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Postbiotic-based recombinant receptor activator of NF-κB ligand enhanced oral vaccine efficiency in chicken

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A.

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gExR.h      CATATGAAGAAGAAGATCATCAGTGCAATTCTTATGTCAACCGTTATTTTATCTGCTGCC   60
gsR.h       CATATGAAGAAGAAGATCATCAGTGCAATTCTTATGTCAACCGTTATTTTATCTGCTGCC   60
            ************************************************************

gExR.h      GCTCCATTGTCTGGTGTGTATGCTGATACAGATCCATCACGTATTTCAAAAGAAGATGCT   120
gsR.h       GCTCCATTGTCTGGTGTGTATGCTGATACA------------------------------   90
            ****************************

gExR.h      CATTGTGTTCGTATGCTTTTTCGTTCACAAGAATCAATTGGTCTTCAAGATACTCCATTT   180
gsR.h       ------------------------------------------------------------   90

gExR.h      GAAAATCAAGAAGTTAAACTTATGCCAGAATCATGTCGTCGTATGAAACGTGCTCTTCAA   240
gsR.h       ------------------------------------------------------------   90

gExR.h      CGTGCTGTTCAAAAAGAAGTTCAACGTATTCTTGGTAAAGAATCACCACGTCCAGAAAAA   300
gsR.h       ------------------------------------GAATCACCACGTCCAGAAAAA   111
                                                ********************

gExR.h      GCTGCTATGGAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACCA   360
gsR.h       GCTGCTATGGAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACCA   171
            ************************************************************

gExR.h      TTTGCTCATCTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCTT   420
gsR.h       TTTGCTCATCTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCTT   231
            ************************************************************

gExR.h      ACTTCATGGCATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGGT   480
gsR.h       ACTTCATGGCATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGGT   291
            ************************************************************

gExR.h      AAACTTATTGTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCAT   540
gsR.h       AAACTTATTGTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCAT   351
            ************************************************************

gExR.h      CATGAAACTTCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAAA   600
gsR.h       CATGAAACTTCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAAA   411
            ************************************************************

gExR.h      ACTAATCTTAAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTGG   660
gsR.h       ACTAATCTTAAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTGG   471
            ************************************************************

gExR.h      TCAGGTAATTCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAAA   720
gsR.h       TCAGGTAATTCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAAA   531
            ************************************************************

gExR.h      ACTGGTGATATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGAA   780
gsR.h       ACTGGTGATATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGAA   591
            ************************************************************

gExR.h      GCTACTTATTTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTGA   840
gsR.h       GCTACTTATTTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTGA   651
            ************************************************************

gExR.h      CTCGAG              846
gsR.h       CTCGAG              657
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B.

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NS.gExR.h       CATATGGATCCATCACGTATTTCAAAAGAAGATGCTCATTGTGTTCGTATGCTTTTTCGT
                60
NS.gsR.h        CATATG------------------------------------------------------
                0
                ******

NS.gExR.h       TCACAAGAATCAATTGGTCTTCAAGATACTCCATTTGAAAATCAAGAAGTTAAACTTATG
                120
NS.gsR.h        ------------------------------------------------------------
                0


NS.gExR.h       CCAGAATCATGTCGTCGTATGAAACGTGCTCTTCAACGTGCTGTTCAAAAAGAAGTTCAA
                180
NS.gsR.h        ------------------------------------------------------------
                0


NS.gExR.h       CGTATTCTTGGTAAAGAATCACCACGTCCAGAAAAAGCTGCTATGGAAGCTATTGGTATG
                240
NS.gsR.h        ---------------GAATCACCACGTCCAGAAAAAGCTGCTATGGAAGCTATTGGTATG
                51
                               *******************************************


NS.gExR.h       GAACTTTATCGTCGTAATAAACCAGAAAAACAACCATTTGCTCATCTTATTATTGATGAT
                300
NS.gsR.h        GAACTTTATCGTCGTAATAAACCAGAAAAACAACCATTTGCTCATCTTATTATTGATGAT
                111
                ************************************************************


NS.gExR.h       AAAAATATTCTTACTGGTACTCGTAAAGTTAATCTTACTTCATGGCATCATAATAAAGGT
                360
NS.gsR.h        AAAAATATTCTTACTGGTACTCGTAAAGTTAATCTTACTTCATGGCATCATAATAAAGGT
                171
                ************************************************************


NS.gExR.h       CAAGCTAATCTTTCAAATATGACTTTTTCAGATGGTAAACTTATTGTTAATCAAGATGGT
                420
NS.gsR.h        CAAGCTAATCTTTCAAATATGACTTTTTCAGATGGTAAACTTATTGTTAATCAAGATGGT
                231
                ************************************************************


NS.gExR.h       TTTTATTATCTTTATGCTAATATTTGTTTTCGTCATCATGAAACTTCAGGTAATCTTACT
                480
NS.gsR.h        TTTTATTATCTTTATGCTAATATTTGTTTTCGTCATCATGAAACTTCAGGTAATCTTACT
                291
                ************************************************************


NS.gExR.h       AAACGTGGTCTTCAACTTATGGTTTATATGACTAAAACTAATCTTAAAATTCGTCGTTCA
                540
NS.gsR.h        AAACGTGGTCTTCAACTTATGGTTTATATGACTAAAACTAATCTTAAAATTCGTCGTTCA
                351
                ************************************************************


NS.gExR.h       GATGTTCTTATGAAAGGTGGTTCAACTAAATATTGGTCAGGTAATTCAGAATTTCATTTT
                600
NS.gsR.h        GATGTTCTTATGAAAGGTGGTTCAACTAAATATTGGTCAGGTAATTCAGAATTTCATTTT
                411
                ************************************************************


NS.gExR.h       TATTCAGTTAATATTGGTGGTTTTCTTAAACTTAAAACTGGTGATATGATTTCAATTCAA
                660
NS.gsR.h        TATTCAGTTAATATTGGTGGTTTTCTTAAACTTAAAACTGGTGATATGATTTCAATTCAA
                471
                ************************************************************


NS.gExR.h       GTTTCAAATCCACTTCTTCTTGATTCATCACAAGAAGCTACTTATTTTGGTGCTTTTAAA
                720
NS.gsR.h        GTTTCAAATCCACTTCTTCTTGATTCATCACAAGAAGCTACTTATTTTGGTGCTTTTAAA
                531
                ************************************************************


NS.gExR.h       GTTCGTGATCTTGATCACCATCATCACCACCATTGACTCGAG    762
NS.gsR.h        GTTCGTGATCTTGATCACCATCATCACCACCATTGACTCGAG    573
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Fig.S1 Insert sequences (A) With signal peptide (USP45) and (B) without signal peptide. gExR.h: Chicken extracellular RANKL sequence with signal peptide. gsR.h: Chicken soluble RANKL sequence with signal peptide. NS.gExR.h: Chicken extracellular RANKL sequence without signal peptide. NS.gsR.h: Chicken soluble RANKL sequence without signal peptide. Red font: Restriction sites. Green font: Signal peptide. Yellow font: His-tag.

A.

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Synthetic_gene      ----------CATATGAAGAAGAAGATCATCAGTGCAATTCTTATGTCAACCGTTATTTT     60
pILPtuf.gsR.h       AGACATTTTTCATATG----------------------------TCAACCGTTATTTT     30
                          ******                                **************

Synthetic_gene      ATCTGCTGCCGCTCCATTGTCTGGTGTGTATGCTGATACAGAATCACCACGTCCAGAAAA     120
pILPtuf.gsR.h       ATCTGCTGCCGCTCCATTGTCTGGTGTGTATGCTGATACAGAATCACCACGTCCAGAAAA     90
                    ************************************************************

Synthetic_gene      AGCTGCTATGGAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACC     180
pILPtuf.gsR.h       AGCTGCTATGGAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACC     150
                    ************************************************************

Synthetic_gene      ATTTGCTCATCTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCT     240
pILPtuf.gsR.h       ATTTGCTCATCTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCT     210
                    ************************************************************

Synthetic_gene      TACTTCATGGCATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGG     300
pILPtuf.gsR.h       TACTTCATGGCATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGG     270
                    ************************************************************

Synthetic_gene      TAAACTTATTGTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCA     360
pILPtuf.gsR.h       TAAACTTATTGTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCA     330
                    ************************************************************

Synthetic_gene      TCATGAAACTTCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAA     420
pILPtuf.gsR.h       TCATGAAACTTCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAA     390
                    ************************************************************

Synthetic_gene      AACTAATCTTAAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTG     480
pILPtuf.gsR.h       AACTAATCTTAAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTG     450
                    ************************************************************

Synthetic_gene      GTCAGGTAATTCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAA     540
pILPtuf.gsR.h       GTCAGGTAATTCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAA     510
                    ************************************************************

Synthetic_gene      AACTGGTGATATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGA     600
pILPtuf.gsR.h       AACTGGTGATATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGA     570
                    ************************************************************

Synthetic_gene      AGCTACTTATTTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTG     660
pILPtuf.gsR.h       AGCTACTTATTTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTG     630
                    ************************************************************

Synthetic_gene      ACTCGAG----------              677
pILPtuf.gsR.h       ACTCGAGGGATCCAGGA              647
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B.

```
Synthetic_gene      ----------CATATGAAGAAGAAGATCATCAGTGCAATTCTTATGTCAACCGTTATTTT    50
pILPtuf.gsR.h       AGACATTTTTCATATGAAGAAGAAGATCATCAGTGCAATTCTTATGTCAACCGTTATTTT    60
                              **************************************************

Synthetic_gene      ATCTGCTGCCGCTCCATTGTCTGGTGTGTATGCTGATACAGAATCACCACGTCCAGAAAA    110
pILPtuf.gsR.h       ATCTGCTGCCGCTCCATTGTCTGGTGTGTATGCTGATACAGAATCACCACGTCCAGAAAA    120
                    ************************************************************

Synthetic_gene      AGCTGCTATGGAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACC    170
pILPtuf.gsR.h       AGCTGCTATGTAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACC    180
                    ********** **************************************************

Synthetic_gene      ATTTGCTCATCTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCT    230
pILPtuf.gsR.h       ATTTGCTCATCTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCT    240
                    ************************************************************

Synthetic_gene      TACTTCATGGCATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGG    290
pILPtuf.gsR.h       TACTTCATGGCATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGG    300
                    ************************************************************

Synthetic_gene      TAAACTTATTGTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCA    350
pILPtuf.gsR.h       TAAACTTATTGTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCA    360
                    ************************************************************

Synthetic_gene      TCATGAAACTTCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAA    410
pILPtuf.gsR.h       TCATGAAACTTCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAA    420
                    ************************************************************

Synthetic_gene      AACTAATCTTAAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTG    470
pILPtuf.gsR.h       AACTAATCTTAAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTG    480
                    ************************************************************

Synthetic_gene      GTCAGGTAATTCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAA    530
pILPtuf.gsR.h       GTCAGGTAATTCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAA    540
                    ************************************************************

Synthetic_gene      AACTGGTGATATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGA    590
pILPtuf.gsR.h       AACTGGTGATATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGA    600
                    ************************************************************

Synthetic_gene      AGCTACTTATTTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTG    650
pILPtuf.gsR.h       AGCTACTTATTTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTG    660
                    ************************************************************

Synthetic_gene      ACTCGAG----------           657
pILPtuf.gsR.h       ACTCGAGGGATCCAGGA           677
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C.

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Synthetic_gene          ---------------CATATGGATCCATCACGTATTTCAAAAGAAGATGCTCATTGTGTT 45
pILPtuf.NS.gExR.h       AGACATTTTTCATAGCATATGGATCCATCACGTATTTCAAAAGAAGATGCTCATTGTGTT 60
                                       ****************************************

Synthetic_gene          CGTATGCTTTTTCGTTCACAAGAATCAATTGGTCTTCAAGATACTCCATTTGAAAATCAA 105
pILPtuf.NS.gExR.h       CGTATGCTTTTTCGTTCACAAGAATCAATTGGTCTTCAAGATACTCCATTTGAAAATCAA 120
                        ************************************************************

Synthetic_gene          GAAGTTAAACTTATGCCAGAATCATGTCGTCGTATGAAACGTGCTCTTCAACGTGCTGTT 165
pILPtuf.NS.gExR.h       GAAGTTAAACTTATGCCAGAATCATGTCGTCGTATGAAACGTGCTCTTCAACGTGCTGTT 180
                        ************************************************************

Synthetic_gene          CAAAAAGAAGTTCAACGTATTCTTGGTAAAGAATCACCACGTCCAGAAAAAGCTGCTATG 225
pILPtuf.NS.gExR.h       CAAAAAGAAGTTCAACGTATTCTTGGTAAAGAATCACCACGTCCAGAAAAAGCTGCTATG 240
                        ************************************************************

Synthetic_gene          GAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACCATTTGCTCAT 285
pILPtuf.NS.gExR.h       GAAGCTATTGGTATGGAACTTTATCGTCGTAATAAACCAGAAAAACAACCATTTGCTCAT 300
                        ************************************************************

Synthetic_gene          CTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCTTACTTCATGG 345
pILPtuf.NS.gExR.h       CTTATTATTGATGATAAAAATATTCTTACTGGTACTCGTAAAGTTAATCTTACTTCATGG 360
                        ************************************************************

Synthetic_gene          CATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGGTAAACTTATT 405
pILPtuf.NS.gExR.h       CATCATAATAAAGGTCAAGCTAATCTTTCAAATATGACTTTTTCAGATGGTAAACTTATT 420
                        ************************************************************

Synthetic_gene          GTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCATCATGAAACT 465
pILPtuf.NS.gExR.h       GTTAATCAAGATGGTTTTTATTATCTTTATGCTAATATTTGTTTTCGTCATCATGAAACT 480
                        ************************************************************

Synthetic_gene          TCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAAAACTAATCTT 525
pILPtuf.NS.gExR.h       TCAGGTAATCTTACTAAACGTGGTCTTCAACTTATGGTTTATATGACTAAAACTAATCTT 540
                        ************************************************************

Synthetic_gene          AAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTGGTCAGGTAAT 585
pILPtuf.NS.gExR.h       AAAATTCGTCGTTCAGATGTTCTTATGAAAGGTGGTTCAACTAAATATTGGTCAGGTAAT 600
                        ************************************************************

Synthetic_gene          TCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAAAACTGGTGAT 645
pILPtuf.NS.gExR.h       TCAGAATTTCATTTTTATTCAGTTAATATTGGTGGTTTTCTTAAACTTAAAACTGGTGAT 660
                        ************************************************************

Synthetic_gene          ATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGAAGCTACTTAT 705
pILPtuf.NS.gExR.h       ATGATTTCAATTCAAGTTTCAAATCCACTTCTTCTTGATTCATCACAAGAAGCTACTTAT 720
                        ************************************************************

Synthetic_gene          TTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTGACTCGAG--- 762
pILPtuf.NS.gExR.h       TTTGGTGCTTTTAAAGTTCGTGATCTTGATCACCATCATCACCACCATTGACTCGAGGGA 780
                        ************************************************************

Synthetic_gene          -------      762
pILPtuf.NS.gExR.h       TCCAGGA      787
```

Fig.S2 Sequencing results of deleted, substituted or inserted insert sequences. A. Deleted gsR.h. B. Substituted gsR.h. C. Inserted NS.gExR.h. Black box: Deletion, substitution or insertion. Black bold font: Vector backbone.

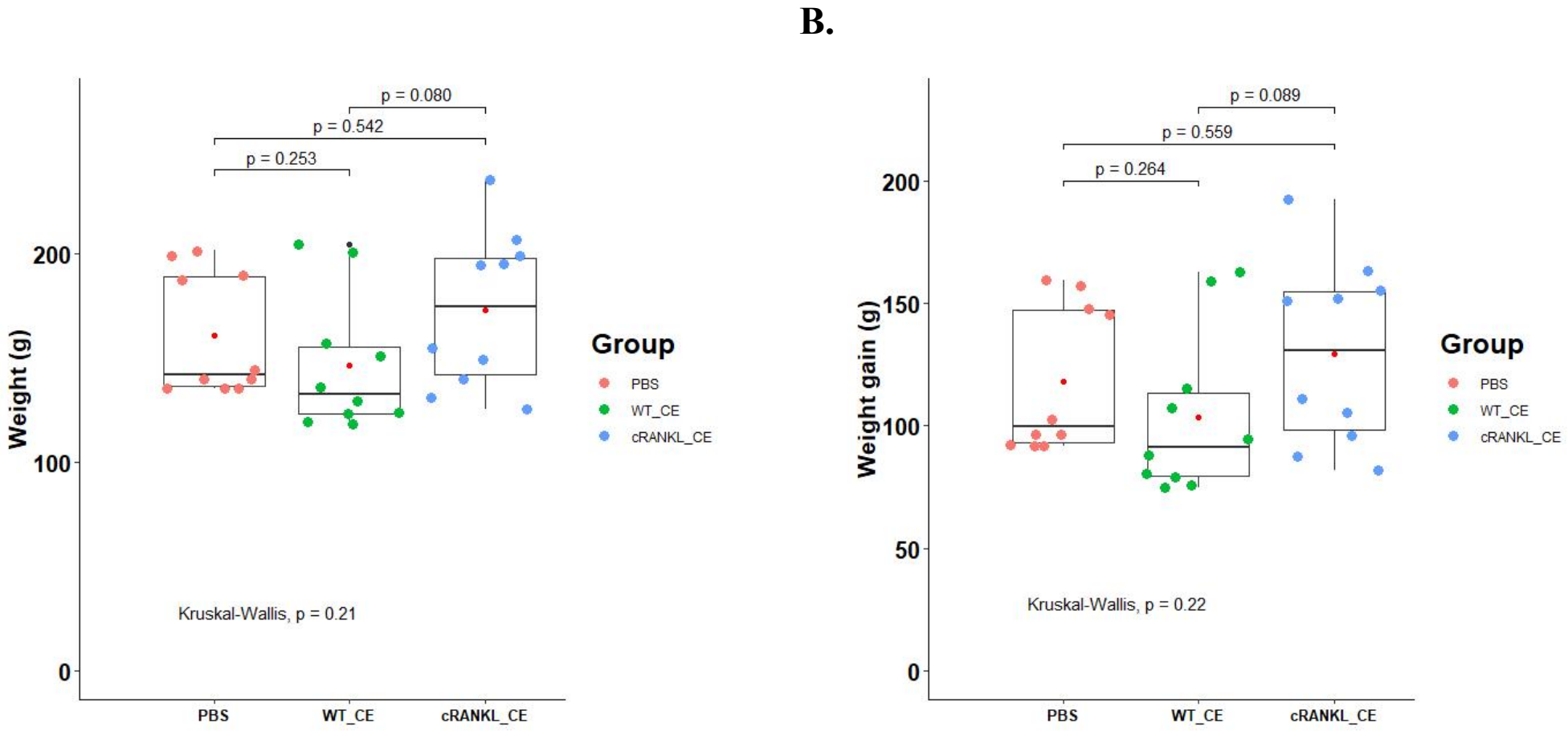

Fig.S3 Weight and weight gain after twelve consecutive days oral administration of cell extracts in chicken (ROSS 308). A. Weight (At day 13). B. Weight gain. For significance tests, Kruskal-Wallis test followed by Dunn's post-hoc test. Mean: Red point; Median: Black line.

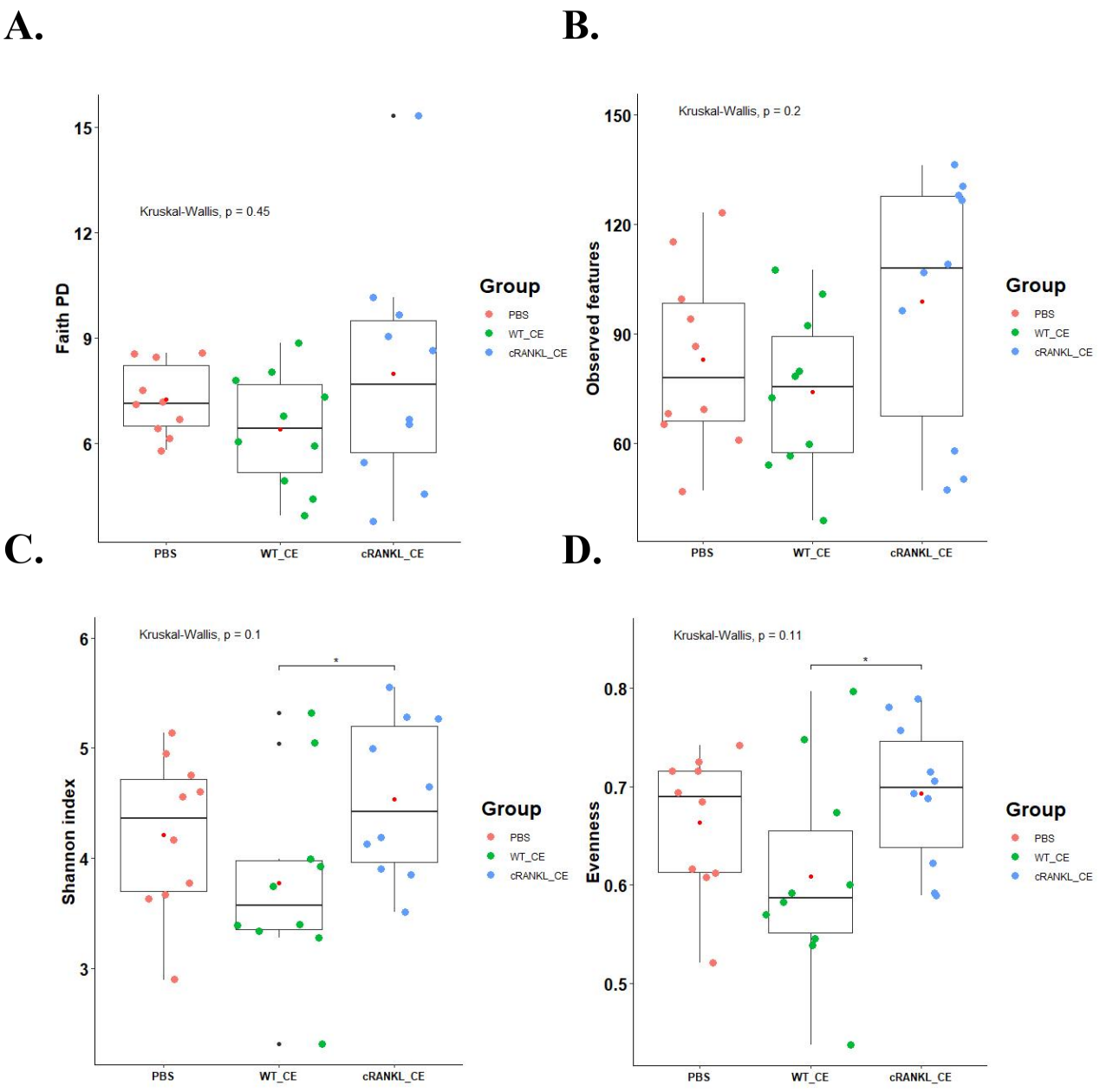

Fig.S4 Box plots of alpha-diversity indices comparing PBS, WT_CE and cRANKL_CE three groups. A. Observed features. B. Pielou evenness. C. Faith's phylogenetic diversity (Faith PD) and D. Shannon's index. *p < 0.05. Mean: Red point; Median: Black line.

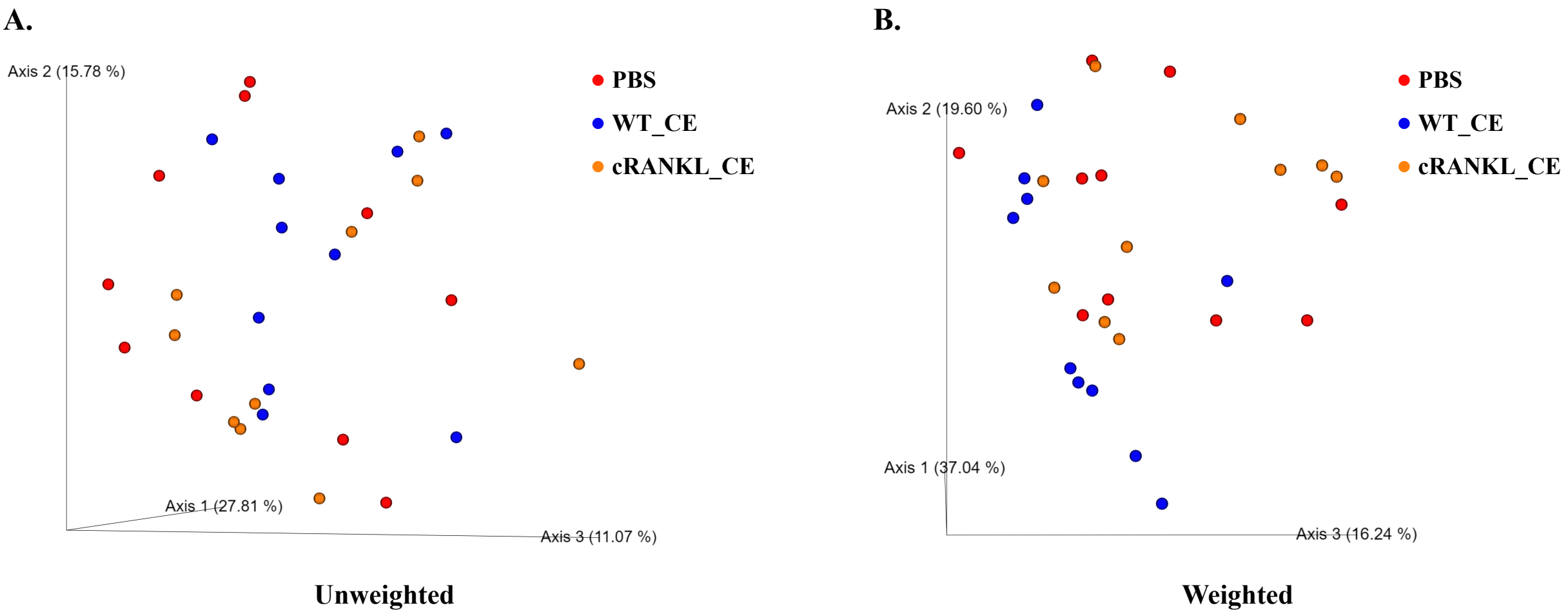

Fig.S5 Principal coordinate analysis of the microbiota among PBS, WT_CE and cRANKL_CE groups. A. Unweighted and (B) weighted based on UniFrac distances. Subject color: red, PBS (n =10); blue, WT_CE (n = 10); orange, cRANKL_CE (n = 10).

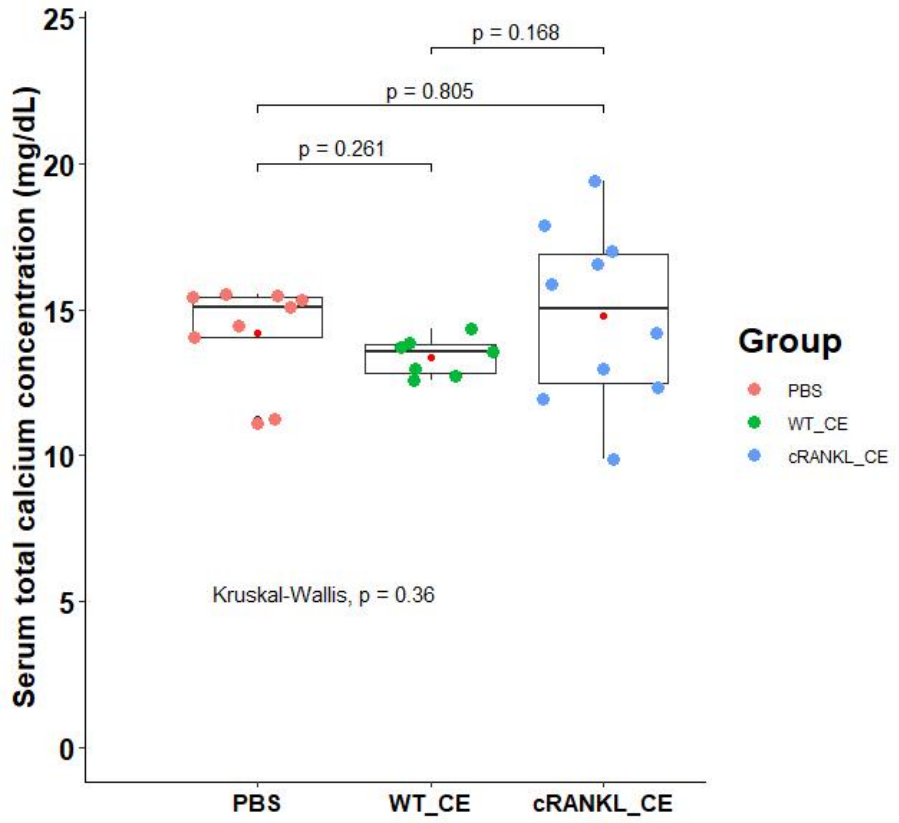

Fig.S6 Serum concentration after twelve consecutive days' oral administration of cell extracts in chicken (ROSS 308). For significance tests, Kruskal-Wallis test followed by Dunn's post-hoc test. Mean: Red point; Median: Black line.