Supplementary information 3

Plasmid DNAs

To construct plasmids coding for the mutant f.Hex1 proteins, the pFLAG-CMV-2.HEXIM1 plasmids were subjected to site directed mutagenesis with the QuickChange II XL Site-Directed Mutagenesis Kit (Cat. No. 200521; Stratagene, La Jolla, CA). To construct the plasmid coding for the mutant f.NLS.Hex1mB1256 protein, pFLAG-CMV-2 vector was first modified by insertion of a cDNA coding for the SV-40 NLS into HindIII-EcoRI sites of the pFLAG-CMV-2 vector, followed by excision of the DNA coding for the Hex1mB1256 protein from pFLAG.HEXIM1mB1256 using EcoRI and SalI restriction enzymes and insertion into EcoRI SalI sites of the modified pFLAG CMV-2 vector. To construct the plasmid coding for the h6.x.Hex1 protein, the cDNA of HEX IM1 was inserted into KpnI-Apal sites of the pcDNA™3.1/HisB (Cat. No. V385-20; Invitrogen, Carlsbad, CA). To construct plasmids coding for the N-terminal deletion mutant h6.x.Hex1 proteins, the PCR-amplified DNA fragments contained the SV-40 NLS at their 3' ends and were inserted into the EcoRI-XbaI sites of the pcDNA™3.1/HisC (Cat. No. V385-20; Invitrogen, Carlsbad, CA). The plasmids coding for the C-terminal deletion mutant h6.x.Hex1 proteins were constructed by using QuickChange II XL Site Directed Mutagenesis Kit. To construct plasmids coding for the GST.Hex1 chimeras, the cDNAs coding for the wild-type or mutant f.Hex1 proteins were inserted into EcoRI-SalI sites of the modified pGEX-4T1 vector. The plasmids coding for the Hex1.YFP and BR.YFP (aa 143-184) chimeras were cloned into the pEYFP-N1 expression vectors (Clontech).