Supplemental material

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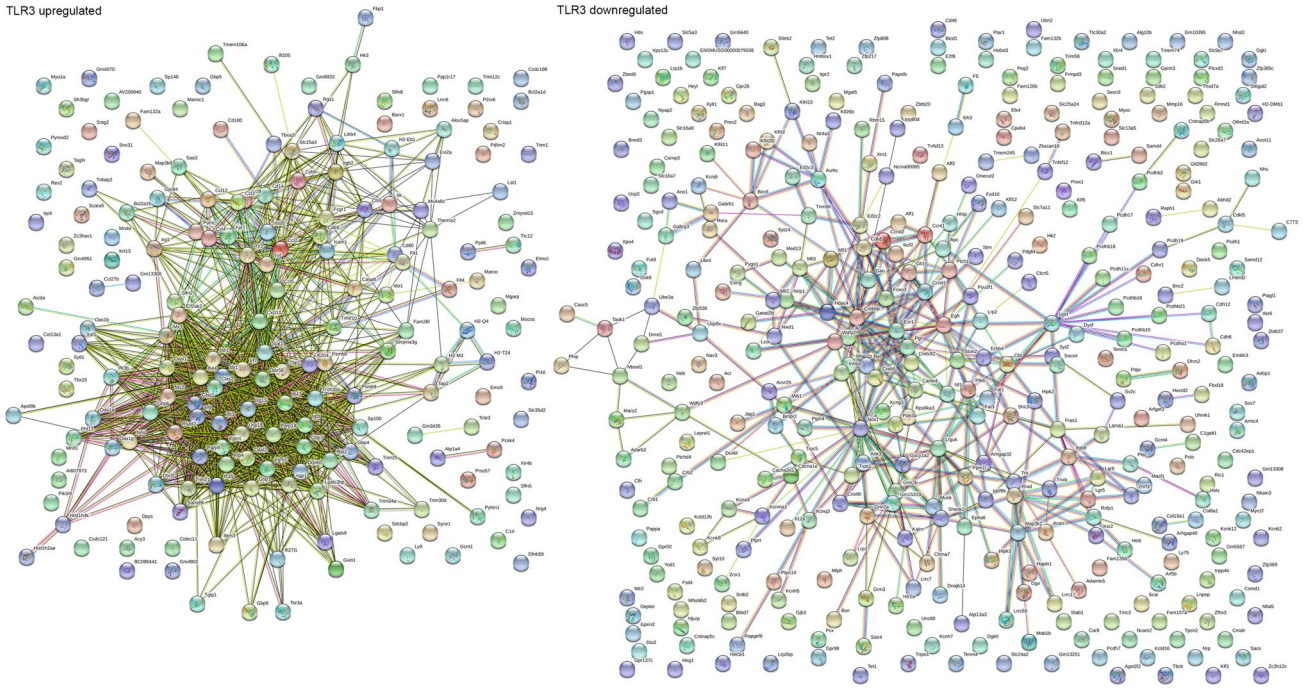

Figure S1. **Interaction map of TLR3-regulated genes.** Genes up- and down-regulated upon TLR3 activation in cultured neurons were analyzed for protein–protein interactions in STRING. TLR3-up-regulated gene products interacted considerably with each other, with innate immune responses as a core function. TLR3-down-regulated genes included function-related transcription factors, signaling molecules, and synaptic ion channels. These data strengthen the role of TLR3 activation in triggering the innate immune response of neurons and impairing their synaptic responses.

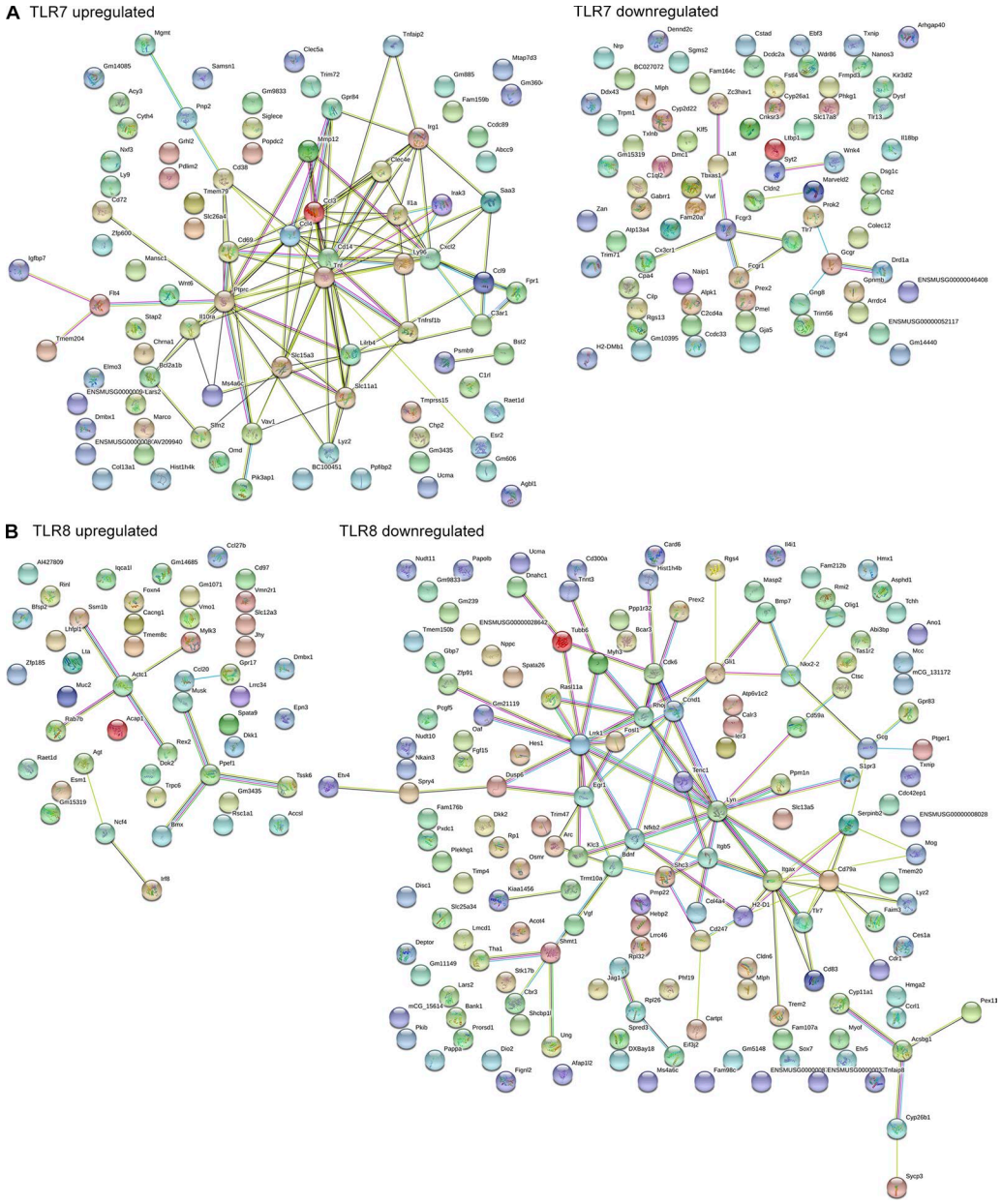

Figure S2. **Interaction maps of TLR7 and TLR8 up-regulated and down-regulated genes. (A and B)** TLR7-regulated genes (A) and TLR8-regulated genes (B) in cultured neurons were analyzed for protein–protein interactions in STRING. **(A)** For TLR7-up-regulated genes, the major protein interactions were related to innate immune responses. There were very few protein interactions among TLR7-down-regulated genes. **(B)** TLR8-up-regulated gene products exhibited very few interactions, whereas TLR8-down-regulated genes presented strong interactions related to several signaling pathways and immediate early genes.

Table S1. **List of genes coregulated by TLR3, TLR7, and TLR8**

TLR	Gene list
Up-regulated	
TLR3, TLR7, and TLR8	*Gm5643_1* and *Gm3435*
TLR3 and TLR7	*Irg1, Tnf, Slfn2, Cd69, Gpr84, Gm5512_1, Ccl4, Ccl3, Marco, Fpr1, Flt4, Cd14, Bcl2a1b, Bst2, Ccl9, Hist1h4k, Ly9, Psmb9, C1rl, Ms4a6c, Lilrb4, Elmo3, Saa3, 2210408F21Rik, Slc15a3, 1700028P14Rik, Col13a1, Tnfaip2, Gm9833, Pdlim2, LOC100504703, Acy3*, and *Mansc1*
TLR3 and TLR8	*Rex2* and *Ccl27b*
TLR7 and TLR8	*Dmbx1*
Down-regulated	
TLR3, TLR7, and TLR8	*Mlph* and *Prex2*
TLR3 and TLR7	*H2-DMb1, Arhgap40, Tmem254c, Nrp, Gm15319, Dysf, Frmpd3, Syt2, Trim56, Crb2, Klf5*, and *Fstl4*
TLR3 and TLR8	*Gli1, Gm15612, Cdk6, Papolb, Ano1, Pappa, Slc13a5, Jag1, Sox7, Fam107a, Dio2, Deptor, Cdc42ep1, Shc3, Nkain3*, and *Ccnd1*
TLR7 and TLR8	*Tlr7, 4930506C21Rik*, and *Txnip*

Table S2. **Gene lists for the top five biological processes regulated by TLR activation in cultured neurons**

GO biological process	Gene list
TLR3-up-regulated	
Innate immune response	*Cd14, Cybb, Fcgr1, Gbp2, H2-Eb1, H2-M3, Irgm1, Ifit1, Ifit3, Acod1, Itk, Lgals9, Cd180, Ly9, Marco, Gbp4, Mx1, Mx2, Eif2ak2, Trim30a, Ccl12, Ccl2, Ccl3, Ccl4, Ccl5, Ccl9, Trim21, Stat1, Tap2, Tgtp1, Tnf, Oasl2, Tlr2, Irf7, Irgm2, Gbp3, Rsad2, Zbp1, Iigp1, Nmi, Ifitm3, Bst2, Ifih1, Zc3hav1, Parp9, Gbp6, Pik3r6, Trim25, Gbp5, Gbp7, Ddx58, Oasl1, C1rl, Ddx60, Gbp9, Oas2, Nlrc5*, and *Isg15*
Response to cytokine	*Casp8, Cd14, Gbp2, H2-Eb1, Irgm1, Cxcl10, Ifi204, Ifi47, Ifit1, Ifit3, Igtp, Acod1, Gbp4, Mx2, Eif2ak2, Saa3, Ccl12, Ccl2, Ccl3, Ccl4, Ccl5, Ccl9, Serpina3g, Trim21, Stat1, Tgtp1, Tnf, Irf7, Irgm2, Gbp3, Zbp1, Iigp1, Nmi, Ifitm3, Bst2, Parp9, Gbp6, Ifi205, Gbp5, Gbp7, Ifi209, Gbp9, Gm4951, Xaf1, Nlrc5*, and *Isg15*
Regulation of defense response	*Alox5ap, Cd14, Ctss, Fcgr1, H2-M3, Ido1, Acod1, Lgals9, Cd180, Gbp4, Trim30a, Ccl3, Ccl5, Tap2, Tnf, Tlr2, Irf7, Rsad2, Zbp1, Nmi, Fam132a, Ifih1, Zc3hav1, Parp9, Pik3r6, Gbp5, Ddx58, Ddx60, Nlrc5, Cybb, Flt4, Irf9, Itk, Ly9, Pomc, Eif2ak2, Ccl2, Ccl4, Isg15, Bcl2a1d, Fpr1, Themis2, C1rl*, and *Trim21*
Inflammatory response	*Alox5ap, Cd14, Ctss, Cybb, Fcgr1, Fpr1, Icam1, Ido1, Cxcl10, Acod1, Itgb2, Lgals9, Cd180, Saa3, Ccl12, Ccl2, Ccl3, Ccl4, Ccl5, Ccl9, Tbxa2r, Tnf, Tlr2, Fam132a, Gbp5, Themis2, Zc3hav1, Ddx60, Gbp4, Scara5*, and *Ddx58*
Response to molecule of bacterial origin	*Aicda, Cd14, Cd80, Gbp2, H2-M3, Ido1, Cxcl10, Acod1, Lgals9, Cd180, Eif2ak2, Ccl12, Ccl2, Ccl5, Stat1, Tap2, Tbxa2r, Tnf, Tlr2, Gbp6, Fcgr1, Irgm2, Gbp3, Iigp1, Gbp7, Gbp9, Isg15*, and *Mgarp*
TLR3-down-regulated	
Chemical synaptic transmission	*Chrna7, Camk4, Egfr, Erbb4, Grin2a, Grin2b, Htr2a, Htr6, Kcnma1, Nf1, Nos1, Musk, Shc3, Sstr4, Syt2, Tnr, Pclo, Syt10, Sv2c, Slc24a2, Pcdhb2, Pcdhb15, Pcdhb16, Pcdhb18, Pcdhb21, Grm3, Ston2, Kmt2a, Pcdh17, Igsf9b, Shank1, Dgki, Kalrn, Cacna1e, Ccnd2, Aff2, Ar, Hipk2, Nr4a3, Prex2, Zfhx3, Pgr, Gcnt4*, and *Strn*
Sensory organ development	*Acvr2b, Apc, Bmpr2, Col8a1, Egfr, Fat1, Lgr5, Grin2b, Hipk2, Inhba, Jag1, Kcnma1, Lama1, Nf1, Nr4a3, Nrp1, Pou2f1, Prox1, Grk1, Adgrv1, Crb1, Nhs, Kmt2c, Sdk2, Bnc2, Fat3*, and *Alg10b*
Homophilic cell adhesion via plasma membrane adhesion molecules	*Cdh6, Pcdha7, Ptprt, Pcdh1, Pcdhb18, Cdhr1, Cdh12, Igsf9b, Sdk2, Fat3, Pcdh19*, and *Pcdhb16*
Response to organic cyclic compound	*Acr, Chrna7, Ar, Cdk6, Egfr, Esr1, Gabrb1, Htr2a, Inhba, Itpr2, Kcnma1, Nr4a3, Nos1, Pappa, Pgr, Ptch1, Rora, Sstr4, Ube3a, Esrrg, Abhd2, Pde3a, Heyl, Ago3, Ago2, Nrip1, Trpa1, Med13, Kmt2d, Kalrn, Il12a, Ptk6, Ccnd1, Erbb4, Zfp536, Sesn3, Phip, Rps6ka3, Rxfp1, Bmpr2*, and *Apc*
Synapse organization	*Chrna7, Ank3, Bsn, Erbb4, Musk, Tnr, Pclo, Pcdhb2, Pcdhb15, Pcdhb16, Pcdhb18, Pcdhb21, Pcdh17, Sdk2, Shank1, Slitrk2*, and *Kalrn*
TLR7-up-regulated	
Innate immune response	*Cd14, Acod1, Ly9, Ly96, Marco, Slc11a1, Ccl3, Ccl4, Ccl9, Tnf, Vav1, Clec5a, Raet1d, Clec4e, Bst2, Irak3, Pik3ap1*, and *C1rl*
Inflammatory response	*C3ar1, Cd14, Fpr1, Il1a, Acod1, Ly96, Slc11a1, Saa3, Ccl3, Ccl4, Ccl9, Cxcl2, Tnf, Tnfrsf1b, Siglece, Pik3ap1, Cd38, Ptprc, Vav1, Clec4e, Samsn1, Irak3, C1rl, Lyz2*, and *Esr2*
Cellular response to IL-1	*Il1a, Acod1, Saa3, Ccl3, Ccl4, Ccl9, Irak3, C3ar1, Cxcl2, Vav1, Cd14, Slc11a1, Ptprc, Tnf, Bst2, Fpr1, Esr2, Flt4, Pik3ap1*, and *Chp2*
Immune effector process	*Ly9, Slc11a1, Ptprc, Abcc9, Tnf, Vav1, Raet1d, Clec4e, Bst2, Irak3, C1rl, Milr1, C3ar1, Samsn1*, and *Psmb9*
Positive regulation of humoral immune response	*Acod1, Ptprc, Tnf, Abcc9, Bst2, Irak3, Slc11a1, Samsn1, Ccl4, Lyz2, Clec4e, Vav1, Ly96, Il1a*, and *Tnfrsf1b*
TLR7-down-regulated	
Antigen processing and presentation of exogenous peptide antigen	*Fcgr1, Fcgr3, H2-DMb1, Nrp1, Zan, Colec12, Lat*, and *Zc3hav1*
Positive regulation of defense response	*Fcgr1, Fcgr3, Zc3hav1, Colec12, Tlr7, Tlr13, Trim56*, and *Cx3cr1*
Telencephalon cell migration	*Cx3cr1, Drd1*, and *Nrp1*
Coronary vasculature development	*Nrp1, Prok2, Ltbp1, Gja5, Klf5, Cx3cr1*, and *Crb2*
Regulation of vasoconstriction	*Drd1, Gja5, Tbxas1, Trpm1, Wnk4, Fam20a*, and *Atp13a4*
TLR8-up-regulated[a]	
Positive regulation of cytokine production	*Agt, Irf8, Lta, Raet1b, Ccl20, Rab7b*, and *Gpr17*
Cardiac muscle cell differentiation	*Actc1, Agt, Dkk1, Mylk3*, and *Tmem8c*

Table S2. **Gene lists for the top five biological processes regulated by TLR activation in cultured neurons *(Continued)***

GO biological process	Gene list
Positive regulation of cellular component biogenesis	*Agt, Irf8, Musk, Adgre5, Mylk3, Dkk1, Lta*, and *Trpc6*
Inflammatory response	*Agt, Lta, Ccl20, Mylk3*, and *Gpr17*
TLR8-down-regulated	
Negative regulation of MAPK activity	*Bmp7, Lyn, Spry4, Dusp6, Spred3, Cd300a, Pkib, Deptor, Prex2, Ier3, Lrrk1, Ccnd1, Gcg, Cartpt, Serpinb2, Fcmr, Timp4, Hmga2, Bank1, Fgf15*, and *Trem2*
Regulation of Wnt signaling pathway	*Ccnd1, Egr1, Gli1, Hmga2, Sox7, Dkk2, Atp6v1c2, Lrrk1, Disc1, Mcc*, and *Bdnf*
Glomerulus development	*Bmp7, Col4a4, Egr1, Hes1, Jag1, Bdnf, Ccnd1, Cdk6, S1pr3, Hmga2, Lyn, Nkx2-2, Nppc, Pmp22, Cartpt, Ucma, Hist1h4b, Timp4, Fgf15, Etv4, Phf19, Tns2, Gli1, Txnip, Cyp26b1, Rp1*, and *Bcar3*
Regulation of osteoblast differentiation	*Bmp7, Cdk6, Gli1, Jag1, Nppc*, and *Ucma*
Negative regulation of cell proliferation	*Bdnf, Bmp7, Cdk6, Fosl1, Hes1, Lyn, Nppc, Etv4, Pmp22, Sox7, Tns2, Cd300a*, and *Mcc*

[a]For TLR8–up-regulated group, only four GO processes were significant.

Table S3. **Primer sequences and probe numbers of the Universal Probe Library for Q-PCR**

Gene	Quantitative RT-PCR primer pair		Probe #
	Forward (5′–3′)	**Reverse (5′–3′)**	
Tlr8	CAAACGTTTTACCTTCCTTTGTCT	ATGGAAGATGGCACTGGTTC	56
Tlr7	TGATCCTGGCCTATCTCTGAC	CGTGTCCACATCGAAAACAC	25
Arc	GGTGAGCTGAAGCCACAAAT	TTCACTGGTATGAATCACTGCTG	79
Egr1	GTCAGCAGCTTCCCGTCT	TGAAAGACCAGTTGAGGTGCT	17
Egr4	GACGCGCTTCTCTCCAAG	AGCCCAGCTCAAGAAGTCG	27
c-Fos	GGGACAGCCTTTCCTACTACC	AGATCTGCGCAAAAGTCCTG	67
Il6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA	6
Il1b	AGTTGACGGACCCCAAAAG	AGCTGGATGCTCTCATCAGG	38
Tnfa	TTGTCTTAATAACGCTGATTTGGT	GGGAGCAGAGGTTCAGTGAT	64
Il12b	GACTCCAGGGGACAGGCTA	CCAGGAGATGGTTAGCTTCTGA	27
Il10	CAGAGCCACATGCTCCTAGA	TGTCCAGCTGGTCCTTTGTT	41
CCL3	TGCCCTTGCTGTTCTTCTCT	GTGGAATCTTCCGGCTGTAG	40
CCL4	GCCCTCTCTCTCCTCTTGCT	GGAGGGTCAGAGCCCATT	1
CCL5	TGCAGAGGACTCTGAGACAGC	GAGTGGTGTCCGAGCCATA	110
Myd88	TGGCCTTGTTAGACCGTGA	AAGTATTTCTGGCAGTCCTCCTC	17
GAPDH	AATGTGTCCGTCGTGGATCT	CCCAGCTCTCCCCATACATA	80
Gm10069	TGCCCAGCAGTTTAGTACCC	TGCTTCCCTGTCTCCACAGT	64