

Martínez-Usatorre et al. The magnitude of T cell receptor stimulation dictates microRNA-155 levels and its target signature correlates with survival in melanoma

Statistical tests main figures

Figure	EXP	Test	p-value	F (DFn, DFd)	Post-Hoc test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary	
1D	7, 42, 47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.3194	F (1, 37) = 1,019	P14 (WT) - gp276 (WT)					
		Row Factor	0.1085	F (1, 37) = 2,705	D7		-0.8547	-1,68 to -0,02968	0.0412	*
		Column Factor	0.0613	F (1, 37) = 3,724	D21		-0.2676	-1,343 to 0,808	0.8112	ns
1F	6, 7,42 and 47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	<0,0001	F (1, 49) = 17,95	P14 (WT) - P14 (MIX)					
		Row Factor	0.0013	F (1, 49) = 11,67	D7		-0.343	-0,9633 to 0,2773	0.3729	ns
		Column Factor	<0,0001	F (1, 49) = 33,16	D21		-2.252	-3,087 to -1,418	<0,0001	****
1G	6, 7,42 and 47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.3138	F (1, 40) = 1,041	gp276 (WT) - gp276 (MIX)					
		Row Factor	0.1095	F (1, 40) = 2,68	D7		0.6259	-0,2138 to 1,466	0.1737	ns
		Column Factor	0.2631	F (1, 40) = 1,288	D21		0.03339	-1,023 to 1,09	0.9966	ns
2A, Right	47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.0248	F (1, 8) = 7,594	SCR - miR-155					
		Row Factor	<0,0001	F (1, 8) = 624,7	WT (D7)		3.148	2,599 to 3,697	<0,0001	****
		Column Factor	0.0425	F (1, 8) = 5,809	MIX (D7)		3.928	3,379 to 4,477	<0,0001	****
2B, D7	47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.2551	F (1, 16) = 1,393	SCR - miR-155					
		Row Factor	<0,0001	F (1, 16) = 116,8	WT		-6.402	-8,722 to -4,082	<0,0001	****
		Column Factor	0.1343	F (1, 16) = 2,488	MIX		-7.972	-10,29 to -5,652	<0,0001	****
					WT - MIX					
					SCR		-0.264	-2,584 to 2,056	0.9527	ns
2B, D21	47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.0436	F (1, 16) = 4,802	SCR - miR-155					
		Row Factor	0.0248	F (1, 16) = 6,138	WT (D21)		-3.943	-6,89 to -0,9971	0.009	**
		Column Factor	0.0439	F (1, 16) = 4,784	MIX (D21)		-0.2417	-3,188 to 2,705	0.9751	ns
					WT (D21) - MIX (D21)					
					SCR		-0.00332	-2,95 to 2,943	>0,9999	ns
2C, left	47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.9566	F (1, 16) = 0,003053	WT - MIX					
		Row Factor	0.3341	F (1, 16) = 0,9917	SCR		-12647	-50103 to 24809	0.6604	ns
		Column Factor	0.2782	F (1, 16) = 1,26	miR-155		-11460	-48916 to 25996	0.7099	ns
2C, right	47	2way anova			Sidak's multiple comparison test		Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.2713	F (1, 16) = 1,298	WT (D20) - MIX (D20)					
		Row Factor	0.1406	F (1, 16) = 2,403	SCR		-21180	-63458 to 21098	0.4139	ns
		Column Factor	0.0107	F (1, 16) = 8,334	miR-155		-48800	-91078 to -6522	0.0232	*

2D	47	2way anova			Sidak's multiple comparison test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.3007		SCR - miR-155				
		Row Factor	<0,0001		WT (D7)	-1038	-1453 to -624,2	<0,0001	****
		Column Factor	0.0077		MIX (D7)	-784.4	-1199 to -370,2	0.0005	***
					WT (D7) - MIX (D7)				
					SCR	234.8	-179,4 to 649	0.3295	ns
			miR-155	488.8	74,6 to 903	0.0203	*		
2E, IFNg	47	2way anova			Sidak's multiple comparison test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.3307	F (1, 16) = 1,006	WT (D7) - MIX (D7)				
		Row Factor	0.0991	F (1, 16) = 3,065	SCR	-0.28	-7,652 to 7,092	0.9946	ns
		Column Factor	0.2728	F (1, 16) = 1,29	miR-155	-4.52	-11,89 to 2,852	0.2774	ns
					SCR - miR-155				
					WT (D7)	-1.58	-8,952 to 5,792	0.8434	ns
			MIX (D7)	-5.82	-13,19 to 1,552	0.1337	ns		
2E, TNFa	47	2way anova			Sidak's multiple comparison test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.3392	F (1, 16) = 0,9704	WT (D7) - MIX (D7)				
		Row Factor	0.008	F (1, 16) = 9,171	SCR	-7.242	-14,59 to 0,1021	0.0535	ns
		Column Factor	0.0004	F (1, 16) = 19,58	miR-155	-11.39	-18,73 to -4,046	0.003	**
					SCR - miR-155				
					WT (D7)	8.45	1,106 to 15,79	0.0236	*
			MIX (D7)	4.302	-3,042 to 11,65	0.3075	ns		
2E, GzB	47	2way anova			Sidak's multiple comparison test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.9175	F (1, 16) = 0,01108	WT (D7) - MIX (D7)				
		Row Factor	<0,0001	F (1, 16) = 92,27	SCR	-1.96	-9,913 to 5,993	0.7991	ns
		Column Factor	0.4616	F (1, 16) = 0,5692	miR-155	-1.48	-9,433 to 6,473	0.8792	ns
					SCR - miR-155				
					WT (D7)	-22.14	-30,09 to -14,19	<0,0001	****
			MIX (D7)	-21.66	-29,61 to -13,71	<0,0001	****		
2F, IFNg	47	2way anova			Sidak's multiple comparison test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.3476	F (1, 16) = 0,9363	WT (D7) - MIX (D7)				
		Row Factor	0.1636	F (1, 16) = 2,132	SCR	-929	-2500 to 642	0.3012	ns
		Column Factor	0.008	F (1, 16) = 9,183	miR-155	-1801	-3372 to -229,6	0.0241	*
					SCR - miR-155				
					WT (D7)	-221.8	-1793 to 1349	0.9283	ns
			MIX (D7)	-1093	-2664 to 477,6	0.1996	ns		
2F, TNFa	47	2way anova			Sidak's multiple comparison test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.9543	F (1, 16) = 0,003394	WT (D7) - MIX (D7)				
		Row Factor	0.007	F (1, 16) = 9,573	SCR	-312.6	-959,3 to 334,1	0.4383	ns
		Column Factor	0.1231	F (1, 16) = 2,65	miR-155	-291	-937,7 to 355,7	0.4865	ns
					SCR - miR-155				
					WT (D7)	-584.4	-1231 to 62,27	0.0794	ns
			MIX (D7)	-562.8	-1209 to 83,87	0.0927	ns		
2F, GzB	47	2way anova			Sidak's multiple comparison test	Mean Diff,	95,00% CI of diff,	Adjusted P Value	Summary
		Interaction	0.2379	F (1, 16) = 1,503	WT (D7) - MIX (D7)				
		Row Factor	<0,0001	F (1, 16) = 120,7	SCR	-34.6	-316,5 to 247,3	0.9453	ns
		Column Factor	0.1176	F (1, 16) = 2,736	miR-155	-232.8	-514,7 to 49,13	0.1137	ns
					SCR - miR-155				
					WT (D7)	-788.8	-1071 to -506,9	<0,0001	****
			MIX (D7)	-987	-1269 to -705,1	<0,0001	****		

Figure	EXP	Test	p-value	F (DFn, DFd)	Post-Hoc test	Mean Diff,	95,00% CI of diff,	Adjusted p-value	Summary
3C	18, 25 and 34	1 way-ANOVA	p-value	F	Tukey's multiple comparison test				
			<0,0001	29.47	D14 S OT-1 eff. vs. D14 T N4 OT-1 eff.	-1.729	-2,735 to -0,7226	<0,0001	****
					D14 S OT-1 eff. vs. D14 T T4 OT-1 eff.	-1.104	-2,136 to -0,07083	0.0294	*
					D14 S OT-1 eff. vs. D21 S OT-1 eff.	1.818	0,6684 to 2,967	0.0002	***
					D14 S OT-1 eff. vs. D21 T N4 OT-1 eff.	-1.823	-2,818 to -0,8279	<0,0001	****
					D14 S OT-1 eff. vs. D21 T T4OT-1 eff.	-0.7211	-1,754 to 0,3116	0.3267	ns
					D14 T N4 OT-1 eff. vs. D14 T T4 OT-1 eff.	0.6252	-0,2457 to 1,496	0.2967	ns
					D14 T N4 OT-1 eff. vs. D21 S OT-1 eff.	3.546	2,54 to 4,553	<0,0001	****
					D14 T N4 OT-1 eff. vs. D21 T N4 OT-1 eff.	-0.09427	-0,9203 to 0,7317	0.9994	ns
					D14 T N4 OT-1 eff. vs. D21 T T4OT-1 eff.	1.008	0,1368 to 1,879	0.0141	*
					D14 T T4 OT-1 eff. vs. D21 S OT-1 eff.	2.921	1,888 to 3,954	<0,0001	****
					D14 T T4 OT-1 eff. vs. D21 T N4 OT-1 eff.	-0.7195	-1,578 to 0,1387	0.1512	ns
					D14 T T4 OT-1 eff. vs. D21 T T4OT-1 eff.	0.3824	-0,519 to 1,284	0.8132	ns
					D21 S OT-1 eff. vs. D21 T N4 OT-1 eff.	-3.641	-4,636 to -2,645	<0,0001	****
					D21 S OT-1 eff. vs. D21 T T4OT-1 eff.	-2.539	-3,571 to -1,506	<0,0001	****
			D21 T N4 OT-1 eff. vs. D21 T T4OT-1 eff.	1.102	0,2438 to 1,96	0.0045	**		
3D N4		Pearsons correlation							
		Pearson r	-0.1239						
		P value	0.5143						
3D T4		Pearsons correlation							
		Pearson r	-0.4835						
		P value	0.0143						
3E N4		Pearsons correlation							
		Pearson r	0.338						
		P value	0.2825						
3E T4	34 and 37	Pearsons correlation							
		Pearson r	-0.1055						
		P value	0.7719						
3F N4		Pearsons correlation							
		Pearson r	0.4327						
		P value	0.2447						
3F T4	25	Pearsons correlation							
		Pearson r	0.5661						
		P value	0.0193						
3G	34 and 37	Shapiro-Wilk normality test	N4 (ISO)	N4 (anti-PD-1)	Unpaired t-test				
		W	0.9409	0.9469	t, df	t=6,248 df=21			
		P value	0.5101	0.6044	P value	<0,0001			
					Unpaired t-test				
		W	0.9086	0.9785	t, df	t=0,987 df=18			
		P value	0.2345	0.9561	P value	0.3367			
3H	34 and 37	Shapiro-Wilk normality test	N4 (ISO)	N4 (anti-PD-1)	Mann-Whitney test				
		W	0.9474	0.7339	Mann-Whitney U	56			
		P value	0.599	0.0013	P value	0.5658			
					Mann-Whitney test				
		W	0.8037	0.9296	Mann-Whitney U	41			
		P value	0.0105	0.4776	P value	0.5516			

Figure	EXP	Test	p-value	F (DFn, DFd)	Post-Hoc test			
4A		2-way RM ANOVA	p-value	F (DFn, DFd)	Sidak's multiple comparisons test	Mean Diff,	95,00% CI of diff.	Summary
		Interaction	0.2688	F (3, 60) = 1,344	HD (N=9) - Melanoma patients (N=13)			
		Time	<0,0001	F (3, 60) = 104,3	NAIVE	-0.2029	-1 to 0,5943	ns
		Column Factor	0.1818	F (1, 20) = 1,914	CM	-0.2451	-1,042 to 0,552	ns
		Subjects (matching)	<0,0001	F (20, 60) = 8,02	EM	-0.3441	-1,141 to 0,453	ns
					EMRA	-0.6846	-1,482 to 0,1126	ns
		2-way RM ANOVA	p-value	F (DFn, DFd)	Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff.	Summary
		Interaction	0.2688	F (3, 60) = 1,344	NAIVE vs. CM	-0.825	-1,171 to -0,4787	****
		Time	<0,0001	F (3, 60) = 104,3	NAIVE vs. EM	-2.045	-2,391 to -1,699	****
		Column Factor	0.1818	F (1, 20) = 1,914	NAIVE vs. EMRA	-1.953	-2,3 to -1,607	****
		Subjects (matching)	<0,0001	F (20, 60) = 8,02	CM vs. EM	-1.22	-1,566 to -0,8737	****
					CM vs. EMRA	-1.128	-1,475 to -0,7822	****
					EM vs. EMRA	0.09156	-0,2547 to 0,4378	ns
		4B		1 way-ANOVA	p-value	F	Dunn's multiple comparisons test	Rank sum diff
	0.036			10.24				
					naive vs. d1	8	ns	
					naive vs. d2	14	*	
					naive vs. d3	10	ns	
					naive vs. d4	7	ns	
					naive vs. d5	6	ns	
					d1 vs. d2	6	ns	
					d1 vs. d3	2	ns	
					d1 vs. d4	-1	ns	
					d1 vs. d5	-2	ns	
					d2 vs. d3	-4	ns	
					d2 vs. d4	-7	ns	
					d2 vs. d5	-8	ns	
4C, miR-155		Shapiro-Wilk normality test	PD-1+	PD-1-	Unpaired t-test			
		W	0.8023	0.9118	t, df	t=5,665 df=4		
		P value	0.0846	0.4787	P value	0.0048		
4C, miR-21		Shapiro-Wilk normality test	PD-1-	PD-1-	Unpaired t-test			
		W	0.945	0.8296	t, df	t=0,1467 df=4		
		P value	0.7014	0.1382	P value	0.8905		
4D		Spearman correlation						
		Spearman r	-0.6264					
		P value	0.0191					
		Number of XY Pairs	14					

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Statistical tests supplementary figures

Figure	EXP	Test								
S1 A	42	Shapiro-Wilk normality test	WT (D6)	MIX (D6)	Unpaired t-test					
		W	0.9663	0.8441	t, df	t=1,607 df=8				
		P value	0.8509	0.1764	P value	0.1468				
		Shapiro-Wilk normality test	WT (D20)	MIX (D20)	Unpaired t-test					
		W	1	0.9704	t, df	t=1,927 df=4				
		P value	>0,9999	0.67	P value	0.1263				
S1 B	7	Shapiro-Wilk normality test	WT (D7)	MIX (D7)	Unpaired t-test					
		W	0.9742	0.882	t, df	t=2,553 df=8				
		P value	0.9013	0.3184	P value	0.034				
		Shapiro-Wilk normality test	WT (D21)	MIX (D21)	Unpaired t-test					
		W	0.9462	0.9661	t, df	t=3,055 df=8				
		P value	0.7098	0.8495	P value	0.0157				
S1 C IFNg	7	Shapiro-Wilk normality test	WT (D7)	MIX (D7)	Unpaired t-test					
		W	0.8837	0.9801	t, df	t=3,701 df=8				
		P value	0.3264	0.9354	P value	0.006				
S1 C TNFa	7	Shapiro-Wilk normality test	WT (D7)	MIX (D7)	Unpaired t-test					
		W	0.9935	0.9031	t, df	t=2,823 df=8				
		P value	0.9904	0.4272	P value	0.0224				
S1 D IFNg	7	Shapiro-Wilk normality test	WT (D21)	MIX (D21)	Unpaired t-test					
		W	0.8689	0.9015	t, df	t=2,98 df=8				
		P value	0.262	0.4183	P value	0.0176				
S1 D TNFa	7	Shapiro-Wilk normality test	WT (D21)	MIX (D21)	Mann Whitney test					
		W	0.6522	0.8652	Mann-Whitney U	5				
		P value	0.0028	0.2476	P value	0.1508				
Figure	EXP	Test	p-value	F (DFn, DFd)	Post-Hoc test	Mean Diff,	95,00% CI of diff,	justed p-va	Summary	
S2E		1 way-ANOVA	p-value	F	Tukey's multiple comparison test					
			<0,0001	30.61	Melanocytes vs. Melanoma cell lines	-1.433	-6,218 to 3,352	0.9504	ns	
					Melanocytes vs. Naive CD8 PB	2.132	-2,63 to 6,895	0.7765	ns	
					Melanocytes vs. EM CD8 PB	4.11	-0,5734 to 8,793	0.118	ns	
					Melanocytes vs. EM CD8 TILN	7.081	2,408 to 11,75	0.0005	***	
					Melanocytes vs. EM CD8 Tumor	7.437	2,166 to 12,71	0.0013	**	
					Melanoma cell lines vs. Naive CD8 PB	3.565	1,139 to 5,992	0.0008	***	
					Melanoma cell lines vs. EM CD8 PB	5.543	3,275 to 7,81	<0,0001	****	
					Melanoma cell lines vs. EM CD8 TILN	8.513	6,269 to 10,76	<0,0001	****	
					Melanoma cell lines vs. EM CD8 Tumor	8.87	5,554 to 12,18	<0,0001	****	
					Naive CD8 PB vs. EM CD8 PB	1.978	-0,2414 to 4,197	0.1079	ns	
					Naive CD8 PB vs. EM CD8 TILN	4.948	2,753 to 7,144	<0,0001	****	
					Naive CD8 PB vs. EM CD8 Tumor	5.304	2,022 to 8,587	0.0002	***	
					EM CD8 PB vs. EM CD8 TILN	2.971	0,9524 to 4,989	0.0007	***	
					EM CD8 PB vs. EM CD8 Tumor	3.327	0,1603 to 6,493	0.0339	*	
					EM CD8 TILN vs. EM CD8 Tumor	0.3562	-2,794 to 3,506	0.9994	ns	