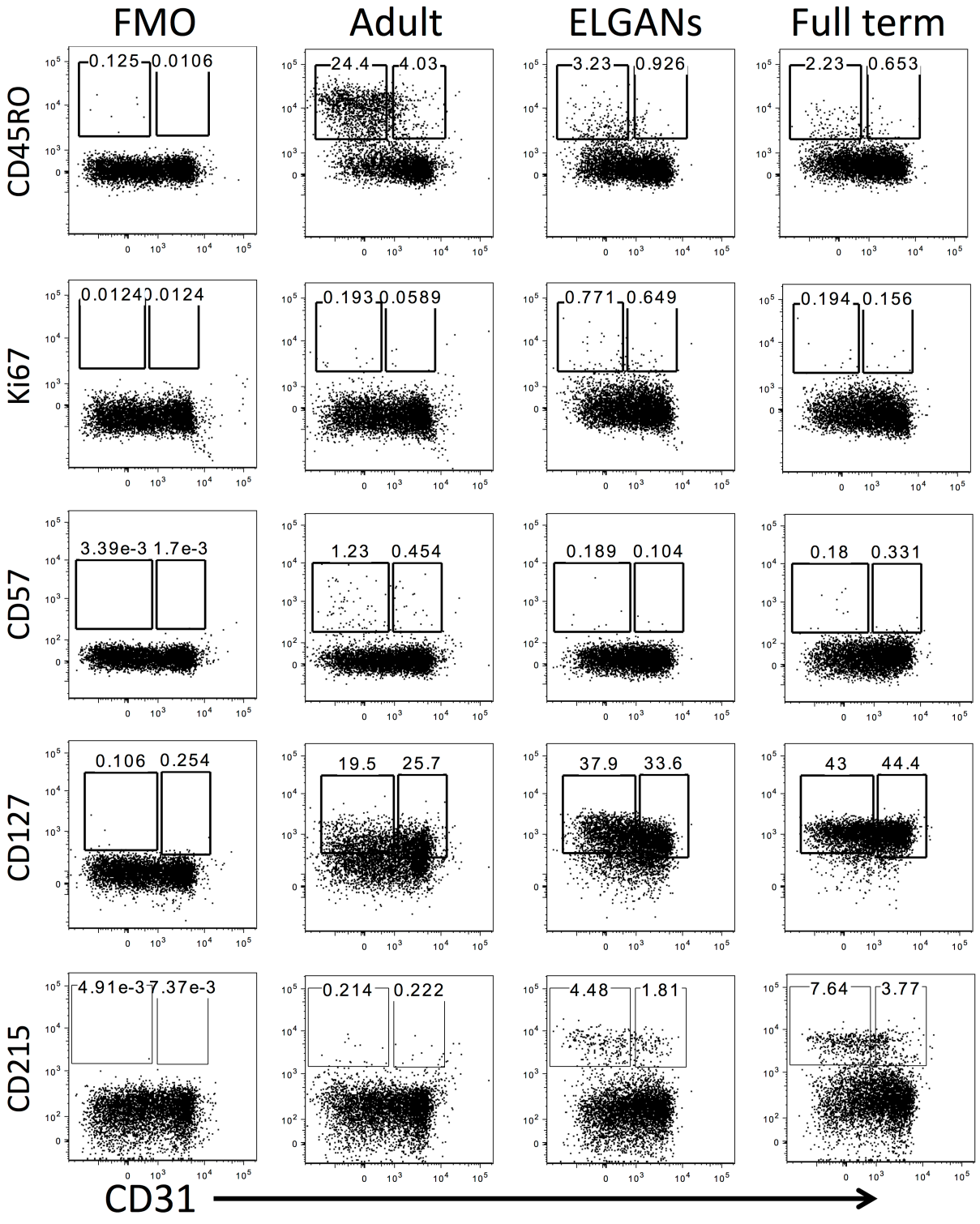
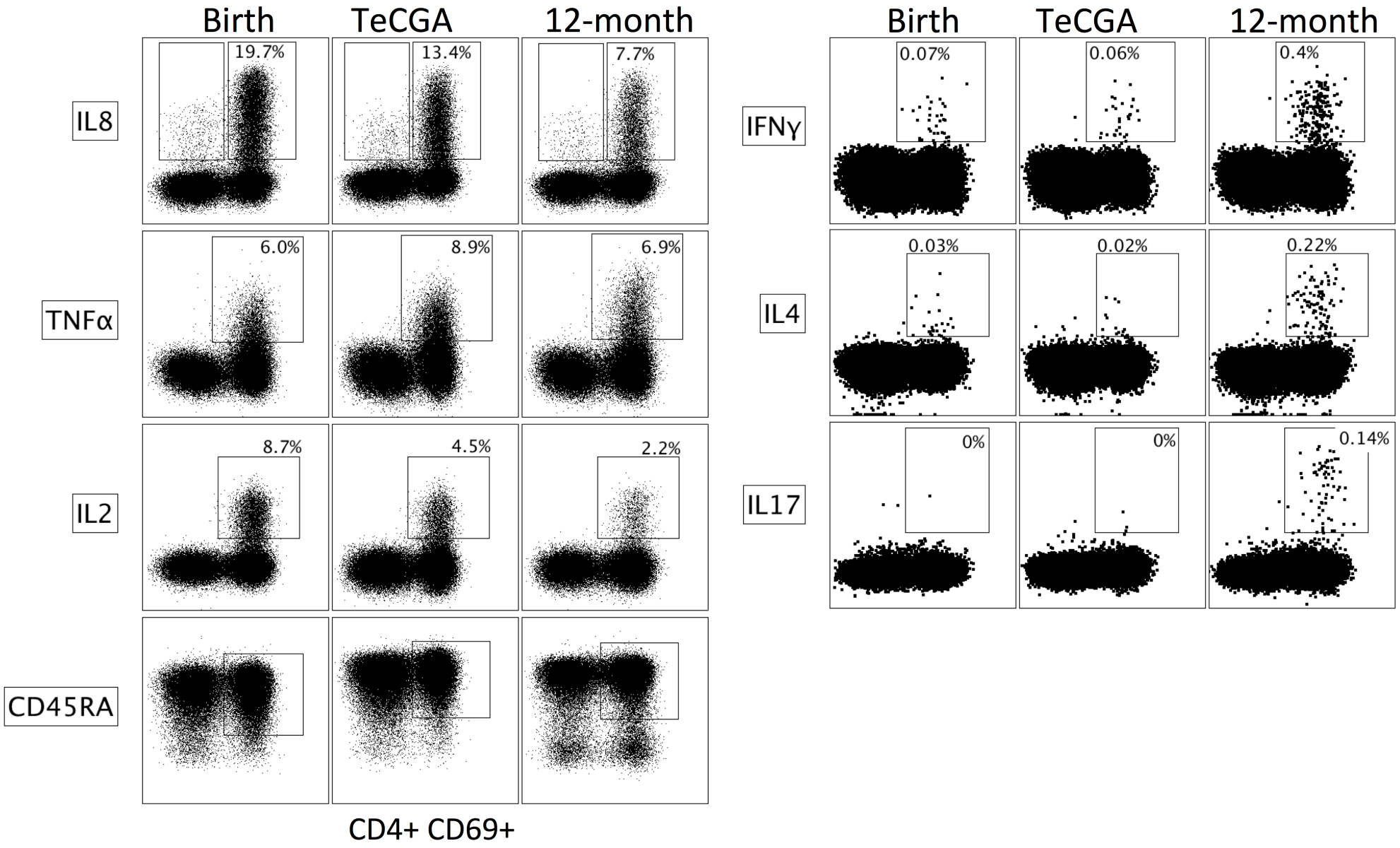
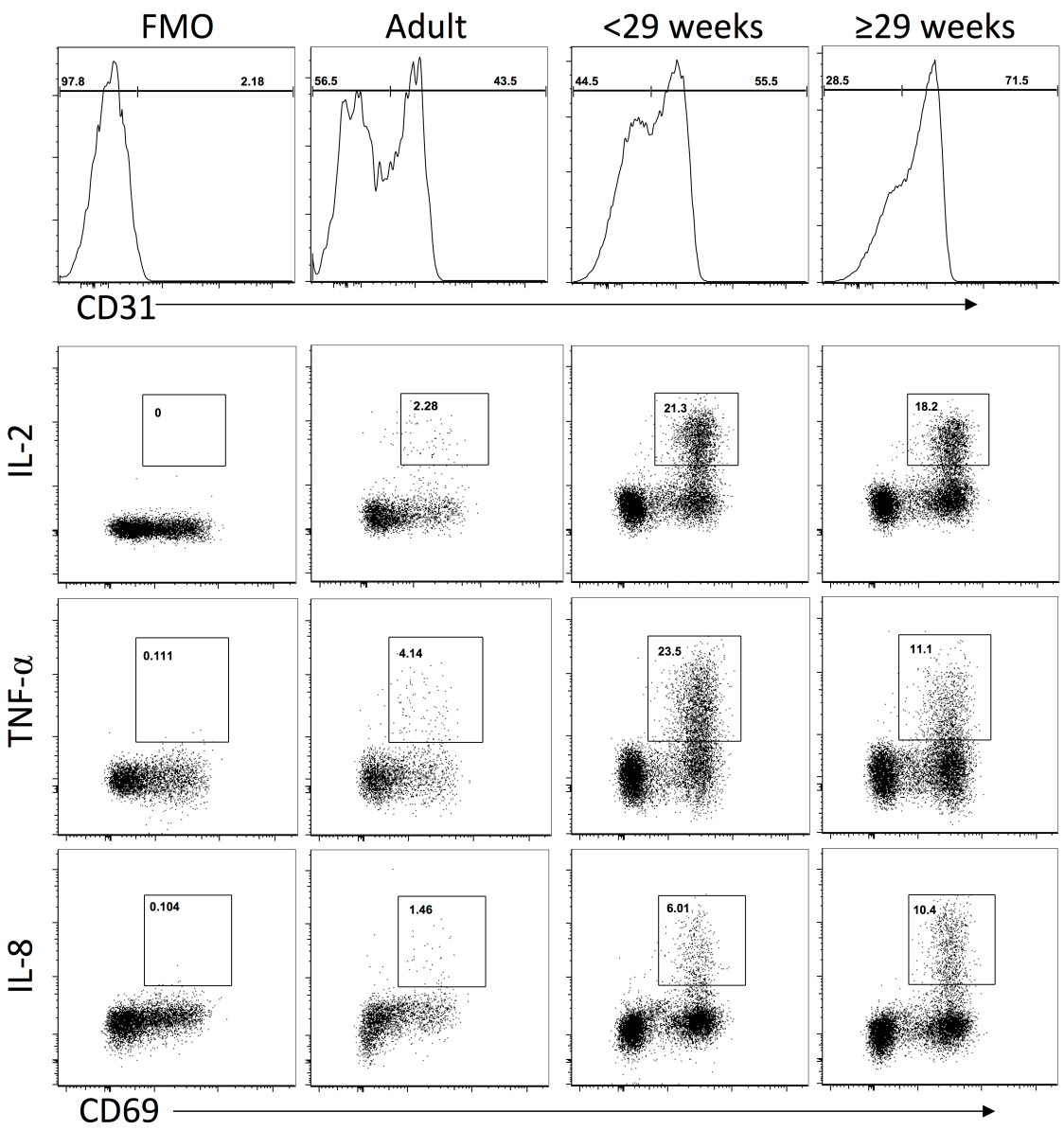
Supplementary Figure 1: Representative flow cytometry plots for each cohort, showing co-staining of CD31, including fluorescence-minus-one control (FMO). ELGANs=extremely low gestational age neonate (born <29 weeks gestational age).

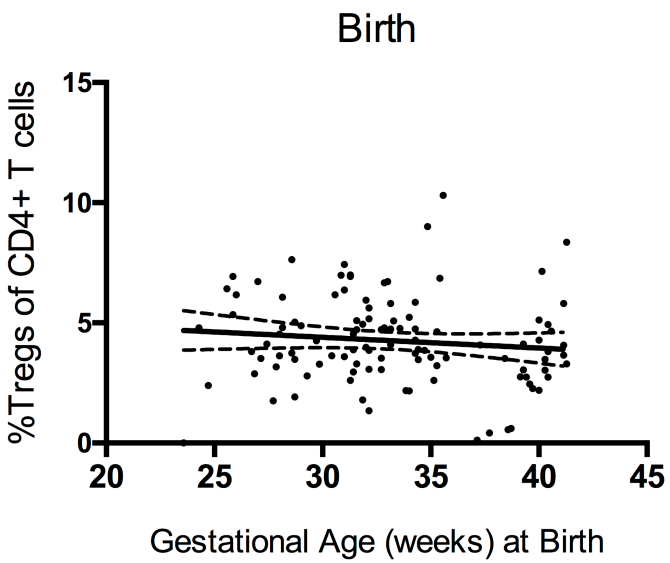


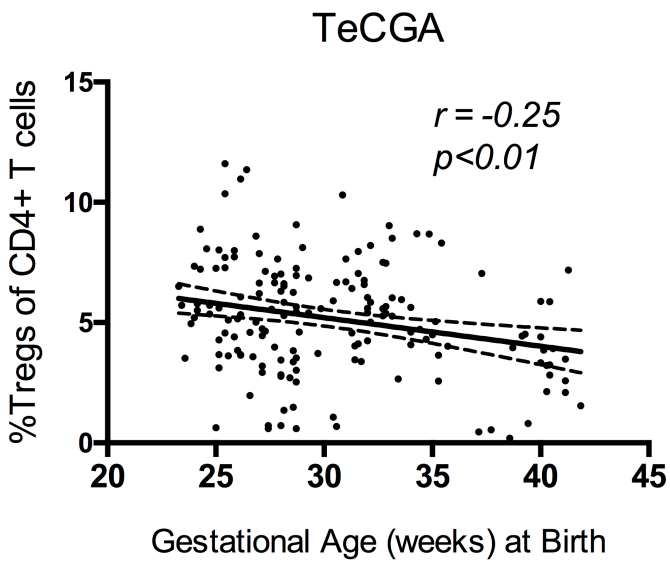


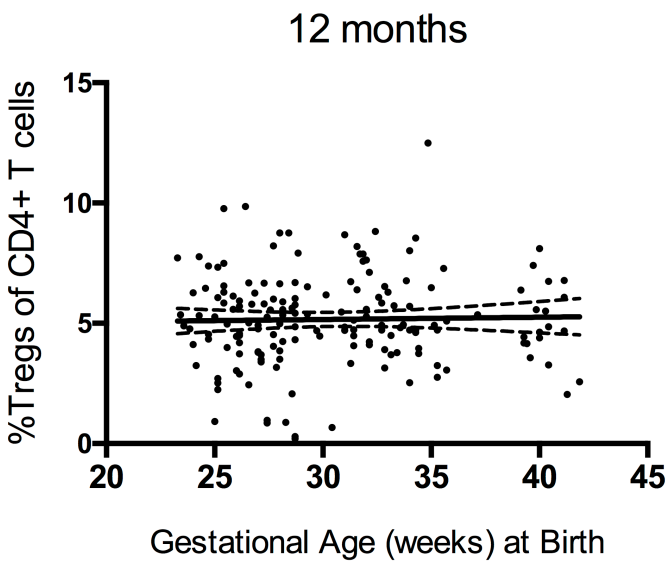
Supplementary Figure 2: Representative flow plots from representative study subject showing changing frequencies of cytokine for all three time points. Umbilical cord blood or PBMC's were stimulated with Staphylococcal enterotoxin B (SEB) in vitro for 2 hours, then in the presence of Golgi blockade for an additional 8 hours. Events shown have been gated based on FSC/SSC/Live/CD14-/CD3+/CD8-/CD4+ marker expression. TeCGA=term-equivalent corrected gestational age (collected when neonate aged to 36-42 weeks post-menstrual age)

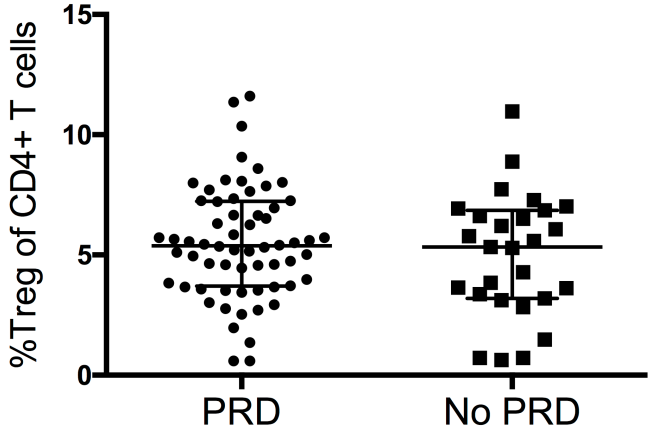


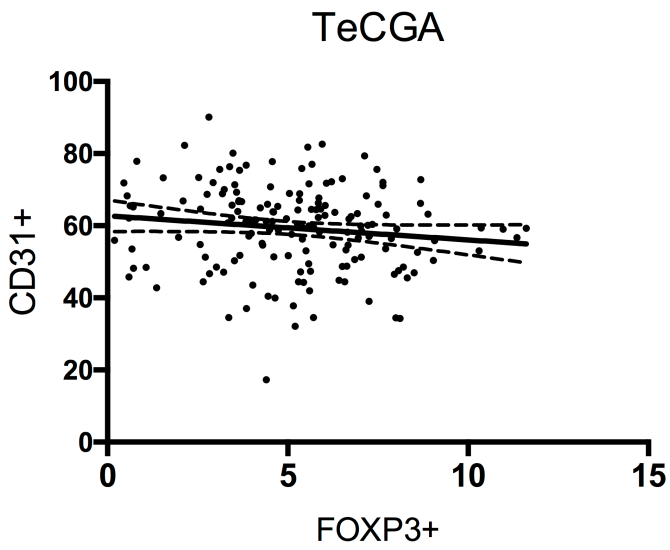
Supplementary Figure 3: Representative flow cytometry plots using co-staining panel for CD31 and intracellular cytokine staining. Umbilical cord blood or adult PBMC's were stimulated with Staphylococcal enterotoxin B (SEB) in vitro for 2 hours, then in the presence of Golgi blockade for an additional 8 hours. Events shown have been gated based on FSC/SSC/ Live/CD14-/CD3+/CD8- marker expression.











Supplementary Figure 4: Tregs are greater in former premature infants at term corrected age, but do not associate with CD31+ T cell frequencies or with PRD. Tregs were identified based on sequential gating of FSC/SSC/Live, CD14-/CD3+/CD8-/CD4+/FOXP3 high/ CD127low. Plots show Tregs as a fraction of CD4 at given gestational age at birth, collected at birth, term-equivalent corrected gestational age (teCGA) and 12 month time points. There was no association with post-prematurity respiratory disease (PRD) or with CD31+ T cell frequencies (Spearman correlation).

	Birth Gestational Age < 29 weeks (N=122)	Birth Gestational Age ≥ 29 weeks (N=111)
Gestational Age at birth (mean (SD))	26.6 (1.6)	34.4 (3.52)
Gender (N (%))		
Male	55 (45.1%)	62 (55.9%)
Female	67 (54.9%)	49 (44.1%)
Race (N (%))		
White	74 (60.7)	71 (64.0%)
>1 race	2 (1.6%)	3 (2.7%)
Black/AA	39 (32.0%)	30 (27.0%)
Asian	3 (2.5%)	0
Other	4 (3.3%)	6 (5.4%)
Unknown	0	1 (0.9%)
Ethnicity (N (%))		
Not Hispanic	113 (92.6%)	104 (93.7%)
Hispanic	9 (7.4%)	7 (6.3%)
Funisitis* (N(%))		
Yes	23 (18.9%)	9 (8.1%)
No	86 (70.5%)	83 (74.8%)
No placental pathology available	13 (10.7%)	19 (17.1%)
Clinical Chorioamnionitis* (N(%))		
Yes	18 (14.8%)	11 (9.9%)
No	104 (85.2%)	100 (90.1%)
Antenatal steroid (N(%))		
Yes	99 (81.1%)	56 (50.5%)
No	23 (18.9%)	55 (49.5%)
Pre-eclampsia (N(%))		
Yes	16 (13.1%)	31 (27.9%)
No	106 (86.9%)	80 (72.1%)
Culture Positive Sepsis or Bell's Stage >2 NEC (N(%))		
No	85 (69.7%)	104 (93.7%)
Yes	37 (30.3%)	7 (6.3%)
Bronchopulmonary Dysplasia (modified Shennan definition§) (N(%))		
Yes	54 (44.3%)	4 (3.6%)
No	68 (55.7%)	107 (96.4%)
Postnatal steroids (N(%))		
No	76 (62.3%)	109 (98.2%)
Yes	46 (37.7%)	2 (1.8%)
PDA requiring surgical ligation (N(%))		
Yes	21 (17.2%)	0
No	101 (82.8%)	111 (100%)
Days on mechanical ventilation¶ (median (IQR))	15.0 (36.0)	0 (0.75)
Post-prematurity Respiratory Disease (PRD)	86 (71%)	43 (39%)

Supplementary Table 1: Subject demographics, grouped by gestational age at birth

*Clinical chorioamnionitis diagnosed by Attending Obstetrician, per ACOG guidelines, typically including at least two of the following: maternal intrapartum fever, maternal or fetal tachycardia, fundal tenderness, purulent/foul-smelling amniotic fluid, maternal leukocytosis. §Bronchopulmonary Dysplasia diagnosed when oxygen requirement >21% at 36 weeks post-menstrual age (PMA) or at discharge if baby was discharged prior to 36 weeks PMA. ¶Days on mechanical ventilation equals the sum of days requiring positive pressure mechanical ventilation breaths (endotracheal or rate provided via non-invasive positive pressure).

	Panel 1		Panel 2		Panel 3	
Fluorochrome	**Marker**	**Clone#**	**Marker**	**Clone#**	**Marker**	**Clone#**
FITC	CD127	HIL-7R-M21	IL-8	E8N1	IL-8	E8N1
APC-CY7			CD69	FN50	CD69	FN50
APC-eFluor 780	CCR7	3D12				
Alexa 700	Ki-67	B56	CD3	UCHT1	IL-2	MQI-17H12
APC			IL-6	MQ2-13A5	CD8	RPA-T8
eFluor 660	FOXP3	236A/E7				
Qdot 800	CD14	Tük4				
BV785			TNFa	MAb11	TNFa	Mab11
Qdot 705	CD8	3B5				
BV711			IL-10	JES3-9D7	IL-10	JES3-9D7
Qdot 655	CD27	CLB-27/1				
BV605			CD45RA	HI100	CD45RA	HI100
BV605	CD25	302632	IL-2	MQ1-17H12	CD31	WM59
BV570			CD8a	RPA-T8		
BV510			CD14	MφP9		
Live/Dead	Aqua	Cat#L34957	Aqua	Cat#L34957	Yellow	Cat#L34959
PacBlue	CD3	S4.1	IL-17	BL168	CD3	S4.1
PE-Cy7	CD31	WM59	IFN-g	B27	IFN-g	B27
PE-Cy5.5	CD4	S3.5	CD4	S3.5		
PE-Cy5	CD45RO	UCHL1			CD56	MEM-188
PE-CF594			CD107a	H4A3		
PETXR	CD215	JM7A4			CD4	S3.5
PE	CD57	HCD57	IL-4	MP4-25D2	CD14	HCD14

Supplementary Table 2: Combinations of antibody conjugates used for flow cytometry

	No PRD (N=36)	PRD (N=86)	*PRD v no PRD (p-value)*	%CD31 ≥ 60 (N=36)	%CD31 < 60 (N=49)	*CD31 low v high (p-value)*
Gestational age at birth (weeks, median (IQR))	27.1 (2.3)	26.6 (2.7)	*0.84*	26.6 (2.7)	27.0 (2.7)	*0.76*
Day of life at discharge (median (IQR))	79.0 (26.0) (3 missing)	78.0 (38.0) (4 missing)	*0.72*	84.5 (45.0)	78.0 (29.0)	*0.89*
Days on mechanical ventilation¶			*0.08*			*1*
≤15 (median)	23 (63.9%)	39 (45.4%)		18 (50.0%)	25 (51.0%)	
>15	13 (36.1%)	47 (54.7%)		18 (50.0%)	24 (49.0)	
Supplemental oxygen exposure day 7 (area under curve∞)			***0.03***			*1*
≤925 (median)	24 (66.7%)	37 (43.0%)		17 (47.2%)	24 (49.0%)	
>925	12 (33.3%)	49 (57.0%)		19 (52.8%)	25 (51.0%)	
Supplemental oxygen exposure day 14 (area under curve)			***0.03***			*0.39*
≤2369 (median)	24 (66.7%)	37 (43.0%)		19 (52.8%)	21 (42.9%)	
>2369	12 (33.3%)	49 (57.0%)		17 (47.2%)	28 (57.1%)	
Gender (N (%))			*0.69*			***0.0004***
Female	21 (58.3%)	46 (53.5%)		27 (75.0%)	17 (34.7%)	
Male	15 (41.7%)	40 (46.5%)		9 (25.0%)	32 (65.3%)	
Race (N (%))			*0.34*			*0.14*
White	21 (58.3%)	53 (43.4%)		20 (55.6%)	34 (69.4%)	
Black/AA	10 (27.8%)	29 (33.7%)		11 (30.6%)	14 (28.6%)	
Asian	2 (5.6%)	1 (1.2%)		3 (8.3%)	0	
Other	2 (5.6%)	2 (2.3%)		2 (5.6%)	1 (2.0%)	
Ethnicity (N (%))						*1*
Not Hispanic	32 (88.9%)	81 (94.2%)	*0.45*	34 (94.4%)	46 (93.9%)	
Hispanic	4 (11.1%)	5 (5.8%)		2 (5.6%)	3 (6.1%)	
Funisitis♯ (N(%))						*0.3*
No	24 (66.7%)	62 (72.1%)	*0.81*	26 (72.2%)	33 (67.4%)	
Yes	8 (22.2%)	15 (17.4%)		8 (22.2%)	8 (16.3%)	
Placental pathology not available	4 (11.1%)	9 (10.5%)		2 (5.6%)	8 (16.3%)	
Clinical Chorioamnionitis* (N(%))						*0.77*
No	29 (80.6%)	75 (87.2%)	*0.4*	30 (83.3%)	42 (85.7%)	
Yes	7 (19.4%)	11 (12.8%)		6 (16.7%)	7 (14.3%)	
Antenatal steroids (N(%))						*1*
No	5 (13.9%)	18 (20.9%)	*0.45*	8 (22.2%)	10 (20.4%)	
Yes	31 (86.1%)	68 (79.1%)		28 (77.8%)	39 (79.6%)	
Pre-eclampsia (N(%))						***0.047***
No	30 (83.3%)	76 (88.4%)	*0.56*	28 (77.8%)	46 (93.9%)	
Yes	6 (16.7%)	10 (11.6%)		8 (22.2%)	3 (6.1%)	
Culture Positive Sepsis or Bell's Stage >2 NEC (N(%))						*0.64*
No	26 (72.2%)	59 (68.6%)	*0.83*	24 (66.7%)	35 (71.4%)	
Yes	10 (27.8%)	27 (31.4%)		12 (33.3%)	14 (28.6%)	
Bronchopulmonary Dysplasia (modified Shennan definition§) (N(%))						*0.27*
No	25 (69.4%)	43 (50.0%)	*0.07*	22 (61.1%)	23 (46.9%)	
Yes	11 (30.6%)	43 (50.0%)		14 (38.9%)	26 (53.1%)	
Postnatal steroids (N(%))						*1*
No	29 (80.6%)	47 (54.7%)	***0.008***	23 (63.9%)	32 (65.3%)	
Yes	7 (19.4%)	39 (45.4%)		13 (36.1%)	17 (34.7%)	
PDA requiring surgical ligation (N(%))						*0.25*
No	30 (83.3%)	71 (82.6%)	*1*	28 (77.8%)	43 (87.8%)	
Yes	6 (16.7%)	15 (17.4%)		8 (22.2%)	6 (12.2%)	

Supplementary Table 3: Univariate comparisons of clinical variables in <29 week subjects with and without post-prematurity respiratory disease (PRD), and in <29 week subjects above (high) and below median CD31+CD4+ T cell frequency at term corrected age

¶Days on mechanical ventilation equals the sum of days requiring positive pressure mechanical ventilation breaths (endotracheal or rate provided via non-invasive positive pressure). ∞Oxygen area under the curve calculated based on methods established by Stevens et al (2010) over a selected duration (seven and 14 days). ♯Funisitis defined as leukocyte infiltration in the perivascular region of the umbilical cord. *Clinical chorioamnionitis diagnosed by Attending Obstetrician, per ACOG guidelines, typically including at least two of the following: maternal intrapartum fever, maternal or fetal tachycardia, fundal tenderness, purulent/foul-smelling amniotic fluid, maternal leukocytosis. §Bronchopulmonary Dysplasia diagnosed when oxygen requirement >21% at 36 weeks post-menstrual age (PMA) or at discharge if baby was discharged prior to 36 weeks PMA.

	Odds Ratio (95% CI)	p-value, Odds Ratio	Sensitivity (% (CI))	Specificity (% (CI))	Error Rate (% (CI))
Continuous					
All subjects (n=233)	0.82 (0.77, 0.88)	<0.0001			
<29 weeks only (n=122)	**0.96**	**0.71**			
Dichotomized					
<33 weeks v ≥33 weeks	6.267 (3.2, 12.16)	<0.0001	88.4 (81.6, 93.3)	45.2 (35.4, 55.3)	30.9 (25.0, 37.3)
<29 weeks v ≥29 weeks	3.78 (2.19, 6.52)	<0.0001	66.7 (57.8, 74.7)	65.4 (55.4, 74.5)	33.9 (27.9, 40.4)

Supplemental Table 4: Performance of gestational age in predicting post-prematurity respiratory disease in neonates