

# Identification of glucocorticoid receptor dependence in metastasis of invasive lobular carcinoma using an in vivo xenograft MIND model

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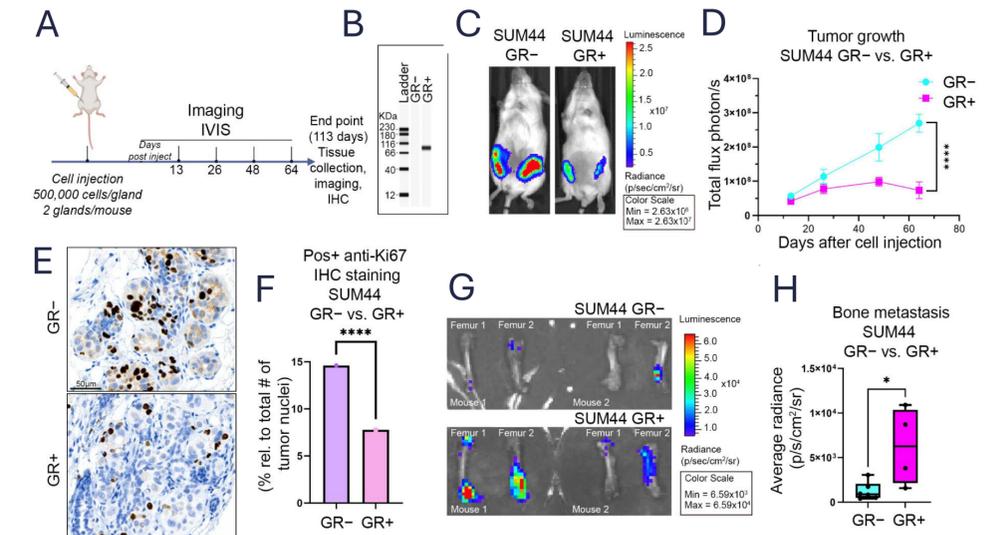
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## Lay abstract

Estrogen receptor (ER)-positive, invasive lobular carcinoma (ILC) is the second most common histological subtype of breast cancer. Although the five-year stage-matched survival is improved compared to infiltrating ductal carcinoma (IDC), ILC late metastatic recurrences (>5 years) are more frequent to bone and brain. Understanding the molecular mechanisms of lobular breast cancer's significantly dormant yet ultimately metastatic phenotype is important to improve clinical outcomes. By examining gene expression following glucocorticoid receptor (GR) activation in metastasis-derived ER+/GR+ human ILC cell lines, we recently uncovered that GR-activation increases integrin expression which may underlie increased GR positive ILC metastasis. Similarly, circulating tumor cells are highly prevalent in lobular carcinoma making it a good model for studying metastasis using the established in vivo xenograft MIND model. We hypothesized that tumor cell GR status may help drive metastasis to secondary organs such as bone and brain. Utilizing in vivo models with ILC cell lines for collection of brain, liver, lung, and femurs at 60, 90, 150 days we can better understand the role of tumor cell GR in early colonization at metastatic sites. Here, we show the presence of tumor cells within the vascularized regions of intact femurs using highly specialized microscopic and tissue clearing techniques. Future studies will include the use of GR modulators in the MIND model to test whether the modulators alter early colonization of GR positive ILC cells.

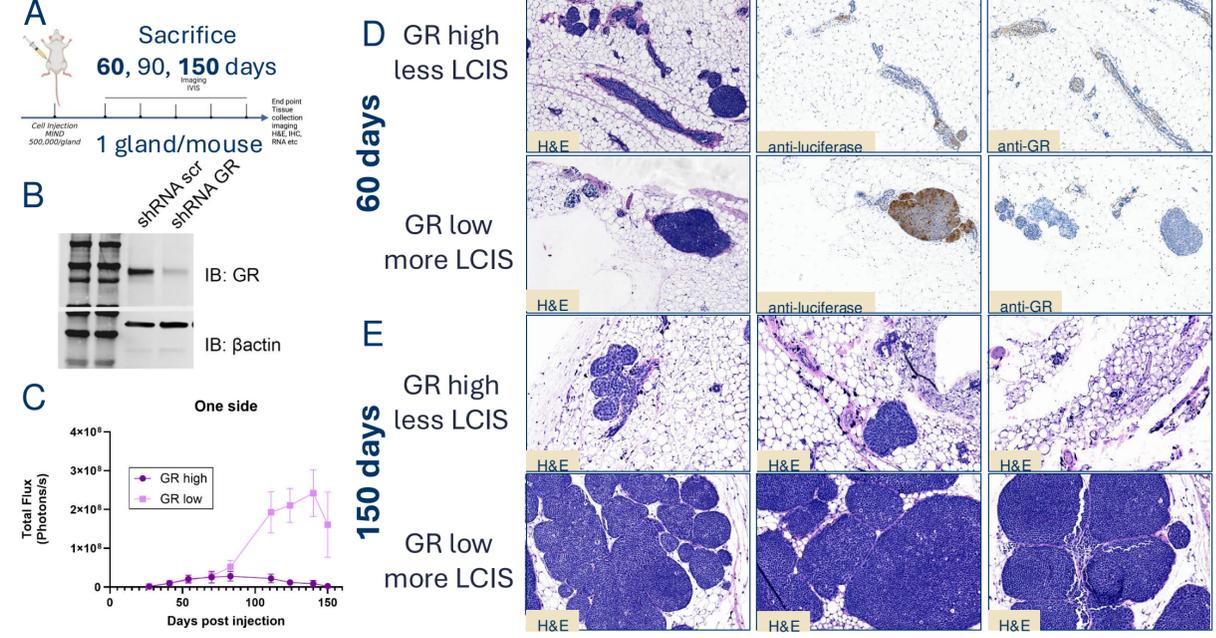
In vivo MIND model reveals that mice engrafted with GR+ ILC cells have increased metastasis to bone

## Objective: Determine how GR affects ILC tumor biology and metastatic phenotypes of disease



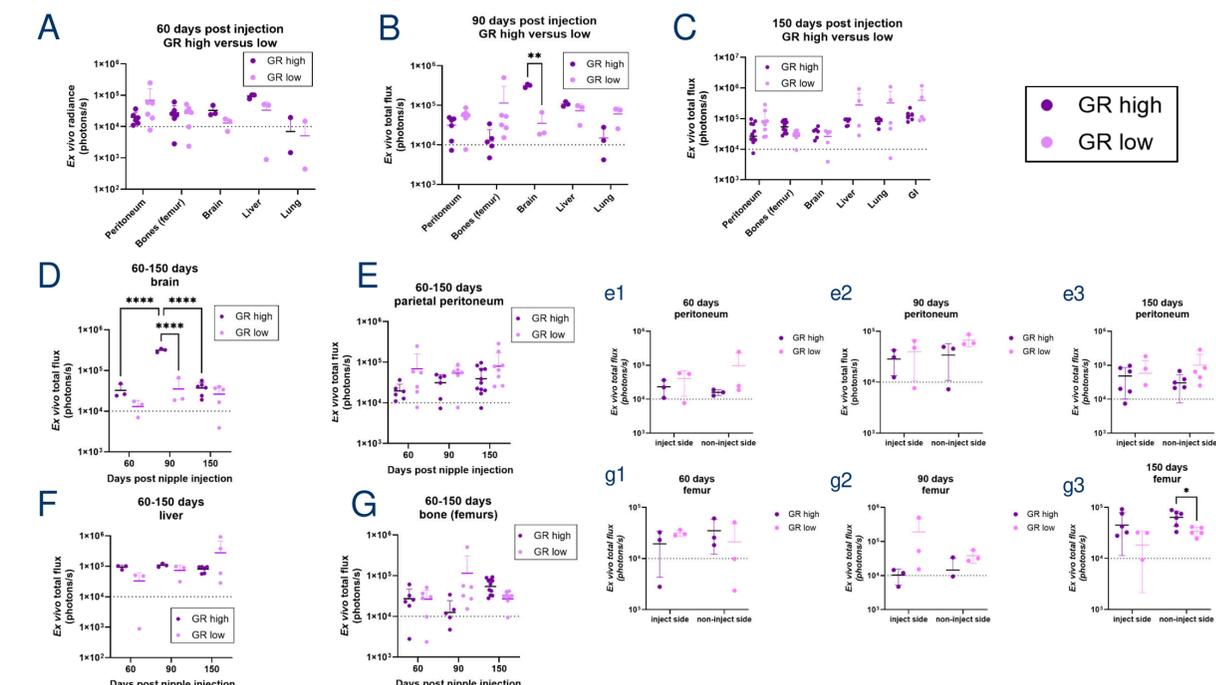
**Figure 1.** A) Schematic illustration of experimental design. B) Immunoblot of SUM44 GR+ or GR null cell line. C) Representative BLI of mice at 64 days post nipple injection, tumor growth shown in D. E) Ki67 proliferative index, quantified in F. G) *Ex vivo* bioluminescence of mice femurs 113 days post injection in SUM44 GR- (top) or SUM44-GR+ (bottom) cells quantified in C.

## Mammary gland tumor 60 & 150 days after nipple injection with GR<sub>high</sub> versus GR<sub>low</sub> ILC cells



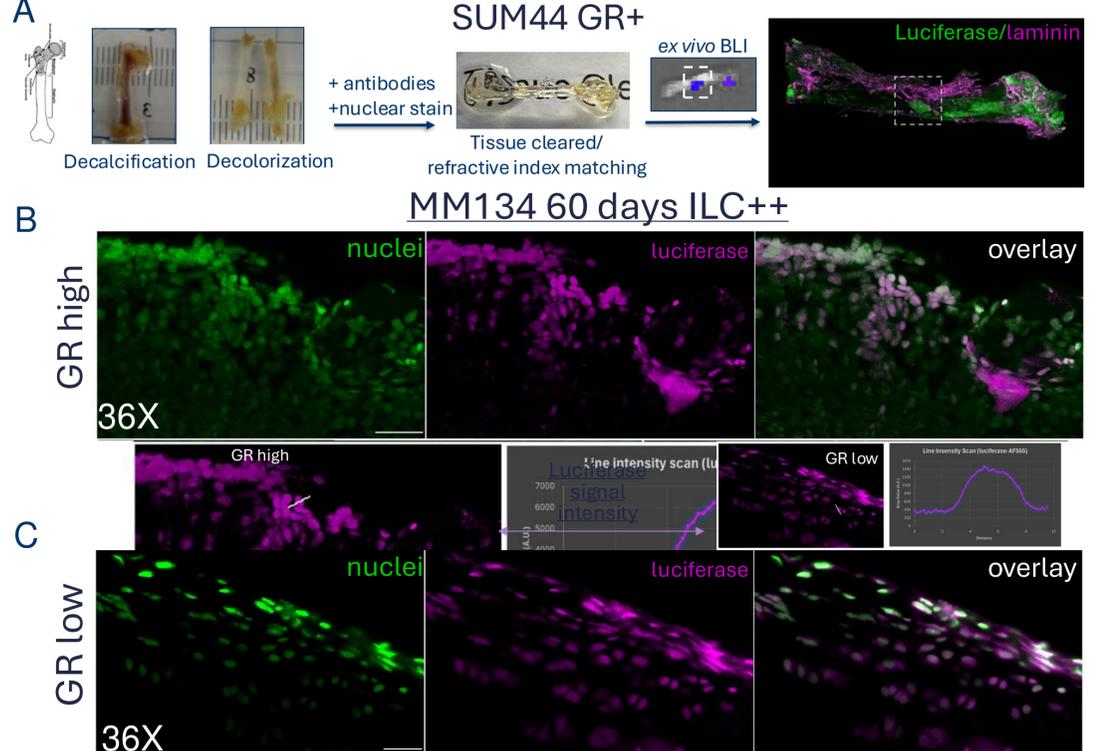
**Figure 2.** A) Experimental design schematic of MIND model time course study. B) Immunoblot of GR KD efficiency in MM134 isogenic cell line. C) Tumor growth plot over 150 days using total flux BLI. D-E) 60 or 150 day mammary gland IHC.

## GR<sub>high</sub> versus GR<sub>low</sub> ex vivo metastasis 60, 90, 150 days post nipple injection with MIND model



**Figure 3.** A-C) 60-150 day *ex vivo* total flux measurements comparing GR KD versus Scrambled MM134 cell line across harvested organs. D-G) *Ex vivo* BLI of brain (D), peritoneum (E, e1-e3), liver (F), and femur (G, g1-g3), 60-150 days.

## BABB Tissue clearing technique for ex vivo ILC bone metastatic evaluation



**Figure 4.** A) Cleared tissue axially swept light sheet microscopic image of ILC SUM44 GR+ cells embedded in bone (Porter et al. 2023) after optimization of BABB tissue clearing method. B-C) MM134 Scrambled (B) or GR KD (C) ILC cells captured using ctALSM in femur 60 days post nipple injection using MIND model.

## Conclusions

- GR expressing ILC cells have reduced LCIS growth in mammary gland compared to GR KD.
- GR expression may change early metastatic colonization phenotype in bone and brain.
- GR expression in ILC MM134 cell line has increased brain metastasis.
- Loss of GR expression may enhance peritoneum metastasis

## Future Studies

Future studies include repeating time course experiment with a second targeting shRNA for GR, performing gene expression analysis on tumor cell isolated from bone marrow to identify GR dependent functional pathways important for metastasis in an in vivo system and testing the effect of GR modulators on tumor growth.

## Funding sources & Acknowledgments

Supported by R01238519, CPRIT RR190037 Scholar Award, Charles Pak Family Cancer & Bone Initiative (CBI) Research Grant CBI-2024-01. REFERENCES: Porter et al. Cancers. 2023.