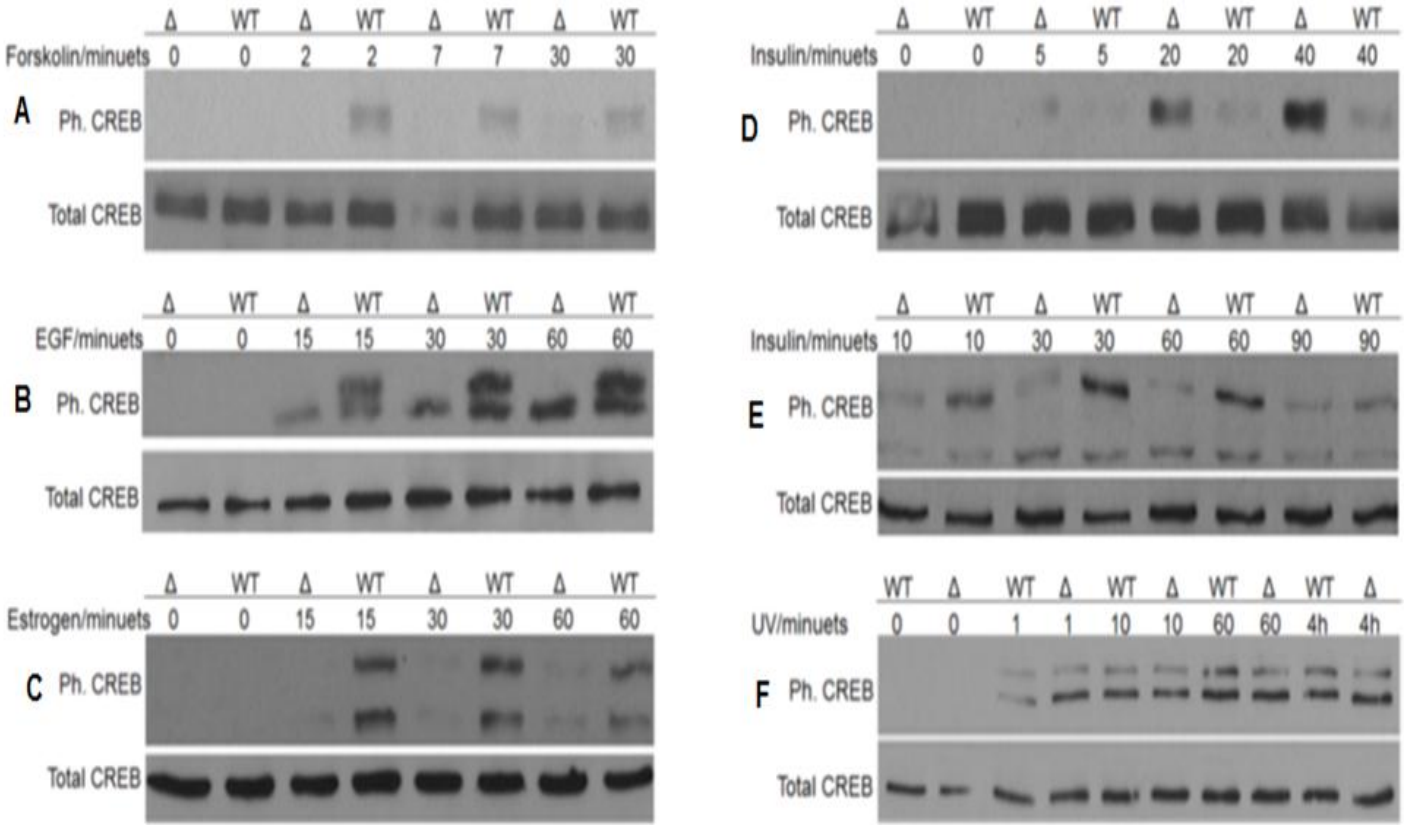
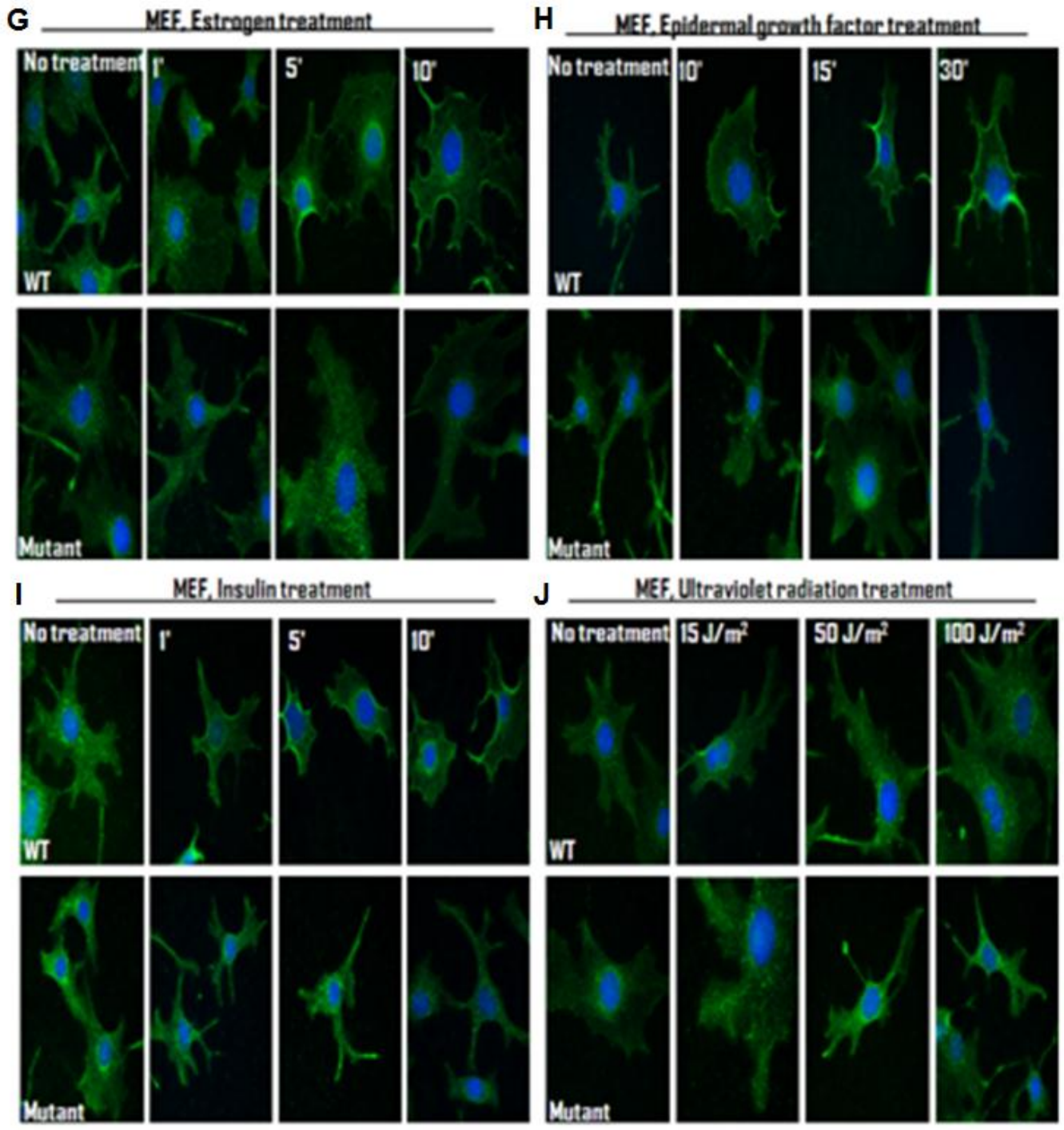
Figure S3


Δ WT Δ WT Δ WT Δ WT
Forskolin/minuets 0 0 2 2 7 7 30 30
A Ph. CREB
Total CREB
Δ WT Δ WT Δ WT Δ WT
EGF/minuets 0 0 15 15 30 30 60 60
B Ph. CREB
Total CREB
Δ WT Δ WT Δ WT Δ WT
Estrogen/minuets 0 0 15 15 30 30 60 60
C Ph. CREB
Total CREB
Δ WT Δ WT Δ WT Δ WT
Insulin/minuets 0 0 5 5 20 20 40 40
D Ph. CREB
Total CREB
Δ WT Δ WT Δ WT Δ WT
Insulin/minuets 10 10 30 30 60 60 90 90
E Ph. CREB
Total CREB
WT Δ WT Δ WT Δ WT Δ WT Δ
UV/minuets 0 0 1 1 10 10 60 60 4h 4h
F Ph. CREB
Total CREB



<u>Figure S3</u>. CC2D1A is specifically involved in cAMP/PKA pathway.

Western blots showing a defect in the early CREB phosphorylation but not in the total amount of CREB protein in CC2D1A mutant MEF cell lysates. Wild-type and CC2D1A

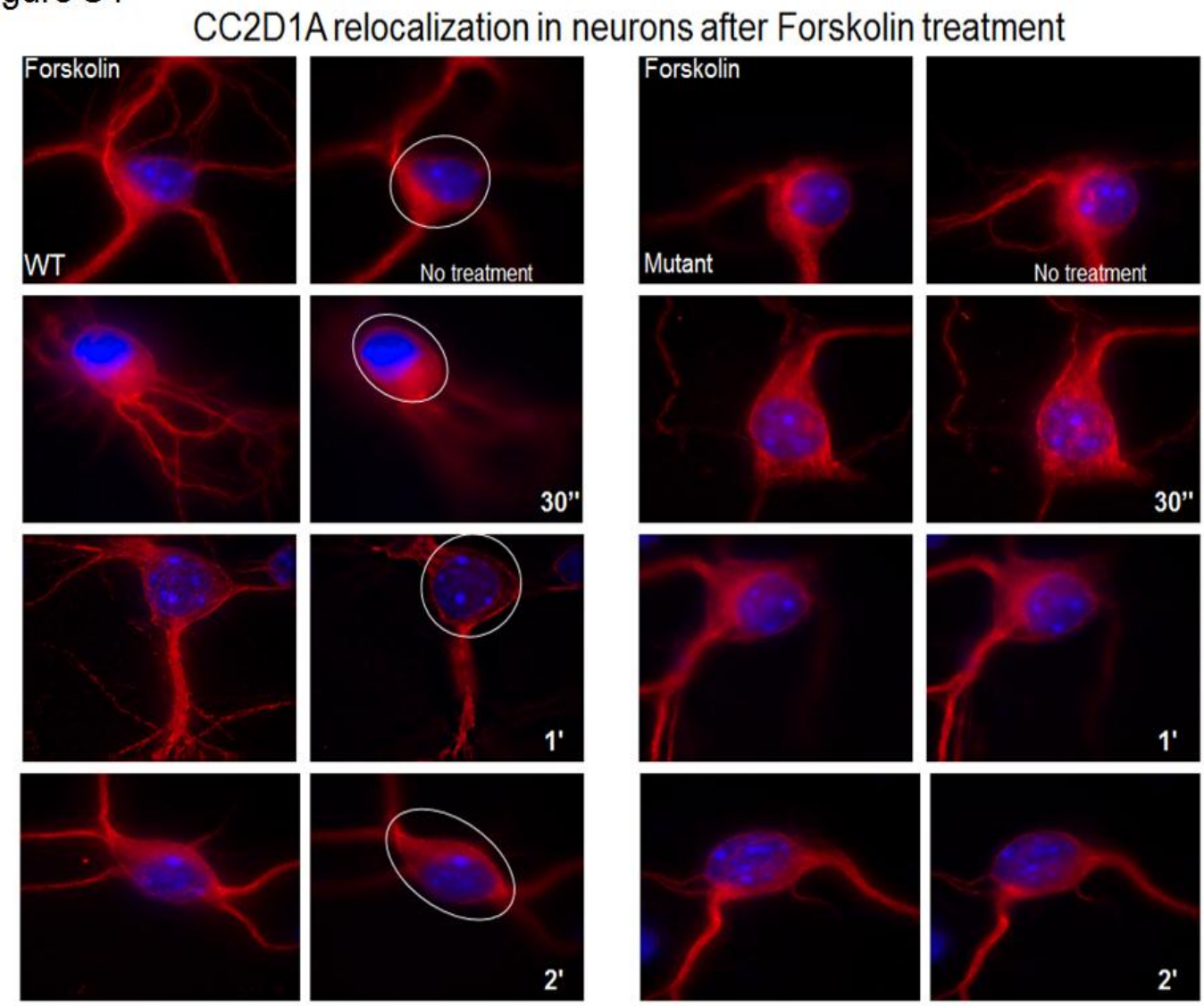
mutant MEF cells were stimulated with (A) Forskolin (50uM) for 0, 2, 7 and 30 minutes, (B) Epidermal growth factor (EGF) (10nM) for 0, 15, 30 and 60 minutes, (C) Estrogen (20nM) for 0, 15, 30 and 60 minutes, (D) Insulin (2uM) for 0, 5, 20 and 40 minutes, (E) Insulin again (2uM) for 10, 30, 60 and 90 minutes.

(F) Western blots showing no defect in the early CREB phosphorylation and a normal total amount of CREB protein in CC2D1A mutant MEF cell lysate after Ultra Violet (UV) treatment. Wild type and CC2D1A mutant MEF cells were treated with UV (100 J/m2) for 0, 1, 10, 60 and 240 minutes (4 hours).

Immunostaining images showing the accumulation of CC2D1A at the cell periphery in wild-type MEF cells after stimulation with (G) Estrogen (20nM) for 0, 1, 5, and 10 minutes (H) EGF (10nM) for 0, 10, 15, and 30 minutes, (I) Insulin (2uM) for 0, 1, 5, and 10 minutes. The localization is not observed in the CC2D1A mutant MEF cells, neither before nor after stimulation with these stimulants.

(J) Immunostaining images showing that CC2D1A does not accumulate at the cell periphery in either in wild-type nor CC2D1A mutant MEF cells after Ultraviolet radiation treatment with 0, 15, 50, 100 J/m2.

Figure S4



<u>Figure S4</u>. CC2D1A relocalizes in neurons after forskolin treatment.

Immunostaining images showing the accumulation of CC2D1A at the cell periphery in wild-type neurons after stimulation with forskolin (50uM) for 0, 30 seconds, 1 and 2 minutes. The localization is not observed in the CC2D1A mutant neurons, neither before nor after stimulation with forskolin.