Table 1. Varieties of chestnuts and procedures for flour preparation in different areas of Tuscany

Area of origin	Varieties	Drying	Temperature	Time	Kind of mill
Caprese Michelangelo(1)	Marroncini, Pistolesi	Traditional	About 30°C	35/40 days	Grindstones powered by electriciy
Caprese Michelangelo (2)	Marroncini, Pistolesi 'Castagne gialle'	Traditional	30-40°C	35/40 days	Grindstones powered by electriciy
Caprese Michelangelo (3)	Marroncini, Pistolesi	Air drying oven	N.D.	From 7 to 15 days	Hammermill powered by electriciy
Garfagnana (1)	Selvana, Mozza e Nerona	Traditional	28-30°C	40 days	Grindstones powered by water
Garfagnana (2)	Cesarucche	Traditional	28-30°C	40 days	Grindstones powered by water
Mugello (1)	Marrone Fiorentino	Traditional	N.D.	40 days	Grindstones powered by water
Mugello (2)	Marrone Fiorentino	Traditional	N.D.	40 days	Grindstones powered by water
Orsigna (1)	Calarese, Pastinese, Ceppa	Traditional	30°C	45 days	Grindstones powered by water
Orsigna (2)	Calarese, Pastinese, Ceppa	No traditional	30°C	45 days	Grindstones powered by water
Alta Maremma	Carpinese, Rossolina, Marrone	Traditional	40°C	30 days	Grindstones powered by water
Lunigiana	Bresciana, Carpanese, Fosetta Marzolina, Moretta, Primaticcia Rigola, Rossella, Rossola	Traditional	N.D.	N.D.	Grindstones powered by electricity
Amiata	Bastarda, Marrone, Cecia, Domestica	Traditional	N.D.	42 days	Grindstones powered by electriciy

N.D. Not declared; Number in areas of origin (1) or (2) or (3) refers to different locations inside the area

Table 2: Main chemical composition of Marrone Fiorentino Chestnut flour.

	Sweet Flour
Moisture (g/100g)	5.4±0.9
Protein (g/100 g, dry weight)	4.0±0.4
Fat (g/100g, dry weight)	5.2±1.0
Saturated Fat (g/100g, dry weight)	0.8±0.2
Monosatured Fat (g/100g, dry weight)	2.4±0.4
Polysaturated Fat (g/100g, dry weight)	1.7±0.5
Sugars (g/100g, dry weight)	29.9±8.3
Starch (g/100g, dry weight)	41.8±5.5
Ash (g/100g , dry weight)	2.5±0.4
K (mg/100 g dry weight)	1076±269
Mg (mg/100 g dry weight)	77.3±21.6
Ca (mg/100 g dry weight)	5.7±2.1
Cu (mg/100 g dry weight)	0.6±0.1
Fe (mg/100 g dry weight)	1.6±0.7
Mn (mg/100 g dry weight)	1.9±0.5
Na (mg/100 g dry weight)	4.8±6.7
Zn (mg/100 g dry weight)	0.9±0.1

Data are means ± SD *(n=24)*

Table 3. Composition in total Polyphenols, Tocopherol and SLs in Tuscan chestnut sweet flour extract obtained from different areas of Tuscany

Origin	**Total Polyphenols** (mg/kg gallic acid)		Total tocopherols (mg/kg)		γ -Tocopherol %	δ –tocopherol %	α-tocopherol %	**Total SL** (mg/kg)	
	mean	SD	mean	SD				mean	SD
Alta Maremma	182 [cd]	241	18 [e]	2	89	3	6	56	4
Amiata	223 [abcd]	129	83 [bcd]	2	96	3	1	49	6
Caprese Michel. (1)	2263 [abcd]	420	22 [e]	1	89	7	3	53	5
Caprese Michel. (2)	1688 [d]	115	103 [ab]	12	97	2	1	52	4
Caprese Michel. (3)	2520 [ab]	27	114 [a]	17	96	3	1	N.D.	
Garfagnana (1)	2602 [ab]	203	64 [d]	3	95	3	1	50	3
Garfagnana (2)	2015 [bcd]	269	64 [d]	5	95	3	1	49	1
Lunigiana	1689 [d]	140	102 [ab]	7	96	3	1	51	1
Mugello (1)	2799 [a]	297	90[bc]	5	97	2	1	49	3
Mugello (2)	2556 [ab]	226	75[cd]	5	96	2	1	48	3
Orsigna (1)	2190 [abcd]	74	82[bcd]	9	94	4	1	52	2
Orsigna (2)	2452 [abc]	184	21[e]	1	91	3	3	52	1

Mean values followed by the same letter are not significantly different (99% level of significance). SL: sphingolipids. N.D. Not determined

Table 4. Effect of total polyphenols or tocopherols on control C2C12 cells and on Dexa- and serum starvation-induced atrophic cells

Cell treatments	Cell diameter (µm)		Number of myonuclei/cell	
	mean	SD	Mean	SD
Vehicle	15	4	11	2
Dexa	8 *	1	6*	2
Dexa + Total Polyphenol extract	7 *	1	6*	2
Dexa + Total Tocopherol extract	12 #	1	9#	2
Starvation (-FCS)	11 *	1	8	2
Starvation + Total Polyphenol extract	9 *§	1	6*§	1
Starvation + Total Tocopherol extract	16	2	11	1
Cycloheximide	9*	2	6*	1

C2C12 myotubes were incubated with vehicle or with polyphenol or tocopherol extracts (100 nM) or cycloheximide (3 µM) for 1 h before the addition of Dexa or serum-starved as indicated min Methods. Thirthy six hours later, cells were observed at phase contrast microscope. Data are reported as mean±standard deviation of at least three independent experiments. Student' *t* test *P<0.05 vs. control cells (vehicle), #P<0.05 vs. specific control (Dexa), §p<0.05 vs. serum-starved cells

Table 5. Effect of total polyphenols or tocopherol on [^{3}H]-leucine incorporation in control cells and in cells induced to atrophy by Dexa-or serum starvation

Cell treatments	[^{3}H]-leucine incorporation (dpm/well)	
	Mean	SD
Vehicle	1500	189
+ Dexa	1350	250
Starvation (– FCS)	780*	315
Total Polyphenol extract	2570*	315
Total Tocopherol extract	2875*	405
Dexa+ Total Polyphenol extract	2175*	185
Dexa+ Total Tocopherol extract	1895*§	205
-FCS + Total Polyphenol extract	1120	200
-FCS + Total Tocopherol extract	1065*	140

C2C12 myotubes were incubated with vehicle or with polyphenol or tocopherol extracts (100 nM) for 1 hour before the addition of Dexamethasone (Dexa) or serum-starved as indicated in Methods. Thirthy six hours later, cells were treated with [^{3}H]-Leucine as reported in Methods. Data are reported as mean and standard deviation of at least three independent experiments. Student' *t* test *$P<0.05$ vs. control cells (vehicle), §$P<0.05$ vs. specific control. –FCS: absence of fetal calf serum.

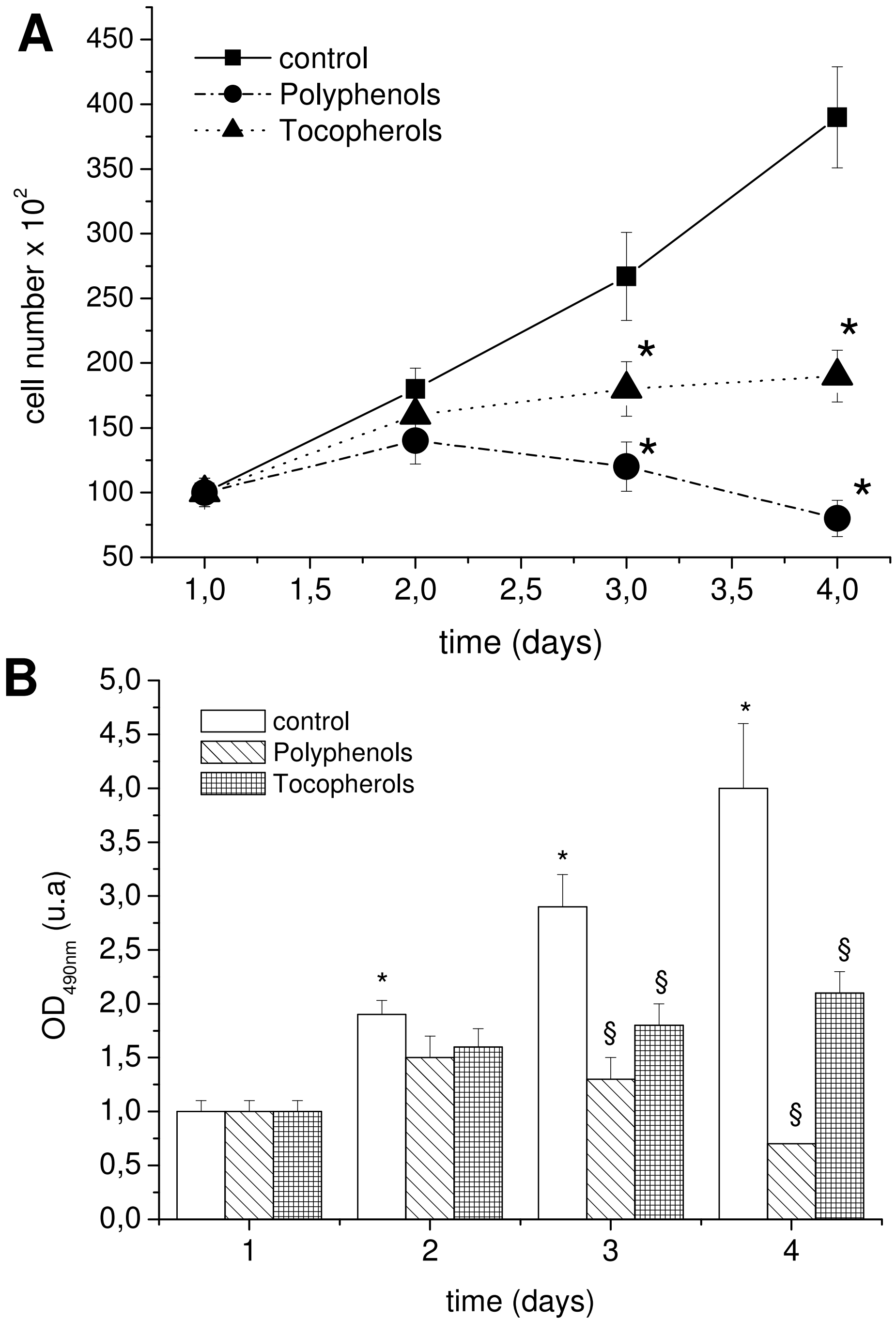

A
control
Polyphenols
Tocopherols
cell number x 10^2
450
400
350
300
250
200
150
100
50
1,0
1,5
2,0
2,5
3,0
3,5
4,0
time (days)
B
control
Polyphenols
Tocopherols
OD$_{490nm}$ (u.a)
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
1
2
3
4
time (days)

Figure 1

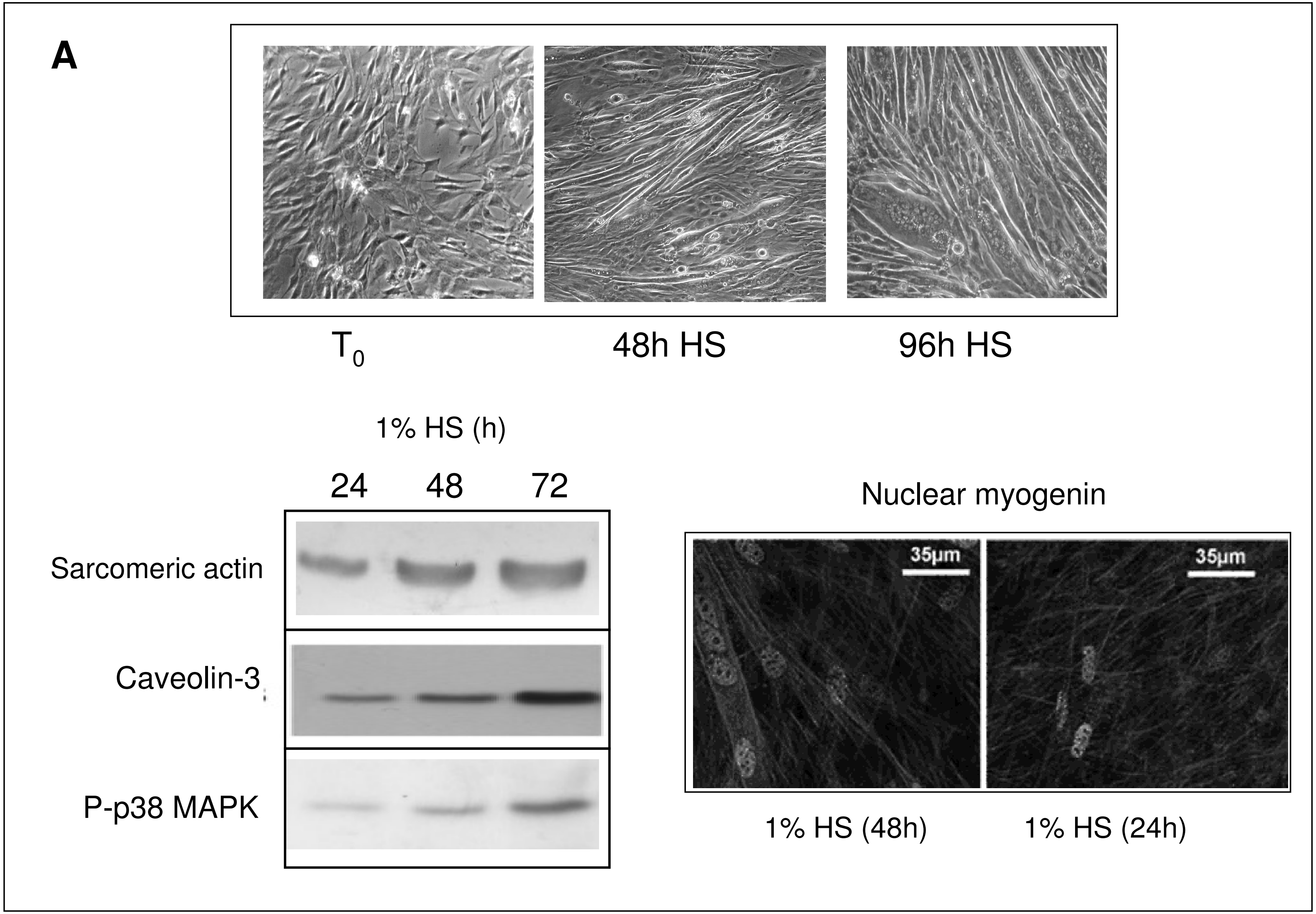

A
T0
48h HS
96h HS
1% HS (h)
24
48
72
Sarcomeric actin
Caveolin-3
P-p38 MAPK
Nuclear myogenin
35µm
35µm
1% HS (48h)
1% HS (24h)

Figure 2A

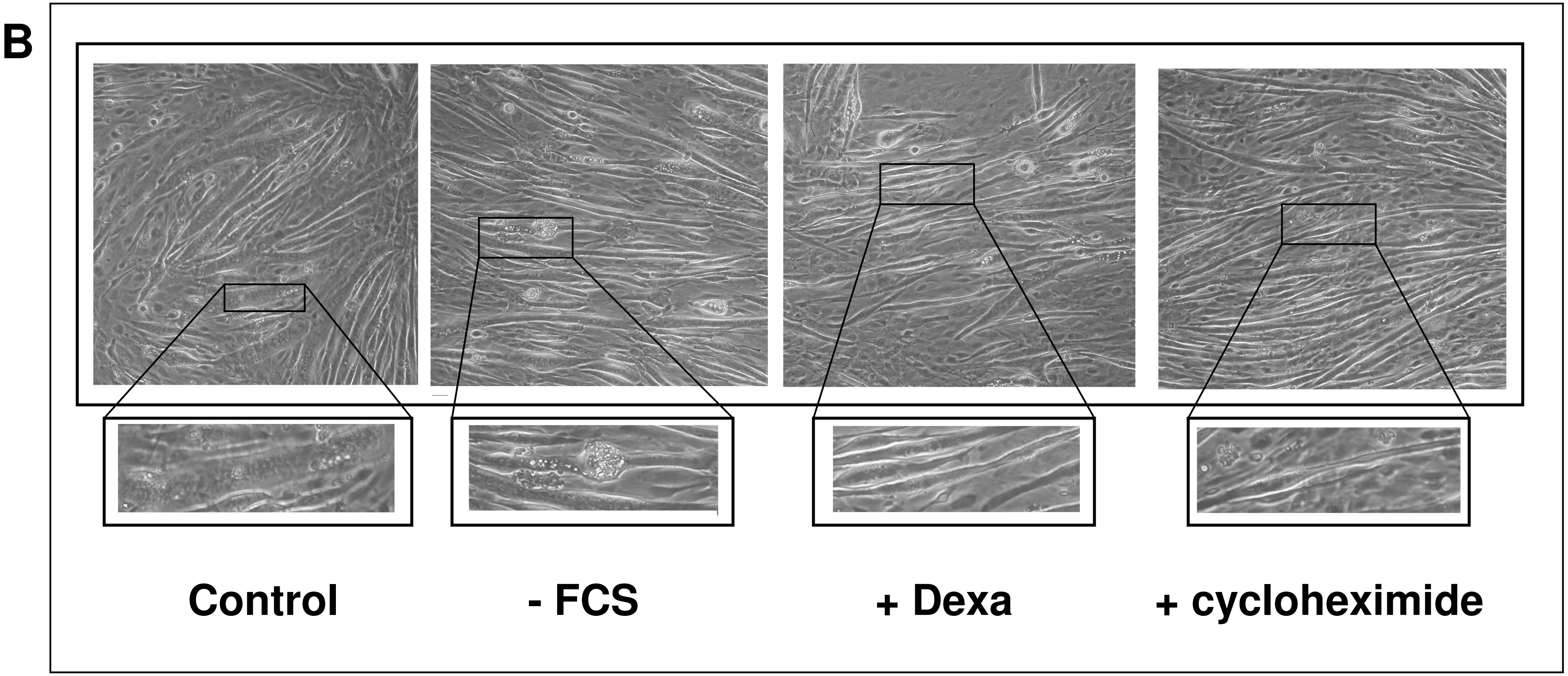


Figure 2B

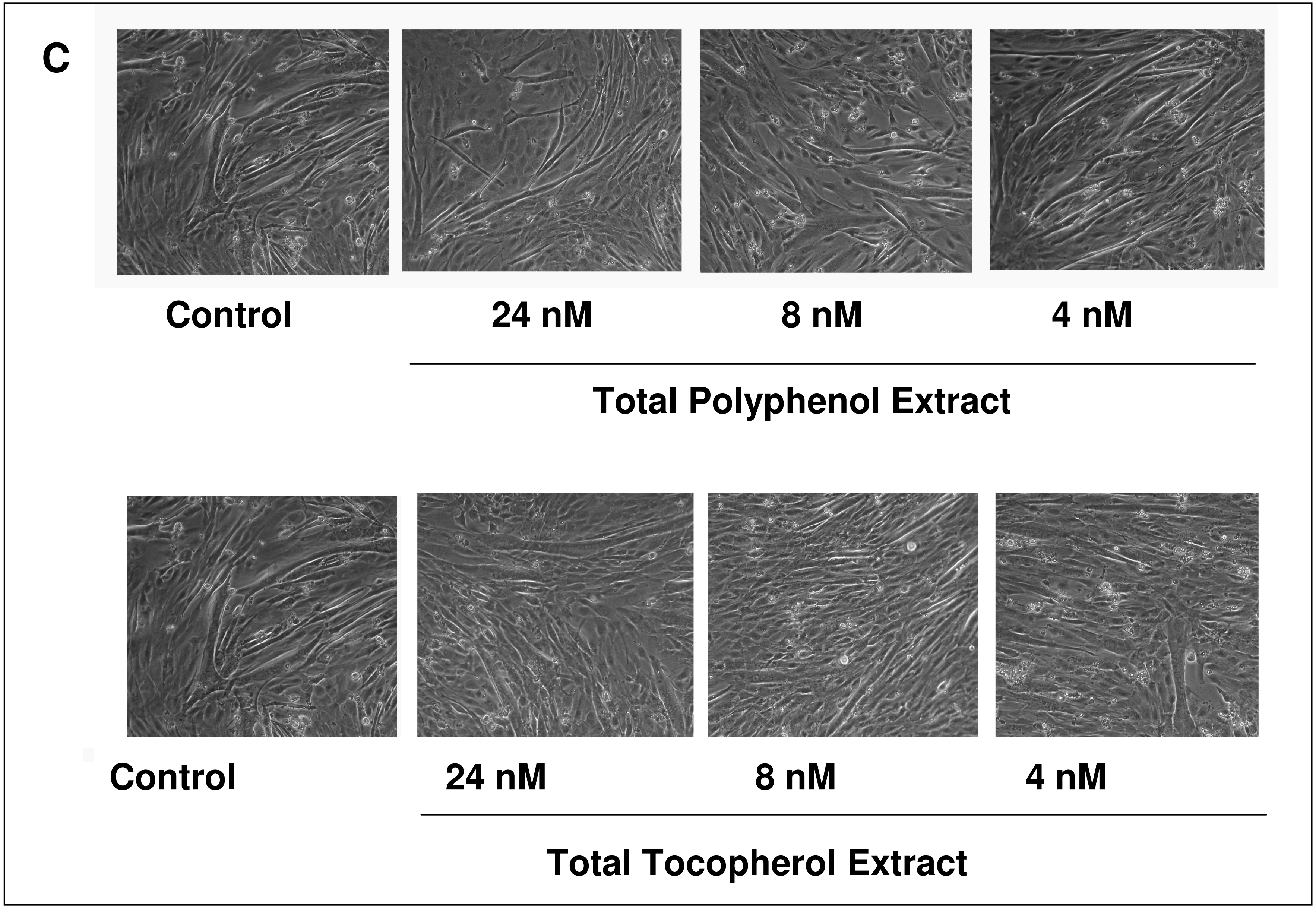

C
Control
24 nM
8 nM
4 nM
Total Polyphenol Extract
Control
24 nM
8 nM
4 nM
Total Tocopherol Extract

Figure 2C

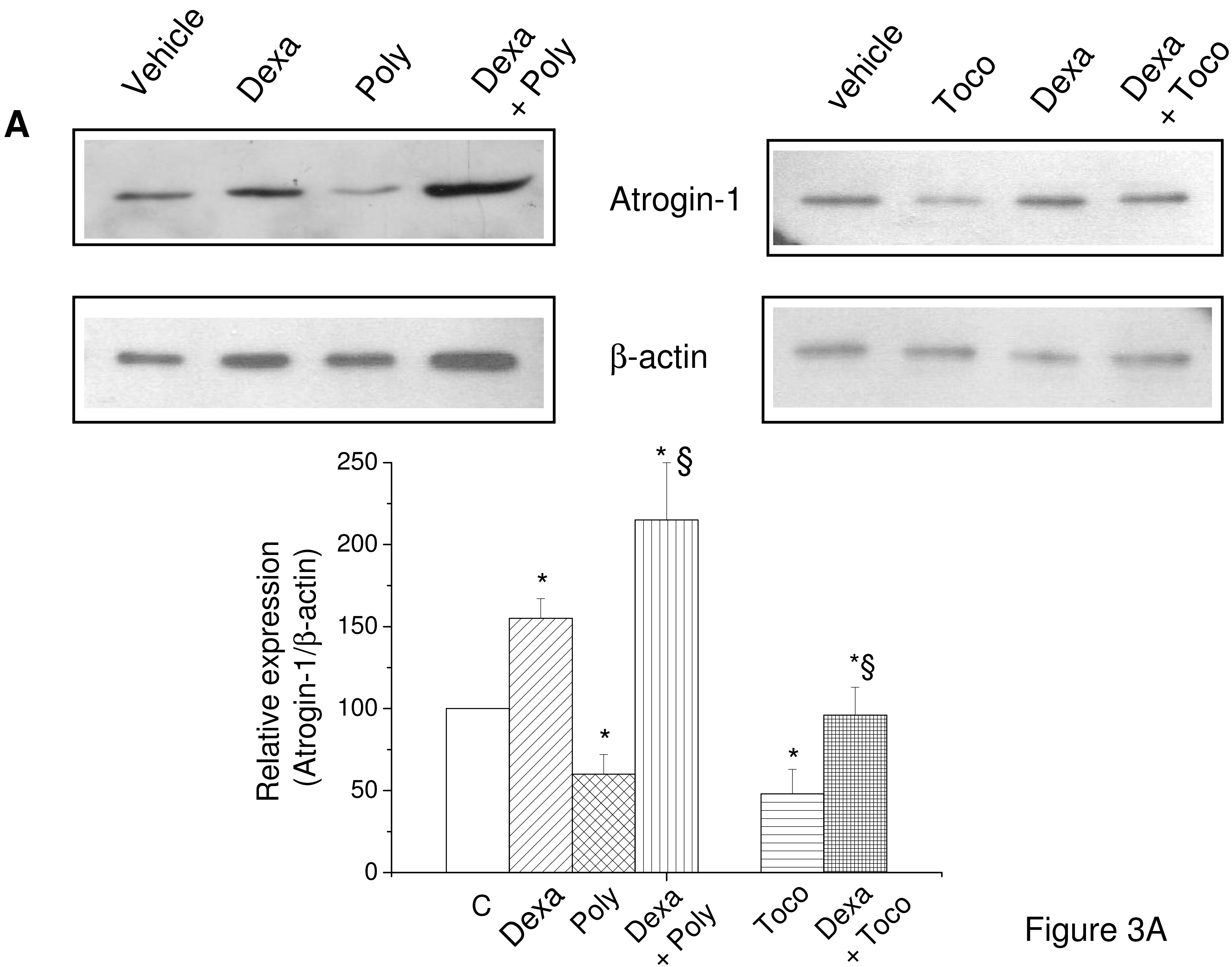

A
Vehicle
Dexa
Poly
Dexa + Poly
vehicle
Toco
Dexa
Dexa + Toco
Atrogin-1
β-actin
Relative expression (Atrogin-1/β-actin)
0
50
100
150
200
250
*
*
* §
*
*§
C
Dexa
Poly
Dexa + Poly
Toco
Dexa + Toco

Figure 3A

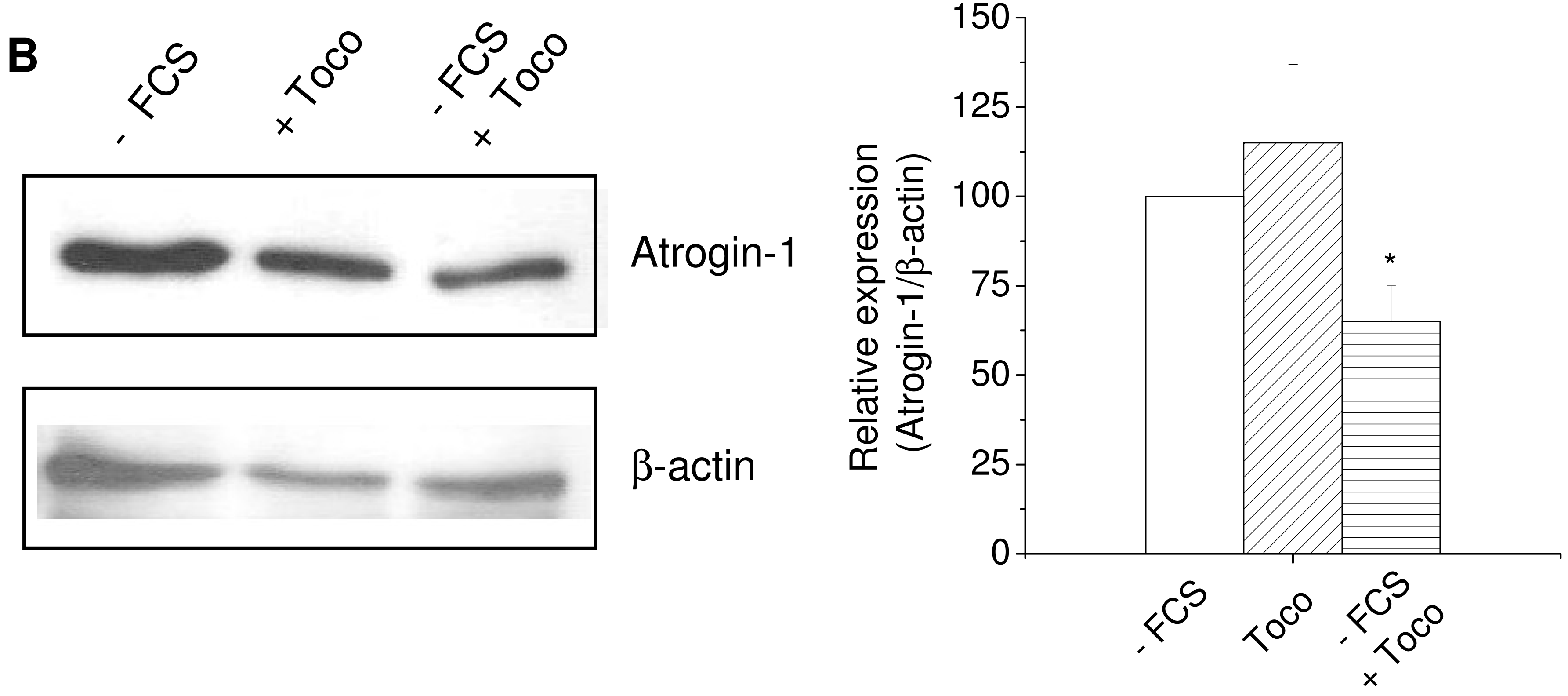

B
- FCS
+ Toco
- FCS
+ Toco
Atrogin-1
β-actin
150
125
100
75
50
25
0
Relative expression
(Atrogin-1/β-actin)
*
- FCS
Toco
- FCS
+ Toco

Figure 3B

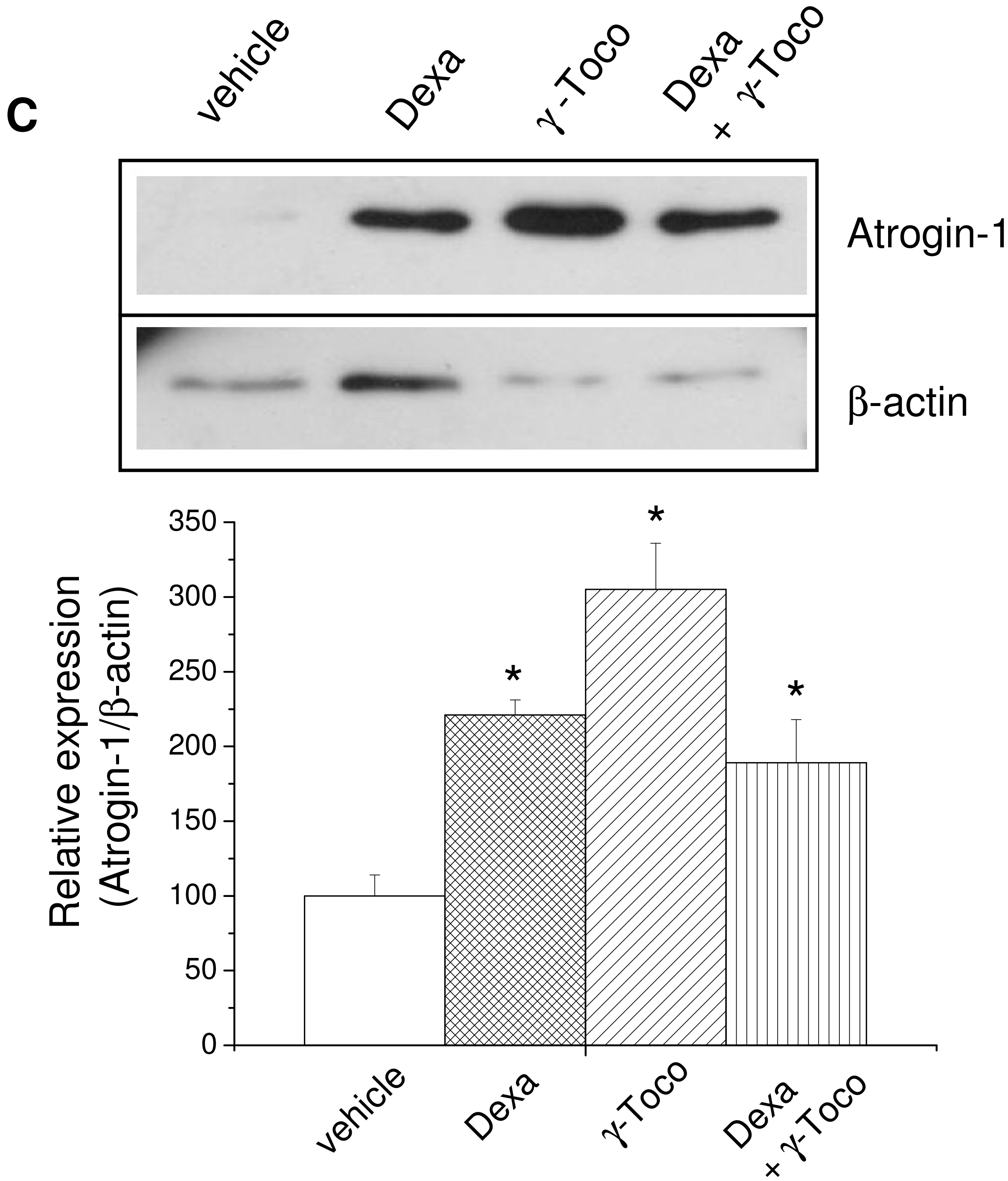
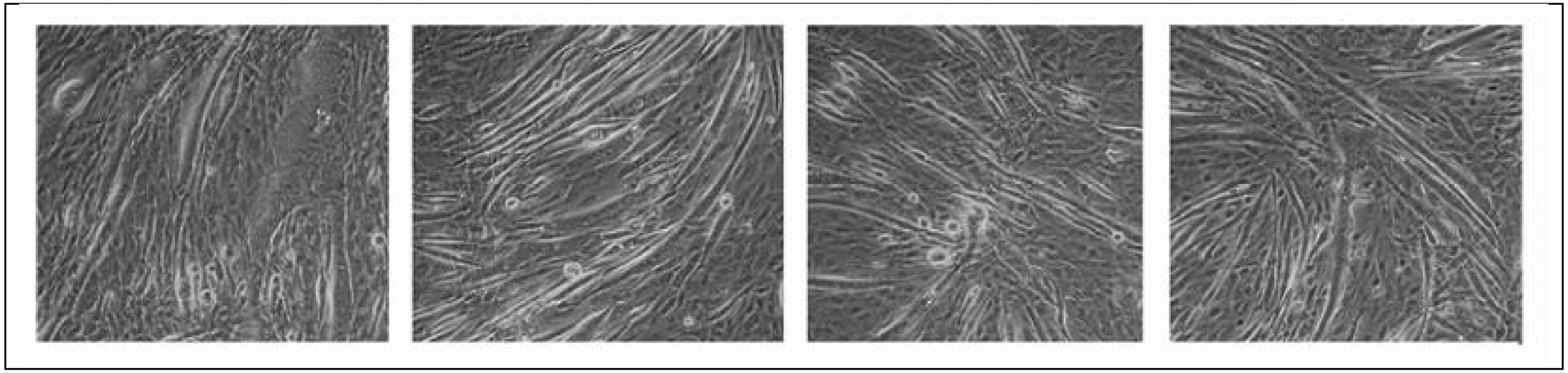

C
vehicle
Dexa
γ-Toco
Dexa + γ-Toco
Atrogin-1
β-actin
Relative expression (Atrogin-1/β-actin)
350
300
250
200
150
100
50
0
*
*
*
vehicle
Dexa
γ-Toco
Dexa + γ-Toco

Figure 3C

D



C Dexa γ-Toco Dexa + γ-Toco

Figure 3D

A

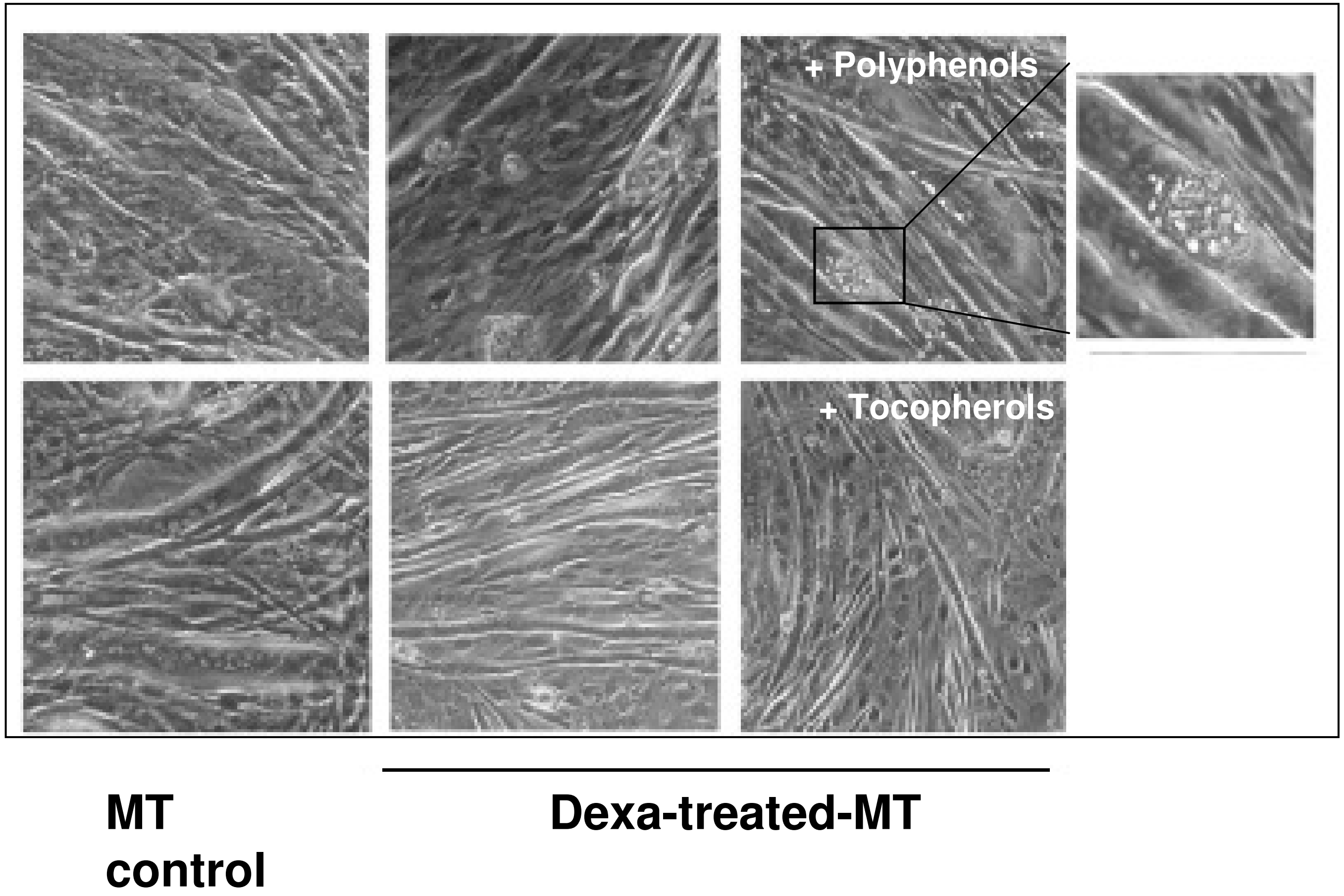


Figure 4A

B

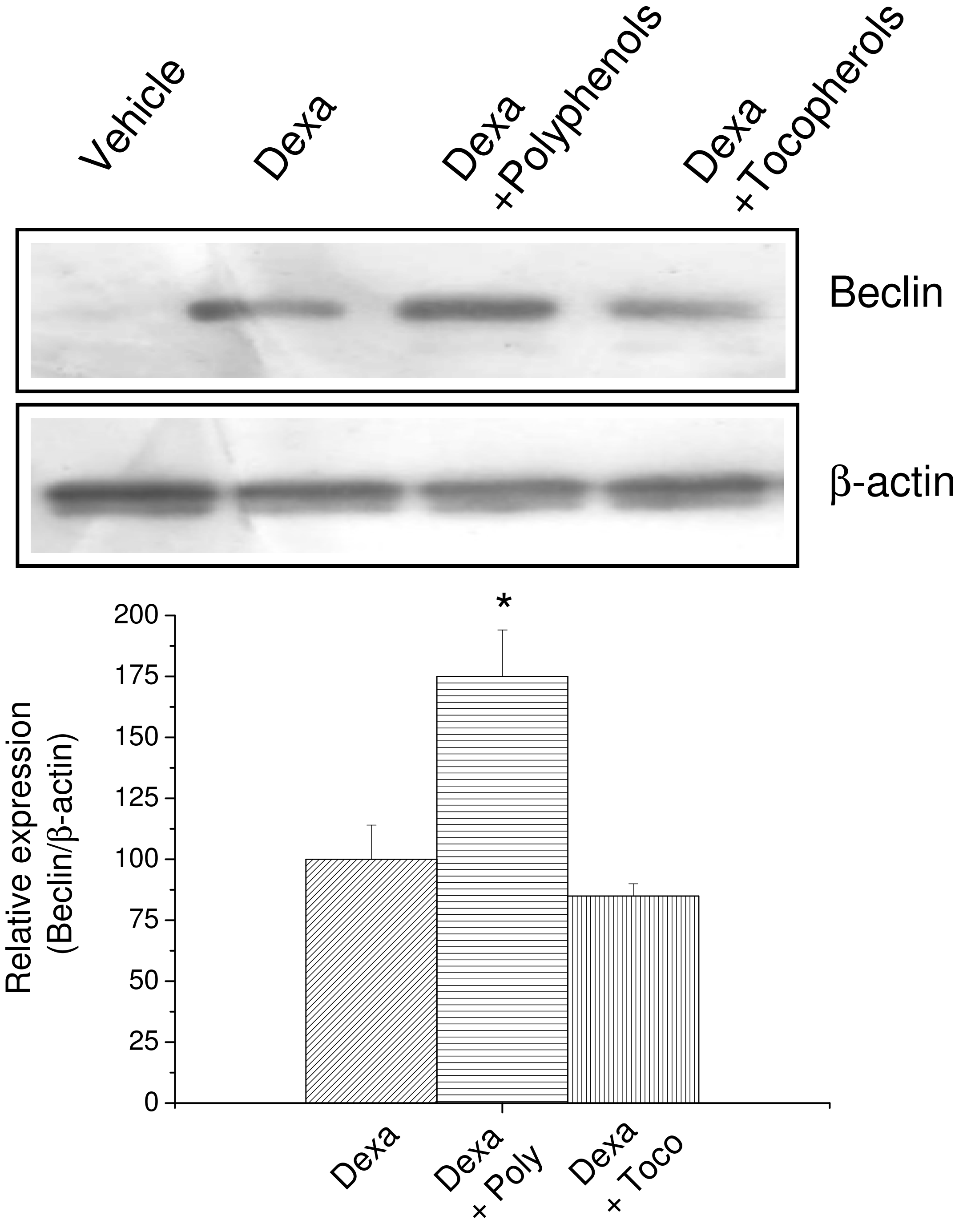
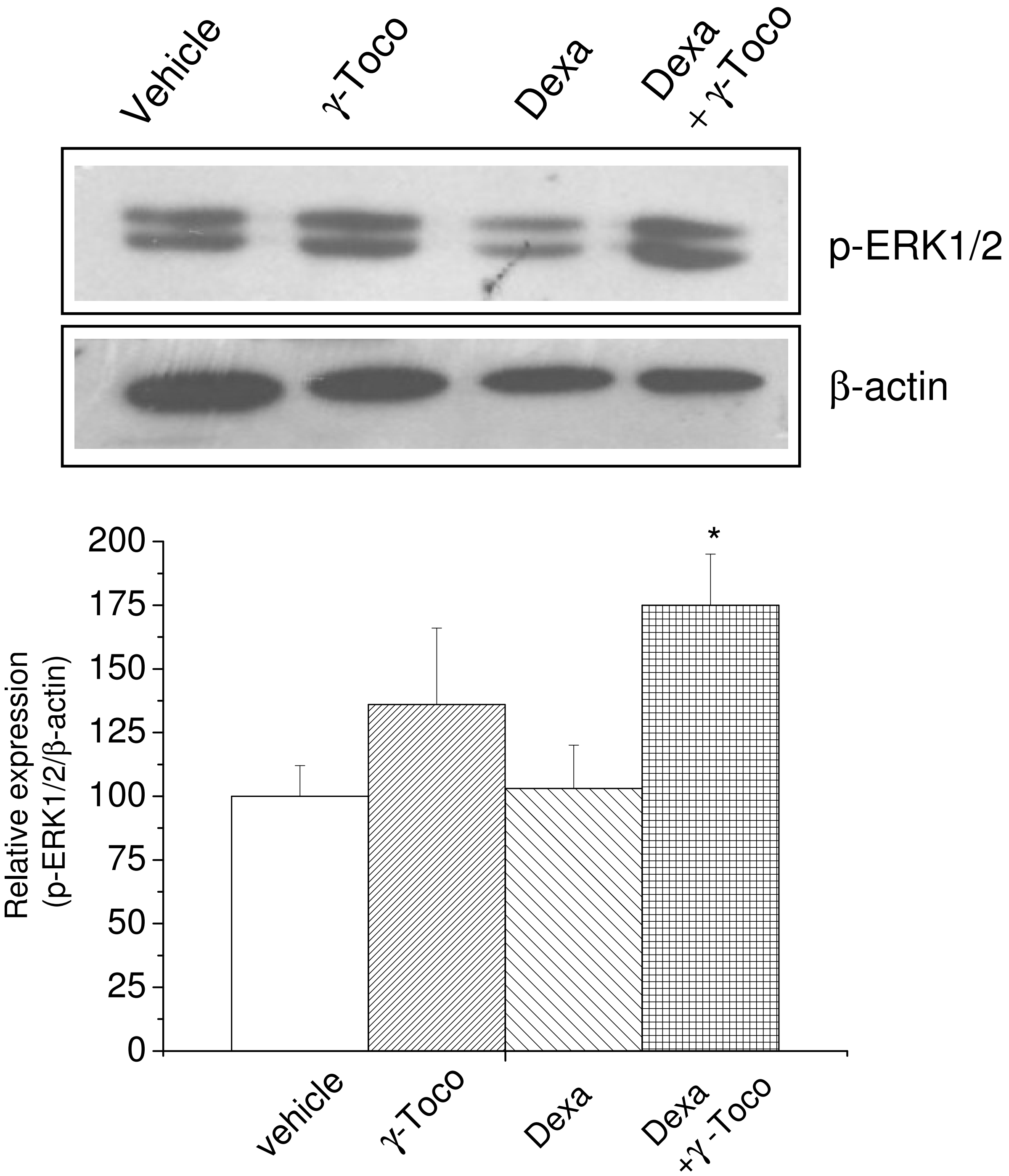

Vehicle
Dexa
Dexa +Polyphenols
Dexa +Tocopherols
Beclin
β-actin
*
200
175
150
125
100
75
50
25
0
Relative expression (Beclin/β-actin)
Dexa
Dexa + Poly
Dexa + Toco

Figure 4B



Vehicle
γ-Toco
Dexa
Dexa + γ-Toco
p-ERK1/2
β-actin
Relative expression (p-ERK1/2/β-actin)
200
175
150
125
100
75
50
25
0
*
vehicle
γ-Toco
Dexa
Dexa +γ -Toco

Figure 5

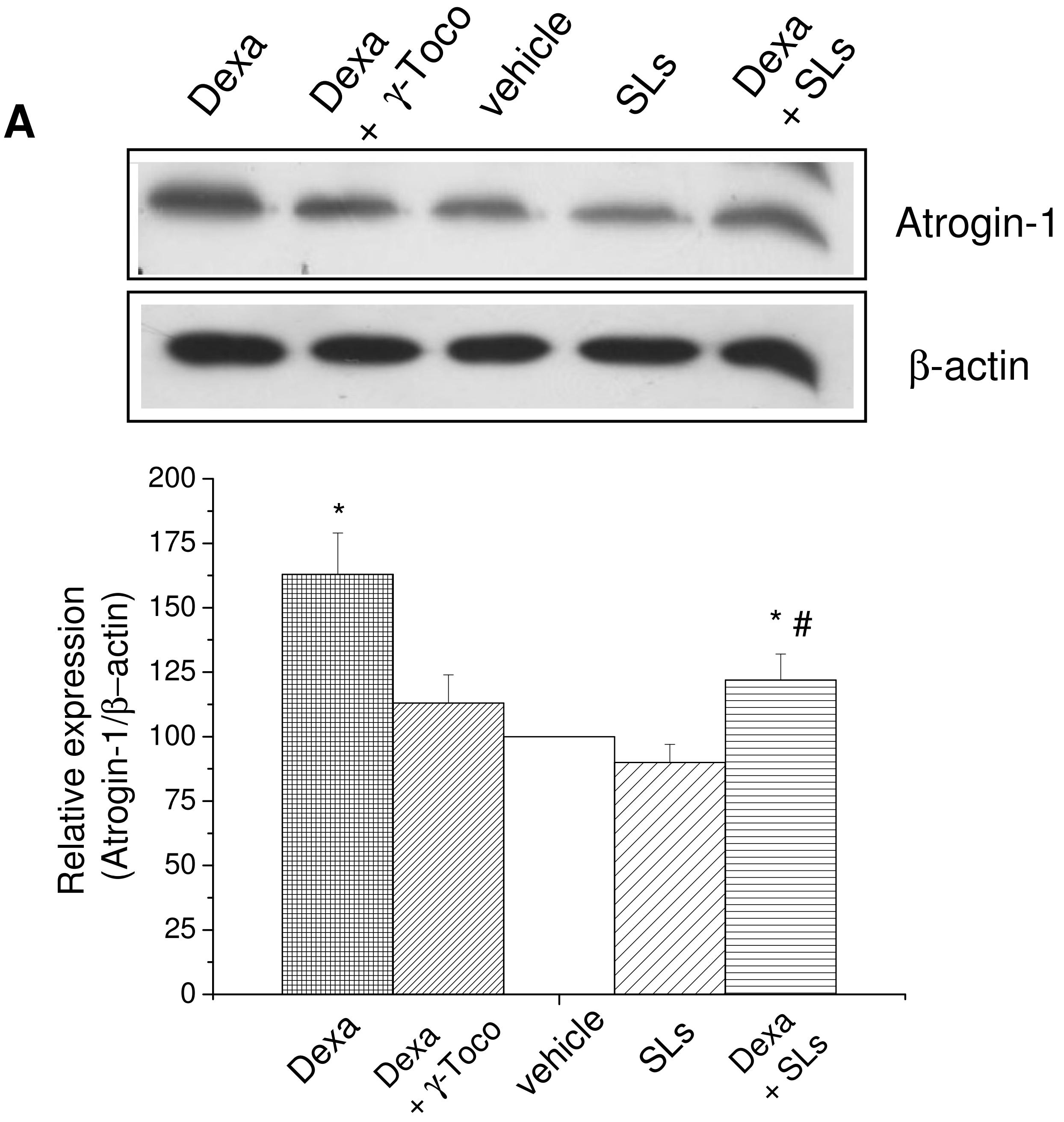

A
Dexa
Dexa + γ-Toco
vehicle
SLs
Dexa + SLs
Atrogin-1
β-actin
Relative expression (Atrogin-1/β–actin)
200
175
150
125
100
75
50
25
0
*
* #

Figure 6A

B

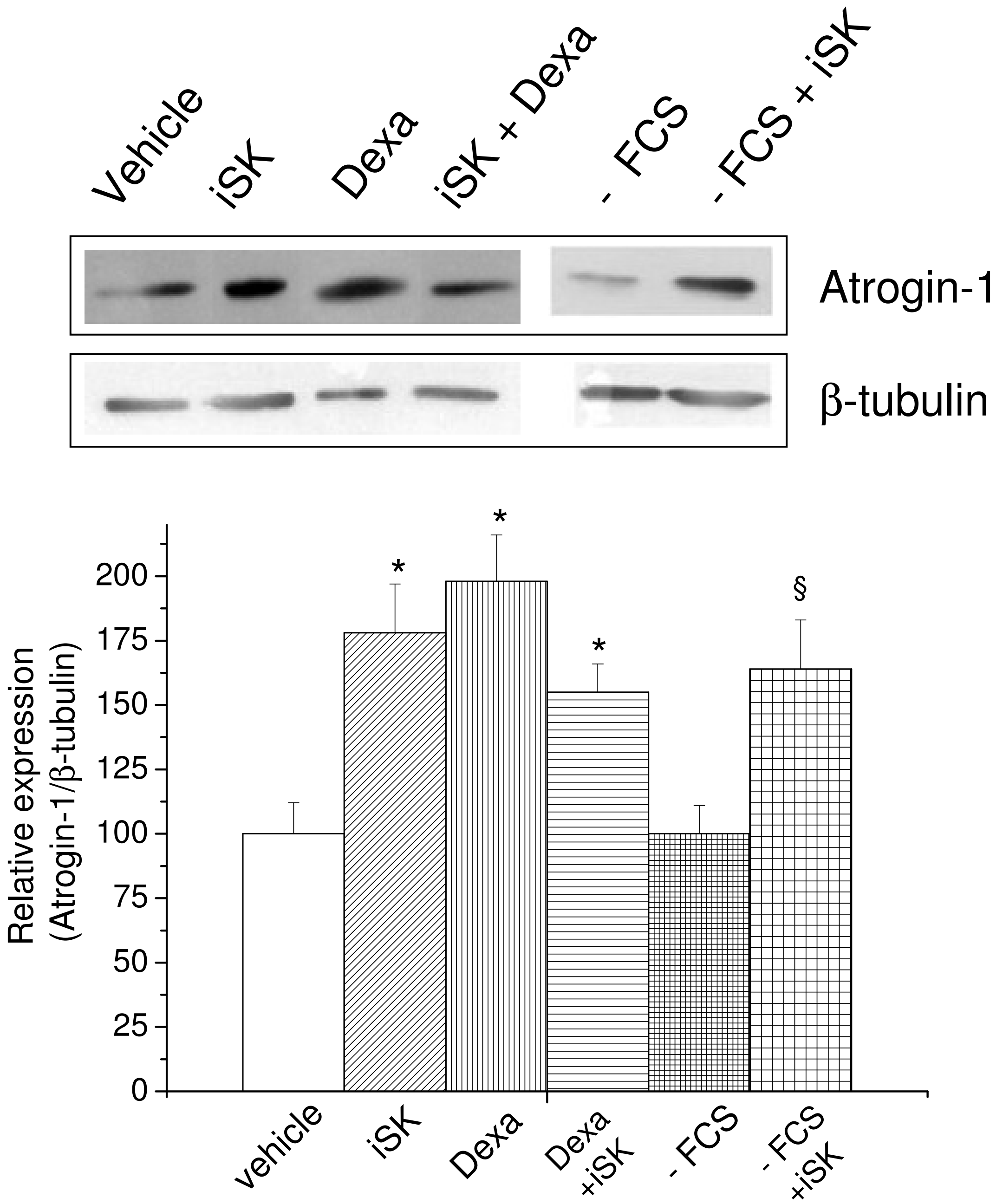

Vehicle
iSK
Dexa
iSK + Dexa
- FCS
- FCS + iSK
Atrogin-1
β-tubulin
Relative expression
(Atrogin-1/β-tubulin)
0
25
50
75
100
125
150
175
200
*
*
*
§
vehicle
iSK
Dexa
Dexa
+iSK
- FCS
- FCS
+iSK

Figure 6B