SUPPLEMENTARY INFORMATION

Supplementary figures

A

<table>
<thead>
<tr>
<th></th>
<th>E.V, shC3G-2</th>
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</thead>
<tbody>
<tr>
<td>C3G</td>
<td>-</td>
</tr>
<tr>
<td>N-Cadherin</td>
<td>- 130KDa</td>
</tr>
<tr>
<td>β-actin</td>
<td>- 35KDa</td>
</tr>
<tr>
<td>Vimentin</td>
<td>- 65KDa</td>
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</table>

C3G/β-actin 1.0 0.5
N-cadherin/β-actin 1.0 5.8
Vimentin/β-actin 1.0 2.0

B

[Graph showing the effect of TGF-β and shC3G-1 on invading cells]

C

[Images showing immunofluorescence staining for Vimentin/DAPI]

D

[Images showing immunofluorescence staining for E-cadherin/DAPI]

E

[Images showing immunofluorescence staining for ZO-1/DAPI]

F

[Images showing immunofluorescence staining for Phallolidin/DAPI]
Supplementary figure 1-Chronic treatment with TGF-β mimicked the effect of C3G knock-down enhancing invasiveness of oval cells. Oval cells (C3G-silenced (shC3G-1, 2 or 3) and non-silenced (EV (transfected with the empty vector)) were maintained untreated in a medium supplemented with 10% FBS or chronically treated with TGF-β to induced EMT. A) Western-blot analysis of C3G, N-Cadherin and Vimentin protein levels normalized with β-Actin. B) Invasion assay through Matrigel using 10% FBS as chemoattractant. Histograms show the mean ± S.E.M. of number of invading cells (n=3). Confocal microscopy images of Vimentin (C), E-cadherin (D), ZO-1 (E) and Phalloidin F-actin (F) staining in cells maintained in the absence of serum. Scale bars: 20μm.

Supplementary figure 2-Effect of C3G knock-down on the expression of cytokeratin 19, albumin and alpha-fetoprotein mRNAs by oval cells. Levels of CK19 (KRT19), Albumin and Afp mRNAs quantified by RT-qPCR in C3G-silenced (shC3G-3) and non-silenced oval cells. Histograms show RQ mean value ± S.E.M. (n=3).

Supplementary figure 3-Lack of a functional MET receptor mimics the reduced adhesion of C3G knock-down in oval cells. Adhesion assay in oval cells with either C3G knock-down (shC3G) or lacking a functional MET receptor (Met-/-) (described in ref 5) and non-silenced wt cells. Histogram showing the mean value ± S.E.M. of the percentage of adhered cells at 15 min (n=3).
Supplementary figure 4-C3G expression is down-regulated in the liver upon chronic damage. (A) Western-blot analysis of C3G protein levels normalized with β-actin in liver samples from 3,5-Diethoxycarbonyl-1,4-Dihydrocollidine (DDC)-treated mice to induce oval cell expansion. Upper panel, images of representative western-blots showing different C3G isoforms with a line separating different blots; lower panel, histogram showing the quantification (mean value ± S.E.M.) of different blots referred to an untreated control, corresponding to 4 independent experiments. (B) Graphic showing RapGEF1 mRNA expression in liver biopsies obtained from healthy normal weight (n=14) and obese (n=12) individuals, non-alcoholic fatty liver disease (NAFLD) with simple steatosis (n=15) and non-alcoholic steatohepatitis (NASH) (n=16) patients expressed as the median TMM (Trimmed Mean of M-values (counts per million normalized to TMM)). These data were extracted from NCBI GEO repository, accession number GSE 126848. ***p≤0.001 versus healthy samples.