Supplementary Materials for

Engineered subtilisin protease degrades active KRAS *in vivo,* leading to differential cell targeting.

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This PDF file includes:

Figs. S1 to S4

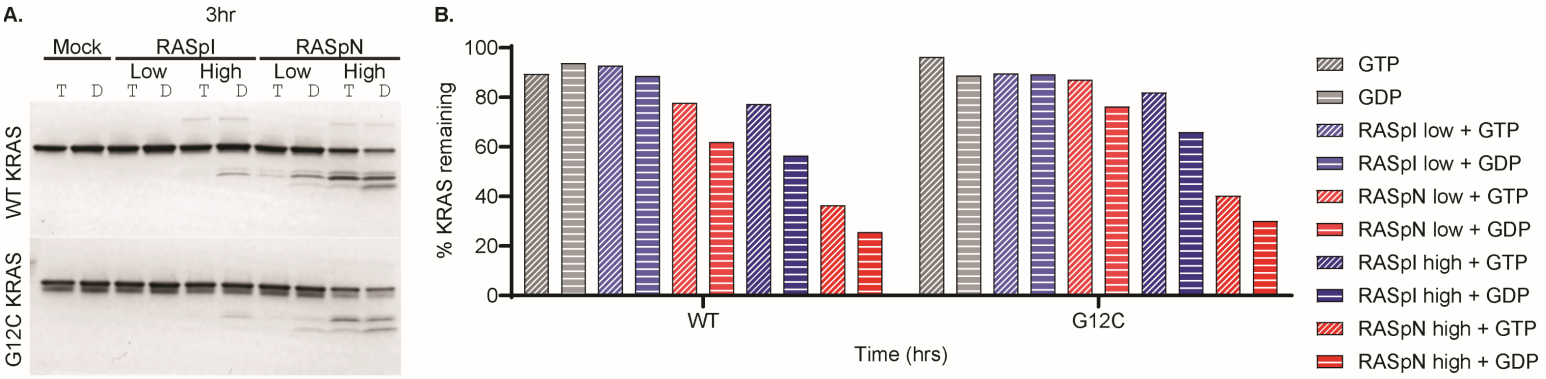

Fig. S1. RASp cleaves purifed KRAS. (A) Coomassie-stained gels of *in vitro* proteolysis of purified GMPPNP- (T) or GDP-bound (D) WT KRAS after incubation with stoichiometric amounts of purified RASpI or RASpN in dose-dependent manner. Proteolysis reactions were allowed to proceed for 3 hr. (B) Quantification of WT KRAS degradation in Coomassie-stained gels in (C) using FIJI.

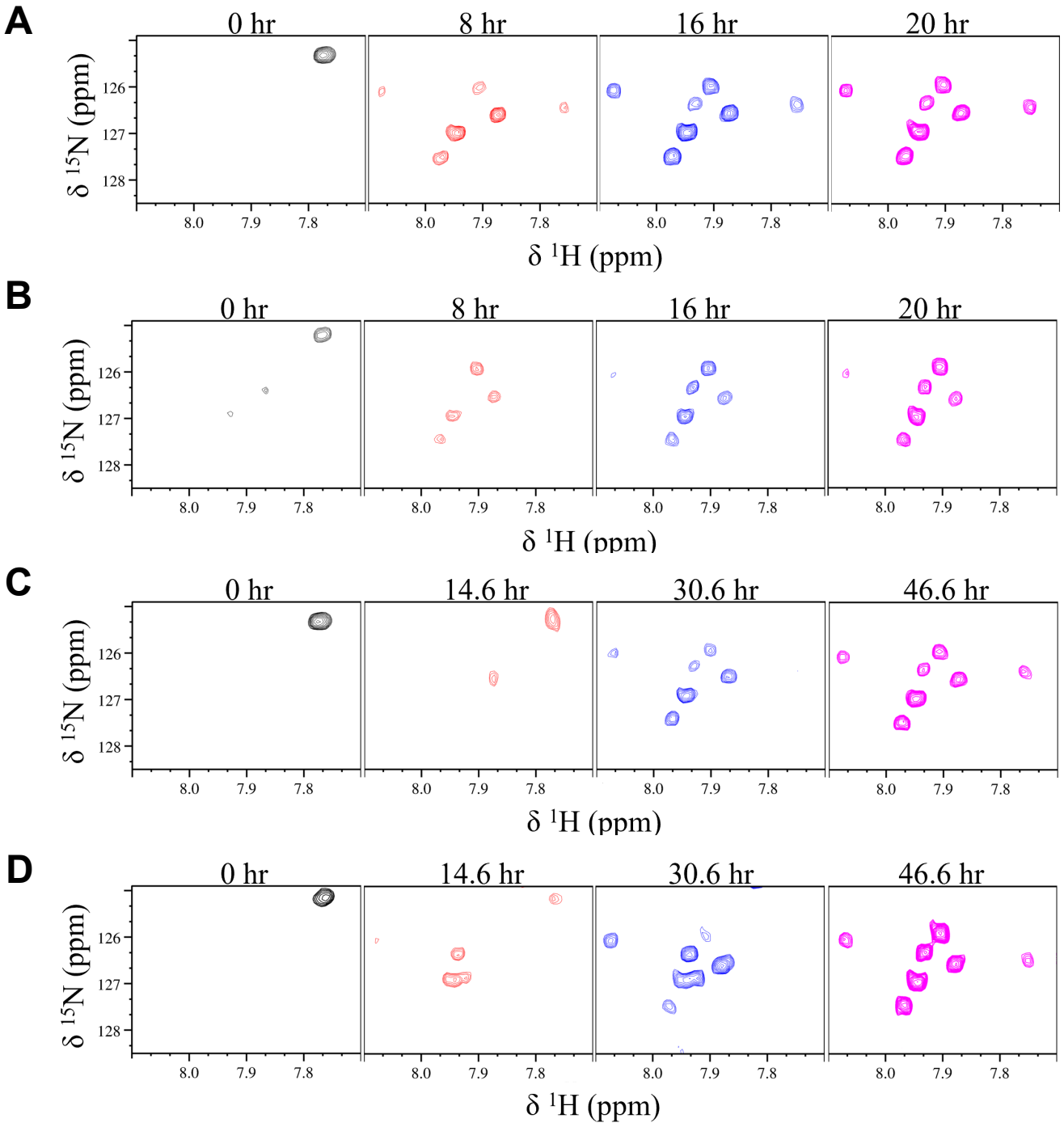

Fig. S2. ^{1}H-^{15}N TROSY-HSQC spectra of uniformly ^{15}N-labeled KRAS WT at four time points in the presence of different concentrations of RASpN. (A) ^{1}H-^{15}N TROSY-HSQC spectrum (^{1}H 7.7-8.1 ppm, ^{15}N 135-129 ppm) of 300 μM ^{15}N labeled KRAS WT (GDP) at time = 0, 8, 16, and 20 hr in the presence of RASpN (stoichiometric ratio) at 37 °C and using 600 MHz NMR. (B) 1H-15N TROSY-HSQC spectrum (^{1}H 7.7-8.1 ppm, ^{15}N 135-129 ppm) of 300 μM ^{15}N labeled KRAS WT (GMPPNP) at time = 0, 8, 16, and 20 hr in the presence of RASpN (stoichiometric ratio). (C) 1H-15N TROSY-HSQC spectrum (^{1}H 7.7-8.1 ppm, ^{15}N 135-129 ppm) of 300 μM ^{15}N labeled KRAS WT (GDP) at time = 0, 14.6, 30.6, and 46.6 hr in the presence of RASpN (10000:1, KRAS WT: RASpN). (D) 1H-15N TROSY-HSQC spectrum (^{1}H 7.7-8.1 ppm, ^{15}N 135-129 ppm) of 300 μM ^{15}N labeled KRAS WT (GMPPNP) at time = 0, 14.6, 30.6, and 46.6 hr in the presence of RASpN (10000:1, KRAS WT: RASpN).

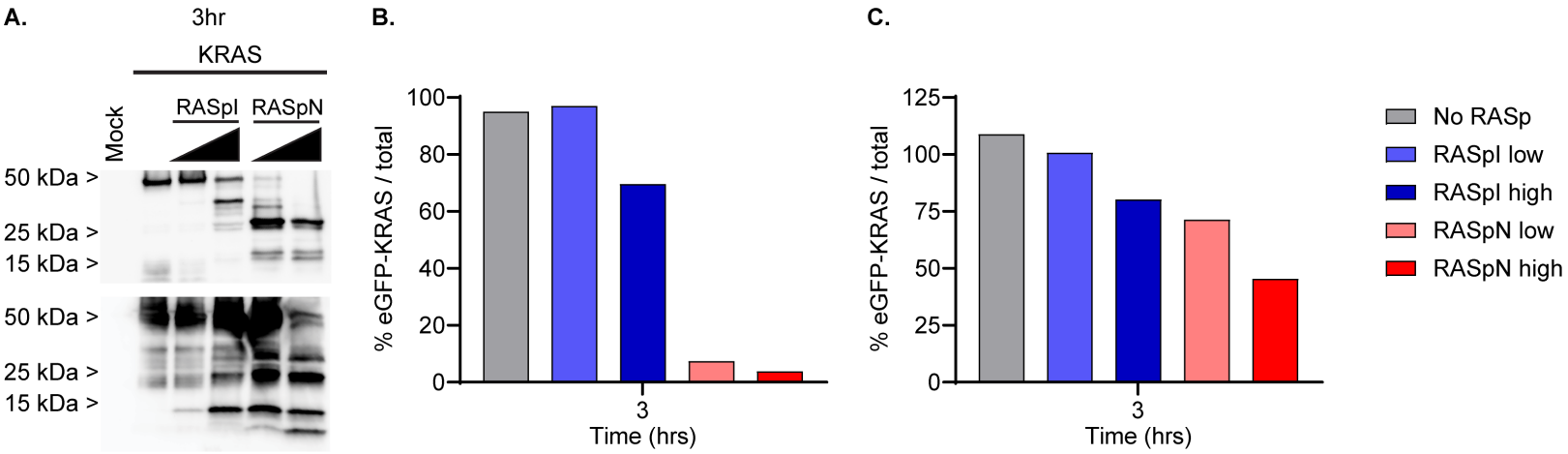

Fig. S3. Purified RASp cleaves exogenous eGFP-KRAS expressed in HEK 293T cell lysates. (A) Western blot shows cleavage of HEK 293T cell lysates expressing eGFP-KRAS after incubation with RASpI or RASpN in a dose-dependent manner for 3 hr. (B) Quantification of KRAS expression signal by eGFP detection in (A) using FIJI. (C) Quantification of KRAS expression signal by KRAS detection in (A) using FIJI.

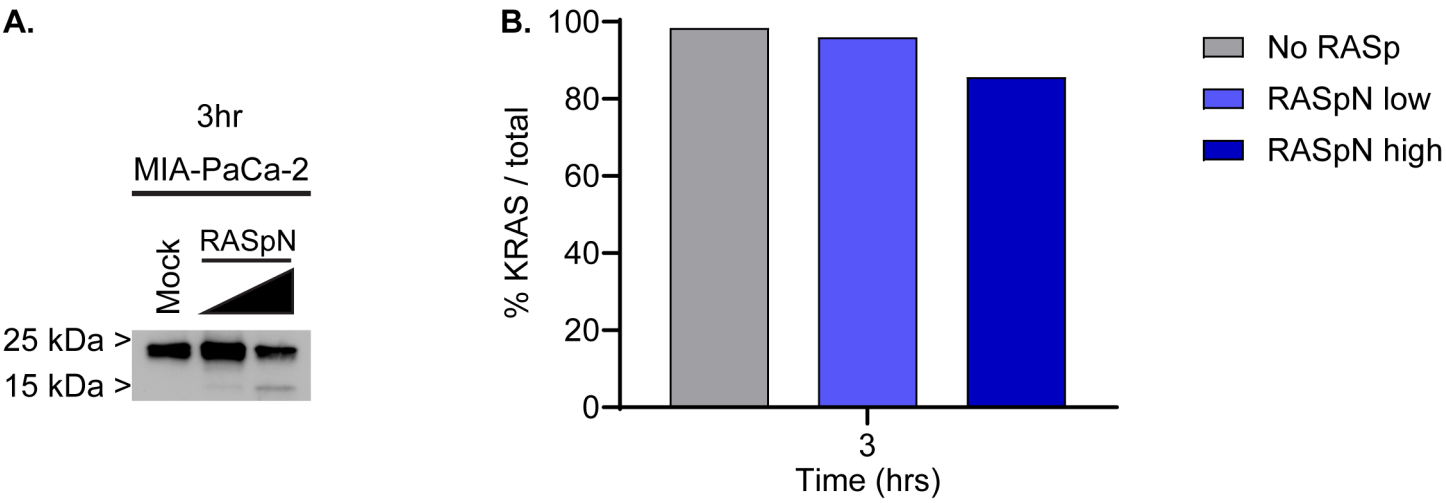

Fig. S4. Purified RASpN cleaves exogenous eGFP-KRAS expressed in MIA PaCa-2 cell lysates. (A) Western blot shows cleavage of HEK 293T cell lysates expressing eGFP-KRAS after incubation with RASpN in a dose-dependent manner for 3 hr. (B) Quantification of remaining KRAS signal in (A) using FIJI.