Tri-Arginine Exosite Patch of Caspase-6 Recruits Substrates for Hydrolysis

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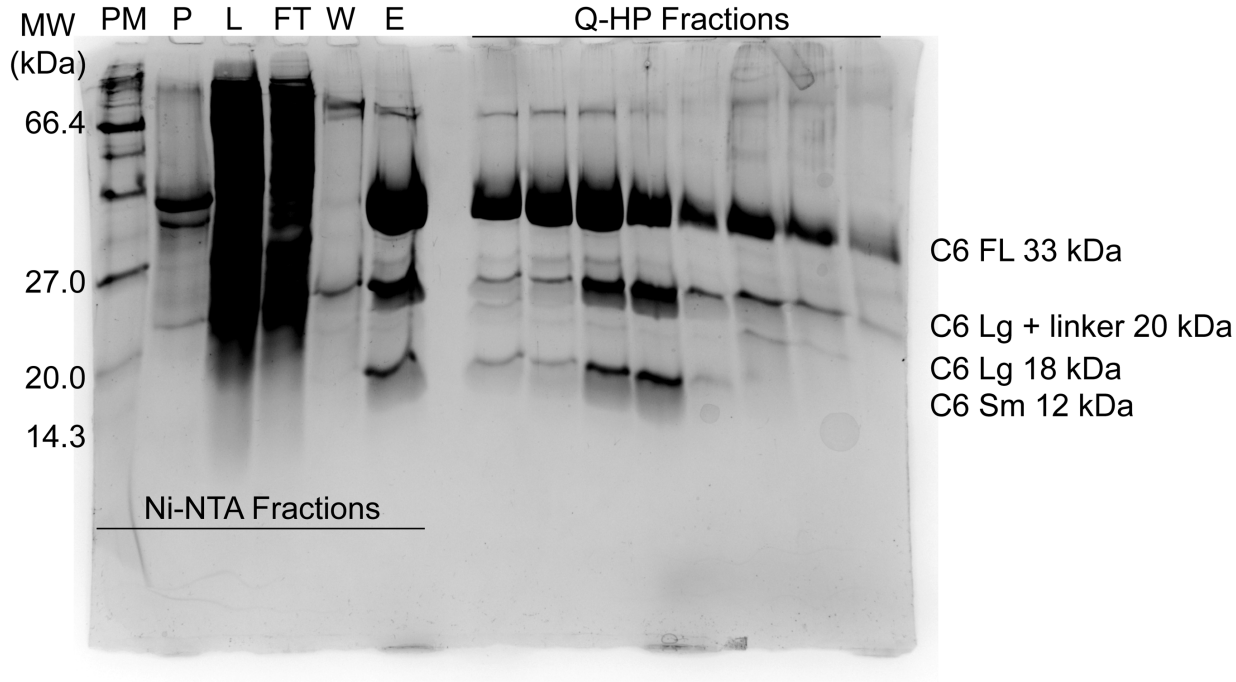
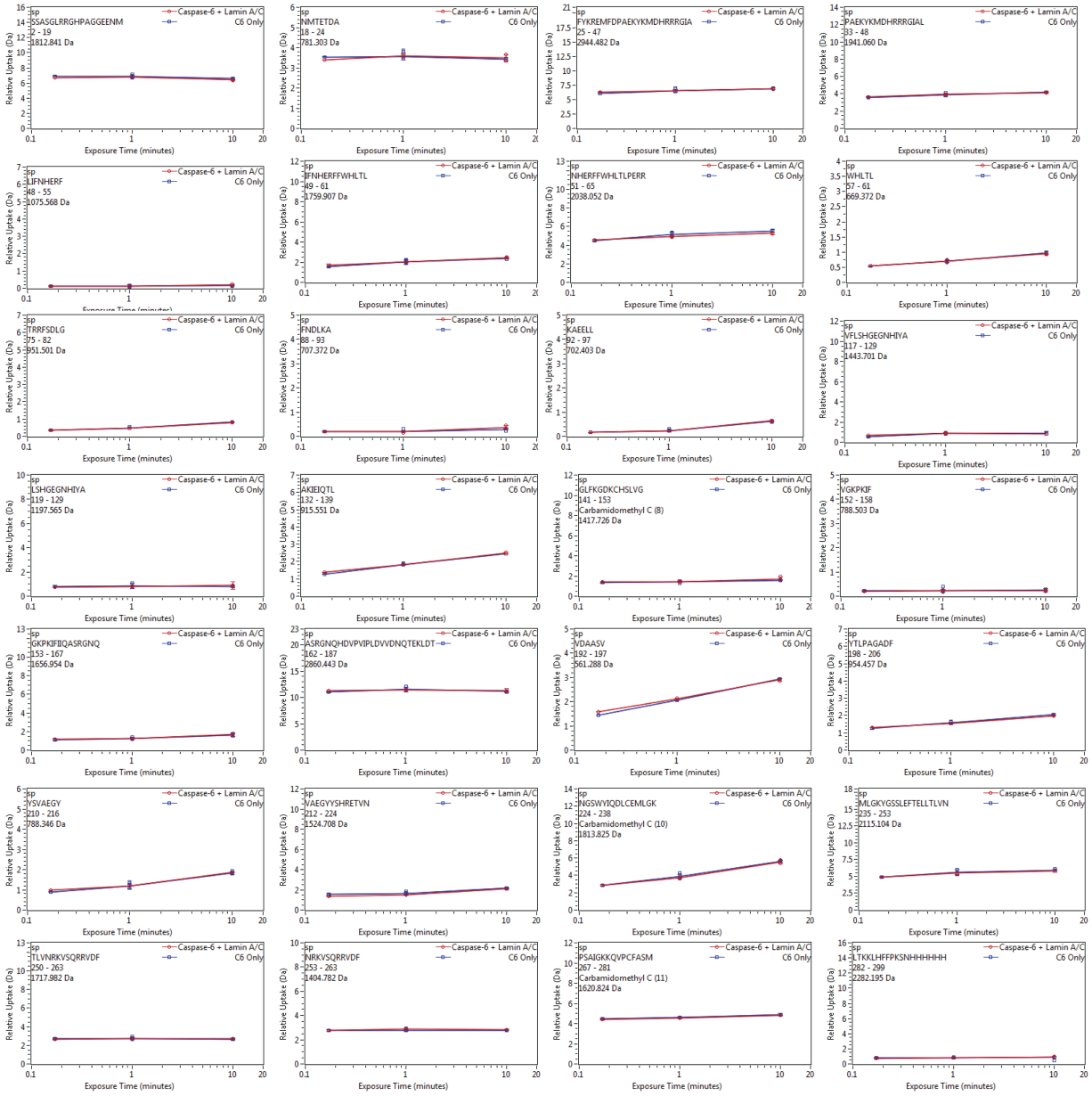
*Supporting Information file contains all supporting figures and figure legends

Supporting Figures and Figure Legends

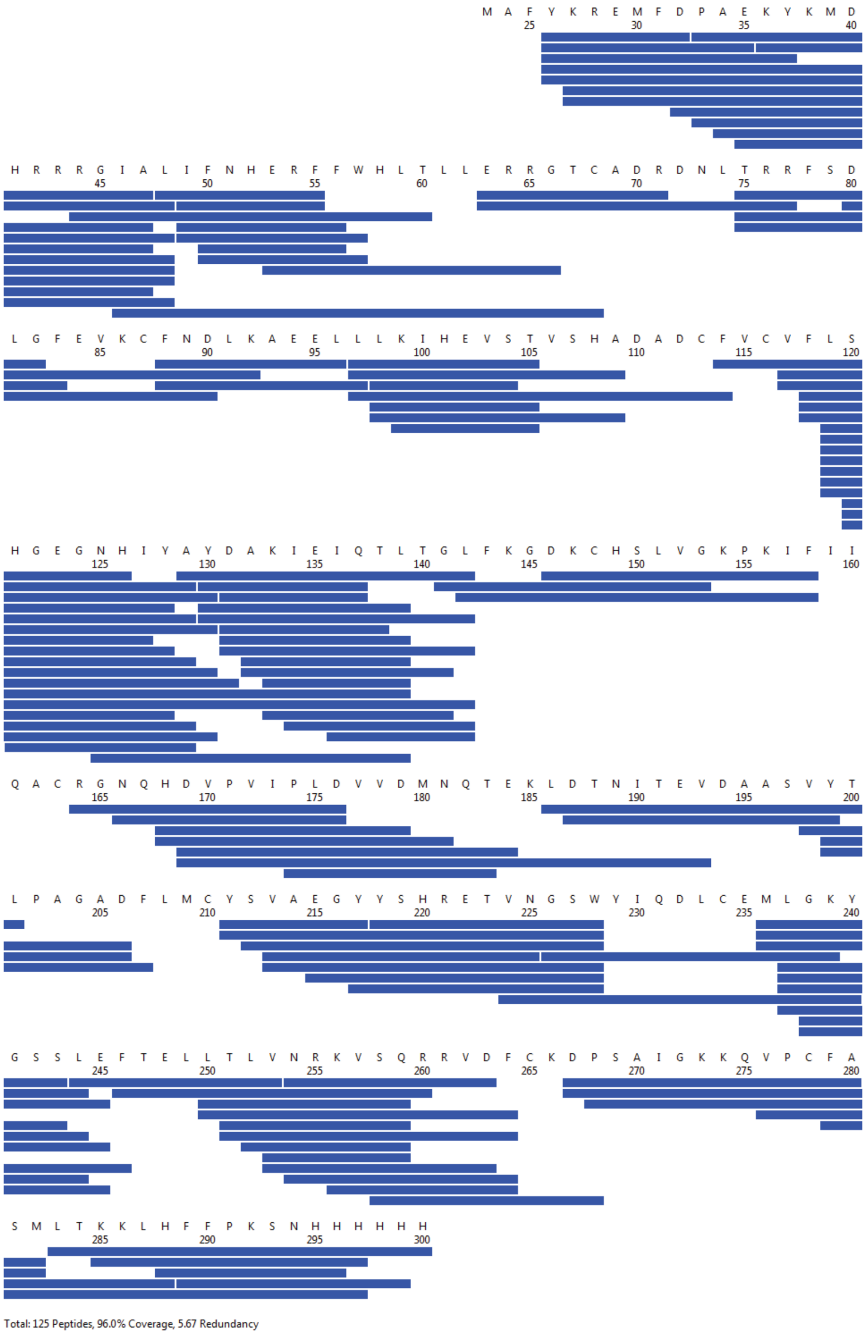


Supporting Figure 1
The Caspase-6 R42-44A FL construct is unable to undergo self-proteolysis after 18 hours of induction. This variant purified by traditional means exists predominantly in the FL state (33 kDa). Mixed protein populations are not suitable to accurately gauge kinetics or substrate hydrolysis, and therefore, the variants were generated in the constitutive CT construct to facilitate production of a homogenous pool of mature protein

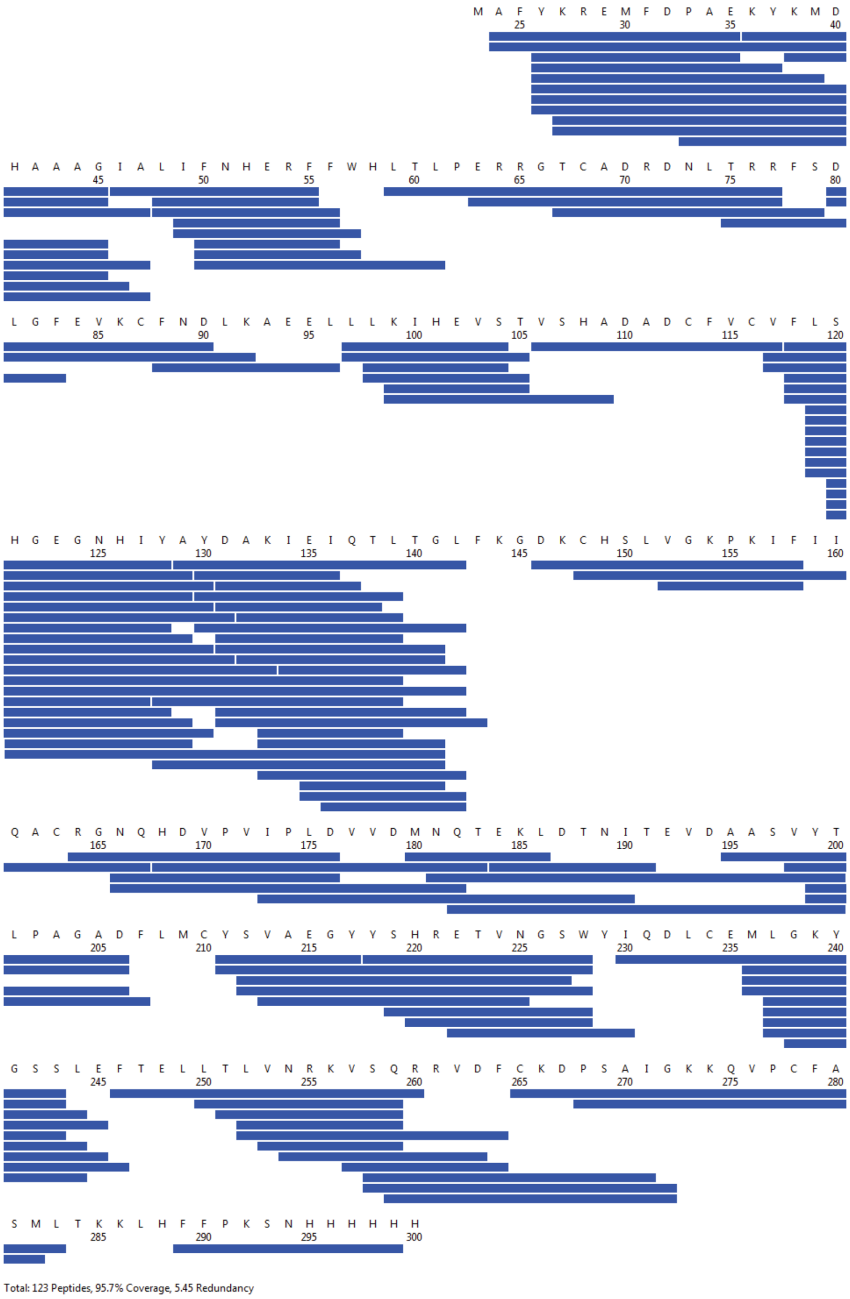


Supporting Figure 2

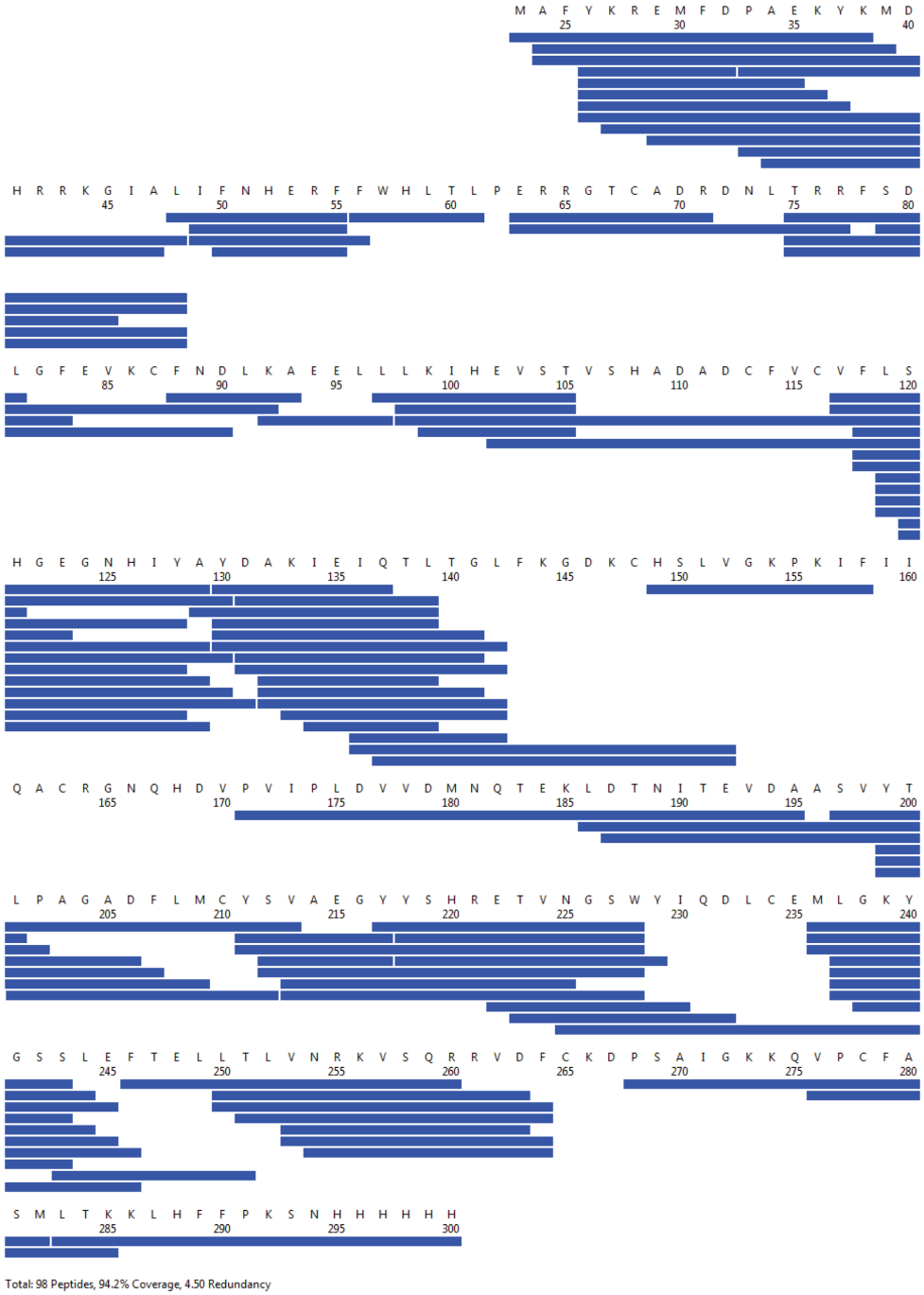
Deuterium Uptake plots of attempts to perform H/Dx in a solution containing both caspase-6 and the cognate caspase-6 substrate lamin C. Uptake plots of 28 peptides that were selected because they span the entire sequence of caspase-6. Both proteins were incubated in a 1:1 ratio at initial concentrations of 30 μM prior to exposure to D_2O. H/Dx analysis was performed and analyzed as previously described. No observable differences between caspase-6 alone (blue) and caspase-6 in the presence of Lamin C (red) were detected under these conditions.



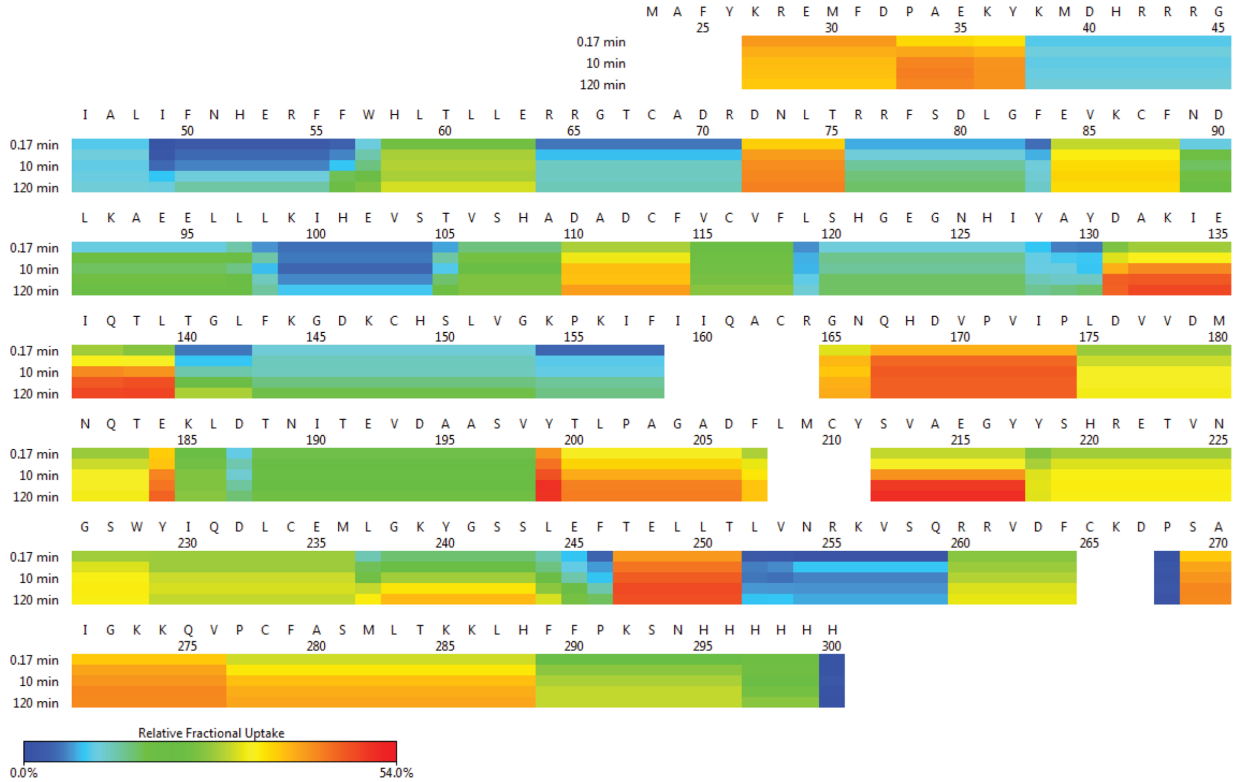
Supporting Figure 3
Peptic peptide map coverage of Caspase-6 ΔN D179 CT, performed in triplicate.



Supporting Figure 4
Peptic peptide map coverage of Caspase-6 ΔN D179 CT R42-44A, performed in triplicate.

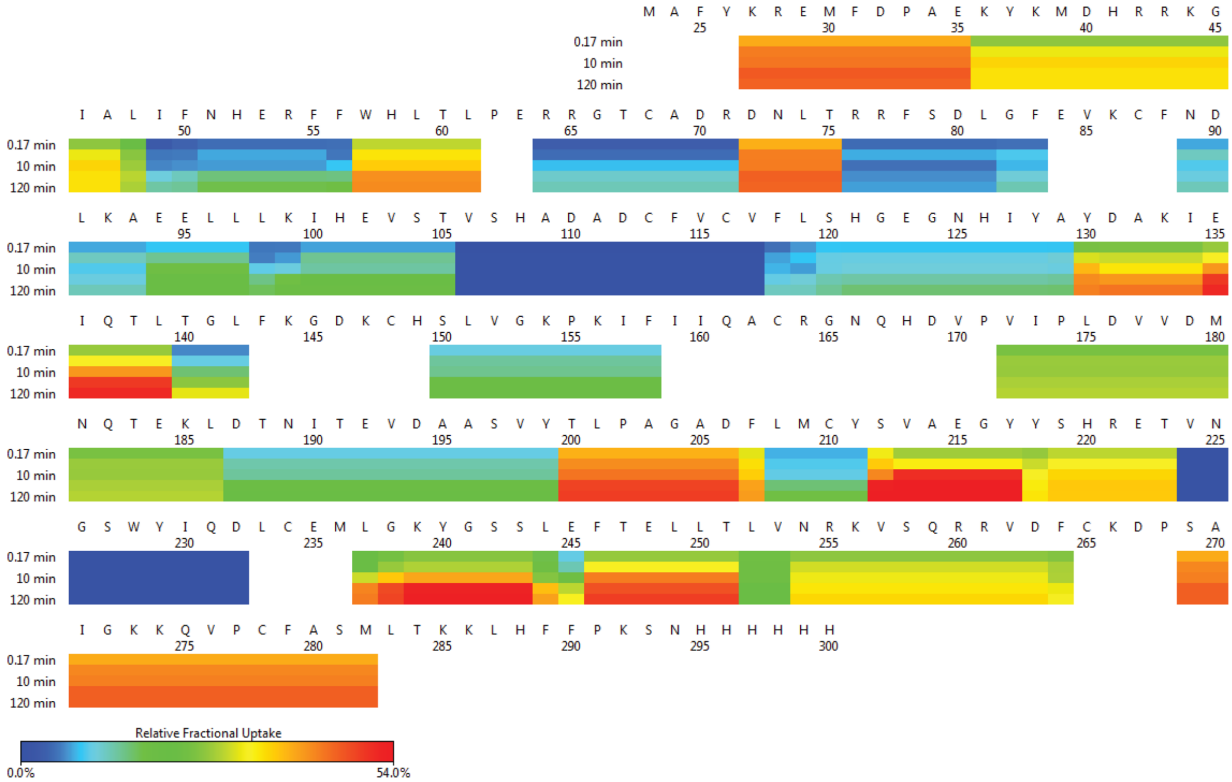


Supporting Figure 5
Peptic peptide map coverage of Caspase-6 ΔN D179 CT R44K, performed in triplicate.



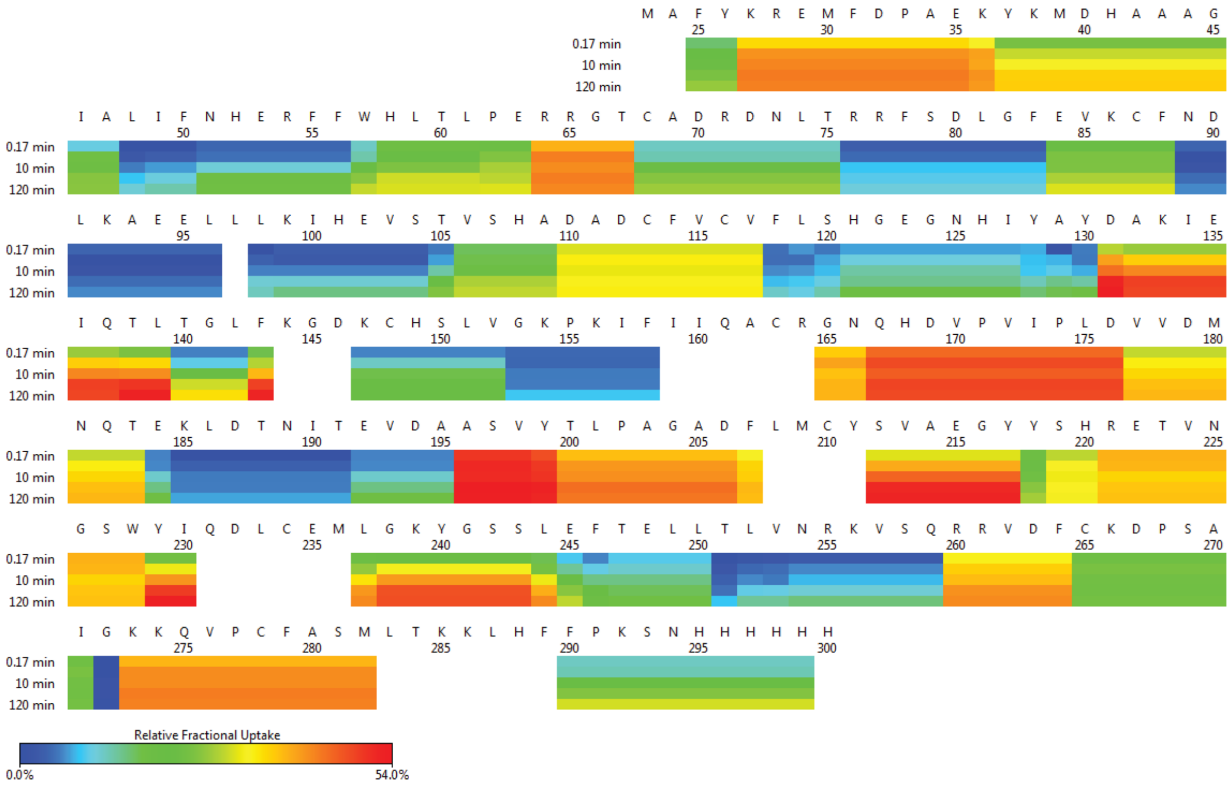
Supporting Figure 6

Deuterium uptake heat map of Caspase-6 ΔN D179 CT. Colors represent the fractional uptake of the total possible deuterium within the identified regions. Scale is set from 0 – 54% which was the lowest maximal uptake observed between all three variants to emphasize differences in regional exchange.



Supporting Figure 7

Deuterium uptake heat map of Caspase-6 ΔN D179 CT R44K. Colors represent the fractional uptake of the total possible deuterium within the identified regions. Scale is set from 0 – 54% which was the lowest maximal uptake observed between all three variants to emphasize differences in regional exchange.



Supporting Figure 8

Deuterium uptake heat map of Caspase-6 ΔN D179 CT R42-44A. Colors represent the fractional uptake of the total possible deuterium within the identified regions. Scale is set from 0 – 54% which was the lowest maximal uptake observed between all three variants to emphasize differences in regional exchange.

Substrate	Analysis of data from Figure	Relative Fold Change Compared to Wild Type Casp-6 R42-44A	R44K
PARP	5A,D	2.4	2.3
Lamin	5B,E	1.3	1.3
DJ-1	5C,F	2.3	1.5
Casp-6 C163S	4C,D	3.6	1.8

Supporting Table 1

Calculated fold differences between the wild-type caspase-6 and the variants R42-44A and R44K in observed of cleavage of casp-6 substrates as function of time. The fold change was calculated by performing a linear regression of the quantified intensities from gels of casp-6 ability to digest of the indicated substrates from 0-6 hours. Slopes (Arbitrary Intensity Units/Time) of the resultant linear regressions were compared to yield relative fold difference between the wild-type and the respective variants. These average values were the result of 5 independent experiments performed on 5 separate days. The level of statistical significance is indicated in Fig 4D and Fig 5D-F.