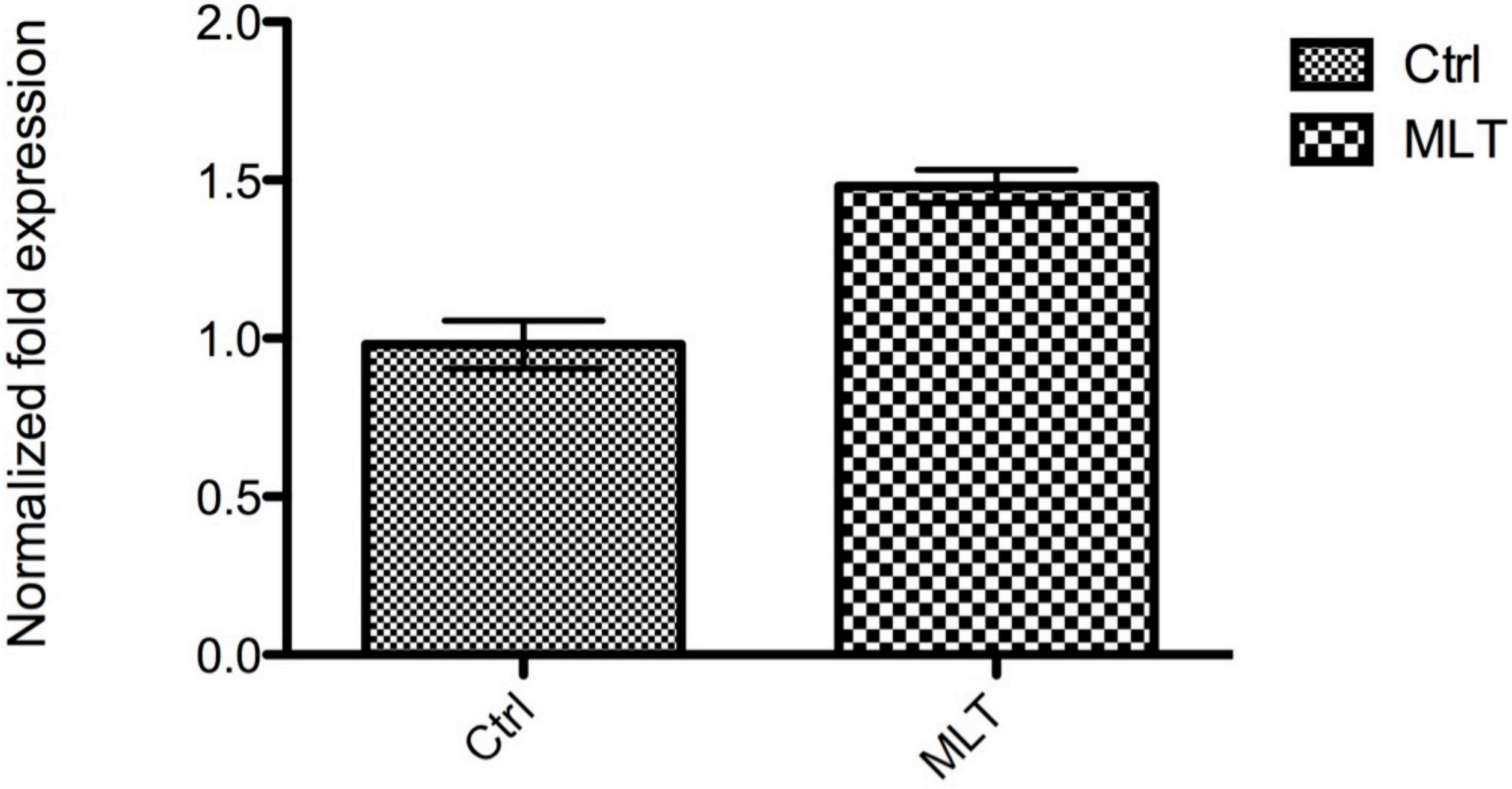
Supplementary Method and Figures 1 & 2

Real-time reverse transcriptase-polymerase chain reaction analysis

Real-time reverse transcriptase-polymerase chain (RT-PCR) analyses were performed to determine the mRNA levels of E-cadherin in MCF-7/CaSrc cells. Total cellular RNA was isolated using the PerfectPure RNA Cultured Cell Kit (5 Prime, Gaithersburg, MD, USA) in accordance with the manufacturer's instructions. Reverse transcription was performed on 1 μg of total RNA using Superscript II RNase H- reverse transcriptase (Invitrogen Corporation) and 200 ng random hexadeoxynulceotide primers in 20-μL reaction volumes containing 3 mM $MgCl_2$, 10 mM DTT, 75 mM KCl, and 0.5 mM dNTP. Real-time PCR was carried out in 20 μL of PCR mixture containing 10 μL of 2× iQ SYBR Green Supermix and 1 μL of each cDNA sample on an iCycler iQ real-time detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) in triplicates and recorded in real time and analyzed using the accompanying program (iCycler iQ real-time PCR detection system software, version 3.0A; Bio-Rad Laboratories, Inc.). The level of the acidic ribosomal phosphoprotein PO 36B4 was also determined by real-time RT-PCR in each cDNA sample to normalize the expression of E-cadherin. The primers used were as follows: human E-cadherin: forward primer, 5'-AGTCCTGGTCCTCT-3', and reverse primer, 5'-ATTCTGATTCTGCTGCTCTTG-3'; 36B4: forward primer, 5'-CGACCTGGAAGTCCAACTA-3', and reverse primer, 5'-ATCTGCTGCATCTGCTTG-3'. Melt curve analysis was performed at the end of each PCR to confirm the specificity of the PCR product. Threshold cycle (Ct) values of E-cadherin among samples were compared after correction for 36B4 expression. The ratio of E-cadherin versus the corresponding 36B4 of each sample was determined on the basis of the equation E-cadherin/36B4 = $2^{Ct(36B4) - Ct(E\text{-}cadherin)}$. The ratio of E-cadherin/36B4 was compared among samples.

Fig 1. MLT increases the mRNA expression of E-cadherin. Serum-starved MCF-7/caSrc cells were treated with MLT (10^{-8} M) or vehicle (0.00001% ethanol) for 6 h. The mRNA levels of E-cadherin were examined by real-time RT-PCR analyses. The mRNA levels of 36B4 were determined to normalize the expression of E-cadherin. $*P < 0.01$ vs diluent-treated cells by two-tailed student's *t* test ($n = 3$). Data are means ± s.d.

Fig 2. Phase-contrast images of MCF-7 cells. Serum-starved MCF-7 cells were treated with TGF-b (10 ng/ml) for 48 h to induce EMT, with or without the presence of MLT (10^{-8} M). (scale bar, 50 uM).



Supplementary Figure. 1

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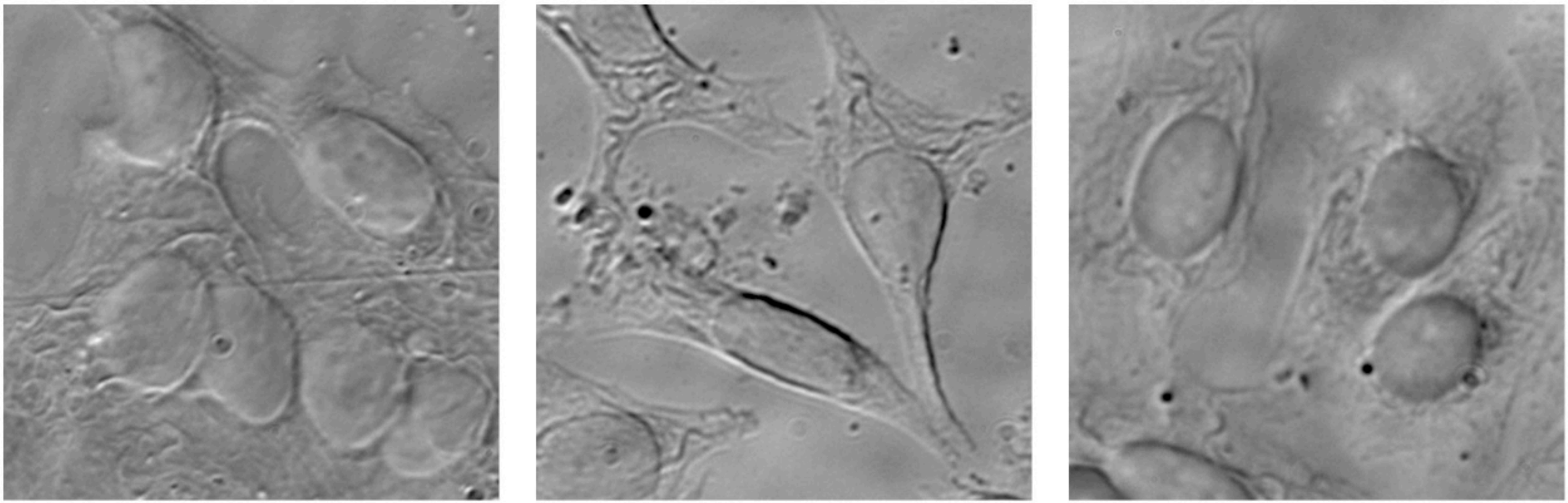
TGF-β

TGF-β+MLT



Supplementary Figure. 2