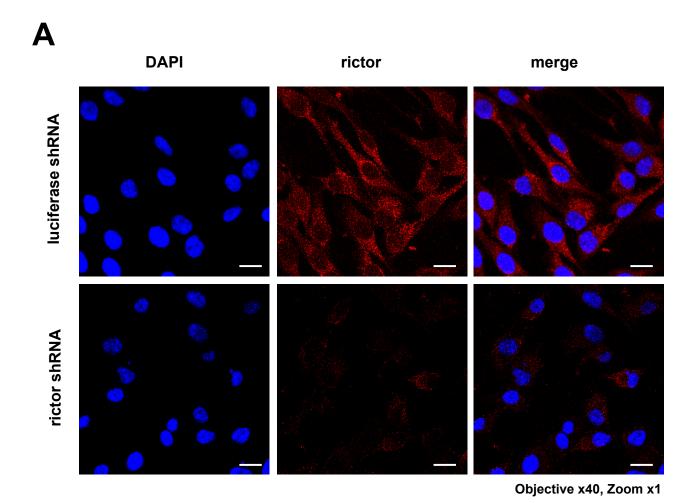
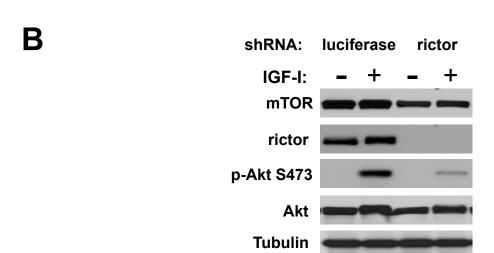
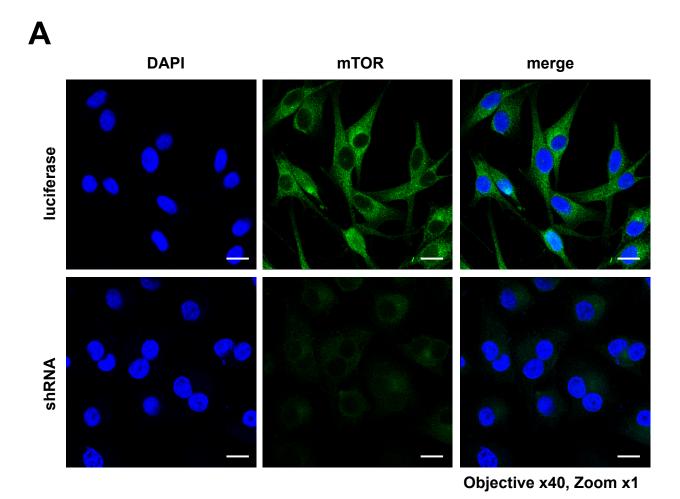
Supplemental Fig. S1. Validation of the rictor staining specificity. (A) MDA-MB-435 cells were infected with lentiviruses expressing shRNA targeting luciferase (control) and rictor. After selection, cells growing in 10% serum were analyzed by immunostaining with the rictor antibody and nuclei were stained with DAPI. Photos were taken using an air x40 objective and a 2.5 zoom. Bar = 20 um. (B) Analysis of the rictor knock down efficiency and the mTORC2 signaling. The cells expressing shRNAs described in (A) were serum starved for 24 hrs and incubated with or without IGFI (50 ng/ml) for 20 min. Following lysis the lysates were analyzed by immunoblotting with the indicated antibodies to detect the rictor abundance and the mTORC2-dependent phosphorylation of Akt on Ser-473.

Supplemental Fig. S2. Validation of the mTOR staining specificity. (A) MDA-MB-435 cells were infected with lentiviruses expressing shRNA targeting luciferase (control) or mTOR. After selection, cells growing in 10% serum were analyzed by immunostaining with the mTOR antibody and nuclei were stained with DAPI. Photos were taken using an air x40 objective and a 2.5 zoom. Bar = 20 um. (B) Analysis of the mTOR knock down efficiency and the mTORC2 signaling. The cells expressing shRNAs described in (A) were growing in 10% serum. Following lysis the lysates were analyzed by immunoblotting with the indicated antibodies to detect the mTOR abundance and the mTORC2-dependent phosphorylation of Akt on Ser-473.

Table 1. The calculated Pearson's corelation coefficient indicating the rictor colocalization with the indicated proteins. The shown values are the average of the Pearson's coefficient obtained by analysis of 10 to 15 images.







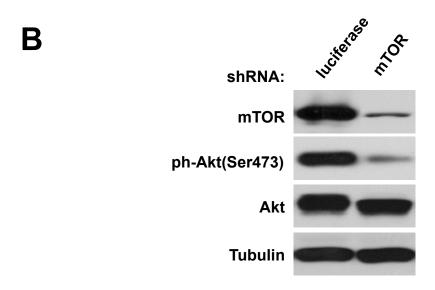


Table 1. Pearson's coefficient of correlation of co-localization between rictor and the indicated proteins.

		Pearson's coefficient
Rictor	mTOR	0.74 ± 0.017
Rictor	S6	0.73 ± 0.016
Rictor	ER488	0.73 ± 0.063
Rictor	Calreticulin	0.66 ± 0.023
Rictor	clathrin	0.52 ± 0.041
Rictor	LAMP2	0.52 ± 0.059
Rictor	EEA1	0.36 ± 0.054
Rictor	GM130	0.19 ± 0.035