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Supplemental Information

Shortening the Half-Life of Cas9 Maintains Its Gene Editing Ability and Reduces Neuronal Toxicity

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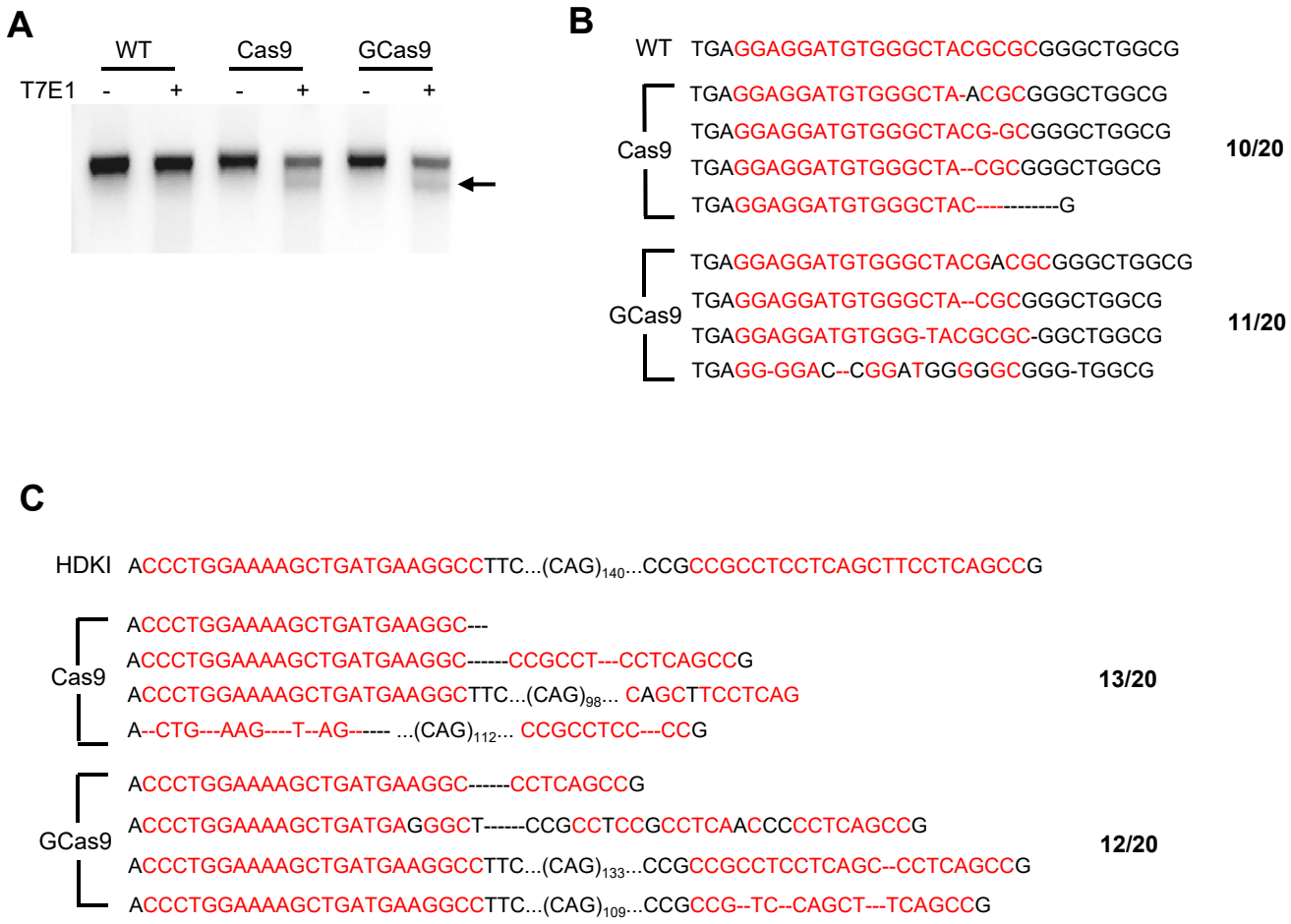

Figure S1. Further characterization of AAV-GCas9 *in vivo* （Related to Figure 2）.
(A) T7 Endonuclease 1 (T7E1) digestion result of the PCR products amplified using *manf* genomic DNA from mouse striatal tissues injected with AAV-Cas9/AAV-*manf*-gRNA or AAV-GCas9/AAV-*manf*-gRNA. Arrow indicates the cleaved products.
(B) Sequencing results of the *manf* genomic DNA from wild type (WT) mouse striatal tissues injected with AAV-Cas9/AAV-*manf*-gRNA or AAV-GCas9/AAV-*manf*-gRNA. Four representative mutant sequences cut by Cas9 or GCas9, and the WT sequence were shown. The gRNA sequence was highlighted in red. The number of clones with mutations versus the total number of clones sequenced is shown to the right of the figure.
(C) Sequencing results of the *htt* genomic DNA from HD140Q KI mouse striatal tissues injected with AAV-Cas9/AAV-*htt*-gRNA or AAV-GCas9/AAV-*htt*-gRNA. Four representative mutant sequences cut by Cas9 or GCas9, and the endogenous HD140Q KI sequence were shown. The gRNA sequences were highlighted in red. The number of clones with mutations versus the total number of clones sequenced is shown to the right of the figure.

Figure S2. Protein interaction network analysis identified that significantly changed genes are enriched in the chemical synaptic transmission and myelin sheath pathway (Related to Figure 3).

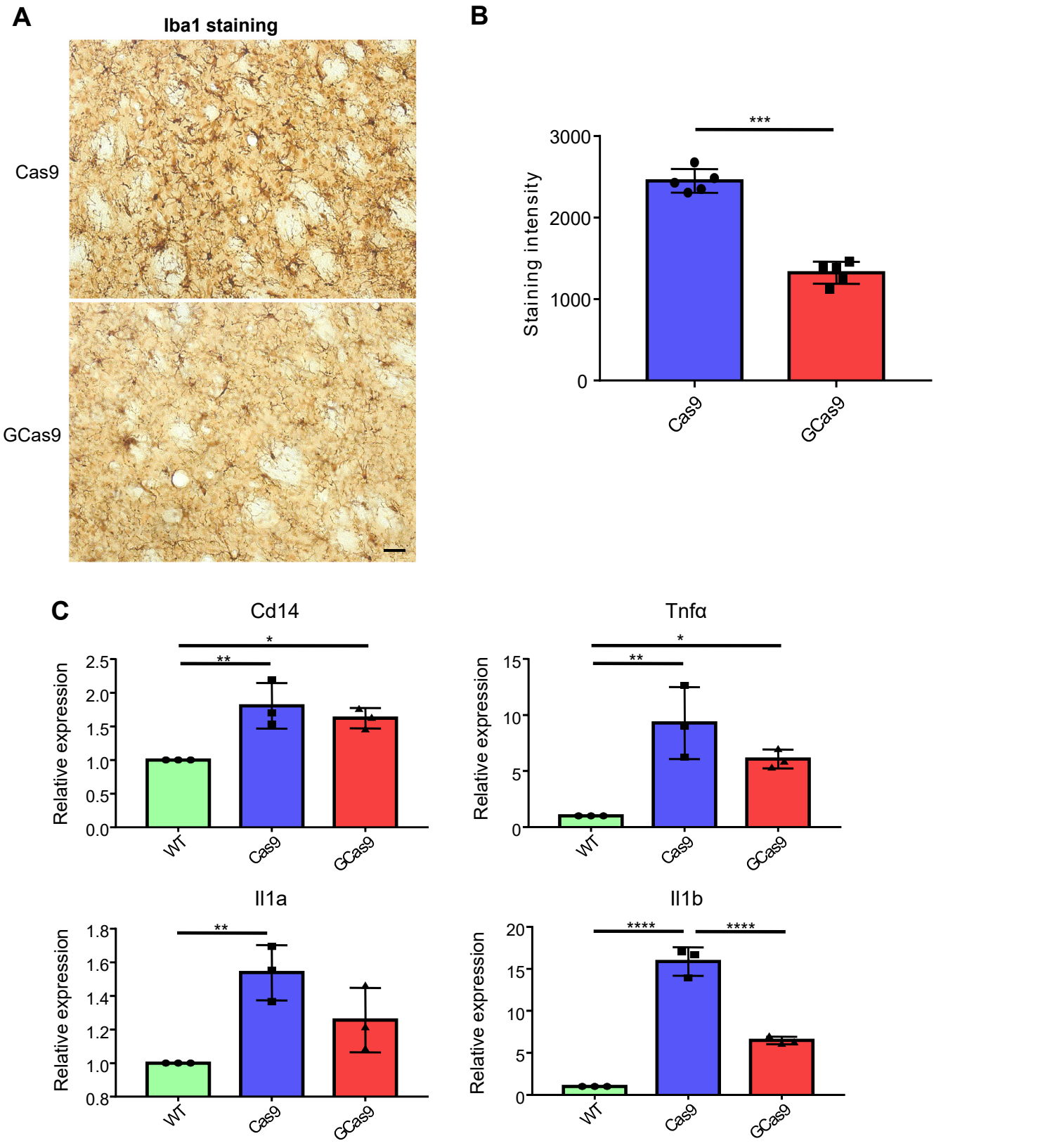

Figure S3. Further analysis of immune response caused by Cas9 expression (Related to Figure 4).
(A) Immunohistochemical results of Iba1 expression in the striatum of wild type (WT) mice 3 weeks after injection of AAV-Cas9 or AAV-GCas9 (Scale bar: 50 μm).
(B) Quantitative analysis of immunohistochemistry results in Figure S4A (n = 5, *** $P < 0.001$, two tailed student t test).
(C) Quantitative RT-PCR results of different cytokines in the striatum of uninjected WT mice, WT mice injected with AAV-Cas9, and WT mice injected with AAV-GCas9 (n = 3, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, one-way ANOVA with Tukey post-tests).

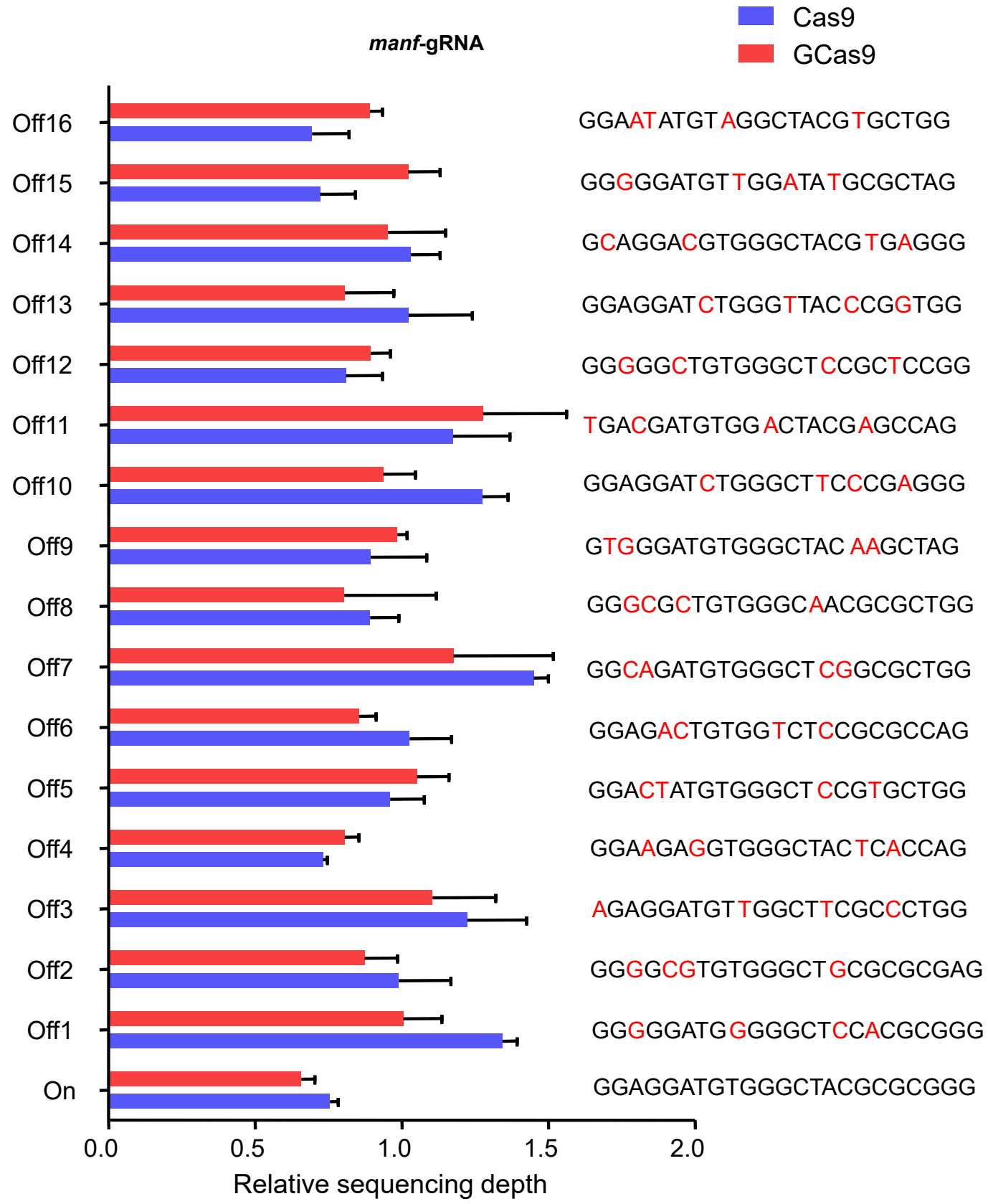

Figure S4. Whole genome sequencing analysis of off-target rates caused by AAV-Cas9 and AAV-GCas9 (Related to Figure 4). Genomic DNA from the striatum of uninjected wild type (WT) mice, or WT mice injected with AAV-Cas9/AAV-*manf*-gRNA or AAV-GCas9/AAV-*manf*-gRNA were subjected to whole genome sequencing. Relative sequencing depth for the *manf* genomic locus targeted by AAV-Cas9/AAV-*manf*-gRNA or AAV-GCas9/AAV-*manf*-gRNA and 16 most likely off-target loci was calculated by normalizing the number of mapped reads in those loci to the uninjected WT control. Mutations caused by Cas9 cutting led to a reduce number of mapped reads, thereby a reduced relative sequencing depth. The letters in red indicate mismatches. The on-target relative sequencing depth for Cas9 is 75.5%, for GCas9 is 65.7% (n = 2).

Table S1. Oligonucleotide sequences, related to STAR Methods

Primer, Cas9, forward	5'-GAAAGTTCGACAATCTGACCAAGG-3'
Primer, Cas9, reverse	5-TGCCACGTGCTTTGTGATCTG-3'
Primer, Sox10, forward	5'-CCCACACTACACCGACCAG-3'
Primer, Sox10, reverse	5'-GGCCATAATAGGGTCCTGAGG-3'
Primer, Olig2, forward	5'-GGGAGGTCATGCCTTACGC-3'
Primer, Olig2, reverse	5'-CTCCAGCGAGTTGGTGAGC-3'
Primer, Olig1, forward	5'-CAGCCACCTATCTCCTCATC-3'
Primer, Olig1, reverse	5'-CGAGTAGGGTAGGATAACTTCG-3'
Primer, Mag, forward	5'-CCCCGAGGATGATGGGGAATACTG-3'
Primer, Mag, reverse	5'-CAGTGTGACTCCAGAAGGATTATG-3'
Primer, Plp1, forward	5'-AGCAAGACCTCTGCCAGTATAGG-3'
Primer, Plp1, reverse	5'-CGCAGCAATAAACAGGTGGAAGG-3'
Primer, Dcx, forward	5'-CTCAAGCCAGAGAGAACAAGGAC-3'
Primer, Dcx, reverse	5'-CAGGACCTGCTCGAAAGAGTGG-3'
Primer, Pkcγ, forward	5'-CAACCAGGGCATCATCTACAGG-3'
Primer, Pkcγ, reverse	5'-AACTCTTCCTCATCTTCCCCATCA-3'
Primer, Hap1, forward	5'-GCGTGCGGCGTTTATTCGAGAG-3'
Primer, Hap1, reverse	5'-GGCTGTGTTCAGGTCCCGTTCT-3'
Primer, Lpar1, forward	5'-ACATGGCACCCCTCTACAGTGAC-3'
Primer, Lpar1, reverse	5'-CCTCATAGTCCTCTGGCGAACATAG-3'
Primer, Wnt7a, forward	5'-GGCAACCTGAGCGACTGT-3'
Primer, Wnt7a, reverse	5'-TGTTCTCCTCCAGGATCTTCCG-3'
Primer, Gprin1, forward	5'-GGACCCTCAGTTGCTTGGAAAGA-3'
Primer, Gprin1, reverse	5'-TGGTTTCACTGGGGACACAAGTTC-3'
Primer, Manf, forward	5'-ATTGACCTGAGCACAGTGGACCTG-3'
Primer, Manf, reverse	5'-TTCAGCACAGCCTTTGCACATCTC-3'

Primer, Cd14, forward	5'-GGACTGATCTCAGCCCTCTG-3'
Primer, Cd14, reverse	5'-GCTTCAGCCCAGTGAAAGAC-3'
Primer, Tnfα, forward	5'-CCCTCACACTCAGATCATCTTCT-3'
Primer, Tnfα, reverse	5'-GCTACGACGTGGGCTACAG-3'
Primer, Il1a, forward	5'-GCACCTTACACCTACCAGAGT-3'
Primer, Il1a, reverse	5'-AAACTTCTGCCTGACGAGCTT-3'
Primer, Il1b, forward	5'-GCAACTGTTCCTGAACTCAACT-3'
Primer, Il1b, reverse	5'-ATCTTTTGGGGTCCGTCAACT-3'
Primer, Actin, forward	5'-TCACTGTCCACCTTCCAGCAGATG-3'
Primer, Actin, reverse	5'-CTCAGTAACAGTCCGCCTAGAAGC-3'