

**Dysregulation of Innate and Adaptive Serum Mediators Precedes Systemic Lupus Erythematosus Classification and Improves Prognostic Accuracy of Autoantibodies**

**by Lu, Munroe et al.**

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**Supplemental Table 1.** Study cohort demographics

	<b>Case (%) n=84</b>	<b>Control (%) n=86</b>	<b>p- value</b>
<b>Gender</b>			
Male	32 (38.1%)	33 (38.4%)	0.9 <sup>a</sup>
Female	52 (61.9%)	53 (61.6%)	-
<b>Ethnicity</b>			
European American	20 (23.8%)	20 (23.2%)	1.0 <sup>a</sup>
African American	50 (59.5%)	51 (59.3%)	-
Hispanic	10 (11.9%)	11 (12.8%)	-
Other	4 (4.8%)	4 (4.7%)	-
<b>Age at classification<sup>c</sup> (SD)</b>	30.4 (6.3)	30.5 (6.3)	0.9 <sup>b</sup>

<sup>a</sup>Chi-square test; <sup>b</sup>Unpaired *t*-test; <sup>c</sup>In cases; controls were matched by gender, race, age ( $\pm$  5 years), and time of sample procurement

**Supplemental Table 2.** Wilcoxon rank-sum test of soluble mediators in each time period. Mediators above the red line were significantly different between cases and controls.

<b>&gt;3.5 years before classification</b>				
<b>Soluble mediator, (Normalized FI)</b>	<b>Case (n = 61), Median (IQR)</b>	<b>Control (n = 56), Median (IQR)</b>	<b>p-value</b>	<b>q-value</b>
IL-5	1.38 (1.24 - 1.62)	0.86 (0.64 - 1.26)	1.36E-07	3.60E-06
IL-6	1.69 (1.4 - 2.15)	1.14 (0.88 - 1.44)	5.16E-07	6.83E-06
IL-4	1.6 (1.31 - 2)	1.25 (1 - 1.61)	1.17E-03	1.00E-02
MIG	0.33 (0.16 - 0.6)	0.54 (0.34 - 0.77)	2.41E-03	1.30E-02
IL12p70	1.5 (1.33 - 1.83)	1.24 (1 - 1.6)	2.15E-03	1.30E-02
IP-10	2.83 (1.89 - 4.67)	2.08 (1.45 - 3.2)	5.55E-03	2.30E-02
TGF-β	1.84 (1.47 - 2.54)	2.69 (1.58 - 4.93)	6.14E-03	2.30E-02
IFN-γ	2.56 (2.2 - 2.98)	2.2 (1.74 - 2.75)	1.05E-02	3.50E-02
IL-1α	0.85 (0.58 - 1.31)	1.12 (0.77 - 1.42)	2.60E-02	7.60E-02
IL-17A	1.87 (1.32 - 2.29)	1.51 (1.25 - 1.9)	4.06E-02	1.07E-01
IL-13	1.32 (1.02 - 1.86)	1.21 (0.91 - 1.63)	4.70E-02	1.13E-01
IL-1β	1.57 (1.33 - 2.07)	1.43 (1 - 1.97)	8.55E-02	1.89E-01
sFasL	5.21 (1.97 - 7.1)	3.88 (2.45 - 5.92)	2.45E-01	4.99E-01
IL-1RA	1.7 (1.2 - 2.84)	1.5 (1.02 - 2.52)	3.01E-01	5.66E-01
IL-23p19	1.65 (1.27 - 2.32)	1.52 (1.14 - 2.1)	3.21E-01	5.66E-01
TNFR II	1.01 (0.92 - 1.21)	1 (0.9 - 1.11)	3.53E-01	5.84E-01
SCF	0.51 (0.31 - 0.8)	0.54 (0.33 - 0.8)	4.55E-01	6.44E-01
IL-21	0.69 (0.51 - 1.08)	0.67 (0.43 - 1.01)	4.62E-01	6.44E-01
APRIL*	5524 (0 - 18877.33)	4869.57 (0 - 14735.83)	4.39E-01	6.44E-01
MCP-3	1.33 (1.06 - 1.75)	1.3 (1.12 - 1.57)	5.46E-01	7.23E-01
Resistin	1.24 (0.81 - 1.87)	1.32 (0.75 - 2.07)	6.41E-01	8.08E-01
PAI-1	1.26 (1.2 - 1.32)	1.26 (1.2 - 1.33)	7.08E-01	8.22E-01
sCD40L	0.04 (0.03 - 0.05)	0.04 (0.03 - 0.06)	7.14E-01	8.22E-01
IL-8	4.53 (2.93 - 19.41)	4.28 (2.97 - 15.24)	8.10E-01	8.76E-01
MIP-1α	2.41 (1.51 - 4.81)	2.13 (1.49 - 3.95)	9.16E-01	8.76E-01
TNFR I	1.05 (0.94 - 1.14)	1.03 (0.91 - 1.17)	9.16E-01	8.76E-01
IL-2	1.4 (1.14 - 2)	1.33 (1.17 - 1.81)	8.80E-01	8.76E-01
BLyS*	1049.55 (883.31 - 1319.46)	1042.96 (905.99 - 1241.23)	9.27E-01	8.76E-01

\*Units pg/ml

**Supplemental Table 2, cont.**

<b>3.5 to 0.9 years before classification</b>				
<b>Soluble mediator, (Normalized FI)</b>	<b>Case (n = 61), Median (IQR)</b>	<b>Control (n = 35), Median (IQR)</b>	<b>p-value</b>	<b>q-value</b>
IL-5	1.5 (1.25 - 1.88)	0.96 (0.74 - 1.09)	7.78E-09	1.43E-07
IL-6	2 (1.38 - 2.6)	1.12 (0.92 - 1.42)	2.68E-06	2.46E-05
IL-4	1.67 (1.17 - 2.1)	1.17 (1 - 1.44)	3.30E-04	1.50E-03
IL-12p70	1.5 (1.29 - 2.14)	1.22 (0.93 - 1.5)	2.56E-04	1.50E-03
IP-10	5.67 (2.67 - 10.33)	2.5 (1.74 - 4.31)	4.82E-04	1.80E-03
IL-17A	2.05 (1.68 - 2.5)	1.58 (1.18 - 2.01)	1.78E-03	5.40E-03
IL-13	1.25 (1.11 - 2.2)	1.11 (0.84 - 1.32)	3.76E-03	9.90E-03
IL-21	0.88 (0.55 - 1.85)	0.54 (0.47 - 0.88)	6.28E-03	1.44E-02
MIP-1 $\alpha$	4 (2.23 - 8.47)	2.2 (1.26 - 4.02)	9.97E-03	2.03E-02
TGF- $\beta$	1.9 (1.41 - 2.62)	2.51 (1.78 - 3.79)	2.60E-02	4.67E-02
MCP-3	1.44 (1.14 - 1.75)	1.22 (0.96 - 1.48)	2.80E-02	4.67E-02
IL-2	1.5 (1.2 - 2.5)	1.25 (1 - 1.5)	3.44E-02	5.26E-02
IL-1RA	1.82 (1.38 - 4.14)	1.61 (0.89 - 2.33)	4.41E-02	6.22E-02
IL-23	1.67 (1.23 - 3)	1.35 (1.06 - 1.98)	5.75E-02	7.54E-02
IL-1 $\beta$	1.83 (1.27 - 3)	1.58 (1.12 - 2.08)	8.33E-02	9.55E-02
IFN- $\gamma$	2.7 (2.11 - 3.38)	2.43 (1.72 - 3.03)	8.00E-02	9.55E-02
APRIL*	7289.3 (2180.54 - 18599.27)	4038.53 (0 - 10773.89)	9.00E-02	9.71E-02
TNFR I	0.99 (0.88 - 1.17)	1.06 (0.99 - 1.22)	1.28E-01	1.31E-01
Resistin	1.25 (0.84 - 1.79)	1.57 (0.9 - 2.14)	2.01E-01	2.94E-01
SCF	0.57 (0.39 - 0.96)	0.72 (0.51 - 0.99)	2.60E-01	2.39E-01
sFasL	6.08 (3.12 - 9.13)	5.15 (3.08 - 7.39)	3.34E-01	2.92E-01
MIG	0.42 (0.2 - 1.08)	0.51 (0.38 - 0.73)	4.65E-01	3.88E-01
sCD40L	0.04 (0.03 - 0.06)	0.03 (0.03 - 0.07)	5.15E-01	4.11E-01
IL-1 $\alpha$	1 (0.66 - 1.33)	1.08 (0.77 - 1.36)	6.64E-01	5.08E-01
IL-8	7.07 (3.05 - 55.23)	9.08 (2.92 - 31.57)	7.81E-01	5.73E-01
PAI-1	1.27 (1.23 - 1.32)	1.28 (1.2 - 1.31)	8.61E-01	6.08E-01
TNFR II	1.01 (0.87 - 1.19)	1.01 (0.91 - 1.12)	9.09E-01	6.17E-01
BLyS*	1095.87 (888.12 - 1448.08)	1127.84 (897.78 - 1271.26)	9.42E-01	6.17E-01

\*Units pg/ml

**Supplemental Table 2, cont.**

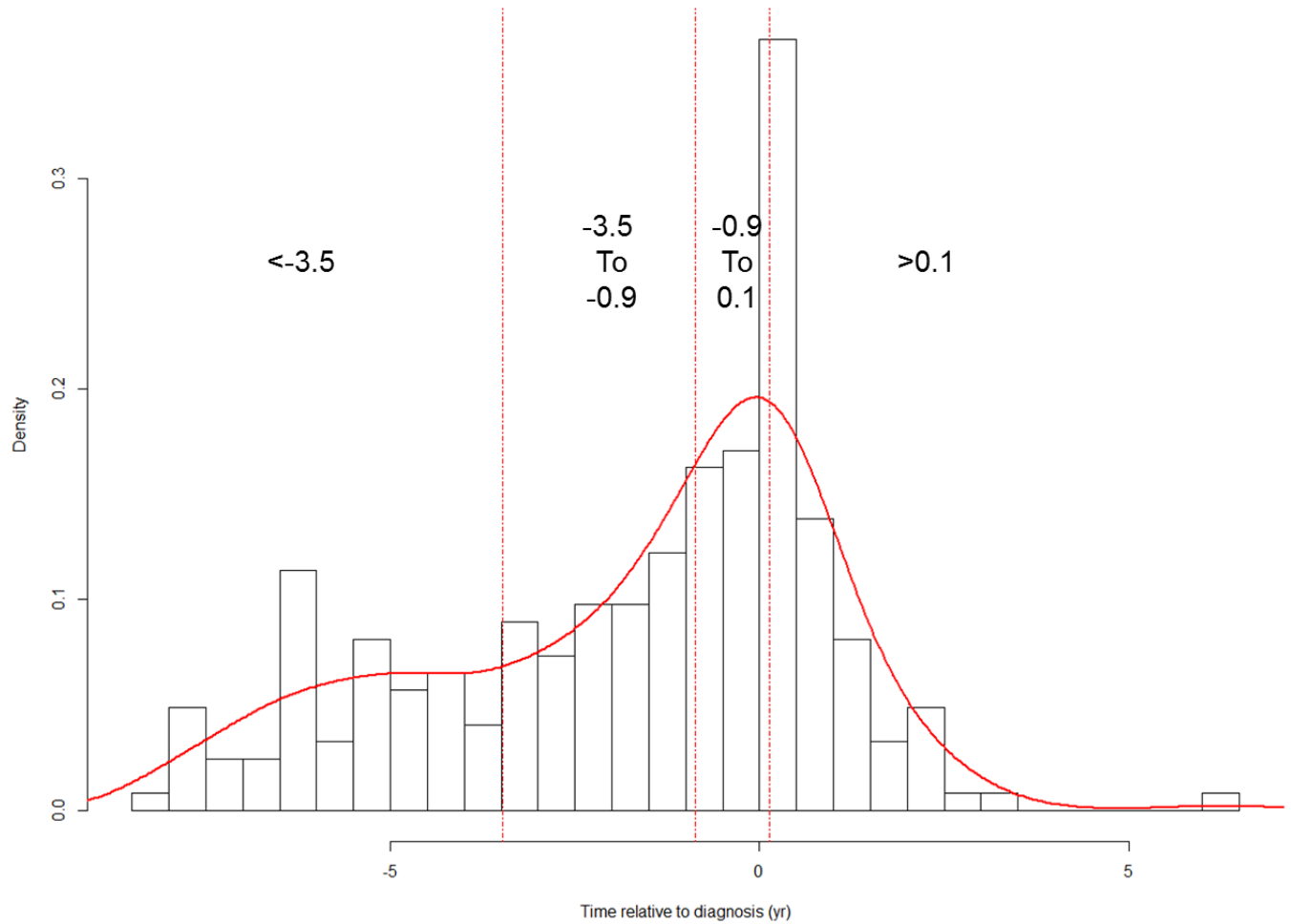
<b>0.9 years before to 0.1 years after classification</b>				
<b>Soluble mediator, (Normalized FI)</b>	<b>Case (n = 63), Median (IQR)</b>	<b>Control (n = 23), Median (IQR)</b>	<b>p-value</b>	<b>q-value</b>
IL-5	1.75 (1.31 - 2.08)	1 (0.61 - 1.14)	2.32E-08	1.37E-07
IL-6	1.86 (1.5 - 3.29)	1.21 (0.81 - 1.43)	1.07E-06	3.15E-06
IP-10	18.75 (6.09 - 51.5)	4.08 (2.63 - 5.79)	8.24E-06	1.62E-05
TNFR II	1.24 (1.09 - 1.42)	0.97 (0.85 - 1.08)	3.01E-05	4.43E-05
TGF-β	2.21 (1.69 - 3)	3.67 (2.47 - 6.54)	3.76E-04	3.69E-04
IFN-γ	3.43 (2.75 - 4.38)	2.06 (1.81 - 2.87)	3.36E-04	3.69E-04
BLyS*	1374.12 (1018.56 - 1788.81)	1014.64 (792.86 - 1225.4)	6.60E-04	5.56E-04
IL-17A	2.15 (1.75 - 2.95)	1.42 (1.25 - 2.02)	2.07E-03	1.50E-03
IL-12p70	1.75 (1.5 - 2.17)	1.39 (1.07 - 1.77)	5.24E-03	3.40E-03
APRIL *	9010.05 (3232.99 - 22773.56)	1615.03 (0 - 7540.67)	6.05E-03	3.60E-03
TNFR I	1.22 (1.02 - 1.5)	1.04 (0.91 - 1.14)	7.85E-03	4.20E-03
sFasL	6.08 (3.12 - 9)	3.44 (2.57 - 4.65)	1.18E-02	5.40E-03
IL-1RA	3.37 (1.62 - 7.68)	1.88 (0.96 - 3.78)	1.15E-02	5.40E-03
IL-13	1.4 (1.11 - 2.33)	1.2 (0.95 - 1.6)	1.43E-02	6.00E-03
IL-23	2 (1.28 - 2.73)	1.33 (1 - 2.1)	2.34E-02	9.20E-03
IL-4	1.8 (1.43 - 2.4)	1.5 (1.17 - 1.9)	3.96E-02	1.37E-02
IL-21	0.99 (0.55 - 2.3)	0.65 (0.4 - 1.34)	3.88E-02	1.37E-02
IL-1β	1.82 (1.36 - 2.43)	1.67 (0.9 - 1.91)	6.42E-02	2.10E-02
sCD40L	0.04 (0.03 - 0.07)	0.06 (0.04 - 0.1)	8.53E-02	2.51E-02
Resistin	1.31 (0.83 - 1.9)	1.74 (0.97 - 2.51)	8.27E-02	2.51E-02
MIP-1α	3.34 (2.16 - 8.52)	2.06 (1.38 - 4.51)	1.31E-01	3.68E-02
PAI-1	1.24 (1.17 - 1.32)	1.27 (1.24 - 1.31)	1.76E-01	4.74 E-02
MCP-3	1.62 (1.22 - 2.12)	1.5 (1.09 - 1.88)	2.15E-01	5.51E-02
SCF	0.73 (0.45 - 1.24)	0.56 (0.41 - 0.94)	2.45E-01	6.02E-02
IL-2	1.8 (1.2 - 2.5)	1.33 (1.12 - 2.62)	4.31E-01	1.02E-01
MIG	0.82 (0.26 - 1.91)	0.48 (0.38 - 1)	4.55E-01	1.03E-01
IL-8	9.23 (4.5 - 33.83)	8.93 (3.89 - 28.07)	6.05E-01	1.32E-01
IL-1α	0.93 (0.61 - 1.56)	0.92 (0.71 - 1.48)	9.48E-01	2.00E-01

\*Units pg/ml

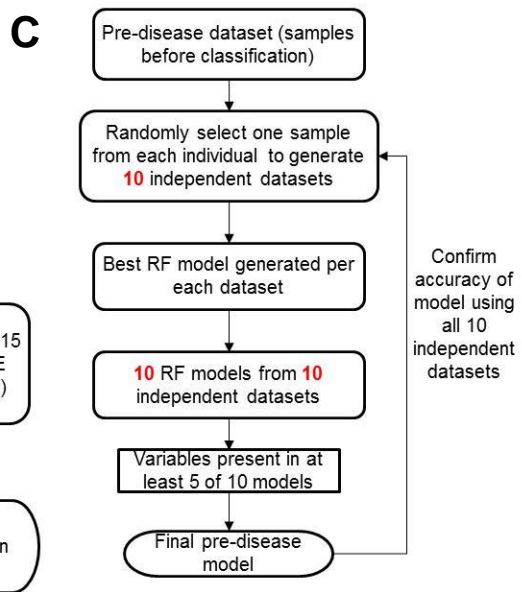
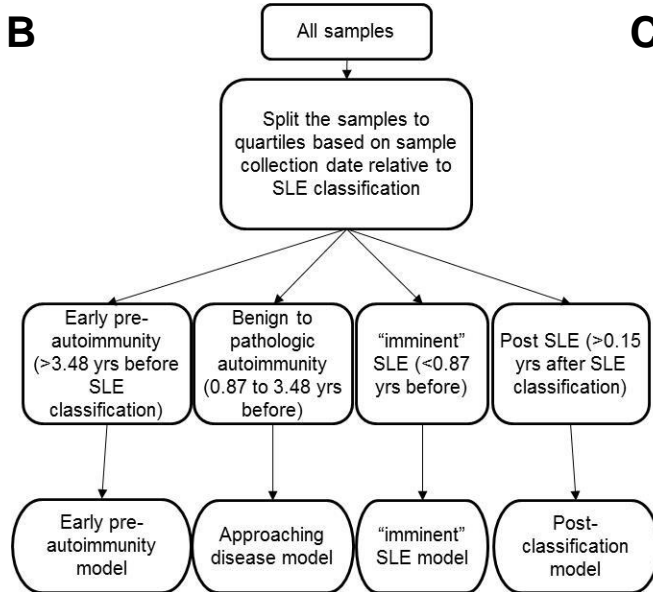
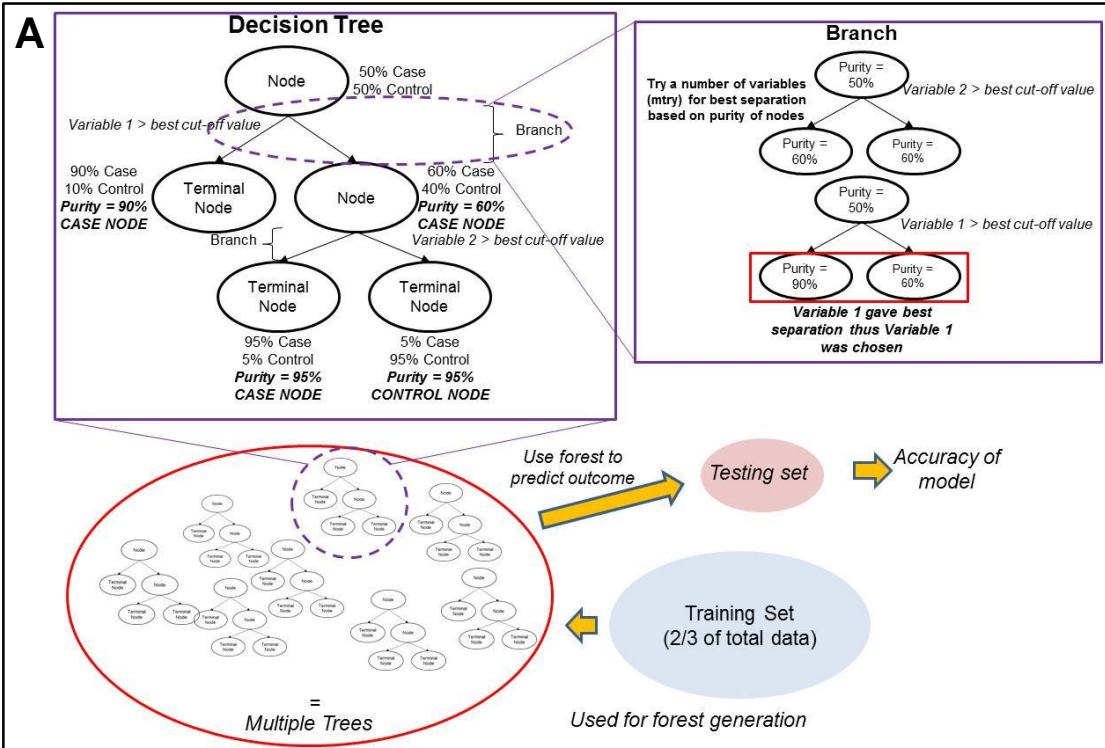
**Supplemental Table 2, cont.**

<b>&gt;0.1 years after classification</b>				
<b>Soluble mediator, (Normalized FI)</b>	<b>Case (n = 61), Median (IQR)</b>	<b>Control (n = 56), Median (IQR)</b>	<b>p-value</b>	<b>q-value</b>
IL-5	1.7 (1.38 - 2.07)	0.9 (0.63 - 1.22)	1.51E-13	1.71E-12
IP-10	13.83 (4.35 - 37.75)	3.26 (2.18 - 5.31)	1.64E-07	4.64E-07
IL-12p70	1.83 (1.5 - 2.32)	1.21 (1.04 - 1.6)	1.16E-07	4.64E-07
IFN- $\gamma$	3.39 (2.81 - 4.86)	2.49 (2 - 2.98)	1.38E-07	4.64E-07
IL-6	2 (1.6 - 3.11)	1.17 (0.99 - 1.7)	5.98E-07	1.35E-06
IL-17A	2.38 (2.04 - 2.87)	1.75 (1.52 - 2)	4.12E-06	7.77E-06
IL-21	1.11 (0.64 - 2.32)	0.61 (0.44 - 1.01)	7.54E-05	1.22E-04
IL-23	2.05 (1.5 - 2.81)	1.5 (1.18 - 1.77)	9.84E-05	1.39E-04
IL-13	1.5 (1.25 - 2.12)	1.2 (0.91 - 1.5)	1.44E-04	1.77E-04
TNFR II	1.14 (0.97 - 1.37)	1.01 (0.91 - 1.11)	1.56E-04	1.77E-04
IL-2	1.63 (1.2 - 2.55)	1.26 (1.08 - 1.67)	5.08E-04	5.23E-04
TNFR I	1.36 (1.06 - 1.51)	1.16 (1.01 - 1.26)	1.14E-03	1.07E-03
IL-4	1.8 (1.24 - 2.73)	1.4 (1.1 - 1.73)	2.04E-03	1.78E-03
TGF- $\beta$	2.09 (1.54 - 3.36)	3.22 (2.26 - 4.92)	2.52E-03	2.04E-03
IL-1RA	2.96 (1.84 - 7.21)	1.93 (1.24 - 4.33)	5.24E-03	3.95E-03
SCF	0.84 (0.55 - 1.53)	0.63 (0.48 - 0.9)	2.85E-02	2.02E-02
MIP-1 $\alpha$	4.6 (2.68 - 10.32)	2.78 (1.94 - 7.2)	3.83E-02	2.55E-02
APRIL*	10989.46 (4931.12 - 27846)	7753.06 (1705.74 - 18667.66)	5.21E-02	3.27E-02
MIG	0.82 (0.28 - 2.5)	0.48 (0.32 - 0.61)	6.34E-02	3.78E-02
BLyS*	1220.72 (909.07 - 2014.15)	1065.02 (917.26 - 1314.42)	8.66E-02	4.88E-02
MCP-3	1.5 (1.33 - 2.08)	1.39 (1.06 - 1.93)	9.05E-02	4.88E-02
PAI-1	1.27 (1.18 - 1.34)	1.28 (1.25 - 1.33)	2.57E-01	1.32E-01
Resistin	1.49 (1.1 - 2.07)	1.62 (1.12 - 2.48)	3.00E-01	1.48E-01
IL-1 $\beta$	1.91 (1.45 - 3.48)	1.62 (1.15 - 2.97)	3.77E-01	1.78E-01
sFasL	6.36 (3.2 - 9.5)	5.13 (3.94 - 7.52)	4.52E-01	2.05E-01
IL-8	8.14 (5.08 - 67.88)	15.1 (4.34 - 87.74)	7.92E-01	3.45E-01
IL-1 $\alpha$	0.97 (0.67 - 1.36)	1 (0.74 - 1.29)	8.76E-01	3.67E-01
sCD40L	0.04 (0.03 - 0.09)	0.04 (0.03 - 0.1)	9.18E-01	3.71E-01

\*Units pg/ml

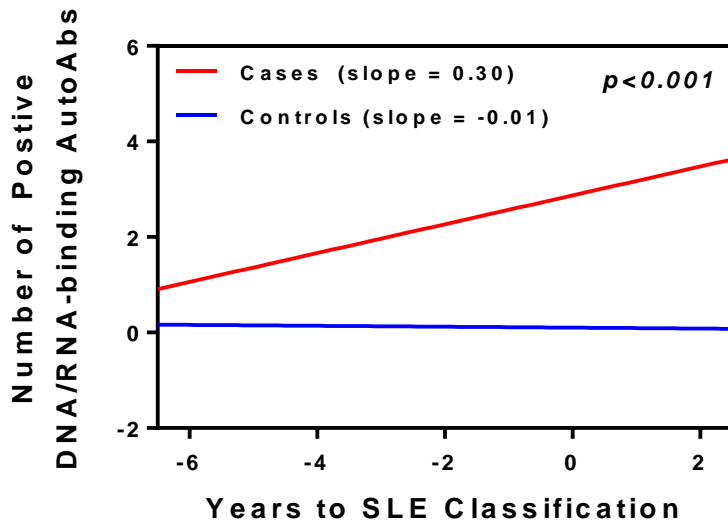


**Supplemental Figure 1. Separation of samples into four time periods relative to SLE classification.** Kernel density of sample drawn date relative to SLE classification based on cases. Quartiles were chosen to insure equal contribution of cases into each quartile.



**Supplemental Figure 2. Overview of Random Forest modeling.** (A) Nomenclature used in random forest algorithm and process of generating RF models. (B-C) Flowchart of RF analysis at different time intervals (B) and for overall pre-disease modeling (C).





**Supplemental Figure 3. Number of SLE-associated autoantibody specificities increases in cases as they approach SLE classification, but not in healthy controls.** Number of positive DNA/RNA-binding autoantibody specificities (binding dsDNA, chromatin, Ro/SSA, La/SSB, Sm, SmRNP, and RNP) over time in patients prior to SLE classification (red), vs. race, gender, age ( $\pm 5$  years) and time of sample procurement-matched healthy controls (blue). P-value for the fixed effect of disease status are show