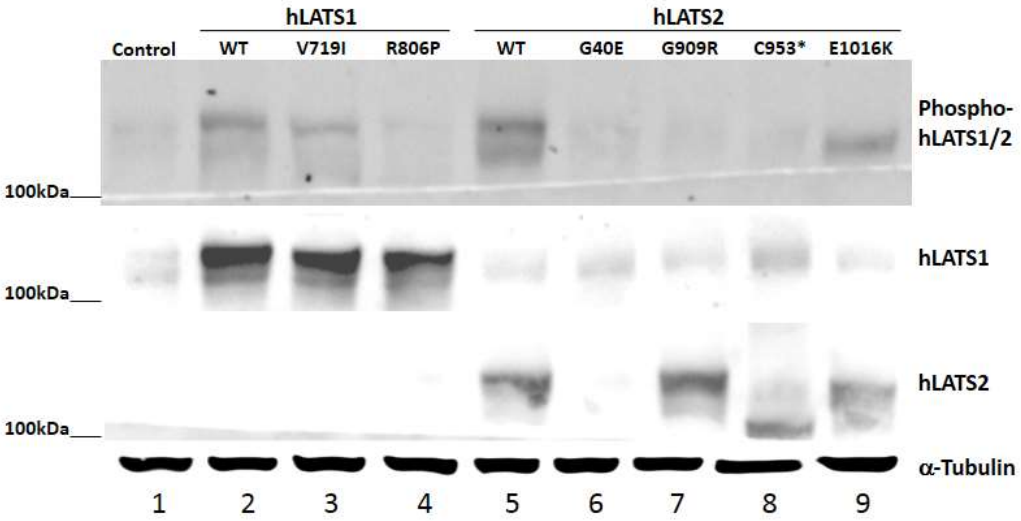

Figure S2 Western blot analysis of human LATS1 and LATS2 proteins in HEK293T cells. HEK293T cells were transfected with wild-type (lane 2 and lane 5) or mutant (lane 3-4 and lane 6-9) *hLATS1/2* DNA constructs as indicated. Anti-hLATS1 and anti-hLATS2 antibodies were used to specifically detect hLATS1 and hLATS2 expression, respectively (the second and third panels). The endogenous hLATS1but not hLATS2 protein was expressed at a detectable level. An anti-Phospho-LATS1/2 (Thr1079/1041) specifically identified activated hLATS1 and hLATS2 kinase proteins (the top panel). As all hLATS1/2 constructs wee tagged with a Flag sequence, their expressions were all verified by an anti-FLAG antibody (data not shown). Expression of a-Tubulin was monitored to ensure equal loading of protein extracts (the bottom panel). Untransfected HEK293T cells were used as a negative control (lane 1). Protein markers are shown on the left side of the panels.

pcDNA3.1-hygro-3xFLAG-hLATS1 and *pcDNA3.1-hygro-3xFLAG-hLATS2* (gift from Dr. Xiaolong Yang) were used for site-directed mutagenesis to generate V719I and R806P mutant constructs for *hLATS1* and G40E, G909R, C953* and E1016K mutants for *hLATS2*. *hLATS1/2* wild-type and mutant variants were cloned into *pUAST-attB* and verified by DNA sequencing.

For Western blot analysis HEK293T cells were transfected using PolyFect (Qiagen). After 36 hours, cells were treated with 1.0 μM okadaic acid (OA) (Sigma) for 30 min at 37°C, and harvested 36 hours later in lysis buffer (150 mM NaCl, 50 mM Tris-HCl [pH = 7.4], 2 mM EDTA [pH = 8.0], 1% Triton-X 100, 10% glycerol, 2 mM DTT, 1 mM PMSF, 10 mM NaF, 2 mM Na_3VO_4, 60 mM Glycerol-2-phosphate, containing Protease inhibitors and phosphatase inhibitor (Sigma). The following antibodies were used: anti-LATS1 (Santa Cruz), anti-LATS2 and anti-Phospho-LATS1/2 (Thr1079/1041) (Cell Signaling), anti-FLAG and anti-a-Tubulin (Sigma).