

Supplementary Information

***Amigo2*-upregulation in Tumour Cells Facilitates
Their Attachment to Liver Endothelial Cells Resulting
in Liver Metastases**

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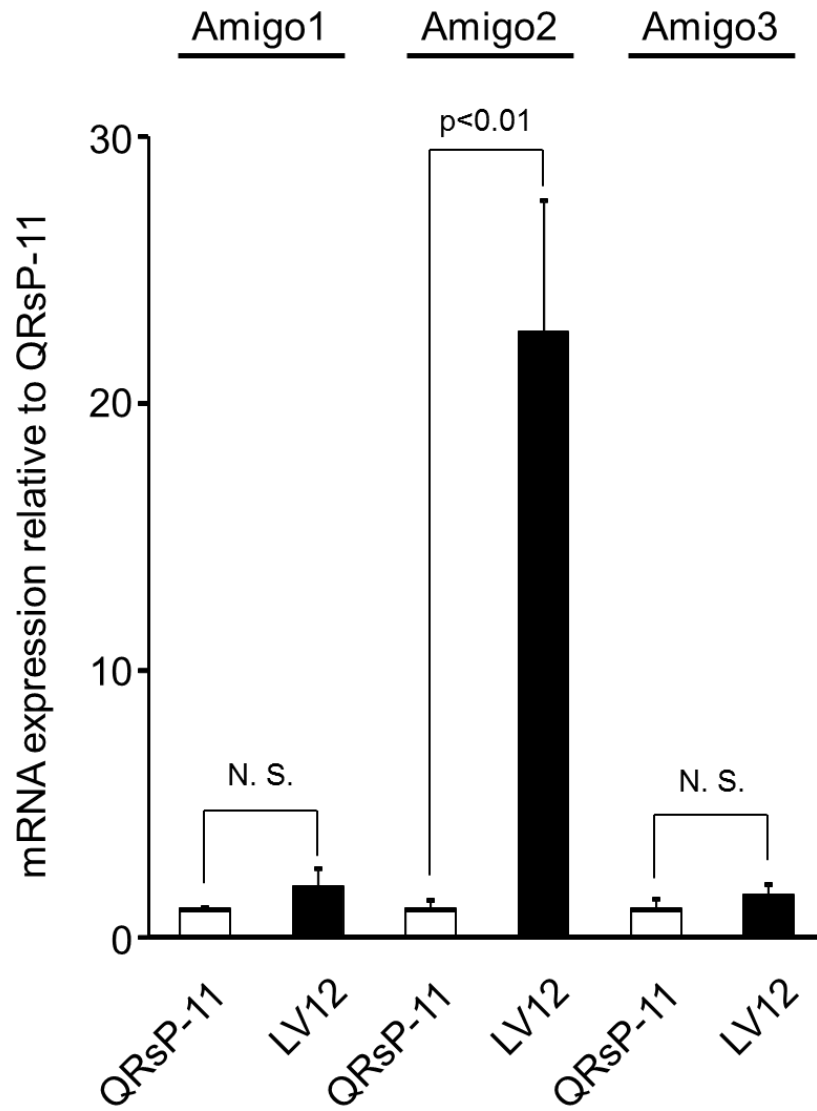
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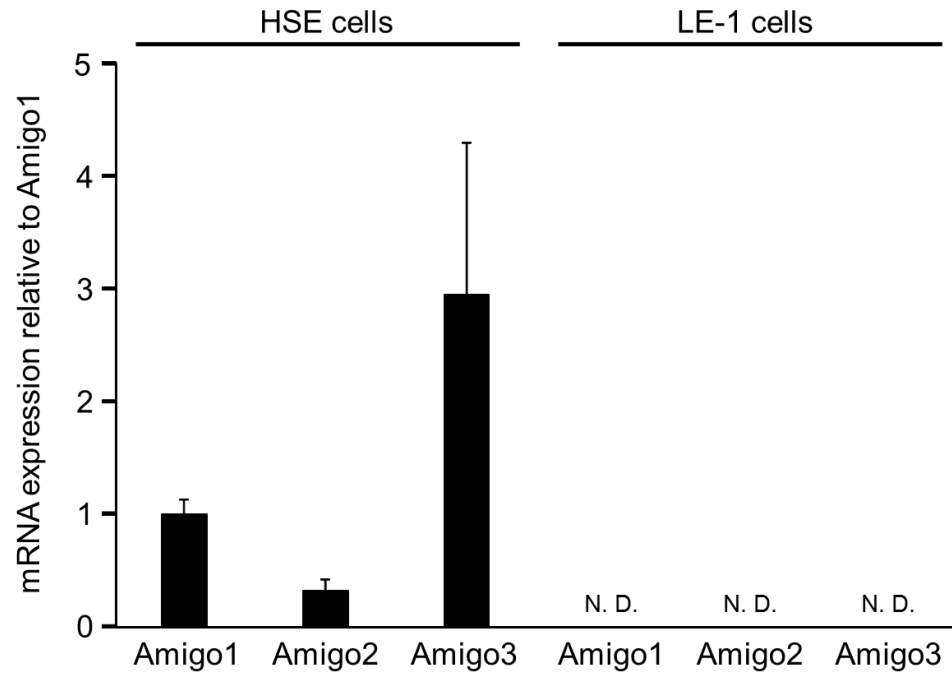
Supplementary Figure S1.



Assessment of Amigo family mRNA expression in LV12 and QRsP-11 cells

The mRNA expression levels of *Amigo1*, *Amigo2* and *Amigo3* in LV12 and QRsP-11 cells were evaluated using qRT-PCR. Bar graphs show means \pm SD (n = 4 in each group)

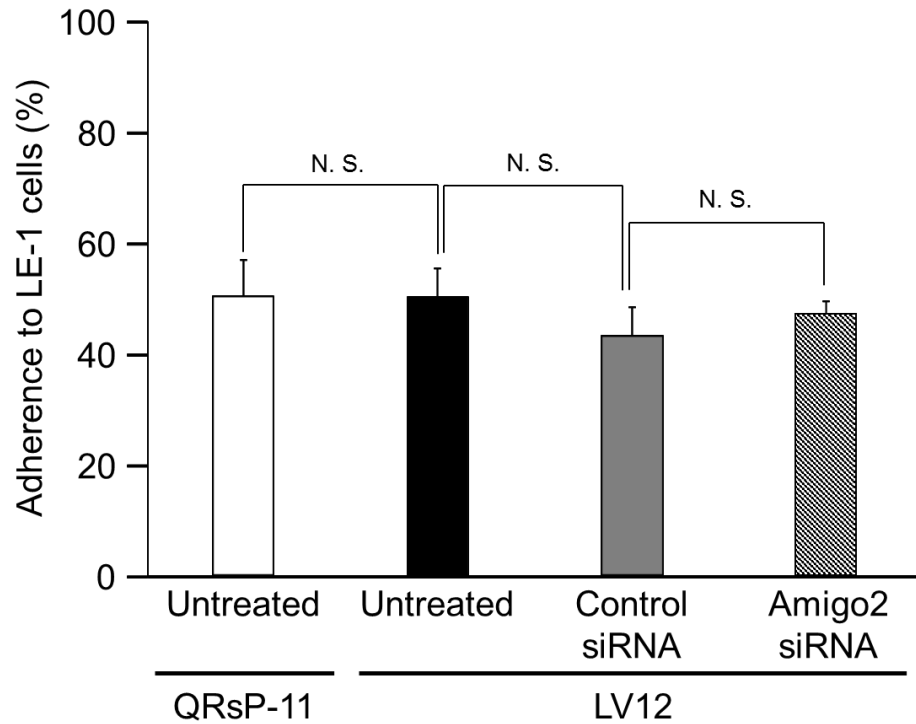
Supplementary Figure S2.



mRNA expression profiles of Amigo family members in HSE and LE-1 cells

Levels of *Amigo1*, *Amigo2* and *Amigo3* mRNAs in HSE and LE-1 cells were measured using qRT-PCR. Bar graphs show means \pm SD (n = 4 in each group).

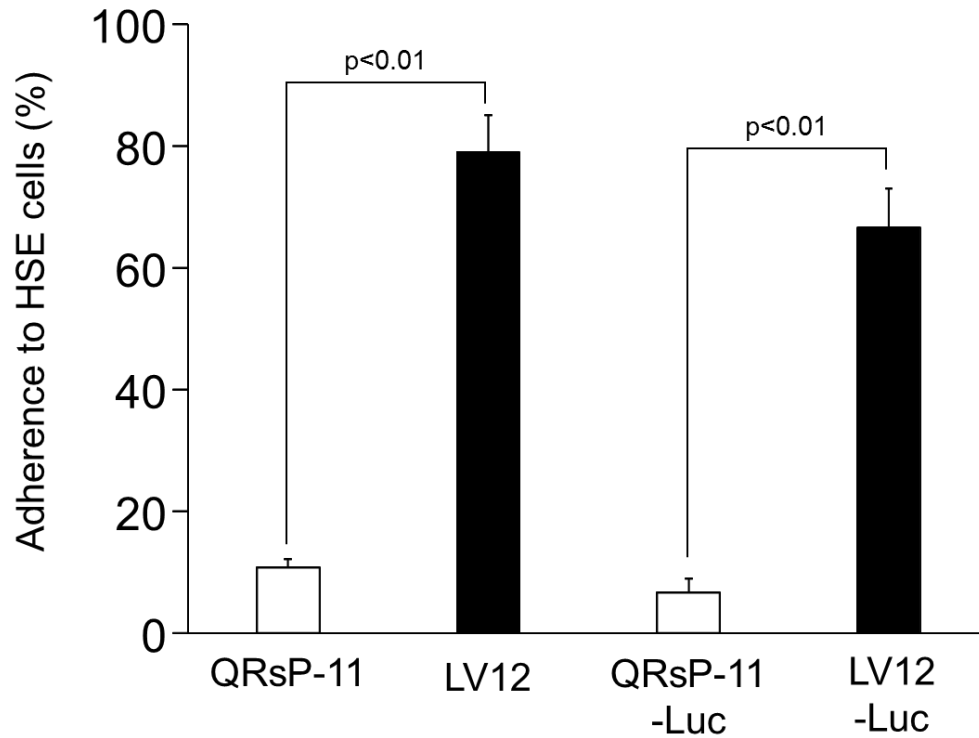
Supplementary Figure S3.



Amigo2 was not required for LV12 cell adhesion to LE-1 lung endothelial cells

LV12 cells were transfected with *Amigo2* or control siRNA and their adhesion to LE-1 lung endothelial cells was then assayed. Bar graphs show means \pm SD (n = 5 in each group).

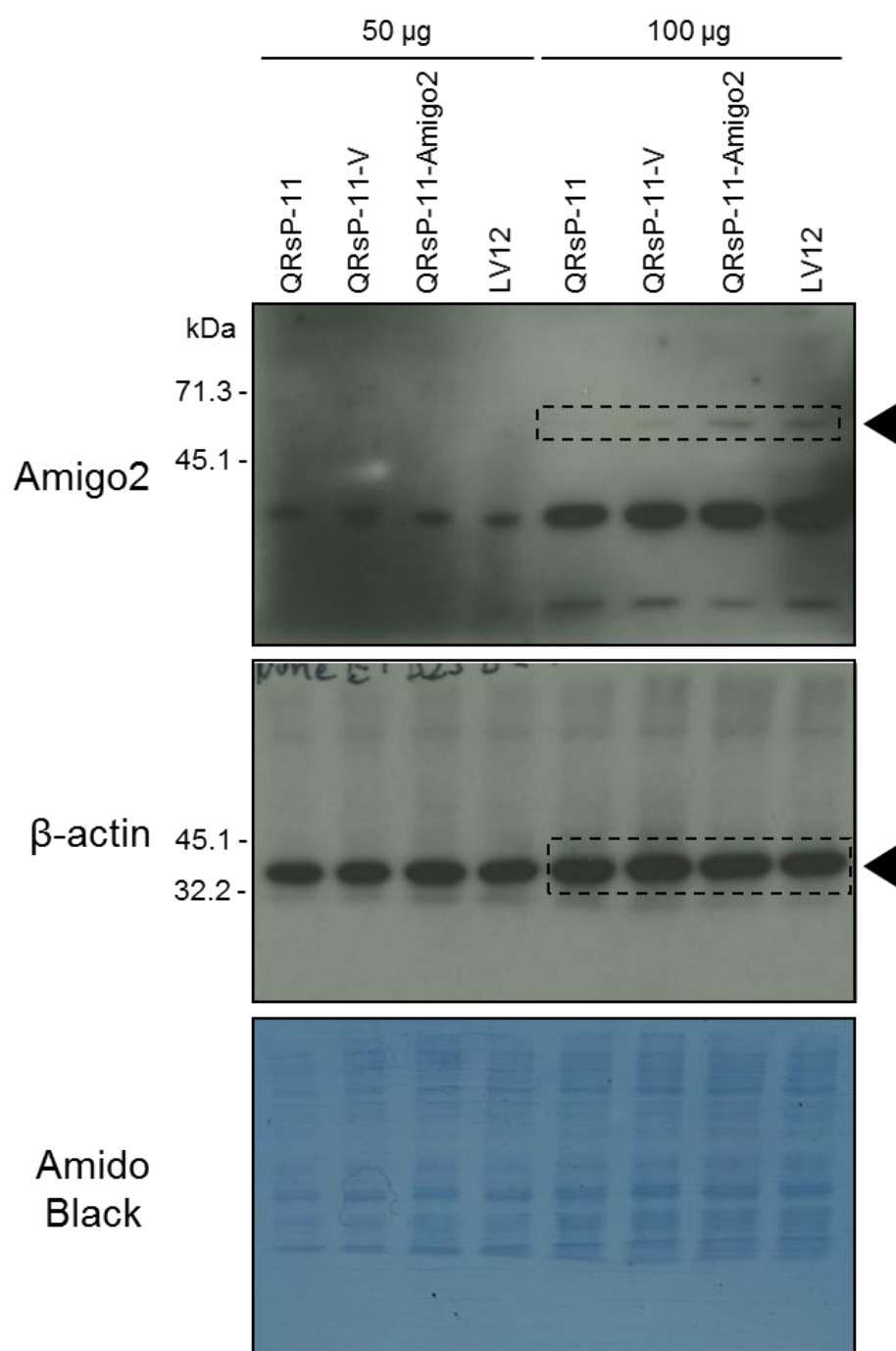
Supplementary Figure S4.



Adhesion properties of tumour cells to HSE liver endothelial cells were not changed by introduction of a luciferase expression vector

The adhesion properties of LV12-Luc or QRsP-11-Luc cells to HSE liver endothelial cells were assessed using an *in vitro* adhesion assay. Bar graphs show means \pm SD (n = 10 in each group).

Supplementary Figure S5.



Whole western blots from Figure 5a in the manuscript

The top panel is the whole gel image for western blot using anti-Amigo2 antibody in Figure 5a. The middle panel is the whole gel image for western blot using anti- β -

actin antibody in Figure 5a. The bottom panel is 0.1% Amido Black 10B (015-02192, Wako, Osaka, Japan) staining for confirmation of equivalence of the loading protein in each lane. The mouse monoclonal anti-Amigo2 antibody is described in the manufacturer's website (https://www.scbt.com/scbt/product/amigo2-antibody-g-7?productCanUrl=amigo2-antibody-g-7&_requestid=916644) and datasheet (<https://datasheets.scbt.com/sc-373699.pdf>).

Supplementary Table S1.

Changes in mean survival in days, liver-to-body weight ratios and Amigo2 expression following in vivo selection of a subline with high liver-metastatic properties

| Cell line ^a | No. of mice examined | Mean survival in days | Liver-to-body weight ratios (%) | Relative Amigo2 mRNA expression ^b |
|------------------------|----------------------|-------------------------|---------------------------------|--|
| QRsP-11 | 5 | 17.0 ± 4.0 | 8.4 ± 4.8 | 1.0 ± 0.2 |
| LV1 | 5 | 18.4 ± 3.4 | 6.5 ± 3.4 | 3.0 ± 0.4 ^d |
| LV2 | 3 | 16.0 ± 0.0 | 20.6 ± 16.4 | 3.1 ± 0.2 ^d |
| LV3 | 4 | 12.8 ± 2.9 | 6.9 ± 1.2 | 1.7 ± 0.4 |
| LV4 | 3 | 21.0 ± 2.6 | 7.0 ± 2.2 | 9.4 ± 0.6 ^d |
| LV5 | 3 | 20.3 ± 2.3 | 20.6 ± 19.0 | 18.0 ± 1.1 ^d |
| LV6 | 5 | 21.4 ± 5.2 | 10.9 ± 8.9 | 15.7 ± 1.0 ^d |
| LV7 | 4 | 20.3 ± 0.5 | 22.8 ± 20.6 | 30.6 ± 3.2 ^d |
| LV8 | 3 | 20.3 ± 3.2 | 32.4 ± 2.3 ^d | 17.1 ± 0.7 ^d |
| LV9 | 5 | 18.4 ± 1.3 | 14.7 ± 13.0 | 17.2 ± 0.8 ^d |
| LV10 | 3 | 18.7 ± 1.2 | 30.7 ± 21.9 | 15.3 ± 0.8 ^d |
| LV11 | 3 | 18.7 ± 2.3 | 28.4 ± 11.4 ^c | 21.1 ± 2.1 ^d |
| LV12 | 7 | 13.3 ± 1.6 ^c | 28.2 ± 9.6 ^d | 19.1 ± 0.3 ^d |

- a. QRsP-11 cells (1×10^6) were injected into mouse spleens. The mice were sacrificed when they appeared moribund. Tumour cells from liver metastases were cultured and used for sequential selection cycles.
- b. *Amigo2* mRNA levels are indicated as fold expression relative to QRsP-11 cells.
- c. $p < 0.05$ versus QRsP-11 cells.
- d. $p < 0.01$ versus QRsP-11 cells.

Supplementary Table S2.

Classification of cell adhesion-related molecules upregulated or downregulated in LV12 cells compared with QRsP-11 cells

| Upregulated gene | | Downregulated gene | |
|--|--------------------------|--|--------------------------|
| Name | Fold change ^a | Name | Fold change ^a |
| Integrin-related gene | | | |
| Integrin beta-like 1 (Itgb1) | 16.5 | Integrin, alpha E, epithelial-associated (Itgae) | 0.3 |
| Integrin beta 7 (Itgb7) | 4.3 | | |
| Immunoglobulin-related gene | | | |
| Immunoglobulin superfamily, member 4A (Igsf4a) | 3.6 | Activated leukocyte cell adhesion molecule (Alcam) | 0.1 |
| Testicular cell adhesion molecule 1 (Tcam1) | 2.9 | Biregional cell adhesion molecule-related/down-regulated by oncogenes (Cdon) binding protein (Boc) | 0.4 |
| Poliovirus receptor (Pvr) | 2.7 | F11 receptor (F11r) | 0.4 |
| CD4 antigen (Cd4) | 2.3 | Coxsackievirus and adenovirus receptor (Cxadr) | 0.4 |
| | | Neural cell adhesion molecule 1 (Ncam1) | 0.5 |
| | | Neighbor of Punc E11 (Nope) | 0.5 |
| | | CD47 antigen (Cd47) | 0.5 |
| Cadherin-related gene | | | |
| Protocadherin 9 (Pcdh9) | 4.4 | Protocadherin beta 14 (Pcdhb14) | 0.2 |
| | | FAT tumor suppressor homolog 1 (Drosophila) (Fat1) | 0.2 |
| | | Protocadherin beta 20 (Pcdhb20) | 0.2 |
| | | Cadherin-like 26 (Cdh26) | 0.4 |
| | | Protocadherin beta 17 | 0.4 |

| | | | |
|--|--|-----------------------|-----|
| | | (Pcdhb17) | |
| | | Protocadherin beta 22 | 0.5 |
| | | (Pcdhb22) | |

Others

| | | | |
|---|------|--|-----|
| Amphoterin-induced gene and open reading frame 2 (Amigo2) | 15.1 | Procollagen, type VI, alpha 1 (Col6a1) | 0.1 |
| Wnt-1-inducible signalling pathway protein 1 (Wisp1) | 7.4 | Palladin, cytoskeletal associated protein (Palld) | 0.1 |
| Nexilin (Nexn) | 5.0 | Endothelial differentiation sphingolipid G-protein-coupled receptor 1 (Edg1) | 0.1 |
| Contactin associated protein-like 4 (Cntnap4) | 3.3 | Neuropilin 1 (Nrp1) | 0.2 |
| Unknown | 3.0 | Spondin 2, extracellular matrix protein (Spon2) | 0.2 |
| Procollagen, type VI, alpha 3 (Col6a3) | 2.9 | Unknown | 0.2 |
| Pinin (Pnn) | 2.8 | Fibronectin 1 (Fn1) | 0.2 |
| Killer cell lectin-like receptor, subfamily A, member 18 (Klra18) | 2.7 | Plakophilin 2 (Pkp2) | 0.2 |
| Phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha) (Pik3r1) | 2.4 | Procollagen, type VI, alpha 2 (Col6a2) | 0.3 |
| Transglutaminase 2, C polypeptide (Tgm2) | 2.4 | Fibronectin leucine rich transmembrane protein 2 (Flrt2) | 0.3 |
| Thrombospondin 4 (Thbs4) | 2.3 | Rho family GTPase 3 (Rnd3) | 0.3 |
| Claudin 3 (Cldn3) | 2.2 | B-cell leukemia/lymphoma 6 (Bcl6) | 0.3 |
| Killer cell lectin-like receptor subfamily A, member 14 (Klra14) | 2.2 | Beta-1,4-N-acetyl-galactosaminyl transferase 2 (B4galnt2) | 0.3 |
| G protein-coupled receptor 98 (Gpr98) | 2.1 | Chemokine (C-X3-C motif) ligand 1 (Cx3cl1) | 0.3 |
| Connective tissue growth factor (Ctgf) | 2.0 | Embryonal Fyn-associated substrate (Efs) | 0.4 |
| Kit ligand (Kitl) | 2.0 | Elastin microfibril interfacier 2 (Emilin2) | 0.4 |
| | | von Willebrand factor A domain containing 1 (Vwal1) | 0.4 |

| | |
|---|-----|
| Procollagen, type V, alpha 2 (Col5a2) | 0.5 |
| LIM domain containing preferred translocation partner in lipoma (Lpp) | 0.5 |
| Scavenger receptor class F, member 2 (Scarf2) | 0.5 |
| Nidogen 1 (Nid1) | 0.5 |
| Procollagen, type V, alpha 1 (Col5a1) | 0.5 |
| Unknown | 0.5 |
| Thrombospondin 1 (Thbs1) | 0.5 |

- a. Cell adhesion-related genes whose expression levels had changed more than 2-fold or less than 0.5-fold in LV12 cells compared with QRsP-11 cells are indicated.

Supplementary Table S3.

Primer sequences used in this study

| Target | Strand | Sequence |
|----------------|---------|-----------------------------|
| Amigo1 | Forward | 5'-GAATGCCTTTGAGGACATGG-3' |
| | Reverse | 5'-CTTCAGCTTGTTGGAGGACAG-3' |
| Amigo2 | Forward | 5'-GAGGCGACCATAATGTCGTT-3' |
| | Reverse | 5'-CGGGCACCTTAGATAGGTTTT-3' |
| Amigo3 | Forward | 5'-CAGCCTCTCAGGATGGTAGC-3' |
| | Reverse | 5'-CAGGGTGGTAAAGCCTGTGT-3' |
| Itgb11 | Forward | 5'-AGCAACCAGATGTGCAAGAA-3' |
| | Reverse | 5'-CCATGGCCTCCACATACTTC-3' |
| Wisp1 | Forward | 5'-TTCTGCAAGTGGCCATAGGT-3' |
| | Reverse | 5'-ACTGGCCAGGGACTCTCAC-3' |
| β -actin | Forward | 5'-AGAGGGAAATCGTGCGTGAC-3' |
| | Reverse | 5'-CAATAGTGATGACCTGGCCGT-3' |