

# Synovial macrophage M1 polarisation exacerbates experimental osteoarthritis partially through R-spondin-2






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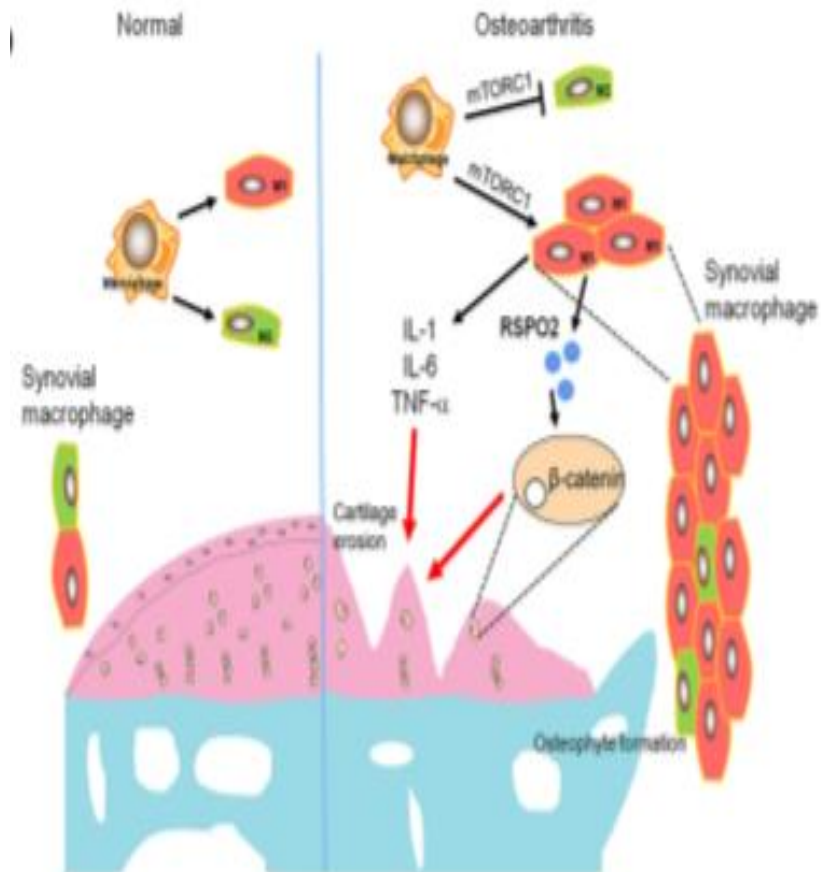
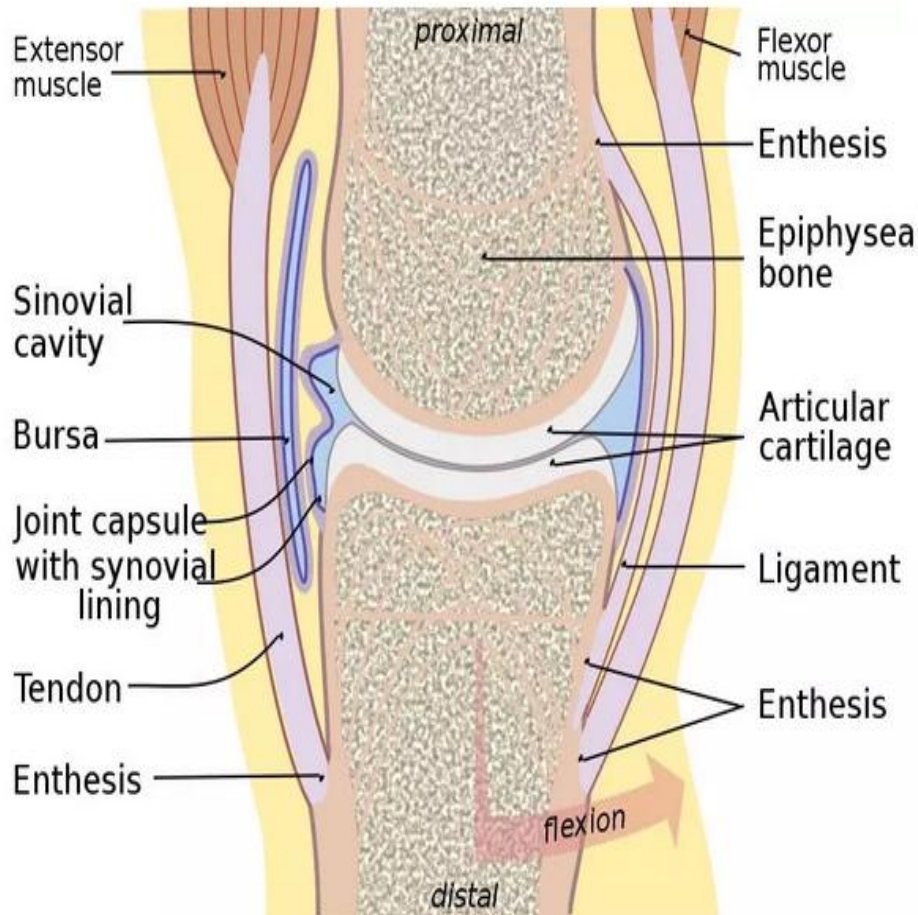
Reporter: Gang Xu

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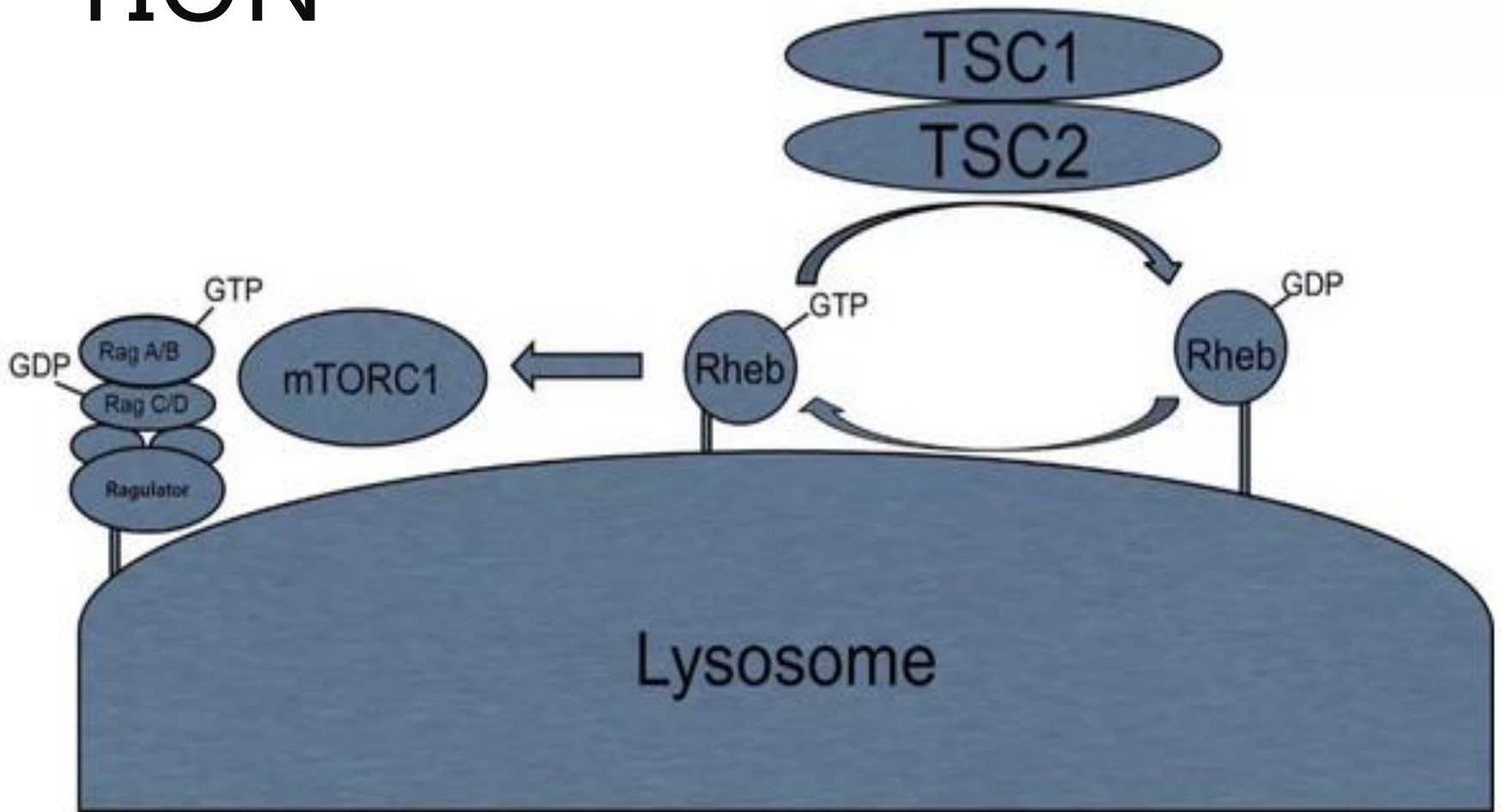
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# Introduction



# INTRODUCTION



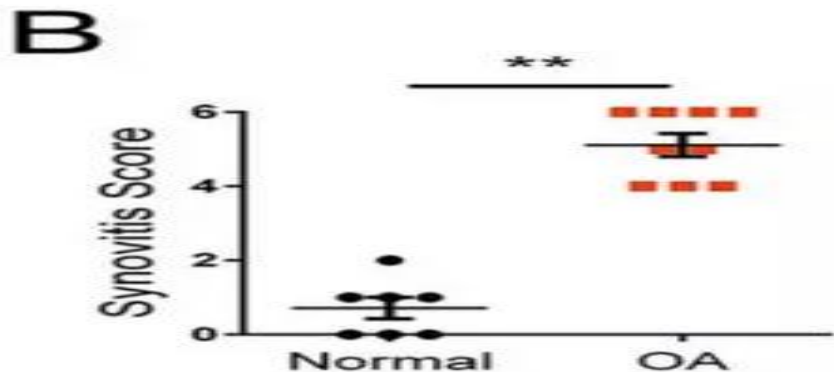
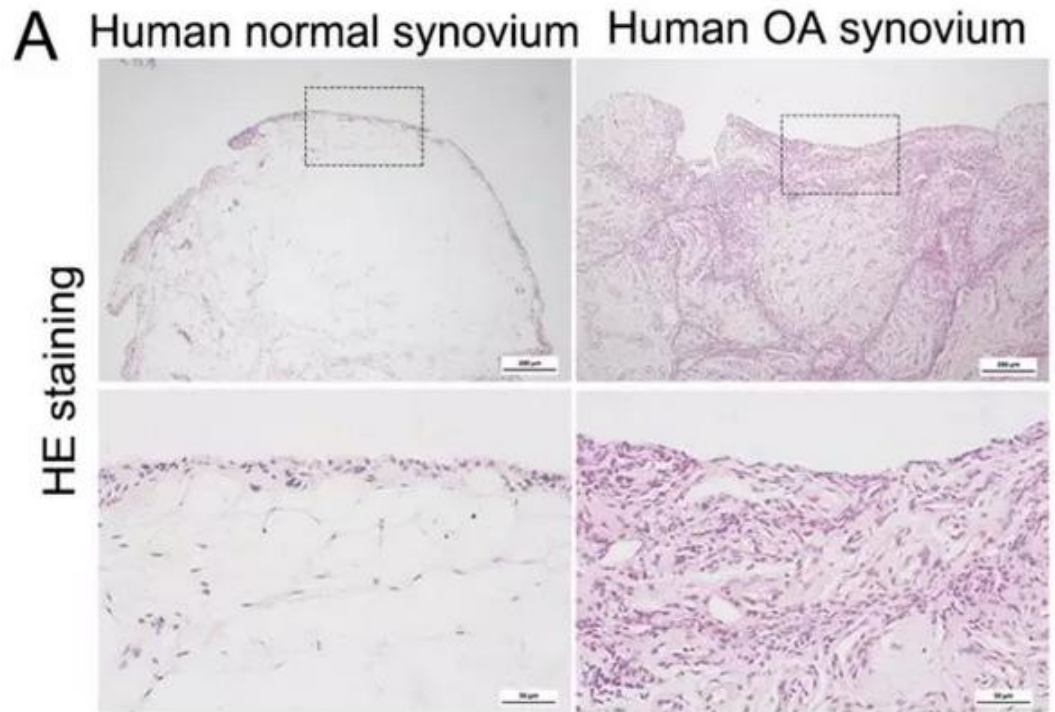
# Materials and Methods

- ❖ 1. Human synovium and KO mice (TSC1 KO and Rheb1 KO)
- ❖ 2. OA model (CIOA)
- ❖ 3. Micro-CT
- ❖ 4. q-PCR
- ❖ 5. Western blotting
- ❖ 6. Enzyme-linked immunosorbent assay (ELISA)
- ❖ 7. Immunohistochemistry

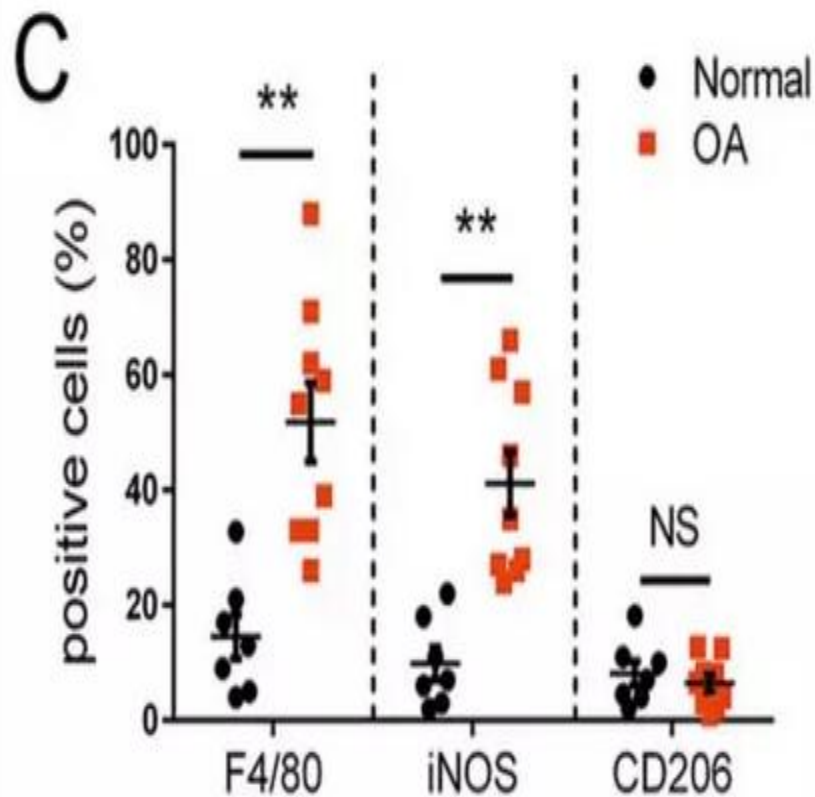
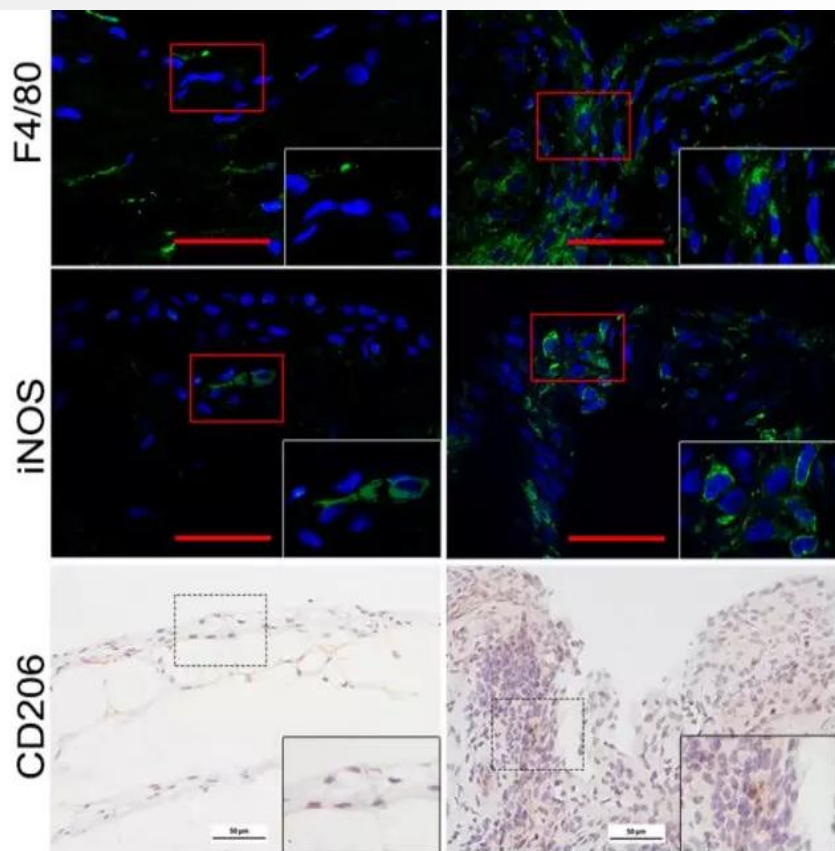
## Results:

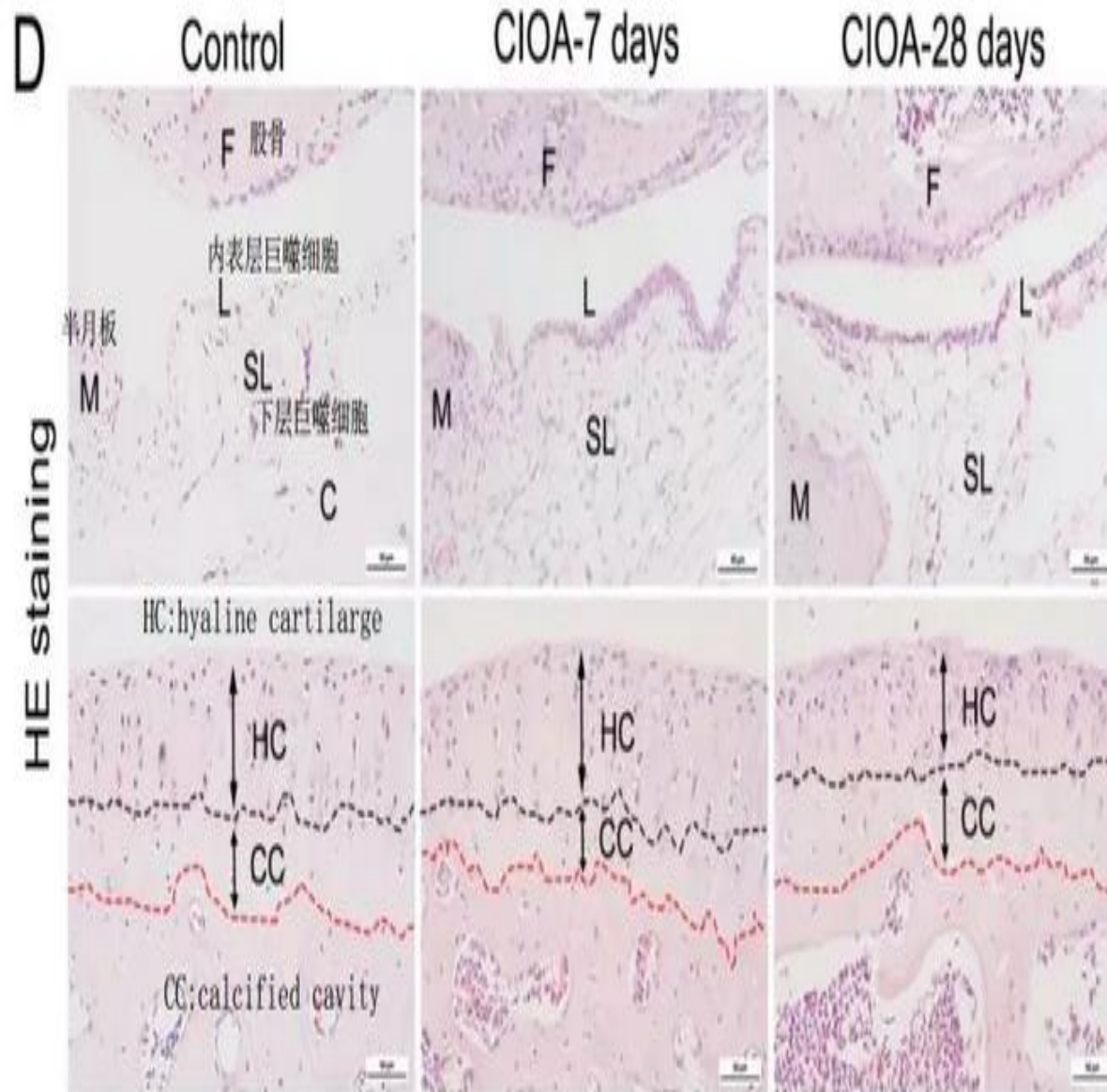
### 1. M1 but not M2-polarised macrophages accumulate in synovial tissue of patients with OA and OA mice

HE staining: In control group, there were only 2 to 3 layers of cells in the inner surface of synovium, and the connective tissue structure of the lower layer was normal without obvious cell infiltration. In OA group, a large number of cells were infiltrated, which accorded with the changes of synovitis.



The cells by immunofluorescence and histochemistry. F4/80 was macrophage marker, iNOS was M1 macrophage marker, and CD206 was M2 macrophage marker. Compared with control group, the fluorescence intensity of F4/80 and iNOS in OA group increased significantly, while CD206 did not increase significantly. It can be seen that macrophages aggregate during the occurrence of OA, and M1 macrophages are the main macrophages.



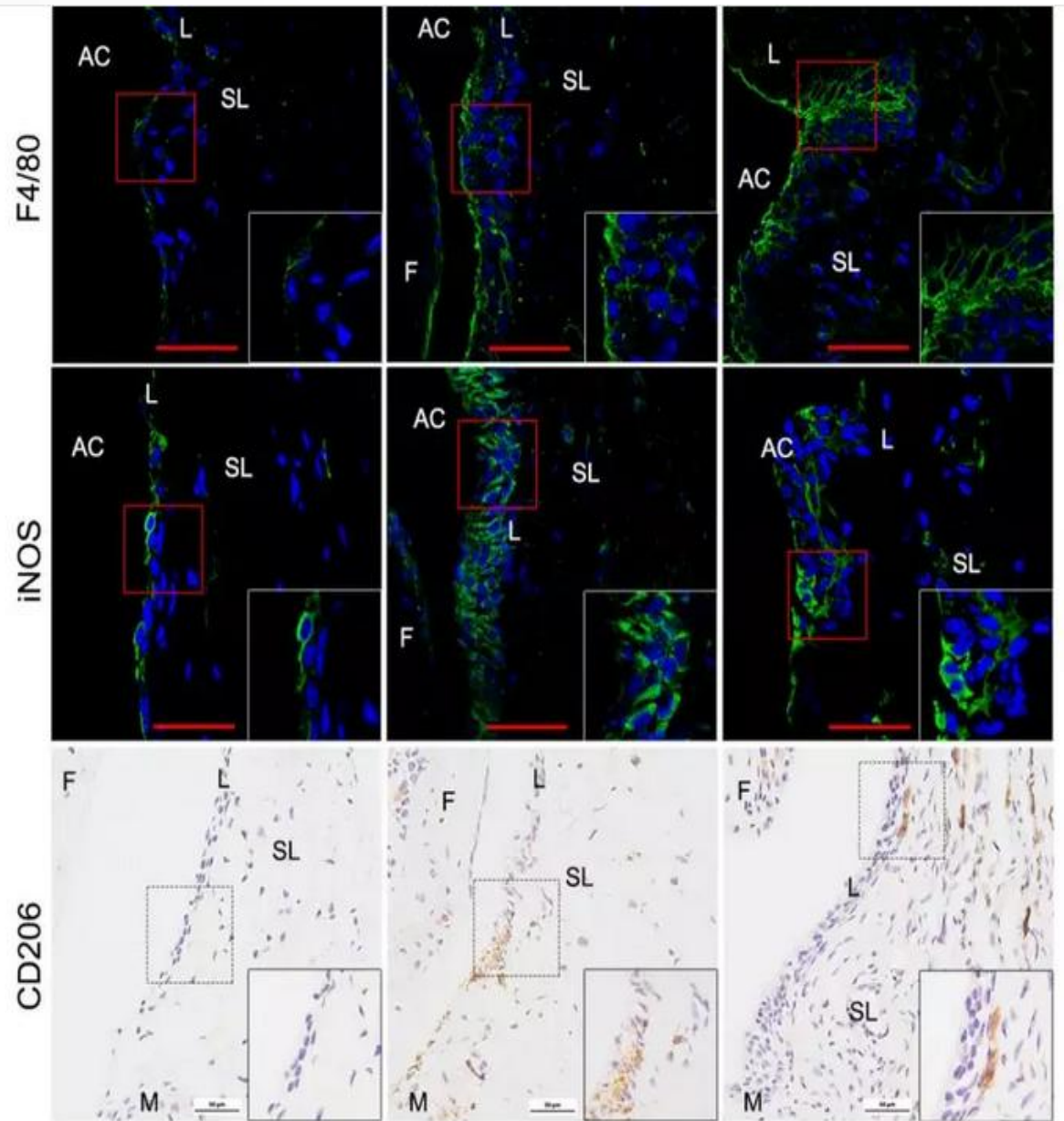


phenotype of collagenase-induced osteoarthritis mouse model (CIOA) was identified. The structure of articular cartilage in control group was normal, and synovium did not increase significantly. In CIOA model mice, synovitis-like changes were observed on 7 and 28 days. Cell infiltration was observed on the inner and outer layers. In addition, the structure of articular cartilage was abnormal, the range of hyaline cartilage was reduced, and the range of calcified cartilage was increased.

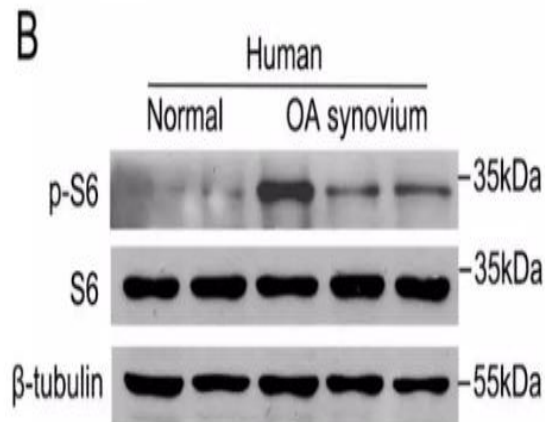
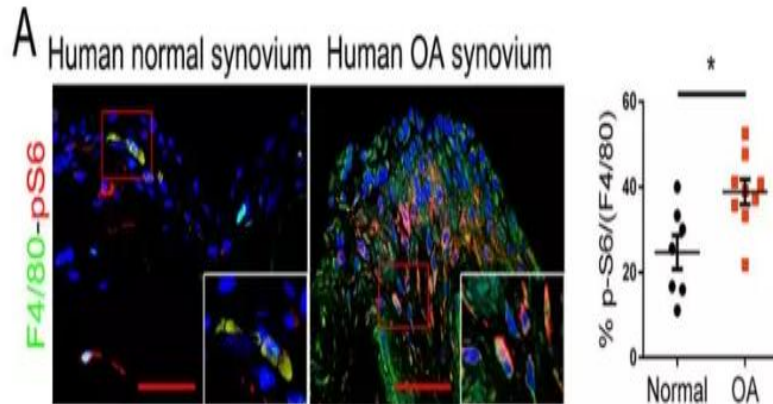


**E**

The macrophages were identified by immunofluorescence and histochemistry. It was found that the number of macrophages and M1-type macrophages increased significantly in the 7-day and 28-day CIOA mice model. The number of M2-type macrophages increased in both the inner and lower layers of the 7-day CIOA model. However, only a few in the 28-day CIOA model were found in the lower layers.



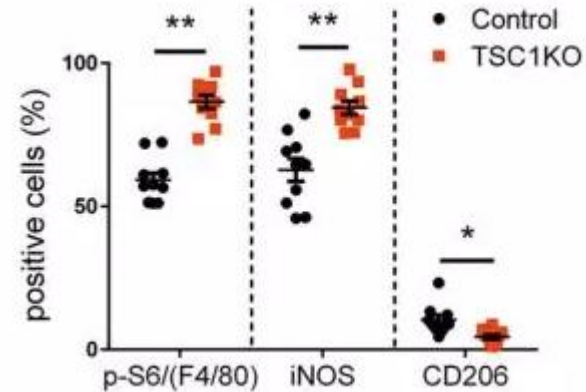
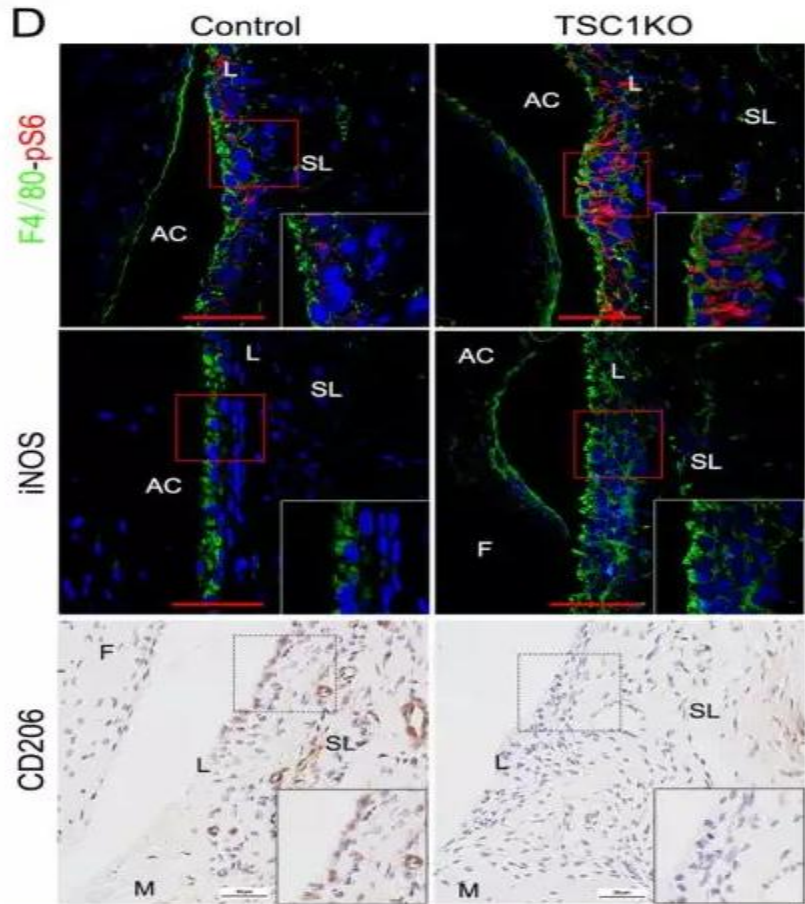
## 2. Constitutive activation of mTORC1 in macrophages enhances their M1 polarisation in NOA synovium



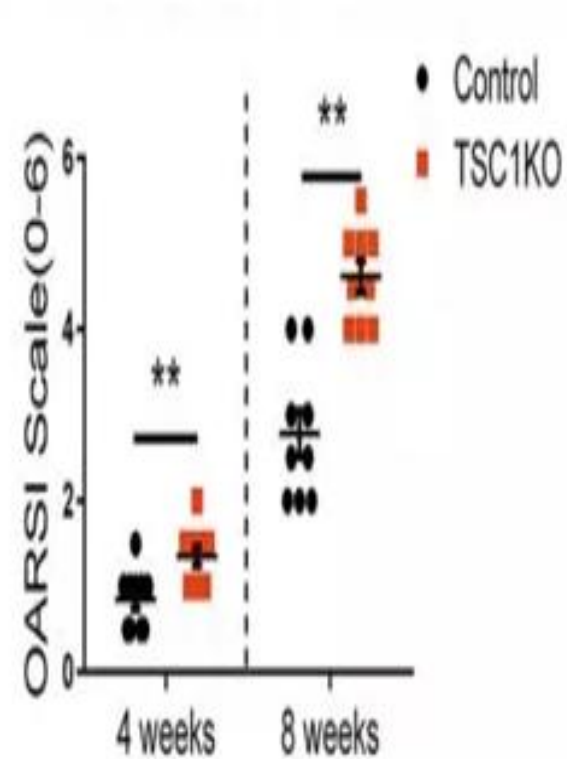
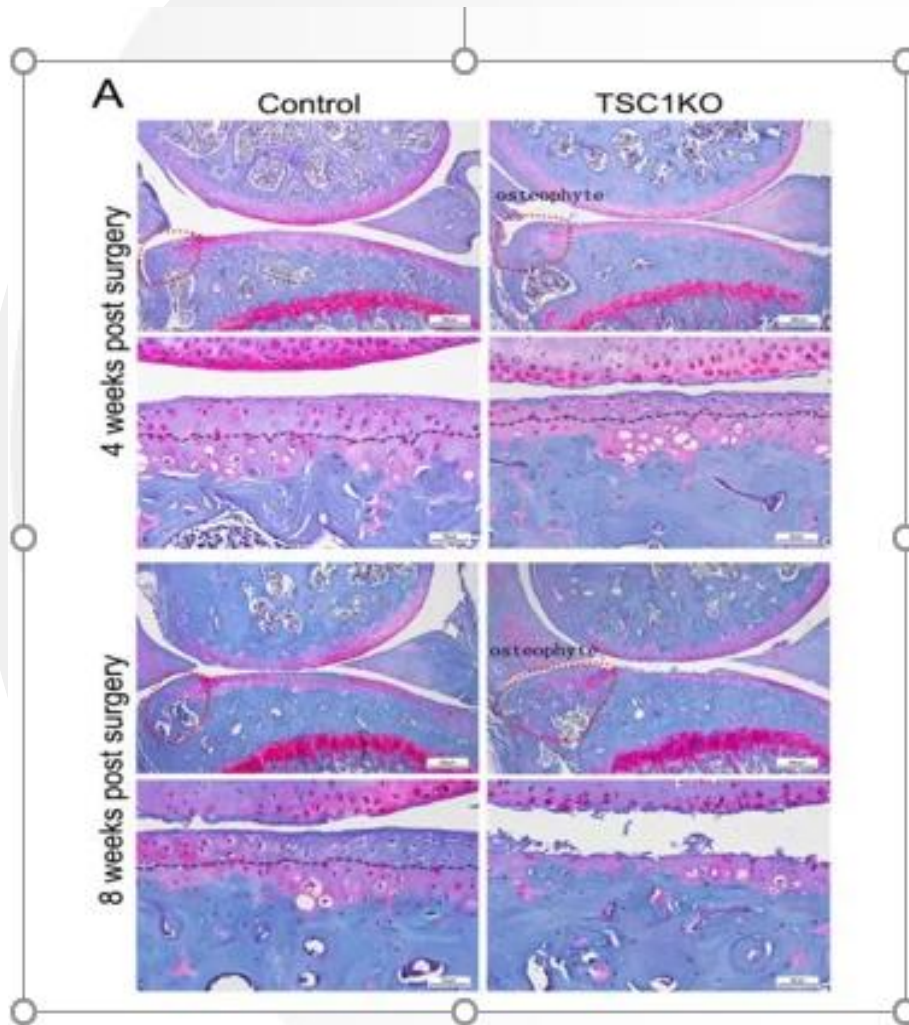
- ❖ S6 protein is the downstream effector molecule of mTORC1. The authors found that phosphorylated p-S6 protein increased significantly in the synovium of human OA. Moreover, p-S6 and F4/80 were co-localized to a certain extent, while S6 level did not change significantly, which excluded the increase of p-S6 phosphorylation caused by the increase of S6 protein. Therefore, the authors directly used p-S6 level to express the activity of mTORC1.



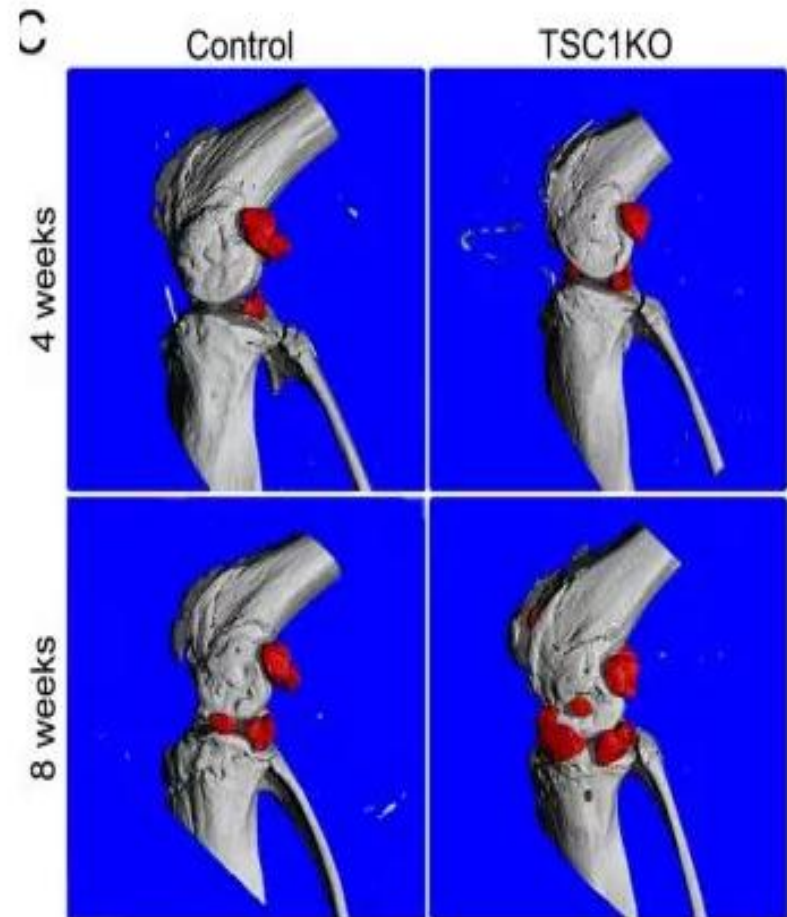
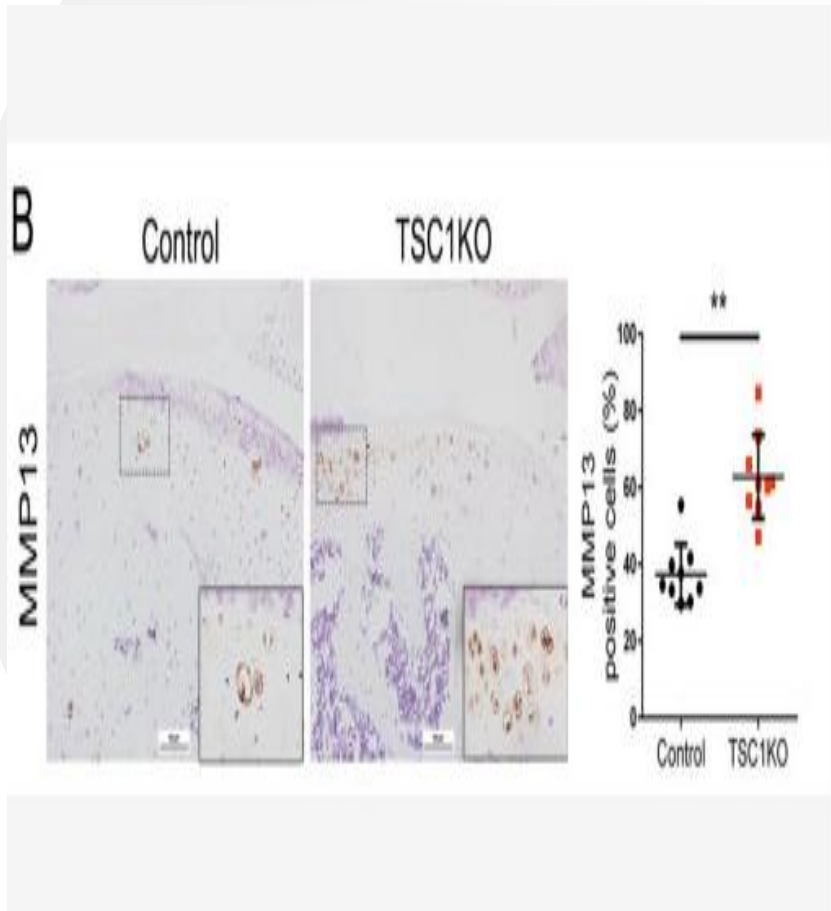
By immunofluorescence, the authors found that the level of p-S6 in TSC1 KO mice increased significantly, which confirmed that the activity of mTORC1 increased. The increased expression of iNOS in the lower layers suggests that the aggregation of M1 macrophages is increased and synovitis is obvious. Immunohistochemistry showed that CD206 was significantly down-regulated, suggesting the decrease of M2 macrophages. This confirms that mTORC1 activation also plays a role in regulating macrophage polarization in the development of OA.



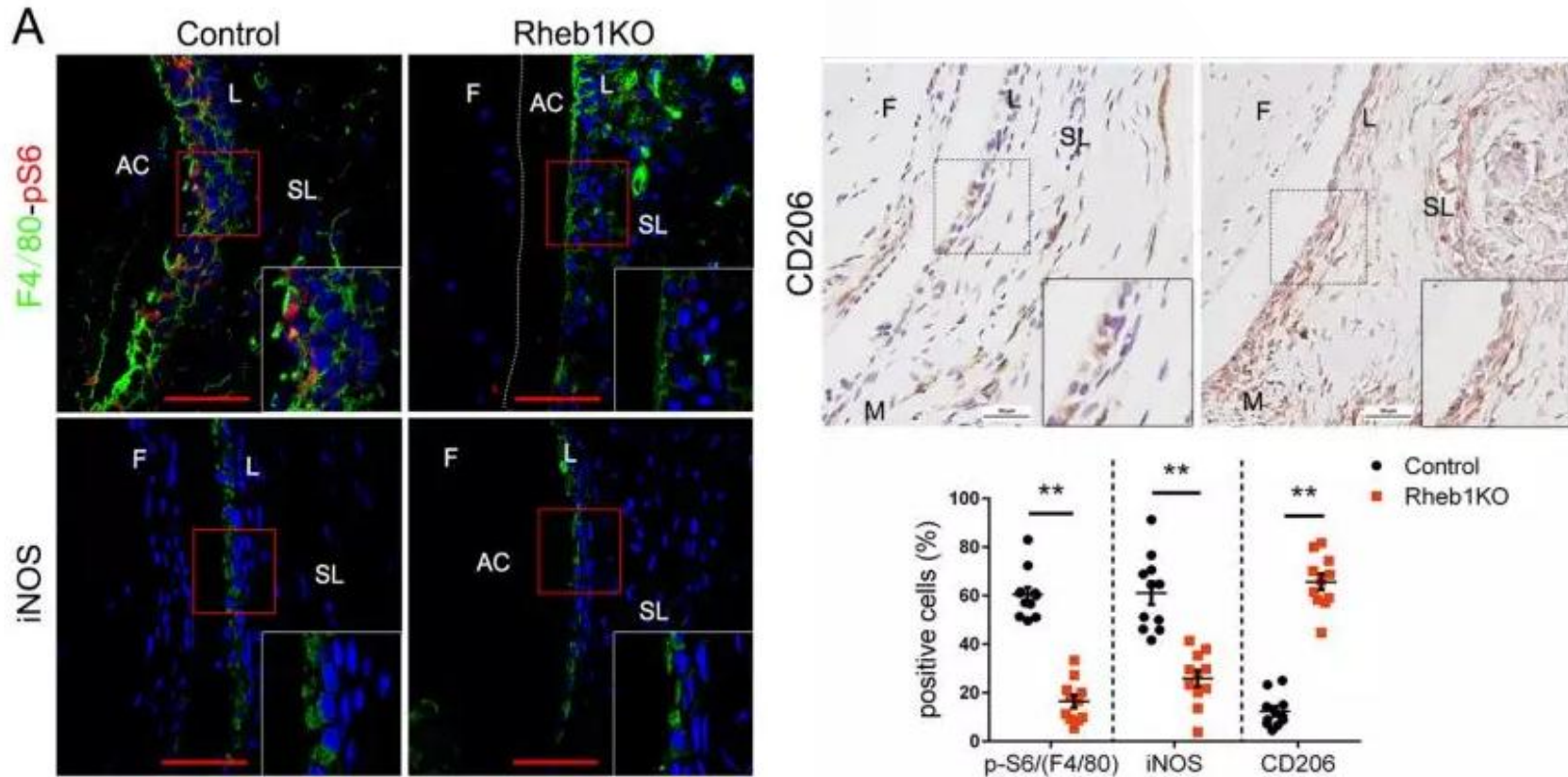
### 3. synovial macrophage M1 polarisation exacerbates cartilage damage and osteophyte formation in collagenase-induced OA



B. Immunohistochemistry of control and TSC1KO 8-week CIOA mice showed that the expression of MMP13 in TSC1KO mice was significantly higher than that in control.  
C. Micro-CT scan showed that the osteophyte volume of TSC1KO mice was more obvious than that of control group.

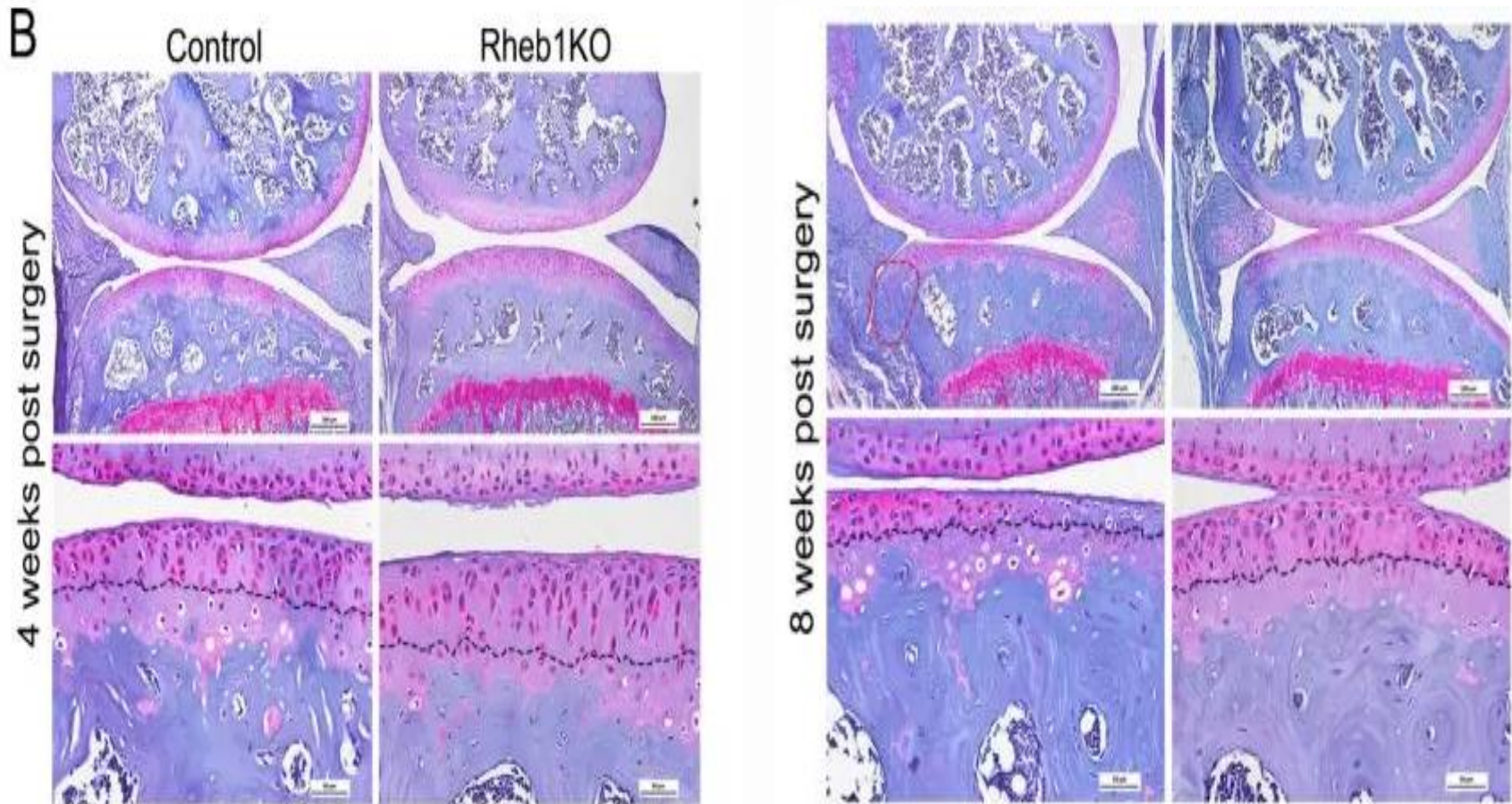


## 4. synovial macrophage M2 polarisation prevents OA development



A. The CIOA model of control and Rheb1KO mice was established. The phenotype of the model was identified after 4 weeks. The p-S6 level of Rheb1KO mice was down-regulated relative to control, which confirmed that the activation of mTORC1 was reduced. The decrease of the fluorescence range in the inner and outer layers of iNOS suggests that the polarization of M1 macrophages decreases, while the proportion of CD206 positive cells in immunohistochemistry increases significantly, suggesting that the polarization of M2 macrophages increases.

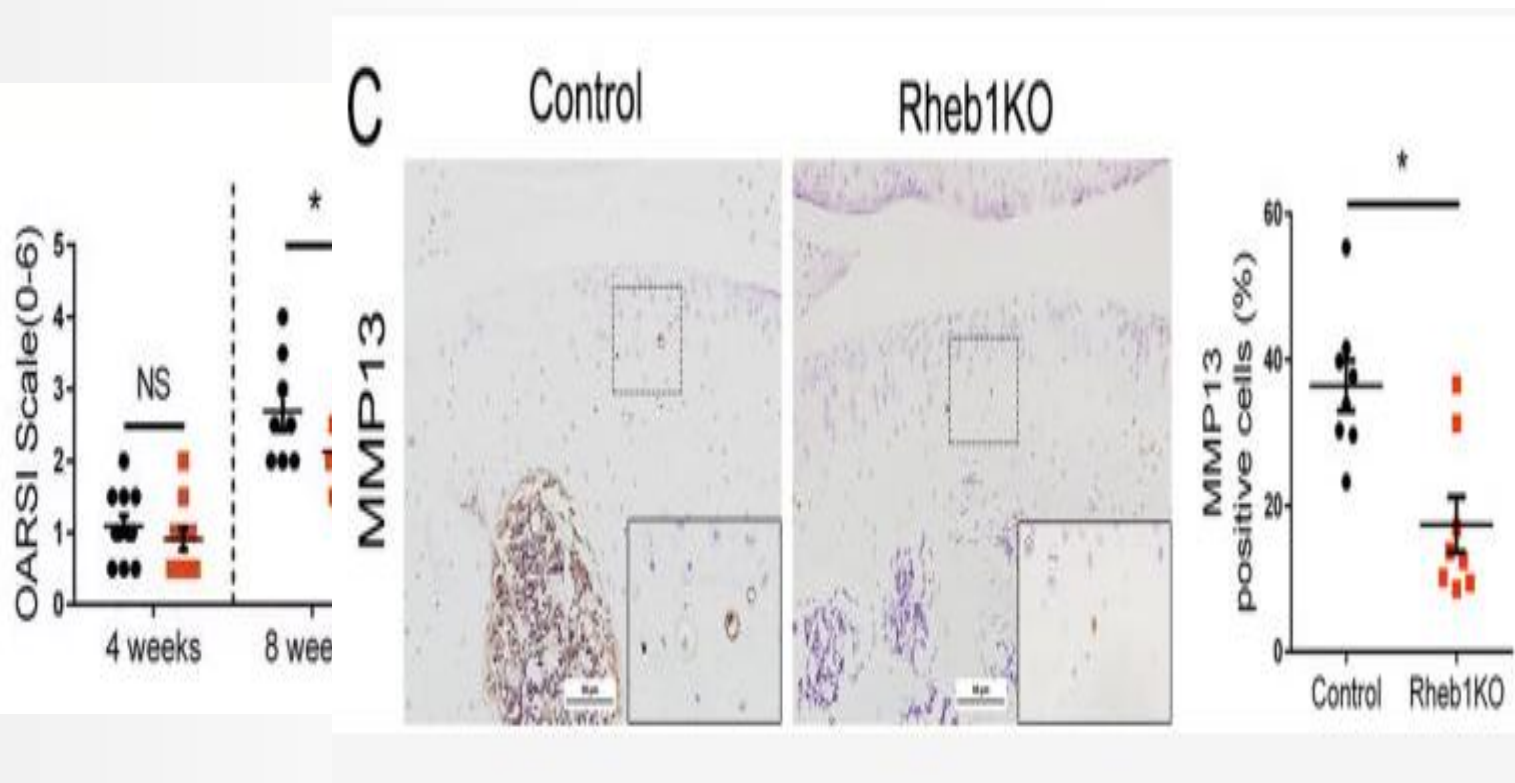
# 4. synovial macrophage M2 polarisation prevents OA development



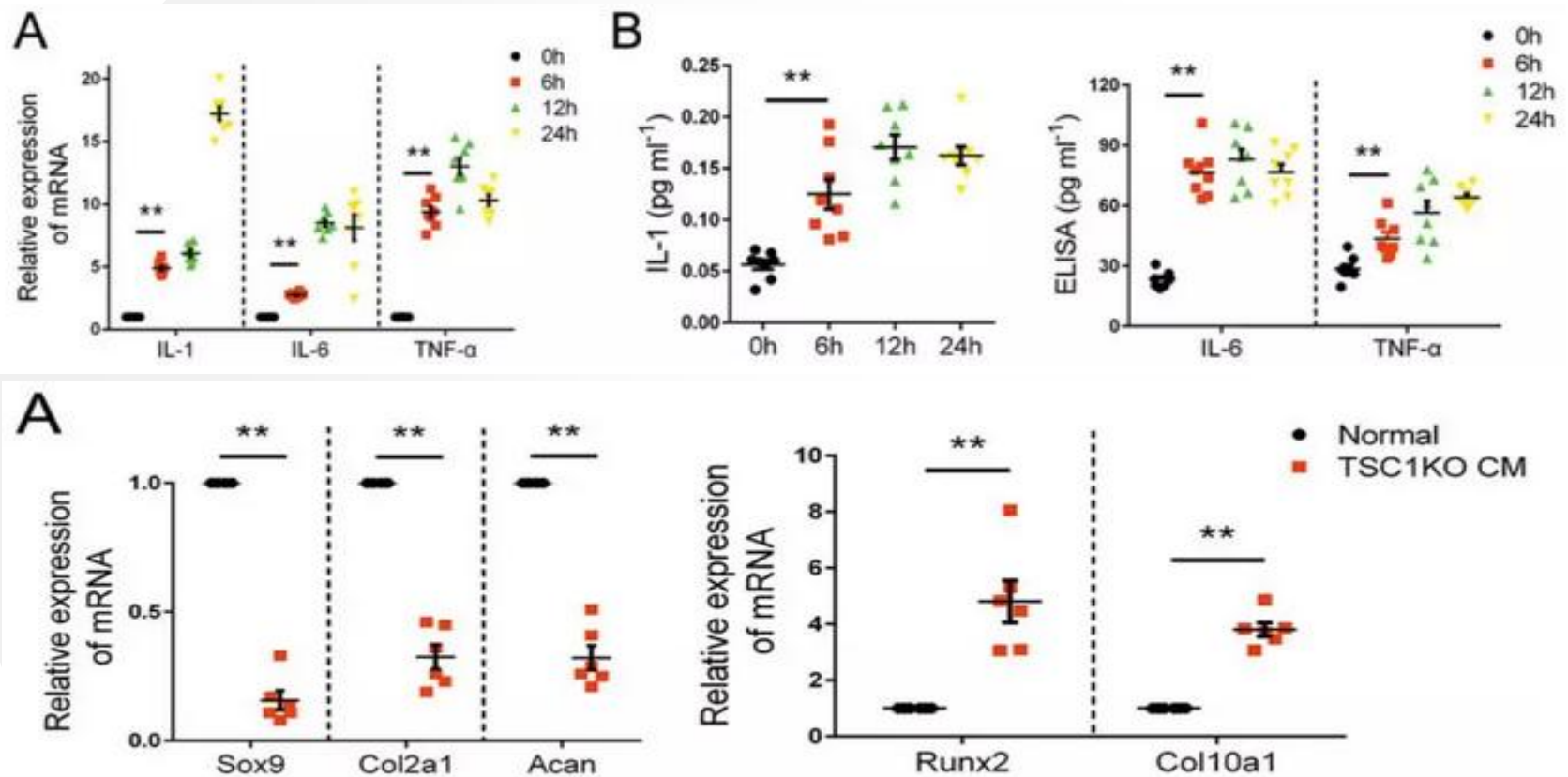


B. Saffron staining was performed on control and Rheb1KO mice at 4 and 8 weeks. It was found that the hyaline cartilage thickness of rheb1KO mice at 4 and 8 weeks was thicker than that of control group, and the synovium of control group was thicker than that of rheb1KO group. At 8 weeks, osteophytes appeared in control group, but not in rheb1KO mice.

C. The articular cartilage of rheb1KO mice was taken for 8 weeks and the expression of MMP13 was detected by immunohistochemistry.



# 5. M1-polarised macrophages produce inflammatory cytokines/enzymes and promote hypertrophic chondrocyte differentiation and maturation in vitro



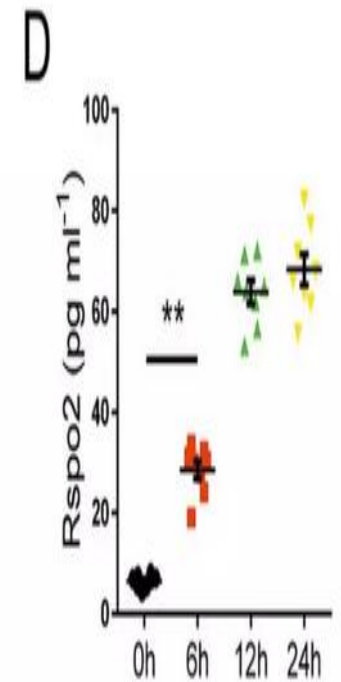
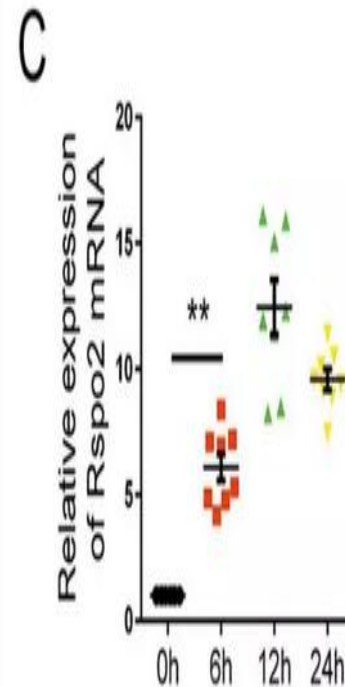
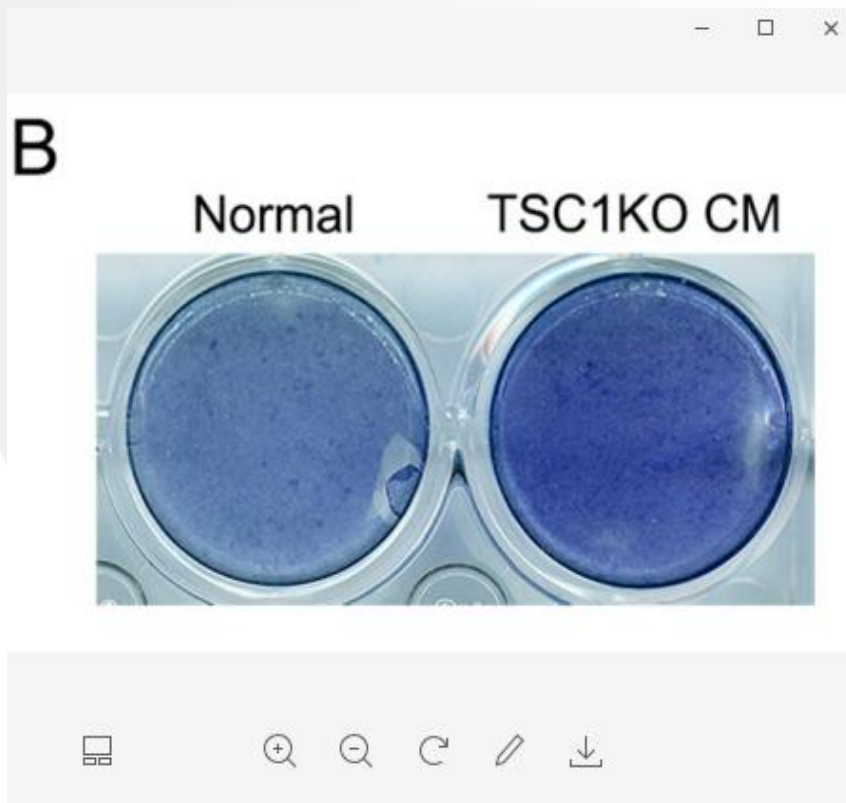
1. RNA sequencing of macrophage samples from control mice and TSC1KO mice showed that IL-1, TNF-alpha and IL-6 expression increased. These three factors contributed to the occurrence of OA, and M1 could secrete these three cytokines. The results were confirmed by QPCR and ELISA. In conclusion, M1 polarized cells can promote the progress of OA by secreting cytokines.

2. The macrophages of control and TSC1KO knockout mice were cultured. The supernatants of the two groups were used to culture ATDC5 chondrocyte progenitor cells, and insulin, transferrin and selenium (ITS) were added to induce cartilage differentiation. Compared with control, the expressions of sox9, COL2A1 and Acan in TSC1KO group were significantly decreased, while the expressions of Runx2 and COL10A1 were significantly increased, suggesting that the expression of ATDC5 chondrocyte progenitor cells was significantly increased. Phagocytes promote cartilage hypertrophy, maturation and mineralization.

B. Toluidine blue staining showed that the mineralization of ATDC5 cells added TSC1KO conditioned medium increased significantly.

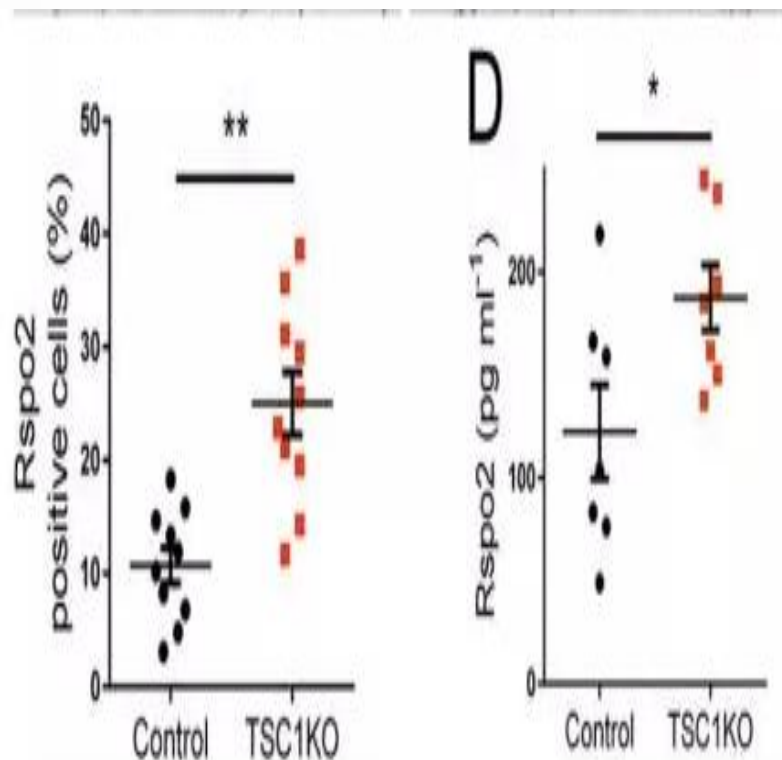
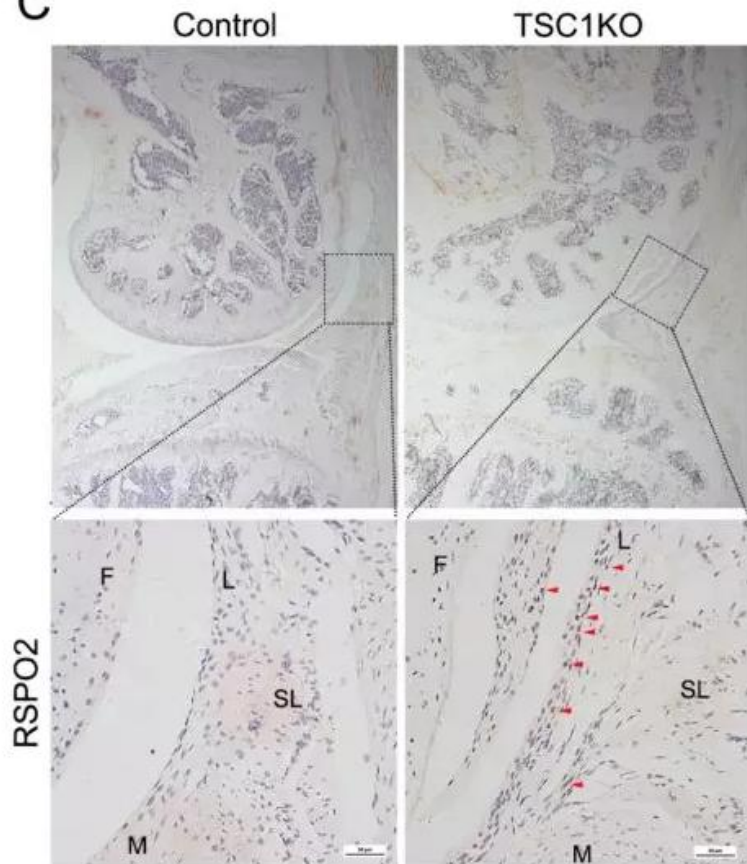
C. The expression of cytokine Rspo2 in TSC1KO macrophages was 33.6 times higher than that in control group, which was confirmed by QPCR and Elisa.

Rspo2 has been shown to have synergistic effects with wnt, promoting beta-catenin activation and promoting cartilage and osteoblast differentiation.



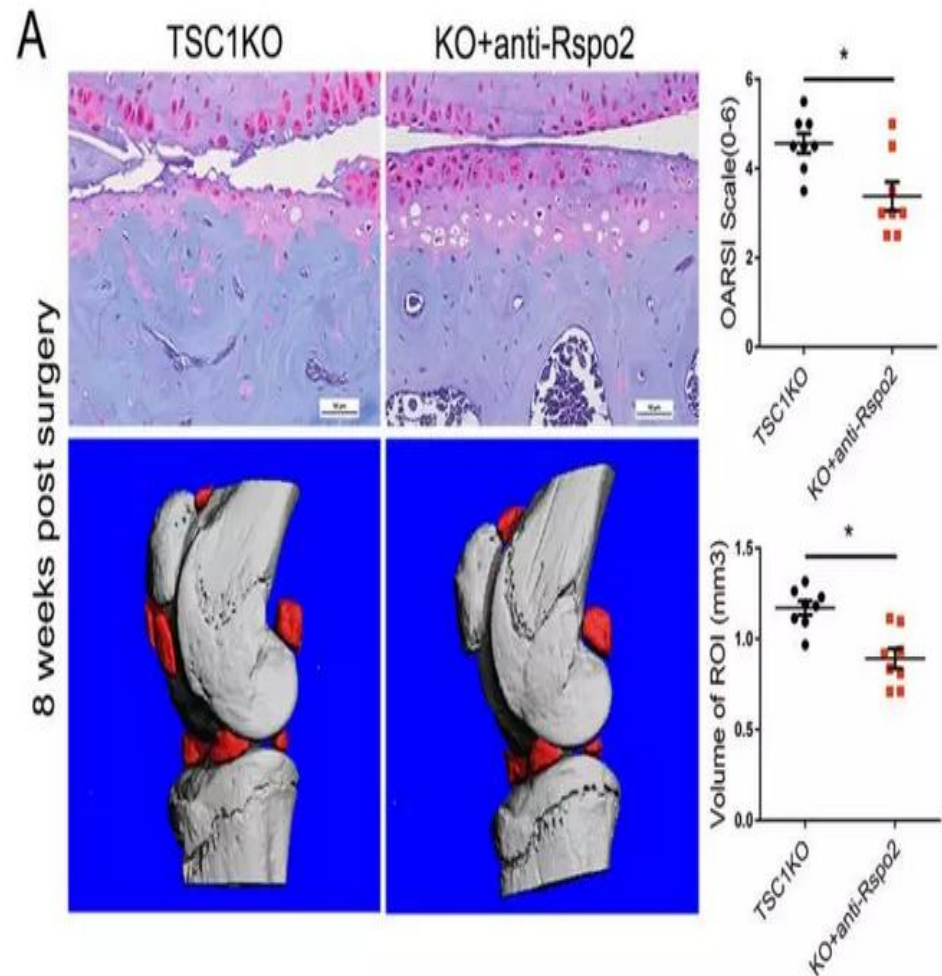
C. The expression of Rspo2 in synovium of 4-week CIOA mice was confirmed by immunohistochemistry. The expression of Rspo2 in synovium of TSC1KO mice was up-regulated. The expression of Rspo2 in serum of 4W control and TSC1KO mice was also detected. The level of Rspo2 in serum was significantly increased.

C



## 6. M1-polarised macrophages produce *rspo2* to promote OA development in mice

- ❖ A. By injecting *Rspo2* antibody into the articular cavity of TSC1KO mice and TSC1KO mice to neutralize *Rspo2*, the authors constructed an 8-week KO and salvage model. It was found that the articular cartilage of TSC1KO mice was worn obviously, the osteophyte volume was larger, and the OA score was higher. The salvage group had relatively complete articular cartilage and smaller osteophyte volume, which indicated that *Rspo2*, as a secretory factor of M1 macrophage, could promote OA. Progress, blocking *Rspo2* can only partially alleviate OA progress.

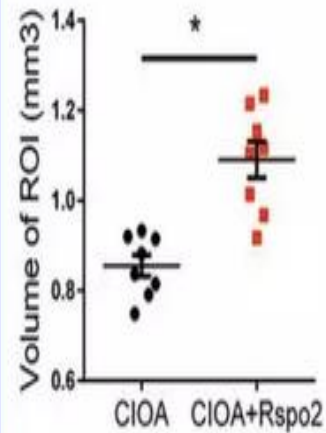
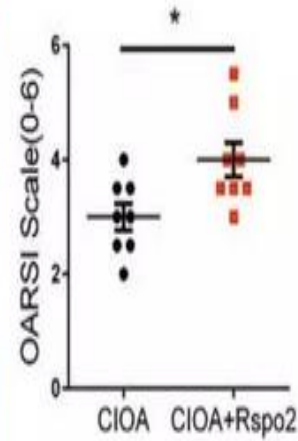
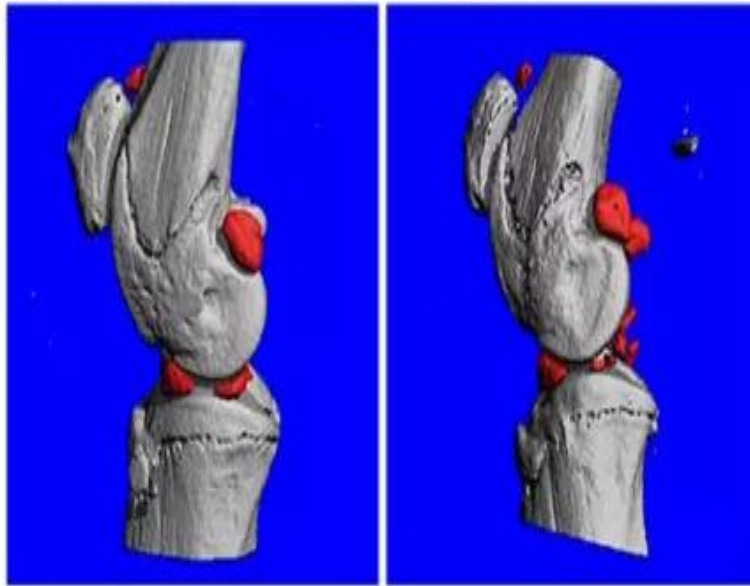
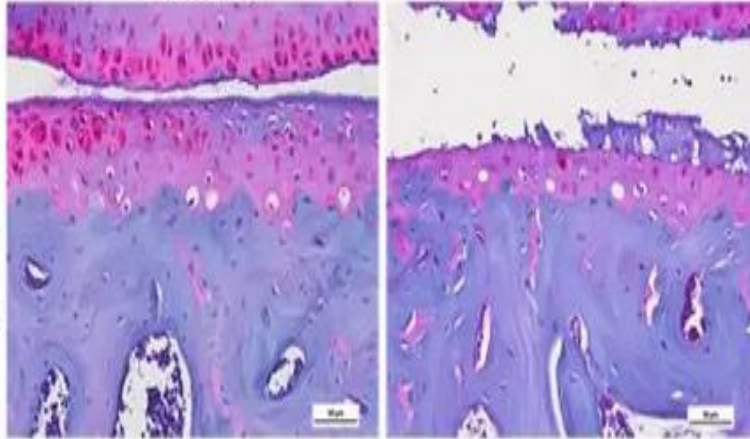


**B**

8 weeks post surgery

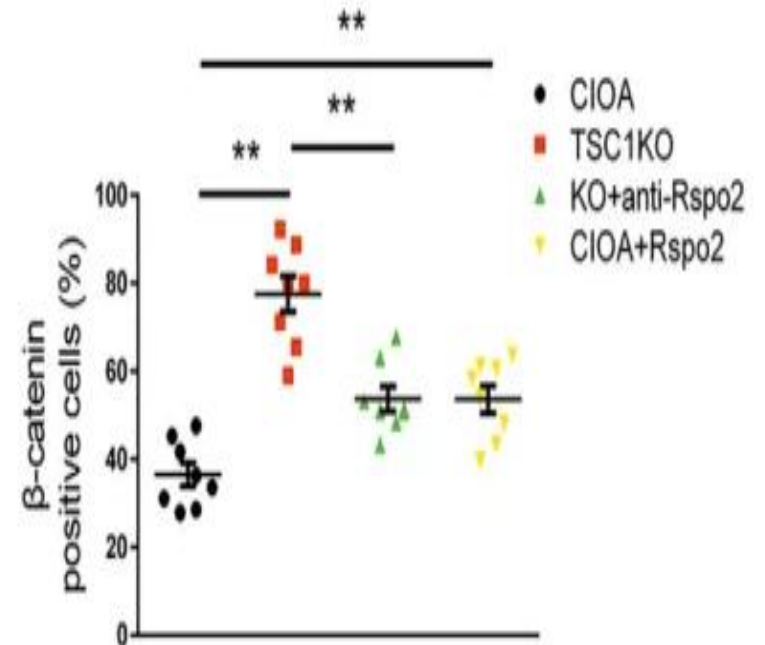
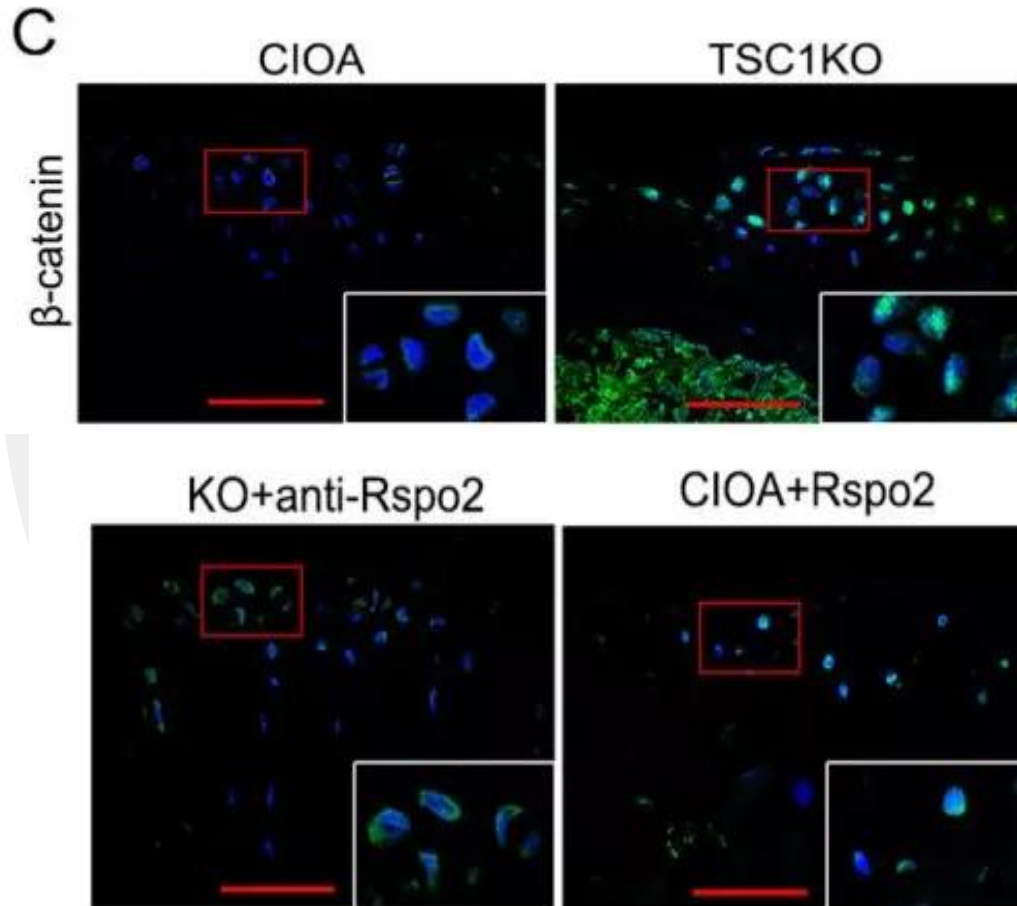
CIOA

CIOA+Rspo2



In addition, CIOA and CIOA + rhrhpo2 (intra-articular injection) mice models were constructed on normal mice. It was found that after 8 weeks of intra-articular injection of rhpo2, the degeneration of articular cartilage was obvious, the OA score was significantly increased, and the volume of osteophyte was also larger, which further demonstrated that Rspo2 could accelerate the progress of OA.addition

C.Rspo2 has been reported to have synergistic effect with wnt. The expression of beta-catenin in articular cartilage of control, TSC1KO, salvage and synergistic groups was detected by immunofluorescence. It was found that TSC1KO and intra-articular injection of Rspo2 could increase beta-catenin expression, while neutralizing Rspo2 in TSC1KO could weaken beta-catenin expression. This shows that M1 macrophage polarization promotes the progress of OA, partly through Rspo2.



# Discussion

- ❖ 1. we found that macrophages accumulated in synovium during the development of OA, especially M1 polarized macrophages. M2 cells accumulated significantly in the early stage and decreased in the late stage, suggesting that both of them may play a role in the occurrence of OA. It has been reported that specific activation of mTORC1 protein in mouse myeloid cells can enhance M1 polarization of macrophages and induce spontaneous M1-related inflammation.
- ❖ 2. Previous experiments have shown that macrophage aggregation exists in the synovium during the development of OA, mainly M1 macrophages, and mTORC1 plays an important role in macrophage polarization.
- ❖ 3. We can find that activated M1 macrophages activated by mTORC1 can promote chondrocyte maturation and mineralization by secreting cytokines, which also suggests that they play an important role in the cartilage degeneration of OA.



# Conclusions

- ❖ By immunofluorescence, immunohistochemistry, QPCR, micro-ct, Elisa, safranin and green fixation techniques, the authors have proved that the activation of mTORC1 in the progress of OA can increase the polarization of M1 macrophages and decrease the polarization of M2 macrophages. M1 macrophages can accelerate the progress of OA, while M2 macrophages can inhibit the progress of OA. M1 macrophages act by secreting IL-1, IL-6, TNF-alpha, or by secreting Rspo2 to activate the Wnt pathway to cause OA.

Thank you for your attention!

