nature

SWISS NETWORK FOR INTERDISCIPLINARY EDUCATION IN CHEMICAL BIOLOGY

Brain-restricted mTOR inhibition with binary pharmacology

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- Chemical genetics to study the **target signaling protein**

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- Small molecules and synthetic Chemistry
- Modulation of antigen presentation

Background

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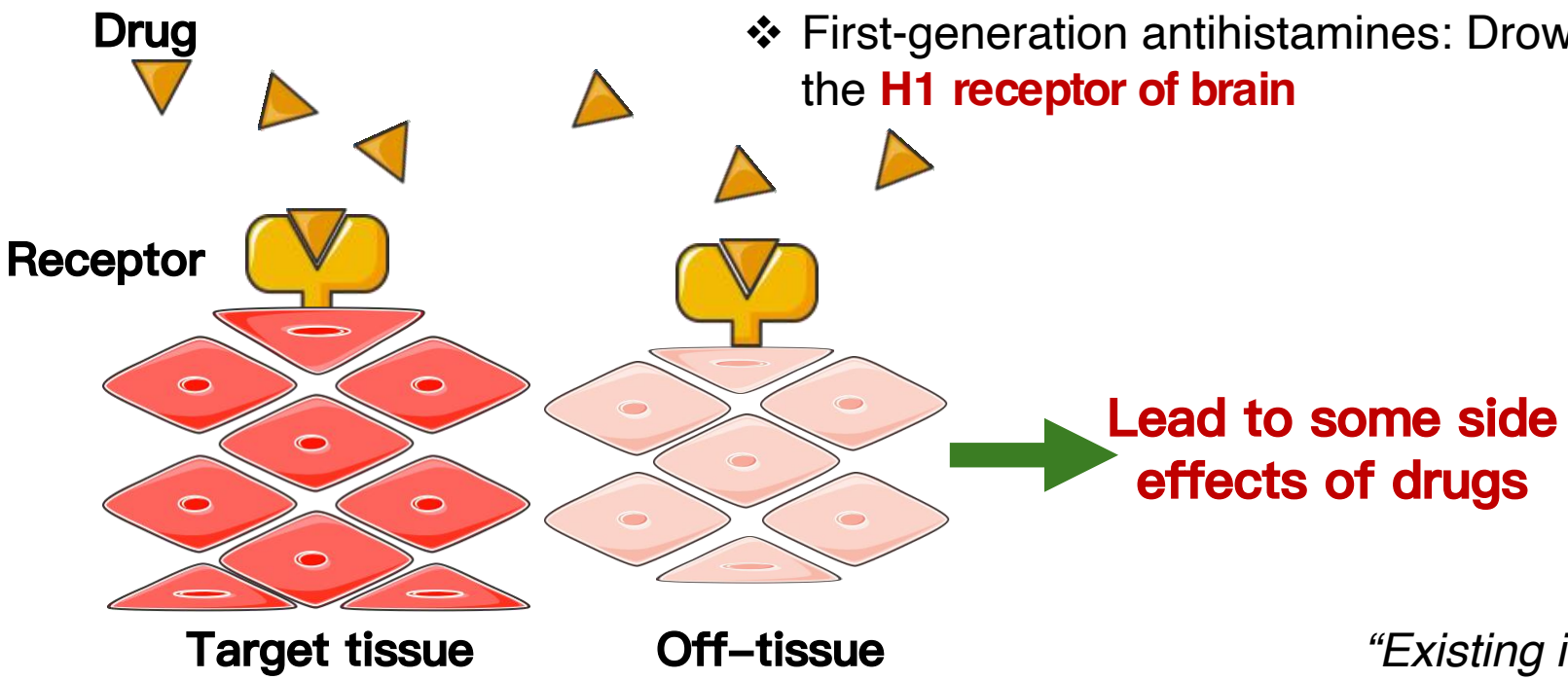
On-Target-Off-Tissue Toxicity

- Satatin: Myopathy caused by inhibition of **HMG-coA reductase**
- First-generation antihistamines: Drowsiness caused by blockade of the **H1 receptor of brain**



"Existing inhibitors of mTOR do not have a confined effect on CNS…"

Background

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❖ 3rd generation TOR kinase inhibitor:

A **brain permeable inhibitor** with FRB domain binding site and mTOR ATP binding site

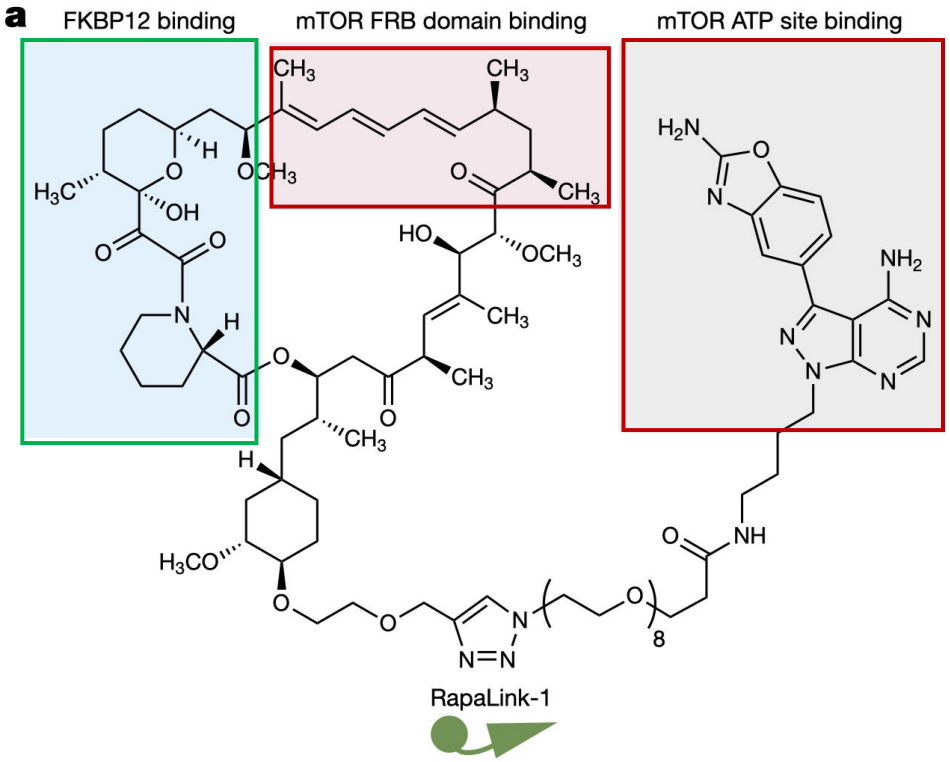
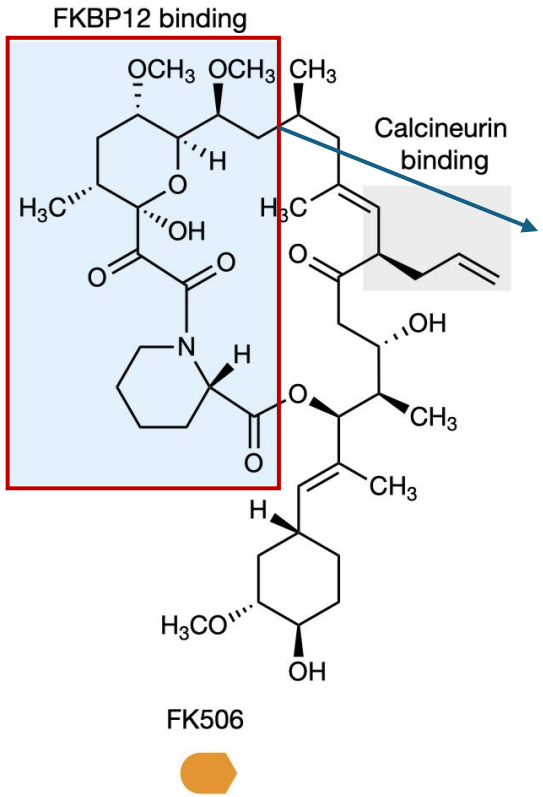


Fig1. Chemical structure of RapaLink–1

❖ Ligand of FKBP12(RapaBlock):

A brain–impermeant FKBP12 ligand



Can compete with the RapaLink-1 outside of the brain **to reduce the inhibition outside of the brain**

Fig2. Chemical structure of FK506

The existence of FKBP12 is essential for RapaLink1 inhibition

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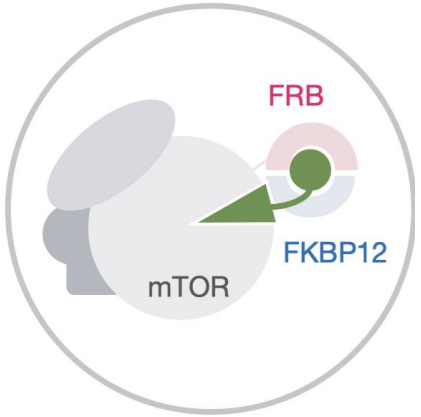
- The RapaLink1 can engage the active site in a **cell-free setting**



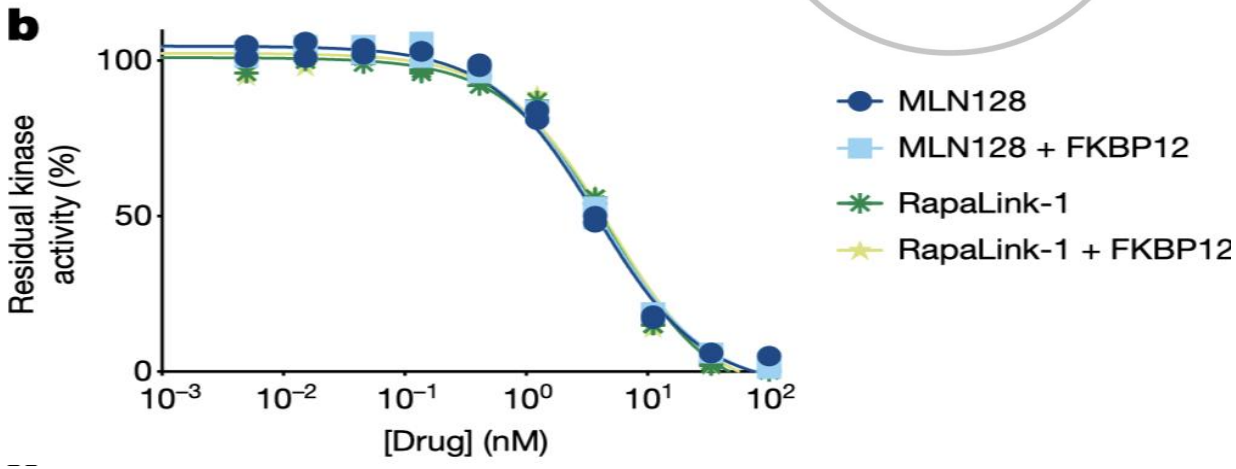


Fig1. Inhibition of mTOR activity by MLN128 and RapaLink with or without FKBP12 in vitro

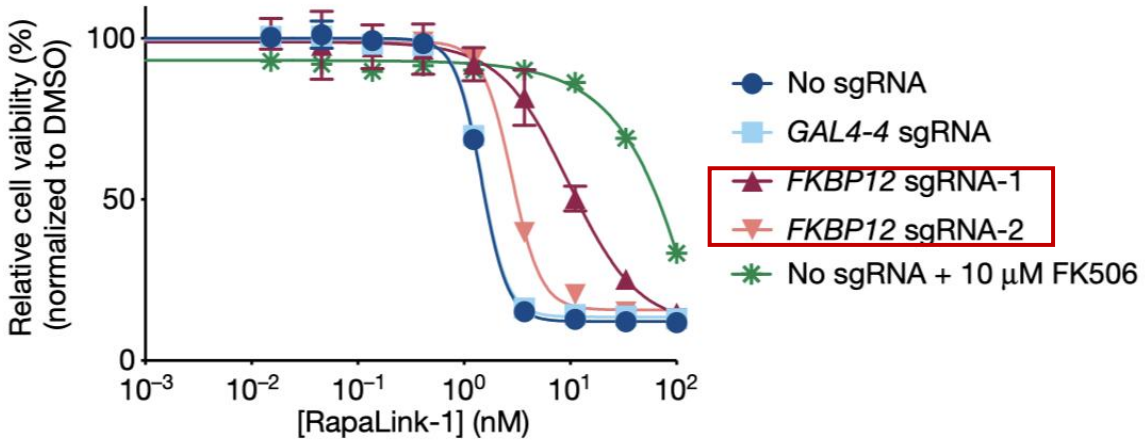


Fig2. Inhibition of mTOR activity by MLN128 and RapaLink with or without FKBP12 in K562 CRISPRi cells

- The knockout of FKBP12 in cells **significantly impede the inhibition of mTOR**

Adding FK506 could significantly reduce the binding between FKBP12 and RapaLink-1

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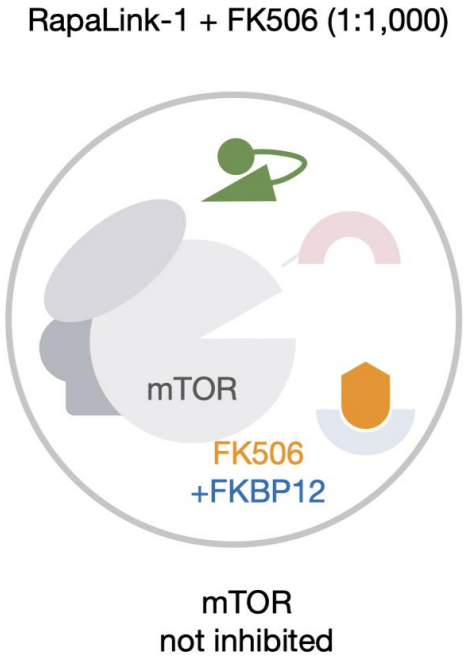

Fig1. Schematics of the proposed working model.

- RapaLink1 alone could **significantly inhibit the phosphorylation of the substrate of mTOR signaling**
- Adding extra FK506 could **prevent this inhibition**

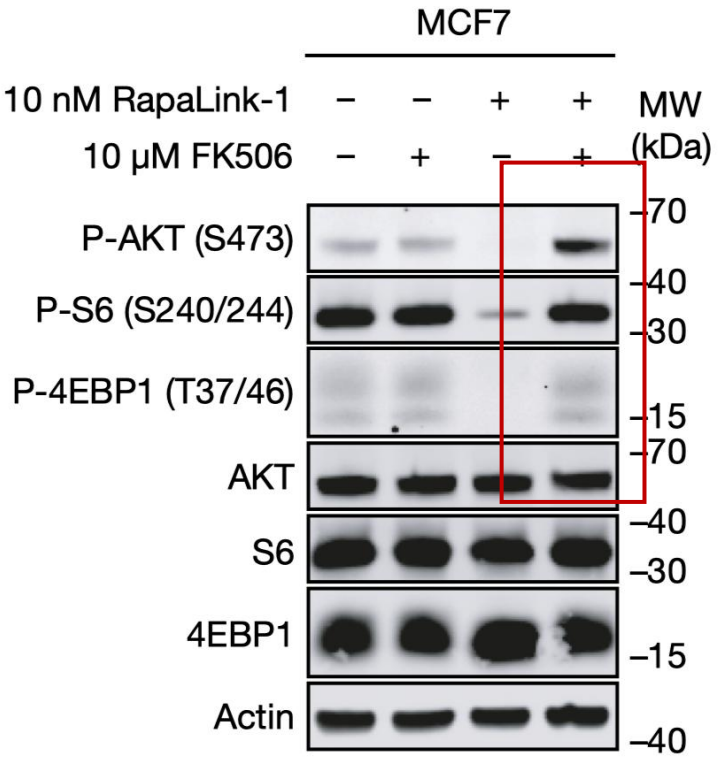

Fig2. Immunoblot analysis of mTOR signalling in MCF7 cells

Synthesis of the Brain-Imperment RapaBlock

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- Known FKBP12 ligands **readily cross the Blood-Brain Barrier(BBB)**
- The FK506 has the advantage of activating **immunosuppression**

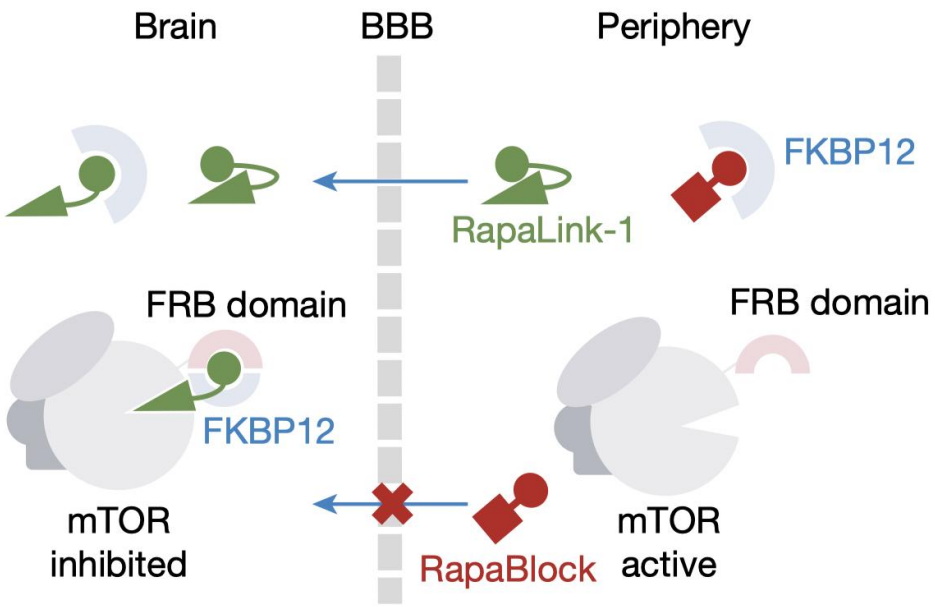

Fig1. Proposed model to achieve brain–specific mTOR inhibition through the combination

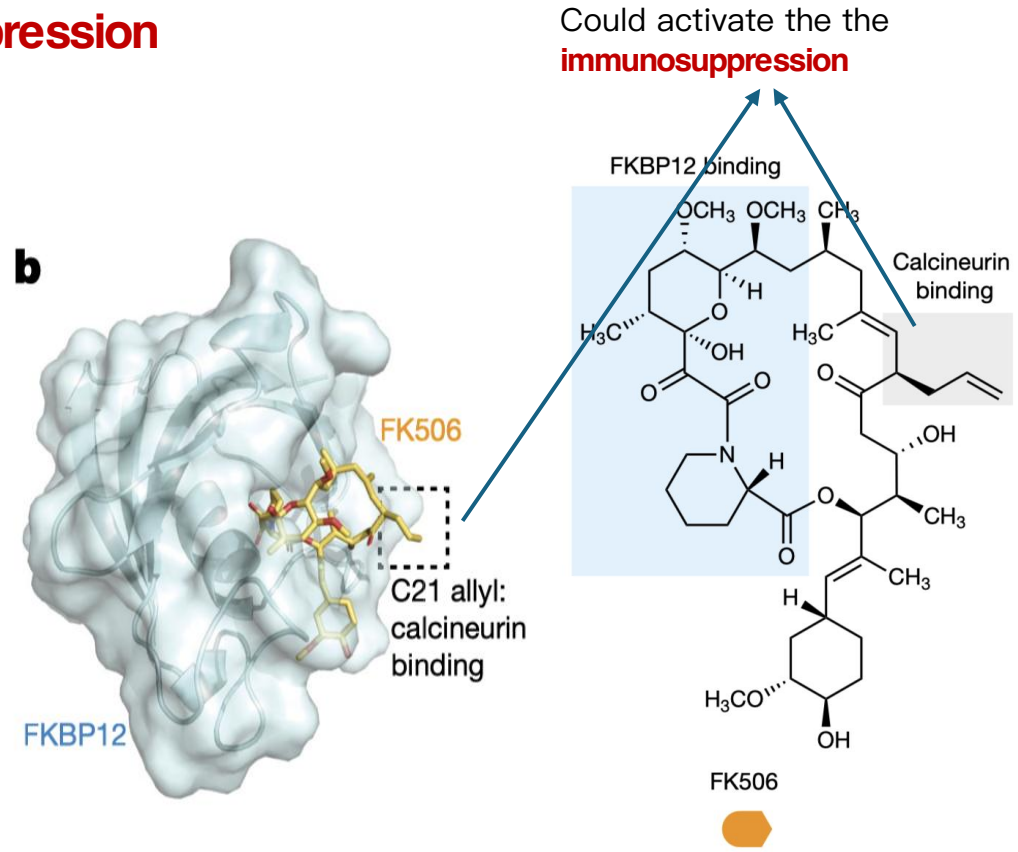

Fig2. FK506—FKBP12 co–crystal structure(left) and Chemical structure(Right) shows the modification on FK506

Synthesis of the Brain-Imperment RapaBlock

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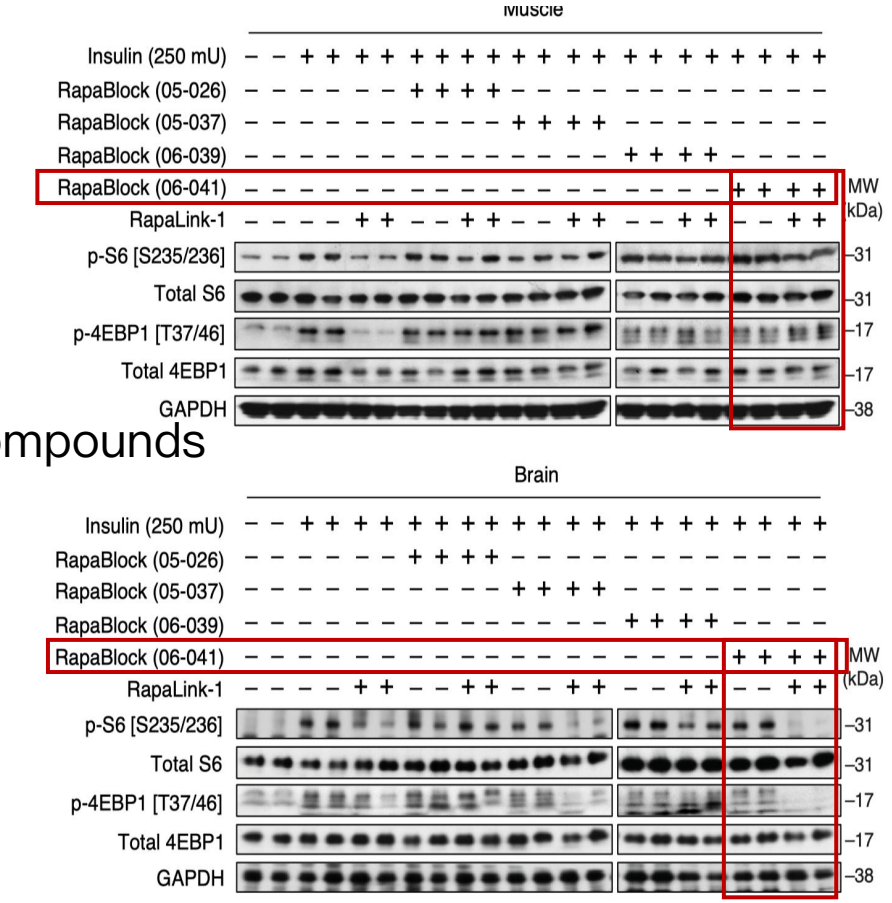
Synthesize the **Shield-1 and FK506 derivatives** which has a potent binding affinity to FKBP12

Four Potential compounds

Screened these compounds in cell, whether they protect mTOR from inhibition by RapaLink-1



FKBP12 Binding moiety

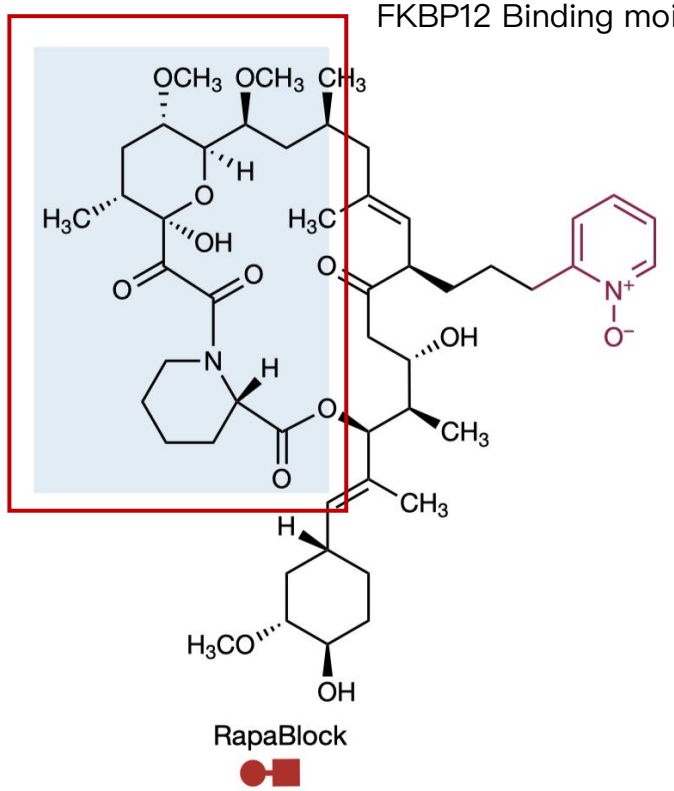


Fig1. Chemical structure of RapaBlock.

RapaBlock showed a comparable affinity with FK506 without affecting mTOR signalling

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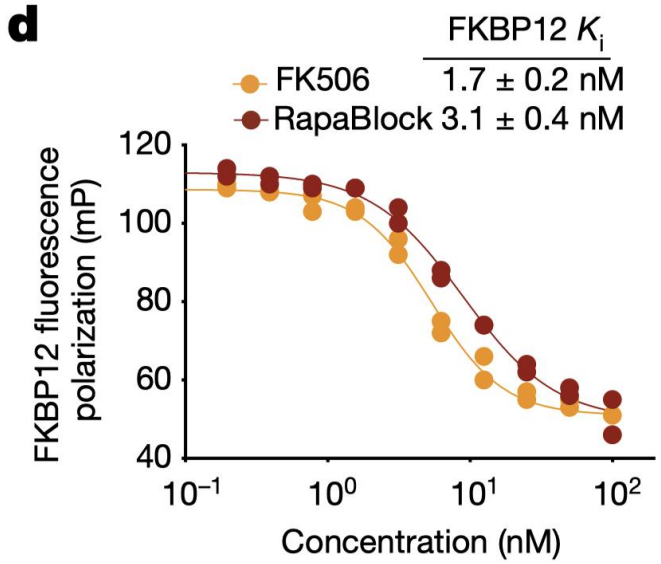

Fig1. Competition fluorescence polarization assay result of FK506 and RepaBlock

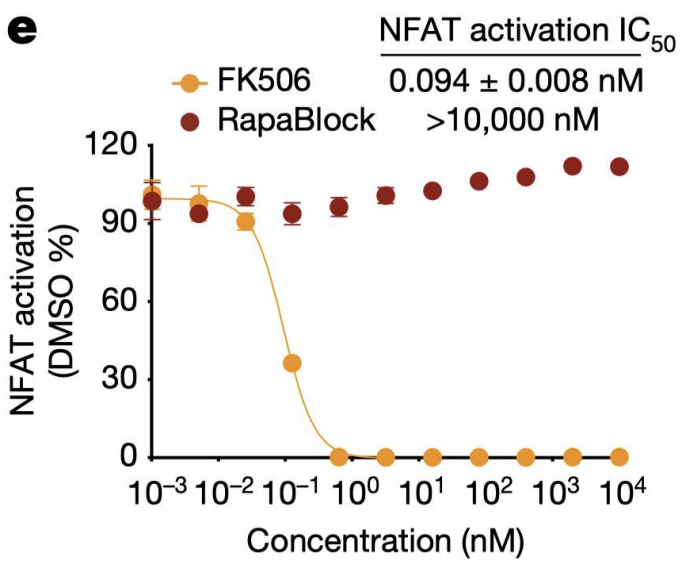

Fig2. Jurkat cells expressing a luciferase under the control of the NFAT transcription response element

- RapaBlock and FK506 bind to FKBP12 with **comparable affinity**
- RapaBlock does not exhibit any inhibitory activity for **calcineurin**

- RapaBlock does **not affect mTOR signalling** but attenuates the pharmacological effects of RapaLink-1 in a **dose-dependent manner**

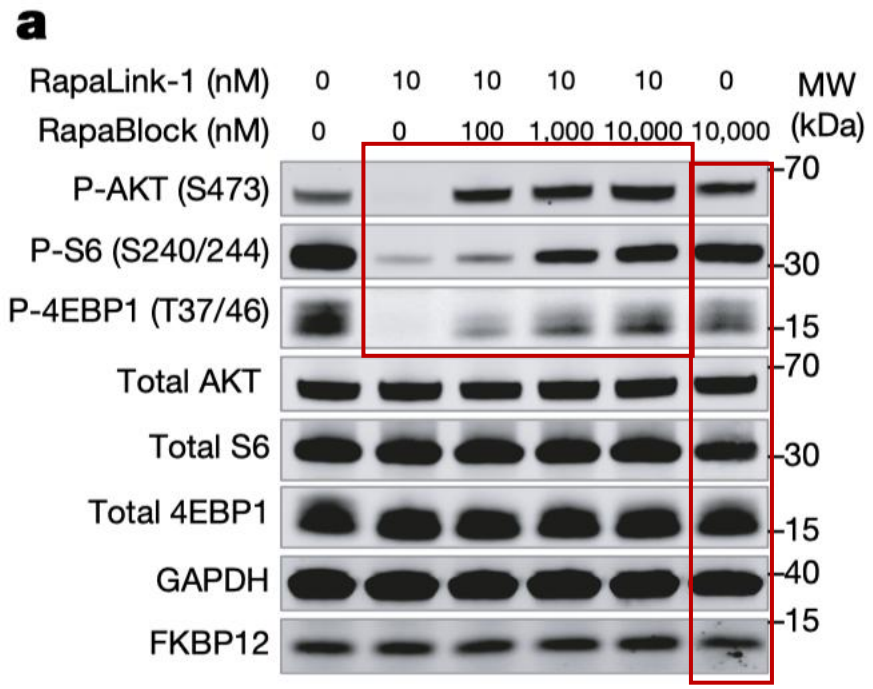

Fig3. MCF7 cells were treated with a combination of RapaLink–1 and RapaBlock

RapaBlock showed a comparable affinity with FK506 without affecting mTOR signalling

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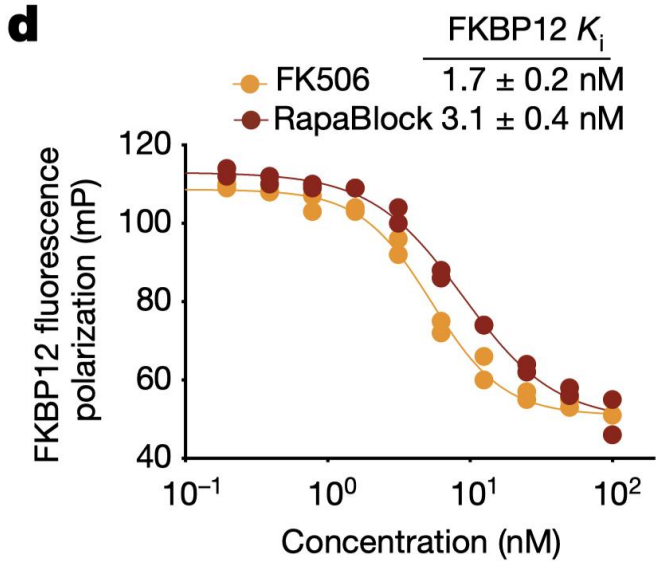

Fig1. Competition fluorescence polarization assay result of FK506 and RepaBlock

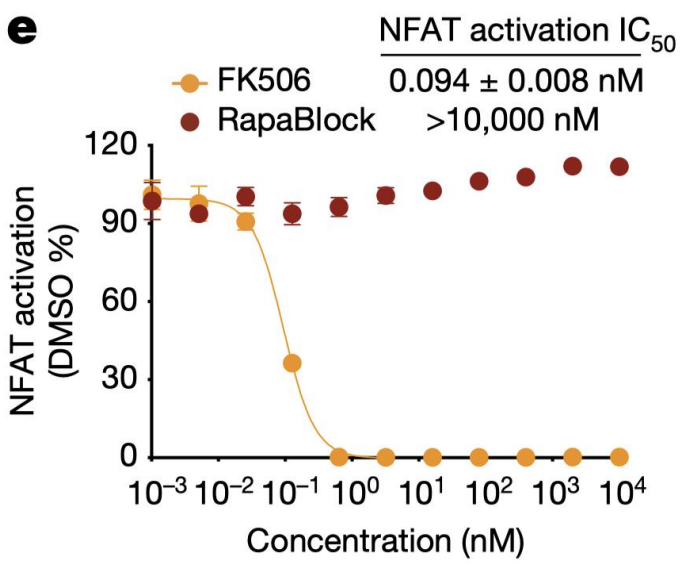

Fig2. Jurkat cells expressing a luciferase under the control of the NFAT transcription response element

- RapaBlock and FK506 bind to FKBP12 with **comparable affinity**
- RapaBlock does not exhibit any inhibitory activity for **calcineurin**

- RapaBlock can also prevent the inhibition caused by **Rapamycin**

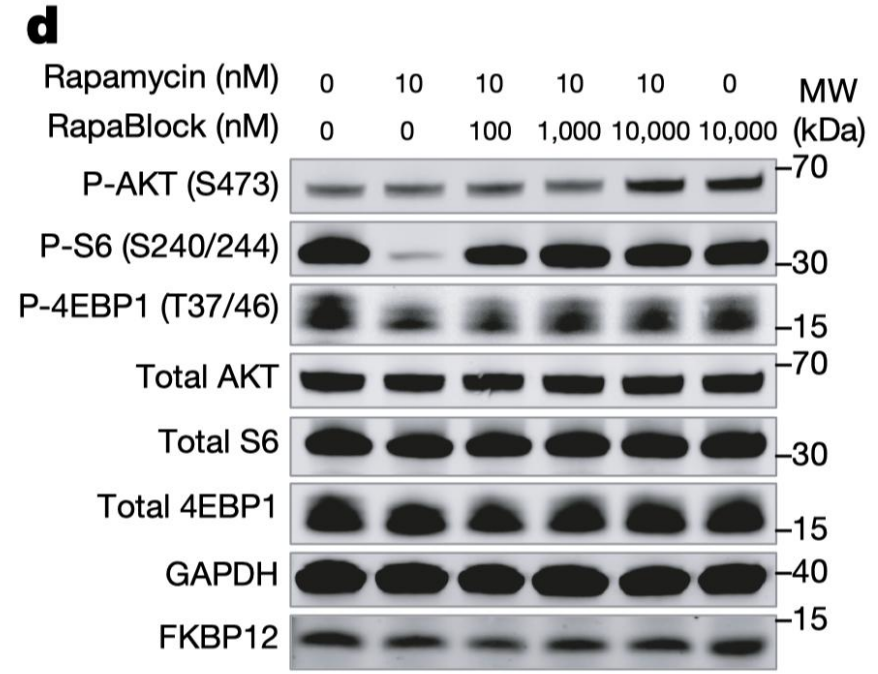

Fig4. . MCF7 cells were treated with a combination of Rapamycin and RapaBlock

RapaBlock protect cell from inhibition by RapaLink1 and Rapamycin

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mTOR signaling *is a central pathway regulating T cell growth, proliferation*

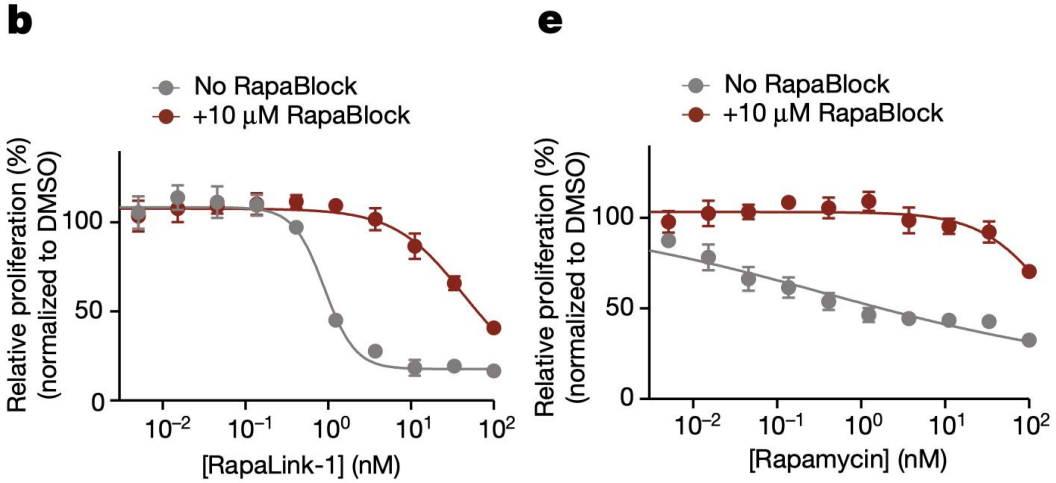

Fig1. Human PBMCs were stimulated with anti–CD3 and anti–CD28 in the presence of varying amounts of RapaLink–1 and RapaBlock(left), rapamycin and RapaBlock(Right)

- RapaLink-1 **inhibited proliferation of PBMCs**
- Addition of **RapaBlock abolished this effect**
- RapaBlock appeared **more effective at protecting PBMCs** from rapamycin-mediated growth inhibition

- RapaBlock renders RapaLink-1 and rapamycin **incapable of inhibiting mTOR**

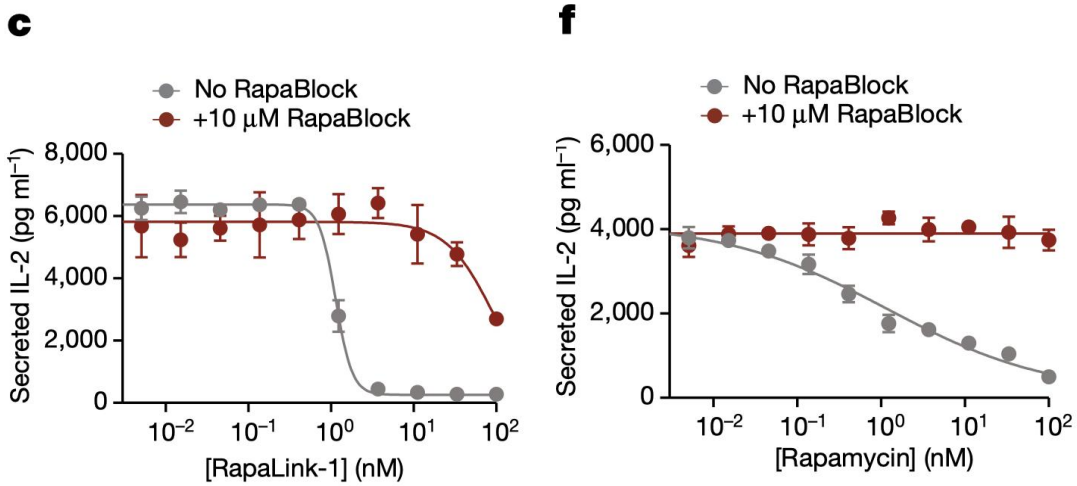

Fig2. Secretion of IL–2 in the cells were stimulated with anti–CD3 and anti–CD28 in the presence of varying amounts of RapaLink–1 and RapaBlock(Left), RapaLink1 and rapamycin

The combination of RapaBlock and RapaLink1 allow the brain-specific inhibition of mTOR

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- The combination of RapaLink1 and RapaBlock **do not affect the mTOR activity in Brain** but can **inhibit this activity in Muscle**
- The fold change before and after adding RapaBlock in **Muscle is significant**, while this fold change is **not significant in brain**

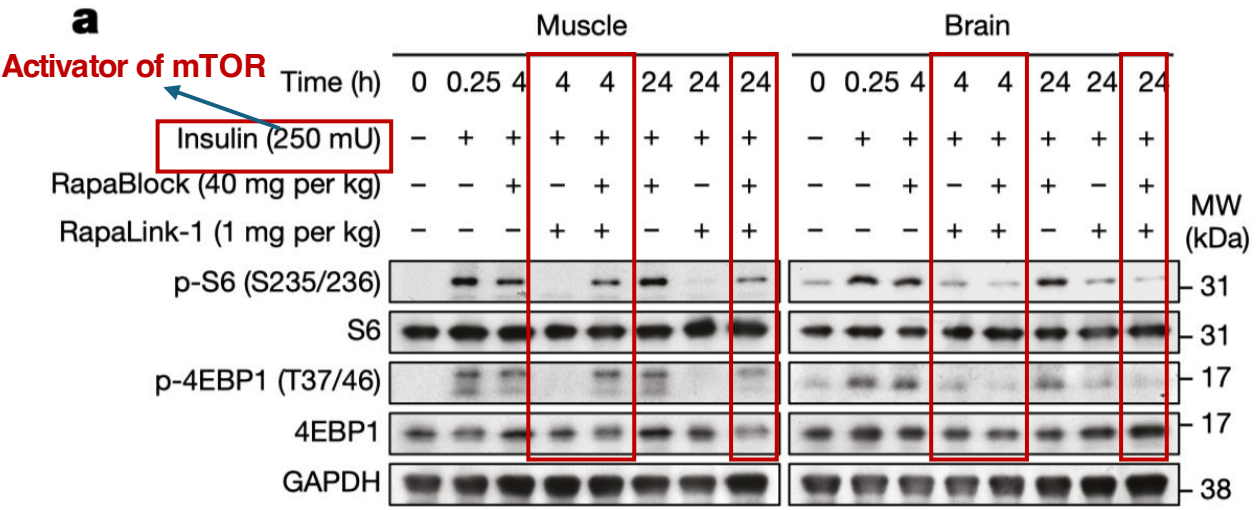

Fig1. immuno blot analysis of mTOR signalling in mouse whole brain and skeletal muscle after treatment of RapaBlock and RapaLink combination

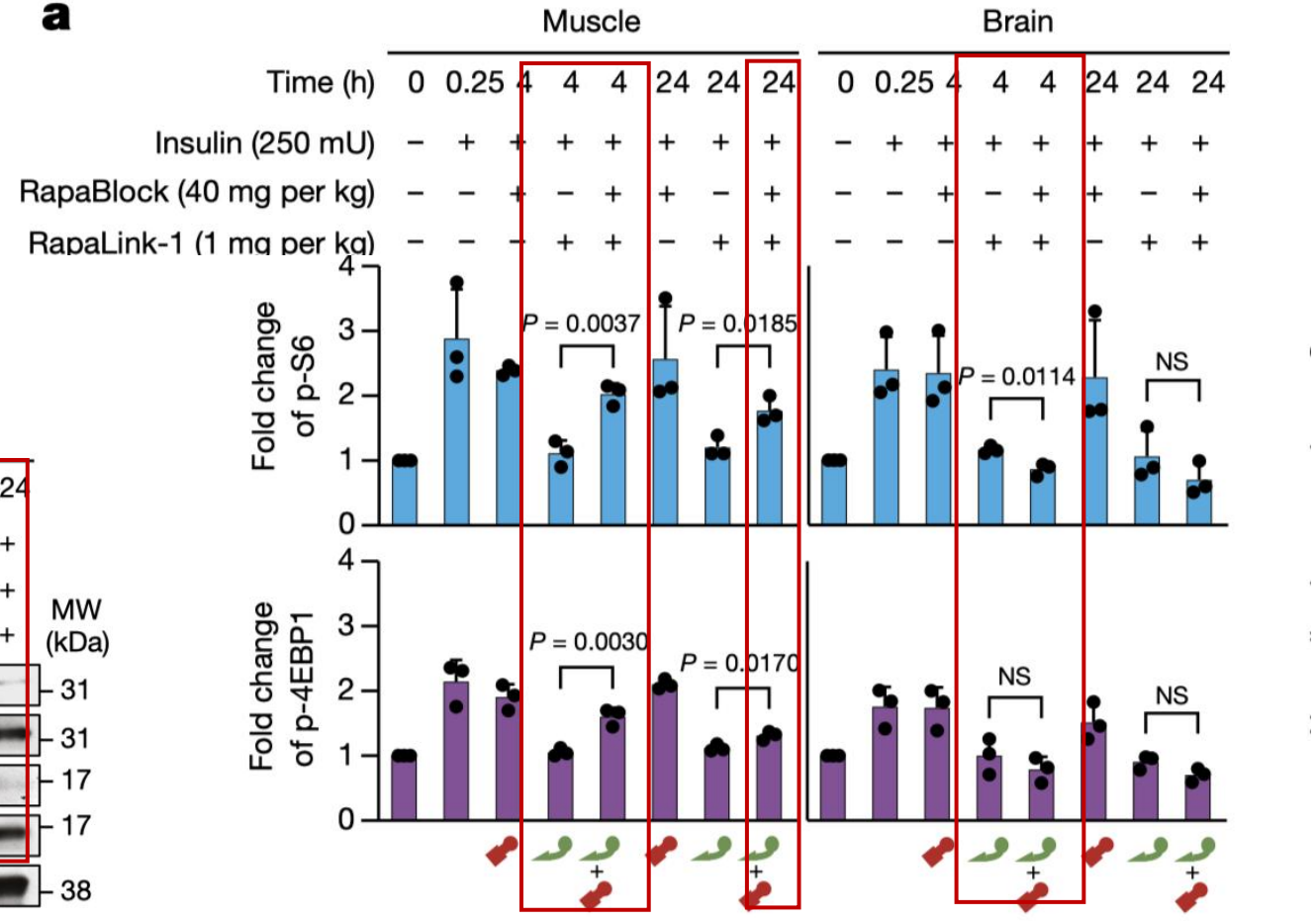

Fig2. Fold change of mTOR signalling in mouse whole brain and skeletal muscle after treatment of RapaBlock and RapaLink combination

The combination of RapaBlock and RapaLink1 could retain the efficiency of RapaLink1 in treating glioblastoma

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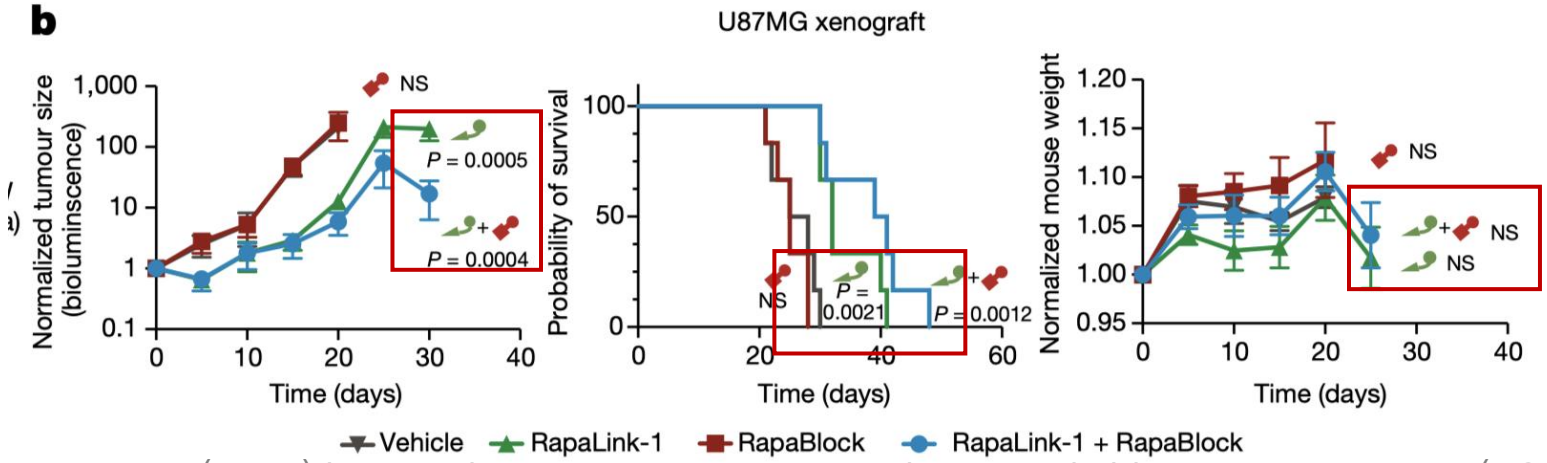

Fig1. Mice (n = 7) bearing luciferase–expressing orthotopic glioblastoma xenografts (U87MG) were treated intraperitoneally every 5 days

- Combination significantly **suppressed tumor growth**
- Combination **conferred the survival rate**
- **No significant weight** changes observed

- **None of the conditions** suppressed the rapid growth of the tumors
- The combination of RapaLink-1 and RapaBlock **conferred a survival benefit**

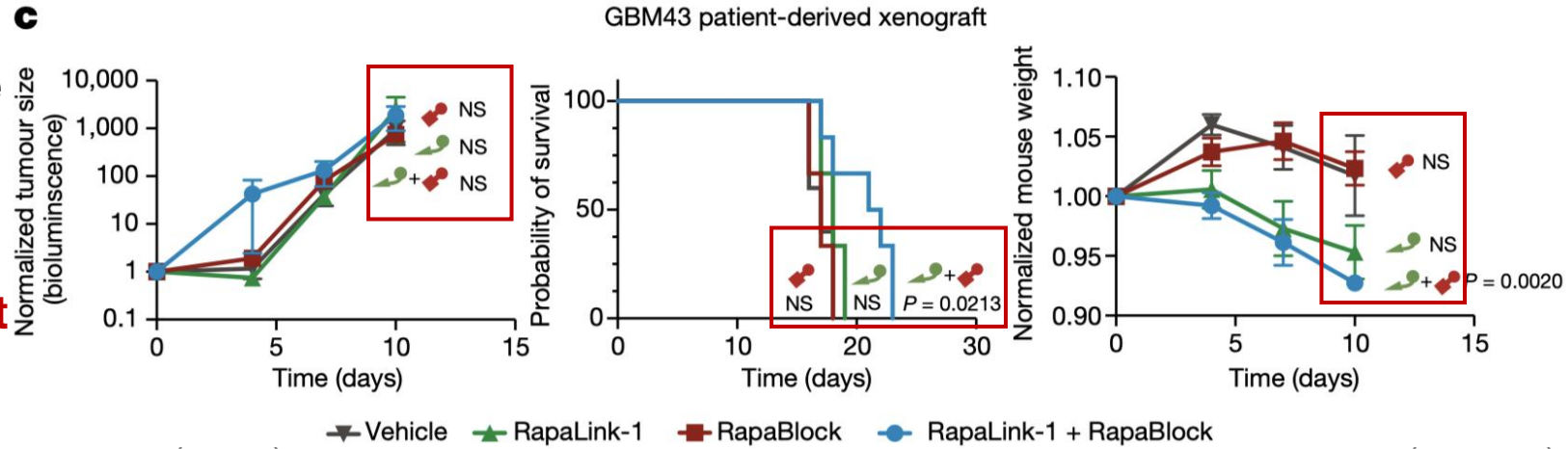

Fig2. Mice (n = 5) bearing luciferase–expressing orthotopic glioblastoma xenografts (GBM43) were treated intraperitoneally every 5 days

The generalizability of this Strategy

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*"**Other bifunctional molecules** consisting of **a FKBP12-binding moiety and a kinase inhibitor moiety** could be conditionally active and amenable to modulation with RapaBlock"*

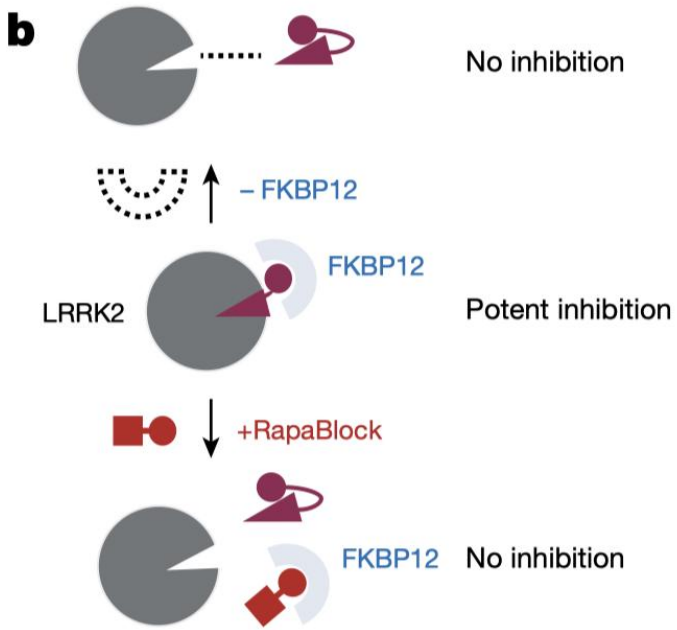

Fig1. Proposed working model for FKBP–dependent kinase inhibitor

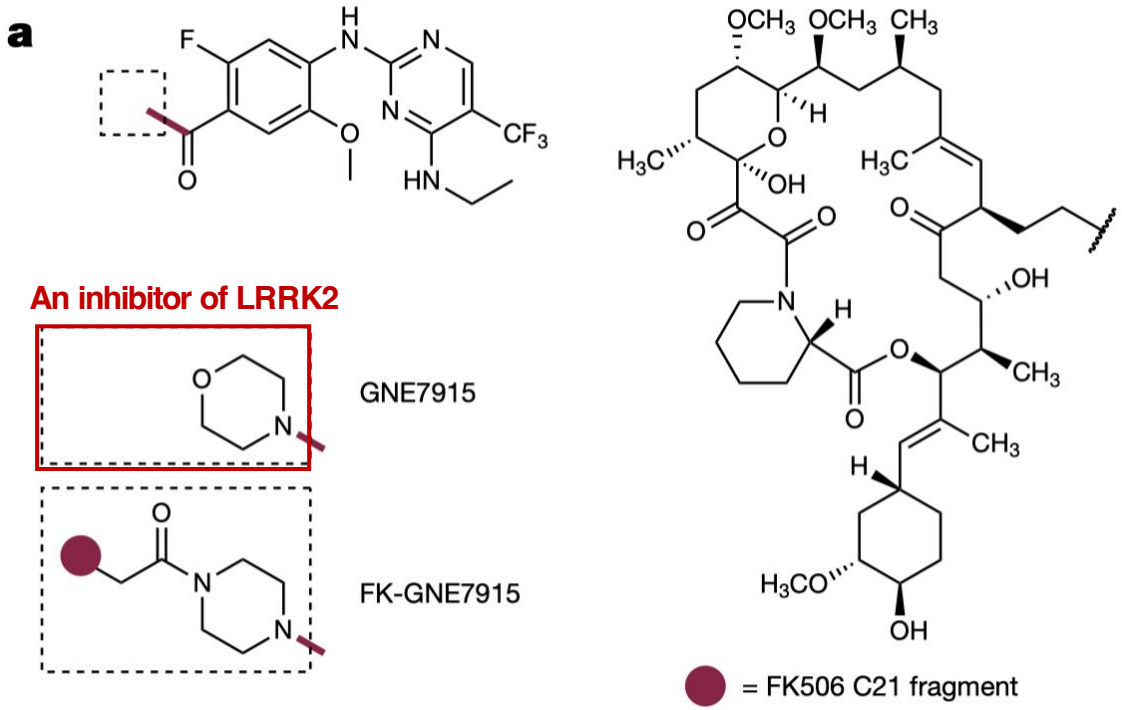

Fig2. Structures of GNE7915 and FK–GNE7915

The strategy of Combining RapaBlock with other FKBP12-dependent inhibitor further proved

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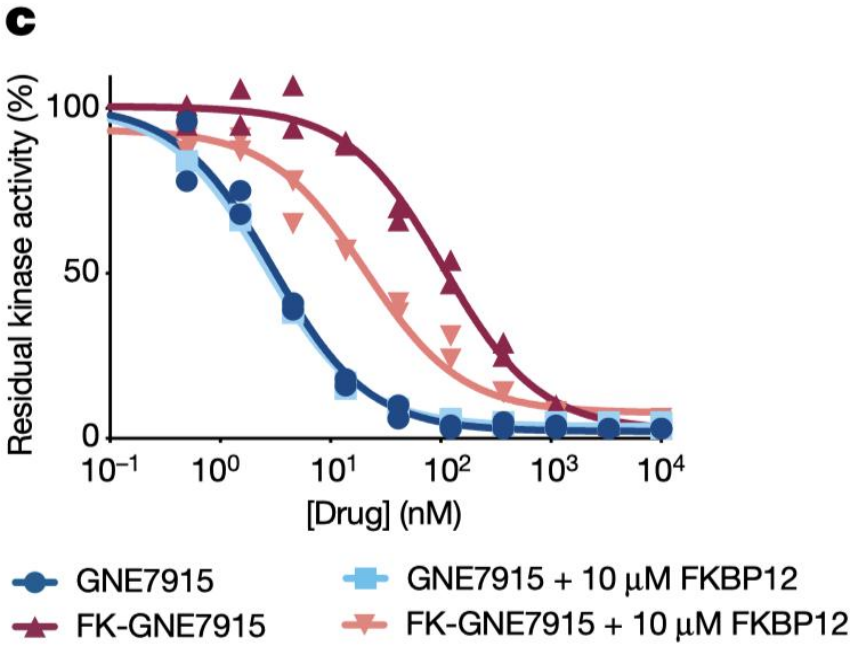

Fig1. Kinase inhibition in the absence or presence of supplemented 10 μM recombinant FKBP12 protein

- In ***in vitro* LRRK2 kinase assays**, FK-GNE7915 was an inferior inhibitor to LRRK2

- But FK-GNE7915 is **more potent** in inhibiting the activity of LRRK ***in vivo***

- Combination of **FK-GNE7915 and RapaBlock** had no effect on LRRK2 activity

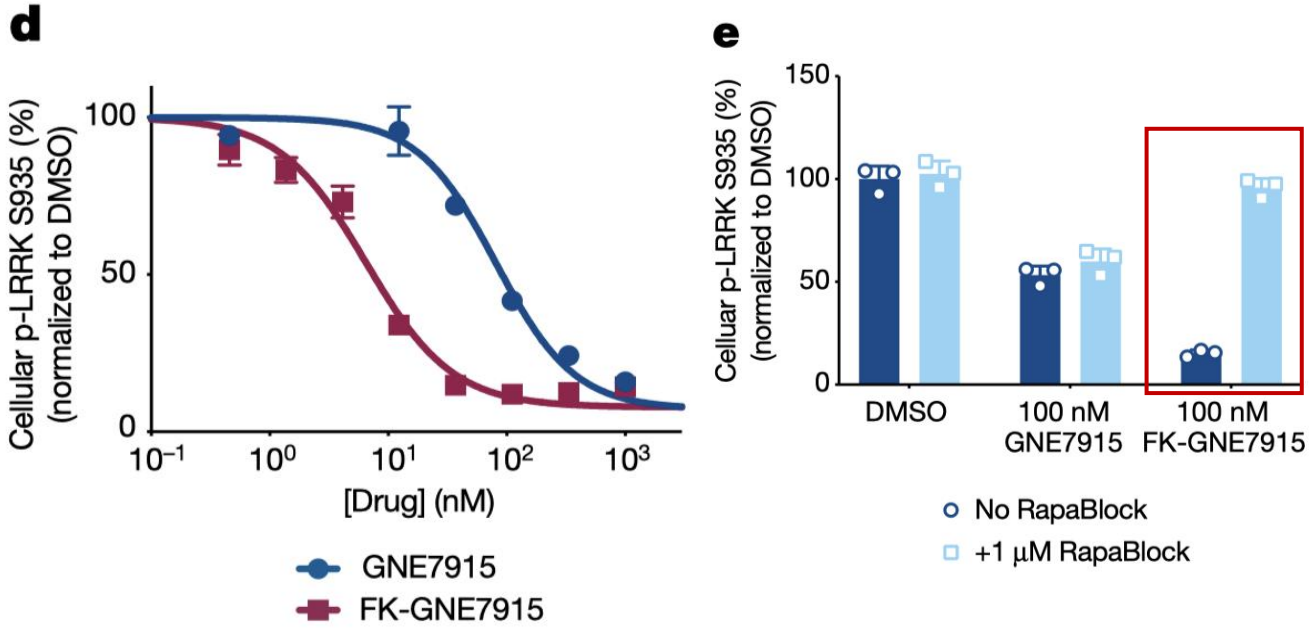

Fig2. RAW264.7 cells were treated with GNE7915, FK–GNE7915 and/or RapaBlock

Conclusion

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Conclusion

- Experiment to prove the FKBP12 is essential for mTOR inhibition
- Prove the FK506 can abolish the inhibition of mTOR by RapaLink
- Extend the FK506 to RapaBlock which can also abolish the inhibition and are brain-imperment
- Vertify the affinity of RapaBlock and whether it will affect the mTOR signaling
- Cell based experiment to further prove the RapaBlock is reliable
- Inject the combination of RapaLink1 and RapaBlock to tissues to see whether it's brain-specific
- Use glioblastoma model to test the combination of RapaLink1 and RapaBlock
- Extend this technology to other FKBP12 dependent inhibitors

Limitation

The limited effect on animal model

Might induce the immune rejection in body

Do not further prove the reliability of the FK–GNE in animal model

Questions

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Why do you think this work can be published on *Nature* ?

Creative

Challenging

Generalizability

Any other strategy to reduce this on-target-off-tissue toxicity?

BBB-permeable acetyl-cholinesterase inhibitor, BBB-impermeable anticholinergic
Global activation + periphery inhibition

BBB-permeable oestrogen, BBB-impermeable oestrogen receptor modulator
Periphery Receptor inhibition