Figure 1: Southern blot analysis of mtDNA. Total DNA was extracted from parental MDA-MB-435 cells (Lanes 1A, 1B) and the clone 7 mtDNA-depleted variant of MDA-MB-435 cells (Lanes 2A, 2B). (A): Total DNA was then digested with PvuII (which cuts a single site in mtDNA generating a single 16.6 Kb fragment), separated by electrophoresis on agarose gel containing ethidium bromide. (B) DNA was transferred to nylon membrane, and the membrane was hybridized to a $^{32}$P-labeled mtDNA-specific DNA probe. The 16.6 Kb mtDNA is present in parental MBA-MD-435 cells (Lane 1B), but absent in Clone 7 (Lane 2B). The 16.6 Kb genome is indicated by arrow.

Figure 2: Rho$^0$ cells do not express mtDNA-encoded COX II protein. Equal amounts of total cell lysates from parental MDA-MB-435 (Lane 1) and Clone 7 Rho$^0$ variant (Lane 2) cells were separated by SDS-PAGE and transferred to a membrane. (A) Blot was hybridized with a monoclonal antibody against cytochrome oxidase II (COXII), and (B) then stripped and rehybridized with a monoclonal antibody against $\alpha$-actin. COXII staining demonstrates the loss of expression of the mtDNA-encoded COXII (Lane 2A). Actin staining shows that protein loading is approximately equal (Lanes 1B, 2B).

Figure 3: Rho$^0$ cells do not grow in galactose medium. Rho$^+$ (MDA-MB-435) cells and Rho$^0$ cells were grown in custom-made medium containing galactose (instead of glucose, purchased from Sigma). While MDA-MB-435 cells continue to grow in this medium (A), Rho$^0$clone 7 cells began to die after 48 hours (B).

Figure 4: Lipid peroxidation is increased due to dysfunction in mitochondria: Subconfluent cultures were collected by trypsinization, washed with ice cold PBS and lysed by freeze/thaw cycles in sterile deionized water. Lipid peroxidation was measured as described in material methods.

Figure 5: RT-PCR analysis: RT-PCR analysis of up-regulated or down regulated genes was carried out as described in material methods.