IP3R2 Orchestrates Calcium-Independent ER-Mitochondrial Signalling to Protect Proximal Tubular Cells from Anoxia–Reoxygenation

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COI Disclosure

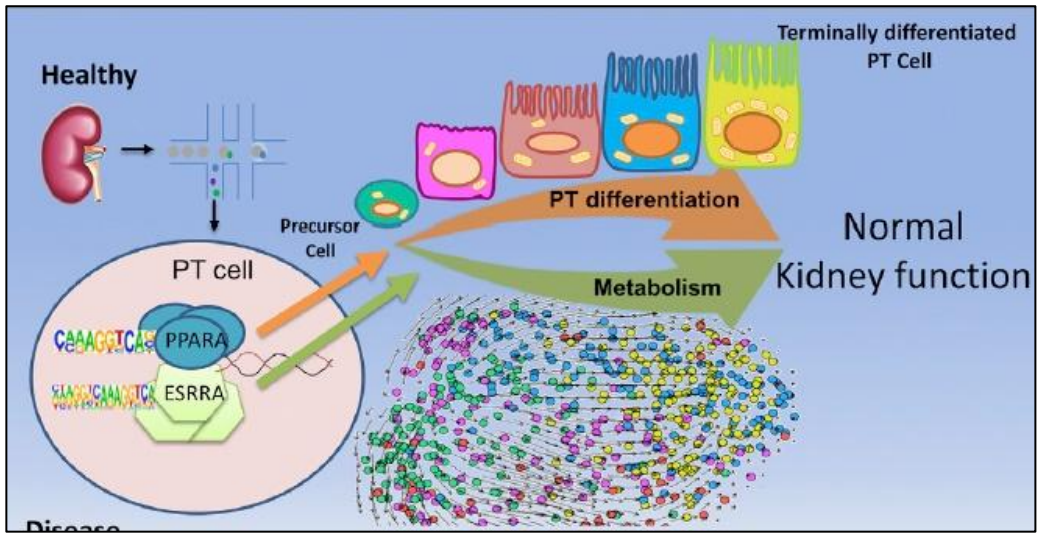
The presenter's department has received research funding from Kyowa Kirin within the past 36 months.
This funding is not directly related to the content of the present study.

Research funding

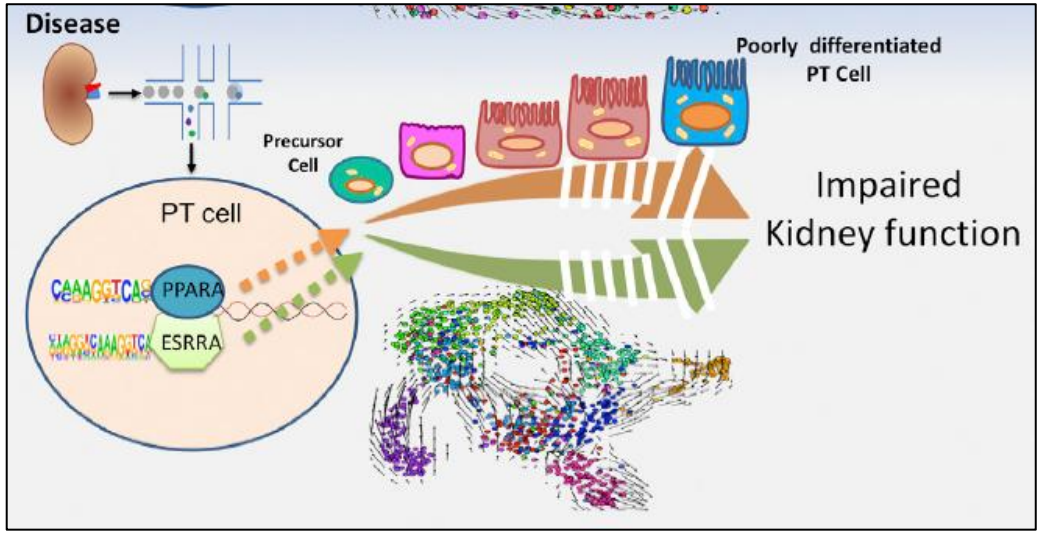
AMED, Kakenhi, Kyowa Kirin

PT cells are highly vulnerable to hypoxic injury, a major driver of AKI progression

Normal kidney function



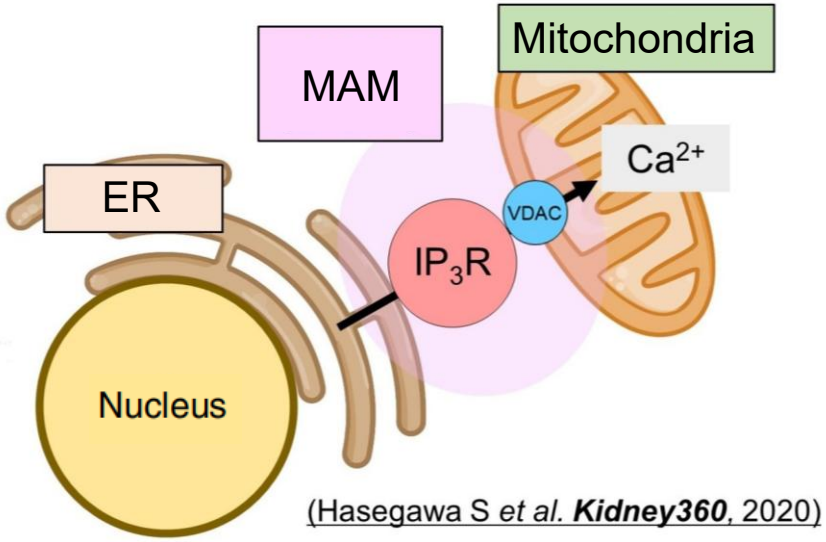
Impaired kidney function



(Susztak, *et al.* ***Cell Metab, 2021***)

Mitochondrial dysfunction in proximal tubules is both a cause and a consequence of renal hypoxia/ ischemia. However, the upstream regulators that govern mitochondrial function during injury and repair remain unclear.

IP3R isoforms and their functions at ER–mitochondria contact sites (MAMs)



(Hasegawa S *et al.* ***Kidney360***, 2020)

IP3R (ITPR)

- Type 1 IP3R (IP3R1; gene: *ITPR1*)
- Type 2 IP3R (IP3R2; gene: *ITPR2*)
- Type 3 IP3R (IP3R3; gene: *ITPR3*)

IP3R	Disease	Result
IP3R1	type-2 diabetes	IP3R1 knockout may represent a target for therapies that aim to reverse nonalcoholic fatty liver disease and type-2 diabetes *Nature, 2020*
IP3R2	aging	ablation of *ITPR2* decreases the number of MAMs and protects the cells against premature senescence *Nat.Communication, 2021*
IP3R3	clear cell renal carcinoma	In tumor cells, IP3R3 has proliferative and anti-apoptotic effect, on contrary to the pro-apoptotic effect of IP3R1 *Cell death and disease, 2019*

➢ **Do IP3Rs protect renal cells from hypoxic stress?**

Methods: In vitro experiment

An in vitro mimic of “in vivo Ischemia-Reperfusion Injury (IRI)”:
24h anoxia followed by 4h reperfusion (AR)

Day1 — HK-2 Cell passage (6-well plate: $15*10^4$cells/well)

Day2 — Transfection: siCtrl / si*ITPR1/2/3*

Day3 (24h) — Anoxia bag 24h:

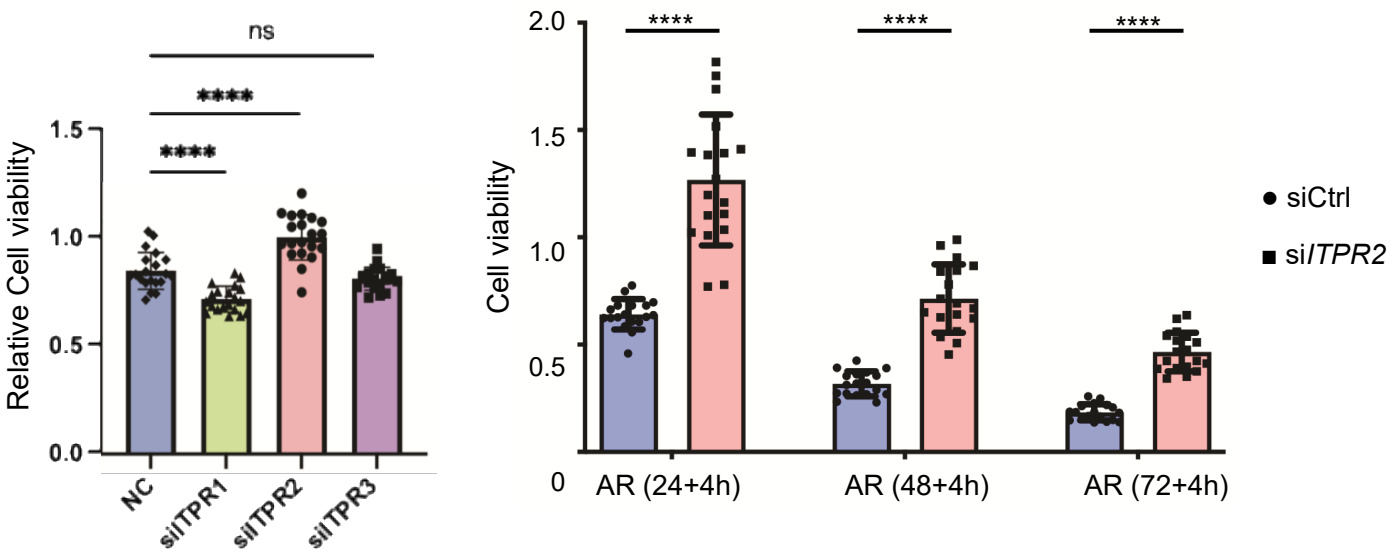
Day (24h) — Reperfusion 4h: culture medium with 10% FBS

Ctrl group: culture medium with 10% FBS

Anoxia group: culture medium without FBS

Result 1:Knocking down ITPR2 increased PT cell viability after AR injury

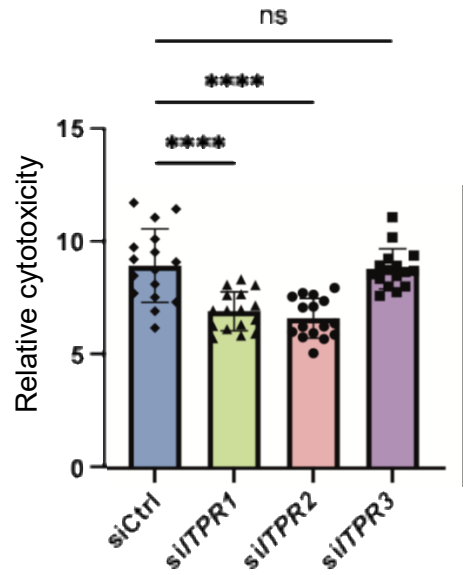
Cell viability: Cell counting kit-8 (CCK-8)



Viability (relative to its own normoxia control)

$$\text{Viability}_X = \frac{OD_{X,\ AR}}{OD_{X,\ Normoxia}}$$

Cytotoxicity: LDH assay



For condition X on the **AR plate**:

$$f_{X,\ AR} = \frac{OD_{X,\ AR}^{\ sample}}{OD_{AR}^{\ HighCtrl}}$$

For the corresponding **Normoxia (control) plate:**

$$f_{X,\ Normoxia} = \frac{OD_{X,\ Normoxia}^{\ sample}}{OD_{Norm}^{\ HighCtrl}}$$

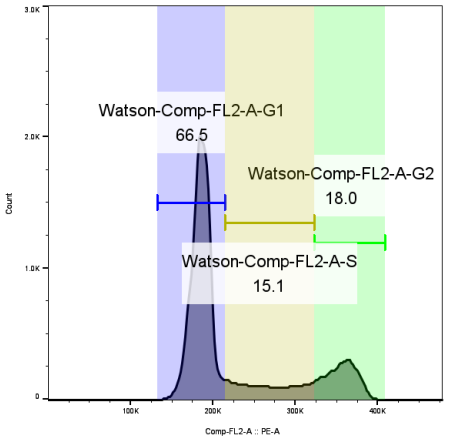
Relative cytotoxicity (AR vs Normoxia):

$$\text{Relative Cytotoxicity}_X = \frac{f_{X,\ AR}}{f_{X,\ Norm}}$$

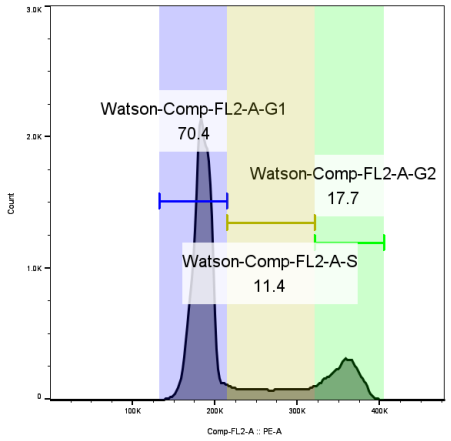
Result 2: Knocking down ITPR2 decreased the proportion of PT cells arrested in G1 phase after AR injury

Cell cycle: Cycletest Plus DNA Reagent Kit

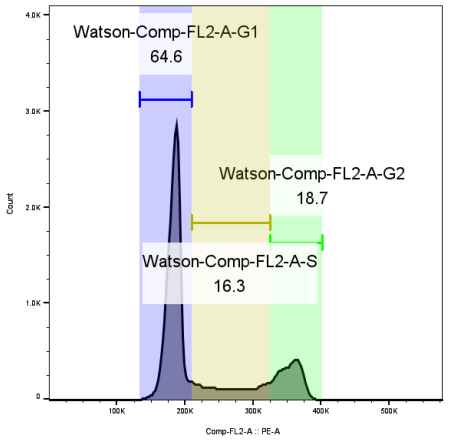
Ctrl (siCtrl)



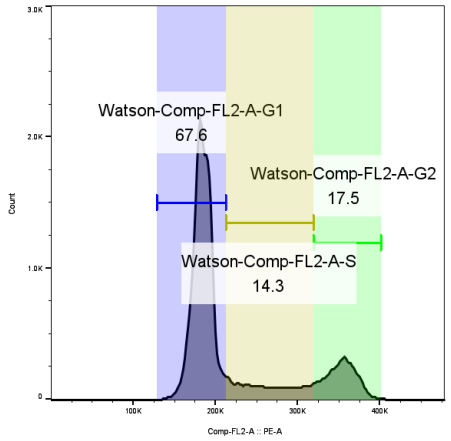
Ctrl (si*ITPR1*)



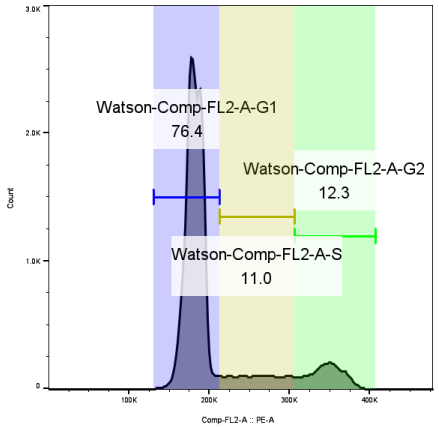
Ctrl (si*ITPR2*)



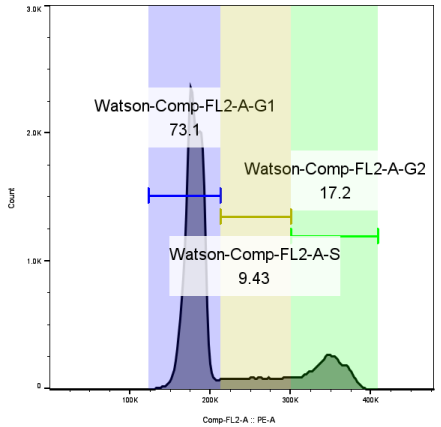
Ctrl (si*ITPR3*)



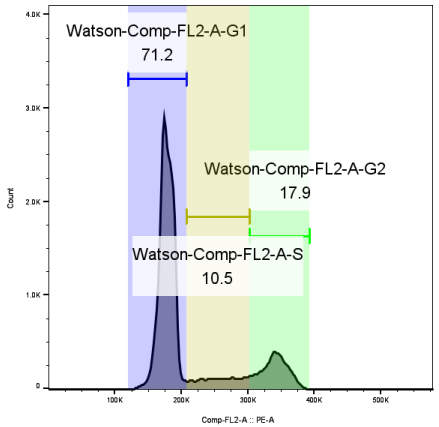
AR (siCtrl)



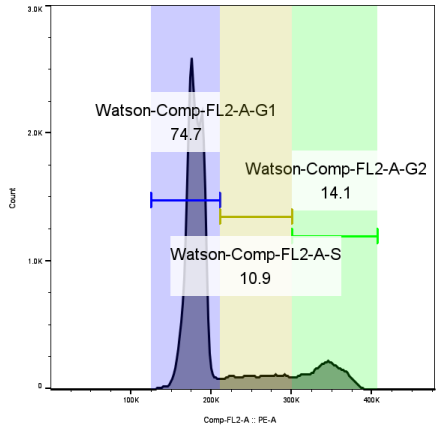
AR (si*ITPR1*)

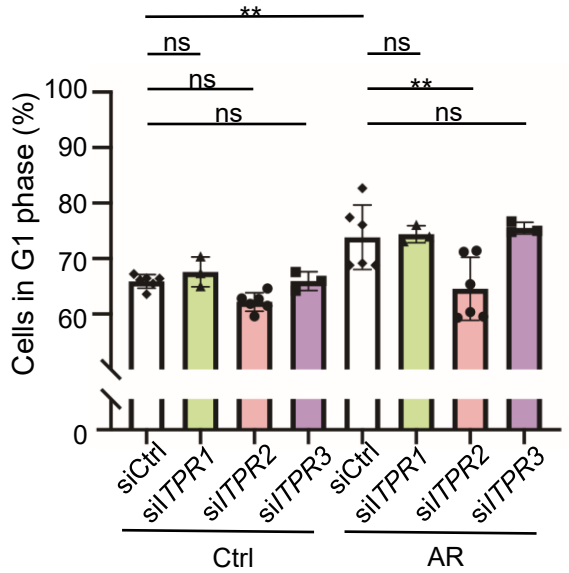


AR (si*ITPR2*)

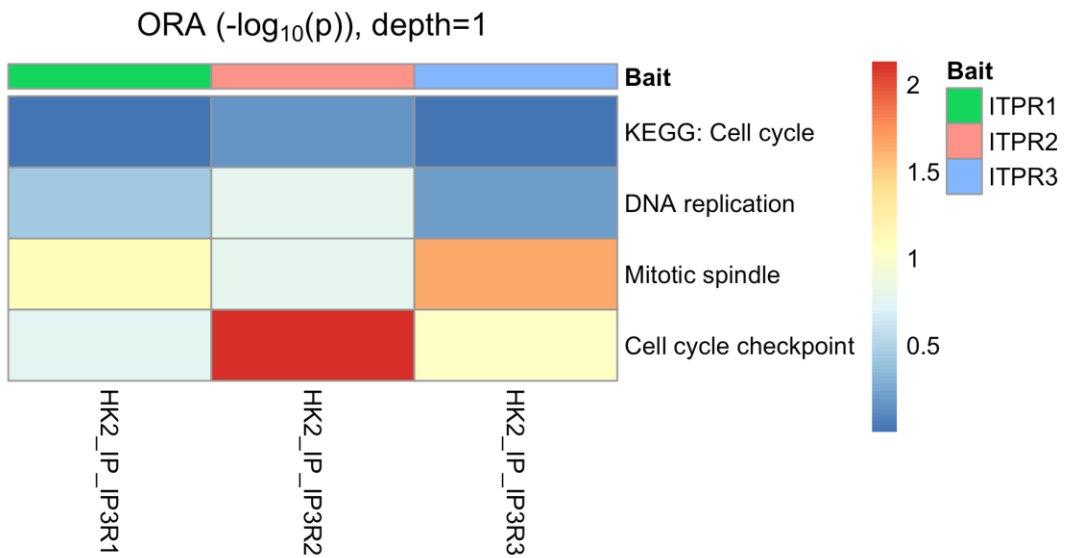


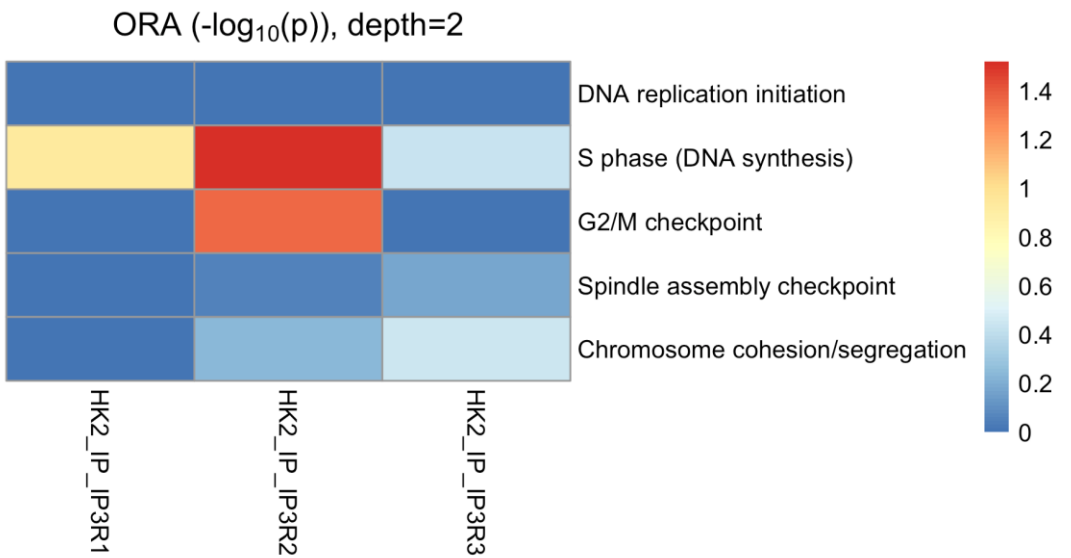
AR (si*ITPR3*)



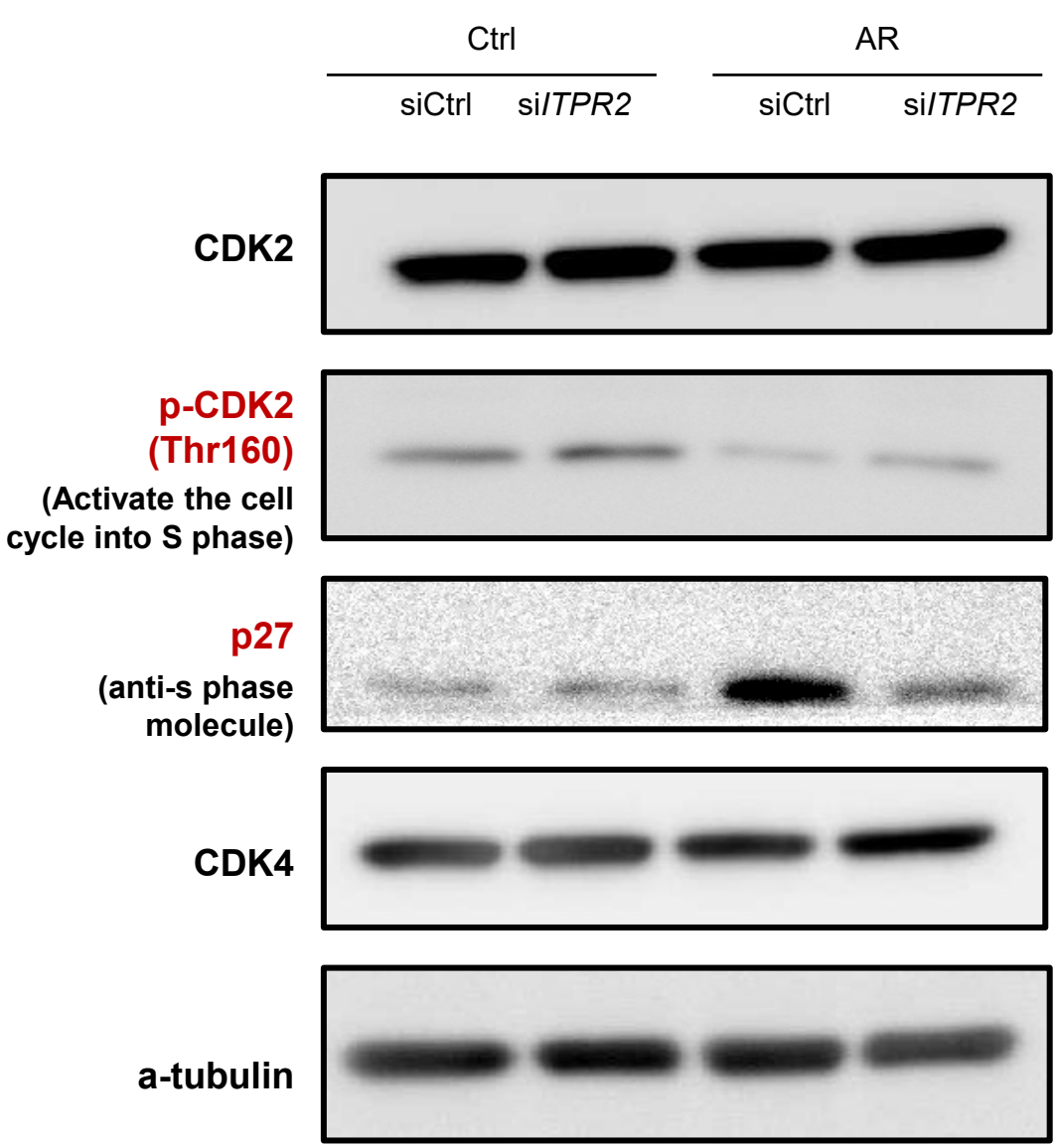


Result 3: Co-IP proteomes revealed IP3R2 interaction with cell-cycle checkpoint proteins

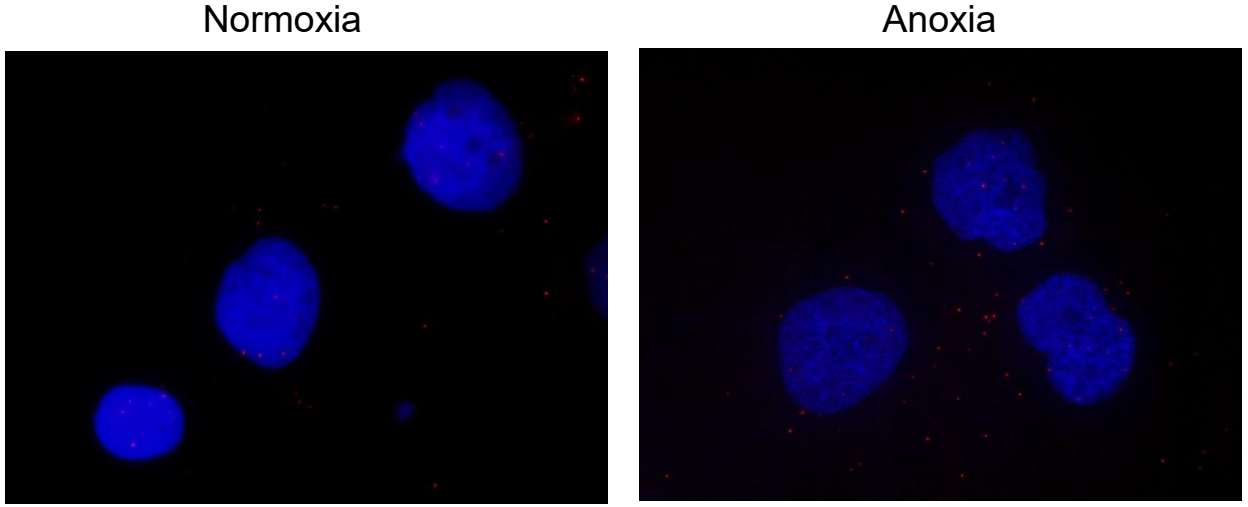


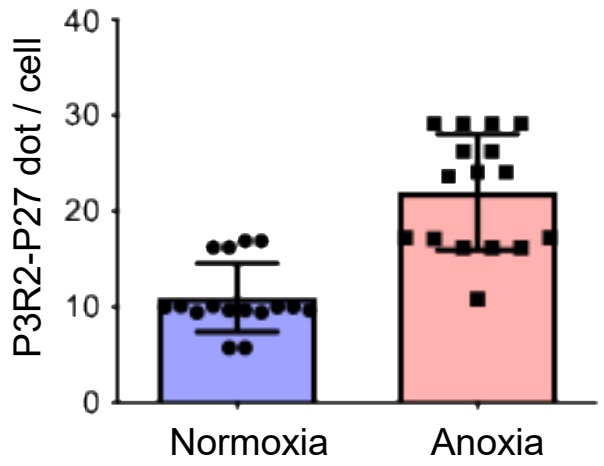


Result 4: IP3R2 knockdown alleviates AR-induced G1 arrest by preserving CDK2 activity



PLA assay (IP3R2-P27: red, nucleus: blue)

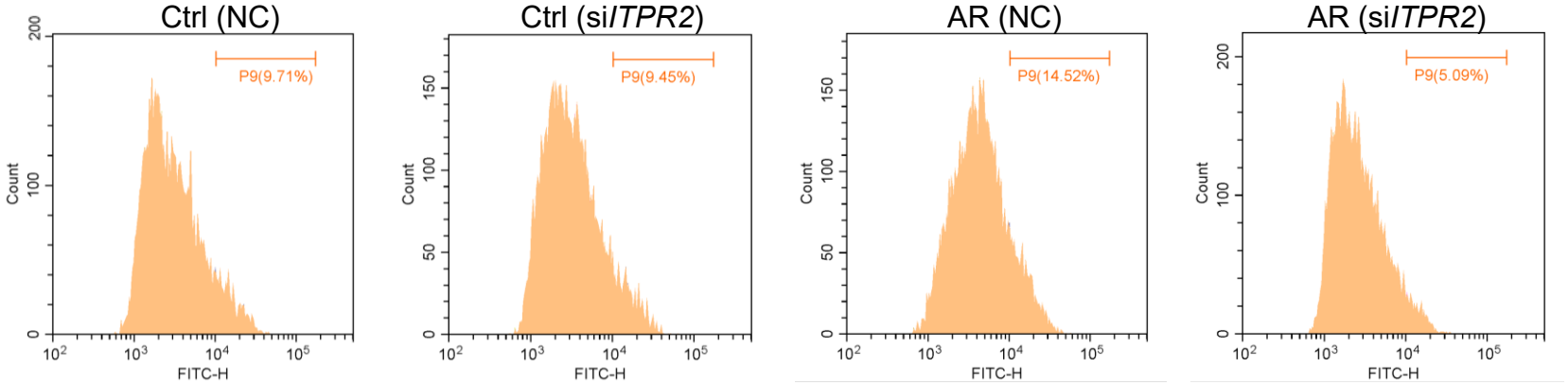


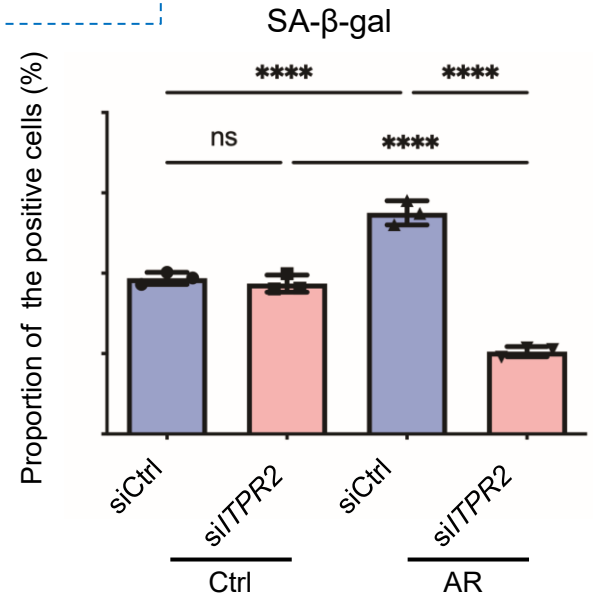


Result 5: ITPR2 knockdown alleviated AR-induced cellular senescence

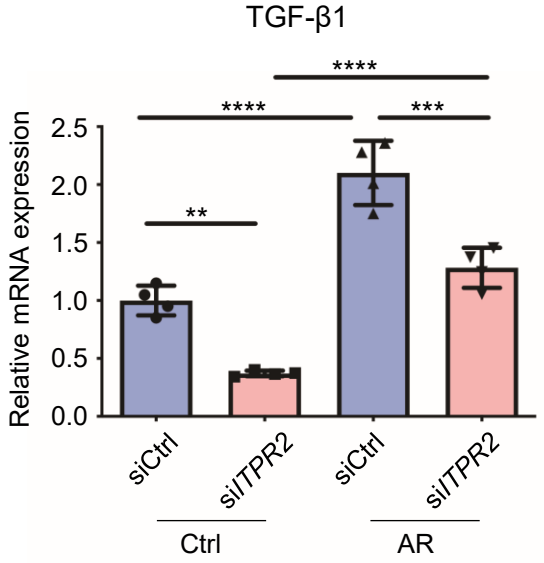
CellEvent™ Senescence Green Flow Cytometry Assay Kit RPTEC cell

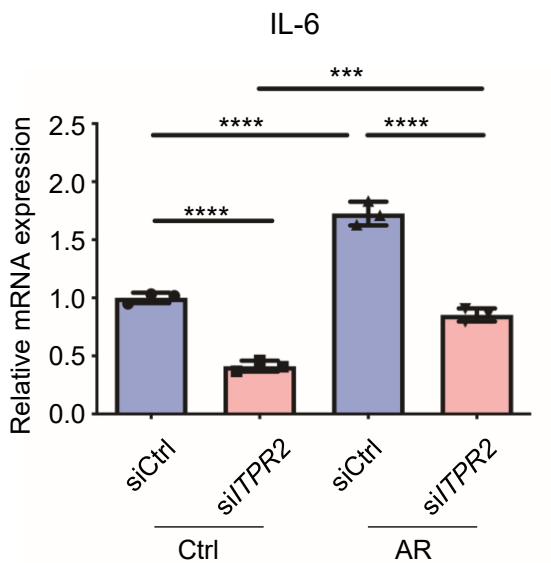
Senescence-Associated β-galactosidase (SA-β-gal) : a well-established biomarker for senescent cells



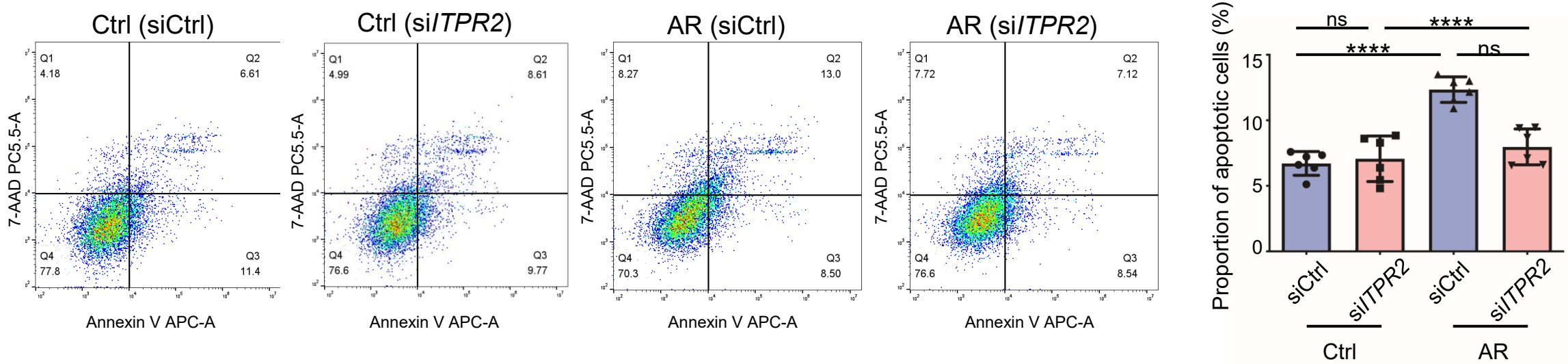


qPCR: Senescence-associated secretory phenotype (SASP)





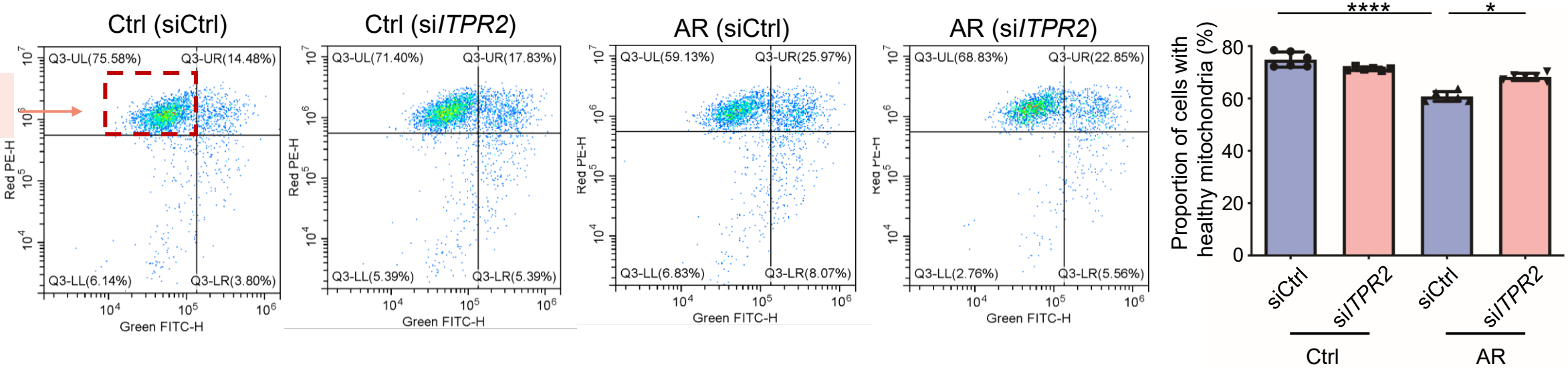
Result 6: ITPR2 knockdown ameliorated apoptosis after AR injury



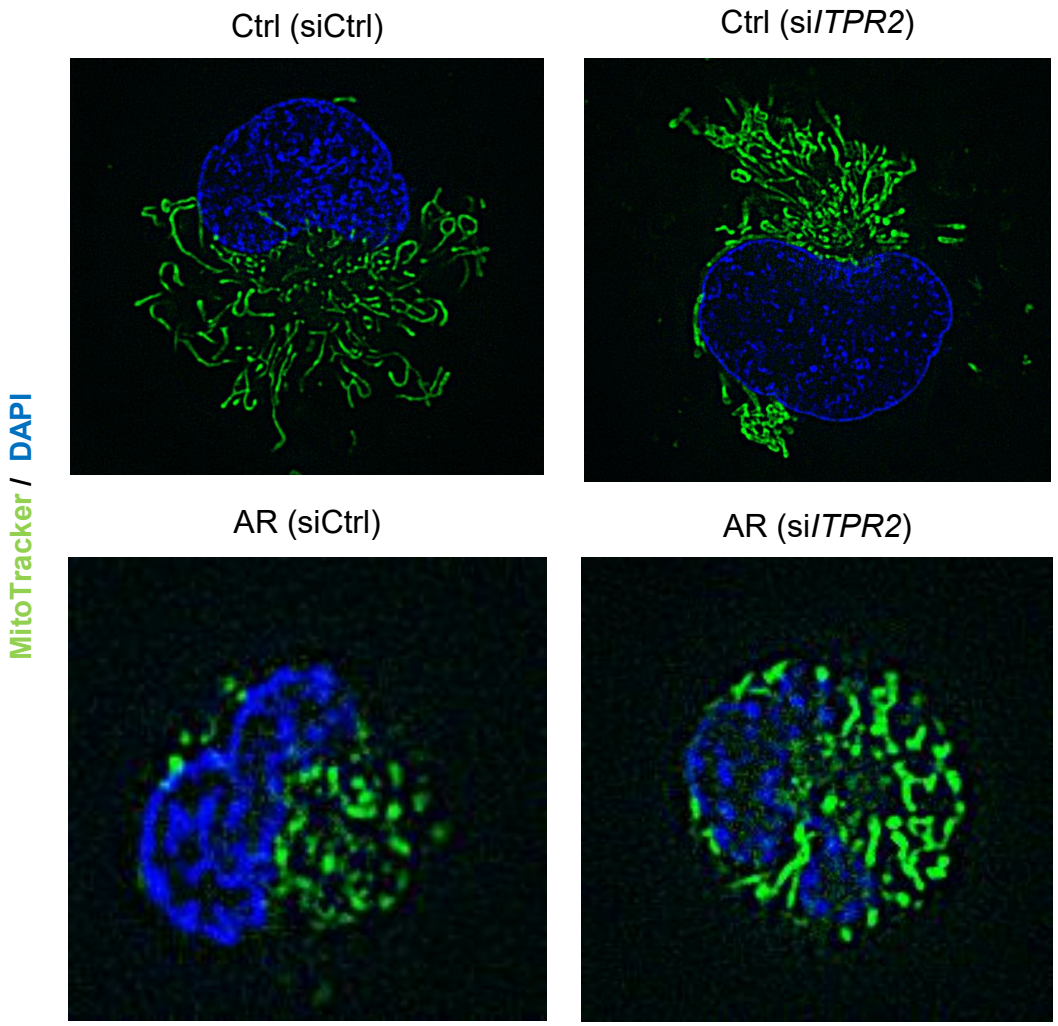
JC-1 staining: the early stage of apoptosis (mitochondrial depolarization)

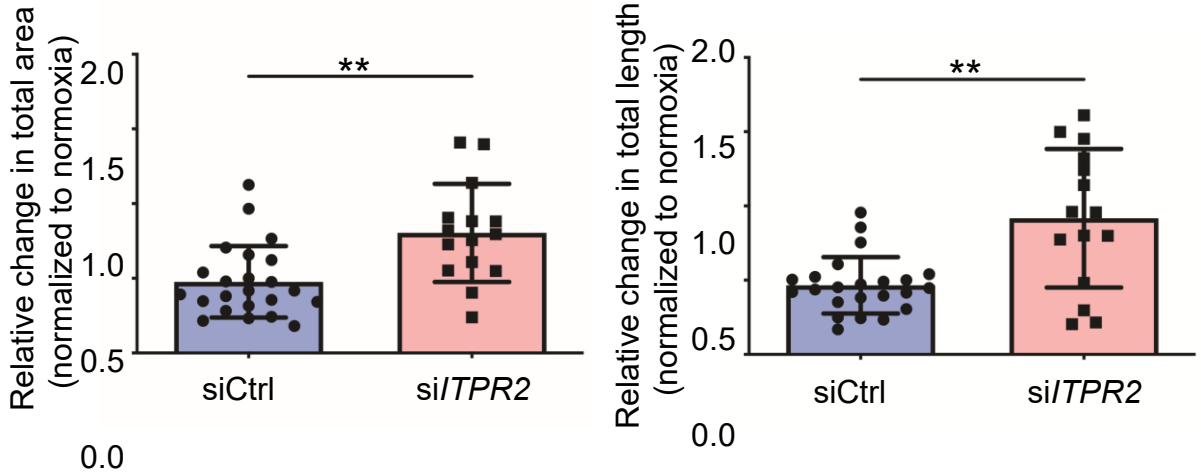
- Indicated by a decrease in the red fluorescence and increase in the green fluorescence

Healthy cells



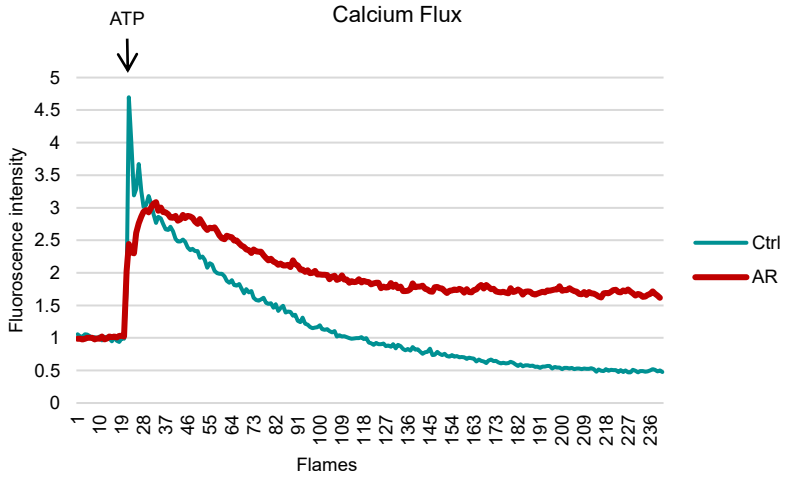
Result 7: ITPR2 knockdown preserved mitochondrial morphology after AR injury

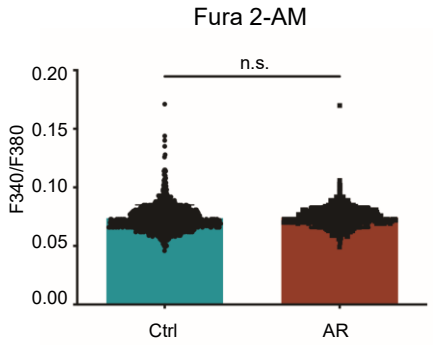


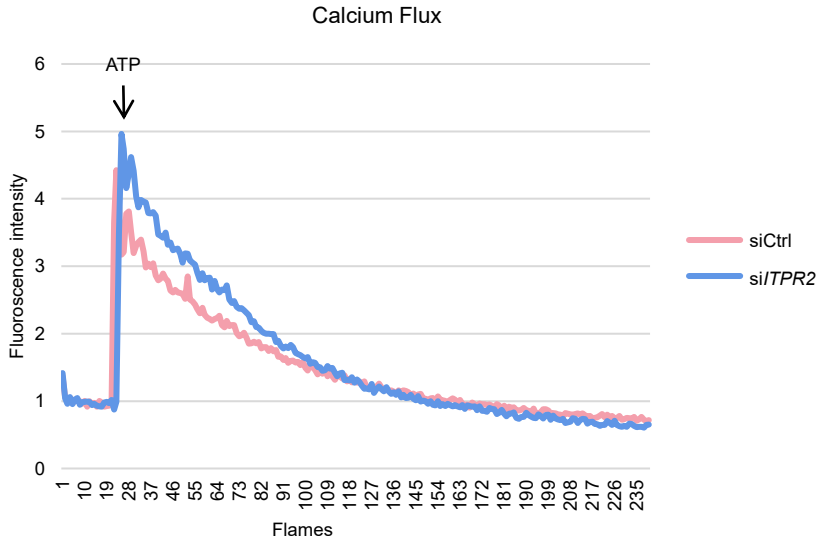


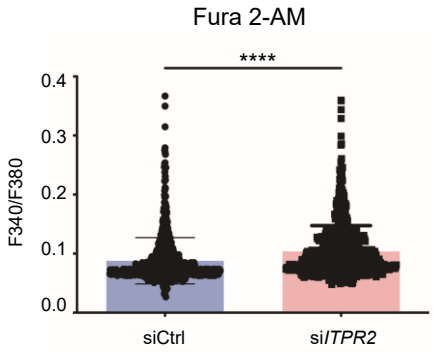
Result 8: Protection by IP3R2 knockdown is calcium-independent and unrelated to MAM number

Calcium flux

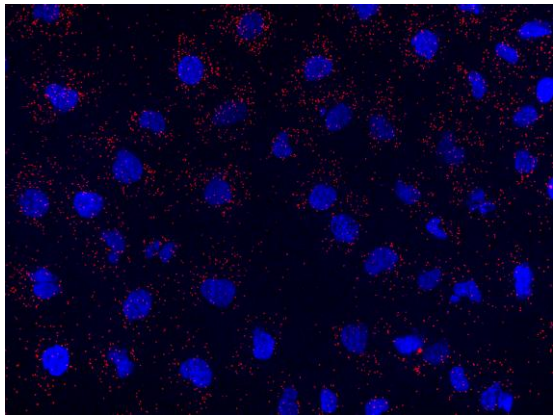


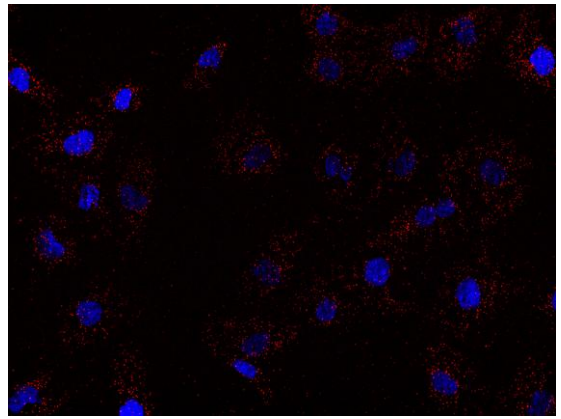


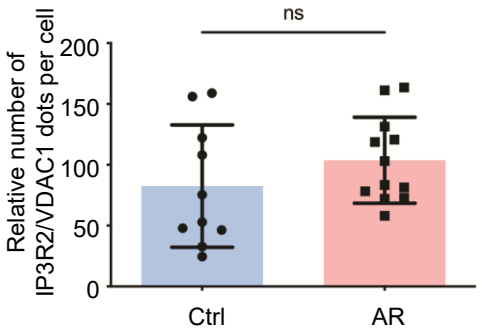




PLA assay (IP3R2-VDAC1:red, nucleus: blue)







Discussion

- **Novel Role of IP3R2:** Our data support a calcium-flux–independent role for IP3R2 in promoting PT cell survival during AR injury..
- **Beyond Calcium Signaling:** Protection occurs without detectable changes in ATP-evoked or basal cytosolic Ca^{2+}, in mitochondrial Ca^{2+}, or MAM number.
- **Cell Survival Mechanisms:** IP3R2 knockdown alleviates AR-induced G1 arrest and **preserves CDK2 activity**, consistent with improved proliferative capacity.
- **Mitochondrial Protection:** IP3R2 knockdown reduces apoptosis and stabilizes mitochondrial polarization and network integrity under stress.
- **Future Directions:** Further in vivo studies are required to validate these findings.

Conclusion

- IP3R2 activity under AR appears largely independent of bulk Ca^{2+} flux and contact abundance.
- Silencing IP3R2 preserves the G1/S transition, improves mitochondrial function, and lowers apoptosis/senescence in PT cells after AR—implicating IP3R2 and its MAM context as regulators of cell fate and mitochondrial homeostasis during AR.