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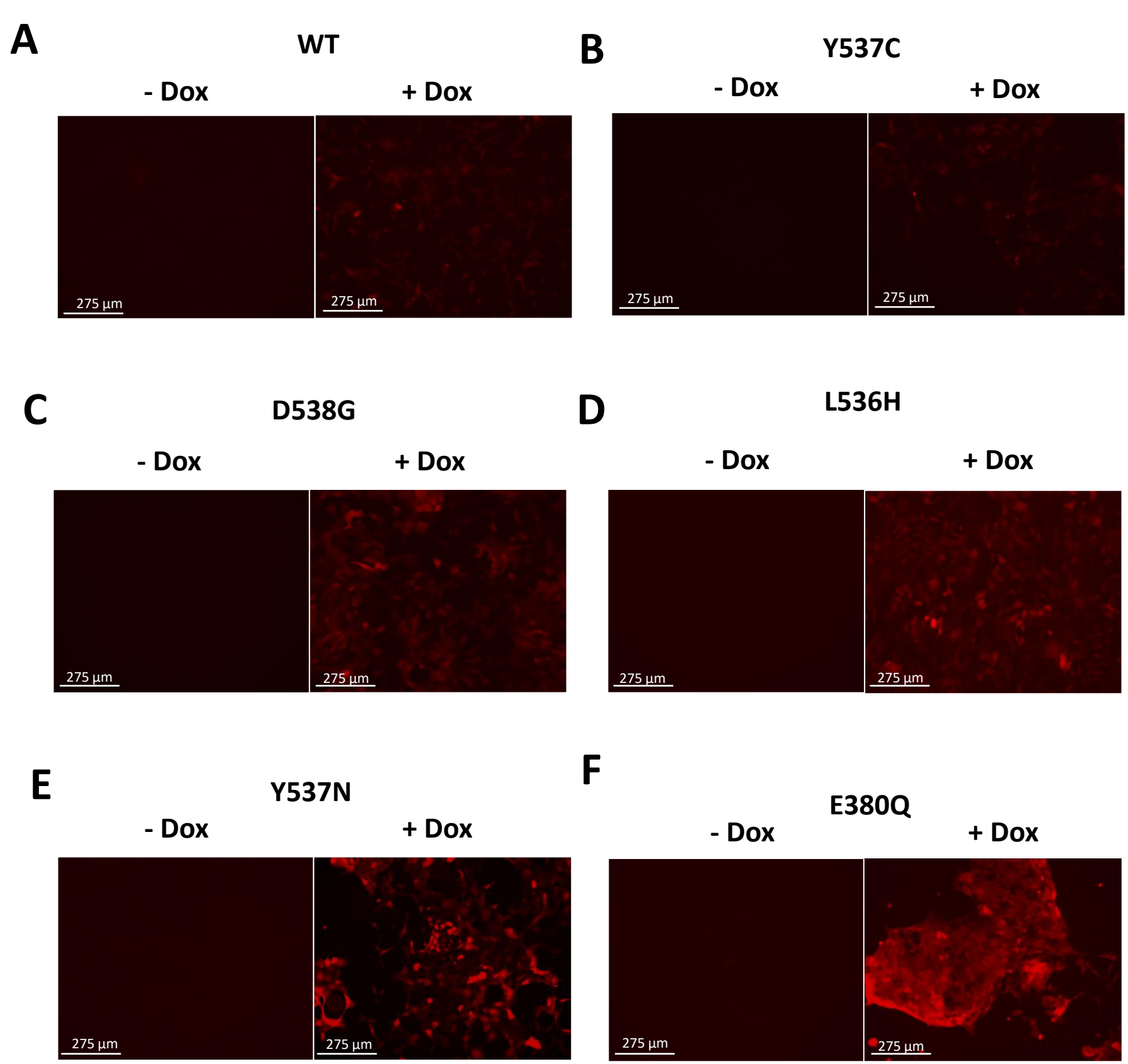
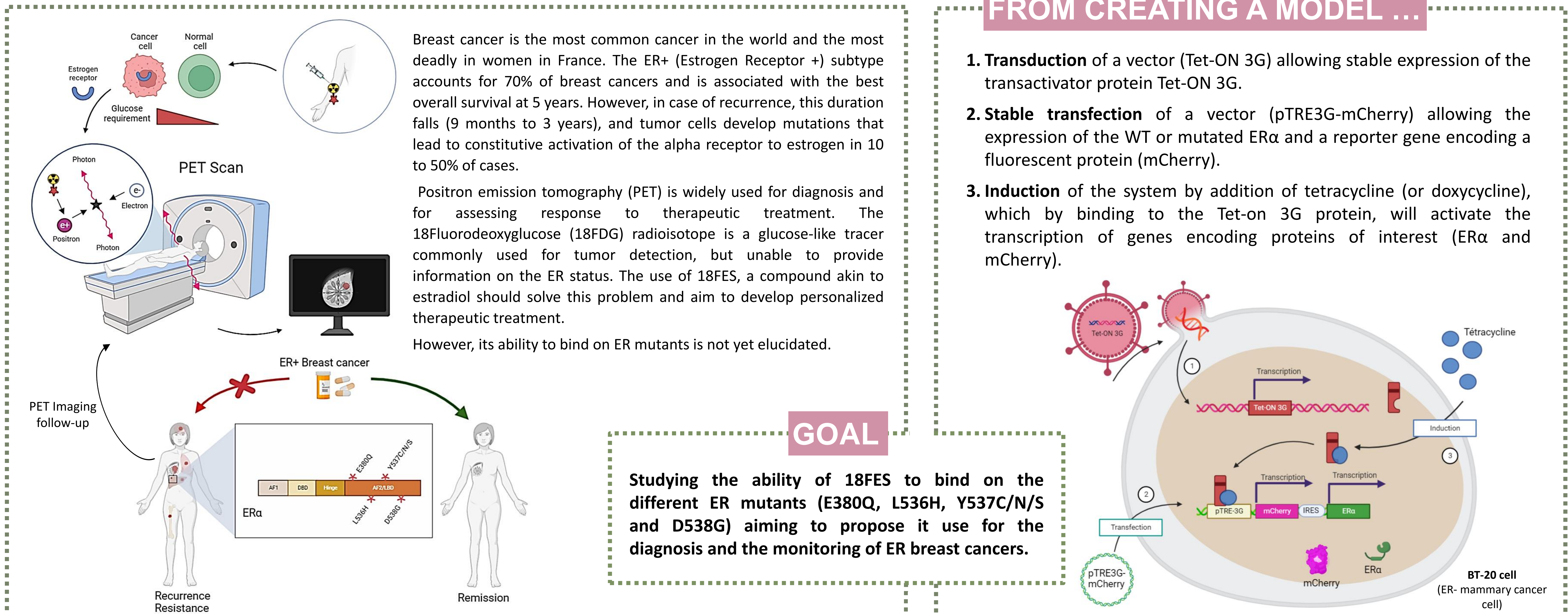
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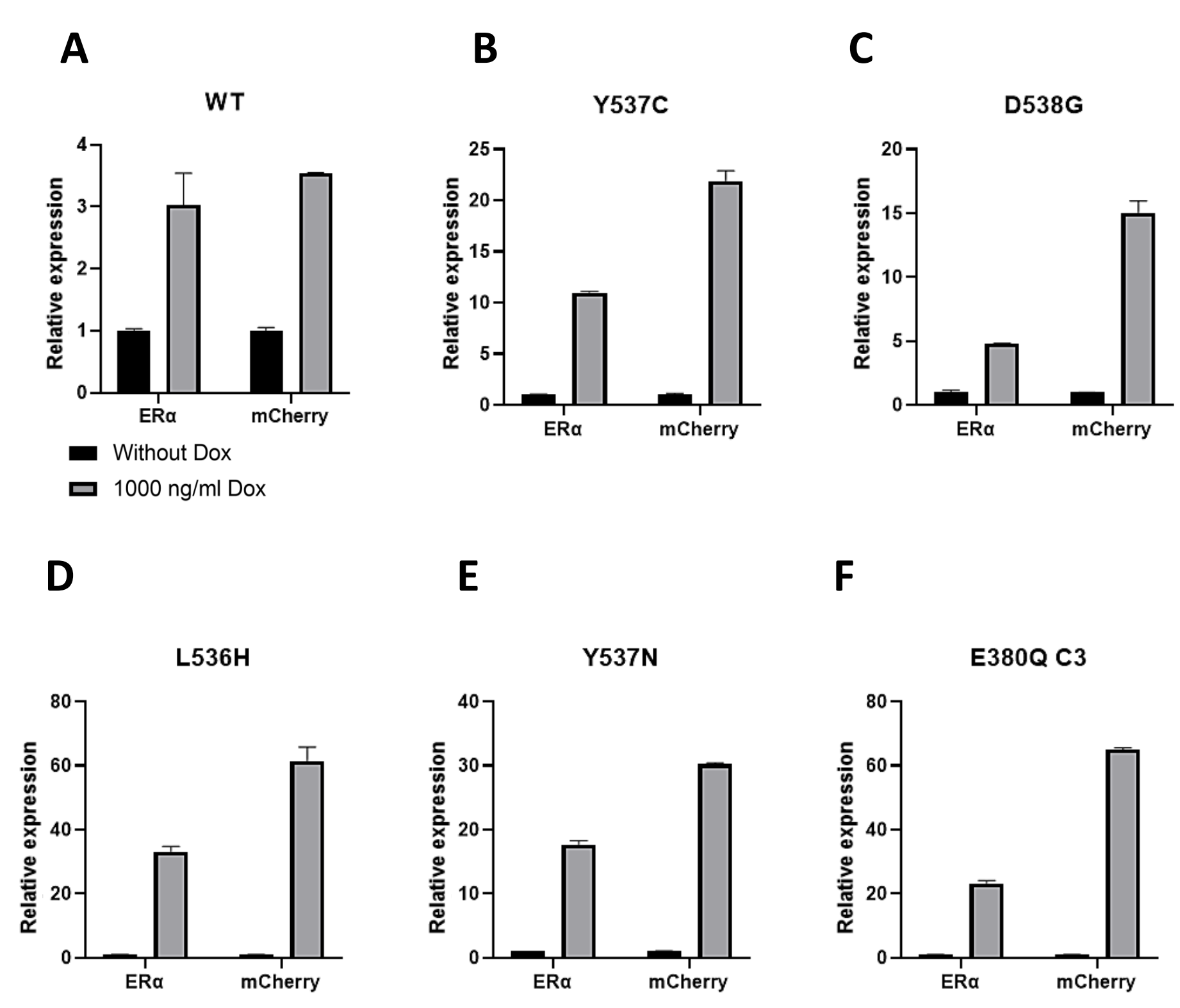
# Development of cellular model to explore the 18FES binding in breast cancers with ER mutations by $\mu$ PET imaging.

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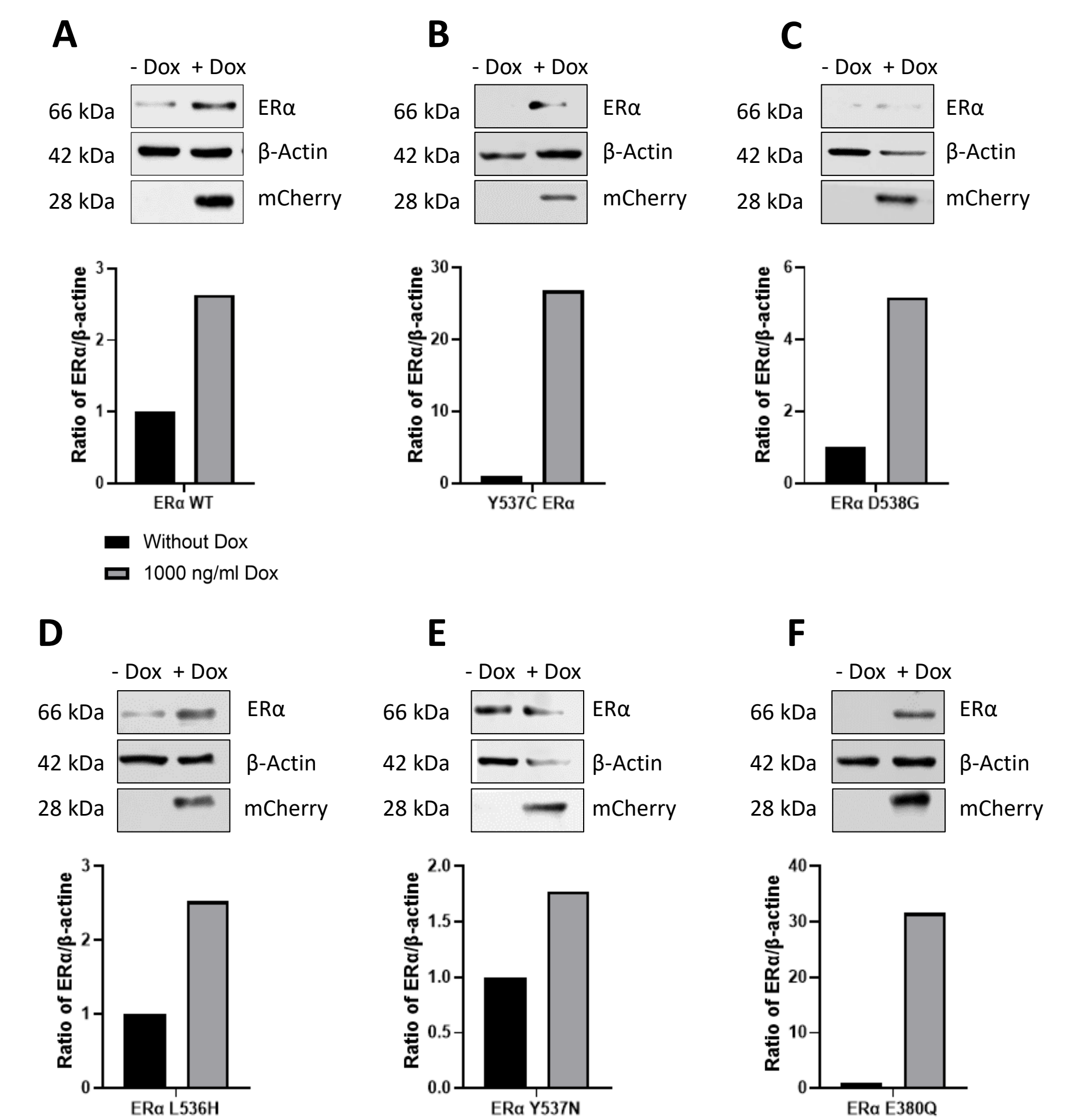
1 - MEDyC CNRS UMR 7369, Extracellular Matrix and Cellular Dynamics, Reims, France 2 - EA7509 – IRMAIC, Godinot Institute, Reims, France 3 - EA 3804, CRESTIC, Reims, France 4 - EA 7319, Institut Godinot, Reims, France 5 – CRAN UMR 7039, Nancy, France  
\* Equal supervision of this work



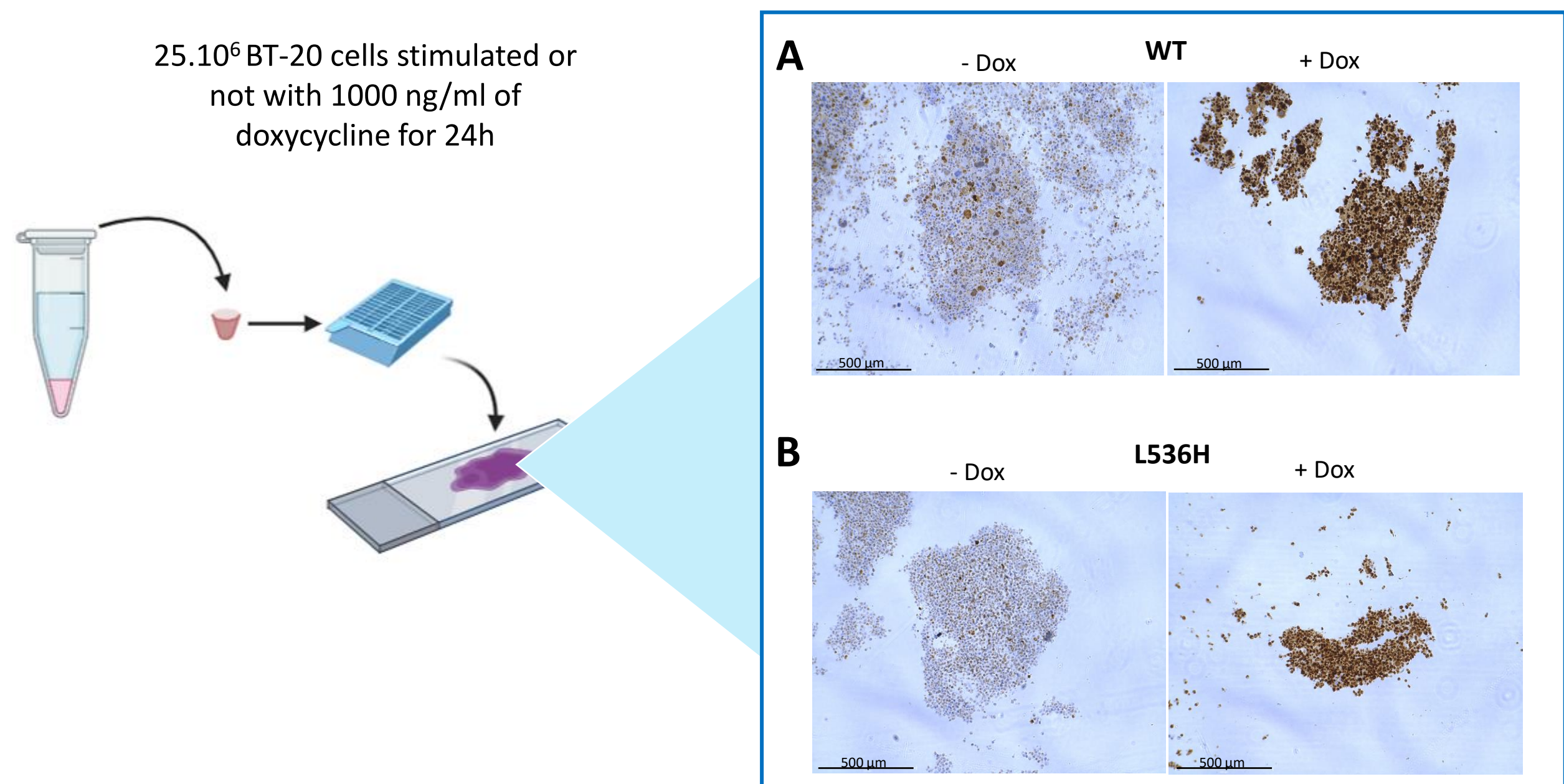
**Figure 1 :** Study of the inducible character of the expression of the mCherry reporter gene expression by fluorescence microscopy. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ER $\alpha$  WT or its mutated variants were stimulated or not with 1000 ng/ml doxycycline (Dox) for 24 hours. Cells expressing ER $\alpha$  WT (A), Y537C (B), D538G (C), L536H (D), Y537N (E) and E380Q (F) were observed and photographed under a fluorescence microscope (EvoS ThermoFisher FI Auto 2).



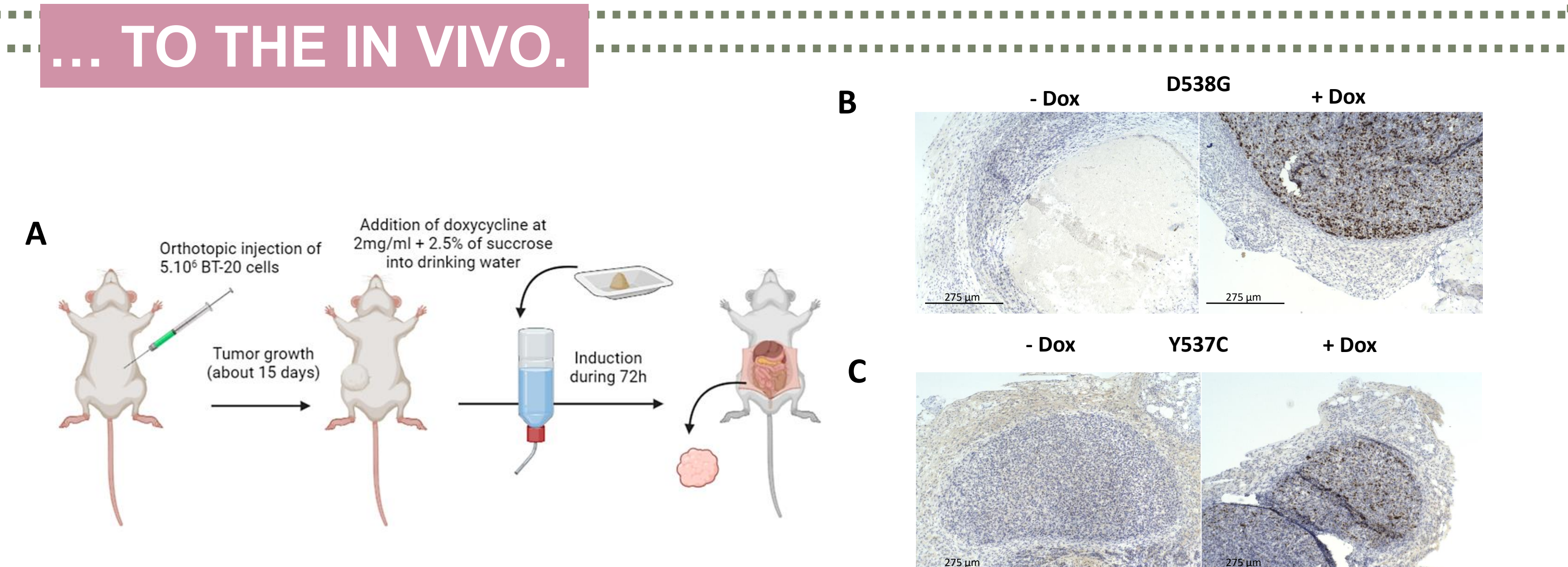
**Figure 2:** Study of the inducible character of the expression of the ER and mCherry variants by RT-qPCR. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ER $\alpha$  WT or its mutated variants were stimulated or not with 1000 ng/ml doxycycline (Dox) for 24 hours. Total RNAs were extracted and analyzed by RT-qPCR. The histograms corresponding to the quantification of the expression of ER $\alpha$  WT (A), Y537C (B), D538G (C), L536H (D), Y537N (E) and E380Q (F) and mCherry are presented and correspond to the relative expression relative to the reference genes (RPS18 and RPL32).



**Figure 3 :** Study of the inducible character of the expression of the ER and mCherry variants by Western Blot. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ER $\alpha$  WT or its mutated variants were stimulated or not with 1000 ng/ml doxycycline (Dox) for 24 hours. The total proteins were extracted and analyzed by SDS-PAGE electrophoresis then western blotted. Western blot results as well as histograms corresponding to the relative quantification of the expression ratio ER $\alpha$ / $\beta$ -Actin WT (A), Y537C (B), D538G (C), L536H (D), Y537N (E) and E380Q (F) between cells treated or not treated with Dox are presented (n=1).



**Figure 4:** Study of the inducible character of the expression of the L536H variant of the ER $\alpha$  by immunohistochemistry. BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ER $\alpha$  WT or mutated variant L536H were stimulated or not per 1000 ng/ml doxycycline (Dox) for 24 hours. The cells were then recovered and centrifuged, and the cell bases were attached with 4% paraformaldehyde for 48 hours. An anti-ER $\alpha$  immunolabeling followed by an HES staining was performed (Dako Omnis Agilent). Cells expressing the ER $\alpha$  WT (A) and L536H (B) were observed and photographed under a microscope (EvoS ThermoFisher FI Auto 2).



**Figure 5:** Study of the inducible character of the expression of ER $\alpha$  variants in vivo by immunohistochemistry. Five millions BT-20 cells expressing the transactivator and pTRE3G-mCherry vector containing ER $\alpha$  WT or its mutated variants were orthotopically injected into the SCID BALB/c (A) mouse fat pad. 15 days post injection, mice were watered with 2 mg/ml of doxycycline + 2.5% sucrose during 3 days. Then, mice were euthanized and the tumors recovered and fixed in formalin for 48 hours. An anti-ER $\alpha$  immunolabeling followed by an HES staining was performed (Dako Omnis Agilent). Tumors expressing ER $\alpha$  D538G (B) and Y537C (C) were observed and photographed under a microscope (EvoS ThermoFisher FI Auto 2).

## CONCLUSION

All the results obtained allowed us to demonstrate the proper functioning of the Tet-ON system for the ER $\alpha$  and its studied variants. After 24 hours of stimulation at 1000 ng/ml doxycycline, we first validated the fluorescence emission of our reporter gene mCherry by fluorescence microscopy (Figure 1). Then the inducible expression of the different ER $\alpha$  variants were validated at the transcriptional level by RT-qPCR (Figure 2), at the protein level by western blot (Figure 3) and immunohistochemistry (Figure 4). Finally, we tested our in vivo model for some variants, which allowed us to validate its inducibility in an orthotopic xenograft fat pad model where doxycycline (2mg/ml) was added in drinking water for 72 hours. This model will soon be used in collaboration with NancycloTEP, to study and quantify the uptake of 18FES in  $\mu$ PET imaging and to clarify the interest of its use in the diagnosis and follow-up of patients with hormone-dependent breast cancer.