SUPPLEMENTARY DATA

Animal models

Athymic Nude-Foxn1nu female mice weighing 22–28 g were purchased from Harlan Laboratories S.A. (Barcelona, Spain) and were housed in the IDIBELL facility in SFP conditions, at 20–24 °C, 60% relative humidity, and 12–12-hour light-dark periods. All animal-related procedures were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals, with the approval of the animal care committee.

Xenografts from primary tumors

Samples were collected at Hospital Universitari de Bellvitge (L’Hospitalet de Llobregat, Barcelona, Spain). The study was approved by the Institutional Review Board. Written informed consent was collected from patients. Non-necrotic tissue pieces (2–3 mm³) from resected ductal breast carcinoma were selected and placed in DMEM (BioWhittaker) supplemented with 10% FBS and penicillin/streptomycin. The xenografts were implanted in animals under isofluorane-induced anesthesia in the intramammary fat path (i.m.f.p). When the i.m.f.p. tumors reached ~1000 mm³, they were excised, dissected into 2–3 mm³ cubes and transplanted into additional mice using the same procedure.

Therapeutic protocols

For brain metastasis treatment, we started therapy on day 14 once the mice had recovered from surgery and after checking the success of cell inoculation. Lenalidomide (LND) obtained from the Celgene Corporation (Summit, NJ) was injected intraperitoneally in DMSO (Sigma-Aldrich) at 50 mg/Kg/day, every day until the end of the experiment. Taxotere (TXT) and NVP-AUY922 (NVP), both from LC Laboratories, were injected intraperitoneally in DMSO at a dosage of 15 mg/Kg/day and 30 mg/Kg/day respectively. Docetaxel was administered every 4 days for 2 weeks and NVP every 2 days for 2 weeks.

Protein expression

Histology and immunohistochemical tumor characterization

The morphology of the engrafted tumors was analyzed by H&E staining in paraffin-embedded sections. Determination of FN14 was performed with anti-FN14 at 1/3000 (Santa Cruz Biotechnology, Santa Cruz, CA) (Tecnique) diluted in Dako Real™ Antibody Diluent Buffer (Dakocytomation): Tris buffer, pH 7.2, 15 mM Na₂N₃LSAB+System-HPR (Dakocytomation) was used, including biotinylated anti-rabbit, anti-mouse and anti-goat immunoglobulins in PBS; streptavidin conjugated to HRP in PBS; and liquid 3’3’ diaminobenzidine in chromogen solution.

Western blotting

Cells were lysed in a 1% SDS (v/v) extraction buffer containing an anti-protease cocktail (Roche, Vilvoorde, Belgium). Protein concentrations were determined using the Bradford assay (MicroBCA, Pierce, Belgium). After resolution by SDS-PAGE, electrophoresed proteins were transferred to polyvinylidene fluoride (PVDF) membranes that were blocked and probed with GRP94 (1/1000, Sta Cruz) and the Peroxidase conjugated Antimouse secondary Ab (Pierce, Perbio Science Ltd., Cheshire, U.K.). Immunoreactive bands were viewed on a VersaDoc™ (Bio-Rad) Imaging System using the Super Signal west-Pico (Pierce). MWs were established with See Blue Plus2 prestained Standford (Invitrogen, San Diego, CA).

Immune-fluorescence analysis

Cells were analyzed for the expression of FN14. Briefly, 15 × 10⁴ or 60 × 10³ cells were seeded in 6 or 24 well-plates containing coverslips. After 24 hours immunofluorescence was performed anti-FN14 primary antibody in PBS1X and SBF 5%, and then fixed with paraformaldehyde at 4% in PBS 1X for 15 min at 4°C.

For IF analysis, coverslips were mounted on slides using Vectashield (Vector laboratories) with DAPI, which was used for nucleus visualization. Preparations were analyzed with the Olympus BX60 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) and images were taken and analyzed using a digital camera and Spot 4.2 software (Diagnostic Instruments, Inc., Sterling Heights, MI).

Cell viability assay

We used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The cells were serum starved for 24 h and exposed for a further 72 h to 0–200 ng/mL of TWEAK (PeproTech, PeproTech EC Ltd., London, U.K.).

Statistical analysis

To evaluate the correlation between protein expression and brain metastasis, immunostained samples were graded on a three-category scale (negative, weak positive, and strong positive). The marker was classed as being overexpressed in strong positive samples. The association with brain metastasis for each marker was tested using a two-sided Fisher exact test and summarized by calculating the sensitivity among tumors that developed metastasis, and specificity among tumors.
without metastasis, for strong positive values. Positive and negative likelihood ratios were also calculated as integrated predictive indexes, as was the area under the ROC curve. Markers were assessed using a multivariate logistic regression model in a forward stepwise procedure to identify the best combination for predicting brain metastasis. Since ErbB2 was already a known metastasis risk factor, an analysis including ErbB2 as the baseline was also performed, as well as a stratified analysis of each candidate marker within ErbB2-positive and -negative tumors. In all analyses, associations were considered significant when $p$ was less than 0.05.

To compare survival times for the control and LND groups, we used the non-parametric Mann-Whitney test and the log-rank test.

The bioluminescence data were transformed using the $\log(1 + x)$ function (where $x = \text{AvR}$), in order to obtain a more regular and positive distribution. Subsequently, these data were normalized by subtracting the first observation (day 14) from each of the following observations. The Student $t$ test was used to compare the treatment groups. Survival curves for each treatment were estimated via the Kaplan-Meier method, and the log-rank test was used to assess the significance of differences.

$P$-values lower than 0.05 were considered significant.
Supplementary Figure S1: Kaplan-Meier survival estimates of overall free survival among patients who received chemoadjuvant therapy, with or without taxanes, according to the GRP94 expression in tumors.

(n = 98). Hazard ratio, HR = 1.69; p = 0.31

(n = 92). Hazard ratio, HR = 1.63; p = 0.29
Supplementary Figure S2: Characterization of two different TNBC engrafted in Athymic Nude-Foxn1nu female mice. A. GRP94 FN14 expression by IHC analysis showing TNBC-EG high positivity of both proteins and TNBC-1070 expressing fewer GRP94-positive cells without FN14 expression. B. Different slope growth of both subcutaneous engrafts treated with taxotere every 4 days for 2 weeks.
Supplementary Figure S3: MDA-MB 435 parental breast cancer cells (435/P) and the brain metastatic variant 435/Br1 were used for in vitro characterization of IMID action. A. Immunofluorescent expression of FN14 (60x) in cells cultured in standard conditions. B. 435/P and 435/Br1 cells cultured for 72 hours in the presence of TWEAK at 2–200 ng/mL. The graph is representative of four different experiments that showed a growth advantage of 435/Br1 cells with regard to 435/P when cells were challenged with TWEAK.
Supplementary Figure S4: Characterization of GRP94 expression in brain metastatic cells. A. Western-blot of 435-P, 435-Brl, BrV5 and BrV5C1 to analyze GRP94 expression (upper panel) and the relative quantification of the protein in cells with regard to the expression of the tubulin (bottom panel). B. Survival assay using BrV5 to assess the IC50 of lenalidomide (LND) and the IC50 of heat-shock 90 inhibitor, NVP-AUY922 (right panel). NVP-AUY922 exerted a cytotoxic effect with an IC50 at 50 nM whereas LND did not have a cytotoxic effect on cultured cells.
Supplementary Figure S5: The two different TNBC engrafted in Athymic Nude-Foxn1nu female mice were used to check the antitumoral effect of LND with regard to docetaxel and NVP-AUY922. A. The upper panels show TNBC-EG (left) and TNBC-1070 (right) tumor growth slopes when treated with LND (50 mg/Kg/day every day), NVP-AUY922 (30 mg/Kg/day every 2 days for 2 weeks), docetaxel (15 mg/Kg/day every 4 days for 2 weeks) and controls injected with vehicle. TNBC-EG tumors decreased with docetaxel treatment with regard to the control ($p = 0.002$). No effect was observed in mice treated with LND ($p = 0.380$), and the tumor volume decrease was not significant ($p = 0.160$) in NVP-AUY922 treated mice. TNBC-1070 tumors treated with LND had similar volume than controls and differences were not statistically significant ($p = 0.118$). Docetaxel and NVP-AUY922 treatment induced significant reduction of tumor volume with regard to control ($p < 0.001$). B. IHC expression of FN14 in tumors treated or not with LND showing the reduced expression in TNBC-EG positive FN14 tumors.

Supplementary Table S1: Brain organ-specific genes/proteins from previously described data based on protein–protein interaction networks analysis (Sanz, et al, 2011 and Sanz et al., 2012)

| Gen Symbol | SwissProt ID | Protein Name | Function | $p$-value * | Network position (linked to) |
|------------|--------------|--------------|----------|-------------|-----------------------------|
| TRAF2      | Q96NT2       | TNF-receptor associated factor 2 | Signal transduction | 0.00007 | 40 S ribosomal protein s12 |
| TNFRSF12A  | Q9NP84       | Fn14         | Receptor  | 0.0001     |                             |
| TRA1       | P14625       | Glucose regulated protein 94 | Protein folding | 0.0009     |                             |
| INHA       | P05111       | Inhibin alpha chain | Signal transduction | < 0.000001 |                             |
| ARFGAP     | Q8N6T3       | ADP-ribosylation factor GTPase-activating protein 1 | Transport | 0.0003     |                             |

(Continued)
| Gen Symbol | SwissProt ID | Protein Name                          | Function               | p-value * | Network position (linked to) |
|------------|--------------|---------------------------------------|------------------------|-----------|-----------------------------|
| HSPCA      | P07900       | heat shock 90kDa protein 1, alpha     | Chaperone              | 0.006     |                             |
| ATF6       | P18850       | ATF-6                                 | ER stress sensors      | 0.256     |                             |
| ERN1       | O75460       | IRE1-alpha                            | ER stress sensors      | 0.441     |                             |
| ERN2       | Q76MJ5       | IRE1-beta                             | ER stress sensors      | 0.038     |                             |
| EIF2AK3    | Q9NZJ5       | PERK                                  | ER stress sensors      | 0.155     |                             |
| DDIT3      | P35638       | CHOP (GADD135)                        | UPR pathways           | 0.006     |                             |
| MAPK8      | P45983       | JNK1                                  | UPR pathways           | 0.009     |                             |
| JUN        | P05412       | c-jun (AP-1)                          | UPR pathways           | 0.006     |                             |
| PRKR       | P19525       | PKR                                   | EIFsK                  | 0.061     |                             |
| SIRT6      | Q8N6T7       | Sirtuin 6                             | Amino acid metabolism  | 0.000004  |                             |
| ABCA1      | O95477       | ATP-binding cassette sub-family A member 1 | Cholesterol  | Cholesterol transporter |     |
| AKT/PKB    | P31749       | RAC-alpha serine/threonine-protein kinase | Protein modification |     |                             |
| SCD-1      | O00767       | Acyl-CoA desaturase                   | Lipid desaturation     |           |                             |
| LXRA       | Q13133       | Oxysterols receptor LXR-alpha         | Lipid metabolism       |           |                             |
| PPARGC1A   | Q9UBK2       | Peroxisome proliferator-activated receptor gamma coactivator 1-alpha | Transcriptional coactivator of nuclear receptors |           |                             |
| mTOR       | P42345       | Serine/threonine-protein kinase mTOR | Cell growth modulator  |           |                             |
| SRC3       | Q9Y6Q9       | Nuclear receptor coactivator 3        | Nuclear receptor coactivator |           |                             |
| SREBF2     | Q12772       | Sterol regulatory binding element 1 (SREBP-1) | Lipid/cholesterol metabolism |           |                             |
| SREBF1     | P36956       | Sterol regulatory binding element 2 (SREBP-2) | Lipid/cholesterol metabolism |           |                             |
| MBTPS1     | Q14703       | Membrane-bound transcription factor site-1 protease (S1P) | Lipid/cholesterol metabolism |           |                             |
| MBTPS2     | O43462       | Membrane-bound transcription factor site-2 protease (S2P) | Lipid/cholesterol metabolism |           |                             |

(Continued)
### UP-REGULATED

| Gen Symbol | SwissProt ID | Protein Name                                      | Function                                    | p-value * | Network position (linked to)                      |
|------------|--------------|---------------------------------------------------|---------------------------------------------|-----------|--------------------------------------------------|
| ACOT7      | O00154       | Cytosolic acyl coenzyme A thioester hydrolase (ACOT7) | Fatty-acil CoA binding                      |           |                                                  |
| ACACA      | Q13085       | Acetyl-CoA carboxylase 1 (ACC1)                    | Fatty acid biosynthesis                     |           |                                                  |
| ACACB      | O00763       | Acetyl-CoA carboxylase 1 (ACC2)                    | Fatty acid biosynthesis                     |           |                                                  |
| FASN       | P49327       | Fatty acid synthase                               | Fatty acid biosynthesis                     |           |                                                  |
| UCP2       | P55851       | Mitochondrial uncoupling protein 2                | Respiratory electron transport chain        |           |                                                  |
| RPS6KB1    | P23443       | Ribosomal protein S6 kinase beta-1 (P70S6K1)      | ApoptosisCell cycleTranslation regulation   |           |                                                  |
| SLC25A1    | P53007       | Citrate transport protein                         | Citrate/malate exchange                     |           |                                                  |
| PCYT2      | Q99447       | Ethanolamine-phosphate cytidyltransferase         | phospholipid biosynthesis                  |           |                                                  |

### DOWN-REGULATED

| Gen Symbol | SwissProt ID | Protein Name                                      | Function                                    | p-value * | Network position (linked to)                      |
|------------|--------------|---------------------------------------------------|---------------------------------------------|-----------|--------------------------------------------------|
| RPS23      | P62266       | 40S ribosomal protein S23                         | Protein biosynthesis                        | 0.000001  | 40 S ribosomal protein s12                       |
| DNM3       | Q6P2G1       | Dynamin 3                                         | Protein biosynthesis                        | 0.0008    |                                                  |
| SERPINB9   | P50453       | Serpin B9                                         | Signal transduction                         | 0.0007    | Tubulin beta-2 chain                             |
| CREB1      | Q53X93       | CAMP responsive element binding protein 1, isoform A | Transcription                              | 0.000005  | Vimentin                                         |
| CREB1      | P16220       | CAMP responsive element binding protein 1, isoform B | Transcription                              | 0.00005   |                                                  |
| AOC3       | Q16853       | vascular adhesion protein-1                       | Cell adhesion                               | 0.0004    | Glyoxalase I                                     |
| PDK1       | Q15118       | Pyruvate dehydrogenase                            | Inhibits the mitochondrial pyruvate dehydrogenase complex |           |                                                  |
| C/EBP      | P49715       | CCAAT/enhancer-binding protein alpha              | Transcription regulation                    |           |                                                  |

(Continued)
| Gen Symbol | SwissProt ID | Protein Name | Function | p-value | Network position (linked to) |
|------------|--------------|--------------|----------|---------|----------------------------|
| SPRC       | P09486       | Basement-membrane protein 40 | Interactions with the extracellular matrix |         | SPARC                      |
| KPCA       | P17252       | Protein kinase C alpha type | Protein modification |         |                            |
| TP53       | P04637       | Cellular tumor antigen p53 | Growth arrest, apoptosis |         |                            |
| SIRT1      | Q96EB6       | NAD-dependent deacetylase sirtuin-1 | Apoptosis |         |                            |
| EGFR       | P00533       | Epidermal growth factor receptor | Growth factor |         |                            |
| SUMO3      | P55854       | Small ubiquitin-related modifier 3 | Protein modification |         |                            |
| BTAF1      | O14981       | TFIID TBP subunit TAF-172(TATA-binding protein-associated factor 172) | Beta tubulin cofactor A |         |                            |

*Only the root proteins have scored from the previous data. The retrieved interacting proteins were unscored.*
Supplementary Table S2: The top 30 genes in the network by GUILD ranking.

| GeneSymbol | Rank |
|------------|------|
| TNFRSF12A  | 1    |
| TRAF2      | 2    |
| TANK       | 3    |
| TP53       | 4    |
| HSP90B1    | 5    |
| SREBF2     | 6    |
| ACACB      | 7    |
| RPS6KB1    | 8    |
| ACACA      | 9    |
| PPARGC1A   | 10   |
| PLSCR4     | 11   |
| ACOT7      | 12   |
| MBTPS2     | 13   |
| MBTPS1     | 14   |
| INHA       | 15   |
| SOX30      | 16   |
| PGRMC1     | 17   |
| TNFRSF18   | 18   |
| USP53      | 19   |
| TRPT1      | 20   |
| FBXO28     | 21   |
| FAM120B    | 22   |
| TNFRSF9    | 23   |
| TNFSF9     | 24   |
| EDARADD    | 25   |
| WDR65      | 26   |
| PPIL5      | 27   |
| TNFSF4     | 28   |
| BANP       | 29   |
| DLGAP5     | 30   |
Supplementary Table S3: GUILD ranks of the BCBrM drug targets and drugs retrieved from DrugBank.

| Gene Symbol | Rank | Drug          |
|-------------|------|---------------|
| TNF         | 194  | Thalidomide   |
| PTGS2       | 246  | Thalidomide   |
| FCGR3B      | 1074 | Bevacizumab   |
| NFKB1       | 1326 | Thalidomide   |
| HDAC3       | 1625 | Vorinostat    |
| HDAC1       | 1859 | Vorinostat    |
| HDAC2       | 2281 | Vorinostat    |
| HDAC6       | 2592 | Vorinostat    |
| POLB        | 2877 | Cytarabine    |
| HDAC8       | 2971 | Vorinostat    |
| FCGR2B      | 3875 | Bevacizumab   |
| DHFR        | 4614 | Methotrexate  |
| FCGR2A      | 5365 | Bevacizumab   |
| C1R         | 5414 | Bevacizumab   |
| FCGR1A      | 5571 | Bevacizumab   |
| FGFR2       | 5800 | Thalidomide   |
| C1QA        | 7462 | Bevacizumab   |
| STMN4       | 7907 | Lomustine     |
| C1QC        | 8290 | Bevacizumab   |
| C1QB        | 8509 | Bevacizumab   |
| VEGFA       | 8659 | Bevacizumab   |