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Role of Yes-associated protein in Interleukin-13 induced nasal remodeling of chronic rhinosinusitis with nasal polyps

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Running title: Role of YAP in IL-13 remodeling of CRSwNP

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Author contributions

De-Yun Wang and Qin-Tai Yang designed the study; Tian Yuan, Rui Zheng, Jing Liu, and Kai Sen Tan wrote the manuscript and conducted most of the experiments; Zhi-qun Huang, Xiang-Min Zhou, Xiao-xue Zi performed the experiments; Hui-jun Qiu, Xin-yue Wang, Wei-hao Wang, Hui-yi Deng, Yu-bin Chen, Wei-feng Kong, Qing-wu Wu, Ying Huang, Hsiao Hui Ong, Xue-kun Huang, and Zhuang-gui Chen collected the clinical and hNECs samples.

Conflict of Interest Statement:

The authors have no conflicts of interest to declare.

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To Editor:

Chronic rhinosinusitis (CRS) is a common otolaryngologic disease. Chronic rhinosinusitis with nasal polyps (CRSwNP) is one of the most frequent CRS phenotypes. Compared with other phenotypes, CRSwNP is associated with higher severity and a poorer response to treatments[1]. Tissue remodeling is closely related to the occurrence and development of CRSwNP[2]. The Hippo pathway plays a role in regulating the balance between stem cells, progenitor cells, and differentiated cells[3]. In our previous study, we reported that Yes-associated protein (YAP) in the Hippo pathway participates in the proliferation and remodeling of CRSwNP[4]. However, its

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specific role in CRSwNP epithelium remodeling remains to be clarified. On the other hand, Interleukin 13 (IL-13) is highly expressed in CRSwNP[5] and is closely related to the remodeling process of the upper airway epithelium[6]. In view that both YAP and IL-13 are part of the upper airway epithelium remodeling process, we hypothesized that interaction between YAP and IL-13 may drive the process. Thus, the present study sought to explore the potential role of the Hippo pathway (YAP) in IL-13 induced nasal epithelial remodeling.

Following our preliminary work, we reconfirmed YAP's expression in CRSwNP with Polymerase Chain Reaction (PCR) and immunofluorescence (IF) (Figure S1A-C) and found that YAP was expressed actively in a variety of epithelial cells (Figure S1D). Compared to healthy control individuals, basal cells and goblet cells were increased in the CRSwNP group, while ciliated cells was decreased (Figure 1A-C, E, G, I). Furthermore, the nuclear YAP (nYAP) ratio of basal cells, goblet cells, and ciliated cells were higher in the CRSwNP group than that in the control group (Figure 1 A-C, D, F, H), indicating the increased YAP activity in these three cell types.

To further study the effect of YAP and IL-13, we used an air-liquid interface (ALI) culture model of human nasal epithelial cells (hNECs)[7]. From the beginning of the ALI culture, on day 0 (d0) to day 24 (d24), we tested the mRNA levels every two days for 13 time points; and performed IF staining at selected timepoints. The PCR and IF results indicated that cell proliferation was dominant from d0 to d2, and the model was fully differentiated on d24 (Figure S2A-C, H). Further, we divided the timepoints in two stages according to ciliogenesis. During stage I, from d0 to d6, no ciliated cell was detected, while ciliated cells were observed in stage II, from d8 to d24. Interestingly, at mRNA levels, Ki-67 was positively correlated to YAP in stage I while FOXJ-1 was positively correlated to YAP in stage II (Figure S2D-G).

To clarify the effect of IL-13 on YAP activity in nasal epithelium, we treated hNECs with IL-13. On d2, IL-13 elevated the mRNA levels of YAP and Ki-67, which were positively correlated (Figure S6A-C). The IF results on d2 and d24, showed that IL-13 increased the YAP activity and proliferation level of basal cells, which were positively correlated, and also the

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percentage of basal cells (Figure 1J-S, Figure S3). Furthermore, IF staining on d24, demonstrated that IL-13 caused goblet cell proliferation while inhibited ciliated cell formation (Figure 1T, U, W). PCR results supported d24 IF results (Figure S6D-G). In addition, IL-13 increased the YAP activity of terminally differentiated goblet cells and ciliated cells (Figure 1T, V, X). These results indicated that IL-13 can cause similar epithelial remodeling and stimulate YAP activity in hNECs.

To identify the effect of active-YAP on the proliferation and differentiation of nasal epithelial cells, we treated hNECs with verteporfin (VP) to suppress YAP activity. After VP treatment on d2 and d24, the IF results showed decreased YAP activity in basal cells, proliferation, and a decreased percentage of basal cells (Figure S4). Moreover, the YAP activity and proliferation in basal cells were positively correlated (Figure S4F, L). In addition, on d24 the IF staining demonstrated that the percentages of goblet cells and ciliated cells were decreased, whereas their YAP activity was also inhibited (Figure S5). These results were supported by PCR (Figure S6H-N). These results indicated that YAP might promote the proliferation and differentiation of nasal epithelium.

We then similarly added VP to inhibit YAP activity in IL-13-treated ALI-cultured hNECs. IF images obtained on d2 and d24, showed that, IL-13+VP treatment resulted in a decreased basal cell percentage, decreased YAP activity in basal cells, and a decreased basal cell proliferation level (Figure 2A-H), demonstrating that VP reversed the IL13-induced proliferation by suppressing the increased YAP of IL-13 treated cells. The IF results on d24 showed that the IL-13-induced increase of goblet cells was inhibited and the ciliated cell ratio rebounded with VP treatment (Figure 2I-M). PCR results supported d24 IF results (Figure S6O-R).

Our results indicated that IL-13 could induce CRSwNP remodeling via promoting YAP activity. IL-13 plays an essential role in CRSwNP remodeling[6], including epithelial goblet cell hyperplasia and ciliogenesis impairment. This study demonstrated YAP is also essential in IL-13 induced high proliferation. Generally, YAP inhibits epithelial differentiation[8]. However, our results demonstrated that YAP can also promote goblet cell differentiation and YAP inhibitor reversed IL-13-induced goblet cell hyperplasia. In contrast, we cannot identify YAP's role in ciliogenesis, and no paper reported the link between YAP and motile cilia. We hypothesize that

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YAP activity in both basal cells and ciliated cells may inhibit ciliogenesis. Another hypothesis is that active-YAP may play different roles in normal conditions and in a Th2 inflammatory environment, warranting additional studies.

In conclusion, our findings partly explain the mechanism underlying IL-13-induced remodeling and demonstrate a possible YAP role in IL-13 induced proliferation and differentiation of the nasal epithelium (Figure S7). Notably, VP, a YAP inhibitor which is also used in ophthalmology[9], can reverse IL-13-induced epithelial remodeling, including goblet cell hyperplasia inhibition and ciliogenesis promotion. VP may have a potential clinical value in controlling symptoms such as excessive nasal discharge and improving nasal epithelial function pre-/post-operation. Furthermore, VP's clinical safety profile renders its clinical application more promising. However, the exact interaction between IL-13 and Hippo pathway in chronic inflammation warrants further studies, such as in allergic rhinosinusitis and asthma.

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Figure legends

Figure 1. IL-13 induce similar nasal epithelial remodeling to CRSwNP, companying with active-YAP overexpression.

Compared with IT, epithelial remodeling exists in CRSwNP (**A-I**). The ratio of basal cells (**A, E**) and goblet cells (**B, G**) in the epithelium of CRSwNP increased, while ciliated cells decreased (**C, I**). The nuclear YAP of the three types of cells in CRSwNP epithelium increased (**D, F, H**). IL-13 induces similar epithelial remodeling to CRSwNP (**J-X**) in ALI-cultured hNECs in vitro. IF results on day 2 and day 24, showed that IL-13 increased the YAP activity (**J, O, K, P**) and proliferation

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level (**J, O, M, R**) of basal cells, which were positively correlated (**N, S**), and also the percentage of basal cells (**J, O, L, Q**). On day 24, IL-13 also caused goblet cell proliferation while inhibited ciliated cell formation (**T, U, W**). After IL-13 treatment, the YAP activity of terminally differentiated goblet cells and ciliated cells increased (**T, V, X**).

A-I, basal cells (marked by P63) are analyzed by tissue biopsy, IT=17, CRSwNP=23; goblet cells (marked by MUC5AC) (IT=16, CRSwNP=26) and ciliated cells (marked by B4-TUBULIN) (IT=16, CRSwNP=17) are analyzed by nasal brush. See also supplementary. J-N, n=8; O-S, n=6; T-X, n=4. IF images, ×400 amplification. IL-13, 10ng/ml.

IL-13, Interleukin 13; CRSwNP, Chronic rhinosinusitis with nasal polyps; YAP, Yes-associated protein; MUC5AC, mucin 5AC; IT, inferior turbinate; ALI, air-liquid interface; hNECs, human nasal epithelial cells; IF, immunofluorescence.

Figure 2. Verteporfin reversed IL-13 induced nasal epithelial remodeling in vitro.

Add verteporfin (VP) to inhibit YAP activity in IL-13-treated ALI-cultured hNECs in vitro (n=6). IF results on day 2 (**A-D**) and day 24 (**E-H**), showed that IL-13+VP treatment resulted in a decreased basal cell percentage (**D, H**), decreased YAP activity in basal cells (**B, F**), and a decreased basal cell proliferation level (**C, G**). The IF results on day 24 (**I-M**) also show that the IL-13-induced increase of goblet cells was inhibited (**K**) and the ciliated cell ratio rebounded (**M**) with VP treatment. And the YAP activity of ciliated cells and goblet cells fall down (**J, L**).

basal cells were marked by P63; goblet cells were marked by MUC5AC; ciliated cells were marked by B4-TUBULIN. IF images: ×400 amplification. IL-13, 10ng/ml; VP, 1μM; DMSO, 1μM.

VP, verteporfin; IL-13, Interleukin 13; YAP, Yes-associated protein; MUC5AC, mucin 5AC; ALI, air-liquid interface; hNECs, human nasal epithelial cells; IF, immunofluorescence.

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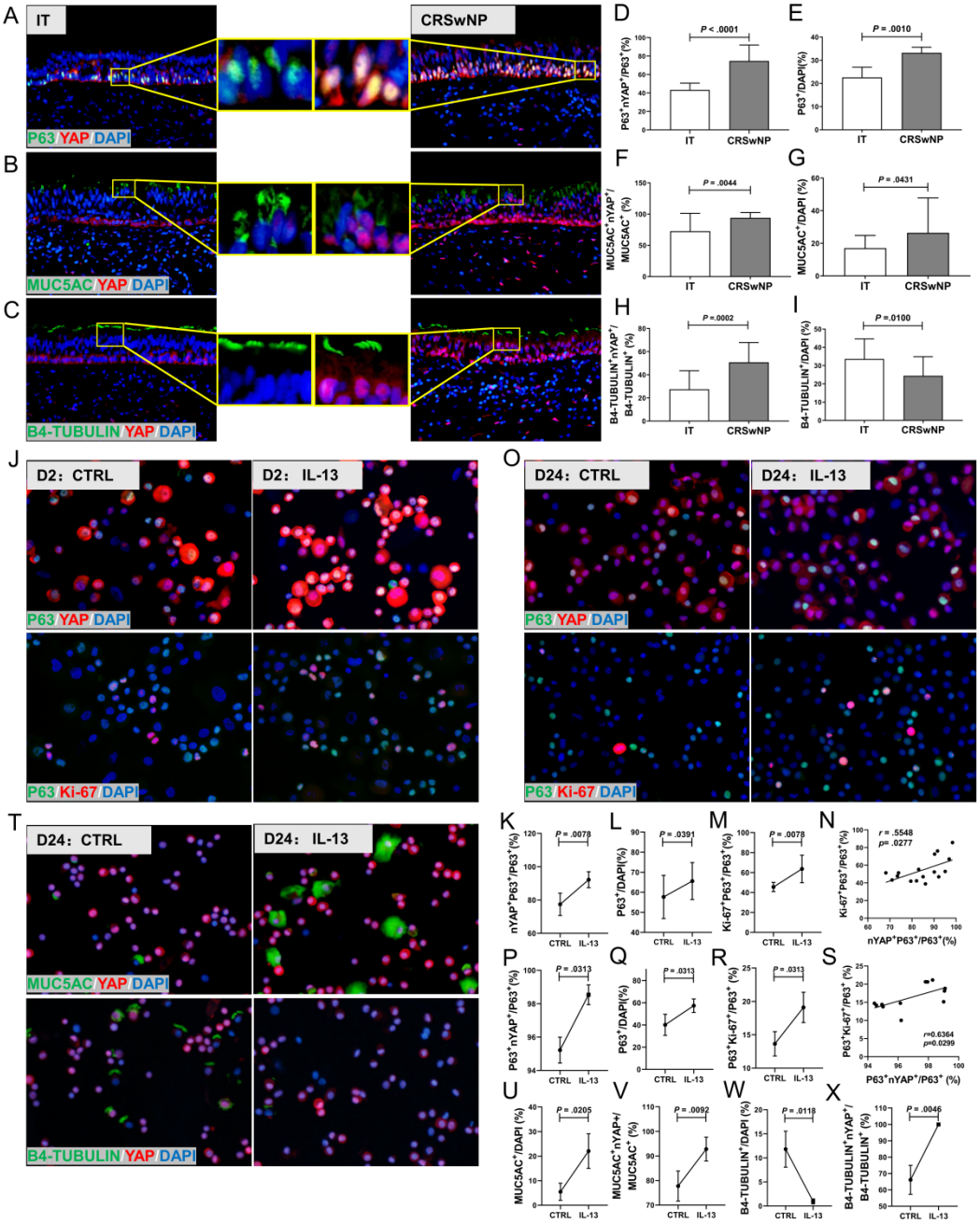

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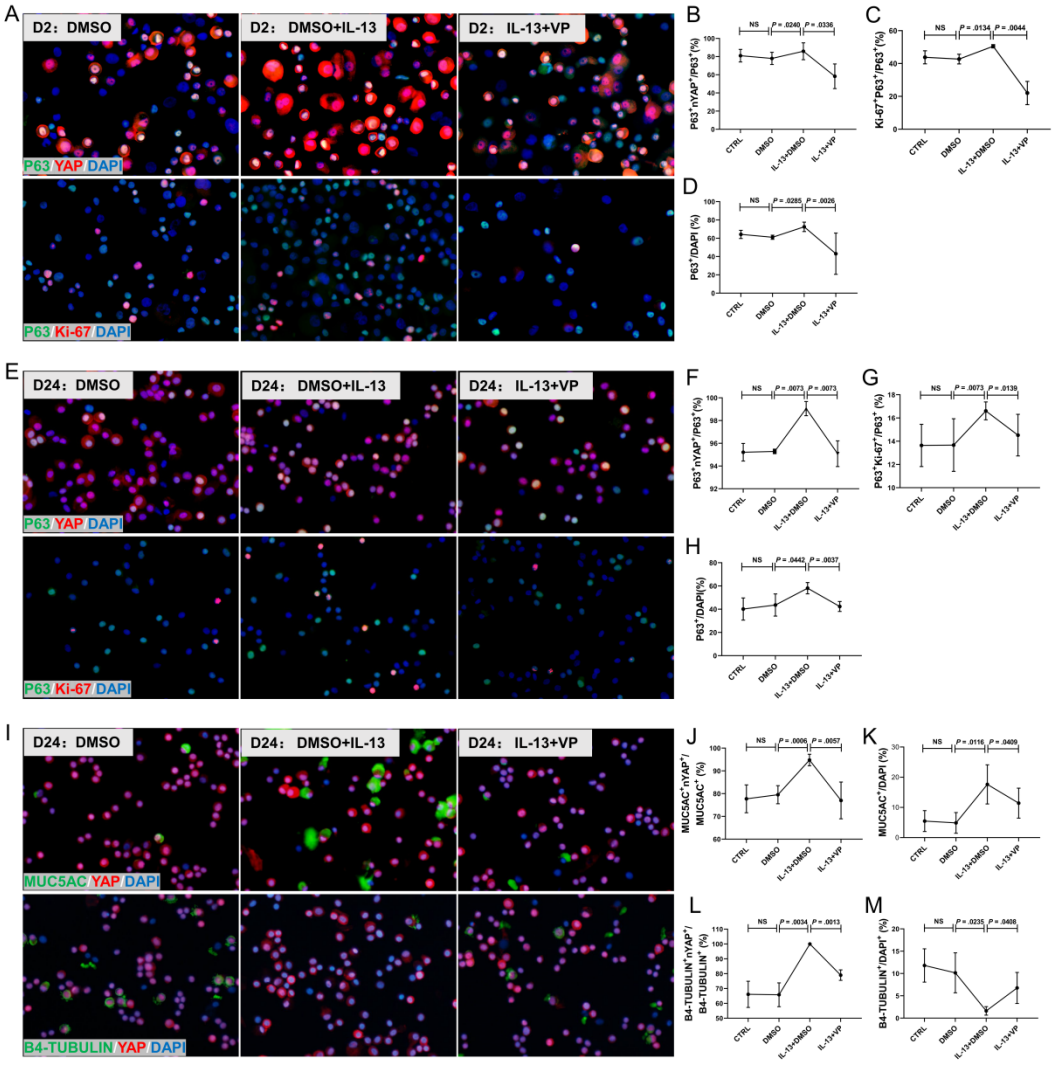

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Supplementary materials

Role of Yes-associated protein in Interleukin-13 induced nasal remodeling of chronic rhinosinusitis with nasal polyps

Tian Yuan, Rui Zheng, Jing Liu, Kai Sen Tan, Zhi-qun Huang, Xiang-Min Zhou, Xiao-xue Zi, Hui-jun Qiu, Xin-yue Wang, Wei-hao Wang, Hui-yi Deng, Yu-bin Chen, Wei-feng Kong, Qing-wu Wu, Ying Huang, Hsiao Hui Ong, Xue-kun Huang, Zhuang-gui Chen, De-Yun Wang, Qin-Tai Yang

Materials and Methods

Patients

Nasal biopsy specimens were obtained from the Department of Otolaryngology, Third Affiliated Hospital of Sun Yat-sen University, and the Department of Otolaryngology, National University Hospital of Singapore. Paraffin-embedded tissue and mRNA were obtained from 23 CRSwNP patients who underwent functional endoscopic sinus surgery, and cytospin samples of nasal brush cells were obtained from 26 outpatients with CRSwNP. The diagnosis of CRSwNP was based on the EPOS 2020 guidelines[1]. The patients had not received corticosteroid or antibiotic treatment for at least 90 days prior to specimen collection, and no respiratory infection or other system diseases were detected. Paraffin-embedded tissue and mRNA were obtained from 17 control patients who underwent septal plastic surgery, whereas nasal brush cells for cytospin samples

were obtained from 16 healthy volunteers. The control individuals did not have a history of sinonasal inflammation or atopic state. The study was approved by the ethics committee of the Third Affiliated Hospital of Sun Yat-sen University and National University Hospital of Singapore. Written informed consent was obtained from all participants before collecting samples.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from surgical nasal tissues soaked in RNAlater, and fresh hNECs cultured in the ALI system, in accordance with the instructions of the mirVana miRNA Isolation Kit (Life Technologies, USA). The double-stranded DNA synthesis system (qScript™ cDNA SuperMix, Quanta BioDesign) consisted of a 20-µl reaction including 1000 ng isolated RNA. The relative gene expression was quantified using SYBR green gene expression assays, and calculated using the comparative $2^{-\Delta\Delta Ct}$ method, with the expression levels being normalized to those of the housekeeping gene RPL13A (ribosomal protein L13a). The primers sequences are shown in Table S1.

Immunofluorescence and assessment

Nasal tissue paraffin sections and cytospin slides were prepared for immunofluorescence (IF) stainings. Following treatment with the pH6 retrieval buffer (Dako A/S), the sections and cytospin slides were blocked at room temperature for 30

min using 10% normal goat serum, and then incubated at 4°C overnight with a primary antibody solution, followed by 1-h incubation with the secondary antibodies (Alexa Fluor 488- or 594-conjugated) in the dark, at room temperature. DAPI (4′ 6-diamidino-2-phenylindole, Life Technologies, Carlsbad, CA) was used to visualize the cell nucleus. The primary antibodies used in this study are shown in Table S2.Immunofluorescence images of tissue sections and cytospin samples were captured at ×400 magnification using the CellSens Standard software (Olympus DP72, Tokyo, Japan).

For every sample except for Figure S1, the IF assessment was based on the average IF of three images, and was evaluated independently by two researchers. In case of disagreement, a third researcher independently provided his evaluation and resolved the disagreement with the other two researchers. As nuclear YAP represents the active-YAP in certain cell types, the activity of YAP in these cells can be assessed based on the percentage of nuclear YAP-positive (nYAP^{+}) cells in the sample. Basal cells are clearly distinguishable in biopsy sections, and goblet cells and ciliated cells are not crowded in cytospin so it's easier to analyze. Therefore, we assessed basal cells (P63-positive, P63^{+}) in biopsy sections, and goblet cells (mucin 5AC [MUC5AC]-positive, MUC5AC^{+}), ciliated cells (B4-TUBULIN positive, B4-TUBULIN^{+}) in cytospin samples by using the following formula:

$$basal\ \ cell\ \ YAP\ \ activity = \frac{number\ \ of\ \ nYAP^{+}\ \ P63^{+}\ \ cells}{number\ \ of\ \ P63^{+}\ \ cells} \times 100\%$$

$$goblet\ \ cell\ \ YAP\ \ activity = \frac{number\ \ of\ \ nYAP^{+}\ \ MUC5AC^{+}\ \ cells}{number\ \ of\ \ MUC5AC^{+}\ \ cells} \times 100\%$$

$$ciliated\ \ cell\ \ YAP\ \ activity = \frac{number\ \ of\ \ nYAP^{+}\ \ B4\text{-}TUBULIN^{+}\ \ cells}{number\ \ of\ \ B4\text{-}TUBULIN^{+}\ \ cells} \times 100\%$$

The ratio of the different types of cells was calculated using the following formula:

$$basal\ \ cell\ \ ratio = \frac{number\ \ of\ \ P63^{+}\ \ cells}{number\ \ of all\ \ cells} \times 100\%$$

$$goblet\ \ cell\ \ ratio = \frac{number\ \ of\ \ MUC5AC^{+}\ \ cells}{number\ \ of all\ \ cells} \times 100\%$$

$$ciliated\ \ cell\ \ ratio = \frac{number\ \ of\ \ B4\text{-}TUBULIN^{+}\ \ cells}{number\ \ of all\ \ cells} \times 100\%$$

For IF assessment in Figure S1, fluorescence intensity was analyzed using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) through calculating the raw mean fluorescence intensity (rMFI) in YAP IF staining and the mean autofluorescence intensity (MAI) in negative controls. YAP expression was measured by corrected mean fluorescence intensity (MFI), which was equals to rMFI minus MAI. For every sample, MFI was the average of 3 images.

hNEC culture and treatment

83 Human nasal epithelial cells (hNECs) were developed from primary human nasal
84 epithelial stem/progenitor cells (hNESPCs)[2,3], obtained from fresh healthy inferior
85 turbinate mucosa. The hNESPCs were transplanted to an air-liquid interface (ALI)
86 system to form a pseudostratified layer within four weeks. A detailed description of the
87 hNEC culture method used is found in our previously published papers[2,3].
88 Regarding verteporfin (VP) treatment, hNECs were treated using different methods: for
89 d2, cells were exposed to VP throughout the 2 days of culture; for d24, cells were
90 transferred onto media containing VP for 1 h before switching back to normal media
91 during every medium change, every two days. Regarding IL-13 treatment (10 ng/mL,
92 R&D System, Minneapolis, MN, USA), as IL-13 was added to the cell culture medium,
93 hNECs were under continuous IL-13 treatment. Since DMSO is the solvent of VP, we
94 set up a control group containing DMSO (1μM) only.
95

96 *Statistics*

97 Statistical significance was analyzed using the GraphPad Prism 8 software. For
98 differences between two groups, the Mann-Whitney U test and Wilcoxon signed-rank
99 test were applied. RM one-way ANOVA and the Friedman test were used to analyze
100 differences between hNECs in multiple groups. Data were presented as the mean ±
101 standard error of the mean (SEM) or median with an interquartile range (25%-75%).
102 Correlation analysis was performed using the Spearman r test. A P value of <.05 was
103 considered statistically significant.

Supplementary References

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125

126 **Supplementary tables**

127 **Table S1. Primer sequences used for RT-qPCR.**

Gene	Forward primer	Reverse primer
YAP	AATTGAGAACAATGACGACC	AGTATCACCTGTATCCATCTC
Ki-67	ACGAGACGCCTGGTTACTATC	GCTCATCAATAACAGACCCATT TAC
MUC5AC	AATGGTGGAGATTTTGACAC	TTCTTGTTCAGGCAAATCAG
FOXJ-1	GTGAAGCCTCCCTACTC	TTCTTGTTCAGGCAAATCAG
RPL13A	GTCTGAAGCCTACAAGAAAG	TGTCAATTTTCTTCTCCACG

128 RT-qPCR: Quantitative Real-Time Polymerase Chain Reaction; YAP, Yes-associated
129 protein; FOXJ-1, Forkhead box protein J1; MUC5AC, mucin 5AC; RPL13A,
130 ribosomal protein L13a.

131

132 **Table S2. Primary antibodies used for IF stainings.**

Antibody	Type	Dilution	Catalog number	Company
YAP	mAb	1/100	#14074	Cell Signaling
Ki-67	mAb	1/200	#12075	Cell Signaling
P63	mAb	1/400	Ab735	Abcam
MUC5AC	pAb	1/500	sc-20118	Santa Cruz Biotechnology
MUC5AC	mAb	1/500	ab24071	Abcam
B4-TUBULIN	mAb	1/800	ab11315	Abcam

IF: immunofluorescence; pAb: rabbit polyclonal antibody; mAb: mouse monoclonal antibody; YAP, Yes-associated protein; MUC5AC, mucin 5AC.

Supplementary Figure legends

Figure S1. Overexpression of total YAP in patients with CRSwNP.

YAP is overexpressed in CRSwNP biopsy on mRNA level (IT=9; CRSwNP=19) (**A**). Mean fluorescence intensity (MFI) of YAP in epithelium of CRSwNP biopsy is also higher than IT (IT=9; CRSwNP=19) (**B, C**). Nuclear YAP can be detected in basal cells (marked by P63, **D left**), goblet cells (marked by MUC5AC, **D middle**), and ciliated cells (marked by B4-TUBULIN, **D right**). IF images, ×400 amplification.

YAP, Yes-associated protein; CRSwNP, Chronic rhinosinusitis with nasal polyps; IT, inferior turbinate; IF, immunofluorescence; MFI, mean fluorescence intensity; MUC5AC, mucin 5AC.

Figure S2. ALI dynamics in normal culture.

In ALI-cultured hNECs in vitro (n=4) on normal condition in ALI culture during day 0 – day 24, measured at 13 timepoints, mRNA trends of Ki-67 (proliferation, purple), FOXJ-1 (ciliated cells, green), and MUC5AC (goblet cells, orange) expression were shown in (**A**), mRNA trends of YAP (red) expression was shown in (**B**). (**C**), representative IF staining of the timepoint when goblet cells were first detected (MUC5AC=red, day 4/6), the timepoint when ciliated cells were first detected (β4-TUBULIN=green, day 8/10), and the timepoint of full differentiation (day 24). Interestingly, at mRNA levels, Ki-67 was positively correlated to YAP (**D, E**) only

155 before ciliated cells can be detected (d0-d6, stage I) while FOXJ-1 was positively
156 correlated to YAP (**F, G**) only after ciliated cells can be detected (d8-d24, stage II). (**H**),
157 From d0 to d2, proliferation was dominant in the culture system. Then, goblet cells and
158 ciliated cells appeared in succession. On d24, the culture system was fully differentiated.
159 IF images, ×400 amplification.
160 ALI, air-liquid interface; hNECs, human nasal epithelial cells; YAP, Yes-associated
161 protein; IF, immunofluorescence; FOXJ-1, Forkhead box protein J1; MUC5AC, mucin
162 5AC.
163
164 **Figure S3. IL-13 induce proliferation in ALI-cultured hNECs in vitro (double**
165 **staining of Fig 1J, O)**
166 For the ease of seeing the double staining, we omitted DAPI in this figure.
167 IL-13, Interleukin 13; ALI, air-liquid interface; hNECs, human nasal epithelial cells.
168
169 **Figure S4. Verteporfin repressed proliferation in ALI-cultured hNECs in vitro.**
170 IF results on d2 (**A, B** is double staining of **A**) and d24 (**G, H** is double staining of **G**),
171 showed that VP (verteporfin) decreased the YAP activity (**C, I**) and proliferation level
172 (**E, K**) of basal cells, which were positively correlated (**F, L**), and also the percentage
173 of basal cells (**D, J**). N=6. IF images, ×400 amplification. VP, 0.5μM, 1μM; DMSO,
174 1μM.
175 VP, verteporfin; ALI, air-liquid interface; YAP, Yes-associated protein; hNECs, human
176 nasal epithelial cells; DMSO, dimethyl sulfoxide.

Figure S5. Verteporfin repressed differentiation in ALI-cultured hNECs in vitro.

On d24, VP (verteporfin) restricted goblet cell (marked by MUC5AC) differentiation and inhibited ciliated cell (marked by B4-TUBULIN) formation (**A, B, D**). The YAP activity of terminally differentiated goblet cells and ciliated cells decreased (**C, E**). N=6. IF images, ×400 amplification. VP, 0.5μM, 1μM; DMSO, 1μM.

VP, verteporfin; ALI, air-liquid interface; hNECs, human nasal epithelial cells; YAP, Yes-associated protein; MUC5AC, mucin 5AC; DMSO, dimethyl sulfoxide.

Figure S6. PCR results of VP and/or IL-13 treatment in ALI-cultured hNECs in vitro.

With IL-13 treatment in ALI-cultured hNECs in vitro, on day 2 (n=8) and day 24 (n=6), IL-13 induce YAP overexpression (**A, D**) and high level of proliferation (Ki-67, **B, E**) at mRNA level, which is positively correlated with each other on day 2 (**C**). On day 24 (n=6), IL-13 restricted FOXJ1 (representing ciliated cells, **F**) but promoted MUC5AC (representing goblet cells, **G**). IL-13, 10ng/ml.

With verteporfin (VP) treatment in ALI-cultured hNECs in vitro, on day 2 (n=6) and day 24 (n=6), VP repressed YAP expression (**H, K**) and the level of proliferation (**I, L**) at mRNA level, which is positively correlated with each other on day 2 (**J**). On day 24 (n=6), VP restricted FOXJ1 (**M**, not significant) and MUC5AC (**N**). VP, 0.5μM, 1μM; DMSO, 1μM.

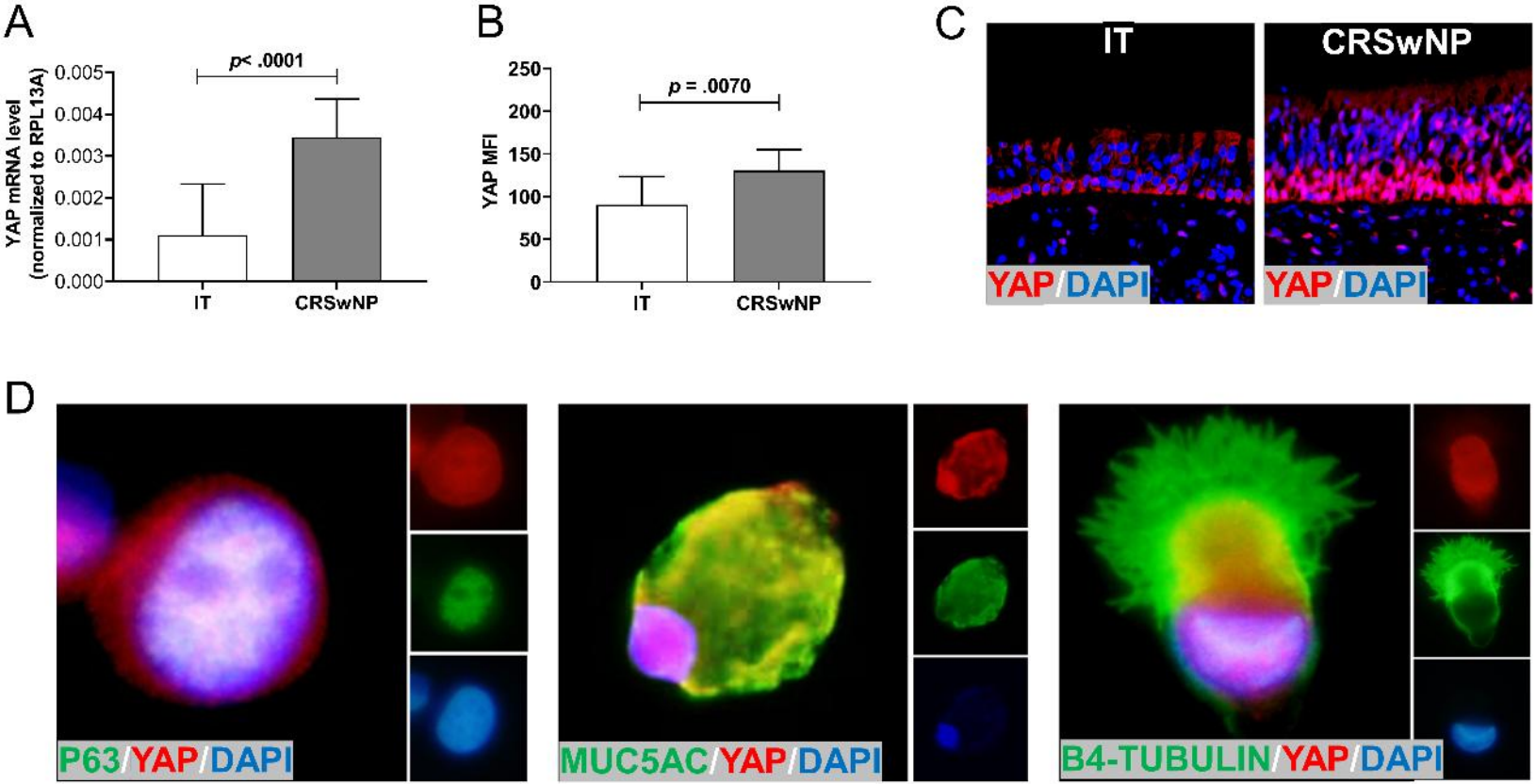
With IL-13+VP treatment in ALI-cultured hNECs in vitro, on day 24 (n=4), PCR results

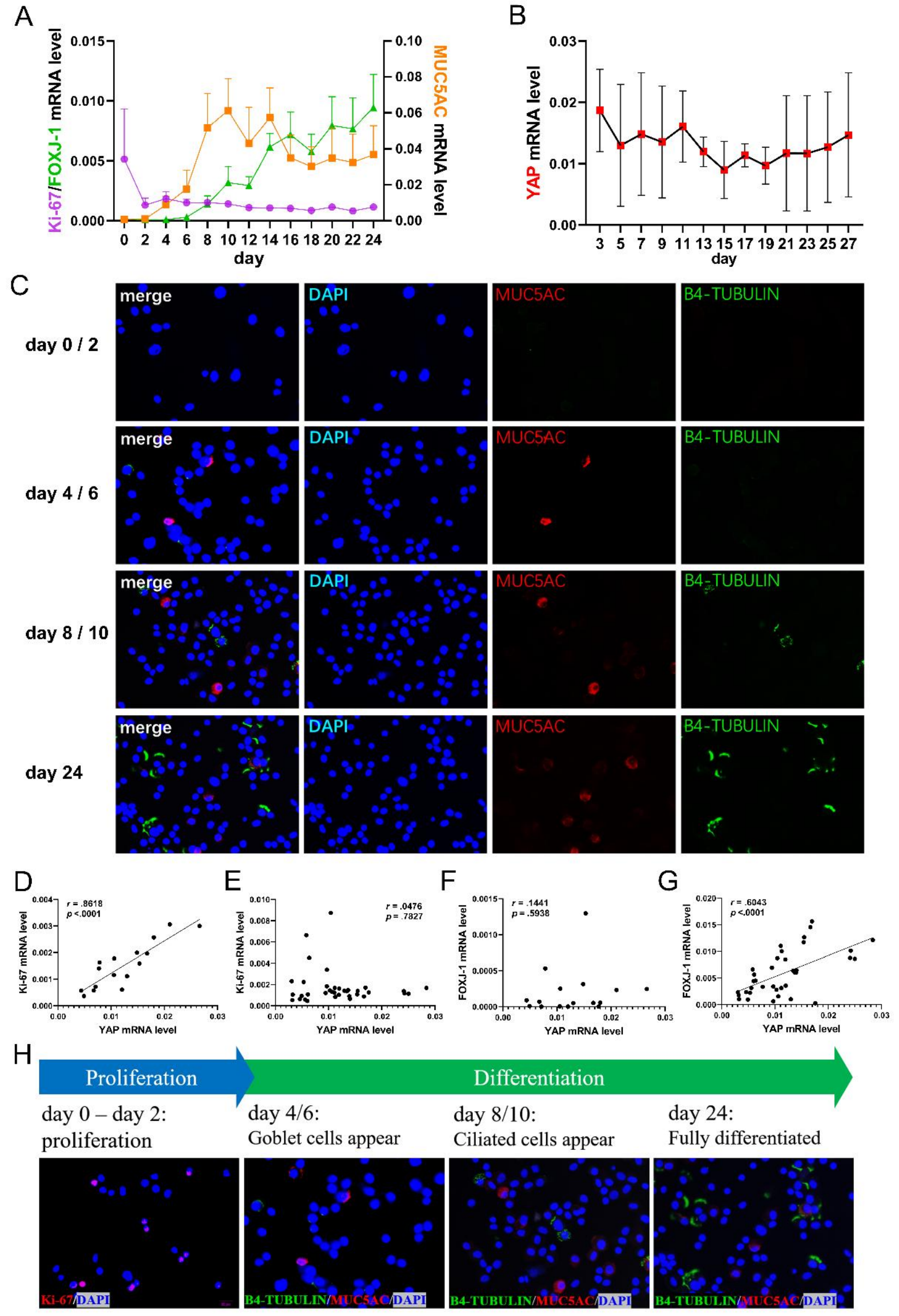
showing the expression of YAP (**O**), Ki-67 (**P**, not significant), FOXJ-1 (**Q**), and MUC5AC (**R**). IL-13, 10ng/ml; VP, 1μM; DMSO, 1μM.

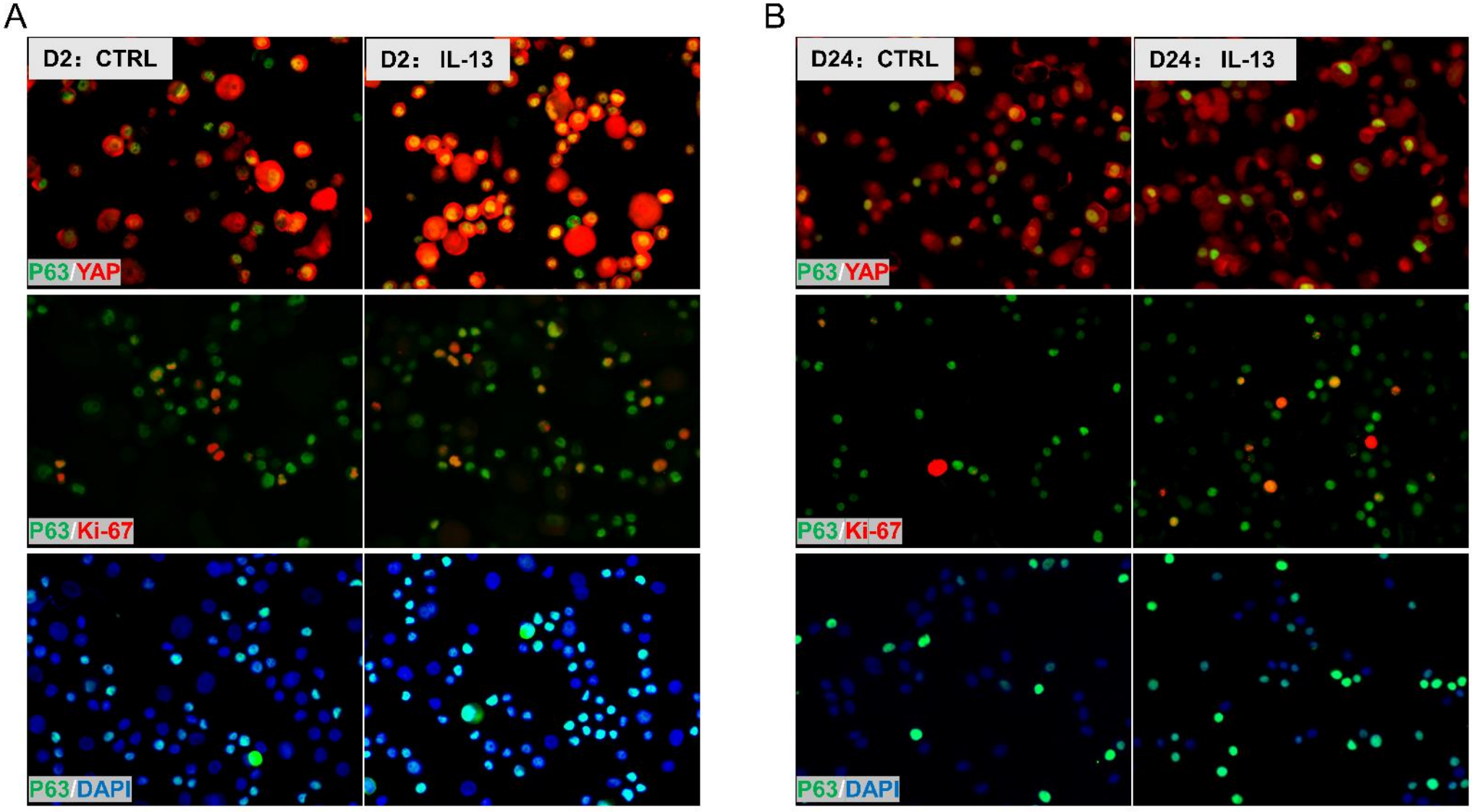
PCR: Quantitative Real-Time Polymerase Chain Reaction; IL-13, interleukin-13; VP, verteporfin; ALI, air-liquid interface; hNECs, human nasal epithelial cells; YAP, Yes-associated protein; FOXJ-1, Forkhead box protein J1; MUC5AC, mucin 5AC; DMSO, dimethyl sulfoxide; NS, not significant.

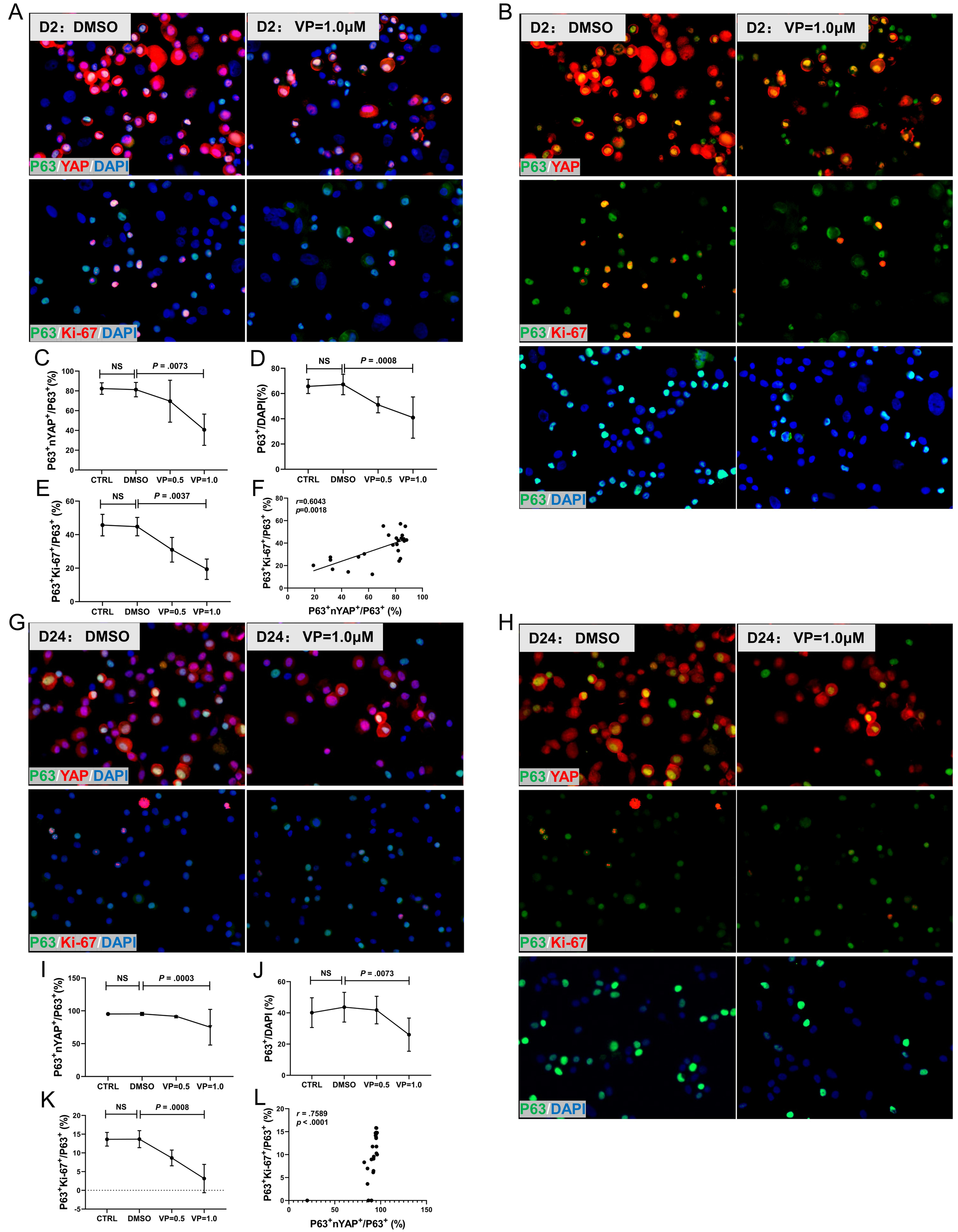
Figure S7. Graphic abstract.

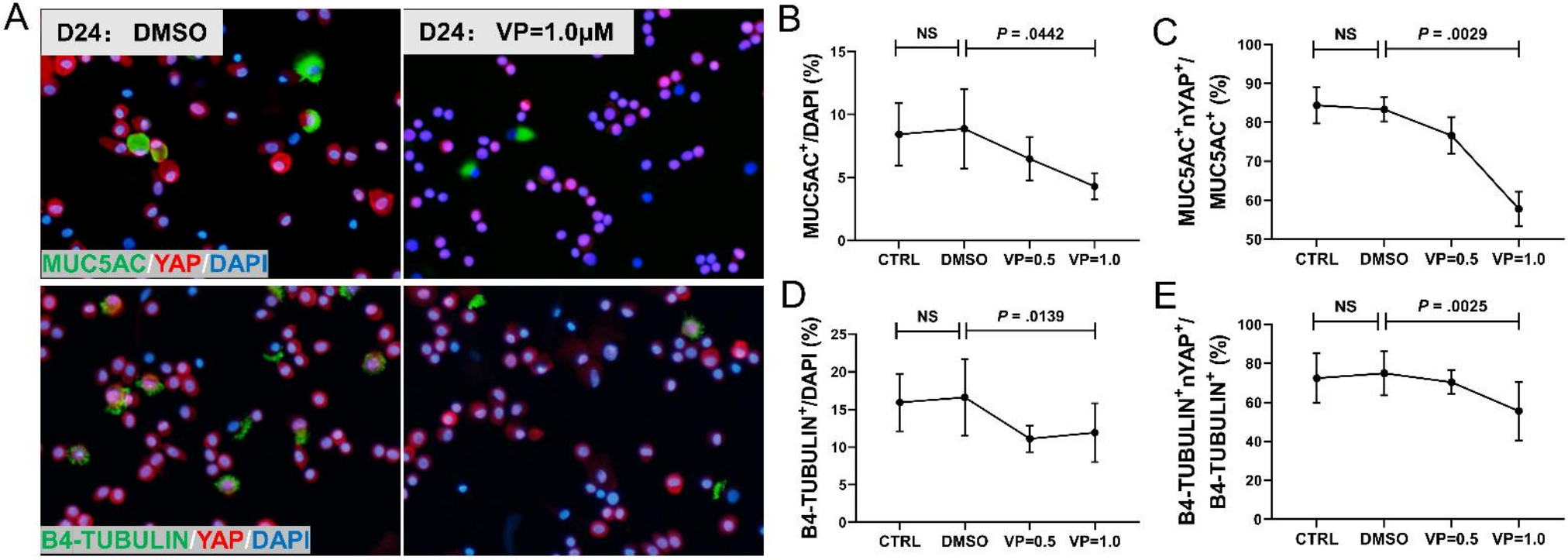
VP could reverse IL-13 induced CRSwNP remodeling via inhibiting YAP activity.

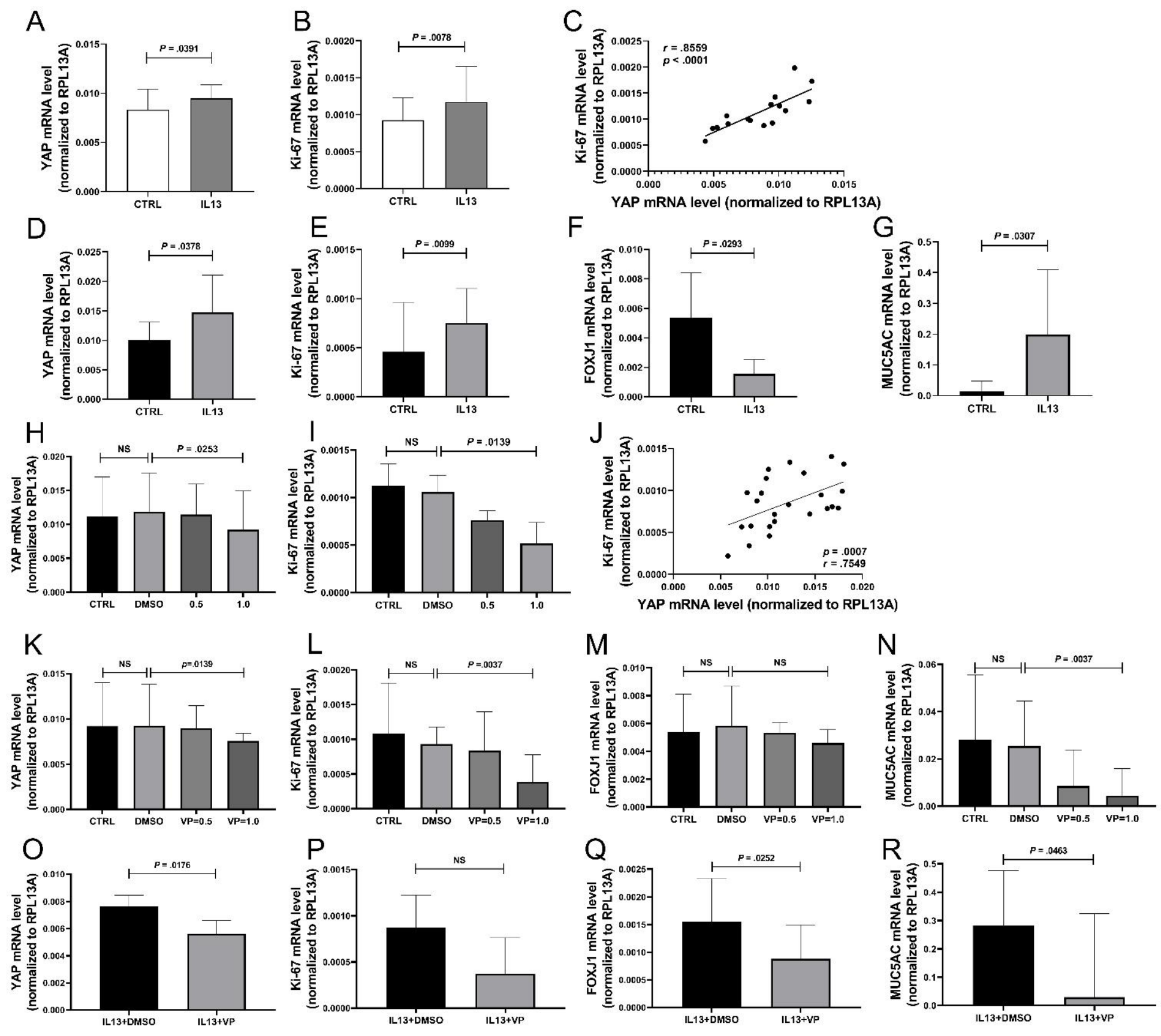

A
p< .0001
YAP mRNA level
(normalized to RPL13A)
0.005
0.004
0.003
0.002
0.001
0.000
IT
CRSwNP
B
p = .0070
YAP MFI
250
200
150
100
50
0
IT
CRSwNP
C
IT
CRSwNP
YAP/DAPI
YAP/DAPI
D
P63/YAP/DAPI
MUC5AC/YAP/DAPI
B4-TUBULIN/YAP/DAPI

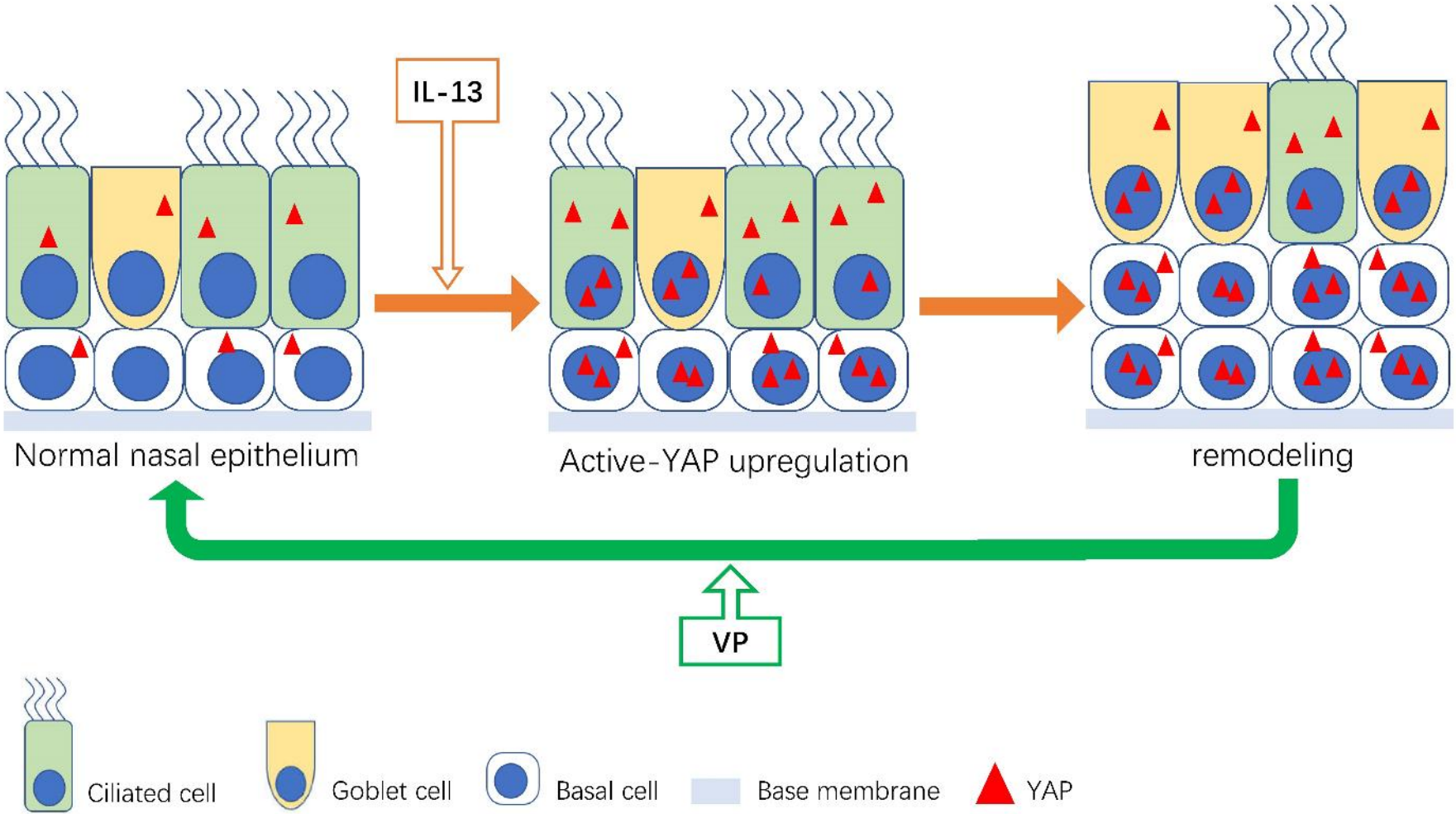

A
Ki-67/FOXJ-1 mRNA level
MUC5AC mRNA level
day
B
YAP mRNA level
day
C
merge
DAPI
MUC5AC
B4-TUBULIN
day 0 / 2
day 4 / 6
day 8 / 10
day 24
D
Ki-67 mRNA level
YAP mRNA level
r = .8618
p <.0001
E
Ki-67 mRNA level
YAP mRNA level
r = .0476
p = .7827
F
FOXJ-1 mRNA level
YAP mRNA level
r = .1441
p = .5938
G
FOXJ-1 mRNA level
YAP mRNA level
r = .6043
p <.0001
H
Proliferation
Differentiation
day 0 – day 2:
proliferation
day 4/6:
Goblet cells appear
day 8/10:
Ciliated cells appear
day 24:
Fully differentiated
Ki-67/DAPI
B4-TUBULIN/MUC5AC/DAPI
B4-TUBULIN/MUC5AC/DAPI
B4-TUBULIN/MUC5AC/DAPI


A
D2：CTRL
D2： IL-13
P63/YAP
P63/Ki-67
P63/DAPI
B
D24：CTRL
D24： IL-13
P63/YAP
P63/Ki-67
P63/DAPI


A
D2：DMSO
D2： VP=1.0μM
P63/YAP/DAPI
P63/Ki-67/DAPI
B
D2： DMSO
D2： VP=1.0μM
P63/YAP
P63/Ki-67
P63/DAPI
C
NS
P = .0073
P63+nYAP+/P63+(%)
CTRL
DMSO
VP=0.5
VP=1.0
D
NS
P = .0008
P63+/DAPI(%)
E
NS
P = .0037
P63+Ki-67+/P63+ (%)
F
r=0.6043
p=0.0018
P63+Ki-67+/P63+ (%)
P63+nYAP+/P63+ (%)
G
D24： DMSO
D24： VP=1.0μM
P63/YAP/DAPI
P63/Ki-67/DAPI
H
D24： DMSO
D24： VP=1.0μM
P63/YAP
P63/Ki-67
P63/DAPI
I
NS
P = .0003
P63+nYAP+/P63+(%)
CTRL
DMSO
VP=0.5
VP=1.0
J
NS
P = .0073
P63+/DAPI (%)
K
NS
P = .0008
P63+Ki-67+/P63+ (%)
L
r = .7589
p < .0001
P63+Ki-67+/P63+ (%)
P63+nYAP+/P63+ (%)


A
D24: DMSO
D24: VP=1.0μM
MUC5AC/YAP/DAPI
B4-TUBULIN/YAP/DAPI
B
NS
P = .0442
MUC5AC+/DAPI (%)
CTRL
DMSO
VP=0.5
VP=1.0
C
NS
P = .0029
MUC5AC+nYAP+/MUC5AC+ (%)
D
NS
P = .0139
B4-TUBULIN+/DAPI (%)
E
NS
P = .0025
B4-TUBULIN+nYAP+/B4-TUBULIN+ (%)


A
YAP mRNA level (normalized to RPL13A)
P = .0391
CTRL
IL13
B
Ki-67 mRNA level (normalized to RPL13A)
P = .0078
CTRL
IL13
C
Ki-67 mRNA level (normalized to RPL13A)
r = .8559
p < .0001
YAP mRNA level (normalized to RPL13A)
D
YAP mRNA level (normalized to RPL13A)
P = .0378
CTRL
IL13
E
Ki-67 mRNA level (normalized to RPL13A)
P = .0099
CTRL
IL13
F
FOXJ1 mRNA level (normalized to RPL13A)
P = .0293
CTRL
IL13
G
MUC5AC mRNA level (normalized to RPL13A)
P = .0307
CTRL
IL13
H
YAP mRNA level (normalized to RPL13A)
NS
P = .0253
CTRL
DMSO
0.5
1.0
I
Ki-67 mRNA level (normalized to RPL13A)
NS
P = .0139
CTRL
DMSO
0.5
1.0
J
Ki-67 mRNA level (normalized to RPL13A)
p = .0007
r = .7549
YAP mRNA level (normalized to RPL13A)
K
YAP mRNA level (normalized to RPL13A)
NS
p=.0139
CTRL
DMSO
VP=0.5
VP=1.0
L
Ki-67 mRNA level (normalized to RPL13A)
NS
P =.0037
CTRL
DMSO
VP=0.5
VP=1.0
M
FOXJ1 mRNA level (normalized to RPL13A)
NS
NS
CTRL
DMSO
VP=0.5
VP=1.0
N
MUC5AC mRNA level (normalized to RPL13A)
NS
P = .0037
CTRL
DMSO
VP=0.5
VP=1.0
O
YAP mRNA level (normalized to RPL13A)
P = .0176
IL13+DMSO
IL13+VP
P
Ki-67 mRNA level (normalized to RPL13A)
NS
IL13+DMSO
IL13+VP
Q
FOXJ1 mRNA level (normalized to RPL13A)
P = .0252
IL13+DMSO
IL13+VP
R
MUC5AC mRNA level (normalized to RPL13A)
P = .0463
IL13+DMSO
IL13+VP


IL-13
Normal nasal epithelium
Active-YAP upregulation
remodeling
VP
Ciliated cell
Goblet cell
Basal cell
Base membrane
YAP