Molecular Mimicry of Human Endothelial Cell Antigen by Autoantibodies to Nonstructural Protein 1 of Dengue Virus

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Supplemental Information

Immunoaffinity purification and identification of the target of DB16-1. COLO 205 cells (1×10^8) were lysed with lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40) supplemented with a protease inhibitor cocktail tablet (Roche) and incubated on ice for 30 min. Cell lysate was prepared at 10,000×g for 15 min at 4°C. The supernatant was applied to protein G sepharose (GE Healthcare Biosciences) coupled with DB16-1. After washing, the proteins binding to DB16-1 were eluted with elution buffer (0.2 M Glycine, pH 2.5, 150 mM NaCl, and 1% NP-40) and the eluates were neutralized with 1 M Tris-HCl, pH 9.1. The eluates were separated in SDS-PAGE and analyzed by LC-MS/MS.