# Supplementary Material: Proteomic Analyses of Fibroblast and Serum Derived Exosomes Identify QSOX1 as A Marker for Non-Invasive Detection of Colorectal Cancer

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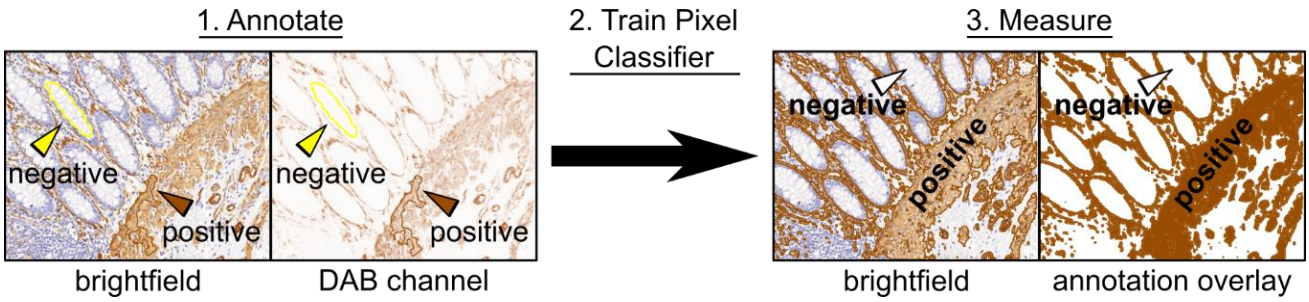



**Figure S1.** IHC analysis in QuPath®.

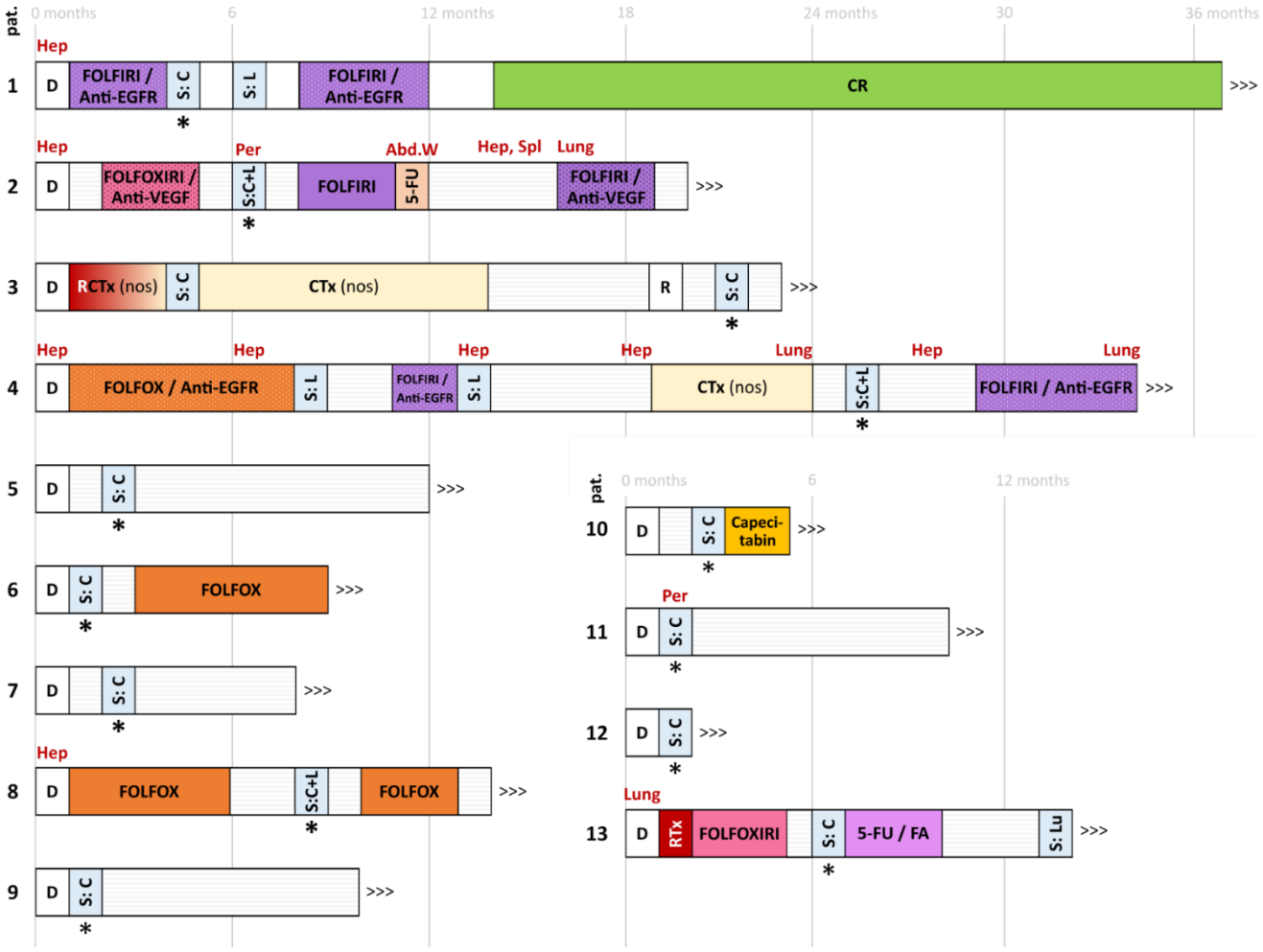



**Figure S2.** Clinicopathologic timelines of patients 1–13. Timelines reflecting disease progression and applied therapy approaches for each patient, with asterisks (*) highlighting the time point of tissue collection. Locations of distant metastasis diagnosed over time are indicated in red letters. Abbreviations: 5-FU: 5-Fluorouracil; Abd.W: abdominal wall; C: colon;

CR: complete remission; CTx: chemotherapy; D: diagnosis; EGFR: epidermal growth factor receptor; FOL: folinic acid; FOLFIRI: FOL/5-FU/Irinotecan combination therapy; FOLFOX: FOL/5-FU/Oxaliplatin combination therapy; FOLFOXIRI: FOL/5-FU/Oxaliplatin/Irinotecan combination therapy; Hep: hepatic; L: liver; Lu: lung; nos: no other specified; pat.: patient; Per: peritoneum; R: local recidive; RCTx: radiochemotherapy; RTx: radiotherapy; S: surgery; Spl: spleen; VEGF: vascular endothelial growth factor.

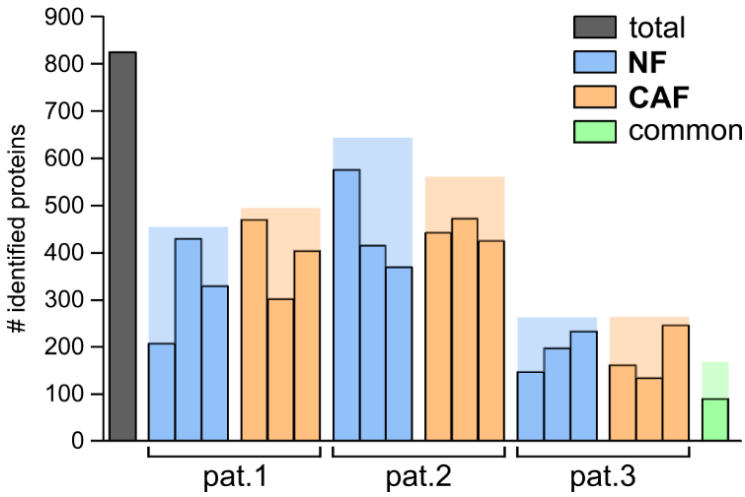



**Figure S3.** Number of identified proteins in mass spectrometry. Primary fibroblast-derived EXOs were subjected to proteomic analysis ($n = 3$). The overall number of identified proteins per sample (proteins identified in at least one replicate) is indicated in faint colors. Abbreviations: CAF: cancer-associated fibroblast; EXO: exosome; NF: normal fibroblast; pat.: patient.

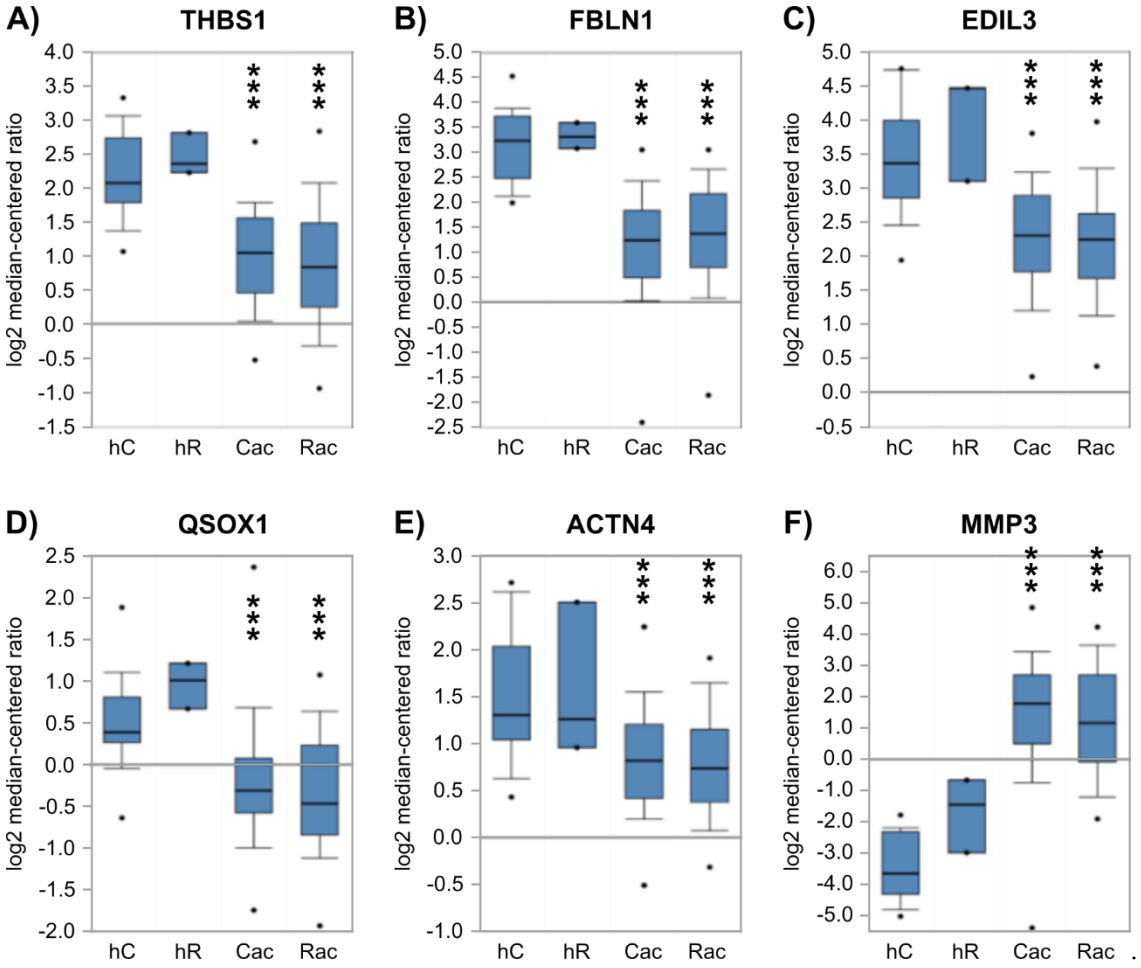



**Figure S4.** mRNA expression of six identified proteins of interest in TCGA CRC, provided by Oncomine**.** Columns display healthy colon (hC, $n$ = 19), healthy rectum (hR, $n$ = 3), colon adenocarcinoma (Cac, $n$ = 101) and rectum adenocarcinoma (Rac, $n$ = 60). Statistical differences as compared to healthy samples (hC and hR): *** $p < 0.001$. (**A)** Thrombospondin 1 (THBS1, reporter A_23_P206210). (**B)** Fibulin 1 (FBLN1, reporter A_23_P211630). (**C)** EGF-like repeats and discoidin domains 3 (EDIL3, reporter A_23_P401606). (**D)** Quiescin sulfhydryl oxidase 1 (QSOX1, reporter A_23_P12463). (**E)** Actinin $\alpha$4 (ACTN4, reporter A_23_P315241). (**F)** Matrix metallopeptidase 3 (MMP3, reporter A_23_P161696). Abbreviations*:* Cac: colon adenocarcinoma; hC: healthy colon; hR: healthy rectum; Rac: rectum adenocarcinoma.

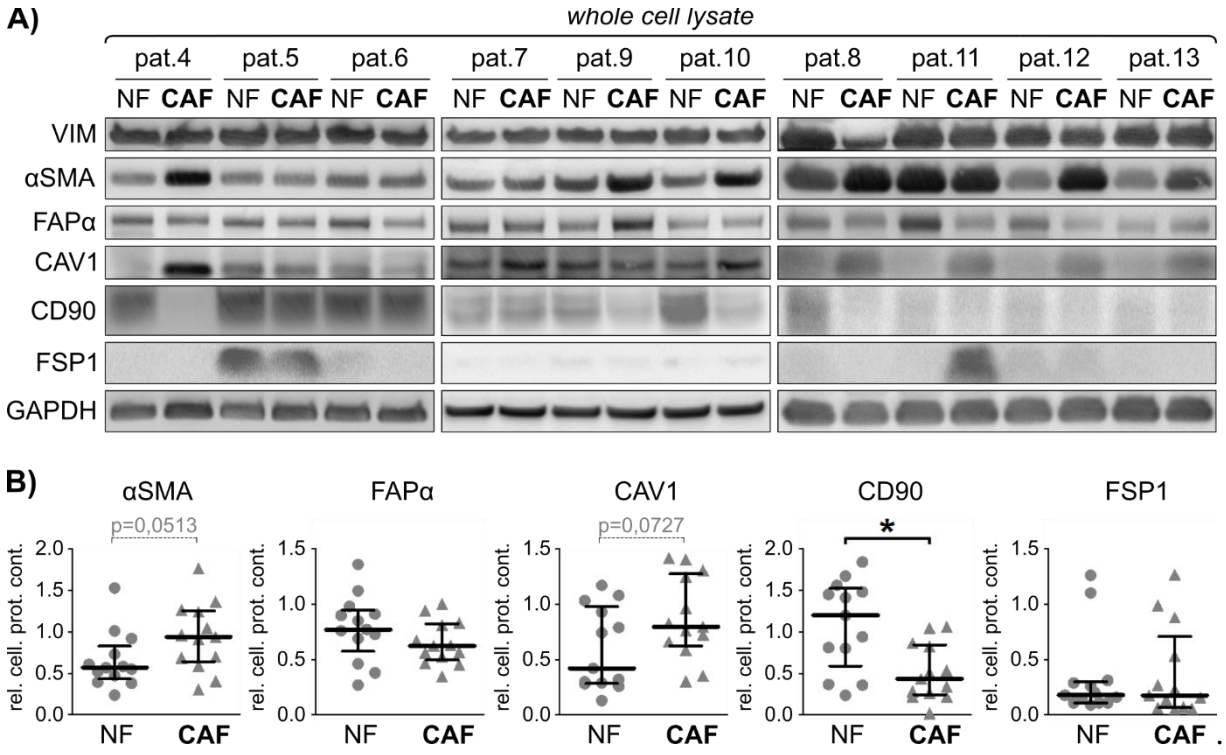
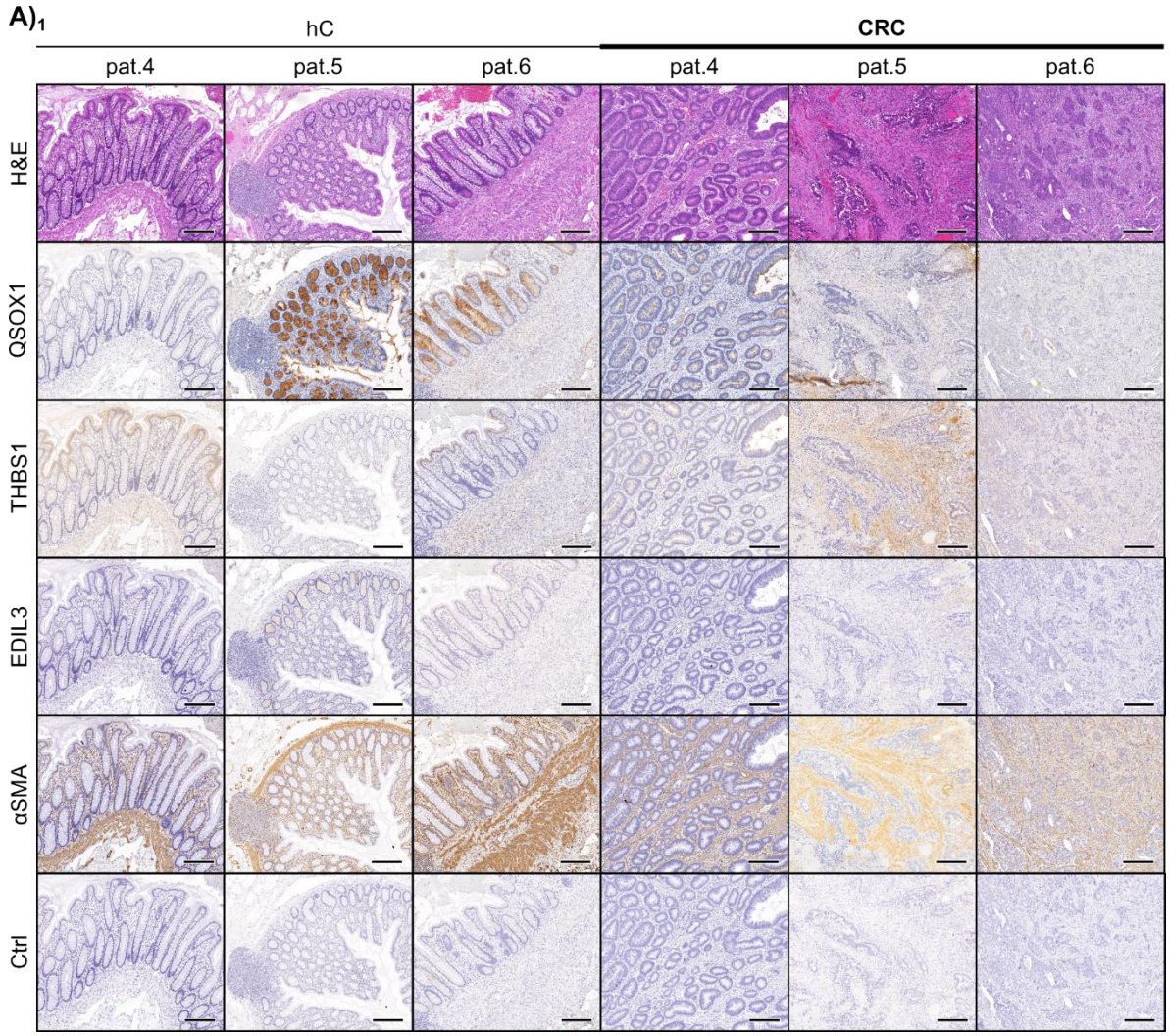



**Figure S5.** Fibroblast activity marker expression in an independent validation cohort. Twenty fibroblast cell lines derived from 10 CRC patients were subjected to cellular protein isolation and subjected to Immunoblot. **(A)** Immunoblot analysis of vimentin (VIM), $\alpha$-smooth-muscle actin ($\alpha$SMA), fibroblast activation protein $\alpha$ (FAP$\alpha$), caveolin 1 (CAV1), cluster of differentiation 90 (CD90) and fibroblast-specific protein 1 (FSP1) in primary fibroblasts, including GAPDH as loading control. **(B)** Graphical analysis of immunoblots shown in (**A**) and (Figure. 1A) using ImageJ, relative to GAPDH. Mann-Whitney-U test: * $p < 0{,}05$. Abbreviations*:* CAF: cancer-associated fibroblast; CRC: colorectal cancer; GAPDH: glyceraldehyde 3 phosphate dehydrogenase; NF: normal fibroblast; pat.: patient; rel. cell. prot. cont.: relative cellular protein content.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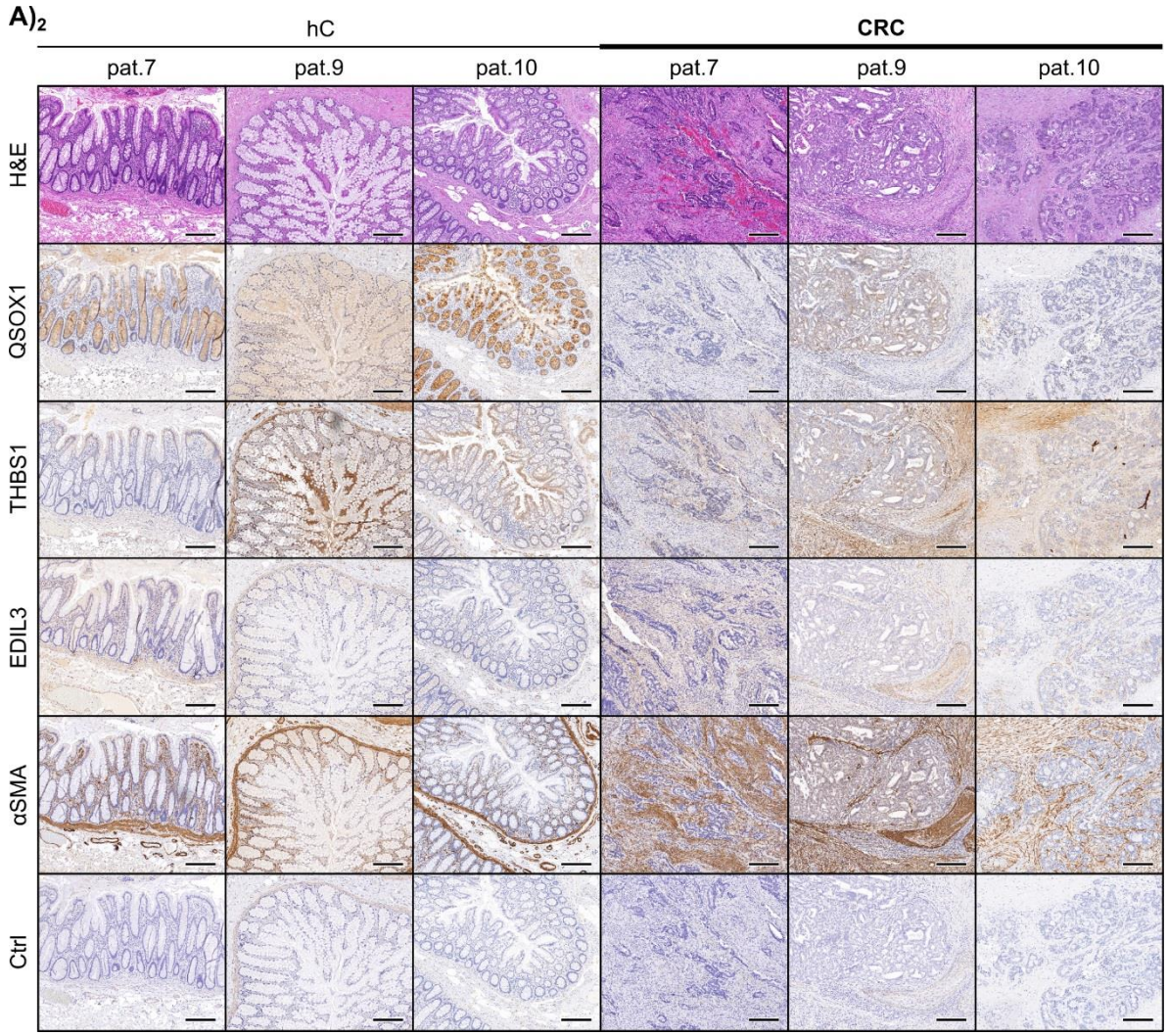
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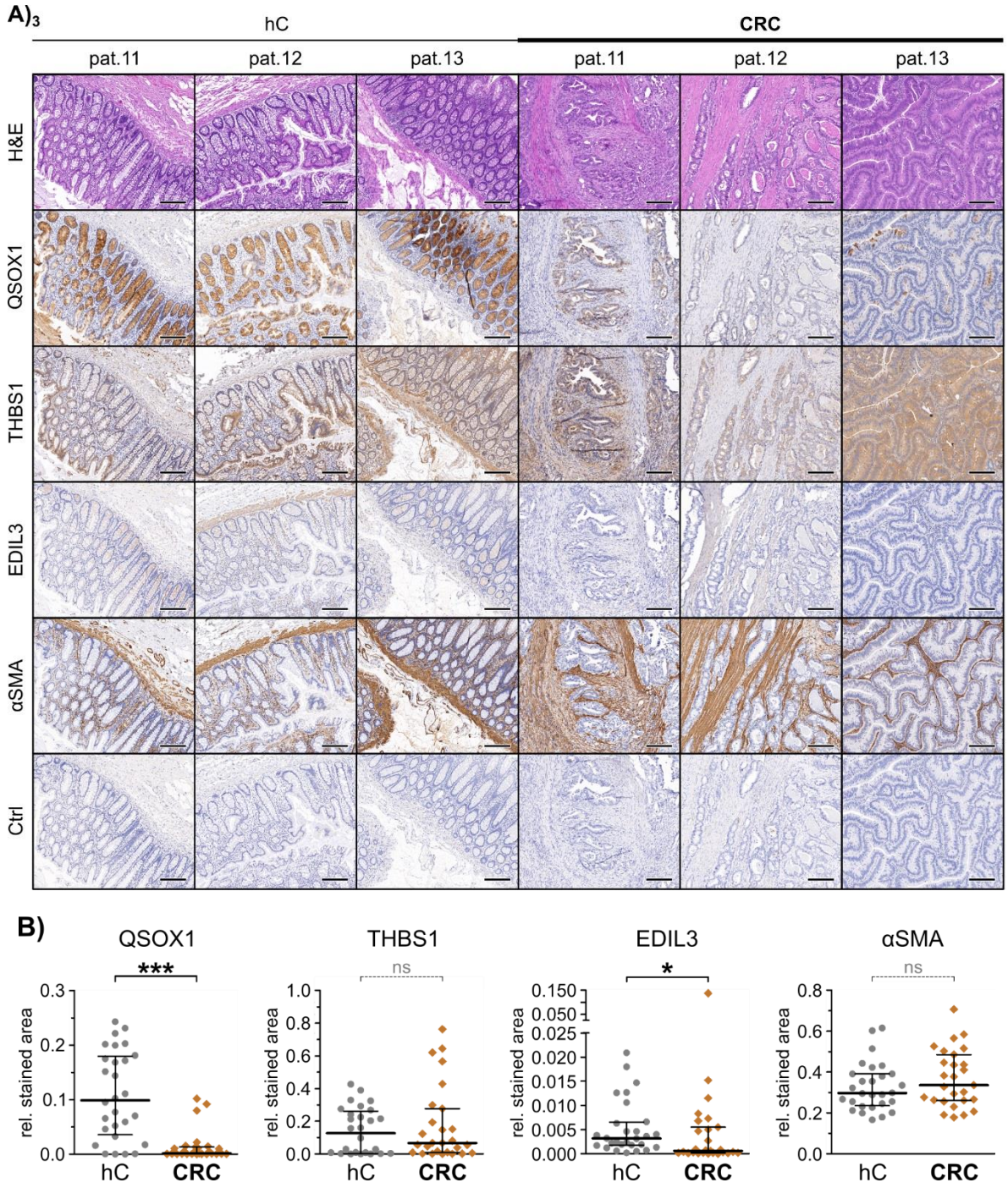


**Figure S6.** In vivo marker expression in patient-matched healthy and malignant colon tissue. **(A)**1-3 Representative images of paraffin embedded tissue slides of healthy and malignant colon tissue derived from patients 4-7 and 9-13, H&E or immunohistochemically stained for the proteins QSOX1, THBS1, EDIL3, αSMA and IgG control (Ctrl). Scale bars equal 250 μm. **(B)** Graphical IHC staining analysis performed in QuPath. From each patient and tissue, a minimum of three representative areas were subjected to graphical and statistical analysis. Mann-Whitney-U test: *** $p < 0.001$, * $p < 0.05$, ns = not significant. Abbreviations: αSMA: α-smooth-muscle actin; CRC: colorectal cancer; Ctrl: control; EDIL3: EGF-like repeats and discoidin domains 3; H&E: hematoxylin and eosin stain; hC: healthy colon; IgG: immunoglobulin G; IHC: immunohistochemistry; pat.: patient; THBS1: thrombospondin 1, QSOX1: quiescin sulfhydryl oxidase 1.

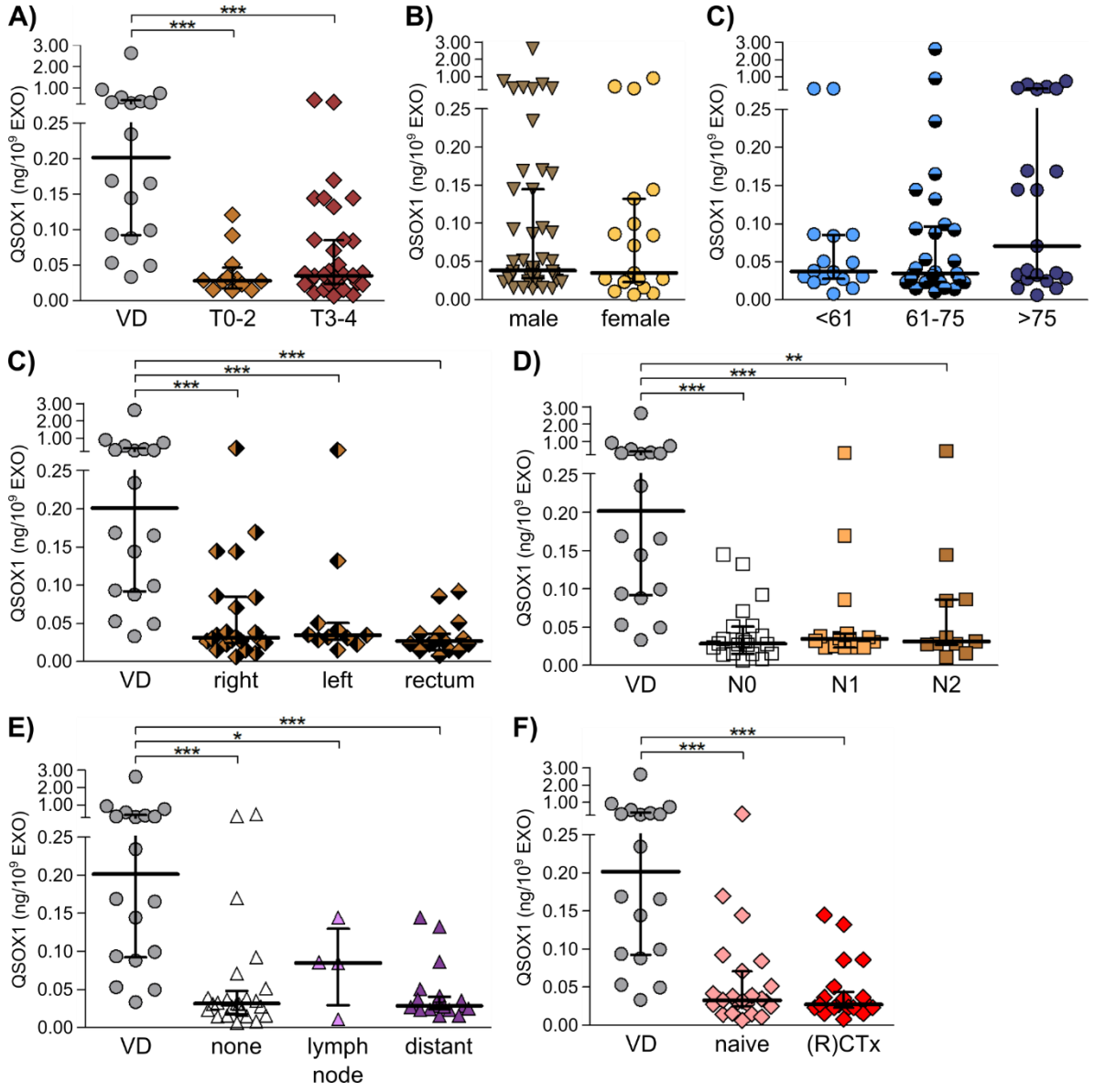


**Figure S7.** Extended patient data correlation on pEXO QSOX1 levels**.** pEXO levels of QSOX1 depending on T stage **(A)**, gender **(B)**, age at time point of surgery and blood sampling **(C)**, primary colorectal tumour site **(D)**, N stage **(E)**, metastasis status **(F)** and pretreatment **(G)**. Mann-Whitney-U test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Abbreviations: pEXO: plasma-derived exosomes; EXO: exosome; QSOX1: quiescin sulfhydryl oxidase 1; (R)CTx: (radio-)chemotherapy; VD: vascular disease