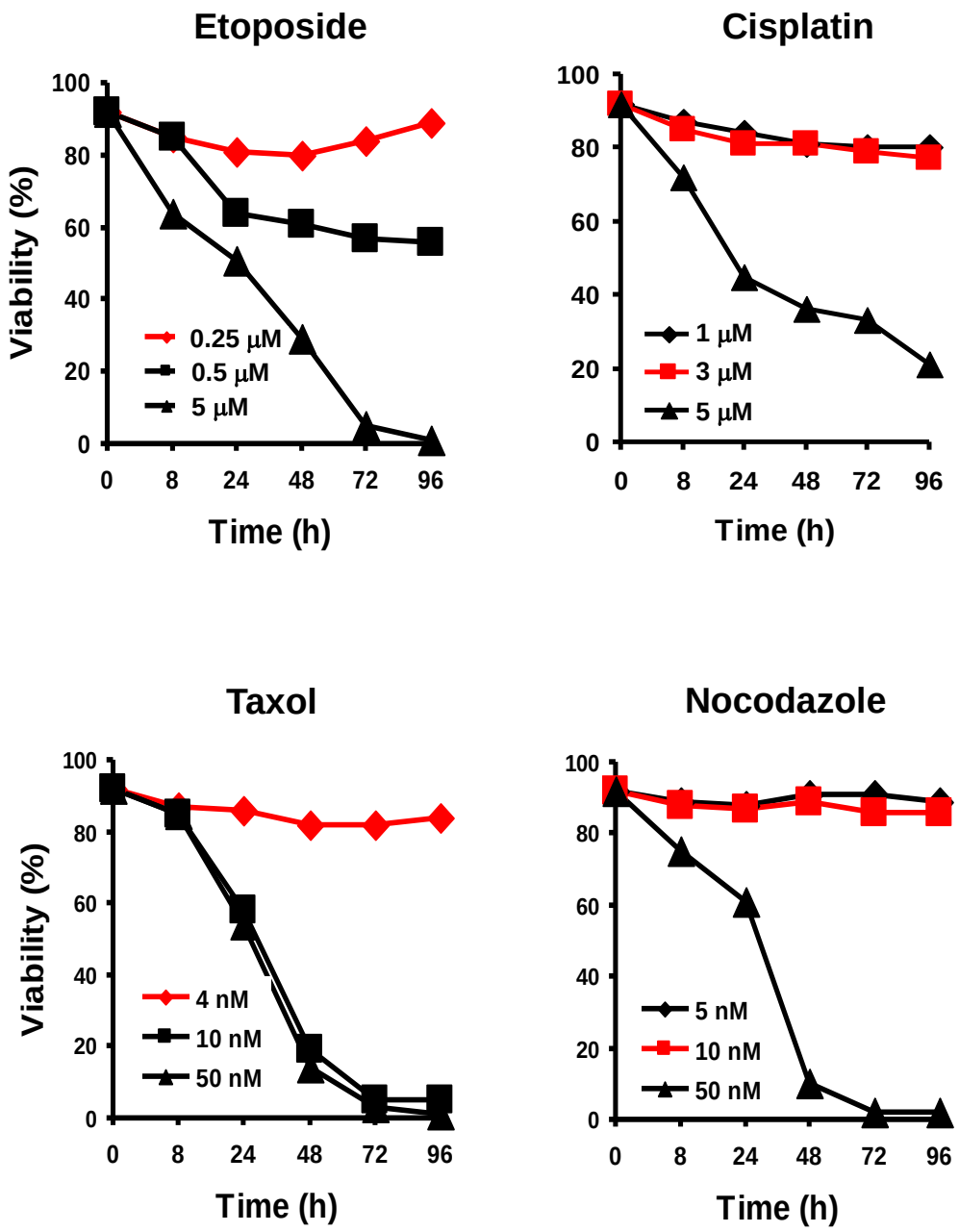
Supplementary Information

ATG5 is induced by DNA-damaging agents and promotes mitotic catastrophe independent of autophagy

Dipak Maskey, Shida Yousefi, Inès Schmid, Inti Zlobec, Aurel Perren, Robert Friis & Hans-Uwe Simon

Correspondence: Hans-Uwe Simon, MD, PhD; E-mail: hus@pki.unibe.ch



Supplementary Figure S1. Concentration- and time-dependent induction of cell death by anticancer drugs in Jurkat T cells. The sublethal concentrations indicated in red were used in subsequent experiments throughout the manuscript. Results are representative of 3 independent experiments.

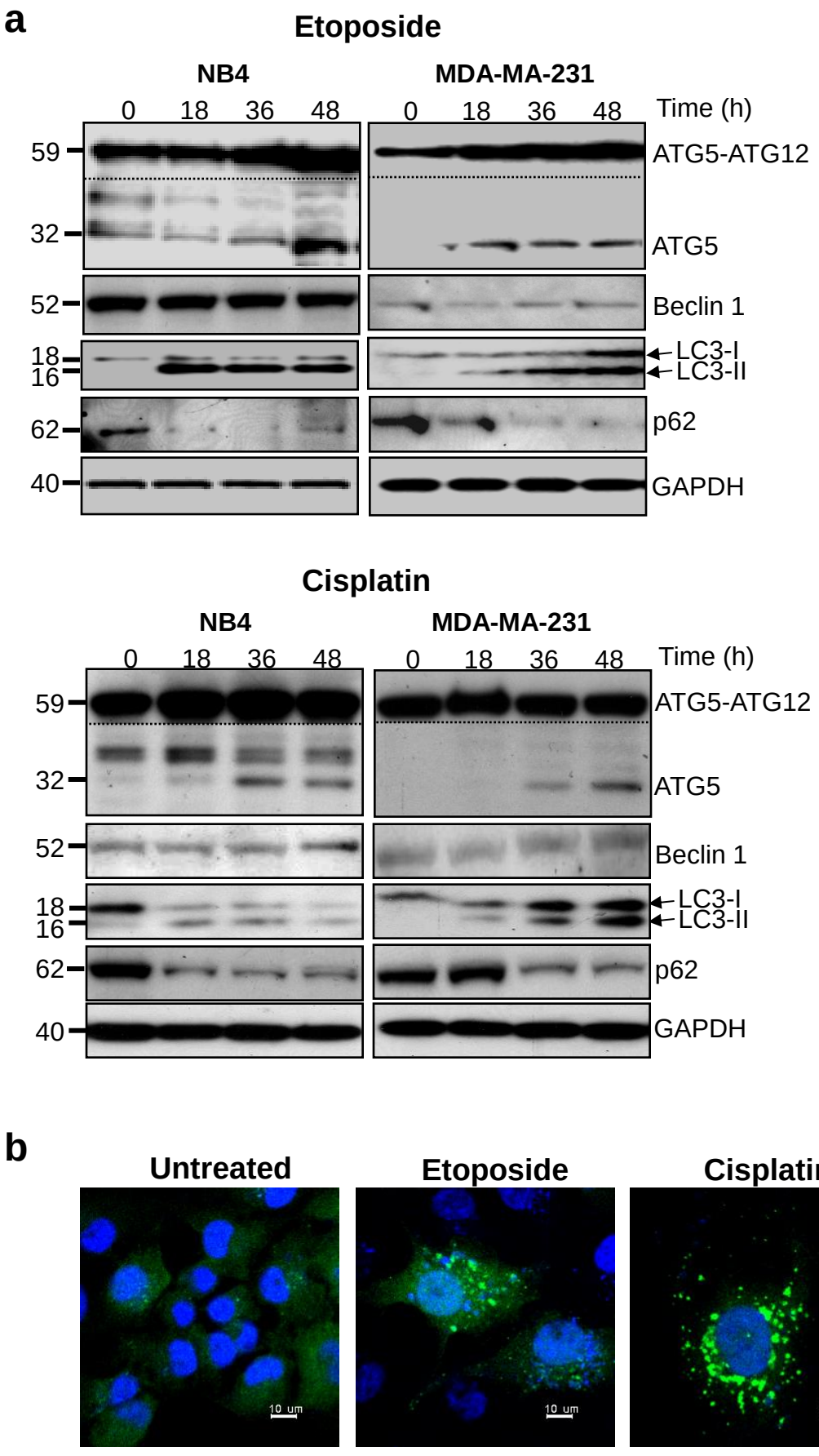
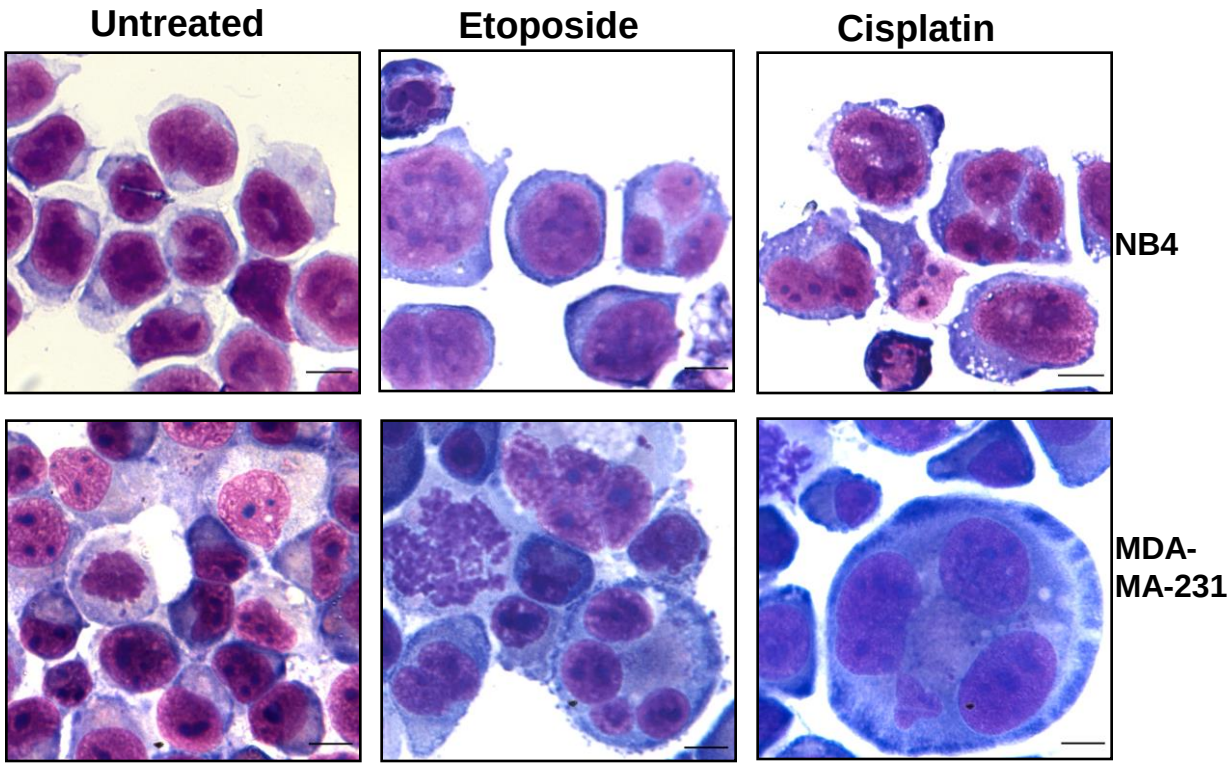
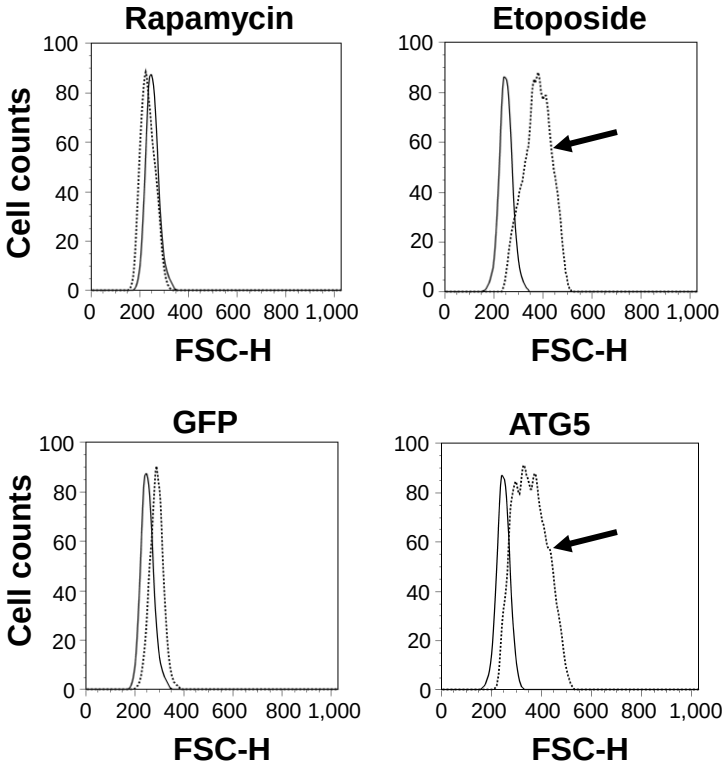


Figure S2, panels a and b

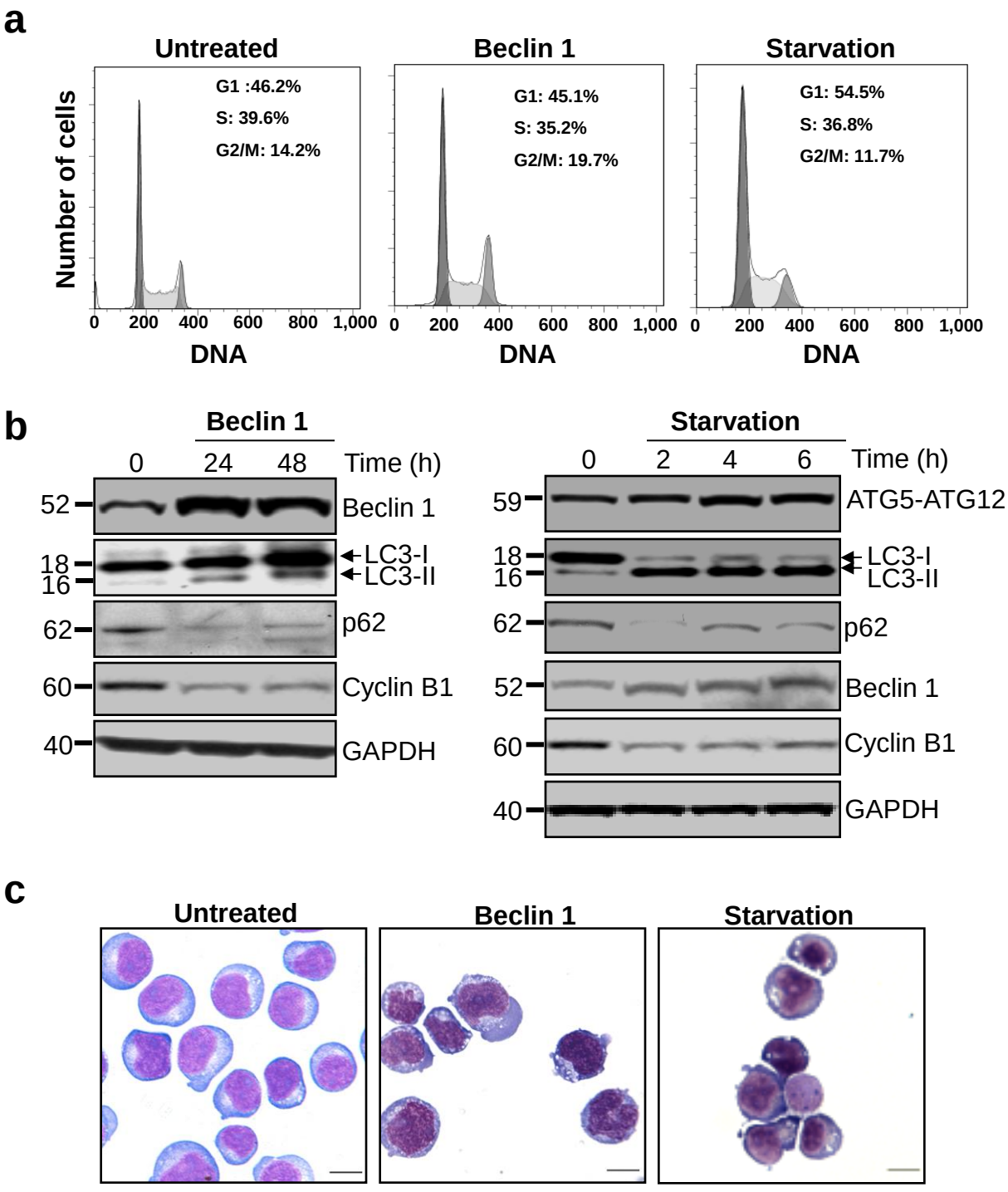
c



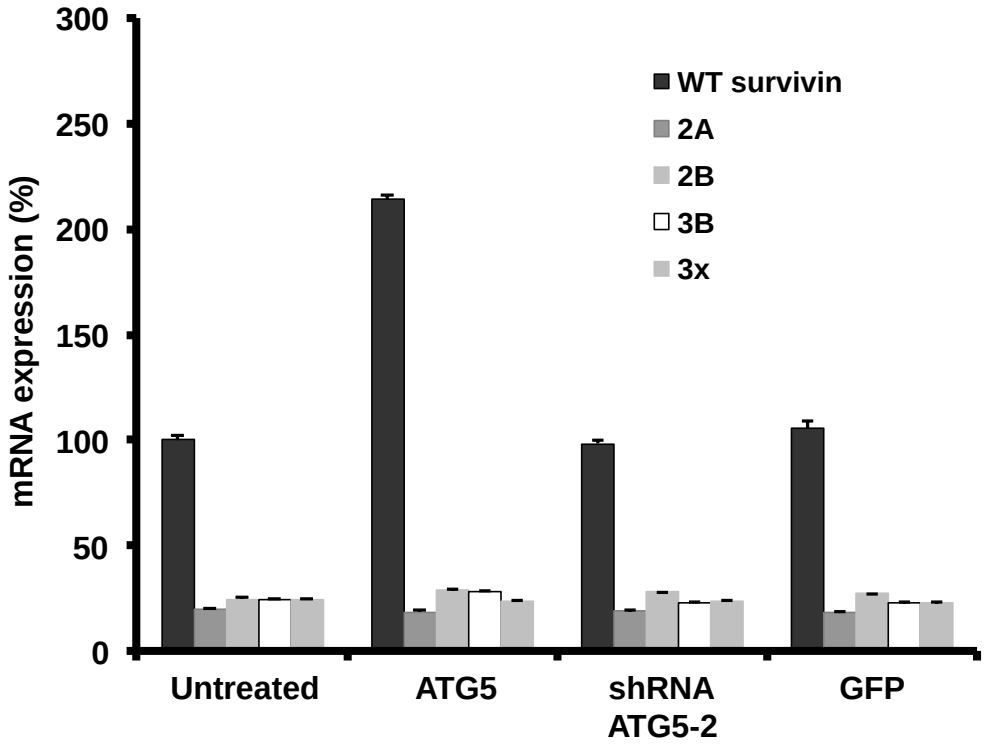
Supplementary Figure S2. Sublethal concentrations of DNA-damaging drugs induce ATG5 gene expression, autophagy, and cells with abnormal nuclei. (**a**) Immunoblotting. NB4 and MDA-MA-231 cells were cultured for the indicated times and ATG5 (monomeric as well as ATG12-conjugated), Beclin 1, LC3 (18 kD as well as 16 kDa), p62, and GAPDH detected. Each immunoblot is representative of at least 3 independent experiments. (**b**) Fluorescence microscopy for GFP-LC3 processing demonstrates autophagy. HeLa cells expressing GFP-LC3 were treated with the indicated DNA-damaging agents for 48 h and then analyzed. Nuclei were stained with DAPI. Both anticancer drugs induced the punctate LC3 pattern typical of autophagy. Bars, 10 μm. (**c**) Giemsa staining morphology. NB4 and MDA-MA-231 cells were cultured for 48 h, stained with Giemsa-May-Grünwald, and examined. Both etoposide and cisplatin treatments resulted in enlarged cells with abnormal nuclei. Bars, 10 μm.



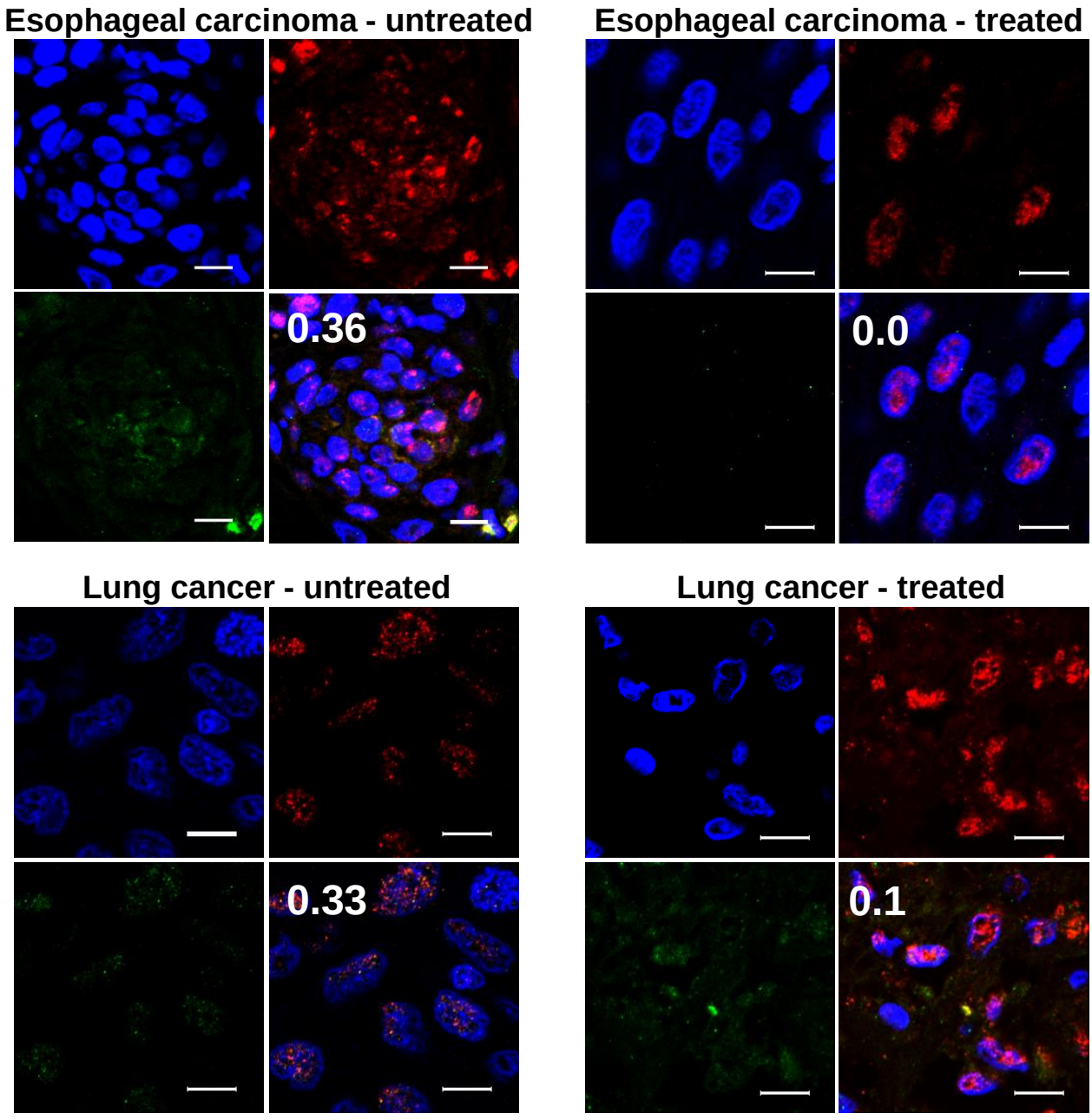
Supplementary Figure S3. Increased cell size following etoposide treatment or lentiviral ATG5 gene transfer. Jurkat T cells were cultured as indicated for 48 h. Cell size was analyzed by forward light scatter versus side light scatter analysis using a flow cytometer. Rapamycin treatment and GFP overexpression were used as negative controls.



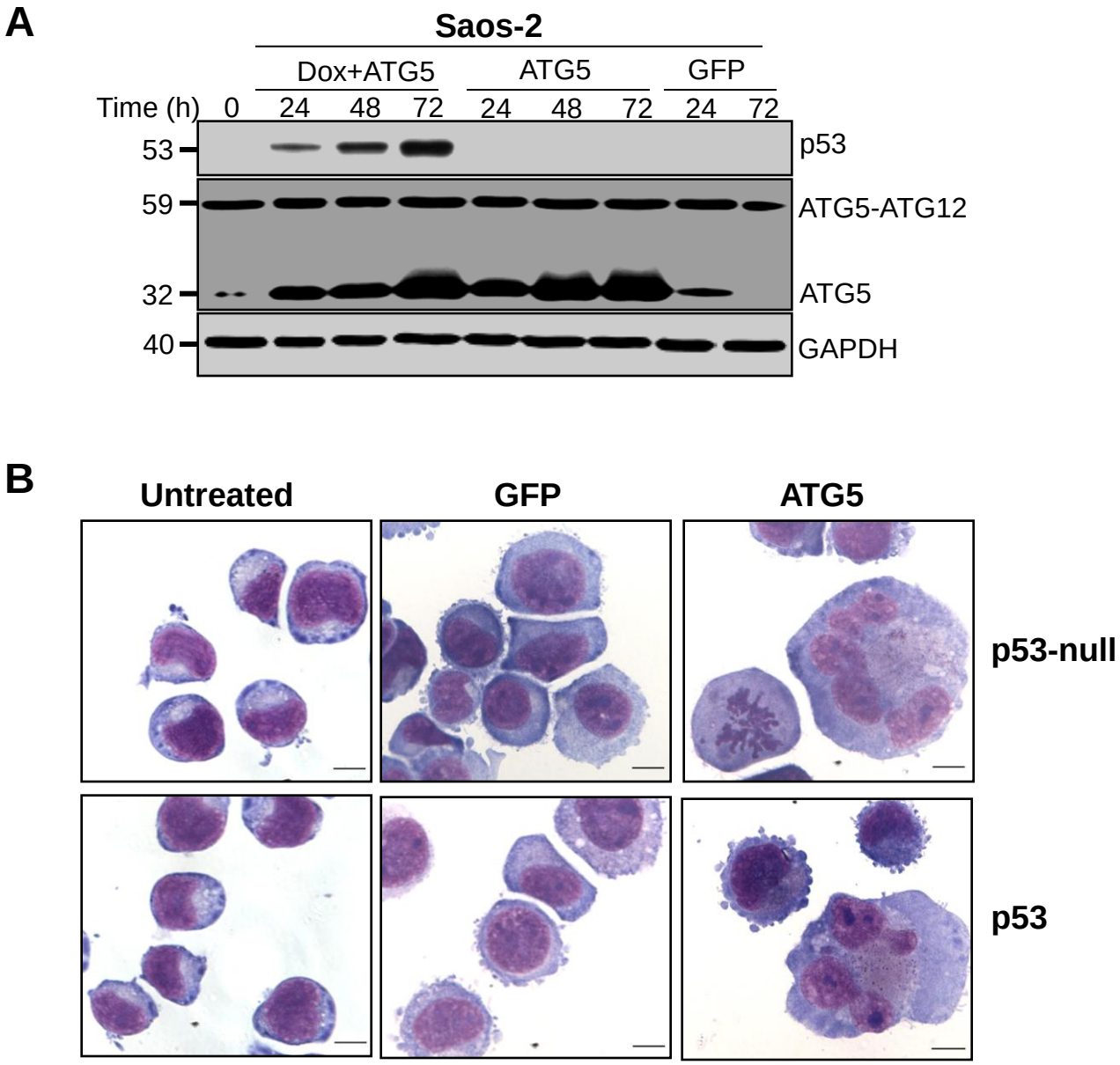
Supplementary Figure S4. Induction of autophagy by Beclin 1 or starvation was not associated with G2 arrest or the appearance of cells with abnormal nuclei. (**a**) Cell cycle analysis. Overexpression of Beclin 1 (18 h) or starvation (6 h) had no influence on the cell cycle of Jurkat T cells. (**b**) Immunoblotting. Jurkat T cells overexpressing Beclin 1 or following starvation exhibited signs of increased autophagic activity. (**c**) Morphological analysis. Same cells as used in panels a and b were stained with Giemsa-May-Grünwald. Bars, 10 μM.



Supplementary Figure S5. Ectopic ATG5 overexpression is associated with increased survivin mRNA expression. Jurkat T cells were transduced as indicated and analyzed 48 h after lentiviral gene transfer by real-time, quantitative PCR. From the different splice variants, the so-called wild-type (WT) survivin was induced as a consequence of ATG5 overexpression, whereas levels of the other known survivin splice variants remained unchanged. Values are means ± S.D. of 2 independent experiments.

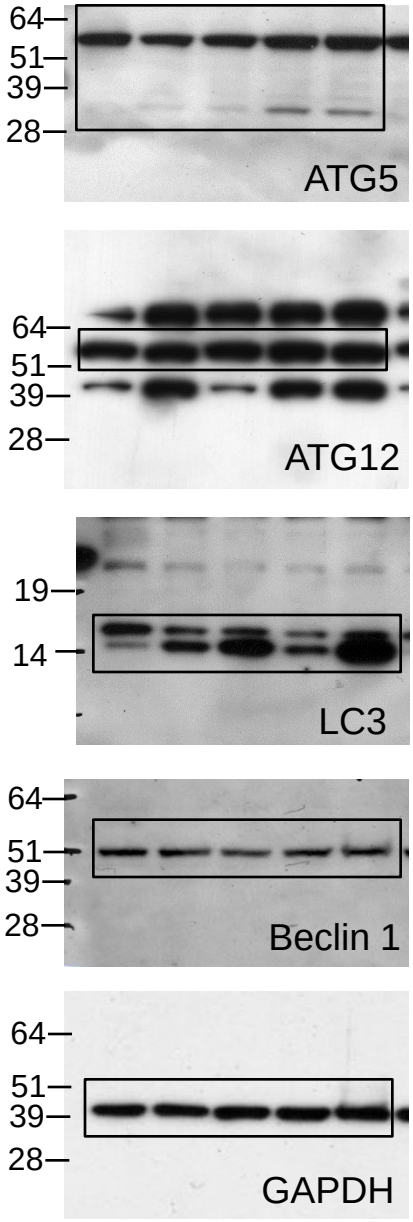


Supplementary Figure S6. Little evidence for co-localization between Aurora B and survivin upon DNA-damaging radio- and/or chemotherapy. Surgically excised cancer tissues were investigated. The numeric value shown in the overlay images is the average Pearson correlation for each tissue section as determined with Imaris. Results are representative of 3 independent experiments. Bars, 10 μm.



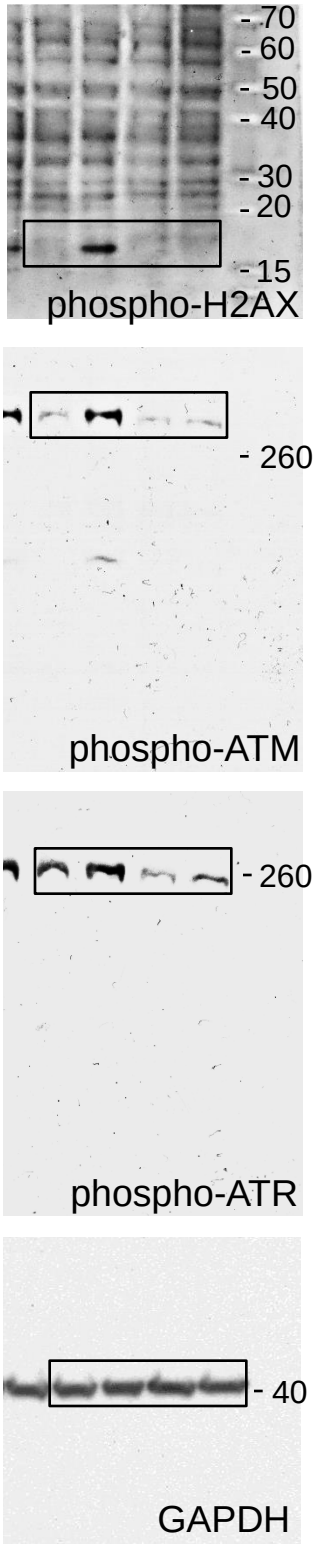
Supplementary Figure S7. The appearance of cells with abnormal nuclei upon ATG5 overexpression is p53-independent. (**a**) Immunoblotting. p53-inducible Saos-2 cells were transduced to express ATG5 by lentiviral gene transfer. As a control, GFP-transduced cells are shown.
(**b**) Morphological analysis. Uninduced and p53-induced Saos-2 cells, untransduced or overexpressing ATG5 or GFP, were stained with Giemsa-May-Grünwald 48 h after lentiviral gene transfer. Bars, 10 μM.

Fig. 1b



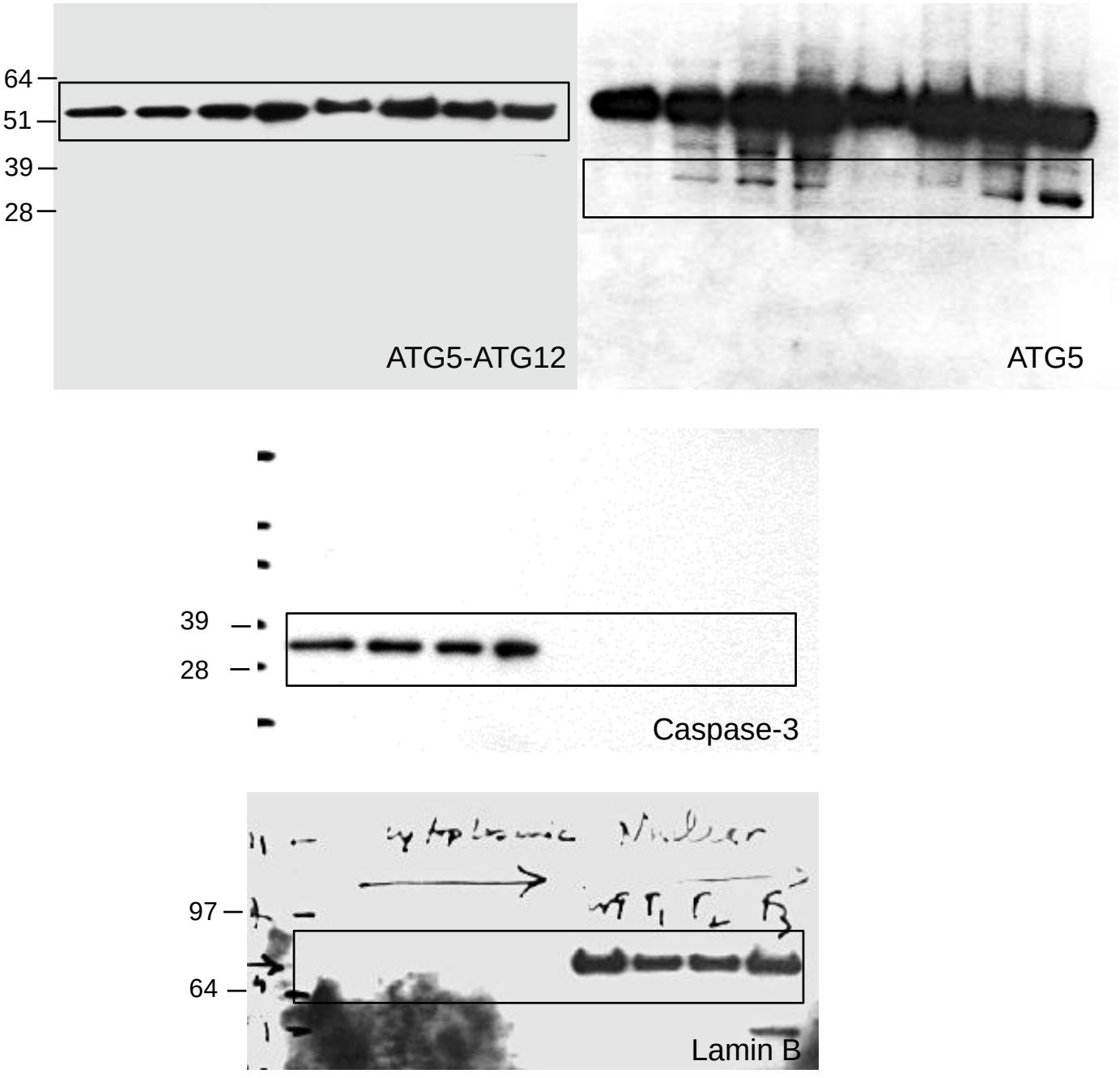
Supplementary Figure S8. Full immunoblot scans are provided for the original Fig. 1b, left panels.

Fig. 5c



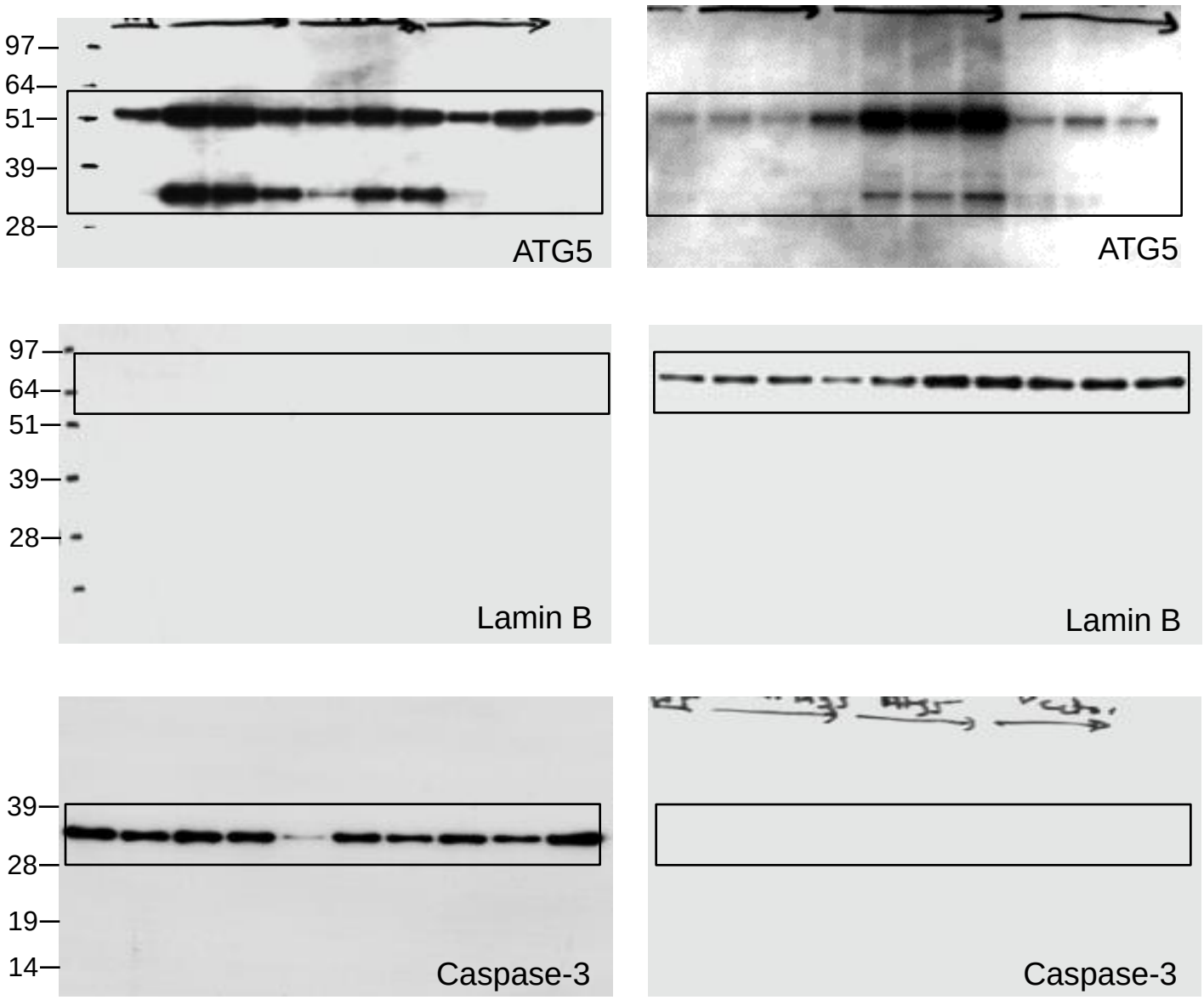
Supplementary Figure S9. Full immunoblot scans are provided for the original Fig. 5c.

Fig. 7a



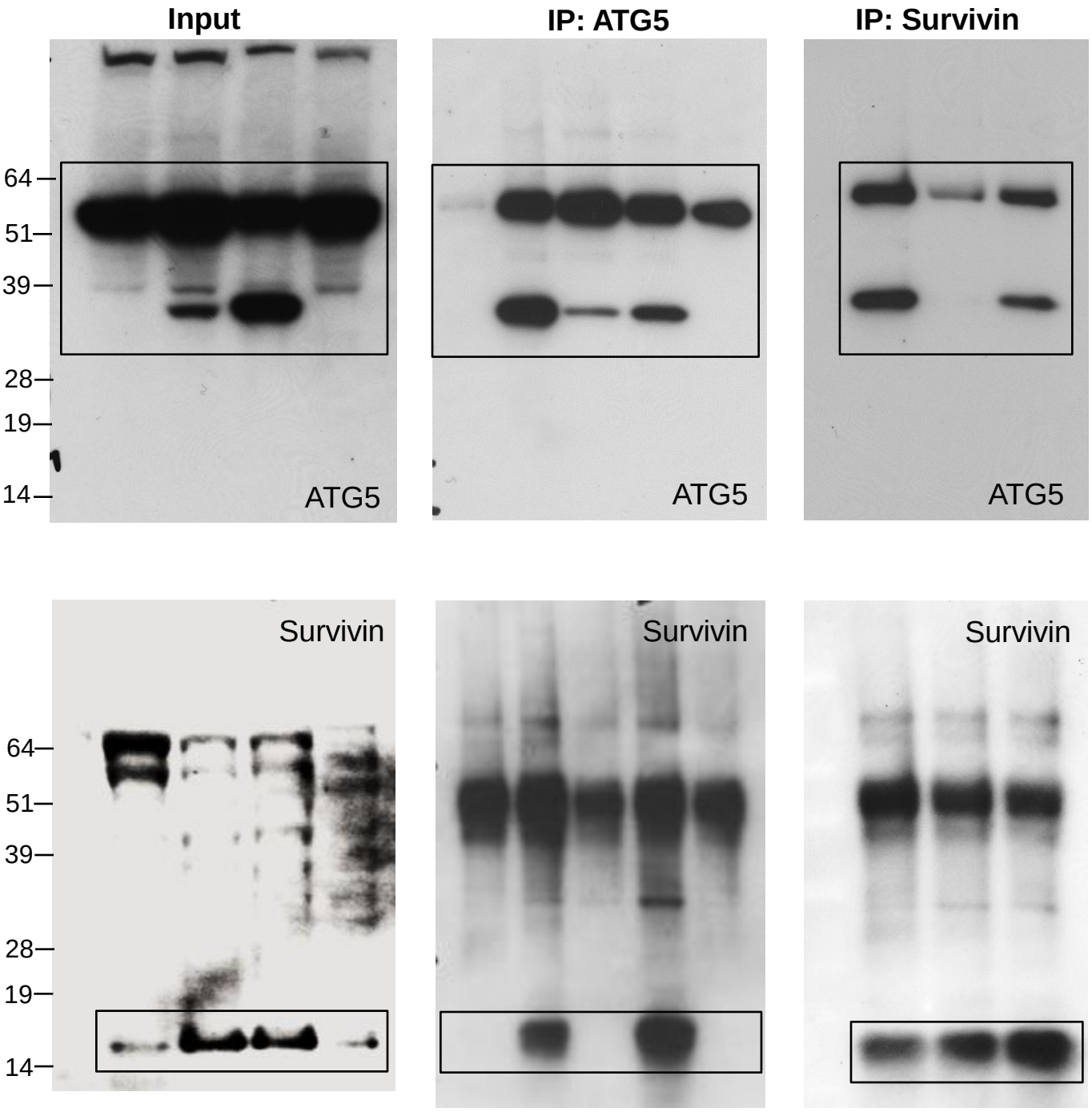
Supplementary Figure S10. Full immunoblot scans are provided for the original Fig. 7a.

Fig. 7d



Supplementary Figure S11. Full immunoblot scans are provided for the original Fig. 7d.

Fig. 8a



Supplementary Figure S12. Full immunoblot scans are provided for the original Fig. 8a.