

Relevance of mortalin to cancer cell stemness and cancer therapy

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Running title: Mortalin contributes to cancer cell stemness

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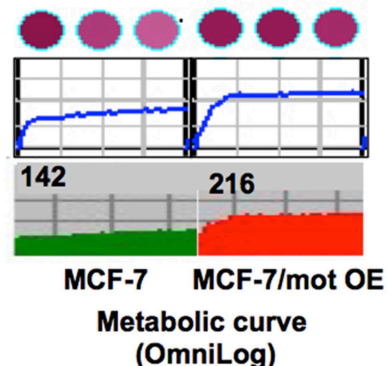
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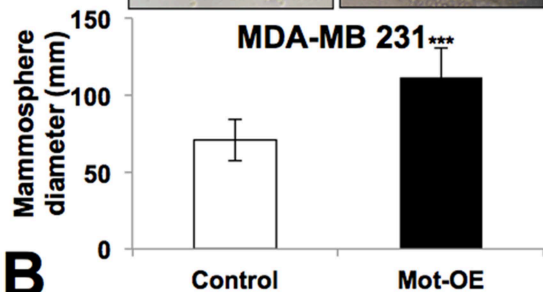
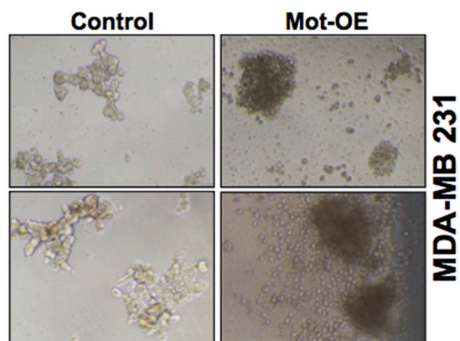
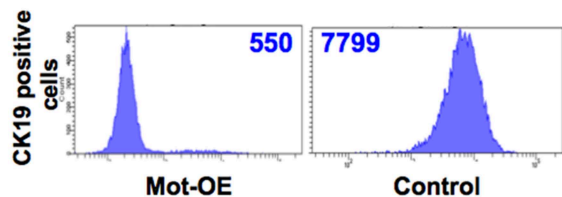
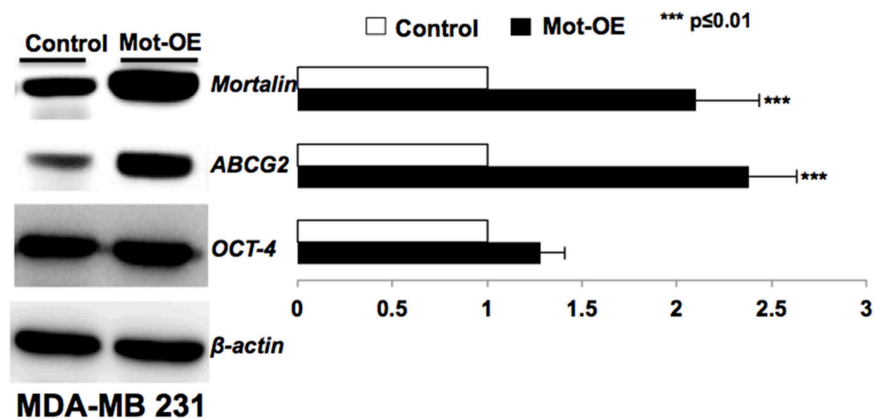
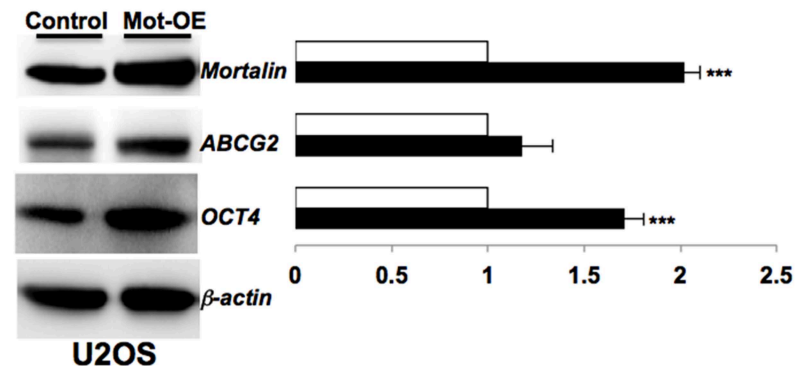
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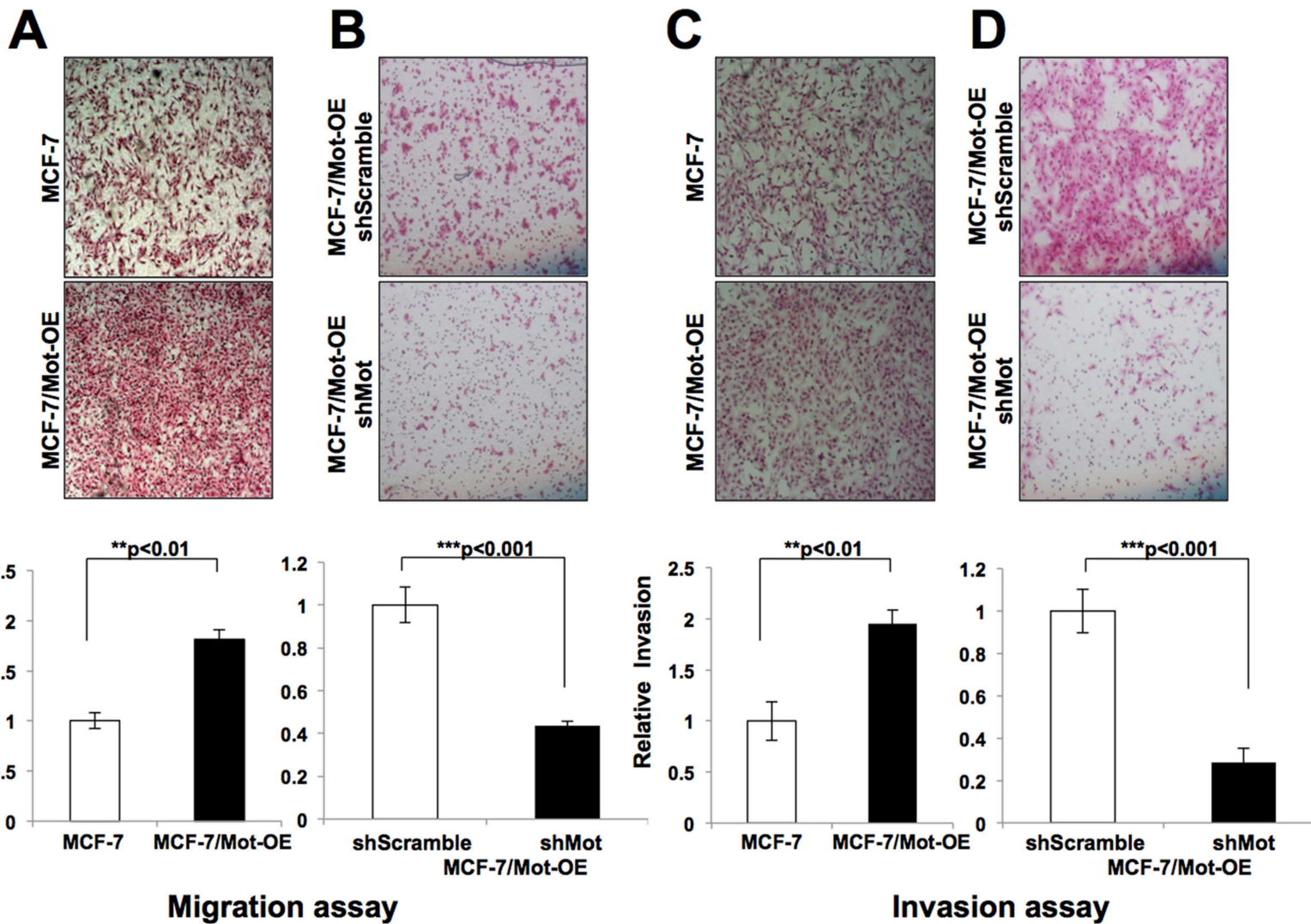
Relative units of mortalin and CD24 expression as determined by Western blotting with specific antibodies. Endogenous β -actin was used as an internal control.

	Cell Lines Name	Description	Mortalin expression (Relative units)	CD24 expression (Relative units)
Normal cells				
1	TIG	Normal Skin Fibroblast	1.0	1.0
2	MRC5	Normal Lung Fibroblast	1.0	1.0
Immortalized cell lines				
3	JFCF-6B	SV 40 Immortalized Fibroblast	2.2	2.1
4	JFCF-4D	SV 40 Immortalized Fibroblast	1.3	1.6
Tumorigenic cell lines				
5	MDA-MB 231	Breast Adenocarcinoma	1.8	0.3
6	Saos-2	Osteogenic Sarcoma	4.5	0.8
7	HeLa	Epithelioid Cervical Carcinoma	4.1	0.5
8	HUH-7	Hepatocellular Carcinoma	1.4	0.6
9	H1299	Non-Small Lung Cell Carcinoma	2.2	0.6
10	MCF-7	Breast Adenocarcinoma	1.8	1.9
11	G361	Skin Malignant Melanoma	2.1	1.4
12	SKOV3	Ovarian Carcinoma	2.2	1.5
13	HUH-6	Human hepatoblastoma	2.0	1.8
14	A549	Adenocarcinoma	3.4	2.8
15	DLD-1	Colorectal Adenocarcinoma	1.4	1.2
16	COLO320	Colorectal Adenocarcinoma	4.0	2.1
17	HCT116	Colorectal Carcinoma	6.7	2.0

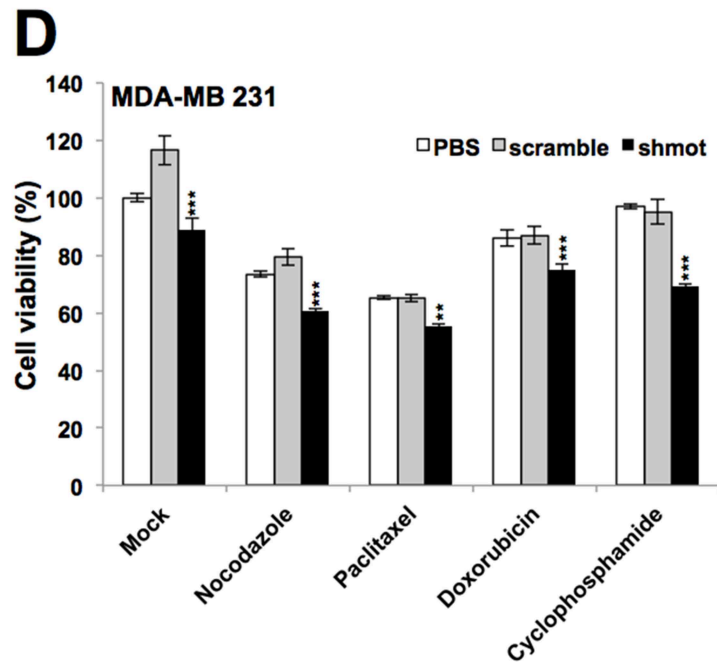
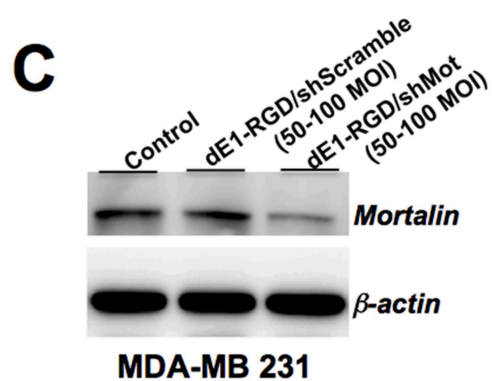
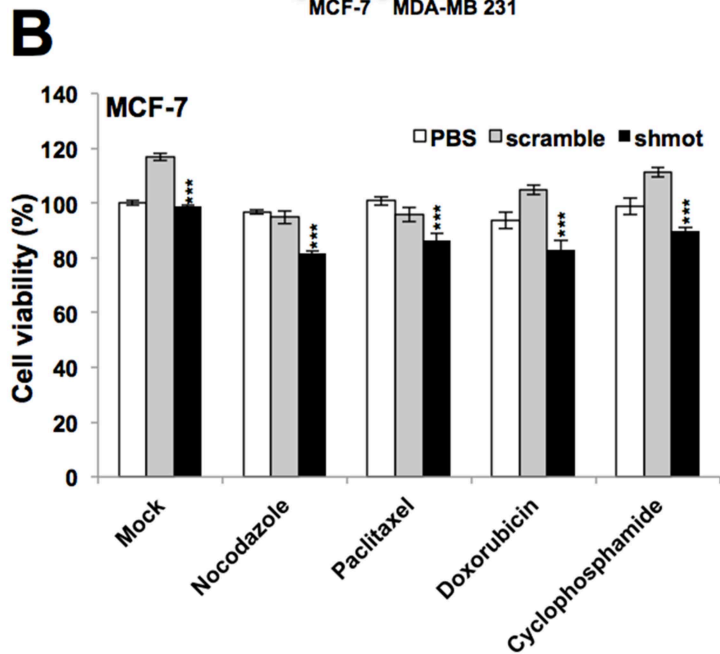
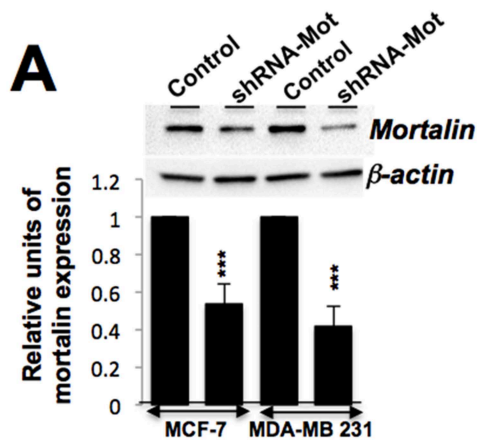
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Supplementary Fig. 1

A**B****C****D**



Supplementary Fig. 3



Supplementary Fig. 4

Supplementary Figure 1. Expression analyses of mortalin and CD24 in human normal and cancer cells. Quantitation of the protein signals obtained by Western blotting with specific anti-mortalin and anti-CD24 antibodies is shown. The expression was normalized against β -actin as a loading control (**A**). Real time metabolic rate of control and mortalin-overexpressing MCF-7 cells as determined by real time metabolic assay in OmniLog incubator (**B**).

Supplementary Figure 2. Mortalin-overexpressing MDA-MB 231 cells show cancer stem cell characteristics. Mortalin-overexpressing MDA-MB 231 cells showed higher degree of spheroid formation expression (**A**), possessed high level of expression of CK19 (**B**), ABCG2, OCT-4 (**C**) as determined by FACS and RT-qPCR analyses, respectively. Mortalin-overexpressing U2OS cells showed high level of expression of ABCG2, OCT-4 as compared to the control cells (**D**).

Supplementary Figure 3. Mortalin knockdown in cancer cells compromised their migration and invasion capacities. Mortalin-overexpressing MCF-7 cells showed higher migration and invasion capacities as compared to control (**A** and **C**, respectively). Mortalin knockdown with specific shRNA compromised their migration and invasion capacities (**B** and **D**).

Supplementary Figure 4. Mortalin knockdown sensitized the cells to drugs. Mortalin knockdown by shRNA plasmid (**A**) and shRNA-expressing adenovirus (**C**) is shown. Mortalin-targeting shRNA-expressing adenovirus treated mortalin-compromised cells showed better response to a variety of drugs (**B** and **D**).