Soluble TIM3 and its ligands Galectin-9 and CEACAM1 are in disequilibrium during Alcohol-related Liver Disease and promote impairment of anti-bacterial immunity.

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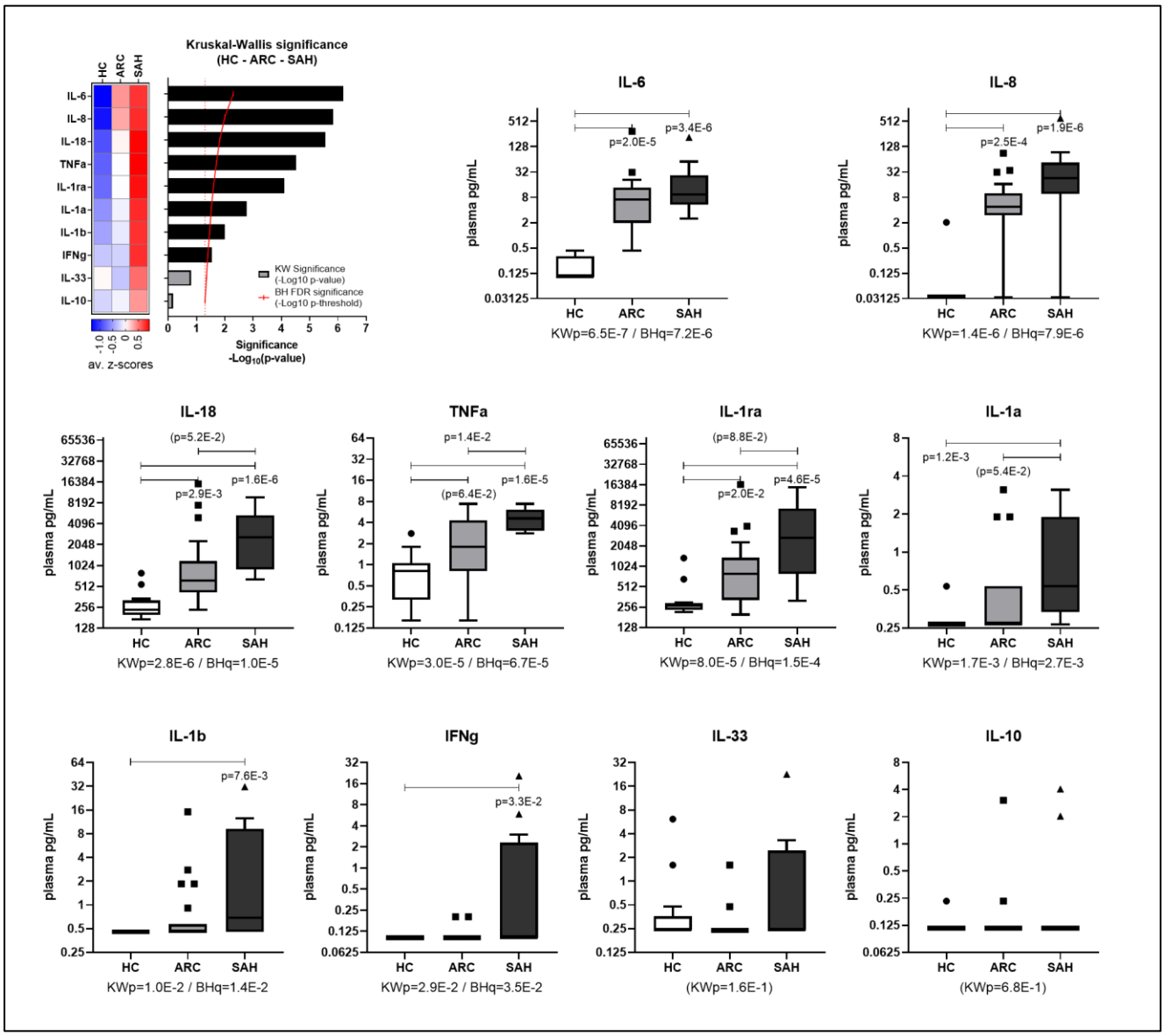
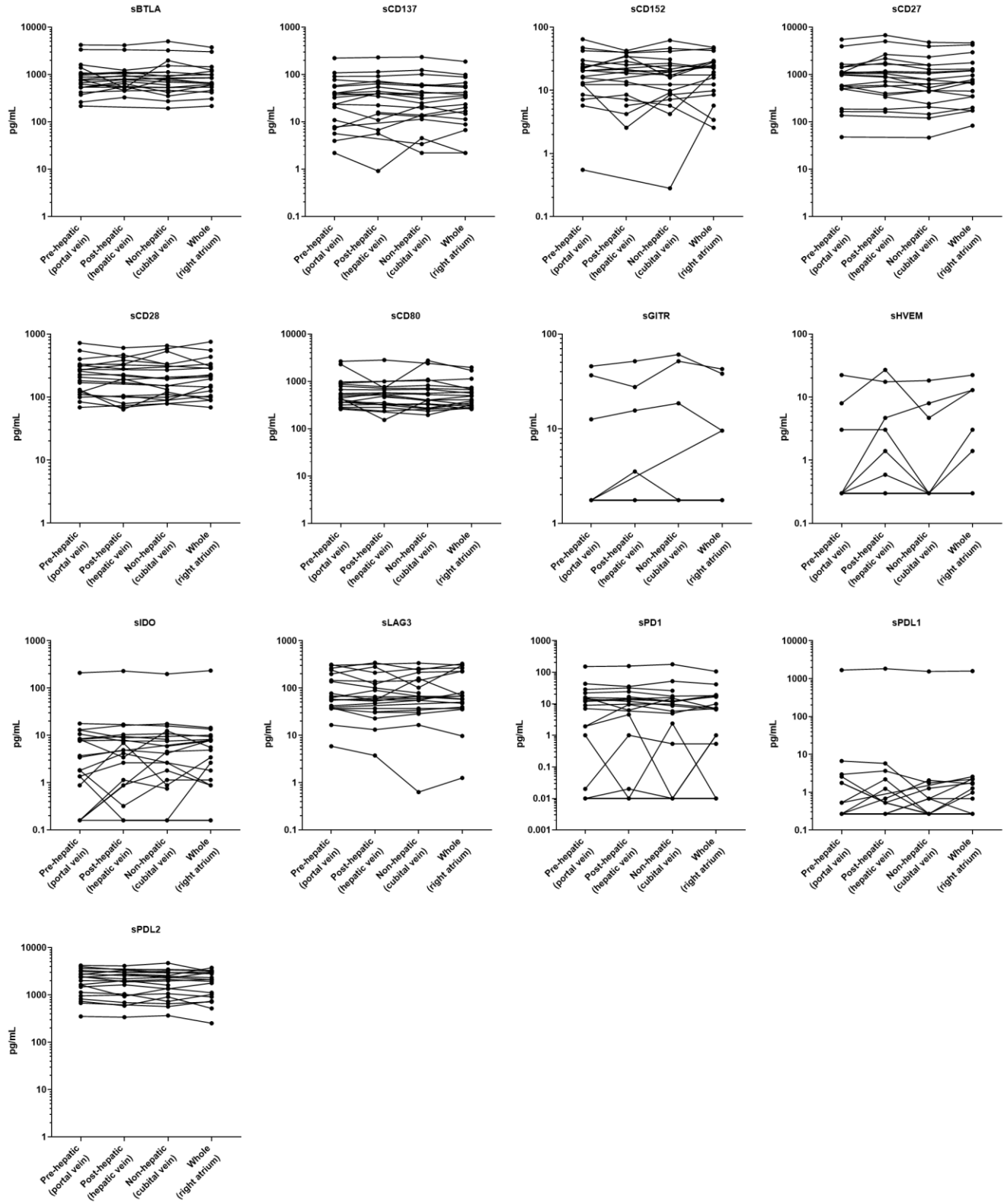
Short title: Soluble immune checkpoints in ALD
Keywords: TIM3; immune checkpoint; alcohol; biomarker

Supplementary Figures and Tables



Supplementary figure 1



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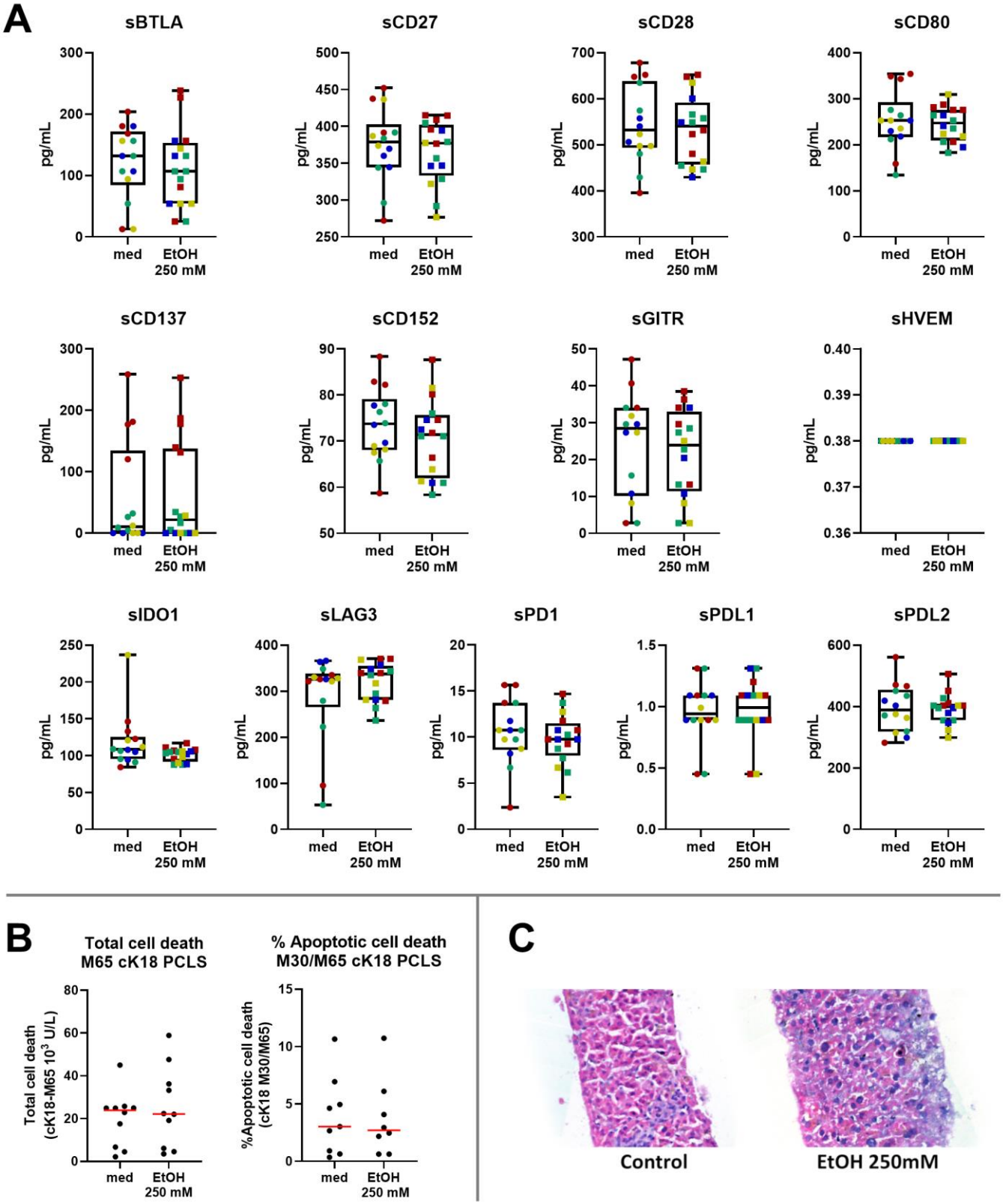

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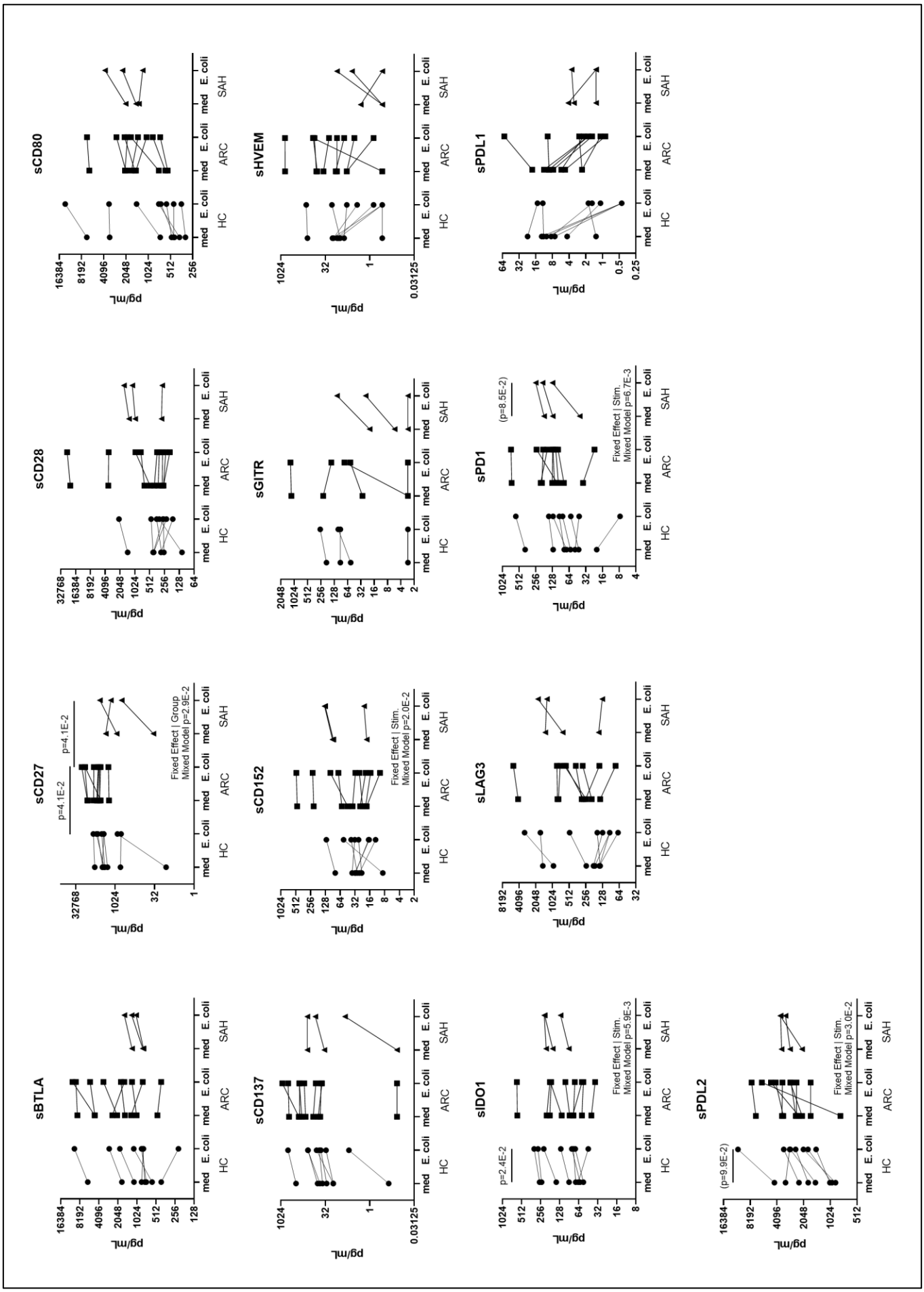

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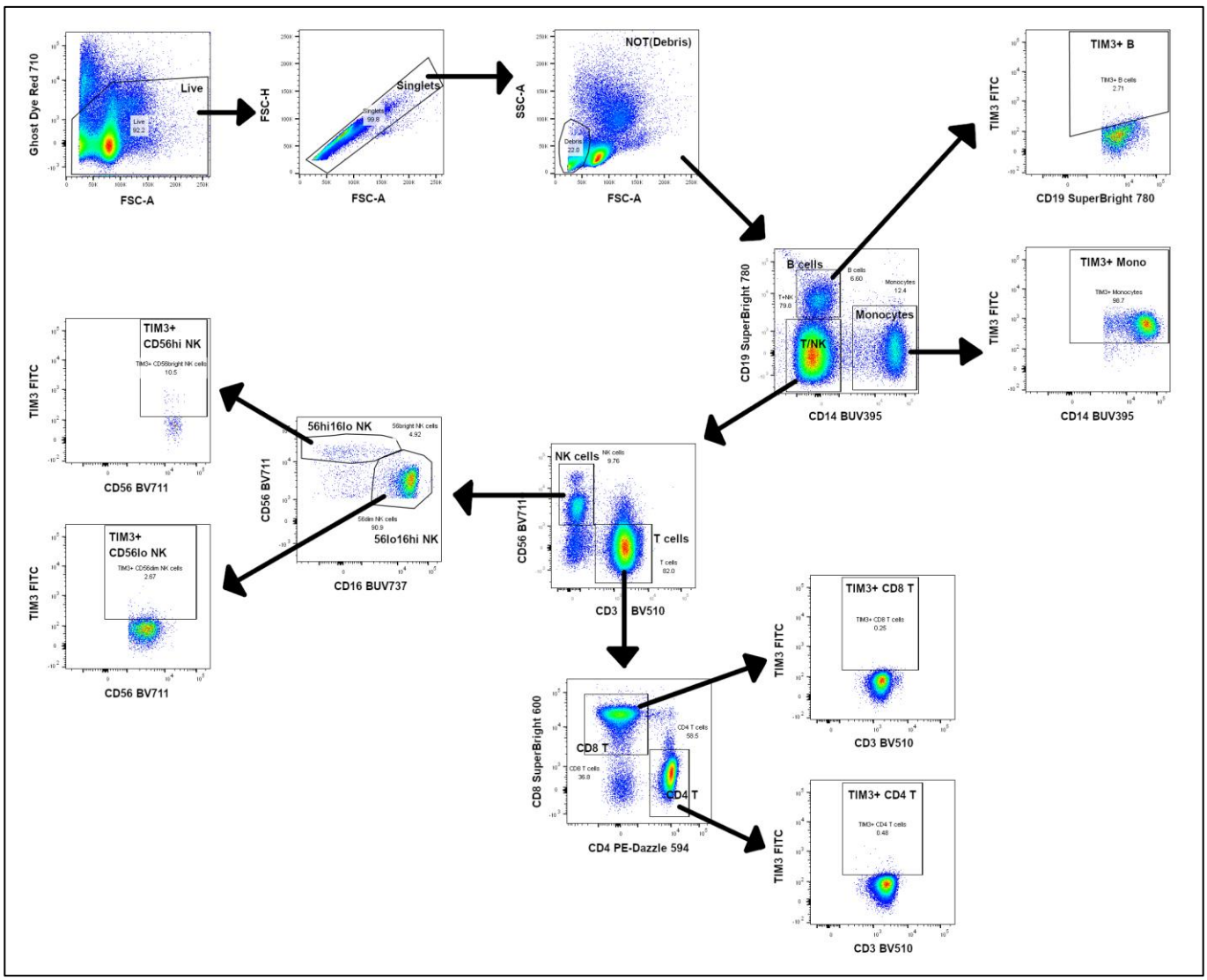

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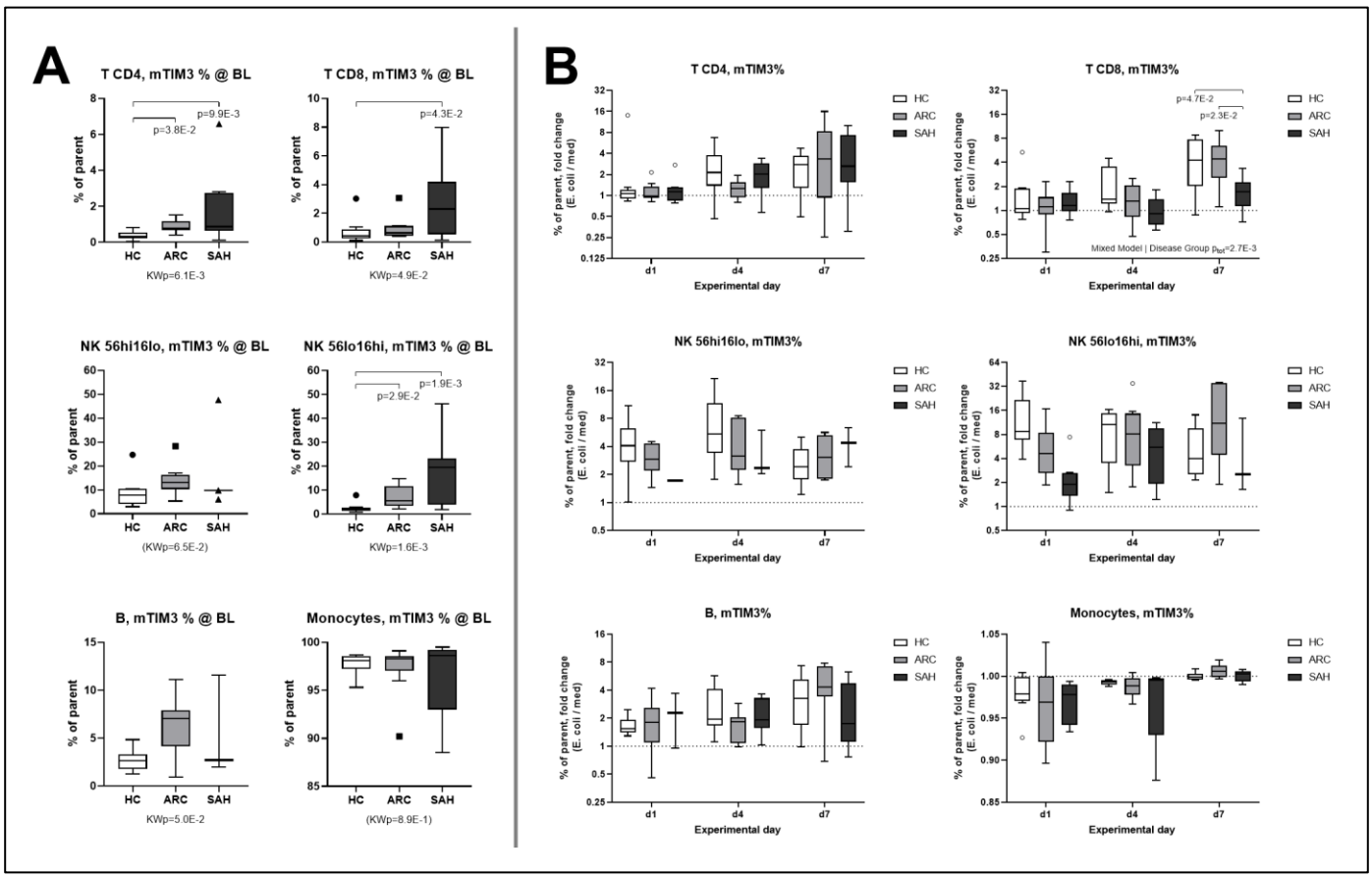

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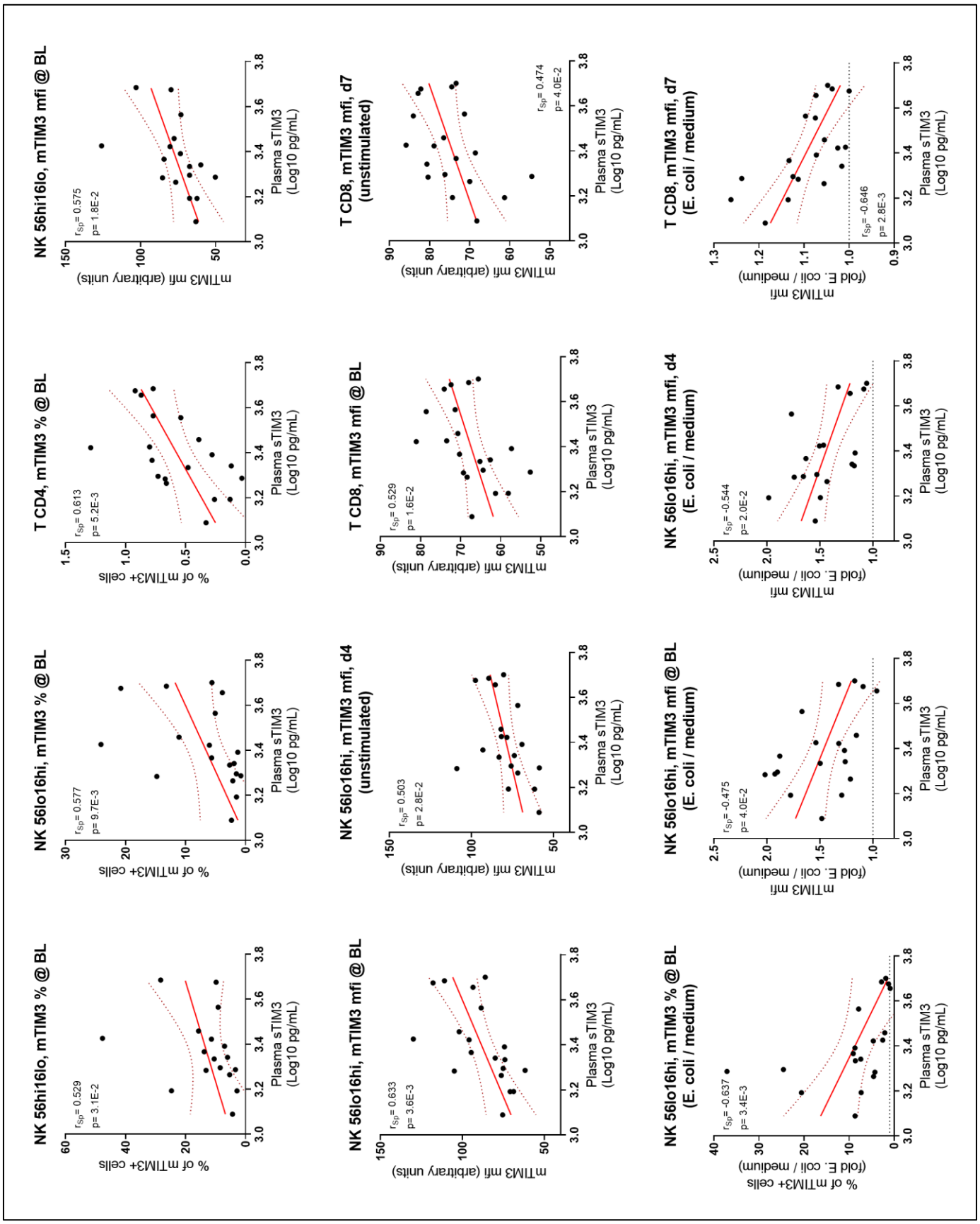

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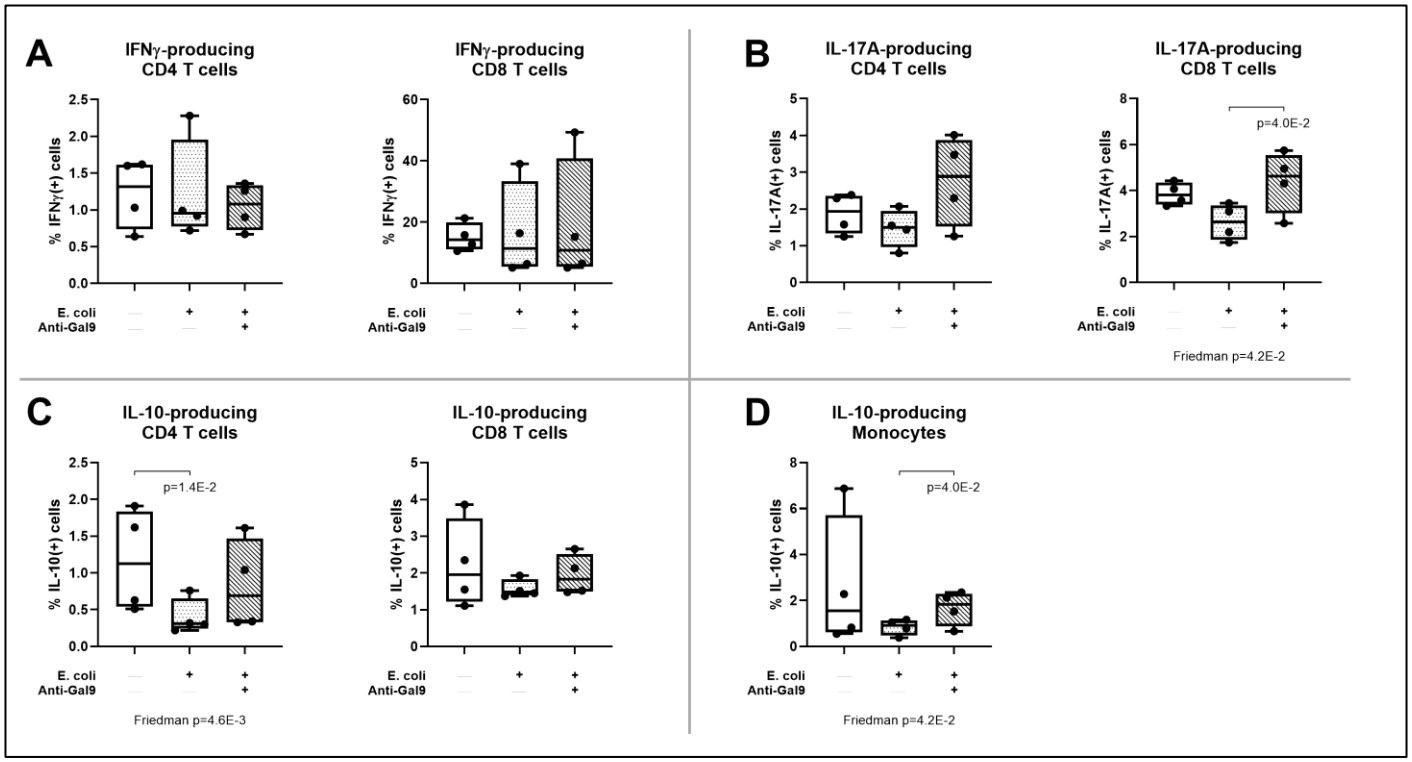

Supplementary figure 8

FACS panel for mTIM3 assessment						
Marker	**Color/Format**	**Clone**	**Laser/Ex Filter**	**Em Filter**	**Company**	**Catalog**
CD14	BUV395	MphiP9	UV	379/28	BD Biosciences	563561
CD16	BUV737	3G8	UV	740/35	BD Biosciences	612786
CD3	Brilliant Violet 510	UCHT1	V	525/50	BioLegend	300448
CD8a	SuperBright 600	SK1	V	610/20	Thermo Fisher Scientific	63-0087-42
CD56	Brilliant Violet 711	HCD56	V	710/50	BioLegend	318336
CD19	SuperBright 780	SJ25C1	V	780/60	Thermo Fisher Scientific	78-0198-42
TIM3	FITC	F38-2E2	B	530/30	Thermo Fisher Scientific	11-3109-42
CD4	PE-Dazzle 594	OKT4	YG	610/20	BioLegend	317448
Ghost Dye Red 710	Ghost Dye Red 710	N/A	R	730/45	Tonbo Biosciences (Cambridge Bioscience)	13-0871-T500

FACS panel for rhsTIM3-Ig cultures						
Marker	**Color/Format**	**Clone**	**Laser/Ex Filter**	**Em Filter**	**Company**	**Catalog**
CD3	Brilliant Violet 510	UCHT1	V	525/50	BioLegend	300448
IFNg	Alexa Fluor 488	4S.B3	B	530/30	BioLegend	502515
IL-10	PE	JES3-9D7	B	585/42	BioLegend	501404
CD8a	PerCP-eFluor710	SK1	B	670 LP	eBioscience	46-0087-42
IL-17	Alexa Fluor 647	SCPL1362	R	660/20	BD	560437
CD4	APC-eFluor780	OKT4	R	780/60	eBioscience	47-0048-42

FACS panel for anti-Galectin-9 antibody cultures						
Marker	**Color/Format**	**Clone**	**Laser/Ex Filter**	**Em Filter**	**Company**	**Catalog**
CD14	BUV395	MphiP9	UV	379/28	BD Biosciences	563561
CD3	Brilliant Violet 510	UCHT1	V	525/50	BioLegend	300448
CD8a	SuperBright 600	SK1	V	610/20	Thermo Fisher Scientific	63-0087-42
IL-17	Brilliant Violet 650	BL168	V	670/30	BioLegend	512304
IL-10	FITC	4S.B3	B	530/30	Thermo Fisher Scientific	BMS131-2FI
IFNg	PE	25723	YG	586/16	R&D	IC285P-100
CD4	PE-Dazzle 594	OKT4	YG	610/20	BioLegend	317448
Ghost Dye Red 710	Ghost Dye Red 710	N/A	R	730/45	Tonbo Biosciences (Cambridge Bioscience)	13-0871-T500

Supplementary table 1

	Stats	HC	ARC	SAH
sTIM3	#5	2057.57 (1663.47 – 2179.89)	3912.93 (2756.22 – 4730.43)	4522.30 (3589.62 – 4726.76)
sCD80	#4	441.21 (337.02 – 470.16)	1172.48 (649.75 – 1938.85)	1493.36 (1009.56 – 2422.43)
sLAG3	#1	51.67 (46.64 – 76.94)	124.67 (66.15 – 196.88)	90.58 (73.67 – 107.46)
sHVEM	#1	141.37 (101.72 – 388.40)	82.18 (56.78 – 141.83)	52.45 (38.73 – 87.48)
sPDL1	(#1)	4.59 (3.96 – 10.22)	10.74 (8.59 – 16.58)	10.22 (7.17 – 11.27)
sCD137		89.18 (60.29 – 121.59)	130.65 (95.05 – 204.27)	126.13 (93.88 – 153.04)
sCD27		1874.70 (1504.41 – 2362.32)	2764.04 (1468.85 – 4952.98)	2774.13 (2631.39 – 4898.27)
sPDL2		1642.96 (1231.25 – 1829.19)	2217.21 (1534.49 – 3303.72)	2800.48 (1857.30 – 3321.46)
sIDO		24.43 (20.84 – 43.22)	34.89 (30.63 – 68.67)	38.26 (26.65 – 47.29)
sGITR		13.68 (7.93 – 77.36)	83.18 (44.02 – 145.72)	36.78 (25.22 – 158.82)
sBTLA		1202.58 (980.29 – 1583.70)	1491.51 (1034.11 – 2129.54)	1118.88 (952.71 – 1555.23)
sCD28		144.26 (126.08 – 233.04)	212.55 (144.26 – 322.07)	156.28 (138.23 – 197.86)
sCD152		44.23 (37.94 – 62.00)	51.36 (43.33 – 65.31)	45.12 (38.84 – 55.80)
sPD1		59.13 (41.16 – 108.18)	89.11 (47.57 – 163.74)	66.00 (48.52 – 105.04)

All measurements are in pg/mL. Data expressed as median (IQR).

= significant difference between HC/ARC/SAH (Benjamini-Hochberg (BH) adjusted Kruskal-Wallis test)

1 symbol: BHq≤0.05 / 2 symbols: BHq≤0.01 / 3 symbols: BHq≤0.005 / 4 symbols: BHq≤0.001 / 5 symbols: BHq≤0.0005

(symbol) = non-significant trend, (p≤0.05) but BH-adjusted q>0.05

Supplementary table 2

Correlations

p-value	Log10_sBTLA Sig. (2-tailed)	Log10_sCD137 Sig. (2-tailed)	Log10_sCD152 Sig. (2-tailed)	Log10_sCD27 Sig. (2-tailed)	Log10_sCD28 Sig. (2-tailed)	Log10_sCD80 Sig. (2-tailed)	Log10_sGITR Sig. (2-tailed)	Log10_sHVEM Sig. (2-tailed)	Log10_sIDO Sig. (2-tailed)	Log10_sLAG3 Sig. (2-tailed)	Log10_sPD1 Sig. (2-tailed)	Log10_sPDL1 Sig. (2-tailed)	Log10_sPDL2 Sig. (2-tailed)	Log10_sTIM3 Sig. (2-tailed)
Log10_sBTLA		1.14E-06	1.18E-06	6.74E-02	2.67E-06	1.45E-01	1.10E-05	8.82E-08	3.71E-02	6.66E-03	7.01E-07	2.29E-04	3.16E-04	2.62E-03
Log10_sCD137	1.14E-06		1.86E-10	1.78E-01	5.03E-11	1.62E-01	1.84E-06	1.61E-05	5.66E-03	3.85E-03	1.28E-06	3.53E-07	3.28E-03	1.04E-02
Log10_sCD152	1.18E-06	1.86E-10		3.65E-02	4.02E-16	4.92E-02	2.27E-07	9.73E-08	4.97E-03	2.68E-03	1.51E-07	2.50E-09	9.43E-05	3.84E-04
Log10_sCD27	6.74E-02	1.78E-01	3.65E-02		2.23E-01	7.21E-01	2.74E-01	1.32E-01	5.40E-01	7.67E-01	2.81E-01	1.26E-02	6.69E-02	5.63E-05
Log10_sCD28	2.67E-06	5.03E-11	4.02E-16	2.23E-01		1.97E-01	4.29E-09	1.12E-07	7.30E-04	4.45E-04	1.95E-08	1.23E-10	3.83E-03	2.81E-03
Log10_sCD80	1.45E-01	1.62E-01	4.92E-02	7.21E-01	1.97E-01		2.27E-01	7.56E-01	9.20E-02	2.91E-02	5.33E-02	1.62E-01	1.47E-03	2.74E-02
Log10_sGITR	1.10E-05	1.84E-06	2.27E-07	2.74E-01	4.29E-09	2.27E-01		3.87E-07	1.27E-03	1.89E-02	2.29E-15	2.16E-06	6.48E-02	2.07E-02
Log10_sHVEM	8.82E-08	1.61E-05	9.73E-08	1.32E-01	1.12E-07	7.56E-01	3.87E-07		2.60E-03	7.99E-02	1.26E-06	5.30E-04	1.81E-02	5.20E-02
Log10_sIDO	3.71E-02	5.66E-03	4.97E-03	5.40E-01	7.30E-04	9.20E-02	1.27E-03	2.60E-03		6.68E-02	3.44E-03	6.58E-03	6.88E-02	1.25E-01
Log10_sLAG3	6.66E-03	3.85E-03	2.68E-03	7.67E-01	4.45E-04	2.91E-02	1.89E-02	7.99E-02	6.68E-02		5.49E-03	3.15E-03	3.29E-02	3.99E-02
Log10_sPD1	7.01E-07	1.28E-06	1.51E-07	2.81E-01	1.95E-08	5.33E-02	2.29E-15	1.26E-06	3.44E-03	5.49E-03		1.04E-05	2.16E-02	2.06E-02
Log10_sPDL1	2.29E-04	3.53E-07	2.50E-09	1.26E-02	1.23E-10	1.62E-01	2.16E-06	5.30E-04	6.58E-03	3.15E-03	1.04E-05		1.13E-02	4.48E-04
Log10_sPDL2	3.16E-04	3.28E-03	9.43E-05	6.69E-02	3.83E-03	1.47E-03	6.48E-02	1.81E-02	6.88E-02	3.29E-03	2.16E-02	1.13E-02		3.51E-05
Log10_sTIM3	2.62E-03	1.04E-02	3.84E-04	5.63E-05	2.81E-03	2.74E-02	2.07E-02	5.20E-02	1.25E-01	3.99E-02	2.06E-02	4.48E-04	3.51E-05	

corr. coeff.	Log10_sBTLA Pearson Correlation	Log10_sCD137 Pearson Correlation	Log10_sCD152 Pearson Correlation	Log10_sCD27 Pearson Correlation	Log10_sCD28 Pearson Correlation	Log10_sCD80 Pearson Correlation	Log10_sGITR Pearson Correlation	Log10_sHVEM Pearson Correlation	Log10_sIDO Pearson Correlation	Log10_sLAG3 Pearson Correlation	Log10_sPD1 Pearson Correlation	Log10_sPDL1 Pearson Correlation	Log10_sPDL2 Pearson Correlation	Log10_sTIM3 Pearson Correlation
Log10_sBTLA	1.000	0.719	0.718	0.313	0.701	0.252	0.669	0.765	0.354	0.450	0.728	0.584	0.574	0.493
Log10_sCD137	0.719	1.000	0.844	0.233	0.857	0.242	0.709	0.660	0.458	0.476	0.717	0.741	0.483	0.428
Log10_sCD152	0.718	0.844	1.000	0.355	0.932	0.335	0.749	0.763	0.464	0.492	0.756	0.815	0.612	0.567
Log10_sCD27	0.313	0.233	0.355	1.000	0.211	-0.063	0.190	0.260	-0.107	-0.052	0.188	0.418	0.313	0.627
Log10_sCD28	0.701	0.857	0.932	0.211	1.000	0.223	0.808	0.761	0.544	0.562	0.788	0.848	0.476	0.490
Log10_sCD80	0.252	0.242	0.335	-0.063	0.223	1.000	0.210	0.054	0.523	0.369	0.329	0.242	0.517	0.373
Log10_sGITR	0.669	0.709	0.749	0.190	0.808	0.210	1.000	0.740	0.493	0.395	0.924	0.706	0.316	0.390
Log10_sHVEM	0.765	0.660	0.763	0.260	0.761	0.054	0.740	1.000	0.493	0.300	0.717	0.556	0.397	0.331
Log10_sIDO	0.354	0.458	0.464	-0.107	0.544	0.289	0.523	0.493	1.000	0.313	0.481	0.451	0.311	0.264
Log10_sLAG3	0.450	0.476	0.492	-0.052	0.562	0.369	0.395	0.300	0.313	1.000	0.459	0.485	0.483	0.349
Log10_sPD1	0.728	0.717	0.756	0.188	0.788	0.329	0.924	0.717	0.481	0.459	1.000	0.671	0.387	0.390
Log10_sPDL1	0.584	0.741	0.815	0.418	0.848	0.242	0.706	0.556	0.451	0.485	0.671	1.000	0.423	0.562
Log10_sPDL2	0.574	0.483	0.612	0.313	0.476	0.517	0.316	0.397	0.311	0.483	0.387	0.423	1.000	0.640
Log10_sTIM3	0.493	0.428	0.567	0.627	0.490	0.373	0.390	0.331	0.264	0.349	0.390	0.562	0.640	1.000

Supplementary table 3

Correlations

p-value	Log10_sBTLA	Log10_sCD137	Log10_sCD152	Log10_sCD27	Log10_sCD28	Log10_sCD80	Log10_sGITR	Log10_sHVEM	Log10_sIDO	Log10_sLAG3	Log10_sPD1	Log10_sPDL1	Log10_sPDL2	Log10_sTIM3
	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)	Sig. (2-tailed)
Log10_sBTLA		6.29E-05	3.65E-08	8.66E-01	9.38E-10	1.83E-05	4.28E-05	1.09E-06	2.67E-05	3.31E-04	7.84E-07	5.30E-03	1.56E-02	9.43E-01
Log10_sCD137	6.29E-05		7.53E-07	4.13E-01	5.51E-06	6.72E-07	2.27E-04	2.36E-03	3.34E-07	1.05E-05	1.88E-05	3.40E-02	5.34E-04	8.90E-01
Log10_sCD152	3.65E-08	7.53E-07		6.43E-01	7.94E-13	8.62E-07	8.57E-06	4.76E-05	2.72E-06	7.19E-05	9.11E-07	8.19E-03	1.53E-03	9.89E-01
Log10_sCD27	8.66E-01	4.13E-01	6.43E-01		7.04E-01	3.83E-01	7.61E-01	7.54E-01	5.91E-01	6.21E-01	6.77E-01	4.62E-02	4.08E-01	7.31E-04
Log10_sCD28	9.38E-10	5.51E-06	7.94E-13	7.04E-01		1.92E-06	1.59E-05	1.34E-05	7.16E-06	1.06E-04	4.20E-07	7.10E-03	3.81E-03	9.95E-01
Log10_sCD80	1.83E-05	6.72E-07	8.62E-07	3.83E-01	1.92E-06		8.81E-04	1.08E-03	3.89E-06	7.55E-07	3.62E-06	3.36E-02	5.08E-04	9.77E-01
Log10_sGITR	4.28E-05	2.27E-04	8.57E-06	7.61E-01	1.59E-05	8.81E-04		1.15E-03	2.95E-05	2.76E-03	1.31E-04	8.78E-04	4.17E-03	4.48E-01
Log10_sHVEM	1.09E-06	2.36E-03	4.76E-05	7.54E-01	1.34E-05	1.08E-03	1.15E-03		3.06E-03	8.48E-03	9.31E-05	4.12E-02	4.17E-02	6.83E-01
Log10_sIDO	2.67E-05	3.34E-07	2.72E-06	5.91E-01	7.16E-06	1.08E-03	2.95E-05	3.06E-03		6.32E-05	2.65E-06	1.41E-02	2.92E-03	8.14E-01
Log10_sLAG3	3.31E-04	1.05E-05	7.19E-05	6.21E-01	7.16E-06	3.89E-06	2.76E-03	8.48E-03	6.32E-05		1.96E-04	1.91E-02	1.33E-03	7.64E-01
Log10_sPD1	7.84E-07	1.88E-05	9.11E-07	6.77E-01	4.20E-07	3.62E-06	1.31E-04	9.31E-05	2.65E-06	1.96E-04		1.36E-02	4.95E-03	9.06E-01
Log10_sPDL1	5.30E-03	3.40E-02	8.19E-03	4.62E-02	7.10E-03	3.36E-02	8.78E-04	4.12E-02	1.41E-02	1.91E-02	1.36E-02		1.12E-01	2.29E-02
Log10_sPDL2	1.56E-02	5.34E-04	1.53E-03	4.08E-01	3.81E-03	5.08E-04	4.17E-03	4.17E-02	2.92E-03	1.33E-03	4.95E-03	1.12E-01		7.06E-01
Log10_sTIM3	9.43E-01	8.90E-01	9.89E-01	7.31E-04	9.95E-01	9.77E-01	4.48E-01	6.83E-01	8.14E-01	7.64E-01	9.06E-01	2.29E-02	7.06E-01	

corr. coeff.	Log10_sBTLA	Log10_sCD137	Log10_sCD152	Log10_sCD27	Log10_sCD28	Log10_sCD80	Log10_sGITR	Log10_sHVEM	Log10_sIDO	Log10_sLAG3	Log10_sPD1	Log10_sPDL1	Log10_sPDL2	Log10_sTIM3
	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation
Log10_sBTLA	1.000	0.883	0.971	-0.052	0.985	0.907	0.891	0.945	0.901	0.839	0.949	0.722	0.653	0.022
Log10_sCD137	0.883	1.000	0.949	-0.249	0.926	0.950	0.851	0.764	0.956	0.917	0.907	0.590	0.824	-0.042
Log10_sCD152	0.971	0.949	1.000	-0.142	0.996	0.948	0.920	0.889	0.935	0.880	0.947	0.696	0.783	0.004
Log10_sCD27	-0.052	-0.249	-0.142	1.000	-0.117	-0.264	0.094	-0.097	-0.165	-0.151	-0.128	0.561	-0.251	0.813
Log10_sCD28	0.985	0.926	0.996	-0.117	1.000	0.939	0.910	0.913	0.922	0.871	0.954	0.705	0.740	0.002
Log10_sCD80	0.907	0.950	0.948	-0.264	0.939	1.000	0.806	0.798	0.931	0.949	0.932	0.591	0.826	-0.009
Log10_sGITR	0.891	0.851	0.920	0.094	0.910	0.806	1.000	0.795	0.899	0.757	0.866	0.806	0.736	0.231
Log10_sHVEM	0.945	0.764	0.889	-0.097	0.913	0.798	0.795	1.000	0.751	0.694	0.874	0.572	0.571	-0.125
Log10_sIDO	0.901	0.956	0.935	-0.165	0.922	0.931	0.899	0.751	1.000	0.883	0.936	0.660	0.754	0.073
Log10_sLAG3	0.839	0.917	0.880	-0.151	0.871	0.949	0.757	0.694	0.883	1.000	0.855	0.638	0.789	0.093
Log10_sPD1	0.949	0.907	0.947	-0.128	0.954	0.932	0.866	0.874	0.936	0.855	1.000	0.663	0.726	0.036
Log10_sPDL1	0.722	0.590	0.696	0.561	0.705	0.591	0.806	0.572	0.660	0.638	0.663	1.000	0.461	0.623
Log10_sPDL2	0.653	0.824	0.783	-0.251	0.740	0.826	0.736	0.571	0.754	0.789	0.726	0.461	1.000	0.116
Log10_sTIM3	0.022	-0.042	0.004	0.813	0.002	-0.009	0.231	-0.125	0.073	0.093	0.036	0.623	0.116	1.000

Supplementary table 4

Coefficients (a,b)

Model	Collinearity Statistics	
	VIF	Tolerance (1 / VIF)
(Intercept)	---	---
Log10_sBTLA	6.068	0.165
Log10_sCD137	6.848	0.146
Log10_sCD152	29.904	0.033
Log10_sCD27	4.419	0.226
Log10_sCD28	34.009	0.029
Log10_sCD80	3.128	0.320
Log10_sGITR	6.595	0.152
Log10_sHVEM	9.329	0.107
Log10_sIDO	2.559	0.391
Log10_sLAG3	3.013	0.332
Log10_sPD1	6.372	0.157
Log10_sPDL1	6.879	0.145
Log10_sPDL2	4.059	0.246
Log10_sTIM3	5.670	0.176

a. Dependent Variable: Group (HC / ARC / SAH)

b. VIF > 5 indicates the presence of multicollinearity (the higher the stronger)

Supplementary table 5

Correlations

p-value	ChildPugh Score	MELD-Score	MaddreyDF	Creatinine (mmol/L)	TotalBilirubin (mmol/L)	AST	GGT	ALP	Albumin (g/dL)	INR	Na (mmol/L)	Platelets (billion/L)	Total Leukocytes (billion/L)	Neutrophils (billion/L)	Lymphocytes (billion/L)	Monocytes (billion/L)	Neutrophil-to-Lymphocyte ratio (NLR)	Monocyte-to-Lymphocyte ratio (MLR)	Log_IL1a	Log_IL1b	Log_IL1ra	Log_IL6	Log_IL8	Log_IL10	Log_IL18	Log_IL33	Log_IFNg	Log_TNFa	Log_Glucose	Log_sCD163
Sig. (2-tailed)	1.4E-03	1.5E-03	1.3E-01	3.7E-01	6.8E-01	9.3E-01	4.0E-01	6.1E-01	4.1E-02	6.5E-02	8.1E-02	9.6E-01	6.6E-01	6.4E-01	1.3E-01	1.7E-01	5.3E-01	2.0E-02	7.5E-02	3.0E-01	1.2E-01	3.6E-01	8.3E-01	7.2E-01	1.5E-01	1.6E-01	1.6E-01	1.0E-01	1.6E-02	3.0E-01
Log_CD80	5.2E-04	3.7E-02	6.2E-01	1.3E-01	2.1E-01	4.4E-01	8.7E-01	9.9E-01	2.4E-05	7.5E-03	8.2E-01	6.2E-01	9.7E-01	7.0E-01	4.5E-01	7.0E-02	8.2E-01	6.4E-02	1.5E-01	1.0E-03	5.2E-02	2.8E-01	2.3E-01	9.7E-01	2.8E-01	1.4E-02	1.2E-01	8.5E-03	2.3E-02
Log_sIA2	4.4E-03	2.3E-02	6.2E-01	5.4E-01	0.94E-01	2.1E-01	3.4E-02	8.9E-01	1.3E-05	1.9E-03	7.1E-01	6.4E-01	3.1E-01	6.4E-01	1.0E-01	1.0E-01	6.4E-01	1.0E-01	3.1E-01	1.5E-01	2.7E-01	1.7E-01	3.2E-01	8.8E-01	9.8E-02	2.1E-01	1.1E-01	3.4E-01	7.7E-01	5.2E-01
Log_sPD1	2.9E-03	1.6E-02	9.3E-01	8.8E-01	7.3E-01	7.3E-01	4.9E-03	2.3E-01	1.1E-01	4.9E-03	6.3E-02	6.8E-02	3.3E-02	8.7E-01	7.4E-01	1.0E-01	8.7E-01	1.4E-01	3.9E-01	1.9E-01	2.9E-03	1.7E-01	3.2E-01	8.8E-01	7.9E-01	3.4E-01	3.4E-01	7.9E-01	1.9E-01	5.2E-01
Log_sPDO	1.2E-01	7.9E-01	8.3E-01	3.2E-01	4.2E-01	1.0E-01	6.1E-01	8.9E-01	3.8E-01	8.3E-01	8.9E-01	3.8E-01	9.0E-01	9.3E-01	7.4E-01	3.6E-01	7.4E-01	3.6E-01	3.9E-01	1.9E-01	2.9E-03	1.2E-01	6.6E-01	5.3E-01	2.9E-03	7.8E-01	5.0E-01	7.9E-01	1.2E-01	8.8E-01
Log_sPDL1	9.6E-02	1.6E-01	8.3E-01	9.3E-01	4.5E-01	1.1E-01	5.5E-02	4.4E-01	1.4E-01	9.6E-02	6.2E-01	6.4E-01	6.4E-01	8.9E-01	9.0E-01	3.8E-01	8.3E-01	4.4E-01	8.9E-01	6.8E-01	9.2E-01	3.3E-01	3.3E-01	4.5E-01	3.8E-01	3.3E-01	3.7E-01	5.3E-01	1.4E-01	1.7E-01
Log_sGITR	1.6E-01	4.8E-01	8.7E-01	6.0E-01	7.5E-01	9.1E-01	4.7E-02	1.6E-01	4.6E-01	2.0E-01	3.8E-01	2.6E-01	6.6E-01	1.0E+00	4.3E-01	4.7E-01	6.5E-01	7.0E-01	5.9E-01	2.3E-01	8.3E-01	9.4E-01	6.5E-01	5.8E-01	2.6E-01	8.7E-01	7.4E-01	6.3E-01	2.6E-01	4.7E-01
Log_sCD28	1.3E-01	7.6E-01	8.3E-01	5.7E-01	6.2E-01	9.3E-01	6.3E-02	5.2E-02	5.3E-01	1.5E-01	5.3E-01	1.4E-01	1.8E-01	1.4E-01	8.3E-01	5.9E-01	3.2E-01	9.4E-01	8.3E-01	7.0E-01	9.4E-01	7.0E-01	4.7E-01	2.0E-01	3.0E-01	9.4E-01	7.4E-01	4.8E-01	3.7E-01	1.2E-01
Log_sHVEM	7.2E-02	2.5E-01	4.0E-01	2.1E-01	8.1E-01	5.0E-01	1.7E-01	2.0E-01	6.5E-01	7.3E-02	9.7E-01	3.2E-01	6.9E-02	2.9E-01	2.6E-01	5.9E-01	9.7E-01	5.9E-01	9.2E-01	4.4E-01	2.3E-01	4.4E-01	1.3E-01	4.8E-01	4.0E-01	6.4E-01	7.7E-01	4.3E-01	7.6E-01	6.8E-01
Log_sBTLA	7.2E-02	5.0E-01	5.3E-01	4.1E-01	7.5E-01	8.9E-01	2.5E-01	2.2E-01	4.0E-01	1.1E-01	4.0E-01	9.3E-01	2.0E-01	1.6E-01	2.0E-01	9.8E-01	2.0E-01	9.8E-01	9.4E-01	9.3E-01	9.3E-01	9.4E-01	4.7E-01	5.8E-01	5.0E-01	8.4E-01	9.5E-01	7.2E-01	2.3E-01	1.9E-01
Log_sCD152	7.2E-02	6.2E-01	7.9E-01	9.4E-01	7.2E-01	7.4E-01	3.1E-01	1.7E-01	5.4E-01	5.1E-01	2.2E-01	7.9E-01	2.5E-01	8.4E-01	2.2E-01	8.8E-01	4.8E-01	8.8E-01	9.2E-01	7.7E-01	7.7E-01	9.4E-01	5.2E-01	5.3E-01	6.1E-01	5.5E-01	8.4E-01	5.5E-01	7.2E-01	8.4E-01
Log_sCD137	7.8E-02	4.1E-01	8.3E-01	7.9E-01	9.4E-01	7.2E-01	7.9E-01	1.7E-01	5.4E-01	2.2E-01	5.1E-01	7.9E-01	2.5E-01	8.4E-01	4.8E-01	8.8E-01	2.2E-01	8.8E-01	9.2E-01	7.7E-01	7.7E-01	9.4E-01	5.2E-01	5.3E-01	6.1E-01	5.5E-01	8.4E-01	5.5E-01	7.2E-01	8.4E-01
Log_Glucose	1.2E-03	2.2E-03	1.0E+00	5.7E-01	4.0E-05	3.5E-01	9.6E-01	3.5E-01	8.4E-02	1.0E-01	3.0E-02	5.5E-01	3.6E-02	4.9E-02	3.2E-01	6.7E-03	1.6E-01	2.6E-01	1.5E-05	9.7E-04	2.2E-04	4.4E-02	1.1E-02	8.2E-03	3.0E-04	4.6E-03	6.8E-03	1.3E-02		
Log_sCD163	7.3E-04	3.6E-02	1.5E-01	5.5E-01	4.8E-02	2.2E-01	7.9E-01	3.4E-01	4.7E-02	7.7E-02	2.3E-01	3.3E-01	6.6E-01	6.5E-01	6.4E-01	8.4E-01	5.8E-01	9.2E-01	4.0E-01	7.8E-01	8.3E-01	1.4E-01	4.7E-02	1.7E-01	2.8E-01	3.2E-01	4.6E-01	2.2E-01		

corr. coeff.	ChildPugh Score	MELD-Score	MaddreyDF	Creatinine (mmol/L)	TotalBilirubin (mmol/L)	AST	GGT	ALP	Albumin (g/dL)	INR	Na (mmol/L)	Platelets (billion/L)	Total Leukocytes (billion/L)	Neutrophils (billion/L)	Lymphocytes (billion/L)	Monocytes (billion/L)	Neutrophil-to-Lymphocyte ratio (NLR)	Monocyte-to-Lymphocyte ratio (MLR)	Log_IL1a	Log_IL1b	Log_IL1ra	Log_IL6	Log_IL8	Log_IL10	Log_IL18	Log_IL33	Log_IFNg	Log_TNFa	Log_Glucose	Log_sCD163
	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation	Pearson Correlation
Log_sTIM3	0.441	0.354	0.153	0.270	0.211	0.155	0.033	0.001	0.042	-0.046	0.102	0.088	-0.083	-0.113	-0.132	0.320	0.087	0.307	0.253	0.434	0.337	0.191	0.210	-0.007	0.391	0.328	0.419	0.269	0.444	0.388
Log_sCD80	0.354	0.170	0.523	0.109	0.001	0.254	0.461	0.296	-0.231	0.001	0.194	0.102	-0.082	-0.121	-0.054	-0.403	-0.156	-0.014	0.133	-0.111	-0.231	-0.243	0.328	-0.233	-0.229	0.041	-0.202	-0.366	0.069	0.224
Log_sIA2	0.525	0.404	0.034	0.195	0.035	0.069	0.071	0.231	0.272	0.429	0.680	0.461	0.121	0.082	0.454	0.403	0.156	0.014	0.133	0.179	0.164	0.196	0.242	0.174	0.044	0.288	0.168	0.169	0.175	0.115
Log_sPD1	0.268	0.178	0.006	0.195	0.002	0.102	0.035	0.034	0.009	0.158	0.015	0.022	0.037	0.013	0.062	0.013	0.055	0.028	0.388	0.064	0.014	0.065	0.099	0.063	0.111	0.053	0.111	0.068	0.175	0.158
Log_sPDL1	0.310	0.244	0.075	0.025	0.010	0.151	0.317	0.333	0.136	0.236	0.330	0.312	0.087	0.069	0.008	0.158	0.022	0.139	0.025	0.086	0.016	0.172	0.173	0.134	0.165	0.154	0.359	0.112	0.257	0.239
Log_sCD27	0.132	0.394	0.132	0.341	0.158	0.007	0.108	0.090	0.072	0.125	0.428	0.149	0.142	0.121	0.013	0.209	0.240	0.222	0.096	0.098	0.016	0.195	0.130	0.234	0.078	0.002	0.069	0.011	0.234	0.011
Log_sPD1	0.304	0.197	0.031	0.139	0.050	0.016	0.286	0.197	0.234	0.139	0.175	0.239	0.077	0.045	0.163	0.125	0.020	0.113	0.065	0.189	0.011	0.021	0.146	0.246	0.210	0.001	0.045	0.008	0.118	0.146
Log_sGITR	0.240	0.253	0.005	0.095	0.005	0.022	0.382	0.344	0.109	0.351	0.138	0.074	0.284	0.255	0.037	0.097	0.175	0.013	0.083	0.176	0.060	0.165	0.280	0.108	0.162	0.051	0.008	0.233	0.032	0.146
Log_sCD28	0.281	0.053	0.067	0.103	0.088	0.017	0.382	0.336	0.109	0.351	0.138	0.074	0.284	0.255	0.037	0.097	0.175	0.013	0.033	0.068	0.013	0.069	0.129	0.225	0.183	0.013	0.068	0.124	0.160	0.272
Log_sHVEM	0.084	-0.096	0.265	-0.055	-0.122	0.127	-0.254	-0.212	0.194	0.061	0.102	0.088	-0.083	-0.113	-0.002	-0.120	-0.176	-0.022	0.083	0.176	0.060	0.165	-0.280	-0.108	0.162	-0.051	0.008	-0.233	0.032	-0.146
Log_sBTLA	0.333	0.193	0.193	0.152	0.148	0.042	0.136	0.273	0.274	0.179	0.397	0.100	0.185	0.311	0.343	0.196	0.050	0.107	0.094	0.005	0.083	0.018	0.148	0.150	0.234	0.149	0.084	0.053	0.053	0.173
Log_sCD152	0.333	0.193	0.193	0.152	0.148	0.042	0.136	0.273	0.274	0.179	0.397	0.100	0.185	0.311	0.343	0.196	0.050	0.107	0.094	0.005	0.083	0.018	0.148	0.150	0.234	0.149	0.084	0.053	0.053	0.173
Log_sCD137	0.83	0.145	0.152	0.447	0.033	0.070	0.20	0.243	0.108	0.313	0.125	0.049	0.195	0.036	0.053	0.139	0.028		0.017	0.015	0.053	0.070	0.115	0.112	0.091	0.107	0.064	0.096	0.138	0.181
Log_Glucose	0.466	0.511	0.001	0.103	0.644	0.187	0.011	0.169	0.300	0.194	0.408	0.114	0.361	0.340	0.174	0.470	0.247	0.036	0.664	0.540	0.586	0.348	0.410	0.466	0.573	0.478	0.456	0.456		
Log_sCD163	0.991	0.960	0.444	0.109	0.341	0.242	0.064	0.137	0.343	0.308	0.231	0.280	0.077	0.080	0.083	0.017	0.098	0.018	0.148	0.051	0.038	0.258	0.343	0.243	0.192	0.177	0.130	0.264		

Supplementary table 6

Correlations

p-value	Log10_IL1a Sig. (2-tailed)	Log10_IL1b Sig. (2-tailed)	Log10_IL1ra Sig. (2-tailed)	Log10_IL6 Sig. (2-tailed)	Log10_IL8 Sig. (2-tailed)	Log10_IL10 Sig. (2-tailed)	Log10_IL18 Sig. (2-tailed)	Log10_IL33 Sig. (2-tailed)	Log10_IFNg Sig. (2-tailed)	Log10_TNFa Sig. (2-tailed)
Log10_sBTLA	5.87E-01	na	4.60E-01	1.63E-01	1.93E-01	5.74E-01	5.70E-01	7.01E-01	na	3.90E-01
Log10_sCD137	9.35E-01	na	7.43E-01	5.28E-01	7.36E-01	4.05E-01	5.35E-01	4.20E-01	na	3.43E-01
Log10_sCD152	6.70E-01	na	6.49E-01	2.96E-01	2.47E-01	4.20E-01	7.03E-01	5.43E-01	na	3.63E-01
Log10_sCD27	8.91E-01	na	1.67E-01	8.55E-01	4.32E-01	8.52E-01	4.13E-01	6.08E-01	na	8.43E-01
Log10_sCD28	6.59E-01	na	5.73E-01	2.47E-01	1.85E-01	4.85E-01	6.92E-01	6.07E-01	na	4.10E-01
Log10_sCD80	8.40E-01	na	4.68E-01	5.00E-01	3.09E-01	6.22E-01	3.59E-01	2.87E-01	na	5.20E-01
Log10_sGITR	6.04E-01	na	8.13E-01	1.88E-01	2.93E-01	2.56E-01	7.05E-01	5.08E-01	na	3.30E-01
Log10_sHVEM	4.31E-01	na	1.42E-01	1.62E-01	1.48E-01	3.32E-01	9.83E-01	9.79E-01	na	2.83E-01
Log10_sIDO	9.32E-01	na	9.43E-01	1.70E-01	5.88E-01	6.84E-01	3.14E-01	3.07E-01	na	4.02E-01
Log10_sLAG3	3.40E-01	na	7.41E-01	8.99E-01	4.26E-01	5.75E-01	1.82E-01	2.24E-01	na	9.58E-01
Log10_sPD1	7.74E-01	na	5.58E-01	1.34E-01	1.86E-01	6.00E-01	5.21E-01	3.54E-01	na	2.51E-01
Log10_sPDL1	7.69E-01	na	3.42E-01	5.04E-01	1.61E-01	6.65E-01	3.27E-01	5.54E-01	na	5.18E-01
Log10_sPDL2	7.11E-01	na	9.80E-01	8.16E-01	3.69E-01	6.16E-02	5.88E-01	1.18E-01	na	3.93E-01
Log10_sTIM3	6.81E-01	na	7.26E-02	6.59E-01	3.42E-01	9.39E-01	8.39E-02	2.17E-01	na	9.48E-01

corr. coeff.	Log10_IL1a Pearson Correlation	Log10_IL1b Pearson Correlation	Log10_IL1ra Pearson Correlation	Log10_IL6 Pearson Correlation	Log10_IL8 Pearson Correlation	Log10_IL10 Pearson Correlation	Log10_IL18 Pearson Correlation	Log10_IL33 Pearson Correlation	Log10_IFNg Pearson Correlation	Log10_TNFa Pearson Correlation
Log10_sBTLA	-0.166	na	-0.225	-0.411	0.385	-0.172	0.174	-0.118	na	-0.260
Log10_sCD137	0.025	na	-0.101	-0.193	0.104	-0.252	0.190	-0.245	na	-0.286
Log10_sCD152	-0.131	na	-0.140	-0.314	0.346	-0.245	0.117	-0.186	na	-0.275
Log10_sCD27	0.042	na	0.408	0.056	0.239	-0.058	0.249	0.157	na	-0.061
Log10_sCD28	-0.136	na	-0.172	-0.346	0.393	-0.213	0.122	-0.158	na	-0.250
Log10_sCD80	0.062	na	-0.221	-0.206	0.306	-0.151	0.277	-0.320	na	-0.197
Log10_sGITR	-0.159	na	0.073	-0.390	0.316	-0.340	0.116	-0.202	na	-0.294
Log10_sHVEM	-0.239	na	-0.430	-0.412	0.424	-0.292	0.006	0.008	na	-0.322
Log10_sIDO	-0.026	na	-0.022	-0.405	0.166	-0.125	0.303	-0.307	na	-0.254
Log10_sLAG3	0.288	na	-0.102	-0.039	0.242	-0.172	0.395	-0.362	na	-0.016
Log10_sPD1	-0.088	na	-0.179	-0.439	0.392	-0.161	0.196	-0.280	na	-0.343
Log10_sPDL1	-0.090	na	0.287	-0.204	0.413	-0.133	0.295	-0.181	na	-0.198
Log10_sPDL2	0.114	na	0.008	0.072	0.272	-0.532	0.166	-0.455	na	-0.259
Log10_sTIM3	0.126	na	0.514	0.135	0.287	-0.023	0.497	-0.368	na	-0.020

Supplementary table 7

Supplementary figure legends.

Supplementary figure 1. Pro-inflammatory and anti-inflammatory cytokines in ALD patients. (A) Significance plot summarising cytokine measurements in HC, ARC and SAH; cytokine measurements: standardised z-scores; significance: $-Log_{10}$(p-value); red continuous line: BH significance threshold; red dotted line: p=0.05; black bars: significantly different cytokines; grey bars: non-significant cytokines. Additionally, individual boxplots for cytokine measurements in HC, ARC and SAH; KWp: raw Kruskal-Wallis p-value; BHq: FDR-adjusted q-value; p: significant multiple comparisons with Dunn's correction. Boxplots (median, IQR, ±Tukey's whiskers/outliers) ordered by decreasing statistical significance.

Supplementary figure 2. Soluble-CR levels in whole blood from four blood compartments in ARC patients. No differences in soluble-CR levels in plasma obtained from four anatomical compartments in 20 ARC patients undergoing TIPS; measurements are matched by subject (Friedman's paired test).

Supplementary figure 3. Soluble-CRs are not released from the liver upon acute ethanol (EtOH) exposure. (A) Lack of soluble-CR production in PCLS treated with ethanol 250 mM for 24 hours (Mann-Whitney test. Each dot represents one slice; each colour represents one subject). Boxplots (median, IQR, ±full range). (B) Lack of cellular toxicity by EtOH, measured as total cell death or % apoptotic cell death by cytokeratin (CK)18 fragments ELISA. Each dot represents one slice; red line: median. (C) Haematoxylin and eosin staining of PCLS, showing maintenance of tissue architecture after EtOH treatment.

Supplementary figure 4. Soluble-CR levels in whole blood upon acute bacterial challenge. Unchanged soluble-CR levels in E. coli-pulsed whole blood (Mixed Model analysis, with significant fixed effect by subject group but not by stimulation).

Supplementary figure 5. Representative FACS gating. Representative FACS plots showing the sequential gating for the measurement of membrane-TIM3 expression on T-cells (CD4/CD8), NK-cells (CD56hi/CD56lo), B-cells and monocytes.

Supplementary figure 6. Expression of membrane-TIM3 on immune subsets and modulation by E. coli. (A) membrane-TIM3 % at baseline (day 1, unstimulated) on T-cells (CD4/CD8), NK-cells (CD56hi/CD56lo), B-cells and monocytes (Kruskal-Wallis test with Dunn's multiple comparison correction). (B) Modulation of membrane-TIM3 % during a 7-day E. coli-stimulated culture; assessments performed at days 1-4-7; data expressed as E. coli fold ratio vs unstimulated medium (Mixed Model analysis, with fixed effect comparisons by group). Boxplots: median, IQR, ±Tukey's whiskers/outliers.

Supplementary figure legends.

Supplementary figure 7. Plasma soluble-TIM3 correlates with membrane-TIM3 expression on T-cells and NK-cells, and with their response to E. coli stimulation. Plasma soluble-TIM3 levels are positively correlated with basal (unstimulated) membrane-TIM3 expression in T-cells and NK-cells. However, plasma soluble-TIM3 levels correlate negatively with membrane-TIM3 upregulation response during antibacterial challenge. Each dot represents one subject; red continuous line: linear regression line; red dotted lines: 95% CI bands for the linear regression line. Correlations evaluated by Spearman's correlation analysis.

Supplementary figure 8. Galectin-9 blockade is not sufficient to rescue anti-bacterial responses in SAH patients. Specific blocking of Galectin-9 in PBMC cultures from SAH patients (n=4) with a neutralising monoclonal antibody was not sufficient to rescue the production of E. coli-stimulated antibacterial T-cell IFNγ (A) or anti-inflammatory T-cell IL-10 (C). Its effects on the production of pro-inflammatory IL-17A by CD8 T cells (B) and anti-inflammatory IL-10 by monocytes (D) were marginal and almost non-significant. Shading identifies different culture conditions as specified along each graph's x-axis. Boxplots: median, IQR, ±Tukey's whiskers/outliers.

Supplementary table legends.

Supplementary table 1. Antibodies used for flow cytometry. All the markers are indicated with their respective fluorochrome, antibody clone, physical excitation/emission lines and manufacturer's details.

Supplementary table 2. Soluble-CR levels measured in HC, ARC and SAH plasma.

Supplementary table 3. Intercorrelation matrix of soluble-CRs in ALD patients. The table lists the p-values and correlation coefficients calculated by Pearson's correlation analysis.

Supplementary table 4. Intercorrelation matrix of soluble-CRs in healthy controls. The table lists the p-values and correlation coefficients calculated by Pearson's correlation analysis.

Supplementary table 5. Variance Inflation factor analysis for plasma soluble-CR measurements in ALD patients and HC. The table lists the 'variance inflation factor' for each soluble-CR, a measurement of multivariate collinearity/correlation. Values above 5 indicate the presence of collinearity.

Supplementary table 6. Correlation analysis for plasma soluble-CR levels, cytokine measurements, clinical parameters and surrogate markers of bacterial translocation (D-lactate and soluble-CD163) in ALD patients. The table lists the p-values and correlation coefficients calculated by Pearson's correlation analysis.

Supplementary table 7. Lack of correlation between plasma soluble-CR levels and pro-/anti-inflammatory cytokines in healthy controls. The table lists the p-values and correlation coefficients calculated by Pearson's correlation analysis.