Silver ions promote blebs growth in U251 glioblastoma cell by activating nonselective cationic current.

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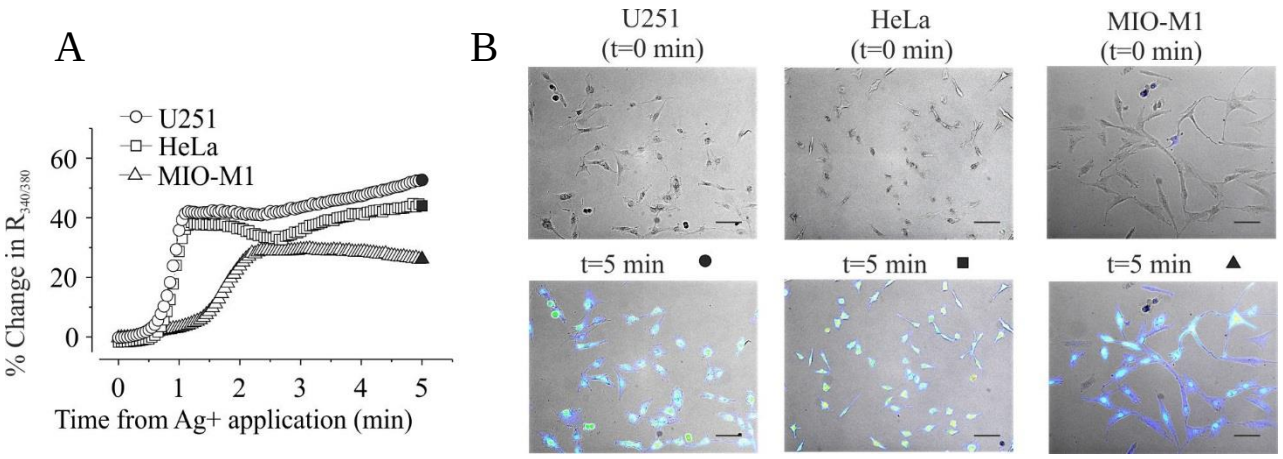
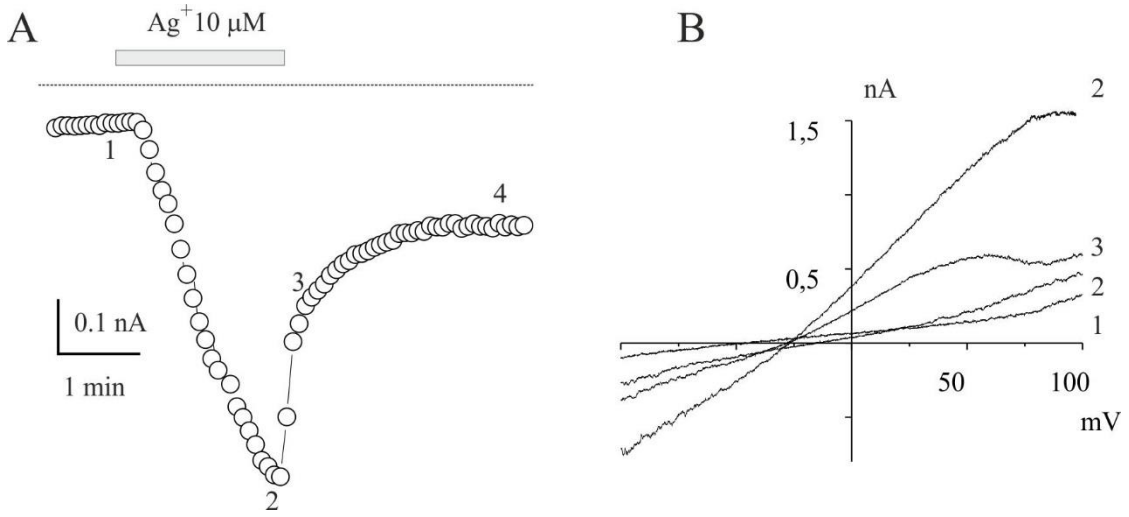
SUPPLEMENTARY FIGURES

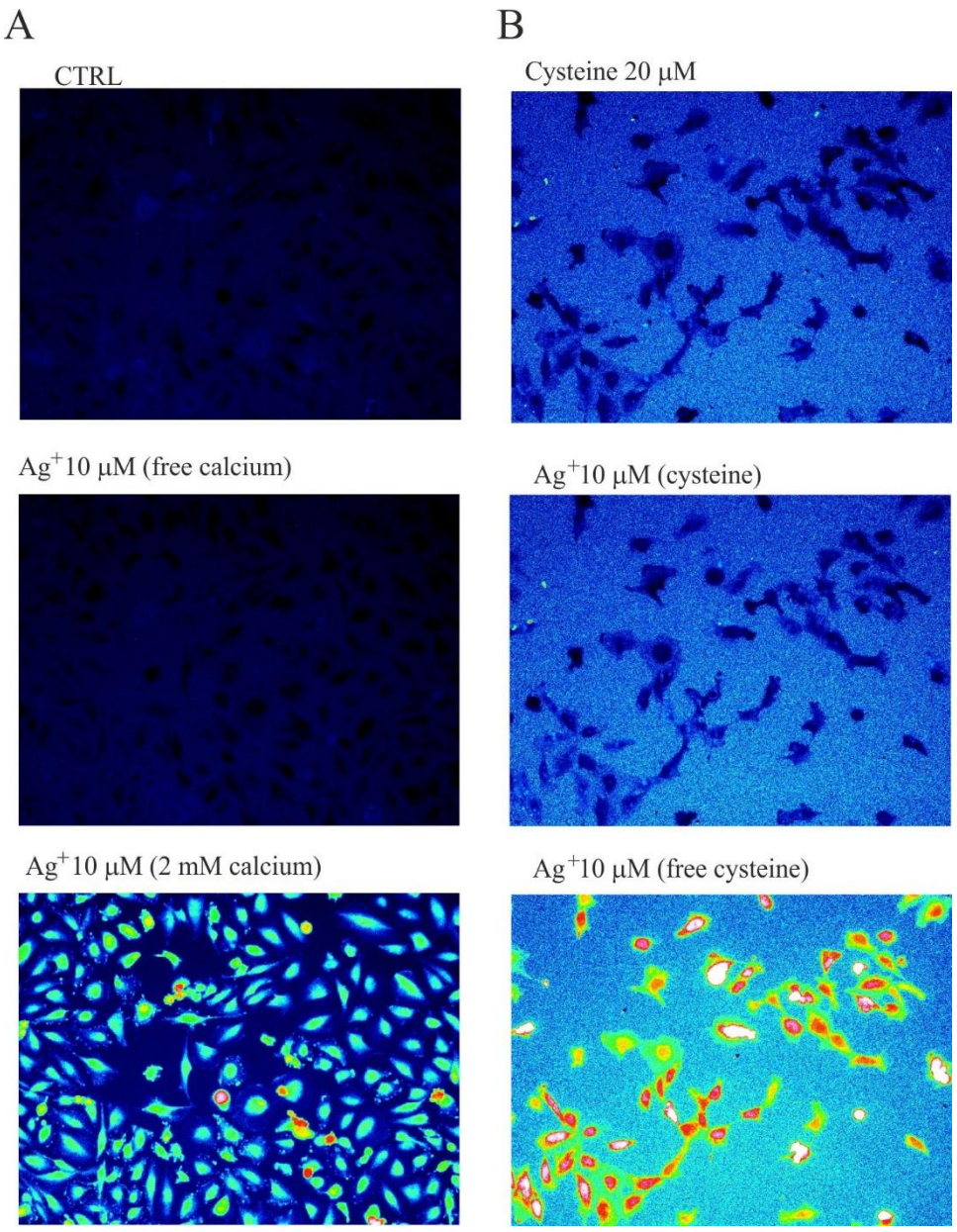


Supplementary Fig. S1. Silver ions increase intracellular Ca^{2+} levels in different human cell lines. A) Intracellular calcium levels evaluated by fluorescent imaging with FURA-2 from 20 cells during application of 3 µM of silver ions in cervical carcinoma HeLa (squares) [1] and immortalized human Müller cell line MIO-M1 (triangles) [2] compared with U251 glioblastoma cell lines (circles). **B)** Merge of brightfield and pseudo-colour of ratio 340/380 emission images of above mentioned cell lines before (0 min) and after 5 minutes application of 3 µM of Ag^{+}. Intracellular calcium levels is indicated on a rainbow scale with blue represent low $[Ca^{2+}]_i$ and orange/red high $[Ca^{2+}]_i$. Scaling bars: 100 µm.

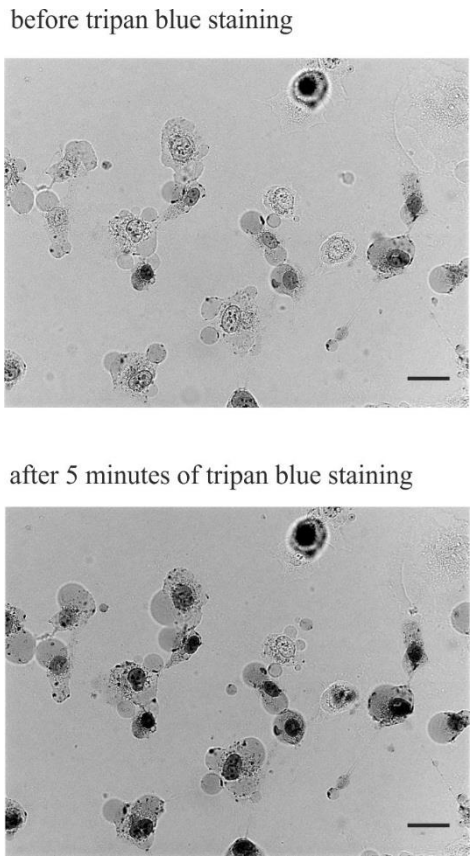
1. Gey G. O., Coffman W. D., Kubicek M. T., 1952. Tissue culture studies of the proliferative capacity of cervical carcinoma and normal epithelium. Cancer Res. 12: 264–265
2. Limb G. A., Salt T. E., Munro P. M., Moss S. E., Khaw P.T. In vitro characterization of aspontaneously immortalized human Müller cell line (MIO-M1). *Invest Ophthalmol Vis Sci*. **43(3)**, 864-9, (2002).



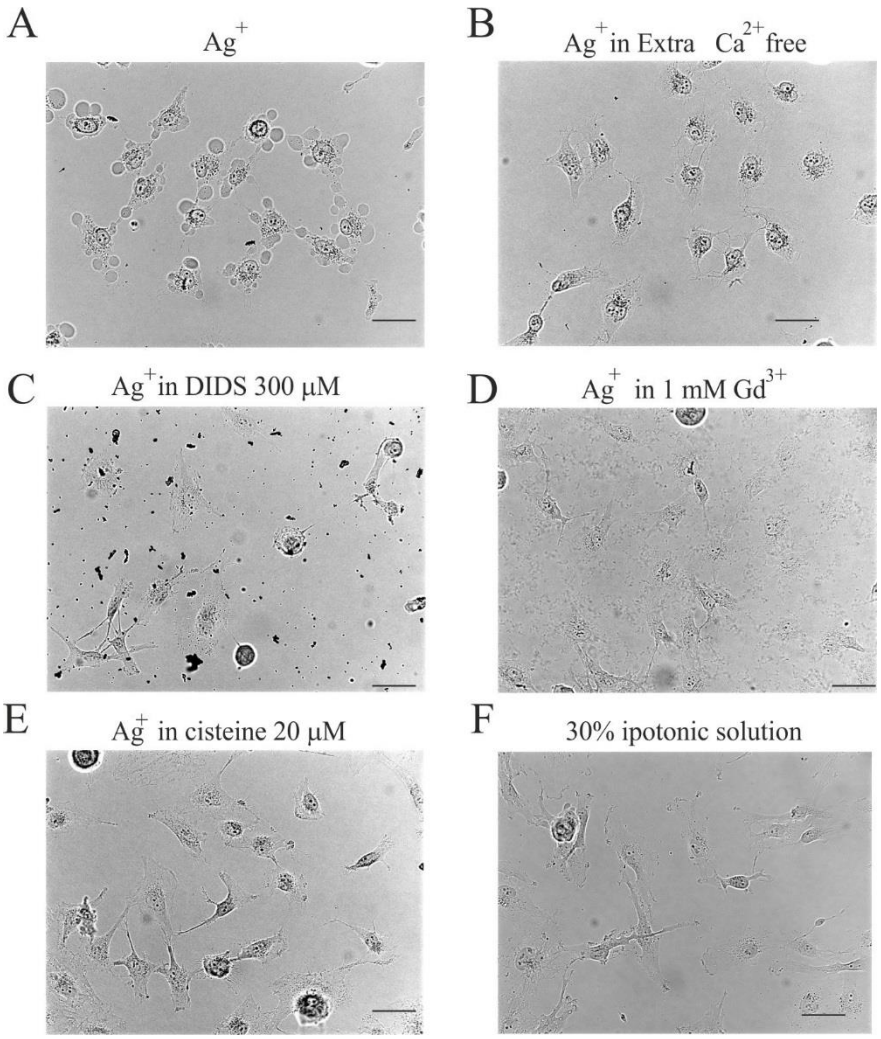
Supplementary Fig. S2. IAg was partially reverted after wash out. A) Time course of inward current at -90 mV before (1), after application 10 µM Ag^+ (2) and during wash-out (3 and 4). The dates points represent the currents recorded at -90 mv obtained during voltage ramp from -100 to 100 mv (1 second duration) from a Vh of 0 mV repeated every 5 s. **B)** Representative current ramps taken at the times indicated in the relative time course shown in A.



Supplementary Fig. S3. Representative pseudo-colours calcium imaging of experimental data from fig. 2. A) and B) Pseudo-colours calcium imaging captures at the time indicating in Fig 2A and 2B respectively. Intracellular calcium levels is indicated on a rainbow scale with blue represent low $[Ca^{2+}]_i$ and orange/red high $[Ca^{2+}]_i$.



Supplementary Fig. S4. Tripan blue assay in Ag^{+} -induced blebbing cells. Images of U251 exposed for 30 min to 3 µM of silver ions before and after 5 min of 10% v/v of tripan blue staining. Scaling bars: 20 µm.



Supplementary Fig. S5. Blockers of IAg abolished silver ions induced morphological changes in U251 cells. A) Blebs formation after 30 minutes of 3 µM silver ions treatment in U251 cells. Blebs formation is abolished when Silver ions is applied in Ca^{2+}-free medium (**B**) and when silver ions was co-applied with 300 µM DIDS (**C**), 1 mM Gd^{3+} (**D**) or 20 µM cysteine (**E**). Ipotonic conditions do not allow blebs formation, although swelling was detect (**F**). Scaling bars: 20 µm.