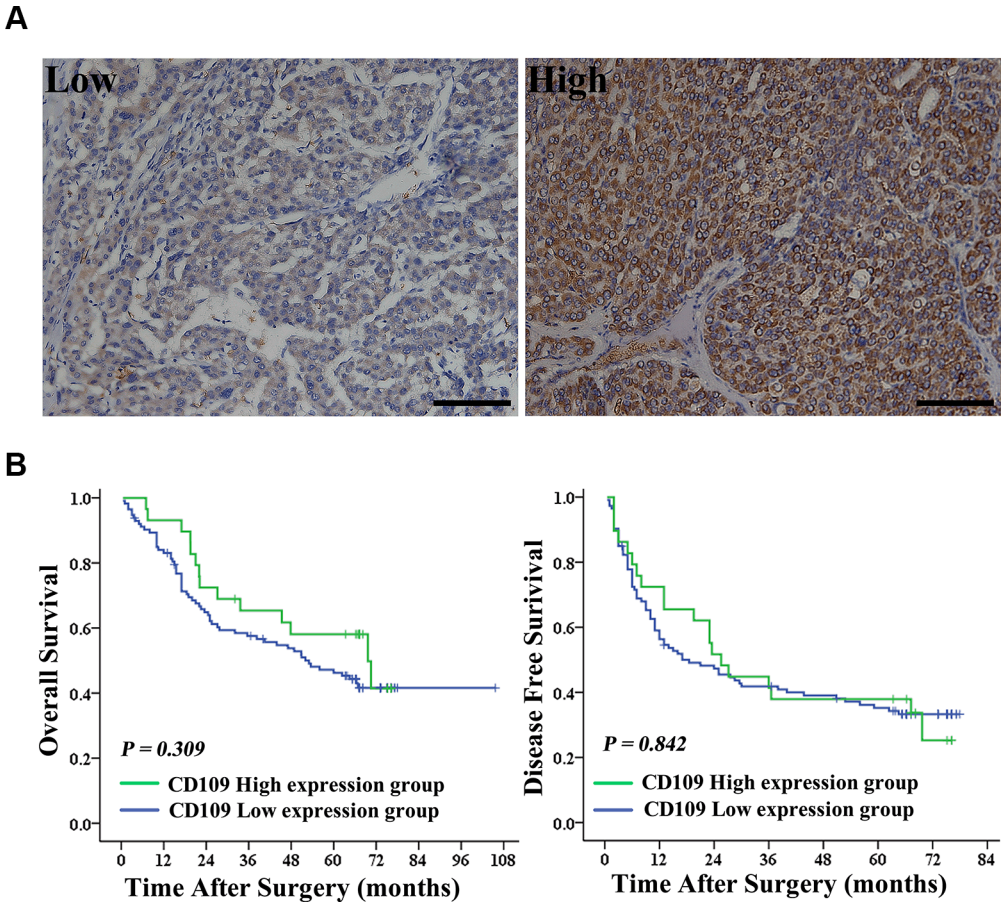
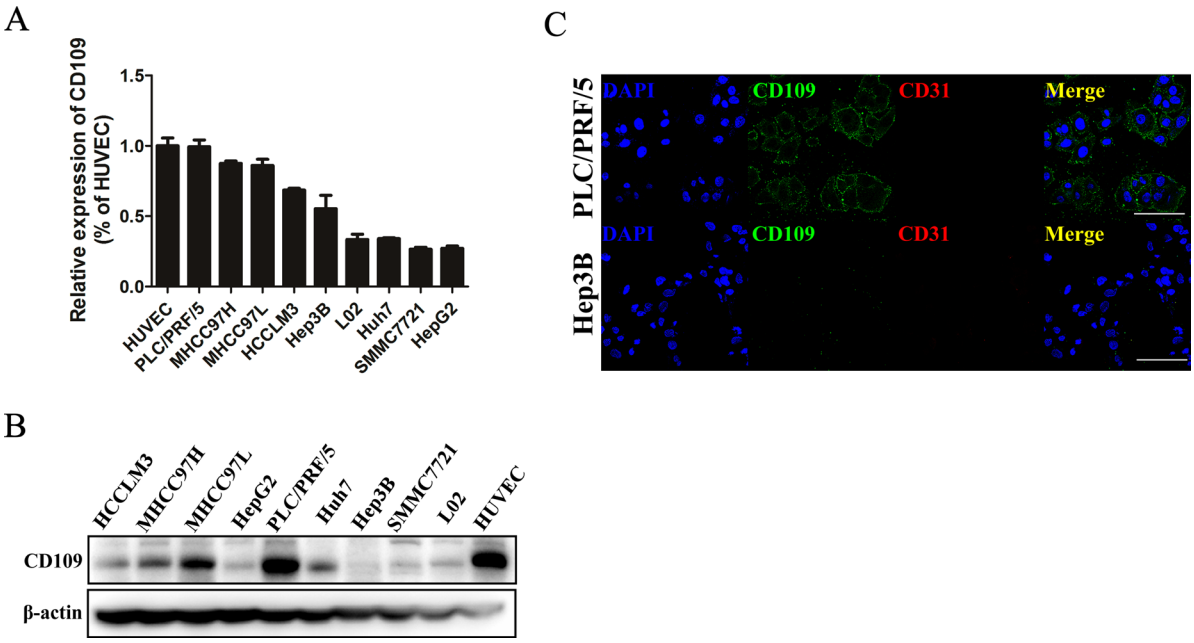
# Reduced expression of CD109 in tumor-associated endothelial cells promotes tumor progression by paracrine interleukin-8 in hepatocellular carcinoma

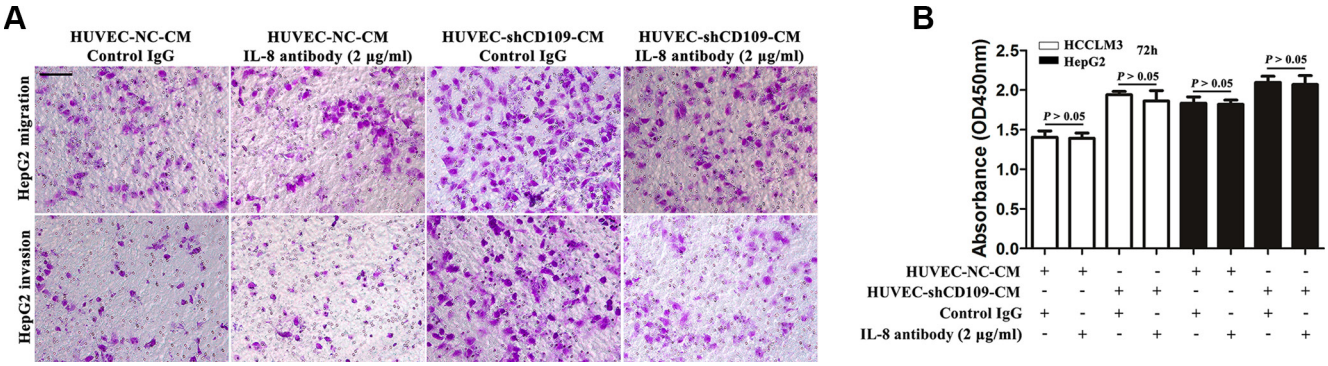
## Supplementary Materials




**Supplementary Figure S1: The expression of CD109 on HCC tumor cells and its association with patient prognosis.** (**A**) Representative images of IHC staining showed the expression of CD109 on HCC tumor cells. (**B**) Kaplan-Meier curves showed that CD109 expression on HCC tumor cells was not associated with overall survival and disease-free survival using log-rank test. Scale bars, 100 μm.




**Supplementary Figure S2: The expression of CD109 on several cell lines.** (**A**) qRT-PCR and (**B**) WB showed the different CD109 mRNA and protein expression in eight human HCC cell lines and the human normal liver cell line (L02) and HUVEC. (**C**) Representative images of double IF staining CD109 (green) and CD31 (red) in PLC/PRF/5 and Hep3B cells. Scale bars, 100 μm. GAPDH was used as internal control for qRT-PCR. β-actin served as a loading control for WB. Data shown as mean ± SD were from triplicates of three independent experiments.




**Supplementary Figure S3: IL-8 mediated the paracrine pro-tumor effects of CD109 knockdown in HUVEC.** (**A**) Representative images of Boyden chamber cell migration and invasion assays showed that HepG2 cell migration and invasion promoted by CD109 knockdown were significantly suppressed when IL-8 was neutralized. (**B**) Cell proliferation assays showed that neutralization of IL-8 did not inhibit HUVEC-shCD109 mediated promotion of tumor cell proliferation. Scale bars, 100 μm. Data shown as mean ± SD were from triplicates of three independent experiments. $P > 0.05$ by $t$ test. CM, conditioned media; NC, negative control.

**Supplementary Table S1: Correlation between CD109 expression on tumor vessels and clinicopathological characteristics**

| | CD109 expression on tumor vessels ($n$ = 142) | | | | |
|---|---|---|---|---|---|
| **Variable** | **High ($n$ = 47)** | | **Low ($n$ = 95)** | | |
| | **No. of patients** | **%** | **No. of patients** | **%** | ***P* value** |
| Age, years[a] | 54.0 ± 9.9 | | 49.6 ± 11.1 | | **0.023** |
| Gender | | | | | 0.615 |
| Male | 38 | 80.9 | 80 | 84.2 | |
| Female | 9 | 19.1 | 15 | 15.8 | |
| HBsAg | | | | | 0.787 |
| Positive | 43 | 91.5 | 84 | 88.4 | |
| Negative | 4 | 8.5 | 11 | 11.6 | |
| AFP | | | | | 0.373 |
| ≤ 20 ng/ml | 26 | 55.3 | 45 | 47.4 | |
| > 20 ng/ml | 21 | 44.7 | 50 | 52.6 | |
| Liver cirrhosis | | | | | |
| Yes | 40 | 85.1 | 73 | 76.8 | 0.250 |
| No | 7 | 14.9 | 22 | 23.2 | |
| Tumor size | | | | | **0.010** |
| ≤ 5 cm | 34 | 72.3 | 47 | 50.5 | |
| > 5 cm | 13 | 27.7 | 48 | 49.5 | |
| Tumor number | | | | | 0.179 |
| Single | 43 | 91.5 | 79 | 83.2 | |
| Multiple | 4 | 8.5 | 16 | 16.8 | |
| Tumor encapsulation | | | | | 0.065 |
| Complete | 27 | 57.4 | 39 | 41.1 | |
| No | 20 | 42.6 | 56 | 58.9 | |
| Microvascular invasion | | | | | **0.036** |
| Yes | 15 | 31.9 | 48 | 50.5 | |
| No | 32 | 68.1 | 47 | 49.5 | |
| Tumor differentiation | | | | | 0.114 |
| I–II | 35 | 74.5 | 58 | 61.1 | |
| III–IV | 12 | 25.5 | 37 | 38.9 | |
| TNM stage | | | | | **0.015** |
| I | 29 | 61.7 | 38 | 40.0 | |
| II | 16 | 34.0 | 39 | 41.1 | |
| IIIA | 2 | 4.3 | 18 | 18.9 | |

Abbreviations: HBsAg, hepatitis B s antigen; AFP, α-fetoprotein; TNM, tumor-nodes-metastasis.
[a]Student's *t*-test.

**Supplementary Table S2: Univariate and multivariate analyses of factors associated with survival and recurrence**

| | OS | | | | DFS | | | |
|---|---|---|---|---|---|---|---|---|
| | | Multivariate | | | | Multivariate | | |
| **Features** | **Univariate *P*** | **HR** | **95% CI** | ***P*** | **Univariate *P*** | **HR** | **95% CI** | ***P*** |
| Age: < 52 vs ≥ 52 years | 0.809 | | | NA | 0.779 | | | NA |
| Gender: female vs male | 0.367 | | | NA | 0.332 | | | NA |
| HBsAg: positive vs negative | 0.320 | | | NA | 0.164 | | | NA |
| Liver cirrhosis: yes vs no | 0.230 | | | NA | 0.302 | | | NA |
| AFP: > 200 ng/dLvs ≤ 200 ng/dL | 0.447 | | | NA | 0.428 | | | NS |
| Tumor size: > 5 cm vs ≤ 5 cm | **0.000** | 2.605 | 1.383–4.909 | **0.003** | **0.002** | | | NS |
| Tumor number: single vs multiple | 0.559 | | | NA | **0.024** | | | NS |
| Tumor encapsulation: complete vs none | 0.317 | | | NA | 0.491 | | | NA |
| Microvascular invasion: no vs yes | **0.006** | | | NS | **0.041** | | | NS |
| Tumor differentiation: III–IV vs I–II | **0.001** | | | NA | **0.000** | **2.451** | **1.410–4.260** | **0.001** |
| TNM stage: IIIA vs II vs I | **0.000** | | | NS | **0.001** | **2.540** | **1.107–5.830** | **0.028** |
| Tumor vessels CD109 high vs low | **0.002** | 0.487 | 0.234–1.014 | 0.054 | **0.000** | **0.322** | **0.166–0.623** | **0.001** |

Abbreviations: OS, overall survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; NA, not adopted; NS, not significant; HBsAg, hepatitis B s antigen; AFP, α-fetoprotein; NS, not significant; TNM, tumor-nodes-metastasis.

**Supplementary Table S3: Primary antibodies for WB, IHC and IF**

| Primary antibody | Concentration for WB | Concentration for IHC | Concentration for IF | Specificity | Molecular weight | Company |
|---|---|---|---|---|---|---|
| CD109 | | 1:100 | | Mouse monoclonal | 159 | R&D |
| CD109 | 1:2000 | | 1:100 | Sheep polyclonal | 159 | R&D |
| CD31 | | | 1:50 | Mouse monoclonal | 130 | Santa Cruz |
| TGF-β RI | 1:1000 | | | Rabbit polyclonal | 56 | Abcam |
| TGF-β RII | 1:500 | | | Rabbit polyclonal | 65 | Abcam |
| p-Smad2 | 1:5000 | | | Rabbit monoclonal | 52 | Abcam |
| Smad2 | 1:5000 | | | Rabbit monoclonal | 52 | Abcam |
| Akt | 1:1000 | | | Rabbit monoclonal | 60 | Cell Signaling Technology |
| p-Akt | 1:1000 | | | Rabbit monoclonal | 60 | Cell Signaling Technology |
| p-ERK | 1:1000 | | | Rabbit monoclonal | 44/42 | Cell Signaling Technology |
| ERK | 1:1000 | | | Rabbit monoclonal | 44/42 | Cell Signaling Technology |
| P65 | 1:5000 | | | Rabbit monoclonal | 65 | Abcam |
| p-P65 | 1:5000 | | | Rabbit monoclonal | 65 | Abcam |
| β-actin | 1:5000 | | | Mouse monoclonal | 43 | Sigma |
| GAPDH | 1:5000 | | | Mouse monoclonal | 36 | Cell Signaling Technology |

Abbreviations: WB, western blotting; IHC, immunohistochemistry; IF, immunofluorescence.