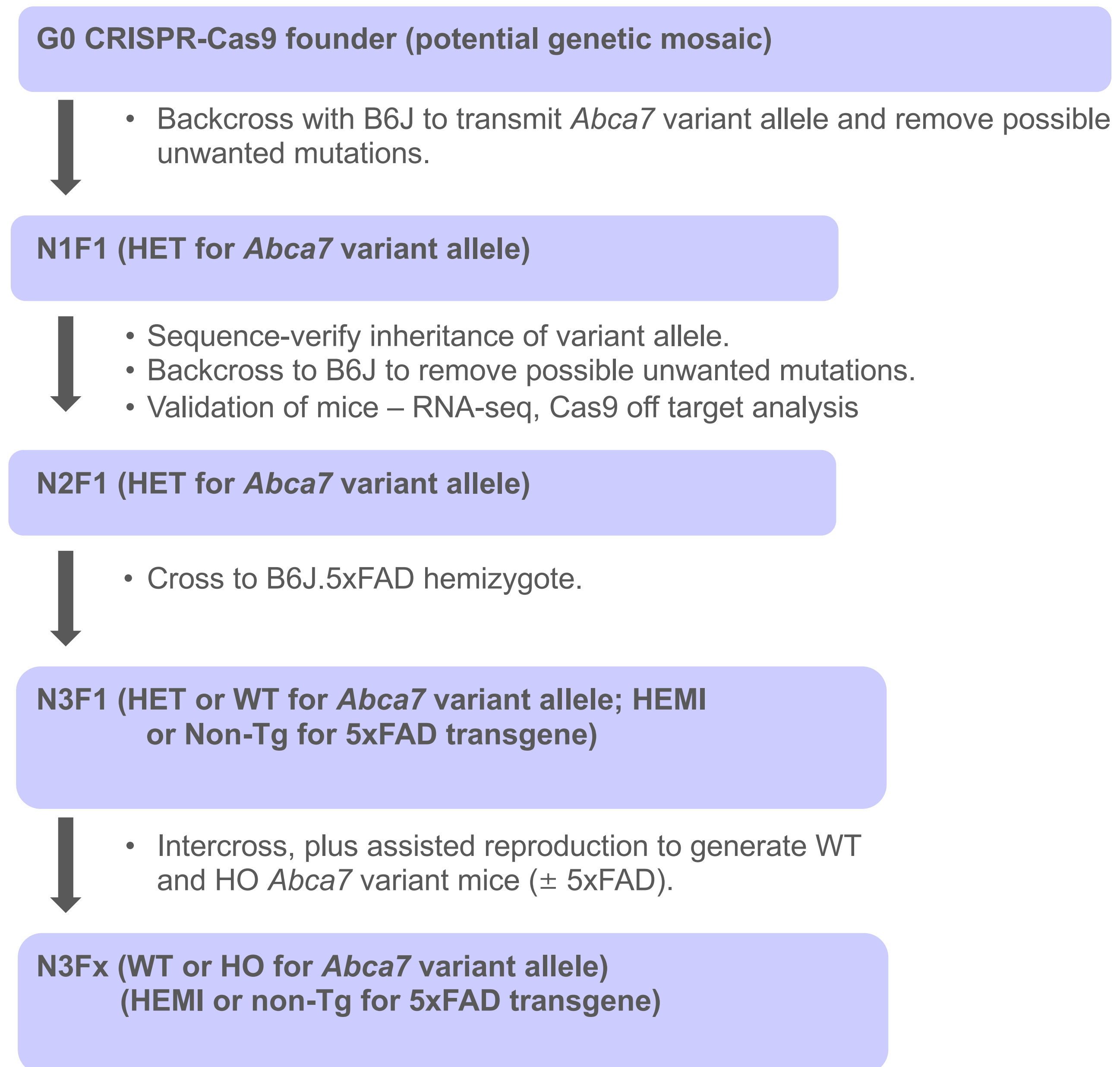
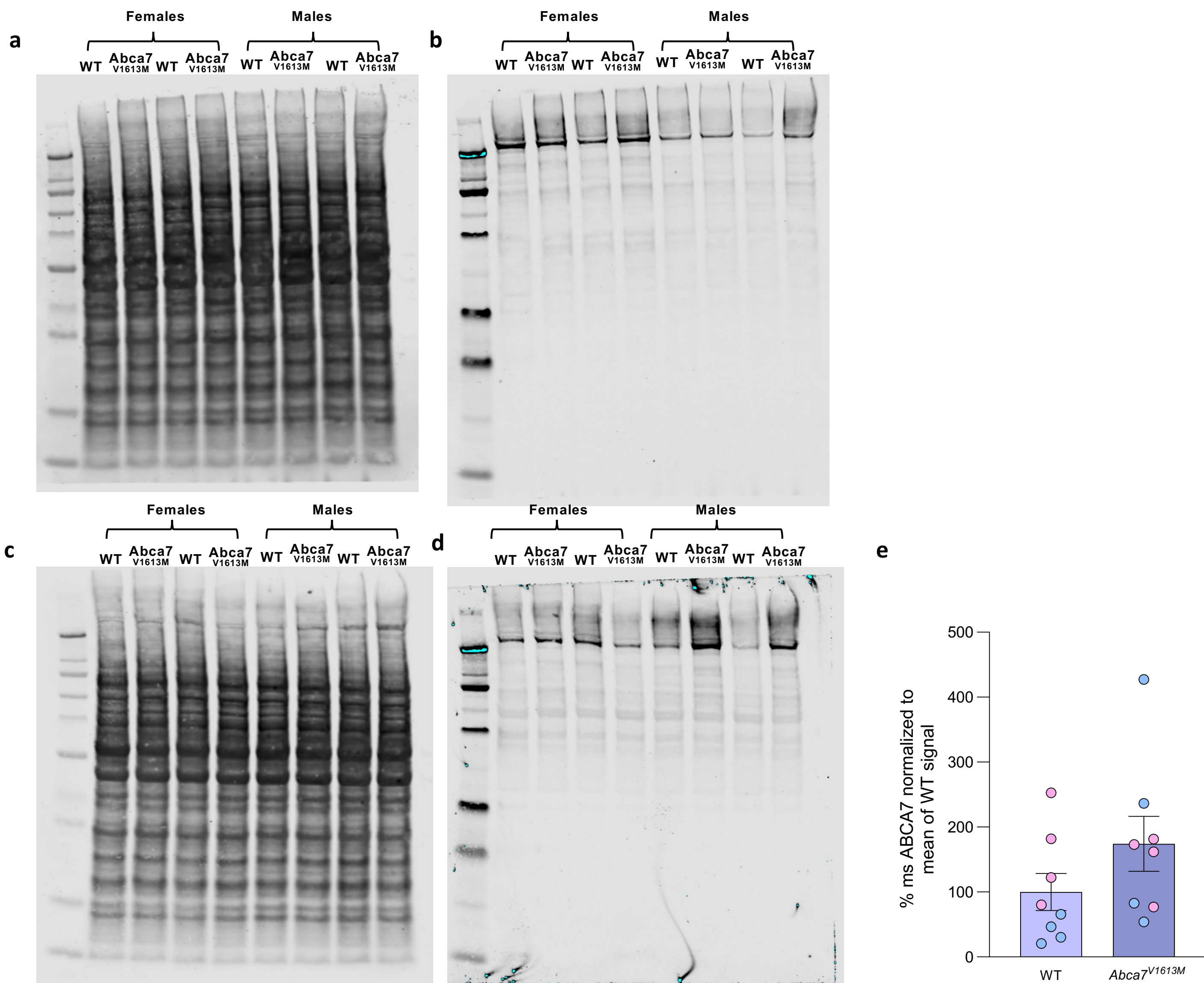
Supplementary figures

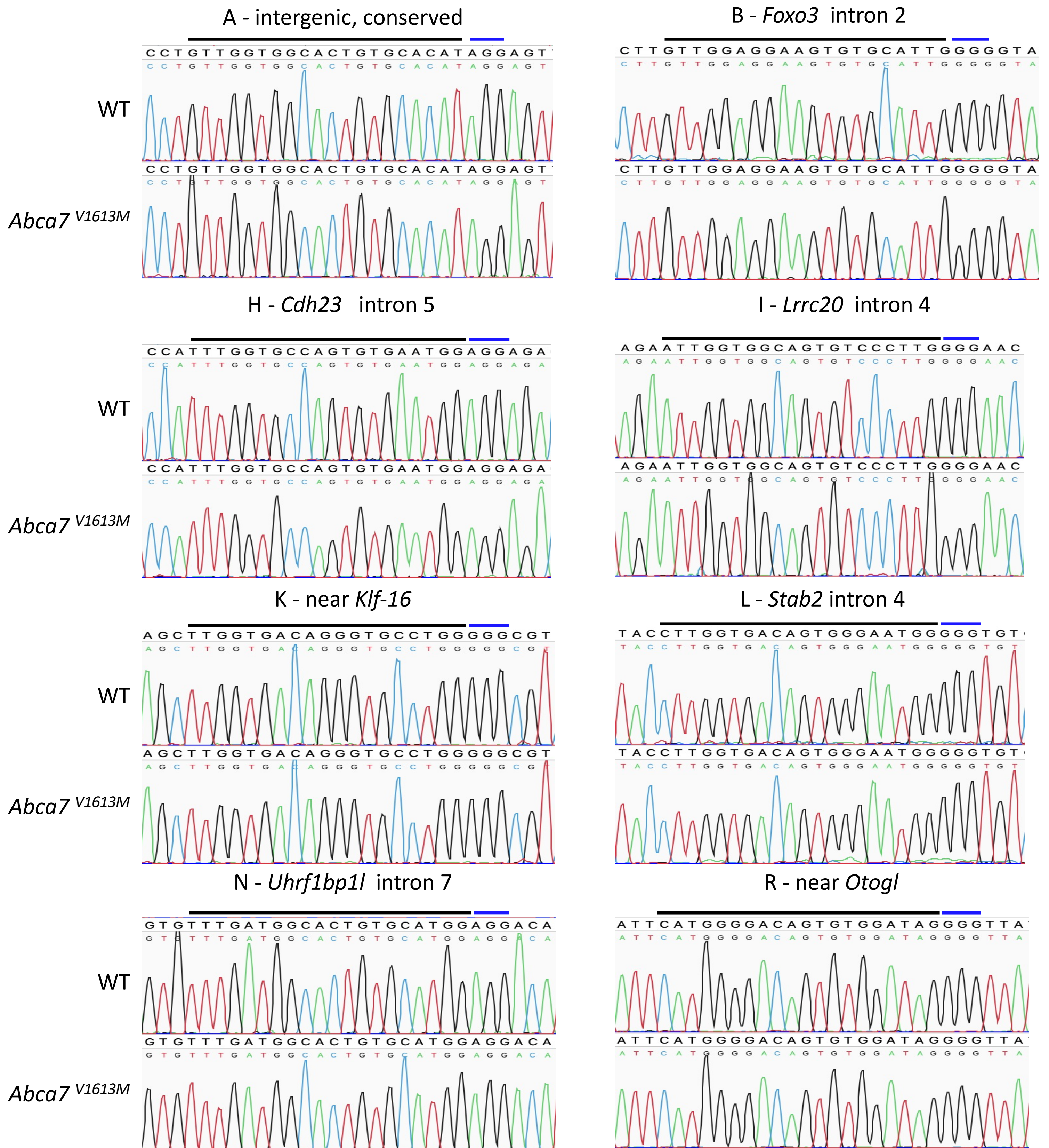
Butler et al., (2023) The *Abca7*^{V1613M} variant reduces Aβ generation, plaque load, and neuronal damage



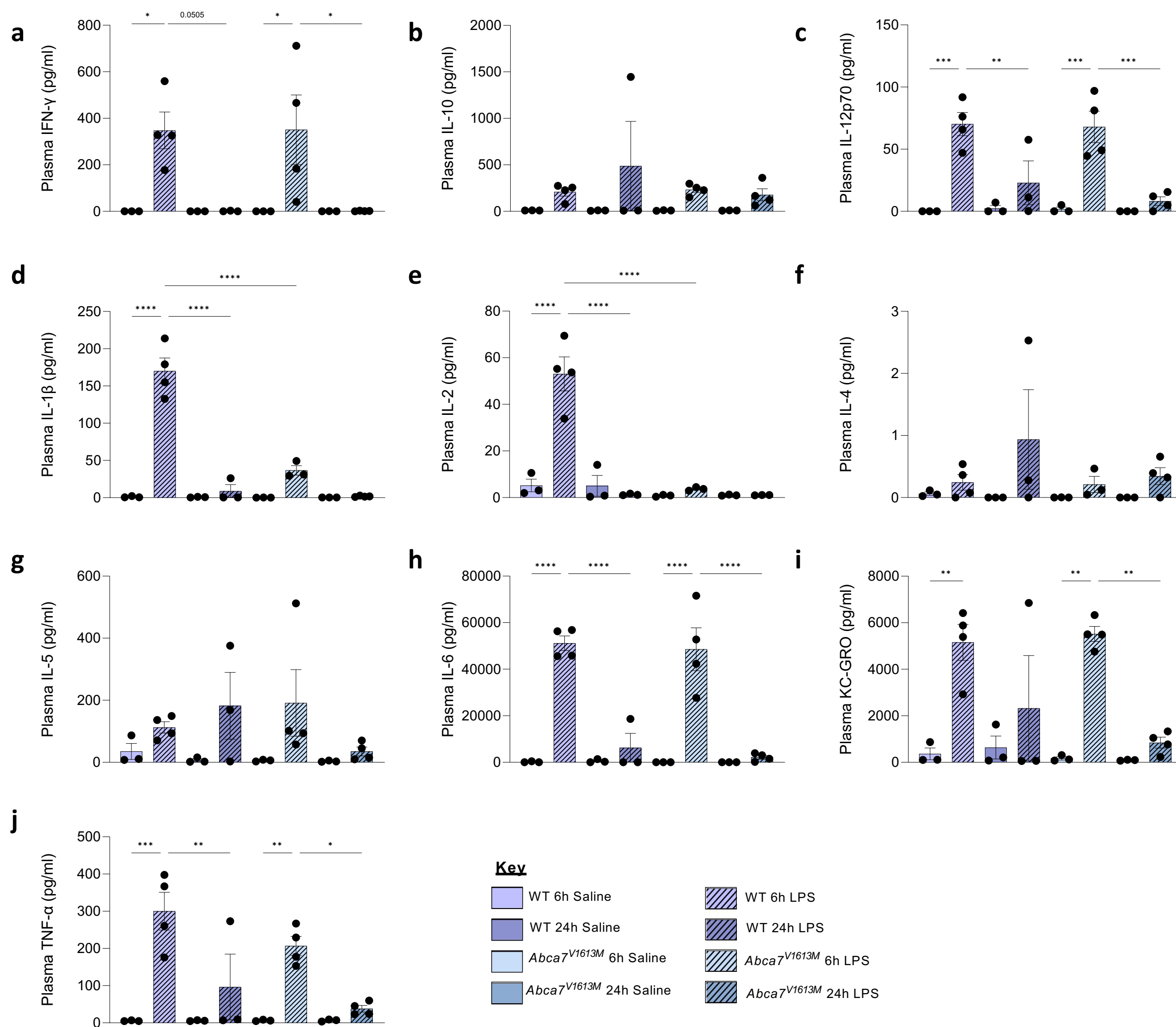
Supplementary Figure 1. Schematic describing breeding and validation of mice used in this study. G0 founder animals containing the desired DNA sequence changes were backcrossed with C57BL/6J mice and N1 *Abca7*^{V1613M} heterozygous mice were sequenced to determine the variant allele. Validation of gene, protein expression, cryptic splicing events and potential off target effects are conducted. N1 *Abca7*^{V1613M} heterozygous mice were again backcrossed with C57BL/6J mice to produce N2F1 *Abca7*^{V1613M} heterozygotes, which were subsequently crossed with 5xFAD hemizygous congenic B6J (B6.CgTg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax, Jackson Lab Stock # 34848, MMRRC) mice to produce N3F1 animals that were heterozygous or wildtype for *Abca7*^{V1613M} and hemizygous or non-transgenic for 5xFAD. These N3F1 animals were used to produce N3Fx experimental and control animals by natural mating or IVF procedures.



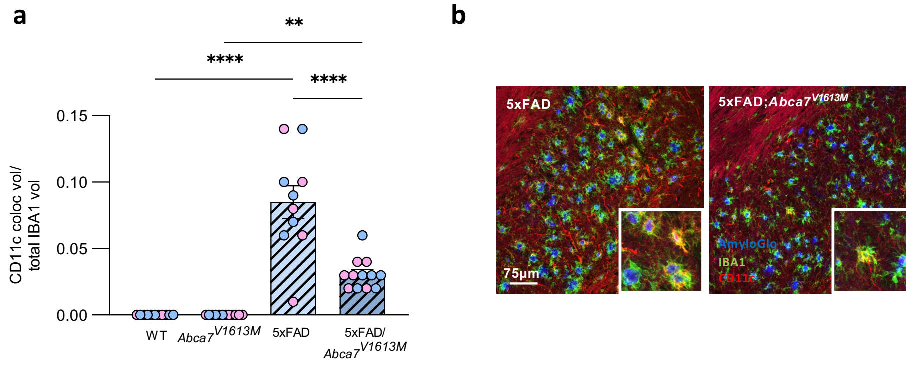
Supplementary Figure 2. Uncropped total protein stains (TPS), western blots, and quantification of mouse ABCA7 in WT (C57BL/6J) and *Abca7*^{V1613M} mice. a) Total protein stain of blot #1 in cortical soluble fraction 4-month-old WT and *Abca7*^{V1613M} mice, of both sexes. b) #1 western blot of mouse ABCA7 in cortical soluble fraction 4-month-old WT and *Abca7*^{V1613M} mice, of both sexes. c) Total protein stain of blot #2 in cortical soluble fraction 4-month-old WT and *Abca7*^{V1613M} mice, of both sexes. d) #2 western blot of mouse ABCA7 in cortical soluble fraction 4-month-old WT and *Abca7*^{V1613M} mice, of both sexes. For all blots Lanes 1, 3, 5, 7, 9 are individual WT mice, Lanes 2, 4, 6, 8, 10 are individual *Abca7*^{V1613M} mice. e) quantification of mouse ABCA7 signal normalized to the mean of WT for each individual blot. N=4 mice per sex/genotype. Data are represented as mean ± SEM. Statistics: Unpaired student's t-test.



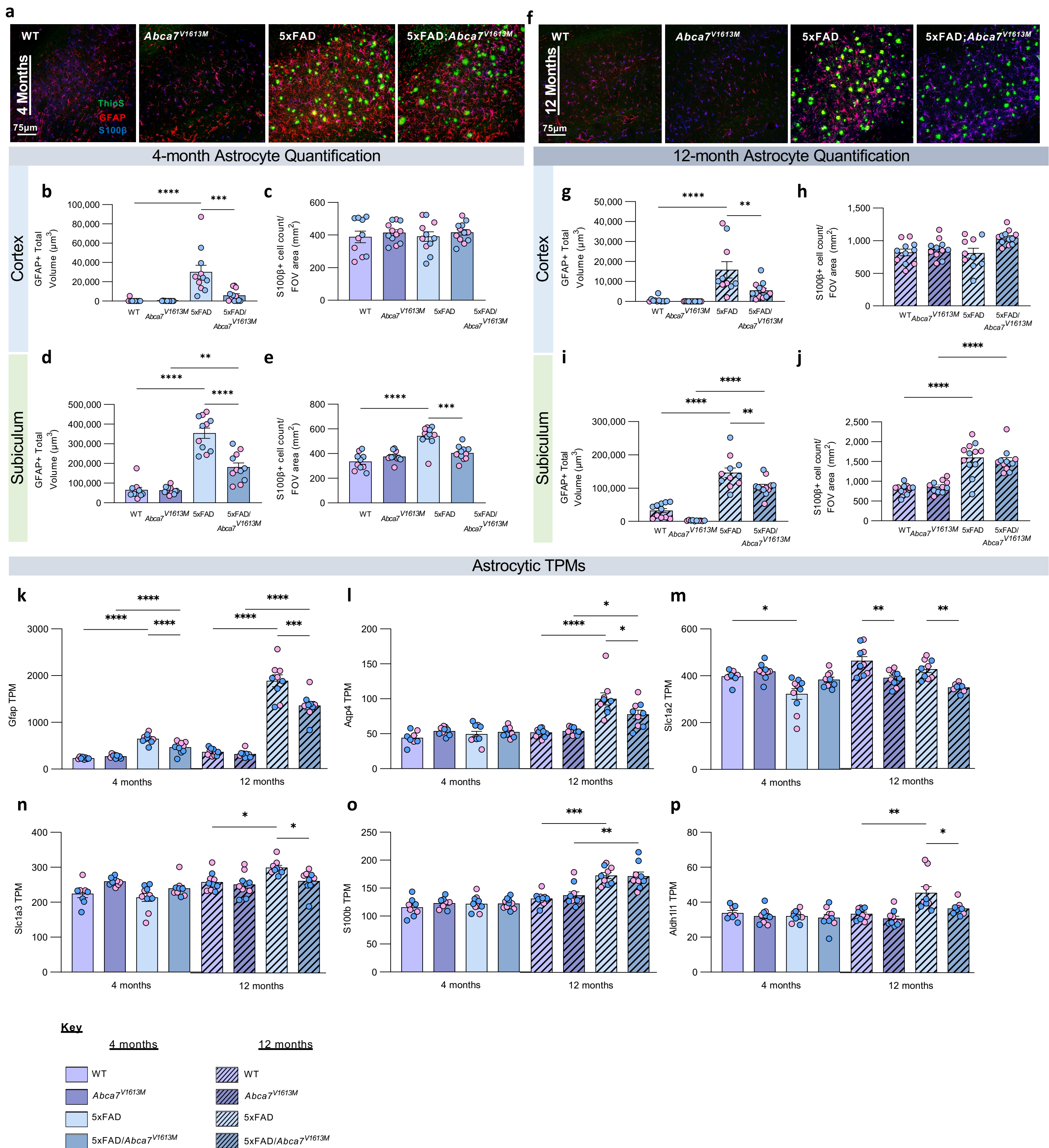
Supplementary Figure 3. Off-target site activity analysis for crRNA TMF1268 on mouse chromosome 10. No difference was found in sequence between the C57BL/6J WT and *Abca7*^{V1613M} alleles at each of the 8 potential off-target sites analyzed. The location of each site is listed in Supplementary Table 1. The black underline denotes the crRNA target sequence while the blue underline denotes the NGG PAM site.



Supplementary Figure 4. LPS induces changes in plasma cytokine levels in WT and *Abca7^{V1613M}* mice. Quantification of a) IFN-γ, b) IL-10, c) IL-12p70, d) IL1β, e) IL-2, f) IL-4, g) IL-5, h) IL-6, i) KC-GRO and j) TNFα in saline and LPS-treated WT and *Abca7^{V1613M}* plasma. N= 3-4 mice per genotype/treatment/timepoint. Data are represented as mean ± SEM. Statistics: Two-way ANOVA, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, **** ≤ 0.0001

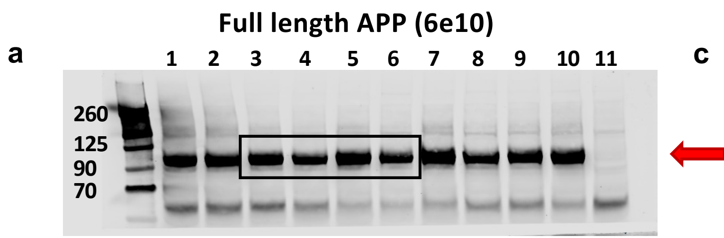


Supplemental Figure 5. 5xFAD/*Abca7*^{V1613M} mice have reduced CD11c expression in subiculum at 12 months. a) Representative images of subiculum stained with Amyloglo (blue) for amyloid beta plaques, IBA1 (green) for microglia and CD11c (red) for disease associated microglia in 5xFAD and 5xFAD/Abca7V1613M mice at 12 months of age. b) Quantification of CD11c+/IBA1+ colocalization volume normalized to total volume of IBA1+ cells. N=4-6 per sex/genotype/age. Data are represented as mean ± SEM. Statistics: Two- way ANOVA, **p ≤ 0.01, **** ≤ 0.0001. Scale bar = 75 microns. 10x digital zoom insert.



Supplementary Figure 6. Reduced astrogliosis in 4- and 12-month-old 5xFAD/*Abca7*^{V1613M} mice. Astrocyte number and size were assessed by staining coronal sections with GFAP (for reactive astrocytes) and S100β for all astrocytes. a) Representative confocal images from subiculum of female WT, *Abca7*^{V1613M} homozygous, 5xFAD, and 5XFAD/*Abca7*^{V1613M} mice displaying changes in GFAP and S100β number and size at 4 months of age. b) Quantification of total volume of GFAP+ cell volume in cortex at 4 months of age. c) Quantification of S100β+ cells in cortex at 4 months of age. d) Quantification of total volume of GFAP+ cell volume in subiculum at 4 months of age. e) Quantification of S100β+ cells in subiculum at 4 months of age. f) Representative confocal images from subiculum of female WT, *Abca7*^{V1613M} homozygous, 5xFAD and 5xFAD/*Abca7*^{V1613M} mice displaying changes in GFAP and S100β number and size at 12 months of age. g) Quantification of total volume of GFAP+ cell volume in cortex at 12 months of age. h) Quantification of S100β+ cells in cortex at 12 months of age. i) Quantification of total volume of GFAP+ cell volume in subiculum at 12 months of age. j) Quantification of S100β+ cells in subiculum at 12 months of age. Transcripts per million (TPMs) of astrocytic markers, k) *Gfap*, l) *Aqp4*, m*)* *Slc1a2*, n) *Slc1a3*, o) *s100b* and p) *Aldh1l1*. N=4-6 per sex/genotype/age. Data are represented as mean ± SEM. Statistics: Two- way ANOVA, $*p \le 0.05$, $**p \le 0.01$, $***p \le 0.001$, $**** \le 0.0001$. Scale bar = 75 microns.

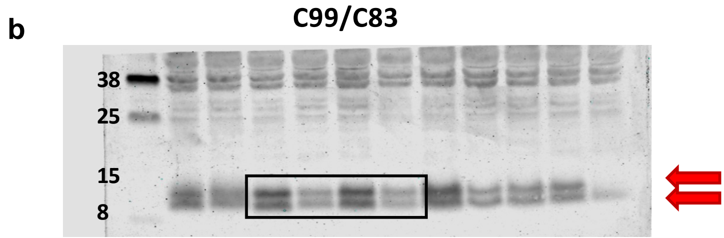
a



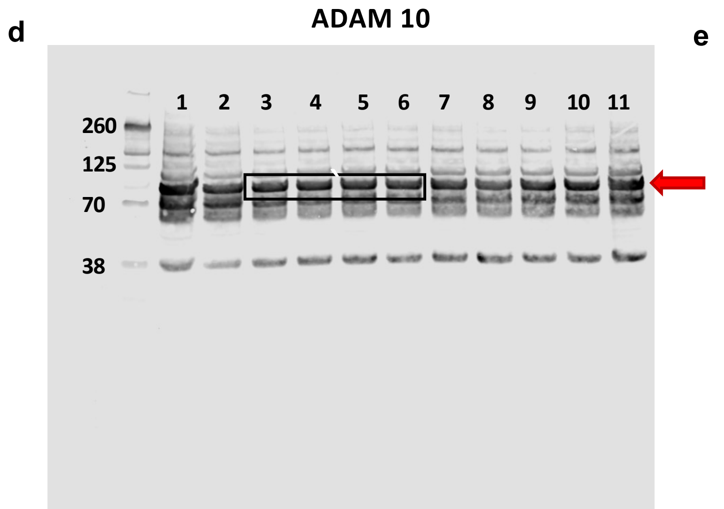
c

TPS for APP blot

b



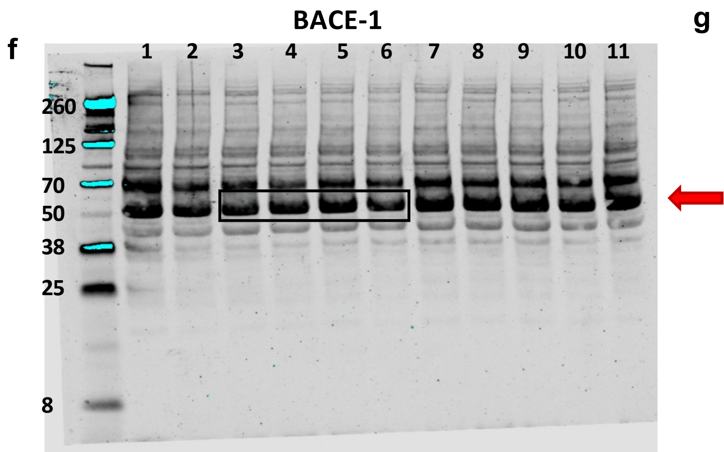
d



e

TPS for ADAM 10 blot

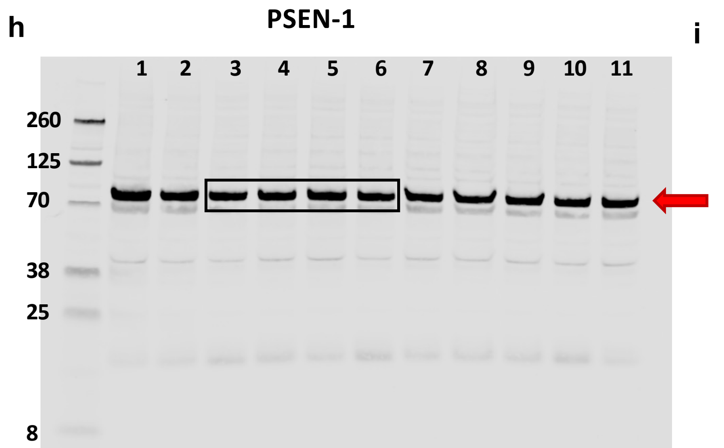
f



g

TPS for BACE-1 blot

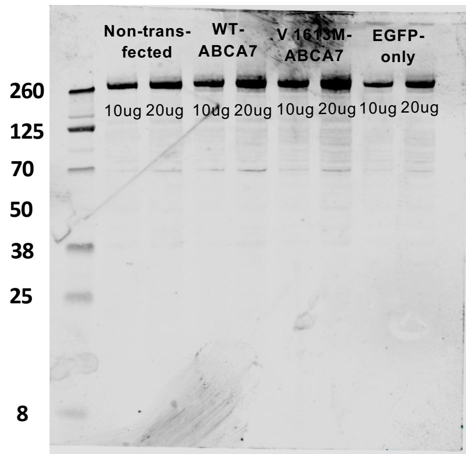
h

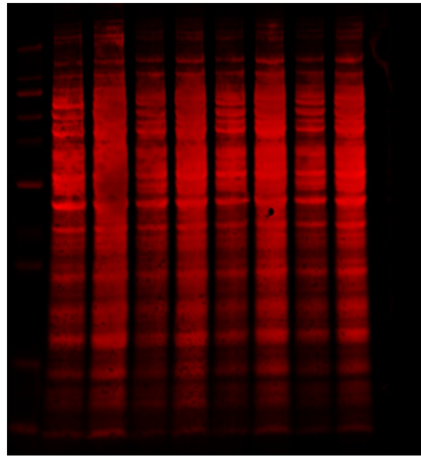


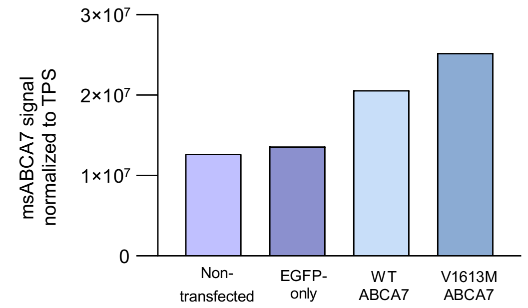
i

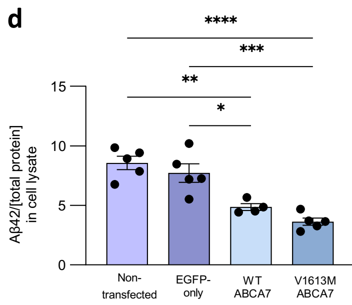
TPS for PSEN-1 blot

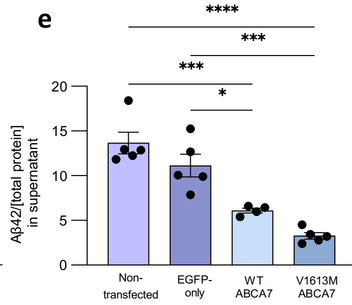
Supplementary Figure 7. Uncropped western blots and associated total protein stains (TPS) for APP processing pathway proteins. Red arrows indicate protein band of interest. Black box indicates cropped section of blot presented in paper. a) Full length APP blot, b) C99/C83 APP c-terminal fragments blot, c) TPS for whole APP blot – used for normalization. d) ADAM-10 blot, e) TPS for ADAM-10 blot, f) BACE-1 blot, g) TPS for BACE-1 blot, h) PSEN-1 blot and i) TPS for PSEN-1 blot. Lanes 1, 3, 5, 7, 9 are individual 5xFAD mice, Lanes 2, 4, 6, 8, 10 are individual 5xFAD/*Abca7*^{V1613M} mice, Lane 11 is a WT mouse as a negative control for APP.

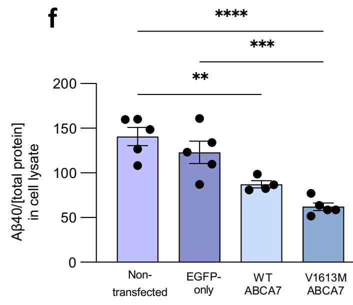

a
Non-trans-fected
WT-ABCA7
V1613M-ABCA7
EGFP-only
260
125
70
50
38
25
8
10ug 20ug 10ug 20ug 10ug 20ug 10ug 20ug

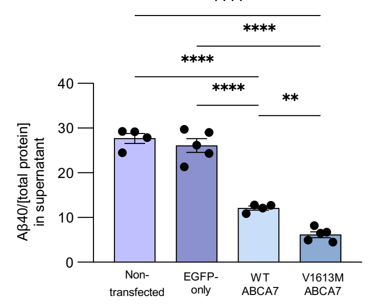

b

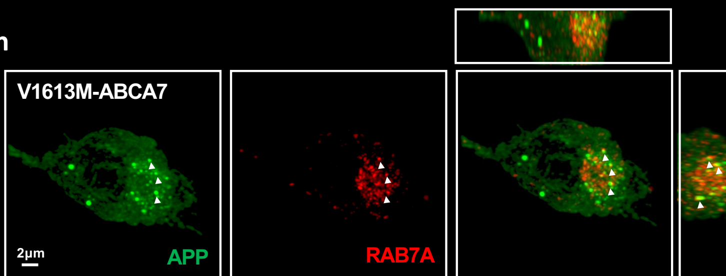

c
msABCA7 signal normalized to TPS
3×10^7
2×10^7
1×10^7
0
Non-transfected
EGFP-only
WT ABCA7
V1613M ABCA7

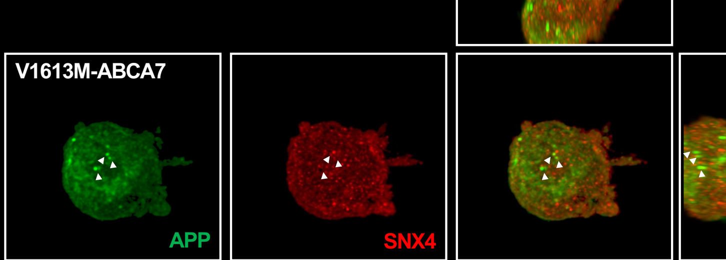

d
Aβ42/[total protein] in cell lysate
Non-transfected
EGFP-only
WT ABCA7
V1613M ABCA7

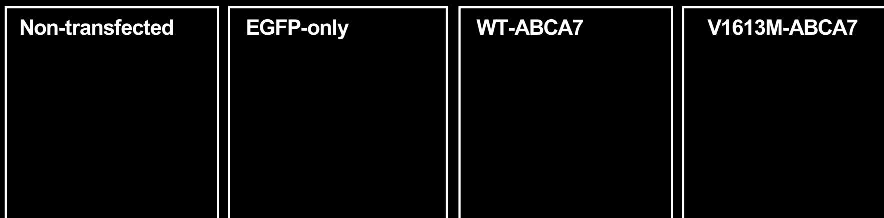

e
Aβ42/[total protein] in supernatant
Non-transfected
EGFP-only
WT ABCA7
V1613M ABCA7

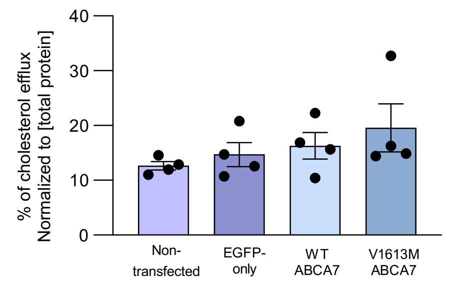

f
Aβ40/[total protein] in cell lysate
Non-transfected
EGFP-only
WT ABCA7
V1613M ABCA7


g
Aβ40/[total protein] in supernatant
Non-transfected
EGFP-only
WT ABCA7
V1613M ABCA7


h
V1613M-ABCA7
2μm
APP
RAB7A


i
V1613M-ABCA7
APP
SNX4


j
Non-transfected
EGFP-only
WT-ABCA7
V1613M-ABCA7


k
% of cholesterol efflux Normalized to [total protein]
40
30
20
10
0
Non-transfected
EGFP-only
WT ABCA7
V1613M ABCA7

Supplementary Figure 8. V1613M ABCA7 overexpressing N2A cells have reduced Aβ40/42 levels, exhibit subcellular colocalization with RAB7+ structures but do not colocalize with SNX4 or effect cholesterol efflux *in vitro*. a) Western blot for mouse ABCA7 in non-transfected, EGFP-only (empty vector), WT-ABCA7 and V1613M-ABCA7 overexpressing N2a cells. b) total protein stain blot for (a), c) Quantification of ABCA7 expression in all four cells lines, indicating higher expression of ABCA7 in WT and V1613M overexpressing lines compared to non-transfected and EGFP- only controls. d) Quantification of Aβ42 normalized to total protein concentration in cell lysate of N2a cell lines, e) Quantification of Aβ42 normalized to total protein concentration in cell supernatant of N2a cell lines, f) Quantification of Aβ40 normalized to total protein concentration in cell lysate of N2a cell lines, g) Quantification of Aβ40 normalized to total protein concentration in cell supernatant of N2a cell lines. Representative confocal images showing 22C11 (APP) and h) RAB7A and i) SNX4 staining in V1613M-ABCA7 overexpressing N2a cells. j) Representative images of secondary antibody only controls for immunocytochemistry staining of N2a cells. No staining is detected. Scale bar = 2 microns, k) Percentage of cholesterol efflux normalized to total protein concentration in N2a cell lines. N=4-5 independent experiments. Data are represented as mean ± SEM. Statistics: One- way ANOVA, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, $^{****} \leq 0.0001$.