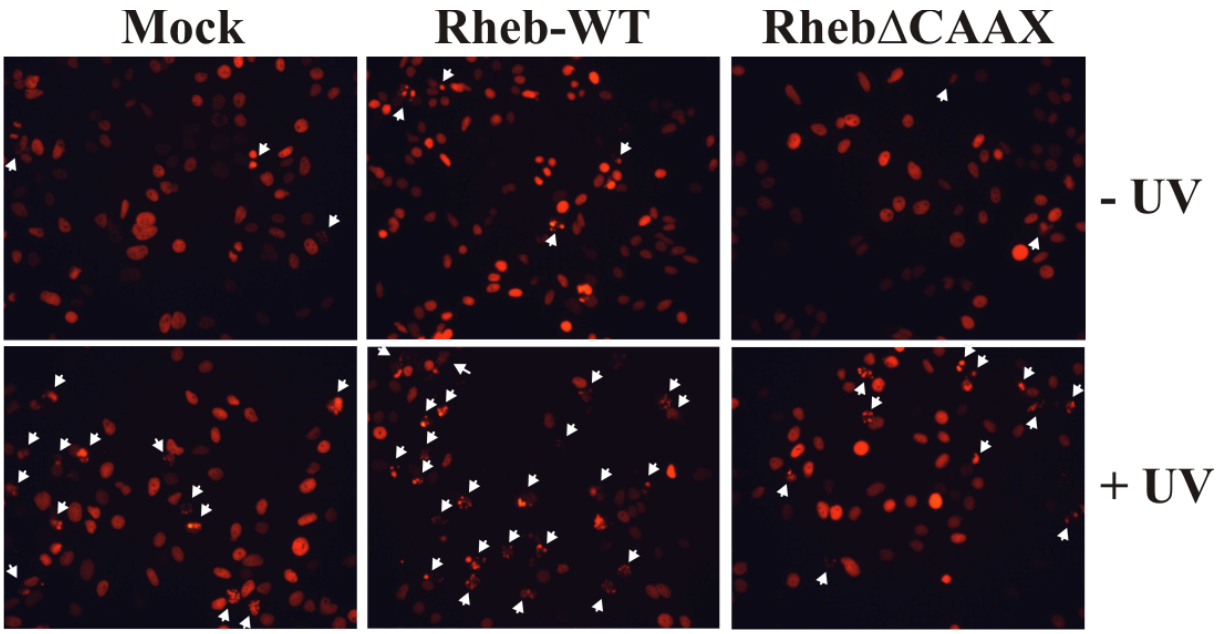
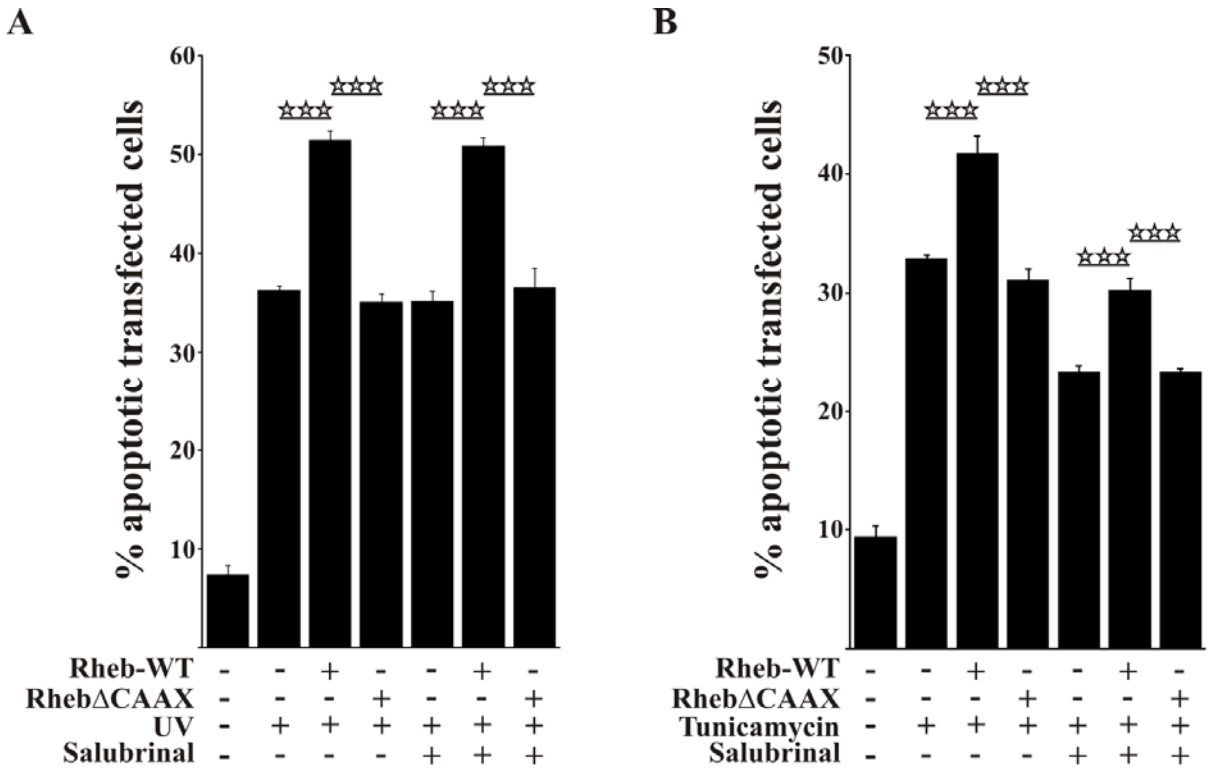
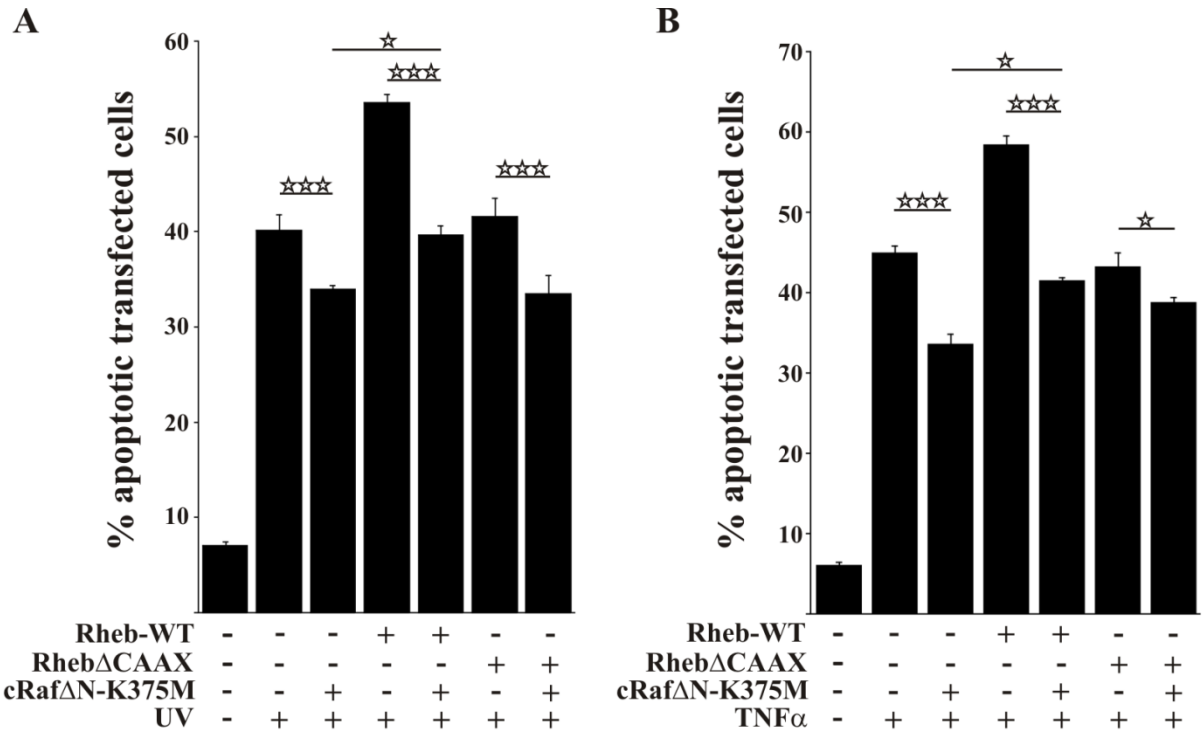


Supplemental Fig. S1: Representative pictures of HeLa cells transfected with Rheb-WT, RhebΔCAAX or vehicle together with a mRFP-H2B reporter plasmid (molar ratio 5:1) before and after UV-light exposure. Arrows showing condensed or fragmented apoptotic nuclei.

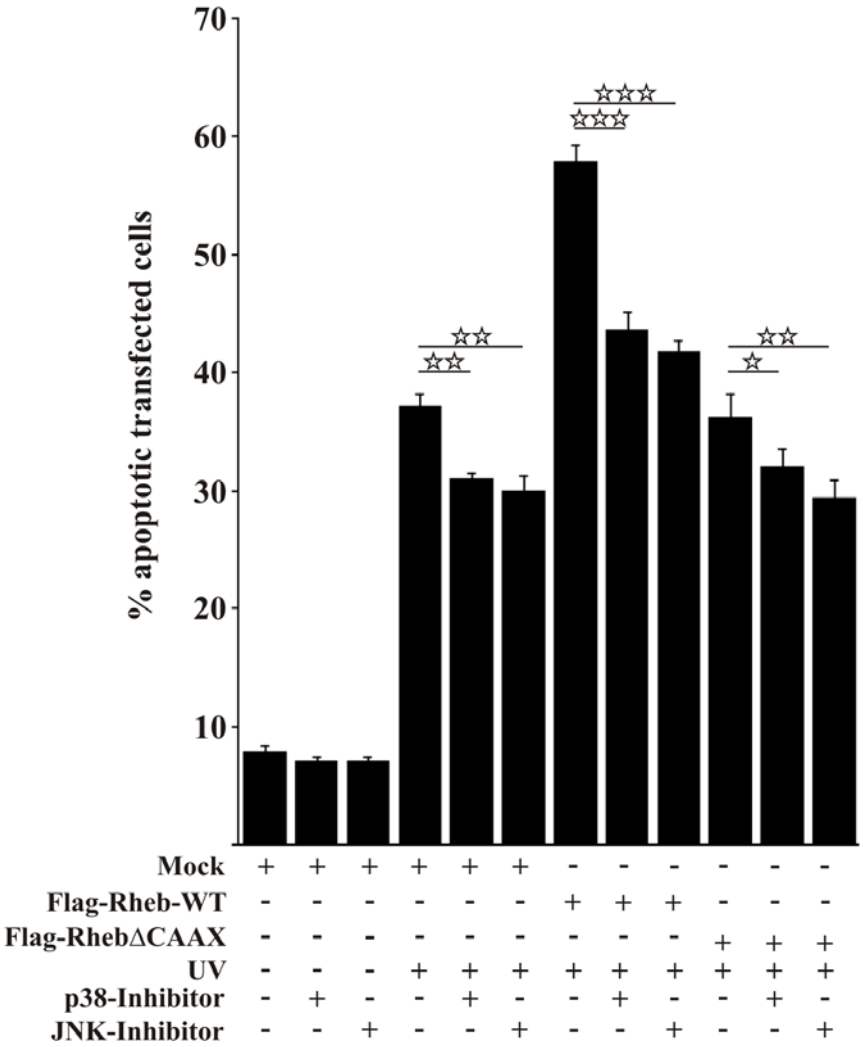


Supplemental Fig. S2: Reduction of ER-Stress with salubrinal could not prevent Rheb enhanced apoptosis. **(A)** Transfected Hela cells were treated with salubrinal 2 h before UV irradiation (0,03 J/cm^2). **(B)** After pretreatment with salubrinal (100 µM) transfected HeLa cells were stimulated with 1,5 µM tunicamycin for 24 h.

Quantification of the percentage of fragmented or pyknotic nuclei within the population of transfected cells was done at the fluorescence microscope. Bars are representing mean ± SEM of four independent experiments. *** $P < 0{,}001$ determined using ANOVA followed by a Bonferroni post-hoc-test.



Supplemental Fig. S3: Coexpression of an inactive cRafΔN-K375M point mutant is still able to significantly protect HeLa cells from toxic stimulation with UV-light **(A)** or TNFα **(B)**. Again Rheb-WT is still able to enhance the rate of apoptotic cells in the presence of overexpressed cRafΔN-K375M. All bars represent mean ± SEM of at least six independent experiments. Determination of significant differences was performed using Anova followed by a Bonferroni post-hoc-test (* $p < 0,05$, *** $p < 0,001$).



Supplemental Fig. S4: Quantification of apoptotic cells showed a significant reduction of the TNFα (data not shown) and UV-light induced cell death in case of pretreatment with p38 MAPK- or JNK-inhibitor. Bars are representing mean ± SEM of four independent experiments. * $P < 0,05$, ** $P < 0,01$, *** $P < 0,001$ determined by ANOVA followed by a Bonferroni post-hoc-test.

Supplemental Table S1:

Table 1: Structural statistics of <SA>[a] for rRheb in solution at 298 K and pH 8.0

Restraints	
Total restraints	*1914*
NOE distance restraints[b]	*1513*
Hydrogen bonds (two restraints per amide bond)	*108*
Dihedral angle restraints (TALOS)	*248*
Φ	*124*
Ψ	*124*
$^3J_{HN-HA}$ (KARPLUS)	*45*
Ramachandran statistics[c] (%)	
Residues in most favoured regions	*76.3*
Residues in additionally allowed regions	*20.0*
Residues in generously allowed regions	*3.2*
Residues in disallowed regions	*0.5*

[a] *<SA> represents the ensemble of the 20 final structures. Force constants used to calculate energy terms are the same as published previously (1-3) .*

[b] *No distance restraint in any of the structure included in the ensemble was violated by > 0.5 Å. (3).*

[c] *According to the programme PROCHECK (4;5).*

Backbone assignments were obtained from three dimensional HNCA, HN(CO)CA, CBCA(CO)NH, and HNCO spectra (6;7). Side chain assignments were obtained from three dimensional HCCH-TOCSY, HCCH-COSY, HNHA, HNHB, HCC(CO)NH, HBHA(CO)NH, ^{15}N-NOESY-HSQC (mixing time of 120 ms), and ^{13}C-NOESY-HSQC (mixing time of 120 ms) spectra as previously published (1;8;9). Water suppression in experiments recorded on samples in H_2O was achieved by incorporation of a Watergate sequence into the various pulse sequences (10-12). 2D ^{1}H-^{15}N HSQC spectra with reduced signal loss due to the fast chemical exchange were recorded using procedures described previously (13).

NOEs were derived from the ^{15}N-edited and the ^{13}C-edited NOESY spectra (1). All protons were explicitly defined in the dynamically simulated annealing calculations; in some cases, however, additional terms were added to the upper bounds as a pseudoatom corrections (14-16). Structures were compared using average RMSD values for Cα, C, and N atoms (XPLOR-NIH). Structure visualisation and superimpositions were done either with PyMol (Delano, W. L., The PyMol Molecular Graphics System (2002) Delano Scientific, Palo Alto, CA, USA) or MOLMOL (17).

Proteins binding studies. NMR titrations using putative ligands of Rheb consisted of monitoring changes in chemical shifts and line widths of the backbone amide resonances of uniformly ^{15}N-enriched Rheb samples as a function of ligand concentration (8;9;18;19). This resulted in a series of ^{1}H-^{15}N-HSQC spectra, following the procedure of 'SAR by NMR' (20;21). Quantitative analysis of

ligand-induced chemical shift pertubation was performed by applying Pythagoras' equation to the weighted chemical shifts:

$$\Delta\delta_{obs}* = \sqrt{\left(\Delta\delta_{^1H_N}\right)^2 + \left(\frac{\Delta\delta_{^{15}N}}{5}\right)^2}$$

Conformational dynamics of Rheb. In Rheb-GDP, the less defined regions in the structure are due to flexibility on the pico- to nanosecond time scale, as seen in the heteronuclear NOE experiment (Fig. 6). Resonances for the first five residues, S1 to K5, and the carboxy-terminal residues S175 to M184 could not be detected in the spectra, presumably because of structural disorder as previously published and corroborated by an independent study (18;19;22). The amide proton resonances of Q72 and T73 are also not detected in the ^{1}H-^{15}N HSQC spectrum. The most prominent differences in the weighted chemical shifts between the inactive and active state of Rheb are found for residues I11 to S20. This region comprises the entire P-loop, a structural element with the GxxxxGKS/T motif that is conserved in all small GTPases. The P-loop interacts with the β- and γ-phosphates of the bound nucleotide (23;24). In Rheb, this P-loop ranges from G13 to S20. In particular, S16 and K19 experience the largest chemical shift differences when switching from the inactive to the active form of Rheb. R15 and S16 in Rheb are equivalent to G12 and G13 in Ras, which are important for the hydrolysis of GTP (25;26). In Rheb, an arginine residue is located at the G12 position in Ras, suggesting a different mechanism of hydrolysis.

Reference List

1. Stoll, R., Renner, C., Zweckstetter, M., Bruggert, M., Ambrosius, D., Palme, S., Engh, R. A., Golob, M., Breibach, I., Buettner, R., Voelter, W., Holak, T. A., and Bosserhoff, A. K. (2001) *EMBO J.* **20,** 340-349
2. Stoll, R., Voelter, W., and Holak, T. A. (1997) *Biopolymers* **41,** 623-634
3. Holak, T. A., Nilges, M., and Oschkinat, H. (1989) *Febs Letters* **242,** 218-224
4. Laskowski, R. A., Rullmann, J. A. C., MacArthur, M. W., Kaptein, R., and Thornton, J. M. (1996) *Journal of Biomolecular Nmr* **8,** 477-486
5. Laskowski, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1993) *Journal of Applied Crystallography* **26,** 283-291
6. Grzesiek, S. and Bax, A. (1992) *Journal of the American Chemical Society* **114,** 6291-6293
7. Grzesiek, S. and Bax, A. (1992) *Journal of Magnetic Resonance* **96,** 432-440
8. Song, J. K. and Markley, J. L. (2001) *Journal of Molecular Recognition* **14,** 166-171
9. Stoll, R., Renner, C., Hansen, S., Palme, S., Klein, C., Belling, A., Zeslawski, W., Kamionka, M., Rehm, T., Muhlhahn, P., Schumacher, R., Hesse, F., Kaluza, B., Voelter, W., Engh, R. A., and Holak, T. A. (2001) *Biochemistry* **40,** 336-344
10. Braunschweiler, L. and Ernst, R. R. (1983) *Journal of Magnetic Resonance* **53,** 521-528
11. Davis, D. G. and Bax, A. (1985) *Journal of the American Chemical Society* **107,** 7197-7198
12. Shaka, A. J., Lee, C. J., and Pines, A. (1988) *Journal of Magnetic Resonance* **77,** 274-293
13. Mori, S., Abeygunawardana, C., Johnson, M. O., and Vanzijl, P. C. M. (1995) *Journal of Magnetic Resonance Series B* **108,** 94-98
14. Brunger, A. T., Adams, P. D., Clore, G. M., Delano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) *Acta Crystallographica Section D-Biological Crystallography* **54,** 905-921
15. Schwieters, C. D., Kuszewski, J. J., and Clore, G. M. (2006) *Progress in Nuclear Magnetic Resonance Spectroscopy* **48,** 47-62
16. Schwieters, C. D., Kuszewski, J. J., Tjandra, N., and Clore, G. M. (2003) *Journal of Magnetic Resonance* **160,** 65-73
17. Koradi, R., Billeter, M., and Wuthrich, K. (1996) *J.Mol.Graph.* **14,** 51-32
18. Berghaus, C., Schwarten, M., Heumann, R., and Stoll, R. (2007) *Biomolecular NMR Assignments* **1,** 45-47

19. Schwarten, M., Berghaus, C., Heumann, R., and Stoll, R. (2007) *Biomolecular NMR Assignments* **1,** 105-108
20. McAlister, M. S. B., Mott, H. R., vanderMerwe, P. A., Campbell, I. D., Davis, S. J., and Driscoll, P. C. (1996) *Biochemistry* **35,** 5982-5991
21. Shuker, S. B., Hajduk, P. J., Meadows, R. P., and Fesik, S. W. (1996) *Science* **274,** 1531-1534
22. Marshall, C. B., Ho, J., Buerger, C., Plevin, M. J., Li, G. Y., Li, Z., Ikura, M., and Stambolic, V. (2009) *Sci.Signal.* **2,** ra3
23. Vetter, I. R. and Wittinghofer, A. (2001) *Science* **294,** 1299-1304
24. Saraste, M., Sibbald, P. R., and Wittinghofer, A. (1990) *Trends Biochem.Sci.* **15,** 430-434
25. Yamagata, K., Sanders, L. K., Kaufmann, W. E., Yee, W., Barnes, C. A., Nathans, D., and Worley, P. F. (1994) *J.Biol.Chem.* **269,** 16333-16339
26. Barbacid, M. (1987) *Annu.Rev.Biochem.* **56,** 779-827