Supplementary methods

Luminex

Experiments were done in triplicate and equal volumes from triplicates were combined into one sample for cytokine measurement by multiplex assays (BioRad) and read with a Bioplex (BioRad).

miR16 analysis and LNA knockdown

miR16-1 stem-loop primer was used to prepare cDNA and miR16 primers used to determine its levels by qPCR. Expression of miR16 was normalized to an ubiquitously expressed small non-coding RNA, RNU6B as performed earlier and sequences provided in table S1 (19). PBECs were plated on 24-well plates (500 µl) and were grown till 30% confluence. Cells were transfected with LNA-miR16 (hsa-miR-16 miRCURY LNA inhibitor probe – Exiqon) or scrambled LNA (LNA-con), with sequences provided in table S1, in the presence of Lipofectamine 2000 transfection reagent (Invitrogen) according to manufacturer's instructions, at a final concentration of 50 nM in serum-free medium (Opti-MEM, Invitrogen). FAM-labelled LNA-miR16 and scrambled (LNA-con) were used at a final concentration of 50 nM as controls for si-RNA and LNA-mediated knockdown, respectively, and to determine transfection efficiency. After transfection, cells were left to recover for 48 hrs followed by stimulation with recombinant human (rh) TNF-α (5 ng/ml) (R&D systems,

rhTNF-α plus rhIL-17A (5 ng/ml and 100 ng/ml, respectively) or no stimulus for 2 hrs for the assessment of RNA and 16 hrs for the assessment of protein production.

mRNA and half-life analysis

Total RNA was extracted from cells with Trizol and cDNA was synthesized with Revert AidTM H Minus Reverse Transcriptase (Fermentas). Quantitative PCR reactions were done with Power Sybr green PCR master mix (Applied Biosystems) and run on Step One Plus Real Time PCR (Applied Biosystems). Expression of the *CXCL8* and *IL6* mRNA was normalized to *GAPDH* mRNA as performed earlier (19), with sequences provided in table Sf1. mRNA half-life was measured by stimulating PBECs for 2 hrs with TNF-α or TNFα and IL-17A followed by addition of actinomycin D (ActD; 5 μg/ml, Sigma) for 1 hr and RNA isolation using Trizol reagent (Invitrogen).

Immunohistochemistry

PBECs were seeded overnight at 5,000 cells in 200 μl per well in chambered slides (Lab-Tek, Thermo Fisher Scientific Inc.). The cells were stimulated with or without TNF-α plus IL-17A for 2 hrs. The cells were then fixed with 4% formaldehyde (Merck) in PBS for 10 minutes, permeabilized with 0.1% Tween-20 (Merck) in 4% formaldehyde for 10 minutes and blocked with 10% donkey serum (#017-000-121 Jackson Immunoresearch) for 1 hr. Primary antibodies were then added: TiAR (#D32D3 – CST; 1:250), eif3η (sc-16377 – Santa Cruz;

1:250), RBP1 (#GTX101844 – GeneTex; 1:250) and incubated at 4°C overnight followed by 1 hr incubation with fluorescent tagged secondary antibody, Alexa Fluor donkey anti-goat 647, red, (A21245 Life Technologies) for TiAR (1:250) and RBP1 (1:400) and Alexa Fluor donkey anti-goat 488, green, (A11055 Life Technologies). Nuclei were counterstained with DAPI (Invitrogen). The samples were then analysed with Leica Confocal Microscope SP-8 X SMD using LAS AF Lite software.

Immuno-purification and western blot

PBECs were plated in T75 (15 ml) flasks and grown until confluent. The cells were then left unstimulated or stimulated with TNF-α and IL-17A for 2 hrs after which cells were harvested and cytoplasmic lysates were prepared on ice using 10% Nonidet P-40 (NP-40) as mentioned elsewhere (24). The cytoplasmic fraction was pre-incubated with an isotype control antibody (Rb-anti-human-α2 macroglobulin, Dako A0033) and protein A beads to remove non-specific bound proteins and loaded on to MAC's separation columns. The pre-cleared lysate then obtained was incubated with rabbit monoclonal TiAR antibody (#D32D3 -CST) and protein A beads. After incubation it was then loaded onto the columns and washed with high and low salt buffers resulting in the flow through. The protein fraction (eluate) with bound RNA was eluted using urea lysis buffer. The detailed description of the protocol and buffers used has been described here (19). Total protein in each fraction was measured by BCA protein assay

kit (Thermo Scientific). Equal amounts of protein were separated by electrophoreses on 13% SDS-PAGE on Bio-Rad Mini Protean II and Hoefer SE600 systems and transferred to PVDF membrane (Millipore). Blots were blocked with 0.4% (w/v) skimmed milk powder in phosphate-buffered saline and incubated with primary antibodies in the ratio rabbit anti-TiAR (1:1000) and with rabbit anti-histone H3 (1:3000) and incubated at 4°C overnight followed by 1 hr incubation at room temperature with IR dye-anti-Rabbit secondary antibodies (1:10,000) (LI-COR Biosciences). The membranes were scanned and quantified using the Odyssey Infrared Imaging System (Li-COR Biosciences).

TiAR knockdown

PBECs were plated on 24-well plates (500 µl) and were grown till 30% confluence. Cells were transduced with the lenti-viral system with pLV-H1-EF1α-green vector ligated with TiAR or scrambled constructs, provided in table S1. The protocols of ligation, transformation, screening inserts in the vector and production of lenti-viral particles were done using BiOSETTIA manual for gene silencing. Polybrene was used to increase the transfection efficiency. Phenotypic screening with green fluorescence showed that 70% of the cells were transfected. The cells were transfected for 72 hrs and stimulated with TNFα and IL-17A to measure the release of cytokine after 16 hrs in the supernatants. To confirm the knockdown

by western blots, whole cell lysis was done with RIPA buffer and analysed for TiAR as mentioned above.

Alveolar macrophages isolation and stimulation

The alveolar macrophages were obtained from adult-onset asthma patients (23). After sputum induction in asthma patients and healthy controls, sputum was processed 1:1 with 10 mM Dithiothreitol (Sigma), for 30 minutes at 4°C on a rotator. DNAse (150 units/ml; Sigma; #D5025-150KU) was added 1:1000 only when there were still some cellular aggregates, 10 minutes on ice after which cells were pelleted (10 min at 400g) and resuspended in 1 ml PBS. Then 3 ml PBS/2% FCS/1 mM EDTA, 30 µl of an erythrocyte pellet and 50 µl RosetteSep Human Monocyte Enrichment Cocktail (Stem cell Technologies) was added for 20 minutes and incubated at room temperature. Subsequently, cells were layered on top of 3 ml LymphoPrep and centrifuged for 22 minutes at 1355g. The mononuclear layer was collected and washed in 25 ml ice-cold IMDM (1% FCS, 100 units penicillin/ml and 10mg/ml streptomycin) and centrifuged for 10 min at 500g) and the pellet was dissolved in 1 ml IMDM (1% FCS, 100 units penicillin/ml and 10mg/ml streptomycin). Macrophages were allowed to adhere to the surface of the plates for 24 hrs and then stimulated with TNF-α and IL-17A, TNF-α alone or not stimulated in fresh medium.

Statistical methods

GraphPad Prism 7 was used for statistical analyses (t-tests, one-way and two-way ANOVA). For t-tests of data with normal distribution parametric t-tests were used whereas Mann-Whitney U non-parametric t-test was performed for data not normally distributed. P values ≤0.05 were considered statistically significant. For associations, Pearson's correlation test was performed with two-tailed analysis. Scatter plot analysis was done in IBM SPSS.

Supplementary tables

Table E1. Oligonucleotide sequences provided for the respective analysis.

Analysis	Oligonucleotide sequences
mRNA analysis	Hu IL-8 PPH00568A, SA Biosciences Hu IL-6 PPH00560B, SA Biosciences Hu GAPDH (forward) : TCA TCT CTG CCC CCT CTG C Hu GAPDH (reverse) : GAG TCC TTC CAG GAT ACC AA
miR 16 analysis	miR16-1 (Stem loop) GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC GCC AAT AT miR 16-1 (Forward) TGC GGT AGC AGC ACG TAA AT miR16-1 (Reverse) TGC AGG GTC CGA GGT AT
miRCURY™ LNA inhibitor	miR16 - CGC CAA TAT TTA CGT GCT GCT A scrambled control – TAA CAC GTC TAT ACG CCC A
BiOSETTIA TiAR knockdown	TiAR 1 - AAAA TGACAGAAGTCCTTATACT TTGGATCCAA AGTATAAGGACTTCTGTCA TiAR 2 - AAAA GAAAGGAGGTCAAAGTAAA TTGGATCCAA TTTACTTTGACCTCCTTTC

	TiAR 3 - AAAA AAGGGCTATTCATTTGTCAGA TTGGATCCAA TCTGACAAATGAATAGCCCTT

Supplementary figure legends

Figure E1. (*A*) **IL-17A with TNF-α distinguishes hyperresponsive PBECs**. PBECs from 43 mild and 16 severe asthmatics and 16 healthy controls were stimulated with TNF-α plus IL-17A for 16 hrs inducing significantly higher levels of CXCL-8 production compared TNF-α or IL-17A alone, in all groups, but more pronounced in asthmatics. Data are shown as mean ± SD. For statistical analyses two-tailed Wilcoxon matched pairs signed rank t-test was used: ****P<0.0001. (*B*). **Correlation between TNF-α and IL-17A-induced CXCL-8 production and the synergistic effect distinguishes hyperresponsive PBECs.** CXCL-8 release upon TNF-α and IL-17A stimulation showed a strong positive correlation with the synergistic effect, calculated as CXCL-8 levels with TNF-α+IL-17A stimulations divided by CXCL-8 levels with TNF-α stimulation alone plus that by IL-17A stimulation alone. Explanations for colored symbols are provided in Figure 1. Pearson's correlation test was performed with two-tailed analysis. (*C*). **TNF-α stimulation alone with dexamethasone reduces CXCL-8 production in bronchial epithelium**. Normo- (in black; *n*=7) and hyperresponsive (in red; *n*=15) PBECs stimulated for 16hrs with TNF-α in the presence or absence of dexamethasone (3.8 μM). For statistical analysis paired t-tests (parametric) were used: *P<0.05. (*D*) **CXCL-10 and MIF levels are not affected by dexamethasone in PBECs.** As (C).

Figure E2. Hyperresponsiveness of PBECs is not associated with PC_{20}. CXCL-8 levels produced by PBECs from mild and severe asthmatics (*n*=35) after TNF-α and IL-17A

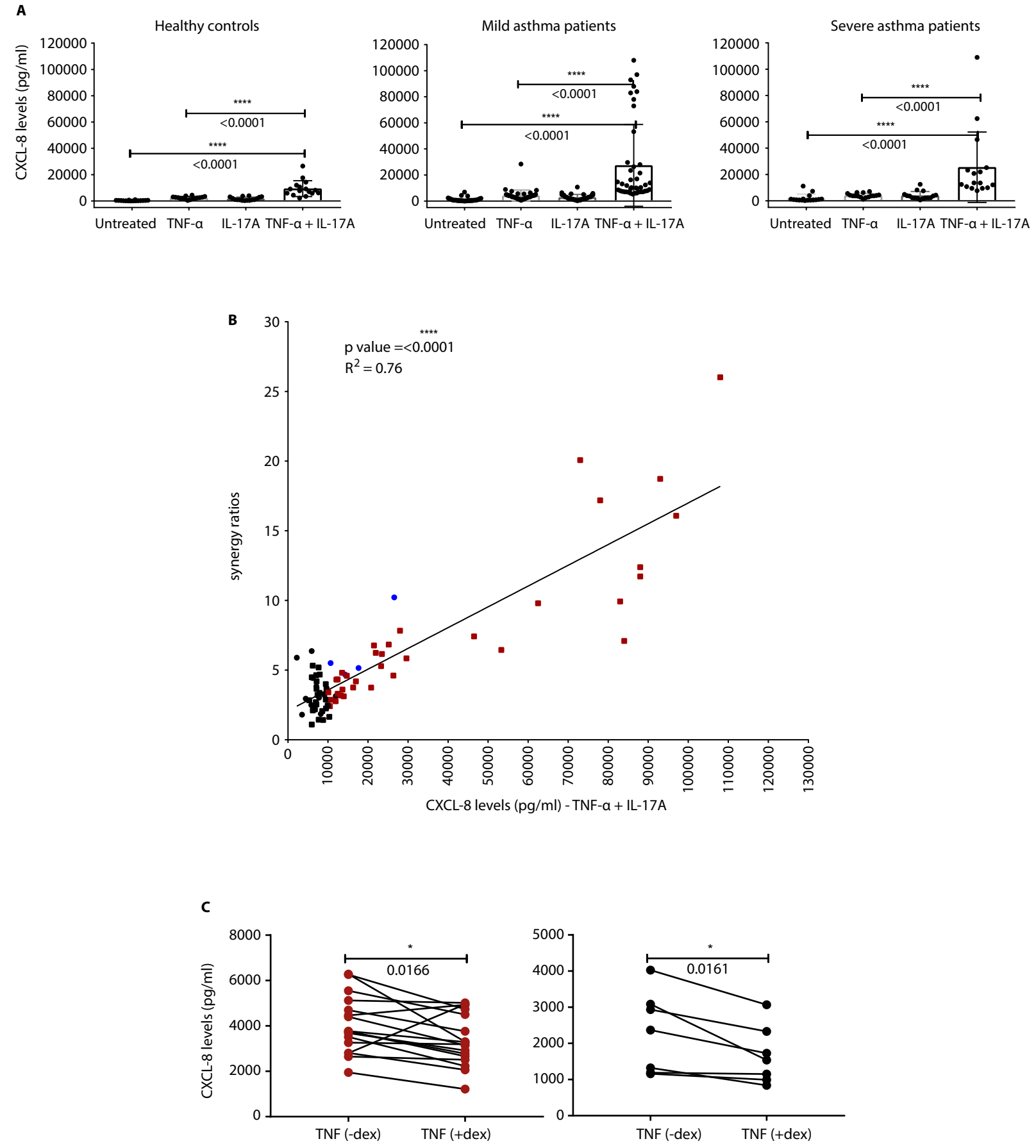
stimulation did not correlate with PC_{20} (mg/ml) measured 3 days prior to collection of bronchial brushes. Pearson's correlation test was performed with two-tailed analysis.

Figure E3. TiAR in hyperresponsive PBECs does not translocate to the cytoplasm with dexamethasone, but translocates upon exposure to arsenite. (*A*) Immunohistochemistry of hyper- (*n*=5 ; upper panel) and normoresponsive (*n*=3 ; lower panel) PBECs shown as TiAR (red) and eiF3η (green) after stimulation with TNF-α and IL-17A for 2 hrs in the presence (right) and absence (left) of dexamethasone. Nuclei are stained with DAPI. (*B*) TiAR (red) is shown by confocal microscopy to translocate to the cytoplasm in hyperresponsive PBECs treated for 30 minutes with 50 μM arsenite and to form stress granules (arrows) with 500 μM after 30 minutes of arsenite. (*n*=3). (*C*) Co-localization of TiAR (red) and eiF3η (green) in cytoplasm of normoresponsive PBECs stimulated for 16 hrs with TNF-α and IL-17A and treatment with 50 μM arsenite for 30 minutes. At 500 μM of arsenite for 30 minutes stress granules are formed. Nuclei are stained with DAPI. (*D*) TiAR is not associated with endoplasmic reticulum as shown by immunohistochemistry of localization of TiAR (green) in the nucleus and RBP1 (red) in cytoplasm stimulated TNF-α and IL-17A for 2 hrs in hyperresponsive PBECs.

Figure E4. (*A*) **IL-17RA mRNA expression remains unchanged between hyper- and normoresponsive PBECs**. IL-17RA mRNA expression is similar in normo- (*n*=3) and hyperresponsive PBECs (*n*=3), from mild asthmatics (*n*=6) and healthy controls (*n*=6), left unstimulated or stimulated with TNF-α, IL-17A or TNF-α with IL-17A for 16 hrs and normalized to GAPDH mRNA expression. (*B*) **Alveolar macrophages from asthmatics and healthy controls do not display hyperresponsiveness.** CXCL-8 measured in healthy controls

(*n*=5) and asthma patients (*n*=6) did not show any synergistic increase with TNF-α and IL-17A compared to TNF-α alone.

Figure E1


A
Healthy controls
Mild asthma patients
Severe asthma patients
CXCL-8 levels (pg/ml)
120000
100000
80000
60000
40000
20000
0
****
<0.0001
Untreated
TNF-α
IL-17A
TNF-α + IL-17A
B
p value =<0.0001
R2 = 0.76
synergy ratios
CXCL-8 levels (pg/ml) - TNF-α + IL-17A
C
CXCL-8 levels (pg/ml)
*
0.0166
0.0161
TNF (-dex)
TNF (+dex)

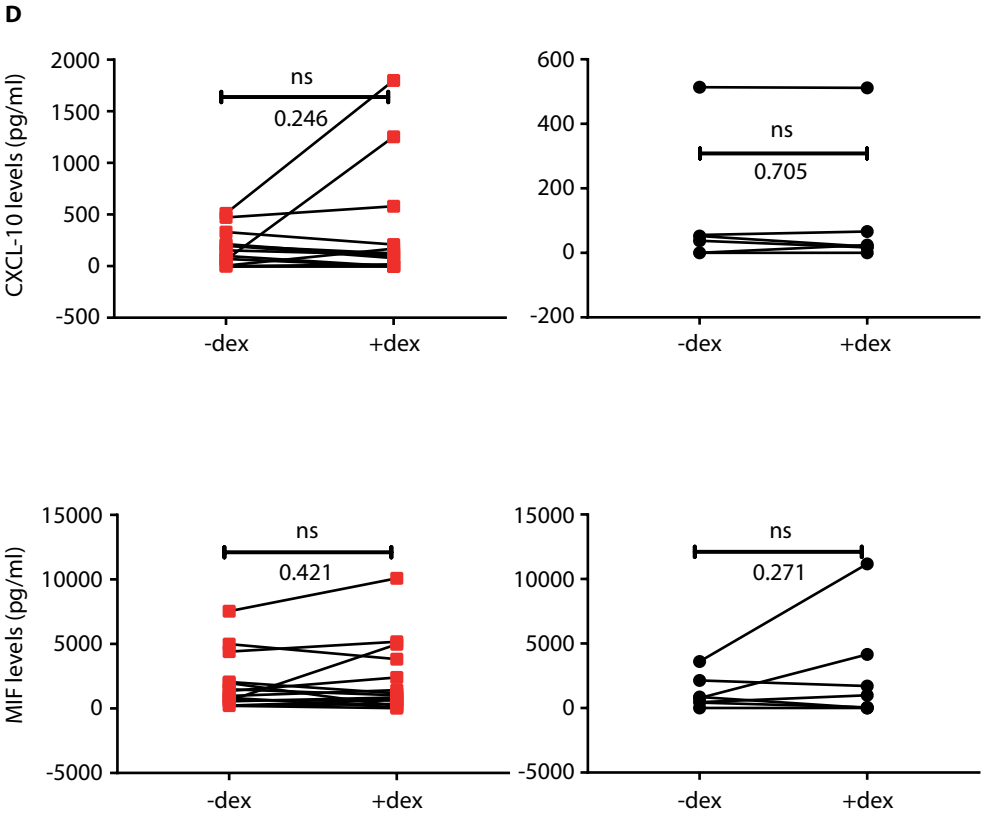

D
CXCL-10 levels (pg/ml)
2000
1500
1000
500
0
-500
ns
0.246
-dex
+dex
600
400
200
0
-200
ns
0.705
-dex
+dex
MIF levels (pg/ml)
15000
10000
5000
0
-5000
ns
0.421
-dex
+dex
15000
10000
5000
0
-5000
ns
0.271
-dex
+dex

Figure E2

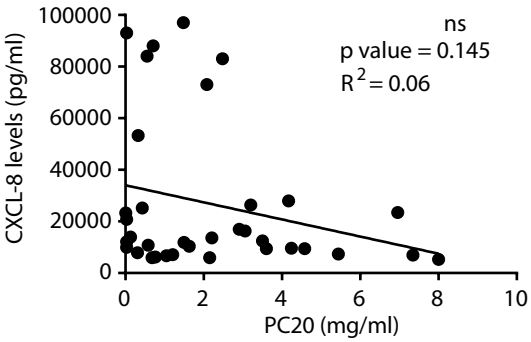

CXCL-8 levels (pg/ml)
100000
80000
60000
40000
20000
0
ns
p value = 0.145
R 2 = 0.06
0
2
4
6
8
10
PC20 (mg/ml)

Figure E3

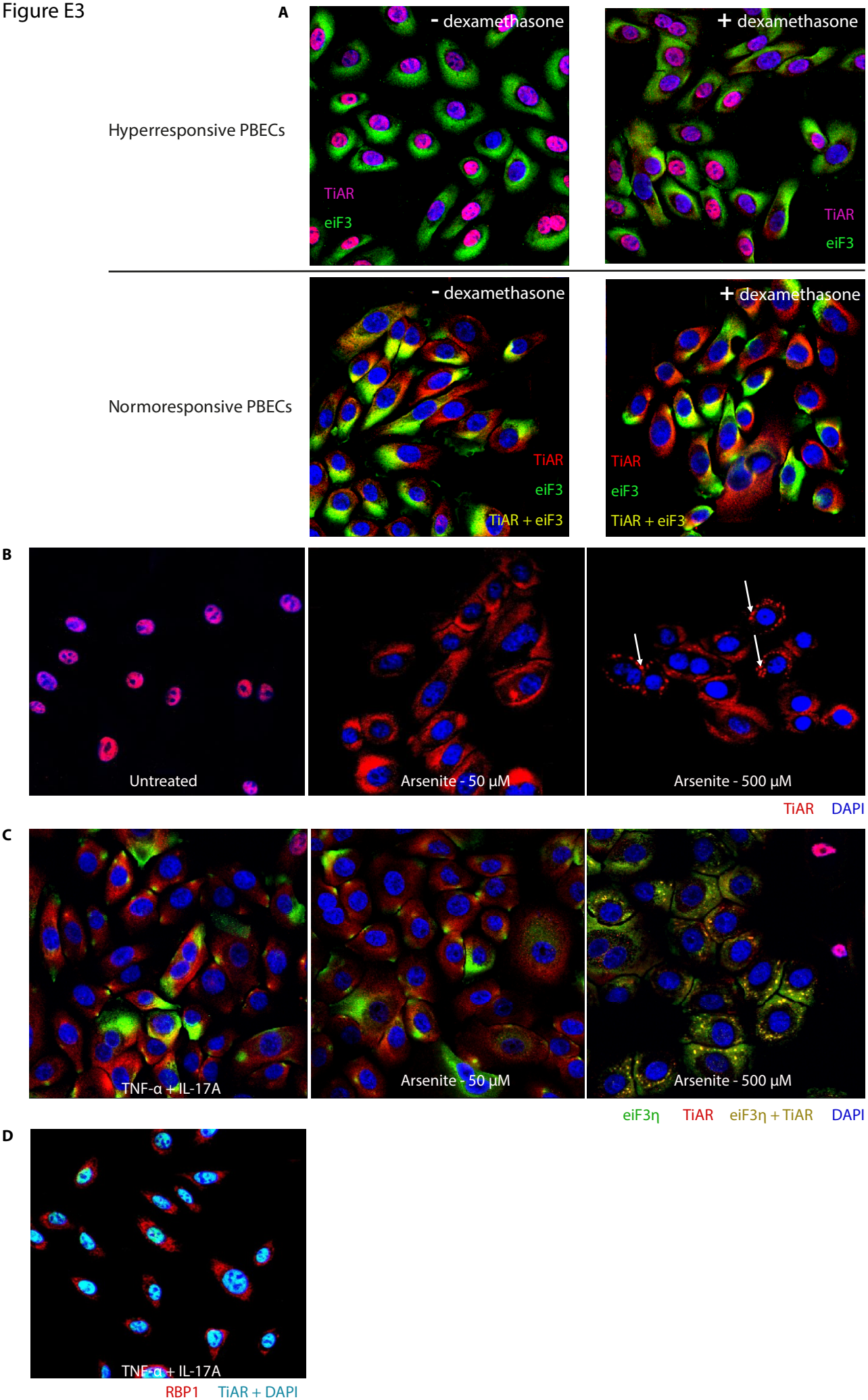
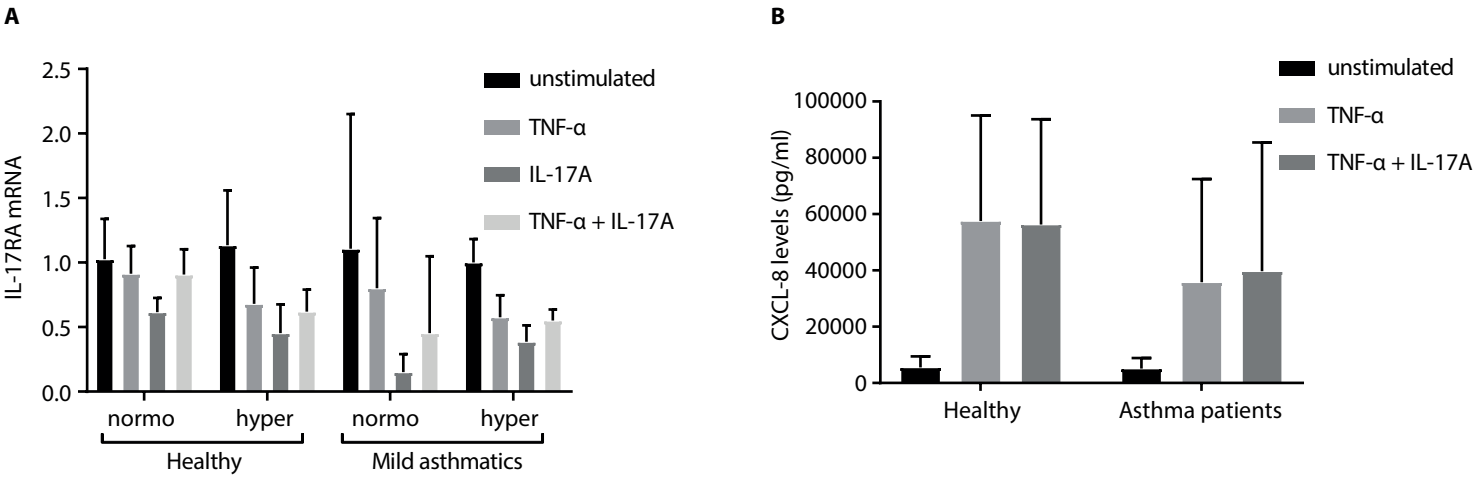

A
Hyperresponsive PBECs
- dexamethasone
TiAR
eiF3
+ dexamethasone
TiAR
eiF3
Normoresponsive PBECs
- dexamethasone
TiAR
eiF3
TiAR + eiF3
+ dexamethasone
TiAR
eiF3
TiAR + eiF3
B
Untreated
Arsenite - 50 µM
Arsenite - 500 µM
TiAR DAPI
C
TNF-α + IL-17A
Arsenite - 50 µM
Arsenite - 500 µM
eiF3η TiAR eiF3η + TiAR DAPI
D
TNF-α + IL-17A
RBP1 TiAR + DAPI

Figure E4


A
IL-17RA mRNA
2.5
2.0
1.5
1.0
0.5
0.0
unstimulated
TNF-α
IL-17A
TNF-α + IL-17A
normo
hyper
normo
hyper
Healthy
Mild asthmatics
B
CXCL-8 levels (pg/ml)
100000
80000
60000
40000
20000
0
unstimulated
TNF-α
TNF-α + IL-17A
Healthy
Asthma patients