Supplemental material

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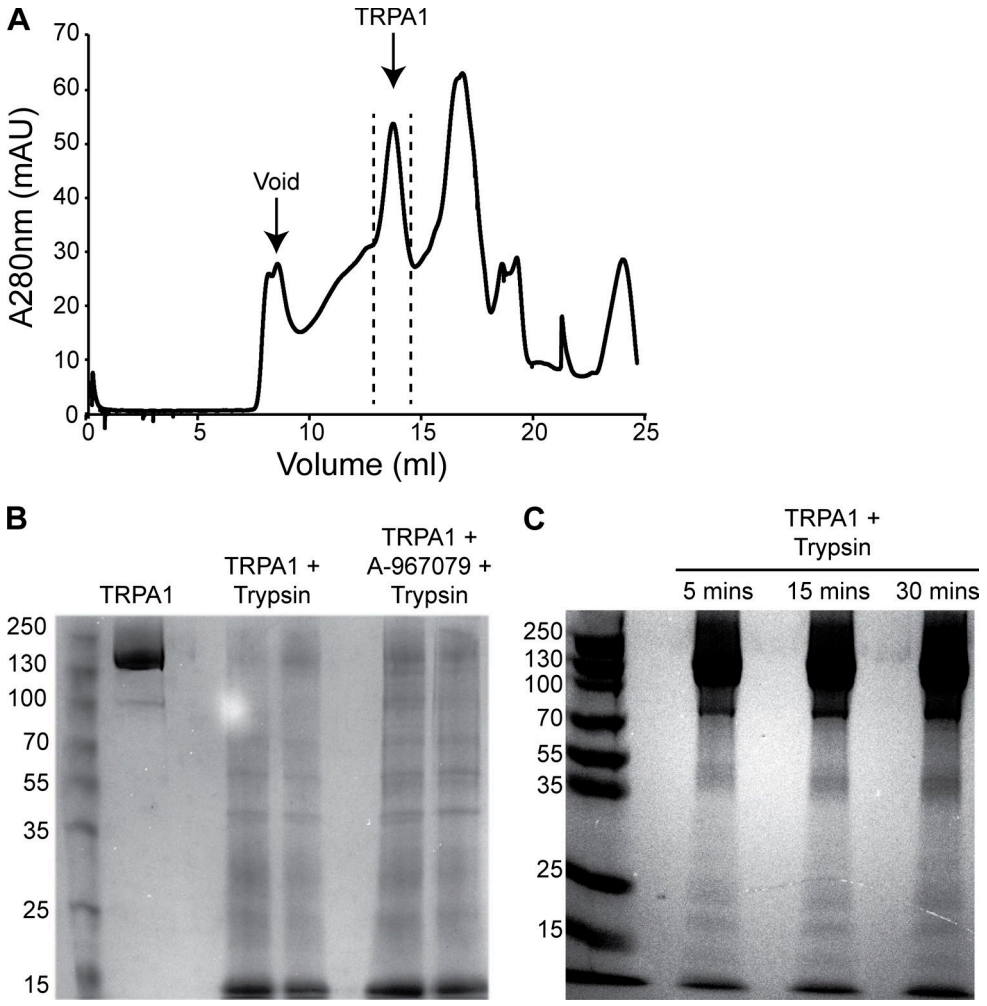

Figure S1. **TRPA1 purification and limited proteolysis of pure TRPA1. (A)** Gel filtration profile of purified mouse TRPA1; the peak corresponding to tetrameric, amphipol-stabilized TRPA1 is shown by the dotted lines. **(B)** Coomassie-stained SDS-PAGE gel showing purified TRPA1, purified TRPA1 cleaved with trypsin for 15 min, and purified TRPA1 treated with A-967079 for 10 min and then cleaved with trypsin for 15 min. **(C)** Coomassie-stained SDS-PAGE gel showing time course of trypsinization of purified TRPA1.

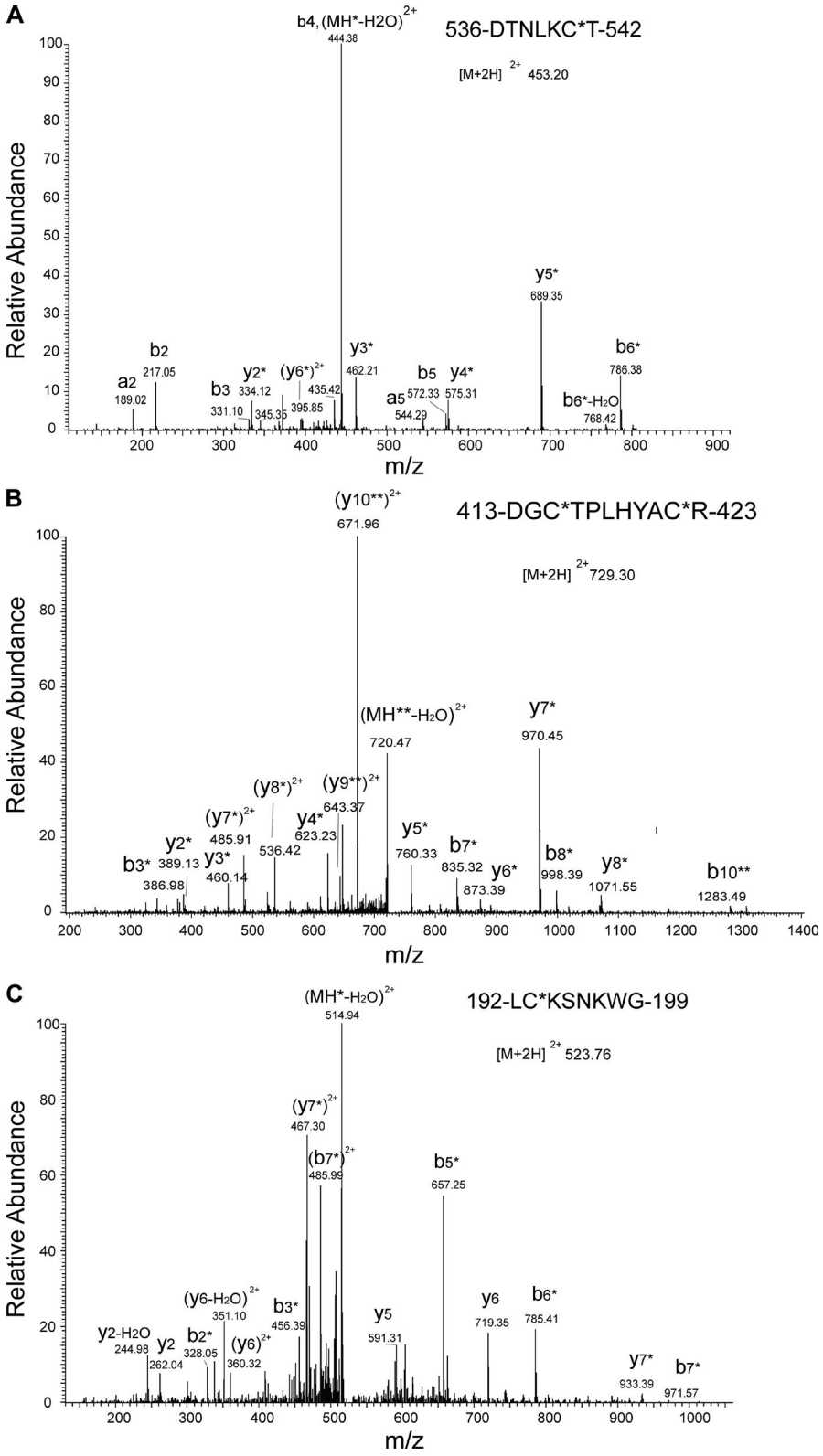

Figure S2. **Nano–LC-MS/MS spectrum of the modified peptides after Asp-N digestion of NMM-activated TRPA1. (A)** MS/MS spectrum of the doubly protonated ion (m/z 453.20) of the 536–542 peptide has mass shift of 111.032 D at Cys-540 residues corresponding to cysteine modification by NMM. The y*- and b*-fragment ions correspond to the y- and b-fragment ions that were modified by NMM. **(B)** MS/MS spectrum of the doubly protonated ion (m/z 729.30) of the 413–423 peptide has mass shift of 222.064 D that corresponds to modification by NMM at both Cys-415 and Cys-422 residues. The y*, b*- and y**, b**-fragment ions correspond to the y- and b-fragment ions that were modified by one and two molecules of NMM, respectively. **(C)** MS/MS spectrum of the doubly protonated ion (m/z 523.76) of the 192–199 peptide has mass shift of 111.032 D at Cys-193 residues corresponding to cysteine modification by NMM. The y*- and b*-fragment ions correspond to the y- and b-fragment ions that were modified by NMM.

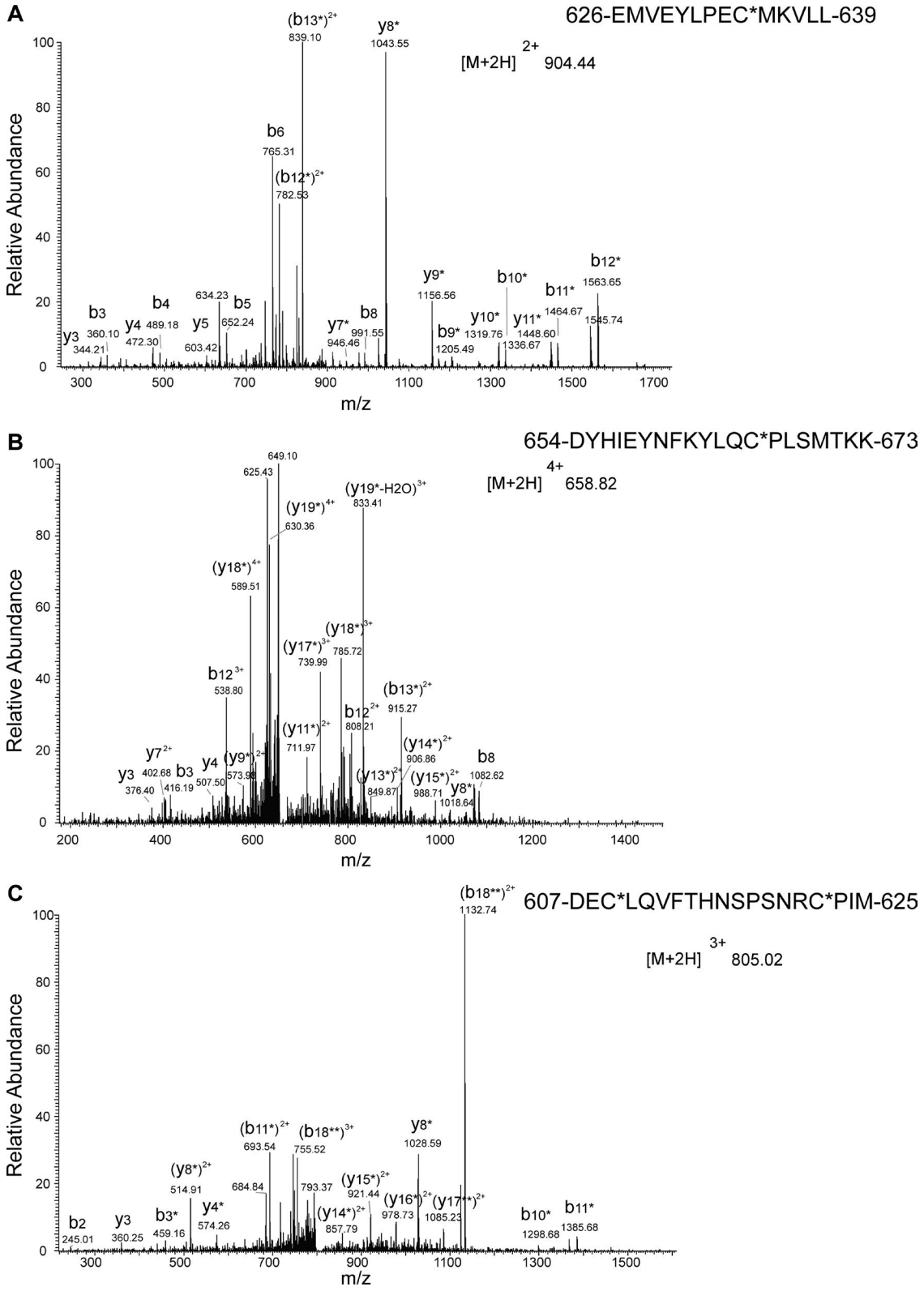

Figure S3. **Nano–LC-MS/MS spectrum of the modified peptides after Asp-N digestion of NMM-activated TRPA1. (A)** MS/MS spectrum of the doubly protonated ion (m/z 904.44) of the 626–639 peptide has mass shift of 111.032 D at Cys-634 residues corresponding to cysteine modification by NMM. The y*- and b*-fragment ions correspond to the y- and b-fragment ions that were modified by NMM. **(B)** MS/MS spectrum of the quadruply protonated ion (m/z 658.82) of the 654–673 peptide has a mass shift of 111.032 D at Cys-666 residues corresponding to cysteine modification by NMM. The y*- and b*-fragment ions correspond to the y- and b-fragment ions that were modified by NMM. **(C)** MS/MS spectrum of the triply protonated ion (m/z 805.02) of the 607–625 peptide has mass shift of 222.064 D that corresponds to modification by NMM at both Cys-609 and Cys-622 residues. The y*, b*- and y**, b**-fragment ions correspond to the y- and b-fragment ions that were modified by one and two molecules of NMM, respectively.