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Effects of TSPO and STAR inhibitors on cell death in a cardiomyocyte model of hypoxia-reoxygenation

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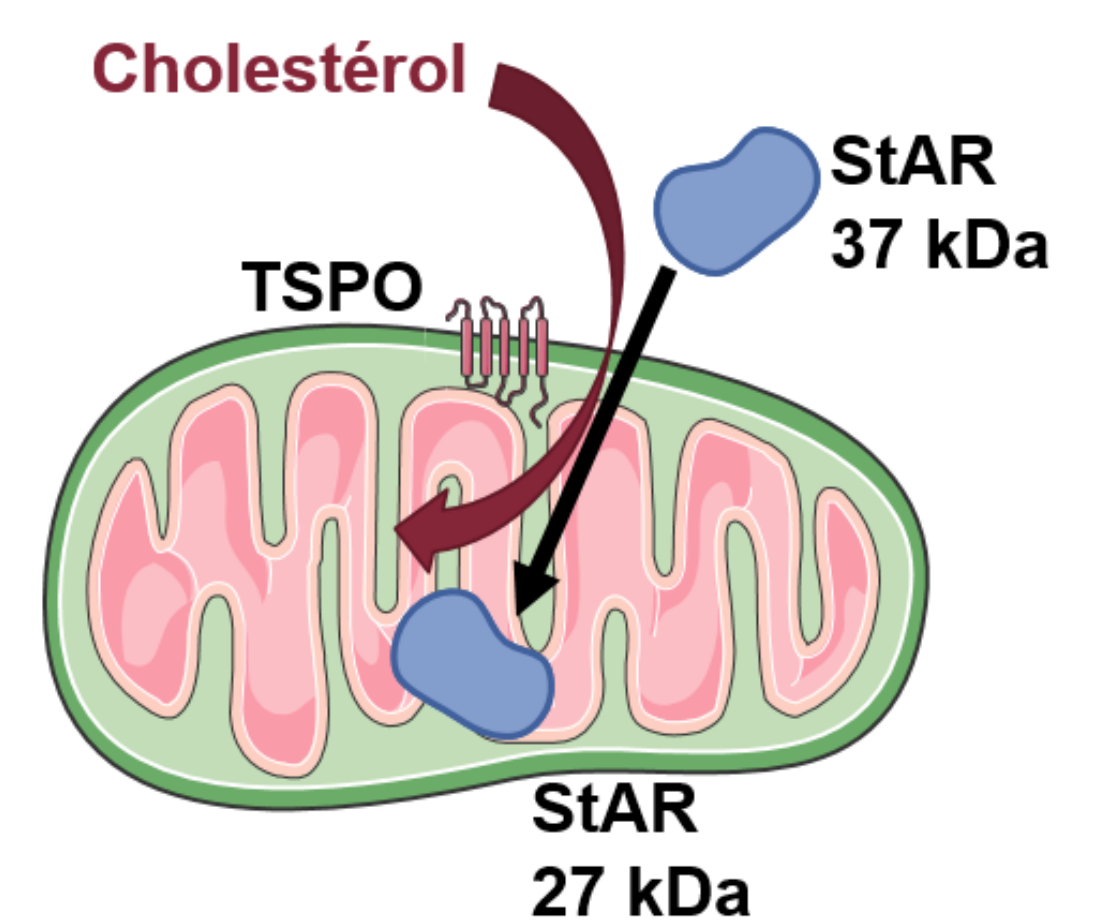
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INTRODUCTION

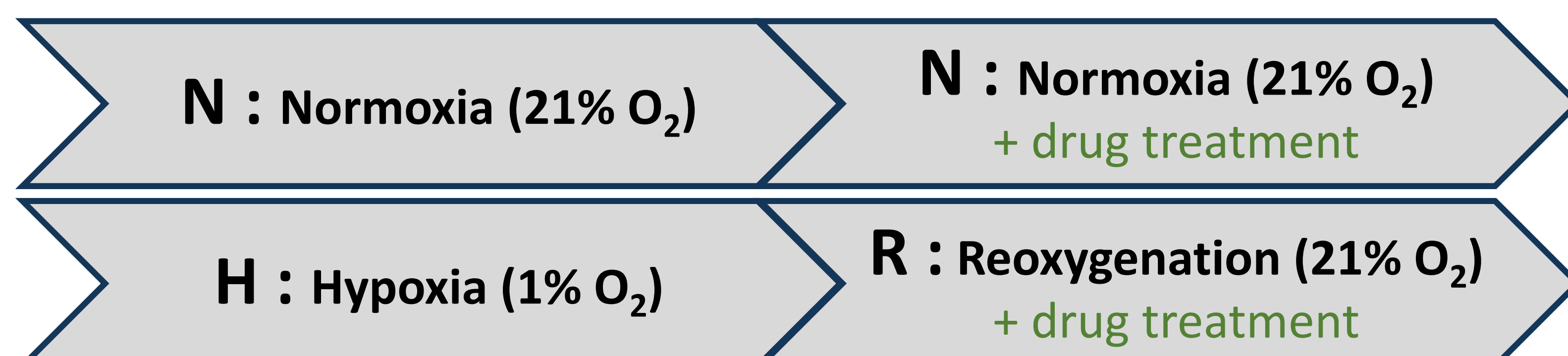
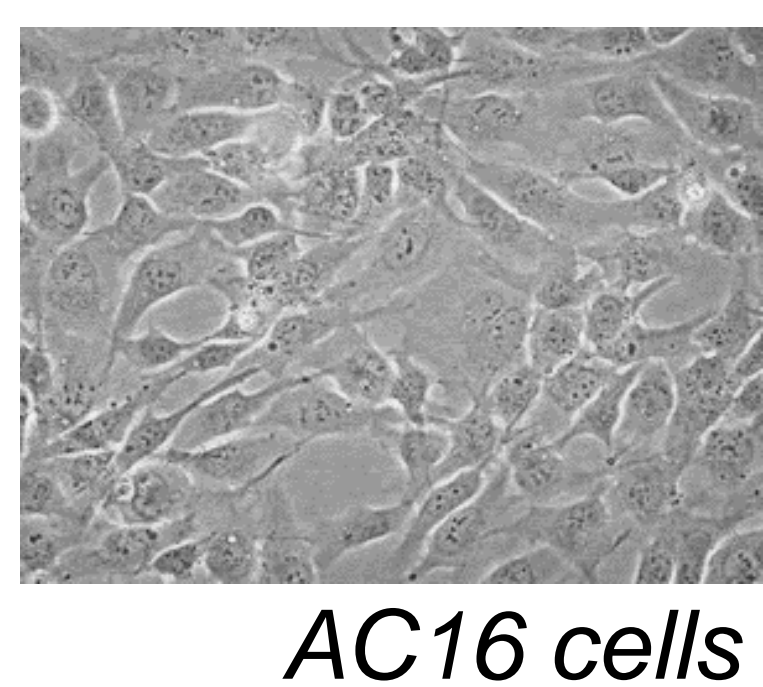
We previously demonstrated that reperfusion of an ischemic myocardium induces an increase in mitochondrial cholesterol (Chol) content accompanied by a generation of oxysterols. The translocator protein (TSPO) and the steroid acute regulatory protein (STAR) are involved in Chol transport at the mitochondrial membrane in steroidogenic tissues but in the heart their roles remain uncertain. The TSPO ligand 4'-chlorodiazepam (4'CDZ) has been demonstrated to inhibit STAR and sterol mitochondrial accumulations and to reduce infarct size. These data suggest that targeting mitochondrial sterol accumulation could participate to the protective effects of TSPO ligands.

➤ The aim of this study was to analyze the mechanisms and the role of the mitochondrial Chol transport in cell death with a cardiomyocyte model of hypoxia-reoxygenation (HR).



METHODS

AC16 human cardiomyocytes were submitted to different durations of hypoxia (1% O₂) followed by reoxygenation (21% O₂) to achieve 50% mortality. Cells were then treated at reoxygenation with 4'CDZ and novel TSPO and STAR inhibitors, known to delay steroidogenesis *in vitro* by targeting Chol specific binding sites of these proteins (CRAC and START, respectively). Cell mortality was assessed with MTT and crystal violet assay and Chol was identified with thin layer chromatography and fluorescent microscopy.



Cell viability :

- MTT Assay
- Crystal violet assay

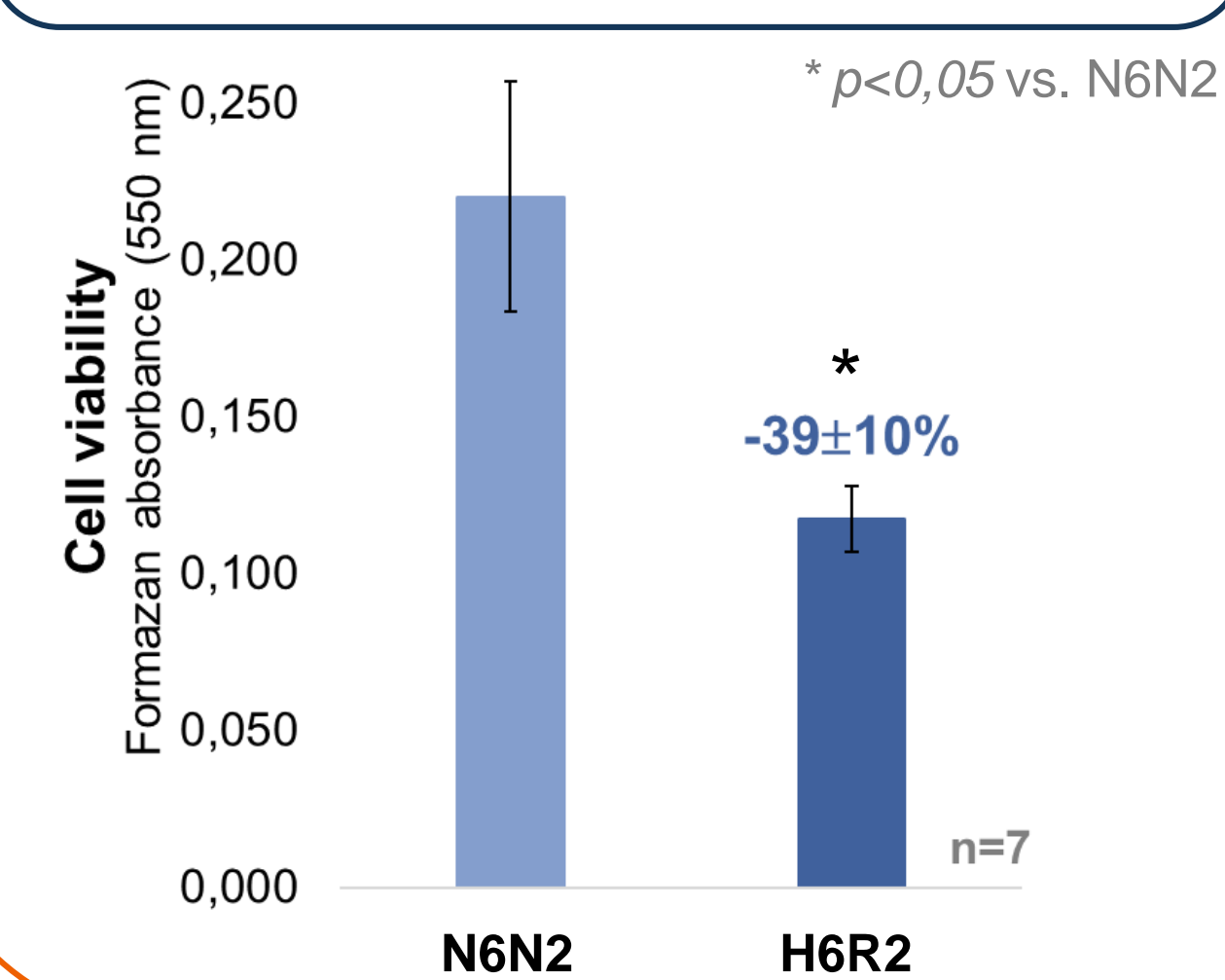
Cholesterol content :

- Thin layer chromatography
- Fluorescent microscopy

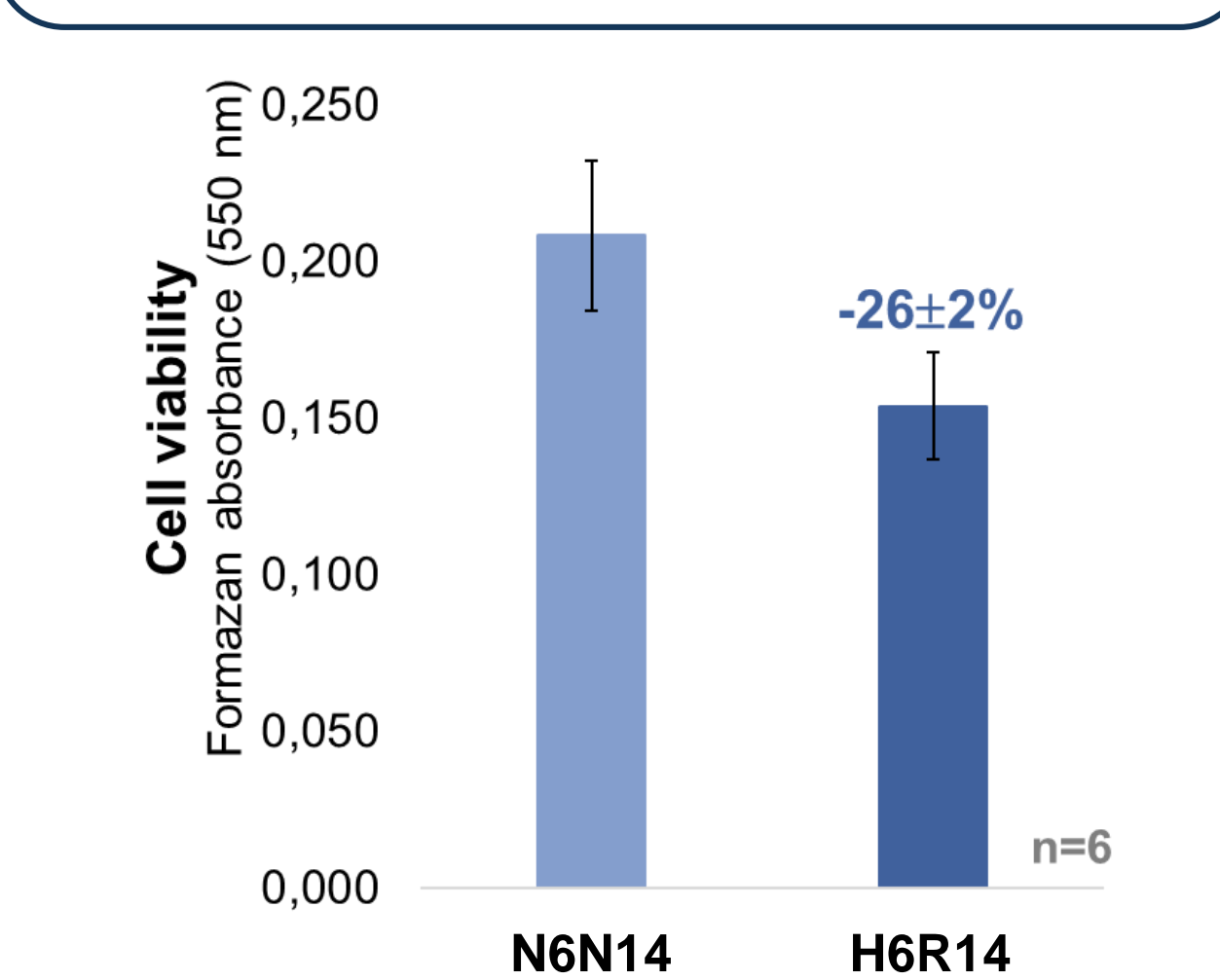
RESULTS

1 – Effect of different HR protocols on cell viability

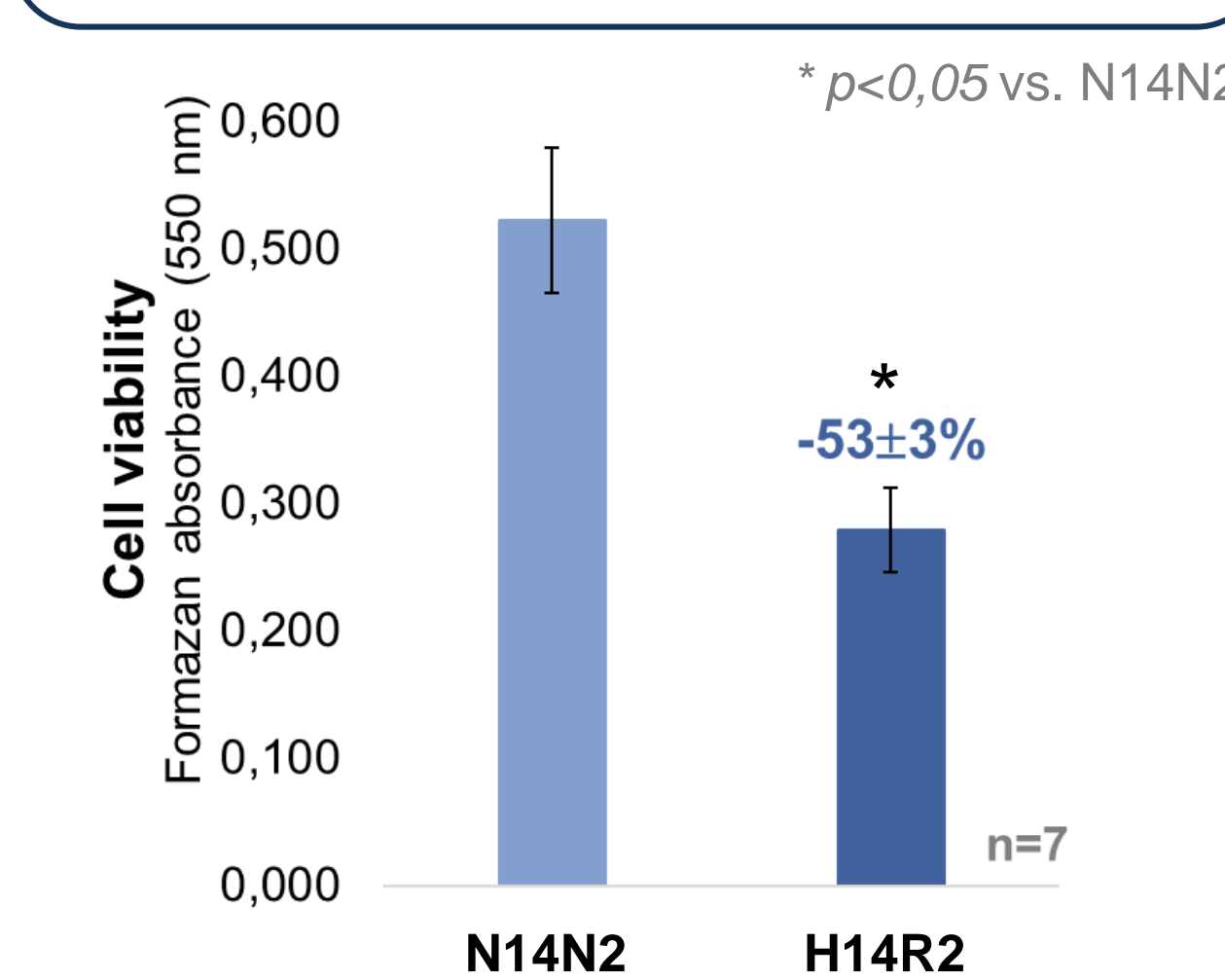
A. 6h hypoxia followed by 2 h reoxygenation induces 39±10% mortality in AC16 cells (MTT assay)



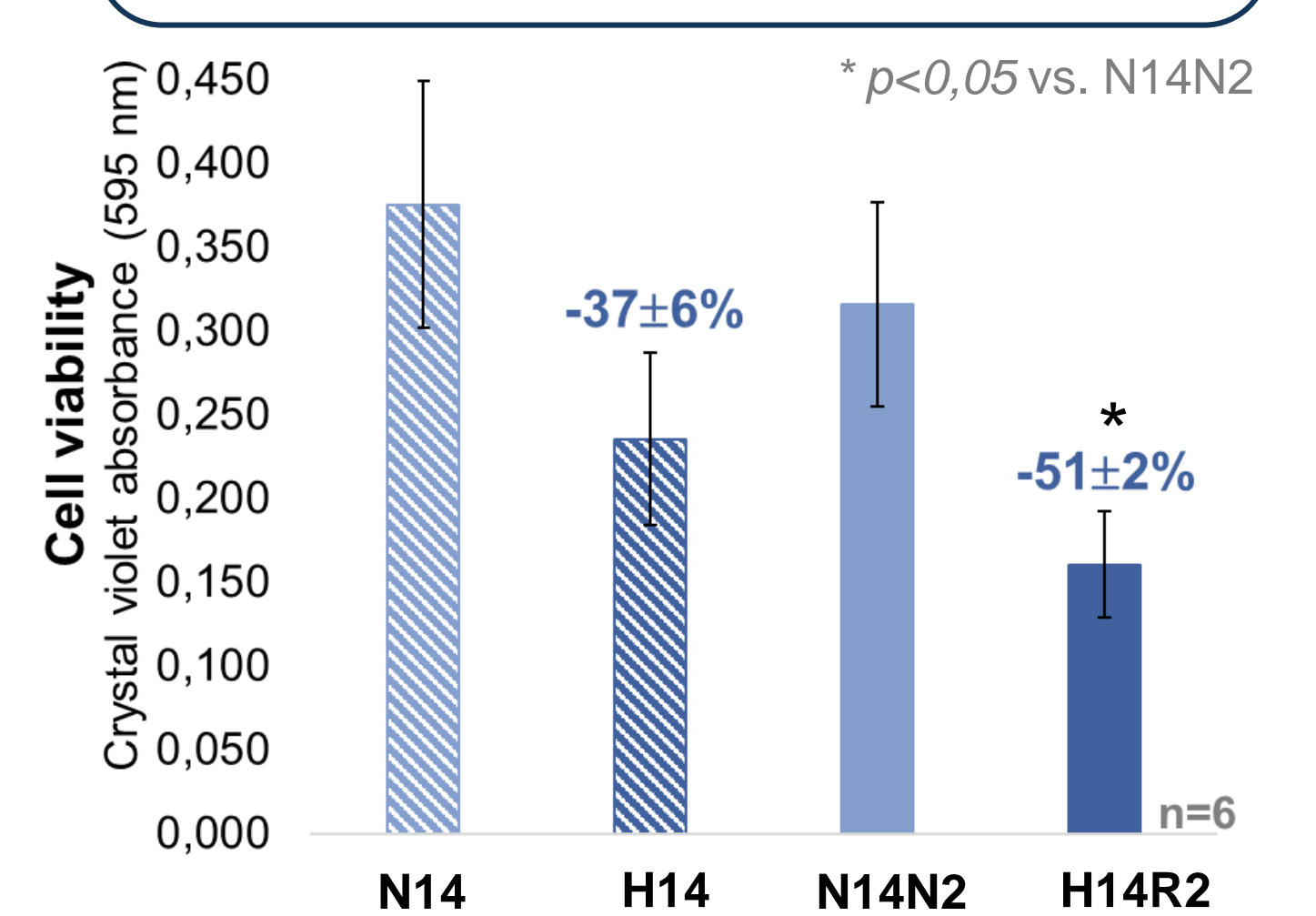
B. 6h hypoxia followed by 14 h reoxygenation induces 26±2% mortality in AC16 cells (MTT assay)



C. 14h hypoxia followed by 2 h reoxygenation induces 53±3% mortality in AC16 cells (MTT assay)

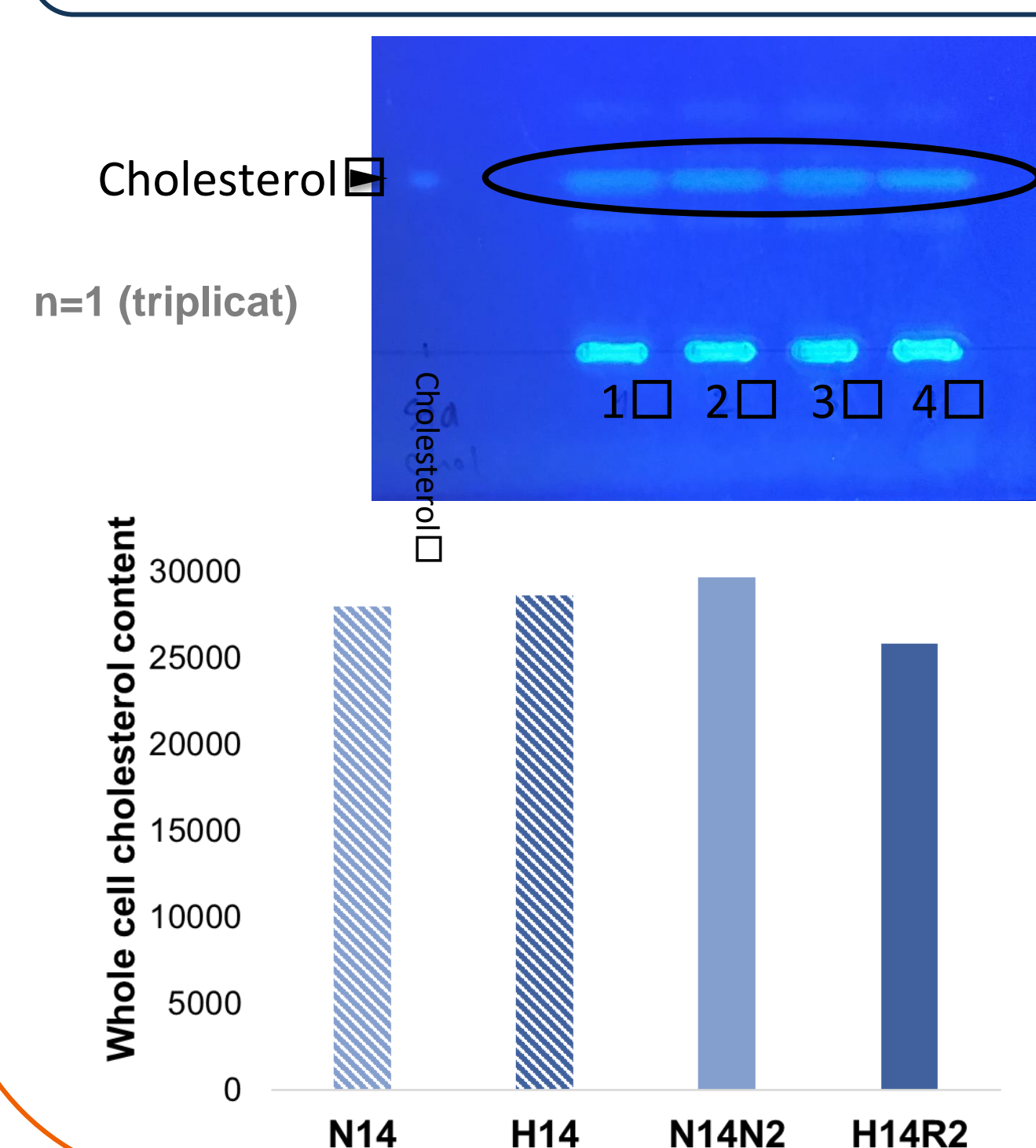


D. 14h hypoxia induces 37±6% mortality, 2h reoxygenation induces further mortality 51±3% in AC16 cells (crystal violet assay)



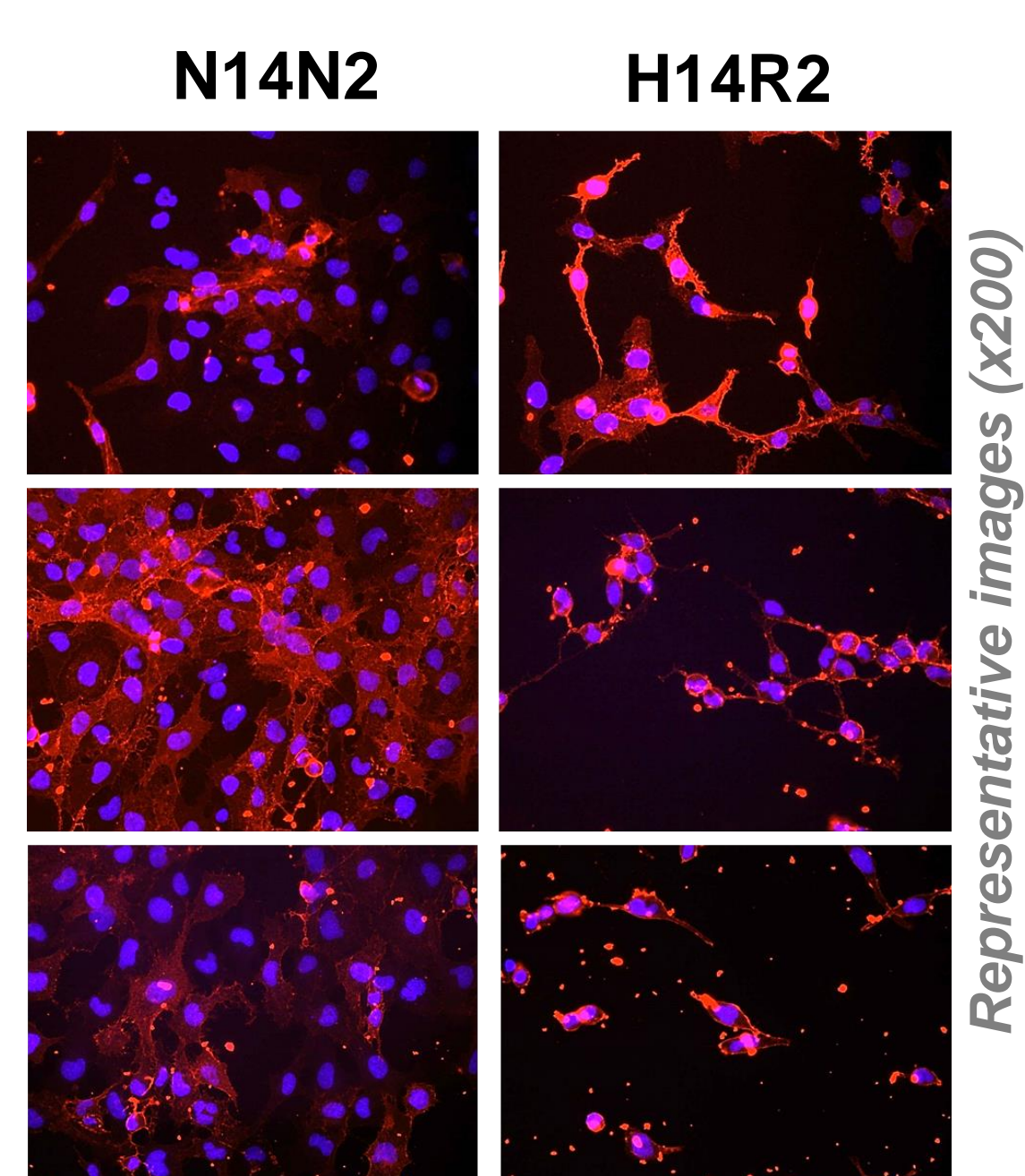
2 – Behavior of cholesterol content during HR

A. Whole cell cholesterol content is not modified by HR



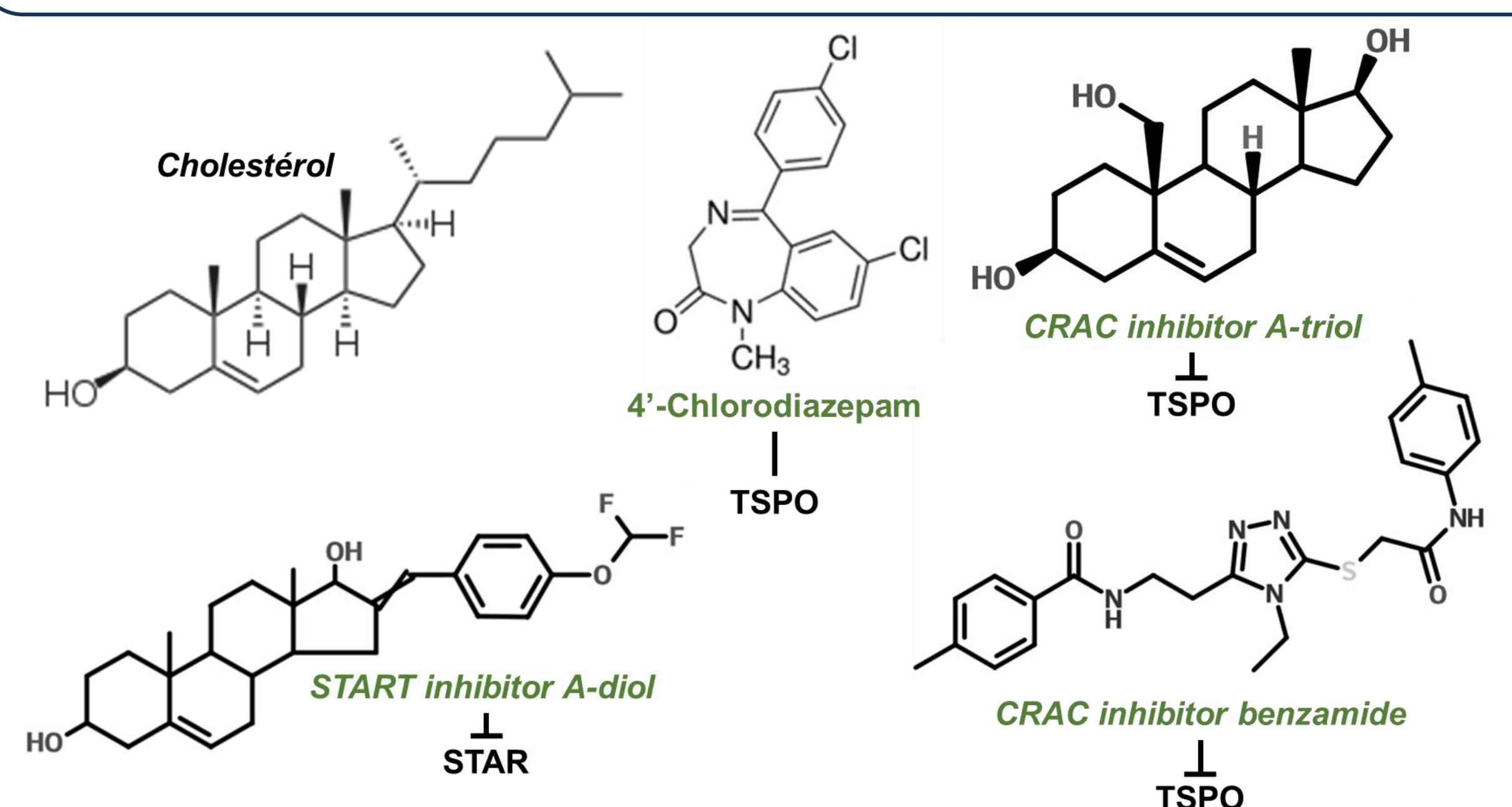
B. Distribution of membrane cholesterol is modified by HR

Red : Membrane cholesterol (D4-mCherry)
Blue : Nucleus (Hoechst)

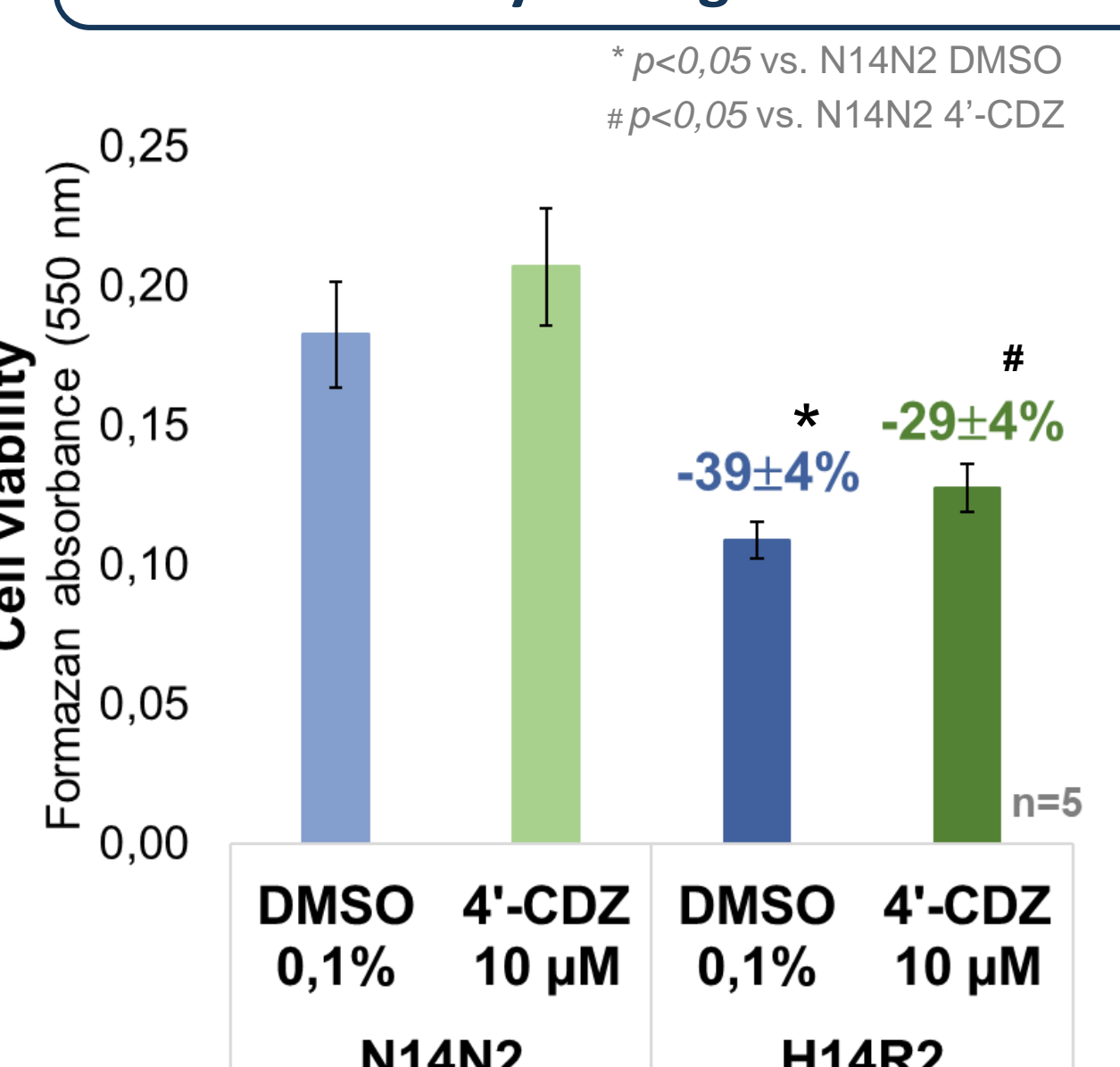


3 – Effect of TSPO and STAR inhibitors on cell mortality at reperfusion

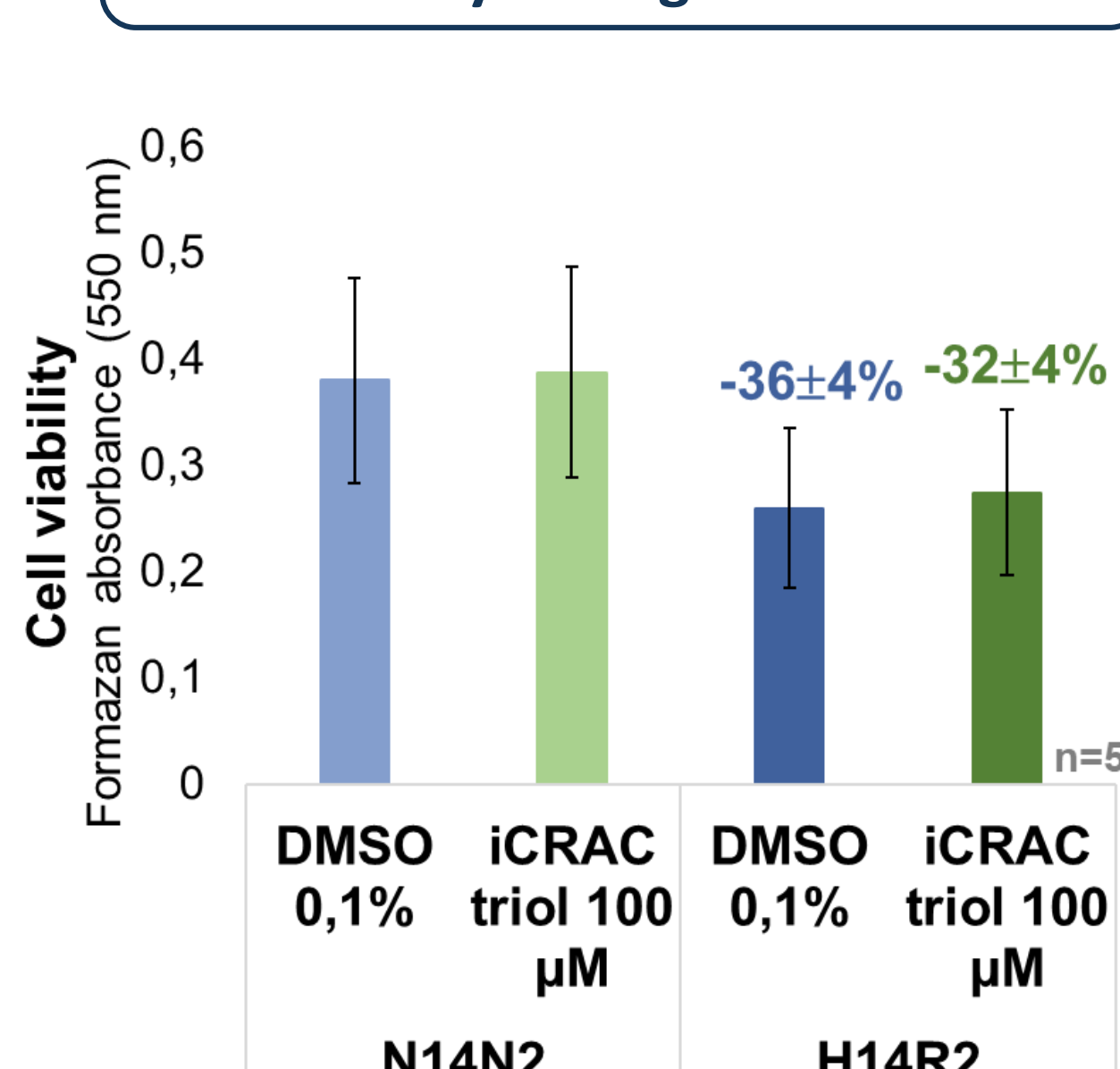
A. Chemical structure of cholesterol (endogen TSPO and STAR ligand), 4'-chlorodiazepam : 4'CDZ (TSPO ligand), CRAC inhibitor A-triol : iCRAC triol (TSPO inhibitor), START inhibitor A-diol : iSTART diol (STAR inhibitor), CRAC inhibitor benzamide : iCRAC benz (TSPO inhibitor)



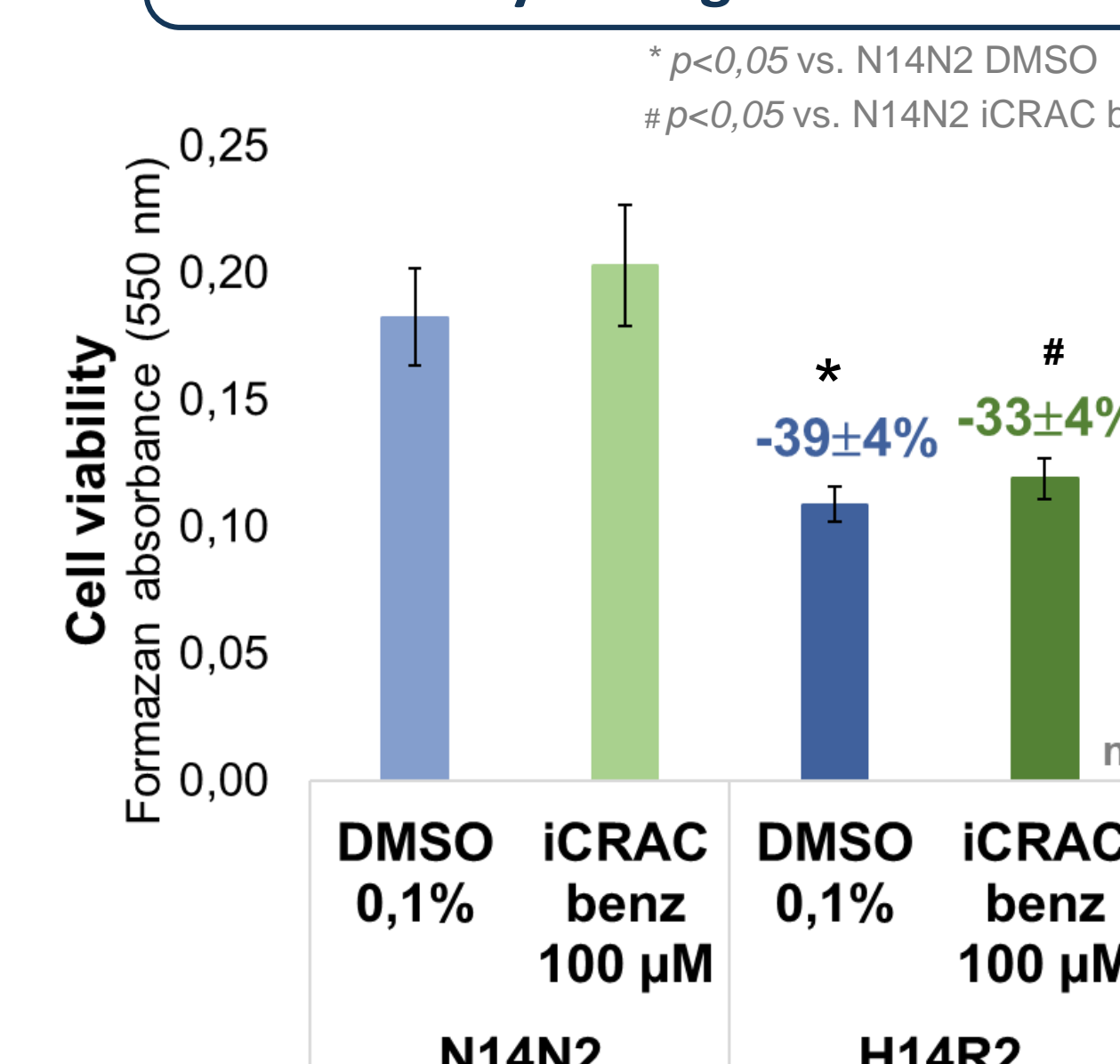
B. Effect of 10 μM 4'-Chlorodiazepam on cell mortality during HR



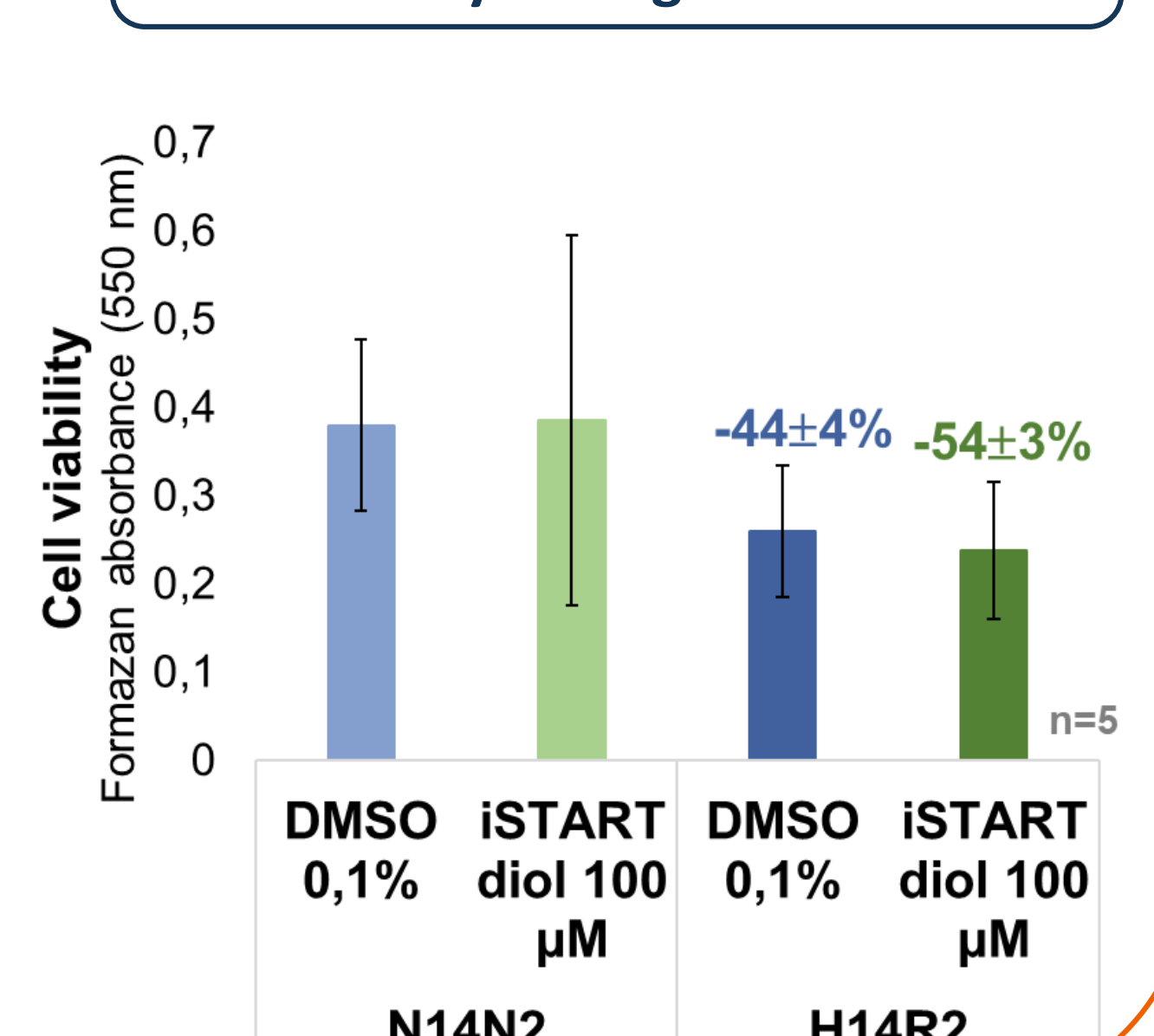
C. Effect of 100 μM iCRAC-triol on cell mortality during HR



D. Effect of 100 μM iCRAC-benz on cell mortality during HR



E. Effect of 10 μM iSTART-diol on cell mortality during HR



CONCLUSION

This preliminary study suggests that TSPO ligands do not exert cardioprotection through a direct action on cardiomyocytes. This conclusion needs to be confirmed with the use of adult primary cardiomyocytes.