## SUPPLEMENTAL DATA

## Supplemental Table I

Data collection and refinement statistics.

| Crystal | M-RasD41E-GppNHp type 1 | M-RasD41E-GppNHp type 2 |
|---|---|---|
| **Data collection** | | |
| X-ray source | SPring-8 (BL38B1) | SPring-8 (BL38B1) |
| Space group | *P*622 | $P2_12_12_1$ |
| Unit-cell parameters (Å) | a = b = 109.42, c = 68.21 | a = 47.11, b = 89.65, c = 41.27 |
| wavelength (Å) | 1.0 | 1.0 |
| Resolution range (Å) | 32.1 - 2.75, (2.85 - 2.75)* | 18.6 - 1.55 (1.63 - 1.55)* |
| No. of unique reflections | 6,653 (634)* | 25,878 (3,685)* |
| Multiplicity | 20.6 (21.4)* | 12.9 (12.3)* |
| I/σ (I) | 66.5 (19.3)* | 7.4 (2.7)* |
| Completeness (%) | 99.7 (100.0)* | 99.2 (98.2)* |
| $R_{merge}$ (%)** | 8.5 (29.3)* | 6.3 (28.3)* |
| **Refinement** | | |
| Resolution range (Å) | 32.1 - 2.75 (2.82 - 2.75)* | 16.0 - 1.55 (1.59 - 1.55)* |
| No. of reflections | 6,335 (454)* | 24,516 (1,780)* |
| *R* factor (%) | 21.4 (24.6)* | 18.6 (22.4)* |
| Free *R* factor [5% set] (%) | 28.7 (35.3)* | 21.3 (23.3)* |
| No. of residues | 168 | 168 |
| No. of nohydrogen atoms | | |
| protein | 1,370 | 1,436 |
| GppNHp | 32 | 32 |
| magnesium | 1 | 1 |
| water | 19 | 159 |
| Average B factor (Å$^2$) | | |
| main chain | 26.4 | 12.4 |
| side chain | 28.2 | 14.9 |
| GppNHp | 12.9 | 7.2 |
| magnesium | 12.7 | 7.2 |
| water | 13.6 | 23.1 |
| Rms deviation from ideality | | |
| bonds (Å) | 0.014 | 0.010 |
| angles (°) | 1.501 | 1.282 |

* Numbers in parentheses refer to data in the highest resolution shell.

** $R_{merge} = \Sigma_{hkl}\Sigma_i|I_i(hkl) - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_i I_i(hkl)$

**Supplemental Table II**

$^{31}$P chemical shifts and conformational states of the GppNHp-bound forms of H-Ras, M-Ras, and their mutants.

| | α-Phosphate | | β-Phosphate | γ-Phosphate | | state 2 |
|---|---|---|---|---|---|---|
| | $\delta_{1,2}$ | | $\delta_{1,2}$ | $\delta_1$ | $\delta_2$ | |
| | ppm | | ppm | ppm | | (%) |
| H-RasWT-GppNHp[a] | -11.2 | -11.7 | -0.25 | -2.6 | -3.3 | 64 ± 2 |
| M-RasWT-GppNHp[b] | -10.4 | | -0.1 | -2.8 | -3.7 | 7 ± 2 |
| M-RasP40D-GppNHp[c] | -11.4 | | -0.4 | -2.9 | -3.9 | 13 ± 3 |
| M-RasD41E-GppNHp[c] | -10.6 | | -0.3 | -2.8 | -3.8 | 21 ± 2 |
| M-RasP40D/D41/L51R/F74Y/E79D-GppNHp[c] | -11.33 | | -0.33 | -2.8 | -3.68 | 23 ± 3 |
| M-RasP40D/D41/L51R/F74Y/E79D/R105Q-GppNHp | -11.3 | | -0.3 | -2.8 | -3.8 | 20 ± 3 |
| M-RasP40D/D41E/L51R/F74Y/E79D/F106Y-GppNHp | -11.3 | | -0.4 | -3.0 | -3.8 | 16 ± 1 |
| M-RasP40D/D41E/L51R/F74Y/E79D/L109Q-GppNHp | -11.6 | | -0.4 | -2.8 | -3.7 | 47 ± 2 |
| M-RasP40D/D41E/L51R/F74Y/E79D/R105Q/F106Y-GppNHp | -11.0 | | -0.4 | -2.9 | -3.9 | 10 ± 1 |
| M-RasP40D/D41E/L51R/F74Y/E79D/F106Y/L109Q-GppNHp | -11.4 | | -0.4 | -3.0 | -4.0 | 30 ± 2 |
| M-RasP40D/D41E/L51R/F74Y/E79D/R105Q/L109Q-GppNHp | -11.7 | | -0.3 | -2.8 | -3.7 | 46 ± 1 |
| M-RasP40D/D41E/L51R/F74Y/E79D/L109Q-GppNHp + c-Raf-1 | -11.7 | | -0.3 | -2.8 | -3.6 | 95 ± 7 |

[a]Data from Ref. 22. [b]Data from Ref. 18. [c]Data from Ref. 21.

## Legends to Supplemental Figures

**Supplemental Fig. 1. Structure-based amino acid sequence alignment of H-Ras, M-Ras, and RalA.** The amino acid sequences of human H-Ras, mouse M-Ras, and human RalA are shown. Residue numbers are shown above the corresponding residues of H-Ras. M-Ras and RalA have 10- and 11-residue extensions at their N termini, respectively. The secondary structures of H-Ras-GppNHp (1CTQ) calculated by the program PROCHECK in the CCP program suit (29) are represented by tubes (α-helices) and arrows (β-strands). Switch I, switch II, and the α3-helix are highlighted by *yellow, green,* and *orange boxes*, respectively. The pre-switch I residues are shown in *blue*, and the residues in switch II and the α3-helix, subjected to amino acid substitutions in this study, are shown in *red* and *light yellow*, respectively.

**Supplemental Fig. 2. Comparison of the hydrogen-bonding networks of the nucleotide-binding sites.** Hydrogen-bonding networks surrounding GppNHp of M-Ras-GppNHp, H-Ras-GppNHp, and M-RasD41E-GppNHp type 1 and type 2 are schematically shown together with $Mg^{2+}$ and water molecules involved. The switch I and pre-switch I residues are shown in *red* while Gly-60/70 (H-Ras/M-Ras) in switch II is shown in *blue*. *Dotted lines* represent hydrogen-bonding interactions with the distance of less than 3.3 Å

between donor and acceptor atoms, regardless of whether main chains or side chains of the residues are involved. The distance was calculated by using the program COOT (Emsley, P., and Cowtan, K. 2004. *Acta Crystallogr.* ***D*** **60**, 2126-2132).

**Supplemental Fig. 3. Comparison of the crystal structures of M-RasP40D/D41E/L51R-GppNHp, RalA-GppNHp, and H-Ras-GppNHp with a special emphasis on switch I, switch II, and the α3-helix.** Superimposition of the backbone structures of M-RasP40D/D41E/L51R-GppNHp, RalA-GppNHp, and H-Ras-GppNHp. Only switch I, switch II, and the α3-helix are colored as indicated. The structure of GppNHp is excerpted from the model of H-Ras-GppNHp. GppNHp and the side chains of Thr-35/45/46 and Gly-60/70/71 (H-Ras/M-Ras/RalA) are shown in the stick model (*red,* oxygen; blue, *nitrogen*; *deep pink,* phosphorus). Direct hydrogen bonds of Thr-35 and Gly-60 with the γ-phosphate in H-Ras-GppNHp are shown by *red dotted lines*, while a $Mg^{2+}$-mediated hydrogen bond (see supplemental Fig. 2) is not shown. The models were generated as described in the Fig. 1 legend.

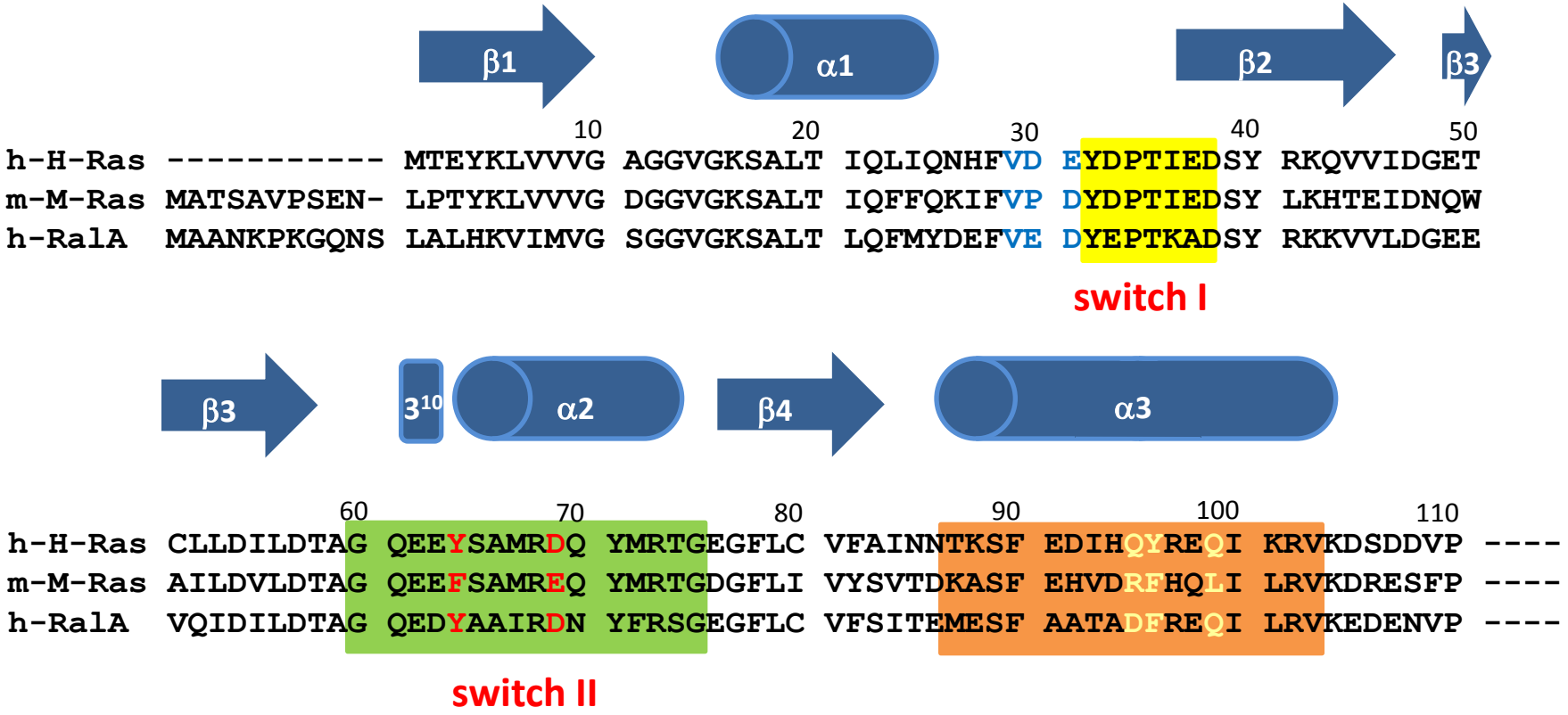
**Supplemental Fig. 4. $^{31}$P-NMR spectrum of M-RasP40D/D41E/L51R/F74Y/E79D/L109Q-GppNHp in the presence of c-Raf-1 RBD.** The spectrum was recorded using 1 mM solution of M-RasP40D/D41E/L51R/F74Y/E79D/L109Q-GppNHp in the presence of 1 mM c-Raf-1 RBD as described in the Fig. 5*A* legend. *α, β,* and *γ* represent the α-, β- and γ-phosphate resonances, respectively. *(1)* and *(2)* represent the γ-phosphate resonance peaks corresponding to state 1 and state 2, respectively. The state 1 and state 2 peaks in the γ-phosphate resonance were fitted by Lorentz curves (blue lines) as described in Fig. 5*A*.

**Supplemental Fig. 5. Comparison of van der Waals contacts between the α3-helix residues and the P-loop residues.** Closed approach of van der Waals distances between Phe-106 and Asp-21 of M-RasD41E-GppNHp type 2 (*upper panel*) and between Tyr-96 and Ala-11 of H-Ras-GppNHp (*lower panel*). The side chain of Phe-106 is located in close proximity to that of Asp-21 in M-RasD41E-GppNHp type 2, indicating that the addition of a hydroxyl group to Phe-106 brings collision. The image of van der Waals distances was generated by the program PyMOL.

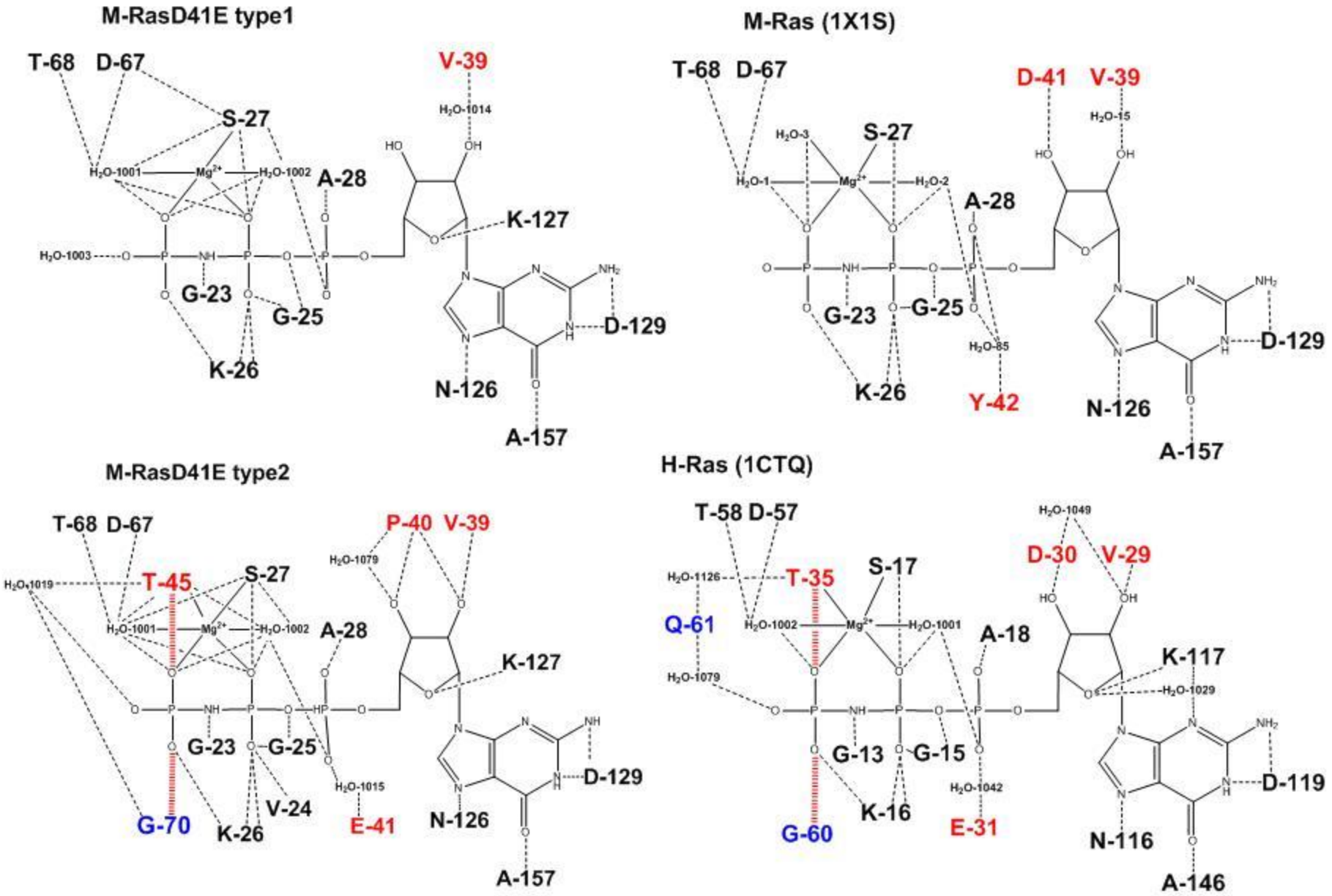
**Supplemental Fig. 6. Comparison of the structures of the pre-switch I and switch I regions between H-Ras-GppNHp and H-RasT35S-GppNHp form 2.** Shown is superimposition of the residues 29-35 of H-Ras-GppNHp (*green*) and H-RasT35S-GppNHp form 2 (*orange*). The structure of GppNHp is excerpted from the model of H-Ras-GppNHp. Oxygen and nitrogen atoms are shown by *red* and *blue colors*, respectively. *Arrows* represent the conformational changes of Val-29, Tyr-32, and Thr-35. A *bidirectional arrow* represents the collision of the side chain of Tyr-32 with that of Val-29. The side chain of Val-29 in H-Ras shows alternative structures. The models were generated as described in the Fig. 2 legend.

**Supplemental Fig. 7. Hydrogen-bonding network between switch II and the α3-helix in RalA-GppNHp.** Switch I, switch II, and the α3-helix of RalA-GppNHp are shown in *yellow, green,* and *orange colors*, respectively. Hydrogen bonds are shown by *red dotted lines*. The models were generated as described in the Fig. 2 legend.

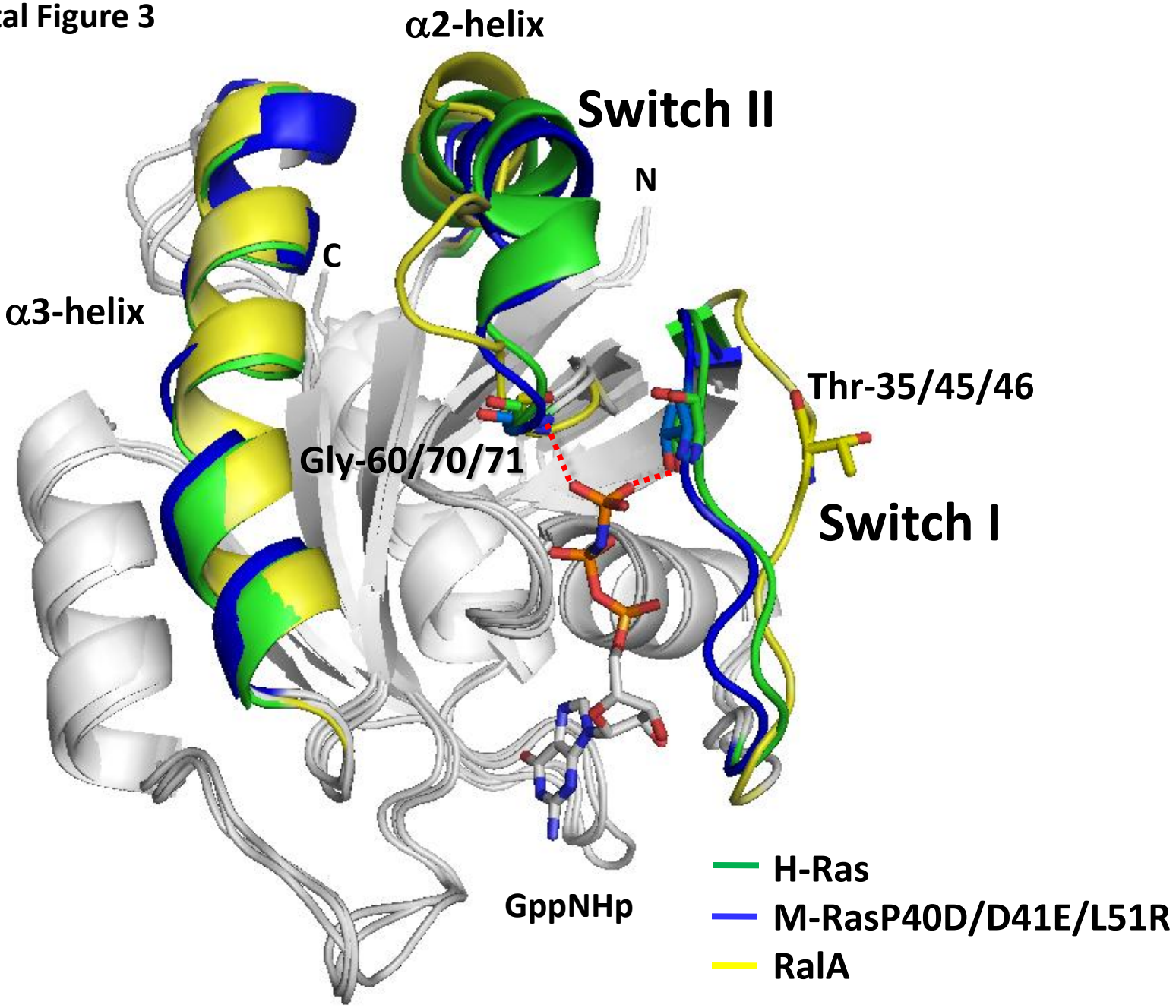
**Supplemental Figure 1**



# Supplemental Figure 2

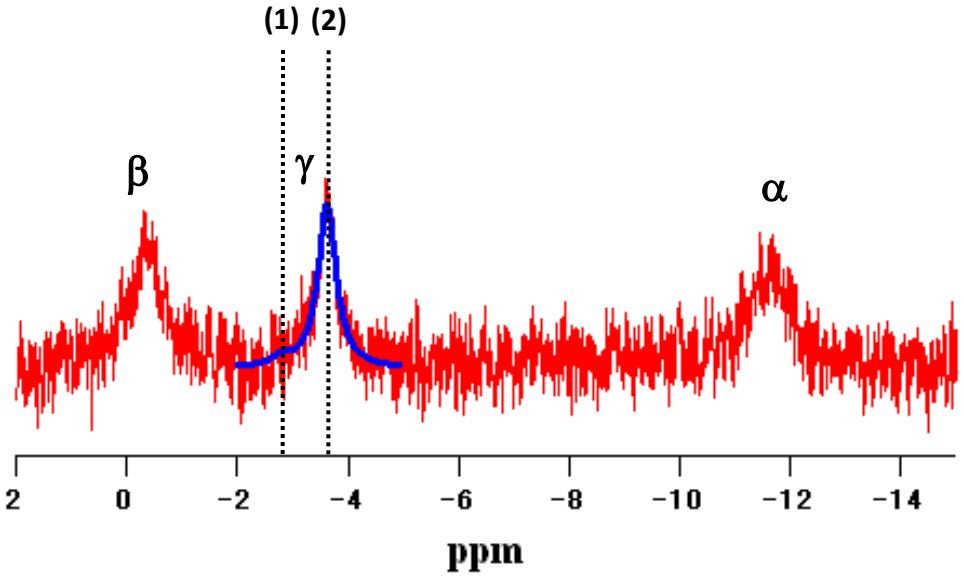


**Supplemental Figure 3**

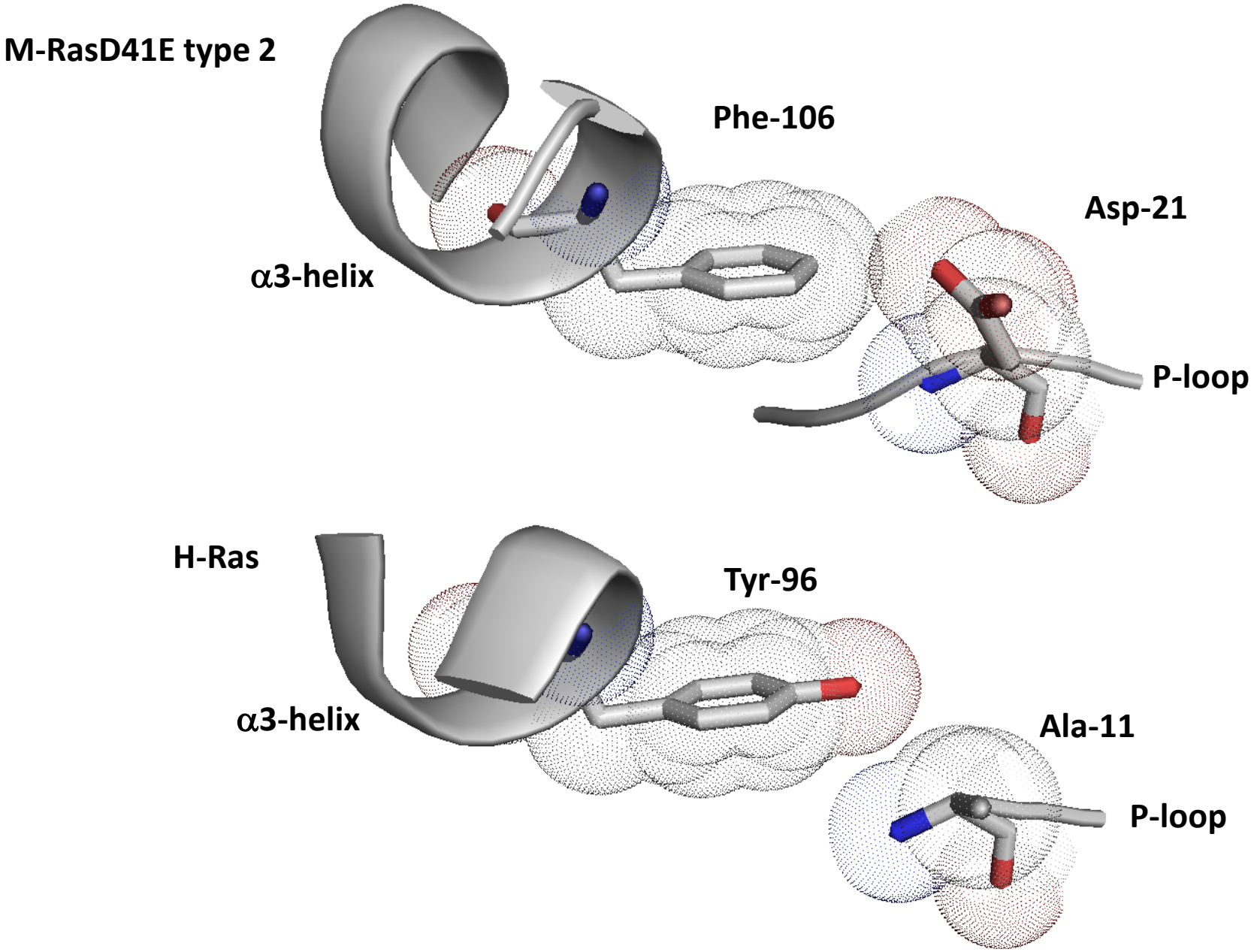


**Supplemental Figure 4**

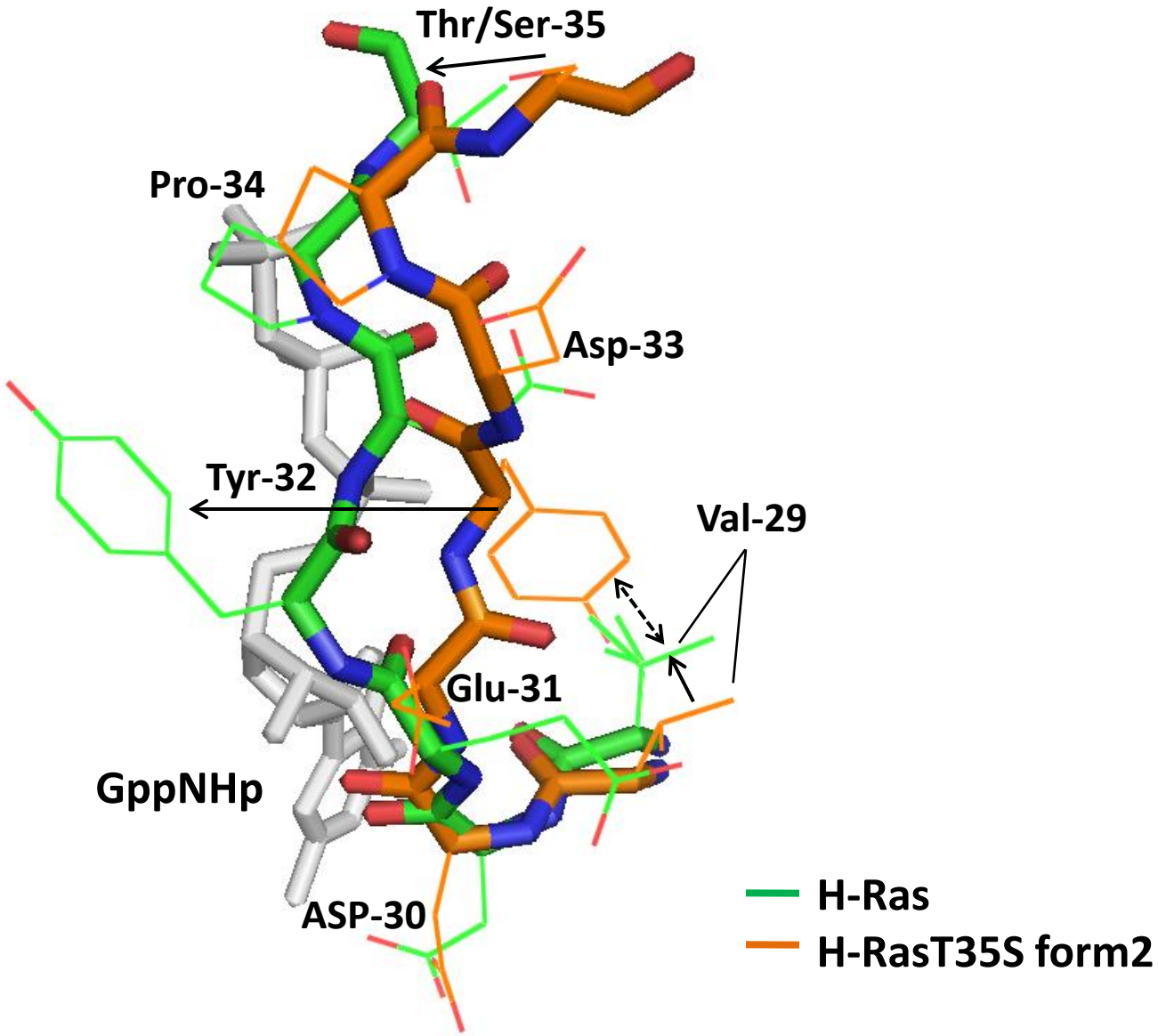
M-RasP40D/D41E/L51R/F74Y/E79D/L109Q-GppNHp + c-Raf-1 RBD



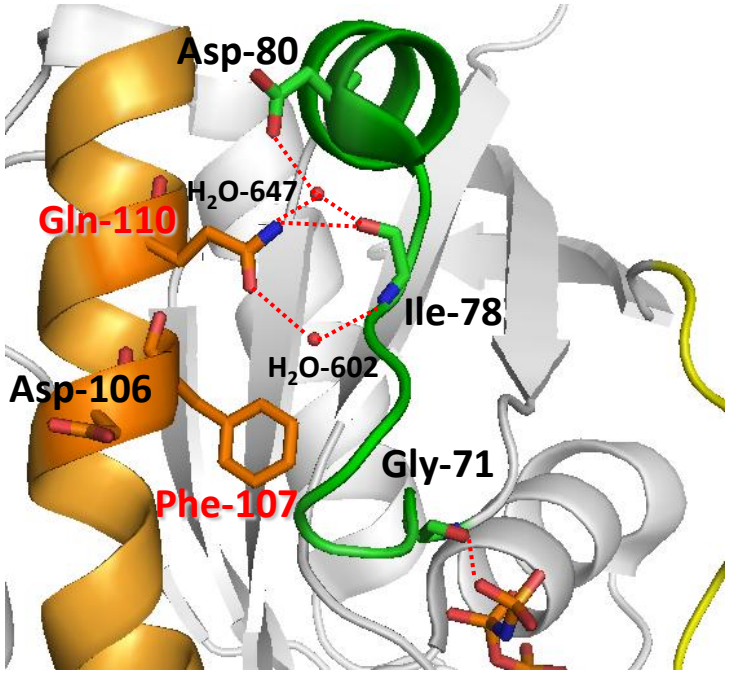
**Supplemental Figure 5**



**Supplemental Figure 6**



**Supplemental Figure 7**




**RalA (state 1)**