

## Supplementary Figure Legends

**Supplementary Figure 1.** Human 3p and 3sp hepatoma cells are diploid. Flow cytometry analysis of cells stained with propidium iodide indicates diploid DNA content of (A) early and (B) late 3p cells as well as (C) early and (D) late 3sp cells.

**Supplementary Figure 2.** Cell invasion of mesenchymal 3sp cells in 3-dimensional (3D) co-cultured 3p/3sp-derived spheroids. A, 3p cells form compact and round spheroids which are unable to invade into the surrounding collagen gel. Co-cultivation of epithelial 3p and mesenchymal 3sp cells (3p + 3sp) showed spheroid formation and strong invasion into the gel (bar, 250  $\mu\text{m}$ ). B, Staining of co-cultivated spheroids with CellTracker™ revealed that only mesenchymal 3sp cells (green) invade into the gel, while epithelial 3p cells (red) reside in the center of the spheroid (bar, 250  $\mu\text{m}$ ). C, Immunofluorescence staining of membrane-bound  $\beta$ -catenin and E-cadherin showing the epithelial organization of 3p cells in a 3D setting, while detached mesenchymal 3sp cells (white arrows) from a co-cultivated spheroid lose these epithelial markers (bar, 25  $\mu\text{m}$ ).

**Supplementary Figure 3.** Whole aCGH profiles and chromosomal aberrations of 3p and 3sp cells. The aCGH signature of 3p and 3sp cells from early cell passages are shown. Blue lines represent 3p cells, whereas magenta lines indicate 3sp cells. Besides deletions of *TRPM3* and *AXINI* (marked with red arrows), major similarities in chromosomal aberrations between 3p and 3sp cells include e.g. chromosome 8, 9p, 13q, 14q and 17.

**Supplementary Figure 4.** Identification of chromosomal break points in *TRPM3* and *AXINI*. PCR discloses chromosomal breaks of (A) *TRPM3* between exon 12 and exon 15 and of (B)

*AXINI* between exon 2 and exon 3 in 3p and 3sp cells. Genomic DNA of primary human hepatocytes (prim hep) were used as control. Ex, exon; bp, 100 basepair ladder.

**Supplementary Figure 5.** Downregulation of liver specific genes in 3sp HCC cells. Gene Set Enrichment Analysis (GSEA) of a whole-genome Affymetrix GeneChip<sup>®</sup> revealed downregulation of liver-specific genes in 3sp cells. Blue and red indicates down- and upregulation, respectively.

**Supplementary Figure 6.** Upregulation of mesenchymal markers in 3sp cells. qRT-PCR analysis of *platelet-derived growth factor receptor beta (PDGF-R $\beta$ )*, *transforming growth factor beta (TGF- $\beta$ )2*, *discoidin domain receptor tyrosine kinase (DDR)2* and *fibroblast activation protein (FAP)*.

**Supplementary Figure 7.** Sorafenib efficiently blocks cell migration of mesenchymal 3sp HCC cells. A, Verification of calculated IC<sub>50</sub> by viability assays. Incubation of 3p and 3sp cells with drugs at their determined IC<sub>50</sub> results in a reduction of viable cells to 50%. B, Migration assays using the Platypus<sup>®</sup> technology shows movement of mesenchymal 3sp cells into the detection zone after 3 days. Treatment of doxorubicin at the IC<sub>50</sub> shows a reduction of the cell number but no impaired migration, whereas treatment of sorafenib at the IC<sub>50</sub> leads to a significantly reduced migratory potential of 3sp cells. C, Quantification of 3sp cell migration by fluorometric analysis. Cells were visualized with green CellTracker<sup>™</sup>. Bar, 250  $\mu$ m; \*\*\*, p<0,005.

**Supplementary Figure 8.** Distinct drug effectiveness due to molecular changes in cells undergoing EMT. A, Downregulation (blue) of multiple drug resistance genes (ABC) in 3sp

cells was determined by Gene Set Enrichment Analysis (GSEA) of a whole-genome Affymetrix GeneChip<sup>®</sup>. The -fold downregulation of mRNA was calculated and depicted by the ratio of 3sp to 3p cells (3sp/3p). B, Phosphoproteomics (human phospho-RTK Proteome Profiler Array, ARY001, R&D Systems, Minneapolis, USA) revealed a higher phosphorylation status of *EGFR* and *ErbB3* in early 3p cells compared to early 3sp cells. Percent in relation to positive control is shown; pRTK, phospho-receptor-tyrosine-kinase array.