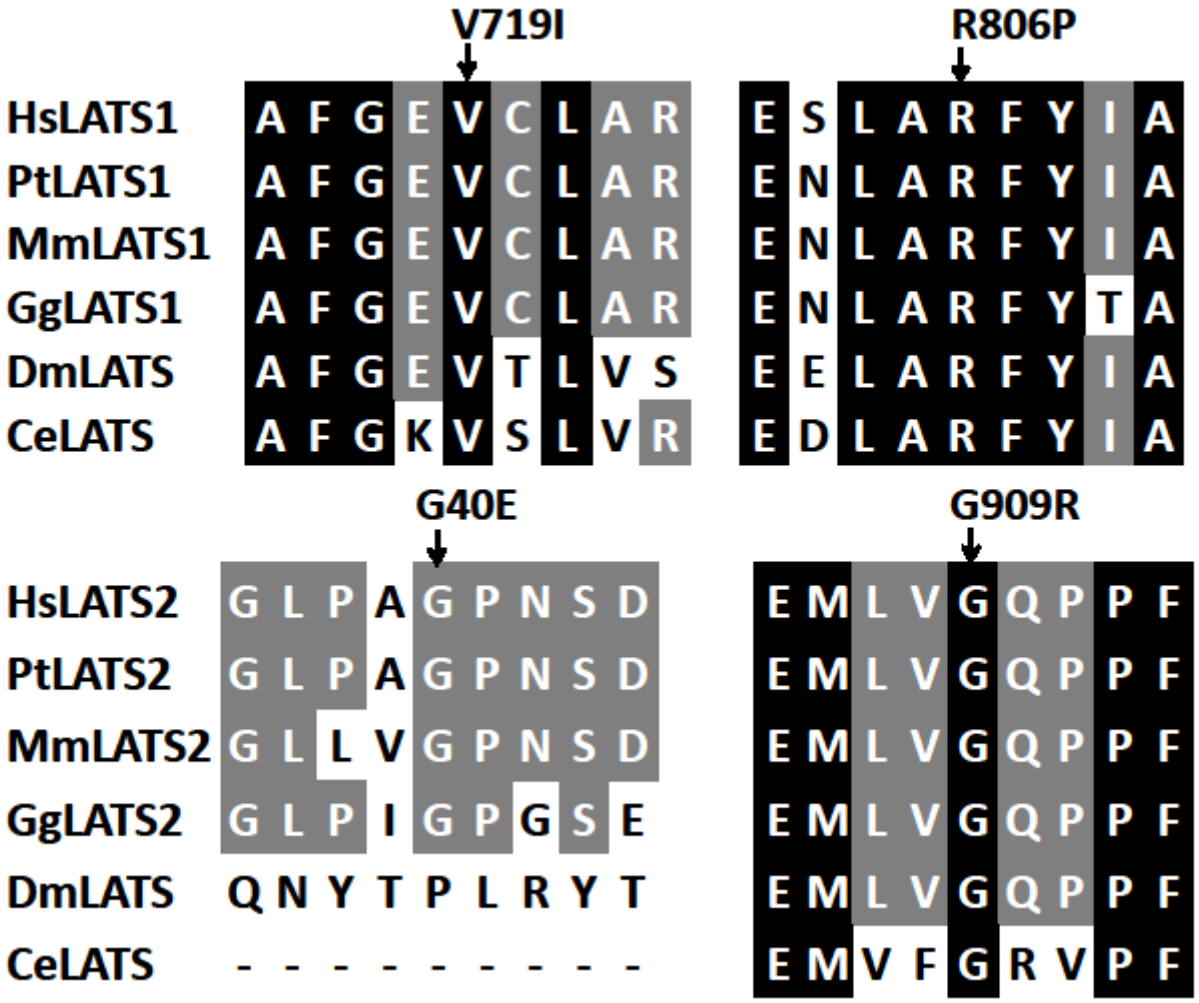

Figure S1 Evolutionary conservation of amino acids that are mutated in human LATS1 and LATS2. Human LATS1 (top) and LATS2 (bottom) protein sequences that cover several mutated amino acids are aligned and compared with homologous sequences from other vertebrates, Drosophila and nematode. For vertebrate Lats1, they include *H. sapiens* NP_004681.1, *P. troglodytes* XP_001173355.1, *M. musculus* NP_034820.1 and *G. gallus* XP_419666.2. Vertebrate Lats2 includes *H. sapiens* NP_055387.2, *P. troglodytes* XP_001149147.1, *M. musculus* NP_056586.2 and *G. gallus* XP_417143.3. For *D. melanogaster* Lats/Warts: NP_733403.1. For *C. elegans* Lats/Wts: NP_492699.1. Protein sequence alignment was performed by using MEGA 5.2.1 (TAMURA *et al*. 2011).

TAMURA, K., D. PETERSON, N. PETERSON, G. STECHER, M. NEI *et al.*, 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evo. **28**: 2731-2739.

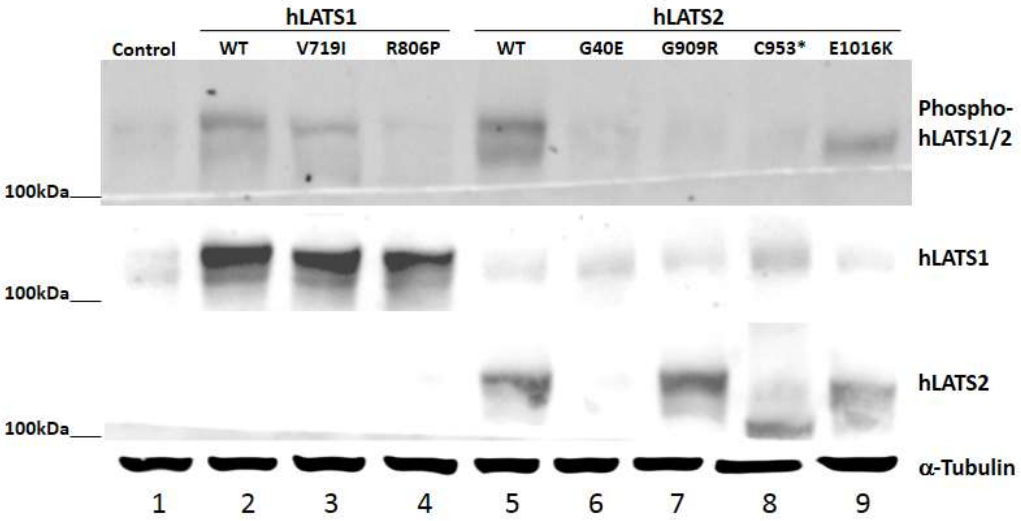

Figure S2 Western blot analysis of human LATS1 and LATS2 proteins in HEK293T cells. HEK293T cells were transfected with wild-type (lane 2 and lane 5) or mutant (lane 3-4 and lane 6-9) *hLATS1/2* DNA constructs as indicated. Anti-hLATS1 and anti-hLATS2 antibodies were used to specifically detect hLATS1 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).

Table S1 Detail information on non-synonymous mutations for human *LATS1* (58 samples) and *LATS2* (43 samples) genes collected in the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/) is presented. Mutations that have been experimentally analyzed in this study are highlighted with bold (hLATS1: V719I and R806P; hLATS2: G40E, G909R and C953*).

Table S1 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156372/-/DC1

Column A: Names of cancer samples.

Column B: Mutations of specific amino acids in hLATS1 or hLATS2 open reading frame. "*" represents a stop codon. "fs*" represents a frame shift followed by certain number of amino acids before a newly introduced stop codon.

Column C: specific nucleotides that have been substituted or deleted.

Column D: Primary tissues where cancers were identified.

Column E: Tissue subtypes.

Column F: Cancer types

Column G: Cancer subtypes

Column H: PubMed identification numbers for relevant references.

Column I: Somatic status - information on a mutation being somatically induced or not.

Column J: Sample source - a sample being derived from tumor tissue or cell line.

Column K: Zygosity - information on whether a mutation is homozygous or heterozygous within the sample.

Table S2 ***in silico*** **analysis of** ***hLATS1*** **and** ***hLATS2*** **mutations from the COSMIC database.** Mutations that have been experimentally analyzed in this study are highlighted with bold (hLATS1: V719I and R806P; hLATS2: G40E, G909R and C953*). While being predicted to be less significant changes, the other ones have been only computationally analyzed. We initially focused on these *hLATS* mutations as they were the only ones available at an early stage of this study.

Table S2 is available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156372/-/DC1

Column A: Gene names - human *LATS1* and *LATS2* genes.

Column B: Cancer sample names.

Column C: Mutations of specific amino acids (AA) in hLATS1 or hLATS2 open reading frames. "*" represents a stop codon.

Column D: LATS protein domains or regions where mutations are located.

Column E: information on site conservation in the *Drosophila warts* gene.

Column F: Counts of the methods shown in Columns G-J, which predicts a mutation to be disrupting or damaging to protein function, based on conservation and structure information.

Column G: The SIFT program (http://sift.jcvi.org/www/SIFT_enst_submit.html) [1].

Column H: The PolyPhen-2 program (http://genetics.bwh.harvard.edu/pph2/) [2].

Column I: The Mutation Assessor program (http://mutationassessor.org/?set=tcga-gbm-nov.2009) [3].

Column J: The SNAP program (https://rostlab.org/services/snap/) [4].

Columns K-M: Information in database on SNP's found in the same amino acid position.

Column N: Reference information.

References:

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