HOXB7 induces oncogenic transformation in NMuMG cells via JAK2-STAT3 signaling

Mai Sakamoto[1,2,+], Jun Nakayama[1,2,3,+,*], Atsuka Matsui[1], Jiro Fujimoto[1,4], Naoki Goshima[5,6,7], Shinya Watanabe[8], Kentaro Semba[1,9,*]

[1] Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering, Waseda University, Tokyo, Japan.

[2] Computational Bio-Big Data Open Innovation Lab. (CBBD-OIL), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan.

[3] Division of Cellular Signaling, National Cancer Center Research Institute, Tokyo, Japan.

[4] Japan Biological Informatics Consortium (JBic), Tokyo, Japan.

[5] Division of Transcriptome Analysis, Translational Research Center, Fukushima Medical University, Fukushima, Japan.

[6] Functional Proteomics Team, Molecular Profiling Research Center for Drug Discovery (molprof), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan.

[7] Department of Human Sciences, Musashino University, Tokyo, Japan.

[8] Department of Biomolecular Profiling, Translational Research Center, Fukushima Medical University, Fukushima, Japan.

[9] Department of Cell Factory, Translational Research Center, Fukushima Medical University, Fukushima, Japan.

[+] These authors equality contributed

[*] Corresponding authors

Correspondence to: Jun Nakayama (junakaya@ncc.go.jp, jnakayama.re@gmail.com) and Kentaro Semba (ksemba@waseda.jp), Department of Life Science and Medical Bioscience, School of Advanced Science and Engineering, Waseda University, TWIns, 2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo, 162-8480, Japan, Tel/Fax: +81-3-5369-7320.

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Abstract:

The homeobox family genes are often dysregulated in various cancer types. In particular, *HOXB7* overexpression contributes to cancer progression by promoting epithelial to mesenchymal transition, anti-cancer drug resistance, and metastasis of breast, hepatocellular and gastric cancer. Although the relationship between *HOXB7* and cancer progression has been described, the role of *HOXB7* in cancer initiation is unclear. In this study, we showed that *HOXB7* overexpression induced oncogenic transformation *in vitro* and *in vivo* through the activation of JAK-STAT signaling and enhanced the expression of *ERBB2* in NMuMG cells. In public data sets, HER2-positive breast cancer highly expressed *HOXB7*, the expression of which was correlated with poor prognosis in breast cancer cohorts. Furthermore, the amplification of *HOXB7* on 17q23.32 was found to be a potential clinical diagnostic marker.

1. Introduction

Cancer is developed by accumulation of proto-oncogenes and tumor suppressor gene mutations. Oncogenic alterations, such as gene amplifications, mutations, chromosomal translocation, and epigenetic dysregulation, cause the transformation of normal cells and the initiation of tumorigenesis (1). Activated oncogenes induce typical phenotypic changes, so-called transformation, in normal cells such as a NIH-3T3 cells and normal murine mammary gland (NMuMG) cells. Therefore, assay systems evaluating such phenotypic changes, namely focus formation, soft agar colonization, and tumorigenesis have been used for the screening of novel oncogenes in previous research (2-7) .

Most oncogenes cause dysregulation of the signal transduction cascades that relate to proliferation, cell cycle, and survival (8,9). Major oncogenic signaling pathways include RAS-MAPK signaling and PI3K-AKT signaling (10). Recent studies showed that the JAK-STAT signaling is also important in cancer initiation and progression. In the initiation of cancer, the transformation activity of *v-src* oncogene is dependent on JAK-STAT signaling, which is constitutively activated in transformed cells (11). In addition, STAT3 is activated in cancer stem cell-like populations (12), and regulates stemness via fatty metabolism in breast cancer (13). In cancer progression, JAK-STAT signaling coactivates and supports cancer growth and progression with the activation of canonical oncogenic signaling pathways, such as RAS-MAPK and PI3K-AKT in various cancer cell types (14). Furthermore, STAT3 activation contributes to chemoresistance, angiogenesis and immune cell regulation (13,15).

The homeobox (HOX) genes encode transcriptional factors, that controls development and organ homeostasis. The HOX superfamily of homeobox genes (HOXA, HOXB, HOXC and HOXD clusters) genes are often dysregulated in breast, prostate, colon, and lung cancer. The differential dysregulation of HOX family genes in various solid tumors provides an opportunity to understand cancer development (16,17). The HOXB clusters is composed of *HOXB1*, *HOXB2*, *HOXB3*, *HOXB4*, *HOXB6*, *HOXB7*, *HO*XB8, *HOXB9*, and *HOXB13* at 17q23.32. HOXB cluster genes are co-amplified by gene amplification in several types of cancer. Notably, overexpression of *HOXB7* contributes to cancer progression and metastasis in breast, hepatocellular, oral, and gastric cancer (18-20). *HOXB7* induces the activation of TGFß signaling, epithelial to mesenchymal transition (EMT) and migration in breast cancer (21,22). High expression of *HOXB7* in hepatocellular carcinoma leads to the proliferation, invasion and metastasis (23). In addition, the luminal subtype breast cancer cell line

MCF7 acquires tamoxifen resistance via *HOXB7* expression (24). In oral cancer, overexpression of *HOXB7* is associated with poor prognosis (25). *HOXB7* as a cofactor of Estrogen Receptor (ER) causes cancer progression through the expression of downstream genes (26). Thus, identification of the specific signal transduction and drug target molecules downstream of *HOXB7* are required to further understand cancer.

Although the relationships between *HOXB7* and breast cancer progression has been reported, the role of *HOXB7* in cancer initiation is unclear. In this study, we focused on the oncogenic function of *HOXB7* in cancer initiation. For this purpose, we performed transformation assays and signal analysis in a normal mammary epithelial cell line, NMuMG, and re-analyzed the public breast cancer cohorts with *HOXB7* expression.

2. Materials and methods

2.1. Cell culture

Normal murine mammary gland (NMuMG) cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Fujifilm Wako, Osaka Japan) supplemented with 10% fetal bovine serum (FBS; NICHIREI BIOSCIENCES INC., Tokyo, Japan), 100 U/mL penicillin G (Meiji-Seika Pharma Co., Ltd., Tokyo, Japan), 100 µg/mL streptomycin (Meiji-Seika Pharma Co., Ltd.), 10 µg/mL insulin (Fujifilm Wako), and 4.5% glucose (Fujifilm Wako) at 37°C with 5% CO_2. Plat-E cells for retroviral packaging were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin G, and 100 µg/mL streptomycin at 37°C with 5% CO_2. Cells were treated with 0.1-1 µM ruxolitinib (LC Laboratories, MA, USA) under experimental conditions.

2.2. Retroviral packaging and infection

Plat-E cells were seeded int 6-well plates and transfected with pMXs-Venus-IRES-Puromycin^R and pMXs-*HOXB7*-IRES-Puromycin^R plasmid DNAs using 6 µg polyethylenimine (PEI; Polysciences, Inc., PA, USA). Two days later, supernatant culture media were collected from transfected Plat-E cells for retroviral infection. NMuMG-Venus and NMuMG-HOXB7 cell lines were established by retroviral infection using the cell supernatants and 800 µg/mL polybrene (SIGMA ALDRICH Co., MO, USA), as

previously described (27). To select the expressing cells, 1 μg/mL puromycin (Fujifilm Wako) was added to the culture medium for 2 days.

2.3. Western blotting

The infected cell lines were lysed with SDS sampling buffer (3.5 % SDS, 12% glycerol, 0.05M Tris-HCl (pH 6.8), 0.003% bromo phenol blue, and 5% 2-mercaptoethanol) for western blotting. Western blotting was performed as previously described (3,28) with the antibodies shown in Supplementary Table 1.

2.4. Cell proliferation assay

A total of 5.0×10^5 NMuMG cells were seeded into a 12-well plate. Cells were harvested with 0.25% trypsin (Thermo Fisher Scientific, MA, USA) and counted at each time point (24, 48 and 72 h) (28,29).

2.5. Transformation assay *in vitro*

For focus formation assay, 7.0×10^4 parental NMuMG cells and 3.5×10^4 HOXB7-expressing NMuMG (NMuMG-HOXB7) cells were mixed and seeded into 12-well plates. Mixed cells were cultured for more than 10 days. For soft agar assay, cells were stained with 0.01% crystal violet/70% methanol to detect the cellular foci. A total of 4.0×10^4 NMuMG cells in 0.37% agarose on the 0.53% bottom agarose were cultured in a 6-well plate for 1 month, as previously described (3).

2.6. Tumorigenesis assay

A total of 1.0×10^6 NMuMG cells were transplanted into 6-8-weeks-old *Rag2*$^{-/-}$ female mice with 100 μL 50% Matrigel (BD Bioscience, CA, USA) as previously described (4). All animal experiments were approved by the Animal Committee of Waseda University (WD19-058, 2019-A068).

2.7. qRT-PCR

Total RNA was extracted using ISOGEN (NIPPON GENE Co., LTD, Tokyo, Japan). cDNA synthesis was performed using the Superscript III first-strand synthesis system (Thermo Fisher Scientific). Quantitative RT-PCR was performed using THUNDER BIRD SYBR qPCR mix (TOYOBO, Osaka,

Japan) and 18S rRNA was used as a relative control. Primers are listed in Supplementary Table 2.

2.8. Clinical database analysis

The genomic profiles and RNA-seq datasets of The Cancer Genome Atlas (TCGA) breast cancer (BRCA) cohorts were subjected to OncoPrint and expression analyses. OncoPrint analyses of *HOXB7* and *ERBB2* was conducted using cBioPortal (30,31). The Kaplan-Meier method was used perform survival analyses of the molecular taxonomy of breast cancer international consortium (METABRIC) (32-34), GSE1456 (35) and GSE9893 (36) data sets, as previously described (28,29,37).

2.9. Statistical analysis

Welch's t-test, Fisher's exact test and log-rank test were performed using R 3.4.1 (https://www.r-project.org/).

3. Results

3.1. HOXB7 induced oncogenic transformation and an EMT-like phenotype in NMuMG cells

We first examined the oncogenic phenotype with HOXB7 expression. HOXB7 was overexpressed in NMuMG cells by retroviral infection. We confirmed the protein levels of vimentin were increased by HOXB7 overexpression (Figure 1A). However, the protein levels of E-cadherin and N-cadherin did not change by HOXB7 expression. The morphology of NMuMG-HOXB7 cells were changed to a spindle-like shape compared with that of Venus-expressing NMuMG cells (Figure 1B). These results showed that HOXB7 expression induced upregulation of a mesenchymal marker of the epithelial to mesenchymal transition (EMT) in NMuMG cells.

Next, we performed focus formation assays and soft agar colony formation assays to examine the *in vitro* transformation activity of HOXB7. HOXB7 expression induced focus-forming activity (Figure 2A). However, colony formation activity was not induced by HOXB7 expression (Supplementary Figure 1). In contrast to the lack of colony-forming activity, NMuMG-HOXB7 cells formed tumors when subcutaneously injected into *Rag2*$^{-/-}$ mice (Figure 2B and C). Our results

suggested that HOXB7 induced transforming activities *in vitro* and *in vivo* as well as a partial EMT-like phenotype in NMuMG cells.

3.2. JAK-STAT signaling induced oncogenic transformation by HOXB7 in NMuMG cells.

To identify the oncogenic signaling pathway of HOXB7, we performed western blotting using antibodies against major cancer-related signaling proteins. HOXB7-expressing cells drastically increased Stat3 phosphorylation and Jak1, Jak2, Stat3 and, Erbb2 expression (Figure 3A). Major proliferative signals, such as Erk and Akt, were not changed by HOXB7 expression. Stat3 and Erbb2 expression were increased at the transcriptional level, while Jak2 was increased at the protein level (Figure 3B).

Ruxolitinib is a kinase inhibitor of JAK1/2 that suppresses the phosphorylation of STAT3. P-STAT3 induced by HOXB7 expression was completely inhibited by ruxolitinib treatment in a dose-dependent manner (Figure 4A, Supplementary Figure 2). Lapatinib, which inhibits the activation of ERBB2, decreased p-Erk and partially p-Stat3 in HOXB7-expressing cells (Figure 4B), suggesting its upstream involvement in the phosphorylation of STAT3. The protein levels of Stat3, Jak2, and Erbb2 were not changed by ruxolitinib treatment, indicating that the transcriptional regulation of Stat3 and Erbb2 was not downstream of Stat3 signaling. Next, to elucidate the contribution of the oncogenic activity of p-Stat3, we performed *in vitro* proliferation assays and focus formation assays following ruxolitinib treatment. HOXB7 decreased the proliferative activity of NMuMG cells *in vitro*; however, ruxolitinib treatment did not affect the proliferative activity of HOXB7-expressing cells (Figure 4C), while inhibiting their focus-forming activity (Figure 4D, Supplementary Figure 3). These results suggested that the activation of JAK-STAT signaling and induction of ERBB2 by HOXB7 induced cellular transformation.

3.3. HOXB7 correlated to poor prognosis in the HER2 positive breast cancer patients.

To elucidate the clinical significance of HOXB7 in breast cancer, we first analyzed RNA-seq data of TCGA BRCA cohorts for *HOXB7* expression. A comparative analysis of normal tissues and breast tumor tissues showed no differences in *HOXB7* expression (Figure 5A). However, expression analysis by breast cancer subtype revealed that the HER2-positive subtype had significantly highly expressed *HOXB7* compared with other subtypes and normal tissue (Figure 5B). To further examine

the genomic profiles of *HOXB7* in TCGA BRCA cohorts, the mutational status of *ERBB2* and *HOXB7* was illustrated as an OncoPrint by cBioPortal. *HOXB7* was amplified in 48 patients (5% of TCGA BRCA). In addition, *HOXB7* was significantly co-amplified with *ERBB2* in 30 patients (Figure 5C, Supplementary Figure 4). These results suggested that *HOXB7* was preferentially amplified and expressed in HER2-positive breast cancer patients.

Next, to examine the relationship between *HOXB7* expression and progression in breast cancer, we performed a survival analysis using several public clinical data sets. In METABRIC cohorts, high expression of *HOXB7* was correlated with poor prognosis in all breast cancer patients (Figure 6A) and in HER2-high patients (Figure 6B). The correlation of poor prognosis and *HOXB7* expression was further confirmed by an analysis of other public breast cancer cohorts that included all subtypes (Figure 6C).

4. Discussion

HOX family genes regulate the development and homeostasis as transcriptional factors (16,17,38). Several HOX genes contribute to cancer malignancy. Notably, accumulating evidence suggests that *HOXB7* is involved in the malignancy of the breast, prostate, hepatocellular and gastric cancer. *HOXB7* overexpression in MCF7 cells (luminal A subtype) promoted acquired resistance to tamoxifen via activation of receptor tyrosine kinases and ERα signaling (24,26). In addition, HOXB7 induced EMT and migration/invasion activity in MCF10A normal human mammary epithelial cells (21). HOXB7 promoted malignant progression through activation of the TGFß signaling in a MMTV-hoxb7/Her2 transgenic mouse model (22). Although *HOXB7* is often amplified in breast cancer patients, the precise mechanism of *HOXB7* in breast cancer initiation is unclear. In this study, we demonstrated that *HOXB7* induced cellular transformation through activation of the JAK-STAT pathway and suggested its role in the poor prognosis of HER2-positive patients.

First, we showed that *HOXB7* induced cellular transformation *in vitro* and tumor formation *in vivo* of normal mammary epithelial cells. *HOXB7* increased the protein levels of Jak2 and Stat3, and concomitantly increased the amount of p-STAT3. Treatment with ruxolitinib, a JAK1/2 inhibitor, suppressed Stat3 phosphorylation and cellular transformation, suggesting the involvement of the

JAK-STAT pathway in HOXB7-mediated transformation. In addition, Jak2 phosphorylation was upregulated by ruxolitinib treatment. This phenomenon was observed in previous research (39), as well as following treatment with pacritinib, another JAK2 inhibitor (40), although the molecular mechanism of p-Jak2 upregulation remains unclear. In general, transcription factors such as HOXB7 are not suitable for molecular targeted therapy. However, it should be noted that HOXB7 activates JAK-STAT signaling. The JAK-STAT pathway is activated in several cancers and is believed to be involved in tumorigenesis. For this reason, kinase inhibitors against JAK have been developed. Even though HOXB7 itself cannot be targeted, a crucial pathway downstream of JAK-STAT signaling may be targeted against cancer with HOXB7 aberrations.

Second, we found that *HOXB7* upregulated the expression of Erbb2. High expression of *ERBB2* is important for the carcinogenesis of HER2-positive breast cancer (41-43). Additionally, previous study showed that *HOXB7* expression induces upregulation of *ERBB2* in MCF7 cells (26). Our results thus suggested that amplification of *HOXB7* has a synergistic effect with the *ERBB2* amplification, and promotes carcinogenesis in HER2-positive breast cancer. In fact, *HOXB7* is preferentially expressed in HER2-positive breast cancer patients. *ERBB2* is a prognostic marker as well as a driver oncogene in HER2-positive cancer. Analysis of clinical data suggests that co-amplification of *HOXB7* with *ERBB2* worsens the prognosis of HER2-positive breast cancer patients.

Our study proposes the importance of the JAK-STAT pathway in *HOXB7*-mediated transformation, indicating that HOXB7 could be an additional prognostic marker of HER2-positive cancer and a potential target for combination therapy with HER2 and JAK inhibitors in HER2- and HOXB7-positive breast cancer patients.

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Authors' contributions

J.N. and K.S. conceived and designed the study. M.S., J.N., and A.M. performed the experiments. M.S. and J.N. analyzed the data. J.F., N.G., S.W. and K.S. interpreted the data. J.N. wrote the manuscript. All authors reviewed and edited the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

1. Hanahan, D., *et al.* (2011) Hallmarks of cancer: the next generation. *Cell*, **144**, 646-74.
2. Saito, M., *et al.* (2012) Expression screening of 17q12-21 amplicon reveals GRB7 as an ERBB2-dependent oncogene. *FEBS Lett*, **586**, 1708-14.
3. Matsui, A., *et al.* (2016) Hepatocyte nuclear factor 1 beta induces transformation and epithelial-to-mesenchymal transition. *FEBS Lett*, **590**, 1211-21.
4. Ihara, T., *et al.* (2017) An in vivo screening system to identify tumorigenic genes. *Oncogene*, **36**, 2023-2029.
5. Hatanaka, H., *et al.* (2007) Transforming activity of purinergic receptor P2Y, G-protein coupled, 2 revealed by retroviral expression screening. *Biochem Biophys Res Commun*, **356**, 723-6.
6. Miyamoto, D., *et al.* (2008) Isolation of a distinct class of gain-of-function SHP-2 mutants with oncogenic RAS-like transforming activity from solid tumors. *Oncogene*, **27**, 3508-15.
7. Alvarez, A., *et al.* (2014) Focus formation: a cell-based assay to determine the oncogenic potential of a gene. *J Vis Exp*, e51742.
8. Kolch, W., *et al.* (2015) The dynamic control of signal transduction networks in cancer cells. *Nat Rev Cancer*, **15**, 515-27.
9. Shortt, J., *et al.* (2012) Oncogenes in cell survival and cell death. *Cold Spring Harb Perspect Biol*, **4**.
10. Hoxhaj, G., *et al.* (2020) The PI3K-AKT network at the interface of oncogenic signalling and cancer metabolism. *Nat Rev Cancer*, **20**, 74-88.
11. Bromberg, J.F., *et al.* (1998) Stat3 activation is required for cellular transformation by v-src. *Mol Cell Biol*, **18**, 2553-8.
12. Marotta, L.L., *et al.* (2011) The JAK2/STAT3 signaling pathway is required for growth of $CD44^{+}CD24^{-}$ stem cell-like breast cancer cells in human tumors. *J Clin Invest*, **121**, 2723-35.
13. Wang, T., *et al.* (2018) JAK/STAT3-Regulated Fatty Acid β-Oxidation Is Critical for Breast Cancer Stem Cell Self-Renewal and Chemoresistance. *Cell Metab*, **27**, 136-150.e5.
14. Harada, D., *et al.* (2014) The Role of STAT3 in Non-Small Cell Lung Cancer. *Cancers (Basel)*, **6**, 708-22.
15. Bishop, J.L., *et al.* (2014) The Multifaceted Roles of STAT3 Signaling in the Progression of Prostate Cancer. *Cancers (Basel)*, **6**, 829-59.
16. Bhatlekar, S., *et al.* (2014) HOX genes and their role in the development of human cancers. *J Mol Med (Berl)*, **92**, 811-23.
17. Li, B., *et al.* (2019) The Role of HOX Transcription Factors in Cancer Predisposition and Progression. *Cancers (Basel)*, **11**.
18. Wang, W.M., *et al.* (2017) HOXB7 promotes tumor progression via bFGF-induced activation of MAPK/ERK pathway and indicated poor prognosis in hepatocellular carcinoma. *Oncotarget*, **8**, 47121-47135.
19. He, X., *et al.* (2017) HOXB7 overexpression promotes cell proliferation and correlates with poor prognosis in gastric cancer patients by inducing expression of

both AKT and MARKs. *Oncotarget*, **8**, 1247-1261.
20. Errico, M.C., *et al.* (2016) The Widening Sphere of Influence of HOXB7 in Solid Tumors. *Cancer Res*, **76**, 2857-62.
21. Wu, X., *et al.* (2006) HOXB7, a homeodomain protein, is overexpressed in breast cancer and confers epithelial-mesenchymal transition. *Cancer Res*, **66**, 9527-34.
22. Liu, S., *et al.* (2015) HOXB7 promotes malignant progression by activating the TGFß signaling pathway. *Cancer Res*, **75**, 709-19.
23. Huan, H.B., *et al.* (2017) HOXB7 accelerates the malignant progression of hepatocellular carcinoma by promoting stemness and epithelial-mesenchymal transition. *J Exp Clin Cancer Res*, **36**, 86.
24. Jin, K., *et al.* (2012) The HOXB7 protein renders breast cancer cells resistant to tamoxifen through activation of the EGFR pathway. *Proc Natl Acad Sci U S A*, **109**, 2736-41.
25. De Souza Setubal Destro, M.F., *et al.* (2010) Overexpression of HOXB7 homeobox gene in oral cancer induces cellular proliferation and is associated with poor prognosis. *Int J Oncol*, **36**, 141-9.
26. Jin, K., *et al.* (2015) HOXB7 Is an ERα Cofactor in the Activation of HER2 and Multiple ER Target Genes Leading to Endocrine Resistance. *Cancer Discov*, **5**, 944-59.
27. Nakayama, J., *et al.* (2017) Comparative analysis of gene regulatory networks of highly metastatic breast cancer cells established by orthotopic transplantation and intra-circulation injection. *Int J Oncol*, **50**, 497-504.
28. Kuroiwa, Y., *et al.* (2020) Proliferative Classification of Intracranially Injected HER2-positive Breast Cancer Cell Lines. *Cancers (Basel)*, **12**, 1811.
29. Han, Y., *et al.* (2020) Establishment and characterization of highly osteolytic luminal breast cancer cell lines by intracaudal arterial injection. *Genes Cells*, **25**, 111-123.
30. Cerami, E., *et al.* (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*, **2**, 401-4.
31. Gao, J., *et al.* (2013) Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*, **6**, pl1.
32. Curtis, C., *et al.* (2012) The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*, **486**, 346-52.
33. Pereira, B., *et al.* (2016) The somatic mutation profiles of 2,433 breast cancers refine their genomic and transcriptomic landscapes. *Nat Commun*, **7**, 11479.
34. Ali, H.R., *et al.* (2020) Imaging mass cytometry and multiplatform genomics define the phenogenomic landscape of breast cancer. *Nat Cancer*, **1**, 163-175.
35. Pawitan, Y., *et al.* (2005) Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. *Breast Cancer Res*, **7**, R953-64.
36. Chanrion, M., *et al.* (2008) A gene expression signature that can predict the recurrence of tamoxifen-treated primary breast cancer. *Clin Cancer Res*, **14**, 1744-52.
37. Yogosawa, S., *et al.* (2021) Carbonic anhydrase 13 suppresses bone metastasis in

breast cancer. *Cancer Treat Res Commun*, **27**, 100332.
38. Rezsohazy, R., *et al.* (2015) Cellular and molecular insights into Hox protein action. *Development*, **142**, 1212-27.
39. Lee, S., *et al.* (2018) Ruxolitinib significantly enhances in vitro apoptosis in Hodgkin lymphoma and primary mediastinal B-cell lymphoma and survival in a lymphoma xenograft murine model. *Oncotarget*, **9**, 9776-9788.
40. Hart, J.R., *et al.* (2011) Essential role of Stat3 in PI3K-induced oncogenic transformation. *Proc Natl Acad Sci U S A*, **108**, 13247-52.
41. Moasser, M.M. (2007) The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene*, **26**, 6469-87.
42. Feng, Y., *et al.* (2018) Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes Dis*, **5**, 77-106.
43. Oh, D.Y., *et al.* (2020) HER2-targeted therapies - a role beyond breast cancer. *Nat Rev Clin Oncol*, **17**, 33-48.

Figure legends

Figure 1. HOXB7 induced an EMT-like phenotype in NMuMG cells.

(A) Western blotting analysis of HOXB7-expressing NMuMG cells. Venus-expressing NMuMG cells were used as a negative control. (B) Bright imaging of HOXB7-expressing cells. (C) Western blotting analysis of an epithelial marker and mesenchymal markers in HOXB7-expressing NMuMG cells.

Figure 2. HOXB7 expression induced transformation and tumorigenesis.

(A) Focus formation assay in HOXB7-expressing NMuMG cells. Left: crystal violet staining. Right: bright field focus. (B) Tumor growth curve with volume. HOXB7-expressing NMuMG cells formed tumors following subcutaneously transplantation of *Rag2*$^{-/-}$ mice (n= 10). (C) Image of HOXB7-positve tumor.

Figure 3. JAK-STAT signaling and ERBB2 signaling were increased by HOXB7 expression.

(A) Western blotting of HOXB7-expressing cells. (B) qRT-PCR of *Stat3*, *Erbb2*, and *Jak2*. Welch's t-test (* $p < 0.001$, $n = 3$).

Figure 4. Transformation by HOXB7 was regulated by Stat3 activation.

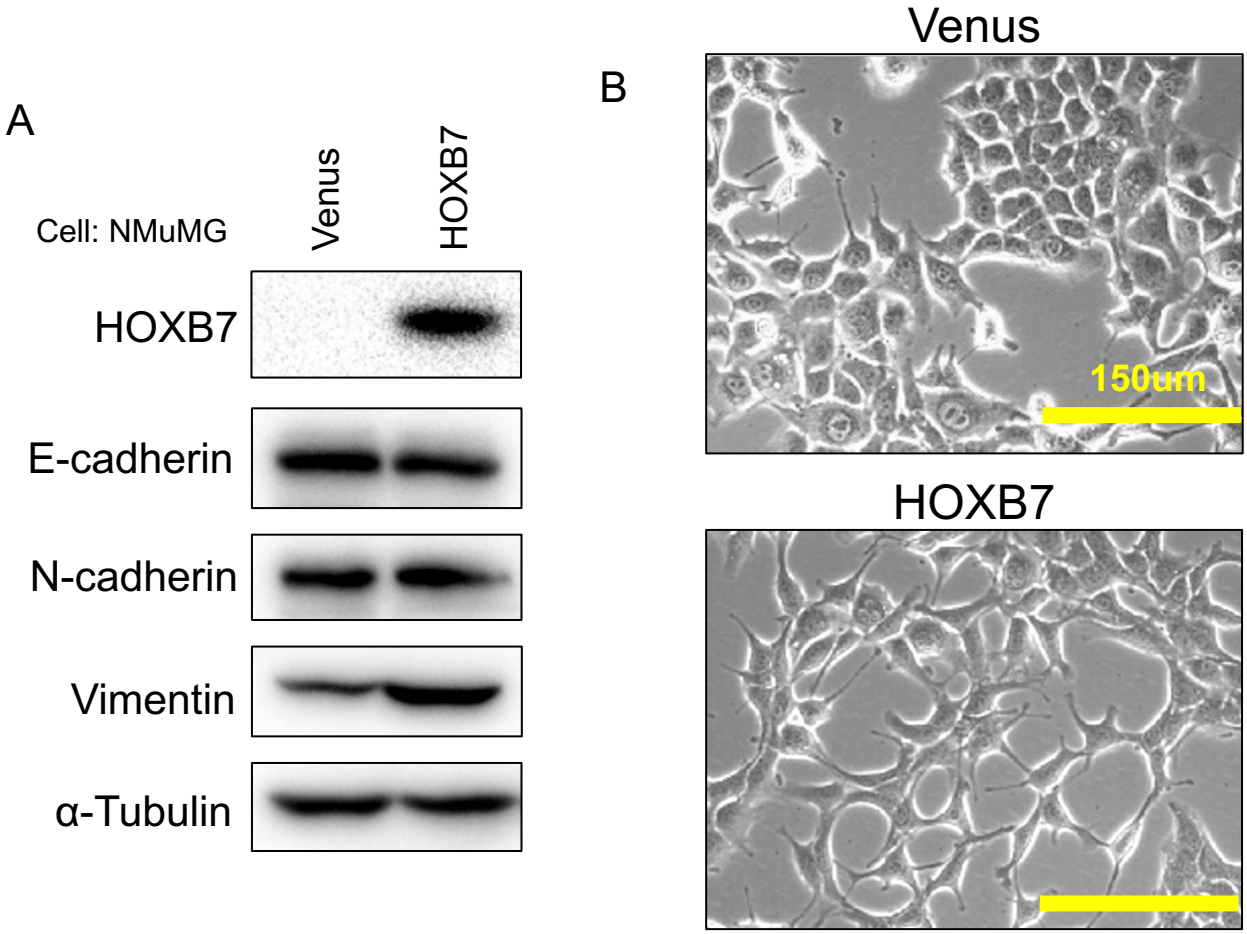
(A) Signal analysis following ruxolitinib treatment of HOXB7-expressing NMuMG cells. (B) Signal analysis following lapatinib treatment of HOXB7-expressing NMuMG cells. (C) *In vitro* growth curve of HOXB7-expressing NMuMG cells treated with ruxolitinib. (n.s.: Not significant, n= 3) (D) Focus formation assay of HOXB7-expressing NMuMG cells treated with ruxolitinib. Foci were stained with crystal violet.

Figure 5. Genomic analysis of TCGA BRCA cohorts.

(A) Comparative expression analysis of *HOXB7* in normal tissue ($n = 112$) and breast cancer tissue ($n = 1093$). Welch's t-test (n.s.: Not significant). (B) Comparative expression analysis of *HOXB7* in breast cancer subtypes of TCGA. Luminal A ($n = 499$), luminal B ($n = 197$), HER2-positive ($n = 78$), basal-like ($n = 171$), normal-like ($n = 36$). Welch's t-test ($p < 0.005$). (C) OncoPrint of *ERBB2* and *HOXB7* genomic status in TCGA.

Figure 6. High expression of HOXB7 correlated to poor prognosis in breast cancer patients. (A) Survival analysis of all METABRIC cohorts with *HOXB7* expression and the overall survival status shown in a Kaplan-Meier plot. Shading along the curves shows 95% confidential interval. Log-rank test ($p = 0.0056$) (B) METABRIC cohorts with high HER2 expression (476 patients; Top 25% of all METABRIC cohorts) Log-rank test ($p = 0.0088$) (C) Survival analysis of other breast cancer cohorts (GSE1456; disease specific survival; GSE9893; overall survival). GSE1456, log-rank test ($p = 0.00022$); GSE9893, log-rank test ($p < 0.0001$).

Figure 1


A
Cell: NMuMG
Venus
HOXB7
HOXB7
E-cadherin
N-cadherin
Vimentin
α-Tubulin
B
Venus
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HOXB7

Figure 2

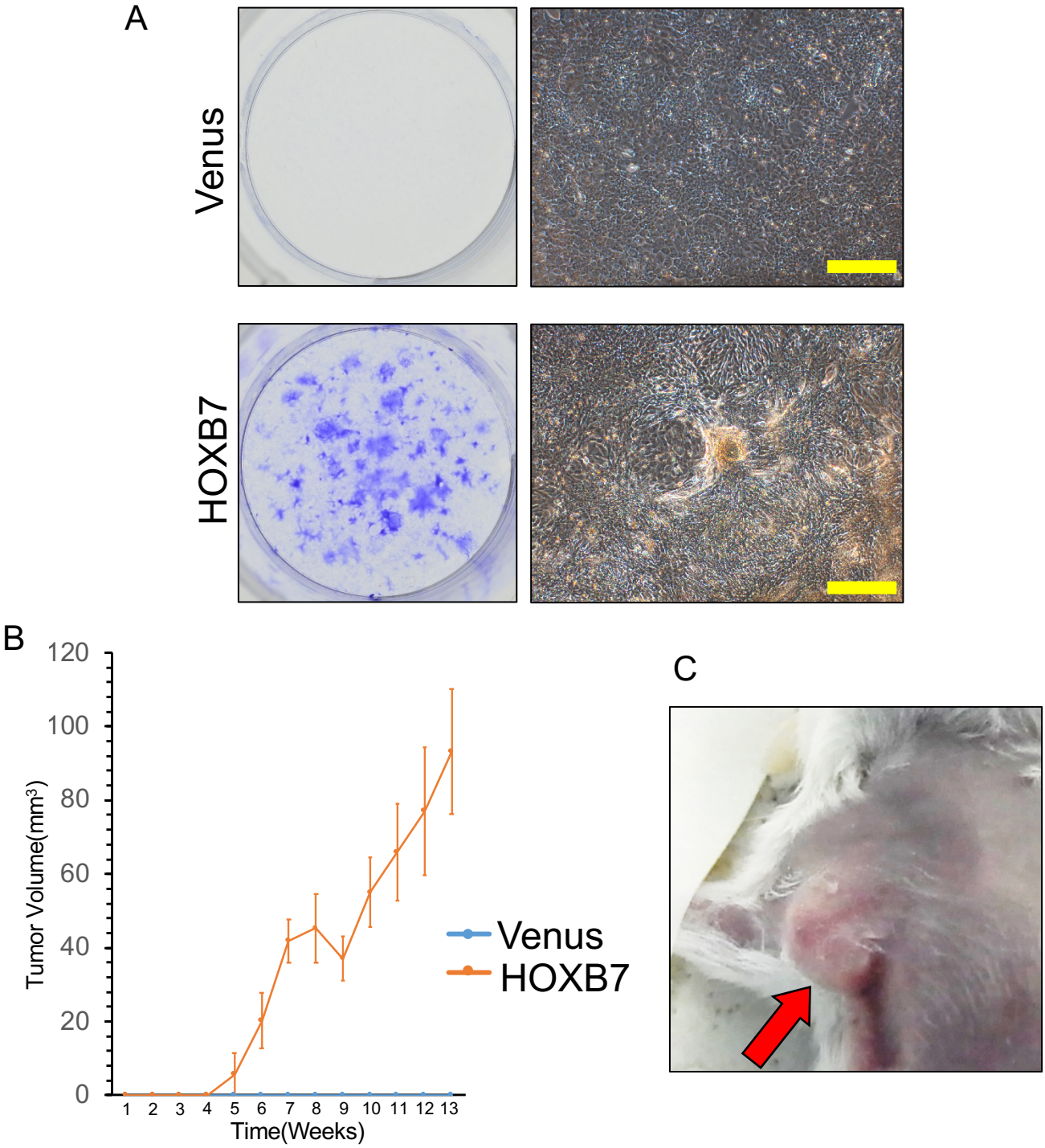

A
Venus
HOXB7
B
Tumor Volume(mm³)
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13
Time(Weeks)
Venus
HOXB7
C

Figure 3

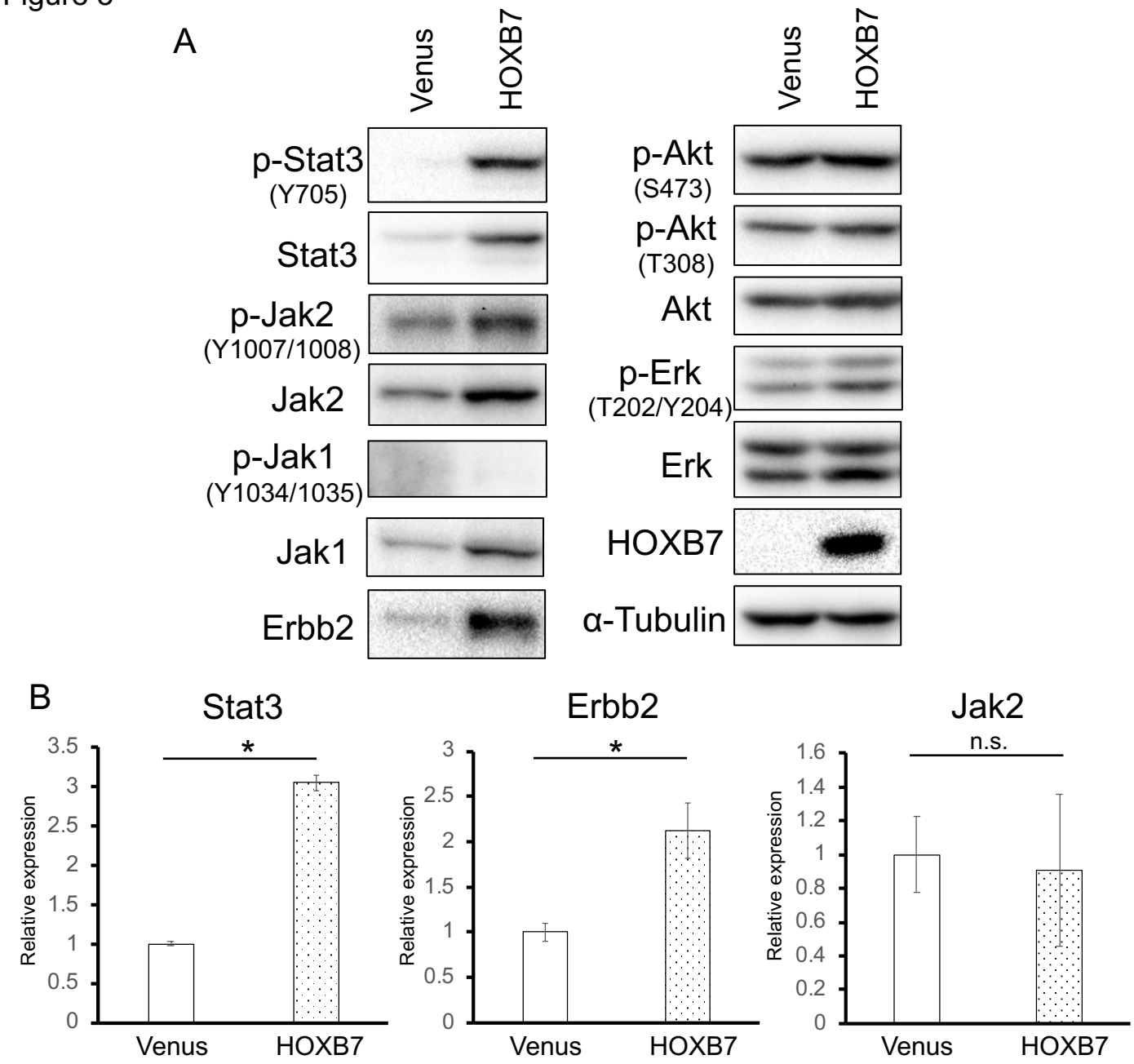
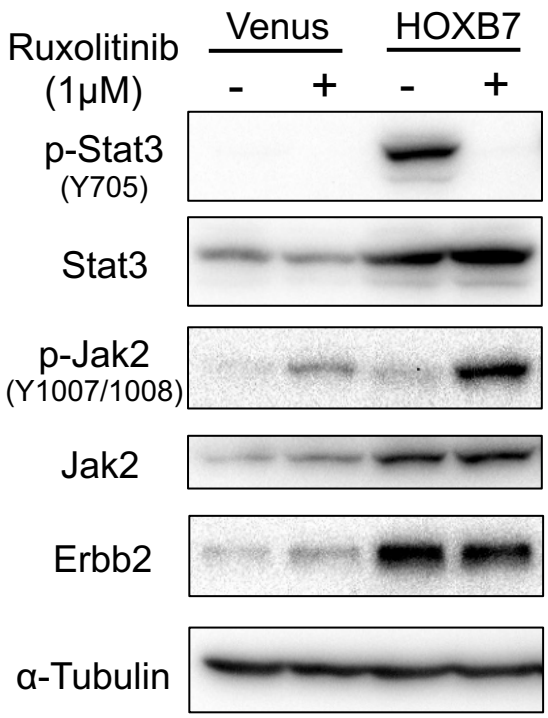
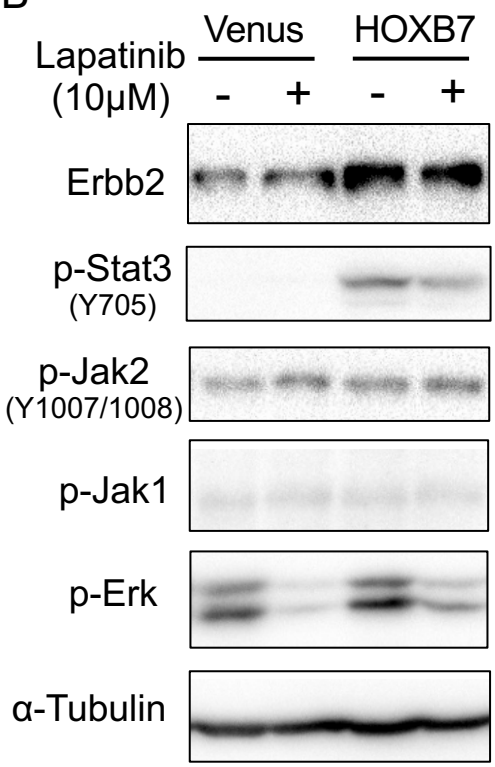

A
Venus
HOXB7
p-Stat3
(Y705)
Stat3
p-Jak2
(Y1007/1008)
Jak2
p-Jak1
(Y1034/1035)
Jak1
Erbb2
p-Akt
(S473)
p-Akt
(T308)
Akt
p-Erk
(T202/Y204)
Erk
HOXB7
α-Tubulin
B
Stat3
Erbb2
Jak2
Relative expression
*
n.s.
Venus
HOXB7

Figure 4

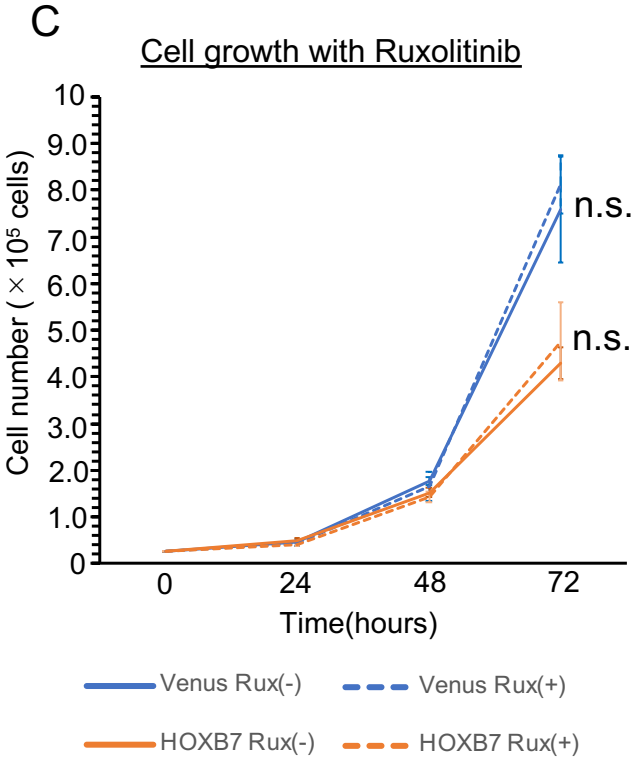
A



B



C



D

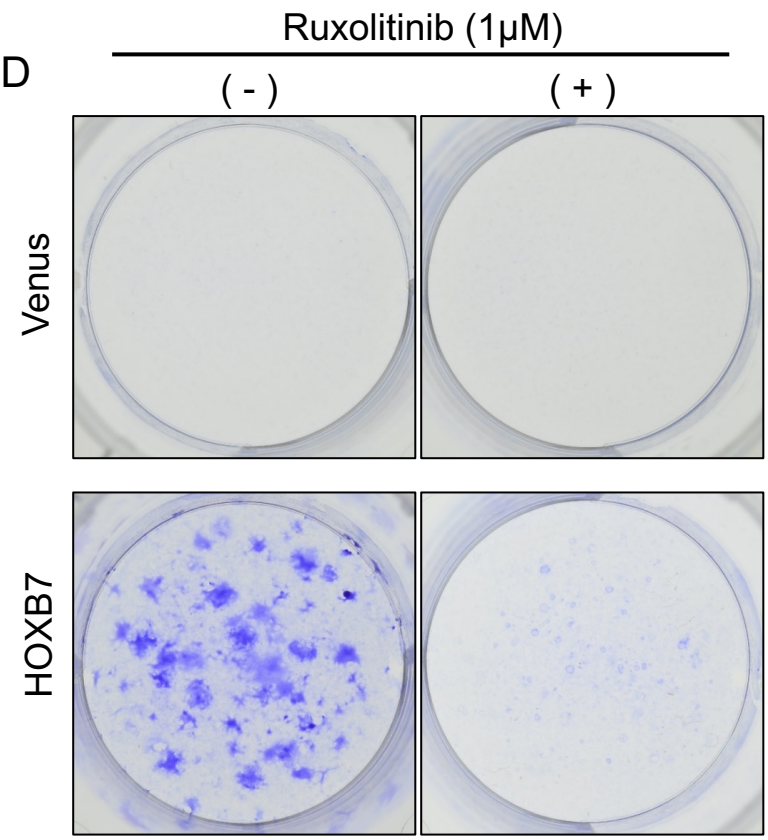


Figure 5

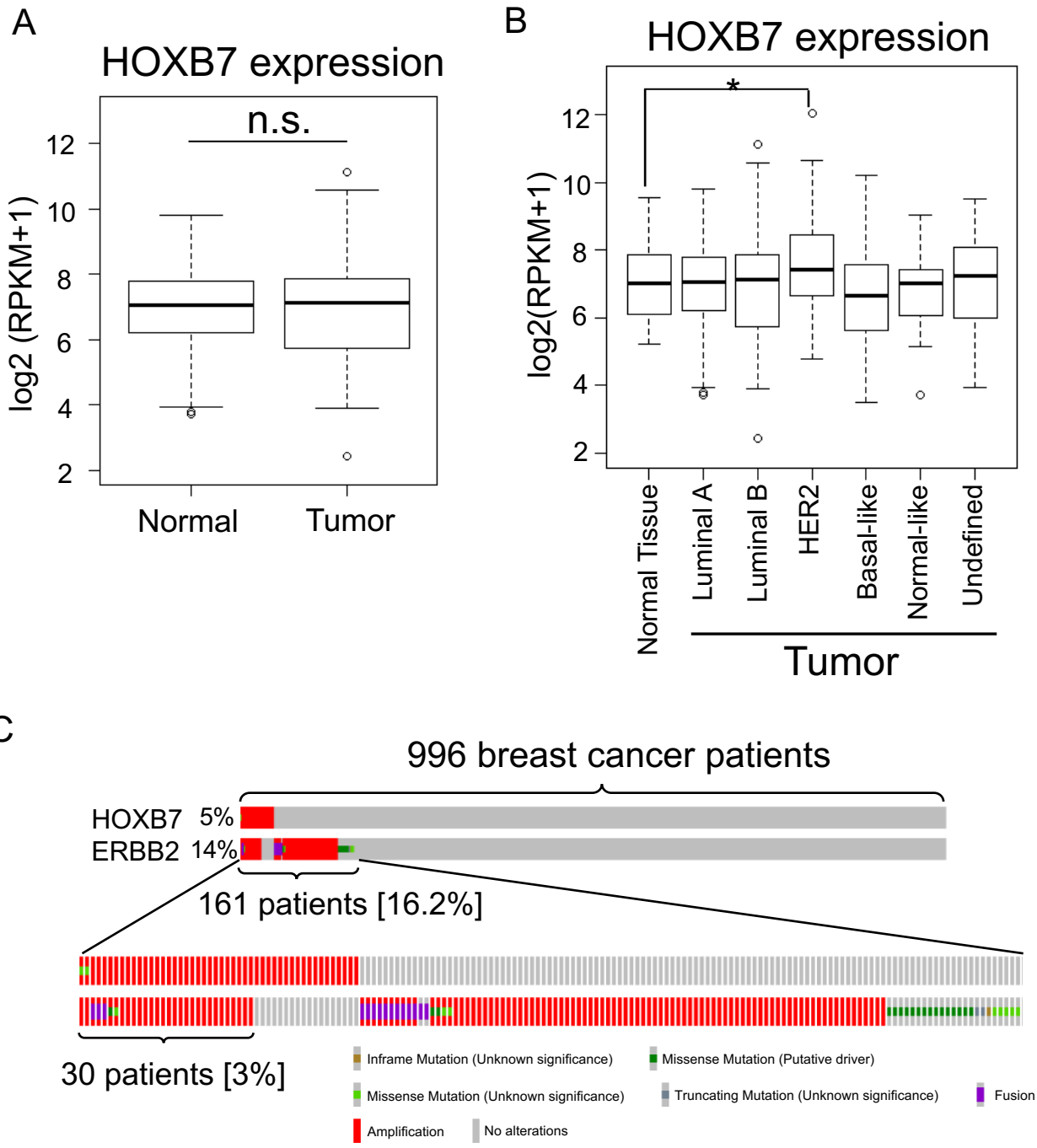
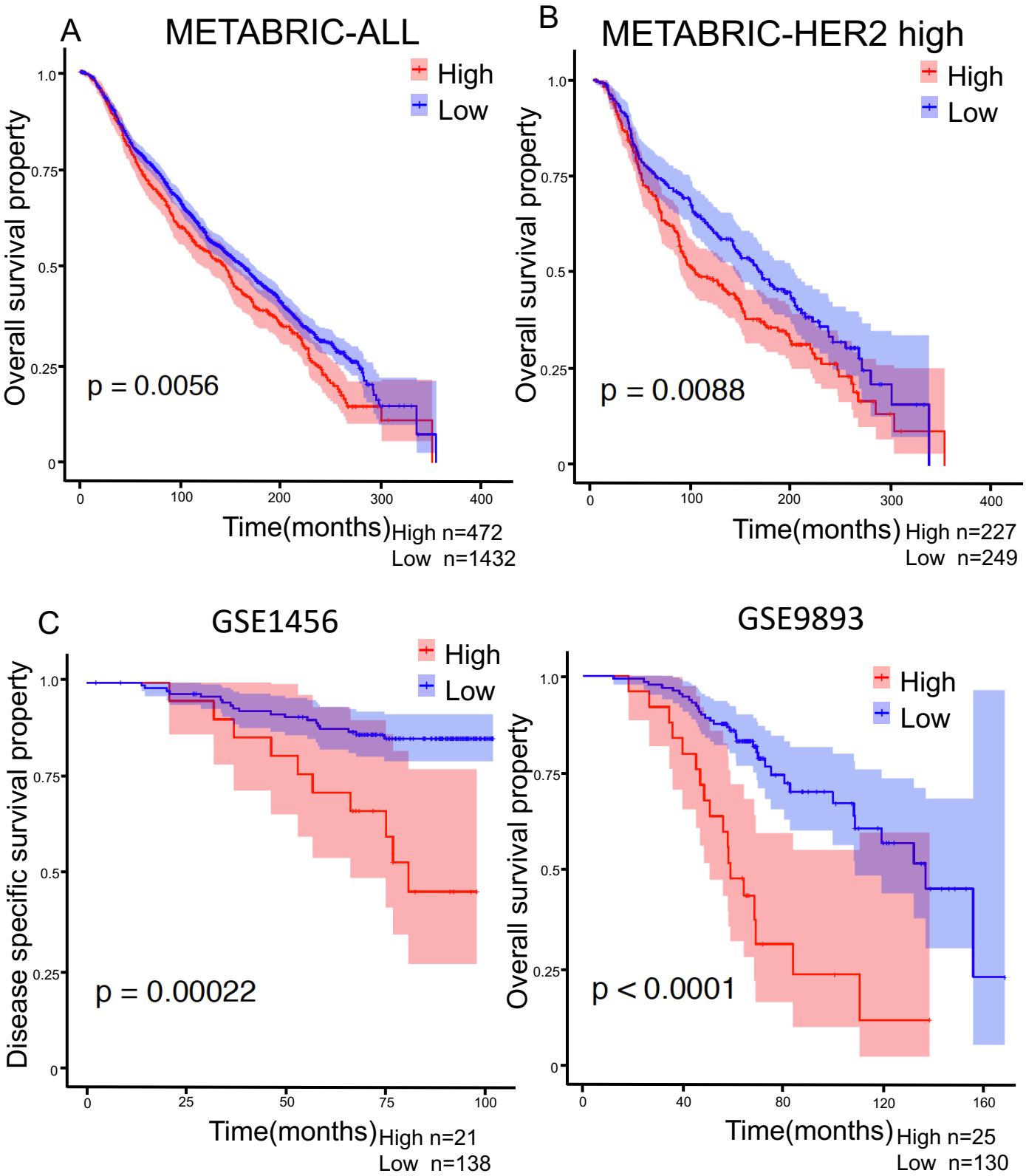

A
HOXB7 expression
n.s.
log2 (RPKM+1)
12
10
8
6
4
2
Normal
Tumor
B
HOXB7 expression
*
log2(RPKM+1)
12
10
8
6
4
2
Normal Tissue
Luminal A
Luminal B
HER2
Basal-like
Normal-like
Undefined
Tumor
C
996 breast cancer patients
HOXB7 5%
ERBB2 14%
161 patients [16.2%]
30 patients [3%]
Inframe Mutation (Unknown significance)
Missense Mutation (Putative driver)
Missense Mutation (Unknown significance)
Truncating Mutation (Unknown significance)
Fusion
Amplification
No alterations

Figure 6


A
METABRIC-ALL
High
Low
Overall survival property
1.0
0.75
0.5
0.25
0
p = 0.0056
0
100
200
300
400
Time(months)
High n=472
Low n=1432
B
METABRIC-HER2 high
High
Low
Overall survival property
1.0
0.75
0.5
0.25
0
p = 0.0088
0
100
200
300
400
Time(months)
High n=227
Low n=249
C
GSE1456
High
Low
Disease specific survival property
1.0
0.75
0.5
0.25
0
p = 0.00022
0
25
50
75
100
Time(months)
High n=21
Low n=138
GSE9893
High
Low
Overall survival property
1.0
0.75
0.5
0.25
0
p < 0.0001
0
40
80
120
160
Time(months)
High n=25
Low n=130