Supplementary Section:

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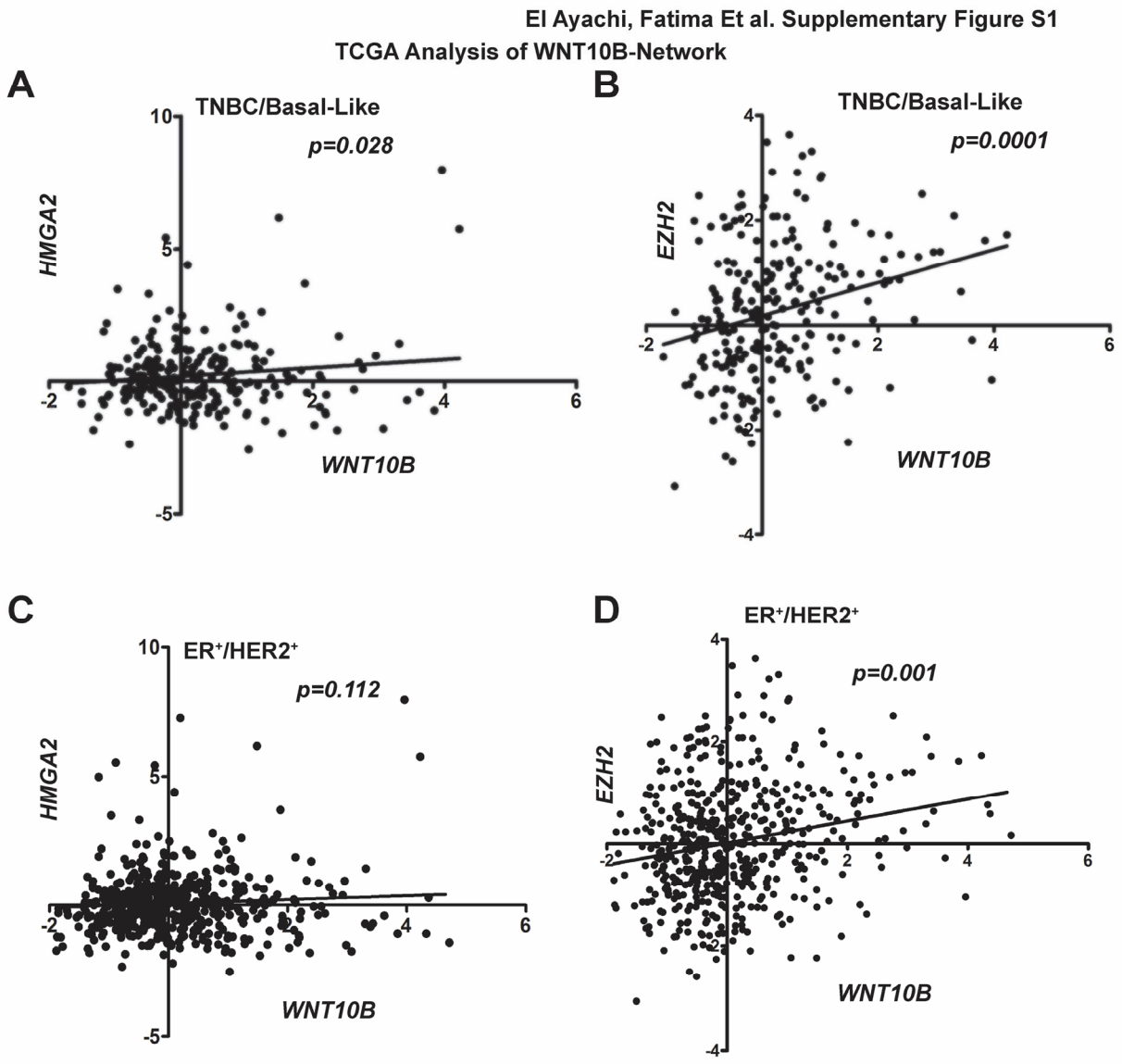
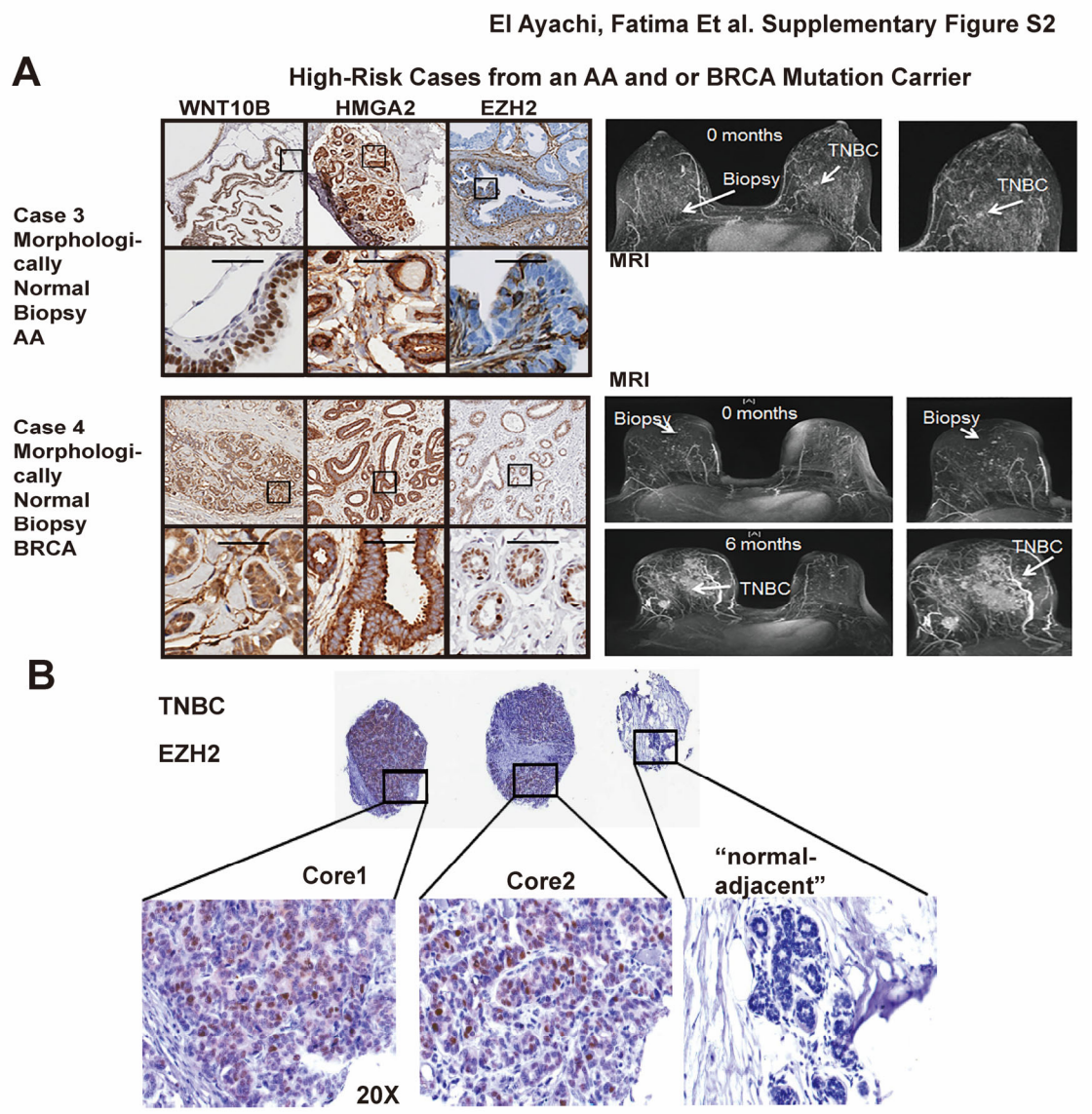
Supplementary Figures and Legends:

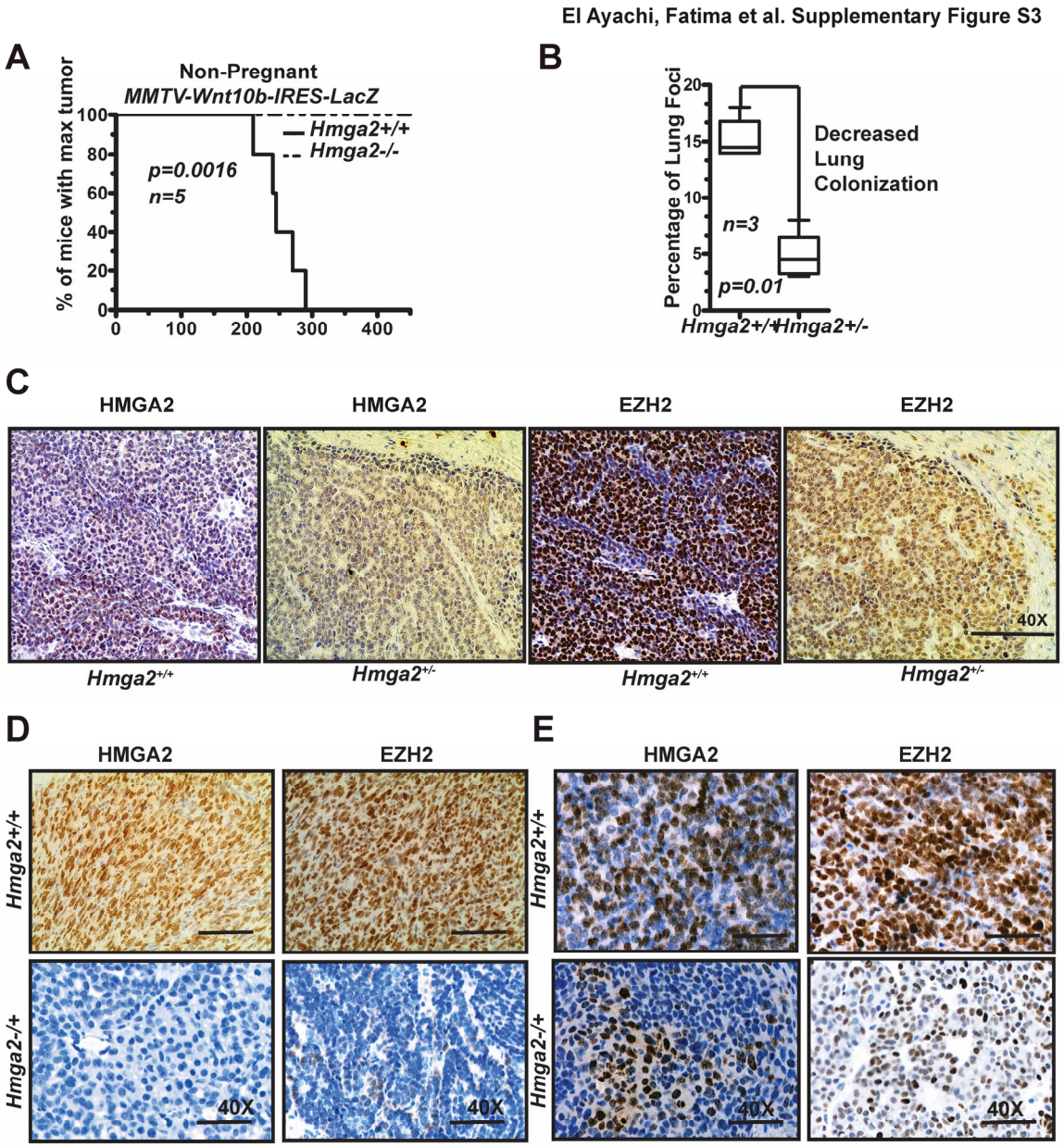


Supplementary Fig. S1. TCGA analysis of WNT10B network.
Gene expression (mRNA) levels of *WNT10B, HMGA2* and *EZH2* were obtained from the TCGA database TNBC samples were from the Nature 2012 Cancer Genome dataset (1) on cBioPortal. Correlations between *WNT10B* and *HMGA2* (**A-B**) or *WNT10B* and *EZH2* (**C-D**). A & C were patients diagnosed with TNBC/Basal-like and B & D were diagnosed with ER+ and or HER2+ tumors. Linear regression analysis were determined using the Best-fit values with 95% confidence interval; R-square values for HMGA2 and WNT10B (R^2=0.004812) and for EZH2 and WNT10B (R^2=0.04436) were calculated. *One-tailed t-test* was used for p-values of (*p=0.0280*) using 239 patient for HMGA2 and WNT10B and for EZH2 and WNT10B (*p<0.0001)* using 242 patient samples. Prism software was used for all of the analyses.



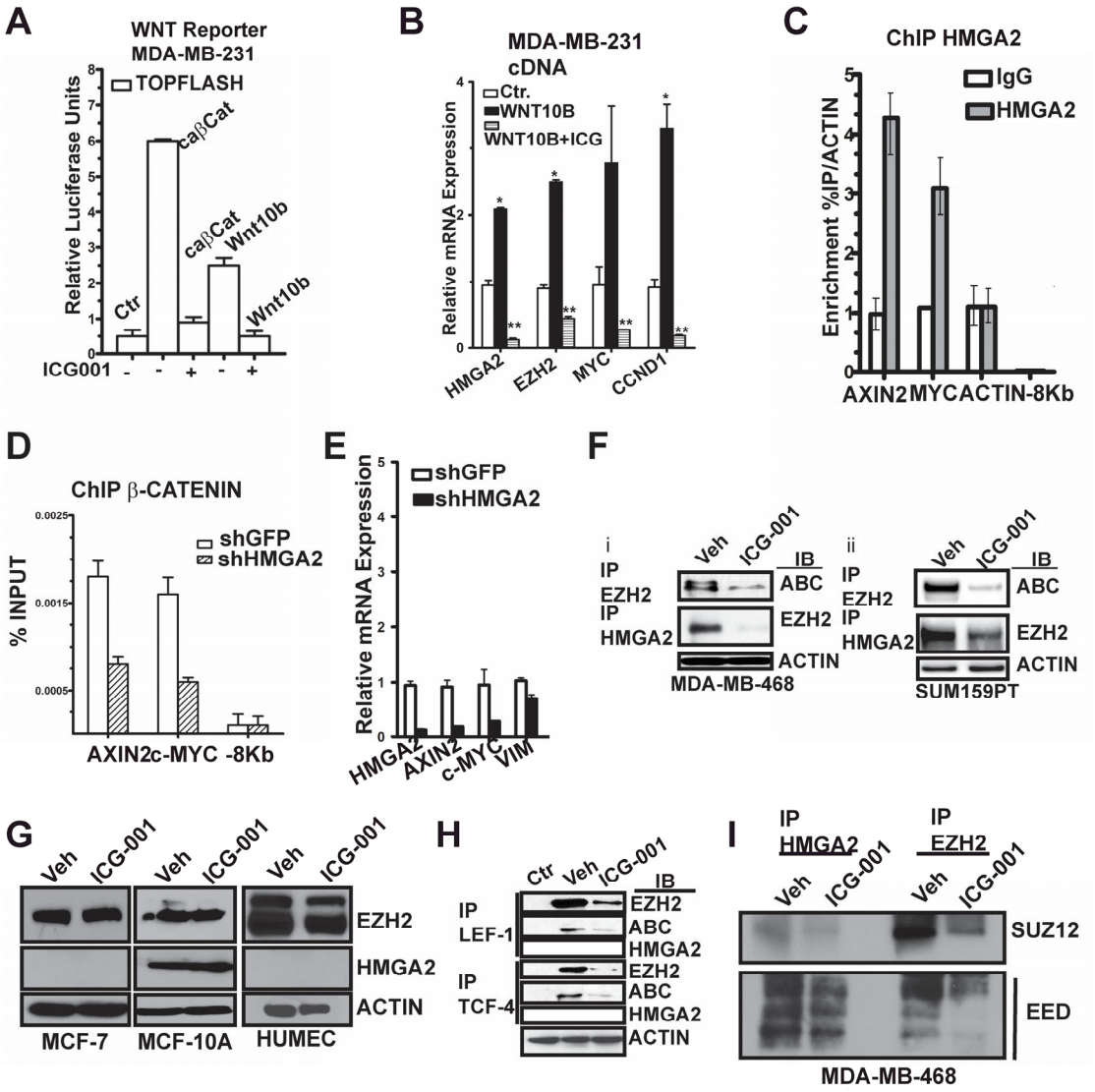
Supplementary Fig. S2. Increased expression of WNT10B, HMGA2, and EZH2 expression in "early" tissues from women at high risk women for TNBC.

Serial breast biopsies from two high-risk women undergoing breast MRI screening who subsequently developed an interval cancer. **A** IHC was conducted on WNT10B, HMGA2 and EZH2 on MRI-guided biopsies on two high risk women for developing triple negative breast cancer. Case 3: is a 47 y/o premenopausal African American female who tested negative for a BRCA mutation. She has a mother who developed premenopausal TNBC and a grandmother who developed high-grade serous ovarian cancer. MRI screening was in 2008. Case 4 is a 43 y/o premenopausal African American female who tested negative for a BRCA mutation. In 04/2012 she had new changes on screening MRI in the right breast with persistent enhancement kinetics. MRI guided biopsy demonstrated benign breast tissue with no evidence of atypia. In 08/2012 the patient developed a left breast mass and overlying erythema. **B** High EZH2 expression was detected in two TNBC from two AA women with metastatic disease, with little to no staining in the "normal" adjacent tissue. See text in supplemental M&M for more patient details**.**



Supplementary Fig. S3. HMGA2-ablation prevented tumor growth in nulliparous female *Wnt10b*^{LacZ} mice.

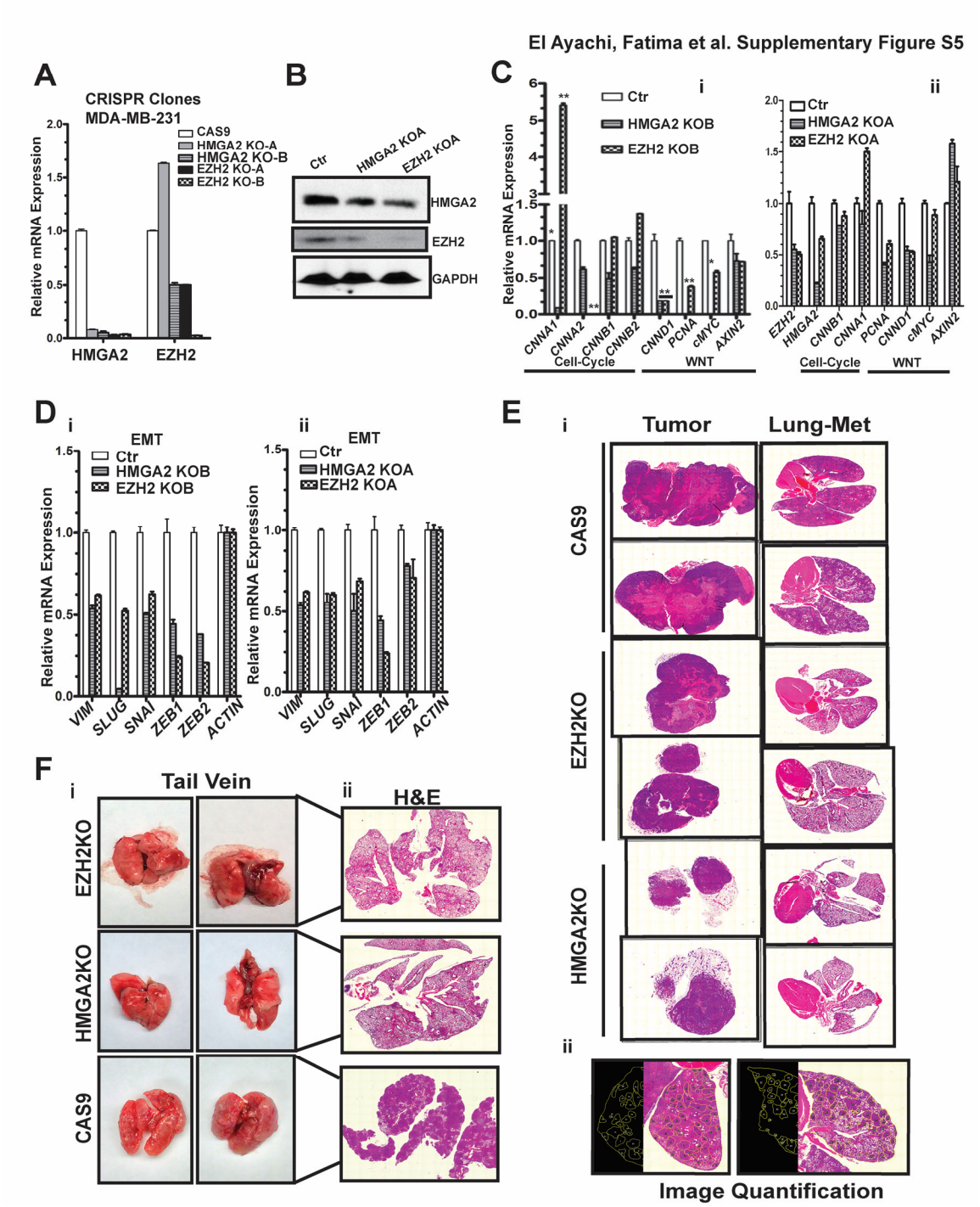
A Kaplan-Meier survival analysis of sister-siblings that were aged matched in nulliparous female *Wnt10bHmga2*$^{+/+}$ and *Wnt10bHmga2*$^{-/-}$. Nulliparous *Wnt10b*^{LacZ} female mice rarely generate tumors, the model is dependent on multiparous events >4 pregnancies. Quantifications n=number of mice, p values generated by Mann Whitney by 1-WAY ANOVA. **B** Incidence of lung metastasis in *Wnt10bHmga2*$^{+/+}$ and *Wnt10bHmga2*$^{+/-}$ in multiparous aged matched female mice. **C-E** IHC of HMGA2 and EZH2 in three pairs of *Wnt10bHmga2*$^{+/+}$ and *Wnt10bHmga2*$^{+/-}$ primary tumors. The age of the mice was at 10 months for C, 12 months for D and 14 months for E for the *Hmga2*$^{-/-}$ mice. Wild type mice ranged from 7-10 months for each group. p-values for C-E by 1-WAY ANOVA (p=0.0001; p=0.0002, respectively), Averages are presented as +/- SEM.



Supplementary Fig. S4. HMGA2-EZH2 protein-protein interactions stabilize nuclear core β-catenin/TCF-4/LEF-1 transcriptional complexes.

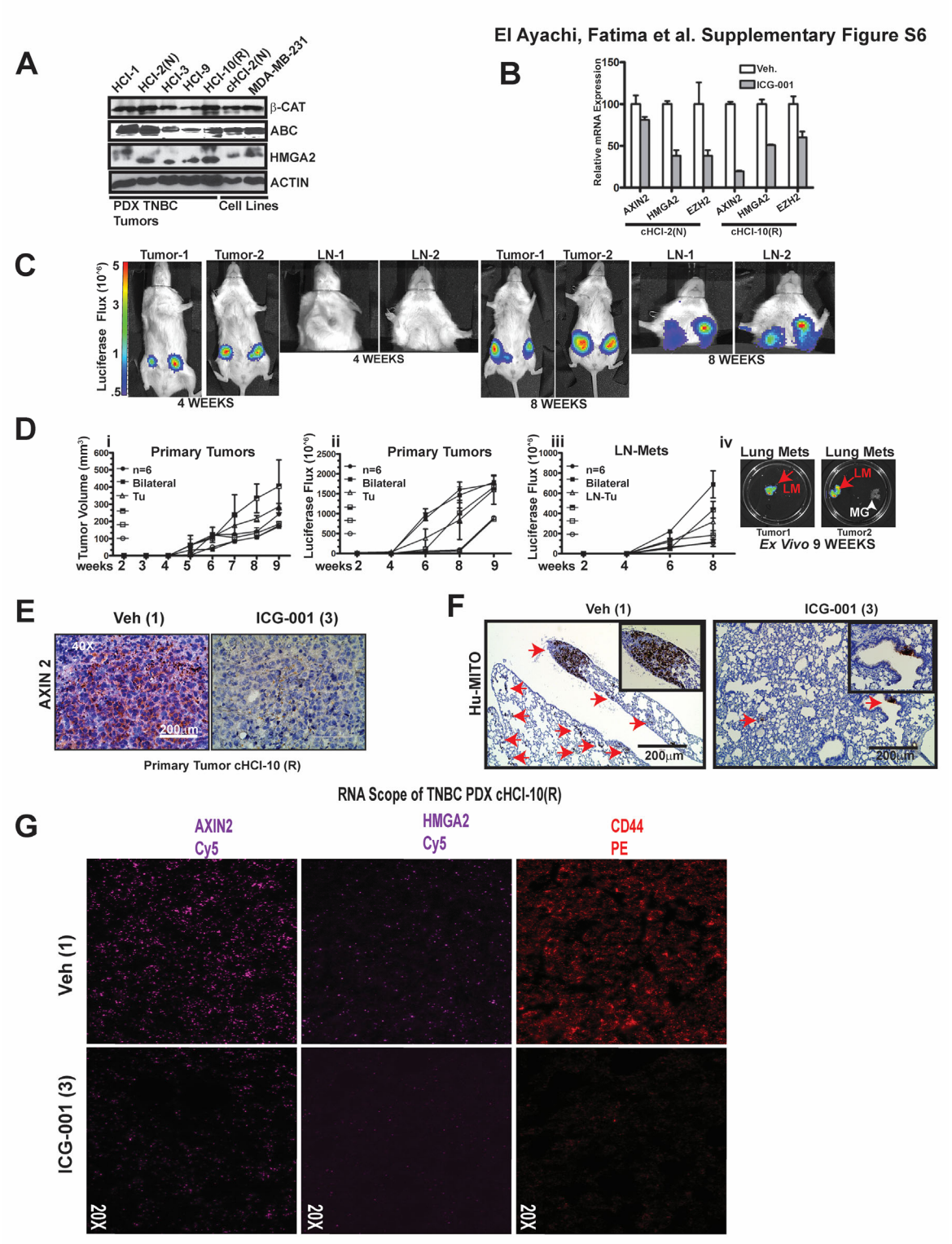
A WNT reporter TOPFLASH assays in the absence and presence of either *WNT10B*, *ca-β-CATENIN* (constitutively active) and with or without the Wnt inhibitor ICG-001 (10 μM; 24 hours) in MDA-MB-231 cells. The firefly luciferase activity was normalized to Renilla luciferase activity. The experiment was performed in biological and technical triplicates. **B** qPCR analysis for relative mRNA expression of *HMGA2, EZH2, MYC and CCND1*. All qPCR *p-values* were generated by Student's *t*-test (*$p=0.05$; **$p=0.005$); the mean of three biological replicate experiments is shown. **C** HMGA2-ChIP experiment in MDA-MB-231 cells on Wnt-responsive elements (WREs) expressed as enrichment relative to the actin promoter control. Controls: IgG, isotype-specific IgG antibody control and -8 Kb (the -8 Kb region upstream of the HMGA2 ORF).**D** β–CATENIN ChIP experiments conducted on *AXIN2*, *MYC* and -8Kb upstream of HMGA2 ORF by qPCR in either *shGFP* and or *shHMGA2* cells. **E** qPCR analysis for *HMGA2, AXIN2, MYC and VIMENTIN* in MDA-MB-231 cells silenced for expression of HMGA2. A short hairpin (sh) for GFP serves as the control. **F** Immunoprecipitation (IP) of EZH2 and IB for ABC and IP HMGA2 and IB for

EZH2 in the absence or presence of ICG-001 for 48hours in MDA-MB-468 (i) and SUM159PT (ii) cells. Straight IB for ACTIN, serving as control. **G** Exposure of ICG-001 for 48 hours in MCF-7, MCF-10A and HUMEC cells and IB for EZH2, HMGA2 and ACTIN. **H** IP of both endogenous LEF-1 and TCF-4 and IB for EZH2, ABC and HMGA2 in the presence or absence of ICG-001 (10μM for 48 hours). ACTIN serves as the control. **I** Immunoprecipitation (IP) of EZH2 and HMGA2 immunoblot for SUZ12 and or EED, in the absence or presence of ICG-001 for 48 hours in MDA-MB-468.



Supplementary Fig. S5. HMGA2-EZH2 expression is necessary for lung primary tumor growth and metastasis.

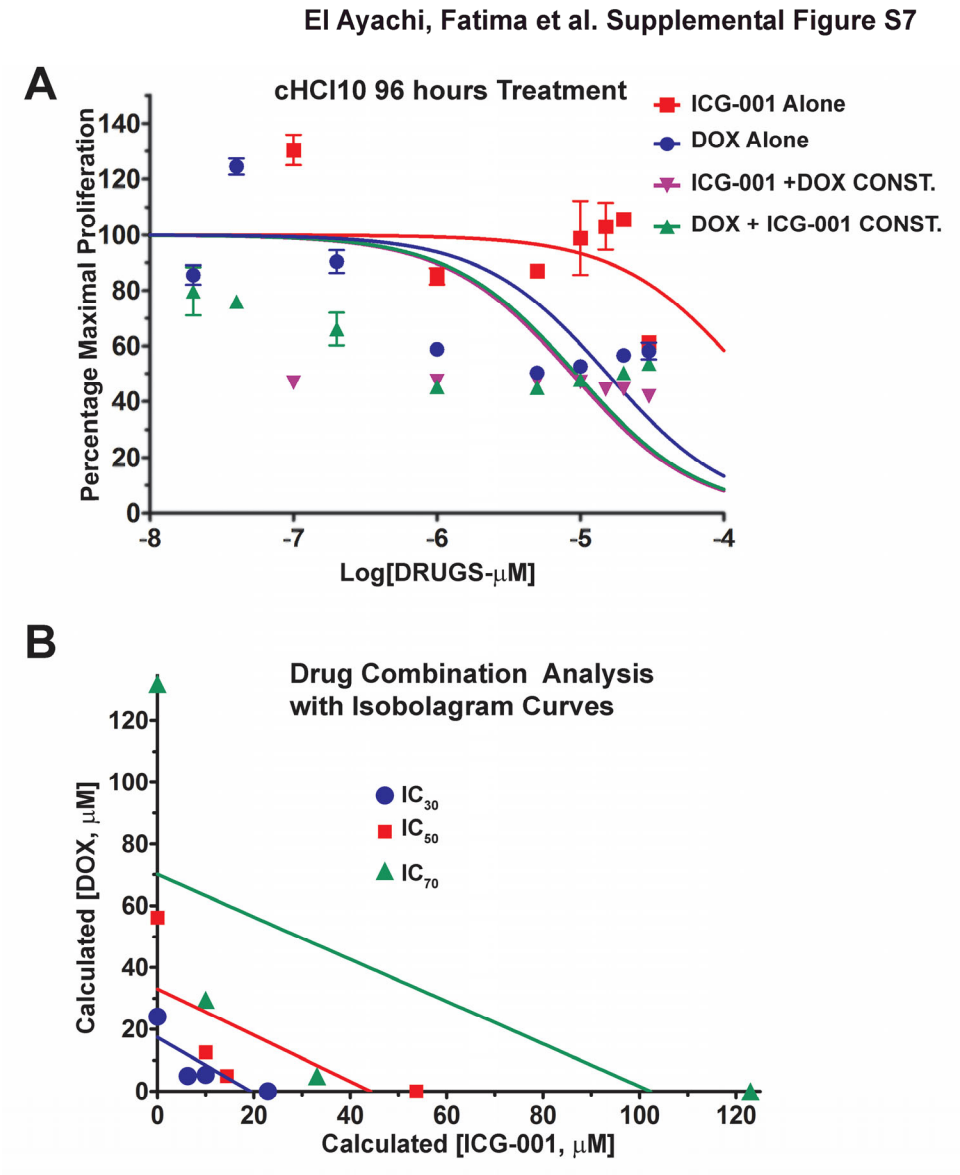
A Generation of two independent CRISPR/CAS9 KO clones A and B, for either HMGA2 and or EZH2. qPCR analysis of mRNA expression for HMGA2 and EZH2. **B** Immunoblot for HMGA2 or EZH2 and in CRISPR/CAS9 KO clones A. Actin serves as the control. **C-D** qPCR analysis of mRNA expression for various cell cycle and WNT-direct targets (Ci-ii) and for EMT markers (D i-ii) in both the CRISPR/CAS9 KO clones A and B, for either HMGA2 and or EZH2. qPCR *p-values* generated by t test (**p=0.05*; ***p=0.005*) was in triplicates. **E** H&E staining of surgically transplanted CAS9 control, HMGA2KO and EZH2KO cells into the mammary fat pad of NSG mice. The lungs and primary tumors are shown for the 3 groups (i). Schematic image quantification of lung met foci/lobe and the foci size in control group with image J software (ii). **F** Images of the lungs from tail-vein injections for the CAS9, HMGA2KO and EZH2KO cells (i) and their corresponding H&E staining (ii). Experiments were conducted with n=6 mice.



Supplementary Fig. S6. TNBC PDX tumors are active for the WNT10B/β-CATENIN/HMGA2 signaling axis.

A Immunoblot analysis for pan-–βCATENIN, transcriptionally activated (Act) –β CATENIN (ABC), and HMGA2 on various EA descent TNBC PDX tumors HCl-1, -2, -3, -9 and -10 with

known sites of metastasis; the HCI-3 PDX is ER+/PR+HER2-negative. MDA-MB-231cells served as a control compared to the cHCI-2 PDX cell line derived from PDX patient HCI-2 and ACTIN serves as the loading control. **B** qPCR of treatment-"naïve" (N) cHCI-2 and "chemoresistant" (R) cHCl-10 PDX cell lines for AXIN2, HMGA2 and or PCNA in the absence or presence of 10 μM (cHCl-2) or 30 μM (cHCl-10) ICG-001 for 48 hrs. The relative mRNA expression is compared to *GADPH*; data is representative of triplicate experiments. **C** TNBC PDX tumor cHCI-10-luciferase tumor fragments were used to re-generate tumors; luciferase signal was tracked in primary tumors (onset at 4 weeks) and the axillary lymph nodes metastasis (LN; onset at 8 weeks). **D (i)** Tumor volume of the HCI-10-Luc2 PDX model was compared over time (n=6 mice and n=12 tumors). **(ii-iii)** Total bioluminescence flux (photons/sec, p/s) was quantified longitudinally using bioluminescence imaging for both primary and LN metastatic tumors **(iv).** Standard deviation is shown. Representative images of the total flux lungs (red arrows) and the thoracic mammary gland #8 serves as non-luciferase signal control (white arrowhead) when imaged *ex vivo*. **E-F** IHC analysis of AXIN2 in primary tumors from the Vehicle (1) and high dosage of ICG-001 (3) cohorts and the Hu-MITO marker in LM-tumors from the Vehicle (1) or ICG-001 (3) cohorts. **G** RNAscope analysis, an *in situ* RNA hybridization assay, was conducted for CD44 (PE), HMGA2 (Cy5) and AXIN2 (Cy5) in Vehicle (1) or ICG-001 (3) treated primary tumors. Images were captured at 40X magnification using the EVOS® imaging system and conducted in biological triplicates. p-values for D by 1-WAY ANOVA (*p=0.0001*; *p=0.0002*, respectively), Averages are presented as +/- SEM.



Supplementary Fig. S7. ICG-001 exposure in cHCl-10 cells sensitizes doxorubicin *in vitro* and determination of IC_{30}, IC_{50} and IC_{70} isobologram curves for ICG-001 and or DOX.

A cHCI-10 cells were plated on 96-well plates and treated with 0.1 μM to 30 μM (0.1 μM, 1 μM, 5 μM, 10 μM, 15 μM, 20 μM and 30 μM) and doxorubicin (DOX) alone at 0.02 μM, 0.04 μM, 0.2 μM, 1 μM, 5 μM, 10 μM, 20 μM and 30 μM) for a total 96 hours. Combinatorial exposure of ICG-001 at either 10 μM constant (CONST.) or 5 μM DOX CONST. with the various concentrations of either ICG-001 or DOX. WST-1 proliferation assays were conducted. Results are from three biological triplicates expressed as mean ± SE. **B** Determination of IC_{30}, IC_{50} and IC_{70} isobologram curves for both ICG-001 and DOX. The results demonstrate synergistic effect of the drugs combination as determined by generation of the combination index (CI; Supplementary Table TS1).

1. Cancer Genome Atlas N. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61-70.

Supplemental Materials and Methods

Human Breast Cancer and Lung Metastasis Tissues: Specimens from patients with primary triple negative breast carcinoma, adjacent normal breast tissue and related lung metastases were evaluated. Archival formalin-fixed paraffin-embedded tissues were obtained from the surgical pathology archive of the University of California Los Angeles and Duke University (Vicky Seewaldt). Tumor staging and grading was performed according to current clinical and pathological classifications by Dr. Cardiff. Informed consent was obtained from all subjects. Studies were approved by the Institutional Review Boards of the collaborating pathologists and the University of California at Los Angeles.

Patients for Magnetic Resonance Imaging (MRI): Breast MRI bilateral dynamic breast 3.0 Tesla (3T) MRI imaging with pre- and dynamic post IV contrast-enhanced imaging was performed on a Siemens Magnetom Trio system (Siemens, Berlin, Germany) with a dedicated breast coil, using Numeris/4 software (Syngo, B13). Studies were assigned an MRI BIRADS final assessment category by a dedicated imaging team at Duke University who were blinded to immunohistochemistry status.

Case 1 is a 27-year-old Caucasian woman with the BRCA1 mutation S868X. Her mother was diagnosed at age 48 with breast cancer and found to carry the BRCA1 mutation, as did two of her mother's sisters. Findings were 1) an oval, well-circumscribed enhancing left breast mass at the 10:00 position, measuring 7 x 4 x 6 mm and demonstrating persistent enhancement, 2) a small enhancing left mass with smooth margins at 12:00 position that measured 6 x 3 x 4 mm and demonstrated persistent enhancement. Ultrasound-guided core needle biopsy of the 12:00 lesion demonstrated a fibroadenoma but no evidence of malignancy or precancerous changes. The patient first noticed a left breast mass in 11/10 at the site of the prior biopsy; she did not follow up until 03/11. Ultrasound guided core biopsy on 03/11 demonstrated grade 3, invasive ductal carcinoma that was ER-/PR-, HER2/neu 1+ by IHC and EGFR 3+ by IHC. She received for four cycles of dose-dense doxorubicin, and cyclophosphamide, followed by two cycles of carboplatin and docetaxel. Mastectomy was performed on 09/11 with a complete pathological response. Radiation therapy to the chest wall was completed 12/11. The patient remains without evidence of recurrence.

Case 2 is a 54 y/o female with the BRCA1 mutation Y1563X. She has a sister who developed breast cancer at age 40. The patient underwent natural menopause at age 40 and risk-reduction salpingo-oophorectomy in 2005. She started MRI screening in 2008. In 08/11 she had new changes on screening MRI in the right breast. There was a 2.3 x 0.3 x 0.6 cm area of non-mass-like enhancement at the 11-12:00 position with persistent enhancement kinetics. MRI guided biopsy 08/11/11 demonstrated benign breast tissue, stromal fibrosis, and chronic inflammation. Six-month follow-up MRI demonstrated a focal area of non-mass-like enhancement at the site of prior MRI biopsy demonstrating fibrosis at the 12:00 position of the right breast

that measured 2 x 1 x 1.3 cm and had significantly increased in size. Biopsy 03/12 demonstrated grade 3 invasive ductal carcinoma that was ER-/PR-, HER2/neu 1+ by IHC and EGFR 3+ by IHC. On 04/12, right mastectomy with sentinel lymph nodes excision and left prophylactic simple mastectomy was performed demonstrating a multifocal tumor measuring 0.4, 0.9, and 1.2 cm and 2 of 4 positive lymph nodes containing invasive cancer, the largest 3.5 mm. She received for four cycles of dose dense doxorubicin, and cyclophosphamide, followed by two cycles of dose-dense paclitaxel. Treatment was complicated by neuropathy. Radiation therapy was completed 11/12. The patient is currently in good health with no evidence of disease recurrence.

Case 3: is a 47 y/o premenopausal African American female who tested negative for a BRCA mutation. She has a mother who developed premenopausal TNBC and a grandmother who developed high-grade serous ovarian cancer. MRI screening was 2008. There was a 1.1 x 0.7 x 0.5 cm area of non-mass-like enhancement in the central left breast with persistent enhancement kinetics. MRI guided biopsy 08/12 demonstrated grade 3 invasive ductal carcinoma that was ER-/PR-, HER2/neu 1+ by IHC and EGFR 3+ by IHC. Left mastectomy with sentinel lymph nodes excision and right prophylactic simple mastectomy was performed in 09/11 demonstrating a multifocal tumor measuring 1.2 x 0.9 x 1.2 cm and 0/3 positive lymph nodes containing invasive cancer. She received for four cycles of doxorubicin, and cyclophosphamide. The patient is currently in good health with no evidence of disease recurrence.

Case 4: is a 43 y/o premenopausal African American female who tested negative for a BRCA mutation. In 04/12 she had new changes on screening MRI in the right breast with persistent enhancement kinetics. MRI guided biopsy 04/12 demonstrated benign breast tissue with no evidence of atypia. In 08/12 the patient developed a left breast mass and overlying erythema. MRI demonstrated a focal area of nonmass- like enhancement at the site of prior MRI biopsy that measured 2 x 1 x 1.3 cm. Punch biopsy 08/12 demonstrated grade 3 invasive ductal carcinoma with dermal and lymphatic involvement that was ER- /PR-, HER2/neu 1+ by IHC and EGFR 3+ by IHC. The patient received neoadjuvant chemotherapy consisting of dose dense doxorubicin, and cyclophosphamide, followed by two cycles of dose-dense paclitaxel. The patient had a complete pathologic response on mastectomy but recurred in 3/13 and died shortly thereafter.

Written informed consent: Women were provided written informed consent to participate in studies approved by the Duke University EMORY University, University of Tennessee Health Science Center and UCLA Institutional Review Boards.

Breast tissue collection and preparation from high-risk women: Breast tissues were collected and prepared within 1 hour after removal from the patient. Upon removal of the breast tissue, samples were marked for orientation purposes and measured. The breast tissues were then positioned with the areola as

the center and then sliced vertically into 1 to 3 centimeter sections with a sterile surgical blade. Breast tissues were then excised from predetermined areas of each of the 1 to 3 centimeter surgically sliced breast tissues. Each excision of breast tissue measured between 2 to 3 cubic centimeters. The breast tissues were then separated into smaller sections in which portions of tissues were placed in tissue cassettes (Leica Microsystem Surgipath) fixed in 10% formalin and paraffin-embedded. Some tissue was frozen-fixed (methanol-acetone) and maintained at -80ºC until needed.

Statistical analysis of publicly available datasets and the high-risk patient samples: For the comparison of HMGA2 and EZH2 gene expression between TNBC and non-TNBC, we mined a dataset of breast cancer samples obtained from 178 women including 58 TNBC patients and 120 non-TNBC patients (9). Continuous variables were compared using the Mann-Whitney U test. For the metastasis free survival analysis of EZH2 in breast cancer patients, we mined a dataset of 286 breast cancer patients, which provided detailed patient follow-up information (10). Patients were dichotomized into two groups using the criterion of above or below the median EZH2 expression value. Plots of the Kaplan-Meier estimates of metastasis-free survival were generated and the log-rank test was applied to compare survival differences between groups; all statistical analysis was performed using SPSS 17.0 software. The same dataset was also used to compare the expression of EZH2 between distant metastatic patients and non-metastatic patient. Two-sided Fisher-Boschloo (11) unconditional exact tests were used to compare the incidence of tumors between different cell populations. Calculations were performed using the R-package "Exact" Calhoun P (2013) http://CRAN.R-project.org/package=Exact. All intended statistical comparisons were reported. No adjustment for multiple testing was performed.

Immunohistochemistry (IHC) and Immunofluorescence (IF): Tissues from breast tumors, adjacent normal breast and lung metastasis were fixed in 4% paraformaldehyde and embedded in paraffin. For in *in vitro* IF assays, cells were cultured in 8-well chamber slides (Fisher) and fixed with 4% paraformaldehyde-PBS for 15 minutes, washed, incubated with blocking buffer (TBS pH 7.8, 3% BSA, 1% NGS, 1% Triton X-100, 0.01% NaAzide) and stained as follows. The following IHC primary antibodies were used: WNT10B (ab91201 5A7, Abcam), EZH2 (Cell Signaling # D2C9), HMGA2 (ab52039, Abcam), non phospho (Active) β-Catenin (#8814, Cell Signaling), Hu anti mitochondria (ab92824, ABCAM), AXIN2 (ab32197, ABCAM) and Ecad (sc-7870, Santa Cruz). For all antibodies, a standard deparafinization and staining procedure was used as described in (1, 2). All IHC was performed manually without the use of automated immunostainers. Antigen retrieval was performed using a Decloaking Chamber (Biocare Medical, Concord, CA; 125ºC, 15 psi) in the presence of citrate buffer (pH 6.0). The total time slides were in the chamber was 45 minutes. Incubations with primary antibodies were performed at room temperature

overnight in a humidified chamber. Normal goat serum was used for blocking. IHC kits anti-rabbit (K4003) or anti-mouse (K4001) secondary antibody with a DAB-CHROMOGEN (K3468) from DAKO were used for amplification and visualization of signal, respectively. Slides were counterstained with Hematoxylin QS (Vector Labs). Tissues known to contain each assessed antigen were used as positive controls.

Mice: *MMTV-Wnt10b-IRES-LacZ* mice were previously described (1) FVB/NJ wild type and transgenic mice were bred in house. All studies were approved by the Office of Animal Research Oversight of the University of California at Los Angeles and the Institutional Animal Care and Use Committee (IACUC) at the University of Tennessee Health Science Center.

Cell Culture Cell Synchronization and Proliferation assay: All cells were maintained in a humidified atmosphere with 5% CO2 in DMEM plus 1% Pen/Strep and 10% FCS. HUMEC (hTERT) and MCF-10A cell grown in DMEM/F12 50/50 plus1% Pen/Strep and 10% FCS, epidermal growth factor, insulin, hydrocortisone and cholera toxin, at standard doses. SUMP159PT RPMI 1% Pen/Strep and 10% FCS. Cells were grown with Synchronization of cell lines at G_1 was performed as previously described (1). ICG-001 (10 μM in 1% DMSO) was used in experiments with synchronized cells as follows: treatment began 16 hours post-release (i.e. S-phase) for an additional 48 hours. Synchronization of cell lines at G_1 for cell cycle progression analysis and MDA-MB-231-shHMGA2 and *WZA*$_{LacZ}$-shHMGA2 cell lines were previously described in (2). Stably passaged PDX cell lines were derived from the Luc2-labeled HCI-2 and HCI- 10 PDX models (cHCI-2 or cHCI-10) as described below using methods published in (4). Cells were cultured in monolayer in a humidified atmosphere with 5% CO2 in M87 complete medium: DMEM/F12 supplemented with 2% FBS, 1x Insulin-transferrin-selenium (ITS), 1x Penicillin-streptomycin glutamine, human epidermal growth factor (5 ng/ml), hydrocortisone (0.3 μg/ml), cholera toxin (0.5 ng/ml) 3,3',5-Triiodo-L-thyronine (5 nM), Isoproterenol hydrochloride (5 μM), Ethanolamine (50 nM) and *O*-Phosphorylethanolamine (50 nM). Cell proliferation was measured using the WST-1 cell proliferation assay (Roche, Basel, Switzerland) according to the manufacturer's protocol.

Generation of the drug combination index by isobole curves from WST-1 assay.

Both the non-adherent and adherent fractions of cHCI-10 Luc2 cells were trypsinized and then seeded at a density of 6,000 cells/well into flat-bottom well 96-well plates and allowed to plate overnight. ICG-001 (ApexBio) master stock (10 mM/100% DMSO, stored -20°C) was diluted immediately prior to use to 1 mM in DMSO as a working stock, which was diluted directly into growth medium. A doxorubicin (Sigma Aldrich) master stock (5 mM/100% DMSO, stored -20°C) and diluted directly into growth medium. The dose range for ICG-001 was 0.1 μM to 30 μM (0.1 μM, 1 μM, 5 μM, 10 μM, 15 μM, 20 μM and 30 μM)

and the dose range for doxorubicin was 0.02 μM to 30 μM (0.02 μM, 0.04μM, 0.2 μM, 1 μM, 5 μM, 10 μM, 20μM, 30 μM). Each drug was added alone or in combination with the other drug to cells (n=3 technical replicates/condition) along with the appropriate vehicle controls (diluted DMSO alone). Drug(s) were not re-added or the medium changed over the course of the experiment (48 hours). At 48h, cells were incubated with WST-1 reagent (EMD Millipore) and read at 440 nm on a spectrophotometer. Combination indices were calculated for the 48-hour time point per (2) , when we observed drug-dependent changes in apoptosis markers by western blotting (Fig. 6).

Tumor biology and metastasis experiments and MDA-MB-231 xenografts: MDA-MB-231 and *WZA*^{LacZ} cells and silenced clones for HMGA2 were prepared for metastatic assays under sterile culturing conditions. 1.25x10^{6} MDA-MB-231 cells (100 μl sterile PBS) were injected into the tail vein of athymic nude (nu/nu) mice (MDA-MB-231) or syngeneic FVB/NJ mice (*WZA*^{LacZ}) and assayed for lung metastasis 4-8 weeks after injection. 1.25x10$_{6}$ *WZA*$_{LacZ}$ cells (200 μl sterile PBS) were injected into the mammary fat pad and assayed for lung metastasis 4-8 weeks after injection. Sequential transplantations of *MMTV-Wnt10b-IRES-LacZ* primary tumors were generated as previously described (2) by injecting 1.25x10^{6} cells were injected into the mammary fat pad. Tumor growth was assessed from 4-6 weeks, 6-8 weeks or 16-24 weeks post-injection, depending on the cell population and the number of cells injected. Experiments were stopped after 6 months if tumor growth could not be detected by palpation. After determination of tumor number and localization, excised tumors and organs were fixed with formaldehyde (4%, 18 hr) and postfixed (70% ethanol, 18 hr) before dehydrating and paraffin-embedding.

Transient transfections: MDA-MB231 cells were transfected using electroporation with an Amaxa Nucleofector (Lonza: pcDNA-GFP) following the manufacturer's protocol with plasmids pCMV-HAhEZH2, (AddGene#24230) or pMIG-HMGA2-FLAG (AddGene#25409). The activity of β-catenin dependent Wnt signaling was measured by the TOPFLASH reporter using the Dual Luciferase Reporter Assay (E1960, Promega, Madison, WI) according to the manufacturer's instructions. Briefly, HEK-293T cells were grown in 24-well plates and were transfected with 0.1 μg of Super8X TOPFLASH plasmid (gift from Randall Moon) and pcDNAWnt10b plasmid (Addgene: WNT open source #302480) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA) per the manufacturer's protocol. Similarly, MDA-MB231 cells were transfected with 0.1 μg of Super8X TOPFLASH plasmid and pcDNAWnt10b plasmid or 0.3 μg of the constitutively active (ca) beta catenin. To normalize transfection efficiency in the reporter assays, cells were cotransfected with 0.1 μg of the internal control reporter, pTK-Renilla luciferase using a polyethylenimine transfection protocol described elsewhere (3). Sixteen hours after transfection, the transfection medium was replaced with DMEM-Hi supplemented with 10% FBS overnight in the presence

or absence of ICG001. At 24 h post-transfection, cells were lysed with commercial reporter lysis buffer (Promega), and luminescence was measured by the Promega Dual-Luciferase Reporter System according to the manufacturer's protocol on a Hidex Plate Chameleon V plate reader. Firefly luciferase signal was normalized to Renilla luciferase expression and data expressed in relative luciferase units (RLU), n=3.

Knockdown experiments: MDA-MB 231 and *WZA*^{LACZ} cells were transduced with either human- or mouse-specific shRNAs (4 target-sets each) to HMGA2 (pLKO.1-ShHMGA2, Thermo-Open Biosystems) or with pLKO.1-shGFP (AddGene) using standard protocols. Clones were selected by puromycin as previously described (2).

RNA and Real-Time PCR: Isolation of total RNA was performed using TRIzol (Invitrogen) according to manufacturer's protocol. RNA was treated with DNA-free kit (Ambion) and converted to cDNA with iScript cDNA Synthesis Kit (BioRad) or Maxima First Strand cDNA Synthesis Kit (Fermentas) according to manufacturer's protocol**.** cDNA was subjected to quantitative PCR (qt-PCR) using the iCycler thermocycler (BioRad) or Realplex2 cycler (Eppendorf). qt-PCR was conducted in a final volume of 20 µl using Maxima SYBR Green/ROX qPCR Master Mix (Fermentas) according to manufacturer's protocols. Amplification conditions were: 95°C (5'), 40 cycles of 95°C (30s), 55°C (60s) and 72°C (60s). Primer pairs for each gene are provided in Supplemental Table S1.

Chromatin immunoprecipitation (ChIP): Cells were grown to confluency for 2–3 days, released from G_1 and then harvested 48hours for ChIP assays performed as described in (7, 8). Briefly, cross-linked chromatin was isolated from each plate and separate precipitation reactions were set up for each target protein complex using specific antisera. DNA was amplified by qPCR using the Power SYBR Green amplification system (Applied Biosystems). IP'd chromatin was amplified in triplicate for each target gene and analyzed on an iCycler thermocycler as described above. Normalization was performed relative to input chromatin, *hACTIN*, -8.0 kb upstream of *hHMGA2* promoter, or to *hHBB* minimal promoters. Antibodies used included HMGA2 (Abgent # AP5359b), EZH2 (Cell Signaling # D2C9), rabbit anti IgG (Santa Cruz #H270) and mouse anti IgG_1 (Santa Cruz # 69786). Following formaldehyde cross-linking and shearing of chromatin using the COVARIS Tru ChIP Chromatin Shearing Kit per the manufacturer's instructions (COVARIS, #520154). Each ChIP experiment was repeated at least 3 times. Primer pairs for each gene regulatory region are provided in Supplemental Table S2.

Cell extraction and Western blotting: Cells were lysed as previously described (1) and 50-250µg of protein was loaded per lane and separated using 10% SDS-PAGE gels. After transfer to Immobilon-P

(Millipore), membranes were immunoblotted (IB) using the following primary antibodies: β-actin (20- 33 A560, Sigma), AXIN2 (#ab32197, Abcam), or antibodies from BD Biosciences: Vimentin (#550513), from Cell Signaling: HMGA2 (#5269), EZH2 (#D2C9), β -catenin non P (active # D13A1) PCNA(#2586), AKT(#9272), pAKT(#4060), Acetylated β –catenin (#9534S), Groucho/TLE (#4681S) SUZ12 (# 3737S), from ThermoFisher Scientific: EED (PA5-34430) or from Santa Cruz: pan- β-Catenin (#H-102, #sc-7199), TUBULIN (#SC 9104), MYC (#SC-764) and CCND1(#SC-718). ImmunoPure-peroxidase conjugated secondary antibodies (Thermo Scientific) were used according to manufacturer's protocols.

Immunoprecipitation: Immunoprecipitation (IP) reactions were conducted on whole-cell lysates prepared in standard RIPA buffer extracted from MDA-MB-231, MCF-7, HUMEC, MCF-10A, SUM159PT, MDA-MB-468 and cells silenced for HMGA2 and or cells that were treated with vehicle control (DMSO) or ICG-001 (10uM, 48h) or transfected 293T cells, as previously described (2). IP's were performed with Santa Cruz antibodies pan-β-catenin (SC7199, H-102), and LEF-1 (SC8591 N-17), ThermoFisher Scientific antibody: EED (PA5-34430) or Cell Signaling antibodies: TCF4 (C959), Act- β -catenin (D13A1), EZH2 (D2C9), HMGA2 (5269), SUZ12 (3737S), HA (C29F4) and FLAG (M2), using 200-750 μg of total input protein per IP.

CRISPR/Cas9 Gene Editing: GeneCopoeia's Complete lentivirus kit was used per manufacturer's instructions to generate Cas9-mediated knockout (KO) models (lentivirus, #CP-LvC9NU-02). MDAMB-231 cells were selected under neomycin (1-3 mg/mL) and transduction verified by eGFP imaging. Subsequently, surviving Cas9-eGFP cells were pooled and then transduced with three independent sgRNAs for either HMGA2 (constructs a-c #HCP219812-LvSG02-3-B-**a,-b** and **–c**) or EZH2 (clones a-c #HCP205051-LvSG02-3-B-**a,-b** and **–c**); these vectors express mCherry, such that all cells surviving selection with puromycin (1-2μg/mL) will be yellow (GFP+/mCherry+). In all cases, Cas9 and sgRNA lentivirus titers were ~10^7/μL and cells were transduced with a MOI of 10:1. Culturing of CRISPR/Cas9 cells in the same media described above as follows: cells initially grown in 12 well plate follow by seeding in to 6 well-plate at very high confluence under 2mg/ml neomycin (neo) and 2 ug/ml puromycin (puro) until 100% confluence. Cell passage into a 6 well-plate and maintain under high selections 2mg/ml neo and 2ug/mL puro with at least 200,000 cell per well at 100% confluence were used for the described assay. All cells were verified for eGFP and mCherry expression before use in each experiment.

Generation of Luciferase2 (Luc2)-labeled HCI PDX models: Cryopreserved tumor fragments derived from previously characterized patient-derived xenograft (PDX) breast tumors (Caucasian, EA patients)

were generously provided by the laboratory of Dr. Alana Welm and the Huntsman Cancer Institute (HCI) tissue resource and application core (HCI-1, HCI-2, HCI-3, HCI-9 and HCI-10). Upon receipt at UTHSC, tumor fragments were thawed, washed and re-implanted into NOD/SCID/ILR2/GAMMA (NSG) immunocompromised female recipients by orthotopic implantation into the right inguinal mammary fat pad(s). Individual PDX tumor lines were then serially re-passaged by transplantation of tumor fragments (2 x 2 mm) into the cleared mammary fat pads of NSG females at 4- 8 weeks of age. Protein extracts were prepared from four triple negative breast cancer (TNBC) lines (HCI-1, HCI-2, HCI-9 and HCI-10) and one Luminal B (HER2-) line, HCI-3, using the protocols described in (5). To facilitate bio-imaging, the metastatic TNBC lines HCI-2 and HCI-10 PDX tumor lines were labeled with firefly luciferase2 (derived from pGL4, Promega). Parental PDX tumors were resected from anesthetized animals, necrotic areas were removed and healthy tumor tissue was chopped to a fine paste and then digested with collagenase III (Worthington Biochemical) and hyaluronidase (Sigma) in DMEM/F12 + 2% FBS using methods outlined in (ref #4). Pelleted large organoids were then digested with dispase II (Roche) to generate a population of single cells/small organoids. Following a series of washes, isolated tumor epithelial cells were plated at a density of 250,000 cells/well into 6- well ultra-low adhesion dishes containing breast stem cell media (DMEM/F12, 1x B27, 20 ng/mL human EGF, 20 ng/mL bFGF and 4 μg/mL heparin). The next day, cells were transduced overnight in the presence of 8 μg/mL polybrene with a m.o.i. of 10 of lentivirus that expressed luciferase2 and puromycin (Lentivirus-EF1alpha-Luciferase-IRES-puro, pEILP, using the pHIV-Luciferase backbone, AddGene plasmid #21375). The next day, the cells were washed with HBSS+ to remove virus and were replated into fresh stem cell media. Forty-eight hours after transduction, cells were selected for luciferase expression by the addition of 1 μg/mL of puromycin for 3-6 days. Transduced cells were centrifuged at low speed to enrich for live cells and the pellet resuspended in a 1:1 mixture of HBSS+/growth-factor reduced Matrigel and transplanted into the cleared mammary fat pads of NSG female mice. After initial tumor formation, which was monitored by bio-imaging (Xenogen IVIS Lumina), the subsequent generation of HCI-2Luc2 or HCI-10-Luc2 TNBC tumors were generated using standard tumor fragment repassaging into NSG recipients. Cell lines were generated by digestion of the second generation of HCI-2Luc2 or HCI-10Luc2 PDX tumors using protocols outlined in (6) and cultured in M87 complete medium in standard tissue culture dishes. Protein extracts were prepared from four triple negative breast cancer (TNBC) lines passaged in NSGγ mice (HCI-1, HCI-2, HCI-9 and HCI-10) using protocols described in (5).

RNAScope *In Situ* Hybridization: ACD RNAScope Multi-Fluorescent Assay for single RNA molecule detection was used following manufacturer's instructions (RNAscope® Multiplex Fluorescent Reagent Kit

V2). Sequences of the probes used in the study are as follows: CD44 (NM_000610, region 157 – 1435), Axin2 (NM_004655.3, region 502 - 1674) and HMGA2 (NM_003483.4, region 2 - 1262).

Briefly, tissue sections were deparaffinized, RNAscope® Hydrogen Peroxide was applied, slides then boiled with target retrieval buffer for 15 minutes, then submitted to protease digestion, followed by hybridization for 2 hours with target probes to human: CD44 (C1 channel), human Axin2 (C2 channel) and human HMGA2 (C3 channel). CD44 specific hybridization signals were detected with Cy3 fluorophores. Axin2 and HMGA2 specific hybridization signals were detected with Cy5 fluorophores.

Slides were mounted using Prolong Gold media containing DAPI (Life Technologies) to highlight nuclei and all the images acquire by EVOS® imaging (Thermo-Fisher).

References:

1. Wend P, Runke S, Wend K, Anchondo B, Yesayan M, Jardon M, et al. WNT10B/beta-catenin signalling induces HMGA2 and proliferation in metastatic triple-negative breast cancer. EMBO Mol Med. 2013;5:264-79.
2. Tallarida RJ. An overview of drug combination analysis with isobolograms. J Pharmacol Exp Ther. 2006;319:1-7.
3. Fatima I, El-Ayachi I, Taotao L, Lillo MA, Krutilina R, Seagroves TN, et al. The natural compound Jatrophone interferes with Wnt/beta-catenin signaling and inhibits proliferation and EMT in human triple-negative breast cancer. PLoS One. 2017;12:e0189864.

Suppl. Information:

Supplementary Table S1. Primer sequences used for qPCR

Primer	Sequence	Orientation
hAXIN2	5'-TCA AGT GCA AAC TTT CGC CAA CCG-3'	S
	5'-TGG TGC AAA GAC ATA GCC AGA ACC-3'	AS
	5'-CTCCGGTTCTTGTCAATGGT-3'	AS
hβ-ACTIN	5'GGACTTCGAGCAAGAGATGG-3'	S
	5'-AGC ACT GTG TTG GCG TAC AG-3'	AS
h-MYC	5'-TCT CCA CAC ATC AGC ACA ACT ACG-3'	S
	5'-TGT GTT CGC CTC TTG ACA TTC TCC-3'	AS
hHMGA2	5'-GCC CCA GGA AGC AGC AGC AA-3'	S
	5'-TCG AAC GTT GGC GCC CCC TA-3'	AS
hVIM	5'-ATGAAAGTGTGGCTGCCAAGAACC-3'	S
	5'-TCTCTTCCATTTCACGCATCTGGC-3'	AS
hCCND1	5'-CAG AAG TGC GAA GAG GAG GTC-3'	S
	5'-TCA TCT TAG AGG CCA CGA ACA T-3'	AS
hSNAIL1	5'-ACTGCAACAAGGAATACCTCAGCC-3'	S
	5'-TTCTTGACATCTGAGTGGGTCTGG-3'	AS
hSNAIL2	5'-TTTCTGGGCTGGCCAAACATAAGC-3'	S
	5'-AATGTGTCCTTGAAGCAACCAGGG-3'	AS
hZEB1	5'-ATGCACAACCAAGTGCAGAAGAGC-3'	S
	5'-AGGCTGATCATTGTTCTTGGCAGG-3'	AS
hEZH2	5'-ATCTAGCATCATAGCTCCAGCTCC-3'	S
	5'-TTTGTGCTATCACACAAGGGCACG-3'	AS
hCCNA1	5'- TGT CTG TTC TGA GAG GGA AAC TGC-3'	S
	5'- AAG GAG AAA CTG GTT GGT GGT TGG-3'	AS
hCCNA2	5'- CCA ATA CTT TCT GCA TCA GCA GCC-3'	S
	5'- AAT GAT TCA GGC CAG CTT TGT CCC-3'	AS
hCCNB1	5'- ATT GTG TGC CCA AGA AGA TGC TGC-3'	S
	5'- TTA GAT GCT CTC CGA AGG AAG TGC-3'	AS
hCCNB2	5'- TTT ACA GGT TCA GCC AGT TTC CCG-3'	S
	5'- TGC TCG CCT TAA GAA GTG TAG TGG-3'	AS
hPCNA	5'- TGT AAA CCT GCA GAG CAT GGA CTC G-	S
	5'- AAA TAC TAG CGC CAA GGT ATC CGC-3'	AS
hZEB2	5'- TCTTCCACCTCAAAGCGCATTTCC-3'	S
	5'- TGTGCTCCATCAAGCAATTCTCCC-3'	AS
hSLUG	5'- TTTCTGGGCTGGCCAAACATAAGC-3'	S
	5'- AATGTGTCCTTGAAGCAACCAGGG-3'	AS

Legend: Antisense (AS), Sense (S) and human (h)

Supplementary Table S2. Primer sequences used for qPCR of ChIP assays.

Primer	Sequence	Orientation
*hAXIN2*promoter	5'-TCTGGTAGCATTATGGCCATCGCA -3'	S
	5'-AAAGTCCTCCAAGCCCAAATTCCC -3'	AS
hMYC -promoter	5'-GCGCCCATTAATACCCTTCTTTCC -3'	S
	5'-ATAAATCATCGCAGGCGGAACAGC -3'	AS
hEZH2 promoter	5'-TAAAACCGTTACCACCCCCG-3'	S
	5'-TTCTTTCGCTGAACACACGG-3'	AS
hACTIN promoter	5'-CAGTGCCTAGGTCACCCACT -3'	S
	5'-AGAAGTCGCAGGACCACACT -3'	AS
-8Kb_hHMGA2prom	5'- TCAGGCCAAAAGCCTCAGAC -3'	S
	5'- AACCACCTGTCTGCTAGGAC-3'	AS

Legend: Antisense (AS), Sense (S), human (h) and mouse (m)

Supplementary Table S3. Drug combination analysis with isobolgrams (1).

DOX (doses vary) +ICG (held constant @ 10 μM)

IC	Dox, μM	ICG-001, μM	Combination Index	Effect
30	24	22.9	0.65	Synergistic
50	56.2	53.7	0.41	Synergistic
70	132	123	0.30	Synergistic

95% CI for dose curves used to calculate inhibitory concentrations
Dox: 29 to 107.4 μM, ICG-001: 38.1 to 74.1 μM, Dox+ ICG held constant at 10 μM: 5.9 to 26.7 μM, ICG+ Dox held constant at 5μM: 8.3 to 24.4 μM

Formula for combination indices:

Drug 1: Doxorubicin
Drug 2: ICG-001
IC= Inhibitory Concentration

$$\frac{[Drug\ 2]}{[\ IC\ Drug\ 2]} + \frac{[IC\ (Drug\ 1 + Drug\ 2)]}{[Drug\ 1]}$$

Reference for isobole method:

1. Tallarida RJ. An overview of drug combination analysis with isobolograms. J Pharmacol Exp Ther. 2006;319:1-7.