

SUPPLEMENTARY TABLES

TABLE S1. List of the antibodies used in the study

| Antibody | PROCEDURE | Dilution | Supplier |
|---------------------------------------|----------------------|-------------|---|
| anti-LRP-1 85k Da | IF | 1:50 | Meridian Life Sciece, Inc, Memphis, USA |
| anti-LRP-1 85k Da | WB | 5 µg/ml | Meridian Life Sciece, Inc, Memphis, USA |
| Anti-LRP-1 515 kDa | Blocking experiments | 0.5-5 µg/ml | Meridian Life Sciece, Inc, Memphis, USA |
| Anti-LRP-5 | IF | 1 ug/ml | BD Bioscience, Franklin Lakes, NJ, USA |
| Anti-LRP-6 | IF | 1:1000 | Cell Signaling, Danvers, MA, USA |
| anti-SERPINB3 | IF | 8 µg/ml | Hepa-Ab, Xeptagen S.p.A., Marghera, VE, Italy |
| anti-vimentin | IF | 1:1000 | GeneTex, Alton Parkway Irvine, CA, USA |
| anti-vimentin | WB | 1:3000 | GeneTex, Alton Parkway Irvine, CA, USA |
| anti-E-cadherin | IF | 1:1000 | BD Bioscience, Franklin Lakes, NJ, USA |
| anti-E-cadherin | WB | 1:3000 | BD Bioscience, Franklin Lakes, NJ, USA |
| Alexa Fluor® 488 Goat Anti-Mouse IgG | IF | 1:500 | Invitrogen Life Technologies, NY, USA |
| Alexa Fluor® 546 Goat Anti-Rabbit IgG | IF | 1:500 | Invitrogen Life Technologies, NY, USA |
| anti-mouse IgG Peroxidase conjugated | WB | 1:1000 | Sigma-Aldrich, St. Louis, MO, USA |
| anti-rabbit IgG Peroxidase conjugated | WB | 1:1000 | Sigma-Aldrich, St. Louis, MO, USA |

WB: Western blot; IF: immunofluorescence

TABLE S2. List of the primers used in the study.

| Target | Orientation | Sequence (5' to 3') |
|------------------|-------------|--------------------------|
| CD274 | Sense | TTGCTGAACGCCCCATACAA |
| | Antisense | GGAATTGGTGGTGGTGGTCT |
| SerpinB3 | Sense | GCAAATGCTCCAGAAGAAAG |
| | Antisense | CGAGGCCAAAATGAAAAGATG |
| Wnt-1 | Sense | CAAACAGCGGCGTCTGATAC |
| | Antisense | AGCCTCGGTTGACGATCTTG |
| Wnt-7a | Sense | AACTTGCACAACAACGAGGC |
| | Antisense | TTGTCCTTGAGCACGTAGCC |
| cMyc | Sense | AAGACAGCGGCAGCCCGAAC |
| | Antisense | TGGGCGAGCTGCTGTCGTTG |
| β -catenin | Sense | TGGTGCCCAGGGAGAACCCC |
| | Antisense | TGTCACCTGGAGGCAGCCCA |
| Axin | Sense | AACGACAGCGAGCAGCAGAG |
| | Antisense | AGCTTGTGACACGGCCCTGG |
| LRP-1 | Sense | AGCAAACGAGGCCTAAGTCA |
| | Antisense | GCTGCTTGTGCTGATGGTAA |
| GAPDH | Sense | TGGTATCGTGGAAGGACTCATGAC |
| | Antisense | ATGCCAGTGAGCTTCCCGTTCAGC |

TABLE S3. Clinical and histological characteristics of the patients included in the study.

| | |
|-------------------------------------|----------------|
| Number of patients: | 38 |
| Age (mean years \pm SD) | 62,7 \pm 9,5 |
| Male sexex (%) | 29/38 (76,3%) |
| Etiology | |
| HBV infection | 7/38 (18,4%) |
| HCV infection | 17/38 (44,7%) |
| HBV+HCV infection | 3/38 (7,9%) |
| Alcohol use | 12/38 (31,6%) |
| Other | 5/38 (11.1%) |
| Pathology | |
| Number of nodules (min-max) | 1,44 (1-3) |
| Nodule diameter mean – mm (min-max) | 54,55 (7-190) |
| Vascular invasion | 19 (50,00%) |
| - Microscopic | 18 (47,37%) |
| - Macroscopic | 1 (2,63%) |
| Grading | |
| GI | 3 (7,9 %) |
| GII | 21 (55,26%) |
| GIII | 14 (36,84%) |

SUPPLEMENTARY FIGURES

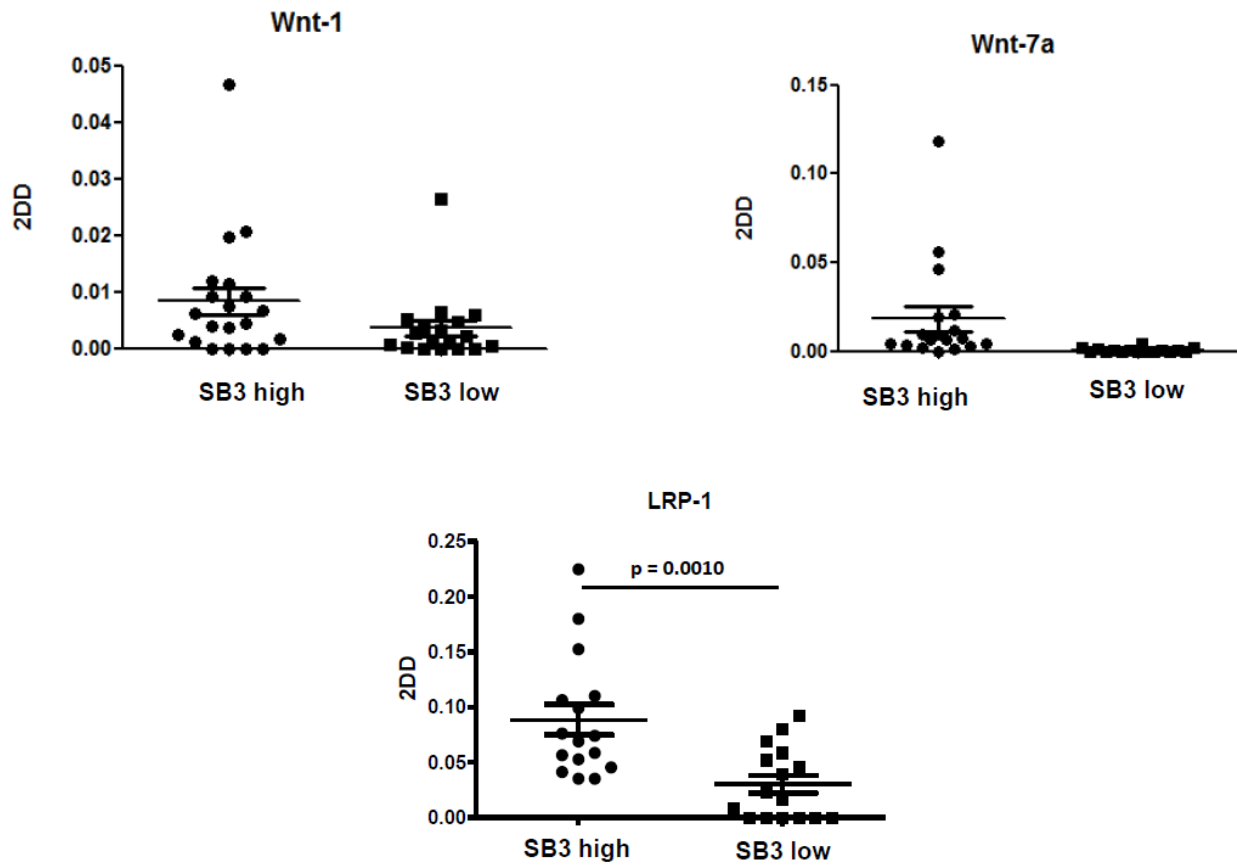


Figure S1 Relative mRNA expression of SerpinB3 compared with Wnt-1, Wnt-7a and LPR-1 in human HCCs. The expression of Wnt-1, Wnt-7a and of LPR-1 was evaluated in 38 tumor specimens from HCC patients, grouped on the basis of the expression of SB3, where high is \geq median value and low is $<$ median value. Experiments were performed in triplicate and data were expressed as mean \pm SD (vertical bars).

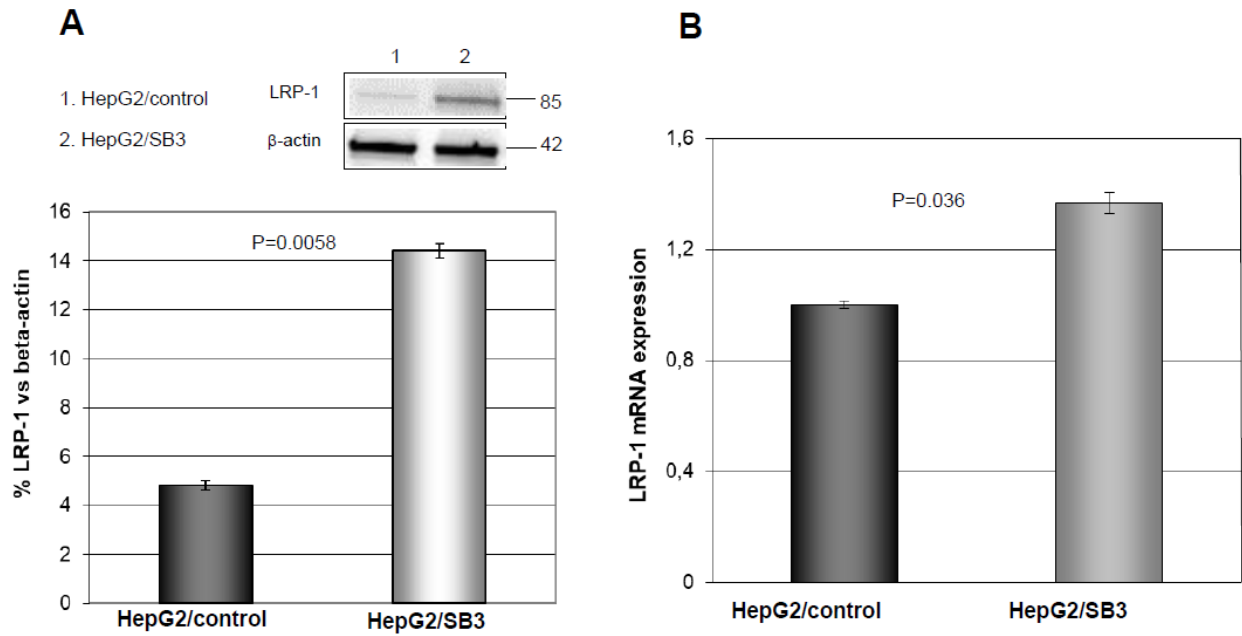


Figure S2 LRP-1 expression in hepatoma cells. A): example of densitometric analysis of Western blot results for LRP-1 protein, normalized to β -actin and the cropped blots of LRP-1 and of the housekeeping β -actin. B): relative LRP-1 mRNA expression in HepG2/SerpinB3 cells. Results are expressed as fold change ($2^{-\Delta\Delta C_t}$) compared to control cells (HepG2/empty vector). Experiments were performed in triplicate and data were expressed as mean \pm SD (vertical bars).

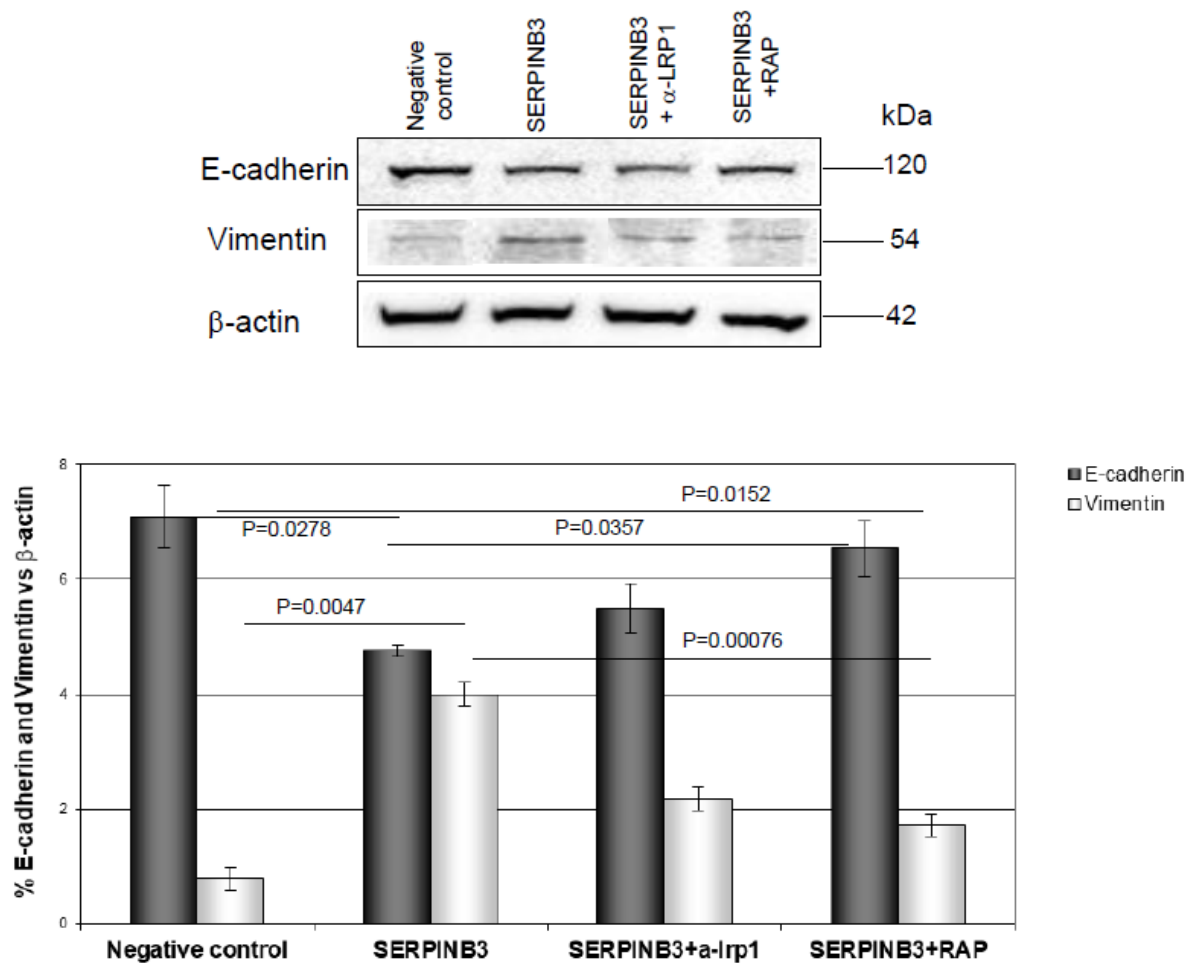


Figure S3 Western blot analysis of E-cadherin and vimentin. Upper panel: example of cropped immunoblot of E-cadherin, vimentin and of the housekeeping β -actin. Samples were loaded in the same gel and run under the same experimental conditions. Lower panel: histogram of quantitative densitometric analysis of the intensity of E-cadherin and vimentin bands, normalized to β -actin obtained in HepG2/empty vector cells incubated overnight with PBS, as negative control, with 100 ng/ml of recombinant SERPINB3 protein, as positive control. Cells were pre-treated with 5 μ g/ml of anti-LRP antibody for 1 hour or overnight incubated with 5 μ g/ml of RAP before treatment with 100ng/ml of recombinant SerpinB3. Experiments were performed in triplicate. Data were expressed as mean \pm SD (vertical bars).