**Supplementary Information for:**
**Determination of Crucial Immunogenic Epitopes in Major Peanut Allergy Protein, Ara h2, via Novel Nanoallergen Platform**

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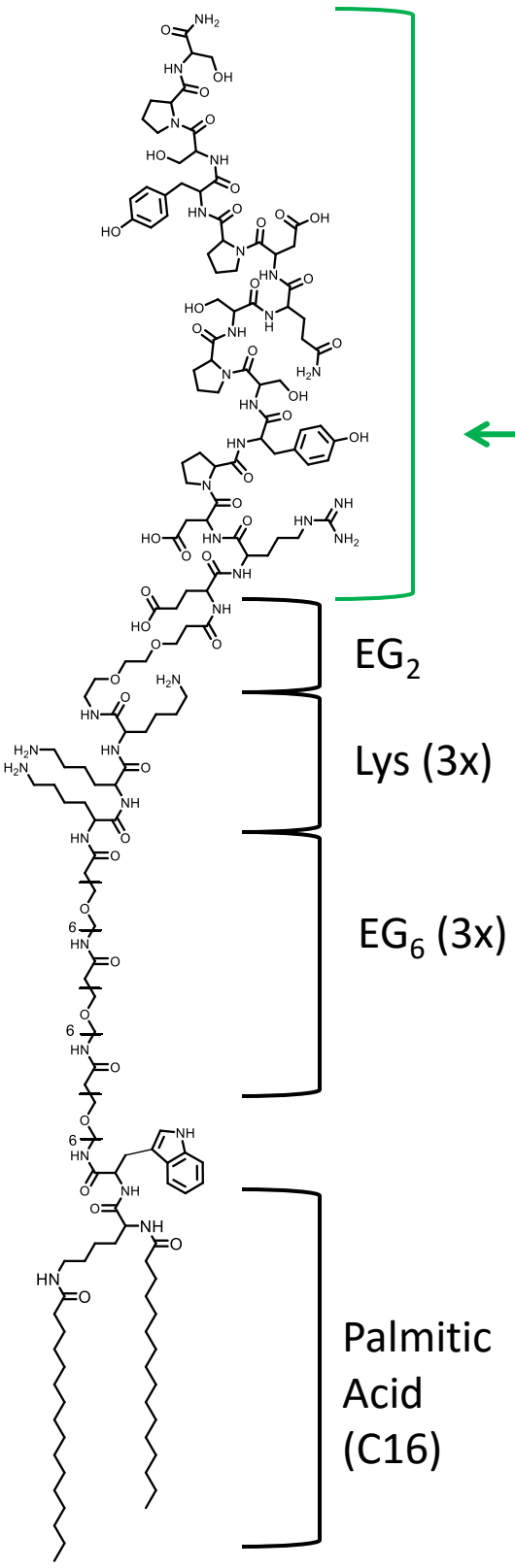
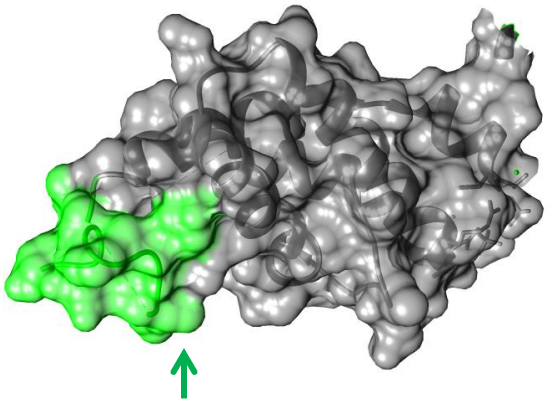
A.




Ara h2 Protein



Ara h2 Epitope

ERDPYSP$^{OH}$SQDPYSP$^{OH}$S

Figure S-1. Schematic of epitope-lipid conjugates . A linear peptide sequence of the peanut protein Ara h2 (residues 79-91) was attached to a lipid using the schematic given. The epitope (shown in green) is shown in context with the native Ara h2 protein (PDB: 3OB4). Note that the schematic was used for all other epitope-lipids.

Table S-1. Listing of Ara h 2 IgE binding epitopes-lipid conjugates.

| Epitope-Lipid | Exact Mass (g/mol) | Purity (%) | Immuno-reactivity (Serum 1) |
|---|---|---|---|
| 1 | 4330.64 | >95% | None |
| 2 | 4094.33 | >95% | High |
| 3 | 3425.16 | >95% | Low |
| 4 | 3498.23 | >95% | None |
| 5 | 3563.21 | >95% | Low |
| 6 | 4563.78 | >95% | Low |
| 7 | 3182.02 | >95% | Low |
| 8 | 3820.28 | >95% | None |

Table S-2. Listing of patient sera and their ImmunoCAP results for peanuts.

| Serum Number | Peanut IgE (kU/L) |
|---|---|
| 1 | 84.4 |
| 2 | 99.3 |
| 3 | 50 |
| 4 | 124.5 |

Table S-3- $EC_{50}$ values and maximum degranulation values for Ara h 2 and 2 % loaded Ara h 2 epitope nanoallergens

| Serum # | 1 | | | | 2 | | | | 3 | | | | 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptide # | $EC_{50}$ (pM) | Error | Max(%) | Error | $EC_{50}$ (pM) | | Max(%) | Error | $EC_{50}$ (pM) | Error | Max(%) | Error | $EC_{50}$ (pM) | Error | Max(%) | Error |
| Ara h2 | 104 | 20 | 35 | 2.1 | 33 | 20 | 35 | 3.5 | 115 | 50 | 52 | 5 | 52 | 12 | 55 | 6 |
| 1 | >5000 | | 0 | | 1400 | 107 | 25.6 | 0.9 | 1340 | 85 | 21.5 | 0.7 | 430 | 35 | 25 | 0.5 |
| 2 | 3.6 | 1.2 | 24 | 1.7 | >5000 | | 24 | 1.2 | 91 | 17 | 8.9 | 0.5 | 5.9 | 6.6 | 21 | 3.3 |
| 3 | 1000 | 110 | 80.8 | 3.4 | 2200 | 390 | 43 | 5.6 | >5000 | | 46 | 2.1 | 1512 | 260 | 37.2 | 4.1 |
| 5 | >2500 | | 79 | 4.9 | 1200 | 170 | 31 | 2.6 | 870 | 150 | 25.1 | 2.1 | 600 | 110 | 37 | 2.5 |
| 6 | >2500 | | 36.1 | 2.7 | 1670 | 220 | 34.9 | 0.9 | 2080 | 170 | 36.6 | 1.9 | 760 | 190 | 46.4 | 4.1 |
| 7 | >2500 | | 21.7 | 0.8 | >2500 | | 15.3 | 1.2 | >5000 | | 10.1 | 2.3 | >2500 | | 20.4 | 3.6 |

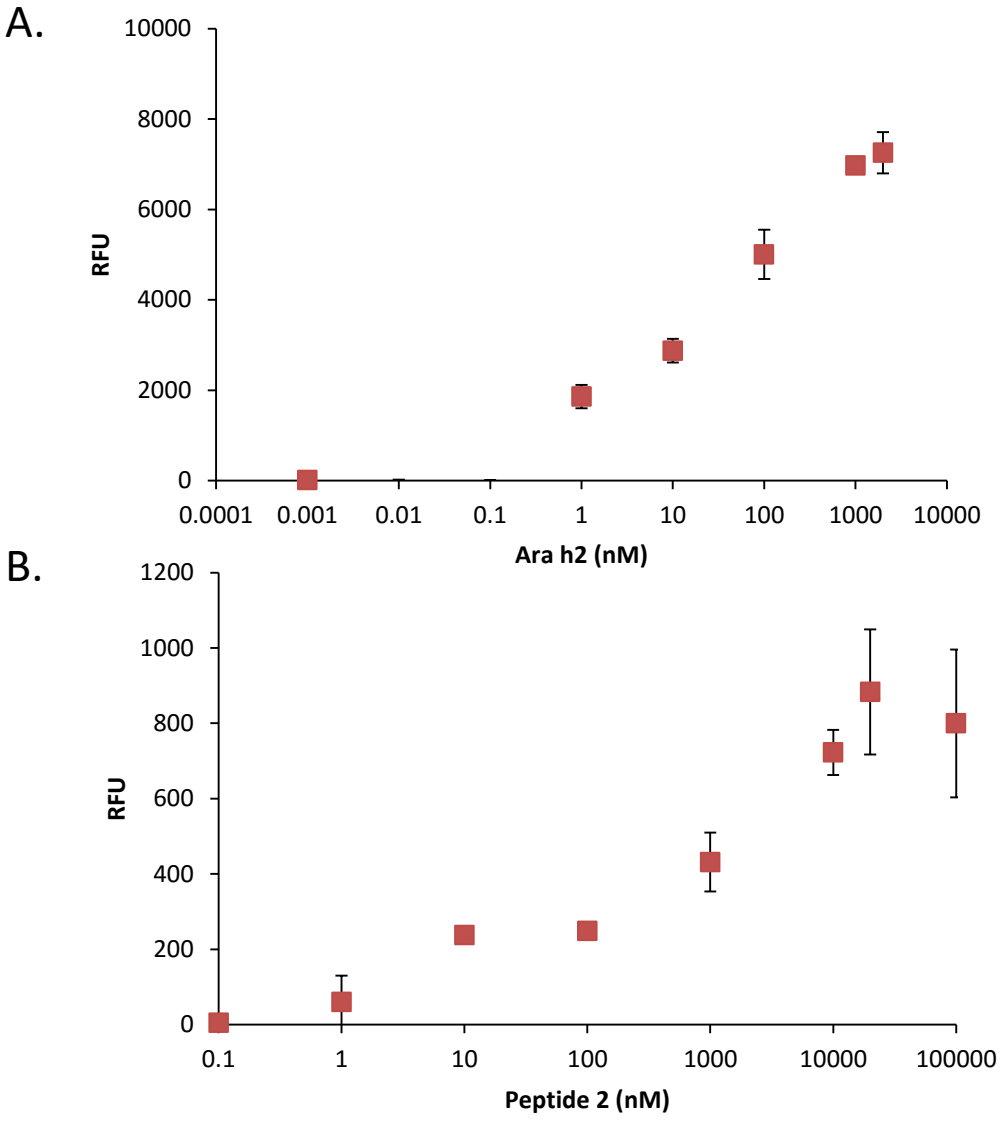



Figure S-2. ELISA binding assay with patient sera. (A) Patient serum 1 was added to a 96 well plate coated with anti-IgE IgG, washed and then either Ara h 2-Biotin or (B) FITC-tagged epitope 2 peptide and the binding was observed using ELISA. Ara h2- $EC_{50}$= 40 ± 27 nM; Epitope 2- $EC_{50}$= 465 ± 200 nM

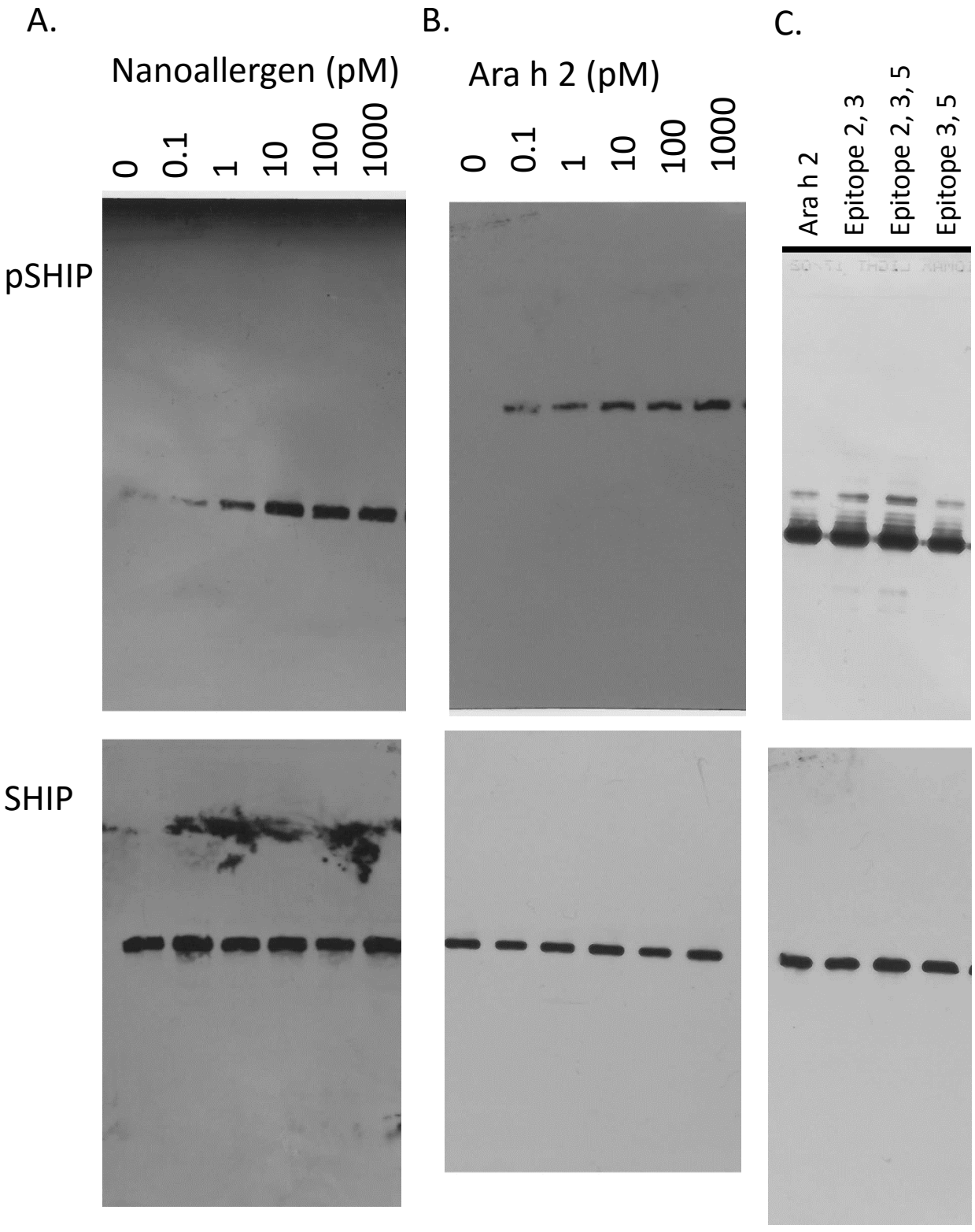



Figure S-3. Full length SHIP blots used in this study. pSHIP shown on top, SHIP at bottom. Note that SHIP-1 protein (MW=133 kDa) appeared between 150 and 100 kDa markers for all gels, with a slight increase in MW for pSHIP.. (A). 2% epitope 2 presenting nanoallergen in Figure 3C, (B) Ara h 2 protein in Figure 3C, (C) multiple nanoallergen formulation and Ara h 2 control in Figure 6. Note bands appearing below pSHIP as possible antibody targeting of SHIP or contamination.

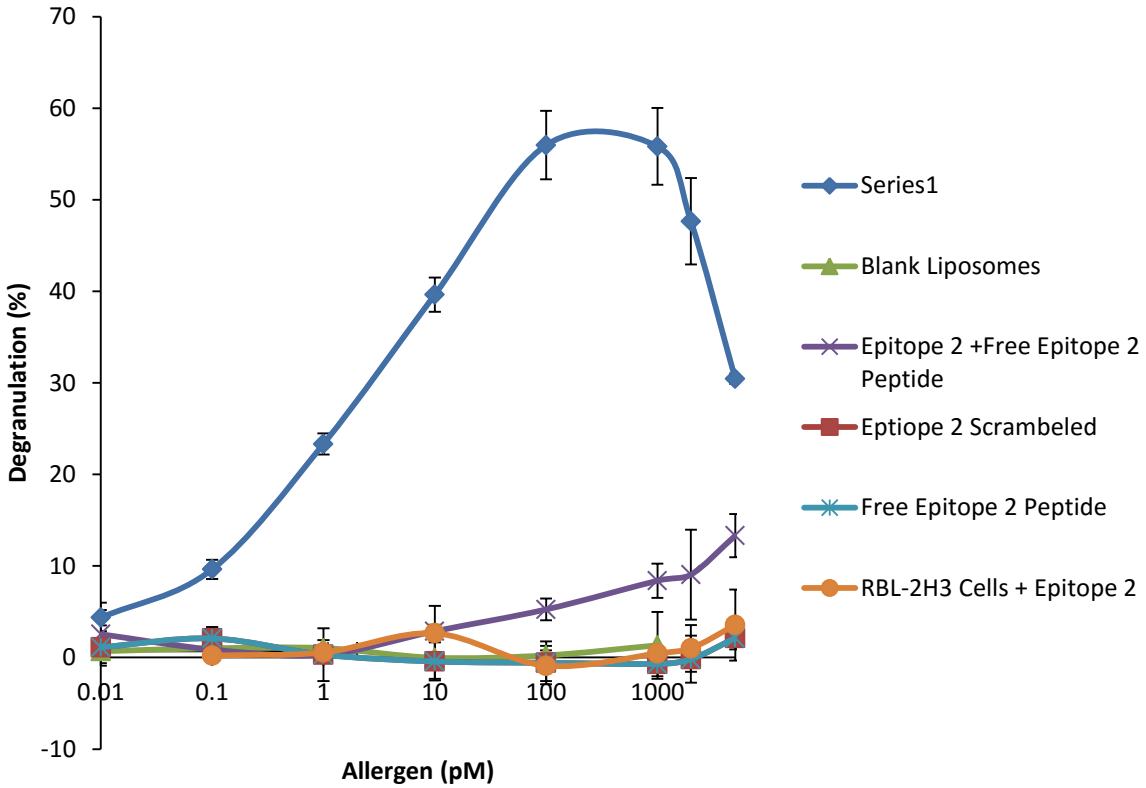



Figure S-4. Nanoallergens demonstrate specificity, as their degranulation response with RBL-SX38 cells can be prevented with addition of 100 μM of free peptide 2 (in purple). Likewise, blank liposomes (in green), liposomes loaded with 2% of epitope 2 sequence that was scrambled (in red) and free epitope 2 alone (in light blue) do not cause a degranulation response. Likewise, cells not expressing the human FcεRI (RBL-2H3 cells) do not demonstrate degranulation when primed with patient serum 1 and incubated with 2% loaded epitope 2 nanoallergens.

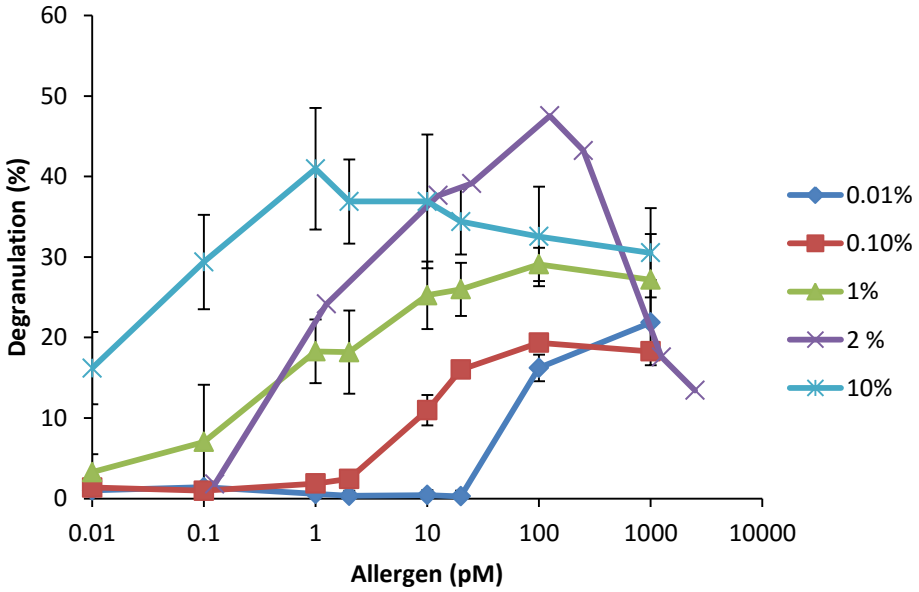



Figure S-5. Nanoallergens can be formed with various epitope loading of epitope 2 and used to trigger degranulation of RBL cells primed with 10% serum 1.

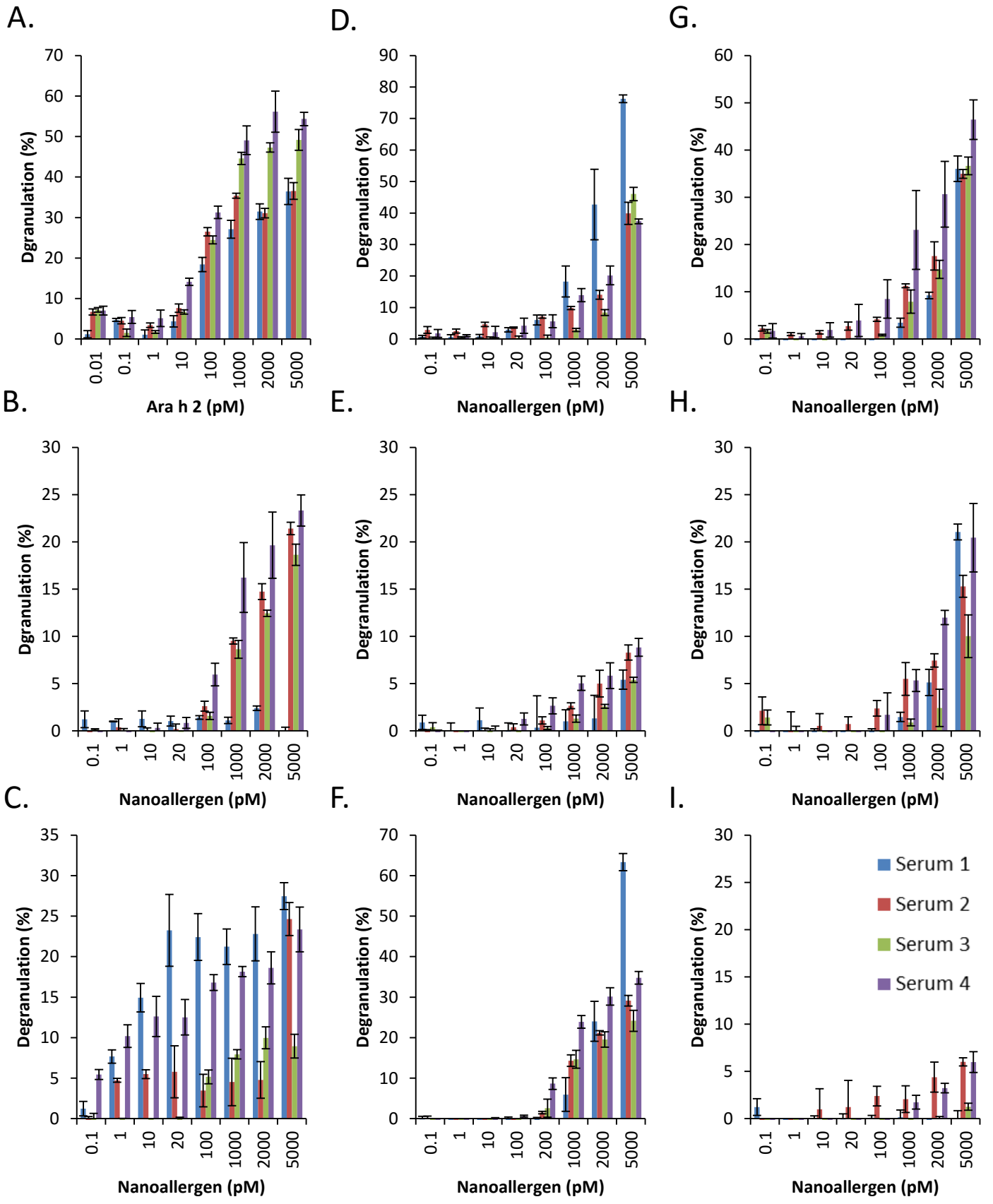



Figure S-6. Raw data for all patient sera/epitope combinations for Ara h 2. Note that 2% epitope loading was used for all assays. (A) is for Ara h 2 protein, while B-I demonstrate epitope 1-8 respectively.

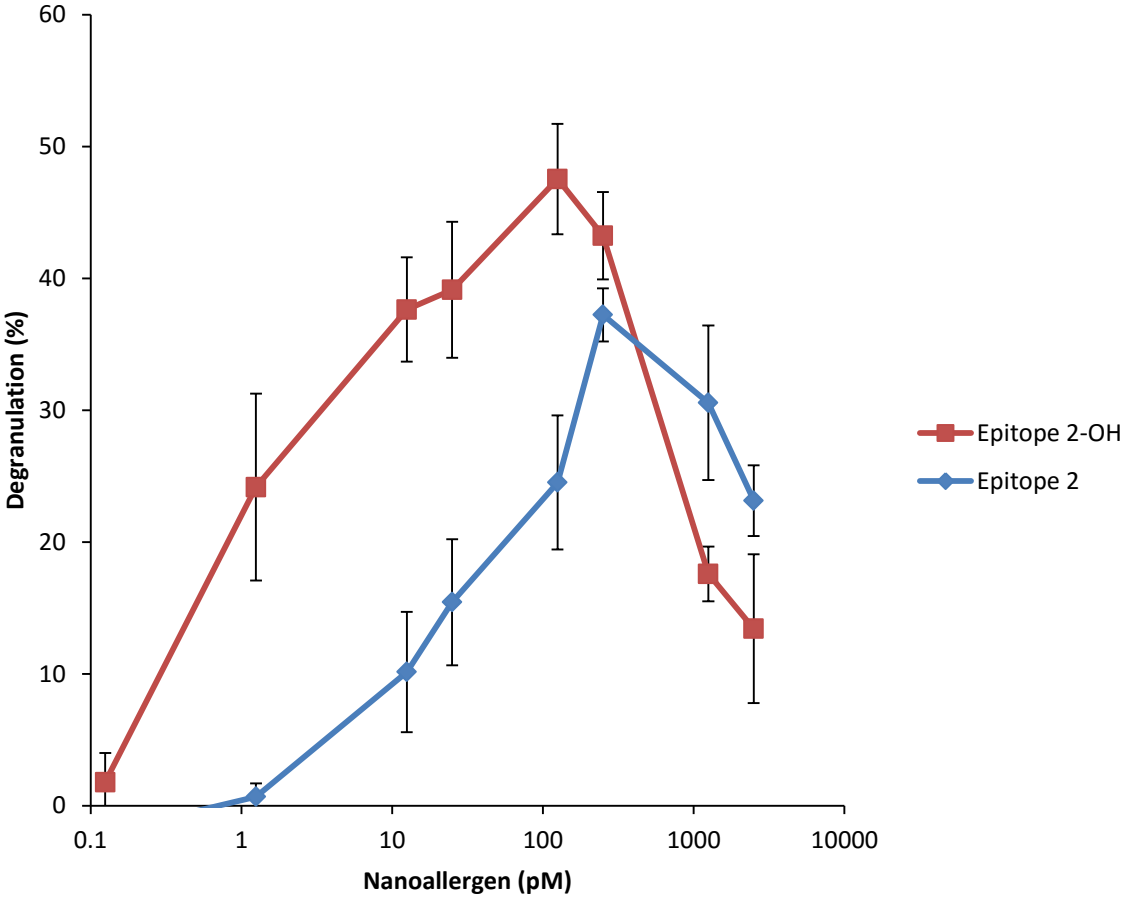



Figure S-7. Epitope peptide 2 demonstrates an increase in response when proline hydroxylations are present. Epitope 2- lipid conjugate was made with and without the addition of proline hydroxylations and loaded at 2% in nanoallergens. Epitope 2, $EC_{50}$ = 21.5 ±8.8 pM; Epitope $2^{OH}$, $EC_{50}$= 0.75±1.5 pM ; $p<0.01$

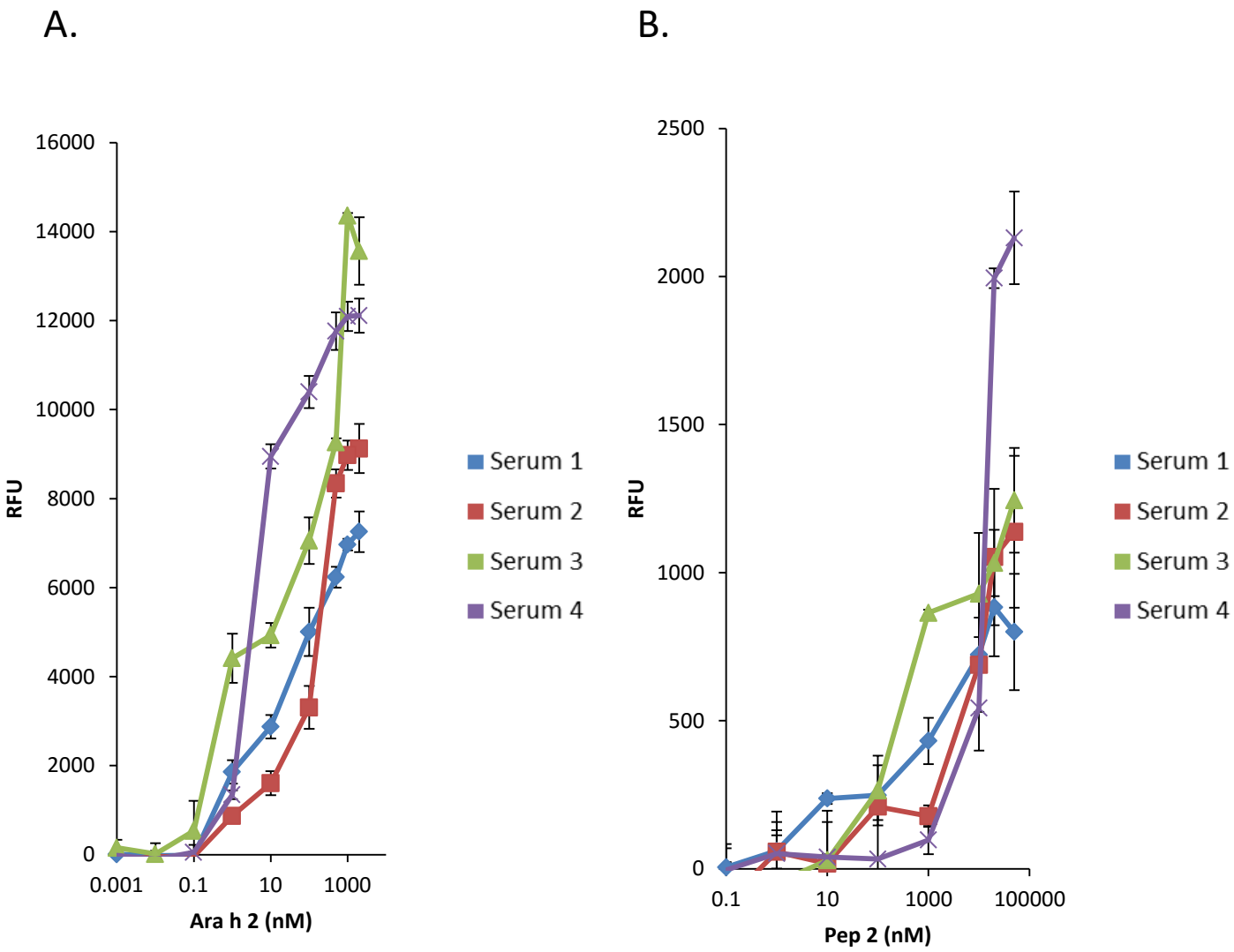



Figure S-8. ELISA binding data for additional sera. (A) Ara h 2 or (B) free epitope peptide 2 was used to bind patient IgE's.

Table S-4: Ara h 6 Epitopes

| Epitope# | Sequence | Number | Notes |
|---|---|---|---|
| 1 | MRRERGRGGDSSSS | 24-37 | |
| 2 | KPCEQHIMQRI | 45-55 | Homology to ara h2 epitope 11 |
| 3 | YDSYDIR | 35-68 | similar to ara h2 epitope 2 |
| 4 | CDELNEMENTQR | 82-93 | Homology to ara h2 epitope 10 |
| 5 | CEALQQIMENQCD | 97-109 | |
| 6 | KRELRMLPQQ | 120-129 | Homology to ara h2 epitope 7 |
| 7 | CNFRAPQRCDLDV | 130-142 | Homology to ara h2 epitope 8 |

Table S-5. Listing of Ara h 6 IgE binding epitopes-lipid conjugates taken from Ostu *et al*.[18]

| Epitope-Lipid | Exact Mass (g/mol) | Purity (%) | Immuno-reactivity (Serum 1) |
|---|---|---|---|
| 1 | 3947.37 | >95% | Moderate |
| 2 | 3733.34 | >95% | Mild |
| 3 | 3110.93 | >95% | High |
| 4 | 3804.23 | >95% | Mild |
| 5 | 3832.45 | >95% | Mild |
| 6 | 3665.35 | >95% | Low |
| 7 | 3728.33 | >95% | Mild |

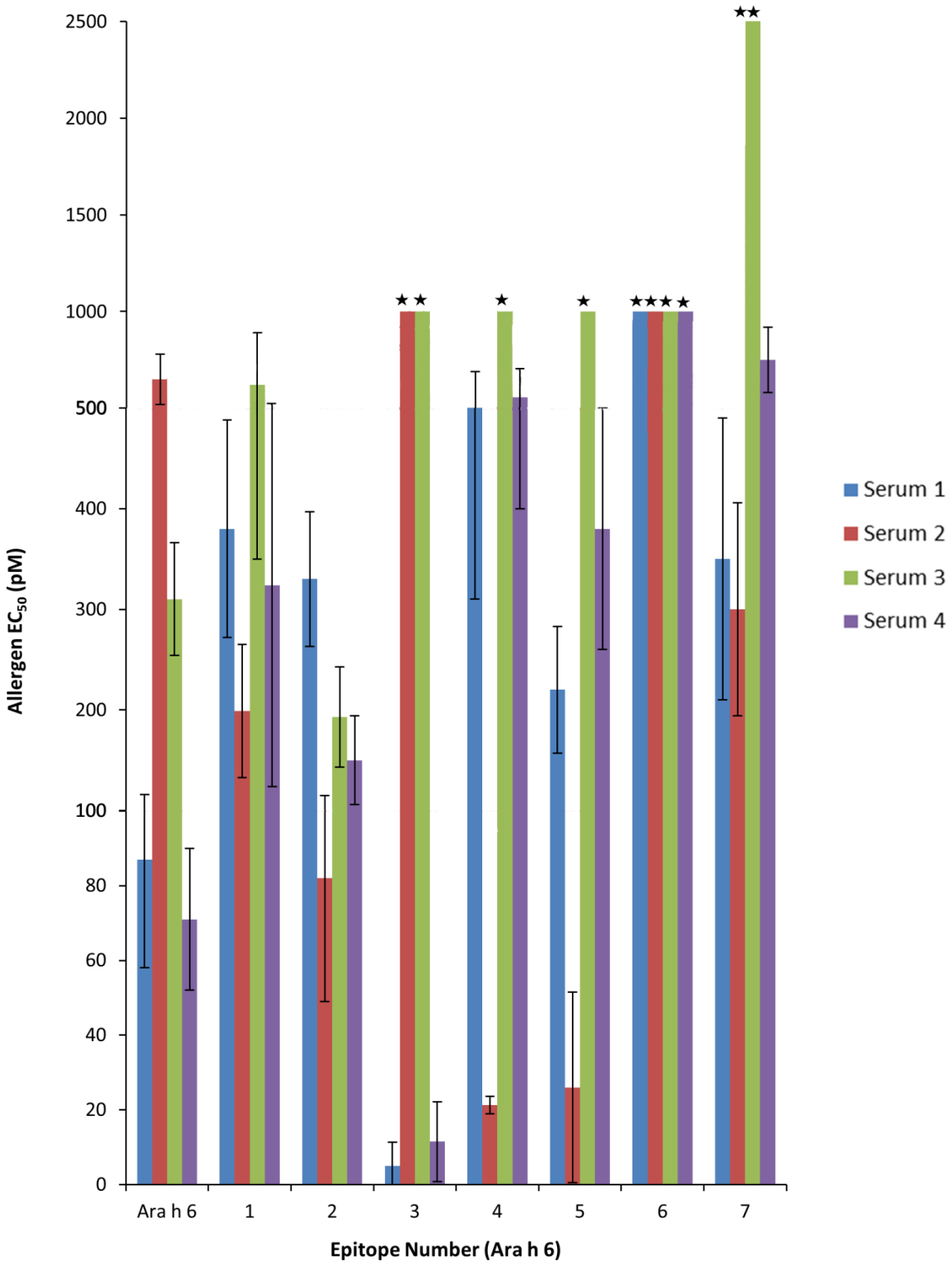



Figure S-9. $EC_{50}$ data for all patient sera/epitope combinations for Ara h 6. Note that 2 % epitope loading was used for all assays.

Table S-6- $EC_{50}$ values and maximum degranulation values for Ara h 6 and 2 % loaded Ara h 6 epitope nanoallergens

| Serum # | 1 | | | | 2 | | | | 3 | | | | 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Peptide # | $EC_{50}$ (pM) | Error | Max(%) | Error | $EC_{50}$ (pM) | | Max(%) | Error | $EC_{50}$ (pM) | Error | Max(%) | Error | $EC_{50}$ (pM) | Error | Max(%) | Error |
| Arah6 | 87 | 29 | 80 | 5.5 | 650 | 130 | 73 | 5 | 310 | 56 | 61 | 3 | 71 | 19 | 91 | 5 |
| 1 | 380 | 108 | 50 | 4 | 199 | 66 | 60 | 4.2 | 620 | 270 | 38 | 4.8 | 324 | 200 | 52 | 8.8 |
| 2 | 330 | 67 | 73 | 3.1 | 82 | 33 | 78 | 6.2 | 193 | 50 | 62 | 3.6 | 150 | 44 | 70 | 4.2 |
| 3 | 5 | 6.4 | 50 | 7.1 | >1000 | | 42.3 | 4.3 | >1000 | | 40.7 | 2.3 | 11.5 | 10.7 | 47 | 6.32 |
| 4 | 500 | 190 | 25.8 | 2.1 | 21.3 | 2.3 | 9.6 | 0.26 | >1000 | | 11.3 | 1.2 | 550 | 150 | 36.2 | 2.5 |
| 5 | 220 | 63 | 18.8 | 4.43 | 26 | 25.5 | 13 | 2 | >1000 | | 8.9 | 0.8 | 380 | 120 | 31.7 | 2.6 |
| 6 | >1000 | | 30.8 | 3.8 | >1000 | | 33.5 | 4.7 | >1000 | | 17.1 | 6.5 | >1000 | | 18.8 | 2.5 |