Supplemental Materials

Molecular Biology of the Cell

Jiao et al.

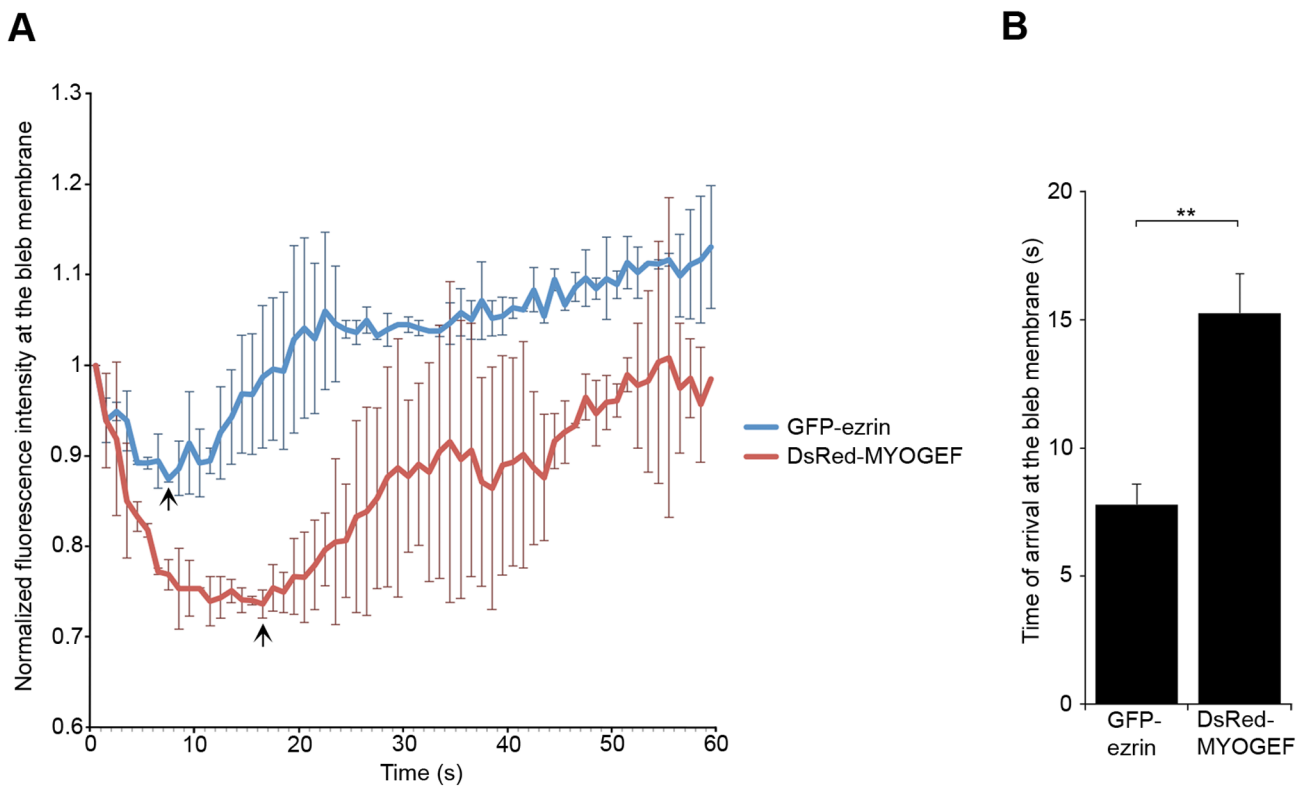


Fig. S1. Quantification of the timing of DsRed-MYOGEF and GFP-ezrin arrival at the bleb membrane during a bleb cycle. (*A*) A plasmid encoding GFP-ezrin was co-transfected into MDA-MB-231 cells with a plasmid encoding DsRed-MYOGEF. The transfected cells were subjected to time-lapse imaging analyses. The fluorescence intensity of GFP-ezrin and DsRed-MYOGEF at the bleb membrane was acquired using the ADAPT tool and normalized to the fluorescence intensity at the cell cortex prior to bleb formation. Black arrows indicate the time point when GFP-ezrin or DsRed-MYOGEF began to be recruited to the bleb membrane. Data are mean ± SD. (*B*) GFP-ezrin is recruited to the bleb membrane prior to DsRed-MYOGEF. The order of GFP-ezrin and DsRed-MYOGEF recruitment to the bleb membrane was quantified based on the normalized intensity of respective proteins at the bleb membrane. When a protein began to show a sustained increase in its fluorescence intensity compared to the lowest intensity at the bleb membrane, it was considered to be the starting point at which the protein was recruited to the bleb membrane. All blebs (n=25) measured in (A) were analyzed. Statistical significance was determined using two-tailed paired Student's t-test. **, $p < 0.01$. Data are mean ± SD.

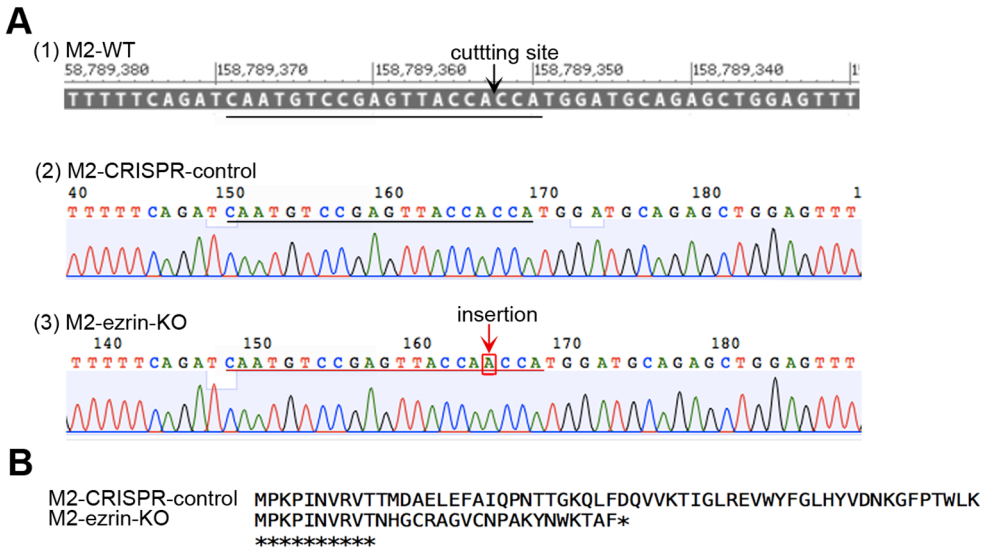


Fig. S2. Premature termination of ezrin translation resulting from CRISPR-mediated ezrin knockout. (*A*) Ezrin-specific CRISPR/Cas9 editing led to an adenine insertion in the third exon of the ezrin gene. A portion of wild type ezrin DNA sequence (A1) is shown with its respective location in the human genome (GI_7430, NCBI). Sequences with a black underline are the target of ezrin-specific single guided RNAs (sgRNAs). The black arrow indicates the cutting site by the DNA endonuclease enzyme Cas9. Genomic DNAs were extracted from CRISPR cells and used as PCR templates. The region flanking the cutting site was amplified by PCR and the resulting PCR products were cloned into T-vector for sequencing. Sequencing results show that an adenine insertion (red square) was present at the ezrin locus in ezrin-KO cells (A3), while InDels were absent in the corresponding region at the ezrin locus in control cells (A2). Note that the DNA sequence with an adenine insertion (red underline) was no longer a good target of ezrin sgRNA. (*B*) Protein alignment shows that insertion of an adenine into the ezrin locus in ezrin-KO M2 melanoma cells introduced a premature stop codon at the 31st amino acid residue as well as led to missense mutations that altered amino acid residues 11-30 in ezrin.

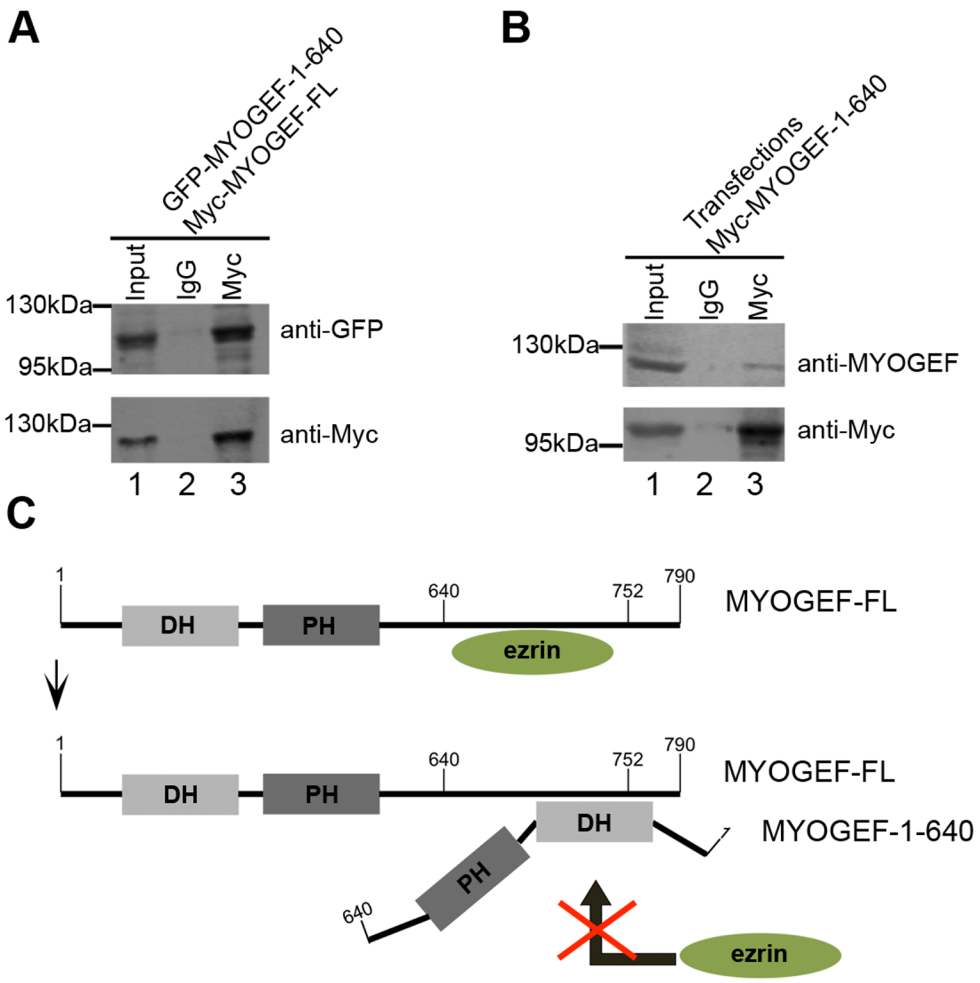


Fig. S3. Interactions between MYOGEF-1-640 and full length MYOGEF. (*A*) Intermolecular interaction between MYOGEF-1-640 and MYOGEF-FL *in vitro*. *In vitro* translated GFP-MYOGEF-1-640 and Myc-MYOGEF-FL were mixed and subjected to co-immunoprecipitation assays with anti-c-Myc agarose beads (compare lane 2 with lane 3). (*B*) Interactions between MYOGEF-1-640 and endogenous MYOGEF in transfected cells. A7 melanoma cells transfected with a plasmid encoding Myc-MYOGEF-1-640 were subjected to co-immunoprecipitation assays with anti-c-Myc agarose beads. Immunoblot analyses showed that endogenous MYOGEF was co-precipitated with Myc-MYOGEF-1-640 (compare lane 2 with lane 3). Note that the antibody for MYOGEF was designed to target the C-terminal region of MYOGEF and does not recognize Myc-MYOGEF-1-640. (*C*) Schematic indicating the impact of MYOGEF-1-640 over-expression on MYOGEF-ezrin interactions. Our results suggest that interactions of MYOGEF-1-640 with endogenous MYOGEF may block the ezrin-binding region, thus disrupting the interaction between ezrin and endogenous MYOGEF.

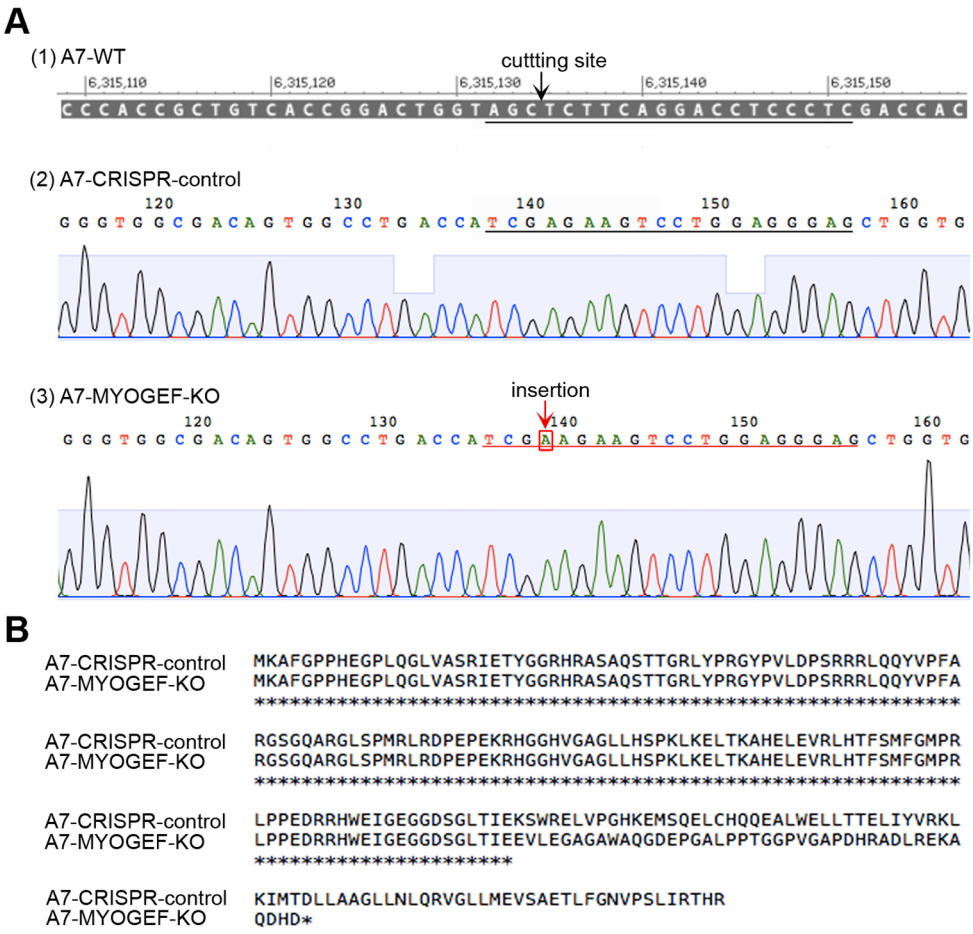


Fig. S4. Premature termination of MYOGEF translation resulting from CRISPR-mediated MYOGEF knockout. (*A*) MYOGEF-specific CRISPR/Cas9 editing led to an adenine insertion in the third coding exon of the MYOGEF gene. A portion of wild type MYOGEF DNA sequence (A1, complementary strand) is shown with its respective location in the human genome (GI_55200, NCBI). The sequence with a black underline is the target of MYOGEF-specific sgRNAs. The black arrow indicates the cutting site by the DNA endonuclease enzyme Cas9. Sequencing results show that an adenine insertion (red square) was present at the MYOGEF locus in MYOGEF-KO cells (A3), while InDels were absent in the corresponding region at the MYOGEF locus in control cells (A2). Note that the DNA sequence with an adenine insertion (red underline) was no longer a good target of MYOGEF sgRNA. (*B*) Protein alignment indicates that insertion of an adenine into the MYOGEF locus in MYOGEF-KO A7 melanoma cells generated a premature stop codon at the 185th amino acid residue as well as led to missense mutations that altered amino acid residues 143-184 in MYOGEF.

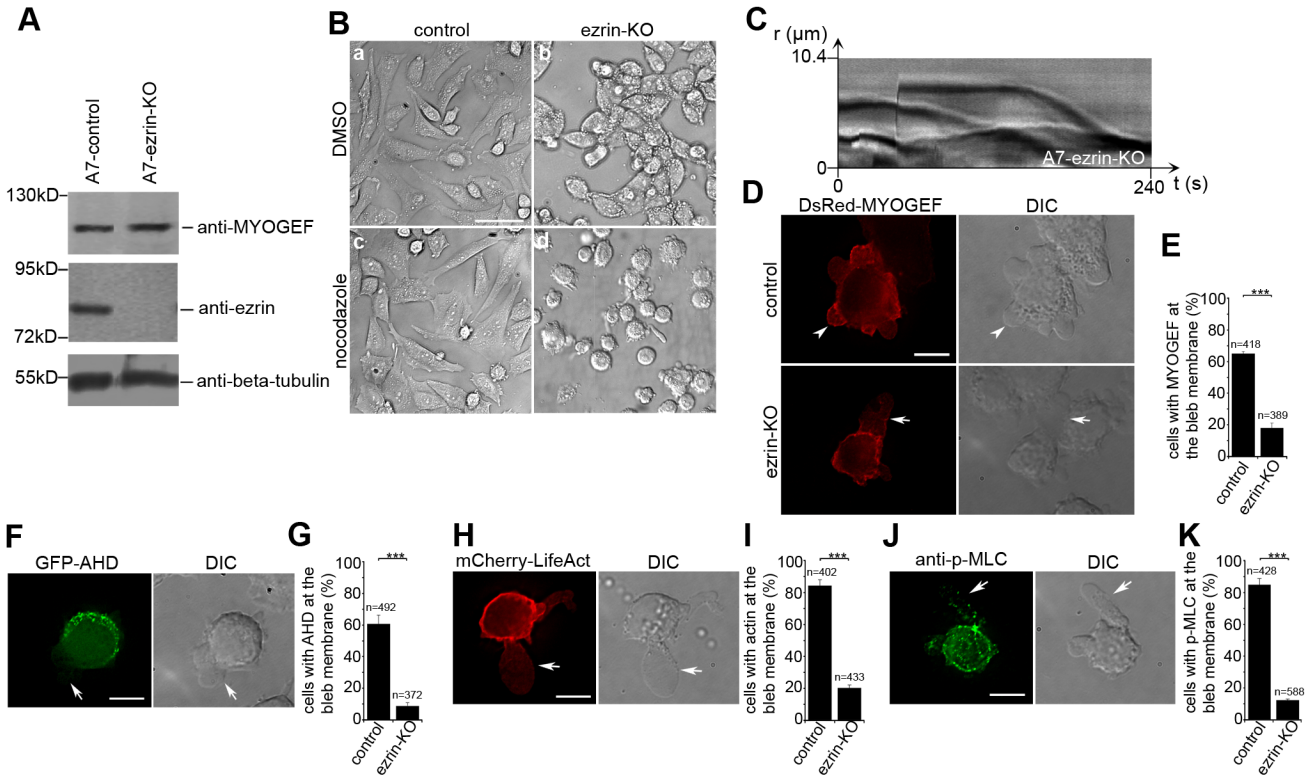
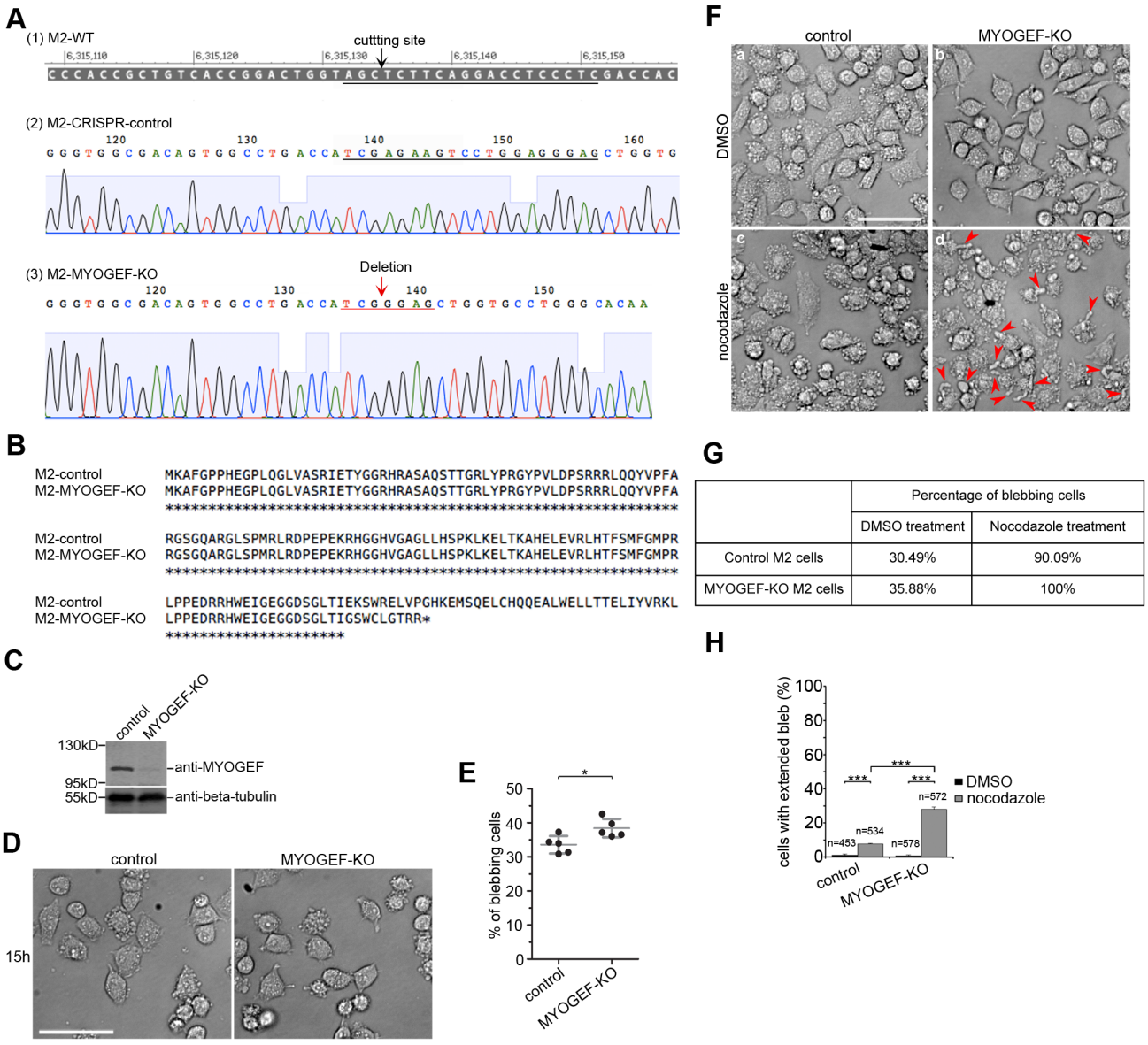


Fig. S5. The effect of ezrin knockout on RhoA activation and cortex reassembly at the bleb membrane. (*A*) Immunoblot showing the protein levels of ezrin and MYOGEF in the control and ezrin-KO A7 melanoma cells. (*B*) Nocodazole induced membrane blebbing in ezrin-KO A7 melanoma cells. Control (Ba and Bc) and ezrin-KO (Bb and Bd) A7 melanoma cells were treated with DMSO (Ba and Bb) or nocodazole (Bc and Bd) for 40 minutes prior to live-cell imaging analyses. Ezrin knockout did not initiate membrane blebbing in A7 melanoma cells (compare panel Ba with panel Bb). However, ezrin-KO A7 melanoma cells showed membrane blebbing after nocodazole treatment (compare panel Bd with panel Bb or Bc). Bar, 50μm. (*C*) Kymograph analysis showing the efficiency of bleb retraction in ezrin-KO A7 melanoma cells. The boundary of bleb membrane was shown vertically along with horizontal time scales. The kymograph showed that bleb retraction was retarded in ezrin-KO cells (compared with the kymograph in A7 control cells in Fig. 5D). (*D-E*) Ezrin knockout interfered with the localization of MYOGEF to the bleb membrane. (*D*) A plasmid encoding DsRed-MYOGEF was transfected into control or ezrin-KO A7 melanoma cells. The transfected cells were treated with nocodazole and then subjected to fluorescence microscopy analyses. Bar, 10μm. (*E*) Quantification of cells with DsRed-MYOGEF recruited to the bleb membrane in control and ezrin-KO cells. Statistical significance was determined using two-tailed paired Student's t-test. ***, $p < 0.001$. Data are mean ± SD. (*F-K*) Ezrin knockout interfered with RhoA activation and cortex reassembly at the bleb membrane. GFP-AHD (*F-G*), mCherry-LifeAct (*H-I*), and phosphorylated-myosin light chain (*J-K*) were

not localized to the bleb membrane in ezrin-KO A7 melanoma cells treated with nocodazole (arrowheads in F, H, and J). Bar, 10μm. Quantification in G, I and K was compiled from three independent experiments. Statistical significance was determined using two-tailed paired Student's t-test. ***, $p < 0.001$. Data are mean ± SD.



	Percentage of blebbing cells	
	DMSO treatment	Nocodazole treatment
Control M2 cells	30.49%	90.09%
MYOGEF-KO M2 cells	35.88%	100%

Fig. S6. The effect of CRISPR-mediated MYOGEF knockout on membrane blebbing in M2 melanoma cells. (*A*) MYOGEF-specific CRISPR/Cas9 editing led to the deletion of 13 nucleotides in the third coding exon of the MYOGEF gene. A portion of wild type MYOGEF DNA sequence (A1, complementary strand) is shown with its respective location in the human genome (GI_55200, NCBI). The sequence with a black underline is the target of MYOGEF-specific sgRNAs. The black arrow indicates the cutting site by the DNA endonuclease enzyme Cas9. Sequencing results show that 13 nucleotides were deleted at the MYOGEF locus in MYOGEF-KO cells (A3; 13 nucleotides downstream of the red arrow were deleted), while the deletion was not observed in the corresponding region at the MYOGEF locus in control cells (A2). Note that the DNA sequence with the deletion was

no longer a good target of MYOGEF sgRNA. (*B*) Protein alignment indicates that deletion of 13 nucleotides in the MYOGEF locus in MYOGEF-KO M2 melanoma cells generated a premature stop codon at the151st amino acid residue as well as led to missense mutations that altered amino acid residues 142-150 in MYOGEF. (*C*) Immunoblot showing a decrease in the protein level of MYOGEF in MYOGEF-KO M2 melanoma cells as compared to that in the control. (*D*) DIC images of control and MYOGEF-KO M2 melanoma cells after culture for 15 h. Bar, 50 μm. (*E*) Quantification of the percentage of blebbing cells from (D). Two hundred cells (represented by a single dot) were analyzed in each experiment per cell line. Five independent experiments were performed. Statistical significance was determined using two-tailed paired Student's t-test. *, $p < 0.05$. Data are mean ± SD. (*F*) DIC images of control (a and c) or MYOGEF-KO (b and d) M2 melanoma cells treated with DMSO (a and b) or nocodazole (c and d). Cells were cultured for 15 h and then treated with DMSO or nocodazole for 40 min. Red arrowheads in panel d indicate the extended blebs. Bar, 50 μm. (*G*) The percentage of blebbing cells from (F). Note that nocodazole treatment increased the percentage of blebbing cells in both control and MYOGEF-KO cells. (*H*) Quantification of extended blebs from (F). Statistical significance was determined using two-way ANOVA test and Tukey's post-hoc test. ***, $p < 0.001$. Data are mean ± SD.

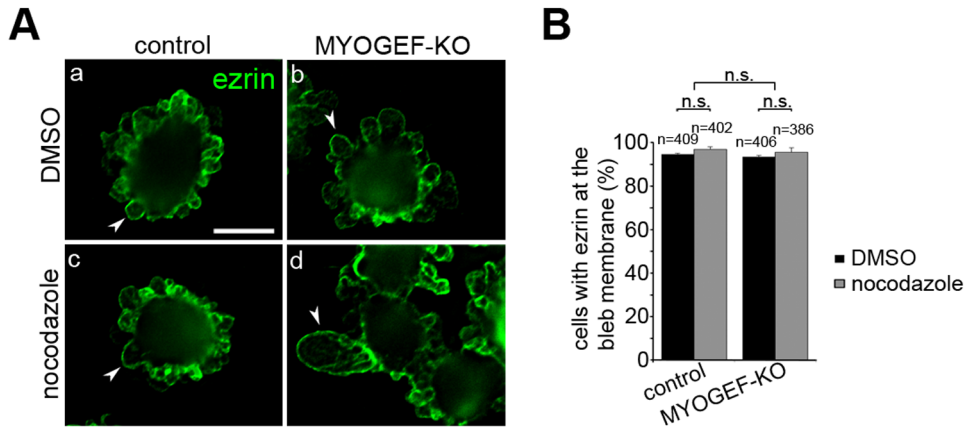


Fig. S7. Effect of MYOGEF knockout on the localization of ezrin at the bleb membrane. (*A*) Control (a and c) and MYOGEF-KO (b and d) M2 melanoma cells were treated with DMSO (a and b) or nocodazole (c and d) for 40 min and then subjected to immunofluorescence staining for ezrin. Arrowheads indicate the localization of ezrin at the bleb membrane. Bar, 10 μm. (*B*) Percentage of cells with ezrin localized to the bleb membrane. Statistical significance was determined using two-way ANOVA test and Tukey's post-hoc test. n.s., non-significant ($p \geq 0.05$). Data are mean ± SD.