Aurintricarboxylic acid as an effective intervention for alveolar epithelial regeneration and tissue repair in emphysematous lung

Kusum Devi[1] and Baisakhi Moharana[1]

[1]CSIR-Central Drug Research Institute

November 21, 2022

Abstract

Background and purpose: Chronic obstructive pulmonary disease (COPD) is a global health burden leading to significant morbidity and mortality worldwide. CS exposure is a crucial driver to various progressive lung diseases including COPD and there is an unmet need of potential therapeutics. The present study examined to decipher the therapeutic role of TWEAK/Fn14 selective inhibitor aurintricarboxylic acid in COPD pathogenesis. Experimental approach: In vitro alveolar type II cells and macrophages were stimulated with cigarette smoke extract (CSE) followed by aurintricarboxylic acid (ATA) treatment. In vivo, rats were injected with CSE intraperitoneally twice a week for 16 weeks followed by ATA (10mg/kg) treatment for 4 weeks. Key results: Increased ROS generation, mitochondrial dysfunction in alveolar type II cells and nuclear translocation of NF-ϰB/p65 in macrophages were abrogated after ATA treatment. In vivo, lung inflammation induced by CSE followed by ATA treatment mitigated respiratory function impairment, immune cell infiltration, emphysema associated morphometric parameters and oxidative stress status. MALDI assessment of rat serum showed decreased CRP and related inflammatory proteins. In addition, ATA inhibited up-regulation of EMT related proteins including vimentin, α-SMA, and downstream transcription factor Snail via inhibition of β-catenin/wnt3a pathway. Furthermore, ATA reduced CSE-induced inflammatory makers including TNF-α, NF-ϰB and MAP kinases including p-p38, p-ERK1/2 and p-JNK in vitro and in vivo. Cumulatively, ATA enhanced lung regeneration and tissue repair by diminishing the inflammation and EMT transition. Hence, ATA can be a novel therapeutic candidate to reduce COPD associated pathological events and promote regeneration.

Hosted file

`BJP Manuscript.docx` available at https://authorea.com/users/526520/articles/596299-aurintricarboxylic-acid-as-an-effective-intervention-for-alveolar-epithelial-regeneration-and-tissue-repair-in-emphysematous-lung

Hosted file

`Table.docx` available at https://authorea.com/users/526520/articles/596299-aurintricarboxylic-acid-as-an-effective-intervention-for-alveolar-epithelial-regeneration-and-tissue-repair-in-emphysematous-lung

Figure 1.

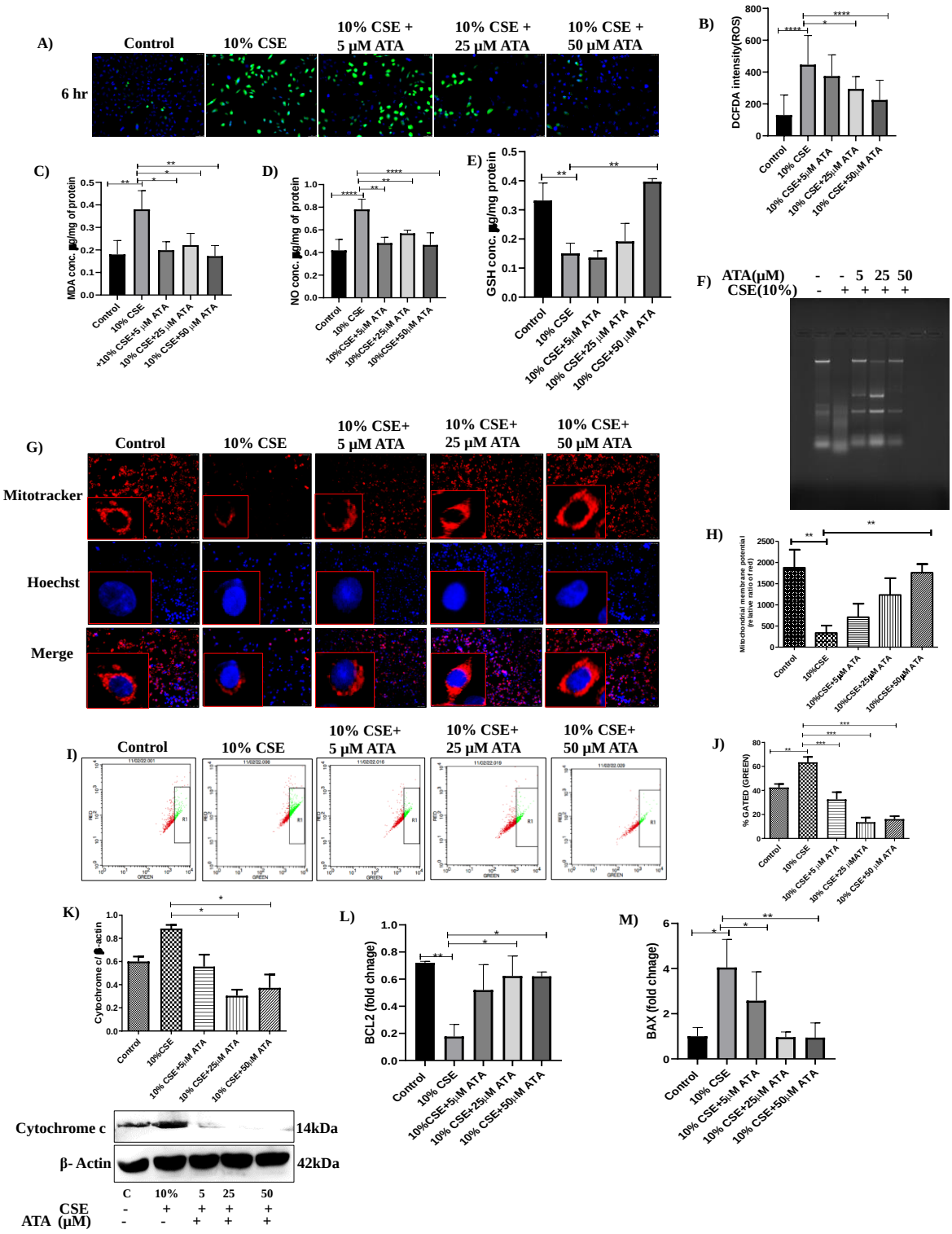

A)
Control
10% CSE
10% CSE + 5 µM ATA
10% CSE + 25 µM ATA
10% CSE + 50 µM ATA
6 hr
B)
DCFDA intensity(ROS)
C)
MDA conc. µg/mg of protein
D)
NO conc. µg/mg of protein
E)
GSH conc. µg/mg protein
Control
10% CSE
10% CSE+5µM ATA
10% CSE+25µM ATA
10% CSE+50µM ATA
F)
ATA(µM) - - 5 25 50
CSE(10%) - + + + +
G)
Control
10% CSE
10% CSE+ 5 µM ATA
10% CSE+ 25 µM ATA
10% CSE+ 50 µM ATA
Mitotracker
Hoechst
Merge
H)
Mitochondrial membrane potential (relative ratio of red)
I)
Control
10% CSE
10% CSE+ 5 µM ATA
10% CSE+ 25 µM ATA
10% CSE+ 50 µM ATA
J)
% GATED (GREEN)
K)
Cytochrome c/ β-actin
L)
BCL2 (fold chnage)
M)
BAX (fold chnage)
Cytochrome c
β- Actin
14kDa
42kDa
C 10% 5 25 50
CSE - + + + +
ATA (µM) - - + + +

Figure 2.

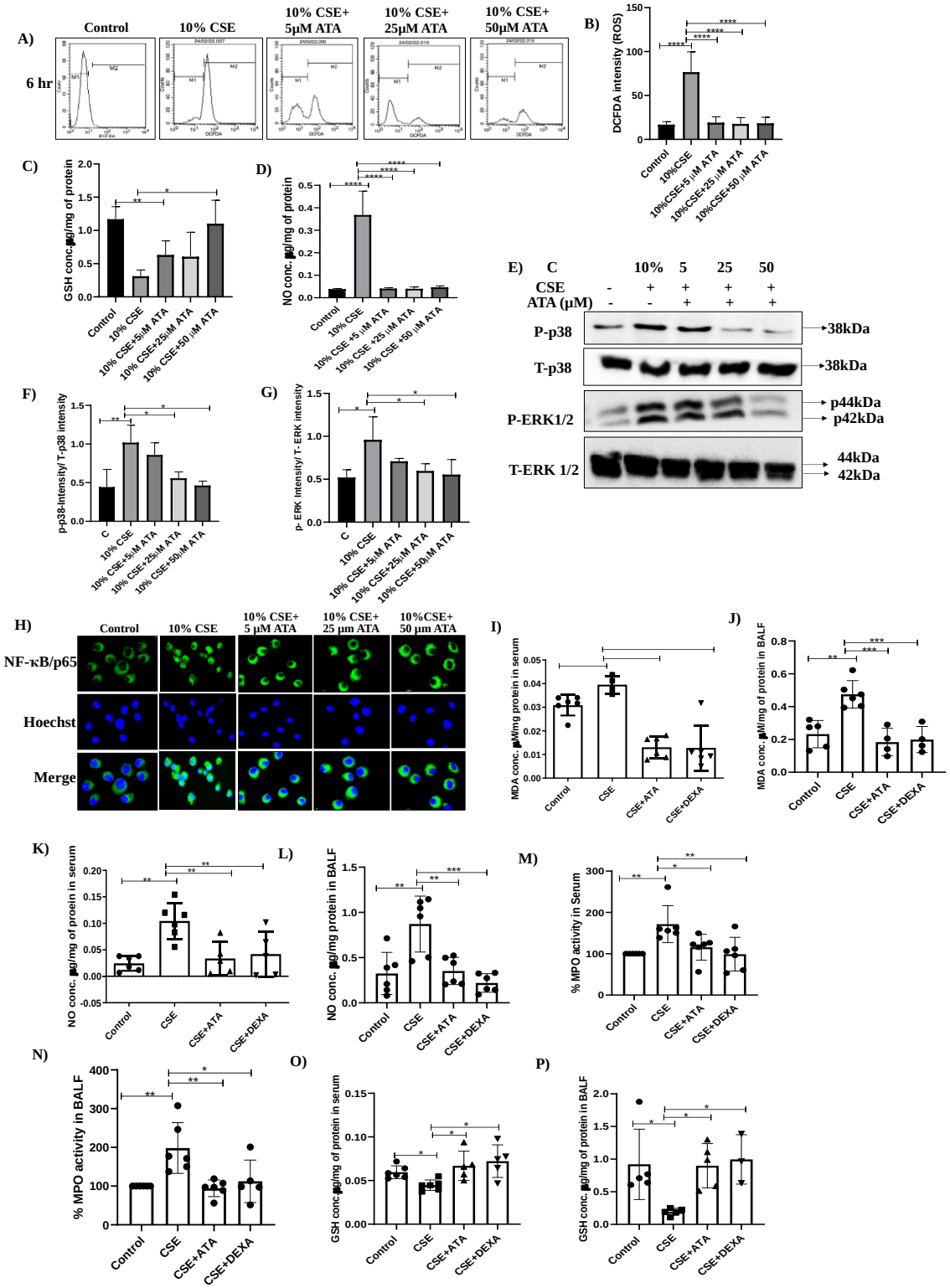

A)
Control
10% CSE
10% CSE+ 5μM ATA
10% CSE+ 25μM ATA
10% CSE+ 50μM ATA
6 hr
B)
DCFDA intensity (ROS)
C)
GSH conc. μg/mg of protein
D)
NO conc. μg/mg of protein
E)
C
10%
5
25
50
CSE
ATA (μM)
P-p38
T-p38
P-ERK1/2
T-ERK 1/2
38kDa
38kDa
p44kDa
p42kDa
44kDa
42kDa
F)
p-p38-Intensity/ T-p38 intensity
G)
p- ERK Intensity/ T- ERK intensity
H)
Control
10% CSE
10% CSE+ 5 μM ATA
10% CSE+ 25 μm ATA
10%CSE+ 50 μm ATA
NF-κB/p65
Hoechst
Merge
I)
MDA conc. μM/mg protein in serum
J)
MDA conc. μM/mg of protein in BALF
K)
NO conc. μg/mg of proein in serum
L)
NO conc. μg/mg protein in BALF
M)
% MPO activity in Serum
N)
% MPO activity in BALF
O)
GSH conc. μg/mg of protein in serum
P)
GSH conc. μg/mg of protein in BALF
Control
CSE
CSE+ATA
CSE+DEXA

Figure 3.

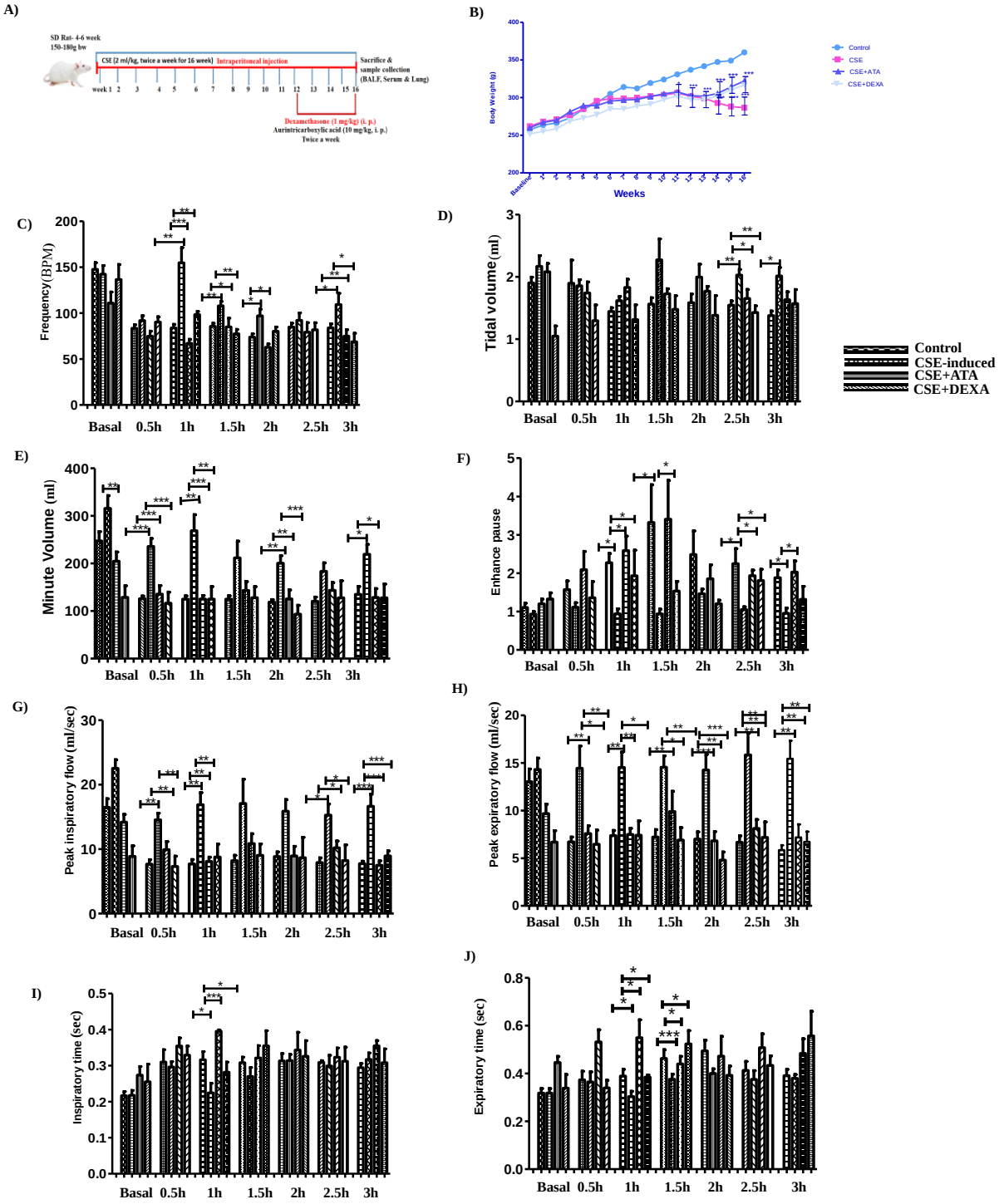

A)
SD Rat- 4-6 week
150-180g bw
CSE (2 ml/kg, twice a week for 16 week)
Intraperitoneal injection
Sacrifice & sample collection (BALF, Serum & Lung)
week 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Dexamethasone (1 mg/kg) (i. p.)
Aurintricarboxylic acid (10 mg/kg, i. p.)
Twice a week
B)
Body Weight (g)
Weeks
Control
CSE
CSE+ATA
CSE+DEXA
C)
Frequency(BPM)
D)
Tidal volume(ml)
E)
Minute Volume (ml)
F)
Enhance pause
G)
Peak inspiratory flow (ml/sec)
H)
Peak expiratory flow (ml/sec)
I)
Inspiratory time (sec)
J)
Expiratory time (sec)
Basal 0.5h 1h 1.5h 2h 2.5h 3h
Control
CSE-induced
CSE+ATA
CSE+DEXA

Figure 4.

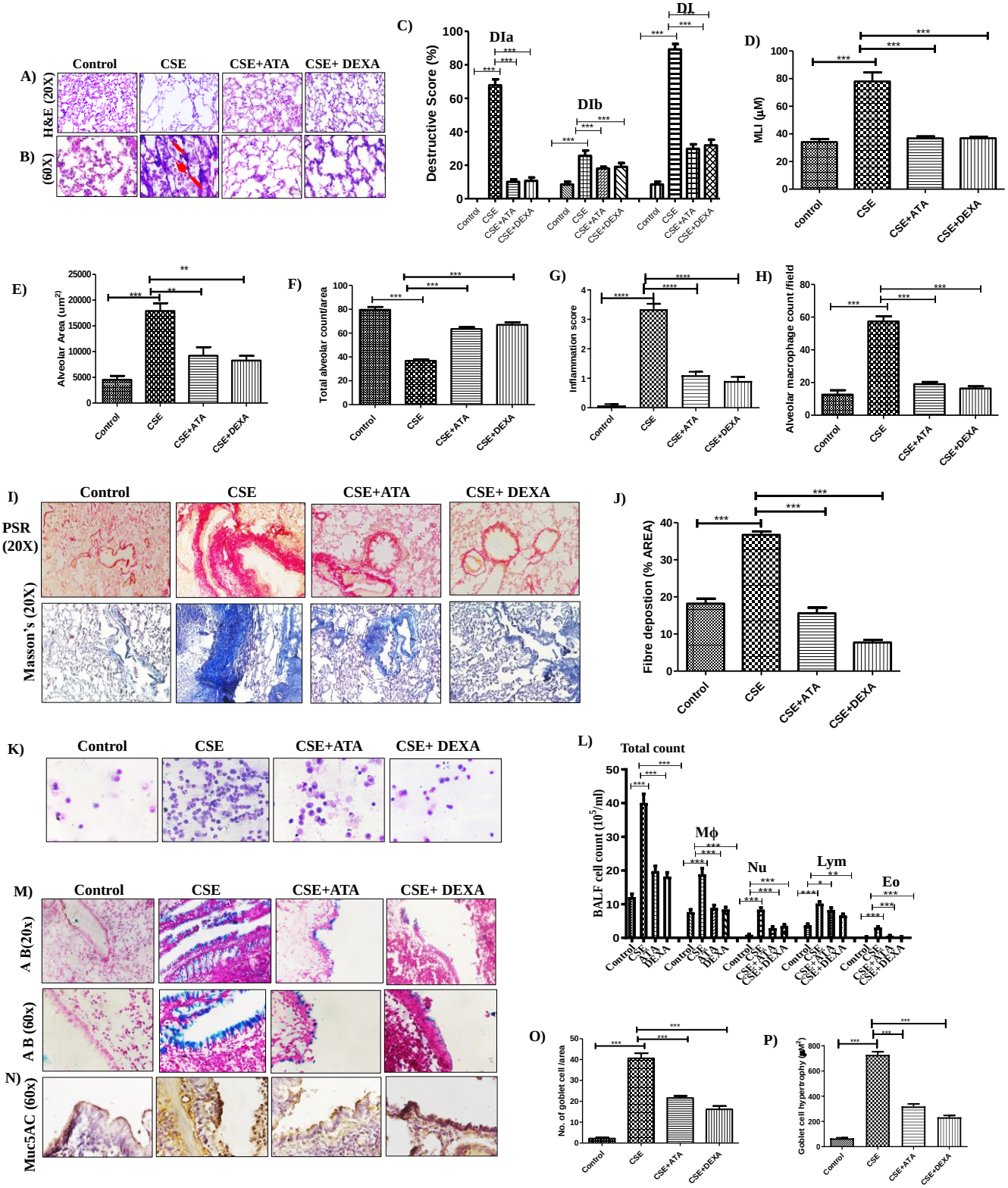

A)
H&E (20X)
B)
(60X)
Control
CSE
CSE+ATA
CSE+ DEXA
C)
Destructive Score (%)
DIa
DIb
DI
D)
MLI (µM)
E)
Alveolar Area (um2)
F)
Total alveolar count/area
G)
Inflammation score
H)
Alveolar macrophage count /field
I)
PSR (20X)
Masson's (20X)
J)
Fibre depostion (% AREA)
K)
L)
Total count
BALF cell count (10^5/ml)
Mϕ
Nu
Lym
Eo
M)
A B(20x)
A B (60x)
N)
Muc5AC (60x)
O)
No. of goblet cell /area
P)
Goblet cell hypertrophy (µm2)

Figure 5.

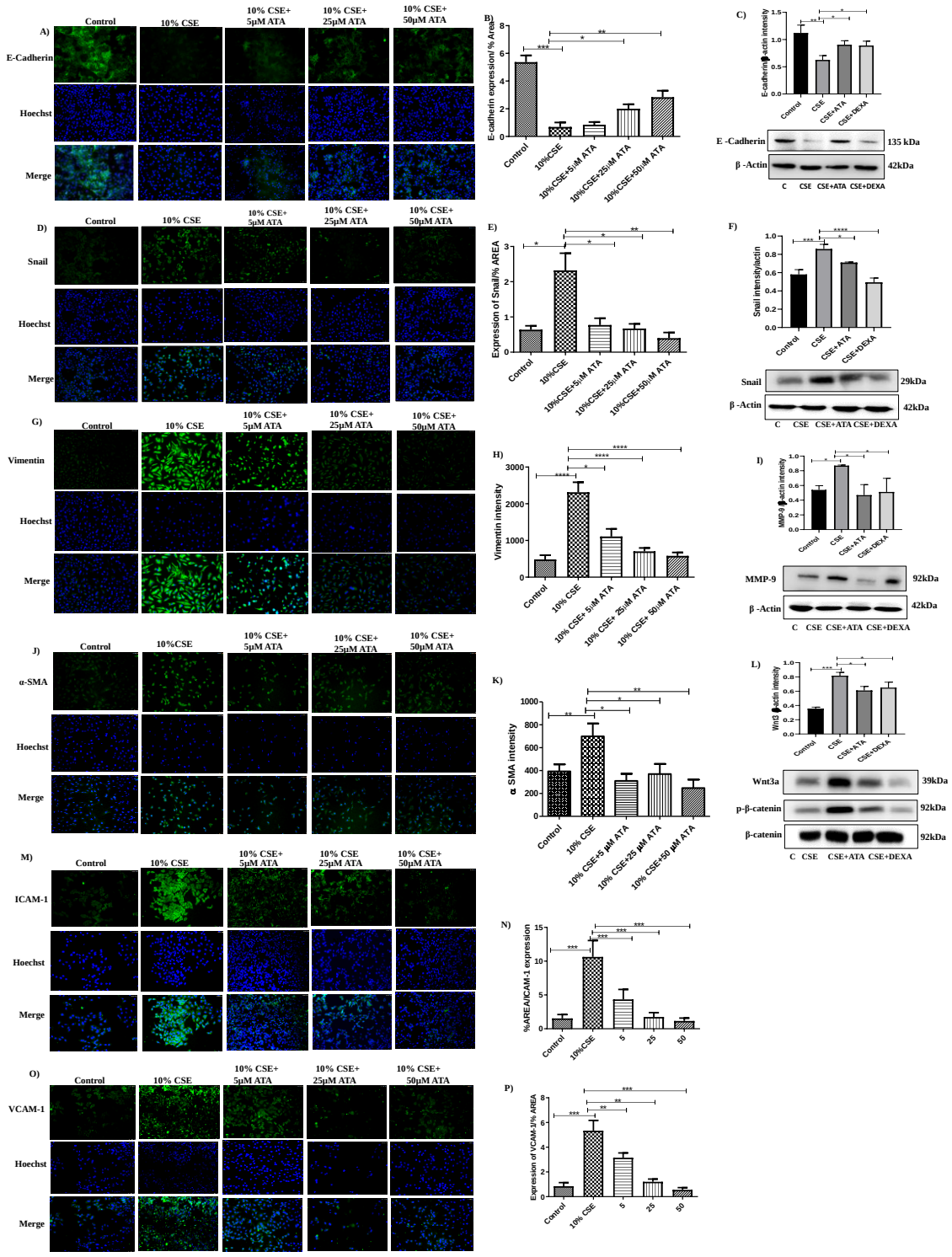

A)
Control
10% CSE
10% CSE+ 5μM ATA
10% CSE+ 25μM ATA
10% CSE+ 50μM ATA
E-Cadherin
Hoechst
Merge
B)
E-cadherin expression/ % Area
Control
10%CSE
10%CSE+5μM ATA
10%CSE+25μM ATA
10%CSE+50μM ATA
C)
E-cadherin/β-actin intensity
Control
CSE
CSE+ATA
CSE+DEXA
E -Cadherin
135 kDa
β -Actin
42kDa
C CSE CSE+ATA CSE+DEXA
D)
Snail
E)
Expression of Snail/% AREA
F)
Snail intensity/actin
Snail
29kDa
G)
Vimentin
H)
Vimentin intensity
I)
MMP-9/β-actin intensity
MMP-9
92kDa
J)
α-SMA
K)
α SMA intensity
L)
Wnt3a/β-actin intensity
Wnt3a
39kDa
p-β-catenin
β-catenin
M)
ICAM-1
N)
%AREA/ICAM-1 expression
O)
VCAM-1
P)
Expression of VCAM-1/% AREA

Figure 6.

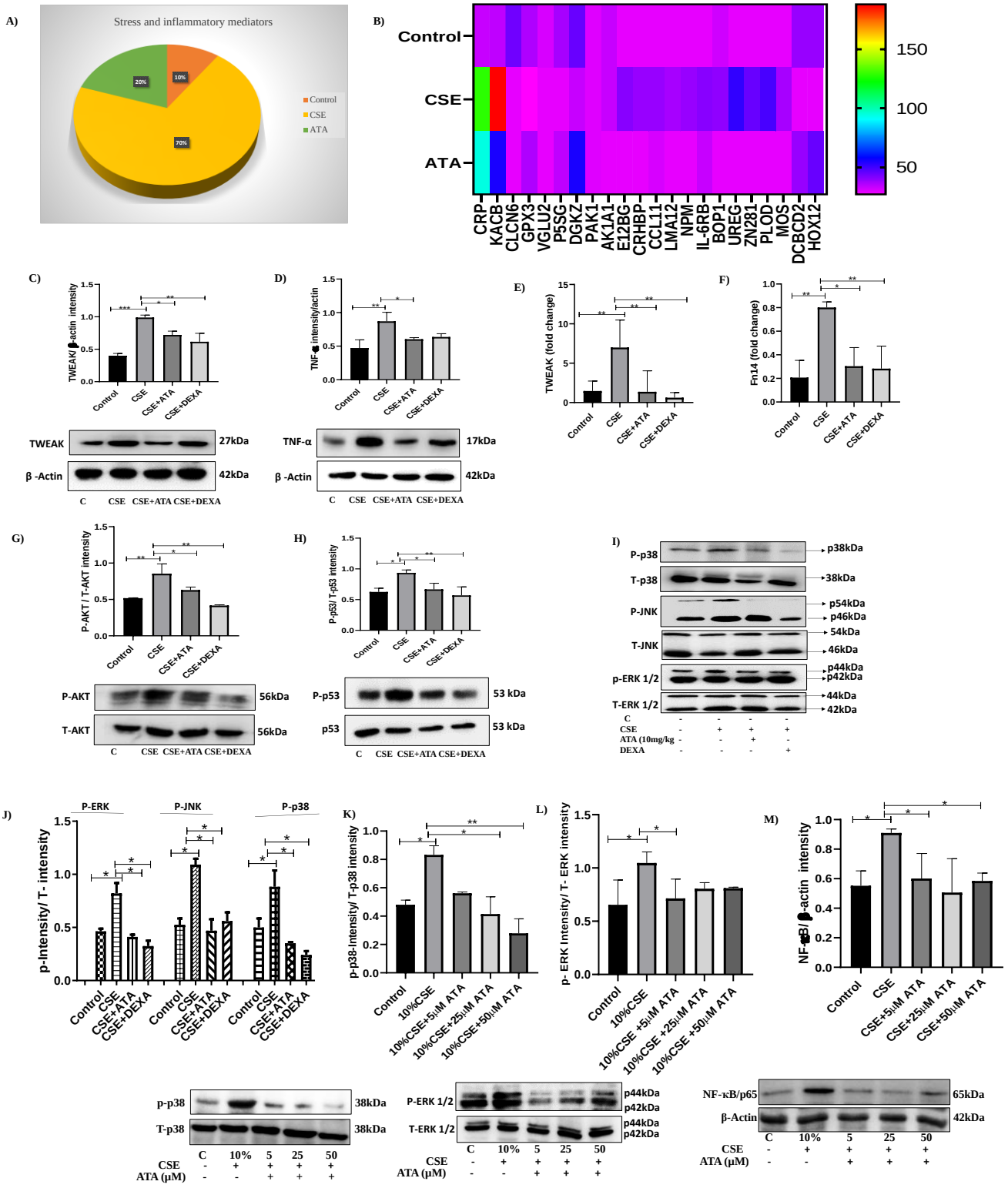
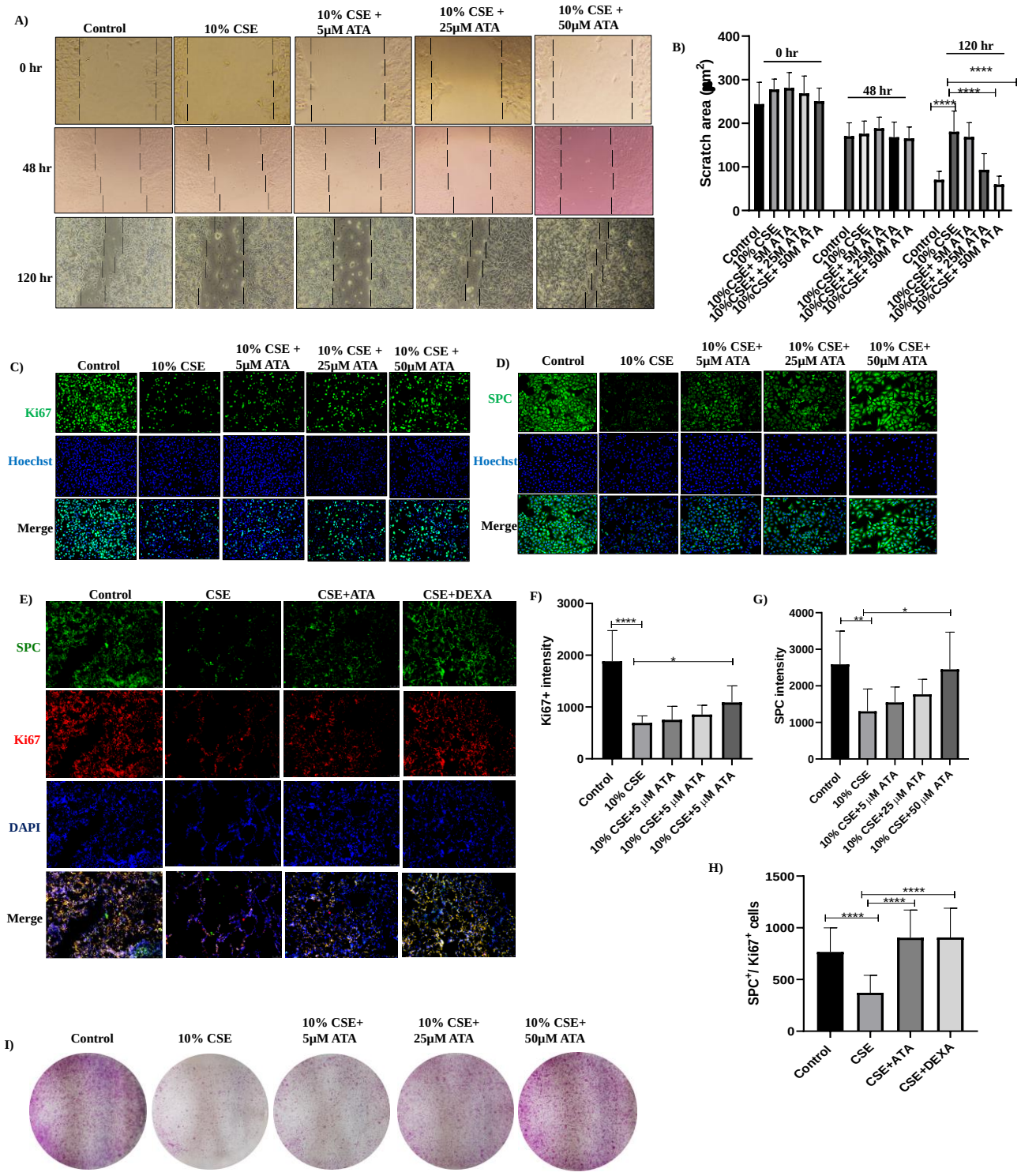

A)
Stress and inflammatory mediators
10%
20%
70%
Control
CSE
ATA
B)
Control
CSE
ATA
150
100
50
CRP
KACB
CLCN6
GPX3
VGLU2
P5SG
DGK2
PAK1
AK1A1
E12BG
CRHBP
CCL11
LMA12
NPM
IL-6RB
BOP1
UREG
ZN281
PLOD
MOS
DCBCD2
HOX12
C)
TWEAK/ β-actin intensity
Control
CSE
CSE+ATA
CSE+DEXA
TWEAK
27kDa
β -Actin
42kDa
C CSE CSE+ATA CSE+DEXA
D)
TNF-α intensity/actin
TNF-α
17kDa
β -Actin
42kDa
C CSE CSE+ATA CSE+DEXA
E)
TWEAK (fold change)
F)
Fn14 (fold change)
G)
P-AKT / T-AKT intensity
P-AKT
56kDa
T-AKT
56kDa
C CSE CSE+ATA CSE+DEXA
H)
P-p53/ T-p53 intensity
P-p53
53 kDa
p53
53 kDa
C CSE CSE+ATA CSE+DEXA
I)
P-p38
T-p38
P-JNK
T-JNK
p-ERK 1/2
T-ERK 1/2
p38kDa
38kDa
p54kDa
p46kDa
54kDa
46kDa
p44kDa
p42kDa
44kDa
42kDa
C
CSE
ATA (10mg/kg
DEXA
J)
P-ERK
P-JNK
P-p38
p-Intensity/ T- intensity
Control
CSE
CSE+ATA
CSE+DEXA
K)
p-p38-Intensity/ T-p38 intensity
Control
10%CSE
10%CSE+5μM ATA
10%CSE+25μM ATA
10%CSE+50μM ATA
L)
p- ERK Intensity/ T- ERK intensity
Control
10%CSE
10%CSE +5μM ATA
10%CSE +25μM ATA
10%CSE +50μM ATA
M)
NF-κB/ β-actin intensity
Control
CSE
CSE+5μM ATA
CSE+25μM ATA
CSE+50μM ATA
p-p38
T-p38
38kDa
38kDa
C 10% 5 25 50
CSE
ATA (μM)
P-ERK 1/2
T-ERK 1/2
p44kDa
p42kDa
p44kDa
p42kDa
C 10% 5 25 50
CSE
ATA (μM)
NF-κB/p65
β-Actin
65kDa
42kDa
C 10% 5 25 50
CSE
ATA (μM)

Figure 7.


A)
Control
10% CSE
10% CSE + 5μM ATA
10% CSE + 25μM ATA
10% CSE + 50μM ATA
0 hr
48 hr
120 hr
B)
Scratch area (μm²)
0 hr
48 hr
120 hr
****
Control
10% CSE
10%CSE+ 5M ATA
10%CSE+ + 25M ATA
10%CSE+ 50M ATA
C)
Ki67
Hoechst
Merge
D)
SPC
E)
CSE
CSE+ATA
CSE+DEXA
DAPI
F)
Ki67+ intensity
G)
SPC intensity
H)
SPC+/ Ki67+ cells
I)