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c-Abl tyrosine kinase is regulated downstream of the cytoskeletal protein synemin in head and neck squamous cell carcinoma radioresistance and DNA repair

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Supplementary Figures

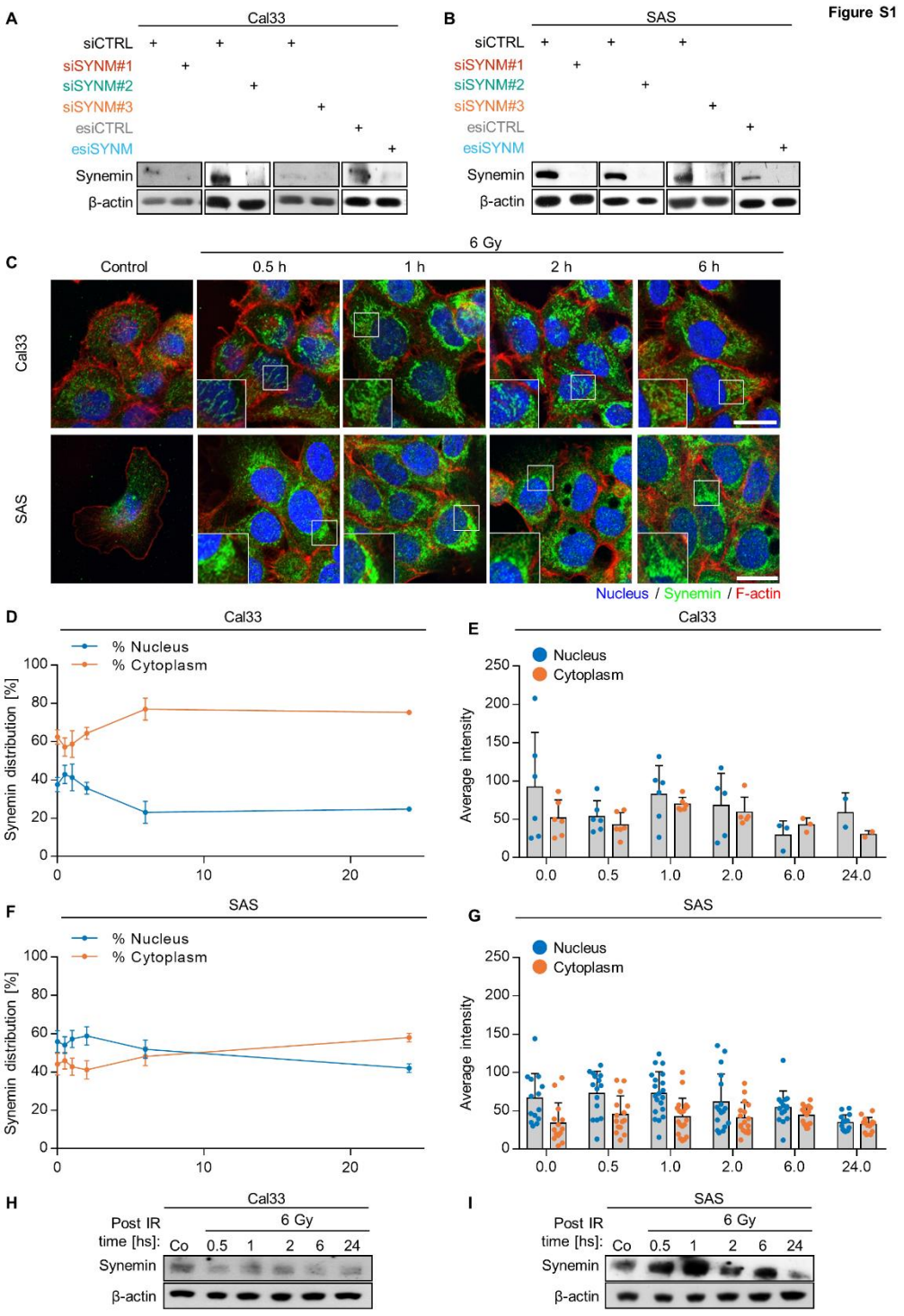

Figure S1. Characterization of synemin upon X-Ray irradiation. A, B, Knockdown efficiencies using three different siRNAs and one esiRNA for synemin silencing in Cal33 (**A**) and SAS (**B**) cells. **C,** Immunofluorescence staining of synemin (green) and its kinetics in Cal33 and SAS cells upon 6 Gy X-rays. Cells were counterstained for F-actin (red) and nucleus (blue) (bar, 20 µm). **D-G,** Kinetic analyses using Fiji software of confocal images showing synemin´s intracellular distribution in Cal33 and SAS cell lines. Ratio of mean fluorescence intensity of nuclear to cytoplasmic localization (**D,F**) and average intensity (**E,G**) were determined using the Intensity Ratio Nuclei Cytoplasm Tool plugin (NIH, USA). **H,I,** Western blots of synemin expression kinetics in whole cell lysates from Cal33 and SAS cells post 6 Gy irradiation. β-actin served as loading control.

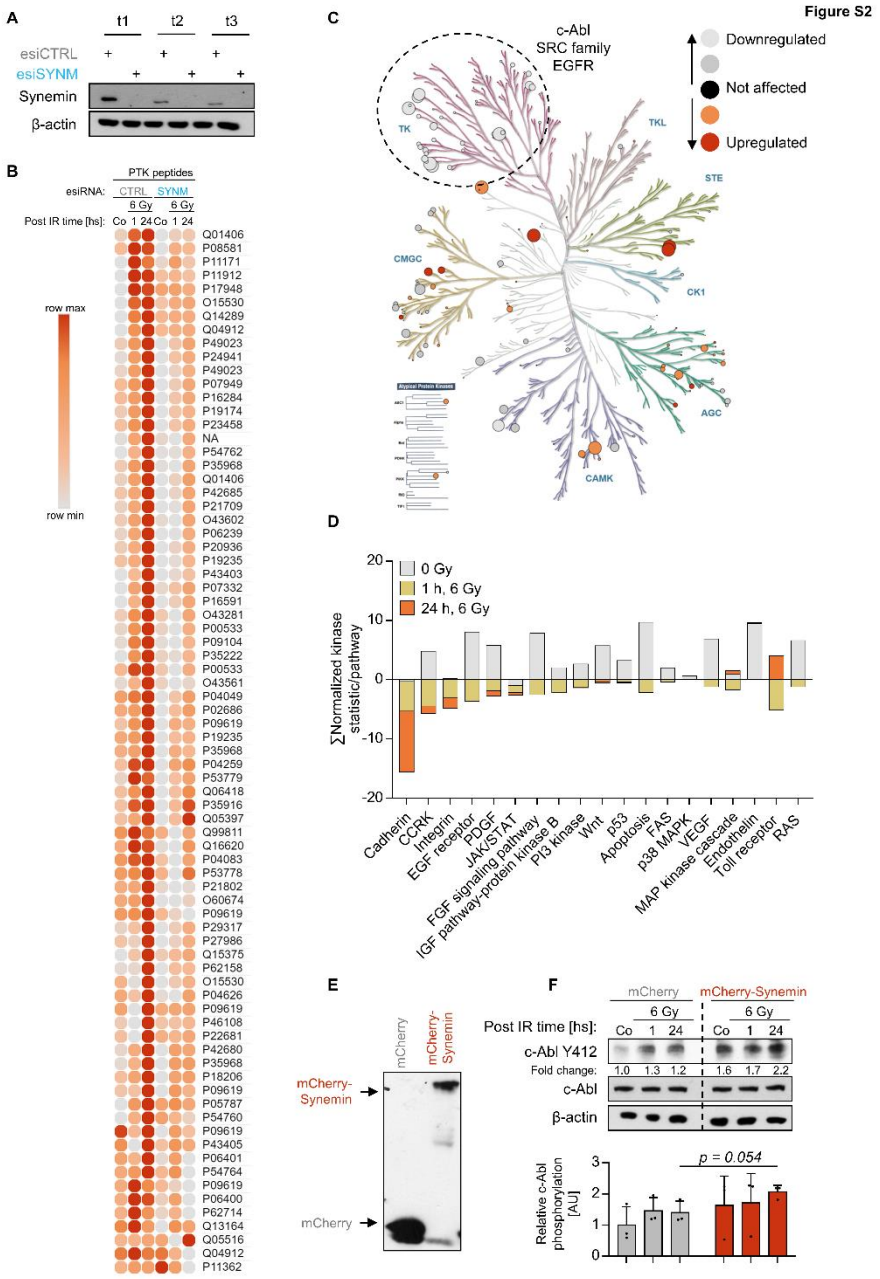

Figure S2. Synemin regulates tyrosine kinase activity, in particular c-Abl kinase. A, Western blots of knockdown efficiencies of samples employed for PAMgene-based kinome analysis. β-actin served as loading control. **B,** Heatmap of phosphorylated peptides from tyrosine kinases of control and synemin knockdown samples before and after 1 and 24 h X-ray irradiation (n=3). **C,** Kinase family tree of down- and up-regulated kinases 24 h post irradiation and synemin knockdown. Black dotted circle shows tyrosine kinases. **D,** Pathway classification of the de-regulated kinases using Panther analysis (http://www.pantherdb.org/). The mean kinase statistic of each kinase involved in the same pathway was summed to obtain an overall pathway related kinase statistic. **E,** Western blot on whole cell lysates from mCherry-Synemin transfectants at different time points post 6 Gy X-ray irradiation. β-actin served as loading control. **F**, Western blot of c-Abl expression and phosphorylation from whole cell lysates of synemin-overexpressing cells. Densitometries of Western blots shown in "E" from synemin-depleted, 6-Gy irradiated cells showing phosphorylated forms of c-Abl (n=3). Phosphorylation levels were calculated relative to the total amount of c-Abl. Data are presented as mean ± SD (n = 3; two-sided t-test).

Figure S3

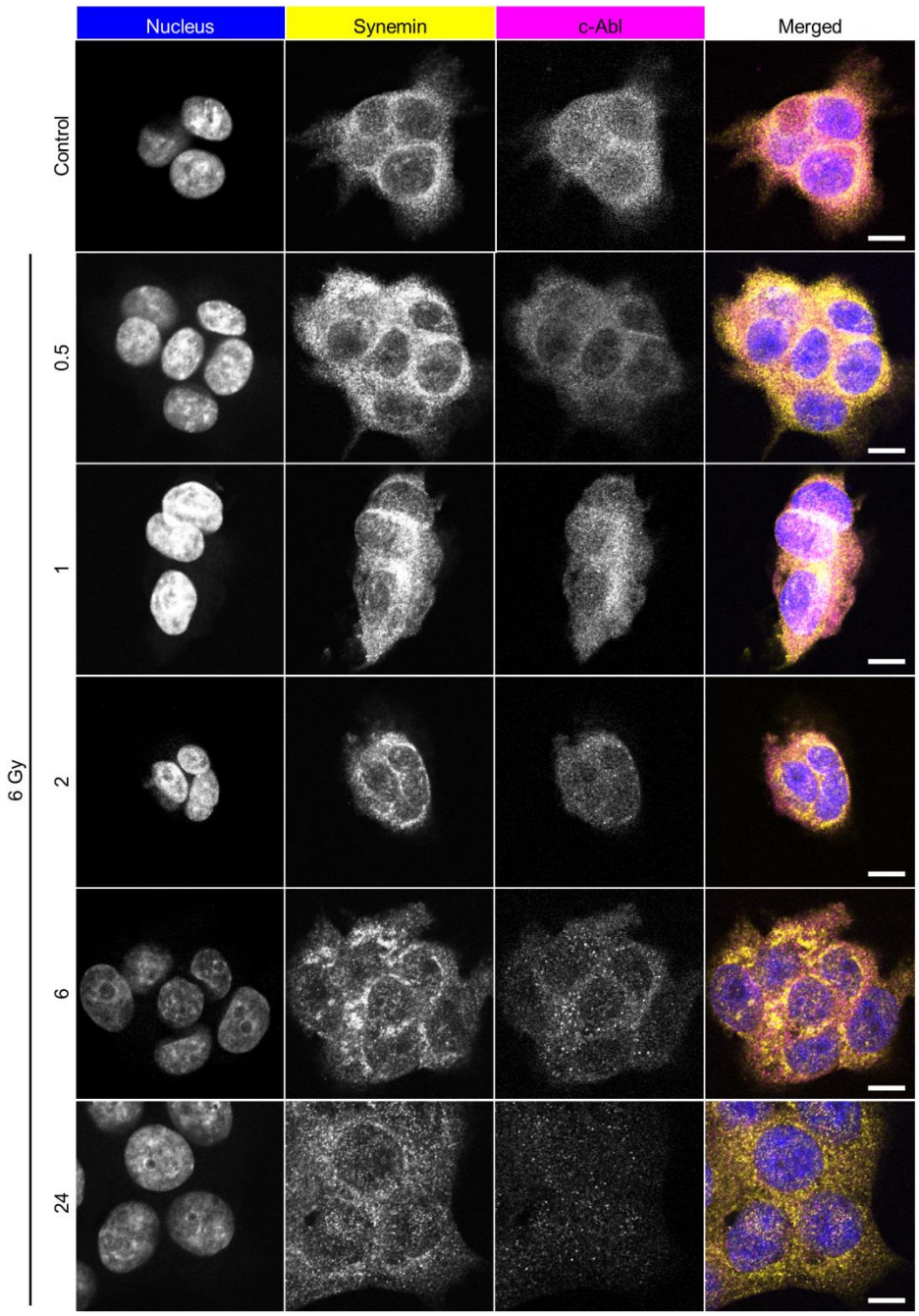

Figure S3. Synemin/c-Abl colocalization after X-ray exposure. **A,** Immunofluorescence staining of synemin and c-Abl in SAS cells after 6 Gy irradiation. Samples were fixed and analyzed at different time points (0.5, 1, 2, 6 and 24 h) post X-ray exposure (bar, 20 μm).

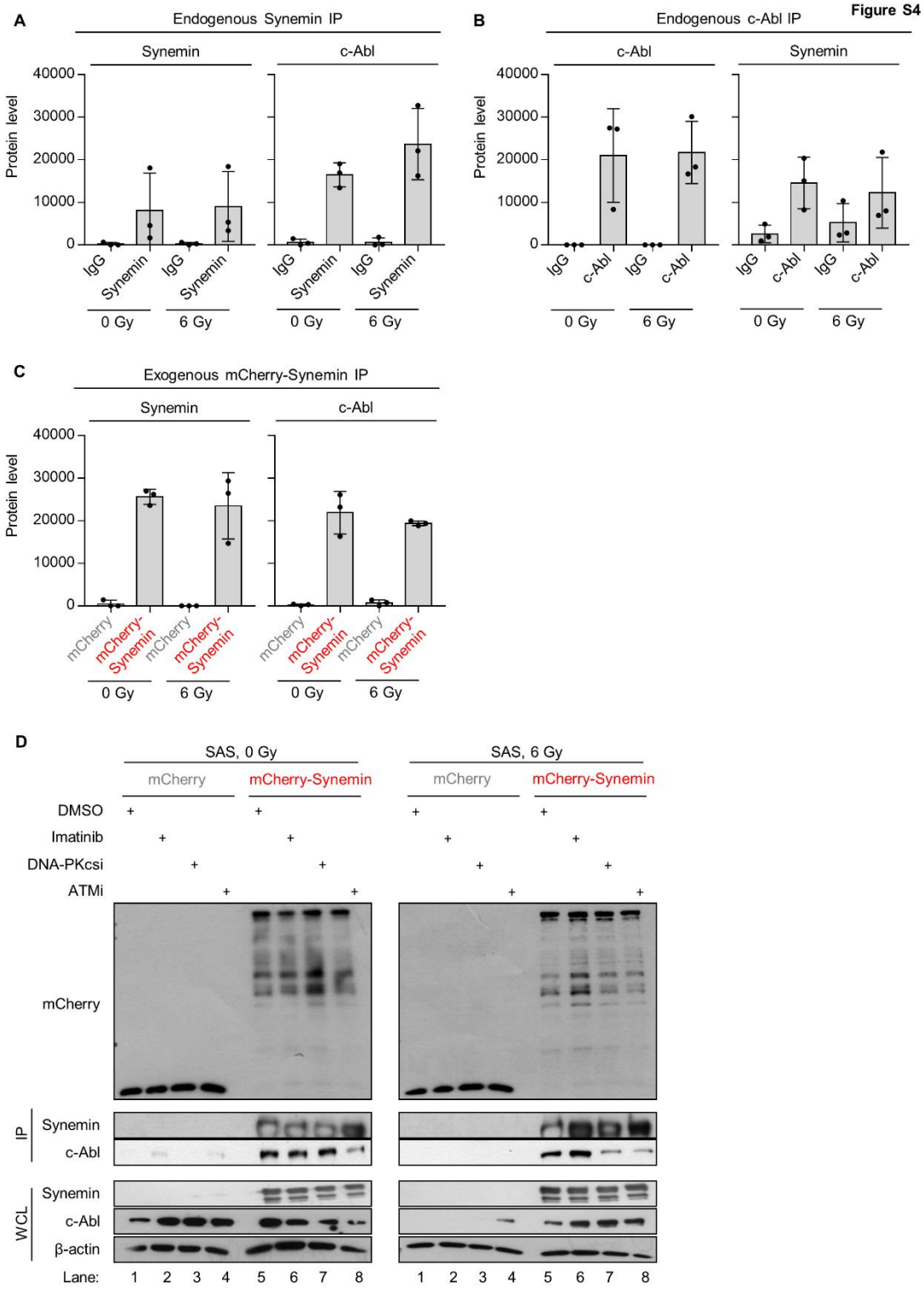

Figure S4. Synemin/c-Abl interact in an ATM-dependent manner. **A**, Densitometries of endogenous synemin immunoprecipitations (IPs) from Figure 4B. **B**, Densitometries of endogenous c-Abl IPs from Figure 4C. **C**, Densitometries of exogenous mCherry (empty vector) and mCherry-Synemin IPs from Figure 4D. **D**, Western blots on mCherry immunoprecipitates from mCherry-SAS and mCherry-Synemin-SAS cells after a 1-h pretreatment with Imatinib, DNA-PKcsi or ATMi alone or in combination with 6 Gy X-rays. IP, immunoprecipitate; WCL, whole cell lysates.