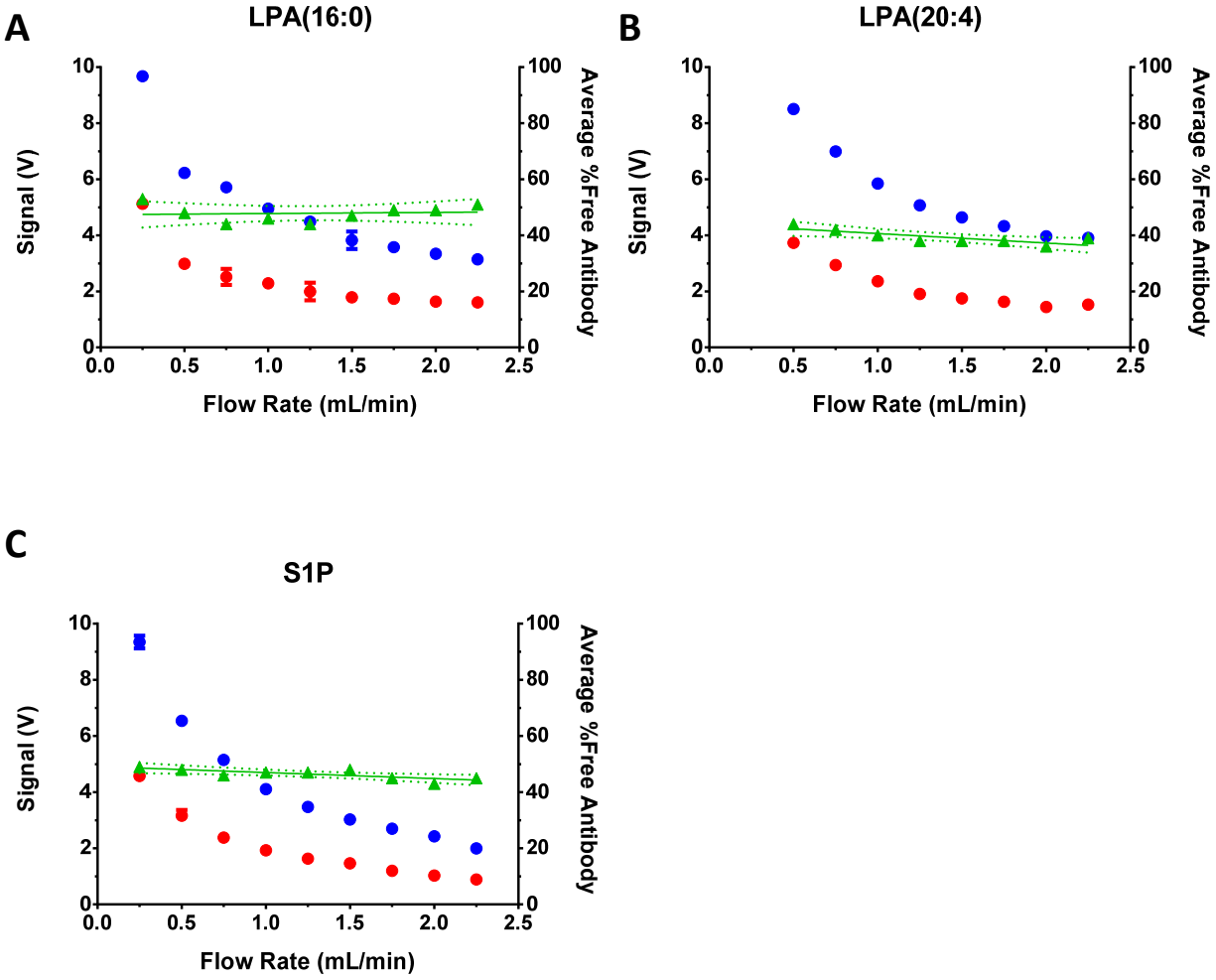
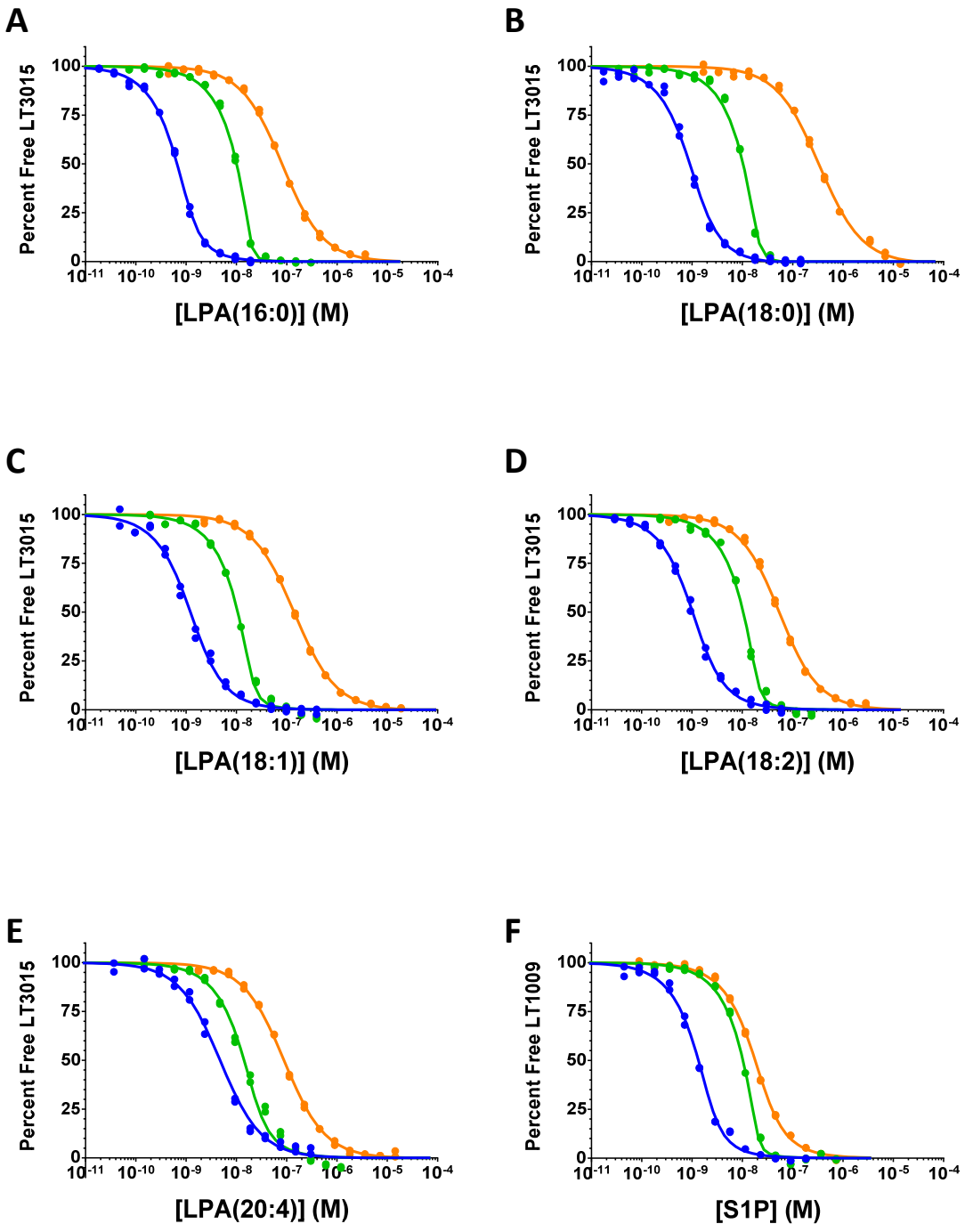
Supplementary Data:



Supplemental Figure S1. Kinetic exclusion determination.

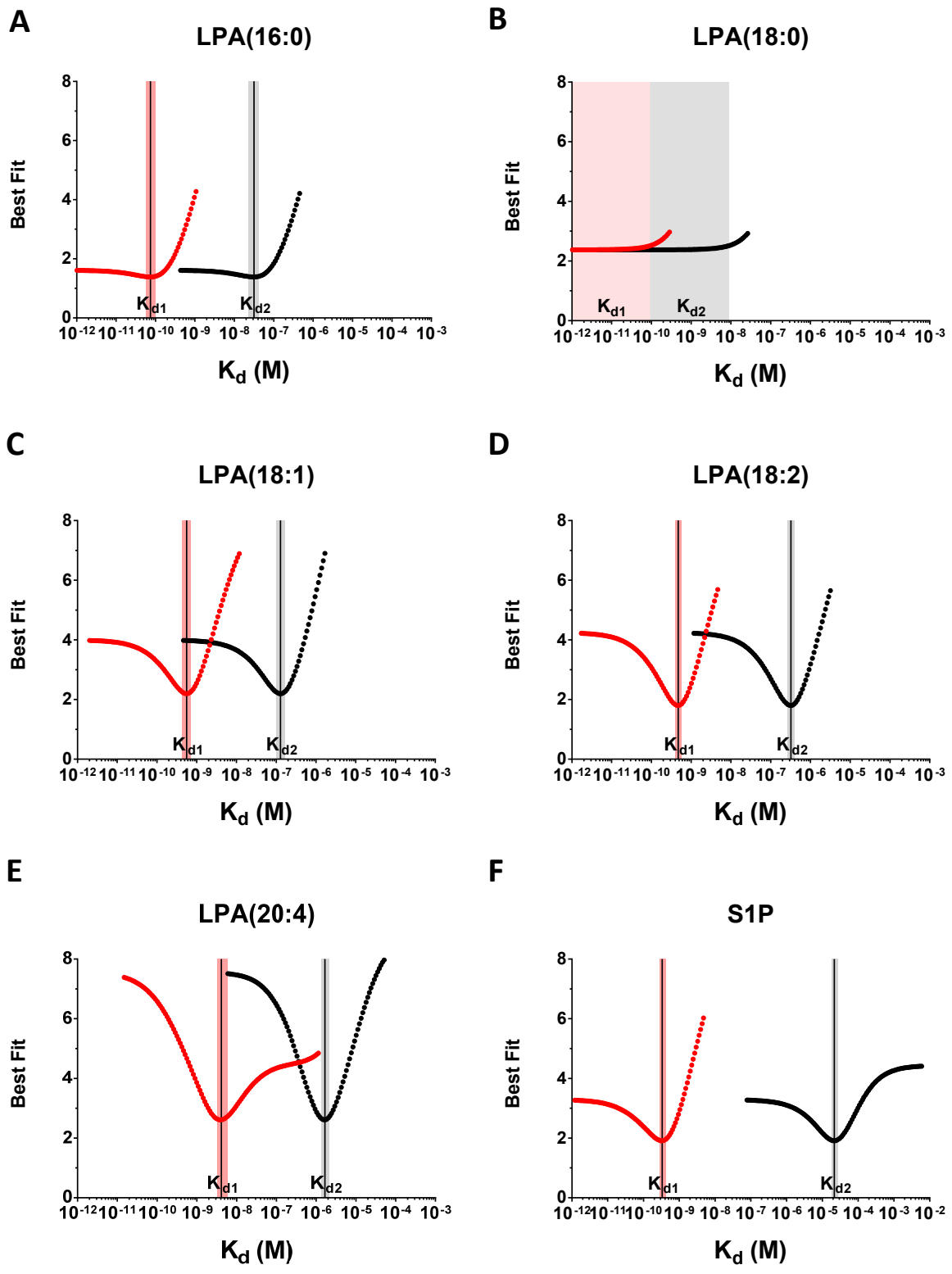
Signal (voltage) of samples consisting of antibody in the absence (blue) and presence (red) of lysophospholipid are shown as circles (mean of duplicates) with standard deviation. The green triangles represent the average percent free antibody and the dotted line the 95% confidence band for the best-fit line. A. 10 nM LT3015, 13 nM BSA, and 13 nM LPA(16:0). B. 10 nM LT3015, 13 nM BSA, and 65 nM LPA(20:4). C. 10 nM LT1009, 1 μM BSA, and 14 nM S1P.



Supplemental Figure S2. Competition affinity binding experiments with FAF-HSA.

Global curve fitting of the three affinity experiments used to determine the equilibrium dissociation constants for the individual LPA species (panels A-E) or S1P (panel F) binding the antibody or FAF-HSA in solution. The percentage of antigen-free binding sites on the antibody (in duplicate) is plotted as a

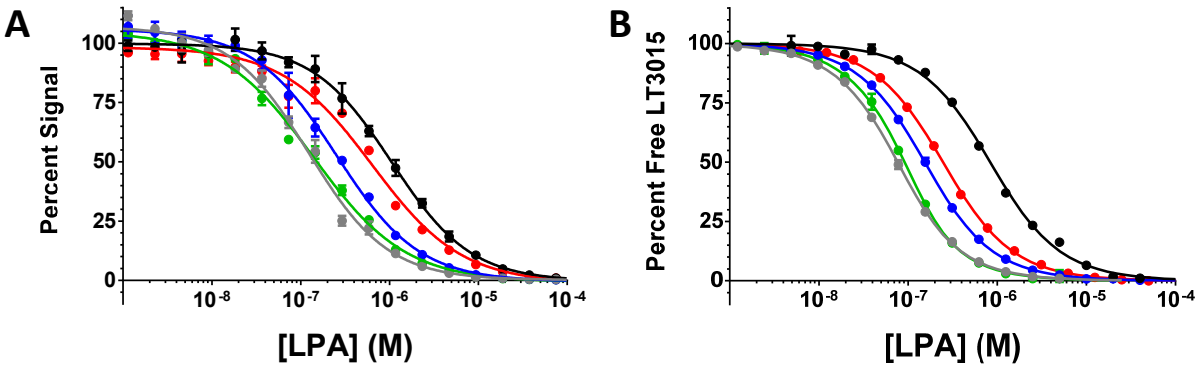
function of the lysophospholipid concentration in each sample. The blue and green curves represent the same experiment described in Figure 2. The LPA (A-E) and S1P (F) experiments shown as orange curves use 10 nM LT3015 and 10 μM FAF-HSA or 10 nM LT1009 and 500 μM FAF-HSA respectively.



Supplemental Figure S3. Error curves for FAF-HSA experiments.

Competition n-curve error curves for the experiments in Supplemental Figure S2. The K_d values for LPA (A-E) and S1P (F) binding either the antibody (K_{d1}, red dotted curve) or FAF-HSA (K_{d2}, black dotted curve) correspond to the position the solid black vertical line intersects the x-axis, and the shaded red and

black bars span the 95% confidence interval for the K_{d1} and K_{d2} value, respectively, except for LPA(18:0) (B) where the lower bound does not resolve. All values are reported in Table 2.



Supplemental Figure S4. Comparison of ELISA and KinExA methods for evaluating LT3015 binding individual LPA species.

A. Overlay of competition ELISA isotherms for LT3015 binding each of the five major LPA species: LPA(16:0), grey; LPA(18:0), black; LPA(18:1), blue; LPA(18:2), green; and LPA(20:4), red in the presence of 10 µM FAF-BSA. B. Overlay of individual KinExA experiments shown in Figure 2 (orange curves) measured in the presence of 10 µM FAF-BSA and LT3015 (procedure described in Materials and Methods) using the same LPA species in (A). The mean and error bars are shown for both graphs. ELISA Method: 96-well polystyrene plates (Greiner) were coated with 100 µL/well of 1.0 µg/mL goat anti-human IgG, Fcγ specific antibody (Jackson ImmunoResearch) in 0.1 M carbonate buffer pH 9.5. Plates were sealed with thermal adhesive and incubated at 4 °C overnight. Following a PBS wash, plates were blocked with 150 µL/well of PBS + 10 µM FAF-BSA for 1 hour at room temperature then washed. 100 µL/well of 680 pM LT3015 in PBS + 10 µM FAF-BSA was added to the plate and allowed to incubate for 1 hour at room temperature. After washing, a two-fold dilution series starting at 300 µM competitor (LPA(16:0), LPA(18:0), LPA(18:1), LPA(18:2), or LPA(20:4)) in PBS + 10 µM FAF-BSA was prepared. All competitors were diluted with tracer (LPA(18:0)-S-PEG2-Biotin), which was prepared by reacting thiolated LPA(18:0) in DMSO with 20-fold excess maleimide-PEG2-Biotin in PBS, in PBS + 10 µM FAF-BSA to 10 nM tracer. 100 µL/well of diluted competitor solution was applied to the plate and allowed to incubate for 17 hours at room temperature. The plates were washed and 100 µL/well of 1:60,000 dilution of peroxidase-conjugated streptavidin (Jackson ImmunoResearch) in PBS + 10 µM

FAF-BSA and allowed to incubate on the plates for 15 minutes. The plates were washed and developed by allowing 100 µL/well cold TMB (Invitrogen) to incubate on the plates for approximately 5 minutes. The reaction was quenched by addition of 100 µL/well 1.0 M H2SO4 then read at 450 nm on a plate reader (Perkin Elmer). Data were normalized and analyzed using a non-linear, 4-paramater fit in GraphPad Prism software.

Supplemental Table 1. K_{d1} and K_{d2} values calculated using 1, 2 or 3 equivalent LPA binding sites on serum albumin.

Lysophosphatidic Acid		1 Binding Site		2 Binding Sites		3 Binding Sites	
		K_{d1}	K_{d2}	K_{d1}	K_{d2}	K_{d1}	K_{d2}
LPA(16:0)	BSA	110 pM	24 nM	110 pM	47 nM	100 pM	68 nM
	HSA	71 pM	10 nM	71 pM	20 nM	74 pM	31 nM
LPA(18:1)	BSA	560 pM	43 nM	560 pM	86 nM	560 pM	130 nM
	HSA	560 pM	44 nM	560 pM	86 nM	560 pM	130 nM
LPA(18:2)	BSA	470 pM	120 nM	470 pM	230 nM	470 pM	350 nM
	HSA	470 pM	110 nM	470 pM	210 nM	470 pM	320 nM
LPA(20:4)	BSA	4.1 nM	720 nM	4.1 nM	1.4 μM	4.2 nM	2.2 μM
	HSA	4.0 nM	540 nM	4.0 nM	1.1 μM	4.1 nM	1.7 μM

Supplemental Table 2. Percent total S1P bound by apoM-HDL, apoM-LDL, HSA, or free at various reported physiological concentrations of components.[a]

apoM-HDL (nM)	apoM-LDL (nM)	HSA (μM)	Total S1P (nM)	apoM-HDL-S1P (%Total S1P)	apoM-LDL-S1P (%Total S1P)	HSA-S1P (%Total S1P)	Free (%Total S1P)
600	10	530	100	49	4.4	45	1.9
600	10	530	400	44	2.0	52	2.2
600	10	530	1200	31	0.8	66	2.7
600	10	670	100	44	4.1	50	1.7
600	10	670	400	39	1.9	57	1.9
600	10	670	1200	28	0.8	69	2.3
600	10	760	100	41	3.9	53	1.5
600	10	760	400	37	1.9	59	1.7
600	10	760	1200	27	0.8	70	2.0
600	25	530	100	46	10.5	42	1.7
600	25	530	400	43	4.9	50	2.1
600	25	530	1200	30	1.9	65	2.7
600	25	670	100	41	9.8	47	1.6
600	25	670	400	38	4.7	55	1.8
600	25	670	1200	28	1.9	68	2.2
600	25	760	100	39	9.4	50	1.5
600	25	760	400	36	4.6	58	1.7
600	25	760	1200	27	1.9	69	2.0
600	40	530	100	43	16.1	39	1.6

600	40	530	400	42	7.7	49	2.0
600	40	530	1200	30	3.1	64	2.7
600	40	670	100	39	15.1	44	1.5
600	40	670	400	38	7.4	53	1.8
600	40	670	1200	28	3.1	67	2.2
600	40	760	100	37	14.5	47	1.4
600	40	760	400	35	7.3	56	1.6
600	40	760	1200	27	3.0	68	2.0
900	10	530	100	59	3.8	36	1.5
900	10	530	400	55	1.9	41	1.7
900	10	530	1200	42	0.8	55	2.3
900	10	670	100	54	3.6	41	1.3
900	10	670	400	50	1.8	46	1.5
900	10	670	1200	39	0.8	58	1.9
900	10	760	100	51	3.5	44	1.3
900	10	760	400	48	1.8	49	1.4
900	10	760	1200	37	0.7	60	1.7
900	25	530	100	56	9.2	34	1.4
900	25	530	400	54	4.6	40	1.7
900	25	530	1200	42	1.9	54	2.2
900	25	670	100	51	8.7	39	1.3
900	25	670	400	49	4.4	45	1.5
900	25	670	1200	39	1.9	57	1.9
900	25	760	100	49	8.4	42	1.2
900	25	760	400	47	4.4	48	1.4

900	25	760	1200	37	1.9	59	1.7
900	40	530	100	53	14.1	32	1.3
900	40	530	400	53	7.3	39	1.6
900	40	530	1200	42	3.1	53	2.2
900	40	670	100	49	13.4	37	1.2
900	40	670	400	48	7.0	43	1.4
900	40	670	1200	39	3.0	57	1.9
900	40	760	100	46	12.9	39	1.1
900	40	760	400	46	6.9	46	1.3
900	40	760	1200	37	3.0	58	1.7
1300	10	530	100	68	3.2	28	1.2
1300	10	530	400	65	1.7	32	1.3
1300	10	530	1200	55	0.7	43	1.8
1300	10	670	100	63	3.1	33	1.1
1300	10	670	400	61	1.7	37	1.2
1300	10	670	1200	51	0.7	47	1.5
1300	10	760	100	61	3.0	35	1.0
1300	10	760	400	58	1.6	39	1.1
1300	10	760	1200	49	0.7	49	1.4
1300	25	530	100	65	7.8	26	1.1
1300	25	530	400	64	4.3	31	1.3
1300	25	530	1200	54	1.9	42	1.8
1300	25	670	100	60	7.5	31	1.0
1300	25	670	400	59	4.1	36	1.2
1300	25	670	1200	50	1.8	46	1.5

1300	25	760	100	58	7.2	34	1.0
1300	25	760	400	57	4.1	38	1.1
1300	25	760	1200	48	1.8	49	1.4
1300	40	530	100	62	12.1	25	1.0
1300	40	530	400	62	6.7	30	1.2
1300	40	530	1200	54	3.0	42	1.7
1300	40	670	100	58	11.6	30	1.0
1300	40	670	400	58	6.5	35	1.1
1300	40	670	1200	50	2.9	46	1.5
1300	40	760	100	55	11.2	32	0.9
1300	40	760	400	55	6.4	37	1.1
1300	40	760	1200	48	2.9	48	1.4

[a] K_{d2} values used for deriving percentages were as reported in this paper (apoM-HDL: 21 nM, apoM-LDL: 2.4 nM, HSA: 22 μM).

Supplemental Table 3. Percent total protein bound to S1P at various reported physiological concentrations of components.[a]

apoM-HDL (nM)	apoM-LDL (nM)	HSA (μM)	Total S1P (nM)	apoM-HDL-S1P (%Total apoM-HDL)	apoM-LDL-S1P (%Total apoM-LDL)	HSA-S1P (%Total HSA)
600	10	530	100	8.2	44	0.008
600	10	530	400	29	80	0.039
600	10	530	1200	62	96	0.149
600	10	670	100	7.3	41	0.007
600	10	670	400	26	76	0.034
600	10	670	1200	56	96	0.124
600	10	760	100	6.8	39	0.007
600	10	760	400	25	76	0.031
600	10	760	1200	54	96	0.111
600	25	530	100	7.7	42	0.008
600	25	530	400	29	78	0.038
600	25	530	1200	60	91	0.147
600	25	670	100	6.8	39	0.007
600	25	670	400	25	75	0.033
600	25	670	1200	56	91	0.122
600	25	760	100	6.5	38	0.007
600	25	760	400	24	74	0.031
600	25	760	1200	54	91	0.109
600	40	530	100	7.2	40	0.007

600	40	530	400	28	77	0.037
600	40	530	1200	60	93	0.145
600	40	670	100	6.5	38	0.007
600	40	670	400	25	74	0.032
600	40	670	1200	56	93	0.120
600	40	760	100	6.2	36	0.006
600	40	760	400	23	73	0.029
600	40	760	1200	54	90	0.107
900	10	530	100	6.6	38	0.007
900	10	530	400	24	76	0.031
900	10	530	1200	56	96	0.125
900	10	670	100	6.0	36	0.006
900	10	670	400	22	72	0.027
900	10	670	1200	52	96	0.104
900	10	760	100	5.7	35	0.006
900	10	760	400	21	72	0.026
900	10	760	1200	49	84	0.095
900	25	530	100	6.2	37	0.006
900	25	530	400	24	74	0.030
900	25	530	1200	56	91	0.122
900	25	670	100	5.7	35	0.006
900	25	670	400	22	70	0.027
900	25	670	1200	52	91	0.102
900	25	760	100	5.4	34	0.006
900	25	760	400	21	70	0.025

900	25	760	1200	49	91	0.093
900	40	530	100	5.9	35	0.006
900	40	530	400	24	73	0.029
900	40	530	1200	56	93	0.120
900	40	670	100	5.4	34	0.006
900	40	670	400	21	70	0.026
900	40	670	1200	52	90	0.102
900	40	760	100	5.1	32	0.005
900	40	760	400	20	69	0.024
900	40	760	1200	49	90	0.092
1300	10	530	100	5.2	32	0.005
1300	10	530	400	20	68	0.024
1300	10	530	1200	51	84	0.097
1300	10	670	100	4.9	31	0.005
1300	10	670	400	19	68	0.022
1300	10	670	1200	47	84	0.084
1300	10	760	100	4.7	30	0.005
1300	10	760	400	18	64	0.021
1300	10	760	1200	45	84	0.077
1300	25	530	100	5.0	31	0.005
1300	25	530	400	20	69	0.023
1300	25	530	1200	50	91	0.095
1300	25	670	100	4.6	30	0.005
1300	25	670	400	18	66	0.021
1300	25	670	1200	46	86	0.082

1300	25	760	100	4.5	29	0.004
1300	25	760	400	18	66	0.020
1300	25	760	1200	44	86	0.077
1300	40	530	100	4.8	30	0.005
1300	40	530	400	19	67	0.023
1300	40	530	1200	50	90	0.095
1300	40	670	100	4.5	29	0.004
1300	40	670	400	18	65	0.021
1300	40	670	1200	46	87	0.082
1300	40	760	100	4.2	28	0.004
1300	40	760	400	17	64	0.019
1300	40	760	1200	44	87	0.076

[a] K_{d2} values used for deriving percentages were as reported in this paper (apoM-HDL: 21 nM, apoM-LDL: 2.4 nM, HSA: 22 μM).