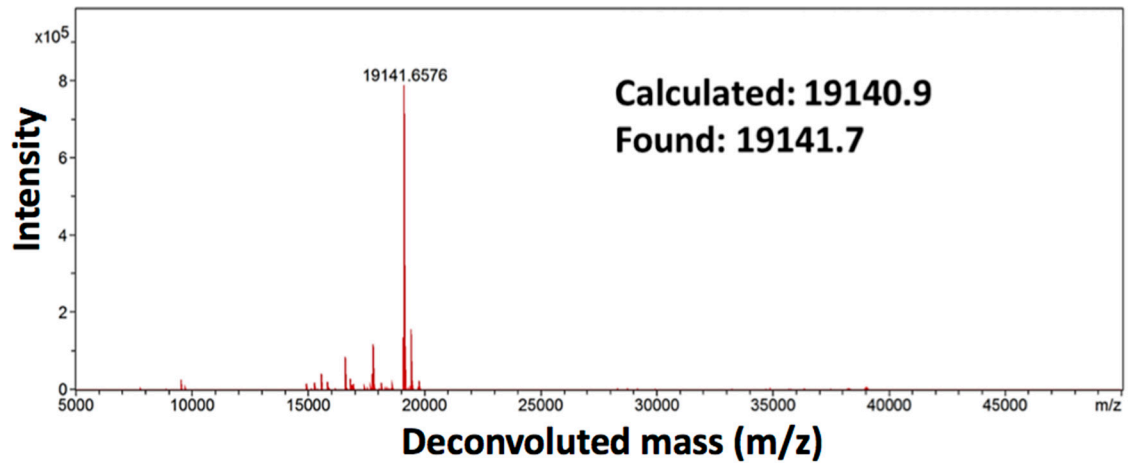
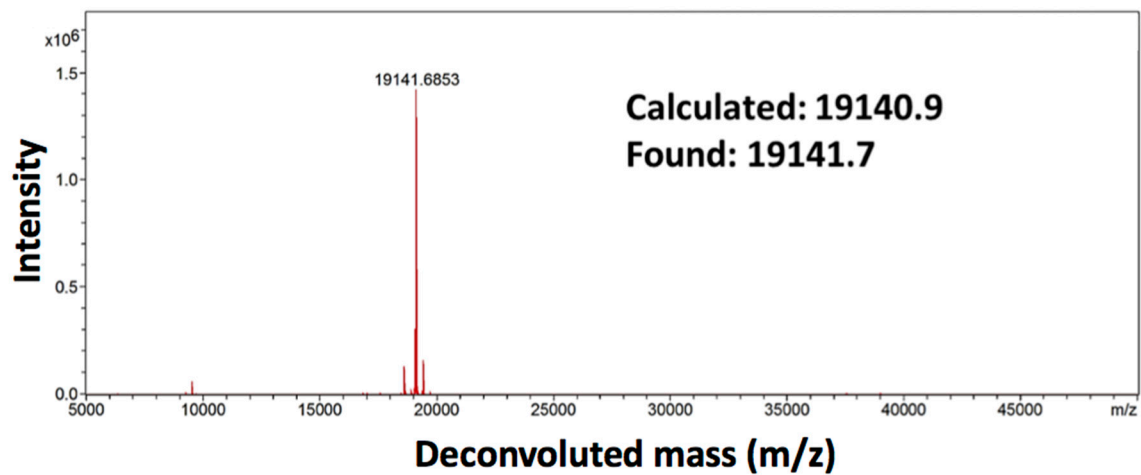


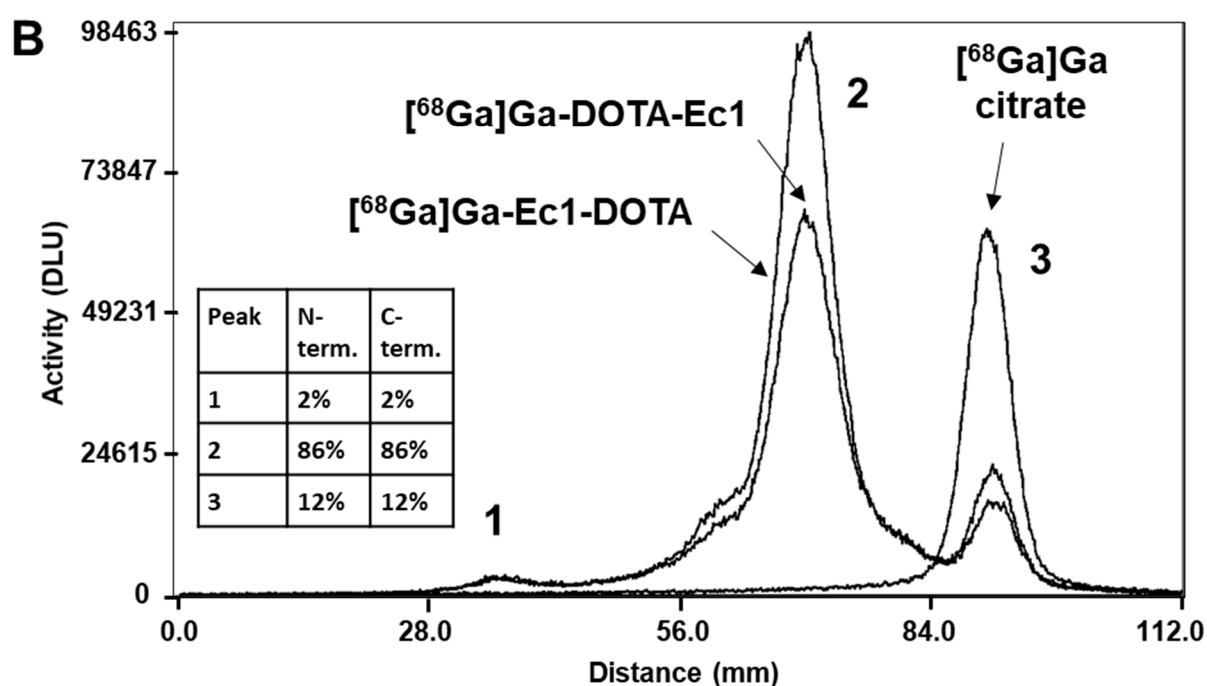
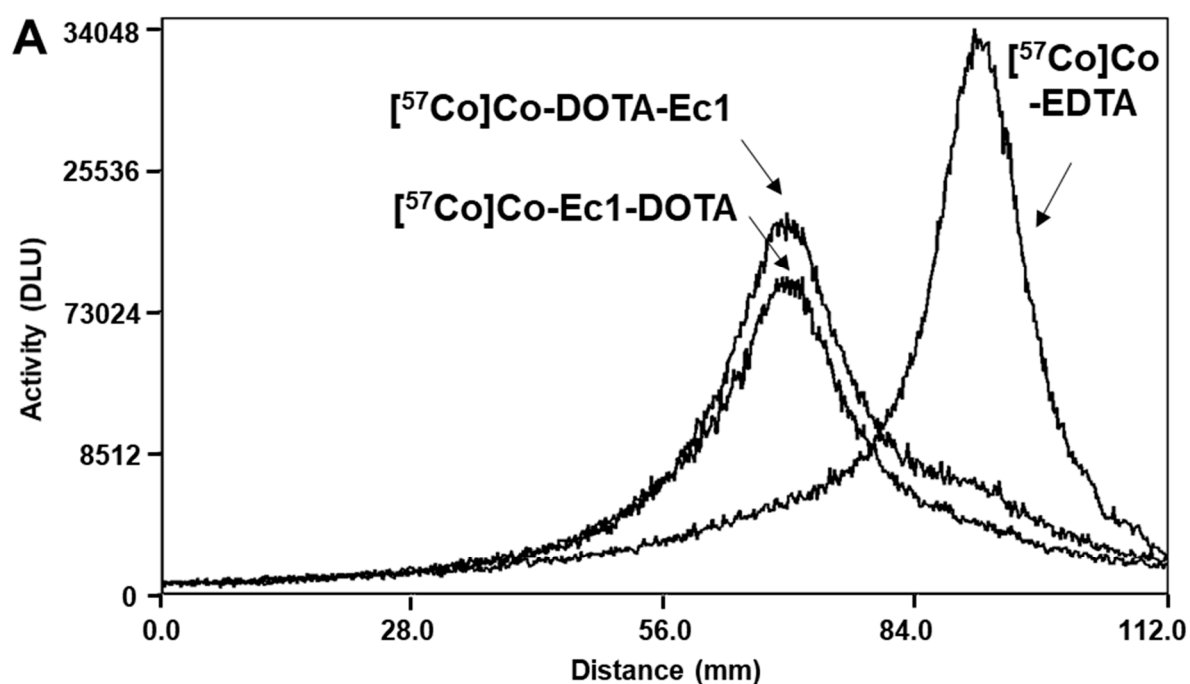
A DOTA-CE₃-Ec1-(HE)₃



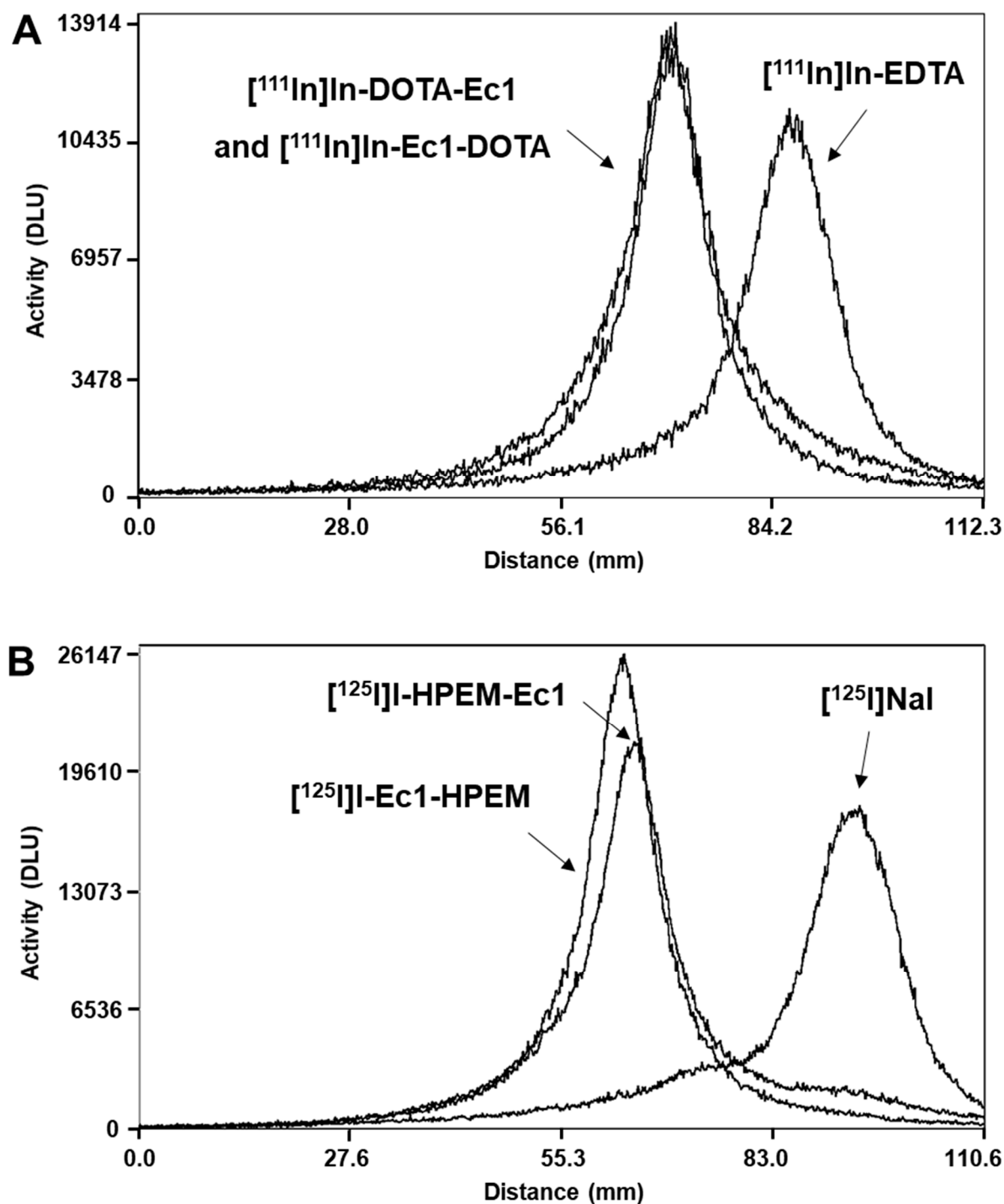
B (HE)₃-Ec1-E₃C-DOTA



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-CE₃-Ec1-(HE)₃ and 19141.7 for (B) C-terminal (HE)₃-CE₃-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.

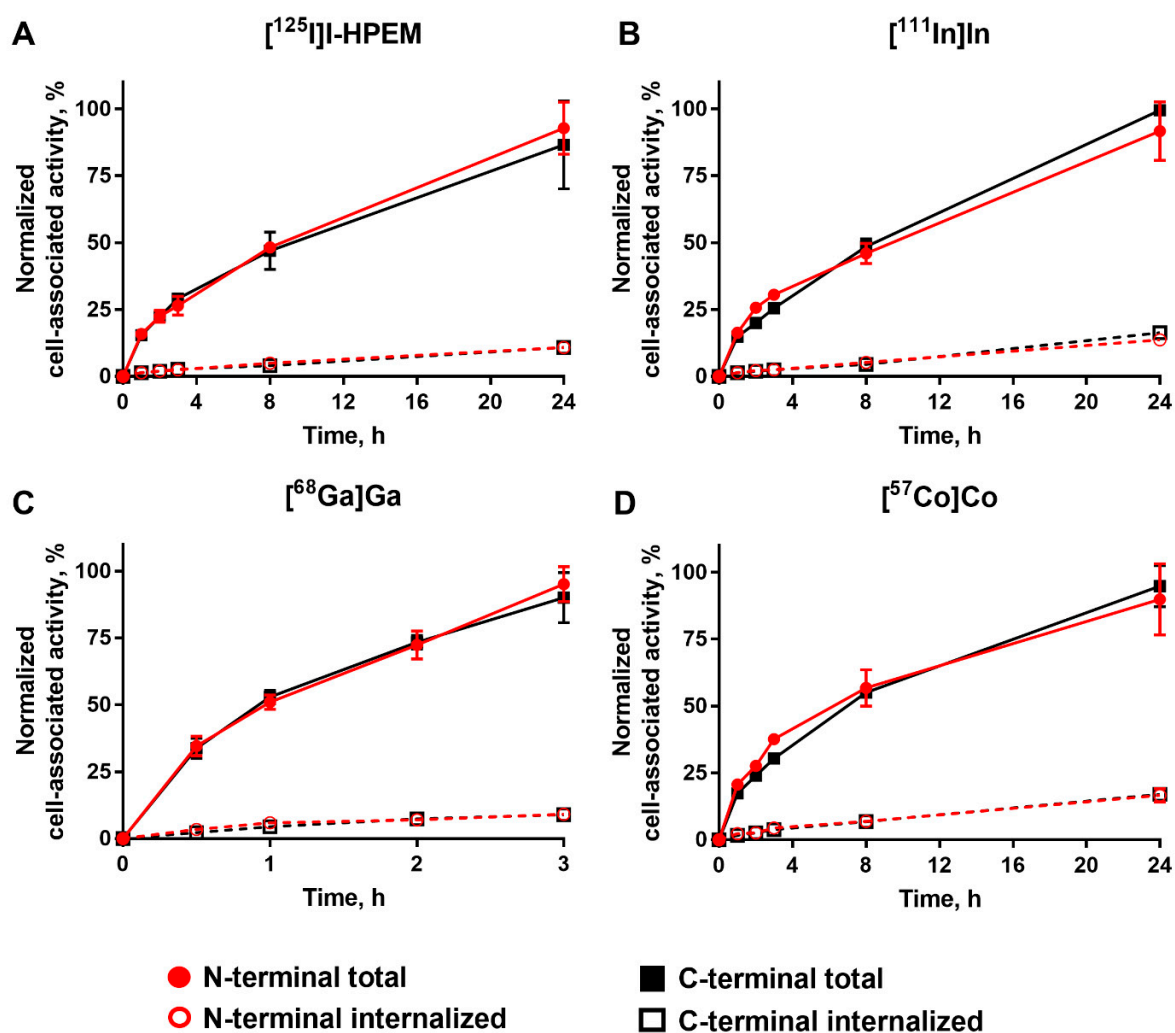


Figure S4. Cellular processing of Ec1 variants radiolabeled with (A) $[^{125}\text{I}]\text{I-HPEM}$, (B) $[^{111}\text{In}]\text{In}$, (C) $[^{68}\text{Ga}]\text{Ga}$ and (D) $[^{57}\text{Co}]\text{Co}$ at N- or C-terminus, by DU145 prostate cancer cells during continuous incubation over 24 h (for $[^{68}\text{Ga}]\text{Ga}$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

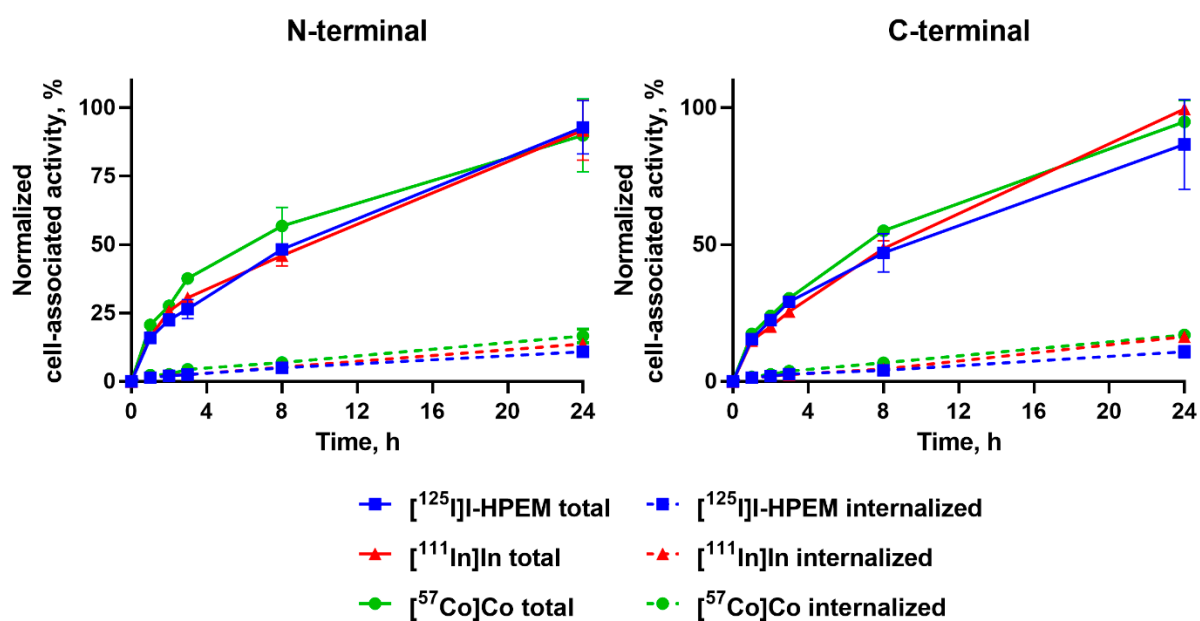


Figure S5. Overlay of cellular processing data for Ec1 variants radiolabeled with $[^{125}\text{I}]\text{I-HPEM}$, $[^{111}\text{In}]\text{In}$, and $[^{57}\text{Co}]\text{Co}$ at N- or C-terminus, by DU145 prostate cancer cells during continuous incubation over 24 h. Data are presented as mean from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