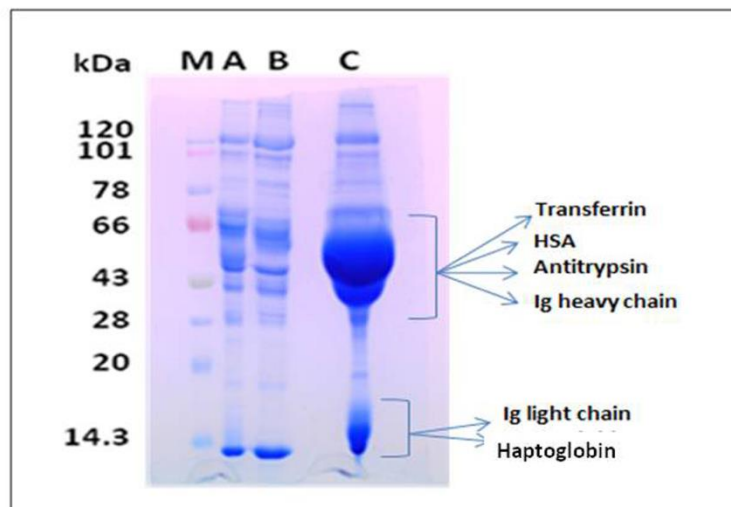1 **Human plasma proteomics study in a pilot scale identifies Apo E and IL-33 as**
2 **markers in atopic asthma**
3
4 # Supplementary Material

5 **Supplementary Figure 1: Snapshot of 1-Dimensional SDS-PAGE of depleted and**
6 **undepleted plasma samples.** Lanes: M denotes prestained protein marker; A and B
7 denotes plasma samples after removal of the high abundant proteins; C denotes
8 undepleted plasma sample.
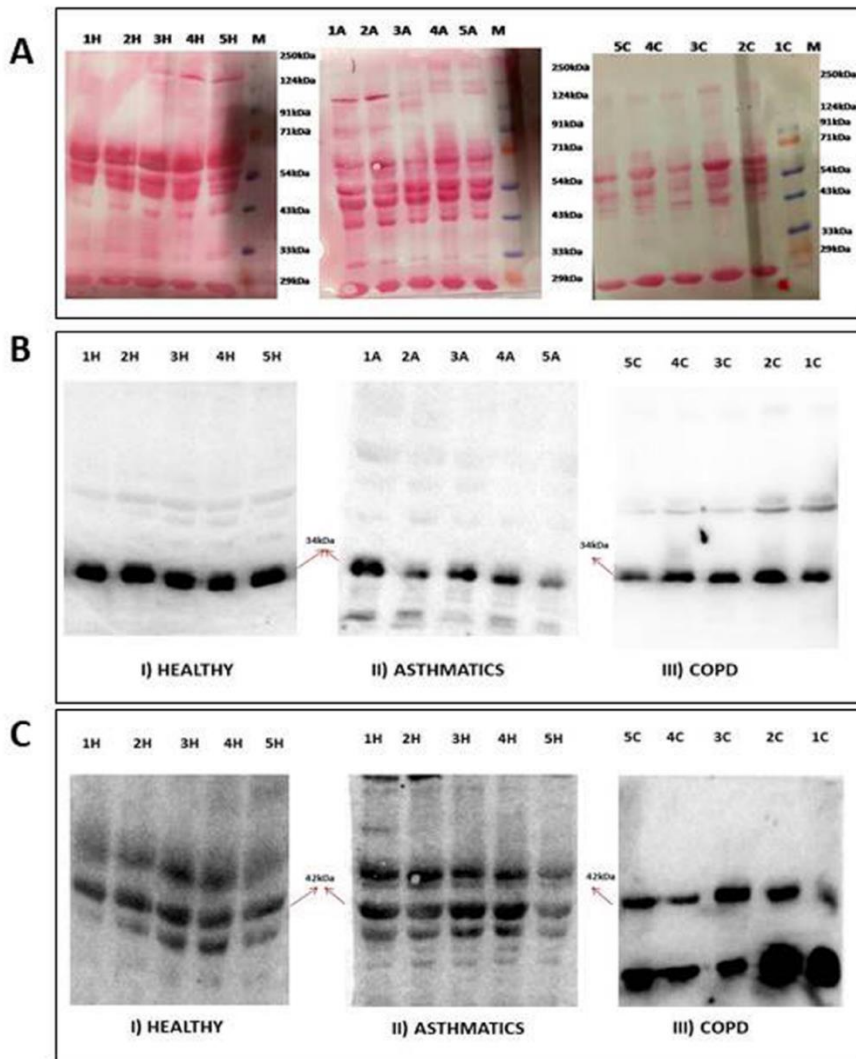

Supplementary Figure 1

9

10

Supplementary Figure 2: Snapshots of the blots for Apo E and beta actin from healthy subjects, atopic asthmatics and COPD Patients. (A) Image of blots stained with Ponceau S. The image shows all the 3 blots for healthy subjects (1H, 2H, 3H, 4H, 5H), atopic asthmatics (1A, 2A, 3A, 4A 5A),and COPD Patients (1C, 2C, 3C, 4C, 5C). M denotes the protein molecular weight ladder with labelled molecular weight. **(B) Blot Image for Apo E in healthy subjects, atopic asthmatics and COPD Patients.** 1H, 2H, 3H, 4H and 5H denotes samples from healthy subjects; 1A, 2A, 3A, 4A and 5A denotes samples from atopic asthmatics; and 1C, 2C, 3C, 4C and 5C denotes samples from COPD patients. The band for Apo E has been labelled at a size corresponding to 34 kDa. **(C) Blot Image for beta actin in healthy subjects, atopic asthmatics and COPD Patients.** 1H, 2H, 3H, 4H and 5H denotes samples from healthy subjects; 1A, 2A, 3A, 4A and 5A denotes samples from atopic asthmatics; and 1C, 2C, 3C, 4C and 5C denotes samples from COPD patients. The same blot used for Apo E was used to develop beta actin. The band for beta actin has been labelled at a size corresponding to 42 kDa.

Supplementary Figure 2

26 **Supplementary Table 1: Detailed information for healthy subjects, atopic**
27 **asthmatics and COPD patients.** The table enlists detailed information of Healthy
28 Volunteers, Atopic Asthma patients and COPD patients including age, sex, smoking
29 habits, IgE Count, % FEV1 and other health complications.
30
31 **Supplementary Table 1**

Category	Sample ID	Age	Sex	Total IgE (IU/mL)	*Aspergillus fumigatus* Positive	*Dermatophagoides* spp. Positive	% Pred FEV1	Smoking Habit	Other Health Complications
Healthy	1H	27	F	56	ND	ND	98	NO	NO
	2H	30	M	70	ND	ND	89	NO	NO
	3H	42	M	64	ND	ND	93	NO	NO
	4H	44	M	210	ND	ND	103	NO	NO
	5H	26	F	70.5	ND	ND	96	NO	NO
Atopic Asthma	1A	76	M	435.7	YES	YES	52	NO	NO
	2A	34	M	386.7	YES	YES	57	NO	NO
	3A	73	F	1993.8	YES	YES	69	NO	NO
	4A	63	F	392	YES	YES	54	NO	NO
	5A	66	F	1785	YES	YES	67	NO	NO
Chronic Obstructive Pulmonary Disorder	1COPD	55	M	ND	ND	ND	40	NO	NO
	2COPD	61	M	ND	ND	ND	37	NO	NO
	3COPD	67	M	ND	ND	ND	21	Presently NO, Previously YES	NO
	4COPD	70	M	ND	ND	ND	30	Presently NO, Previously YES	NO
	5COPD	58	M	ND	ND	ND	45	NO	NO

32

33

34 **Supplementary Table 2: List of the different plasma proteins identified by LC-**
35 **MS/MS.** The attached MS Excel file enlists the total unique peptides identified and total
36 spectral count in healthy Controls (n=5) and atopic asthma patients (n=5) for the 167
37 different plasma proteins identified by mass spectrometry. The first sheet provides an
38 overview of the average of total unique peptide and average of spectral count for the
39 five healthy and five atopic asthma patient samples. The subsequent sheets (Nos. 2 to 5)
40 provide the total unique peptide and the total spectral count for each run of LC-MS/MS.

41 **Supplementary Table 2 is available from:**
42 https://www.dovepress.com/get_supplementary_file.php?f=211569.xlsx
43

44 **Supplementary Table 3: Details of the 14 Apolipoproteins identified by mass**
45 **spectrometry. (A)** The table enlists the UniProt IDs, gi number, total unique peptides
46 identified and total spectral count in healthy Controls (n=5) and atopic asthma patients
47 (n=5) for the 14 different Apolipoproteins identified by LC-MS/MS. **(B)** The table enlists
48 the Spectral Count for the 14 different Apolipoproteins identified in healthy subjects
49 (n=5) and atopic asthma patients (n=5). **(C)** The table enlists the computed Normalized
50 Spectral Abundance Factor (NSAF) values for the 14 different Apolipoproteins identified
51 in healthy subjects (n=5) and atopic asthma patients (n=5). * denotes that the p-value of
52 Apo E was computed for healthy subjects (n=4) and atopic asthma patients (n=4) after
53 excluding the NSAF obtained for sample 3H and 5A which appears to be outliers after
54 normalization. Apo A1 and Apo B showed high abundance in terms of spectral count, but
55 the NSAF value for Apo B was low owing to the large size of protein. NSAF for Apo A1
56 was higher as the size of the protein was smaller compared to Apo B.
57 **Supplementary Table 3A**

Protein Name	UniProt ID	Gene ID	Total Unique Peptide Mean	Total Unique Peptide Mean	Total Spectral Count Mean	Total Spectral Count Mean
			Healthy Subjects (n=5)	Atopic Asthmatics (n=5)	Healthy Subjects (n=5)	Atopic Asthmatics (n=5)
Apolipoprotein A-I	P02647	335	43	41	275	282
Apolipoprotein B	P04114	338	125	125	169	159
Apolipoprotein A-IV	P06727	337	31	24	65	39
Apolipoprotein E	P02649	348	17	15	34	26
Apolipoprotein D	P05090	347	6	5	12	7
Apolipoprotein A-II	P02652	336	10	12	32	41
Apolipoprotein C-I	P02654	341	5	4	6	8
Apolipoprotein C-II	P02655	344	5	5	6	7
Apolipoprotein C-III	P02656	345	2	3	3	5
Apolipoprotein C-IV	P55056	346	3	4	5	6
Apolipoprotein L1	O14791	8542	4	4	6	5
Apolipoprotein M	O95445	55937	2	2	4	3
Apolipoprotein F	Q13790	319	1	2	1	2
Apolipoprotein a	P08519	4018	2	2	2	3

58

59 **Supplementary Table 3B**

Protein	Spectral Count for Healthy Subjects					Spectral Count for Atopic Asthmatics				
	1H	2H	3H	4H	5H	1A	2A	3A	4A	5A
Apo A1	206	280	481	249	159	131	378	503	256	140
Apo B	126	274	262	103	80	205	203	193	93	10
Apo E	35	58	31	28	20	23	30	32	21	26
Apo A4	68	70	105	54	27	25	53	65	36	15
Apo D	10	14	7	14	13	5	15	2	6	7
Apo A2	0	41	68	9	8	0	64	86	5	8
Apo C1	0	8	12	2	2	0	10	19	1	1
Apo C2	0	4	7	0	0	0	7	7	0	0
Apo C3	0	7	1	0	1	0	9	1	0	0
Apo C4	0	5	0	0	0	0	6	0	0	0
Apo L1	5	12	9	2	2	3	16	2	3	1
Apo M	0	1	2	7	4	0	2	2	1	7
Apo F	0	2	0	1	1	0	3	1	0	0
Apo a	0	3	1	2	2	0	5	3	1	1

60

61

62 **Supplementary Table 3C**

Protein	NSAF Values for Healthy Subjects					NSAF Values for Atopic Asthma Patients					P-value
	1H	2H	3H	4H	5H	1A	2A	3A	4A	5A	
Apo A1	20.063	15.753	21.380	23.170	21.845	14.646	20.683	24.067	26.524	28.407	0.153
Apo B	0.718	0.902	0.681	0.561	0.643	1.341	0.650	0.540	0.564	0.119	0.388
Apo E	2.871	2.748	1.161	2.194	2.314	2.166	1.382	1.290	1.833	4.443	0.009*
Apo A4	4.465	2.655	3.147	3.388	2.501	1.884	1.955	2.097	2.515	2.052	0.019
Apo D	1.376	1.113	0.439	1.840	2.523	0.790	1.159	0.135	0.878	2.006	0.024
Apo A2	0	6.159	8.070	2.236	2.935	0	9.350	10.986	1.383	4.334	0.084
Apo C1	0	1.448	1.716	0.599	0.884	0	1.760	2.924	0.333	0.653	0.246
Apo C2	0	0.595	0.823	0	0	0	1.012	0.885	0	0	0.151
Apo C3	0	1.062	0.119	0	0.370	0	1.328	0.129	0	0	0.430
Apo C4	0	0.591	0	0	0	0	0.690	0	0	0	0.187
Apo L1	0.327	0.453	0.268	0.125	0.184	0.225	0.587	0.064	0.208	0.136	0.341
Apo M	0	0.079	0.126	0.925	0.781	0	0.155	0.136	0.147	2.017	0.376
Apo F	0	0.092	0	0.076	0.112	0	0.134	0.039	0	0	0.265
Apo a	0	0.009	0.002	0.011	0.016	0	0.016	0.008	0.006	0.012	0.409

63

64

65 **Supplementary Table 4: Computed NSAF values of Apolipoprotein E in Healthy**
66 **subjects and Atopic asthma patients.** The table enlists the Normal Spectral Abundance
67 Factor (NSAF) for Apolipoprotein E across the different healthy and atopic asthma
68 patient samples computed from the LC-MS/MS data and the average NSAF for healthy
69 controls and asthmatics. ⧧ and * denotes that the average NSAF of Apo E for healthy
70 subjects and atopic asthma patients respectively have been computed excluding the NSAF
71 obtained for sample 3H and 5A which appears to be outliers after normalization.

Healthy Controls	1H	2H	3H	4H	5H	Average
NSAF for Apo E	2.871153	2.748472	1.160608	2.194512	2.314409	2.257831⧧
Atopic Asthmatics	1A	2A	3A	4A	5A	Average
NSAF for Apo E	2.165921	1.382578	1.289601	1.832651	4.443451	1.667688*

72
73

74 **Supplementary Table 5: Details of densitometric analysis from Western Blot of**
75 **Apolipoprotein E.** The table enlists the densities of Apolipoprotein E (Apo E) bands
76 obtained from western blot across healthy controls, atopic asthma and COPD patient
77 samples and the standard errors across the samples. Apo E expression level has been
78 analyzed with respect to Beta Actin (internal control).1H, 2H, 3H, 4H, 5H denotes the
79 healthy samples; 1A, 2A, 3A, 4A and 5A denotes the atopic asthma patient samples and
80 1COPD, 2COPD, 3COPD, 4COPD, 5COPD denotes the COPD patient samples.

Category	Samples	Apo E expression level with respect to Beta Actin	Average Apo E expression level with respect to Beta Actin	Standard Error
Healthy	1H	1.712	1.626	0.176
	2H	1.369		
	3H	1.301		
	4H	1.473		
	5H	2.274		
Atopic Asthma	1A	0.847	0.801	0.081
	2A	0.949		
	3A	0.862		
	4A	0.865		
	5A	0.483		
COPD	1COPD	1.708	1.258	0.139
	2COPD	1.208		
	3COPD	1.354		
	4COPD	1.165		
	5COPD	0.854		

81
82

83 **Supplementary Table 6: List of pro-inflammatory cytokines analyzed by ELISA**
84 **and their outcomes.** Only IL-33 was within the detection limit while the other
85 interleukins tested namely, IL-4, IL-5 and IL-13 were not within the detection limit.

Cytokines Studied	Outcome
IL-4	Not Detectable
IL-5	Not Detectable
IL-13	Not Detectable
IL-33	Detected (in pg/ml)

86

87 **Supplementary Table 7: (A) OD values at 450 nm and corresponding concentration**
88 **of IL-33 (pg/ml) in healthy volunteers and atopic asthmatics.** IL-33 level was
89 measured thrice using Quantikine ELISA kit for Human IL-33 (R&D systems; Lot No.:
90 P118763). The plasma concentration of IL-33 was computed from the OD values at 450
91 nm using the standard curve equation: $y = 0.004x$. 1H, 2H, 3H, 4H, 5H denotes the healthy
92 samples; 1A, 2A, 3A, 4A and 5A denotes the atopic asthma patient samples.
93 **(B) OD values at 450 nm and corresponding concentration of IL-33 (pg/ml) in COPD**
94 **patients.** IL-33 level was measured thrice using Quantikine ELISA kit for Human IL-33
95 (R&D systems; Lot No.: P177042). The plasma concentration of IL-33 was computed
96 from the OD values at 450 nm using the standard curve equations $y = 0.0118x + 0.005$.
97 1COPD, 2COPD, 3COPD, 4COPD, 5COPD denotes the COPD patient samples. The
98 tables show the individual and mean IL-33 concentration (in pg/ml) and the standard error
99 computed across the healthy subjects and patients.
100
101 **Supplementary Table 7A**

Category	Samples	Average OD values at 450 nm	Concentration (pg/ml)	Average Concentration (pg/ml)	Standard Error
Healthy Subjects	1H	0.011	2.75	2.35 pg/ml	0.308
	2H	0.006	1.417		
	3H	0.008	1.917		
	4H	0.010	2.5		
	5H	0.013	3.167		
Atopic Asthmatics	1A	0.038	9.417	9.033 pg/ml	0.585
	2A	0.044	10.917		
	3A	0.031	7.667		
	4A	0.032	7.917		
	5A	0.037	9.25		

102
103 **Supplementary Table 7B**

Category	Samples	Average OD values at 450 nm	Concentration (pg/ml)	Average Concentration (pg/ml)	Standard Error
COPD Patients	1COPD	0.0683	5.757	5.92 pg/ml	0.089
	2COPD	0.0677	5.697		
	3COPD	0.0727	6.152		
	4COPD	0.072	6.091		
	5COPD	0.0703	5.939		

104