Supplemental materials for

The crystal structure of the plant small GTPase OsRac1 reveals its mode of binding to NADPH oxidase

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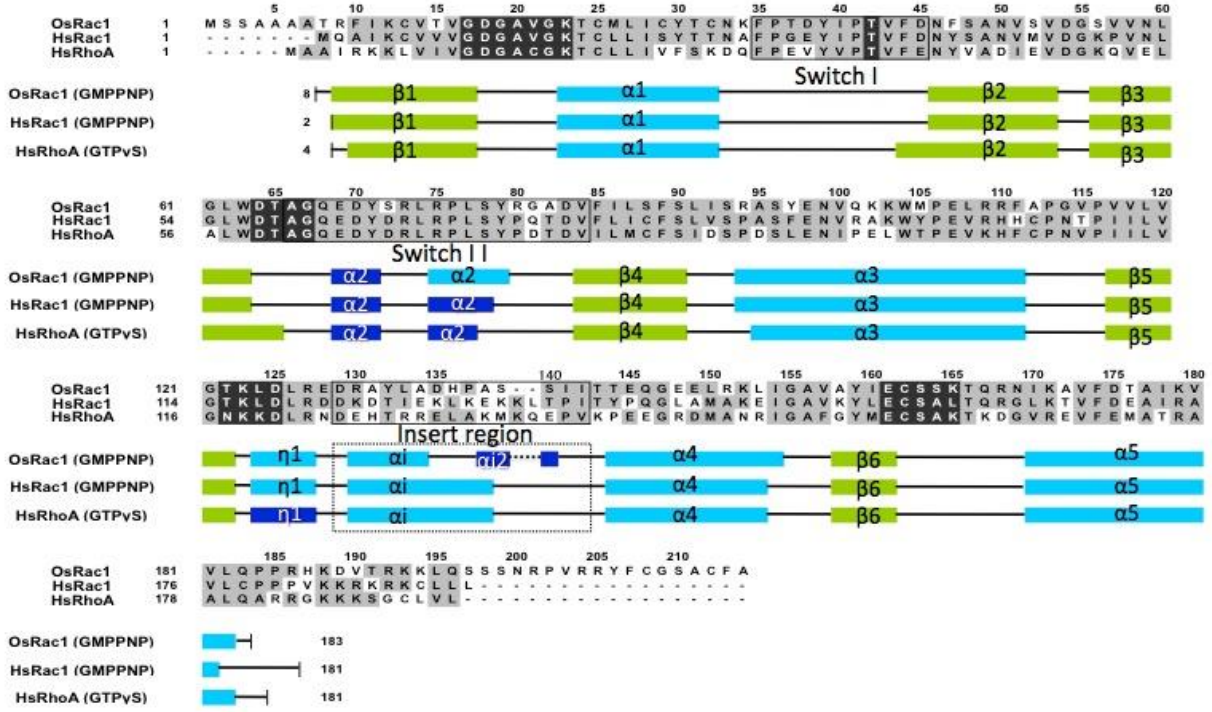

FIGURE S1. Comparison of primary sequences of OsRac1, HsRac1 and HsRhoA by multiple sequence alignment. Secondary structural elements are calculated from structures in Protein Data Bank (HsRac1: 1MH1; HsRhoA: 1A2B) and indicated under the amino acid sequences (β-strand: green; α-helix: cyan; 3_{10}-helix: blue). The sequences of the Switch I, Switch II and Insert regions are boxed by dashed black lines, and secondary structure of the Insert regions are boxed by black dashed-line. Conserved motifs which are important for GTP hydrolysis are highlighted by white letters on a black background. The dotted line indicates disordered region.

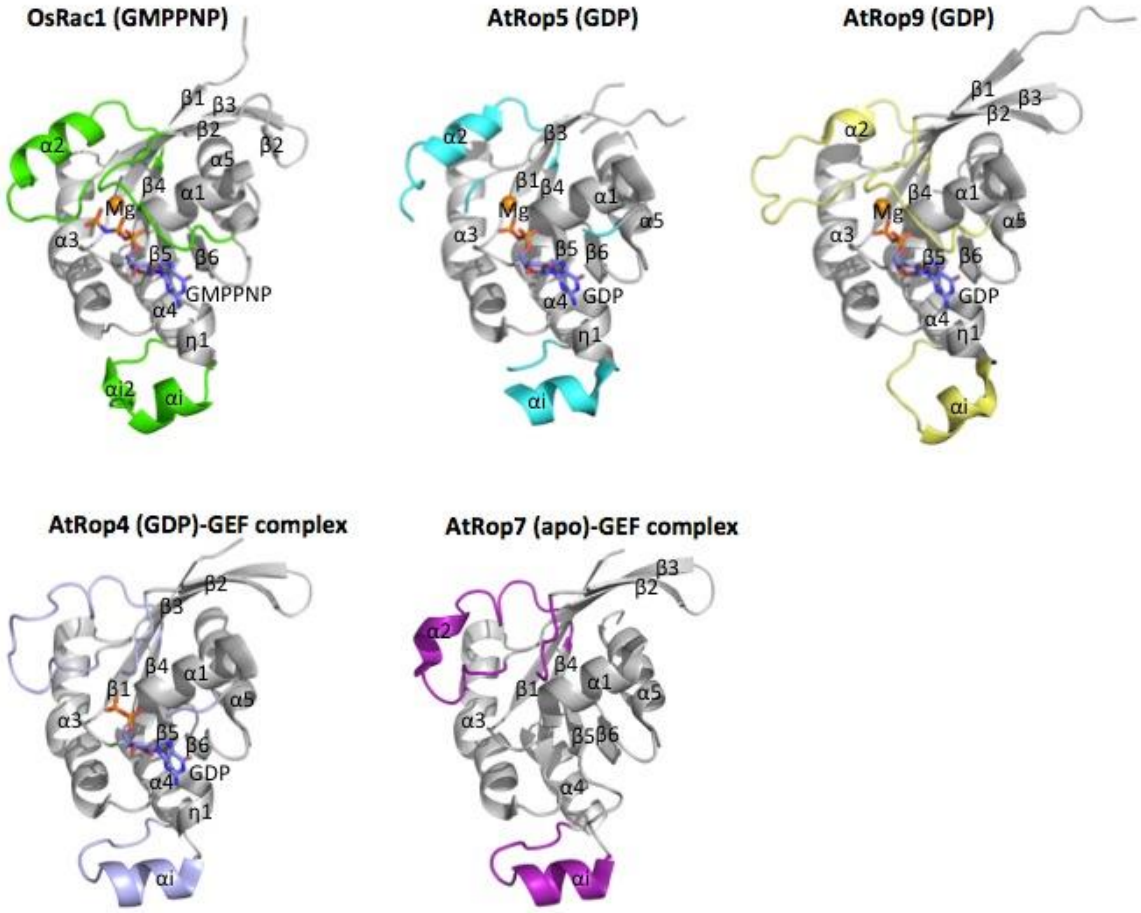

FIGURE S2. Comparison of the structures of OsRac1(GMPPNP), AtRop5(GDP) (PDB code: 3BWD), AtRop9(GDP) (PDB code: 2J0V, chain B), the AtRop4(GDP)-GEF(PRONE) complex (PDB code: 2NTY, chain B) and the AtRop7(apo)-GEF(PRONE) complex (PDB code: 2WBL, chain C). Switch I, Switch II and the Insert region are in non-gray colors. GMPPNP and GDP are shown as stick models (red, deep blue, and orange indicate O, N and P atoms, respectively). The Mg^{2+} ion is shown as an orange sphere. Models were generated using PyMOL.

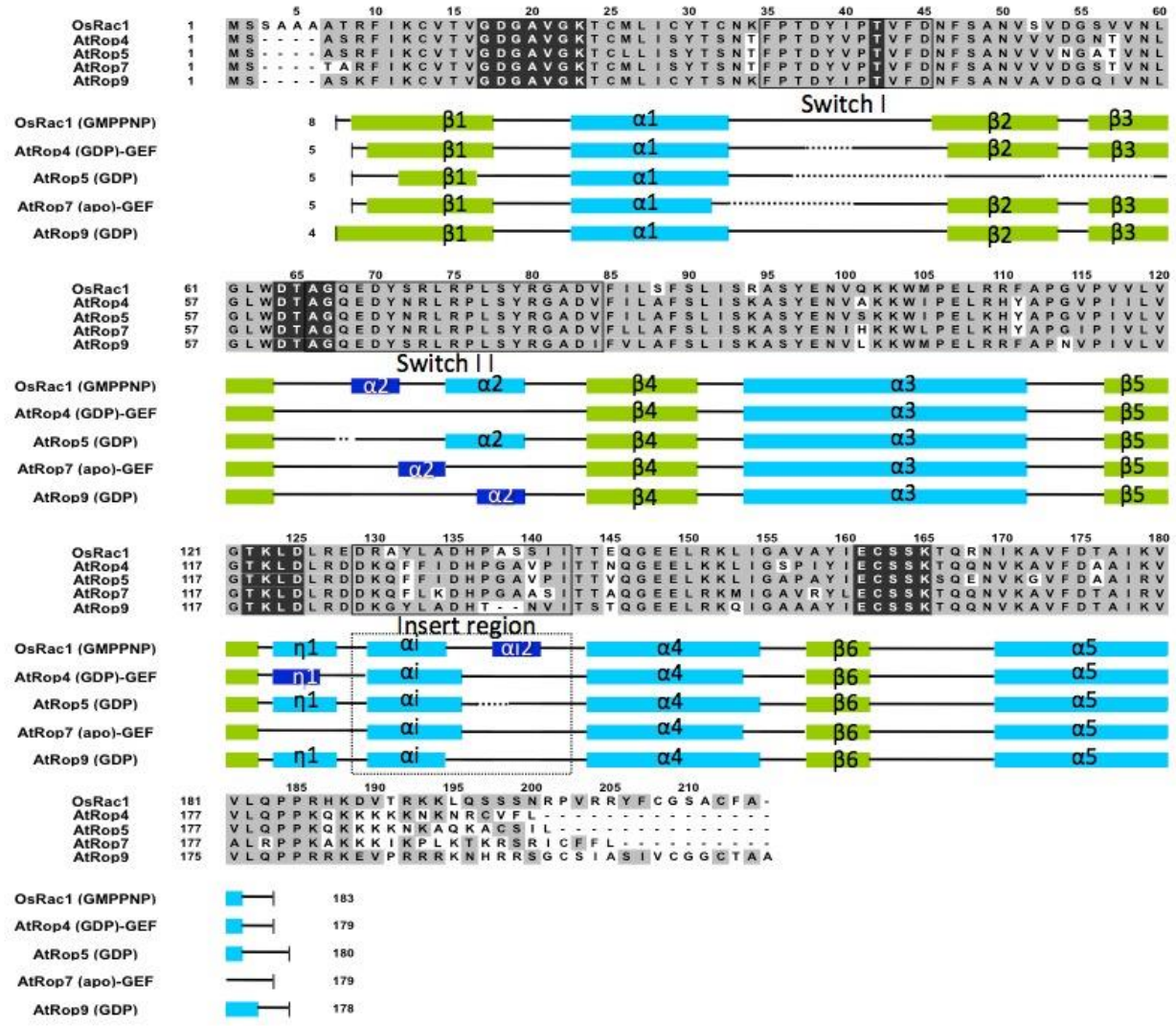

FIGURE S3. Comparison of primary sequences of plant Rac/Rops by multiple sequence alignment. Secondary structural elements calculated from structures of OsRac1, AtRop4(PDB code: 2NTY), AtRop5 (PDB code: 3BWD), AtRop7(PDB code: 2WBL) and AtRop9(PDB code: 2J0V) are drawn under the amino acid sequences (β-strand: green; α-helix: cyan; 3_{10}-helix: blue). The sequence of the Switch I, Switch II and Insert regions are boxed by thin black lines, and secondary structures of the Insert regions are indicated by dashed black lines. Conserved motifs which are important for GTP hydrolysis are highlighted by white letters on a black background. The dotted lines indicate disordered regions.

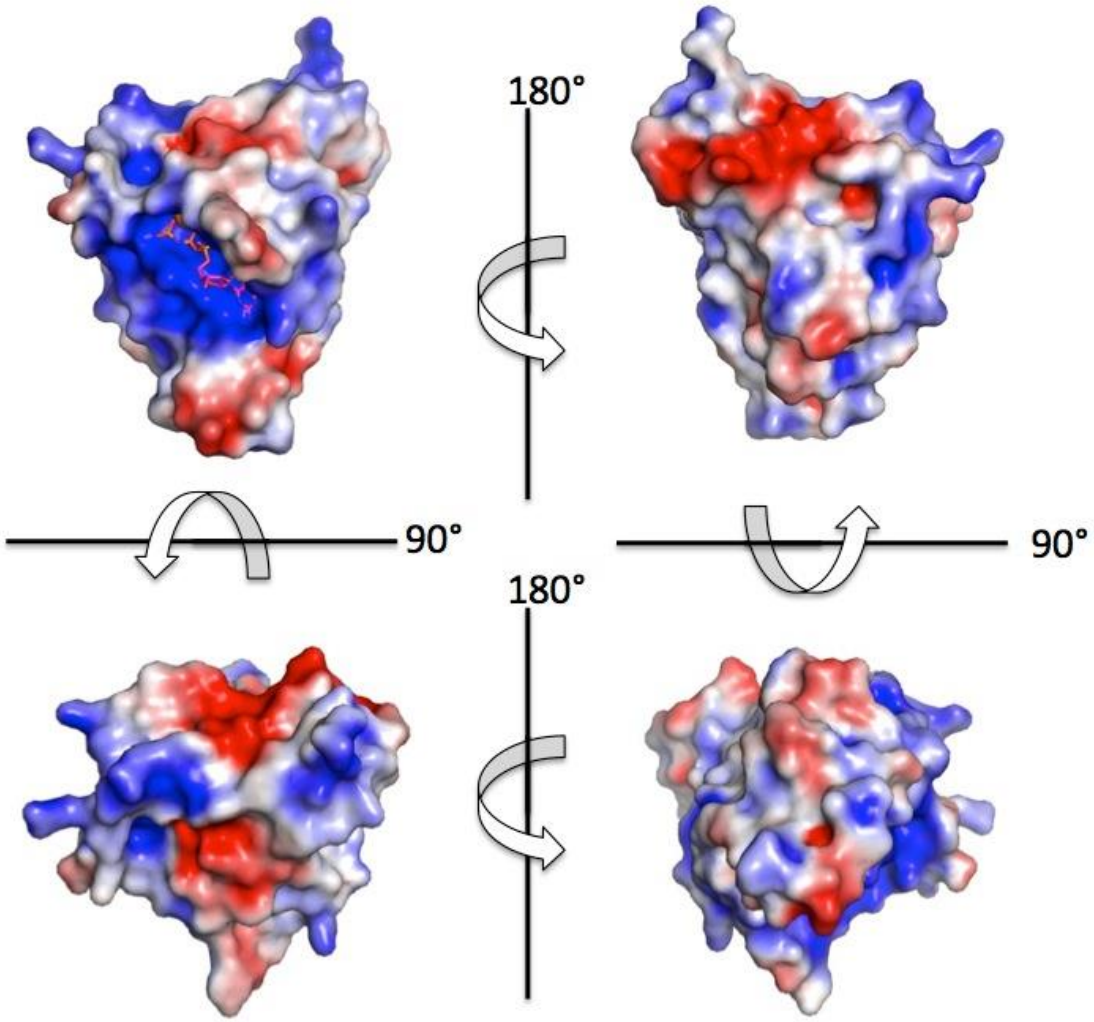

FIGURE S4. Mapping of electrostatic potential of OsRac1-GMPPNP. Positive and negative charges on the molecular surface are colored blue and red, respectively. GMPPNP is shown in the stick model and the Mg^{2+} ion is shown as an orange sphere. The surface potential was calculated using APBS tools.

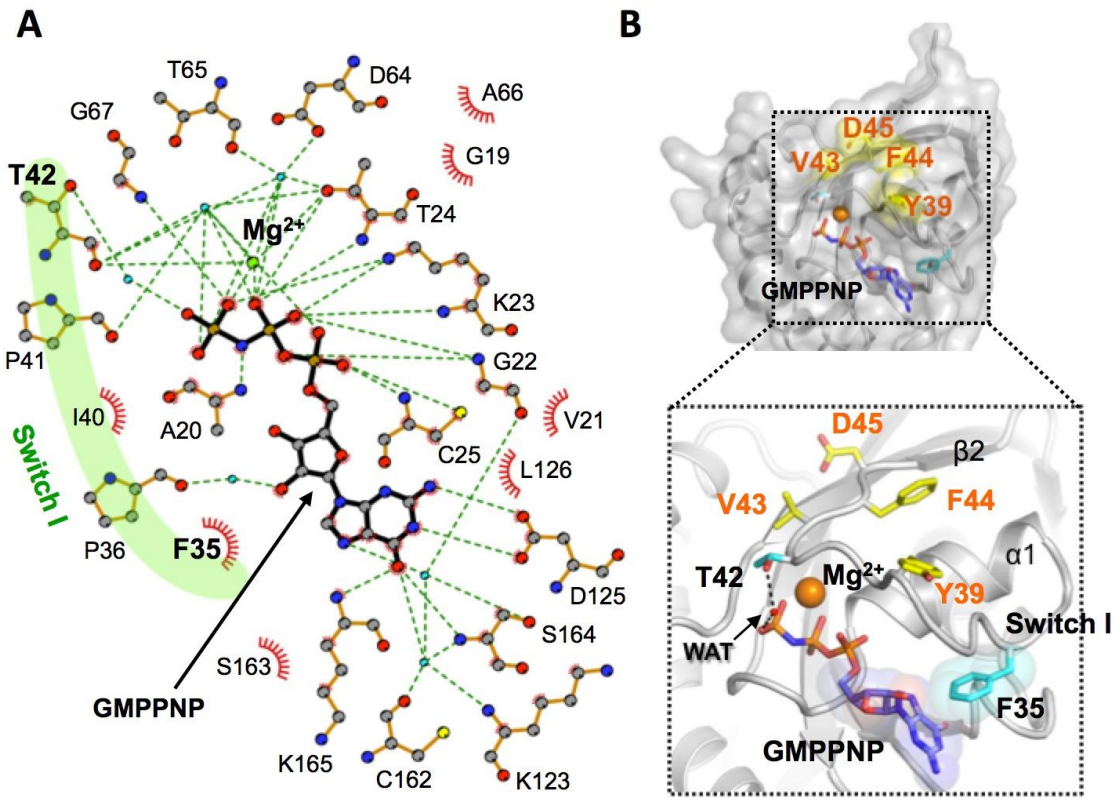

FIGURE S5. Interaction between OsRac1 and GMPPNP. (A) 2D scheme of interaction between OsRac1 and GMPPNP. OsRac1 residues, which contact with GMPPNP or the Mg^{2+} ion, and the GMPPNP molecule are shown in the ball-and-stick model (red, oxygen; blue, nitrogen; orange, phosphorus; gray, carbon; yellow, sulfur). The Mg^{2+} ion and water molecules (WAT) are shown in sphere colored green and cyan, respectively. Hydrogen bonds are indicated by dashed green lines and the OsRac1 residues which form hydrophobic interactions with GMPPNP are represented by arcs with spokes. The figure was generated by LIGPLOT software (Wallace *et al.*, Protein Eng., 1995). (B) Critical residues in Switch I for $OsRbohB^{138-313}$ binding are shown as a stick representation colored with yellow (Tyr39, Val43, Phe44 and Asp45). Other Switch I residues which form direct interactions with GMPPNP are colored cyan (Thr42 (hydrogen bond) and Phe35 (hydrophobic contact)). The Mg^{2+} ion and the water molecule (WAT) are shown as an orange sphere and small red sphere, respectively. The hydrogen bond is indicated by dashed black line.

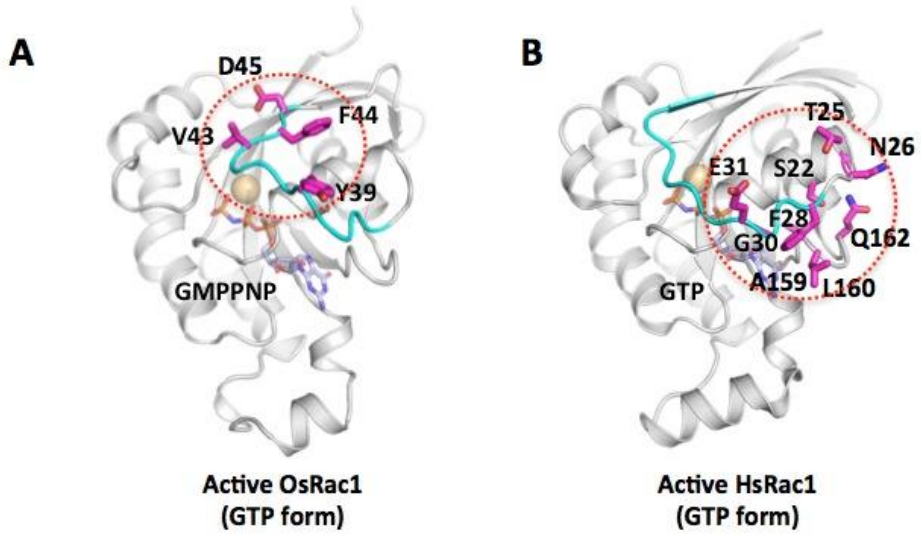

FIGURE S6. Differences in the NADPH oxidase-binding sites in plant and animal. OsRac1(GMPPNP) (A) and HsRac1(GTP) (B) are shown in ribbon representation. Side chains of amino acid residues that are important for the interaction with the NADPH oxidases (OsRbohB$^{138\text{-}313}$ or p67^{phox}) are shown in the stick model (magenta) and demarcated by the orange dashed circles. Switch I regions are colored in cyan. Although the Switch I regions are critical for the activation of NADPH oxidase in both plant and animal, molecular details of the interacting regions are slightly different.