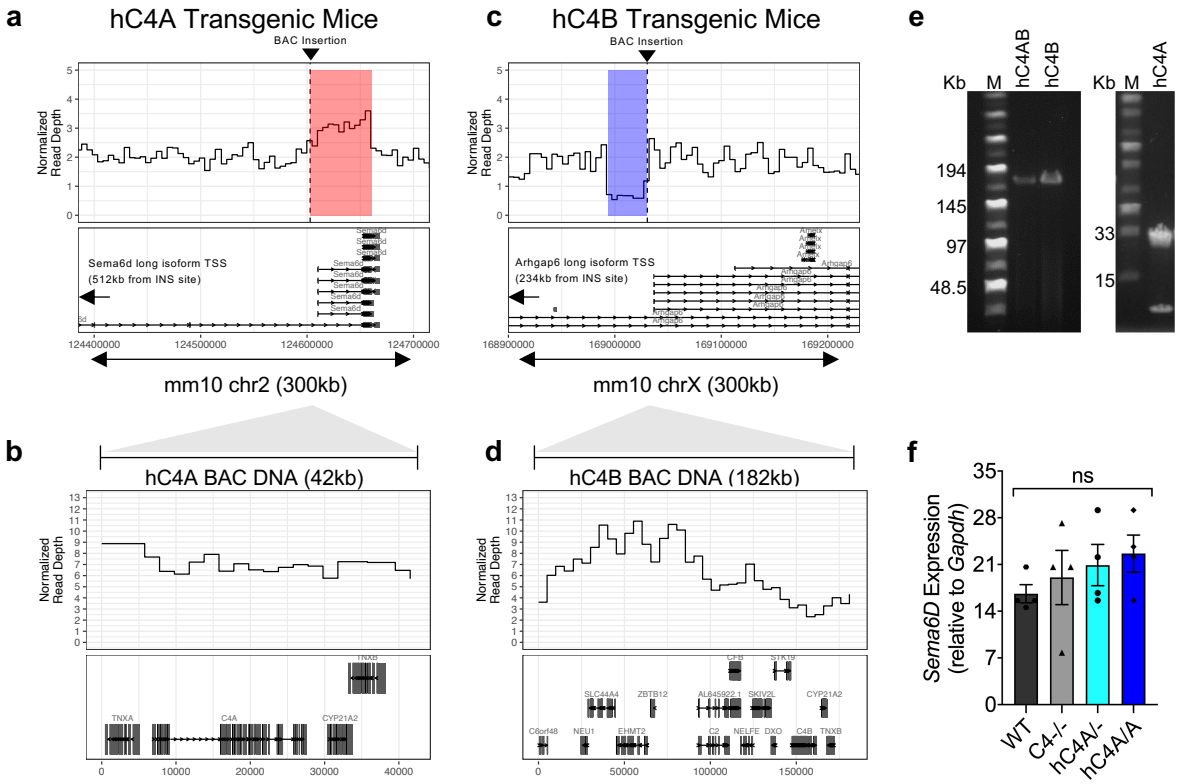
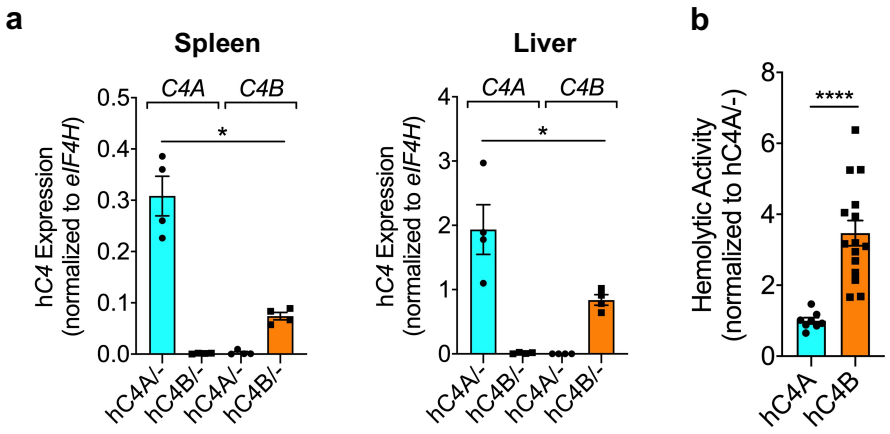


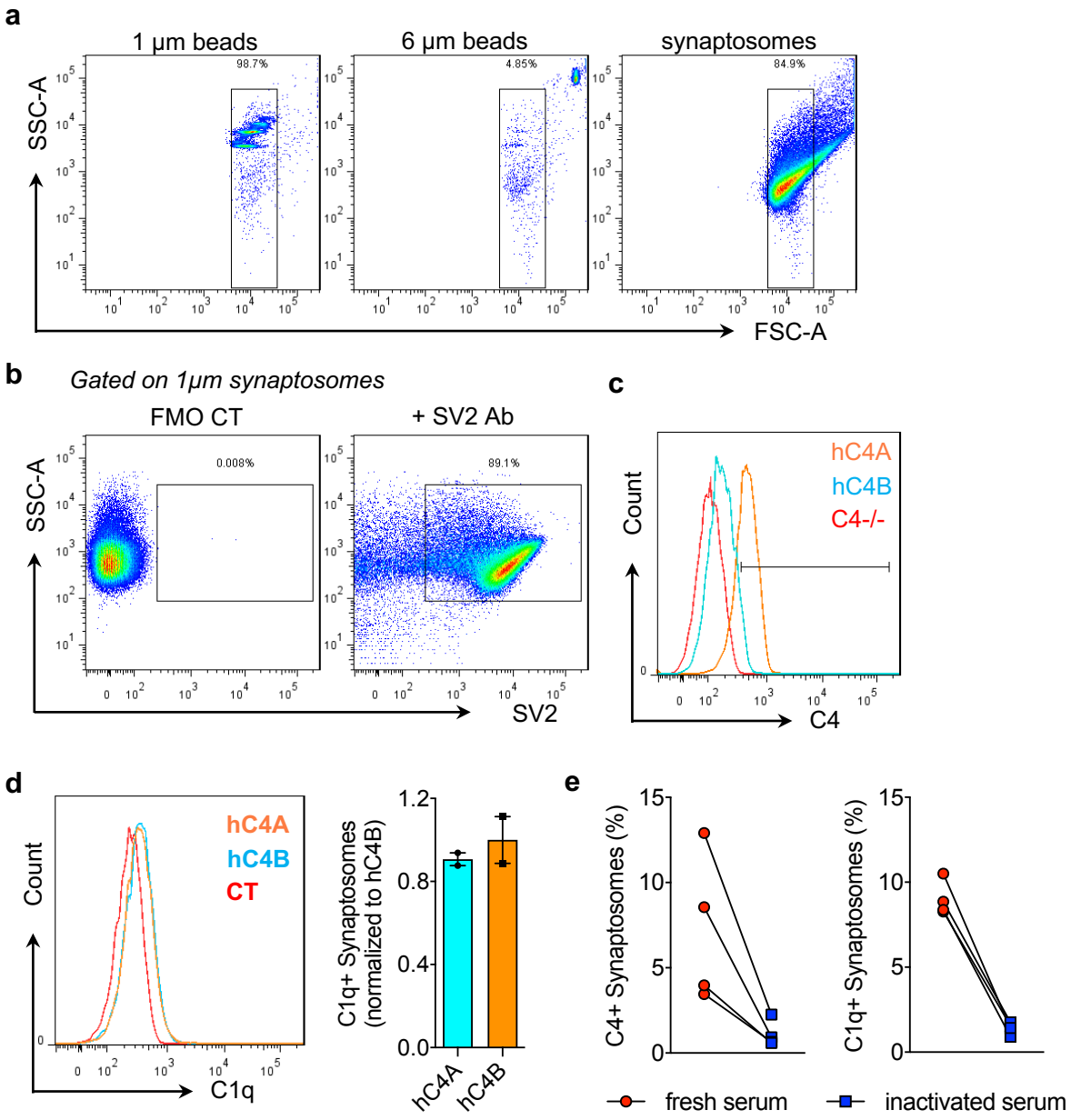
Extended Figure 1: Characteristics of C4 BAC DNA integration

a-d, Insertion sites and associated rearrangements are shown for each mouse strain. **a, c**, The BAC DNA insertion site is shown for each mouse strain. Normalized read depth in 2-kb genomic windows at the insertion sites of the BACs. Dashed vertical lines indicate the insertion site. The hC4A insertion was associated with a duplication, and the hC4B insertion was associated with a small deletion. **b, d**, The BAC DNA–associated rearrangements are shown for each mouse strain. Normalized read depth in 1-kb genomic windows shows the copy number of the inserted constructs. Gene models are shown beneath the read depth plots. Human C4A was likely inserted into hC4A transgenic mice at a normalized read depth between 5 and 9. Human C4B was likely inserted into hC4B transgenic mice at a copy number between 2 and 5. The inserted hC4B BAC constructs contain additional internal copy number variants. **e**, Pulse-field gel showing linearized hC4B and hC4A BAC DNA used for microinjection into zygotes (representative of 3 independent experiments). **f**, *Sema6D* mRNA expression level in cortex was not changed by the region duplication caused by hC4A BAC DNA insertion. *Gapdh* was used as a control housekeeping gene (n = 4 mice per group; ns = $P > 0.05$, Kruskal-Wallis test with Dunn's multiple comparisons test). Bar graph shows mean ± s.e.m



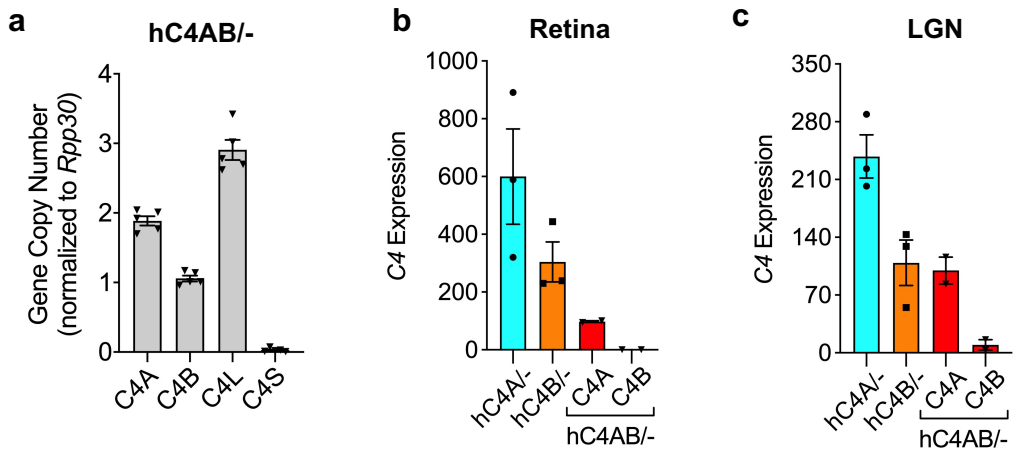
Extended Figure 2: Peripheral expression and function of C4 in hC4 transgenic mice

a, *C4A*- and *C4B*-specific mRNA level was measured by ddPCR in the spleen (left) and liver (right) of hC4A/- and hC4B/- mice. *eiF4H* was used as the control housekeeping gene (n = 4 mice per group; * P_{Spleen} = 0.0286, * P_{Liver} = 0.0286; two-tailed Mann-Whitney test). **b**, C4 hemolytic activity was measured with equal amounts of hC4A or hC4B protein using sensitized sheep red blood cells. Data were normalized to hC4A samples (n = 8 hC4A and n = 15 hC4B combined from 3 independent experiments; **** $P < 0.0001$, Unpaired, two-tailed t test). Bar graphs show mean ± s.e.m.



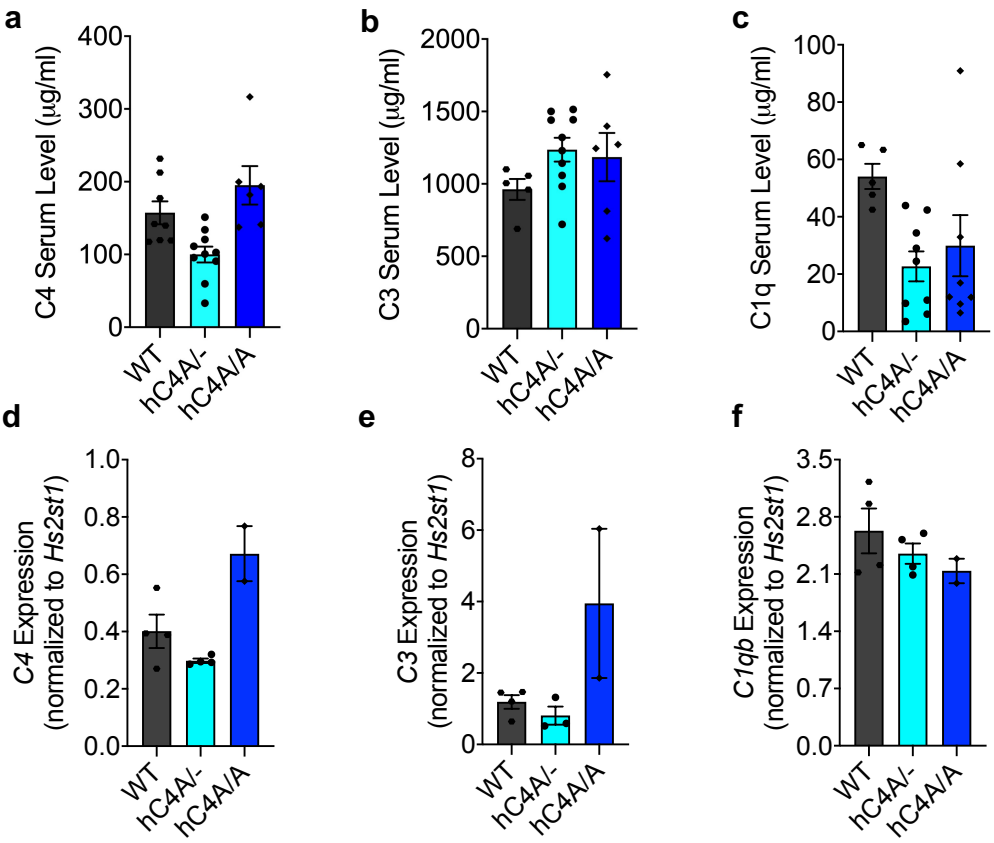
Extended Figure 3: Complement activation and binding to synaptosomes

a, Representative dot plots showing the FSC-A / SSC-A of 1-µm beads, 6-µm beads, and synaptosomes. Further analysis will be gated on 1-µm synaptosomes. **b**, Synaptosomes were permeabilized and stained with anti-SV2 antibody (+ SV2 Ab) or no antibody (FMO CT). More than 85% of the particles analyzed contain SV2 protein. **c,** Representative histogram plot of C4 staining on synaptosomes isolated from C4-/- mice and incubated with serum from C4-/- (red), hC4A (orange), and hC4B (blue) mice. **d**, C1q deposition is shown and quantified using serum from hC4A mice (orange; n = 2), hC4B mice (blue; n = 2), or no serum CT (red). **e**, C4 (left) and C1q (right) deposition was detected on synaptosomes using fresh (red) or heat-inactivated (blue) serum. Bar graph shows mean ± s.e.m.



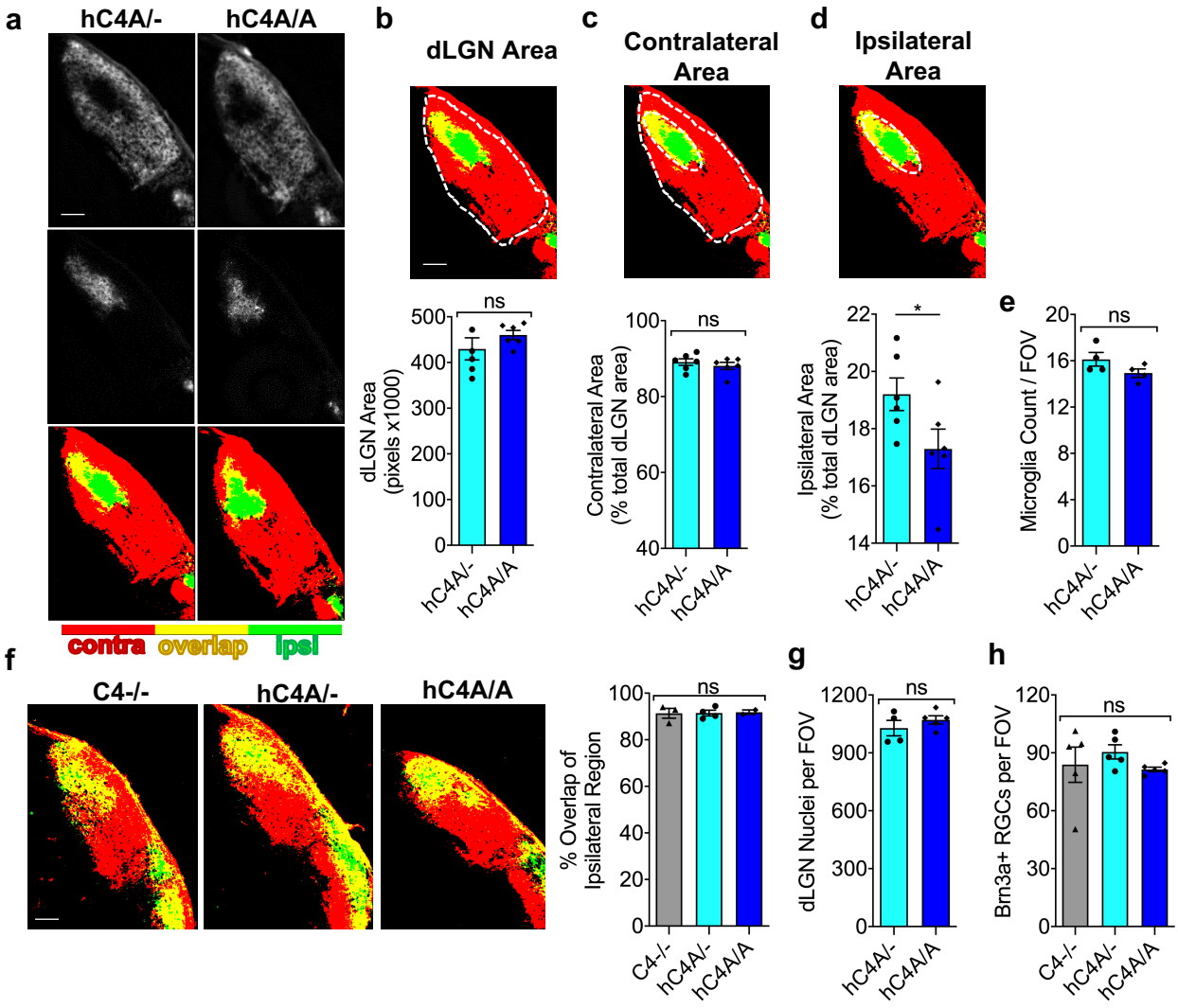
Extended Figure 4: Human C4 in the retinogeniculate system

a, hC4AB/- mice: gene copy numbers for *C4A*, *C4B*, *C4L,* and *C4S* were determined by ddPCR using *Rpp30* as a reference gene. It showed an insertion of two *C4AL* genes and one *C4BL* gene (n = 5 representative mice). Whole-genome sequencing revealed the hC4AB BAC DNA was inserted in chromosome 3 and that one C4A copy was truncated resulting in only one C4A coding copy (data not shown). **b-c**, Absolute quantification by ddPCR was used to measure *C4A*- and *C4B*-specific mRNA level in the retina and LGN from P5 hC4A/- (n = 3), hC4B/- (n = 3), and hC4AB/- mice (n = 2). Bar graphs show mean ± s.e.m.



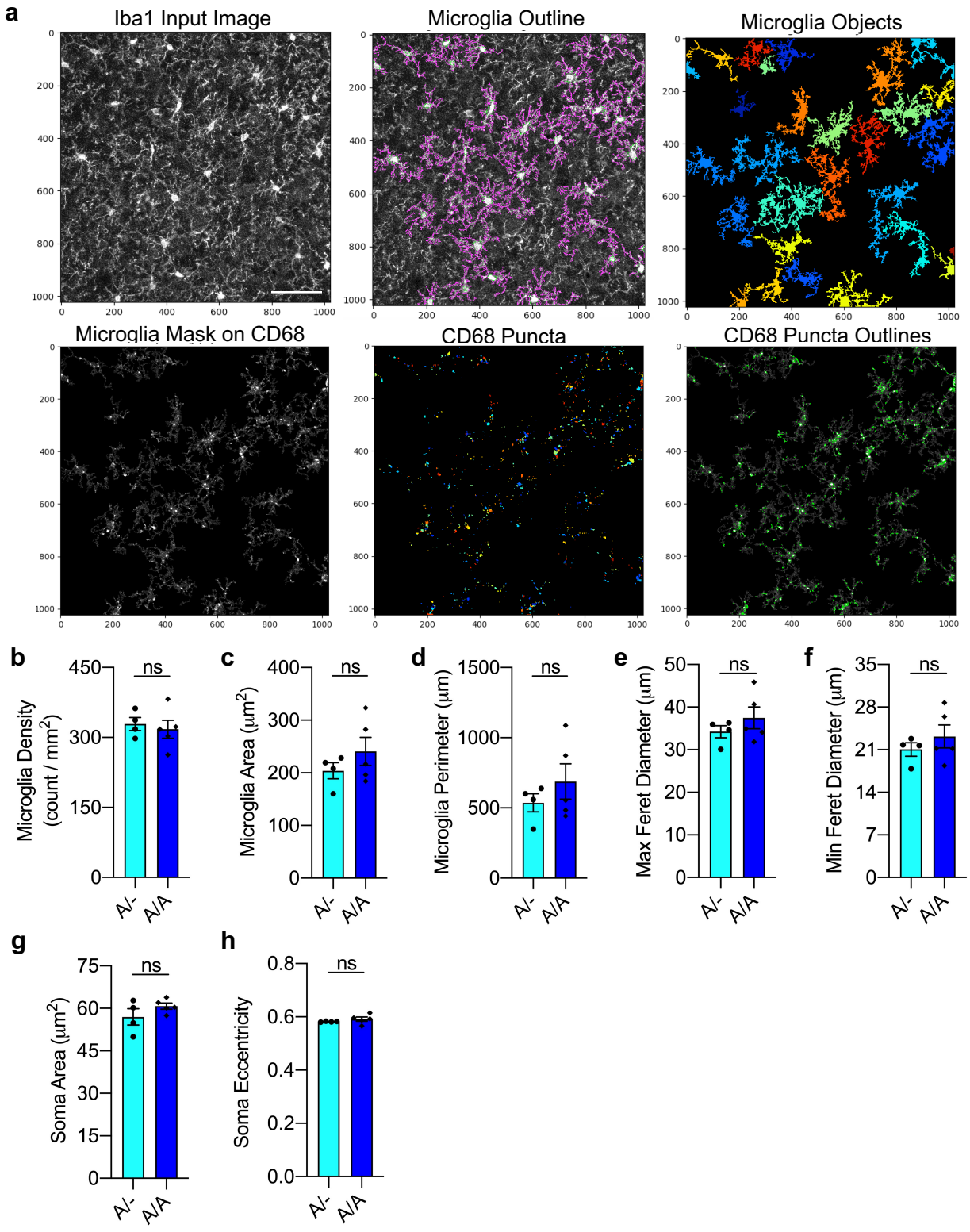
Extended Figure 5: Complement profile in hC4 transgenic mice during adolescence (P40)

a-c, Level of classical complement cascade proteins were measured in serum by ELISA in adolescent (P40) WT, hC4A/-, and hC4A/A mice. **a**, C4 serum level was measured in WT (n = 8), hC4A/- (n = 10), and hC4A/A mice (n = 6). **b**, C3 serum level was measured in WT (n = 5), hC4A/- (n = 10), and hC4A/A mice (n = 6). **c**, C1q serum level was measured in WT (n = 5), hC4A/- (n = 9), and hC4A/A mice (n = 8). **d-f**, RNA expression of classical complement cascade components in the FC were measured by ddPCR in adolescent (P40) WT, hC4A/-, and hC4A/A mice. All RNA measurements were normalized to *Hs2st1* expression level. **d**, *C4* mRNA expression level in FC was measured in WT (n = 4), hC4A/- (n = 4), and hC4A/A mice (n = 2). **e**, *C3* mRNA expression level in FC was measured in WT (n = 4), hC4A/- (n = 3), and hC4A/A mice (n = 2). **f**, *C1qb* mRNA expression level in FC was measured in WT (n = 4), hC4A/- (n = 4), and hC4A/A mice (n = 2). Bar graphs show mean ± s.e.m.



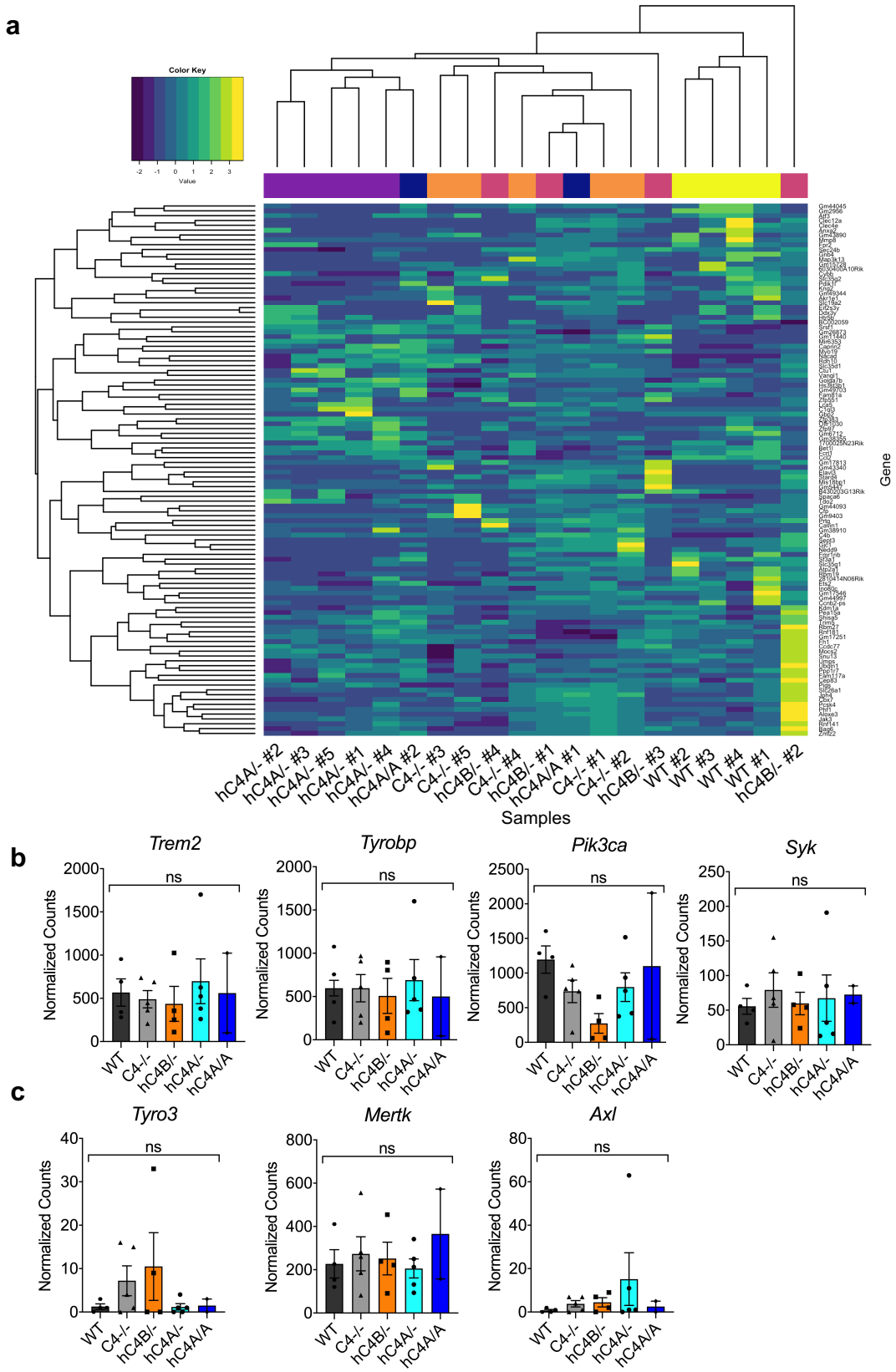
Extended Figure 6: C4A overexpression doesn't change LGN cellularity or morphology

a, Representative images of contralateral (top), ipsilateral (middle), and overlapping regions (bottom; scale = 20 µm). **b-d**, dLGN size (**b**), contralateral area (**c**), and ipsilateral area (**d**) were measured in P10 hC4A/- and hC4A/A mice (n = 6 hC4A/- and n = 6 hC4A/A littermates from 3 independent cohorts; two-tailed Mann-Whitney test; ns = $P > 0.05$, * $P = 0.0411$; scale = 20 µm). **e**, Iba1 staining was used to count the total number of microglia in the dLGN FOV (n = 4 mice per group; two-tailed Mann-Whitney test; ns = $P > 0.05$). **f**, Mice were injected with fluorescent-conjugated cholera toxin subunit-B (CTB) in each eye at P4 and brains were harvested at P5. Representative pseudo-colored images of P5 dLGN from C4-/-, hC4A/-, and hC4A/A littermates of contralateral (red), ipsilateral territories (green), and overlap (yellow) between the two territories (scale = 20 µm). Percent of overlap in ipsilateral region was compared in C4-/- (n = 3), hC4A/- (n = 4), and hC4A/A mice (n = 2; littermates; Kruskal-Wallis test with Dunn's multiple comparisons test; ns = $P > 0.05$). **g**, DAPI was used to count the total number of cells in the dLGN field of view (FOV) (n = 6 hC4A/- and n = 5 hC4A/A littermates from 3 independent cohorts; two-tailed Mann-Whitney test; ns = $P > 0.05$). **h**, The number of Brn3a+ RGCs was compared between C4-/-, hC4A/-, and hC4A/A mice (n = 5 mice per group; Kruskal-Wallis test with Dunn's multiple comparisons test; ns = $P > 0.05$; scale = 20 µm). Bar graphs show mean ± s.e.m.



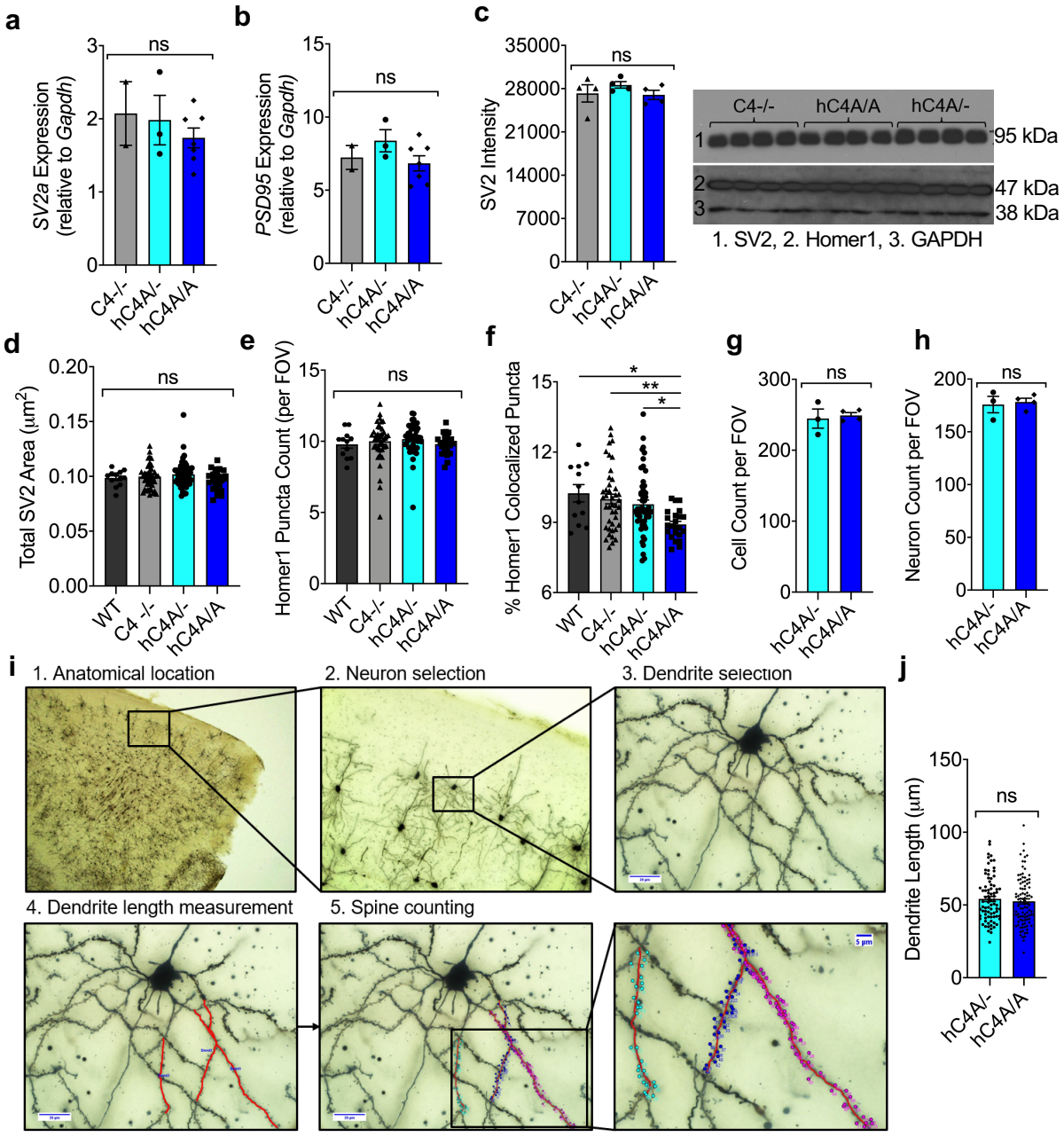
Extended Figure 7: Cell Profiler morphological microglia analysis

a, Cell Profiler software was used to analyze microglia morphology and lysosomal activity in the frontal cortex of P40 hC4A/- and hC4A/A mice. Microglial soma and processes are identified by Iba1 signature by using the image-based watershed method (top row). Microglia are used as a mask to select and quantify intracellular CD68 puncta (bottom row; scale = 50 µm). **b-h**, Morphological parameters for mPFC microglia (**b-f**) and their soma (**g-h**) from hC4A/- (n = 4) and hC4A/A (n = 5) mice were calculated by Cell Profiler software at P40 timepoint (ns = P > 0.05, two-tailed Mann-Whitney test). Bar graphs show mean ± s.e.m.



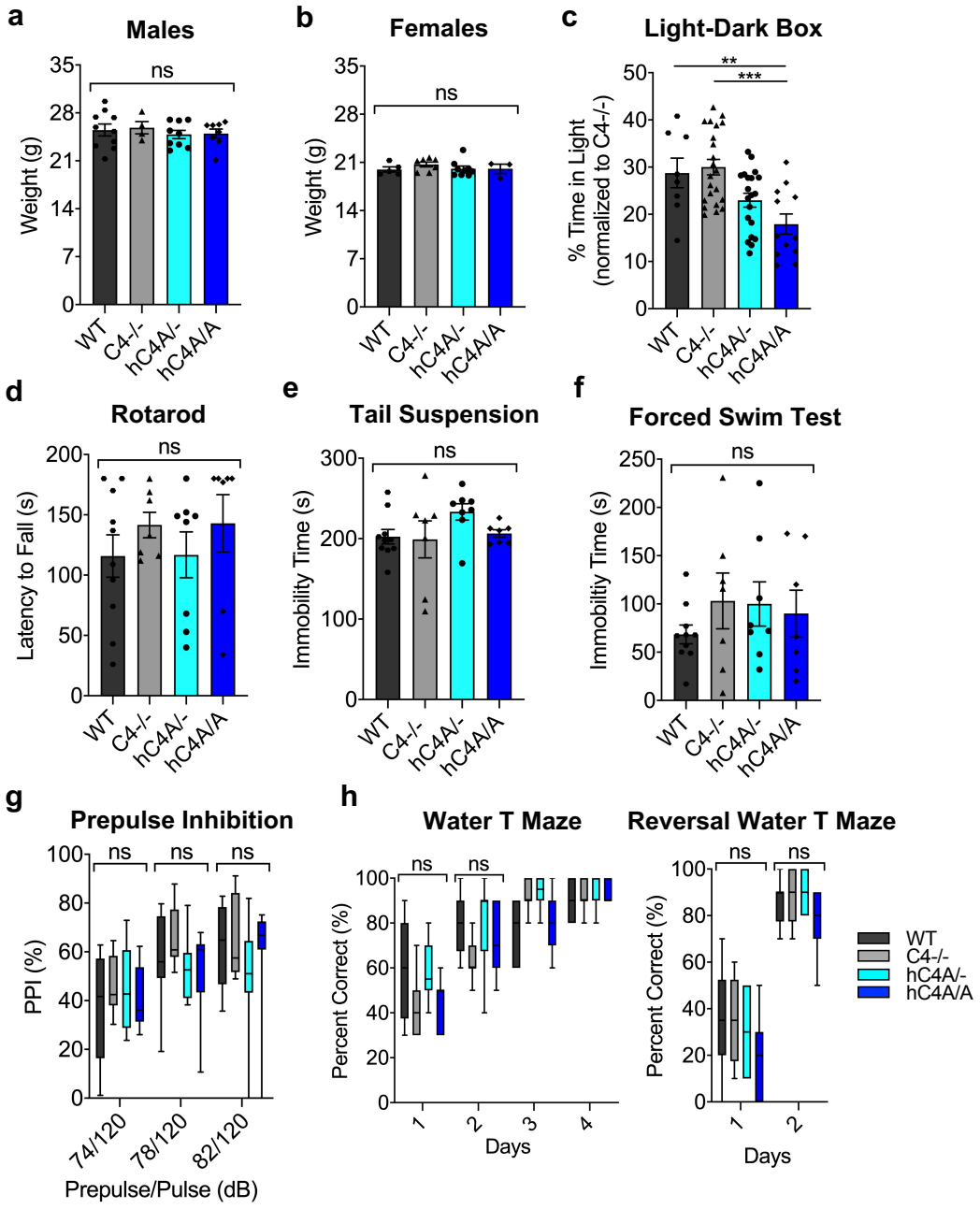
Extended Figure 8: Microglial RNA sequencing analysis reveals no transcriptomic alterations in hC4 transgenic mice

a, Bulk RNA sequencing analysis of microglia isolated from the frontal cortex of adolescent (P40) mice from WT (n = 4), C4-/- (n = 5), and hC4A/- (n = 5), hC4B/- (n = 4), and hC4A/A (n = 2) groups. Heatmap representation of differentially expressed genes between all experimental groups shows no significant transcriptional profile difference between any two groups. **b-c,** Normalized gene counts for the TREM2/DAP12 signaling pathway (**b**) and TAM receptor genes (**c**) were calculated for WT (n = 4), C4-/- (n = 5), hC4A/- (n = 5), hC4B/- (n = 4), and hC4A/A (n = 2; ns = $P > 0.05$, Kruskal-Wallis test with Dunn's test). Bar graphs show mean ± s.e.m.



Extended Figure 9: Synaptic protein expression is not affected by C4A overexpression in mice

a-b, rt-PCR was used to measure mRNA expression of *Sv2a* (**a**) and *Psd95* (**b**) in the FC in adult mice (C4-/- n = 2, hC4A/- n = 3, and hC4A/A littermates n = 7; Kruskal-Wallis test with Dunn's multiple comparisons test; ns = $P > 0.05$). RNA expression was normalized to *Gapdh* expression. **c**, SV2 and PSD95 protein level were analyzed by western blot. GAPDH was used as a loading control protein (C4-/- n = 4, hC4A/- n = 4, and hC4A/A n = 4 littermates from one experiment; Kruskal-Wallis test with Dunn's multiple comparisons test; ns = $P > 0.05$). **d**, Total SV2 area per FOV in the mPFC was calculated from immunofluorescence staining between WT (n = 3), C4-/- (n = 11), hC4A/- (n = 13), and hC4A/A (n = 6) groups (Kruskal-Wallis test with Dunn's multiple comparisons test). **e-f**, Total Homer1 puncta and percentage of colocalized Homer1 puncta from the mPFC in adult mice were calculated for WT (n = 3), C4-/- (n = 11), hC4A/- (n = 13), and hC4A/A (n = 6) groups (Kruskal-Wallis test with Dunn's multiple comparisons test; ns = $P > 0.05$, * $P_{WT\ vs\ A/A} = 0.0139$, ** $P_{WT\ vs\ A/A} = 0.0139$). **g-h**, Brain sections were stained with DAPI and NeuN, and cellularity was measured in frontal cortex at P180. Total cells (g) and neurons (h) per FOV in frontal cortex is represented for hC4A/- (n = 3) and hC4A/A mice (n = 4; two-tailed Mann-Whitney test; ns = $P > 0.05$). **i**, Method used for the manual counting of dendritic spines in mPFC of hC4 mice. **j**, Length of dendrites that have been used for spine density analysis for hC4A/- (80 dendrites) and hC4A/A (96 dendrites; two-tailed Mann-Whitney test; ns = $P > 0.05$). Bar graphs show mean ± s.e.m.



Extended Figure 10: Human C4A overexpression alters mouse behavior

a-h, WT, C4-/-, hC4A/-, and hC4A/A mice were subjected to a battery of behavioral tests. **a-b**, Weight of male and female mice were compared between WT (n = 10), C4-/- (n = 4), hC4A/- (n = 9), and hC4A/A mice (n = 8; Kruskal-Wallis test with Dunn's multiple comparisons test; ns = $P > 0.05$). **c**, Anxiety levels were measured in the light-dark box test by time spent in the light-zone between WT (n = 8), C4-/- (n = 22), hC4A/- (n = 20), and hC4A/A (n = 12) groups (two-tailed, unpaired t test; ** $P = 0.0085$; two-tailed Mann-Whitney test, *** $P = 0.0009$). Results were normalized to the C4-/- group to retain littermate controls. **d**, In the rotarod test, latency to fall was measured in seconds for WT (n = 10), C4-/- (n = 7), hC4A/- (n = 8), and hC4A/A (n = 7) groups (Kruskal-Wallis test with Dunn's multiple comparisons test, ns = $P > 0.05$). **e-f**, Immobility time in seconds was compared in the tail suspension test (**e**) and the forced swim test (**f**) for WT (n = 10), C4-/- (n = 7), hC4A/- (n = 8), and hC4A/A (n = 7) groups (Kruskal-Wallis test with Dunn's multiple comparisons test, ns = $P > 0.05$). **g**, Prepulse inhibition was measured between WT (n = 10), C4-/- (n = 7), hC4A/- (n = 8), and hC4A/A (n = 7) groups (two-way ANOVA with Tukey's multiple comparisons test; ns = $P > 0.05$). **h,** Percent of correct decisions were recorded in the water t maze and the reversal water t maze for WT (n = 10), C4-/- (n = 7), hC4A/- (n = 8), and hC4A/A (n = 7) groups (two-way ANOVA with Tukey's multiple comparisons test; ns = $P > 0.05$). Bar graphs show mean ± s.e.m. Box-and-whisker plots display the median (center line), 25th to 75th percentile (box), and minimum to maximum values (whiskers).