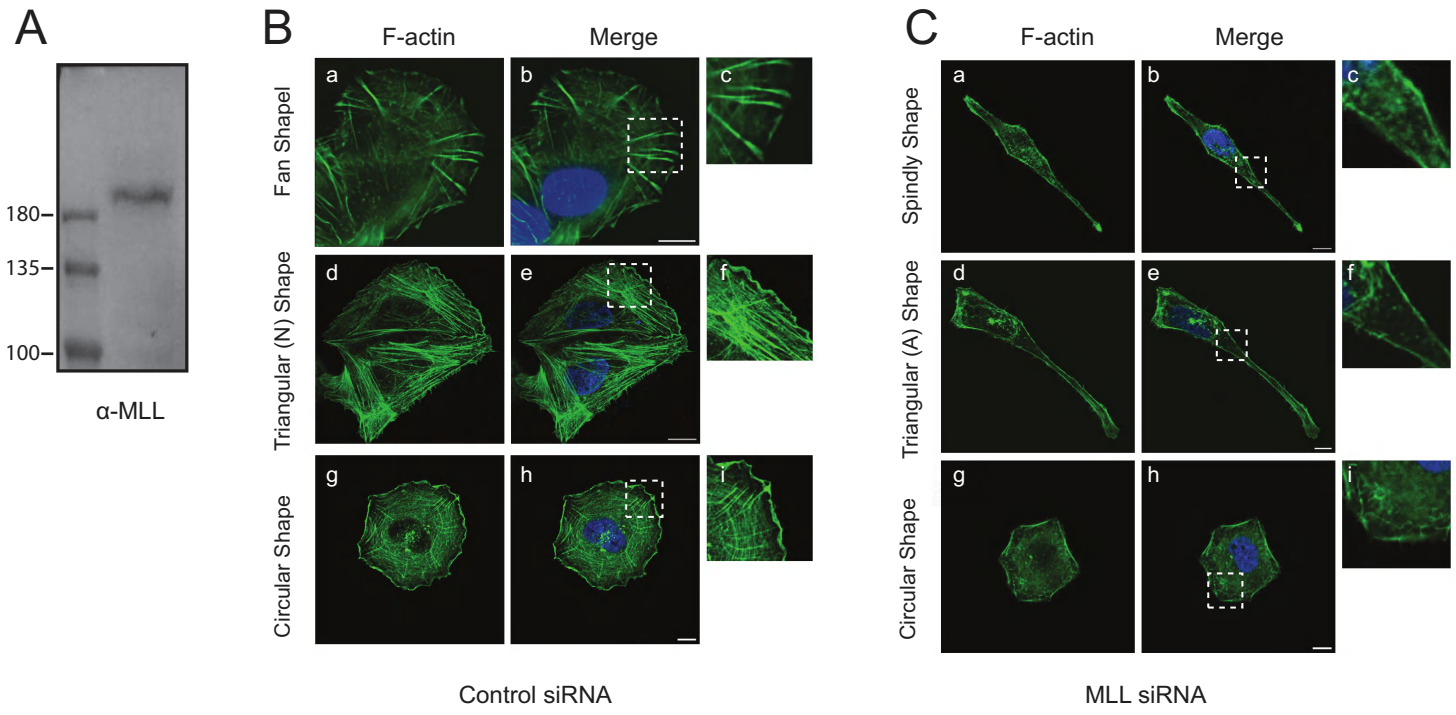

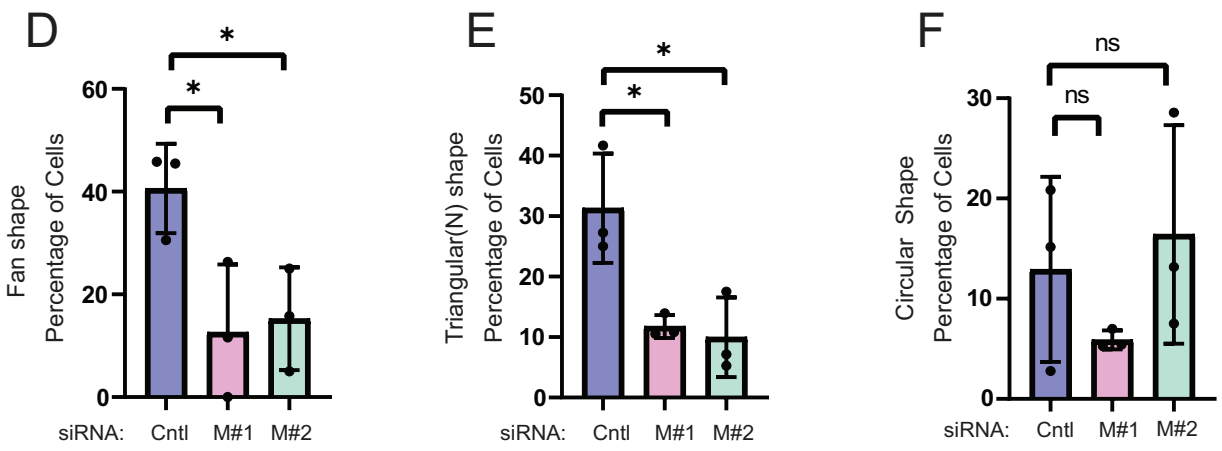

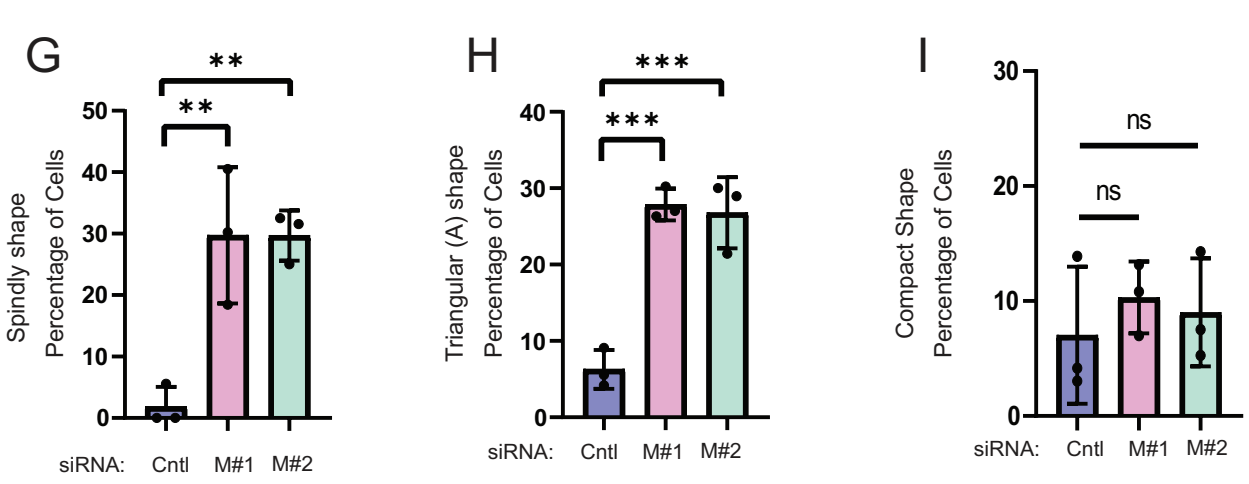

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Fig. S1. Loss of MLL affects cell shape and actin cytoskeleton.

(A) U2OS cells were lysed and immunoblotted. Blot was then probed with in-house generated MLL antibody. Molecular weights are indicated on the left. **(B-C)** U2OS cells treated with control or MLL siRNA were fixed and used for immunofluorescence and stained with rhodamine conjugated phalloidin to mark filamentous actin (green). Nucleus was stained using DAPI (blue). Representative images from control (**B**) and MLL (**C**) siRNA-treated cells exhibiting three major shapes are displayed, which are quantified in Figure **1D** as well as here. Images from individual MLL siRNA treatments are shown in Figure **1C**. Panels c,f,I, show magnified views of the boxed regions in panels b,e,h, respectively. Scale bars: 10 µm. **(D-I)** Quantification of percentage of cells showing different cell shapes from **B-C**. Data represents mean ± SD. *P = 0.0339 and 0.0493 **(D)**, *P = 0.0188 and 0.0128 **(E)**, ns P = 0.5140 and 0.8304 **(F)**, **P = 0.0052 each **(G)**, *** P = 0.0004 and 0.0005 **(H)**, ns P =0.6284 and 0.8323 **(I)** for M#1 and M#2 respectively. One way ANOVA with Dunnett's multiple comparisons test was performed. F-actin, filamentous-actin; Cntl, control; M#1, MLL siRNA1; M#2, MLL siRNA#2; Triangular (N), Triangular (Normal); Triangular (A), Triangular (Abnormal).

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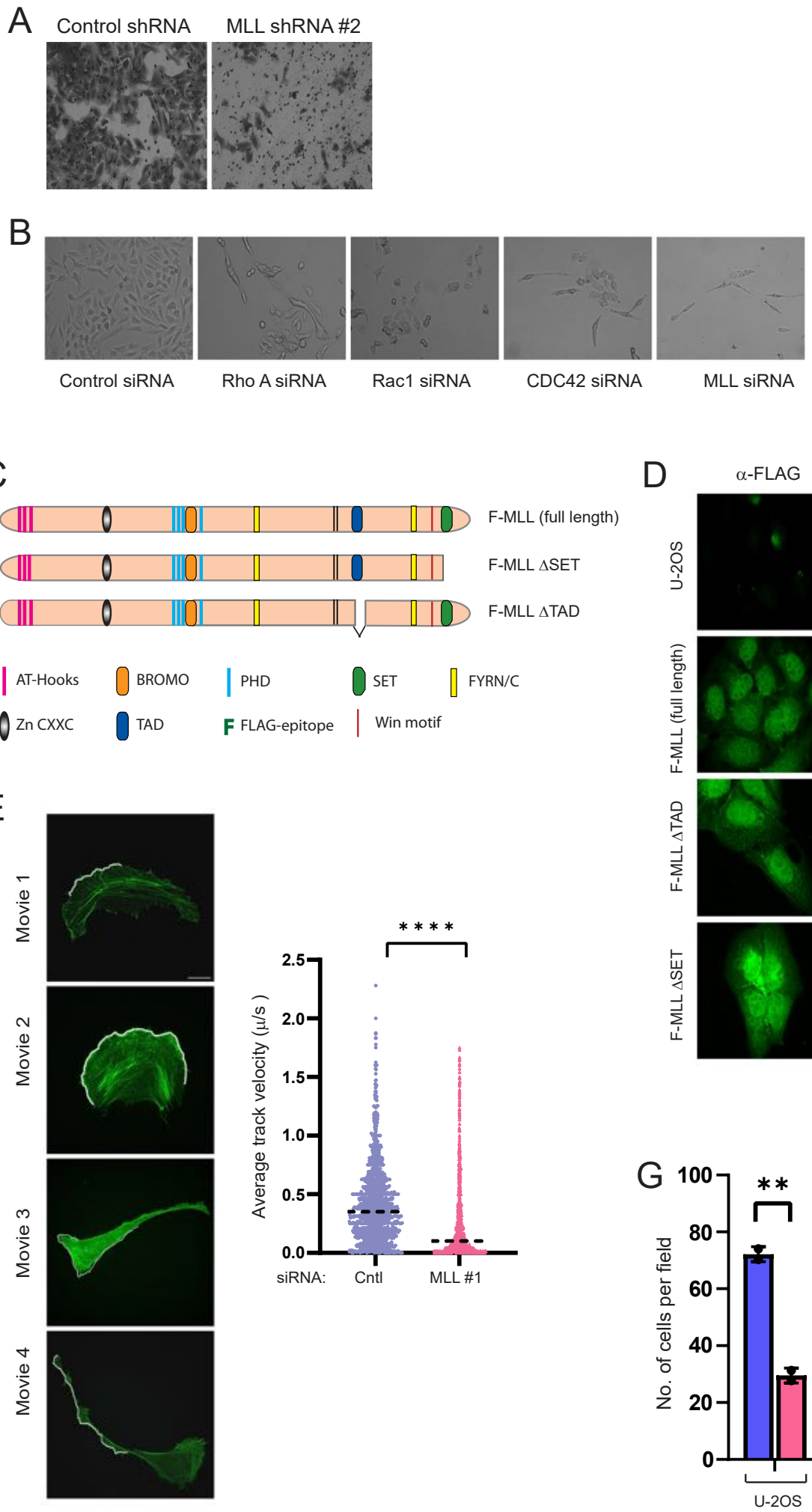

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Fig. S2. Depletion of MLL alters cell-spreading and cell migration.

(A) U2OS cells were treated with control or MLL shRNA#2 for 48 h and transwell migration (Boyden chamber) assay was performed. Bright field images show migrated cells upon treatment with respective shRNAs as indicated. (**B**) Bright field images of U2OS cells treated with various siRNAs (indicated below the images) for 72 h. Images were captured in Zeiss Axiovert 40 CFL at 10X magnification. (**C**) Schematic of domain architecture of FLAG epitope tagged full length MLL and its different mutants used for the rescue experiments is shown. F-MLL (full length) denotes FLAG epitope tagged MLL protein with all its domains intact whereas F-MLLΔSET and ΔTAD denote recombinant MLL protein devoid of its transcription effector— SET or transactivation —domain respectively. Domains of MLL which impart it chromatin binding capabilities are shown as indicated: AT hooks bind to minor grove of DNA, Zinc finger (Zn) CXXC domain helps in recognition of unmethylated CpG islands, Bromodomains (Bromo) are essential for protein-protein interactions, plant homeodomain (PHD) of MLL help it to read histone marks. The 'FY' rich N-terminal (FYRN) and 'FY' rich C-terminal (FYRC) domains are required for heterodimerization of MLL_N with MLL_C subunits. WDR5 interacting (Win) motif is responsible for interacting with WDR5. (**D**) U2OS and U2OS cells expressing full length MLL and its various mutants tagged with FLAG epitope tag were fixed for immunofluorescence. Cells were stained with anti-FLAG antibody (green) to detect recombinant MLL expression and nucleus was stained with DAPI. Scale bars: 10 µm. (**E**) Live cell movies of U2OS cells expressing GFP–Lifeact from Figure **3G** were utilised for generating kymographs shown in panel on the left. Region used for kymograph generation are marked in white. Scale bars: 10 µm. Average track velocities from same time-lapse images in Figure **3G** are shown. ****P <0.0001, (Student's unpaired two-tailed t test; n = 44 movies for each siRNA from 3 experiments). **(F)** U2OS cells and U2OS expressing various mutants of MLL were treated with control and MLL shRNA#2 for 48 h and 10^5 cells were seeded for transwell migration assay. Quantifications for number of cells migrated per field of image is shown. Data are represented as mean ± SD. **P =0.004 (U2OS) and 0.003 (SET) ns, P =0.4 respectively (Student's unpaired two-tailed t test; m=2 experiments). ns, not significant; cntl, control.

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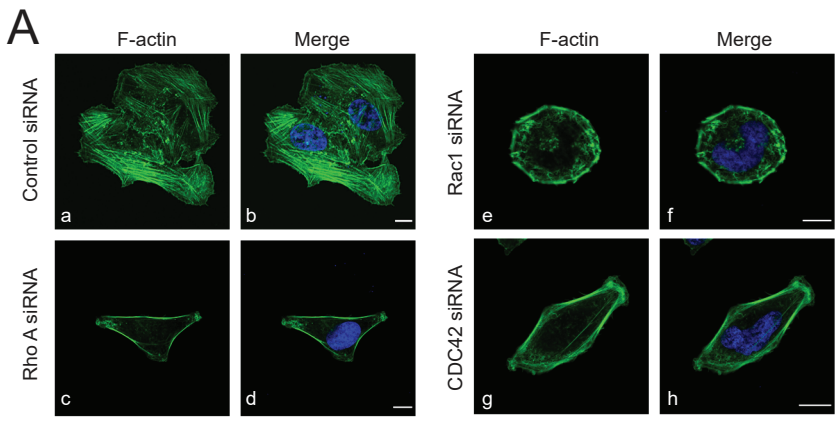

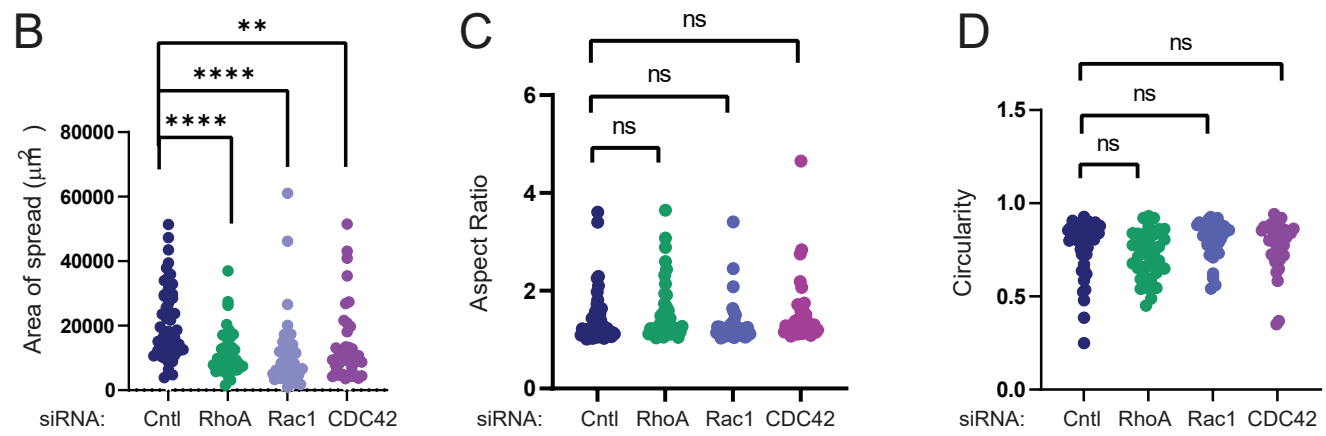

Fig. S3. Rho GTPases depletion alters cell-spreading.

(**A**) Fibronectin spreading of control U2OS cells (panels a and b) and U2OS cells upon knock down of Rho GTPases: RhoA (panels c and d), RAC1 (panels e and f) and CDC42 (panels g and h). The cells were stained with rhodamine-conjugated phalloidin to mark F-actin (green) and DAPI to mark nucleus (blue). Scale bars: 10 μm. (**B**) Quantification of area of spread of cells treated with respective siRNAs. Data represents violin plot with all the data points. ***P =0.0005 for RhoA siRNA, ****P <0.0001 for RAC1 siRNA and **P =0.001 for CDC42 siRNA-treated cells respectively. (**C**) Quantification of aspect ratio of cells treated with respective siRNAs. Data represents violin plot with all the data points. ns, P =0.72, 0.62, 0.90 for RhoA, RAC1 and CDC42 siRNA treated cells respectively. (**D**) Quantification of circularity of cells treated with respective siRNAs. Data represents violin plot with all the data points. ns, P =0.09, 0.22, 0.99 for RhoA, RAC1 and CDC42 siRNA treated cells respectively. (**B-D**) One-way ANOVA with Dunnett's multiple comparisons test was performed. n = 40 cells and m = 2 experiments. F-actin, filamentous actin; Cntl, control. ns, not significant.

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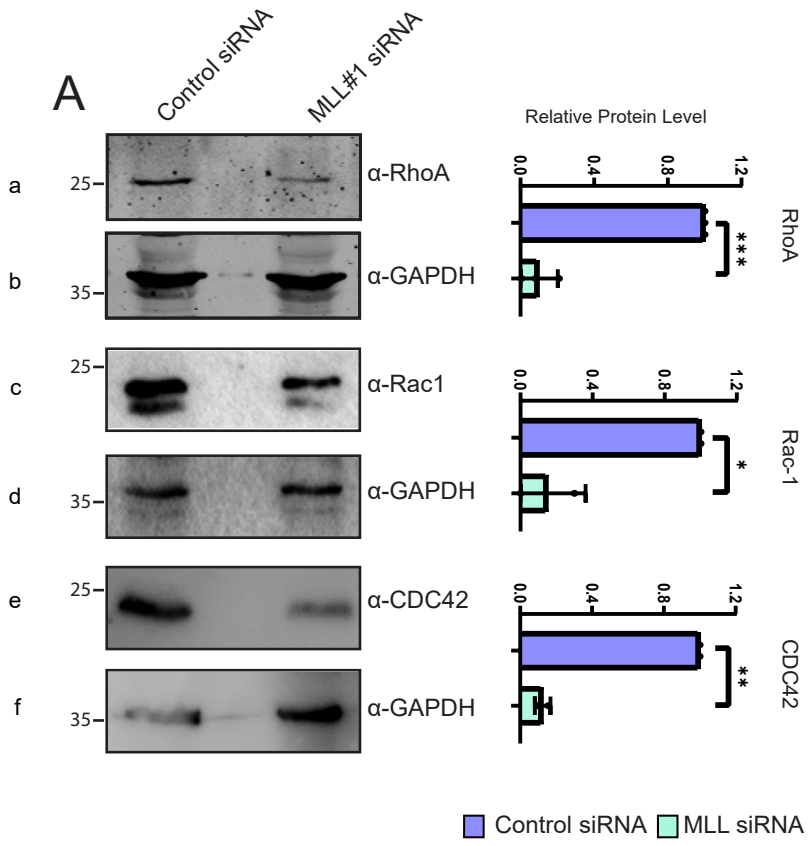

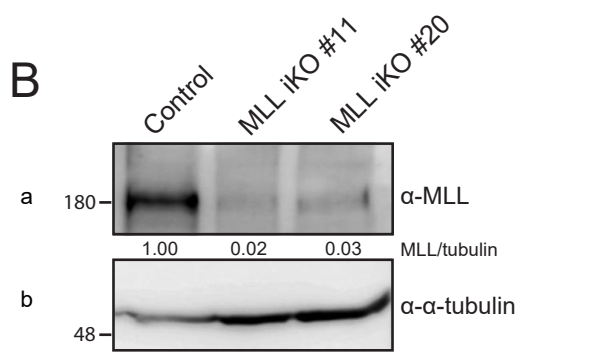

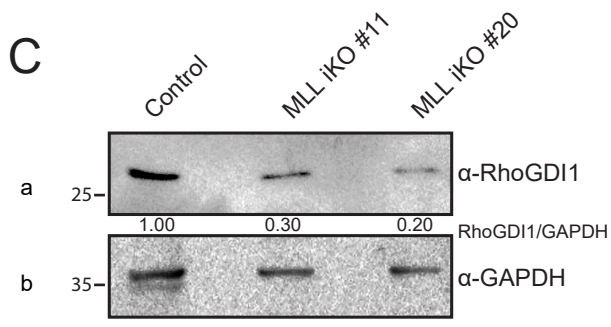

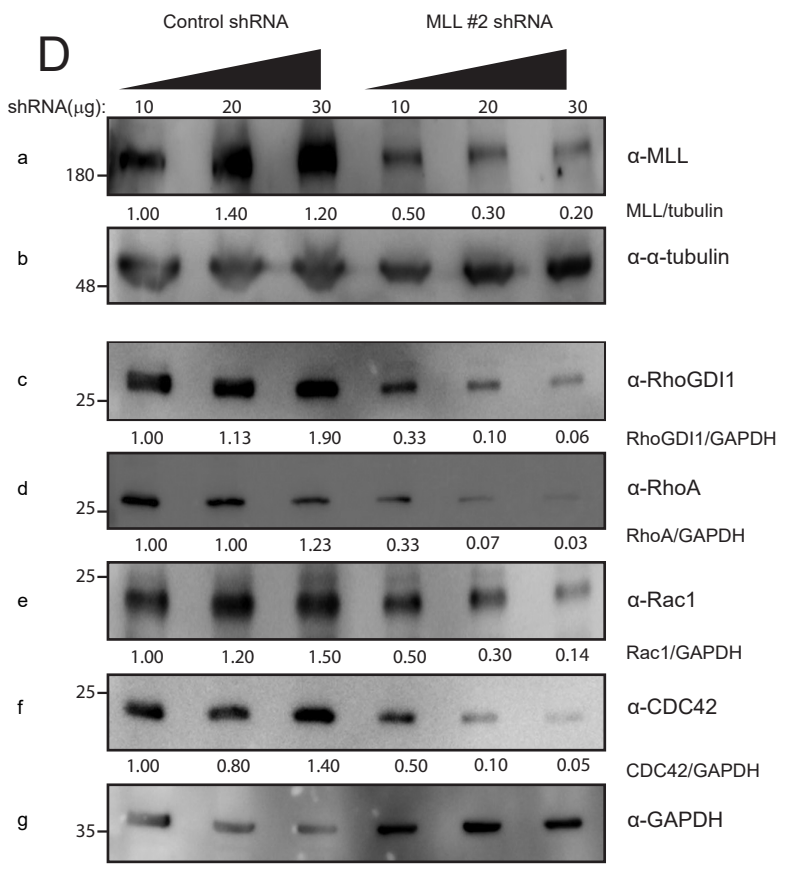

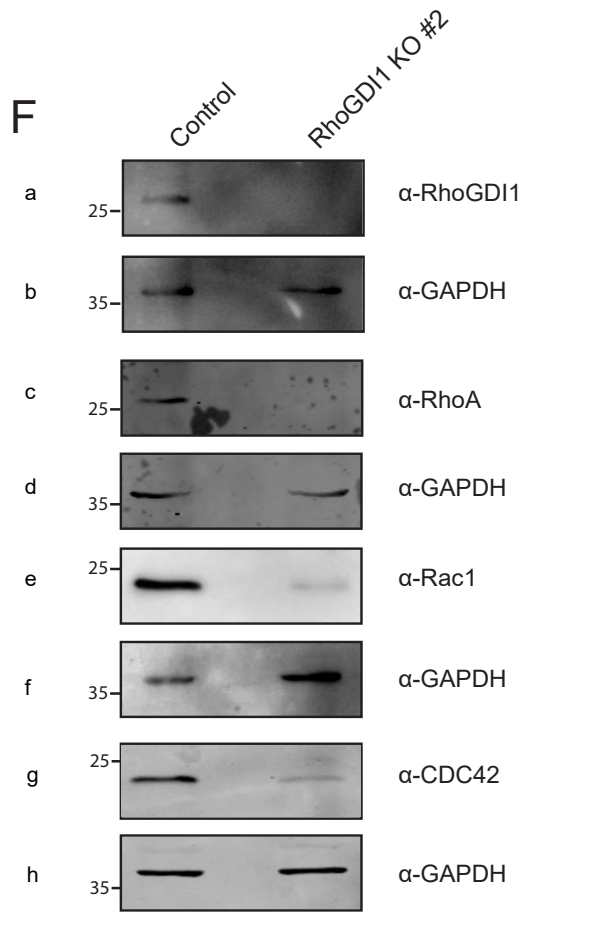

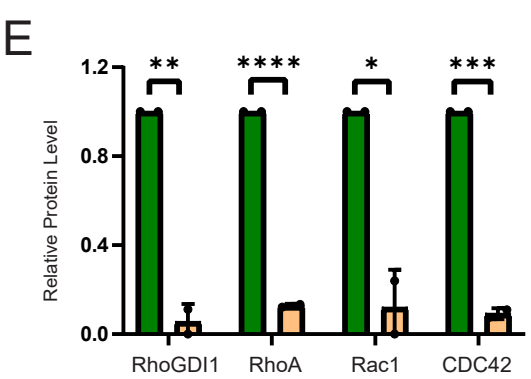

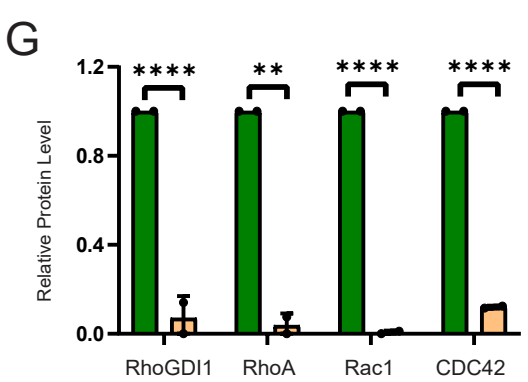

Chinchole Supplementary Figure 4

Fig. S4. MLL regulates the protein levels of RhoGDI1.

(A) MDA-MB-231 cells treated with either control or MLL#1 siRNAs, were harvested after 72 h, lysed and immunoblotted. The blots were probed with various antibodies as indicated on the right. Panels a, c and e show the endogenous protein levels of RhoA, Rac1 and CDC42 respectively upon MLL depletion. The blots shown in panels b, d and f were probed with anti-GAPDH antibody. Numbers on the left indicate molecular weight markers in kDa. Quantifications of protein levels relative to loading control are shown on the right of the blots. Data represents mean ± SD. ***P = 0.0001, *P = 0.0296 and ** P = 0.0012 for RhoA, Rac1 and CDC42 respectively. (Student's unpaired two-tailed t test; m=2 experiments). **(B, C)** Control HEK293 and MLL knock out cell lines #11 and #20 were lysed in NETN buffer and immunoblotted. The blots were probed with antibodies against MLL (**B**, panel a), RhoGDI1 (**C** panel a), and loading control, α-tubulin (**B** panel b) and GAPDH (**C**, panel b). The MLL/tubulin (**B**) and RhoGDI1/GAPDH (**C**) ratios are indicated. **(D)** U2OS cells treated with 10, 20 or 30 µg of control or MLL shRNA#2 were lysed and immunoblotted. Blots were probed with antibodies against MLL, RhoGDI1, RhoA, Rac1 and CDC42 in panels, a, c, d, e and f respectively, as indicated. The blots shown in panels b and g were probed with anti- α-tubulin and ani-GAPDH antibodies respectively. The MLL/tubulin and Rho GTPases/GAPDH ratios are indicated. **(E)** Quantification of protein levels relative to loading control of immunoblots from Figure **5E** are shown. Data represents mean ± SD. **P = 0.0035, ****P <0.0001, *P = 0.018, ***P= 0.0004. Student's unpaired two-tailed ttest; m= 2. **(F)** Control U2OS and RhoGDI1 knock out #2 cells were lysed and immunoblotted. The blots were probed with anti RhoGDI1 (panel a), RhoA (panel c), RAC1 (panel e) and CDC42 (panel g) antibodies respectively. Loading control, GAPDH is shown in panels b, d, f and h for respective blots. **(G)** Quantification of relative protein levels from immunoblots in **F** are shown. Data represents mean ± SD. ****P <0.0001 for RhoGDI1, Rac1 and CDC42. **P = 0.002 for RhoA. Student's unpaired two-tailed t test; m= 2. (A-D, F) Numbers on the left indicate molecular weight markers in kDa; KO, knock out.

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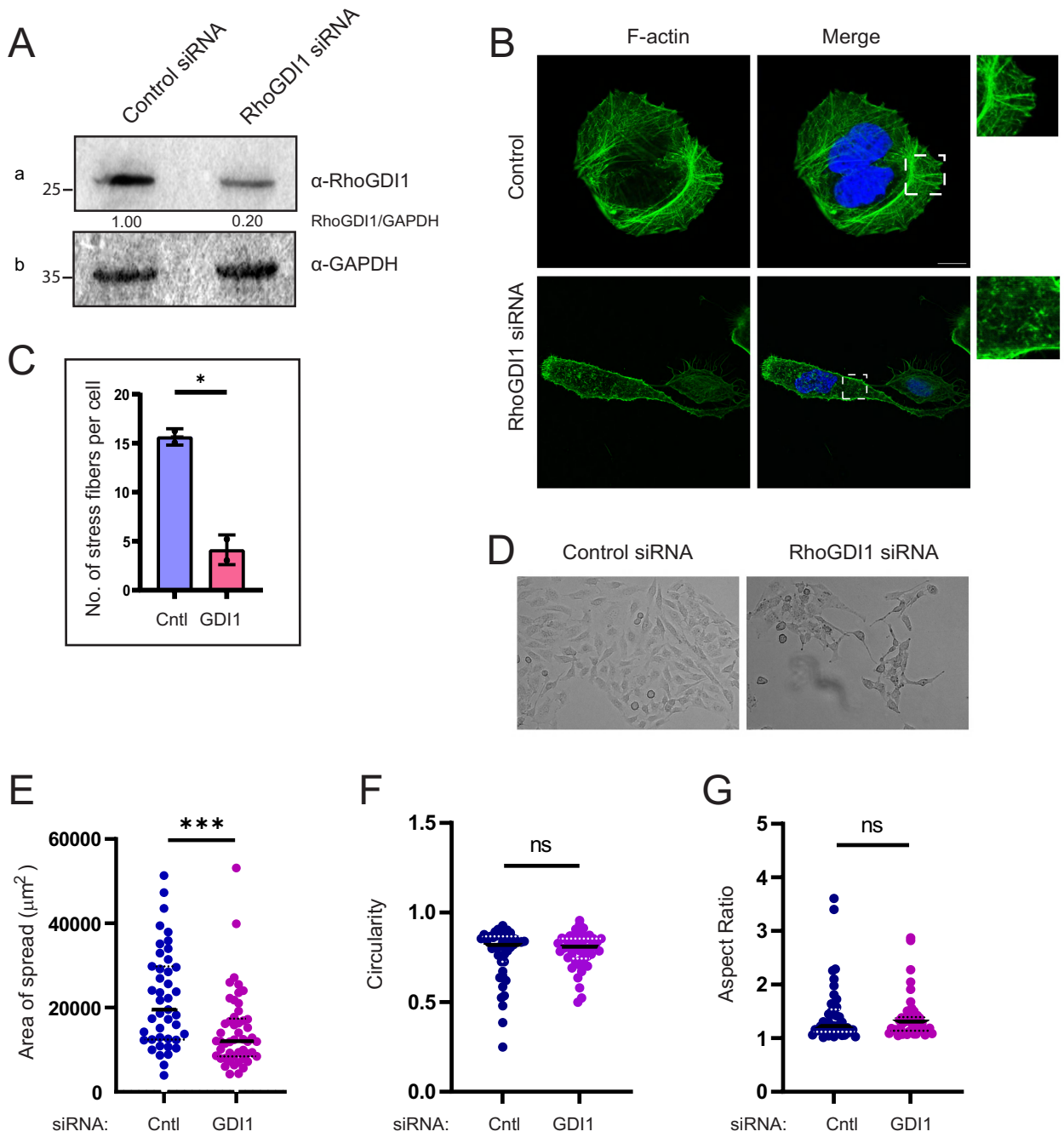

Fig. S5. Depletion of RhoGDI1 affects actin stress fiber formation and cell spreading.

(A) U2OS cells were treated with control or RhoGDI1 siRNAs for 72 h, lysed and immunoblotted. Blots were probed with RhoGDI1 (panel a) and GAPDH (panel b) antibodies. Numbers on the left indicate molecular weight markers in kDa. The RhoGDI1/GAPDH ratio is indicated **(B)** U2OS cells were seeded on cover slips and treated with either control or RhoGDI1 siRNA for 72 h prior to fixation for immunofluorescence. Actin stress fibers stained with stained with rhodamine-conjugated phalloidin is shown in control siRNA (upper panel) and RhoGDI1 siRNA (lower panel) treated cells respectively. Insets are shown adjacent to IF images. White squares indicate region highlighted in insets. Scale bars: 10 μm. **(C)** Quantification of number of stress fibers per cell from **B** is shown. Data represents mean ± SD.

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*P = 0.01 (Student's unpaired two-tailed t test; n = 40 cells, m = 2 experiments). Data in wild type U2OS is same as Figure **3E**, as these experiments were done together. **(D)** U2OS cells were treated with control and RhoGDI1 siRNA for 72 h and bright field images were captured at 10x magnification in Zeiss Axiovert CFL 40 microscope. **(E)** U2OS cells treated with control and RhoGDI1 siRNA were spread on fibronectin coated cover slips for 4 h and the area of spread of the cells was quantified. ***P =0.0005. **(F)** Quantification of circularity of control and RhoGDI1 siRNA treated U2OS cells. ns P =0.43. **(G)** Quantification of aspect ratio of control and RhoGDI1 siRNA treated U2OS cells. ns P =0.50 **(E-G)** Data represents violin plot with all data points. Student's unpaired two-tailed t test; n = 40 cells and m = 2 experiments. F-actin, filamentous actin; Cntl, control; GDI1, RhoGDI1; No., number; ns, not significant.

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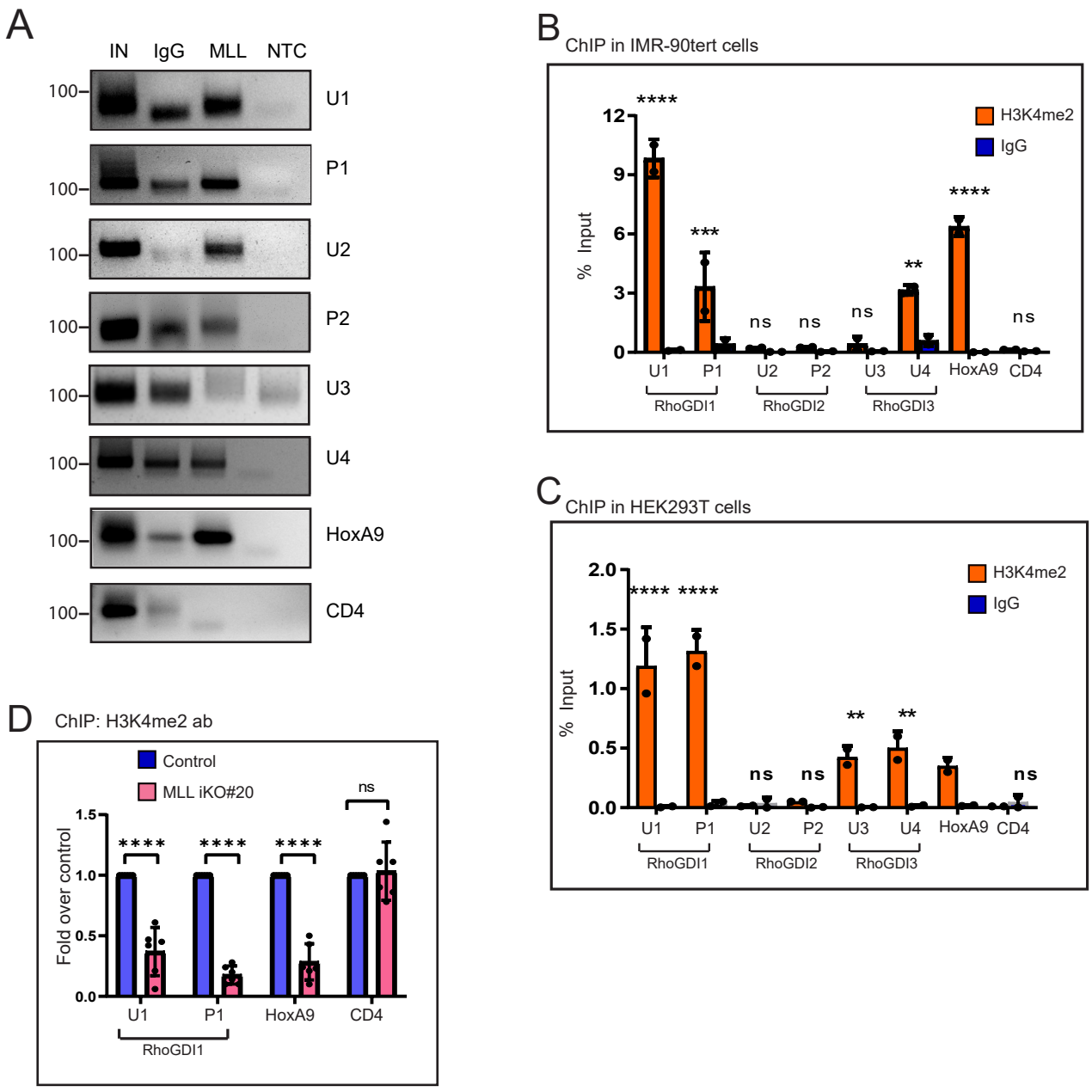

Fig. S6. MLL binds to RhoGDI1 promoter to deposit H3K4 trimethylation marks.

(A) HEK293 cells were subjected to ChIP PCR analysis using anti-MLL and IgG antibodies. The ChIP samples were subsequently used for RT q-PCR amplification. Agarose gel image of the amplicons obtained from RTq-PCR are shown. Primers used for the amplification are indicated on the right. Numbers indicated on the right are molecular weight marker in base pair. **(B-C)** ChIP was performed cells using H3K4me2 and IgG antibody in IMR-90tert **(B)** and HEK293T **(C)** cells. (**D**) ChIP assay was performed in HEK293 control (Cas-9 expressing) and MLL knockout cell lines using H3K4me2 antibody. The immunoprecipitated DNA was quantified with RT-qPCR and the results are plotted as the percentage enrichment relative to the input in **B and C** and as fold change with respect to control in **D,** as described in main text. The antibodies used are indicated in the top right corner of each box. The error bars represent mean ± S.D. two-way ANOVA, *P ≤0.05, **P ≤0.01, *** P ≤0.001, ****P ≤0.0001, ns: not significant, P > 0.05. All the experiments are done at least twice. IN, input; NTC, no template control.

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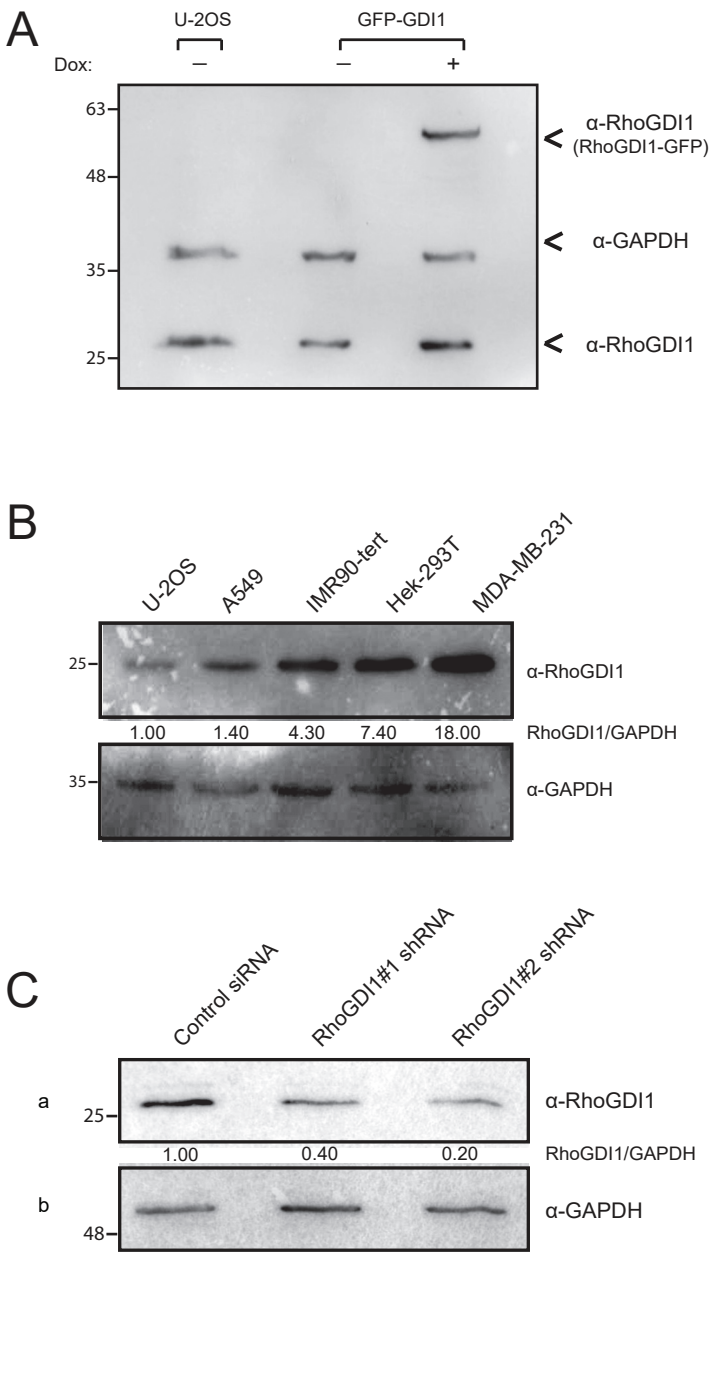

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Fig. S7. Endogenous expression of RhoGDI1 in different cell lines.

(A) Western blot analysis of whole cell lysates from U2OS cells as well as U2OS cells over-expressing GFP tagged RhoGDI1 (GFP-GDI1) is shown. The expression of GFP-RhoGDI1 is induced by Doxycycline (Dox). The immunoblots were probed with anti-RhoGDI1 antibody (which detected both recombinant RhoGDI1-GFP and endogenous RhoGDI1) and anti-GAPDH antibody is shown. (**B**)Whole cell lysates were prepared from U2OS, A549, IMR- 90tert, HEK293T and MDA-MB-231 cell lines and immunoblotted. Blots were probed using anti-RhoGDI1 antibody (upper panel) and anti-GAPDH antibody (lower panel). **(C)** MDA-MB-231 cells were treated with control shRNA or RhoGDI1 shRNA#1 or #2 and incubated for 48 h. Cells were harvested, lysed and immunoblotted. Blots were probed with anti-RhoGDI1 (panel a) and anti-GAPDH (panel b) antibodies. Numbers indicated on the left are the molecular weight markers. (**B-C**) The RhoGDI1/GAPDH ratios are indicated. (**D**) U2OS cells constitutively expressing GFP-RhoGDI1 for 72 h were fixed for IF and stained using F-actin (green). Nucleus was stained with DAPI (blue). Expression of GFP-RhoGDI1 is shown with 'red' pseudo-colour. Magnified views of the dashed boxes are shown. White arrows indicate stress fibers. Scale bar: 10 µm.

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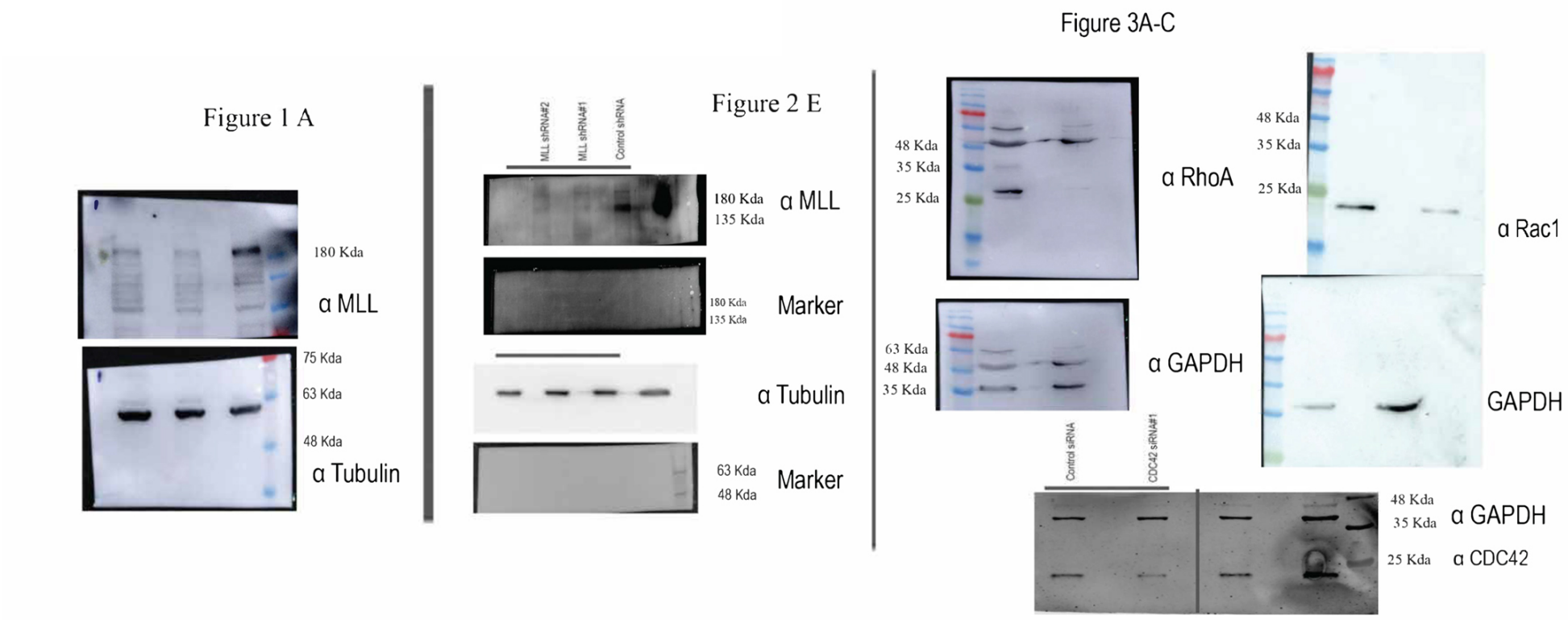
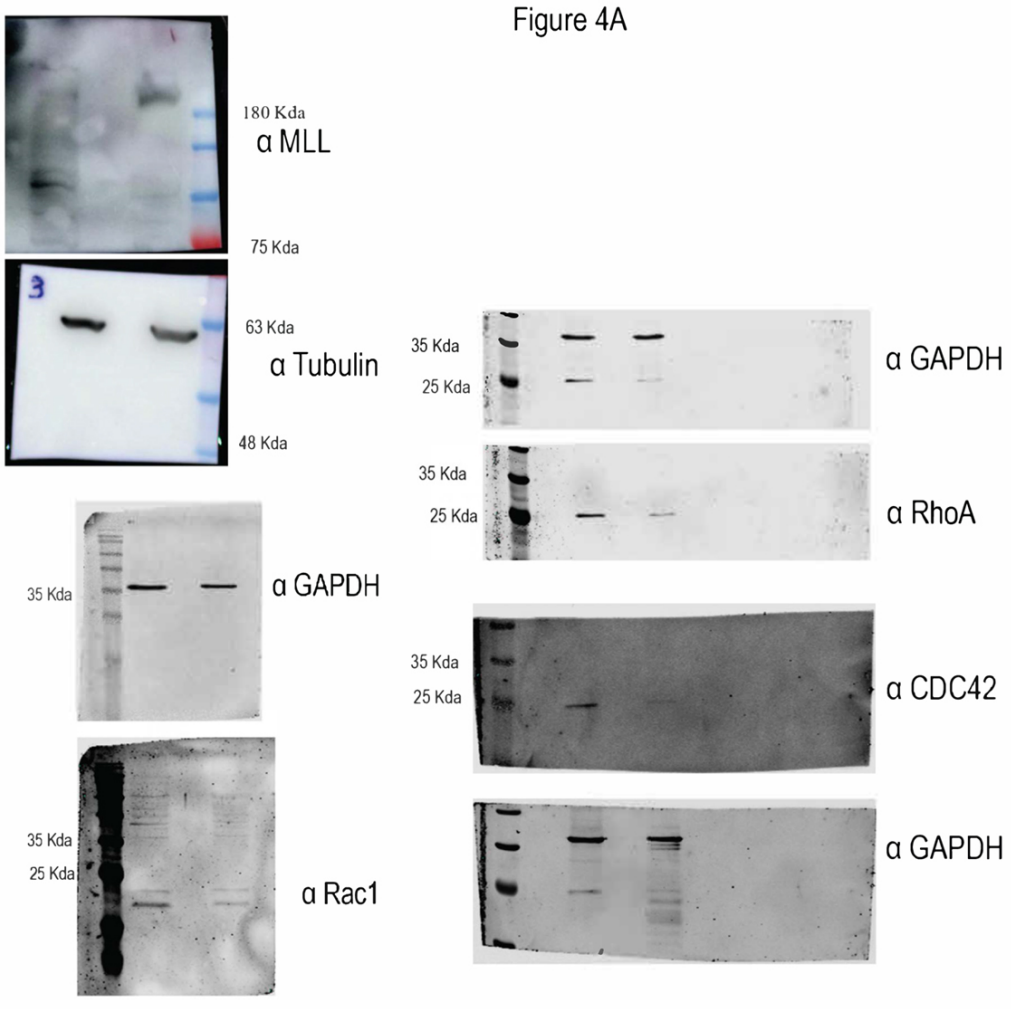
Fig. S8. Blot Transparency





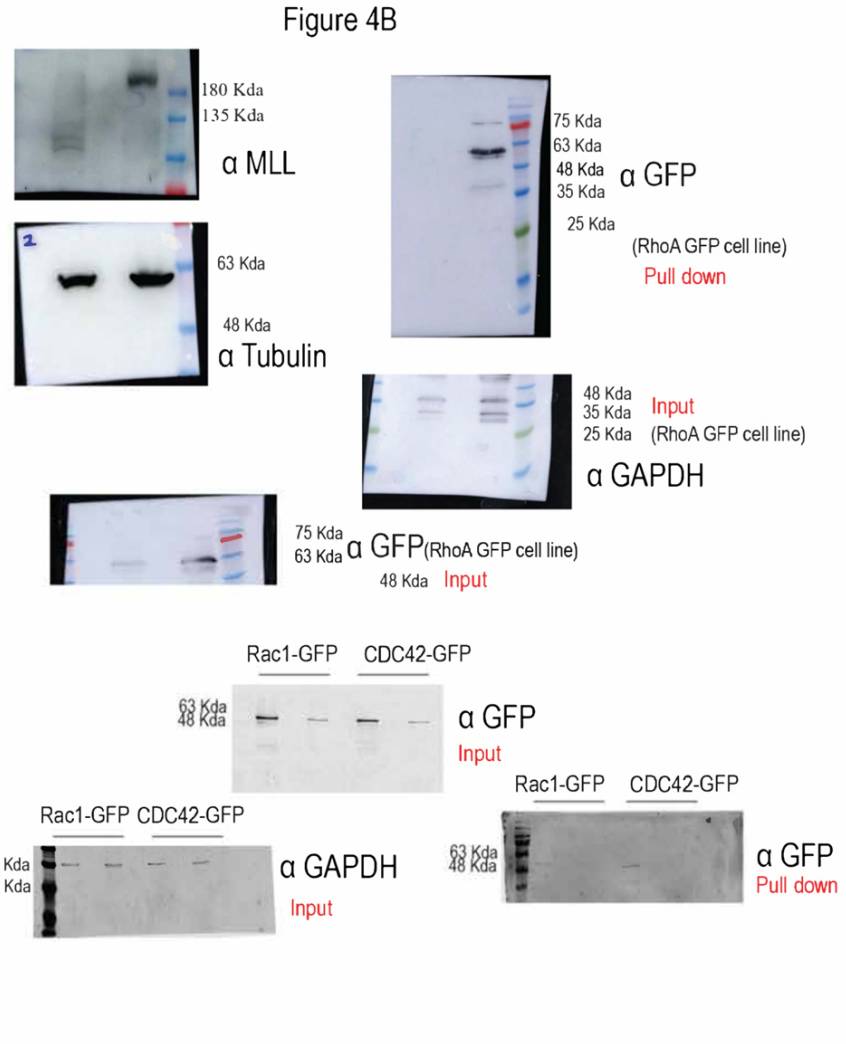


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Figure 5A

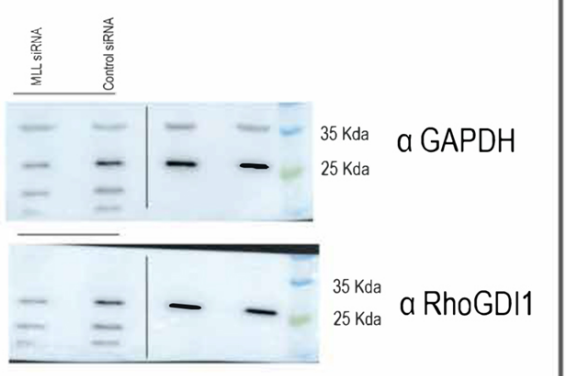


Figure 5B

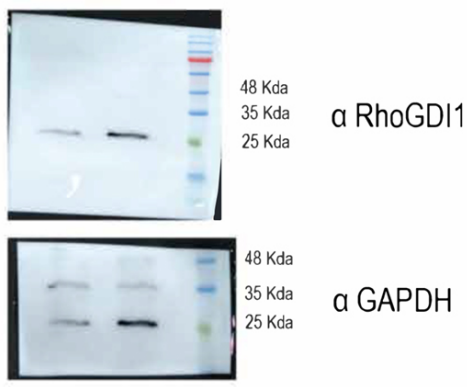
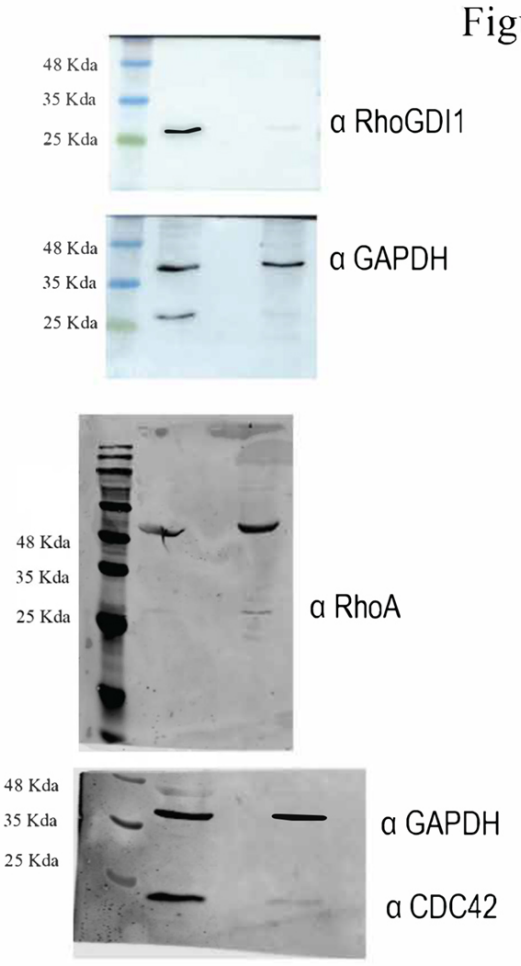
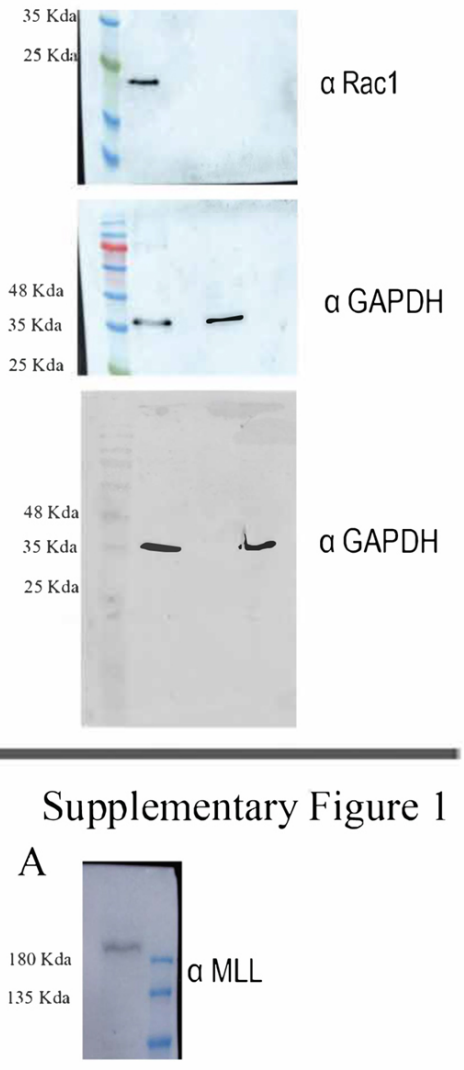


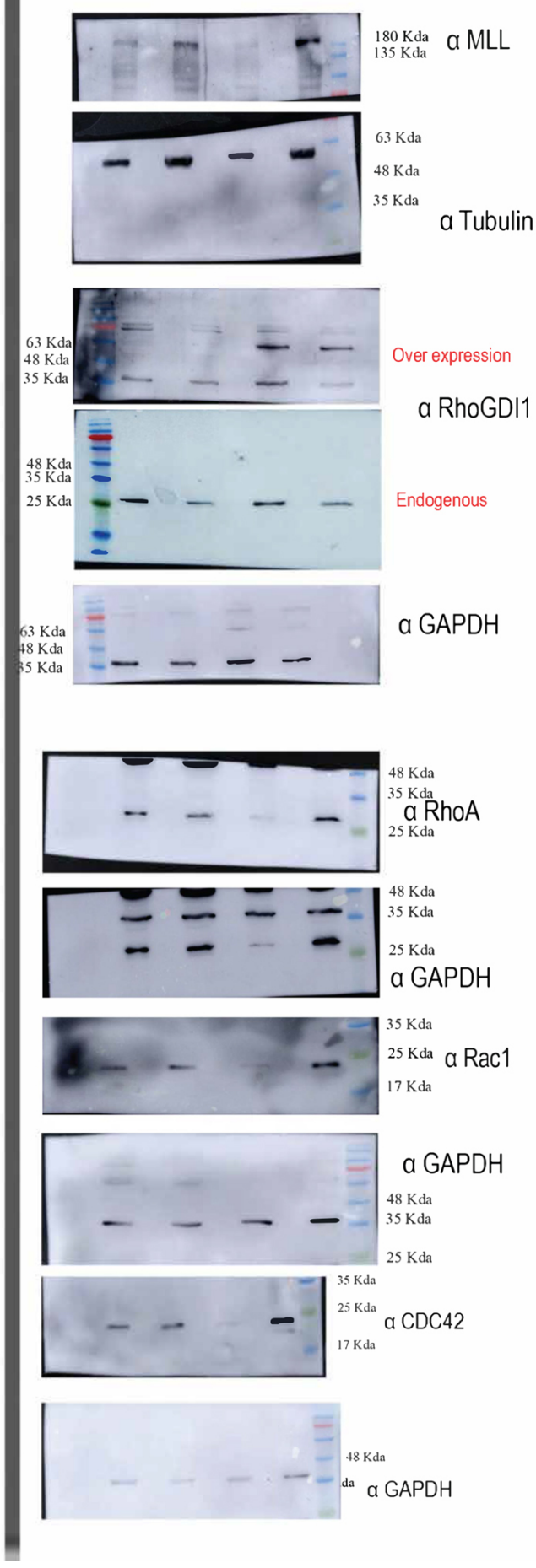
Figure 5E





Supplementary Figure 1

Figure 7A



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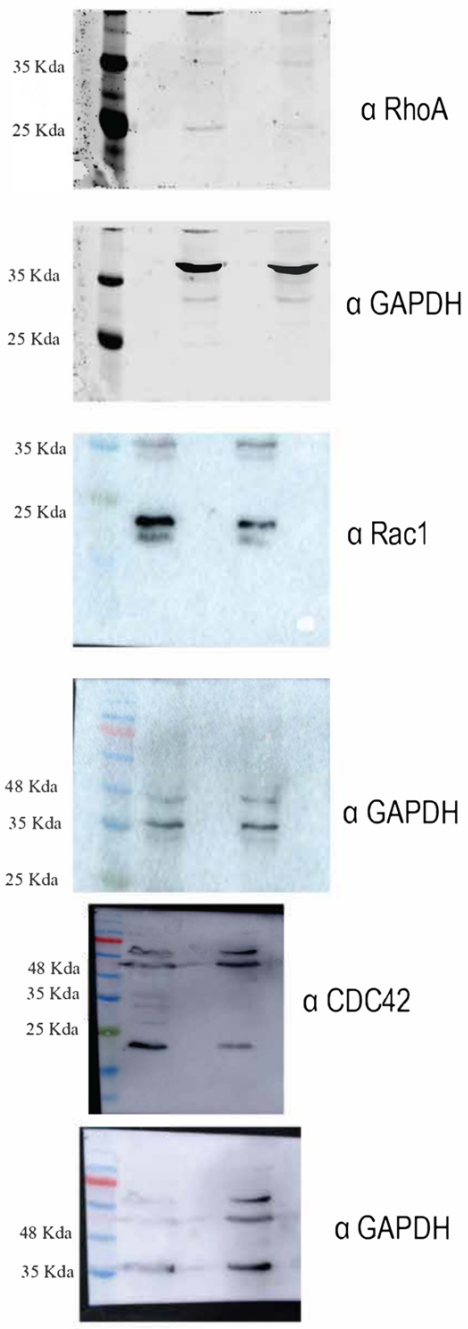
Supplementary Figure 4A



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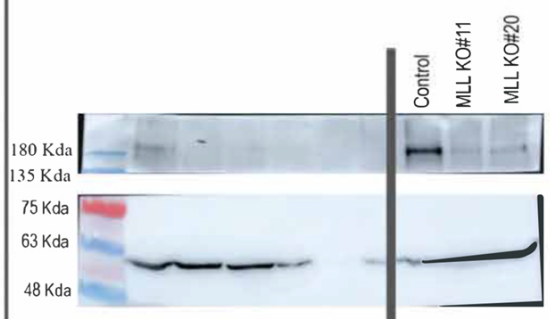
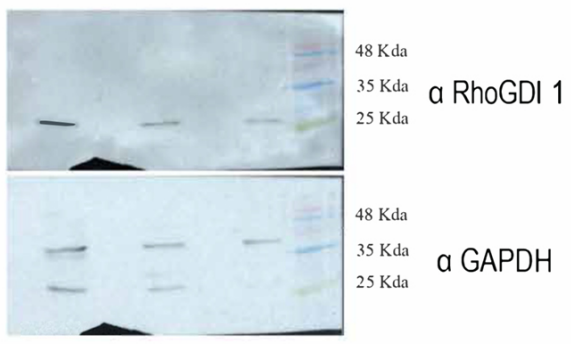
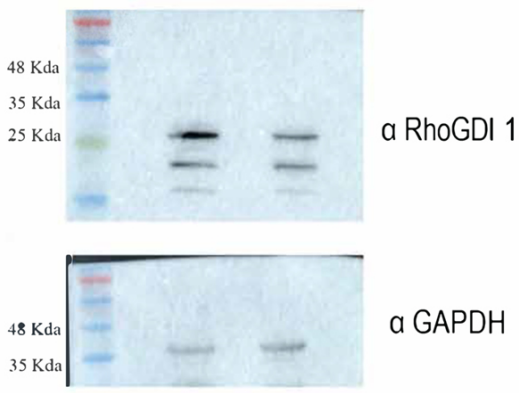


Supplementary Figure 4C



Supplementary Figure 5A



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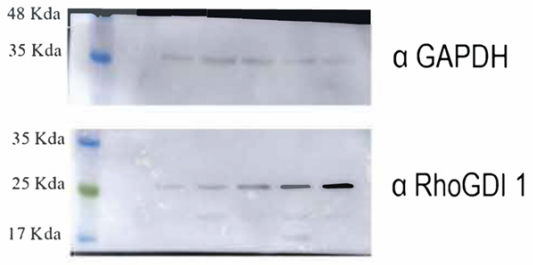

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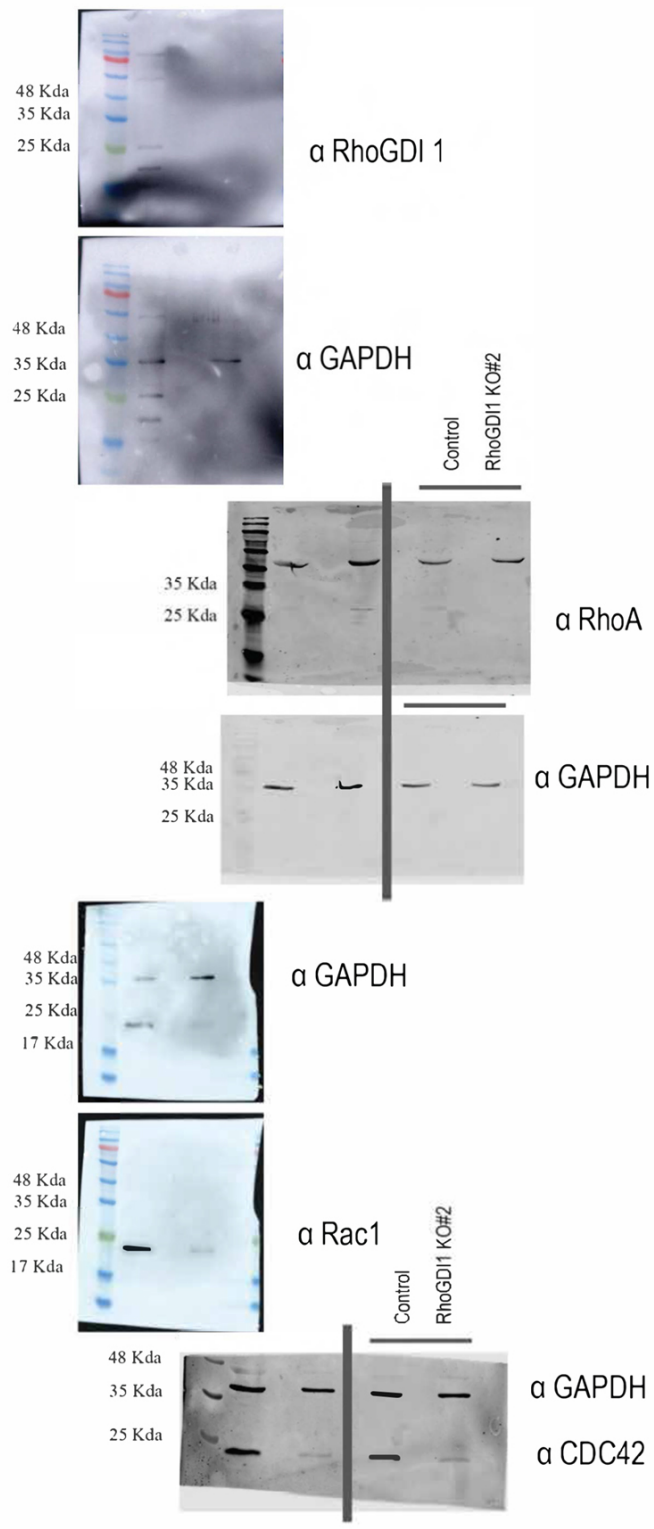

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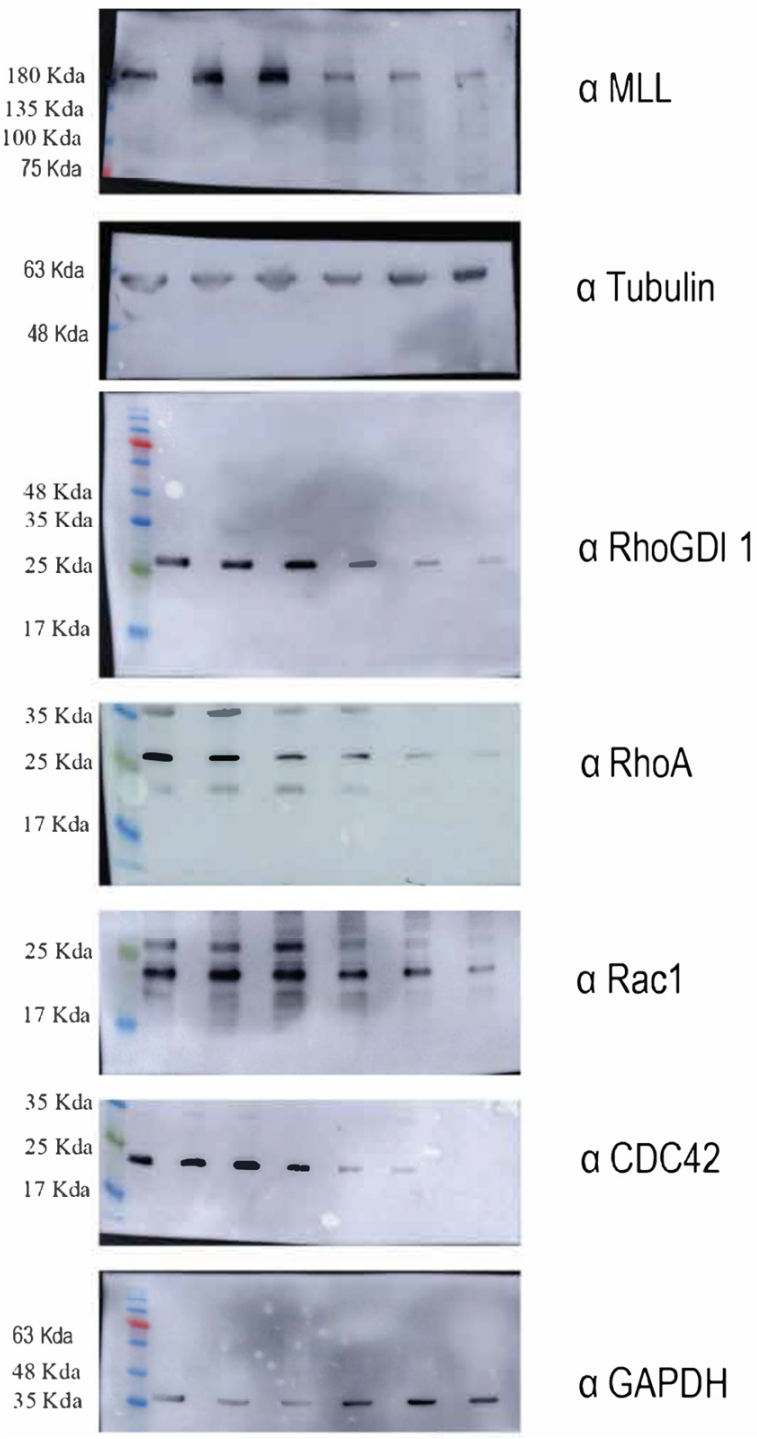
Supplementary Figure 4D



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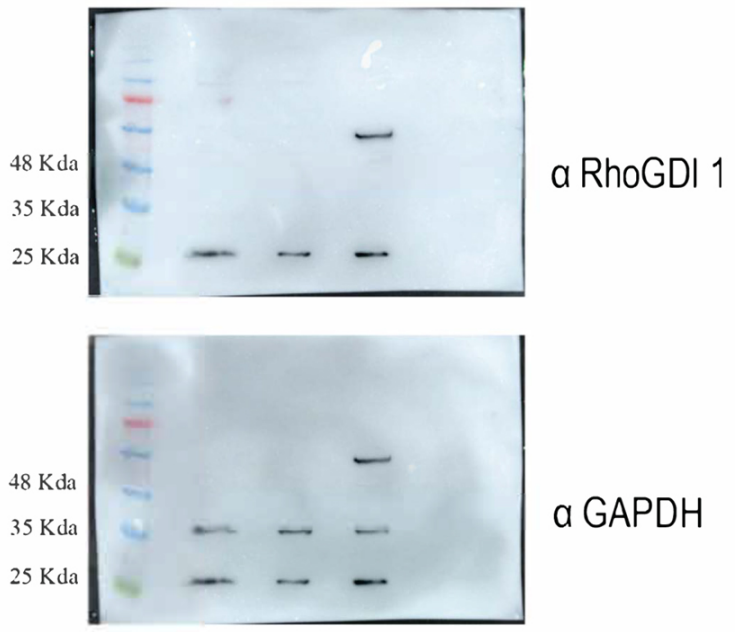

Supplementary Figure 7C

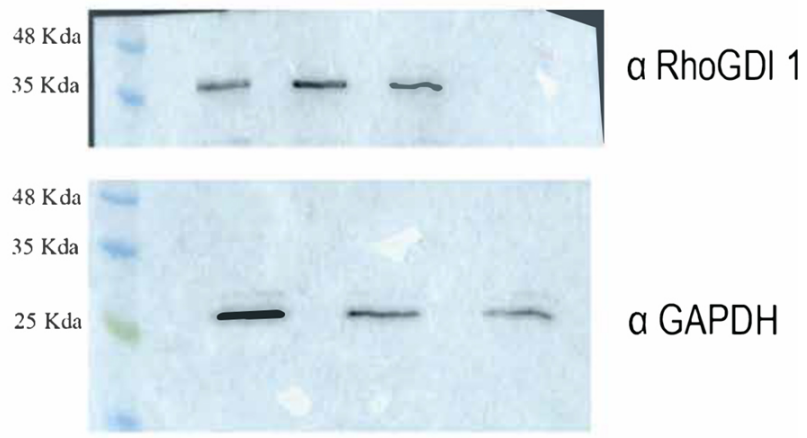

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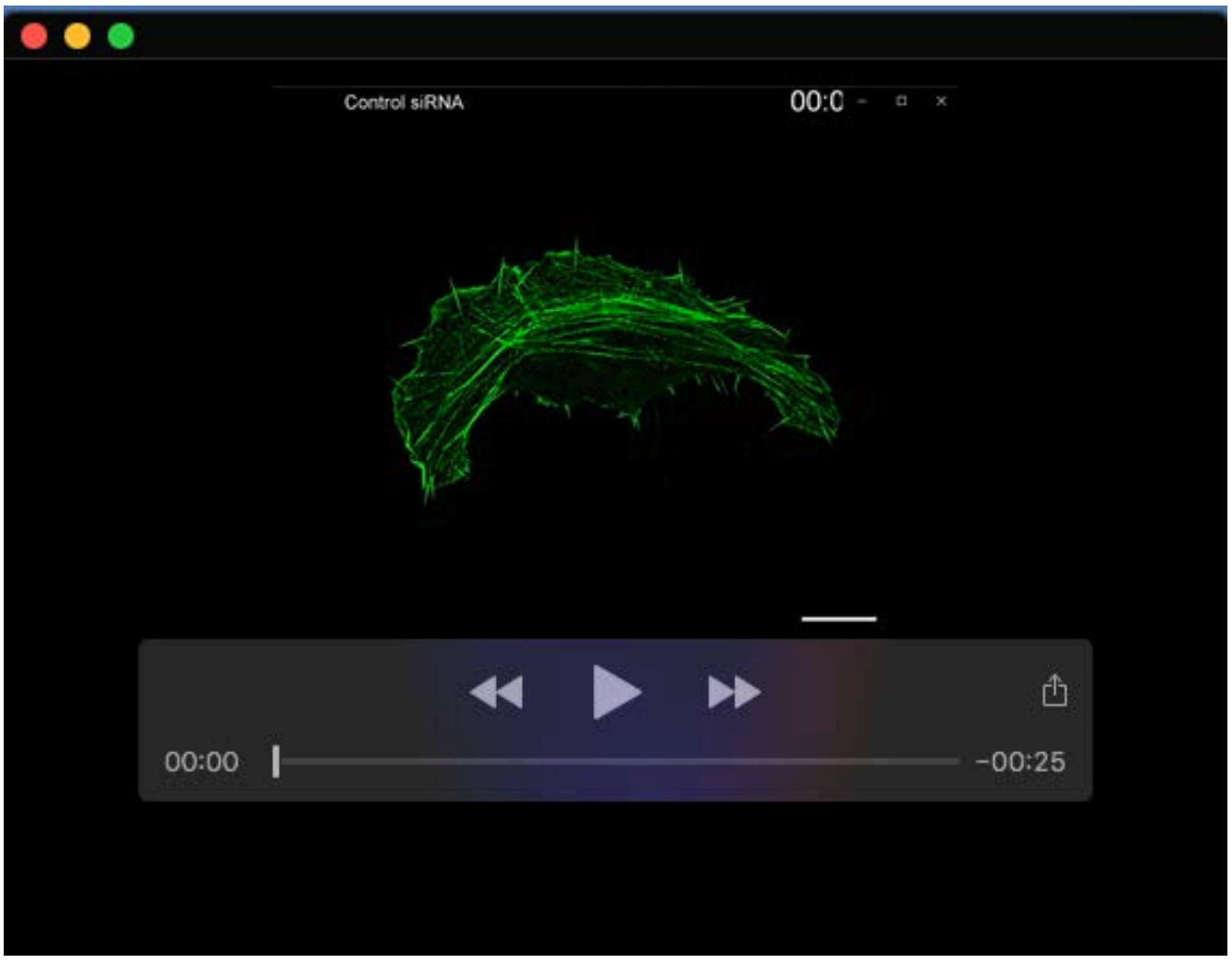
Table S1. Primers used for transcript analysis

Primer Names	Sequences
GAPDH qRT	F: 5'-ATGTTCGTCATGGGTGTGAA-3' R: 5'-GAGGCAGGGATGATGTTCTG-3'
MLL qRT	F: 5'-ATCGTCCACCGCAAATGCTTCTA-3' R: 5'-AGCCATGCCAATCTCATCTTGTT-3'
RhoA qRT	F: 5'-AAGGACCAGTTCCCAGAGGT-3' R: 5'-TTCTGGGGTCCACTTTTCTG-3'
Rac1 qRT	F: 5-'CGCAAACAGATGTGTTCTTA-3' R: 5'-CTAGGATGATGGGAGTGTTG-3'
CDC42 qRT	F: 5'-CTGAAGGCTGTCAAGTATGT-3' R: 5'-GAGAGATGTTCATAGCAGCA-3'
RhoGDI1 qRT	F: 5'-TAGGATCCCGGCGCCTAC-3' R: 5'-TTGGGGTCTGCGGAAACG-3'
RhoGDI2 qRT	F: 5'-ACAAAGCAGGGAAGTGTCAGA-3' R: 5'-GTCAGAGTTGAGAGACAGAGGC-3'
RhoGDI3 qRT	F: 5'-GGACCAGGTGTTTGTCCTGA-3' R: 5'-CCACGGGAGTCACAAACT-3'

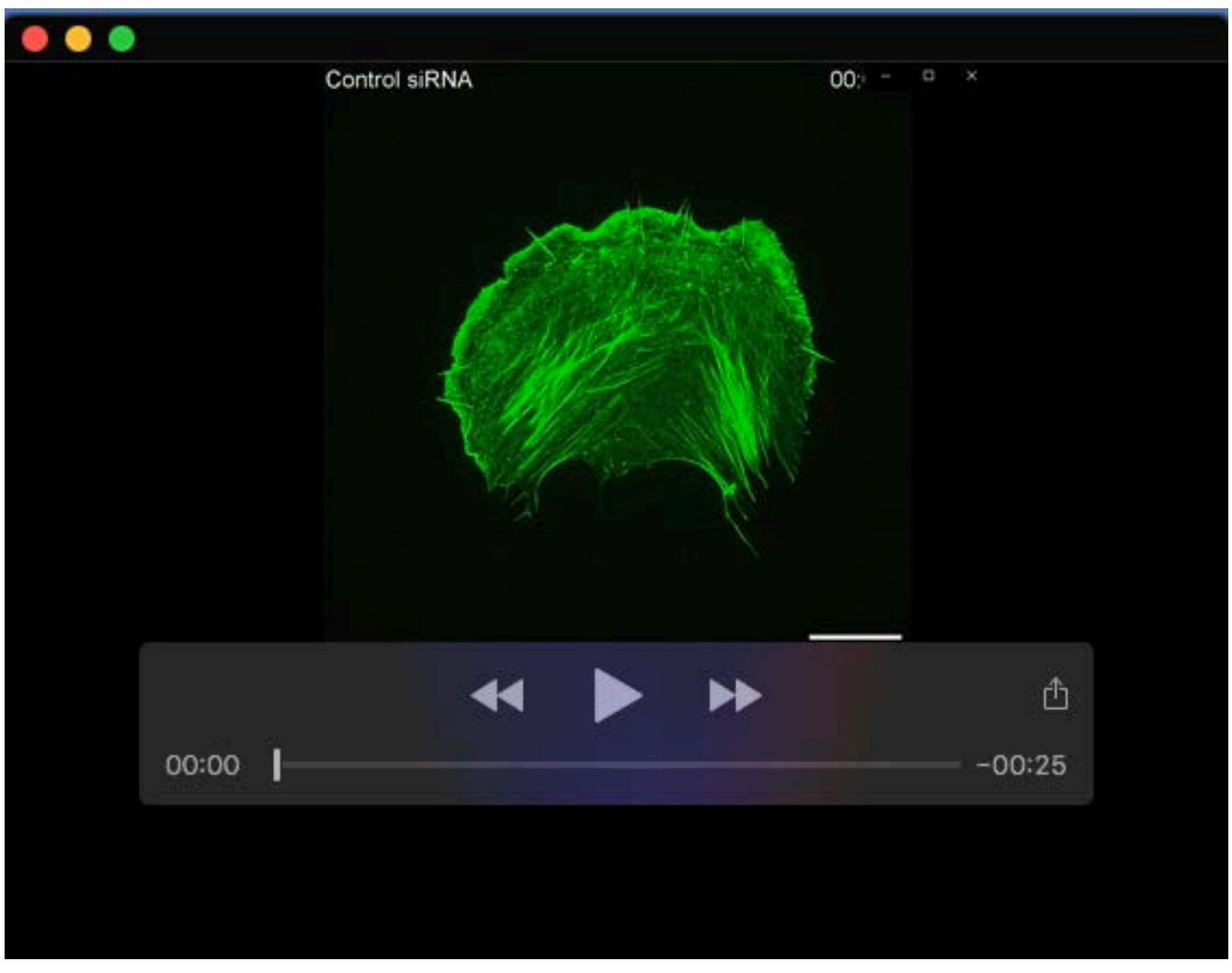
Table S2. Primers used for ChIP assay

Primer names	Sequence
RhoGDI1 PROMOTER (P1)	F: 5'-GTGAGCGGAAGTCTCGTG-3' R: 5'-GCCGCGCGGTTCAGGATC-3'
RhoGDI1 UPSTREAM (U1)	F: 5'- GGGCGAATGTGTGGAATCTC-3' R: 5'-CACCGTGAGCAGATGAGGG-3'
RhoGDI2 PROMOTER (P2)	F: 5'-CCAGGGTTTCCTCTTCAAGTAG-3' R: 5'-CTGTCTCTCAACTCTGACTTC-3'
RhoGDI2 UPSTREAM (U2)	F: 5'-GAAGAAAAATCAGCCAAAACTA-3' R: 5'-TCCTTAAACAGATTTTTGGAGT-3'
RhoGDI3 NEGATIVE (U3)	F: 5'-AAGGGCGGGTCTAATTTCTG-3' R: 5'-CTGCGTCTGGATAAGGGAG-3'
RhoGDI3 POSITIVE (U4)	F: 5'-TACTGATGACCGTGAACCTG-3' R: 5'-CTGAGGGAGCTTCTGTCTG-3'
HOXA9	F: 5'-CTCCGCCGCTCTCATTCTCAG-3' R: 5'-GCCAGAAGGGGTGACTGTCC-3'
CD4	F: 5'-TGTGCTCTGCCCAGTTGTCT-3' R: 5'-GCTCATGACCAGTTCCAAGAGAA-3'

Journal of Cell Science • Supplementary information

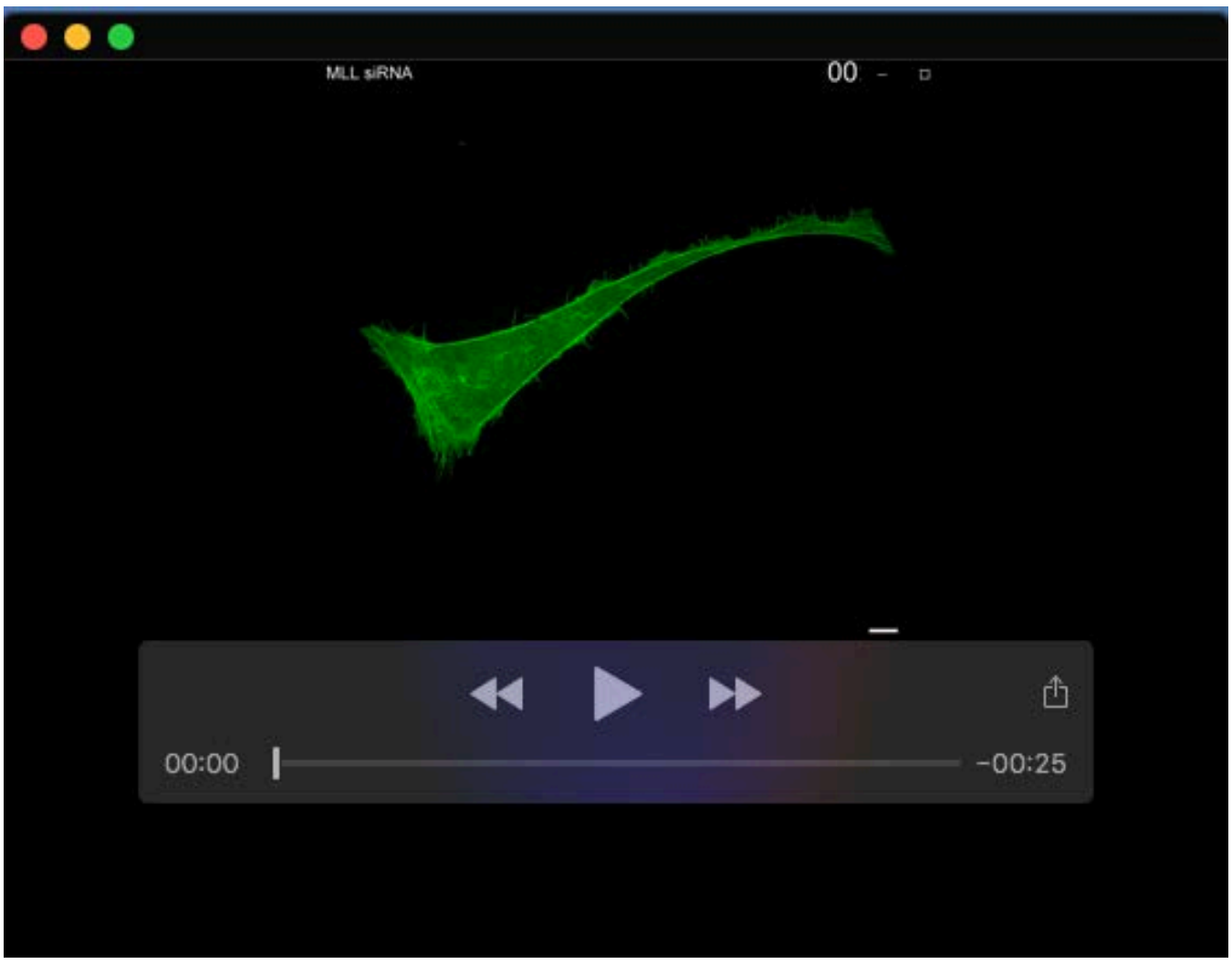


Movie 1. U-2OS cell expressing GFP-Lifeact (green) treated with control siRNA, corresponding to Figure 3G panel a, is shown here. Frame size is 73x73 µm and frame step is 5 s. Scale bar, 10 µm.

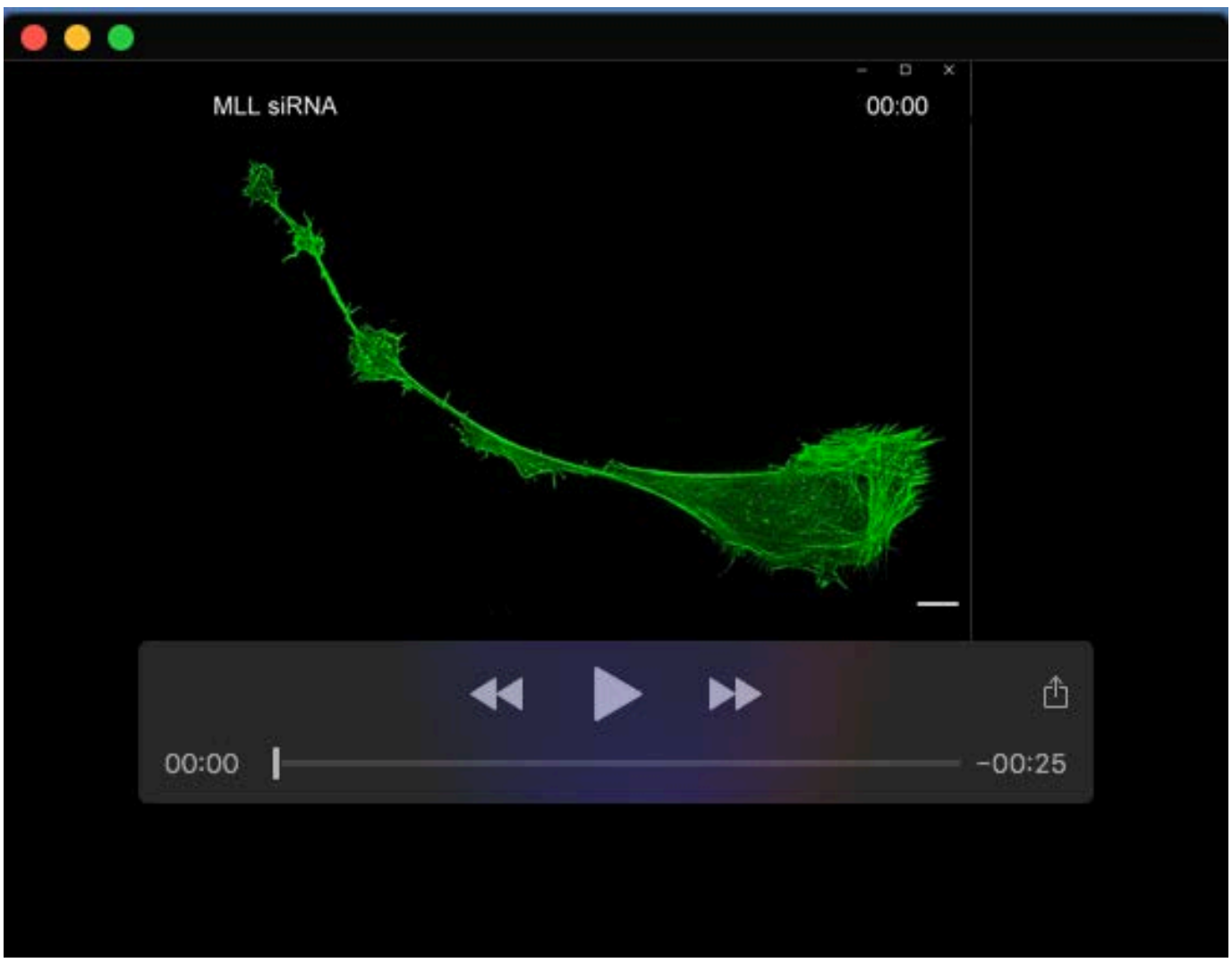


Movie 2.Shown here is U-2OS cell expressing GFP-Lifeact treated with control siRNA, corresponding to Figure 3G panel b, Frame size is 64x64 µm and frame step is 5 s. Scale bar, 10 µm.

Journal of Cell Science • Supplementary information



Movie 3. U-2OS cell expressing GFP-Lifeact (green) treated with MLL siRNA#1, corresponding to Figure 3G panel c is shown. Frame size is 128x128 µm and frame step is 5s. Scale bar, 10 µm.



Movie 4. U-2OS cell expressing GFP-Lifeact (green) treated with MLL siRNA#1, corresponding to Figure 3G panel d is shown. Frame size is 186x130 µm and frame step is 5 s. Scale bar, 10 µm.

Journal of Cell Science • Supplementary information