Drug-based modulation of endogenous stem cells promotes functional remyelination *in vivo*

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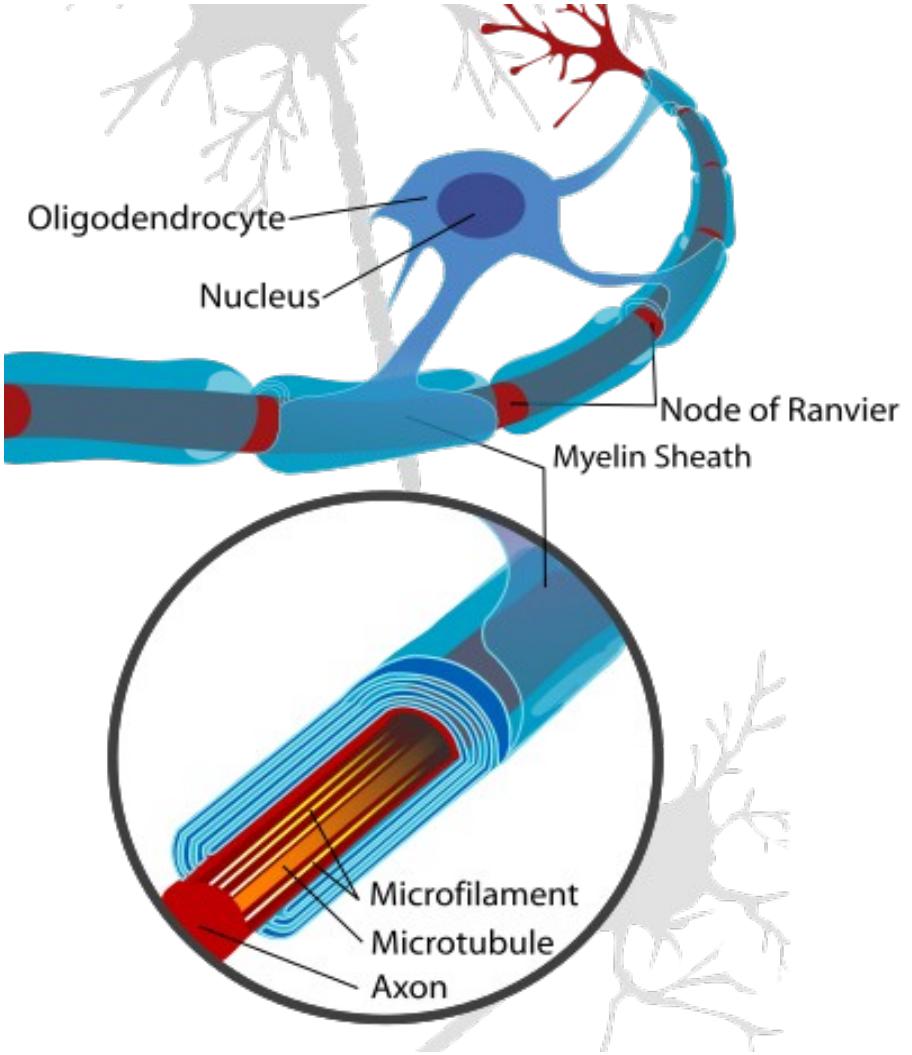
Jun. 11. 15. Nature

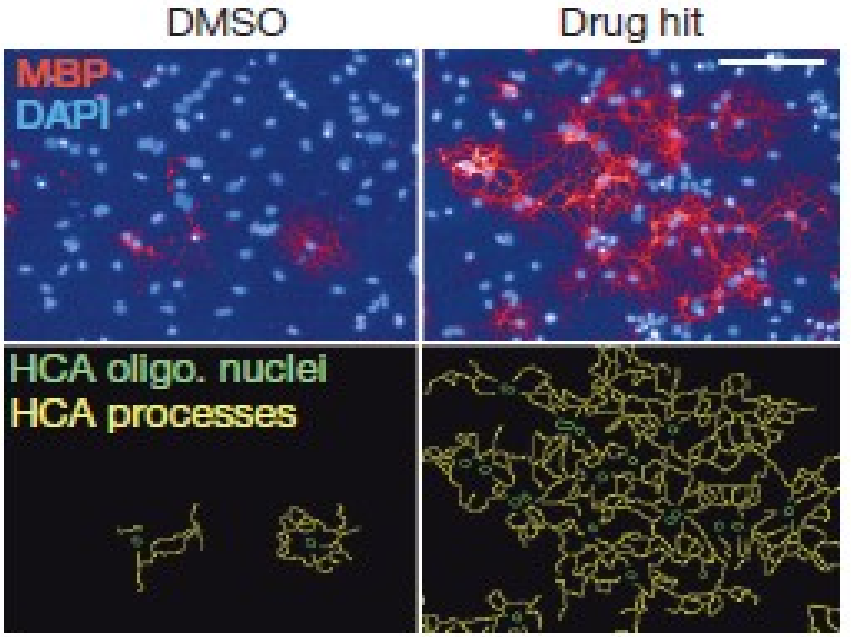
Background

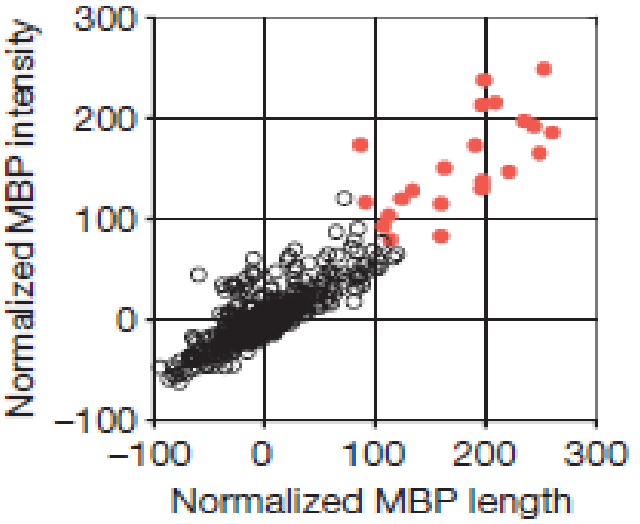
- Remyelination is the process of propagating oligodendrocyte precursor cells to form oligodendrocytes to create new myelin sheaths on demyelinated axons in the CNS.
- Remyelination may provide therapeutic benefit in multiple sclerosis and demyelination disorder.



HTS screen for inducer of oligodendrocyte differentiation

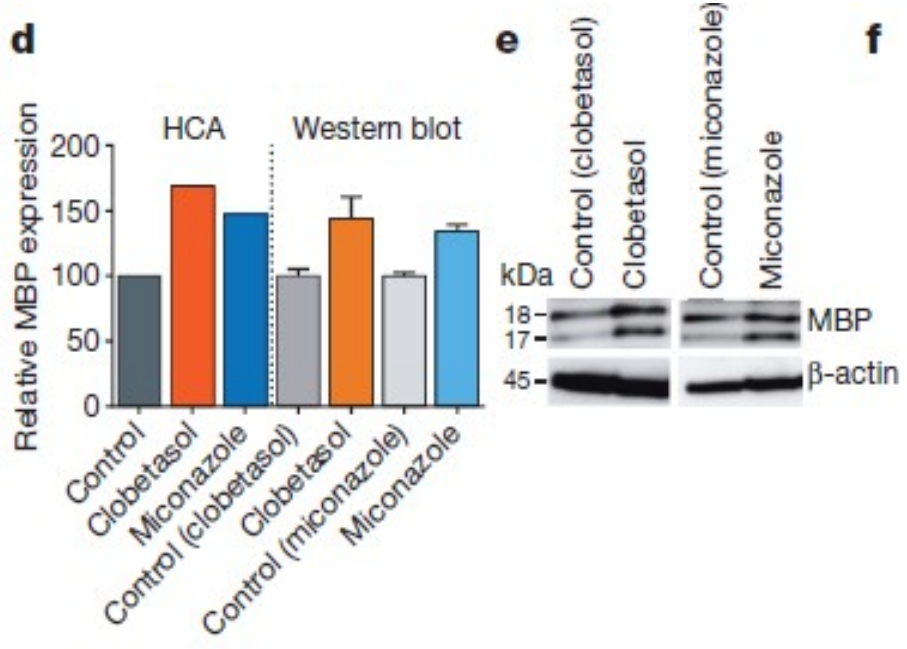
- Cell: mouse epiblast stem cell (EpiSC)-derived OPCs.
- Detection: Myelin basic protein (MBP) expression.
- Detection method: High concent analysis.

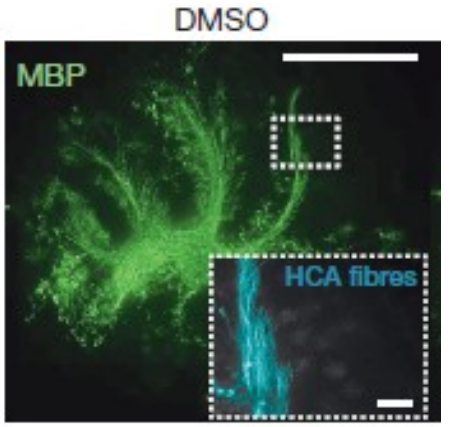


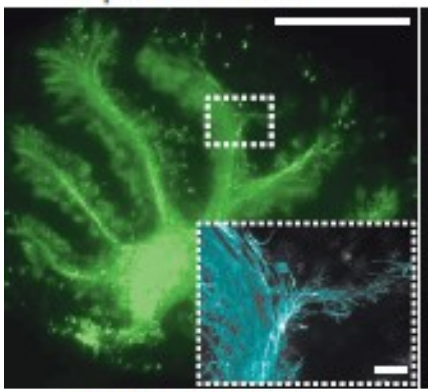


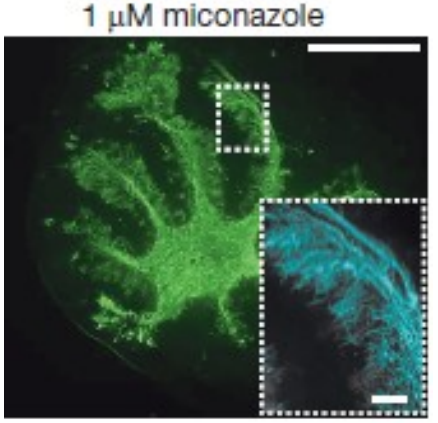
To test the effect of drug hits on nativ OPC

- Screen material: cerebellar slice from postnatal day 7 mice.
- Treat with drug hits from the first round screen.
- MBP antibody to detect the maturation of native OPCs.



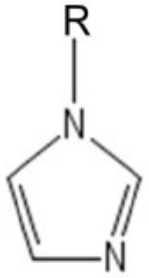




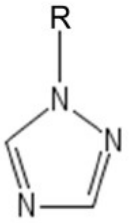


SAR analysis

a

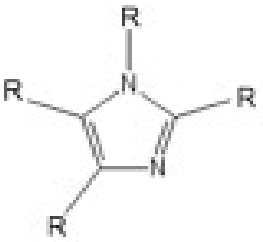


(1,3) Diazoles, mono-substituted at the 1-position	Primary Screen Rank
econazole	3
ketoconazole	4
miconazole	5
clotrimazole	6
bifonazole	9
oxiconazole	10
ozagrel	428
1-benzylimidazole	616



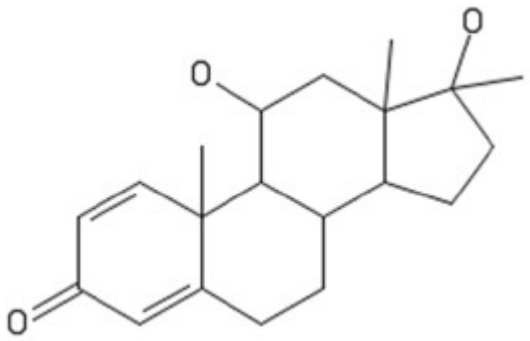
(1,2,4) Triazoles, mono-substituted at the 1-position	Primary Screen Rank
rizatriptan	214
voriconazole	219
letrozole	271
itraconazole	413
fluconazole	414
anastrazole	571

b



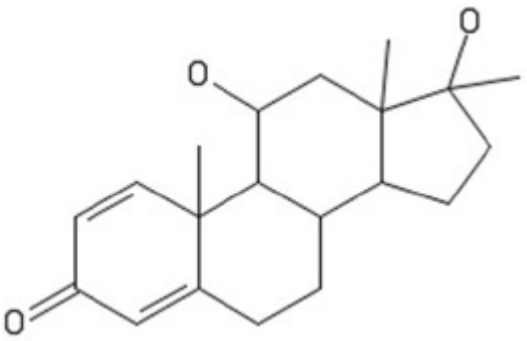
(1,3) Diazoles, poly-substituted	Primary Screen Rank
telithromycin	27
metronidazole	60
ondansetron	116
azathioprine	315
(+)-etomidate	322
ornidazole	327
pilocarpine	473
methimazole	483
tinidazole	589

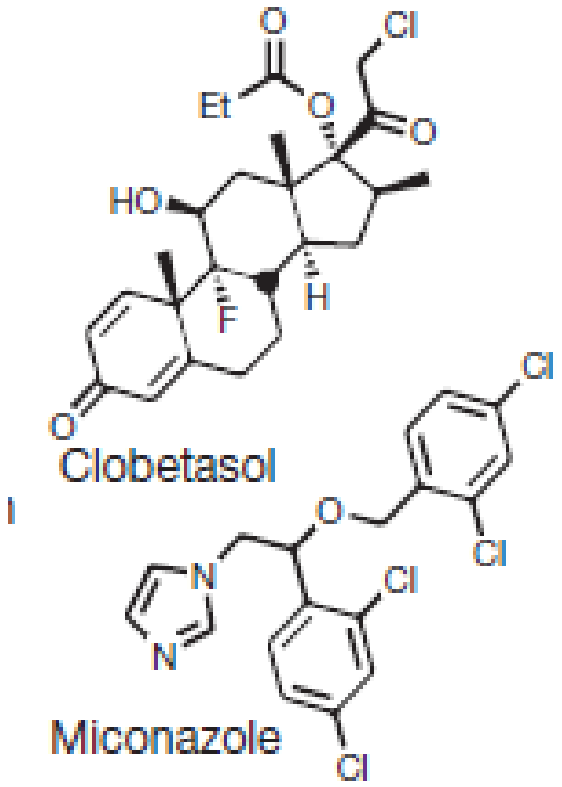
SAR analysis



Steroids	Primary Screen Rank
clobetasol	7
betamethasone	19
methylprednisolone	21
budesonide	28
triamcinolone	29
amcinonide	35
fluticasone	37
fluorometholone	38
depo-medrol	41
beclomethasone	44
loteprednol	45
fluocinolone	47

SAR analysis





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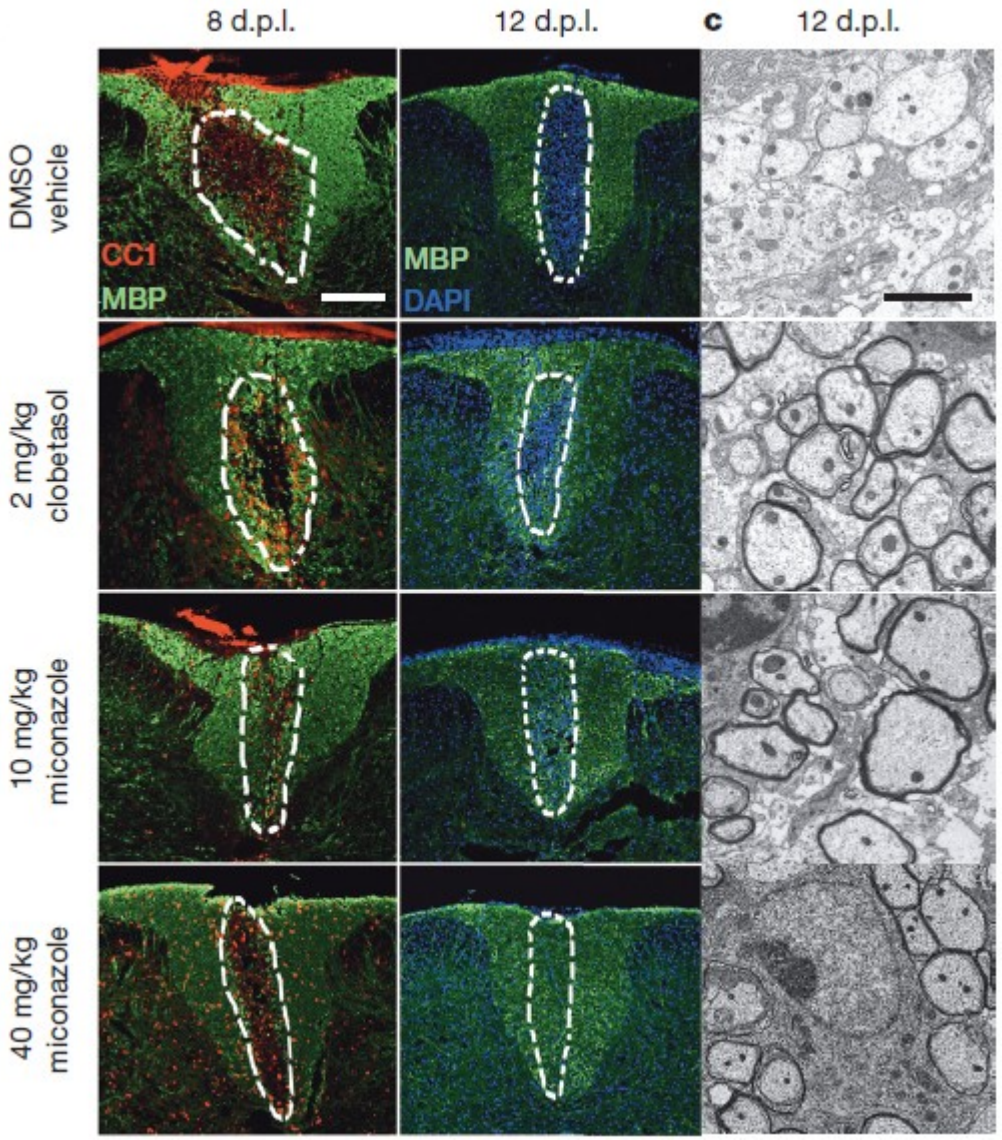
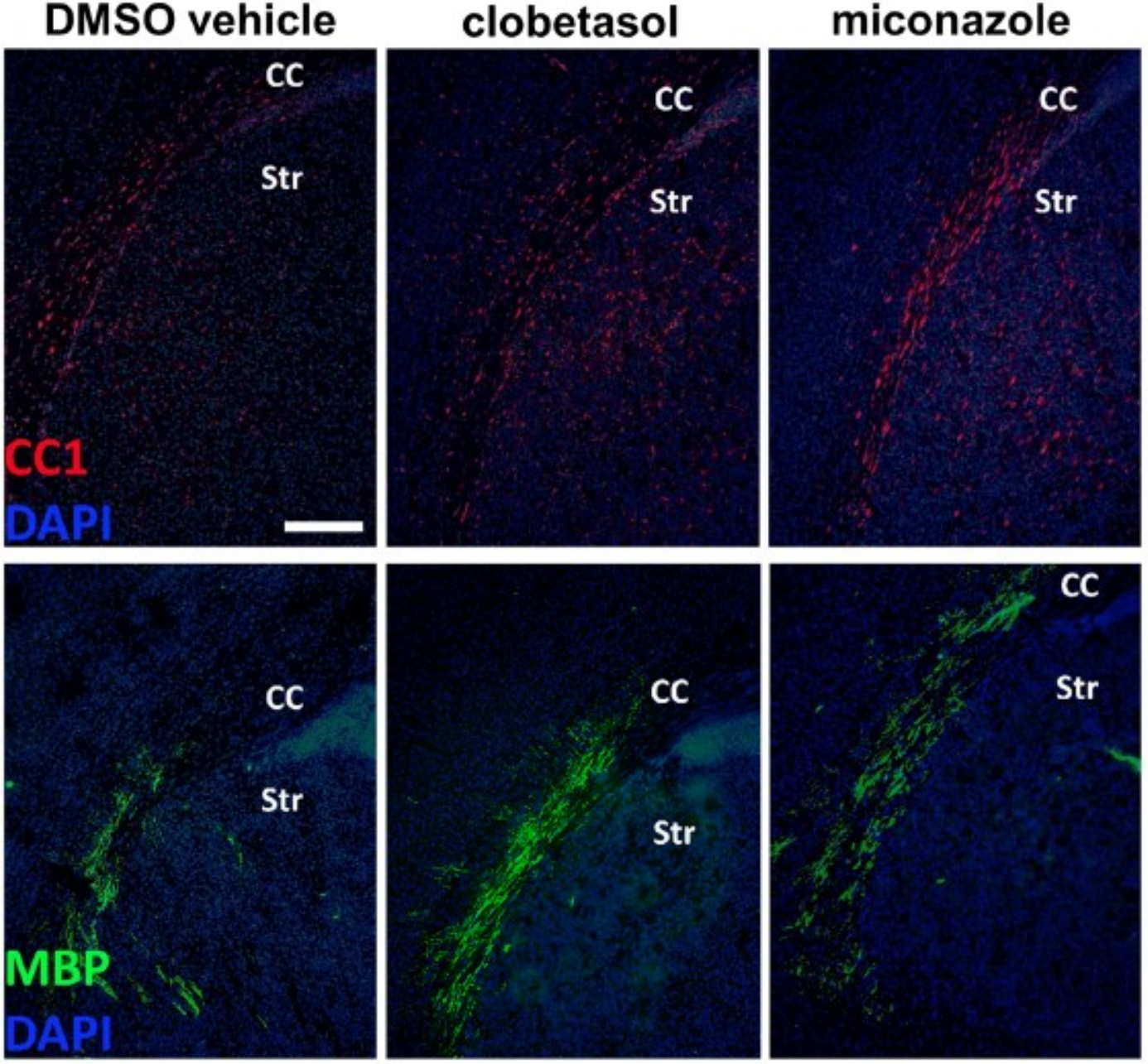
beclomethasone	44
loteprednol	45
fluocinolone	47

To test the effect of drug hits *in vivo*

- Toxin induce model: focal demyelinated lesions are generated in dorsal white matter of the spinal cord of adult mice by localized injection of lysolecithin (lysophosphatidylcholine (LPC))
- Demyelination is generated 4 days after injection.
- Remyelination normally start 14-21 days after injection.
- Therefore, 4-10 days after injection is a good window to observe the effect of drugs.

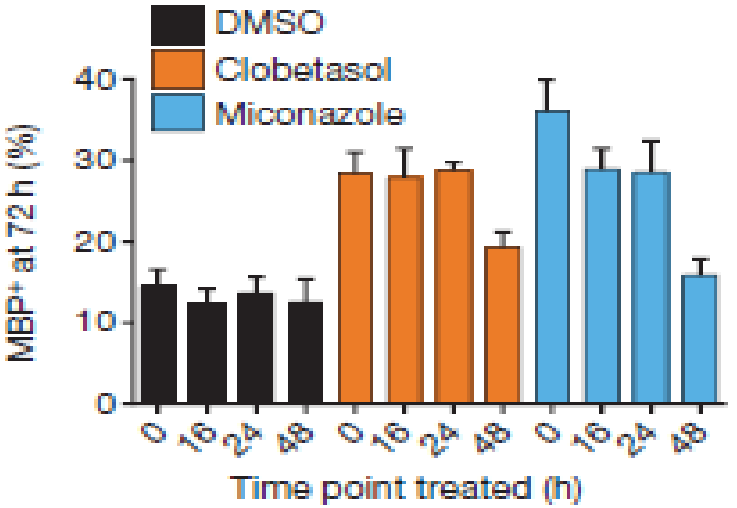


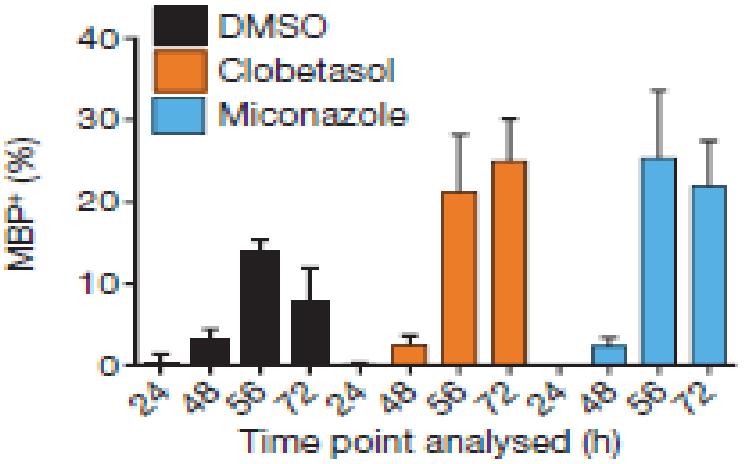
The effect of drug on healthy animal

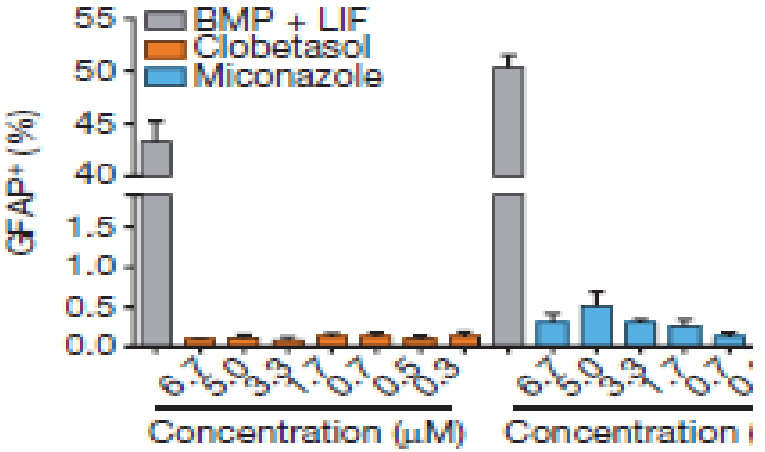


Stage when drugs works

- OPC in differentiation conditions was treated with drugs and DMSO at different time point. MBP expression was assayed at 72h.
- But neigher drug works on astrocyte formation.
- Therefore, these two drugs might be direct inducer of oligodentrocyte differentiation.

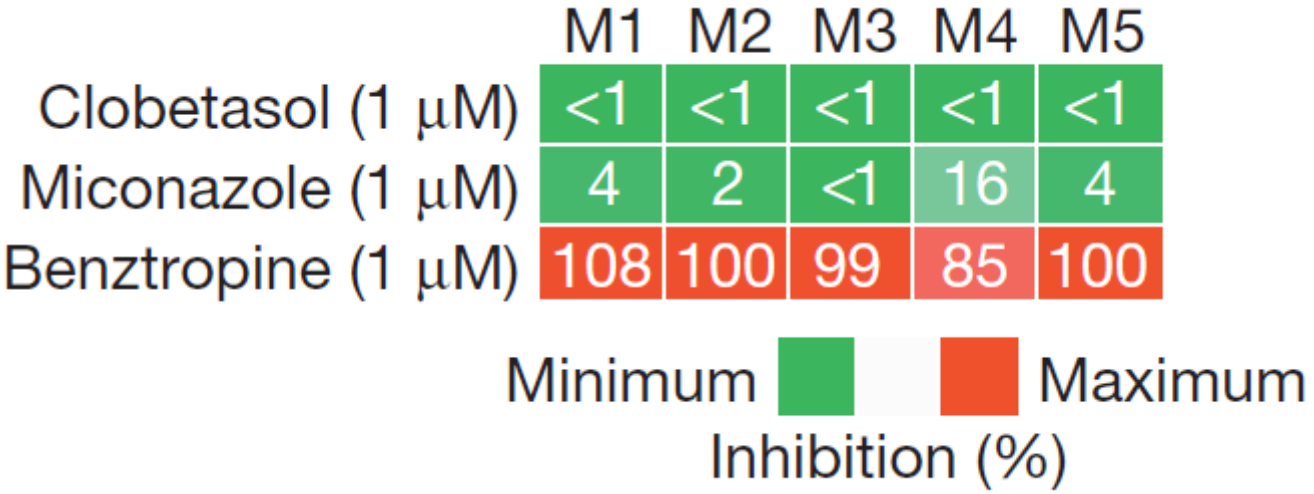






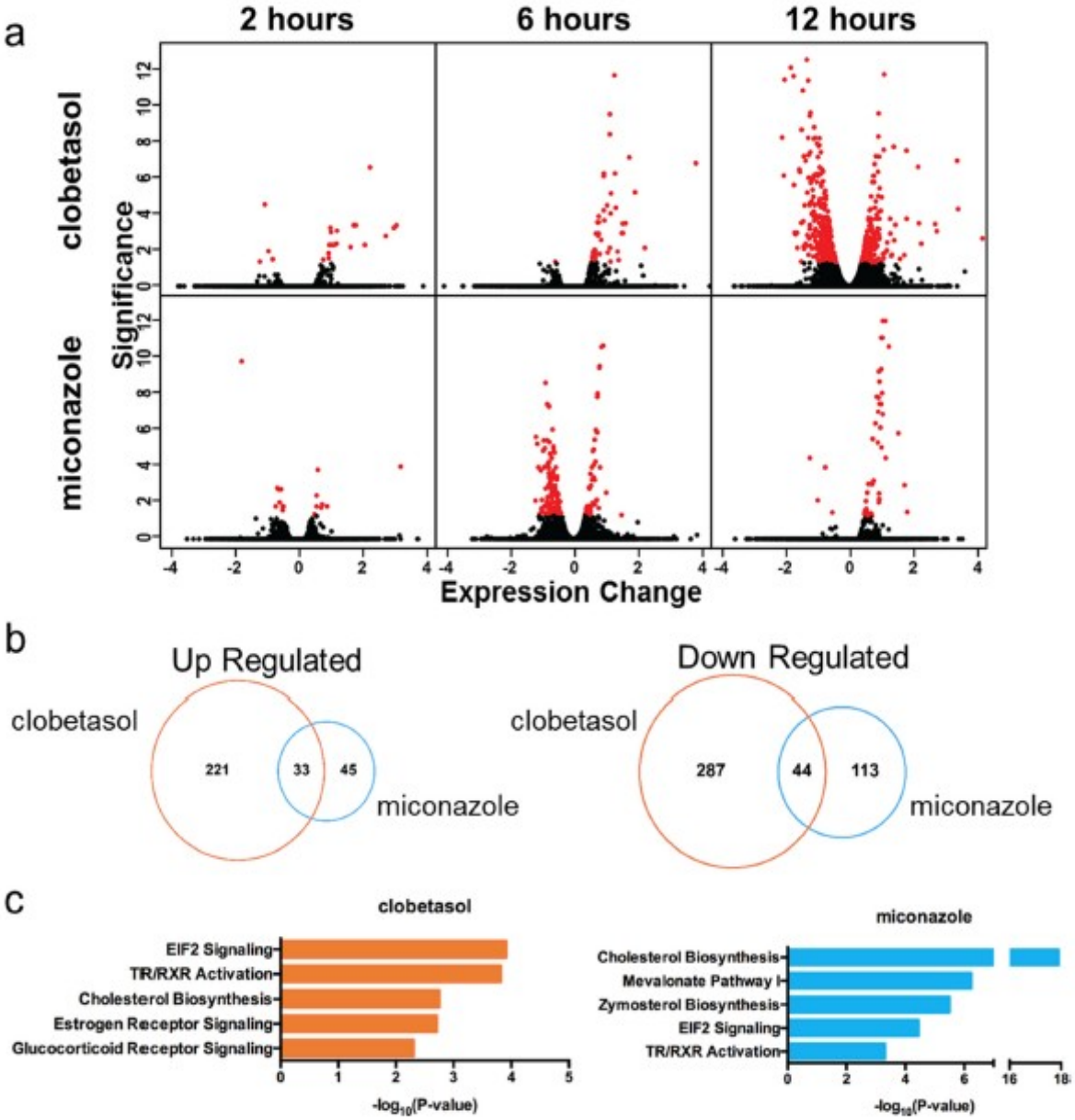
Drugs do not target the muscarinic receptor

- There are compounds induce the remyelination through inhibition of muscarinic receptor.
- Maybe these two hits also induce the remyelination through the same mechanism.
- Clobetasol and miconazole didn't inhibit activity of any kinase.



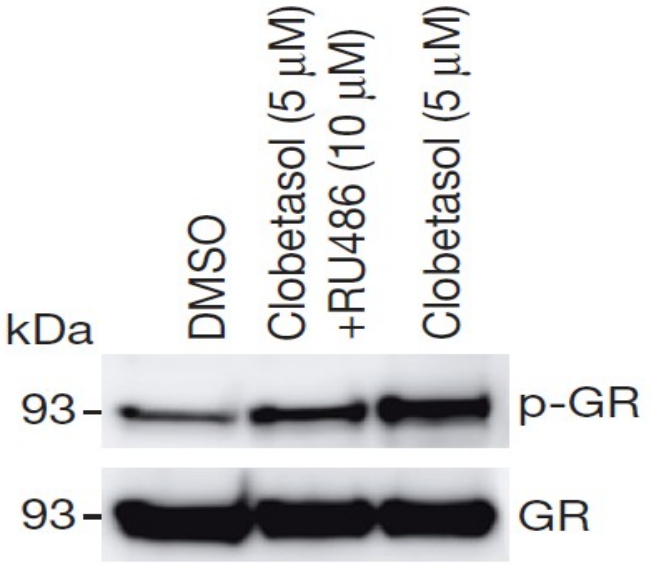
Genome-wide RNA sequencing to find the mechanism of Clobetasol and miconazole

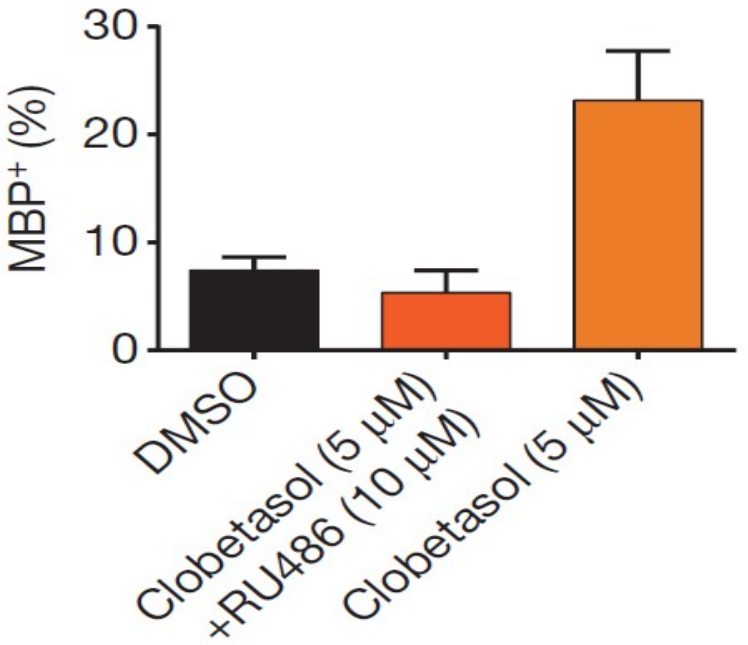
- Both compounds influence the pathway within hours.
- Red dots shows differentially expressed gene.
- Clobetasol causes more gene change than miconazole. There changing profile have a few overlap.
- Clobetasol and miconazole target at different signaling pathway.



Clobetasol induce the remylenation through glococorticoid receptor signaling pathway

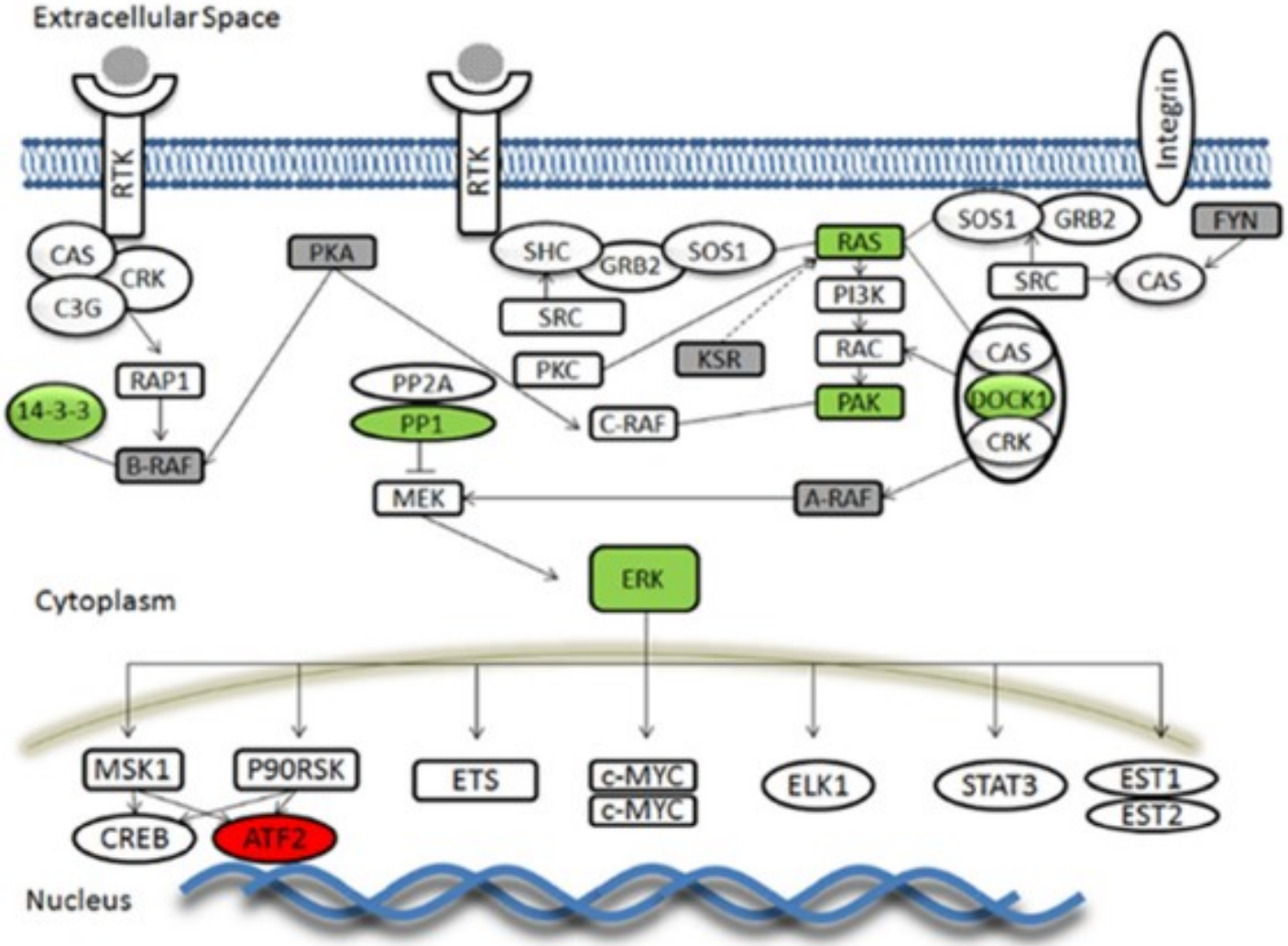
- Glococorticoid receptor signaling pathway is known be important regulator of myeling gene expression.
- Glococorticoid receptor pathway can also enhance Schwann-cell-mediated myelination in the peripheral nervous system





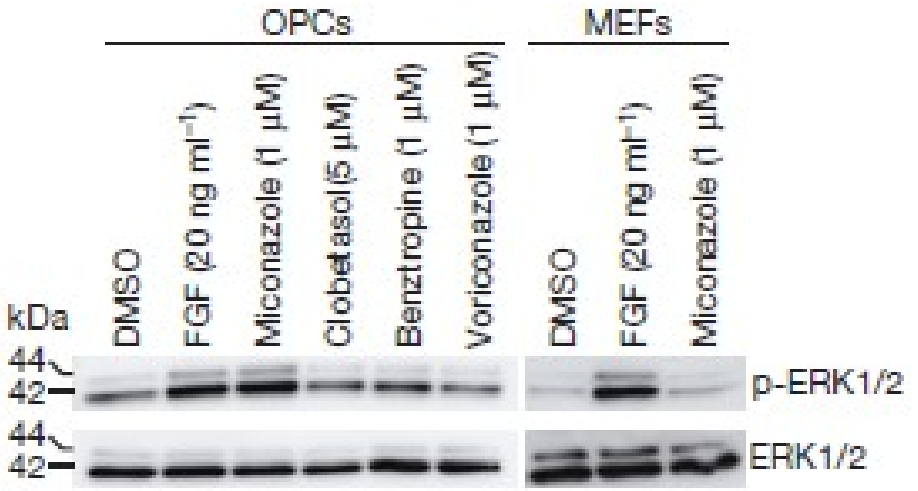
The mechanism of miconazole is identified through phosphoproteomic analysis

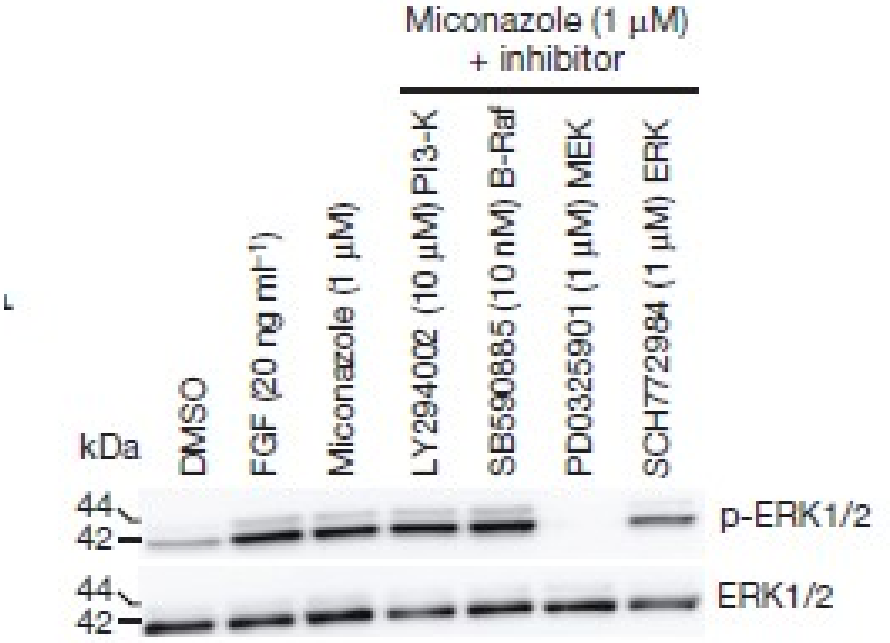
- Phosphoproteomic analysis



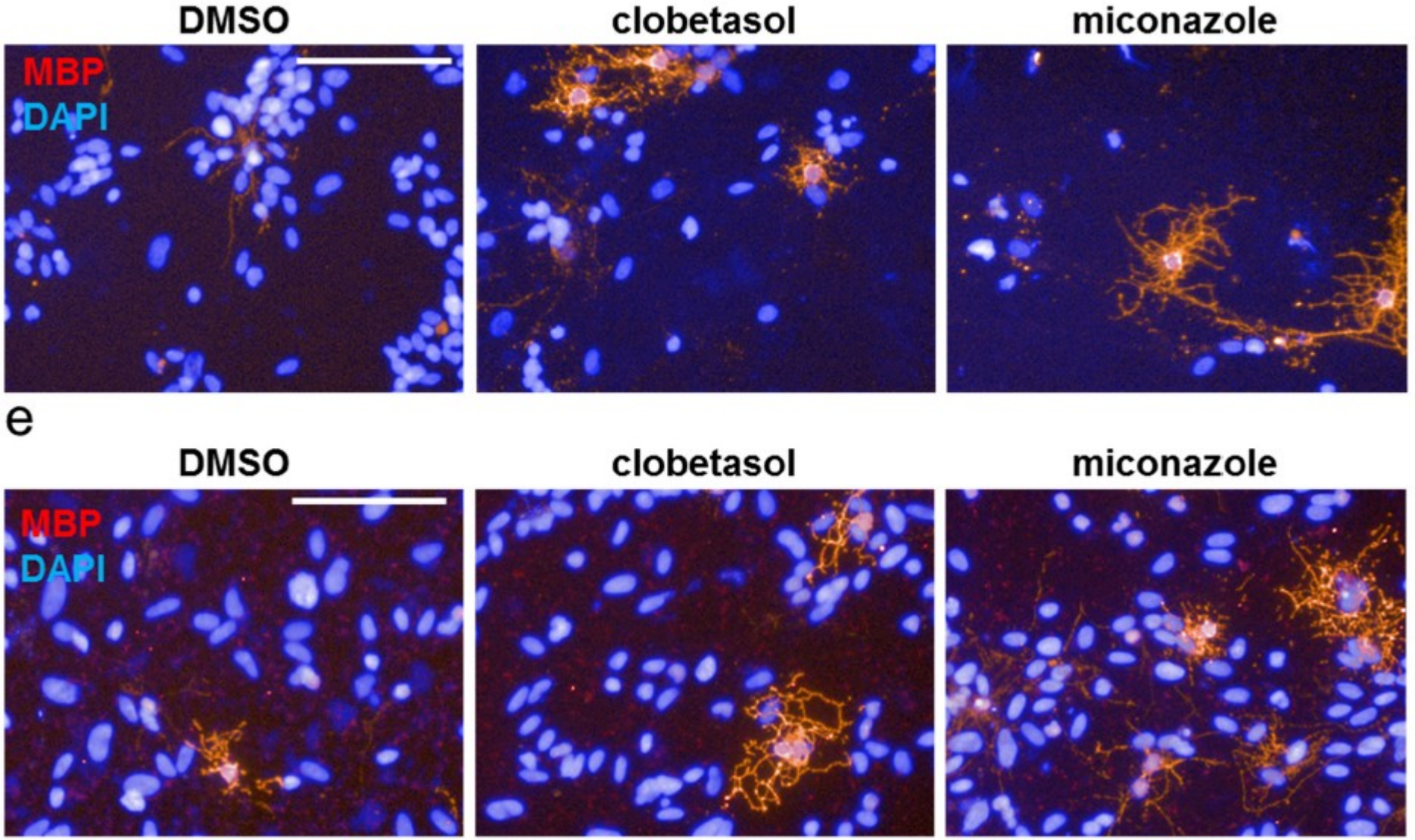
Miconazole works through ERK1/2 pathway

- Miconazole treated OPCs shows strong and sustained phosphorylated ERK1/2.
- But clobetasol and benztropine cannot induce ERK1/2 phosphorylation.
- The phosphorylation of ERK1/2 is blocked by inhibit its upstream MAPK pathway.
- An analog to miconazole, voriconazole, failed to induce phosphorylation of ERK1/2.



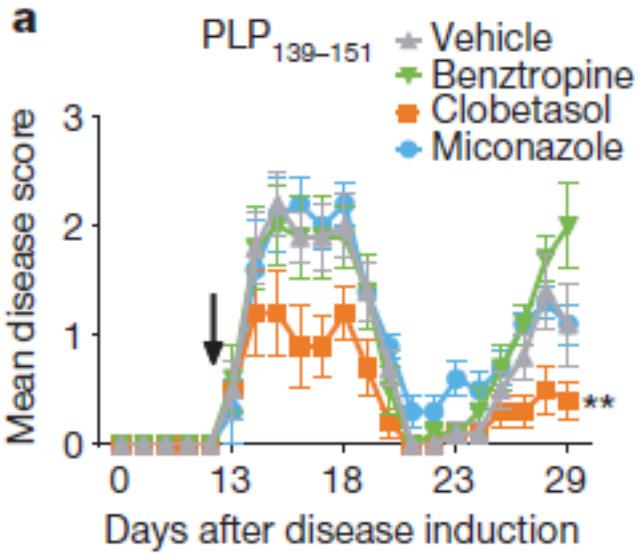


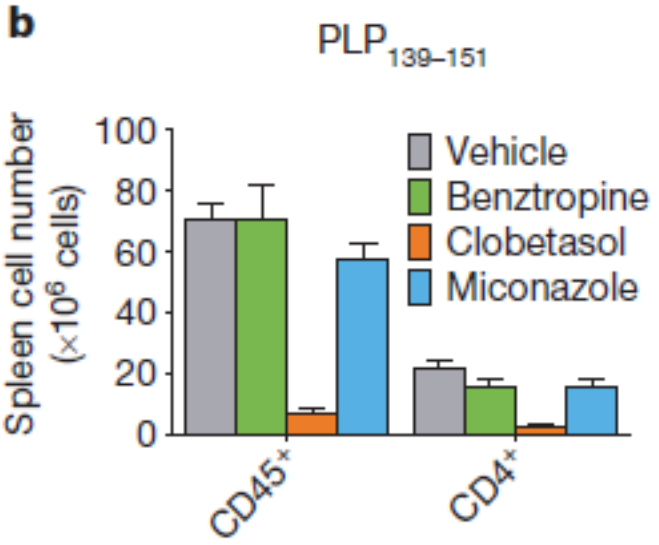
The effect of clobetasol and miconazole on human OPC



The effect of clobetasol and miconazole on immune-cell survival and function

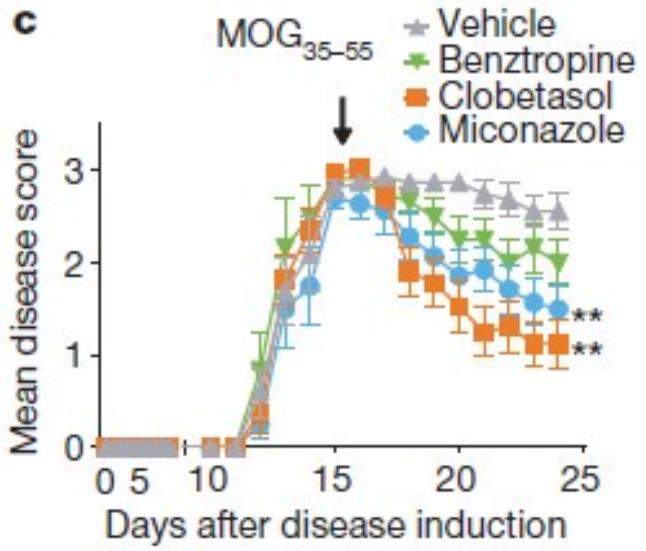
- Clobetasol can alter the differentiation of T-cell.
- This is due to its corticosteroid property.
- This result is confirmed by the evidence that it can resude the T-cell number in spleen.
- Miconazole and benztropine cannot inhibit T-cell proliferation.

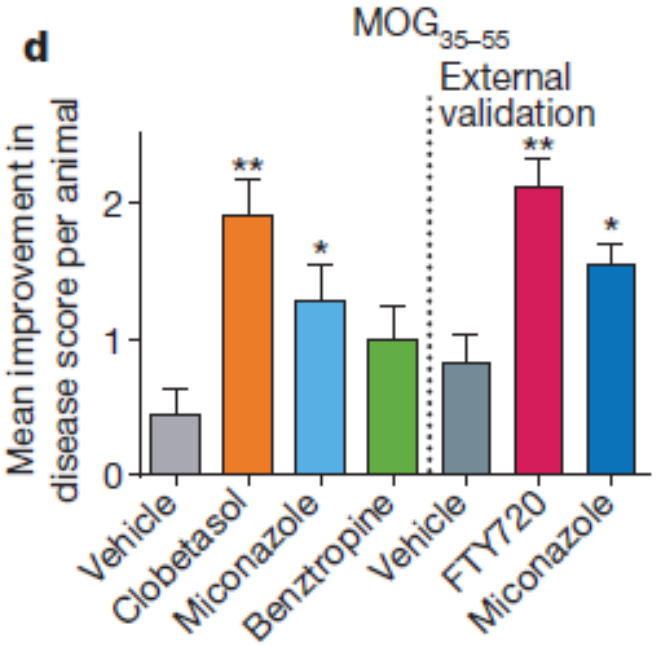




Encephalomyelitis(EAE) model to test whether drugs can reverse the disease

- MOG induced EAE mouse model was used to test the effect of clobetasol and miconazole.
- Animals in both treatment group regain one or both hindlimb.
- Benztropine also works but with lower efficiency.
- The histological immunostain shows the functional recovery by MBP expression.





Summary

- This group generated a high-content screening with the purpose of looking for effective inducer of remyelination.
- Two drugs, miconazole and clobetasol, were discovered to modulate OPC differentiation directly, enhance remyelination, and significantly reduce disease severity in mouse models of MS.
- These drugs use different mechanism to induce the remylination. Clobetasol can alter the gene expression in glococorticoid receptor pathway, while miconazole use a MEK dependent pathway to alter the phosphorylation of ERK1/2.
- Clobetasol can inhibit the differentiation of T cell. But miconazole only works as an inducer of remylination.