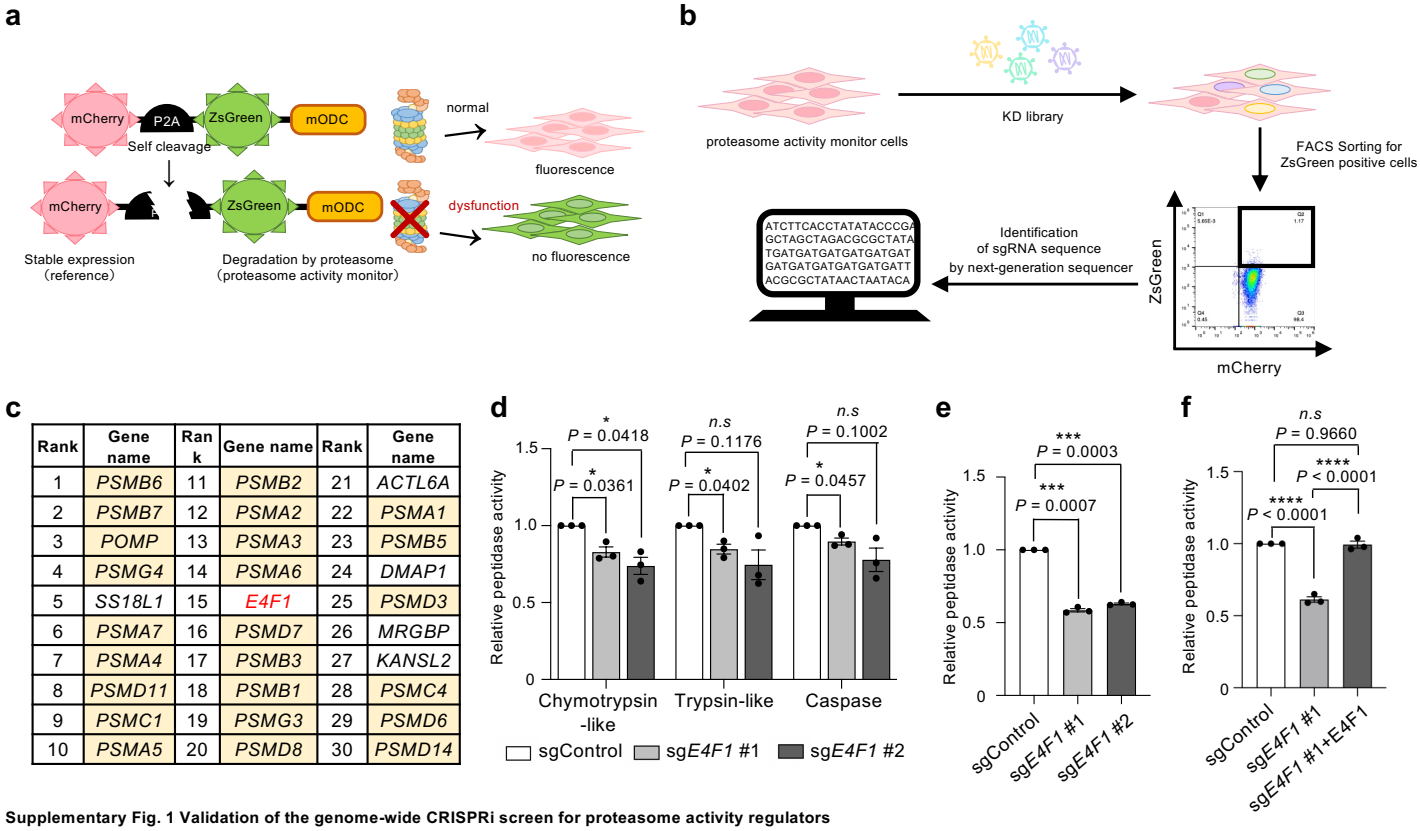

c

Rank	Gene name	Rank	Gene name	Rank	Gene name
1	*PSMB6*	11	*PSMB2*	21	*ACTL6A*
2	*PSMB7*	12	*PSMA2*	22	*PSMA1*
3	*POMP*	13	*PSMA3*	23	*PSMB5*
4	*PSMG4*	14	*PSMA6*	24	*DMAP1*
5	*SS18L1*	15	*E4F1*	25	*PSMD3*
6	*PSMA7*	16	*PSMD7*	26	*MRGBP*
7	*PSMA4*	17	*PSMB3*	27	*KANSL2*
8	*PSMD11*	18	*PSMB1*	28	*PSMC4*
9	*PSMC1*	19	*PSMG3*	29	*PSMD6*
10	*PSMA5*	20	*PSMD8*	30	*PSMD14*

Supplementary Fig. 1 Validation of the genome-wide CRISPRi screen for proteasome activity regulators

a Schematic representation of the proteasome activity monitor. The fluorescent protein ZsGreen was fused to the mODC sequence (ZsGreen-mODC). mCherry, which serves as a reference, was linked with ZsGreen-mODC via the self-cleavage sequence P2A. **b** Schematic overview of the genome-wide CRISPRi screen. The fluorescence intensity of ZsGreen relative to that of mCherry increases when proteasome function is impaired. The cell population in the upper right-hand quadrant of the flow cytometry plot was collected and subjected to next-generation sequencing analysis. **c** List of the top 30 hit genes identified in the screen. **d** Proteasome chymotrypsin-, trypsin-, and caspase-like activities of HEK293T cells transduced with sgControl or sg*E4F1*. The peptidase activities were measured in the absence of SDS to assess 26S proteasome activity. **e** Proteasome caspase-like activity of HEK293T cells transfected with sgControl or sg*E4F1*. The peptidase activities were measured in the presence of 0.025% SDS to assess core particle activity. **f** Chymotrypsin-like proteasome activity of the E4F1-knockdown HEK293T cells following re-expression of E4F1 cDNA. The peptidase activities were measured in the absence of SDS. Data represent mean ± s.e.m. (n = 3 biologically independent experiments). Statistical significance was determined using an unpaired two-tailed Student's t-test with Welch's correction (**d**, **e**) or one-way ANOVA followed by Tukey's multiple-comparison test (**f**). **P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; n.s., not significant.* All experiments were performed at least three biologically independent times with similar results. Source data are provided as a Source Data file.