Supplementary Materials: Transcytosis, Antitumor Activity and Toxicity of Staphylococcal Enterotoxin C2 as an Oral Administration Protein Drug

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Formula of HBSS: A buffer: 16 g NaCl, 0.8 g KCl, 0.2 g $MgSO_4.7H_2O$, 0.2 g $MgCl_2.6H_2O$, 0.28 g $CaCl_2$ dissolved in 1 L ultra-pure water and adjusted pH to 7.4.B buffer: 0.3 g $Na_2HPO_4.12H_2O$, 0.12 g KH_2PO_4, 2 g glucose dissolved in 1 L ultra-pure water and adjusted pH to 7.4. A buffer and B buffer were sterilized and mixed in equal volumes.

Sandwich ELISA: 96-well plates were coated by adding 100 μL of anti-his monoclonal antibody (500 μg/mL in carbonate-bicarbonate buffer, pH 9.6) overnight at 4 °C. After that, the plates were blocked with defatted milk in PBST buffer (PBS, pH 7.4, containing 0.05% of Tween-20) for 2 h at 37 °C. After blocking, each well was incubated with standard SEC2-His or samples that were diluted to serial fine concentration by blocking buffer for 2 h at 37 °C. 100 μL of rabbit anti-SEC2 polyclonal antibody or biotinylated anti-SEC2 monoclonal antibody (1 μg/mL in blocking buffer) was added to each well for 2 h at 37 °C, following by incubation with 100 μL HRP-tagged antibody or streptavidin (diluted 1:2000 in blocking buffer) for 1 h at 37 °C. 100 μL of tetramethylbenzidine (TMB, 75 μg/mL) substrate solution was added to each well and then incubated for 30 min at 37 °C. After that, 100 μL of stop solution (2 M H_2SO_4) was added to each well. Finally, the absorbance was measured at 450 nm in an ELISA plate reader.

Colorimetric method: Standard HRP solution or samples were diluted to serial fine concentration and added into 96-well plates. 50 μL of TMB (75 μg/mL) was added to each well and then incubated for 5 min at 37 °C. After that, 50 μL of stop solution (2 M H_2SO_4) was added to each well. Finally, the absorbance was measured at 450 nm in an ELISA plate reader.

Western-blotting: Standard SEC2-His or samples were separated on 12% SDS-polyacrylamide gel using a Protean Minigel apparatus (BioRad, Hercules, CA, USA), and proteins were transferred to a polyvinyldifluoride membrane (Millipore, Billerica, MA, USA). After blocking the membrane with defatted milk in PBST buffer (PBS, pH 7.4, containing 0.1% of Tween-20) at 4 °C overnight, the membrane was incubated with anti-his monoclonal antibody at 37 °C for 2 h. After that, membrane was washed 4 times using PBST buffer and then incubated with HRP tagged goat anti mouse antibody at 37 °C for 2 h followed by washing membrane 4 times. At last, membrane was incubated with ECL reagent (Biological Industries, Nahariya, Israel) and light emission detected by exposing the membrane to an X-ray film (Kodak, Rochester, NY, USA).