Circulating levels of soluble urokinase plasminogen activator receptor predict outcome after resection of biliary tract cancer

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Table S1. Serum levels of various laboratory markers (validation cohort only)

	BTC patients median [range]	**Healthy controls** median [range]
suPAR pre-OP [ng/ml]	4.47 [1.36-66.48]	1.55 [0.0-4.38]
suPAR post-OP [ng/ml]	4.80 [1.57-12.60]	-
CEA [µg/l]	3.10 [0.71-333.0]	1.25 [0.4-6.30]
CA 19-9 [U/ml]	83.2 [0.60-22911.0]	5.1 [0.0-31.0]
Leucocyte count [cells/nl]	8.00 [2.90-21.60]	-
CRP [mg/l]	18.0 [0.0-230.0]	-
AST [U/l]	45.5 [18.0-1587.0]	28.0 [20.0-78.0]
ALT [U/l]	43.0 [7.0-1097.0]	20.0 [5.0-82.0]
GGT [U/l]	305.0 [36.0-1794.0]	16.0 [8.0-120.0]
ALP [U/l]	219.5 [53.0-1055.00]	62.0 [36.0-102.0]
LDH [U/l]	255.0 [100.3-877.0]	160.0 [60.0 -204.0]
Bilirubin [mg/dl]	0.97 [0.05-4.17]	0.41 [0.10-1.46]
Creatinine [mg/dl]	0.84 [0.43-2.08]	-
Sodium [mmol/l]	140.0 [131.0-146.0]	-
Potassium [mmol/l]	4.35 [2.90-5.50]	-
Haemoglobin [g/l]	12.5 [7.80-17.10]	-
Platelets [cells/nl]	275.5 [65.0-931.0]	-
CXCL5 pre-OP [pg/ml]	464.5 [0.0 - 2866.1]	
CX3CL1 pre-OP [pg/ml]	257.61 [83.33-1092.5]	
IL-8 pre-OP [pg/ml]	39.23 [7.1-489.85]	
IL-8 post-OP [pg/ml]	27.63 [5.95-1415.34]	
IL-1ß post-OP [pg/ml]	1.35 [0.8-7.48]	
TNF-α post-OP [pg/ml]	29.34 [13.93-66.09]	
CCL3 post-OP [pg/ml]	8.47 [2.5-44.62]	

suPAR: soluble urokinase plasminogen activator receptor, CEA: carcinoembryonic antigen, CA 19-9: carbohydrate-Antigen 19-9, CRP: C-reactive protein, AST: aspartate transaminase, ALT: alanine transaminase, GGT: γ-Glutamyl transpeptidase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase, CXCL5: C-X-C motif chemokine 5, CX3CL1: chemokine C-X3-C motif ligand 1 (Fractalkine), IL-8: interleukin 8, IL-1ß: interleukin 1 beta, TNF-α: tumor necrosis factor alpha, CCL3: chemokine CC motif ligand 3

Table S2. Clinicopathological characteristics of BTC patients included into the IHC tumoral uPAR expression analysis.

	IHC cohort
BTC patients	n = 108
Relevant uPAR expression [%]: Yes (IRS >1) No (IRS 0 or 1)	 54.6 45.4
Gender [%]: male-female	 54.6 - 45.4
Age [years, median and range]	69 [35 - 85]
BMI [kg/m^2, median and range]	24.15 [17.72 – 43.91]
Anatomic location of BTC [%]: Intrahepatic Klatskin Distal Gallbladder	 49.5 43.9 0.9 5.6
Tumor characteristics [%]: T1 - T2 - T3 - T4 N0 - N1 M0 - M1 Perineural invasion: No - Yes Lymphatic invasion: No - Yes Vascular invasion: No - Yes G1 - G2 - G3 R0 - R1 - Rx	 18.2 - 50.5 - 26.3 - 5.1 56.5 - 43.5 90.5 - 9.5 32.4 - 67.6 79.8 - 20.2 75.6 - 24.4 1.0 - 72.0 - 27.0 76.7 - 19.4 - 3.9
ECOG PS [%]: ECOG 0 ECOG 1 ECOG 2	 42.5 48.1 9.4

BTC: biliary tract cancer, BMI: body mass index, ECOG PS: „Eastern Cooperative Oncology Group“ performance status, G: tumor grading, IRS: immunoreactive score, TNM: TNM tumor stage, R: resection status

Table S3. Correlation analysis between pre-operative suPAR serum levels and various laboratory markers

Parameter	Correlation coefficient (r_S)	p-value
ALT	0.196	0.141
Bilirubin	0.390	< 0.001
GGT	0.185	0.078
ALP	0.331	0.001
CRP	0.262	0.012
CXCL5	0.210	0.042
CX3CL1	0.562	< 0.001
IL-8	0.497	< 0.001

ALT: alanine transaminase, GGT: γ-Glutamyl transpeptidase, ALP: alkaline phosphatase, CRP: C-reactive protein, CXCL5: C-X-C motif chemokine 5, CX3CL1: chemokine C-X3-C motif ligand 1 (Fractalkine), IL-8: interleukin 8

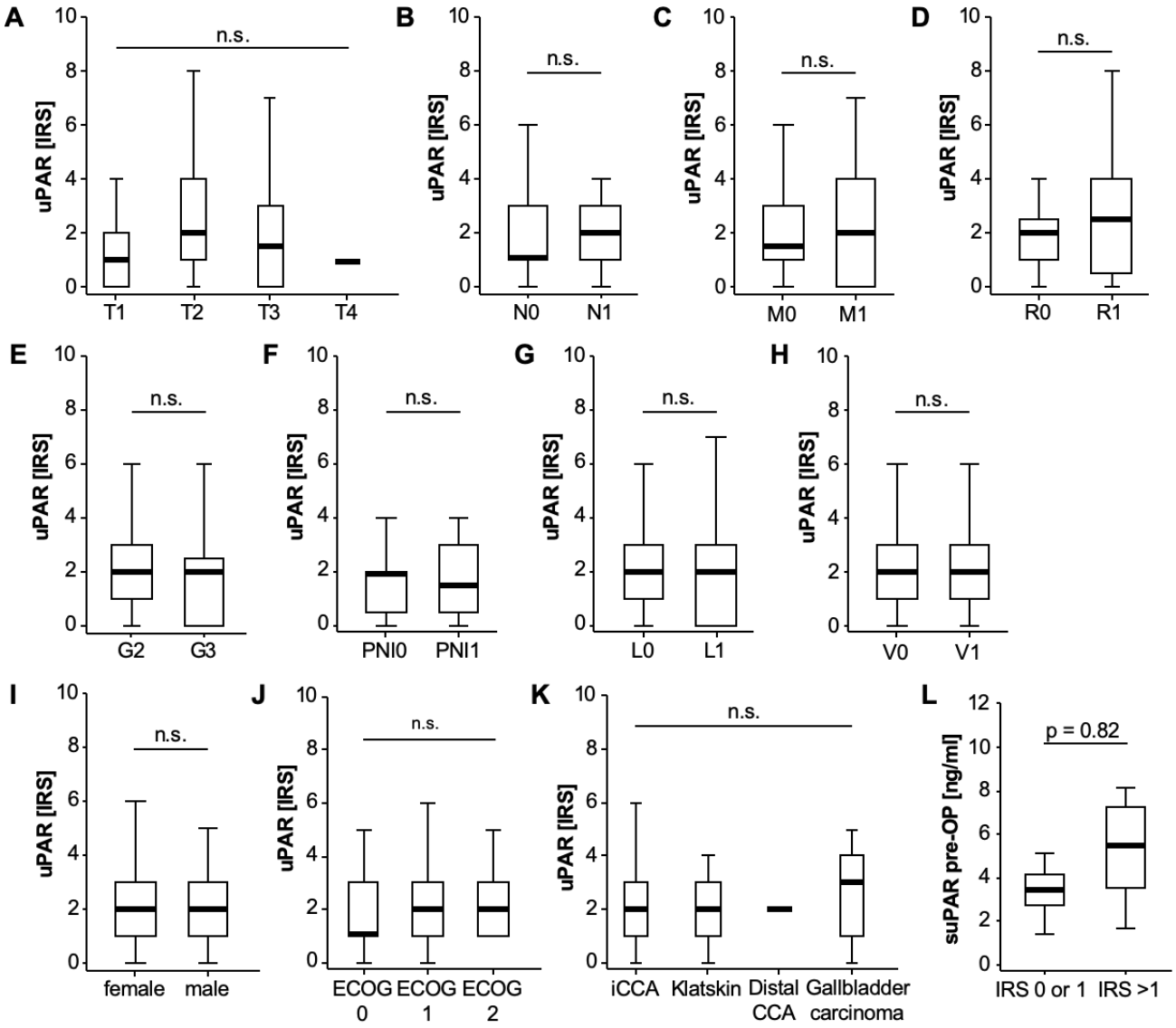

Fig. S1. Tumoral uPAR expression and patient characteristics

(A-K) Tumoral uPAR expression is unaltered between patients with different tumor (TNM stage, resection status, tumor grading, perineural, vascular or lymphatic invasion and tumor localization) or clinical characteristics (sex and ECOG performance status). (L) In an exploratory setting of n=23 patients, circulating suPAR levels showed a strong trend towards higher levels in patients with relevant (IRS >1) uPAR tissue expression compared to patients with irrelevant (IRS 0 or 1) tumoral uPAR expression.

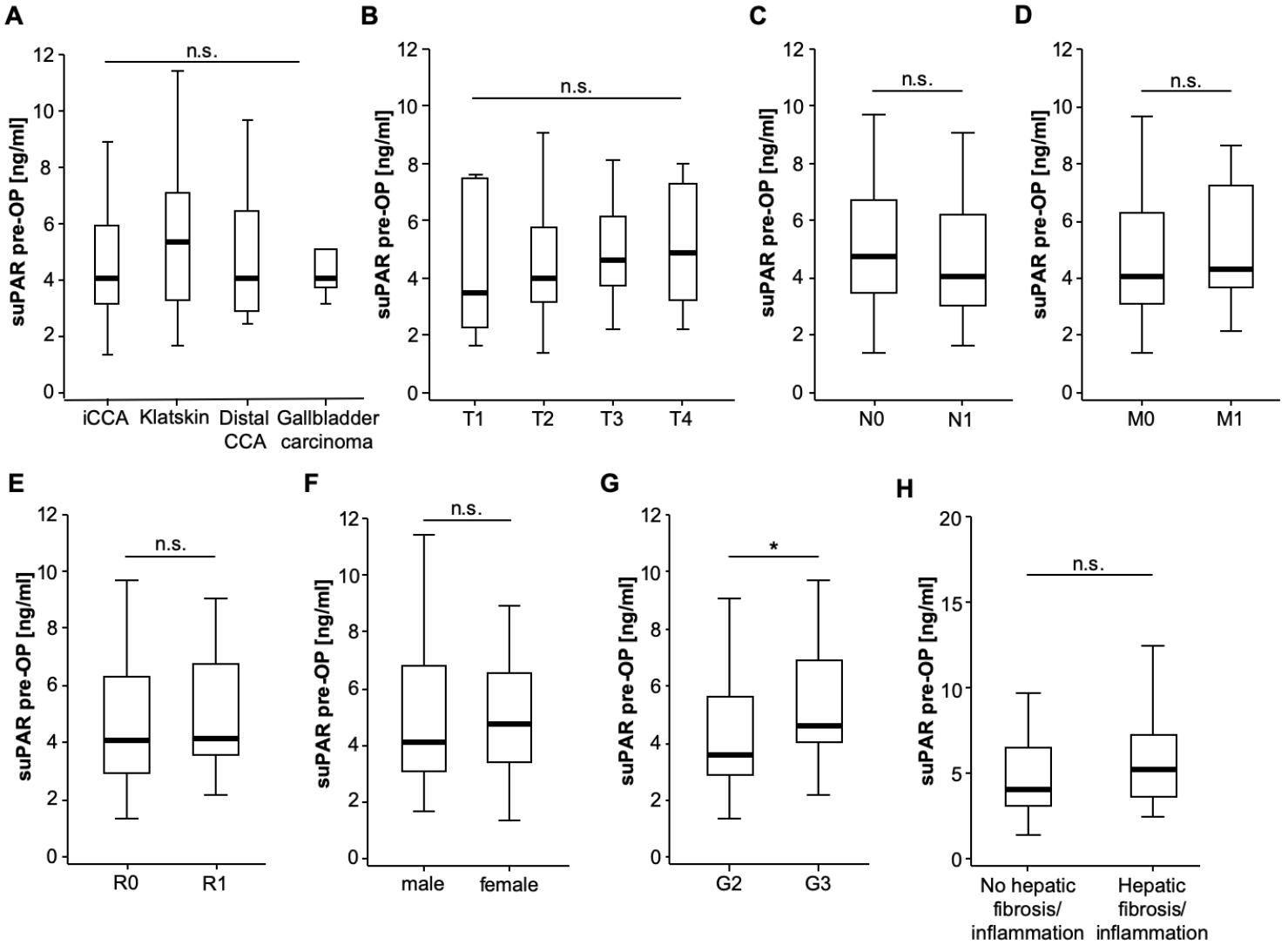

Fig. S2. Preoperative suPAR levels and patient characteristics

(A) SuPAR serum levels are unaltered between different subtypes of BTC (intrahepatic CCA, Klatskin tumors, distal CCA, and gallbladder carcinoma). Patients with T_1- to T_4-tumor stage (B), with or without lymph node involvement (C) as well as non-metastasized or metastasized patients who were still resectable (D). Initial suPAR levels do not differ between patients with R_0 or R_1 tumor resection status or male and female patients (E and F). Patients with poorly differentiated BTC (G3) have significantly higher suPAR level compared to moderately differentiated tumors (G2). (H) Circulating suPAR is unaltered between patients with histologically relevant sings of liver inflammation or liver fibrosis in the resected non-tumorous liver samples and patients without any signs of chronic liver disease.

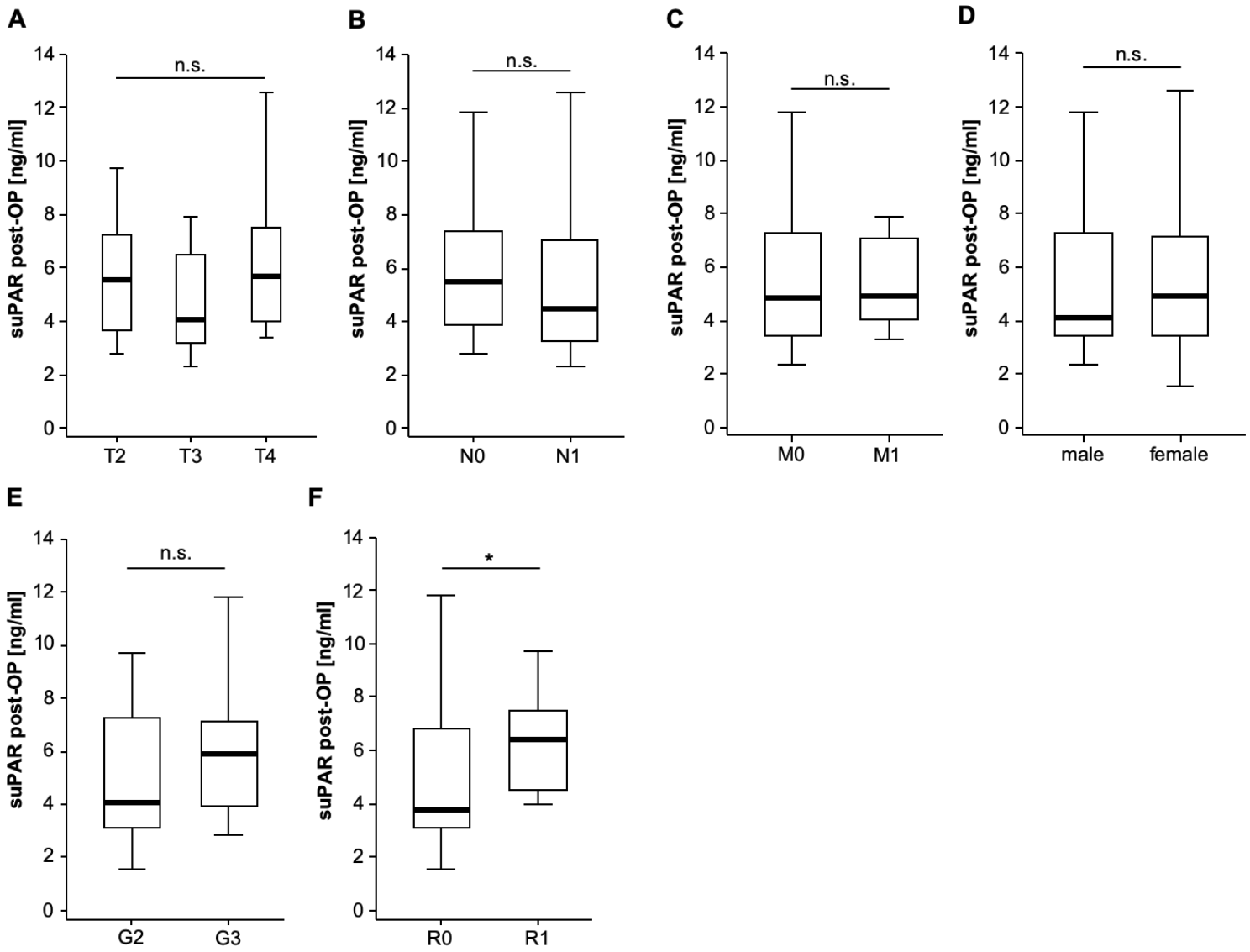

Fig. S3. Postoperative suPAR levels and patient characteristics
Postoperative suPAR serum levels are similar in patients with T_2 - to T_4-tumor stage (A), with or without lymph node involvement (B) as well as non-metastasized or metastasized patients who were still resectable (C). (E) Postoperative suPAR levels do not differ between male and female patients (D) and G2 or G3 differentiated patients. (F) Patients with R_1 resection status have significantly higher postoperative suPAR levels compared to R_0-resected patients.

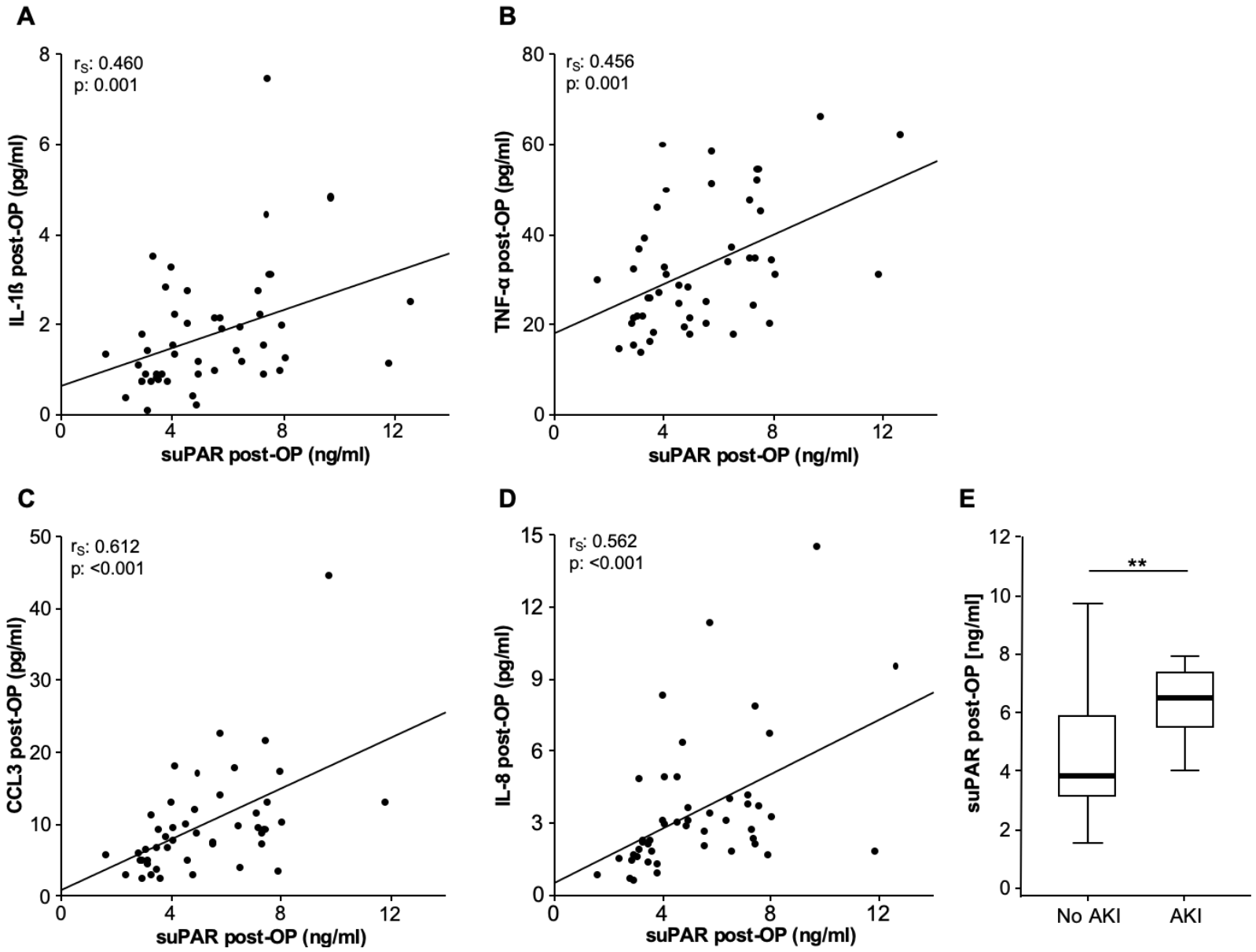

Fig. S4. Drivers of elevated postoperative suPAR levels

Postoperative suPAR serum levels positively correlate with pro-inflammatory cytokine levels such as IL-1ß (A), TNF-α (B), CCL3 (C) and IL-8 (D). (E) Postoperative suPAR levels are significantly higher in patients who presented with an impaired renal function after surgery (Supp. Figure 4E).