

Identificación de genes diana de microARNs asociados al pronóstico funcional del ictus isquémico

Aina Medina Dols

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Contacto:

aina.medo@gmail.com

Background

Mecanismos para reparar la lesión isquémica



Angiogénesis

Neurogénesis

**Neuritogénesis
& sinaptogénesis**

Plasticidad neuronal



microARNs

Pequeños ARNs no codificantes

19-22 nt

Capacidad de silenciar el ARN mensajero (ARNm)

1 miRNA → capacidad de silenciar múltiples ARNm

~ 1900 miRNA en humanos → Reguladores de más del 60% del genoma humano

Imagen modificada de María Ángeles Moro. Unidad de Investigación neurovascular. Universidad Complutense de Madrid

Importantes reguladores génicos implicados en diferentes procesos biológicos

Objetivo

Identificar microARNs asociados al pronóstico funcional del ictus isquémico y determinar sus dianas terapéuticas

Reclutamiento de pacientes

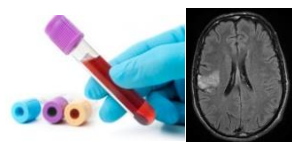
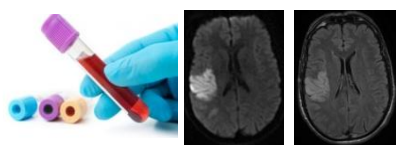


Imagen modificada de Saver JL et al. Stroke. 2021;52:3054–3062

Proyecto miRO

Criterios de inclusión

Pacientes ≤ 60 años
 Ictus en territorio anterior (PACI - TACI)
 mRS previo: 0
 NIHSS en el momento de inclusión (día 10): 4-14
 Demostración del infarto en neuroimatgen a los 10 días

Criterios de exclusión

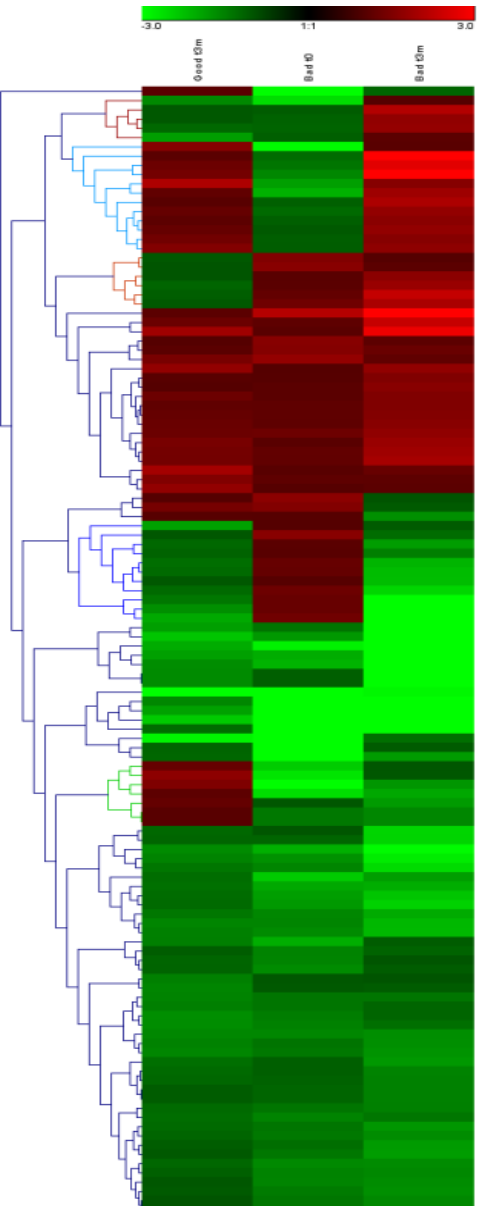
Pacientes en coma
 Ictus en territorio vascular posterior (POCI)
 Neoplasia cerebral - Edema - HIC - HSA
 Adicción a drogas
 Recurrencia durante el periodo de seguimiento

mRS=0-2

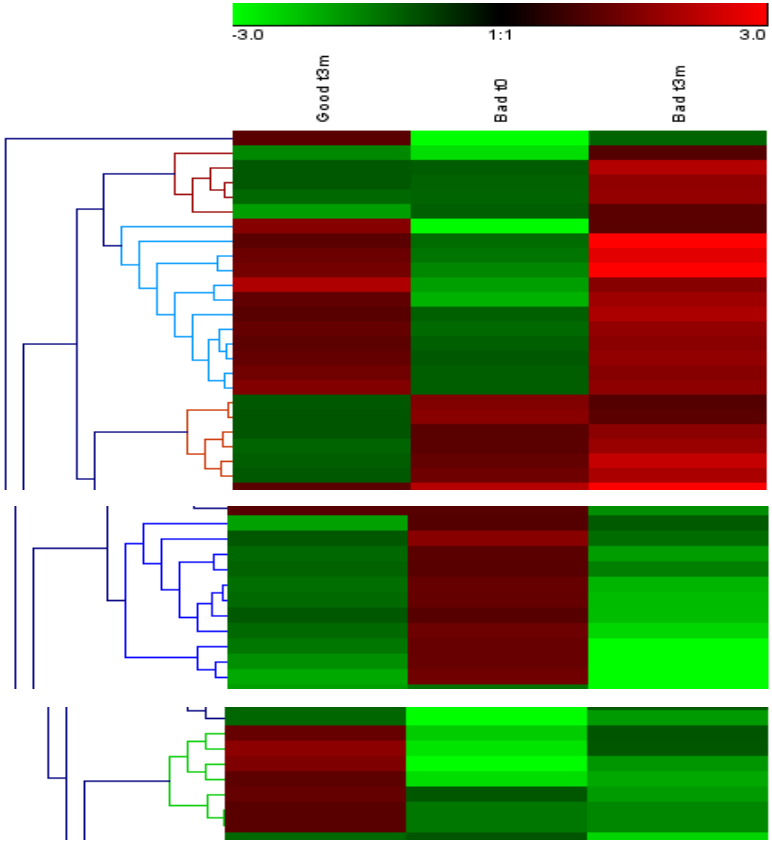


mRS=4-5





Affymetrix® Human miRNA 3.0

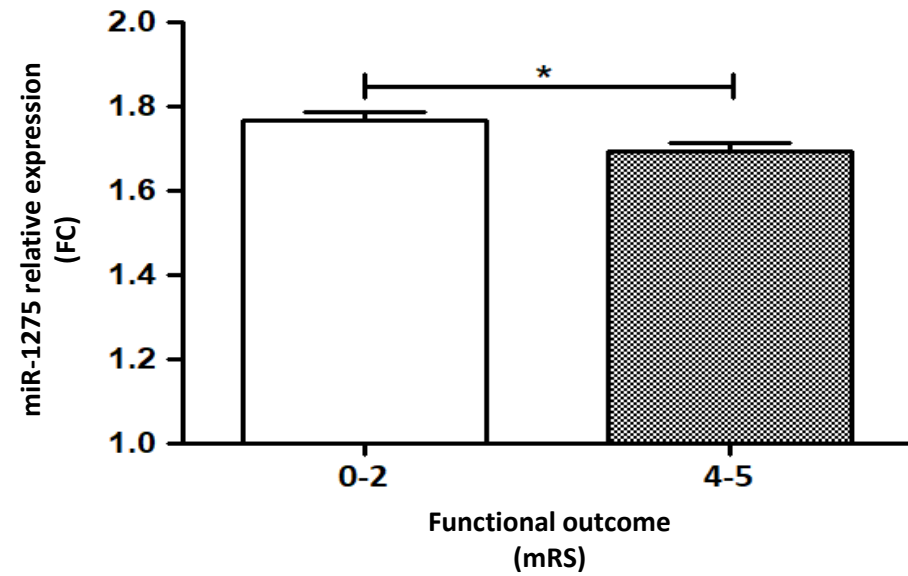
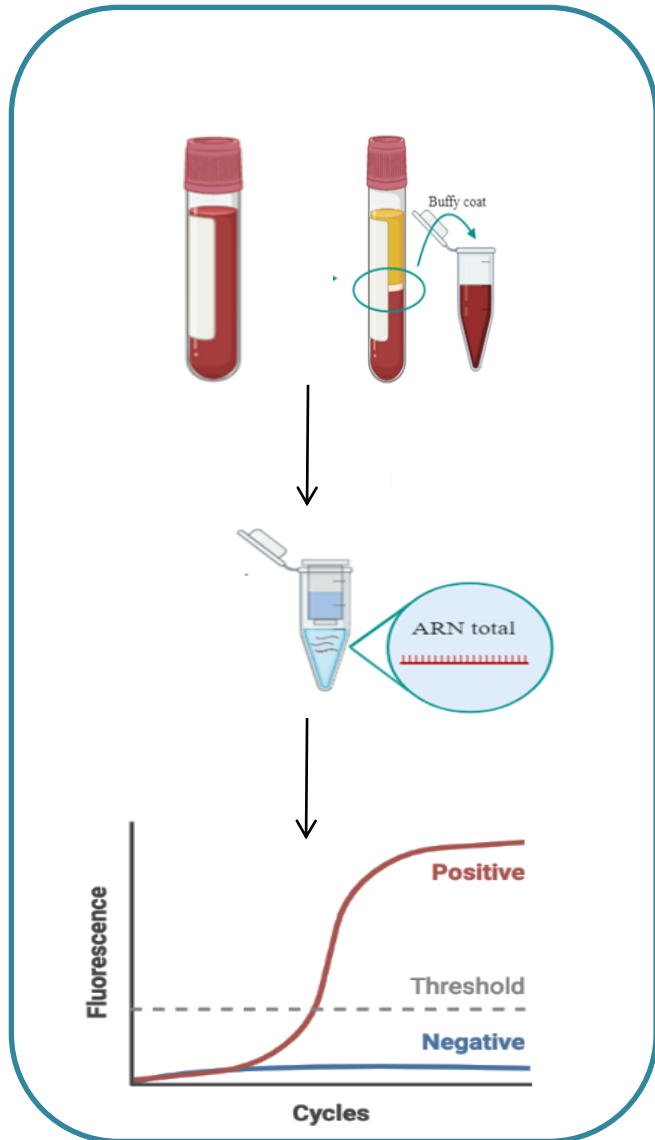


- hsa-miR-1275_st
- hsa-miR-181b_st
- hp_hsa-miR-3200_st
- hsa-miR-3646_st
- hsa-miR-4274_st
- hsa-miR-3175_st
- hsa-miR-20b-2-star_st
- hsa-let7a_st
- hsa-miR-331-5p_st
- hsa-miR-4634_st
- hsa-miR-671-5p_st
- hp_hsa-miR-4605_st
- hsa-let7d_st
- hsa-miR-1299_st
- hsa-miR-1343_st
- hsa-miR-3154_st
- hsa-miR-943_st
- ACA41_st
- hsa-miR-4264_st
- hsa-miR-4510_st
- hsa-miR-4793-5p_st
- hsa-miR-2276_st
- hsa-miR-548q_st
- hsa-miR-628-3p_st
- hp_hsa-miR-3759_x_st
- hsa-miR-15b_st
- hp_hsa-miR-20b-2_x_st
- hsa-miR-3924_st
- hsa-miR-4784_st
- hsa-miR-4721_st
- hp_hsa-miR-3183_st
- hp_hsa-miR-5461_st
- hsa-miR-219-1-3p_st
- hp_hsa-miR-4451_st
- hsa-miR-143_st
- hsa-miR-33b-star_st
- hp_hsa-miR-29a-2_x_st
- hsa-miR-3917_st
- hsa-miR-188-5p_st
- hsa-miR-675_st
- hsa-miR-4289_st
- ACA37_x_st
- U35B_st
- hsa-miR-146b-5p_st
- hsa-miR-103b-star_st
- U104_st
- hsa-miR-191-star_st
- hsa-miR-4706_st
- hsa-miR-22_st
- hsa-miR-4707-3p_st
- HBII-142_st
- HBII-142_x_st
- mgh28S-2409_st
- U43_st
- U43_x_st
- U34_st
- U46_st
- hsa-miR-3613-3p_st
- hsa-miR-496b-5p_st
- hp_hsa-miR-5461_st
- hp_hsa-miR-5461_x_st
- U67_st
- U65_st
- U62_st
- hsa-miR-17-star_st
- HBII-289_st
- HBII-85-26_st
- HBII-85-6_x_st
- hsa-miR-5201_st
- hsa-miR-602a_st
- hsa-miR-602-3p_st
- hsa-miR-941_st
- hsa-miR-331-3p_st
- hsa-miR-4646-5p_st
- hsa-miR-3609_st
- hsa-miR-625_st
- U103B_st
- U59B_st
- mgh28S-2411_st
- U25_st
- U41_x_st
- HBII-259_st
- U54_st
- hp_hsa-miR-320a-1_st
- hp_hsa-miR-3010-1_st
- HBII-202_st
- snR36C_st
- HBII-85-5_x_st
- U41_st
- U66B_x_st
- hsa-miR-4306_st
- hsa-miR-595_st
- hp_hsa-miR-491_st
- mglU12-22-U4-9_st
- hp_hsa-miR-4857_st
- hsa-miR-2116-star_st
- hp_hsa-miR-4274_st
- hsa-miR-1470_st
- hsa-miR-1237_st
- hsa-miR-149_st
- hp_hsa-miR-9-3_st
- HBII-85-2_x_st
- hsa-miR-3175_st
- U103_s_st
- U105_st
- U33_st
- U95_st
- U95_st
- U95_st
- ACA32_st
- HBII-85-4_x_st
- hp_hsa-miR-520g_x_st
- hsa-miR-4725-3p_st
- hp_hsa-miR-4970-1_st
- U95_st
- HBII-85-26_x_st
- hp_hsa-miR-320a-1_x_st
- U106B_st
- U95_st
- U95_x_st
- U95_x_st

11 microARNs asociados al pronóstico funcional del ictus criptogénico

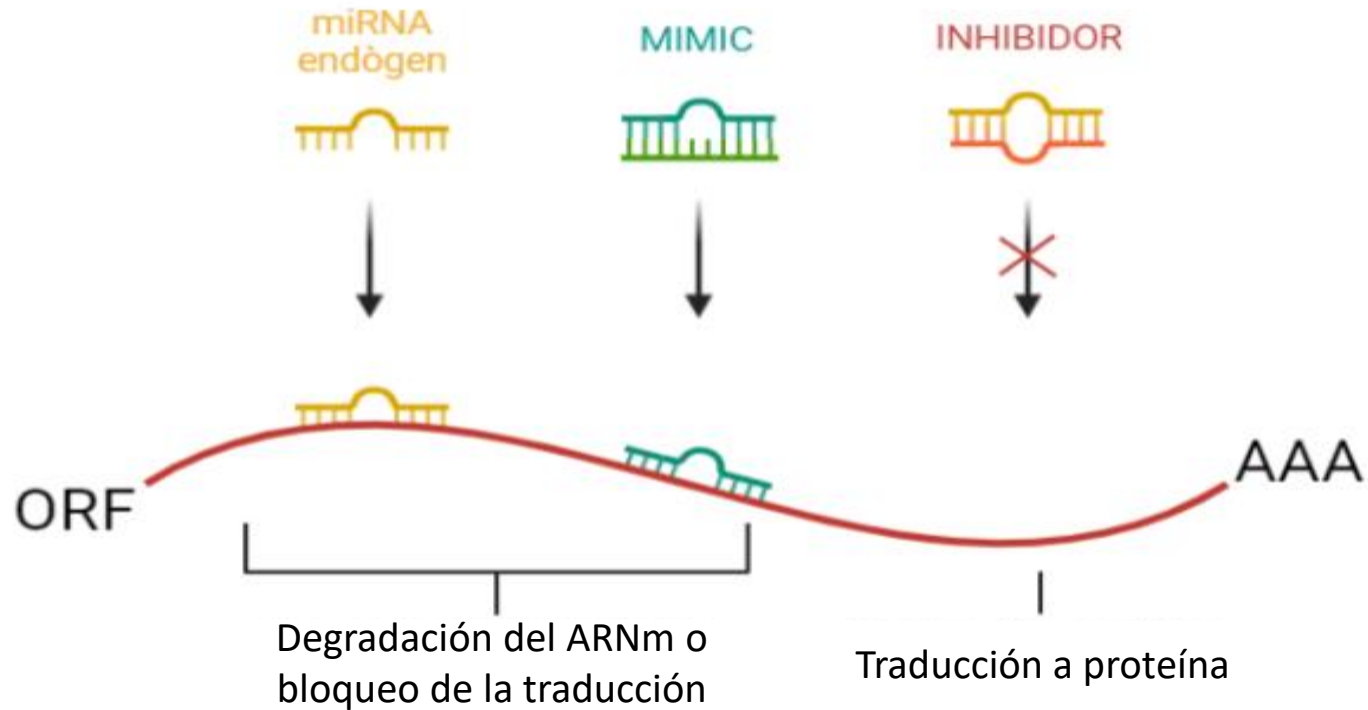
miRNA	Fold Change (bad vs. good)	ANOVA p value (bad vs. good)
hsa-miR-675*	1.76	0.043
hsa-miR-4746-5p	1.69	0.010
hsa-miR-647	-1.51	0.035
SNORD84	-1.56	0.007
SNORD41	-1.58	0.030
hsa-miR-181b	-1.63	0.047
SNORD116-8	-1.69	0.009
SNORD1C	-1.7	0.013
SCARNA17	-1.77	0.017
hsa-miR-4321	-1.83	0.028
SNORD100	-1.9	0.032
SNORD57	-2.01	0.009
ENSG00000252543	-1.13	0.049
hsa-miR-17*	-2.59	0.047
SNORD35A	-2.83	0.026
hsa-miR-625	-2.88	0.026
hsa-miR-941	-3.13	0.047
hsa-miR-3609	-3.34	0.032
hsa-miR-3201	-3.66	0.007
hsa-miR-1275	-6.68	0.014

miR-1275 infra-expresado en pacientes con discapacidad

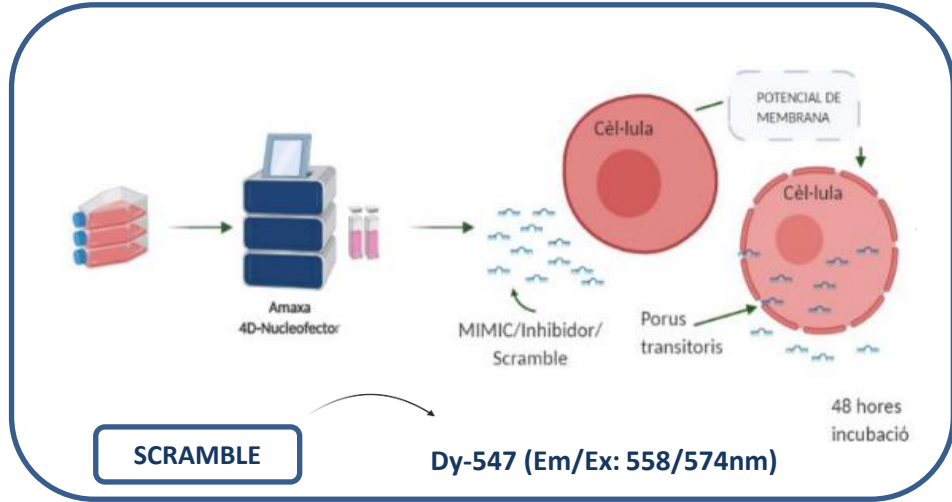


Modulación *in vitro* del miR-1275

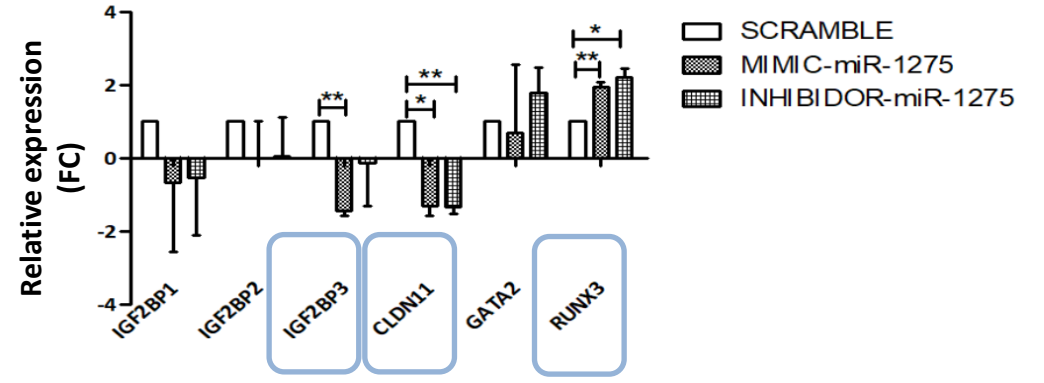
Células
hCMEC/D3



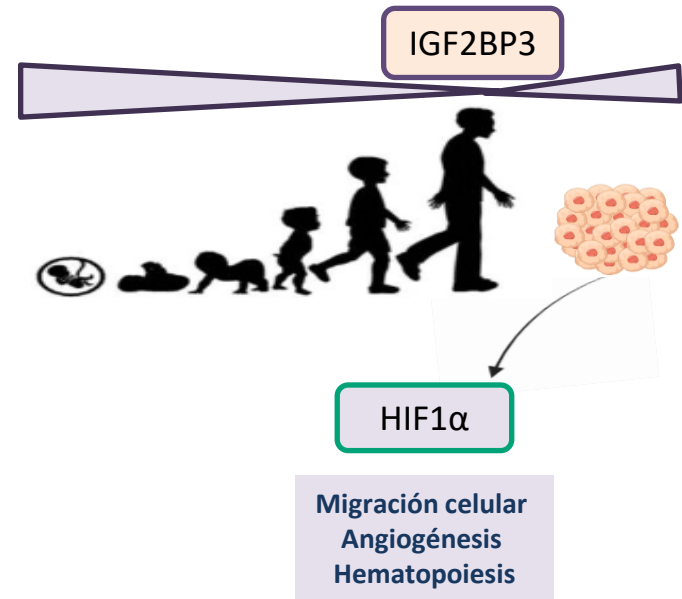
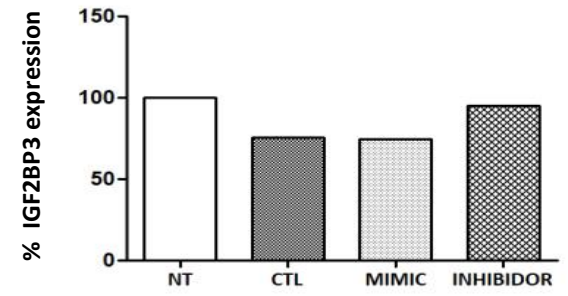
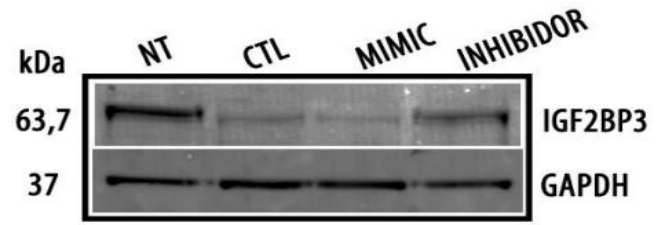
Modulación *in vitro* del miR-1275



RT-PCR

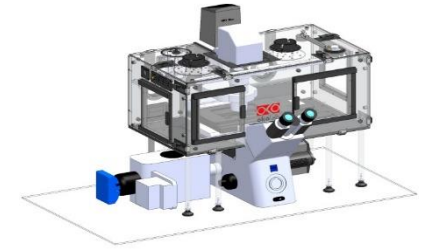


WB



Privación de oxígeno (OD)

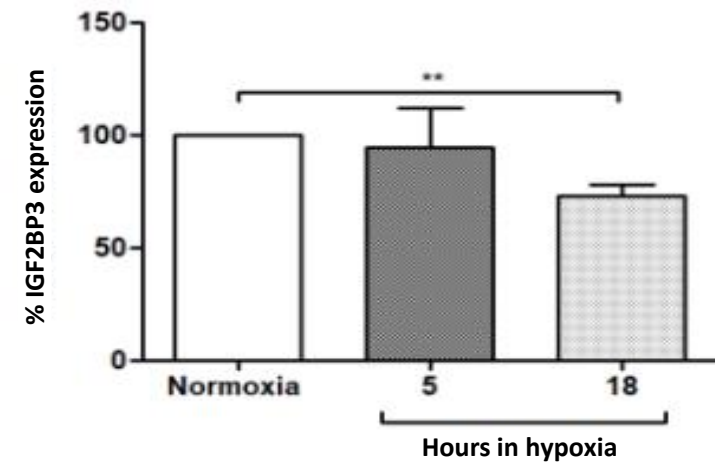
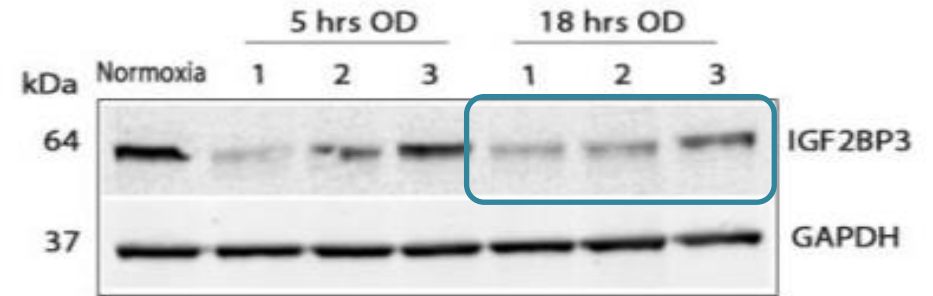
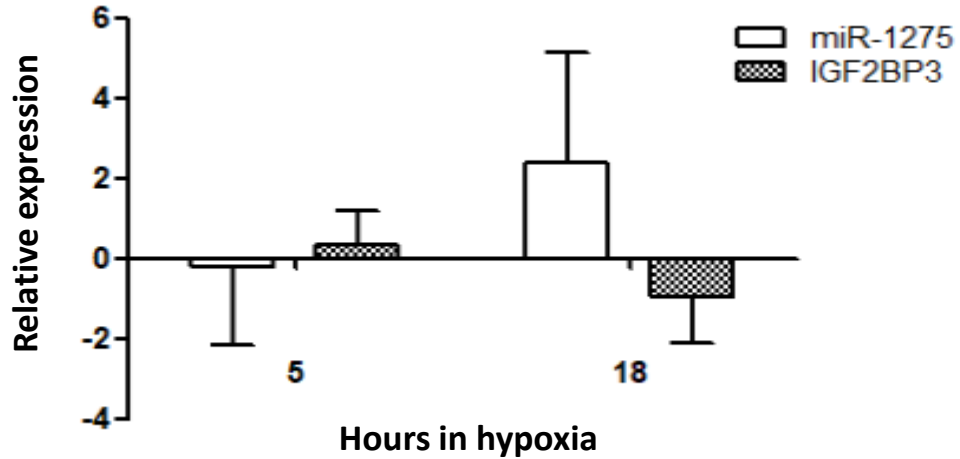
Privación de oxígeno (OD)
95%N₂- 5%CO₂



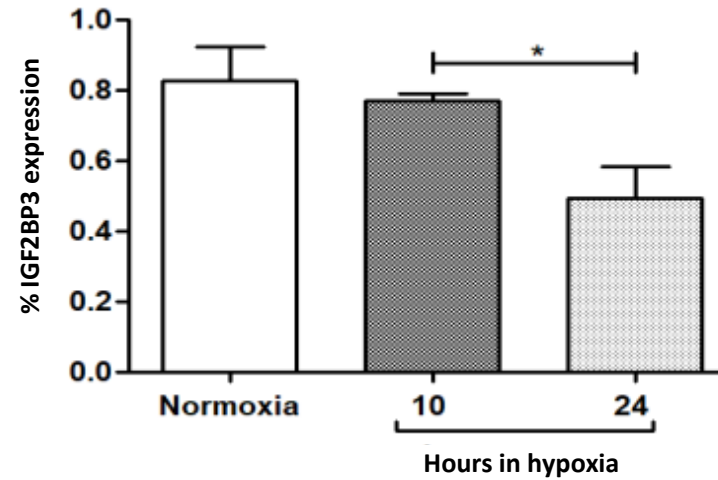
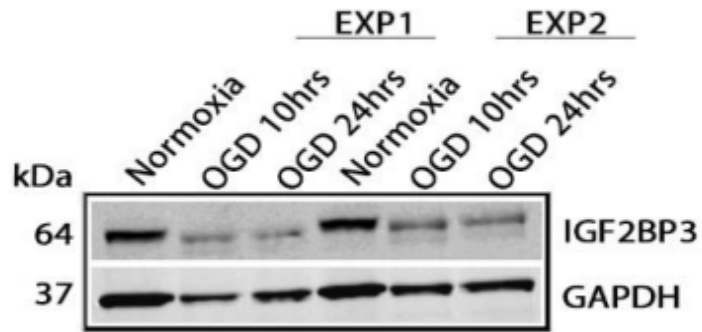
HIF1 α



miR-1275



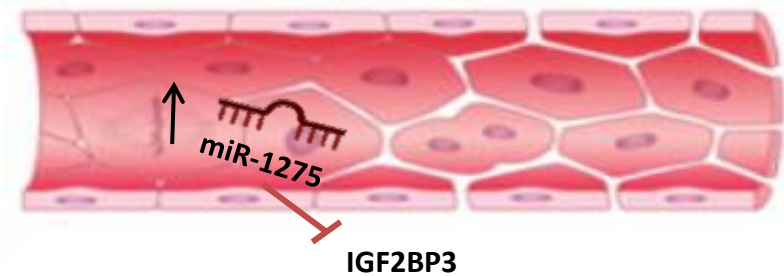
Privación de oxígeno y glucosa (OGD)



Glioma Endothelial Cells

↓ IGF2BP3 ↑ Permeabilidad de la BTB

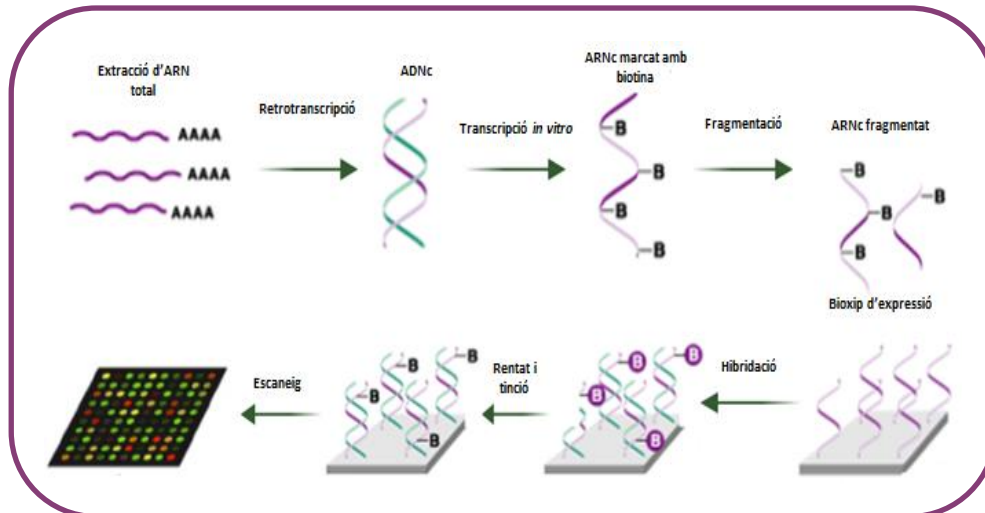
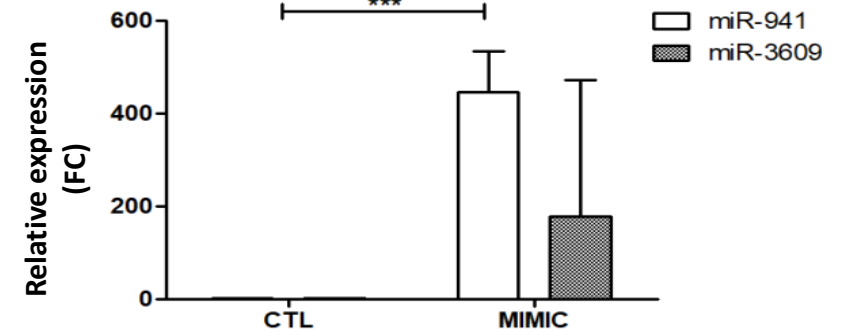
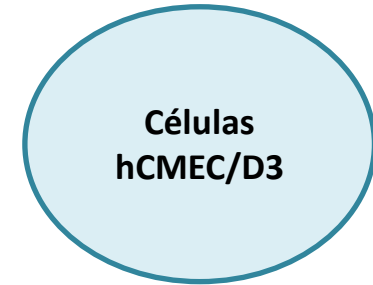
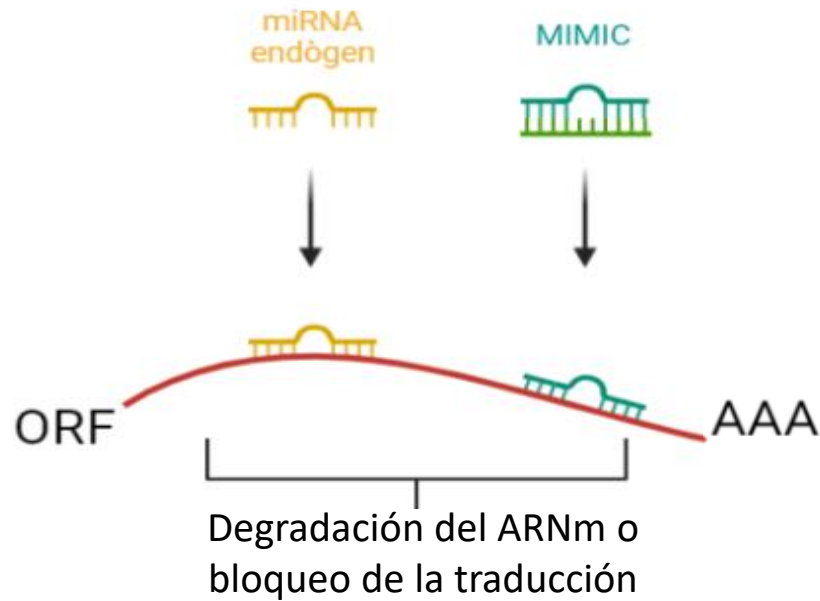
Pacientes con buena recuperación funcional



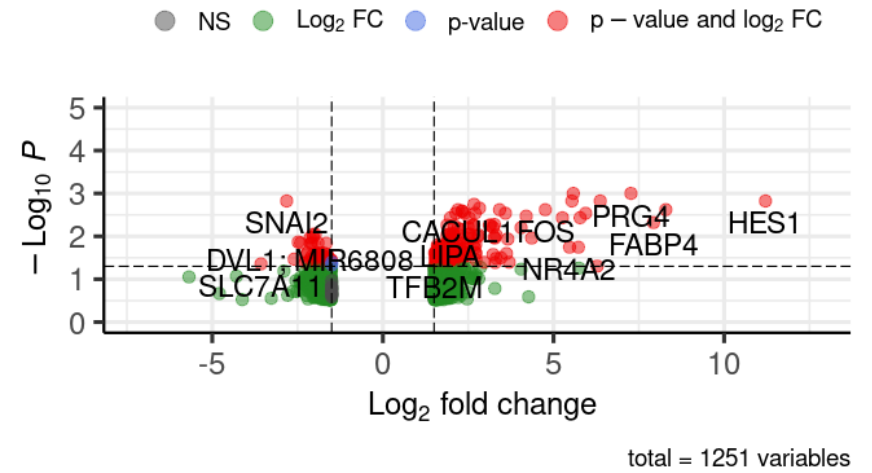
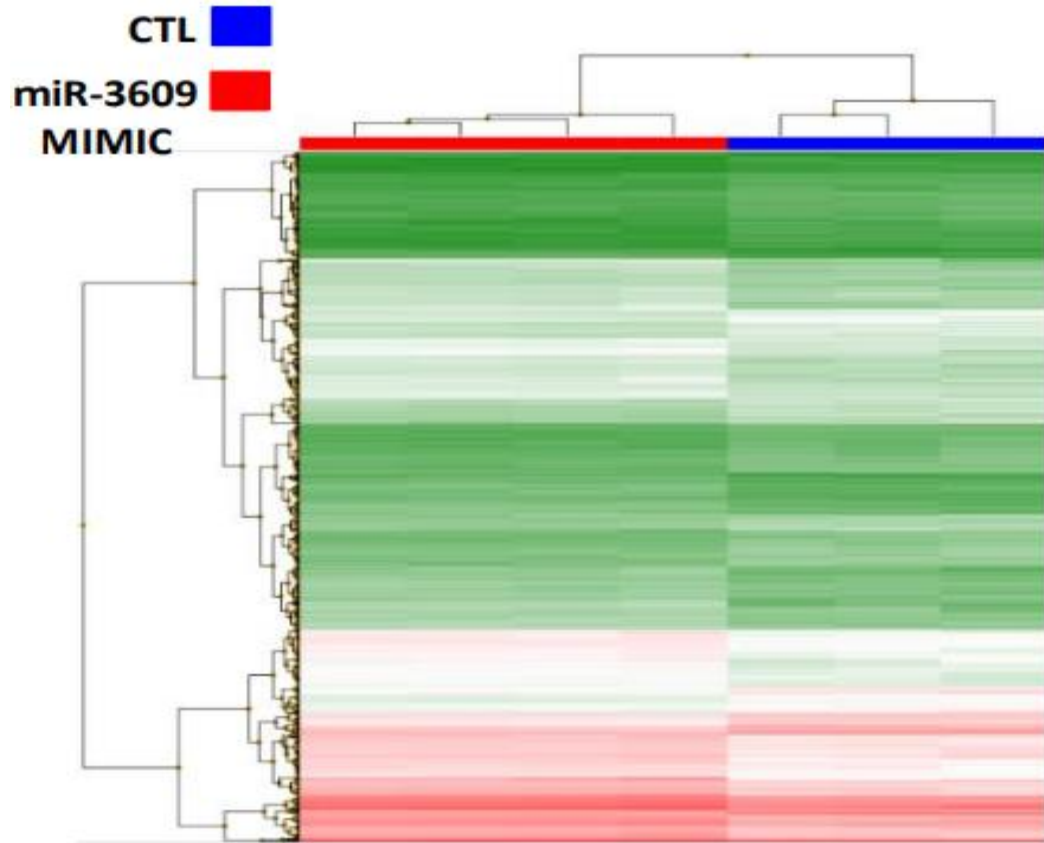
Modificació de Macka Julia J. et al. Hematology. 2018.

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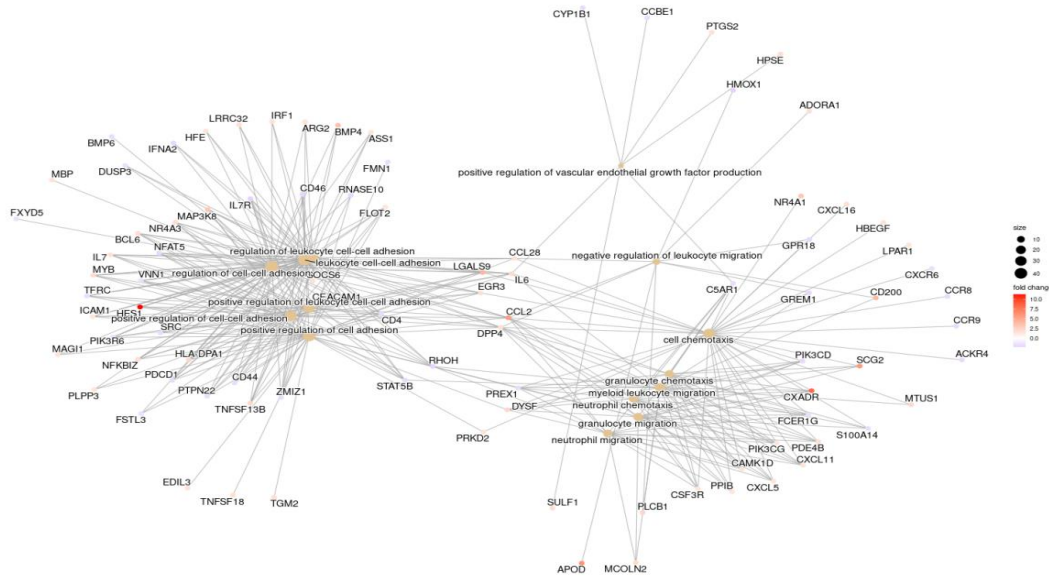
Modulació *in-vitro* de la expressió del miR-3609 y miR-941



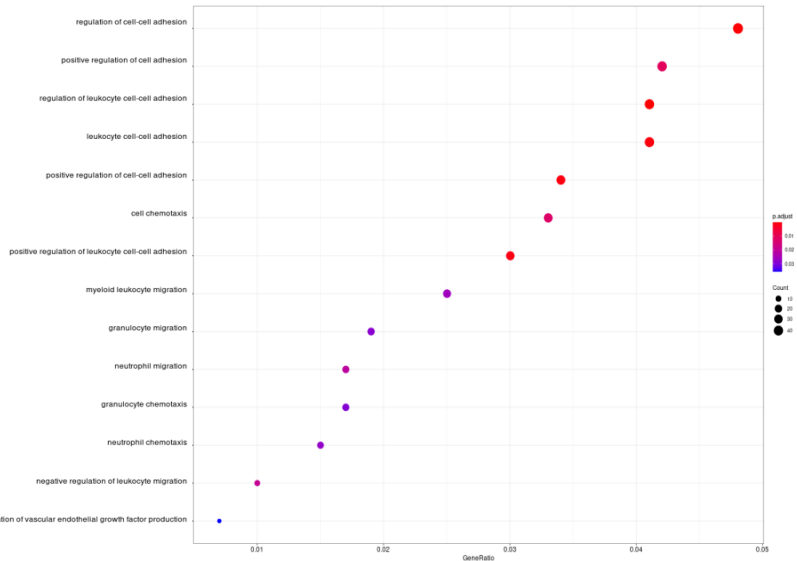
Resultados miR-3609



Resultados miR-3609

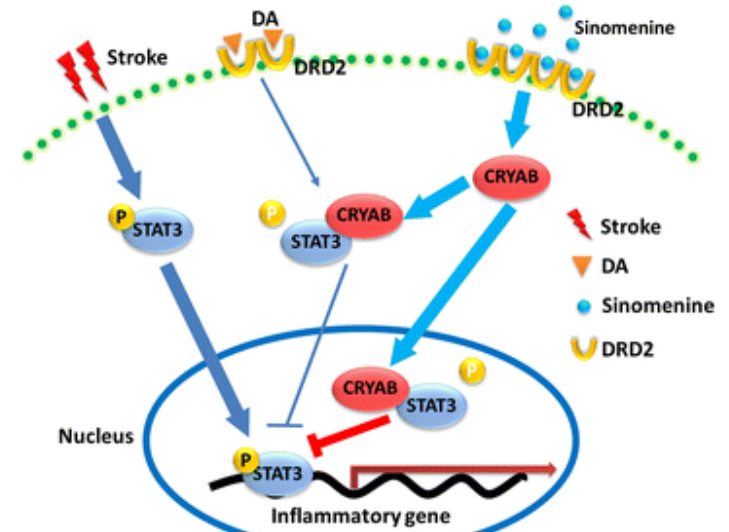
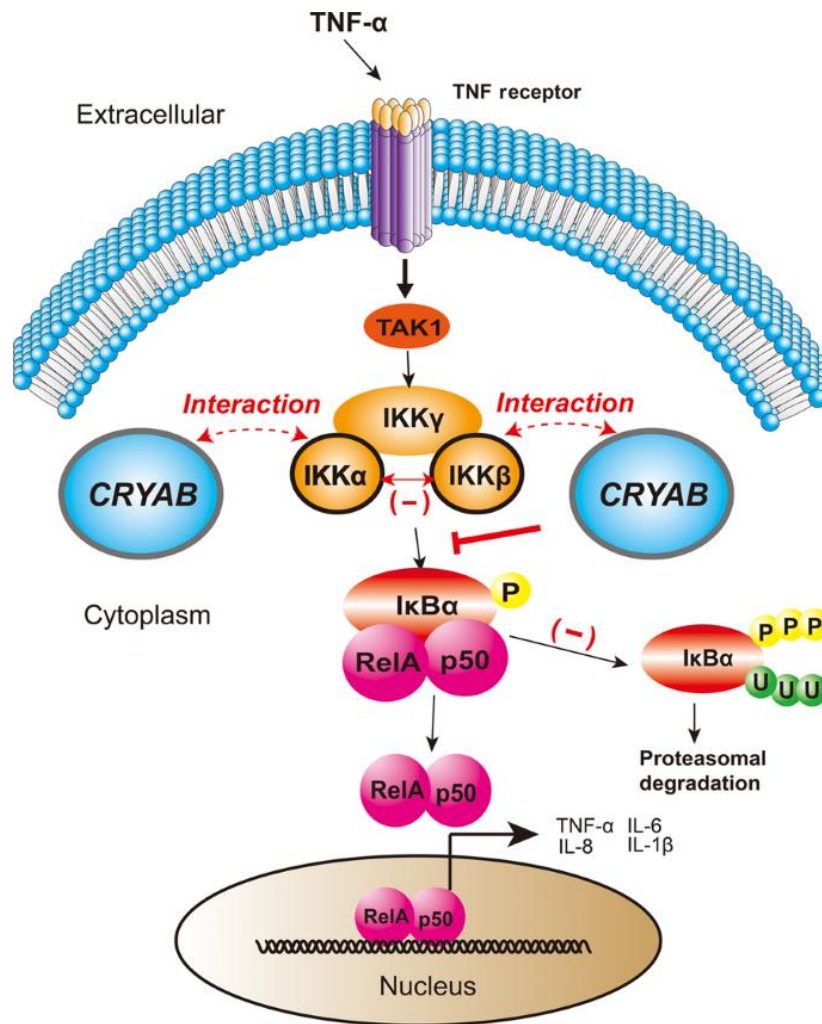


1. Regulación de la adhesión célula-célula
2. Migración de neutrófilos
3. Proliferación de VEGF

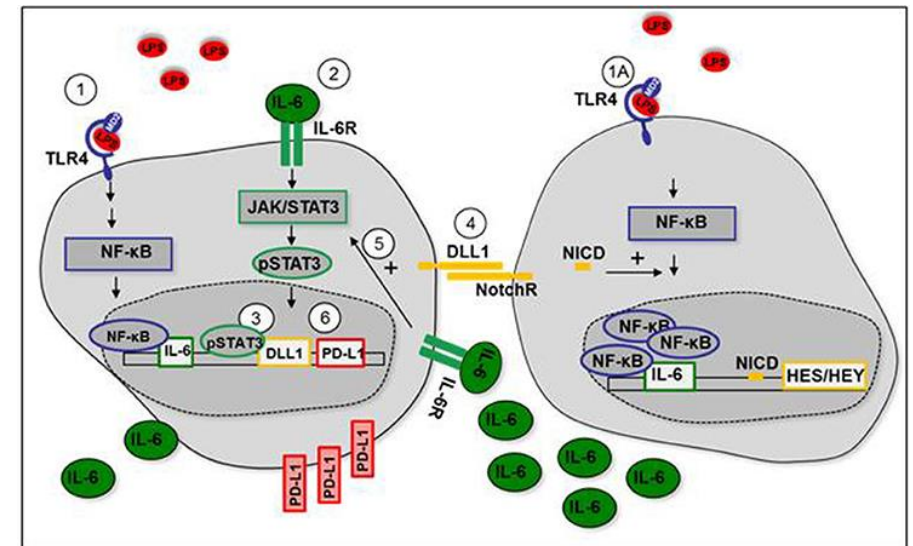


miR3609-Mimic	Scramble	Fold Change	P-val	Gene Symbol
Avg (log2)	Avg (log2)			
6.07	8.79	-6.58	0.0188	CRYAB
8.79	11.43	-6.2	0.0046	LURAP1L
11.02	13.38	-5.14	0.0001	SLC7A11
11.15	13.17	-4.06	0.0426	ASNS
7.71	9.42	-3.29	0.0167	TM4SF19
6.49	8.2	-3.27	1.63E-05	LAMB3; MIR4260
9.03	10.69	-3.16	0.0032	JAZF1
6.81	8.44	-3.08	0.0366	NSG1; D4S234E
13.04	14.64	-3.04	0.0014	STC2

Resultados miR-3609



Qiu, J. *et al.* Sinomenine activates astrocytic dopamine D2 receptors and alleviates neuroinflammatory injury via the CRYAB/STAT3 pathway after ischemic stroke in mice. *J Neuroinflammation* 13, 263 (2016).

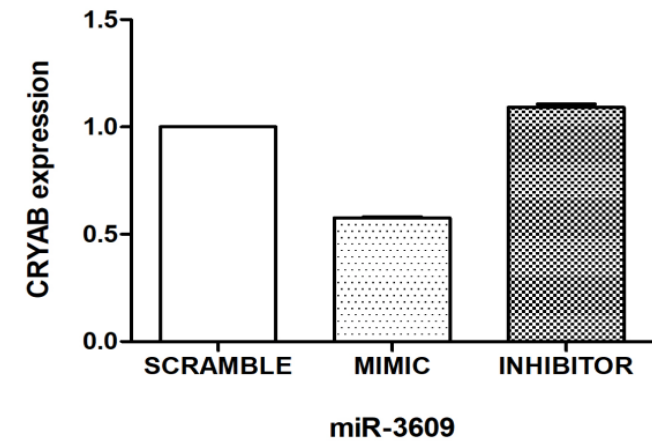
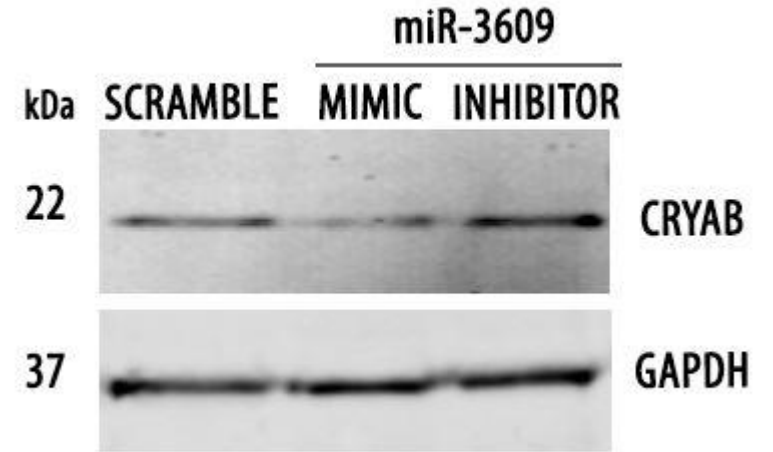
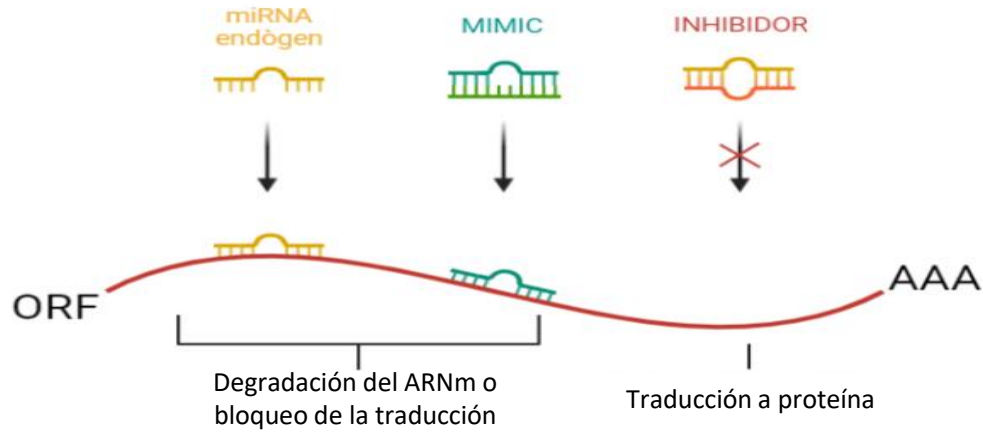


Hildebrand D. *et al.* *Front. Cell. Infect. Microbiol.* (2018)

Xu, W. *et al.* Small heat shock protein CRYAB inhibits intestinal mucosal inflammatory responses and protects barrier integrity through suppressing IKKβ activity. *Mucosal Immunol* 12, 1291–1303 (2019).

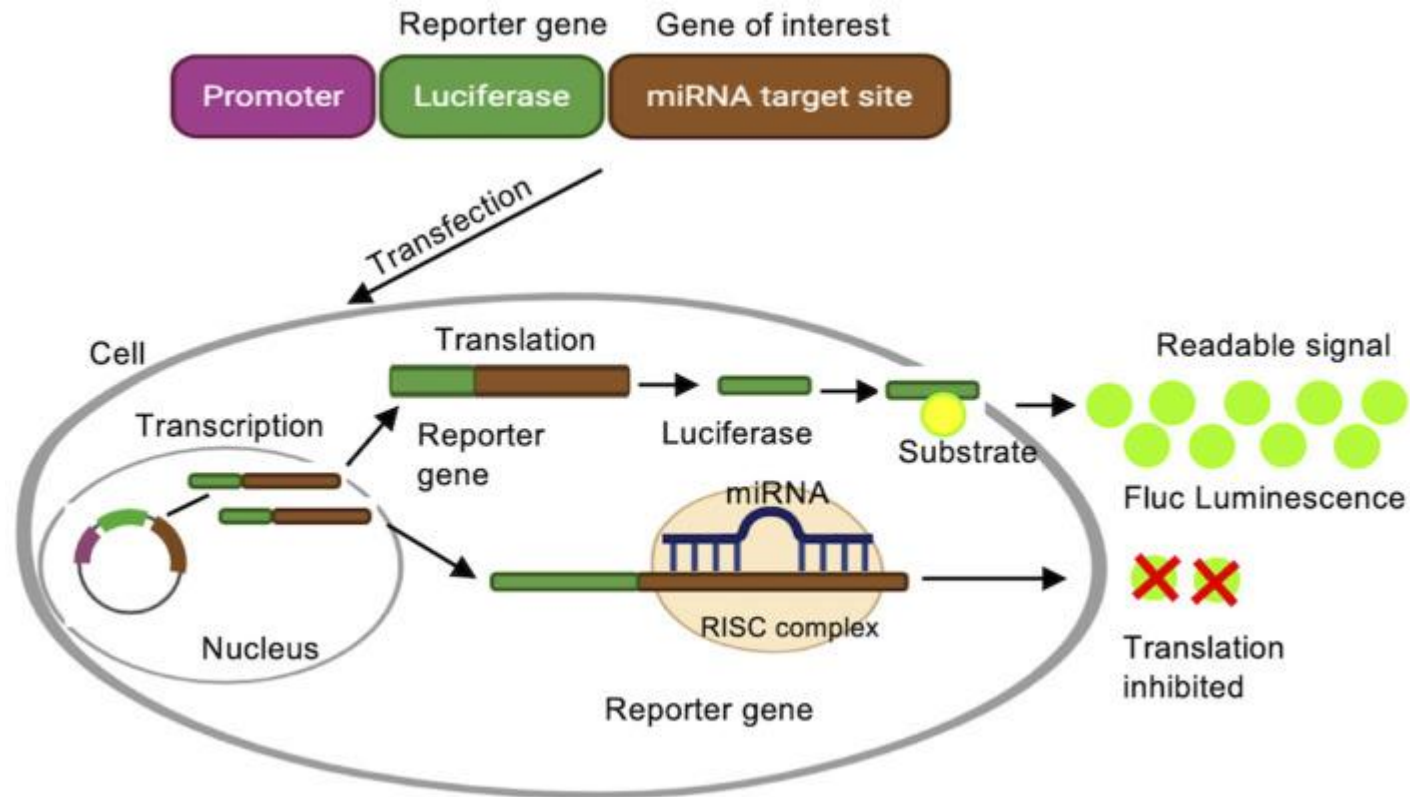
Resultados miR-3609

WB

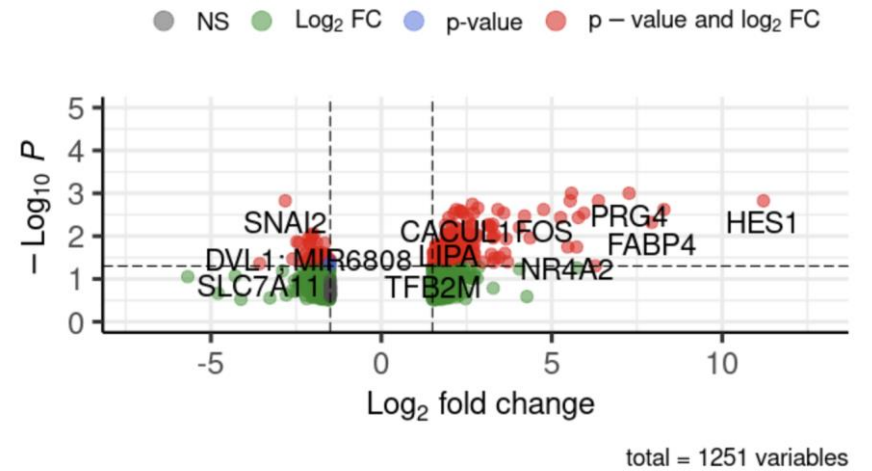
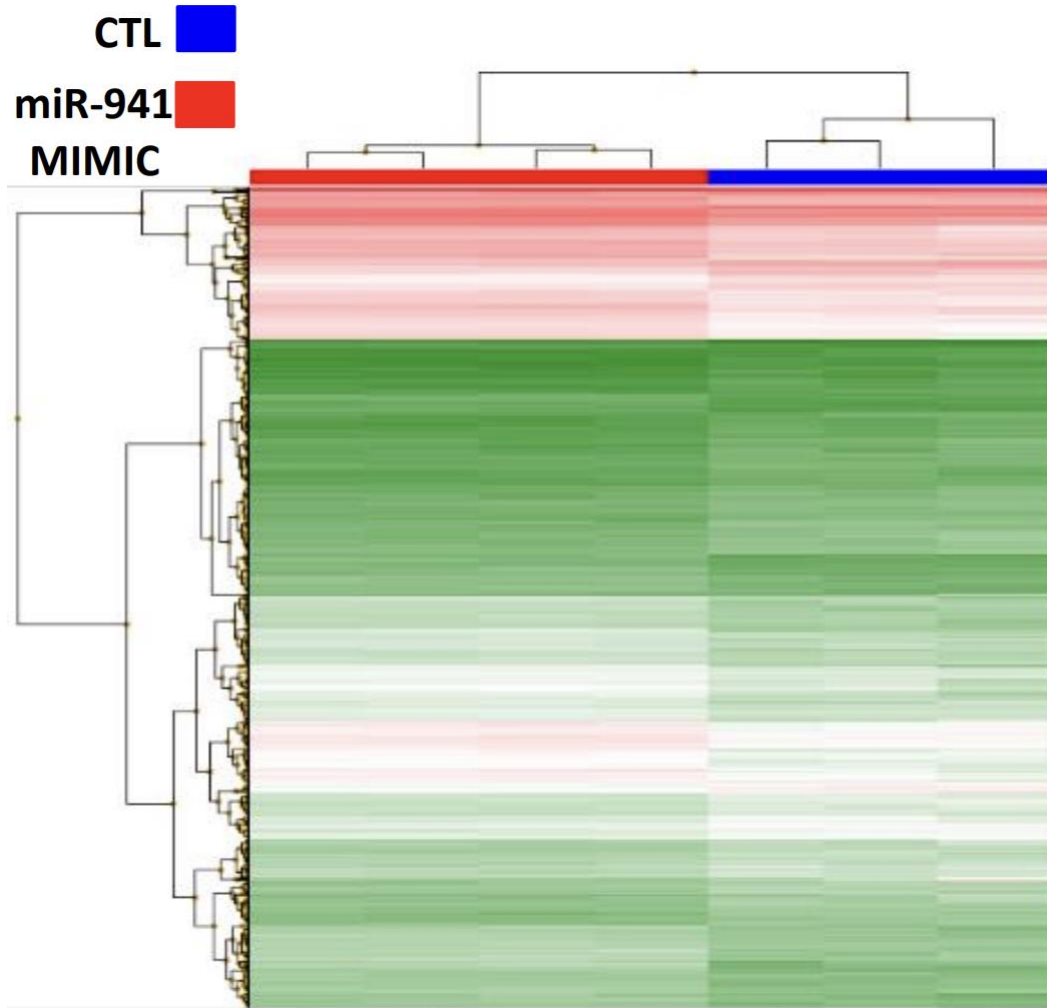


Ensayo de luciferasa

Position 231-261: 5'..... gaccccTCAC ACTCA... ATG.....GAC ... 3' CRYAB 5'UTR
3' caaAGUGaTGAGUaaUACTggCUG 5' miR-3609



Resultados miR-941



Conclusiones

- Se han identificado un total de 11 microARNs como potenciales biomarcadores para predecir el pronóstico funcional del ictus isquémico.
- miR-1275 se infra-expresa tanto a las 24 horas cómo a los 10 días después del ictus en pacientes con marcada discapacidad (mRS=4-5). Este miRNA, en células de endotelio cerebral vascular humano (hCMEC/D3) se sobre-expresa en respuesta a hipoxia y modula la expresión del gen IGF2BP3.
- El miR-3609 se infra-expresa a los 10 días después del ictus en pacientes con marcada discapacidad (mRS=4-5). Tiene la capacidad de regular la expresión de CRYAB, un modulador de la neuroinflamación y la angiogénesis después de un daño cerebral.
- Los pacientes con marcada discapacidad presentan una menor expresión del miR-941 frente a pacientes sin discapacidad.



GeneStroke



Universitat de les Illes Balears

