Supplemental Data for

Temporal Response of MicroRNA Expression in Blood Leukocytes from Patients with the Acute Respiratory Distress Syndrome

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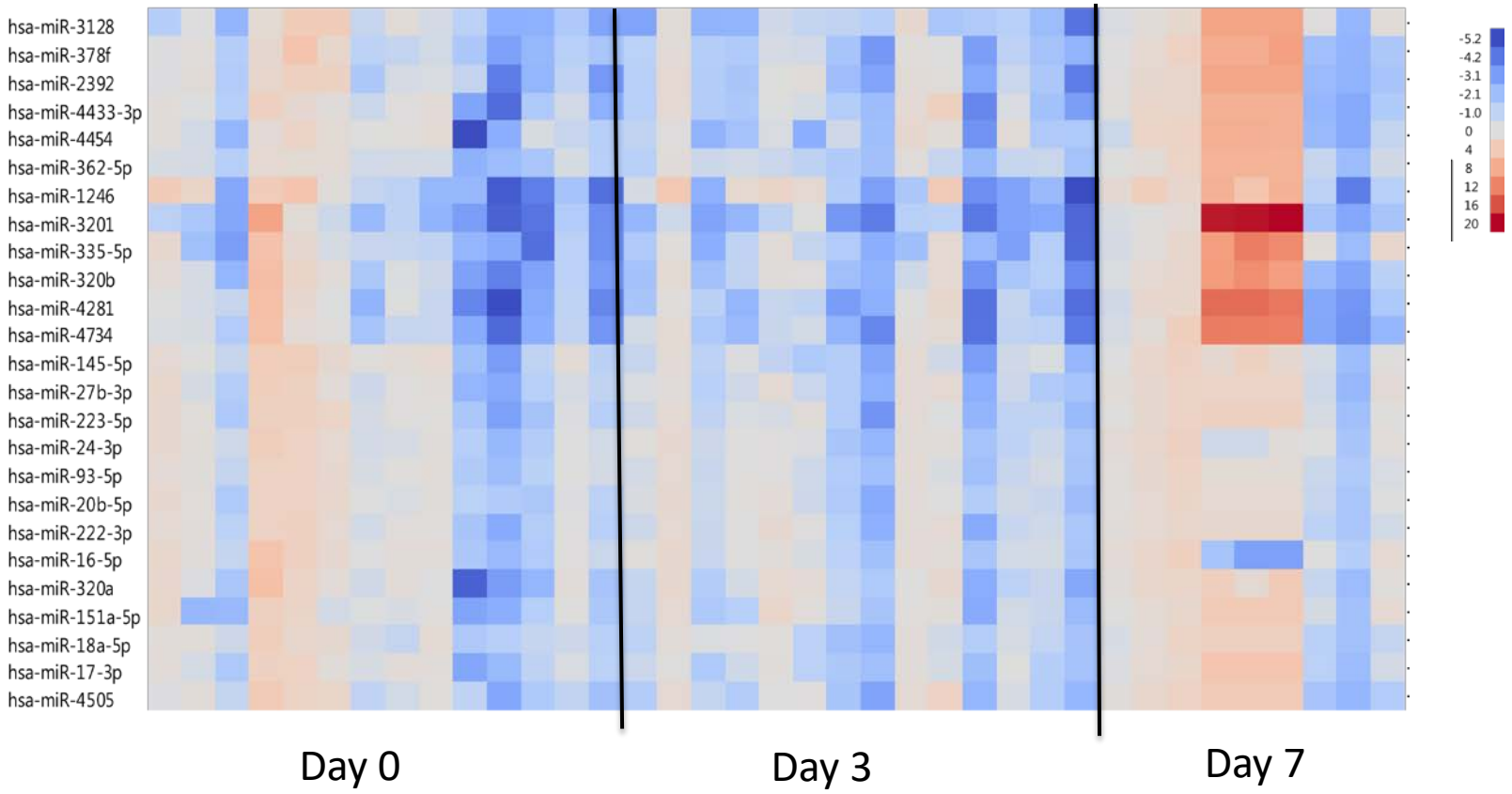
Supplemental Table 1. Clinical characteristics of patients with available sequential blood samples for miRNA analysis compared to those without samples.

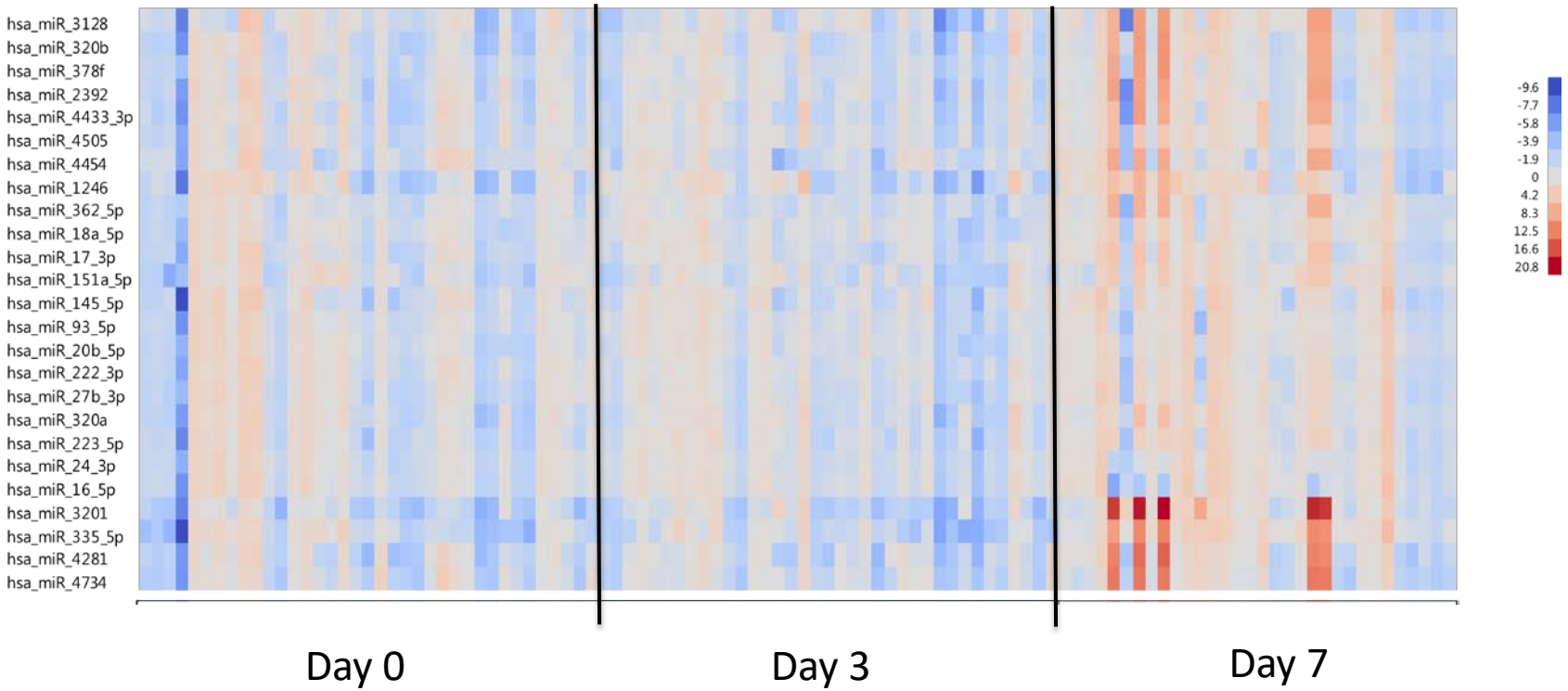
Variables	Patients with buffy coat cells available n = 51	Patients without buffy coat cells n = 41	P value
Age[a]	50.4 ± 17.4	52.5 ± 13.0	0.51
Sex[b] (men/women)	25/26	23/18	0.53
APACHE 3[a]	60.4 ± 17.7	62.2 ± 20.4	0.66
PaO2/FiO2 day 0[a]	117.1 ± 48.8	124.4 ±45.9	0.46
WBC[a] (x $10^3/mm^3$)	15.5 ± 7.3	15.3 ± 7.5	0.88
Hospital mortality (%)[b]	12 (23.5)	15 (36.5)	0.25

Summary data displayed as mean ± sd, a – t-test, b – Fisher's exact test



Supplemental Figure 1. Heat map of miRNA expression evaluated using real-time PCR of blood leukocytes on day 0, 3, and 7 from ARDS patients randomized to placebo/usual.

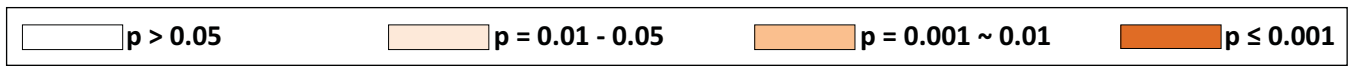
Blue color represents downregulation and red represents upregulation. The heat map shows real-time PCR data of miRNA expression from day 0, 3 and 7 of ARDS in 14 patients who received usual care for ARDS. There was no significant change in any of the 25 miRNA when comparing day 0 and day 3. However, by day 7, miRNA expression was upregulated in 14 of 25 miRNA targets at day 7 compared to day 3 or day 0.



Supplemental Figure 2. Heat map of miRNA expression evaluated using real-time PCR of blood leukocytes on day 0, 3, and 7 from ARDS patients treated with corticosteroids/usual care.

Each column represents a patient while each row represents a miRNA as indicated. Blue color represents downregulation and red represents upregulation. The heat map shows real-time PCR data of miRNA expression from day 0, 3 and 7 of ARDS in 37 patients who received corticosteroids for ARDS. There was no significant change in any of the 25 miRNA when comparing day 0 and day 3. However, by day 7, miRNA expression was upregulated in 22 of 25 miRNA targets.

	Kovach *et al* (9)	Kovach *et al* (9)	Kovach *et al* (9)	Kovach *et al* (9)	Kovach *et al* (9)	Kovach *et al* (9)	Kovach *et al* (9)	Kovach *et al* (9)	Kovach *et al* (9)	Howrylak *et al* (10)	Howrylak *et al* (10)	Kangelaris et al (12)	Kovach *et al* (1)	Kovach et al (9) Kangelaris et al (12)	Howrylak *et al* (10)	Kovach *et al* (9)	Kovach et al (9) Kangelaris et al (12)	Kangelaris et al (12)	Howrylak *et al* (10)
		AM down	AM up	AM down	AM down	AM up	AM down	AM down	AM down				AM up	AM up		AM up	AM up		
	PBL up	PBL down	PBL up	PBL down	PBL down	PBL up	PBL down	PBL down	PBL down	PBL up	PBL down	PBL down	PBL up	PBL up	PBL up	PBL up	PBL up	PBL down	PBL down
	CEACAM6	CCDC50	MRVI1	RORA	SASH1	DYSF	EPB41L4A	ICA1L	YES1	ARF3	PNPLA2	MME	MGAM	OLM4	BTG2	CXCR1	MMP8	HCAR3	NQO2
hsa-miR-4505	1015	3040	1207		1466	5334	3599	1619	3191	2093	2060	4366	1253	215	2239	1360		1038	465
hsa-miR-4505	226	240	2206		4871	5345	3235		3242	2115	603		2539			1152		1049	
hsa-miR-4505	3219		2218		5189	4592			3251	1064	43								
hsa-miR-4505	1082																		
hsa-miR-2392	2056	1510	3629	1437	4867	4248	3408		3257	2097		5185	1416	179	1692	2243		1038	
hsa-miR-2392	1640	4981	986	3386	5456		3432			1064		4355	2226	216		1359			
hsa-miR-2392			2117	397			3462			1205		4068				1151			
hsa-miR-4433-3p	2486	8499	3798	10284	6411	4804	2567	3177	1347		1944				123	1394	2651		690
hsa-miR-4433-3p	2050	4755	1202	5690	3420				3261		2308		2220		2225				698
hsa-miR-4433-3p	233		2602	391	1166				3311		2324								
hsa-miR-4433-3p									1412										
hsa-miR-4734	676	4759	990	436	5501	4594	3424	1446	3198	2084	617		2231	123			670	1077	467
hsa-miR-4734	692	3047	1789	7505	3115	6411				2097	666							1131	
hsa-miR-4734	1719	608	1060		1471									1599				1114	
hsa-miR-4734	179		2117															979	
hsa-miR-4734	1868																		
hsa-miR-4281			1775			2390	2592	7714	3287			27	3561				1015		913
hsa-miR-4281			2131				3442										627		
hsa-miR-378f	1088	7624		9915		4646		6678						212					
hsa-miR-378f	2051							4200						231					
hsa-miR-18a-5p		7180	2598			4810	3274	4149	1543			4352			2393				
hsa-miR-27b-3p	587		2136		5229		1120					1169		1886	364				
hsa-miR-27b-3p																			
hsa-miR-145-5p			2083			717							2967						707
hsa-miR-145-5p						5926													
hsa-miR-151a-5p	52				3267	4782	3502												701
hsa-miR-320b		405		5662				1357		1201						1357	654		
hsa-miR-320b		5895		8233															
hsa-miR-320b		3307																	
hsa-miR-320a		5895		5662				1357		1201						1357	654		
hsa-miR-320a		3307		8233															
hsa-miR-4454				6867			2590								2391				
hsa-miR-1246					3403	5889						8							
hsa-miR-1246					3131														
hsa-miR-1246					5179														
hsa-miR-17-3p	676																206		
hsa-miR-17-3p	1717																		
hsa-miR-17-3p	1714																		
hsa-miR-20b-5p								4149			2067								
hsa-miR-20b-5p											1951								
hsa-miR-3128		966					1147	4873											
hsa-miR-335-5p		4747																	
hsa-miR-335-5p		4752																	
hsa-miR-3201												4393							
hsa-miR-223-5p																			
number of binding sites per mRNA	**21**	**19**	**18**	**17**	**16**	**14**	**14**	**11**	**11**	**10**	**10**	**9**	**9**	**8**	**8**	**8**	**7**	**7**	**7**



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	AM down				AM up			AM down										
	PBL down	PBL up	PBL up	PBL down	PBL up	PBL up	PBL down	PBL down	PBL up	PBL up	PBL up	PBL up	PBL up	PBL up	PBL up	PBL down	PBL down	PBL up
	ZNF124	LCN2	CDKN1A	NPEPL1	CD177	FTH1 (ferritin)	CNTNAP3	ENPP5	NFKB (p65)	IL-18	IL-1R2	CASP1	CD24	RETN	TCN1	RBP7	CREBZF	ANXA3
hsa-miR-4505	2030	34	1899	2051	1471		3364			890								
hsa-miR-4505	1958		1647	137														
hsa-miR-4505			1709															
hsa-miR-4505																		
hsa-miR-2392		348	1698		979						1026	402		62	953			426
hsa-miR-2392					1453													
hsa-miR-2392																		
hsa-miR-4433-3p	1989	350		1971		608	6260	143										
hsa-miR-4433-3p	2039			1403														
hsa-miR-4433-3p																		
hsa-miR-4433-3p																		
hsa-miR-4734		742														460		
hsa-miR-4734																		
hsa-miR-4734																		
hsa-miR-4734																		
hsa-miR-4734																		
hsa-miR-4281	2003	147			847	616												
hsa-miR-4281						731												
hsa-miR-378f						1010				885			1415					
hsa-miR-378f																		
hsa-miR-18a-5p	817																	
hsa-miR-27b-3p				2052			1256											
hsa-miR-27b-3p							483											
hsa-miR-145-5p	1999		1294								740							
hsa-miR-145-5p																		
hsa-miR-151a-5p					432			276										
hsa-miR-320b																		
hsa-miR-320b																		
hsa-miR-320b																		
hsa-miR-320a																		
hsa-miR-320a																		
hsa-miR-4454																	3465	
hsa-miR-1246																		
hsa-miR-1246																		
hsa-miR-1246																		
hsa-miR-17-3p									204									
hsa-miR-17-3p																		
hsa-miR-17-3p																		
hsa-miR-20b-5p								272										
hsa-miR-20b-5p																		
hsa-miR-3128																		
hsa-miR-335-5p																		
hsa-miR-335-5p																		
hsa-miR-3201																		
hsa-miR-223-5p									198									
number of binding sites per mRNA	7	6	5	5	5	4	4	3	2	2	2	1	1	1	1	1	1	1

Supplemental Figure 3: miRNA heterodimer formation with leukocyte mRNA described in prior studies of patients with ARDS.
Messenger RNA found in either peripheral blood leukocytes (PBL) or alveolar macrophages (AM) described in previous reports of ARDS (6 – 9) patients are listed across the top of the figure. MiRNA that were significantly upregulated at day 7 compared to baseline values are listed in the first column. A pattern-based methodology was used to identify putative miRNA binding sites and the corresponding heteroduplex formation (23). The number within the cell is related to the binding location to the leftmost position of the predicted target site. Nominal p values are provided with the binding location on the mRNA (p > 0.05 – blank, p = 0.01 - 0.05 light orange, p = 0.001 ~ 0.01 orange, p = < 0.001 dark orange). Heteroduplexes were not found for the following mRNA: IL-1beta, SNORD1A, SNORD64, S100A12, IMMP2L, CEACAM, UPAR, VEGF A, VEGF C, NFKB50.

Gene Abbreviations: ANXA3 - Annexin A3, ARF3 - ADP ribosylation factor 3, BTG2 - BTG family member 2, CASP1 - Caspase 1, CCDC50 - Coiled coil domain containing protein 50, CD177 - CD177 molecule, CD24 - CD24 molecule, CDKN1A - Cyclin-dependent kinase inhibitor 1A, CEACAM1 - Carcinoembryonic Antigen-Related Cell Adhesion Molecule 1, CEACAM6 - Carcinoembryonic antigen-related cell adhesion molecule 6, CNTNAP3 - Contactin associated protein-like 3, CREBZF - CREB/ATF bZIP transcription factor, CXCR1 - Chemokine (C-X-C motif) receptor 1, DYSF – Dysferlin, ENPP5 - Ectonucleotide pyrophosphatase/phosphodiesterase 5, EPB41L4A - Erythrocyte membrane protein band 4.1 like 4A, FTH1 - Ferritin, heavy polypeptide 1, HCAR3 - Hydroxycarboxylic acid receptor 3, ICA1L - Islet cell autoantigen 1 like, IL-18 - Interleukin -18, IL-1b - Interleukin 1b, IL-1R2 - Interleukin 1 receptor, type 2, IMMP2L - Inner Mitochondrial Membrane Peptidase Subunit 2, LCN2 - Lipocalin2, MGAM - Maltase-glucoamylase, MME - Membrane metallo-endopeptidase, MMP8 - Matrix metallopeptidase 8, MRVI1 - Murine retrovirus integration site 1 homolog, NFKB(p65) - Nuclear Factor NF-Kappa-B P65 Subunit, NFKB50 - Nuclear Factor of Kappa Light Polypeptide Gene Enhancer in B-Cells 1, NPEP - Aminopeptidase-like 1, NQO2, - NAD(P)H dehydrogenase, quinone 2, OLM4 - Olfactomedin 4, PNPLA2 - Patatin like phospholipase domain containing 2, RBP7 - Retinol binding protein 7, RETN – Resistin, RORA - Retinoid-related orphan receptor alpha gene, S100A12 - S100 Calcium Binding Protein A12, SASH1 - SAM- and SH3-domain containing 1 gene, SNORD1A - Small Nucleolar RNA, C/D Box 1A, SNORD64 - Small Nucleolar RNA, C/D Box 64, TCN1 - Transcobalamin 1, UPAR - Plasminogen Activator, Urokinase Receptor, VEGF A - Vascular Endothelial Growth Factor A, VEGF C - Vascular Endothelial Growth Factor C, YES1 - YES proto-oncogene 1, Src family tyrosine kinase, ZNF124 - Zinc finger protein 124