Supplementary information

Immunoinformatics based designing of a multi-epitope chimeric vaccine from multi-domain outer surface antigens of Leptospira

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**Table S1: Ramachandran statistics and ProSA Z score**

| Protein | Favoured Region (%) | Allowed Region (%) | Outlier Region (%) | ProSA Z-Score |
|---------|---------------------|-------------------|-------------------|---------------|
| LigA7   | 94.3                | 5.7               | 0                 | -3.64         |
| LigA8   | 92.7                | 6.1               | 1.2               | -4.47         |
| LigA9   | 93                  | 7                 | 0                 | -4.47         |
| LigA10  | 95.3                | 3.5               | 1.2               | -4.18         |
| LigA11  | 94.1                | 5.9               | 0                 | -4.66         |
| LigA12  | 92                  | 6.9               | 1.1               | -5.17         |
| LigA13  | 92                  | 6.9               | 1.1               | -4.35         |
| LigB1   | 95.3                | 4.7               | 0                 | -4.96         |
| LigB2   | 96.4                | 2.4               | 1.2               | -3.88         |
| LigB3   | 96.3                | 3.7               | 0                 | -4.01         |
| LigB4   | 96.5                | 2.3               | 1.2               | -4.10         |
| LigB5   | 97.6                | 1.2               | 1.2               | -3.50         |
| LigB6   | 97.7                | 1.1               | 1.1               | -3.82         |
| LigB7   | 94.3                | 4.5               | 1.1               | -2.98         |
| LigB8   | 94                  | 6                 | 0                 | -3.10         |
| LigB9   | 94.3                | 4.5               | 1.1               | -3.73         |
| LigB10  | 94.3                | 4.5               | 1.1               | -3.17         |
| LigB11  | 95.3                | 3.5               | 1.2               | -2.77         |
| LigB12  | 95.5                | 3.4               | 1.1               | -4.70         |
### Table S2: List of predicted conformation BCL epitopes

| Domain | BEPro | DiscoTope 2.0 | ElliPro | Score |
|--------|-------|---------------|---------|-------|
| LigA7  | 1-2, 30-33, 36, 54, 56-59 | 2, 5, 29-36, 56, 58, 59 | 13 | 0.791 |
|        |       |               | A:A1, A:K2, A:L3, A:V4, A:E5, A:F29, A:T30, A:D31, A:N32, A:S33, A:N34, A:G78, A:G79, A:N55, A:T56, A:N57, A:A58, A:K59, A:A11, A:A12, A:A13, A:S14, A:K15, A:A16, A:K17, A:G18, A:S46, A:N47, A:T48, A:D49, A:I50, A:L66, A:K67, A:Q68, A:G69, A:T70, A:T87, A:T89, A:Q90 | 21 | 0.67  |
| LigA8  | 1, 12, 24-32, 51-54, 74 | 1, 22, 24-32, 36, 52-54 | 15 | 0.833 |
|        |       |               | A:S1, A:I24, A:F25, A:T26, A:D27, A:H28, A:S29, A:K30 | 8 | 0.72  |
| LigA9  | 1, 26-31, 51, 53-56 | 2, 4, 23, 25, 27-33, 54, 55 | 13 | 0.725 |
|        |       |               | A:T1, A:S2, A:I25, A:Y26, A:S27, A:D28, A:N29, A:S30, A:S31, A:K32, A:I34, A:T35, A:S36, A:S51, A:N52, A:T53, A:K54, A:G55, A:Y56, A:G75, A:N76 | 21 | 0.647 |
| LigA10 | 13, 27-30, 54-56 | 13, 28, 29, 87 | 4 | 0.652 |
| LigA10 | 13, 27-30, 54-56 | 13, 28, 29, 87 | 4 | 0.561 |

**Score**: calculated based on the predicted epitopes and their binding scores.
| LigA11 | 13-15, 27-30, 47, 55, 65, 67, 87 | 13-15, 28, 29, 47, 65, 67, 87 | 9 | A:T8, A:S9, A:S10, A:H11, A:K12, A:A13, A:K14, A:G15, A:S43, A:N44, A:T45, A:A46, A:Y47, A:L63, A:S64, A:K65, A:G66, A:T67, A:Q85, A:T87 | 20 | 0.699 |
| ------ | ---------------------------------- | ----------------------------- | --- | -------------------------------------------------------------------------------- | ---- | ------ |
| LigA11 | 13-15, 27-30, 47, 55, 65, 67, 87 | 13-15, 28, 29, 47, 65, 67, 87 | 9 | A:A13, A:K14, A:G15, A:T87 | 4 | 0.784 |
| LigA12 | 1-2, 4, 6, 13, 25-33, 53-55, 76-78, 89 | 1, 2, 4, 23-33, 35-37, 55, 73, 75-79 | 24 | A:R1, A:Y2, A:T25, A:Y26, A:S27, A:D28, A:Q29, A:S30, A:T31, A:K32, A:L34, A:T35, A:K35, A:K56, A:K57, A:Y74, A:D75, A:H76, A:H77, A:Q79 | 24 | 0.682 |
| LigA13 | 1, 15, 29-32, 56-57, 89 | 1, 29-31, 1 | 1, 29-31 | 4 | A:S53, A:N54, A:V55, A:D56, A:D57, A:E58 | 6 | 0.732 |
| LigB1 | 1-6, 18-19, 29-37, 40-49-50, 55-56, 74-76, 86-87 | 1-6, 27, 29-37, 40, 41, 56, 74-77 | 23 | A:N1, A:P2, A:T3, A:T5, A:R6, A:I29, A:F30, A:D31, A:N32, A:G33, A:T34, A:N35, A:Q36 | 13 | 0.8 |
| LigB1 | 1-6, 18-19, 29-37, 40-49-50, 55-56, 74-76, 86-87 | 1-6, 27, 29-37, 40, 41, 56, 74-77 | 23 | A:S14, A:S15, A:I16, A:A17, A:N18, A:G19, A:S48, A:Q49, A:S50, A:V51, A:I62, A:A63, A:S64, A:G65, A:S66, A:T83, A:V84, A:T85, A:P86, A:A87 | 20 | 0.647 |
| LigB2 | 1, 14-15, 25-32, 38, 48, 73-75, 85-86 | 1, 14,15, 25-32, 38, 48, 73, 74 | 15 | A:S1, A:Q3, A:I25, A:F26, A:S27, A:D28, A:G29, A:S30, A:H31 | 9 | 0.832 |
| LigB2 | 1, 14-15, 25-32, 38, 48, 73-75, 85-86 | 1, 14,15, 25-32, 38, 48, 73, 74 | 15 | A:S9, A:G10, A:I11, A:L12, A:P13, A:K14, A:G15, A:T16, A:N17, A:S45, A:N46, A:P47, A:D48, A:L49, A:I61, A:N62, A:L63, A:G64, A:T65, A:E80, A:T82, A:V83, A:G84, A:D85, A:A86, A:I34, A:S35, A:N36, A:D37, A:P38, A:L39, A:D53, A:D54, A:S55, A:G56, A:Q73, A:S74, A:K75 | 25 | 0.623 |
| LigB3 | 1-2, 6-7, 9, 14-15, 17, 19, 23, 25-33, 36, 45-46, 51-53, 55, 71-72, 83-84 | 1, 2, 7, 9, 14, 15, 17, 23-33, 36, 46, 51-53, 59, 71, 72, 83, 84 | 28 | A:S1, A:Q2, A:I11, A:Y26, A:S27, A:D28, A:G29, A:S30, A:N31, A:R32, A:I34 | 11 | 0767 |
| LigB3 | 1-2, 6-7, 9, 14-15, 17, 19, 23, 25-33, 36, 45-46, 51-53, 55, 71-72, 83-84 | 1, 2, 7, 9, 14, 15, 17, 23-33, 36, 46, 51-53, 59, 71, 72, 83, 84 | 28 | A:E71, A:N72, A:I73, A:I74, A:N8, A:N9, A:P10, A:N11, A:I12, A:P13, A:L14, A:G15, A:K16, A:K17, A:S43, A:N44, A:S45, A:T46, A:I47, A:A59, A:D60, A:T61, A:G62, A:I63, A:I80, A:T82, A:P83, A:R84, A:S35, A:S36, A:S37, A:Q51, A:N52, A:N53 | 4 | 0.726 |
| LigB4 | 1, 26-31, 46, 53-55, 66, 75-76, 88 | 25, 27-31, 54, 55, 76 | 9 | A:V1, A:S2, A:I25, A:F26, A:T27, A:D28, A:N29, A:S30, A:N31, A:S32, A:I34 | 11 | 0.758 |
| LigB4 | 1, 26-31, 46, 53-55, 66, 75-76, 88 | 25, 27-31, 54, 55, 76 | 9 | A:G75, A:G76, A:Q77, A:Q78, A:S51, A:N52, A:A53, A:S54, A:D55, A:S56, A:H57 | 4 | 0.716 |
| LigB5 | 1, 25-32, 36, 52-55, 74-75 | 25-32, 52-54, 25-32, 52-54, | 11 | A:T1, A:S2, A:I25, A:F26, A:T27, A:D28, A:N29, A:S30, A:K31 | 9 | 0.854 |
| LigB5 | 1, 25-32, 36, 52-55, 74-75 | 25-32, 52-54, | 11 | A:G74, A:K75, A:V76, A:S77 | 4 | 0.713 |
|  |  |  |  | A:V8, A:L9, A:P10, A:S11, A:I12, A:A13, A:K14, A:G15, A:L16, A:S43, A:S44, A:A45, A:K46, A:V47, A:H62 | 24 | 0.610 |
|    | LigB6 1-2, 7, 25-33, 36, 53-56, 76, 90 | LigB6 1-2, 7, 25-33, 36, 53-56, 76, 90 | LigB7 1, 25-32, 36, 45-46, 53-56, 76, 89-90 | LigB7 1, 25-32, 36, 45-46, 53-56, 76, 89-90 | LigB8 27-30, 45-46, 52, 73, 85 | LigB8 27-30, 45-46, 52, 73, 85 |
|----|------------------------------------------|-------------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------|------------------------------------------|
|    | A:63, A:V64, A:G65, A:D66, A:T83, A:V84, A:V85, A:P86, A:A8 | A:T35, A:D36, A:Q37, A:S48, A:V49, A:S50, A:N51, A:L52, A:D53, A:D54, A:N55, A:K56 | A:T1, A:S2, A:I25, A:Y26, A:S27, A:D28, A:N29, A:S30, A:N31, A:K32 | A:G75, A:K76, A:V77, A:S78 | A:V1, A:E2, A:I25, A:F26, A:T27, A:D28, A:N29, A:S30, A:N31, A:S32, A:I34 | A:P7, A:I8, A:N9, A:P10, A:S11, A:V12, A:A13, A:K14, A:G15, A:L16, A:S43, A:N44, A:T45, A:D46, A:I47, A:E49, A:L63, A:T64, A:P65, A:G66, A:S67, A:K85, A:V86, A:T87, A:P88, A:A89, A:K90 |
|    | 18 | 18 | 11 | 11 | 3 | 3 |
|    | A:G75, A:K76, A:V77, A:S78 | A:S36, A:S51, A:N52, A:A53, A:Q54, A:K55, A:N56, A:Q57 | A:V1, A:E2, A:I25, A:F26, A:T27, A:D28, A:N29, A:S30, A:N31, A:S32, A:I34 | A:I1, A:S2, A:T25, A:Y26, A:T27, A:D28, A:H29, A:S30, A:V31, A:Q32, A:V34, A:T35, A:A36, A:L37, A:A38, A:N51, A:N52, A:V53, A:T54, A:G55 | A:P7, A:I8, A:N9, A:P10, A:S11, A:V12, A:A13, A:K14, A:G15, A:L16, A:S43, A:N44, A:P45, A:R46, A:K47, A:V60, A:A61, A:T62, A:G63, A:N64, A:N81, A:V82, A:T83, A:P84, A:A85 | A:P7, A:I8, A:N9, A:P10, A:S11, A:V12, A:A13, A:K14, A:G15, A:L16, A:S43, A:N44, A:P45, A:R46, A:K47, A:V60, A:A61, A:T62, A:G63, A:N64, A:N81, A:V82, A:T83, A:P84, A:A85 |
|    | 10 | 10 | 11 | 24 | 3 | 3 |
|    | 0.831 | 0.573 | 0.768 | 0.653 | 0.776 | 0.640 |
|    | 0.697 | 0.670 | 0.697 | 0.579 | 0.579 | 0.625 |
### Supplementary Material

| LigB9   | 1-2, 7, 9, 14-15, 26-31, 45-46, 51, 53-56, 66, 75, 76, 81, 89-90 | 2, 7, 9, 13-15, 26-31, 33, 44-46, 51, 53-56, 63, 64, 66, 67, 88-90 | 28 | A:T1, A:S2, A:I25, A:F26, A:S27, A:D28, A:K29, A:S30, A:T31, A:Q32 | 10 | 0.832 |
|---------|-------------------------------------------------|-------------------------------------------------|----|-------------------------------------------------|----|------|
| LigB9   | 1-2, 7, 9, 14-15, 26-31, 45-46, 51, 53-56, 66, 75, 76, 81, 89-90 | 2, 7, 9, 13-15, 26-31, 33, 44-46, 51, 53-56, 63, 64, 66, 67, 88-90 | 28 | A:F76, A:I77, A:Q78 | 3 | 0.719 |
|         |                                                 |                                                 |    | A:T35, A:Q36, A:L37, A:E51, A:N52, A:T53, A:S54, A:G55, A:K56, A:K57 | 10 | 0.627 |
|         |                                                 |                                                 |    | A:T8, A:I9, A:N10, A:S11, A:I12, A:T13, A:H14, A:G15, A:L16, A:S43, A:D44, A:P45, A:S46, A:K47, A:S63, A:K64, A:L65, A:G66, A:S67, A:V86, A:T87, A:D88, A:L89 | 23 | 0.611 |
| LigB10  | 1-2, 7, 25-31, 53-55, 75-77, 90 | 1, 2, 25-31, 54, 55, 76 | 12 | A:K1, A:S2, A:T25, A:F26, A:I27, A:D28, A:G29, A:S30, A:F31, A:Q32 | 10 | 0.844 |
| LigB10  | 1-2, 7, 25-31, 53-55, 75-77, 90 | 1, 2, 25-31, 54, 55, 76 | 12 | A:N75, A:S76, A:K77 | 3 | 0.770 |
|         |                                                 |                                                 |    | A:S10, A:S11, A:I12, A:A13, A:K14, A:G15, A:L16, A:S43, A:K44, A:S45, A:D46, A:V47, A:A48, A:A62, A:L63, A:S64, A:I65, A:G66, A:S67, A:N83, A:N85, A:V86, A:S87, A:A88, A:A89, A:T90 | 26 | 0.607 |
|         |                                                 |                                                 |    | A:T35, A:N36, A:L37, A:I50, A:N51, A:N52, A:A53, A:A54, A:N55, A:E56, A:K57 | 11 | 0.600 |
| LigB11  | 1-2, 4, 7, 9, 15, 25-32, 45-46, 53-56, 75-77, 80, 87, 88 | 1-4, 6, 7, 9, 23-33, 36, 37, 46, 53-56, 67, 73, 75-78, 80, 82 | 33 | A:D1, A:S2, A:K4, A:V25, A:Y26, A:S27, A:D28, A:S29, A:T30, A:I31, A:Q32 | 11 | 0.806 |
| LigB11  | 1-2, 4, 7, 9, 15, 25-32, 45-46, 53-56, 75-77, 80, 87, 88 | 1-4, 6, 7, 9, 23-33, 36, 37, 46, 53-56, 67, 73, 75-78, 80, 82 | 33 | A:N75, A:S76, A:K77 | 3 | 0.754 |
|         |                                                 |                                                 |    | A:V8, A:N9, A:N10, A:N11, A:I12, A:A13, A:K14, A:G15, A:L16, A:S43, A:N44, A:S45, A:S46, A:S47, A:L63, A:Q64, A:I65, A:G66, A:N67, A:T84, A:V85, A:S86, A:A87, A:A88 | 24 | 0.642 |
|         |                                                 |                                                 |    | A:S35, A:D36, A:S37, A:S51, A:N52, A:S53, A:T54, A:E55, A:T56, A:K57, A:G58 | 11 | 0.544 |
### Supplementary Material

| LigB12 | **14-15**, 27-30, 45-47, 53-55, 64, 66-67, 76, **86-90** | **14, 15**, 46, 63, 64, 66, 67, **86-90** | 12 | A:S1, A:S2, A:G24, A:T25, A:Y26, A:S27, A:A28, A:G29, A:T30, A:K31, A:A32, A:L34 | 12 | 0.749 |
|--------|------------------------------------------------|---------------------------------|----|----------------------------------------------------------------------------------|----|------|
| LigB12 | **14-15**, 27-30, 45-47, 53-55, 64, 66-67, 76, **86-90** | **14, 15**, 46, 63, 64, 66, 67, **86-90** | 12 | A:G75, A:S76, A:V77, A:S78 | 4 | 0.708 |
|         | A:P7, A:I8, A:N9, A:T10, A:N11, A:I12, A:N13, **A:T14, A:T15**, A:S43, A:N44, A:Q45, A:S46, A:Q47, A:K49, A:I63, A:A64, A:S65, A:G66, A:N67, A:T84, A:V85, A:N86, A:K87, A:T88, A:D89, **A:T90** | 27 | 0.622 |
|         | A:T35, A:S36, A:S37, A:S51, A:N52, A:A53, A:S54, A:E55, A:T56, A:K57 | 10 | 0.614 |
Table S3: List of predicted linear BCL epitopes

| Domain | BepiPred 2.0 | BCPred | ABCPred, |
|--------|-------------|--------|----------|
|        | Peptide     | Position | Peptide  | Position | Score | Peptides | Position | Score |
| LigA7  | ASKAKGLT    | 12-19   | FTDNSNSDITNQ | 28-39   | 0.992 | IQITPAASKAKGL | 5-18 | 0.87 |
|        | FTDNSNSDI   | 28-36   | TWNSSNTDILTV | 41-52   | 0.916 | AKGLTERFKATGIF | 15-28 | 0.87 |
|        | VSNTNAKRGLG | 52-62   | GLGSTLKQGTVK | 60-71   | 0.695 | QGTVKVTASMMGGE | 67-80 | 0.80 |
|        | EDS         | 79-81   | DSVDFTVQTATL | 81-92   | 0.583 | LGSTLQGTVKTA | 61-74 | 0.80 |
|        |             |         |           |         |       | SNSDITNQVTWNSS | 32-45 | 0.80 |
|        |             |         |           |         |       | IQITPAASKAKGL | 5-18 | 0.87 |
| LigA8  | VSPTRASIAKG | 5-15    | TSIEVSPTRASI | 1-12    | 0.898 | NISITATEKLSGK | 66-79 | 0.86 |
|        | TDHSKKNIT   | 27-35   | KKNITEQVTWKS | 31-42   | 0.741 | SSSKALSMNAPGE | 42-55 | 0.81 |
|        | KSSSKALS    | 41-48   | LSGKTDITVTPA | 76-87   | 0.715 | VSPTRASIAKGMTQ | 5-18 | 0.80 |
|        |             |         |           |         |       | SMLNAPGEEGTGKA | 48-61 | 0.79 |
|        |             |         |           |         |       | KGMTQKFTATGI | 14-25 | 0.307 |
| LigA9  | ISPVKHCLVKGLT | 5-17 | TLGNVSSQVSKL | 73-84   | 0.999 | NTKGYQGQAHGTGT | 52-65 | 0.94 |
|        | YSDNNSKDI   | 26-34   | AHGTGTGTVDIK | 60-71   | 0.946 | GIYSDNSSKDTSA | 24-37 | 0.78 |
|        | SSNN        | 42-45   | VATISNTKGYQG | 47-58   | 0.616 | SKDITSAVTWSSN | 31-44 | 0.75 |
|        | NTKGYQGQ    | 52-59   | TGJYSDNNSKDI | 23-34   | 0.373 |         |         |      |
| LigA10 | NPTSSHKAKGLTE | 6-18 | FTDNSTKIDTDQ | 26-37   | 0.948 | TDNSTKIDTDQVTW | 27-40 | 0.88 |
|        | TDNSTKIDTD  | 27-36   | ISNATGSKGVVN | 50-61   | 0.720 | LNPTSSHKAKGLTE | 5-18 | 0.85 |
|        | SS          | 42-43   | SSANATFQVTPA | 78-89   | 0.411 | ITDQVTWKSNTAY | 34-47 | 0.84 |
|        | NATGSK      | 52-57   | HKAkGLTENFKA | 11-22   | 0.338 | SHKAKGLETENFKAT | 10-23 | 0.80 |
|        |             |         |           |         |       | LTENFKATGVFTDN | 16-29 | 0.77 |
|        |             |         |           |         |       | KGTSHISATLGSIS | 65-78 | 0.75 |
| LigA11 | LigA12 | LigA13 | LigB1 | LigB2 | LigB3 |
|--------|--------|--------|-------|-------|-------|
| IPNNISFAKGNs | YTDHSEADI | S | YAGIEKGYT | S | SSISKTKGST |
| 6-17 | 26-34 | 43 | 9-17 | 29-37 | 10-19 |
| AKGNYSQFKATG | AKGNYSQFKATG | AKGNYSQFKATG | YAGIEKGYT | YAGIEKGYT | SSISKTKGST |
| 13-24 | 13-24 | 13-24 | 13-24 | 13-24 | 13-24 |
| 0.639 | 0.639 | 0.639 | 0.85 | 0.85 | 0.85 |
| VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | GTYSQDSTKLDT | GTYSQDSTKLDT | GTYSQDSTKLDT |
| 60-71 | 60-71 | 60-71 | 24-35 | 24-35 | 24-35 |
| 0.855 | 0.855 | 0.855 | 0.920 | 0.920 | 0.920 |
| EADITEQVTWSSSN | FKTGITYTDHSEAD | GSTNITAKLSDTV | SDQSTKLTEDVTW | SDQSTKLTEDVTW | SDQSTKLTEDVTW |
| 31-44 | 20-33 | 66-79 | 27-40 | 27-40 | 27-40 |
| VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT |
| 6-17 | 6-17 | 6-17 | 6-17 | 6-17 | 6-17 |
| 0.751 | 0.751 | 0.751 | 0.751 | 0.751 | 0.751 |
| VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs |
| 5-18 | 5-18 | 5-18 | 5-18 | 5-18 | 5-18 |
| 0.81 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 |
| YTDHSEADI | YTDHSEADI | YTDHSEADI | YTDHSEADI | YTDHSEADI | YTDHSEADI |
| 26-34 | 26-34 | 26-34 | 26-34 | 26-34 | 26-34 |
| 0.85 | 0.85 | 0.85 | 0.85 | 0.85 | 0.85 |
| AKGNYSQFKATG | AKGNYSQFKATG | AKGNYSQFKATG | AKGNYSQFKATG | AKGNYSQFKATG | AKGNYSQFKATG |
| 13-24 | 13-24 | 13-24 | 13-24 | 13-24 | 13-24 |
| 0.639 | 0.639 | 0.639 | 0.639 | 0.639 | 0.639 |
| VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT |
| 60-71 | 60-71 | 60-71 | 60-71 | 60-71 | 60-71 |
| 0.855 | 0.855 | 0.855 | 0.855 | 0.855 | 0.855 |
| EADITEQVTWSSSN | FKTGITYTDHSEAD | GSTNITAKLSDTV | SDQSTKLTEDVTW | SDQSTKLTEDVTW | SDQSTKLTEDVTW |
| 31-44 | 20-33 | 66-79 | 27-40 | 27-40 | 27-40 |
| VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT | VSTTNIGSTNIT |
| 6-17 | 6-17 | 6-17 | 6-17 | 6-17 | 6-17 |
| 0.751 | 0.751 | 0.751 | 0.751 | 0.751 | 0.751 |
| VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs | VIPNNISFAKGNs |
| 5-18 | 5-18 | 5-18 | 5-18 | 5-18 | 5-18 |
| 0.81 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 |
### Supplementary Material

| LigB4 | SPTNSTVAKGLQ | 6-17 | GSGILPKG | 9-20 | 0.553 | LIATGIYSNDTNRD | 20-33 | 0.77 |
|-------|--------------|------|-----------|-------|--------|----------------|--------|------|
|       | FTDNSNDIDTDQ | 26-37 | 0.972 | TGDSSNTDILSI | 39-50 | 0.851 | NQGNNVKVTASIGG | 64-77 | 0.81 |
|       | T               | 45 | ISVSPSTNVAK | 3-14 | 0.83 | FKATGIFTDNSNSD | 20-33 | 0.79 |
|       | LSISNASDSHG | 48-58 | ASTLNOGNVKVT | 60-71 | 0.352 | VSISVSPSTNVAK | 1-14 | 0.77 |
|       | GGIQGSTDFTVT | 75-86 | 0.316 |                | | | |
| LigB5 | SPVLPSIAKGLT | 6-17 | GIGTNYSDNSND | 20-37 | 0.954 | SVSILDDNKGLGKA | 48-61 | 0.84 |
|       | FTDNSKSKIDTDQ | 26-37 | 0.954 | GDTTITATLV | 65-76 | 0.944 | KGLTQKFTAIGIFT | 14-27 | 0.83 |
|       | SSAIVSVSNLDDNK | 43-59 | LDDNKGLGKAHA | 52-63 | 0.764 | TDNSKSKIDTDQVTW | 27-40 | 0.77 |
|       | LG               | | | | | | |
| LigB6 | NPVNPSLAKGLT | 6-17 | AQKNQGNYAA | 53-64 | 0.973 | ISNAQKNQGNYAA | 50-63 | 0.87 |
|       | YSDNSNKIDT | 26-35 | TFGKVSSVPVSTL | 83-94 | 0.847 | GIYSDNSNKIDTSA | 24-37 | 0.82 |
|       | SSDS | 42-45 | FSATGIYSDNSN | 20-31 | 0.503 | QKFSATGIYSDNSN | 18-31 | 0.78 |
|       | NAQKNQG | 52-58 | VTWFSSDSSIAT | 38-49 | 0.469 | KGLTQKFTAIGIFT | 14-27 | 0.83 |
|       | TQPAAASKAKGLT | 6-17 | ITNTSGSKG | 50-61 | 0.999 | ITPAAASKAKGLTE | 5-18 | 0.89 |
|       | FTDNSNDIT | 26-35 | FTDNSNDITNQ | 26-37 | 0.992 | TDNSNDITNQVTW | 27-40 | 0.83 |
|       | NTSGSKGI | 52-59 | | | | | |
| LigB7 | TPDAAASKAKGLT | 6-17 | TPDAAASKAKGLTE | 5-18 | 0.89 | | | |
|       | FTDNSNDIT | 26-35 | FTDNSNDITNQ | 26-37 | 0.992 | TPDAAASKAKGLTE | 5-18 | 0.89 |
|       | NTSGSKGI | 52-59 | | | | | |
| LigB8 | TPINSVAKGLI | 6-17 | VNNVTGSVTTVA | 50-61 | 0.989 | VQDVTALATWSSN | 31-44 | 0.89 |
|       | YTDHSVQVDVT | 26-35 | IAVTPINPSVAK | 3-14 | 0.979 | FKATGTYTDHSVQD | 20-33 | 0.84 |
|       | SSSNPRKAMV | 41-50 | ATGTYTDHSVQD | 22-33 | 0.911 | KGLIRQFKATGTYT | 14-27 | 0.83 |
|       | SNTDIAEIINTSG | 43-56 | 0.79 | | | | |
|       | NQVTVNSNTDIAE | 36-49 | 0.76 | | | | |
| LigB9 | PTINSITHGLT | 7-17 | FSDKSTQNLTQ | 26-37 | 0.986 | TQNLQTVTW | 31-44 | 0.88 |
|       | FSDKSTQNLT | 26-35 | IENTSQGKG | 50-61 | 0.899 | SDPSKIEIEN | 43-56 | 0.85 |
|       | | | | | | | |
| Supplementary Material |
|-------------------------|
| **ISSDP**SKIEIENTSGKKGIA | 41-60 | IQSSPIPITVTD | 77-89 | 0.595 | PTINSITHGLTKQF | 7-20 | 0.82 |
| L | 65 | EITPTINSITHG | 4-15 | 0.497 | KATGIFSDKSTQNL | 21-34 | 0.79 |
| FI | 76-77 | THGLTKQFKATGIF | 13-26 | 0.77 | NTSGKKGIATASKL | 52-65 | 0.76 |
| **LigB10** |  |  |  |  |  |  |  |
| PSSSSIAKGLTQ | 7-18 | VAPINNAANEK | 47-58 | 1 | QQFKAIGTFIDGSE | 18-31 | 0.90 |
| **FID**GSEQEI | 26-34 | GSEQEITNLVTW | 29-40 | 0.741 | ITNLVTWYSSKSDV | 34-47 | 0.75 |
| YSSKS | 41-45 |  |  |  |  |  |  |
| AANEKGLA | 53-60 |  |  |  |  |  |  |
| **LigB11** |  |  |  |  |  |  |  |
| INPVNNIIAKGLT | 5-17 | ISNSTETKKGAT | 50-61 | 0.97 | QQYTALGVYSDSTI | 18-31 | 0.90 |
| **YSDSTIQDIS** | 26-35 | YSDSTIQDISDS | 26-37 | 0.865 | ALQIGNSKITATYN | 62-75 | 0.85 |
| SNS | 43-45 | IKINPVNNIIAK | 3-14 | 0.853 | SSNSSISISNTE | 42-55 | 0.84 |
| **STETKGK** | 53-59 |  |  |  |  |  |  |
|  |  | SDSTIQDISDSVTW | 27-40 | 0.82 |  |  |  |
|  |  | NSTETKKGKATALQI | 52-65 | 0.79 |  |  |  |
|  |  | AKGLTQQYTALGVY | 13-26 | 0.77 |  |  |  |
| **LigB12** |  |  |  |  |  |  |  |
| ISPINTNINTTVS | 5-17 | SPINTNINTTVS | 6-17 | 0.936 | KADLTSSVTWSSN | 31-44 | 0.91 |
| SAGTKADL | 27-34 | VTGIASGNPTII | 60-71 | 0.916 | VTGIASGNPTIIAT | 60-73 | 0.90 |
| SNQ | 43-45 | QAKVSNASETKG | 47-58 | 0.339 | FFAVGTYSAGTKAD | 20-34 | 0.85 |
| ETK | 55-57 | GTYSAGTKADLT | 24-35 | 0.327 | KVSNASETKGLVTG | 49-62 | 0.84 |
|  |  | PINTNINTTVSKQF | 7-20 | 0.76 |  |  |  |
Table S4: List of predicted CTL epitopes

| Domain | IEDB       | NetMHC 4.0       | ProPred       |
|--------|------------|------------------|---------------|
| LigA7  | FTDNSNSDI  | FTDNSNSDI       | FTDNSNSDI     |
|        | GTVKVTASM  | GTVKVTASM       | GTVKVTASM     |
|        | LTVSNTNAK  | SMGGIEDSV       | SMGGIEDSV     |
|        | STLQGTVK   | VEIQITPAA       | VEIQITPAA     |
|        | LTVSNTNAK  | LTVSNTNAK       | LTVSNTNAK     |
| LigA8  | ATLEKLSGK  | ATLEKLSGK       | ATLEKLSGK     |
|        | TWKSSSKAL  | TWKSSSKAL       | TWKSSSKAL     |
|        | EVSPTRASI  | EVSPTRASI       | EVSPTRASI     |
|        | KSSSKALSM  | KSSSKALSM       | KSSSKALSM     |
|        | KNITEQVTW  | KNITEQVTW       | KNITEQVTW     |
|        | QVTWKSSSK  | QVTWKSSSK       | QVTWKSSSK     |
|        | SIAKGMTQK  | SIAKGMTQK       | SIAKGMTQK     |
|        | GIFTDHSKK  | GIFTDHSKK       | GIFTDHSKK     |
| LigA9  | ATISNTKGY  | ATISNTKGY       | ATISNTKGY     |
|        | SVATISNTK  | SVATISNTK       | SVATISNTK     |
|        | TLGNVSSQV  | TLGNVSSQV       | TLGNVSSQV     |
|        | KDITSAVTW  | KDITSAVTW       | KDITSAVTW     |
|        | WHSSNNSVA  | WHSSNNSVA       | WHSSNNSVA     |
|        | SSKDITSAV  | SSKDITSAV       | SSKDITSAV     |
|        | GTVDIKATL  | GTVDIKATL       | GTVDIKATL     |
|        | YSDNSSKDI  | YSDNSSKDI       | YSDNSSKDI     |
| LigA10 | SSANATFQV  | SSANATFQV       | SSANATFQV     |
|        | GVFTDNSTK  | GVFTDNSTK       | GVFTDNSTK     |
|        | KISNATGSK  | KISNATGSK       | KISNATGSK     |
| LigA11  |  |  |
|---|---|---|
| LigA12  |  |  |
| LigA13  |  |  |
| LigB1     | HQFKATGKF | HQFKATGKF |
|-----------|-----------|-----------|
|           | WSSSNPTVV | WSSSNPTVV |
|          FENGAEIDL | FENGAEIDL |
|           SSNPTVVS | SSNPTVVS |
|          ISSSIDFEV | ISSSIDFEV |
|           TISLSSISK | TISLSSISK |
| LigB1     | IANGTSTTL | IANGTSTTL |
|           | TTTLEVTAIF | TTTLEVTAIF |
|           | IKAEYNGLY | IKAEYNGLY |
| LigB2     | HIRASFQSK | HIRASFQSK |
|           | RQFSAIGIF | RQFSAIGIF |
|           | EEMTVGDAV | EEMTVGDAV |
|           | GTAHIRASF | GTAHIRASF |
|           | LPKGTNRQF | LPKGTNRQF |
|           | HQDISNDPL | HQDISNDPL |
|           | ISNDPLIVW | ISNDPLIVW |
|           | FSDGSHQDI | FSDGSHQDI |
| LigB3     | KQKLIATGI | KQKLIATGI |
|           | IPLGKKQKL | IPLGKKQKL |
|           | YSDNSNRDI | YSDNSNRDI |
|           | TADTGIVTI | TADTGIVTI |
| LigB4     | FTDNSNSDI | FTDNSNSDI |
|           | IQGSTDFTV | IQGSTDFTV |
| LigB5     | TSIEVSPVL | TSIEVSPVL |
|           | TTITATLGK | TTITATLGK |
| LigB6     | AQKNQGNAY | AQKNQGNAY |
|           | STLSVTAAK | STLSVTAAK |
|           | QKFSATGIY | QKFSATGIY |
|           | KVSSPVSTL | KVSSPVSTL |
|           | KDISAVTW | KDISAVTW |
|           | GATDIKATF | GATDIKATF |
| LigB7  | FTDNSNSDI | FTDNSNSDI | FTDNSNSDI |
|-------|-----------|-----------|-----------|
|       | EISAALGSI | EISAALGSI | EISAALGSI |
|       | ERFKATGIF | ERFKATGIF | ERFKATGIF |
|       | VEIQITPAA | VEIQITPAA | VEIQITPAA |
| LigB8 | RQFKATGTY | RQFKATGTY | RQFKATGTY |
|       | MVNNVTGSV | MVNNVTGSV | MVNNVTGSV |
|       | SVLNVT PAL | SVLNVT PAL | SVLNVT PAL |
|       | ATWSSSNPR | ATWSSSNPR | ATWSSSNPR |
|       | YTDHSVQDV | YTDHSVQDV | YTDHSVQDV |
|       | HSVQDVTA L | HSVQDVTA L | HSVQDVTA L |
| LigB9 | FS DKSTQNL | FS DKSTQNL | FS DKSTQNL |
|       | TSIEITPTI | TSIEITPTI | TSIEITPTI |
|       | QSSPIPI TV | QSSPIPI TV | QSSPIPI TV |
|       | FIQSSPIPI | FIQSSPIPI | FIQSSPIPI |
|       | YKFIQSSPI | YKFIQSSPI | YKFIQSSPI |
|       | TINSITHGL | TINSITHGL | TINSITHGL |
|       | KQFKATGIF | KQFKATGIF | KQFKATGIF |
|       | ITHGLTKQF | ITHGLTKQF | ITHGLTKQF |
|       | SNIKAVYKF | SNIKAVYKF | SNIKAVYKF |
|       | EITPTINSI | EITPTINSI | EITPTINSI |
|       | KSTQNLTQL | KSTQNLTQL | KSTQNLTQL |
|       | SSNIKAVYK | SSNIKAVYK | SSNIKAVYK |
| LigB10| GSSDIYAIY | GSSDIYAIY | GSSDIYAIY |
|       | FIDGSEQEI | FIDGSEQEI | FIDGSEQEI |
|       | LSIGSSDIY | LSIGSSDIY | LSIGSSDIY |
|       | NEKGLATAL | NEKGLATAL | NEKGLATAL |
|       | YAIYNSISS | YAIYNSISS | YAIYNSISS |
|       | QEI TNLVTW | QEI TNLVTW | QEI TNLVTW |
|       | EI TNLVTWY | EI TNLVTWY | EI TNLVTWY |
|       | D I YAIYNSI | D I YAIYNSI | D I YAIYNSI |
|       | SSN KINFNV | SSN KINFNV | SSN KINFNV |
| LigB11  | QQFKAIGTF | QQFKAIGTF | YSDSTIQDI | YSDSTIQDI | QQYTALGVTY | QQYTALGVTY | WSSNSSL | WSSNSSL | TIQDISDSV | TIQDISDSV | ETKGKATAL | ETKGKATAL | KITATYNSI | KITATYNSI |
|--------|-----------|-----------|-----------|-----------|------------|------------|----------|----------|-----------|-----------|------------|------------|-----------|-----------|
| LigB12 | QQFKAIGTF | QQFKAIGTF | KQFFAVGTY | KQFFAVGTY | TVSKQFFAV  | TVSKQFFAV  | ETKGLVTGI | ETKGLVTGI | YSAGTKADL | YSAGTKADL |           |            |           |            |
Table S5: List of predicted HTL epitopes

| Domain | ProPred-I       | NetMHC 2.3       | IEDB       |
|--------|----------------|-----------------|------------|
| LigA7  | VEIQITPAA      | VEIQITPAA       |            |
|        | ILTVSNTNA      | ILTVSNTNA       |            |
|        | VKVTASMGG      | VKVTASMGG       |            |
|        | LVEIQITPAA     | LVEIQITPAA      |            |
|        | ILTVSNTNA      | ILTVSNTNA       |            |
|        | IQITPAADAS     | IQITPAADAS      |            |
| LigA8  | VTWKSSTK       | VTWKSSTK        |            |
|        | ISITATLEK      | ISITATLEK       |            |
|        | IEVSPTRAS      | IEVSPTRAS       |            |
| LigA9  | ITSAVTWHG      | ITSAVTWHG       |            |
|        | ITSAVTWHSSNS   | ITSAVTWHSSNS   |            |
|        | VDIKATLGNVS    | VDIKATLGNVS     |            |
|        | IKATLGNVSSQVS  | IKATLGNVSSQVS   |            |
|        | SNNSVATISNTKG  | SNNSVATISNTKG   |            |
| LigA10 | IVLNPTSSH      | IVLNPTSSH       | IVLNPTSSH |
|        | WKSSNTAYA      | WKSSNTAYA       |            |
|        | IEIVLNPTS      | IEIVLNPTS       |            |
|        | IVLNPTSSHKCA   | IVLNPTSSHKCA    |            |
|        | LGSISSANA      | LGSISSANA       |            |
| LigA11 | SVLNVPALL      | SVLNVPALL       |            |
| Lig | VIPNNISFA       | VIPNNISFA       | VIPNNISFA       |
|-----|----------------|----------------|----------------|
|     | IEVIPNNIS      | IEVIPNNIS      | IEVIPNNIS      |
|     | YQFKATGIY      | YQFKATGIY      | YQFKATGIY      |
|     | LVSTTNIGS      | LVSTTNIGS      | LVSTTNIGS      |
| LigA12 | YIMITPSYA   | YIMITPSYA   | YIMITPSYA   |
|       | IMITPSYAG     | IMITPSYAG     | IMITPSYAG     |
|       | WFSSNPSSV     | WFSSNPSSV     | WFSSNPSSV     |
|       | QSSYTPVTVES    | QSSYTPVTVES    | QSSYTPVTVES    |
|       | TVVFYDHHTQS    | TVVFYDHHTQS    | TVVFYDHHTQS    |
|       | VTFWSSNPSSV    | VTFWSSNPSSV    | VTFWSSNPSSV    |
| LigA13 | TVVSISNVDD   | TVVSISNVDD   | TVVSISNVDD   |
|       | LVTWSSSNPT    | LVTWSSSNPT    | LVTWSSSNPT    |
|       | IVNITISLS     | IVNITISLS     | IVNITISLS     |
|       | VNITISLSS     | VNITISLSS     | VNITISLSS     |
|       | ITISLSSIS     | ITISLSSIS     | ITISLSSIS     |
| LigB1  | VVTIQGNRV     | VVTIQGNRV     | VVTIQGNRV     |
|       | VTIQGRNRVR    | VTIQGRNRVR    | VTIQGRNRVR    |
|       | IQGNVRGI      | IQGNVRGI      | IQGNVRGI      |
| LigB3  | VKLIVTPAA     | VKLIVTPAA     | VKLIVTPAA     |
|       | IVTISASSE     | IVTISASSE     | IVTISASSE     |
|       | IWNSSNSTIA    | IWNSSNSTIA    | IWNSSNSTIA    |
|       | IQVTSNPNPI    | IQVTSNPNPI    | IQVTSNPNPI    |
|       | VKLIVTPAAL    | VKLIVTPAAL    | VKLIVTPAAL    |
|       | GIVTISASSE    | GIVTISASSE    | GIVTISASSE    |
| LigB5 | LigB6 | LigB7 | LigB8 | LigB9 | LigB10 |
|-------|-------|-------|-------|-------|-------|
| IVTISASSEN | IVTISASSEN | IVTISASSEN | IVTISASSEN | IVTISASSEN | IVTISASSEN |
| WLTVVPAVL | WLTVVPAVL | WLTVVPAVL | WLTVVPAVL | WLTVVPAVL | WLTVVPAVL |
| WNSSSAIVS | WNSSSAIVS | WNSSSAIVS | WNSSSAIVS | WNSSSAIVS | WNSSSAIVS |
| VLPSIAKGL | VLPSIAKGL | VLPSIAKGL | VLPSIAKGL | VLPSIAKGL | VLPSIAKGL |
| IQINPVNPS | IQINPVNPS | IQINPVNPS | IQINPVNPS | IQINPVNPS | IQINPVNPS |
| ITSAVTFWS | ITSAVTFWS | ITSAVTFWS | ITSAVTFWS | ITSAVTFWS | ITSAVTFWS |
| WFSSDSSIA | WFSSDSSIA | WFSSDSSIA | WFSSDSSIA | WFSSDSSIA | WFSSDSSIA |
| VNPSLAKGL | VNPSLAKGL | VNPSLAKGL | VNPSLAKGL | VNPSLAKGL | VNPSLAKGL |
| ILKVTPAQL | ILKVTPAQL | ILKVTPAQL | ILKVTPAQL | ILKVTPAQL | ILKVTPAQL |
| VEIQITPAA | VEIQITPAA | VEIQITPAA | VEIQITPAA | VEIQITPAA | VEIQITPAA |
| VILKVTPAQ | VILKVTPAQ | VILKVTPAQ | VILKVTPAQ | VILKVTPAQ | VILKVTPAQ |
| IKSSKVILK | IKSSKVILK | IKSSKVILK | IKSSKVILK | IKSSKVILK | IKSSKVILK |
| IQITPAAAS | IQITPAAAS | IQITPAAAS | IQITPAAAS | IQITPAAAS | IQITPAAAS |
| VLNVPALL | VLNVPALL | VLNVPALL | VLNVPALL | VLNVPALL | VLNVPALL |
| LIRQFKATGTY | LIRQFKATGTY | LIRQFKATGTY | LIRQFKATGTY | LIRQFKATGTY | LIRQFKATGTY |
| MVNNVTGSV | MVNNVTGSV | MVNNVTGSV | MVNNVTGSV | MVNNVTGSV | MVNNVTGSV |
| VTPINPSVATWSS | VTPINPSVATWSS | VTPINPSVATWSS | VTPINPSVATWSS | VTPINPSVATWSS | VTPINPSVATWSS |
| AVYKFIQSSPIPI TV | AVYKFIQSSPIPI TV | AVYKFIQSSPIPI TV | AVYKFIQSSPIPI TV | AVYKFIQSSPIPI TV | AVYKFIQSSPIPI TV |
| FIQSSPIPI | FIQSSPIPI | FIQSSPIPI | FIQSSPIPI | FIQSSPIPI | FIQSSPIPI |
| SIEITPTINS | SIEITPTINS | SIEITPTINS | SIEITPTINS | SIEITPTINS | SIEITPTINS |
| WISSDPSKI | WISSDPSKI | WISSDPSKI | WISSDPSKI | WISSDPSKI | WISSDPSKI |
| SDIYAIYNSISSNK | SDIYAIYNSISSNK | SDIYAIYNSISSNK | SDIYAIYNSISSNK | SDIYAIYNSISSNK | SDIYAIYNSISSNK |
| SSDIYAIYNSISSNK | SSDIYAIYNSISSNK | SSDIYAIYNSISSNK | SSDIYAIYNSISSNK | SSDIYAIYNSISSNK | SSDIYAIYNSISSNK |
| LVTWYSSKS | LVTWYSSKS | LVTWYSSKS | LVTWYSSKS | LVTWYSSKS | LVTWYSSKS |
| LigB11 | LigB12 |
|--------|--------|
| IYAIYNSIS | IYAIYNSIS |
| YAIYNSISS | YAIYNSISS |
| FKAIGTFID | FKAIGTFID |
| ITISPSSSS | ITISPSSSS |
| VTWSSSNSS | VTWSSSNSS |
| IKINPVNNN | IKINPVNNN |
| FFAVGTYSA | FFAVGTYSA |
| VTWSSNQS | VTWSSNQS |
| ISPINTNINTTV | ISPINTNINTTV |
Figure legends

Figure S1: Separate RMSD plot for the vaccine construct and TLR4 at 100ns.

Figure S2: RMSF plot for vaccine construct only at 50ns of MDS.

Figure S3: Separate Rg plot for TLR4 and vaccine construct at 100ns of MDS.
Figure S1
Supplementary Material

Figure S2

RMS fluctuation
Figure S3