Supplementary Figure 1. Omomyc expression levels in BC cells. A. Western blot of Omomyc 3 days after addition of 0.6 µg/mL doxycycline. B. Representative density plots of Omomyc levels in MDA-MB-231-Omomyc cells untreated (-Omomyc) or treated with 0.6 µg/mL doxycycline for 3 days (+Omomyc) measured by flow cytometry. C. Quantification of relative cell number after GFP expression. Graph shows mean ± SD; Statistical significance was determined via two-tailed Mann–Whitney test. D. Linear regression of relative cell number vs. Omomyc expression levels in each cell line. Graph shows mean ± standard error of the mean (SEM); statistical significance was determined via two-tailed Pearson correlation.
Supplementary Figure 2. Omomyc expression reduces the capacity BC cells to induce angiogenesis and to migrate. A-F. Quantification of number of nodes (A), number of junctions (B), number of segments (C), number of branches (D), total branching length (E) and total segments length (F) in HUVEC cells exposed to conditioned media from MDA-MB-231-GFP cells untreated (-GFP) or treated with 0.6 µg/mL doxycycline for 3 days (+GFP) and from MDA-MB-231-Omomyc cells untreated (-Omomyc) or treated for 3 days with 0.6 µg/mL doxycycline (+Omomyc). Graphs show mean ± SD; statistical significance was determined via two-tailed unpaired T test. G-I. Quantification of migrated BT-549 (G), MCF7 (H) and CAL-51 (I) cells corrected for the total cell number in a migration Boyden chamber assay. Cells were plated on top of a Boyden chamber in 0.5% FBS and migrated for 24 or 48 hours through a porous membrane towards wells containing 10% FBS. Graphs show mean ± SD; statistical significance was determined via two-tailed unpaired T test.
Supplementary Figure 3. Mouse weight change upon Omomyc expression. A. % of weight change in mice bearing orthotopic MDA-MB-231-Omomyc tumors treated with 5% sucrose (-Omomyc) or with 2 g/L doxycycline diluted in 5% sucrose (+Omomyc). Graph shows mean ± SD.
Supplementary Figure 4. Omomyc expression reduces proliferation and lung tumor burden. 

A. Representative images of double immunofluorescence for Omomyc and Ki67 in tumors from sucrose- and doxycycline-treated mice (left panels) with its quantification (right panel). Graph shows mean ± SD; statistical significance was determined via two-tailed unpaired T test.

B. Representative hematoxylin and eosin (H&E)-stained lung sections in a lung colonization mouse model after expression of Omomyc for 5 weeks.

C. Quantification of the mean tumor area in the lungs of mice treated with sucrose (-Omomyc) or doxycycline (+Omomyc). Graph shows mean ± SD; statistical significance was determined via two-tailed unpaired T test.

D. Number of tumors in the lungs of mice treated with sucrose (-Omomyc) or doxycycline (+Omomyc). Graph shows mean ± SD; statistical significance was determined via two-tailed unpaired T test.
Supplementary Figure 5. The Omomyc mini-protein spontaneously penetrates into MDA-MB-2321 and MCF7 cells in a dose-dependent manner. A. Flow cytometry analysis of MDA-MB-231 and MCF7 cells untreated or treated with Omomyc-Alexa Fluor 488 (Omomyc-A488) at 0.5 µM, 1 µM and 5 µM for 15 minutes.
Supplementary Figure 6. Expression of the Omomyc transgene and treatment with the Omomyc mini-protein regulate common genes and gene sets in MDA-MB-231-Omomyc cells. A. Venn diagram of downregulated (left) and upregulated (right) genes after expression of the Omomyc transgene (1 µg/mL doxycycline for 4 days) or treatment with the Omomyc mini-protein (20 µM for 3 days). B. Venn diagram of downregulated (left) and upregulated (right) gene sets after expression of the Omomyc transgene or treatment with the Omomyc mini-protein. C. Western blot showing cytoplasmic (C) and nuclear (N) Omomyc after subcellular fractionation of cells expressing the Omomyc transgene or treated with the Omomyc mini-protein. CDK4 was used as a cytoplasmic marker and Histone H3 was used as a nuclear marker. D. Relapse-free survival (RFS) plots of 8 selected genes significantly downregulated or upregulated by expression of the Omomyc transgene and by treatment with the Omomyc mini-protein. YAF9 = YEATS4, CDGG1 = TGFB1. Source: Kaplan-Meier Plotter for breast cancer. E. Type and frequency of genomic alterations in TGFB1, SKP2, YEAT4, MAD2L1, CHAF1B, SCN5A and DEPDC1B in BC patients. Source: cBioPortal.
**Supplementary Table 1:** Top 10 genes whose expression is altered in patients with amplification on at least 1 of the 7 genes shown in Fig. 6C.

| Gene   | Cytoband | Amplified group | Unamplified group | Log Ratio | p-Value   | q-Value   | Enriched in       |
|--------|----------|-----------------|-------------------|-----------|-----------|-----------|------------------|
| MYC    | 8q24.21  | 119 (37.19%)    | 761 (15.59%)      | 1.25      | 1.63E-19  | 7.02E-18  | Amplified group  |
| CASC8  | 8q24.21  | 118 (36.88%)    | 589 (12.07%)      | 1.61      | 1.12E-27  | 8.94E-26  | Amplified group  |
| POU5F1B| 8q24.21  | 118 (36.88%)    | 589 (12.07%)      | 1.61      | 1.12E-27  | 8.94E-26  | Amplified group  |
| CCAT1  | 8q24.21  | 117 (36.56%)    | 570 (11.68%)      | 1.65      | 3.01E-28  | 2.42E-26  | Amplified group  |
| PVT1   | 8q24.21  | 116 (36.25%)    | 580 (11.89%)      | 1.61      | 4.53E-27  | 3.48E-25  | Amplified group  |
| FRS2   | 12q15    | 114 (35.63%)    | 3 (0.06%)         | 9.18      | 7.19E-143 | 5.59E-139 | Amplified group  |
| PCAT1  | 8q24.21  | 114 (35.63%)    | 561 (11.50%)      | 1.63      | 4.57E-27  | 3.49E-25  | Amplified group  |
| PRNCR1 | 8q24.21  | 114 (35.63%)    | 561 (11.50%)      | 1.63      | 4.57E-27  | 3.49E-25  | Amplified group  |
| LYZ    | 12q15    | 113 (35.31%)    | 0 (0.00%)         | >10       | 7.68E-147 | 1.79E-142 | Amplified group  |
| CPSF6  | 12q15    | 112 (35.00%)    | 1 (0.02%)         | >10       | 2.04E-143 | 2.38E-139 | Amplified group  |

**Supplementary Table 2:** Sequences of primers used for RT-qPCR analysis.

| Target gene | Forward primer | Reverse primer |
|-------------|----------------|----------------|
| SKP2        | 5'-TACAGAAAGAATCTCCAGAAATCAGATC-3' | 5'-GGAAAAATTCTGAAAGCAGTCA-3' |
| YEATS4      | 5'-TGAAAGACCTGTAACCCCTGTATC-3' | 5'-CATCATTGCTGTTGGGTCTTG-3' |
| SCN5A       | 5'-GAGCTCTGTCAGATTTGAGG-3' | 5'-GAAGATGAGGCAGACGAGGA-3' |
| CHAF1B      | 5'-TGACGGTGCCCTCTGACTGT-3' | 5'-GGCACCGTTTCTACTTCTCAA-3' |
| TGFBI       | 5'-ATCCCCAGACTCACGCAAGAC-3' | 5'-CGCTCAGTCCAGAGATG-3' |
| MAD2L1      | 5'-GACATTCTGCGACTCTGTTG-3' | 5'-AACTGTTGCCACCTCTTC-3' |
| DEPDC1B     | 5'-TGGTACCGCAACTGATGG-3' | 5'-GACGGCAAAATGATGGAGCA-3' |
| EGR1        | 5'-CAGCACCCTTCAACCTCAG-3' | 5'-CACAAGGTTTGGCCACTGT-3' |
| GAPDH       | 5'-CAAGAGCAACAAGAGGAGAGAG-3' | 5'-CTACATGGCAACTGTTGAGG-3' |
| β-Tubulin   | 5'-TCTACCTCCCTCAGCTCAG-3' | 5'-CCAGAGTCAGGGGTGTT-3' |