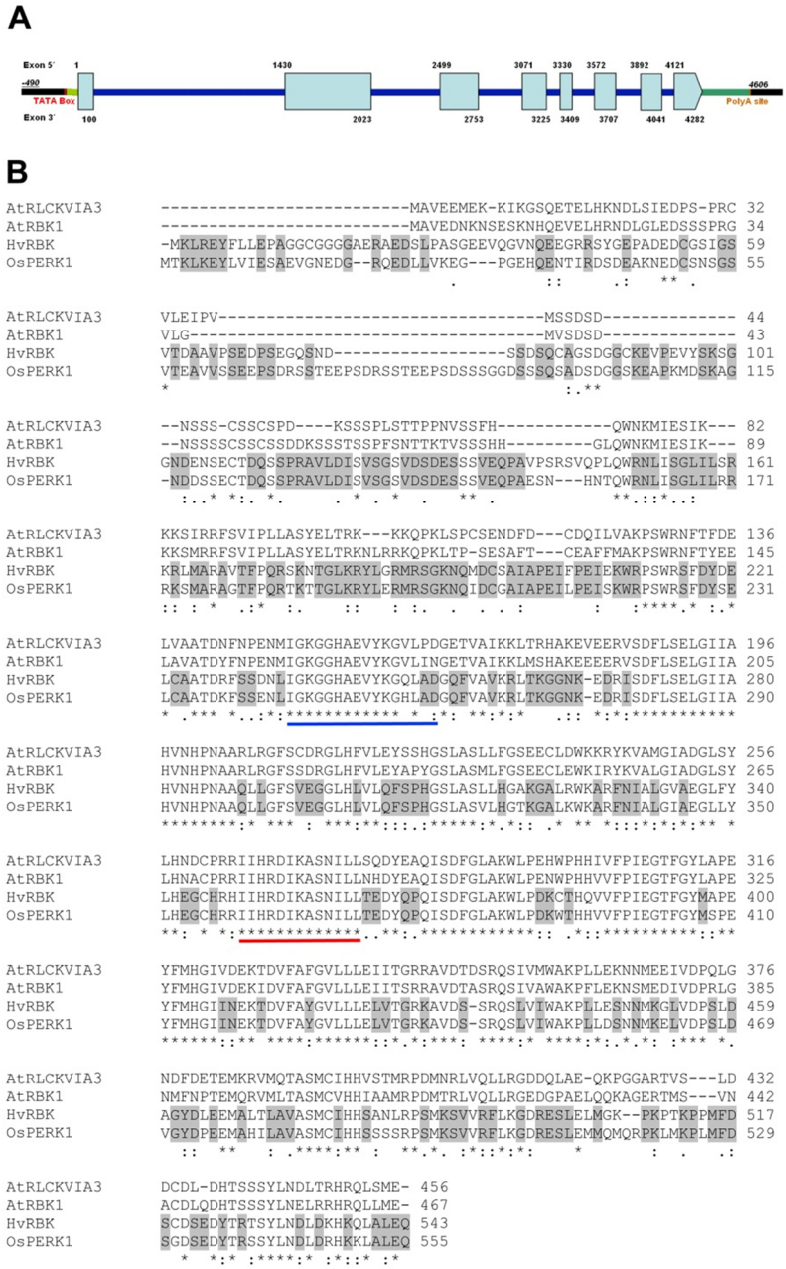

Figure S1. Genomic structure of the HvRBK1-coding gene and the comparison of the deduced amino acid sequence of HvRBK1 to related proteins. A, Structure of the genomic sequence (> contig_6719 length=7372 rbca=7HL at http://webblast.ipk-gatersleben.de/barley/index.php) of HvRBK1. TATA Box and poly-A sites are indicated in red. Transcribed untranslated regions are indicated in green. Light blue boxes indicate exons. Blue lines indicate introns. ATG starts at indicated nucleotide position 1 and stop codon TGA ends at indicated position 4282. 5`cis region is limited by -490 base pairs. 3´ region beyond the poly-A signal is neglected. B, Alignment of full-length HvRBK1, AtRLCK

VIA3, AtRBK1 (AtRLCK VIA4) and rice OsPERK1 protein sequences (accession numbers are given before the references). Sequence alignment revealed high similarity of kinases at the C-terminus (kinase domain) and limited similarity at the N-terminus. The latter one is, however, serine-rich in all four proteins and in particular in OsPERK1, AtRLCK VIA3, and AtRBK1. Grey shaded letters show amino acids identical in OsPERK1 and HvRBK1 only. Asterisks indicate amino acids identical in all four proteins. Blue underlined letters mark the highly conserved protein kinase ATP-binding region signature and red underlined letters mark the serine/threonine protein kinases active-site signature. Alignment was made using the ClustalW program (Thompson et al., 1994).

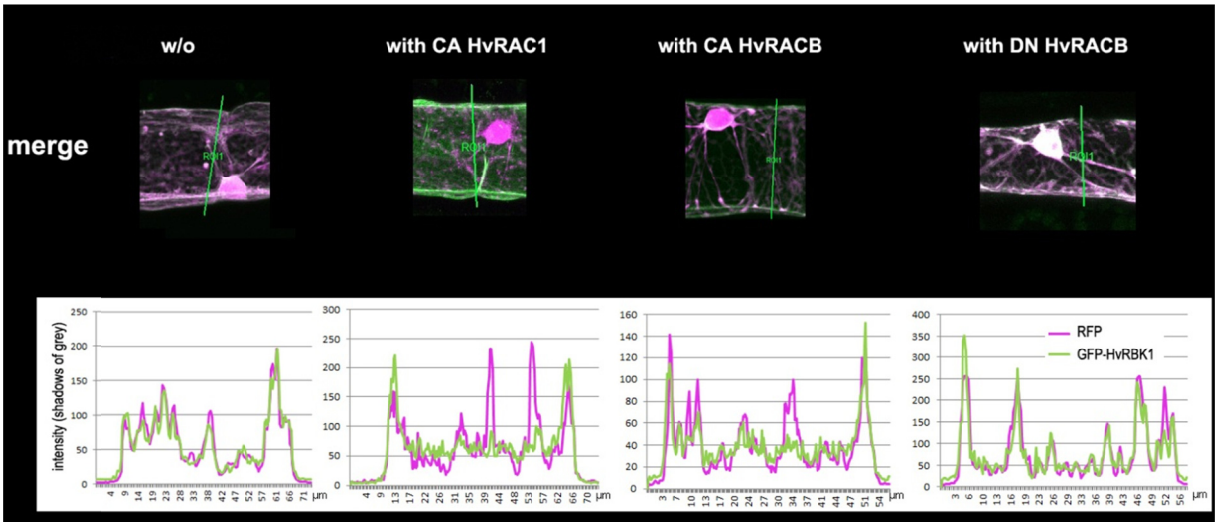

Figure S2. Quantification of GFP-HvRBK1 signal intensity in cells co-expressing different versions of HvROPs. Merged channel pictures represent magnifications of the same cells as in Figure 4. Pixel intensity was measured along the green line in a maximum projection of 25 optical sections at 2 µm increments. GFP-HvRBK1 signal is high in the cell periphery but weak in cytoplasmic strands when CA HvRACB or CA HvRAC1 are coexpressed. By contrast GFP-HvRBK1 signal is higher in the cytoplasm and weaker in the cell periphery without co-expression of a CA HvROP or with co-expression of DN HvRACB. RFP signal is always high in the cytoplasm.

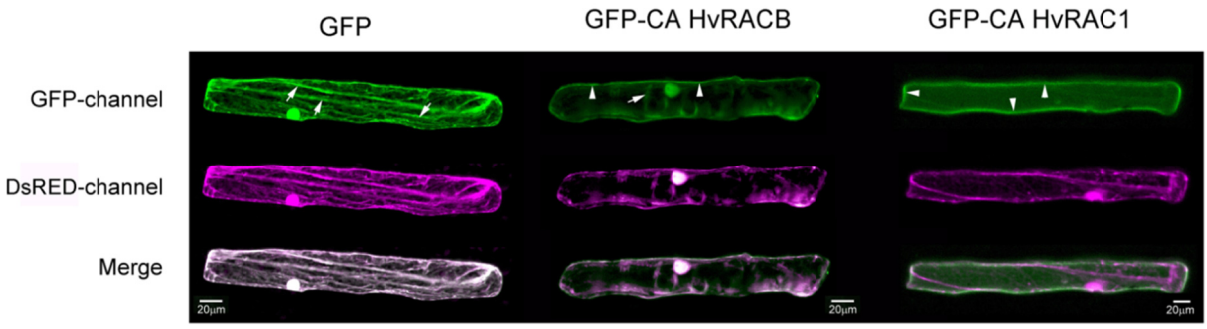

Figure S3. Subcellular localization of GFP-CA HvRACB and GFP-CA HvRAC1. To demonstrate subcellular localization of CA HvRACB and CA HvRAC1, both proteins were fused to GFP and co-expressed with DsRED as a marker for cytoplasmic and nuclear localization. Pictures represent whole cell maximum projection of 25 optical sections at 2 µm increments. As indicated in GFP and merged channels, GFP-CA RACB has a more peripheral localization (arrow heads) as compared to free GFP (left) that is detectable in cytoplasmic strands (arrows) and nucleoplasm. By contrast, GFP-CA HvRAC1 is more exclusively associated with the cell periphery (arrow heads), when compared to GFP-CA HvRACB.

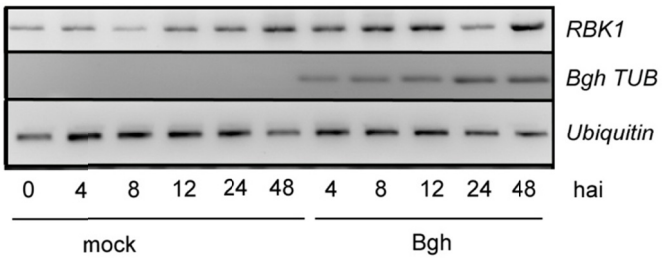

Figure S4. *HvRBK1* expression pattern in the interaction of barley with *B. graminis* f.sp. *hordei*. RNA was isolated from *B. graminis* f.sp. *hordei* or mock inoculated barley leaves harvested at 4, 8, 12, 24, 48 hpi, and reverse transcribed to cDNA, which was used as template in PCR reactions. Constitutively expressed *HvUBIQUITIN* served as quality control of the cDNA synthesis. Fungal *Bgh TUBULIN* (*TUB*) served as a positive control for successful inoculation.

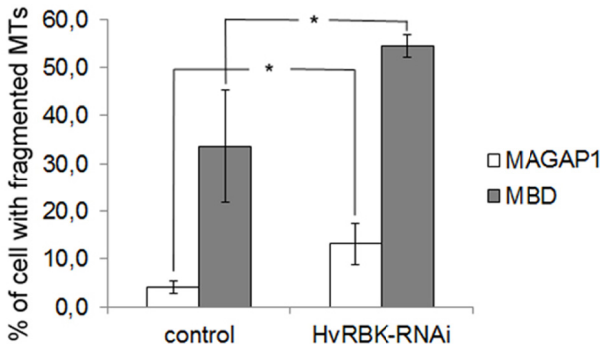

Figure S5. Influences of TIGS of HvRBK1 on microtubule organization in epidermal cells of barley. Epidermal cells were transiently transformed with the MT-markers RFP-HvMAGAP1 (full length) (MAGAP1) or DsRED-MBD (MBD) and the HvRBK1 RNAi construct or the empty RNAi vector control. Columns represent means ± standard deviations of at least five independent experiments (50 cells for each plasmid combination were investigated per experiment), with significantly more fragmented MTs after TIGS of HvRBK1 when compared to controls (two-sided student´s t-test $p<0.05$ for both MT markers*). Noteworthy, when experiments were performed with the MT marker DsRED-MBD (MAP4), we observed a high basal level of fragmented MT in control cells.