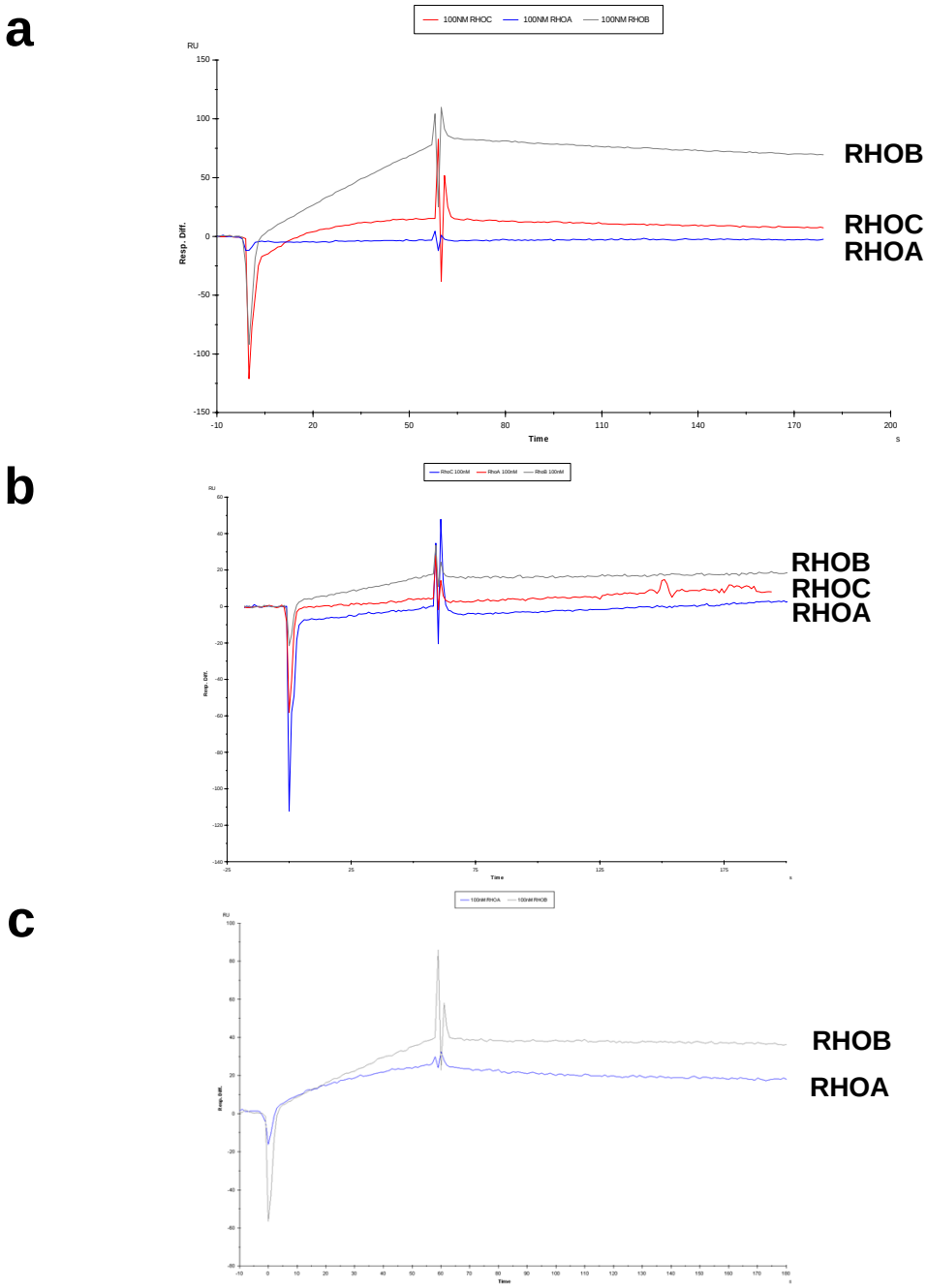


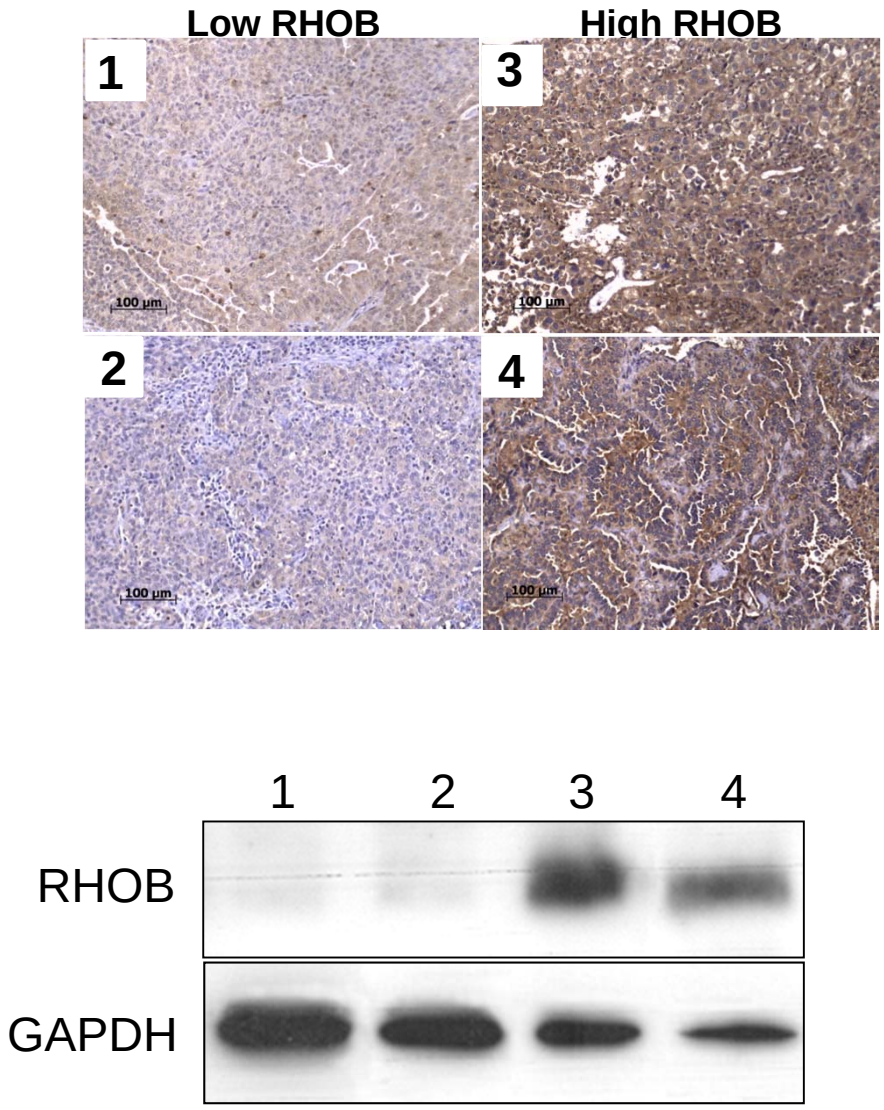
Sup. Fig. 1. Specificity of different RHOB antibodies by surface plasmon resonance (SPR).

Different anti-RHOB antibodies were immobilized on a CM5 chip and binding of 100 nM human recombinant RHOA, B and C was monitorized. A representive experiment out of three independent repeats was shown. RU, resonance units; s, seconds.

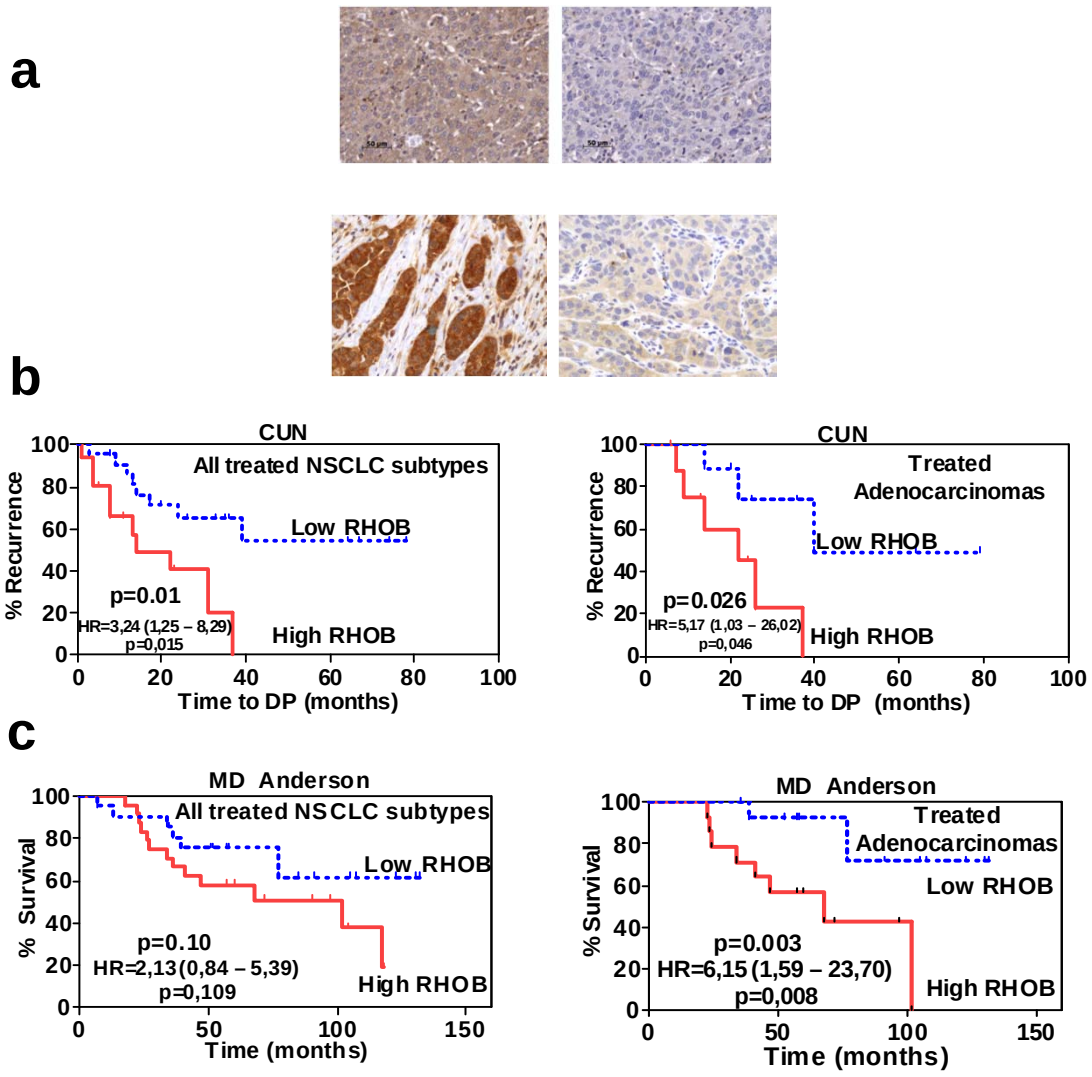
a. Monoclonal anti-RHOB antibody (C-5, sc-8048, Santa Cruz Biotechnology)

b. Polyclonal anti-RHOB antibody (#2098, Cell Signaling)

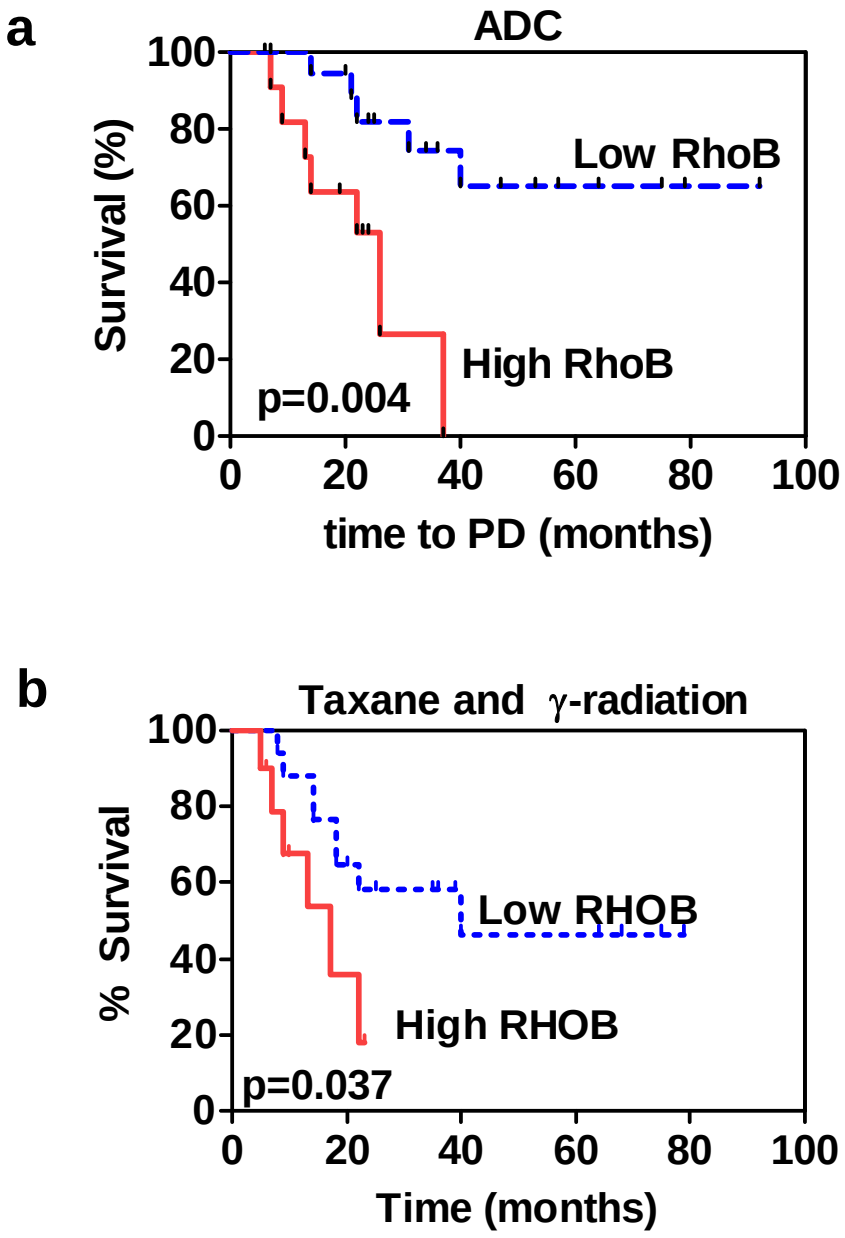
c. Polyclonal anti-RHOB antibody (119, sc-180, Santa Cruz Biotechnology)



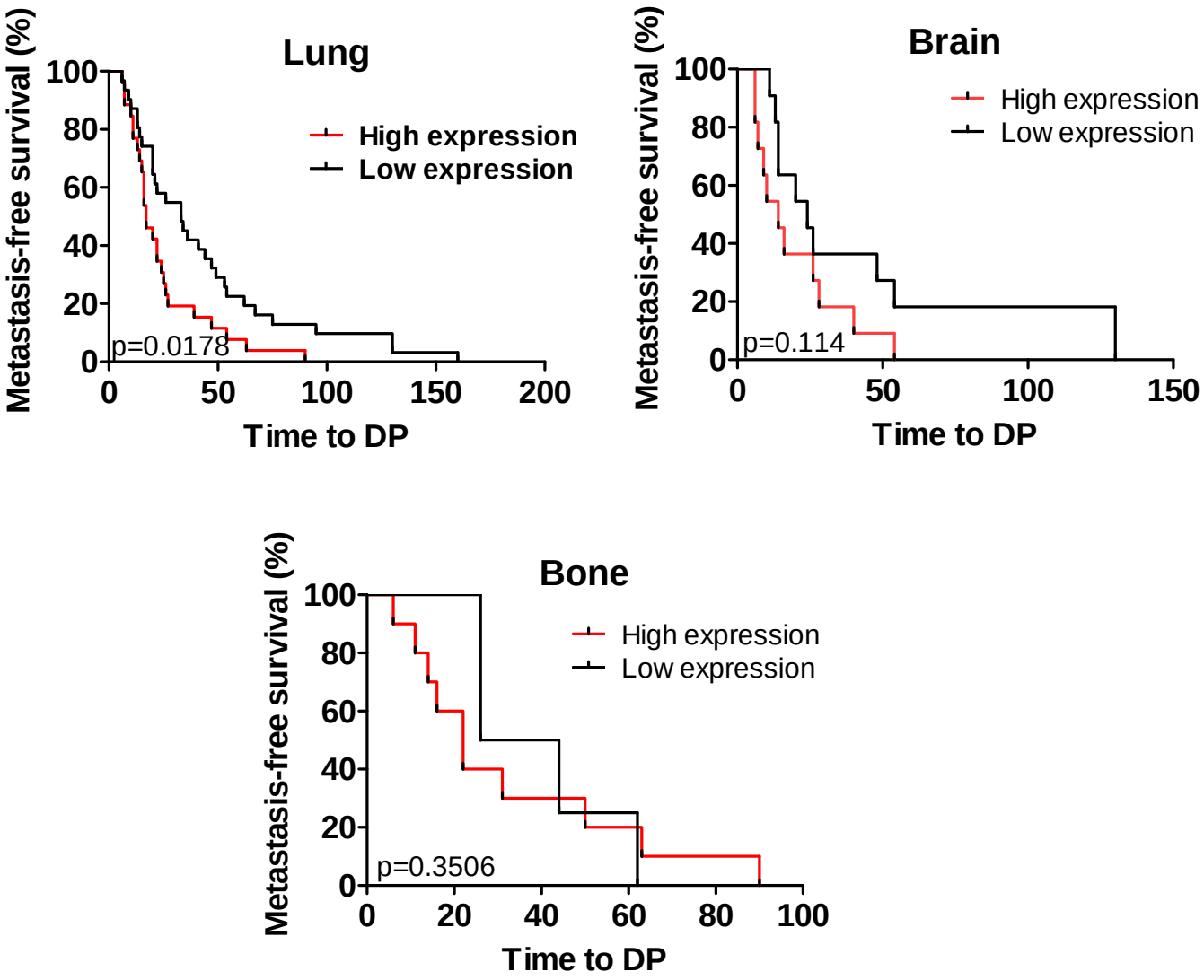
Sup. Fig. 2. Correlation between RHOB expression in western blot and immunohistochemistry in human tumor samples. Several tumor samples displaying low (1 and 2) or high RHOB levels (3 and 4) by immunohistochemistry (*upper panels)* were subjected to westernblot (*lower panels*) using the same RHOB antibody. A correlation between westernblot staining and immunohistochemical reactivity was shown.



Sup Fig 3. a. ***Top:*** Representative images of RHOB immunohistochemical analysis of serial slices of lung tumor biopsies (40X magnification) incubated without (left) or with neutralization RhoB peptide at 25 ng/ml (right). ***Bottom:*** Representative images of immunohistochemical analysis of two different lung ADCs with high (*right*) and low (*left*) RHOB levels. **b**. Recurrence of patients with high and low RHOB levels assessed by immunohistochemistry in relation to time to disease progression (DP) in the data set from CUN (University Hospital, Clínica Universidad de Navarra) for all treated histological subtypes of NSCLC (left panel, n=38) and treated ACs only (n=18, right panel). **c**. Similar assessment as in **b**. was performed in the cohort of patients from M.D. Anderson Cancer Center (treated NSCLC, n=45; treated ADC, n=30).



Sup Fig. 4. a. Recurrence of ADC patients with high and low RHOB levels assessed by immunohistochemistry in relation to time to progressive disease (PD) in the CUN cohort (n=31).**b.** Recurrence of NSCLC in patients treated with adjuvant radiotherapy and/or taxane-based chemotherapy (n=27), with high and low RHOB levels, assessed by immunohistochemistry



Sup. Fig. 5. Analysis of recurrence of patients (MD Anderson cohort) in different metastasis target organs according to RHOB levels by immunohistochemistry. Kaplan-Meier curves showing time to disease progression (DP) to lung (top left panel, n=57), brain (top right panel, n=22) and bone (bottom panel, n=14). Patients with tumors displaying high RHOB expression showed higher risk of lung relapse compared to those with low RHOB levels. This result was consistent with a similar trend found in bone and brain metastasis, but they do not reach statistical significance. Thus, RHOB expression seems to be linked to a poor outcome in disease progression, not specifically to a risk of relapse due to metastasis on a specific organ.

Table S1. Histopathological and clinical features of the patients included in the study.

		CUN series (n=78)	MD Anderson series (n=234)
Age (years)	**Mean ±SD**	**63±10**	**66±11**
Gender	**Male**	**67**	**114**
	Female	**11**	**120**
Histology	**Squamous cell carcinoma**	**31**	**88**
	Adenocarcinoma	**37**	**143**
	Others	**10**	**3**
pT	**T1**	**24**	**90**
	T2	**46**	**117**
	T3	**6**	**12**
	T4	**2**	**15**
pN	**N0**	**53**	**164**
	N1	**17**	**44**
	N2	**8**	**26**
Follow up time (months)	**(Mean ±SD) [Median]**	**29±22 [23]**	**66±34 [65]**
Vital Status	**Alive**	**64**	**119**
	Dead	**14**	**115**
Disease Status	**DF**	**50**	**149**
	PD	**28**	**85**
Chemotherapy		**30**	**26**
Radiotherapy		**14**	**28**
Both Chemotherapy + Radiotherapy		**14**	**7**

Supplementary Results and Discussion

Specificity of three available antibodies was assessed by Surface Plamon Resonance (SPR) by immobilizing the antibody to the chip and binding to recombinant RhoA, RHoB and RhoC. None of the antibodies studied showed a total specificity (Sup Fig. 1). The most specific anti-RHOB antibody (Cell signaling) was not suitable for immunohistochemistry (according to manufacturer´s recommendations and data not shown). The other antibodies have been previously used for immunohistochemistry (Sato et al. and Mazières et al.).

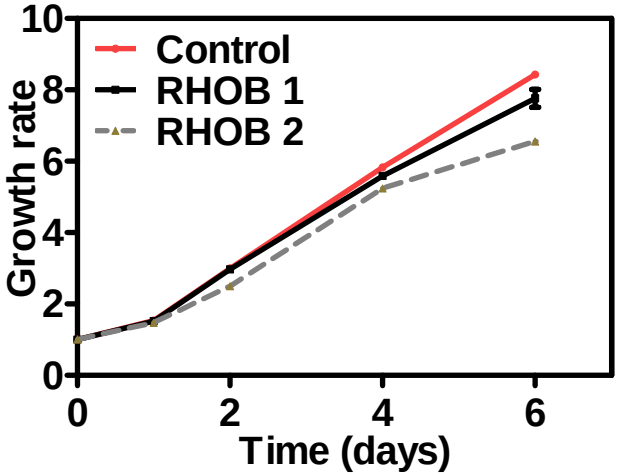
Next, we showed a correlation between the Western blot and immunohistochemical analyses of RHOB levels in paraffin-embedded tumors of human lung cancer (sup. fig. 2). As an additional control experiment, we used a specific peptide for RHOB competing with the antibody in adsorption control experiments by immunohistochemistry (sup. fig. 3, Top).

Immunohistochemical analysis in a cohort of the "Clínica Universidad de Navarra" (CUN) showed no statistically significant differences when all histological subtypes were included after checking antibody specificity with rigorous controls (Sup. fig. 3). In the study of the AC subset, high RHOB tumor staining was associated with a dramatic decrease in time to progression (TP) ($p=0.004$, Sup. fig.3).

Patients were separated into two subgroups: non-treated and treated with adjuvant therapy. No significant differences were found in non-treated patients (data not shown). In treated patients of all histological subtypes, TP was significantly shorter for high RHOB tumors than for low RHOB tumors ($p=0.01$, Sup Fig. 3). Moreover, considering only the treated ADC patients, high RHOB expression was significantly associated with decreased TP ($p=0.026$, Sup Fig. 3). In both cohorts, univariate Cox analysis revealed significant risk of recurrence in high RHOB treated NSCLC and ADC tumors. These data suggest a potential association between RHOB levels and TP in treated ADC patients.

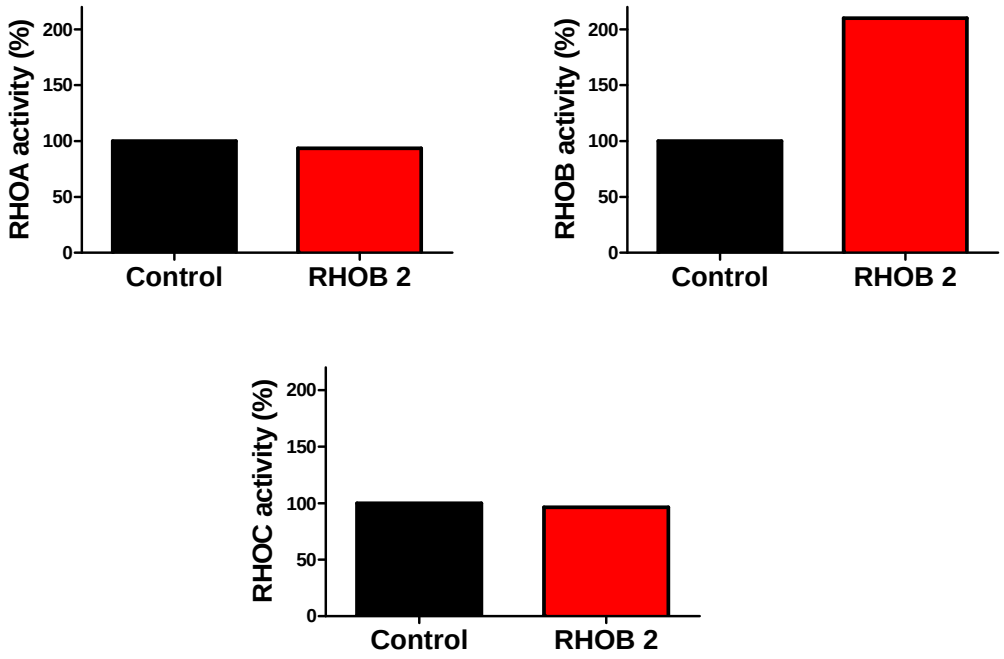
We further investigated these results in a new independent series of 234 NSCLC tumors from the M.D. Anderson Cancer Center. No statistically significant differences were found when all patients or only the ADC subset were analyzed (data not shown). In accordance with the CUN series, and considering all histological subtypes of treated tumors, survival of patients with high RHOB tumors was lower than those with low RHOB tumors, although it did not reach statistical significance (Sup. Fig. 3, left). More importantly, considering only treated AC patients, a significantly shorter survival time was found in patients with high RHOB tumors as compared with low RHOB tumors (Sup Fig. 3, right). In both cohorts, univariate Cox analysis revealed significant risk of recurrence in high RHOB treated NSCLC and ADC tumors. Taken together, these data suggest that a strong association exists between RHOB expression levels and survival time in ADC patients treated with adjuvant therapy.

The evidence presented here also underscores the validity of RHOB as a factor with potential predictive value in lung ADC patients. However, future studies with large cohorts should be carried out to validate this hypothesis. Nevertheless, one could consider its use alone or in combination with other predictive markers of response to chemotherapy. The relevance of RHOB in the histological subset of lung ADCs might have other translational implications. For instance, one could also anticipate a stratification of patients according to RHOB expression levels, and the application of a tailored therapeutic regimen to improve patient survival. Similarly, decreasing RHOB levels or activity before or in combination with radio- and chemotherapy, according to our findings, may increase therapeutic efficacy.



Sup Fig 6. Cell proliferation assay for different RHOB overexpressors.

a



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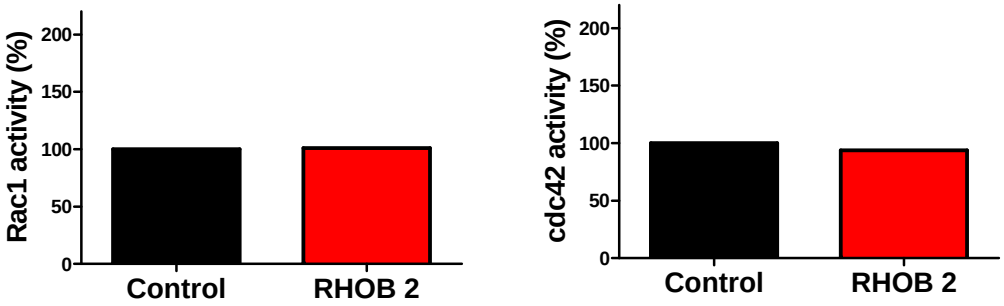
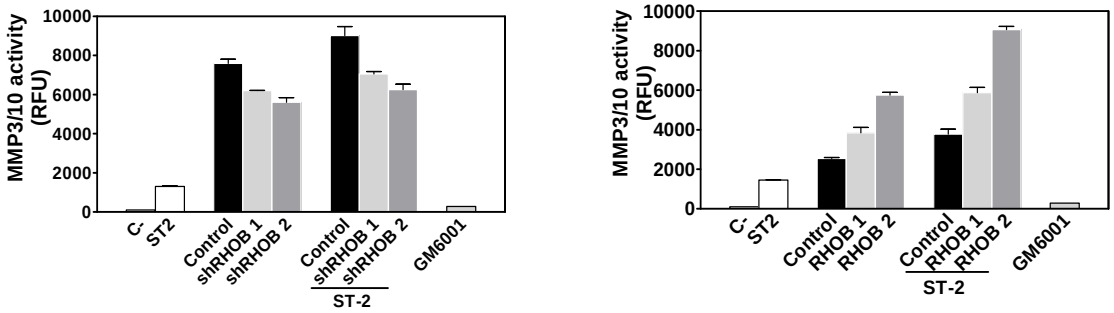
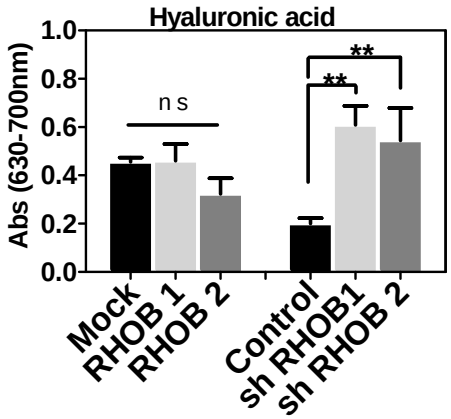


Sup. Fig. 7. a. Densitometric analysis of active RHO GTPases family including RHOA, RHOB and RHOC. **b.** and other family members, Rac1 and CDC42 Signal was normalized to total RHO.



Sup. Fig. 8. Global MMP activity assessed in the conditioned medium of shRHOB cells (*left panel*) and RHOB overexpressing cells(*right panel)* in coculture with ST-2 cells for 3 days using a fluorogenic substrate M-2110 (Bachem) which is recognized by MMP3/MMP10. RFU: Relative fluorescence units.



Sup. Fig. 9. Adhesion assay of cells with different RHOB levels to hyaluronic acid. Attached cells were fixed, stained with toluidine blue and solubilized to measure absorbance at 630 nm. Experiments were performed three times with similar results. Data are presented as the fold change compared to control transduced cells (mean ± SD, ANOVA followed by Dunnett test).