Toll-Like Receptor 8 Stability is Regulated by Ring Finger 216 in response to circulating MicroRNAs

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ONLINE DATA SUPPLEMENT

ONLINE SUPPLEMENTARY MATERIAL

Supplemental Figure 1: (A) THP-1 cells were treated with mir-mimics for 2 h and immunoblotted for TLR2 or Actin. (B) TLR8 Gene expression from GEO GSE47625 analyzed by GEO2R (NCBI). NS = not significant. (C) Human PBMC's were treated with R848 (1µg/µL) for 2 h and immunoblotted for TLR8 or Actin. (D) THP-1 cells were treated for various time points with R848 (1µg/µL) and immunoblotted for TLR2 or TLR4.

Supplemental Figure 2: (A-E) Plasma cytokine levels from "control" or "ARDS" subjects. (F-J) Plasma cytokine levels were plotted against TLR8 OD. No significant associations were observed. All statistical analyses were performed in Graphpad.

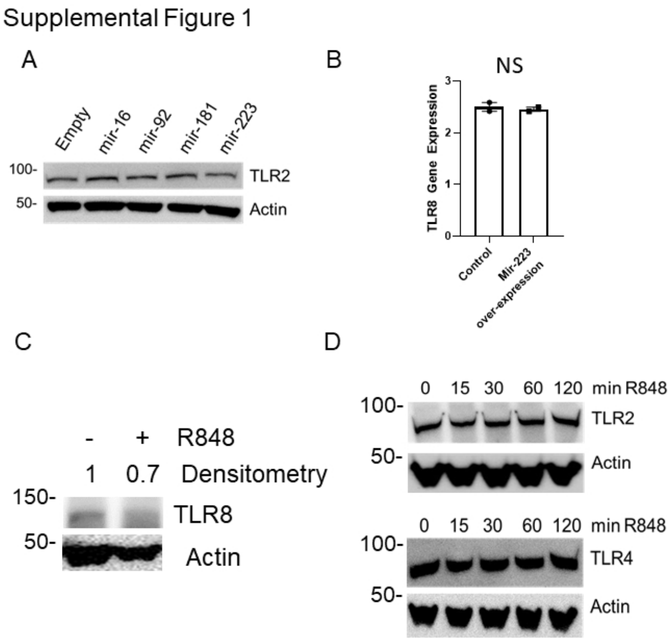
Supplemental Figure 3: (A-C) RNF216 gene expression in three separate cohorts of critically ill subjects with or at risk for ARDS. Gene expression values were generated from GEO2R and plotted in Graphpad. Datasets were analyzed by Student's T test.

Supplemental Table 1: Demographic Characteristics of subjects with ARDS for which Nanostring miRNA array was performed.

Supplemental Table 2: Clinical and Demographic characteristics of subjects for which plasma samples were assayed for ability to activate TLR8.

Supplemental Table 3: miRNA counts above baseline from Nanostring miRNA array from n=6 ARDS subjects

Supplemental Table 4: RNF216 Gene expression is down-regulated in three separate gene array datasets from critically ill humans at risk or with ARDS compared to control. The table summarizes findings from three separate studies examining gene expression changes in human subjects with risk factors for or with ARDS. Gene expression data was analyzed via GEO2R (NCBI).



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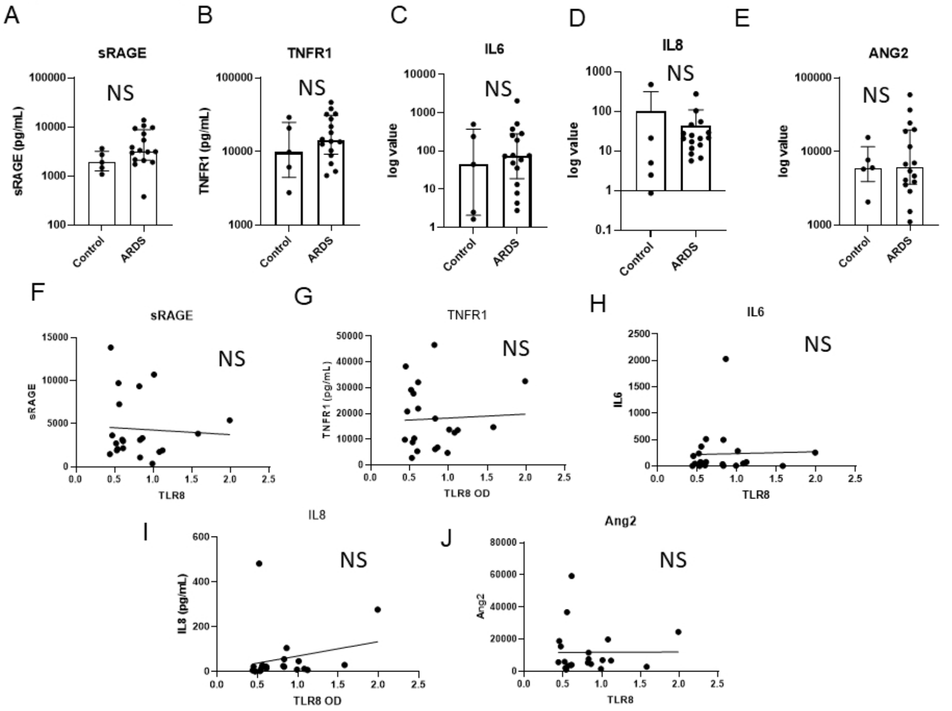
Supplemental Figure 2



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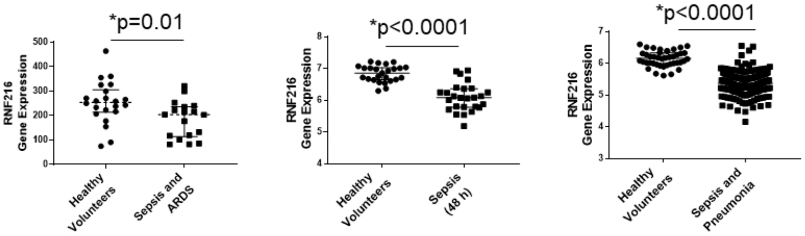

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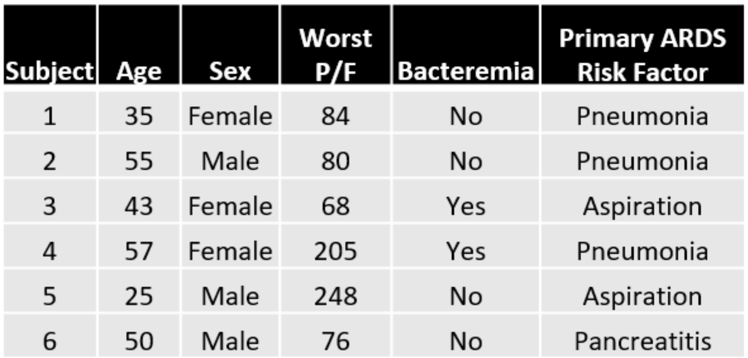
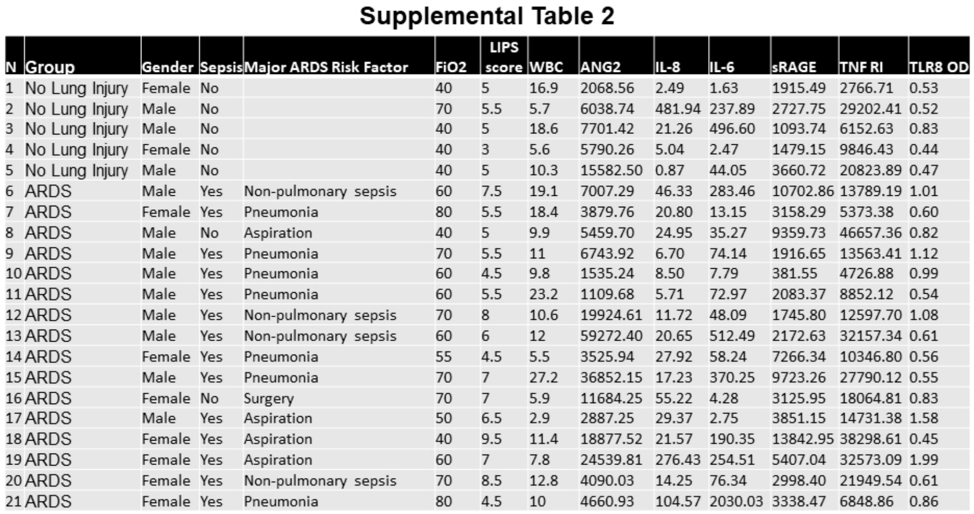
Supplemental Table 1



Subject	Age	Sex	Worst P/F	Bacteremia	Primary ARDS Risk Factor
1	35	Female	84	No	Pneumonia
2	55	Male	80	No	Pneumonia
3	43	Female	68	Yes	Aspiration
4	57	Female	205	Yes	Pneumonia
5	25	Male	248	No	Aspiration
6	50	Male	76	No	Pancreatitis

Supplemental Table 1: Demographic Characteristics of subjects with ARDS for which Nanostring miRNA array was performed



Supplemental Table 2

N	Group	Gender	Sepsis	Major ARDS Risk Factor	FiO2	LIPS score	WBC	ANG2	IL-8	IL-6	sRAGE	TNF RI	TLR8 OD
1	No Lung Injury	Female	No		40	5	16.9	2068.56	2.49	1.63	1915.49	2766.71	0.53
2	No Lung Injury	Male	No		70	5.5	5.7	6038.74	481.94	237.89	2727.75	29202.41	0.52
3	No Lung Injury	Male	No		40	5	18.6	7701.42	21.26	496.60	1093.74	6152.63	0.83
4	No Lung Injury	Female	No		40	3	5.6	5790.26	5.04	2.47	1479.15	9846.43	0.44
5	No Lung Injury	Male	No		40	5	10.3	15582.50	0.87	44.05	3660.72	20823.89	0.47
6	ARDS	Male	Yes	Non-pulmonary sepsis	60	7.5	19.1	7007.29	46.33	283.46	10702.86	13789.19	1.01
7	ARDS	Female	Yes	Pneumonia	80	5.5	18.4	3879.76	20.80	13.15	3158.29	5373.38	0.60
8	ARDS	Male	No	Aspiration	40	5	9.9	5459.70	24.95	35.27	9359.73	46657.36	0.82
9	ARDS	Male	Yes	Pneumonia	70	5.5	11	6743.92	6.70	74.14	1916.65	13563.41	1.12
10	ARDS	Male	Yes	Pneumonia	60	4.5	9.8	1535.24	8.50	7.79	381.55	4726.88	0.99
11	ARDS	Male	Yes	Pneumonia	60	5.5	23.2	1109.68	5.71	72.97	2083.37	8852.12	0.54
12	ARDS	Male	Yes	Non-pulmonary sepsis	70	8	10.6	19924.61	11.72	48.09	1745.80	12597.70	1.08
13	ARDS	Male	Yes	Non-pulmonary sepsis	60	6	12	59272.40	20.65	512.49	2172.63	32157.34	0.61
14	ARDS	Female	Yes	Pneumonia	55	4.5	5.5	3525.94	27.92	58.24	7266.34	10346.80	0.56
15	ARDS	Male	Yes	Pneumonia	70	7	27.2	36852.15	17.23	370.25	9723.26	27790.12	0.55
16	ARDS	Female	No	Surgery	70	7	5.9	11684.25	55.22	4.28	3125.95	18064.81	0.83
17	ARDS	Male	Yes	Aspiration	50	6.5	2.9	2887.25	29.37	2.75	3851.15	14731.38	1.58
18	ARDS	Female	Yes	Aspiration	40	9.5	11.4	18877.52	21.57	190.35	13842.95	38298.61	0.45
19	ARDS	Female	Yes	Aspiration	60	7	7.8	24539.81	276.43	254.51	5407.04	32573.09	1.99
20	ARDS	Female	Yes	Non-pulmonary sepsis	70	8.5	12.8	4090.03	14.25	76.34	2998.40	21949.54	0.61
21	ARDS	Female	Yes	Pneumonia	80	4.5	10	4660.93	104.57	2030.03	3338.47	6848.86	0.86

Supplemental Table 2: Clinical and Demographic characteristics of subjects for which plasma samples were assayed for ability to activate TLR8.

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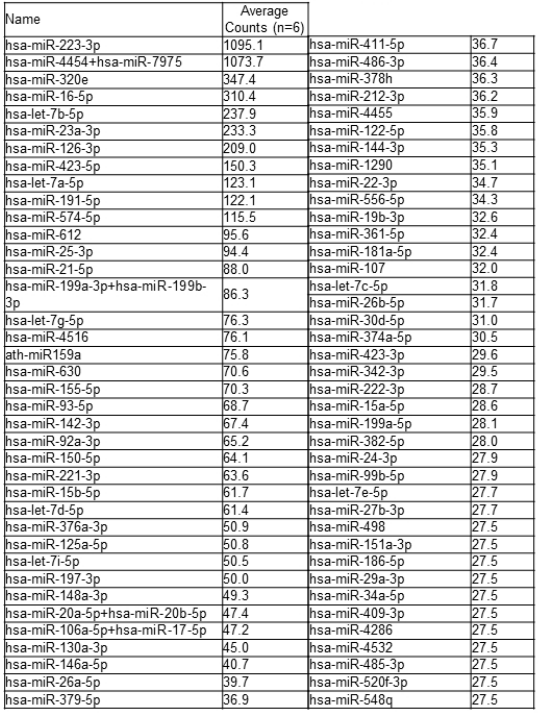

Name	Average Counts (n=6)
hsa-miR-223-3p	1095.1
hsa-miR-4454+hsa-miR-7975	1073.7
hsa-miR-320e	347.4
hsa-miR-16-5p	310.4
hsa-let-7b-5p	237.9
hsa-miR-23a-3p	233.3
hsa-miR-126-3p	209.0
hsa-miR-423-5p	150.3
hsa-let-7a-5p	123.1
hsa-miR-191-5p	122.1
hsa-miR-574-5p	115.5
hsa-miR-612	95.6
hsa-miR-25-3p	94.4
hsa-miR-21-5p	88.0
hsa-miR-199a-3p+hsa-miR-199b-3p	86.3
hsa-let-7g-5p	76.3
hsa-miR-4516	76.1
ath-miR159a	75.8
hsa-miR-630	70.6
hsa-miR-155-5p	70.3
hsa-miR-93-5p	68.7
hsa-miR-142-3p	67.4
hsa-miR-92a-3p	65.2
hsa-miR-150-5p	64.1
hsa-miR-221-3p	63.6
hsa-miR-15b-5p	61.7
hsa-let-7d-5p	61.4
hsa-miR-376a-3p	50.9
hsa-miR-125a-5p	50.8
hsa-let-7i-5p	50.5
hsa-miR-197-3p	50.0
hsa-miR-148a-3p	49.3
hsa-miR-20a-5p+hsa-miR-20b-5p	47.4
hsa-miR-106a-5p+hsa-miR-17-5p	47.2
hsa-miR-130a-3p	45.0
hsa-miR-146a-5p	40.7
hsa-miR-26a-5p	39.7
hsa-miR-379-5p	36.9
hsa-miR-411-5p	36.7
hsa-miR-486-3p	36.4
hsa-miR-378h	36.3
hsa-miR-212-3p	36.2
hsa-miR-4455	35.9
hsa-miR-122-5p	35.8
hsa-miR-144-3p	35.3
hsa-miR-1290	35.1
hsa-miR-22-3p	34.7
hsa-miR-556-5p	34.3
hsa-miR-19b-3p	32.6
hsa-miR-361-5p	32.4
hsa-miR-181a-5p	32.4
hsa-miR-107	32.0
hsa-let-7c-5p	31.8
hsa-miR-26b-5p	31.7
hsa-miR-30d-5p	31.0
hsa-miR-374a-5p	30.5
hsa-miR-423-3p	29.6
hsa-miR-342-3p	29.5
hsa-miR-222-3p	28.7
hsa-miR-15a-5p	28.6
hsa-miR-199a-5p	28.1
hsa-miR-382-5p	28.0
hsa-miR-24-3p	27.9
hsa-miR-99b-5p	27.9
hsa-let-7e-5p	27.7
hsa-miR-27b-3p	27.7
hsa-miR-498	27.5
hsa-miR-151a-3p	27.5
hsa-miR-186-5p	27.5
hsa-miR-29a-3p	27.5
hsa-miR-34a-5p	27.5
hsa-miR-409-3p	27.5
hsa-miR-4286	27.5
hsa-miR-4532	27.5
hsa-miR-485-3p	27.5
hsa-miR-520f-3p	27.5
hsa-miR-548q	27.5

Supplemental Table 3: miRNA counts above baseline from Nanostring miRNA array from n=6 ARDS subjects

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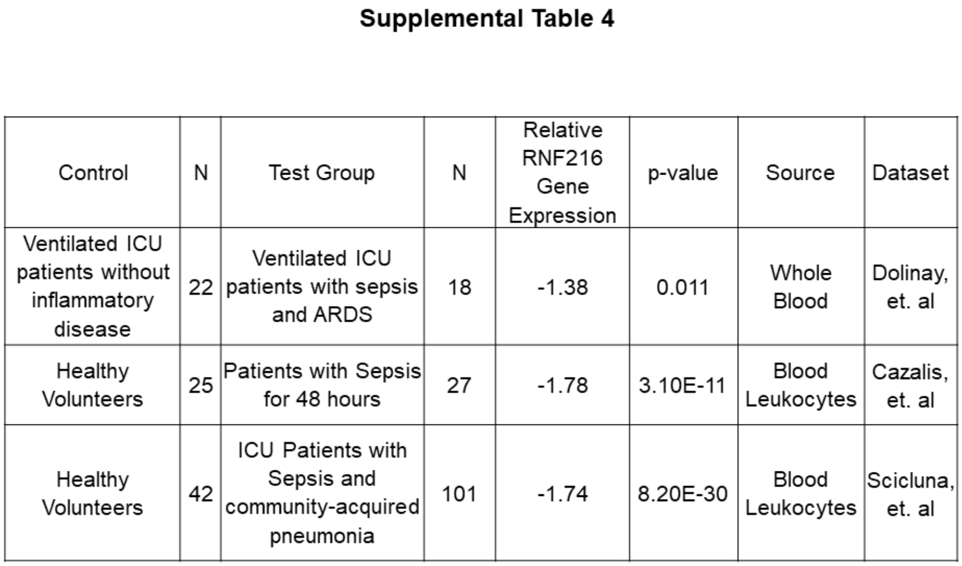

Supplemental Table 4

Control	N	Test Group	N	Relative RNF216 Gene Expression	p-value	Source	Dataset
Ventilated ICU patients without inflammatory disease	22	Ventilated ICU patients with sepsis and ARDS	18	-1.38	0.011	Whole Blood	Dolinay, et. al
Healthy Volunteers	25	Patients with Sepsis for 48 hours	27	-1.78	3.10E-11	Blood Leukocytes	Cazalis, et. al
Healthy Volunteers	42	ICU Patients with Sepsis and community-acquired pneumonia	101	-1.74	8.20E-30	Blood Leukocytes	Scicluna, et. al

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