Supplemental Figures:

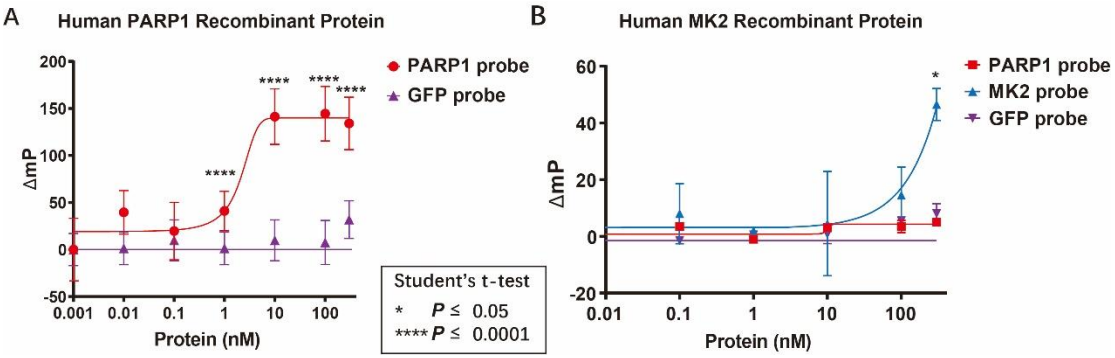


Fig. S1. CFAST probes bind to their protein targets. (A) FA measurements of PARP1 and EGFP probes with varying concentrations of recombinant PARP1 protein. **(B)** FA measurements of PARP1, MK2, and EGFP probes with varying concentrations of recombinant MK2 protein. ΔmP values were obtained by normalizing to mP values of the corresponding 0.001 nM protein sample. Data are presented as mean ± SD, n = 2.

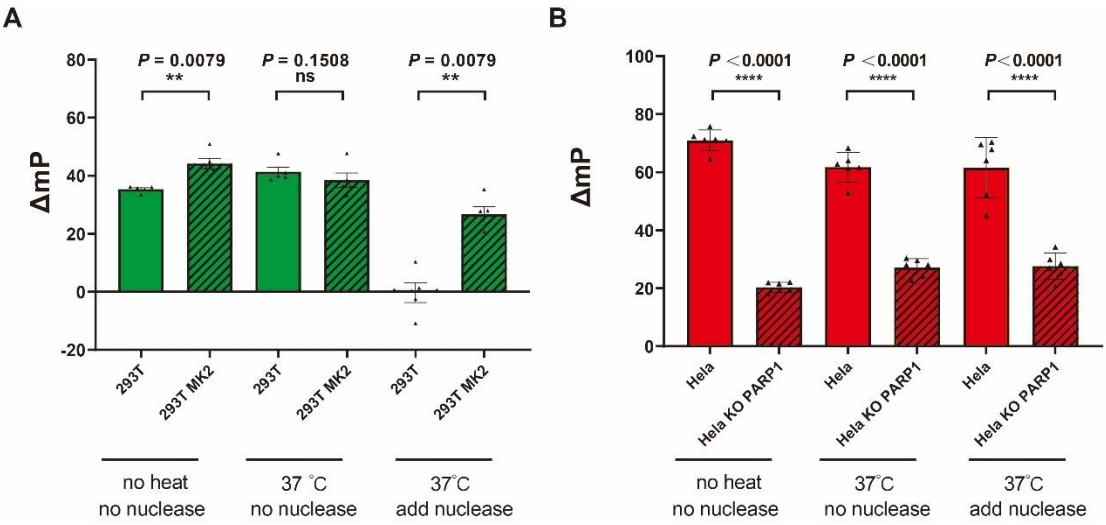


Fig. S2. Nuclease incubation reduces background. **(A)** FA measurements of MK2 probe in HEK293T and HEK293T expressing MK2 cell lysate, with and without heat and nuclease treatment. ΔmP values were obtained by normalizing to FA measurements of EGFP probe added to aliquots of the experimental samples. **(B)** FA measurements of PARP1 probe in Hela and Hela PARP1 knockout cell lysate, with and without heat and nuclease treatment. Data are presented as mean ± SD, n = 5.

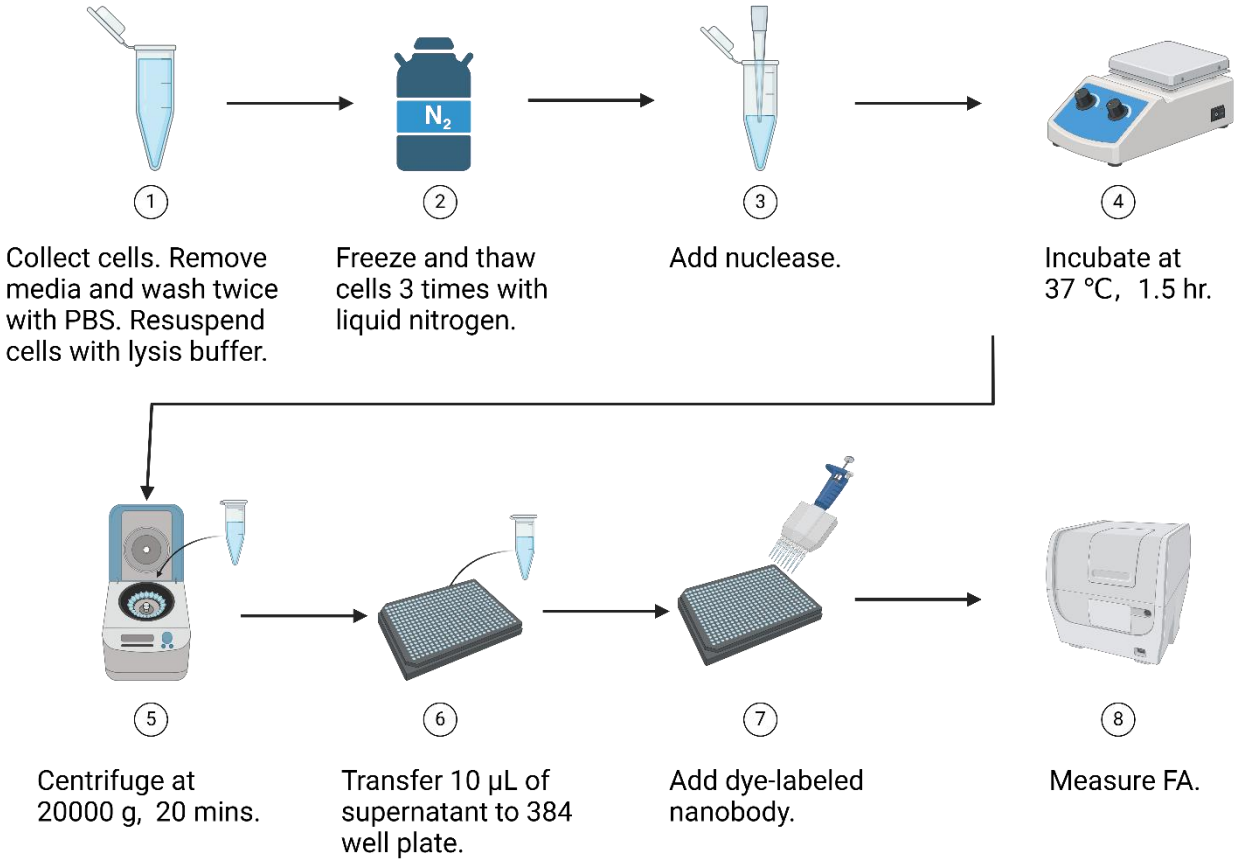


Fig. S3. CFAST workflow for cellular protein quantification (1.5 ml tube). Created with BioRender.com/y88t205.

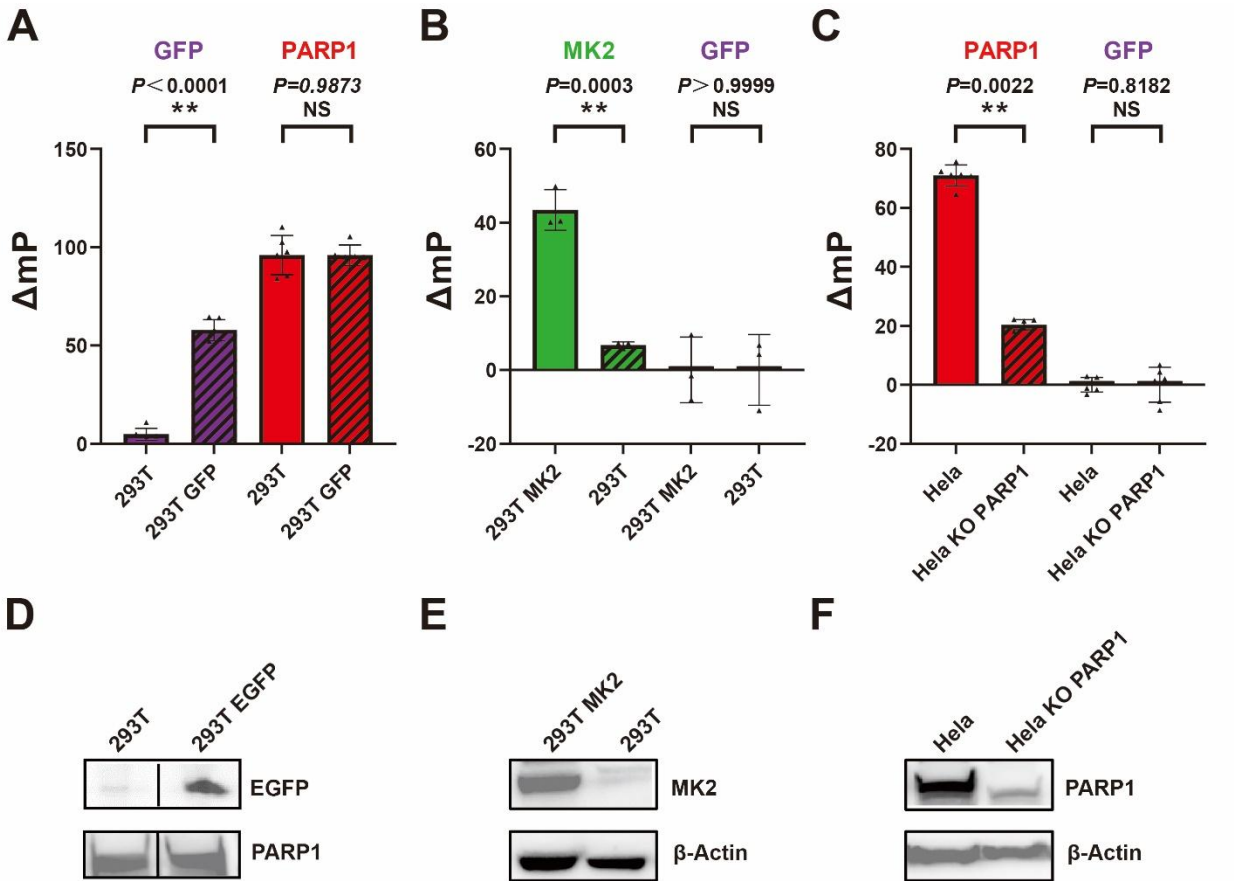
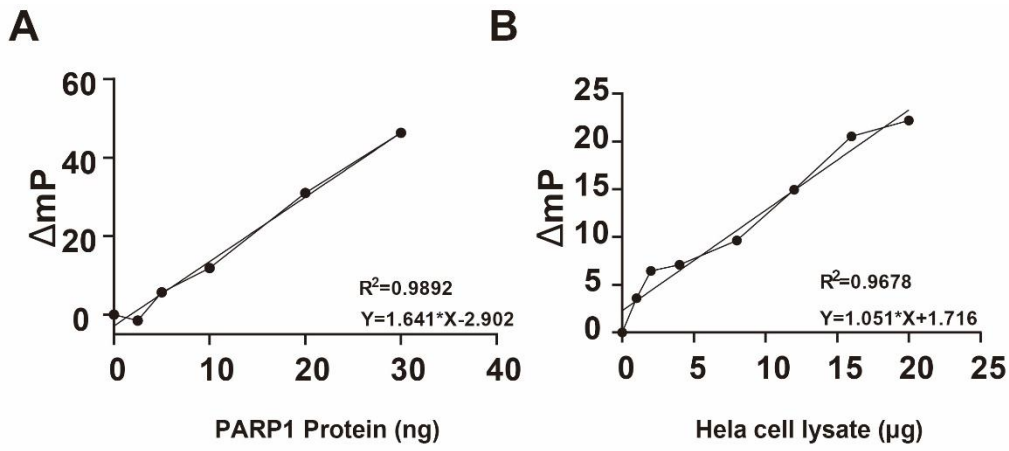
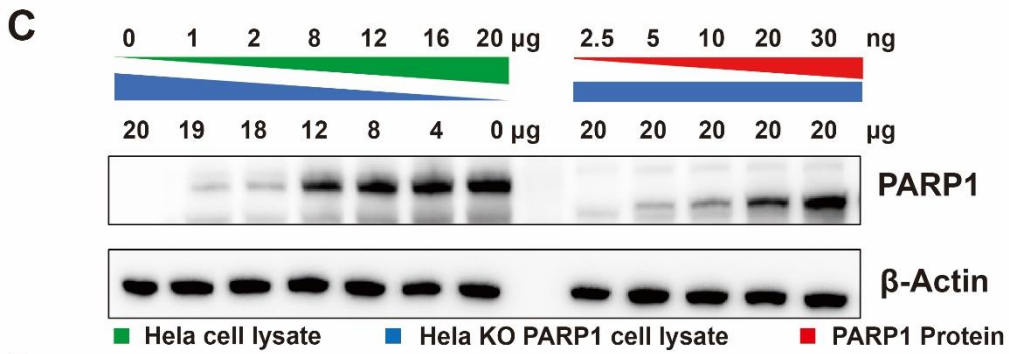


Fig. S4. CFAST probes can quantify cellular protein levels. (A) FA measurements of EGFP and PARP1 probes in HEK293T and HEK293T expressing EGFP cell lysates. ΔmP values were obtained by normalizing to the HEK293T-EGFP probe sample. **(B)** FA measurements of MK2 and EGFP probes in HEK293T and HEK293T expressing MK2 cell lysates. ΔmP values were obtained by normalizing to mP values of the EGFP probe samples. **(C)** FA measurements of PARP1 and EGFP probes in Hela and Hela PARP1 knockout cell lysates. ΔmP values were obtained by normalizing to mP values of the EGFP probe samples. Western blots for **(D)** EGFP and PARP1 in HEK293T and HEK293T expressing EGFP cells, **(E)** MK2 and ACTB in HEK293T expressing MK2 and HEK293T cells, and **(F)** PARP1 and ACTB in Hela and Hela PARP1 knockout cells. Data are presented as mean ± SD, n = 5.





D

Absolute quantitative analysis of PARP1 protein in Hela by CFAST				
Hela cell lysate/ μg	8	12	16	20
Mean mP value	9.625	14.9375	20.53125	22.1875
Corresponding PARP1 pure protein/ ng	7.6337	10.8711	14.2798	15.2891
PARP1 protein/ Hela cell lysate (ng/μg)	0.9542	0.9059	0.8924	0.7644
Mean ± SD	0.879225 ± 0.081017			

Fig. S5. CFAST can quantify absolute amounts of cellular PARP1. (A) FA measurements of PARP1 probe in varying amounts of recombinant PARP1 protein added to the same amount of Hela PARP1 knockout cell lysate. **(B)** FA measurements of PARP1 probe in increasing amounts of Hela cell lysate balanced with decreasing amounts of Hela PARP1 knockout lysate. **(C)** Western blot for PARP1 and ACTB in increasing amounts of Hela cell lysate balanced with decreasing amounts of Hela PARP1 knockout lysate, and in increasing amounts of recombinant PARP1 protein balanced with the same amount of Hela PARP1 knockout cell lysate. **(D)** Tabulation of values used to determine the absolute amount of PARP1 in Hela cell lysate. ΔmP values were obtained by normalizing to FA measurements of EGFP probe added to aliquots of the experimental samples. Data are presented as mean ± SD, n = 4.

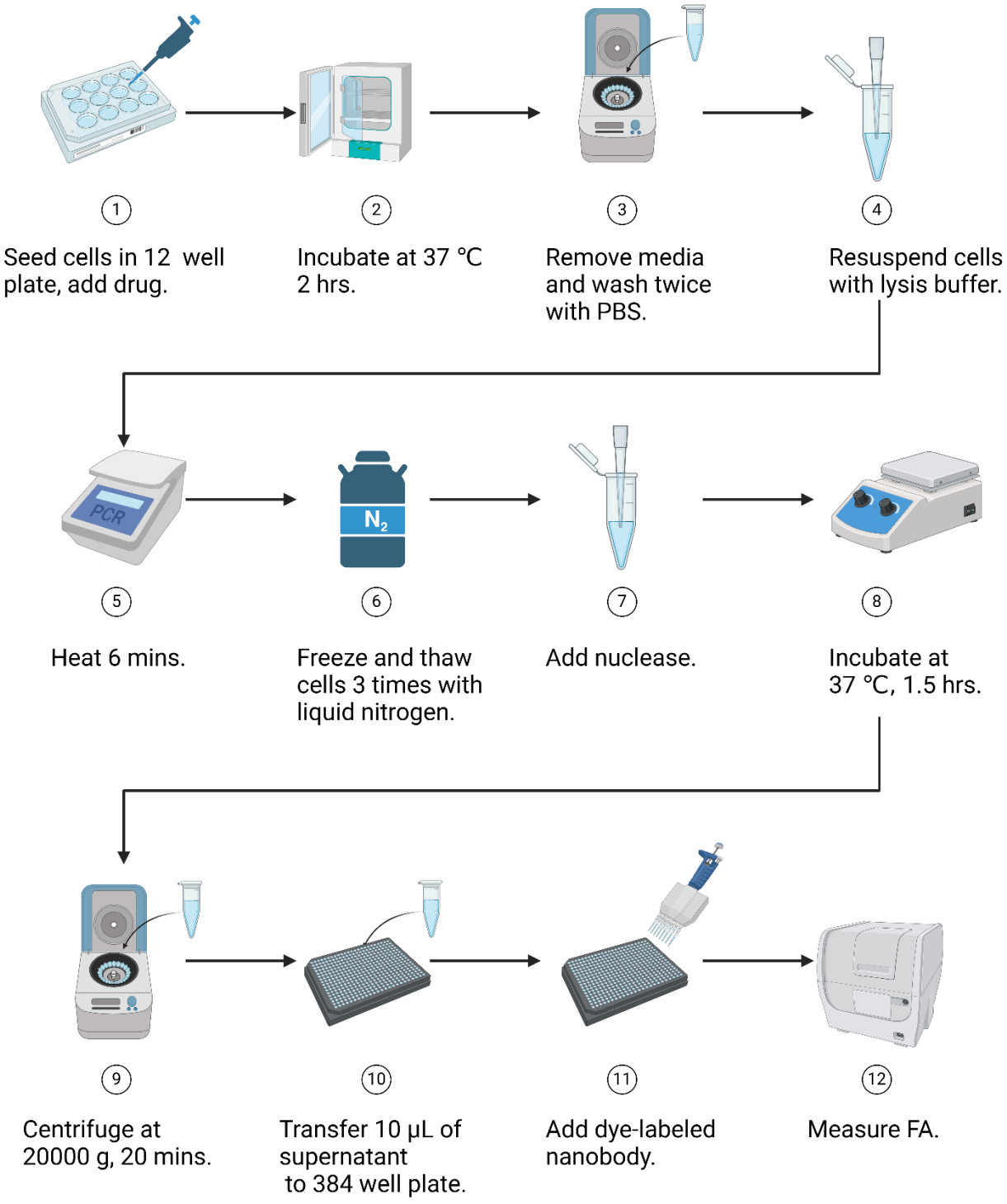


Fig. S6. CFAST workflow for detecting cellular protein-small molecule interactions (1.5 ml tube). Created with BioRender.com/r71o252.

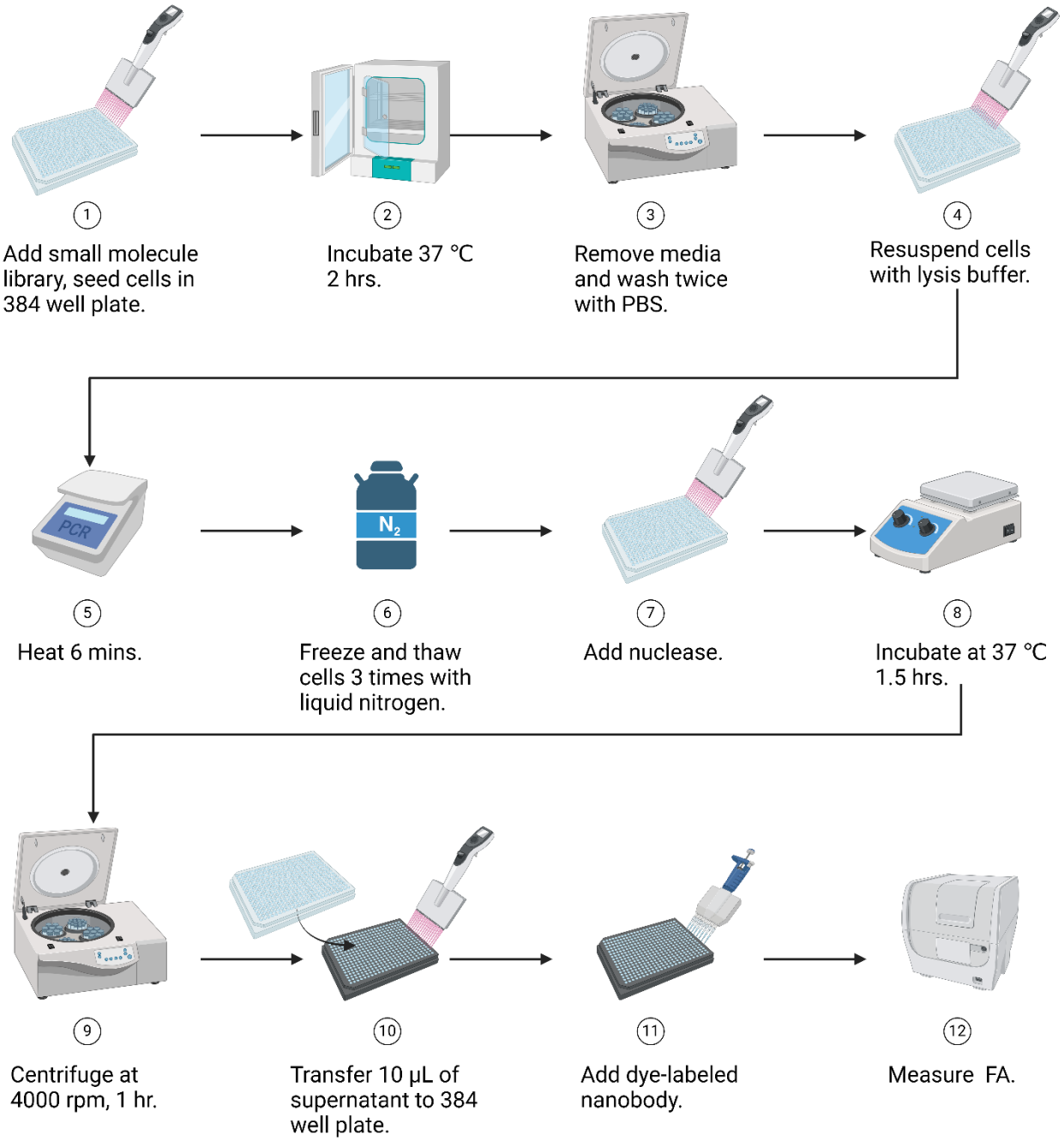


Fig. S7. CFAST workflow for high-throughput screening (384-well plate). Created with BioRender.com/f66w260.

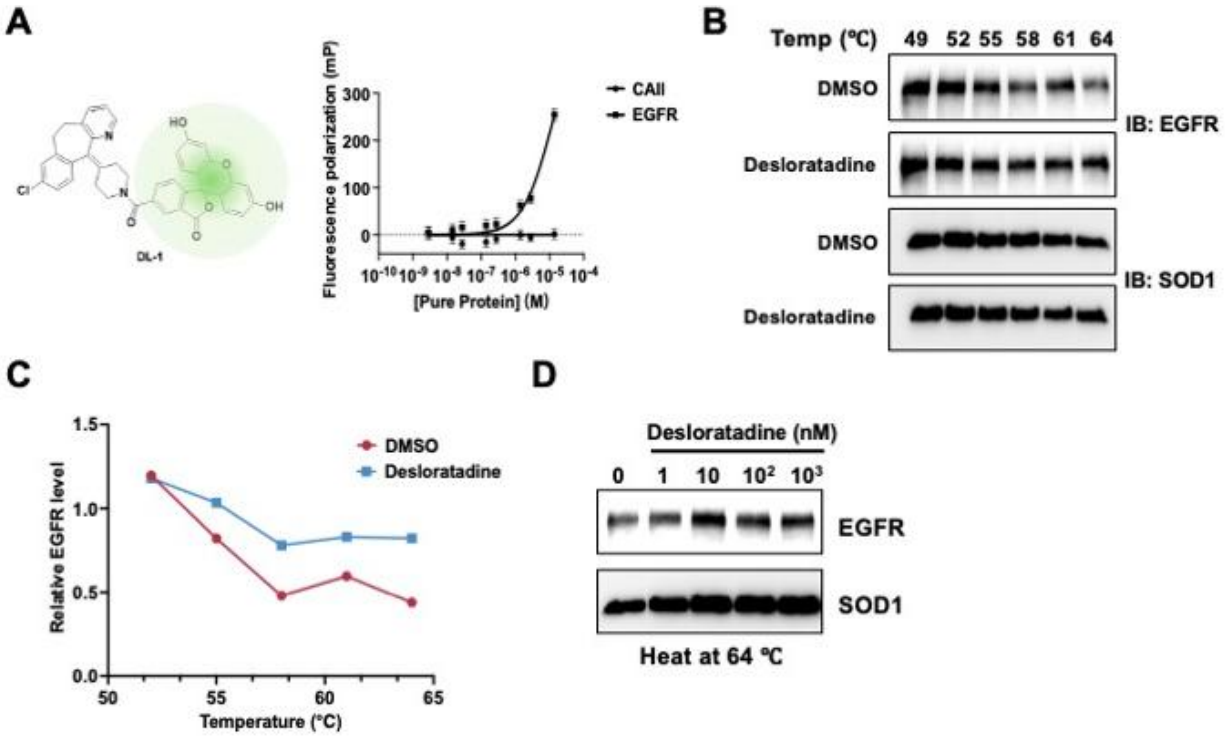


Fig. S8. Validation of desloratadine as an EGFR binder. (A) Fluorescence polarization assay of fluorescent dye-labeled desloratadine with purified EGFR or CAII protein, n = 6. **(B)** Temperature gradient CETSA Western blot of EGFR and SOD1 in DMSO- and desloratadine-treated A431 cells. **(C)** Quantified EGFR protein levels in **(B)**. **(D)** Dose-response CETSA Western blot of EGFR and SOD1 in DMSO- and desloratidine-treated A431 cells, heated at 64 ℃.

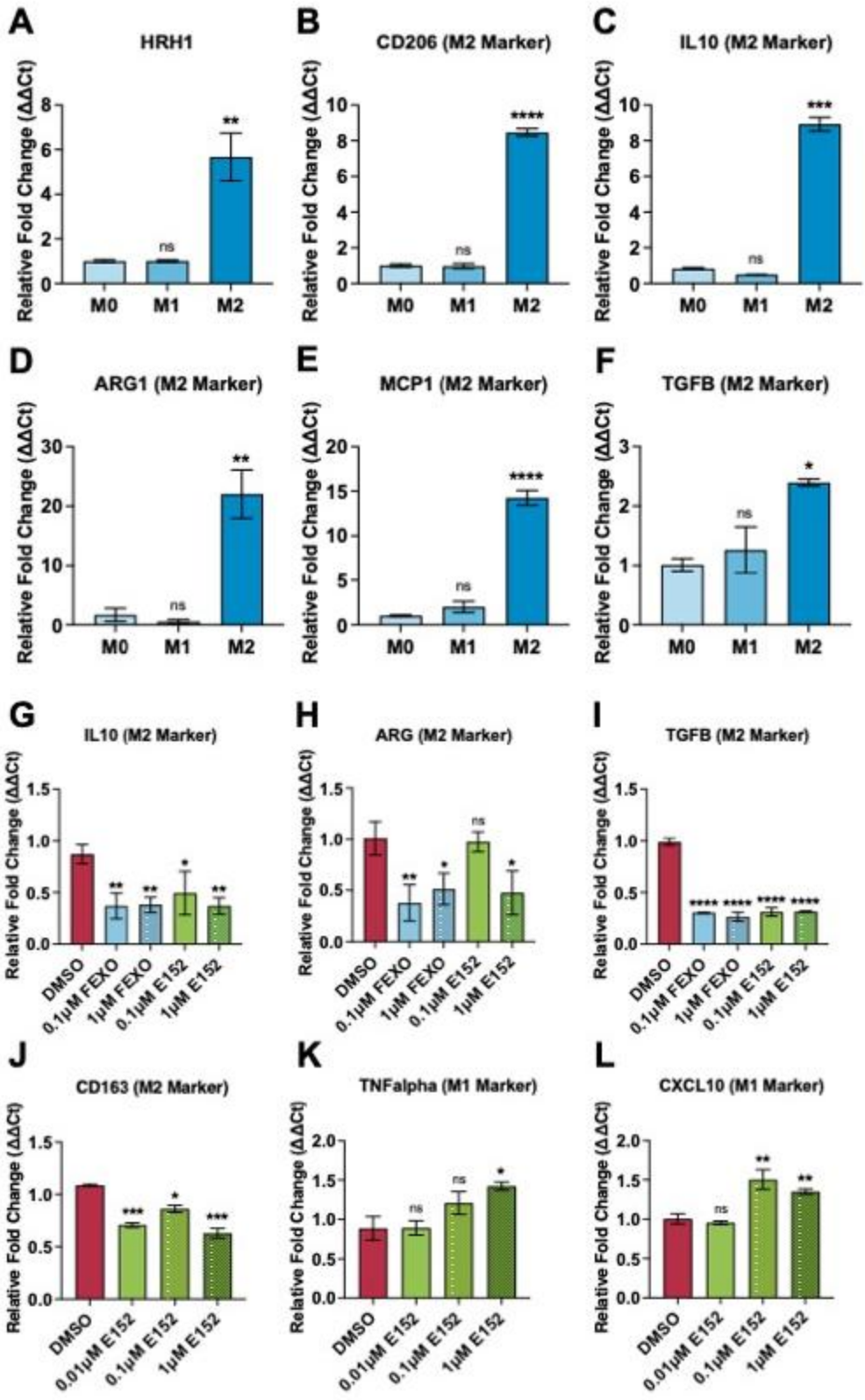


Fig. S9. PROTACs E151 and E152 alter macrophage subtype gene expression signatures. (A-F) qRT-PCR determined relative mRNA level of HRH1 and M2 macrophage markers (CD206,

IL-10, ARG, MCP1, and TGFB) in human monocyte THP-1 induced M1-like or M2-like macrophages. Significance was computed by one-way ANOVA. **(G-L)** qRT-PCR determined relative mRNA level of M2 macrophage markers (IL-10, ARG, TGFB, and CD163) and M1 macrophage markers (TNFalpha and CXCL10) in DMSO-, fexofenadine-, or E152-treated induced M2-like macrophages. Significance was computed by one-way ANOVA.