Supplementary data

‘T2-high’ in severe asthma related to blood eosinophil, exhaled nitric oxide and serum periostin

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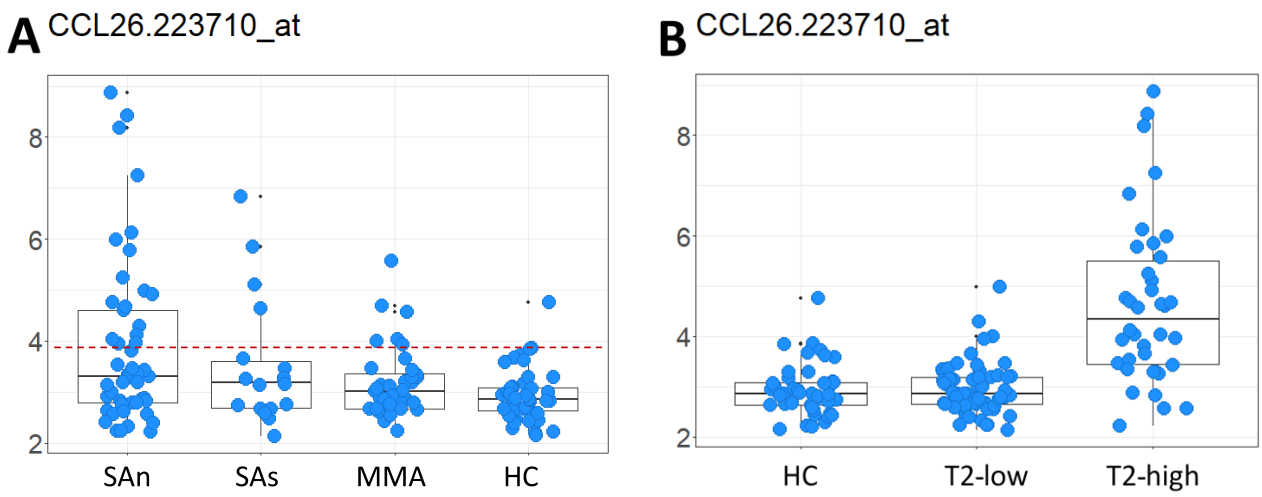
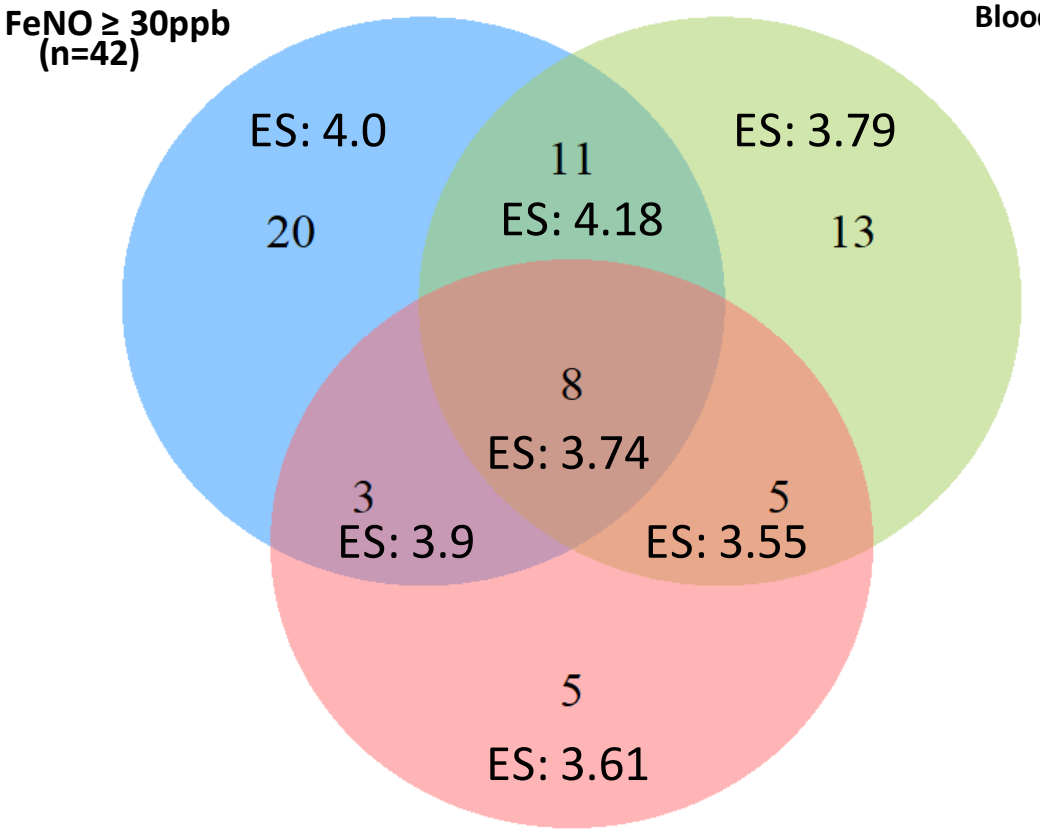
Supplementary Figs 1-4

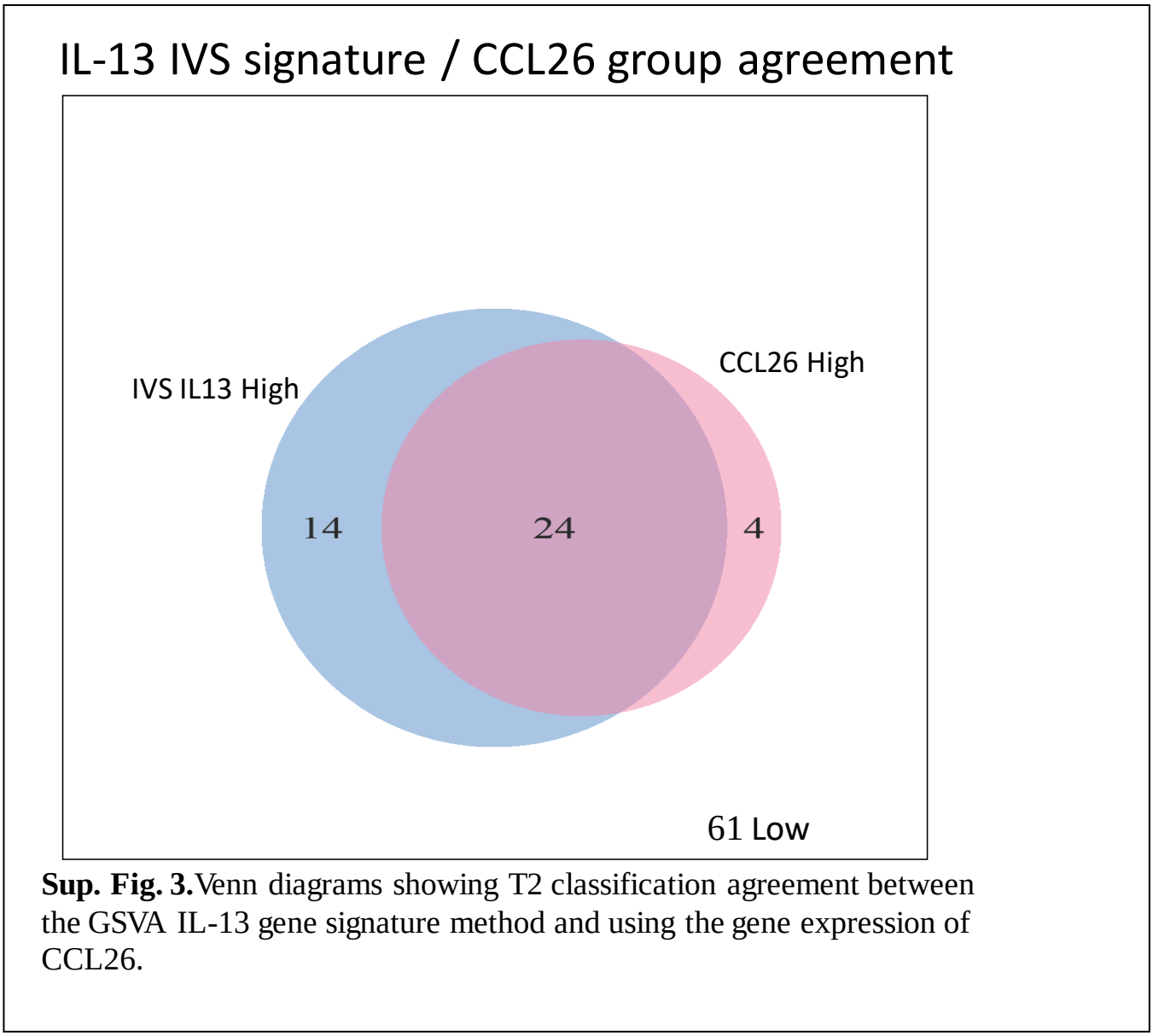
Supplementary Tables 1-3



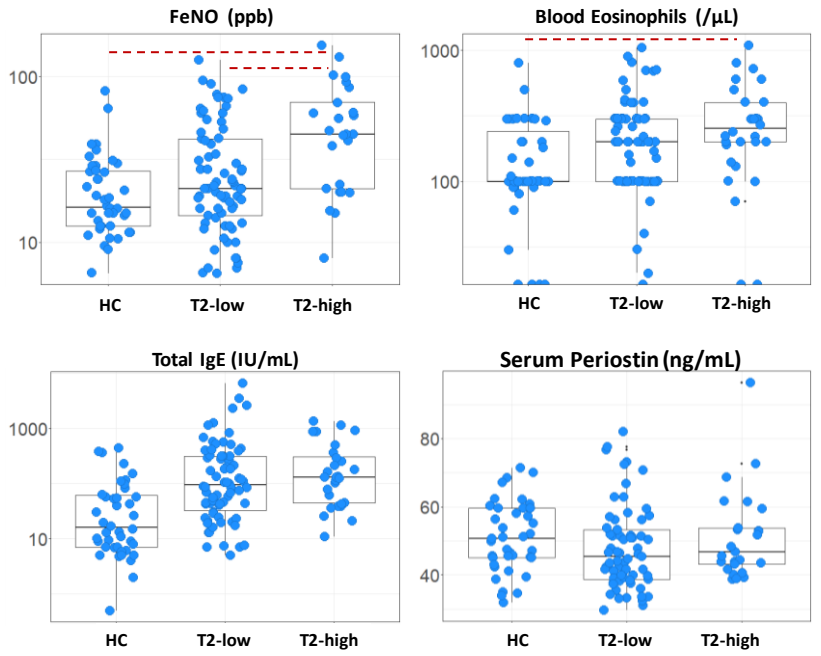
Sup. Fig 1. T2-high CCL26-based definition. A. CCL26 gene expression in bronchial brushings. The 95th percentile of the ES score in healthy controls (HC), estimated at 3.82, was used to classify the samples into T2-high and T2-Low. B. CCL26 Gene expression in the T2-high/low groups as originally defined by the ES score of the IL-13 T2 signature. HC: Healthy controls; MMA: Mild/Moderate non-smoking asthmatics; SAn: Severe non-smoking asthma; SAs: Smokers and ex-smokers with severe asthma.



Sup. Fig 2. A. Venn Diagram displaying the overlap of patients with high levels of FeNO (≥ 30ppb), blood eosinophils (≥300/µL), and serum periostin (≥55µg/L) and CCL26 average gene expression in each group.



Sup. Fig. 3. Venn diagrams showing T2 classification agreement between the GSVA IL-13 gene signature method and using the gene expression of CCL26.



Sup. Fig. 4. Box plots of T2 biomarkers. Distribution in healthy controls (HC) and patients classified as T2-low and T2-high by CCL26 gene expression.

Supplementary Table 1. Co-morbidities between T2-high and T2-low

Co-morbidities	T2-high	T2-low	p-value
Sinusitis	15 (39.5) [38]	10 (15.4) [65]	0.009
Psychiatric Disease	3 (7.9) [38]	14 (21.5) [65]	0.099
Coronary artery disease	0 (0) [38]	0 (0) [65]	1
Non Allergic Rhinitis	9 (23.7) [38]	8 (12.3) [65]	0.164
Nasal Polyp Surgery	10 (26.3) [38]	11 (16.9) [65]	0.313
Vocal Cord dysfunction	0 (0) [38]	1 (1.5) [65]	1
Allergic Rhinitis	23 (60.5) [38]	27 (41.5) [65]	0.097
Diabetes	0 (0) [38]	1 (1.5) [65]	1
Gastrooesophageal reflux symptoms	12 (31.6) [38]	34 (52.3) [65]	0.064
Osteoporosis	4 (10.5) [38]	6 (9.2) [65]	1
Hay Fever	24 (63.2) [38]	39 (60) [65]	1
Nasal Polyps	11 (28.9) [38]	16 (24.6) [65]	0.64
Eczema	18 (47.4) [38]	26 (40) [65]	0.538
Sinus Surgery	10 (26.3) [38]	4 (6.2) [65]	0.007
Hypertension	8 (21.1) [38]	14 (21.5) [65]	1

Supplementary Table 2. Comparison of clinical and inflammatory parameters between severe asthma with T2-high versus T2-low

	T2-high	**T2-low**	p-value
Age (Mean, SD) [N]	50.8 (13.6) [28]	48.6 (12.7) [39]	0.52
Female (N/%)[N]	11 (39.3%) [28]	21 (53.8.5%) [39]	0.32
BMI (Mean, SD) [N]	28.2 (5.2) [28]	31.7(6.5) [39]	0.03
Atopy (N/%)[N]	19 (79.2%) [24]	28 (73.7%) [38]	0.76
Total IgE IU/mL (Median, IQR) [N]	187.5 (517.4) [28]	85.2 (344.9) [38]	0.19
Current smokers	3 (10.1%) [28]	3 (7.7%) [39]	0.97
Ex-smokers	8 (28.6%) [28]	13 (33.3%) [39]	0.97
On oral corticosteroids (N/%)[N]	15 (55.5%) [27]	13 (37.1%) [35]	0.15
Anti IgE therapy	2 (8.0%) [25]	3 (9.7%) [31]	1.0
FEV_1 % (Median, IQR) [N]	69.9 (22.5) [28]	76.4 (33.5) [39]	0.07
FEV_1/FVC (%) (Median, IQR) [N]	62.4 (16.9) [28]	66.3 (19.9) [39]	0.30
FVC % predicted (Median, IQR) [N]	88.3 (13.8) [28]	95.3 (26.2) [39]	0.13
FEV_1 % increase post-salbutamol (Median, IQR) [N]	12.98 (16.5) [28]	6.7 (12.3) [39]	0.03
FeNO ppb (Median, IQR) [N]	43.0 (43.0) [26]	23.7 (35.2) [36]	0.20
Exacerbations (Median, IQR) [N]	2.0 (3.0) [28]	2.0 (2.0) [38]	0.54
Nasal Polyps diagnosed (N/%)[N]	11 (42.3%) [26]	14(36.8%) [38]	0.79
ACQ5 (Mean, SD) [N]	2.1 (1.0) [24]	1.9 (1.2) [28]	0.64
Blood Eosinophils /mL (Median, IQR) [N]	259 (243) [28]	199 (199) [39]	0.02
Blood Neutrophils /mL	5913 (3653) [28]	4299 (2549) [39]	0.21
Sputum Eosinophils % (Median, IQR) [N]	14.5 (39.9) [8]	0.6 (2.8) [9]	0.11
Sputum Neutrophils % (Median, IQR) [N]	66.5 (36.5) [8]	52.7 (31.2) [9]	0.56
Sputum Lymphocytes % (Median, IQR) [N]	0.3 (1.0) [8]	1.0 (0.6) [9]	0.08
Sputum Macrophages % (Median, IQR) [N]	10.1 (10.6) [8]	39.9 (41.6) [9]	0.02
Serum CCL18 pg/ml (Median, IQR) [N]	170.5 (82.3) [25]	156.3 (99.9) [32]	0.19
Serum IL17A pg/ml (Median, IQR) [N]	0.28 (0.23) [25]	0.38 (0.23) [32]	0.18
Serum periostin ng/ml (Median, IQR) [N]	45.6 (20.1) [25]	46.1 (12.7) [33]	0.92
Serum IL-1α pg/ml (Median, IQR) [N]	37.7 (6.6) [28]	34.1 (10.9) [38]	0.01
Plasma CCL11 pg/ml (Median, IQR) [N]	102.5 (33.3) [26]	89.5 (67.9) [36]	0.32
Plasma CCL26 (Median, IQR) [N]	21.8 (14.9) [26]	12.3 (16.9) [36]	0.002

Supplementary Table 3. Contingency tables for biomarker cut-off points with T2-high and T2-low asthma defined by CCL26 gene expression

Group	T2 high	T2 low	P value
FeNO ≥30ppb	17	25	0.007
FeNO <30ppb	8	48	
Blood Eos ≥300/μl	12	25	0.51
Blood Eos <300/μl	16	50	
Serum Periostin ≥55μg/L	6	15	1.0
Serum Periostin <55μg/L	19	50	
Blood Eos ≥300/μl & FeNO ≥30ppb	8	11	0.12
Blood Eos <300/μl & FeNO <30ppb	17	62	
Blood Eos ≥300/μl & Serum Periostin ≥55μg/L	3	10	1.0
Blood Eos <300/μ & Serum Periostin <55μg/L	22	63	
FeNO ≥30ppb & Serum Periostin ≥55μg/L	3	8	1.0
FeNO <30ppb & Serum Periostin <55μg/L	22	65	
Blood Eos ≥300/μl & FeNO ≥30ppb& Serum Periostin ≥55μg/L	2	6	0.02
Blood Eos <300/μl & FeNO <30ppb & Serum Periostin <55μg/L	23	67	