Supplementary Figure Legends

Supplementary Figure S1. Correlation of genomic loss and mRNA expression in breast cancer patients. The relative level of genomic M KK3 and M KK3 mRNA level in mammary tumor tissues is shown for 5 patients. Relative M KK3 mRNA expression was determined using the ΔΔCt method with the average of PPIA and GAPDH serving as an endogenous reference. Data was normalized to the sample with highest relative M KK3 expression level. Data for genomic copy number was determined as in Fig. 1C, with further detail in Materials and Methods, wherein data was normalized to the average of those samples from random normal control patients (2 copies). Analysis gave an R^2 value of 0.9491 suggesting a strong positive correlation.

Supplementary Figure S2. Generation of stable MDA-MB-468 cell lines expressing MKK3dn or MKK3ca. (A) MDA-MB-468 cells were transfected with plasmids encoding dominant negative (MKK3dn) or constitutively active (MKK3ca) mutant MKK3 proteins. Colonies that displayed resistance to the selection antibiotic G418 were analyzed for expression of the Flag-tagged MKK3 mutant protein by immunofluorescence using an anti-Flag antibody and an Alexa-594-conjugated secondary antibody while nuclei were stained with DAPI. Colonies with the most stable expression were selected for further analysis. (B) Selected MKK3dn and MKK3ca colonies were assessed for degree and purity of transgene expression by flow cytometry following intracellular staining with FITC-conjugated Flag antibodies. Representative histograms are shown for each cell type: untransfected MDA-MB-468 cells (open) and MKK3dn and MKK3ca cells (grey) were stained with FITC-conjugated anti-FLAG antibodies. (C) Cell lines were finally assessed for population purity by flow cytometric analysis of FITC
staining and side scatter. A representative scatter plot showing distinct single populations is shown.

**Supplementary Figure S3. M KK3 signalling inhibits Akt activation in MDA-MB-468 cells.** Western blotting results from MKK3dn, MKK3ca, and untransfected MDA-MB-468 cells untreated (NT) or stimulated with stem cell factor (SCF) demonstrates impaired Akt signalling in MKK3ca cells. A representative blot is shown for phospho (p-) and total Akt levels, $n = 4$ independent experiments.