Supporting Information

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

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TACCTTTCAGGTAGTAGTTGAATTCAGGTAGTATTTGTTCAGTTTTTTTTTTTTCCCTTCATGT
TCTAAGACCAGCTTGAGAGGCAAAGGTTGTACCACTGAGCTCTAGTTGTTGTTACCTAAAAA
GGCCTTGTTTTAAATTTCTGTGATACCTAAGAATTTCAAATCTGGGTTGTCATGGATTCTTTA
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GCAATAATTCTACATCATTTCTCCCCTTCCCTTCCACTTGTTTAGACTAAGATATGTTAGAGA
GGGAAAGGGTCGTTGTTTTAGTAAATACTATTGCTGTTGACATGTTAATACTATTGCTGTTGA
CATGTTTACTGATGGGCTGTGTTCCATAATTTTGTTTTAGGTCTTTTGTTTGAAACAGTTTACT
GTTTTTATCAGTTTTGGTCCCTAATTTTTCCTAACCTACAGTTTTTCTCTGAGTACATATGGTT
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AAGCTTGAAGGGAAGCTAAATGTTTGTTACAAATTTAAGACAGTATTTTAATGCCGTTTGCA
TTTTTCTAAGAATTTTCTATAAAGCTAATTCTGTTATTTTTTGTCTCTAAATTAGGGAACTGTC
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ACCAACAGGTACTCTCATTTCTCAGAATAAGGGGCATTCCTAAATTTTAAAAGTAGGTCAAC
TATTGTCATGGAATAATGTGACTGGTAAATAATTCATTTTTTCTTGAATTTATTTATAGACCT
GATAGCAAGAACTGGCAGTACCAAGAAACTATCAAGAAAGGAGATCTGCTACTAAACAGAG
TTCAAAAACTTTCCAGAGTAATTAATATG.

The sequence of the donor 964 bp is:

TAAAGCCATGTAACTAACAAATGATTTGCTTTAGAGATAATTATTTGGAATTTTTATAGCTTA
CTTCACAATGTGCCCAGGTCAGCTGTATAAAATAAATACTGCATTGTTGTTTCTTTCCATTTA
TTTTTTTCTTAAACACATAGGCCTTCTAGAATCGGGTCCACTTATGTTAAAATAATGTCACTT
GAATTATTCATTAATGAAAATCTGGTTTATAGGGTAAGGTTGCAGCTGGGTTCAGCTAGGTT
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TATCGCTTACCTTTAAATCTCTTACCTGTAAGCTTCCCGATTTTAGAAAGAAGAGTAGTGGCC
TAAAATTAAACAAACAGTGTCTCCCACTTTTAGTATACAAAAGATAGAAGATAAAGAAAAT
GTGTAATAAGCTTTCCCTCAGAAACAAGTAACCATATAAGGCAGGAAGTTACTCTTTTTTAG
GAAGCTGACATAACCTGGATTACAGAATCTACCAAGGCTGATCTCAGTTTTCTAAAACCTTA
TATGTAAGTATTTATTAGCAAACTGAATCTTTTTTCCAAAAGTAGGACCATAGTAATAATAG
CAGTGAGGAAAACAAAGTTGTTCCTCTGTTTGTTTGAAACAGTTTATTAGTTTTATCAGTTTT
ATTCAGTGTGTCAGATAGGAATAAGTGCTATAAGAAGTATAAAACCAGATAGACAGACAGA
TAGATAGATAGATAGATAGAGATACAGGGTATAGGCCGTCTTCTGAGGTGTTGTGGAAGGC
TTCTATGGTAAGGTGACATTTAAGTGAAAAGGAGGGAGAGCCCATGAACGTATGGAGGGAA
GAAAGACGAGGAAAGTCACTGGCAATTTTAAGTGCGGATTTGGCTTACCTTTTACGTCTCAC
TTACGGCATTCGGTGGCATGGATTGTACCGCGG.

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

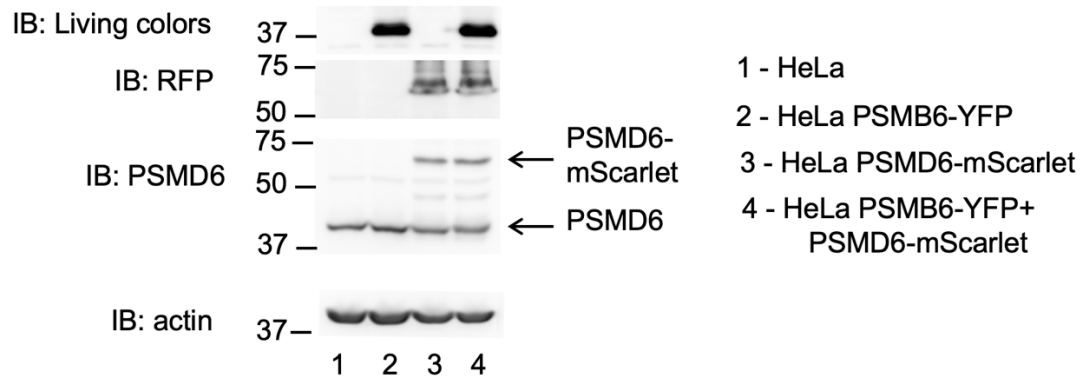

Figure S2: Evidence for heterozygosity of the PSMD6-mScarlet cells. Extracts from the indicated cells were prepared and analyzed by western blotting using the indicated antibodies.

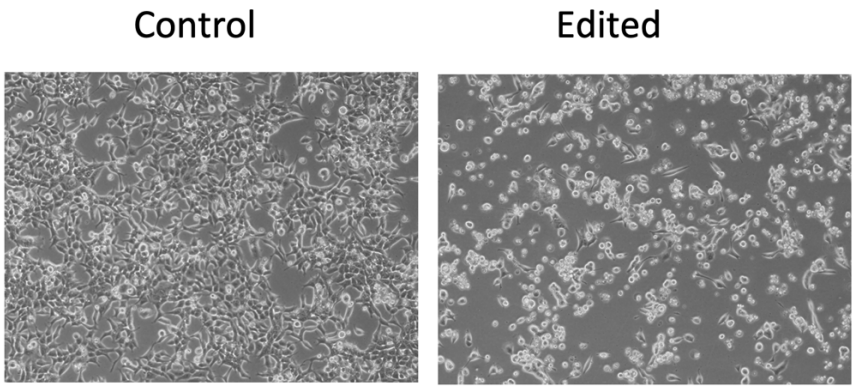

Fig S3: Microscopic images of control and C-terminus truncated PSMA3 edited cells after 6 days.

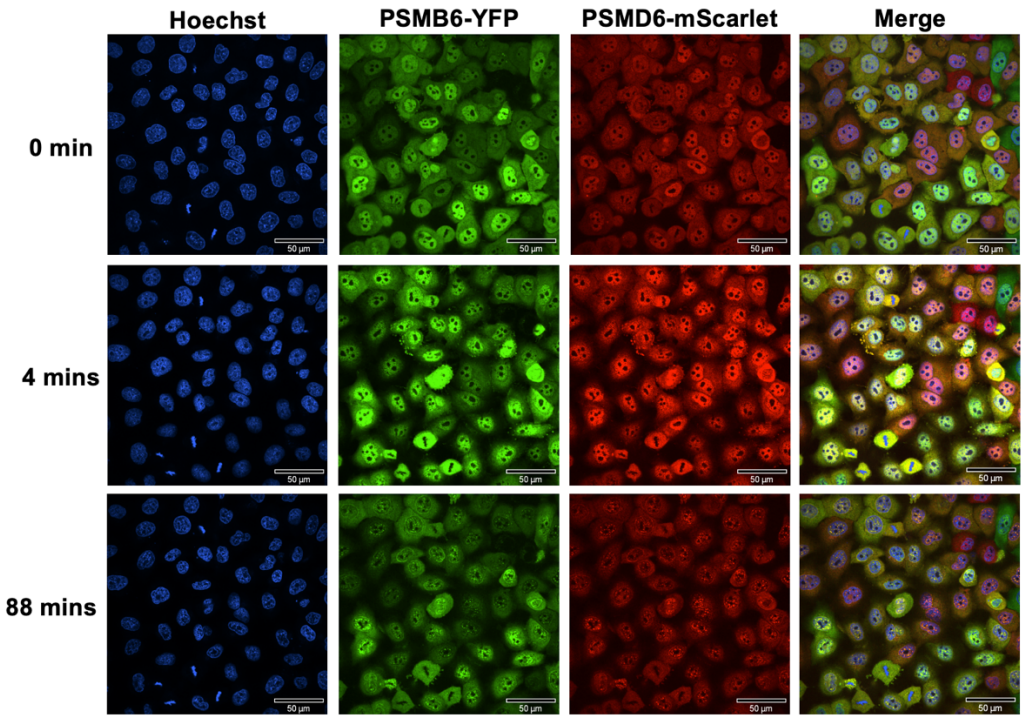

Figure S4: Wide-field images of proteasome granules under sucrose osmotic stress at different time points. Time-lapse images of HeLa PSMB6-YFP PSMD6-mScarlet cells treated with 200 mM sucrose. The green live-imaging marker represents all proteasomes, the red live-imaging marker represents 19S RP and 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 10µm.

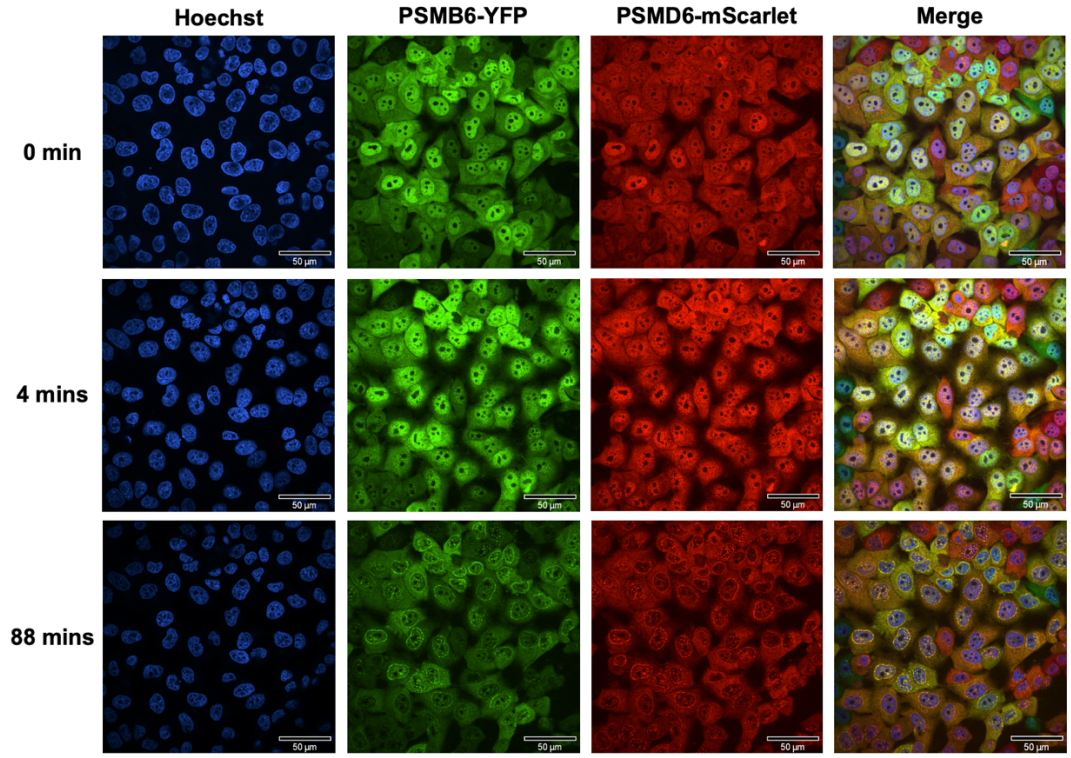

Figure S5: Wide-field images of proteasome granules under NaCl osmotic stress at different time points. Time-lapse images of HeLa PSMB6-YFP PSMD6-mScarlet cells treated with 150 mM NaCl. The green live-imaging marker represents all proteasomes, the red live-imaging marker represents 19S RP and the yellow spots in the merge panel represent 26S proteasomes. The nuclei were Hoechst stained (blue). Selected time points are shown. The scale bar is 10µm.

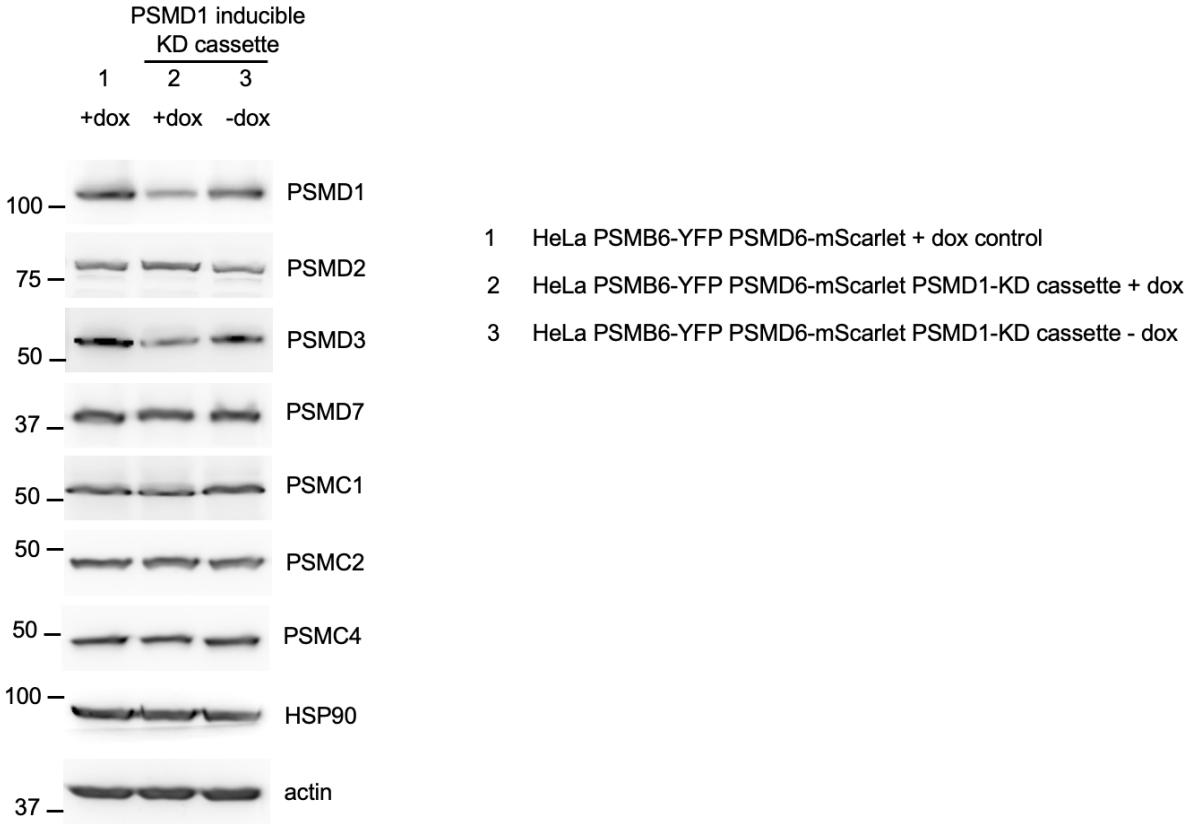

Figure S6: Western blot analysis of the components of 19S in PSMD1 knockout cells. HeLa PSMB6-YFP PSMD6-mScarlet PSMD1-KD cassette cells were treated (lane 2) or untreated (lane 3) with doxycycline for 3.5 days. As an additional control, HeLa PSMB6-YFP PSMD6-mScarlet cells without PSMD1-KD cassette were also doxycycline-treated and analyzed after 3.5 days (lane 1).

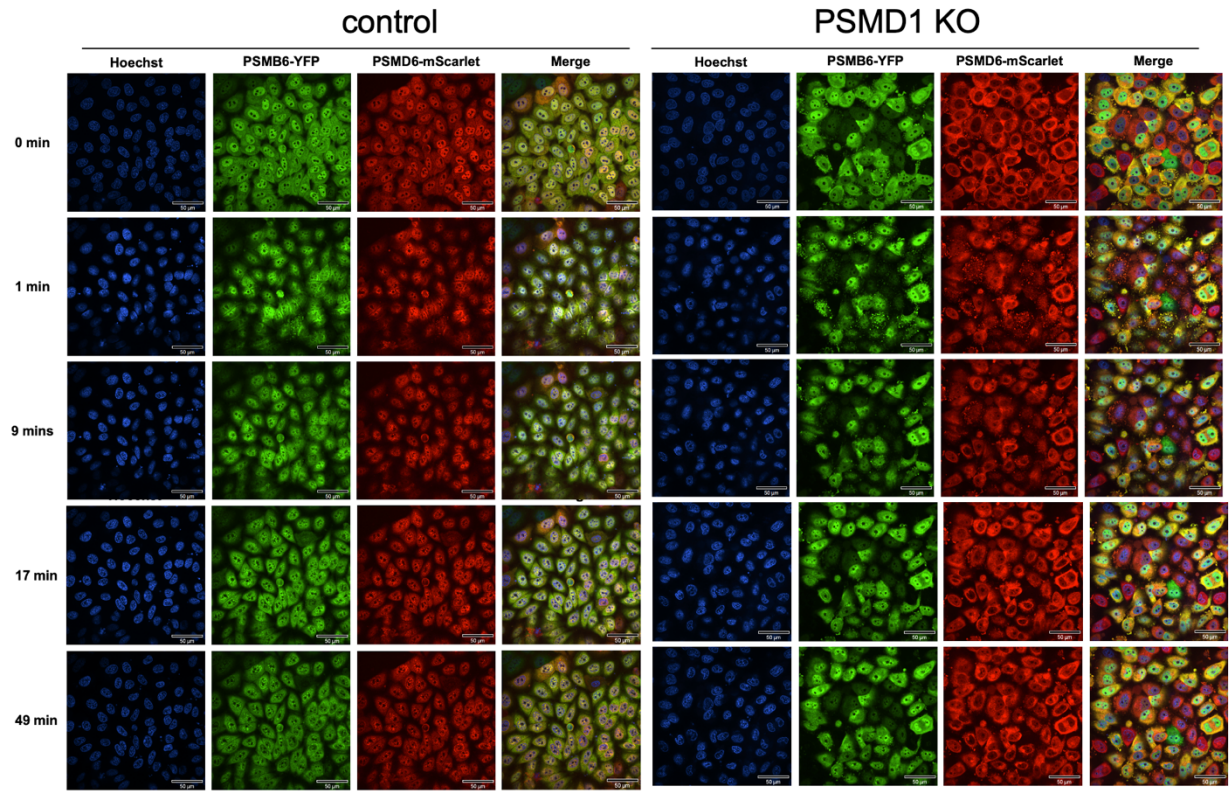

Figure S7: Wide-field images of proteasome granules under osmotic stress. Wide-fields time-lapse images of HeLa PSMB6-YFP PSMD6-mScarlet cells and HeLa PSMB6-YFP PSMD6-mScarlet PSMD1-KD cells treated with 100 mM NaCl. The scale bar is 10µm.