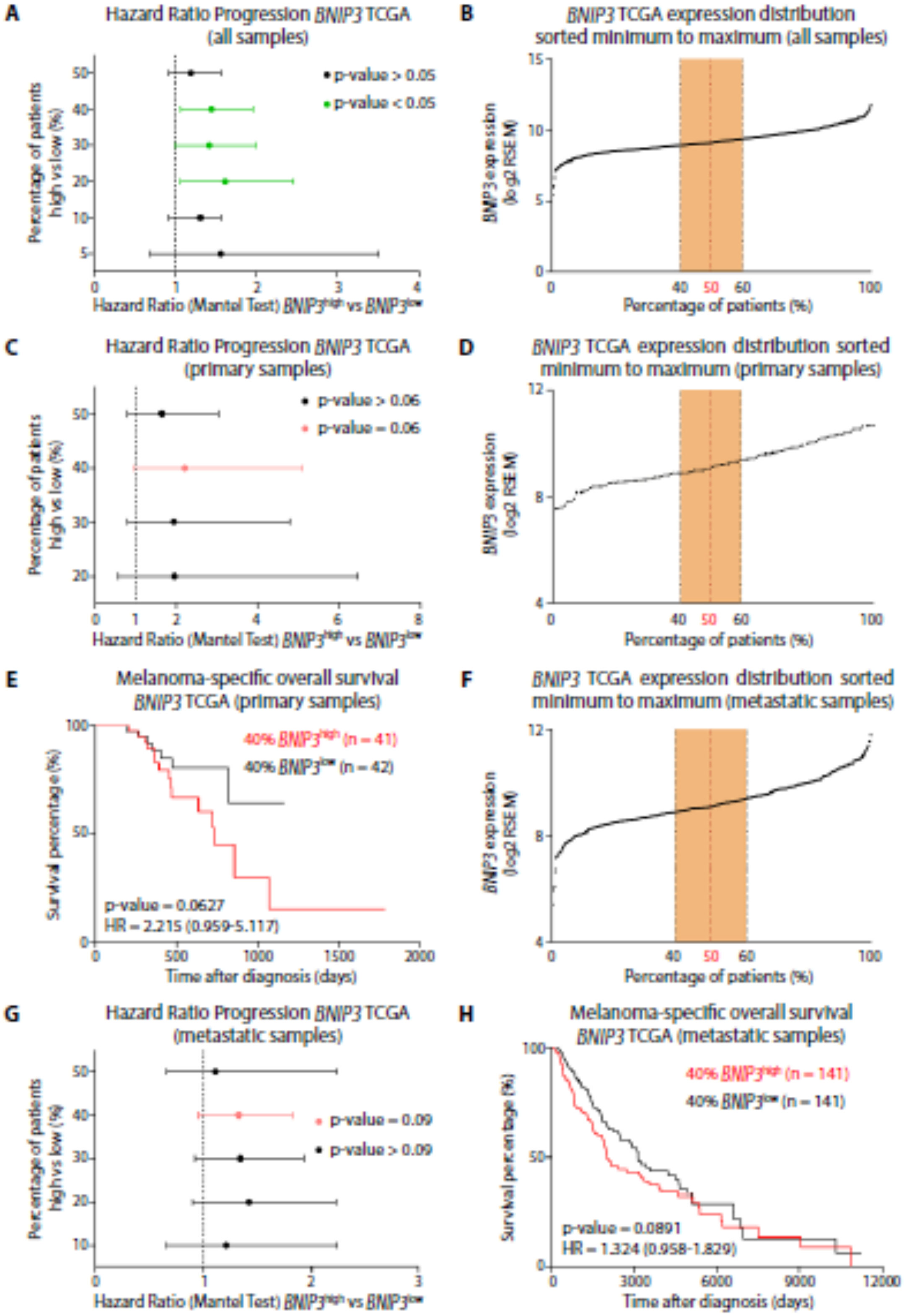
Appendix of the manuscript: **BNIP3 promotes HIF-1α-driven melanoma growth by curbing intracellular iron homeostasis** by Vara-Perez M. *et al*.

Appendix Figures S1-3
Appendix Tables S1-5
References


A
Hazard Ratio Progression BNIP3 TCGA (all samples)
Percentage of patients high vs low (%)
50
40
30
20
10
5
p-value > 0.05
p-value < 0.05
0
1
2
3
4
Hazard Ratio (Mantel Test) BNIP3high vs BNIP3low
B
BNIP3 TCGA expression distribution sorted minimum to maximum (all samples)
BNIP3 expression (log2 RSEM)
15
10
5
0
0
40
50
60
100
Percentage of patients (%)
C
Hazard Ratio Progression BNIP3 TCGA (primary samples)
Percentage of patients high vs low (%)
50
40
30
20
p-value > 0.06
p-value = 0.06
0
1
2
4
6
8
Hazard Ratio (Mantel Test) BNIP3high vs BNIP3low
D
BNIP3 TCGA expression distribution sorted minimum to maximum (primary samples)
BNIP3 expression (log2 RSEM)
12
8
4
0
40
50
60
100
Percentage of patients (%)
E
Melanoma-specific overall survival BNIP3 TCGA (primary samples)
Survival percentage (%)
100
50
0
40% BNIP3high (n = 41)
40% BNIP3low (n = 42)
p-value = 0.0627
HR = 2.215 (0.959-5.117)
0
500
1000
1500
2000
Time after diagnosis (days)
F
BNIP3 TCGA expression distribution sorted minimum to maximum (metastatic samples)
BNIP3 expression (log2 RSEM)
12
8
4
0
40
50
60
100
Percentage of patients (%)
G
Hazard Ratio Progression BNIP3 TCGA (metastatic samples)
Percentage of patients high vs low (%)
50
40
30
20
10
p-value = 0.09
p-value > 0.09
0
1
2
3
Hazard Ratio (Mantel Test) BNIP3high vs BNIP3low
H
Melanoma-specific overall survival BNIP3 TCGA (metastatic samples)
Survival percentage (%)
100
50
0
40% BNIP3high (n = 141)
40% BNIP3low (n = 141)
p-value = 0.0891
HR = 1.324 (0.958-1.829)
0
3000
6000
9000
12000
Time after diagnosis (days)

Appendix Figure S1. BNIP3 levels in melanoma inversely correlate with patient's prognosis (related to Figure 1).

(**A**) Hazard ratio analysis of all the specimens of the SKCM cohort (n=469) from the TCGA, segregated in *BNIP3* highest and lowest levels using different cut-offs (50% cut-off takes the 50% of the samples harboring the highest *BNIP3* levels and compares them against the other 50% with the lowest *BNIP3* levels, 40% compares the 40% highest with the 40% lowest, and so forth).

(**B**) Distribution of *BNIP3* levels within the SKCM cohort (n=469) from the TCGA, sorted from minimum to maximum.

(**C**) Hazard ratio analysis of the primary samples of the SKCM cohort (n=103) from the TCGA, segregated in *BNIP3* highest and lowest levels using different cut-offs (alike in Fig. S1A).

(**D**) Distribution of *BNIP3* levels within the primary samples of the SKCM cohort (n=103) from the TCGA, sorted from minimum to maximum.

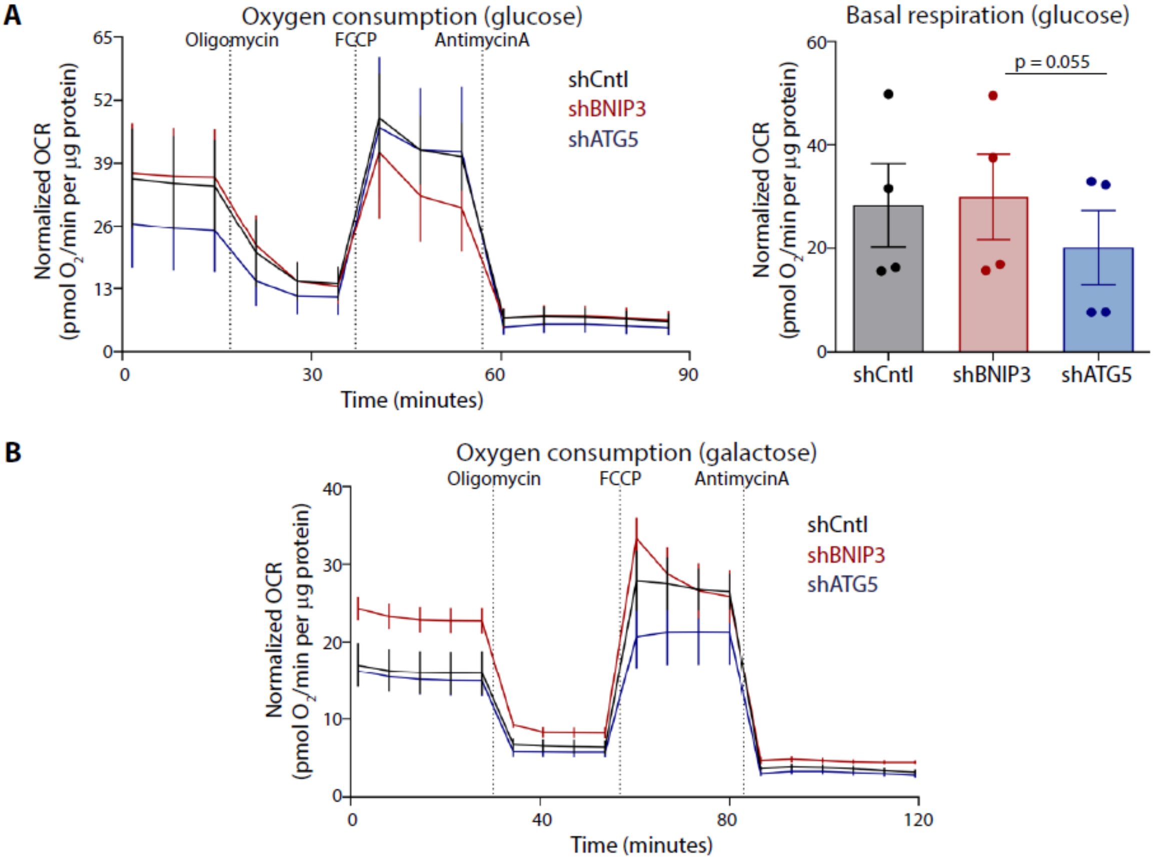
(**E**) Kaplan Meier representing the survival difference between the SKCM primary samples with the 40% highest *BNIP3* values against the samples with the lowest 40% *BNIP3* levels.

(**F**) Hazard ratio analysis of the metastatic samples of the SKCM cohort (n=366) from the TCGA, segregated in *BNIP3* highest and lowest levels using different cut-offs (alike in Fig. S1A).

(**G**) Distribution of *BNIP3* levels within the metastatic samples of the SKCM cohort (n=366) from the TCGA, sorted from minimum to maximum.

(**H**) Kaplan Meier plot representing the survival difference between the SKCM metastatic samples with the 40% highest *BNIP3* values against the samples with the lowest 40% *BNIP3* levels.

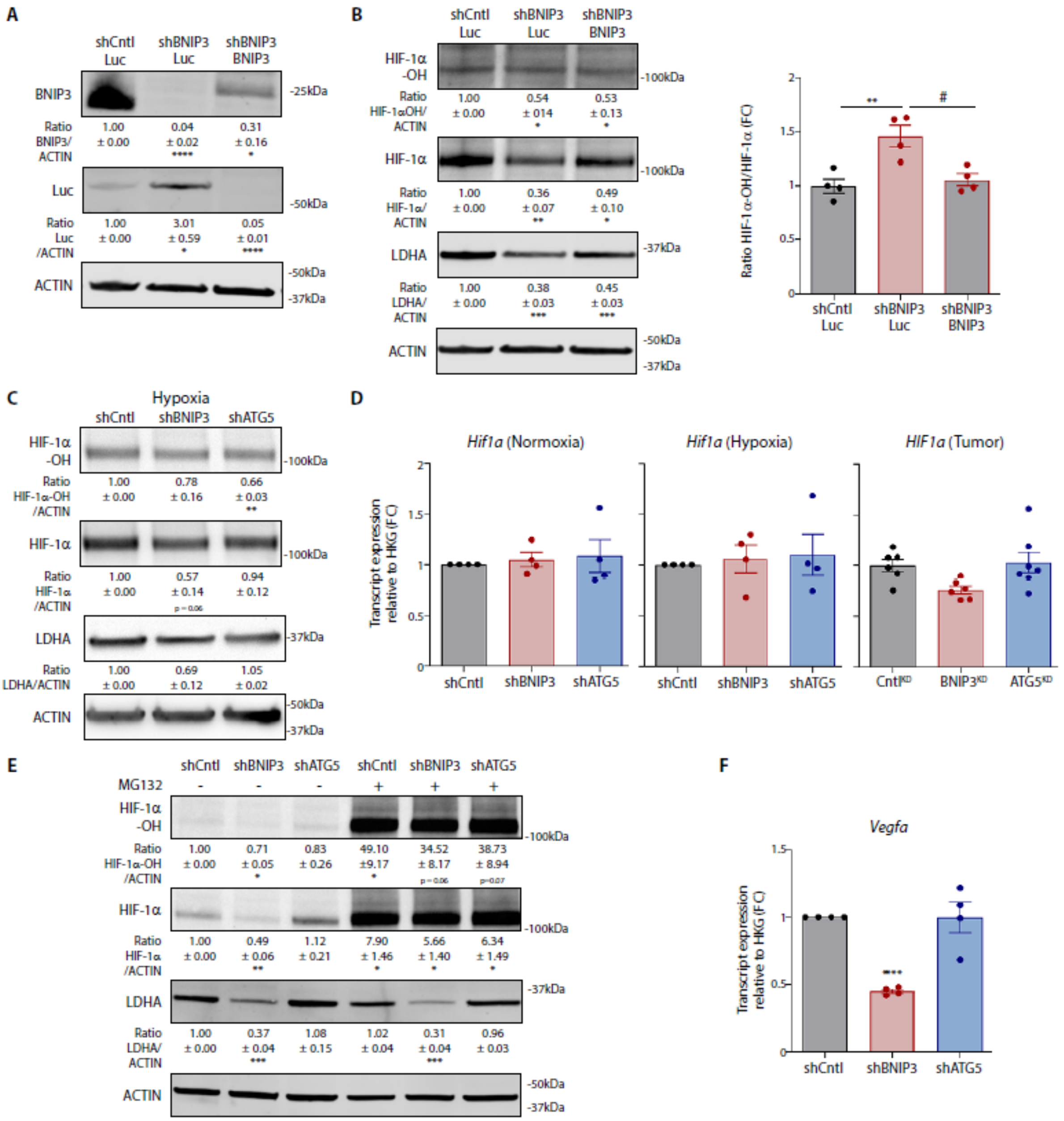
Data information: Hazard ratios were calculated using the Mantel-Haenszel test and corresponding p-values using the log-rank (Mantel-Cox) test. Both Kaplan Meier curves were analyzed using the log-rank (Mantel-Cox) test.



Appendix Figure S2. BNIP3 loss rewires melanoma cell's metabolism (related to Figure 3).

(**A-B**) OCR per µg of protein of B16-F10 cells assayed in either glucose (A; 25mM glucose, 2mM glutamine; n=4) or galactose (B; 25mM galactose, 2mM glutamine; n=3) medium using the Seahorse technology. Basal respiration (glucose) was analyzed using a RM one-way ANOVA (Geisser-Greenhouse correction) with Holm-Sidak's multiple comparisons test

Data information: All quantitative data are mean ± SEM. # $p < 0.05$ when compared against shCntl.



Appendix Figure S3. BNIP3 stabilizes normoxic HIF-1α on a post-translational level in murine melanoma cells (related to Figure 4).

(**A-B**) Immunoblot detection of BNIP3, luciferase (Luc) and ACTIN (A) or hydroxylated HIF-1α (HIF-1α-OH), total HIF-1α, LDHA and ACTIN (B) protein levels from lysates of B16-F10 cells lentivirally transduced with either Luc (shCntl Luc, shBNIP3 Luc) or a BNIP3-FL (shBNIP3 BNIP3) construct. The HIF-1α-OH /HIF-1α ratio is shown in a bar graph below. Densitometric quantifications relative to ACTIN levels are shown below each corresponding band.

(**C**) Immunoblot detection of hydroxylated HIF-1α (HIF-1α-OH), total HIF-1α, LDHA and ACTIN protein levels from lysates of B16-F10 cells collected after 24h of hypoxic (1% O_2) exposure.

(**D**) *Hif1a* transcript levels from lysates of normoxic, hypoxic or tumor B16-F10 cells [Cntl^{KD} (n=6), BNIP3^{KD} (n=6) and ATG5^{KD} (n=7)].

(**E**) Immunoblot detection of hydroxylated HIF-1α (HIF-1α-OH), total HIF-1α, LDHA and ACTIN protein levels from normoxic lysates of B16-F10 cells cultured in the presence or absence of the proteasomal inhibitor MG132 (10nM) for 6h.

(**F**) *Vegfa* transcript levels in lysates from normoxic B16-F10 cells.

Data information: All quantitative data are mean ± SEM. Densitometric quantifications of all protein levels are shown below the corresponding band $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ when compared against shCntl. Unless otherwise stated, each graph represents n=4 biologically independent experiments analyzed using a one-sample T-test against shCntl.

Appendix Table S1. Patient information (related to Figure 1).

Patient data Figure 1C - paired primary and metastases

Patient	Age at diagnosis	Gender	Type	TILS	Breslow from primary (mm)	Ulceration	Mitoses	Regression
1	87	F	SSMM	non-brisk	1,73	no	1-6	no
2	74	M	ALMM	non-brisk	3,25	yes	1-6	no
3	64	F	NMM	non-brisk	1,58	no	1-6	no
4	66	F	ALMM	non-brisk	4,00	yes	> 6	no
5	47	M	SSMM	non-brisk	1,44	no	1-6	no
6	58	M	SSMM	non-brisk	3,68	yes	> 6	no
7	58	M	SSMM	non-brisk	3,20	yes	> 6	no

Patient data Figure 1D - TMA

Sample	Age at time of sampling	Gender	Type 1st metastases	Site primary	Type primary	Breslow primary	Ulceration primary	Mitoses primary	TILS primary
BNIP3 score = 0									
1	88	F	in transit metastases	foot	ALMM	6,2	NA	NA	NA
BNIP3 score = 1									
2	55	F	LN metastases	lower leg	SSMM	0,7	+	NA	NA
3	44	F	lung, spleen adrenal, LN metastases	shoulder	MMnos	NA	NA	NA	NA
4	47	F	LN metastases	trunk	SSMM	1	NA	NA	NA
5	89	F	LN metastases	conjunctiva	MMnos	NA	NA	NA	NA
6	38	F	LN metastases	arm	SSMM	1	NA	NA	NA
7	88	F	in transit metastases	foot	ALMM	1,8	+	NA	NA
8	30	F	LN metastases	unknown	NMM	20	NA	NA	NA
9	69	F	local cutaneous metastases	unknown	SSMM	1,92	NA	NA	NA
10	52	F	LN metastases	foot	SSMM	3,68	+	>6	0
11	60	F	LN metastases	arm	NMM	2,8	+	>1 <6	non-brisk

12	50	F	LN metastases	knee	SSMM	1,6	NA	NA	NA
13	76	F	LN metastases	lower leg	SSMM	1,25	NA	NA	NA
BNIP3 score = 2									
14	75	F	LN metastases	lower leg	SSMM	NA	NA	NA	NA
15	68	F	LN metastases	lower leg	NMM	1,9	NA	NA	NA
16	61	F	local recurrence	foot	MMnos	1	NA	NA	NA
17	70	F	abdominal metastases	trunk	SSMM	1,4	NA	NA	NA
18	61	F	local recurrence	foot	MMnos	1	NA	NA	NA
19	36	F	LN metastases	lower leg	NMM	1,1	NA	NA	NA
20	43	F	LN metastases	shoulder	SSMM	0,39	NA	NA	NA
21	30	F	LN metastases	thigh	MMnos	12	NA	NA	NA
22	27	F	LN metastases	leg	NMM	2,01	NA	NA	NA
23	62	F	in transit, LN, lung metastases	nail toe	ALMM	NA	NA	NA	NA
24	34	F	LN metastases	back	SSMM	1,1	NA	NA	NA
25	68	F	LN metastases	lower leg	SSMM	18	+	NA	microsatellite
26	43	F	LN metastases	back	SSMM	3,06	NA	NA	NA
27	34	F	local cutaneous metastases	lower leg	NMM	2,1	NA	NA	NA
28	51	F	LN metastases	unknown	LN metastases	NA	NA	NA	NA
29	51	F	LN metastases	unknown	LN metastases	NA	NA	NA	NA
30	38	F	LN metastases	arm	SSMM	1	NA	NA	NA
31	63	F	NA	upper leg	SSMM	1,6	NA	NA	NA

32	77	F	local cutaneous metastases	lower leg	SSMM	1,9	NA	NA	NA
33	61	F	intestinal metastases	lower leg	MMnos	NA	NA	NA	NA
34	43	F	LN metastases	shoulder	SSMM	1,4	NA	NA	NA
35	51	F	LN metastases	arm	SSMM	1,125	-	++	non-brisk
36	36	F	LN metastases	lower leg	NMM	5	NA	NA	NA
37	71	F	local cutaneous recurrence	lower leg	MMnos	5,5	NA	NA	NA
38	48	F	LN metastases	unknown	MMnos	NA	NA	NA	NA
39	52	F	LN metastases	trunk	SSMM	0,44	NA	NA	NA
40	52	F	LN metastases	lower leg	SSMM	1,06	NA	NA	NA
41	55	F	LN metastases	lower leg	MMnos	1,6	NA	NA	NA
42	55	F	LN metastases	lower leg	MMnos	1,6	NA	NA	NA
43	58	F	LN metastases	vulva	SSMM	4,5	NA	NA	NA
44	70	F	LN metastases	unknown	MMnos	4,1	NA	NA	NA
45	39	F	LN metastases	lower leg	NMM	12	+	>6	0
46	36	F	LN metastases	lower leg	SSMM	2,08	NA	NA	NA
47	69	F	in transit metastases	lower leg	SSMM	1,9	-	unknown	unknown
48	40	F	breast metastases	arm	SSMM	0,69	-	unknown	brisk
49	39	F	brain metastases	back	SSMM	1,28	-	>1 <6	brisk
50	80	F	LN metastases	arm	MMnos	2,75	+	>6	brisk
51	69	F	LN metastases	arm	SSMM	10,9	-	>6	non-brisk
52	55	F	local recurrence	ear	NMM	2,4	NA	NA	NA

53	51	F	LN metastases	lower leg	ALMM	1,92	NA	NA	NA
54	65	F	LN metastases	upper leg	NMM	3,1	NA	NA	NA
55	49	F	LN metastases	back	NMM	5,6	NA	NA	NA
56	75	F	LN metastases	trunk	NMM	3,3	NA	NA	NA
57	39	F	LN metastases	upper arm	SSMM	1,25	NA	NA	NA
58	59	F	LN metastases	trunk	SSMM	1,39	NA	NA	NA
59	86	F	in transit metastases	lower leg	MMnos	8	NA	NA	NA
60	71	F	LN metastases	lower leg	SSMM	0,93	-	0	NA
61	54	F	in transit metastases	foot	SSMM	3,5	+	NA	NA
62	61	F	in transit metastases	lower leg	SSMM	2,6	NA	NA	NA
63	81	F	in transit metastases	teen	SSMM	1,06	-	>1 <6	0
64	76	F	LN metastases	face	SSMM	0,55	NA	NA	NA
65	88	F	salivary gland metastases	face	MMnos	NA	NA	NA	NA
66	61	F	LN metastases	lower leg	SSMM	2,24	NA	NA	NA
BNIP3 score = 3									
67	71	F	local skin metastases	lower leg	SSMM	NA	NA	NA	NA
68	34	F	LN metastases	back	SSMM	1,1	NA	NA	NA
69	71	F	local skin metastases	lower leg	SSMM	NA	NA	NA	NA
70	58	F	in transit metastases	foot	ALMM	NA	NA	NA	NA
71	53	F	LN metastases	unknown	SSMM	1	NA	NA	NA
72	46	F	in transit metastases	lower leg	MMnos	2,75	NA	NA	NA
73	79	F	LN metastases	arm	NMM	2,4	NA	NA	NA
74	77	F	LN metastases	face	MMnos	1,36	NA	NA	NA

75	76	F	bone metastases	lower leg	NA	4	NA	NA	NA
76	47	F	LN metastases	upper leg	MMnos	5,6	NA	NA	NA
77	36	F	subcutaneous, LN metastases	lower leg	SSMM	1,04	NA	NA	NA
78	72	F	local cutaneous metastases	lower leg	SSMM	2,4	NA	NA	NA
79	84	F	nasal	nasal mucosa	MMnos	NA	NA	NA	NA
80	90	F	LN metastases	toe	ALMM	NA	NA	NA	NA
81	56	F	nodal metastases	thigh	SSMM	3,26	unknown	unknown	unknown
82	61	F	NA	axilla	LN metastases	NA	NA	NA	NA
83	39	F	LN metastases	lower leg	SSMM	1,1	NA	NA	NA
84	49	F	LN metastases	arm	SSMM	1,125	-	++	non-brisk
85	71	F	cutaneous metastases	lower leg	NMM	3	+	NA	NA
86	60	F	LN metastases	nail toe	ALMM	3,8	NA	NA	NA
87	36	F	local cutaneous metastases	lower leg	NMM	2,1	NA	NA	NA
88	72	F	cutaneous metastases	lower leg	NMM	3	+	NA	NA
89	40	F	lung, bone, LN metastases	lower leg	LN metastases	NA	NA	NA	NA
90	79	F	local cutaneous metastases	unknown	SSMM	3,46	+	>6	absent
91	22	F	in transit metastases, LN metastases	back	NMM	11	NA	NA	NA
92	42	F	LN metastases	rectum	MMnos	NA	NA	NA	NA

93	60	F	local cutaneous metastases	lower leg	MMnos	NA	NA	NA	NA
94	80	F	local cutaneous metastases	unknown	SSMM	3,46	+	>6	absent
95	88	F	local skin metastases	lower leg	NMM	unknown	unknown	unknown	unknown
96	80	F	local cutaneous metastases	unknown	SSMM	3,46	+	>6	absent
97	45	F	abdominal metastases	upper arm	SSMM	1,2	NA	NA	NA
98	76	F	local skin metastases	lower leg	SSMM	NA	NA	NA	NA
99	66	F	in transit metastases	lower leg	SSMM	1,9	-	unknown	unknown
100	62	F	LN metastases	lower leg	SSMM	0,8	NA	NA	NA
101	93	F	in transit metastases	unknown	NMM	5	NA	NA	NA
102	50	F	in transit metastases	lower leg	MMnos	2,75	NA	NA	NA
103	88	F	local skin metastases	lower leg	NMM	unknown	unknown	unknown	unknown
104	41	F	lung metastases	vulva	SSMM	5,1	+	NA	NA
105	62	F	local cutaneous metastases	lower leg	MMnos	NA	NA	NA	NA
106	72	F	local cutaneous recurrence	lower leg	MM nos	5,5	NA	NA	NA
107	81	F	local cutaneous metastases	unknown	SSMM	3,46	+	>6	absent
108	42	F	lung metastases	vulva	SSMM	5,1	+	NA	NA
109	43	F	LN metastases	unknown	MMnos	7,2	NA	NA	NA
110	89	F	local skin metastases	lower leg	NMM	unknown	unknown	unknown	unknown

111	38	F	local cutaneous metastases	lower leg	NMM	2,1	NA	NA	NA
112	75	F	cutaneous metastases	lower leg	NMM	3	+	NA	NA
113	72	F	in transit metastases	lower leg	MMnos	NA	NA	NA	NA
114	75	F	cutaneous metastases	lower leg	NMM	3	+	NA	NA
115	33	F	LN metastases	hand	MMnos	2,75	-	>6	non-brisk
116	69	F	cardiac metastases	hand	SSMM	5,12	+	>6	0
117	25	F	lung metastases	unknown	MMnos	NA	NA	NA	NA
118	37	F	LN metastases	neck	SSMM	2,81	unknown	unknown	unknown
119	71	F	in transit metastases	unknown	SSMM	1,3	NA	NA	NA
120	30	F	LN metastases	lower leg	MMnos	NA	NA	NA	NA
121	69	F	LN metastases	lower leg	SSMM	0,93	-	0	NA
122	42	F	LN metastases	upper arm	NMM	1,99	NA	NA	NA
123	77	F	cutaneous metastases	lower leg	NMM	3	+	NA	NA
124	77	F	cutaneous metastases	lower leg	NMM	3	+	NA	NA
125	41	F	local cutaneous metastases	lower leg	NMM	2,1	NA	NA	NA
126	41	F	local cutaneous metastases	lower leg	NMM	2,1	NA	NA	NA
127	68	F	local recurrence	neck	MMnos	NA	NA	NA	NA
128	34	F	LN metastases	lower leg	SSMM	0,75	NA	NA	NA
129	60	F	LN metastases	face	MMnos	NA	NA	NA	NA
130	51	F	lung metastases	brain metastases	NA	NA	NA	NA	NA

131	68	F	LN metastases, satellite	lower leg	MMnos	3	NA	NA	NA
132	81	F	vulvar recurrence	vulva	MMnos	10	NA	NA	NA
133	73	F	LN metastases	upper leg	SSMM	1,5	NA	NA	NA
134	52	F	cutaneous metastases	lower leg	MMnos	3,84	NA	NA	NA
135	49	F	local recurrence	lower leg	SSMM	4,4	NA	NA	NA
136	67	F	in transit metastases	foot	ALMM	NA	NA	NA	NA
137	59	F	LN metastases	trunk	SSMM	1,39	NA	NA	NA
138	79	F	LN metastases	brain metastases	no primary	NA	NA	NA	NA
139	63	F	nodal, in transit metastases	lower leg	NMM	3,5	unknown	unknown	unknown
140	49	F	local recurrence	lower leg	SSMM	4,4	NA	NA	NA
141	79	F	LN metastases	brain metastases	no primary	NA	NA	NA	NA
142	79	F	local cutaneous metastases	lower leg	MMnos	NA	NA	NA	NA
143	81	F	vulvar recurrence	vulva	MMnos	10	NA	NA	NA
144	56	F	local cutaneous recurrence	lower leg	SSMM	1,9	NA	NA	NA
145	56	F	LN metastases	sole of foot	ALMM	6,8	+	NA	microsatellite
146	92	F	local cutaneous metastases	lower leg	NMM	3,2	NA	NA	NA
147	92	F	local cutaneous metastases	lower leg	NMM	3,2	NA	NA	NA
148	42	F	LN metastases	back	SSMM	1,2	-	NA	regressive

149	57	F	in transit, LN metastases	lower leg	SSMM	1	NA	NA	NA
150	57	F	local recurrence	lower leg	NMM	5,7	NA	NA	microsatellite
151	80	F	local cutaneous metastases	lower leg	MMnos	NA	NA	NA	NA
152	64	F	local recurrence	lower leg	SSMM	1,73	NA	NA	NA
153	51	F	abdominal metastases	upper arm	SSMM	1,2	NA	NA	NA
154	78	F	local cutaneous metastases	lower leg	SSMM	NA	+	NA	NA
155	78	F	local cutaneous metastases	lower leg	SSMM	NA	+	NA	NA
156	64	F	local recurrence	arm	cutaneous metastases (no primary)	NA	NA	NA	NA
157	69	F	in transit metastases	foot	ALMM	NA	NA	NA	NA
158	63	F	lung, liver, bone, adrenal metastases	shoulder	SSMM	4,1	+	NA	NA

Legend: F (female), ALMM (acral lentiginous malignant melanoma), LN (lymph node), SSMM (superficial spreading malignant melanoma), NMM (nodular melanoma), MMnos (malignant melanoma not otherwise specified), NA (no data available)

Appendix Table S2. Western blot quantifications from Figure 5 and Figure 6

	Figure 5B (n=3)					
	shCntl IOX2 -	shCntl IOX2 +	shBNIP3 IOX2 -	shBNIP3 IOX2+	shATG5 IOX2-	shATG5 IOX2+
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
HIF-1a-OH/ACTIN	1.00 ± 0.00	0.06 ± 0.03***	0.44 ± 0.10*	0.21 ± 0.12*	0.51 ± 0.06*	0.31 ± 0.14*
HIF-1a/ACTIN	1.00 ± 0.00	3.89 ± 2.20	0.28 ± 0.03**	3.32 ± 0.86	0.95 ± 0.09	5.40 ± 1.63
LDHA/ACTIN	1.00 ± 0.00	1.49 ± 0.34	0.28 ± 0.02***	1.06 ± 0.27	0.87 ± 0.06	1.78 ± 0.21
HIF-1a-OH/HIF-1a	1.00 ± 0.00	0.03 ± 0.01***	1.56 ± 0.28	0.06 ± 0.03***	0.55 ± 0.07*	0.08 ± 0.05**

	Figure 5D (n=3)					
	shCntl DFO -	shCntl DFO +	shBNIP3 DFO -	shBNIP3 DFO+	shATG5 DFO-	shATG5 DFO+
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
HIF-1a-OH/ACTIN	1.00 ± 0.00	0.33 ± 0.18 (p=0.06)	0.91 ± 0.27	0.27 ± 0.09*	1.03 ± 0.54	0.39 ± 0.24
HIF-1a/ACTIN	1.00 ± 0.00	2.82 ± 0.69	0.60 ± 0.07	3.10 ± 0.44*	1.18 ± 0.25	3.68 ± 0.88
LDHA/ACTIN	1.00 ± 0.00	1.57 ± 0.18	0.34 ± 0.03**	0.81 ± 0.02*	0.86 ± 0.14	1.49 ± 0.40
HIF-1a-OH/HIF-1a	1.00 ± 0.00	0.10 ± 0.03**	1.48 ± 0.32	0.08 ± 0.02***	0.76 ± 0.25	0.09 ± 0.04**

	Figure 5E (n=3)					
	shCntl siScr	shCntl siPHD2	shBNIP3 siScr	shBNIP3 siPHD2	shATG5 siScr	shATG5 siPHD2
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
HIF-1a-OH/ACTIN	1.00 ± 0.00	0.81 ± 0.06	0.89 ± 0.26	0.99 ± 0.35	1.03 ± 0.07	0.74 ± 0.20
HIF-1a/ACTIN	1.00 ± 0.00	2.58 ± 0.31*	0.73 ± 0.12	2.39 ± 0.86	1.12 ± 0.25	2.21 ± 0.38
LDHA/ACTIN	1.00 ± 0.00	2.03 ± 0.48	0.28 ± 0.10*	0.85 ± 0.20	1.12 ± 0.11	2.32 ± 0.42
PHD2/ACTIN	1.00 ± 0.00	0.14 ± 0.02	1.63 ± 0.31	0.28 ± 0.02**	1.11 ± 0.17	0.17 ± 0.04**
HIF-1a-OH/HIF-1a	1.00 ± 0.00	0.32 ± 0.04**	1.20 ± 0.19	0.42 ± 0.03**	1.08 ± 0.37	0.36 ± 0.13*

	Figure 6C (n=3)					
	shCntl BafA -	shCntl BafA +	shBNIP3 BafA -	shBNIP3 BafA+	shATG5 BafA-	shATG5 BafA+
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
NCOA4/ACTIN	1.00 ± 0.00	11.44± 1.13*	1.41 ± 0.10*	21.98 ± 5.43 (p=0.06)	1.16 ± 0.04 (p=0.07)	11.88 ± 1.17*
FTL/ACTIN	1.00 ± 0.00	0.24 ± 0.07**	1.16 ± 0.50	0.85 ± 0.47	0.84 ± 0.27	0.32 ± 0.15*

	Figure 6F (n=4)					
	shCntl siScr	shCntl siNCOA4	shBNIP3 siScr	shBNIP3 siNCOA4	shATG5 siScr	shATG5 siNCOA4
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
HIF-1a-OH/ACTIN	1.00 ± 0.00	0.85 ± 0.18	0.53 ± 0.06**	0.55 ± 0.10*	1.12 ± 0.27	0.60 ± 0.15
HIF-1a/ACTIN	1.00 ± 0.00	1.13 ± 0.15	0.52 ± 0.10*	0.74 ± 0.10	1.25 ± 0.22	0.74 ± 0.08*
LDHA/ACTIN	1.00 ± 0.00	1.19 ± 0.06*	0.22 ± 0.02****	0.32 ± 0.03***	1.08 ± 0.11	1.15 ± 0.09
NCOA4/ACTIN	1.00 ± 0.00	0.22 ± 0.05***	2.22 ± 0.36*	0.36 ± 0.14*	1.13 ± 0.23	0.15 ± 0.10**
HIF-1a-OH/HIF-1a	1.00 ± 0.00	0.85 ± 0.18	1.29 ± 0.36	0.84 ± 0.16	0.88 ± 0.11	0.73 ± 0.14

All data was analyzed using a one-sample T test against shCntl untreated (5B, 5D, 6C) or shCntl siScr (5E, 6F) except shBNIP3 BafA- and shBNIP3 BafA+ in 6C that were analyzed using a Wilcoxon rank test against shCntl. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

In 6C, FTL shBNIP3 conditions were analyzed using a Wilcoxon test against shCntl Baf- whereas the rest were analyzed using a one sample T test against shCntl Baf-.

Appendix Table S3. CE-ICP-MS values related to Figure 6B

Condition	Biological Replicate	Fe^{3+} (µg/L)	Fe^{2+} (µg/L)	Fe^{2+}/Fe^{3+} ratio
shCntl	E1	288.44	86.51	0.30
shCntl	E2	212.06	85.95	0.41
shCntl	E3	347.54	131.03	0.38
shBNIP3	E1	22.27	96.30	4.32
shBNIP3	E2	41.47	118.33	2.85
shBNIP3	E3	31.52	82.97	2.63
shATG5	E1	68.82	47.29	0.69
shATG5	E2	60.93	16.98	0.28
shATG5	E3	55.17	18.43	0.33

Appendix Table S4. CE-ICP-MS values related to Figure 6E

Condition	Biological Replicate	Fe^{3+} (µg/L)	Fe^{2+} (µg/L)	Fe^{2+}/Fe^{3+} ratio
shCntl siScr	E1	25.60	16.34	0.64
shCntl siScr	E2	42.19	13.49	0.32
shCntl siScr	E3	53.64	19.44	0.36
shCntl siScr	E4	18.96	10.98	0.58
shBNIP3 si Scr	E1	11.55	38.48	3.33
shBNIP3 si Scr	E2	12.99	31.85	2.45
shBNIP3 si Scr	E3	56.02	62.48	1.12
shBNIP3 si Scr	E4	15.50	36.41	2.35
shATG53 si Scr	E1	10.66	3.21	0.30
shATG53 si Scr	E2	13.32	8.54	0.64
shATG53 si Scr	E3	37.72	21.42	0.57
shATG53 si Scr	E4	28.31	15.16	0.54
shCntl siNCOA4	E1	33.29	13.70	0.41
shCntl siNCOA4	E2	24.95	10.34	0.41
shCntl siNCOA4	E3	43.07	26.58	0.62
shCntl siNCOA4	E4	29.39	24.29	0.83
shBNIP3 siNCOA4	E1	38.36	28.77	0.75
shBNIP3 siNCOA4	E2	39.74	11.79	0.30
shBNIP3 siNCOA4	E3	38.44	10.29	0.27
shBNIP3 siNCOA4	E4	42.00	17.97	0.43
shATG53 siNCOA4	E1	8.30	2.51	0.30
shATG53 siNCOA4	E2	51.98	44.84	0.86
shATG53 siNCOA4	E3	50.97	32.46	0.64
shATG53 siNCOA4	E4	15.71	12.01	0.76

Appendix Table S5. Sequences of the qPCR primers

Gene	Primer orientation	Sequence	Source
		MURINE	
Hprt	FW	TCCTCCTCAGACCGCTTTT	Inhouse
	RV	CCTGGTTCATCATCGCTAATC	Inhouse
Ppib	FW	GGAGATGGCACAGGAGGAA	Inhouse
	RV	GCCCGTAGTGCTTCAGCTT	Inhouse
Bnip3	FW	CCTGTCGCAGTTGGGTTC	Inhouse
	RV	TTGCCATTGCTGAAGTGC	Inhouse
Hif1a	FW	GCACTAGACAAAGTTCACCTGAGA	Inhouse
	RV	CGCTATCCACATCAAAGCAA	Inhouse
Glut1	FW	GACCCTGCACCTCATTGG	Inhouse
	RV	GATGCTCAGATAGGACATCCAAG	Inhouse
Hk2	FW	AAGAGAACAAGGGCGAGGAG	Inhouse
	RV	GGCAAAATGGGGATGTTTC	Inhouse
Pkm1	FW	TGGACATGGTGTTTGCATCT	Inhouse
	RV	CCTTGGCTGTTTCTCCAGAC	Inhouse
Pkm2	FW	GTGACCTGGGCATTGAGATT	Inhouse
	RV	CGGAGTTCCTCGAATAGCTG	Inhouse
Pdk1	FW	GTGTTTGCTGAAGCTCCTAAAGG	Inhouse
	RV	TGTTCAAAACCACGCCCAAT	Inhouse
Mct1	FW	TTGCCCCTTTGTCTACAACC	Inhouse
	RV	CCTCCGCTTTCTGTTCTTTG	Inhouse
Ldha	FW	ATCTTAATGAAGGACTTGGCGGAT	Inhouse
	RV	AGCTTGGAGTTCGCAGTTACA	Inhouse
Ldhb	FW	CATTGCGTCCGTTGCAGATG	Inhouse
	RV	GGAGGAACAAGCTCCCGTG	Inhouse
Mct4	FW	GACAGAGGCAGATACAGCGG	Inhouse
	RV	GAGGGCTGCTTTCACCAAGA	Inhouse
Vegfa	FW	TTAAACGAACGTACTTGCAGATG	Inhouse
	RV	AGAGGTCTGGTTCCCGAAA	Inhouse
Egln2 (PHD1)	FW	GGTCCGTTGAGTGTAGAGCTG	Inhouse
	RV	CAAGAGGCCATGACTCACCT	Inhouse
Egln1 (PHD2)	FW	CTGTGGAACAGCCCTTTTTG	Inhouse
	RV	CGAGTCTCTCTGCGAATCCT	Inhouse
Egln3 (PHD3)	FW	TGTCTGGTACTTCGATGCTGA	Inhouse
	RV	AGCAAGAGCAGATTCAGTTTTTC	Inhouse
Ncoa4	FW	TCCCAGTGTGATTTGGCCATGTGTTTAGT	Inhouse
	RV	TGCACTGCAGGGTGCATAACATGGTAAA	Inhouse

Gene	**Primer orientation**	**Sequence**	**Source**
		MURINE	
Map1lc3b (LC3B)	FW	AGATGTTACCATACGCCCTTCT	Inhouse
	RV	TCAGAACCACCCTTACAGAGAC	Inhouse
Sqstm1 (p62)	FW	CTTCTGGGCAAGGAGGAGG	Inhouse
	RV	CAACCAAGTCCCCATCCTCA	Inhouse

Gene	**Primer orientation**	**Sequence**	**Source**
		HUMAN	
HPRT	FW	GACCAGTCAACAGGGGACAT	Inhouse
	RV	GTGTCAATTATATCTTCCACAATCAAG	Inhouse
PPIB	FW	CCAACGCAGGCAAAGACACCAA	(Capomaccio *et al*, 2011)
	RV	GCTCTCCACCTTCCGCACCA	
BNIP3	FW	CTGGACGGAGTAGCTCCAAG	Inhouse
	RV	CCGACTTGACCAATCCCATA	Inhouse
HIF1A	FW	TTTTTCAAGCAGTAGGAATTGGA	Inhouse
	RV	GTGATGTAGTAGCTGCATGATCG	Inhouse
SLC2A1 (GLUT1)	FW	ATGATGCGGGAGAAGAAGGT	Inhouse
	RV	TCGAAGATGCTCGTGGAGTA	Inhouse
HK2	FW	AAGGCTTCAAGGCATCTG	(Jin *et al*, 2017)
	RV	CCACAGGTCATCATAGTTCC	
PKM2	FW	GTGCGAGCCTCAAGTCACTCCACA	(Chu *et al*, 2015)
	RV	TATAAGAAGCCTCCACGCTGCCCA	
PDK1	FW	CTGTGATACGGATCAGAAACCG	(Velpula *et al*, 2013)
	RV	TCCACCAAACAATAAAGAGTGCT	
LDHA	FW	AGCCCGATTCCGTTACCT	(Daniele *et al*, 2015)
	RV	CACCAGCAACATTCATTCCA	
LDHB	FW	CTAGCTAGCCTAGTGAGCTCTAGGCTGTAG	(Luo *et al*, 2013)
	RV	ACGCGTCGACGCACACTACAATAGTTAATTTTA	
SLC16A3 (MCT4)	FW	GGGACTTCTACCCGGTTTGG	Inhouse
	RV	CATGTGCCTCTGGACCATGT	Inhouse
VEGFA	FW	TTAACCGAACGTACTTGCAGATG	Inhouse
	RV	GAGAGATCTGGTTCCCGAAA	Inhouse
EGLN1 (PHD2)	FW	AGGCGATAAGATCACCTGGAT	Inhouse
	RV	TTCGTCCGGCCATTGATTTTG	Inhouse

Appendix references

Capomaccio S, Cappelli K, Spinsanti G, Mencarelli M, Muscettola M, Felicetti M, Verini Supplizi A & Bonifazi M (2011) Athletic humans and horses: comparative analysis of interleukin-6 (IL-6) and IL-6 receptor (IL-6R) expression in peripheral blood mononuclear cells in trained and untrained subjects at rest. *BMC Physiol.* **11:** 3

Chu B, Wang J, Wang Y & Yang G (2015) Knockdown of PKM2 induces apoptosis and autophagy in human A549 alveolar adenocarcinoma cells. *Mol. Med. Rep.* **12:** 4358–4363

Daniele S, Giacomelli C, Zappelli E, Granchi C, Trincavelli ML, Minutolo F & Martini C (2015) Lactate dehydrogenase-A inhibition induces human glioblastoma multiforme stem cell differentiation and death. *Sci. Rep.* **5:** 15556

Jin F, Wang Y, Zhu Y, Li S, Liu Y, Chen C, Wang X, Zen K & Li L (2017) The miR-125a/HK2 axis regulates cancer cell energy metabolism reprogramming in hepatocellular carcinoma. *Sci. Rep.* **7:** 3089

Luo D, Wilson JM, Harvel N, Liu J, Pei L, Huang S, Hawthorn L & Shi H (2013) A systematic evaluation of miRNA:mRNA interactions involved in the migration and invasion of breast cancer cells. *J. Transl. Med.* **11:** 57

Velpula KK, Bhasin A, Asuthkar S & Tsung AJ (2013) Combined targeting of PDK1 and EGFR triggers regression of glioblastoma by reversing the Warburg effect. *Cancer Res.* **73:** 7277–7289