Journal club

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Potential Application of Intestinal Organoids in Intestinal Diseases

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Stem Cell Reviews and Reports

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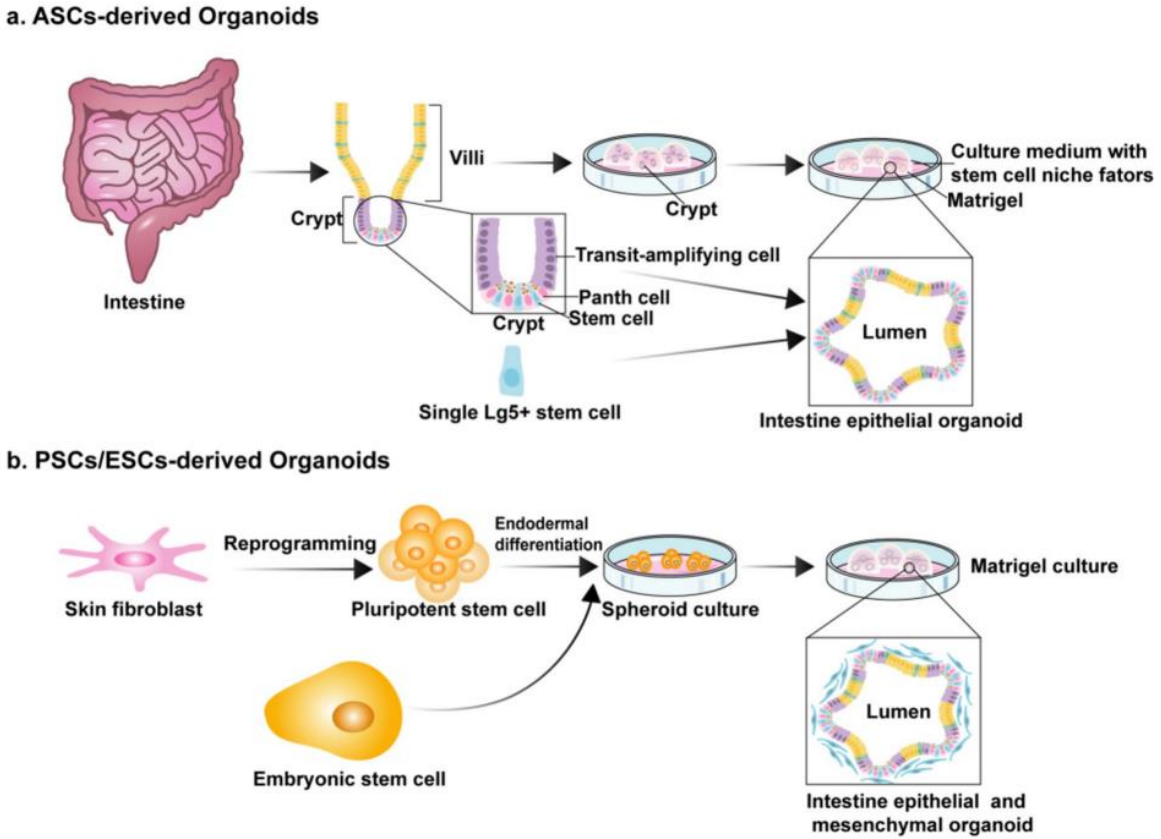
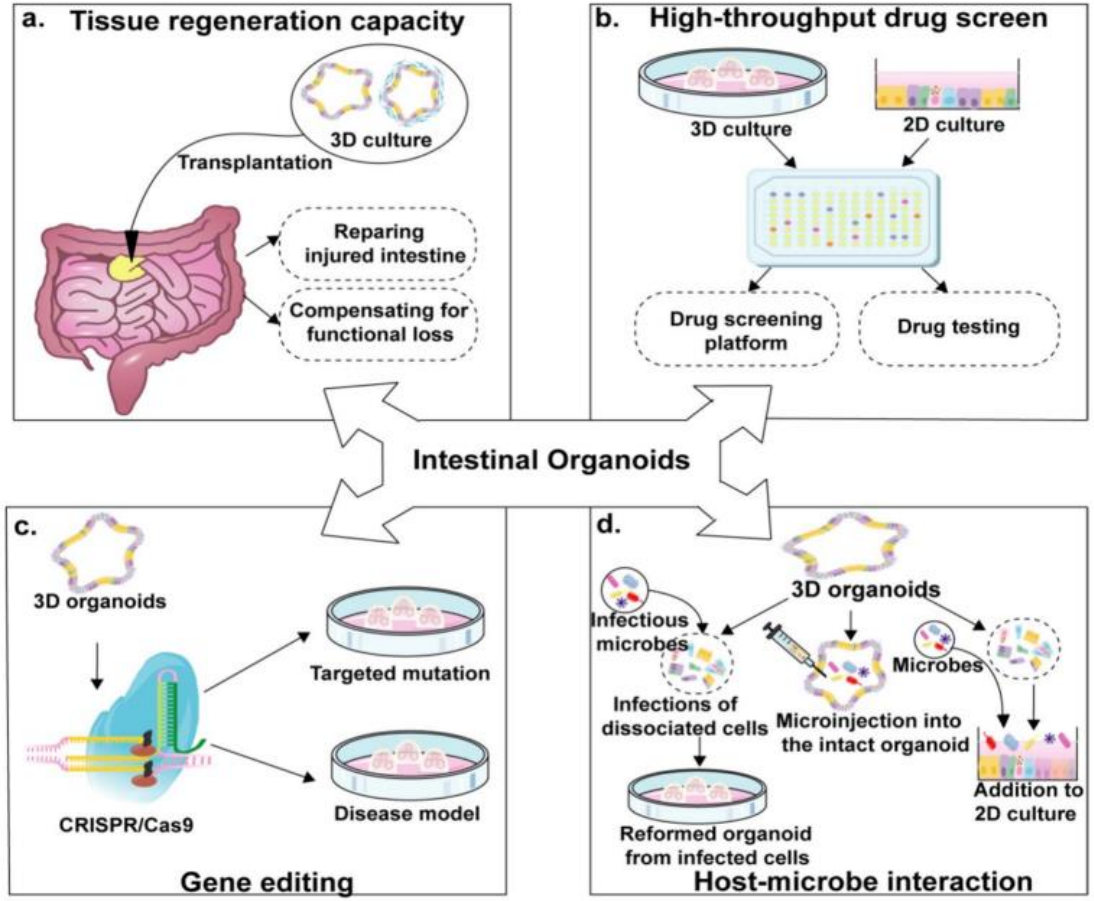
Hyewon Lee

Intestinal organoid





Intestinal organoids are derived from self-renewal and self-organization intestinal stem cells (ISCs), which can replicate the genetic characteristics, functions, and structures of the original tissues.

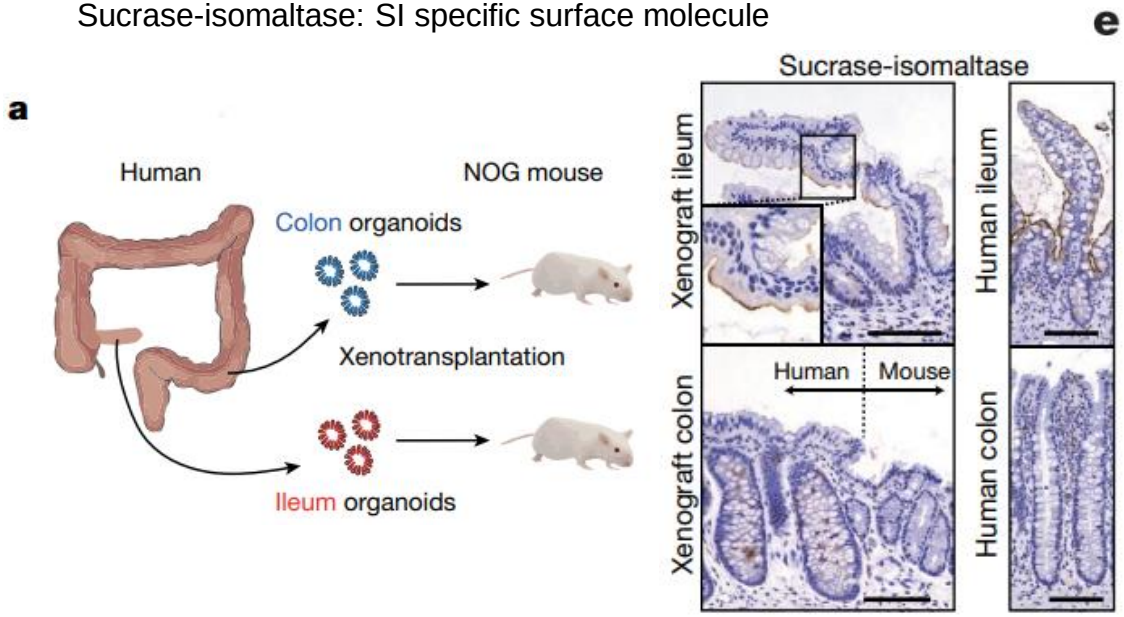
Potential Application of Intestinal Organoids in Intestinal Diseases

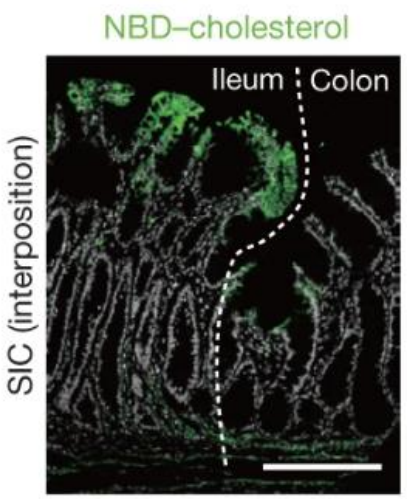
1. Application of intestinal organoids in **regenerative medicine**

Xenotransplanted human colon & ileum organoids into native colonic epithelium (mouse, rat)

NOG: NOD/Shi-scid IL2rgamma(null) (NOG) mice
Sucrase-isomaltase: SI specific surface molecule

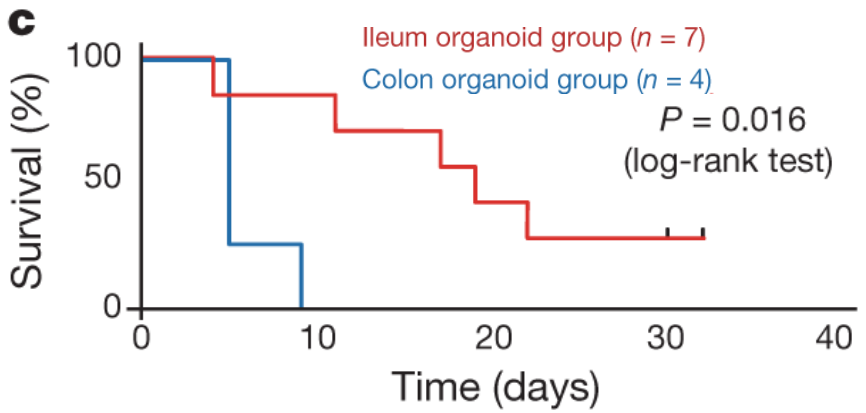
SBS: short bowel syndrome





Therapeutic effect of SIC in rat SBS models

► Cholesterol absorption↑, Survival rate ↑



Generation of small intestinalized colon

① Epithelium removal (EDTA treatment)

② Organoid infusion (Syringe filled with organoid suspension)

③ Cell retension

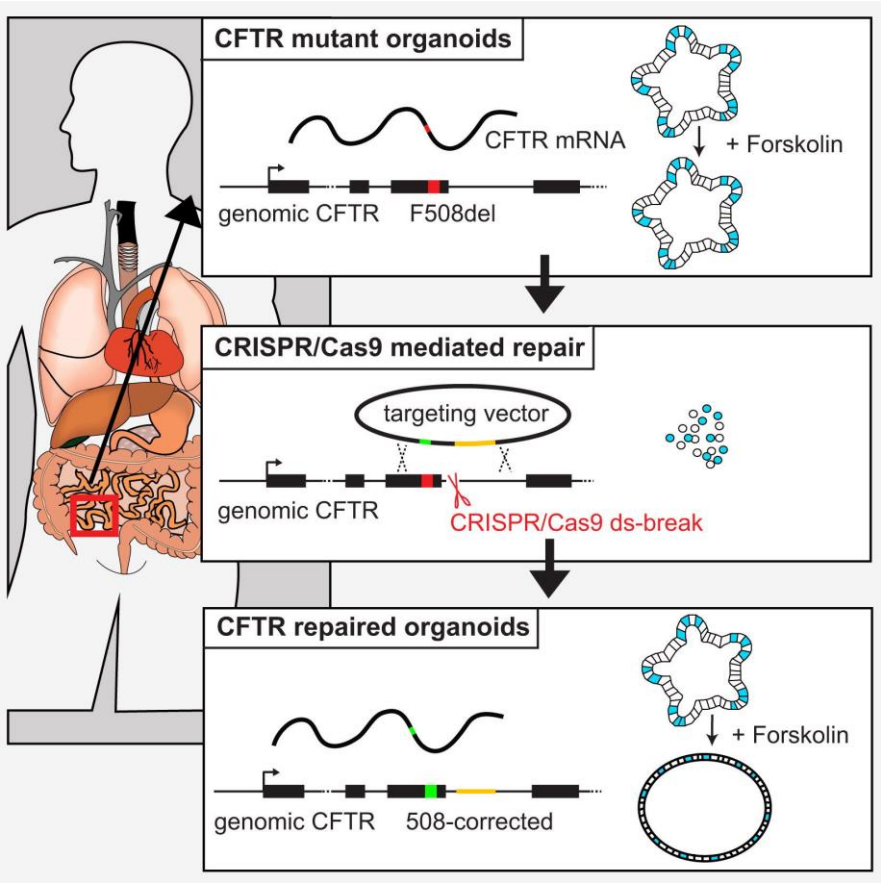
- Colonic epithelium를 human colon- or ileum-derived organoid로 대체
- ileum-derived organoid: functional한 small intestinalized colon (SIC) 형성
- SIC는 SBS의 Rat model에서 장 기능 개선
- ileum organoid 대신 colon organoid 이식하면 사망률↑

Sugimoto, S., Kobayashi, E., Fujii, M., et al. (2021). An organoid-based organ-repurposing approach to treat short bowel syndrome. Nature, 592, 99–104.

Potential Application of Intestinal Organoids in Intestinal Diseases

2. Application of intestinal organoids in **genetic engineering**

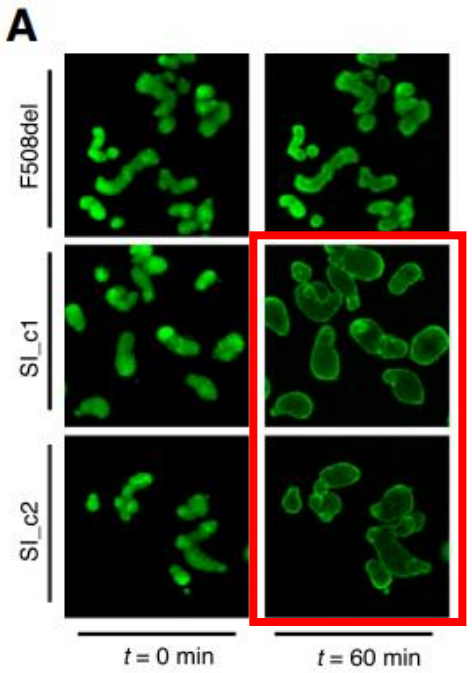
CRISPR/Cas9-Mediated Genome Editing in Adult Stem Cells



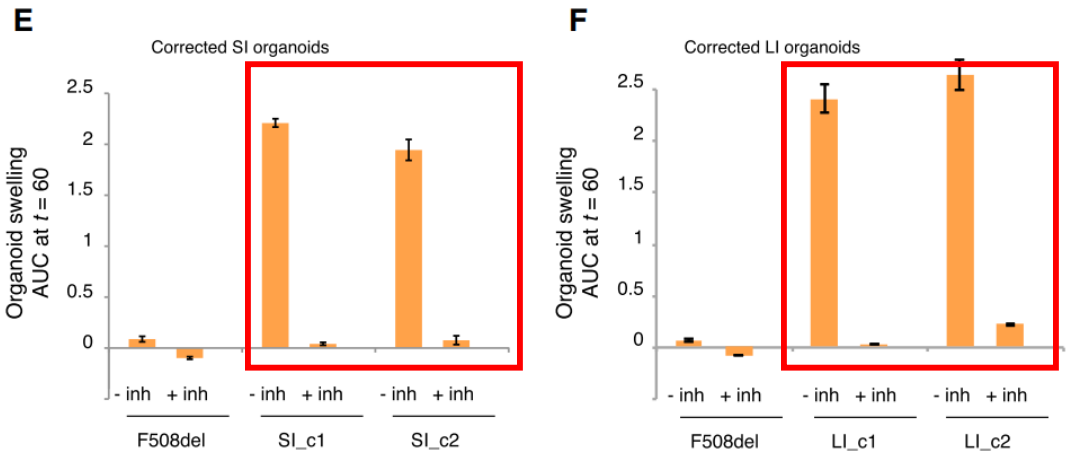
CFTR: cystic fibrosis transmembrane conductor receptor

F508del: CFTR 돌연변이; exon 11의 508번에서 Phe 결손

Forskolin: cAMP증가→CFTR 활성화→organoid 부풀게 됨



Forskolin-induced organoid swelling assay



► swelling↑

SI: small intestine / LI: large intestine

inh; chemical CFTR inhibitor

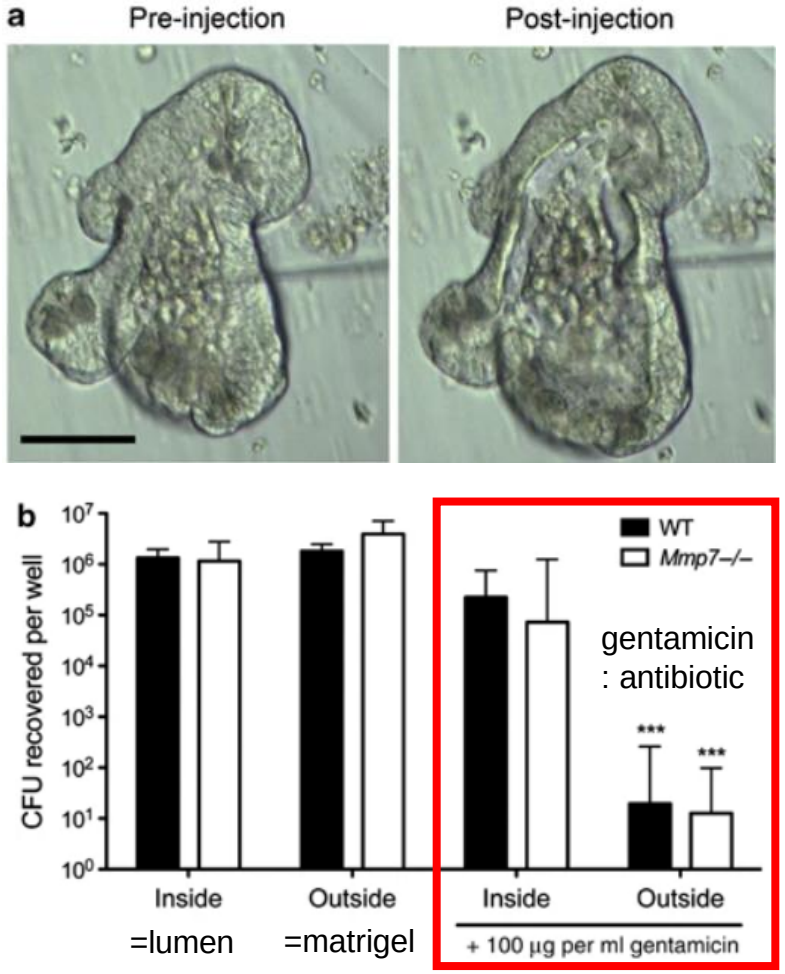
- Cystic fibrosis patients의 CFTR mutant organoid: cAMP-induced swelling X
- CFTR correction by CRISPR/Cas9 system
- CFTR 기능 회복: Forskolin treatment → organoid swelling ↑

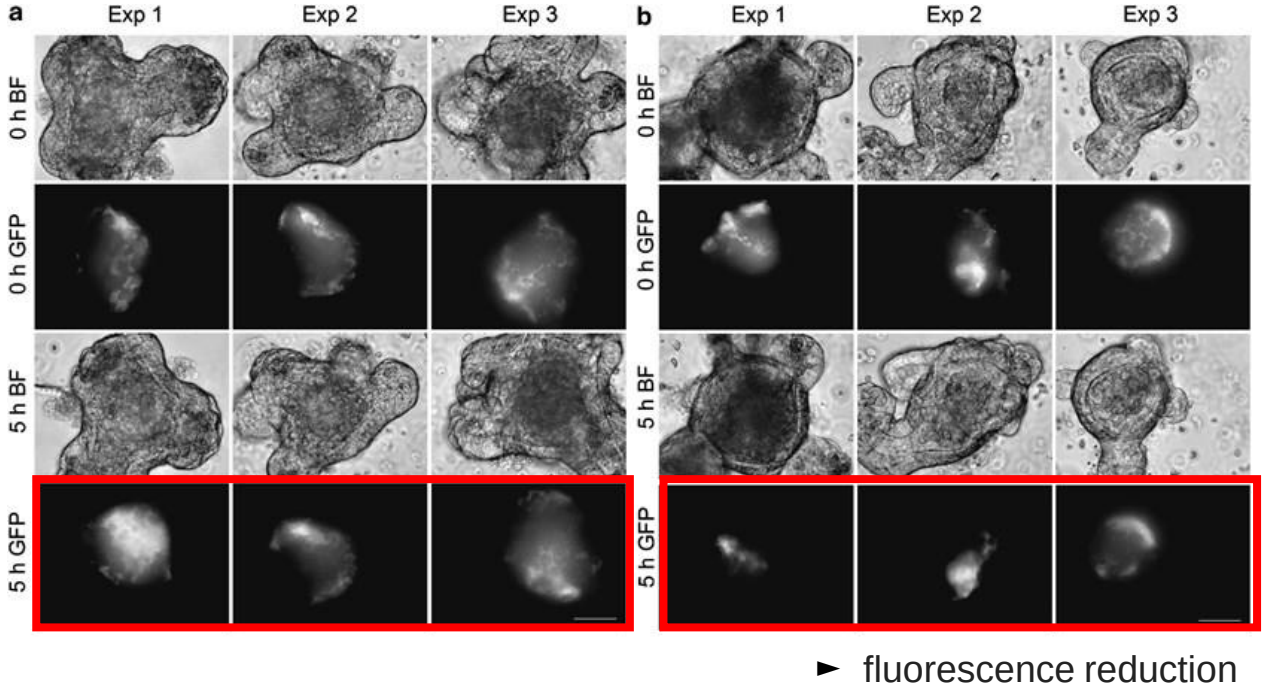
Schwank, G., Koo, B. K., Sasselli, V., et al. (2013). Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell, 13, 653–658.

Potential Application of Intestinal Organoids in Intestinal Diseases

3. Application of intestinal organoids in **host-microbial interactions**

Microinjection of organoids to mimic enteric infection





► fluorescence reduction

- WT & *Mmp7*−/− mice SI에 *Salmonella enterica* serovar Typhimurium microinjection
- MMP7 did not alter the integrity of the organoid lumen
- WT mice produced mature α-defensins (*Mmp7*−/− mice did not)
- WT organoid가 α-defensin 있는 sealed lumen 형성 → Bacterial growth↓

MMP7: protease (mouse pro-α-defensins→mature form)
α-defensin: antimicrobial peptide → *S. enterica* Typhimurium 성장 제한

Wilson, S. S., Tocchi, A., Holly, M. K., et al. (2015). A small intestinal organoid model of non-invasive enteric pathogen-epithelial cell interactions. Mucosal Immunology, 8, 352–361.

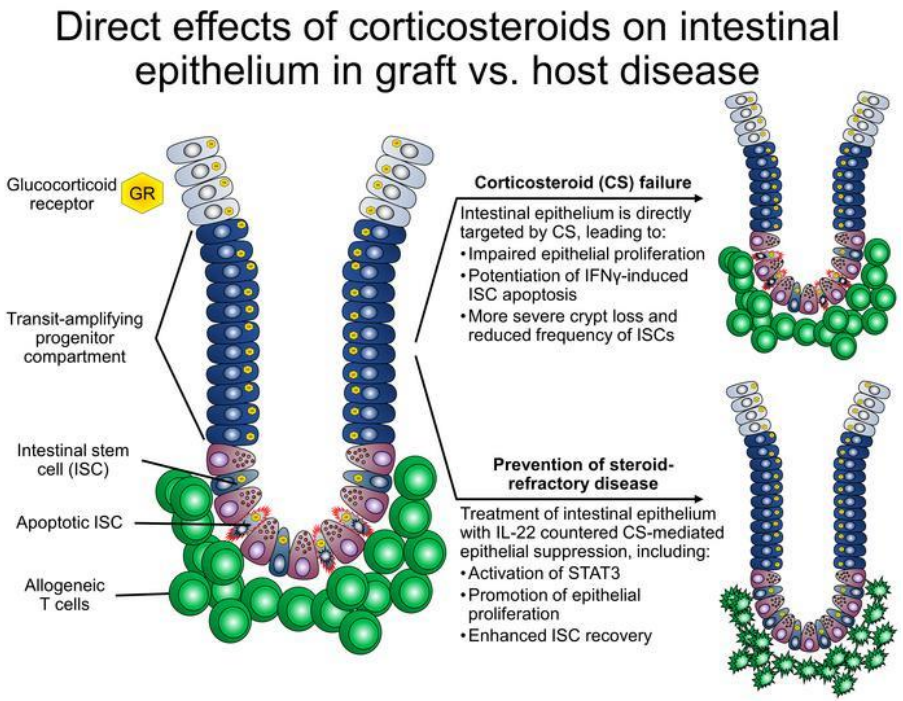
Research

JCI The Journal of Clinical Investigation

Corticosteroids impair epithelial regeneration in immune-mediated intestinal damage

Viktor Arnhold, … , Caroline A. Lindemans, Alan M. Hanash

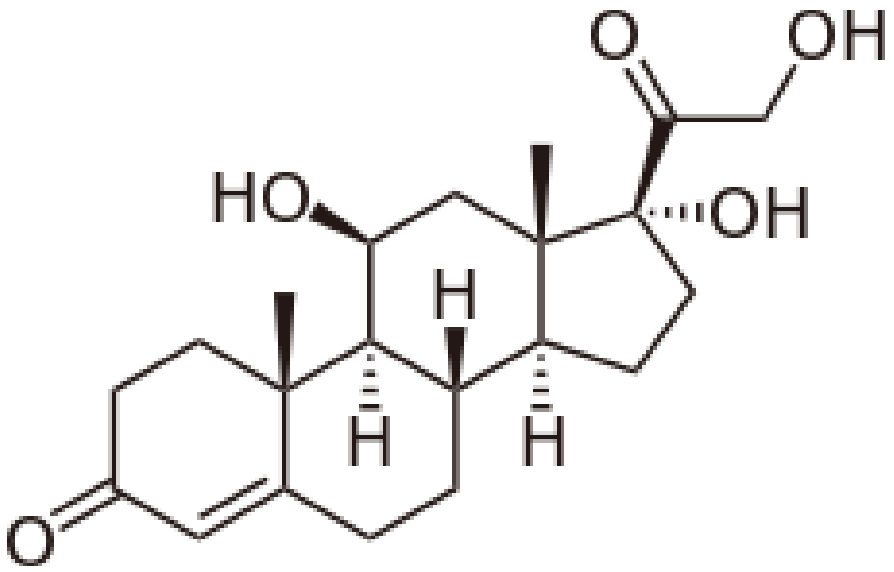
J Clin Invest. 2024. https://doi.org/10.1172/JCI155880.



IF = 15.9

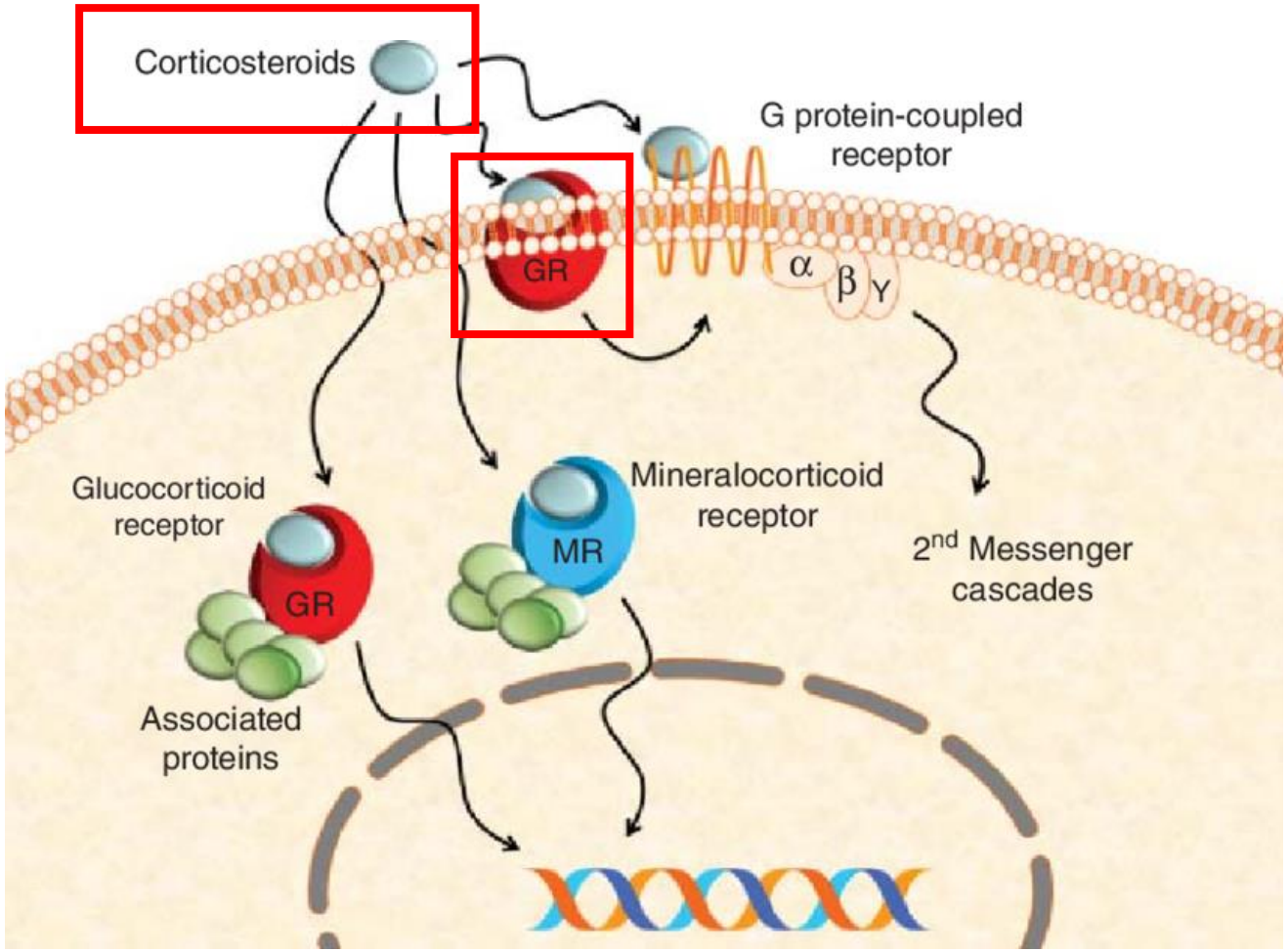
2024. 02. 29.
Hyewon Lee

Corticosteroid (CS)



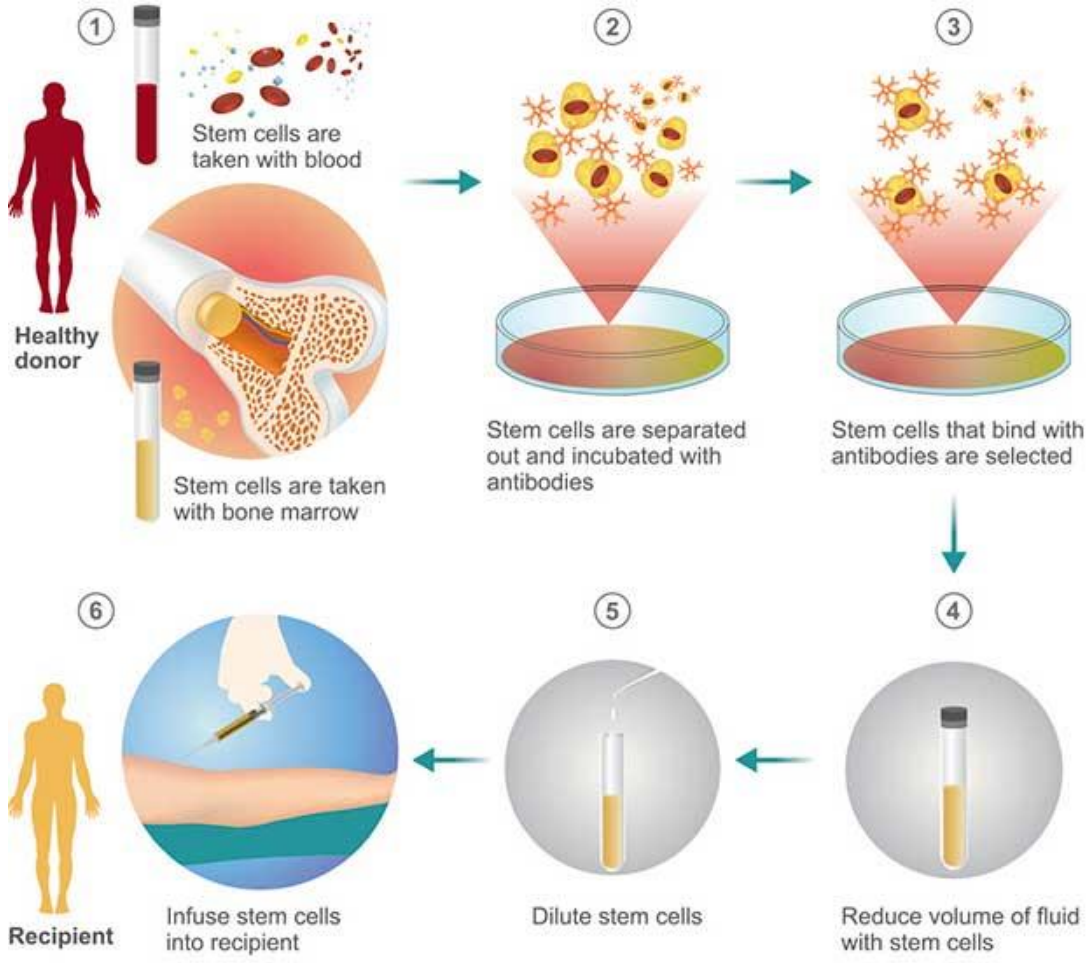
- Anti-inflammatory medicines used to treat a range of conditions
- Target: Glucocorticoid receptor
- prednisolone, methylprednisolone, dex, budesonide ...

Glucocorticoid receptor (GR)



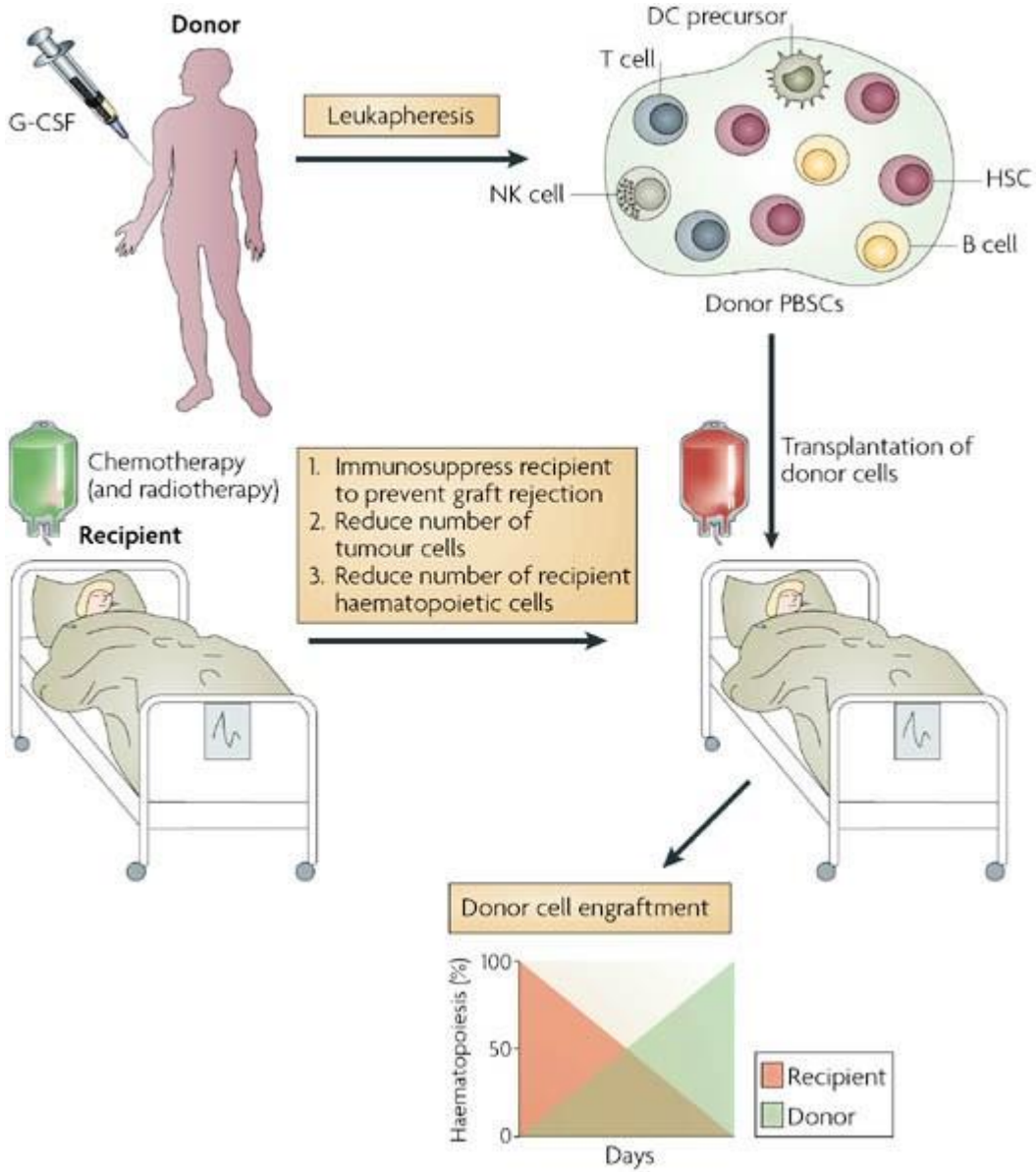
- **Glucocorticoid receptor** is widely expressed, including within the intestines
- GR protein is encoded by ***NR3C1*** gene which is located on chromosome 5

Allogeneic hematopoietic/bone marrow transplantation (allo-BMT)



Acute GVHD, which occurs in 30-70% of patients undergoing allo-BMT,
is an immune-mediated complication arising from donor T-cell-mediated responses against recipient tissues

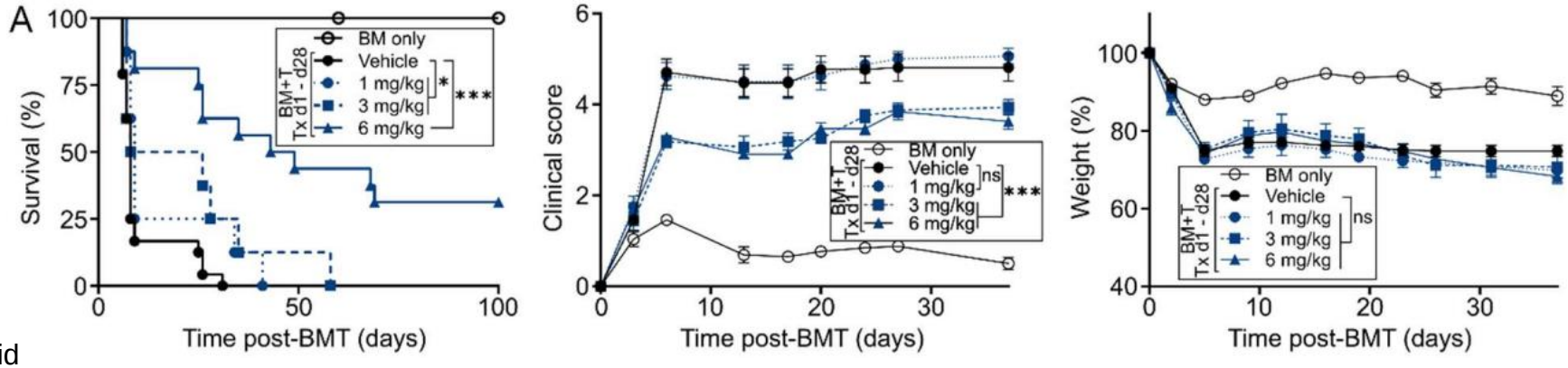
Graft-versus-Host Disease (GvHD)



Corticosteroids reduce epithelial proliferation in vivo

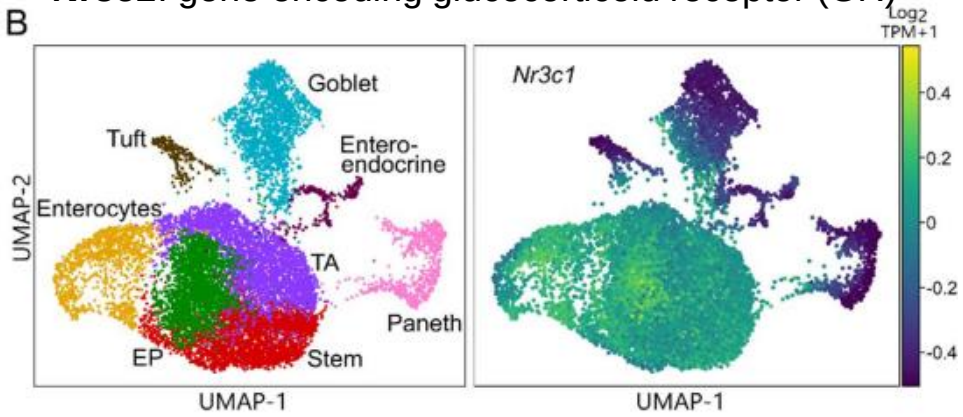
Murine GVHD models
- B6-into-BALB/c BMT recipients
- +/- prednisolone
- 1, 3, 6 mg/kg i.p. daily
  from day 1 to 28 post-BMT

BMT: Bone marrow transplantation
Prednisolone: 임상에서 사용되는 corticosteroid



scRNA-seq
- SI epithelial cells from WT B6 mice

Nr3c1: gene encoding glucocorticoid receptor (GR)



► stem cell, TA cell, enterocyte lineage에서 발현↑

Immunohistochemical (IHC) staining
for GR in the SI of WT mice

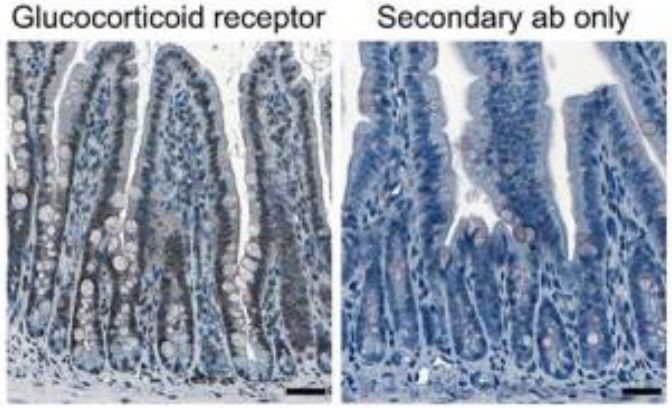
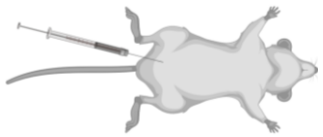


Fig. 1A-C

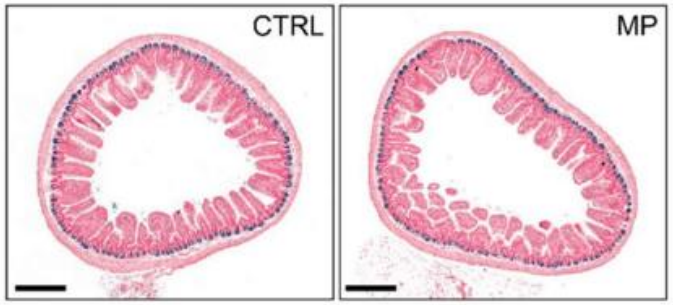
Corticosteroids reduce epithelial proliferation in vivo



WT B6 mice
- treated w/ MP
- 2 mg/kg i.p. daily for 7 days

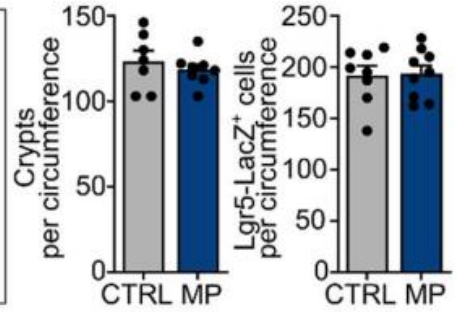
MP(Methylprednisolone)
: 임상에서 사용되는 corticosteroid

D



250 μm

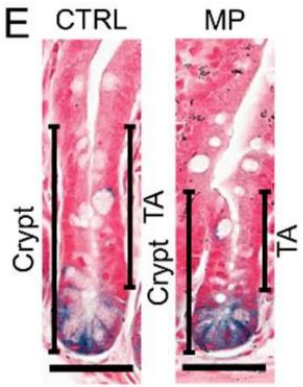
Lgr5: intestinal stem cell marker



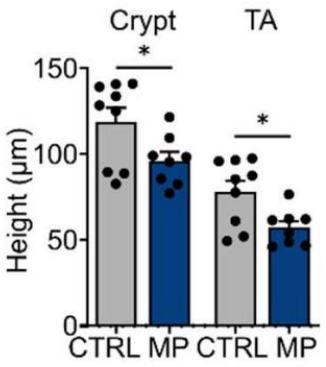
► intestinal toxicity X

TA: transit amplifying cell

E

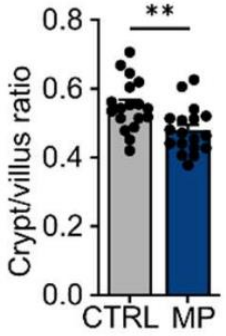


50 μm

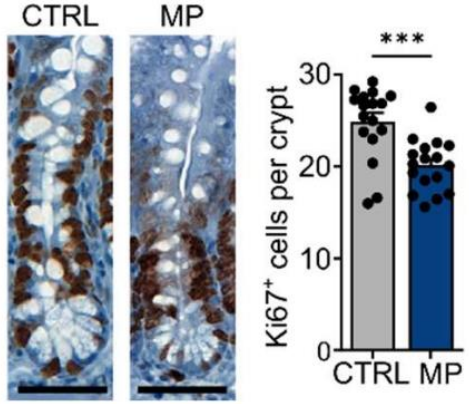


► significant height reduction

F

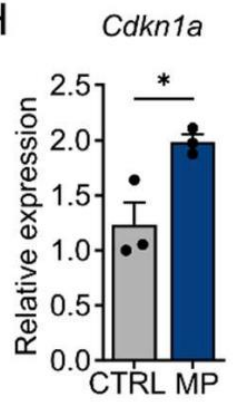


G



Ki67: proliferation marker

H

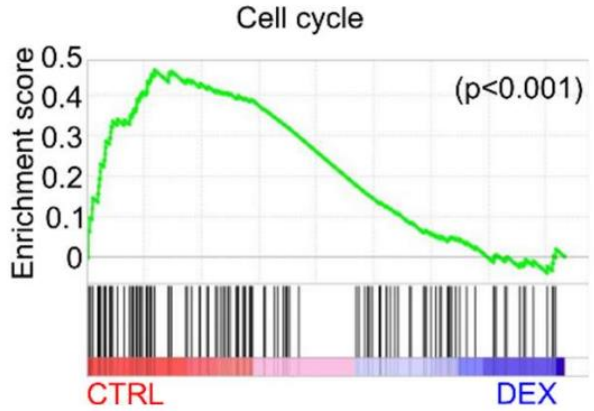


► ileal epithelium의 proliferation↓

Cdkn1a: cell cycle arrest
(encoding p21)

GSEA (Gene set enrichment analysis)
SI epithelial cells of WT mice

I

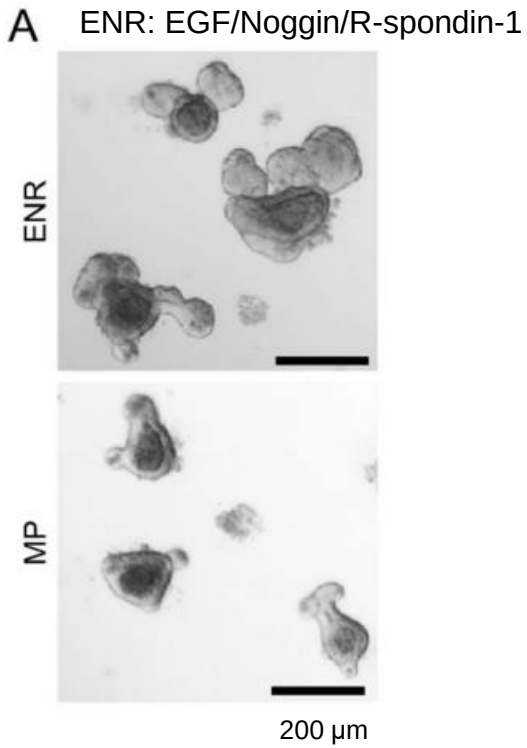


DEX(Dexamethasone) : 임상에서 사용되는 corticosteroid

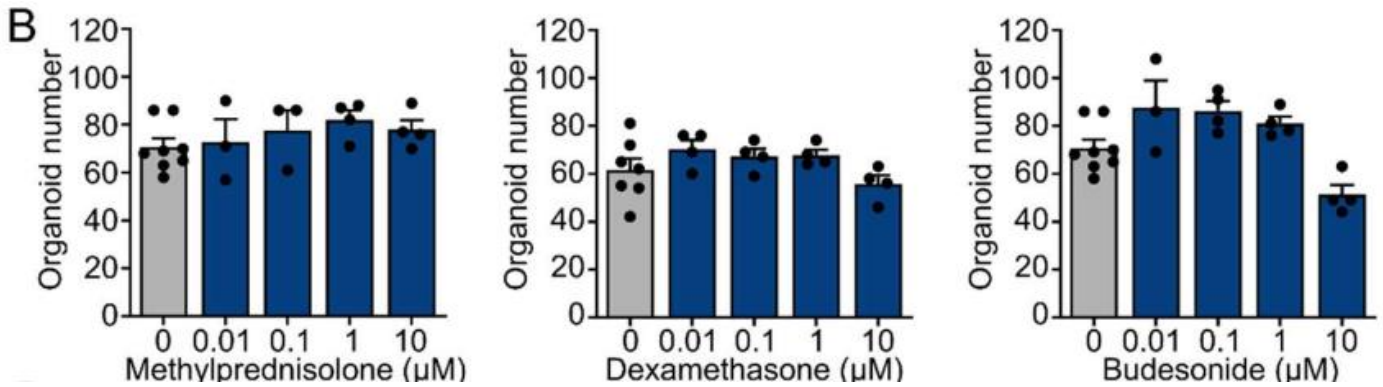
Fig. 1D-I

Corticosteroid exposure reduces mouse and human organoid cell proliferation

Murine SI organoids
- cultured in ENR +/- MP, DEX, Budesonide
- for 7 days
- n= 3-8 wells per group

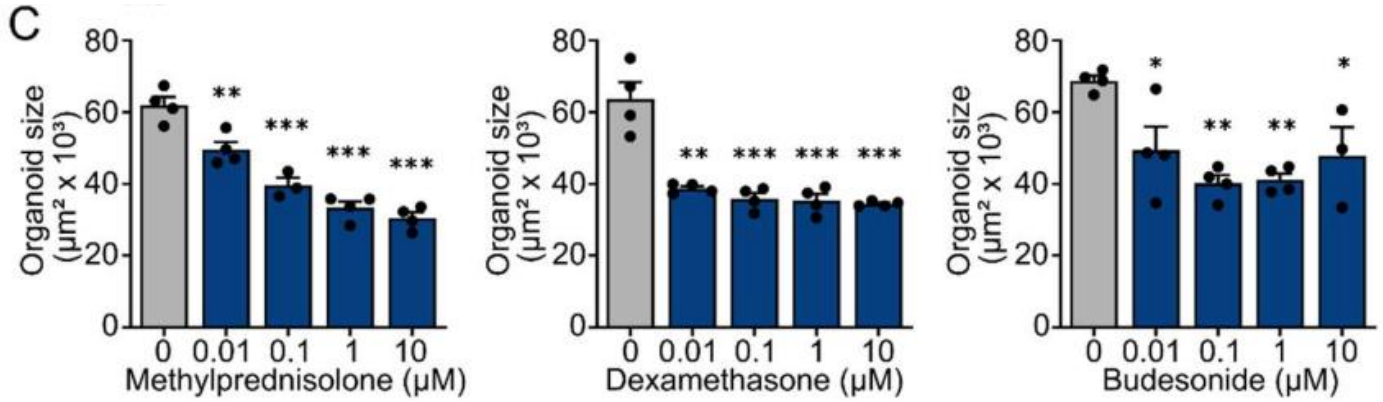


Organoid number (frequency)



► vialibility 변화 X

Organoid size



► eplithelial proliferation↓

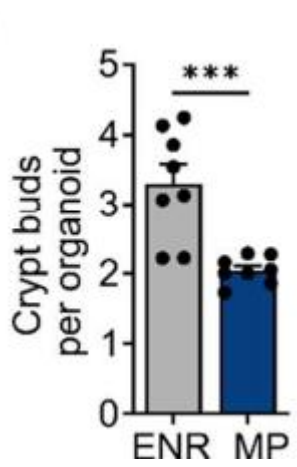
Fig. 2A-C

Corticosteroid exposure reduces mouse and human organoid cell proliferation

Frequency of crypt bud formation (*ex vivo*)

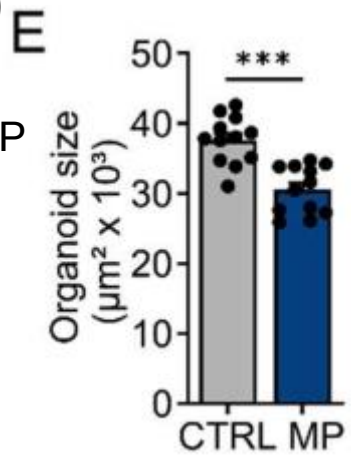
Murine SI organoids
- cultured +/- MP for 5 days
- n= 8 wells per group

► attenuated crypt bud formation

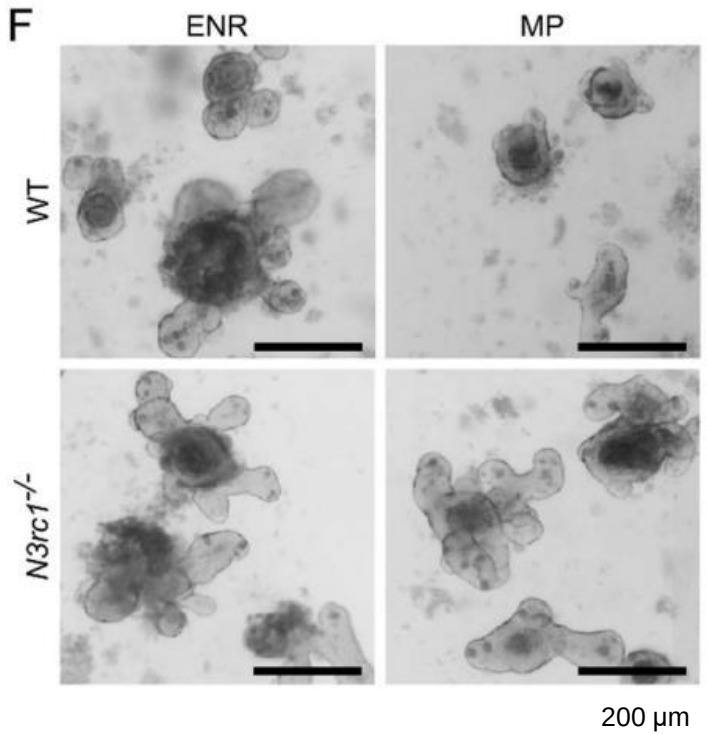


Size of SI organoids (*in vivo*)

Murine SI organoids (WT)
- crypts of WT mice i.p. w/ MP
- cultured in ENR for 6 days
- n= 12 wells per group

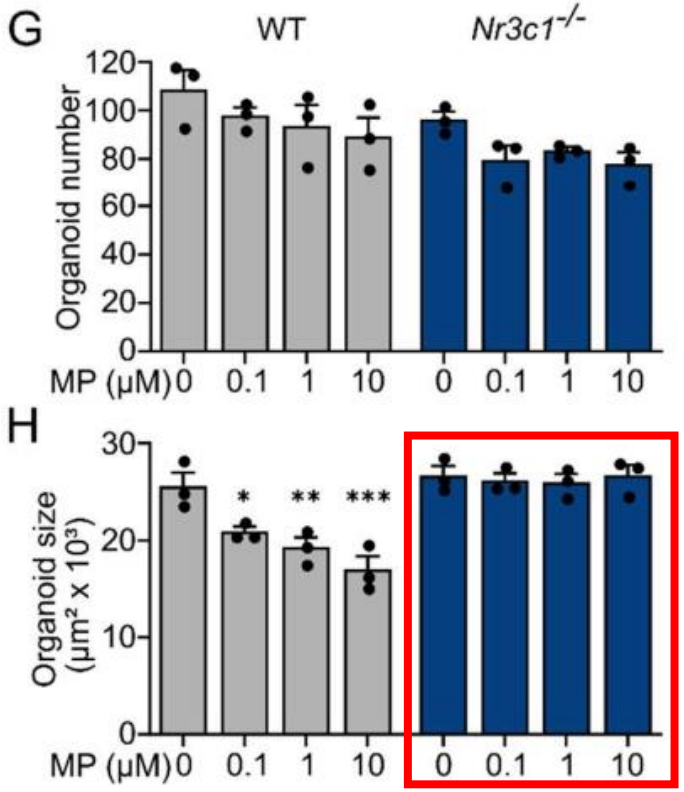


Murine SI organoids (WT or *Nr3c1-/-*) ➡ *Nr3c1-/-* organoid
- cultured +/- MP
- for 4 days
- n= 3 wells per group
- tamoxifen 2 mg i.p. inj for 5 days



Nr3c1: gene encoding glucocorticoid receptor (GR)

Organoid number (frequency) & size

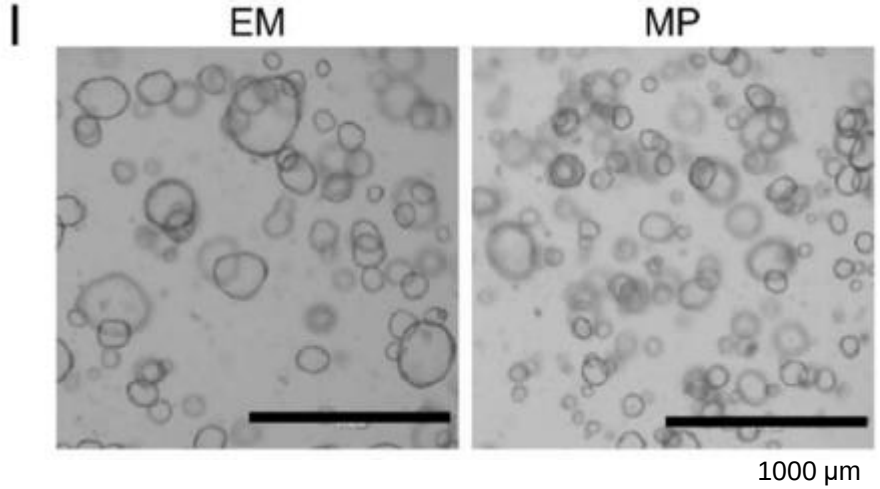


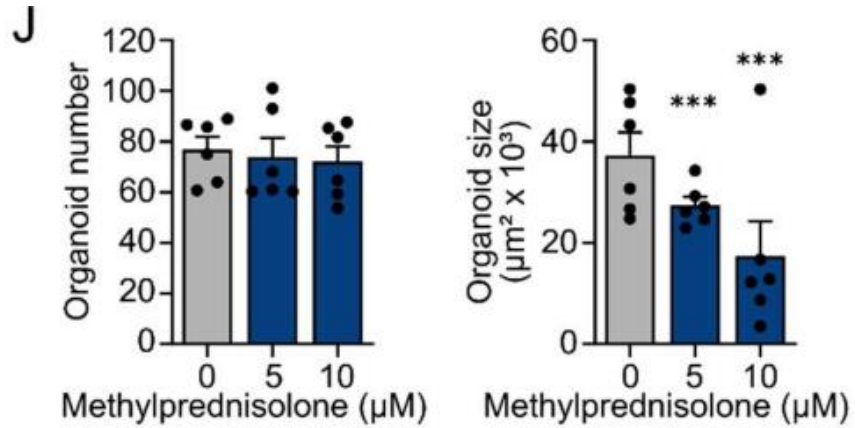
► resistant to growth inhibition by MP

Fig. 2D-H

Corticosteroid exposure reduces mouse and human organoid cell proliferation

Human SI organoids
- primary duodenal tissue
- cultured +/- MP for 5 days
- n= 6 wells per group

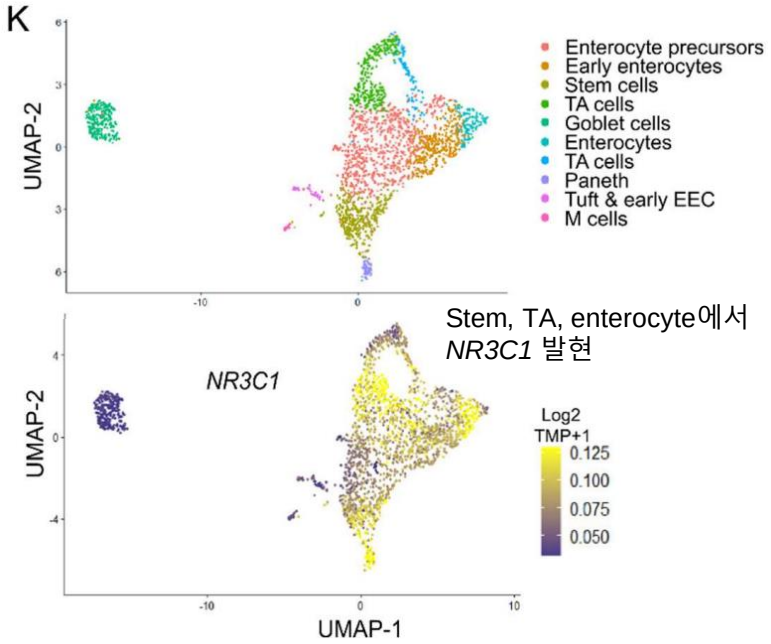




► frequency 변화 없이 proliferation↓

scRNA-seq

Human



Stem, TA, enterocyte에서 *NR3C1* 발현

Murine data (previous)

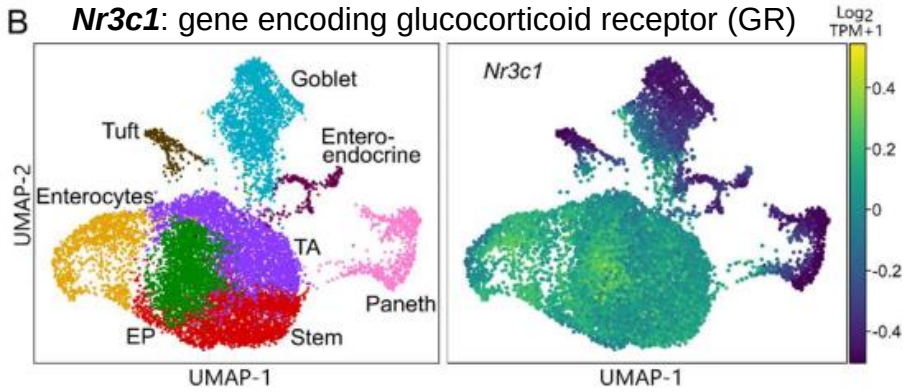


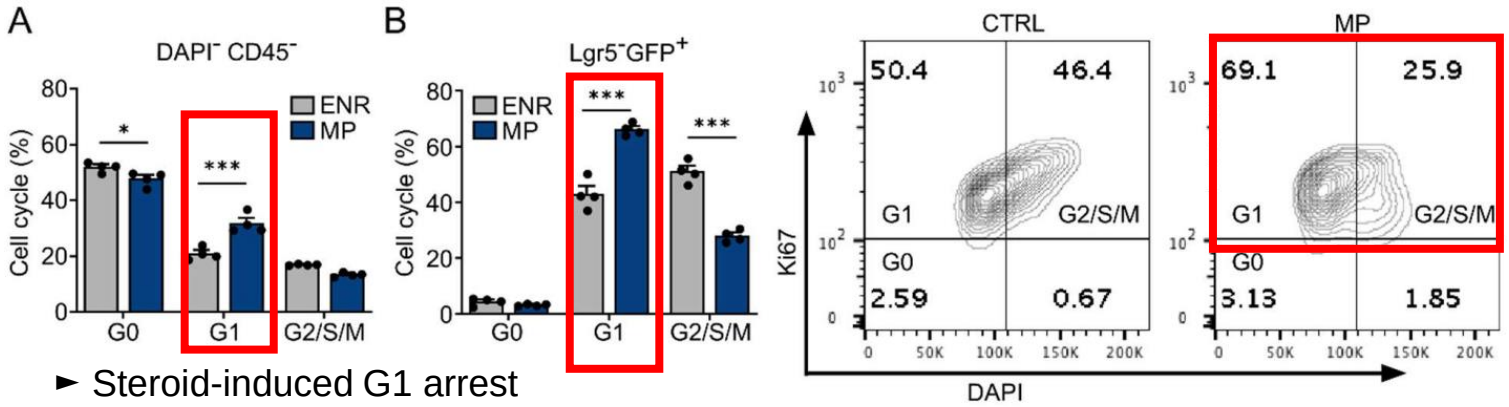
Fig. 2I-K, 1B

Corticosteroid reduce the proliferation of murine and human organoid cells

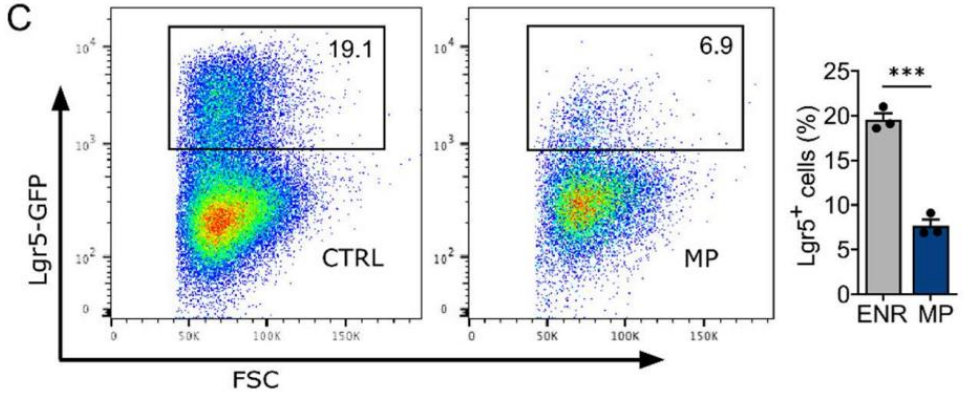
Quantifications of intracellular Ki67-DAPI cell cycle analysis

- <u>murine</u> SI organoids cultured +/- MP(10µM) for 5 days

Ki67: proliferation marker



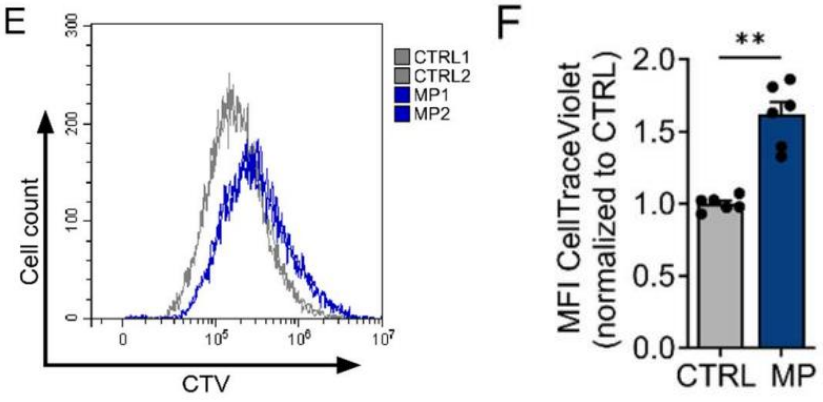
► Steroid-induced G1 arrest



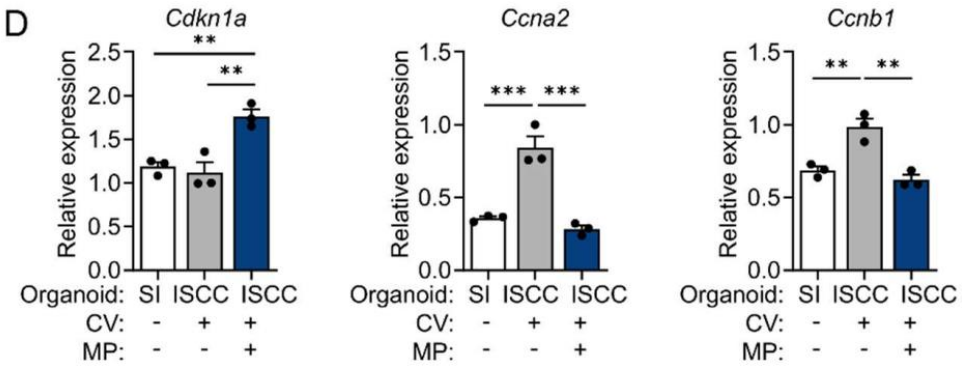
Lgr5: intestinal stem cell marker

Flow cytometry & Quantifications of CTV

- <u>human</u> SI organoids cultured +/- MP for 5 days



qPCR



ISCC: Intestinal stem cell colonies
Cdkn1a: cell cycle arrest
Ccna2, Ccnb1: cyclin gene

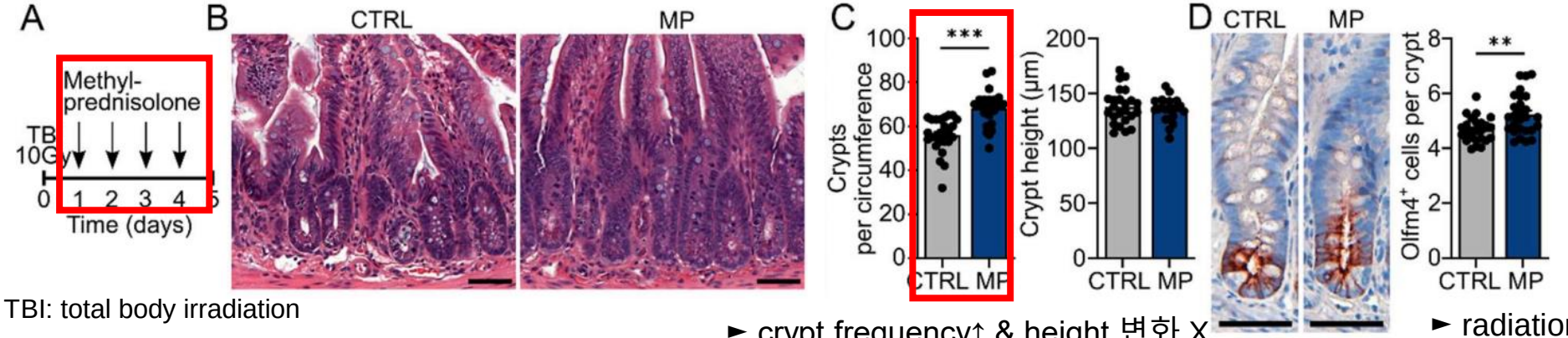
Fig. 3A-F

Epithelial effects of corticosteroid treatment after irradiation are timing dependent.

Early steroid administration (WT B6 mice)
- MP i.p. daily starting 24hr post-TBI

MP(Methylprednisolone)
: 임상에서 사용되는 corticosteroid

Olfm4: SI ISC marker
(target gene for the Notch signaling pathway)

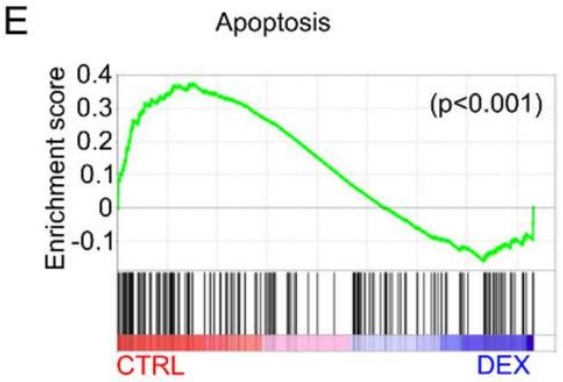


TBI: total body irradiation

► crypt frequency↑ & height 변화 X

► radiation injury로부터 stem cell 보호

GSEA (WT mice treated w/ DEX)

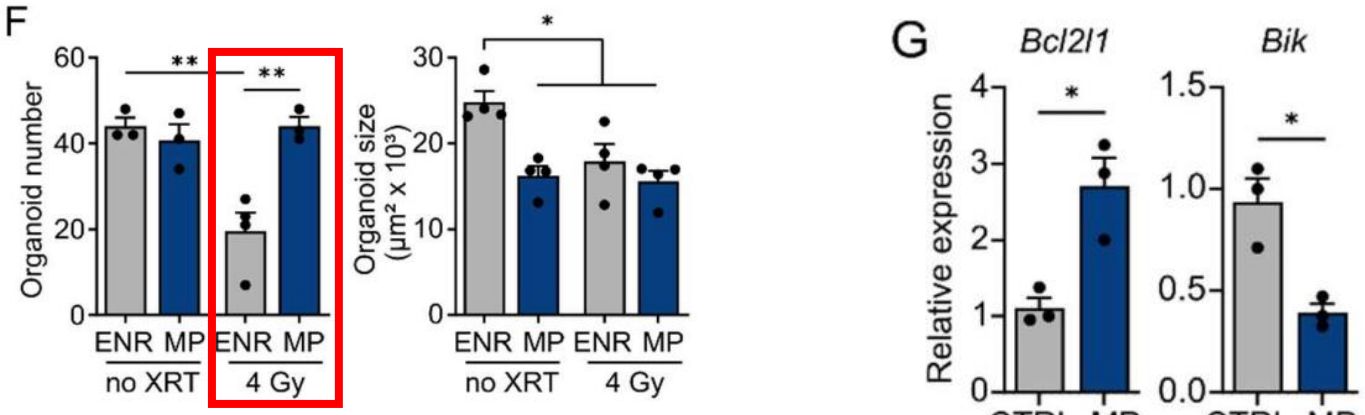


► DEX 처리 시 pro-apoptotic gene 발현↓

► reduce intestinal radiation injury

Organoid (SI crypts isolation)
- SI crypts isolation→irradiation→CS treatment

Bcl2l1: anti-apoptotic gene
Bik: pro-apoptotic gene



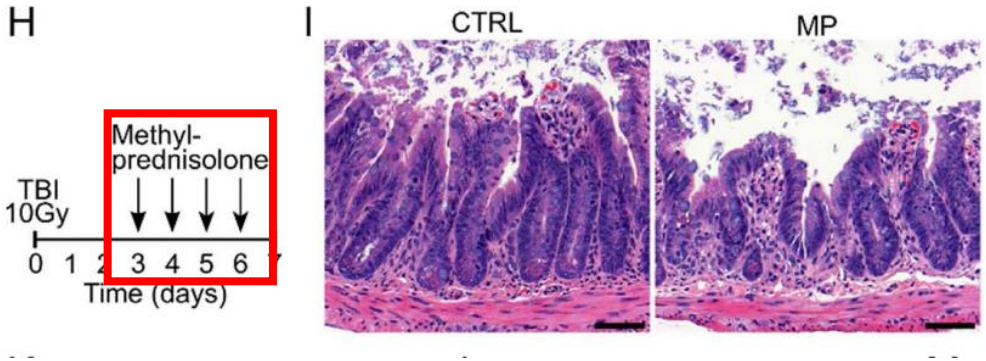
► organoid frequency↑ & size 변화 X

Fig. 4A-G

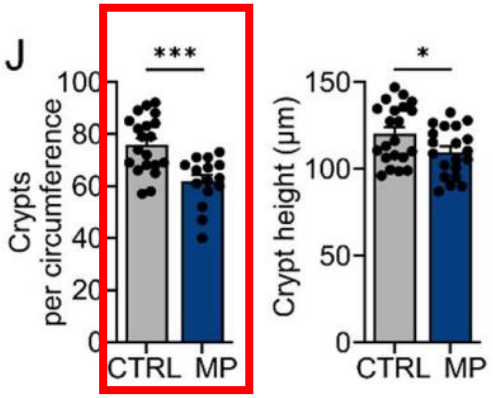
Epithelial effects of corticosteroid treatment after irradiation are timing dependent.

Delayed steroid administration (WT B6 mice)
- MP i.p. days 3-6 post-TBI

MP(Methylprednisolone)
: 임상에서 사용되는 corticosteroid

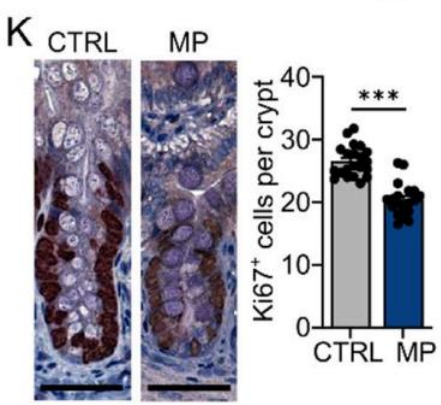


TBI: total body irradiation



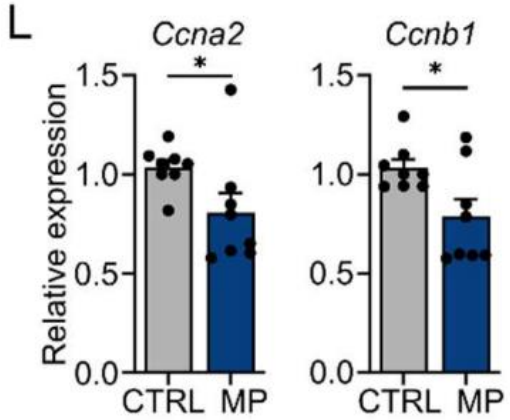
► ileal crypt loss

Ki67: proliferation marker

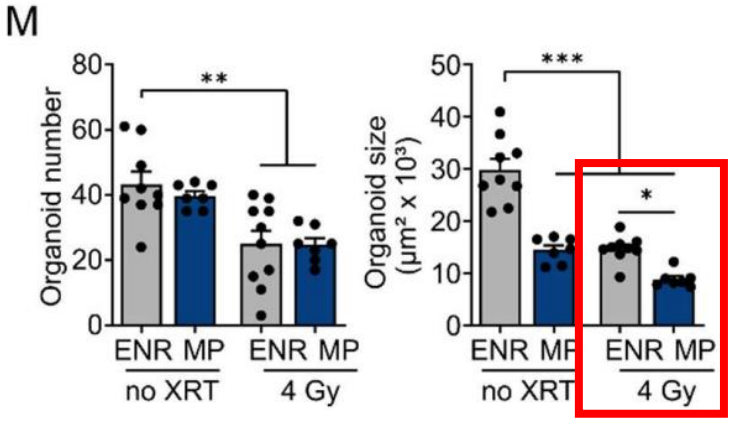


► epithelial proliferation↓

Organoid (SI crypts isolation)
- SI crypts isolation→irradiation→CS treatment



Ccna2, Ccnb1: cyclin gene



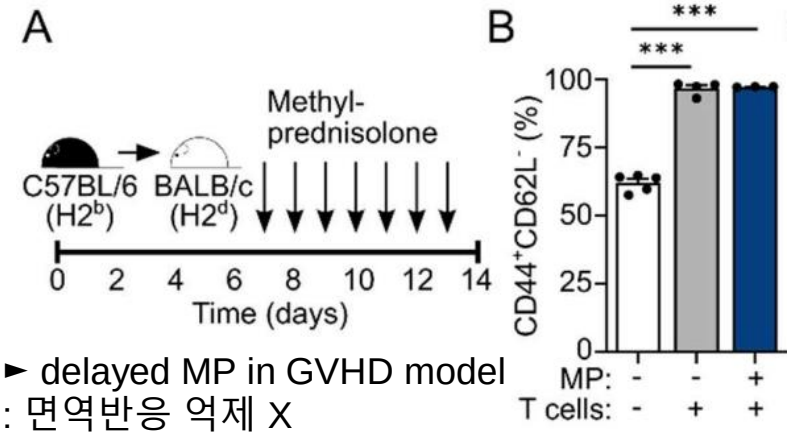
► organoid frequency 변화X & Size↓
► delayed treatment는 regenerative response 악화

Fig. 4I-M

Corticosteroids impair the epithelial response to immune-mediated GI damage

Bone marrow transplantation (BM +/- T cells)

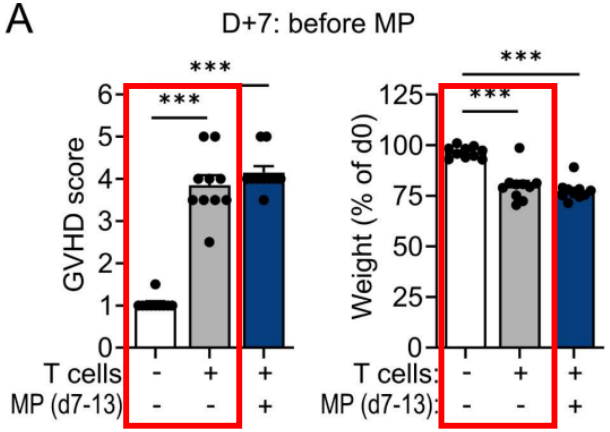
- MP i.p. daily from day 7-14 post-BMT



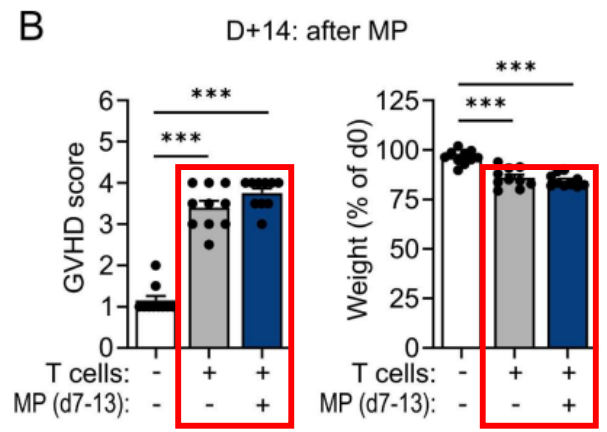
► delayed MP in GVHD model
: 면역반응 억제 X

CD44+CD65L-: effector memory T cell

MP(Methylprednisolone)
: 임상에서 사용되는 corticosteroid

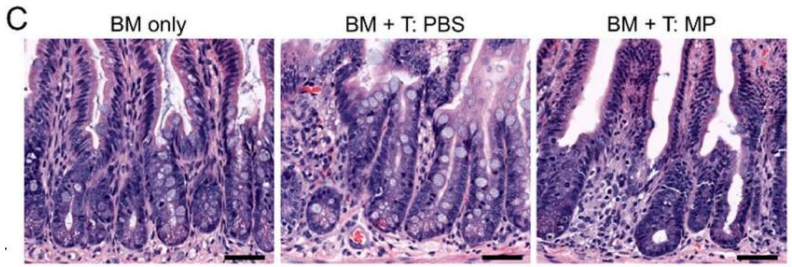


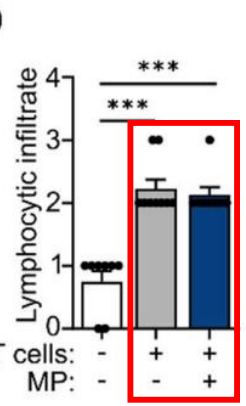
► acute GVHD



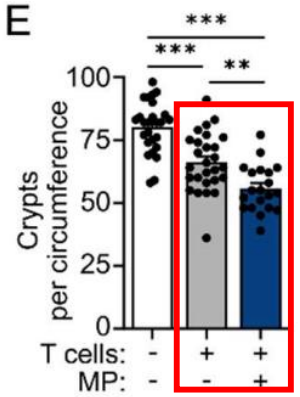
► Steroid-refractory GVHD (SR GVHD)

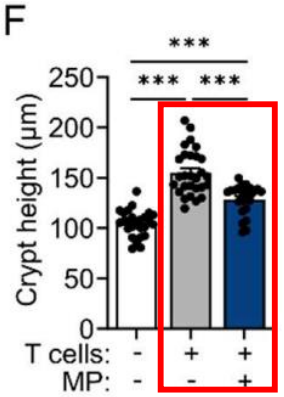
Images of ileum

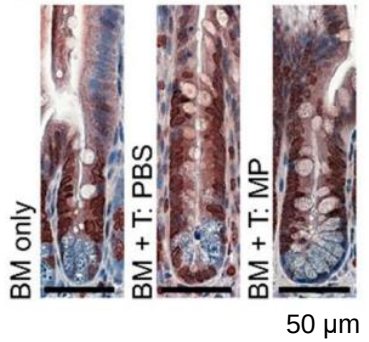


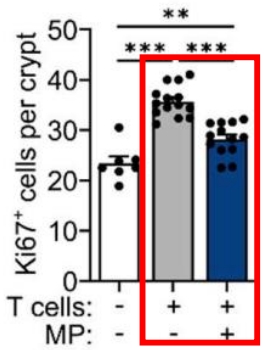


► lymphocytic infiltration 지속
► immune response 억제 X









► more severe GVHD pathology
► crypt loss, height↓, Ki67+ ↓

Ki67: proliferation marker

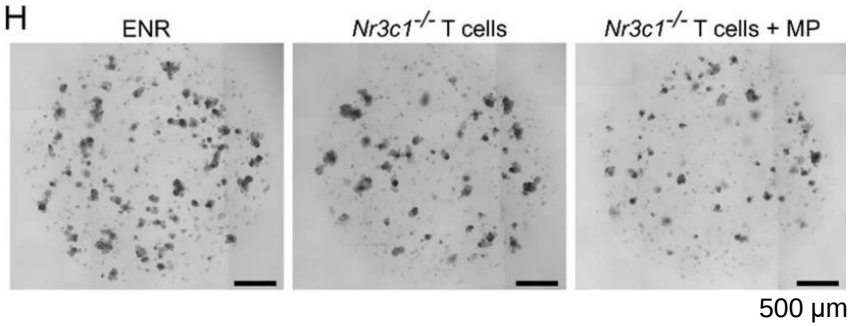
Fig. 5A-G, S1A, S1B

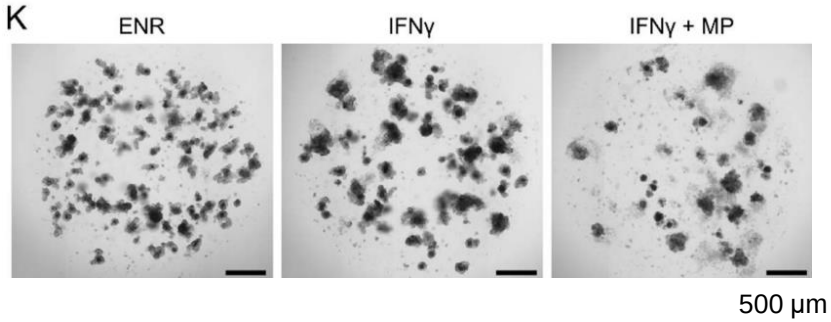
Corticosteroids augment immune-mediated GI damage induced by T cells and their effector cytokines ex vivo

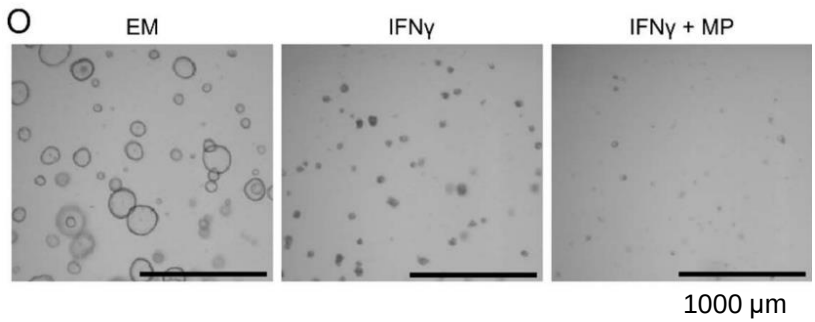
SI organoid (B6)
- cultured with +/- T cells & MP

SI organoid (B6)
- cultured +/- MP & rmIFNγ

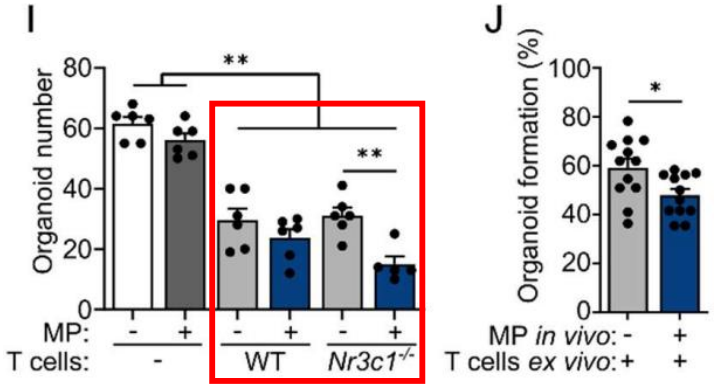
SI organoid (Human)
- cultured +/- MP & rhIFNγ





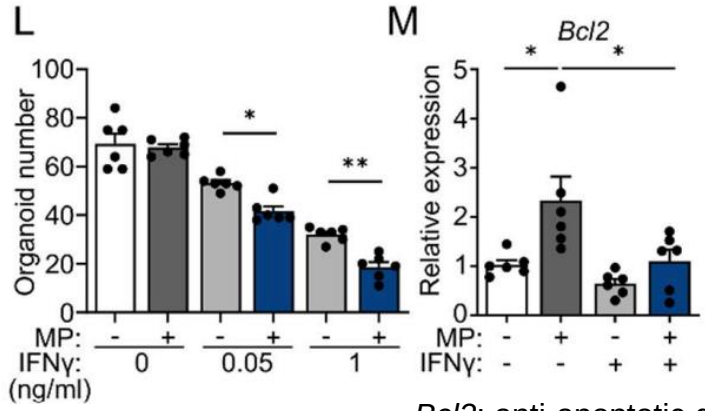


Nr3c1: gene encoding glucocorticoid receptor (GR)

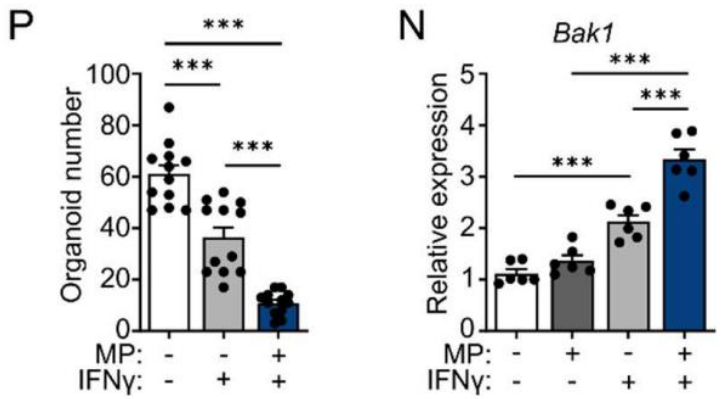


GR-intact GR-deficient

► GR-deficient T cell co-culture & MP treatment
→ more severe organoid reduction



Bcl2: anti-apoptotic gene



Bak1: pro-apoptotic gene

► IFNγ → crypt loss in GVHD & organoid toxicity
► IFNγ & MP treatment → reduced viable SI organoid

Fig. 5H-P

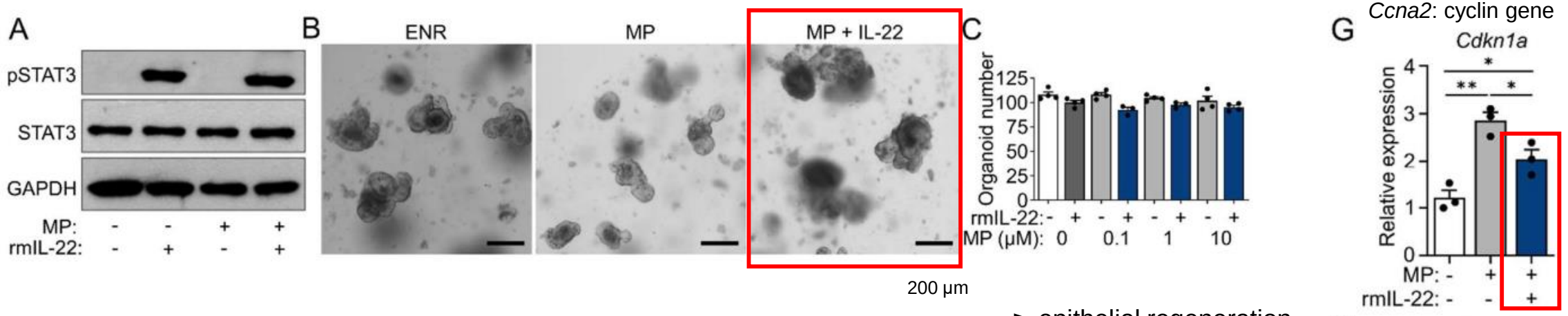
IL-22 treatment overcomes corticosteroid-induced inhibition of epithelial proliferation

Western blot (murine SI organoids)
- treated MP for 24 hours & rmIL-22 for 2 hours

STAT3: epithelial regeneration & recovery
(GR can interfere with STAT3 function)
IL-22: activate STAT3 & promote recovery

SI organoid (B6)
- cultured +/- MP & rmIL-22

Cdkn1a: cell cycle arrest
Ccna2: cyclin gene



► epithelial regeneration

human SI organoids
- cultured +/- MP & rhIL-22 for 6 days

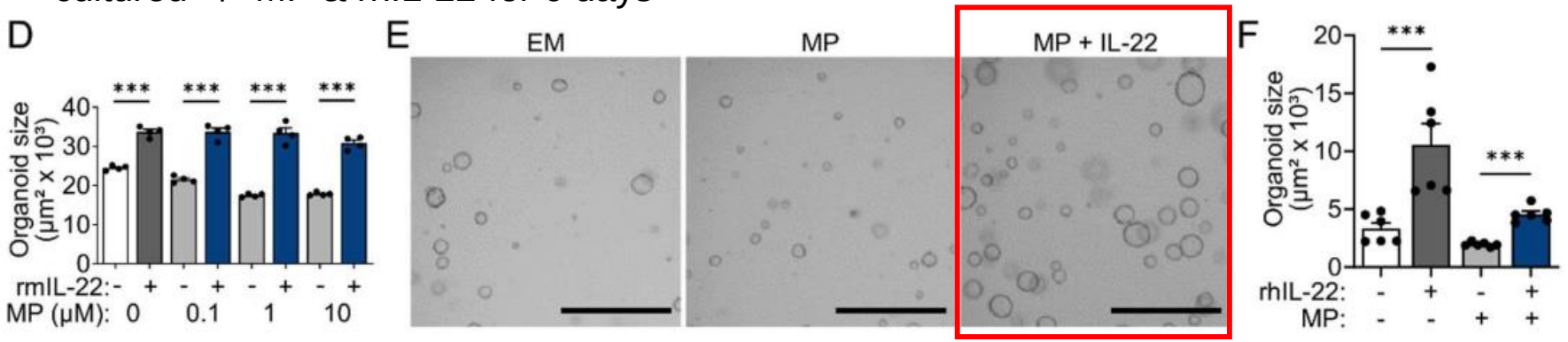
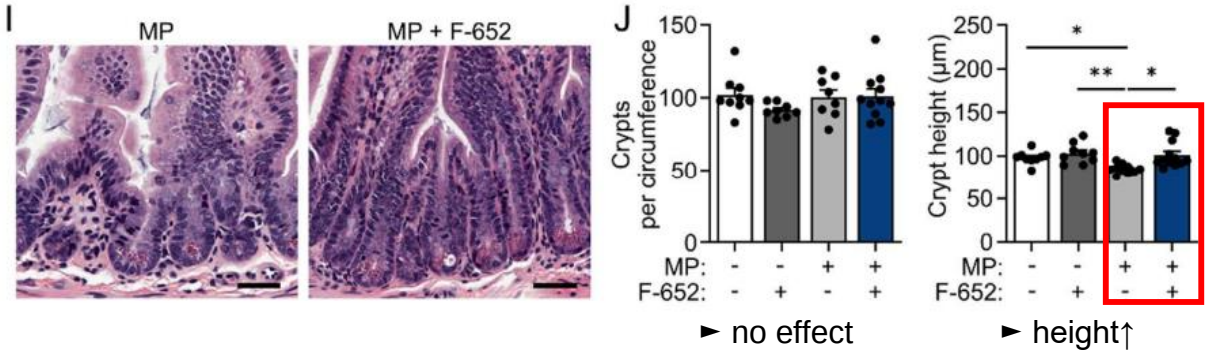


Fig. 6A-H

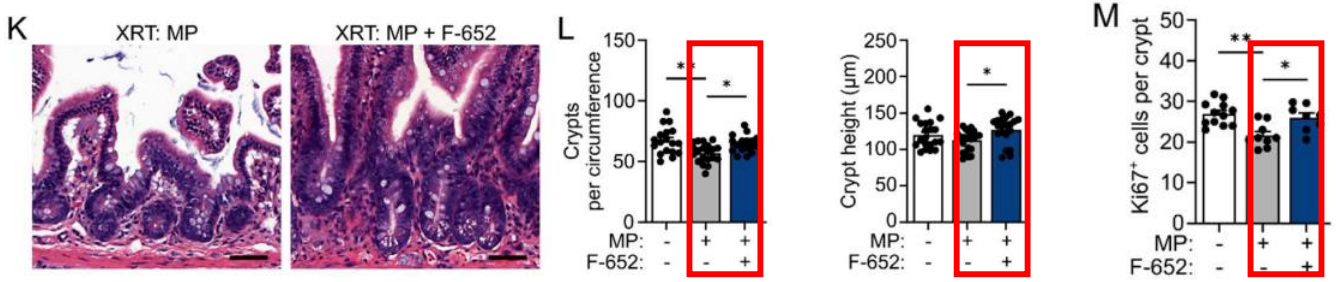
IL-22 treatment overcomes corticosteroid-induced inhibition of epithelial proliferation

F-652 administration (*in vivo*) (WT B6 mice)
- (+/-) MP & F-652

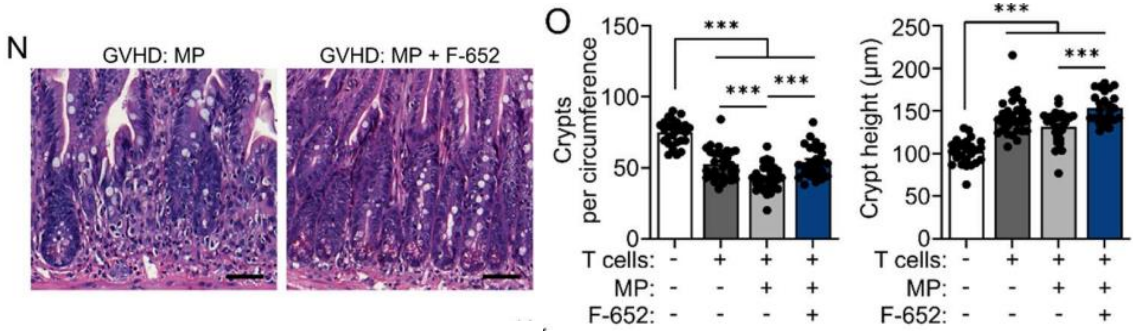
F-652: rhIL-22-dimer/Fc-fusion protein



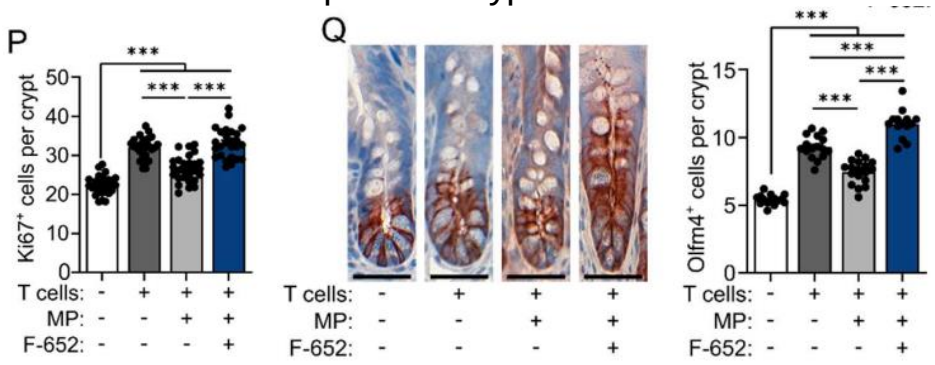
Delayed steroid administration (*in vivo*) (WT B6 mice)
- (+/-) MP & F-652 i.p. starting 72 hours post-TBI



Combined CS & F-652 treatment in GVHD
- B6-into-BALB/c transplant of BM +/- T cells
- (+/-) MP & F-652 starting on day 7 post-TBI



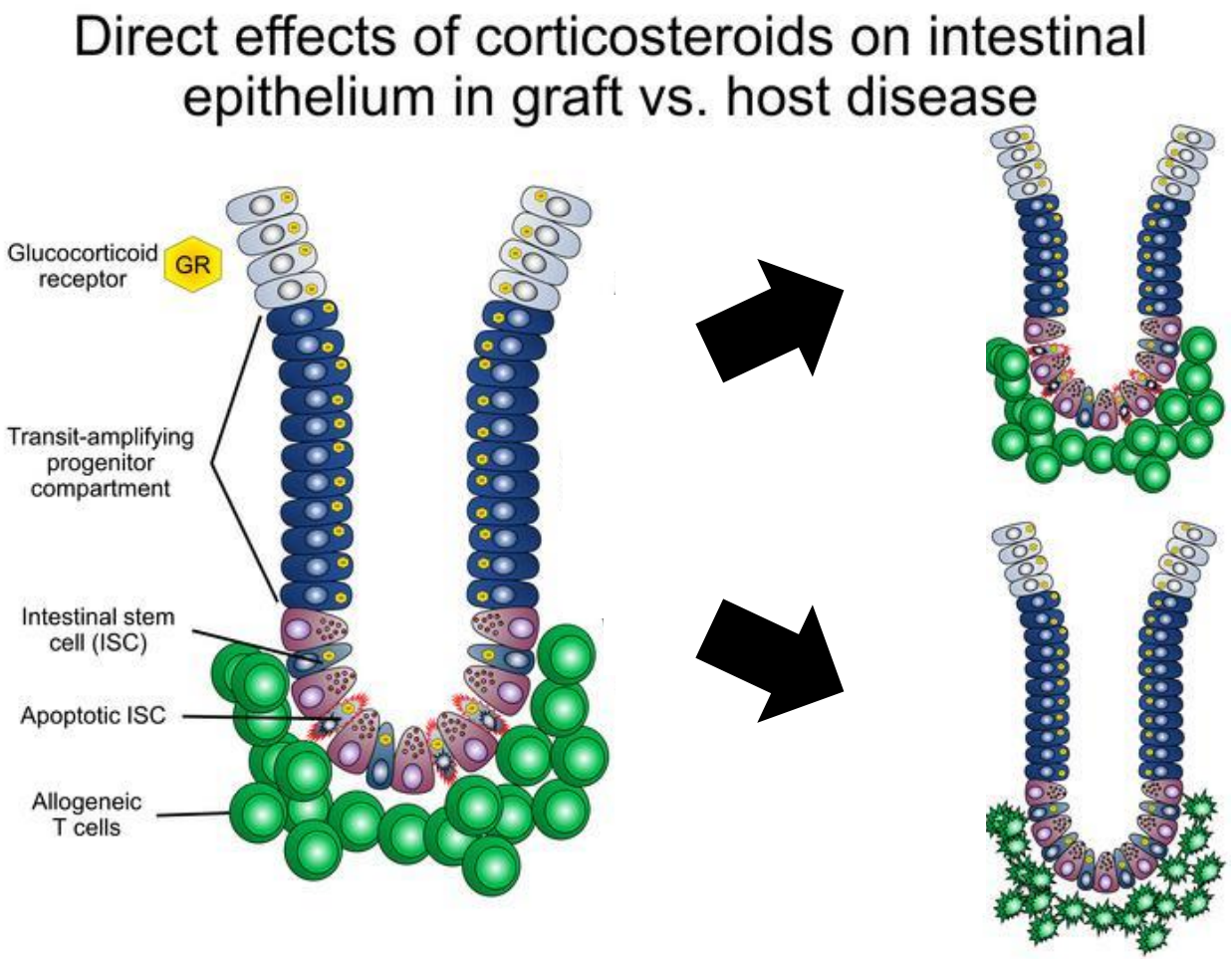
► prevent crypt loss



Olfm4: SI ISC marker

Fig. 6I-Q

Summary



- Intestinal epithelium is directly targeted by CS
  - Impaired epithelial proliferation
  - Potentiation of IFNγ-induced ISC apoptosis
  - More severe crypt loss and reduced frequency of ISCs
- Treatment of intestinal epithelium with IL-22 countered CS-mediated epithelial suppression
  - Activation of STAT3
  - Promotion of epithelial proliferation
  - Enhanced ISC recovery