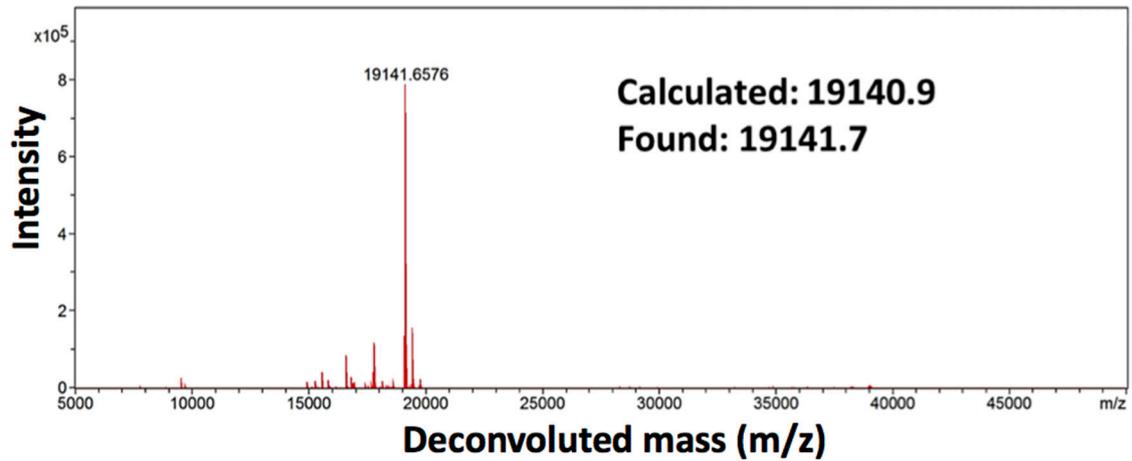
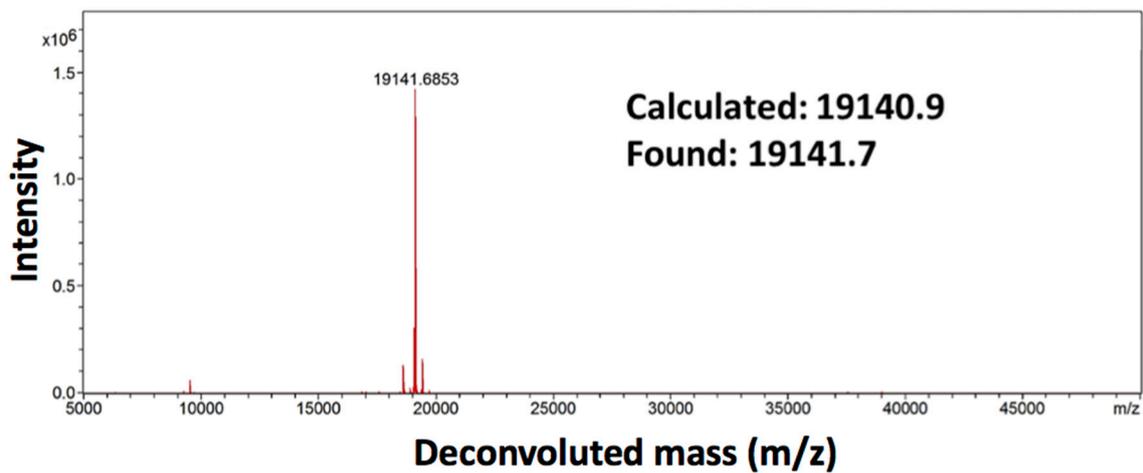


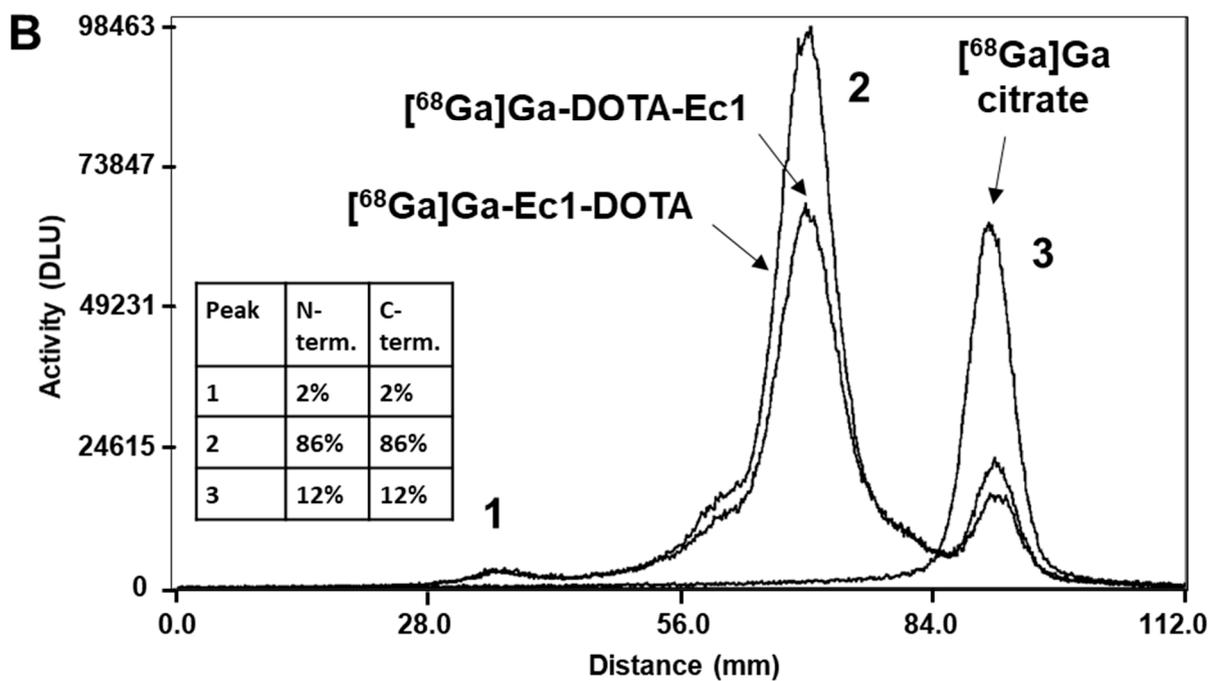
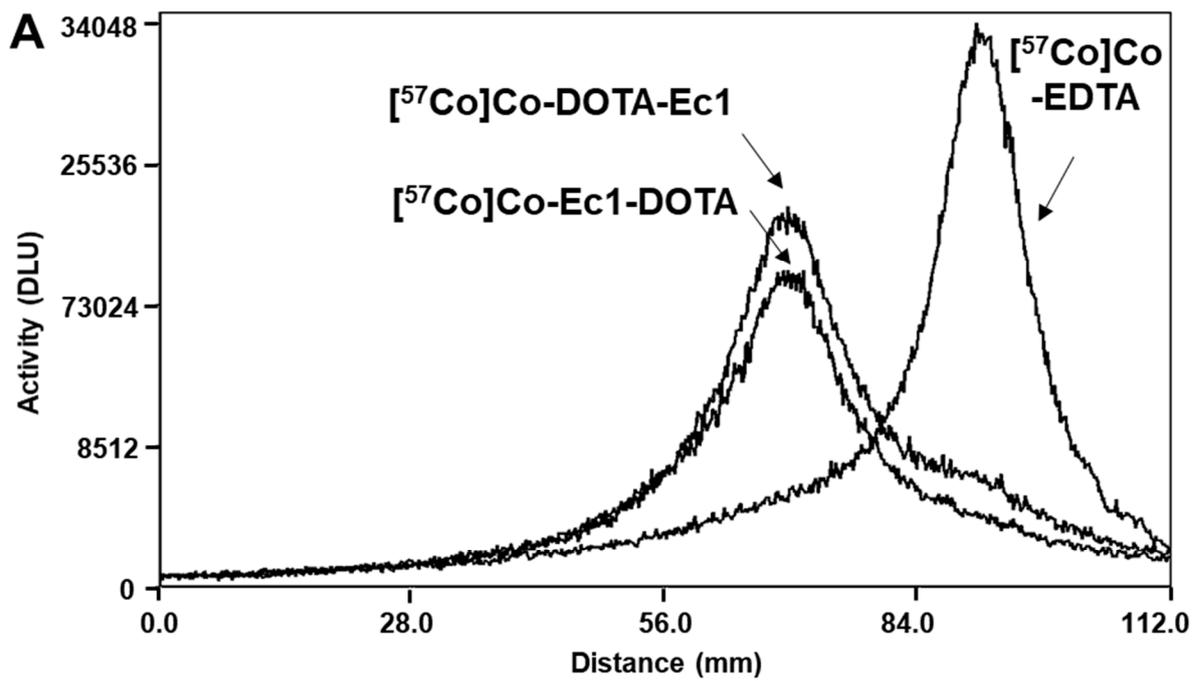
**A** DOTA-CE<sub>3</sub>-Ec1-(HE)<sub>3</sub>



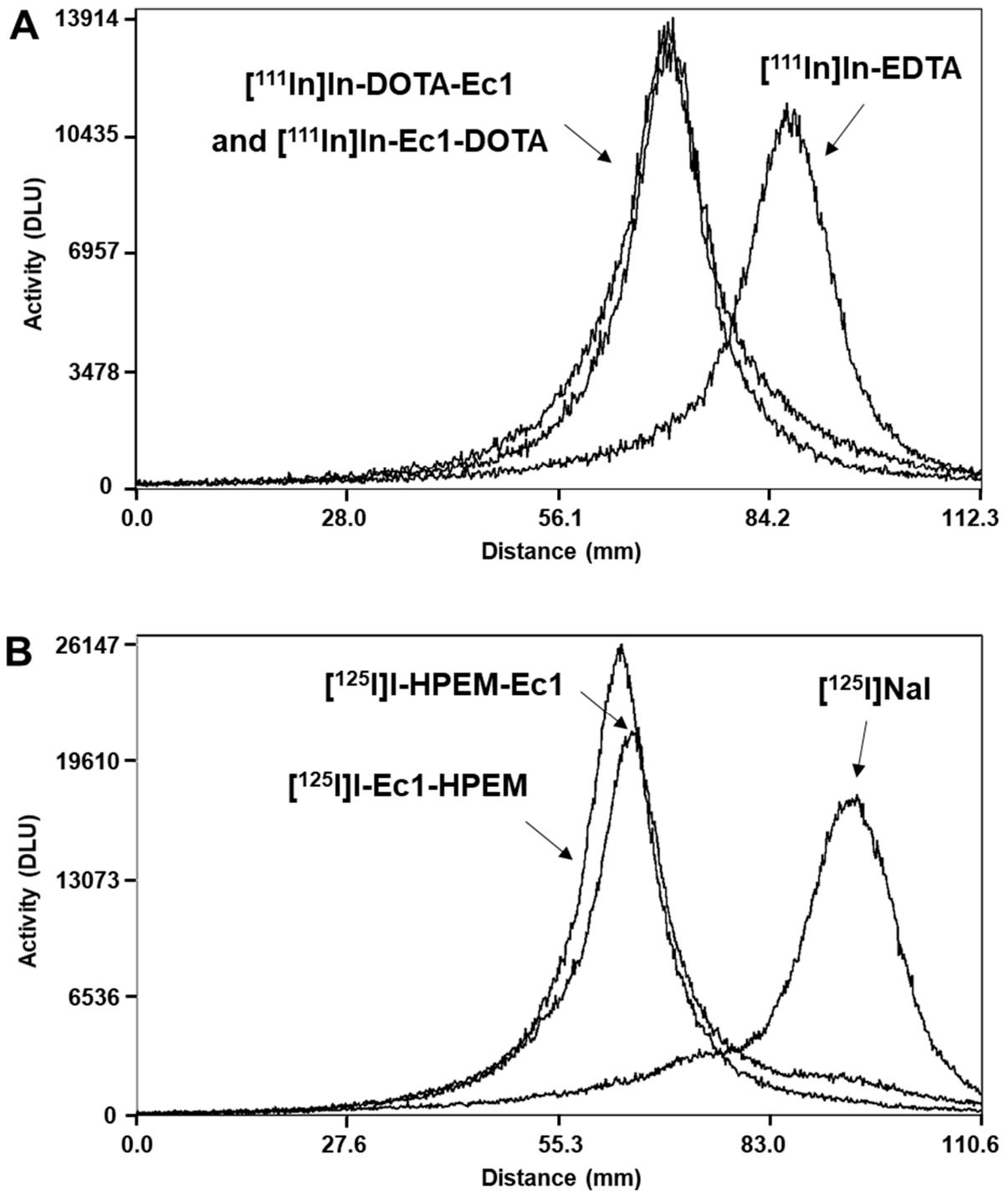
**B** (HE)<sub>3</sub>-Ec1-E<sub>3</sub>C-DOTA



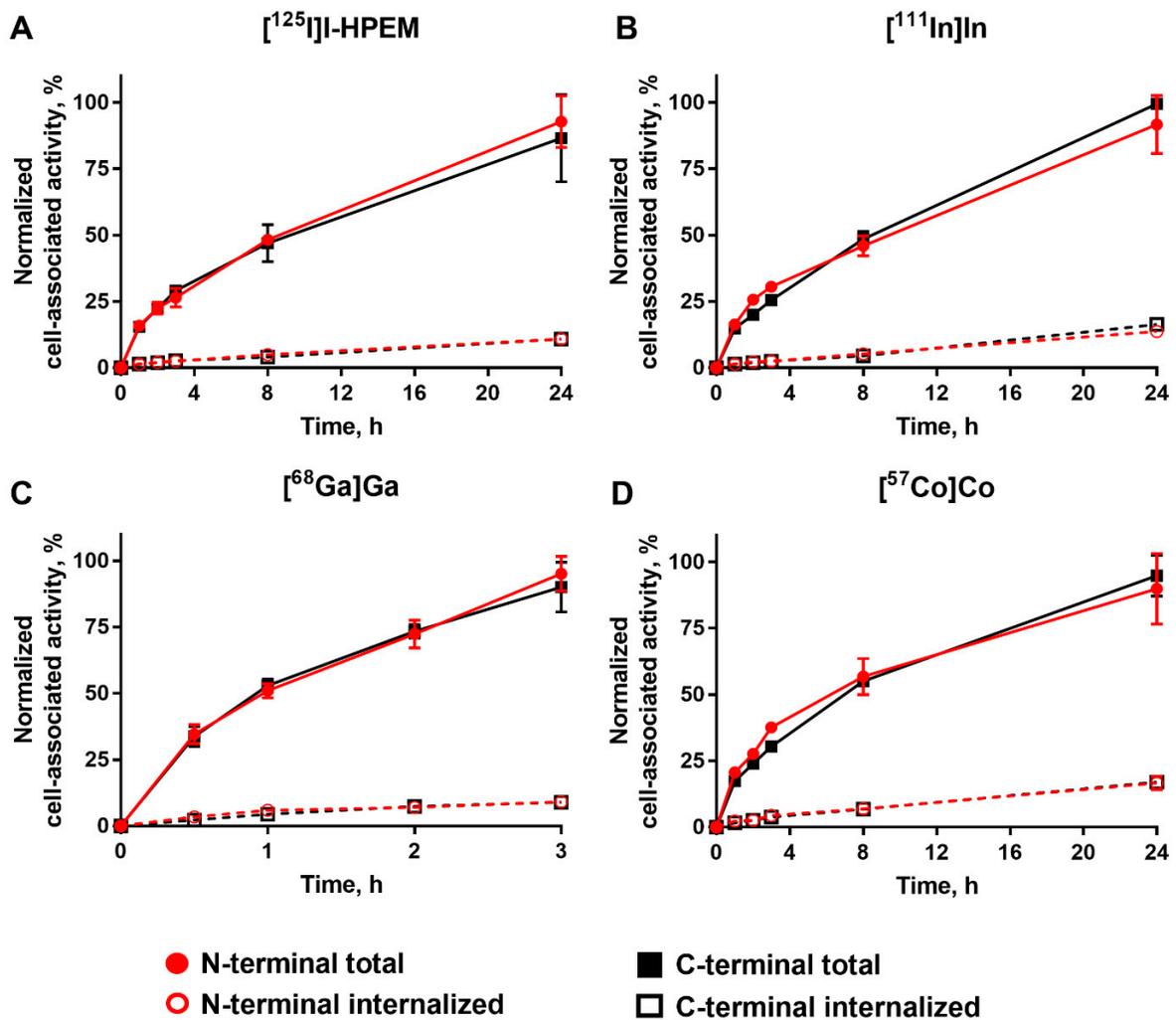
**Supplementary Figure S1.** ESI-MS spectra of Ec1 variants conjugated to DOTA chelator at N- or C-terminus. Calculated molecular weight for both variants 19140.9, found 19141.7 for (A) N-terminal DOTA-CE<sub>3</sub>-Ec1-(HE)<sub>3</sub> and 19141.7 for (B) C-terminal (HE)<sub>3</sub>-CE<sub>3</sub>-Ec1-DOTA.



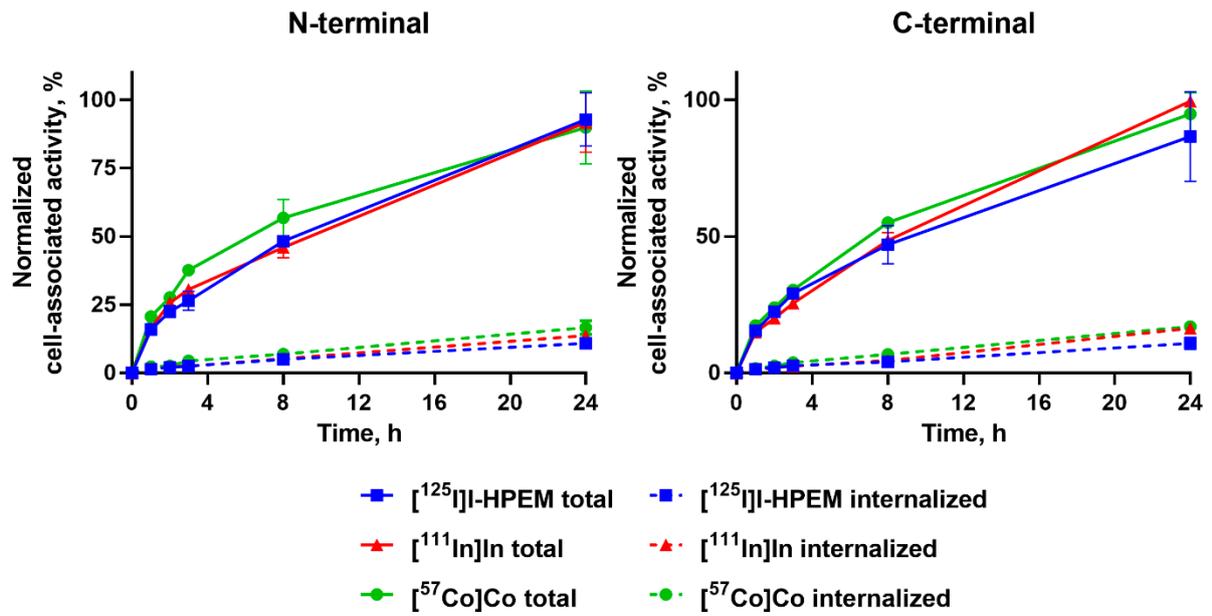
**Supplementary Figure S2.** SDS-PAGE analysis of (A)  $[^{57}\text{Co}]\text{Co-DOTA-Ec1}$  and  $[^{57}\text{Co}]\text{Co-Ec1-DOTA}$ , and (B)  $[^{68}\text{Ga}]\text{Ga-DOTA-Ec1}$  and  $[^{68}\text{Ga}]\text{Ga-Ec1-DOTA}$ .  $[^{57}\text{Co}]\text{Co-EDTA}$  or  $[^{68}\text{Ga}]\text{Ga-citrate}$  were used as markers for low molecular weight compounds.



**Supplementary Figure S3.** SDS-PAGE analysis of (A)  $[^{111}\text{In}]\text{In-DOTA-Ec1}$  and  $[^{111}\text{In}]\text{In-Ec1-DOTA}$ , and (B)  $[^{125}\text{I}]\text{I-HPEM-Ec1}$  and  $[^{125}\text{I}]\text{I-Ec1-HPEM}$ .  $[^{111}\text{In}]\text{In-EDTA}$  or  $[^{125}\text{I}]\text{NaI}$  were used as markers for low molecular weight compounds.



**Figure S4.** Cellular processing of Ec1 variants radiolabeled with (A) [<sup>125</sup>I]I-HPEM, (B) [<sup>111</sup>In]In, (C) [<sup>68</sup>Ga]Ga and (D) [<sup>57</sup>Co]Co at N- or C-terminus, by DU145 prostate cancer cells during continuous incubation over 24 h (for [<sup>68</sup>Ga]Ga over 3 h). Data are presented as mean from three samples ± SD; error bars may not be visible when they are smaller than symbols.



**Figure S5.** Overlay of cellular processing data for Ec1 variants radiolabeled with [<sup>125</sup>I]I-HPEM, [<sup>111</sup>In]In, and [<sup>57</sup>Co]Co at N- or C-terminus, by DU145 prostate cancer cells during continuous incubation over 24 h. Data are presented as mean from three samples ± SD; error bars may not be visible when they are smaller than symbols.