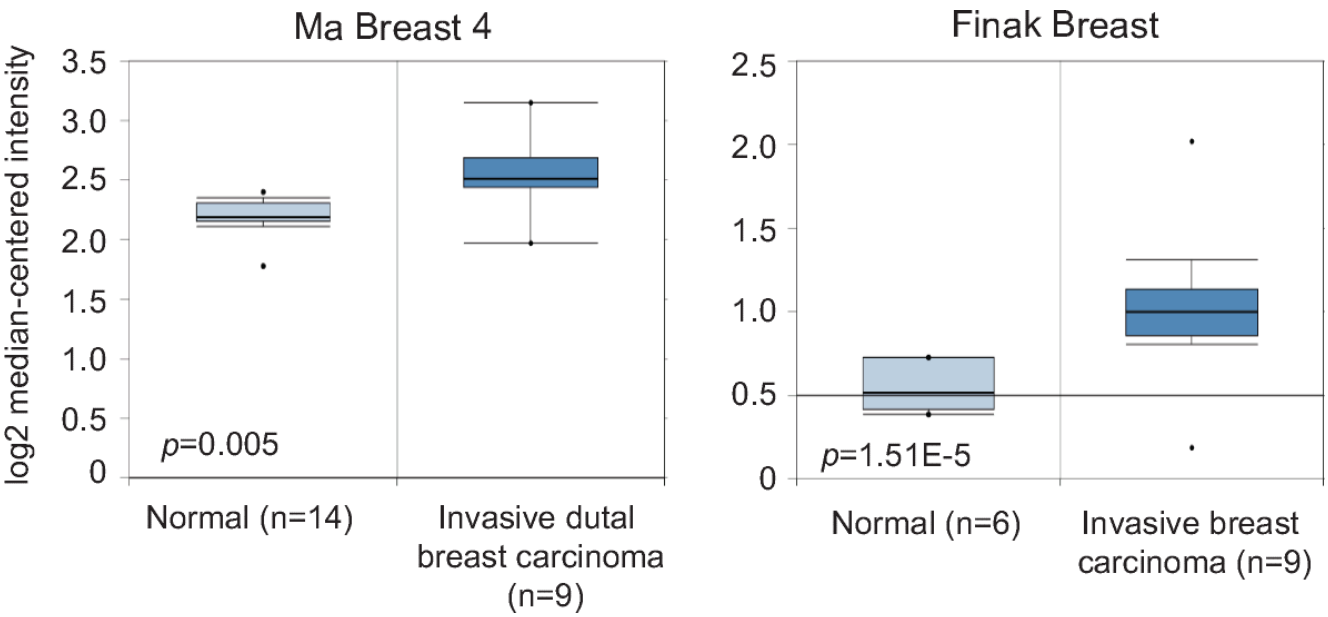
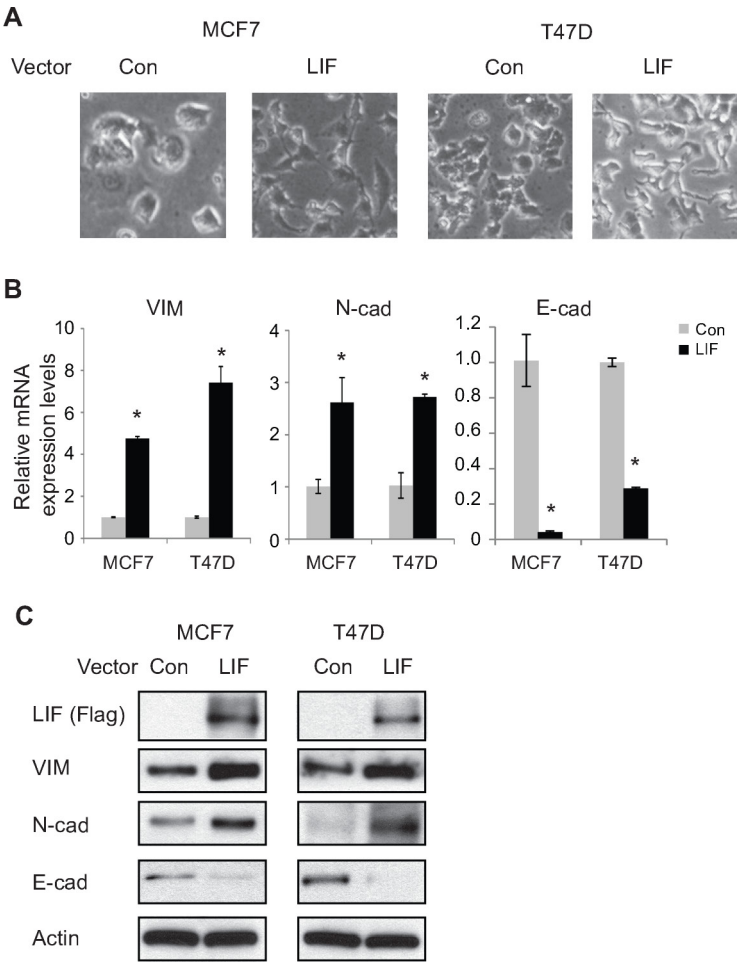
# Leukemia inhibitory factor promotes EMT through STAT3-dependent miR-21 induction

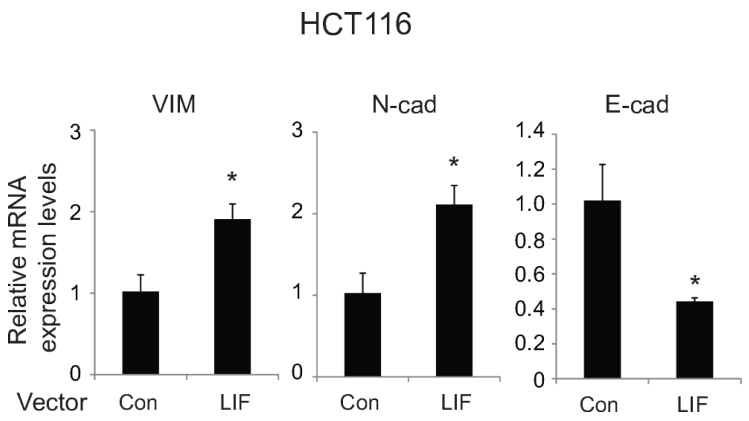
## Supplementary Materials



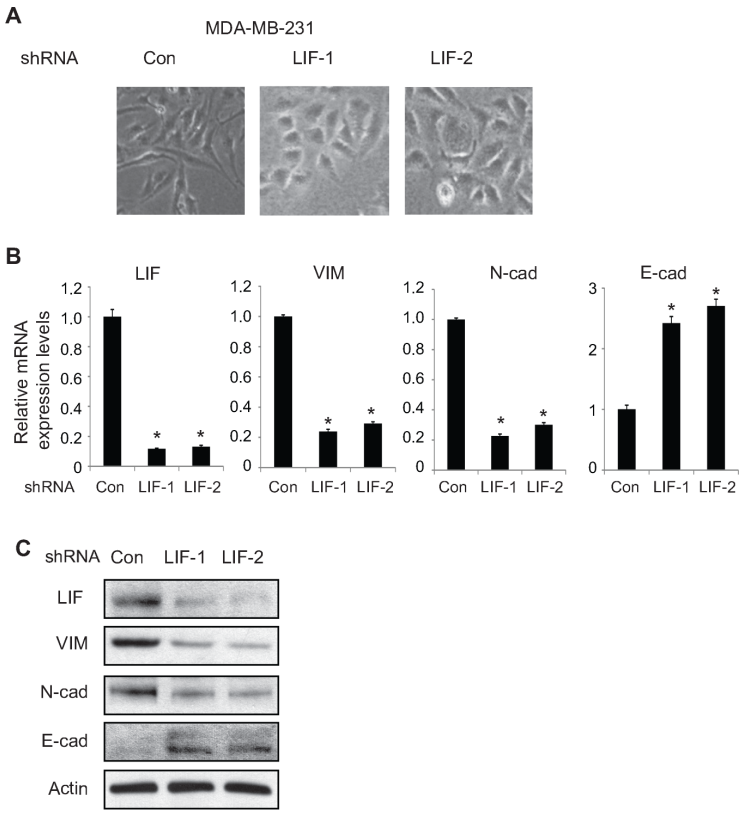

**Supplementary Figure S1: LIF mRNA levels are frequently elevated in tumors.** LIF mRNA levels are elevated in invasive breast carcinomas in 2 different datasets from the Oncomine database (GSE14548 and GSE9014). LIF mRNA levels are presented as box plots and expressed in terms of a log2 median-centered intensity which is calculated by normalizing the intensity of LIF probe to the median of the probe intensities across the entire array [15, 16].



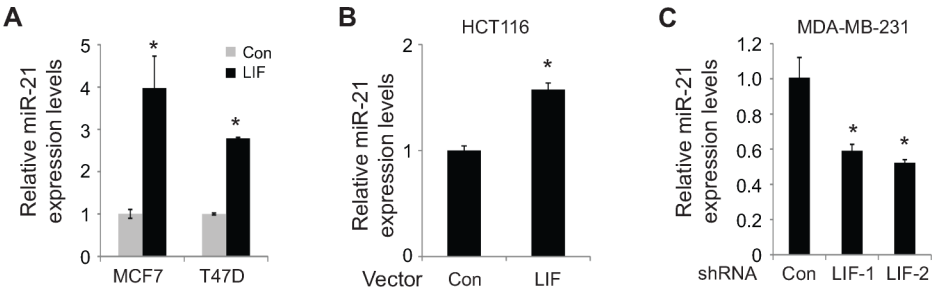

**Supplementary Figure S2: Stable ectopic LIF expression promotes EMT in MCF7 and T47D cells.** (**A**) EMT morphological change was examined by phase-contrast photomicrographs in MCF7 and T47D cells with stable ectopic LIF expression and control cells stably transfected with control expression vectors (Con). (**B and C**) The mRNA (B) and protein (C) levels of mesenchymal markers (VIM, N-cad) and epithelial marker (E-cad) were determined in cells by real-time PCR and Western blot assays, respectively. The mRNA levels of these genes were normalized to β- actin. Data are presented as mean ± s.d. ($n = 3$). *$p < 0.05$; student *t*-test.



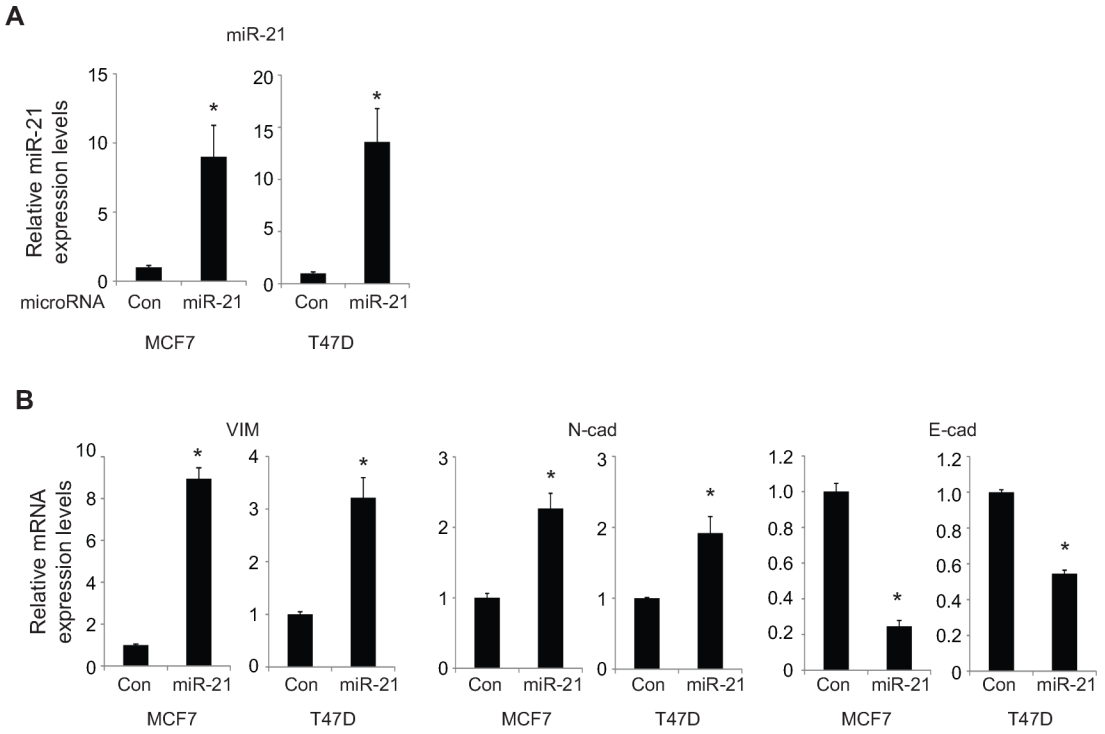

**Supplementary Figure S3: Ectopic LIF expression changes the expression levels of EMT markers in human colorectal cancer HCT116 cells.** HCT116 cells were transiently transfected with LIF expression vectors or control vectors (Con). The mRNA expression levels of mesenchymal markers (VIM, N-cad) and epithelial marker (E-cad) were determined in cells at 48 h after transfection by real-time PCR. The mRNA levels of these genes were normalized to β-actin. Data are presented as mean ± s.d. ($n = 3$). *$p < 0.05$; student *t*-test.



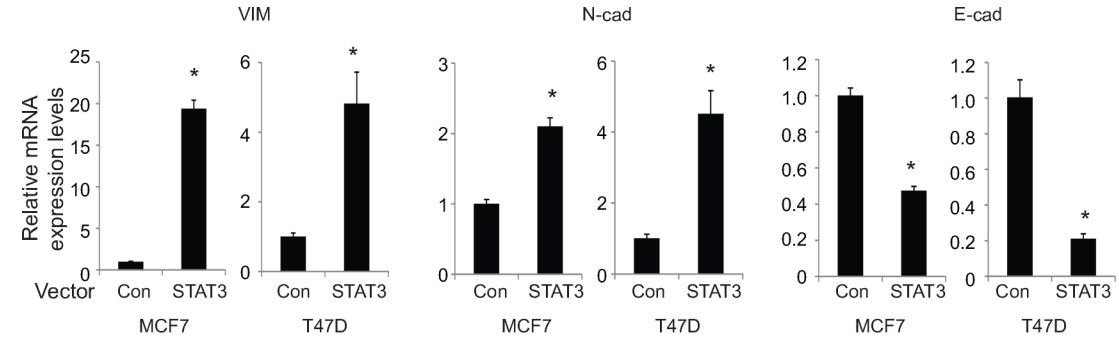

**Supplementary Figure S4: Stable knockdown of endogenous LIF reverses EMT in MDA-MB-231 cells.** (**A**) MDA-MB-231 cells with stable knockdown of LIF by shRNA vectors and their control cells were employed to examine EMT morphological change by phase-contrast photomicrographs. (**B and C**) Stable knockdown of LIF changed the mRNA (B) and protein (C) levels of EMT markers in MDA-MB-231 cells. In (B) data are presented as mean ± s.d. ($n = 3$). *$p < 0.05$; student $t$-test.



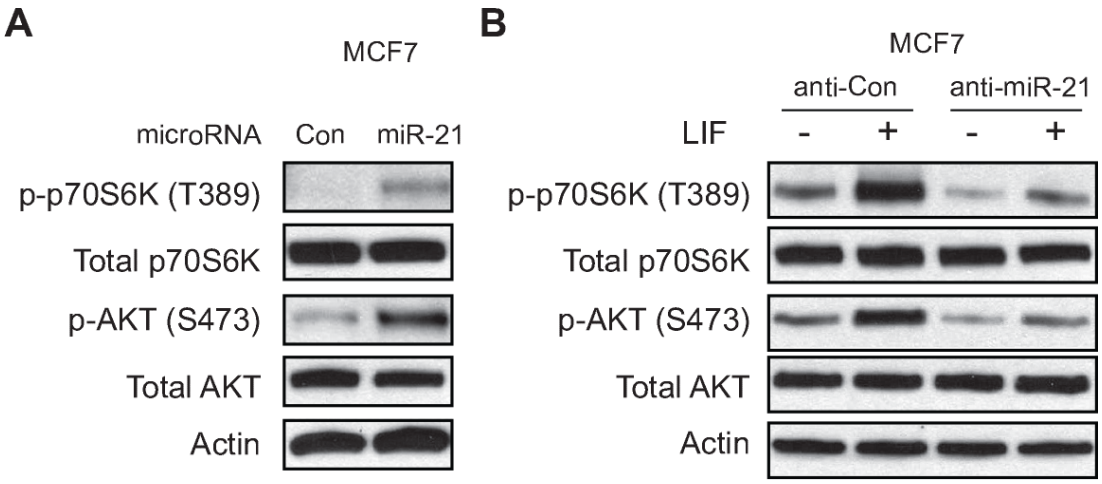

**Supplementary Figure S5: LIF induces the expression of miR-21 in cells.** (**A**) The expression of miR-21 was determined in MCF7 and T47D cells with ectopic stable LIF expression and their control cells. (**B**) The expression of miR-21 was determined in HCT116 cells with ectopic expression of LIF. (**C**) The expression of miR-21 was determined in MDA-MB-231 cells with stable knockdown of LIF by shRNA vectors targeting LIF and their control cells. The expression of miR-21 was normalized to the U6 snRNA. Data are presented as mean ± s.d. ($n = 3$). *$p < 0.05$, student $t$-test.




**Supplementary Figure S6: miR-21 increases the mRNA expression levels of mesenchymal markers and decreased the expression levels of epithelial marker.** MCF7 and T47D cells were transfected with miR-21 mimics or microRNA controls. The expression levels of mesenchymal markers (VIM and N-cad), and epithelial marker (E-cad) were determined by real-time PCR. The mRNA levels of these genes were normalized to β-actin. Data are presented as mean ± s.d. ($n = 3$). *$p < 0.05$; student *t*-test.




**Supplementary Figure S7: STAT3 increases the mRNA expression levels of mesenchymal markers and decreased the expression levels of the epithelial marker.** MCF7 and T47D cells were transfected with STAT3 expression vectors or control vectors. The expression levels of mesenchymal markers (VIM and N-cad) and epithelial marker (E-cad) were determined by realtime PCR. The mRNA levels of these genes were normalized to β-actin. Data are presented as mean ± s.d. ($n = 3$). *$p < 0.05$; student *t*-test.




**Supplementary Figure S8: Blocking miR-21 greatly inhibits the activation of the AKT/mTOR signaling by LIF.** (**A**) miR-21 increased the phosphorylation levels of AKT and p70S6K in MCF7 cells. MCF7 cells were transfected with miR-21 mimics or microRNA controls, and the levels of phosphorylated AKT at S473 (p-AKT), total AKT, phosphorylated p70S6K at T389 (p-p70S6K) and total p70S6K were determined by Western blot assay. (**B**) Blocking miR-21 by anti-miR-21 oligos greatly inhibited the activation the p-AKT and p-p70S6K by LIF in MCF7 cells. MCF7 cells with and without stable ectopic expression of LIF were transfected with anti-miR-21 RNA or control RNA oligos.