

## **Supplemental Material to:**

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Hernando and Michele Pagano**

**FBH1 protects melanocytes from transformation and is  
deregulated in melanomas**

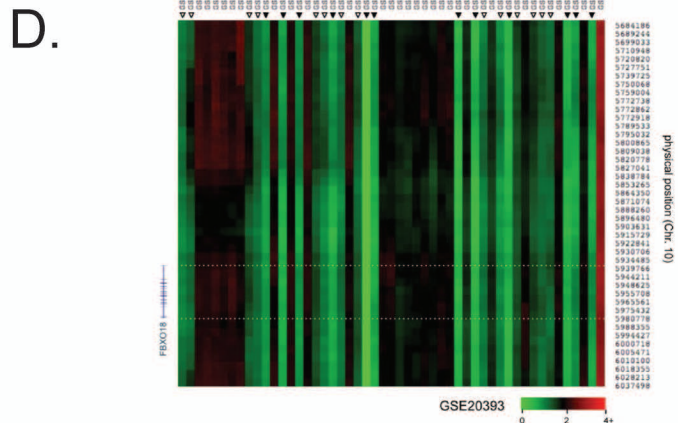
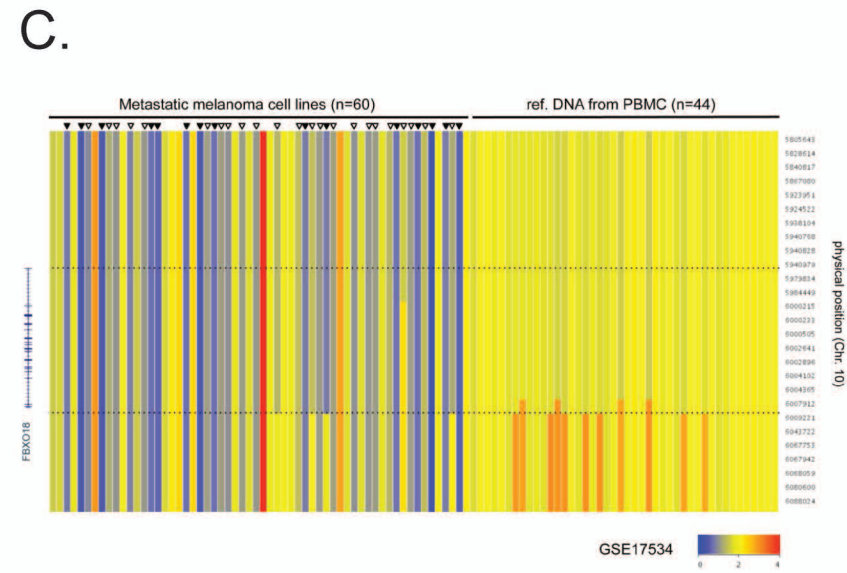
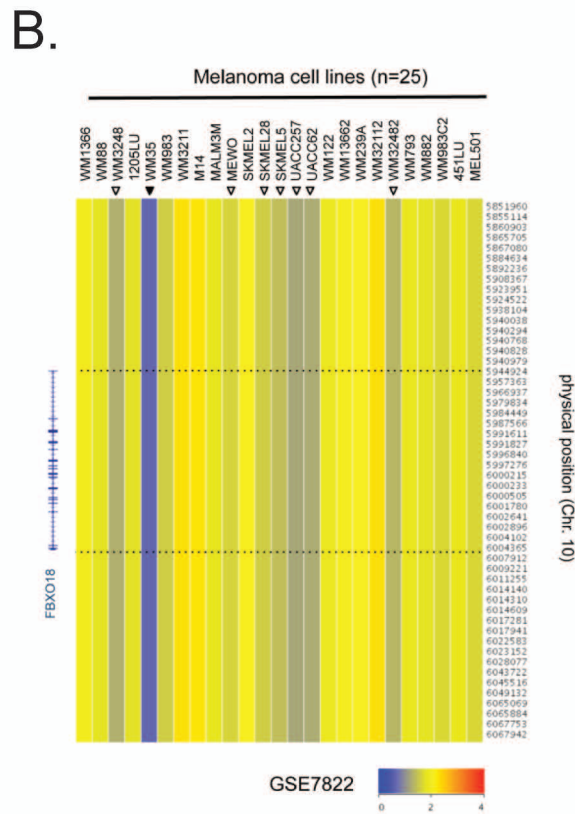
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**<http://www.landesbioscience.com/journals/cc/article/24165>**

**A.**

TISSUE (CELL LINE ORIGIN)	SAMPLES #	DEL.#
HEMATOPOIETIC	77	3 (4%)
BLADDER	10	0
BRAIN	7	1 (14%)
BREAST	21	0
CNS	11	2 (18%)
CERVIX	7	1 (14%)
COLON	19	0
CONNECTIVE T.	4	0
ESOPHAGUS	4	0
EYE	1	0
KIDNEY	8	0
LIVER	9	0
LUNG	81	19 (23%)
MUSCLE	4	0
OVARY	7	1 (14%)
PANCREAS	9	0
PHARYNX	2	0
PLACENTA	3	0
PROSTATE	6	1 (16%)
RECTUM	2	0
SARCOMA	2	0
<b>SKIN (melanocytes)</b>	<b>12</b>	<b>4 (33%)</b>
STOMACH	5	0
SYNOVIUM	1	0
THYROID	4	0
UTERUS	9	0
VULVA	3	0

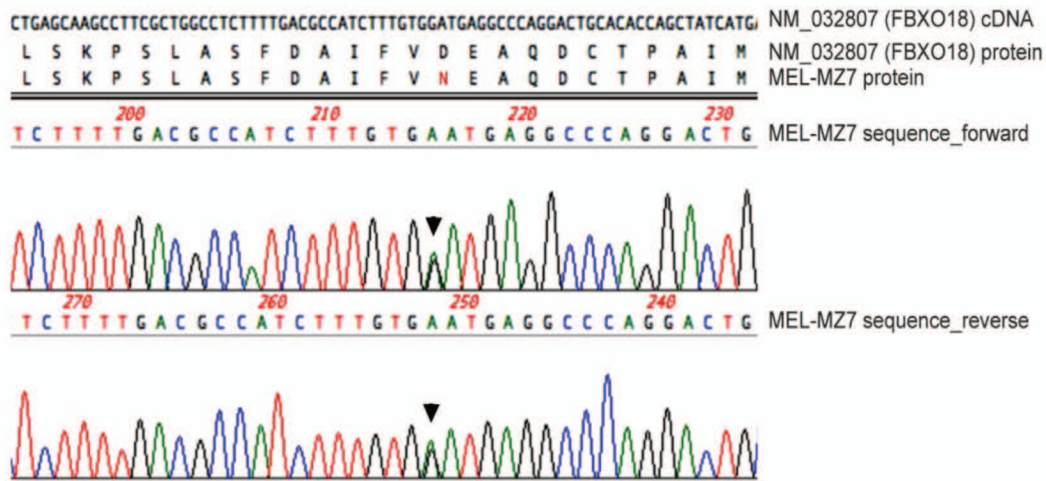


**E.**

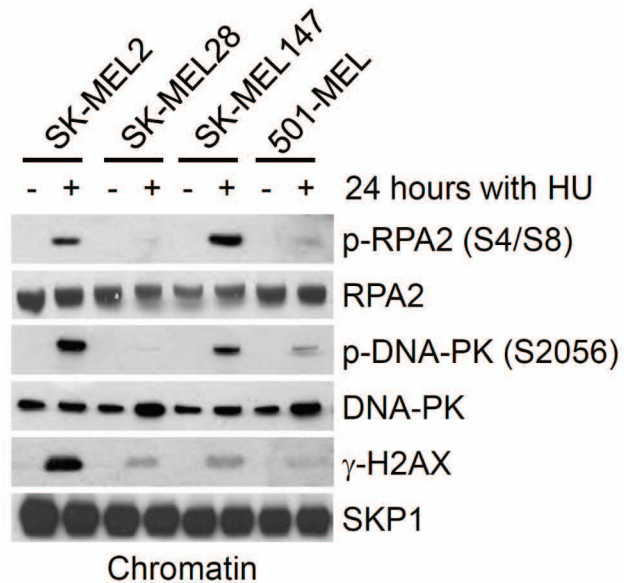
ARRAY		DELETIONS IN <i>FBXO18</i> LOCUS	
		HEMIZYGOUS	HOMOZYGOUS
GSE7822	25 melanoma cell lines	7 (28%)	1 (4%)
GSE22305	19 melanoma cell lines	9 (47%)	1 (5%)
GSE17534	60 metastatic melanoma cell lines	23 (38%)	15 (25%)
GSE20393	51 melanomas	14 (28%)	12 (23%)

A.

## MZ7-MEL (D698N)



B.



Supplementary Figure S2

## Supplementary Information

### Figure S1. The *FBH1* gene is deleted in melanoma cell lines and primary melanomas.

- (A) A High-Density, Single Nucleotide Polymorphism (HD-SNP) inferred copy number heat map of the *FBH1* locus in human cell lines. The data were gathered from the GSK cancer cell line genome profiling database ([https://cabig.nci.nih.gov/caArray\\_GSKdata/](https://cabig.nci.nih.gov/caArray_GSKdata/)) and analyzed via the Cancer Genome Atlas data portal (<http://cancergenome.nih.gov/>). The table summarizes the number of *FBH1* (*FBXO18*) deletions identified in 332 cell lines. Of 12 melanoma cell lines, four (HMCB, WM115, C32TG, and A7) display hemizygous deletions.
- (B) Affymetrix Genome-Wide Mapping 500K array copy number data of the *FBH1* locus in 25 melanoma cell lines is shown. The data are accessible at the National Center for Biotechnology Information-Gene Expression Omnibus (NCBI-GEO; <http://www.ncbi.nlm.nih.gov/geo/>) database <sup>1</sup>, accession # GSE7822 <sup>2</sup>.
- (C) Affymetrix Genome-Wide Human HD-SNP 250K StyI array copy number data for the *FBH1* locus in 60 malignant melanoma cell lines derived from metastatic tumors of melanoma patients. In addition, DNA from peripheral blood mononuclear cells was available for 44 of the 60 samples. The data are accessible at the NCBI-GEO (<http://www.ncbi.nlm.nih.gov/geo/>) database <sup>1</sup>, accession # GSE17534 <sup>3</sup>.
- (D) Agilent Genome-Wide Human 244A aCGH array copy number data for the *FBH1* locus in 51 primary melanomas is shown. The data are accessible at the NCBI-GEO (<http://www.ncbi.nlm.nih.gov/geo/>) database <sup>1</sup>, accession # GSE20393 [ENREF 4](#)<sup>4</sup>.
- (E) The table on the right summarizes the number of *FBH1* deletions identified by the studies reported in panels B-E.

### Figure S2. Melanoma cells expressing either low levels of *FBH1* or mutated *FBH1* are resistant to hydroxyurea.

- (A) The chromatogram displays the point mutation in *FBH1* (indicated by an arrow) in the MZ7-MEL melanoma cell line, as reported by the COSMIC database (<http://www.sanger.ac.uk/genetics/CGP/cosmic/>) and confirmed by sequencing of a PCR product from MZ7-MEL melanoma cell cDNA.
- (B) The indicated melanoma cells were treated with either HU (+) or vehicle (-) for 24 hours. After harvesting, cells were fractionated into soluble and chromatin fractions, and these fractions were immunoblotted for the indicated proteins.

## References

- 1 Edgar, R., Domrachev, M. & Lash, A. E. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res* **30**, 207-210, (2002).
- 2 Greshock, J. *et al.* A comparison of DNA copy number profiling platforms. *Cancer Res* **67**, 10173-10180, (2007).
- 3 Gast, A. *et al.* Somatic alterations in the melanoma genome: a high-resolution array-based comparative genomic hybridization study. *Genes Chromosomes Cancer* **49**, 733-745, (2010).

- 4 Kan, Z. *et al.* Diverse somatic mutation patterns and pathway alterations in human cancers. *Nature* **466**, 869-873, (2010).