cancers

MDPI

Supplementary Materials: Combined De-Repression of Chemoresistance Associated Mitogen-Activated Protein Kinase 14 and Activating Transcription Factor 2 by Loss of microRNA-622 in Hepatocellular Carcinoma

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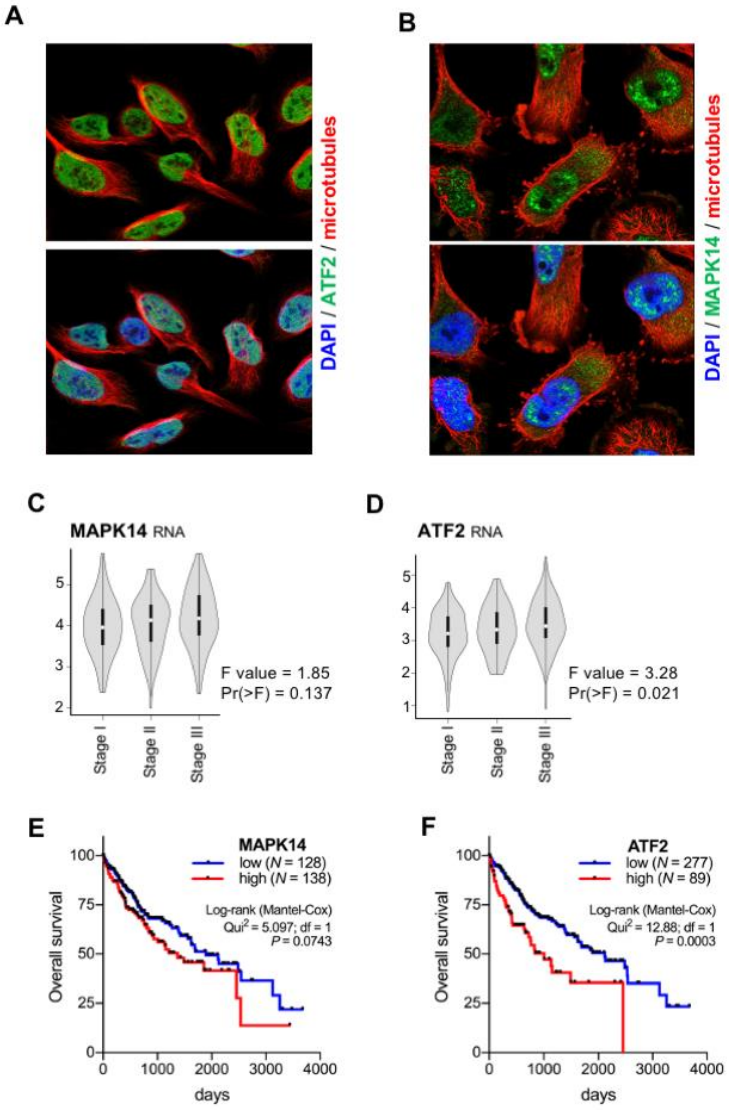


Figure S1. Expression patterns of MAPK14 and ATF2 and correlation with tumor stages and survival in HCC. (**A**,**B**) Immunofluorescence image confirming exclusive nuclear localization of ATF2 (**A**) also in non-HCC cancer cell lines (e.g., the glioblastoma cell line "U-251 MG") (40-fold magnification). (**B**) Immunofluorescence image revealing cellular MAPK14 expression patterns (both cytoplasmatic and nuclear) in non-HCC cancer cell lines (e.g., the glioblastoma cell line "U-251 MG") (40-fold magnification). (**C**,**D**) MAPK14 (**C**) and ATF2 (**D**) RNA expression (log_2(TPM)) different HCC tumor stages (I–III). TCGA-derived data were used applying the Gene Expression Profiling Interactive Analysis (GEPIA) database. (**E**,**F**) "The Cancer Genome Atlas" (TCGA) derived dataset provided by the ProteinAtlas database was used for overall survival analysis comparing high and low MAPK14 (**E**) and ATF2 (**F**) levels, respectively, in HCC patients. Survival analysis was performed computationally applying log-rank testing (Mantel-Cox) (**E**,**F**).

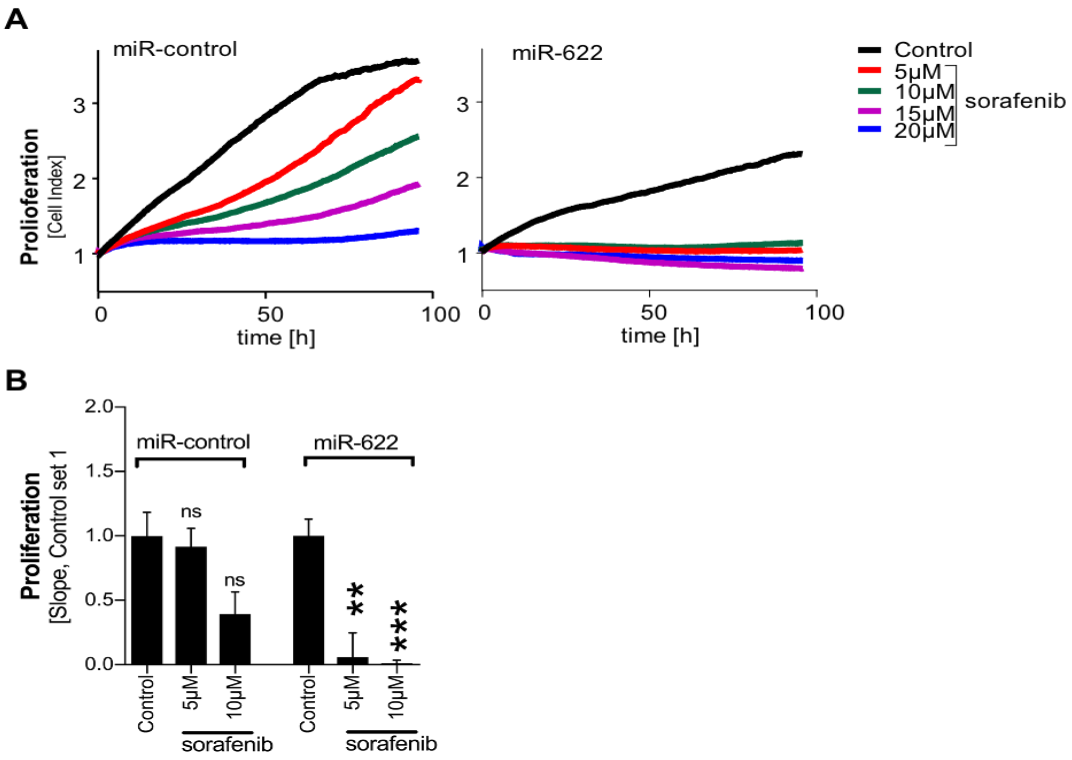
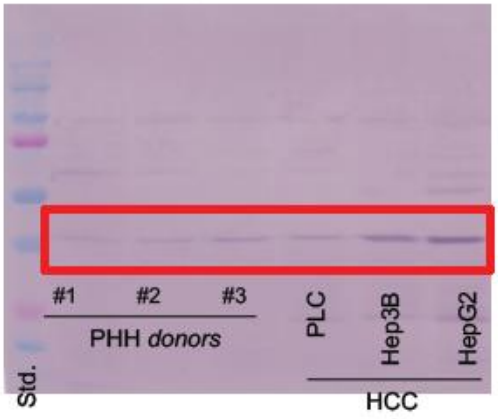


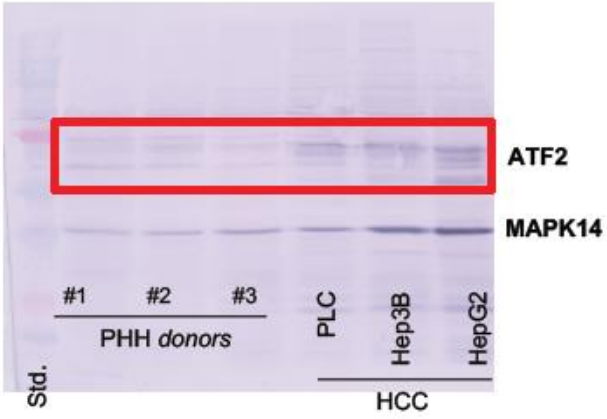
Figure S2. Real-time cell proliferation analysis (RTCA) of sorafenib-resistant HCC cells. (**A**,**B**) Real-time cell proliferation analyis (RTCA) of sorafenib-resistant HCC cells (PLC) treated with different doses of sorafenib (0, 5, 10, 15, 20µM), with (miR-622) or without (miR-control) prior transfection of miR-622. (A) reveals representative proliferation curves. (B) summarizes proliferation (calculated by the mean "slope" of the proliferation curves) ($n = 4$). Data are presented as the mean ± SEM. Statistical significance was determined by 2-tailed, unpaired t-test (**B**). $^{**}P < 0.01$ vs Control, $^{***}P < 0.001$ vs Control, ns: non-significant vs Control.

Uncropped Western blots

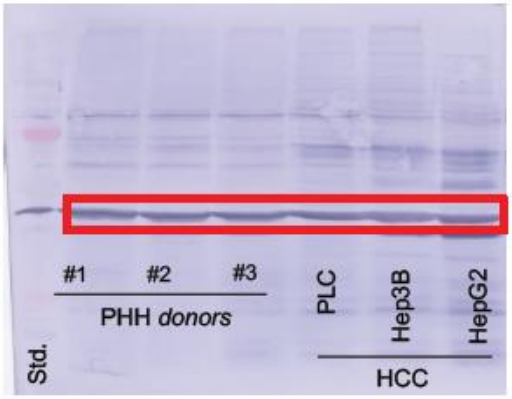
MAPK14 staining



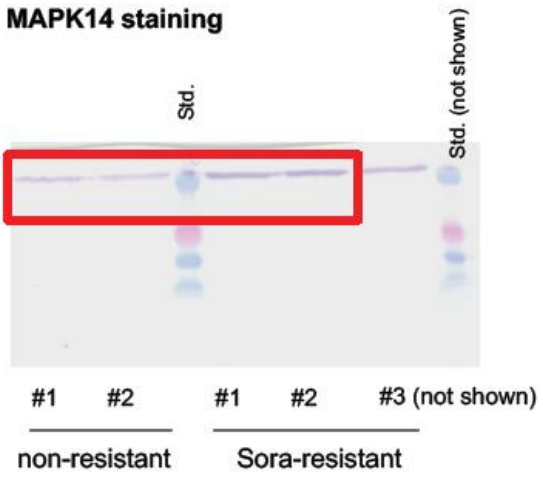
ATF2 staining

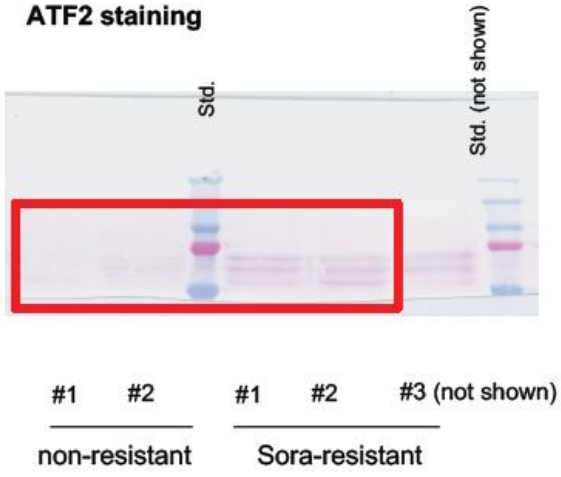


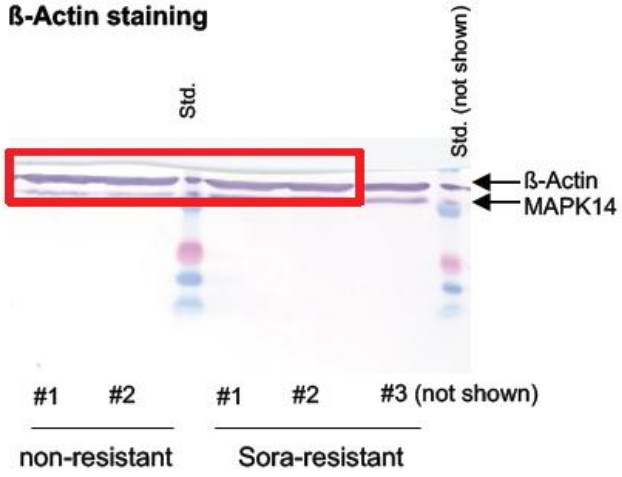
ß-Actin staining



Uncropped Western blot

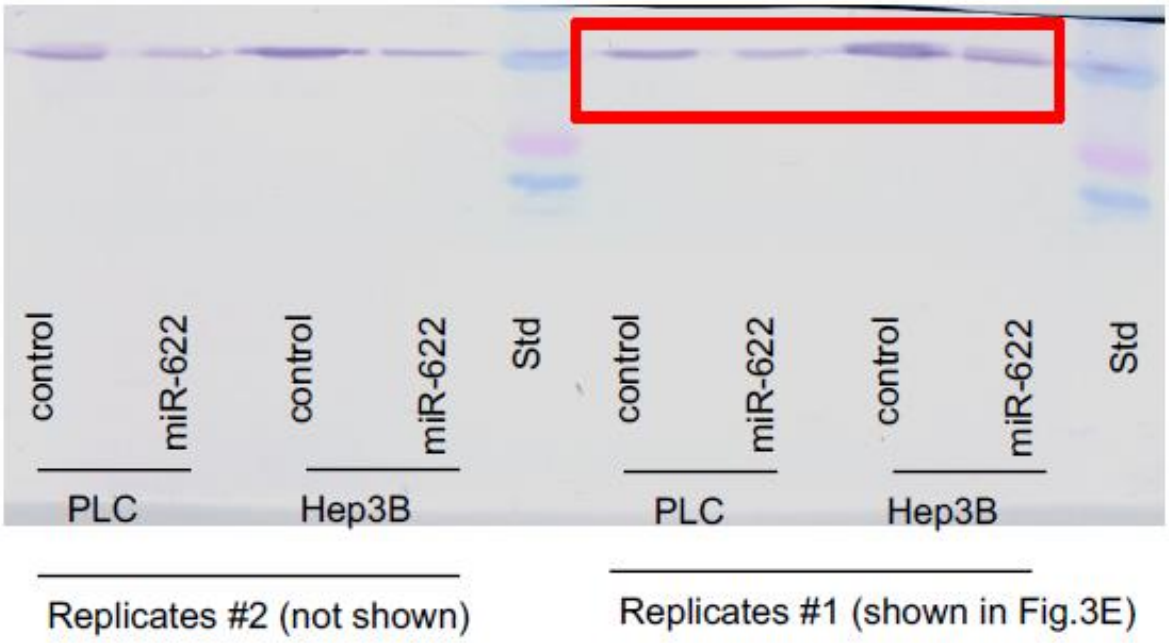




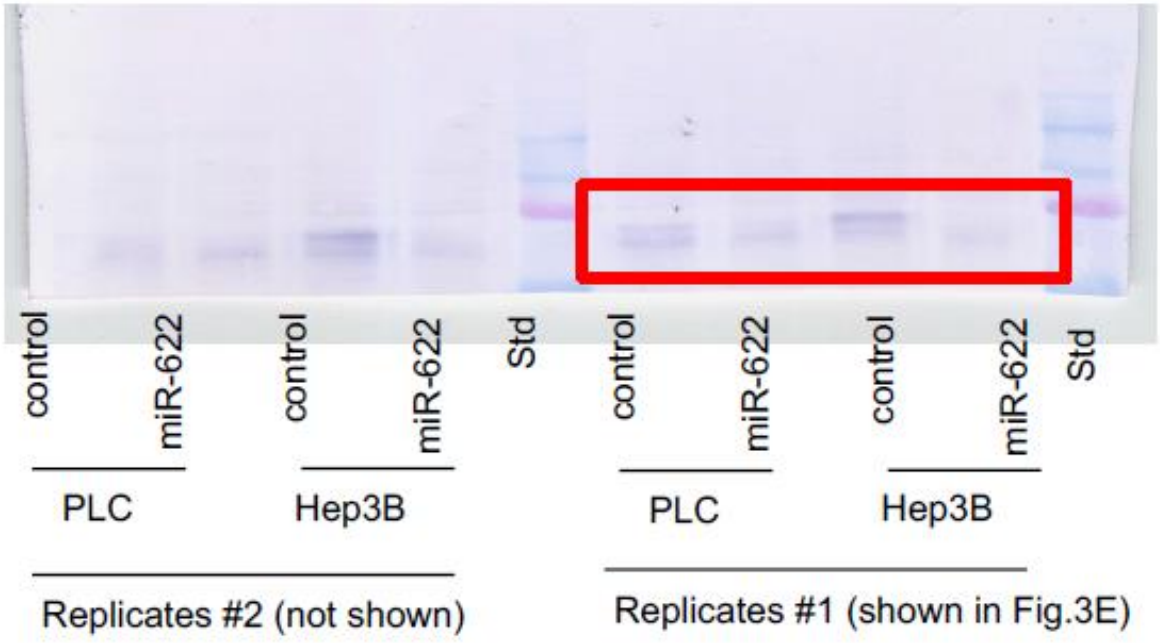


Uncropped Western blot

MAPK14 staining



ATF2 staining



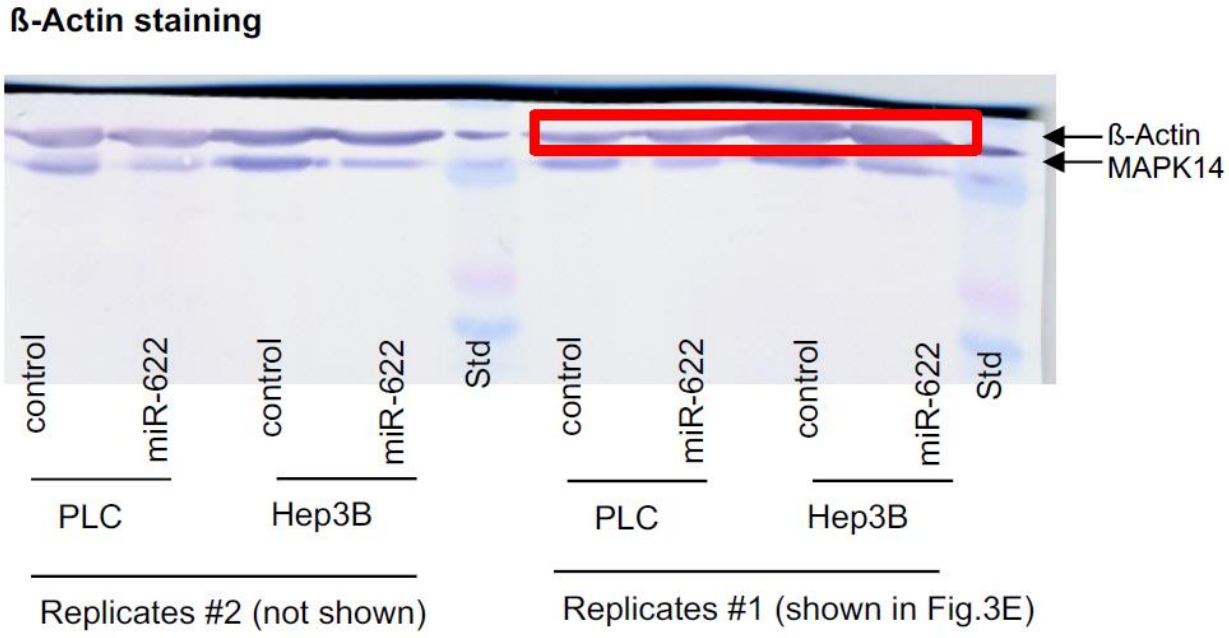


Figure S3. The uncropped Western blots.

Table S1. Gene expression in paired peri-HCC vs. HCC patient tissues.

Gene Name	IR-Score	*n* (%)	peri-HCC	HCC	*P* *	*R* ** *P* **
MAPK14	negative/low (0)	43 (46.7)	26	17	**0.006**	0.300 **0.004**
	moderate (1)	34 (36.9)	16	18		
	strong (2)	15 (16.3)	2	13		
ATF2	negative/low (0)	28 (32.2)	17	11	**0.028**	0.030 **0.029**
	moderate (1)	39 (44.8)	22	17		
	strong (2)	20 (23.0)	5	15		

* Fisher's exact test (two-sided); bold face representing *P*-values <0.05. **Spearman correlation (*R*) and according *P*-value (*P*). IR, immunoreactivity. Y5R, NPY, TGFβ and DPP4 staining scores were analyzed qualitatively according to both staining intensity (describing "negative/low" ("0"), "moderate" ("1"), or "strong" ("2")) and percentage of positive cells ("0": <5 %; "1": 5-20%; "2": 20-40%, "3":>40% positive cells).

Table S2. Clinicopathological characteristics and MAPK14 immunoreactivity in human HCC tissues.

Clinico-Pathological Characteristic	Categorisation	MAPK14 IR–Tumor Site					
		n (%)	Negative/Low (0)	Moderate (1)	Strong (3)	*P**	*R* ** *P* **
Age at diagnosis	<60 years	12 (25.0)	6	4	2	0.492	0.185
	≥60 years	36 (75.0)	11	14	11		0.224
Gender	Female	4 (8.30)	0	3	1	0.238	−0.136
	Male	44 (91.7)	17	15	12		0.398
Fibrosis (Desmet score)	1	7 (14.9)	3	2	2	0.786	−0.153
	2	12 (25.5)	2	5	5		0.303
	3	22 (46.8)	8	9	5		
	4	6 (12.8)	3	2	1		
Aetiology	alcohol						
	non-alcohol (NAFLD, HBV, HCV)	12 (25.0)	3	6	3	0.645	0.067
		36 (75.0)	14	12	10		0.610
Histological grade	G1	18 (36.5)	9	7	2	**0.047**	0.403
	G2	22 (45.8)	8	8	6		**0.005**
	G3	8 (17.7)	0	3	5		
Tumor size	<5 cm	29 (63.0)	8	11	10	0.325	−0.222
	≥5 cm	17 (37.0)	8	6	3		0.167
ATF2 IR (tumor site)	negative/low (0)	11 (25.6)	8	3	0	**<0.0001**	0.75
	moderate (1)	17 (39.5)	8	9	0		**<0.0001**
	strong (2)	15 (34.9)	0	5	10		

* Fisher's exact test (two-sided); bold face representing *P*-values <0.05. ** Spearman correlation (R) and according *P*-value (P). IR, immunoreactivity; ND, not determined. MAPK14 and ATF2 staining scores were analyzed qualitatively according to both staining intensity (describing "negative/low" ("0"), "moderate" ("1"), or "strong" ("2")) and percentage of positive cells ("0": <5 %; "1": 5-20%; "2": 20-40%, "3":>40% positive cells). The Ki-67 staining score was analyzed qualitatively according to both staining intensity (describing "negative" ("0"), "low" ("1"), "moderate" ("2"), or "strong" ("3")) and percentage of positive cells ("0": <5 %; "1": 5-20%; "2": 20-40%, "3":>40% positive cells). Fibrosis was determined applying the "Desmet" Score System. Ns: non-significant.

Table S3. Clinicopathological characteristics and ATF2 immunoreactivity in human HCC tissues.

Clinico-Pathological Characteristic	Categorisation	ATF2 IR–Tumor Site					
		***n* (%)**	**Negative/Low (0)**	**Moderate (1)**	**Strong (3)**	***P****	***R**** ***P****
Age at diagnosis	<60 years	11 (25.6)	2	5	4	0.907	−0.064
	≥60 years	32 (74.4)	9	12	11		0.803
Gender	Female	4 (9.30)	0	1	3	0.347	−0.275
	Male	39 (90.7)	11	16	12		0.099
Fibrosis (Desmet score)	1	7 (16.7)	2	3	2	0.966	−0.060
	2	9 (21.4)	2	3	4		0.703
	3	20 (47.6)	5	7	8		
	4	6 (14.3)	2	3	1		
Aetiology	alcohol	11 (25.6)	2	4	5	0.745	0.137
	non-alcohol (NAFLD, HBV, HCV)	32 (74.4)	9	13	10		0.455
Histological grade	G1	17 (39.5)	6	9	2	**0.046**	0.411
	G2	18 (41.9)	4	7	7		**0.007**
	G3	8 (18.6)	1	1	6		
Tumor size	<5 cm	24 (58.5)	5	8	11	0.373	−0.203
	≥5 cm	17 (41.5)	5	8	4		0.189
MAPK14 IR (tumor site)	negative/low (0)	16 (37.2)	8	8	0	**<0.0001**	0.75
	moderate (1)	17 (39.5)	3	9	5		**<0.0001**
	strong (2)	10 (23.3)	0	0	10		

* Fisher's exact test (two-sided); bold face representing *P*-values <0.05. **Spearman correlation (R) and according *P*-value (P). IR, immunoreactivity; ND, not determined. MAPK14 and ATF2 staining scores were analyzed qualitatively according to both staining intensity (describing "negative/low" ("0"), "moderate" ("1"), or "strong" ("2")) and percentage of positive cells ("0": <5 %; "1": 5-20%; "2": 20-40%, "3":>40% positive cells). The Ki-67 staining score was analyzed qualitatively according to both staining intensity (describing "negative" ("0"), "low" ("1"), "moderate" ("2"), or "strong" ("3")) and percentage of positive cells ("0": <5 %; "1": 5-20%; "2": 20-40%, "3":>40% positive cells). Fibrosis was determined applying the "Desmet" Score System. Ns: non-significant.