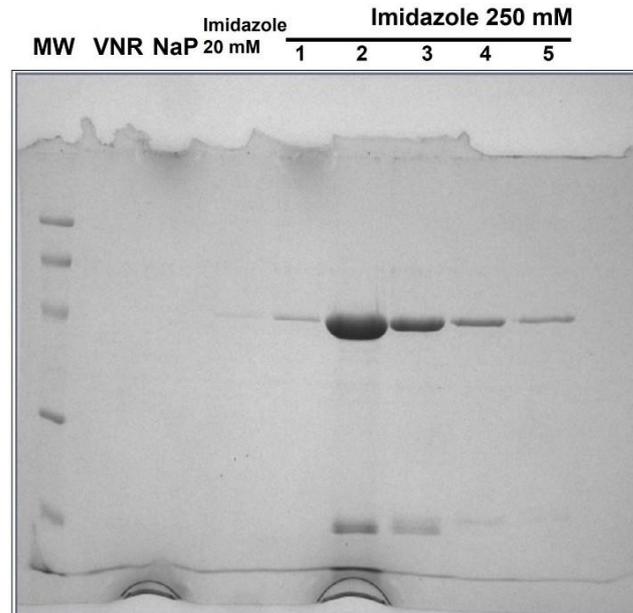
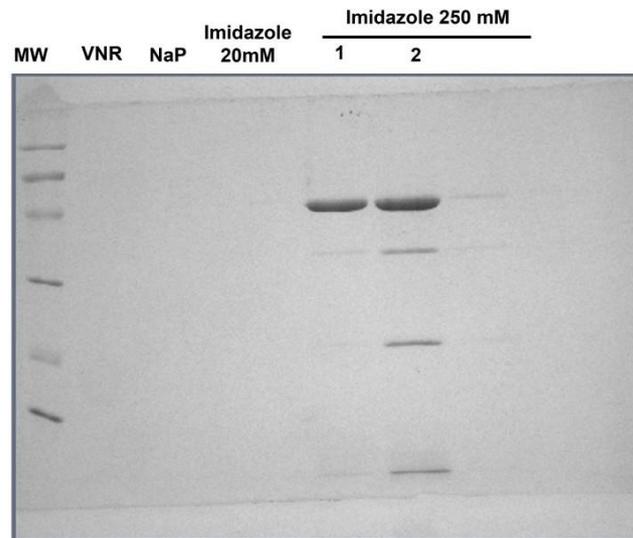
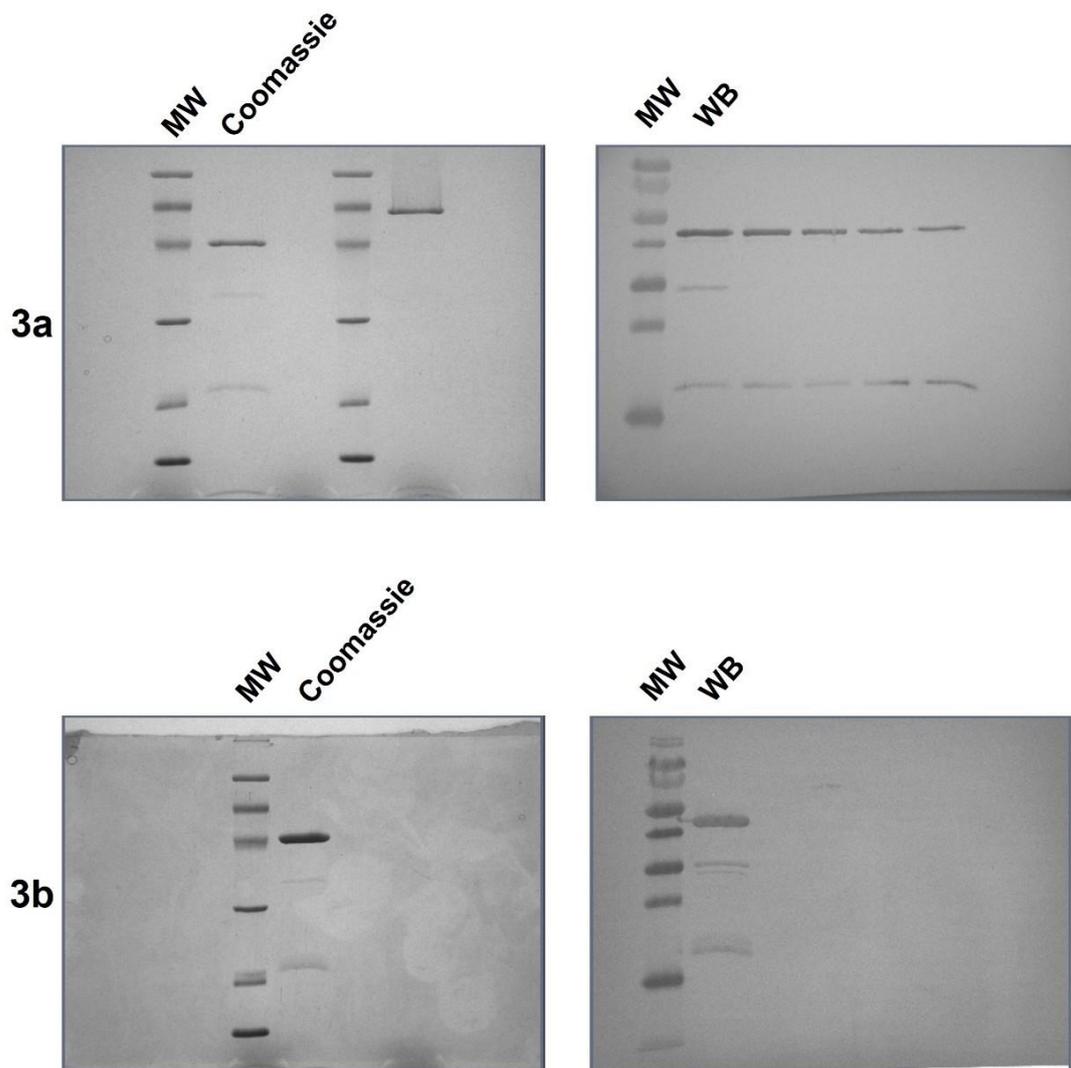


# Supplementary Materials: A New Optimized Version of a Colorectal Cancer-Targeted Immunotoxin Based on a Non-immunogenic Variant of the Ribotoxin $\alpha$ -Sarcin

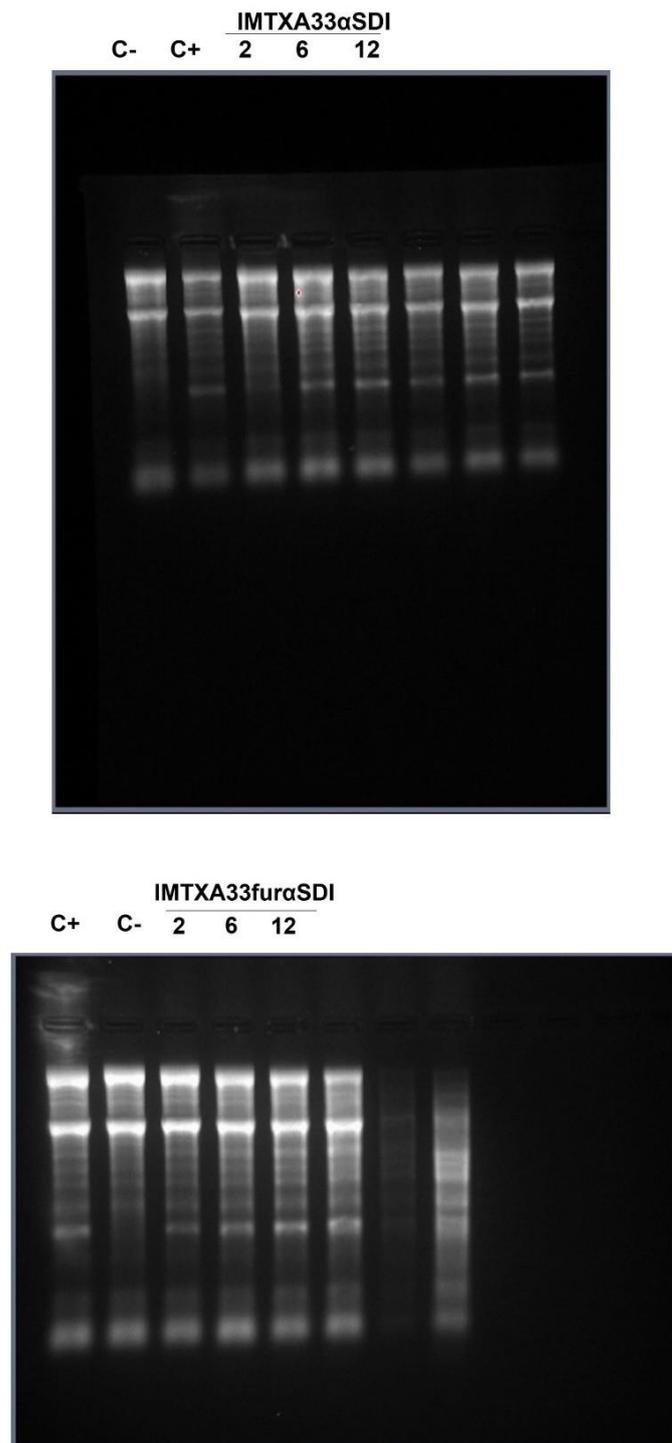
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**Figure S1. Original SDS-PAGE of both purified immunotoxins.** Original full-length gels from Figure 2 are presented. Coomassie blue stained SDS-PAGE analysis of the different pools obtained during the  $\text{Ni}^{2+}$ -NTA affinity chromatography of IMTXA33 $\alpha$ SDI (a) and IMTXA33fura $\alpha$ SDI (b). Lines shown correspond to: MW, prestained molecular weight standard (kDa); VNR, nor retained fraction; NaP, washed fraction eluted with sodium phosphate buffer; Imidazole 20 mM, washed fraction eluted with sodium phosphate buffer containing imidazole 20 mM; and different 1ml fractions eluted with 250 mM imidazole. In both immunotoxins, the arrow indicates the expected molecular weight of 45 kDa for both immunotoxins.



**Figure S2. SDS-PAGE and Western Blot analysis of both purified immunotoxins.** Original full-length gels and western blots from Figure 3a and 3b are presented. In all cases lines corresponding to information appeared in Figure 3 are indicated. SDS-PAGE followed by Coomassie blue staining (left) and Western Blot analysis (right) of the purified final fraction of IMTXA33 $\alpha$ SDI. (a) and IMTXA33furaSDI (b). Western blot analysis was carried out using rabbit anti- $\alpha$ -sarcin serum. MW corresponds to prestained molecular weight standards. Images were acquired and analyzed using the Quantity One 1-D analysis software (BioRad).



**Figure S3. In vitro functional characterization.** Ribonucleolytic activity of the toxic domain: Rabbit reticulocytes assays were made in order to test the ribonucleolytic activity of  $\alpha$ -sarcin within both constructs. Original full-length gels from Figure 4a are presented. (a) Ribonucleolytic activity of the toxic domain of both immunotoxins, carried out by a rabbit reticulocyte assay. The arrow indicates the release of the  $\alpha$ -fragment, produced by the cleavage of the SRL due to the  $\alpha$ SDI. In both gels, 2, 6 and 12 pmoles of both IMTXA33 $\alpha$ SDI and IMTXA33furaSDI were assayed. C+ represents 2 pmoles of fungal wild-type  $\alpha$ -sarcin, whereas in C-, the protein sample was replaced by buffer. Images were acquired and analyzed using the Gel Doc XR Imaging System and the Quantityone software (Bio-Rad).