**Low dose paclitaxel reduces S100A4 nuclear import to inhibit invasion and hematogenous metastasis of cholangiocarcinoma**

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**Supplementary Figure Legends**

**Supplementary Figure 1. Schedule of treatment with low dose metronomic PTX and assessment by bioluminescence analysis in SCID mice xenografted with EGI-1 cells.** See text for detail.

**Supplementary Figure 2.** **Low dose PTX reduced motility and invasiveness of CCA-TV3 cells. A.** Consistent with data obtained with EGI-1 cells,by wound-healing assay, cell motility of CCA-TV3 cells significantly decreased, in a dose-dependent fashion, following PTX 1.5 (dotted line) and 15nM (dashed line) exposure compared with untreated controls (continue line) (n=4). **B.** Similar to EGI-1 cells, the same PTX dose regimens significantly blunted the invasive abilities of CCA-TV3 cells, with respect to controls in Boyden chambers coated with Matrigel, (n=3). Representative images of scratch and transwell filter are shown below their respective plot. \*p<0,05 vs Ctrl; \*\*p<0.01 vs Ctrl.

**Supplementary Figure 3.** **In contrast with high doses, low dose PTX did not affect cell proliferation, viability, and apoptosis of CCA-TV3 cells. A-C.** Concomitant effects of low dose PTX (1.5 and 15nM) on cell proliferation (BRDU incorporation, A), viability (MTS assay, B), and apoptosis (CC3 immunofluorescence in cultured cells, C) were also evaluated in CCA-TV3 cells and compared with the effects of higher doses (150 and 1500nM) and with untreated controls. In contrast with high doses, these cell activities were not affected by low dose PTX (n=3 in all experiments), in accordance with what observed in EGI-1 cells. \*\*p<0.01 vs Ctrl.

**Supplementary Figure 4. Low dose PTX did not affect Rac-1 GTP levels, while it reduced the MT1-MMP membrane expression. A.** By G-LISA assay, low dose PTX (1.5 and 15nM) did not modify Rac-1 GTP levels in EGI-1 cells, with respect to untreated controls (A, n=3, in duplicate). **B.** In contrast, the same PTX doses significantly blunted the expression of MT1-MMP (red) at the membrane level of EGI-1 cells, as shown by immunofluorescence analysis of cultured cells; representative plots are given above each micrograph. Red line depicts the fluorescence intensity profile of MT1-MMP through the cell area, while blue line represents the intensity of nuclear staining (DAPI); peak of red line corresponds to the lowest level of blue staining, according to a membrane localization of MT1-MMP. Original magnification: 400x.

**Supplementary Figure 5. Low dose PTX did not affect cytoskeletal integrity of EGI-1 cells. A.** By phalloidin staining, only a small percentage of EGI-1 cells challenged with low dose PTX (1.5, 15nM), displayed structural alterations of the actin filaments, which instead were observed in a larger subset of EGI-1 cells exposed to high dose PTX (150, 1500nM). No differences in damaged cells between low dose PTX and untreated cells could be observed (n=3, percentage of apoptotic cells expressed as black area of the column). **B.** Representative immunofluorescence for FITC-conjugated phalloidin (green) in cultured cells treated with increasing doses of PTX are shown below each column plot; perinuclear condensation, a hallmark of cytoskeletal disaggregation, is clearly observed in single cells treated with PTX 150nM (white arrow), whereas cytoplasm shrinkage is evident in cells treated with PTX 1500nM. Original magnification, M=400x. **C.** WB for β-tubulin in microtubule fractions purified by ultracentrifugation shows lack of expression indicating cytoskeletal damage in cells exposed to PTX 150 e PTX 1500nM.

**Supplementary Figure 6. EGI-1 cells but not TFK-1 cells contained SUMOylated S100A4; inhibitory effects of low dose PTX on SUMOylating complex did not associate with any deregulation of SUMO E subunits. A.** By SUMOylation assay, SUMOylated S100A4 could be detected only in EGI-1 cells (expressing S100A4 in the nucleus), whereas it was absent in TFK-1 cells, a CCA cell line where S100A4 expression is limited to the cytoplasm. Accordingly, un-SUMOylated S100A4 could be detected in either cell lines, as shown by the band present in the flow through fraction. **B-D.** By Real time PCR, mRNA expression levels of each SUMOylating complex subunit in EGI-1 cells (B, E1; C, E2; D, E3), were not affected by treatment with low dose PTX.

**Supplementary Figure 7. LDM PTX did not affect the splenic tumor mass in SCID mice xenotransplanted with EGI-1 cells, *in vivo*.** **A-C.** SCID mice xenografted by intrasplenic injection of EGI-1 cells transduced with a lentiviral vector encoding the firefly luciferase gene were treated with LDM PTX or vehicle after confirming tumor engraftment by bioluminescence imaging. No statistically significant differences between LDM PTX treated mice and controls were observed both in the level of photon emission (A, representative images taken at the beginning (T0) and at the end (T2) of treatment; B, bioluminescence curve during the treatment) and in the size of the splenic tumor mass measured at necroscopic examination (C).