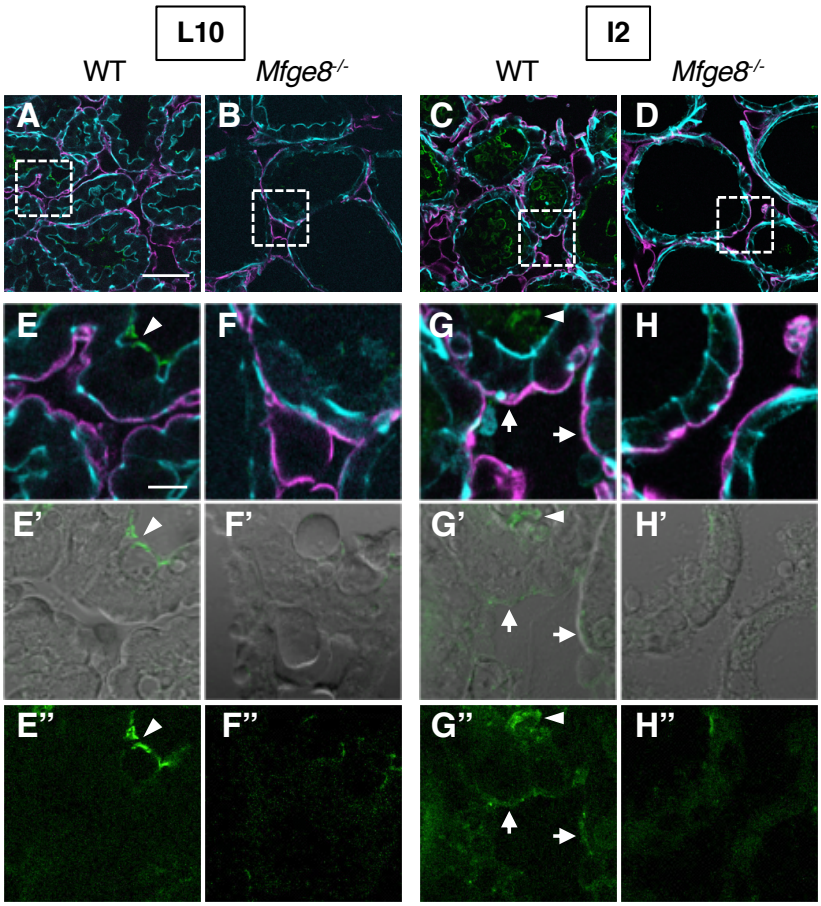
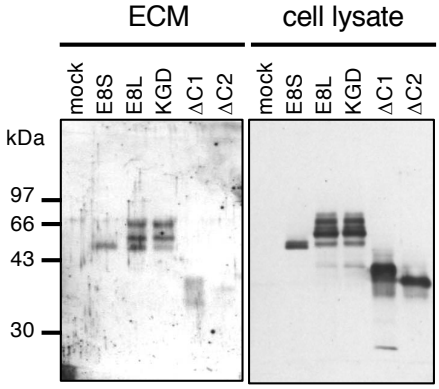
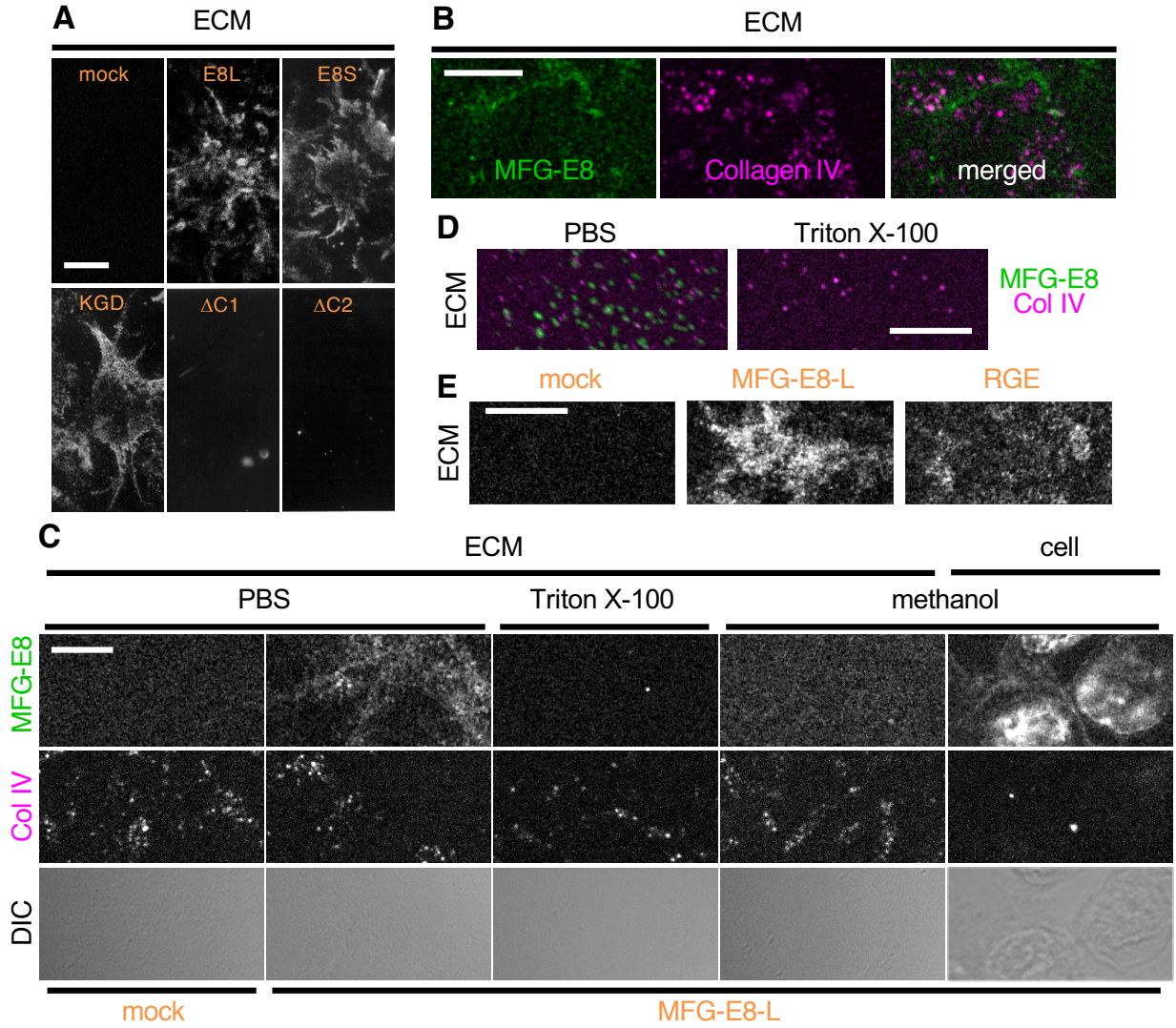


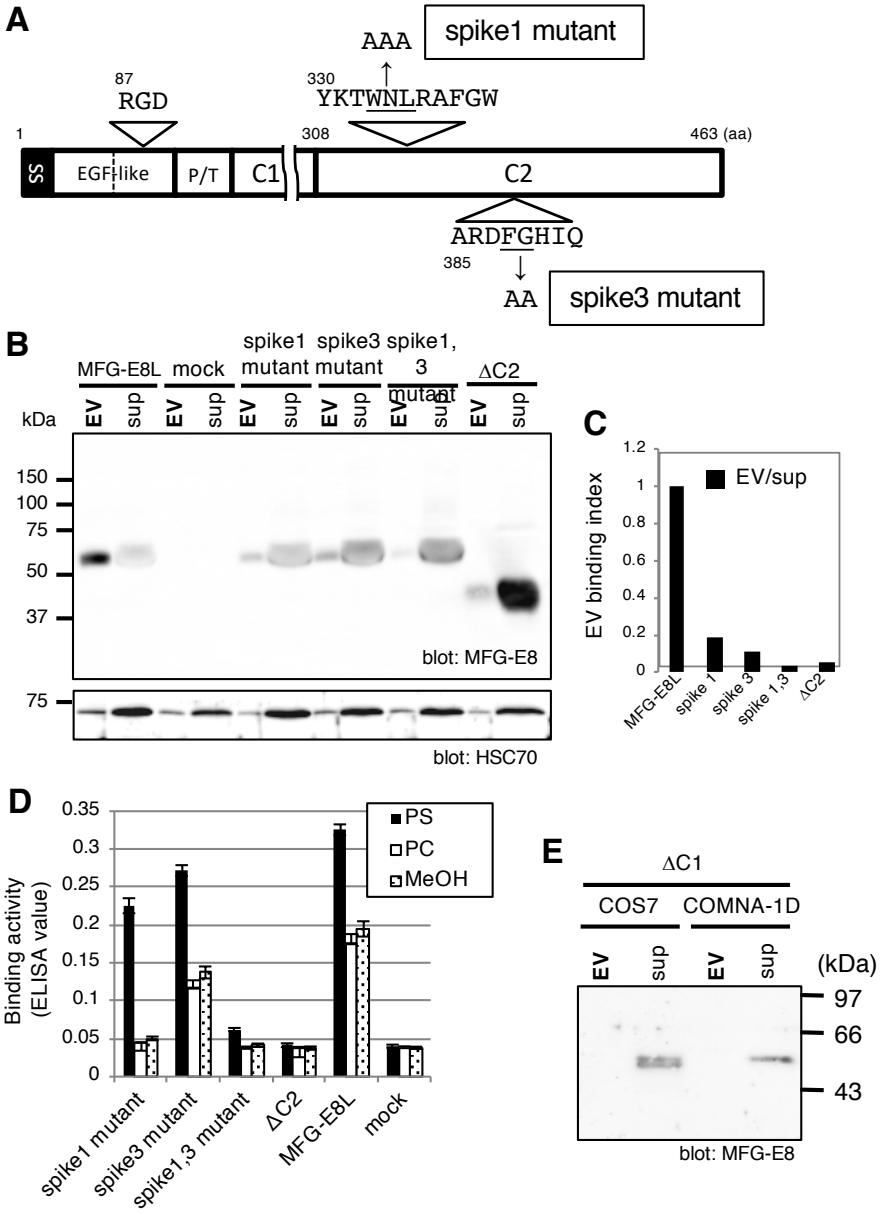
MFG-E8 F-actin Collagen IV

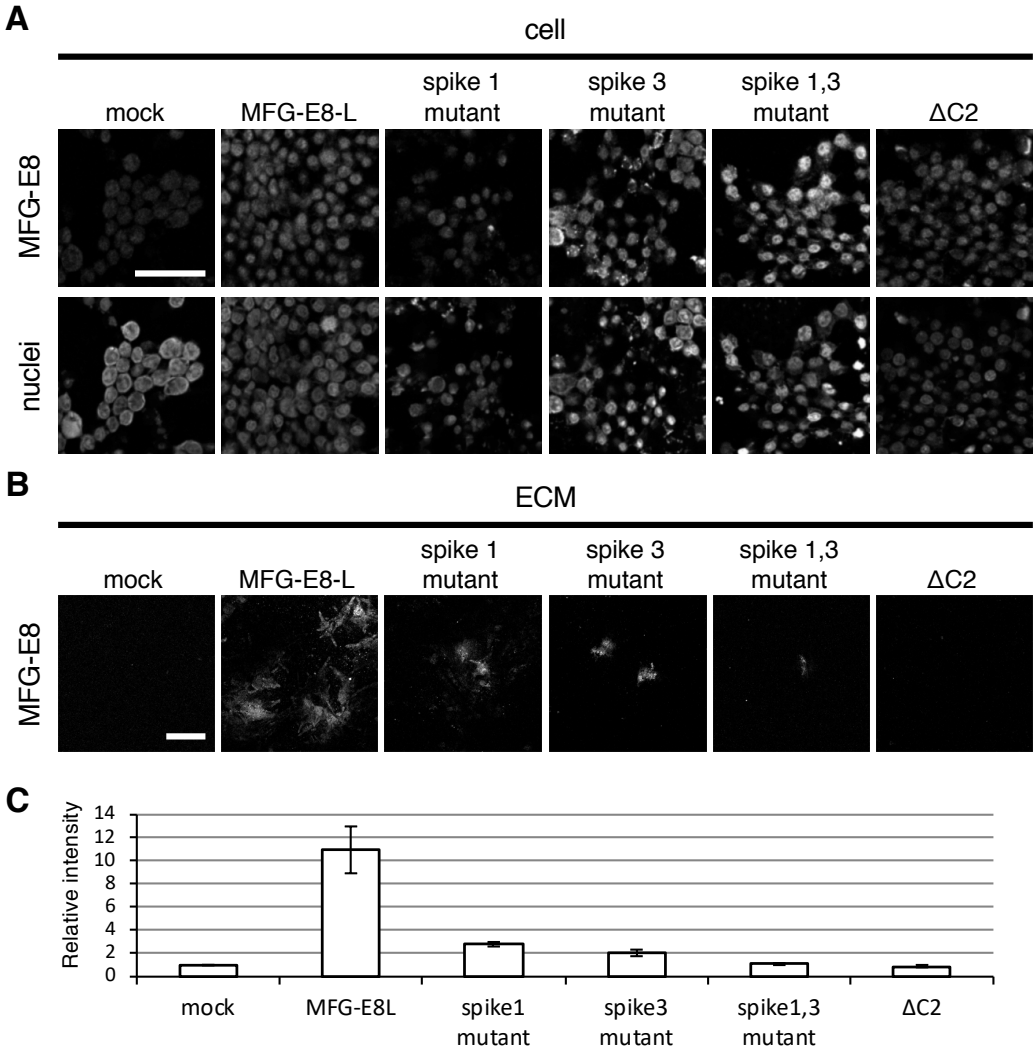
Ooishi et al. Fig.2

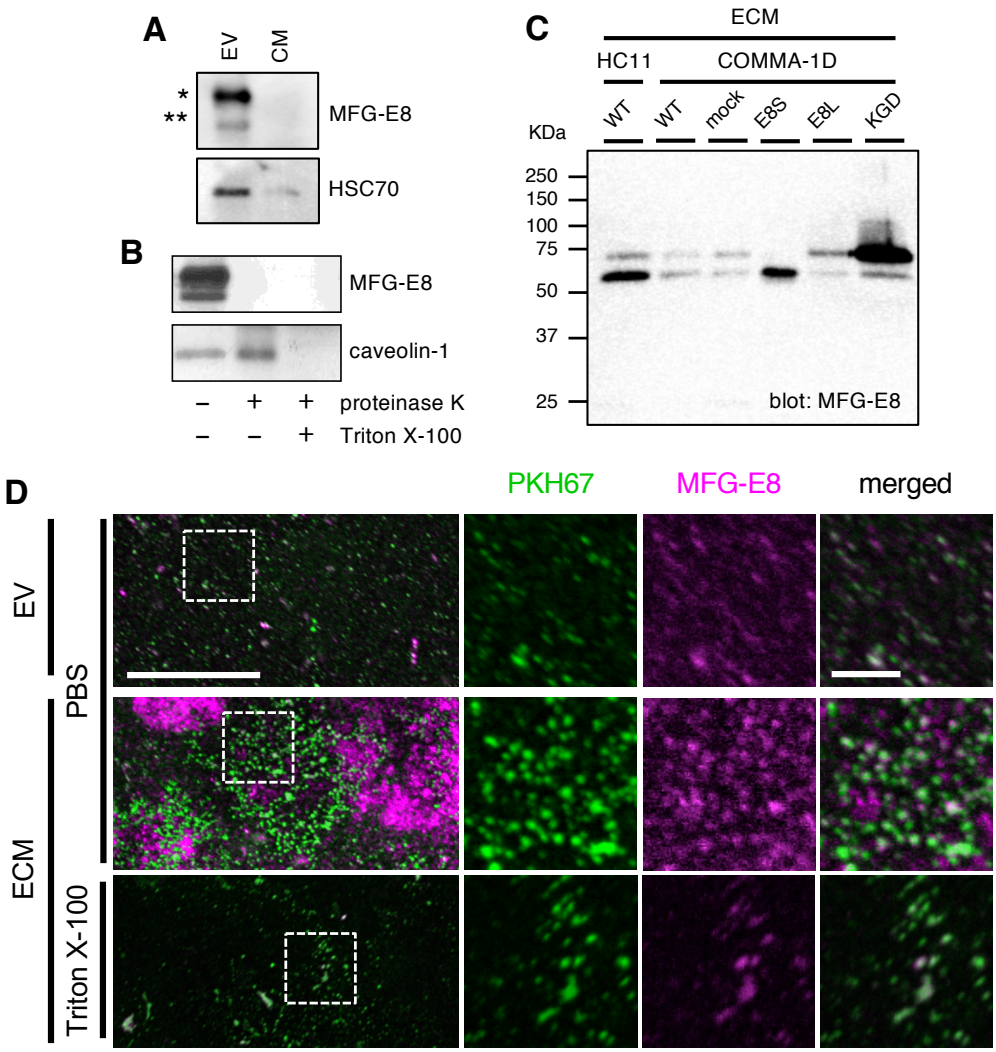


blot: MFG-E8

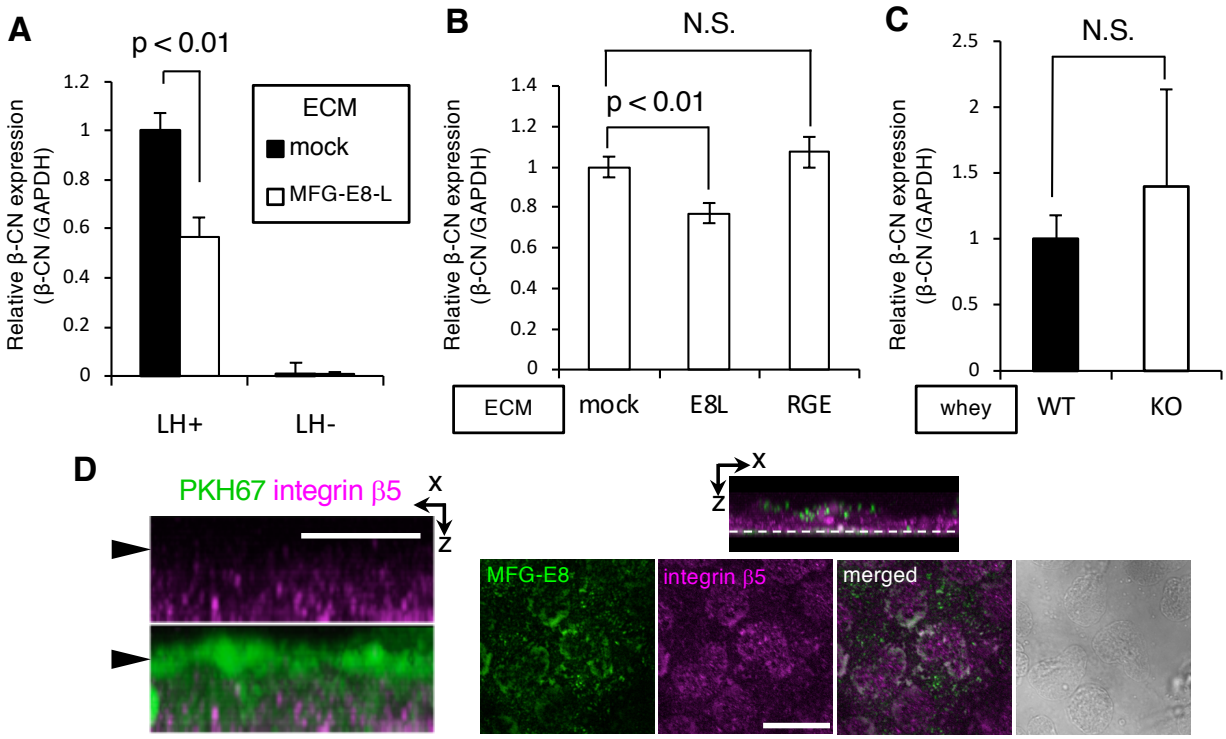

A
ECM
mock
E8L
E8S
KGD
ΔC1
ΔC2
B
ECM
MFG-E8
Collagen IV
merged
D
PBS
Triton X-100
ECM
MFG-E8
Col IV
E
mock
MFG-E8-L
RGE
ECM
C
ECM
cell
PBS
Triton X-100
methanol
MFG-E8
Col IV
DIC
mock
MFG-E8-L


A
spike1 mutant
AAA
87
RGD
330
YKTWNLRAFGW
1
308
463 (aa)
SS
EGF-like
P/T
C1
C2
ARDFGHIQ
385
AA
spike3 mutant
B
MFG-E8L
mock
spike1 mutant
spike3 mutant
spike1, 3 mutant
ΔC2
EV
sup
kDa
150
100
75
50
37
blot: MFG-E8
75
blot: HSC70
C
EV/sup
EV binding index
1.2
1
0.8
0.6
0.4
0.2
0
MFG-E8L
spike 1
spike 3
spike 1,3
ΔC2
D
PS
PC
MeOH
Binding activity (ELISA value)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
spike1 mutant
spike3 mutant
spike1,3 mutant
ΔC2
MFG-E8L
mock
E
ΔC1
COS7
COMNA-1D
EV
sup
(kDa)
97
66
43
blot: MFG-E8


A
cell
mock
MFG-E8-L
spike 1 mutant
spike 3 mutant
spike 1,3 mutant
ΔC2
MFG-E8
nuclei
B
ECM
mock
MFG-E8-L
spike 1 mutant
spike 3 mutant
spike 1,3 mutant
ΔC2
MFG-E8
C
Relative intensity
14
12
10
8
6
4
2
0
mock
MFG-E8L
spike1 mutant
spike3 mutant
spike1,3 mutant
ΔC2


A
EV
CM
*
**
MFG-E8
HSC70
B
MFG-E8
caveolin-1
– + + proteinase K
– – + Triton X-100
C
ECM
HC11
COMMA-1D
WT
WT
mock
E8S
E8L
KGD
KDa
250
150
100
75
50
37
25
blot: MFG-E8
D
PKH67
MFG-E8
merged
EV
ECM
PBS
Triton X-100

Ooishi et al. Fig.7



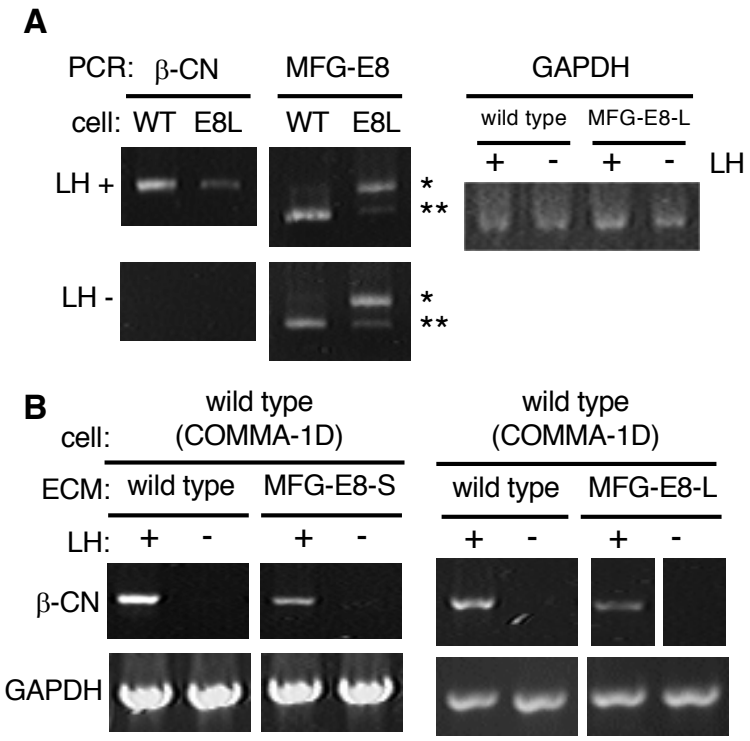


Figure S1. Suppressive effect of the conditioned ECM prepared from COMMA-1D cells overexpressing MFG-E8 on β-casein gene expression. (A) The MFG-E8-L stable clone (E8L) and parental wild type COMMA-1D cells (WT) were cultured in the presence (+) or the absence (-) of the lactogenic hormones (LH) for 5 days. Expression of β-casein (β-CN), MFG-E8, and GAPDH were analyzed by RT-PCR. Single and double asterisks (* and **) respectively indicate the positions of MFG-E8-L and MFG-E8-S. (B) Conditioned dishes were prepared from wild type COMMA-1D cells and the MFG-E8-L and -S transformant clones. Wild type COMMA-1D cells on the conditioned culture dishes were cultured in the presence (+) or the absence (-) of the lactogenic hormones for 40 h. Total RNAs were subjected to RT-PCR analysis for the expression of β-casein and GAPDH.

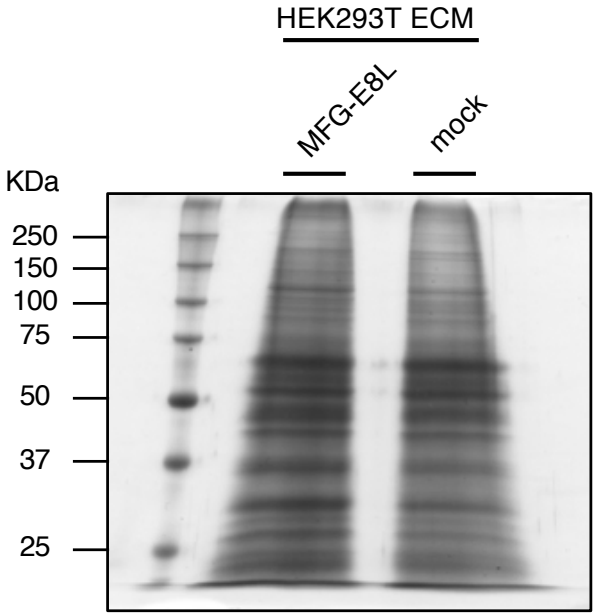


Figure S2. Total proteins in the conditioned ECM produced by HEK293T cells overexpressing MFG-E8. HEK293T cells in 100 mm dishes (1.2 x 10^6 cells/dish) were transfected with the MFG-E8-L expression vector and an empty vector (mock). After 48-hour culture, the conditioned ECM was harvested. Solubilized proteins in the same volume of ECM solutions were separated by SDS-PAGE and then silver-stained.

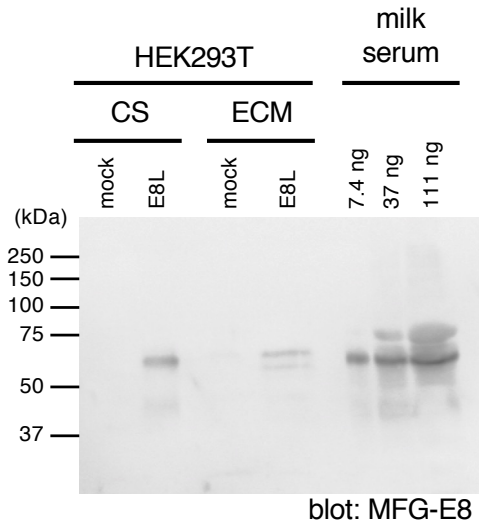


Figure S3. Quantitative comparison of MFG-E8 originated from mouse milk and overexpressing cells. HEK293T cells were transiently transfected with MFG-E8-L in a 100 mm dish culture with 6 ml of culture medium. MFG-E8 protein expressed in the halves of cellular substrata (ECM) and 3 ml of culture supernatants (CS) were compared with 7.4, 37, and 111 ng of MFG-E8 in mouse milk serum, which represented approximately 40, 200, and 600 pM when they were diluted in 3 ml of medium, respectively.

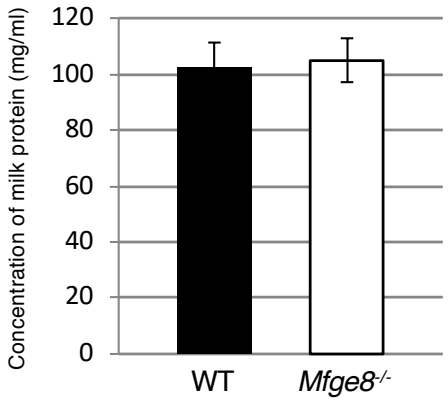


Figure S4. Quantitative comparison of the milk protein concentrations collected from the involuting mammary glands. Milk samples were obtained from the wild type (WT) and *MFG-E8*-deficient (*Mfge8*$^{-/-}$) mice at 48 hours after withdrawal of suckling pups. Protein concentration of whole milk was measured by Bradford assay.