SUPPLEMENTAL FILES

Figure legend for Supplemental Data

Supplemental Figure 1 *Structure of ceramide, sphingomyelin, and S18 (N-oleoyl serinol)*

Ceramide shown here is N-palmitoyl sphingosine or C16 ceramide. In C18:1 ceramide (N-oleoyl sphingosine), the fatty acid residue is oleic acid (C18:1) instead of palmitic acid (C16:0). Oleic acid is also the fatty acid portion used for synthesis of S18. Sphingolipids were drawn using the tools kit of www.lipidmaps.org, S18 was drawn using ChemDraw.

Supplemental Figure 2 *GC-MS of ceramide*

The figure shows the GC-MS analysis of C18:1 ceramide. A, GC profile (arrow indicates peak of C18:1 fatty acid). B, MS analysis of the C18:1 peak (fatty acid after methanolysis). C, oleic acid standard. Note that the identity of the mass fragmentation products between in B and C. A similar analysis was performed to identify other ceramide species, in particular C16 ceramide. No sphingoid base was found in the hexane layer.

Supplemental Figure 3 *Anti-ceramide antibody induces distribution of membrane-bound aPKC to the cytosol and obliterates microtubule extension to the membrane.*

A. Incubation of C17.2 cells with control IgG (pre-immune serum). Endogenous aPKC (red) and α-tubulin (green) was detected using the respective antibodies. F-actin was stained with Alexa 647-conjugated phalloidin (blue). Note that aPKC and the plus ends of microtubules are co-localized at membrane protrusions. A similar distribution is observed with anti-GA1 or anti-FGFR1 rabbit IgG used as additional control antibodies. **B**. Same as in upper left panel, but after incubation with anti-ceramide rabbit IgG (20 μg/ml protein A sepharose eluate) for 4 h. **C**.

Same as in upper right panel, but simultaneous addition of 1 μM C16 cermide. **D**. Same as in lower left panel, but simultaneous addition of 40 μM S18. Bars in A-D = 5 μm. **E and F**. Phase contrast images at lower magnification than fluorescence images. **E.** ES cell-derived NPs after incubation with pre-immuneserum IgG. **F.** Anti-ceramide antibody. Bar in F = 20 μm. Note that cells round up, form clusters, and retract processes after treatment with anti-ceramide antibody.

Supplemental Figure 4 *Ceramide co-localizes with phosphorylated aPKC and Cdc42.*

Confocal laser scanning microscopy was performed with fixed NPs using antibodies against ceramide (Cy2, green), pPKCζ/λ (Cy5, blue), and Cdc42 (Cy3, red). Arrow indicates co-distribution.

Supplemental Figure 5 *Ceramide is associated with aPKC and α-tubulin in processes of NPs*.

Confocal laser scanning microscopy was performed using antibodies against ceramide (Cy5, red) and α-tubulin (Cy3, blue) on fixed NPs that expressed PKCζ-GFP (green). **A**. Ectopic expression of PKCζ-GFP induces elongated processes that are strongly stained for ceramide at their tips (arrow). **B**. The Cy3-to-Cy5 FRET analysis in these tips and acceptor bleaching was achieved as described in (18). Note that the FRET signal (arrow) was detectable at a site where ceramide co-distributed with aPKC. Also note that this site showed enhanced Cy3 fluorescence after acceptor bleaching by repeated scanning with the 633 nm laser.

Supplemental Figure 6 *Ceramide depletion disturbs cortical lamination of newborn mice.*

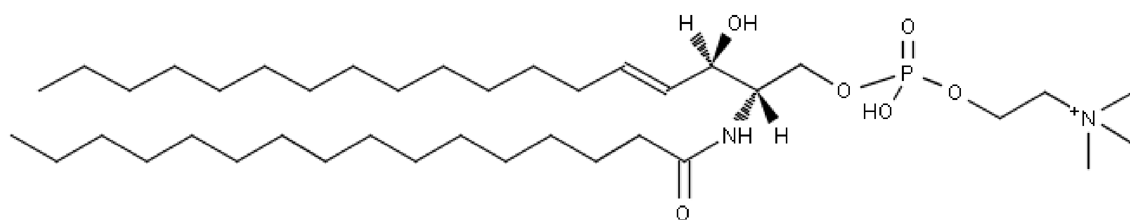
Pregnant mice were treated with myriocin from gestational day E11.5 – E15.5 as described in Experimental Procedures. Treatment was then stopped until delivery. Brains of neonates were

extracted, fixed, and subjected to cryo-sectioning. Immunocytochemistry was performed for Map-2 (pseudo-colored in red) and nuclei counterstained with Topro-3 (pseudocolored in blue). Note thickened cortex, abnormal distribution of nuclei, and severely disturbed cortical layers. **A**, Hoechst staining, overview, epifluorescence. Box frames part of the section shown in B. **B**. Confocal fluorescence microscopy, bar = 500 µm. **C**. same as in B, but higher magnification.

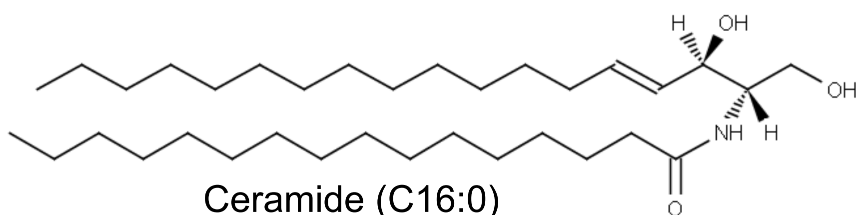
Supplemental Videos *Ceramide depletion impairs NP migration shown by real time live cell imaging.*

Immediately after generating the gap in a scratch migration assay, time lapse photographs were taken every 10 minutes with a microscope equipped with an environmental chamber, which was equilibrated to 37 °C and 5% CO_2 atmosphere. The video was generated using “Quick Time Player” with 6 frames per second and then converted to mov files. **Video a**, control NPs. **Video b**, myriocin-treated NPs.

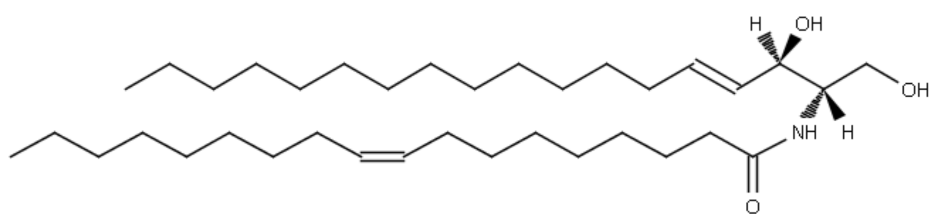
Supplemental Figure 1



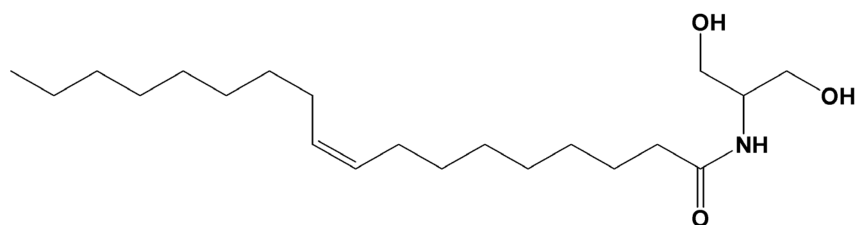
Sphingomyelin (SM)



Ceramide (C16:0)



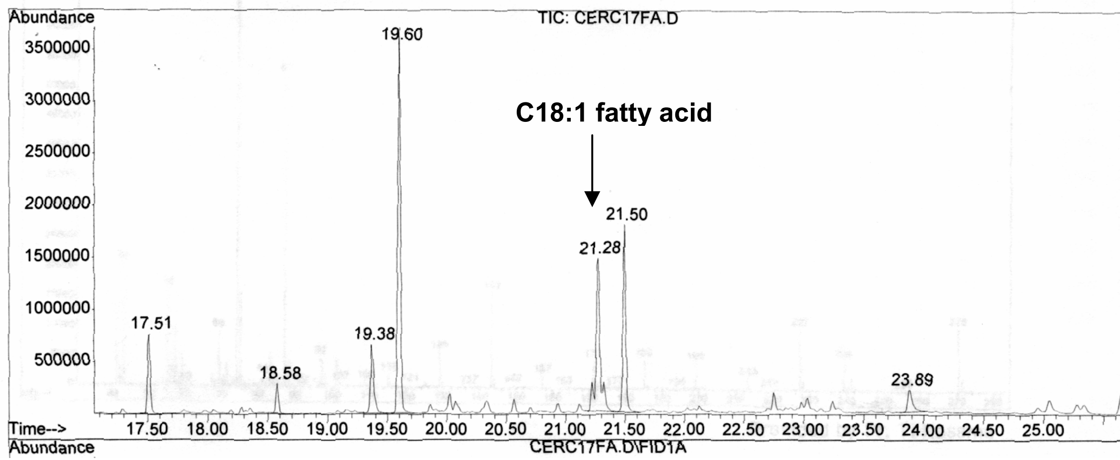
Ceramide (C18:1)



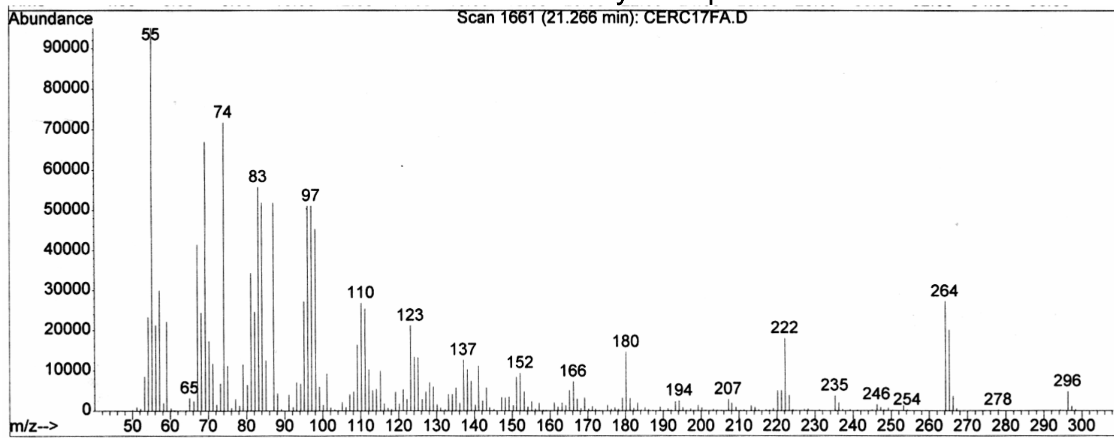
N-oleoyl serinol (S18)

Supplemental Figure 2

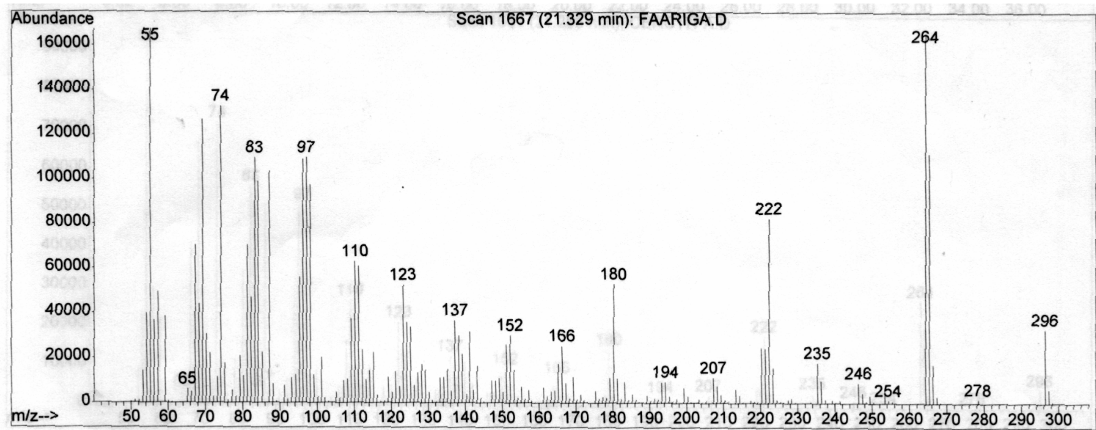
A GC for fatty acids from ceramide



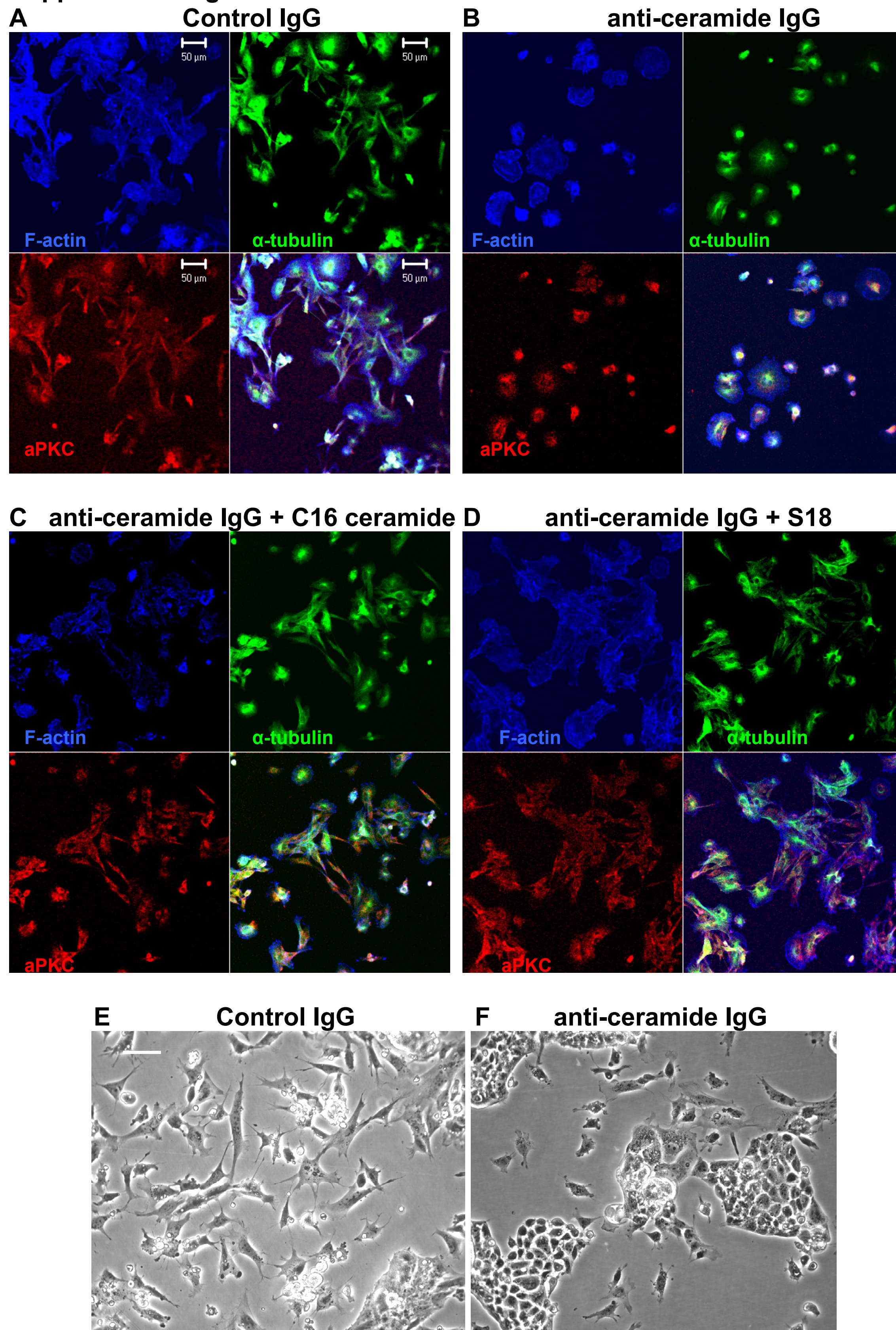
B MS for C18:1 fatty acid peak



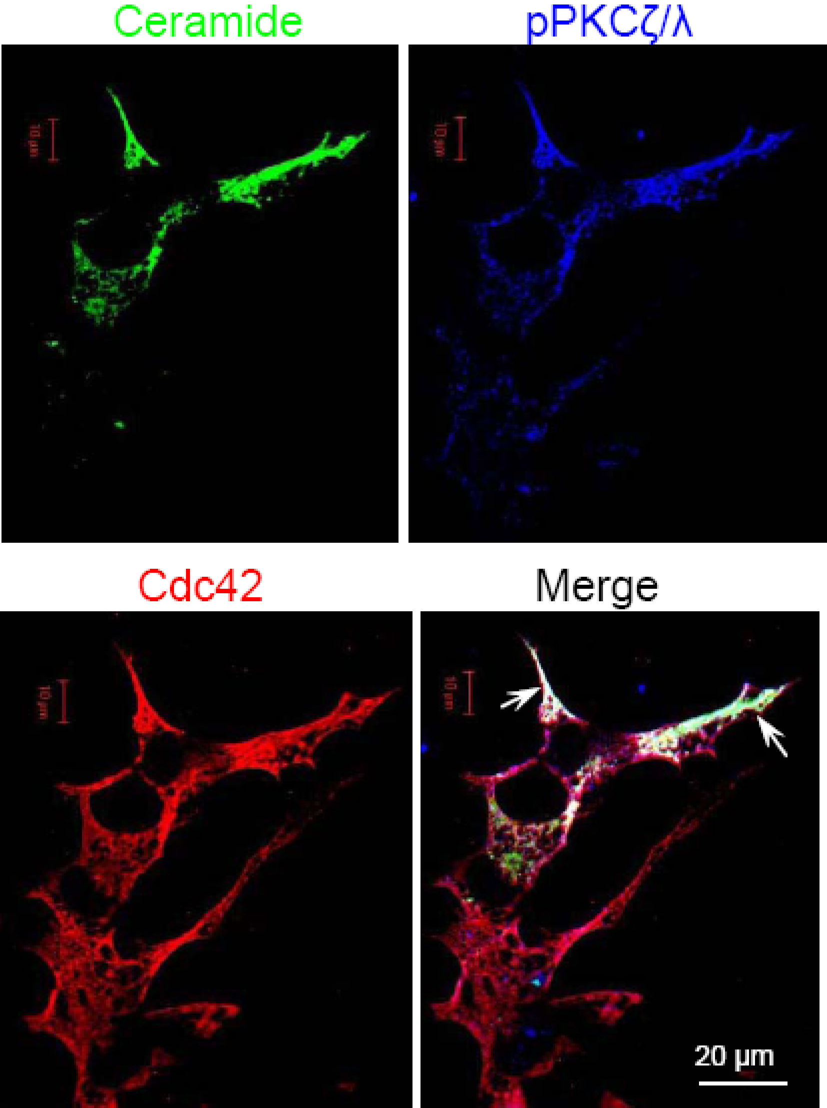
C C18:1 standard



Supplemental Figure 3

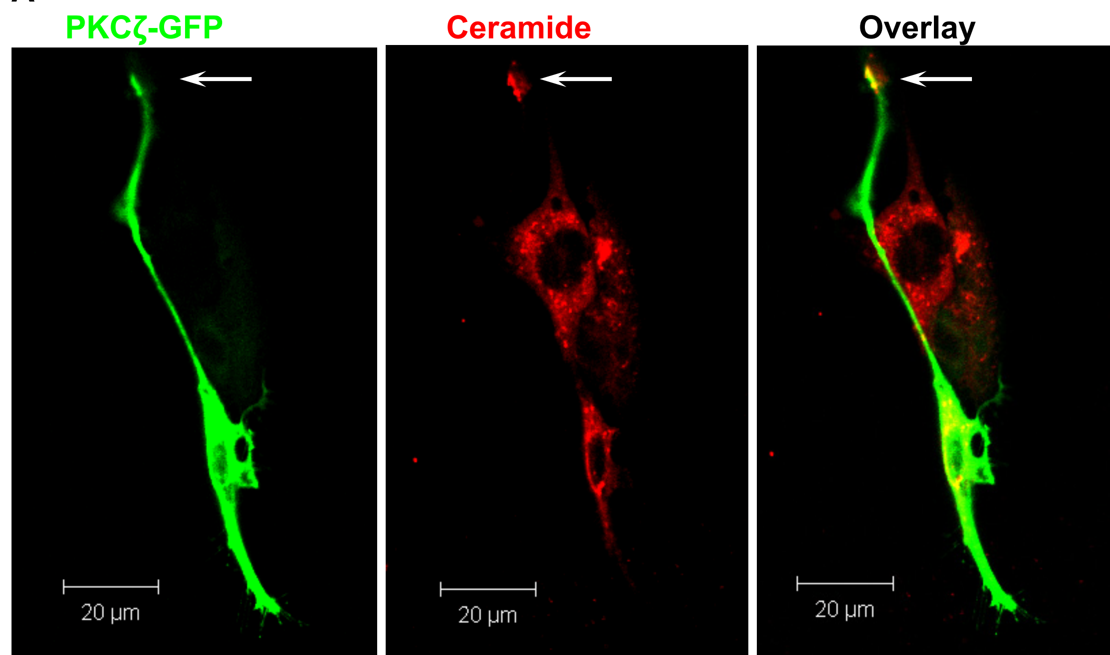


Supplemental Figure 4



Supplemental Figure 5

A



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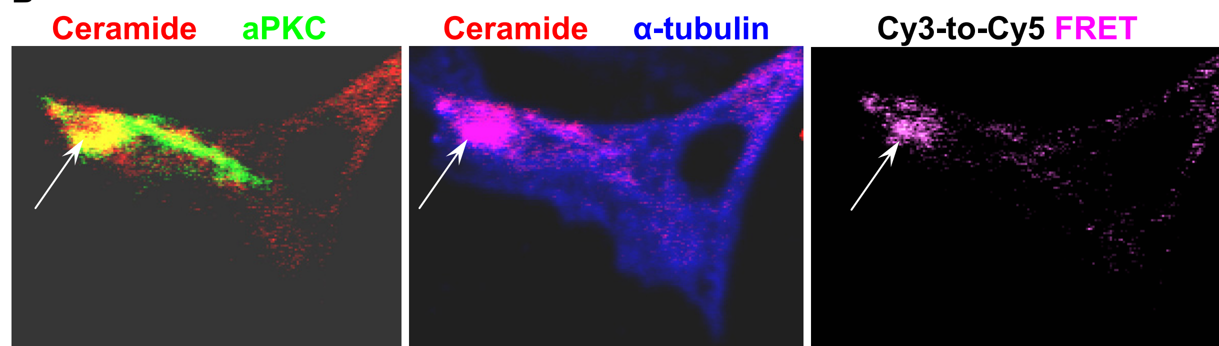
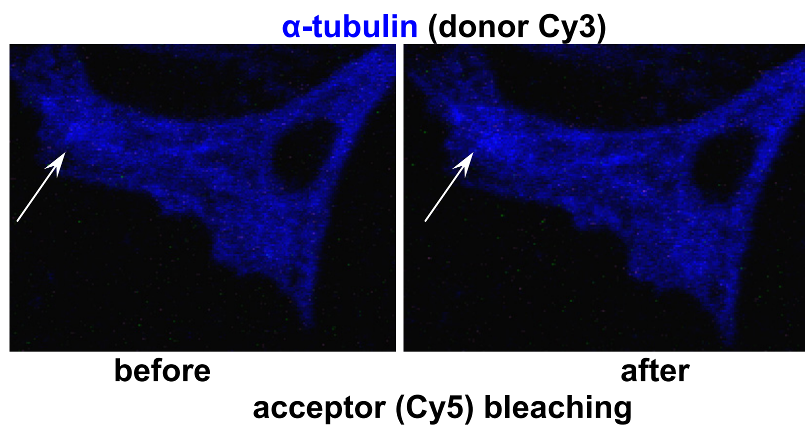
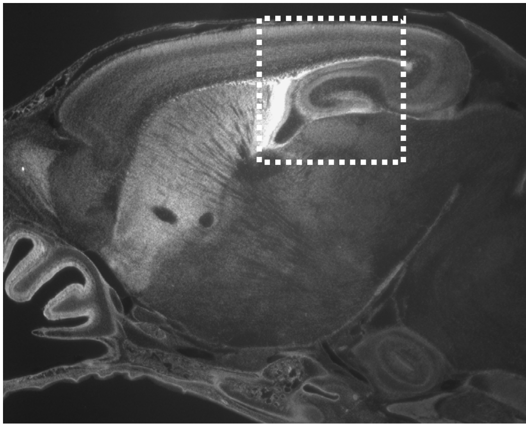
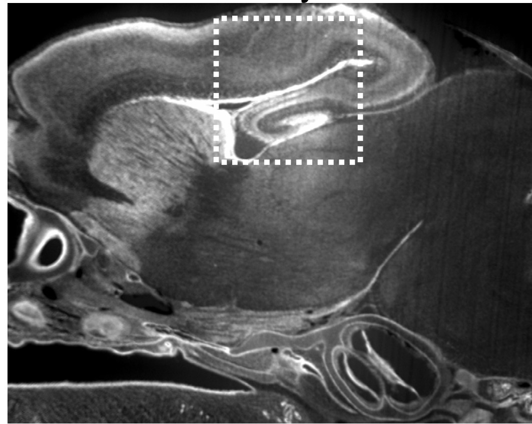




Supplemental Figure 6

A





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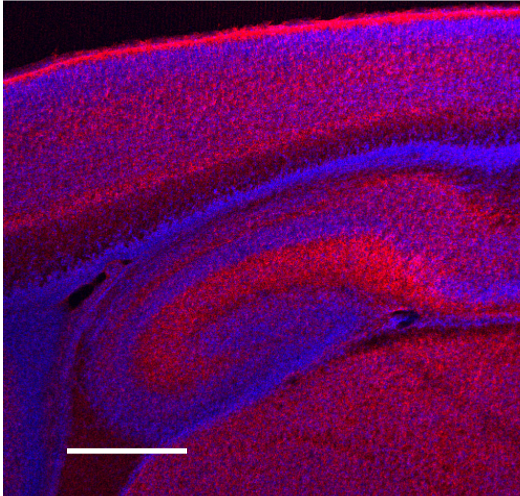

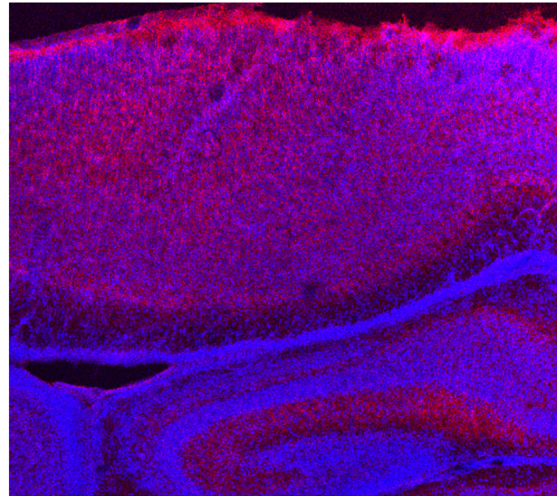

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