Expanded View Figures

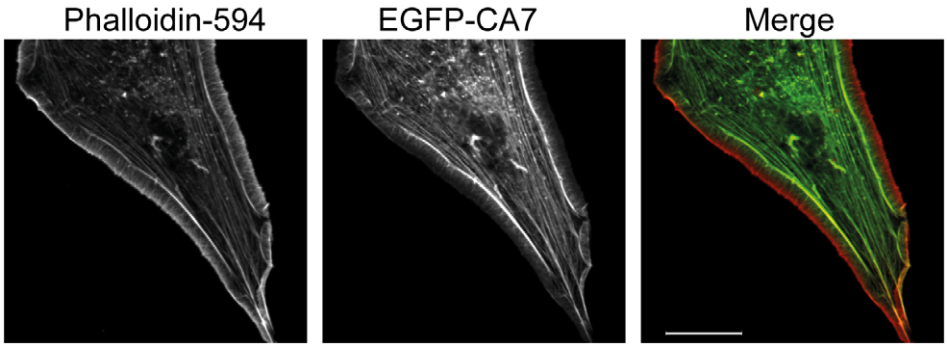

Figure EV1. CA7 co-localizes with selective actin filaments.

EGFP-CA7 co-localizes with subcellular F-actin structures (visualized with Phalloidin-594) except at the very edges of NIH3T3 fibroblast lamellipodia. Scale bar 20 μm.

Figure EV2. CA7 directly interacts with F-actin and enhances actin filament polymerization.

A, B Actin co-sedimentation assay was carried out at five different concentrations of β/γ-actin and with 1 μM (A) CA7 or (B) CA2 at two different pH (7.4 or 6.5). After centrifugation, the supernatant (S) and pellet (P) fractions were separated and resolved by SDS-PAGE. Staining the gels with Coomassie Blue showed that CA7 co-sedimented in the pellets with actin, whereas CA2 was found only in the supernatant fraction. CA7; three repetitions and CA2; one repetition at each of the four actin concentrations/pH.

C Analysis of the CA2 gels confirmed that the isoform does not interact with actin at either pH tested.

D In the presence of mCA7, existing filaments assemble thus increasing elongation velocity in a stepwise manner. Fluorescence time-lapse images of F-actin bundling in the *in vitro* bundling assay. A mixture of unlabeled and Rhodamine labeled non-muscle actin was polymerized in the absence (PBS control, upper row) or in the presence of mCA7 (1.12 μM, lower row). Numbers above images indicate the time after the onset of the experiment (images every 10 s) (First frame 200 s = 3 min 20 s, last frame 500 = 8 min 20 s). The filament/bundle ends that were followed over the experiment are indicated with white arrows. Intensity-based fire-coloring (Fiji) was used. Scale bar 5 μm.

E Quantification of the filament/bundle length for shown frames. Measured lengths were plotted to Excel x = time in seconds, y = length in μm.

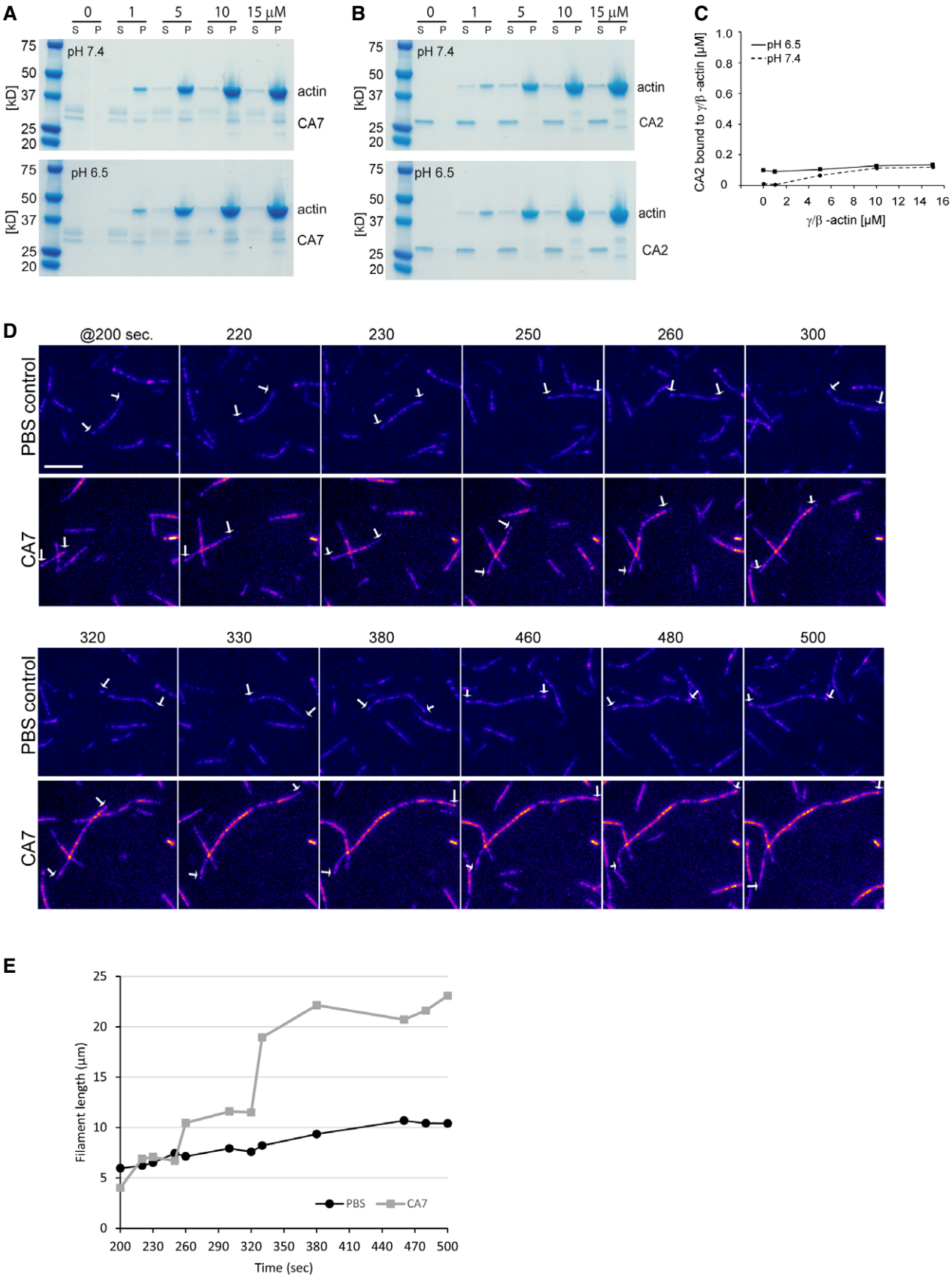
A
0 1 5 10 15 µM
S P S P S P S P S P
75
50
37
25
20
[kD]
pH 7.4
actin
CA7
pH 6.5
actin
CA7
B
0 1 5 10 15 µM
S P S P S P S P S P
75
50
37
25
20
[kD]
pH 7.4
actin
CA2
pH 6.5
actin
CA2
C
pH 6.5
pH 7.4
CA2 bound to γ/β -actin [µM]
1.0
0.8
0.6
0.4
0.2
0
0 2 4 6 8 10 12 14 16
γ/β -actin [µM]
D
@200 sec.
220
230
250
260
300
PBS control
CA7
320
330
380
460
480
500
PBS control
CA7
E
Filament length (µm)
25
20
15
10
5
0
PBS
CA7
200 230 260 290 320 350 380 410 440 470 500
Time (sec)

Figure EV2.

Figure EV3. The identified actin interaction sites are unique to CA7 among human cytosolic CAs.

Sequence alignment of the catalytically active (CA1, CA2, CA3, CA5A, CA5B, CA7, and CA13) and catalytically inactive (CA8 and CA10) human cytosolic CA protein sequences generated using the Clustal O (1.2.1) multiple sequence alignment. The amino acids that were characterized as part of a putative actin-binding site of CA7 (highlighted in red) are not conserved in the other cytosolic CA isoforms (highlighted in gray). An asterisk below the aligned sequences indicates fully conserved residues, a colon indicates residues with strongly similar properties (scoring > 0.5 in the Gonnet PAM 250 matrix) and a period indicates residues with weakly similar properties (scoring ≤ 0.5 in the Gonnet PAM 250 matrix).

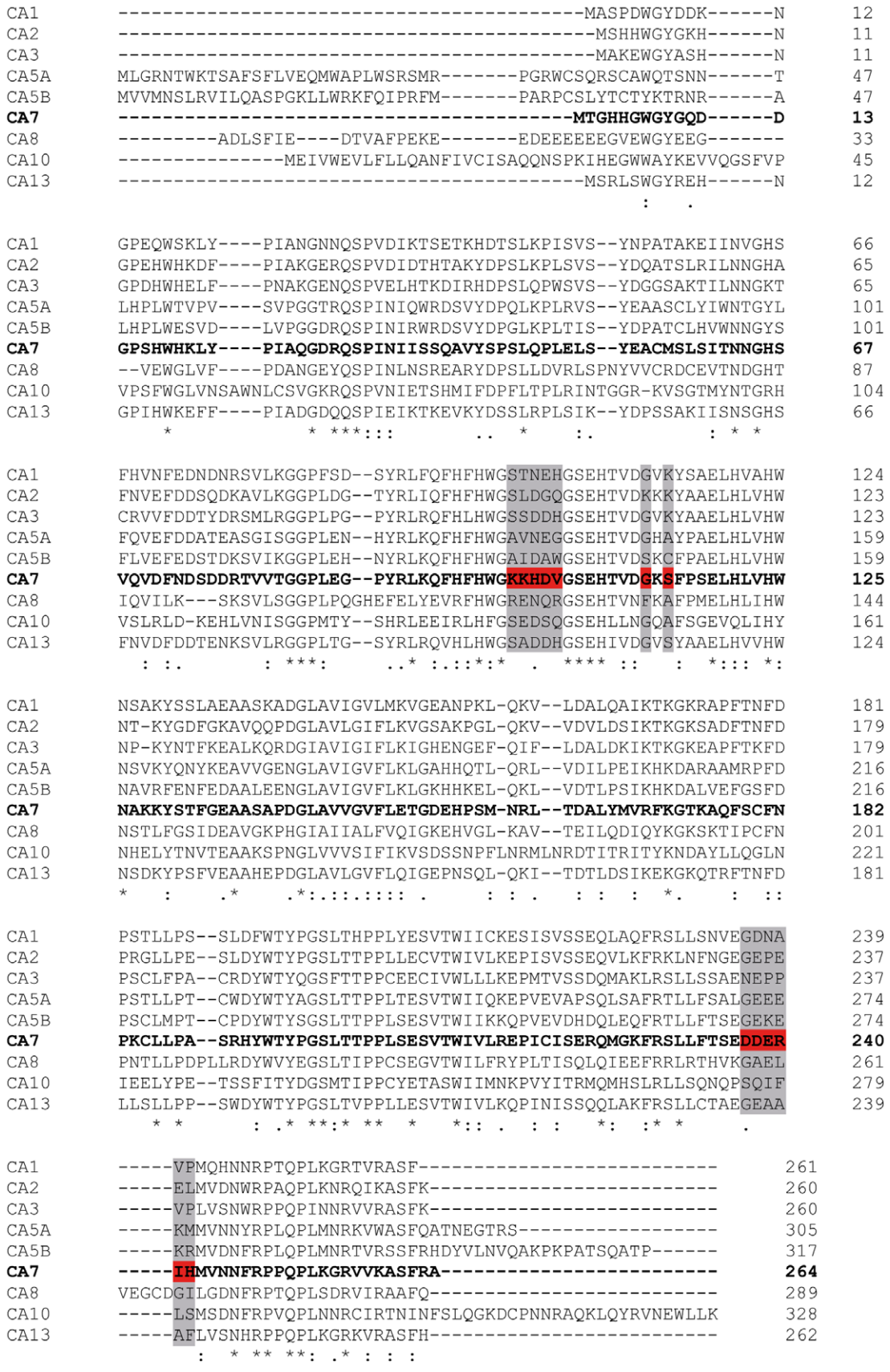
CA1 ----------MASPDWGYDDK------N 12
CA2 ----------MSHHWGYGKH------N 11
CA3 ----------MAKEWGYASH------N 11
CA5A MLGRNTWKTSAFSFLVEQMWAPLWSRSMR-------PGRWCSQRSCAWQTSNN------T 47
CA5B MVVMNSLRVILQASPGKLLWRKFQIPRFM-------PARPCSLYTCTYKTRNR------A 47
CA7 ----------MTGHHGWGYGQD------D 13
CA8 ---------ADLSFIE----DTVAFPEKE-------EDEEEEEEGVEWGYEEG------- 33
CA10 --------------MEIVWEVLFLLQANFIVCISAQQNSPKIHEGWWAYKEVVQGSFVP 45
CA13 ----------MSRLSWGYREH------N 12
CA1 GPEQWSKLY----PIANGNNQSPVDIKTSETKHDTSLKPISVS--YNPATAKEIINVGHS 66
CA2 GPEHWHKDF----PIAKGERQSPVDIDTHTAKYDPSLKPLSVS--YDQATSLRILNNGHA 65
CA3 GPDHWHELF----PNAKGENQSPVELHTKDIRHDPSLQPWSVS--YDGGSAKTILNNGKT 65
CA5A LHPLWTVPV----SVPGGTRQSPINIQWRDSVYDPQLKPLRVS--YEAASCLYIWNTGYL 101
CA5B LHPLWESVD----LVPGGDRQSPINIRWRDSVYDPGLKPLTIS--YDPATCLHVWNNGYS 101
CA7 GPSHWHKLY----PIAQGDRQSPINIISSQAVYSPSLQPLELS--YEACMSLSITNNGHS 67
CA8 --VEWGLVF----PDANGEYQSPINLNSREARYDPSLLDVRLSPNYVVCRDCEVTNDGHT 87
CA10 VPSFWGLVNSAWNLCSVGKRQSPVNIETSHMIFDPFLTPLRINTGGR-KVSGTMYNTGRH 104
CA13 GPIHWKEFF----PIADGDQQSPIEIKTKEVKYDSSLRPLSIK--YDPSSAKIISNSGHS 66
CA1 FHVNFEDNDNRSVLKGGPFSD--SYRLFQFHFHWGSTNEHGSEHTVDGVKYSAELHVAHW 124
CA2 FNVEFDDSQDKAVLKGGPLDG--TYRLIQFHFHWGSLDGQGSEHTVDKKKYAAELHLVHW 123
CA3 CRVVFDDTYDRSMLRGGPLPG--PYRLRQFHLHWGSSDDHGSEHTVDGVKYAAELHLVHW 123
CA5A FQVEFDDATEASGISGGPLEN--HYRLKQFHFHWGAVNEGGSEHTVDGHAYPAELHLVHW 159
CA5B FLVEFEDSTDKSVIKGGPLEH--NYRLKQFHFHWGAIDAWGSEHTVDSKCFPAELHLVHW 159
CA7 VQVDFNDSDDRTVVTGGPLEG--PYRLKQFHFHWGKKHDVGSEHTVDGKSFPSELHLVHW 125
CA8 IQVILK---SKSVLSGGPLPQGHEFELYEVRFHWGRENQRGSEHTVNFKAFPMELHLIHW 144
CA10 VSLRLD-KEHLVNISGGPMTY--SHRLEEIRLHFGSEDSQGSEHLLNGQAFSGEVQLIHY 161
CA13 FNVDFDDTENKSVLRGGPLTG--SYRLRQVHLHWGSADDHGSEHIVDGVSYAAELHVVHW 124
CA1 NSAKYSSLAEAASKADGLAVIGVLMKVGEANPKL-QKV--LDALQAIKTKGKRAPFTNFD 181
CA2 NT-KYGDFGKAVQQPDGLAVLGIFLKVGSAKPGL-QKV--VDVLDSIKTKGKSADFTNFD 179
CA3 NP-KYNTFKEALKQRDGIAVIGIFLKIGHENGEF-QIF--LDALDKIKTKGKEAPFTKFD 179
CA5A NSVKYQNYKEAVVGENGLAVIGVFLKLGAHHQTL-QRL--VDILPEIKHKDARAAMRPFD 216
CA5B NAVRFENFEDAALEENGLAVIGVFLKLGKHHKEL-QKL--VDTLPSIKHKDALVEFGSFD 216
CA7 NAKKYSTFGEAASAPDGLAVVGVFLETGDEHPSM-NRL--TDALYMVRFKGTKAQFSCFN 182
CA8 NSTLFGSIDEAVGKPHGIAIIALFVQIGKEHVGL-KAV--TEILQDIQYKGKSKTIPCFN 201
CA10 NHELYTNVTEAAKSPNGLVVVSIFIKVSDSSNPFLNRMLNRDTITRITYKNDAYLLQGLN 221
CA13 NSDKYPSFVEAAHEPDGLAVLGVFLQIGEPNSQL-QKI--TDTLDSIKEKGKQTRFTNFD 181
CA1 PSTLLPS--SLDFWTYPGSLTHPPLYESVTWIICKESISVSSEQLAQFRSLLSNVEGDNA 239
CA2 PRGLLPE--SLDYWTYPGSLTTPPLLECVTWIVLKEPISVSSEQVLKFRKLNFNGEGEPE 237
CA3 PSCLFPA--CRDYWTYQGSFTTPPCEECIVWLLLKEPMTVSSDQMAKLRSLLSSAENEPP 237
CA5A PSTLLPT--CWDYWTYAGSLTTPPLTESVTWIIQKEPVEVAPSQLSAFRTLLFSALGEEE 274
CA5B PSCLMPT--CPDYWTYSGSLTTPPLSESVTWIIKKQPVEVDHDQLEQFRTLLFTSEGEKE 274
CA7 PKCLLPA--SRHYWTYPGSLTTPPLSESVTWIVLREPICISERQMGKFRSLLFTSEDDER 240
CA8 PNTLLPDPLLRDYWVYEGSLTIPPCSEGVTWILFRYPLTISQLQIEEFRRLRTHVKGAEL 261
CA10 IEELYPE--TSSFITYDGSMTIPPCYETASWIIMNKPVYITRMQMHSLRLLSQNQPSQIF 279
CA13 LLSLLPP--SWDYWTYPGSLTVPPLLESVTWIVLKQPINISSQQLAKFRSLLCTAEGEAA 239
CA1 -----VPMQHNNRPTQPLKGRTVRASF---------- 261
CA2 -----ELMVDNWRPAQPLKNRQIKASFK---------- 260
CA3 -----VPLVSNWRPPQPINNRVVRASFK---------- 260
CA5A -----KMMVNNYRPLQPLMNRKVWASFQATNEGTRS---------- 305
CA5B -----KRMVDNFRPLQPLMNRTVRSSFRHDYVLNVQAKPKPATSQATP------ 317
CA7 -----IHMVNNFRPPQPLKGRVVKASFRA---------- 264
CA8 VEGCDGILGDNFRPTQPLSDRVIRAAFQ---------- 289
CA10 -----LSMSDNFRPVQPLNNRCIRTNINFSLQGKDCPNNRAQKLQYRVNEWLLK 328
CA13 -----AFLVSNHRPPQPLKGRKVRASFH---------- 262

Figure EV3.

Figure EV4. Subcellular localization and expression levels of the chimeric CA fusion proteins EGFP-CA7-mutant2, EGFP-CA2-revCA7, EGFP-CA7-R223E, and EGFP-CA7-H96/98C in fibroblasts.

A–G (A) Transfection with EGFP-CA7-mutant2 modified cellular F-actin structures in a similar manner than CA7. (B) Introduction of KKHDV and DDERIH motifs to CA2 (EGFP-CA2-revCA7) did not affect the diffuse cytosolic localization of the isoform 2. (C) EGFP-CA7-R223E and (D) the catalytically loss-of-function mutant EGFP-CA7-H96/98C co-localized with F-actin. F-actin is visualized with Phalloidin-594 in (A–D). The normalized fluorescence emission intensity profiles of (E) EGFP-CA2-revCA7 (F) EGFP-CA7-R223E, and (G) EGFP-CA7-H96/98C (black lines) and F-actin (red line). The yellow line in (B–D) indicates the cross-section of the cell from which the pixel intensities were measured. Analysis of co-localization is shown in lower panels of (E–G). Representative single-cell pixel intensities of EGFP and phalloidin-594 channels were plotted against each other and the Pearson's correlation coefficient value (r) was calculated. $n = 3$–4 independent transfections/construct. Scale bars in (A–D) is 20 μm.

H A representative Western blot showing the expression levels of EGFP-CA fusion proteins in 10 μg of lysate collected 24 h after transfection. The EGFP-tagged CA fusion proteins are visible at approximately 60 kDa, and the β-actin loading control is visible at 42 kDa.

I Quantification of the fusion proteins expression levels in NIH3T3 cells. Expression level of EGFP-CA7 was set at 1 for each Western blot. $n = 5$ independent transfections for all fusion proteins except for CA7-mutant2, for which $n = 4$ (data passed Shapiro–Wilk test for normality). Transfections' efficacy of different mutant fusion proteins was compared to CA7 WT using one-way ANOVA with Dunnett's multiple comparisons test. Data are given as mean + SEM.

Source data are available online for this figure.

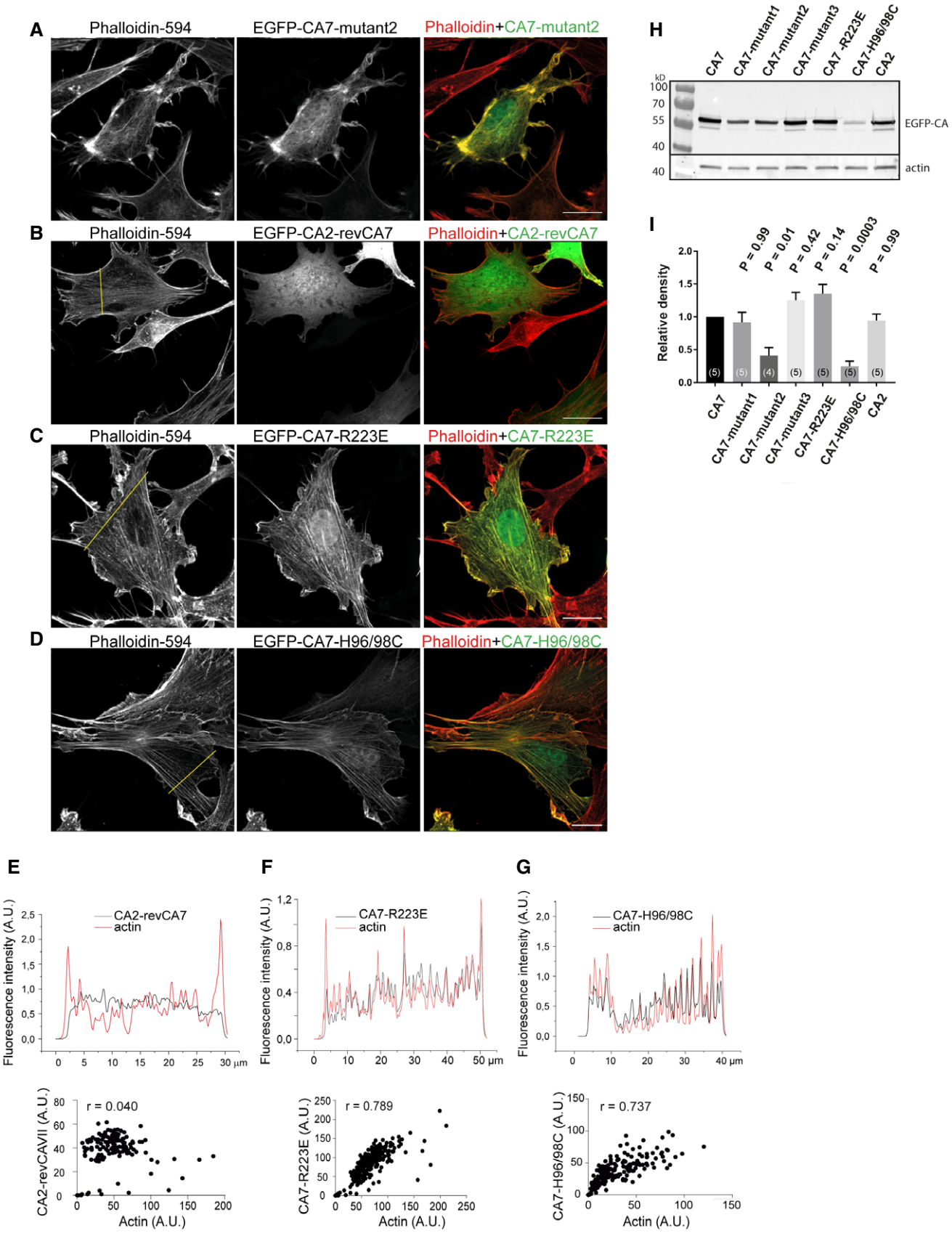
A
Phalloidin-594
EGFP-CA7-mutant2
Phalloidin+CA7-mutant2
B
Phalloidin-594
EGFP-CA2-revCA7
Phalloidin+CA2-revCA7
C
Phalloidin-594
EGFP-CA7-R223E
Phalloidin+CA7-R223E
D
Phalloidin-594
EGFP-CA7-H96/98C
Phalloidin+CA7-H96/98C
H
CA7
CA7-mutant1
CA7-mutant2
CA7-mutant3
CA7 -R223E
CA7-H96/98C
CA2
kD
100
70
55
40
40
EGFP-CA
actin
I
Relative density
P = 0.99
P = 0.01
P = 0.42
P = 0.14
P = 0.0003
P = 0.99
(5)
(5)
(4)
(5)
(5)
(5)
(5)
CA7
CA7-mutant1
CA7-mutant2
CA7-mutant3
CA7-R223E
CA7-H96/98C
CA2
E
CA2-revCA7
actin
Fluorescence intensity (A.U.)
30 µm
CA2-revCAVII (A.U.)
r = 0.040
Actin (A.U.)
F
CA7-R223E
actin
Fluorescence intensity (A.U.)
50 µm
CA7-R223E (A.U.)
r = 0.789
Actin (A.U.)
G
CA7-H96/98C
actin
Fluorescence intensity (A.U.)
40 µm
CA7-H96/98C (A.U.)
r = 0.737
Actin (A.U.)

Figure EV4.

Figure EV5. Localization of WT and chimeric CA EGFP-fusion proteins and their effect on dendritic spines in cultured hippocampal neurons.

A–H (A) Control experiment with neurons co-expressing mCherry-actin and EGFP. (B) Neuron transfected with mCherry-actin and EGFP-CA2. Compared to the spine-targeted mCherry-actin, CA2 localizes more diffusely along dendritic shafts and spines. Both (C) EGFP-CA7 and (D) EGFP-CA7-mutant2 show a highly overlapping localization with mCherry-actin and disruption of dendritic spine morphology. Spines were replaced by thick, filopodia-like dendritic protrusions, which lack spine heads. The loss-of-function constructs (E) EGFP-CA7-mutant3 and (F) EGFP-CA7-mutant1 are more homogenously present in both dendrites and spines. (G) EGFP-CA7-R223E and (H) the catalytically inactive EGFP-CA7-H96/98C showed overlapping localization with mCherry-actin. Scale bar 5 μm (A–C, G, H), 10 μm (D–F).

I EGFP-CA7 expression disrupted normal spine morphology in cultured neurons. Control, only mCherry-actin: spines with head 0.34 ± 0.04, thin spines/filopodia 0.21 ± 0.02, abnormal spines 0.00 ± 0.00, total 0.54 ± 0.05 spines/μm; $n = 10$ cells, 509 spines, 973 μm analyzed dendrite. EGFP-CA2: spines with head 0.35 ± 0.04, thin spines/filopodia 0.17 ± 0.02, abnormal spines 0.00 ± 0.00, total 0.53 ± 0.06 spines/μm; $n = 10$ cells, 535 spines, 992 μm analyzed dendrite; EGFP-CA7: spines with head 0.05 ± 0.02, thin spines /filopodia 0.10 ± 0.02, abnormal spines 0.34 ± 0.03, total 0.49 ± 0.05 spines/μm; $n = 10$ cells, 493 spines, 10,256 μm dendrite. Analyzed cells were pooled from two independent experiments. The bar diagrams show the mean.

Source data are available online for this figure.

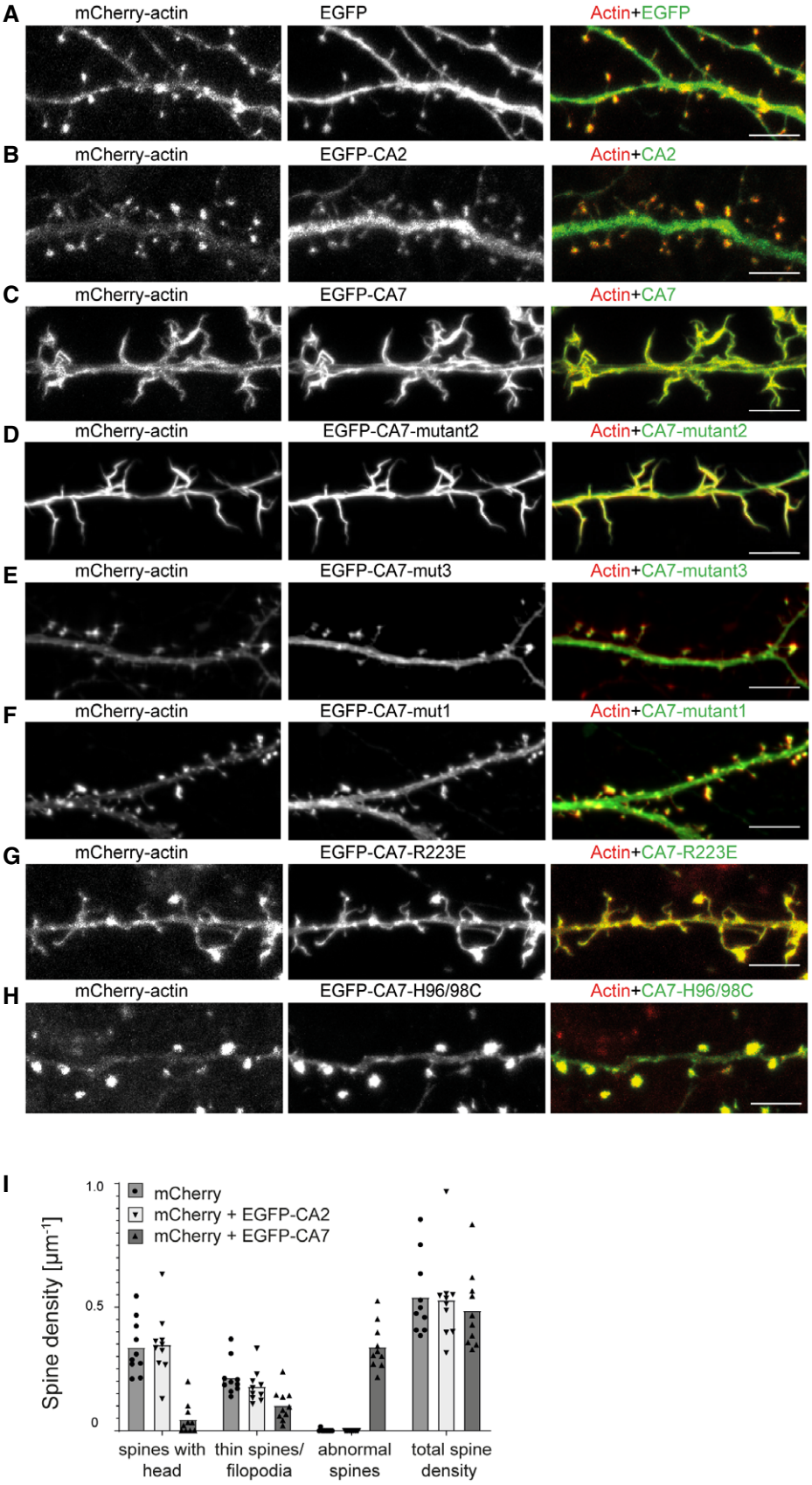
A
mCherry-actin
EGFP
Actin+EGFP
B
mCherry-actin
EGFP-CA2
Actin+CA2
C
mCherry-actin
EGFP-CA7
Actin+CA7
D
mCherry-actin
EGFP-CA7-mutant2
Actin+CA7-mutant2
E
mCherry-actin
EGFP-CA7-mut3
Actin+CA7-mutant3
F
mCherry-actin
EGFP-CA7-mut1
Actin+CA7-mutant1
G
mCherry-actin
EGFP-CA7-R223E
Actin+CA7-R223E
H
mCherry-actin
EGFP-CA7-H96/98C
Actin+CA7-H96/98C
I
mCherry
mCherry + EGFP-CA2
mCherry + EGFP-CA7
Spine density [µm-1]
1.0
0.5
0
spines with head
thin spines/ filopodia
abnormal spines
total spine density

Figure EV5.