Supplemental Section

Supplementary Figure 1 Leptin induces the expression of mesenchymal markers and nuclear localization of Snail and Slug in MCF7 cells. A, MCF7 cells were treated with 100 ng/ml leptin for 3 and 5 days (3d, 5d). Total lysates were immunoblotted for Snail, Slug, Zeb1, Twist and Fibronectin. Actin was used as control. **B,** MCF7 cells were treated as in A. Fluorescent confocal microscopy showed the nuclear localization of Snail and Slug in leptin-treated cells. Nuclei were visualized with DAPI staining.

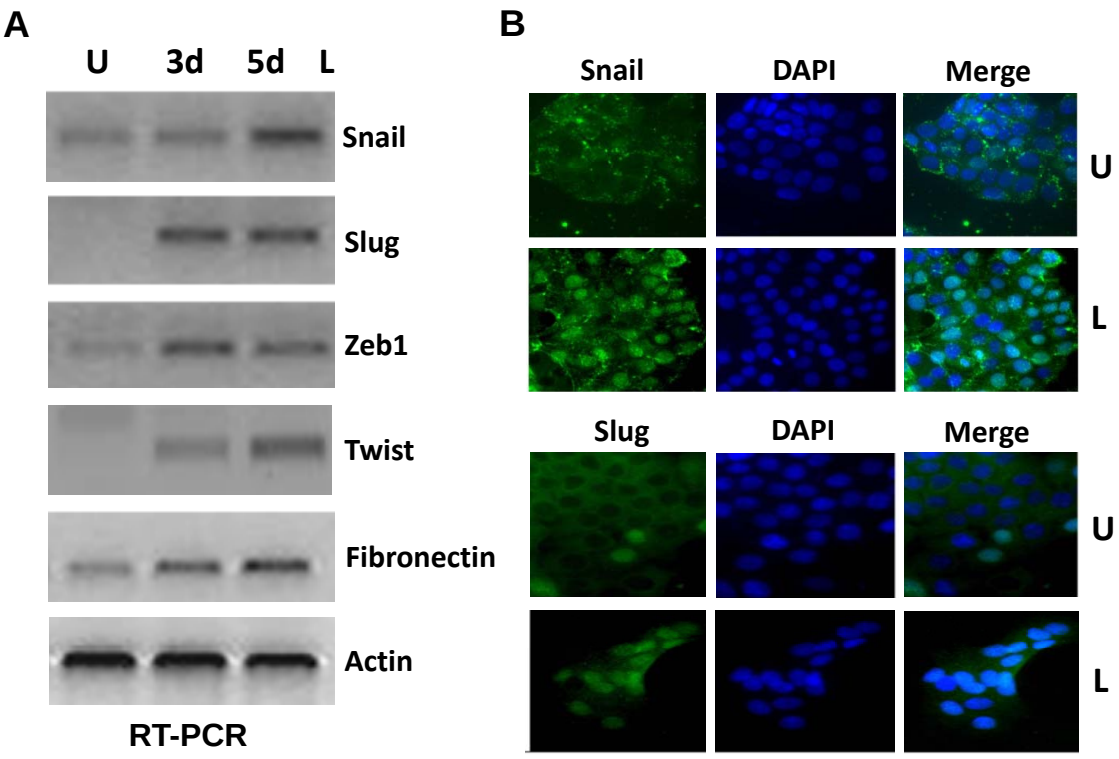
Supplementary Figure 2 Leptin induces epithelial to mesenchymal transition in breast cancer cells. A, MDA-MB-468 and MDA-MB-231 cells were serum-starved for 16 hours followed by treatment with 100 ng/ml of leptin (L) or untreated (U) for 3 days. Morphological changes associated with EMT are shown in phase-contrast images. MDA-MB-468 cells show increased intracellular separation and detached mesenchymal-like morphology in the presence of leptin. MDA-MB-231 cells displayed the presence of spindle-shaped cells, increased intercellular separation and pseudopodia upon leptin treatment. **B,** MDA-MB-468 and MDA-MB-231 cells were treated as in A. Expression level of Fibronectin, Keratin 18, Slug and Snail were examined using RT-PCR analysis. Leptin treatment increases Vimentin, Snail and Slug expression and decreases Keratin 18 expression. **C,** MDA-MB-468 and MDA-MB-231 cells were cultured in Matrigel invasion chambers followed by treatment with leptin for 24 h. The number of cells that invaded through the Matrigel was counted in five different regions. The slides were blinded to remove counting bias. The results show mean of three independent experiments performed in triplicates. * $P < 0.005$ compared with untreated controls. (U). **D,** MDA-MB-468 and MDA-MB-231 cells were subjected to scratch migration assay in the presence (L) or absence (U) of leptin. The histogram shows fold-change in migration. * $P < 0.001$ compared with untreated controls.

Supplementary Figure 3 Leptin increases Cyclin D1 expression and recruitment of phospho-Stat3 to cyclin D1 promoter. A, MCF7 and MDA-MB-231 cells were treated with 100 ng/ml leptin for indicated time intervals. Cyclin D1 expression level was analyzed using RT-PCR analysis. Actin expression level was used as control. Leptin treatment increases cyclin D1 expression. **B,** MCF7 and MDA-MB-231 cells were treated with 100 ng/ml leptin for indicated time intervals. Total lysates were immunoblotted for Cyclin D1 expression. The membranes were re-blotted using actin antibody as control. The blots are representative of multiple independent experiments. Leptin increases the expression of Cyclin D1. **C,** Soluble chromatin was prepared from MCF7 and MDA-MB-231 breast cancer cells treated and untreated with leptin as indicated and immunoprecipitated with 5 μg of specific antibodies against p-Stat3 overnight at 4^0C. The immune complexes were pulled down with protein A Agarose/salmon sperm DNA beads and washed extensively as described in materials and methods, and cross-linking was reversed. The purified DNA was analyzed by PCR using primers spanning the putative Stat3 binding site (GAS sites) at cyclin D1 promoter. EBAG9 was used as a control gene. Leptin increases recruitment of p-Stat3 to responsive gene promoters.

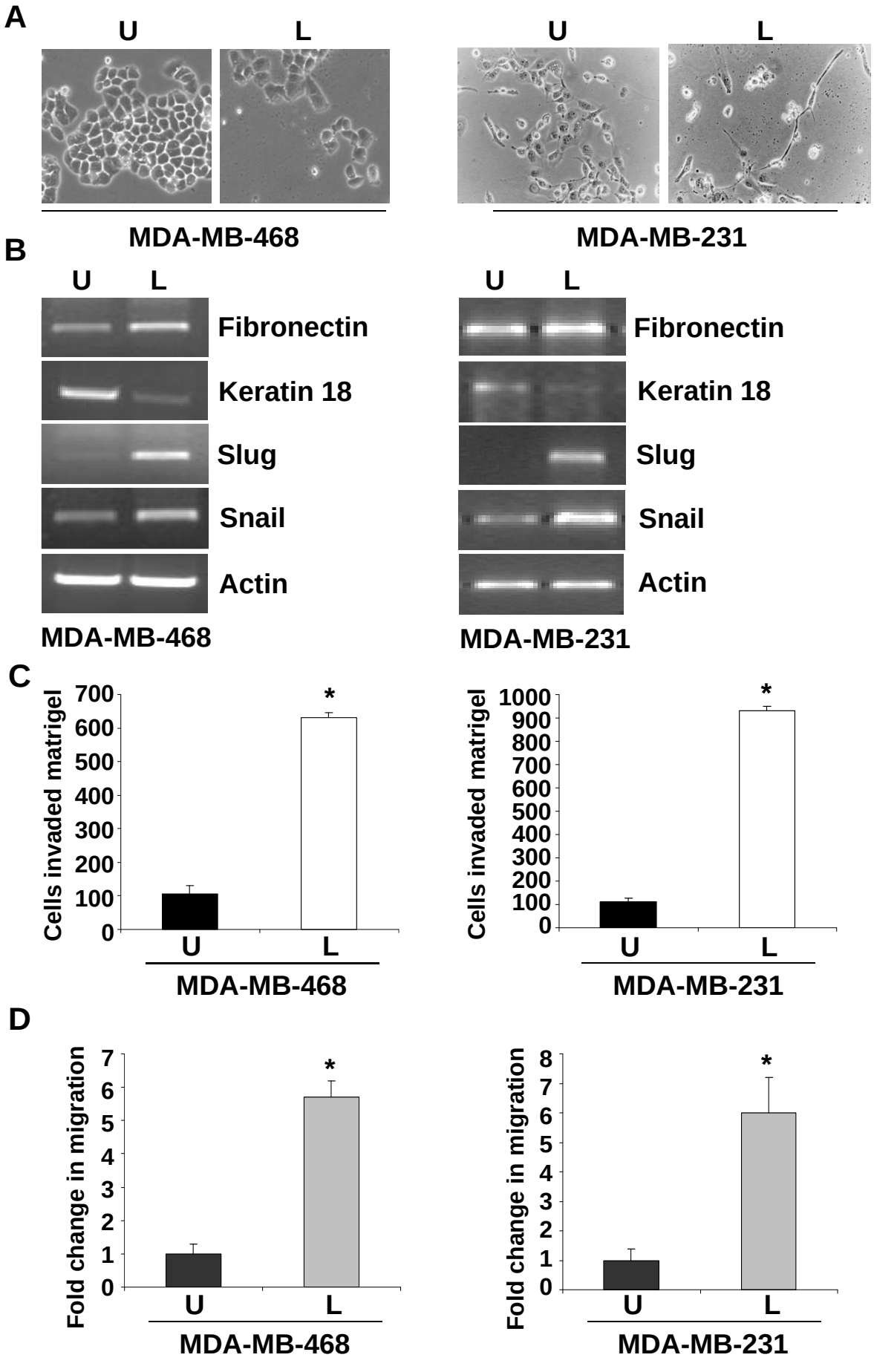
Supplementary Figure 4 Leptin increases Fibronectin expression. Breast cancer cells were treated with 100 ng/ml leptin for indicated time intervals. Fibronectin expression level was analyzed using RT-PCR analysis. Actin expression level was used as control. Leptin treatment increases fibronectin expression.

Supplementary Figure 5 Leptin increases MTA1 expression. MDA-MB-231 breast cancer cells were treated with 100 ng/ml leptin for indicated time intervals. Total lysates were immunoblotted for MTA1 expression. The membranes were re-blotted using actin antibody as control. The blots are representative of multiple independent experiments. Leptin increases the expression of MTA1.

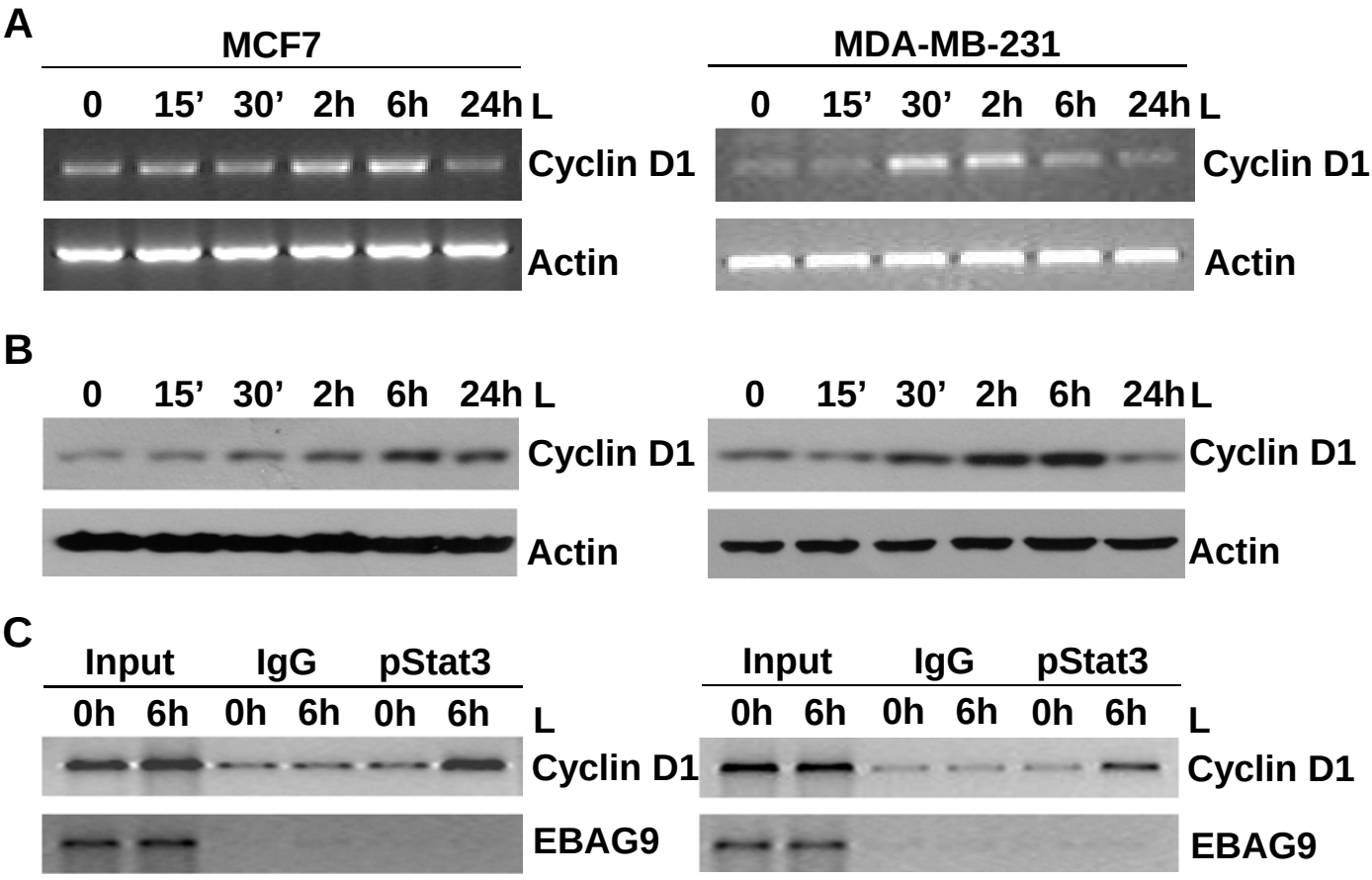
Supplementary Figure 1



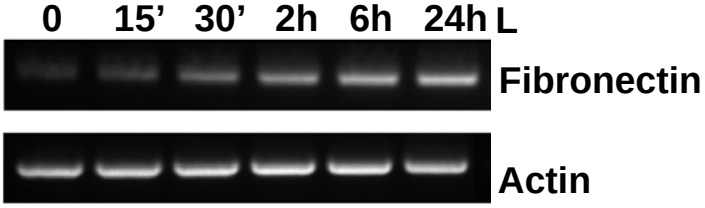
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5