

**Supplementary Information for:**

**Profiling of polyphenolic compounds of *Leontopodium alpinum* Cass. callus cultures using UPLC/ESI-IM-HRMS and screening of *in-vitro* effects**

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**Abstract**

*Leontopodium alpinum* Cass. (edelweiss) is recognized as a frequent constituent of anti-aging skin care products, providing increased antioxidant and anti-inflammatory defense. Considering the growing demand and the protected status of edelweiss in many countries, alternative ways of production have been developed, one of them being callus culturing. This study reports the phytochemical composition of a methanolic extract of *L. alpinum* callus cultures, characterized by liquid chromatography coupled to ion-mobility high resolution mass spectrometry (UPLC/IM-HRMS). The methanolic extract exhibited strong free radical scavenging activity ( $122.19 \pm 7.28$  [mg AAE/g dw]), while the quantitative evaluation revealed that four major constituents (phenylpropanoid derivatives) represent 57.13% (m/m) of the extract. Consequently, a screening of antiproliferative effects was performed on ten cancer cell lines, representative to prostate, colon, lung and breast cancer, showing inhibition of colony formation in all cases. These results provide a comprehensive phytochemical characterization of *L. alpinum* callus cultures using advanced IM-HRMS, while the *in-vitro* explorations confirmed the potent antioxidant properties of edelweiss which are worth exploring further in cancer prevention.

**Keywords:** callus cultures, edelweiss, phytochemical characterization, antioxidants, ion-mobility mass spectrometry

**Figure S1.** Diagnostic product ion - glucaric acid

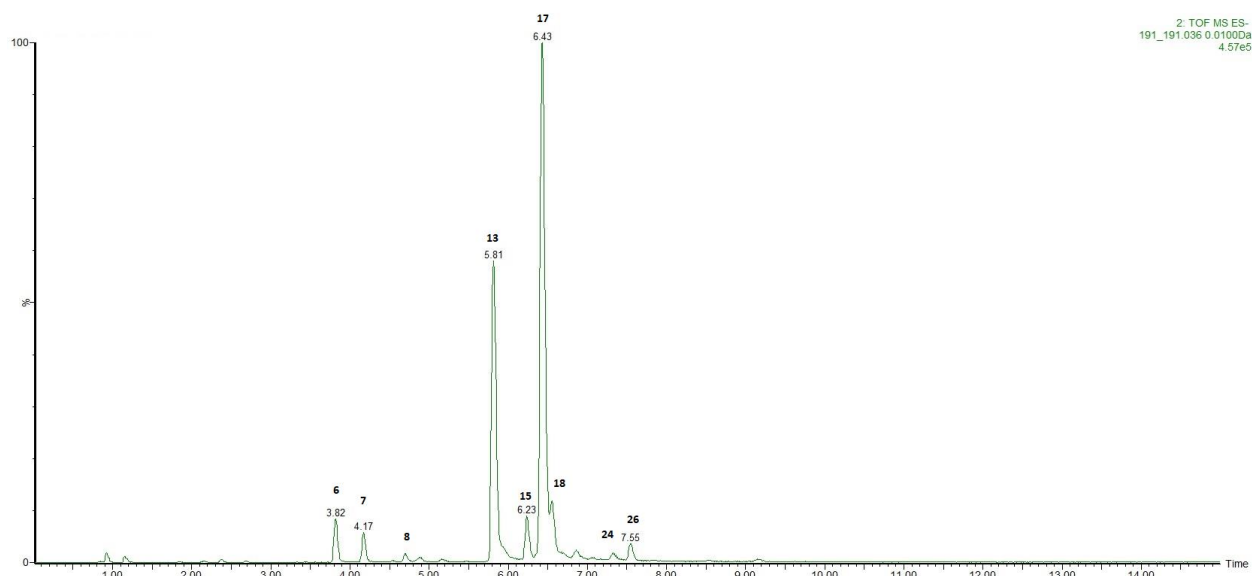
**Figure S2.** Diagnostic product ion – quinic acid

**Figure S3.** Morphological analysis

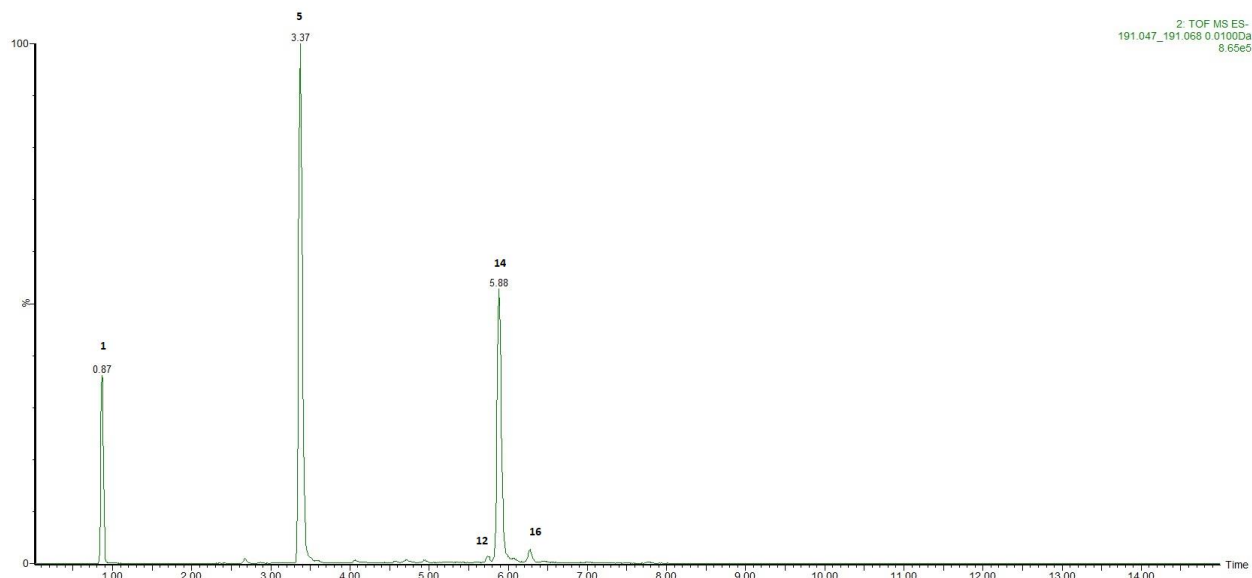
**Figure S4.** Colony assay microscopy images and measurements



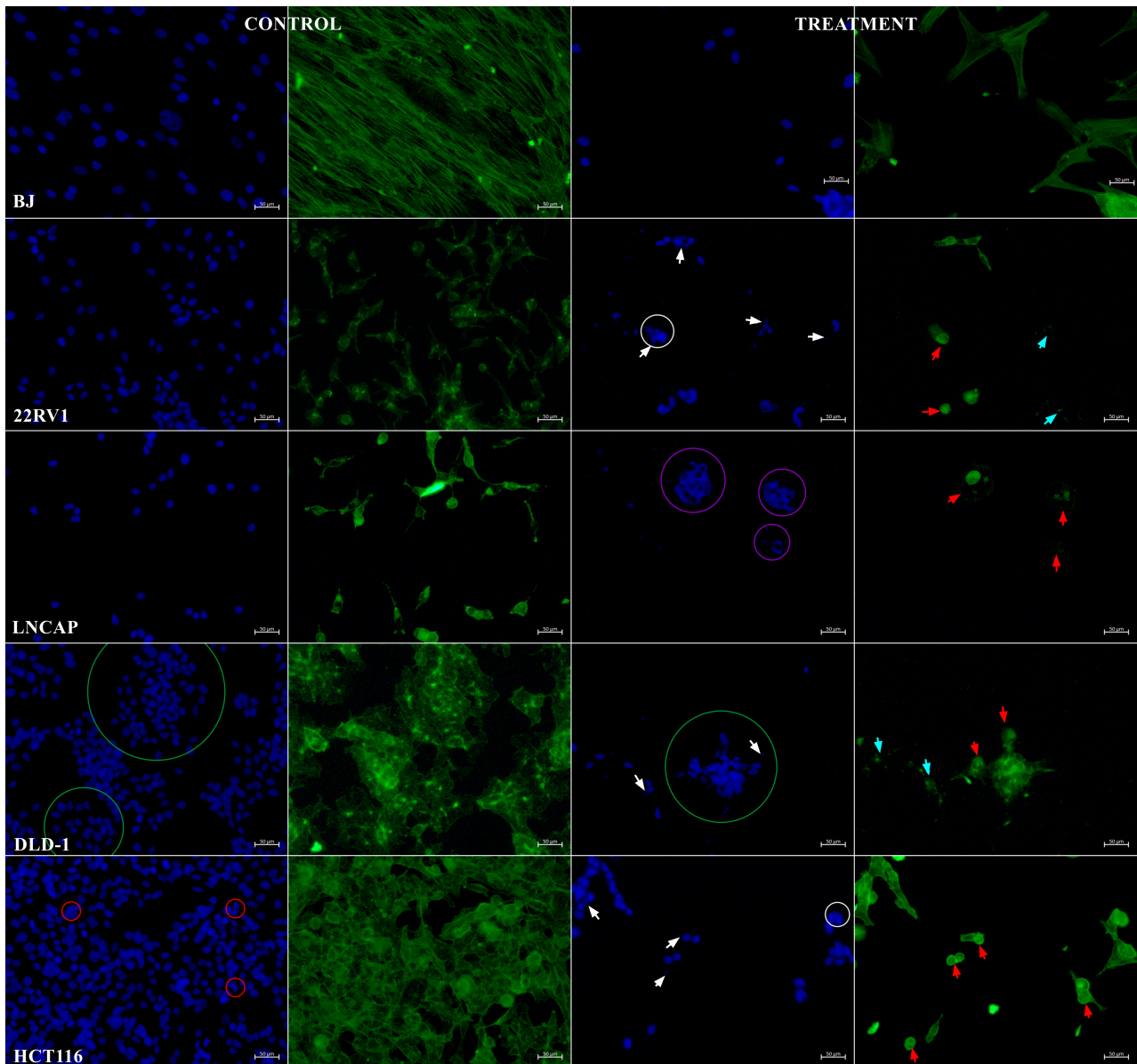
**Figure S1.** Diagnostic product ion - glucaric acid



**Figure S2.** Diagnostic product ion – quinic acid



**Figure S3. Morphological analysis.** Effect of *L. alpinum* extract on human normal skin fibroblast, prostate, colon, lung and breast cancer cell lines. Morphological aspects highlighted with DAPI (Blue – for the nucleus) and Phalloidin-FITC (Green – actin filaments). Scale bar of 200  $\mu$ m, without digital zoom. White arrows – fragmented nucleus; Red arrows – round shaped cells; Turquoise arrows – fragmented cytoskeleton; Green circle – colony size; White circle – multilobular nucleus; Red circle – dividing cells.



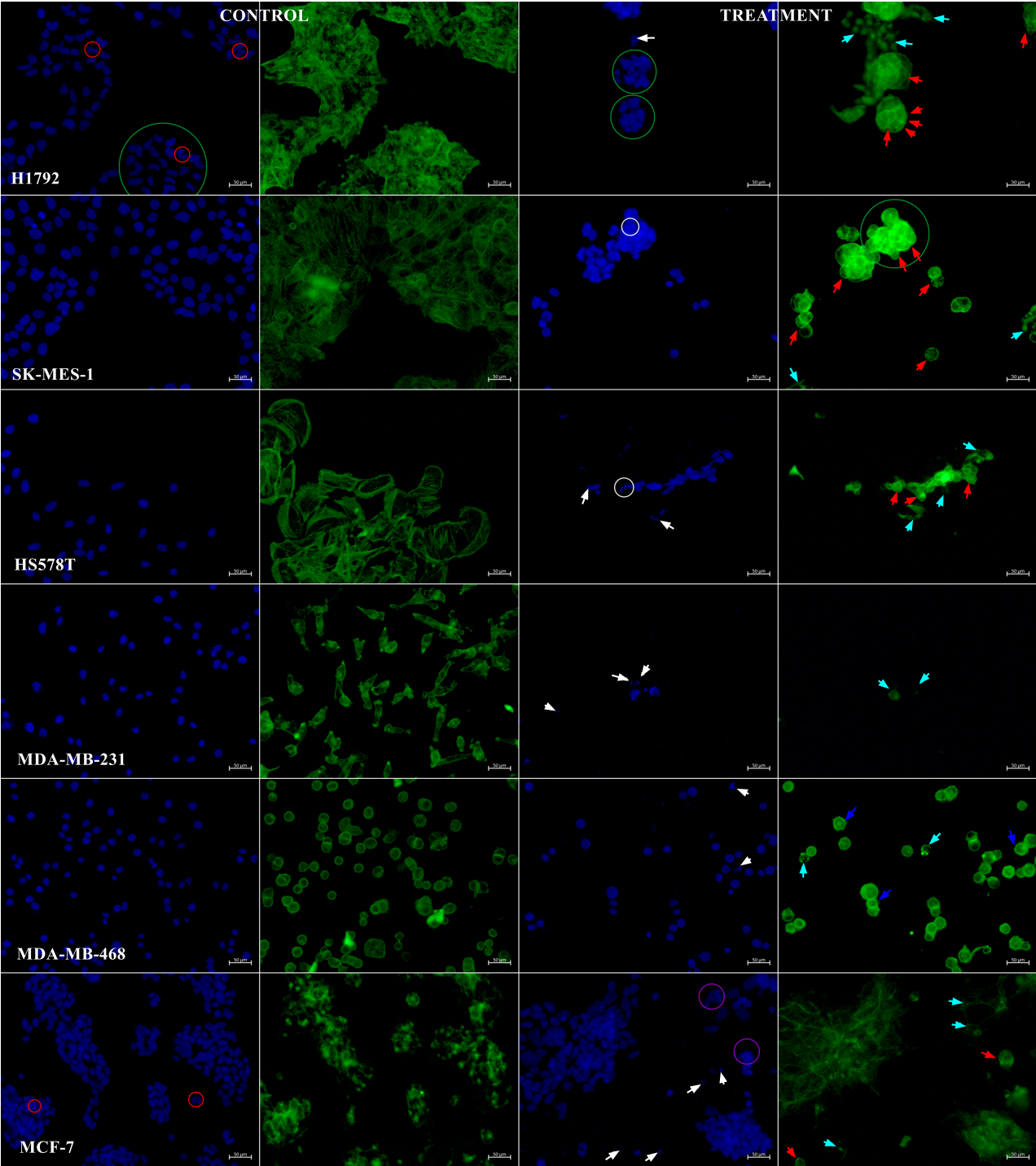


Figure S4. Colony assay. Microscopy images and measurements (n=3)

