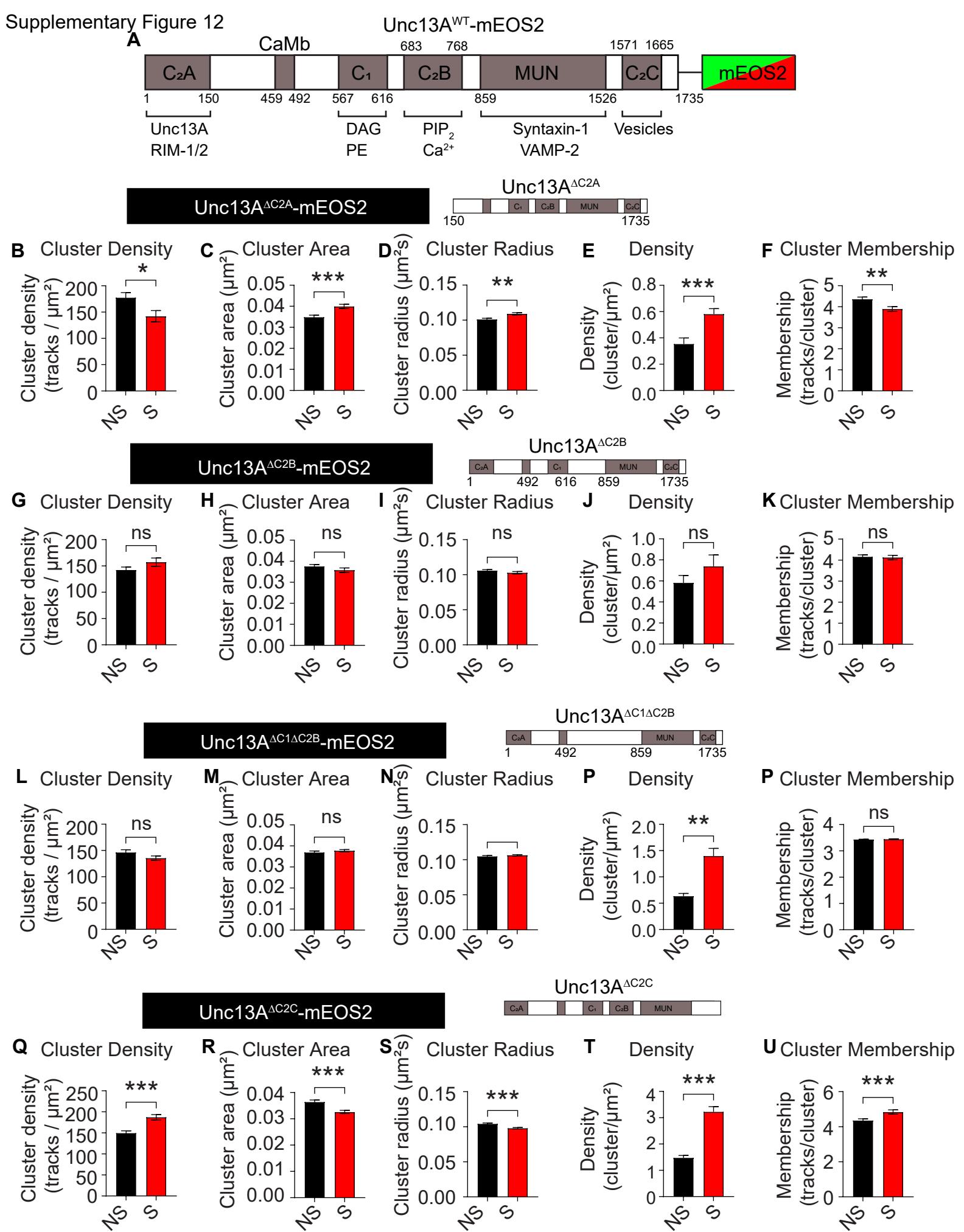
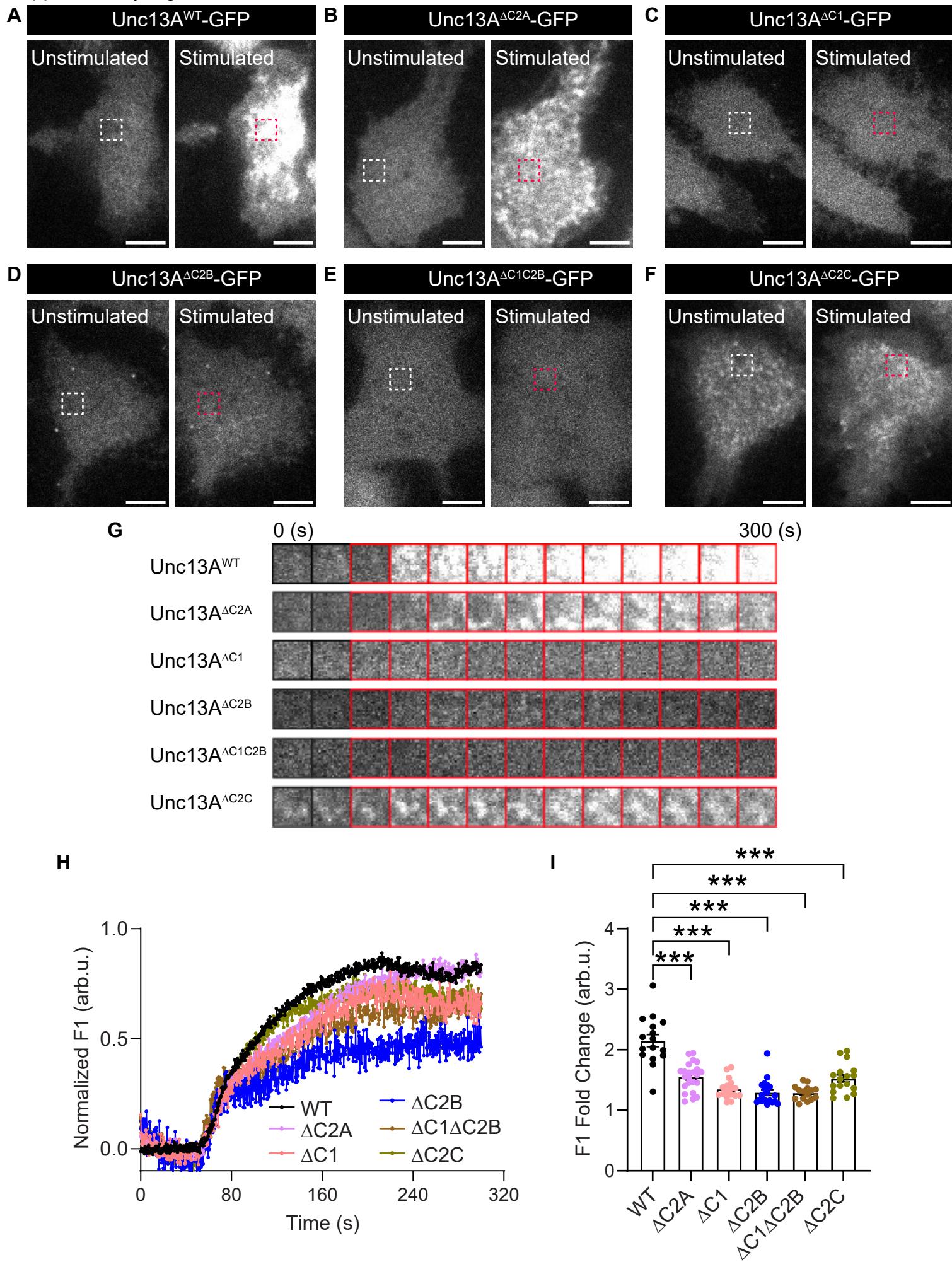


Figure 7

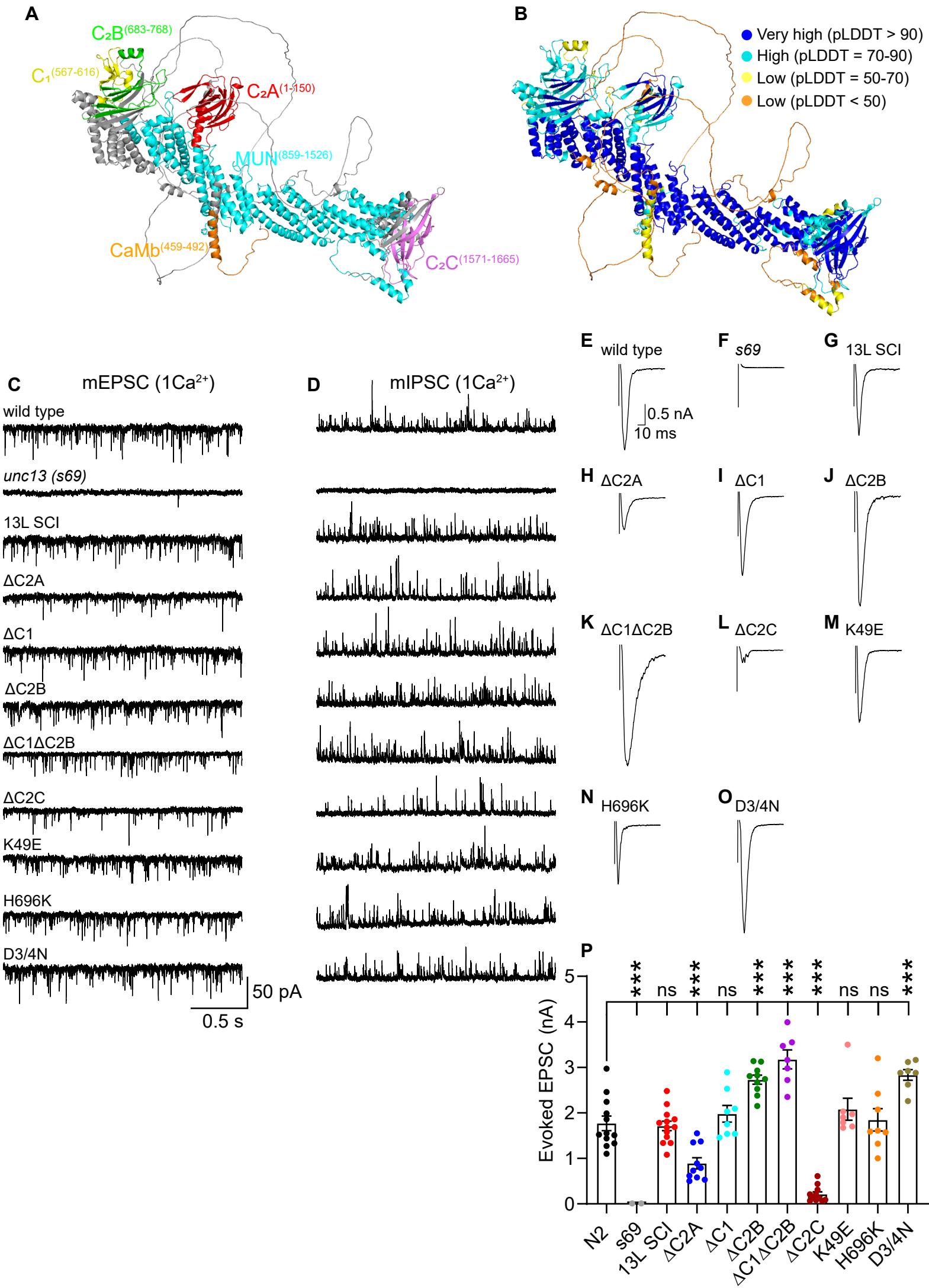
Supplementary Figure 12



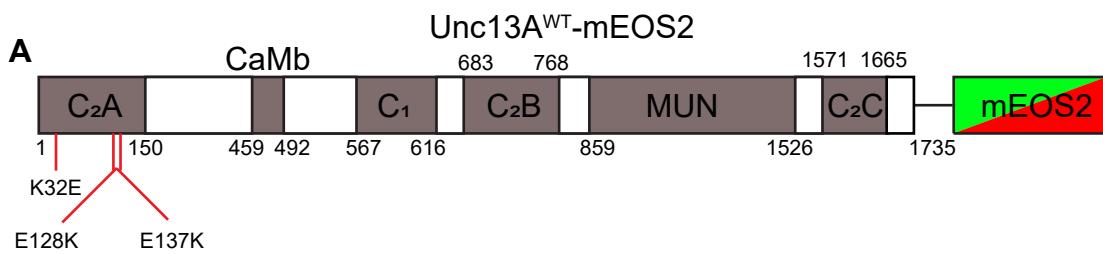
Supplementary Figure 7



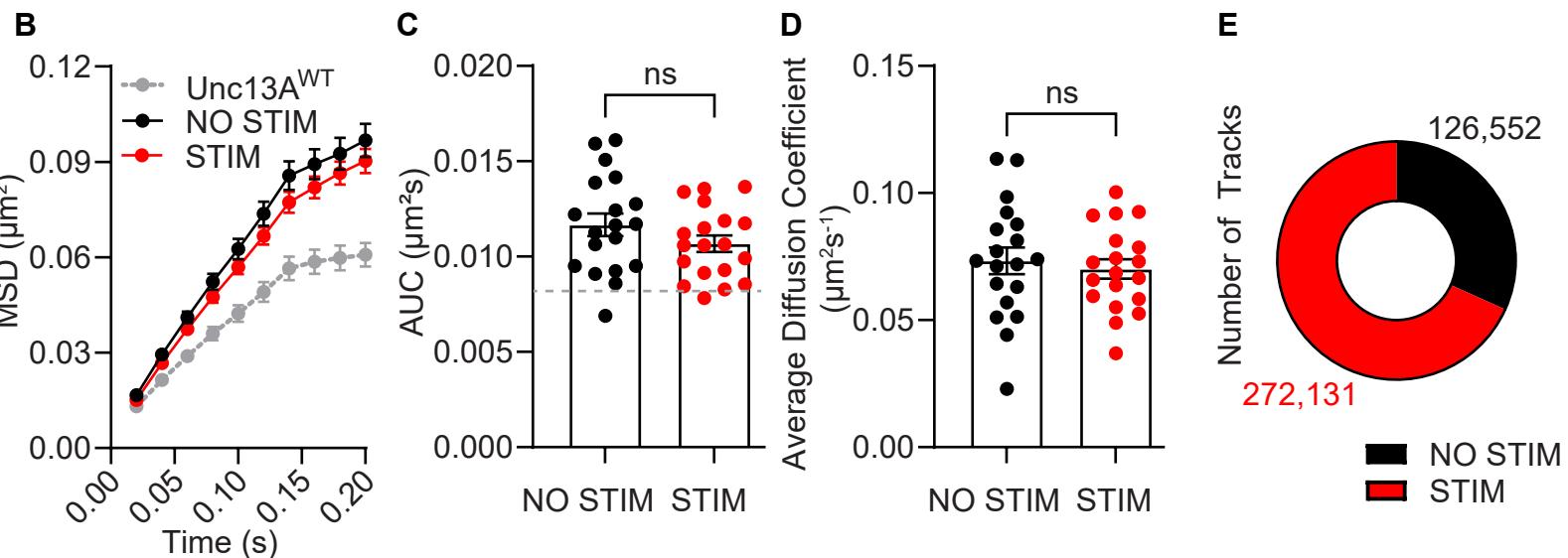
Supplementary Figure 8



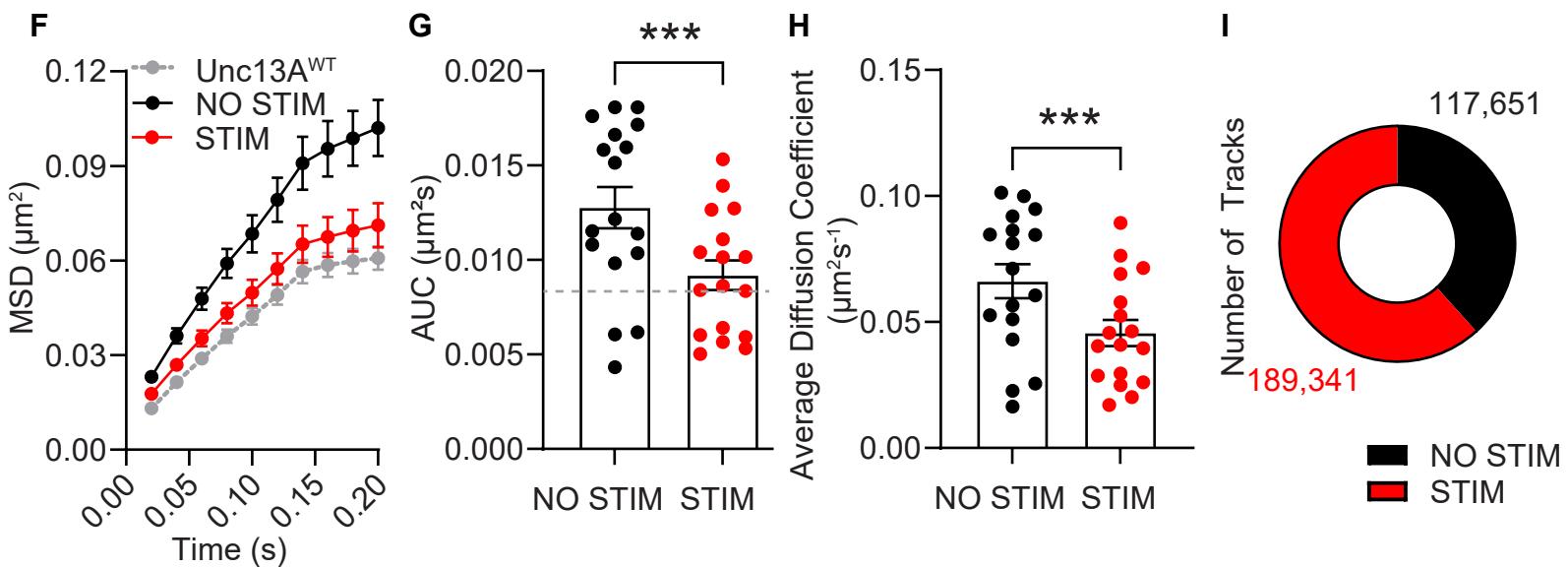
Supplementary Figure 9



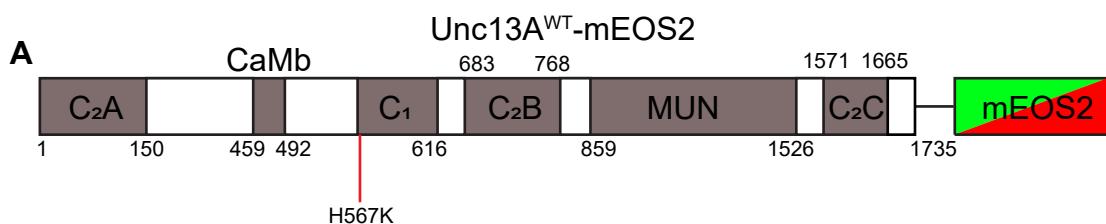
Unc13A^{K32E}-mEOS2 (Non-homodimerising mutant)



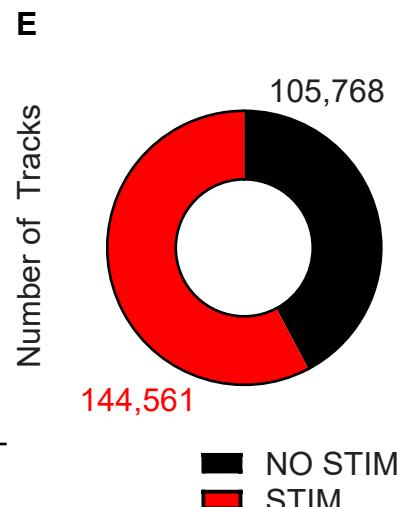
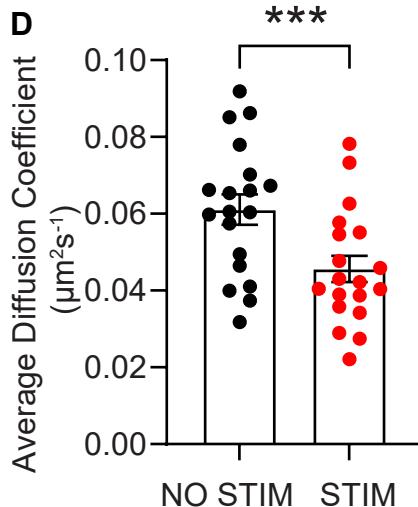
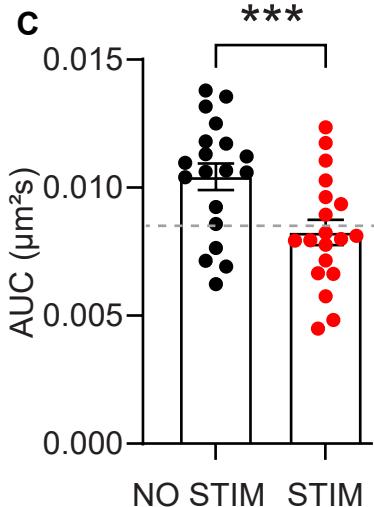
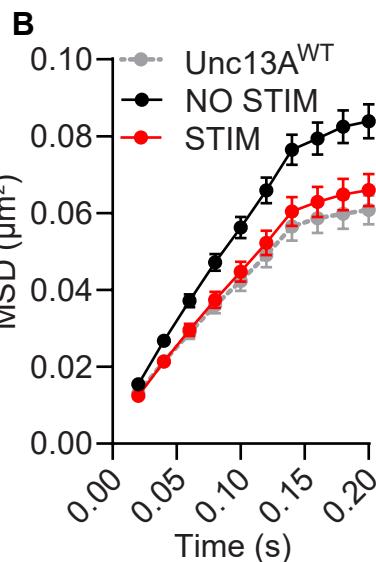
Unc13A^{E128/137K}-mEOS2 (Non-heterodimerising mutant)



Supplementary Figure 10



Unc13A^{H567K}-mEOS2 (Diacylglycerol-insensitive mutant)



Supplementary Figure 11

Unc13A^{WT}-mEOS2 + Phorbol Esters (PMA)

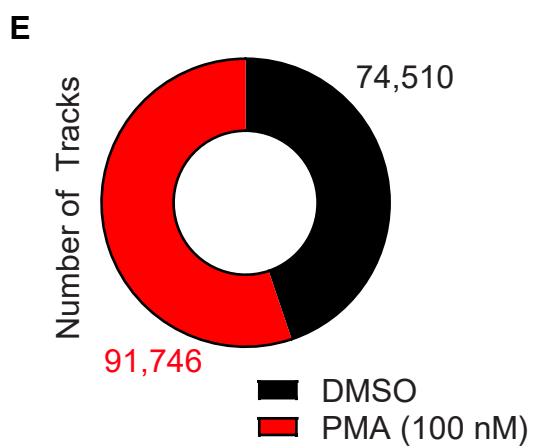
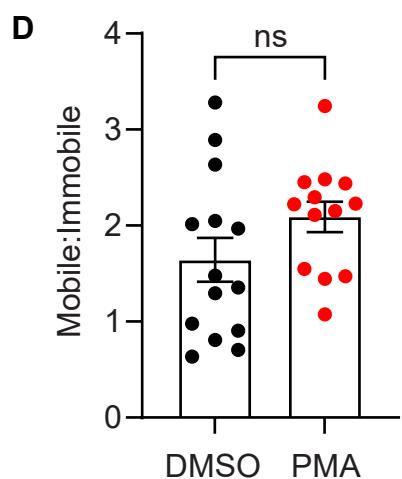
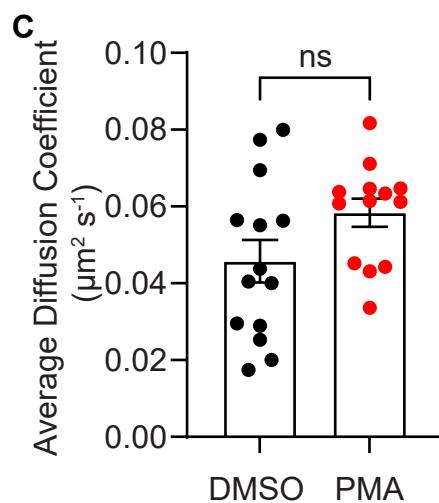
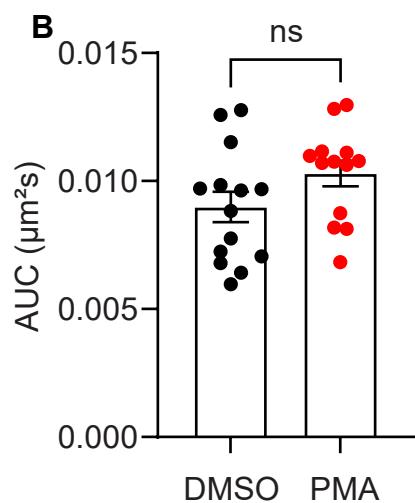
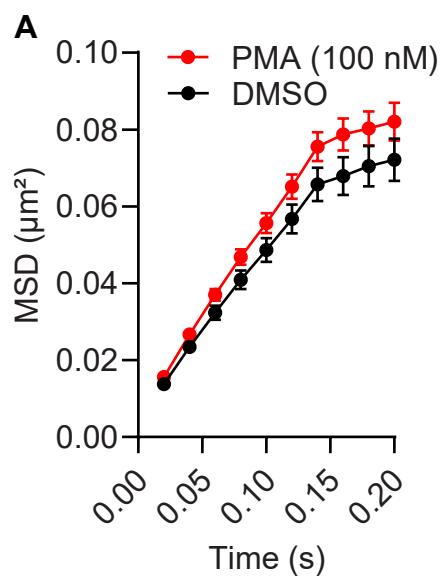
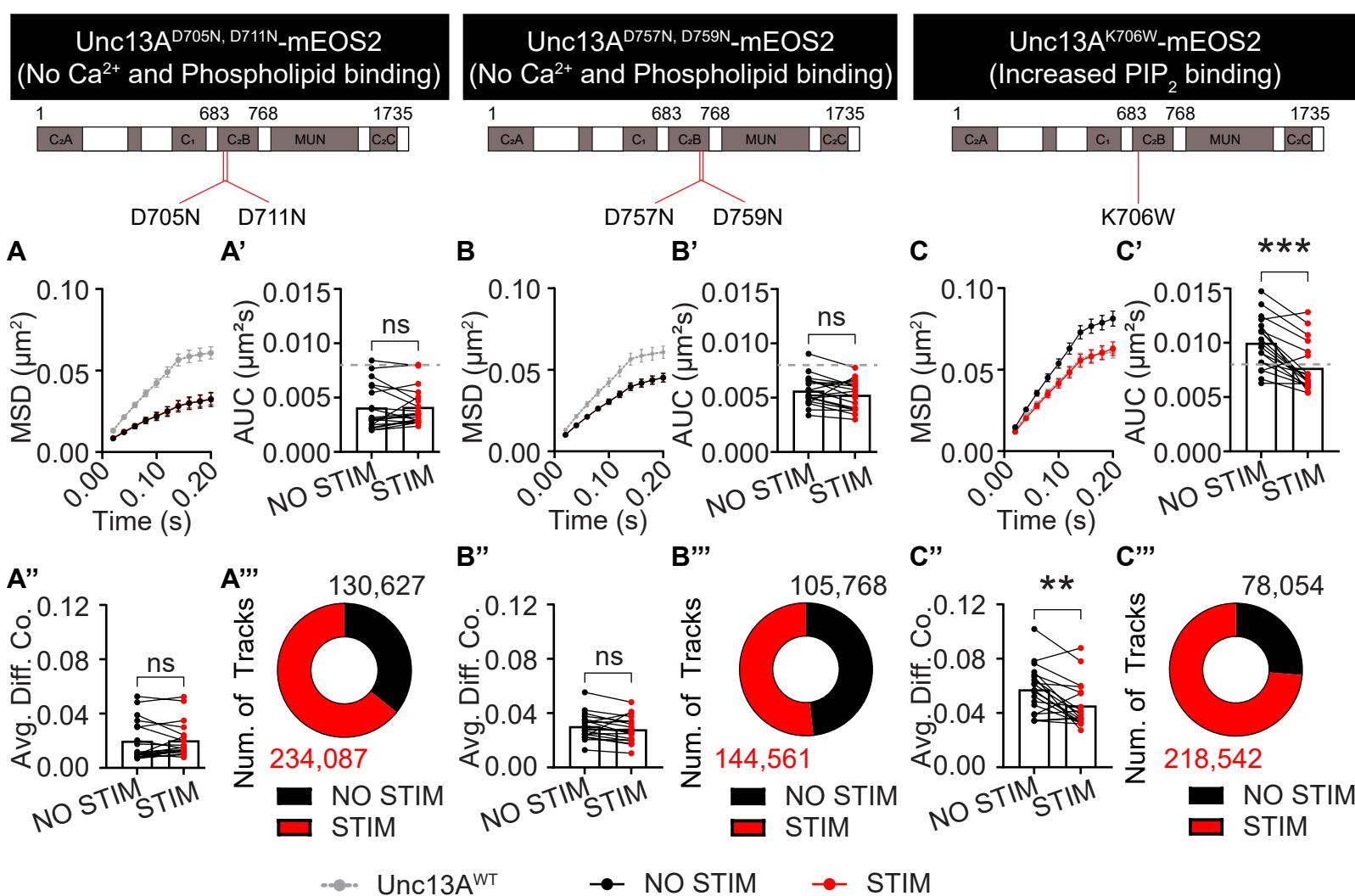


Figure 8**D****Schema - Paper summary / Modelling Data**

Munc13-1^{WT::mEOS2} + PI3K δ Inhibitor (CAL-101)

