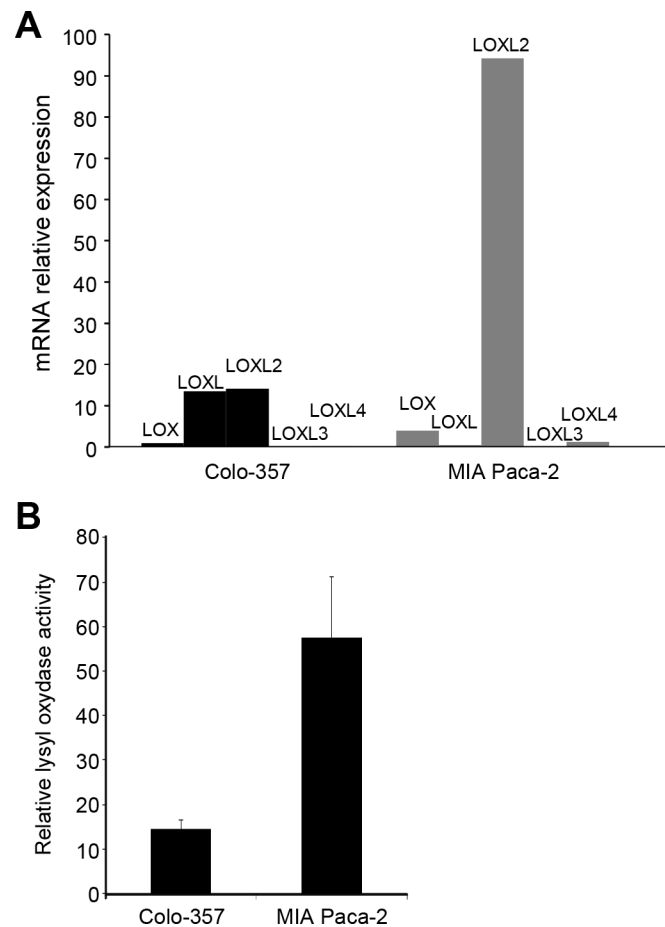
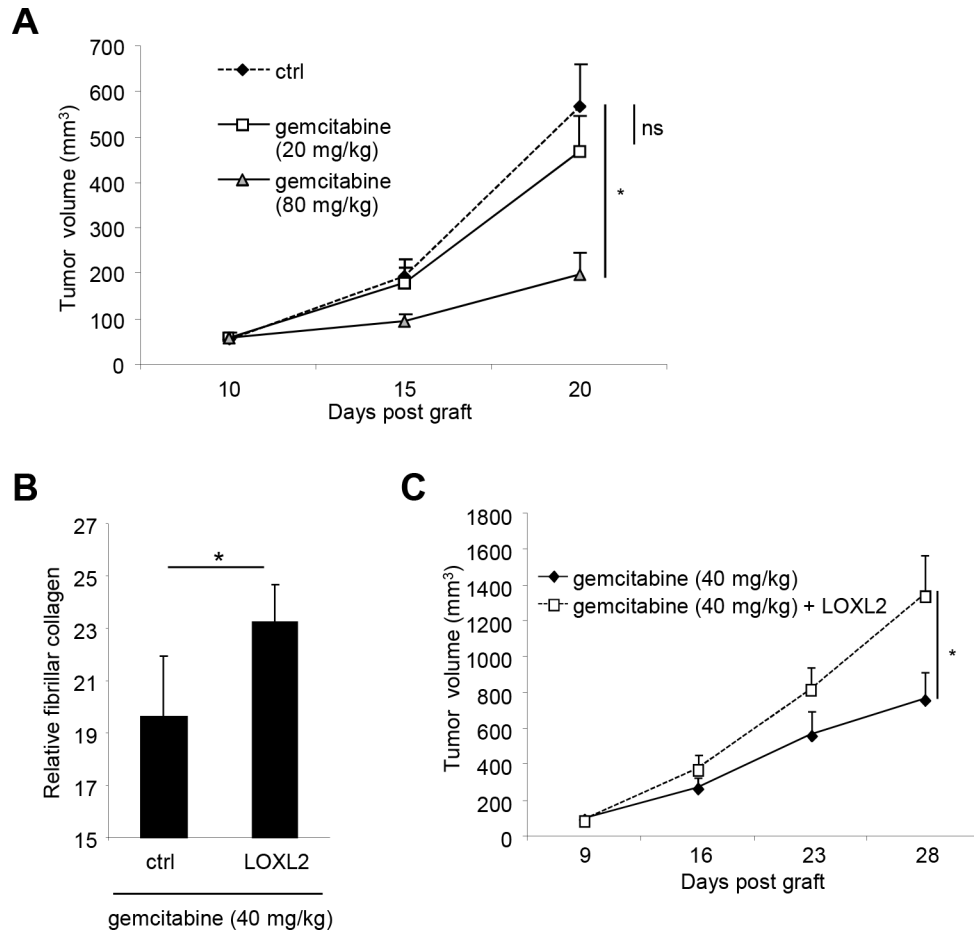


Lysyl oxidase family activity promotes resistance of pancreatic ductal adenocarcinoma to chemotherapy by limiting the intratumoral anticancer drug distribution

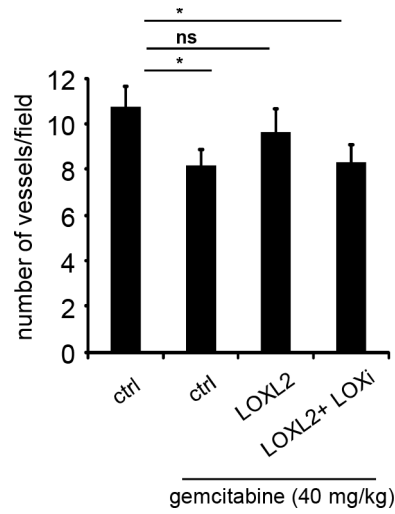
Supplementary Materials



Supplementary Figure S1: Expression and activity of LOX family members. (A) RNAs were extracted from the indicated PDAC-derived cell lines and LOX, LOXL1, LOXL2, LOXL3, and LOXL4 transcript levels were assayed by qRT-PCR and normalized against HPRT1. (B) Supernatant of the indicated cell lines were removed and LOX family members activity measured.



Supplementary Figure S2: Response to gemcitabine treatment depends of lysyl oxidase level activity. Five million Colo-357 pancreatic cancer cells per mouse were grafted into the right flanks of 6-week-old nude mice. The mice were subjected to the indicated treatment when the tumors reached a mean size of 50 mm³ (A) or of 100 mm³ (B–C). (A) Mice were intravenously injected every 3 days with the indicated dose of gemcitabine and tumor volumes were calculated ($n = 12$ per group). Values are means \pm SEM. (B–C) Mice were treated every two days by IV injection of 40 mg/kg gemcitabine, alone or in combination with 10 ng recombinant LOXL2 injected directly into the tumor ($n = 5$ per group). (B) Tissues were picosirius red stained and microscopically analyzed under polarized light to quantify cross-linked collagen. Relative levels of cross-linked collagen are presented as means \pm SEM. (C) Tumor volumes were calculated and means \pm SEM are presented. In each panel, values are means \pm SEM and the statistical test used was Mann-Whitney *U*.



Supplementary Figure S3: LOX family activity increases vessel number. One million Colo-357/Luc pancreatic cancer cells per mouse were orthotopically grafted into 6-weekold nude mice. The mice were left untreated or received IV injections of 40 mg/kg gemcitabine, alone or with IP-injected LOXL2 or LOXL2 + LOXi, when the tumors reached a mean luminescence of 800 cts/s ($n = 7$ per group). Sixty-three days after grafting, the tumors were processed for immunohistochemical CD31 staining. The mean number of vessels per field was calculated and statistical analysis was performed with the Mann-Whitney U test.

Supplementary Table S1: Patients' characteristics

Variable	No. of Patients	%
Gender		
Female	147	47.6
Male	162	52.4
Age, years		
Median	62.3	
range	37–87	
Mean	63.2	
SD	10.1	
Resection margins		
R0	239	77.3
R1	70	22.7
Tumor Differentiation		
Well		
Moderate	135	43.7
Poor	121	39.2
Unknown	40	12.9
Tumor Differentiation		
Well/moderate	256	82.8
Poor	40	17.2
Tumor stage		
T1/T2	57	18.4
T3	252	81.6
Lymph Nodes		
Negative, N0	78	25.2
Positive, N1	231	74.8
Tumor Stage		
I	7	2.3
IB	13	4.2
IIA	51	16.5
IIB	238	77.0
Perineural invasion		
Negative	94	30.4
Positive	213	69.6
Lymphovascular invasion		
Negative	125	40.5
Positive	184	59.5
Adjuvant therapy		
Yes	212	70.6
No	91	29.4
Type of adjuvant therapy		
Gemcitabine-based chemotherapy	181	85.4
Other	31	14.6

abbreviations: SD, standard deviation.

Supplementary Table S2: Univariate analysis in the subgroup of patients who received an adjuvant “Gemcitabine-based chemotherapy”

Variable	<i>n</i> = 181			<i>n</i> = 174		
	No. events	Median OS (months)	Log-Rank <i>p</i> -value	No. events	Median DFS (months)	Log-Rank <i>p</i> -value
Gender						
Female	45/89	33.1		53/86	15.5	
Male	46/92	33.6	0.720	63/89	14.7	0.611
Resection margins						
R0	61/133	35.1		79/130	16.8	
R1	30/48	20.5	< 0.001	37/45	11.3	< 0.001
Tumor Differentiation						
Well	36/78	35.1		46/77	18.6	
Moderate	37/70	32.7		47/68	14.3	
Poor	14/27	22.9	0.241	21/26	11.3	0.026
Tumor Differentiation						
Well/moderate	73/148	33.6		93/145	16.6	
Poor	14/27	22.9	0.182	21/26	11.3	0.020
Tumor stage						
T1/T2	16/25	33.6		17/25	15.1	
T3	75/156	33.3	0.642	99/150	12.6	0.820
Lymph Nodes						
Negative, N0	17/37	35.1		19/35	20.0	
Positive, N1	74/144	32.7	0.268	97/140	14.5	0.049
Perineural invasion						
Negative	28/61	34.3		35/60	18.4	
Positive	63/120	30.5	0.699	81/115	14.5	0.103
Lymphovascular invasion						
Negative	28/61	34.3		26/43	17.9	
Positive	63/120	31.8	0.160	89/130	14.1	0.138
Level of fibrillary collagen P50 (median)						
High (> P50)	46/89	26.9		56/84	12.8	
Low (< P50)	44/91	34.3	0.025	59/90	16.9	0.035