Supporting Information

Investigating the Regulatory Interaction of Linker Region of *Ciona intestinalis* Voltage-sensitive Phosphatase with Lipid Membrane

Sungjae Kim,[†] Md. Mahbubur Rahman,[‡] Kwangmo Noh,[‡] Jae-Joon Lee,[‡] and Young Jun Kim[†,‡,*]

[†]*Department of Applied Biochemistry and* [‡]*Nanotechnology Research Center, Konkuk University, Chung-ju 380-701, Korea*
[*]*E-mail: ykim@kku.ac.kr*
Received July 15, 2014, Accepted July 31, 2014

Materials and Method

Materials. *Escherichia coli* Rosetta 2 (DE3) competent cells were purchased from Novagen (Darmstadt, Germany). Ampicillin, chloramphenicol, and isopropyl-β-D-thiogalactopyranoside (IPTG) were obtained from Biosesang (Sungnam, Korea). Ni-NTA agarose resin was purchased from Qiagen (Venlo, Netherlands). *para*-nitrophenyl phosphate (*p*NPP), malachite green oxalate salt, ammonium molybdate tetrahydrate, and 11-mercaptoundecanoic acid (MUA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Di-C_4-phosphoinositides were purchased from Echelon Bioscience (Salt Lake City, UT, USA). Di-C_{16}-phosphoinositides and 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS) were from Cayman Chemicals (Ann Arbor, MI, USA) and Avanti Polar Lipids (Alabaster, AL. USA), respectively. Malachite green reagent was prepared as previously described (Taylor, G. S.; Dixon, J. E. *Methods Enzymol.* **2003**, *366*, 43-56).

Purification of Recombinant Ci-VSP(248-576) Wild-type and Mutants. Truncated version of the wild-type ΔCi-VSP(248-576) was subcloned into pET21a(+) vector and expressed in the *E. coli* Rosetta 2 (DE3) strain. We designed a series of ΔCi-VSP mutants, where each positively charged residue (K252, R253, and R254) was mutated to either a neutral charge (Ala, A) or a negative charge (Glu, E) residue. The expression of wild-type or mutants of recombinant ΔCi-VSP(248-576) was induced in *E. coli* at 16 °C with 0.4 mM IPTG and allowed to accumulate at high levels overnight. Cells were harvested and resuspended in lysis buffer consisting off 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM imidazole, 0.05% β-mercaptoethnaol (BME), and protease inhibitors. Cells were disrupted by probe sonication, and the lysate was passed onto a Ni-NTA agarose column. The C-terminal 6× histidine-tagged protein was purified by washing the column using elution buffer containing 50 mM Tris-HCl (pH 8.0), 25 mM NaCl, 100 mM imidazole, and 0.05% BME). The eluate was dialyzed into the elution buffer without imidazole. The protein dialysate was added to a buffer containing 20% glycerol and stored at −70 °C. When necessary, further purification was perfumed through size-exclusion chromatography.

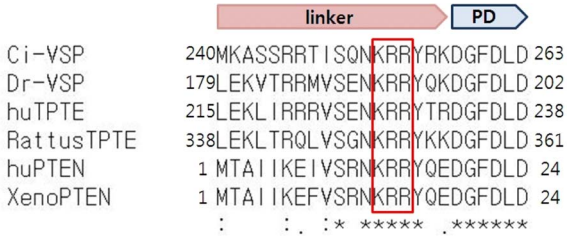

Figure S1. Sequence alignment of key region, including the phospholipid-binding motif (PBM) of the linker region of VSP- and PTEN-homologs. The linker region of Ci-VSP carries positively charged residues (K252, R253, and R254), which were individually mutated to either the neutral (Ala) or negative charge (Glu) amino acid residue.

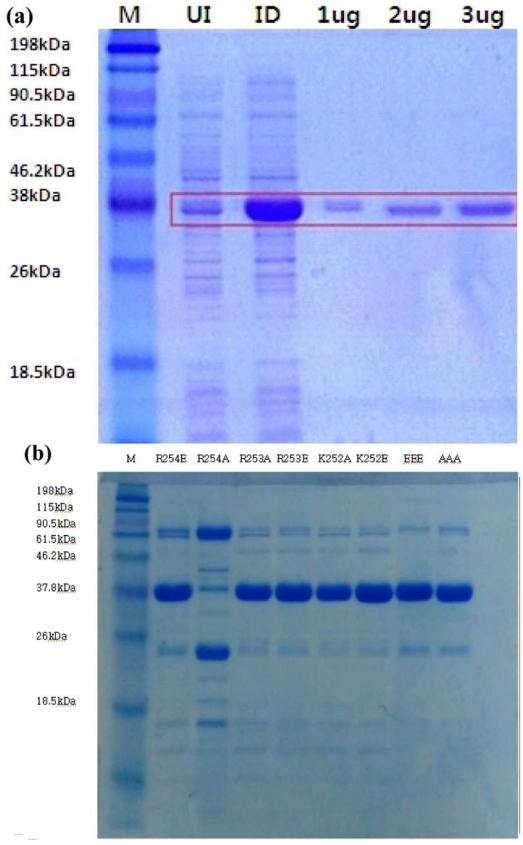

Figure S2. Characterization of purified ΔCi-VSP(248-576) WT and mutants using SDS-PAGE. (a) Purity of ΔCi-VSP(248-576) WT (1 μg to 3 μg), UI: uninduced, ID: induced by IPTG (b) Expression profile of purified ΔCi-VSP(248-576) mutants

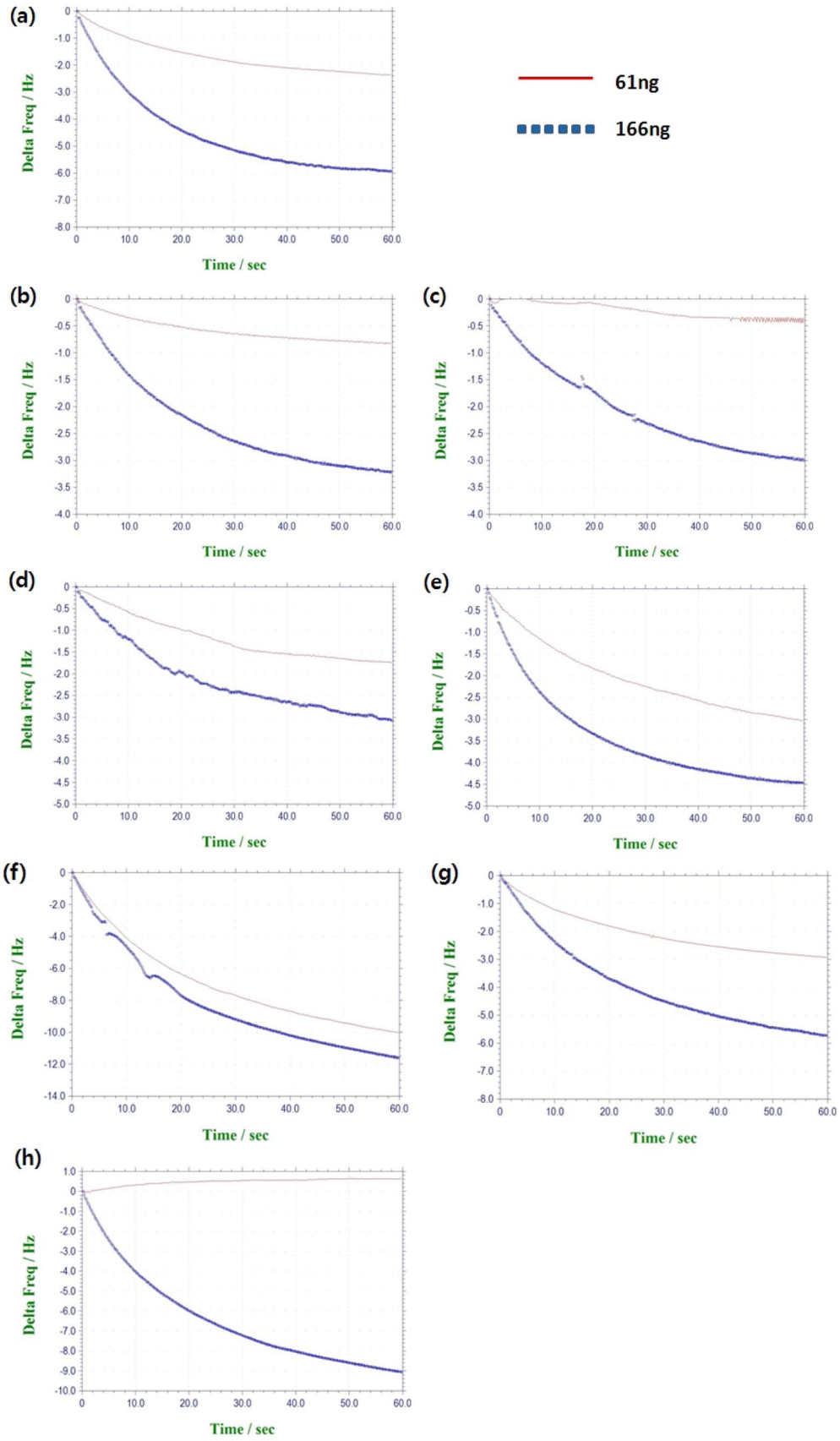

Figure S3. Electric sensorgram recordings (60 s) were measured by QCM for two different amounts of protein: 61 ng (red trace) and 166 ng (blue dotted trace) for ΔCi-VSP and its mutants. (a) ΔCi-VSP, (b) ΔCi-VSP K252A, (c) ΔCi-VSP K252E, (d) ΔCi-VSP R253A, (e) ΔCi-VSP R253E, (f) ΔCi-VSP R254E, (g) ΔCi-VSP K252A/R253A/R254A, and (h) ΔCi-VSP K252E/R253E/R254E.