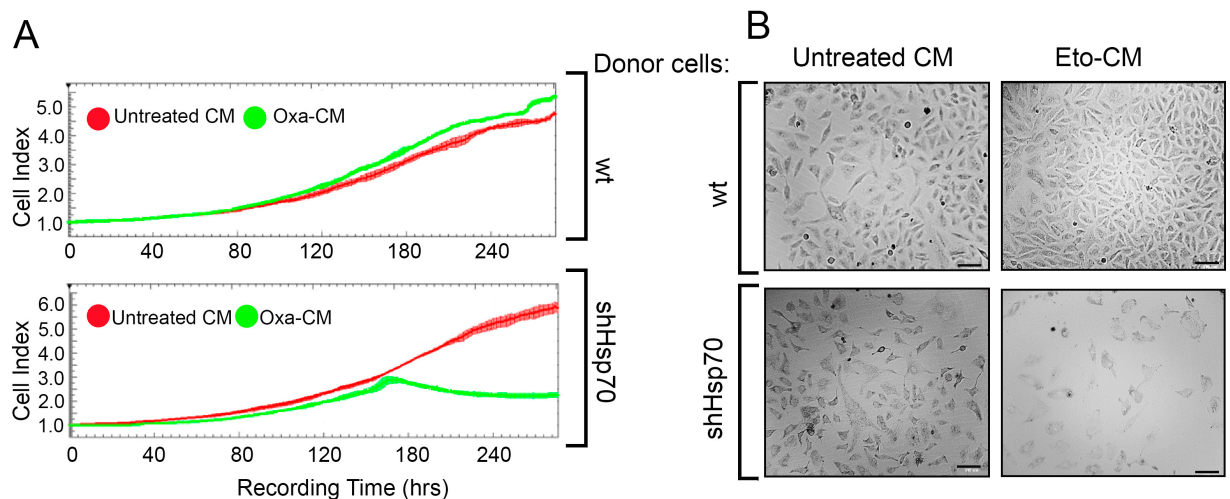


Supplementary Material  
to the manuscript “Supplementary Material  
to the manuscript “Autocrine regulation of tumor cell repopulation by  
Hsp70-HMGB1 alarmin complex”  
by Sverchinsky et al



**Figure 1S. Hsp70 occurring in CM enhances the repopulation of H1299 human lung carcinoma cells.**

(A) Data of xCELLigence experiment on H1299 cell growth in Oxa-CM from H1299wt and H1299shHsp70 cells. (B) The microscopy of H1299 cells growing in Eto-CM from H1299wt and H1299shHsp70 cells for 14 days. Scale bar - 50  $\mu$ m.

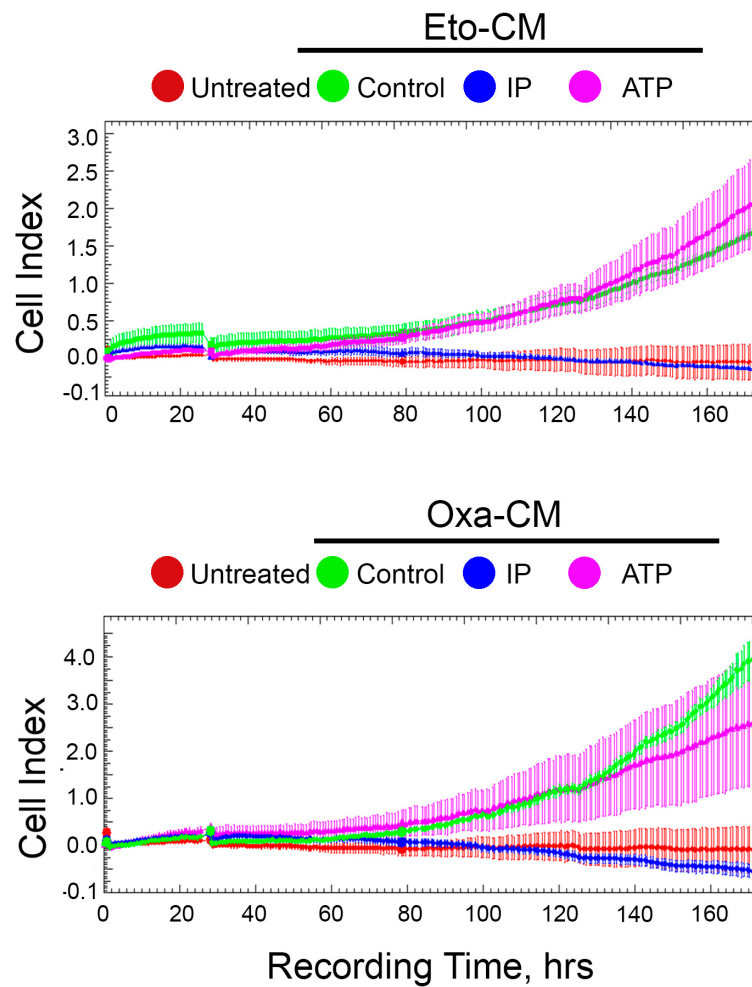


Figure 2S. **Depletion of Hsp70 from Eto-CM or Oxa-CM with the aid of ATP or IP with anti-Hsp70 results in distinct effect on DLD1 cell repopulation rate.** ATP depletion did not affect the increase of cell growth caused by Eto-CM or Oxa-CM whereas the depletion with specific antibody canceled growth-stimulating properties of CM.

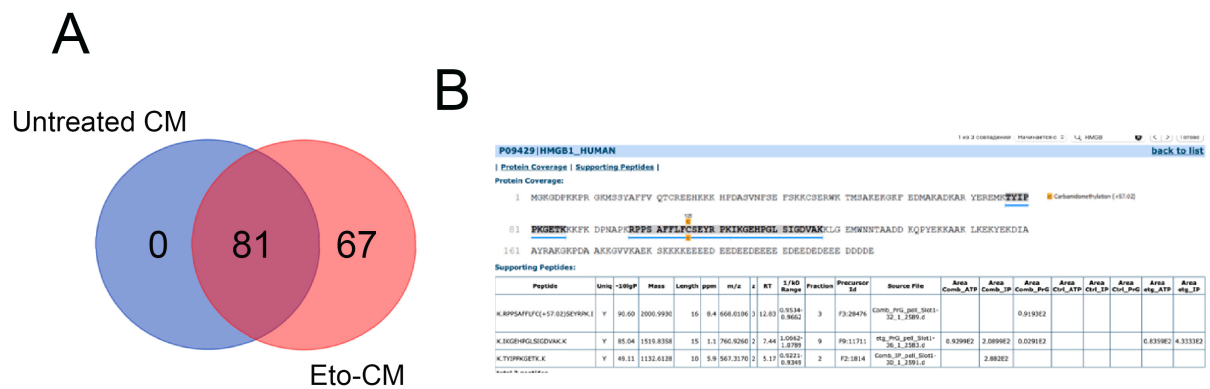


Figure 3S. Proteomic analysis revealed High mobility box group B1(HMGB1) among 67 proteins bound to Hsp70 released from Eto-treated A549 cells but not to Hsp70 released from untreated cells.

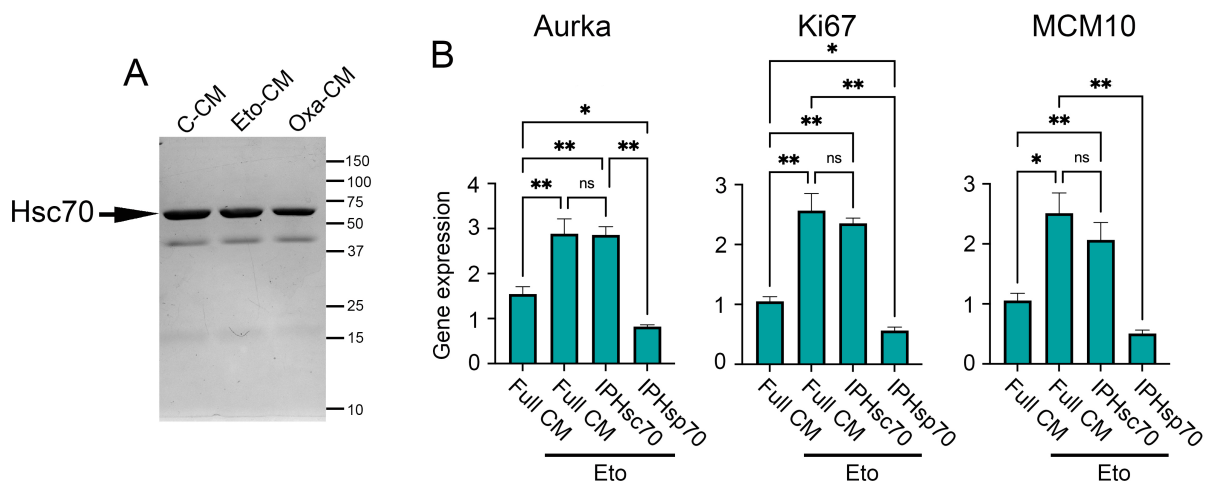
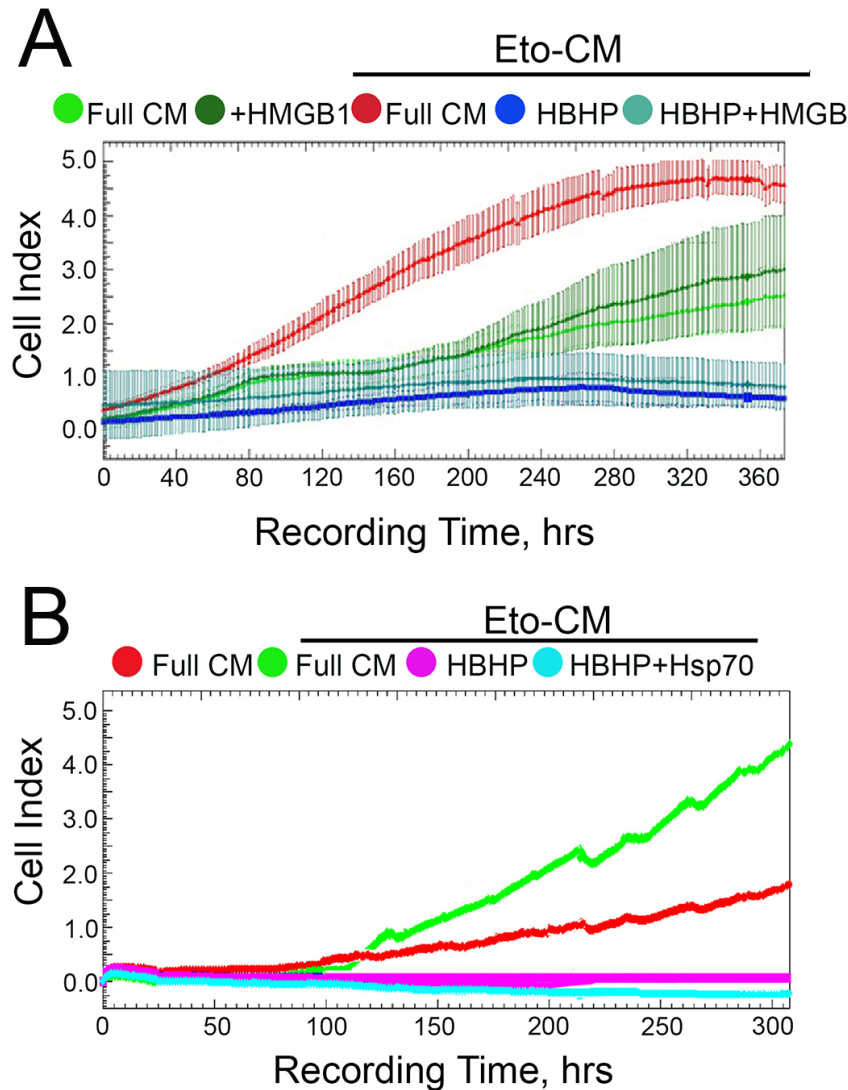


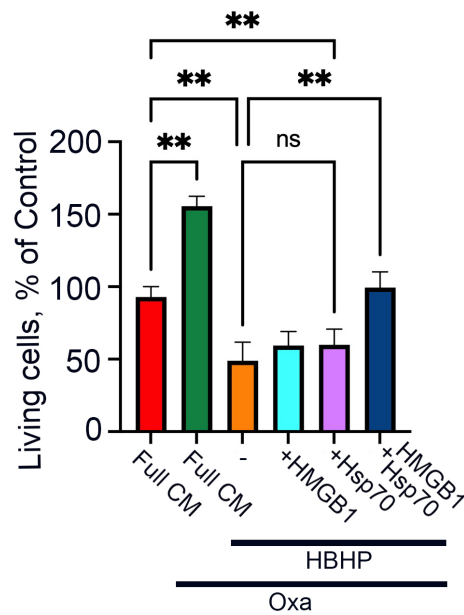
Figure 4S. **Removal of Hsc70 from the conditioned medium of A549 cells treated with etoposide does not lead to the abolition of repopulation.**

(A) Ag-stained gel from CM from untreated A549 cells and from Eto-CM and Oxa-CM performed with antibodies against Hsc70. (B) Expression of repopulation marker genes in A549 cells in presence of Eto-CM depleted with Hsc70. \* $p < 0.01$ ; \*\* $p < 0.001$ .



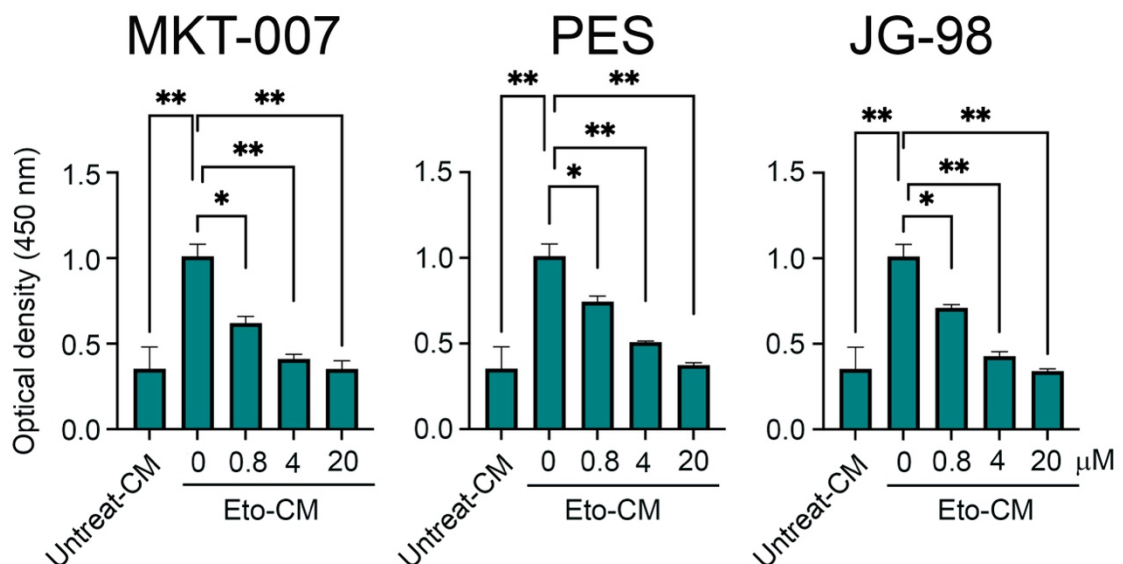
**Figure 5S. Hsp70 or HMGB1 separately do not stimulate tumor cell repopulation**

HMGB1-Hsp70 complex formed in Eto-CM from A549 cells was removed by the addition of HBHP-Biotin-Avidin gel, then purified HMGB1 (A) or purified Hsp70 (B) were added and these media were applied to A549 seeded to E-plates at a low concentration. Cell growth in real time was estimated using the xCELLigence technique.



**Fig. 6S. To stimulate tumor cell repopulation, Hsp70-HMGB1 complex is needed.**

HMGB1-Hsp70 complex was depleted from Eto-CM from A549 cells with aid of HBHP-Bio-Avidin gel and then purified HMGB1 or purified Hsp70 or Hsp70-HMGB1 complex were added and these media were applied to A549 seeded to plates at a low concentration. Twelve days later, cell growth was assessed using the MTT method. \*\* $p < 0.001$ .



**Figure 7S. Uncouplers of Hsp70-HMGB1 complex reduced the Hsp70-HMGB1 complex formation dose-dependently.** Data of PPI assay with purified Hsp70 and HMGB1 in the same mode as on Fig. 4E.

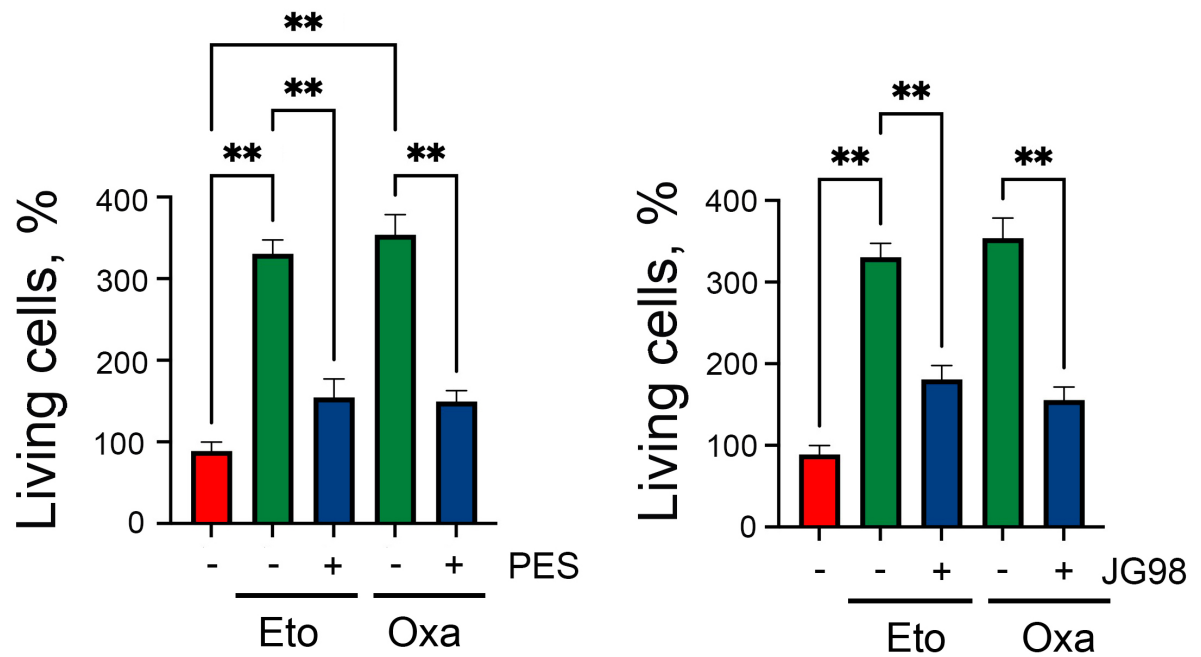


Figure 8S. **Hsp70 inhibitors able to reduce Hsp70-HMGB1 complexes formation in CM suppress tumor cells growth *in vitro***

A549 cells were seeded into wells of 96-well plates at a low concentration and incubated in Eto-CM or in Oxa-CM in presence of PES (left) or JG-98 (right) during 14 days. Data of MTT assay. \*\*  $p < 0.001$ .