

Onconase cytotoxicity relies on the distribution of its positive charge

Rebecca F. Turcotte, Luke D. Lavis and Ronald T. Raines

Page	Contents
S1	Table of Contents
S2	Table S1. Mass analysis of ONC and its variants.
S3	Fig. S1. Effect of ONC triple variants on the proliferation of K-562 cells.
S4	Fig. S2. Effect of ONC double variants on the proliferation of K-562 cells.

Table S1. Mass analysis of ONC and its variants.

ONC	Mass ^a (Da)	
	expected	observed
wild-type	11,820	11,828
<i>Patch variants</i>		
K45A/K49A/K55A (<i>Patch 1</i>)	11,649	11,657
R15A/K80A/K81A (<i>Patch 2</i>)	11,621	11,626
R73A/K76A/K78A (<i>Patch 3</i>)	11,621	11,625
<i>Distributed variants</i>		
R15A/K49A/R73A	11,593	11,595
R15A/K45A/K76A	11,621	11,622
R15A/K55A/K76A	11,621	11,629
<i>Double variants</i>		
K49A/R73A	11,678	11,675
R73A/K78A	11,678	11,678
K76A/K78A	11,706	11,710
R15A/R73A	11,650	11,653
K80A/K81A	11,706	11,711
R15A/K49A	11,678	11,677
R73A/K76A	11,678	11,678
R15A/K80A	11,678	11,683
R15A/K81A	11,678	11,684
<i>Single variants</i>		
R15A	11,735	11,735
K49A	11,763	11,770
R73A	11,735	11,728
K76A	11,763	11,783
K78A	11,763	11,776
K80A	11,763	11,781
K81A	11,763	11,780
<i>Labeled variants</i>		
S61C-TML	12,615	12,619
K9A/K31A/S61C-TML	12,501	12,490
R15A/K80A/K81A/S61C-TML	12,416	12,413
R15A/K55A/K76A/S61C-TML	12,416	12,418
K49A/R73A/S61C-TML	12,470	12,465
R15A/K81A/S61C-TML	12,470	12,477

^a Values of m/z ($\pm 0.05\%$) were determined by MALDI-TOF mass spectroscopy.

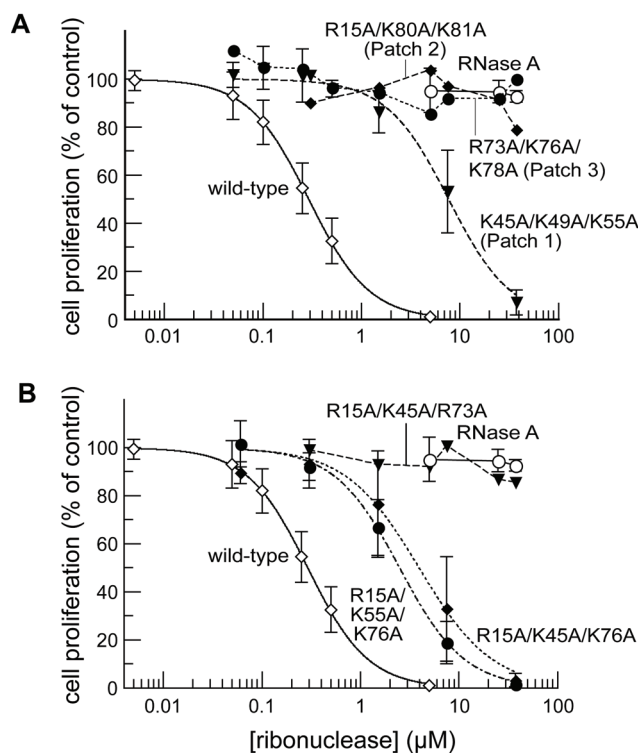


Fig. S1. Effect of ONC triple variants on the proliferation of K-562 cells. Cell proliferation was measured by monitoring the incorporation of [*methyl*- ^3H]thymidine into genomic DNA. Data points indicate the mean ($\pm\text{SE}$) of three separate experiments carried out in triplicate. Wild-type ONC and RNase A controls are shown with open diamonds and circles, respectively. Values of IC_{50} are listed in Table 1. (A) Effect of replacing Patches 1, 2, or 3. (B) Effect of replacing three residues distributed across the three patches.

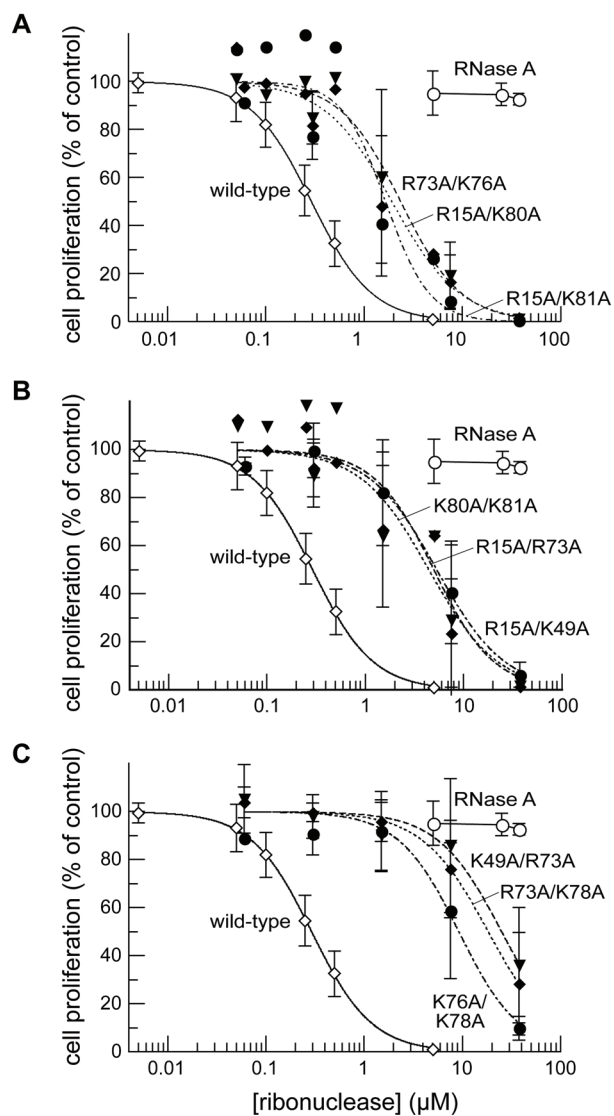


Fig. S2. Effect of ONC double variants on the proliferation of K-562 cells. Cell proliferation was measured by monitoring the incorporation of [*methyl*- ^3H]thymidine into genomic DNA. Data points indicate the mean ($\pm\text{SE}$) of three separate experiments carried out in triplicate. Values of IC_{50} are listed in Table 1. (A) Compilation of data for variants that retained high cytotoxicity. (B) Compilation of data for variants that exhibited moderate cytotoxicity. (C) Compilation of data for variants that exhibited low cytotoxicity.