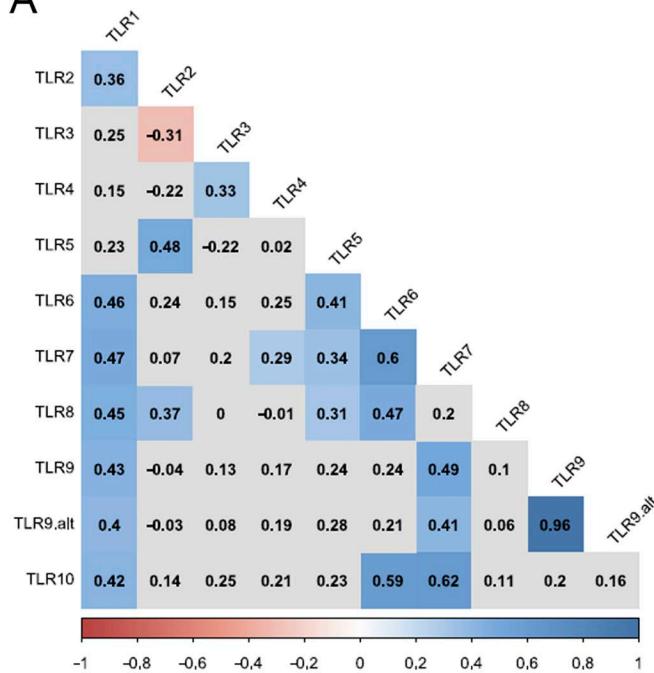


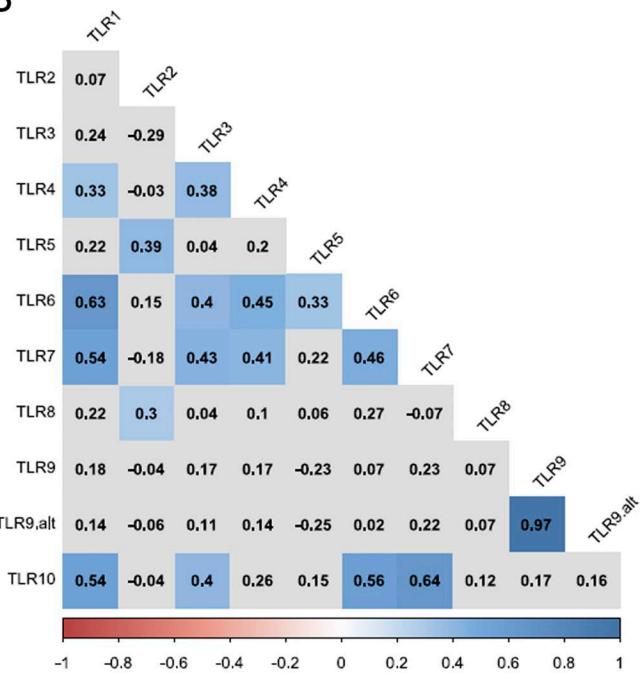
Supplemental material

Chakrabarty et al., <https://doi.org/10.1084/jem.20180484>

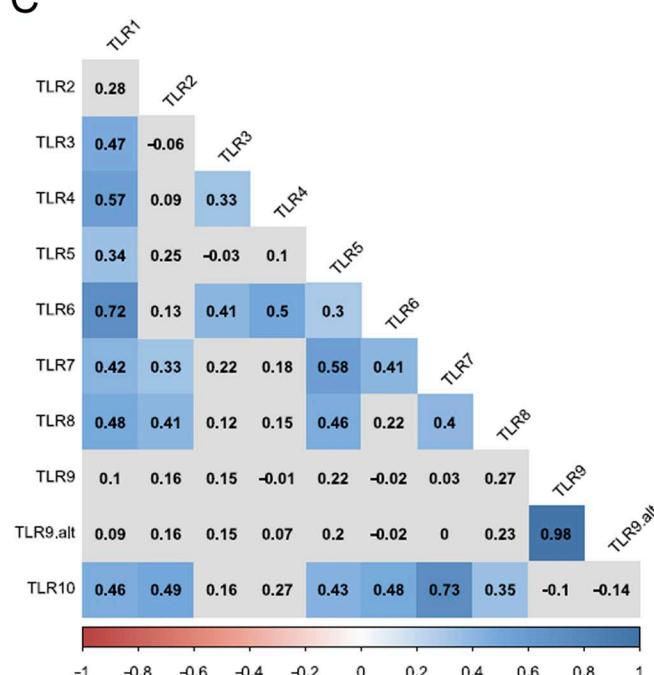
A



B



C



D

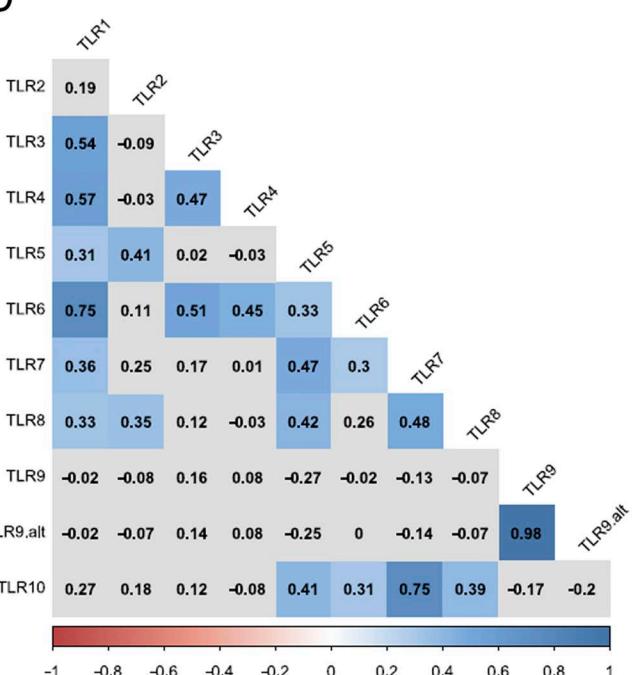


Figure S1. TLR coexpression network in human AD. (A-D) Brain expression correlations of TLR family genes in the TCX of subjects with AD (A); elderly control subjects without neurodegenerative conditions (B); CER of subjects with AD (C); and controls (D). Pairwise Pearson correlations were conducted using gene expression residuals obtained after regressing out the covariates in the comprehensive model (see Tables S3–S6). Those pairwise comparisons that are statistically significant at $P < 0.05$ are colored. Colors depict the correlation coefficients ranging between -1 and 1. Numbers in the squares are the correlation coefficient values for each pairwise comparison. Genes are depicted in the same order to enable comparisons across the four analyses. Correlation plots generated using “corrplot” R-Package (Wei and Simko, 2016).

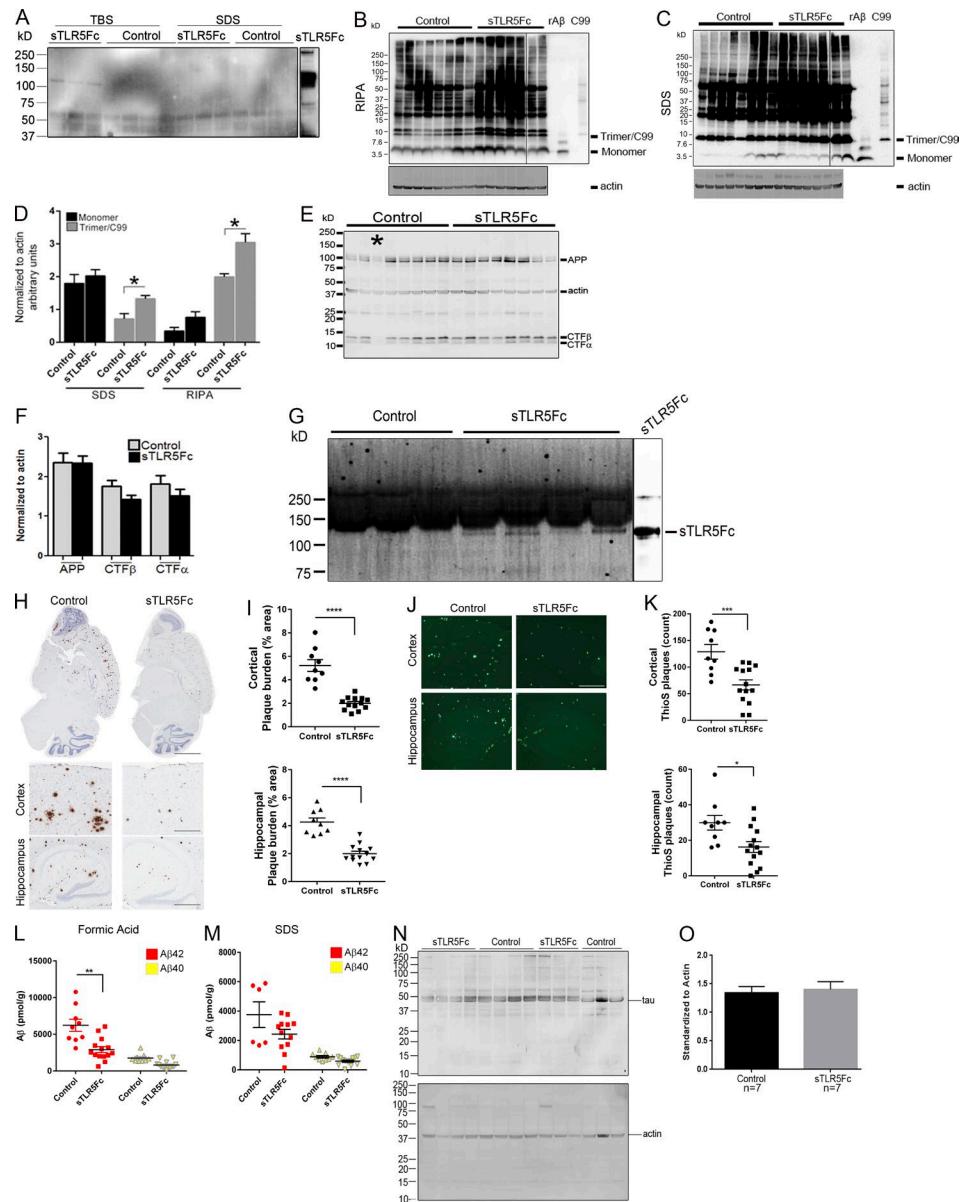


Figure S2. sTLR5Fc alters Aβ plaques but does not affect APP levels. **(A)** sTLR5Fc protein could be detected in the Tris-buffered saline-extracted brains of sTLR5Fc-expressing CRND8 mice, but not in control mice. The right lane depicts sTLR5Fc-recombinant protein preparation purified from Chinese hamster ovary stable cell line. The panels of the immunoblot (divided by a perpendicular black line) depict dissimilar image manipulations performed on the immunoblot, which allowed for better depiction of the sTLR5Fc band in the mouse brains without over-exposing the lanes (on the right) containing recombinant sTLR5Fc protein. **(B and C)** Representative 82E1 immunoblots of 6-mo-old sTLR5Fc-expressing mice show significant changes in a band corresponding to Aβ trimer/C99 compared with control mice in SDS- and RIPA-extracted cellular fractions. The right two lanes show a representative aggregated Aβ42 preparation (rAβ42) and a Chinese hamster ovary cell lysate overexpressing APP CTF β (C99). The lower panel represents the 82E1 blot reprobed with anti-actin antibody to depict loading amount. Molecular weight markers are indicated on the left (kilodaltons). Black lines indicate that intervening lanes have been spliced out. **(D)** Quantitative densitometric analysis of the Aβ trimer/C99 immunoreactive band normalized to actin is depicted. Data represent mean \pm SEM; $n = 7-8$ /group. * $P < 0.05$; one-way ANOVA. **(E and F)** No significant change was observed in APP, CTF α , and CTF β levels in sTLR5Fc compared with control mice. Data represent mean \pm SEM; $n = 7-8$ /group. **(G)** sTLR5Fc protein could be immunoprecipitated from plasma of sTLR5Fc-expressing TgCRND8 mice using human anti-Fc antibody and detected with anti-V5 antibody. Recombinant sTLR5Fc is depicted in the right lane (separated by a black line) for determining molecular weight of the plasma-derived material. Molecular weight markers are indicated on the left (kilodaltons). **(H–M)** An independent cohort confirms that rAAV2/1-sTLR5Fc reduces Aβ plaque deposition in TgCRND8 mice. Neonatal CRND8 mice were injected with AAV2/1-sTLR5Fc in the cerebral ventricles and analyzed after 6 mo for Aβ plaques (H–K) and biochemical Aβ levels (L and M). **(H–K)** Both anti-Aβ mAb Ab5 (biotinylated; H and I) and thioflavin S (ThioS) staining (J and K) depict reduced Aβ deposition in the hippocampus and cortex of sTLR5Fc mice (4F, 9M) compared with naive controls (4F, 5M). Scale bars, 125 μ m. Data represent mean \pm SEM; $n = 9-14$ /group. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$. **(L and M)** Biochemical analyses of sequentially extracted Aβ42 and Aβ40 levels by end-specific sandwich ELISA showed that sTLR5Fc reduces formic acid-extractable insoluble Aβ42 levels. Three mice from the SDS/control group were excluded from Aβ42 analysis for technical issues. Data represents mean \pm SEM; $n = 9-14$ /group. ** $P < 0.01$; one-way ANOVA. **(N and O)** Endogenous mouse tau levels are unaffected in sTLR5Fc-expressing TgCRND8 mice. Total mouse tau protein levels were analyzed from cohort 1 (described in Fig. 2). **(O)** Quantitative densitometric analysis of the tau immunoreactive band in the RIPA-soluble fraction, normalized to actin levels, is depicted. Data represent mean \pm SEM; $n = 7$ /group.

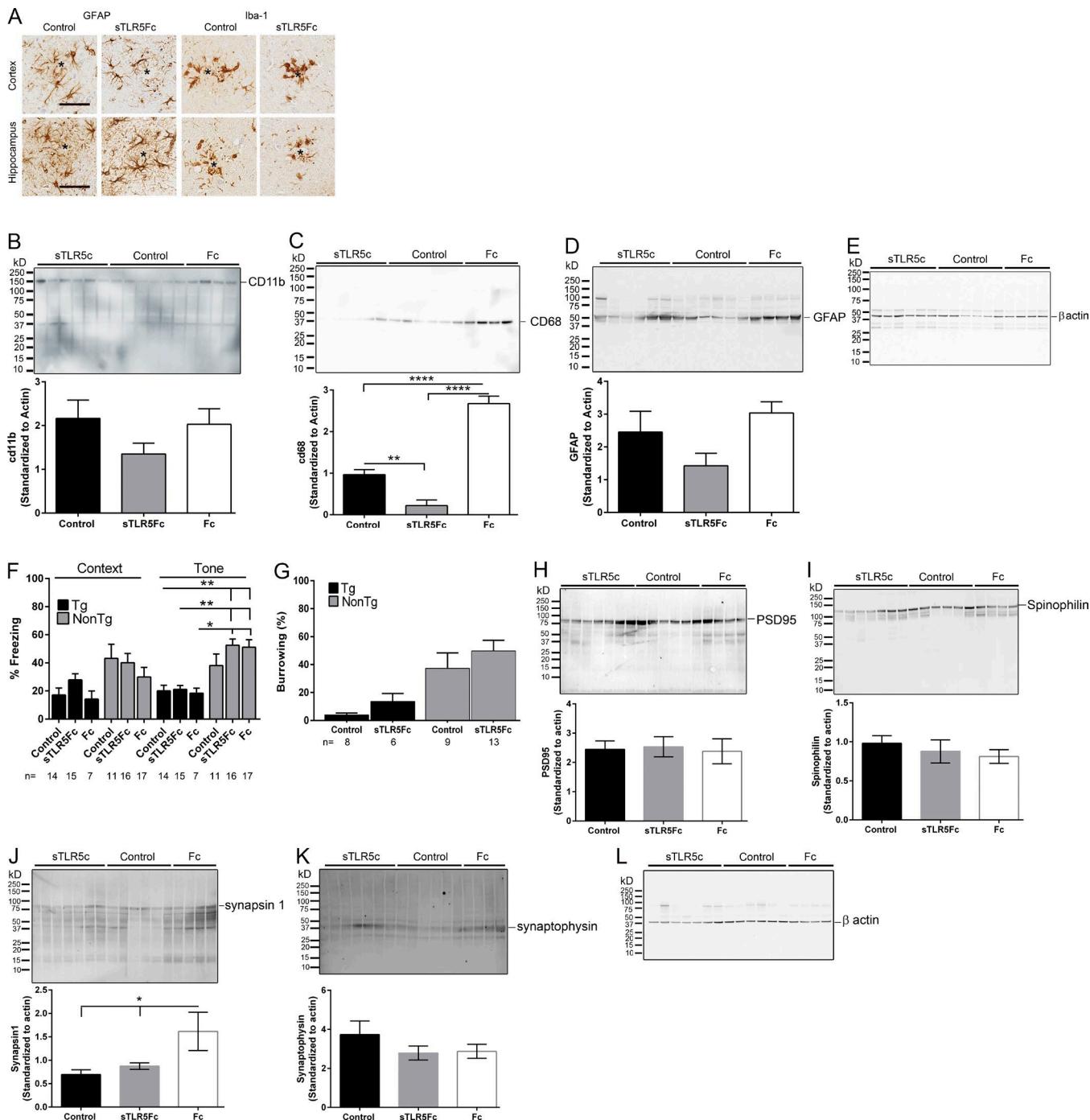


Figure S3. sTLR5Fc expression in transgenic CRND8 mice alters immune activation but does not alter cognitive impairment. **(A)** Representative plaque-associated GFAP and Iba-1 immunostaining in cortex and hippocampus of sTLR5Fc and control mice. Asterisks denote A β plaque core. Scale bars, 50 μ m. **(B-D)** CD11b (B), CD68 (C), and GFAP (D) immunoblotting shows decreasing levels of astrogliosis and microgliosis in sTLR5Fc mice compared with control mice and Fc-expressing mice. Molecular weight standards are depicted in the left panel in kilodaltons. **(E)** Quantitative densitometric analysis of immunoreactive bands of interest is depicted following normalization to actin levels. $n = 4-6/\text{group}$. ** $P < 0.01$; *** $P < 0.001$; one-way ANOVA. **(F-L)** Age-related increase in A β plaque burden is associated with impairment in conditioned fear memory in CRND8 mouse model of amyloidosis as represented by freezing immediately following the presentation of a foot shock. **(F)** Overall, the tone fear memory of transgenic CRND8 mice (Tg) was impaired compared with control nontransgenic mice (NonTg) in the three treatment groups. The sTLR5Fc-expressing transgenic mice showed a trend toward improved context fear memory compared with control transgenic mice. No changes were seen in tone fear memory. For Tg mice, control = 14, sTLR5Fc = 15, and Fc = 7; for NonTg mice, control = 11, sTLR5Fc = 16, and Fc = 17. * $P < 0.05$, ** $P < 0.01$; one-way ANOVA. **(G)** No significant effect on burrowing was noticed across genotype and treatment groups. For Tg mice, control = 8 and sTLR5Fc = 6; for NonTg mice, control = 9 and sTLR5Fc = 13 (burrowing test, B). **(H-L)** Immunoblotting for different synaptic proteins (PSD95, spinophilin, synapsin 1, and synaptophysin) was done for sTLR5Fc, Fc, and control transgenic mice. Quantitative densitometric analysis of the immunoreactive band(s) normalized to actin (L) is depicted below the corresponding immunoblotting panel. Data represent mean \pm SEM. $n = 4-7/\text{group}$. * $P < 0.05$; one-way ANOVA.

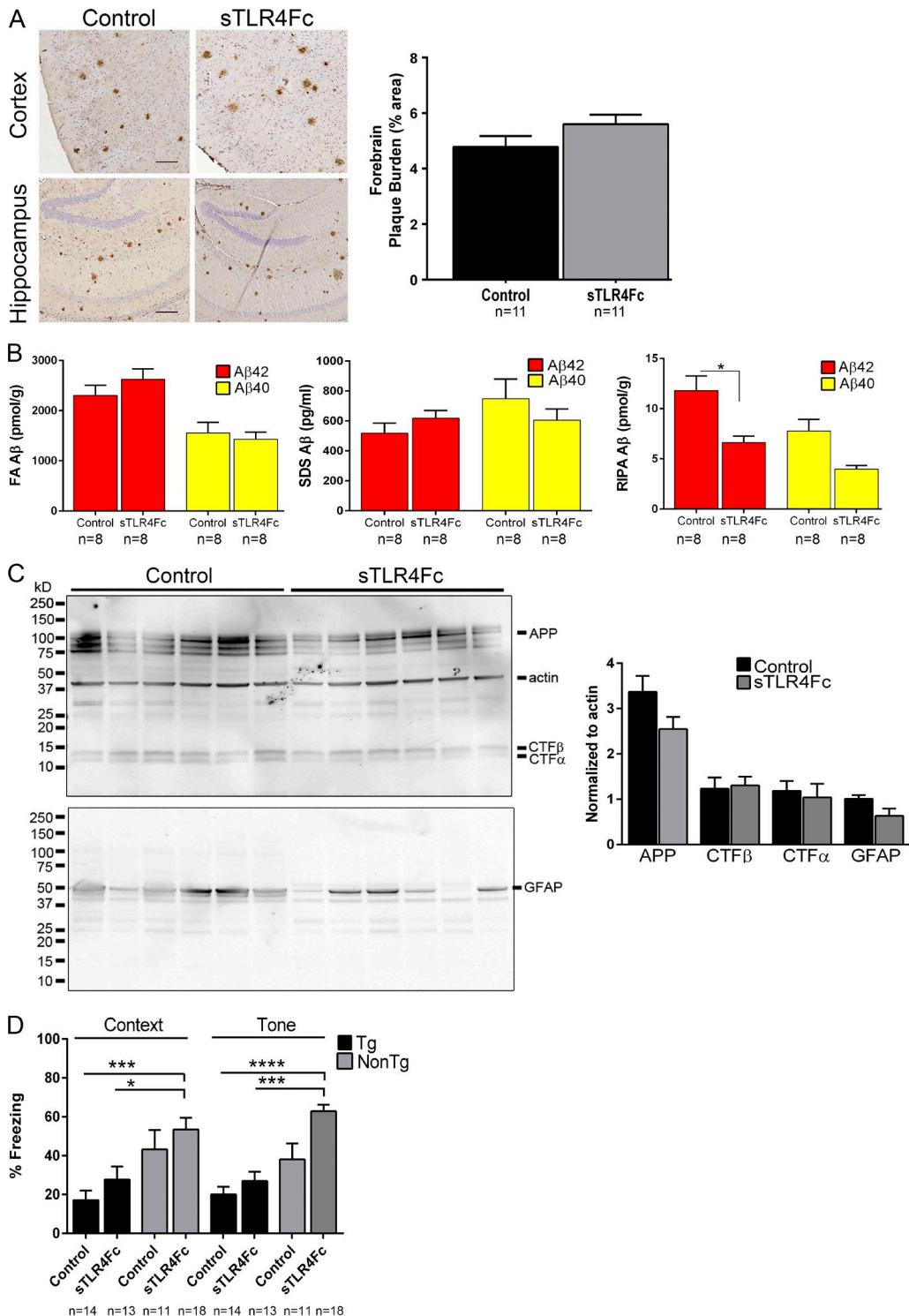


Figure S4. rAAV2/1-sTLR4Fc does not alter A β plaque deposition. **(A)** Neonatal CRND8 mice were injected with either AAV2/1-sTLR4Fc or AAV2/1-EGFP (control) in the cerebral ventricles and analyzed after 6 mo for A β plaque pathology and plaque burden in the cortex and hippocampus using anti-A β mAb 33.1.1. Scale bars, 125 μ m (cortex and hippocampus). n = 11/group. Data represent mean \pm SEM. **(B)** Biochemical analyses of sequentially extracted A β 42 and A β 40 levels by end-specific sandwich ELISA show unaltered formic acid- and SDS-extractable insoluble A β levels in sTLR4Fc-expressing mice compared with EGFP-expressing mice. RIPA-extractable A β 42 levels (but not A β 40) are reduced in sTLR4Fc-expressing mice. Data represent mean \pm SEM. n = 8/group. *P < 0.05; one-way ANOVA. **(C)** No significant change was noticed in APP, CTFs, and astrogliosis marker (GFAP) in sTLR4Fc-expressing mice compared with controls. Quantitative densitometric analysis of the immunoreactive band normalized to actin is depicted. Molecular weight standards are depicted in the left panel in kilodaltons. Data represent mean \pm SEM n = 6/group. **(D)** No significant change was noted in fear-conditioning test (context and tone fear) in transgenic (Tg) or nontransgenic (NonTg) cohorts following expression of sTLR4Fc, though the transgenic control mice showed significant impairment compared with nontransgenic mice in both the control and treatment groups. Data represent mean \pm SEM. For Tg mice, control = 14 and sTLR4Fc = 13; for NonTg mice, control = 11 and sTLR4Fc = 18. *P < 0.05, ***P < 0.001, ****P < 0.0001; one-way ANOVA.

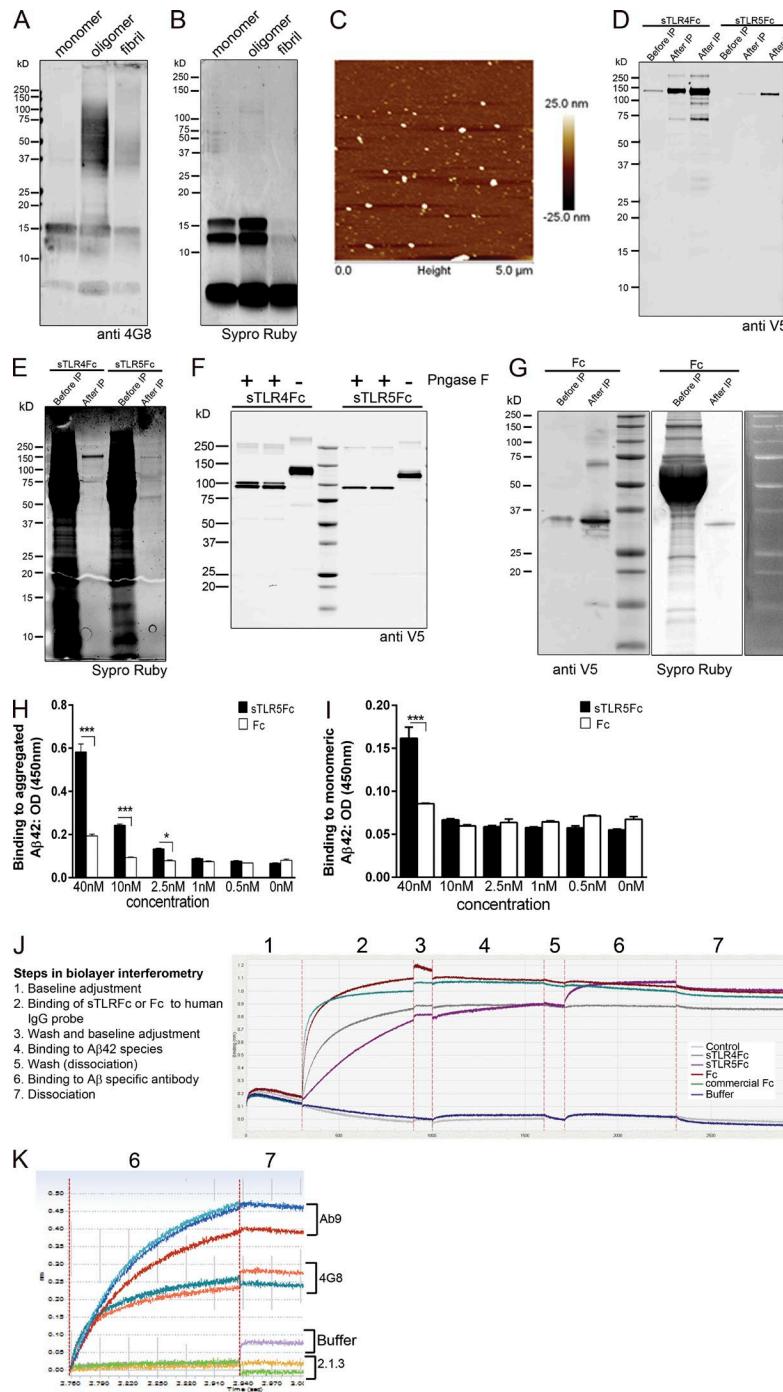


Figure S5. Delineating the binding parameters of recombinant sTLR5Fc to A β . **(A and B)** Characterization of the recombinant A β preparations on a 4G8 immunoblot (A) and on a Sypro Ruby-stained gel (B) following separation on reducing PAGE. **(C)** A representative picture of oligomeric A β 42 analyzed by atomic force microscopy is shown. **(D-G)** Recombinant sTLR4Fc, sTLR5Fc, or Fc used for in vitro binding experiments was purified by immunoprecipitation (IP) of corresponding clonal cell line media using anti-human IgG. Representative pictographs of the recombinant protein preparations on a V5 immunoblot (D and G) and on a Sypro Ruby-stained gel (E and G) following separation on reducing PAGE before and after immunoprecipitation purification are shown. Recombinant sTLR4Fc and sTLR5Fc proteins migrate at a molecular weight higher than the expected size (~99 kD). **(F)** This is likely a result of posttranslational glycosylation, as deglycosylation with PNGaseF results in both sTLR4Fc and sTLR5Fc banding at the expected molecular weight. Molecular weight standards are depicted in the left panel in kilodaltons. **(H and I)** In an ELISA-based assay, recombinant sTLR5Fc showed stronger association with fibrillar A β 42 (H) than monomeric A β 42 (I) in a dose-dependent manner. In this assay, sTLR5Fc was allowed to bind to different A β species on an ELISA plate, and the bound molecules were detected by an anti-V5 tag antibody. *P < 0.05, ***P < 0.001. Data represent mean \pm SEM. **(J)** Biolayer interferometry was used in a second assay to detect sTLR5Fc-A β interaction, as shown stepwise. Using bilayer interferometry, A β 42 could be shown to bind to recombinant sTLR5Fc (purple), but not to recombinant sTLR4Fc (dark gray), recombinant Fc protein (red), or commercially available recombinant Fc protein (green). **(K)** sTLR5Fc-A β complex bound to Octet sensors was detected in step 6 using anti-A β 1-16 antibody (Ab5). This complex (sTLR5Fc-A β) could also be detected using the anti-A β 16-25 antibody, 4G8, but not by a C-terminal A β 42 end-specific antibody (2.1.3).

Table S1. Human subjects used in TLR coexpression analysis

Variables	AD (n = 84)	Control (n = 80)	AD (n = 86)	Control (n = 80)
Mean age ± SD (range)	82.4 ± 7.7 (60–90)	82.6 ± 8.8 (53–90)	82.5 ± 7.7 (60–90)	82.5 ± 8.3 (58–90)
APOE4 positive/negative/null (% APOE4 positive)	43/41 (51%)	10/70 (13%)	43/43 (50%)	11/69 (14%)
Female (%)	48 (57%)	39 (49%)	49 (57%)	39 (49%)
Mean RIN ± SD (range)	8.6 ± 0.5 (7.7–10.0)	7.6 ± 1.0 (5.3–9.7)	8.3 ± 0.8 (5.7–10.0)	7.6 ± 1.0 (5.5–9.7)

Table S2. Differential expression of TLR genes in the human TCX

Gene name	CQN_AD	CQN_CT	Model	Dx.Beta ± SE	Effect direction	Dx.P value	Dx.q Value
	Mean ± SEM	Mean ± SEM					
<i>TLR1</i>	1.8059 ± 0.08	1.5703 ± 0.14	Comprehensive	0.0440 ± 0.15	UpInAD	7.75E-01	8.96E-01
			Simple	0.7972 ± 0.23	UpInAD	8.63E-04	6.02E-03
<i>TLR2</i>	3.4546 ± 0.08	3.1565 ± 0.13	Comprehensive	0.2888 ± 0.15	UpInAD	5.91E-02	3.38E-01
			Simple	0.9230 ± 0.23	UpInAD	9.16E-05	1.16E-03
<i>TLR3</i>	0.9502 ± 0.09	0.9555 ± 0.1	Comprehensive	-0.4218 ± 0.14	DownInAD	2.75E-03	9.14E-02
			Simple	0.4277 ± 0.20	UpInAD	3.12E-02	6.99E-02
<i>TLR4</i>	5.5387 ± 0.08	4.8019 ± 0.1	Comprehensive	0.3507 ± 0.15	UpInAD	2.09E-02	2.23E-01
			Simple	1.1496 ± 0.19	UpInAD	1.83E-08	2.66E-06
<i>TLR5</i>	2.145 ± 0.07	1.8712 ± 0.1	Comprehensive	0.2182 ± 0.13	UpInAD	8.46E-02	3.80E-01
			Simple	0.6814 ± 0.18	UpInAD	1.89E-04	1.99E-03
<i>TLR6</i>	1.2308 ± 0.09	1.1047 ± 0.12	Comprehensive	-0.0990 ± 0.12	DownInAD	4.14E-01	6.67E-01
			Simple	0.6898 ± 0.23	UpInAD	3.16E-03	1.41E-02
<i>TLR7</i>	1.248 ± 0.1	1.3926 ± 0.15	Comprehensive	0.03805 ± 0.17	UpInAD	8.19E-01	9.18E-01
			Simple	0.6063 ± 0.26	UpInAD	2.12E-02	5.21E-02
<i>TLR8</i>	-0.5576 ± 0.13	-1.0422 ± 0.16	Comprehensive	0.17590 ± 0.23	UpInAD	4.40E-01	6.87E-01
			Simple	1.1801 ± 0.32	UpInAD	3.25E-04	2.97E-03
<i>TLR9</i>	3.8658 ± 0.04	3.8829 ± 0.06	Comprehensive	-0.0271 ± 0.06	DownInAD	6.76E-01	8.41E-01
			Simple	-0.2505 ± 0.08	DownInAD	3.01E-03	1.37E-02
<i>TLR9.alt</i>	3.906 ± 0.04	3.8984 ± 0.05	Comprehensive	-0.0386 ± 0.06	DownInAD	5.42E-01	7.59E-01
			Simple	-0.2844 ± 0.09	DownInAD	1.20E-03	7.55E-03
<i>TLR10</i>	0.8639 ± 0.09	1.2294 ± 0.13	Comprehensive	-0.2299 ± 0.20	DownInAD	2.63E-01	5.47E-01
			Simple	0.1592 ± 0.24	UpInAD	5.05E-01	6.07E-01

Gene expression measurements were done using RNAseq in TCX of subjects with AD and old control subjects without neurodegenerative neuropathology ($n = 156$). Simple model includes covariates' age at death, gender, RIN, tissue source, and flowcell. Comprehensive model includes these and also five cell type markers to account for cell count differences between AD and control tissue. These markers are for the following genes (cell types): CD68 (microglia), CD34 (endothelial), OLIG2 (oligodendroglia), GFAP (astrocyte), and ENO2 (neuron). AD, Alzheimer's disease patient; CT, nondemented aged patient; CQN, conditional quartile normalization; Dx.Beta, effect size for diagnosis (AD = 1, control = 0); Dx.Beta ± SE, standard error for effect size; Dx.P value, uncorrected P value for association with diagnosis; Dx.q Value, false rates of discovery-corrected q value for association with diagnosis. Effect direction is based on the direction of Beta and does not take into consideration the statistical significance. Bold q values <0.05 ; bold italic q values >0.05 and <0.1 .

Table S3. Differential expression of TLR genes in human CER

Gene name	CQN_AD	CQN_CT	Model	Dx.Beta ± SE	Effect direction	Dx.P value	Dx.q value
	Mean ± SEM						
<i>TLR1</i>	-1.031 ± 0.09	-1.1435 ± 0.11	Comprehensive	0.4291 ± 0.18	UpInAD	2.05E-02	7.17E-02
			Simple	0.5786 ± 0.18	UpInAD	1.65E-03	8.55E-03
<i>TLR2</i>	0.6978 ± 0.06	0.5785 ± 0.07	Comprehensive	-0.0049 ± 0.11	DownInAD	9.65E-01	9.79E-01
			Simple	0.2369 ± 0.13	UpInAD	7.70E-02	1.51E-01
<i>TLR3</i>	-1.7025 ± 0.08	-1.3625 ± 0.08	Comprehensive	-0.0517 ± 0.13	DownInAD	6.98E-01	8.03E-01
			Simple	0.1231 ± 0.13	UpInAD	4.13E-01	5.37E-01
<i>TLR4</i>	2.4245 ± 0.09	2.2198 ± 0.09	Comprehensive	0.4562 ± 0.13	UpInAD	3.84E-03	2.35E-02
			Simple	0.6883 ± 0.13	UpInAD	6.94E-05	1.04E-03
<i>TLR5</i>	-1.6374 ± 0.08	-1.7783 ± 0.1	Comprehensive	0.1080 ± 0.13	UpInAD	4.89E-01	6.37E-01
			Simple	0.3519 ± 0.13	UpInAD	5.47E-02	1.16E-01
<i>TLR6</i>	-1.7512 ± 0.1	-1.8561 ± 0.11	Comprehensive	0.2731 ± 0.13	UpInAD	1.72E-01	3.13E-01
			Simple	0.5307 ± 0.13	UpInAD	9.36E-03	3.05E-02
<i>TLR7</i>	-2.5722 ± 0.11	-2.5085 ± 0.14	Comprehensive	-0.0697 ± 0.13	DownInAD	7.58E-01	8.45E-01
			Simple	0.1989 ± 0.13	UpInAD	4.09E-01	5.33E-01
<i>TLR8</i>	-4.3613 ± 0.13	-4.5885 ± 0.13	Comprehensive	0.4219 ± 0.13	UpInAD	1.13E-01	2.33E-01
			Simple	0.5621 ± 0.13	UpInAD	3.28E-02	7.90E-02
<i>TLR9</i>	2.8813 ± 0.03	2.7909 ± 0.04	Comprehensive	-0.0546 ± 0.13	DownInAD	4.62E-01	6.14E-01
			Simple	-0.0625 ± 0.13	DownInAD	3.74E-01	4.99E-01
<i>TLR9.alt</i>	2.9214 ± 0.03	2.8176 ± 0.04	Comprehensive	-0.0537 ± 0.13	DownInAD	4.38E-01	5.93E-01
			Simple	-0.0784 ± 0.13	DownInAD	2.33E-01	3.49E-01
<i>TLR10</i>	-2.6543 ± 0.09	-2.364 ± 0.11	Comprehensive	-0.2299 ± 0.13	DownInAD	2.63E-01	4.19E-01
			Simple	-0.2206 ± 0.13	DownInAD	2.67E-01	3.88E-01

Gene expression measurements were done using RNAseq in CER of subjects with AD and old control subjects without neurodegenerative neuropathology. Simple model includes covariates' age at death, gender, RIN, tissue source, and flowcell. Comprehensive model includes additional covariates, such as cell type markers to account for cell count differences between AD and control tissue. AD, Alzheimer's disease patient; CT, nondemented aged patient; CQN, conditional quartile normalization; Dx.Beta, effect size for diagnosis (AD = 1, control = 0); Dx.Beta ± SE, standard error for effect size; Dx.P value, uncorrected P value for association with diagnosis; Dx.q value, false rates of discovery-corrected q value for association with diagnosis. Effect direction is based on the direction of Beta and does not take into consideration the statistical significance. Bold q values <0.05; bold italic q values >0.05 and <0.1.

Table S4. TLR family gene expression correlations in TCX of AD subjects

TLR5	TLR9	TLR9.alt	TLR10	TLR1	TLR6	TLR2	TLR3	TLR4	TLR7	TLR8	
TLR5	1	3.51E-02	1.25E-02	4.02E-02	3.62E-02	1.59E-04	6.40E-06	5.13E-02	8.58E-01	2.20E-03	4.74E-03
TLR9	0.24	1	0.00E+00	7.66E-02	7.68E-05	3.47E-02	7.26E-01	2.43E-01	1.24E-01	4.75E-06	3.97E-01
TLR9.alt	0.28	0.96	1	1.49E-01	2.25E-04	5.58E-02	8.16E-01	4.64E-01	8.34E-02	1.54E-04	6.02E-01
TLR10	0.23	0.2	0.16	1	1.00E-04	7.94E-09	2.05E-01	2.59E-02	5.88E-02	1.12E-09	3.36E-01
TLR1	0.23	0.43	0.4	0.42	1	2.16E-05	8.76E-04	2.58E-02	1.95E-01	1.39E-05	2.96E-05
TLR6	0.41	0.24	0.21	0.59	0.46	1	3.03E-02	1.95E-01	2.42E-02	5.41E-09	1.38E-05
TLR2	0.48	-0.04	-0.03	0.14	0.36	0.24	1	5.11E-03	5.06E-02	5.43E-01	7.98E-04
TLR3	-0.22	0.13	0.08	0.25	0.25	0.15	-0.31	1	2.53E-03	6.92E-02	9.82E-01
TLR4	0.02	0.17	0.19	0.21	0.15	0.25	-0.22	0.33	1	8.35E-03	9.20E-01
TLR7	0.34	0.49	0.41	0.62	0.47	0.6	0.07	0.2	0.29	1	6.91E-02
TLR8	0.31	0.1	0.06	0.11	0.45	0.47	0.37	0	-0.01	0.2	1

Combined table (lower diagonal = Pearson correlation; upper diagonal = correlation P values).

Table S5. TLR family gene expression correlations in TCX of control subjects

	TLR5	TLR9	TLR9.alt	TLR10	TLR1	TLR6	TLR2	TLR3	TLR4	TLR7	TLR8
TLR5	1	4.30E-02	2.86E-02	1.86E-01	5.12E-02	3.22E-03	5.05E-04	7.30E-01	9.05E-02	5.81E-02	6.26E-01
TLR9	-0.23	1	0.00E+00	1.47E-01	1.18E-01	5.41E-01	7.12E-01	1.53E-01	1.41E-01	4.28E-02	5.49E-01
TLR9.alt	-0.25	0.97	1	1.76E-01	2.11E-01	8.95E-01	5.97E-01	3.23E-01	2.39E-01	5.50E-02	5.56E-01
TLR10	0.15	0.17	0.16	1	5.57E-07	1.07E-07	7.15E-01	2.98E-04	2.31E-02	5.55E-10	3.20E-01
TLR1	0.22	0.18	0.14	0.54	1	1.41E-09	5.20E-01	3.94E-02	3.91E-03	5.37E-07	5.69E-02
TLR6	0.33	0.07	0.02	0.56	0.63	1	2.10E-01	3.92E-04	4.54E-05	2.46E-05	1.66E-02
TLR2	0.39	-0.04	-0.06	-0.04	0.07	0.15	1	1.14E-02	7.80E-01	1.24E-01	9.19E-03
TLR3	0.04	0.17	0.11	0.4	0.24	0.4	-0.29	1	7.26E-04	1.11E-04	7.46E-01
TLR4	0.2	0.17	0.14	0.26	0.33	0.45	-0.03	0.38	1	2.79E-04	4.05E-01
TLR7	0.22	0.23	0.22	0.64	0.54	0.46	-0.18	0.43	0.41	1	5.62E-01
TLR8	0.06	0.07	0.07	0.12	0.22	0.27	0.3	0.04	0.1	-0.07	1

Combined table (lower diagonal = Pearson correlation; upper diagonal = correlation P values).

Table S6. TLR family gene expression correlations in CER of AD subjects

	TLR5	TLR9	TLR9.alt	TLR10	TLR1	TLR6	TLR2	TLR3	TLR4	TLR7	TLR8
TLR5	1	5.21E-02	7.81E-02	8.05E-05	2.32E-03	7.60E-03	2.61E-02	7.76E-01	3.73E-01	1.92E-08	1.44E-05
TLR9	0.22	1	0.00E+00	4.00E-01	3.98E-01	8.93E-01	1.67E-01	1.86E-01	9.49E-01	7.75E-01	1.71E-02
TLR9.alt	0.2	0.98	1	2.28E-01	4.33E-01	8.72E-01	1.62E-01	1.89E-01	5.50E-01	9.86E-01	3.76E-02
TLR10	0.43	-0.1	-0.14	1	1.77E-05	7.75E-06	5.08E-06	1.62E-01	1.72E-02	1.55E-14	1.57E-03
TLR1	0.34	0.1	0.09	0.46	1	2.98E-14	1.10E-02	8.78E-06	3.93E-08	1.22E-04	8.04E-06
TLR6	0.3	-0.02	-0.02	0.48	0.72	1	2.53E-01	1.79E-04	2.90E-06	1.60E-04	5.28E-02
TLR2	0.25	0.16	0.16	0.49	0.28	0.13	1	5.77E-01	4.40E-01	3.12E-03	1.59E-04
TLR3	-0.03	0.15	0.15	0.16	0.47	0.41	-0.06	1	3.19E-03	4.90E-02	2.92E-01
TLR4	0.1	-0.01	0.07	0.27	0.57	0.5	0.09	0.33	1	1.11E-01	1.86E-01
TLR7	0.58	0.03	0	0.73	0.42	0.41	0.33	0.22	0.18	1	2.19E-04
TLR8	0.46	0.27	0.23	0.35	0.48	0.22	0.41	0.12	0.15	0.4	1

Combined table (lower diagonal = Pearson correlation; upper diagonal = correlation P values).

Table S7. TLR family gene expression correlations in CER of control subjects

	TLR5	TLR9	TLR9.alt	TLR10	TLR1	TLR6	TLR2	TLR3	TLR4	TLR7	TLR8
TLR5	1	1.84E-02	2.77E-02	2.19E-04	7.24E-03	4.02E-03	2.63E-04	8.95E-01	7.64E-01	1.74E-05	1.66E-04
TLR9	-0.27	1	0.00E+00	1.45E-01	8.93E-01	8.76E-01	4.93E-01	1.76E-01	5.00E-01	2.69E-01	5.33E-01
TLR9.alt	-0.25	0.98	1	8.42E-02	8.94E-01	9.90E-01	5.57E-01	2.36E-01	5.00E-01	2.15E-01	5.28E-01
TLR10	0.41	-0.17	-0.2	1	1.71E-02	6.76E-03	1.27E-01	2.90E-01	4.76E-01	1.20E-14	4.96E-04
TLR1	0.31	-0.02	-0.02	0.27	1	7.55E-15	9.60E-02	4.19E-07	7.95E-08	1.53E-03	3.93E-03
TLR6	0.33	-0.02	0	0.31	0.75	1	3.43E-01	2.85E-06	4.15E-05	8.50E-03	2.07E-02
TLR2	0.41	-0.08	-0.07	0.18	0.19	0.11	1	4.52E-01	8.30E-01	3.11E-02	1.69E-03
TLR3	0.02	0.16	0.14	0.12	0.54	0.51	-0.09	1	2.14E-05	1.40E-01	2.99E-01
TLR4	-0.03	0.08	0.08	-0.08	0.57	0.45	-0.03	0.47	1	9.27E-01	7.83E-01
TLR7	0.47	-0.13	-0.14	0.75	0.36	0.3	0.25	0.17	0.01	1	9.80E-06
TLR8	0.42	-0.07	-0.07	0.39	0.33	0.26	0.35	0.12	-0.03	0.48	1

Combined table (lower diagonal = Pearson correlation; upper diagonal = correlation P values).

Table S8. DEG in TgCRND8 mice expressing sTLR5Fc compared to control mice

Gene ID	foldchange	P value	log10 P value	q value
MMP11	-0.47453333	0.000353839	3.451194184	0.057645818
CR4/ITGAX	-1.12495	0.000453904	3.343035918	0.057645818
MMP2	-0.69745	0.001436008	2.842843083	0.121582027
MAPT	-1.08576667	0.002929173	2.533255038	0.169111367
CX3CR1	-0.2853	0.003328964	2.477690911	0.169111367
Clu/ApoJ	-0.152	0.004708167	2.327148101	0.198933179
Transferrin	-0.573	0.005565048	2.254531052	0.198933179
Tubb5	-0.17916667	0.006265612	2.203036508	0.198933179
Cd55	0.3427	0.007804225	2.107670209	0.220252577
FcRn	0.378116667	0.012905351	1.889230167	0.232775815
JAK3	-0.44906667	0.013010487	1.885706438	0.232775815
BDNF	0.370583333	0.013423791	1.872124832	0.232775815
Csf1	-0.31038333	0.013428608	1.871969002	0.232775815
ApoE4-2	-0.16866667	0.013746603	1.861804601	0.232775815
BACE1	-0.11673333	0.019180273	1.717145216	0.304486834
Sox10	-0.41053333	0.020439265	1.689534721	0.305386669
Il12a	0.45525	0.029198782	1.534635268	0.39492568
ECE2	-0.34803333	0.030907329	1.509938525	0.39492568
Catalase	-0.22691667	0.034285506	1.464889437	0.403467204
Csf1R/cd115	-0.20426667	0.035374405	1.451310858	0.403467204
Dock2	-0.6087	0.036534432	1.437297642	0.403467204
GRIN2a	0.200516667	0.040201036	1.395762754	0.416557067
PTGDS	0.732666667	0.041325002	1.383787112	0.416557067
Il1rn	-0.8816	0.0426397	1.370185861	0.416557067
Gusb	-0.20351667	0.047026175	1.327660345	0.427760124
Mrc1	0.5939	0.047154659	1.326475388	0.427760124
betalll Tubulin	-0.143	0.051476723	1.28838911	0.43321118
S100A11	0.427183333	0.052345558	1.281120163	0.43321118
c2	0.372566667	0.055504831	1.255669214	0.43321118
C3ar1	0.252533333	0.058217174	1.23494888	0.43321118
B2M	0.289	0.058905237	1.229846095	0.43321118
Gls	0.116166667	0.059694454	1.224066017	0.43321118
Myl2	-0.73005	0.065496887	1.183779341	0.462116925
CXCR7	0.329216667	0.067402006	1.171327175	0.462247419
Mapk8	0.191783333	0.069155126	1.160175625	0.462247419
CASP1	0.4644	0.079072818	1.101972783	0.499072917
SerpinG1	0.9997	0.080389097	1.094802852	0.499072917
Fos	-0.40855	0.080559014	1.093885857	0.499072917
SOD1	0.673083333	0.086008484	1.065458707	0.511622356
Creb1	0.160166667	0.088340993	1.053837723	0.511622356
VWF	0.376233333	0.090641756	1.04267169	0.511622356
Lysozyme M (Lyz2)	0.294983333	0.093071368	1.031183901	0.513915816
Cxcl8	-0.96218333	0.095471172	1.020127746	0.515950589
Hspb1	0.4285	0.109025496	0.962471927	0.573228368
Msr1	0.4987	0.110583425	0.956309962	0.573228368

Table S8. DEG in TgCRND8 mice expressing sTLR5Fc compared to control mice (Continued)

Gene ID	foldchange	P value	log10 P value	q value
PTGS2	0.313	0.114751308	0.940242357	0.575575566
UbC	-0.20983333	0.115568322	0.937161191	0.575575566
STAT3	-0.11491667	0.119003972	0.924438543	0.578621605
ABCA1	-0.11811667	0.120736004	0.918163202	0.578621605
Ccl7	-0.955	0.137061019	0.863086043	0.644694424
IGFBP2	0.189033333	0.142116786	0.847354621	0.653061861
ADAM 10	0.067583333	0.143982143	0.841691368	0.653061861
Tyrosine hydroxylase	-0.63906667	0.152820595	0.815818114	0.680990019
Ltbr	-0.19223333	0.157367126	0.803085987	0.684569868
Prkcb1	0.166833333	0.159014261	0.798563925	0.684569868
Synuclein	0.179733333	0.174671451	0.757778072	0.716161302
Myeloperoxidase	-0.60836667	0.180233533	0.744164404	0.716161302
APP-2	-0.06533333	0.181949801	0.740048416	0.716161302
Lrrk2	0.227616667	0.183962397	0.73527094	0.716161302
Tlr4	0.239433333	0.18955025	0.722275638	0.716161302
Ccl8	-0.5143	0.202324376	0.69395179	0.716161302
CD39/ENTPD1	0.11395	0.203797011	0.690802191	0.716161302
IGF1	-0.28876667	0.205811323	0.686530736	0.716161302
PTGER4	-0.38871667	0.206551019	0.684972658	0.716161302
TDP43-2	0.079416667	0.208808737	0.680251333	0.716161302
Aif	0.084216667	0.209700119	0.678401324	0.716161302
PTAFR/PAFR	0.248533333	0.209824144	0.678144541	0.716161302
CD14	0.294133333	0.211221094	0.675262712	0.716161302
eif2alpha	0.099183333	0.211831168	0.67401014	0.716161302
IGFBP4	0.157866667	0.216926606	0.663687179	0.716161302
Tgfb1	0.094783333	0.217104017	0.663332142	0.716161302
Ubiquilin1/2	-0.07021667	0.22249132	0.652686927	0.724523018
Il10rb	0.137983333	0.226814299	0.644329569	0.724797882
Connexin47	-0.39545	0.228282797	0.641526814	0.724797882
Mapt	-0.12471667	0.237373176	0.62456836	0.727434498
PPP1R2	0.087333333	0.237882829	0.623636905	0.727434498
Neprilysin	0.1621	0.23801619	0.6233935	0.727434498
Mef2c	0.112333333	0.249217841	0.603420871	0.727434498
CD33	-0.19546667	0.25072758	0.600797892	0.727434498
C1s	0.482216667	0.25202455	0.598557151	0.727434498
Cfb	0.443383333	0.258050784	0.588294817	0.73645954
TREML4	-0.4239	0.276151618	0.558852407	0.754373261
Limk1	-0.13751667	0.277195943	0.557213131	0.754373261
TNFSF11	-0.48421667	0.277333202	0.556998134	0.754373261
S100A8	0.545416667	0.277617299	0.556553476	0.754373261
GRN	-0.65083333	0.279177506	0.554119577	0.754373261
Lrrk2-2	0.170533333	0.288462133	0.53991119	0.771256649
IDE	0.065433333	0.295291472	0.529749095	0.773620075
alpha-syn	0.1433	0.295437588	0.529534252	0.773620075
ADAM 17/TACE	0.085983333	0.299666797	0.523361374	0.776687413

Table S8. DEG in TgCRND8 mice expressing sTLR5Fc compared to control mice (Continued)

Gene ID	foldchange	P value	log10 P value	q value
ACE	0.287833333	0.304447744	0.516487239	0.781108354
c4a	-0.1702	0.310484625	0.507959902	0.788630946
GBA	0.152066667	0.317212093	0.498650265	0.795659815
FcgRIII	0.176383333	0.321466575	0.492864177	0.795659815
Ccl11	0.438416667	0.326073702	0.486684225	0.795659815
Nos2	0.37065	0.328759756	0.48312135	0.795659815
Arc/arg3_1	-0.32921667	0.328914491	0.482916993	0.795659815
TREML1	-0.19975	0.340931761	0.467332538	0.803793592
TNFSF9	-0.29928333	0.34724431	0.459364861	0.803793592
Mertk	0.1217	0.347572925	0.458954062	0.803793592
H2Ea	0.8091	0.348665653	0.457590833	0.803793592
Cxcl10	-0.39838333	0.350412064	0.455420951	0.803793592
Tlr6	-0.26361667	0.359669267	0.444096671	0.808638689
Cfl1	0.0655	0.364504813	0.438296733	0.808638689
NPY	0.134483333	0.369989217	0.431810933	0.808638689
ECE1	-0.111566667	0.373421565	0.427800605	0.808638689
Il8rb	-0.59483333	0.375293988	0.425628392	0.808638689
Fcrls	0.370116667	0.375666793	0.425197193	0.808638689
Flii	-0.107266667	0.383661423	0.416051867	0.813348194
Cxcl5	-0.37075	0.386161103	0.413231474	0.813348194
Pink1	-0.08613333	0.387461147	0.41177184	0.813348194
C5AR1	0.261466667	0.4060802	0.391388186	0.841492197
Ctsc	0.097783333	0.407494253	0.389878512	0.841492197
C1qa	0.113666667	0.413094056	0.383951054	0.846176535
GRN-2	-0.08128333	0.425901325	0.370691009	0.863559095
Pgk1	0.051	0.428379709	0.368171109	0.863559095
HO-1	-0.14361667	0.434244132	0.362266041	0.868488264
protein phosphatase 2A	0.043833333	0.443459796	0.353145747	0.878853082
MMP3	0.415216667	0.446346644	0.350327726	0.878853082
APP	-0.0435	0.464426472	0.333083034	0.899277778
ALOX5	-0.168366667	0.465159423	0.332398177	0.899277778
Itgb2/MAC-1	0.148566667	0.467341208	0.330365924	0.899277778
Ccl19	0.22845	0.478548167	0.320074343	0.913919055
IL-4	-0.41801667	0.487191863	0.312299974	0.923170251
Hprt1	0.041333333	0.496200092	0.30434316	0.923170251
Lrp1	-0.12125	0.498062028	0.302716568	0.923170251
CCND1	-0.10863333	0.508959574	0.293316712	0.923170251
Ms4a4a	0.335833333	0.512225773	0.290538573	0.923170251
C1r	-0.12033333	0.512527975	0.290282425	0.923170251
synaptophysin	-0.0395	0.515166925	0.288052027	0.923170251
Chi3l3 (YM1)	0.18325	0.516474391	0.286951207	0.923170251
CCL5	-0.267	0.518304077	0.285415375	0.923170251
IFN-gamma	0.3123	0.529797074	0.275890445	0.923170251
Ctsb	-0.04275	0.532871075	0.273377854	0.923170251
ARG1	0.2542	0.535059033	0.2715983	0.923170251

Table S8. DEG in TgCRND8 mice expressing sTLR5Fc compared to control mice (Continued)

Gene ID	foldchange	P value	log10 P value	q value
Tlr2	0.17455	0.541628117	0.266298799	0.923170251
Map2k4	0.0315	0.542937173	0.265250422	0.923170251
CASP8	0.0911	0.545853154	0.262924176	0.923170251
CRHR1	0.0865	0.546148454	0.262689292	0.923170251
Ccl17	0.154183333	0.546744709	0.262215411	0.923170251
FcgRI	0.09335	0.548813811	0.260574968	0.923170251
Ccl3	-0.21118333	0.554436309	0.256148337	0.926492253
Cxcl9	-0.34561667	0.565382554	0.247657597	0.927416974
cd163	0.19945	0.568187442	0.245508369	0.927416974
Maff	0.17685	0.569340909	0.24462761	0.927416974
TLr8	0.268483333	0.574620107	0.240619181	0.927416974
CD36	0.165483333	0.578274225	0.237866165	0.927416974
P2ry12	0.065483333	0.580905146	0.235894776	0.927416974
Mfn1	0.036716667	0.591330664	0.2281696	0.927416974
IL-6	0.199216667	0.596504568	0.224386226	0.927416974
tPA	-0.07366667	0.598582817	0.222875754	0.927416974
GAPDH	0.029833333	0.605006135	0.218240221	0.927416974
Ly86	0.0846	0.605128861	0.218152134	0.927416974
STAT6	-0.06278333	0.605693382	0.217747172	0.927416974
CRH	-0.10143333	0.610688099	0.214180544	0.927416974
MMP9	-0.17555	0.614700661	0.21133632	0.927416974
IGFBP5	-0.06906667	0.630493569	0.200319339	0.927416974
Il6ra	0.0838	0.632529503	0.198919213	0.927416974
IGFBP3	0.094016667	0.635227136	0.197070958	0.927416974
Cltc	0.033333333	0.636941785	0.195900259	0.927416974
Ptpn6	-0.0529	0.637388463	0.195595802	0.927416974
TNF α	-0.25858333	0.641536422	0.192778682	0.927416974
IFIH1	0.067983333	0.642914274	0.191846932	0.927416974
CCL2	0.110683333	0.644427423	0.190825987	0.927416974
Il1r1	0.057416667	0.646185641	0.189642696	0.927416974
Ncf1	-0.06356667	0.646270884	0.18958541	0.927416974
Plasmin	0.242433333	0.654104789	0.184352672	0.933385485
Snap91	-0.03033333	0.659865005	0.180544903	0.933772773
Il7	-0.14385	0.6682224	0.17507897	0.933772773
Fcer1g	-0.07406667	0.671623732	0.172873966	0.933772773
Trem-1	0.242516667	0.67395615	0.17136836	0.933772773
Tlr1	-0.09386667	0.674586507	0.17096235	0.933772773
zif268	0.097116667	0.67643382	0.169774687	0.933772773
Ccr4	-0.20545	0.687528861	0.162709066	0.943339745
SERPINA3	0.054983333	0.693791159	0.158771238	0.943339745
Calbindin	0.04655	0.694506033	0.158323977	0.943339745
Axi	0.038733333	0.703824704	0.152535493	0.94402793
PSD95	0.041416667	0.711123203	0.148055151	0.94402793
Cxcr4	0.253283333	0.711305788	0.147943657	0.94402793
Mef2b	-0.11316667	0.71693124	0.144522495	0.94402793

Table S8. DEG in TgCRND8 mice expressing sTLR5Fc compared to control mice (Continued)

Gene ID	foldchange	P value	log10 P value	q value
FcgRIIb	-0.06205	0.720447884	0.14239743	0.94402793
C1qb	0.062916667	0.738554027	0.13161773	0.961521797
ALOX5AP	-0.04828333	0.744685436	0.12802714	0.961521797
C3	-0.11471667	0.75032305	0.124751712	0.961521797
MARCO	-0.19405	0.751630138	0.123995814	0.961521797
PTPRC/CD45	-0.05098333	0.753318258	0.123021506	0.961521797
Crp	-0.12151667	0.75804486	0.120305093	0.962716972
Hck	0.122733333	0.764321476	0.116723938	0.965858979
TNFRSF1A	-0.0497	0.768160333	0.114548123	0.965904577
Peripherin	-0.07511667	0.776385963	0.109922325	0.966990972
TIMP1	0.126716667	0.778113749	0.108956911	0.966990972
SERPINA1	-0.10721667	0.782237728	0.106661242	0.966990972
MAPT-2	0.0892	0.78425252	0.105544077	0.966990972
P2RY6	-0.04813333	0.790258713	0.102230707	0.969581762
ITM2b-2	0.096083333	0.793988215	0.100185944	0.969581762
TLR9	-0.07976667	0.802238213	0.095696655	0.970741744
TDP43	-0.15735	0.802581757	0.095510716	0.970741744
Mafk	-0.04036667	0.813866416	0.089446872	0.970901886
TNFRSF1B	-0.0962	0.821411024	0.085439473	0.970901886
Mbl2	0.137816667	0.832399188	0.079668352	0.970901886
Il1a	-0.07163333	0.834712856	0.078462898	0.970901886
Aquaporin4	0.026983333	0.842495305	0.074432511	0.970901886
Myc	0.039933333	0.842804585	0.074273111	0.970901886
ZBP1	-0.05925	0.845597824	0.072836143	0.970901886
Ly96	-0.03298333	0.847748394	0.071733024	0.970901886
Park9 (ATP13A2)	-0.03065	0.848872673	0.071157447	0.970901886
Cd68	-0.0266	0.849042485	0.071070578	0.970901886
Cebpd	0.0418	0.852371071	0.069371298	0.970901886
Drp1	0.0105	0.852405986	0.069353509	0.970901886
PTGER1	-0.06571667	0.856828398	0.067106148	0.97091442
Hspb2	0.0384	0.860061986	0.065470247	0.97091442
Mapt-2	-0.01653333	0.8670791	0.061941282	0.970966966
Tgfb1	0.03355	0.868520077	0.061220138	0.970966966
NLRP3	0.0302	0.871576646	0.059694415	0.970966966
Tgfb3	-0.03176667	0.878237549	0.056387999	0.972212437
IL-10	0.091733333	0.888055829	0.051559731	0.972212437
FTL-2	0.011316667	0.888302886	0.051438927	0.972212437
H2-Eb1	0.071733333	0.891279128	0.049986264	0.972212437
ApoE4	-0.07311667	0.891832669	0.049716623	0.972212437
Ctsd	-0.015	0.896758032	0.047324725	0.973404018
Map2k6	-0.01551667	0.908320588	0.041760842	0.977212052
TyroBP/DAP12	0.024066667	0.910092133	0.04091464	0.977212052
Ccr1	0.059633333	0.911808096	0.040096556	0.977212052
Bsn	-0.01421667	0.918065753	0.037126213	0.979784459
CARD9	0.011866667	0.927929157	0.032485179	0.986167389

Table S8. DEG in TgCRND8 mice expressing sTLR5Fc compared to control mice (Continued)

Gene ID	foldchange	P value	log10 P value	q value
Trem-2	0.017633333	0.932444601	0.030376961	0.98637202
Ccl4	-0.04026667	0.944856949	0.024633939	0.992417383
FTL	-0.03103333	0.947718463	0.023320659	0.992417383
Ccl21b	0.013133333	0.955135875	0.019934842	0.992417383
Ms4a6a	0.018816667	0.956754691	0.0191994	0.992417383
TREML2	-0.021433333	0.957252987	0.01897327	0.992417383
IL-1b	0.014283333	0.963449457	0.016171064	0.994781147
ITM2b	0.001666667	0.97297855	0.011896734	0.997874662
Masp2	-0.01005	0.977266395	0.009987035	0.997874662
Tlr7	-0.005983333	0.98314115	0.007384126	0.997874662
CR3/ITGAM	-0.00238333	0.987416897	0.005499445	0.997874662
FcgRIV	0.00625	0.989082664	0.00476741	0.997874662
S100A9	-0.00638333	0.990017381	0.004357181	0.997874662
GFAP	-0.00058333	0.997081059	0.001269534	0.997981314
SOD1-2	-0.00016667	0.997981314	0.00087759	0.997981314

Table S9. Human subjects used for analysis of protein-coding TLR variants in AD and controls

Series	Study cohort				AAD (years)				Females				APOE ε4 subjects			
	AD		CON		AD		CON		AD		CON		AD		CON	
	n	%	n	%	Mean	SD	Mean	SD	n	%	n	%	n	%	n	%
Jacksonville	670	47.1	752	52.9	74.8	5.6	76.4	7.5	397	59.3	438	58.2	454	67.8	206	27.4
Autopsy ^a	1,170	76.6	358	23.4	81.3	8.7	75.5	8.3	671	57.4	155	43.3	718	61.4	81	22.6
Rochester	578	19.7	2,359	80.3	79.9	7.8	78.3	5.6	348	60.2	1,264	53.6	322	55.7	553	23.4
Total	2,418	41.1	3,469	58.9	79.2	8.2	77.6	6.4	1,416	58.6	1,857	53.5	1,494	61.8	840	24.2

AAD, anticipated age at the time of death; CON, control.

^aAutopsy controls, unlike the clinical controls, who were neurologically normal, include some non-AD degenerative disorders.

Table S10. Association of protein-coding variants of TLR5 with risk for AD

Hn	Haplotype	Encoded TLR5 protein	Freq	OR	L95	U95	P
H1	111111	TLR5	0.555	1.00	(referent haplotype)		
H2	112111	TLR5 p.F616L	0.364	0.91	0.82	1.01	0.067
H3	112122	TLR5 p.Q181K-R392X	0.059	1.06	0.87	1.29	0.57
H4	222211	TLR5 p.L478I-F616L-I644F-D846G	0.010	0.53	0.33	0.85	0.0083
All			0.988	Global P value			0.012

Hn, haplotype; Freq, allele frequency; L95, lower bound of 95% confidence interval for OR; U95, upper bound of 95% confidence interval for OR; P, P value.

Table S11. Analysis of TLR5-associated SNPs in human brain

CHR	SNP	Base pair	AAChange.refGene	n	OR	L95	U95	P
1	rs5744177	223283837	TLR5:NM_003268:exon6:c.A2537G:p.D846G	5,815	0.55	0.34	0.88	0.013
1	rs5744175	223284444	TLR5:NM_003268:exon6:c.A1930T:p.I644F	5,861	0.55	0.34	0.88	0.014
1	rs5744174	223284528	TLR5:NM_003268:exon6:c.T1846C:p.F616L	5,764	0.92	0.84	1.01	0.084
1	rs5744171	223284915	TLR5:NM_003268:exon6:c.C1459A:p.L487I	5,748	0.55	0.34	0.88	0.019
1	rs5744168	223285200	TLR5:NM_003268:exon6:c.C1174T:p.R392X	5,850	1.10	0.91	1.33	0.328
1	rs45528236	223285833	TLR5:NM_003268:exon6:c.C541A:p.Q181K	5,823	1.10	0.90	1.33	0.350

CHR, chromosome; L95, lower bound of 95% confidence interval for OR; U95, upper bound of 95% confidence interval for OR; P, P value.

Reference

Wei, T., and V. Simko. 2016. Corrplot. Available at: <https://github.com/taiyun/corrplot> (accessed August 29, 2017).