Supplementary information

Preparation and analysis of cell fractions

HeLa cells were stably transfected with pCB7-VSV-BSPRY or the empty pCB7 vector using Lipofectamine 2000 (Invitrogen, Breda, The Netherlands) DMEM supplemented with 10 % (v/v) fetal calf serum. Clones were selected by culture in the presence of 400 μg/ml hygromycin. Subsequently, cells were grown to confluency in a T75 flask and homogenized by brief sonication in homogenization buffer (250 mM sucrose, 10 mM Tris, pH 7.4). After a 10 min spin at 400 g (input fraction), the particulate or membrane fraction was obtained by centrifugation at 100,000 g for 30 min. The supernatant was collected (cytosol fraction) and the pellet was resuspended in homogenization buffer and centrifuged at 100,000 g for 30 min to remove cytosolic contaminants of the membrane fraction. The expression of VSV-BSPRY in the membrane and cytosol fraction was analyzed by immunoblot analysis.

Supplementary figure

The subcellular localization of BSPRY was determined stably transfected HeLa (A) or MDCK (B) cells. Cell homogenates were centrifuged at 400 g (input) and subsequently at 100,000 g. The cytosolic (supernatant) and membranous (pellet) pools of BSPRY were quantified using two clones of HeLa cells (1 & 2) or a pooled fraction of MDCK cells, consisting of three independent clones, stably expressing VSV-BSPRY. A large fraction of the protein was present in the soluble cytosolic fraction whereas a significant amount of the protein localized in the membrane/particulate fraction. Analysis

of Na^+, K^+-ATPase or β-actin as markers for intrinsic membrane proteins or cytosolic proteins, respectively, indicated the purity of the cytosol and membrane fraction. None-transfected (NT) cells showed no signal, demonstrating the specificity of the signal.

Supplementary figure