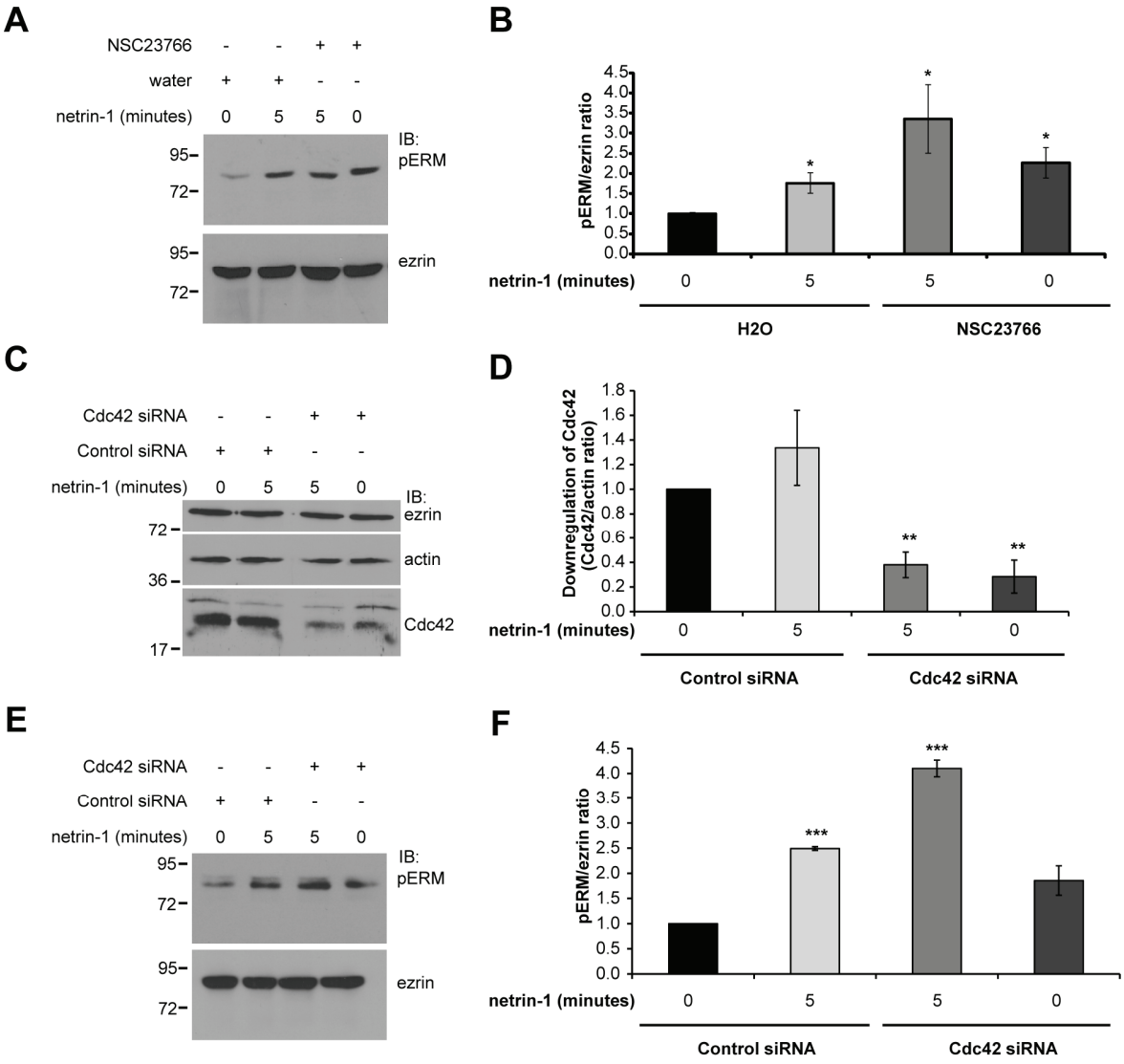
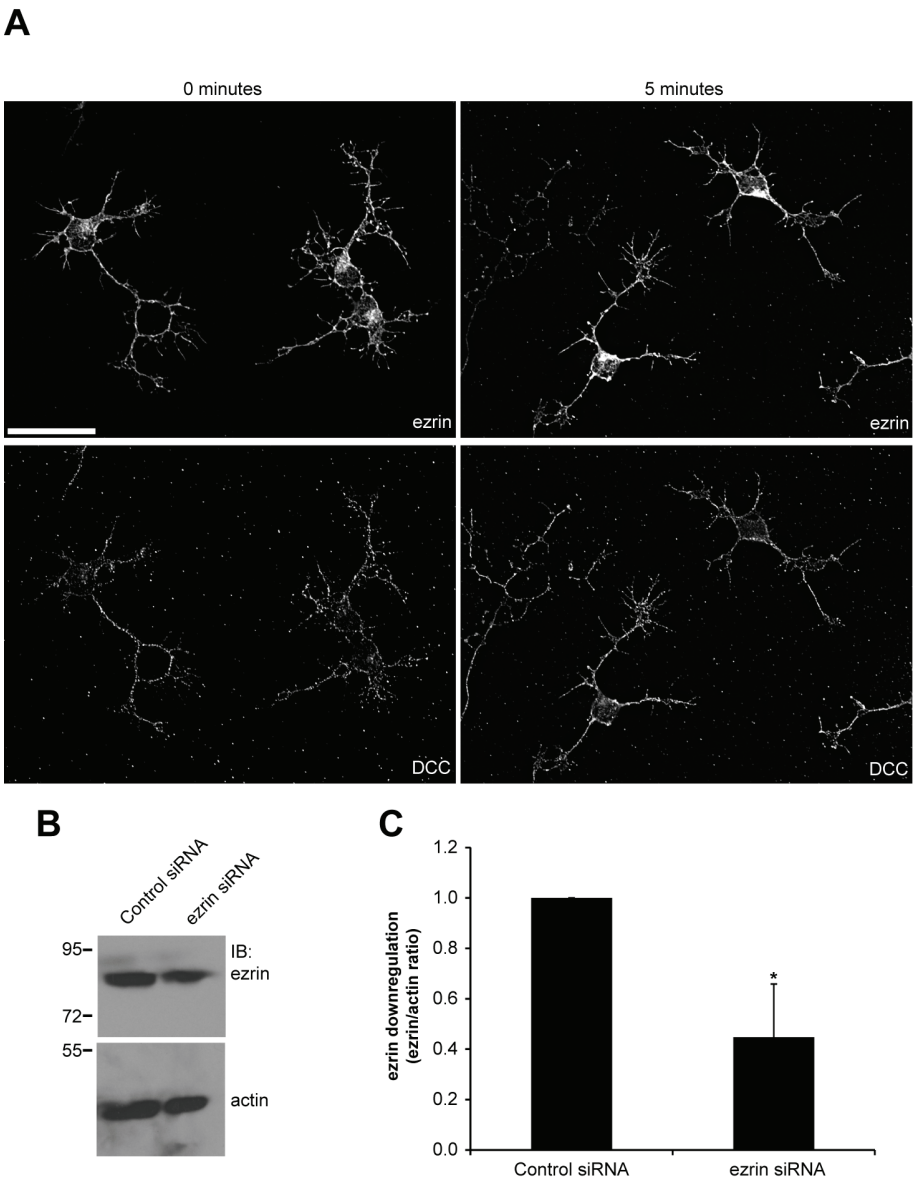
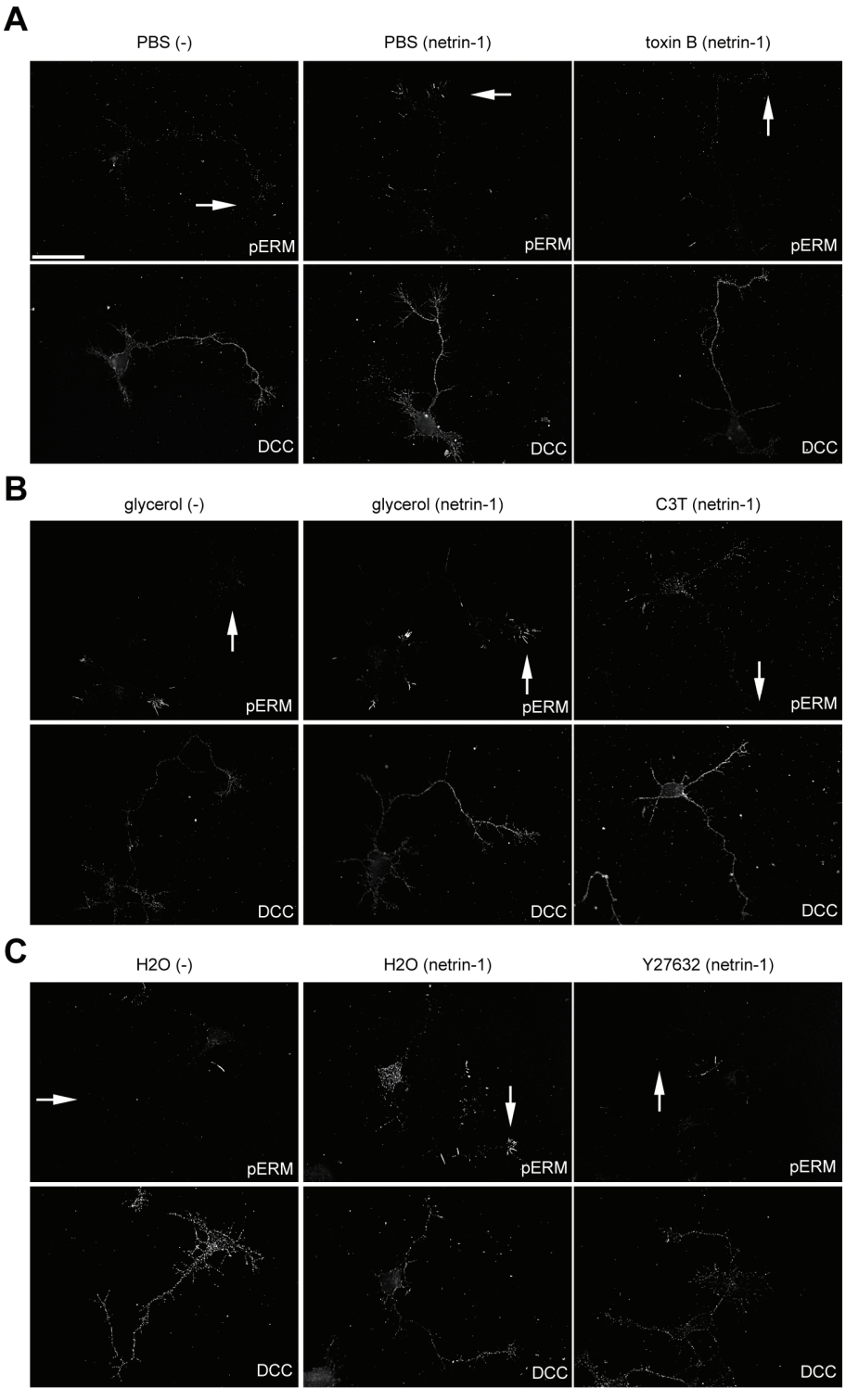


Supplemental Figure 1. Netrin-1-dependent ERM phosphorylation is not blocked by Rac1 inhibition or Cdc42 downregulation.

Embryonic rat cortical neurons (E18, 2DIV) were incubated with netrin-1 for the indicated times following treatment with either: (A and B) Rac1 inhibitor NSC23766 or water as a negative control or (C-F) Cdc42 siRNA or Control Negative siRNA electroporation. Protein lysates were resolved by SDS-PAGE and immunoblotted with anti-pERM, –ezrin, -actin, or –Cdc42 antibodies. (D) Quantitative densitometry of Cdc42 downregulation is represented as the ratio of Cdc42 over actin. Cdc42 expression is downregulated by at least 62%. (F) Quantitative densitometry is represented as the ratio of pERM over total ezrin and corresponds to the average of at least three independent experiments. Error bars represent SEM (*p<0.05, **p<0.01, ***p<0.005).



Supplemental Figure 2. Subcellular localization and downregulation of ezrin in cortical neurons.
(A) Embryonic rat cortical neurons (E18, 2DIV) were incubated with netrin-1 for 0 or 5 minutes, fixed and immunostained with antibodies against DCC and ezrin. Scale bar, 5 μm. Total ezrin proteins, unlike phosphorylated ERM proteins, do not accumulate in growth cones following netrin-1 stimulation. (B) Embryonic rat cortical neurons were electroporated with Negative Control or ezrin siRNA and protein lysates were resolved by SDS-PAGE and immunoblotted with anti–ezrin and –actin antibodies. (C) Quantitative densitometry of ezrin donregulation in (B) is represented as the ratio of ezrin over actin. Ezrin expression is downregulated by 55%. Error bars represent SEM (*$p<0.05$).


A
PBS (-)
PBS (netrin-1)
toxin B (netrin-1)
pERM
DCC
B
glycerol (-)
glycerol (netrin-1)
C3T (netrin-1)
C
H2O (-)
H2O (netrin-1)
Y27632 (netrin-1)

Supplemental Figure 3. Toxin B, C3 transferase and Y27632 inhibit netrin-1-induced accumulation of phosphorylated ERM proteins in growth cones.
Embryonic rat cortical neurons (E18, 2DIV) were incubated with netrin-1 for 0 or 5 minutes following treatment with either: (A) Rho GTPase inhibitor toxin B or PBS as a negative control, (B) RhoA inhibitor C3 Transferase (C3T) or glycerol as a negative control or (C) Rho kinase inhibitor Y27632 or water (H2O) as a negative control. Neurons were fixed and immunostained with anti-pERM and –DCC antibodies. The quantification of the accumulation of phosphorylated ERM proteins (pERM) in growth cones (arrows) is represented in Figure 6. Scale bar, 5 μm.