

Supplementary Information

Methods

Analysis of apoptosis

Cells undergoing apoptosis were detected in cells and tissue sections by the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) method, using the *in situ* apoptosis detection TUNEL kit (Takara, Japan) according to the manufacturer's recommended protocol. The frequency of apoptosis was calculated as an apoptotic index, in which the proportion of cells undergoing apoptosis was expressed as a percentage of all carcinoma cells observed. The apoptotic index of cells was calculated as the number of TUNEL-positive cells to all cells counted in the whole fields of the slide; the apoptotic index of tissues was calculated as the number of TUNEL-positive cells to cells counted in $100 \times$ high magnification fields (HPF) of the slide, each field was subjected to two independent counts. The *in situ* apoptosis detection TUNEL kit contained tissue sections that served as a positive control.

Immunofluorescence

Approximately 1×10^5 of LLC, LLC-miPS CM or LLC-MEF cells were seeded on a slide in 12-well plate coated with 3% gelatin previously. After 24 hours, the cells were washed with phosphate-buffered saline (PBS) twice and permeabilized and fixed in 4% paraformaldehyde and 0.1% Triton X100 in PBS buffer at 4°C for 30 min. The cells were then washed 3 times with PBS and incubated with the blocking solution (10% goat serum in PBS). The cells were then incubated with the primary antibodies of β -catenin (BD Biosciences) diluted 1:100 for 2 hours, washed 3 times with PBS for 15 min and finally incubated with FITC-labelled anti-mouse IgG Fc antibody (Invitrogen) diluted 1:1000 for 2 hours. The slides were washed extensively with PBS and mounted with PBS. All slides were photographed using confocal microscopy, LSM 510 Meta (Carl Zeiss, Germany) equipped with Argon laser and LSM software (Carl Zeiss, Germany).

miPS microenvironment generates EMT in mouse LLC cells

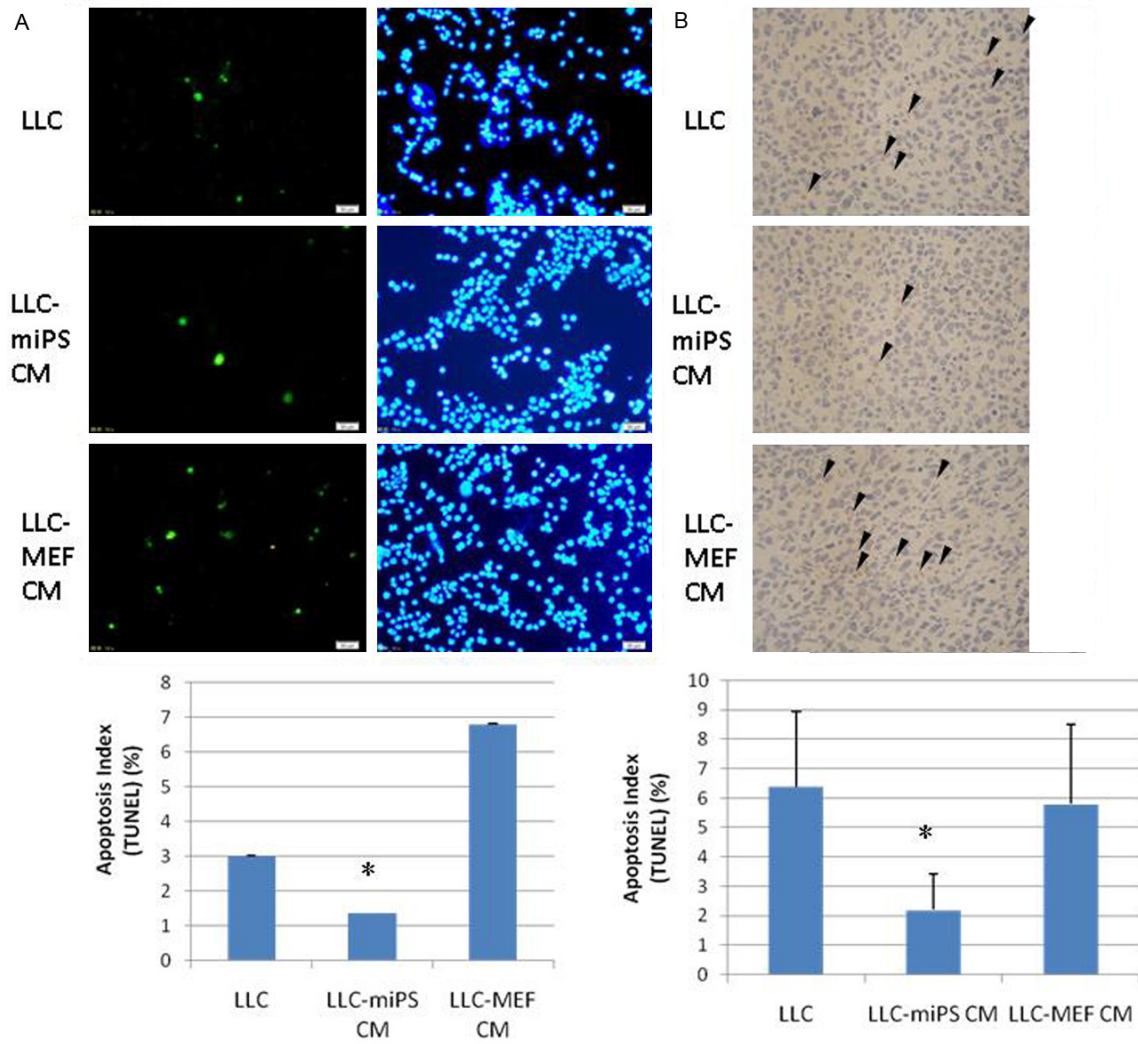


Figure S1. Cell apoptosis in vivo and in vitro. A. Cell apoptosis in LLC, LLC-miPS and LLC-MEF cells was determined by TUNEL staining. Representative micrographs of apoptotic cells are shown in the upper panel (Scale bar: 50 μ m). The apoptotic index of cells was calculated as the number of TUNEL-positive cells to all cells counted in the whole fields of the slide. Values indicated by an asterisk (*) are significantly different ($P < 0.05$). B. Tumor cell apoptosis in LLC, LLC-miPS and LLC-MEF cells derived from tumors as determined by TUNEL staining. Representative micrographs of the apoptosis cells are shown in the upper panel in 100 \times high magnification fields (HPF). The apoptotic index of cells was calculated as the number of TUNEL-positive cells to all cells counted in 100 \times HPF of the slide. Values indicated by an asterisk (*) are significantly different ($P < 0.05$).

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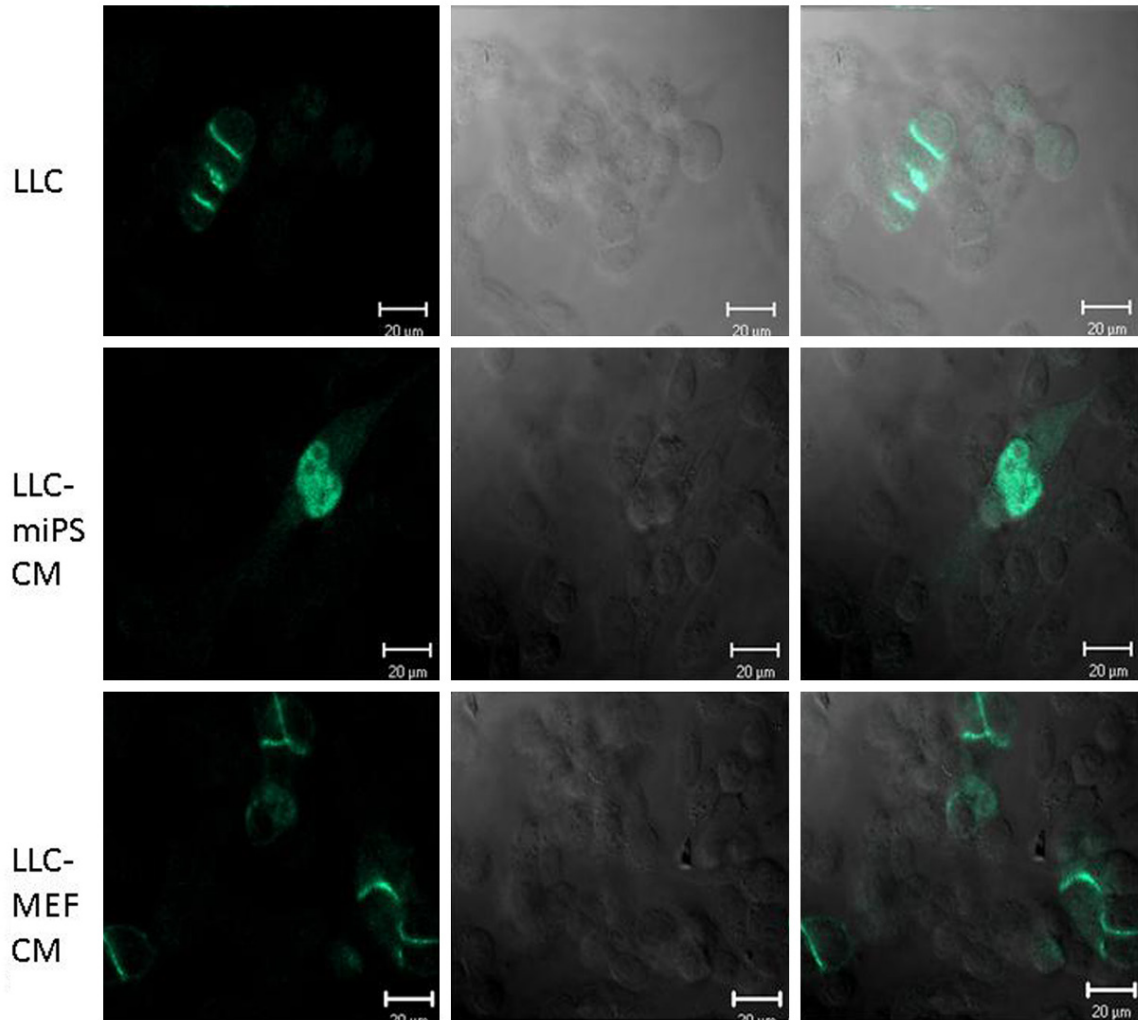


Figure S2. Immunofluorescence localization of β -catenin. Immunofluorescence localization of β -catenin in LLC, LLC-miPS CM and LLC-MEF CM cells were checked by confocal microscopy. Scale bar: 20 μ m.