**Supplementary Figure and Table Legends**

**Supplementary Figure S1:** Analysis of EVI1 expression within a BC patient cohort. **A,** Kaplan-Meier overall survival curves according to EVI1 expression in all BC cases as well as in ER+, ER-, ER-HER2+ and triple-negative BC subgroups (top), and more detailed clinico-pathological information according to EVI1 expression in all BC cases and ER-negative BC subgroups (bottom). **B,** FISH analysis indicating *EVI1* copy gain in 2 out of 515 analyzed BC samples.

**Supplementary Figure S2:** Validation of effects using an alternative *EVI1* shRNA. **A,** qRT-PCR analysis of *EVI1* and *MDS1/EVI1* expression in control vs. *EVI1* knockdown MDA-MB-231 cells using an alternative shRNA construct (*EVI1* shRNA alternate). Indicated are *EVI1* and *MDS1/EVI1* expression levels relative to *GAPDH* (Ct method) and normalized to control cells (100%). **B,** Corresponding immunoblots verifying efficient reduction of EVI1 protein expression also for the alternative shRNA construct. Anti--actin staining is shown for loading control. **C,** Growth curves illustrating a growth defect in *EVI1* knockdown MDA-MB-231 cells treated with the alternative shRNA vs. respective control lentiviral particles. **D,** Elevated basal apoptosis in MDA-MB-231 cells transduced with the alternative *EVI1* shRNA. Cells were stained with PI and apoptosis deduced from the percentage of sub-G1 cells.

**Supplementary Figure S3:** Verification of *EVI1*-related phenotypic observations in Hs 578T cells. **A,** Immunoblots of whole cell lysates derived from control vs. *EVI1* knockdown Hs 578T cells. Anti--actin staining is shown for loading control. **B,** Growth curves illustrating a growth defect in *EVI1* knockdown Hs 578T cells vs. respective controls. **C,** Elevated basal apoptosis in *EVI1* knockdown Hs 578T cells as compared to controls. Cells were stained with PI and apoptosis deduced from the percentage of sub-G1 cells. **D,** *EVI1* knockdown sensitizes Hs 578T cells to TRAIL-induced apoptosis. **E/F,** EVI1 affects cell cycle progression.Induction of a G0/G1 defect (E) and reduced BrdU incorporation (F) in *EVI1* knockdown Hs 578T cells.

**Supplementary Figure S4:** EVI1 affects cell cycle regulatory molecules in BC. **A,** Immunoblots of whole cell lysates derived from control vs. *EVI1* knockdown MDA-MB-231 cells. Note an induction of p21Cip1 and p27Kip1 and a decrease in CDK2 expression in response to *EVI1* knockdown. Anti-ß-actin staining is shown for loading control. **B,** qRT-PCR analysis of *CDKN1A* (p21) and *CDKN1B* (p27) gene expression in *EVI1* knockdown MDA-MB-231 cells. Respective expression levels in control cells were set to 1.

**Supplementary Figure S5:** MAPK inhibition modulates BC cell cycle progression and abrogates estrogen-mediated growth rescue in *EVI1* knockdown cells. **A,** Immunoblot analysis verifying downregulation of pERK in MDA-MB-231 (left) and T-47D cells (right) treated with two or three different *EVI1* shRNAs [*EVI1* shRNA; *EVI1* shRNA (alternate); *EVI1* shRNA(alternate2)] as compared to lentivirally transduced control shRNAs. **B-E,** Effects of MEK inhibitors and pERK suppression on growth, cell cycle and apoptosis. **B,** Growth curves of MDA-MB-231 (left) and T-47D (right) cells treated with the MEK inhibitor CI-1040 (5 μM) *vs.* DMSO control. **C,** Cell cycle analyses of MDA-MB-231 (left) and T-47D (right) cells treated with different concentrations of MEK inhibitor CI-1040. Note a dose-dependent induction of a G0/G1-defect in MEK inhibitor-treated cells. **D,** Verification of a G0/G1-defect in MDA-MB-231 cells treated with two alternative MEK inhibitors (1 μM Trametinib or 4 μM AZD6244). **E,** Elevated specific apoptosis in MEK inhibitor-treated MDA-MB-231 cells vs. DMSO-treated control cells. Cells were stained with PI and apoptosis was deduced from the percentage of sub-G1 cells. **F,** Immunoblot analysis of pERK levels in T-47D cells treated with different MEK inhibitors: CI-1040 (5 μM), AZD6244 (4 μM) and Trametinib (1 μM). **G,** Estrogen supplementation does not rescue growth of *EVI1* knockdown cells treated with tamoxifen (10 μM) or CI-1040 (5 μM). Indicated are cell numbers derived from equally plated control (sh-noncoding) and *EVI1*-shRNA-treated T-47D cells cultured over 9 days with vehicle control, β-estradiol (100 nM), or β-estradiol together with tamoxifen (10 μM) or CI-1040 (5 μM).

**Supplementary Figure S6:** Effect of *EVI1* knockdown on tumorigenicity *in vivo* and clonogenicity *in vitro*. **A,** Immunoblot analysis of whole cell lysates derived from tumors excised from xenotransplanted NSG mice, documenting persistent EVI1 knockdown and pERK inhibition *in vivo*. **B,** Quantification of tumor formation from control and *EVI1* knockdown MDA-MD-231 cells in zebrafish. Note that, in contrast to ER+ BC cells, tumor formation could not be restored from transplanted *EVI1* knockdown ER- cells exposed to 100 nM estradiol for 72 hours before and after transplantation. P-values were calculated by a Mann-Whitney test. **C,** Quantification of tumor formation from *EVI1* knockdown ER+ T-47D cells treated with β-estradiol (100 nM), CI-1040 (200 nM) or the combination of both drugs in zebrafish. P-values were calculated by the application of a Chi-square test. **D,** *EVI1* knockdown T-47D cells show an impaired formation of mammospheres in an *in vitro* surrogate assay of BC tumorigenicity.

**Supplementary Figure S7:** Gene expression patterns associated with EVI1 knockdown or overexpression in BC. **A/B,** Gene expression profiles from MDA-MB-231 cells transduced either with control or EVI1 shRNA lentiviral particles. Shown are genes involved in GPCR signaling (A) and cell cycle regulation (B). Heat maps illustrate genes with EVI1-dependent expression differences of >1.5 fold. **C,** qRT-PCR verification of differential expression for 15 candidate genes as identified from the microarray data set. **D,** qRT-PCR analysis of candidate genes in EVI1-overexpressing vs. control Hs 578T cells indicate *KISS1* as the most robustly regulated gene. Indicated are fold changes in gene expression in EVI1-modified versus control cells (C-D). **E,** qRT-PCR documenting co-depletion of *EVI1* and *KISS1* mRNA in two primary ER- BC cell samples (P3 and P4) treated for 48 hours with *EVI1*-specific or control siRNAs. Shown are fold changes in mRNA expression in *EVI1* knockdown vs. control cells for each patient sample.

**Supplementary Figure S8:** EVI1 ChIP analyses of the *KISS1* promoter. **A,** Schematic illustration of *KISS1* promoter regions with potential EVI1-binding sites (*KISS1-1* to *KISS1-4*) therein. **B,** To provide a positive control for the ChIP results presented in Figure 7D, and a respective negative control using non-specific primers, ChIP analyses were performed to analyze occupancy of the *BCL2L1* promoter by EVI1 protein using primer sets as described.

**Supplementary Figure S9:** Verification of the effects of KISS1 overexpression and respectively RKI-1447 treatment on BC cell migration using an alternative cell line (Hs 578T). **A,** Migration (“wound healing”) assays of control and *EVI1*-knockdown Hs 578T BC cells with or without concomitant KISS1 overexpression. Stable EVI1 knockdown or control cells were obtained via lentiviral transduction of Hs578T cells engineered to conditionally express KISS1 upon doxycycline treatment. Doxycyline was added at 1 μg/ml 24 hours before and during (12 hours) of the migration assay. Note that KISS1 overexpression indeed induces migration and rescues the impaired motility of EVI1 knockdown cells. **B,** Semi-quantitative analysis of the migration effects depicted in panel A at 12 hours after start of the migration assay. **C,** Migration assays of control and EVI1-overexpressing Hs 578T BC cells with or without concomitant treatment with the RHO/ROCK inhibitor RKI-1447 (2 μM). Note that EVI1 overexpression enhances cell migration, whereas RKI-1447 treatment impairs this effect. **D,** Semi-quantitative analysis of the migration effects depicted in panel C at 12 hours after start of the migration assay.

**Supplementary Figure S10.** Differential role of KISS1 in *EVI1*-mediated cell migration, cell growth and ERK activation. **A,** Stimulatory effect of Kisspeptin (Kp-10, left) and KISS1 overexpression (*KISS1* OE, right) on the migration of MDA-MB-231 cells. **B,** qRT-PCR confirmation of *KISS1* mRNA induction in MDA-MB-231 and Hs 578T cells transduced with the Teton lentiviral vector system and cultured for 24 hours in the presence of doxycycline (1 μg/ml). **C,** Growth curves of MDA-MB-231 (ER-) and T-47D (ER+) cells transduced either with control or *EVI1* shRNA lentiviral particles and grown in the absence or presence of Kp-10 (1 µM). Note that Kp-10 supplementation cannot restore the growth defects imposed by knockdown of *EVI1.* **D,**Neither supplementation with Kp-10 (500 nM or 1000 nM) nor KISS1 overexpression induces pERK in MDA-MB-231 cells. **E,** qRT-PCR analysis of *KISS1* mRNA in *EVI1*-overexpressing MDA-MB-231 cells treated with MEK inhibitor CI-1040. **F-G,** Treatment with the MEK inhibitor CI-1040 (5 µM) does not alter EVI1-dependent migration. Migration assays of control, *EVI1*-overexpressing (F) and *KISS1*-overexpressing (G) MDA-MB-231 cells in the presence or absence of CI-1040 (5 μM).

**Supplementary Table 1:** Stratification of BC-TMA patient samples according to EVI1 expression levels in relation to histological, ER and HER2 status.

**Supplementary Table 2:** Index list ofprimer sequences as used for qRT-PCR, ChIP analyses and the cloning of *KISS1* from cDNA DNA.