

Figure S1

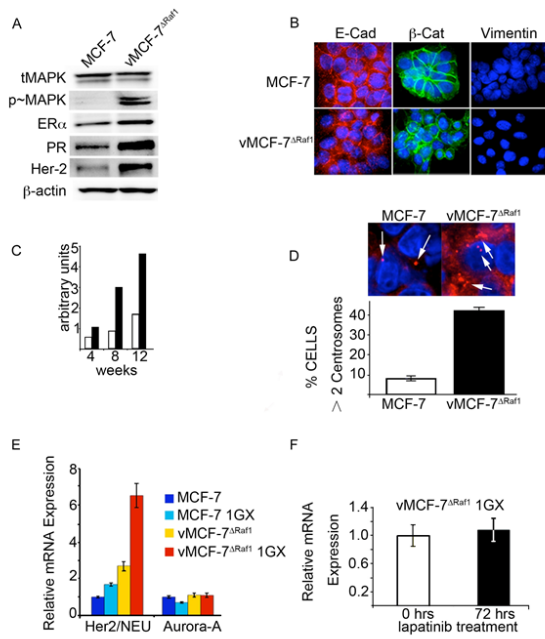


Figure S2

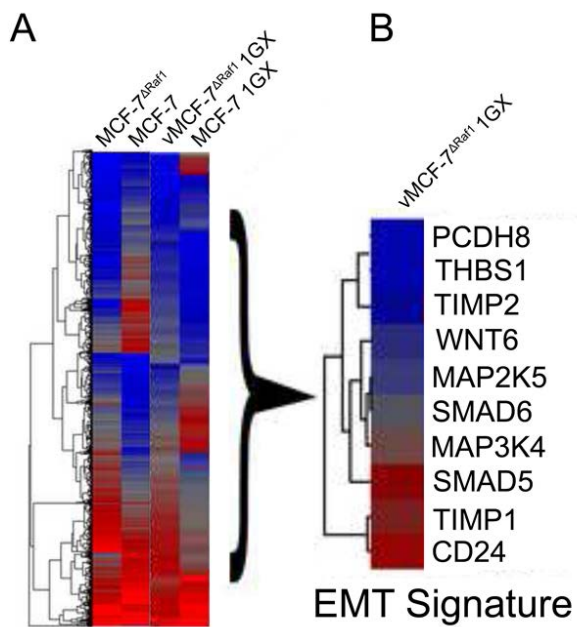


Figure S3

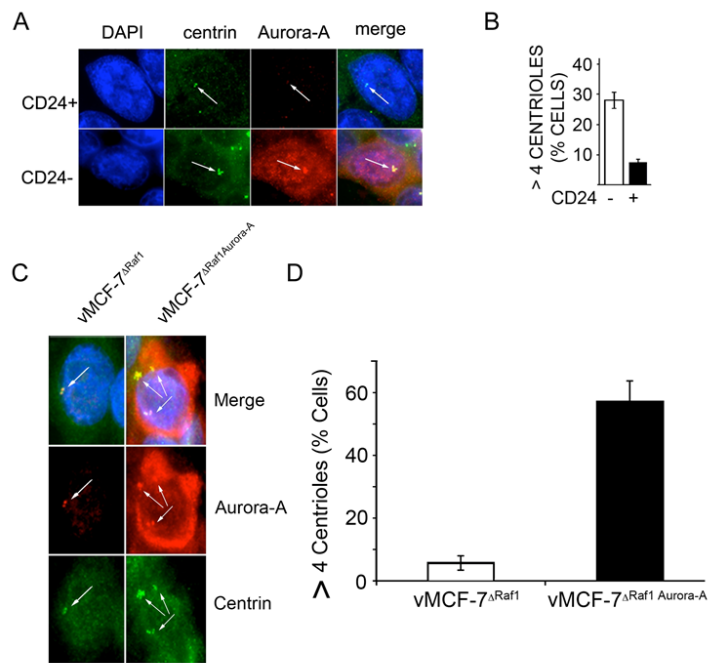


Figure S4

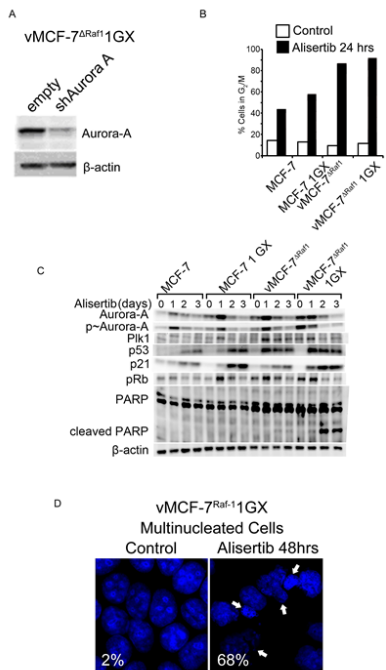


Figure S5

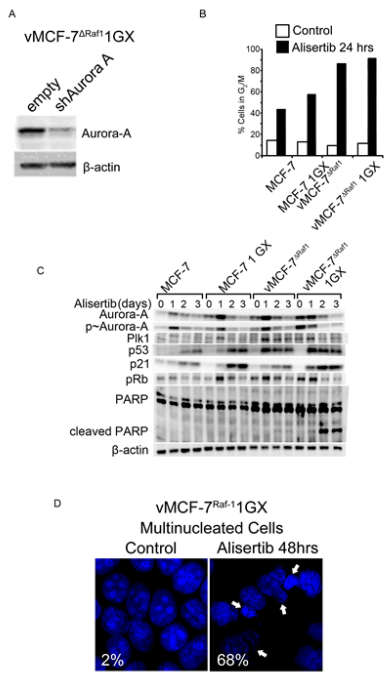


Figure S6

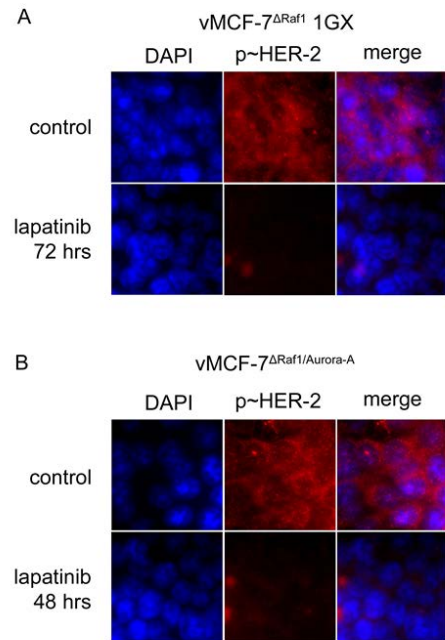


Figure S7

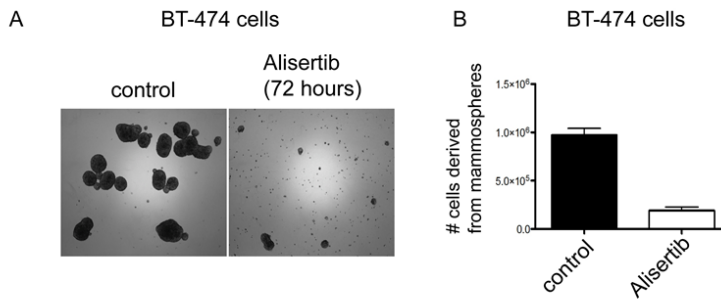


Figure S1. Molecular Characterization Of Breast Cancer Cells. (A) Immunoblot analysis of MCF-7 and vMCF-7^{ΔRaf-1} cells showing MAPK constitutive phosphorylation, expression of ER and PR and HER-2/Neu over-expression. (B) Immunofluorescence analysis showing expression of E-cadherin (red) and B-catenin (green) and lack of vimentin (green) in MCF-7 and vMCF-7^{ΔRaf-1} cells. DNA was labeled in blue with Hoechst dye. (C) Graph showing the area of tumor growth by *NIH Image J program* from three independent experiments. (D) Immunofluorescence analysis showing centrosome amplification in vMCF-7^{ΔRaf-1} xenografts. Centrioles were labeled in red with γ -tubulin and DNA was labeled in blue with Hoechst dye. Graph showing the percentage of cells with amplified centrosomes from three independent experiments (+/- s.d.). (E) Graph showing relative mRNA expression by real-time PCR of HER-2/Neu and Aurora-A genes in MCF-7 and variant cells from three independent experiments (+/- s.d.). (F) Graph showing relative mRNA expression by real-time PCR of Aurora-A gene in vMCF-7^{ΔRaf-1} 1GX cells before and after treatment with 1 μ M lapatinib from three independent experiments (+/- s.d.).

Figure S2. Transcriptome Analysis Of Breast Cancer Cells. (A) Heat map representing the unsupervised cluster analysis of global gene expression in MCF-7, MCF-7 1GX, vMCF-7^{ΔRaf-1} and vMCF-7^{ΔRaf-1} 1GX cells. (B) Heat map representing the identification of an EMT signature in vMCF-7^{ΔRaf-1} 1GX cells.

Figure S3. Characterization of Centrosome Phenotype In Breast Cancer Cells. (A) Immunofluorescence analysis showing the centrosome phenotype in CD24⁺ and CD24^{-low} cancer cells. The centrosome protein centrin was labeled in green, the mitotic kinase Aurora-A was labeled in red and DNA was labeled in blue with Hoechst dye. (B) Graph showing the percentage of CD24⁺ and CD24^{-low} cancer cells with amplified centrosomes from three independent experiments (+/- s.d.). (C) Immunofluorescence analysis showing the centrosome phenotype in vMCF-7^{ΔRaf-1} and vMCF-7^{ΔRaf-1/Aurora-A} cells. The Centrosome protein centrin was labeled in green, the mitotic kinase Aurora-A was labeled in red and DNA was labeled in blue with Hoechst dye. (D) Graph showing the percentage of vMCF-7^{ΔRaf-1} and vMCF-7^{ΔRaf-1/Aurora-A} cells with amplified centrosomes (> 4 centrioles) from three independent experiments (+/- s.d.).

Figure S4. Molecular Inhibition Of Aurora-A Kinase Activity *In Vitro*. (A) Immunoblot analysis showing down-regulation of Aurora-A expression by an shRNA vector in vMCF-7^{ΔRaf-1} 1GX cells. (B) Graph displaying cell cycle analysis (FACS) of breast cancer cells showing that treatment with 1μM Alisertib for 24 hours arrested cells in the G2/M phase of the cell cycle. (C) Immunoblot analysis of MCF-7, MCF-7 1GX, vMCF-7^{ΔRaf-1} and vMCF-7^{ΔRaf-1} 1GX cells showing that treatment with 1μM Alisertib suppressed Aurora-A kinase activity, reduced PLK1 expression and activated the p53 mediated G1/S cell cycle checkpoint. Activation of apoptosis was detected by cleaved PARP only in vMCF-7^{ΔRaf-1} 1GX cells. (D) Immunofluorescence analysis showing the development of multinucleated cells following treatment with 1μM Alisertib of vMCF-7^{ΔRaf-1} 1GX cells.

Figure S5. Inhibition of p~HER-2/Neu in Breast Cancer Cells. (A) Immunofluorescence analysis showing inhibition of p~HER-2/Neu following treatment with 1 μ M Lapatinib of vMCF-7 ^{Δ Raf-1} 1GX cells. (B) Immunofluorescence analysis showing inhibition of p~HER-2/Neu following treatment with 1 μ M Lapatinib of vMCF-7 ^{Δ Raf-1/Aurora-A} cells.

Figure S6. Inhibition of Mammospheres Growth in BT-474 Breast Cancer Cells. (A) Light microscopy analysis showing inhibition of mammospheres growth derived from BT-474 breast cancer cells after treatment with 1 μ M Alisertib for 72 hours. (B) Graph showing the percentage of cells derived from mammospheres before and after treatment with 1 μ M Alisertib. Experiments were performed in triplicate (+/- s.d.).