**Supplementary figure legends**

**Supplementary Figure 1 – Autofluoresence as a biomarker for pancreatic cells stem cells. (A)** mRNA levels for pluripotency-associated genes in Fluo+ vs Fluo– sorted cells; PDAC-185 and -A6L, n=6, \* p<0.05. **(B)** Nanog protein expression in Fluo+ and Fluo– cells. Representative Western blot using β-ACTIN as loading control. **(C)** H&E histology of the PDAC-185 parental tumor (upper left). Representative FACS plot for sorting of single Fluo+ cells (upper right). Arising tumors following injection of single cells into immunocompromised mice were digested and sorted for Fluo+ and Fluo– cells. Representative FACS plot is shown (lower right). **(D)** mRNA levels for pluripotency-associated genes for the parental 185 tumor, the single Fluo+ cell-derived tumor, and for A6L tumors are shown. \* p<0.05, n=3 (lower left).

**Supplementary Figure 2 – PDAC CSCs over-express DNMT1 and bear higher 5mC levels. (A)** Representative images of dot blots showing levels of 5mC in marker-positive (Fluo-positive and CD133-positive) versus marker-negative (Fluo-negative and CD133-negative) cells. Quantification of 5mC levels was performed by densitometry. **(B)** qRT-PCR analysis of DNMT1 in sorted PDAC-185 sphere-derived autofluorescent-positive (Fluo+) and -negative (Fluo–) cells (\* P<0.05; n=4).

**Supplementary Figure 3 – DNMT1 inhibitor Zebularine decreases CSC phenotype.** (**A**) Representative FACS plots for the CSC marker CD133 in spheres versus adherent primary PDAC cells. **(B)** mRNA levels for the pluripotency-associated gene NANOG in CD133+ vs CD133– sorted cells; n=4, \* p<0.05. NANOG and DNMT1 protein expression in CD133+ and CD133– PDAC-354 sorted cells. Representative Western blot using β-ACTIN as loading control. **(C)** Graph illustrating cytotoxicity following 24h treatment with increasing concentrations of Zebularine; n=3, \* p<0.05. **(D)** mRNA levels for DNMT1 gene in control (Ctrl) versus Zebularine (Zeb) treated cells n=3, \* p<0.05. **(E)** Representative flow cytometry plots showing the percentage of Fluo+ cells from Control and Zebularine-treated cells (left), together with quantification (right). Data are represented as fold change compared to control cells; n=4, \* p<0.05.

 **Supplementary Figure 4 – DNMT1 inhibitor Decitabine decreased CSC phenotype.** (**A**) Graph illustrating cytotoxicity following 24h treatment with increasing concentrations of Decitabine (n=3, \* p<0.05). **(B)** Representative Western blot showing DNMT1 protein level in control and Decitabine-treated spheres together with densitometric quantification. **(C)** Number of spheres per ml in 1st and 2nd generation cultures from various primary PDAC tumors (A6L, 185, and 354; \* P<0.05, n=3). **(D)** qRT-PCR analysis of pluripotency-associated genes (NANOG, OCT3/4, SOX2, and KLF4) in 1st generation spheres. Data are normalized to β-ACTIN and presented as fold change compared to untreated cells, (\* P<0.05, n=3). **(E)** Representative flow cytometry plots for E-CADHERIN and Pan-CYTOKERATIN (Pan-CK) in control and Decitabine-treated cells (upper panel) together with quantification (lower panel). Data are represented as fold change compared to control cells (n=4, \* p<0.05).

**Figure 5 – DNMT1 inhibitor Zebularine promotes CSC differentiation.** (**A**) Representative images of pan-CYTOKERATIN (Pan-CK) staining for Ctrl versus Zeb-treated cells. **(B)** Representative flow cytometry plots for E-CADHERIN and Pan-CK surface expression in control and Zebularine-treated cells (left) together with quantification (right). Data are represented as fold change compared to control cells; n=4, \* p<0.05. **(C)** Representative flow cytometry plots for Pan-CK surface expression in Cas9 only (control) and Cas9+sgDNMT1(DNMT1-KO) cells.

 **Supplementary Figure 6 – DNMT1 inhibition modulates expression of miR-203, miR-205,
and the miR-17-92 cluster.** (**A**) qRT-PCR analysis of miR-203 and -205 expression in control and Zebularine (Zeb)-treated cells. Data are normalized to Snord44 and presented as fold change compared to untreated cells. **(B)** qRT-PCR analysis of miR-203 and miR-205 in control (Cas9) and DNMT1-KO (Cas9+sgDNMT1) cells. Data are normalized to Snord44 and presented as fold change compared to control cells (n=4, \* p<0.05). **(C)** qRT-PCR analysis of members of the miR-17-92 cluster (miR17, 18a, 19a, 19b, and 20a) in control and DNMT1-KO sphere-derived cells (\* p<0.05, n=3).