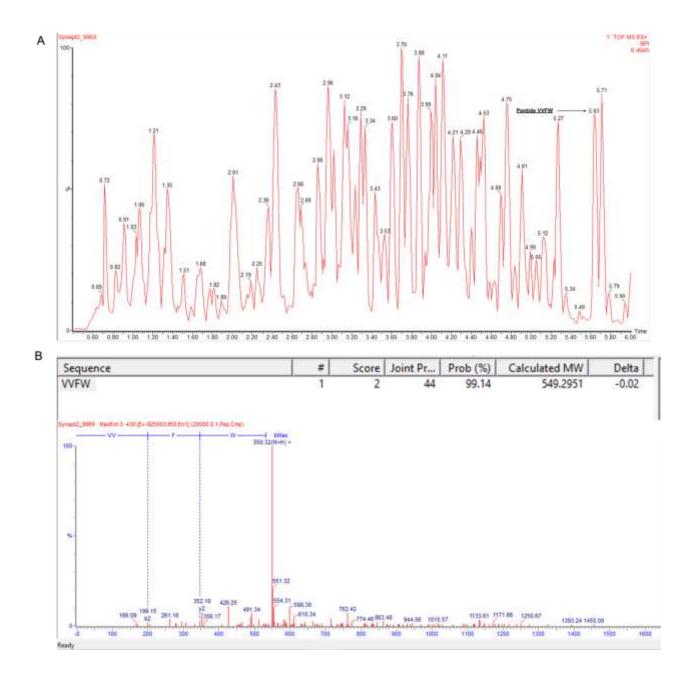
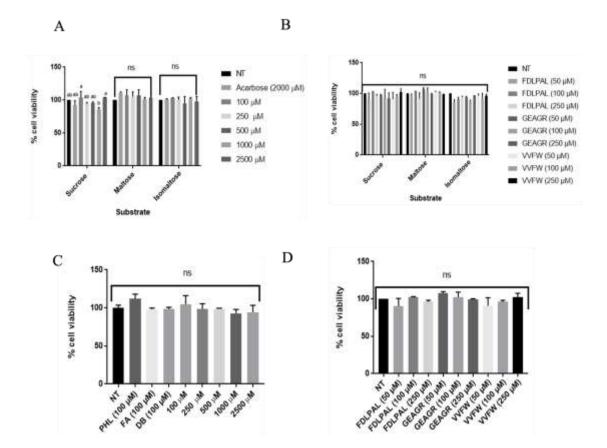
Electronic Supplementary Material (ESI) for Food & Function. This journal is © The Royal Society of Chemistry 2023

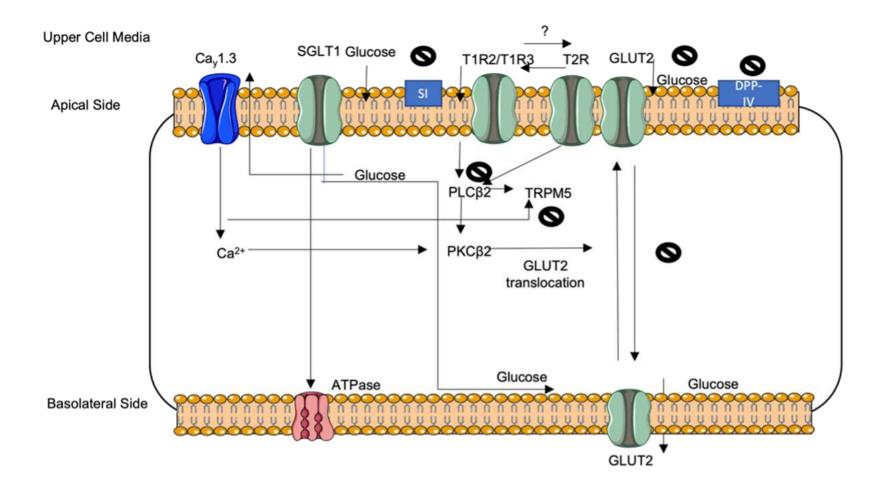


Supplementary Figure 1. LC-ESI-MS/MS technique used for peptide sequencing, specifically for peptide Valine-Valine-Phenylalanine-Tryptophan (VVFW). A. Mass spectrum of germinated chickpea protein hydrolysate; B. Final results of peptide sequencing.



Supplementary Figure 2. **A.** Cell viability of Caco-2 cells treated with germinated chickpea protein hydrolysate or positive control Acarbose (2 mM) and further stimulated with sucrose, maltose or isomaltose. **B.** Cell viability of Caco-2 cells treated with pure peptides FDLPAL, GEAGR, or VVFW and further stimulated with sucrose, maltose or isomaltose. **C.** Cell viability of Caco-2 cells treated with germinated chickpea protein hydrolysate or positive controls phloretin (PHL), flufenamic acid (FA) and denatonium benzoate (DB) and further stimulated with glucose. **D.** Cell viability of Caco-2 cells treated with pure peptides FDLPAL, GEAGR, or VVFW and further stimulated with glucose. Letters indicate significant differences (p < 0.05). NT: Nontreated cells; NS: Non-significant (p > 0.05). Bars indicate mean value obtained and error bars show standard deviation.

Supplementary Figure 3. Proposed mechanism of action.



Supplementary Table 1. Physicochemical, bioactive and bitterness properties of peptides identified in germinated chickpea protein hydrolysate from metabolic sources.

| Peptide | Length | Mass<br>(g/mol) | pI    | Net<br>charge | Hydrophobicity (kcal/mol) | Bioactive<br>Properties                              | Bioactive<br>peptide<br>fragment(s) | Bitter fragments       | Predicted<br>activation of<br>bitter receptors                                   |
|---------|--------|-----------------|-------|---------------|---------------------------|--|-------------------------------------|------------------------|--|
| STSA    | 4      | 364.16          | 5.51  | 0             | 9.57                      | ACE-inhibition                                       | ST                                  | NI/A                   | hT2R1  |
| 515A    | 4      | 304.10          | 3.31  | U             | 9.57                      | DPP-IV inhibition                                    | TS                                  | N/A                    | 1112K1   |
|         |        |                 |       |               |                           | ACE-inhibition                                       | GA, GG,<br>PG, AR, TP               |                        | hT2R1, hT2R14,   |
|         |        |                 |       |               |                           | DPP-IV inhibition                                    | TP, GA,<br>GG, PG, VT               |                        | hT2R16,<br>hT2R47,<br>hT2R41,  |
| VTPGGAR | 7      | 656.36          | 11.10 | 1             | 12.44                     | Peptide regulating stomach mucosal membrane activity | PG                                  | R, P,<br>PGG, V,<br>PG | hT2R41,<br>hT2R10,<br>hT2R43,<br>hT2R46, hT2R7,<br>hT2R44,<br>hT2R38,<br>hT2R39, |
|         |        |                 |       |               |                           | Antithrombotic peptide                               | PG                                  |                        | hT2R40, hT2R4  |
|         |        |                 |       |               |                           | ACE-inhibition                                       | VG, TP                              |                        | hT2R1, hT2R16,   |
| TPVG    | 4      | 372.20          | 5.49  | 0             | 8.98                      | DPP-IV inhibition                                    | TP, PV, VG                          | P, V, VG               | hT2R10,<br>hT2R14,<br>hT2R46,<br>hT2R41,<br>hT2R43                               |
| DAGLG   | 5      | 431.20          | 3.13  | -1            | 13.09                     | ACE-inhibition                                       | GL, AG,<br>DA, LG                   |                        | hT2R14, hT2R1,<br>hT2R16,  |

|             |    |        |       |   |       | DPP-IV inhibition a-Glucosidase inhibition           | GL, AG,<br>DA, AG, GE<br>EA                                 | LG, GL,<br>L, GLG,<br>DA                           | hT2R41,<br>hT2R39   |
|-------------|----|--------|-------|---|-------|--|---|--|---|
|             |    |        |       |   |       | ACE-inhibition                                       | GP, KG,<br>GS, GV,<br>GK, GG,<br>SG, PG,<br>GPP, PP,<br>SGP |  |   |
|             |    |        |       |   |       | Antithrombotic peptide                               | GP, PG  |  | hT2R7, hT2R14,  |
| GKGSGPPGGVA | 11 | 882.45 | 10.16 | 1 | 17.23 | Peptide regulating stomach mucosal membrane activity | GP, PG  | P, V, K,<br>PP, GP,<br>PPG,<br>PGG,<br>GV,<br>GGV, | hT2R47,<br>hT2R43,<br>hT2R40,<br>hT2R16,<br>hT2R44,<br>hT2R46, hT2R1, |
|             |    |        |       |   |       | Antioxidative peptide                                | GPP   | KG, VA,<br>PG                                      | hT2R38,<br>hT2R39,<br>hT2R41,   |
|             |    |        |       |   |       | DPP-IV inhibition                                    | GP, PP, VA,<br>PPG, GG,<br>GV, KG, PG                       |  | hT2R41,<br>hT2R10, hT2R4  |
|             |    |        |       |   |       | a-Glucosidase inhibition                             | PP  |  |   |
|             |    |        |       |   |       | HMG-CoA<br>reductase<br>inhibitor                    | GGV   |  |   |
| VPGGGSR     | 7  | 628.33 | 11.11 | 1 | 13.30 | ACE-inhibition                                       | VP, GS,<br>GG, PG   | R, P, V,<br>PGG, PG                                | hT2R1, hT2R16,<br>hT2R14,   |

|            |    |        |       |   |       | DPP-IV inhibition Antithrombotic Peptide regulating stomach mucosal membrane activity | VP, GG, PG PG PG      |                           | hT2R41,<br>hT2R43,<br>hT2R10,<br>hT2R46,<br>hT2R47, hT2R7,<br>hT2R39,<br>hT2R38,<br>hT2R44,<br>hT2R40 |
|------------|----|--------|-------|---|-------|---|-----------------------|---------------------------|---|
|            |    |        |       |   |       | ACE-inhibition  | VG, GK,<br>GG, PP     |                           | hT2R7, hT2R14,<br>hT2R47, hT2R1,<br>hT2R16,<br>hT2R40,<br>hT2R43,                                     |
| GKSPPVGGGK | 10 | 882.49 | 10.65 | 2 | 18.38 | DPP-IV inhibition   | SP, GG, KS,<br>PV, VG | P, V, K,<br>PP, VG        | hT2R38,   |
|            |    |        |       |   |       | $\alpha$ -Glucosidase inhibition  | PP                    | 11, 10                    | hT2R46,<br>hT2R39,<br>hT2R41,<br>hT2R44,<br>hT2R10, hT2R4   |
|            |    |        |       |   |       | ACE-inhibition  | VG, GR,<br>GG, PP, TP |                           | hT2R7, hT2R14,<br>hT2R47,   |
|            |    |        |       |   |       | DPP-IV inhibition   | PP, TP, GG,<br>PV, VG | DDV                       | hT2R43,<br>hT2R16,<br>hT2R46,   |
| TPPVGGGGR  | 9  | 796.42 | 10.79 | 1 | 14.38 | α-Glucosidase inhibition  | PP                    | R, P, V,<br>PP, VG,<br>GR | hT2R40,<br>hT2R40, hT2R1,<br>hT2R44,<br>hT2R38,<br>hT2R39,<br>hT2R10,<br>hT2R41, hT2R4                |

| SPLAPG    | 6 | 540.29 | 5.51  | 0 | 9.04  | ACE-inhibition  DPP-IV inhibition  Antithrombotic  Peptide regulating stomach mucosal membrane activity | PL, LAP,<br>AP, LA, PG<br>LA, AP,<br>APG, SP,<br>PL, PG<br>PG      | P, L, PL,<br>LA, PG            | hT2R1, hT2R14,<br>hT2R16,<br>hT2R43, hT2R7,<br>hT2R41,<br>hT2R40,<br>hT2R10,<br>hT2R46,<br>hT2R47,<br>hT2R39,<br>hT2R39,<br>hT2R44,<br>hT2R38 |
|-----------|---|--------|-------|---|-------|---|--|--------------------------------|---|
| LSPAGGK   | 7 | 628.35 | 10.14 | 1 | 12.85 | DPP-IV inhibition  ACE-inhibition   | PA, SP, AG,<br>GG<br>LSP, LSPA,<br>AG, GK,<br>GG                   | P, L, K,<br>PA                 | hT2R1, hT2R16,<br>hT2R14,<br>hT2R41,<br>hT2R47,<br>hT2R43, hT2R7,<br>hT2R10,<br>hT2R46,<br>hT2R38,<br>hT2R39,<br>hT2R44,<br>hT2R44,           |
| AKPSGGPVG | 9 | 768.41 | 10.21 | 1 | 14.93 | ACE-inhibition  DPP-IV inhibition  Peptide regulating   | GP, GPV,<br>VG, GG,<br>SG, KP<br>GP, KP,<br>GG, PS, PV,<br>VG, GPV | P, V, K,<br>KP, GP,<br>GGP, VG | hT2R7, hT2R14,<br>hT2R47,<br>hT2R40,<br>hT2R43, hT2R1,<br>hT2R44,<br>hT2R16,<br>hT2R46,<br>hT2R41,  |

|                |    |         |       |   |       | stomach mucosal membrane activity Antioxidative Antithrombotic | KP<br>GP                                |                    | hT2R38,<br>hT2R10,<br>hT2R39, hT2R4                   |
|----------------|----|---------|-------|---|-------|--|---|--------------------|---|
| TVAGKG         | 6  | 531.30  | 9.82  | 1 | 13.29 | ACE-inhibition   | AG, KG,<br>GK                           | V, K,              | hT2R1, hT2R16,<br>hT2R14,<br>hT2R41,                  |
| TVAGRO         | Ü  | 331.30  | 7.02  | 1 | 13.27 | DPP-IV inhibition  | VA, AG,<br>KG, TV                       | KG, VA             | hT2R39,<br>hT2R10                                     |
|                |    |         |       |   |       | ACE-inhibition   | AA, GA,<br>GH, GG,<br>SG, LG, HP        |                    | hT2R40, hT2R7,  |
|                |    |         |       |   |       | Glucose uptake stimulation                                     | LV                                      |                    | hT2R39,<br>hT2R14,                                    |
| TLGHPSGGGGAALV | 14 | 1192.62 | 7.57  | 0 | 14.87 | Hypotensive  | AA                                      | P, V, L,<br>LG, LV | hT2R41,<br>hT2R47,                                    |
|                |    |         |       |   |       | DPP-IV inhibition  | GA, AL,<br>AA, GG,<br>GH, LV, PS,<br>TL | LG, LV             | hT2R47,<br>hT2R38, hT2R5,<br>hT2R43, hT2R1,<br>hT2R44 |
|                |    |         |       |   |       | Antioxidative  | GAA                                     |                    |   |
|                |    |         |       |   |       | ACE-inhibition   | AP, KG,<br>GS, GV,<br>GK, SG            | P, V, L,           | hT2R7, hT2R14,<br>hT2R40,<br>hT2R47, hT2R1,           |
| GKGSAPSSGVA    | 11 | 916.46  | 10.16 | 1 | 16.21 | DPP-IV inhibition  | VA, AP,<br>GV, KG, PS                   | GV, KG,<br>VA      | hT2R39,<br>hT2R41,<br>hT2R43,<br>hT2R38,<br>hT2R16,   |

|           |   |        |       |   |       |   |                                   |                    | hT2R44,<br>hT2R46   |
|-----------|---|--------|-------|---|-------|---|-----------------------------------|--------------------|---|
|           |   |        |       |   |       | ACE-inhibition  | AP, GA,<br>AG, PG                 |                    | hT2R1, hT2R14,  |
| SPAGAPG   | 7 | 555.26 | 5.51  | 0 | 11.94 | Peptide<br>regulating<br>stomach<br>mucosal<br>membrane<br>activity | PG                                | P, PA,<br>PG       | hT2R16,<br>hT2R46,<br>hT2R43,<br>hT2R10,<br>hT2R41,<br>hT2R39,  |
|           |   |        |       |   |       | Antithrombotic  | PG                                |                    | hT2R47, hT2R7,  |
|           |   |        |       |   |       | DPP-IV inhibition   | AP, PA,<br>APG, SP,<br>GA, AG, PG |                    | hT2R44  |
|           |   |        |       |   |       | ACE-inhibition  | AG, KG,<br>GK, PT, HP             |                    | hT2R7, hT2R14,<br>hT2R47,   |
| HPTASAGKG | 9 | 824.41 | 9.94  | 1 | 17.18 | DPP-IV inhibition   | HP, TA,<br>AG, AS,<br>KG, PT      | P, K, KG           | hT2R40, hT2R1,<br>hT2R43,<br>hT2R41,<br>hT2R16,<br>hT2R38,<br>hT2R39,<br>hT2R44,<br>hT2R46,<br>hT2R10 |
|           |   |        |       |   |       | ACE-inhibition  | GL, KG,<br>GK, SF, GL             | E I V              | hT2R14,<br>hT2R16, hT2R1,   |
| GKGLSF    | 6 | 607.33 | 10.16 | 1 | 10.50 | DPP-IV inhibition   | KG, SF                            | F, L, K,<br>KG, GL | hT2R38,<br>hT2R46,<br>hT2R43,   |

|          |   |        |      |   |       |   |                                   |                 | hT2R47, hT2R7,<br>hT2R39,<br>hT2R10,<br>hT2R44, hT2R4,<br>hT2R40,<br>hT2R41, hT2R9                            |
|----------|---|--------|------|---|-------|---|-----------------------------------|-----------------|---|
|          |   |        |      |   |       | ACE-inhibition  | KG, GK,<br>GG, SG, HP             |                 | hT2R7, hT2R14,<br>hT2R16, hT2R1,  |
| HPSGGKGG | 8 | 695.33 | 9.94 | 1 | 18.23 | DPP-IV inhibition   | HP, GG,<br>KG, PS                 | P, K, KG        | hT2R43,<br>hT2R38,<br>hT2R47,<br>hT2R39,<br>hT2R46,<br>hT2R41,<br>hT2R44,<br>hT2R10,<br>hT2R40, hT2R4         |
| TKATAS   | 6 | 577.31 | 9.82 | 1 | 12.66 | ACE-inhibition  DPP-IV inhibition   | KA<br>KA, TA,<br>AS, AT, TK       | K               | hT2R1, hT2R14,<br>hT2R41,<br>hT2R16,<br>hT2R39  |
| LPLL     | 4 | 454.31 | 5.58 | 0 | 4.29  | ACE-inhibition Glucose uptake stimulation Antioxidative DPP-IV inhibition DPP-IV inhibition | LL  LPL  LL, LPL,  LP, PL  VV, VF | P, L, LL,<br>PL | hT2R14,<br>hT2R40, hT2R1,<br>hT2R10,<br>hT2R43,<br>hT2R41,<br>hT2R39, hT2R4,<br>hT2R46,<br>hT2R16,<br>hT2R47, |

|         |   |        |       |    |       |                   |                       |                           | hT2R38, hT2R7,<br>hT2R44  |
|---------|---|--------|-------|----|-------|-------------------|-----------------------|---------------------------|---|
| FLDPAL  | 6 | 674.36 | 3.12  | -1 | 7.97  | DPP-IV inhibition | PA, FL, AL,<br>DP     | P, F, L,<br>FL, LD,<br>PA | hT2R14, hT2R7,<br>hT2R43,<br>hT2R40,<br>hT2R47,<br>hT2R46,<br>hT2R44, hT2R1,<br>hT2R38,<br>hT2R10, hT2R4,<br>hT2R39,<br>hT2R16,<br>hT2R41,<br>hT2R41, |
|         |   |        |       |    |       | ACE-inhibition    | GL, KG,<br>GK, SF     |                           | hT2R14,<br>hT2R16, hT2R1,   |
| GKGLSF  | 6 | 607.33 | 10.16 | 1  | 10.50 | DPP-IV inhibition | GL, KG, SF            | F, L, K,<br>GL, KG        | hT2R38,<br>hT2R46,<br>hT2R43,<br>hT2R47, hT2R7,<br>hT2R39,<br>hT2R10,<br>hT2R44, hT2R4,<br>hT2R44, hT2R4,<br>hT2R40,<br>hT2R41, hT2R9                 |
|         |   |        |       |    |       | ACE-inhibition    | AG, GR,<br>GG, TP     |                           | hT2R1, hT2R14,<br>hT2R16,   |
| VTPAGGR | 7 | 656.36 | 11.11 | 1  | 12.44 | DPP-IV inhibition | PA, TP, AG,<br>GG, VT | V, R, P,<br>GR, PA        | hT2R47,<br>hT2R46,<br>hT2R43, hT2R7,<br>hT2R41,<br>hT2R10,  |

|                |    |         |       |   |       |  |   |   | hT2R44,<br>hT2R38,<br>hT2R39,<br>hT2R40, hT2R4   |
|----------------|----|---------|-------|---|-------|--|---|---|--|
| TPPAGGAAR      | 9  | 796.42  | 10.79 | 1 | 14.04 | ACE-inhibition  Antioxidative Hypotensive  DPP-IV inhibition | AA, GA,<br>AG, GG,<br>AR, PP, TP<br>GAA<br>AA<br>PP, PA, TP,<br>GA, AA,<br>AG, GG | R, P, PP,<br>PA                                   | hT2R7, hT2R14,<br>hT2R47, hT2R1,<br>hT2R43,<br>hT2R16,<br>hT2R40,<br>hT2R46,<br>hT2R44,<br>hT2R41,<br>hT2R38,          |
|                |    |         |       |   |       | α -Glucosidase inhibition                                    | PP  |   | hT2R10,<br>hT2R39, hT2R4   |
|                |    |         |       |   |       | ACE-inhibition   | GL, KG,<br>GG, LG   |   | hT2R7, hT2R14,<br>hT2R47,  |
| PSPKGGGLG      | 9  | 768.41  | 10.59 | 1 | 14.79 | DPP-IV inhibition  | SP, GL, GG,<br>KG, PK, PS   | P, L, K, PK, LG, GL, GLG, GGL, GGLG, GGGLG, GGGLG | hT2R16,<br>hT2R43, hT2R1,<br>hT2R38,<br>hT2R46,<br>hT2R40,<br>hT2R44,<br>hT2R39,<br>hT2R41,<br>hT2R10, hT2R4,<br>hT2R9 |
| SPHLAGSGGGAALV | 14 | 1192.62 | 7.63  | 0 | 14.43 | ACE-inhibition   | LA, AA,<br>GA, AG,<br>HL, GS,<br>GG, SG,<br>PH, AGS                               | P, V, L,<br>LV, LA                                | hT2R40, hT2R7,<br>hT2R39,<br>hT2R41,<br>hT2R14, hT2R5,<br>hT2R47,  |

|           |   |        |      |   |       | Glucose uptake stimulation Antioxidative Hypotensive  DPP-IV inhibition | LV HL, GAA AA LA, SP, GA, HL, AL, AA, AG, GG, LV, PH |                  | hT2R38,<br>hT2R43   |
|-----------|---|--------|------|---|-------|---|--|------------------|---|
|           |   |        |      |   |       | ACE-inhibition  | GPA, GA,<br>AG, PG,<br>AGP, GAGP                     |                  | hT2R1, hT2R14,  |
|           |   |        |      |   |       | Antithrombotic  | GP, PG   |                  | hT2R16,   |
| SPGAGPA   | 7 | 555.26 | 5.51 | 0 | 11.94 | Peptide<br>regulating<br>stomach<br>mucosal<br>activity                 | GP, PG   | P, GP,<br>PA, PG | hT2R46,<br>hT2R43,<br>hT2R10,<br>hT2R41,<br>hT2R39,<br>hT2R47, hT2R7, |
|           |   |        |      |   |       | DPP-IV inhibition   | GP, PA, SP,<br>GPA, GA,<br>AG, PG                    |                  | hT2R44  |
|           |   |        |      |   |       | ACE-inhibition  | KG, GK,<br>GG, TG, HP                                |                  | hT2R40, hT2R7,<br>hT2R39,   |
| HPATTGGKG | 9 | 824.41 | 9.94 | 1 | 17.62 | DPP-IV inhibition   | AT, GG,<br>KG, TG,<br>TT, HP, PA                     | P, K, KG,<br>PA  | hT2R14,<br>hT2R41,<br>hT2R38, hT2R5,<br>hT2R47,<br>hT2R43, hT2R1      |
| TKSGVS    | 6 | 577.31 | 9.82 | 1 | 12.56 | ACE-inhibition DPP-IV inhibition  | GV, SG<br>GV, KS,<br>TK, VS                          | V, K, GV         | hT2R1, hT2R14,<br>hT2R16,   |

|  |  |  | DPP-IV     | PA, LP, AL   | hT2R41, |
|--|--|--|------------|--------------|---------|
|  |  |  | inhibition | 171, L1, 71L | hT2R39  |

Supplementary Table 2. Molecular docking of peptides identified by LC-ESI-MS/MS from germinated chickpea protein hydrolysate present in legumin.

|        |                               | T2R4   | T2R14                         |   |  |  |
|--------|-------------------------------|--|-------------------------------|---|--|--|
|        | Energy of Affinity (kcal/mol) | Amino acid residues  | Energy of Affinity (kcal/mol) | Amino acid residues   |  |  |
| VVFW   | -5.3                          | LEU181 [4.19], PHE189 [3.97], SER186 [3.27], SER243 [1.79], TYR250 [5.25], VAL182 [5.44, 5.45]   | -10.5                         | ASN157 [2.25, 2.37],<br>GLU255 [2.35], ILE148<br>[5.25], ILE262 [4.63,<br>5.41], PHE175 [3.79],<br>SER169 [1.97], SER250<br>[2.64], SER254 [2.74],<br>TRP89 [3.65, 3.76]  |  |  |
| FDLPAL | -5.4                          | LEU181 [4.78], PHE189 [4.88], SER243 [2.03], TYR250 [4.28], VAL178 [5.25], VAL193 [4.99]   | -9.7                          | ASN157 [2.03, 2.43,<br>2.95], GLU259 [3.07],<br>ILE148 [4.07], LEU261<br>[5.02], PHE175 [3.63],<br>PHE243 [3.78], PHE247<br>[4.81], SER167 [3.01,<br>3.12], SER169 [2.19],<br>TRP66 [4.14], TRP89<br>[4.58, 5.22] |  |  |
| GEAGR  | -5.9                          | GLN249 [2.78],<br>LEU177 [2.90],<br>LYS262 [2.84],<br>MET89 [3.03],<br>SER176 [2.73],<br>SER180 [2.48,<br>3.19], SER184<br>[2.45], THR166<br>[2.70, 2.81, 3.39],<br>TYR147 [2.86,<br>2.94] | -8.2                          | ASN157 [2.42, 2.73],<br>GLU255 [2.27], PHE175<br>[3.61], SER167 [2.84],<br>SER169 [2.24, 2.73],<br>THR86 [2.35], TRP89<br>[3.90]  |  |  |