Supporting Information

Figure S1: ***Schematics of constructs that are used in the study.***

A, Summary of expression constructs used for myristoylated GFP or mCherry reporters. **B,** Summary of expression constructs used for axonally targeted or cell body restricted mCherry-GAP43 fusion protein mRNAs.

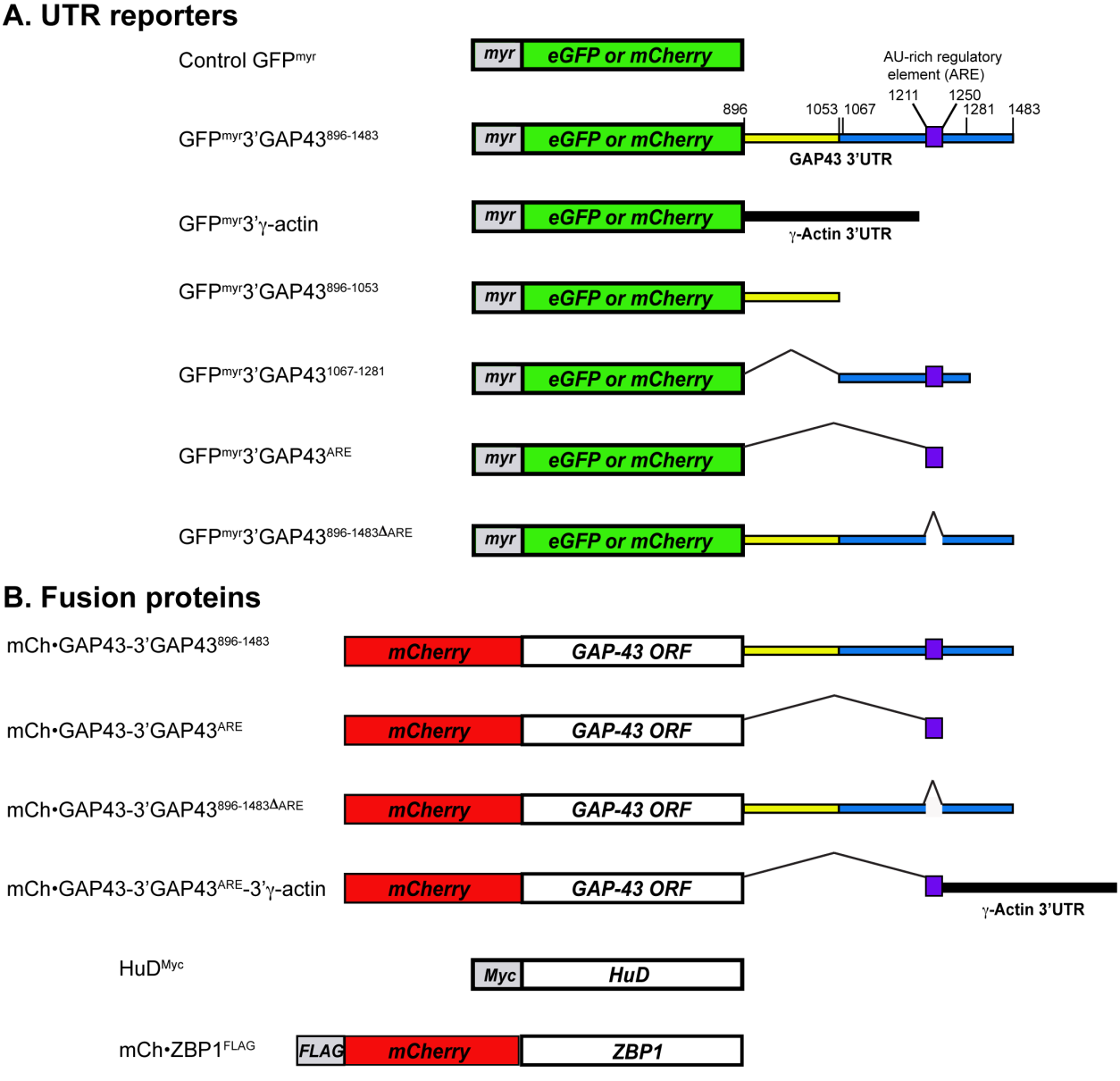

Figure S2: ***Axotomy triggers an increase of axonal GAP-43 mRNA levels that reduce axonal β-actin mRNA.***

A, Quantitative analyses were performed to show endogenous axonal levels of β-actin, GAP-43 and amphoterin mRNAs in sciatic nerve using quantitative FISH/IF technique. Only axonal FISH signals manifested by overlapping with axonal markers (NF + peripherin) were analyzed from proximal to the crush site (grey columns) and compared with those from naïve nerve (white columns). There is a significant increase in axonal GAP-43 mRNA and depletion of axonal β-actin mRNA with no significant change in axonal amphoterin mRNA with injury (* $p \leq 0.05$, ** $p \leq 0.01$ by Student's T-test for 7 d injured *vs*. naïve).

B, Quantitative FISH/IF analyses for indicated mRNAs in cultures of injury conditioned (grey columns) *vs*. naïve (white columns) is shown. Signals were analyzed as in A. As with the nerve *in vivo*, axons of the injury conditioned neurons show reduction in β-actin mRNA and enrichment of GAP-43 mRNA (* $p \leq 0.05$, ** $p \leq 0.01$ by Student's T-test for injury conditioned vs. naïve).

C, RTqPCR results from L4-5 DRGs at 0 and 7 days post nerve crush injury are shown (white and grey columns, respectively). The axotomy causes a significant increase in levels of GAP-43 mRNA by 7 days post injury, but no significant change in β-actin or amphoterin mRNAs (** $p \leq 0.01$ by one-way ANOVA for crush *vs*. control).

D, Representative FISH/IF images of distal axon shaft for control and transfected cultures. DRG neurons were transfected with $GFP^{myr}3'\gamma$-actin or $GFP^{myr}3'GAP43^{ARE}$ construct and processed for FISH/IF for endogenous β-actin mRNA (red) and neurofilament and peripherin proteins (NF+P, blue). Exposure was matched for each FISH/IF mages. Scale bars = 10 μm.

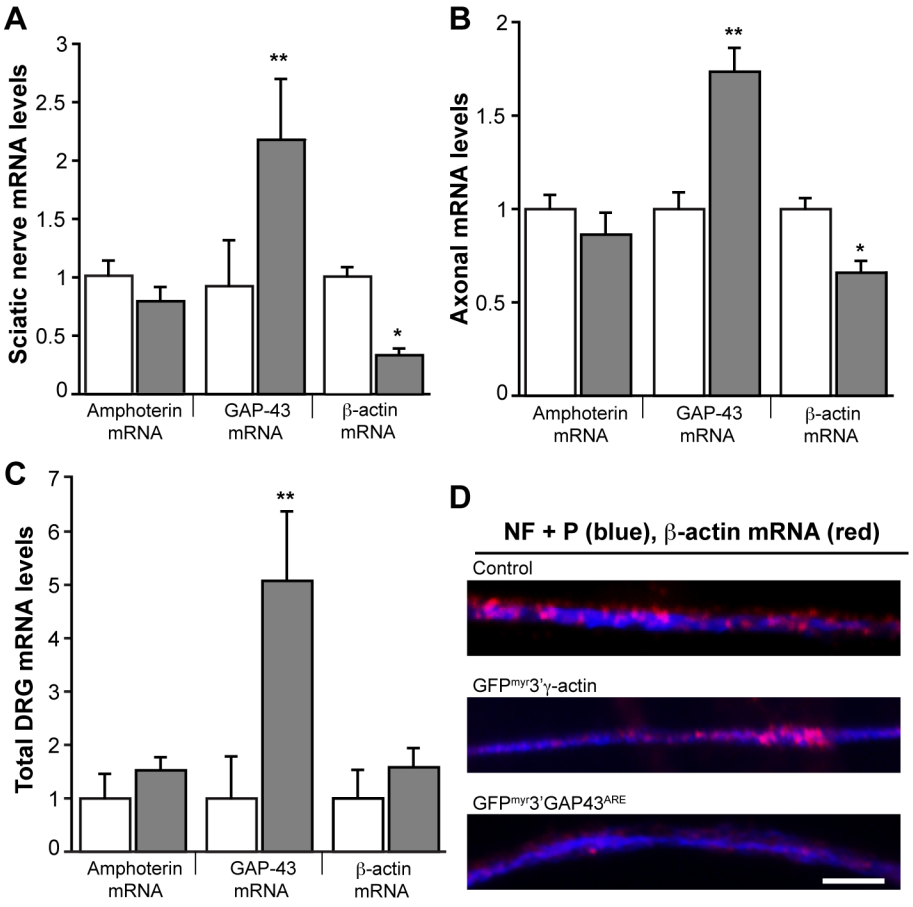
A
Sciatic nerve mRNA levels
3
2.5
2
1.5
1
0.5
0
**
*
Amphoterin mRNA
GAP-43 mRNA
β-actin mRNA
B
Axonal mRNA levels
2
1.5
1
0.5
0
**
*
Amphoterin mRNA
GAP-43 mRNA
β-actin mRNA
C
Total DRG mRNA levels
7
6
5
4
3
2
1
0
**
Amphoterin mRNA
GAP-43 mRNA
β-actin mRNA
D
NF + P (blue), β-actin mRNA (red)
Control
GFPmyr3'γ-actin
GFPmyr3'GAP43ARE

Figure S3: ***Sequences in the 3'UTR of GAP-43 support reporter mRNA translation in axons.***

A-**C,** Representative time-lapse images for FRAP analyses of adult DRG neurons transfected with the indicated constructs are shown for sequences in standard medium (**A**-**B**) and medium with 50 μM anisomycin (**C**). The boxed regions represent the ROIs that were photobleached followed by fluorescence recovery being monitored over 20 min and arrows indicate growth cones. Note that no clear recovery of fluorescent signal was observed with anisomycin pretreatment, indicating a protein synthesis-dependent recovery (**C**).

D,E, Quantification of FRAP experiments over multiple time-lapse sequences is shown as normalized signal intensity relative to pre- and post-bleach (average ± SEM, $n \geq 7$ neurons over > 3 separate transfection experiments for each construct; ** = $p \leq 0.01$ and *** = $p \leq 0.001$ by repeated measures ANOVA with Bonferroni post-hoc comparisons) [Scale bars = 50 μm].

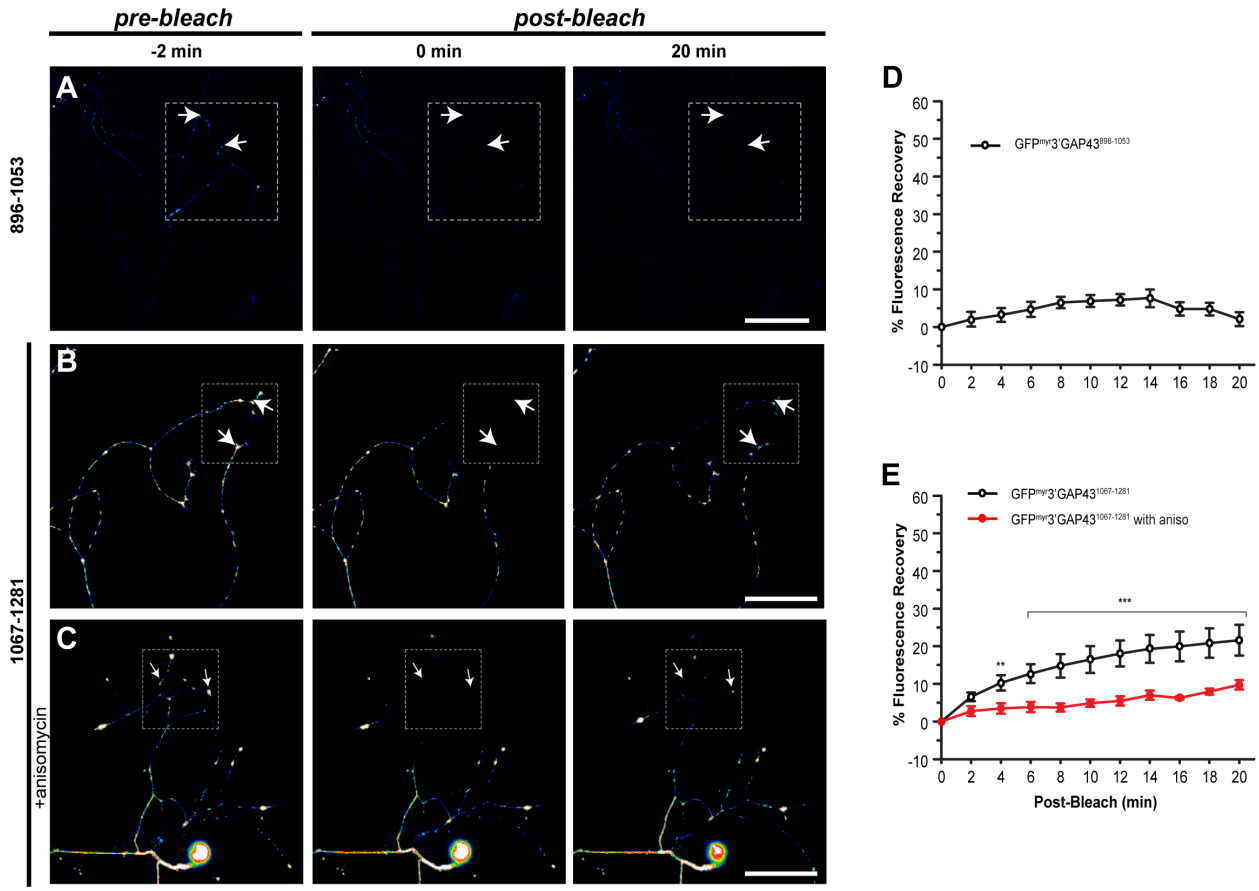
pre-bleach
post-bleach
-2 min
0 min
20 min
A
B
C
896-1053
1067-1281
+anisomycin
D
% Fluorescence Recovery
GFPmyr3'GAP43898-1053
E
GFPmyr3'GAP431067-1281
GFPmyr3'GAP431067-1281 with aniso
**
***
Post-Bleach (min)

Figure S4: ***Both localizing and nonlocalizing reporter mRNAs are expressed in cell body of neurons.***

A-D. Representative images of transfected adult DRG neurons in culture. DRG neurons were transfected with localizing mCh^{myr}3'GAP43$^{896\text{-}1483}$ construct (**A)** or mCh^{myr}3'GAP43^{ARE} (**B**) construct, and nonlocalizing mCh^{myr}3'GAP43$^{896\text{-}1053}$ (**C**) or mCh^{myr}3'GAP43$^{896\text{-}1483\Delta ARE}$ (**D**), and processed for immunocytochemistry for neurofilament and peripherin proteins (NF+P, blue). Note that each reporter mRNA are clearly expressed in the cell body (asterisks). Scale bar = 50 µm.

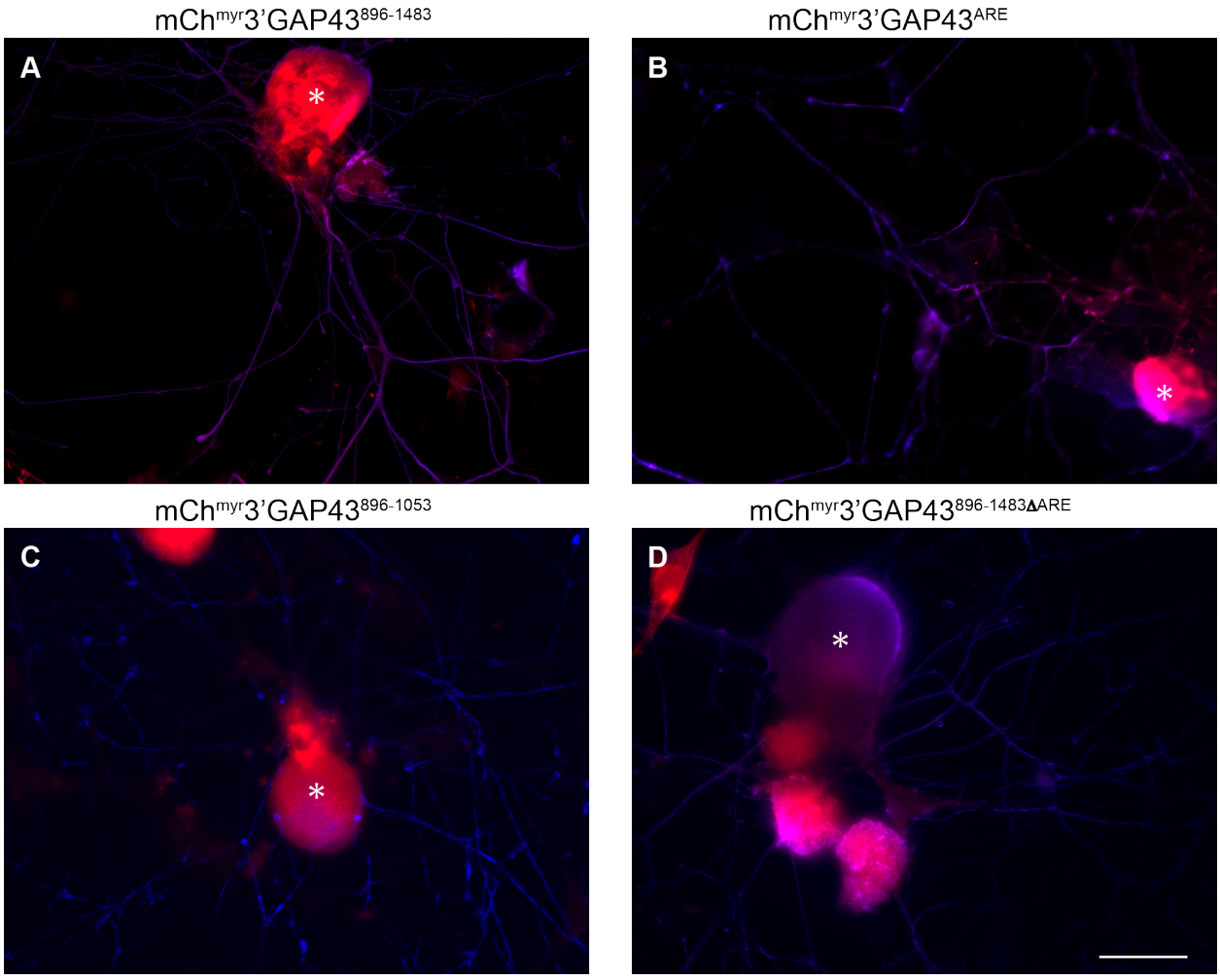

Figure S5: Overexpression of GAP-43's 3'UTR attenuates axon outgrowth when the ARE is included

Representative images of DRG neurons transfected with the indicated axonally targeted ($GFP^{myr}3'GAP43^{896\text{-}1483}$, $GFP^{myr}3'GAP43^{1211\text{-}1250}$) or cell body restricted GFP^{myr} constructs are shown ($GFP^{myr}3'\gamma$-actin, $GFP^{myr}3'GAP43^{896\text{-}1483}\Delta^{ARE}$) compared to control mCherry-transfected neurons (Scale bar = 200 μm).

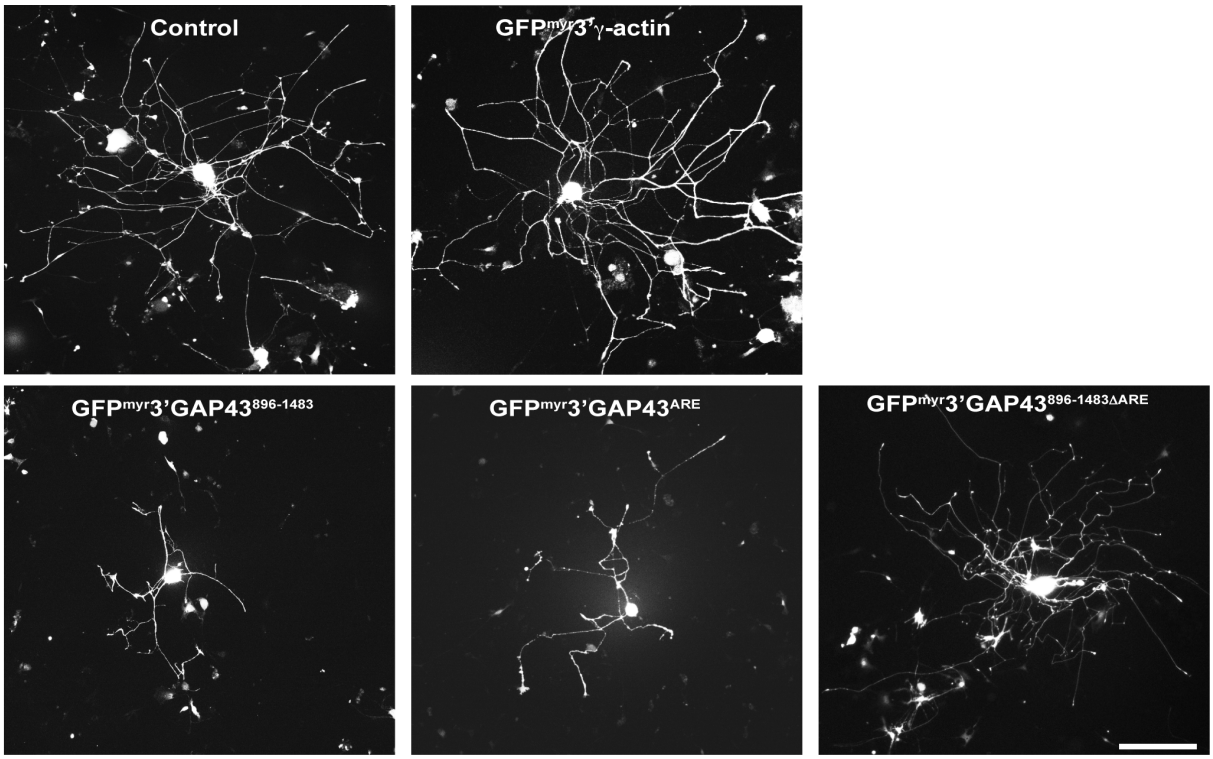

Figure S6: Overexpression of axonally targeted GAP-43 ORF increases axonal growth when the ARE is included

Representative images of DRG neurons transfected with the indicated ARE-containing GAP-43 ORF (mCh•GAP43-3'GAP43$^{1211\text{-}1250}$, mCh•GAP43-3'GAP43$^{1210\text{-}1250}$-3'γ-actin) vs. ARE-lacking GAP-43 ORF constructs (mCh•GAP43-3'γ-actin, mCh•GAP43-3'GAP43$^{896\text{-}1483\Delta^{ARE}}$) are shown compared to control mCherry-transfected neurons (Scale bar = 200 μm).