

Supplemental Data

Supplemental Figure S1. Characterization of Commercial Endophilin Antibodies

Myc tagged Endophilin 1, 2 and 3 full length were transfected in HEK293 cells and blots were probed with antibodies for Endophilin 1(S-20), Endophilin 2(E-15) and Endophilin 3(K-17) from Santa Cruz Biotechnology. These antibodies were used to blot brain coIP samples in figure 1E and for ImmunoEM studies in Figure 2A.

Supplemental Figure S2. Arc-endophilin Vesicles Co-localize with Early Endosomal Markers and are Present Near the Plasma Membrane

- (A) Arc-endophilin vesicular structures co-localize with transferrin receptors, with some puncta also co-localizing with Rab11, but not with LAMP1. Scale bar is 30 μm)
- (B) Hela cells were transfected with GFP-Arc, GFP vector and GFP-Vinculin (top, middle and bottom panel respectively). Images of live cells were taken using Olympus IX81 microscope with a 60x objective in wide field (WF, left panels) and total internal reflection microscopy (TIRF) modes (right panels). Arc protein is present on the plasma membrane as it is visible in TIRF while GFP is not. Vinculin, a focal adhesion molecule, was used as a positive control. (GFP-vinculin plasmid is a generous gift from Dr. Susan Craig of Johns Hopkins University). Scale bar is 20 μm .
- (C) Hela cells were transfected with GFP-Arc and endophilin 3 (172-347). Live images of Arc-endophilin vesicles were taken in wide field and TIRF modes (top panels). Time laps images at 1 min intervals (lower panels). Red arrowheads indicate the movement, overlap and possible fusion of two vesicles, where blue arrowheads indicate stationary vesicles.

Supplemental Figure S3. Arc Binding Region in Endophilin 2

- (A) Arc 89-100 peptide mimics Arc and binds endophilin 3 full length (En3 FL), endophilin 3 with SH3 domain mutation (En3 FL W322A) and endophilin 2(172-368), but not endophilin 3(221-347).
- (B) Full length Arc and chimeras between endophilin 1 and 3 were transfected in HEK293 cells. Cell lysates were IPed with myc antibody and probed with Arc antibody. A chimera that includes the C-terminal BAR and the variable region of endophilin 3(172-285), together with the SH3 domain of endophilin 1 (291-352) showed robust binding to Arc. By contrast, a chimera with the C-terminal BAR and variable region of endophilin 1, together with the SH3 domain of endophilin 3 did not bind (not shown). Thus, the SH3 domain does not contribute to specificity of binding to Arc. Chimeras that begin with endophilin 1(172-N) and fuse with endophilin 3 at different positions in the variable domain did not bind Arc unless they include with the C-terminal 8 aa of the BAR domain (aa 240). Thus, endophilin 1(172-239)/3(240-347) binds Arc while endophilin 1(172-247)/3(248-347) does not. Chimeric constructs of endophilin 1 and 3 show co-IP with Arc if they include the C-terminus of the BAR and variable domains of endophilin 3.
- (C) A similar analysis of endophilin1/2 chimeras indicates an identical requirement for endophilin 2 (aa 240-247) is required to bind Arc. Chimeras that include the C-terminal BAR and the variable region of endophilin 2 (aa 172-306), together with the

SH3 domain of endophilin 1 (291-352) coIP with Arc. By contrast, a chimera with the C-terminal BAR and variable region of endophilin 1, together with the SH3 domain of endophilin 2 does not co-IP.

Supplemental Table S1. Summary of Arc-Endophilin binding and Vesicle Formation

- A) Summary of wild type and mutant Arc binding to endophilin 3(172-347), and vesicle association in HeLa cells.
- B) Summary of full length Arc binding to endophilins, and Arc-endophilin vesicle association in HeLa cells.

Supplemental Figure S4. Arc and Endophilin 3 Transgenes Co-localize with Excitatory Synaptic Proteins in Neurons

- (A) Arc transgene co-localizes with PSD-95 in spines. Endophilin 3 full-length transgene localize to large puncta at synapses, which co-localize with surface GluR1. Large puncta in the dendritic shaft can also be seen.

Supplemental Figure S5. Arc localization with Endophilin 3 Mutant Transgenes in Neurons

Arc and endophilin 3(W322A) [En3-FL SH3 W322A)] bind in biochemical assays, but the W322A mutation interrupts endophilin 3 binding to dynamin, and endophilin 3(W322A) does not associate with vesicles in HeLa cells (Supplemental Table T1B). When co-expressed in neurons, Arc and endophilin 3(W322A) co-localize in dendrites and spines but compared to Wt proteins, show diffuse localization without prominent association in vesicles.

Arc Δ 91-100 does not bind endophilin and does not associate with vesicles in HeLa cells (Supplemental Table T1A). When co-expressed in neurons, Arc Δ 91-100 is present in dendrites but does not co-localize with endophilin 3.

Supplemental Figure S6. Endophilin 2 Co-localizes with Arc but Endophilin 1 Does Not in Neurons.

Endophilin 1 transgene expression is diffuse and localizes to dendrites, spines and axons and does not co-localize with Arc in vesicular puncta. By contrast, endophilin 2 forms large vesicular structures that co-localize with Arc, similar to endophilin 3-Arc co-expressed transgenes.

Supplemental Figure S7. Endophilin and Dynamin-CT Transgenes Co-localize with Endogenous AMPARs.

- (A) Endophilin 3 full-length puncta co-localize with total GluR2 and GluR1 (using C-terminal antibodies) but do not co-localize with mGluR5 receptors.
- (B) Dynamin2-CT (503-871) puncta co-localize with GluR1.

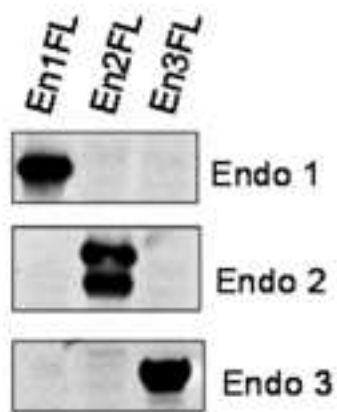
Supplemental Figure S8. Effect of Arc Expression on NMDA Receptors

Arc expression increased the total intensity of NR1 puncta as compared to GFP transfected neurons (n = 30 regions from 10 cells, p < 0.005)

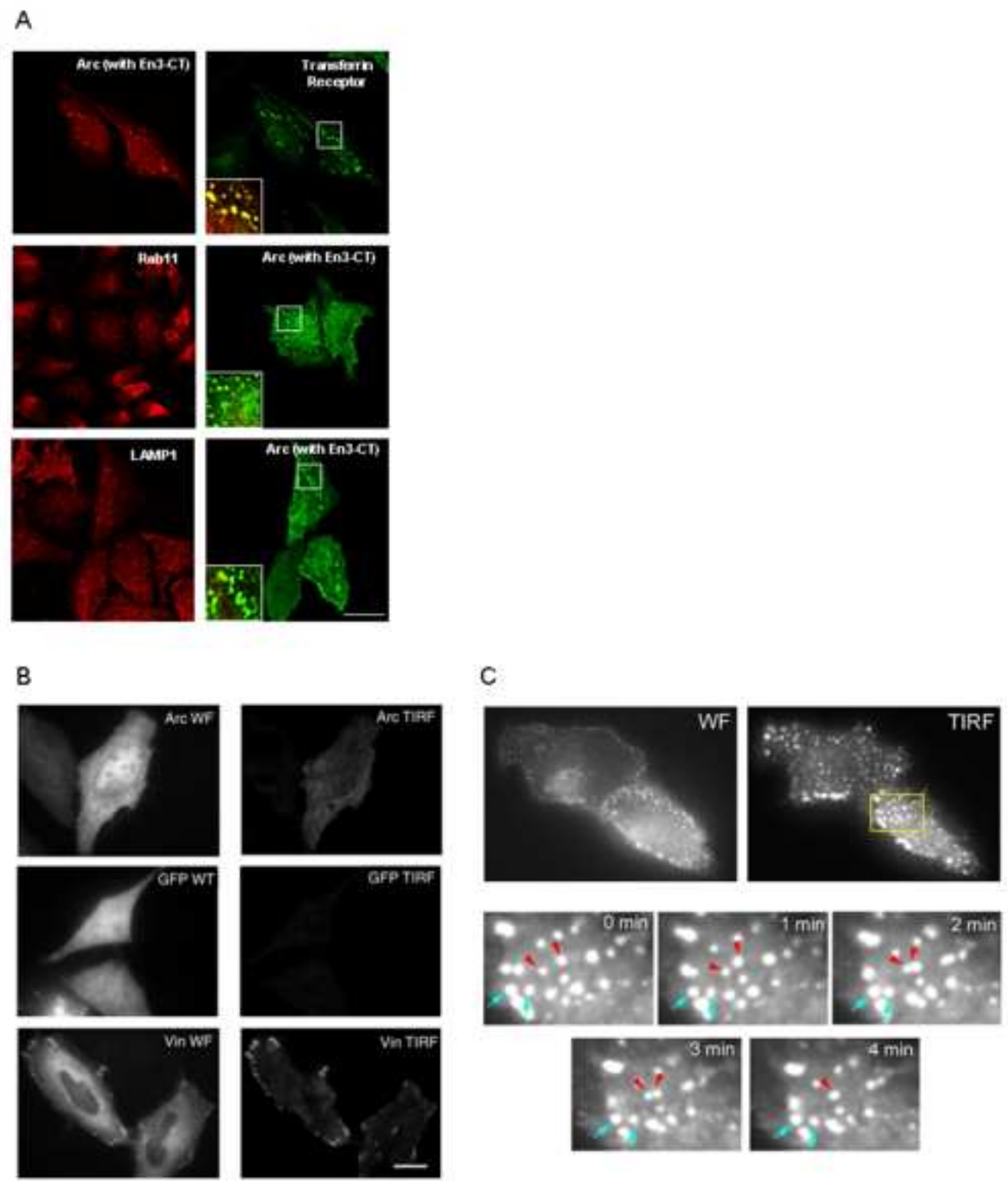
Supplemental Figure S9. Properties of Surface and Internalized GluR1 in Arc Transfected and Arc KO Neurons

Quantitation of surface and internalized GluR1 in Arc overexpression and KO experiments. These data were used to show the ratios observed in Figure 8.

Antibody specificity for Endophilins



Supplemental Figure S2 - Chowdhury et al, 2006



Supplemental Table T1 - Chowdhury et al, 2006

A

Summary of Endophilin 3 (172-347) interaction with Arc wt and mutants in heterologous cells

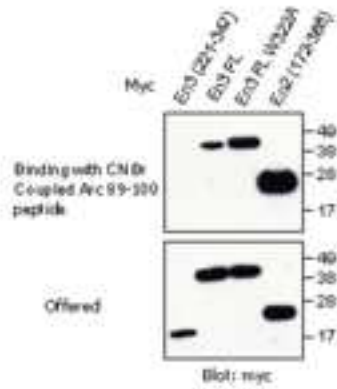
	<u>Binding</u>	<u>Vesicle</u>
Arc FL	+++	+++
Arc Δ 50-80	++++	++++
Arc Δ 81-90	+	+
Arc Δ 91-100	-	-
Arc Δ 101-130	+	+
Arc 1-154	+++	-
Arc 155-396	-	-

B

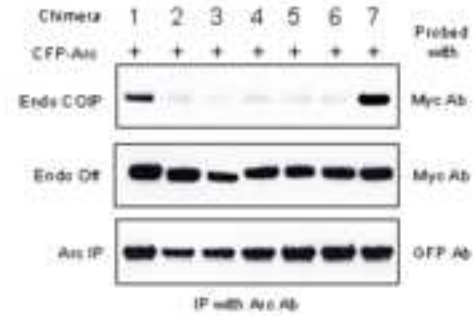
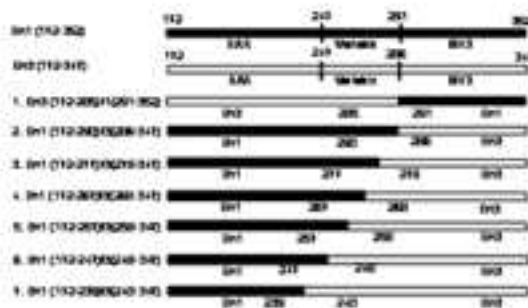
Summary of Arc interaction with Endophilins in heterologous cells

	<u>Binding</u>	<u>Vesicle</u>
Endophilin 1 FL	-	-
Endophilin 2 FL	-	-
Endophilin 3 FL	+	-
Endophilin 1(172-352)	-	-
Endophilin 2 (172-368)	++++	++++
Endophilin 3 (172-347)	+++	+++
Endo1(172-290)Endo3(286-347)	-	-
Endo3(172-285)Endo1(291-352)	+++	+++
Endophilin 3 (W322A)	+	-
Endophilin 3(172-347; W322A)	+++	-
Endophilin 1 (172-352;W322A)	-	-
Endophilin 3 (201-347)	+++	+++
Endophilin 3 (221-347)	-	-

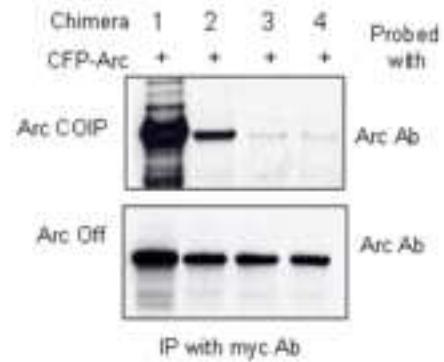
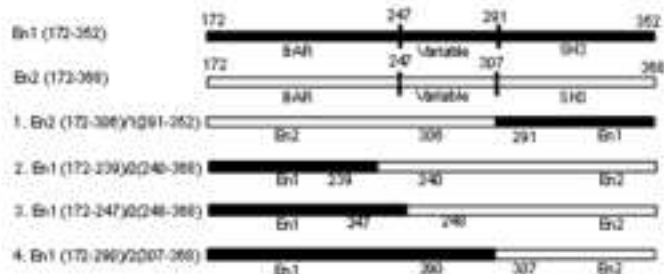
A



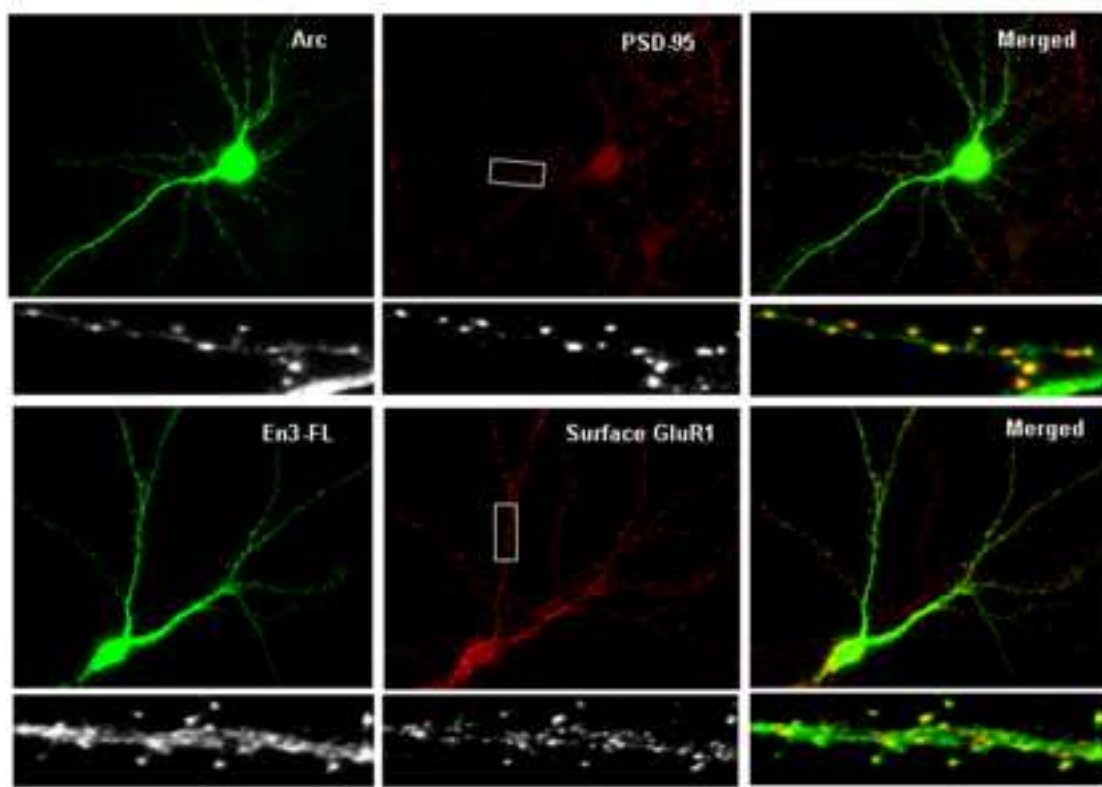
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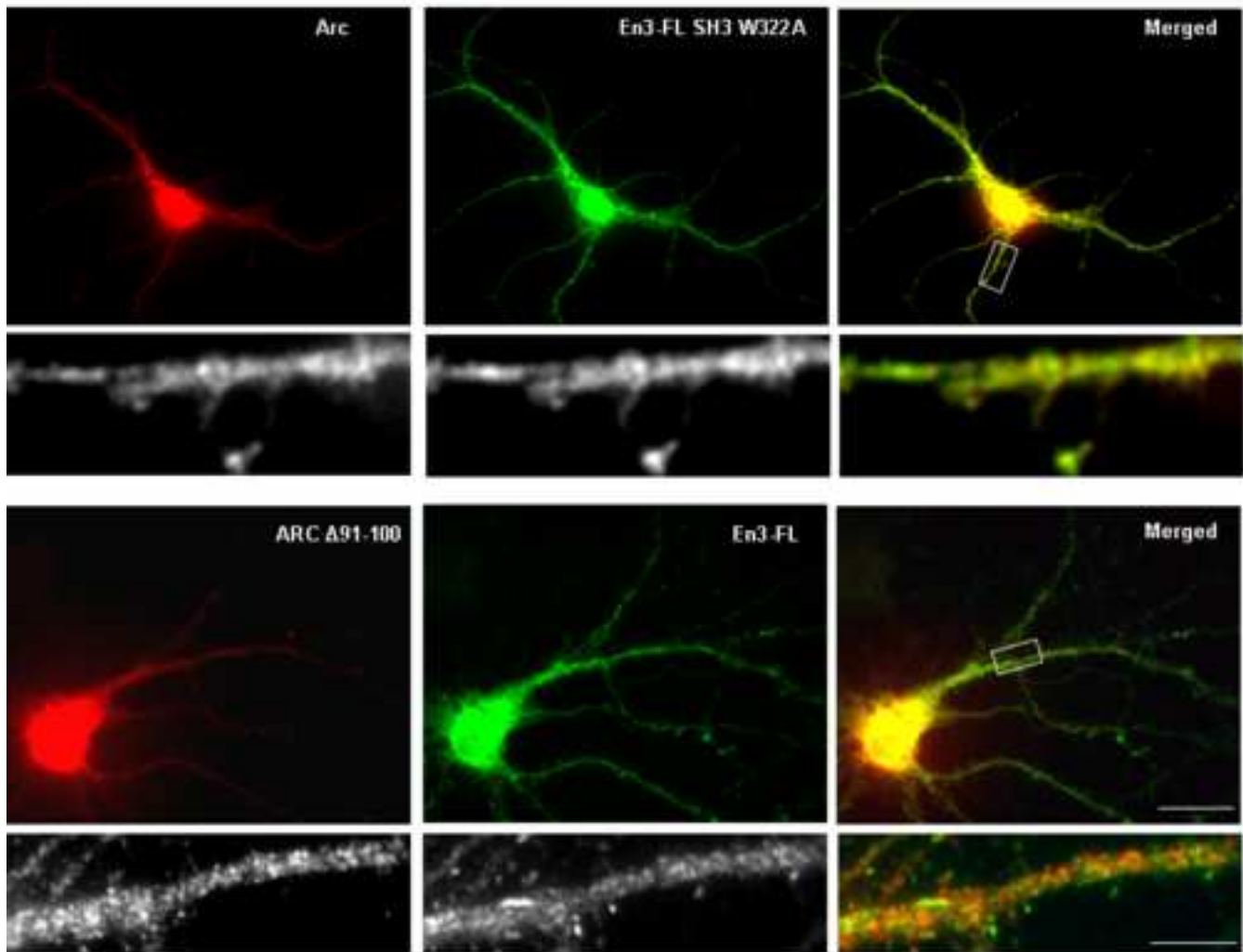
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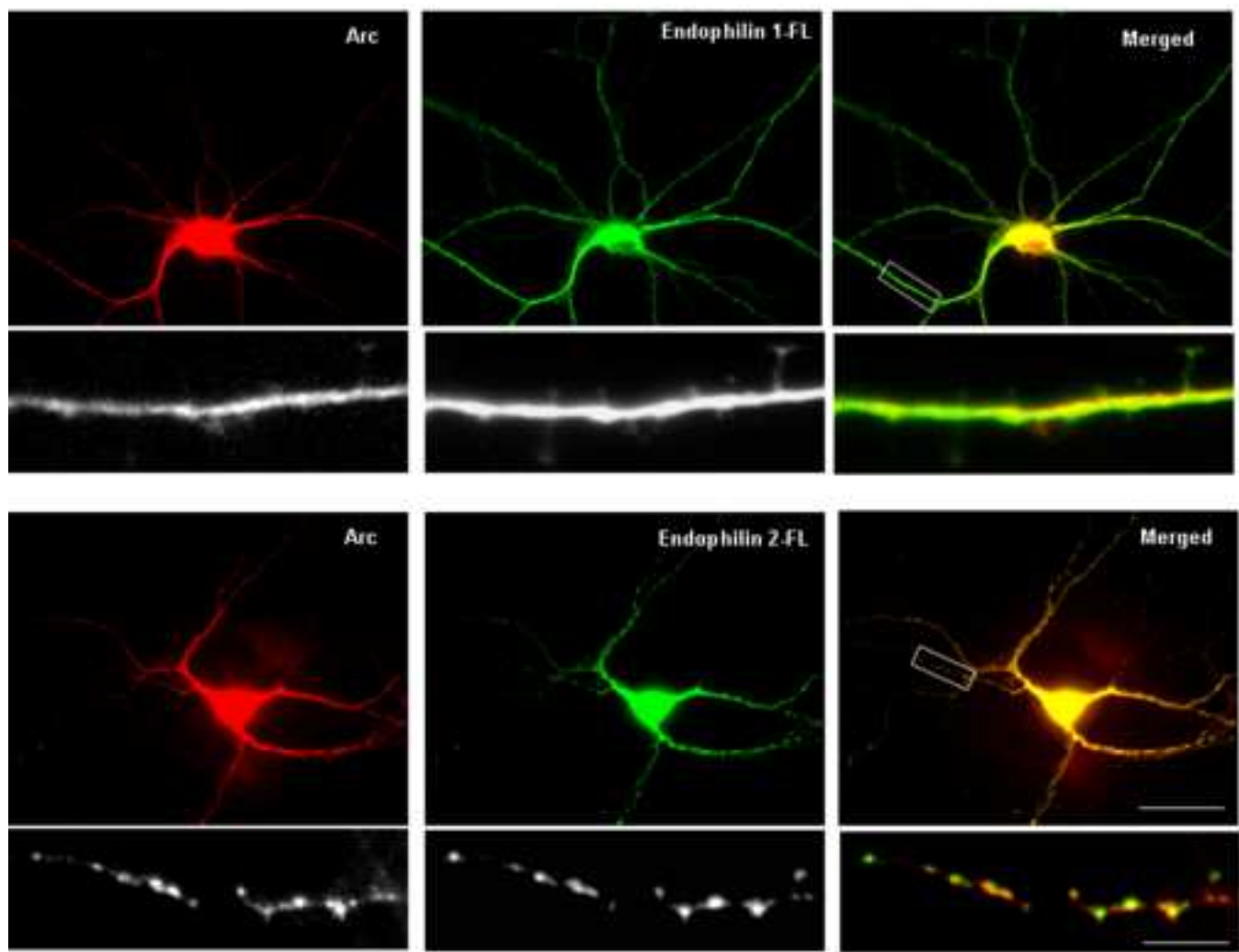
Supplemental Figure S4 - Chowdhury et al, 2006



Supplemental Figure S5 - Chowdhury et al, 2005

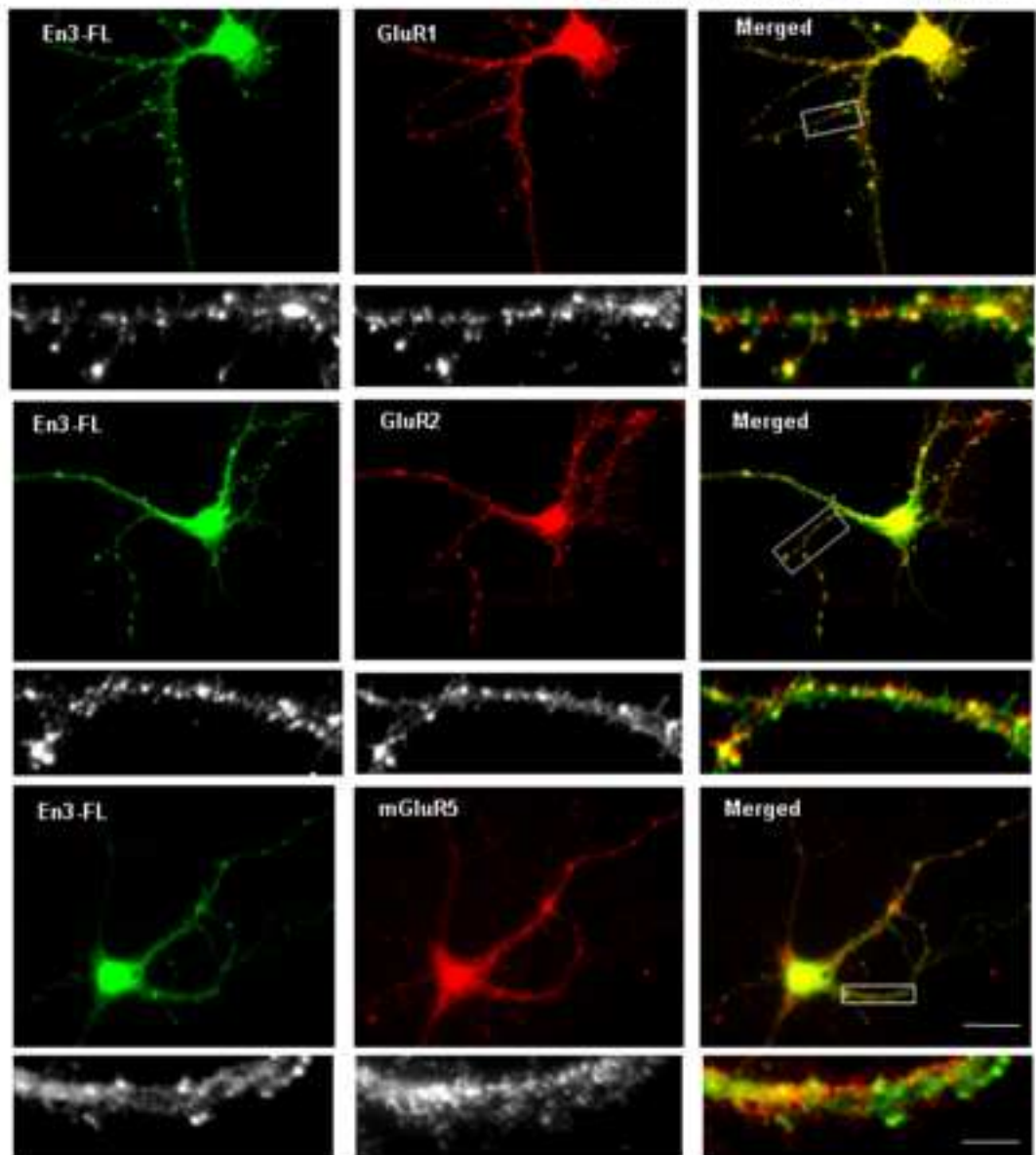


Supplemental Figure S6 - Chowdhury et al, 2006

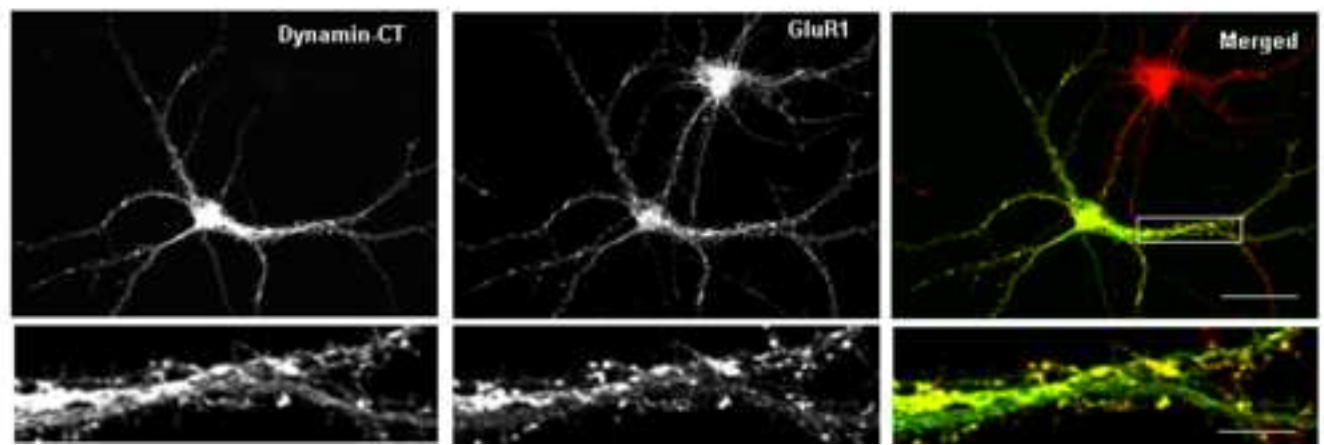


Supplemental Figure S7 - Chowdhury et al, 2006

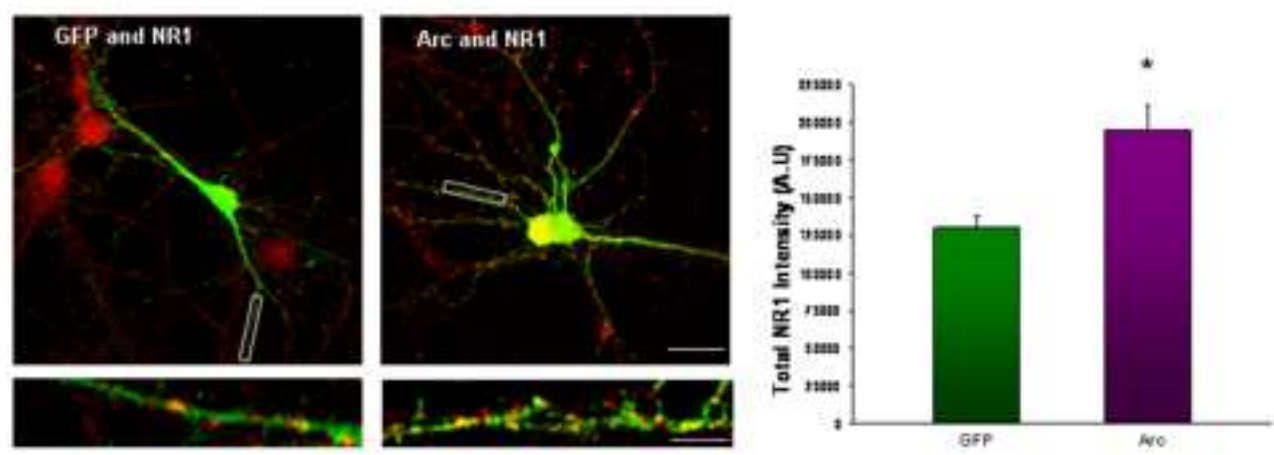
A



B



Supplemental Figure S8 - Chowdhury et al, 2006



Supplemental Figure 9 - Chowdhury et al, 2006

