APPENDIX

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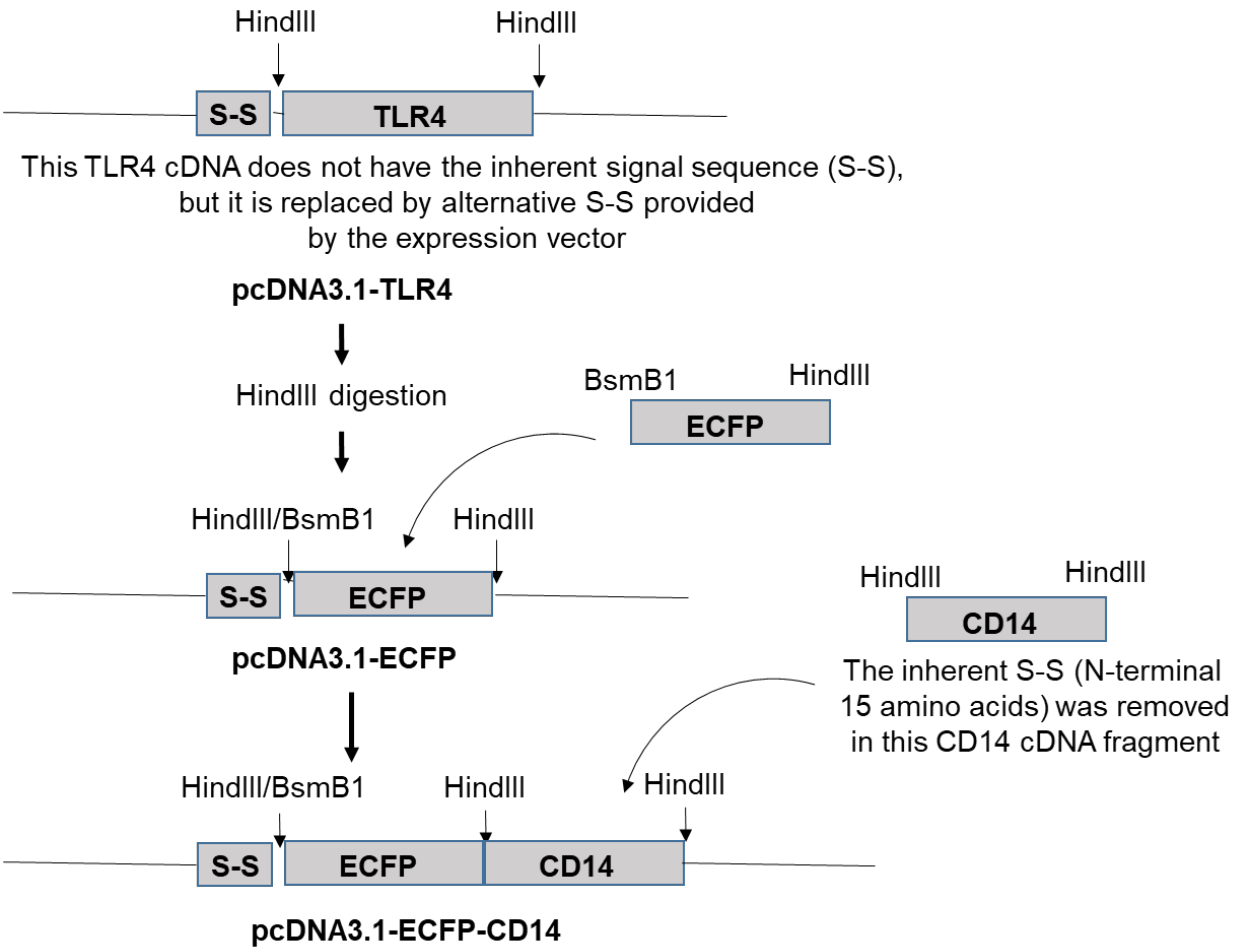

Appendix Figure S1. Cloning scheme of ECFP-CD14 expression construct.

The BsmB1 and HindIII fragment of ECFP was generated by PCR with Pfu DNA polymerase using the primers: 5'TCTA ACA CGT CTC GAG CTG GTG AGC AAG GGC GAG GAG C3' and 5'TTGG AAG AAG CTT GTA CAG CTC GTC CAT GCCG3'. (Underlining indicates the compatible cohesive end generated by BsmBI enzyme digestion at the former primer, and HindIII enzyme digestion at the latter; bolding indicates the relevant restriction enzyme site). From the pcDNA3.1-TLR4 encoding construct harboring the signaling sequence (S-S) at the N-terminus, HindIII enzyme digestion removes the entire TLR4-encoding DNA sequence but leaves the S-S sequence in the plasmid. The BsmB1 and HindIII fragment of ECFP was inserted in the HindIII site of the plasmid backbone, resulting in a pcDNA3.1-ECFP construct.

The mouse CD14 encoding fragment was PCR amplified with Pfu DNA polymerase using the following primers: CD14-Forward 5'ATGTTG AAG CTT TCT CCC GCC CCA CCA GAG CCC3' and CD14-Reverse 5'CGTAG AAG CTT TTA AAC AAA GAG GCG ATC TCC3' (underlining indicates the HindIII enzyme site; bolding indicates the stop codon). With these primers,

PCR amplification generated the CD14 fragment without the N-terminal signal sequence (15 amino acids). The PCR fragment was treated with HindIII restriction enzyme and incorporated into the HindIII-cleaved site of the pcDNA3.1-Tlr4 plasmid to replace the entire Tlr4 encoding region with the CD14 DNA fragment, generating a pcDNA3.1-Cd14 expression construct.

Subsequently, the HindIII fragment obtained from pcDNA3.1-CD14 was inserted into the HindIII site of pcDNA3.1-ECFP, leading to a pcDNA3.1-CD14-ECFP expression construct with the GPI-linkage sequence preserved.

At each step of subcloning, we performed DNA sequencing to confirm the integrity of the complete cDNA sequence and to verify the sequence in frame. The expression of each construct was also confirmed by western blotting after transfecting them into HEK293 cells.