**Supplementary Figure Legends**

**Supplementary Figure S1. Related to Figure 1. AKT1 is downregulated in aggressive breast cancer.** **(A)** Downregulation of AKT1 in basal like breast cancer cell line in CCLE data set. **(B)** Correlation between AKT3 expression level and EMT phenotype in CCLE cell lines. **(C)** Nonsupervised clustering of 54 breast cancer cell lines based on 10 genes. A subset of 10 genes showing distinct expression pattern in basal- versus luminal-breast cancer cell lines in the heatmap. Expressions of BLBC gene signature (ESR1*,* ERBB2andPgR) are also shown. **(D)** Box plots showing the average expression level of AKT1/2/3 or individual AKT isoforms gene in basal and luminal breast cancer cell lines (left) and (middle) from **(A)**. **(E)** myr-AKT1 and myr-AKT2 produce similar kinase activity in cells measuring by GSK3-p status.

**Supplementary Figure S2. Related to Figure 2. AKT1 preferentially interacts with Twist1. (A)** Representative silver-stained gel after immunoprecipitation. Heavy chain and light chain from IgG were labeled. **(B)**Lysates fromMDA-MB-435 cell were immunoprecipitated with anti-AKT1, anti-Twist1 or normal mouse/rabbit IgG. Western blot was performed to test for their interaction. Molecular masses are indicated in kDa. **(C)** Rabbit anti-AKT1 shows positive complexes with mouse anti-Twist1 cells after DuoLink labeling (red dots). Cells stained with either anti-AKT1 or anti-Twist1 antibodies are shown as negative controls. Error bars represent ±SD (n = 4). **(D)** GST pull-down assay of [35S] methionine-labeled Twist1 and GST-AKT1, GST-AKT2 or GST alone. After extensive washes, the bound proteins were collected, resolved on SDS-PAGE and autoradiography. Coomassie blue staining of GST fusion proteins is shown in the lower panel. **(E)** HEK-293T cells were co-transfected with HA-AKT1 and Flag-Twist1, Flag-Snail, Flag-Foxc2 or Flag-E12. Cell lysates were immunoprecipitated with anti-Flag antibody and blotted with anti-HA antibody. The asterisks indicate heavy chain and light chain from IgG.

**Supplementary Figure S3. Related to Figure 2. AKT1 phosphorylates Twist1 at two putative motifs.** **(A)** Lysates from HEK-293T cells expressing empty vector (-), HA-Twist1 and Flag-AKT1, Flag-GSK3, Flag-IKK or Flag-IKK were immunoprecipitated with HA antibody. Bound proteins were analyzed by Western blot. Input is 1% of total extracts. **(B)** Sequencing alignment of two AKT phosphorylation motifs in different species. **(C)** Approximately 1 mg of protein extracts obtained from HEK-293T cells coexpressing Flag-Twist1 and empty vector (-), HA-AKT1 WT, HA-AKT1 DN, or HA-myr-AKT1 were immunoprecipitated with monoclonal anti-Flag antibody. The total amounts of IP were analyzed by Western blot with rabbit anti-phospho-AKT-substrate and anti-phospho-RXRXXS/T antibodies. **(D)** Similar experiment as described in **(C)** was performed to test AKT1-mediated phosphorylation on Twist1, Snail, FOXC2, and E12. **(E)** Analysis of Twist1 phosphorylation using Phos-tag/SDS–polyacrylamide gel (PAGE). HEK-293 cells transfected with HA-Twist1 WT or HA-Twist1 AVA together with HA-myr-AKT1 were separated by Phos-tag/SDS–PAGE, under conditions in which phosphorylated proteins migrate more slowly than corresponding nonphosphorylated proteins, and analyzed by Western blotting using an anti-HA antibody. \*non-specific band. **(F)** *In vitro* kinase assay was conducted as described in the Material and Method. Coomassie blue staining represents equal amount of GST proteins used in the experiment. **(G)** Selection of pGIPz mediated shAKT1, shAKT2 and shAKT3 clones. The red labels indicated the shRNA clones being used for later study. **(H)** Cloning strategy for generating CMV-driven AKT1 overexpression in shAKT1 construct.

**Supplementary Figure S4. Related to Figure 3. AKT1 induces -TrCP mediated Twist1 degradation.** **(A and B)** Pulse-chase analysis of Twist1 in **(A)** overexpression and **(B)** knockdown of -TrCP cells. **(C)** HeLa cells were treated with the indicated concentrations of CG, TG and resveratrol (-TrCP inducers) for 48 h. **(D)** Complete Western blots of Figure 4D. HeLa cells were synchronized by double-thymidine block and released at indicated time points. Cell cycle progression was determined using propidium iodine (50 μg/ml) staining followed by FACS analysis. **(E)**-TrCP controls Twist1 stability during cell cycle progression. HeLa cells carrying siCTRL or si-TrCP were synchronized using thymidine/nocodazole block. Protein expression was visualized by Western blot. S.E., short exposure; L.E., long exposure.

**Supplementary Figure S5. Related to Figure 5. Twist1 AVA induces a stronger EMT phenotypic change.** **(A)** Immunofluorescence staining of Twist1 in myr-AKT1 DN stable clones. **(B)** Myc-Twist1 WT, AVA and DDD were transfected into MCF7 cells and selected with neomycin. Total of 69 neomycin resistant clones were isolated. Expression of Twist1 and E-cadherin in these cells was examined by Western blot with the indicated antibodies. **(C)** Confocal images of Twist1 stable clones. Twist1 stable transfectants were stained with indicated EMT markers and analyzed using confocal microscopy. **(D)** Twist1 variants stable transfectants in MDCK cells were created using a retrovirus based protein expression and analyzed by Western blot with the indicated antibodies. **(E and F)** Monolayer cell proliferation and growth rates of MCF7 Twist1 variants were determined by the MTT assay **(E)** and cell counting **(F).**

**Supplementary Figure S6. Related to Figure 5. Twist1 AVA induces a stronger EMT phenotypic change.** **(A)** Monolayer cell cultures of MCF7 Twist1 stable transfectants were gently scratched with a pipette tip to create a wound. Photographs were taken immediately after the incision, and recorded for every 2 h in culture under a time-lapse microscope at 10x magnification. Image showing the cellular migration at 0, 12, and 24 h. **(B)** Quantification of the migrating cells from **(A).** **(C)** E-cadherin or a vector control was co-expressed with GFP in Twist1 AVA cells. After 24 h, the migration of GFP-positive cells was scored under a time-lapse fluorescent microscope and the expression of GFP and E-cadherin in these cells was measured by Western blot. **(D)** MCF7 stable transfectants were treated with adriamycin for 16 hr. Cell apoptosis was measured using Tunnel assay. **(E)** Western blot showing Twist1 but not Snail is required for shAKT1 mediated EMT.

**Supplementary Figure S7. Related to Figure 5. AKT1 switches Twsit1 from transcription activator to protein degradation by phosphorylating Twist1 at T121 and S123. (A)** Protein stabilities of Twist1 WT, S42A, VA and AVA were determined as described in Fig. 3D. **(B)** HEK-293T cells were transfected with HA-Twist1 variants together with plasmid expressing Histidine-tag (6x-His) fused ubiquitin or HA-myr-AKT1. Covalently conjugated His-ubiquitin of Twist1 was pulled down by Ni2+ agarose beads under denaturing condition and analyzed by Western blot with indicated antibodies. **(C)** MCF7 stable transfectants were subjected to qChIP assay. Mouse IgG was used as a negative control. **(D)** Cells were transiently transfected with E-cadherin-luc reporter together with a *Renilla* luciferase expression vector pRL-TK as an internal control. The relative luciferase activities were determined from three independent samples after normalization to the internal control. **(E)** Total RNA was extracted from MCF7-Twist1 stable transfectants and subjected to qPCR with indicated primers sets. **(F)** Transwell invasion assay of MCF7-Twist1 stable transfectants. **(G)** A model showing that additional phosphorylation of Twist1 T121 and S123 by AKT1 induces -TrCP mediated protein degradation and thereby inhibits EMT in breast cancer cells.

**Supplementary Figure S8. Related to Figure 6. Clinical association of AKT1, -TrCP, Twist1 and E-caherin expression in breast cancer patients.** Representative IHC staining results for AKT1, -TrCP, Twist1 and E-cadherin in human breast cancer tissues.

**Supplementary Figure S9. Related to Figure 6. MK-2206 induces EMT phenotypic change.** **(A)** MCF 10A were continuously treated with 100 nM TGF and 0.2 M MK-2206. One fifth of the cells were split every three days (one passage). Phase contrast images represent the morphology changes. **(B)** Western blot analysis of EMT in MCF 10A WT and shAKT2/3 cells treated with MK-2206. **(C)** Western blot analysis of EMT in 4T1 cells treated with MK-2206 alone or co-treated with resveratrol for 10 days.