



FIGURE S2. Activity profiling of female rat liver cytosolic DUBs after SEC. A female rat liver cytosol was subjected to SEC and the collected fractions (*lanes 1-14*) were incubated with HA-UbVME and analyzed by SDS-PAGE/Western blot with an anti-HA antibody (*panel 1*). Fractions were also assayed for deubiquitinating activity using the indicated monoubiquitinated PEX5 proteins (*panels 3-5*). The reactions were terminated with 20 mM NEM, and analyzed by SDS-PAGE. Autoradiographs (*panels 3 and 4*) and Coomassie blue-stained gels (*panel 5*) are presented. The elution profile of USP9X was also assessed using the anti-USP9X antibody (*panel 2*). The asterisk in *panel 1* indicates an HA-UbVME-reactive DUB that co-elutes with the hydrolytic activity detected towards monoubiquitinated PEX5 proteins. The elution positions and molecular masses of the protein standards used to calibrate the SEC column, as well as the column void volume (V_0) are indicated. Reactions using the initial cytosolic extract (*lanes Cyt*) and the monoubiquitinated PEX5 proteins alone (*lanes 1*) are also presented. Numbers to the left in *panel 1* indicate the molecular masses of protein standards in kDa.