

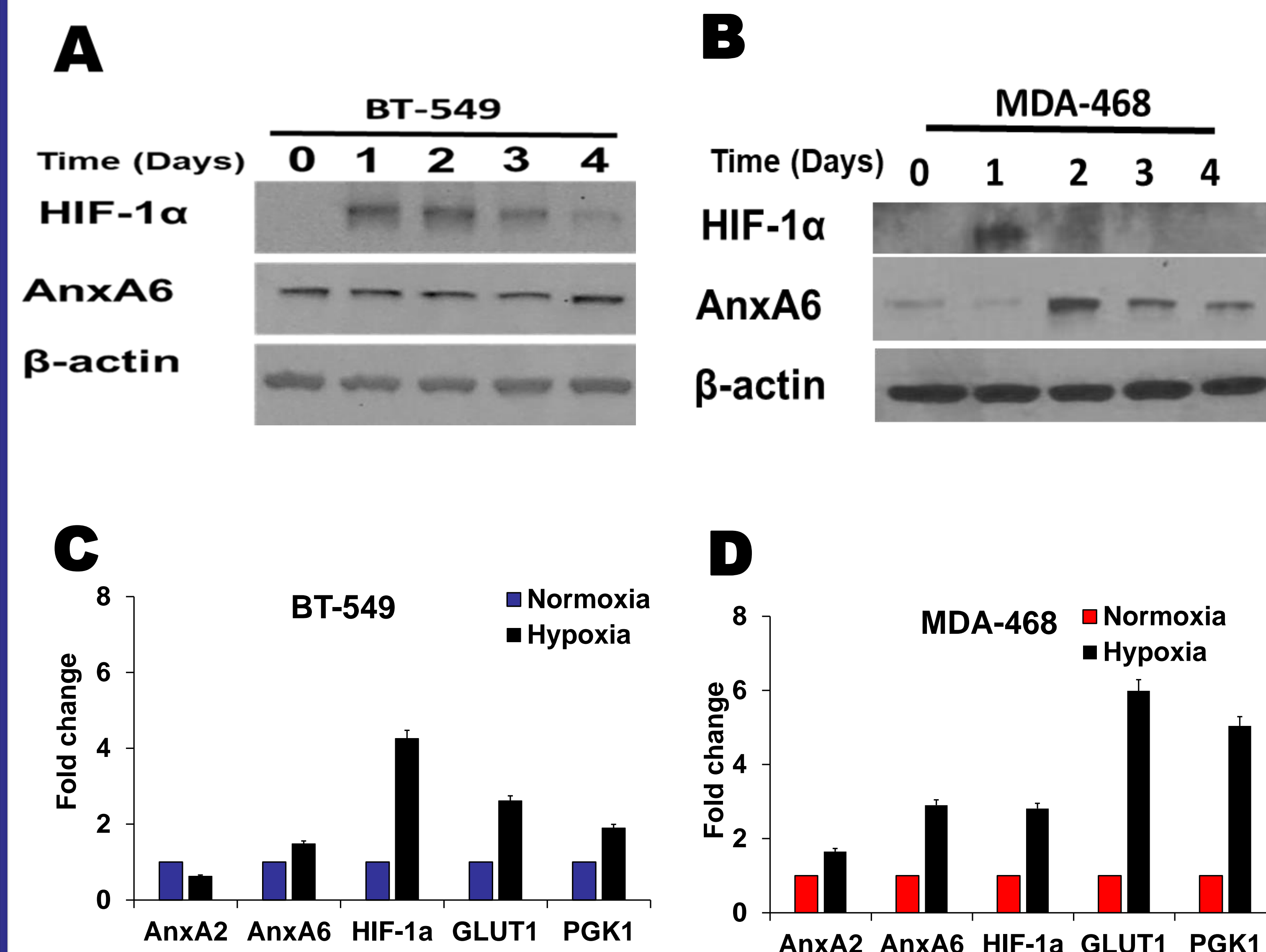
## Summary

Metabolic reprogramming is an emerging hallmark of cancer cells, in which cells exhibit metabolic phenotypes to fuel their proliferation and progression. The significant advancements made in the area of metabolic reprogramming make possible new strategies for overcoming malignant cancer, including triple-negative breast cancer. Triple-negative breast cancer (TNBC), is associated with high histologic grade, aggressive phenotype, and poor prognosis. While TNBC responds well to initial chemotherapy, most patients suffer from dismal clinical outcomes including quick relapse and metastatic spread. Although no effective therapies currently exist against metastatic TNBC, the epidermal growth factor receptor (EGFR) is commonly overexpressed, making it a potential therapeutic target. However, TNBC clinical trial outcomes for EGFR-targeted tyrosine kinase inhibitors (TKIs) have proven disappointing, and our *in vitro* studies reveal that while TNBC cell lines have initial sensitivity to TKIs, resistance rapidly develops accompanied by the up-regulation of the Ca<sup>2+</sup>-dependent membrane binding protein, Annexin A6 (AnxA6). AnxA6 is a multifunctional protein that plays relevant roles tumor cell motility, invasive and proliferative properties in TNBC. Preliminary studies reveal that exposure of TNBC cells to acute hypoxia (1% O<sub>2</sub>; ≤24 h) is associated with down-regulation of AnxA6 while chronic hypoxia (1% O<sub>2</sub>; >24 h) leads to a persistent up-regulation of AnxA6, hypoxia-inducible factors 1/2 alpha, and other downstream hypoxia response genes. In the presence of hypoxia, TNBC cells were less sensitized to EGFR-TKIs. Down-regulation of AnxA6 in TNBC cells is associated with reduced number and size of lipid droplets, while treatment of AnxA6 depleted cells with EGFR-TKIs reversed the utilization of lipids. Together, these data suggest that the physiological stress of hypoxia may mediate AnxA6 expression status and consequently, enhanced cell renewal, survival, and TKI resistance in TNBC tumors. We hypothesize that hypoxia-mediated differential expression of AnxA6 in TNBC cells is associated with differential metabolic reprogramming that promotes distinct patterns of resistance of TNBC cells to TKIs.

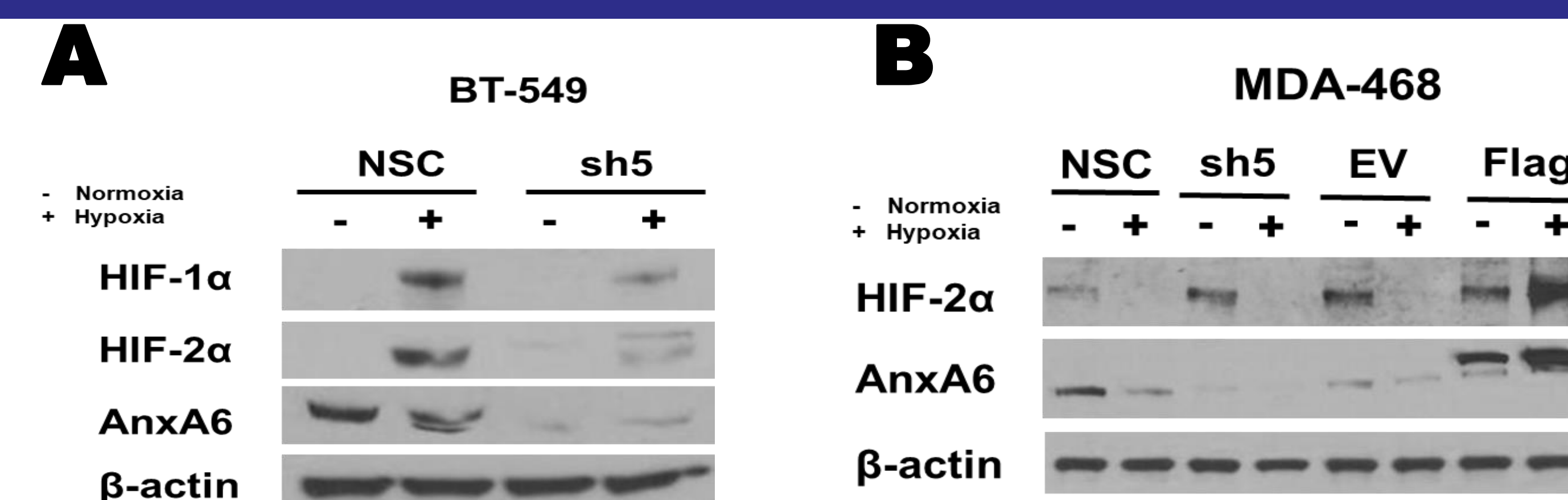
## Methods

To test this hypothesis, TNBC cell lines were incubated in normoxic (37° C, 20% O<sub>2</sub>, 5% CO<sub>2</sub>) and hypoxic (37° C, 1% O<sub>2</sub>, 5% CO<sub>2</sub>, 94% N<sub>2</sub>) conditions to assess protein and mRNA expressions, as well as cell viability. Metabolic studies were conducted using commercial kits from ABCAM and Sigma Aldrich. Lipid droplet analysis was conducted using Oil Red staining. A Seahorse XF Analyzer was used to measure the basal oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of TNBC cell lines varying AnxA6 expression.

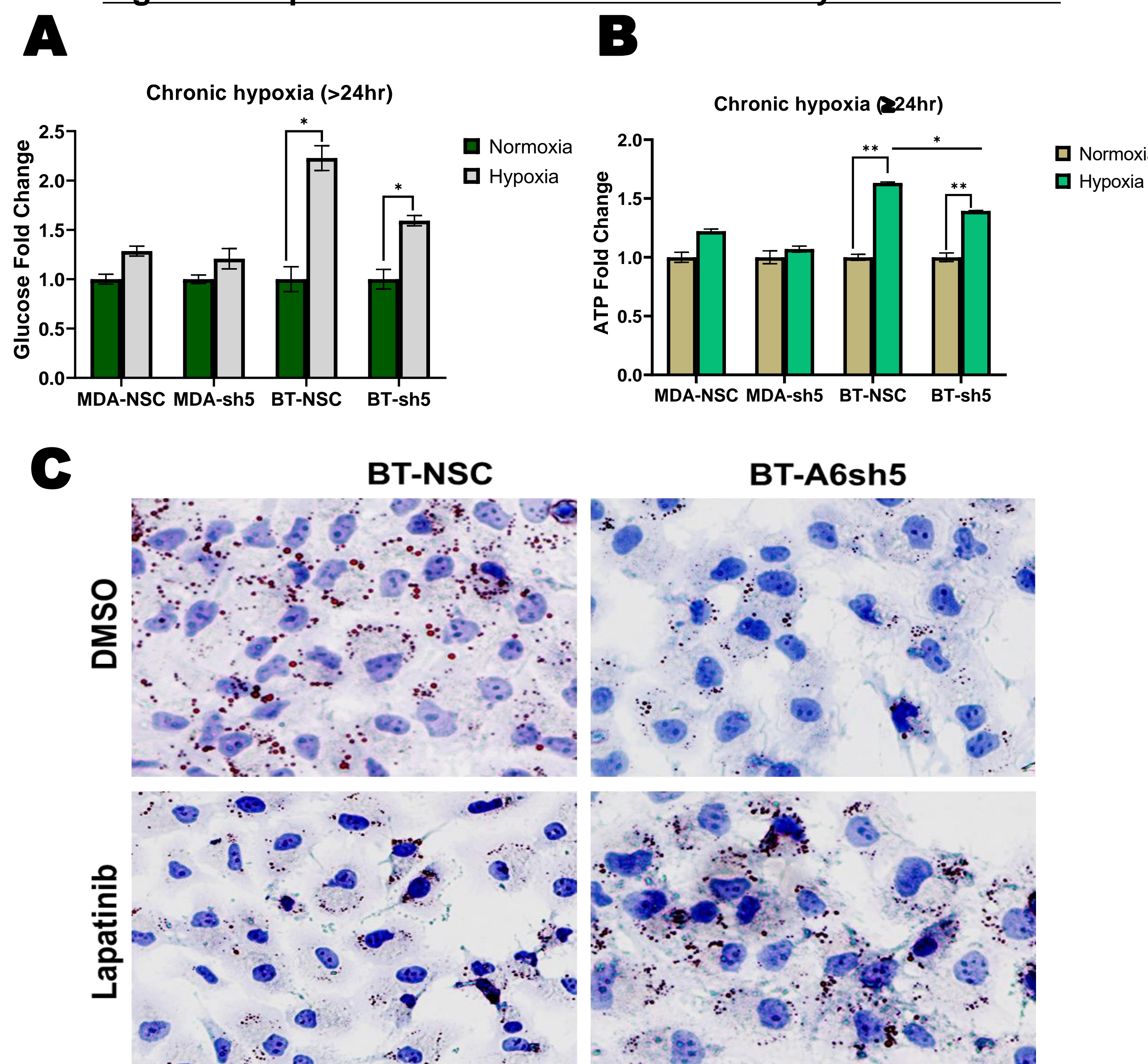
## Results



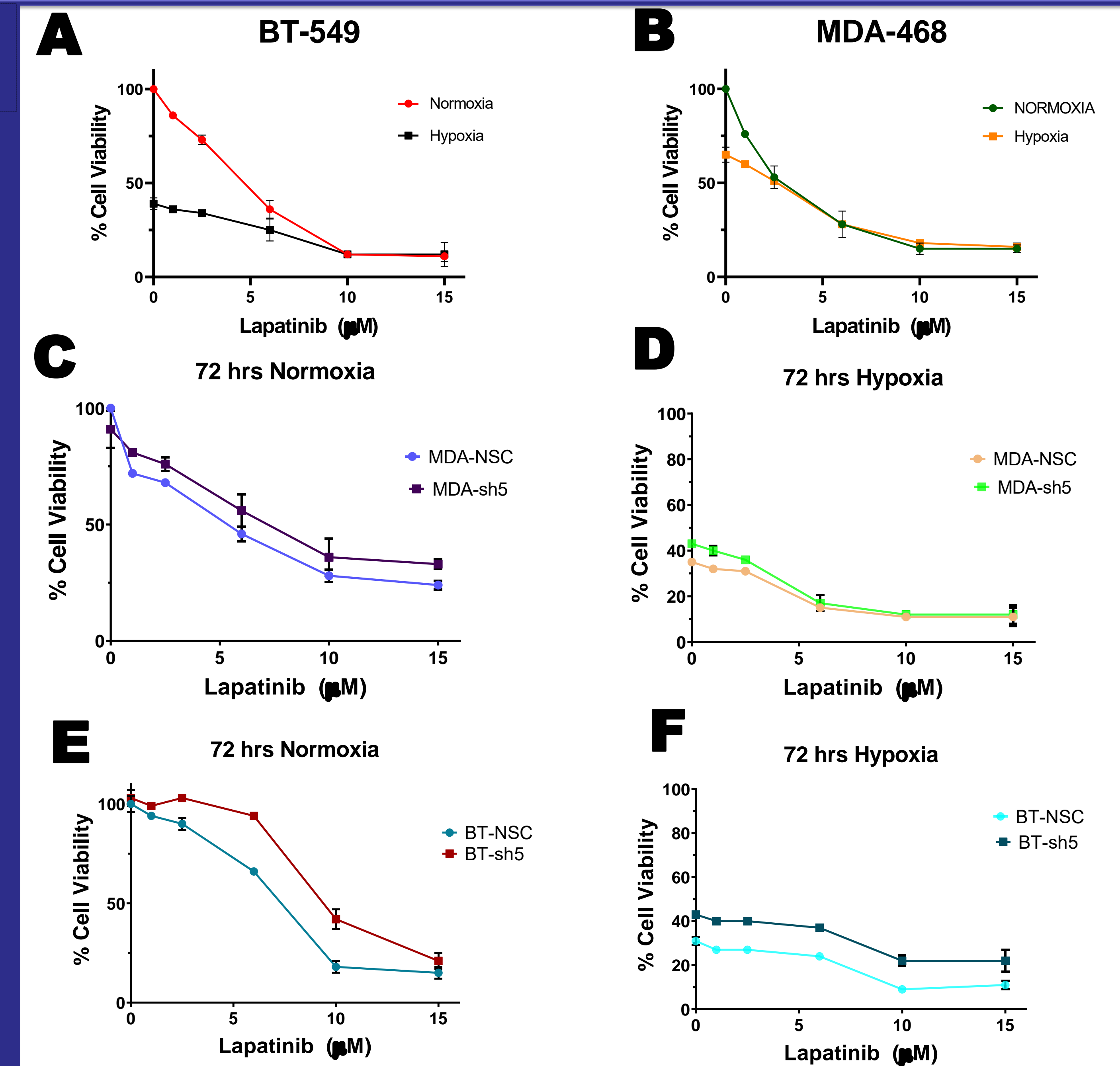
**Figure 1. TNBC cell responses to acute and chronic hypoxia.**



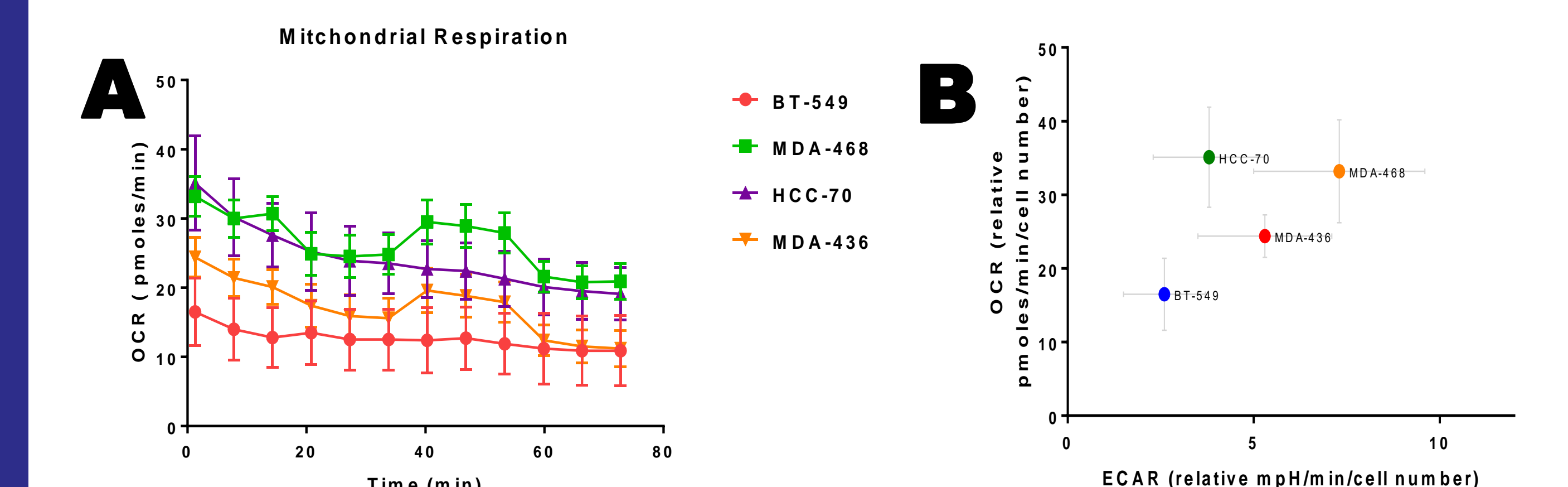
**Figure 2. Depletion of AnxA6 alters HIFs stability in TNBC cells.**



**Figure 3. AnxA6 expression underlies responses to metabolic vulnerabilities.**



**Figure 4. Chronic hypoxia sensitizes TNBC cells to Lapatinib.**



**Figure 5. TNBC basal metabolic profiles.**

## Conclusions

- Chronic hypoxia differentially influences AnxA6 protein stability in TNBC cells.
- Depletion of AnxA6 in TNBC cells alters the stability of HIFs.
- AnxA6 expression underlies response to metabolic vulnerabilities
- AnxA6 depletion in TNBC cells is associated with decreased lipid droplets abundance and Lapatinib treatment reverses lipid utilization
- AnxA6-depleted TNBC cells are sensitized to Lapatinib under chronic hypoxia.
- Relative basal OCR and ECAR data highlight potential AnxA6-dependence
- Overall, AnxA6 expression status affects the metabolic vulnerabilities of TNBC cells by influencing extracellular acidification rates and oxygen consumption rates via utilization of lipids and/or glycolysis.

## Acknowledgements

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