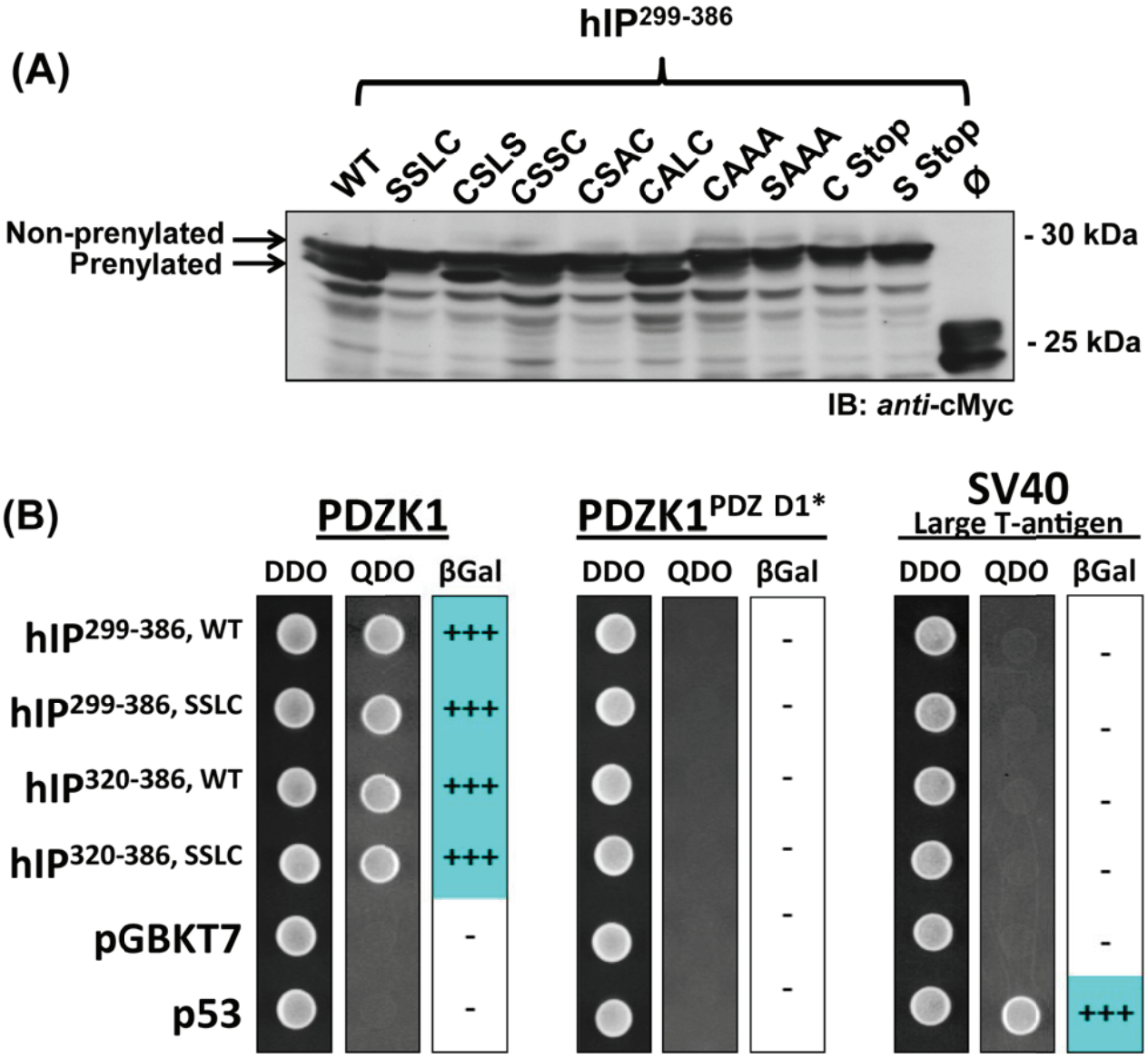
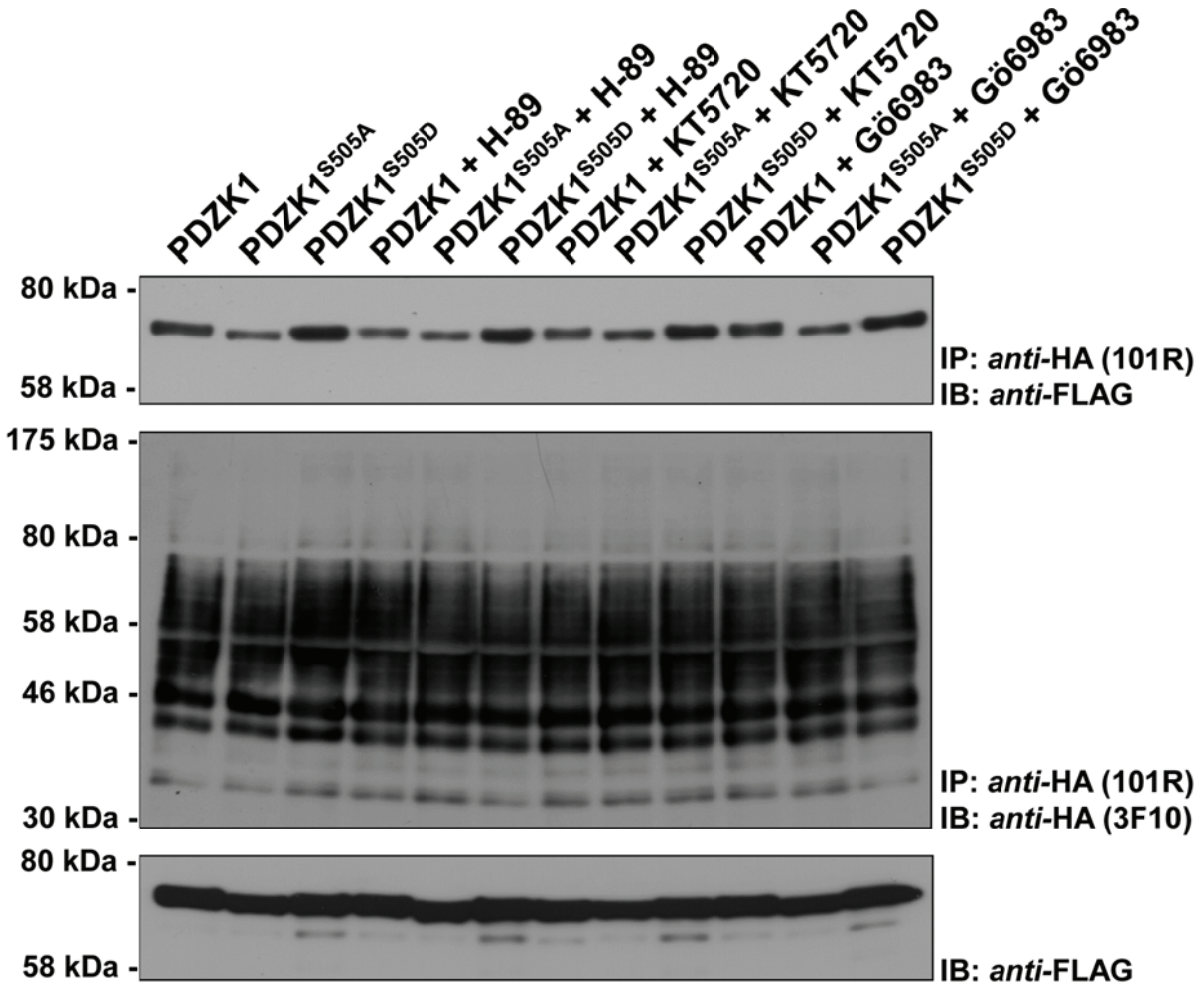
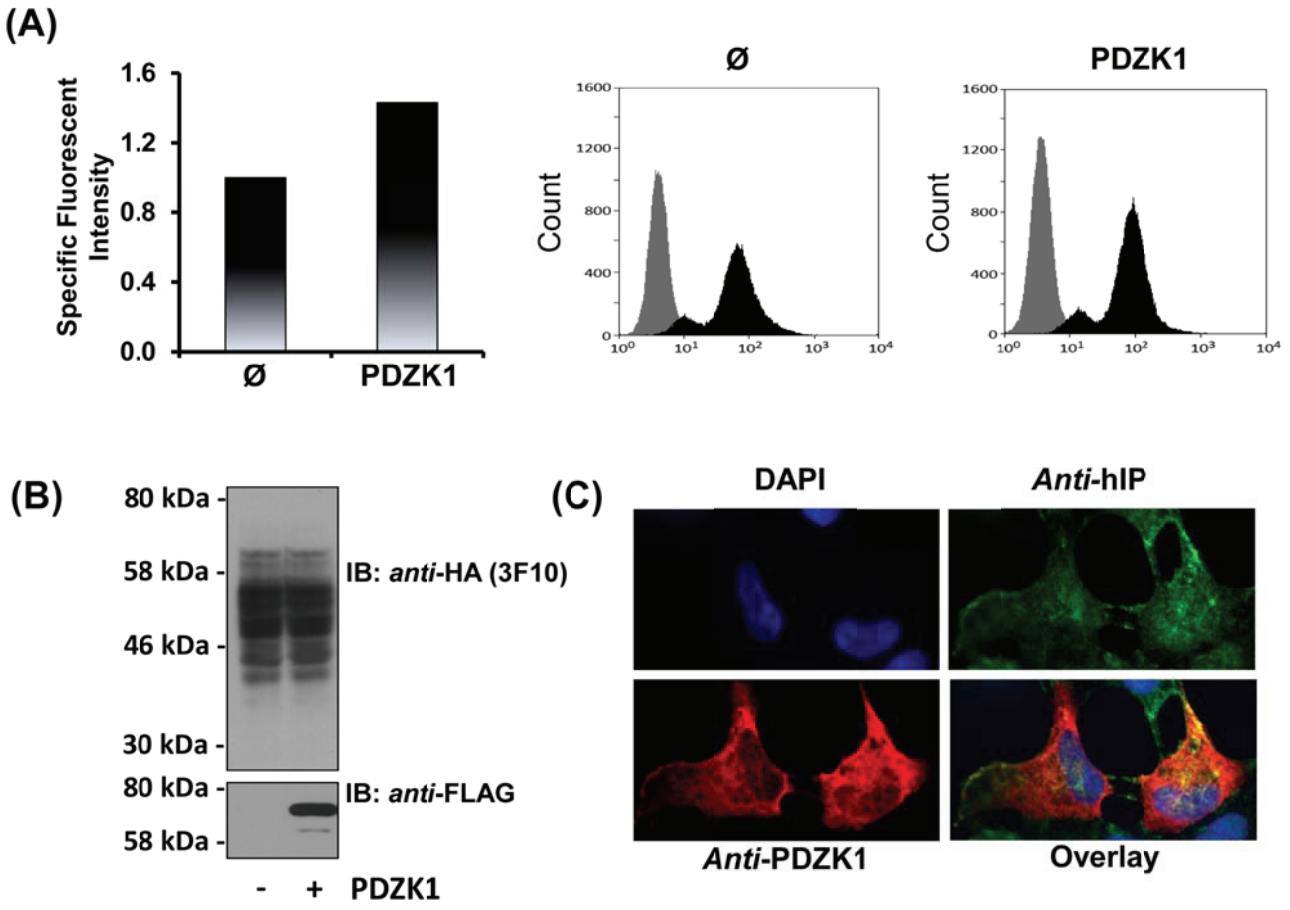


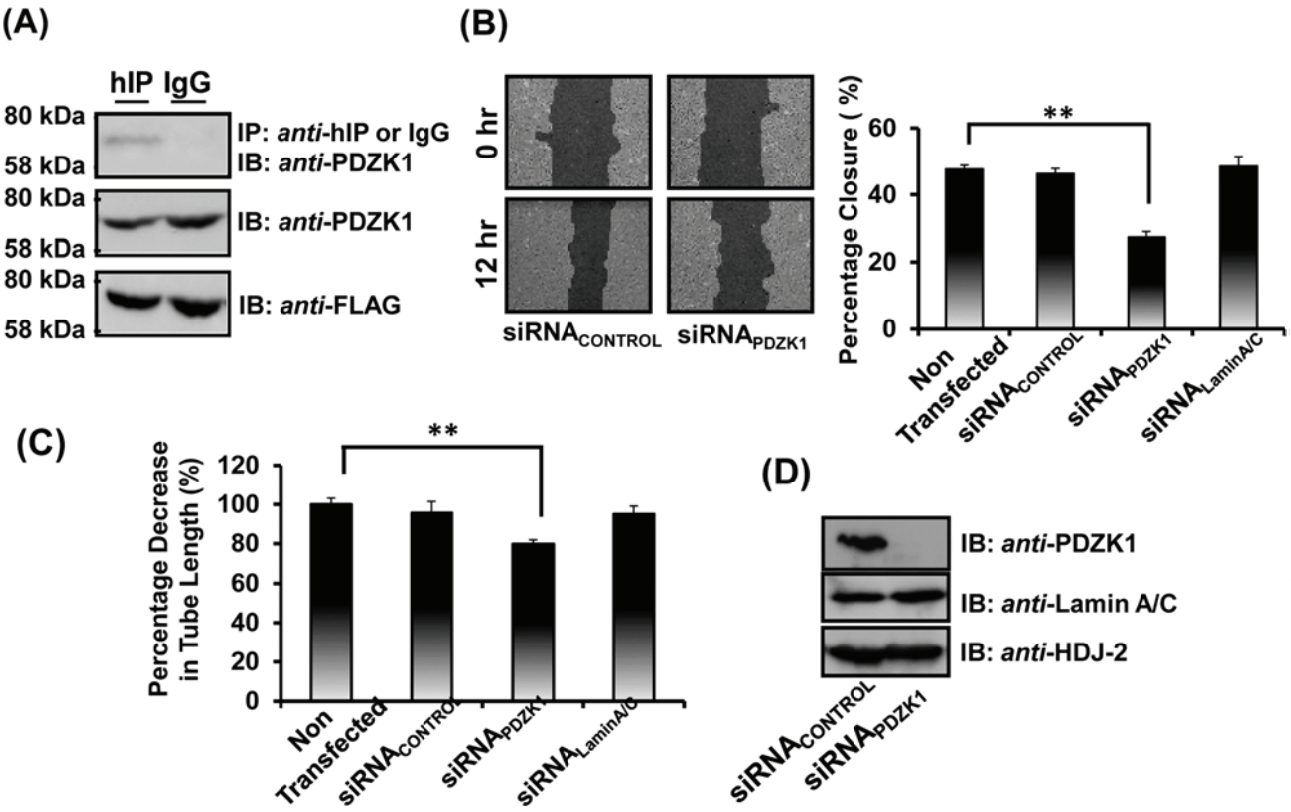
Supplemental Figure 1. Western Blots Analysis of hIP Bait Proteins Expressed in *S.c* AH109.
Panel A: Immunoblot analysis of protein extracted from the *S.c* AH109 bait strains transformed with recombinant pGBKT7 encoding the hIP$^{299\text{-}386}$ subfragment with its wild type (-C^{383}SLC^{386}) or the listed mutated variant sequences at its carboxyl terminus and, as a control, with the vector pGBKT7 (ø) alone. Proteins were resolved by SDS-PAGE and immunoblotted with *anti*-Myc (9B11) antibody. The arrows to the left of the panel indicate the non-isoprenylated and isoprenylated forms of the hIP. **Panel B:** *S.c* Y187 (pACT2:PDZK1), *S.c* Y187 (pACT2:PDZK1$^{PDZ\ D1*}$) and, as controls, *S.c* Y187 (pTD1-1) prey strains were mated with *S.c* AH109 bait strains transformed with recombinant pGBKT7 encoding the listed hIP subfragments and, as controls, p53 or with the vector pGBKT7 alone. Diploids were selected on DDO medium, whereas interactants were selected on QDO medium and by their ability to express β-Gal. Data: n ≥ 3.



Supplemental Figure 2. Effect of Kinase Inhibition on the Interaction of PDZK1 with the hIP.
HEK.hIP cells, transiently transfected with pCMVTag2C encoding PDZK1^{FL}, PDZK1^{S505A} or PDZK1^{S505D} were pre-incubated with vehicle (0.01 % DMSO), H-89 (10 μM; 10 min), KT5720 (5 μM; 10 min) or with GÖ6983 (1 μM; 10 min), as indicated. HA-tagged hIPs were immunoprecipitated with *anti*-HA 101R antibody; immunoprecipitates (IP) were resolved by SDS-PAGE and immunoblotted (IB), as indicated. Uniform expression of the Flag-tagged PDZK1 proteins was verified by immunoblot analysis of whole cell lysates (50 μg/lane) with *anti*-FLAG antibody (lower panels). Data: $n \geq 3$.



Supplemental Figure 3. Effect of PDZK1 on the expression of and co-localization with the hIP.
Panels A & B: Flow cytometric and immunoblot analysis of the effect of PDZK1 on hIP expression: HEK.hIP cells were transiently transfected with pCMVTag2C:PDZK1 or, as control, pCMVTag2C vector (Ø) alone. Cell surface HA-tagged hIP expression was examined by flow cytometry (**Panel A**), using *anti*-HA 101R antibody and AlexaFluor 488 goat *anti*-mouse IgG secondary antibody. Fluorescent intensity was corrected for background fluorescence using a control isotype IgG. Bar charts represent fold increases in fluorescent intensity upon PDZK1 over-expression where levels upon control transfection (Ø) are expressed as 1. Representative flow cytometry histograms show the specific cell surface expression of HA-tagged hIP (black) relative to background (grey), where the horizontal axis shows fluorescent intensity and the vertical axis represents cell count. Expression of the HA-tagged hIP and Flag-tagged PDZK1 proteins were verified by immunoblot analysis of the respective whole cell lysates (50 µg/lane) using *anti*-HA 3F10 and *anti*-FLAG (upper and middle panels, respectively; **Panel B**). Data: $n \geq 3$. **Panel C:** HEK.hIP cells were immune-labelled with *anti*-hIP and *anti*-PDZK1 antibodies under permeabilizing conditions followed by detection with *anti*-Rabbit AlexaFluor488 (*anti*-hIP) and *anti*-Mouse AlexaFluor594 (*anti*-PDZK1) conjugated secondary antibodies, respectively, or both (overlay) and then counterstained with DAPI. Data: $n \geq 3$.



Supplemental Figure 4. Effect of Disruption of PDZK1 Expression on Endothelial Cell Migration and Tube Formation under Basal Conditions.

Panel A: 1° HUVECs, transiently transfected with pCMVTag2C:PDZK1^{FL}, were subject to immunoprecipitation with *anti*-hIP antibody or, as a control, with the pre-immune IgG. Immunoprecipitates (IP) were resolved by SDS-PAGE and immunoblotted (IB), as indicated. Expression levels of PDZK1 were verified by immunoblotting of whole cell lysates with *anti*-PDZK1 (middle panel) and *anti*-FLAG (lower panel) antisera. Data: n ≥ 3. **Panel B & C:** 1° HUVECs were transfected with *si*RNA$_{PDZK1}$, *si*RNA$_{LaminA/C}$ or *si*RNA$_{CONTROL}$. Some 36 hr later, transfected or, as a control, non-transfected cells were either scratched and wounds analysed immediately (0 hr) or after 12 hr (**Panel B**), seeded on Matrigel™ and tube formation evaluated after 12 hr (**Panel C**) or immunoblot analysis (**Panel C)** assessed PDZK1 (Upper panel) and Lamin A/C (Middle Panel) abundance 36 hr post-transfection. Analysis of HDJ-2 expression (Lower panel) was used as a loading control. Bar charts represent mean percentage closure at 12 hr (% ± SEM; n = 3; **Panel B**) or percentage decrease in basal tube length at 12 hr (% ± SEM; n = 3; **Panel C**). The asterisks indicate significant *si*RNA$_{PDZK1}$ -mediated decreases in migration (**Panel B**) or tube formation (**Panel C**), where ** indicates $p < 0.01$ for post-hoc Dunnett's multiple comparison t-test analysis.

SUPPLEMENTAL TABLE 1. PRIMERS USED FOR SITE-DIRECTED MUTAGENESIS OF THE 'GLGF MOTIFS' WITHIN PDZ DOMAIN (PDZD) 1 TO PDZD4 OF PDZK1 & SERINE 505 MUTANTS.

WILD TYPE 'GLGF MOTIF'	MUTATED 'GLGF MOTIF'	RECOMBINANT PLASMID GENERATED	OLIGONUCLEOTIDE PRIMER*
PDZD1: $N^{19}YGF^{22}$	$\underline{K}^{19}Y\underline{RS}^{22}$	pACT2: $PDZK1^{PDZD1*}$/ pCMVTag2C:$PDZK1^{PDZD1*}$	5'GGCAAGCAAGAAGGGCAAAAGTAT***GGCTT***CTTCCTGCGAATTGAGAAGG -3'
PDZD2: $S^{144}YGF^{147}$	$S^{144}Y\underline{RE}^{14}$	pCMVTag2C:$PDZK1^{PDZD2*}$	5'CTGGAAGGAAGGAGGCAGCTAT***C***GC***GAG***TCTCTGAAAACTGTCCAA -3'
PDZD3: $G^{252}YGF^{255}$	$G^{252}Y\underline{RE}^{255}$	pCMVTag2C:$PDZK1^{PDZD3*}$	5'GAAGAAAGGAAGCAATGGCTAT***C***GT***GAG***TATCTGAGGGCAGGCTCAGAA -3'
PDZD4: $G^{387}YGF^{390}$	$G^{387}Y\underline{RE}^{390}$	pCMVTag2C:$PDZK1^{PDZD4*}$	5'GGCTAAAGGTGAAAATGGCTAT***C***GC***GAG***CACTTAAATGCGATTCGGGGTC-3'

RECOMBINANT PLASMID GENERATED	OLIGONUCLEOTIDE PRIMER*
pCMVTag2C:$PDZK1^{S505A}$	5'-GCAAAAGAACGGGCCCAC***GC***TACAGCCTCACATTCTTC-3'
pCMVTag2C:$PDZK1^{S505D}$	5'-GCAAAAGAACGGGCCCAC***GA***TACAGCCTCACATTCTTC-3'

* Sequences given correspond to those of the sense primer only where the identity of the mutator codon(s) is in boldface italics and antisense primer sequences are inferred.