Expanded View Figures

Figure EV1. CAD cells are responsive to Wnt signals.

A mRNA expression levels of 10 Fzd receptors in CAD cells were determined by qPCR analysis. *RPLP0* mRNA expression was used for normalization. Efficiency (eff) was calculated for each set of primers and used for the calculation of the expression by $-\Delta\Delta C_T$ method.

B Representing confocal images of CAD cells treated for 4 h with 200 μM H_2O_2, 200 ng/ml Wnt7a, or 200 ng/ml Wnt5a. Cells were fixed and then stained with WGA (green) and DAPI (blue).

C Percentage of TNT-connected CAD cells with each of the indicated treatments.

D Average transferred vesicle per acceptor cell with indicated treatments.

E Immunoblot of activated β-catenin in CAD cells cultured in control conditions or treated for 4 h with 200 μM H_2O_2; 10 mM LiCl; or 50, 100, and 200 ng/ml Wnt7a. GAPDH was used as loading control.

F Immunoblot of activated β-catenin and phosphorylated GSK3β (S9) in CAD cells treated in the presence or absence of Wnt7a for the indicated time points. Immunoblots show that 200 ng/ml Wnt7a could not activate β-catenin, while 10 mM LiCl effectively activated β-catenin after 60 min of incubation. GAPDH was used as loading control.

G Representative confocal images of control CAD cells and cells treated with 200 μM H_2O_2, LiCl, or NaCl for 4 h. Cells were stained with WGA (green) and Hoechst (blue).

H Percentage of TNT-connected CAD cells with each of the indicated treatments.

Data information: In (B, G), scale bars represent 10 μm. In (A, C, D, H), graph represents the average of three independent experiments ± SEM. Statistical significance was calculated with respect to control (Ctrl); $*P \leq 0.05$, NS = not significant (one-way ANOVA).

Source data are available online for this figure.

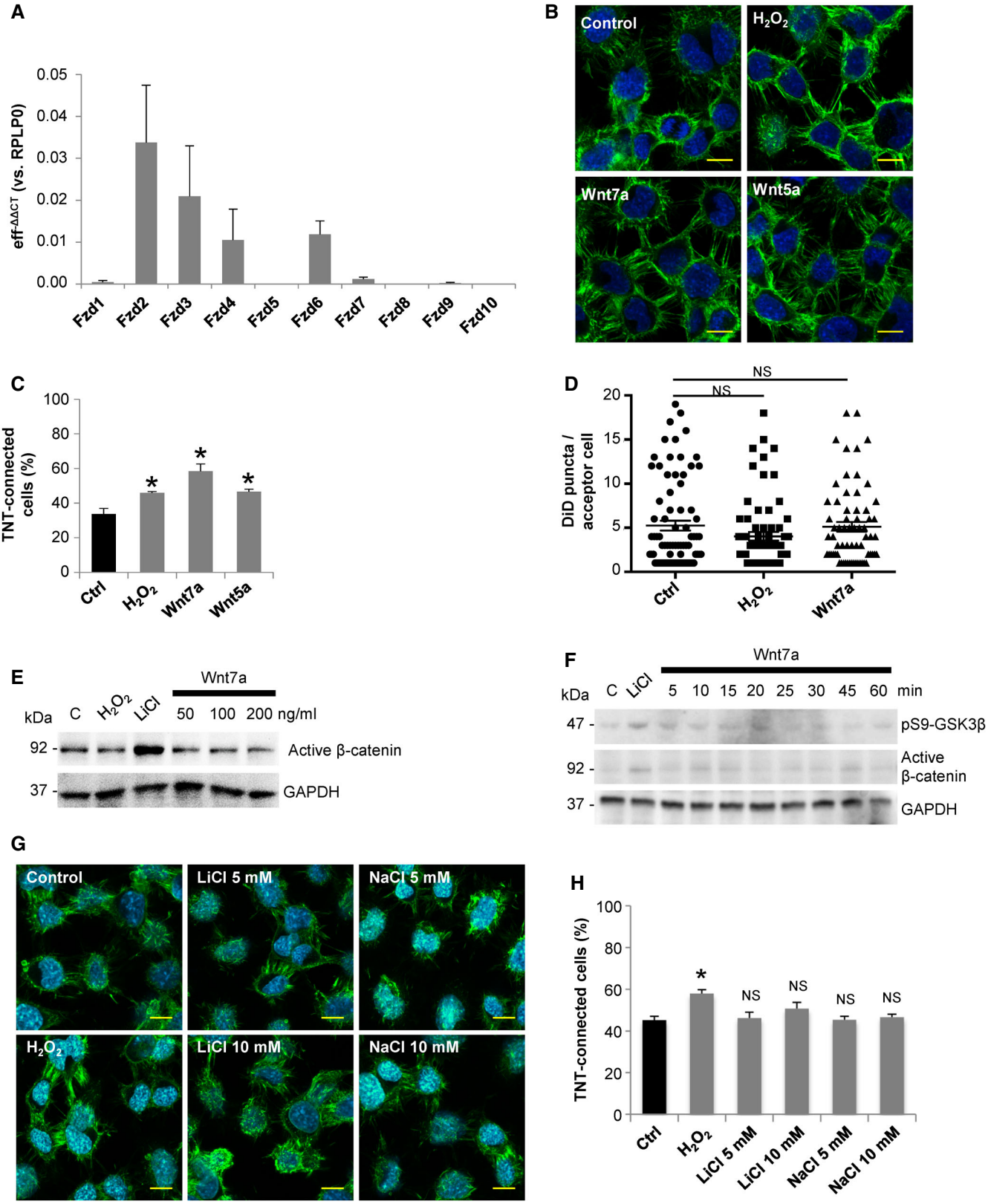
A
eff-ΔΔCT (vs. RPLP0)
0.05
0.04
0.03
0.02
0.01
0.00
Fzd1
Fzd2
Fzd3
Fzd4
Fzd5
Fzd6
Fzd7
Fzd8
Fzd9
Fzd10
B
Control
H2O2
Wnt7a
Wnt5a
C
TNT-connected cells (%)
100
80
60
40
20
0
Ctrl
H2O2
Wnt7a
Wnt5a
D
NS
NS
DiD puncta / acceptor cell
20
15
10
5
0
Ctrl
H2O2
Wnt7a
E
Wnt7a
kDa
C
H2O2
LiCl
50
100
200 ng/ml
92
Active β-catenin
37
GAPDH
F
Wnt7a
kDa
C
LiCl
5
10
15
20
25
30
45
60
min
47
pS9-GSK3β
92
Active β-catenin
37
GAPDH
G
Control
LiCl 5 mM
NaCl 5 mM
H2O2
LiCl 10 mM
NaCl 10 mM
H
TNT-connected cells (%)
100
80
60
40
20
0
NS
NS
NS
NS
Ctrl
H2O2
LiCl 5 mM
LiCl 10 mM
NaCl 5 mM
NaCl 10 mM

Figure EV1.

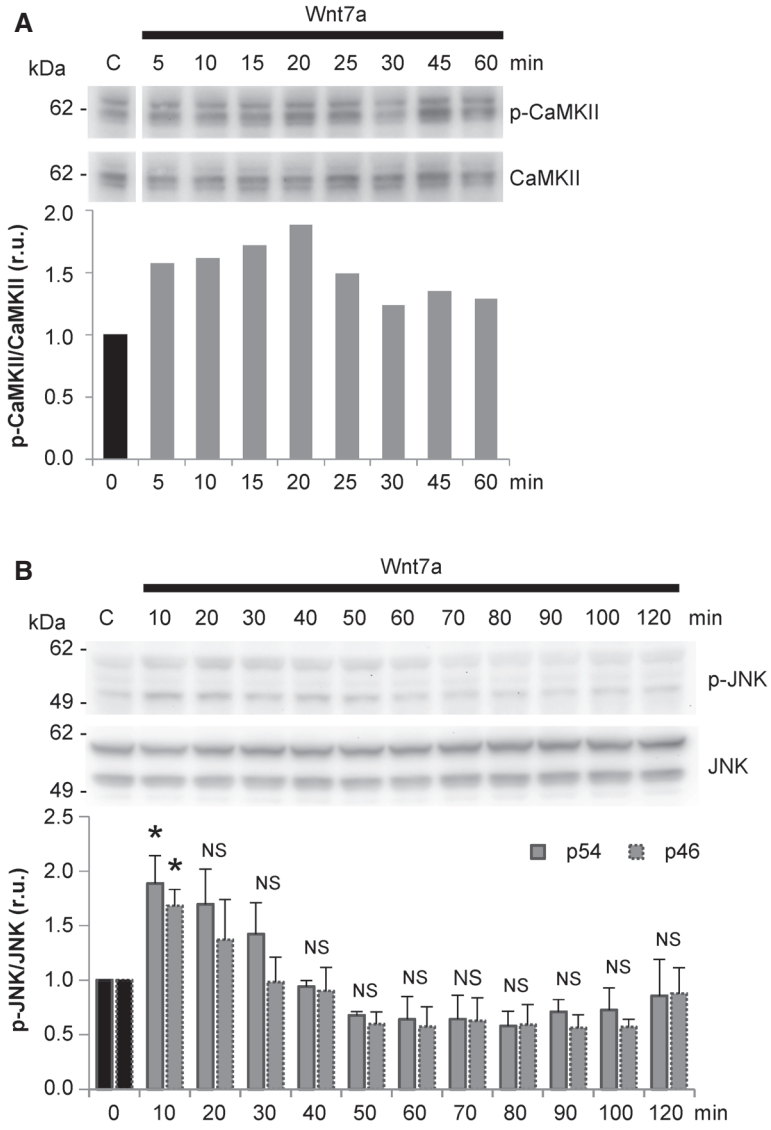

Figure EV2. Wnt7a induces the activation of Wnt/β-catenin-independent pathways in CAD cells.

A Western blot analysis of CaMKII and p-CaMKII in CAD cells treated in the presence or absence of Wnt7a for the indicated time points. Immunoblot and bar graph show that 200 ng/ml Wnt7a activated CaMKII in CAD cells. The graph shows p-CaMKII/CaMKII values (r.u., relative units) for each time point normalized to time 0.

B Western blot analysis of JNK and p-JNK in CAD cells treated in the presence or absence of Wnt7a for the indicated time points. Immunoblot and bar graph show that 200 ng/ml Wnt7a activated JNK in CAD cells. The graph shows p-JNK/JNK values (r.u., relative units) for each time point normalized to time 0. The data are presented as the average of three independent experiments ± SEM. Statistical significance was calculated with respect to control (Ctrl); $*P \leq 0.05$, NS = not significant (one-way ANOVA).

Source data are available online for this figure.

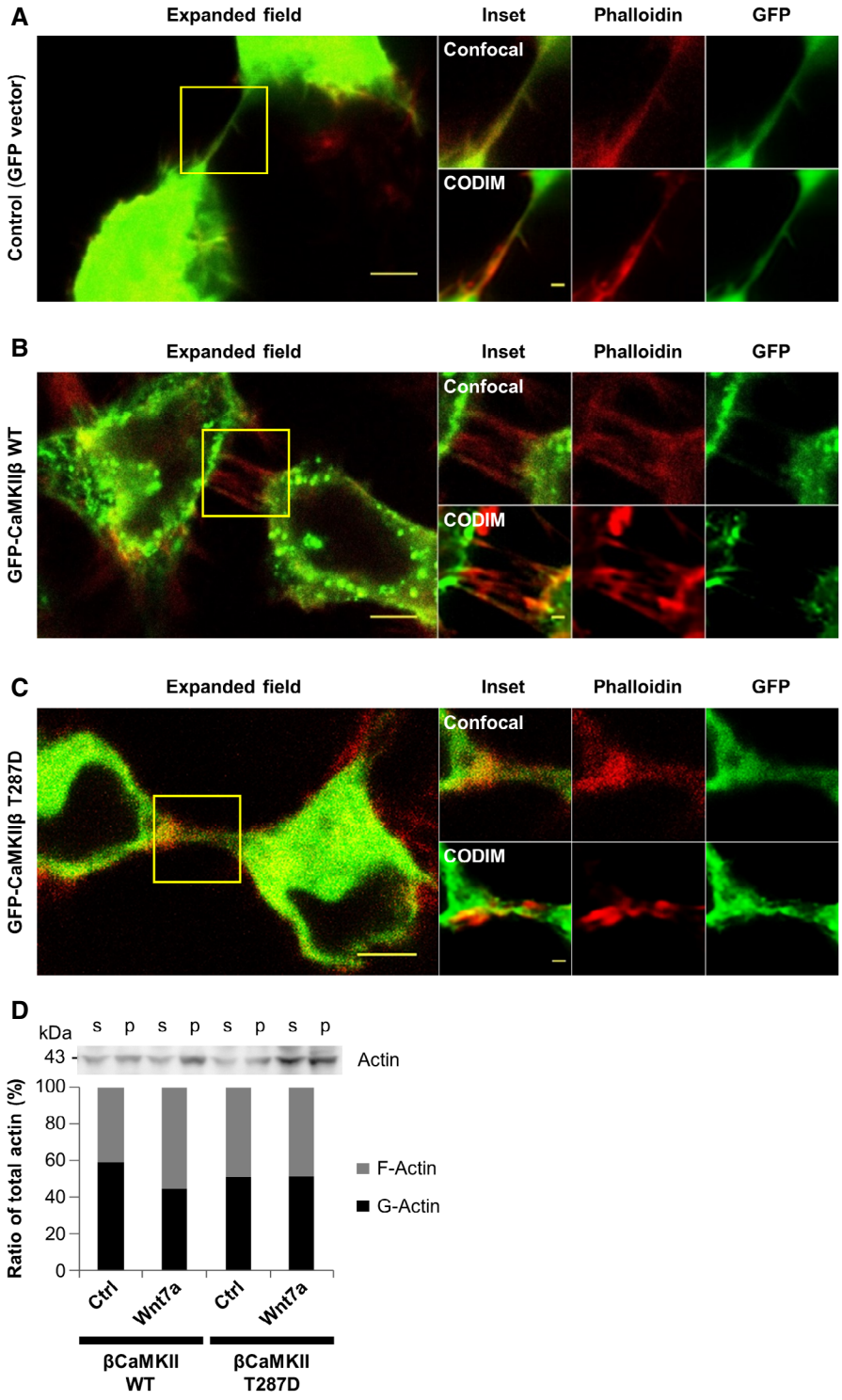

Figure EV3. Actin-binding activity of βCaMKII modulates actin dynamics.

A–C Image resolution comparison between images obtained with CODIM super-resolution and confocal microscopy techniques. Confocal (3 upper panels at the right) and CODIM super-resolution images (3 bottom panels at the right) of the area depicted in the confocal expanded field images (left panels) of transfected cells (green) showing expression of GFP-vector (A), GFP-βCaMKII WT (B), or GFP-βCaMKII T287D (C). Cells were stained for F-actin with phalloidin (red). Note: CODIM images presented here are the same as those in Fig 4C. Scale bars in the expanded field represent 5 μm and 1 μm in the inset.

D Anti-actin immunoblot and bar graph showing changes in the ratio between G-actin (in supernatant fraction, s) and F-actin (in the pellet fraction, p) of CAD cells transfected with either GFP-βCaMKII WT or GFP- βCaMKII T287D plasmids and treated or not with 200 ng/ml Wnt7a.

Source data are available online for this figure.

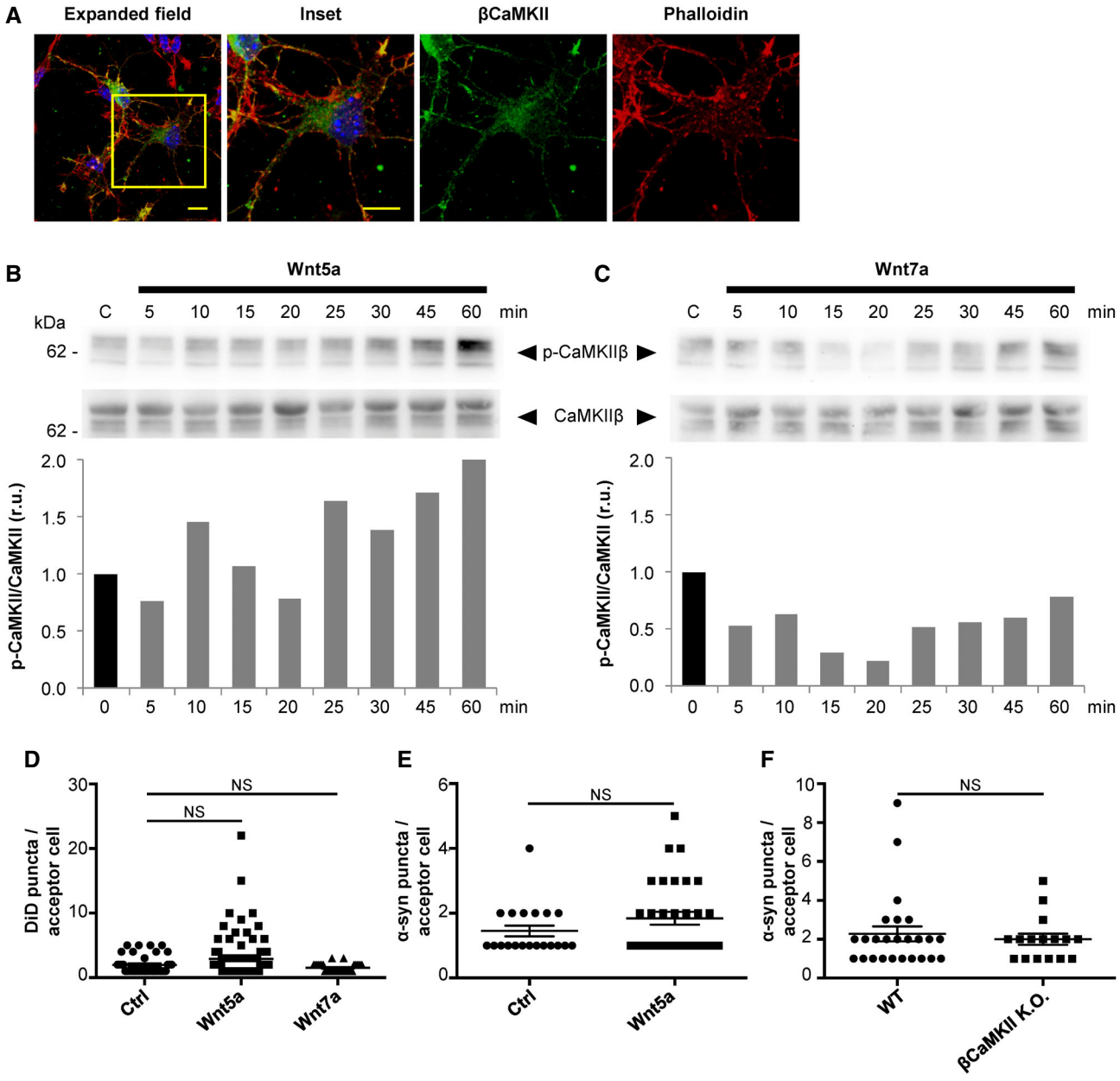

Figure EV4. Wnt5a, but not Wnt7a, activates CaMKII in cortical neurons.

A Confocal images representing the intracellular localization of endogenous βCaMKII in primary mouse cortical neurons of 1 DIV. Cells were also stained with DAPI (blue) and phalloidin (red). Scale bars represent 10 μm.

B, C Western blot analysis of CaMKII and p-CaMKII in CAD cells treated with Wnt5a (B) or Wnt7a (C) for the indicated time points. The graphs show p-CaMKII/CaMKII values (r.u., relative units) for each time point normalized to time 0.

D–F Average number of transferred vesicles or α-syn puncta per acceptor neurons for the indicated treatment or genotypes. Graphs represent the average of three independent experiments ± SEM. Statistical significance was calculated with respect to control (Ctrl); NS = not significant (one-way ANOVA for D and Student's *t*-test for E, F).

Source data are available online for this figure.