Supplementary Figures 1-7

Ocular instillation of conditioned medium from mesenchymal stem cells is effective for dry eye syndrome by improving corneal barrier function

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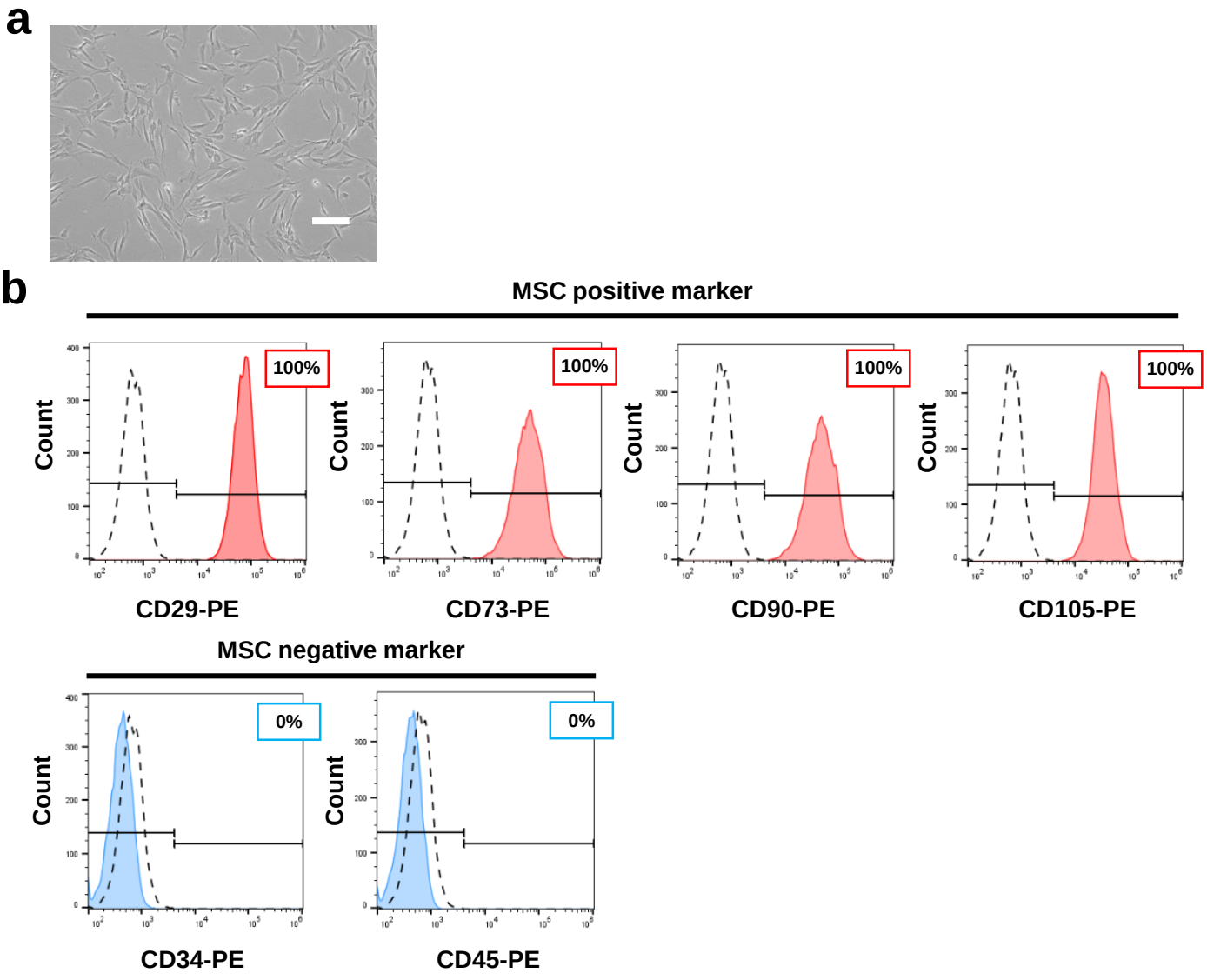
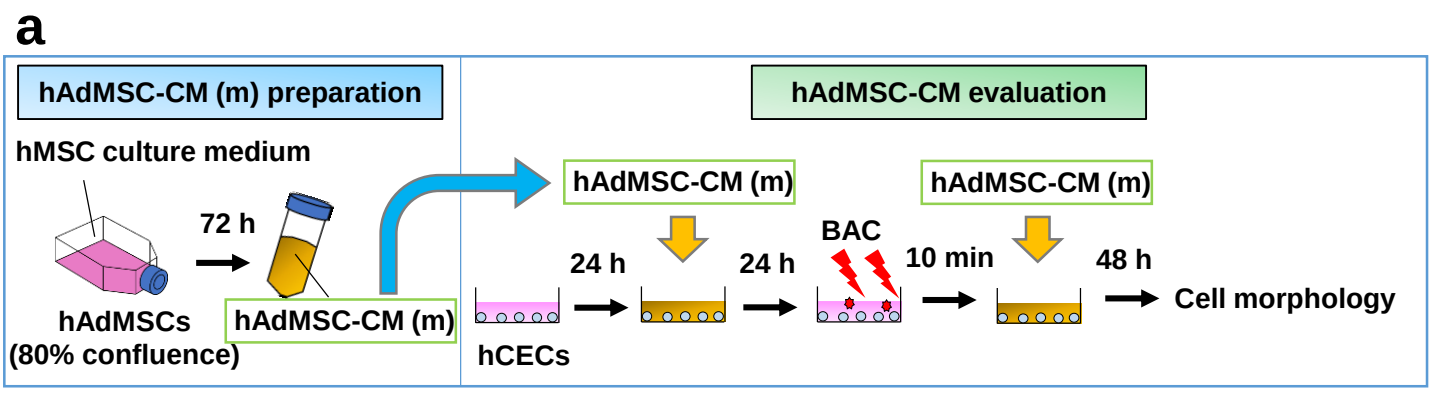
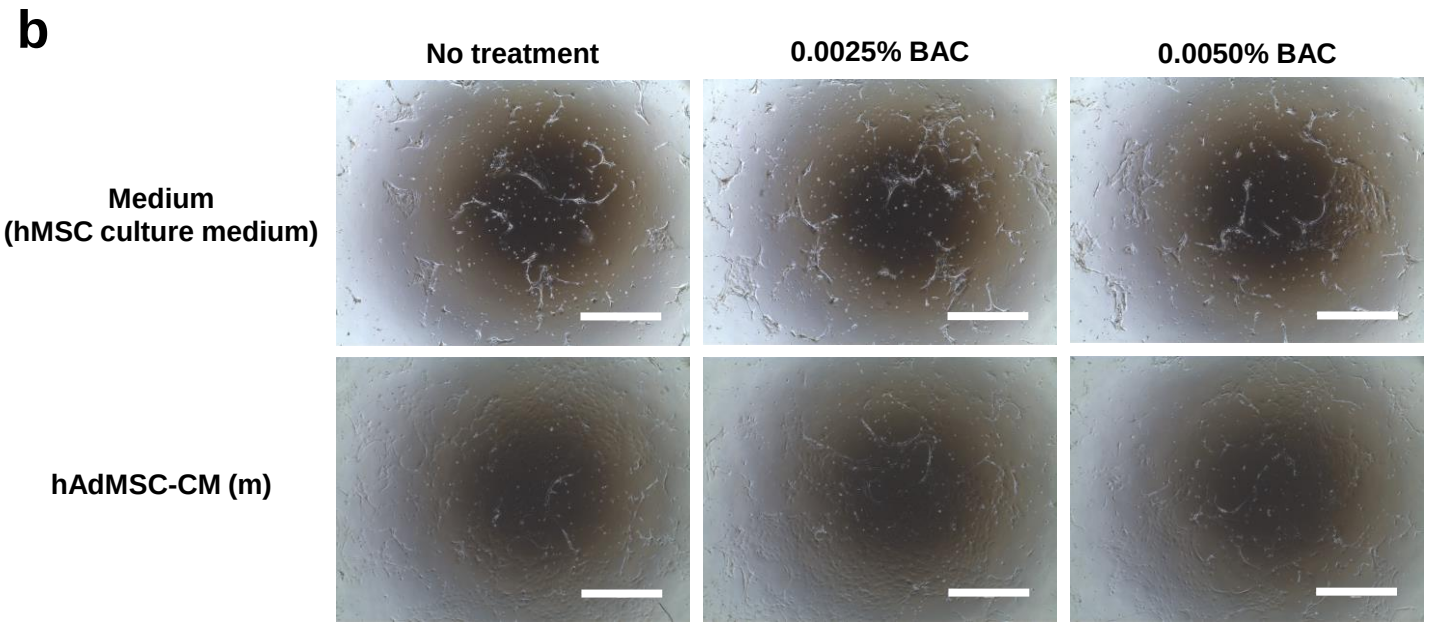
Supplementary Figure S1



Supplementary Figure S1. Characteristics of hAdMSCs. (a) Phase images of hAdMSCs. Scale bar, 200 μm. *n* = 12 biological replicates. (b) Expression of hMSC positive and negative markers. *n* = 3 biological replicates. hAdMSCs; human adipose-derived mesenchymal stem cells.

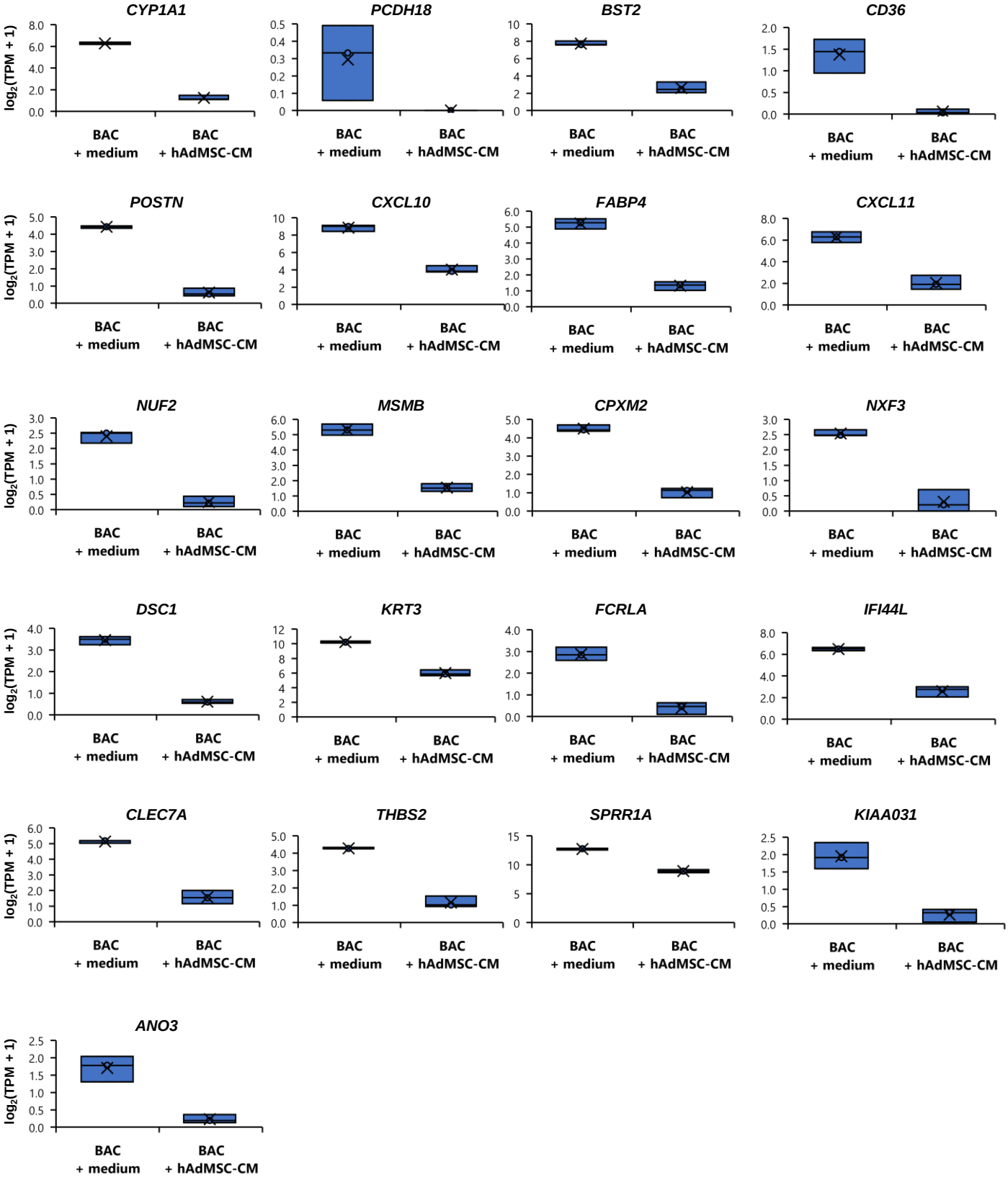
Supplementary Figure S2





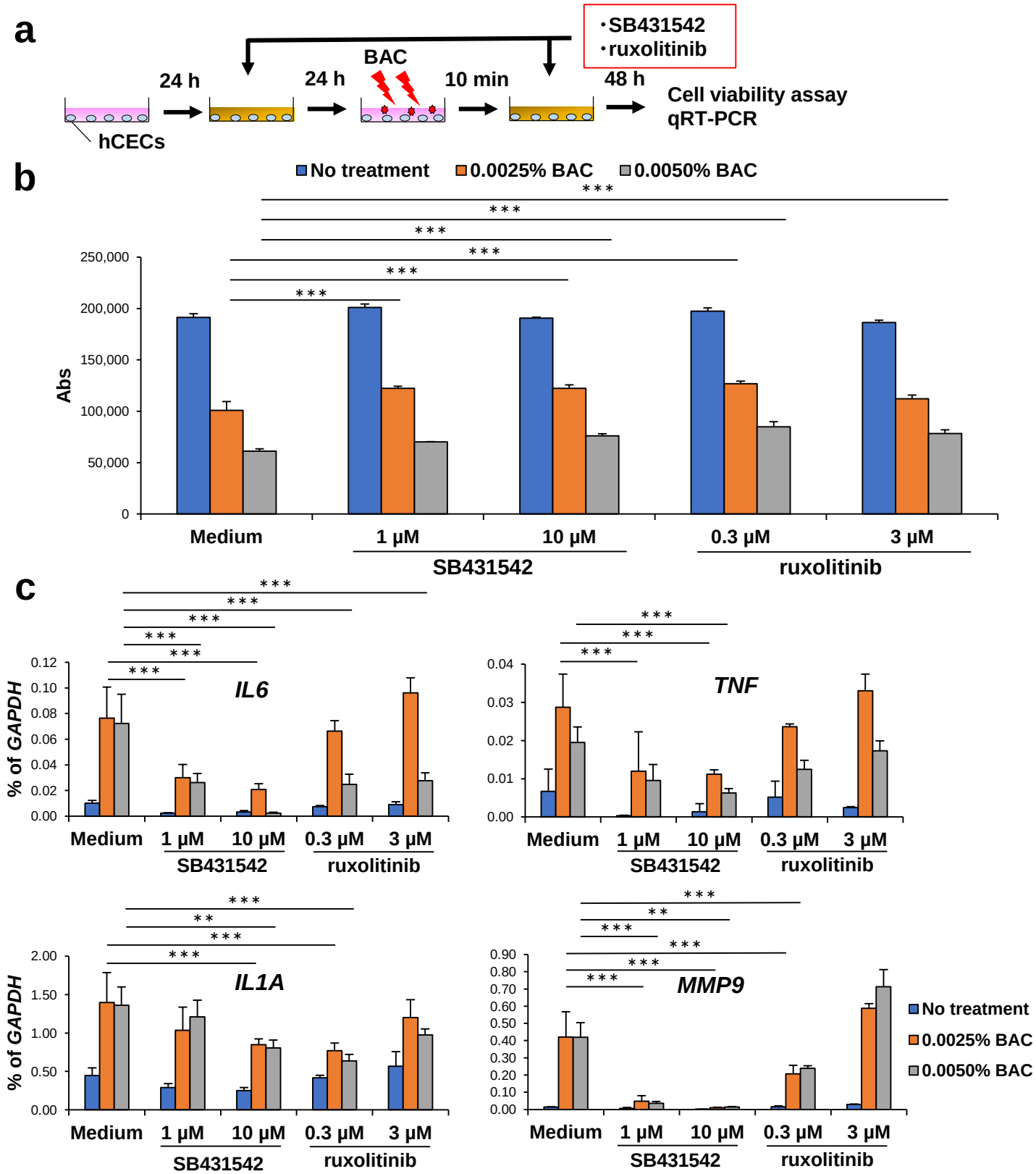
Supplementary Figure S2. Morphology of hCECs could not be maintained by hAdMSC-CM derived from hMSC culture medium. (a) Schematic representation of hAdMSC-CM preparation using hMSC culture medium (hAdMSC-CM (m))and the evaluation of hAdMSC-CM (m) on hCECs. (b) Phase images of hCECs 2 days after treatment with or without BAC. Scale bar, 1000 μm. *n* = 9 biological replicates. hAdMSC-CM; conditioned medium of human adipose-derived mesenchymal stem cells, BAC; benzalkonium chloride, hCECs; human corneal epithelial cells.

Supplementary Figure S3



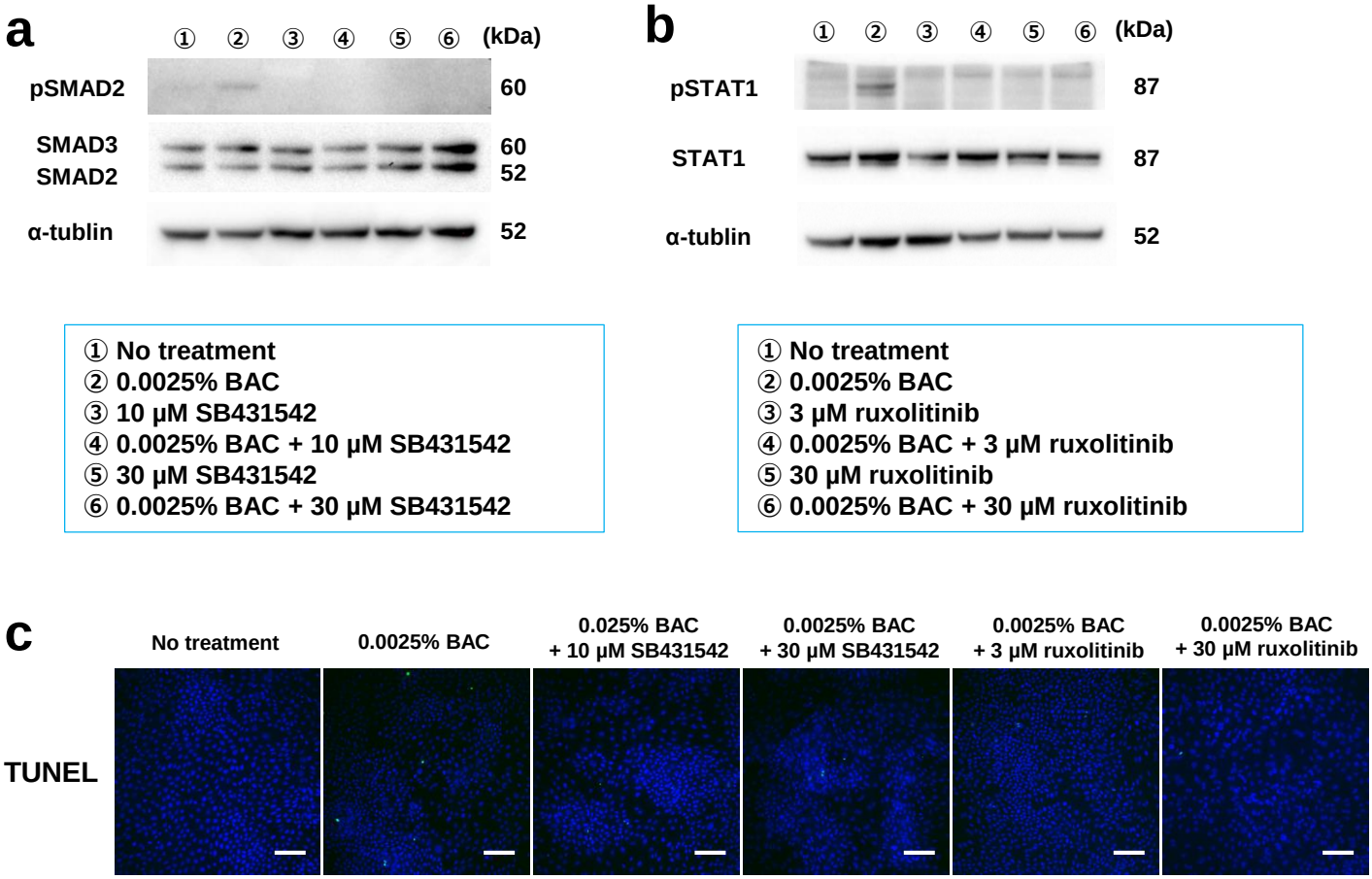
Supplementary Figure S3. Expressions levels of TGFβ or JAK-STAT signalling pathways-related genes. Box plots of TGFβ or JAK-STAT signalling pathways-related genes in the top 30 genes with significant fold reductions in expression levels due to hAdMSC-CM. *n* = 3 biological replicates.

Supplementary Figure S4



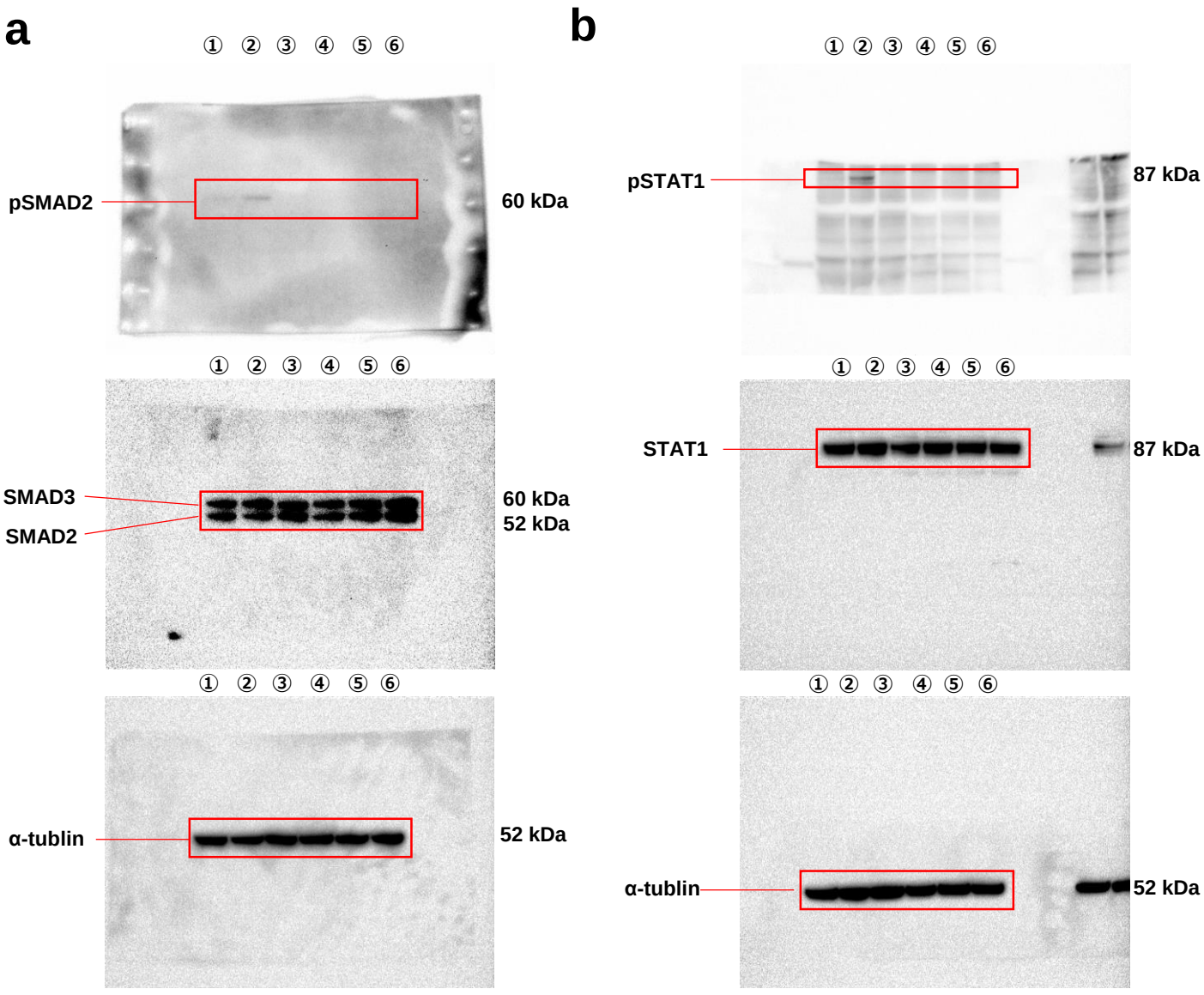
Supplementary Figure S4. BAC-induced cytotoxicity and inflammation of hCECs are suppressed by inhibition of TGFβ and JAK-STAT signalling pathways. (a) Schematic representation of the evaluation of SB431542 and ruxolitinib on BAC-induced cytotoxicity and inflammation of hCECs. (b) Cell viability assay of hCECs 2 days after treatment with or without BAC. The results are presented as the mean ± SD; $n = 4$ biological replicates. ***$p < 0.001$. (c) Expression levels of inflammation-related genes in hCECs 2 days after treatment with or without BAC. The results are presented as the mean ± SD; $n = 4$ biological replicates. **$p < 0.01$, and ***$p < 0.001$.

Supplementary Figure S5



Supplementary Figure S5. Number of apoptotic cells was not increased by inhibition of TGFβ and JAK-STAT signalling pathways. (a) Expression of TGFβ signalling-related proteins. *n* = 3 biological replicates. (b) Expression of JAK-STAT signalling-related proteins. *n* = 3 biological replicates. (c) Fluorescein staining images of TUNEL in hCECs 1 day after treatment with or without BAC. Scale bar, 100 μm. *n* = 4 biological replicates. TUNEL; Terminal deoxynucleotidyl transferase dUTP nick end labelling.

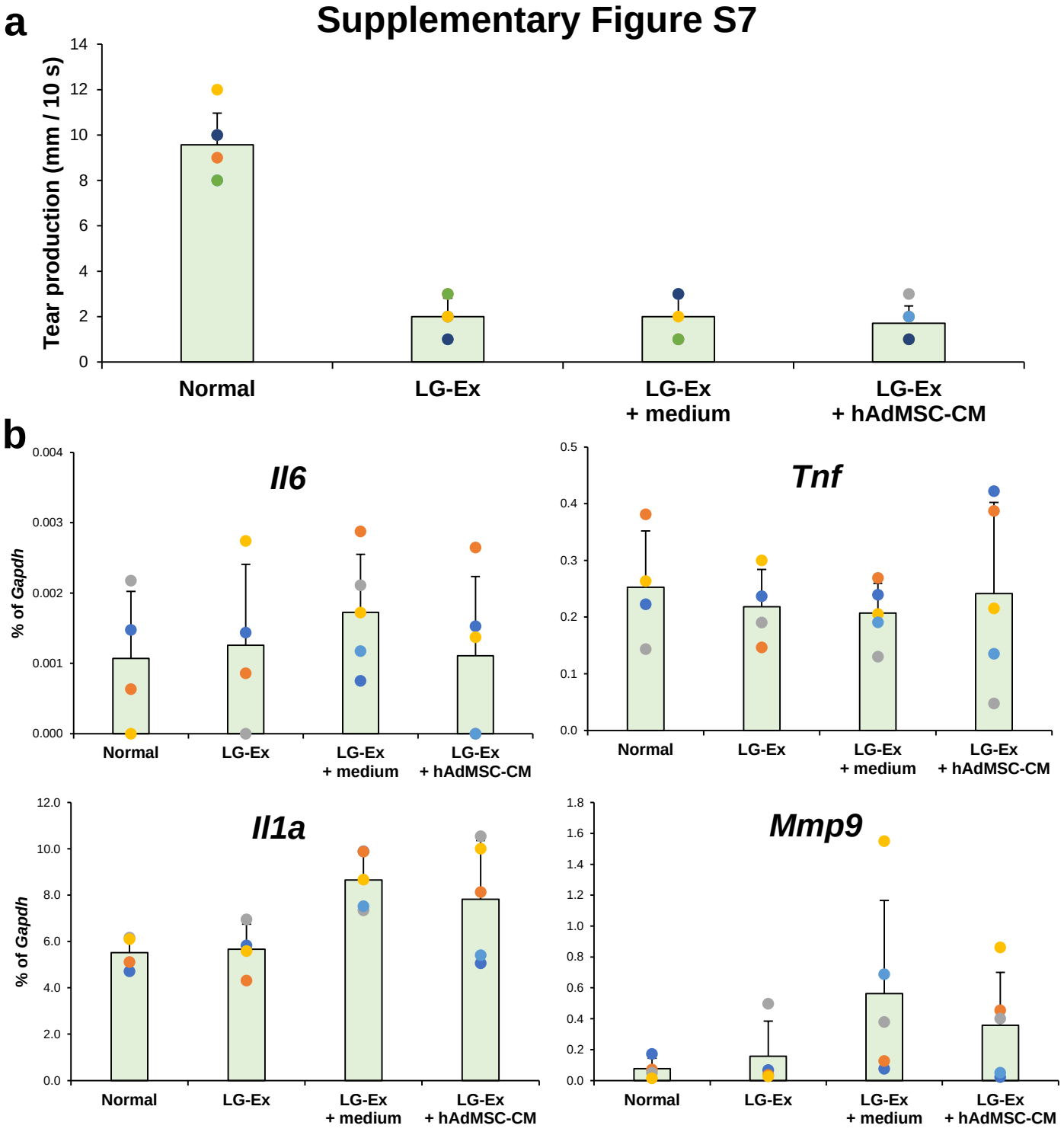
Supplementary Figure S6



① No treatment
② 0.0025% BAC
③ 10 μM SB431542
④ 0.0025% BAC + 10 μM SB431542
⑤ 30 μM SB431542
⑥ 0.0025% BAC + 30 μM SB431542

① No treatment
② 0.0025% BAC
③ 3 μM ruxolitinib
④ 0.0025% BAC + 3 μM ruxolitinib
⑤ 30 μM ruxolitinib
⑥ 0.0025% BAC + 30 μM ruxolitinib

Supplementary Figure S6. Uncropped western blots used for the preparation of supplementary Figure S5. (a) Expression of TGFβ signalling-related proteins. *n* = 3 biological replicates. (b) Expression of JAK-STAT signalling-related proteins. *n* = 3 biological replicates.



Supplementary Figure S7. Examination of tear production and corneal inflammation in LG-Ex rats.
(a) Measurement of tear production in SD (Normal), LG-Ex, LG-Ex with ocular instillation of medium (LG-Ex + medium), and LG-Ex with ocular instillation of hAdMSC-CM (LG-Ex + hAdMSC-CM) rats 1 week after ocular instillation. The results are presented as the mean ± SD; *n* = 7 biological replicates. (b) Expression levels of inflammation related-genes in the cornea of Normal, LG-Ex, LG-Ex + medium, and LG-Ex + hAdMSC-CM rats. The results are presented as the mean ± SD; *n* = 4 (Normal and LG-Ex) and n = 5 (LG-Ex + medium and LG-Ex + hAdMSC-CM) biological replicates.