

Thrombus proteomic markers associated with first-pass reperfusion in acute stroke therapy

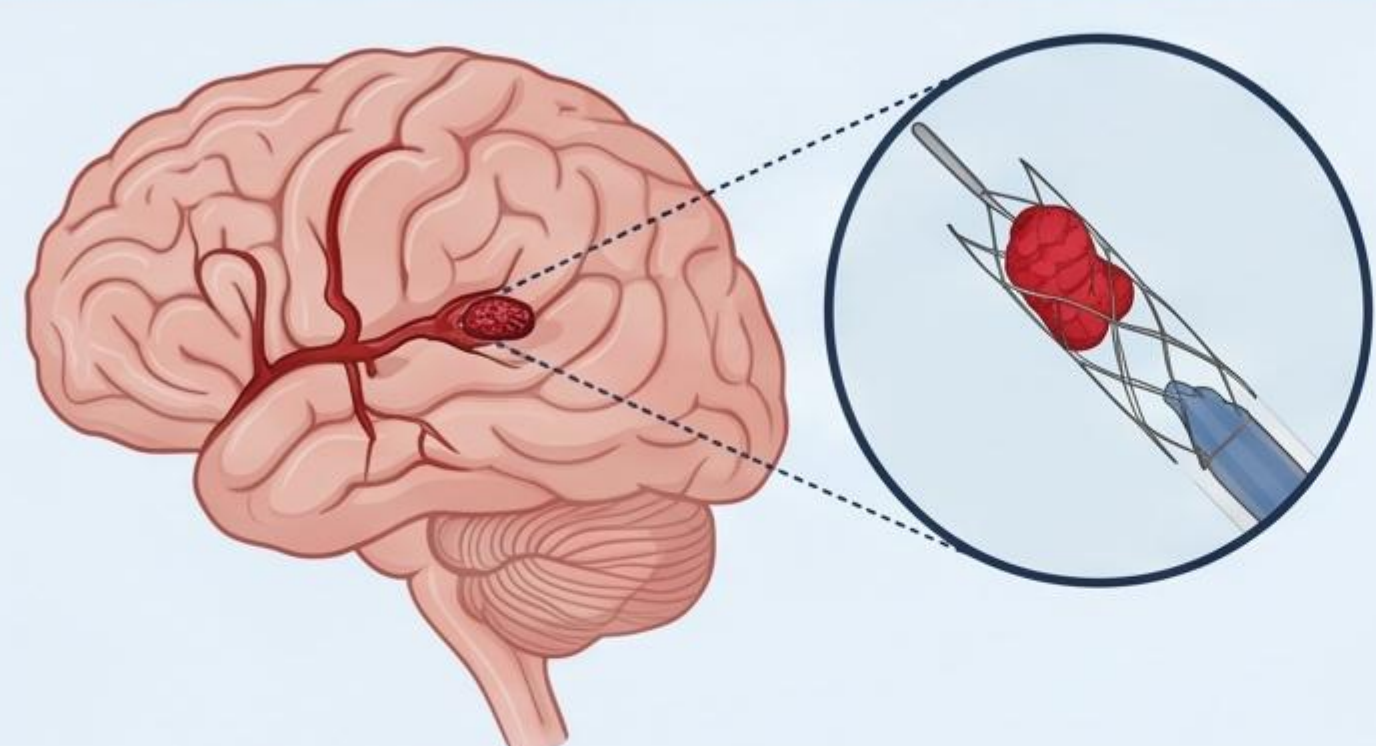
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INTRODUCTION

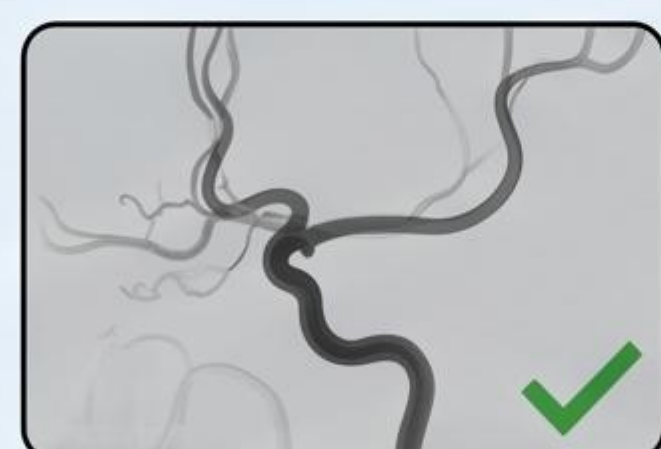
INVESTIGATING THE PROTEOMIC PROFILING OF STROKE THROMBI: COMPARING mFPE SUCCESS & FAILURE

MECHANICAL THROMBECTOMY (MT)



MODIFIED FIRST-PASS EFFECT (mFPE) ACHIEVEMENT

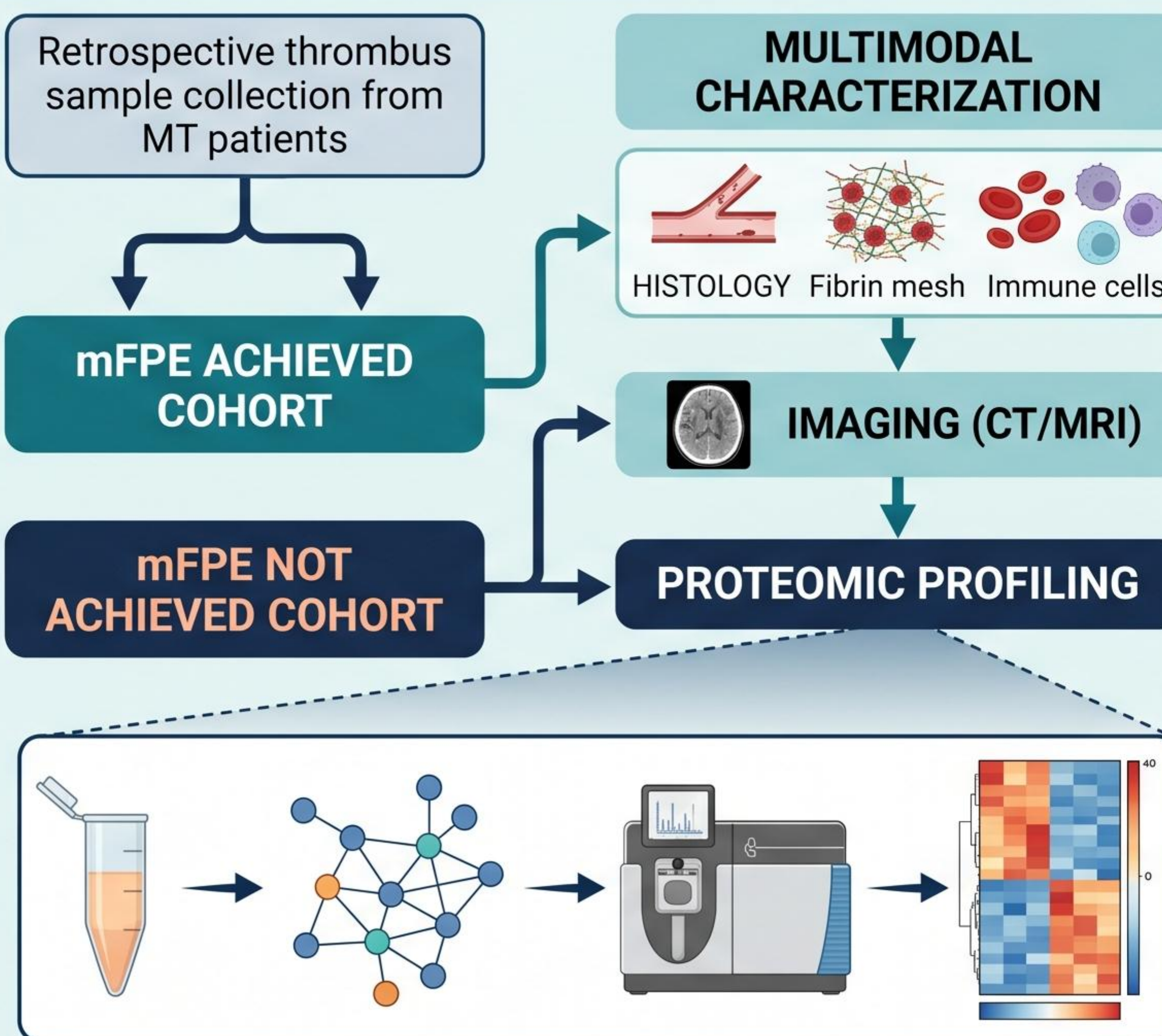
mFPE achieved
(100% Recanalization,
single pass)



mFPE not achieved
(Partial or incomplete
Recanalization after
multiple passes)

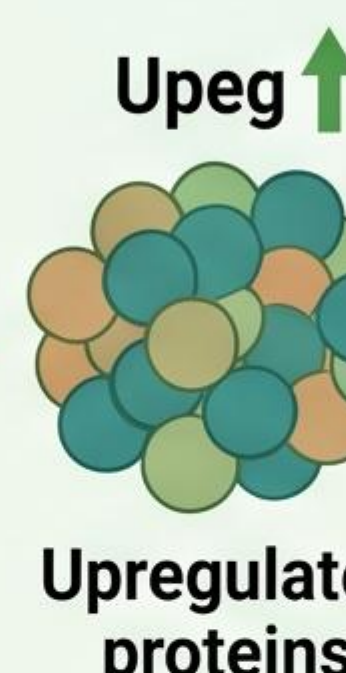


STUDY DESIGN: PROTEOMIC PROFILING



EXPECTED OUTCOMES

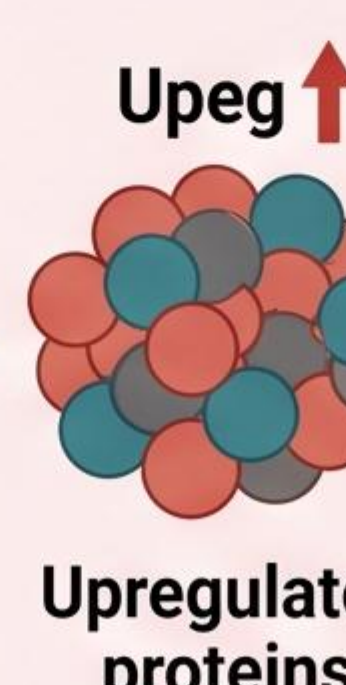
BIOMARKERS FOR mFPE SUCCESS



Functional enrichment

- Efficient Lysis
- Reduced Inflammation
- Enhanced Reperfusion

BIOMARKERS FOR mFPE FAILURE



Functional Enrichment

- Thrombectomy Resistance
- Fibrin Organization
- Platelet Aggregation

KEY QUESTIONS & OBJECTIVES

1. IDENTIFY THROMBUS BIOMARKERS FOR TREATMENT RESPONSE

2. EXPLAIN mFPE HETEROGENEITY IN ACUTE ISCHEMIC STROKE

3. INFORM FUTURE TARGETED THERAPIES & STRATEGIES

OBJECTIVE

- Mechanical thrombectomy (MT) has improved disability outcomes after ischemic stroke (IS), mainly when the so-called modified first-pass effect (mFPE) is achieved. This study aims to explore the proteomic profiling of thrombi composition, comparing patients who achieved mFPE and those who did not.

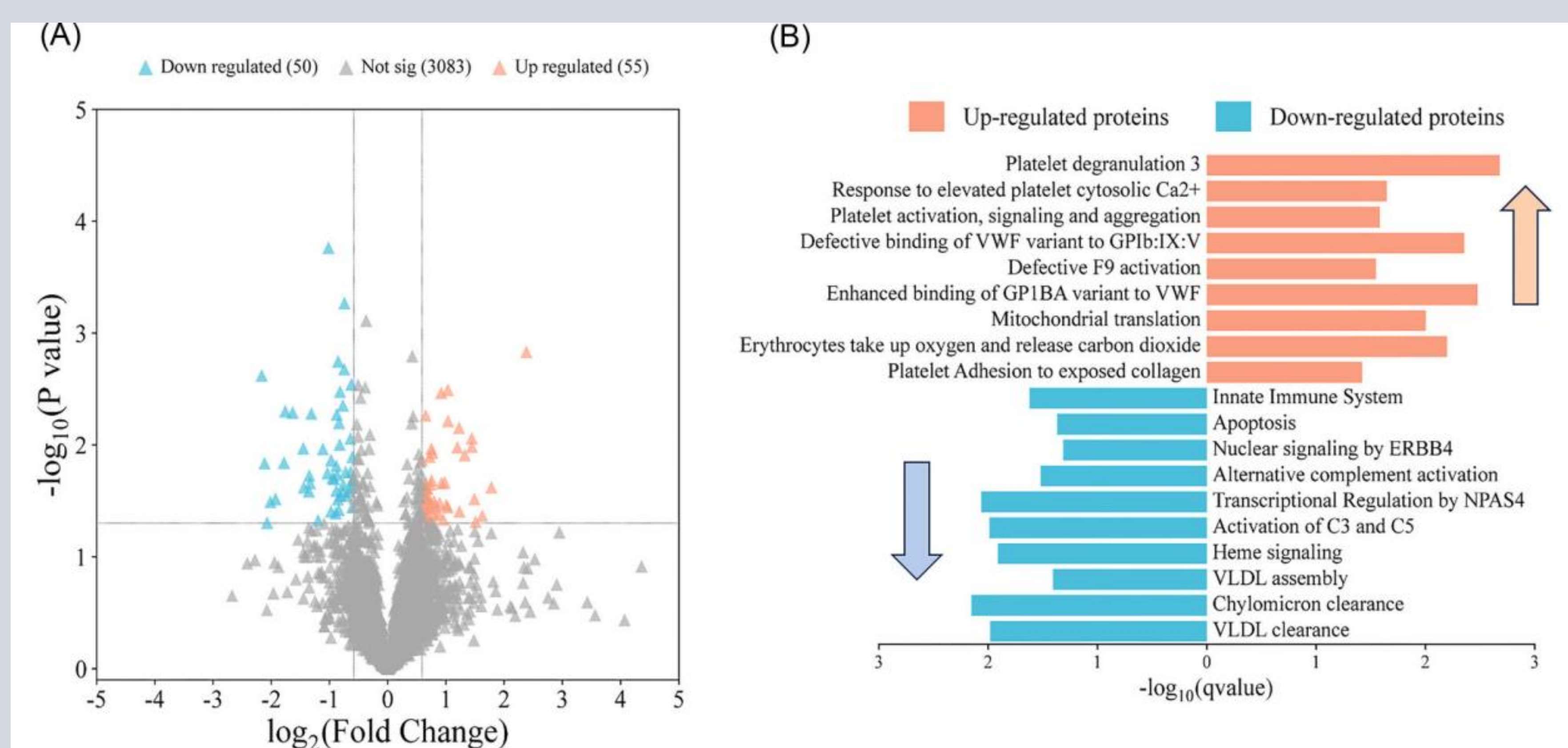
MATERIALS AND METHODS

- A prospective study was conducted based on consecutive IS patients who underwent MT for proteomic analysis of the thrombi. mFPE was defined as a reperfusion grade with a thrombolysis in cerebral infarction scale $\geq 2b$ after the first pass of the thrombectomy device. A qualitative and quantitative protein analysis was performed using LC-MS/MS for DDA and SWATH-MS analysis, respectively (*Precis Clin Med.* 2026 Feb 24;9(1):pbag008).

RESULTS

A total of 88 patients were included, with a median age of 75 years (± 16) and 55.7% females (49 patients). mFPE was presented in 51.1% (45 patients), and presented a higher proportion of functional independence at 90 days (62.2% vs. 37.8%; $p=0.02$).

Proteomic analyses revealed distinct protein expression profiles in thrombi from patients who achieved the modified first-pass effect, mainly related to platelet activation and haemostasis (ACTN1, ACTN4, FLNA, TLN1, ITGB3, PPBP), atherosclerosis and vascular remodelling (SCPEP1, LGALS1, ADAP1, HBA1), and inflammatory and stress-related responses (ANXA1, S100A9, HSP90AB1, HSP90B1).



CONCLUSION

This exploratory proteomic study provides new insights into the biological complexity of IS, revealing distinct protein expression patterns in those who achieved the mFPE, and deserves further investigation in future research.

ACKNOWLEDGEMENTS

Xunta de Galicia (Axencia Galega de Innovación: IN607A202203)(Axencia Galega para a Xestión do Coñecemento en Saúde:SA304D-PRIS-T-2024/03), (ISCIII/PI21/01256/, PI24/00813). RICORS-ICTUS. (RD24/0009/0017). R. Iglesias-Rey (CP22/00061) Programa Miguel Servet ISCIII y Co-financiado por la EU. EBRAINS 2.0, European Union's Research and Innovation Program Horizon Europe No.101147319.

The role of the NLRP3 inflammasome in the maintenance of blood-brain barrier integrity during ischemic stroke

María Álvarez Rubal¹, Kiara Anaide Pinto¹, Esther Fuertes Yebra¹, Laura Ávila Poza¹, Clara Martín Fernández¹, Inés Valencia Fernández², Céline Decouty Pérez¹, And Javier Egea^{1,2}
¹ Laboratorio de Neuroinflamación Molecular y Plasticidad Neuronal, Unidad de Investigación, Hospital Universitario Santa Cristina. Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, España.

INTRODUCTION

Cerebral ischemia is the second leading cause of death and the main cause of adult disability worldwide. It occurs when a brain vessel is obstructed, which results in reduced blood flow to the affected area. This reduction of perfusion triggers a rapid **neurovascular unit (NVU) damage**. As an important part of the NVU, **pericytes provide structural and functional support to it**, which are **tightly connected to endothelial cells** through gap junctions along the basal lamina. They contribute to the preservation of the BBB, forming blood vessels, regulating blood flow, and modulation of the immune response, playing an **important role in inflammation, angiogenesis, and neurogenesis**. A key component of the ischemia-induced inflammatory response is the activation of the **NLRP3 inflammasome**, a pivotal multiprotein complex in the innate immunity implicated in **maturation and secretion of important proinflammatory cytokines**. Therefore, we hypothesized that **protecting the BBB and attenuating the inflammatory response following ischemia** is crucial for maintaining communication and functionality among the various components of the NVU. In particular, our study focuses on the interaction between two main components of the NVU: endothelial cells and pericytes. Furthermore, we aim to visualize the involvement of the NLRP3 inflammasome in cerebral pericytes and its role in maintaining blood-brain barrier (BBB) integrity during cerebral ischemia.

Pericyte coverage is reduced after 24 hours post-ischemia in Wild-type (WT) animals, an effect that is reversed following treatment with MCC950

In NLRP3 knockout animals, pericyte coverage is slightly diminished at 24 hours of reoxygenation

The inhibition or genetic depletion of the NLRP3 inflammasome counteracts the loss of pericyte coverage at 24 hours post-ischemia

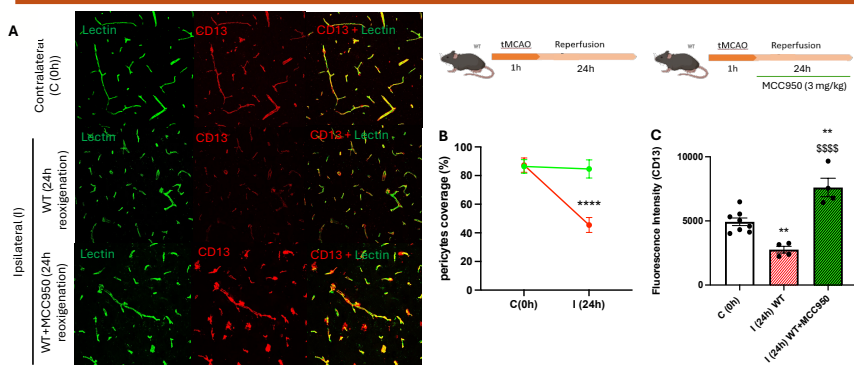


Fig. 1. A. Representative immunofluorescence of CD13 (pericytes) /Lectin (endothelium) on the ipsilateral (I) and contralateral (C) hemisphere at 24 h of reperfusion followed by a scheme of the protocol done in WT animals and WT animals treated with MCC950 (3 mg/kg) 1h post-ischemia. B. Quantification of the pericyte coverage on endothelial cells on the ipsilateral and contralateral hemisphere of WT animals (red) and WT animals treated with MCC950 (green) at 24 h of reoxygenation. C. Fluorescence intensity of CD13 of WT animals and WT animals treated with MCC950 at 24 h of reoxygenation. **p<0.01, ****p<0.0001 vs C (0h); \$\$\$p<0.0001 vs I (24h) WT, n=4-8.

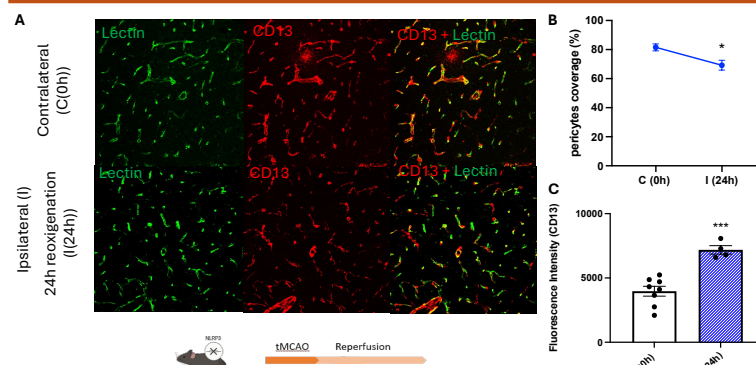


Fig. 2. A. Representative immunofluorescence of CD13/Lectin on the ipsilateral and contralateral hemisphere at 24 h of reperfusion followed by a scheme of the protocol done in NLRP3 KO animals. B. Quantification of the pericyte coverage on endothelial cells on the ipsilateral and contralateral hemisphere at 24 h post-ischemia. C. Fluorescence intensity of CD13 after 24 h of reoxygenation. *p<0.05, ***p<0.001 vs C (0h), n=4-8.

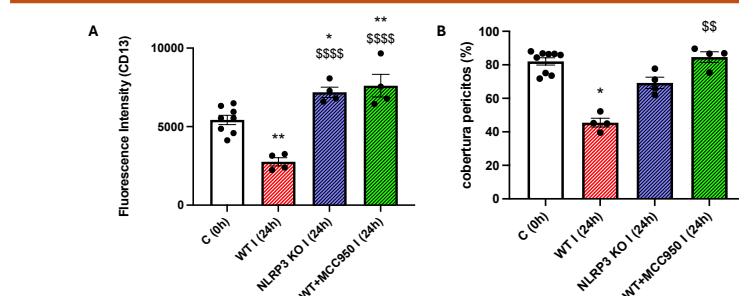


Fig. 3. A. Comparative quantification of the pericyte coverage on endothelial cells on the ipsilateral and contralateral hemisphere at 24 h post-ischemia in WT, NLRP3 KO and MCC950 treated animals. B. Comparative quantification of the fluorescence intensity of CD13 after 24 h of reoxygenation in WT, NLRP3 KO and MCC950 treated animals. (*p<0.05, **p<0.01 vs C (0h); \$\$\$p<0.0001 vs I (24h) WT, n=4-8.

Pre-treatment with MCC950 before hypoxia reduced segment formation in Matrigel co-cultures of pericytes and endothelial cells

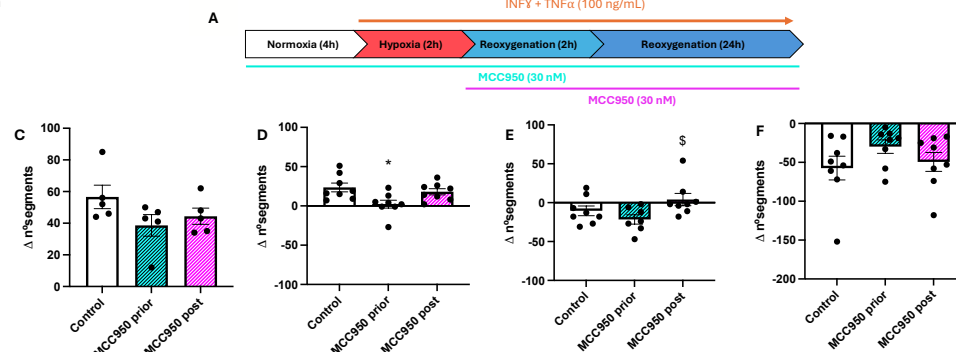
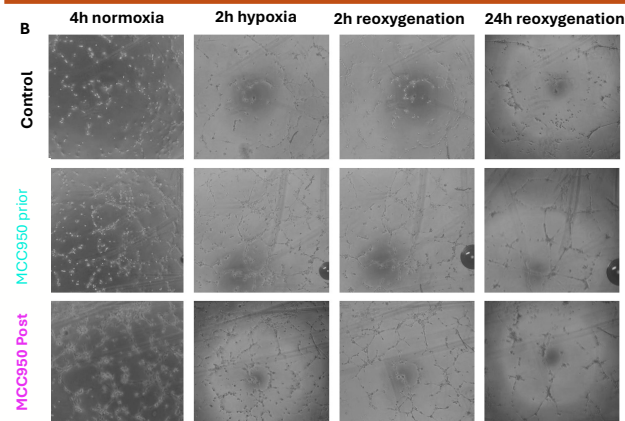


Fig. 4. A. Graphical representation of the experimental protocols performed in three-dimensional co-cultures. B. Representative image of the protocols performed in co-cultures under normoxic, hypoxic and reoxygenation conditions. C. Variation in segment formation in co-cultures over 0 to 4 h under normoxia. D. Variation in segment formation in co-cultures during 2 h reoxygenation period. E. Variation in segment formation in co-cultures during 2 h reoxygenation to 24 h of reoxygenation. *p<0.05 vs Control; \$p<0.05 vs MCC950 prior hypoxia, n=8.

The NLRP3 inflammasome modulates the expression of various markers in human pericytes

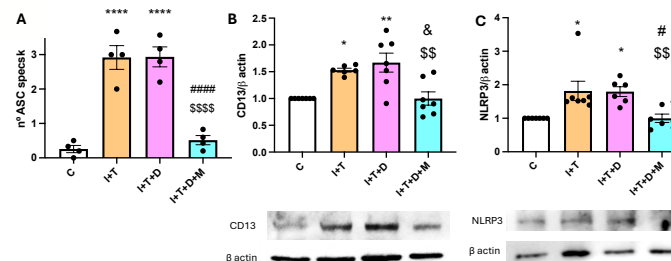
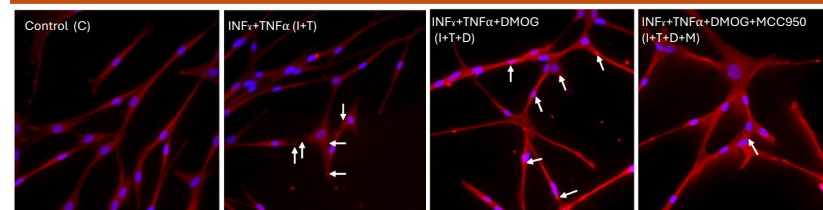


Fig. 5. A. Representative immunofluorescence and quantification of ASC specks formed in pericytes with inflammatory, hypoxic and treated conditions. B. CD13 expression with inflammatory, hypoxic and treated conditions. *p<0.05, **p<0.01, ****p<0.0001 vs C; \$\$\$p<0.0001 vs I+T; #####p<0.0001 vs I+T+D, n=4-7.

Inhibición de NCLX en el ictus isquémico y hemorrágico: papel en la ferroptosis

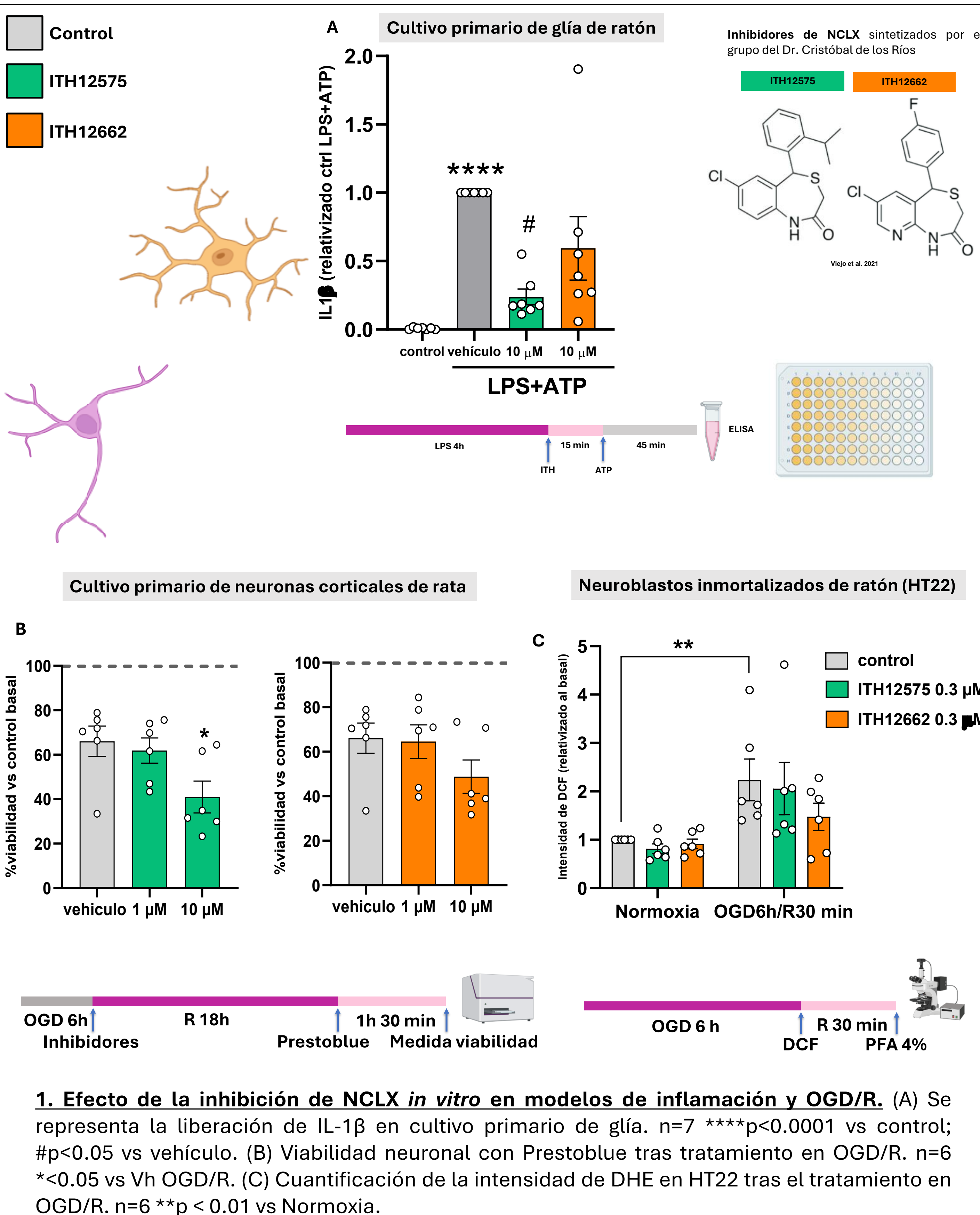
Icíar Polo-Fernández¹, Júlia Baixauli-Martín¹, Céline Decouty-Pérez¹, Susana Delgado-Martín¹, Ana Belén López-Rodríguez^{1,2}, Javier Egea¹, Antonio Martínez-Ruiz¹

¹Unidad de Investigación, Hospital Universitario Santa Cristina, Instituto de Investigación Sanitaria Princesa (IIS-Princesa), Madrid, España

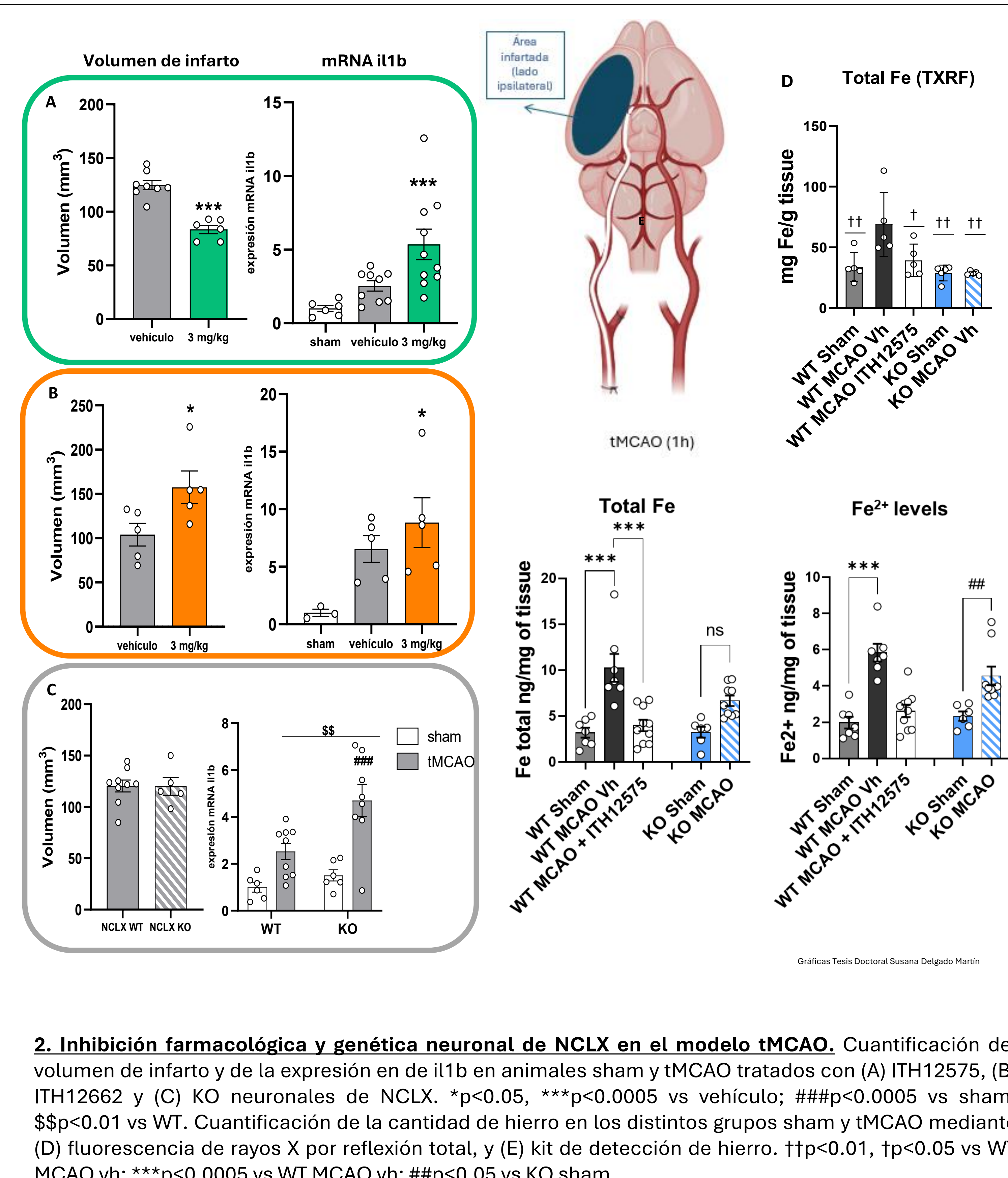
²Servicio de Neurología, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-Princesa), Madrid, España.

La entrada mitocondrial de Na⁺ mediada por el intercambiador mitocondrial Na⁺/Ca²⁺ (**NCLX**) participa en la producción de especies reactivas de oxígeno (**ROS**) en condiciones de **isquemia**, lo que sugiere un papel clave de NCLX en el **daño oxidativo** asociado al **ictus**. Durante la **reperfusión**, la recuperación del oxígeno favorece un **aumento** de la **generación mitocondrial de ROS**, proceso que podría **atenuarse** mediante la **inhibición de NCLX**. Por otro lado, el **ictus hemorrágico** se caracteriza por una acumulación intracelular de **hierro** que desencadena **peroxidación lipídica** y muerte celular por **ferroptosis**. En este contexto, se ha descrito que la **inhibición de NCLX** ejerce un efecto **neuroprotector** frente a este tipo de muerte celular.

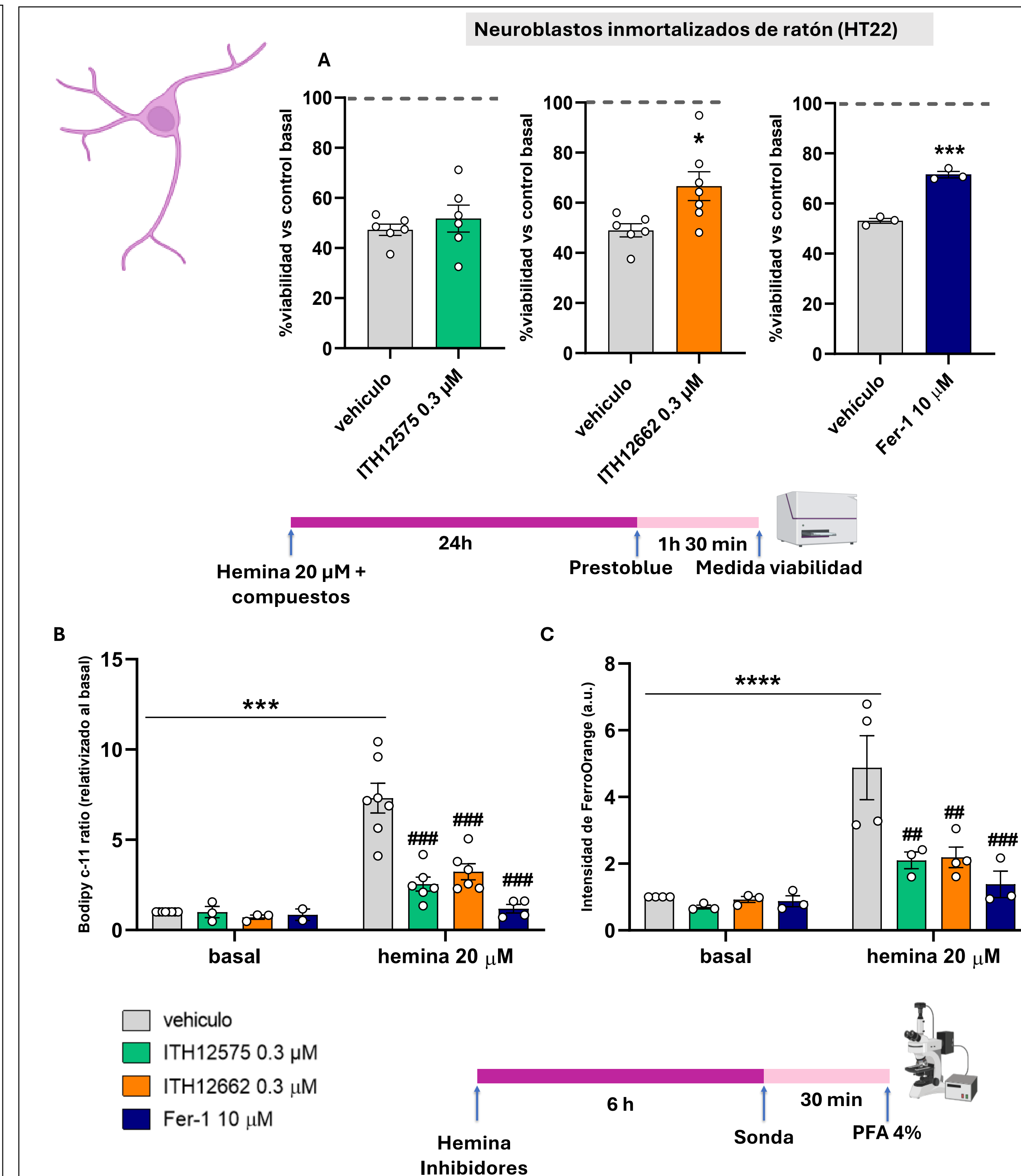
Modelos *in vitro* inflamación y OGD/R



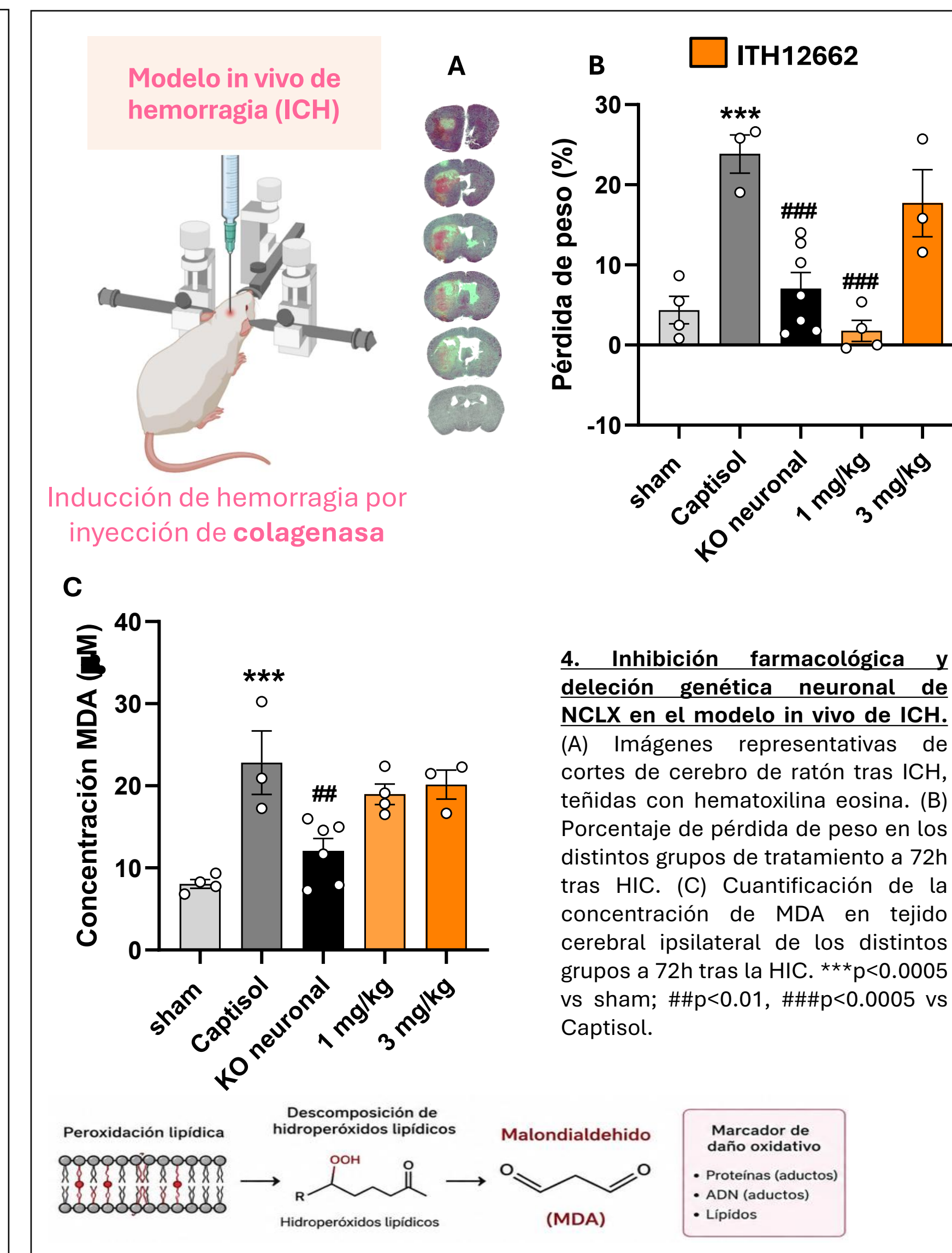
Inhibición de NCLX en el modelo tMCAO



Modelo *in vitro* de hemorragia intracerebral (HIC)



Inhibición de NCLX en el modelo HIC *in vivo*



CONCLUSIONES

NCLX juega un papel clave en la muerte celular por **ferroptosis**. Sin embargo, su **inhibición** parece ser **perjudicial** en los modelos de ictus con **reperfusión**. Por ello, muestra **potencial** en el **ictus hemorrágico**, donde la ferroptosis es clave, apoyando su estudio como **estrategia terapéutica**.

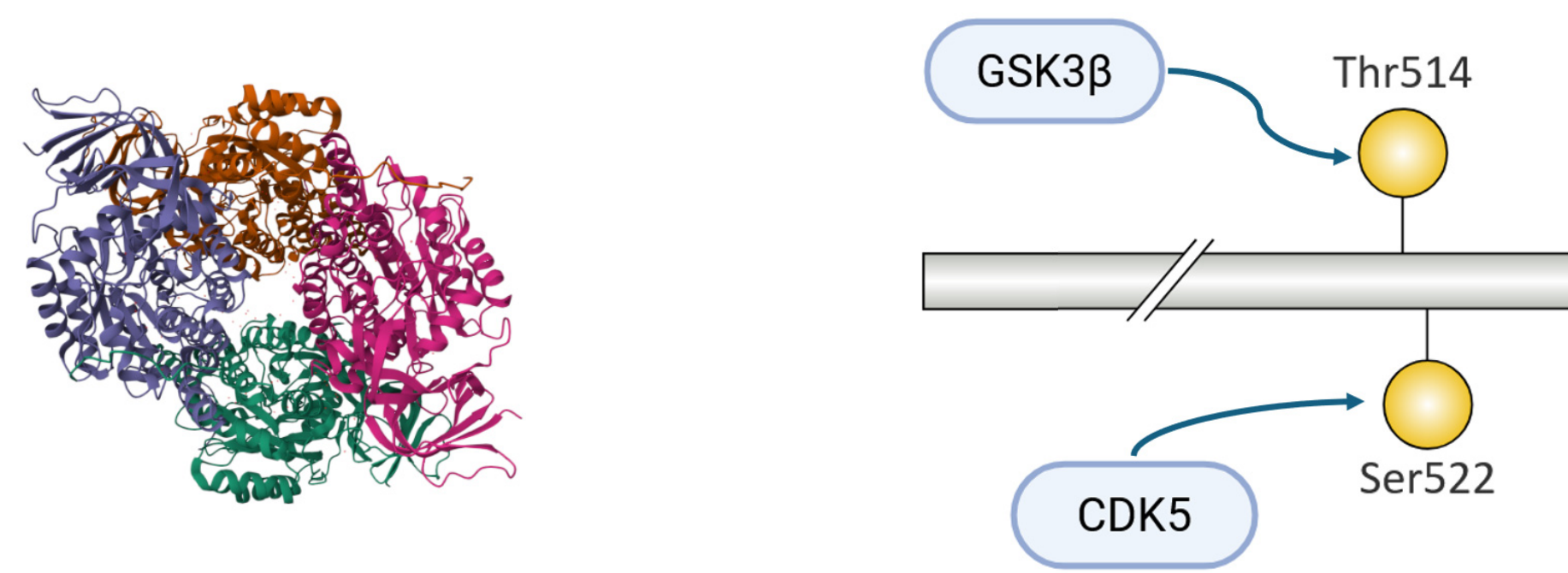
La fosforilación de CRMP2 como nuevo marcador de daño celular en ischemia cerebral

Marcel Roca¹, Emma Martínez-Alonso¹, Mikahela A. López-Morales², Juan B. Salom², Alberto Alcázar¹

(1) Departamento de Investigación, IRYCIS, Hospital Ramón y Cajal, Madrid, España. (2) Unidad Mixta de Investigación Cerebrovascular, IIS La Fe, Universidad de Valencia, Valencia, España.

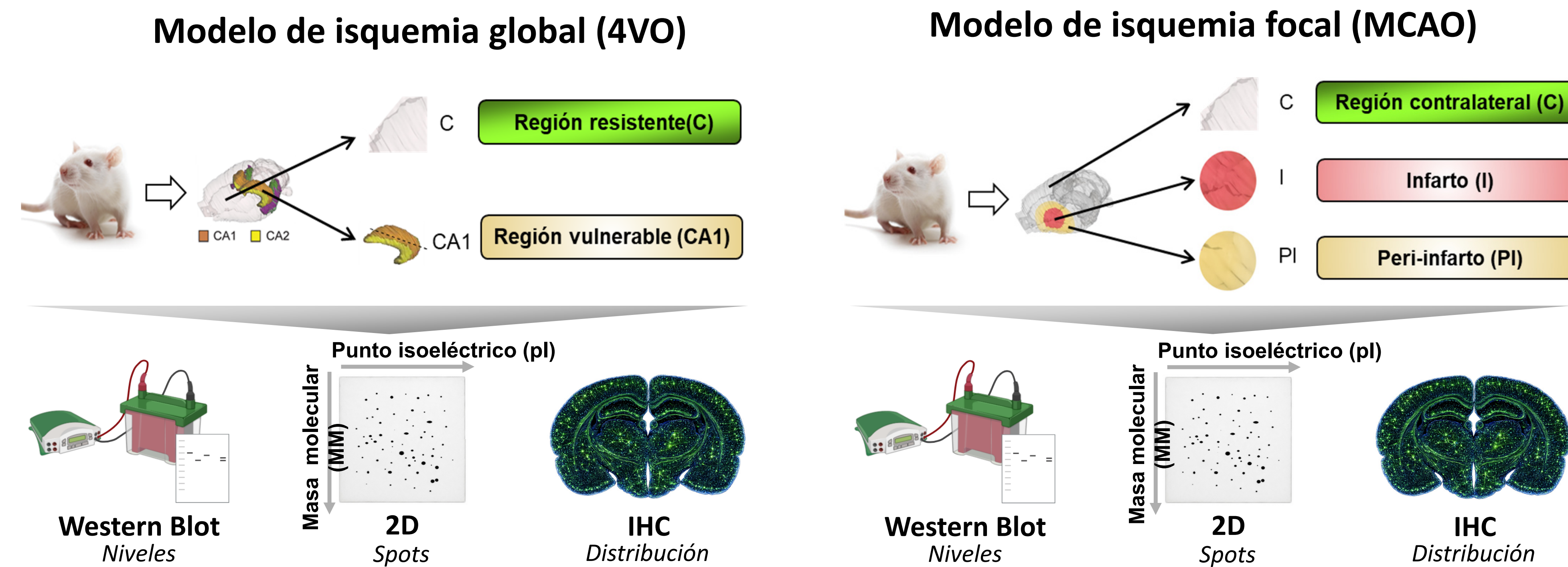
Introducción

CRMP2 (collapsin response mediator protein 2, o DRP2) es una proteína citosólica presente en el SNC. Su principal función es modular el crecimiento axonal y la plasticidad sináptica, desempeñando un papel clave en la **recuperación tras lesión**. Esta función está **regulada por fosforilación**, destacando los residuos Thr514 y Ser522 (pSer522 y pThr514) como sitios de fosforilación por GSK3 β y Cdk5, respectivamente. Además, su desregulación se ha relacionado con trastornos neurológicos, sugiriéndola como posible diana terapéutica. Por todo ello, el objetivo fue **caracterizar la fosforilación de CRMP2 tras la ischemia cerebral** e investigar su papel en la fisiopatología del ictus.



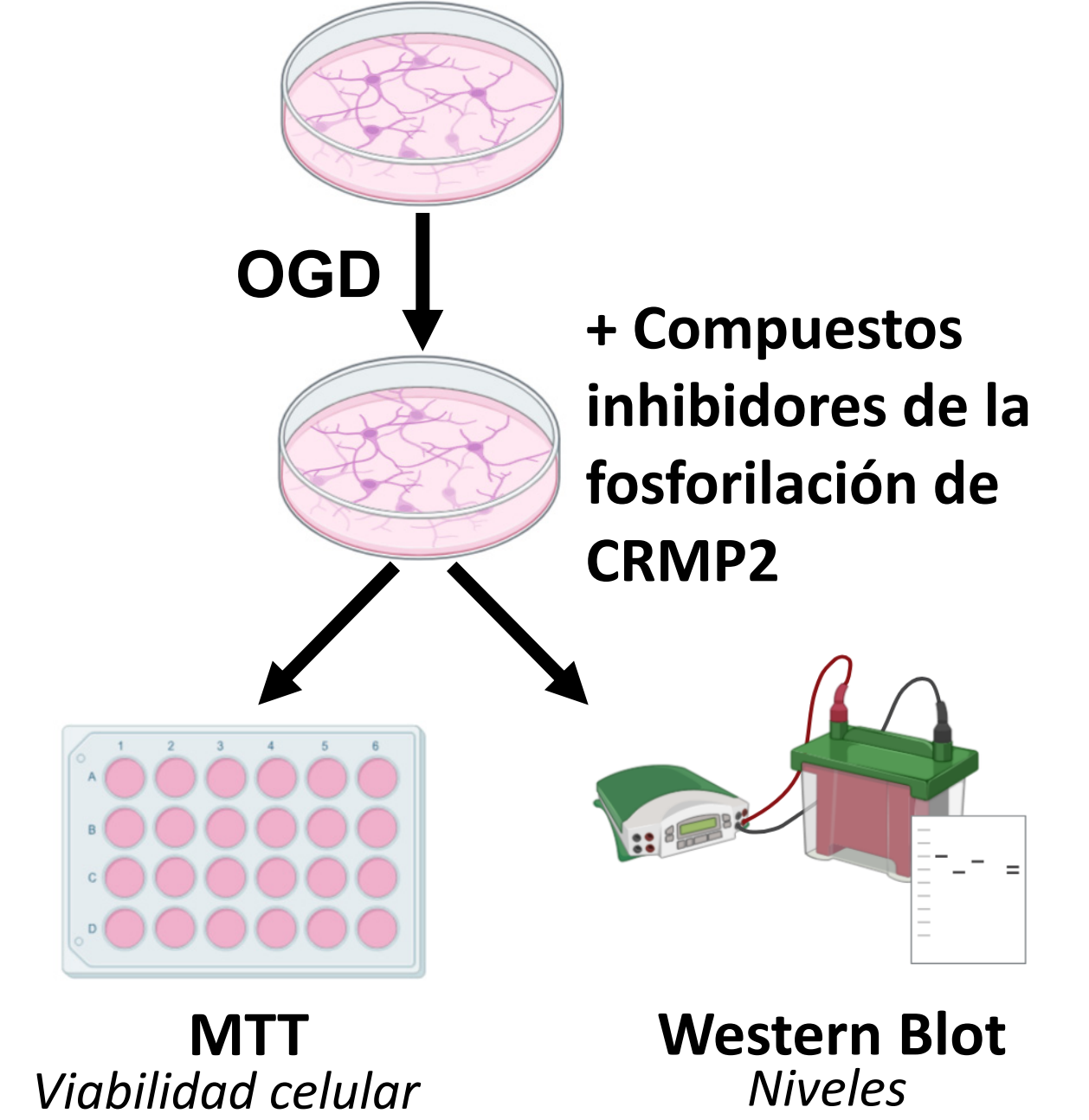
Métodos

Caracterización de las fosforilaciones en Thr514 y Ser522



Ensayo de inhibidores de la fosforilación de CRMP2

Cultivo neuronal primario



Resultados

Modelo de ischemia global

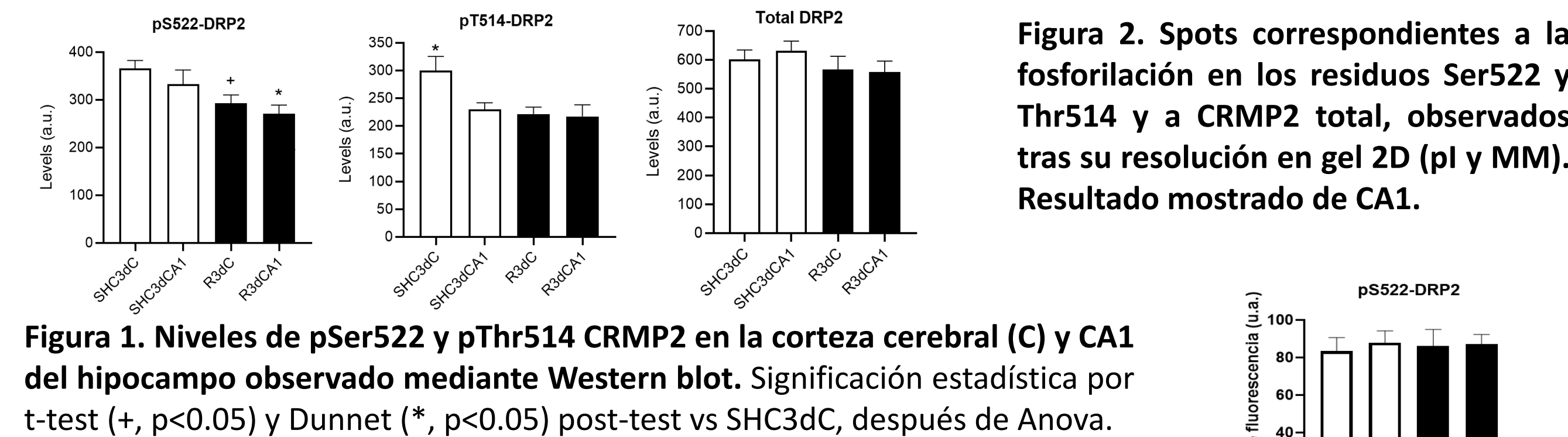
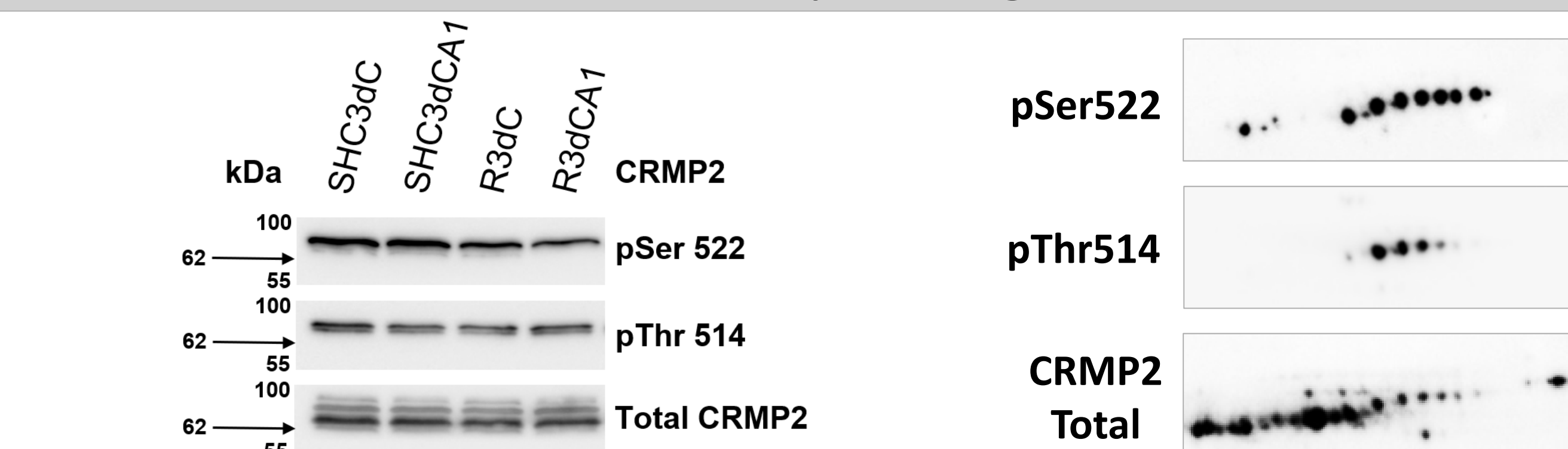


Figura 1. Niveles de pSer522 y pThr514 CRMP2 en la corteza cerebral (C) y CA1 del hipocampo observado mediante Western blot. Significación estadística por t-test (+, p<0.05) y Dunnet (*, p<0.05) post-test vs SHC3dC, después de Anova.

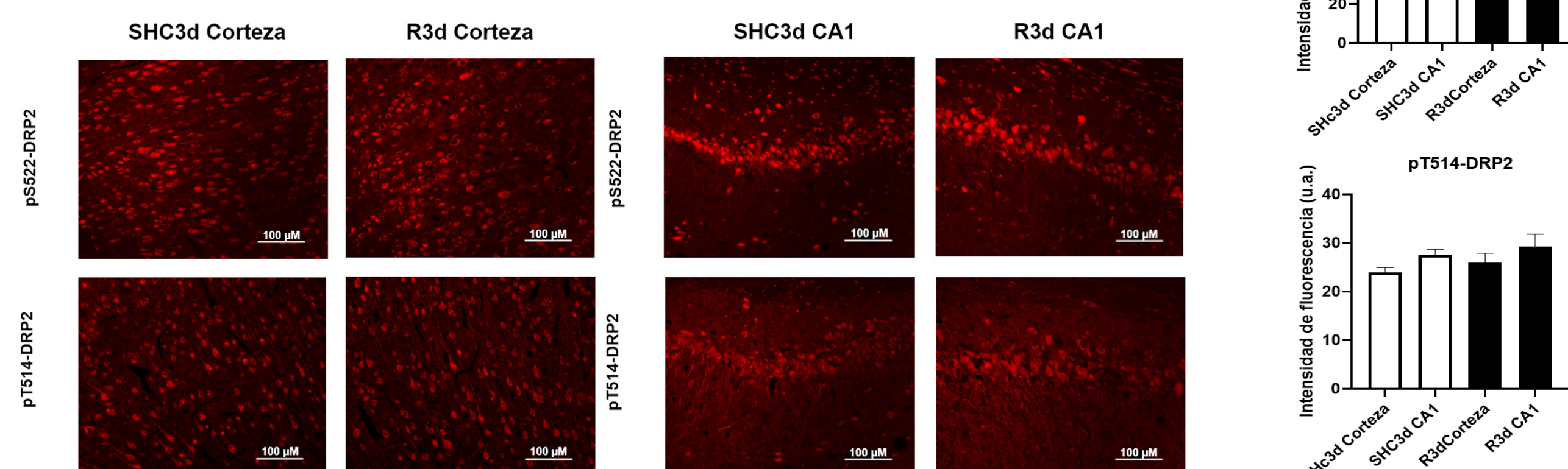


Figura 3. Distribución e intensidad de fluorescencia de pSer522 y pThr514 CRMP2 en corteza y CA1.

Modelo de ischemia focal

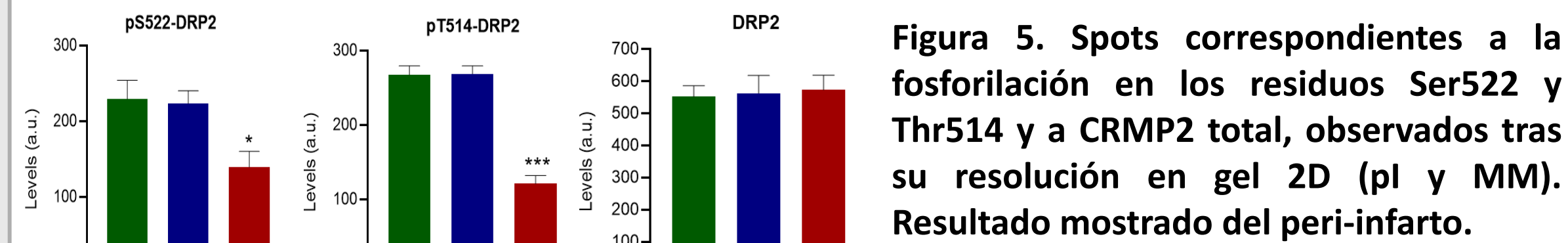
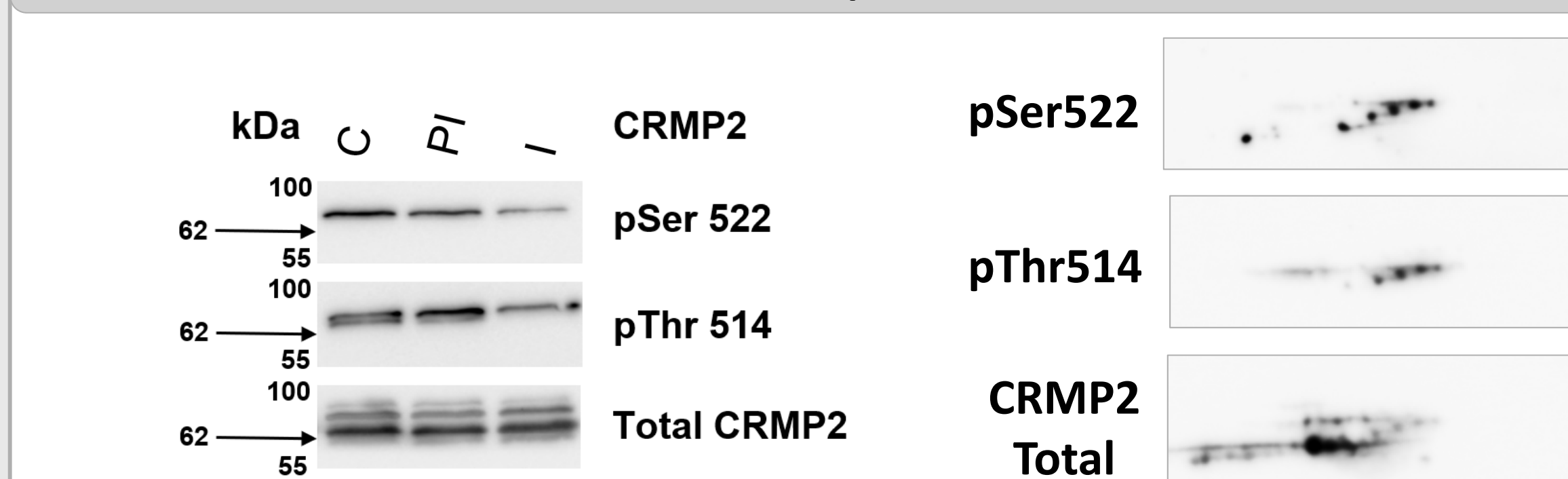


Figura 4. Niveles de pSer522 y pThr514 CRMP2 en corteza contralateral (C), peri-infarto (PI) e infarto (I) observado mediante Western blot. Significación estadística por Dunnet post-test (*, p<0.05; ***, p<0.001) vs C y PI, después de Anova.

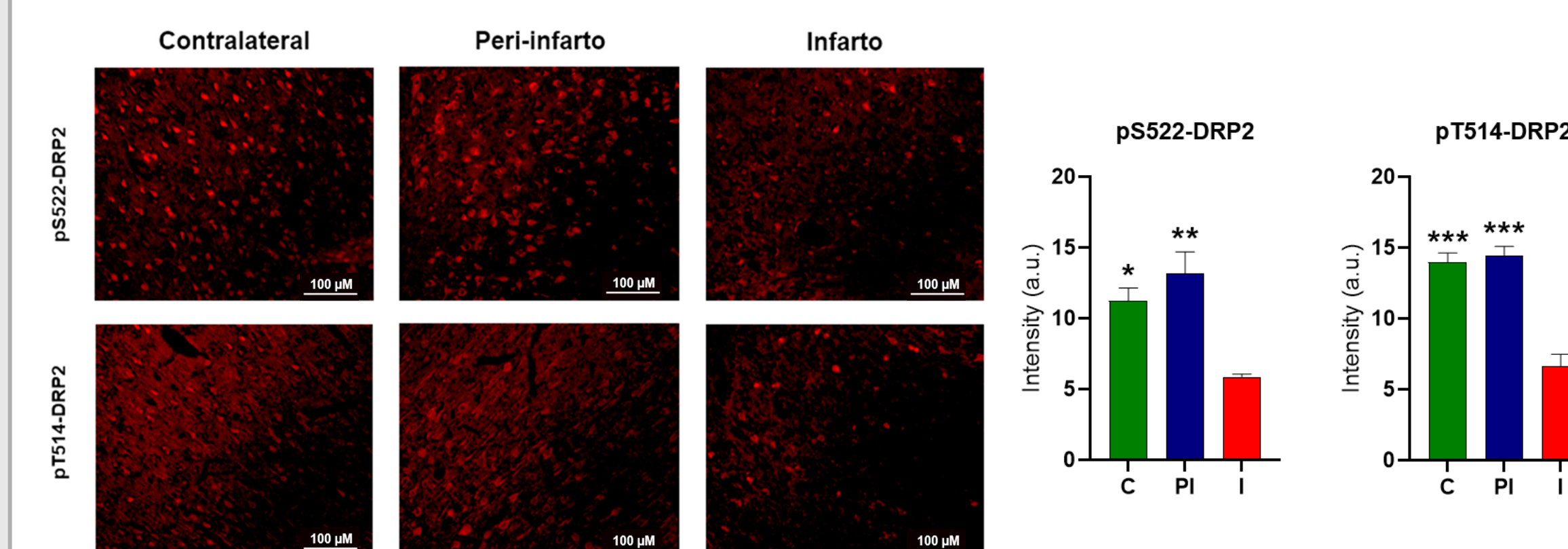


Figura 6. Distribución e intensidad de fluorescencia de pSer522 y pThr514 CRMP2 en la región contralateral (C), peri-infarto (PI) e infarto (I). Significación estadística por Dunnet post-test (*, p<0.05; **, p<0.01; ***, p<0.001) vs Infarto (I), después de Anova.

Cultivos neuronales primarios

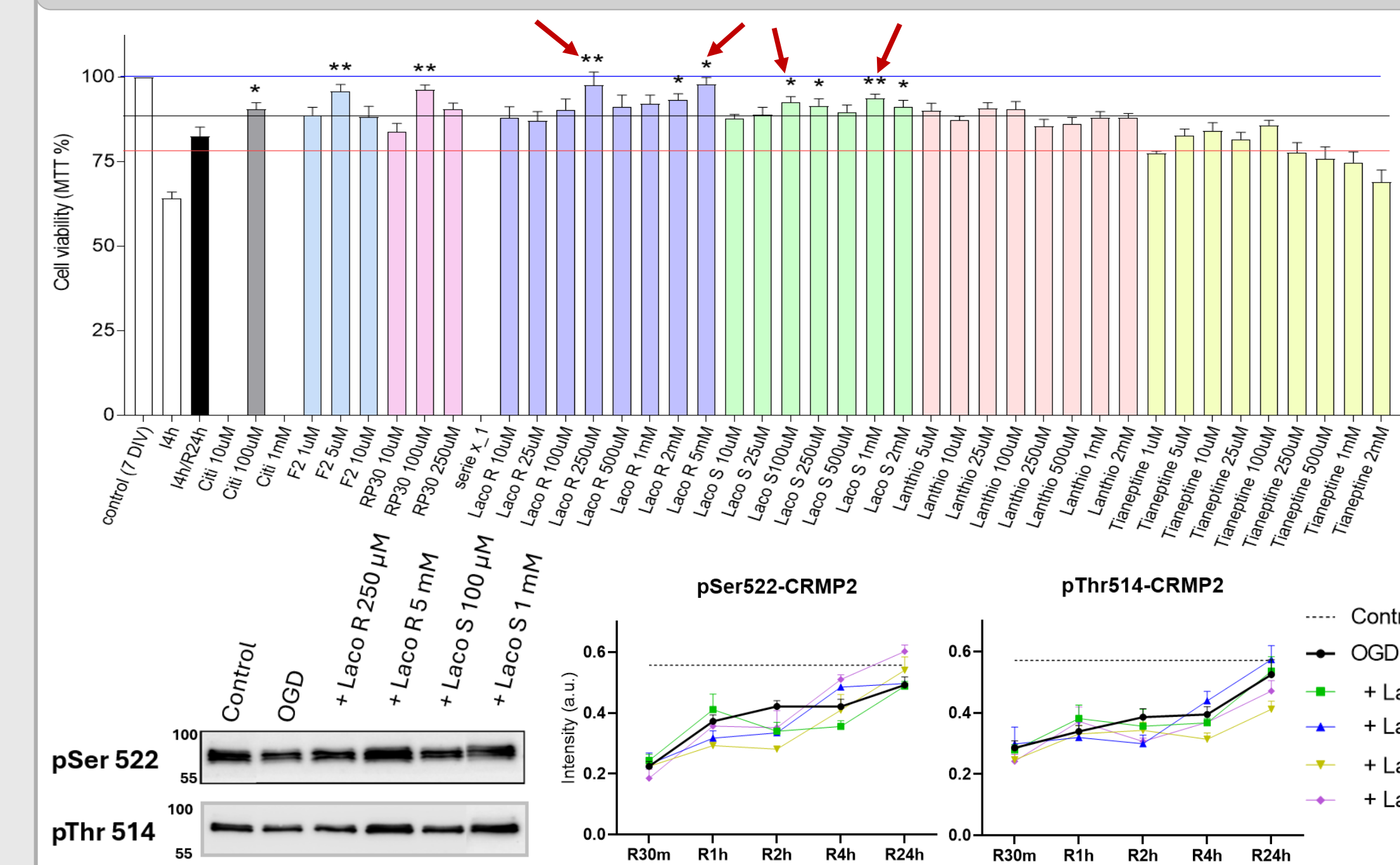
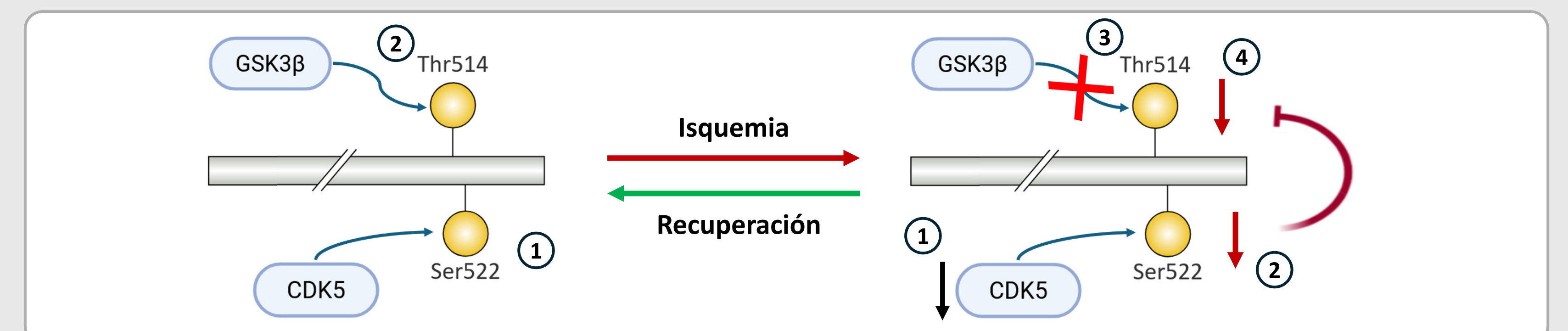


Figura 7. Ensayo de viabilidad celular en cultivos neuronales primarios sometidos a OGD y posterior tratamiento con inhibidores de la fosforilación de CRMP2 al inicio de la reperfusion. Las flechas señalan las concentraciones con mayor recuperación de la viabilidad celular. *, p<0.05; y **, p<0.01; por Dunnet post-test vs 24 h de recuperación sin tratamiento (14h/R24h), después de Anova.

Figura 8. Efecto sobre la fosforilación de Thr514 y Ser522 en cultivos neuronales primarios tratados con los inhibidores seleccionados en la Figura 7. No se encontraron resultados significativos.

Conclusiones

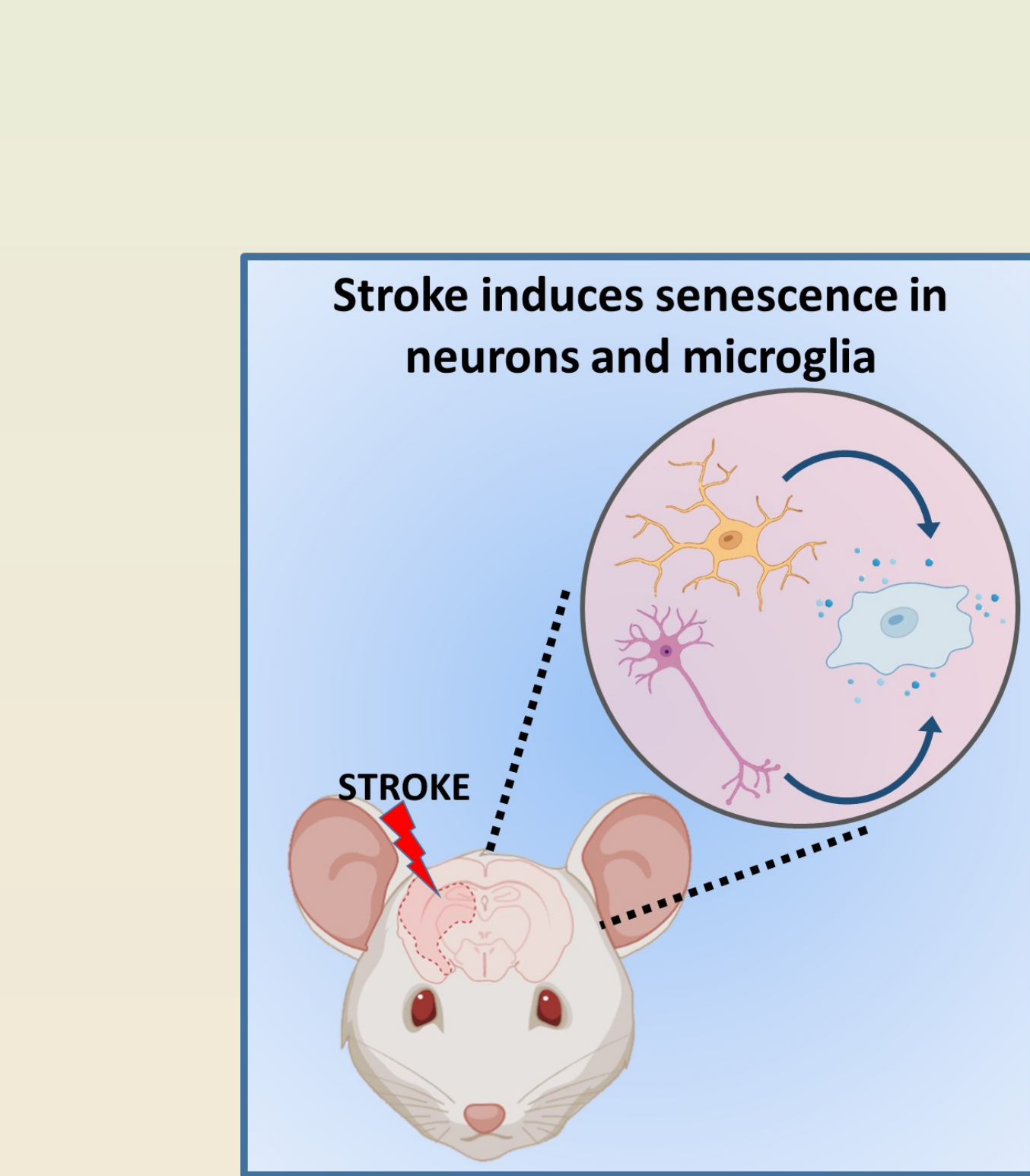


TARGETING CELLULAR SENESCENCE AFTER ISCHEMIC STROKE:
THERAPEUTIC EFFECT OF THE SENOLYTIC NAVITOCCLAXMikahela A. López-Morales^{1,2}, Júlia Baixauli-Martín¹, María C. Burguete^{1,3}, Alicia Aliena-Valero¹, José E. Peris-Ribera^{1,4}, Juan B Salom^{1,3}.¹ Unidad Mixta de Investigación Cerebrovascular, IIS La FE, 46026 Valencia, Spain.² Departamento de Fisioterapia, Universidad de Valencia, 46010 Valencia, Spain.³ Departamento de Biotecnología, Universidad Politécnica de Valencia, 46022 Valencia, Spain.⁴ Departamento de Fisiología, Universidad de Valencia, 46100 Burjassot, Valencia, Spain.

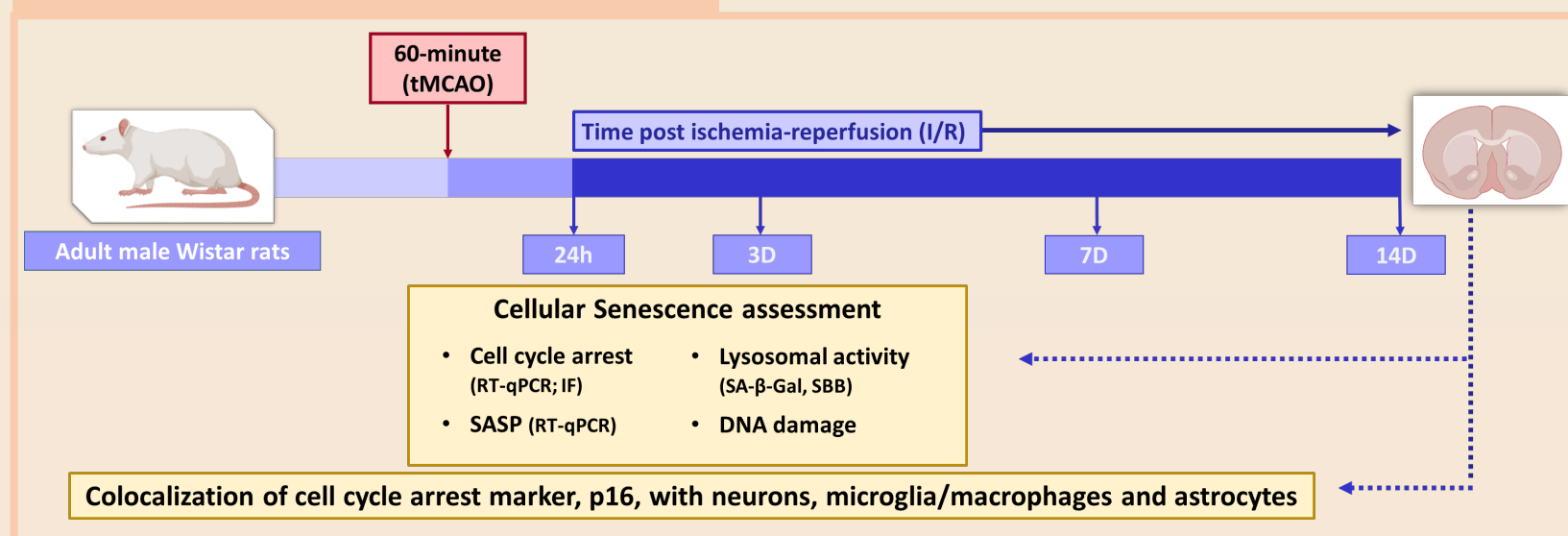
*mikahela_lopez@iislafe.es

BACKGROUND AND OBJECTIVES

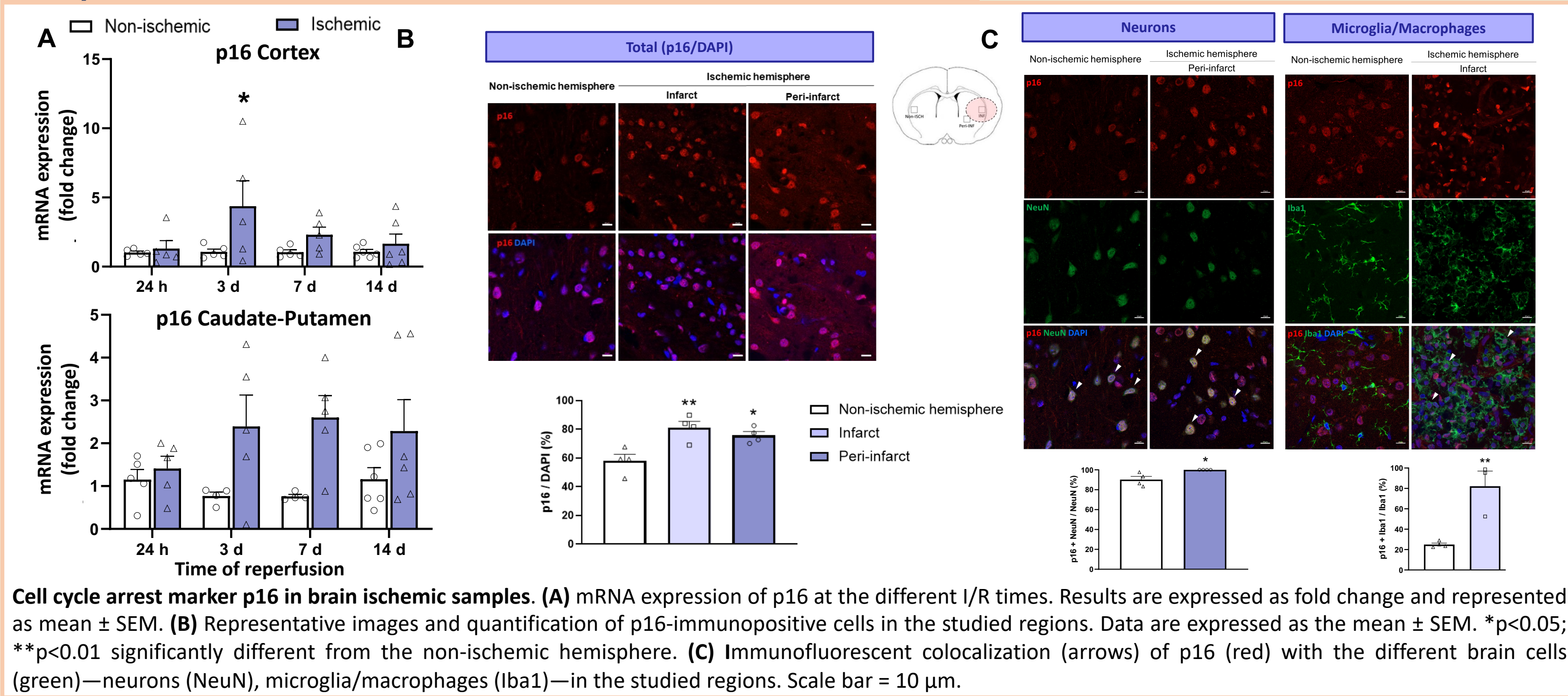
Brain cellular senescence has been suggested as a pathophysiological mechanism in ischaemic stroke (IS). We and others have recently described its occurrence in brain cells following IS, pointing to the potential of targeting senescence as a cerebroprotective therapeutic approach. **This study aimed to further characterise cellular senescence following IS, to identify the cell types involved, and to evaluate the effects of the senolytic drug navitoclax.**



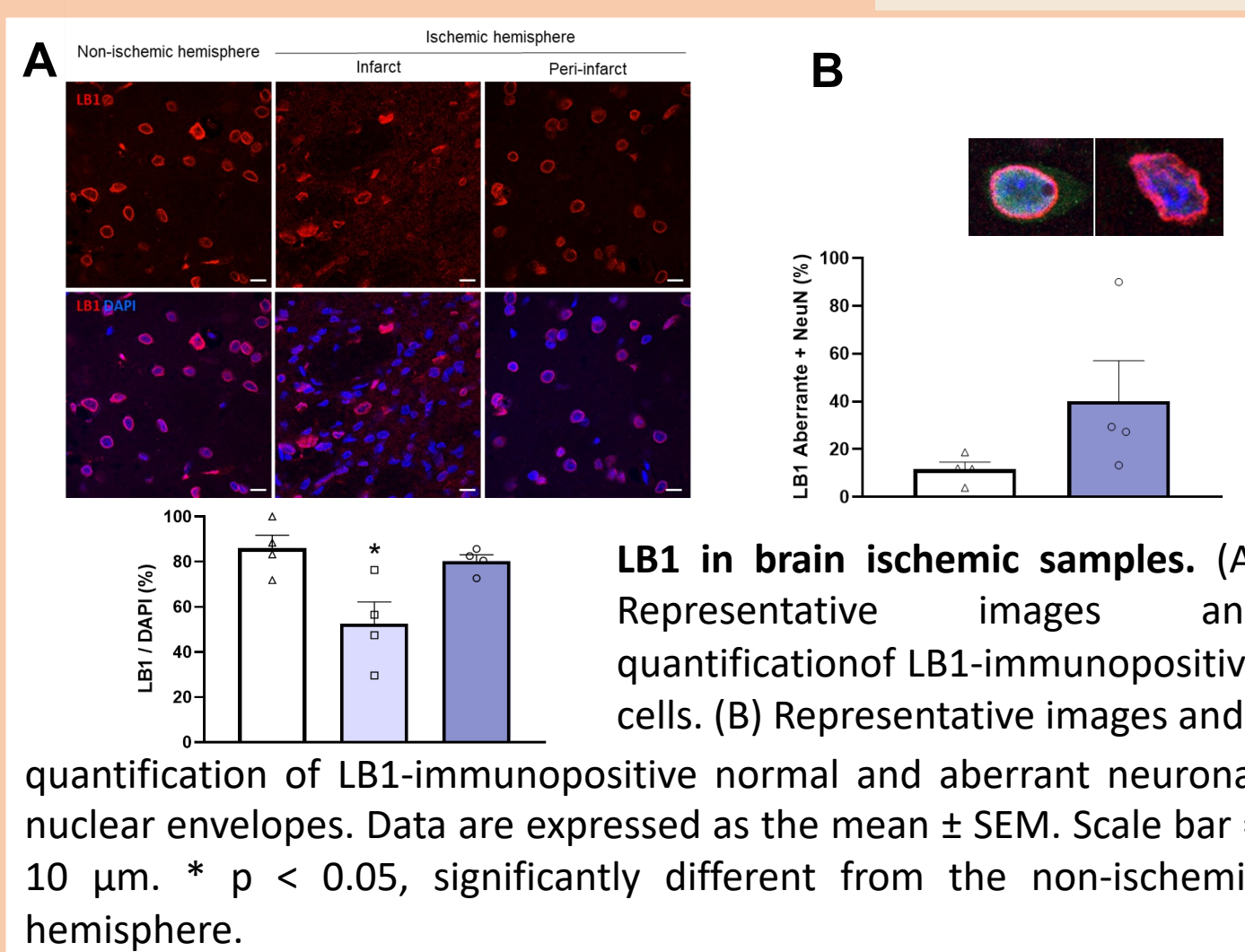
Methods



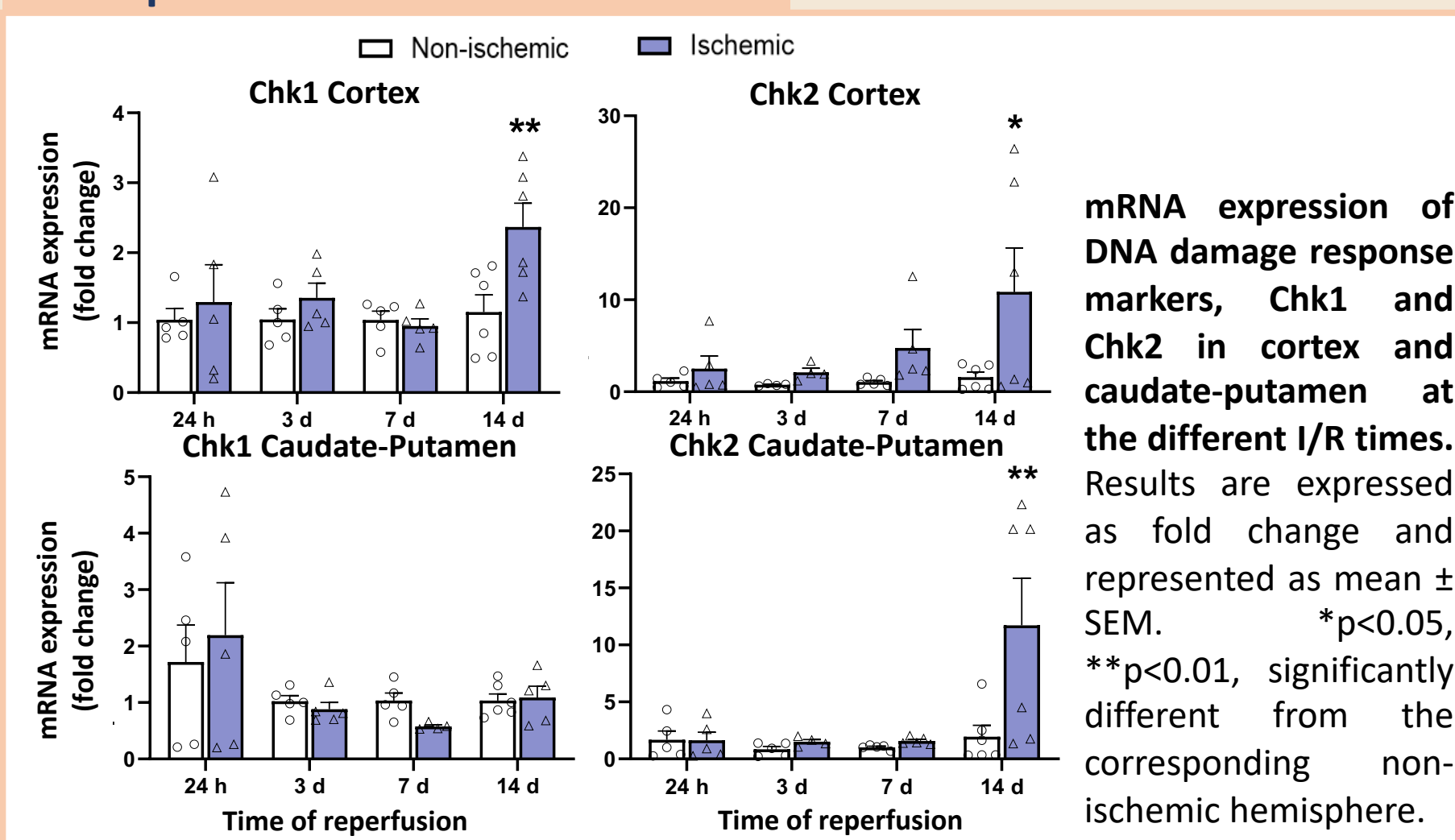
Cell cycle arrest



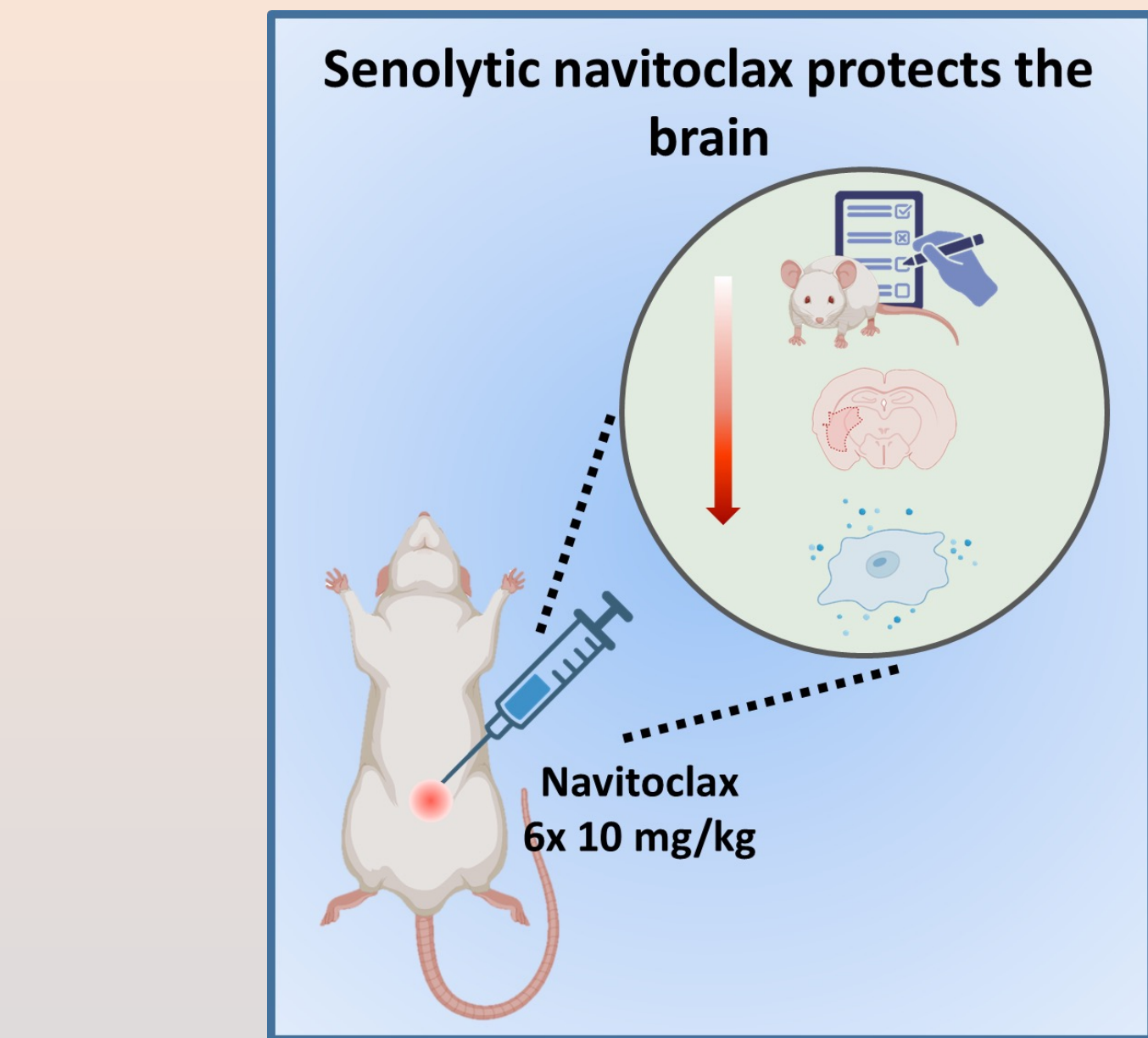
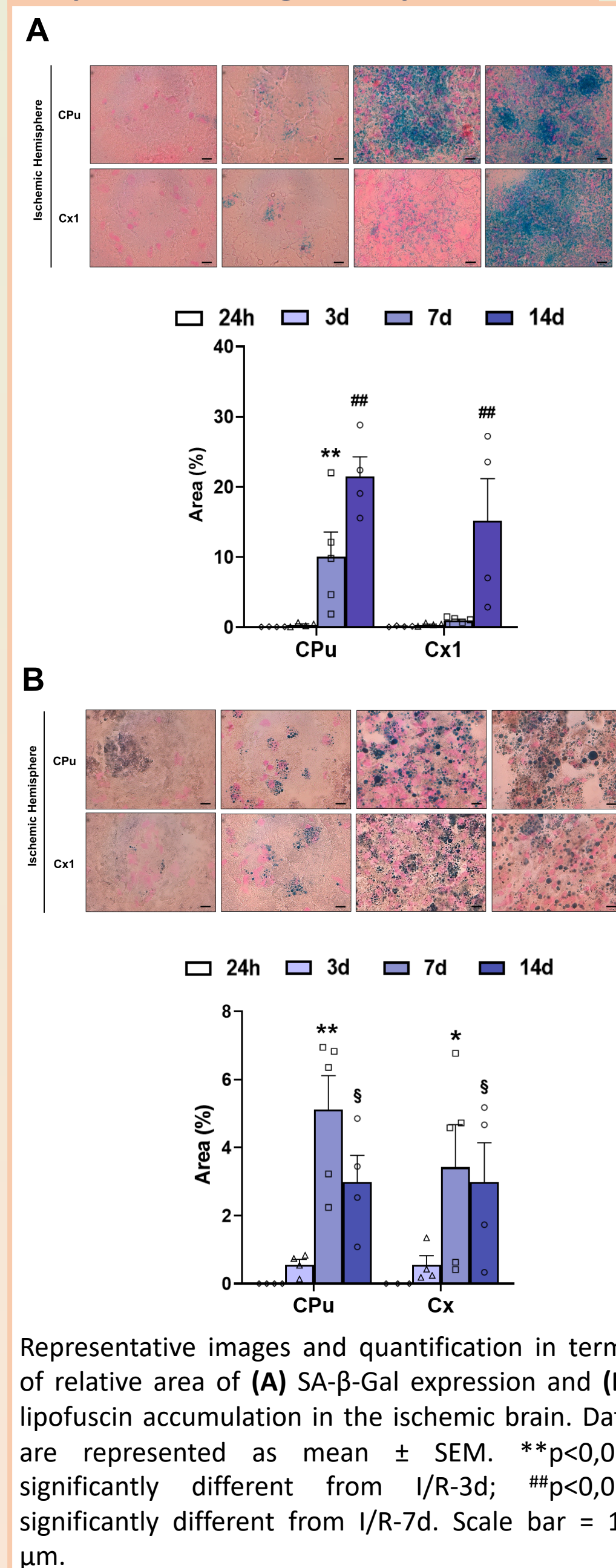
Lamin B1



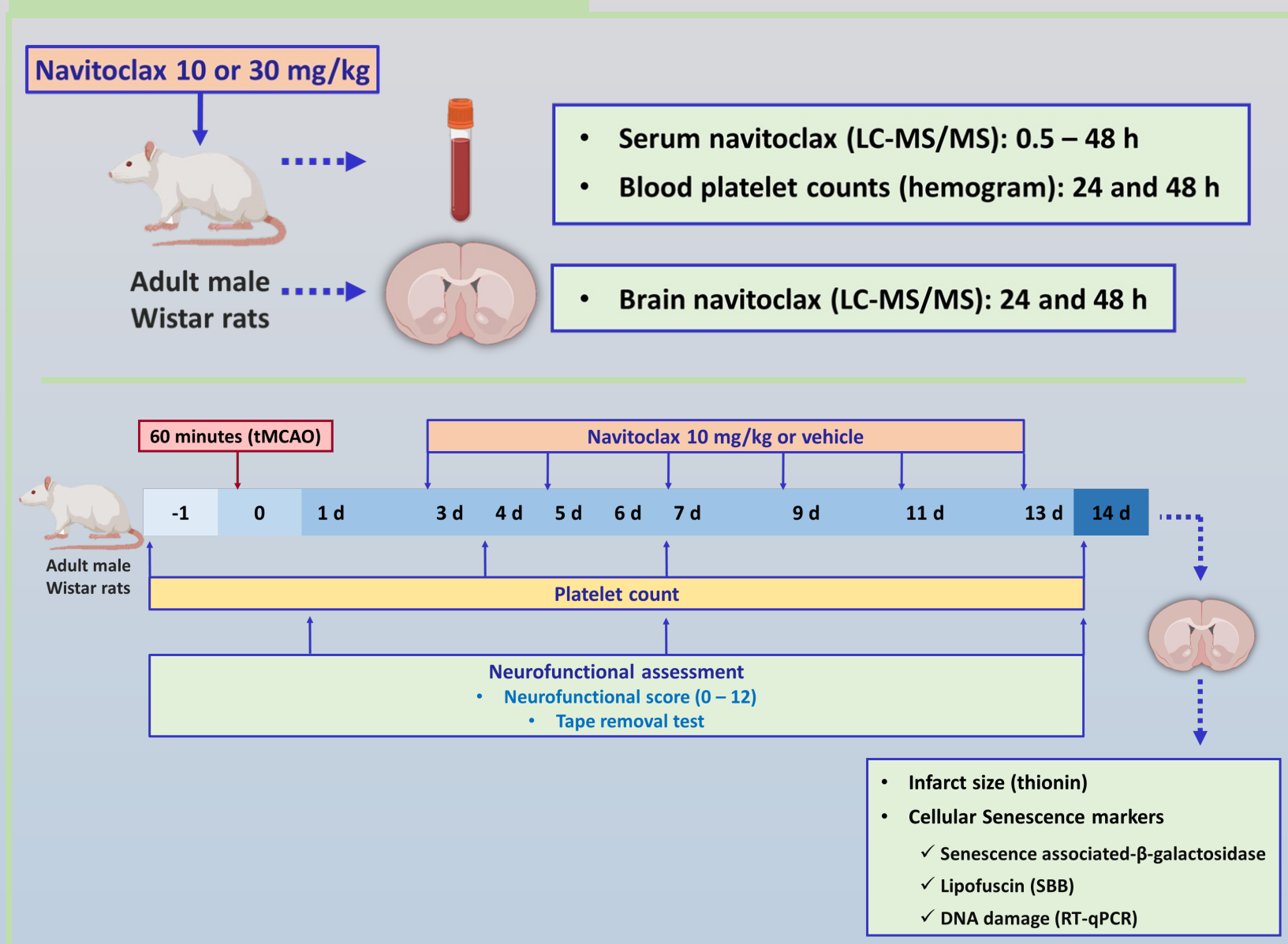
Checkpoint kinases 1 and 2



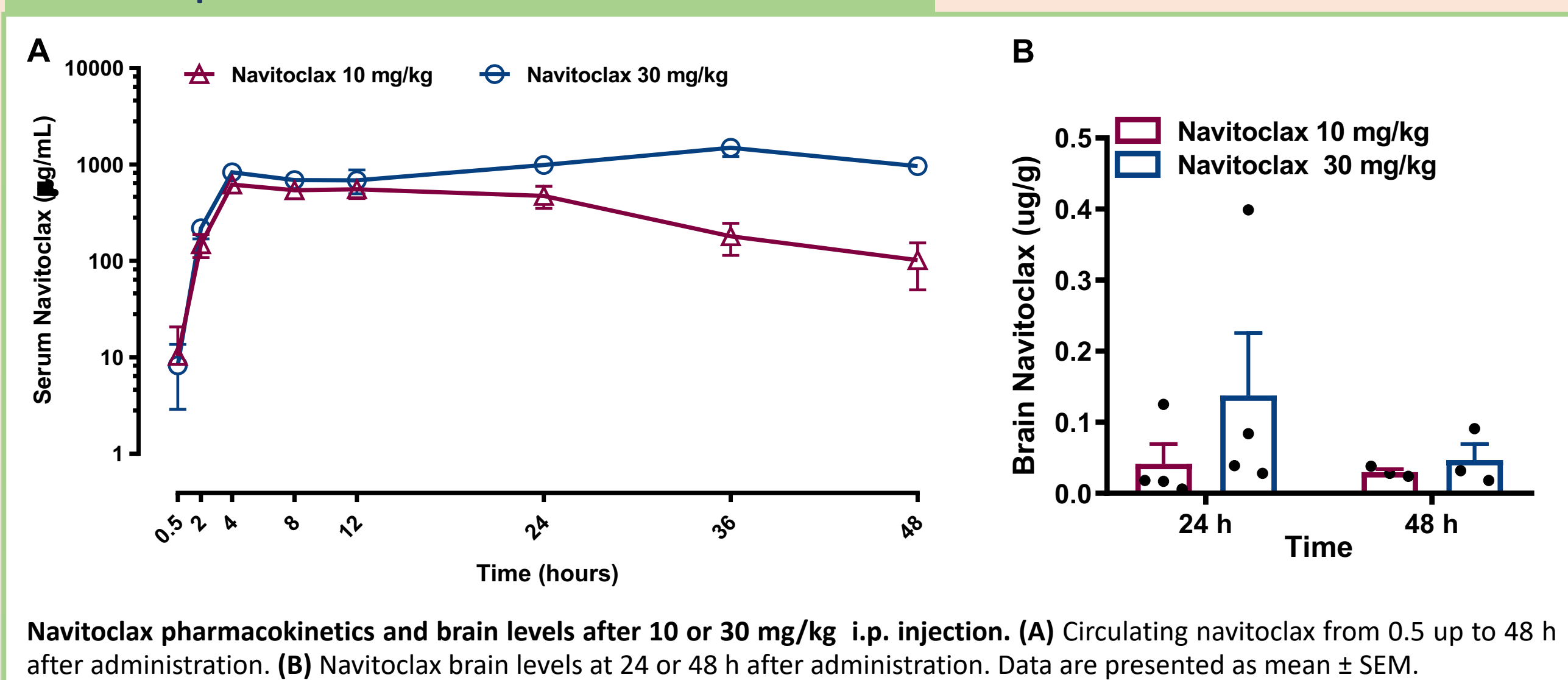
SA-β-Gal Staining and Lipofuscin



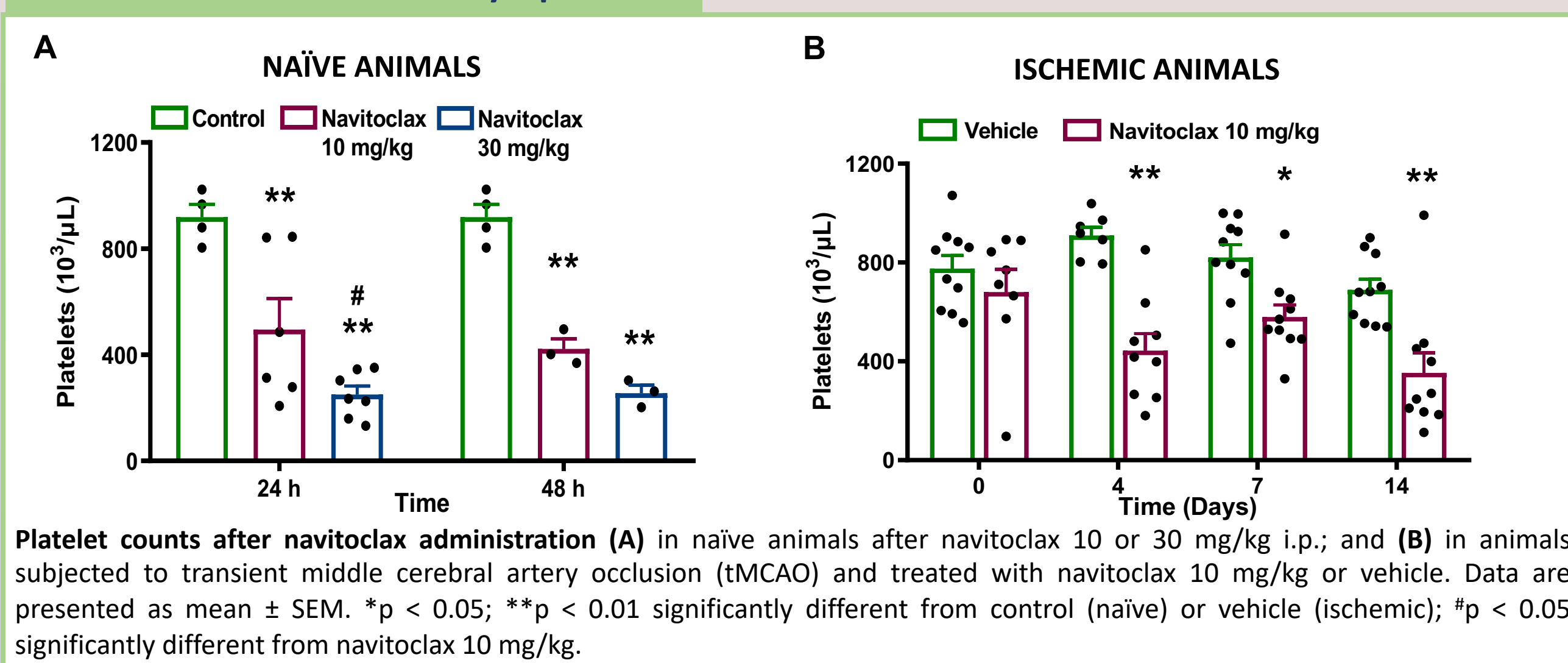
Methods



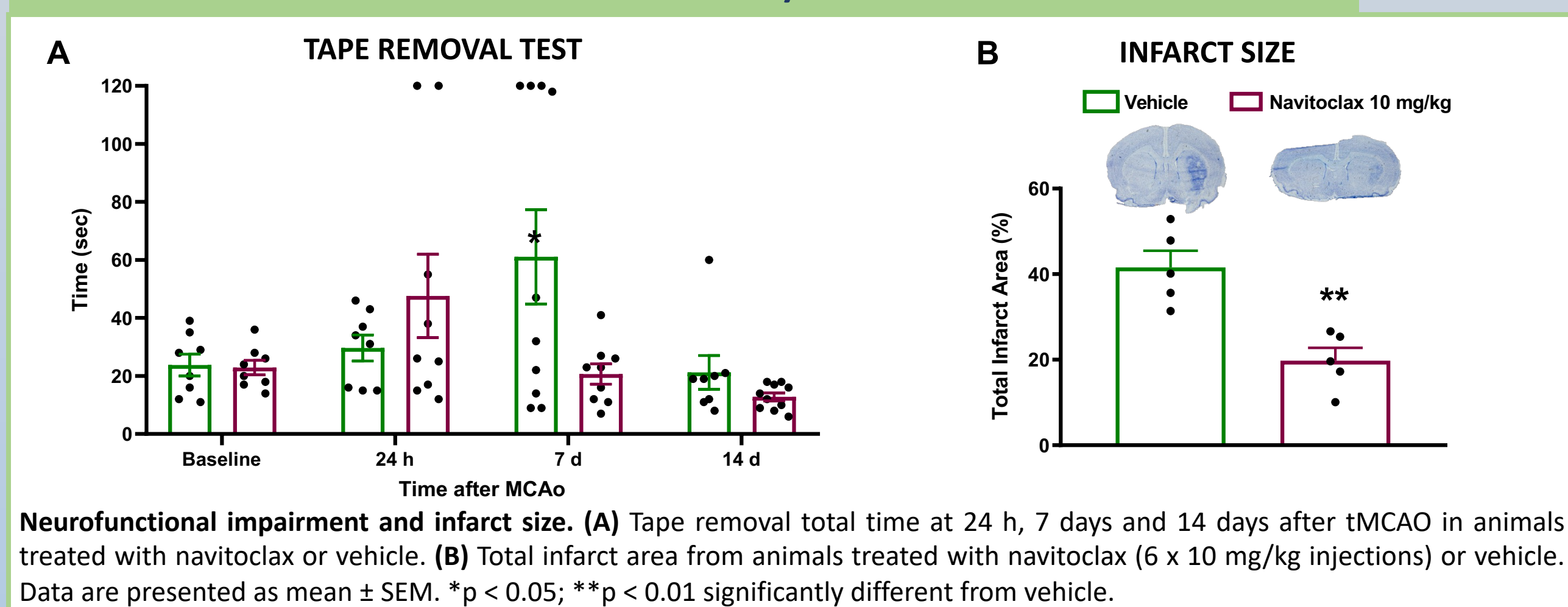
Navitoclax pharmacokinetics and brain levels



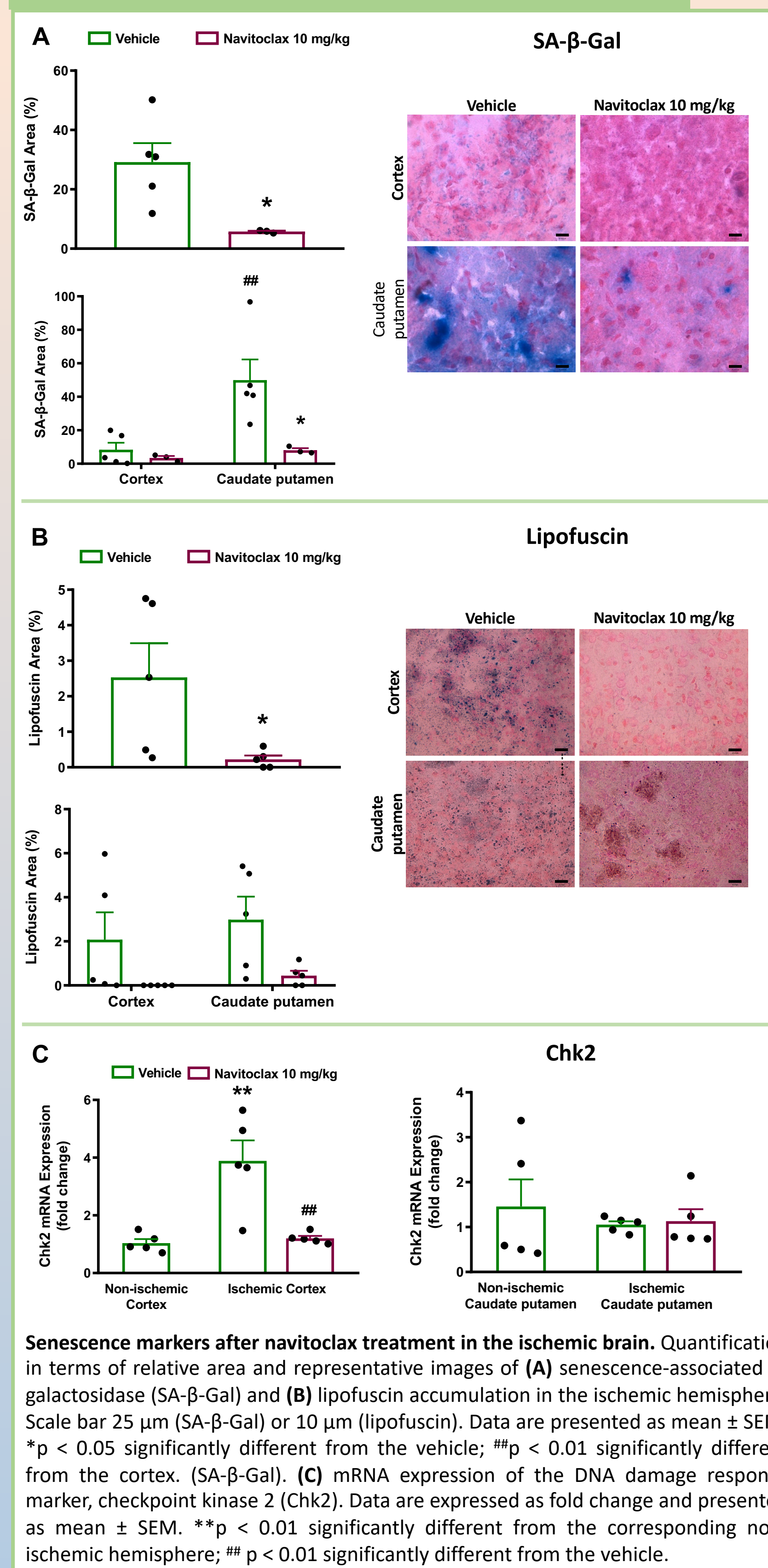
Navitoclax induces thrombocytopenia



Navitoclax accelerates neurofunctional recovery and reduces infarct size after IS



Navitoclax decreases senescence markers in the ischemic brain



CONCLUSIONS

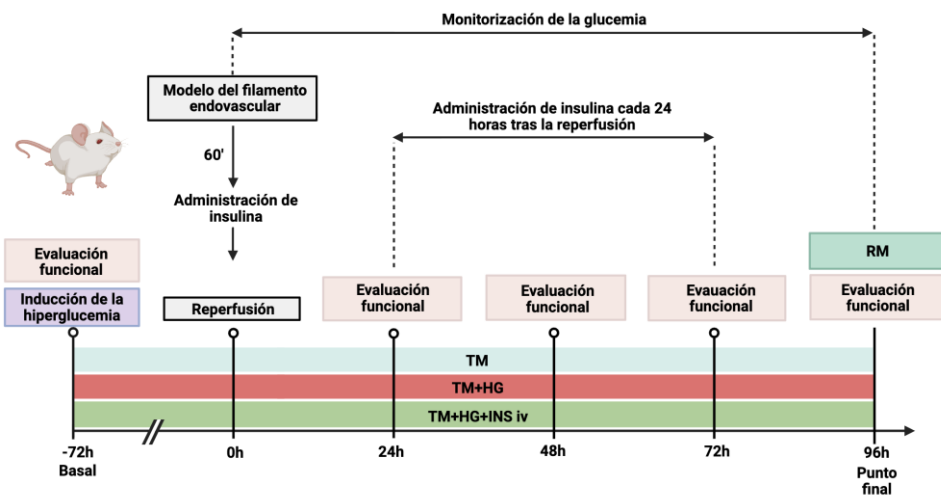
- Cellular senescence is triggered after ischemic stroke in neurons and microglia/macrophages.
- Navitoclax confers protection after ischemic stroke by improving functional recovery and reducing infarct size, which is associated with decreased senescence markers.
- Although moderate thrombocytopenia warrants caution, targeting senescence emerges as a promising therapeutic strategy for ischemic stroke.

FUNDING: This study was supported by Ministerio de Ciencia e Innovación (MICIU/AEI /10.13039/501100011033), grant PID2020-119603RB-I00, and by Conselleria de Educació, Cultura, Universitats y Empleo de la Generalitat Valenciana (GE 2025), grant CIGE/2024/81.

Objetivo del estudio: investigar si una menor variabilidad glucémica, mediante la administración de insulina intravenosa, se asocia a una mejor recuperación en un modelo de ictus isquémico con reperusión en rata.

DISEÑO DEL ESTUDIO

Ratas Sprague-Dawley. 3 grupos experimentales aleatorizados: **Machos y hembras 1:1 N=20**



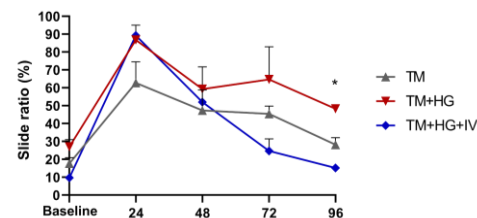
CONCLUSIÓN

Reducir la variabilidad glucémica mediante insulina intravenosa se asocia a una mejor recuperación funcional y protección cerebral en un modelo experimental en ictus con reperusión.

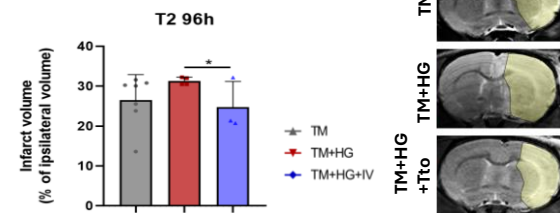
RESULTADOS

La reducción de la VG por medio de la administración de insulina IV:

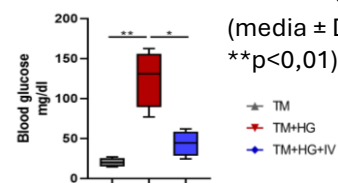
✓ **Mejora la recuperación funcional a 96h.** (media $\pm$ DE; * $p < 0.05$).



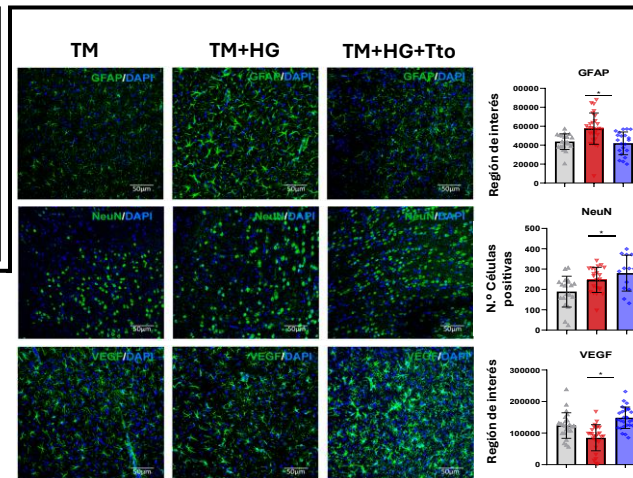
✓ **Reduce el volumen del infarto.**
(media $\pm$ DE; *p<0.05).



✓ Reduce la variabilidad glucémica.



✓ Favorece la **angiogénesis**, reduce la **astrogliosis** y contribuye a la **preservación del tejido cerebral**. (media $\pm$ DE; *p<0.05).



NOVEL INSIGHTS INTO WRAP53 NUCLEAR TRANSLOCATION AND ITS NEUROPROTECTIVE EFFECT IN STROKE

Irene Sánchez-Morán^{1,2}, Sandra Martínez-Peralta^{1,2}, Cristina Rodríguez^{1,2}, Ángeles Almeida.

Instituto de Biología Funcional y Genómica (IBFG), CSIC, Universidad de Salamanca, Salamanca, Spain

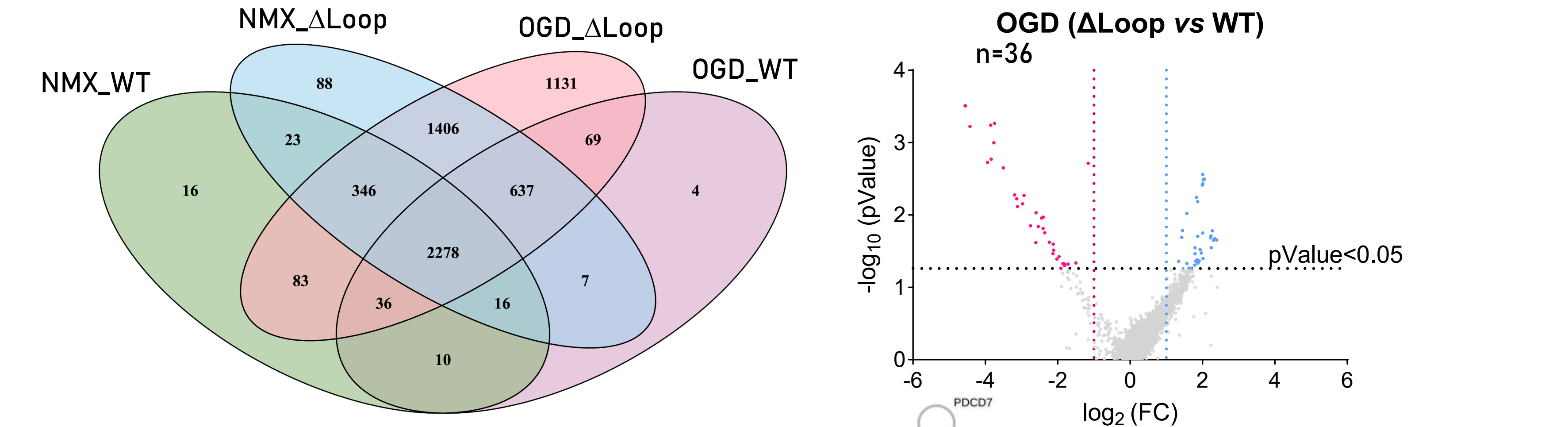
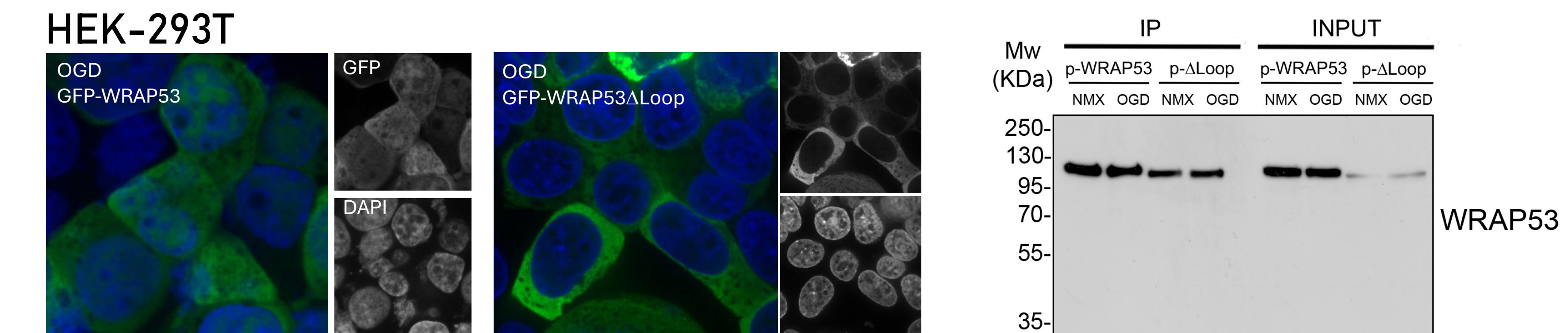
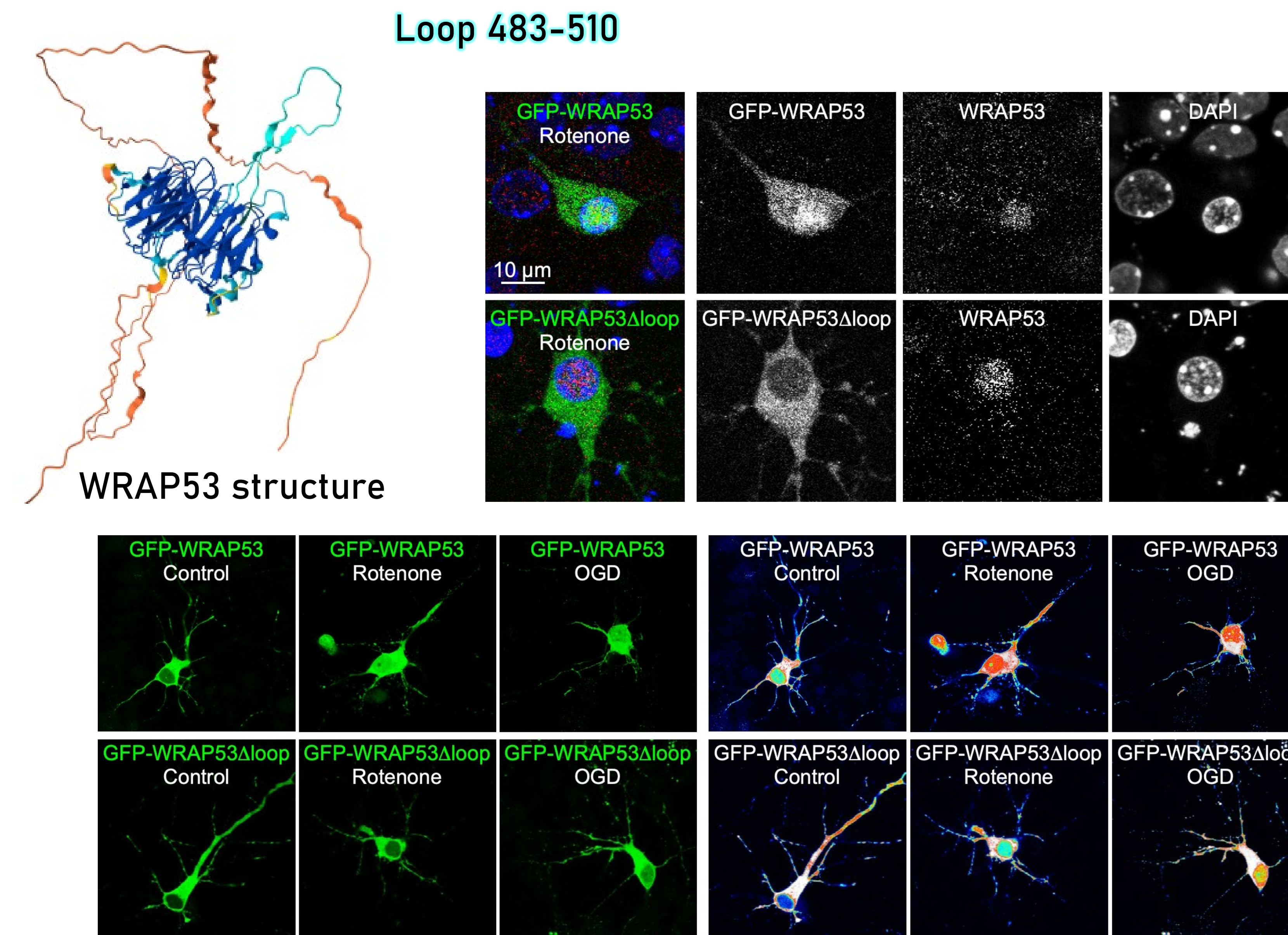
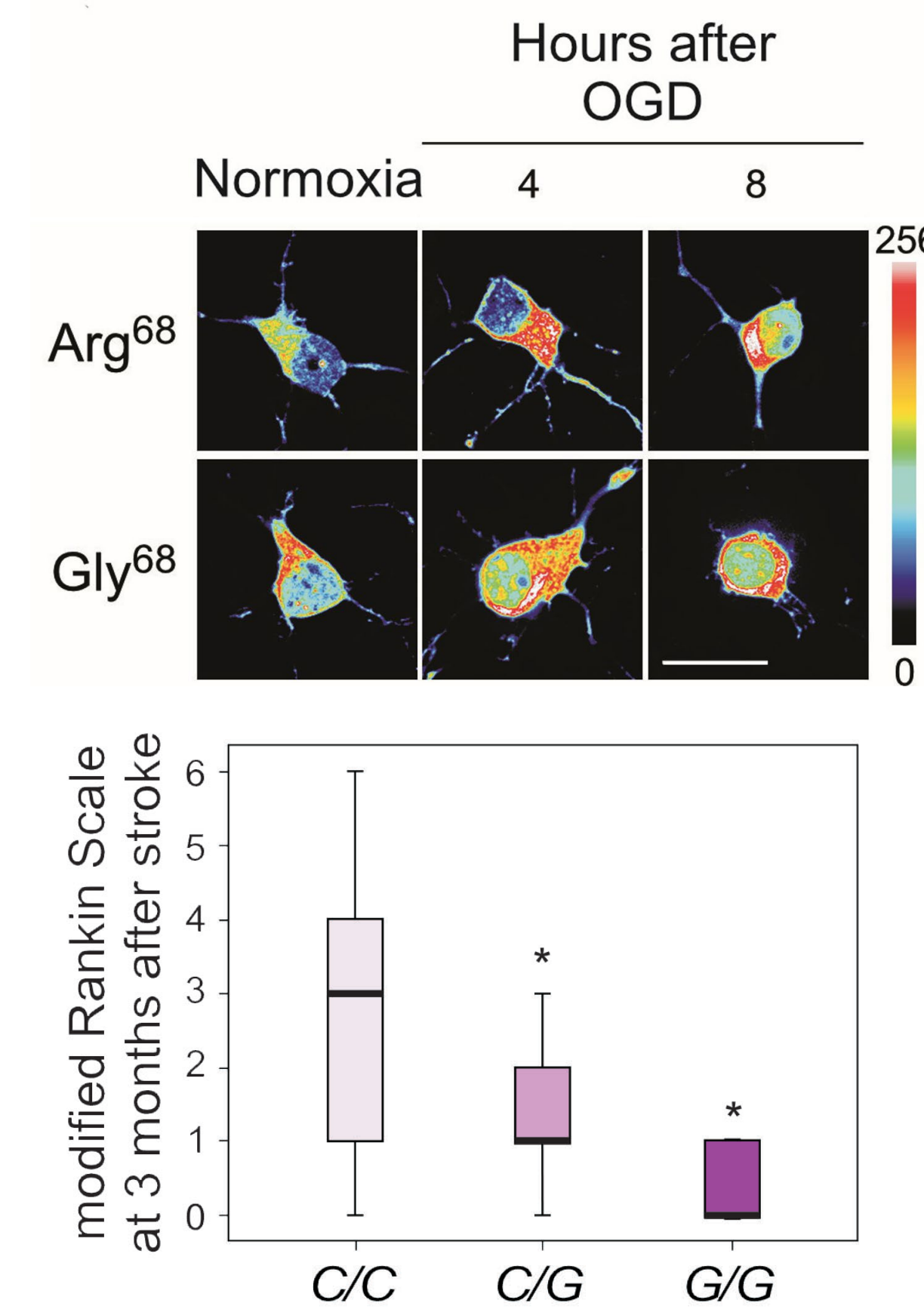
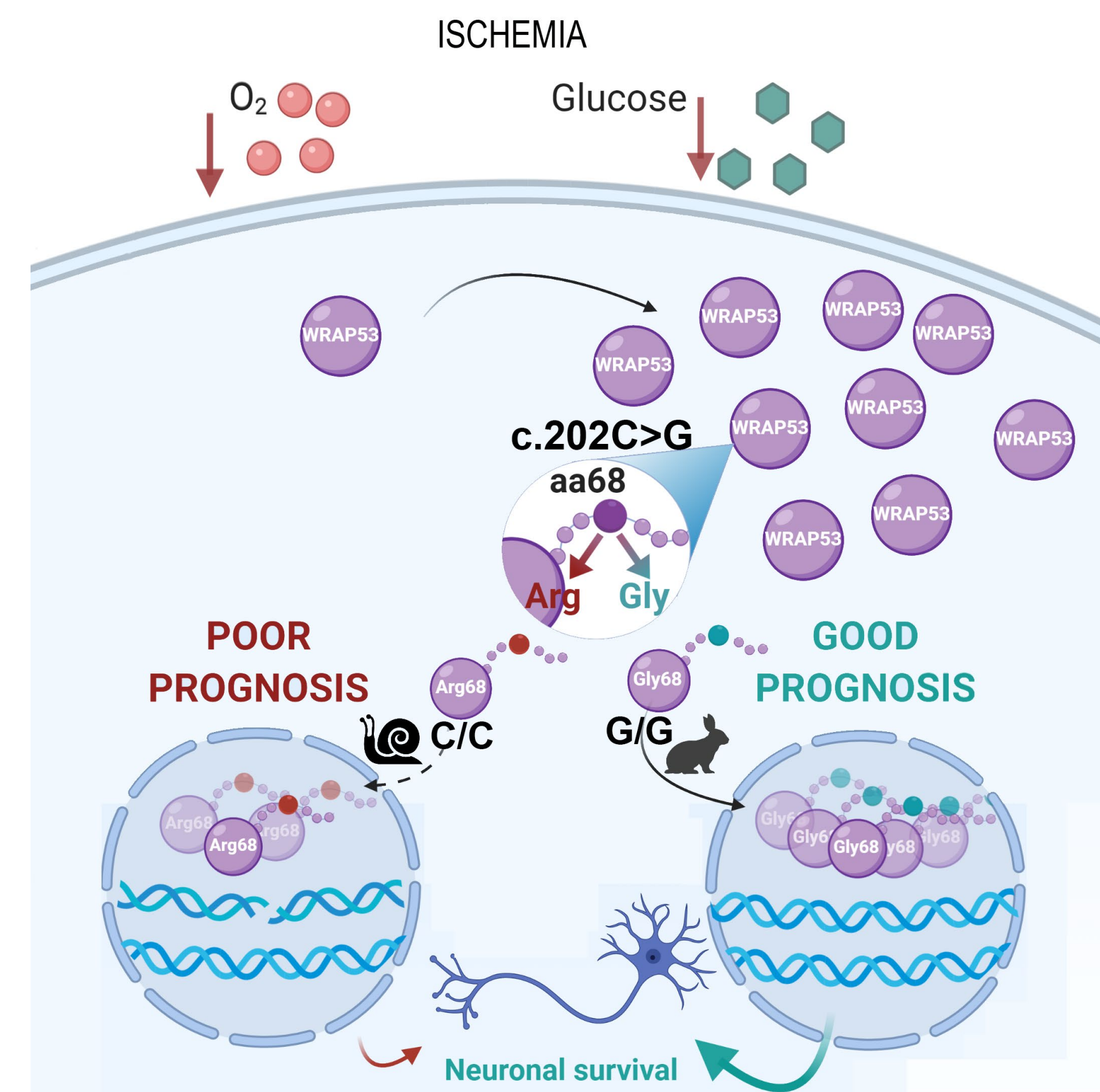
Instituto de Investigación Biomédica de Salamanca (IBSAL), Hospital Universitario de Salamanca, Universidad de Salamanca, CSIC, Salamanca, Spain

2. The amino acid sequence 483–510 governs WRAP53 translocation driven by ischemia-induced oxidative stress

3. The study of the interactome of WRAP53 revealed putative modulators of WRAP53 translocation

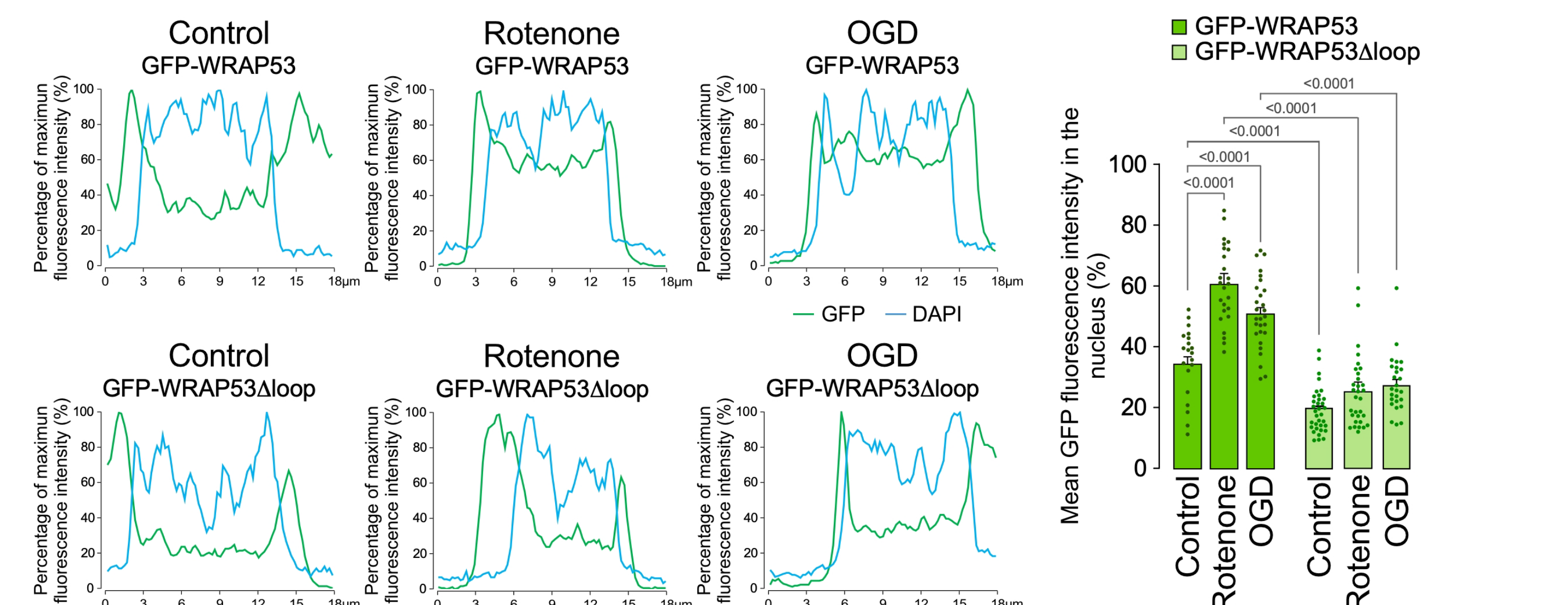
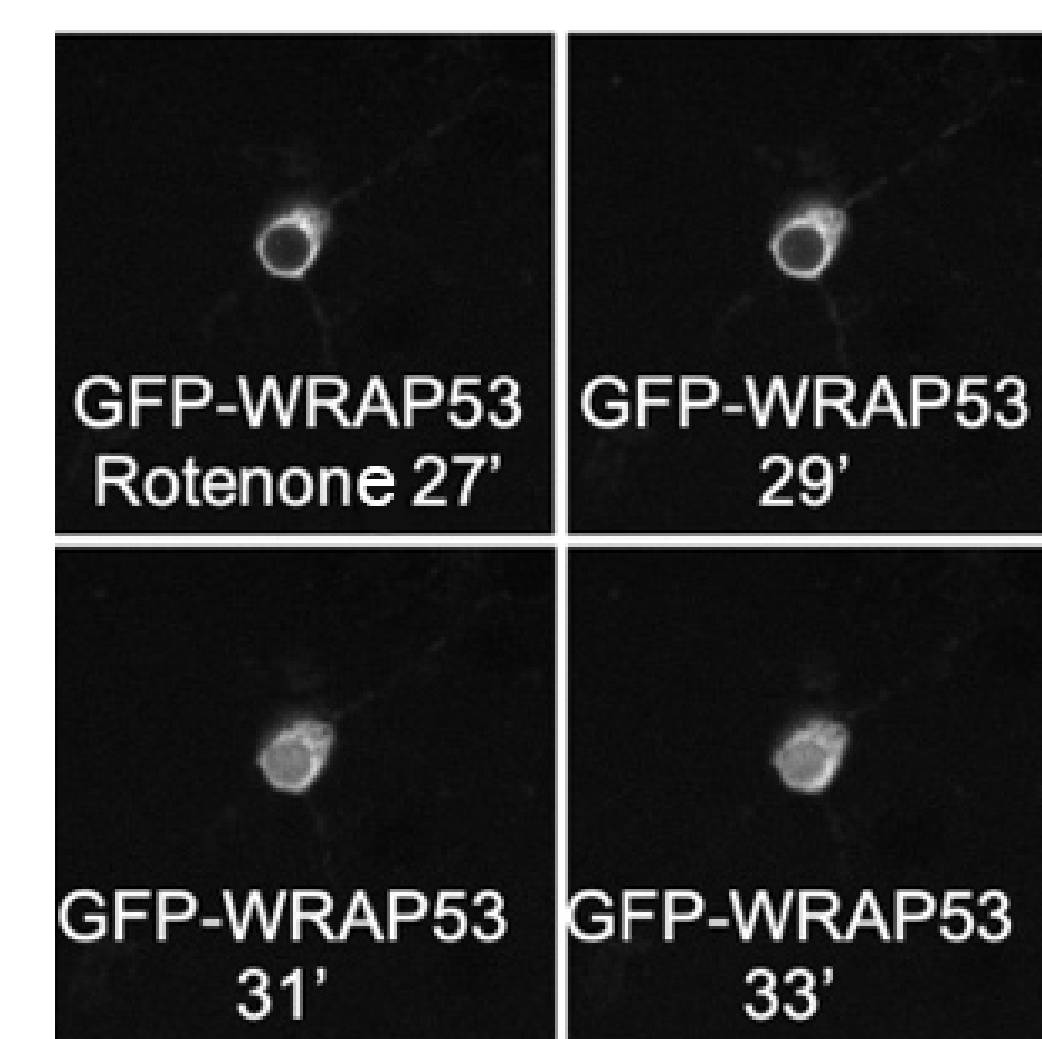
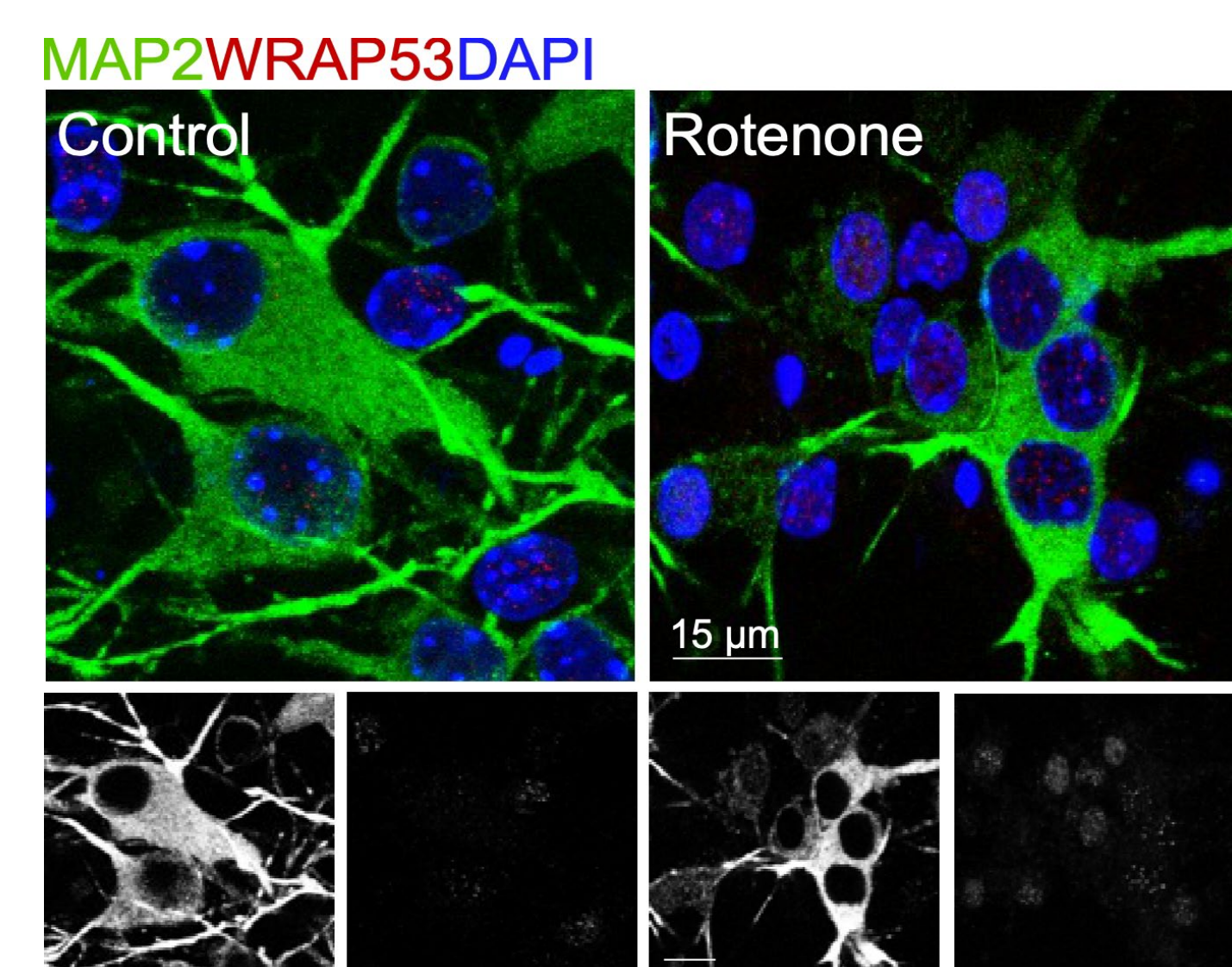
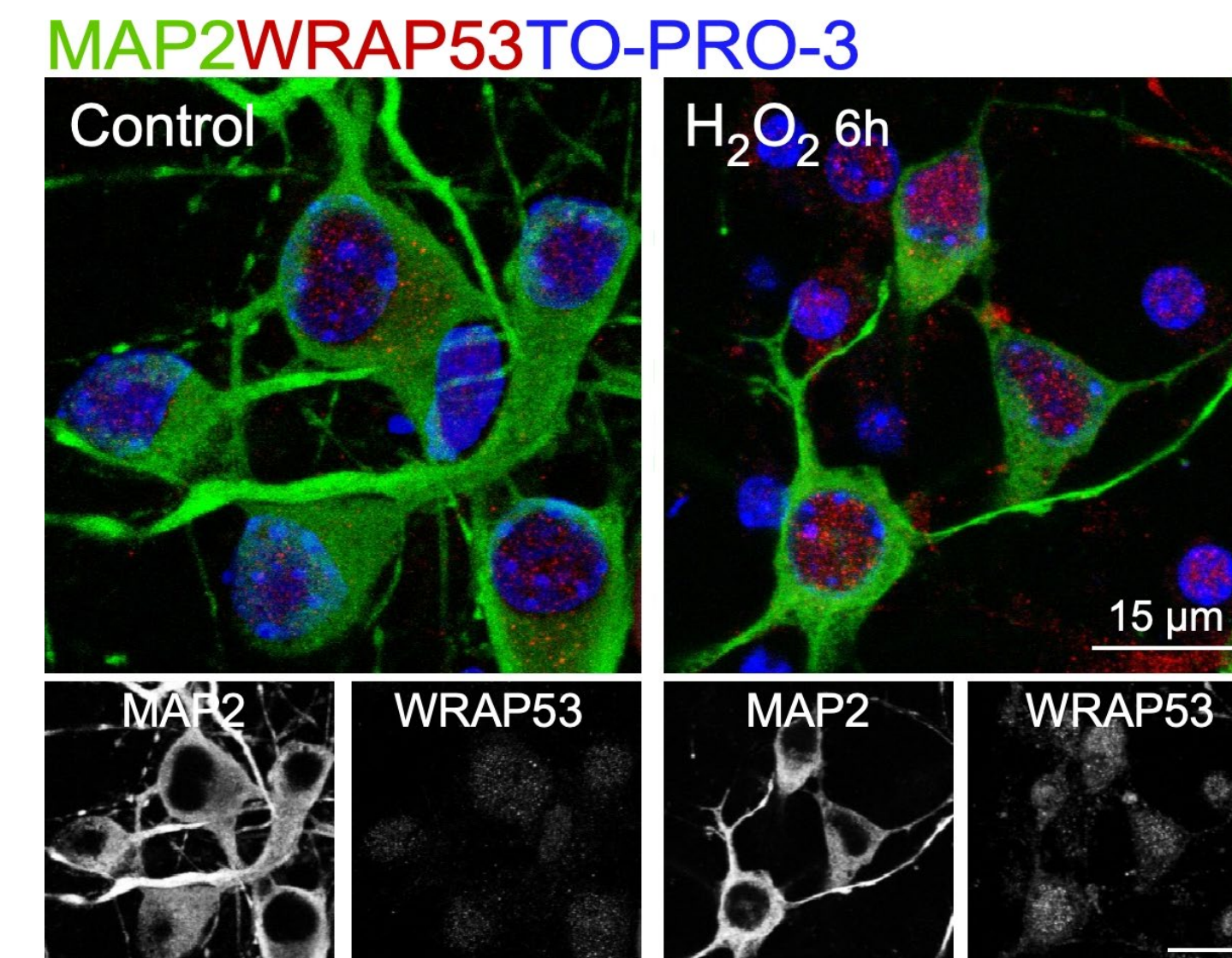
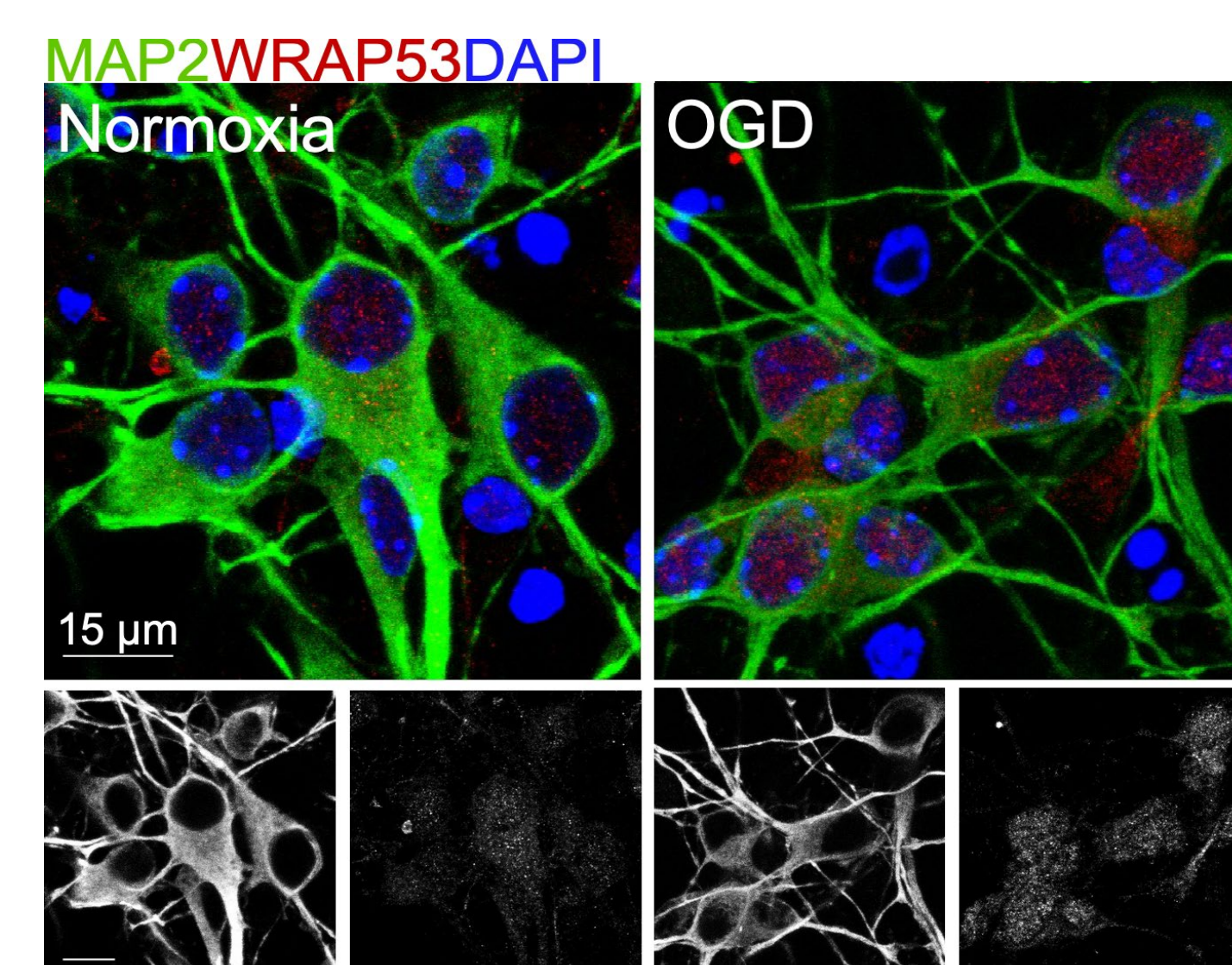
Background

Ischemia induces nuclear accumulation of WRAP53, promoting DNA repair in neurons. The human Wrap53 c.202C>G SNP (Arg68Gly) determines protein nuclear translocation rate leading to better outcomes in stroke patients



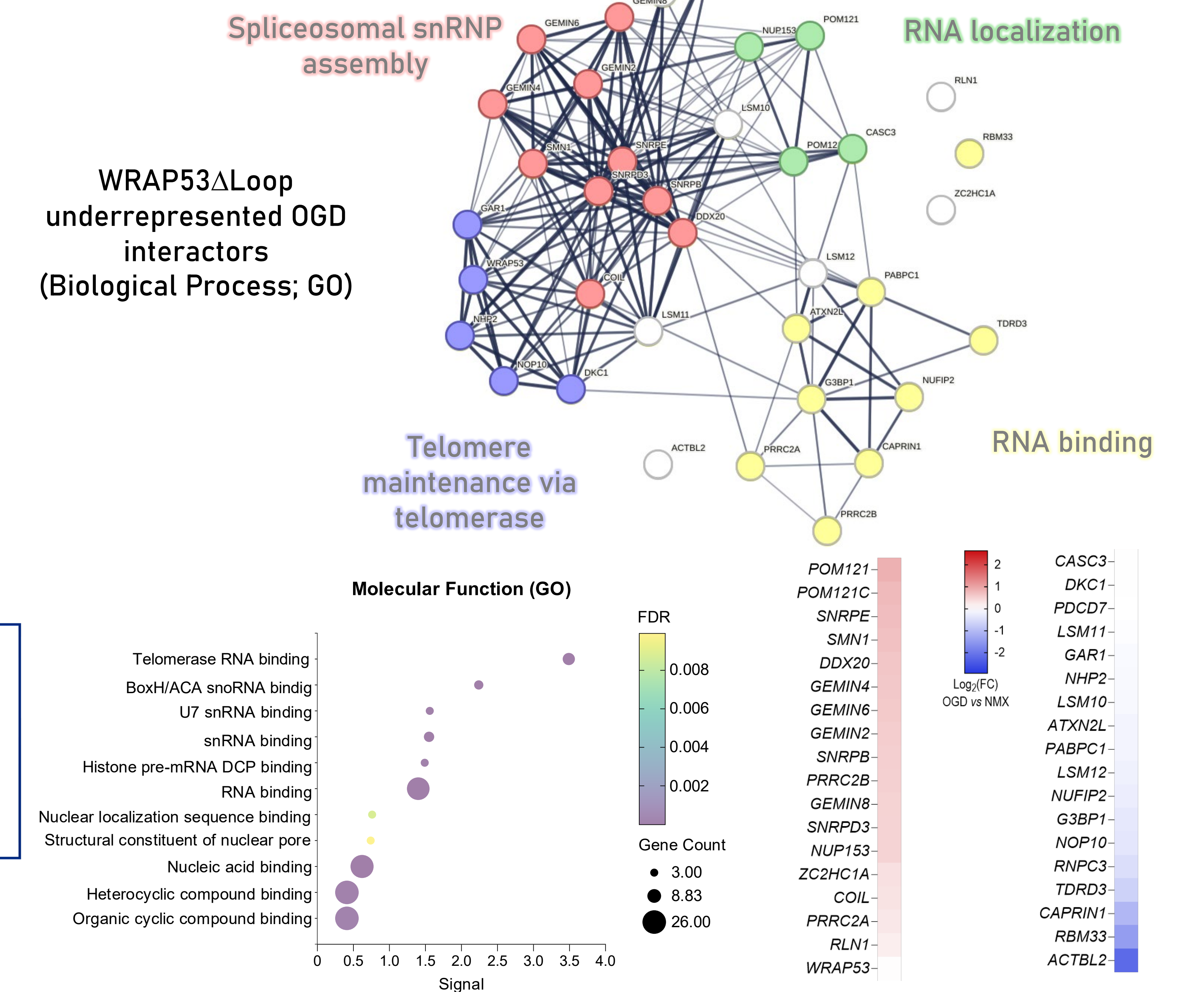
Results

1. Oxidative stress mimicked the effect of ischemia on the nuclear accumulation of WRAP53. Specifically, mitochondrial ROS triggered a rapid WRAP53 translocation



We characterize mitochondrial ROS as the driving force in WRAP53 nuclear transport, acting alongside the amino acid sequence 483–510 that is essential for this process. These findings uncover novel molecular targets as potentials enhancers of DNA repair, thereby offering potential avenues for the development of neuroprotective therapeutic strategies against stroke.

Acknowledgments Funded by ISCIII (PI24/00810; RD24/0009/0005, co-funded by the European Union) and Junta de Castilla y León (CSI011P23)



Modelización experimental de la tolerancia isquémica humana

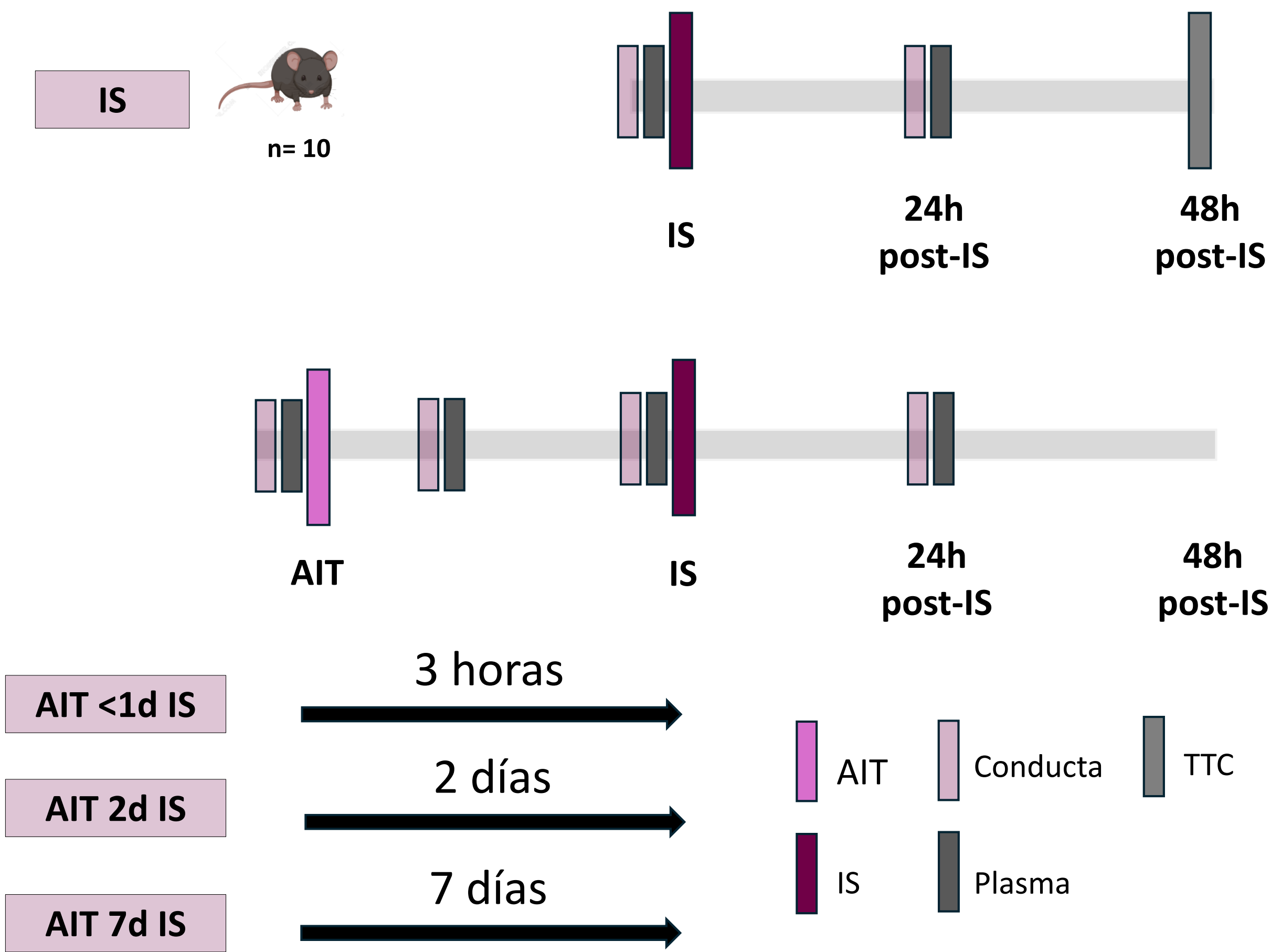
Sara Salvany ^{1,2}, Alberto Martínez ^{1,2}, Cristina Pereira ^{1,2}, Glòria Arqué ^{1,2}, Francisco Purroy ^{1,2,3}

¹ Grupo de Investigación en Neurociencias Clínicas, Institut de Recerca Biomèdica de Lleida (IRBLleida)
² Universitat de Lleida
³ Hospital Universitari Arnau de Vilanova de Lleida (HUAV)

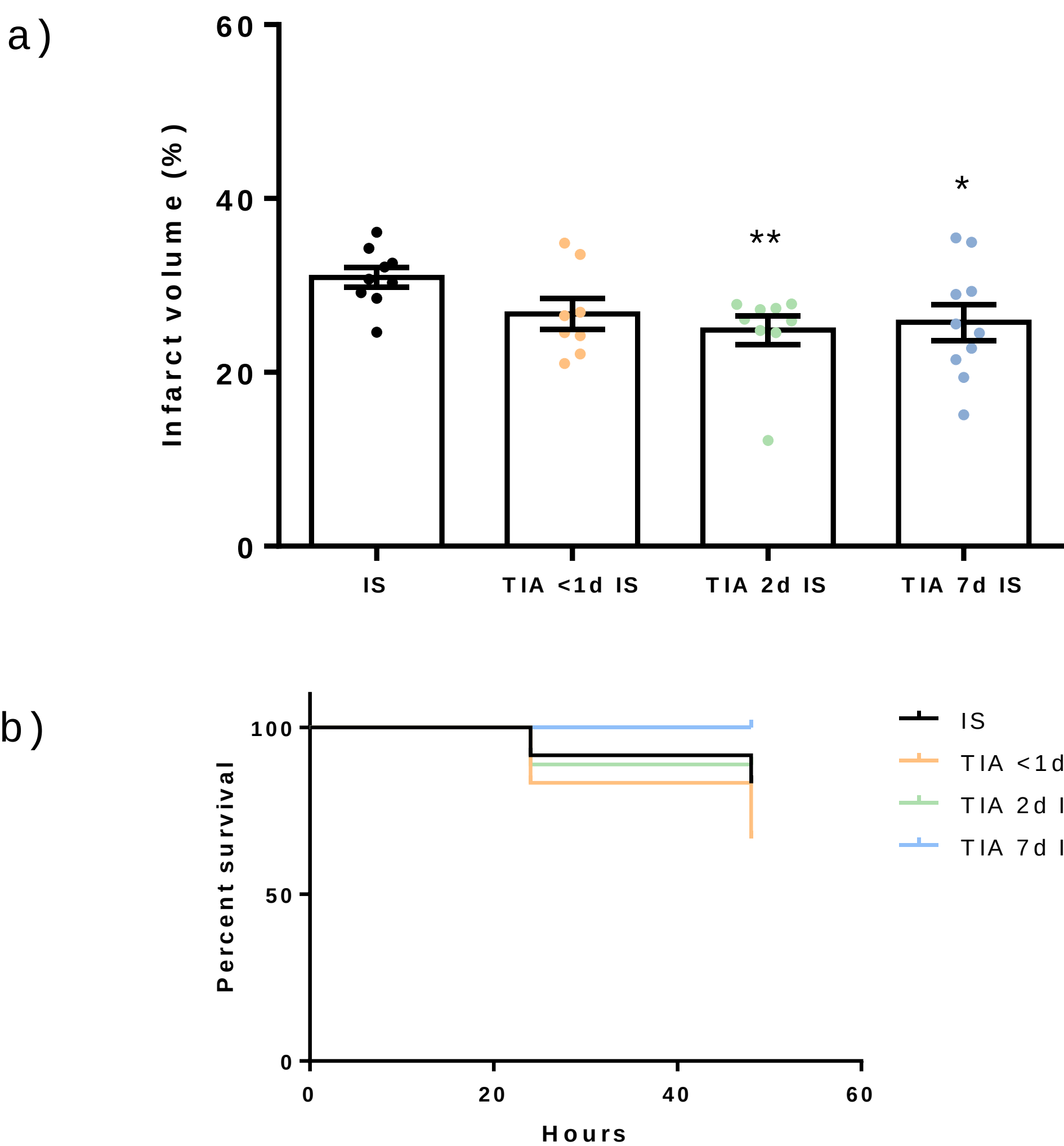
CONTEXTO

La **tolerancia isquémica** (TI) se define como un estado de **protección** celular inducido por breves episodios previos de isquemia. En el contexto de la patología cerebrovascular, un **accidente isquémico transitorio** (AIT) previo se asocia con menores volúmenes lesionales y mejores resultados funcionales tras un ictus isquémico (IS).

MÉTODOS



RESULTADOS



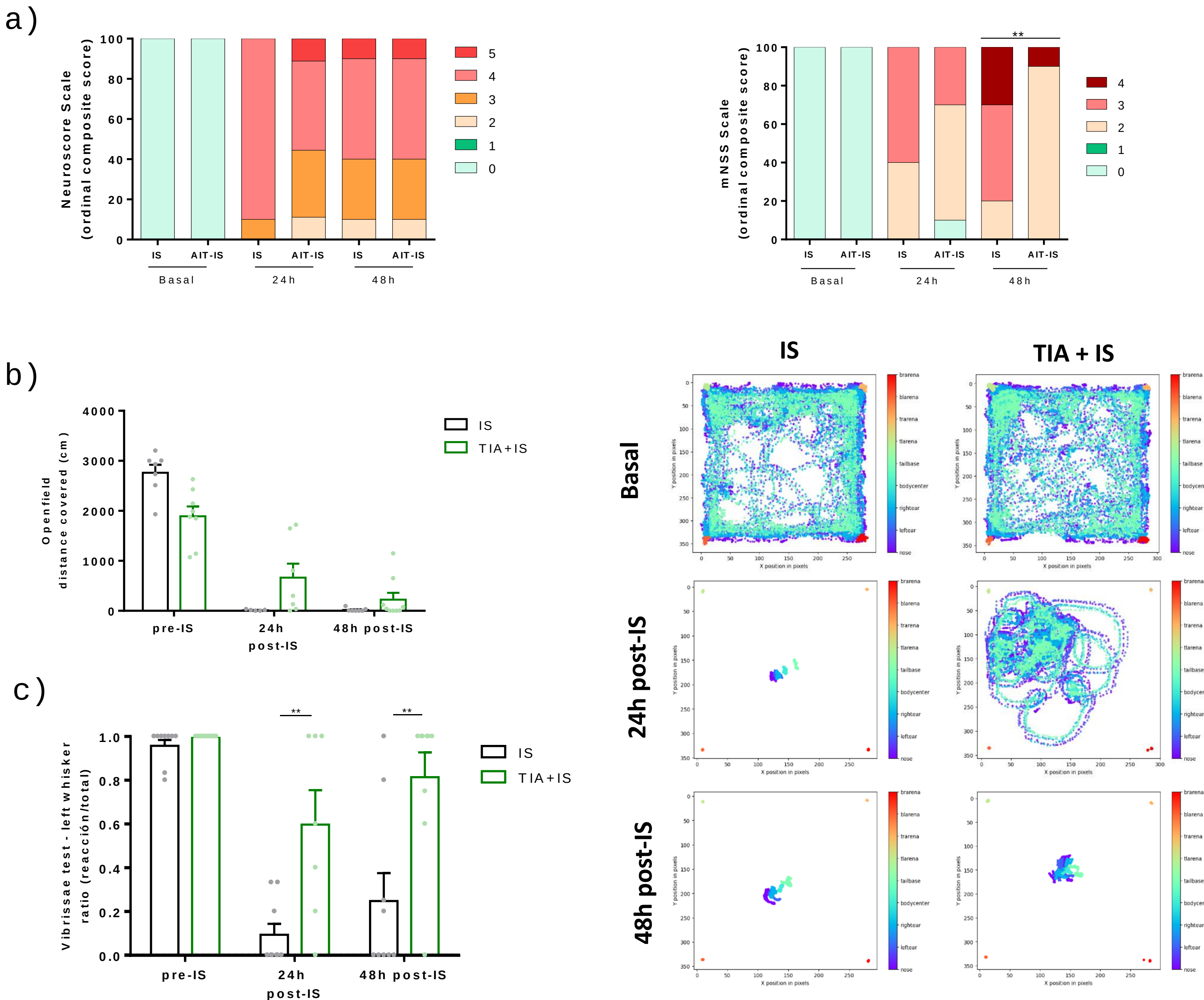
a) La aplicación del AIT 2 o 7 días antes del ictus isquémico (IS) **redujo significativamente el volumen de infarto** en comparación con la condición IS. b) La **supervivencia** de los animales en los grupos precondicionados con AIT fue **similar** a la observada en los animales sometidos únicamente a IS.

n = 8–10 animales por condición. El análisis estadístico se realizó mediante una prueba t no apareada (unpaired t-test), y la significación estadística se muestra respecto a la condición IS

CONCLUSIONES

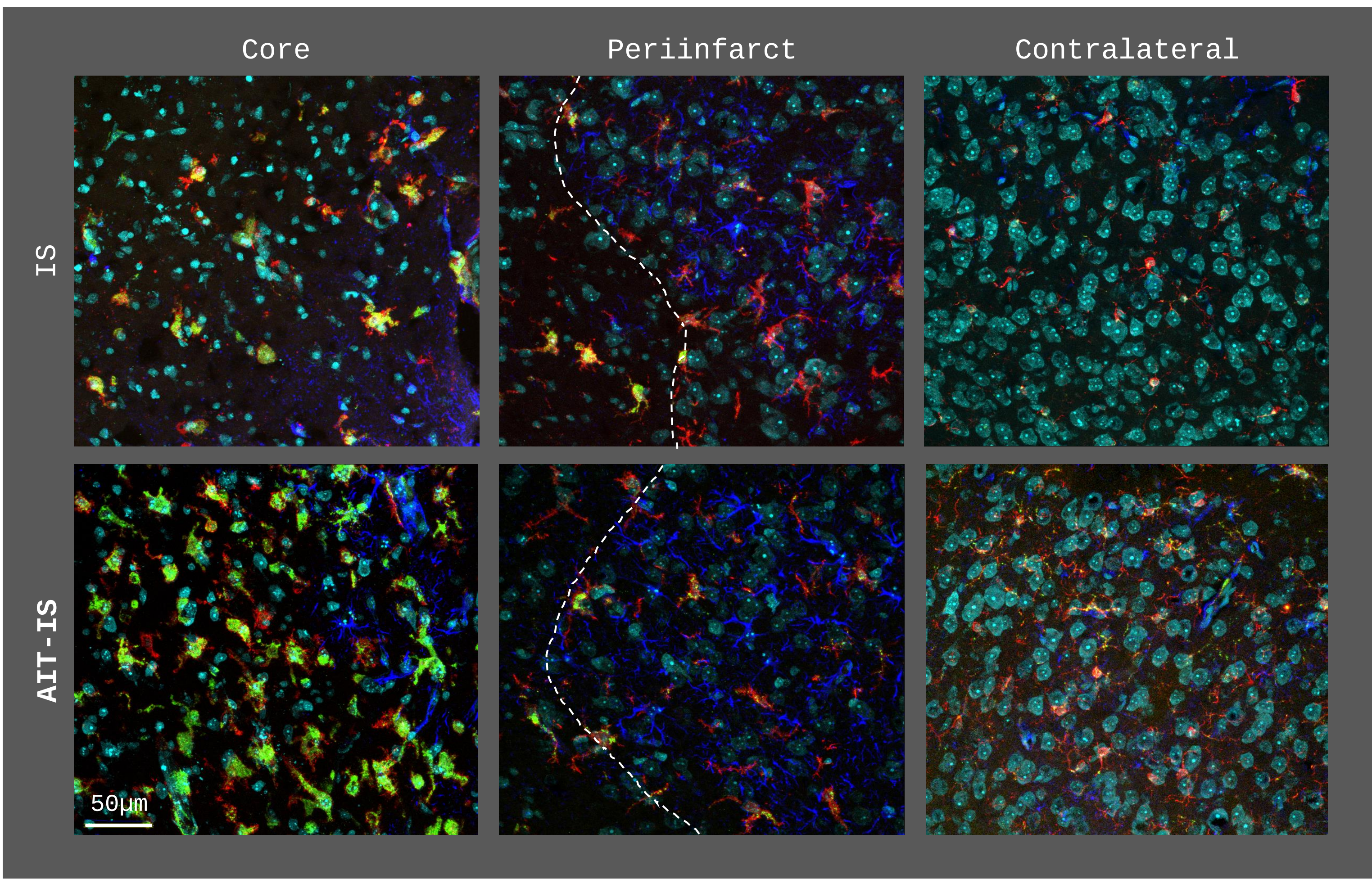
Estos resultados demuestran la posibilidad de modelizar en un modelo animal la TI descrita en humanos, identificando una ventana temporal de neuroprotección. Este modelo abre nuevas vías para estudiar los mecanismos neuroprotectores de la TI y su base en la unidad neurovascular.

RESULTADOS



a) se observa una tendencia a presentar **puntuaciones inferiores en el NeuroScore** y una reducción significativa de la puntuación **mNSS** a las 48 horas post-IS en los animales sometidos a un AIT previo. b) se detectan diferencias significativas en la **conservación de la sensibilidad** en la condición AIT 2d IS en comparación con el grupo sin AIT. c) se aprecia una tendencia clara hacia una mejor **preservación motora** en el grupo con AIT previo respecto al grupo sin AIT, hallazgo que se ve respaldado por el mapa de intensidad de píxeles obtenido mediante el seguimiento del desplazamiento de los animales (d).

n = 8–10 animales por condición. El análisis estadístico se realizó mediante una ANOVA no paramétrica, y la significación estadística se muestra respecto a la condición IS



La cuantificación del área de neuropilo cubierta por **GFAP**, **IBA1**, **CD68** o **Nissl** no muestra diferencias estadísticamente significativas entre las condiciones IS y AIT-IS

n = 3 animales por condición. El análisis estadístico se realizó mediante una prueba t no apareada (unpaired t-test), y la significación estadística se muestra respecto a la condición IS

CONTACTO

¿Te interesa colaborar o explorar oportunidades de financiación juntos?

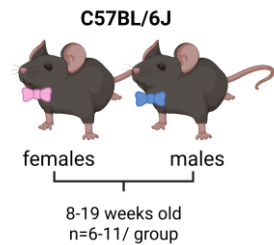
Escríbenos:
sara.salvany@udl.cat



INTRODUCTION

Stroke is a major global health disease affecting both sexes, **yet women remain underrepresented in stroke clinical trials** and poorly studied in preclinical animal studies. This study evaluates **the feasibility of incorporating both sexes in preclinical stroke research**, comparing outcomes in different ischemic and haemorrhagic models in mice.

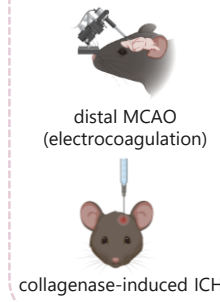
METHODS



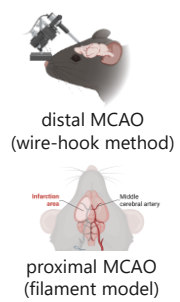
Outcomes measurement



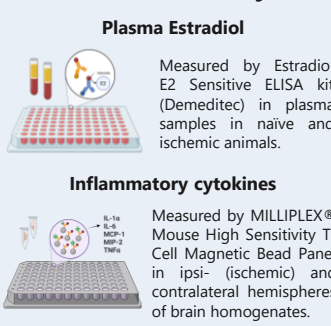
Permanent models



Transient models



Molecular assays



Study timelines

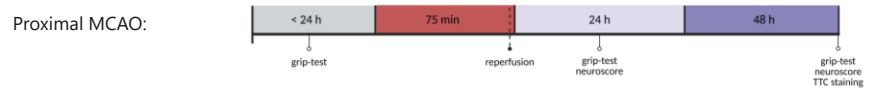
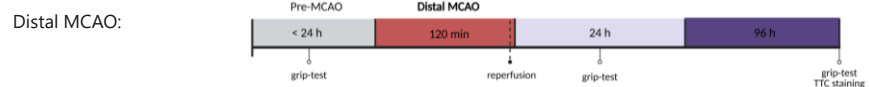
Permanent models



Collagenase-induced ICH:

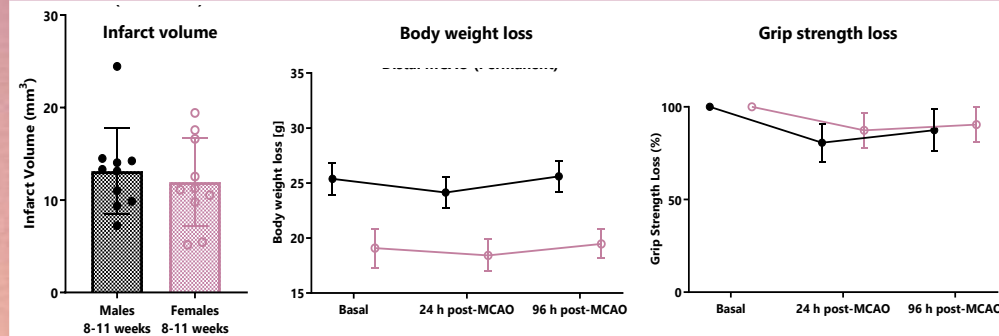


Transient models



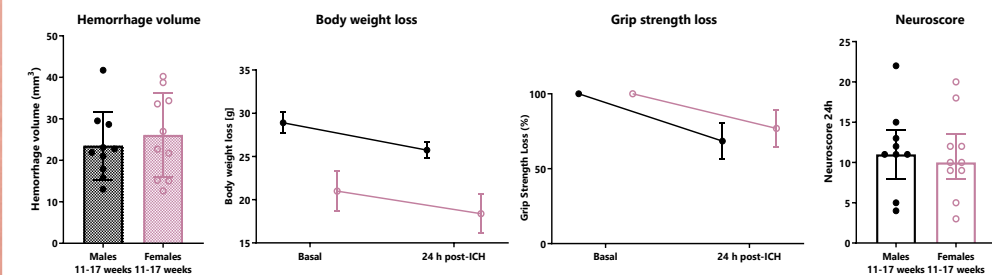
RESULTS: Permanent models

Permanent Distal MCAO



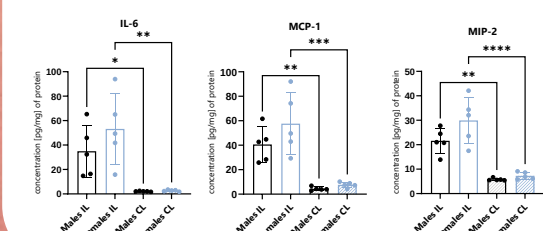
No sex differences were detected in lesion volume after permanent distal MCAO ($p>0.05$, unpaired t-test), as well as in body weight and grip strength loss over time ($p>0.05$, RM 2-way ANOVA, $n=10$ /group).

Collagenase-induced ICH



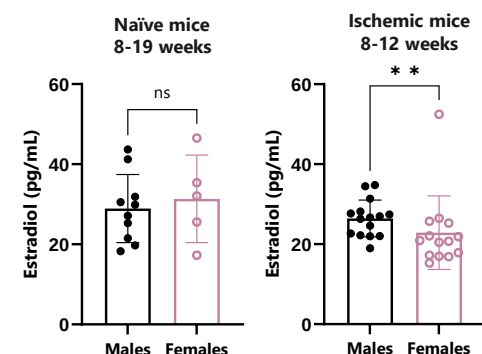
No sex differences were observed in haemorrhage volume and neurological score after ICH ($p>0.05$, unpaired t-test or Mann Whitney U test, respectively), as well as in body weight and grip strength loss over time ($p>0.05$, RM 2-way ANOVA, $n=10$ /group).

Inflammatory cytokines after ICH



IL-6, MCP-1, and MIP-2 were markedly increased in the ipsilateral (IL) versus contralateral (CL) hemisphere in both sexes in ICH mice. IL-1 α and TNF- α levels were similar in both hemispheres and comparable between sexes (not shown). ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, one-way ANOVA; $n=5$ /group. Data represent individual values, mean $\pm$ SD.

RESULTS: Estradiol levels

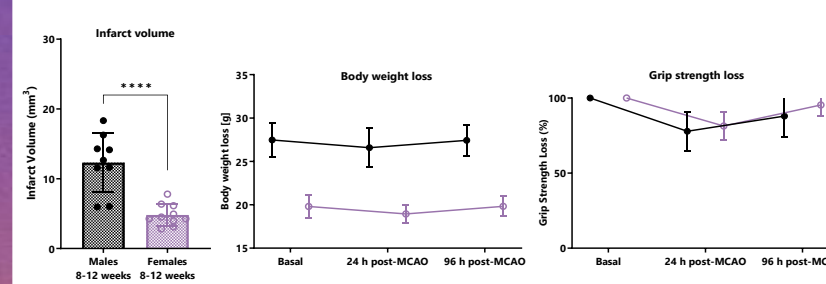


In naïve mice (8–19 weeks), **plasma estradiol concentrations did not differ significantly between males and females** (means 28.9 vs 31.4 pg/mL, unpaired t-test $p=0.64$).

In ischemic mice (proximal transient MCAO, 8–12 weeks), males exhibited higher estradiol levels than females (with means 26.4 vs 22.9 pg/mL, respectively; Mann–Whitney U test $p=0.0043$).

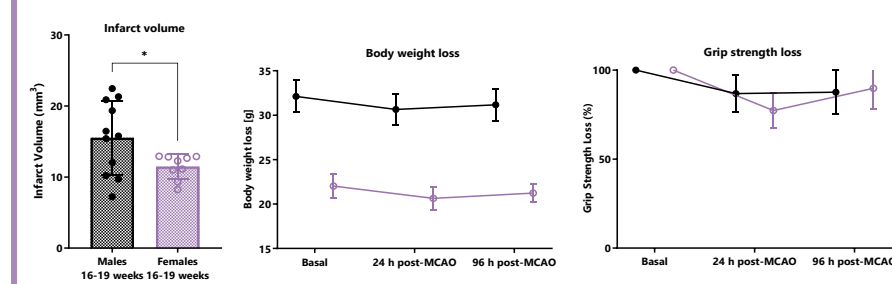
RESULTS: Transient models

Transient Distal MCAO



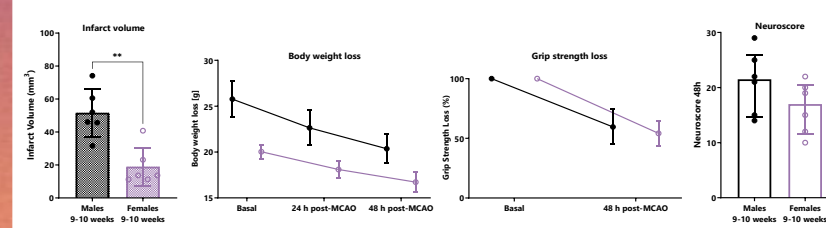
In mice of 8–12 weeks (mean body weight of 19.8 $\pm$ 1g for females, 27.5 $\pm$ 2g for males), **females had smaller infarct volumes than males** ($p<0.0001$, unpaired t-test, $n=9/10$ /group), and female body weight correlated with infarct volume ($r=0.71$, $p=0.006$, Spearman correlation, data not shown).

Transient Distal MCAO (optimized)



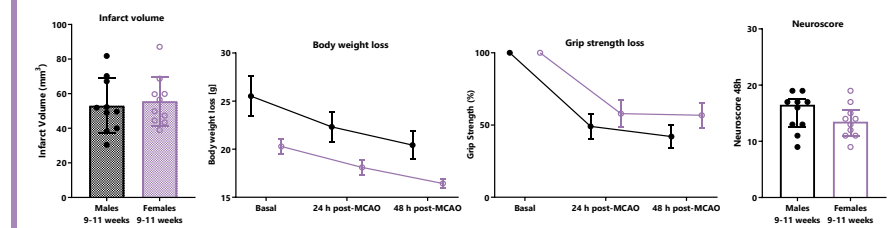
In mice aged 16–19 weeks (mean body weight 22 $\pm$ 1g for females, 32 $\pm$ 2g for males), **sex differences were reduced, but remained significant** ($p<0.05$, unpaired t-test, $n=9/11$ /group). Body weight loss and grip strength loss continued without differences over time (RM 2 way ANOVA).

Transient Proximal MCAO



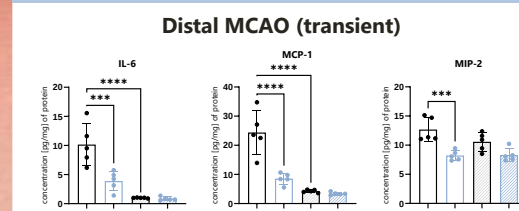
Females exhibited significantly smaller infarct volumes than males after transient proximal MCAO ($p<0.01$, t-test, $n=6$ /group). Neuroscore was reduced, although not significantly, in females vs. in males ($p=0.3$, Mann Whitney U test). Body weight and grip strength loss were similar in males and females over time ($p>0.05$, RM 2-way ANOVA).

Transient Proximal MCAO (optimized)



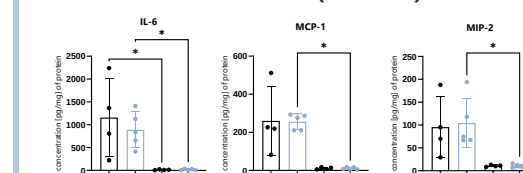
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Inflammatory cytokines after transient models (optimized)



Females showed lower IL-6, MCP-1 and MIP-2 levels than males in ischemic hemispheres, whereas IL-1 α and TNF- α levels were similar in both hemispheres and comparable between sexes (not shown). **** $p<0.0001$; $n=5$ /group. Data represent individual values, mean $\pm$ SD.

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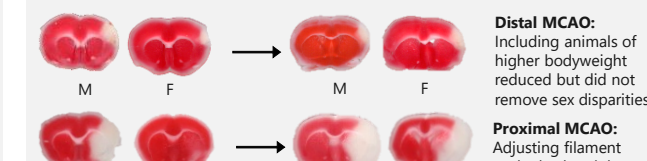
CONCLUSIONS

Permanent Models



No significant sex difference in lesion volume or neurological outcomes in young mice aged 8–17 weeks.

Transient Models



Transient models **require protocol adjustments to ensure reproducible infarct** induction across sexes.

Estradiol levels

in naïve 8–19-week-old mice do not differ between males and females, indicating that **baseline circulating estrogen is unlikely to account for sex differences** in transient distal MCAO.

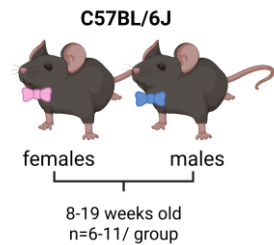
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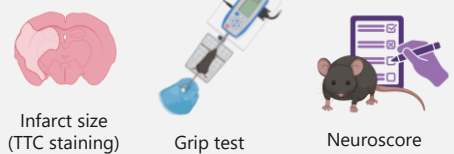
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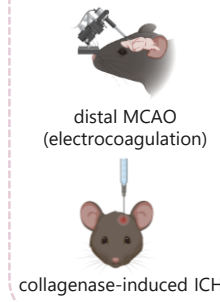
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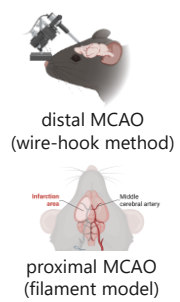
Outcomes measurement



Permanent models



Transient models



Molecular assays

Plasma Estradiol

Measured by Estradiol E2 Sensitive ELISA kit (Demeditec) in plasma samples in naïve and ischemic animals.

Inflammatory cytokines

Measured by MILLIPLEX® Mouse High Sensitivity T-Cell Magnetic Bead Panel in ipsi- (ischemic) and contralateral hemispheres of brain homogenates.

Study timelines

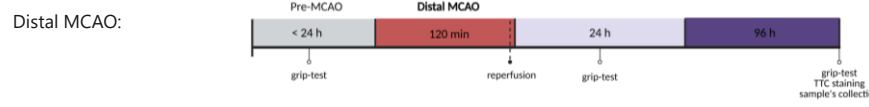
Permanent models



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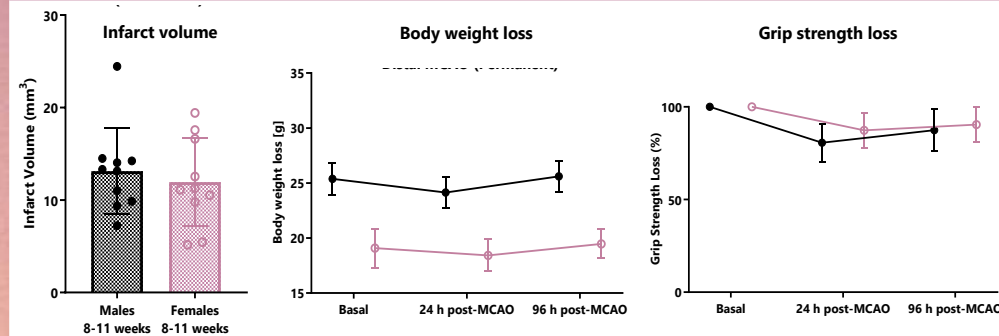


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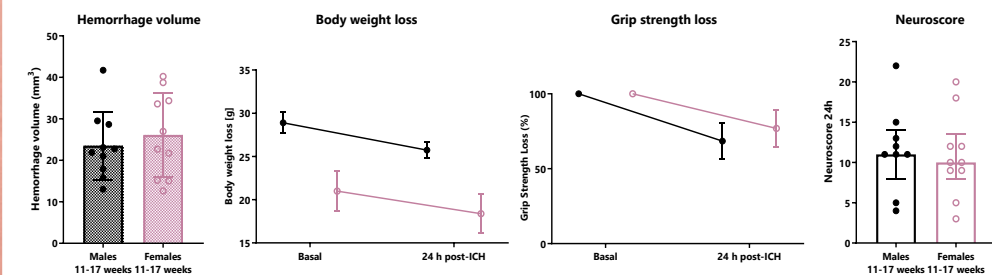
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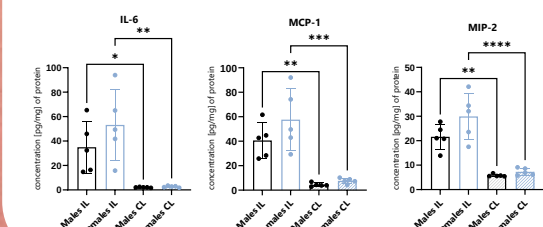
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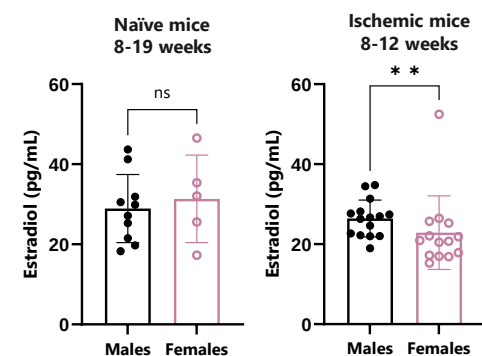
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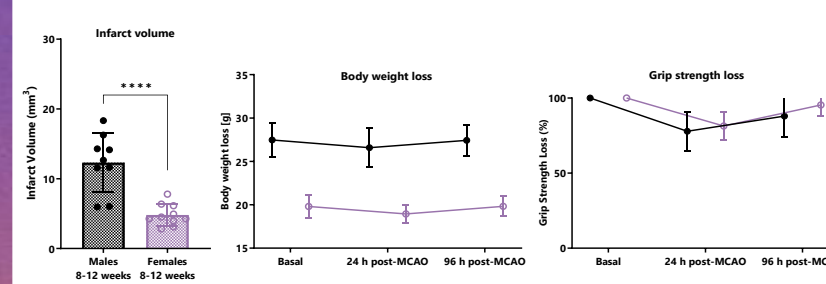


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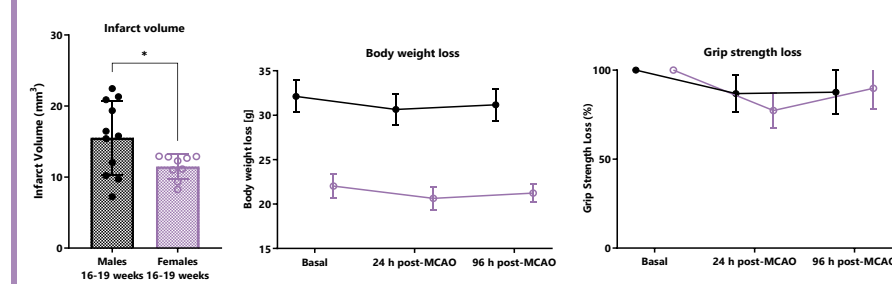
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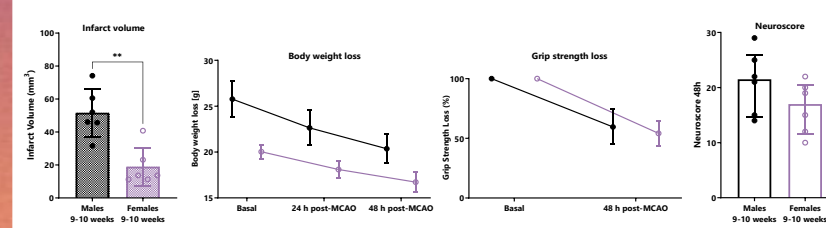
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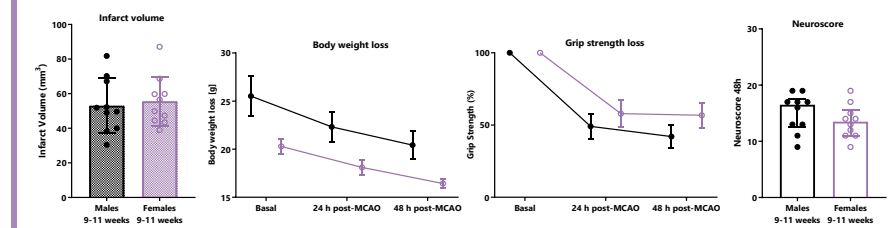
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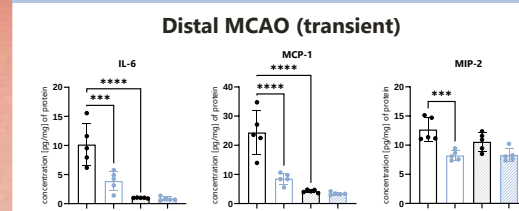
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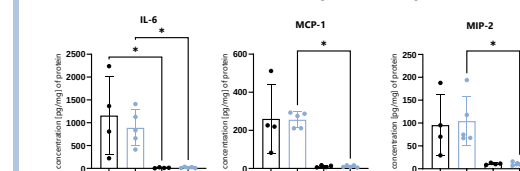
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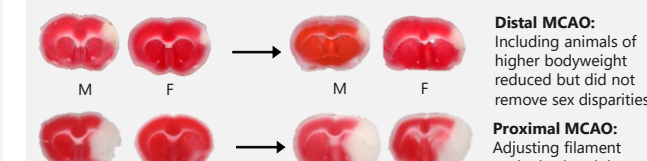
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