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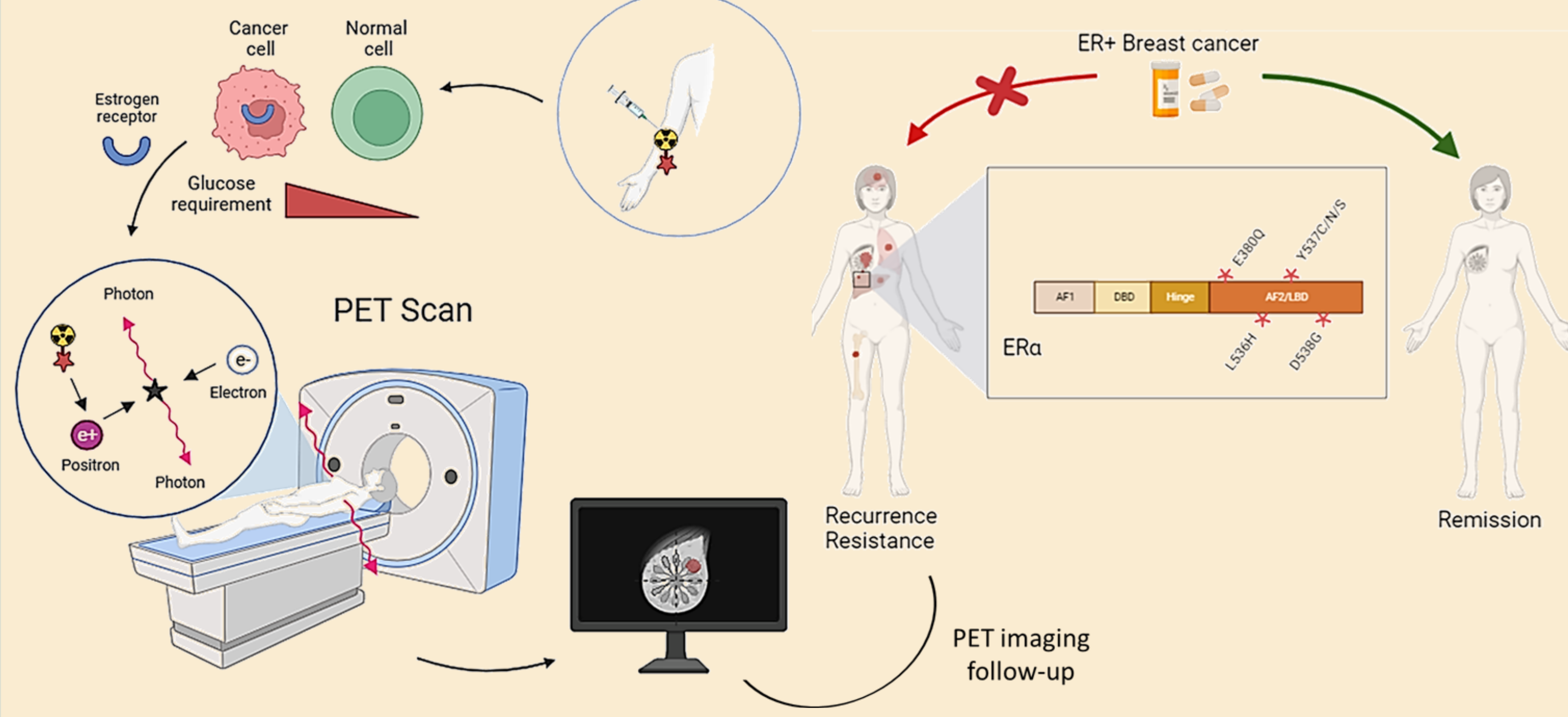
ASSESSMENT OF [18F]-FES PERFORMANCE IN PET IMAGING FOR ER MUTATED BREAST CANCER

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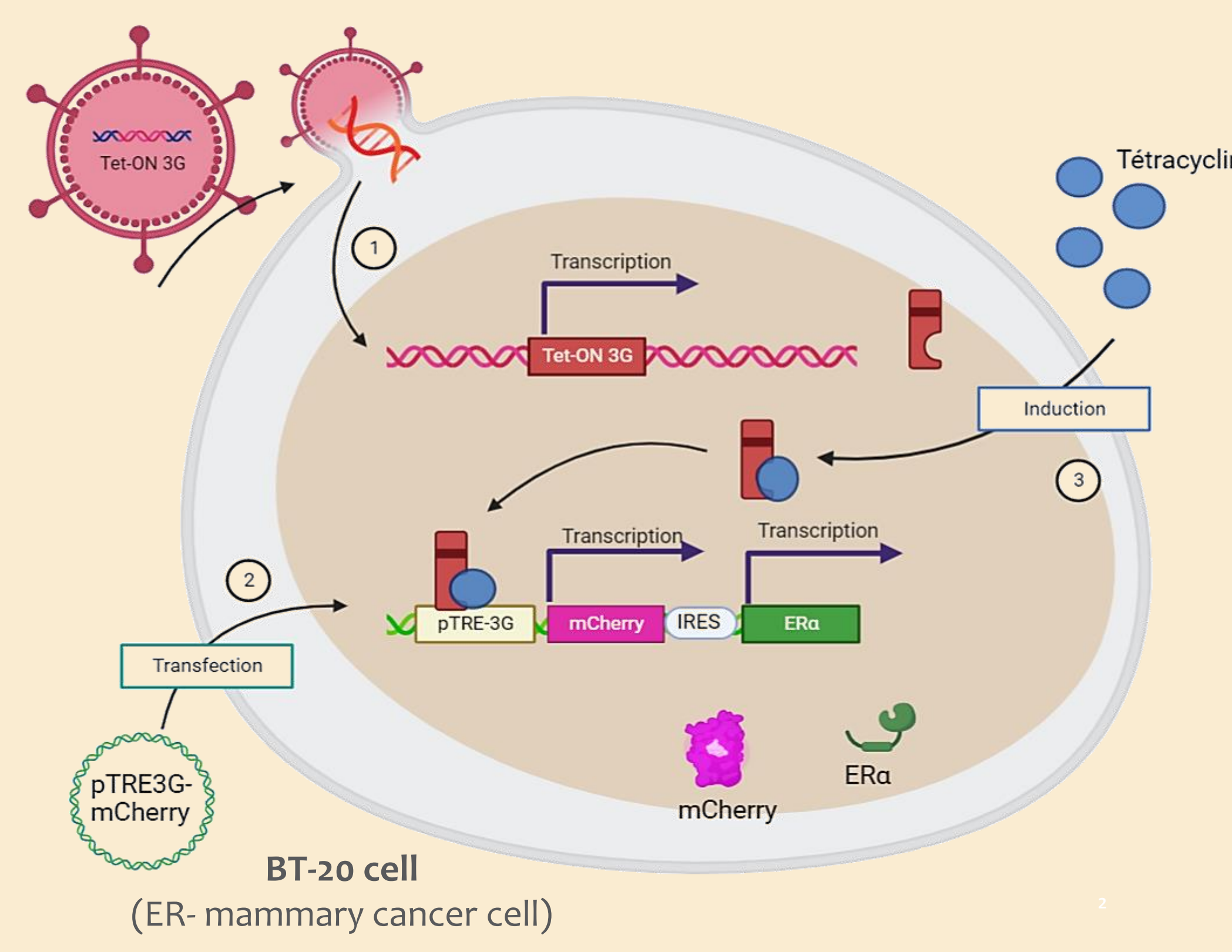
BACKGROUND

Breast cancer is the most common cancer in the world and the most deadly in women in France. The ER+ (Estrogen Receptor +) subtype accounts for 70% of breast cancers and is associated with the best overall survival at 5 years. However, in case of recurrence, this duration falls (9 months to 3 years), and tumor cells develop mutations that lead to constitutive activation of the alpha receptor to estrogen in 10 to 50% of cases. Positron emission tomography (PET) is widely used for diagnosis and for

assessing response to therapeutic treatment. The 18Fluorodeoxyglucose ([18F]-FDG) radioisotope is a glucose-like tracer commonly used for tumor detection, but unable to provide information on the ER status. The use of 18Fluoroestradiol ([18F]-FES), a compound akin to estradiol should solve this problem and aim to develop personalized therapeutic treatment. However, its ability to bind on ER mutants is not yet elucidated.



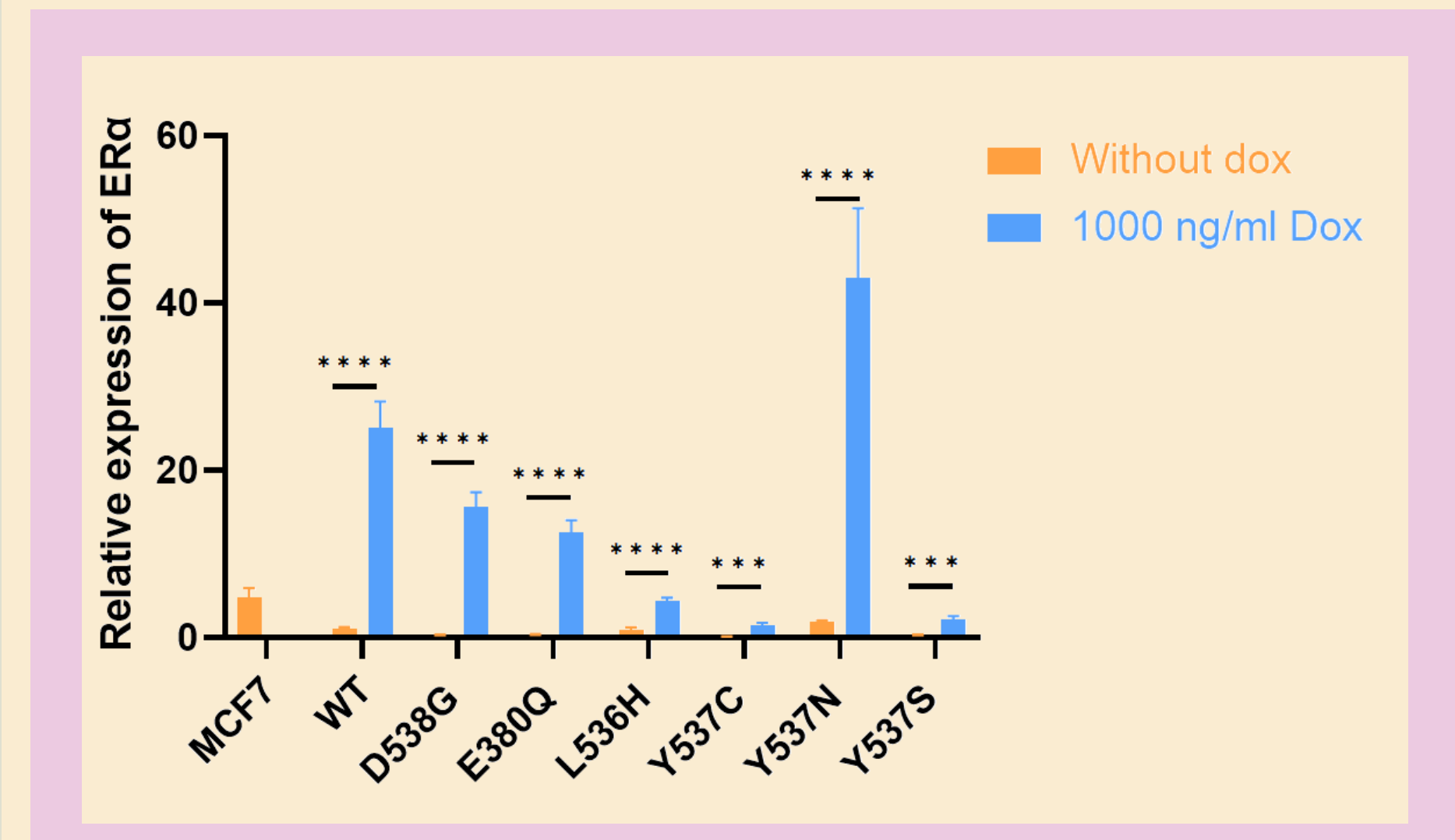
01 MODEL



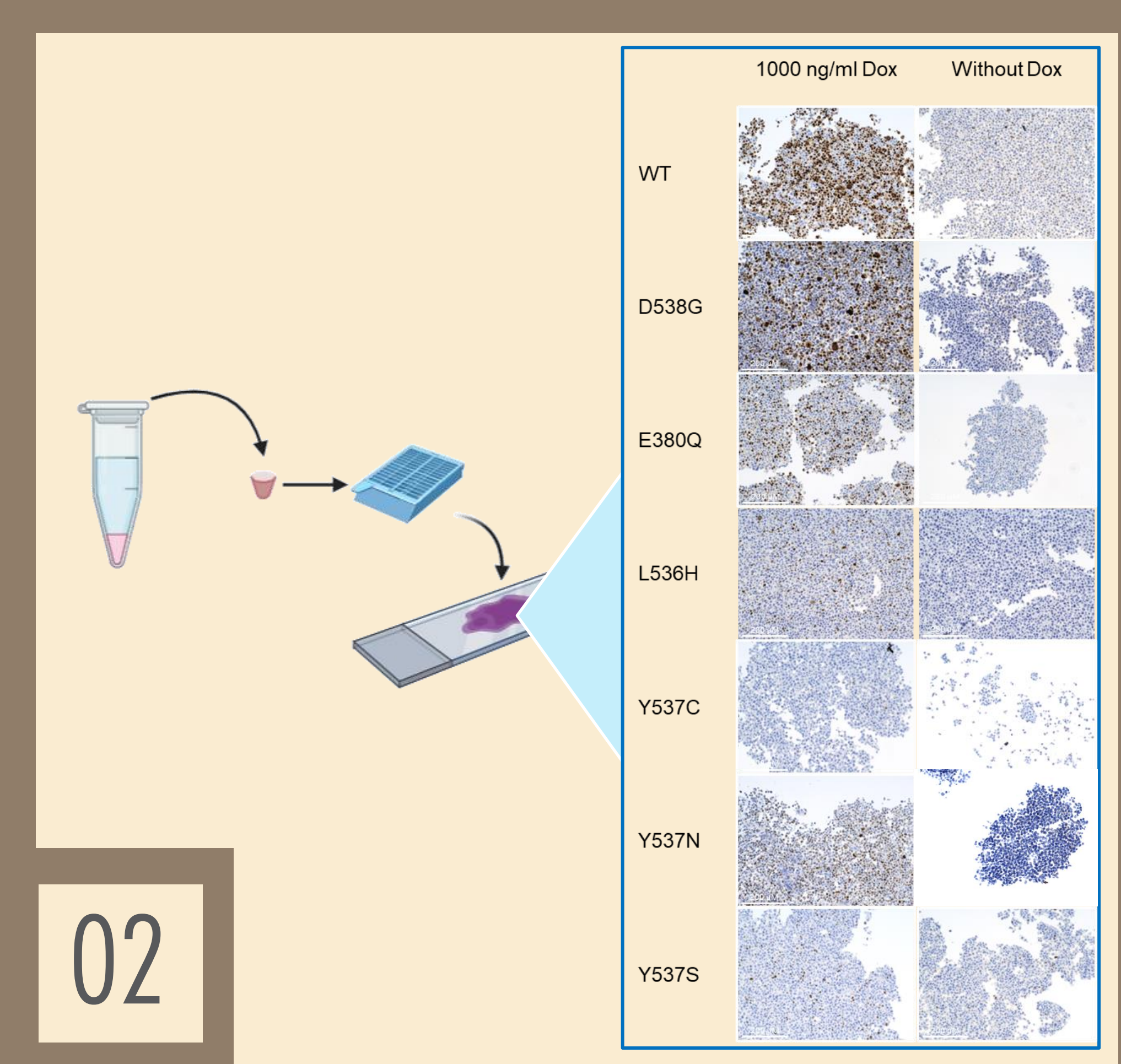
- 1. Transduction**
Transduction of a vector (Tet-ON 3G) allowing stable expression of the transactivator protein Tet-ON 3G.
- 2. Stable transfection**
Stable transfection of a vector (pTRE3G-mCherry) allowing the expression of the WT or mutated ERα and a reporter gene encoding a fluorescent protein (mCherry).
- 3. Induction**
Induction of the system by addition of tetracycline (or doxycycline), which by binding to the Tet-on 3G protein, will activate the transcription of genes encoding proteins of interest (ERα and mCherry).

Our goal is: Studying the ability of [18F]-FES to bind on the different ER mutants (E380Q, L536H, Y537C/N/S and D538G) aiming to propose its use for the diagnosis and the monitoring of ER breast cancers.

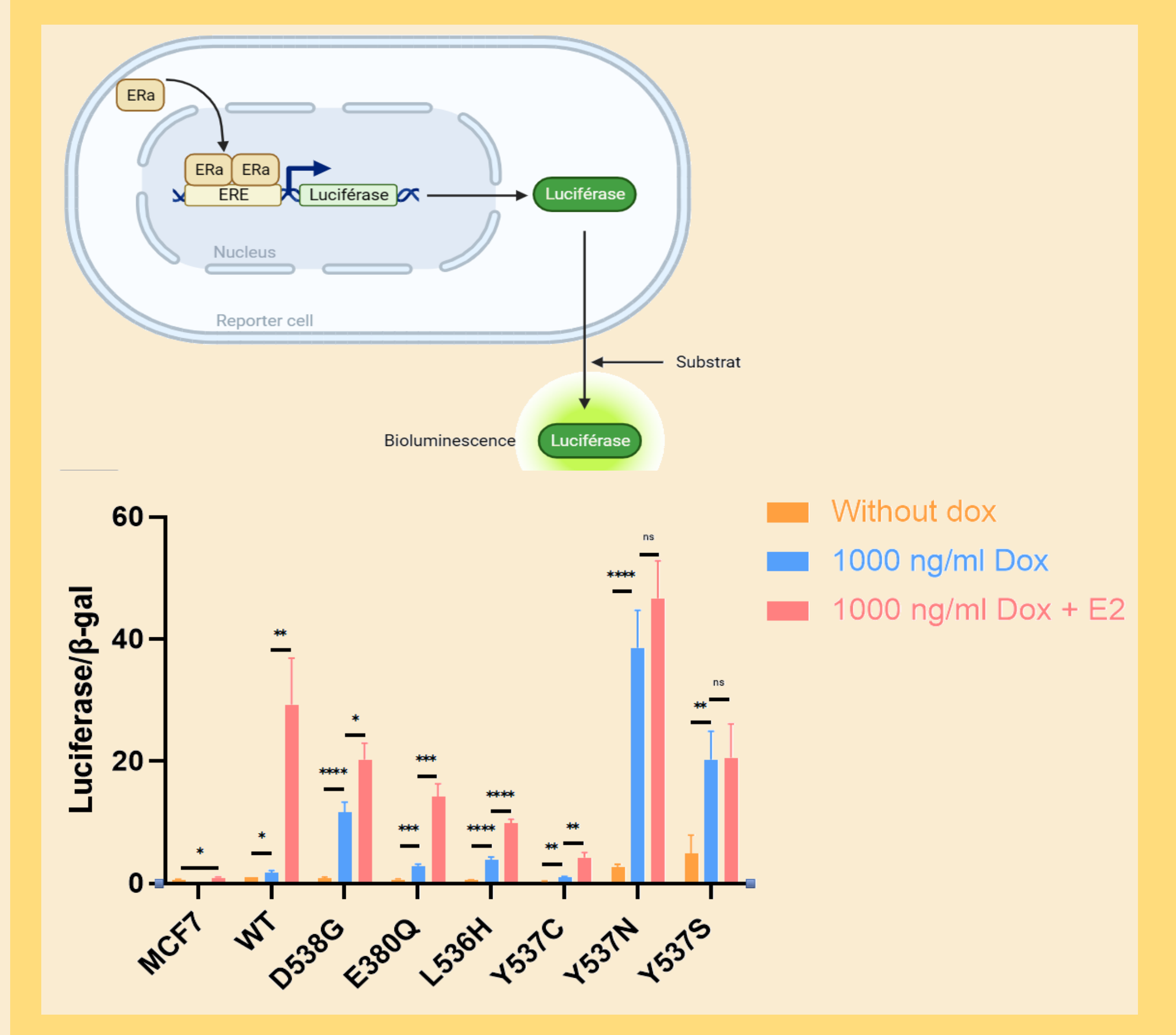
02 RESULTS



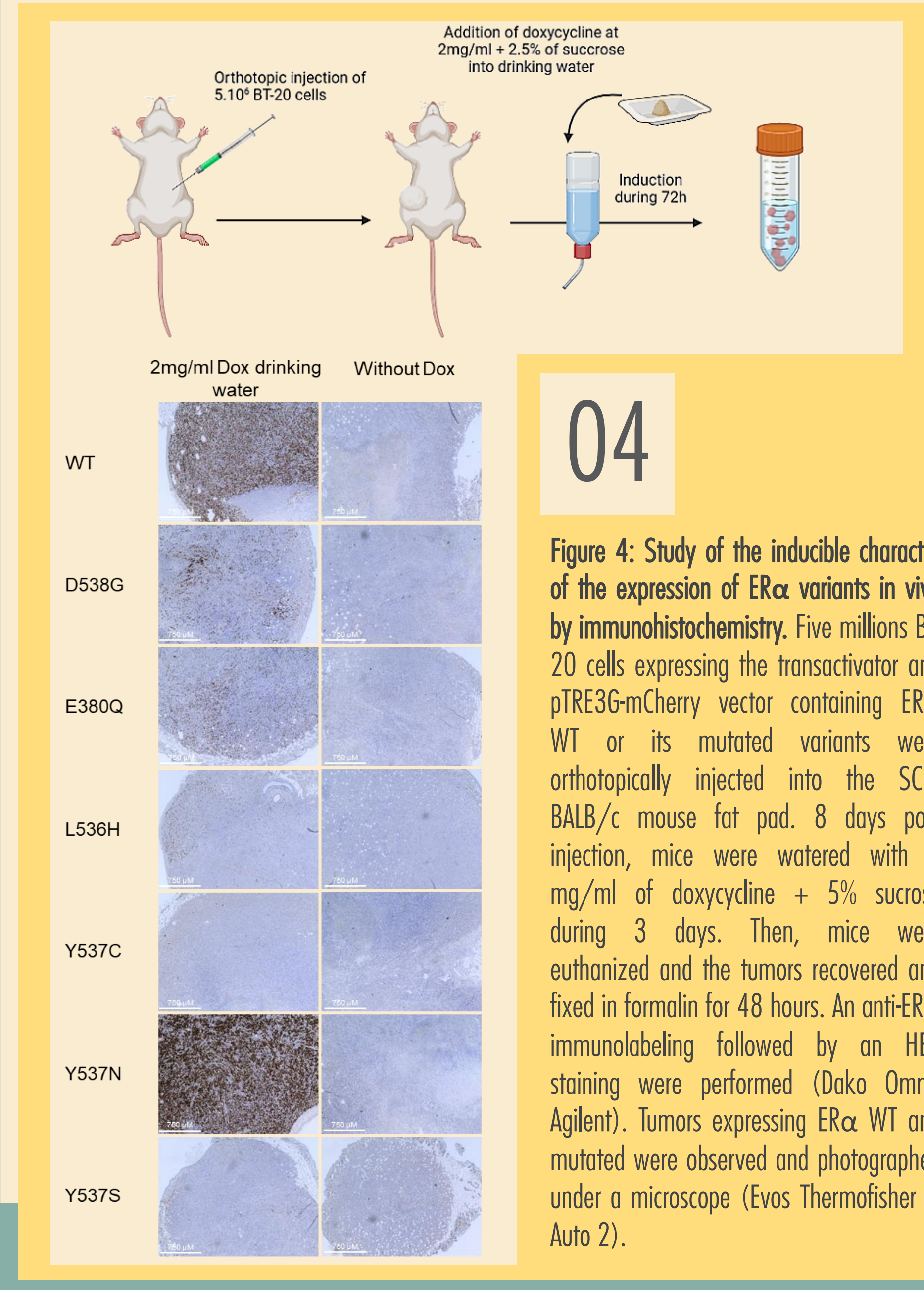
01 Figure 1: Study of the inducible character of the expression of the ER variants by RT-qPCR. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ERα WT or its mutated variants were stimulated or not with 1000 ng/ml doxycycline (Dox) for 24 hours. Total RNAs were extracted and analyzed by RT-qPCR. The histogram corresponding to the quantification of the expression of Eralpha correspond to the relative expression to the BT-20 WT uninduced and to the reference genes (RS18 and RPL32). N=10



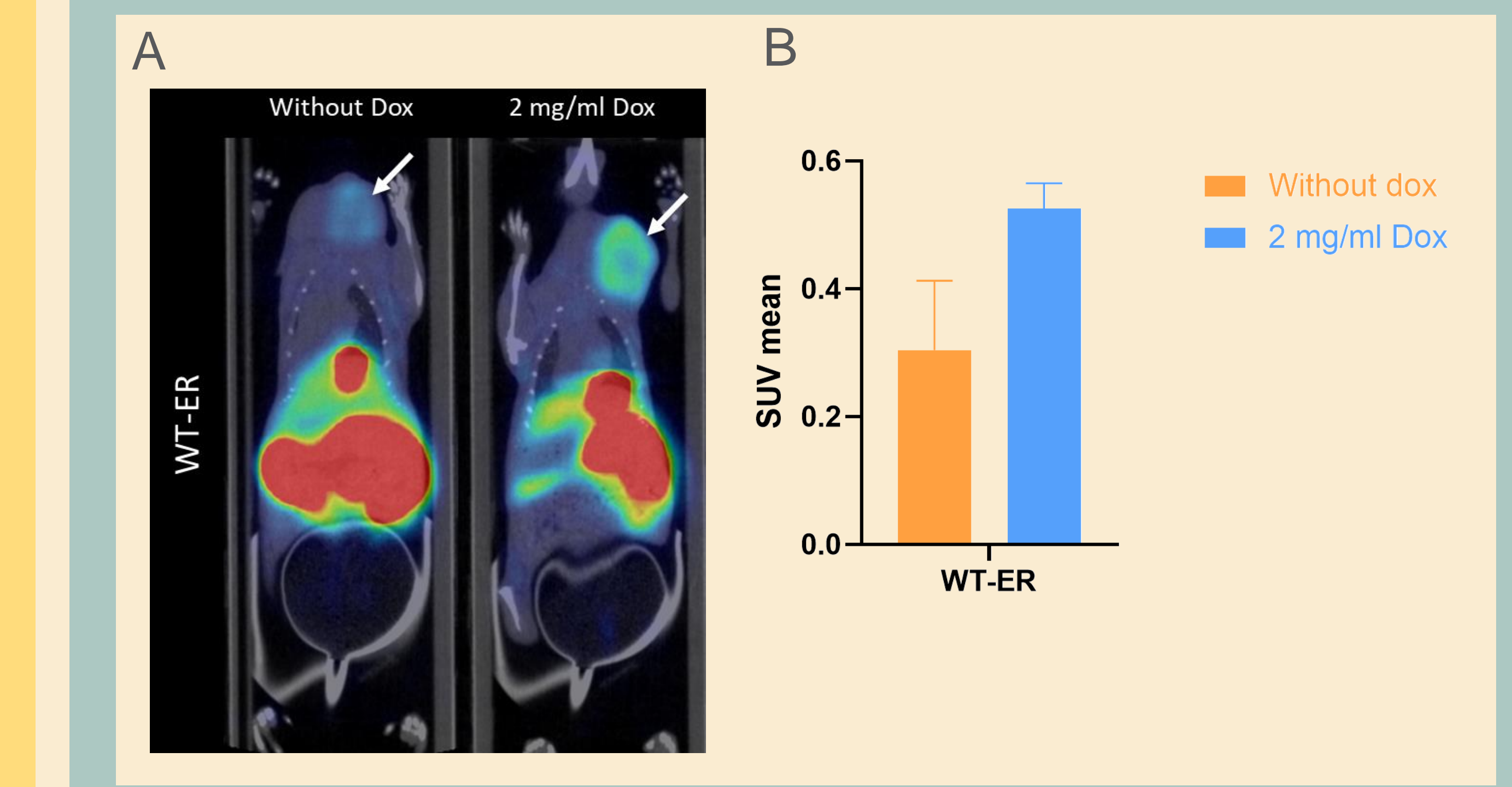
02 Figure 2: Study of the inducible nature of the expression of the ERα variants by immunohistochemistry. BT-20 cells expressing the transactivator and the pTRE3G-mCherry vector containing either the WT ERα or its mutated variants were stimulated or not with 1000 ng/ml doxycycline for 24 hours. The cells were then collected and centrifuged, and the cell pellets were fixed with 4% paraformaldehyde for 48 hours. An anti-ERα immunostaining followed by HES counterstaining was performed (Dako Omnis Agilent). Cells expressing the WT ERα and mutants were observed and photographed under a microscope (Evos Thermofisher FI Auto 2).



03 Figure 3: Functional Study of ER Variants Using Reporter Gene System. BT-20 cells with transactivator and pTRE3G-mCherry (ERα WT or mutants) were transfected with plasmids: one with an estrogen response element (ERE) and luciferase, another with β-galactosidase. Cells were treated with 1000 ng/ml doxycycline for 24 hours and 10 nM estradiol for 12 hours. Protein extracts were tested with luciferase substrate, and bioluminescence was normalized to beta-galactosidase activity. The histogram shows luciferase activity normalized to beta-galactosidase and uninduced BT-20 WT. N=6



04 Figure 4: Study of the inducible character of the expression of ERα variants in vivo by immunohistochemistry. Five millions BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ERα WT or its mutated variants were orthotopically injected into the SCID BALB/c mouse fat pad. 8 days post injection, mice were watered with 2 mg/ml of doxycycline + 5% sucrose during 3 days. Then, mice were euthanized and the tumors recovered and fixed in formalin for 48 hours. An anti-ERα immunolabeling followed by an HES staining were performed (Dako Omnis Agilent). Tumors expressing ERα WT and mutated were observed and photographed under a microscope (Evos Thermofisher FI Auto 2).



05 Figure 5: Study of the uptake of [18F]-FES by PET/CT imaging of WT-ER tumor xenografts. (A) Representative coronal PET/CT images of mice bearing inducible WT-ER tumors imaged 1 hour after tail vein injection with 5.5 MBq of [18F]-FES. Mice were imaged 11 days after tumor cell injection and were given water containing 2 mg/ml doxycycline + 5% sucrose or only 5% of sucrose without doxycycline for 3 days (n = 2 and 2 respectively). Tumors are indicated by arrows. (B) [18F]-FES uptake in tumor xenografts quantified as SUV mean. Values represent mean ± SEM

03 CONCLUSION

We demonstrated the inducible nature of our Tet-ON system with 1000 ng/ml of doxycycline. Initial tests validated the inducible expression of ERα variants using qPCR and Immunohistochemistry. The functionality of our protein of interest was confirmed using a reporter gene technique. We then tested our in vivo model with various variants using 2 mg/ml of doxycycline for 72 hours. All this data validates our model, allowing us to manipulate [18F]-FES, and study its in vivo binding capacity to ER variants.

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