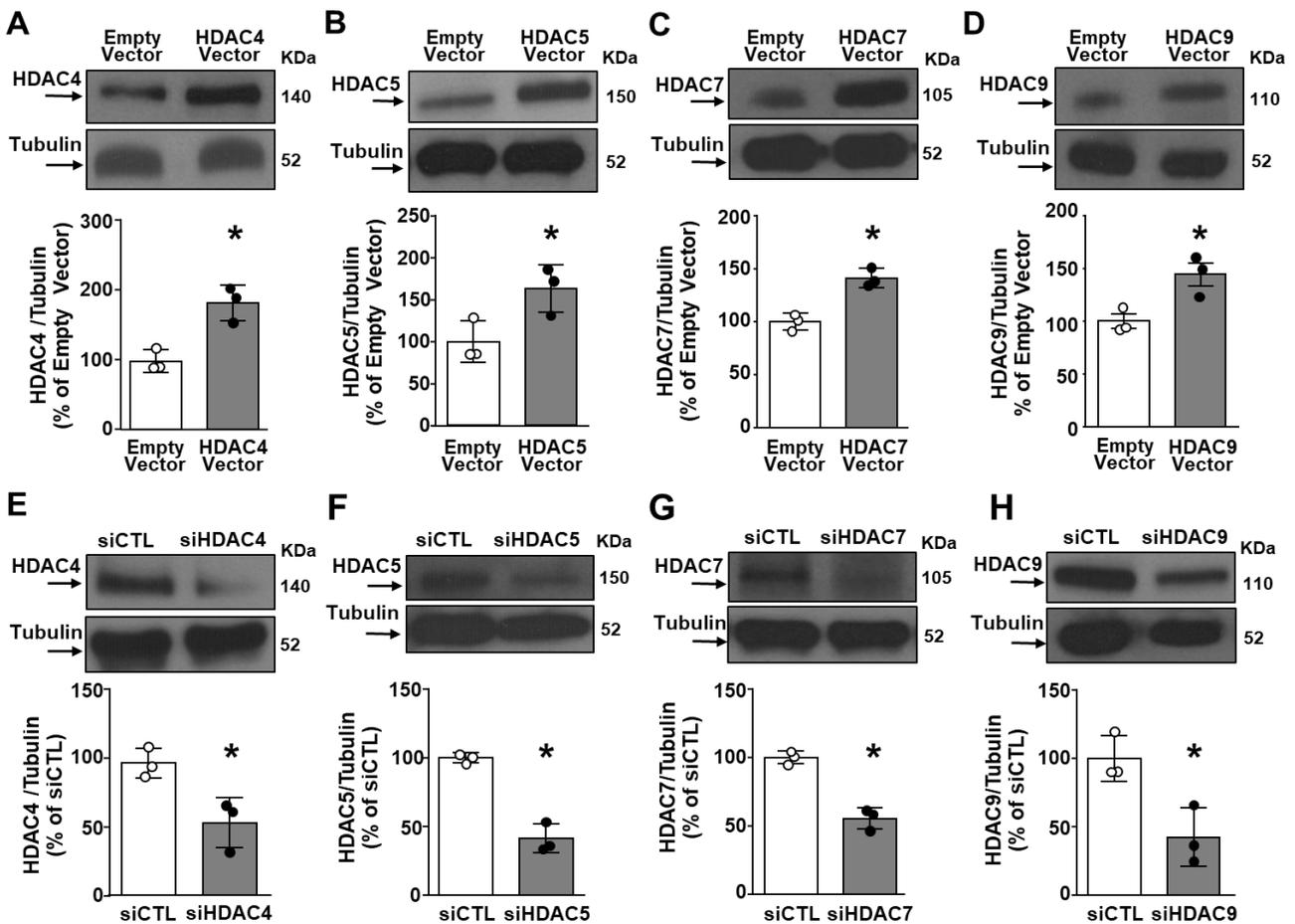


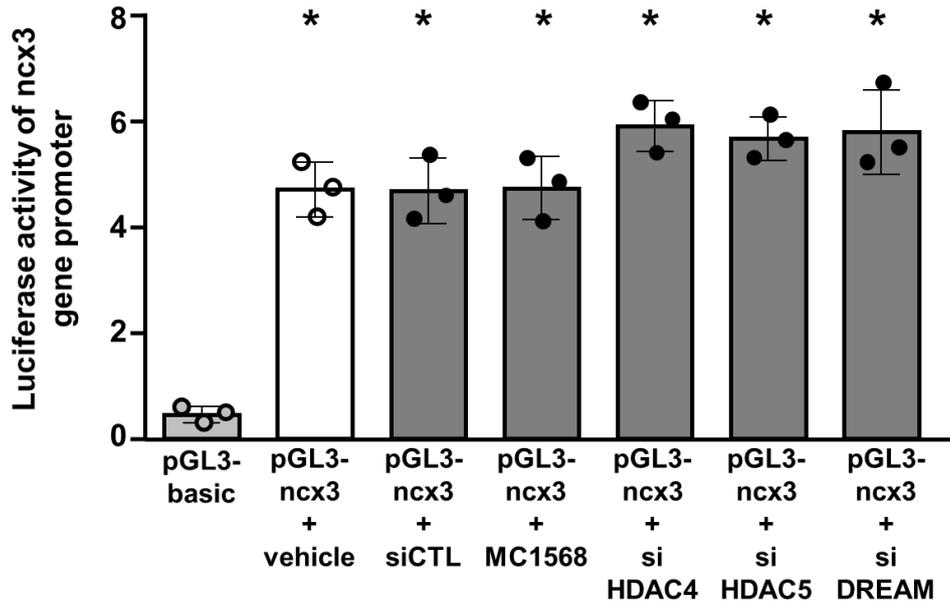
Supplementary material



Supplemental Fig. 1

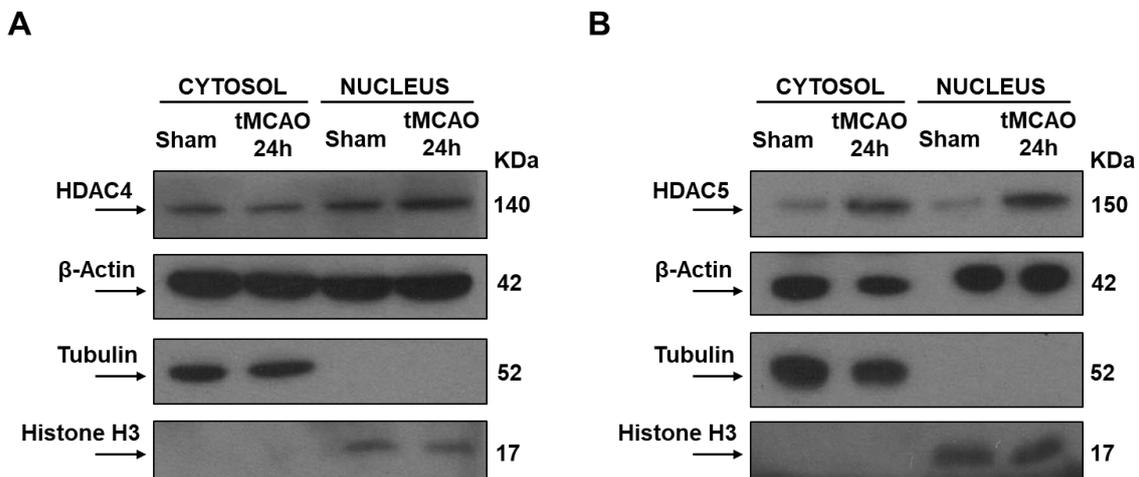
Supplementary Fig 1. Cortical neurons transfected with constructs or siRNAs for HDAC4, HDAC5, HDAC7, and HDAC9.

(A-D) Representative WB with quantification in cortical neurons after 24 h of transfection with vectors overexpressing HDAC4 (n=3), HDAC5 (n=3), HDAC7 (n=3) and HDAC9 (n=3). *p < 0.05 vs Empty Vector, Student t test. (E-H) Representative WB with quantification in cortical neurons after 24 h of transfection with siRNAs against HDAC4 (n=3), HDAC5 (n=3), HDAC7 (n=3), HDAC9 (n=3). *p < 0.05 vs siCTL, Student t test.



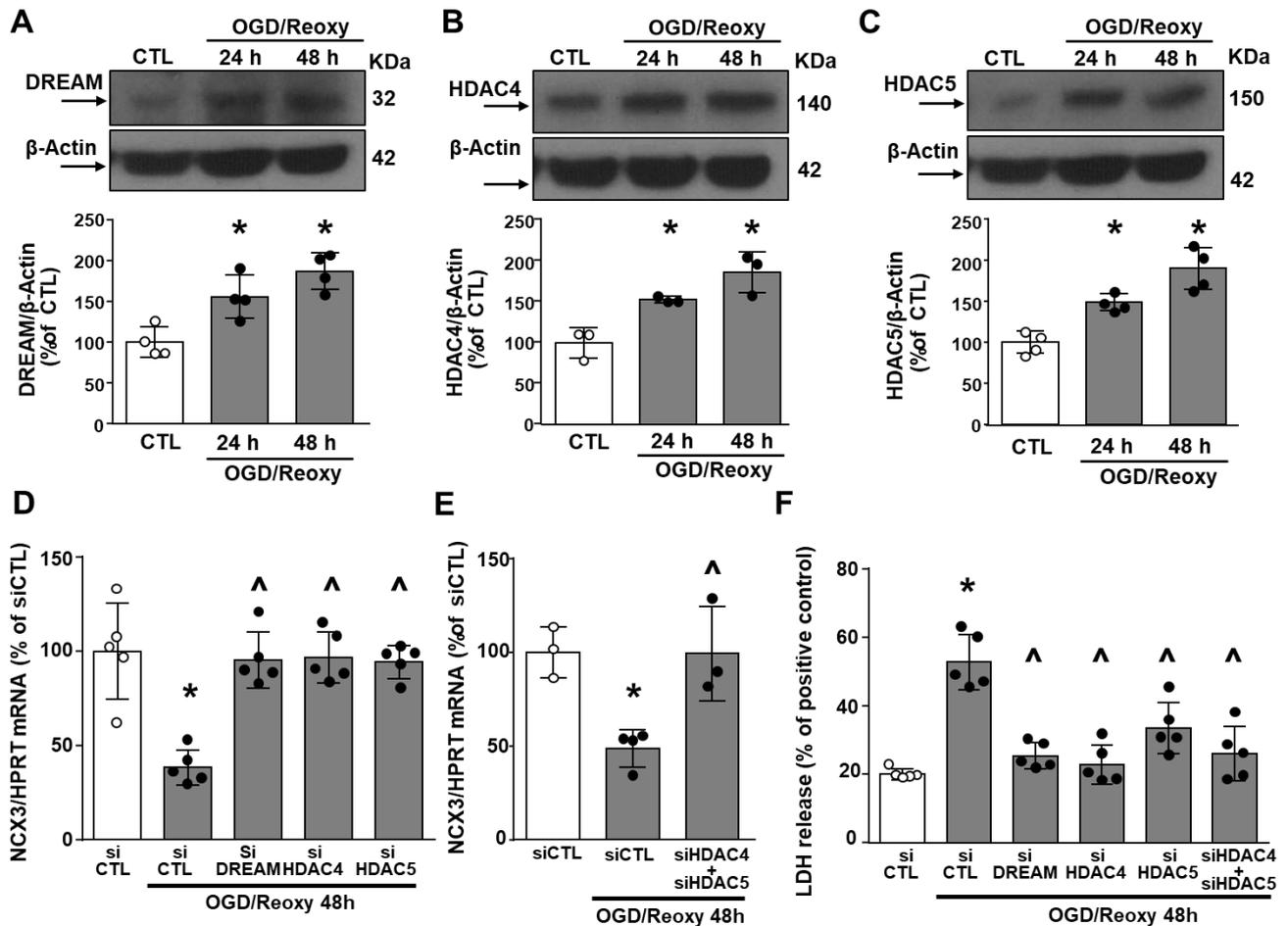
Supplementary Fig 2. Effect of MC1568, siHDAC4 siHDAC5 and siDREAM on *ncx3* promoter activity in U87 glial cells.

(A) Luciferase activity of *ncx3* gene promoter WT (pGL3-*ncx3*) in the following experimental conditions: **(1)** pGL3-basic, **(2)** pGL3-*ncx3*+vehicle, **(3)** pGL3-*ncx3*+siCTL **(4)** pGL3-*ncx3*+MC1568, **(5)** pGL3-*ncx3*+siHDAC4, **(6)** pGL3-*ncx3*+siHDAC5 and **(7)** pGL3-*ncx3*+siDREAM. Cells were treated with MC1568 or vehicle for a 2-h pulse (n=3). *p < 0.05 vs pGL3-basic, one-way ANOVA, followed by Turkey's multiple comparison test.



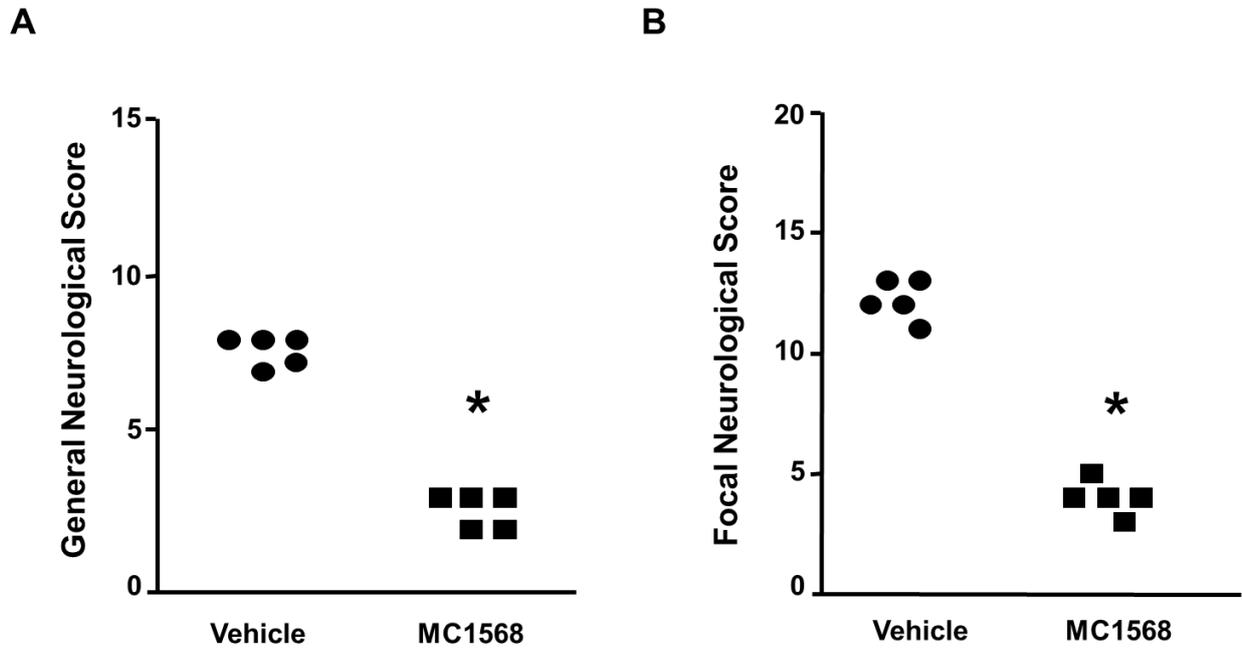
Supplementary Fig 3. Effect of 24 h tMCAO on cytosol/nucleus localization of HDAC4 and HDAC5.

(A, B), Western blots of HDAC4 and HDAC5 protein levels after 24 h of tMCAO. β -actin was used as loading control, tubulin and histone H3 (H3) were used to verify the integrity of the cytoplasmic and nuclear fraction of the samples.



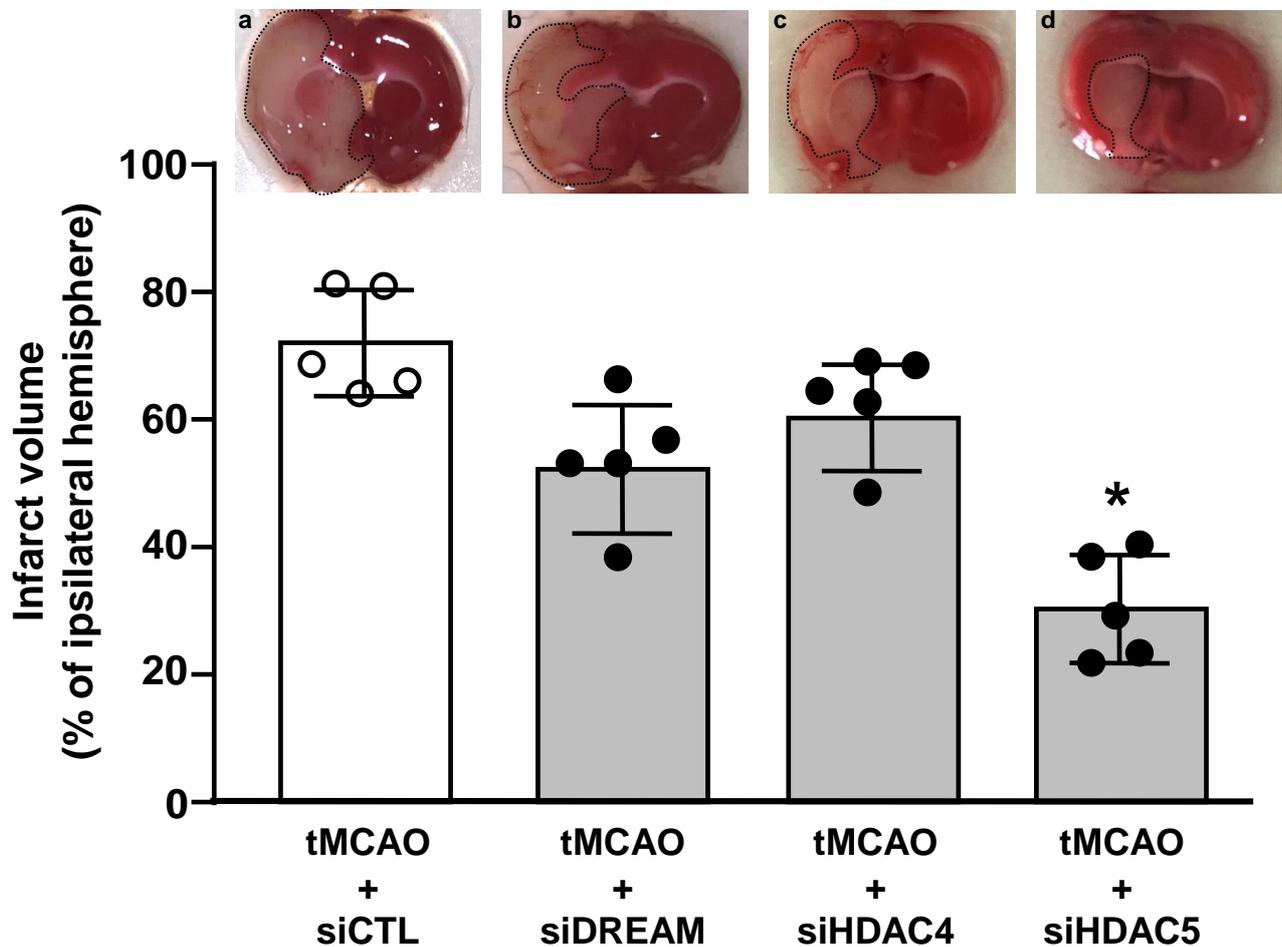
Supplementary Fig 4. Effect of DREAM, HDAC4 and HDAC5 knockdown on cell survival and *ncx3* gene expression in cortical neurons exposed to OGD/Reoxy.

(A-C) Western blots of DREAM, HDAC4 and HDAC5 protein levels after 12 h and 24 h of OGD/Reoxy (n=3/4). *p < 0.05 vs CTL. The same filter immunoblotted with anti-HDAC5 was stripped and re-probed with anti-DREAM antibody. **(D,F)** Effect of siDREAM, siHDAC4, siHDAC5 and siHDAC4+siHDAC5, transfected in cortical neurons exposed to 48 h of OGD/Reoxy on: **(D,E)** *ncx3* gene (n=3/5) and **(F)** LDH release (n=5). *p \leq 0.05 vs siCTL and Δ p \leq 0.05 vs siCTL+OGD/Reoxy 48h, one-way ANOVA, followed by Turkey's multiple comparison test.



Supplementary Fig 5. Evaluation of general and focal neurological deficits after MC1568 icv injected in ischemic rats.

(A,B) Effect of MC1568 icv administered on general and focal scores evaluated when rats were euthanized 24 h after tMCAO. * $p < 0.05$ vs Vehicle ($n=5$). Kruskal-Wallis test, followed by Dunn's multiple comparison test.



Supplementary Fig 6. Effect of siRNAs targeting HDAC4, HDAC5 and DREAM on ischemic damage induced in male rats by tMCAO. % of brain infarct volume in male rats subjected to tMCAO after icv administration of: (a) siCTL, (b) siDREAM, (c) siHDAC4, and (d) siHDAC5. Representative brain slices from each experimental group are shown at the top of each column (n=5). Ischemic rats were euthanized 24 h after tMCAO. % of the infarct volume was compared to the ipsilateral hemisphere. *p ≤0.05 vstMCAO+siCTL+Vehicle; two-way ANOVA, followed by Newman-Keuls test.