SUPPLEMENTARY MATERIALS

TITLE: Activator protein transcription factors coordinate human IL-33 expression from non-canonical promoters in chronic airway disease

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SUPPLEMENTARY FIGURES

IL33A2 (new transcript) promoter region, [exon 1A2'], exon 1A2, exon 1A1 and exon 2

CCCCTCCCTCCTATTTATTGCACACAGCTCAATTCCTGATTCATTCTTGTCATGTTGGCATCCT
GTCCGCTTGCTTCACTGCTTTATCCAAAGGGCAGACATAGCCATCTTTCTTCATTACTCACAAA
TGCAACACTATGGCTTGTAGCAGATGATTGCTCAGGTTTGGGGATGATTAAAGGGGATTACTGC
ACAGAAAATGAAACAACTTTAAACAAGATAACAGAGTTCCTTAGTTGTTTTTTCCAAAGGGGAC
TCCATCATAGTCACATTTTCCCAAGCCTGGTCAGCCATCTGTTGCTGTCTCATTTTCTCTTTCC
CCTGGATTCCAGAACCCTAACATTCTATCCTTCAACATGCCATGAGAAAAACTAAAAAATTCCT
ATTTCATTTTACAGTAAACTTTTTTCTGCTGCCCCTTTGTTTCAAGGTCACCCTTCTAATCTTG
TAGATTGAATGGATGTAGAGGCTTGCTAGGGTAACAGTCCATCACAATATCCTTCTAAGTACAG
CAGCAGTCTTCCTTCCAATGTGTGATCTTAGGATGTGGAATCAGTCCAAGTCT**TAAAA**GAGCAT
TACTTCATCCCTTTATTTTCTGCT[ACAGATGCCAAACGAGATGGAGAGAGG]ATCTCCCTTCT
AAGGCAATTTGGGTCTCTGCCAAACTTTGGCTAATAAAAAGAGTCTACAGACTCCTCCGAACAC
AGAGCTGCAGCTCTTCAGGGAAGAAATCAAAACAAGATCACAAGAATACTGAAAAATGAAGCCT
AAAATGAAGTATTCAACCAACAAAATTTCCACAGCAAAGTGGAAGAACACAGCAAGCAAAGCCT
TGTGTTTCAAGCTGGGA

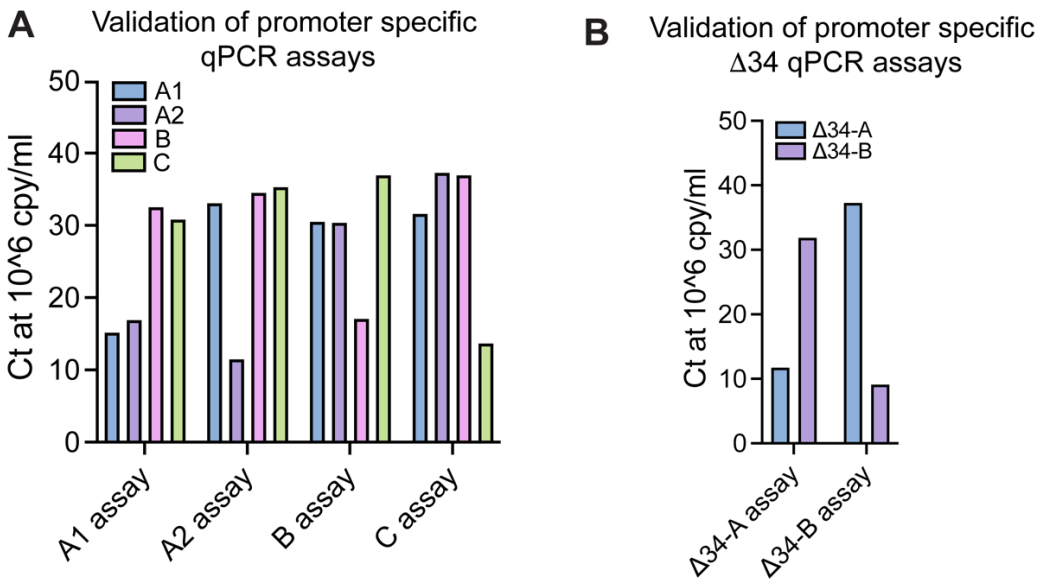
IL33A1 (NM_033439.4) promoter region, exon 1A1 and exon 2

GCAAAATTTCTCATGAGGTAAGAAGAACAACTTCTCATGGGATCTATAGTACAGGATTTGCTTT
TGTGATAAAGTATTATCTTCTGCGAGATTTTTGAGATGAATAGAAAGAAAAGATGAACAGTGGG
GAGCAGGAAAGCCCGTCAGATATGTTGGAATACAGAGTATTTCAGAGCTTTTATTTGCAGACGA
GCTGCTTATTACTGATAGGCCAAAGGGGGTCGCAAATTTCTATTATAAAGGAATTTGGAAGAAT
ACACTGATCTCTTAATGAAGTTTGTAAATTAAGAAGCCAACGGCCAAAAGTGAATATATAAATG
GCAACAGAATTTCAAAATGGACAGTGCTGAAGTTGAAGTTTAGGAGTTCAACTCCAAAGAGAGC
CAAAACATAAAGTTTAGGGGCAGAAGAAGAATCATAATTGCTGGTTTAAAATATTCAGATGGAG
GGAGGACGCAGAAAGTAGTGAGCCTTAGATGTTGACAGAATTGTAACTCTGTTGGCTCTTTACA
TGAGATTTCAAGCCTGCTAAAATCTCACCCGCCCAGATCTCCCTTCTAAGGCAATTTGGGTCTC
TGCCAAACTTTGGC**TAATAA**AAAGAGTCTACAGACTCCTCCGAACACAGAGCTGCAGCTCTTCA
GGGAAGAAATCAAAACAAGATCACAAGAATACTGAAAAATGAAGCCTAAAATGAAGTATTCAAC
CAACAAAATTTCCACAGCAAAGTGGAAGAACACAGCAAGCAAAGCCTTGTGTTTCAAGCTGGGA

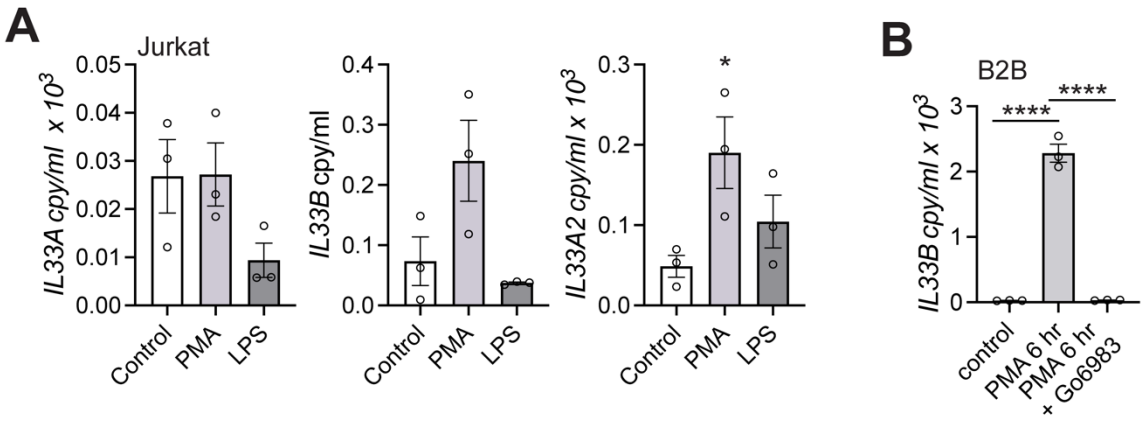
IL33B (NM_001314045.2) promoter region, exon 1B and exon 2

CCTTCTATATTATATGTTATGCAATAAACACTGCCTAGAGCCTATATTGCAAAGATTTTATAAA
AGTTAATGATTAACTAAGTGTAACTCTTTGACAGTGCTACAACTAGGCCGGGTGCAGTGGCTCA
CGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGCAGGAGGATCACTTGCGGTCAGGAGTTCAGG
ACCAGCCTGGCCAACATGGTGAAACCCCATCTCTATAAAAATACAAAAAAAATTGGCCAGGTGT
GGTGGCATACACCTTGTAGTCCCAGCTACTTGGGAGGCTGAGACAGAAGAATTGCTTTAATCTG
GGAGGTGGAGGCTGCAGTGAGCTGAGATCGTGCCACTGCACTCCAGCCTGGGCAGCAGAGGGAG
ACTCTGTCTCAAAAAATAAATAAATAAATAAAATGAAAGTGCTACAGCTAAAATAATCTCTGTT
TAGGATCTGACGGTAGGAAAAAGATAAGCTCCCACACGTTCTAATGCATTTAAGTAGCTCCATC
TGCATTGCCTCATACATCAATACTGTCATCCAATGTTGCTTAATCCATGTGTGTGTGTGTCTA**T**
ATAAGTAGAACTGGGATGTAACTGCCTTAAATACTACAATTGCTGACTACAGGAAACCTCATCA
TCTGAGACCAGCACTTTATAAATTAGAATACTGAAAAATGAAGCCTAAAATGAAGTATTCAACC
AACAAAATTTCCACAGCAAAGTGGAAGAACACAGCAAGCAAAGCCTTGTGTTTCAAGCTGGGA

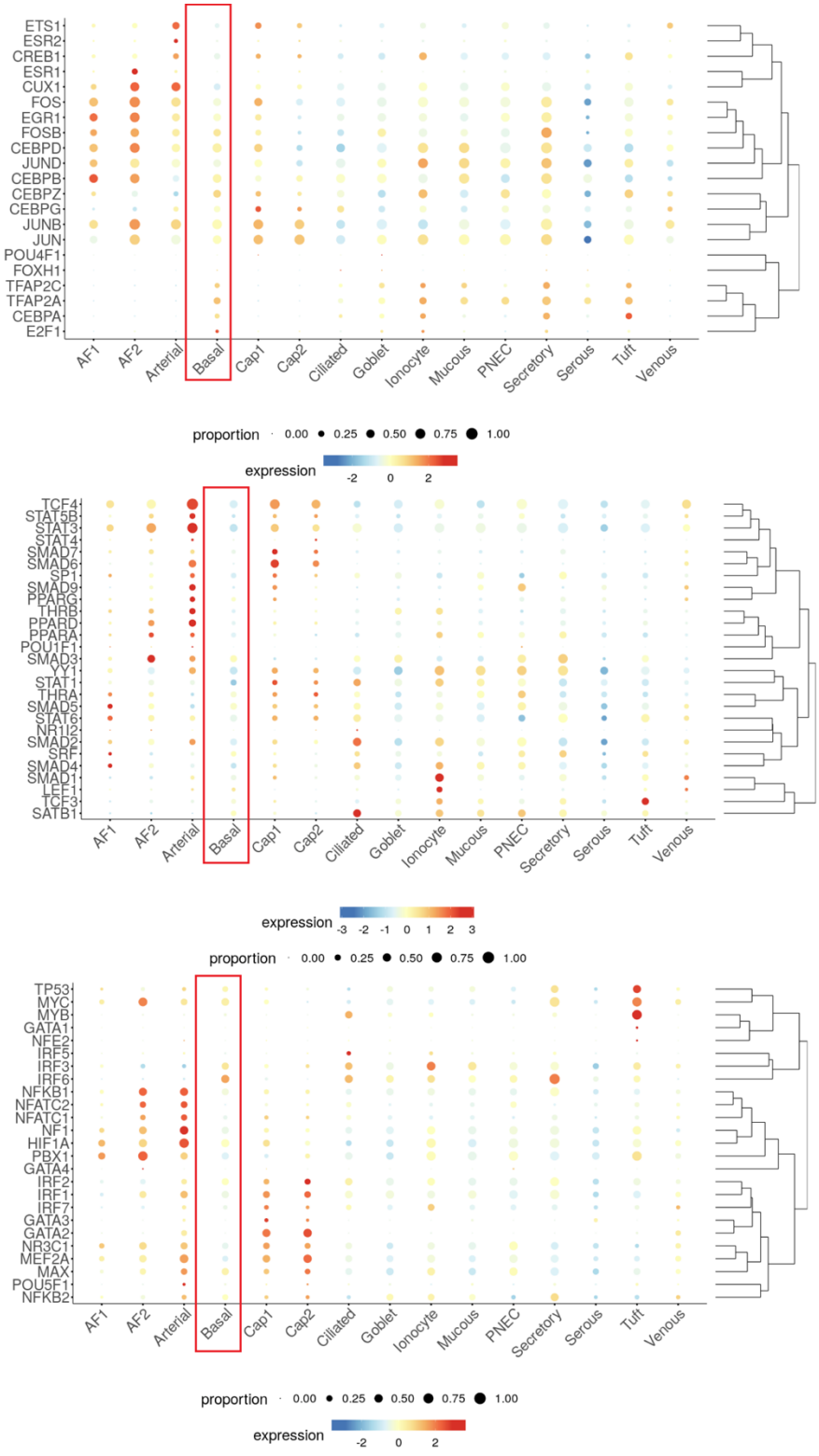
Supplementary Figure 1. Cloned *IL33* transcript sequences. Core promoter sequences used in transcription factor binding array with black text, confirmed by Sanger sequencing. 5' UTR exon 1A1, 1A2-1A1, and 1B are shown based on cloning from COPD airway cells.



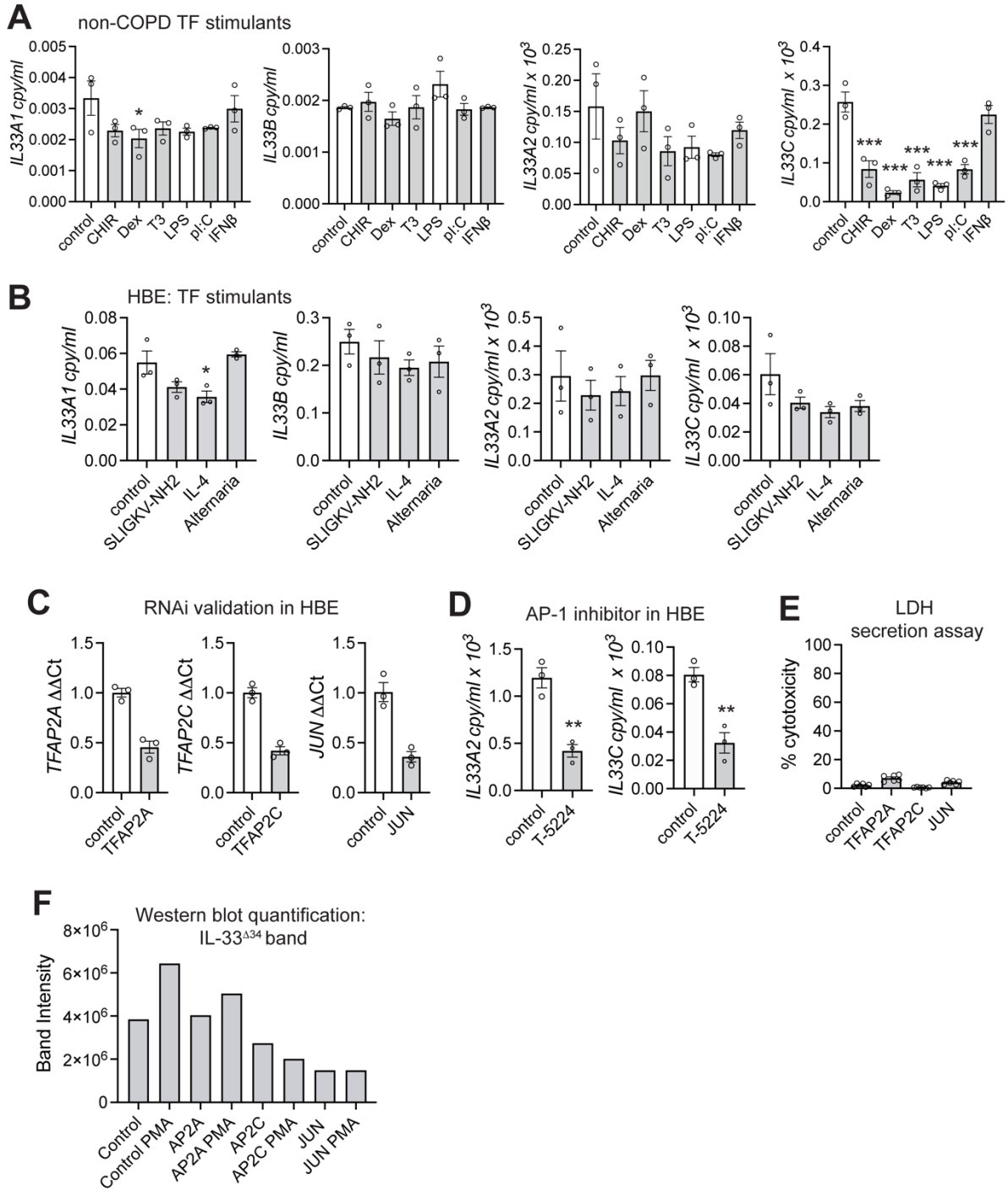
Supplementary Figure 2. Validation of isoform-specific assays. A) Cycling threshold (Ct) at 10^6 cpy/ml using plasmid standards for promoter-specific transcripts demonstrating specificity for *IL33B*, *IL33C*, and *IL33A2* assays, but amplification on both *IL33A1* and *IL33A2* for the *IL33A1* assay. **B)** Comparative expression at 10^6 cpy/ml performed using plasmid standards for *IL33*$^{\Delta 34}$ based on promoter specific transcripts for *IL33A1*, *IL33B*. Experiments are representative of duplicate repeats.



Supplementary Figure 3. Jurkat PMA expression data and B2B PKC inhibitor data. **A)** Jurkat expression in response to PMA measured for *IL33A*, *IL33B*, and *IL33A2* assays, *IL33C* was not detected. **B)** PMA induction of *IL33B* in B2B cells treated with or without the PKC inhibitor Go6983, other *IL33* promoter-specific isoforms were not detected. Statistical analysis: one-way ANOVA (A, B), *P*-value limits: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Experiments are representative of duplicate repeats.


ETS1
ESR2
CREB1
ESR1
CUX1
FOS
EGR1
FOSB
CEBPD
JUND
CEBPB
CEBPZ
CEBPG
JUNB
JUN
POU4F1
FOXH1
TFAP2C
TFAP2A
CEBPA
E2F1
AF1
AF2
Arterial
Basal
Cap1
Cap2
Ciliated
Goblet
Ionocyte
Mucous
PNEC
Secretory
Serous
Tuft
Venous
proportion 0.00 0.25 0.50 0.75 1.00
expression -2 0 2
TCF4
STAT5B
STAT3
STAT4
SMAD7
SMAD6
SP1
SMAD9
PPARG
THRB
PPARD
PPARA
POU1F1
SMAD3
YY1
STAT1
THRA
SMAD5
STAT6
NR1I2
SMAD2
SRF
SMAD4
SMAD1
LEF1
TCF3
SATB1
expression -3 -2 -1 0 1 2 3
proportion 0.00 0.25 0.50 0.75 1.00
TP53
MYC
MYB
GATA1
NFE2
IRF5
IRF3
IRF6
NFKB1
NFATC2
NFATC1
NF1
HIF1A
PBX1
GATA4
IRF2
IRF1
IRF7
GATA3
GATA2
NR3C1
MEF2A
MAX
POU5F1
NFKB2
proportion 0.00 0.25 0.50 0.75 1.00
expression -2 0 2

Supplementary Figure 4. LungMap single-cell TF expression for candidates from TF binding array. Bubble plots generated for transcription factors relevant to the TF array in Figure 4A using the LungMap shinycell explorer. This is based on data generated by the LungMap Consortium [U01HL122642] and downloaded from www.lungmap.net on December 14, 2023. Bubble plots displaying TF expression levels for human structural lung cells are shown for comparison to basal cells highlighted by red boxes.



Supplementary Figure 5. HBE and basal cell response to TF activators and inhibitors. A) Follow up *IL33* expression analysis for TFs with > 30% inhibition in array performed in non-COPD airway basal cells, working concentrations are reported in

Supplementary Table 4. **B)** Additional TF activators tested for *IL33* expression to address endogenous disease-relevant upstream signals mimicked by PMA in HBE cells. **C)** Validation of RNAi with relative expression for AP-1 (*JUN*) and AP-2 (*TFAP2A, TFAP2C*) transcription factors under control and shRNA knockdown conditions. **D)** Expression levels for *IL33A2* and *IL33C* under conditions of AP-1 inhibitor treatment. **E)** LDH release assay to demonstrate minimal cellular toxicity in setting of IL-33 secretion assay post-PMA treatment in Figure 4E. **F)** Quantitative summary of band intensity for lower ~24 kDa band on anti-IL-33 CTD western blot under RNAi conditions in HBE cells treated with and without PMA. Statistical analysis: one-way ANOVA (A, B), *P*-value limits: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Data shown is representative of experiments performed in duplicate.

Supplementary Table 1. Clinical characteristics of non-COPD and severe (GOLD Stage IV) COPD lung specimens.

Characteristics	Non-COPD	COPD
Number per group	18	20
Mean age (range)	32.13 (18-67)	61.4 (52-70)
Male:Female	7:2:9	7:13
FVC (L)	–	1.97 (0.21)
FEV1 (L)	–	0.52 (0.04)
FEV_1/FVC (%)	–	24.95
Pack-years (range)	3.75 (0-15)	42.74 (10-96)

Supplementary Table 2: List of reagents and materials.

Reagent	Supplier	Reference/Iden
Antibodies		
Human IL-33 rabbit polyclonal (1:50 IF)	Sigma	HPA024426
Human IL-33 (CTD) goat monoclonal (1:1000 WB)	R&D systems	MAB36253
Human IL-33 (NTD) goat polyclonal (1 ug/mL)	R&D systems	AF4810
Human IL-33 mouse monoclonal (Nessy-1) (1:100 IHC)	Enzo	ALX-804-840PF
AP2 alpha monoclonal (3B5) (1:100 IHC)	Invitrogen	MA1-872
AP2 gamma monoclonal (3A11A5) (1:100 IHC)	Invitrogen	MA5-31928
Phospho-AP2-gamma (Serr434) polyclonal (1:50 IHC)	Invitrogen	PA5-105357
c-Jun monoclonal	Invitrogen	MA5-15881
c-Fos monoclonal	Invitrogen	MA5-15055
IF = immunofluorescence, WB = western blot		
Commercial lentiviruses		
TFAP2A shRNA	Santa Cruz	sc-105074-V
TFAP2C shRNA	Santa Cruz	sc-29696-V
JUN shRNA	Santa Cruz	sc-29223-V
Scrambled control shRNA	Santa Cruz	sc-108080

Biological Samples		
Primary human basal cells	BJH	N/A
Control and COPD lung tissue	BJH	N/A
Chemicals, Peptides, and Recombinant Proteins		
Dexamethasone	Sigma	D4902
Interferon-β	R&D Systems	8499-IF
CHIR99021	Stem Cell	72052
Lipopolysaccharide (LPS)	Millipore-Sigma	LPS25
Poly I:C (lipofectamine transfected)	Enzo	ALX-746-021
IL-4	R&D systems	BT-004
Cobalt chloride	Sigma	C8861
SLIGKV-NH2	Tocris	3010
Alternaria alternata extract	Greer	XPM1D3A2.5
Triiodothyronine (T3)	Sigma	T6397
T-5224 (AP-1 inhibitor)	Tocris	7317
Phorbol-12-myristate 13-acetate (PMA)	Tocris	1201
Commercial Assays		
TF binding array	Signosis	FA-2001
Cell Lines		

HBE-1	UNC Marisco Lung Institute	Yankaskas et al, 1993
Beas2B	Millipore	95102433
16HBE14o-	Millipore	SCC150

Oligonucleotides	
Cloning primers	
IL33A promoter 5'Kpn	GGGGTACCGCAAAATTTCTCATGAGG
IL33A promoter 3'Xho	CCGCTCGAGCTTTTTATTAGCCAAAGTTTGG
IL33B promoter 5'Kpn	GGGGTACCCCTTCTATATTATATGTTATGC
IL33B promoter 3'Xho	CCGCTCGAGAAGGCAGTTACATCCCAGTTC
IL33A2 promoter 5' Kpn	GGGGTACCCCCCTCCCTCCTATTTATTGC

IL33A2 promoter 3' Xho	CCGCTCGAGAGCAGAAAATAAAGGGATGAAGTAATGC
IL33C promoter 5'Kpn	GGGGTACCTGCTTGTCCTACTAG
hIL33C promoter 3'Xho	CCGCTCGAGAGAGAGGAAATCCG
IL33C 5’Nde	GGGAATTCGACTTACTCACTGCTGCCTTCC
IL33A2 5’Nde	GGGAATTCACGAGATGGAGAGAGGGTGAGTAGGAGCAAAATTTCTCATGAGAATACTGAAAAATGTAC
IL33A 5’Nde	GGGAATTCCATATGACACAGAGCTGCAGCTCTTCAGGGAAG

IL33B 5'Nde	GGGAATTCCATATGAAATACTACAATTGCTGACTACAGGAAACC
IL33 3'Xho	CCGCTCGAGTCATCAGTTTCAGAGA
qPCR primer-probe sets	
IL33 ∆34 specific	
Forward Primer	CACAGCAAAGTGGAAGAACAC
Probe	AGCTTGAAACACAAGGCTTTGCTTGC
Reverse Primer	TACTCTGTAATAGGTGAAATTCTTCCC
IL33B ∆34 specific	
Forward Primer	CATCATCTGAGACCAGCACTT

Probe	TGGAAGAACACA GCAAGCAAAGCC
Reverse Primer	TAGGTGAAATTCT TCCCAGCTT
IL33A1 ∆34 specific	
Forward Primer	AGCTCTTCAGGG AAGAAATCAAA
Probe	TGGAAGAACACA GCAAGCAAAGCC
Reverse Primer	AGGTGAAATTCTT CCCAGCTT
IL33 full specific	
Forward Primer	GGTGGTTTCTCT CCTAAAGTAACA G
Probe	TTTATGAAGCTCC GCTCTGGCCTT
Reverse Primer	CCCAACAGAAGG CCAAAGAA
IL33A	

Forward Primer	CAGCTCTTCAGG GAAGAAATCA
Probe	TGCTTGCTGTGTT CTTCCACTTTGC
Reverse Primer	TCCCAGCTTGAA ACACAAGG
IL33A2	
Forward Primer	CAGATGCCAAAC GAGATGGA
Probe	AGTTTGGCAGAG ACCCAAATTGCC
Reverse Primer	TGTGTTCGGAGG AGTCTGTA
IL33B	
Forward Primer	AATACTACAATTG CTGACTACAGGA
Probe	ACCTCATCA/ZEN/ TCTGAGACCAGC ACT
Reverse Primer	CTTGCTGTGTTCT TCCACTTTG

IL33C	
Forward Primer	CTCACCAGATGC CAGCATAATA
Probe	CCAACAGAAGGC CAAAGAAGTTTG CC
Reverse Primer	GAGCGGAGCTTC ATAAAGTACA
TFAP2A IDT predesigned assay	Hs.PT.58.5602
TFAP2C IDT predesigned assay	Hs.PT.58.2610271 0
JUN IDT predesigned assay	Hs.PT.58.2509471 4.g
FOS IDT predesigned assay	
SYBR Green IDT PrimeTime qPCR Primers	
IL-33 A1 Set	

Primer 1	5'- CGTCCTCCCTCC ATCTGAATA-3'
Primer 2	5'- TGGACAGTGCTG AAGTTGAAG-3'
IL-33 A2 Set	
Primer 1	5'- TCATCTGCTACAA GCCATAGTG-3'
Primer 2	5'- CGCTTGCTTCAC TGCTTTATC-3'
IL-33 B Set	
Primer 1	5'- TCCCAAAGTGCT GGGATTAC-3'
Primer 2	5'- CACTGCCTAGAG CCTATATTGC-3'

IL-33 C Set	
Primer 1	5'- AACCAAACCACT CCCATGATAA-3'
Primer 2	5'- CTATCTCAGCTCA CTGCAACC-3'
IL-33 Intron 1 enhancer set	
Primer 1	5'- CACAGTGCAGAG GAGGAAATAC-3'
Primer 2	5'- GTCCTGTGGCTG CATACAA-3'
IL-33 A1 upstream enhancer set	
Primer 1	5'- AGATGGAGTCTT GCTTCATCAC-3'

Primer 2	5'- GAGGCAGGAGAA TTGCTTGA-3'

Supplementary Table 3: List of reagents and working concentrations for *IL33* expression screening.

Agent	Working Concentration	Source
PMA	20ng/ml	Tocris
ionomycin	500 ng/ml	Tocris
ATP	10 uM	Sigma
AMP	10 uM	Sigma
epinephrine	27 uM	Sigma
uric acid	6 uM	Sigma
DAPT	1 uM	Sigma
DLK1	1000 ng/ml	R&D systems
HGF	100 ng/ml	Peprotech
R-Spondin	5 ng/ml	Stem Cell technologies
retinoic acid	50 nM	Sigma
IFN-beta	100 ng/ml	R&D systems
IFN-gamma	100 ng/mL	R&D systems
IL-13	100 ng/ml	R&D systems
IL-17A	100 ng/ml	R&D systems
IL-1a	100 ng/ml	R&D systems
IL-33	100 ng/ml	R&D systems
IL-4	100 ng/ml	R&D systems

TNF	100 ng/ml	Sigma
trefoil factor 2	100 ng/ml	Peprotech
trefoil factor 1	100 ng/ml	Peprotech
pI:C- lipofectamine transfected	1 ug/ml	Sigma
LPS	1 ug/ml	Sigma
Alternaria alternata extract	20 microgram/ml	Greer
House dust mite extract	20 microgram/ml	Greer
PBS		Sigma

Supplementary Table 4: List of reagents and working concentrations for follow up on hits from TF array.

Agent	Working Concentration	Source
PMA	20ng/ml	Tocris
T5524 (AP-1 inhibitor)	10 μM	Tocris
dexamethasone	100 nM	Tocris
Interferon-β	10 μM	R&D systems
Poly I:C (transfected)	1 μg/ml	Enzo
LPS	1 μg/ml	Sigma
Cobalt chloride	50 μM	Sigma
IL-4	100 ng/ml	R&D systems
CHIR99021	1000 ng/ml	Stem Cell Technologies
T3	100 nM	Sigma
Alternaria extract	20 μg/ml	Greer
SLIGKV-NH2	100 μM	Tocris