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Italiadomani
PIANO NAZIONALE
DI RIPRESA E RESILIENZA

Inibitori di ERO1 a piccola molecola per il trattamento del tumore al seno

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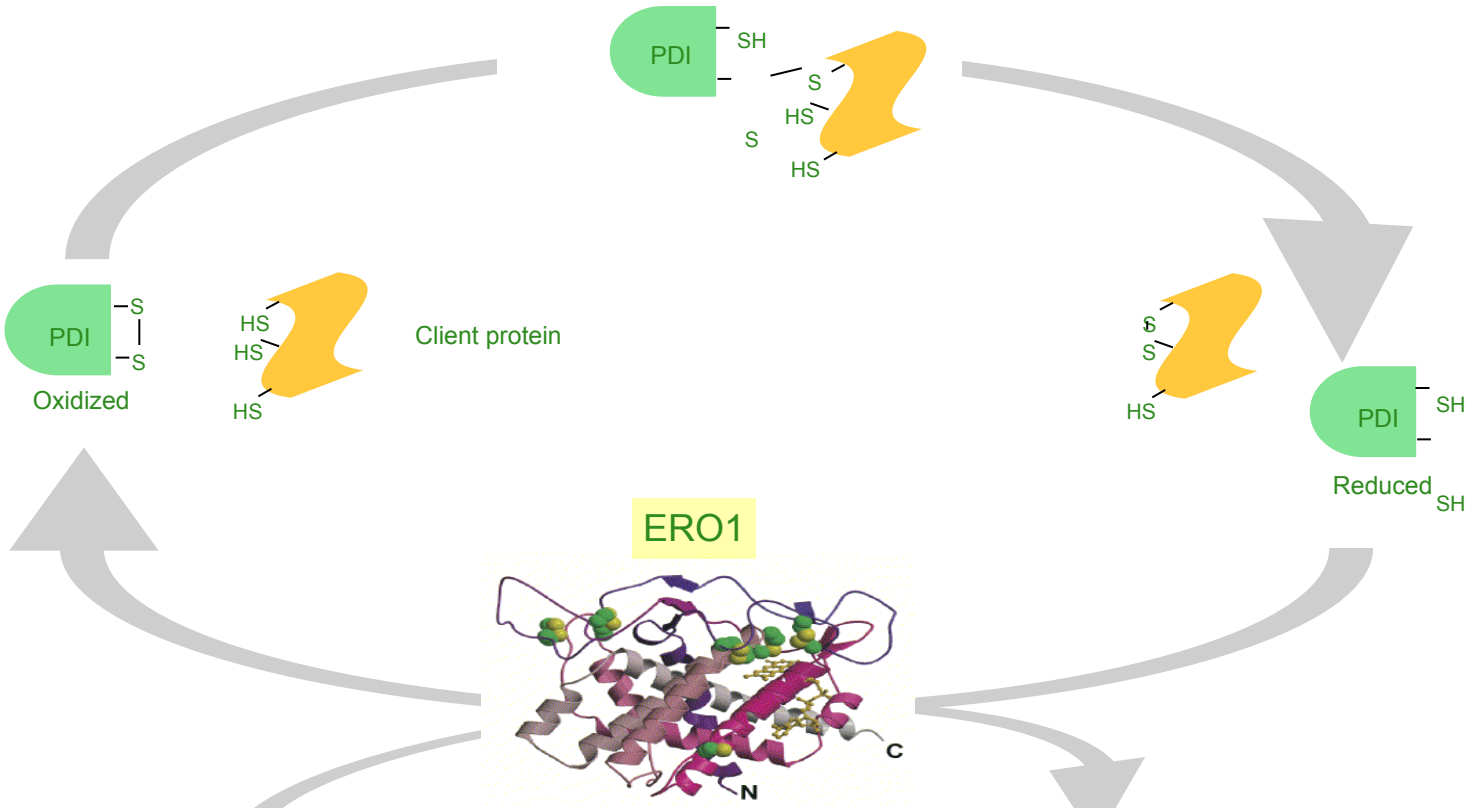
Overview:

- ERO1: an oxidoreductase localized in the Endoplasmic Reticulum (ER)
- ERO1 activity is dispensable in healthy cells
- ERO1 plays a role in the Unfolded Protein Response (UPR)
- ERO1 links hypoxia and VEGF signaling in breast cancer
- Genetic inhibition of ERO1 reduces breast cancer growth and metastasis by impairing angiogenesis
- ERO1 inhibitors hold therapeutic potential in cancer due to the selective requirement for ERO1 activity in tumors—but not in healthy cells
- Development of novel pyrazolone-based ERO1 inhibitors

ERO1, an ER oxido-reductase



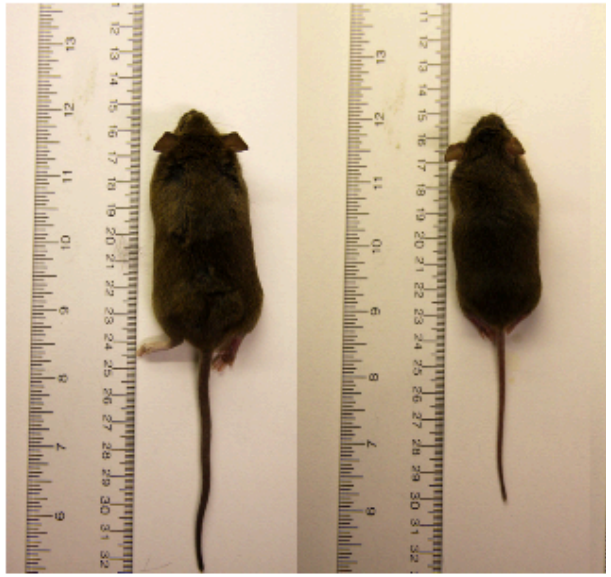
O_2 + 2 electrons -> H_2O_2

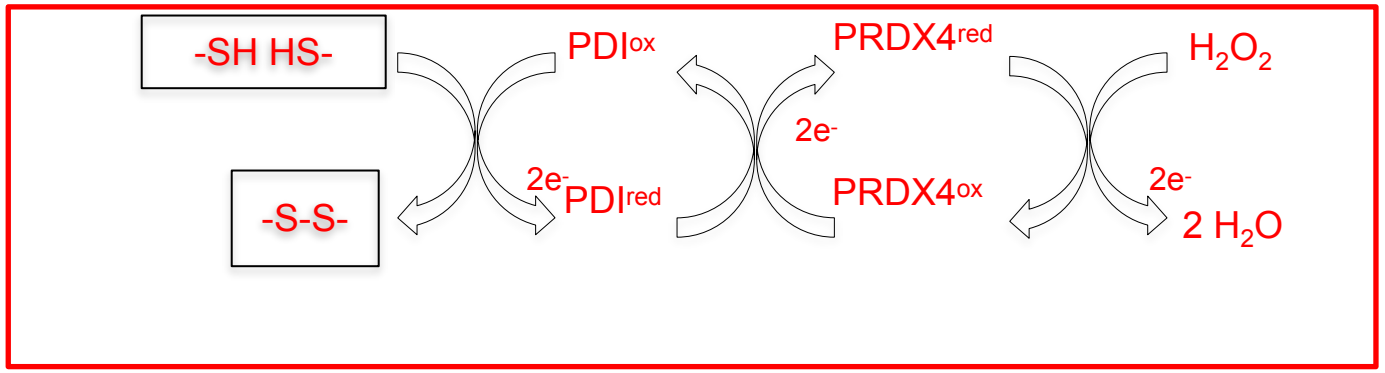
Pollard Mg, *et al Mol Cell* 1998
Frand AR, *et al Mol Cell* 1998
Zito E, *et al Free Radic Biol Med 2015*

Mice lacking ERO1A and ERO1B are viable and fertile

Wild-type

Double mutant
(ERO1A, ERO1B)



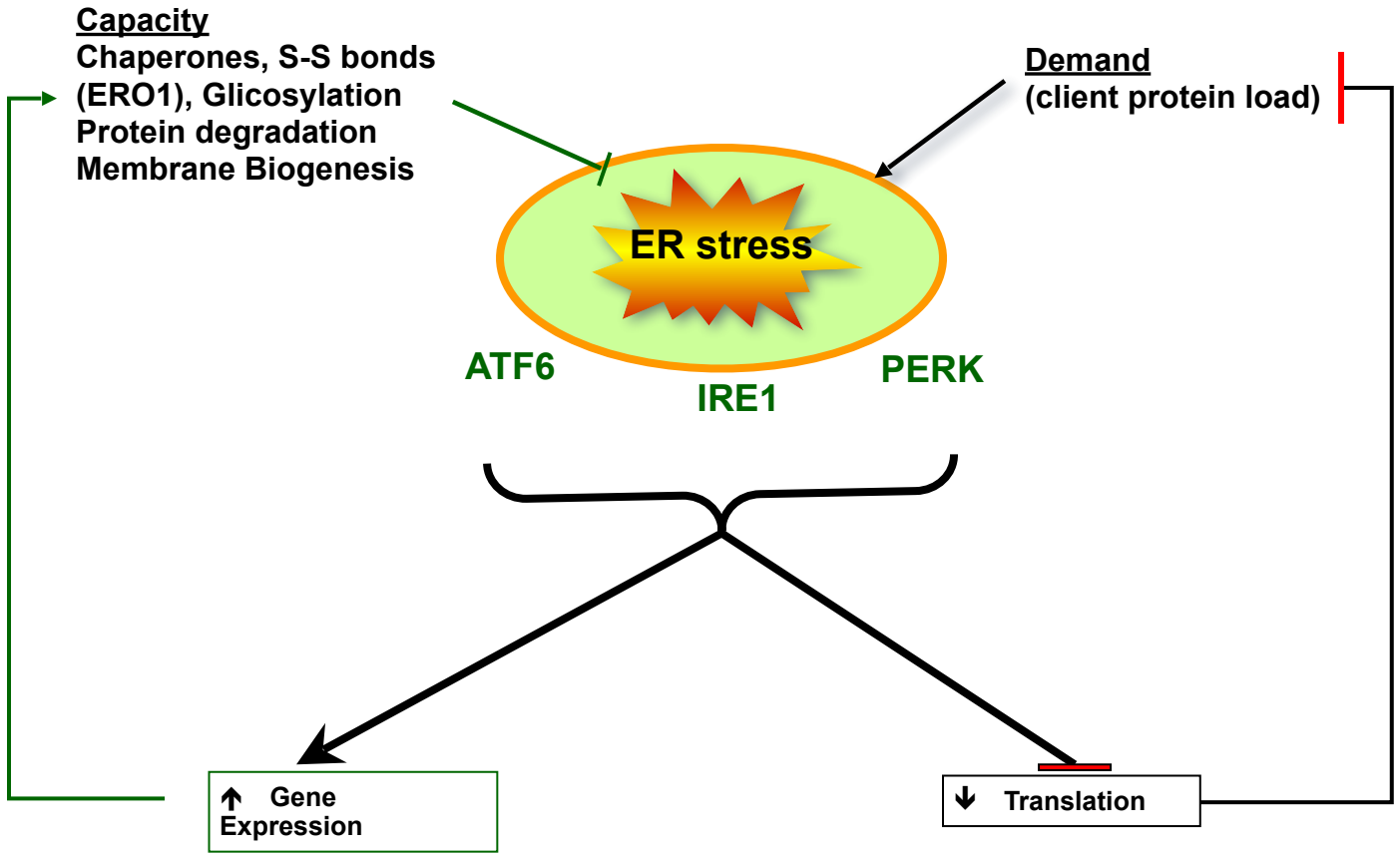


Tavender TJ et al. EMBO J 2010

Zito E et al. *Molecular Cell* 2010

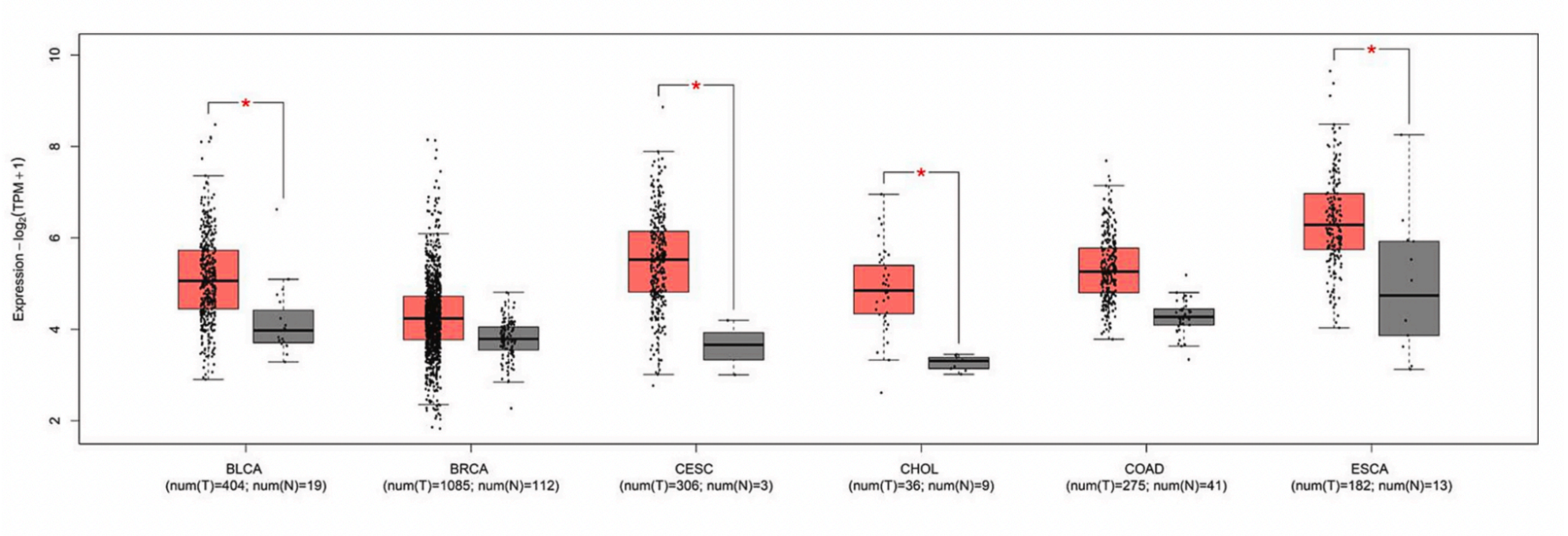
Zito E et al. *Molecular Cell* 2012

ERO1 is part of UPR



Walter P & Ron D *Science* 2011

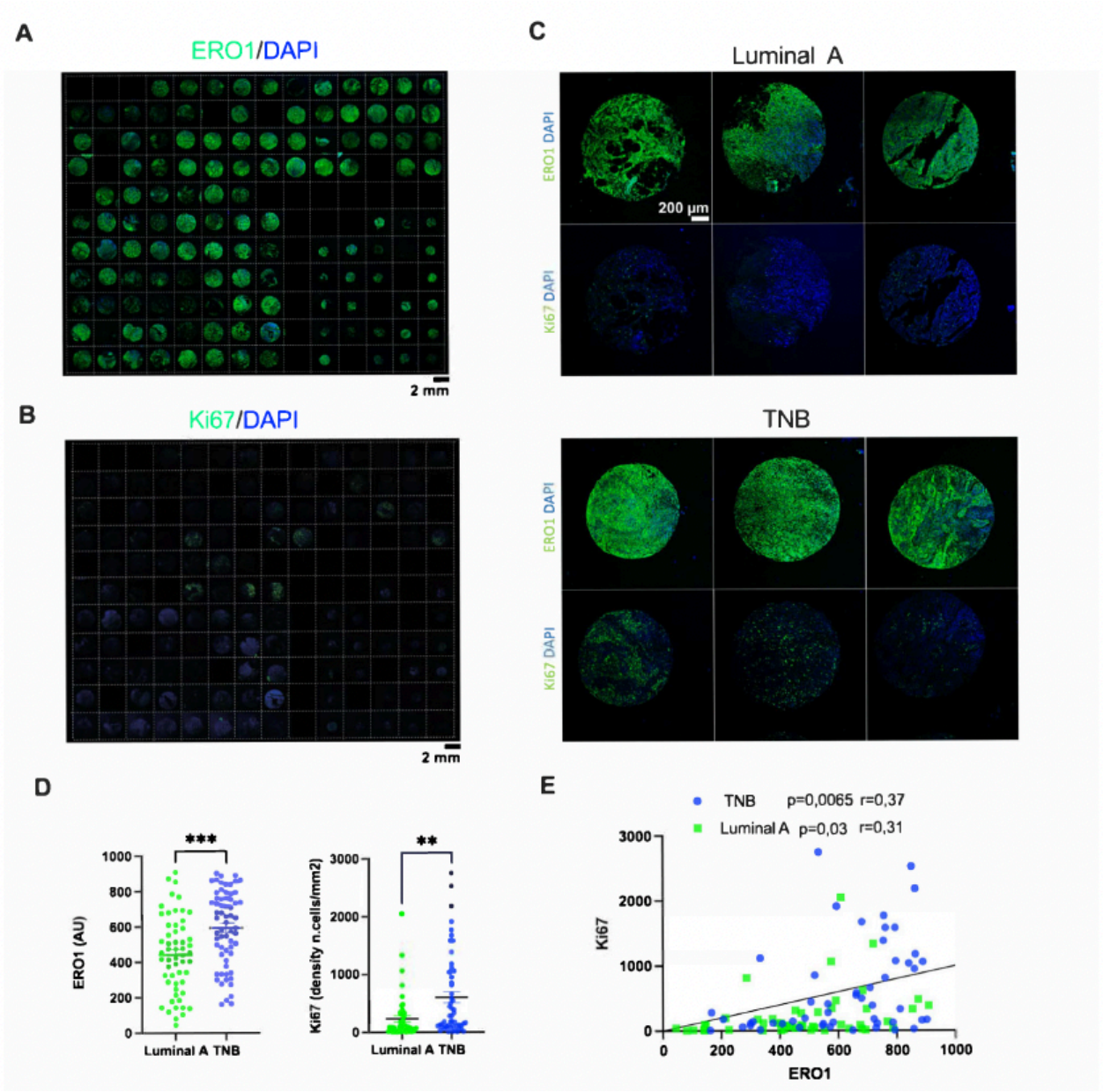
ERO1 expression is higher in tumors



Data were sourced from the GEPIA2 platform, utilizing the TCGA dataset.
Red: tumor, Gray: adjacent normal tissue;

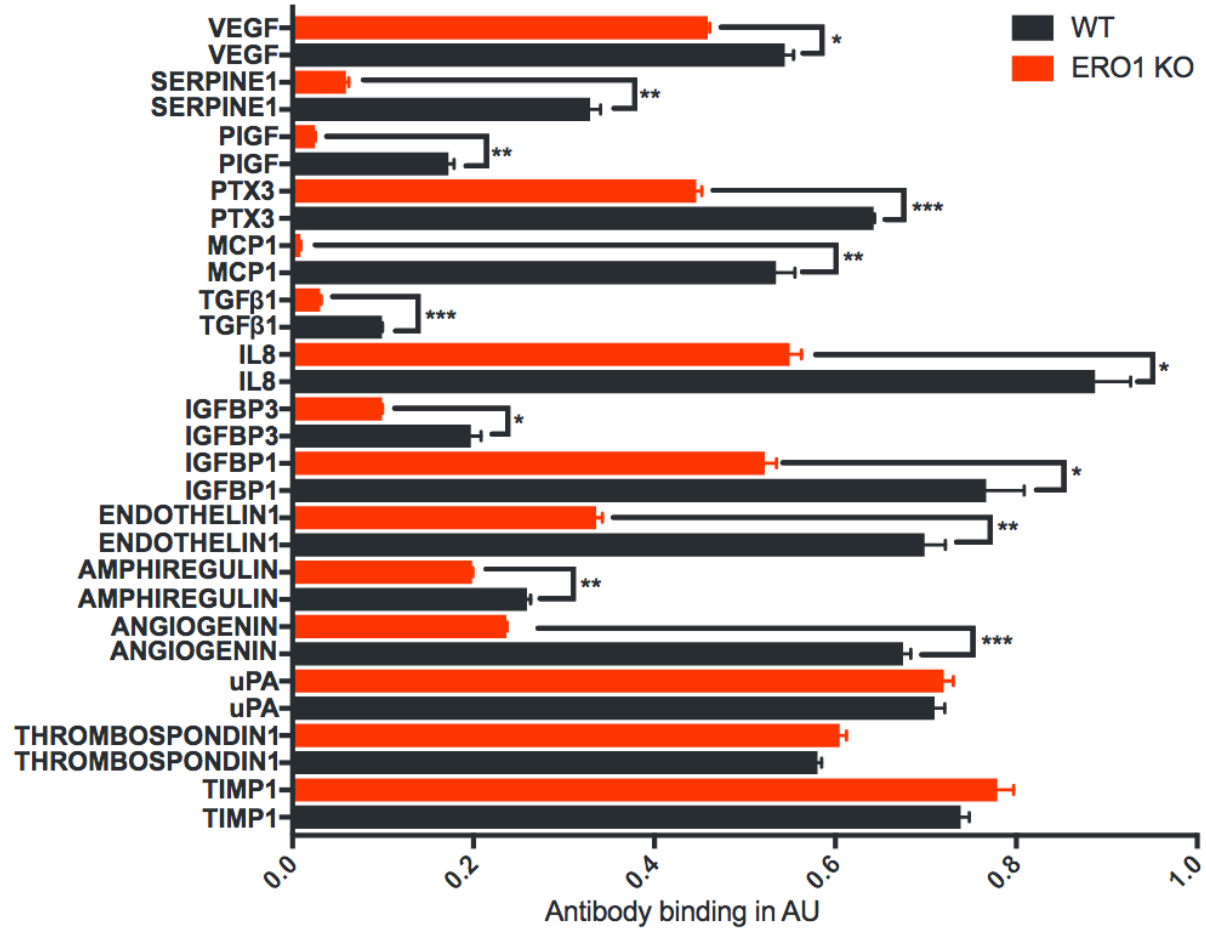
Zito E et al. *BBA-Reviews on cancer* 2024

ERO1 expression is higher in aggressive Triple negative breast tumors



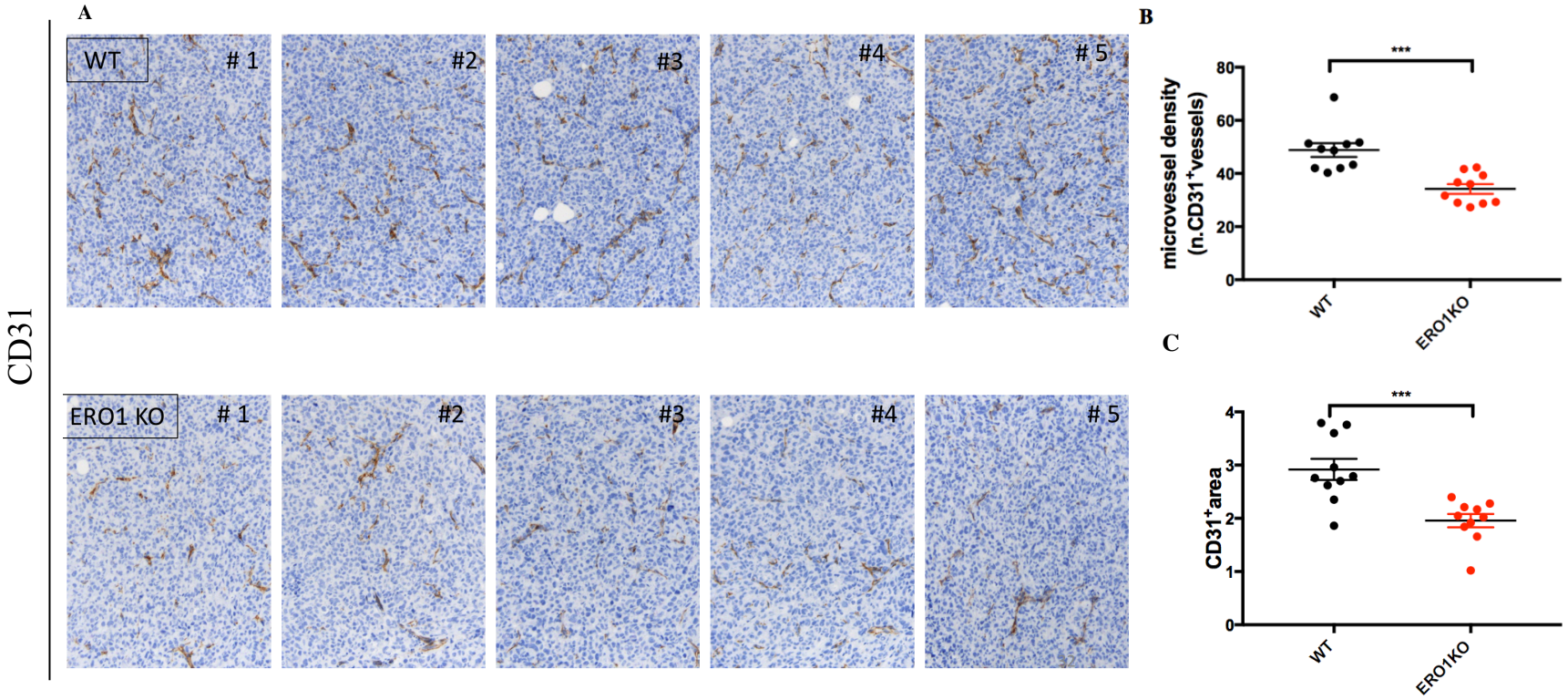
Varone E et al. *Cell Death & Disease 2025*

Reduced secretion of angiogenic-related factors in ERO1 KO breast cancer cells under hypoxia



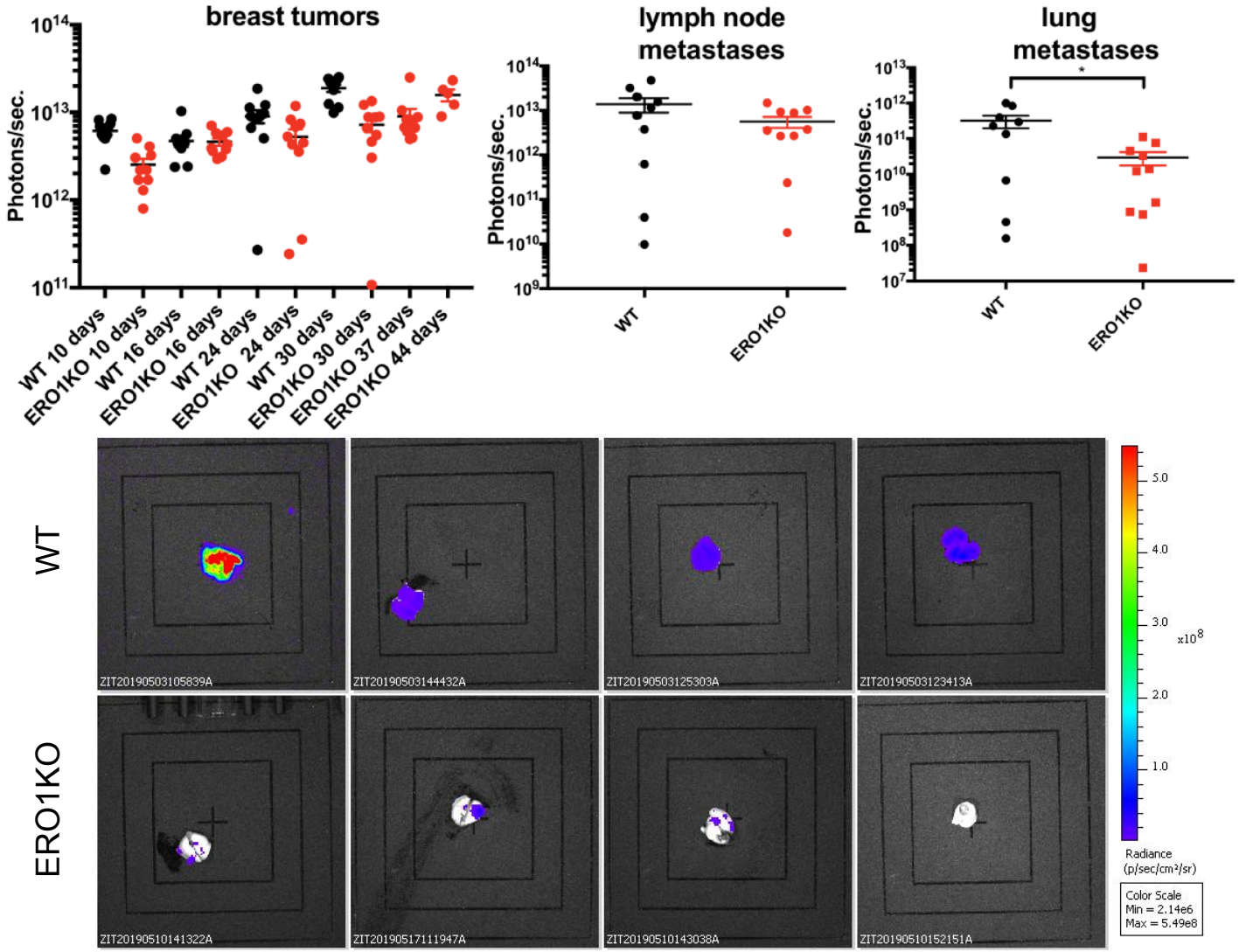
Varone E. et al. *Oncogene* 2021

Lack of ERO1 in primary tumors impairs angiogenesis



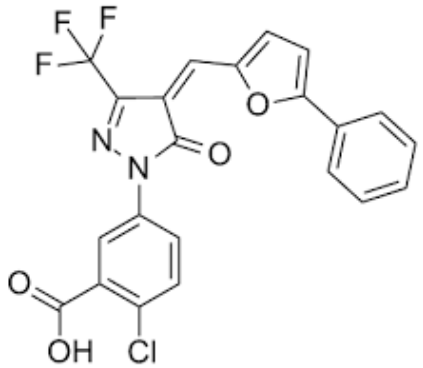
Varone E. et al. *Oncogene* 2021

Lack of ERO1 in the primary tumor impairs lung metastases



Varone E. et al. *Oncogene* 2021

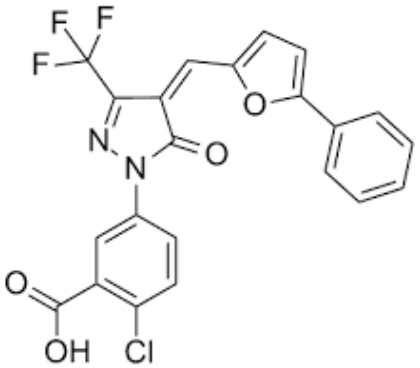
EN460: Early small-molecule ERO1 inhibitor



EN460

Blais J. et al. *JBC* 2010

EN460: Early small-molecule ERO1 inhibitor

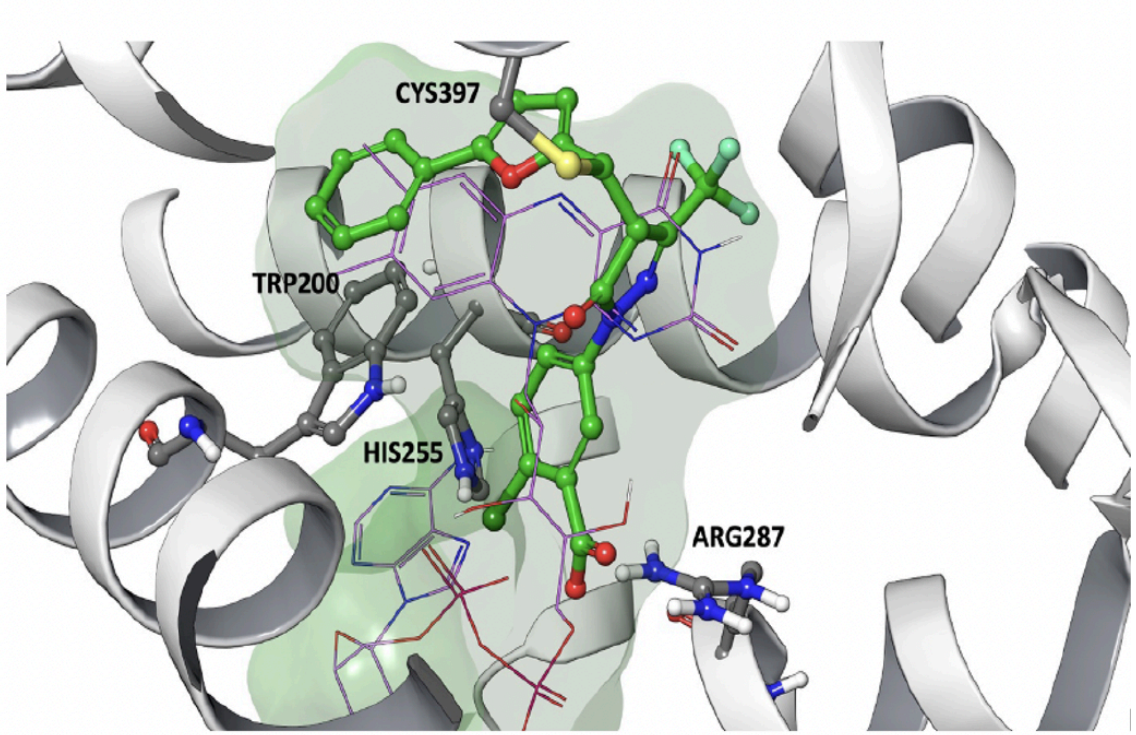


EN460

- Serves as a starting point for ERO1 inhibition
- We conducted a structure-activity relationship (SAR) campaign to improve:
  - Poor water solubility
  - Inhibitory potency (IC_{50})

Blais J. et al. *JBC* 2010

Computational modeling of EN460 binding to ERO1

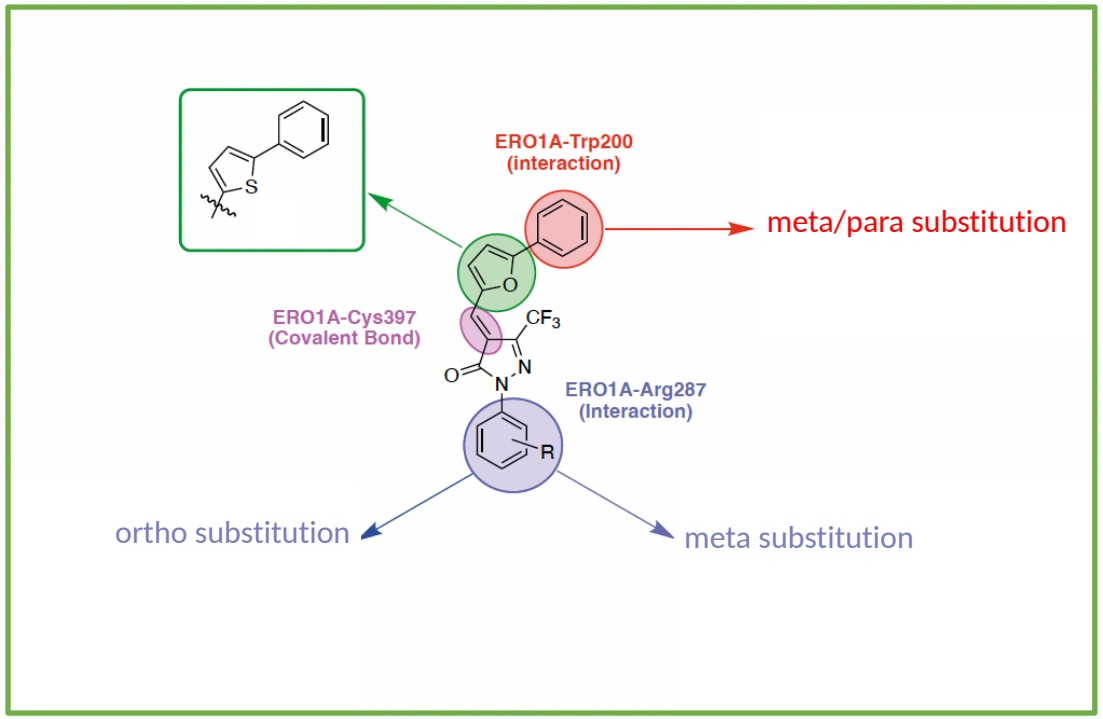


EN460 Binding to ERO1

• Forms a covalent bond via its electrophilic Michael acceptor and Cys397
• Engages in key non-covalent interactions:
    - The phenyl-furan moiety forms a π-stacking interaction with Trp200
    - The carboxylate group on the phenyl ring interacts with Arg287
    - The trifluoromethyl group occupies a sub-pocket near the backbone of Glu186

Varone E et al. *Cell Death & Disease 2025*

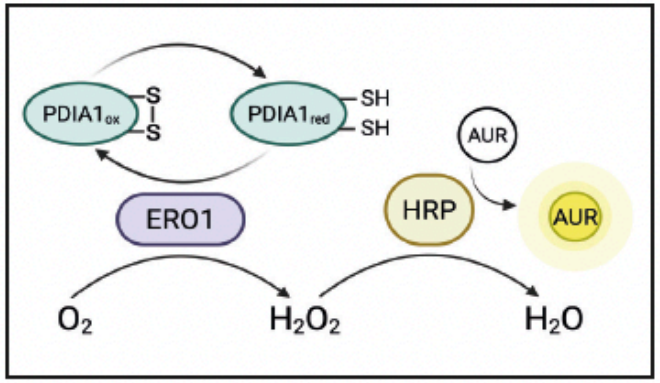
Synthesis of forty EN460 derivatives and three salts

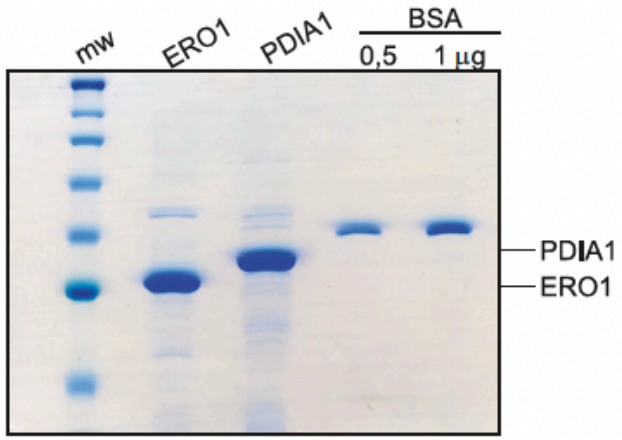


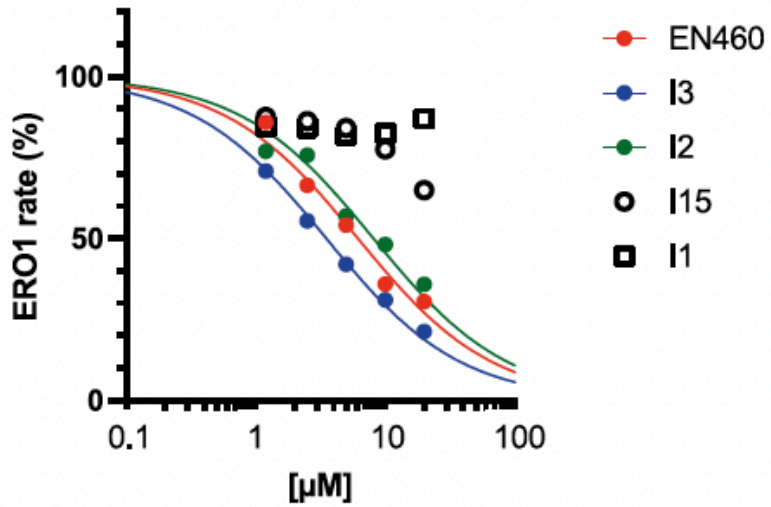
- 40 EN460 derivatives obtaining by substitution on the indicated pharmacophoric groups

- three salts of the carboxylate anion on the phenyl ring

Kinetic AUR-based assay of ERO1 activity for in vitro screening of EN460 derivatives

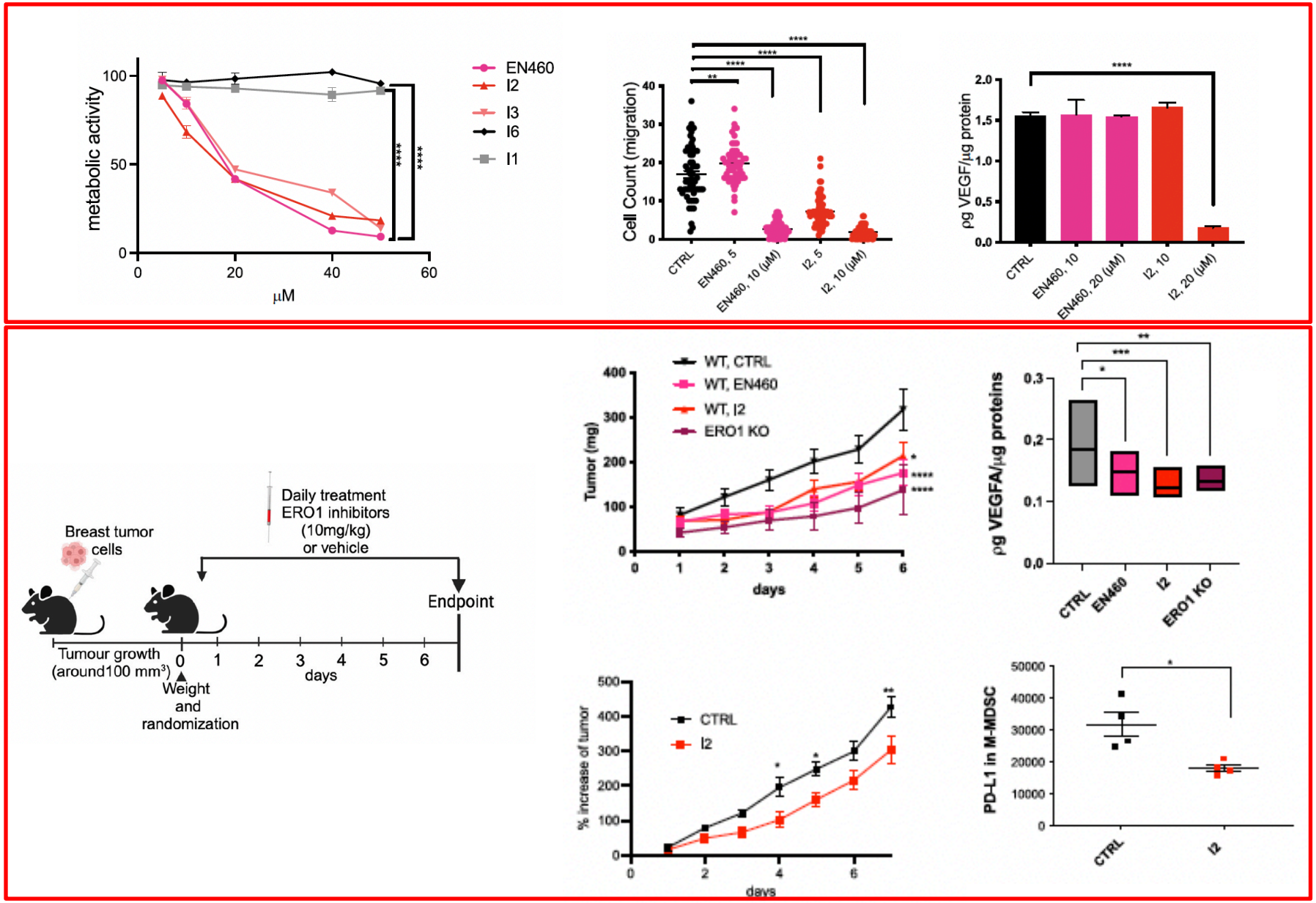






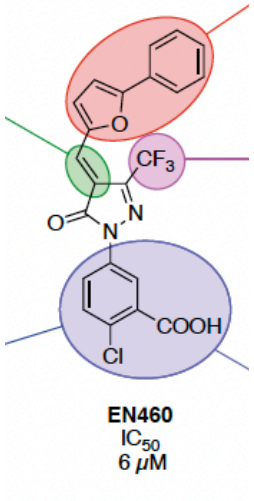
Varone E et al. *Cell Death & Disease 2025*

Anticancer effects of ERO1 inhibition in TNBC-bearing mice



Varone E et al. *Cell Death & Disease 2025*

Structure–Activity Relationship (SAR) summary of EN460 derivatives:



EN460
IC_{50}
6 μM

1. Essential role of Michael acceptor

- The Michael acceptor moiety is critical for ERO1 inhibition. Removing or modifying this group leads to loss of activity.

2. Solubility vs. Activity

- Salt formation or acidic sulfonate substitution on the phenyl ring increases water solubility but completely abolishes biological activity. This indicates that enhancing aqueous solubility in this chemical class is unlikely without compromising potency.

3. Phenyl-Furan moiety requirement

- The phenyl-furan fragment is indispensable for activity. Its removal or replacement significantly reduces or eliminates ERO1 inhibition.

4. Phenyl ring distortion enhances activity

- Distortion of the phenyl ring is necessary for activity.
- Mono ortho-fluorine substitution enhances this distortion, resulting in a twofold increase in potency.

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