

# **NMR analysis of the backbone dynamics of the small GTPase Rheb and its interaction with the regulatory protein FKBP38**

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## **Supplementary Information**

### **Supplementary Results**

*Comparison of the backbone dynamics of two different GTP analogue-bound states of Rheb $\Delta$ CT.*

The backbone dynamic behavior of Rheb $\Delta$ CT bound to GTP-analogues Rheb $\Delta$ CT-GppNHp and -GppCp is overall similar (SI Fig. S4). The average <sup>15</sup>N-T<sub>1</sub>, -T<sub>2</sub>, and {<sup>1</sup>H}-<sup>15</sup>N NOE values for Rheb $\Delta$ CT-GppCp are 725 ± 77 ms and 94 ± 21 ms, and 0.77 ± 0.14, respectively compared to 716 ± 41 ms 87 ± 17 ms, and 0.79 ± 0.11, respectively for the GppNHp-bound state. The values for all analyzable residues for the GppCp-bound state are listed in SI table S3. Differences to the GppNHp-bound state were generally observed for residues involved in nucleotide-binding (based on Uniprot-ID Q15382: 16-21 in G1/P-loop, 32-38 in G2/switch 1, 119-122 in G4, 149-150 in G5) or spatially close to the nucleotide binding region. This include residues near the β and γ phosphate group of the nucleotide, i. e. in the P-loop T14, K19 and S20, and several residues in as well as between the G4 and G5 regions. In addition, the conformational differences due to the different chemical bond between the β and γ phosphate group of the nucleotide appeared to slightly modulate the dynamics of residues in the two β-strands following the switch 1 region (≈ residues 45-55)

and the loop region around residues 85-90 that is spatially close to the nucleotide binding region.

### Supplementary Tables

**Supplementary Table S1:**  $^{15}\text{N}$ -relaxation times  $T_1$  and  $T_2$ , and  $\{^1\text{H}\}$ - $^{15}\text{N}$  NOE values and corresponding errors for the GDP-bound form of Rheb $\Delta$ CT

| Residue | $^{15}\text{N}$ - $T_1$ [ms] $\pm$ error | $^{15}\text{N}$ - $T_2$ [ms] $\pm$ error | $\{^1\text{H}\}$ - $^{15}\text{N}$ NOE $\pm$ error |
|---------|--|--|--|
| 6       | 724 $\pm$ 2                              | 100 $\pm$ 0                              | 0.70 $\pm$ 0.03                                    |
| 7       | 673 $\pm$ 1                              | 95 $\pm$ 0                               | 0.80 $\pm$ 0.03                                    |
| 8       | 690 $\pm$ 1                              | 100 $\pm$ 1                              | 0.81 $\pm$ 0.03                                    |
| 9       | 641 $\pm$ 1                              | 100 $\pm$ 1                              | 0.76 $\pm$ 0.03                                    |
| 10      | 614 $\pm$ 1                              | 94 $\pm$ 1                               | 0.81 $\pm$ 0.04                                    |
| 11      | 655 $\pm$ 1                              | 91 $\pm$ 1                               | 0.74 $\pm$ 0.03                                    |
| 12      | 651 $\pm$ 1                              | 88 $\pm$ 1                               | 0.75 $\pm$ 0.03                                    |
| 14      | 666 $\pm$ 2                              | 88 $\pm$ 1                               | 0.78 $\pm$ 0.04                                    |
| 15      | 657 $\pm$ 5                              | 96 $\pm$ 1                               | 0.87 $\pm$ 0.10                                    |
| 16      | 677 $\pm$ 2                              | 81 $\pm$ 1                               | 0.80 $\pm$ 0.04                                    |
| 18      | 616 $\pm$ 2                              | 87 $\pm$ 1                               | 0.78 $\pm$ 0.04                                    |
| 19      | 677 $\pm$ 3                              | 86 $\pm$ 1                               | 0.92 $\pm$ 0.05                                    |
| 20      | 694 $\pm$ 2                              | 76 $\pm$ 0                               | 0.86 $\pm$ 0.03                                    |
| 21      | 729 $\pm$ 2                              | 78 $\pm$ 1                               | 0.92 $\pm$ 0.03                                    |
| 22      | 650 $\pm$ 1                              | 85 $\pm$ 0                               | 0.85 $\pm$ 0.02                                    |
| 24      | 665 $\pm$ 2                              | 78 $\pm$ 0                               | 0.85 $\pm$ 0.03                                    |
| 25      | 651 $\pm$ 2                              | 84 $\pm$ 0                               | 0.93 $\pm$ 0.04                                    |
| 26      | 663 $\pm$ 2                              | 79 $\pm$ 0                               | 0.91 $\pm$ 0.03                                    |
| 27      | 670 $\pm$ 1                              | 77 $\pm$ 0                               | 0.83 $\pm$ 0.03                                    |
| 28      | 698 $\pm$ 2                              | 80 $\pm$ 1                               | 0.80 $\pm$ 0.03                                    |
| 29      | 644 $\pm$ 2                              | 90 $\pm$ 0                               | 0.83 $\pm$ 0.04                                    |
| 31      | 721 $\pm$ 1                              | 95 $\pm$ 0                               | 0.74 $\pm$ 0.03                                    |
| 32      | 720 $\pm$ 2                              | 96 $\pm$ 0                               | 0.74 $\pm$ 0.05                                    |
| 33      | 670 $\pm$ 1                              | 108 $\pm$ 0                              | 0.71 $\pm$ 0.02                                    |
| 34      | 671 $\pm$ 1                              | 114 $\pm$ 0                              | 0.73 $\pm$ 0.03                                    |

|    |         |         |             |
|----|---------|---------|-------------|
| 35 | 694 ± 1 | 143 ± 0 | 0.48 ± 0.03 |
| 36 | 764 ± 4 | 103 ± 1 | 0.78 ± 0.04 |
| 38 | 684 ± 2 | 81 ± 1  | 0.78 ± 0.03 |
| 39 | 640 ± 6 | 85 ± 0  | 0.62 ± 0.08 |
| 40 | 668 ± 1 | 86 ± 1  | 0.78 ± 0.06 |
| 41 | 755 ± 2 | 86 ± 1  | 0.71 ± 0.03 |
| 42 | 726 ± 2 | 76 ± 0  | 0.86 ± 0.04 |
| 43 | 654 ± 2 | 78 ± 1  | 0.76 ± 0.03 |
| 44 | 717 ± 1 | 85 ± 0  | 0.82 ± 0.03 |
| 45 | 665 ± 1 | 81 ± 0  | 0.75 ± 0.03 |
| 46 | 703 ± 1 | 78 ± 0  | 0.81 ± 0.02 |
| 47 | 675 ± 1 | 84 ± 0  | 0.78 ± 0.03 |
| 48 | 692 ± 1 | 79 ± 0  | 0.83 ± 0.03 |
| 51 | 769 ± 4 | 80 ± 1  | 0.81 ± 0.02 |
| 52 | 745 ± 1 | 90 ± 0  | 0.77 ± 0.03 |
| 53 | 735 ± 1 | 84 ± 1  | 0.74 ± 0.03 |
| 54 | 667 ± 1 | 95 ± 0  | 0.82 ± 0.03 |
| 55 | 693 ± 1 | 96 ± 0  | 0.82 ± 0.03 |
| 56 | 670 ± 1 | 108 ± 0 | 0.77 ± 0.03 |
| 57 | 662 ± 1 | 114 ± 0 | 0.78 ± 0.03 |
| 58 | 655 ± 1 | 143 ± 0 | 0.83 ± 0.03 |
| 59 | 667 ± 1 | 103 ± 1 | 0.84 ± 0.04 |
| 60 | 701 ± 2 | 97 ± 1  | 0.70 ± 0.07 |
| 61 | 677 ± 4 | 103 ± 1 | 0.85 ± 0.03 |
| 62 | 708 ± 1 | 90 ± 0  | 0.58 ± 0.05 |
| 63 | 664 ± 2 | 118 ± 1 | 0.85 ± 0.08 |
| 64 | 634 ± 3 | 90 ± 1  | 0.87 ± 0.03 |
| 65 | 720 ± 2 | 86 ± 0  | 0.84 ± 0.05 |
| 66 | 718 ± 3 | 88 ± 1  | 0.76 ± 0.03 |
| 68 | 668 ± 1 | 86 ± 0  | 0.78 ± 0.03 |
| 69 | 708 ± 2 | 89 ± 0  | 0.79 ± 0.04 |
| 74 | 609 ± 2 | 73 ± 0  | 0.77 ± 0.03 |
| 75 | 609 ± 1 | 92 ± 0  | 0.80 ± 0.04 |

|     |         |         |             |
|-----|---------|---------|-------------|
| 76  | 680 ± 3 | 92 ± 0  | 0.45 ± 0.05 |
| 77  | 670 ± 1 | 71 ± 0  | 0.78 ± 0.05 |
| 78  | 669 ± 2 | 92 ± 0  | 0.81 ± 0.05 |
| 79  | 666 ± 1 | 89 ± 1  | 0.76 ± 0.03 |
| 83  | 664 ± 1 | 93 ± 0  | 0.72 ± 0.03 |
| 84  | 647 ± 1 | 92 ± 1  | 0.77 ± 0.03 |
| 85  | 650 ± 1 | 87 ± 1  | 0.87 ± 0.03 |
| 86  | 701 ± 2 | 89 ± 1  | 0.80 ± 0.04 |
| 87  | 682 ± 1 | 74 ± 0  | 0.79 ± 0.03 |
| 88  | 675 ± 2 | 85 ± 0  | 0.91 ± 0.04 |
| 89  | 673 ± 1 | 79 ± 0  | 0.83 ± 0.02 |
| 90  | 660 ± 1 | 85 ± 0  | 0.83 ± 0.03 |
| 91  | 643 ± 1 | 87 ± 0  | 0.92 ± 0.02 |
| 92  | 675 ± 1 | 83 ± 0  | 0.83 ± 0.03 |
| 93  | 639 ± 2 | 85 ± 0  | 0.78 ± 0.04 |
| 94  | 701 ± 1 | 80 ± 0  | 0.87 ± 0.03 |
| 95  | 660 ± 1 | 81 ± 0  | 0.84 ± 0.03 |
| 96  | 64 ± 1  | 68 ± 0  | 0.86 ± 0.04 |
| 97  | 650 ± 4 | 72 ± 0  | 0.81 ± 0.07 |
| 98  | 674 ± 3 | 86 ± 1  | 0.76 ± 0.06 |
| 99  | 687 ± 1 | 83 ± 0  | 0.82 ± 0.03 |
| 101 | 656 ± 3 | 77 ± 0  | 0.82 ± 0.05 |
| 105 | 688 ± 1 | 82 ± 0  | 0.78 ± 0.03 |
| 106 | 100 ± 1 | 89 ± 0  | 0.74 ± 0.03 |
| 107 | 686 ± 1 | 89 ± 0  | 0.77 ± 0.03 |
| 108 | 672 ± 1 | 96 ± 0  | 0.75 ± 0.03 |
| 109 | 733 ± 2 | 109 ± 1 | 0.66 ± 0.04 |
| 110 | 729 ± 1 | 133 ± 0 | 0.43 ± 0.03 |
| 111 | 736 ± 1 | 122 ± 0 | 0.56 ± 0.03 |
| 112 | 771 ± 1 | 125 ± 0 | 0.43 ± 0.03 |
| 115 | 647 ± 1 | 95 ± 1  | 0.74 ± 0.03 |
| 116 | 647 ± 1 | 93 ± 1  | 0.89 ± 0.03 |
| 118 | 659 ± 2 | 90 ± 1  | 0.83 ± 0.04 |

|     |         |         |             |
|-----|---------|---------|-------------|
| 119 | 661 ± 1 | 85 ± 1  | 0.78 ± 0.02 |
| 120 | 746 ± 6 | 84 ± 1  | 0.95 ± 0.08 |
| 121 | 685 ± 1 | 79 ± 0  | 0.78 ± 0.03 |
| 122 | 622 ± 1 | 80 ± 0  | 0.86 ± 0.03 |
| 123 | 692 ± 2 | 90 ± 0  | 0.89 ± 0.04 |
| 124 | 665 ± 2 | 88 ± 0  | 0.79 ± 0.03 |
| 125 | 735 ± 2 | 85 ± 1  | 0.74 ± 0.03 |
| 127 | 647 ± 3 | 87 ±    | 0.75 ± 0.05 |
| 128 | 670 ± 2 | 79 ± 1  | 0.86 ± 0.05 |
| 129 | 690 ± 3 | 98 ± 1  | 0.81 ± 0.05 |
| 130 | 694 ± 1 | 88 ± 0  | 0.84 ± 0.03 |
| 131 | 654 ± 2 | 88 ± 0  | 0.88 ± 0.03 |
| 132 | 683 ± 1 | 83 ± 0  | 0.88 ± 0.02 |
| 133 | 691 ± 1 | 85 ± 0  | 0.80 ± 0.03 |
| 134 | 654 ± 3 | 93 ± 1  | 0.74 ± 0.6  |
| 135 | 649 ± 2 | 83 ± 0  | 0.85 ± 0.03 |
| 136 | 658 ± 1 | 79 ± 0  | 0.84 ± 0.03 |
| 137 | 610 ± 3 | 88 ± 0  | 0.74 ± 0.05 |
| 138 | 649 ± 1 | 82 ± 0  | 0.86 ± 0.03 |
| 139 | 676 ± 1 | 80 ± 0  | 0.83 ± 0.02 |
| 140 | 650 ± 1 | 80 ± 0  | 0.80 ± 0.03 |
| 141 | 677 ± 2 | 94 ± 1  | 0.75 ± 0.04 |
| 142 | 711 ± 1 | 82 ± 0  | 0.44 ± 0.07 |
| 143 | 714 ± 1 | 81 ± 0  | 0.86 ± 0.03 |
| 144 | 720 ± 1 | 100 ± 0 | 0.73 ± 0.03 |
| 145 | 685 ± 1 | 93 ± 0  | 0.87 ± 0.03 |
| 146 | 653 ± 2 | 89 ± 0  | 0.86 ± 0.03 |
| 147 | 678 ± 2 | 93 ± 0  | 0.78 ± 0.04 |
| 148 | 666 ± 1 | 83 ± 1  | 0.91 ± 0.03 |
| 149 | 737 ± 2 | 84 ± 0  | 0.85 ± 0.04 |
| 150 | 689 ± 1 | 90 ± 0  | 0.90 ± 0.03 |
| 151 | 660 ± 3 | 86 ± 0  | 0.79 ± 0.05 |
| 152 | 660 ± 2 | 90 ± 0  | 0.78 ± 0.04 |

|     |         |         |             |
|-----|---------|---------|-------------|
| 154 | 753 ± 2 | 88 ± 0  | 0.83 ± 0.03 |
| 155 | 646 ± 3 | 96 ± 0  | 0.81 ± 0.05 |
| 156 | 654 ± 1 | 93 ± 0  | 0.86 ± 0.05 |
| 157 | 677 ± 1 | 82 ± 0  | 0.76 ± 0.02 |
| 158 | 654 ± 3 | 81 ± 0  | 0.83 ± 0.02 |
| 159 | 650 ± 5 | 82 ± 0  | 0.77 ± 0.05 |
| 160 | 661 ± 1 | 83 ± 1  | 0.77 ± 0.10 |
| 161 | 686 ± 5 | 83 ± 0  | 0.82 ± 0.02 |
| 163 | 626 ± 5 | 82 ± 0  | 0.70 ± 0.03 |
| 164 | 684 ± 1 | 81 ± 1  | 0.83 ± 0.08 |
| 165 | 637 ± 1 | 82 ± 0  | 0.72 ± 0.03 |
| 166 | 657 ± 1 | 83 ± 0  | 0.81 ± 0.03 |
| 167 | 649 ± 1 | 94 ± 0  | 0.81 ± 0.02 |
| 168 | 649 ± 1 | 95 ± 0  | 0.67 ± 0.03 |
| 169 | 606 ± 0 | 117 ± 0 | 0.50 ± 0.02 |
| 170 | 614 ± 0 | 194 ± 0 | 0.37 ± 0.02 |

**Supplementary Table S2:** <sup>15</sup>N-relaxation times T<sub>1</sub> and T<sub>2</sub>, and {<sup>1</sup>H}-<sup>15</sup>N NOE values and corresponding errors for the GppNHp-bound form of RhebΔCT

| Residue | <sup>15</sup> N-T <sub>1</sub> [ms] ± error | <sup>15</sup> N-T <sub>2</sub> [ms] ± error | { <sup>1</sup> H}- <sup>15</sup> N NOE ± error |
|---------|---|---|--|
| 4       | 87 ± 3                                      | 170 ± 1                                     | 0.12 ± 0.06                                    |
| 5       | 773 ± 3                                     | 124 ± 1                                     | 0.39 ± 0.06                                    |
| 6       | 756 ± 1                                     | 94 ± 0                                      | 0.74 ± 0.04                                    |
| 7       | 709 ± 1                                     | 95 ± 0                                      | 0.86 ± 0.05                                    |
| 8       | 702 ± 1                                     | 93 ± 0                                      | 0.81 ± 0.05                                    |
| 9       | 668 ± 1                                     | 88 ± 0                                      | 0.74 ± 0.05                                    |
| 10      | 696 ± 2                                     | 93 ± 1                                      | 0.69 ± 0.07                                    |
| 11      | 671 ± 1                                     | 87 ± 0                                      | 0.77 ± 0.06                                    |
| 12      | 725 ± 1                                     | 90 ± 0                                      | 0.84 ± 0.05                                    |
| 13      | 682 ± 1                                     | 97 ± 0                                      | 0.78 ± 0.05                                    |
| 14      | 655 ± 2                                     | 80 ± 1                                      | 0.89 ± 0.10                                    |
| 16      | 638 ± 1                                     | 79 ± 0                                      | 0.88 ± 0.07                                    |
| 17      | 675 ± 2                                     | 95 ± 0                                      | 0.77 ± 0.06                                    |

|    |             |             |                 |
|----|-------------|-------------|-----------------|
| 18 | $828 \pm 3$ | $88 \pm 1$  | $0.82 \pm 0.08$ |
| 20 | $710 \pm 3$ | $73 \pm 0$  | $0.79 \pm 0.05$ |
| 21 | $728 \pm 2$ | $68 \pm 1$  | $0.87 \pm 0.10$ |
| 22 | $715 \pm 1$ | $85 \pm 1$  | $0.81 \pm 0.06$ |
| 23 | $794 \pm 6$ | $77 \pm 0$  | $0.86 \pm 0.05$ |
| 26 | $697 \pm 1$ | $75 \pm 0$  | $0.82 \pm 0.04$ |
| 27 | $732 \pm 1$ | $75 \pm 0$  | $0.78 \pm 0.06$ |
| 28 | $698 \pm 1$ | $73 \pm 0$  | $0.78 \pm 0.05$ |
| 29 | $698 \pm 1$ | $91 \pm 0$  | $0.88 \pm 0.05$ |
| 43 | $695 \pm 2$ | $77 \pm 1$  | $0.81 \pm 0.07$ |
| 44 | $708 \pm 1$ | $91 \pm 0$  | $0.30 \pm 0.04$ |
| 45 | $705 \pm 1$ | $88 \pm 0$  | $0.77 \pm 0.04$ |
| 46 | $724 \pm 1$ | $99 \pm 0$  | $0.76 \pm 0.04$ |
| 47 | $708 \pm 1$ | $83 \pm 0$  | $0.76 \pm 0.03$ |
| 48 | $760 \pm 1$ | $94 \pm 0$  | $0.81 \pm 0.03$ |
| 49 | $735 \pm 1$ | $97 \pm 0$  | $0.75 \pm 0.04$ |
| 51 | $823 \pm 1$ | $98 \pm 0$  | $0.70 \pm 0.04$ |
| 52 | $804 \pm 1$ | $82 \pm 0$  | $0.74 \pm 0.03$ |
| 53 | $764 \pm 1$ | $92 \pm 0$  | $0.73 \pm 0.03$ |
| 54 | $755 \pm 1$ | $87 \pm 0$  | $0.69 \pm 0.04$ |
| 55 | $734 \pm 1$ | $90 \pm 0$  | $0.78 \pm 0.04$ |
| 56 | $689 \pm 1$ | $89 \pm 0$  | $0.71 \pm 0.05$ |
| 57 | $698 \pm 1$ | $87 \pm 0$  | $0.73 \pm 0.06$ |
| 58 | $699 \pm 1$ | $93 \pm 0$  | $0.86 \pm 0.06$ |
| 59 | $674 \pm 1$ | $91 \pm 0$  | $0.88 \pm 0.06$ |
| 63 | $827 \pm 6$ | $82 \pm 1$  | $0.96 \pm 0.13$ |
| 65 | $775 \pm 1$ | $91 \pm 0$  | $0.71 \pm 0.04$ |
| 66 | $733 \pm 4$ | $81 \pm 1$  | $0.72 \pm 0.16$ |
| 67 | $684 \pm 1$ | $81 \pm 0$  | $0.85 \pm 0.04$ |
| 68 | $781 \pm 1$ | $87 \pm 0$  | $0.80 \pm 0.03$ |
| 77 | $713 \pm 2$ | $91 \pm 1$  | $0.77 \pm 0.10$ |
| 79 | $695 \pm 1$ | $90 \pm 0$  | $0.67 \pm 0.06$ |
| 80 | $661 \pm 1$ | $100 \pm 0$ | $0.65 \pm 0.05$ |

|     |         |         |             |
|-----|---------|---------|-------------|
| 81  | 690 ± 1 | 81 ± 0  | 0.88 ± 0.06 |
| 82  | 654 ± 1 | 85 ± 0  | 0.77 ± 0.05 |
| 83  | 668 ± 1 | 94 ± 0  | 0.84 ± 0.05 |
| 84  | 693 ± 1 | 89 ± 0  | 0.90 ± 0.05 |
| 85  | 670 ± 1 | 81 ± 0  | 0.87 ± 0.05 |
| 86  | 692 ± 2 | 83 ± 0  | 0.98 ± 0.08 |
| 87  | 702 ± 1 | 66 ± 0  | 0.89 ± 0.05 |
| 88  | 721 ± 1 | 80 ± 0  | 0.84 ± 0.05 |
| 90  | 702 ± 1 | 86 ± 0  | 0.79 ± 0.05 |
| 92  | 695 ± 1 | 86 ± 0  | 0.85 ± 0.04 |
| 93  | 714 ± 1 | 84 ± 0  | 0.86 ± 0.05 |
| 94  | 699 ± 1 | 78 ± 0  | 0.85 ± 0.04 |
| 95  | 708 ± 1 | 79 ± 0  | 0.80 ± 0.04 |
| 96  | 717 ± 1 | 72 ± 0  | 0.77 ± 0.04 |
| 97  | 673 ± 1 | 79 ± 0  | 0.77 ± 0.06 |
| 98  | 678 ± 1 | 75 ± 0  | 0.92 ± 0.05 |
| 99  | 710 ± 1 | 74 ± 0  | 0.80 ± 0.05 |
| 100 | 782 ± 1 | 71 ± 0  | 0.84 ± 0.04 |
| 101 | 692 ± 1 | 80 ± 0  | 0.98 ± 0.06 |
| 102 | 732 ± 1 | 79 ± 0  | 0.85 ± 0.04 |
| 107 | 744 ± 1 | 79 ± 0  | 0.66 ± 0.04 |
| 108 | 752 ± 1 | 102 ± 0 | 0.66 ± 0.04 |
| 109 | 774 ± 2 | 91 ± 1  | 0.72 ± 0.10 |
| 110 | 801 ± 1 | 129 ± 0 | 0.44 ± 0.03 |
| 111 | 725 ± 1 | 119 ± 0 | 0.54 ± 0.04 |
| 112 | 805 ± 0 | 124 ± 0 | 0.40 ± 0.03 |
| 114 | 701 ± 1 | 93 ± 0  | 0.73 ± 0.05 |
| 115 | 668 ± 1 | 87 ± 0  | 0.74 ± 0.04 |
| 116 | 653 ± 1 | 96 ± 0  | 0.73 ± 0.05 |
| 117 | 651 ± 1 | 90 ± 0  | 0.82 ± 0.04 |
| 118 | 734 ± 2 | 83 ± 0  | 0.97 ± 0.06 |
| 119 | 687 ± 1 | 84 ± 0  | 0.82 ± 0.03 |
| 120 | 732 ± 2 | 84 ± 0  | 0.81 ± 0.07 |



|     |         |        |             |
|-----|---------|--------|-------------|
| 121 | 723 ± 1 | 74 ± 0 | 0.90 ± 0.04 |
| 122 | 647 ± 1 | 77 ± 0 | 0.92 ± 0.05 |
| 123 | 704 ± 1 | 82 ± 0 | 0.93 ± 0.04 |
| 124 | 738 ± 1 | 85 ± 0 | 0.81 ± 0.03 |
| 126 | 748 ± 1 | 85 ± 0 | 0.70 ± 0.04 |
| 127 | 692 ± 1 | 85 ± 0 | 0.83 ± 0.05 |
| 128 | 681 ± 2 | 70 ± 1 | 0.86 ± 0.08 |
| 129 | 712 ± 1 | 96 ± 0 | 0.73 ± 0.06 |
| 130 | 742 ± 1 | 88 ± 0 | 0.79 ± 0.04 |
| 131 | 724 ± 1 | 86 ± 0 | 0.82 ± 0.04 |
| 132 | 736 ± 1 | 82 ± 0 | 0.86 ± 0.03 |
| 133 | 724 ± 1 | 79 ± 0 | 0.81 ± 0.04 |
| 134 | 682 ± 1 | 91 ± 0 | 0.85 ± 0.05 |
| 135 | 707 ± 1 | 76 ± 0 | 0.84 ± 0.04 |
| 136 | 718 ± 1 | 76 ± 0 | 0.84 ± 0.03 |
| 137 | 679 ± 1 | 75 ± 0 | 0.81 ± 0.04 |
| 138 | 706 ± 1 | 80 ± 0 | 0.86 ± 0.04 |
| 139 | 710 ± 0 | 76 ± 0 | 0.83 ± 0.03 |
| 140 | 748 ± 1 | 78 ± 0 | 0.81 ± 0.03 |
| 141 | 732 ± 1 | 88 ± 0 | 0.85 ± 0.04 |
| 142 | 745 ± 1 | 76 ± 0 | 0.81 ± 0.03 |
| 143 | 742 ± 1 | 78 ± 0 | 0.77 ± 0.03 |
| 144 | 753 ± 1 | 99 ± 0 | 0.71 ± 0.03 |
| 145 | 720 ± 1 | 88 ± 0 | 0.90 ± 0.04 |
| 146 | 709 ± 1 | 84 ± 0 | 0.79 ± 0.03 |
| 147 | 729 ± 1 | 89 ± 0 | 0.77 ± 0.03 |
| 148 | 700 ± 1 | 79 ± 0 | 0.80 ± 0.04 |
| 149 | 777 ± 1 | 84 ± 0 | 0.84 ± 0.05 |
| 150 | 705 ± 1 | 86 ± 0 | 0.91 ± 0.05 |
| 151 | 716 ± 1 | 84 ± 0 | 0.79 ± 0.06 |
| 152 | 718 ± 1 | 82 ± 0 | 0.81 ± 0.04 |
| 154 | 727 ± 2 | 84 ± 0 | 0.76 ± 0.04 |
| 155 | 693 ± 1 | 80 ± 0 | 0.76 ± 0.05 |

|     |         |         |             |
|-----|---------|---------|-------------|
| 156 | 707 ± 1 | 89 ± 0  | 0.77 ± 0.04 |
| 157 | 720 ± 1 | 80 ± 0  | 0.81 ± 0.04 |
| 158 | 713 ± 1 | 77 ± 0  | 0.85 ± 0.03 |
| 159 | 693 ± 1 | 77 ± 0  | 0.85 ± 0.04 |
| 160 | 644 ± 2 | 82 ± 0  | 0.89 ± 0.07 |
| 162 | 692 ± 1 | 75 ± 0  | 0.80 ± 0.04 |
| 164 | 706 ± 1 | 80 ± 0  | 0.83 ± 0.06 |
| 165 | 690 ± 1 | 78 ± 0  | 0.75 ± 0.04 |
| 166 | 699 ± 1 | 83 ± 0  | 0.79 ± 0.03 |
| 167 | 696 ± 0 | 84 ± 0  | 0.82 ± 0.03 |
| 168 | 723 ± 0 | 87 ± 0  | 0.73 ± 0.02 |
| 169 | 683 ± 0 | 98 ± 0  | 0.60 ± 0.01 |
| 170 | 660 ± 0 | 186 ± 0 | 0.38 ± 0.02 |

**Supplementary Table S3:**  $^{15}\text{N}$ -relaxation times  $T_1$  and  $T_2$ , and  $\{^1\text{H}\}$ - $^{15}\text{N}$  NOE values and corresponding errors for the GppCp-bound form of Rheb $\Delta$ CT

| Residue | $^{15}\text{N}$ - $T_1$ [ms] ± error | $^{15}\text{N}$ - $T_2$ [ms] ± error | $\{^1\text{H}\}$ - $^{15}\text{N}$ NOE ± error |
|---------|--------------------------------------|--------------------------------------|--|
| 5       | 863 ± 7                              | 151 ± 3                              | 0.58 ± 0.12                                    |
| 6       | 746 ± 4                              | 105 ± 1                              | 0.73 ± 0.08                                    |
| 7       | 657 ± 3                              | 85 ± 2                               | 0.77 ± 0.09                                    |
| 8       | 725 ± 5                              | 118 ± 3                              | 0.91 ± 0.13                                    |
| 9       | 806 ± 7                              | 104 ± 3                              | 0.82 ± 0.12                                    |
| 10      | 680 ± 4                              | 105 ± 3                              | 0.86 ± 0.15                                    |
| 11      | 672 ± 4                              | 92 ± 3                               | 0.81 ± 0.13                                    |
| 13      | 640 ± 6                              | 113 ± 3                              | 0.67 ± 0.13                                    |
| 14      | 857 ± 12                             | 83 ± 3                               | 0.62 ± 0.13                                    |
| 17      | 644 ± 3                              | 99 ± 2                               | 0.76 ± 0.13                                    |
| 18      | 606 ± 5                              | 103 ± 3                              | 0.79 ± 0.11                                    |
| 20      | 804 ± 7                              | 70 ± 1                               | 0.65 ± 0.12                                    |
| 22      | 731 ± 4                              | 78 ± 1                               | 0.73 ± 0.12                                    |
| 23      | 763 ± 5                              | 77 ± 1                               | 0.78 ± 0.13                                    |
| 26      | 665 ± 5                              | 73 ± 2                               | 0.92 ± 0.09                                    |
| 29      | 656 ± 3                              | 115 ± 3                              | 0.79 ± 0.11                                    |

|     |         |         |             |
|-----|---------|---------|-------------|
| 44  | 815 ± 4 | 92 ± 2  | 0.73 ± 0.09 |
| 46  | 767 ± 4 | 116 ± 2 | 0.83 ± 0.13 |
| 47  | 716 ± 3 | 85 ± 1  | 0.71 ± 0.09 |
| 49  | 750 ± 4 | 113 ± 3 | 0.75 ± 0.10 |
| 52  | 773 ± 4 | 93 ± 1  | 0.76 ± 0.14 |
| 53  | 747 ± 3 | 93 ± 1  | 0.60 ± 0.12 |
| 54  | 694 ± 4 | 117 ± 3 | 0.70 ± 0.14 |
| 55  | 733 ± 6 | 94 ± 2  | 0.91 ± 0.13 |
| 56  | 718 ± 5 | 92 ± 2  | 0.94 ± 0.13 |
| 58  | 627 ± 5 | 104 ± 3 | 0.83 ± 0.13 |
| 59  | 682 ± 5 | 95 ± 2  | 0.79 ± 0.12 |
| 79  | 623 ± 4 | 92 ± 3  | 0.75 ± 0.12 |
| 80  | 678 ± 4 | 104 ± 2 | 0.70 ± 0.12 |
| 81  | 722 ± 6 | 79 ± 2  | 0.89 ± 0.12 |
| 82  | 596 ± 3 | 102 ± 3 | 0.77 ± 0.13 |
| 85  | 788 ± 8 | 90 ± 2  | 0.63 ± 0.11 |
| 87  | 681 ± 7 | 70 ± 1  | 0.89 ± 0.12 |
| 88  | 621 ± 4 | 95 ± 2  | 0.75 ± 0.11 |
| 90  | 852 ± 5 | 105 ± 3 | 0.89 ± 0.11 |
| 93  | 738 ± 6 | 94      | 0.71 ± 0.11 |
| 94  | 697 ± 3 | 91 ± 2  | 0.79 ± 0.10 |
| 95  | 722 ± 4 | 103 ± 2 | 0.93 ± 0.17 |
| 96  | 735 ± 4 | 66 ± 1  | 0.72 ± 0.13 |
| 97  | 611 ± 5 | 69 ± 2  | 0.86 ± 0.12 |
| 98  | 699 ± 4 | 83 ± 2  | 0.93 ± 0.11 |
| 99  | 750 ± 5 | 89 ± 2  | 0.82 ± 0.09 |
| 101 | 610 ± 3 | 102 ± 2 | 0.75 ± 0.10 |
| 102 | 709 ± 4 | 80 ± 2  | 0.77 ± 0.10 |
| 103 | 690 ± 4 | 78 ± 1  | 0.99 ± 0.08 |
| 105 | 810 ± 6 | 90 ± 2  | 0.66 ± 0.07 |
| 107 | 732 ± 3 | 82 ± 1  | 0.86 ± 0.07 |
| 108 | 743 ± 3 | 96 ± 1  | 0.81 ± 0.08 |
| 109 | 782 ± 5 | 103 ± 2 | 0.86 ± 0.13 |

|     |          |         |             |
|-----|----------|---------|-------------|
| 110 | 764 ± 2  | 139 ± 1 | 0.52 ± 0.13 |
| 111 | 805 ± 5  | 127 ± 3 | 0.44 ± 0.15 |
| 112 | 767 ± 2  | 119 ± 1 | 0.36 ± 0.11 |
| 114 | 753 ± 3  | 111 ± 1 | 0.89 ± 0.10 |
| 115 | 805 ± 5  | 94 ± 2  | 0.78 ± 0.11 |
| 116 | 730 ± 7  | 106 ± 4 | 0.80 ± 0.08 |
| 117 | 703 ± 5  | 103 ± 3 | 0.77 ± 0.16 |
| 120 | 672 ± 9  | 69 ± 2  | 0.68 ± 0.13 |
| 121 | 685 ± 4  | 69 ± 1  | 0.90 ± 0.08 |
| 122 | 755 ± 5  | 93 ± 2  | 1.02 ± 0.07 |
| 123 | 631 ± 4  | 84 ± 1  | 0.95 ± 0.09 |
| 124 | 737 ± 4  | 79 ± 1  | 0.79 ± 0.12 |
| 125 | 406 ± 17 | 85 ± 1  | 0.71 ± 0.10 |
| 126 | 792 ± 4  | 80 ± 1  | 0.77 ± 0.08 |
| 127 | 954 ± 10 | 91 ± 2  | 0.61 ± 0.12 |
| 128 | 787 ± 6  | 69 ± 1  | 0.69 ± 0.10 |
| 129 | 798 ± 7  | 74 ± 2  | 0.76 ± 0.10 |
| 132 | 802 ± 4  | 89 ± 1  | 0.87 ± 0.07 |
| 133 | 657 ± 3  | 78 ± 1  | 0.84 ± 0.10 |
| 134 | 698 ± 6  | 102 ± 2 | 0.69 ± 0.07 |
| 135 | 752 ± 4  | 82 ± 1  | 0.87 ± 0.11 |
| 136 | 715 ± 3  | 81 ± 1  | 0.70 ± 0.10 |
| 137 | 861 ± 6  | 87 ± 1  | 0.92 ± 0.11 |
| 138 | 725 ± 3  | 80 ± 2  | 0.89 ± 0.11 |
| 139 | 861 ± 6  | 75 ± 1  | 0.90 ± 0.10 |
| 140 | 725 ± 3  | 78 ± 1  | 0.80 ± 0.16 |
| 141 | 765 ± 3  | 91 ± 2  | 0.69 ± 0.11 |
| 143 | 728 ± 5  | 69 ± 1  | 0.74 ± 0.11 |
| 144 | 836 ± 4  | 105 ± 1 | 0.91 ± 0.09 |
| 145 | 781 ± 4  | 85 ± 1  | 0.98 ± 0.12 |
| 146 | 747 ± 3  | 87 ± 2  | 0.89 ± 0.10 |
| 148 | 621 ± 2  | 113 ± 3 | 0.83 ± 0.08 |
| 149 | 711 ± 3  | 96 ± 2  | 0.83 ± 0.13 |

|     |         |         |             |
|-----|---------|---------|-------------|
| 150 | 778 ± 6 | 99 ± 2  | 0.81 ± 0.20 |
| 152 | 729 ± 4 | 81 ± 1  | 0.85 ± 0.07 |
| 154 | 741 ± 4 | 89 ± 1  | 0.69 ± 0.16 |
| 155 | 759 ± 5 | 89 ± 2  | 0.86 ± 0.09 |
| 156 | 608 ± 4 | 81 ± 1  | 0.90 ± 0.08 |
| 157 | 673 ± 3 | 84 ± 2  | 0.74 ± 0.06 |
| 158 | 799 ± 3 | 80 ± 1  | 0.80 ± 0.04 |
| 159 | 732 ± 4 | 71 ± 1  | 0.82 ± 0.06 |
| 162 | 736 ± 4 | 93 ± 2  | 0.77 ± 0.00 |
| 164 | 699 ± 8 | 80 ± 2  | 0.80 ± 0.01 |
| 165 | 681 ± 3 | 80 ± 1  | 0.78 ± 0.12 |
| 166 | 672 ± 4 | 132 ± 3 | 0.68 ± 0.08 |
| 167 | 698 ± 2 | 94 ± 1  | 0.69 ± 0.09 |
| 168 | 678 ± 1 | 84 ± 1  | 0.72 ± 0.13 |
| 169 | 667 ± 1 | 99 ± 0  | 0.58 ± 0.12 |
| 170 | 638 ± 1 | 192 ± 1 | 0.29 ± 0.15 |

**Supplementary Table S4:**  $^{15}\text{N}$ -relaxation times  $T_1$  and  $T_2$ , and  $\{^1\text{H}\}$ - $^{15}\text{N}$  NOE values and corresponding errors for the GppNHp-bound form of Rheb $\Delta$ CT in the presence of FKBP38-BD

| Residue | $^{15}\text{N}$ - $T_1$ [ms] ± error | $^{15}\text{N}$ - $T_2$ [ms] ± error | $\{^1\text{H}\}$ - $^{15}\text{N}$ NOE ± error |
|---------|--------------------------------------|--------------------------------------|--|
| 4       | 726 ± 2                              | 170 ± 0                              | 0.43 ± 0.08                                    |
| 5       | 663 ± 2                              | 118 ± 0                              | 0.67 ± 0.10                                    |
| 6       | 771 ± 2                              | 93 ± 0                               | 0.69 ± 0.06                                    |
| 7       | 710 ± 2                              | 88 ± 1                               | 0.80 ± 0.06                                    |
| 8       | 755 ± 2                              | 93 ± 1                               | 0.89 ± 0.06                                    |
| 9       | 720 ± 2                              | 90 ± 1                               | 0.84 ± 0.07                                    |
| 10      | 700 ± 3                              | 106 ± 1                              | 0.81 ± 0.08                                    |
| 12      | 665 ± 2                              | 89 ± 1                               | 0.84 ± 0.07                                    |
| 13      | 756 ± 3                              | 97 ± 1                               | 0.80 ± 0.07                                    |
| 14      | 718 ± 8                              | 98 ± 2                               | 0.92 ± 0.12                                    |
| 16      | 795 ± 6                              | 88 ± 1                               | 0.73 ± 0.09                                    |
| 17      | 570 ± 2                              | 84 ± 1                               | 1.08 ± 0.08                                    |
| 18      | 574 ± 4                              | 77 ± 1                               | 1.01 ± 0.11                                    |

|    |         |         |             |
|----|---------|---------|-------------|
| 20 | 705 ± 3 | 75 ± 1  | 0.73 ± 0.07 |
| 21 | 844 ± 7 | 75 ± 2  | 0.84 ± 0.12 |
| 22 | 620 ± 3 | 87 ± 1  | 0.69 ± 0.07 |
| 23 | 728 ± 3 | 71 ± 1  | 0.90 ± 0.07 |
| 24 | 717 ± 3 | 60 ± 1  | 0.59 ± 0.08 |
| 25 | 704 ± 1 | 74 ± 0  | 0.92 ± 0.05 |
| 26 | 706 ± 2 | 76 ± 0  | 0.78 ± 0.05 |
| 27 | 719 ± 3 | 74 ± 1  | 0.81 ± 0.08 |
| 28 | 707 ± 2 | 90 ± 1  | 0.95 ± 0.07 |
| 29 | 688 ± 2 | 117 ± 5 | 0.66 ± 0.06 |
| 44 | 777 ± 1 | 91 ± 0  | 0.91 ± 0.05 |
| 46 | 703 ± 1 | 97 ± 1  | 0.77 ± 0.06 |
| 47 | 672 ± 1 | 85 ± 0  | 0.83 ± 0.05 |
| 48 | 713 ± 1 | 92 ± 0  | 0.81 ± 0.04 |
| 49 | 711 ± 2 | 90 ± 0  | 0.68 ± 0.05 |
| 51 | 801 ± 3 | 95 ± 0  | 0.85 ± 0.08 |
| 52 | 820 ± 2 | 81 ± 0  | 0.68 ± 0.04 |
| 53 | 717 ± 1 | 87 ± 0  | 0.74 ± 0.05 |
| 54 | 693 ± 2 | 86 ± 0  | 0.70 ± 0.07 |
| 56 | 689 ± 2 | 85 ± 1  | 0.78 ± 0.04 |
| 57 | 686 ± 3 | 92 ± 1  | 0.73 ± 0.07 |
| 58 | 588 ± 2 | 85 ± 1  | 0.87 ± 0.08 |
| 59 | 652 ± 2 | 91 ± 1  | 0.81 ± 0.08 |
| 63 | 770 ± 8 | 86 ± 2  | 0.84 ± 0.07 |
| 65 | 703 ± 2 | 88 ± 1  | 0.77 ± 0.07 |
| 67 | 645 ± 2 | 76 ± 0  | 0.67 ± 0.05 |
| 68 | 722 ± 2 | 88 ± 0  | 0.86 ± 0.03 |
| 78 | 786 ± 4 | 90 ± 1  | 0.75 ± 0.07 |
| 79 | 669 ± 2 | 81 ± 1  | 0.69 ± 0.06 |
| 84 | 655 ± 2 | 81 ± 1  | 0.83 ± 0.07 |
| 85 | 635 ± 2 | 76 ± 1  | 0.83 ± 0.06 |
| 86 | 763 ± 5 | 79 ± 1  | 0.90 ± 0.10 |
| 87 | 691 ± 2 | 68 ± 0  | 0.86 ± 0.06 |

|     |         |         |             |
|-----|---------|---------|-------------|
| 88  | 701 ± 2 | 76 ± 0  | 0.82 ± 0.06 |
| 90  | 686 ± 2 | 80 ± 0  | 0.83 ± 0.06 |
| 92  | 720 ± 2 | 78 ± 0  | 0.82 ± 0.06 |
| 93  | 727 ± 3 | 76 ± 1  | 0.83 ± 0.06 |
| 94  | 711 ± 2 | 74 ± 0  | 0.78 ± 0.05 |
| 95  | 691 ± 2 | 74 ± 0  | 0.81 ± 0.05 |
| 96  | 687 ± 2 | 68 ± 0  | 0.89 ± 0.05 |
| 97  | 632 ± 3 | 29 ± 1  | 0.73 ± 0.08 |
| 98  | 718 ± 2 | 77 ± 1  | 0.71 ± 0.07 |
| 99  | 607 ± 3 | 74 ± 1  | 0.84 ± 0.06 |
| 100 | 618 ± 2 | 63 ± 0  | 0.71 ± 0.05 |
| 101 | 754 ± 2 | 72 ± 1  | 0.80 ± 0.07 |
| 106 | 700 ± 1 | 85 ± 1  | 0.80 ± 0.03 |
| 107 | 703 ± 1 | 76 ± 0  | 0.85 ± 0.05 |
| 108 | 662 ± 1 | 84 ± 0  | 0.77 ± 0.06 |
| 110 | 756 ± 1 | 121 ± 0 | 0.42 ± 0.04 |
| 112 | 777 ± 1 | 114 ± 0 | 0.48 ± 0.04 |
| 114 | 755 ± 3 | 89 ± 1  | 0.98 ± 0.08 |
| 115 | 651 ± 1 | 83 ± 0  | 0.76 ± 0.06 |
| 116 | 707 ± 2 | 83 ± 1  | 0.84 ± 0.07 |
| 117 | 673 ± 1 | 86 ± 0  | 0.75 ± 0.06 |
| 118 | 643 ± 2 | 80 ± 0  | 0.95 ± 0.08 |
| 121 | 722 ± 2 | 69 ± 0  | 0.85 ± 0.05 |
| 122 | 628 ± 2 | 75 ± 1  | 0.73 ± 0.06 |
| 123 | 731 ± 2 | 79 ± 0  | 0.70 ± 0.06 |
| 124 | 650 ± 1 | 81 ± 0  | 0.84 ± 0.05 |
| 127 | 658 ± 2 | 81 ± 0  | 0.74 ± 0.07 |
| 128 | 820 ± 4 | 70 ± 1  | 0.80 ± 0.10 |
| 129 | 691 ± 2 | 92 ± 1  | 0.86 ± 0.07 |
| 131 | 681 ± 2 | 82 ± 0  | 0.75 ± 0.05 |
| 132 | 738 ± 1 | 73 ± 0  | 0.74 ± 0.04 |
| 133 | 730 ± 2 | 74 ± 0  | 0.72 ± 0.05 |
| 134 | 634 ± 2 | 84 ± 0  | 0.88 ± 0.07 |

|     |         |         |             |
|-----|---------|---------|-------------|
| 135 | 705 ± 2 | 71 ± 0  | 0.85 ± 0.05 |
| 136 | 702 ± 1 | 68 ± 0  | 0.89 ± 0.04 |
| 137 | 636 ± 2 | 72 ± 0  | 0.83 ± 0.06 |
| 139 | 752 ± 1 | 71 ± 0  | 0.79 ± 0.04 |
| 140 | 724 ± 1 | 73 ± 0  | 0.79 ± 0.04 |
| 141 | 708 ± 2 | 82 ± 0  | 0.82 ± 0.05 |
| 142 | 757 ± 1 | 76 ± 0  | 0.74 ± 0.04 |
| 143 | 738 ± 1 | 77 ± 0  | 0.84 ± 0.05 |
| 144 | 754 ± 1 | 91 ± 0  | 0.77 ± 0.05 |
| 146 | 682 ± 2 | 82 ± 0  | 0.72 ± 0.04 |
| 147 | 671 ± 1 | 86 ± 0  | 0.88 ± 0.06 |
| 148 | 710 ± 2 | 77 ± 0  | 0.78 ± 0.06 |
| 149 | 782 ± 3 | 78 ± 0  | 0.72 ± 0.07 |
| 150 | 662 ± 2 | 86 ± 1  | 0.75 ± 0.06 |
| 152 | 700 ± 1 | 82 ± 0  | 0.91 ± 0.06 |
| 154 | 807 ± 3 | 79 ± 0  | 0.69 ± 0.07 |
| 155 | 689 ± 2 | 80 ± 0  | 0.94 ± 0.07 |
| 156 | 694 ± 2 | 87 ± 0  | 0.78 ± 0.06 |
| 157 | 739 ± 1 | 79 ± 0  | 0.80 ± 0.05 |
| 158 | 676 ± 1 | 74 ± 0  | 0.79 ± 0.04 |
| 159 | 690 ± 2 | 74 ± 0  | 0.86 ± 0.06 |
| 160 | 709 ± 3 | 78 ± 0  | 0.75 ± 0.10 |
| 161 | 729 ± 2 | 78 ± 0  | 0.83 ± 0.09 |
| 162 | 705 ± 3 | 73 ± 0  | 0.77 ± 0.05 |
| 164 | 778 ± 1 | 73 ± 0  | 0.79 ± 0.09 |
| 165 | 696 ± 1 | 75 ± 0  | 0.77 ± 0.05 |
| 166 | 662 ± 1 | 82 ± 0  | 0.87 ± 0.05 |
| 167 | 660 ± 1 | 86 ± 0  | 0.82 ± 0.05 |
| 169 | 688 ± 1 | 97 ± 0  | 0.65 ± 0.02 |
| 170 | 640 ± 0 | 178 ± 0 | 0.42 ± 0.03 |

**Supplementary Table S5:** Results from the Lipari and Szabo model-free analysis of the <sup>15</sup>N-relaxation data of RhebΔCT bound to GDP with TENSOR2



| Residue | Model | $\chi^2$ | $S^2 \pm \text{error}$ | $\tau_i [\text{ns}] \pm \text{error}$ | $R_{\text{ex}} [\text{s}^{-1}] \pm \text{error}$ |
|---------|-------|----------|------------------------|---------------------------------------|--|
| 6       | 5     | 0.00(0)  | $0.94 \pm 0.01$        | $0.940 \pm 0.451$                     | $0.0 \pm 0.0$                                    |
| 7       | 5     | 0.00(0)  | $0.94 \pm 0.01$        | $5.140 \pm 3.320$                     | $0.0 \pm 0.0$                                    |
| 8       | (6)5  | 0.17(4)  | $0.90 \pm 0.01$        | $9.900 \pm 3.390$                     | $0.0 \pm 0.0$                                    |
| 9       | 5     | 0.00(0)  | $0.87 \pm 0.02$        | $3.730 \pm 2.920$                     | $0.0 \pm 0.0$                                    |
| 10      | (6)5  | 0.13(8)  | $0.84 \pm 0.02$        | $9.900 \pm 3.050$                     | $0.0 \pm 0.0$                                    |
| 11      | 5     | 0.00(0)  | $0.95 \pm 0.01$        | $1.460 \pm 2.420$                     | $0.0 \pm 0.0$                                    |
| 12      | 5     | 0.00(0)  | $0.97 \pm 0.01$        | $1.080 \pm 2.560$                     | $0.0 \pm 0.0$                                    |
| 14      | 1     | 0.67(7)  | $0.85 \pm 0.00$        | $0.000 \pm 0.000$                     | $0.0 \pm 0.0$                                    |
| 15      | (6)5  | 0.51(5)  | $0.89 \pm 0.02$        | $9.900 \pm 3.880$                     | $0.8 \pm 0.0$                                    |
| 16      | 3     | 0.00(6)  | $0.84 \pm 0.00$        | $0.000 \pm 0.000$                     | $1.1 \pm 0.1$                                    |
| 18      | 5     | 0.00(0)  | $0.95 \pm 0.01$        | $3.000 \pm 3.430$                     | $0.0 \pm 0.0$                                    |
| 19      | (6)5  | 5.68(0)  | $1.03 \pm 0.01$        | $9.900 \pm 4.150$                     | $0.0 \pm 0.0$                                    |
| 20      | (6)3  | 3.54(0)  | $0.82 \pm 0.00$        | $0.000 \pm 0.000$                     | $2.2 \pm 0.1$                                    |
| 21      | (3)3  | 11.40(0) | $0.78 \pm 0.00$        | $0.000 \pm 0.000$                     | $2.3 \pm 0.0$                                    |
| 22      | 1     | 4.59(0)  | $0.87 \pm 0.00$        | $0.000 \pm 0.000$                     | $0.0 \pm 0.0$                                    |
| 24      | 3     | 1.57(0)  | $0.85 \pm 0.00$        | $0.000 \pm 0.000$                     | $1.2 \pm 0.1$                                    |
| 25      | (3)3  | 10.70(0) | $0.87 \pm 0.00$        | $0.000 \pm 0.000$                     | $0.2 \pm 0.0$                                    |
| 26      | (6)5  | 10.50(0) | $1.09 \pm 0.01$        | $9.900 \pm 3.130$                     | $0.0 \pm 0.0$                                    |
| 27      | 3     | 1.04(0)  | $0.84 \pm 0.00$        | $0.000 \pm 0.000$                     | $1.5 \pm 0.1$                                    |
| 28      | 3     | 0.01(8)  | $0.81 \pm 0.00$        | $0.000 \pm 0.000$                     | $1.5 \pm 0.1$                                    |
| 29      | (6)5  | 0.69(2)  | $0.93 \pm 0.01$        | $9.840 \pm 3.760$                     | $0.0 \pm 0.0$                                    |
| 31      | 2     | 0.99(4)  | $0.78 \pm 0.00$        | $0.010 \pm 0.004$                     | $0.0 \pm 0.0$                                    |
| 32      | 2     | 0.24(5)  | $0.77 \pm 0.00$        | $0.021 \pm 0.007$                     | $0.0 \pm 0.0$                                    |
| 33      | 5     | 0.00(0)  | $0.84 \pm 0.01$        | $2.330 \pm 0.472$                     | $0.0 \pm 0.0$                                    |
| 34      | 5     | 0.00(0)  | $0.81 \pm 0.01$        | $3.330 \pm 1.350$                     | $0.0 \pm 0.0$                                    |
| 35      | 5     | 0.00(0)  | $0.65 \pm 0.00$        | $1.580 \pm 0.075$                     | $0.0 \pm 0.0$                                    |
| 36      | 5     | 0.00(0)  | $0.97 \pm 0.01$        | $1.720 \pm 3.760$                     | $0.0 \pm 0.0$                                    |
| 38      | 3     | 0.61(8)  | $0.83 \pm 0.00$        | $0.000 \pm 0.000$                     | $1.2 \pm 0.1$                                    |
| 39      | 2     | 1.30(0)  | $0.87 \pm 0.00$        | $0.054 \pm 0.023$                     | $0.0 \pm 0.0$                                    |
| 40      | 1     | 1.69(0)  | $0.85 \pm 0.00$        | $0.000 \pm 0.000$                     | $0.0 \pm 0.0$                                    |
| 41      | 4     | 0.00(0)  | $0.73 \pm 0.01$        | $0.019 \pm 0.006$                     | $1.7 \pm 0.1$                                    |
| 42      | 3     | 2.14(0)  | $0.78 \pm 0.00$        | $0.000 \pm 0.000$                     | $2.6 \pm 0.1$                                    |

|    |       |         |                 |                   |               |
|----|-------|---------|-----------------|-------------------|---------------|
| 43 | 3     | 1.94(0) | $0.87 \pm 0.00$ | $0.000 \pm 0.000$ | $1.1 \pm 0.1$ |
| 44 | 3     | 0.44(7) | $0.79 \pm 0.00$ | $0.000 \pm 0.000$ | $1.1 \pm 0.1$ |
| 45 | 3     | 3.83(0) | $0.85 \pm 0.00$ | $0.000 \pm 0.000$ | $0.8 \pm 0.1$ |
| 46 | 3     | 0.01(7) | $0.81 \pm 0.00$ | $0.000 \pm 0.000$ | $1.9 \pm 0.1$ |
| 47 | 3     | 1.21(0) | $0.84 \pm 0.00$ | $0.000 \pm 0.000$ | $0.6 \pm 0.1$ |
| 48 | 3     | 0.62(1) | $0.82 \pm 0.00$ | $0.000 \pm 0.000$ | $1.6 \pm 0.1$ |
| 51 | 3     | 0.03(3) | $0.74 \pm 0.00$ | $0.000 \pm 0.000$ | $2.5 \pm 0.1$ |
| 52 | 3     | 1.59(0) | $0.76 \pm 0.00$ | $0.000 \pm 0.000$ | $0.8 \pm 0.1$ |
| 53 | 4     | 0.00(0) | $0.76 \pm 0.01$ | $0.015 \pm 0.007$ | $1.7 \pm 0.1$ |
| 54 | (6)5  | 0.83(4) | $0.92 \pm 0.01$ | $9.900 \pm 3.00$  | $0.0 \pm 0.0$ |
| 55 | (6)5  | 0.47(7) | $0.94 \pm 0.01$ | $9.900 \pm 3.500$ | $0.0 \pm 0.0$ |
| 56 | 5     | 0.00(0) | $0.84 \pm 0.03$ | $5.500 \pm 2.840$ | $0.0 \pm 0.0$ |
| 57 | 5     | 0.00(0) | $0.76 \pm 0.03$ | $7.530 \pm 2.780$ | $0.0 \pm 0.0$ |
| 58 | (6)5  | 2.73(0) | $0.48 \pm 0.05$ | $9.890 \pm 2.100$ | $0.0 \pm 0.0$ |
| 59 | (6)5  | 1.66(0) | $0.83 \pm 0.02$ | $9.900 \pm 3.000$ | $0.0 \pm 0.0$ |
| 60 | 5     | 0.00(0) | $0.94 \pm 0.03$ | $0.794 \pm 2.000$ | $0.0 \pm 0.0$ |
| 61 | (6)5  | 4.06(0) | $0.85 \pm 0.02$ | $9.900 \pm 2.940$ | $0.0 \pm 0.0$ |
| 62 | 4     | 0.00(0) | $0.76 \pm 0.01$ | $0.055 \pm 0.009$ | $0.8 \pm 0.1$ |
| 63 | (6)5  | 0.73(0) | $0.69 \pm 0.04$ | $9.890 \pm 3.000$ | $0.0 \pm 0.0$ |
| 64 | (6)5  | 4.94(0) | $0.92 \pm 0.01$ | $9.900 \pm 3.420$ | $0.0 \pm 0.0$ |
| 65 | 3     | 0.52(9) | $0.79 \pm 0.00$ | $0.000 \pm 0.000$ | $1.0 \pm 0.1$ |
| 66 | 3     | 2.49(0) | $0.79 \pm 0.00$ | $0.000 \pm 0.000$ | $0.7 \pm 0.1$ |
| 68 | 3     | 0.12(1) | $0.80 \pm 0.00$ | $0.000 \pm 0.000$ | $0.4 \pm 0.1$ |
| 69 | 6 (4) | 1.06(0) | $0.20 \pm 0.07$ | $9.090 \pm 1.740$ | $8.3 \pm 0.5$ |
| 74 | 3     | 0.73(6) | $0.93 \pm 0.00$ | $0.000 \pm 0.000$ | $1.2 \pm 0.1$ |
| 75 | (6)5  | 0.08(9) | $0.86 \pm 0.02$ | $9.900 \pm 3.080$ | $0.0 \pm 0.0$ |
| 76 | 4     | 0.00(0) | $0.77 \pm 0.01$ | $0.097 \pm 0.012$ | $0.4 \pm 0.1$ |
| 77 | 3     | 0.36(5) | $0.84 \pm 0.00$ | $0.000 \pm 0.000$ | $2.7 \pm 0.1$ |
| 78 | (6)5  | 0.04(6) | $0.95 \pm 0.01$ | $9.840 \pm 4.050$ | $0.0 \pm 0.0$ |
| 79 | 2     | 3.52(0) | $0.83 \pm 0.00$ | $0.029 \pm 0.008$ | $0.0 \pm 0.0$ |
| 83 | 5     | 0.00(0) | $0.93 \pm 0.01$ | $1.270 \pm 1.370$ | $0.0 \pm 0.0$ |
| 84 | 5     | 0.00(0) | $0.94 \pm 0.01$ | $2.560 \pm 3.000$ | $0.0 \pm 0.0$ |
| 85 | (6)5  | 5.09(0) | $0.98 \pm 0.01$ | $9.810 \pm 4.000$ | $0.0 \pm 0.0$ |

|     |      |          |             |               |           |
|-----|------|----------|-------------|---------------|-----------|
| 86  | 3    | 0.00(5)  | 0.81 ± 0.00 | 0.000 ± 0.000 | 0.3 ± 0.1 |
| 87  | 3    | 0.18(4)  | 0.83 ± 0.00 | 0.000 ± 0.000 | 2.2 ± 0.1 |
| 88  | (3)3 | 8.03(0)  | 0.84 ± 0.00 | 0.000 ± 0.000 | 0.5 ± 0.0 |
| 89  | 3    | 1.53(0)  | 0.84 ± 0.00 | 0.000 ± 0.000 | 1.3 ± 0.0 |
| 90  | 3    | 0.81(7)  | 0.86 ± 0.00 | 0.000 ± 0.000 | 0.2 ± 0.1 |
| 91  | (6)5 | 21.30(0) | 0.97 ± 0.00 | 9.900 ± 3.790 | 0.0 ± 0.0 |
| 92  | 3    | 0.68(1)  | 0.84 ± 0.00 | 0.000 ± 0.000 | 0.7 ± 0.1 |
| 93  | 2    | 3.29(0)  | 0.87 ± 0.00 | 0.037 ± 0.011 | 0.0 ± 0.0 |
| 94  | (6)3 | 5.98(0)  | 0.81 ± 0.00 | 0.000 ± 0.000 | 1.6 ± 0.1 |
| 95  | 3    | 2.03(0)  | 0.86 ± 0.00 | 0.000 ± 0.000 | 0.8 ± 0.1 |
| 96  | 3    | 1.96(0)  | 0.87 ± 0.00 | 0.000 ± 0.000 | 2.9 ± 0.1 |
| 97  | 3    | 0.00(3)  | 0.87 ± 0.01 | 0.000 ± 0.000 | 2.1 ± 0.1 |
| 98  | 3    | 0.48(4)  | 0.84 ± 0.00 | 0.000 ± 0.000 | 0.3 ± 0.1 |
| 99  | 3    | 0.32(3)  | 0.82 ± 0.00 | 0.000 ± 0.000 | 0.9 ± 0.1 |
| 101 | 3    | 0.12(2)  | 0.86 ± 0.00 | 0.000 ± 0.000 | 1.3 ± 0.0 |
| 105 | 3    | 0.41(4)  | 0.82 ± 0.00 | 0.000 ± 0.000 | 1.1 ± 0.0 |
| 106 | 3    | 4.00(0)  | 0.81 ± 0.00 | 0.000 ± 0.000 | 0.3 ± 0.0 |
| 107 | 3    | 0.94(5)  | 0.82 ± 0.00 | 0.000 ± 0.000 | 0.1 ± 0.0 |
| 108 | 5    | 0.00(0)  | 0.93 ± 0.01 | 2.000 ± 0.741 | 0.0 ± 0.0 |
| 109 | 5    | 0.00(0)  | 0.89 ± 0.01 | 1.280 ± 0.240 | 0.0 ± 0.0 |
| 110 | 5    | 0.00(0)  | 0.71 ± 0.00 | 1.200 ± 0.062 | 0.0 ± 0.0 |
| 111 | 5    | 0.00(0)  | 0.79 ± 0.01 | 1.340 ± 0.117 | 0.0 ± 0.0 |
| 112 | 5    | 0.00(0)  | 0.79 ± 0.00 | 0.899 ± 0.055 | 0.0 ± 0.0 |
| 115 | 5    | 0.00(0)  | 0.91 ± 0.01 | 2.130 ± 2.210 | 0.0 ± 0.0 |
| 116 | (6)5 | 6.86(0)  | 0.90 ± 0.01 | 9.900 ± 3.280 | 0.0 ± 0.0 |
| 118 | (6)5 | 0.43(7)  | 0.96 ± 0.01 | 9.900 ± 3.910 | 0.0 ± 0.0 |
| 119 | 3    | 1.32(0)  | 0.86 ± 0.00 | 0.000 ± 0.000 | 0.2 ± 0.1 |
| 120 | 3    | 2.99(0)  | 0.76 ± 0.01 | 0.000 ± 0.000 | 1.7 ± 0.1 |
| 121 | 3    | 0.54(5)  | 0.83 ± 0.00 | 0.000 ± 0.000 | 1.5 ± 0.1 |
| 122 | (6)5 | 4.11(0)  | 1.01 ± 0.01 | 7.680 ± 4.110 | 0.0 ± 0.0 |
| 123 | (6)5 | 5.54(0)  | 1.01 ± 0.01 | 3.880 ± 4.480 | 0.0 ± 0.0 |
| 124 | 2    | 1.54(0)  | 0.84 ± 0.00 | 0.016 ± 0.006 | 0.0 ± 0.0 |
| 125 | 3    | 3.19(0)  | 0.77 ± 0.00 | 0.000 ± 0.000 | 1.4 ± 0.1 |

|     |      |          |             |               |           |
|-----|------|----------|-------------|---------------|-----------|
| 127 | 2    | 2.25(0)  | 0.85 ± 0.00 | 0.051 ± 0.013 | 0.0 ± 0.0 |
| 128 | 3    | 1.43(0)  | 0.84 ± 0.00 | 0.000 ± 0.000 | 1.3 ± 0.1 |
| 129 | (6)5 | 0.12(2)  | 0.91 ± 0.01 | 9.900 ± 3.730 | 0.0 ± 0.0 |
| 130 | 3    | 1.18(0)  | 0.82 ± 0.00 | 0.000 ± 0.000 | 0.4 ± 0.1 |
| 131 | (6)5 | 9.35(0)  | 0.97 ± 0.00 | 9.760 ± 3.860 | 0.0 ± 0.0 |
| 132 | (6)5 | 13.40(0) | 1.07 ± 0.01 | 9.900 ± 3.240 | 0.0 ± 0.0 |
| 133 | 3    | 0.00(0)  | 0.82 ± 0.00 | 0.000 ± 0.000 | 0.6 ± 0.0 |
| 134 | 5    | 0.00(0)  | 0.93 ± 0.01 | 1.850 ± 2.590 | 0.0 ± 0.0 |
| 135 | 3    | 1.56(0)  | 0.87 ± 0.00 | 0.000 ± 0.000 | 0.3 ± 0.1 |
| 136 | 3    | 2.37(0)  | 0.86 ± 0.00 | 0.000 ± 0.000 | 1.1 ± 0.0 |
| 137 | 5    | 0.00(0)  | 0.93 ± 0.01 | 1.830 ± 3.000 | 0.0 ± 0.0 |
| 138 | (6)5 | 3.74(0)  | 1.04 ± 0.01 | 9.900 ± 3.680 | 0.0 ± 0.0 |
| 139 | 3    | 2.14(0)  | 0.84 ± 0.00 | 0.000 ± 0.000 | 1.2 ± 0.0 |
| 140 | 3    | 0.06(1)  | 0.87 ± 0.00 | 0.000 ± 0.000 | 0.8 ± 0.0 |
| 141 | 5    | 0.00(0)  | 0.95 ± 0.01 | 1.720 ± 2.850 | 0.0 ± 0.0 |
| 142 | 4    | 0.00(0)  | 0.73 ± 0.01 | 0.082 ± 0.012 | 2.2 ± 0.1 |
| 143 | (6)3 | 4.15(0)  | 0.79 ± 0.00 | 0.000 ± 0.000 | 1.6 ± 0.1 |
| 144 | 5    | 0.00(0)  | 0.94 ± 0.01 | 1.300 ± 0.463 | 0.0 ± 0.0 |
| 145 | (6)5 | 4.74(0)  | 0.96 ± 0.01 | 9.870 ± 3.660 | 0.0 ± 0.0 |
| 146 | (6)5 | 2.80(0)  | 0.96 ± 0.01 | 9.900 ± 3.830 | 0.0 ± 0.0 |
| 147 | 5    | 0.00(0)  | 0.96 ± 0.01 | 2.540 ± 3.770 | 0.0 ± 0.0 |
| 148 | (3)3 | 11.00(0) | 0.85 ± 0.00 | 0.000 ± 0.000 | 0.6 ± 0.0 |
| 149 | 3    | 1.79(0)  | 0.77 ± 0.00 | 0.000 ± 0.000 | 1.5 ± 0.1 |
| 150 | (6)5 | 13.20(0) | 1.00 ± 0.01 | 9.900 ± 4.480 | 0.0 ± 0.0 |
| 151 | 1    | 2.40(0)  | 0.86 ± 0.00 | 0.000 ± 0.000 | 0.0 ± 0.0 |
| 152 | 5    | 0.00(0)  | 0.97 ± 0.01 | 2.340 ± 3.940 | 0.0 ± 0.0 |
| 154 | 3    | 0.73(2)  | 0.75 ± 0.00 | 0.000 ± 0.000 | 1.2 ± 0.1 |
| 155 | (6)5 | 0.06(4)  | 0.87 ± 0.02 | 9.900 ± 3.450 | 0.0 ± 0.0 |
| 156 | (6)5 | 1.17(0)  | 0.92 ± 0.01 | 9.900 ± 3.700 | 0.0 ± 0.0 |
| 157 | 3    | 3.25(0)  | 0.84 ± 0.00 | 0.000 ± 0.000 | 0.9 ± 0.1 |
| 158 | 3    | 1.32(0)  | 0.87 ± 0.00 | 0.000 ± 0.000 | 0.6 ± 0.1 |
| 159 | 3    | 0.49(6)  | 0.87 ± 0.01 | 0.000 ± 0.000 | 0.5 ± 0.1 |
| 160 | 3    | 0.11(1)  | 0.86 ± 0.00 | 0.000 ± 0.000 | 0.5 ± 0.1 |

|     |      |         |                 |                   |               |
|-----|------|---------|-----------------|-------------------|---------------|
| 161 | 3    | 0.71(5) | $0.83 \pm 0.01$ | $0.000 \pm 0.000$ | $0.9 \pm 0.1$ |
| 163 | 4    | 0.00(0) | $0.88 \pm 0.01$ | $0.059 \pm 0.016$ | $0.2 \pm 0.1$ |
| 164 | 3    | 0.13(6) | $0.83 \pm 0.00$ | $0.000 \pm 0.000$ | $1.2 \pm 0.1$ |
| 165 | 4    | 0.00(0) | $0.87 \pm 0.01$ | $0.044 \pm 0.013$ | $0.4 \pm 0.1$ |
| 166 | 3    | 0.19(9) | $0.86 \pm 0.00$ | $0.000 \pm 0.000$ | $0.5 \pm 0.0$ |
| 167 | (6)5 | 0.27(5) | $0.89 \pm 0.01$ | $9.900 \pm 3.000$ | $0.0 \pm 0.0$ |
| 168 | 5    | 0.00(0) | $0.90 \pm 0.01$ | $1.200 \pm 0.175$ | $0.0 \pm 0.0$ |
| 169 | 5    | 0.00(0) | $0.69 \pm 0.01$ | $1.540 \pm 0.102$ | $0.0 \pm 0.0$ |
| 170 | 5    | 0.00(0) | $0.39 \pm 0.00$ | $1.810 \pm 0.085$ | $0.0 \pm 0.0$ |

**Supplementary Table S6:** Results from the Lipari and Szabo model-free analysis of the  $^{15}\text{N}$ -relaxation data of Rheb $\Delta$ CT bound to GppNHp with TENSOR2

| Residue | Model | $\chi^2$ | $S^2 \pm \text{error}$ | $\tau_i [\text{ns}] \pm \text{error}$ | $R_{\text{ex}} [\text{s}^{-1}] \pm \text{error}$ |
|---------|-------|----------|------------------------|---------------------------------------|--|
| 4       | 5     | 0.00(0)  | $0.59 \pm 0.01$        | $0.95 \pm 0.07$                       | $0.0 \pm 0.0$                                    |
| 5       | 5     | 0.00(0)  | $0.74 \pm 0.01$        | $1.00 \pm 0.12$                       | $0.0 \pm 0.0$                                    |
| 6       | 2     | 4.46(0)  | $0.75 \pm 0.00$        | $0.03 \pm 0.00$                       | $0.0 \pm 0.0$                                    |
| 7       | (6)5  | 1.61(0)  | $0.88 \pm 0.02$        | $10.40 \pm 3.67$                      | $0.0 \pm 0.0$                                    |
| 8       | (6)5  | 0.06(5)  | $0.86 \pm 0.02$        | $10.40 \pm 3.62$                      | $0.0 \pm 0.0$                                    |
| 9       | 5     | 0.00(0)  | $0.92 \pm 0.01$        | $1.82 \pm 2.57$                       | $0.0 \pm 0.0$                                    |
| 10      | 5     | 0.00(0)  | $0.90 \pm 0.01$        | $1.36 \pm 2.64$                       | $0.0 \pm 0.0$                                    |
| 11      | 5     | 0.00(0)  | $0.94 \pm 0.01$        | $2.33 \pm 3.66$                       | $0.0 \pm 0.0$                                    |
| 12      | (6)5  | 0.57(5)  | $0.96 \pm 0.01$        | $10.40 \pm 4.23$                      | $0.0 \pm 0.0$                                    |
| 13      | 5     | 0.00(0)  | $0.87 \pm 0.02$        | $5.90 \pm 3.56$                       | $0.0 \pm 0.0$                                    |
| 14      | (6)5  | 0.85(0)  | $0.97 \pm 0.01$        | $10.40 \pm 4.54$                      | $0.0 \pm 0.0$                                    |
| 16      | (6)5  | 1.35(0)  | $0.79 \pm 0.03$        | $10.40 \pm 3.38$                      | $0.0 \pm 0.0$                                    |
| 17      | 5     | 0.00(0)  | $0.94 \pm 0.01$        | $2.86 \pm 4.10$                       | $0.0 \pm 0.0$                                    |
| 18      | 3     | 0.03(0)  | $0.71 \pm 0.00$        | $0.00 \pm 0.00$                       | $1.9 \pm 0.1$                                    |
| 20      | 3     | 0.15(7)  | $0.83 \pm 0.00$        | $0.00 \pm 0.00$                       | $2.9 \pm 0.1$                                    |
| 21      | 3     | 0.37(2)  | $0.81 \pm 0.00$        | $0.00 \pm 0.00$                       | $0.3 \pm 0.1$                                    |
| 22      | 3     | 0.00(0)  | $0.83 \pm 0.00$        | $0.00 \pm 0.00$                       | $1.3 \pm 0.0$                                    |
| 23      | 3     | 1.37(0)  | $0.75 \pm 0.01$        | $0.00 \pm 0.00$                       | $0.5 \pm 0.2$                                    |
| 26      | 3     | 0.12(4)  | $0.85 \pm 0.00$        | $0.00 \pm 0.00$                       | $1.4 \pm 0.1$                                    |

|    |      |         |                 |                  |               |
|----|------|---------|-----------------|------------------|---------------|
| 27 | 3    | 0.15(2) | $0.81 \pm 0.00$ | $0.00 \pm 0.00$  | $2.2 \pm 0.1$ |
| 28 | 5    | 0.00(0) | $0.94 \pm 0.01$ | $4.00 \pm 0.00$  | $0.0 \pm 0.0$ |
| 29 | (6)5 | 2.62(0) | $0.91 \pm 0.01$ | $10.40 \pm 3.84$ | $0.0 \pm 0.0$ |
| 43 | 3    | 0.01(2) | $0.85 \pm 0.00$ | $0.00 \pm 0.00$  | $0.9 \pm 0.1$ |
| 44 | (6)5 | 7.07(0) | $0.92 \pm 0.01$ | $10.40 \pm 3.90$ | $0.0 \pm 0.0$ |
| 45 | 5    | 0.00(0) | $0.97 \pm 0.01$ | $1.86 \pm 3.84$  | $0.0 \pm 0.0$ |
| 46 | 5    | 0.00(0) | $0.91 \pm 0.02$ | $2.93 \pm 3.51$  | $0.0 \pm 0.0$ |
| 47 | 3    | 2.14(0) | $0.84 \pm 0.00$ | $0.00 \pm 0.00$  | $0.2 \pm 0.0$ |
| 48 | (6)5 | 0.05(4) | $0.97 \pm 0.00$ | $10.40 \pm 4.14$ | $0.0 \pm 0.0$ |
| 49 | 5    | 0.00(0) | $0.93 \pm 0.01$ | $2.08 \pm 2.81$  | $0.0 \pm 0.0$ |
| 51 | 4    | 0.00(0) | $0.70 \pm 0.01$ | $0.02 \pm 0.01$  | $0.3 \pm 0.1$ |
| 52 | 3    | 4.46(0) | $0.74 \pm 0.00$ | $0.00 \pm 0.00$  | $1.7 \pm 0.0$ |
| 53 | 2    | 0.13(5) | $0.76 \pm 0.00$ | $0.01 \pm 0.00$  | $0.0 \pm 0.0$ |
| 54 | 4    | 0.00(0) | $0.76 \pm 0.01$ | $0.03 \pm 0.00$  | $0.7 \pm 0.1$ |
| 55 | 5    | 0.00(0) | $0.98 \pm 0.01$ | $1.61 \pm 0.00$  | $0.0 \pm 0.0$ |
| 56 | 5    | 0.00(0) | $0.93 \pm 0.01$ | $1.25 \pm 0.00$  | $0.0 \pm 0.0$ |
| 57 | 5    | 0.00(0) | $0.96 \pm 0.01$ | $0.91 \pm 3.00$  | $0.0 \pm 0.0$ |
| 58 | (6)5 | 1.20(0) | $0.89 \pm 0.01$ | $10.40 \pm 3.89$ | $0.0 \pm 0.0$ |
| 59 | (6)5 | 1.94(0) | $0.88 \pm 0.02$ | $10.40 \pm 3.76$ | $0.0 \pm 0.0$ |
| 63 | 3    | 1.37(0) | $0.72 \pm 0.00$ | $0.00 \pm 0.00$  | $2.0 \pm 0.1$ |
| 65 | 4    | 0.00(0) | $0.75 \pm 0.01$ | $0.02 \pm 0.01$  | $0.4 \pm 0.1$ |
| 66 | 3    | 0.28(4) | $0.81 \pm 0.01$ | $0.00 \pm 0.00$  | $0.9 \pm 0.2$ |
| 67 | 1    | 4.42(0) | $0.87 \pm 0.00$ | $0.00 \pm 0.00$  | $0.0 \pm 0.0$ |
| 68 | 3    | 0.08(0) | $0.76 \pm 0.00$ | $0.00 \pm 0.00$  | $0.7 \pm 0.0$ |
| 77 | 5    | 0.00(0) | $0.95 \pm 0.01$ | $2.11 \pm 4.14$  | $0.0 \pm 0.0$ |
| 79 | 5    | 0.00(0) | $0.92 \pm 0.01$ | $1.00 \pm 0.64$  | $0.0 \pm 0.0$ |
| 80 | 5    | 0.00(0) | $0.83 \pm 0.01$ | $1.77 \pm 0.63$  | $0.0 \pm 0.0$ |
| 81 | 3    | 1.74(0) | $0.86 \pm 0.00$ | $0.00 \pm 0.00$  | $0.2 \pm 0.1$ |
| 82 | 5    | 0.00(0) | $0.94 \pm 0.01$ | $2.48 \pm 3.63$  | $0.0 \pm 0.0$ |
| 83 | (6)5 | 0.58(6) | $0.84 \pm 0.02$ | $10.40 \pm 3.63$ | $0.0 \pm 0.0$ |
| 84 | (6)5 | 3.59(0) | $0.93 \pm 0.01$ | $10.40 \pm 4.14$ | $0.0 \pm 0.0$ |
| 85 | (6)5 | 1.79(0) | $0.99 \pm 0.01$ | $10.40 \pm 4.67$ | $0.0 \pm 0.0$ |
| 86 | 1    | 6.31(0) | $0.85 \pm 0.00$ | $17 \pm 0.00$    | $0.0 \pm 0.0$ |

|     |      |          |             |              |           |
|-----|------|----------|-------------|--------------|-----------|
| 87  | (6)3 | 3.49(0)  | 0.84 ± 0.00 | 0.00 ± 0.00  | 3.2 ± 0.1 |
| 88  | 3    | 0.42(3)  | 0.82 ± 0.00 | 0.00 ± 0.00  | 0.9 ± 0.0 |
| 90  | 5    | 0.00(0)  | 0.98 ± 0.01 | 2.17 ± 4.56  | 0.0 ± 0.0 |
| 92  | (6)5 | 1.13(0)  | 0.96 ± 0.01 | 10.40 ± 4.17 | 0.0 ± 0.0 |
| 93  | 3    | 1.22(0)  | 0.83 ± 0.00 | 0.00 ± 0.00  | 0.2 ± 0.0 |
| 94  | 3    | 1.47(0)  | 0.85 ± 0.00 | 0.00 ± 0.00  | 0.8 ± 0.0 |
| 95  | 3    | 0.00(1)  | 0.84 ± 0.00 | 0.00 ± 0.00  | 0.9 ± 0.0 |
| 96  | 3    | 0.70(5)  | 0.83 ± 0.00 | 0.00 ± 0.00  | 2.2 ± 0.0 |
| 97  | 3    | 0.45(5)  | 0.88 ± 0.00 | 0.00 ± 0.00  | 0.2 ± 0.1 |
| 98  | 5    | 0.00(0)  | 1.07 ± 0.01 | 1.09 ± 1.78  | 0.0 ± 0.0 |
| 99  | 3    | 0.01(5)  | 0.83 ± 0.00 | 0.00 ± 0.00  | 1.7 ± 0.0 |
| 100 | 3    | 0.70(2)  | 0.76 ± 0.00 | 0.00 ± 0.00  | 3.4± 0.0  |
| 101 | (3)3 | 10.60(0) | 0.86 ± 0.00 | 0.00 ± 0.00  | 0.5 ± 0.0 |
| 102 | 3    | 1.37(0)  | 0.81 ± 0.00 | 0.00 ± 0.00  | 1.2 ± 0.0 |
| 107 | 4    | 0.00(0)  | 0.77 ± 0.01 | 0.03 ± 0.01  | 1.8 ± 0.1 |
| 108 | 5    | 0.00(0)  | 0.89 ± 0.01 | 1.23 ± 0.26  | 0.0 ± 0.0 |
| 109 | 3    | 0.67(1)  | 0.76 ± 0.00 | 0.00 ± 0.00  | 0.2 ± 0.1 |
| 110 | 5    | 0.00(0)  | 0.75 ± 0.01 | 1.11 ± 0.07  | 0.0 ± 0.0 |
| 111 | 5    | 0.00(0)  | 0.75 ± 0.01 | 1.47 ± 0.17  | 0.0 ± 0.0 |
| 112 | 5    | 0.00(0)  | 0.77 ± 0.01 | 0.92 ± 0.06  | 0.0 ± 0.0 |
| 114 | 5    | 0.00(0)  | 0.92 ± 0.01 | 1.71 ± 3.21  | 0.0 ± 0.0 |
| 115 | 5    | 0.00(0)  | 0.93 ± 0.01 | 1.66 ± 2.44  | 0.0 ± 0.0 |
| 116 | 5    | 0.00(0)  | 0.86 ± 0.02 | 2.80 ± 2.78  | 0.0 ± 0.0 |
| 117 | (6)5 | 0.20(0)  | 0.86 ± 0.02 | 10.40 ± 3.56 | 0.0 ± 0.0 |
| 118 | (6)3 | 7.98(0)  | 0.81 ± 0.00 | 0.00 ± 0.00  | 0.7 ± 0.1 |
| 119 | (6)5 | 0.29(5)  | 0.98 ± 0.00 | 10.40 ± 4.25 | 0.0 ± 0.0 |
| 120 | 3    | 0.01(3)  | 0.81 ± 0.00 | 0.00 ± 0.00  | 0.0 ± 0.5 |
| 121 | (6)3 | 5.65(0)  | 0.82 ± 0.00 | 0.00 ± 0.00  | 1.9± 0.0  |
| 122 | (6)5 | 3.12(0)  | 1.02 ± 0.04 | 0.19 ± 3.84  | 0.0 ± 0.0 |
| 123 | (3)3 | 8.72(0)  | 0.84 ± 0.00 | 0.00 ± 0.00  | 0.3 ± 0.0 |
| 124 | 3    | 0.00(9)  | 0.80 ± 0.00 | 0.00 ± 0.00  | 0.4 ± 0.0 |
| 126 | 4    | 0.00(0)  | 0.77 ± 0.01 | 0.03 ± 0.01  | 0.8 ± 0.1 |
| 127 | (6)5 | 0.28(0)  | 0.98± 0.00  | 10.30 ± 4.17 | 0.0 ± 0.0 |

|     |      |         |             |              |           |
|-----|------|---------|-------------|--------------|-----------|
| 128 | 3    | 0.61(6) | 0.87 ± 0.00 | 0.00 ± 0.00  | 1.9 ± 0.1 |
| 129 | 5    | 0.00(0) | 0.91 ± 0.01 | 1.91 ± 3.32  | 0.0 ± 0.0 |
| 130 | 3    | 0.25(2) | 0.80 ± 0.00 | 0.00 ± 0.00  | 0.1 ± 0.0 |
| 131 | 1    | 4.94(0) | 0.82 ± 0.00 | 0.00 ± 0.00  | 0.0 ± 0.0 |
| 132 | 3    | 2.69(0) | 0.80 ± 0.00 | 0.00 ± 0.00  | 0.8 ± 0.0 |
| 133 | 3    | 0.05(3) | 0.82 ± 0.00 | 0.00 ± 0.00  | 1.1 ± 0.0 |
| 134 | (6)5 | 1.34(0) | 0.89 ± 0.02 | 10.40 ± 3.62 | 0.0 ± 0.0 |
| 135 | 3    | 0.81(1) | 0.84 ± 0.00 | 0.00 ± 0.00  | 1.3 ± 0.0 |
| 136 | 3    | 1.36(0) | 0.82 ± 0.00 | 0.00 ± 0.00  | 1.5 ± 0.0 |
| 137 | 3    | 0.00(2) | 0.87 ± 0.00 | 0.00 ± 0.00  | 1.0 ± 0.0 |
| 138 | 3    | 2.73(0) | 0.84 ± 0.00 | 0.00 ± 0.00  | 0.6 ± 0.0 |
| 139 | 3    | 0.66(0) | 0.83 ± 0.00 | 0.00 ± 0.00  | 1.3 ± 0.0 |
| 140 | 3    | 0.03(5) | 0.79 ± 0.00 | 0.00 ± 0.00  | 1.7 ± 0.0 |
| 141 | 1    | 1.89(0) | 0.81 ± 0.00 | 0.00 ± 0.00  | 0.0 ± 0.0 |
| 142 | 3    | 0.01(6) | 0.79 ± 0.00 | 0.00 ± 0.00  | 1.9 ± 0.0 |
| 143 | 3    | 1.06(0) | 0.80 ± 0.00 | 0.00 ± 0.00  | 1.5 ± 0.0 |
| 144 | 5    | 0.00(0) | 0.92 ± 0.01 | 1.39 ± 0.67  | 0.0 ± 0.0 |
| 145 | (6)5 | 6.19(0) | 0.98 ± 0.00 | 10.40 ± 4.23 | 0.0 ± 0.0 |
| 146 | 3    | 0.19(4) | 0.84 ± 0.00 | 0.00 ± 0.00  | 0.1 ± 0.0 |
| 147 | 5    | 0.00(0) | 0.98 ± 0.01 | 1.06 ± 3.25  | 0.0 ± 0.0 |
| 148 | 3    | 0.03(5) | 0.85 ± 0.00 | 0.00 ± 0.00  | 0.7 ± 0.0 |
| 149 | 3    | 0.49(7) | 0.76 ± 0.00 | 0.00 ± 0.00  | 1.1 ± 0.1 |
| 150 | (6)5 | 4.01(0) | 0.98 ± 0.01 | 10.40 ± 4.53 | 0.0 ± 0.0 |
| 151 | 3    | 0.09(7) | 0.83 ± 0.00 | 0.00 ± 0.00  | 0.3 ± 0.0 |
| 152 | 3    | 0.03(7) | 0.82 ± 0.00 | 0.00 ± 0.00  | 0.5 ± 0.0 |
| 154 | 3    | 1.05(0) | 0.81 ± 0.00 | 0.00 ± 0.00  | 0.5 ± 0.1 |
| 155 | 3    | 0.96(6) | 0.85 ± 0.00 | 0.00 ± 0.00  | 0.5 ± 0.0 |
| 156 | 5    | 0.00(0) | 0.96 ± 0.01 | 2.00 ± 3.44  | 0.0 ± 0.0 |
| 157 | 3    | 0.00(2) | 0.82 ± 0.00 | 0.00 ± 0.00  | 0.9 ± 0.0 |
| 158 | 3    | 2.50(0) | 0.83 ± 0.00 | 0.00 ± 0.00  | 1.3 ± 0.0 |
| 159 | 3    | 1.12(0) | 0.85 ± 0.00 | 0.00 ± 0.00  | 0.9 ± 0.0 |
| 160 | (6)5 | 1.38(0) | 0.94 ± 0.01 | 10.40 ± 4.26 | 0.0 ± 0.0 |
| 162 | 3    | 0.02(4) | 0.86 ± 0.00 | 0.00 ± 0.00  | 1.2 ± 0.0 |



|     |      |         |                 |                  |               |
|-----|------|---------|-----------------|------------------|---------------|
| 164 | 3    | 0.14(7) | $0.84 \pm 0.00$ | $0.00 \pm 0.00$  | $0.6 \pm 0.0$ |
| 165 | 3    | 1.78(0) | $0.86 \pm 0.00$ | $0.00 \pm 0.00$  | $0.8 \pm 0.0$ |
| 166 | 3    | 0.13(9) | $0.85 \pm 0.00$ | $0.00 \pm 0.00$  | $0.1 \pm 0.0$ |
| 167 | (6)5 | 0.31(4) | $0.99 \pm 0.00$ | $10.40 \pm 4.54$ | $0.0 \pm 0.8$ |
| 168 | 2    | 1.28(0) | $0.81 \pm 0.00$ | $0.02 \pm 0.00$  | $0.0 \pm 0.0$ |
| 169 | 5    | 0.00(0) | $0.84 \pm 0.00$ | $1.23 \pm 0.06$  | $0.0 \pm 0.0$ |
| 170 | 5    | 0.00(0) | $0.42 \pm 0.00$ | $1.77 \pm 0.06$  | $0.0 \pm 0.0$ |

### Supplementary Figure Legends

**Fig. S1:**  $^1\text{H}$ - $^{15}\text{N}$ (1,2) spectrum of Rheb $\Delta$ CT in the GDP-bound state. The assignments are indicated by the one letter amino acid code and the sequence position. Side chain amide proton resonances of glutamine and asparagine residues are connected by black lines. The resonances of some residues (assignment labels in brackets) are only visible at lower contour level. Negative peaks (red) correspond to residues that are spectrally folded.

**Fig. S2:**  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum of Rheb $\Delta$ CT in the GppNHp-bound state. The assignments are indicated by the one letter amino acid code and the sequence position. Side chain amide proton resonances of glutamine and asparagine residues are connected by black lines. The resonances of some residues (assignment labels in brackets) are only visible at lower contour level. Negative peaks (red) correspond to residues that are spectrally folded.

**Fig. S3:** (A) Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of Rheb $\Delta$ CT in the GppNHp- (red) and GppCp- (green) bound states. (B) Larger  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectrum of Rheb $\Delta$ CT in the GppCp-bound state. The assignments are indicated by the one letter amino acid code and the sequence position. Side chain amide proton resonances of glutamine and asparagine residues are connected by black lines. The resonances of some residues (assignment labels in brackets) are only visible at lower contour level. Negative peaks (red) correspond to residues that are spectrally folded.

**Fig. S4:** Comparison of the backbone dynamics of Rheb $\Delta$ CT in the GppNHp- (red) and GppCp- (green) bound states. Shown are plots of the  $^{15}\text{N}$ -relaxation data including  $^{15}\text{N}$ - $T_1$  (top panel),  $-T_2$  (second panel), and  $\{^1\text{H}\}$ - $^{15}\text{N}$  NOE (third panel) values. The location of the

G-boxes and the switch 1 and 2 (SW1 & 2) regions (1,2) and the secondary structure elements are indicated at the top. Filled grey arrows represent  $\beta$ -sheet conformation and grey spirals helical conformation. The secondary structure content was derived from the solution structure of rat Rheb-GDP (PDB-ID 2L0X) (3). That of mouse Rheb-GppNHp is almost identical (PDB- ID 4O25) (4). A superposition of the respective  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra is shown in SI Fig. S3A.

**Fig. S5:** More NMR data recorded to characterize the interaction between Rheb $\Delta$ CT and FKBP38-BD. **(A)** View of the structure of mouse Rheb-GppNHp with the spectral changes observed in Fig. 3D mapped onto it (magenta – weak to medium and orange – very weak chemical shift changes. The representation corresponds to a view from the top (=  $90^\circ$  rotation around a horizontal axis) on the structure as shown in Fig. 3E (left side). **(B)** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -Rheb $\Delta$ CT-GppNHp in the presence of increasing concentrations of unlabeled FKBP38-BD. The color coding and the molar ratios of the two proteins are indicated in the upper left. The residues that showed medium (magenta) or weak (orange) shifts comparing  $^{15}\text{N}$ -Rheb $\Delta$ CT-GppNHp in the absence (black) and presence of unlabeled FKBP38-BD (red) in Fig. 3C are labeled in the same color coding. The boxed region contains peaks for the side chain  $-\text{NH}_2$  groups of glutamines and asparagines. **(C-E)** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ - FKBP38-BD in the presence of increasing concentrations of unlabeled Rheb $\Delta$ CT-GDP or –GppNHp or a 13mer peptide corresponding to the switch 1 region of Rheb, respectively. The color coding and the molar ratios of the two components are indicated in the upper left. **(F)** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -Rheb $\Delta$ CT-GppCp in the absence (black) and presence of unlabeled FKBP38-BD (red). That  $^{15}\text{N}$ -Rheb $\Delta$ CT-GppCp did not show the same shifts as the GppNHp state may be explained by the fact that it was not fully exchanged, which is indicated by a few remaining peaks of the GDP state (most black peaks with no red peaks on top). Since the latter disappeared upon addition of FKBP38-BD, its presence appeared to have stimulated the further exchange from the GDP to the GppCp state of Rheb $\Delta$ CT. Note that the exchange of GDP to the GTP-analogue GppNHp occurred generally faster during the sample preparation for the NMR studies (see methods) than to GppCp using otherwise the same conditions.

**Fig. S6:** NMR data recorded to characterize a potential influence of the addition of  $\text{CaCl}_2$  on the interaction between FKBP38-BD and Rheb $\Delta$ CT. **(A)** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC

spectra of  $^{15}\text{N}$ - FKBP38-BD in the absence (black) and presence of 2 mM  $\text{CaCl}_2$  (red) in the buffer. Two of the residues of FKBP38-BD that have been suggested to weakly interact with  $\text{Ca}^{2+}$  (5) are labeled with the one letter amino acid code and the sequence position in the full-length protein and in brackets in the protein used in the published study (5). **(B)** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ - FKBP38-BD in the presence of only 2 mM  $\text{CaCl}_2$  in the buffer in the absence (black) and presence of unlabeled Rheb $\Delta$ CT-GppNHp (red). **(C)** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -Rheb $\Delta$ CT-GppNHp in the absence (black) and presence of unlabeled FKBP38-BD and 2 mM  $\text{CaCl}_2$  (red). The residues that showed medium (magenta) or weak (orange) shifts comparing  $^{15}\text{N}$ -Rheb $\Delta$ CT-GppNHp in the absence (black) and presence of unlabeled FKBP38-BD (red) in Fig. 3C are labeled in the same color coding. The boxed region contains peaks for the side chain  $-\text{NH}_2$  groups of glutamines and asparagines. Most of the medium shifting ones such as G101, H100, K97 or I99 show also shifts here. However R7 and I47 did not show significant shifts. The peak of the side chain amide proton of W141 has presumably a slightly different peak position as in Fig. 3C and overlaps here with L12. Most of the residues showing weak shifts in the Fig. 3C (orange labels) do not show a significant shift here. Presumably because of the slightly different buffer conditions ( $\pm$  2 mM  $\text{CaCl}_2$ ) and slight differences in the used protein concentrations and corresponding molar ratios.

**Fig. S7:** Comparison of the backbone dynamics of Rheb $\Delta$ CT in the GppNHp-bound state in the absence (red) and presence (blue) of unlabeled FKBP38-BD. Shown are plots of the  $^{15}\text{N}$  relaxation data including  $^{15}\text{N}$ - $T_1$  (top panel),  $-T_2$  (second panel), and  $\{^1\text{H}\}$ - $^{15}\text{N}$  NOE (third panel) values. The location of the G-boxes and the switch 1 and 2 (SW1 & 2) regions and the secondary structure elements are indicated at the top. Filled grey arrows represent  $\beta$ -sheet conformation and grey spirals helical conformation. The secondary structure content was derived from the solution structure of rat Rheb-GDP (PDB-ID 2L0X) (3). That of mouse Rheb-GppNHp is almost identical (PDB- ID 4O25) (4). A superposition of the respective  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra is shown in Fig. 3C.

**Fig. S8:** More NMR data regarding the effect of FKBP38-BD on the GDP to GppNHp exchange of Rheb $\Delta$ CT. (A-F) Superpositions of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -Rheb $\Delta$ CT-GDP in the presence of GppNHp and if indicated at the top of unlabeled FKBP38-BD at a molar rate of 1:102 and/or catalytic amounts of Antarctic phosphatase (= PPase) after

different incubation times. The color coding is indicated in upper left of each plot. The  $^1\text{H}$ - $^{15}\text{N}$  crosspeaks of the residues of Rheb $\Delta$ CT-GDP showing strong chemical shift changes to the GppNHp-bound state and that have been considered for the rate analysis are labeled with the one letter amino acid code and the sequence position. The corresponding analysis for the shown series **A** and **B** (**A1/B1** - only FKBP38-BD present, **A2/B2** - FKBP38-BD and PPase present, **A3/B3** only PPase present) are displayed as a function of the residue sequence position in SI Fig. S10 (C) and (D), respectively.

**Fig. S9:** More NMR data regarding the effect of FKBP38-BD on the GDP to GppNHp exchange of Rheb $\Delta$ CT. (A-E) Superpositions of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -Rheb $\Delta$ CT-GDP in the presence of GppNHp and if indicated at the top of unlabeled FKBP38-BD at a molar ratio of 1:102 (series **C**) or 1:96 (series **F**) and or catalytic amounts of Antarctic phosphatase (= PPase) after different incubation times. The color coding is indicated in the upper left of each plot. The  $^1\text{H}$ - $^{15}\text{N}$  crosspeaks of the residues of Rheb $\Delta$ CT-GDP showing strong chemical shift changes to the GppNHp-bound state and that have been considered for the rate analysis are labeled with the one letter amino acid code and the residue number. The corresponding analysis for the shown series **C** and **F** (**C1/F1**, only FKBP38-BD present, **C2/F2** - FKBP38-BD and PPase present, **C3/F3** only PPase present) are displayed as a function of the residue sequence position in SI Fig. S10 (E) and (F), respectively.

**Fig. S10:** Superposition of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -Rheb $\Delta$ CT-GDP in the presence of GppNHp and unlabeled FKBP38-BD at a molar ratio of 1:15 (series **H**) and either catalytic amounts of Antarctic phosphatase (= PPase) (A) or no Antarctic phosphatase (B) after different incubation times. The color coding is indicated in the upper left of each plot. If FKBP38-BD is present in such high concentrations compared to Rheb the exchange is so fast that the peaks of the starting GDP state are mostly not visible anymore. Thus the peaks of the target GppNHp and not of the starting GDP state have been labeled with the one letter amino acid code and the sequence position. (**C-F**) Plots of the determined exchange times  $\tau_{\text{ex}}$  as a function of the residue sequence position for the series A-C and F (see also Fig. 4A-B, SI Fig. S8-S9, and table 1).

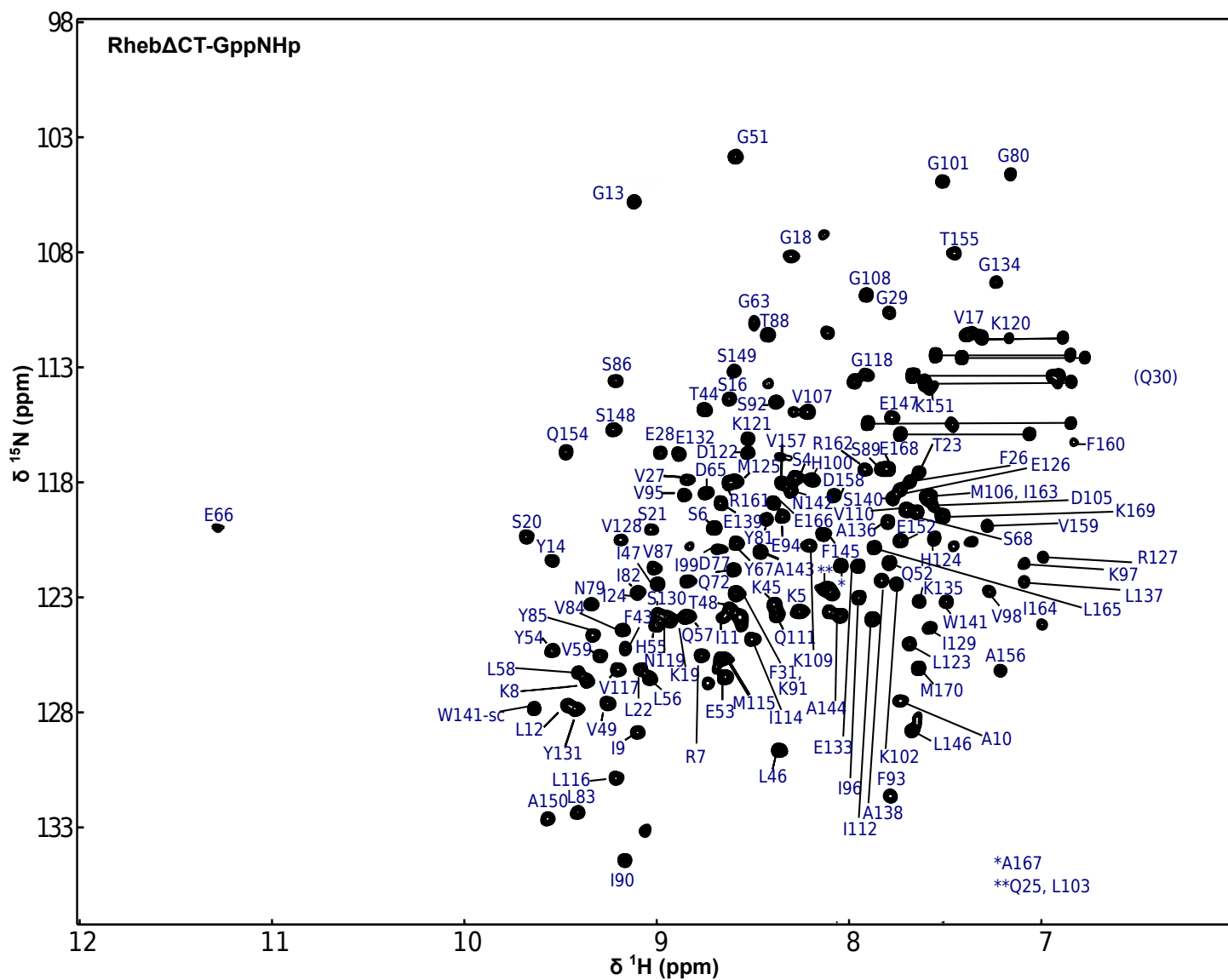
**Fig. S11:** Picture of the alignment of the amino acid sequences of human Rheb, Ras (p21ras), and Cdc42. The respective Uniprot codes are given at the beginning of each line. The figure

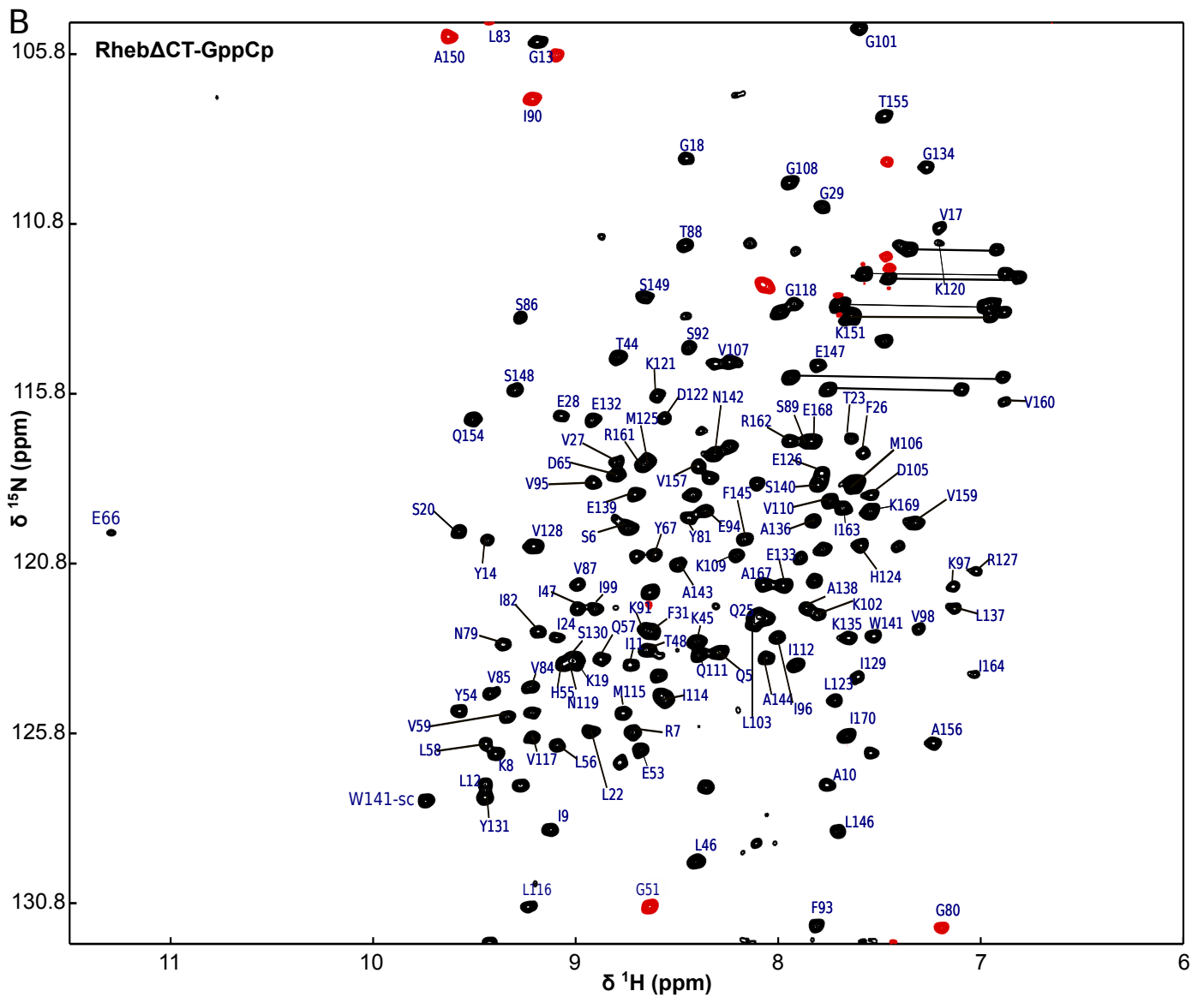
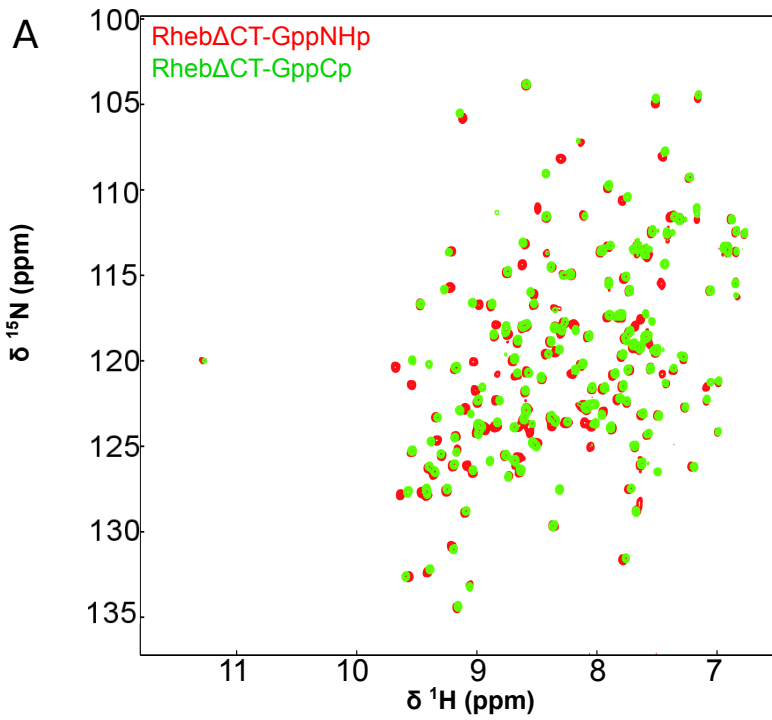
was made using MultAlin (6) for the alignment and ESPript (7) for the illustration of the alignment.

### Supplementary References

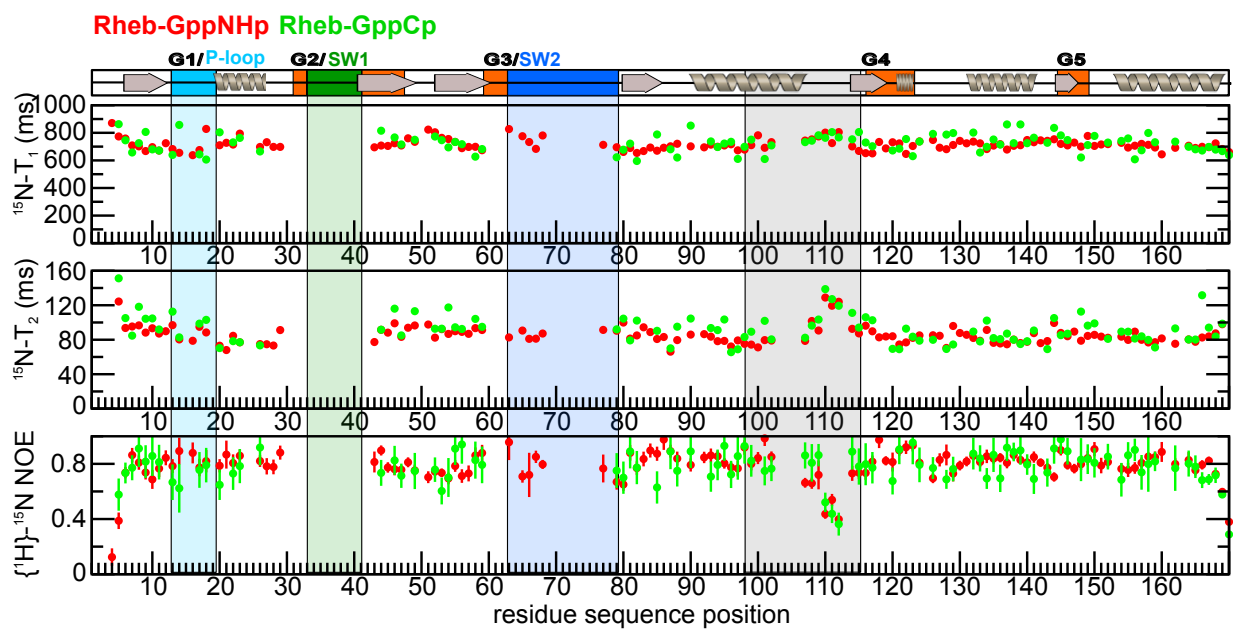
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2. Heard, J. J., Fong, V., Bathaie, S. Z., and Tamanoi, F. (2014) Recent progress in the study of the Rheb family GTPases. *Cellular signalling* **26**, 1950-1957
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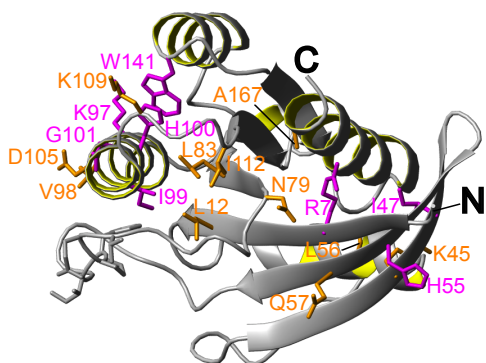




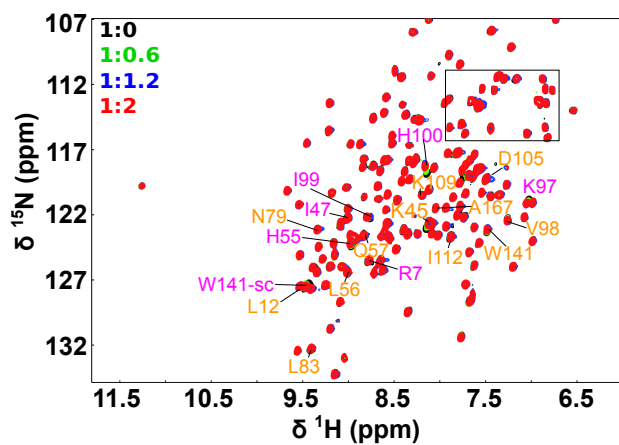




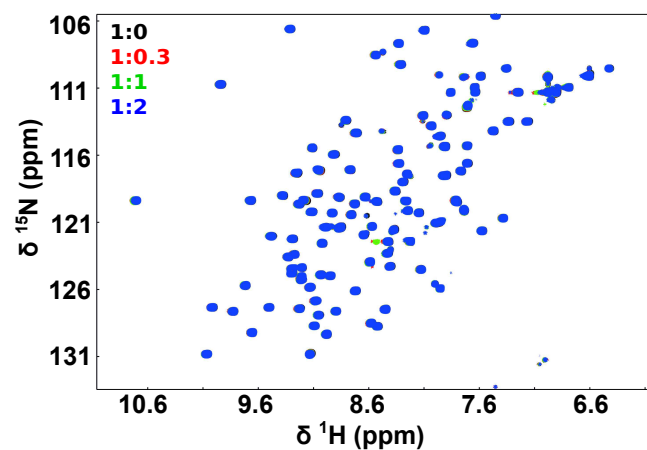
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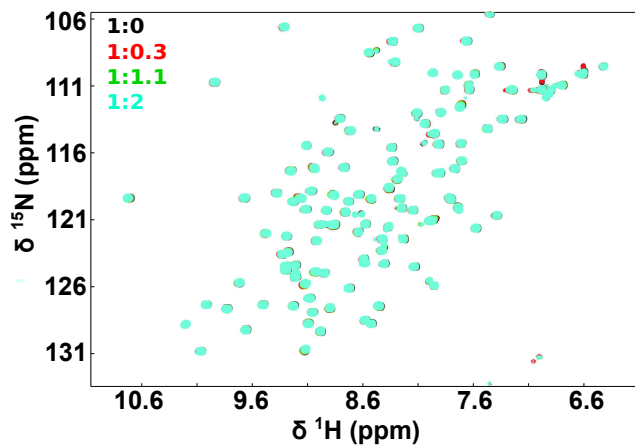
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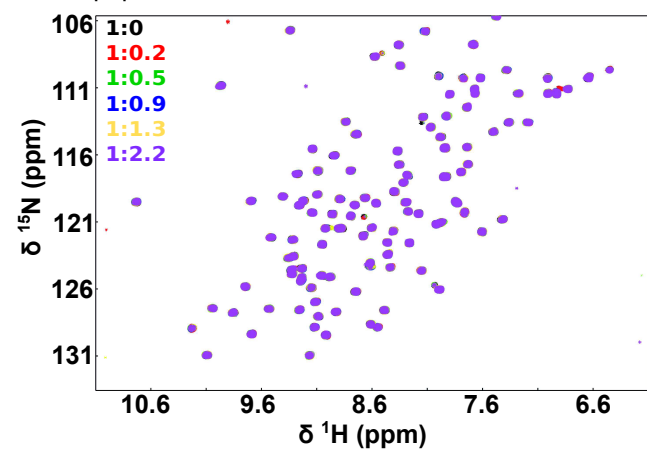
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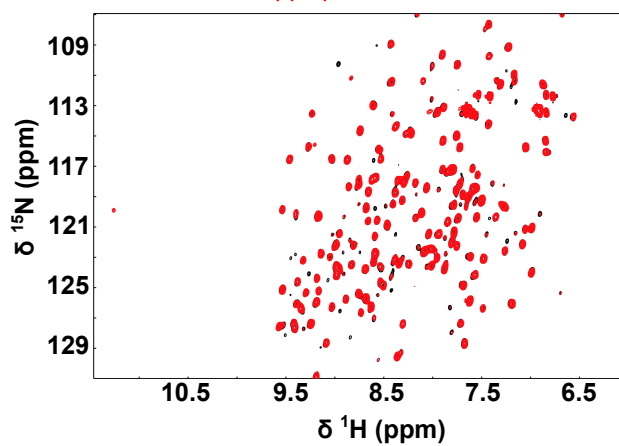
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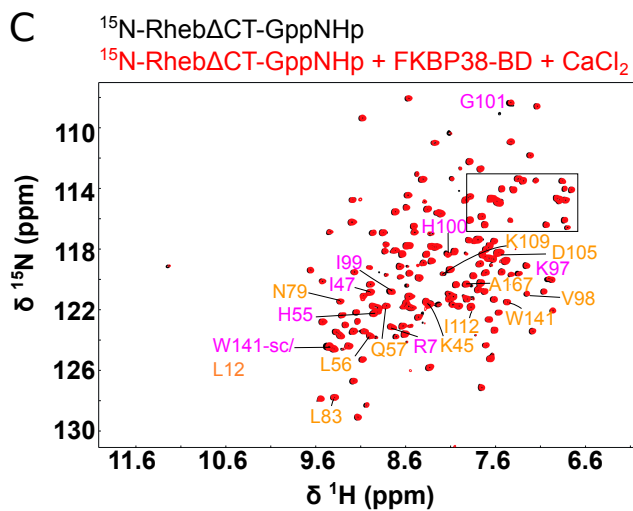
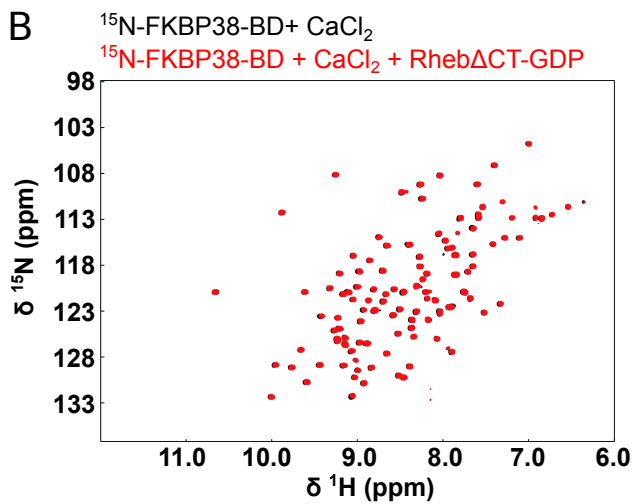
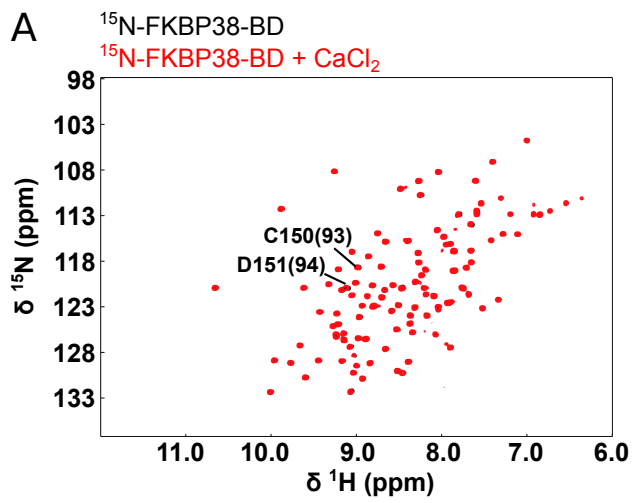


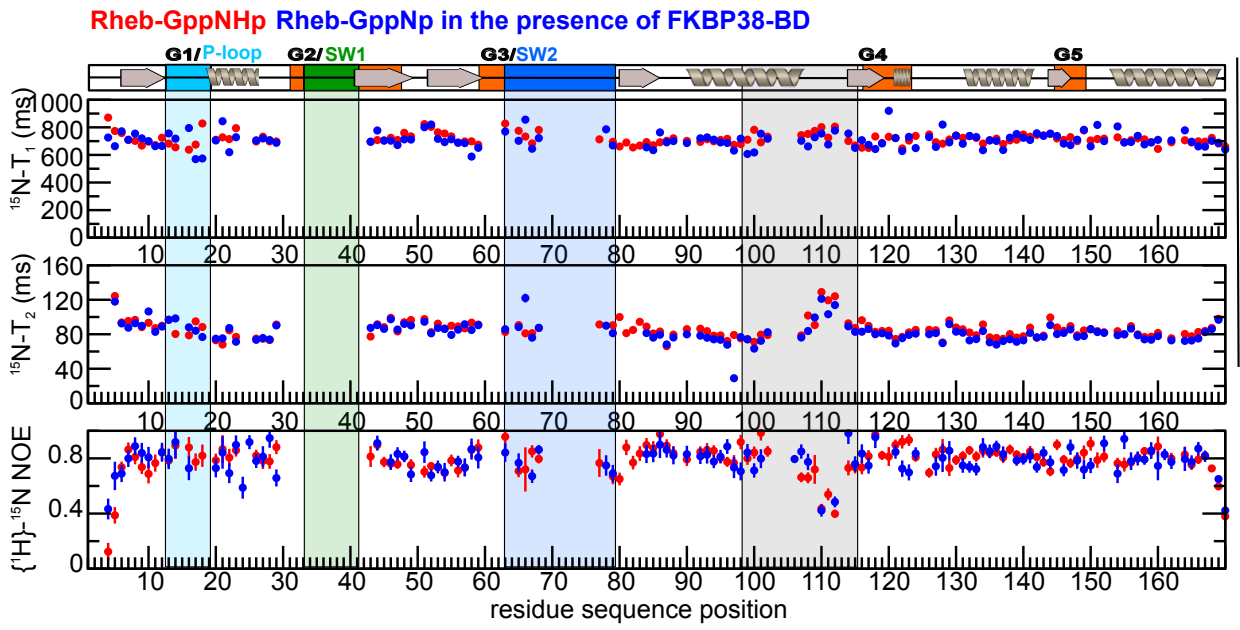
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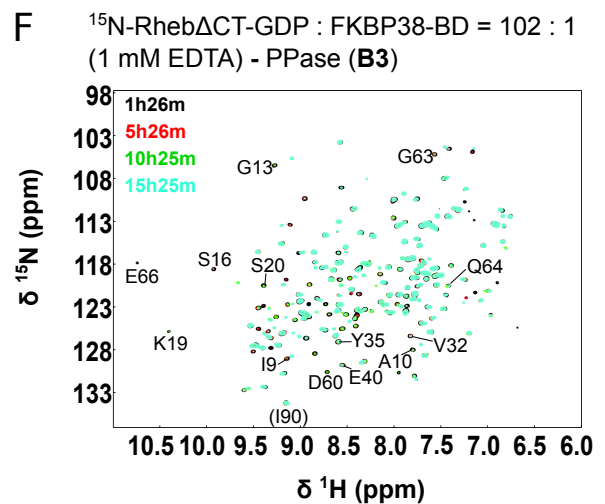
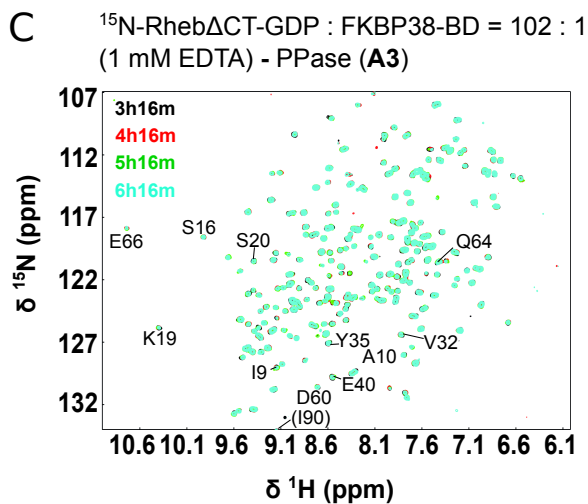
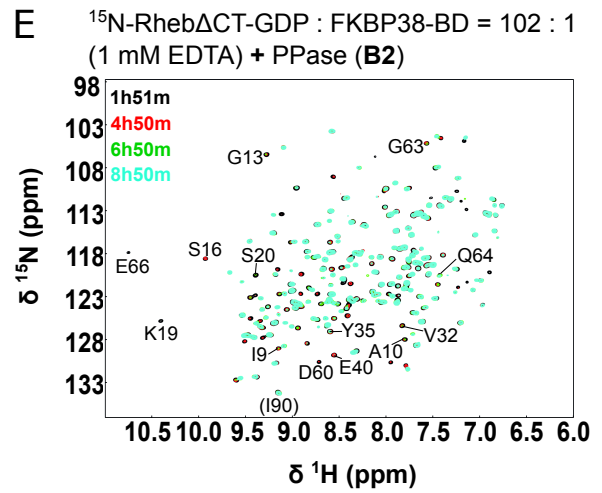
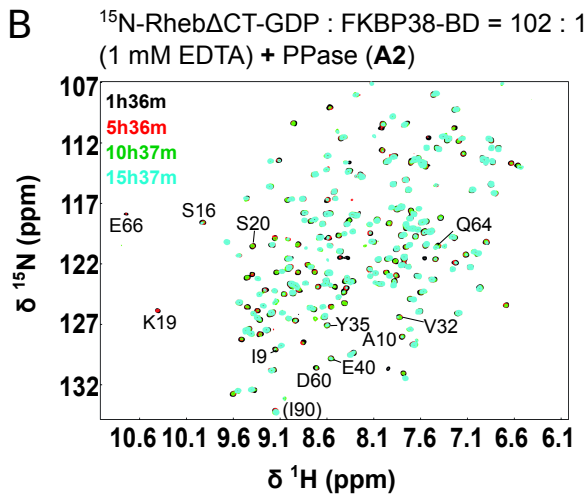
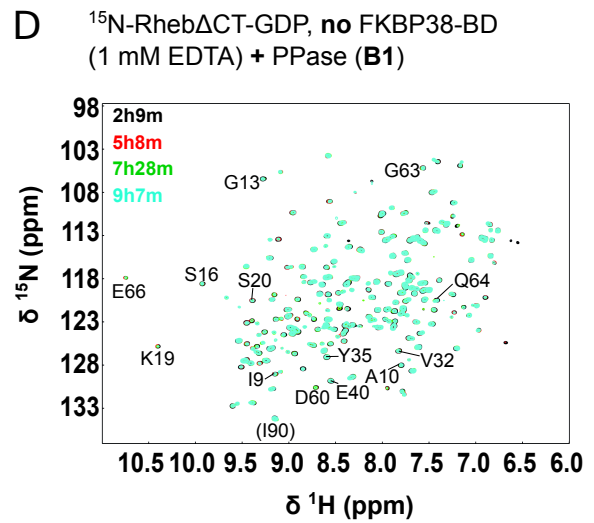
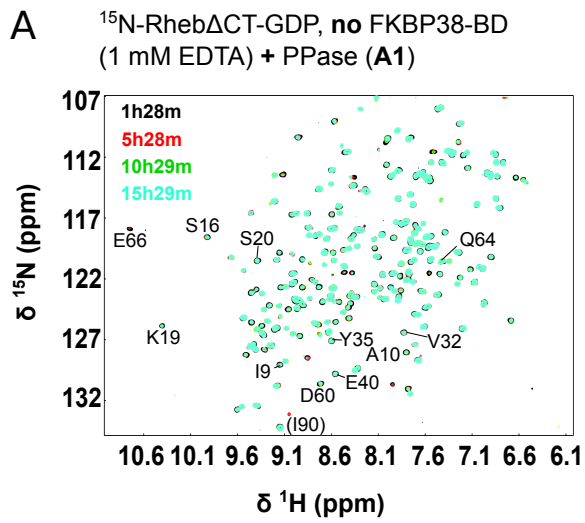


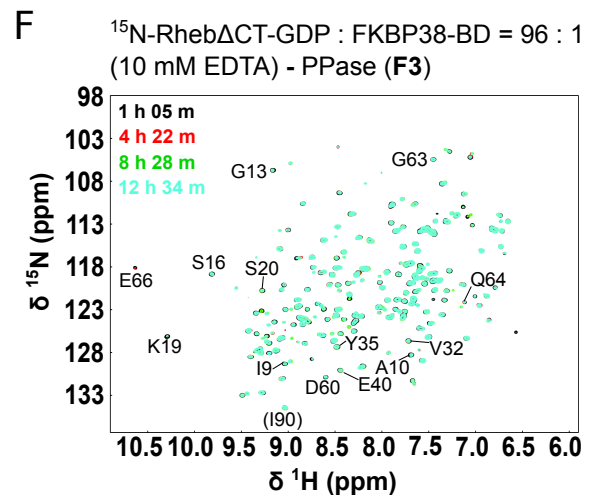
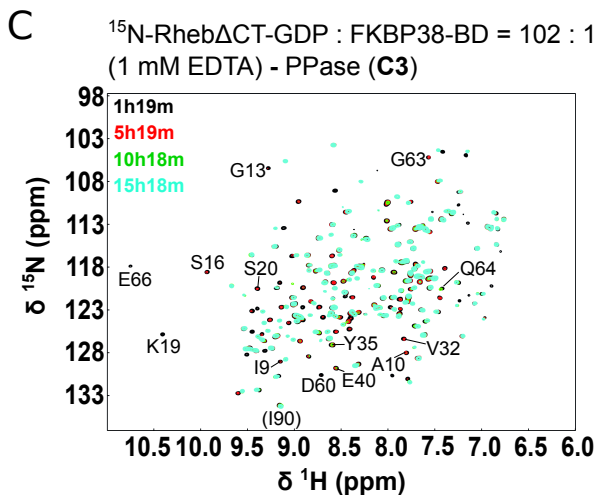
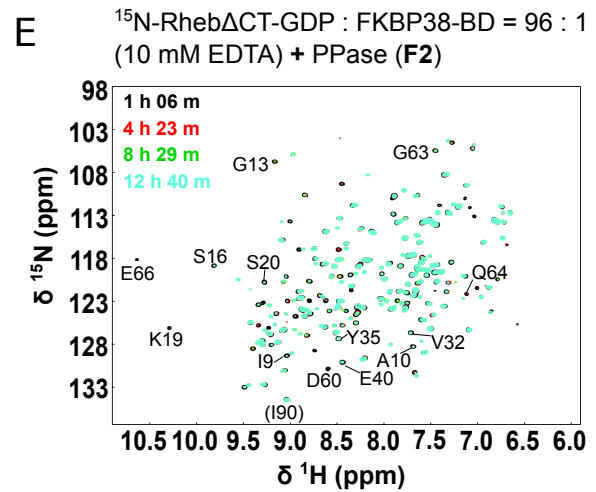
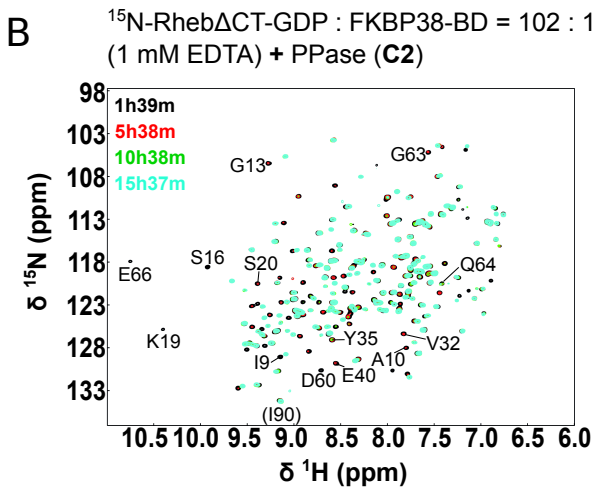
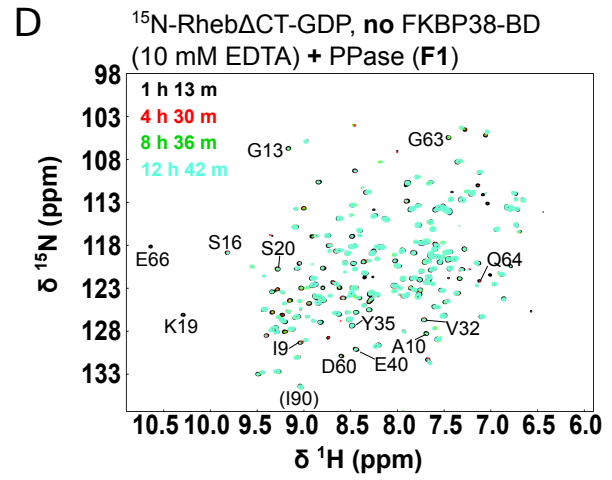
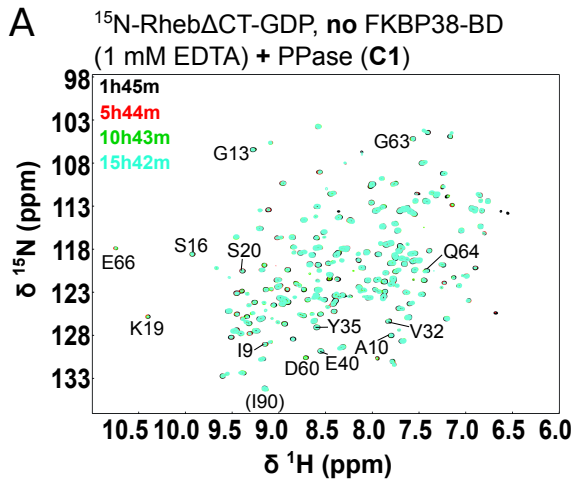
F  $^{15}\text{N}$ -Rheb $\Delta$ CT-GppCp free  
 $^{15}\text{N}$ -Rheb $\Delta$ CT-GppCp + FKBP38-BD

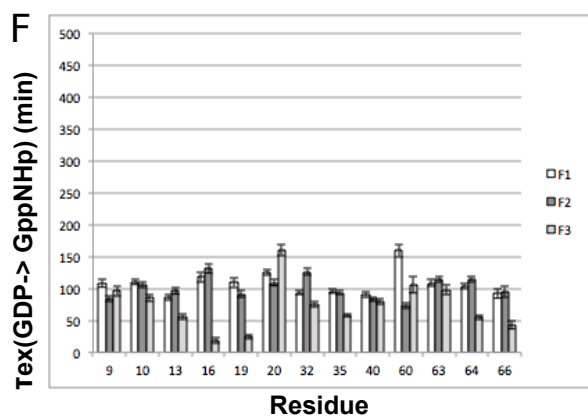
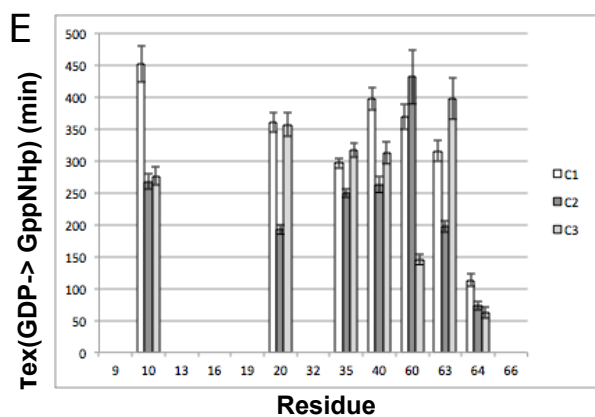
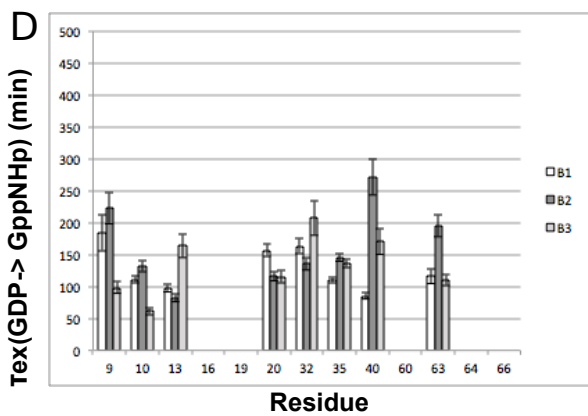
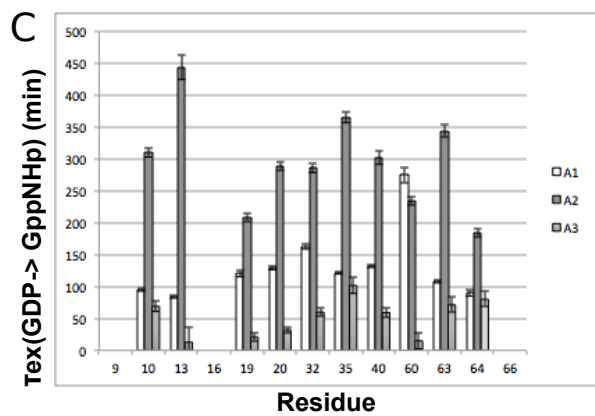
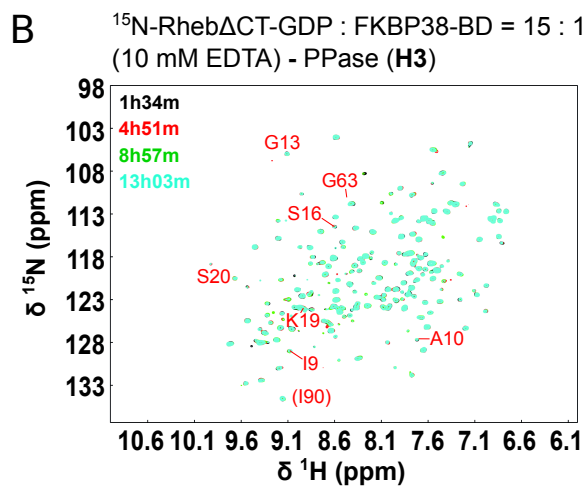
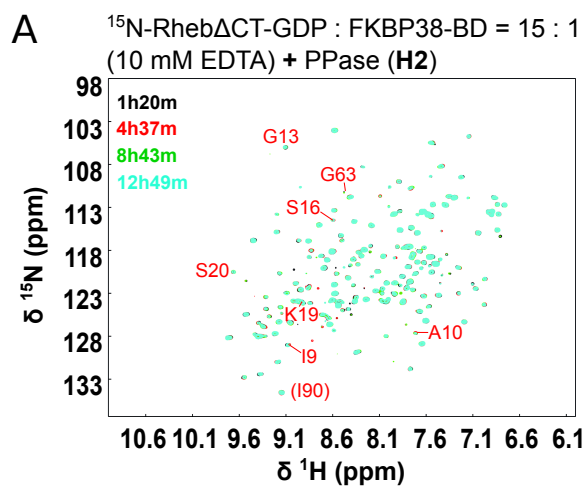












De Cicco et al.  
SI Fig. S11

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1           10           20           30           40           50           60
sp|Q15382|RHEB_HUMAN MPQS KSRK IAILG YRS VGKSS LTIQFVEGQ FVDSYDPTIENT FTKLITVNGQEH LQLVD
sp|P01112|RASH_HUMAN ... MTEYKLVVVGAGG VGKSA LTIQLIQNH FVDEYDPTIED SYRKQVVVIDGETCLLDILD
sp|P60953|CDC42_HUMA ... MQTIKCVVVG DGA VGKTC LLSYTTNKF PSEYVPTVFDN YAVTVMI GEPYT LGLFD
consensus>50 ... m...K.v!vG.g.VGKs.LtIqfven.FvdeYdPT!e#.%k!!!nG#.y.Lql.D

```

```

70           80           90           100          110          120
sp|Q15382|RHEB_HUMAN TAGQDEYSIFPQTYSIDINGYILVYSVTSISKSFVVIKVIHGKLLDMVGKVQIPIMLVGNK
sp|P01112|RASH_HUMAN TAGQEEYSAMRDQYMRTEGEGFLCVFAINNTKSFEDIHQYREQIKRVKDSDDVPMVLVGNK
sp|P60953|CDC42_HUMA TAGQEDYDRLRPLSYPTDVFVCFVSVSPS SFENVKE.KWVPEITHHCPKTFLLVGTQ
consensus>50 TAGQ##Ys.mrq.y..d.#g%l.v%s!.s.kSFEd!kq.....qiP.mLVGnk

```

```

130          140          150          160          170
sp|Q15382|RHEB_HUMAN KDIHMERVTSYE EGKALAE SWNAAELES SAKENQTAV DVFRRITL EAE..KM..DGAASQ
sp|P01112|RASH_HUMAN CDI.AARTVESRQAQDLARSYGIPYIETS AKTRQGVEDAFYTLVREIRQHKL..RKLNP
sp|P60953|CDC42_HUMA IDLRDDEPSTIEK LAKNKQKPIITPETAEKLARDLKA VKYVECSALTQKGLKNVFEAILAA
consensus>50 .DL..er.i...eakdla.s....f.E.sAke.q.v.dvf...i.#....km..d.....

```

```

180
sp|Q15382|RHEB_HUMAN GKS SCSVM...
sp|P01112|RASH_HUMAN DESGPGCMSCKCVLS
sp|P60953|CDC42_HUMA LEPPEPKSRR CVLL
consensus>50 .es....ms..cvl.

```