## SUPPLEMENTAL MATERIAL



**Fig. S1.** Fluorescent cell labeling of Leo cells with FAM-X-CTT54 at 37 °C for 30 mins. FAM-X-CTT54 concentration (nM): (a) 0, and (b) 300.



**Fig. S2.** Fluorescent cell labeling with 3C6-TAMRA. LNCaP (A), Leo (B), and PC-3 (C) at 4 °C for 1 h with 3C6-TAMRA. 3C6-TAMRA concentration: (a) 0, (b) 50X-dilution.



**Fig. S3.** Enzymatic activity recovery profiles for PSMA inhibition by CTT-54 and FAMX-CTT54. Enzymatic activity was determined after 100-fold dilution of PSMA that was first pre-incubated with inhibitor at 10-fold the  $IC_{50}$  value. The x-axis represents incubation time (min) after rapid dilution of the enzyme-inhibitor complex with a saturating concentration of substrate and the y-axis represents product formation (pmol) of PSMA enzymatic activity. On the basis of recovery profiles, both CTT-54 and FAMX-CTT54 are reversible. Uninhibited PSMA served as a control.