Tissue and plasma levels of galectins in patients with high grade serous ovarian carcinoma as new predictive biomarkers

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Supplementary File

Supplementary Table I

Supplementary table I: Patients characteristics (multi-subtype tissue microarray (n=63))

	Clear cell (n=12)	Endometrioid (n=21)	Mucinous (n=14)	Serous (n=16)
Age range (mean)	47-73 (57)	43-80 (59)	28-82 (54)	33-79 (57)
Grade				
Borderline	0	0	12	3
I (Low)	0	11	1	2
II	4	5	1	5
III (High)	8	5	0	6
Stage				
I	6	13	11	2
II	2	1	1	3
III	4	6	2	10
IV	0	1	0	1

Supplementary Table II

Supplementary table II: Patients characteristics (HGSC tissue microarray (n=209))

Age range (median)	36-89 (64.5)
Figo Stage	
I	13
II	23
III	148
IV	25
CA-125 range (median)	5-13763 (667)
Residual disease	
No residual disease	40
≤ 1 cm	51
>1 cm ≤ 2 cm	30
> 2 cm	50
Unknown	38
Chemotherapy	
Paclitaxel/carboplatin	147
Other	60
Unknown	2
Reccurence	
Yes	172
No	37
Mortality	
Yes	140
No	69

Supplementary Table III

Supplementary Table III: Correlation between the patients characteristics and galectins expression in cancer cells and stroma of HGSC.

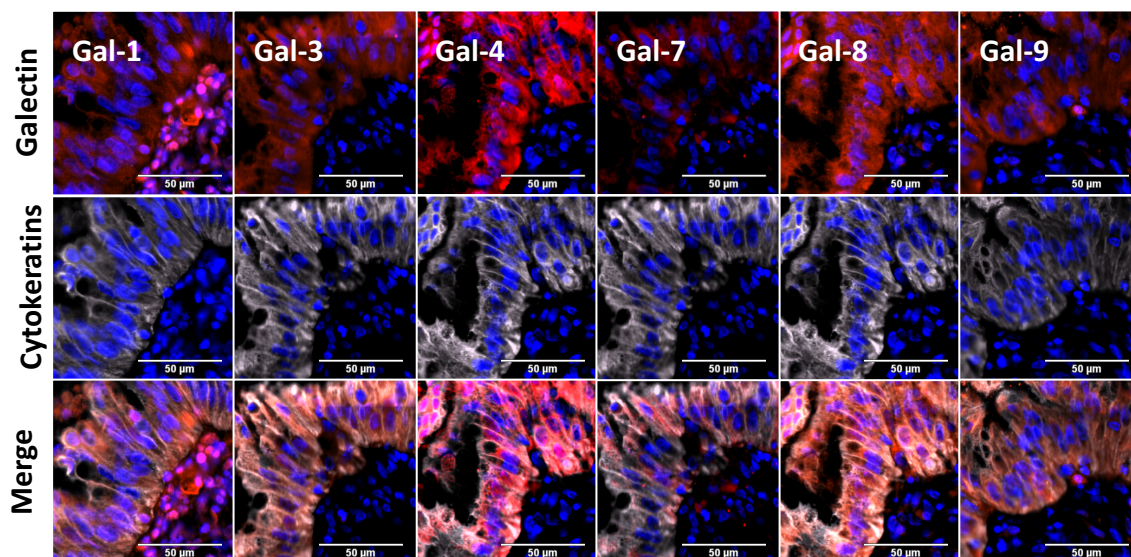|	Gal-1 Low	Gal-1 High	Gal-3 Low	Gal-3 High	Gal-7 Low	Gal-7 High	Gal-8 Low	Gal-8 High	Gal-9 Low	Gal-9 High
Cancer cells										
Age										
Mean	61	62	64	59	59	63	63	61	62	61
Figo Stage	$P = 0.056$									
Low (I-II)	29 (14)	13 (6)	13 (7)	20 (11)	13 (7)	20 (11)	17 (9)	17 (9)	16 (8)	18 (9)
High (III-IV)	84 (41)	**77 (38)**	58 (31)	98 (52)	71 (38)	85 (45)	67 (34)	98 (49)	82 (43)	74 (39)
CA-125	$P = 0.016$									
Median	453.36	**809.5**	587.9	697.44	765.5	559.5	530.32	618	553	697.44
Residual disease										
No	21 (13)	15 (9)	12 (8)	25 (16)	17 (11)	20 (13)	18 (11)	20 (12)	21 (13)	16 (10)
Yes	63 (40)	59 (37)	50 (32)	69 (44)	51 (33)	68 (44)	58 (35)	70 (42)	60 (38)	60 (38)
Recurrence	$P = 0.092$						$P = 0.065$			
No	23 (12)	12 (6)	15 (8)	20 (11)	18 (10)	17 (9)	21 (11)	16 (8)	21 (11)	14 (8)
Yes	75 (40)	**78 (41)**	56 (30)	94 (51)	63 (34)	87 (47)	62 (32)	**96 (49)**	76 (41)	75 (40)
Death										
No	49 (28)	36 (21)	30 (16)	56 (30)	41 (22)	45 (24)	36 (18)	53 (27)	47 (25)	38 (20)
Yes	53 (31)	34 (20)	41 (22)	62 (33)	43 (23)	60 (32)	48 (24)	62 (31)	51 (27)	54 (28)
Stroma										
Age										
Mean	61	61	60	62	61	1	61	61	61	62
Figo Stage										
Low (I-II)	13 (7)	19 (10)	16 (9)	16 (9)	31 (17)	1 (1)	22 (12)	11 (6)	10 (5)	23 (13)
High (III-IV)	49 (26)	111 (58)	92 (49)	63 (34)	141 (75)	14 (7)	109 (58)	47 (25)	51 (28)	100 (54)
CA-125	$P = 0.125$								$P = 0.074$	
Median	453.4	793	548.5	828.5	589.5	769.5	618	587.9	510	**767.5**
Residual desease										
No	10 (6)	26 (16)	20 (13)	17 (11)	35 (23)	2 (1)	23 (15)	15 (9)	13 (9)	23 (15)
Yes	42 (27)	80 (51)	72 (47)	45 (29)	107 (69)	10 (6)	85 (54)	35 (22)	34 (23)	81(54)
Recurrence	$P = 0.008$									
No	18 (10)	17 (6)	19 (10)	16 (9)	31 (17)	4 (2)	24 (13)	11 (6)	11 (6)	23 (13)
Yes	41 (22)	112 **(60)**	86 (47)	62 (34)	137 (75)	11 (6)	103 (56)	47 (25)	46 (26)	100 (56)
Death								$P = 0.027$		
No	31 (16)	54 (28)	46 (25)	38 (20)	77 (41)	7 (4)	53 (28)	**78 (41)**	24 (13)	58 (32)
Yes	31 (16)	76 (40)	62 (33)	41 (22)	95 (51)	8 (4)	34 (18)	24 (13)	37 (20)	65 (35)

Supplementary Table IV

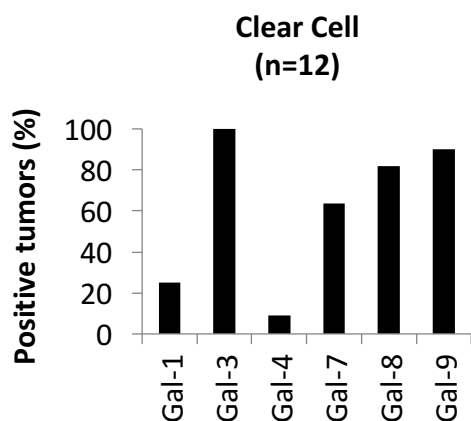**Supplementary table IV:** Correlation between the presence of galectins in the tumor and the plasma.

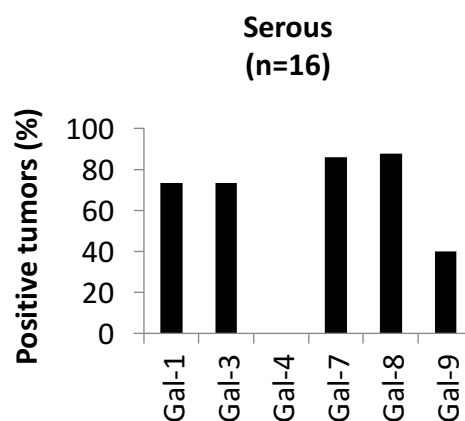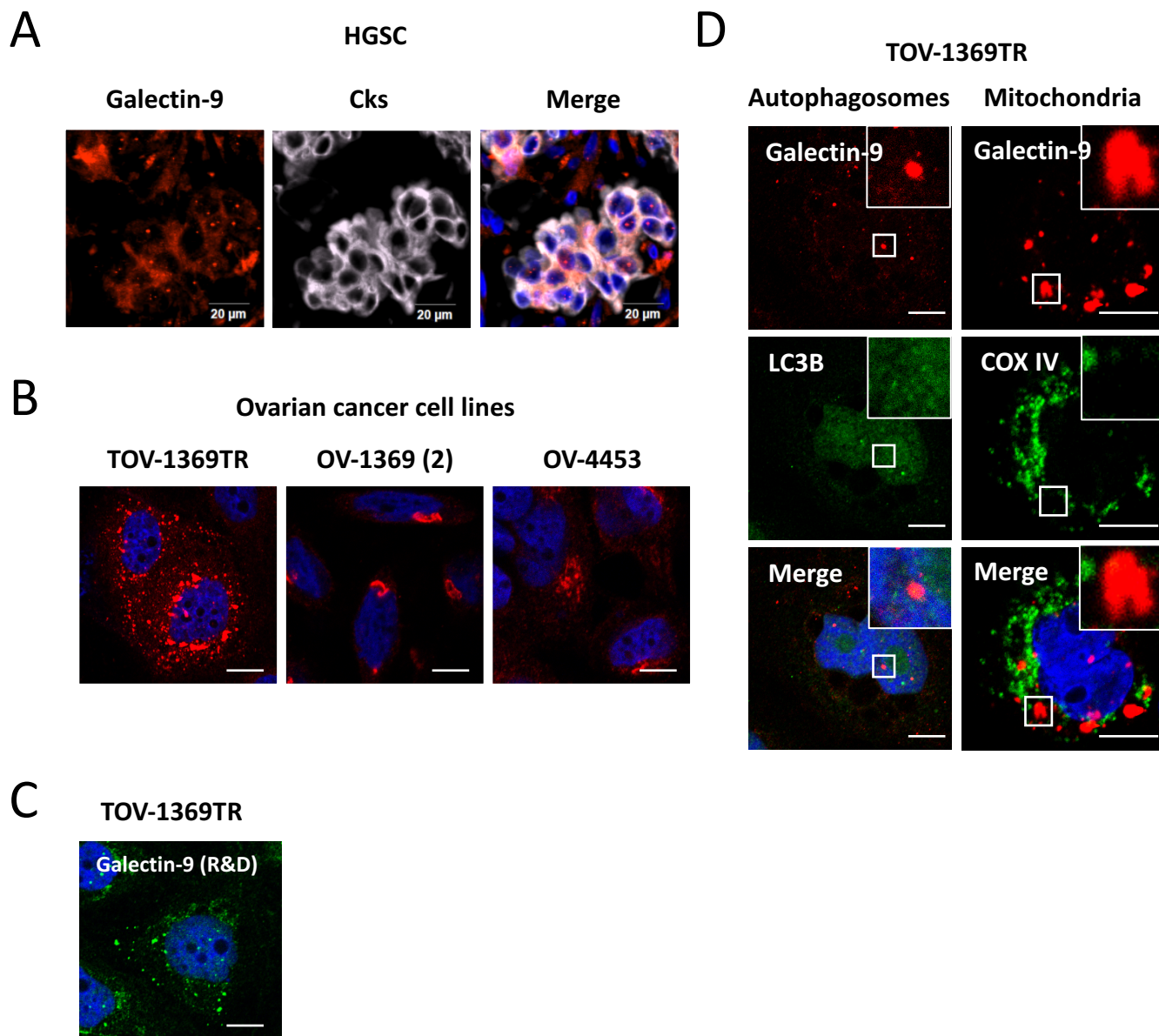|			Epithelium		Stroma	
			Negative	Positive	Negative	Positive
Galectin-8			P = 0.280		P = 0.007	
ELISA	Negative	n	28	54	46	33
		%	34.1	65.9	58.2	41.8
	Positive	n	24	30	41	9
		%	44.4	55.6	82	18
Galectin-9			P = 0.152		P = 0.689	
ELISA	Negative	n	34	21	17	36
		%	61.8	38.2	32.1	67.9
	Positive	n	35	38	19	50
		%	47.9	52.1	27.5	72.5

Supplementary figure 1

Supplementary figure 1: Galectins expression in different histological subtypes of ovarian cancer. (A) Positive IHF staining of gal-1, -3, -4, -7 -8 and -9 in a single mucinous tumor. Cytokeratin staining was used to identify epithelial cancer cells. Bar represent 50 µm. Percentage of positive tumors displaying expression of gal-1, -3, -4, -7, -8 and -9 in (B) clear cell, (C) endometrioid, (D) mucinous and (E) serous ovarian tumors.
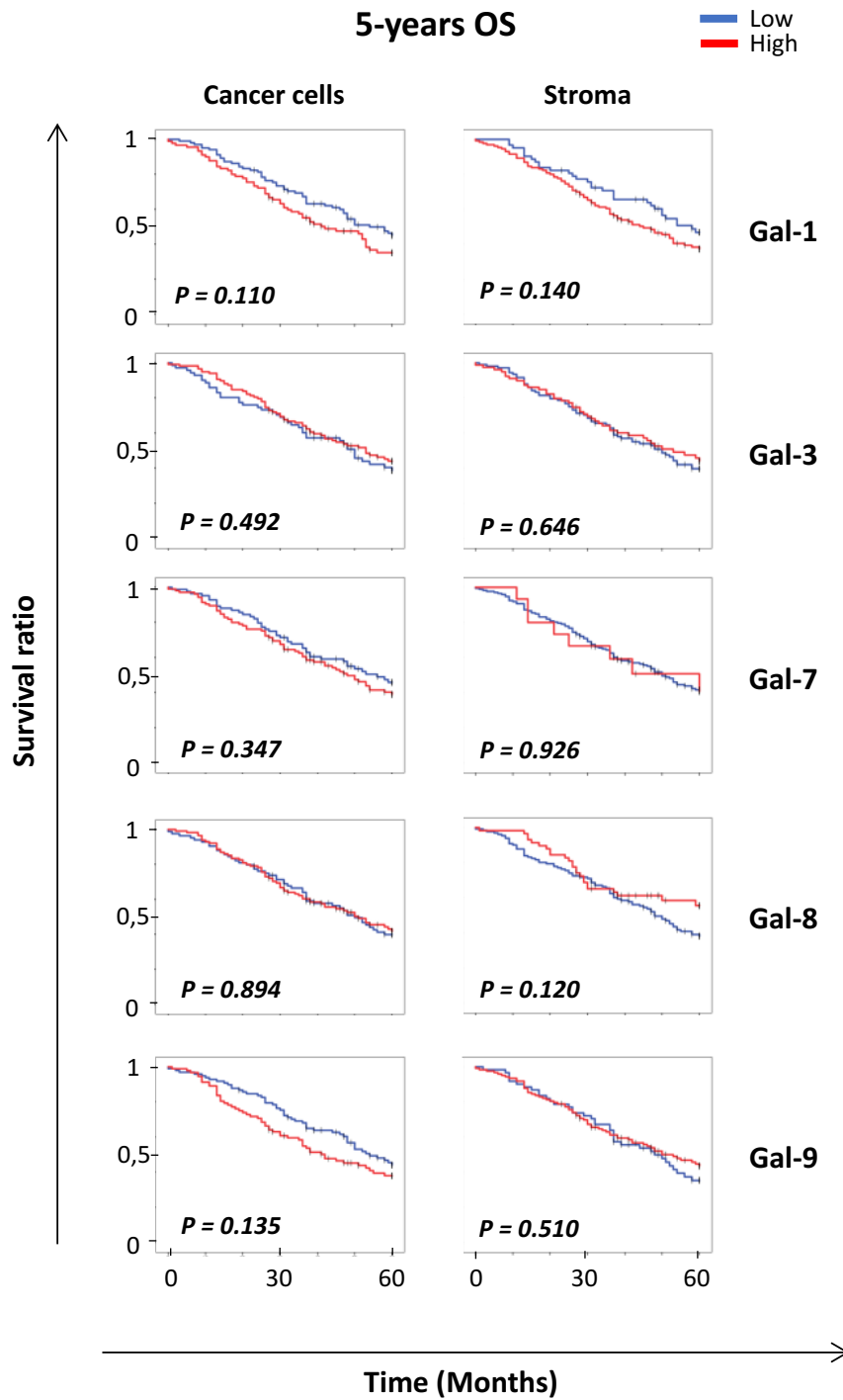
Supplementary figure 2

A

HGSC

Galectin-9 | Cks | Merge

B

Ovarian cancer cell lines

TOV-1369TR | OV-1369 (2) | OV-4453

C

TOV-1369TR

D

TOV-1369TR

Autophagosomes | Mitochondria

Supplementary figure 2: Gal-9P in ovarian cancer cells. (A) IHF staining of gal-9P in HGSC. Cytokeratin staining was used to identify epithelial cancer cells. Bar represent 20 μm. (B) IF staining of gal-9 (red) was detected by confocal imagery in Tov-1369TR, Tov-1369(2) and OV-4453 ovarian cancer cell lines. Nuclei were stained with DAPI (blue). (C) IF staining of gal-9 (red) was detected by confocal imagery in Tov-1369TR with a goat anti-gal-9 antibody (R&D). (D) IF staining of gal-9 (red), LC3B (green, left panel) and COX IV (green, right panel) in TOV-1369TR cells. LC3B and Cox IV antibodies were used to stain autophagosome and mitochondria, respectively. Nuclei were stained with DAPI (blue). Bars represent 10 μm.
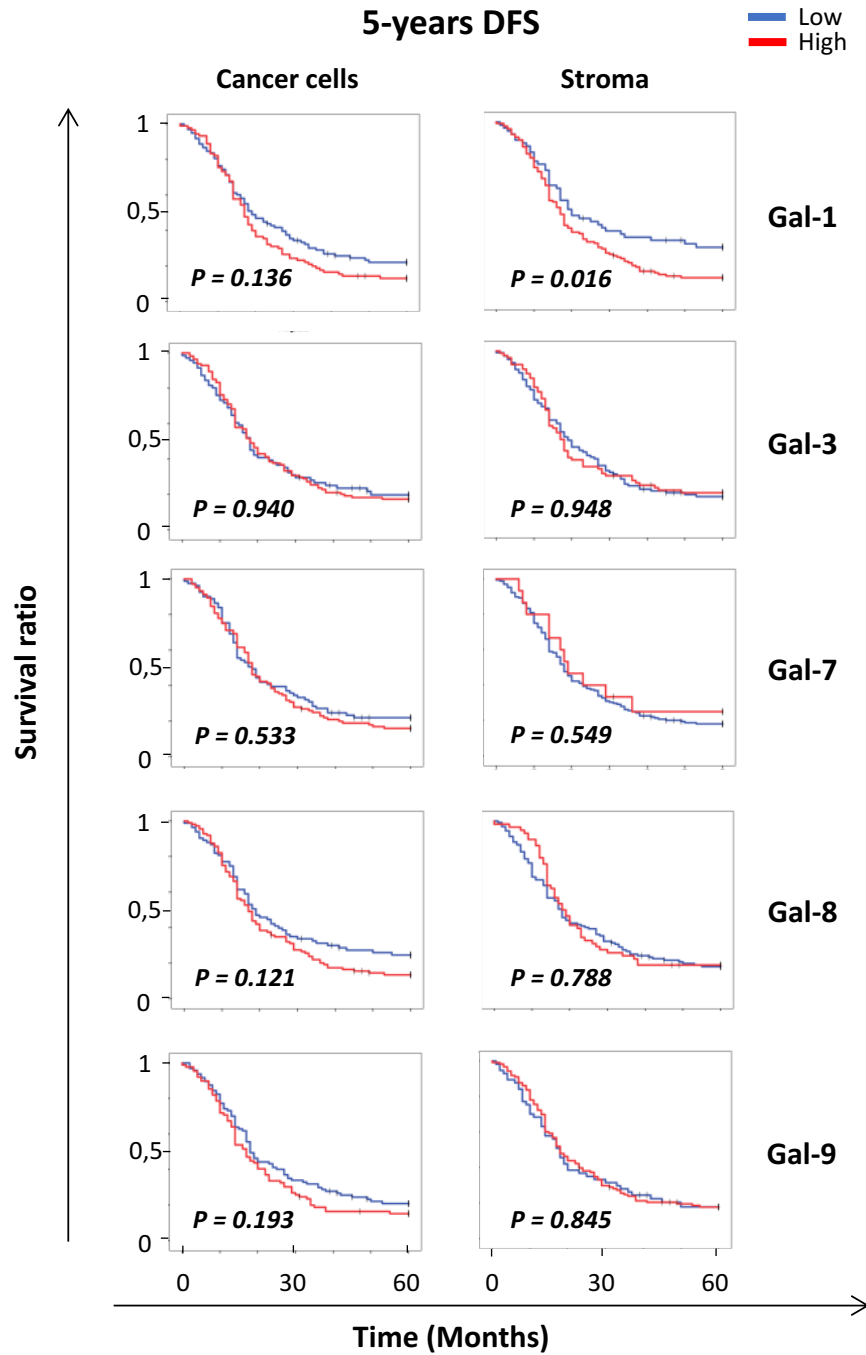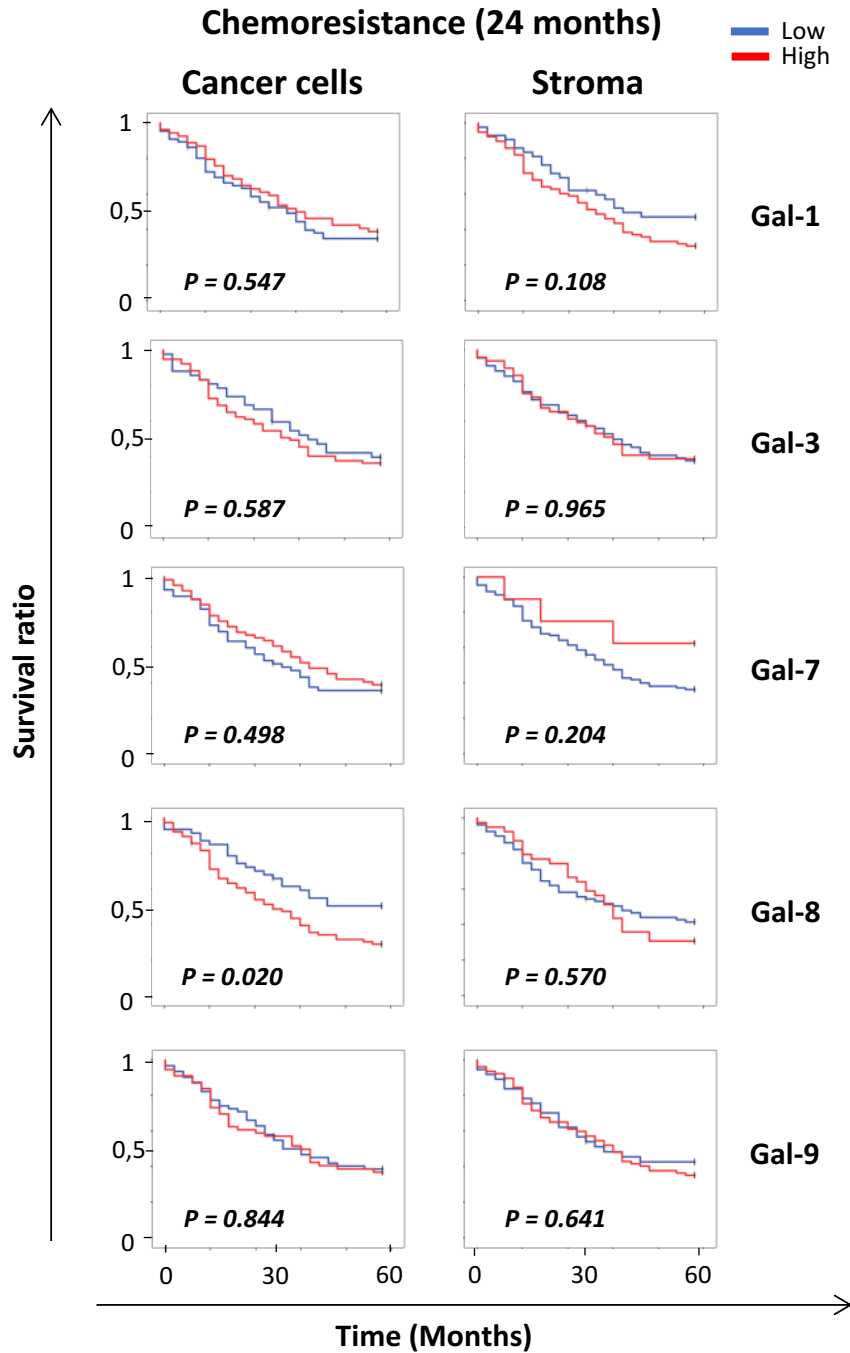
Supplementary figure 3

A

Supplementary figure 3

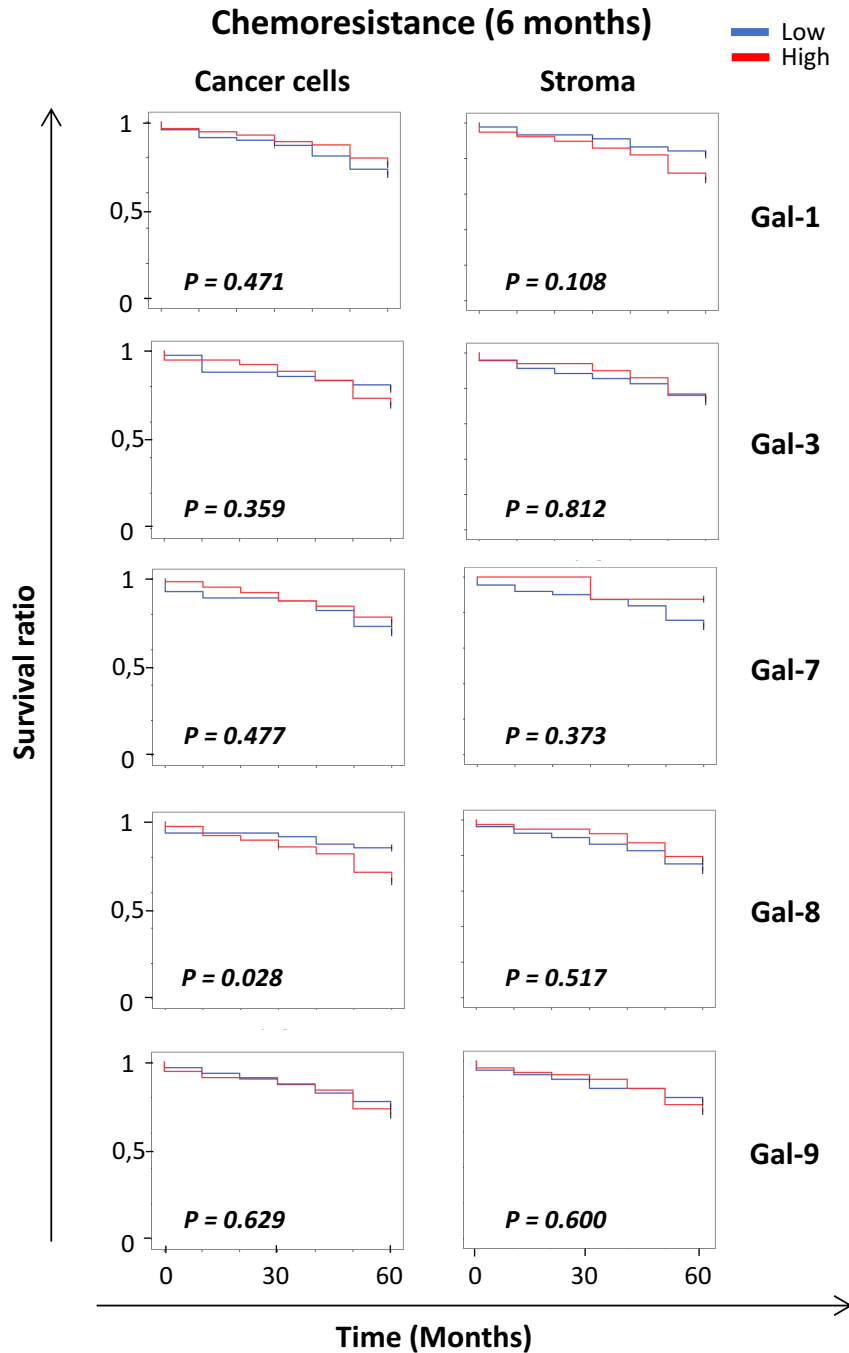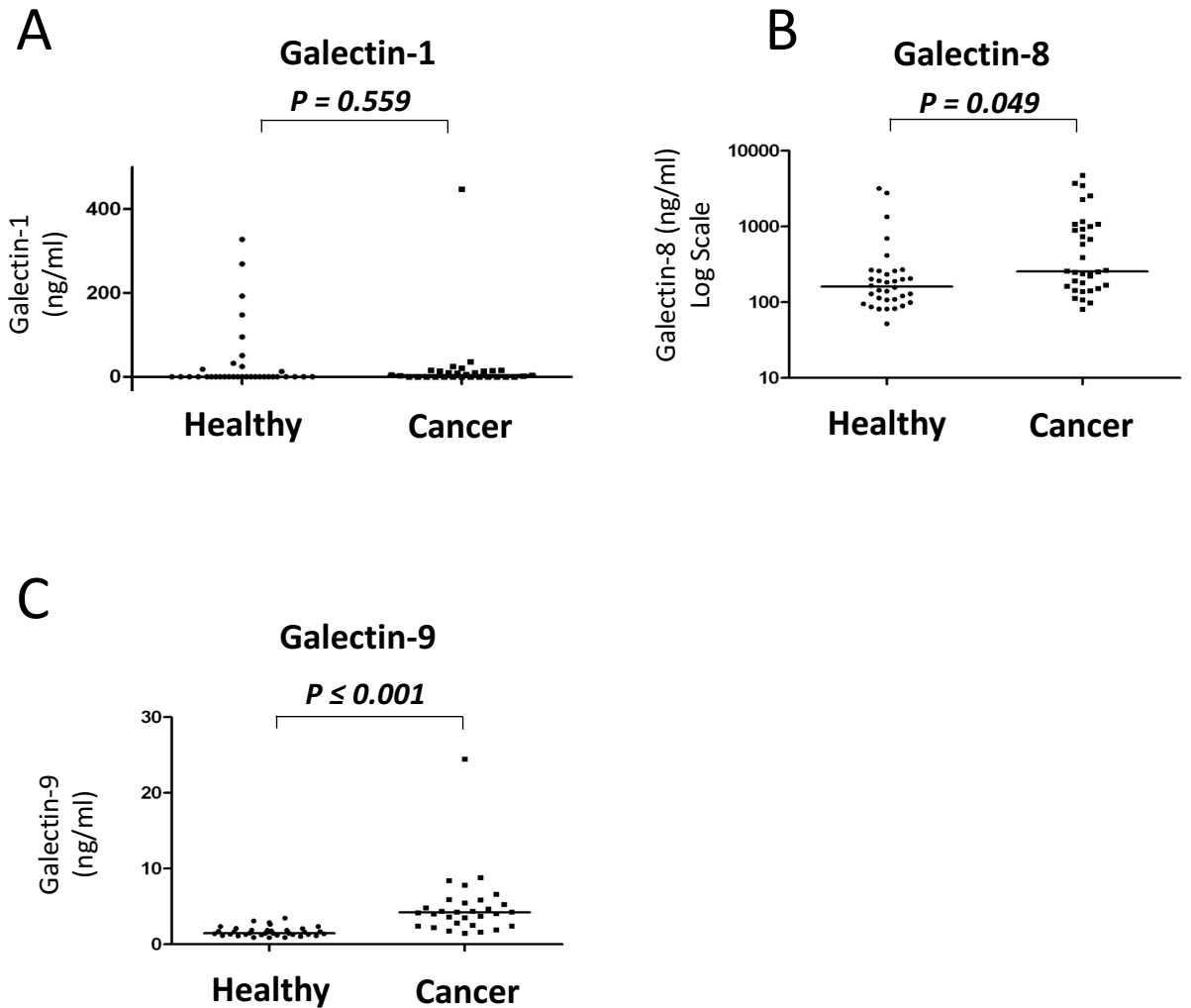

B

5-years DFS

Supplementary figure 3

Supplementary figure 3

Supplementary figure 3: Galectins predictive value of OS, DFS and chemoresistance. Kaplan-Meier curves of (A) 5-years OS, (B) 5-years DFS and chemoresistance analyzed at (C) 24 months and (D) 6 months post-treatment according to epithelial or stromal presence of gal-1, -3, -7, -8 and -9. Blue bar: galectin negative tumors, red bar: galectin positive tumors.

Supplementary figure 4

A

Galectin-1

P = 0.559

B

Galectin-8

P = 0.049

C

Galectin-9

P ≤ 0.001

Supplementary figure 4: Galectins concentration in the plasma of healthy donors and ovarian cancer patients. Levels of circulating gal-1, gal-8 and gal-9 in 35 healthy donors and 35 cancer patients.