Supplementary Information for

Insights into ultra-low affinity lipase-antibody noncovalent complex binding mechanisms

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**Figure S1 Caption**: Mass spectra of (A) LPLA2, (B) LPLA2-Lot 2 (C) PLBL2 (D) PLBL2-lot 2 and overlays of the deconvoluted masses from (E) LPLA2 (F) PLBL2 (G) deglycosylated LPLA2 (reference in black, lot 2 in red) and (H) deglycosylated PLBL2 (reference in black, lot 2 in red).

**Figure S1 Alt Text**: The figure contains raw mass spectrometric profiles of both lots of LPLA2 and PLBL2. The deconvoluted masses (with and without deglycosylation) indicate differences in the spectra between lots.

| Masses | Reference | Lot 2 |
|--------|-----------|-------|
| LPLA2, C-Term (39.9 kDa) | $2.2 \times 10^4$ | $4.8 \times 10^4$ |
| LPLA2, C-Term + 28 Da (40.0 kDa) | $1.4 \times 10^4$ | $1.3 \times 10^5$ |
| PLBL2, N-Term (21.8 kDa) | $1.09 \times 10^5$ | $4.02 \times 10^5$ |
**Figure S2 Caption:** Reference intact spectra of (A) IgG4-B, (B) IgG1-B and (C) IgG1-A.

**Figure S2 Alt Text:** The figure depicts raw mass spectrometric profiles for all three mAbs used in the study.
**Figure S3 Caption:** LPLA2-IgG4-B formation at a (A) 10:1 and (B) 1:10 molar ratio, respectively. The antibody (Ab) peak remains the most intense species regardless of the solution ratio. However, the reduced concentrations at the 1:10 molar ratio prevent significant overlapping distributions from the Ab and Ab dimer.

**Figure S3 Alt Text:** mAb-lipase complex formation was observed using native mass spectrometry. While both 10:1 and 1:10 molar ratios enabled complex formation, 1:10 molar ratio resulted in the cleanest observation with minimal overlap.
Figure S4 Caption: Schematic of the non-MS atmospheric ion mobility technology for detecting native state proteins and complexes. Samples flow through an electrospray emitter at 300 nL/min producing charged droplets, which pass through a charge reducing field. Droplets emerge with a single charge and evaporate yielding singly charged ions. At a given voltage, proteins of different collisional cross-sectional areas take different trajectories around a central rod, where only ions with a certain inverse mobility will hit and be detected on the ring. A sweep of the voltage enables the relative proportion of sample components to be identified in a single two-minute run.

Figure S4 Alt Text: Schematic of the non-MS atmospheric ion mobility technology for detecting native state proteins and complexes. Samples flow through an electrospray emitter at 300 nL/min producing charged droplets, which pass through a charge reducing field. Droplets emerge with a single charge and evaporate yielding singly charged ions. At a given voltage, proteins of different collisional cross-sectional areas take different trajectories around a central rod, where only ions with a certain inverse mobility will hit and be detected on the ring. A sweep of the voltage enables the relative proportion of sample components to be identified in a single two-minute run.
Figure S5 Caption: Desialylated LPLA2 bound to IgG4-B with similar affinity as detected by native MS.

Figure S5 Alt Text: Native MS spectra of desialylated LPLA2 bound to IgG4-B did not show any difference in binding affinity compared to untreated LPLA2.
Figure S6 Caption: Comparison of glycans pre-storage and post-storage of PLBL2-reference, PLBL2-lot 2, and LPLA2-lot 2.

Figure S6 Alt Text: Three bar graphs of different lots of PLBL2 and LPLA2 depict the change in glycosylation upon storage. Mannosylation levels dropped significantly upon storage for all lipases and lots while there were small changes in sialylation and fucosylation in some instances.
Figure S7 Caption: Low resolving power (4375 RP @ 200 m/z) of (A) PLBL2 and (B) LPLA2 binding to IgG4-B mutants, where the complex is highlighted in green. All intensity is relative to the base peak of the maximum charge state of the free antibody. The +21 peak of IgG4-B, at about 7095 m/z and the +20 peak at 7450 m/z overlap the complex distributions, but may be distinguished on the basis of exact mass. For the mutant 47L198A-LPLA2 complex, the +29 peak could be observed by SIM, but the complex distribution was not above the noise in the full spectrum.
**Figure S7 Alt Text:** Native MS spectra of IgG4-B mutants in the presence of PLBL2 and LPLA2. IgG4-B complexes were not observed for some mutants indicating disruption of binding.

**Figure S8 Caption:** Binding dissociation curves were calculated for (A) LPLA2-IgG4-B mutants or (B) PLBL2-IgG4-B mutants using a four-parameter logistic growth curve, with outliers detected by the Tukey method removed from calculations (highlighted in red). L198A did not fit an S-curve shape, as the complex was already unstable at the lowest energy setting available.

**Figure S8 Alt Text:** This figure displays the binding dissociation curves between the mAbs and lipases to obtain the VC50 data.
Figure S9 Caption: Ion mobility analysis of mutant IgG4-B species against LPLA2. Fit curves are labeled with the peak assignment, where LPLA2 is the remaining free lipase, the Ab is the remaining free Ab, 2xAb is a gas-phase dimer artifact, 2xLPLA2 is a gas-phase dimer artifact, and complex is the peak of interest. The fit sum is the curve created from the overlay of the Gaussian-A plots, and the thick lines plotted show the mean +/- standard deviation at each inverse mobility. Fits were performed in MagicPlot Pro as described in the methods.
Figure S9 Alt Text: Raw ion mobility curves depicting the peaks representing free lipase, free mAb and the mAb-lipase complex respectively.

Figure S10 Caption: Electrostatic surface map (red = electronegative; white = neutral; blue = electropositive) of PLBL2 (panel A) and LPLA2 (panel C). Hydrophobic surface
map (red = hydrophobic; white = neutral; blue = hydrophilic) of PLBL2 (panel B) and LPLA2 (panel D). mAb binding peptides are displayed as spheres.

Figure S10 Alt Text: Surface maps of PLBL2 and LPLA2 with the electrostatic and hydrophobic surface displayed. No correlation between potential interacting residues and charge or hydrophobic patches.

Table S1. The reduced heterogeneity of desialylated LPLA2 permitted deconvolution of the spectra. The mass shift is reported against the experimentally determined deglycosylated intact mass (44491.5 Da). Where identifications were not possible due to the large combinatorial space of 14 different glycans permitted across four N-glycosylation sites, the relative glycan unit shifts compared to the most intense mass, labeled reference A, is given. Of 38 deconvolved masses, only three were not found to be associated with a predicted glycosylation pattern.

| Identification          | Average Mass | Intensity | Delta Mass to Deglycosylated Intact Protein |
|-------------------------|--------------|-----------|--------------------------------------------|
| Ref A                   | 51919.13     | 2.87E+04  | 7427.63                                    |
| A+Hex                   | 52286.90     | 2.01E+04  | 7795.40                                    |
| A+2Hex-HexNac           | 52042.92     | 1.96E+04  | 7551.42                                    |
| A+2Hex-2HexNac          | 51840.66     | 1.75E+04  | 7349.16                                    |
| A+2HexNac-Hex           | 52161.78     | 1.75E+04  | 7670.28                                    |
| A+2Hex+HexNac           | 52448.30     | 1.72E+04  | 7956.80                                    |
| A+2Hex+2HexNac          | 52651.66     | 1.60E+04  | 8160.16                                    |
| A-2Hex-HexNac-Fuc       | 51245.45     | 1.54E+04  | 6753.95                                    |
| A+3Hex-HexNac           | 52204.03     | 1.52E+04  | 7712.53                                    |
| A-2Hex+2HexNac          | 52000.66     | 1.49E+04  | 7509.16                                    |
| A+Hex                   | 52082.84     | 1.38E+04  | 7591.34                                    |
| A+2Hex+HexNac+Fuc       | 52594.37     | 1.27E+04  | 8102.87                                    |
| A-Hex-HexNac            | 51554.44     | 1.25E+04  | 7062.94                                    |
| A+3Hex+2HexNac+Fuc      | 52960.11     | 1.24E+04  | 8468.61                                    |
| A+HexNac                | 52122.14     | 1.09E+04  | 7630.64                                    |
| A-Fuc                   | 51775.95     | 1.07E+04  | 7284.45                                    |
| A+4Hex+Fuc              | 51121.84     | 1.03E+04  | 6630.34                                    |
| A+3Hex+3HexNac          | 52814.34     | 1.02E+04  | 8322.84                                    |
| A-2HexNac+Hex           | 51677.66     | 1.01E+04  | 7166.16                                    |
| A+4Hex+3HexNac+Fuc      | 53324.30     | 1.00E+04  | 8832.80                                    |
| A+4Hex                  | 52570.86     | 9.64E+03  | 8079.36                                    |
| A-3Hex-2HexNac-Fuc      | 50878.42     | 8.97E+03  | 6386.92                                    |
| A-Hex                   | 51756.77     | 8.97E+03  | 7265.27                                    |
| A-3Hex                  | 52408.32     | 8.72E+03  | 7916.82                                    |
| A-Hex-HexNac-Fuc        | 51409.79     | 8.36E+03  | 6918.29                                    |
| A+2Hex+HexNac+2Fuc      | 52736.13     | 7.83E+03  | 8244.63                                    |
| A+HexNac+Fuc            | 51979.77     | 7.60E+03  | 7488.27                                    |
| A+5Hex+4HexNac+Fuc      | 53690.83     | 6.68E+03  | 9199.33                                    |
| A-3Hex+HexNac           | 51636.53     | 6.44E+03  | 7145.03                                    |
| A-3HexNac               | 51312.33     | 6.14E+03  | 6820.83                                    |
| A-HexNac                | 51716.11     | 5.84E+03  | 7224.61                                    |
| A-4Hex-HexNac           | 52772.68     | 5.62E+03  | 8281.18                                    |
| A+2Hex+HexNac+3Fuc      | 52883.36     | 4.35E+03  | 8391.86                                    |
### Table S2. Glycan composition of PLBL2-reference, PLBL2-Lot 2, and LPLA2-Lot 2 at time point zero and six months (inactive).

| Glycan Name | Rel. % T0 | Rel. % T6 mo. | Mass | Composition | Sialylation T0 | Sialylation T6 mo. | Mannosylation T0 | Mannosylation T6 mo. | Fucosylation T0 | Fucosylation T6 mo. |
|-------------|-----------|---------------|------|-------------|----------------|-------------------|-----------------|-------------------|----------------|-------------------|
| Man3        | 2.9 1     | 0.00          | 910.3| Hex3HexNAc2 | 2.91           | 0.00              |                 |                   |                 |                   |
| Man4        | 1.1 2     | 0.33          | 1072.4 | Hex4HexNAc2 | 1.12           | 0.33              |                 |                   |                 |                   |
| Man5        | 4.3 7     | 0.00          | 1234.4 | Hex5HexNAc2 | 4.37           | 0.00              |                 |                   |                 |                   |
| 2000 OA 0G | 0.8 2     | 0.59          | 1316.5 | Hex3HexNAc4 | 0.83           |                   |                 |                   |                 |                   |
| 2100 OA 0G | 9.9 7     | 0.00          | 1462.5 | Hex3HexNAc4dHex1 | 9.97 | 0.00 | 0.00 |                   |                 |                   |
| 2010 OA 0G | 0.0 0     | 1.62          | 1478.5 | Hex4HexNAc4 | 0.00           | 0.00              | 0.00            | 0.00              | 0.00            |                   |
| Man7        | 0.8 7     | 0.00          | 1558.5 | Hex7HexNAc2 | 0.87           | 0.00              |                 |                   |                 |                   |
| 2110 OA 0G | 1.8 2     | 1.30          | 1624.6 | Hex4HexNAc4dHex1 | 1.82 | 1.30 |                   |                   |                   |                   |
| 3100 OA 0G | 16. 31    | 0.00          | 1665.6 | Hex3HexNAc5 | 16.3           |                   | 0.00            |                   |                   |                   |
| 2120 OA 0G | 0.0 0     | 0.94          | 1786.7 | Hex5HexNAc4dHex1 | 0.94 | 1.36 | 0.00            |                   |                   |                   |
| 3110 OA 0G | 6.9 6     | 11.5 4        | 1827.7 | Hex4HexNAc5 | 6.96           | 11.54             |                 |                   |                   |                   |
| Man9        | 3.4 0     | 5.48          | 1882.6 | Hex9HexNAc2 | 3.40           | 5.48              |                 |                   |                 |                   |
| 2111 1A 0G | 0.9 9     | 1.75          | 1915.7 | Hex4HexNAc4dHex1 | 0.99 | 1.75 | 0.99            |                   |                   |                   |
| 2021 1A 0G / 2111 0A 1G | 0.0 0 | 1.36 | 1931.7 | Hex5HexNAc4dHex1 | 0.00 | 1.36 | 0.00            |                   |                   |                   |
| 3011 1A 0G | 2.7 9     | 1.78          | 1972.7 | Hex4HexNAc5 | 2.79           | 1.78              |                 |                   |                 |                   |
| 3120 OA 0G | 5.1 3     | 0.58          | 1989.7 | Hex5HexNAc5| 5.13           |                   |                 |                   |                 |                   |
| Man9Glc1    | 0.0 0     | 1.56          | 2044.7 | Hex10HexNAc2 | 0.00           |                   |                 |                   |                 |                   |
| 2121 1A 0G | 14. 26    | 0.00          | 2077.7 | Hex5HexNAc4dHex1 | 14.26 | 0.00 | 14.2 6 |                   |                   |                   |
| 3111 1A 0G | 0.0 0     | 19.9 1        | 2118.8 | Hex4HexNAc5 | 0.00           | 19.9              | 0.00            |                   | 0.00            |                   |
### PLBL2-Reference

| Glycan Name | Rel. % T0 | Rel. % T6 mo. | Mass     | Composition                                                                 | Sialylation | Mannosylation | Fucosylation |
|-------------|-----------|---------------|----------|-----------------------------------------------------------------------------|-------------|---------------|--------------|
| Man3        | 1.5       | 0.00          | 910.3    | Hex3HexNAc2                                                                | 1.59        | 0.00          |              |
| Man4        | 3.4       | 2.12          | 1072.4   | Hex4HexNAc2                                                                | 3.45        | 2.12          |              |
| Man5        | 3.4       | 0.00          | 1234.8   | Hex5HexNAc2                                                                | 3.41        | 0.00          |              |
| 1100 0A 0G  | 0.0       | 1.62          | 1259.5   | Hex3HexNAc3dHex1                                                          | 0.00        | 0.00          | 0.00 1.62    |
| 1010 0A 0G  | 0.0       | 3.29          | 1275.5   | Hex4HexNAc3 nonspecific hexose is Man or Gal                               | 0.00        | 0.00          |              |
| 2000 0A 0G  | 1.4       | 1.15          | 1316.5   | Hex3HexNAc4 G0                                                             |              |               |              |
| Man6        | 3.9       | 2.51          | 1396.5   | Hex6HexNAc2                                                                | 3.91        | 2.51          |              |
| 2100 0A 0G  | 1.2       | 0.00          | 1462.5   | Hex3HexNAc4dHex1 G0F                                                       | 1.28        | 0.00          |              |
| 2010 0A 0G  | 2.1       | 0.00          | 1478.5   | Hex4HexNAc4 G1                                                             |              |               |              |
| Man7        | 1.8       | 0.00          | 1558.5   | Hex7HexNAc2                                                                | 1.82        | 0.00          |              |
| 1011 1A 0G  | 0.9       | 2.71          | 1566.5   | Hex4HexNAc3NeuAc1                                                          | 0.90        | 2.71          |              |
| 2110 0A 0G  | 2.2       | 2.32          | 1624.6   | Hex4HexNAc4dHex1 G1F                                                       | 2.21        | 2.32          |              |
| 2020 0A 0G  | 13.25     | 13.09         | 1640.6   | Hex5HexNAc4 G2                                                             |              |               |              |
| Man8        | 0.6       | 0.00          | 1720.6   | Hex8HexNAc2                                                                | 0.62        | 0.00          |              |
| 1111 0A 1G  | 2.0       | 0.00          | 1728.6   | Hex4HexNAc3dHex1NeuG c1                                                    | 2.06        | 0.00          | 2.06 0.00    |
| 2011 1A 0G  | 0.7       | 0.00          | 1769.6   | Hex4HexNAc4 NeuAc1                                                          | 0.78        | 0.00          |              |
| 2120 0A 0G  | 3.2       | 11.25         | 1786.7   | Hex5HexNAc4dHex1G2F                                                       | 3.24        | 11.25         |              |
| 2111 1A 0G  | 1.2       | 2.84          | 1915.7   | Hex4HexNAc4dHex1NeuAc1                                                     | 1.22        | 2.84          | 1.22 2.84    |
| Glycan Name | Rel. % | T0 | Rel. % | T6 mo. | Mass  | Composition | Sialylation | Mannosylation | Fucosylation |
|-------------|-------|----|-------|-------|-------|-------------|-------------|---------------|--------------|
|             |       |    |       |       |       |             | T0          | T6 mo.        | T0           | T6 mo.       |
| Man3        | 8.5   | 9  | 0.00  | 910.3 | Hex3HexNAc2 |             | 8.59        | 0.00          |              |              |
| 0100 0A 0G  | 0.0   | 0  | 0.31  | 1056.4| Hex3HexNAc2dHex1 |             | 0.00        | 0.00          | 0.31         |              |
| Man4        | 15.4  | 14 | 0.00  | 1072.4| Hex4HexNAc2 |             | 15.14       |               |              |              |
| 1000 0A 0G  | 0.34  | 1113.4 | 0.00  | Hex3HexNAc3 |             | 0.00        | 0.00        | 0.00          |              |
| Man5        | 16.26 | 1234.4 | 0.00  | Hex5HexNAc2 |             | 16.26       | 0.00        |               |              |
| 1100 0A 0G  | 4.51  | 1259.5 | 0.00  | Hex3HexNAc3dHex1 |             | 0.00        | 0.00        | 0.00          | 4.51         |
| 1010 0A 0G  | 1275.5 | 0.00 | Hex4HexNAc3 | nonspecific hexose is Man or Gal | 0.00        | 0.00        | 0.00          |              |
| 2000 0A 0G  | 3.3  | 0.00 | 1316.5 | Hex3HexNAc4 | G0           | 0.00        | 0.00        | 0.00          |              |
| Man6        | 2.2  | 0.00 | 1396.5 | Hex6HexNAc2 |             | 2.20        |             |               |              |
| 2100 0A 0G  | 0.00  | 1462.5 | 0.00  | Hex3HexNAc4dHex1 | G0F          | 0.90        | 0.00        |               |              |
| 2010 0A 0G  | 1.5  | 0.00 | 1478.5 | Hex4HexNAc4 | G1           |             | 0.00        | 0.00          |              |
| 3000 0A 0G  | 0.25  | 1519.6 | 0.00  | Hex3HexNAc5 |             | 0.00        | 0.00        | 0.00          |              |
| 1011 1A 0G  | 3.10  | 0.00 | 1566.6 | Hex4HexNAc3NeuAc1 |             | 0.00        | 3.10        | 0.00          | 0.00         |
| 2110 0A 0G  | 3.6  | 0.00 | 1624.6 | Hex4HexNAc4dHex1 | G1F          | 3.68        |             |               |              |
| 2020 0A 0G  | 14.36 | 0.00 | 1640.6 | Hex5HexNAc4 | G2           |             | 0.00        | 0.00          |              |
|   | 3010 0A 0G | 0.0 0 | 0.66 | 1681.6 | Hex4HexNAc5 | 0.00 | 0.00 | 0.00 |
|---|------------|------|------|--------|--------------|------|------|------|
| Man8 | 0.8 3 | 0.00 | 1720.6 | Hex8HexNAc2 | 0.00 | 0.00 | 0.00 |
| 1111 0A 1G | 1.1 5 | 0.00 | 1728.6 | Hex4HexNAc3dHex1NeuGc1 | 1.15 | 0.00 | 1.15 0.00 |
| 2120 0A 0G | 3.8 0 | 0.00 | 1786.7 | Hex5HexNAc4dHex1G2F | 3.80 | 0.00 | 0.00 |
| 3110 0A 0G | 0.0 0 | 0.85 | 1827.7 | Hex4HexNAc5dHex1 | 0.00 | 0.00 | 0.00 0.85 |
| 2111 1A 0G | 1.3 5 | 0.00 | 1915.7 | Hex4HexNAc4dHex1NeuAc1 | 1.35 | 1.35 |
| 2021 1A 0G / 2111 0A 1G | 16.31 0.00 | 1931.7 | Hex5HexNAc4NeuAc1 / Hex4HexNAc4dHex1NeuGc1 | 16.31 | 0.00 | 0.00 0.28 |
| 2130 0A 0G | 0.0 0 | 0.28 | 1948.7 | Hex6HexNAc4dHex1 | 0.00 | 0.00 | 0.00 0.28 |
| 2121 1A 0G | 7.1 8 | 0.00 | 2077.7 | Hex5HexNAc4dHex1NeuAc1 | 7.18 | 0.00 | 7.18 0.00 |
| 3111 1A 0G | 0.0 0 | 0.73 | 2118.8 | Hex4HexNAc5dHex1NeuAc1 | 0.00 | 0.73 | 0.00 0.00 0.73 |
| 3130 0A 0G | 0.0 0 | 2.71 | 2151.8 | Hex6HexNAc5dHex1 | 0.00 | 0.00 | 0.00 2.71 |
| 2022 2A 0G | 1.3 9 | 0.00 | 2222.8 | Hex5HexNAc4NeuAc2 | 1.39 |
| 2122 2A 0G | 1.0 0 | 0.00 | 2367.9 | Hex5HexNAc4dHex1NeuAc2 | 1.00 | 0.00 | 1.00 0.00 |
| 3131 1A 0G | 0.0 0 | 10.7 6 | 2442.9 | Hex6HexNAc5dHex1NeuAc1 | 0.00 | 10.7 6 | 0.00 0.00 10.76 |
Table S3. Screening of IgG4-B alanine mutants against PLBL2 by SPR

| Mutation | $k_a$ | $k_d$ | $K_D$ | Fold decrease in $K_D$ |
|----------|-------|-------|-------|-----------------------|
| WT       | 4.73E+03 | 0.01671 | 3.53E-06 |                        |
| F154A    | 5.47E+02 | 0.01908 | 3.49E-05 | 10                    |
| P155A    | 4.60E+02 | 0.04123 | 8.96E-05 | 25                    |
| E156A    | 6.12E+04 | 0.09252 | 1.51E-06 | 0                     |
| P157A    | 4.03E+02 | 0.007351 | 3.49E-05 | 5                     |
| V158A    | 4.36E+02 | 0.007987 | 1.83E-05 | 5                     |
| T159A    | 1.67E+02 | 0.008762 | 5.24E-05 | 15                    |
| G170A    | 5.69E+02 | 0.009909 | 1.74E-05 | 5                     |
| V171A    | 2.04E+02 | 0.01245 | 6.12E-05 | 17                    |
| T173A    | 1.83E+02 | 0.009921 | 5.42E-05 | 15                    |
| F174A    | 1.53E+02 | 0.0378 | 2.47E-04 | 70                    |
| P175A    | 9.78E+01 | 0.01397 | 1.43E-04 | 40                    |
| V177A    | 2.45E+02 | 0.01264 | 5.17E-05 | 15                    |
| L178A    | 2.64E+02 | 0.01197 | 4.53E-05 | 13                    |
| Q179A    | 1.02E+02 | 0.02355 | 2.32E-04 | 66                    |
| S180A    | 2.62E+02 | 0.01583 | 6.04E-05 | 17                    |
| S181A    | 1.99E+02 | 0.01292 | 6.51E-05 | 18                    |
| G182A    | 1.47E+02 | 0.0143 | 9.71E-05 | 27                    |
| Y184A    | 6.60E+02 | 0.008499 | 1.29E-05 | 4                     |
| L186A    | 6.37E+02 | 0.01634 | 2.57E-05 | 7                     |
| S187A    | 1.09E+03 | 0.01262 | 1.16E-05 | 3                     |
| V189A    | 1.46E+02 | 0.01302 | 8.95E-05 | 25                    |
| V190A    | 1.71E+02 | 0.007244 | 4.25E-05 | 12                    |
| T191A    | 1.23E+02 | 0.00831 | 6.78E-05 | 19                    |
| V192A    | 1.48E+02 | 0.01682 | 1.14E-04 | 32                    |
| P193A    | 1.27E+02 | 0.007293 | 5.73E-05 | 16                    |
| S194A    | 3.59E+02 | 0.006978 | 1.94E-05 | 5                     |
| S195A    | 1.56E+02 | 0.006177 | 3.95E-05 | 11                    |
| S196A    | 1.58E+02 | 0.008049 | 5.11E-05 | 14                    |
| L197A    | 1.05E+03 | 0.01176 | 1.12E-05 | 3                     |
| L198A    | 1.88E+02 | 0.02024 | 1.08E-04 | 30                    |
| T199A    | 8.24E+02 | 0.007519 | 9.13E-06 | 3                     |
| K200A    | 1.12E+02 | 0.0129 | 1.16E-04 | 33                    |
Table S4. MS Parameters optimized for the detection of free lipase, free antibody, lipase antibody complexes at 17,500 RP, and SIM experiments at 4375 RP (settings used for binding dissociation experiments).

|                      | Free Lipase | Free Antibody | Complex @ 17,500 RP | SIM complex @ 4375 RP |
|----------------------|-------------|---------------|---------------------|-----------------------|
| Injection Time       | 100         | 50            | 100                 | 1000                  |
| MS2 isolation window | N/A         | N/A           | N/A                 | 20                    |
| S-lens voltage       | 21          | 21            | 21                  | 21                    |
| Skimmer voltage      | 15          | 15            | 15                  | 15                    |
| In-Source Trapping   | -50         | -150          | -50                 | -10                   |
| Injection flatapole offset (V) | 5    | 5             | 5                   | 8                     |
| Bent flatapole DC    | 2           | 2             | 2                   | 2                     |
| Quadrupole transmission RF mode | High | High | High | High |
| Trans multipole DC   | 0           | 0             | 0                   | 0                     |
| C-trap entrance lens | 2           | 2             | 2                   | 2                     |
| Orbitrap detector mode | Low          | Low            | Low                 | Low                   |
| Extended Trapping Voltage | 10            | 3             | 0                   | 3-300                 |
| Pressure             | 4           | 1             | 6                   | 1                     |
Table S5. VC50 values and confidence intervals (CI) for binding dissociation native MS experiments of LPLA2 and PLBL2 against IgG4-B mutants.

| Mutant | LPLA2 VC50 | Lower CI, VC50 | Lower CI, VC50 | PLBL2 VC50 | Lower CI, VC50 | Lower CI, VC50 |
|--------|------------|----------------|----------------|------------|----------------|----------------|
| 174    | 39.28      | 37.23          | 41.44          | 58.18      | 44.71          | 75.71          |
| 175    | 37.4       | 36.04          | 38.82          | 25.49      | 16.72          | 38.87          |
| 179    | 44.05      | 41.91          | 46.3           | 47.29      | 32.28          | 69.3           |
| 192    | 42.21      | 40.59          | 43.88          | 46.63      | 33.83          | 64.27          |
| 198    | Too low to quantify | 51.18          | 38.23          | 68.52      |                |                |
| 200    | 37.65      | 36.01          | 39.36          | 94.52      | 49.56          | 180.28         |
| WT     | 45.18      | 43.35          | 47.09          | 111.43     | 68.76          | 171.98         |