# Supplementary Material

## Involvement of the nuclear proteasome activator PA28γ in the cellular response to DNA double-strand breaks

Adva Levy-Barda[1], Yaniv Lerenthal[1], Anthony J. Davis[2], Young Min Chung[3], Jeroen Essers[4], Zhengping Shao[2], Nicole van Vliet[4], David J. Chen[2], Mickey C-T. Hu[3], Roland Kanaar[4], Yael Ziv[1], and Yosef Shiloh[1]

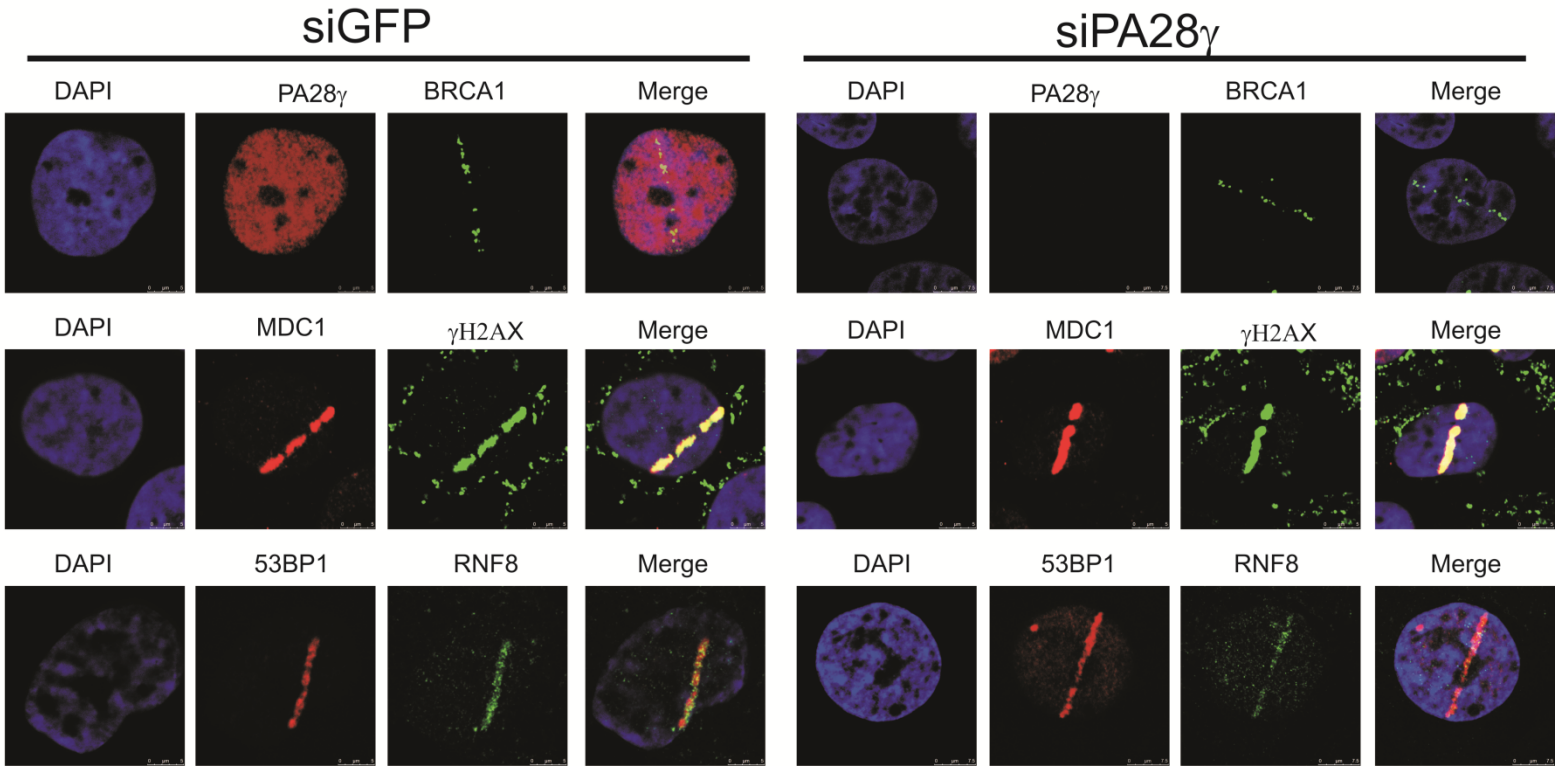
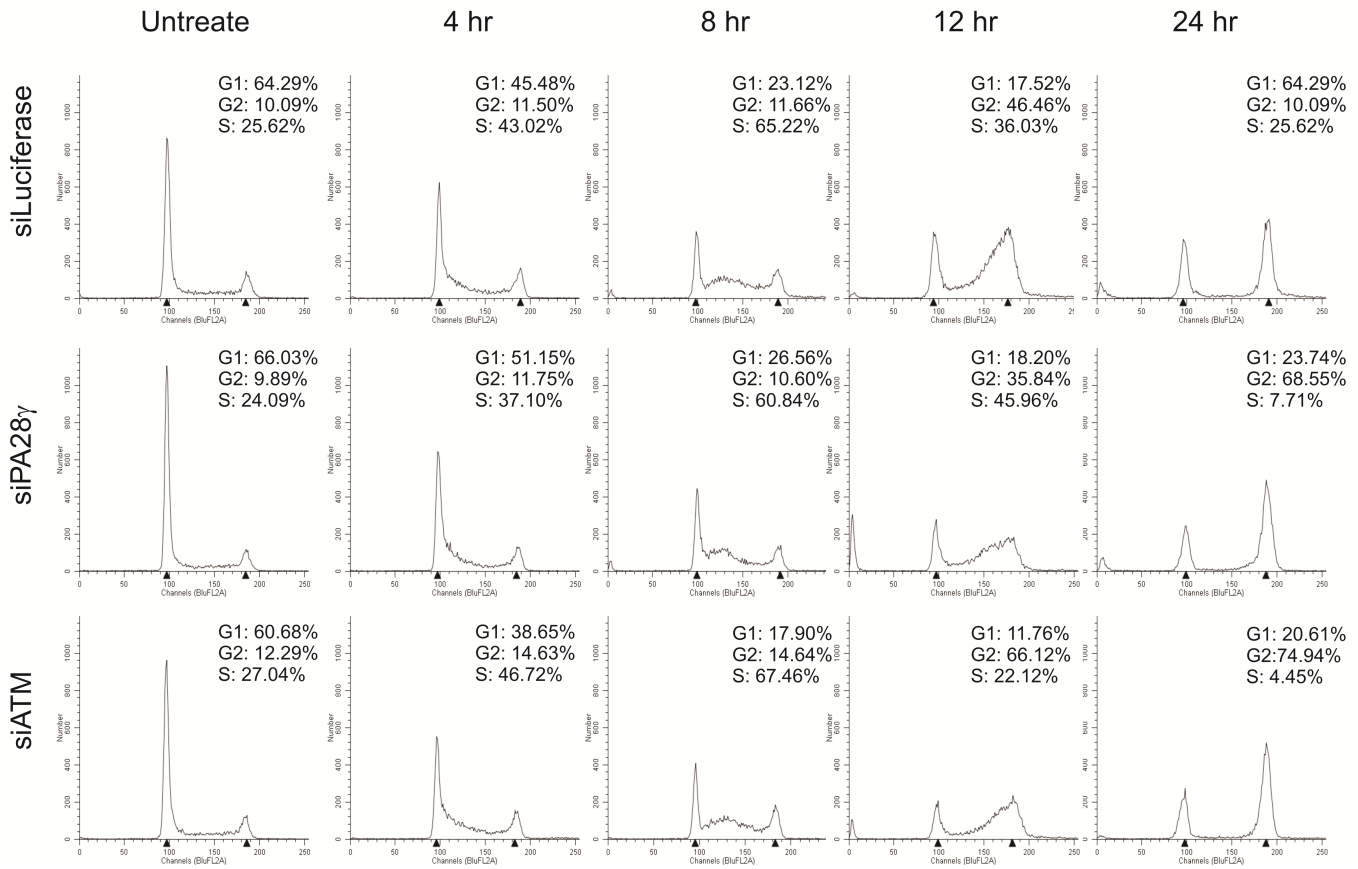



**Figure S1. Depletion of PA28γ does not influence the recruitment of MDC1, RNF8 or BRCA1 to DNA damage sites.** Localized DNA damage was induced in U2OS cells using a focused laser microbeam. The cells were fixed 5 min after damage induction and stained with the indicated antibodies.



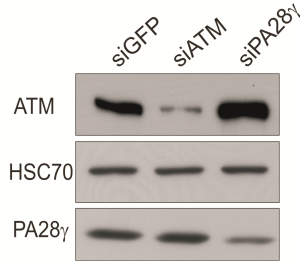




**Figure S2. Involvement of PA28γ in the S-phase checkpoint.** Left panel: Cell cycle analysis of U2OS cells 72 hr after transfection with siPA28γ or siLuciferase and siATM as control. Cells were treated with 50 ng/ml of NCS 72 hr after siRNA transfection, and analyzed at various time points using flow cytometry. Right panel: Western blotting analysis showing the extend of protein depletion 72 hr after siRNA transfection. Results of one of three independent experiments are shown

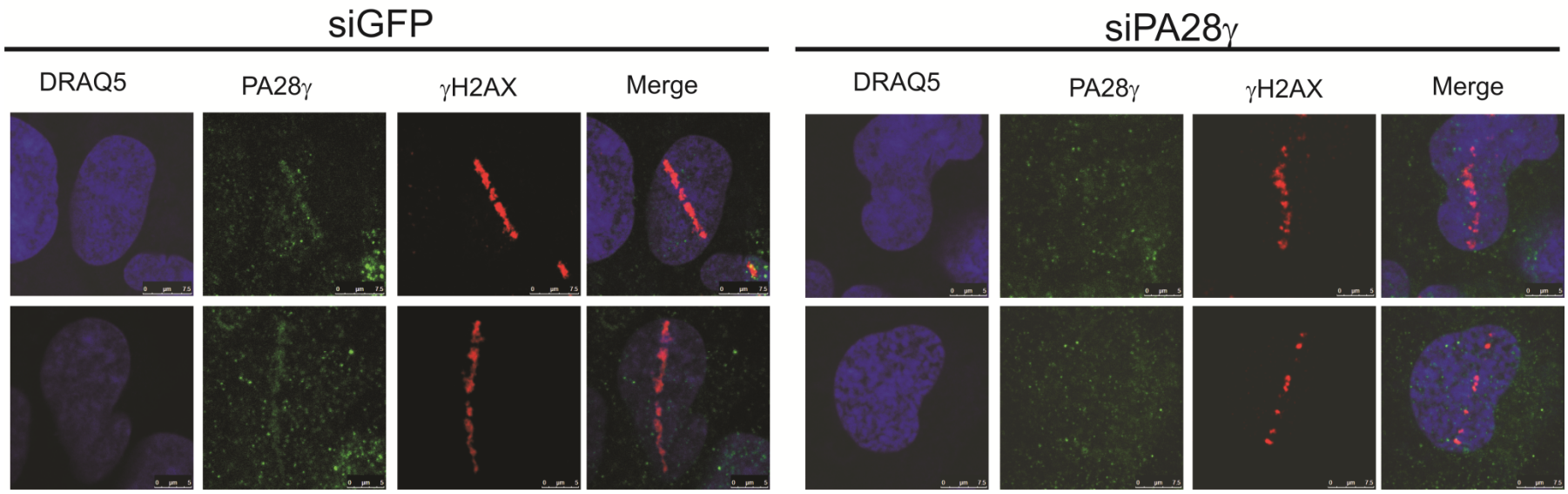



**Figure S3. Specificity of the antibody against PA28γ.** U2OS cells were transfected with siRNAs against GFP or PA28γ, and localized DNA damage was induced using a laser microbeam 72 hr later. The cells were treated with 0.25% NP40, fixed and co-stained with antibodies against PA28γ and γH2AX.

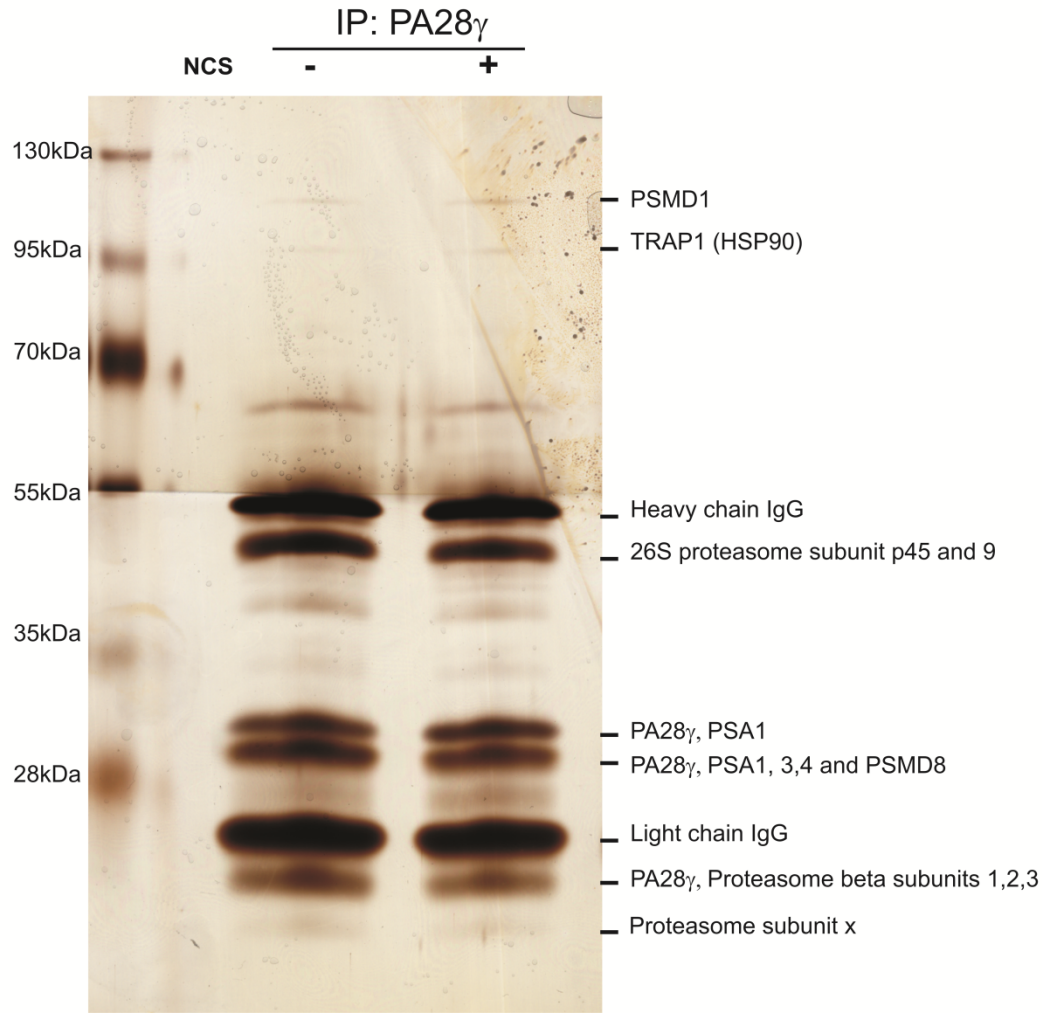



**Figure S4. Stable interaction between PA28γ and proteasome subunits is not affected by DNA damage.** HEK293 cells were treated with 200 ng/ml of NCS for 1 hr, endogenous PA28γ was immunoprecipitated and the immune complexes were separated using SDS-PAGE. Proteins were identified by mass spectrometry.

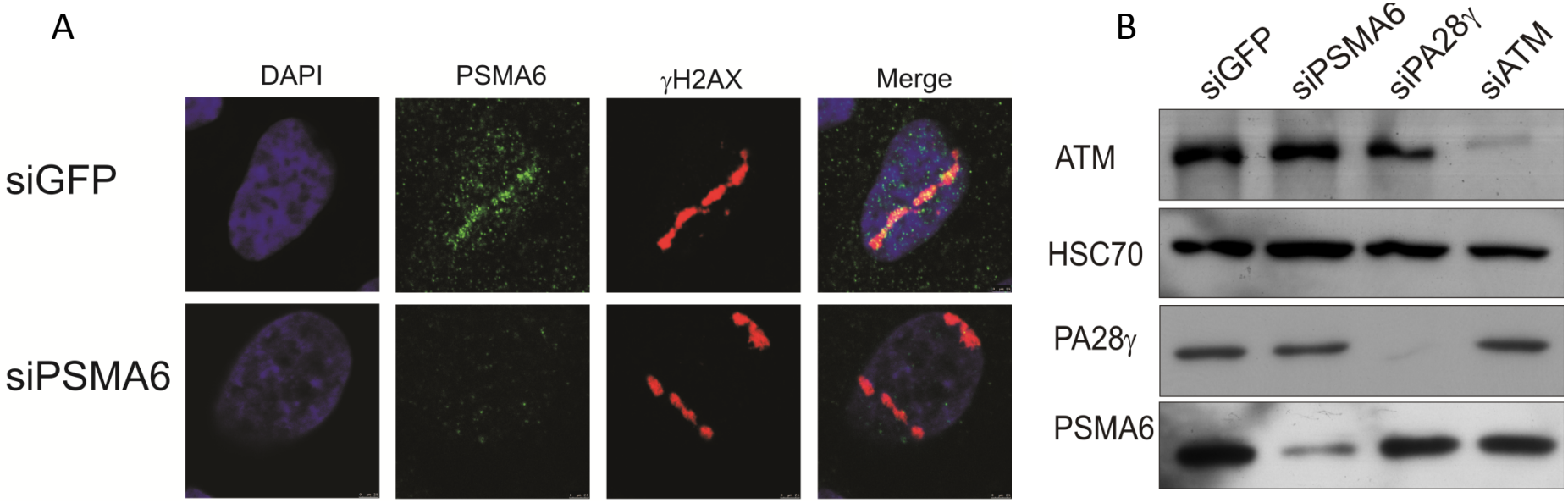



**Figure S5. Specificity of the antibody against PSMA6. (**A) U2OS cells were transfected with siRNA against GFP or PSMA6, localized DNA damage was induced using a laser microbeam 72 hr later, and the cells were co-stained with antibodies against PSMA6 and γH2AX. (B) Western blotting analysis shows the extent of gene silencing in this experiment.

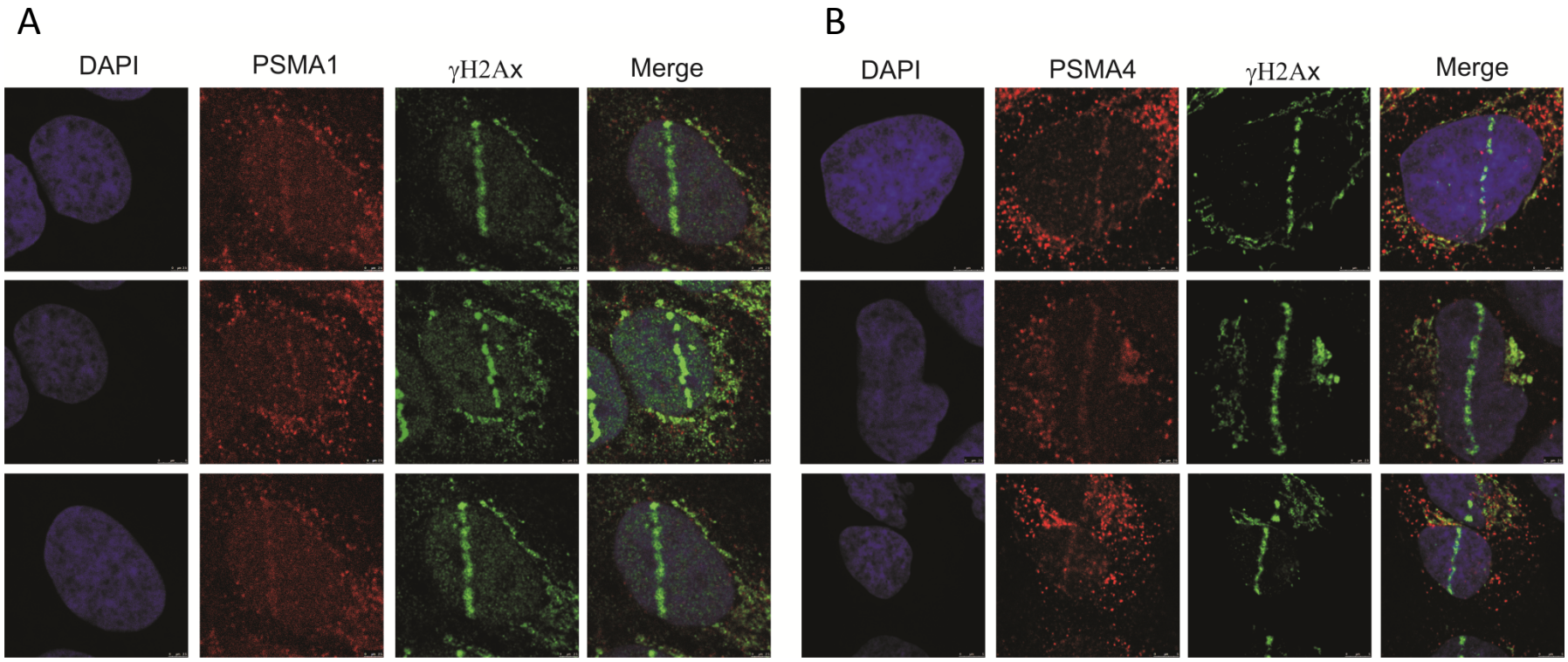



**Figure S6. Recruitment of the 20S proteasome subunits to DNA damage sites.**
Localized DNA damage was induced using a laser microbeam in U2OS cells. The cells were co-stained with antibodies against 20S proteasome subunits PSMA1 (A), PSMA4 (B) and γH2AX.