

## Supplementary Information

### **CpG-oligodeoxynucleotides developed for grouper toll-like receptor (TLR) 21s effectively activate mouse and human TLR9s mediated immune responses**

Da-Wei Yeh<sup>1\*</sup>, Chao-Yang Lai<sup>1\*</sup>, Yi-Ling Liu<sup>1</sup>, Chih-Hao Lu<sup>1,2</sup>, Ping-Hui Tseng<sup>3</sup>,  
Chiou-Hwa Yuh<sup>4</sup>, Guann-Yi Yu<sup>5</sup>, Shih-Jen Liu<sup>5</sup>, Chih-Hsiang Leng<sup>5</sup>, & Tsung-Hsien Chuang<sup>1,6</sup>.

<sup>1</sup> Immunology Research Center, National Health Research Institutes, Miaoli, Taiwan

<sup>2</sup> Department of Life Sciences, National Central University, Taoyuan, Taiwan

<sup>3</sup> Institute of Biochemistry and Molecular Biology, National Yang-Ming University, Taipei, Taiwan

<sup>4</sup> Institute of Molecular and Genomic Medicine, National Health Research Institutes, Miaoli, Taiwan

<sup>5</sup> National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli, Taiwan.

<sup>6</sup> Program in Environmental and Occupational Medicine, Kaohsiung Medical University Kaohsiung, Taiwan

\*These authors contributed equally to this work.

Correspondence and requests for materials should be addressed to T.-H.C. (email: thchuang@nhri.org.tw)

**Supplementary Table 1. Nucleotide sequences of the primers used for reverse transcription-quantitative polymerase chain reaction (RT-qPCR).**

**Supplementary Figure 1. Alignment of toll-like receptor (TLR) 21 protein sequences for orange-spotted grouper (*Epinephelus coioides*; osgTLR21A and osgTLR21B) and giant grouper (*E. lanceolatus*; ggTLR21).** LRR, leucine-rich repeat; LRR-CT, C-terminal LRR; TM, transmembrane domain; Box1–3, box 1–3 in the cytosolic Toll/interleukin receptor (TIR) domain. Amino acids are color-coded to indicate their chemical properties: blue, acidic; pink, basic; green, hydroxyl/amine/basic/Q; red, hydrophobic (including aliphatic Y). Asterisk, identical residues; single dot, conservative substitutions; two dots, highly conservative substitutions.

**Supplementary Figure 2. Phylogenetic analysis of grouper (*Epinephelus* spp.) toll-like receptor (TLR) 21s and zebrafish (*zeb*, *Danio rerio*) TLRs.** Numbers in the left column represent the percentage protein identities of each TLR to giant grouper (gg, *E. lanceolatus*) TLR21. The GenBank accession numbers of these TLR protein sequences are listed in the right column.

**Supplementary Figure 3. Phylogenetic analysis of fish toll-like receptor (TLR) 21s.** Numbers in the left column represent the percentage protein identities of each fish TLR21 to giant grouper (gg, *E. lanceolatus*) TLR21. The GenBank accession numbers of these TLR protein sequences are listed in the right column.

**Supplementary Figure 4. Full length immunoblots of figure 1D.** Total cell lysates were collected, separated by SDS-PAGE, blotted to polyvinylidene fluoride (PVDF) membrane, and probed with primary and secondary antibodies as described in the methods section.

Supplementary table 1

Grouper Primers used in RT-qPCR

β-actin	forward 5'-GACATGGTGCGGTTTCTCTT-3'
	reverse 5'-GCCTCTGCTGTGCTGATGTA-3'
IL-1β	forward 5'-GACATGGTGCGGTTTCTCTT-3'
	reverse 5'-GCCTCTGCTGTGCTGATGTA-3'
IL-6	forward 5'-CCTGAAGGACCTCGACAATC-3'
	reverse 5'-TCCTGACAGCCAGACTTCCT-3'
IL-8	forward 5'-GAGCTGCACTGTCGCTGIAT-3'
	reverse 5'-TGTTGGCCATGATCCTGTTA-3'
IFNγ	forward 5'-GACCACCAAGATGGAGGCTA-3'
	reverse 5'-TACCGGTGTTTCCTCAGGTC-3'

Mouse Primers used in RT-qPCR

gapdh	forward 5'-ACCCAGAAGACTGTGGATGG-3'
	reverse 5'-CACATTGGGGGTAGGAACAC-3'
tnf-α	forward 5'-GGATCTGGCGCTACTCAGAC-3'
	reverse 5'-TCCGATAGCTGGTTGGTTTC-3'
il-1β	forward 5'-CAGGCAGGCAGTATCACTCA-3'
	reverse 5'-AGCTCATATGGGTCCGACAG-3'
il-6	forward 5'-AGTTGCCTTCTTGGGACTGA-3'
	reverse 5'-TCCACGATTCCAGAGAAC-3'
cxcl1	forward 5'-GCTGGGATTCACCTCAAGAA-3'
	reverse 5'-CTTGGGGACACCTTTAGCA-3'

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                                LRR1
osgTLR21A MASLTYQLLSVTLALCAVHLSGYSFRNCIEDPPYPYQGSFKCILRKE SNLSALI GDLFPQT 60
osgTLR21B MASLTYQLLSVTLALCAVHLSGYSFRNCIEDPPYPYQGSFKCILRKE SNLSALI GDLFPQT 60
ggTLR21   MASLTYQLLSVTLALCAVHLSGYSFRNCIEDPPYPYQGSFKCILRKE SNLSAVI GDLFPQT 60
          *****:*****

                                LRR2                                LRR3                                LRR4
osgTLR21A AVNLTITINPVWHIPSMSEFVNVTNLEYLRLDHNLYLRKIDPFAFQNLTRLKSLNVSSNNIS 120
osgTLR21B AVNLTITINPVWHIPSMSEFVNVTNLEYLRLDHNLYLRKIDPFAFQNLTRLKSLNVSSNNIS 120
ggTLR21   AVNLTITINPVWHIPSMSEFVNVTNLEYLRLDHNLYLRKIDPFAFQNLTRLKSLNVSSNNIS 120
          *****:*****

                                LRR5
osgTLR21A QLNPNYVFKDLHNLTYLSLTNNSLKQLPVGIFSTLLNLDLIMRQNLLTNFSGIAESVSHL 180
osgTLR21B QLNPNYVFKDLHNLTYLSLTNNSLKQLPVGIFSTLLNLDLIMRQNLLTNFSGIAESVSHL 180
ggTLR21   QLNPNYVFKDLHNLTYLSLTNNSLKQLPVGIFSTLLNLDLIMRQNLLTNFSGIAESVSHL 180
          *****:*****

                                LRR6                                LRR7
osgTLR21A PKLRVLDLFCFNNLTNLKHSNASLPKSLTTLTYICRNNLLTLGCHQSFLGFIQLLDLSYNPR 240
osgTLR21B PKLRVLDLFCFNNLTNLKHSNASLPKSLTTLTYICRNNLLTLGCHQSFLGFIQLLDLSYNPR 240
ggTLR21   PKLRVLDLFCFNNLTNLKHSNASLPKSLTTLTYICRNNLLTLGCHQSFLGFIQLLDLSYNPR 240
          *****:*****

                                LRR8                                LRR9
osgTLR21A LPTMAFQGVVDSLHINYLRLRSTSVKVVDFLNI SNVNAGSVDFSGMGLKNDILLTELCTSL 300
osgTLR21B LPTMAFQGVVDSLHINYLRLRSTSVKVVDFLNI SNVNAGSVDFSGMGLKNDILLTELCTSL 300
ggTLR21   LPTMAFQGVVDSLHINYLRLRSTSVKVVDFLNI SNVNAGSVDFSGMGLKNDILLTELCTSL 300
          *****:*****

                                LRR10                               LRR11
osgTLR21A KGKVKSIKRMGLSNNNGIKNLTNNALQYCPITITGSLDLSRNNLKSTGCFKFLDKHTQIKSL 360
osgTLR21B KGKVKSIKRMGLSNNNGIKNLTNNALQYCPITITGSLDLSRNNLKSTGCFKFLDKHTQIKSL 360
ggTLR21   KGKVKSIKRMGLSNNNGIKNLTNYALQYCPITITGSLDLSRNNLKSTGCFKFLDKHTQIKSL 360
          *****:*****

                                LRR12                               LRR13                               LRR14
osgTLR21A NAEHNHITSLQSCKTQNMVYLNHLEELSRYRNIILSVNAYAFSHTPNIKTLKLNINTISF 420
osgTLR21B NAEHNHITSLQSCKTQNMVYLNHLEELSRYRNIILSVNAYAFSHTPNIKTLKLNINTISF 420
ggTLR21   NAEHNHITSLQSCKTENMVYLNHLEELSRYRNIILSVNAYAFSHTPNIKTLKLNINTISF 420
          *****:*****

                                LRR15                               LRR16
osgTLR21A LHRKALKGLKSLEMLRLDNNLLTDLFNDTFEDNVNLQTLNLRNNRIAVIFNGTFLSLRNL 480
osgTLR21B LHRKALKGLKSLEMLRLDNNLLTDLFNDTFEDNVNLQTLNLRNNRIAVIFNGTFLSLRNL 480
ggTLR21   LHRKALKGLKSLEMLRLDNNLLTDLFNDTFEDNVNLQTLNLRNNRIAVIFNGTFLSLRNL 480
          *****:*****

                                LRR17                               LRR18
osgTLR21A TTLDLGGNKITHEFEQSGLDGLTSLSKFYLDGNNLKEIDTSLYRVFQDTLTVLDLKSNIY 540
osgTLR21B TTLDLGGNKITHEFEQSGLDGLTSLSKFYLDGNNLKEIDTSLYRVFQDTLTVLDLKSNIY 540
ggTLR21   TTLDLGGNKITHEFEQSGLDGLTSLSKFYLDGNNLKEIDTSLYRVFQDTLTVLDLKSNIY 540
          *****:*****

                                LRR19                               LRR20
osgTLR21A FFHKVITSSPFMNL SKLTDLKLKGQRP HGLSVLPQNFRRGLHSLKSLYL TNNNIYYLAPDA 600
osgTLR21B FFHKVITSSPFMNL SKLTDLKLKGQRP HGLSVLPQNFRRGLHSLKSLYL TNNNIYYLAPDA 600
ggTLR21   FLHKVITSSPFMNL SKLSDLKLKGQRP HGLSVLPQNFRRGLHSLKSLYLANNNIYYLAPDA 600
          *****:*****

                                LRR21                               LRR22
osgTLR21A FDDLNLTLFSLSEGCCVGAQLQPGIFKNLRLNLSMENMGIQTFSEVFGNLTKLHKL 660
osgTLR21B FDDLNLTLFSLSEGCCVGAQLQPGIFKNLRLNLSMENMGIQTFSEVFGNLTKLHKL 660
ggTLR21   FDDLNLTLFSLSECCVGAQLQPGIFKNLRLNLSMENMGIQTFSEVFGNLTKLHKL 660
          *****:*****

                                LRR23                               LRR CT
osgTLR21A QLNRRNVMQSIHYEILDSLP ELHYLDLRDTPLSCTCKNHLLQNWTLHNRVQVVLYNMKC 720
osgTLR21B QLNRRNVMQSIHYEILDSLP ELHYLDLRDTPLSCTCKNHLLQNWTLHNRVQVVLYNMKC 720
ggTLR21   QLNRRNVMQSIHYEILDSLP ELHYLDLRDTPLSCTCKNHLLQNWTLHNRVQVVLYNMKC 720
          *****:*****

                                TM
osgTLR21A QDDNQHNFYNFKTNVCYIDLGEYFLSTAI VIFLFTVTPLLYVKLYWKMKYGYVFRSWF 780
osgTLR21B QDDNQHNFYNFKTNVCYIDLGEYFLSTAI VIFLFTVTPLLYVKLYWKMKYGYVFRSWF 780
ggTLR21   FDDNQHNFYNFKTNVCYIDLGEYFLSTAI VIFLFTVTPLLYVKLYWKMKYNYVFRSWF 780
          *****:*****

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osgTLR21B SDQWRRLREQEENCKYDAFISYNS SDEQWVIEQLMPNLEGNSSFKLCLHHRDFELGRDI 840
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          *****:*****

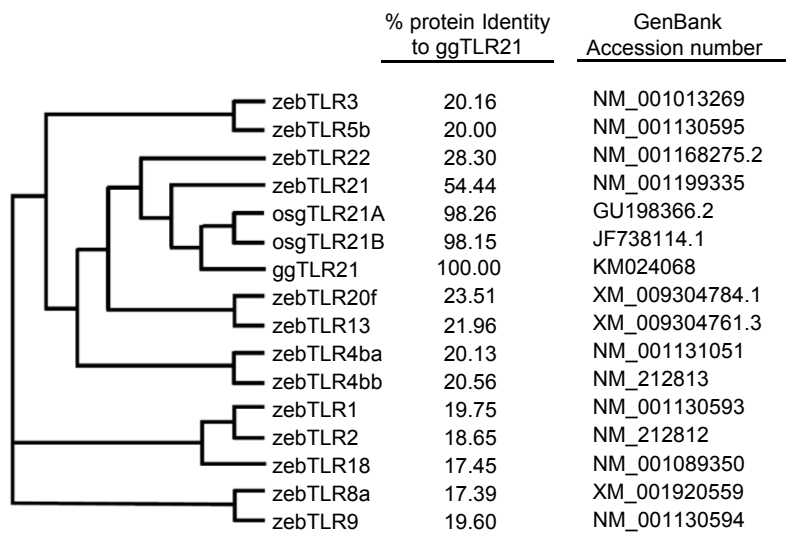
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ggTLR21   VDNIVSAVYSSRRTICVVS RNFLTSEWCSLEIQ LASYRLFDEHRDVLLLVFLEPISERQL 900
          *****:*****

                                Box3
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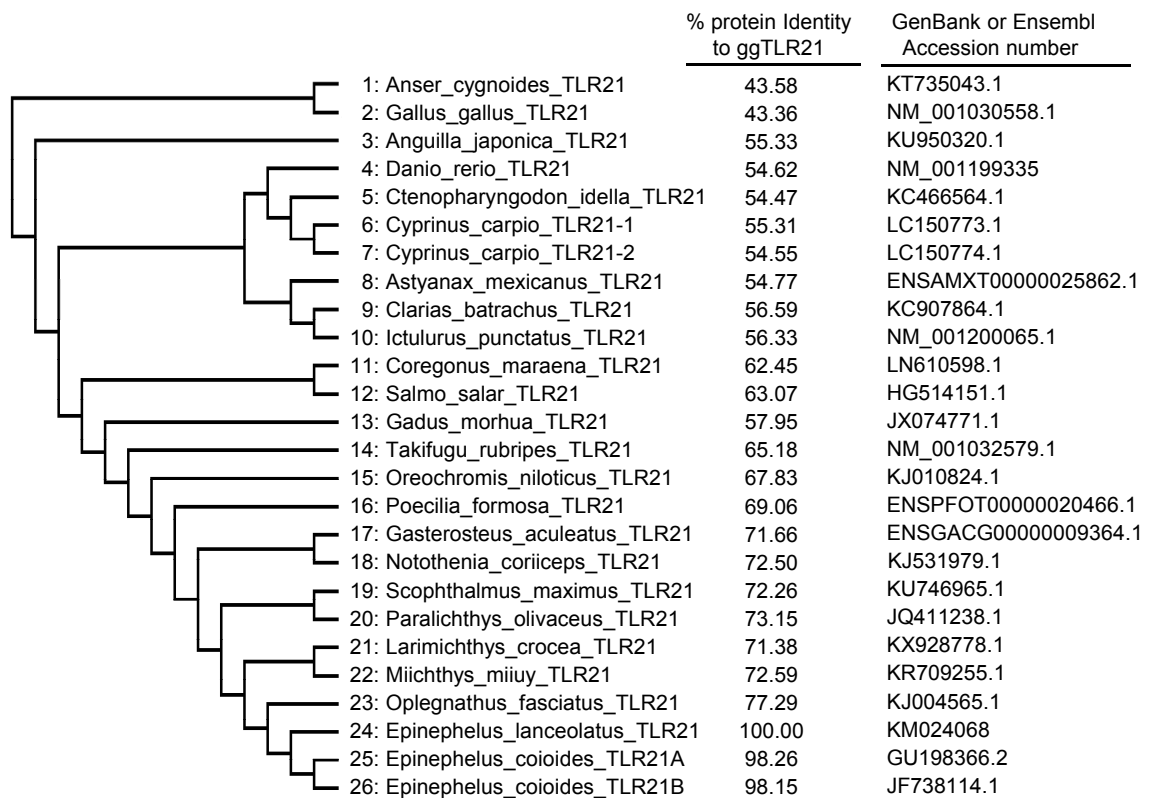
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ggTLR21   GRTEHVDARTSDENYLLP 979
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Supplementary figure 2



Supplementary figure 3



Supplementary figure 4

