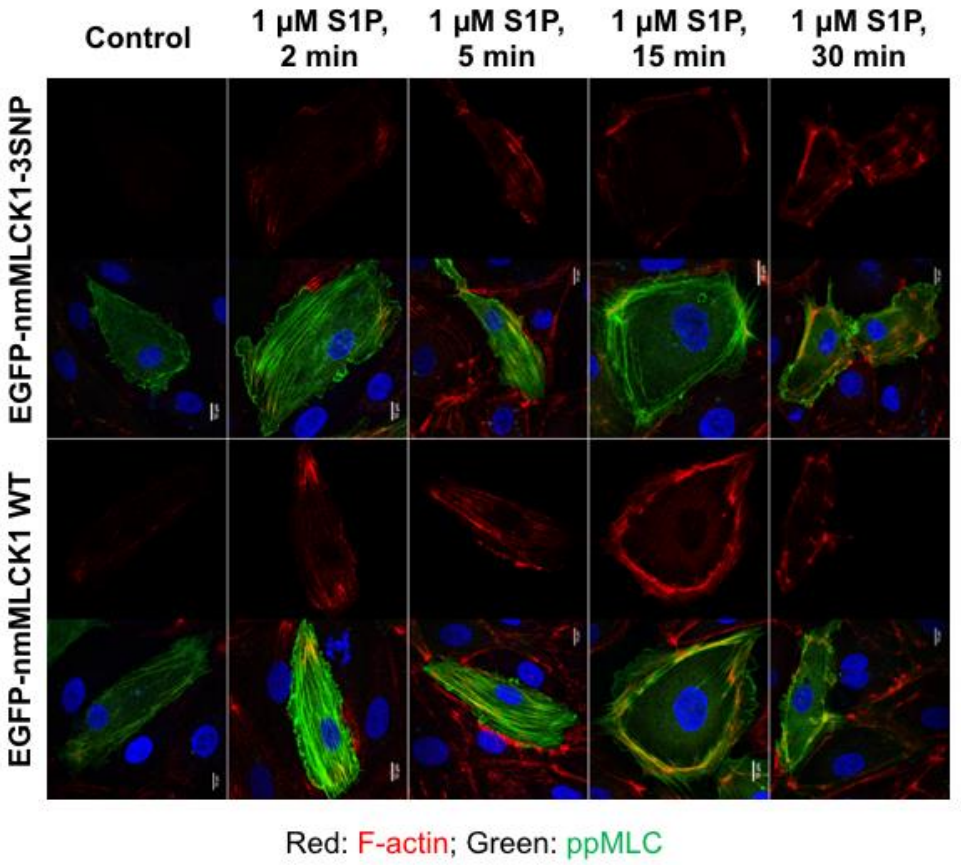
Supplementary Table S1. Protrusion kymography dynamics in response to S1P in ECs transfected with nmMLCK2.

nmMLCK2 Variants	WT-nmMLCK2	S147P-nmMLCK2	3SNP-nmMLCK2
Speed (nm/s)	50.7 ± 5.32	70.0 ± 5.93 *	43.0 ± 5.16 *#
Distance (nm)	3804 ± 437	2412 ± 242 *	2197 ± 224 *
Time to Max Distance (sec)	90.7 ± 11.7	37.1 ± 5.04 *	57.4 ± 10.4 *#

*, $p<0.05$ compared to WT-nmMLCK2 transfected cells. #, $p<0.05$ compared to S147P-nmMLCK2. n=20-30.

Supplementary Figure S1. MLC phosphorylation and cellular localization upon S1P challenge. Human pulmonary microvascular ECs were transfected with EGFP-nmMLCK1 constructs with wild type or 3SNP. Then ECs were challenged with S1P (1 μM) for 2-30 min. Immunofluorescence assays were performed to visualize MLC phosphorylation (green), and F-actin (red). These representative images were selected from more than 5 independent assays.



Supplementary Figure S2. Kymographs of EGFP-nmMLCK2 transfected cells. Representative kymographs of EGFP-labelled nmMLCK2 (WT or mutated) in cortical regions from S1P-treated cells.