

Supplemental Material

Supplementary Figures

Figure S1: The secretome of senescent melanoma cells triggers melanoma initiating-cell phenotype. (A) Immunofluorescence experiments with MITF and E-cadherin antibodies of 501mel cells exposed to secretome of control (Cont) or of senescent (SSMC) melanoma cells. (B) Human melanoma cell lines (501mel, WM9, MeWo, G361), exposed to the secretome of control melanoma cells (Cont) or to SSMC were analyzed for MITF content by flow cytometry as described in Fig.1E. (C) Cell count of human melanoma cell lines (501mel, WM9) and cells freshly isolated from human biopsy (Patient#1) exposed to secretome of control melanoma cells (Cont) or to SSMC. (D) Effect of secretome of control melanoma cells (Cont) or SSMC on 501mel cell viability as illustrated by FACS analysis of violet blue staining. Staurosporine, (1 μ M) for 16 hrs, was used as a positive control.

Figure S2: IL6 triggers STAT3 activation. Immunofluorescence experiments with MITF and phospho-STAT3 (p-STAT3) antibodies of 501mel cells exposed or not to IL6 (20 ng/ml).

Figure S3: STAT3 inhibition prevents the decrease in MITF level and the activation of STAT3 mediated by the secretome of senescent melanoma cells. Human melanoma cell lines (501mel, WM9) and cells freshly isolated from human biopsy (Patient#1) were transfected with control or STAT3 siRNA and were exposed to the secretome of control melanoma cells (Cont) or SSMC. Cell lysates were analyzed by western blotting with the indicated antibodies.

Figure S4: Effect of the secretome of senescent melanoma cells on genes implicated in mesenchymal and stemness phenotype. (A) QRT-PCR analysis of MITF, AXL, mesenchymal-related (FN1, CCL2, TGFBI, DKK3, THBS2) and stemness (NANOG, OCT4, ALDH1A3) genes in melanoma cells exposed to SSMC versus control medium. (B) Ingenuity pathway analysis (IPA®) was performed on the 52 genes specifically regulated in three melanoma cell types by the SSMC.

Supplementary Tables

Division	Cont,%	SSMC,%
0	0,9	1,9
1	7,9	26,9
2	53,6	57,1
3	29,9	11,8
4	6,6	2,2

Table S1: Effect of the secretome from control (Cont) or senescent (SSMC) melanoma cells on melanoma populations according to the number of divisions. Quantification by the MACSQuant software.

Division	Cont,%	IL6, %
0	0,6	1,4
1	5,15	17,9
2	38,6	63,9
3	43,6	14,1
4	11,9	2,7

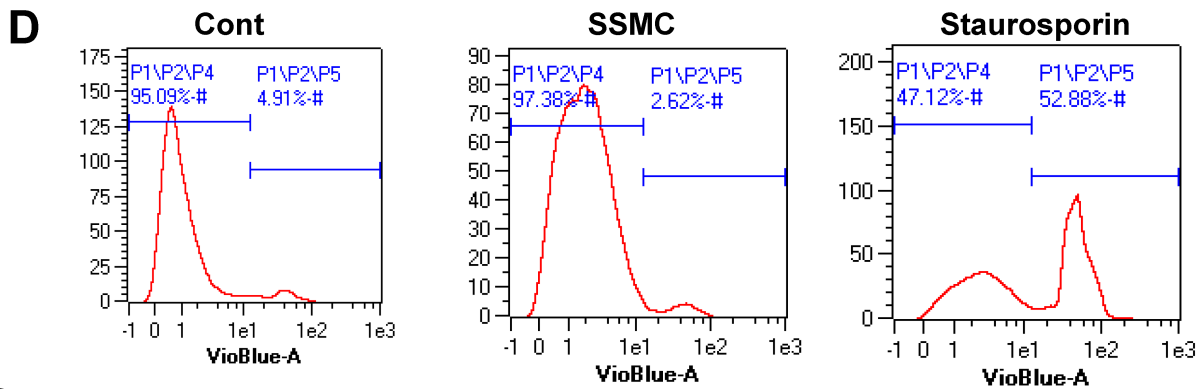
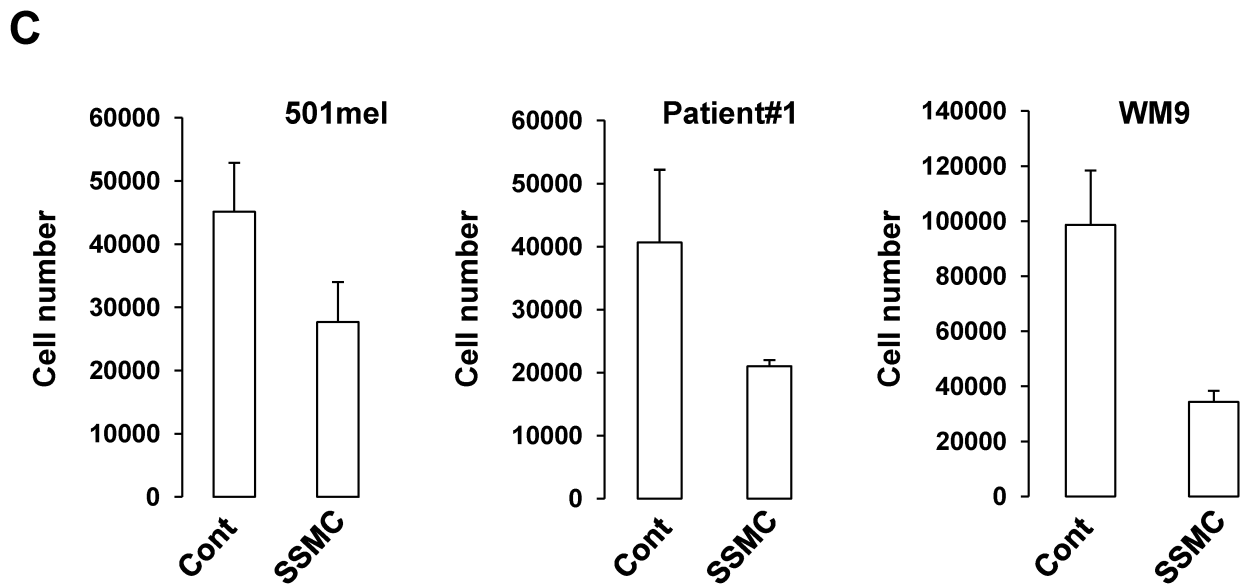
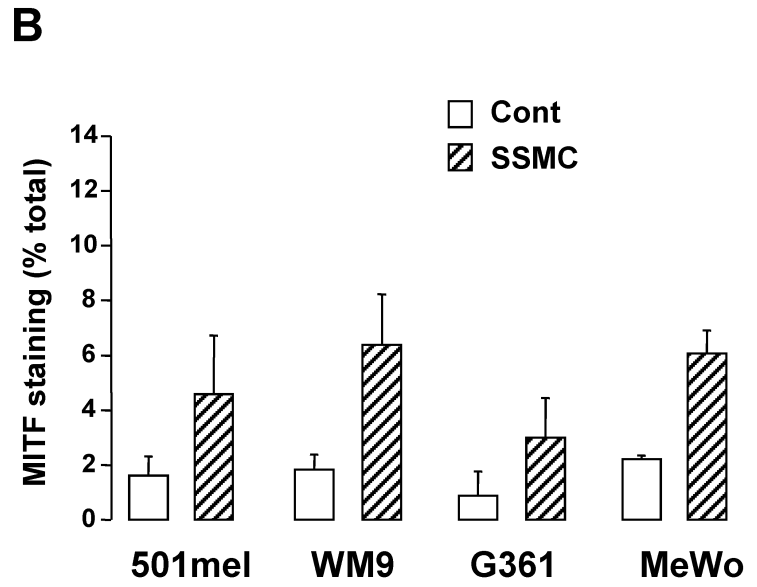
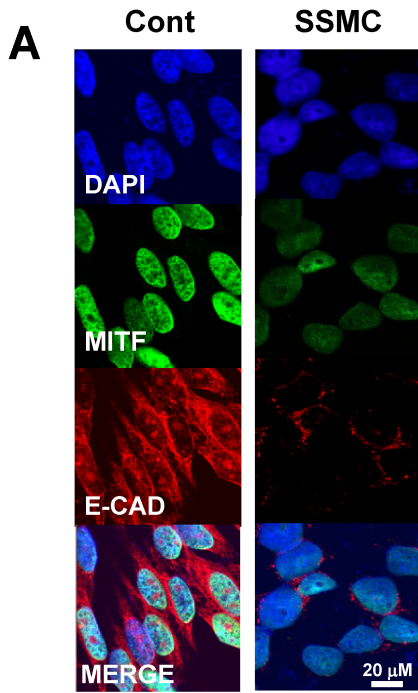
Table S2: Effect of the secretome from control (Cont) or IL6-treated melanoma cells on melanoma populations according to the number of divisions. Quantification by the MACSQuant software.

Gene symbol	Inhibition (%)	p-value
HPS4	42.50	0.0384
MBP	41.67	0.0422
MITF	28.22	0.0006
MLANA	40.26	0.0024
MLPH	43.16	0.0481
OSTM1	24.02	0.0219
RAB27A	39.49	0.0167
RAB38	37.45	0.0008
SLC24A5	36.28	0.0124

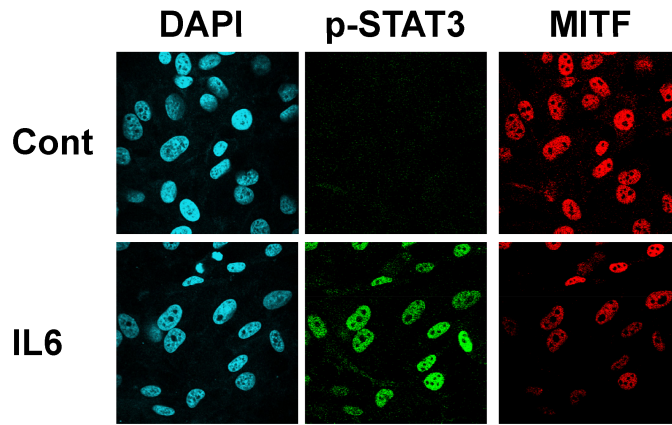
Table S3: Inhibition of MITF and some MITF target genes in 501mel, WM9 and melanoma cells from patient#1 exposed to SSMC. Values were extracted from Illumina BeadChips Arrays.

Symbol	Entrez Gene Name	Cont/SSMC, present study		Down regulated by MITF, Bertolotto et al, 37		Increased in the invasive phenotype, Widmer et al, 36
		Log (FC)	p-value	Log(FC)	p-value	
DKK3	dickkopf 3 homolog (Xenopus laevis)	5.489	6.39E-03	-1.5355	0.0408	yes
LOXL4	lysyl oxidase-like 4	3.679	5.74E-03	-1.510	0.0011	yes
ALDH1A3	aldehyde dehydrogenase 1 family, member A3	3.495	4.17E-03	-2.1818	0.0006	yes
TIMP3	TIMP metalloproteinase inhibitor 3	2.796	2.36E-03	-1.8110	0.0008	yes
PXDN	peroxidase homolog (Drosophila)	2.767	1.13E-03	-1.7038	0.0213	yes
IL1B	interleukin 1, beta	2.096	1.55E-02	-5.7419	0.0196	yes
AXL	AXL receptor tyrosine kinase	2.072	4.65E-03	-1.5008	0.0022	yes
PMEPA1	prostate transmembrane protein, androgen induced 1	1.889	4.80E-03	-4.4740	0.0385	yes
COL8A1	collagen, type VIII, alpha 1	1.79	3.02E-03	-3.8748	2.81E-05	yes
TMEM171	transmembrane protein 171	1.698	1.65E-02	-2.1069	0.0012	yes
CCL2	chemokine (C-C motif) ligand 2	1.686	2.00E-02	-4.8434	0.0404	yes
TMEM47	transmembrane protein 47	1.66	4.74E-03	-3.3295	2.74E-05	yes
MYOF	myoferlin	1.234	1.21E-02	-1.8567	0.0013	yes
EFEMP2	EGF containing fibulin-like extracellular matrix protein 2	1.048	1.36E-02	-1.7690	0.0069	yes
GNG2	guanine nucleotide binding protein (G protein), gamma 2	1.025	4.23E-03	-1.5772	0.0266	yes

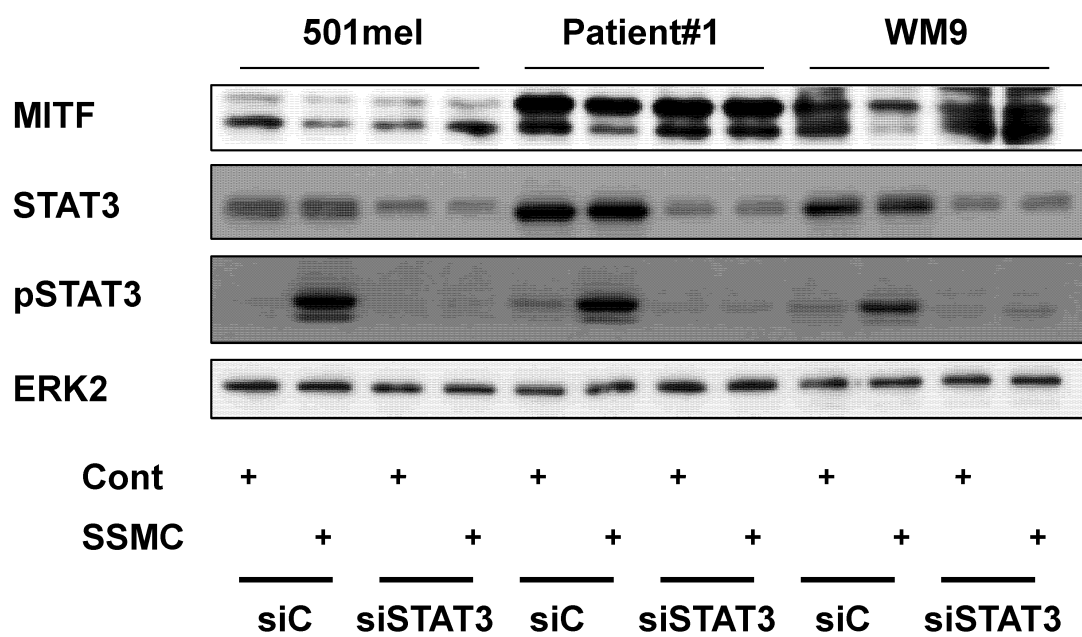
Table S4: Genes that are both up-regulated in melanoma cell lines with invasive phenotype³⁶ and down-regulated in A375 melanoma cells over-expressing MITF.



Sup Figure 1

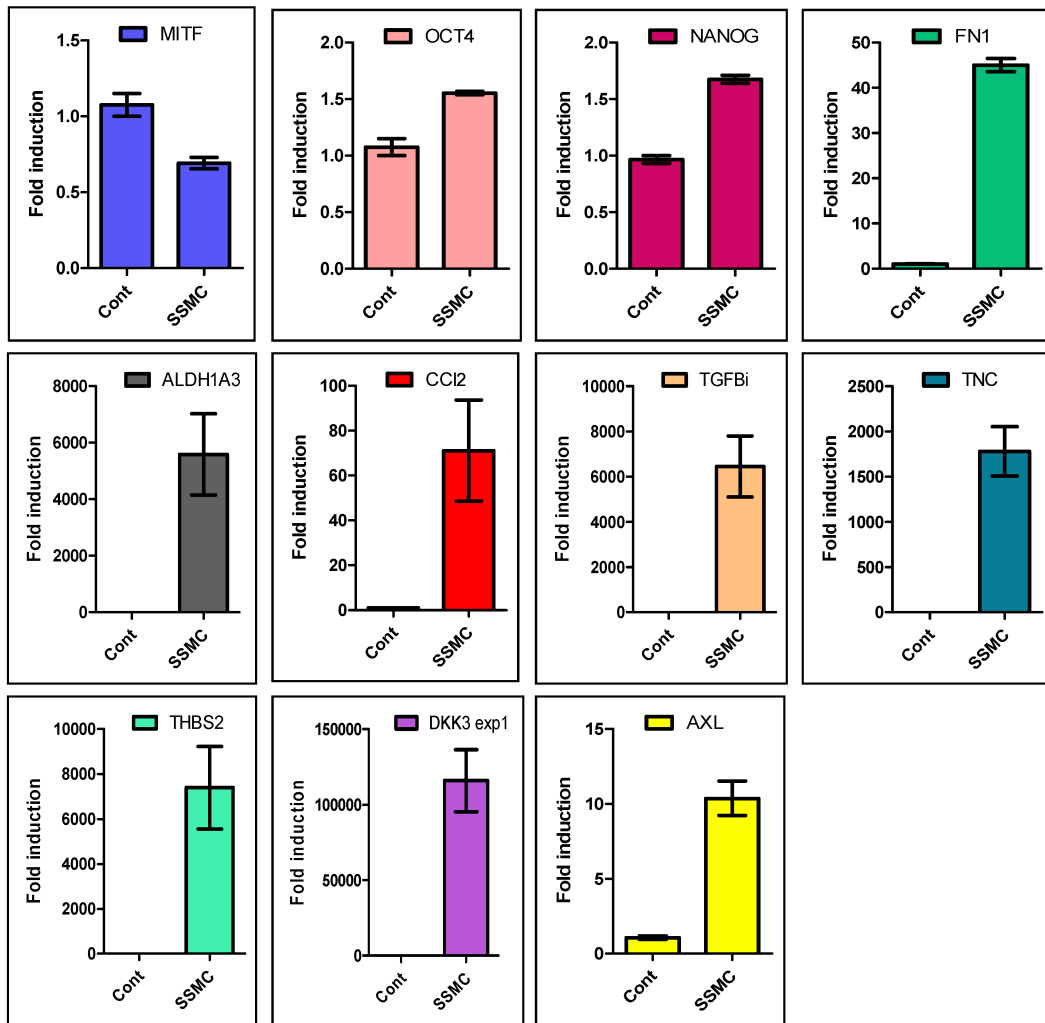


Sup Figure 2



Sup Figure 3

A



B

Molecular and Cellular Functions

Name	p-value	# Molecules
Cellular Movement	2,78E-07 - 1,35E-02	19
Cell-To-Cell Signaling and Interaction	1,09E-06 - 1,22E-02	16
Cellular Growth and Proliferation	1,95E-06 - 1,22E-02	28
Cellular Assembly and Organization	2,74E-05 - 1,22E-02	22
Cellular Function and Maintenance	2,74E-05 - 1,22E-02	18

Figure S4