

Supplemental material

Lazic et al., <https://doi.org/10.1084/jem.20181035>

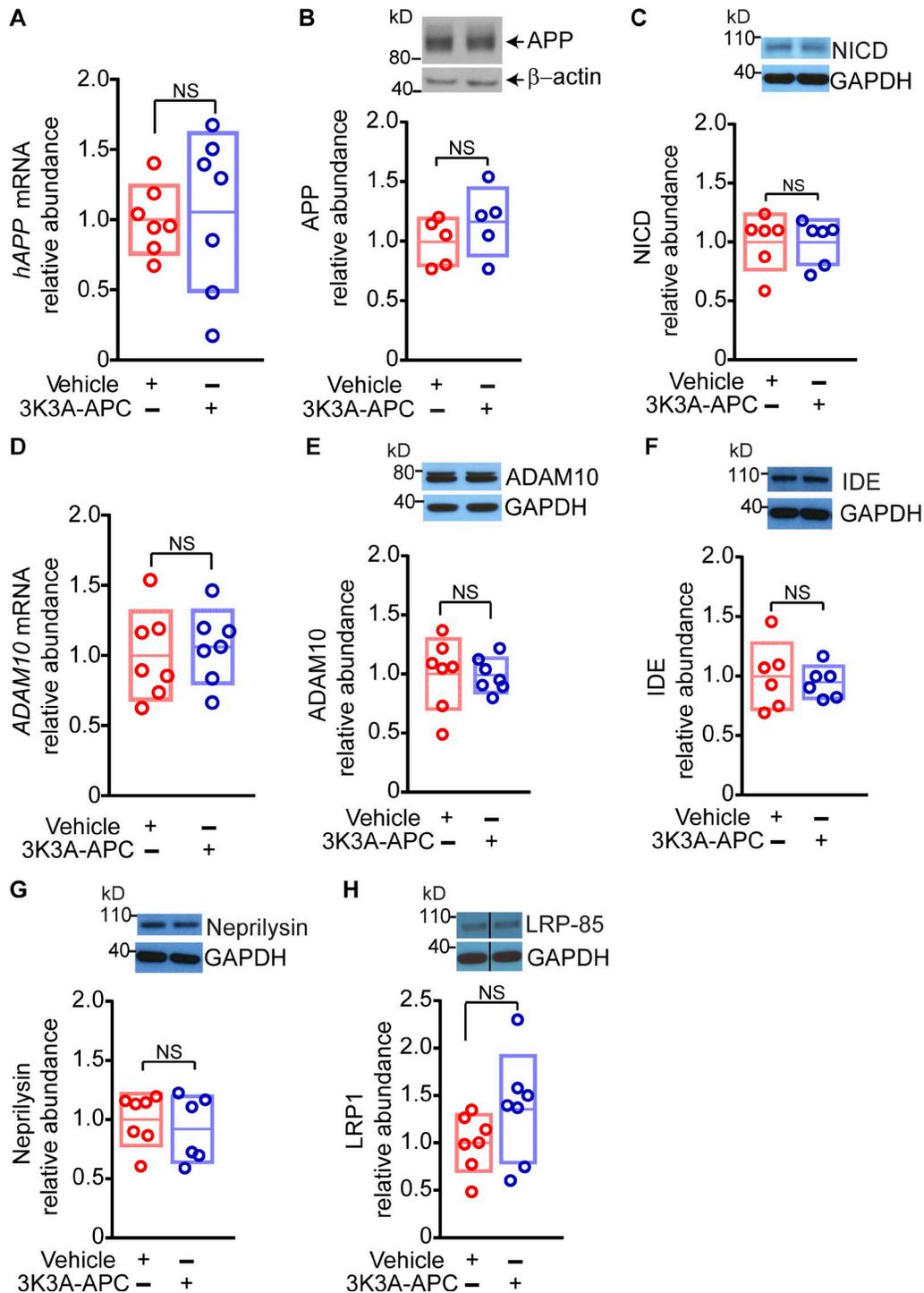


Figure S1. **3K3A-APC treatment does not affect APP levels, APP processing, or A β clearance.** (A–H) *hAPP* mRNA levels, normalized by *Gapdh* mRNA (A). APP levels with β -actin used as a loading control (B), Notch intracellular domain (NICD) protein levels (C), disintegrin and *ADAM10* mRNA levels normalized by *Gapdh* mRNA (D), ADAM10 protein levels (E), IDE protein levels (F), and neprilysin protein levels (G) in the cortex, and LRP1 levels in cortical microvesicles (H) in 5XFAD mice treated with vehicle or 3K3A-APC. In C and E–H, GAPDH was used as a loading control. Data are shown as single points per mouse, with boxplots representing means \pm SD. In A, $n = 7$ mice per group; in B, $n = 5$ mice per group; in C–H, $n = 6$ –7 mice per group (statistical significance by two-tailed Student's *t* test).

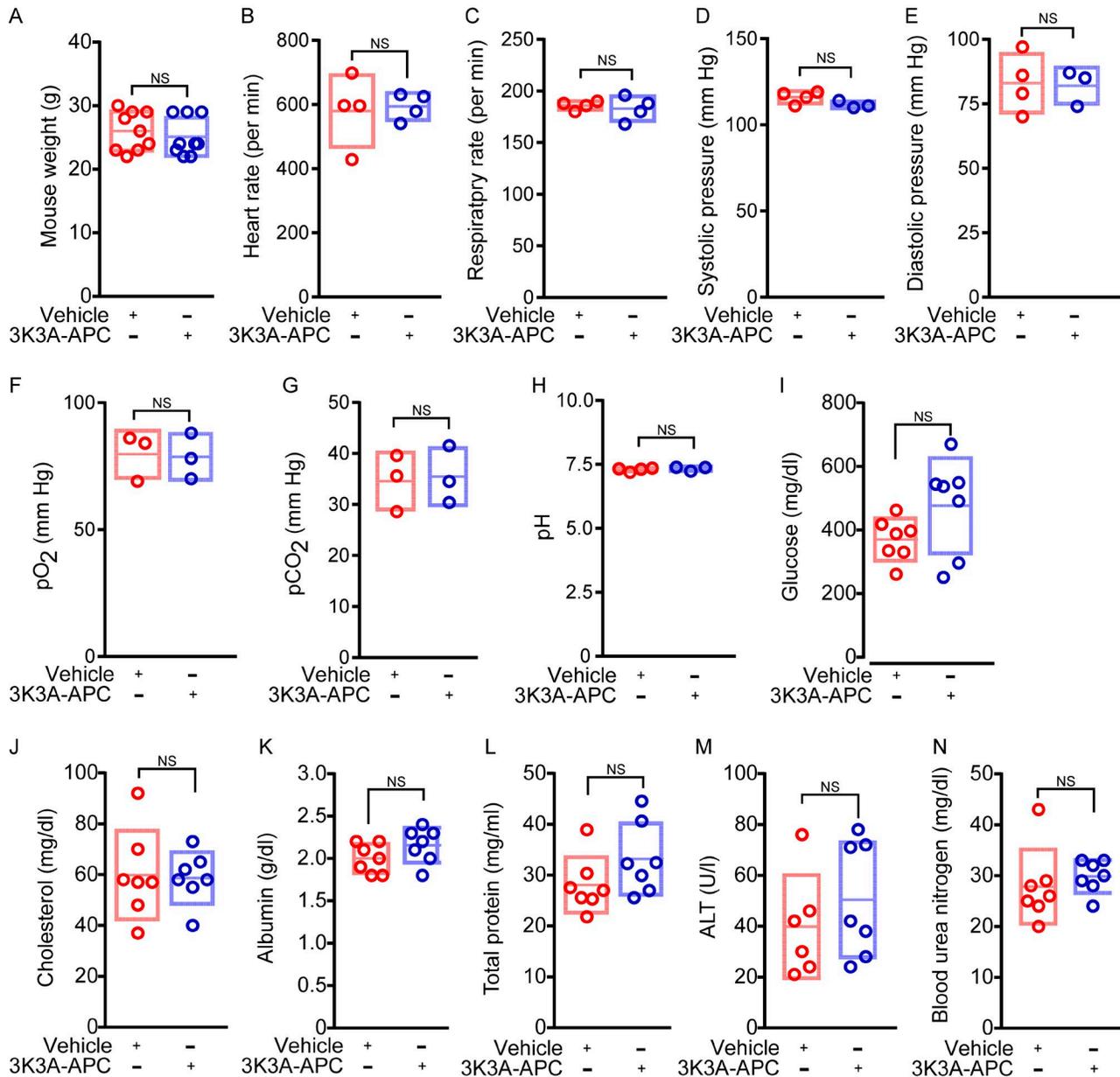


Figure S2. **3K3A-APC treatment does not affect systemic physiological and biochemical parameters.** (A–N) No differences in mouse body weight (A), heart rate (B), respiratory rate (C), systolic arterial pressure (D), diastolic arterial pressure (E), arterial pO₂ (F), arterial pCO₂ (G), arterial pH (H), plasma glucose levels (I), plasma cholesterol levels (J), plasma albumin levels (K), total protein levels (L), plasma alanine aminotransferase (ALT) levels (M), and blood urea nitrogen levels (N) in 5XFAD mice treated with vehicle or 3K3A-APC. Data are shown as single points per mouse, with boxplots representing means ± SD. In A, *n* = 9 mice per group; in B–H, *n* = 3–4 mice per group; in I–N, *n* = 6–7 mice per group (statistical significance by two-tailed Student's *t* test).

Figure 2B

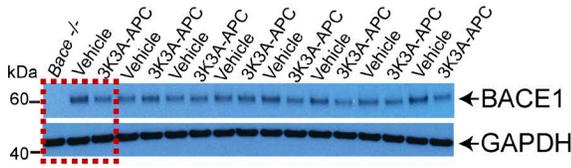


Figure 3E

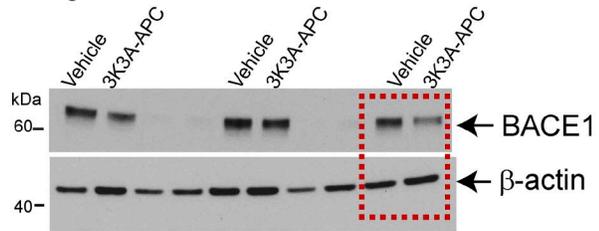


Figure 2F

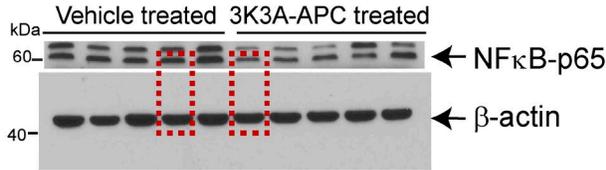


Figure 3I

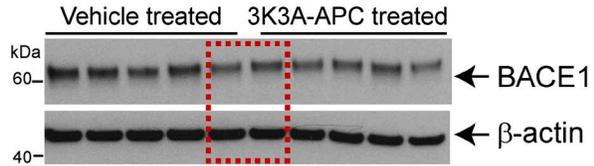


Figure 2G

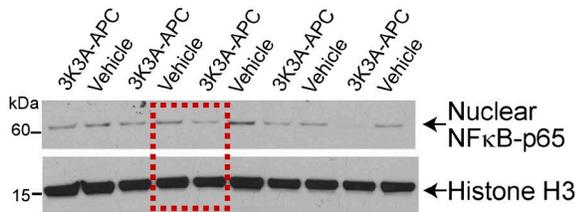


Figure 4B

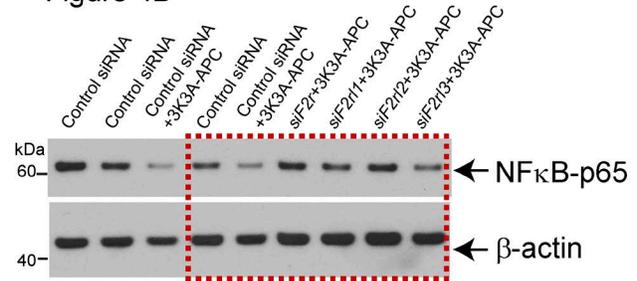


Figure 3B

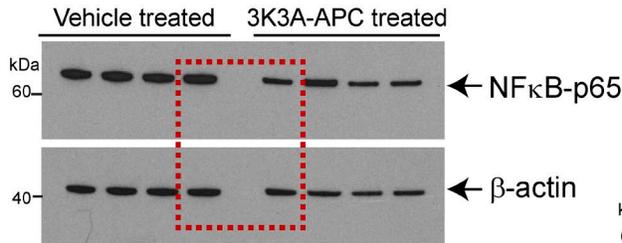


Figure 4C

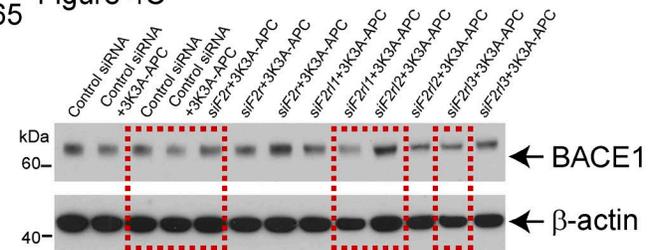


Figure 3C

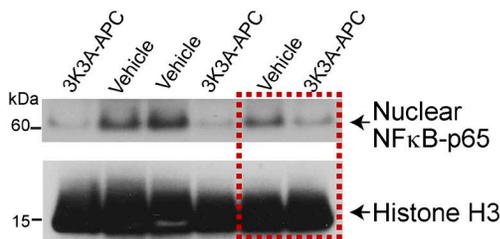


Figure 5D

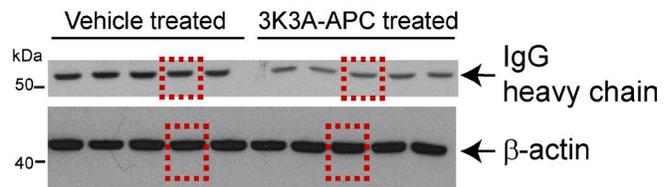


Figure S3. Full gels for all Western blot images used for quantification in Figs. 2, 3, 4, and 5. Red dotted squares indicate lanes presented as representative blots in the main figures.

Table S1. **Primary and secondary antibodies used for IHC**

| Primary antibody/lectin (manufacturer, catalog number, and dilution used) | Secondary antibody/IgG (manufacturer, catalog number, and dilution used) |
|--|---|
| Human Aβ | |
| Rabbit anti-human A β (Cell Signaling, 8243S, 1:500) | Alexa Fluor 647–conjugated donkey anti-rabbit (Invitrogen, A-31573, 1:500) |
| Smooth muscle cells | |
| Mouse FITC-conjugated α SMA (Sigma, clone 1A4, F3777, 1:500) | N/A |
| p-NFκB | |
| Rabbit anti-human NF κ B-p65 (phospho S536), cross-reacts with mouse NF κ B-p65 (phospho-S536; Abcam, ab86299, 1:00) | Alexa Fluor 647–conjugated donkey anti-rabbit (Invitrogen, A-31573,1:500) |
| Neurons | |
| Mouse anti-mouse NeuN (Millipore, MAB377, 1:500) | Alexa Fluor 488–conjugated donkey anti-mouse (Invitrogen, A-21202, 1:500) |
| Vasculature | |
| Dylight 488–conjugated <i>L. esculentum</i> lectin (Vector Labs, DL-1174, 1:200) | N/A |
| Fibrinogen/fibrin | |
| Rabbit anti-human fibrinogen, cross-reacts with mouse fibrinogen (Montagne et al., 2018 ; Dako, A0080, 1:400) | Alexa Fluor 568–conjugated donkey anti-rabbit (Invitrogen, A-10042, 1:500) |
| IgG | |
| N/A | Alexa Fluor 647–conjugated donkey anti-mouse (Invitrogen, A-31571, 1:500) |
| Microglia | |
| Rabbit anti-mouse Iba1 (Wako, 019-19741; 1:500) | Alexa Fluor 568–conjugated donkey anti-rabbit (Invitrogen, A-10042, 1:500) |
| Astrocytes | |
| Rabbit anti-bovine GFAP, cross-reacts with mouse GFAP (Montagne et al., 2018 ; Dako, Z0334, 1:500) | Alexa Fluor 568–conjugated donkey anti-rabbit (Invitrogen, A-10042, 1:500) |

N/A, not applicable.

Table S2. **Primary and secondary antibodies used for immunoblotting**

| Primary antibody (manufacturer, catalog number, and dilution used) | Respective secondary antibody/ IgG (manufacturer, catalog number, and dilution used) |
|---|---|
| BACE1 | |
| Rabbit anti-human BACE1, cross-reacts with mouse BACE1 (Cell Signaling, 5606, 1:1,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| NFκB | |
| Rabbit anti-human NFκB-p65, cross-reacts with mouse NFκB-p65 (Abcam, ab16502, 1:1,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| PARs | |
| Mouse anti-human PAR1, cross-reacts with mouse PAR1 (Santa Cruz, sc-13503) | HRP-conjugated donkey anti-mouse (Cell Signaling, 7076S, 1:3,000) |
| Rabbit anti-human PAR2, cross-reacts with mouse PAR2 (Abcam, ab124227) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| Rabbit anti-human PAR3, cross-reacts with mouse PAR3 (Santa Cruz, sc-5598) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| Rabbit anti-human PAR4, cross-reacts with mouse PAR4 (Thermo Fisher, PA5-72392) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| IgG | |
| N/A | HRP-conjugated donkey anti-mouse (Santa Cruz, 2096, 1:3,000) |
| APP | |
| Mouse anti-APP N terminus 66–81, cross reacts with human and mouse APP (Millipore, MAB348, 1:1,000) | HRP-conjugated horse anti-mouse (Cell Signaling, 7076S, 1:3,000) |
| ADAM10 | |
| Rat anti-mouse ADAM10 (R&D Systems, AF1126, 1:1,000) | HRP-conjugated goat anti-rat (Cell Signaling, 7077, 1:3,000) |
| Neprilysin | |
| Goat anti-mouse neprilysin (R&D Systems, AF1126, 1:1,000) | HRP-conjugated donkey anti-goat (Invitrogen, A16005, 1:3,000) |
| IDE | |
| Rabbit anti-rat IDE, cross-reacts with mouse IDE (Millipore Sigma, PC730, 1:1,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| NICD | |
| Rabbit anti-human NICD cross-reacts with mouse NICD (Millipore Sigma, 07-1232, 1:1,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| LRP1 | |
| Rabbit anti-human LRP1 antibody (EPR3724), cross-reacts with mouse LRP1 (Abcam, ab92544, 1:5,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| Loading controls | |
| Rabbit anti-human histone H3, cross-reacts with mouse histone H3 (Abcam, ab1791, 1:1,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| Rabbit anti-human β-actin, cross-reacts with mouse β-actin (Cell Signaling, 4970S, 1:2,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |
| Rabbit anti-human GAPDH, cross-reacts with mouse GAPDH (Cell Signaling, 2118, 1:2,000) | HRP-conjugated donkey anti-rabbit (Invitrogen, A16023, 1:3,000) |

N/A, not applicable; NICD, Notch 1 intracellular domain.

References

Montagne, A., A.M. Nikolakopoulou, Z. Zhao, A.P. Sagare, G. Si, D. Lazic, S.R. Barnes, M. Daiyanu, A. Ramanathan, A. Go, et al. 2018. Pericyte degeneration causes white matter dysfunction in the mouse central nervous system. *Nat. Med.* 24:326–337. <https://doi.org/10.1038/nm.4482>