Simulated microgravity triggers epithelial mesenchymal transition in human keratinocytes.

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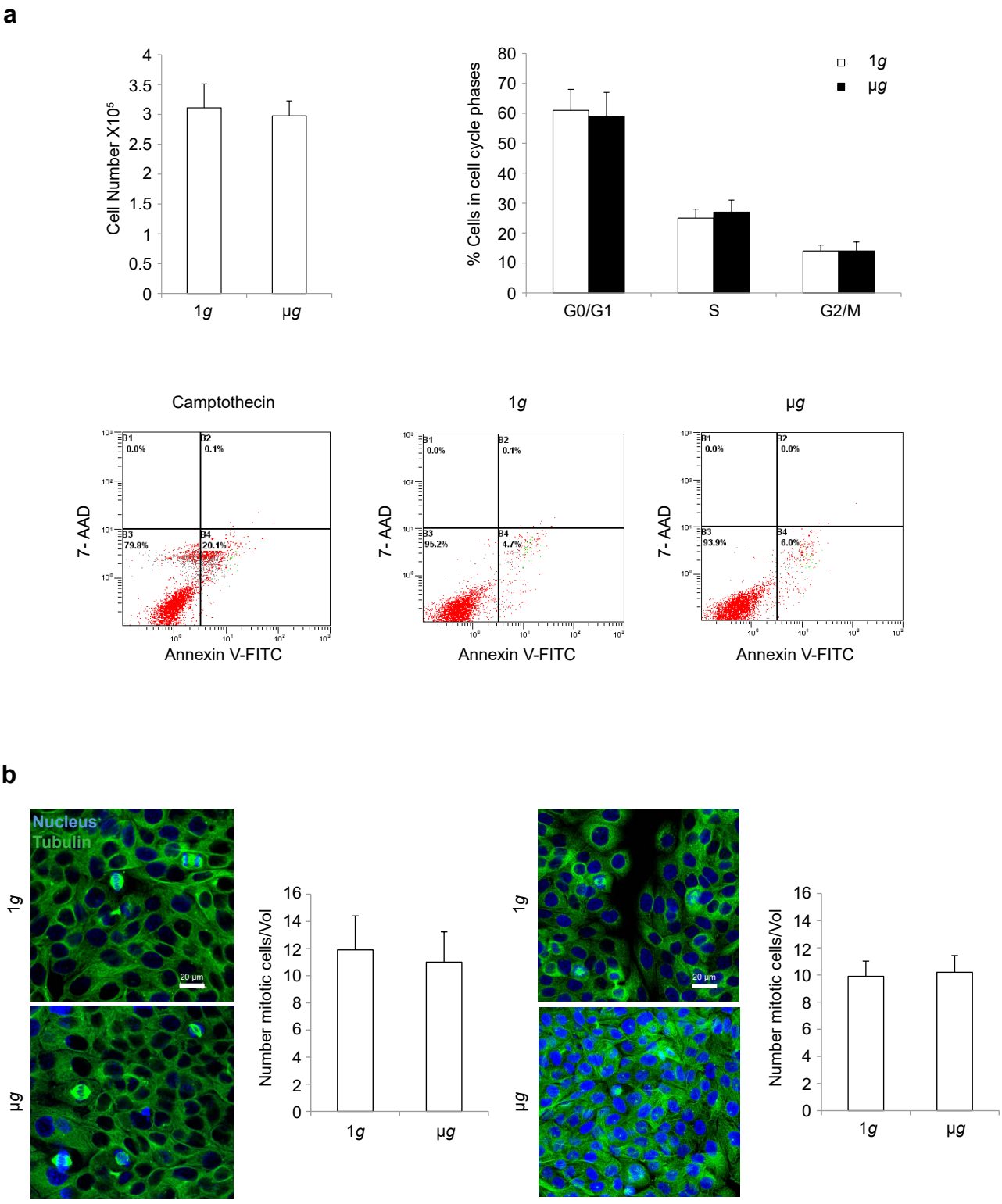
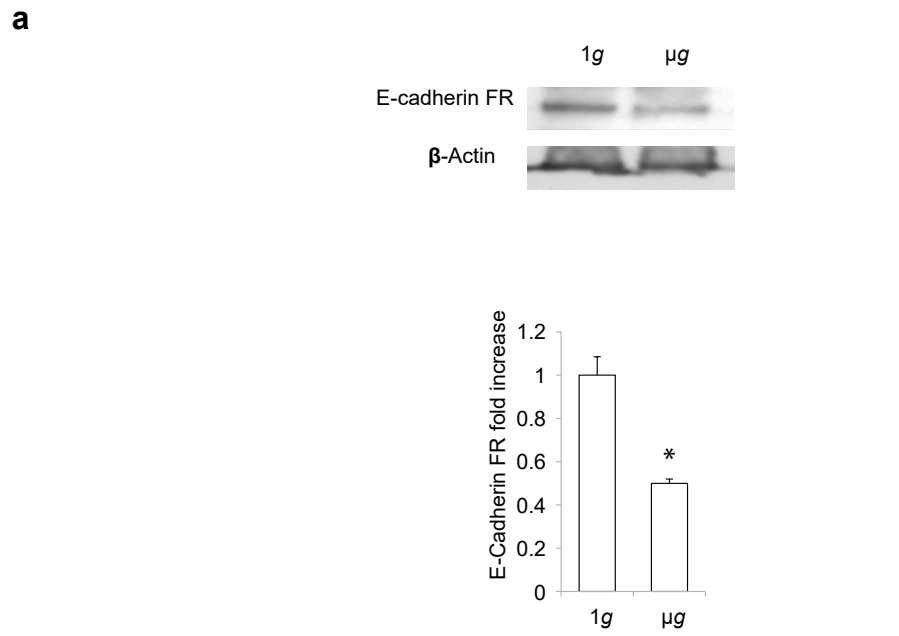
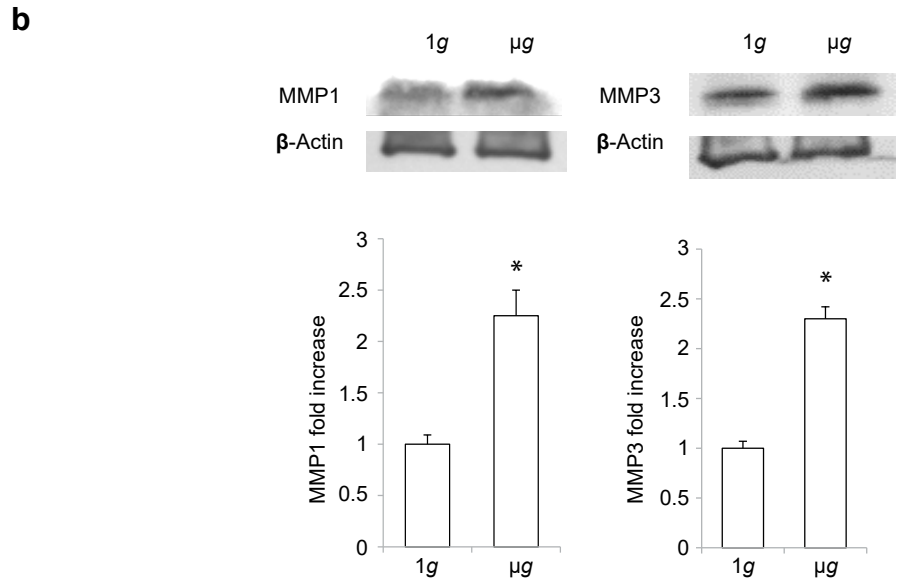


Supplementary Figure S1. Simuated microgravity does not affect cell proliferation or apoptosis. (**a**) Proliferation assay, cytofluorimetric analysis of the percentage of cell cycle phase distribution and Annexin V cytofluorimetric assay using camptothecin treatment as positive control for apoptosis were performed in HaCaT cells exposed to simulated microgravity for 24 hours (µ*g*) or kept at 1*g*. No significant differences in cell number increase (top left chart), in cell cycle phase distribution (top right chart) or in apoptotic rate are found in µ*g* cells respect to control cells (bottom panels). Results are expressed as mean ± SD from three different experiments in duplicate. (**b**) Immunofluorescence analysis using anti-β tubulin antibody in µ*g* or 1*g* HaCaT cells. Nuclei were stained with TOPRO-3. Mitotic spindles and midbodies count shows no significant differences in the number of mitotic cells/fields in µ*g* cells respect to control cells. Results are expressed as mean ± SD from three different experiments.





Supplementary Figure S2. Simulated microgravity treatment induces metalloproteases and E-cadherine fragment (FR) protein expression in HaCaT cells. Cells were exposed to μ*g* or kept at 1*g* for 24 hours. Western blot analysis shows a reduction of E-cadherin FR (**a**) and an increase of MMP1 and MMP3 protein levels (**b**) in simulated microgravity-treated cells compared to cells kept at 1*g*. The equal loading was assessed using anti-β actin antibody. For the densitometric analysis, the values from three independent experiments were normalized, expressed as fold increase and reported in graph as mean values ± SD. Student's *t* test was performed and significance levels have been defined as follows: * $p<0.05$ vs 1*g* cells.

Supplementary Table S1. Primers used for target and housekeeping genes.

Gene	Primer sequence (5'-3') Forward-Reverse	Lenght	T_m
Snail1	5'-GCTGCAGGACTCTAATCCAGA-'3 5'-ATCTCCGGAGGTGGGATG-3'	21 18	58.24 57.70
Snail2	5'-TGGTTGCTTCAAGGACACAT-'3 5'-AGCAAATGCTCTGTTGCAGTG-3'	20 21	58.60 59.20
ZEB2	5'-AAGCCAGGGACAGATCAGC-'3 5'-CCACACTCTGTGCATTTGAACT-3'	19 22	59.39 59.38
N-cadherin	5'-CTCCATGTGCCGGATAGC-'3 5'-CGATTTCACCAGAAGCCTCTAC-3'	18 22	57.63 58.81
MMP1	5'-ACTGAGAAAGAAGAC AAAGGCAAG-3' 5'-TGGGCTGCTTCATCACCTTC-3'	24 20	59.42 60.32
MMP2	5'- CCTGATGTCCAGCGAGTGG-3' 5'- TCTTCTTCACCTCATTGTATCTCCAG-3'	19 26	60.15 60.13
MMP3	5'- TGGACAAAGGATACAACAGGGAC-3' 5'- TGTGAGTGAGTGATAGAGTGGGT-3'	23 23	60.24 60.50
MMP9	5'-CGCGCTGGGCTTAGATCATT-3' 5'- GGGCGAGGACCATAGAGGT-3'	20 19	60.88 60.46
18s	5'-AACCAACCCGGTCAGCCCCT-3' 5'-TTCGAATGGGTCGTCGCCGC-3'	20 20	66.14 65.80