

Expanded View Figures

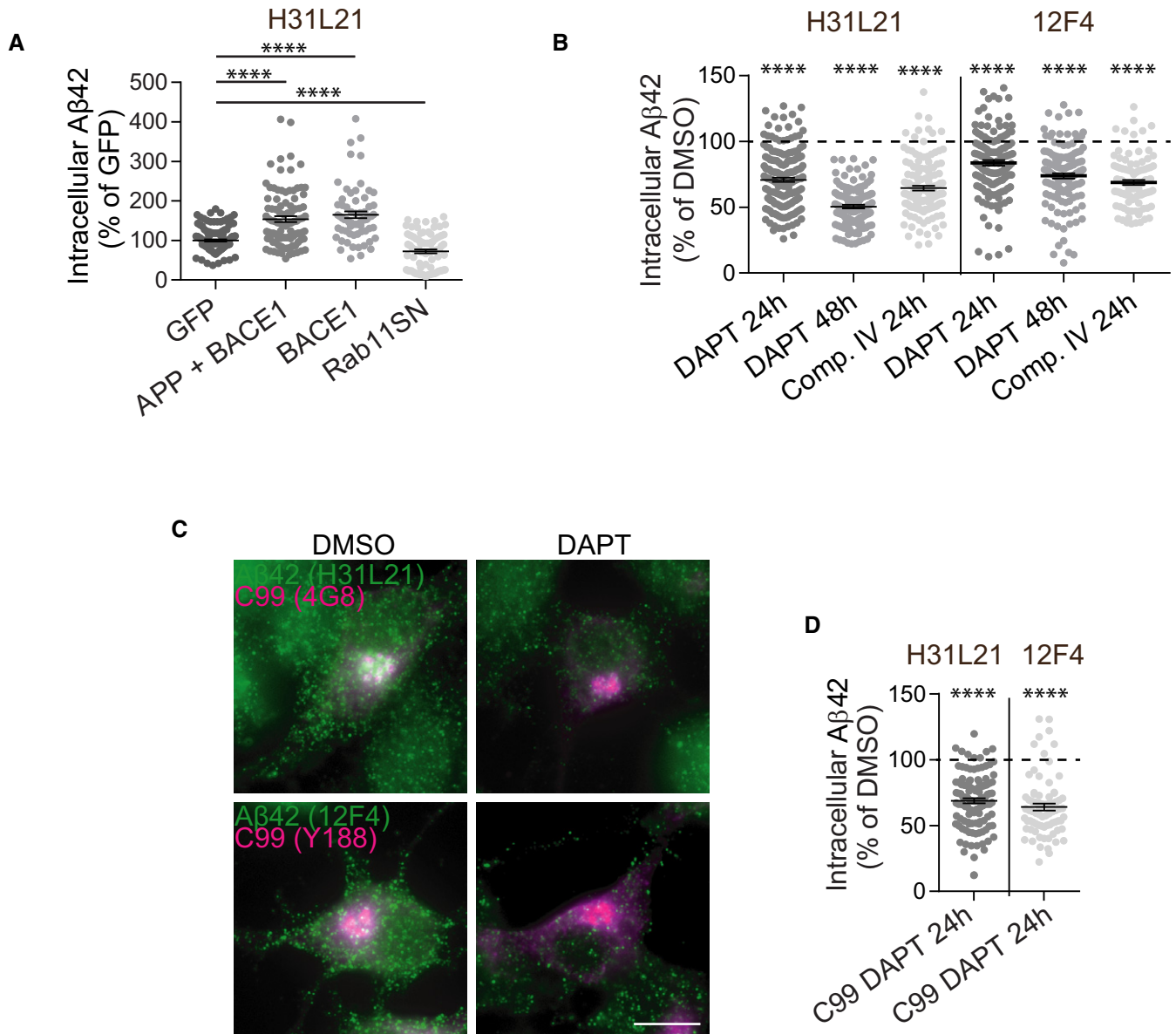


Figure EV1. Validation of intracellular endogenous A β 42 semi-quantitative assay.

- A** Intracellular endogenous A β 42 changes positively induced by overexpressing APP-RFP and BACE1-GFP or BACE1-GFP alone; or negatively induced by overexpressing Rab11SN, a dominant-negative mutant of Rab11 [89] in N2a cells immunolabelled with anti-A β 42 (clone H31L21). Single-cell semi-quantitative analysis of A β 42 immunofluorescence normalised to A β 42 fluorescence intensity in GFP-transfected cells ($n = 3-5$, $N_{\text{GFP}} = 133$, $N_{\text{APP + BACE1}} = 98$, $N_{\text{BACE1}} = 65$, $N_{\text{Rab11SN}} = 101$; **** $P < 0.0001$ vs. GFP, Mann-Whitney test, mean \pm SEM).
- B** Intracellular endogenous A β 42 changes upon the indicated treatment with inhibitors of A β generation, DAPT that blocks γ -secretase [90], or compound IV that blocks BACE1 [91], or their vehicle DMSO in N2a cells immunolabelled with anti-A β 42 (clones H31L21 or 12F4). Single-cell semi-quantitative analysis of A β 42 immunofluorescence normalised to A β 42 fluorescence intensity in cells treated with DMSO ($n = 2-3$, H31L21: $N_{\text{DMSO 24 h}} = 195$, $N_{\text{DMSO 48 h}} = 145$, $N_{\text{DAPT 24 h}} = 178$, $N_{\text{DAPT 48 h}} = 128$, $N_{\text{Comp. IV}} = 141$, 12F4: $N_{\text{DMSO 24 h}} = 243$, $N_{\text{DMSO 48 h}} = 138$, $N_{\text{DAPT 24 h}} = 139$, $N_{\text{DAPT 48 h}} = 128$, $N_{\text{Comp. IV}} = 97$; **** $P < 0.0001$ vs. DMSO, t -test, mean \pm SEM).
- C** Intracellular endogenous A β 42 (green) and C99 (magenta) in N2a cells expressing C99 treated with DAPT or its vehicle DMSO, immunolabelled with anti-A β 42 (clones H31L21 or 12F4 as indicated) and with anti-C-terminal APP (Y188 or 4G8), analysed by epifluorescence microscopy. Scale bar, 10 μm .
- D** Intracellular endogenous A β 42 changes upon expression of C99 and treatment with DAPT or its vehicle DMSO in N2a cells immunolabelled with anti-A β 42 (clones H31L21 or 12F4). Single-cell semi-quantitative analysis of A β 42 immunofluorescence normalised to A β 42 fluorescence intensity in cells treated with DMSO ($n = 3$, H31L21: $N_{\text{C99 + DMSO}} = 70$, $N_{\text{C99 + DAPT}} = 116$, 12F4: $N_{\text{C99 + DMSO}} = 84$, $N_{\text{C99 + DAPT}} = 80$; **** $P < 0.0001$ vs. C99 + DMSO, t -test, mean \pm SEM).

Figure EV2. CD2AP and Bin1 knockdown efficiency, intracellular A β and APP-CTFs levels upon DAPT treatment.

- A Bin1 and CD2AP knockdown efficiency in siBin1-, siCD2AP- and siControl-treated neurons by Western blot analysis with anti-Bin1, anti-CD2AP and anti-tubulin as a loading control. The quantification of Bin1 and CD2AP levels normalised to tubulin levels is shown on the right ($n_{\text{siBin1}} = 5$, **** $P < 0.0001$, t -test, mean \pm SEM; $n_{\text{siCD2AP}} = 3$, ** $P = 0.075$, t -test, mean \pm SEM).
- B Bin1 and CD2AP knockdown efficiency in siBin1-, siCD2AP- and siControl-treated N2a cells by Western blot analysis with anti-Bin1, anti-CD2AP and anti-tubulin as a loading control. Quantification of Bin1 and CD2AP levels normalised to tubulin levels is shown on the right ($n_{\text{siBin1}} = 3$; * $P = 0.0137$, t -test, mean \pm SEM; $n_{\text{siCD2AP}} = 3$; ** $P = 0.0028$, t -test, mean \pm SEM).
- C Intracellular endogenous A β 42 immunolabelled with anti-A β 42 (clone H31L21) in N2a cells treated with siBin1, siCD2AP or siControl alone or upon expression of the siRNA-resistant plasmids: CD2AP-GFP, neuronal Bin1 or ubiquitous Bin1 (right insets), analysed by epifluorescence microscopy. Scale bars, 10 μm . The quantification of A β 42 intensity per cell is shown on the right ($n = 3$, $N_{\text{siControl}} = 197$, $N_{\text{siCD2AP}} = 208$, $N_{\text{siCD2AP} + \text{CD2AP-GFP}} = 170$, $N_{\text{siBin1}} = 186$, $N_{\text{siBin1} + \text{neuronal Bin1}} = 117$, $N_{\text{siBin1} + \text{ubiquitous Bin1}} = 203$; **** $P < 0.0001$ siCD2AP vs. siControl, siBin1 vs. siControl and siBin1 + ubiquitous Bin1 vs. siControl, ##### $P < 0.0001$ siCD2AP + CD2AP-GFP vs. siCD2AP and siBin1 vs. siBin1 + neuronal Bin1 and siBin1 + ubiquitous Bin1 vs. siBin1 + neuronal Bin1, one-way ANOVA with Tukey's test; mean \pm SEM).
- D Intracellular endogenous A β 40 in N2a cells treated with siBin1, siCD2AP or siControl, immunolabelled with anti-A β 40, analysed by epifluorescence microscopy. Scale bar, 10 μm . The quantification of A β 40 intensity per cell is shown on the right ($n = 3$, $N_{\text{siControl}} = 268$, $N_{\text{siBin1}} = 234$, $N_{\text{siCD2AP}} = 257$; **** $P < 0.0001$ siBin1 vs. siControl, t -test, mean \pm SEM).
- E Endogenous APP and APP C-terminal fragments (APP-CTFs) levels by Western blot analysis with anti-APP antibody (Y188) of siBin1-, siCD2AP- or siControl-treated neurons upon DAPT treatment. The graph on the right shows the quantification of APP-CTFs normalised to APP ($n = 4$; ** $P = 0.0057$ siBin1 vs. siControl, t -test, mean \pm SEM).
- F Endogenous nicastrin levels by Western blot analysis with anti-nicastrin antibody (PA1-758) and tubulin as a loading control in siBin1-, siCD2AP- or siControl-treated neurons. The quantification of nicastrin levels normalised to tubulin levels is shown on the right ($n = 3$, mean \pm SEM).

Source data are available online for this figure.

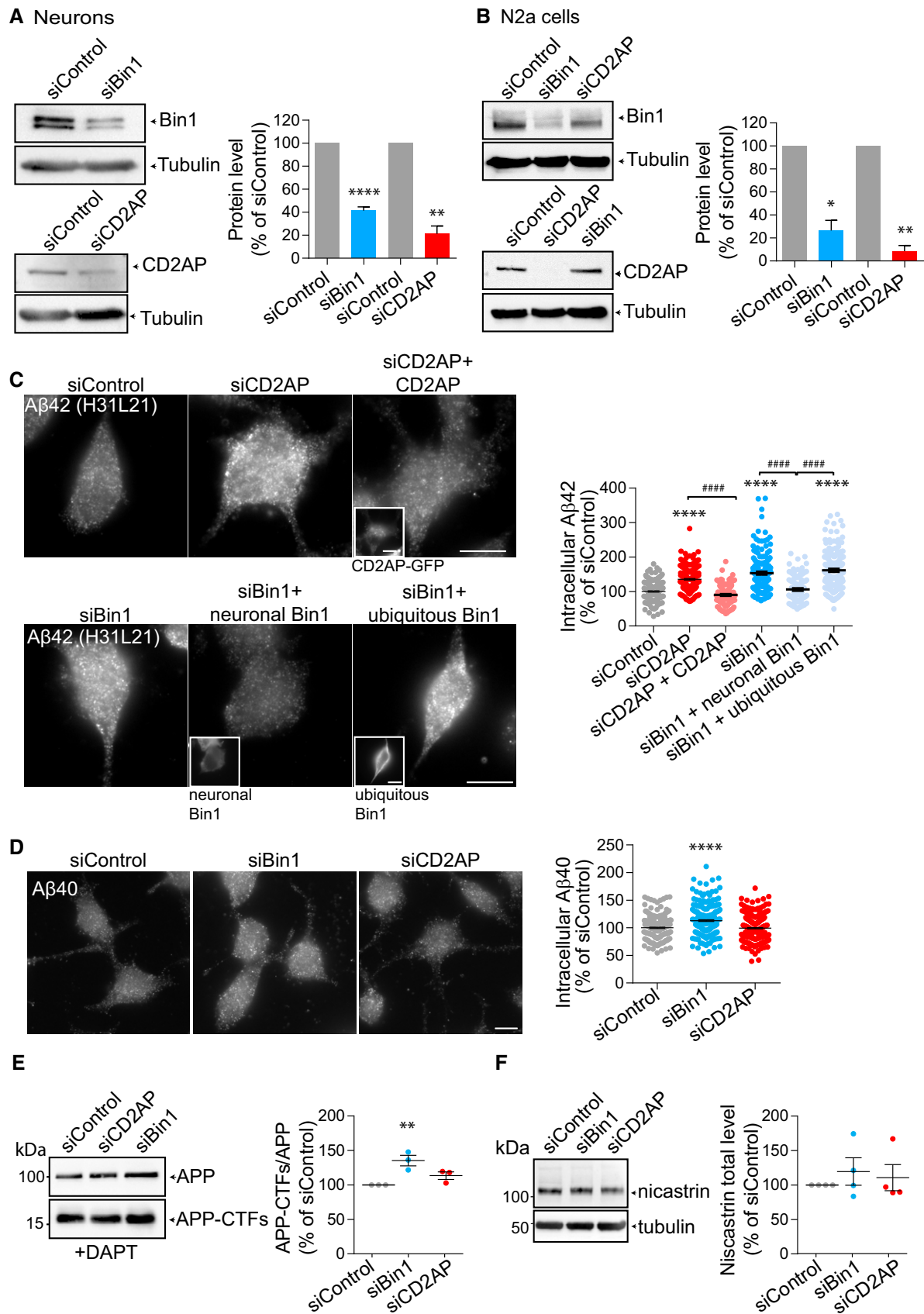


Figure EV2.

Figure EV3. APP trafficking assays upon CD2AP and Bin1 depletion.

N2a cells treated with siBin1, siCD2AP or siControl

- A Surface APP detected after a 4-min pulse with anti-N-terminal APP (22C11) and immunofluorescence of non-permeabilised N2a cells expressing APP-RFP (insets), analysed by epifluorescence microscopy. Scale bars, 10 μ m. The graph on the right shows the amount of cell surface APP fluorescence per cell quantified and normalised to APP-RFP fluorescence ($n = 4$, $N_{\text{siControl}} = 91$, $N_{\text{siBin1}} = 49$, $N_{\text{siCD2AP}} = 85$; mean \pm SEM).
- B Recycled APP detected with 22C11 at the plasma membrane of non-permeabilised cells expressing APP-RFP (insets), upon a 10-min pulse with 22C11, membrane acid stripping and 20-min chase, analysed by epifluorescence microscopy. Scale bars, 10 μ m. The graph on the right shows the amount of recycled APP fluorescence per cell quantified and normalised to APP-RFP fluorescence ($n = 4$, $N_{\text{siControl}} = 75$, $N_{\text{siBin1}} = 61$, $N_{\text{siCD2AP}} = 63$; mean \pm SEM).
- C Endocytosed APP detected with 22C11 upon 10-min pulse (left panels) and a 60-min chase (right panels) in DMSO- or leupeptin-treated N2a cells expressing APP-RFP (insets), analysed by epifluorescence microscopy. Scale bars, 10 μ m. The graph on the right shows APP degradation assessed by the decrease in the amount of endocytosed APP fluorescence at 60 min relative to time 0 (10-min pulse) in DMSO-treated cells normalised to APP-RFP fluorescence ($n = 3$, $N_{\text{DMSO 10 min}} = 111$, $N_{\text{DMSO 60 min}} = 109$, $N_{\text{Leu 10 min}} = 118$, $N_{\text{Leu 60 min}} = 99$, **** $P_{60 \text{ min}} < 0.0001$, t -test, mean \pm SEM).
- D APP levels by Western blot with anti-APP (Y188) of DMSO- or leupeptin-treated neurons at 11–12DIV. Quantification of APP levels normalised to tubulin levels is shown on the right ($n = 3$, * $P = 0.0296$ leupeptin vs. DMSO, t -test, mean \pm SEM).
- E Non-degraded APP detected with 22C11 (10-min pulse and 60-min chase) in N2a cells expressing APP-RFP (left insets) treated with siCD2AP, siControl alone or upon expression of siRNA-resistant CD2AP-GFP (right insets), analysed by epifluorescence microscopy. Scale bars, 10 μ m. The amount of endocytosed APP fluorescence per cell quantified and normalised to APP-RFP fluorescence is shown on the right ($n = 3$, $N_{\text{siControl}} = 109$, $N_{\text{siCD2AP}} = 82$, $N_{\text{siCD2AP + CD2AP-GFP}} = 83$; **** $P < 0.0001$ siCD2AP vs. siControl, **** $P < 0.0001$ siCD2AP+CD2AP-GFP vs. siCD2AP, one-way ANOVA with Tukey's test, mean \pm SEM).

Source data are available online for this figure.

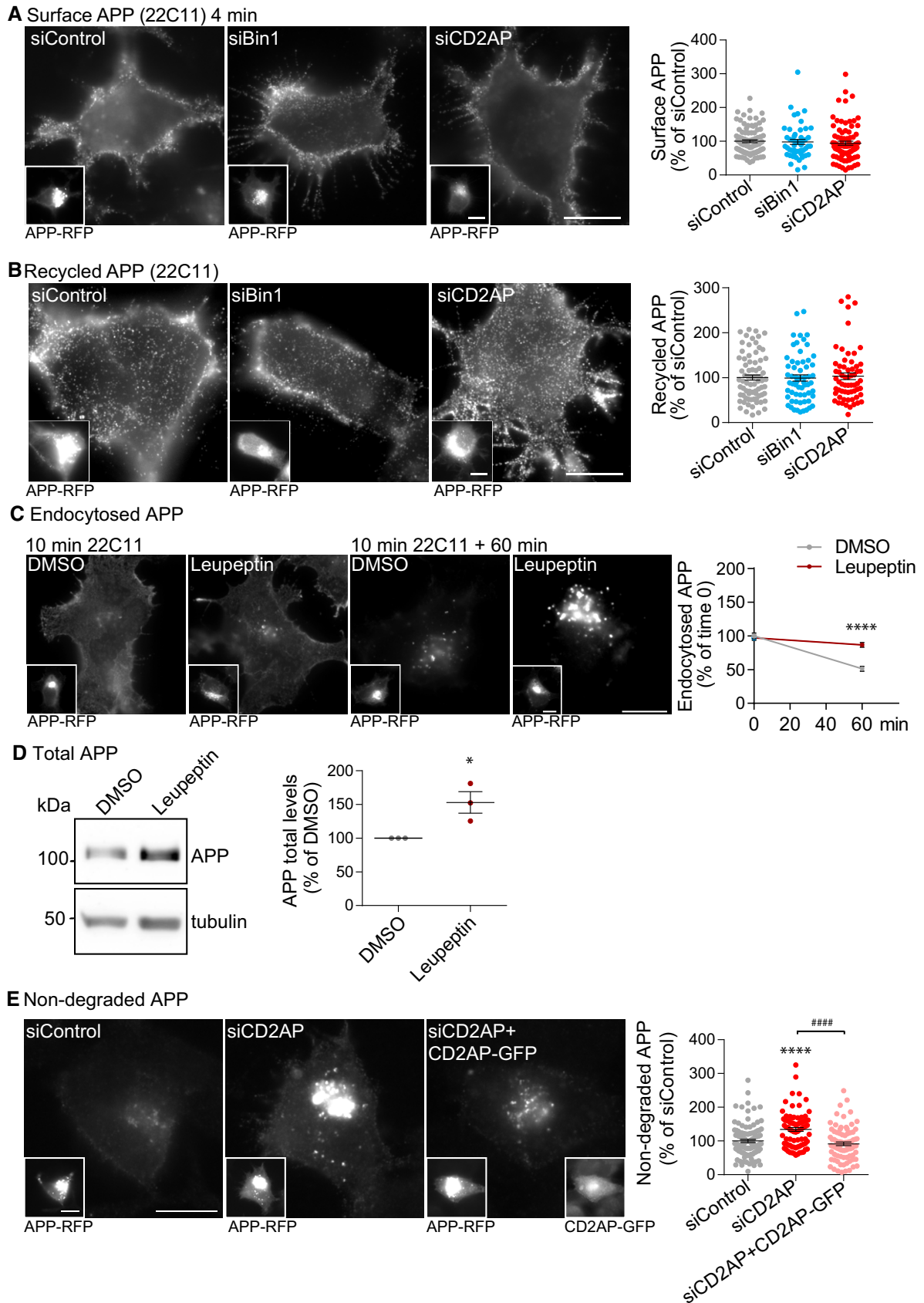


Figure EV3.

Figure EV4. BACE1 trafficking assays upon CD2AP and Bin1 depletion.

N2a cells treated with siBin1, siCD2AP or siControl

- A Surface BACE1 detected after a 4-min pulse with anti-FLAG (M1) and immunofluorescence of non-permeabilised cells expressing BACE1-GFP (insets), analysed by epifluorescence microscopy. Scale bars, 10 μ m. The graph on the right shows the amount of cell surface BACE1 fluorescence per cell quantified and normalised to BACE1-GFP fluorescence ($n = 4$, $N_{\text{siControl}} = 70$, $N_{\text{siBin1}} = 43$, $N_{\text{siCD2AP}} = 68$; mean \pm SEM).
- B Surface BACE1 detected as in (A) before and after acid stripping, analysed by epifluorescence microscopy. Scale bars, 10 μ m.
- C Endocytosed BACE1 detected with M1 (10-min pulse and 60-min chase) and BACE1-GFP (insets), analysed by epifluorescence microscopy. Scale bars, 10 μ m. The graph on the right shows the amount of endocytosed BACE1 fluorescence per cell quantified and normalised to BACE1-GFP fluorescence ($n = 4$, $N_{\text{siControl}} = 82$, $N_{\text{siBin1}} = 72$, $N_{\text{siCD2AP}} = 76$; mean \pm SEM).
- D Non-recycled BACE1 detected with M1 (10-min pulse, acid stripping and 20-min chase) in acid-stripped permeabilised N2a cells expressing BACE1-GFP (left insets) treated with siBin1 or siControl alone or upon expression of neuronal Bin1 or ubiquitous Bin1 (right insets), analysed by epifluorescence microscopy. Scale bars, 10 μ m. The graph on the right shows the amount of recycled BACE1 fluorescence per cell quantified and normalised to BACE1-GFP fluorescence ($n = 3$, $N_{\text{siControl}} = 95$, $N_{\text{siBin1}} = 120$, $N_{\text{siBin1} + \text{neuronal Bin1}} = 99$, $N_{\text{siBin1} + \text{ubiquitous Bin1}} = 89$; **** $P < 0.0001$ siBin1 vs. siControl and siBin1 + ubiquitous Bin1 vs. siControl, **** $P < 0.0001$ siBin1 vs. siBin1 + neuronal Bin1 and siBin1 + ubiquitous Bin1 vs. siBin1 + neuronal Bin1, one-way ANOVA with Tukey's test; mean \pm SEM).
- E Endogenous BACE1 levels by Western blot analysis with anti-BACE1 antibody and tubulin as a loading control in siBin1-, siCD2AP- or siControl-treated neurons. Quantification of BACE1 levels normalised to tubulin levels is shown on the right ($n = 5$, mean \pm SEM).

Source data are available online for this figure.

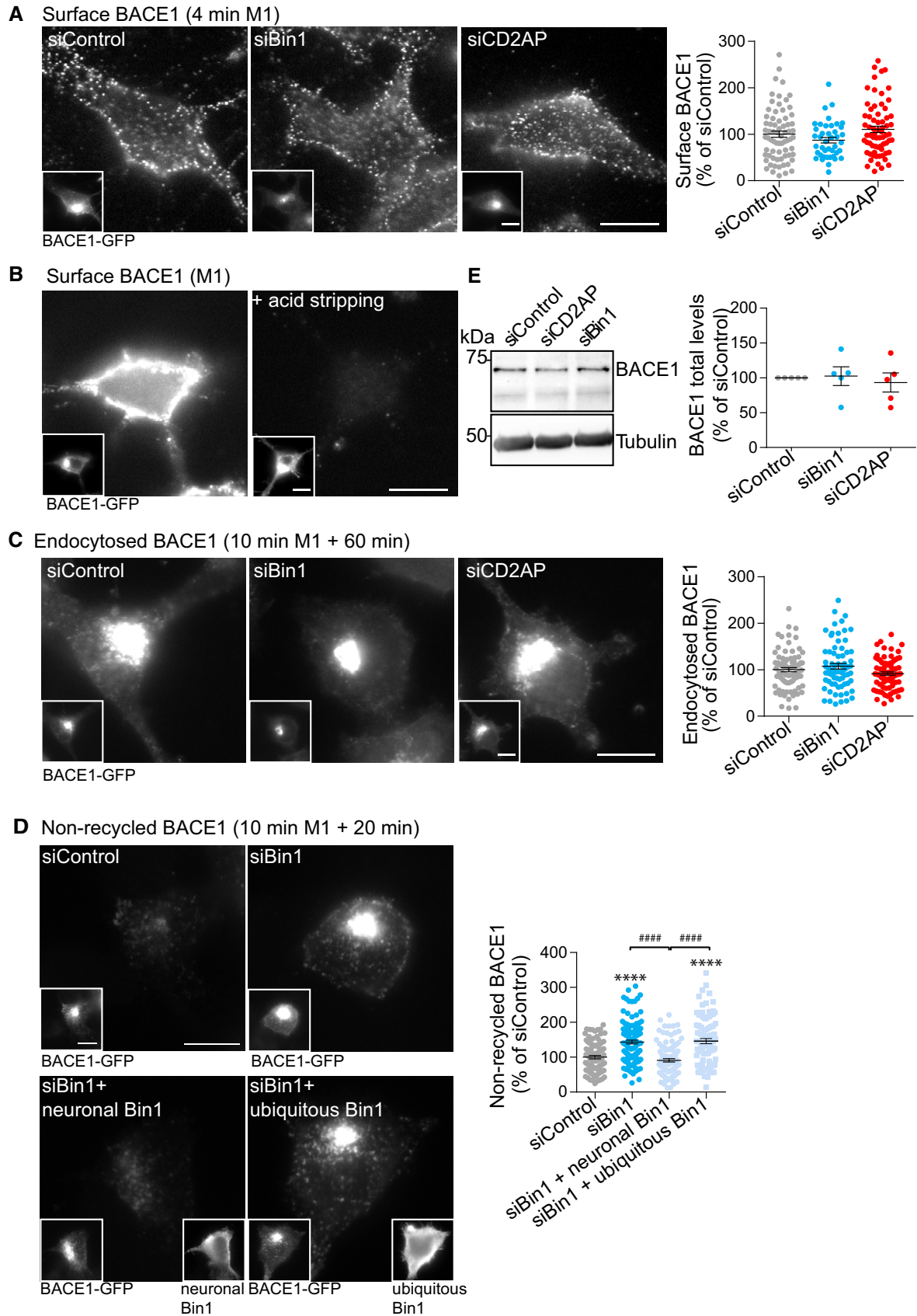


Figure EV5. CD2AP and Bin1 localisation, BACE1 and APP endocytosis and early endosomes alterations upon knockdown of Bin1 and CD2AP.

- A CD2AP localisation in neurons immunolabelled at 21 DIV with anti-CD2AP (green), anti-AnkG (magenta) and anti-MAP2 (blue) to identify dendrites (shown in magnified dendrites), analysed by epifluorescence microscopy. The white rectangle indicates the magnified dendrite shown in the bottom panels. Scale bars, 10 μ m.
- B Bin1 localisation in neurons immunolabelled at 21 DIV with anti-Bin1 (green) and anti-AnkG (magenta) to identify axons, analysed by epifluorescence microscopy. The white rectangle indicates the magnified axon shown in the lower panels. Scale bars, 10 μ m.
- C The axon/dendrite ratio of Bin1 and CD2AP calculated to quantify polarisation is shown ($N_{CD2AP} = 23$, $N_{Bin1} = 25$; **** $P < 0.0001$, t -test; mean \pm SEM).
- D–E BACE1 endocytosis detected with M1 in siBin1- and siControl-treated neurons expressing BACE1-GFP N-terminally tagged with FLAG, analysed by epifluorescence microscopy. (D) Endocytosed BACE1 (top panels) detected with M1 (5-min pulse) and BACE1-GFP (bottom panels). Arrowheads identify axons (Ax) and dendrites (Dd) magnified on the right. Scale bars, 10 μ m. (E) The amount of endocytosed BACE1 per cell body, dendrite and axons was normalised to BACE1-GFP fluorescence in the cell body and quantified as percentage of siControl ($n = 3$, $N_{siControl} = 14$, $N_{siBin1} = 14$; mean \pm SEM).
- F Endocytosed APP (green) detected with 22C11 (60-min chase) in EEA1-positive early endosomes (magenta) in siCD2AP- and siControl-treated N2a cells expressing APP-RFP, analysed by spinning-disc confocal microscopy. Scale bar, 10 μ m. The white squares are magnified on the right. Scale bar, 1 μ m.
- G The quantification of colocalisation between endocytosed APP and EEA1 is shown ($n = 3$, $N_{siControl} = 21$, $N_{siCD2AP} = 21$; **** $P < 0.001$, t -test, mean \pm SEM).
- H Non-recycled BACE1 (green) detected with M1 (15-min pulse, acid stripping and 20-min chase) in EEA1-positive early endosomes (magenta) in siControl- and siBin1-treated N2a cells expressing BACE1-GFP, analysed by spinning-disc confocal microscopy. Scale bar, 10 μ m. The white squares indicate EEA1-positive endosomes magnified on the right. Scale bar, 1 μ m.
- I Quantification of colocalisation between non-recycled BACE1 and EEA1 is shown ($n = 3$, $N_{siControl} = 26$, $N_{siBin1} = 26$; **** $P < 0.001$, t -test, mean \pm SEM).
- J Early endosomes immunolabelled with anti-EEA1 in siCD2AP- and siControl-treated neurons analysed by epifluorescence microscopy. Scale bars, 10 μ m. The quantification of the average area of EEA1-positive early endosomes in dendrites is shown on the right ($n = 4$, $N_{siControl} = 34$, $N_{siCD2AP} = 28$; **** $P < 0.0001$, t -test, mean \pm SEM).
- K Rab5-positive early endosomes detected in siBin1- and siControl-treated neurons expressing Rab5-GFP, analysed by spinning-disc confocal microscopy. Scale bars, 10 μ m. The quantification of the average area of Rab5-positive early endosomes in axons is shown on the right ($n = 3$, $N_{siControl} = 14$, $N_{siBin1} = 19$; **** $P < 0.001$, t -test, mean \pm SEM).

