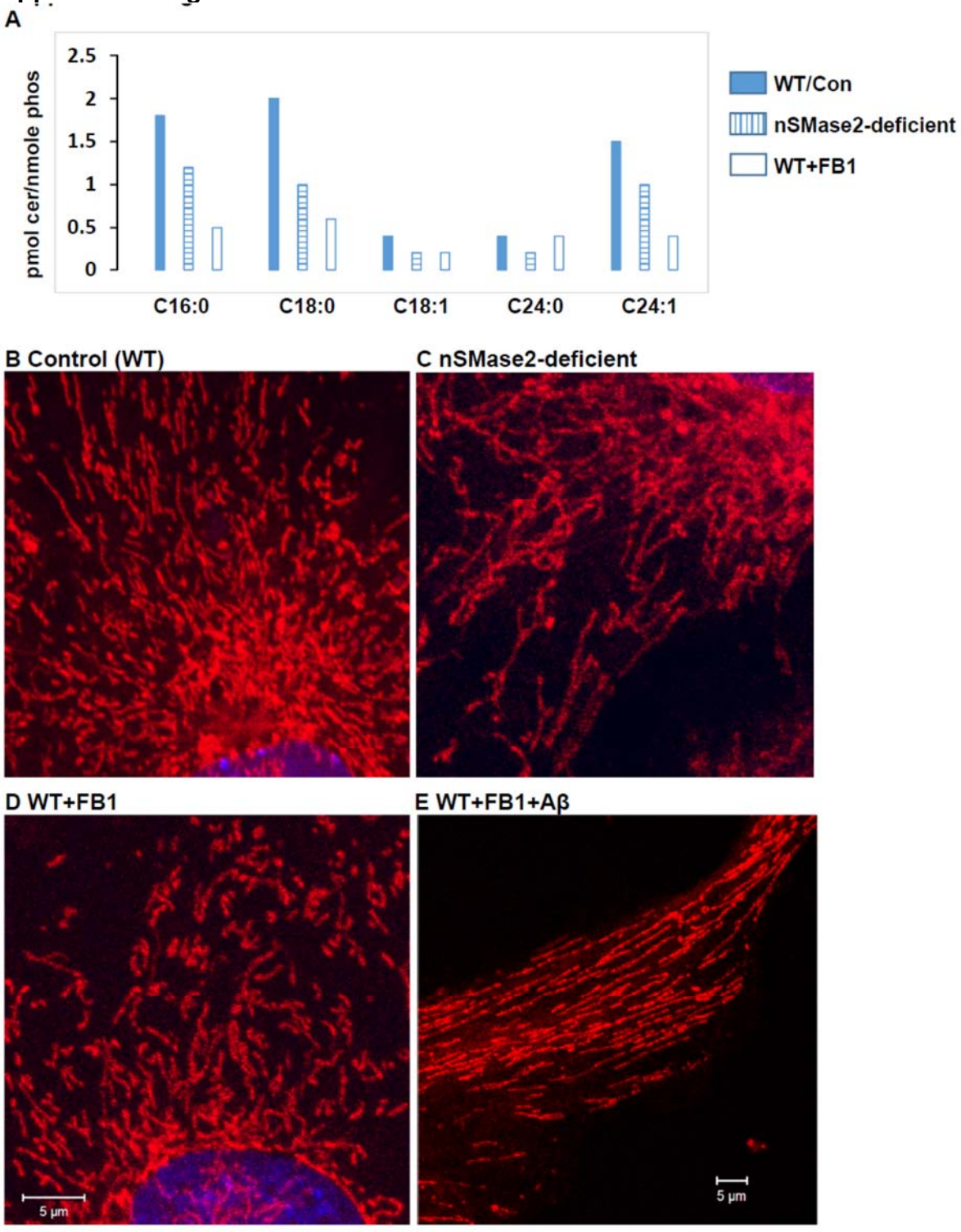
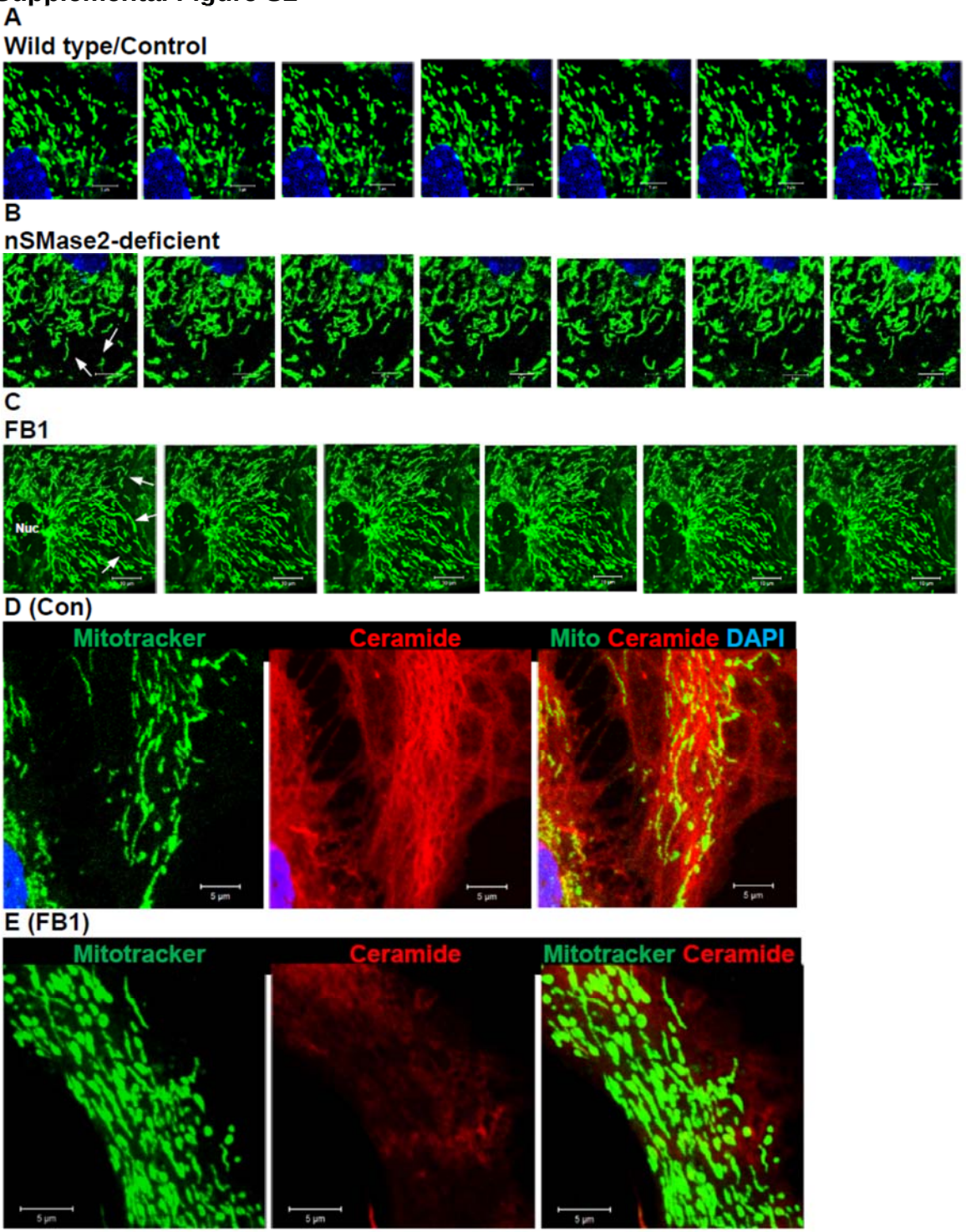
Supplemental Figures
Supplemental Figure S1



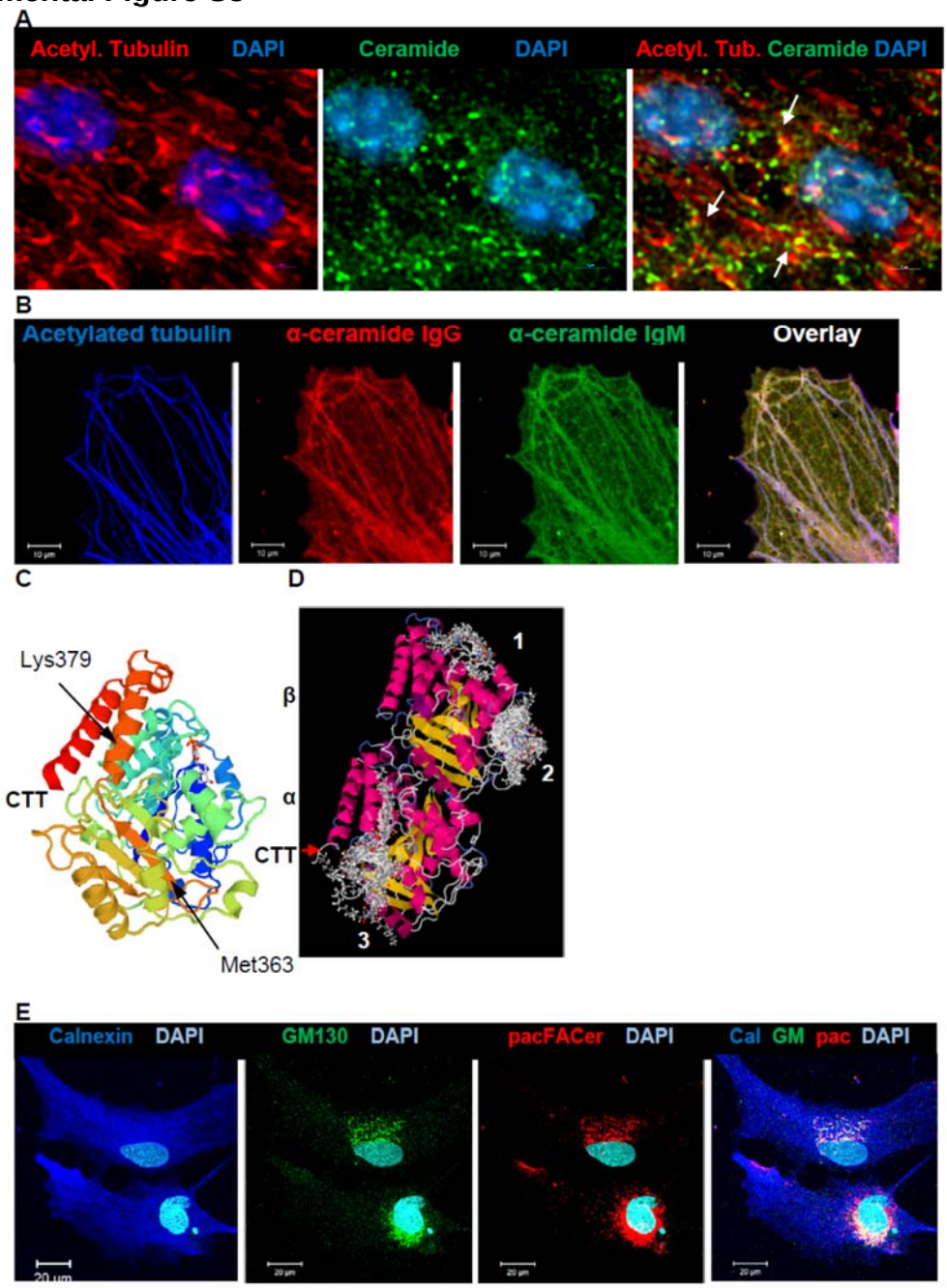
A. Composition of ceramides in primary cultures of astrocytes normalized to lipid phosphates (WT, wild-type; FB1, Fumonisin B1. **B-E.** Mitochondria in WT control (B), nSMase2-deficient (C), WT+FB1 (D) and WT+FB1+Aβ (E) treated astrocytes. Bar = 5 µm.

Supplemental Figure S2



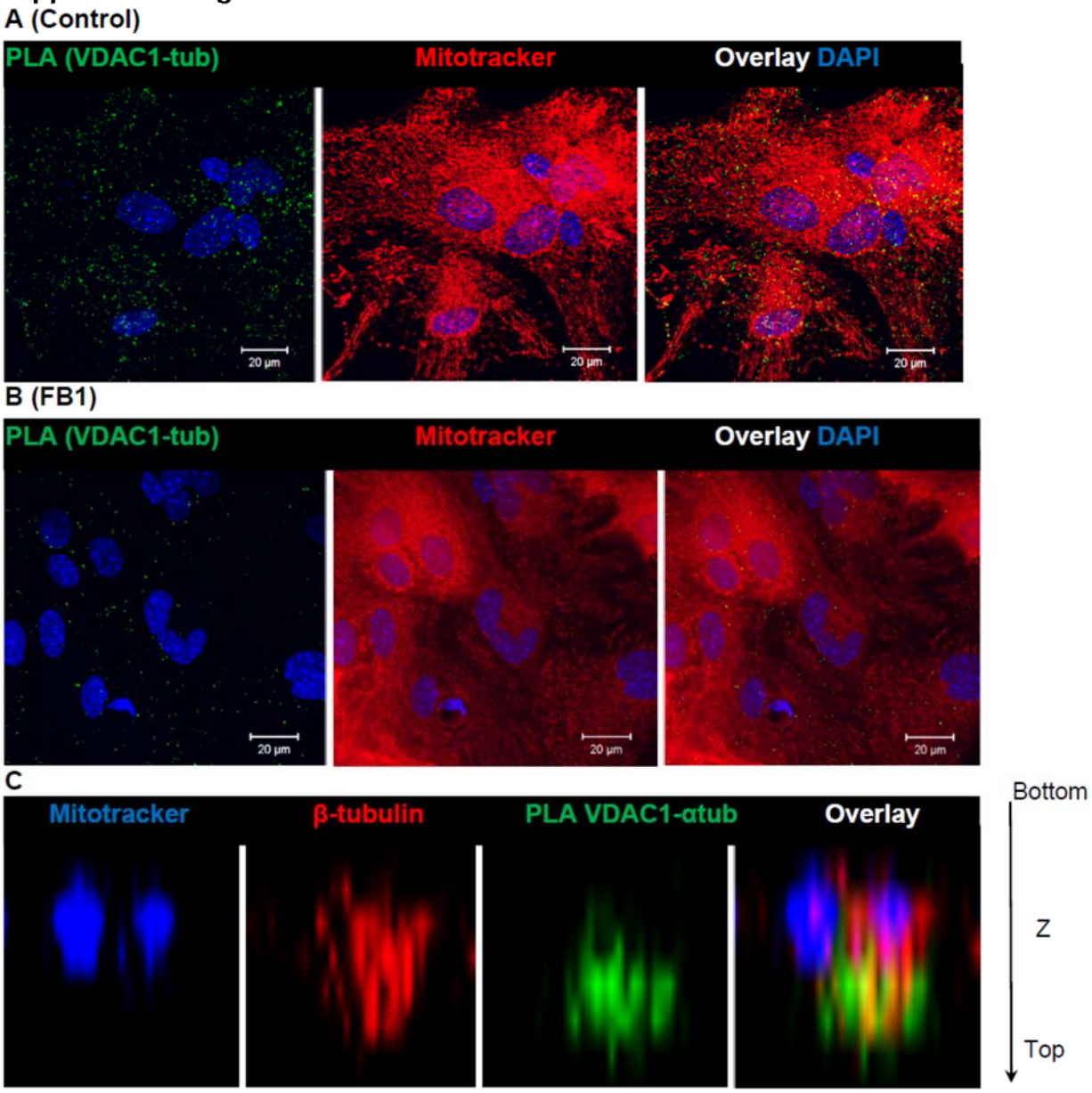
A-C. Real-time imaging of CytoPainter-labeled mitochondria (pseudocolored in green) in wild type (A), nSMase2-deficient (B), and FB1-treated (C) astrocytes. Arrows point at fast moving mitochondria. See also supplemental movies. **D, E.** Primary cultured astrocytes treated with FB1 (D is control) and labeled for ceramide (anti-ceramide IgG, pseudocolored in red) and mitochondria (mitotracker, pseudocolored in green).

Supplemental Figure S3



A. Labeling of cryosectioned WT mouse brain for acetylated tubulin (red) and ceramide (green). Arrows point at sites of colocalization. **B.** Immunocytochemistry using anti-acetylated tubulin mouse IgG (pseudocolored in blue), anti-ceramide rabbit IgG (pseudocolored in red), and anti-ceramide mouse IgM MAB0014 (pseudocolored in green). **C.** Location of pacFACer-cross-linked peptide sequence on β-tubulin. Beta-sheet S10 and helix 11 are colored in orange. Arrows point at amino acids flanking the cross-linked peptide. CTT, C-terminal tail. **D**. Molecular modeling using αβ tubulin dimer (ijff.pdb) as the target and C16:0 ceramide (zinc_40164304.mol2) as the ligand in Swissdock. Image shows binding sites for association of ceramide with binding energies >8 kcal/mol. **E.** Immunocytochemistry using anti-GM130 mouse IgG (pseudocolored in green) with pacFACer-labeled (pseudocolored in red) astrocytes 0 h post-fixation.

Supplemental Figure S4



Immunocytochemistry using control (**A**) and FB1-treated (**B**, 48 h 20 µM FB1) astrocytes and antibodies against VDAC1 (rabbit IgG) and α-tubulin (mouse IgG) for PLA (pseudocolored in green). Mitochondria are labeled with mitotracker (pseudocolored in red). **C**, Z-scan cross-section showing PLA for VDAC1 and α-tubulin (pseudocolored in green) at mitochondria (mitotracker, pseudocolored in blue) and colocalization with β-tubulin (pseudocolored in red).