**Supplementary Figure Legends**

**Supplementary figure S1.** Suppression of GPx2 reduces proliferation and clone forming potential of colon CSCs.

**A,** Immunoblot analysis of GPx2 expression in HT29 colonosphere lines stably expressing scrambled shRNAs, shGPx2#1 or shGPx2#2.

**B,** Western blot analysis of GPx2 and cleaved caspase-3 expression after single cell making of L145 colonospheres expressing scrambled shRNAs, and different clones of shGPx2#1 or shGPx2#2.

**C,** Analysis of cell viability in L145 colonospheres expressing either scrambled or GPx2-targeting shRNAs after treatment with Elesclomol and Cisplatin using the Multitox-Fluo probe (Promega). Data are shown as means +SD and represent data from 3 independent experiments. \*p≤ 0.05.

**D,** Clone forming assay of L145 and HT29 colonosphere lines expressing scrambled shRNAs, or shGPx2#1 using matrigel 3D-culture system. Graphs show mean ± SEM, \*p<0.05.

**E,** qPCR analysis of mRNA levels of the proliferation cell marker Ki67 in L145 colonosphere line expressing scrambled shRNAs, shGPx2#1 or shGPx2#2, 72 hours after treatment with 0.2mM of N‐acetylcyteine (NAC). Graphs show mean ± SEM, \*p<0.05, \*\*p<0.01.

**F,** qPCR analysis of mRNA levels of Ki67 in L145 colonosphere line expressing the indicated shRNAs 72 hours after treatment with 50µM H2O2. Graphs show mean ± SEM, \*p<0.05.

**Supplementary figure S2.** Loss of GPx2 impairs differentiation of CSCs**.**

**A,** Immunoblot analysis of GPx2 protein levels in CRC29 cells following short-hairpin RNA-mediated GPx2 silencing.

**B,** qPCR analysis of mRNA levels of GPx2, and the indicated stem cell markers in CRC29 colonosphere line stably expressing scrambled shRNAs or shGPx2#1. Graphs show mean ± SEM, \*p<0.05.

**C,** qPCR analysis of mRNA levels of the stem cell marker OLFM4 in L145 colonospheres expressing scrambled shRNAs, shGPx2#1 or shGPx2#2, 72 hours after treatment with 0.2mM of N‐acetylcyteine (NAC) (left graph) or 50µM H2O2 (right graph). Graphs show mean ± SEM, \*p<0.05, \*\*\*p<0.001.

**D,** qPCR analysis of mRNA levels for GPx2 inL145 colonosphere line stably expressing scrambled shRNAs or shGPx2#1. Graphs show mean ± SEM.

**E,** qPCR analysis of mRNA levels for the indicated differentiation markers in CRC29 colonosphere line stably expressing scrambled shRNAs or shGPx2#1. Graphs show mean ± SEM, \*p<0.05, ND=Not detectable.

**F,** Immunoblot analysis of GPx2 protein and the indicated differentiation markers in HT29 colonosphere lines expressing scrambled shRNAs, shGPx2#1 or shGPx2#2.

**G,** Immunoblot of GPx2 and MUC2 expression in HT29 cells expressing scrambled shRNAs, or shGPx2#1 reconstituted with either FLAG (control) or shRNA-insensitive FLAG-GPx2.

**H,** Western Blot analysis of GPx2, MUC2, cleaved caspase-3, p16 and p21 expression in HT29 cells colonospheres lines expressing scrambled shRNAsor shGPx2#1, 24 and 48 hours after single cell making, treated with or without the general apoptosis inhibitor z-VAD.

**I,** qPCR analysis of mRNA levels for the senescence marker p16 in L145 colonosphere line stably expressing scrambled shRNAs or shGPx2#1. Graphs show mean ± SEM.

**Supplementary figure S3.** Loss of GPx2 reduces tumor growth.

**A,** Subcutaneous tumor formation by L145 colonospheres expressing scrambled shRNAs, shGPx2#1-2, or shGPx2#2. The equivalent of 3000 cells was inoculated subcutaneously into immunodeficient mice and tumor volume was determined over time. The photograph shows tumors formed in all three groups. Graphs show mean ± SEM, \*p<0.05.

**B,** FACS-analysis using the Aldefluor® assay in L145 cells isolated from scrambled, shGPx2#1-2, or shGPx2#2 subcutaneous tumors. Cells incubated with the Aldefluor® substrate BAAA and the ALDH specific inhibitor DEAB were used to set the gate.

**Supplementary figure S4.** Overexpression of GPx2 promotes differentiation of CSCs and accelerates tumor growth.

**A,** Immunoblot analysis of GPx2 protein and the indicated differentiation markers in CRC29 colonosphere lines stably expressing Flag- or YFP-tagged GPx2. \*Indicates endogenous GPx2 protein.

**B,** qPCR analysis of mRNA levels of the indicated differentiation markers was performed on CRC29 colonosphere line stably expressing control YFP or YFP‐GPx2 protein. Data are shown as means +SEM and represent data from 3 independent experiments. \*p≤ 0.05.

**C,** Immunofluorescence analysis of the differentiation marker MUC2 and the stem cell marker OCT4 in control or YFP‐GPx2-expressing CRC29 cells.

**D,** q-PCR analysis of mRNA levels of the indicated stem cell genes in CRC29 colonosphere line stably expressing control or YFP‐GPx2 protein. Data are shown as means +SEM and represent data from 3 independent experiments. \*p≤ 0.05.

**E,** Tumor forming capacity was performed by injecting L145 and L167 colonospheres expressing control or YFP‐GPx2 subcutaneously injected into immunodeficient mice. Tumor volume was measured over time. Graphs show mean ± SEM, \*p<0.05.

**Supplementary figure S5.** GPx2 expression in human colon tumor cohorts correlates with epithelial differentiation and low H2O2 stress.

**A,** Comparative clustering by using the GPx2, epithelial and CCS classifiers in the de Sousa and MVRM cohorts shows a near-complete overlap between the CCS1, epithelial-high and GPx2-high subgroups.

**B,** Expression levels of GPx2 in CCS1 *versus* CCS3 and in epithelial-high *versus* epithelial-low tumors in the de Sousa and MVRM cohorts. The box plots show a significantly higher expression of GPx2 in the CCS1 and epithelial-high tumor subgroups.

**C,** XY plot showing the inverse correlation of the GPx2 co-expression signature with the H2O2 metagene in the De Sousa cohort. CCS subgroups are color-coded, revealing high GPx2 and low H2O2 stress in CCS1 tumors.

**Supplementary figure S6.** High GPx2 expression in CCS3 subtype colon tumors is associated with increased metastatic risk.

**A,** Expression levels of the GPx2 co-expression gene set in histologically well–differentiated metastases (n=16) and poorly differentiated metastases (n=17) in the Snoeren dataset (p<0.05).

**B,** Kaplan-Meier curves of 74 patients with metastasis-prone CCS3-type tumors in the MVRM dataset. Time to recurrence in patients with high GPx2 expression and low GPx2 expression is shown (log rank p=2.7e-3).

**C,** Kaplan-Meier curves of 24 patients with metastasis-prone CCS3-type tumors in the AMC-90 dataset. Time to recurrence in patients whit high GPx2 expression and low GPx2 expression is shown (log rank p=3.9e-3)