

Supporting Information

Method of monitoring 26S proteasome in cells revealed the crucial role of PSMA3 C-terminus in 26S integrity

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TACCTTTCAGGTAGTAGTTGAATTCAGGTAGTATTTGTTTCAGTTTTTTTTTTTCCCTTCATGT
TCTAAGACCAGCTTGAGAGGCCAAAGGTTGTACCACTGAGCTCTAGTTGTTGTTACCTAAAAA
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GCAATAATTCTACATCATTTCTCCCCTTCCCTTCCACTTGTTTAGACTAAGATATGTTAGAGA
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ACCAACAGGTACTCTCATTTCTCAGAATAAGGGGCATTCTAAATTTTAAAAGTAGGTCAAC
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TTCAAAAACCTTCCAGAGTAATTAATATG.

The sequence of the donor 964 bp is:

TAAAGCCATGTAACTAACAATGATTGCTTTAGAGATAATTATTTGGAATTTTTATAGCTTA
CTTACAATGTGCCCAGGTCAGCTGTATAAAATAAATACTGCATTGTTGTTTCTTTCCATTTA
TTTTTTCTTAAACACATAGGCCTTCTAGAATCGGGTCCACTTATGTTAAAATAATGTCACCT
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TATCGCTTACCTTTAAATCTCTTACCTGTAAGCTTCCCGATTTTAGAAAGAAGAGTAGTGGCC
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GTGTAATAAGCTTTCCCTCAGAAACAAGTAACCATATAAGGCAGGAAGTTACTCTTTTTTAG
GAAGCTGACATAACCTGGATTACAGAATCTACCAAGGCTGATCTCAGTTTTCTAAAACCTTA
TATGTAAGTATTTATTAGCAAACCTGAATCTTTTTTCCAAAAGTAGGACCATAGTAATAATAG
CAGTGAGGAAAACAAAGTTGTTCCCTCTGTTTGTGTTGAAACAGTTTATTAGTTTTATCAGTTTT
ATTCAGTGTGTCAGATAGGAATAAGTGCTATAAGAAGTATAAAACCAGATAGACAGACAGA
TAGATAGATAGATAGATAGAGATACAGGGTATAGGCCGTCTTCTGAGGTGTTGTGGAAGGC
TTCTATGGTAAGGTGACATTTAAGTGAAAAGGAGGGAGAGCCCATGAACGTATGGAGGGAA
GAAAGACGAGGAAAGTCACTGGCAATTTTAAGTGCGGATTTGGCTTACCTTTTACGTCTCAC
TTACGGCATTCCGGTGGCATGGATTGTACCGCGG.

Figure S1: The sequence of the donor DNA in CRISPR-Cas9 editing of PSMD6.

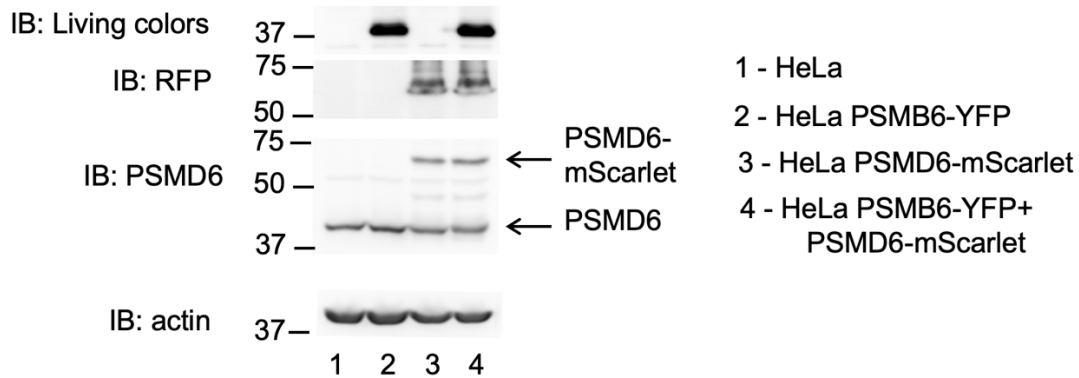


Figure S2: Evidence for heterozygosity of the PSMD6-mScarlet cells. Extracts from the indicated cells were prepared and analyzed by western blotting using the indicated antibodies.

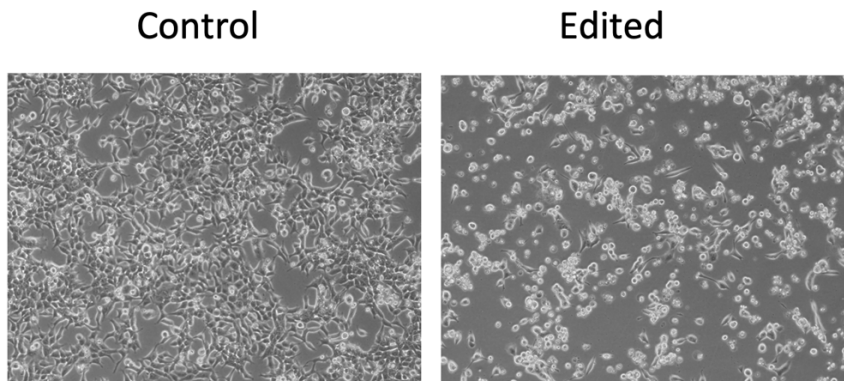


Figure S3: Microscopic images of control and C-terminus truncated PSMA3 edited cells after 6 days.

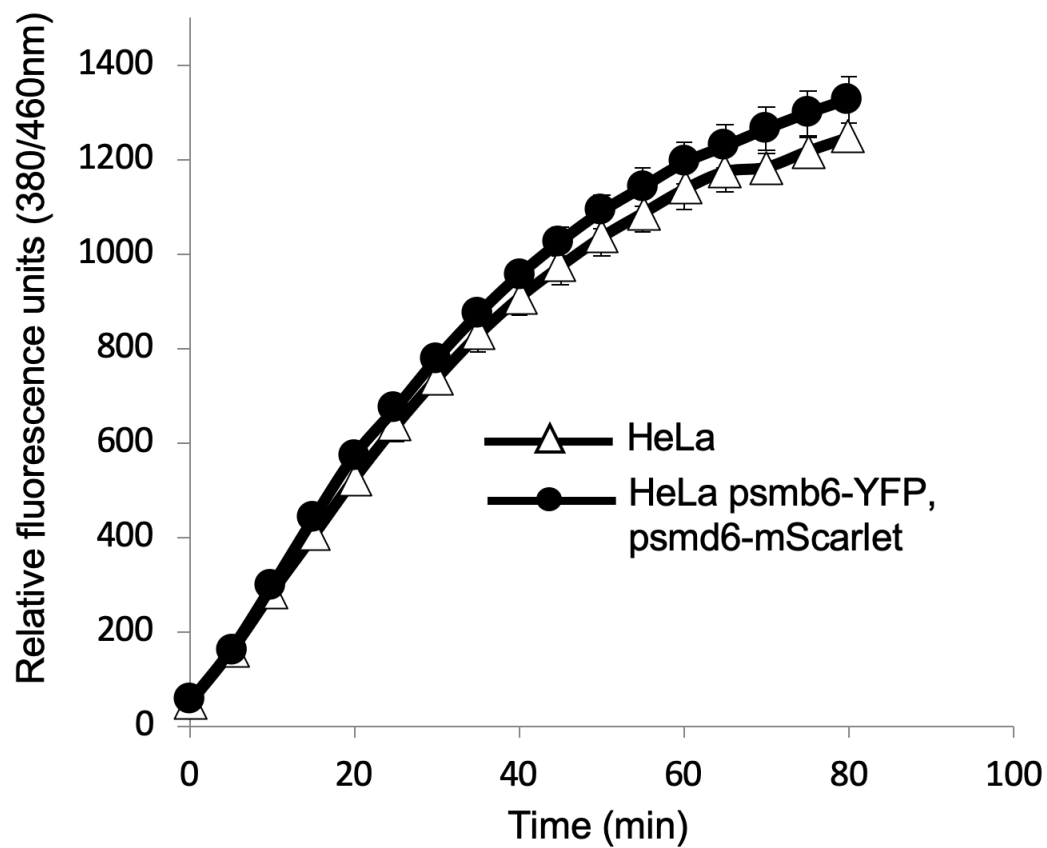


Figure S4: Proteasome activity of HeLa cells edited for PSMB6-YFP and PSMD6-mScarlet. Extracts of control HeLa cells and the CRISPR edited cells were assayed for Chymotrypsin-like activities over time using the fluorescent substrate Suc-LLVY-AMC.

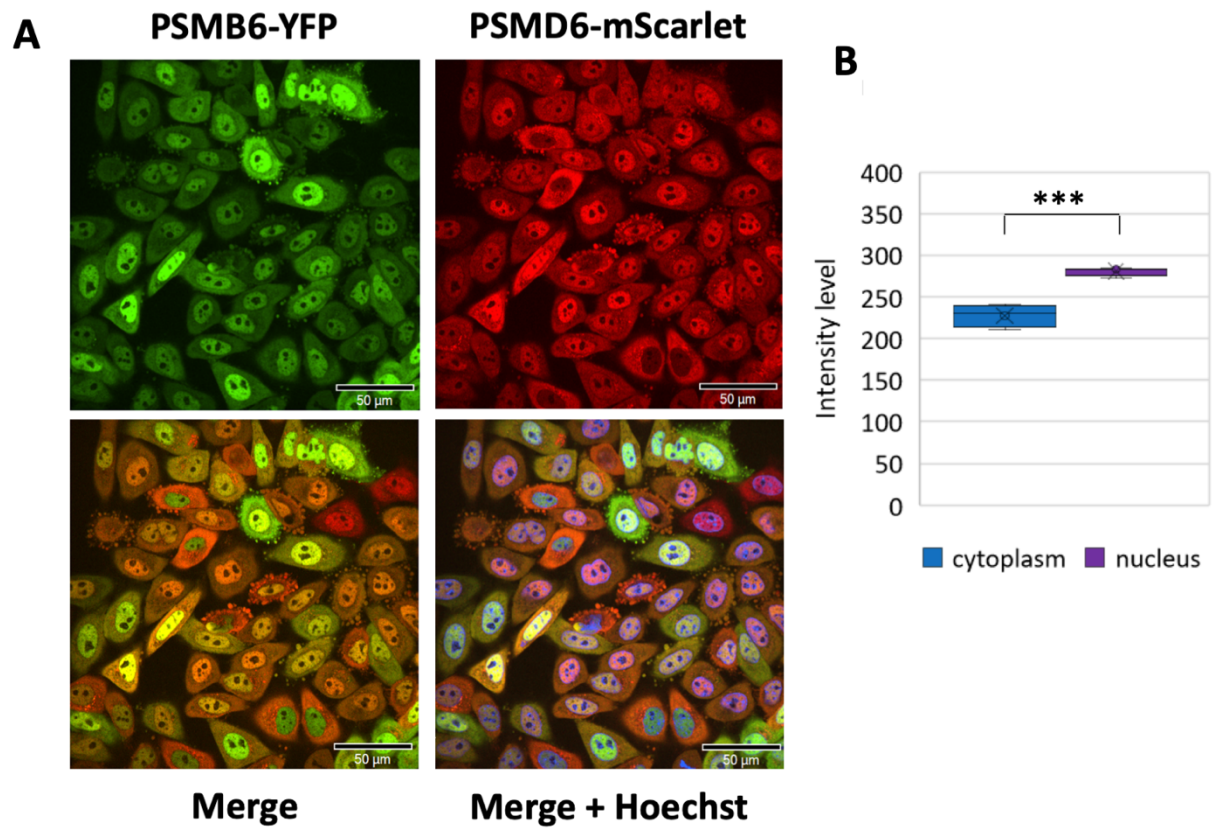


Figure S5: Establishment of a double reporter cell line. A. HeLa PSMB6-YFP PSMB6-mScarlet cells – a wide-field view. Green live-imaging marker represents 20S, red marker 19S and hence 26S, blue marker represents the nucleus. Scale bar is 10 μ m. B. Boxplot analysis of the intensity level in the cytoplasm and the nucleus. ***P < 0.001 by 2-tailed unpaired t-test.

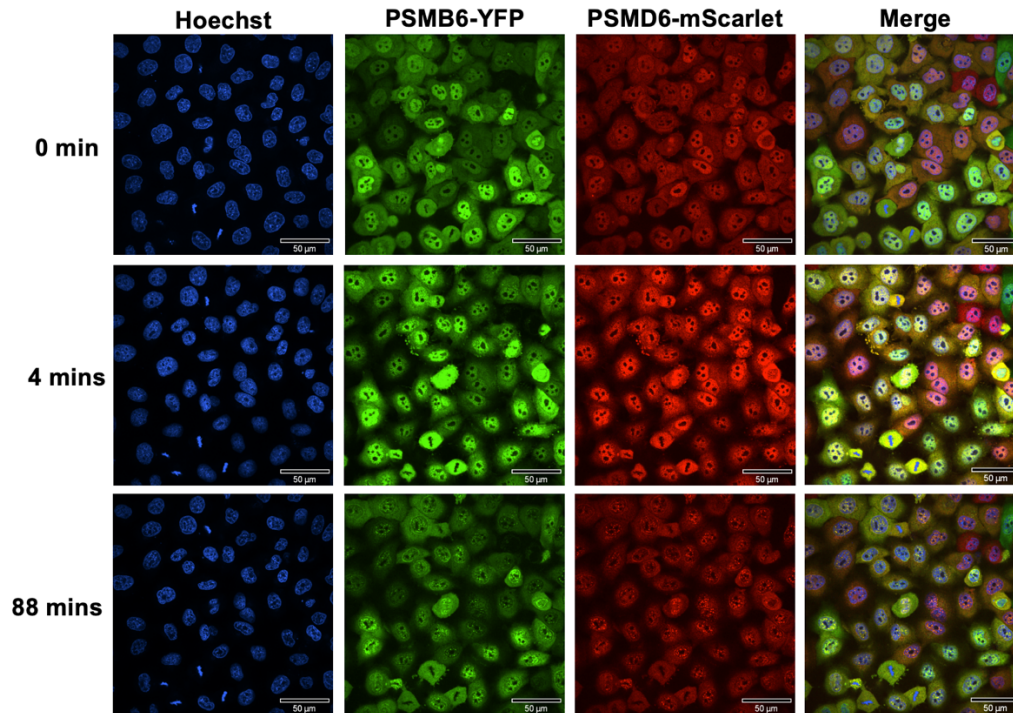


Figure S6: Wide-field images of proteasome granules under sucrose osmotic stress at different time points. Time-lapse images of HeLa PSMB6-YFP PSMD6-mScarlet cells treated with 200 mM sucrose. The green live-imaging marker represents all proteasomes, the red live-imaging marker represents 19S RP and the yellow spots in the merge panel represent 26S proteasomes. The nuclei were Hoechst stained (blue). Selected time points are shown. The scale bar is 10 μ m.

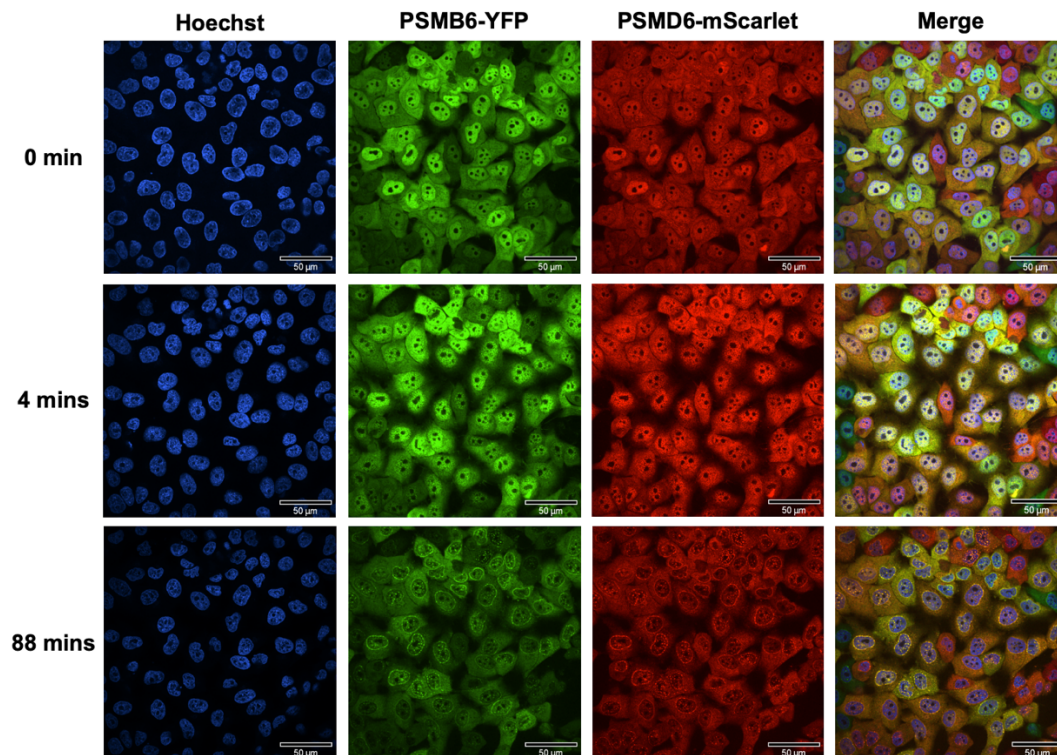


Figure S7: Wide-field images of proteasome granules under NaCl osmotic stress at different time points. Time-lapse images of HeLa PSMB6-YFP PSMD6-mScarlet cells treated with 150 mM NaCl. The green live-imaging marker represents all proteasomes, the red live-imaging marker represents 19S RP and the yellow spots in the merge panel represent 26S proteasomes. The nuclei were Hoechst stained (blue). Selected time points are shown. The scale bar is 10 μ m.

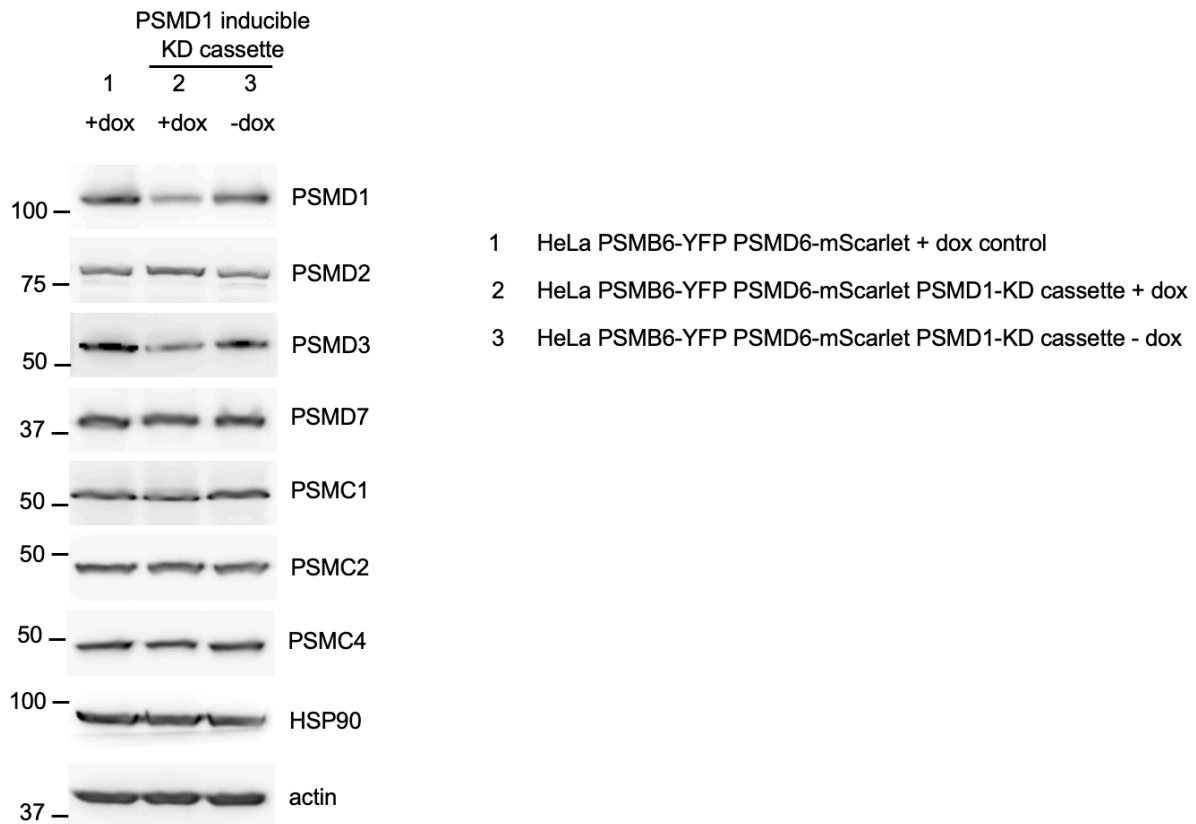


Figure S8: Western blot analysis of the components of 19S in PSMD1 knockout cells. HeLa PSMB6-YFP PSMD6-mScarlet PSMD1-KD cassette cells were treated (lane 2) or untreated (lane 3) with doxycycline for 3.5 days. As an additional control, HeLa PSMB6-YFP PSMD6-mScarlet cells without PSMD1-KD cassette were also doxycycline-treated and analyzed after 3.5 days (lane 1).

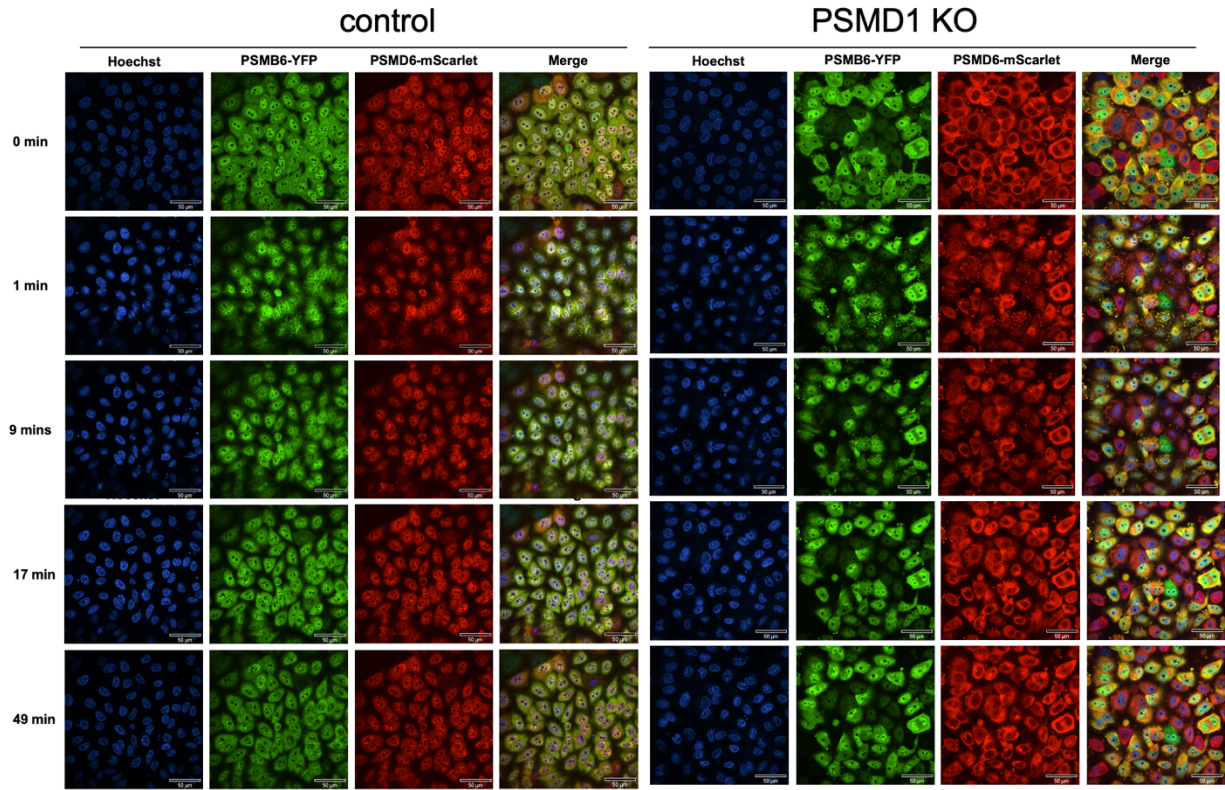


Figure S9: Wide-field images of proteasome granules under osmotic stress. Wide-fields time-lapse images of HeLa PSMB6-YFP PSMD6-mScarlet cells and HeLa PSMB6-YFP PSMD6-mScarlet PSMD1-KD cells treated with 100 mM NaCl. The scale bar is 10 μ m.