Supplemental Materials

Molecular Biology of the Cell

Silkworth et al.

Supplementary Data for

The Neuron Specific Formin Delphilin Nucleates Non-Muscle Actin but Does Not Enhance Elongation

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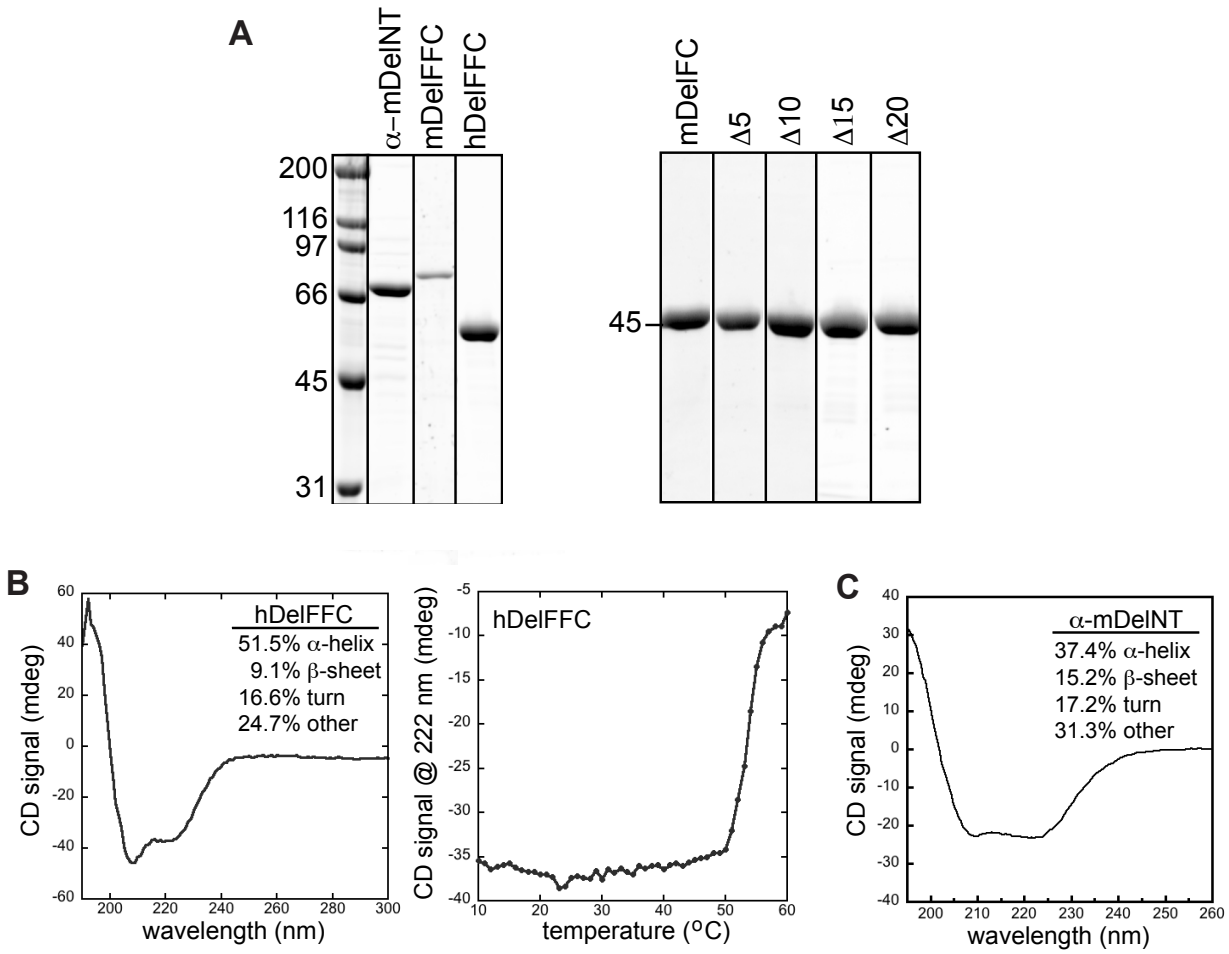

Figure S1. Proteins used in this study. A) SDS-PAGE gels of the constructs used in this paper: α-mDelNT, mDelFFC, and hDelFFC are grouped together. mDelFC, and truncations thereof are grouped separately. B) Circular dichroism analysis of secondary structure of 0.06 mg/ml hDelFFC. Loss of structure as a function of temperature demonstrates that hDelFFC is relatively thermal stable compared to other formins. The T_m (~55 °C) is about 5° higher than mDelFC, 14° higher than mDia1-FH2 (Kupi *et al.*, 2013), and 20° higher than CapuFFC (Vizcarra *et al.*, 2014). C) Circular dichroism analysis of secondary structure of 0.2 mg/ml α-mDelNT. Most of the secondary structure detected is α-helical, consistent with a PDZ domain. More than 50% of the construct has secondary structure, suggesting that it is folded.

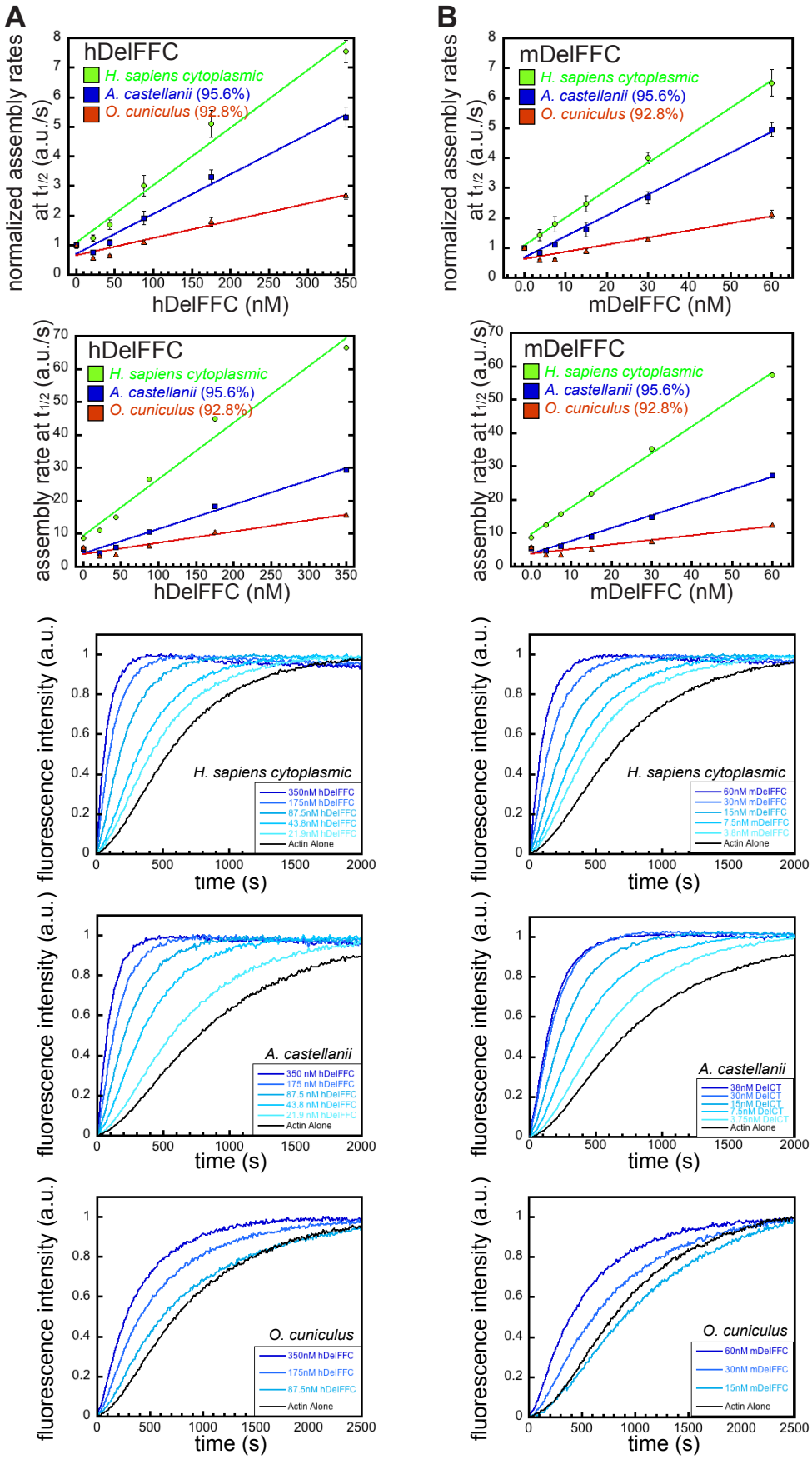
A
B
hDelFFC
mDelFFC
H. sapiens cytoplasmic
A. castellanii (95.6%)
O. cuniculus (92.8%)
normalized assembly rates at t1/2 (a.u./s)
assembly rate at t1/2 (a.u./s)
hDelFFC (nM)
mDelFFC (nM)
fluorescence intensity (a.u.)
time (s)
H. sapiens cytoplasmic
A. castellanii
O. cuniculus
350nM hDelFFC
175nM hDelFFC
87.5nM hDelFFC
43.8nM hDelFFC
21.9nM hDelFFC
Actin Alone
60nM mDelFFC
30nM mDelFFC
15nM mDelFFC
7.5nM mDelFFC
3.8nM mDelFFC
350 nM hDelFFC
175 nM hDelFFC
87.5 nM hDelFFC
43.8 nM hDelFFC
21.9 nM hDelFFC
38nM DelCT
30nM DelCT
15nM DelCT
7.5nM DelCT
3.75nM DelCT

Figure S2. Delphilin-mediated assembly rates depend on actin isoform. A) Comparison of hDelFFC-stimulated assembly rates normalized to actin alone (top) or not. Below are raw pyrene actin assembly data with hDelFFC as indicated and 4 μM actin (source indicated). B) Comparison of mDelFFC-stimulated assembly rates normalized to actin alone (top) or not. Below are raw pyrene actin assembly data with mDelFFC as indicated and 4 μM actin (source indicated).

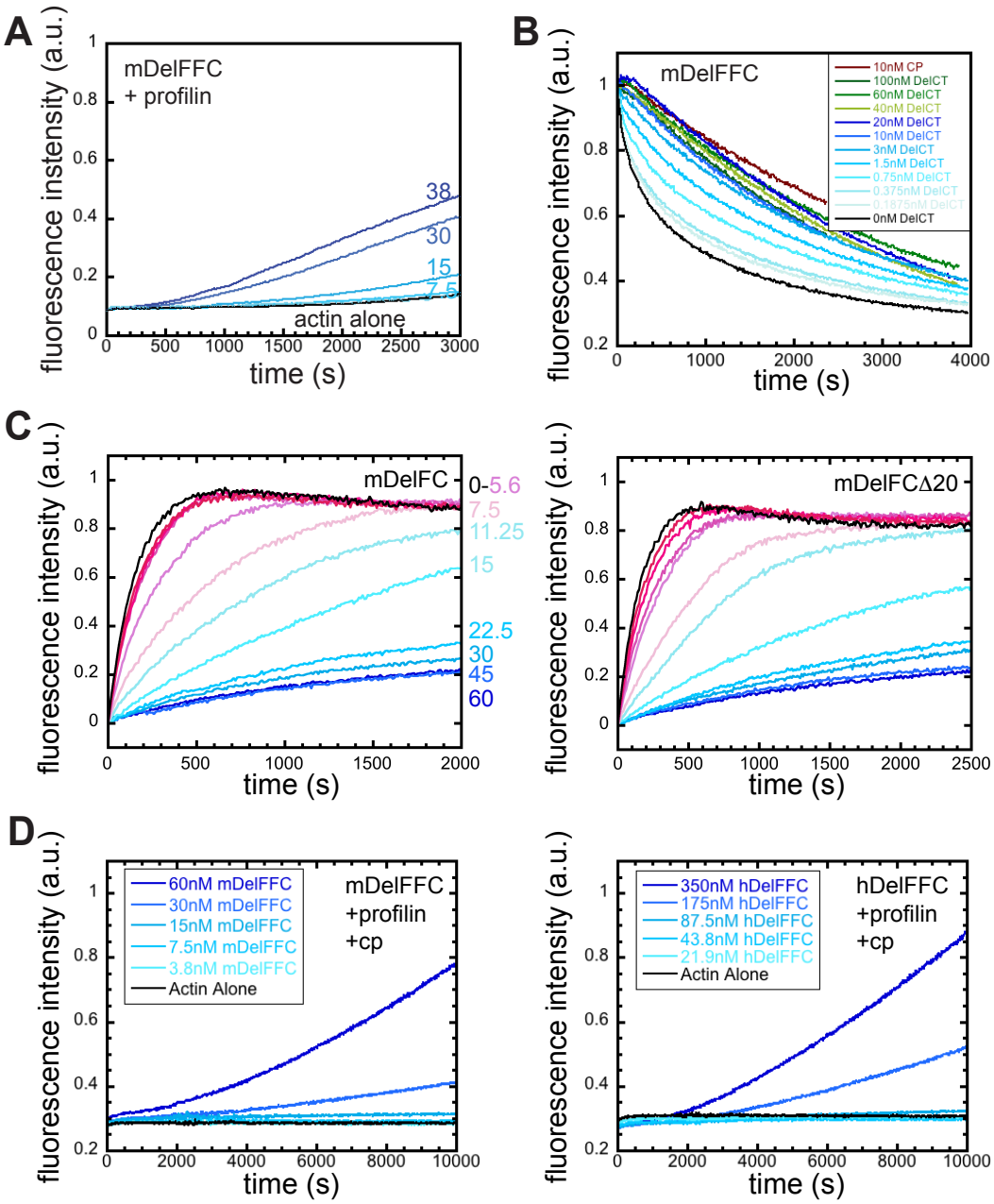

Figure S3. Actin polymerization and depolymerization in the presence of Delphilin. A) Pyrene actin assembly assay with mDelFFC concentrations as indicated (nM), 4 μM actin and 12 μM *S. pombe* profilin. B) Representative normalized depolymerization data. Filamentous actin was diluted to 0.1 μM in the presence of increasing concentrations of Delphilin. Capping protein is shown as a control. Analysis of three independent repeats of these assays is shown in Figure 3A. The apparent affinity of mDelFFC for barbed ends is 0.6 nM. C) Representative seeded elongation assays in the presence of increasing concentrations of mDelFC (left) or mDelFCΔ20 (right). The IC_{50} is 7 nM and elongation is ~97% inhibited in both cases, demonstrating that the C-terminus has little influence on the affinity of Delphilin for barbed ends. This value is also similar to that of mDelFFC under the same conditions (IC_{50} = 13 nM). D) Delphilin stimulated actin assembly in the presence of profilin and capping protein. Nucleation is weak but filament elongation at higher concentrations of mDelFCC demonstrates that Delphilin can protect the barbed end in the presence of capping protein.