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Functional coupling of TRPM2 and extrasynaptic NMDARs exacerbates excitotoxicity in ischemic brain injury

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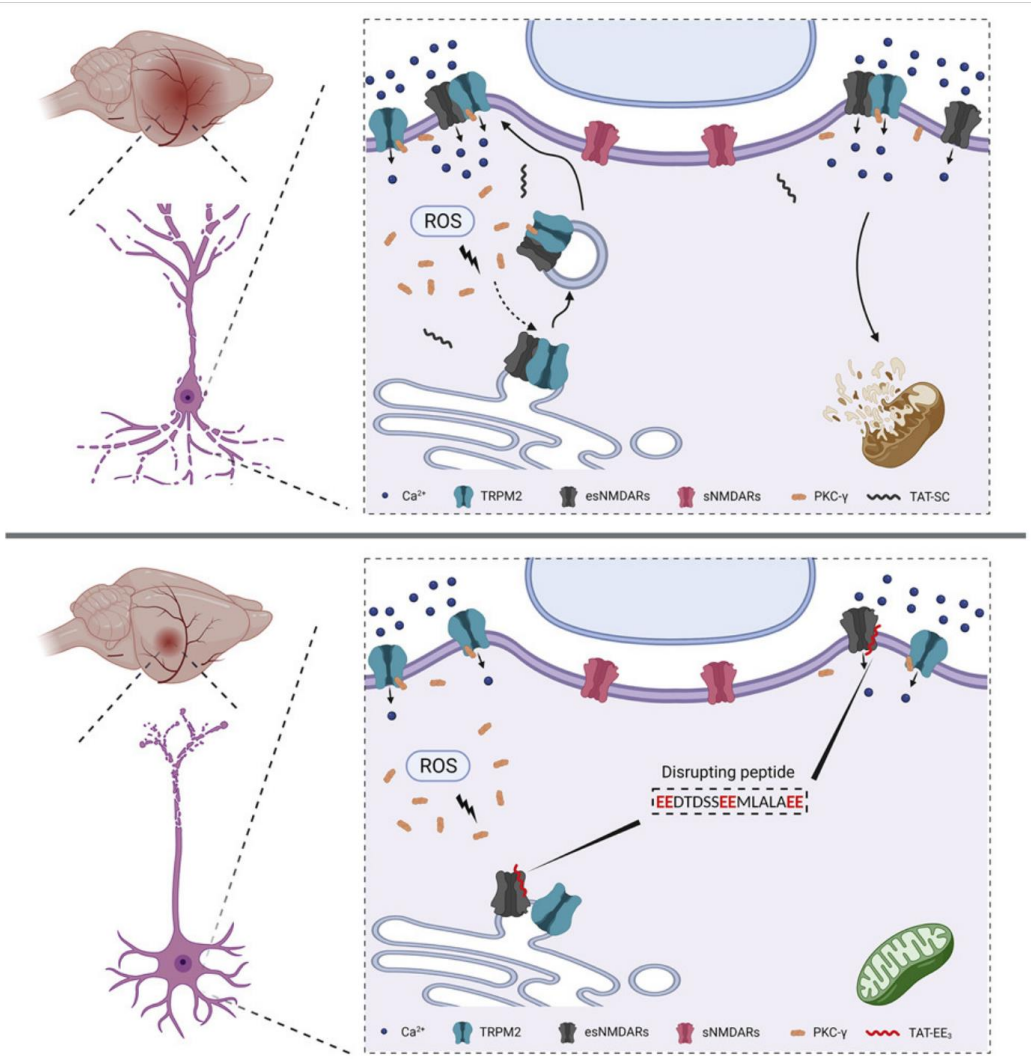
최하경

논문 선정 이유

✓Stroke 발생 시 나타나는 damage 공부

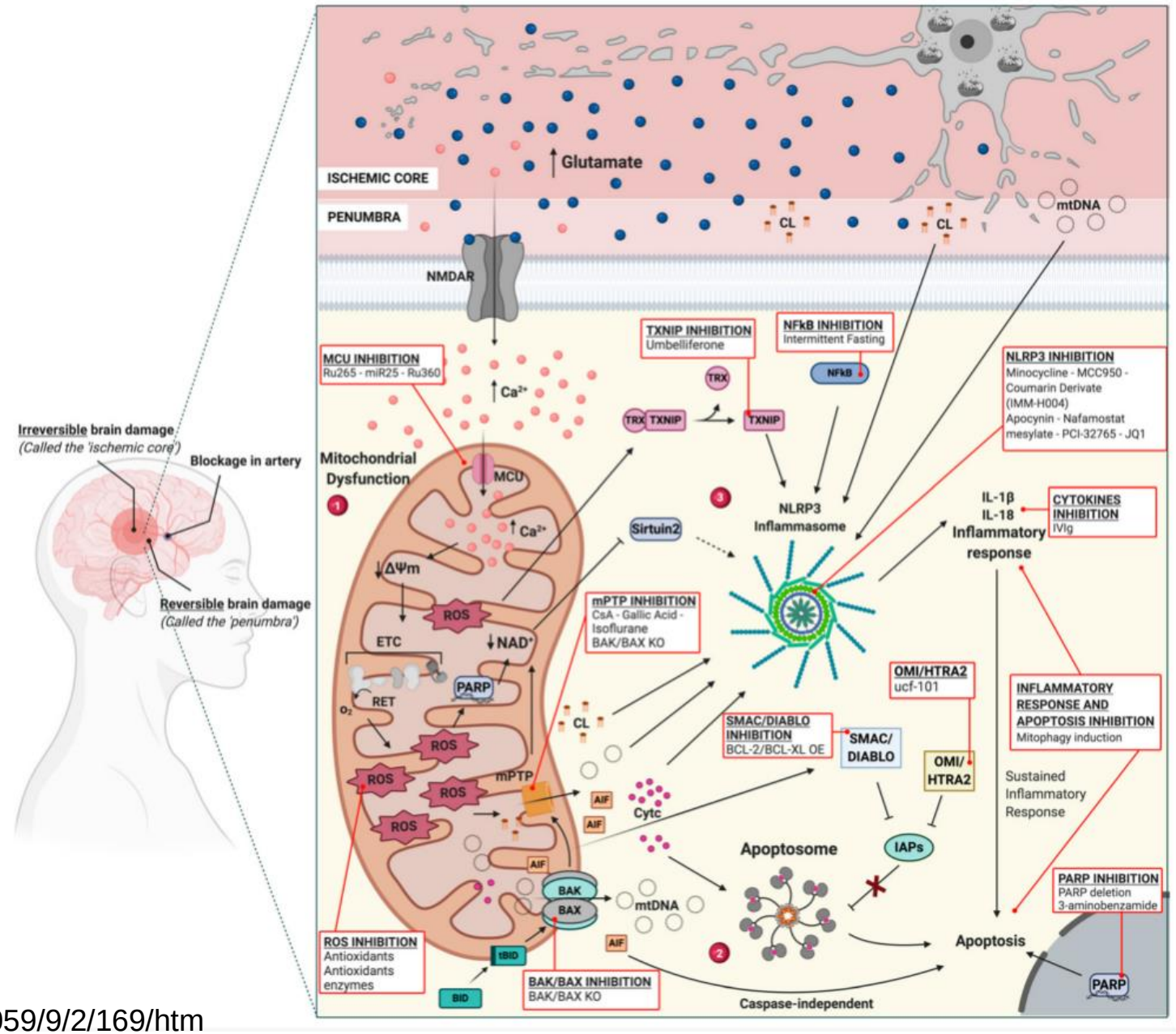
✓ ischemic damage를 일으키는 mechanism 공부

Abstract



Excitotoxicity induced by NMDA receptor (NMDAR) activation is a major cause of neuronal death in ischemic stroke. However, past efforts of directly targeting NMDARs have unfortunately failed in clinical trials. Here, we reveal an unexpected mechanism underlying NMDAR-mediated neurotoxicity, which leads to the identification of a novel target and development of an effective therapeutic peptide for ischemic stroke. We show that NMDAR-induced excitotoxicity is enhanced by physical and functional coupling of NMDAR to an ion channel TRPM2 upon ischemic insults. TRPM2-NMDAR association promotes the surface expression of extrasynaptic NMDARs, leading to enhanced NMDAR activity and increased neuronal death. We identified a specific NMDAR-interacting motif on TRPM2 and designed a membrane-permeable peptide to uncouple the TRPM2-NMDAR interaction. This disrupting peptide protects neurons against ischemic injury *in vitro* and protects mice against ischemic stroke *in vivo*. These findings provide an unconventional strategy to mitigate excitotoxic neuronal death without directly targeting NMDARs.

Introduction – Stroke



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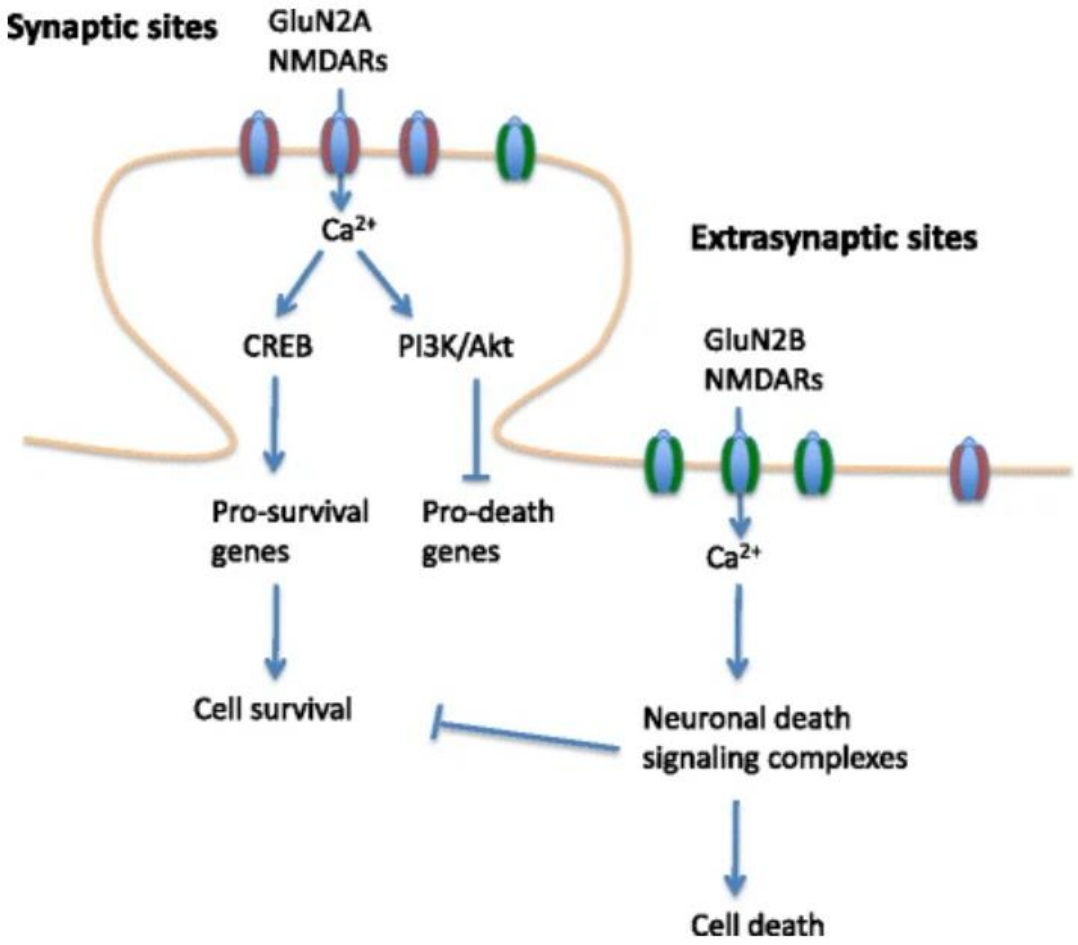
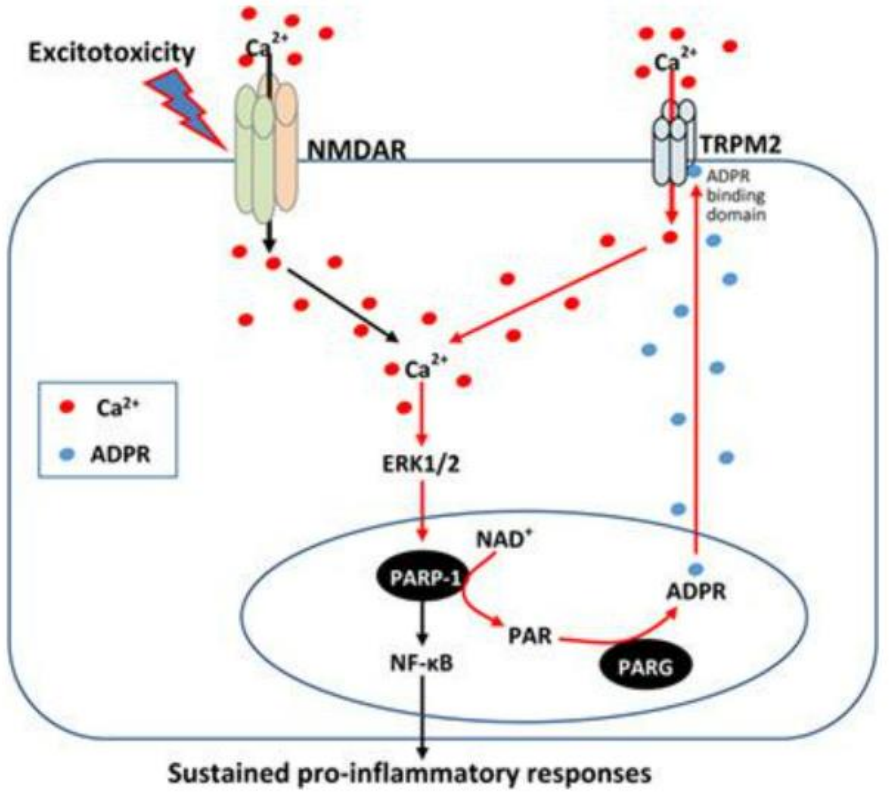
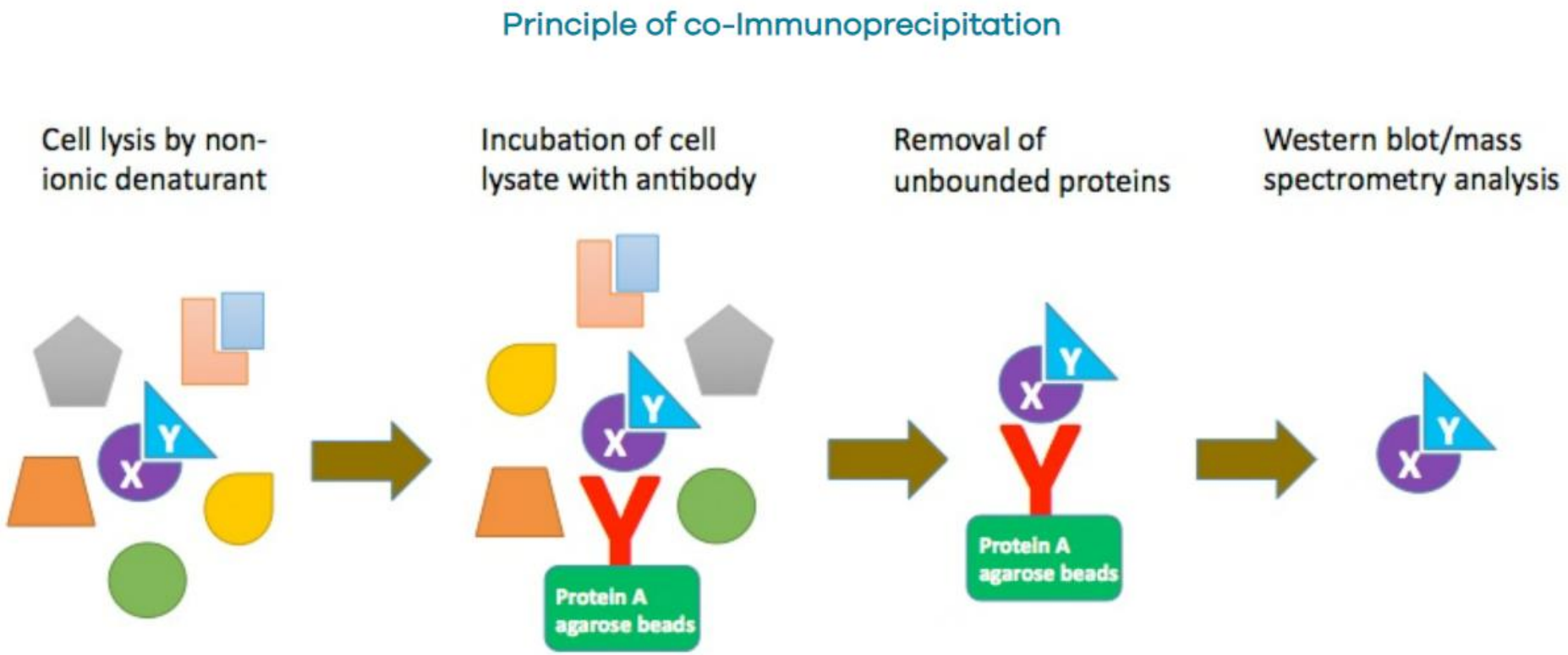
Introduction – NMDAR & TRPM2





https://molecularbrain.biomedcentral.com/articles/10.1186/s13041-018-0357-8

Introduction – coimmunoprecipitation



https://www.profacgen.com/Co-Immunoprecipitation-Co-IP.htm

Hypothesis & Purpose

- **Hypothesis: Intervention of TRPM2-NMDAR coupling may represent a promising therapeutic strategy for ischemic stroke**

- **Purpose: To investigate mechanism between TRPM2 and NMDAR in ischemic stroke**

❖ Specific Aims

Q1. Does TRPM2 deletion protect mice against ischemic stroke?

A1. TRPM2 has critical role in aggregating NMDAR-mediated Ca2+ overload, mitochondrial dysfunction, and neuronal death.

Q2. Does TRPM2 interact with NMDARs?

A2. EE3 of TRPM2 and KKR of NMDAR are essential for both physical and functional coupling between TRPM2 and NMDARs.

Q3. Does disruption of TRPM2-NMDAR interaction protect neurons against OGD?

A3. TAT-EE3 disrupts TRPM2-NMDAR coupling and reduces surface expression of NMDARs , preventing ischemic stroke.

Figure flow

1. Does TRPM2 deletion protect mice against ischemic stroke?

Figure 1. Neuron-specific *Trpm2* deletion protects the brain against ischemic damage

2. Does TRPM2 interact with NMDARs?

Figure 2. TRPM2 physically and functionally interacts with NMDARs

Figure 3. EE3 motif in TRPM2 mediates TRPM2-NMDARs coupling

Figure 4. KKR motif in GluN2a and GluN2b is required for the direct binding to the EE3 motif in TRPM2

Figure 5. N-tail of TRPM2 interacts with PKC- γ

3. Does disruption of TRPM2-NMDAR interaction protect neurons against OGD?

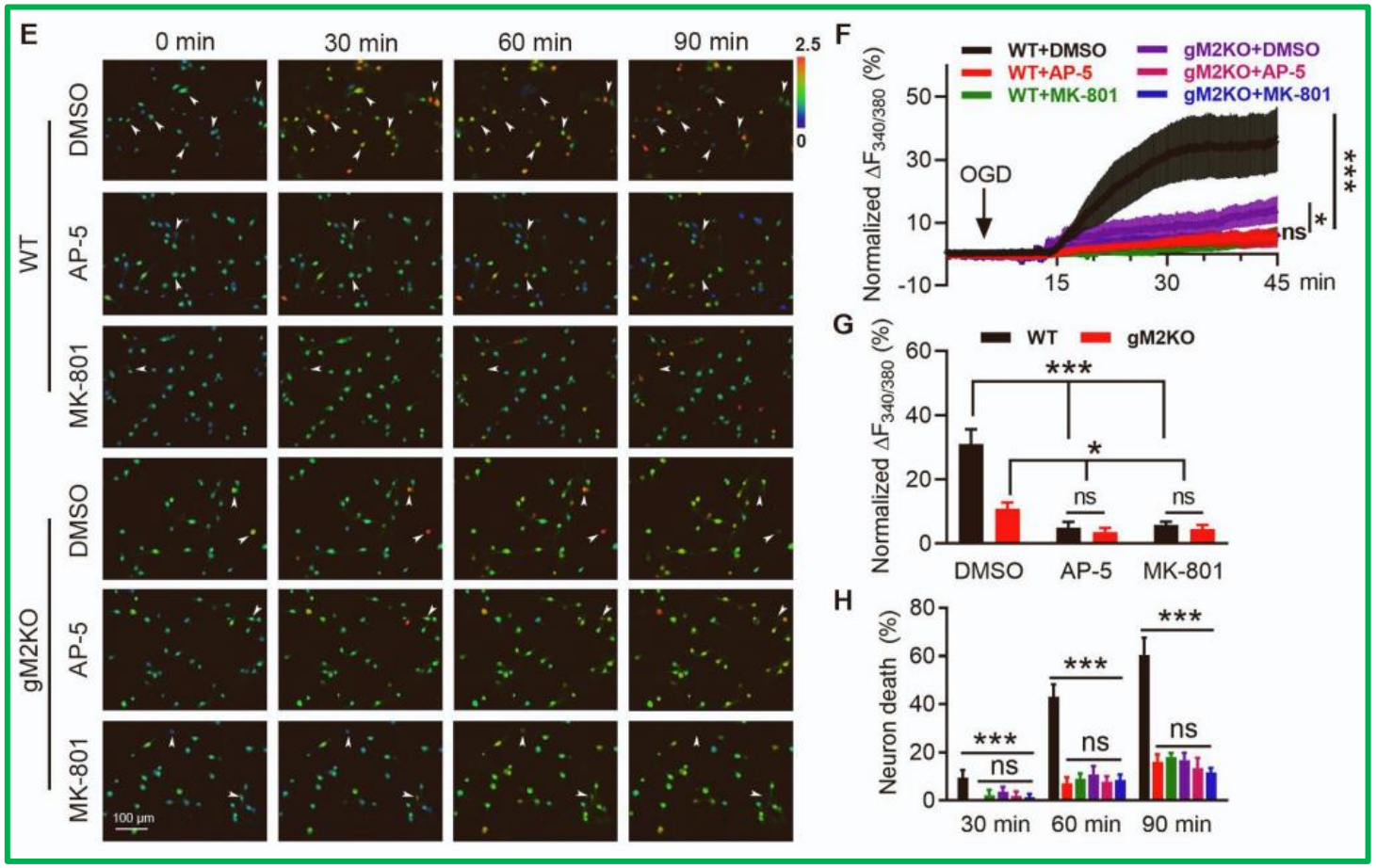
Figure 6. TAT-EE3 disrupts the physical and functional interaction between TRPM2 and NMDAR

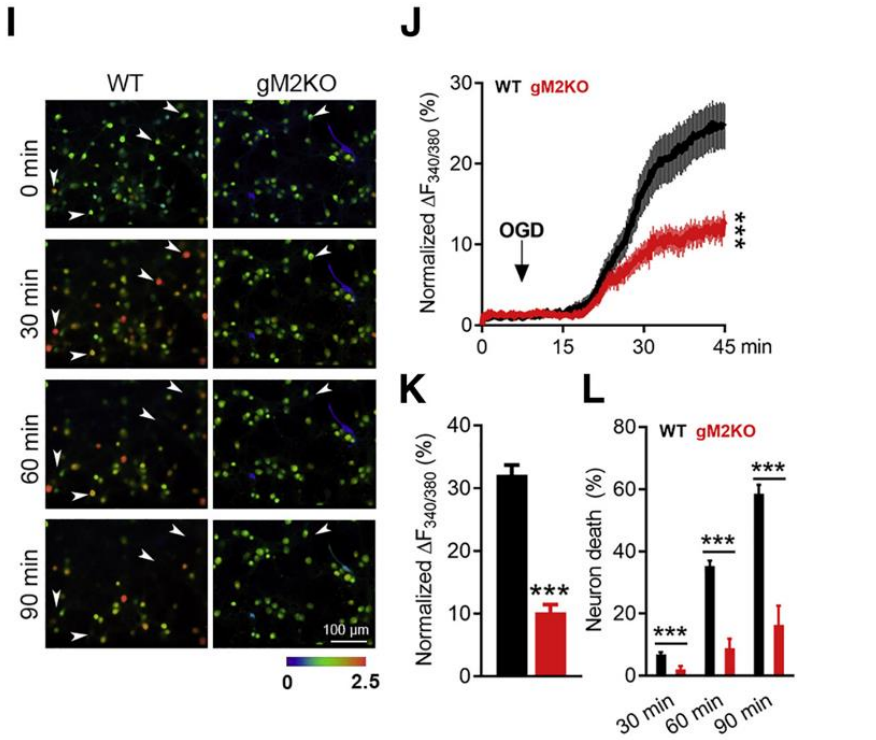
Figure 7. Uncoupling TRPM2 and NMDARs by TAT-EE3 protects neurons against OGD-induced injury

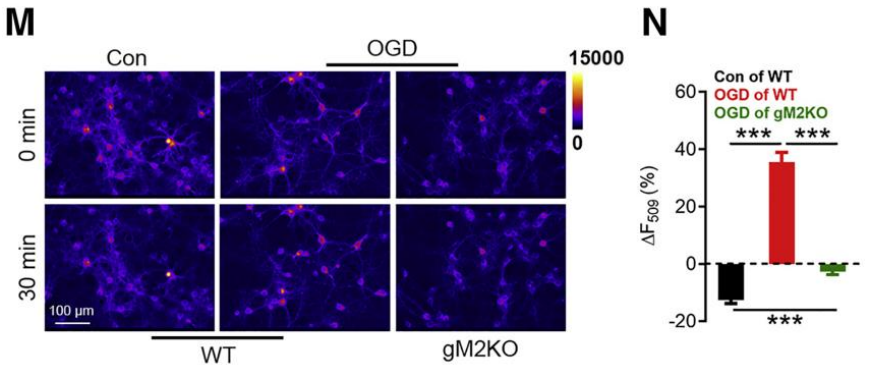
Figure 8. TAT-EE3 alleviates ischemic stroke by preserving prosurvival signaling

Figure 1. Neuron-specific *Trpm2* deletion protects the brain against ischemic damage

Q. To investigate whether TRPM2 is involved in ischemic brain damage







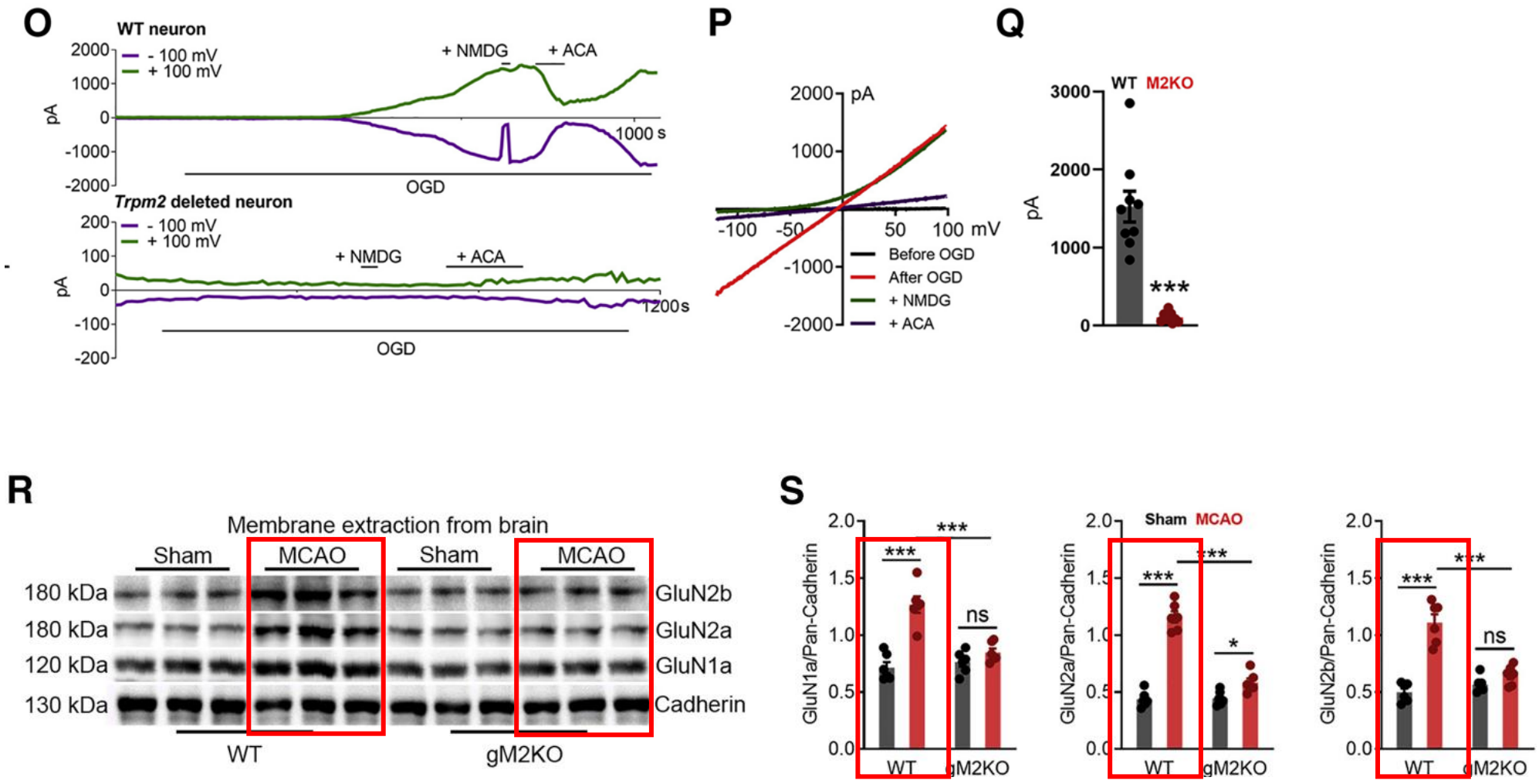
Supplementary Figure 2

- ✓ gM2KO : global *Trpm2* knockout
- ✓ nM2KO : neuron-specific *Trpm2* deletion
- ✓ AP5, MK801 : NMDAR inhibitor

A. TRPM2 plays critical role in aggregating NMDAR-mediated Ca2+ overload, mitochondrial dysfunction, and neuronal death during OGD.

Figure 1. Neuron-specific *Trpm2* deletion protects the brain against ischemic damage

Q. To examine whether TRPM2 in neurons can be activated by OGD.

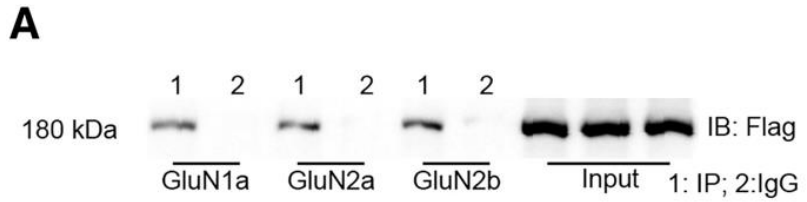


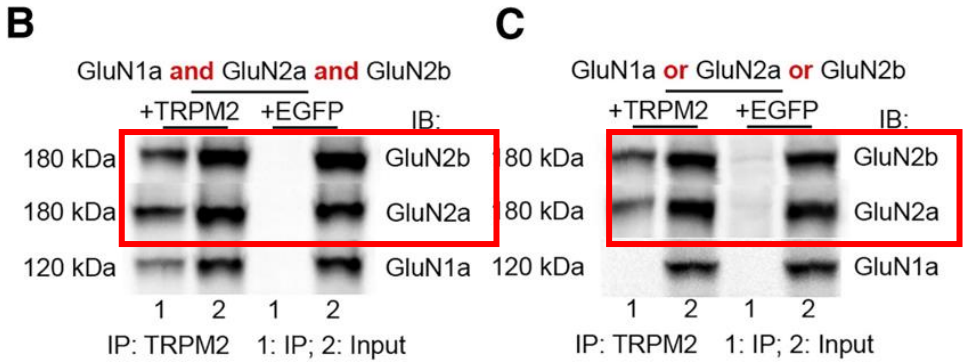
A. Upregulation of TRPM2 expression is important for the increase of NDMARs' surface expression after ischemic stroke.

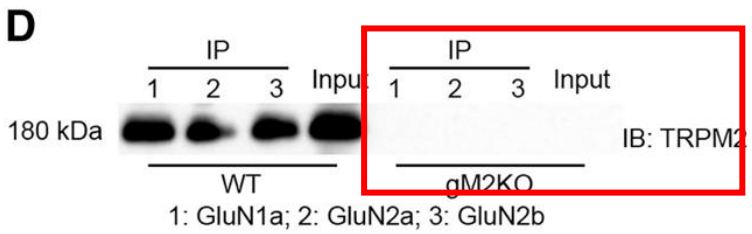
Figure 2. TRPM2 physically and functionally interacts with NMDARs

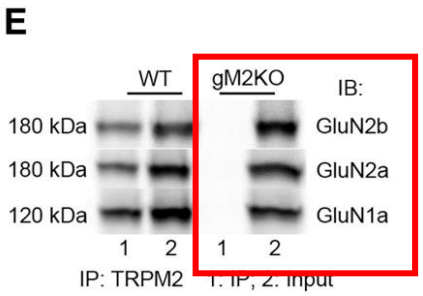
Q. To examine whether TRPM2 interacts with NMDARs

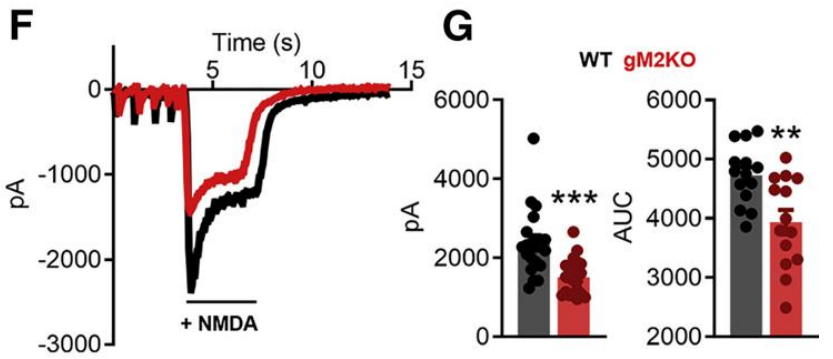
✓ EGFP : reporter protein

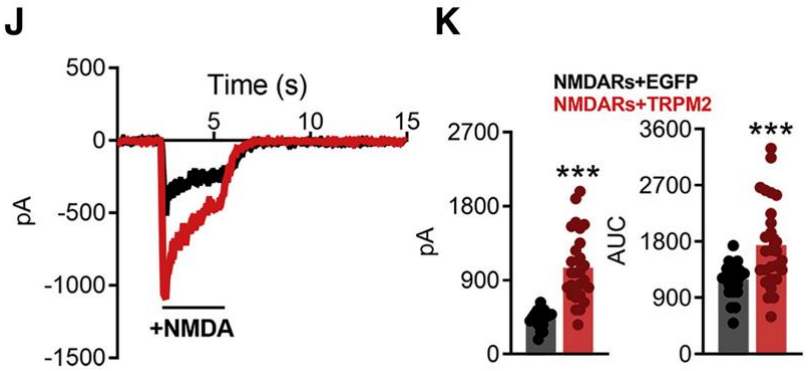


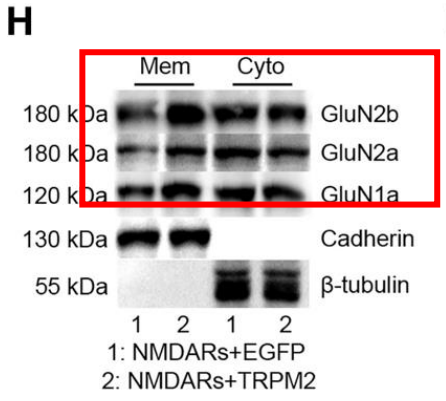


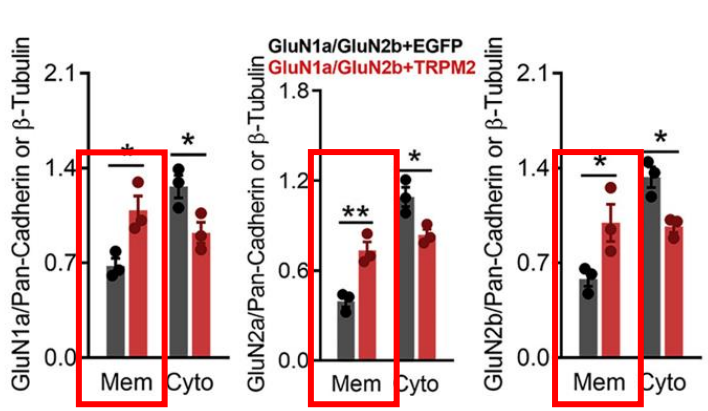








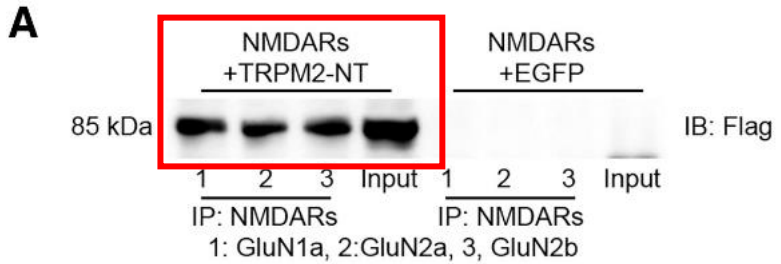


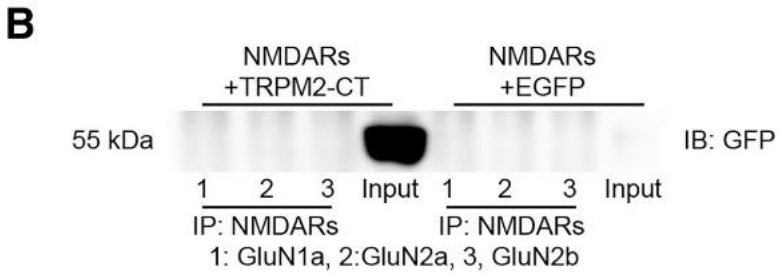


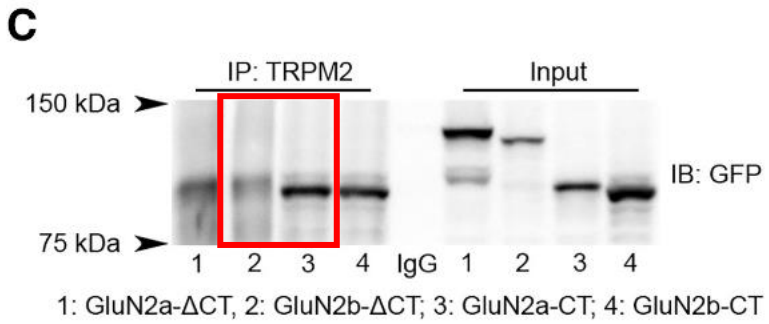
A. Physical interaction between TRPM2 and NMDARs produces functional consequences.

Figure 3. EE3 motif in TRPM2 mediates TRPM2-NMDARs coupling

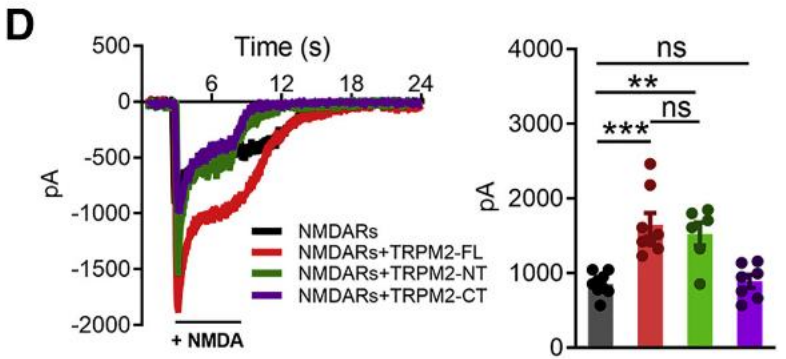
Q. To identify interaction motifs in NMDARs and TRPM2



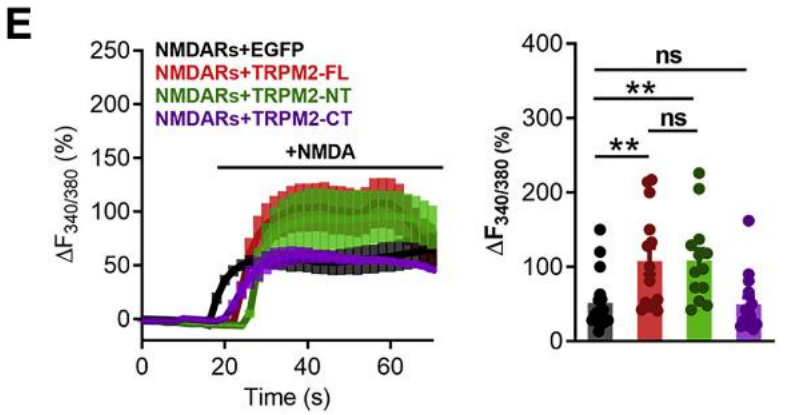




✓ EGFP : reporter protein



NMDAR current

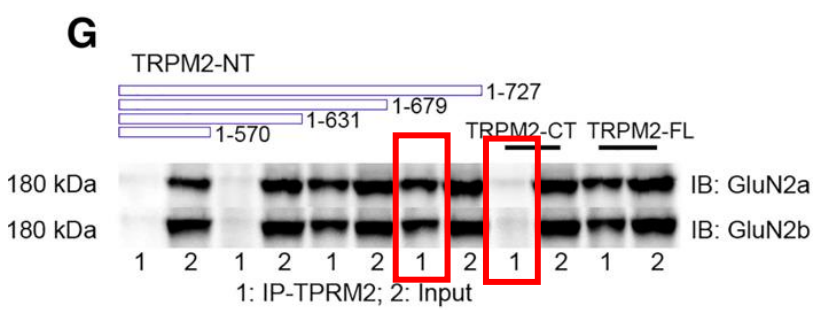


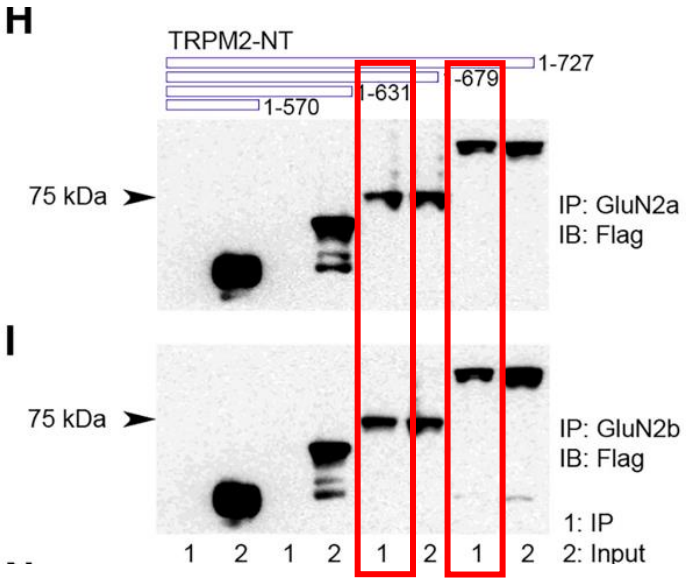
Ca2+ influx

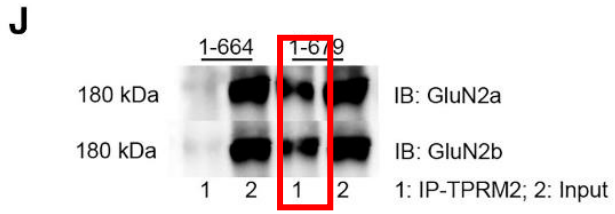
A. EE3 motif of TRPM2 and KKR motif of GluN2/GluNb in NMDAR interact directly, producing functional coupling.

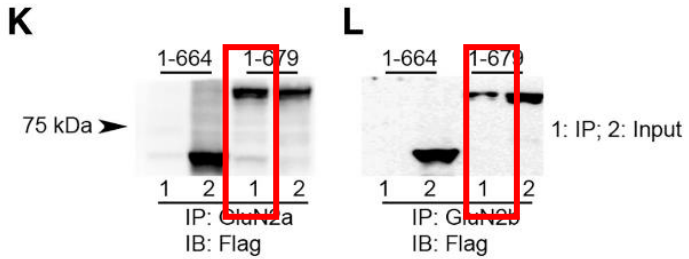
Figure 3. EE3 motif in TRPM2 mediates TRPM2-NMDARs coupling

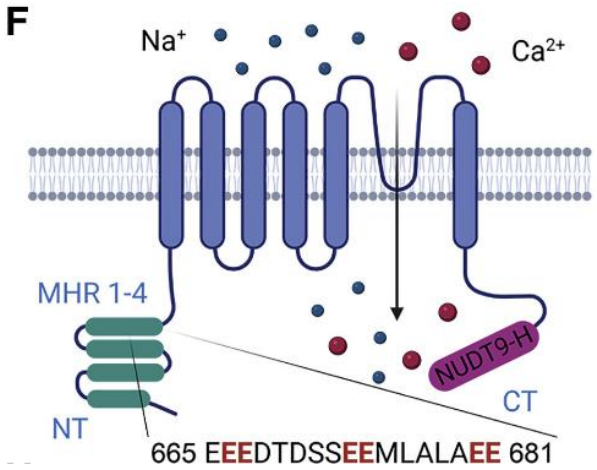
Q. To identify interaction motifs in NMDARs and TRPM2

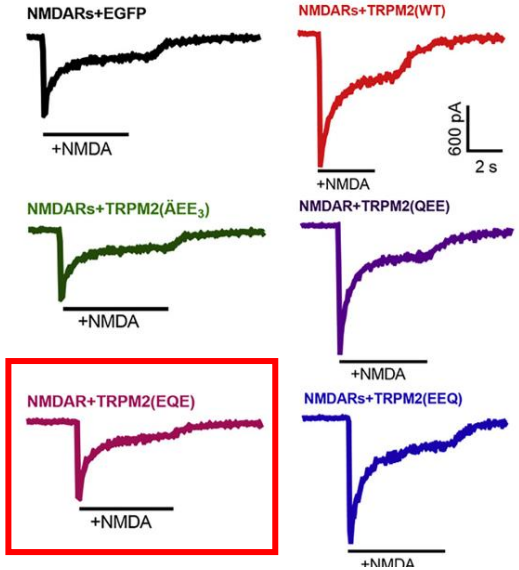


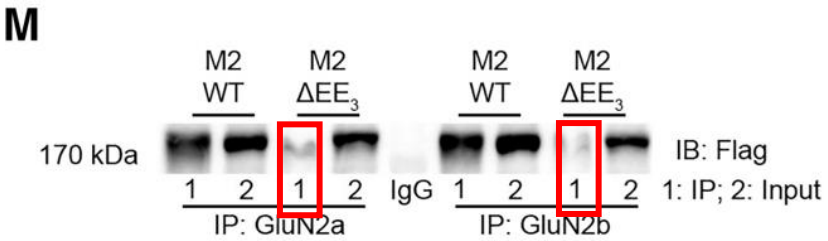


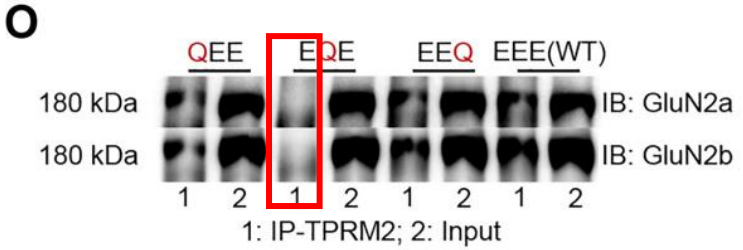


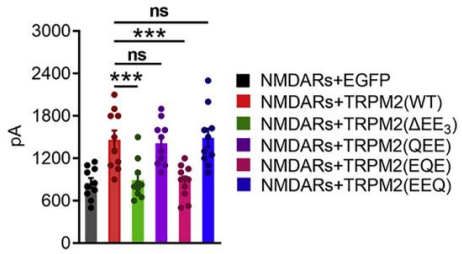










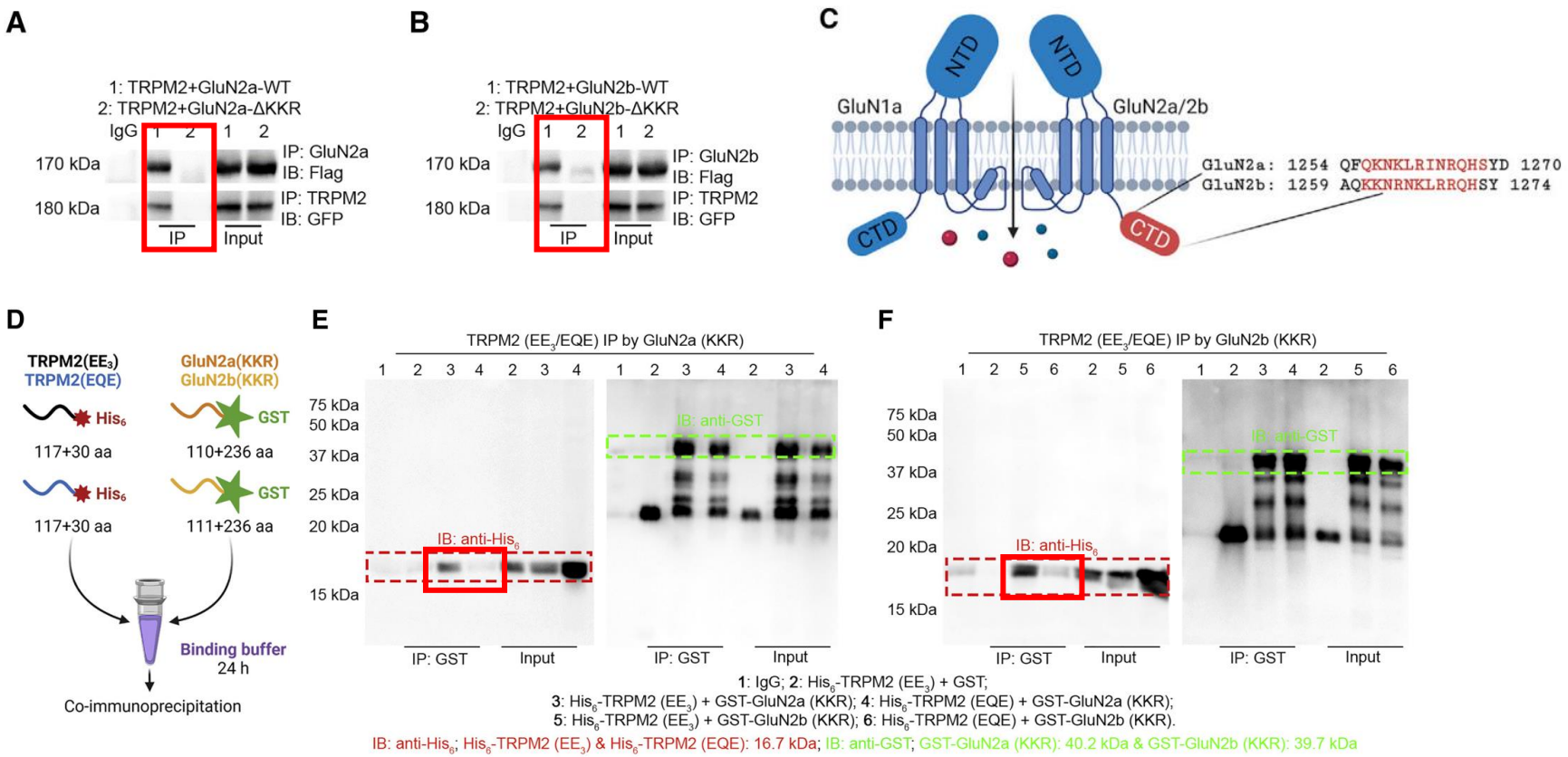


→ ① EE3 motif of TRPM2

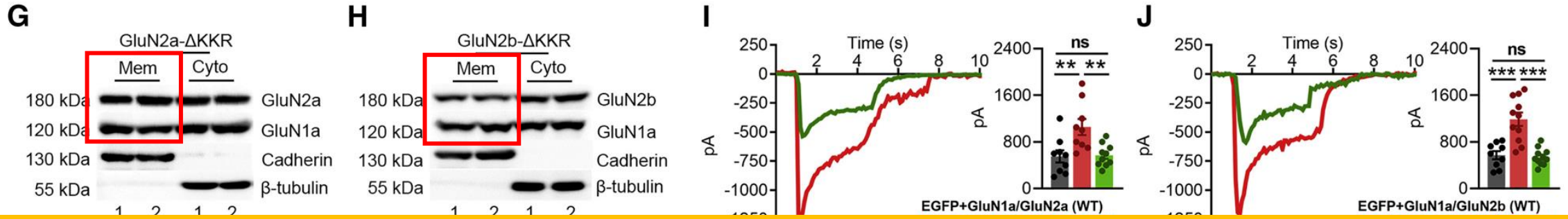
A. EE3 motif of TRPM2 and KKR motif of GluN2/GluNb in NMDAR interact directly, producing functional coupling.

Figure 4. KKR motif in GluN2a and GluN2b is required for direct binding to EE3 motif

Q. To identify interaction motifs in NMDARs and TRPM2



→ ② KRR motif



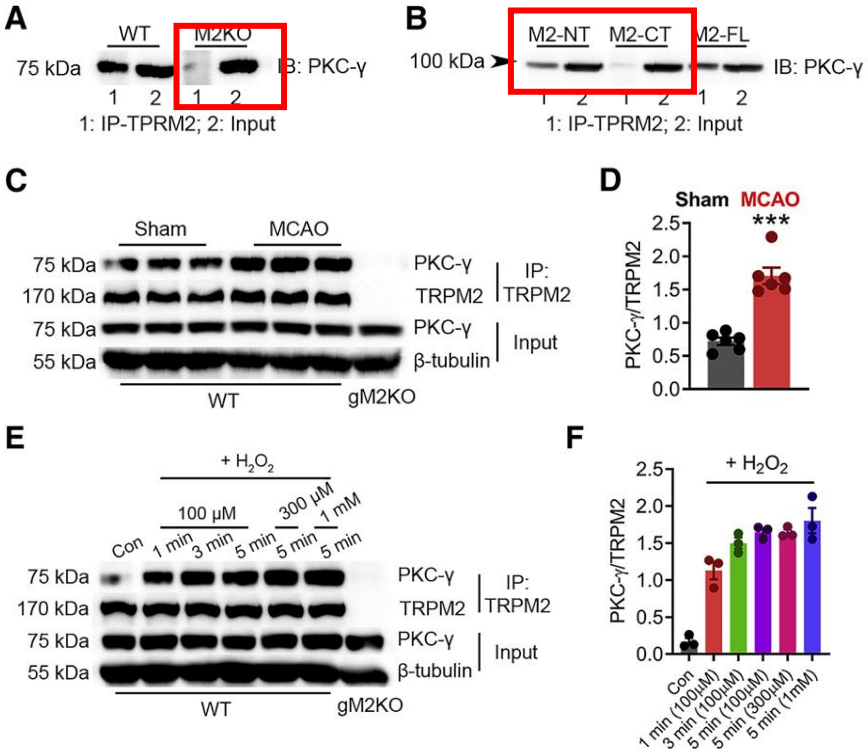
A. EE3 motif of TRPM2 and KKR motif of GluN2/GluNb in NMDAR interact directly, producing functional coupling.

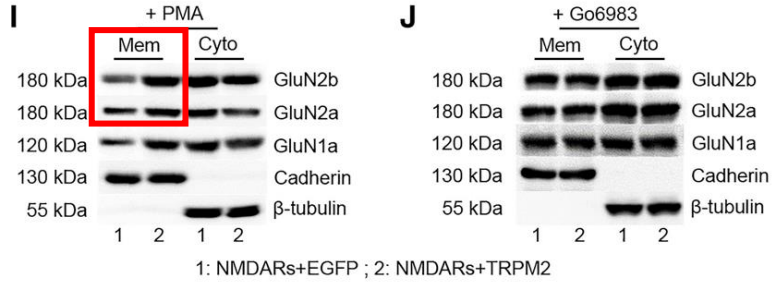
Figure 5. N-tail of TRPM2 interacts with PKC-γ

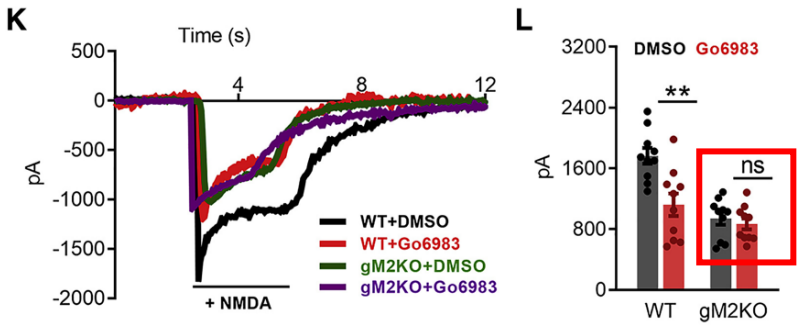
Q. To investigate how TRPM2 potentiates NMDAR currents

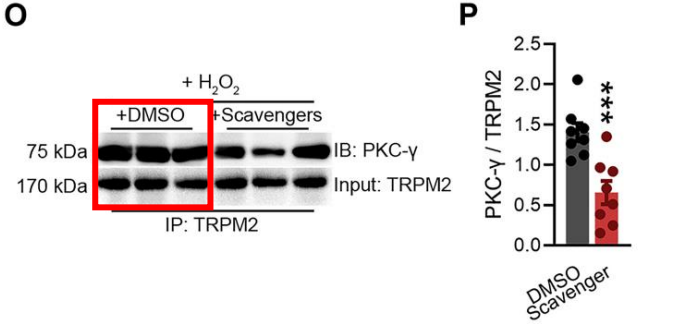
- ✓ PKC γ : neuron specific PKC
- ✓ PKC γ – DN : dominant negative PKC γ
- ✓ EGFP : reporter protein
- ✓ PMA : PKC activator
- ✓ Go6983 : PKC inhibitor
- ✓ Endosidin : exocyst inhibitor

① Interaction between TRPM2 and PKC- γ

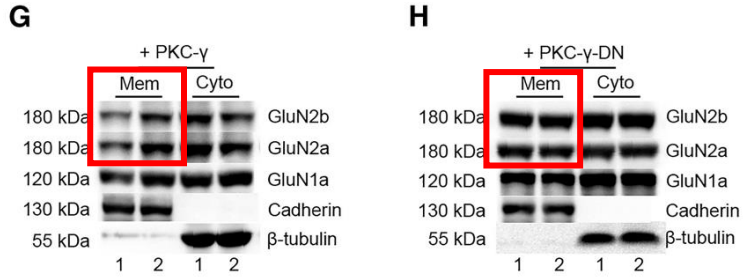


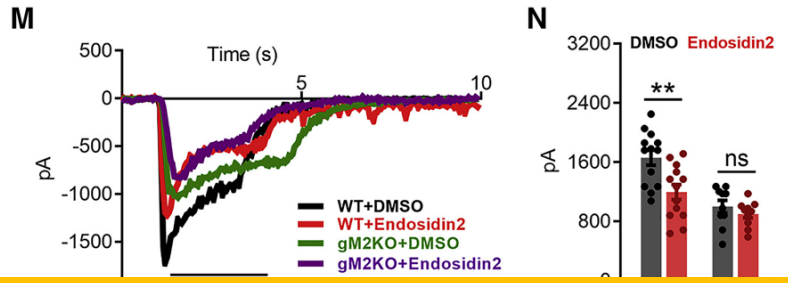




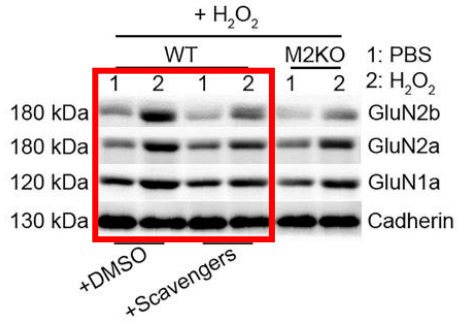


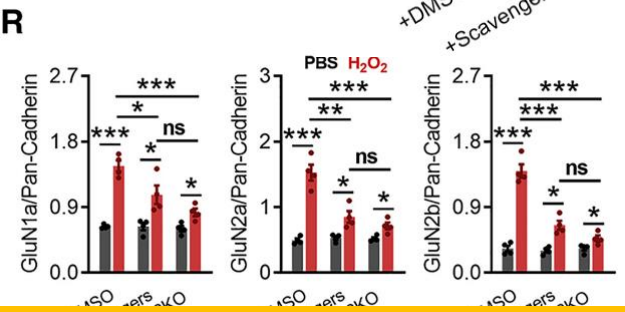
② exocyst and NMDAR trafficking





③ oxidative stress



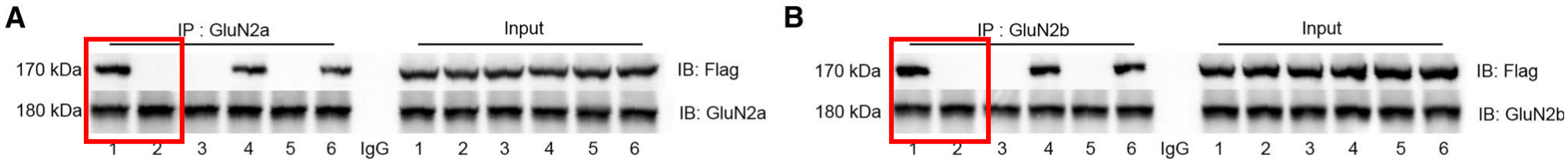


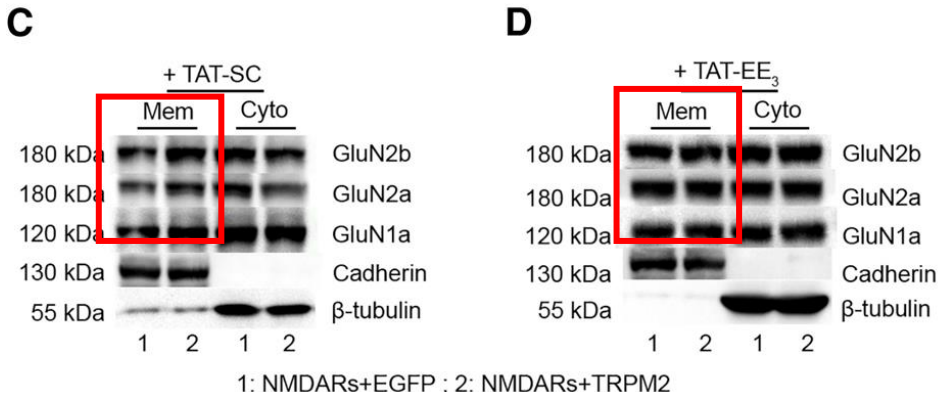
A. Interaction between PKC-γ and TRPM2, promoted by oxidative stress, may be required for functional coupling of TRPM2 and NMDAR.

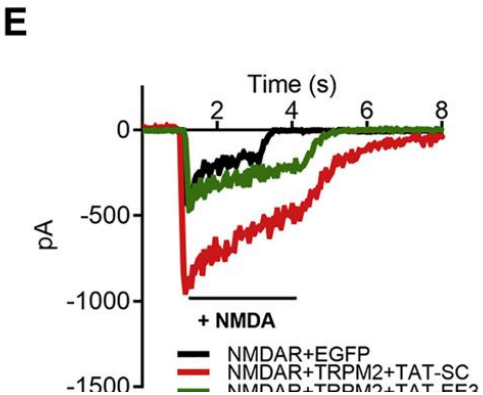
Figure 6. TAT-EE3 disrupts the physical and functional interaction between TRPM2 and NMDAR

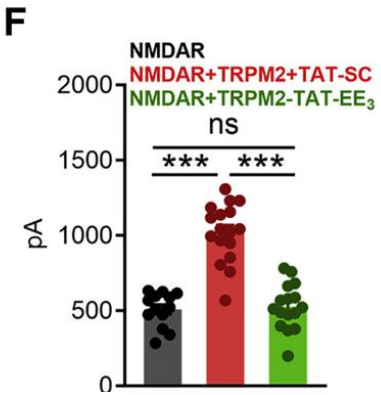
Q. To investigate whether disruption of physical interaction influences their functional coupling

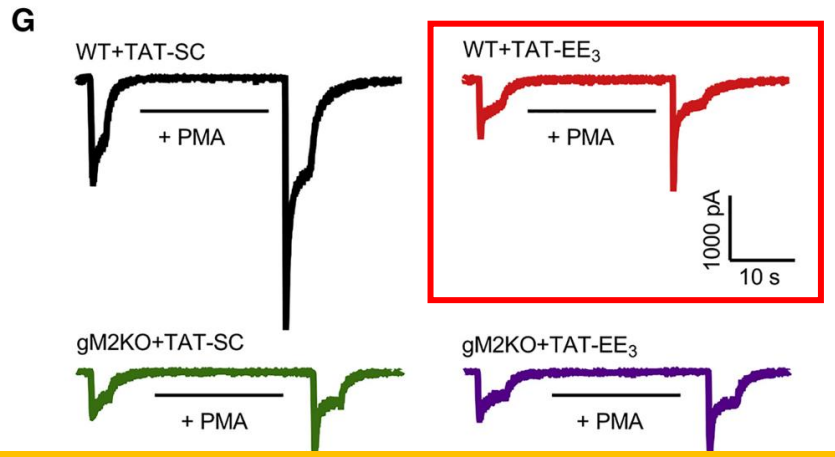
✓ TAT-EE3 : disrupting TRPM2/NMDAR physical binding

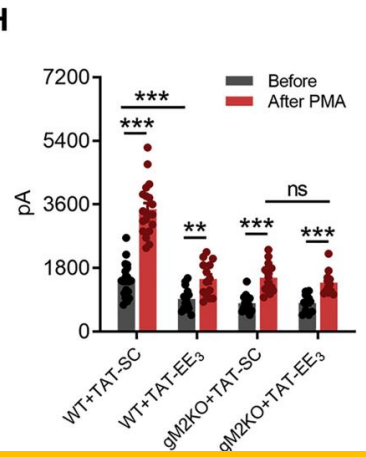


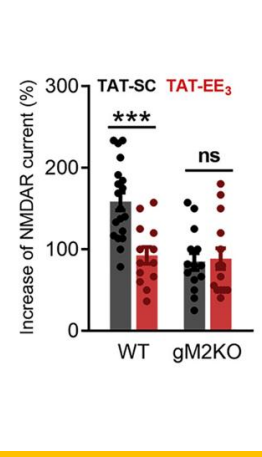








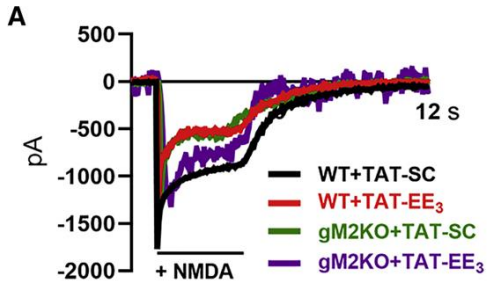


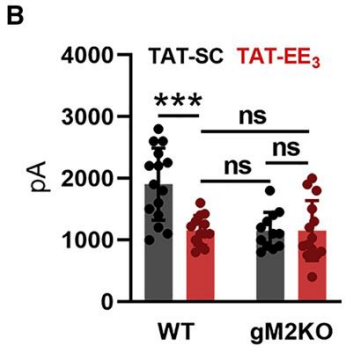


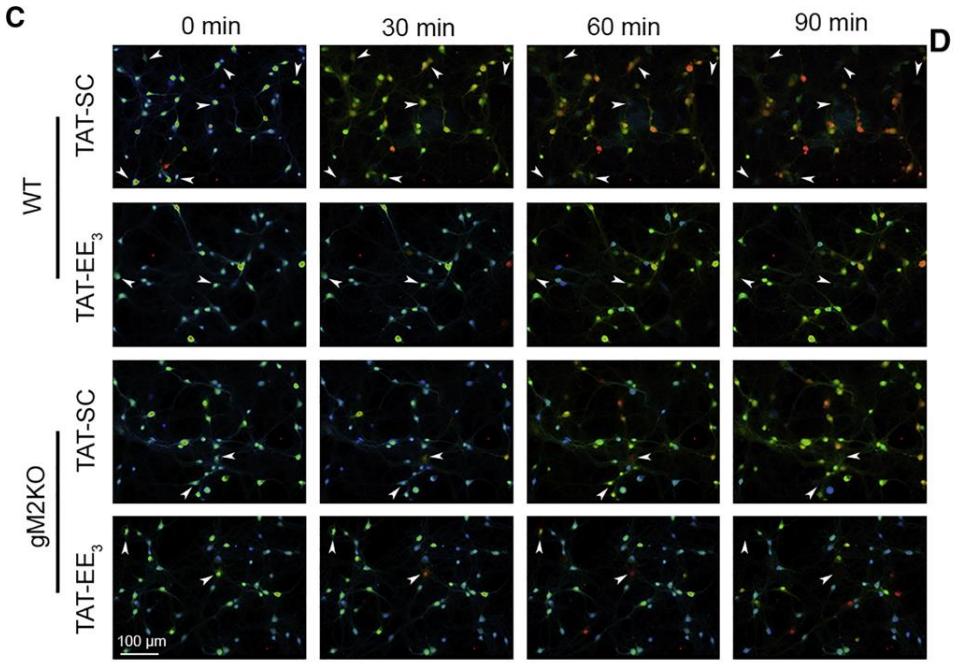
A. TAT-EE3 inhibits exacerbation of excitotoxicity caused by TRPM2-NMDAR coupling and protects neuron against OGD.

Figure 7. Uncoupling TRPM2 and NMDARs by TAT-EE3 protects neurons against OGD-injury

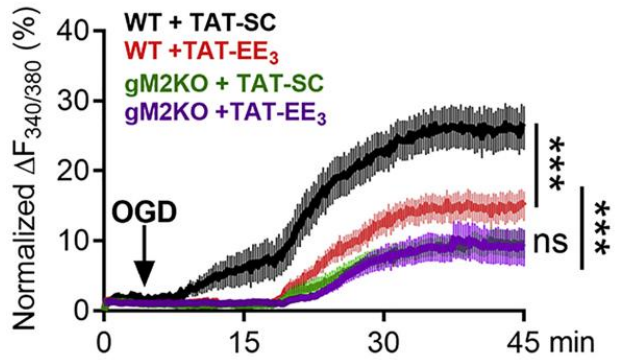
Q. To investigate TAT-EE3's potential protective effect against ischemic injury



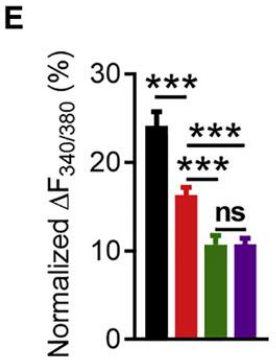




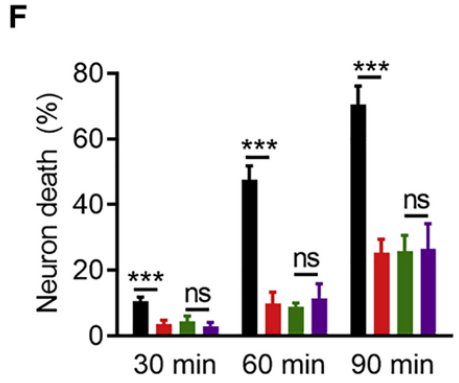
Intracellular Ca2+ ratio imaging after OGD

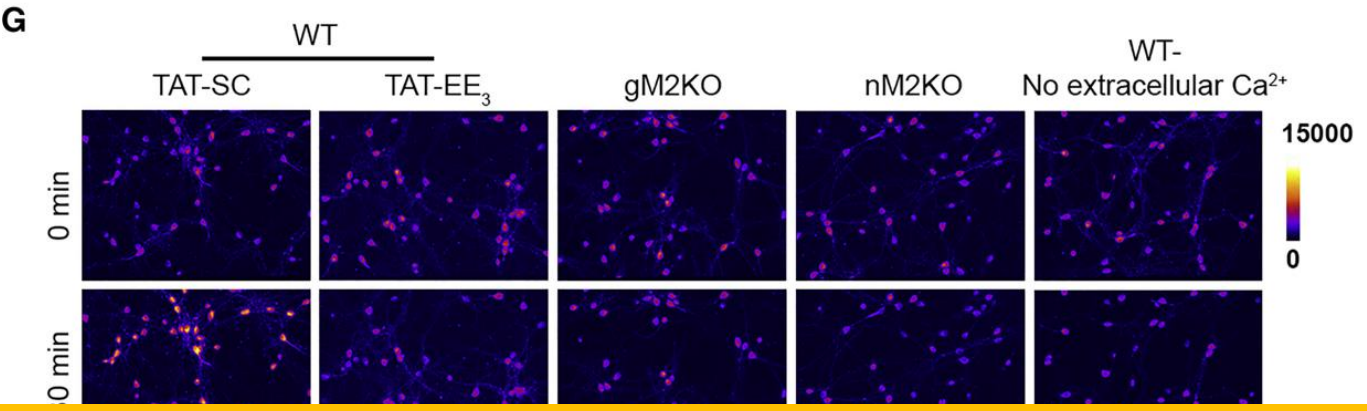


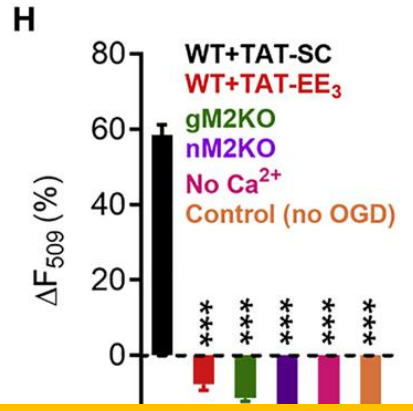
OGD-induced intracellular Ca2+ influx changes



OGD-induced neural death





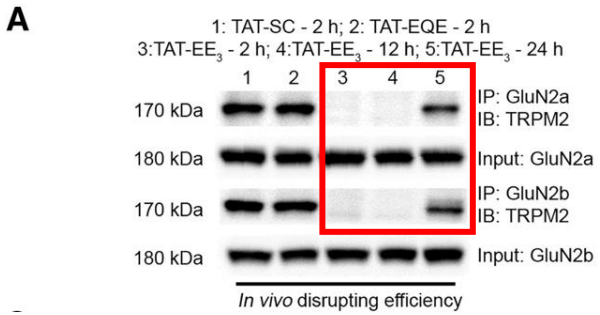


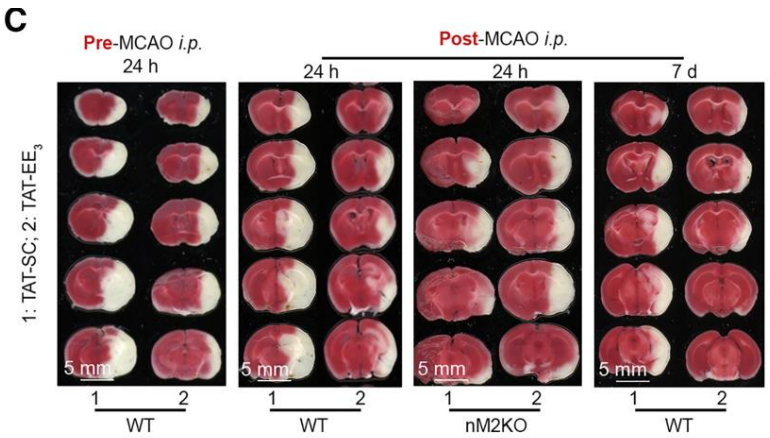
OGD-induced mitochondria depolarization

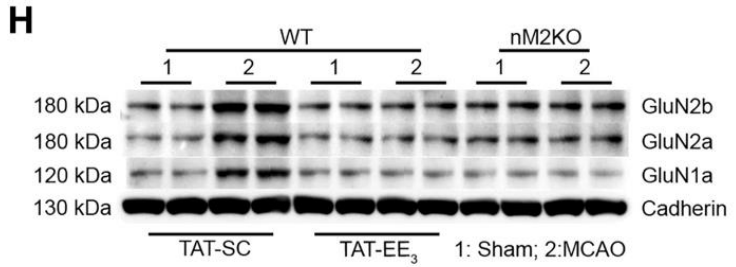
A. TAT-EE3 inhibits exacerbation of excitotoxicity caused by TRPM2-NMDAR coupling and protects neuron against OGD.

Figure 8. TAT-EE3 alleviates ischemic stroke by preventing prosurvival signal

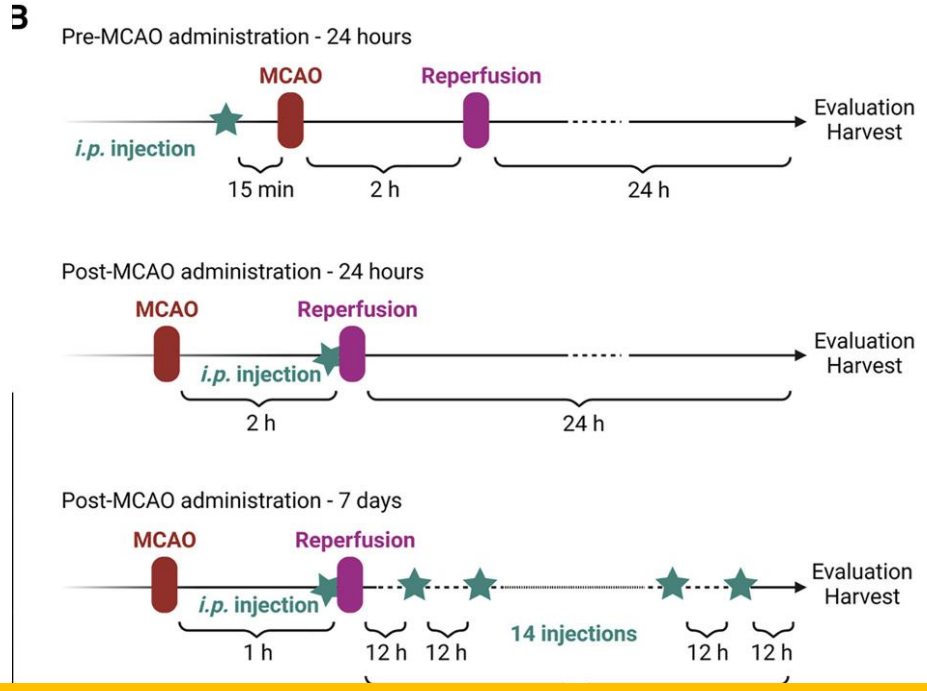
Q. To investigate TAT-EE3's potential protective effect against ischemic injury

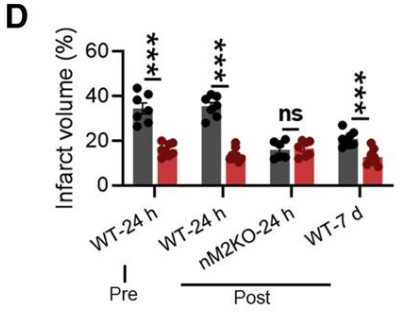


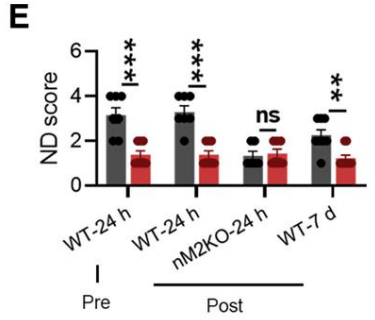


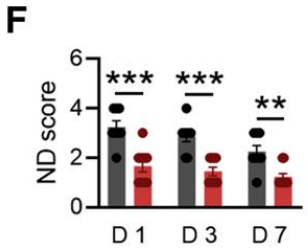


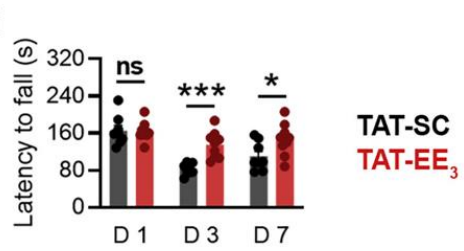
Injection strategy

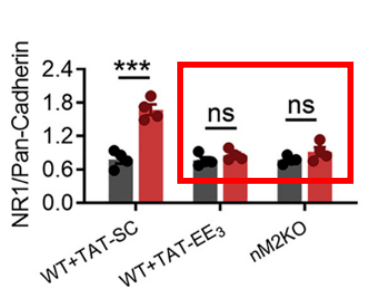


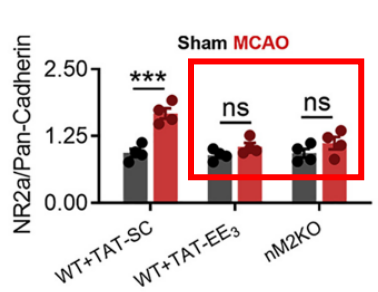


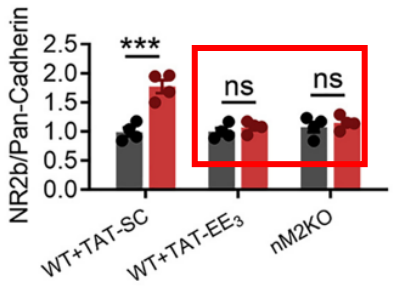










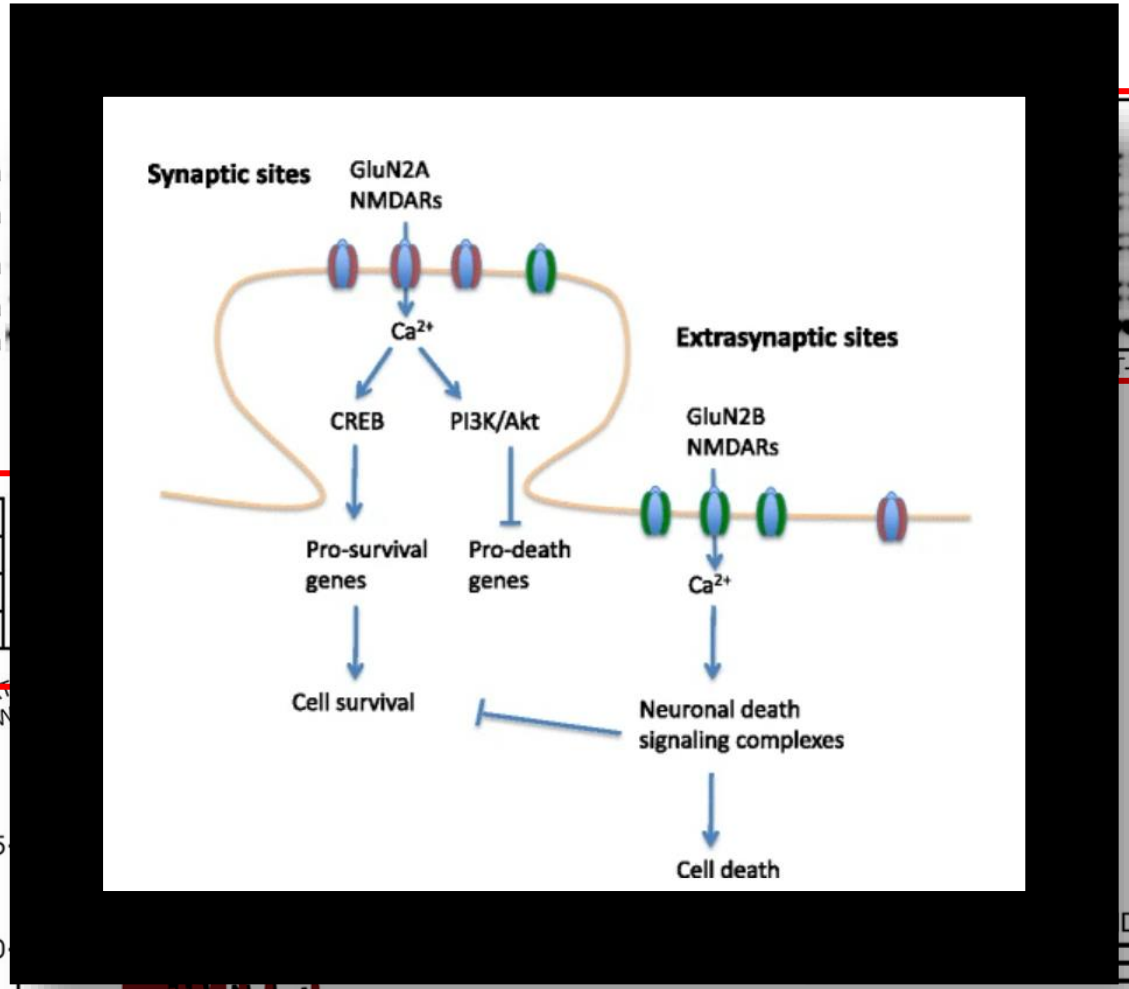


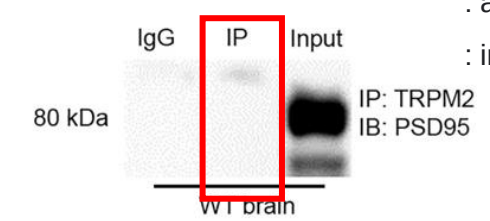
A. TAT-EE3 disrupts TRPM2-NMDAR coupling and reduces surface expression of NMDARs, thereby protecting mice from ischemic stroke.

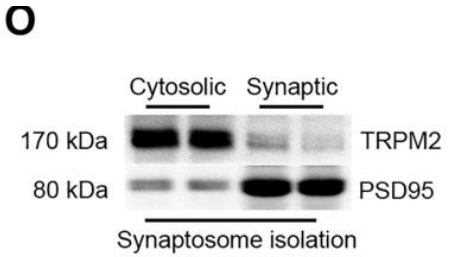
Figure 8. TAT-EE3 alleviates ischemic stroke by preventing prosurvival signal

Q. To investigate TAT-EE3's potential protective effect against ischemic injury

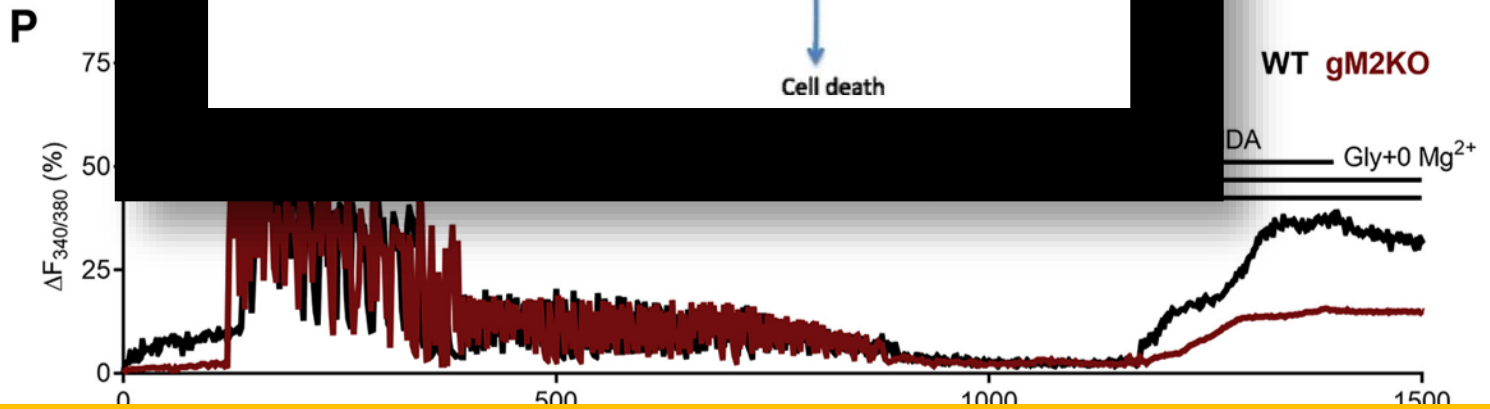
- ✓ sNMDAR : promote neural survival
  - : activated by 4-AP/Bic
  - : activate ERK1/2 & CREB
- ✓ esNMDAR : inhibit neural survival
  - : activated by NMDA
  - : inhibit pERK1/2 & pCREB

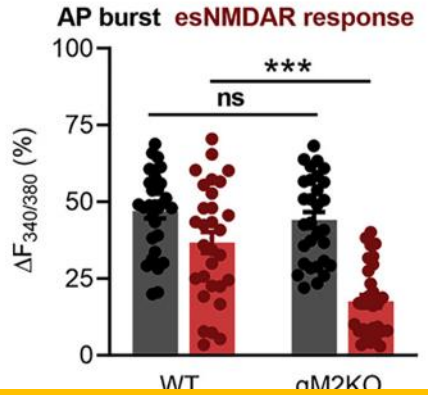






- ✓ PSD95 : synaptic protein associating with sNMDAR





A. TAT-EE3 disrupts TRPM2-NMDAR coupling and reduces surface expression of NMDARs, thereby protecting mice from ischemic stroke.

Discussion

In this study, we revealed a potential therapeutic strategy for ischemic stroke by targeting TRPM2-NMDAR association. Previous studies have shown the protective effects of global Trpm2 deletion. Our neuron-specific Trpm2 deletion results establish that neuronal TRPM2 plays a key role in aggregating excitotoxic neuronal apoptosis and necrosis in ischemic stroke through a previously unknown mechanism: physical and functional coupling with NMDARs. TRPM2 directly interact with GluN2a/b through the unique EE3 motif in its N-tail and the KKR motifs in the C-tail of GluN2a/b. The EE3 motif is evolutionally conserved in TRPM2 of different species but absent in other subfamilies of TRP channels, including TRPM4, which was shown to interact with GluN2a/2b. We showed that TRPM2 selectively enhances the activity of esNMDARs by increasing surface expression, which is mediated by recruitment of PKCg to the TRPM2-NMDAR complex. PKC regulates NMDAR's surface trafficking via different mechanisms. Some studies demonstrated that PKC phosphorylates serine residues (Ser896 and Ser897) on GluN1, whereas others showed the regulation of PKC on NMDARs is not mediated by directly phosphorylation but by triggering autophosphorylation of CaMKII that associates with NMDARs. Interestingly, CaMKII inhibitor can reduce the enhanced surface expression of NMDARs in our study. Moreover, we found that TRPM2-induced increase of NMDARs' surface expression can be inhibited by the exocyst inhibitor endosidin 2. NMDARs have been shown to interact with the exocyst for PKC-induced surface trafficking. Although we do not know the exact mechanism by which PKCg mediates trafficking of the TRPM2-NMDAR complex, we propose the following working model. Under oxidative stress conditions, TRPM2 recruits PKCg to the TRPM2/NMDARs to bring PKCg to the close proximity of NMDAR-interacting partners such as CaMKII and consequently promotes NMDARs' surface trafficking, leading to enhanced excitotoxicity. Nonetheless, future investigations are needed to understand where PKCg begins to bind to the TRPM2-NMDAR complex. We found that TRPM2 exacerbates excitotoxicity by preferentially enhancing the function of esNMDARs. Disruption of TRPM2-NMDAR interaction by TAT-EE3 prevented the inhibition on phosphorylation of ERK1/2 and CREB, the prosurvival signaling that can be shut off by esNMDAR activation during ischemic stroke, indicating that disruption of TRPM2-NMDAR coupling largely eliminates the esNMDAR-mediated excitotoxicity. It is not surprising that TRPM2 preferentially influence esNMDARs because we found that TRPM2 could not be detected in the synaptosomes or immunoprecipitated by anti-PSD-95. Moreover, Trpm2 deletion significantly reduced extrasynaptic, but not the synaptic, NMDA-induced Ca2+ response. Similarly, other studies have demonstrated a predominantly extrasynaptic distribution of TRPM2 in cultured hippocampal neurons and that TRPM2 is absent in the synaptic proteins.

Discussion

Importantly, TRPM2 only enhances NMDAR function during ischemic stroke, as TRPM2-NMDAR interaction and increase of NMDARs' surface trafficking are promoted by PKCg under oxidative stress conditions. Thus, disrupting the TRPM2-NMDAR interaction to specifically target esNMDARs will unlikely generate similar side effects caused by inhibition of synaptic NMDARs by conventional NMDAR antagonists. The most exciting result in our study is that the TRPM2-derived interfering peptide TAT-EE3 protects mice against ischemic stroke in both short- and long-term MCAO. Cell-permeable peptides such as TAT-fused peptides have been well characterized and are considered powerful tools for both clinical applications and basic research studies. We found that TAT-EE3 disrupts TRPM2-NMDAR interaction in vitro and in vivo, effectively inhibits excitotoxicity, and prevents the reduction of phosphorylated CREB and ERK1/2 levels. Our results indicate that peptide-based uncoupling of TRPM2-NMDAR association is a promising therapeutic strategy for ischemic stroke. NMDARs interact with various proteins. A recent study showed that interaction of GluN2a/b with the Ca2+-impermeable channel TRPM4 through a 57-aa domain (TwinF) in the N-tail of TRPM4 enhances excitotoxicity. Interestingly, interaction of TRPM4 with NMDARs does not influence NDMAR currents or Ca2+ signaling but promotes prodeath signaling. The authors demonstrated that the physical coupling of TRPM4 and NMDARs is required to promote excitotoxicity. Similar to TRPM2, TRPM4 preferentially influences esNMDARs. It would be exciting to speculate that like scaffolding proteins for synaptic NMDARs' trafficking, TRPM2 and TRPM4 may represent a family of proteins that are able to form interacting complex with NMDARs and may exclusively localize NMDARs to the extrasynaptic sites. Moreover, as shown by previous studies that GluN2b is preferentially localized at extrasynaptic sites, it will be of a great interest to understand how TRPM2 in our study and TRPM4 in the previous study only influence esNMDARs but interact with both GluN2a and GluN2b. In summary, we found that TRPM2 in neurons promotes neuronal death during ischemic stroke by coupling with esNMDARs via its EE3 motif. Targeting TRPM2-NMDAR interaction could be a promising strategy for developing more effective and safer therapies for ischemic stroke.

Conceptual Advance

- ✓ Found that TRPM2 in neurons promotes neuronal death during ischemic stroke by coupling with esNMDARs via its EE3 motif
- ✓ Targeting TRPM2-NMDAR interaction could be a promising strategy for developing more effective and safer therapies for ischemic stroke.

Limitation

- ✓ how TRPM2 only influence esNMDARs but interact with both GluN2a and GluN2b
- ✓ No description about Figure8 P and Q