

**Chromosome 8q24 amplification associated with human hepatocellular carcinoma
predicts MYC/ZEB1/MIZ1 transcriptional regulation**

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Table S1. Comparison of MYC, Ki-67, ZEB1 and MIZ1 (nuclear only) expression patterns with HCC clinicopathologic parameters.

Variables	n	MYC expression		Ki-67 expression		ZEB1 expression		MIZ1 nuclear-only	
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Total HCC cases	147	27 (18%)	120	49 (33%)	98	14 (10%)	133	14 (10%)	133
Grade									
Low Grade I	14	1 (7%)	13	4 (29%)	10	0 (0%)	14	0 (0%)	14
Intermediate Grade II	85	8 (9%)	77	12 (14%)	73	1 (1%)	84	0 (0%)	85
High Grade III	33	10 (30%)	23	19 (58%)	14	6 (18%)	27	5 (15%)	28
High Grade IV	15	8 (53%)	7	14 (93%)	1	7 (47%)	8	9 (60%)	6
Viralhepatitis history									
Present	94	23 (24%)	71	36 (38%)	58	13 (14%)	81	13 (14%)	81
Low Grade I	6	1 (17%)	5	1 (17%)	5	0 (0%)	6	0 (0%)	6
Intermediate Grade II	61	5 (8%)	56	9 (15%)	52	0 (0%)	61	0 (0%)	61
High Grade III	19	9 (17%)	10	18 (95%)	1	6 (46%)	13	5 (36%)	14
High Grade IV	8	8 (100%)	0	8 (100%)	0	7 (88%)	1	8 (100%)	0
Absent	53	4 (8%)	49	13 (24%)	40	1 (2%)	52	1 (2%)	52
Cirrhosis									
Present	87	24 (28%)	63	34 (39%)	53	12 (14%)	75	13(15%)	74
Low Grade I	5	1 (20%)	4	1 (20%)	4	0 (0%)	5	0 (0%)	5
Intermediate Grade II	56	6 (11%)	50	8 (17%)	48	0 (0%)	56	0 (0%)	56
High Grade III	18	9 (50%)	9	17 (94%)	1	5 (28%)	13	5 (28%)	13
High Grade IV	8	8 (100%)	0	8 (100%)	0	7 (88%)	1	8 (100%)	0
Absent	60	3 (5%)	57	15 (25%)	45	2 (3%)	58	1 (2%)	59
Alcohol consumption									
Present	113	26 (23%)	87	42 (37%)	71	13 (12%)	100	14 (12%)	99
Low Grade I	7	1 (14%)	6	3 (43%)	4	0 (0%)	7	0 (0%)	7
Intermediate Grade II	63	7 (11%)	56	8 (13%)	55	1 (2%)	62	0 (0%)	63
High Grade III	28	10 (36%)	18	17 (61%)	11	5 (18%)	23	5 (18%)	23
High Grade IV	15	8 (53%)	7	14 (93%)	1	7 (47%)	8	9 (60%)	6
Absent	34	1 (3%)	33	7 (21%)	27	1 (3%)	33	0(0%)	34
Metastasis									
Present	51	26 (51%)	25	47 (92%)	4	14 (27%)	37	14 (27%)	37
Low Grade I	2	1 (50%)	1	2 (100%)	0	0 (0%)	2	0 (0%)	2
Intermediate Grade II	14	7 (50%)	7	12 (86%)	2	1 (7%)	13	0 (0%)	14
High Grade III	20	10 (50%)	10	18 (90%)	2	6 (30%)	14	5 (25%)	15
High Grade IV	15	8 (53%)	7	14 (93%)	1	7 (47%)	8	9 (60%)	6
Absent	96	1 (1%)	95	3 (3%)	93	0 (0%)	96	0 (0%)	96

Table S2. The individuals with HCC with high nuclear ZEB1 expression were also positive for metastasis.

Case	Nodes Examined	Nodes Involved/ (% positive)	Metastasis
1	12	4 (33%)	Yes
2	28	16 (57%)	Yes
3	14	6 (43%)	Yes
4	9	6 (67%)	Yes
5	18	12 (67%)	Yes
6	20	9 (45%)	Yes
7	8	4 (50%)	Yes
8	9	5 (56%)	Yes
9	23	12 (52%)	Yes
10	19	12 (63%)	Yes
11	21	11 (52%)	Yes
12	16	11 (69%)	Yes
13	8	6 (75%)	Yes
14	13	8 (62%)	Yes

Patient lymph nodes from all ZEB1 positive cases identified in the extended cohort were examined for the presence of tumor cells and metastasis confirmed.

Table S3. Patient response to treatment when diagnosed with Grade I HCC.

Case #	Grade	Treatment	Overall Survival
HCC-1	Grade I	Sorafenib	4 Years 8 Months
HCC-2	Grade I	Sorafenib	N/A
HCC-3	Grade I	Sorafenib	5 years 6 Months
HCC-4	Grade I	Sorafenib	5 Years 4 Months
HCC-5	Grade I	Sorafenib	6 Years 8 Months
HCC-6	Grade I	Sorafenib	N/A
HCC-7	Grade I	Sorafenib	5 Years 11 Months
HCC-8	Grade I	Sorafenib	6 Years 9 months
HCC-9	Grade I	Sorafenib	5 Years 2 Months
HCC-10	Grade I	Sorafenib	7 Years 3 Months
HCC-11	Grade I	Sorafenib	6 Years 8 Months
HCC-12	Grade I	Sorafenib	N/A
HCC-13	Grade I	Sorafenib	6 Years 3 months
HCC-14	Grade I	Sorafenib	5 Years 8 Months

Time to disease progression refers to the time from tumor excision and treatment initiation to disease progression. Overall survival refers to the time from diagnosis the day the individual was declared deceased.

Table S4. Patient response to treatment when diagnosed with Grade II HCC.

Case #	Grade	Initial Treatment	Time to Disease Progression	Overall Survival Time
HCC-15	Grade II	Sorafenib	2 Years 6 Months	3 Years 9 Months
HCC-16	Grade II	Levatinib	3 Years 9 Months	4 Years 7 Months
HCC-17	Grade II	Sorafenib	3 Years 4 Months	4 Years 8 Months
HCC-18	Grade II	Sorafenib	3 Years 10 Months	4 Years 9 Months
HCC-19	Grade II	HIAC + Sorafenib	3 Years 6 Months	4 Years 5 Months
HCC-20	Grade II	HIAC + Sorafenib	4 Years 6 Months	4 Years 8 Months
HCC-21	Grade II	Sorafenib	2 Years 6 Months	3 Years 8 Months
HCC-22	Grade II	HIAC + Levatinib	2 Years 8 Months	3 Years 7 Months
HCC-23	Grade II	Sorafenib	3 Years 10 Months	4 Years 11 Months
HCC-24	Grade II	HIAC + Sorafenib	3 Years 4 Months	4 Years 4 Months
HCC-25	Grade II	HIAC + Sorafenib	2 Years 6 Months	3 Years 9 Months
HCC-26	Grade II	Sorafenib	2 Years 8 Months	3 Years 7 Months
HCC-27	Grade II	Sorafenib	4 Years 6 Months	5 Years 5 Months
HCC-28	Grade II	HIAC + Sorafenib	3 Years 8 Months	4 Years 7 Months
HCC-29	Grade II	Sorafenib	2 Years 10 Months	3 Years 8 Months
HCC-30	Grade II	Sorafenib	2 Years 8 Months	3 Years 6 Months
HCC-31	Grade II	HIAC + Levatinib	3 Years 10 Months	4 Years 9 Months
HCC-32	Grade II	Sorafenib	3 Years 6 Months	4 Years 5 Months
HCC-33	Grade II	Sorafenib	2 Years 8 Months	3 Years 6 Months
HCC-34	Grade II	HIAC + Sorafenib	4 Years 2 Months	5 Years 1 Month
HCC-35	Grade II	HIAC + Sorafenib	3 Years 10 Months	4 Years 9 Months
HCC-36	Grade II	Sorafenib	4 Years 7 Months	5 Years 6 Months
HCC-37	Grade II	Sorafenib	4 Years 5 Months	5 Years 3 Months
HCC-38	Grade II	HIAC + Sorafenib	3 Years 8 Months	4 Years 6 Months
HCC-39	Grade II	HIAC + Sorafenib	2 Years 4 Months	3 Years 1 Month
HCC-40	Grade II	Sorafenib	3 Years 10 Months	4 Years 6 Months
HCC-41	Grade II	HIAC + Levatinib	2 Years 11 Months	3 Years 9 Months
HCC-42	Grade II	Sorafenib	4 Years 6 Months	5 Years 3 Months
HCC-43	Grade II	Sorafenib	3 Years 8 Months	4 Years 5 Months
HCC-44	Grade II	Sorafenib	4 Years 4 Months	5 Years 2 Months
HCC-45	Grade II	HIAC + Sorafenib	2 Years 10 Months	3 Years 6 Months
HCC-46	Grade II	HIAC + Sorafenib	2 Years 11 Months	3 Years 6 Months
HCC-47	Grade II	Sorafenib	4 Years 6 Months	5 Years 4 Months
HCC-48	Grade II	Sorafenib	3 Years 8 Months	4 Years 6 Months
HCC-49	Grade II	HIAC + Sorafenib	4 Years 3 Months	5 Years 1 Month
HCC-50	Grade II	Sorafenib	2 Years 6 Months	3 Years 5 Months
HCC-51	Grade II	HIAC + Sorafenib	2 Years 10 Months	3 Years 9 Months
HCC-52	Grade II	Sorafenib	2 Years 11 Months	3 Years 9 Months
HCC-53	Grade II	Sorafenib	3 Years 6 Months	4 Years 5 Months

HCC-54	Grade II	HIAC + Levantinib	4 Years 10 Months	5 Years 8 Months
HCC-55	Grade II	Sorafenib	4 Years 8 Months	5 Years 6 Months
HCC-56	Grade II	HIAC + Sorafenib	3 Years 8 Months	4 Years 6 Months
HCC-57	Grade II	Sorafenib	4 Years 10 Months	5 Years 8 Months
HCC-58	Grade II	HIAC + Levantinib	3 Years 11 Months	4 Years 9 Months
HCC-59	Grade II	Sorafenib	4 Years 7 Months	5 Years 5 Months
HCC-60	Grade II	HIAC + Sorafenib	2 Years 8 Months	3 Years 6 Months
HCC-61	Grade II	Sorafenib	3 Years 8 Months	4 Years 7 Months
HCC-62	Grade II	HIAC + Levantinib	4 Years 4 Months	5 Years 2 Months
HCC-63	Grade II	Sorafenib	3 Years 11 Months	4 Years 10 Months
HCC-64	Grade II	Sorafenib	3 Years 8 Months	4 Years 9 Months
HCC-65	Grade II	HIAC + Sorafenib	3 Years 10 Months	4 Years 11 Months
HCC-66	Grade II	Sorafenib	3 Years 8 Months	4 Years 8 Months
HCC-67	Grade II	Sorafenib	3 Years 6 Months	4 Years 5 Months
HCC-68	Grade II	HIAC + Levantinib	2 Years 10 Months	3 Years 9 Months
HCC-69	Grade II	HIAC + Levantinib	2 Years 11 Months	3 Years 8 Months
HCC-70	Grade II	Sorafenib	3 Years 8 Months	4 Years 9 Months
HCC-71	Grade II	Sorafenib	4 Years 2 Months	5 Years
HCC-72	Grade II	HIAC + Sorafenib	4 Years 7 Months	5 Years 8 Months
HCC-73	Grade II	Sorafenib	3 Years 8 Months	4 Years 6 Months
HCC-74	Grade II	HIAC + Sorafenib	3 Years 2 Months	4 Years 1 Month
HCC-75	Grade II	Sorafenib	3 Years 10 Months	4 Years 9 Months
HCC-76	Grade II	Sorafenib	3 Years 11 Months	4 Years 9 Months
HCC-77	Grade II	HIAC + Levantinib	2 Years 11 Months	3 Years 9 Months
HCC-78	Grade II	Sorafenib	4 Years 2 Months	5 Years
HCC-79	Grade II	HIAC + Levantinib	3 Years 10 Months	4 Years 11 Months
HCC-80	Grade II	Sorafenib	4 Years 2 Months	5 Years 1 Month
HCC-81	Grade II	Sorafenib	3 Years 8 Months	4 Years 7 Months
HCC-82	Grade II	HIAC + Sorafenib	4 Years 2 Months	4 Years 11 Months
HCC-83	Grade II	HIAC + Sorafenib	3 Years 11 Months	4 Years 10 Months
HCC-84	Grade II	Sorafenib	2 Years 4 Months	3 Years 2 Months
HCC-85	Grade II	Sorafenib	3 Years 10 Months	4 Years 6 Months
HCC-86	Grade II	HIAC + Levantinib	2 Years 9 Months	3 Years 7 Months
HCC-87	Grade II	HIAC + Levantinib	3 Years 2 Months	4 Years
HCC-88	Grade II	Sorafenib	3 Years 7 Months	4 Years 6 Months
HCC-89	Grade II	HIAC + Sorafenib	2 Years 5 Months	3 Years 2 Months
HCC-90	Grade II	Sorafenib	3 Years 7 Months	4 Years 5 Months
HCC-91	Grade II	Sorafenib	3 Years 8 Months	4 Years 6 Months
HCC-92	Grade II	Sorafenib	4 Years 3 Months	4 Years 8 Months
HCC-93	Grade II	Levantinib	2 Years 10 Months	3 Years 6 Months

HCC-94	Grade II	Sorafenib	3 Years 3 Months	3 Years 11 Months
HCC-95	Grade II	Sorafenib	4 Years 2 Months	4 Years 10 Months
HCC-96	Grade II	Levatinib	3 Years 6 Months	4 Years 4 Months
HCC-97	Grade II	Sorafenib	3 Years 1 Month	3 Years 10 Month
HCC-98	Grade II	Levatinib	2 Years 7 Months	3 Years 5 Months
HCC-99	Grade II	Sorafenib	3 Years 4 Months	4 Years 3 Months

Time to disease progression refers to the time from tumor excision and treatment initiation to disease progression. Overall survival refers to the time from diagnosis the day the individual was declared deceased. HIAC, hepatic intra- arterial chemotherapy

Table S5. Patient response to treatment when diagnosed with Grade III HCC.

Case #	Grade	Treatment	Time to Disease Progression	Overall Survival
HCC-100	Grade III	HIAC + Levantinib	8 Months 12 Days	14 Months 6 Days
HCC-101	Grade III	HIAC + Levantinib	9 Months 6 Days	14 Months 12 Days
HCC-102	Grade III	HIAC + Sorafenib	6 Months 2 Days	11 Months 26 Days
HCC-103	Grade III	HIAC + Levantinib	8 Months 8 Days	13 Months 14 Days
HCC-104	Grade III	Sorafenib + Erlotinib	6 Months 2 Days	12 Months 8 Days
HCC-105	Grade III	HIAC + Levantinib	7 Months 18 Days	13 Months 2 Days
HCC-106	Grade III	Sorafenib + Doxorubicin	5 Months 22 Days	11 Months 12 Days
HCC-107	Grade III	Sorafenib + Doxorubicin	7 Months 4 Days	12 Months 18 Days
HCC-108	Grade III	Sorafenib	7 Months 29 Days	12 Months 18 Days
HCC-109	Grade III	HIAC + Levantinib	8 Months 3 Days	13 Months 16 Days
HCC-110	Grade III	Sorafenib + Doxorubicin	7 Months 12 Days	13 Months 20 Days
HCC-111	Grade III	HIAC + Sorafenib	8 Months 20 Days	12 Months 22 Days
HCC-112	Grade III	HIAC + Sorafenib	6 Months 4 Days	11 Months 24 Days
HCC-113	Grade III	HIAC + Levantinib	6 Months 24 Days	12 Months 2 Days
HCC-114	Grade III	HIAC + Levantinib	8 Months 2 Days	14 Months 20 Days
HCC-115	Grade III	Sorafenib + Doxorubicin	5 Months 2 Days	11 Months 6 Days
HCC-116	Grade III	Sorafenib	7 Months 4 Days	13 Months 4 Days
HCC-117	Grade III	HIAC + Levantinib	7 Months 13 Days	13 Months 4 Days
HCC-118	Grade III	Sorafenib + Doxorubicin	5 Months 28 Days	10 Months 24 Days
HCC-119	Grade III	Sorafenib + Doxorubicin	7 Months 24 Days	12 Months 12 Days
HCC-120	Grade III	HIAC + Levantinib	8 Months 16 Days	13 Months 17 Days
HCC-121	Grade III	Sorafenib + Doxorubicin	6 Months 21 Days	11 Months 28 Days
HCC-122	Grade III	Sorafenib + Erlotinib	5 Months 10 Days	11 Months 4 Days
HCC-123	Grade III	Sorafenib + Doxorubicin	7 Months 22 Days	13 Months 30 Days
HCC-124	Grade III	Levantinib	6 Months 28 Days	12 Months 28 Days
HCC-125	Grade III	Sorafenib + Doxorubicin	6 Months 11 Days	12 Months 18 Days
HCC-126	Grade III	HIAC + Sorafenib	5 Months 23 Days	11 Months 2 Days
HCC-127	Grade III	Sorafenib + Erlotinib	6 Months 26 Days	10 Months 24 Days
HCC-128	Grade III	Sorafenib + Doxorubicin	7 Months 24 Days	13 Months 28 Days
HCC-129	Grade III	Levantinib	7 Months 14 Days	13 Months 4 Days
HCC-130	Grade III	Sorafenib	7 Months 12 Days	12 Months 4 Days
HCC-131	Grade III	Sorafenib + Erlotinib	6 Months 10 Days	11 Months 15 Days
HCC-132	Grade III	HIAC + Levantinib	7 Months 14 Days	12 Months 12 Days

Time to disease progression refers to the time from tumor excision and treatment initiation to disease progression. Overall survival refers to the time from diagnosis the day the individual was declared deceased. The individuals highlighted in yellow were confirmed for *c-myc* amplification, displayed MYC, ZEB1, MIZ1 and Ki-67 nuclear labeling and had cirrhosis, confirmed metastases

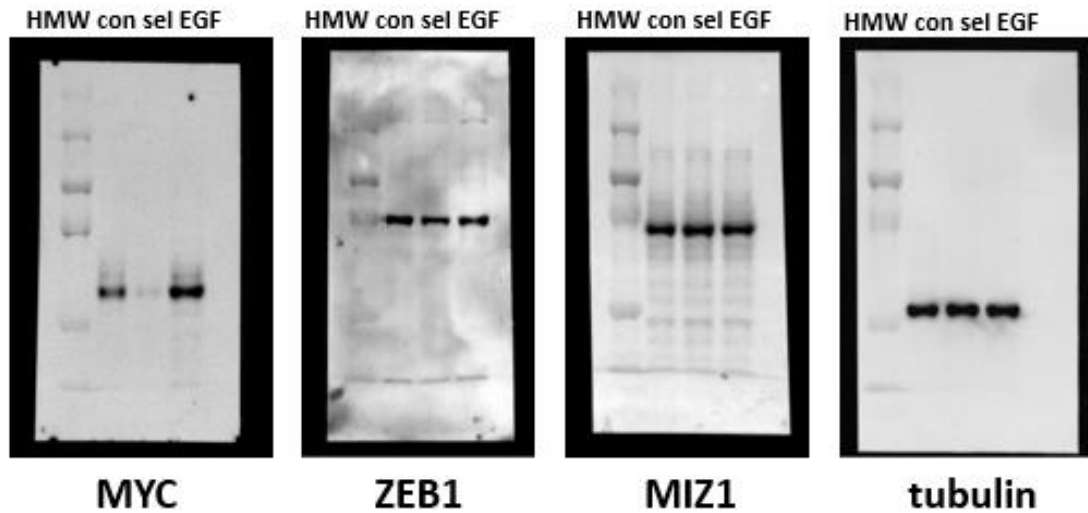
and had histories of alcohol consumption and viral hepatitis. HIAC, hepatic intra- arterial chemotherapy

Table S6. Patient response to treatment when diagnosed with Grade IV HCC.

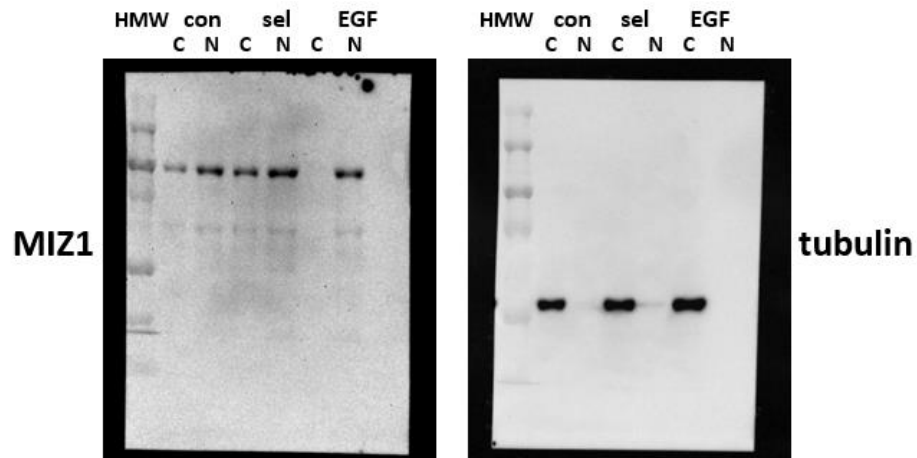
Case #	Grade	Treatment	Time to Disease Progression	Overall Survival
HCC-133	Grade IV	HIAC + Sorafenib	7 Months 10 Days	12 Months 12 Days
HCC-134	Grade IV	HIAC + Levantinib	8 Months 4 Days	12 Months 18 Days
HCC-135	Grade IV	Sorafenib + Doxorubicin	5 Months 12 Days	10 Months 8 Days
HCC-136	Grade IV	HIAC + Sorafenib	6 Months 4 Days	10 Months 1 Day
HCC-137	Grade IV	HIAC + Levantinib	8 Months 6 Days	11 Months 4 Days
HCC-138	Grade IV	Sorafenib + Doxorubicin	7 Months 13 Days	12 Months 3 Days
HCC-139	Grade IV	Levantinib	6 Months 11 Days	10 Months 7 Days
HCC-140	Grade IV	Sorafenib + Erlotinib	4 Months 8 Days	9 Months 26 Days
HCC-141	Grade IV	Sorafenib + Doxorubicin	5 Months 21 Days	11 Months 2 Days
HCC-142	Grade IV	HIAC + Sorafenib	6 Months 23 Days	11 Months 4 Days
HCC-143	Grade IV	Levantinib	6 Months 10 Days	9 Months 27 Days
HCC-144	Grade IV	HIAC + Sorafenib	6 Months 28 Days	10 Months 14 Days
HCC-145	Grade IV	HIAC + Levantinib	5 Months 29 Days	10 Months 17 Days
HCC-146	Grade IV	Sorafenib + Erlotinib	5 Months 11 Days	10 Months 14 Days
HCC-147	Grade IV	HIAC + Sorafenib	7 Months 12 Days	11 Months 16 Days

Time to disease progression refers to the time from tumor excision and treatment initiation to disease progression. Overall survival refers to the time from diagnosis the day the individual was declared deceased. The individuals highlighted in yellow were confirmed for *c-myc* amplification, displayed MYC, ZEB1, MIZ1 and Ki-67 nuclear labeling and had cirrhosis, confirmed metastases and had histories of alcohol consumption and viral hepatitis. HIAC, hepatic intra- arterial chemotherapy.

Fig S1.

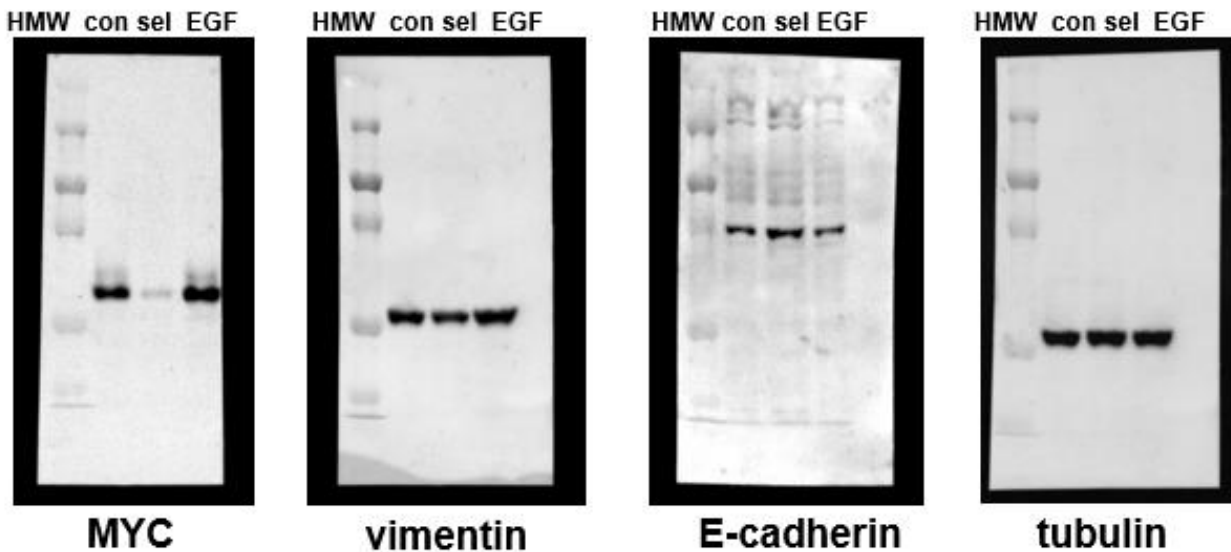


Uncropped, uncut Western Blots of lysates from control or treated cells (from Fig. 6E) were immunoblotted with the indicated antibodies. The proteins were transferred from a 15 well, 9% gel to the membrane. High molecular weight (HMW) standards are shown in the far left-hand lanes of each blot.



Uncropped, uncut Western Blots of cytosolic and nuclear fractions prepared from control or treated cells (from Fig. 6I) were immunoblotted with the indicated antibodies. The proteins were transferred from a 15 well, 9% gel to the membrane. High molecular weight (HMW) standards are shown in the far left-hand lanes of each blot.

Fig S2.



Uncropped, uncut Western Blots of lysates from control or treated cells (from Fig. 7B) were immunoblotted with the indicated antibodies. The proteins were transferred from a 15 well, 9% gel to the membrane. High molecular weight (HMW) standards are shown in the far left-hand lanes of each blot.