FIG. 1

Experimental asthma induction and anti-HH treatments		Analysis
Preventive treatment		
Groups:		
8 weeks untreated	(UT)	Lung function (resistance and compliance)
8 weeks OVA	(OVA)	
8 weeks OVA + anti-hedgehog	(OVA + αHH)	
8 weeks OVA + isotype control	(OVA + Iso)	
4 weeks untreated	(UT)	Lung histopathology (epithelium, mucus, smooth muscle, inflammation)
4 weeks OVA	(OVA)	
4 weeks OVA + anti-hedgehog	(OVA + αHH)	
4 weeks OVA + isotype control	(OVA + Iso)	
4 weeks untreated	(UT)	BAL and lung immune cell infiltrates
4 weeks HDM	(HDM)	
4 weeks HDM + anti-hedgehog	(HDM + αHH)	
4 weeks HDM + isotype control	(HDM + Iso)	
Therapeutic treatment		Cytokine production, IgE
Groups:		
8 weeks untreated		RNA and protein expression for injury, remodeling and inflammatory genes
8 weeks OVA		
4 weeks OVA followed by 4 weeks OVA+ anti-hedgehog		
4 weeks OVA followed by 4 weeks OVA+ isotype control		

FIG. 2

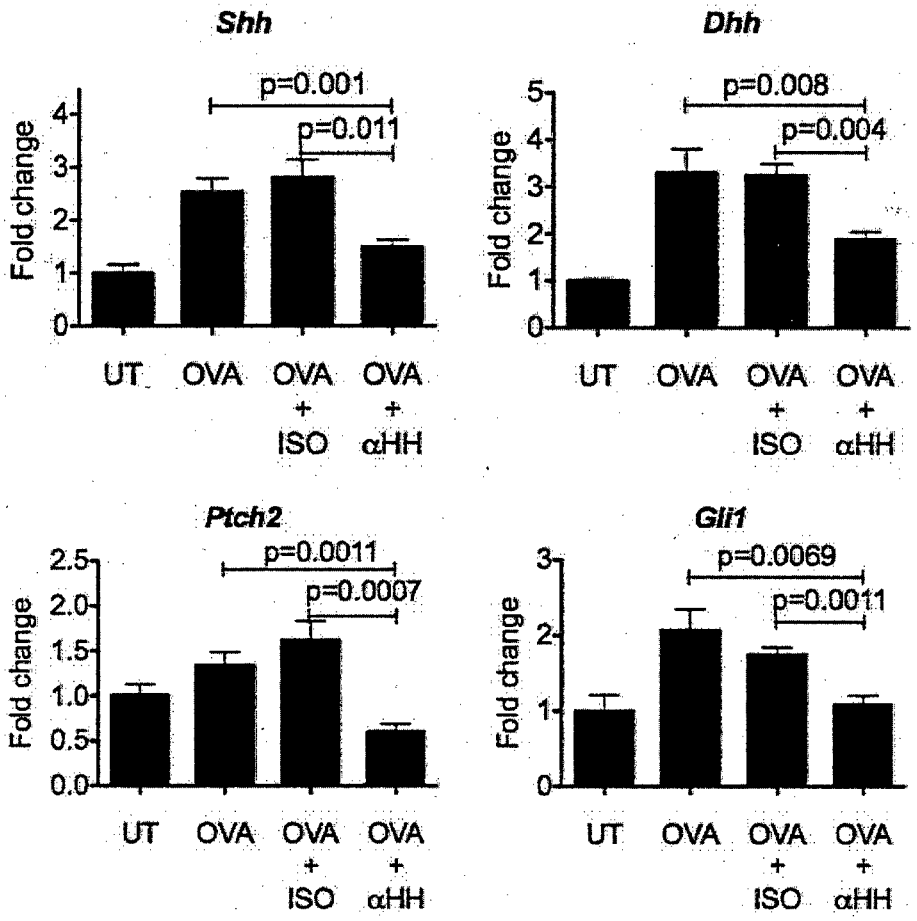
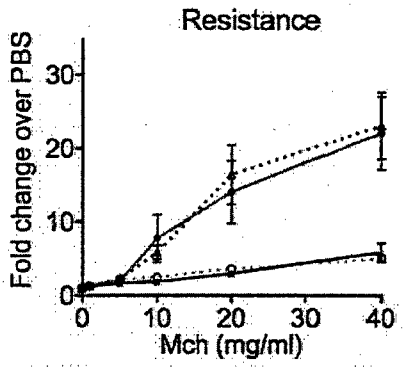
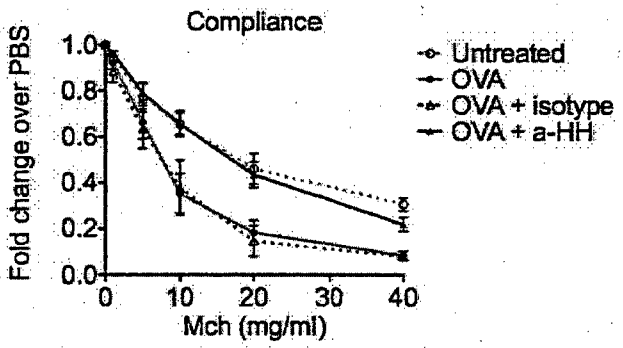

Shh
p=0.001
p=0.011
Fold change
0
1
2
3
4
UT
OVA
OVA + ISO
OVA + αHH
Dhh
p=0.008
p=0.004
Fold change
0
1
2
3
4
5
UT
OVA
OVA + ISO
OVA + αHH
Ptch2
p=0.0011
p=0.0007
Fold change
0.0
0.5
1.0
1.5
2.0
2.5
UT
OVA
OVA + ISO
OVA + αHH
Gli1
p=0.0069
p=0.0011
Fold change
0
1
2
3
UT
OVA
OVA + ISO
OVA + αHH

FIG. 3



p-value:	10	20	40 mg/ml
OVA vs UT	ns	0.042	0.003
OVA vs a-HH	ns	0.046	0.009
Iso vs UT	ns	0.006	0.001
Iso vs a-HH	ns	0.008	0.005



p-value:	10	20	40 mg/ml
OVA vs UT	0.013	0.006	<0.001
OVA vs a-HH	0.016	0.008	0.002
Iso vs UT	0.049	0.007	<0.001

FIG. 4

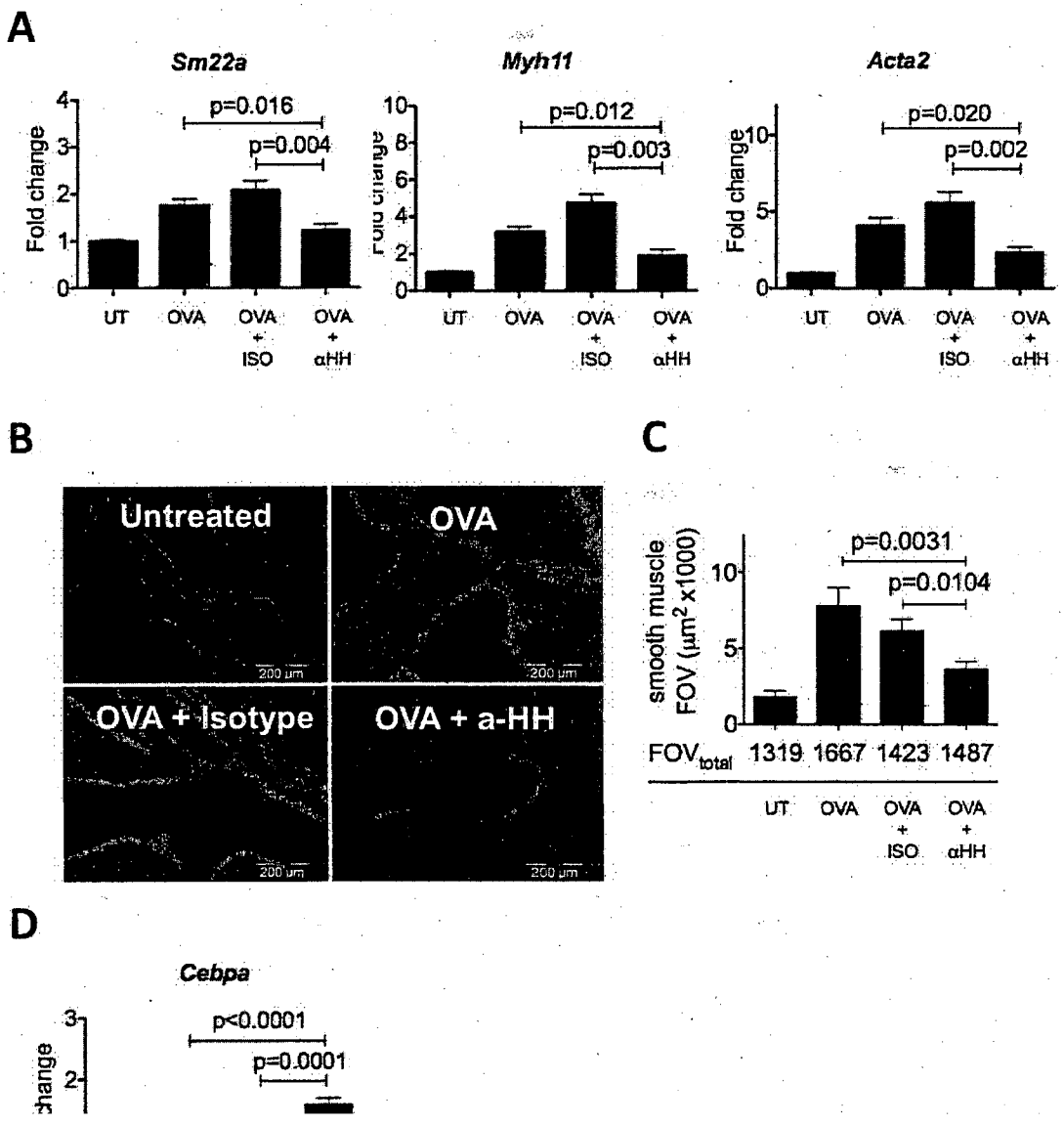

A
Sm22a
Myh11
Acta2
Fold change
p=0.016
p=0.004
p=0.012
p=0.003
p=0.020
p=0.002
UT
OVA
OVA + ISO
OVA + αHH
B
Untreated
OVA
OVA + Isotype
OVA + a-HH
200 μm
C
smooth muscle FOV (μm² x1000)
p=0.0031
p=0.0104
FOV_total 1319 1667 1423 1487
D
Cebpa
p<0.0001
p=0.0001

FIG. 5

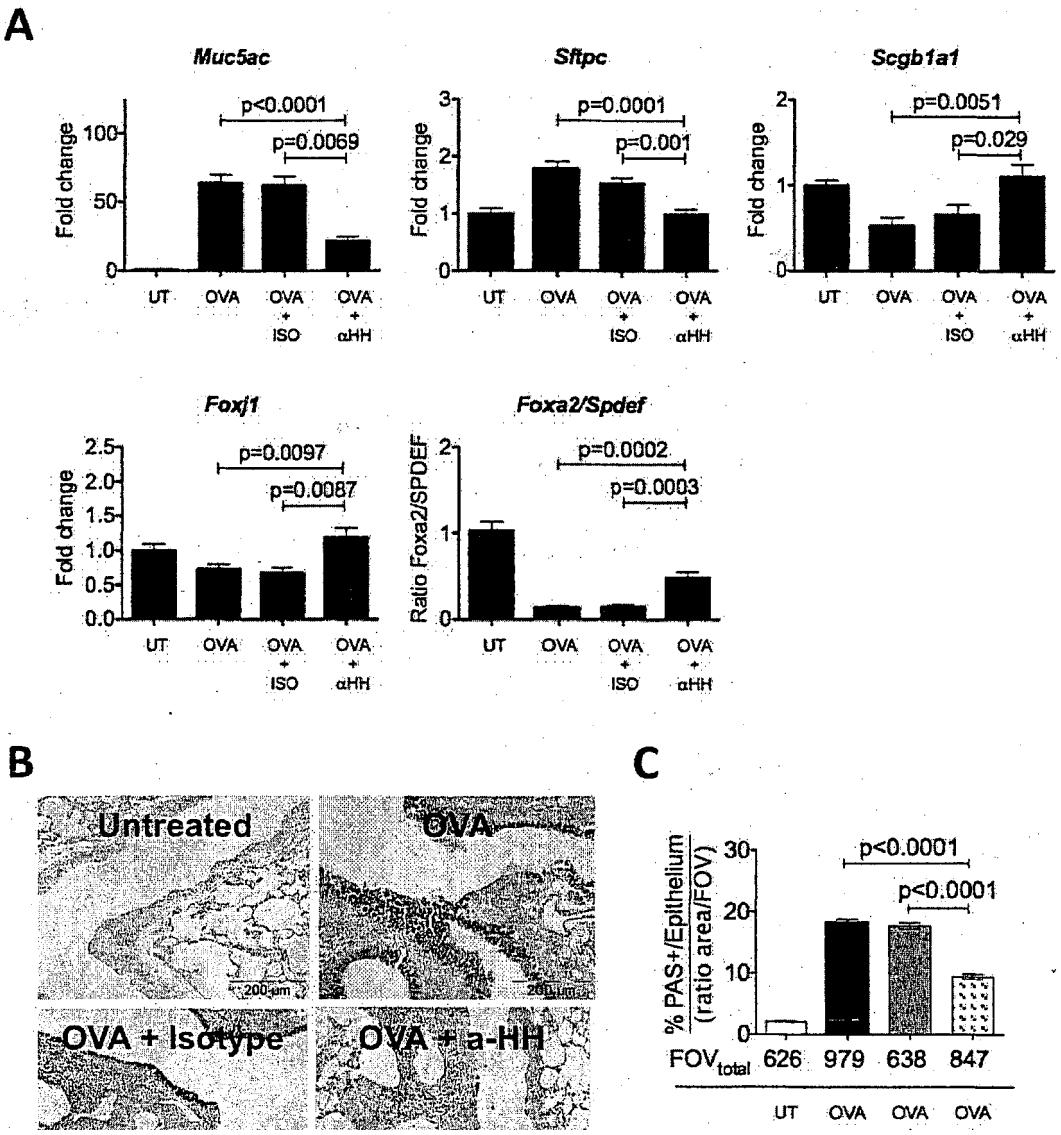

A
Muc5ac
Sftpc
Scgb1a1
Foxj1
Foxa2/Spdef
Fold change
Ratio Foxa2/SPDEF
p<0.0001
p=0.0069
p=0.0001
p=0.001
p=0.0051
p=0.029
p=0.0097
p=0.0087
p=0.0002
p=0.0003
UT
OVA
OVA + ISO
OVA + αHH
B
Untreated
OVA
OVA + Isotype
OVA + a-HH
200 μm
C
% PAS+/Epithelium (ratio area/FOV)
p<0.0001
p<0.0001
FOV total 626 979 638 847
UT OVA OVA OVA

FIG. 6

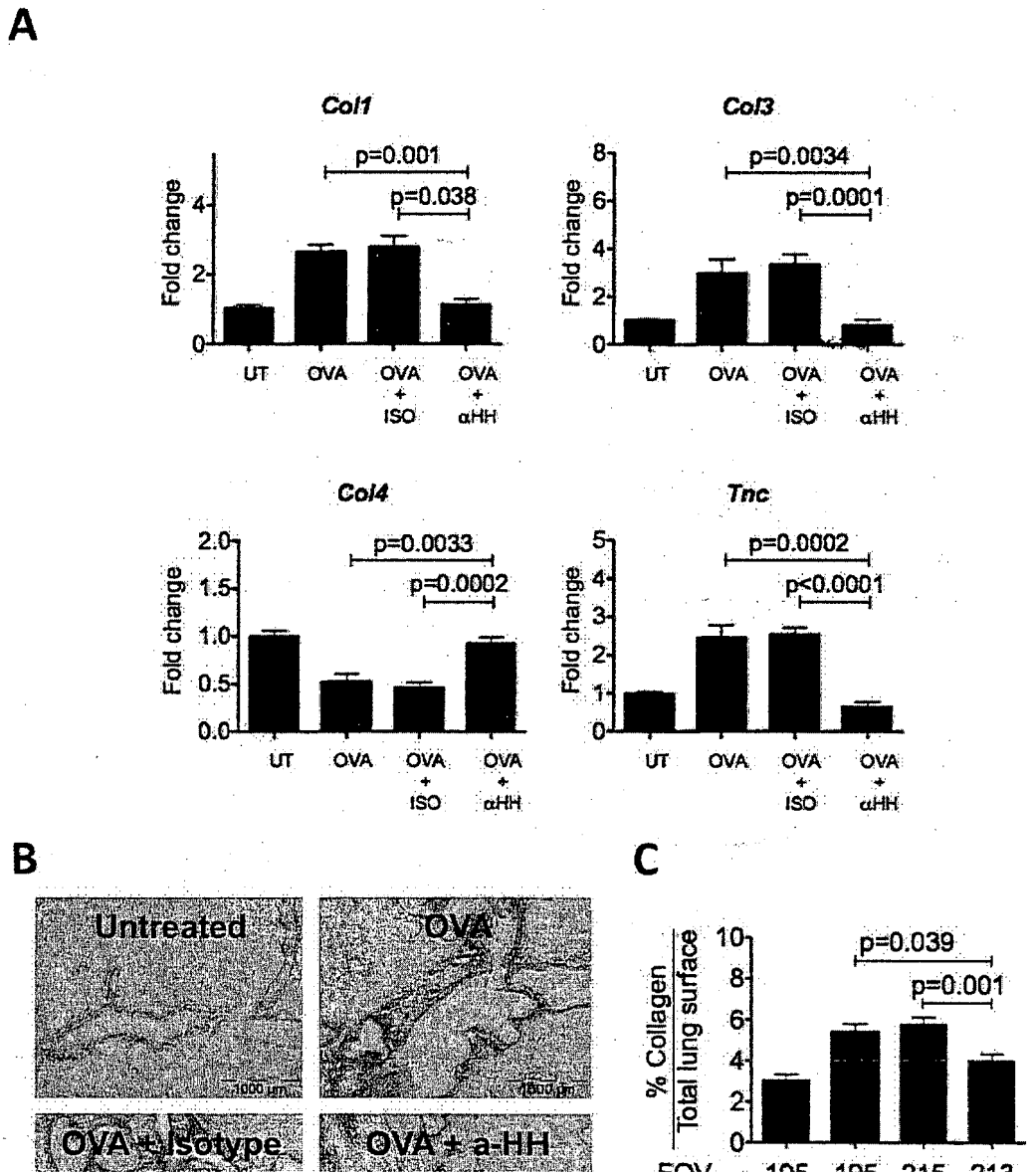

A
Col1
p=0.001
p=0.038
Fold change
UT
OVA
OVA + ISO
OVA + αHH
Col3
p=0.0034
p=0.0001
Col4
p=0.0033
p=0.0002
Tnc
p=0.0002
p<0.0001
B
Untreated
OVA
OVA + Isotype
OVA + a-HH
C
% Collagen / Total lung surface
p=0.039
p=0.001

FIG. 7

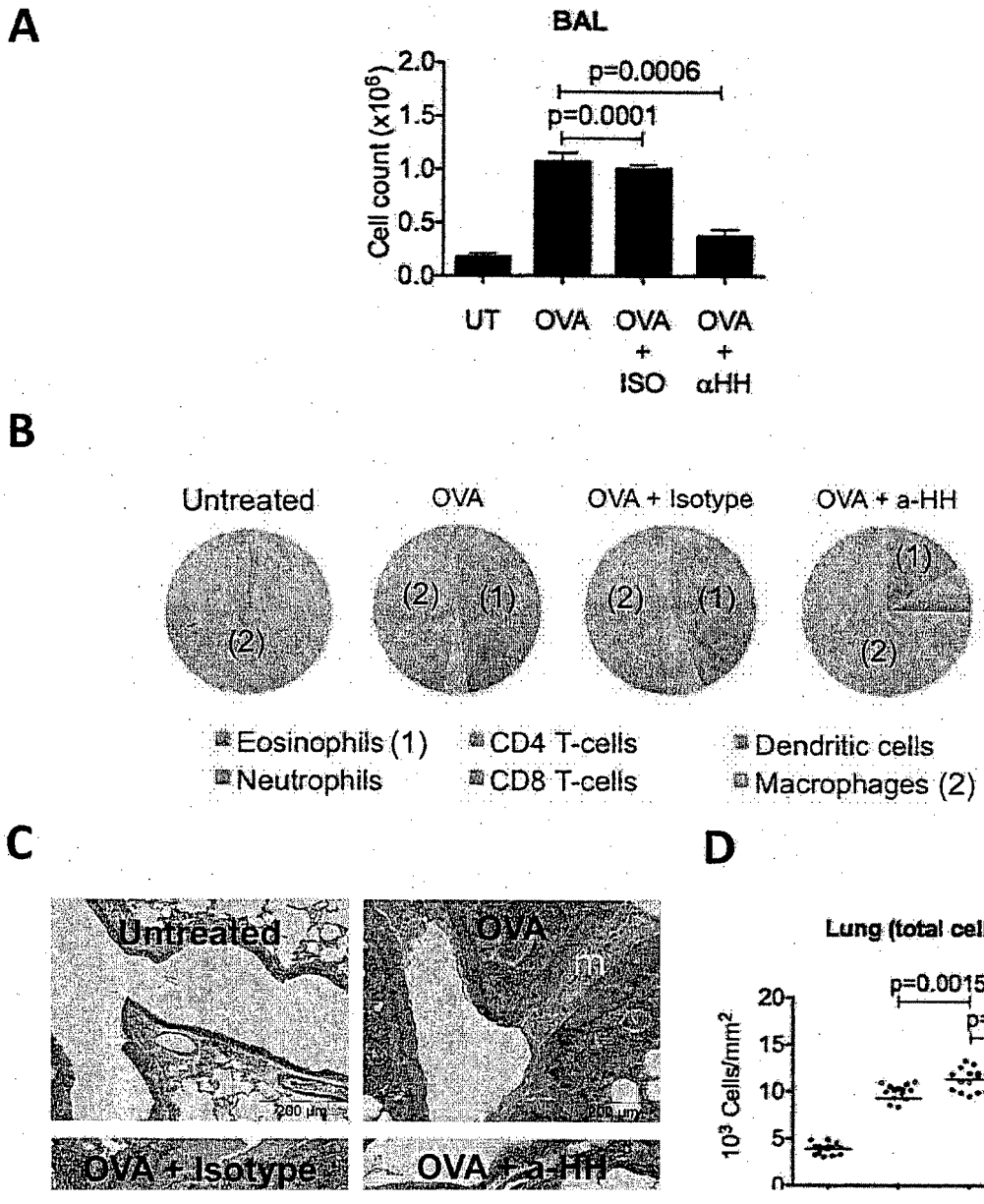

A
BAL
Cell count (x10^6)
2.0
1.5
1.0
0.5
0.0
p=0.0006
p=0.0001
UT
OVA
OVA + ISO
OVA + αHH
B
Untreated
OVA
OVA + Isotype
OVA + a-HH
(1)
(2)
Eosinophils (1)
Neutrophils
CD4 T-cells
CD8 T-cells
Dendritic cells
Macrophages (2)
C
Untreated
OVA
OVA + Isotype
OVA + a-HH
200 µm
D
Lung (total cells)
p=0.0015
p=0.008
10^3 Cells/mm^2
20
15
10
5
0

FIG. 8

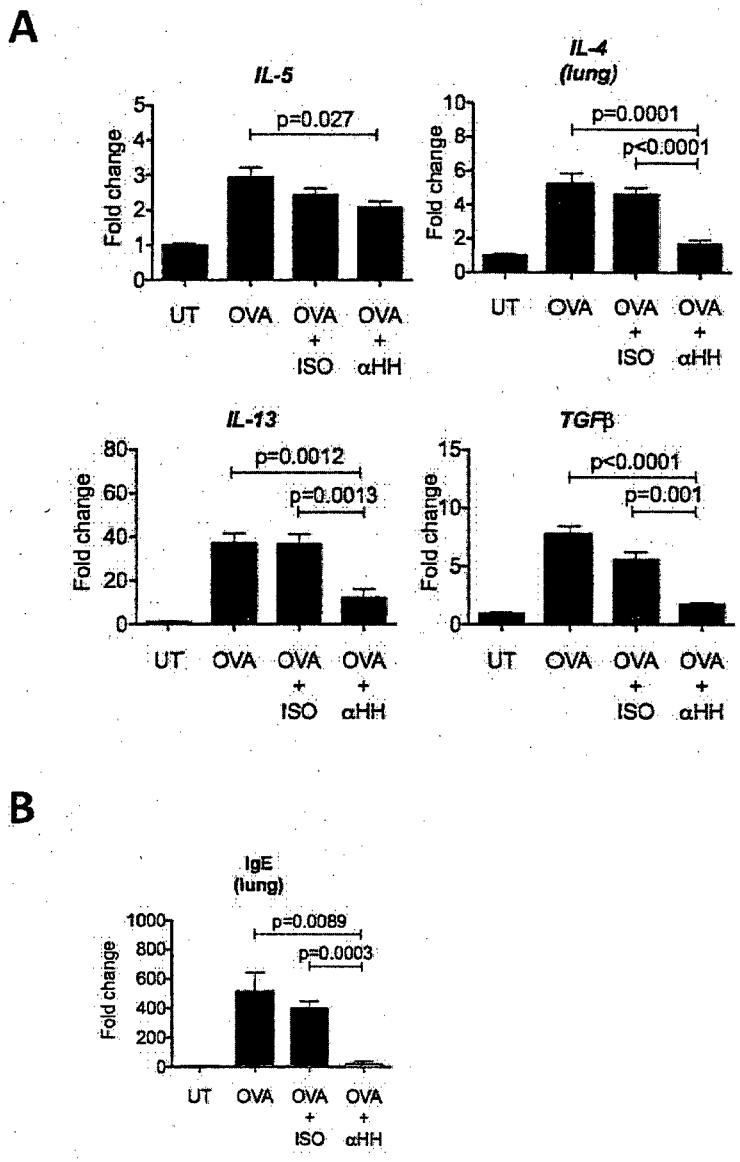

A
IL-5
p=0.027
Fold change
UT
OVA
OVA + ISO
OVA + αHH
IL-4 (lung)
p=0.0001
p<0.0001
IL-13
p=0.0012
p=0.0013
TGFβ
p<0.0001
p=0.001
B
IgE (lung)
p=0.0089
p=0.0003

FIG. 9

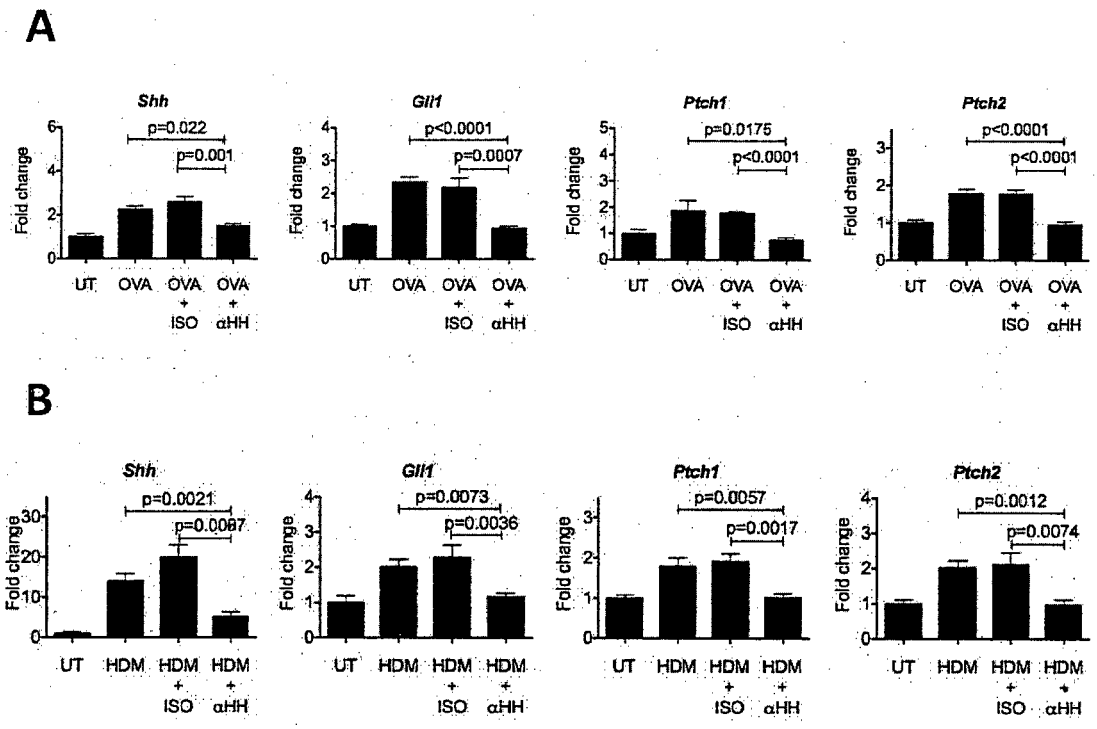
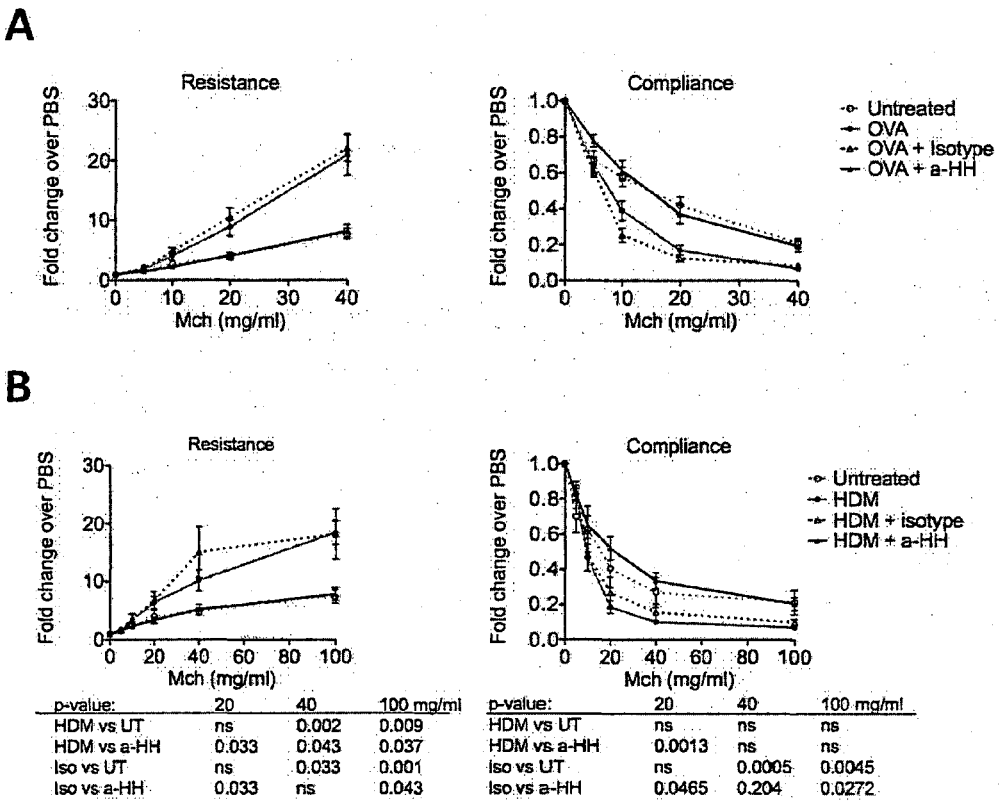

A
Shh
p=0.022
p=0.001
Gli1
p<0.0001
p=0.0007
Ptch1
p=0.0175
p<0.0001
Ptch2
p<0.0001
p<0.0001
Fold change
UT
OVA
OVA + ISO
OVA + αHH
B
Shh
p=0.0021
p=0.0087
Gli1
p=0.0073
p=0.0036
Ptch1
p=0.0057
p=0.0017
Ptch2
p=0.0012
p=0.0074
Fold change
UT
HDM
HDM + ISO
HDM + αHH

FIG. 10



p-value:	20	40	100 mg/ml
HDM vs UT	ns	0.002	0.009
HDM vs a-HH	0.033	0.043	0.037
Iso vs UT	ns	0.033	0.001
Iso vs a-HH	0.033	ns	0.043

p-value:	20	40	100 mg/ml
HDM vs UT	ns	ns	ns
HDM vs a-HH	0.0013	ns	ns
Iso vs UT	ns	0.0005	0.0045
Iso vs a-HH	0.0465	0.204	0.0272

FIG. 11

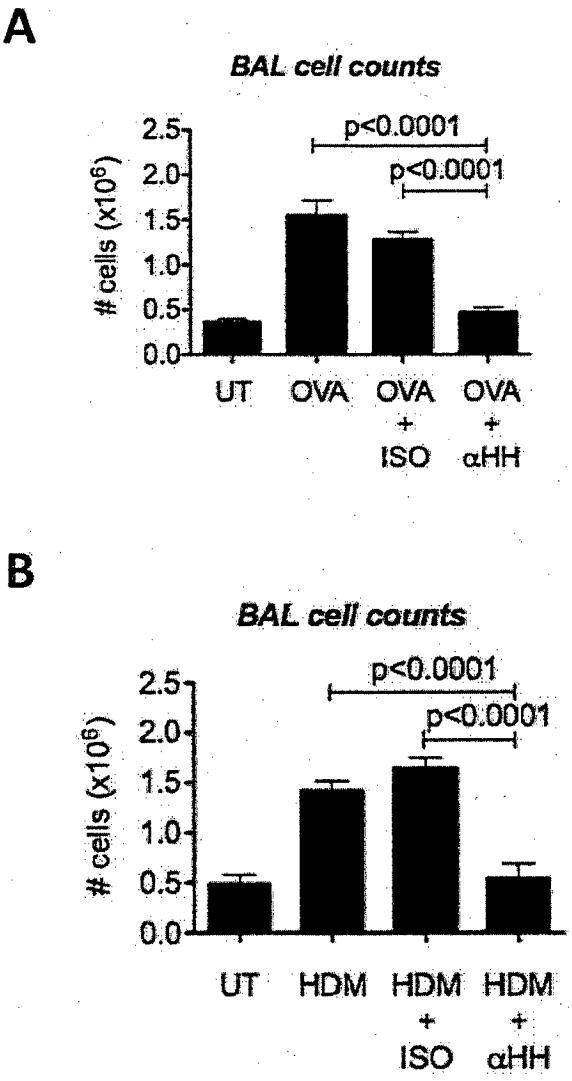

A
BAL cell counts
cells (x10^6)
2.5
2.0
1.5
1.0
0.5
0.0
p<0.0001
p<0.0001
UT
OVA
OVA + ISO
OVA + αHH
B
BAL cell counts
cells (x10^6)
2.5
2.0
1.5
1.0
0.5
0.0
p<0.0001
p<0.0001
UT
HDM
HDM + ISO
HDM + αHH

FIG. 12

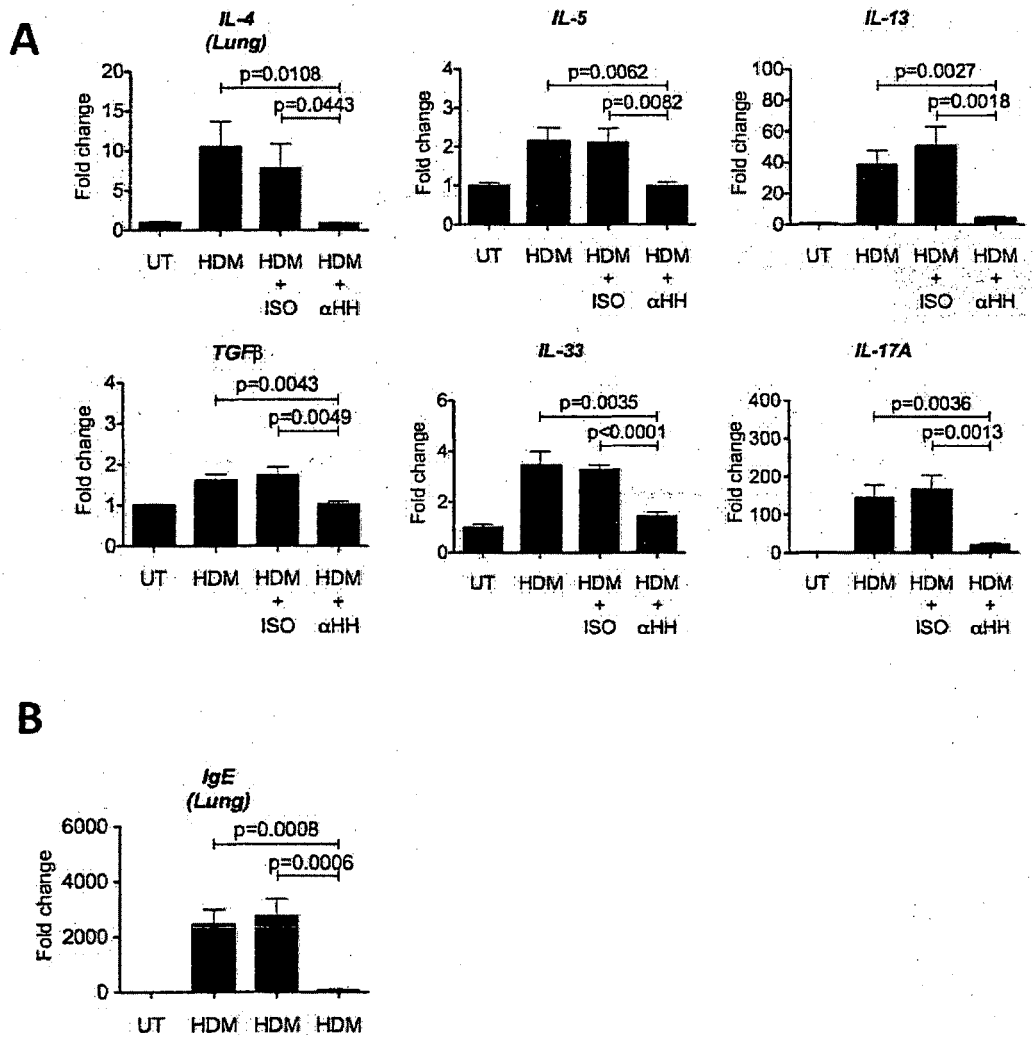

A
IL-4 (Lung)
p=0.0108
p=0.0443
Fold change
UT HDM HDM + ISO HDM + αHH
IL-5
p=0.0062
p=0.0082
IL-13
p=0.0027
p=0.0018
TGFβ
p=0.0043
p=0.0049
IL-33
p=0.0035
p<0.0001
IL-17A
p=0.0036
p=0.0013
B
IgE (Lung)
p=0.0008
p=0.0006

FIG. 13

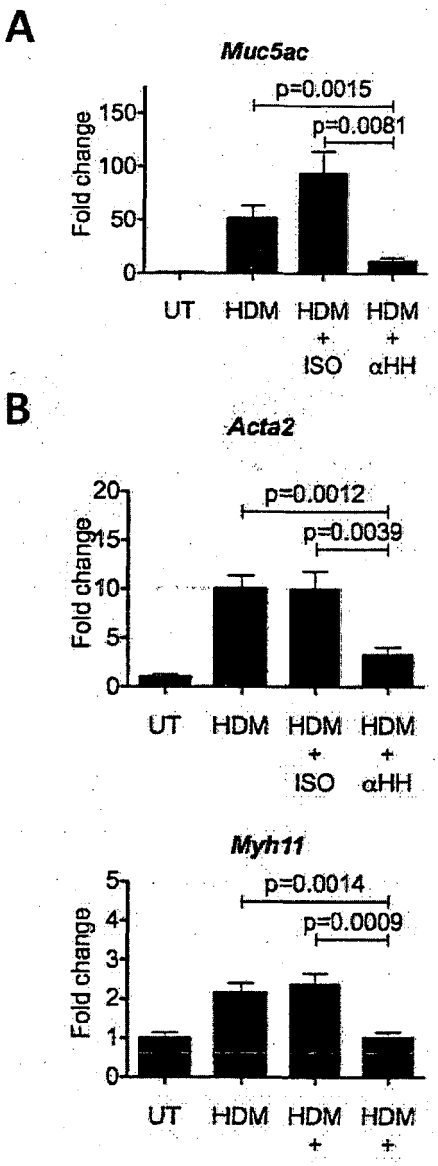

A
Muc5ac
p=0.0015
p=0.0081
Fold change
150
100
50
0
UT
HDM
HDM + ISO
HDM + αHH
B
Acta2
p=0.0012
p=0.0039
Fold change
20
15
10
5
0
UT
HDM
HDM + ISO
HDM + αHH
Myh11
p=0.0014
p=0.0009
Fold change
5
4
3
2
1
0
UT
HDM
HDM +
HDM +

FIG. 14

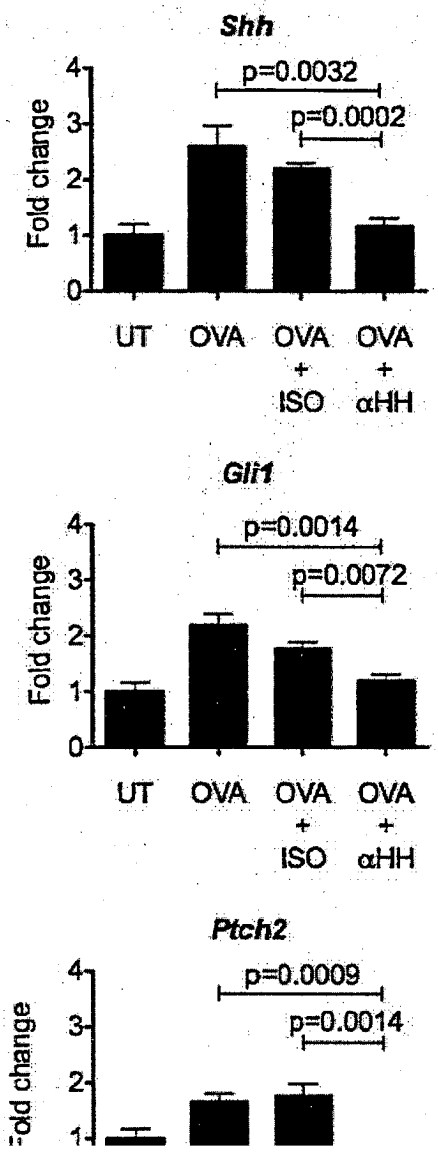

Shh
p=0.0032
p=0.0002
Fold change
0
1
2
3
4
UT
OVA
OVA + ISO
OVA + αHH
Gli1
p=0.0014
p=0.0072
Fold change
0
1
2
3
4
UT
OVA
OVA + ISO
OVA + αHH
Ptch2
p=0.0009
p=0.0014
Fold change
1
2
3
4

FIG. 15

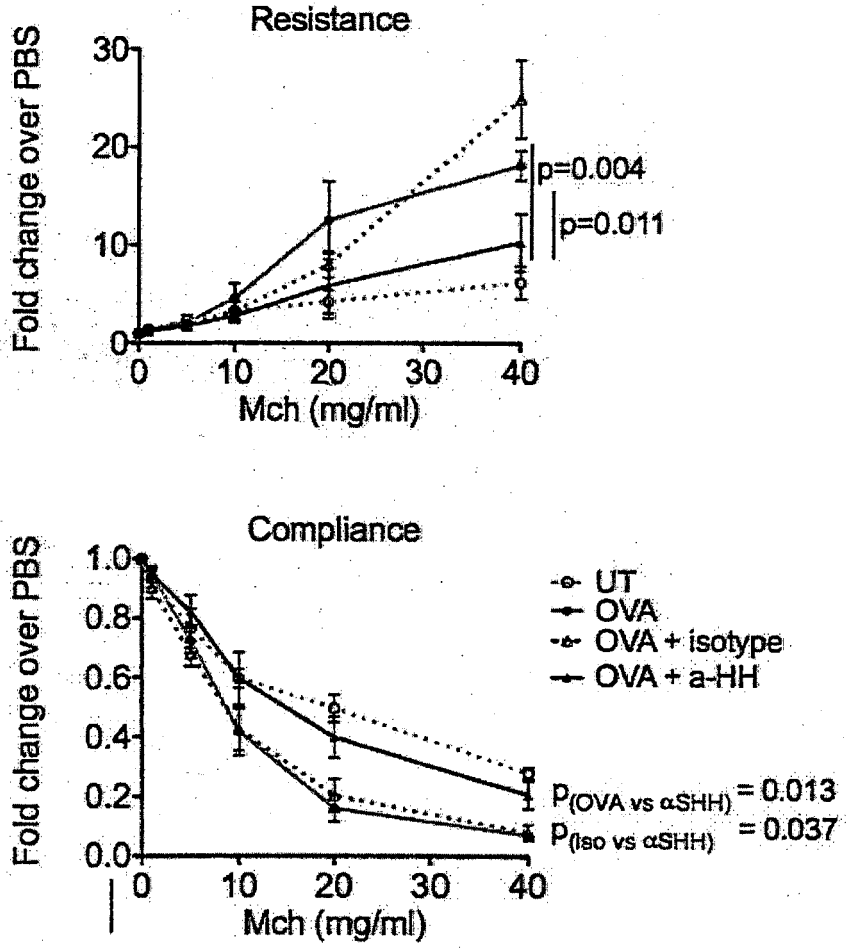

Resistance
Fold change over PBS
30
20
10
0
0
10
20
30
40
Mch (mg/ml)
p=0.004
p=0.011
Compliance
Fold change over PBS
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
Mch (mg/ml)
UT
OVA
OVA + isotype
OVA + a-HH
P(OVA vs αSHH) = 0.013
P(Iso vs αSHH) = 0.037

FIG. 16

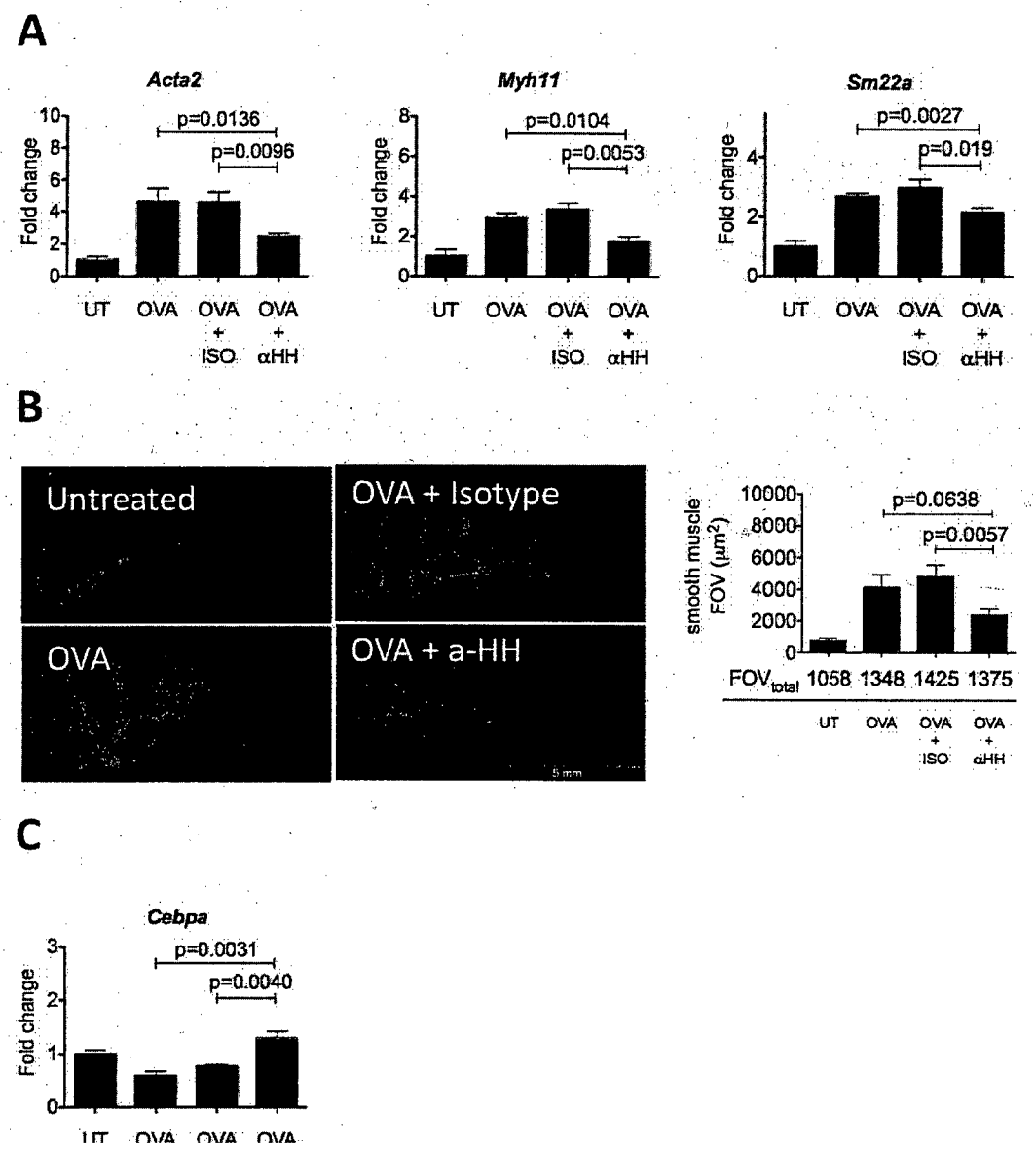

A
Acta2
Myh11
Sm22a
Fold change
p=0.0136
p=0.0096
p=0.0104
p=0.0053
p=0.0027
p=0.019
UT
OVA
OVA + ISO
OVA + αHH
B
Untreated
OVA + Isotype
OVA
OVA + a-HH
5 mm
smooth muscle FOV (μm²)
p=0.0638
p=0.0057
FOVtotal 1058 1348 1425 1375
C
Cebpa
p=0.0031
p=0.0040

FIG. 17

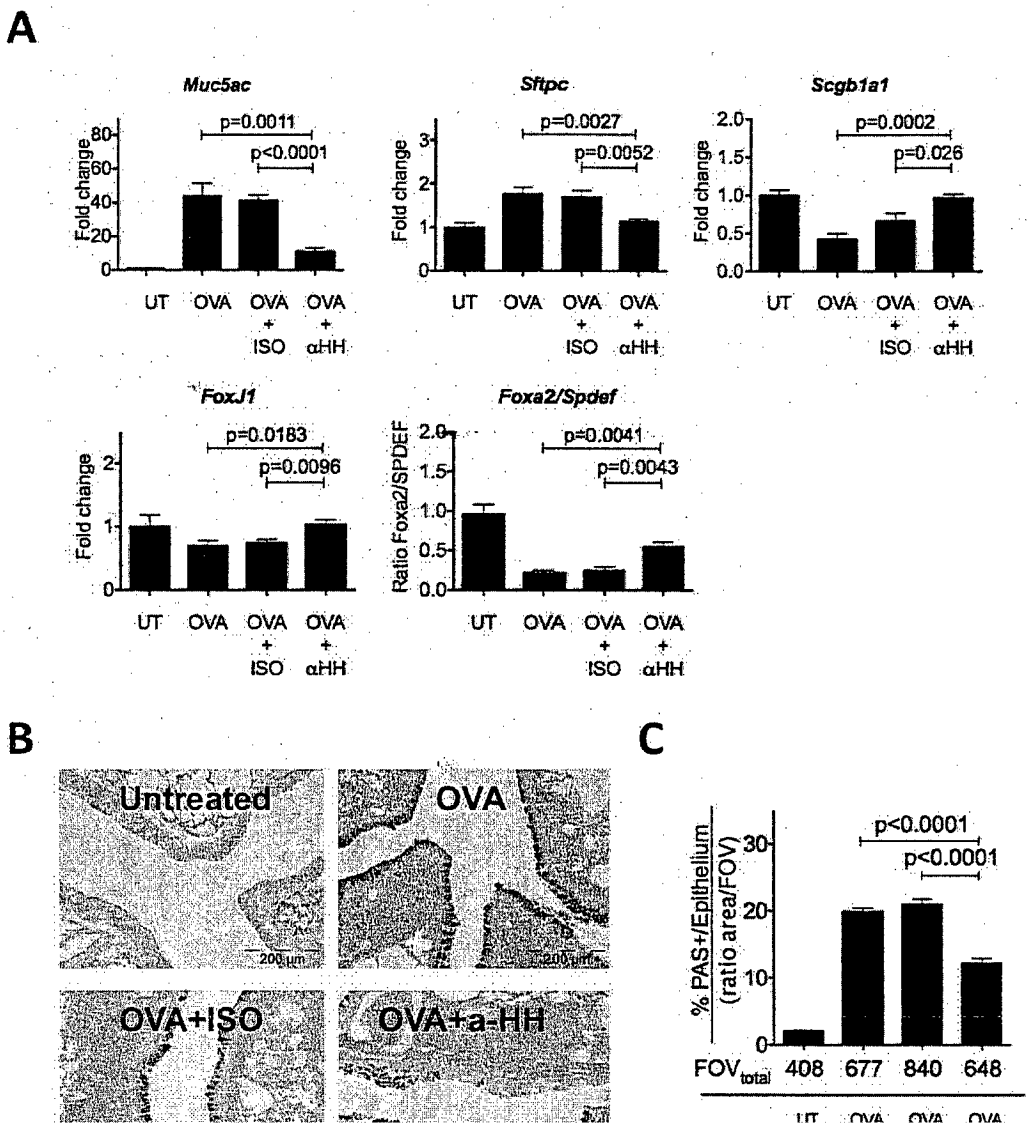

A
Muc5ac
p=0.0011
p<0.0001
Fold change
UT
OVA
OVA + ISO
OVA + αHH
Sftpc
p=0.0027
p=0.0052
Scgb1a1
p=0.0002
p=0.026
FoxJ1
p=0.0183
p=0.0096
Foxa2/Spdef
p=0.0041
p=0.0043
Ratio Foxa2/SPDEF
B
Untreated
OVA
OVA+ISO
OVA+a-HH
200 μm
C
% PAS+/Epithelium (ratio area/FOV)
p<0.0001
p<0.0001
FOV total
408
677
840
648

FIG. 18

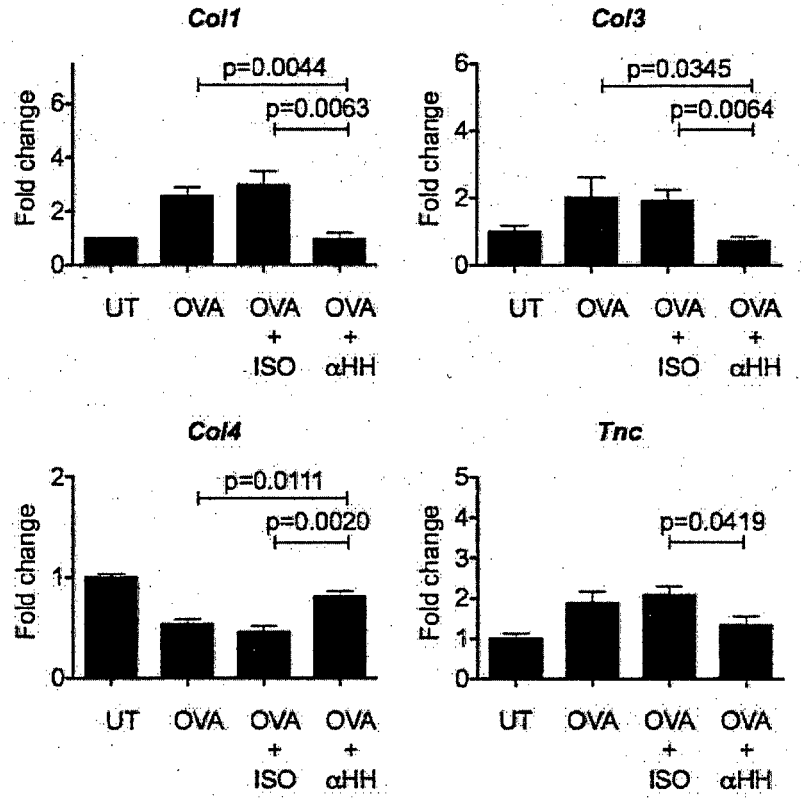

Col1
p=0.0044
p=0.0063
Fold change
0
2
4
6
UT
OVA
OVA + ISO
OVA + αHH
Col3
p=0.0345
p=0.0064
Fold change
0
2
4
6
UT
OVA
OVA + ISO
OVA + αHH
Col4
p=0.0111
p=0.0020
Fold change
0
1
2
UT
OVA
OVA + ISO
OVA + αHH
Tnc
p=0.0419
Fold change
0
1
2
3
4
5
UT
OVA
OVA + ISO
OVA + αHH

FIG. 19

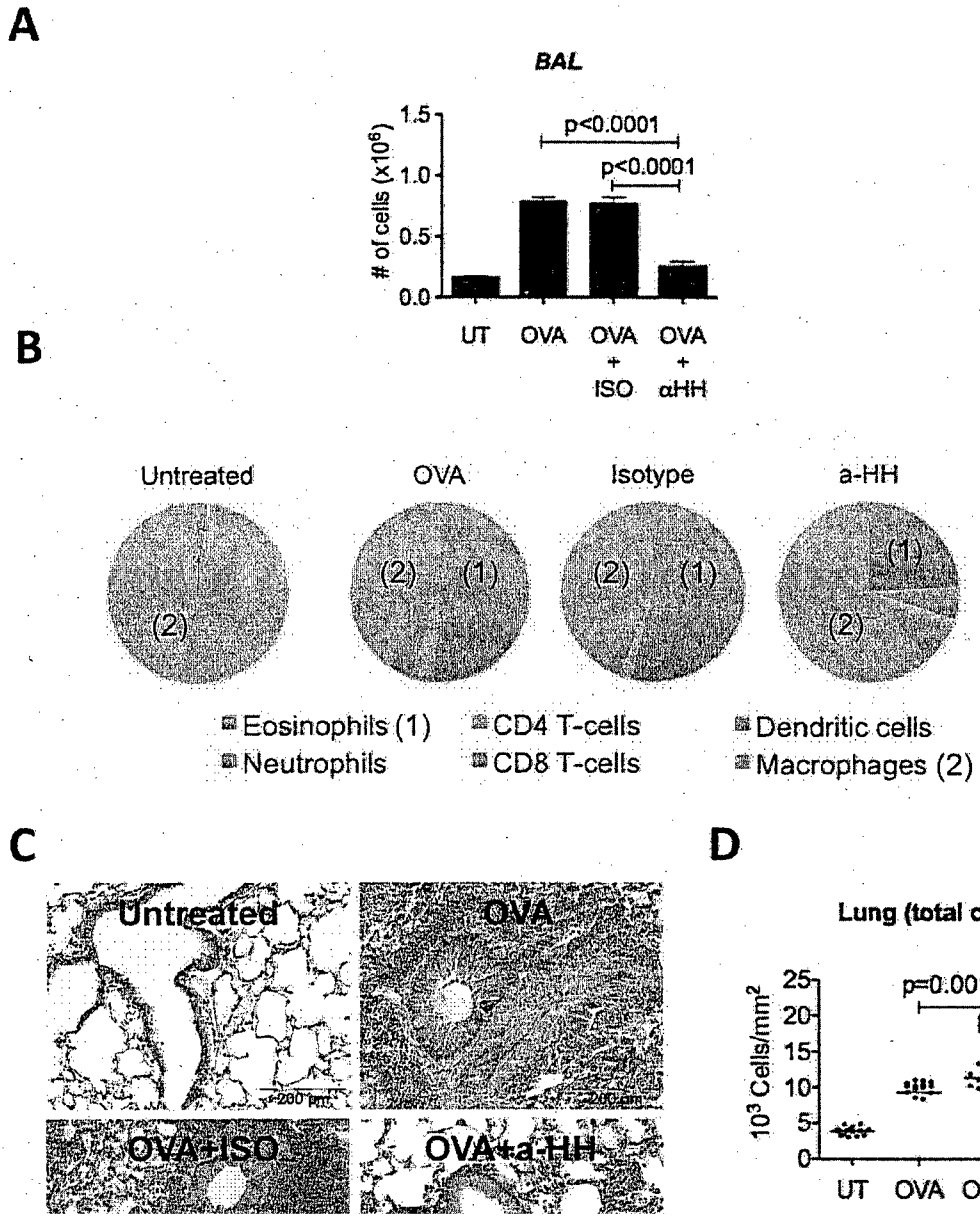

A
BAL
# of cells (x10^6)
1.5
1.0
0.5
0.0
p<0.0001
p<0.0001
UT
OVA
OVA + ISO
OVA + αHH
B
Untreated
OVA
Isotype
a-HH
(1)
(2)
Eosinophils (1)
Neutrophils
CD4 T-cells
CD8 T-cells
Dendritic cells
Macrophages (2)
C
Untreated
OVA
OVA+ISO
OVA+a-HH
D
Lung (total cells)
10^3 Cells/mm^2
25
20
15
10
5
0
p=0.0015
p=0.008
UT
OVA
OVA
OVA

FIG. 20

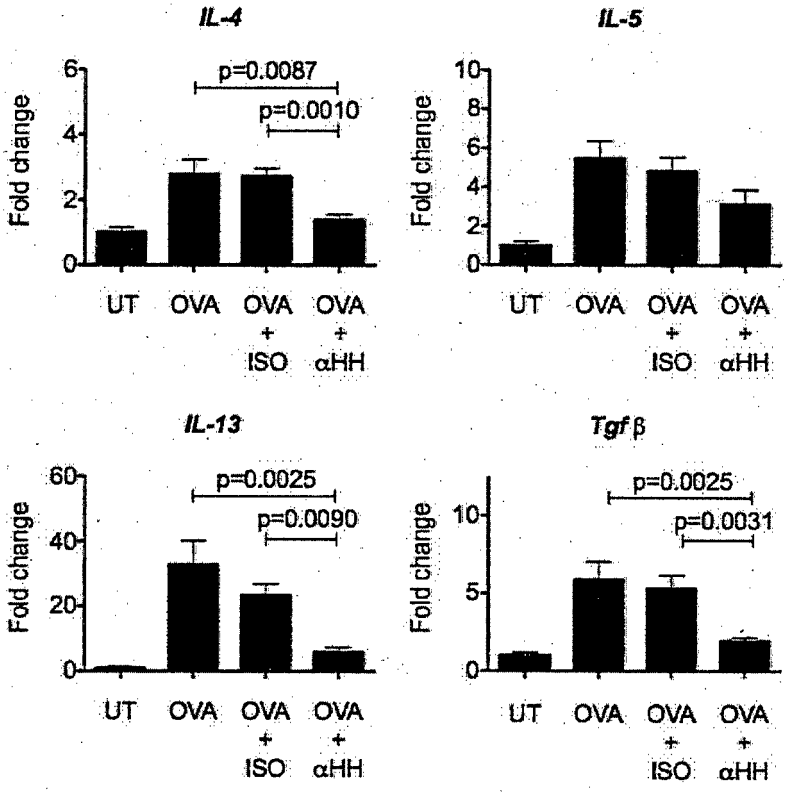
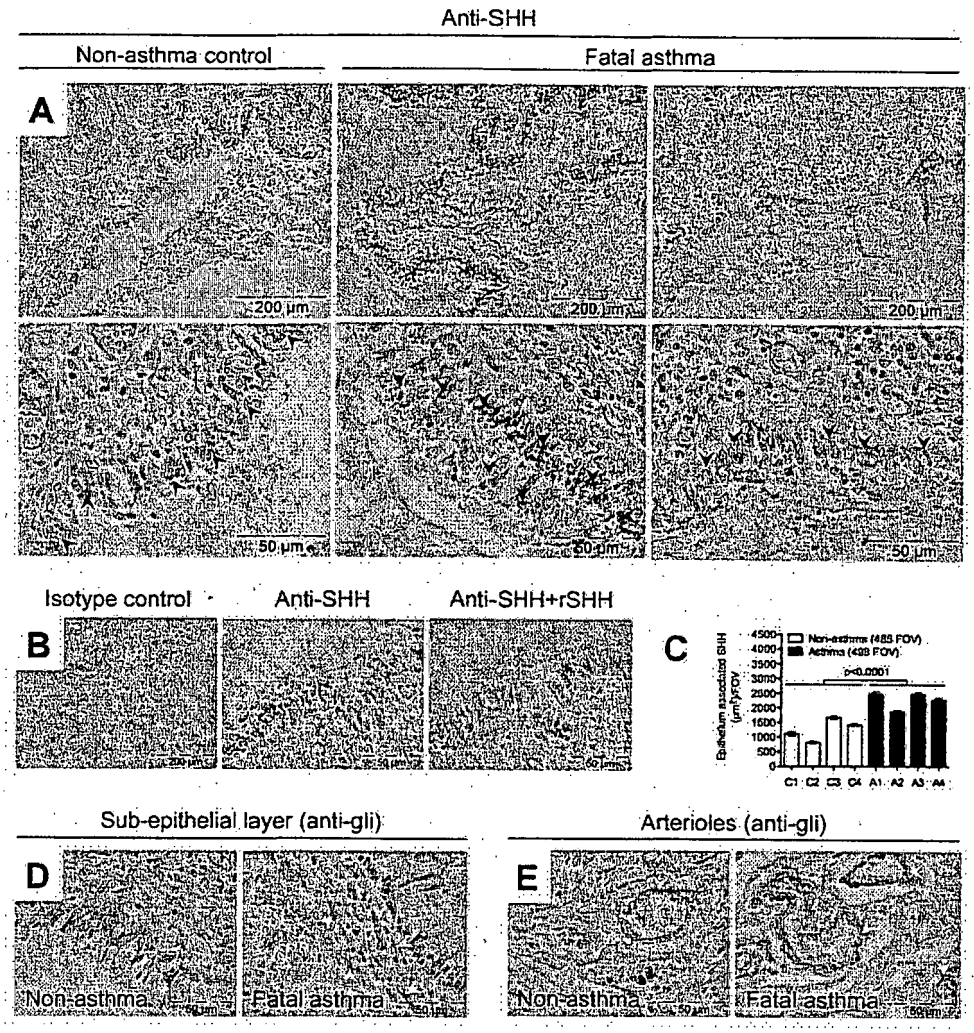

IL-4
p=0.0087
p=0.0010
Fold change
6
4
2
0
UT
OVA
OVA + ISO
OVA + αHH
IL-5
10
8
6
4
2
0
IL-13
p=0.0025
p=0.0090
60
40
20
Tgf β
p=0.0025
p=0.0031
5

FIG. 21


Anti-SHH
Non-asthma control
Fatal asthma
A
200 µm
50 µm
Isotype control
Anti-SHH
Anti-SHH+rSHH
B
C
Non-asthma (485 FOV)
Asthma (499 FOV)
p<0.0001
C1 C2 C3 C4 A1 A2 A3 A4
Sub-epithelial layer (anti-gli)
Arterioles (anti-gli)
D
E
Non-asthma
Fatal asthma