Supplemental Information

WDR5 modulates cell motility and morphology and controls nuclear changes induced by a 3D environment

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Supplemental Inventory

SI Material and Methods.

7 Supplementary Figures.

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Supplemental Experimental Procedures

Reagents and antibodies. The mouse antibody anti-H3K9me2/3, and the rabbit antibodies anti-H3K4me3, -phospho-MLC, -phospho-ERM, -ERM, -WASP, -Cofilin, -ASH2L and myosin 2A were from Cell Signaling. The mouse antibodies anti-β-tubulin, -talin and -vinculin were from Sigma-Aldrich. The mouse antibodies anti-α4 (HP2/1), -αL (TS1/11), -β1 (HUST21), -β2 (TS1/18) and -αE-selectin (Tea2/1) were a generous gift from Prof. Sánchez-Madrid. The anti-RbBP5 antibody was from Biorbyt. WDR5 was detected with antibodies from Santacruz and Bethyl Laboratories. The rabbit antibodies anti-RhoGDI and -Vav1 were from Santacruz. The rabbit anti-lamin B1 was from Abcam. Tetramethylrhodamine (TRITC) Phalloidin, CellTrace™ CFSE, CellTrace™ Far Red, secondary antibodies Alexa-488, -594, -647 for immunofluorescence analysis, Hoechst 33342 and DAPI were obtained from Thermo Scientific. Secondary antibodies (ID680 and ID800) for blot detection were from Rockland. Bovine collagen solution type I (Purecol) was from Advanced Biomatrix. VCAM1 was obtained from Martin Humphries and from Peprotech. OICR-9429 was from Tocris Bioscience and GM6001 from Merk Millipore. ML-7, Blebbistatin, Y27632, EHT 1864 and actinomycin D were from Sigma-Aldrich. Glass bottomed plates from Mattek and Ibidi.

Transplantation assay Adult zebrafish (*Danio rerio*) were maintained at the University of Manchester Biological Services Unit according to National Home Office regulations under the Animals (Scientific Procedures) Act 1986. Casper strain (*roy*$^{-/-}$, *nacre*$^{-/-}$) zebrafish were used to generate embryos completely lacking pigment, which can otherwise obscure imaging. Vital-dye-labeled control shRNA and WDR5 or RbBP5 depleted cells were resuspended at $7.5x10^7$ cells/ml in PBS supplemented with 0.5 % polyvinylpyrrolidinone K60 solution (PVP, Sigma-Aldrich) on ice. 48 h post fertilization (hpf) embryos were anaesthetized with 0.1 mg/ml MS-222 and approximately $2x10^3$ of a 1:1 mixture of control and WDR5 or RbBP5 depleted were injected into the common cardinal vein, also referred to as the Duct of Cuvier,

using a micropipette and pump (World Precision Instruments). Engrafted embryos were maintained at 34°C for 24 h post injection (hpi). Lymphocyte extravasation was then assessed by live-imaging embryos.

Live-imaging 24 hpi engrafted zebrafish embryos were anaesthetized in 0.1 mg/ml MS-222 and xenografts imaged using a Leica TCS SP5 AOBS upright confocal (Leica Microsystems) using a 20x 0.50 Plan Fluotar dipping objective and 1.0 x confocal zoom. Images (format 512 x 512) were collected sequentially and the maximum intensity projections of these 3D stacks are shown in the results.

Nuclear swelling experiments. Control or WDR5 depleted cells were cultured in suspension, 2D and 3D conditions. The nuclei were isolated and sedimented on poly-

Lysine coated coverslips in TKMC buffer or in swelling conditions (TKMC with or without KCl 10 μM; EDTA, at 1 and 10 μM) for 5 min. Then nuclei were fixed with 4% formaldehyde (10 min), permeabilized with 0.5% Tx-100 PBS (5 min) and stained by Hoechst 33342. Nuclear area was analyzed by confocal microscopy. Quantification of nuclear area was determined with ImageJ and 3D movies rendered with LAS-AF3D software (Leica).

High-Velocity Video Microscopy. Nuclei from cells cultured in suspension were isolated, and sedimented onto poly-Lysine coated slides. Nuclei were imaged in a phase contrast inverted microscope (NikonEclipse2000Ti) equipped with a 100 W TI-12 DH Pillar Illuminator, an LWD 0.52 collimator, and a 100× oil immersion objective (PlanApoVC, N.A. 1.4; Nikon). Tracking movies were captured with a FASTCAM SA3 camera (Photron), with an effective pixel size of 50 × 50 nm2. To provide optimal signal-to-noise ratio (SNR), the movies were recorded during 1 s of tracking time at a sampling frequency of 500 Hz (n = 500 frames).

Multiple particle tracking of nuclear particles. Images from nuclei were analyzed with a code generated with Mathematica software (Wolfram Research). Dense nuclear grains were

identified as highly-contrasted objects with a 2D-Gaussian intensity profile of intensity significantly larger than the averaged background (3). In every frame, the instantaneous state of every nuclear particle is determined by fitting the intensity profiles to a Gaussian function; for a particle i, we record as a function of tim: i) two dimensional coordinate r_i = (x_i, y_i) (corresponding to center of the Gaussian profile), ii) a circular-like diameter a_i (corresponding to the Gaussian width), and iii) the intensity I_i (as the integrated area of the Gaussian). The instantaneous centroid of these particles is evaluated as the center-of-mass R = suma_i(r_i x A_i)/suma_i(A_i), using I_i as weighting factor. Troubleshooting is performed by discarding particles with consecutive coordinates varying larger than a 10% of the previous displacement, and more than 2% in the apparent size characteristics (both diameter and intensity). Larger variations in the apparent size are interpreted as either spurious particle exchanges, or offplane defocusing leading to 3D-contributions to the particle displacements (4). The 2D acceptable Brownian trajectories are processed to get the mean square displacements as a function of time MSD(t) = suma_j(deltar_j^2 – deltar^2)/n, where the average displacement deltar = suma_j(deltar_j)/n is calculated for every grain i along a time series j = 1, 2…n. Using the 2D-diffusion equation MSD(t) = 4Dt, the diffusion coefficient corresponding to every trajectory is computed as the slope D of the linear fit. Further, the apparent viscosity eta is estimated using the Stokes-Einstein relationship for sticking conditions, D = kBT/6pi eta a, where kB is the Boltzmann's constant, and the apparent size of the nuclear particle. The average calculated in a given specimen over a collection of acceptable particle (normally higher than 10), is the quantity assumed with phenotyping value.

Cell migration assay. We coated glass-bottomed plates with VCAM1 (2μg/ml). PBL, or Jurkat (control or WDR5 depleted) cells were plated onto wells at 37°C and 5% CO2. Time-lapse images were acquired with a sCMOS Orca-Flash 4.0LT camera (Hamamatsu) coupled

to an inverted DMi8 microscpe (Leica). Images were collected every 30 sec over 30 min (sparsely plated cells). Cell morphology was determined using ImageJ.

Cell migration through a 3D collagen matrix was assayed according to the manufacturer's instructions (Ibidi). Control and WDR5 or RbBP5 depleted Jurkat cells were labeled with vital-dyes. Then, cells were washed and both populations mixed at 1:1 prior embedded in a 3D collagen matrix. Random cell migration was analyzed from images taken every 5 min during 3 h (for WDR5 cells) and 2 h (for RbBP5). Images were acquired on a Nikon TE2000 PFS microscope using a 20x/ 0.5 Plan Fluor objective and the Sedat filter set Chroma (89000). The images were collected using a Cascade II EMCCD camera (Photometrics) with a Z optical spacing of 0.2 μm. Cell migration was tracked using Imaris.

Atomic Force Microscopy (AFM). Nuclei from cells cultured in suspension, 2D or 3D conditions were isolated using a hypotonic buffer A (10 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% (v/v) glycerol, 1 mM DTT and Roche protease inhibitor) and 0.5% of NP-40 followed by vortexing for 15 sec and centrifugation for 5 min at 4°C at 3,500 g (1). Nuclei were resuspended in TKMC buffer (50 mM Tris pH 7.5, 25 mM KCl, 3 mM $MgCl_2$, 3 mM $CaCl_2$, and proteinase inhibitors) and sedimented onto poly-Lysine coated slides (Thermo Scientific). Then nuclei were fixed with PBS 2% glutaraldehyde for 10 min, washed with PBS and water, and allowed to air dry prior to analysis. AFM was performed on a JPK NanoWizard IV (JPK Instruments AG, Berlin Germany) system mounted on a Zeiss AX10 (Carl Zeiss Microscopy GmbH, Jena, Germany) inverted microscope. Experiments were performed using a Bruker ScanAsyst-Fluid Probe (nominal spring constant 0.7N/m, Bruker AXS, S.A.S, France). The instrument was operated using JPK recommended protocols and calibration of the tip was performed via the Auto Calibration option in the JPK SPM Control Software (version 6.0.45). The sample was rehydrated in double distilled deionised water prior to imaging. Images were captured at 512x512 pixel resolution in QI mode, the

topography and adhesion channels were used for analysis. The images were processed in the JPK Data Processing (version 6.0.45) analysis software. For nanomechanical testing, 9 separate measurements were taken for each nucleus using a ramp size of 500 nm and a trigger threshold of 5 nN. The force curves were analyzed using the JPK analysis software (as above). The approach curve was fitted using a contact point based corrected Hertzian Model. From this, the Young's Modulus of each point was taken and averaged for each nucleus.

Immunofluorescence. For stainings in 2D, PBL, control or WDR5 depleted Jurkat cells were cultured for 20 min on VCAM1 coated coverslips. Cells were fixed in 4% formaldehyde (10 min), permeabilized with 0.5% Tx-100 in PBS (5 min), blocked in 10% horse serum and incubated with appropriated primary antibodies for 1 h at RT. After several washes, samples were incubated with secondary antibodies (1 h at RT). Samples were washed and mounted in Dako. Images were acquired using a Leica SP5 and SPE confocal microscopes with an objective ACS-APO 40x NA 1.30 oil immersion. Quantification of trailing edge length was determined using ImageJ.

Production of lentivirus Short hairpin RNA sequences encoding sequences targeting WDR5 (WDR5.1: GCTCAGAGGATAACCTTGTTT; WDR5.2: GAATGAGAAATACTGCATA), RbBP5 (RbBP5.1: TAAAGGTGCAAGTCAAAGC; RbBP5.2: TATCAGTGGAAGCACTCAC) and MYH9 (MYH9.1: CCGCGAAGTCAGCTCCCTAAA; MYH9.2: GCCGTACAACAAATACCGCTT) were purchased from Sigma-Aldrich and inserted in the pVenus lentiviral transfer vector (pVLVTHM). For Rac constructs we subclone those obtained from Pat Caswell (University of Manchester) into pVLVTHM vector. All recombinant lentiviruses were generated by transient transfection of 293T cells using Pei according to manufacturer's protocol. Briefly, subconfluent HEK293T cells were co-transfected with the pVLVTHM vector and the packaging vectors pPsPax2, pMD2G. After 24 h, the medium was replaced with fresh medium with or without10 mM sodium butyrate

and media changed after 6h. 48 h (for WDR5) or 72 h (for RbBP5) later virus containing supernatants were harvested and filtered through 0.45 μm pore-sized membranes. Infection was performed by adding the lentiviral containing media to Jurkat cells at $1x10^6$ cells/ml, with 10 μg/ml Polybrene (Millipore). The media was changed after 24 hours, and cells were passaged over two weeks. Stably infected cells were sorted by FACS based upon GFP fluorescence.

MNase digestion. To analyze the nucleosomal profile, cells of interest were collected, lysed in lysis buffer (10 mM Tris [pH 7.5], 10 mM NaCl, 2 mM $MgCl_2$, 0.5% NP-40, 1 mM $CaCl_2$) with protease inhibitors (Roche) and MNase digestion performed with 100U of MNase (New England Biolabs) at 37°C. Reactions were stopped adding EDTA, incubated for 10 min at 37°C with RNaseA (Life Technologies) and then incubated overnight at 65°C with 0.01% SDS and proteinase K (New England Biolabs). Digested DNA was washed with phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated by 3 M sodium acetate and 2.5 volumes of ethanol. DNA pellet was dissolved in water and nucleosomal releasing resolved in 2% agarose gel. DNA digestion profiles were quantified with ImageJ.

RT-qPCR. Total RNA was extracted using the RNeasy kit (Qiagen), and cDNA was synthesized from 1.0 μg of total RNA using SuperScript II First-Strand Synthesis System and oligo(dT) (Invitrogen). cDNA concentrations were quantified using a NanoDrop ND-1000 Spectrophotometer (Fisher Scientific). Oligonucleotides for MYC were designed according to the Roche software (Universal Human Probe Roche Library): MYC 5'-gctgcttagacgctggattt-3' and 5'-taacgttgaggggcatcg-3'. Quantitative real-time PCR was performed on a Roche LightCycler® 480 following the manufacturer's instructions. Assays were made in triplicates and results normalized according to the expression levels of TBP (Roche Real Time Ready Single Assay ID 101145). Melt curve analysis was performed at the end of PCR to confirm

the presence of a single, specific product. The results were expressed using the ΔΔCT method for quantification.

Cell adhesion assay. Control or WDR5 depleted cells were cultured on 96-well plates coated with collagen type I (10 μg/ml), ICAM1 and VCAM1 (both at 5 μg/ml). Cell adhesion was analyzed as in (2). The wells were gently washed with PBS twice and the adherent cells were fixed with formaldehyde (4%) in PBS. Cells were stained with 0.5% crystal violet in 20% methanol, extensively washed in water, and stain solubilized with 0.1% sodium citrate in 50% ethanol. The absorbance at 570 nm was read on an Opsys MR (Dynex) plate reader.

H3K4me3 quantification and Immunoblotting. Histones were extracted from cells according to the manufacturer's instructions (Epigentek), and H3K4me3 levels determined using a colorimetric EpiQuik Global Tri-Methyl Histone H-K4 Quantification Kit. Also, cells were lysed in RIPA buffer for 30 min at 4C and then sonicated using a Microson XL2000 (Misonix). For cellular fractionation, the cytoplasmic fraction was extracted in buffer A with NP-40 and centrifugation for 5 min at 4°C at 3,500 g. Supernatant was the cytoplasmic fraction. The pellet (consisting of nuclei) was then resuspended in buffer C containing 20 mM HEPES, 1 mM EDTA, 1 mM EGTA, 0.4 M NaCl, 1 mN DTT, 0.5 mM PMSF and protease inhibitor to obtain the nuclear proteins after high-speed centrifugation. The pellet (chromatin bound fraction) was resuspended in sample buffer and sonicated for its extraction. Samples were boiled and proteins resolved in 8-12.5% polyacrylamide gels. Gels were transferred to nitrocellulose membranes then blocked in 5% low fat milk in TBS-Tween (0.5%) for 1 hour at room temperature. Membranes were incubated with primary antibodies in 5% low fat milk in TBS-tween (0.5%) at the appropriate dilution at 4°C overnight. Membranes were washed in TBS-Tween (0.5%) and incubated with appropriated IRDye or HRP-conjugated secondary antibodies (Li-cor) in 5% low fat milk in TBS-Tween (0.5%) for 1 hour at room temperature. Protein signal was analyzed by Odyssey (Li-cor) or Alliance 4.7 (UVITEC).

Transwell invasion Collagen gels were prepared on the upper chamber of transwell plate inserts (Corning Costar, 6.5 mm diameter, 3 μm, 5 μm and 8 μm pore sizes). 600 μL of medium with serum and 50 μL of a cell suspension (2×10^5 cells/well) were added to the lower chamber and upper chamber, respectively. The chambers were incubated in a CO2 incubator at 37 °C for 6 h (cell invasion in the collagen gel) or 24 h (cell migration across the transwell filter). Migrated cells from the lower chamber were collected and counted.

Flow cytometry. Cellular phenotypic analysis was carried out by indirect immunofluorescence. Cells were blocked with human IgG (50ug/ml; 30 min), incubated with primary antibodies (5-10ug/ml; 30 min), followed by appropriated fluorochrome-conjugated secondary antibodies for 30 min (Jackson ImmunoResearch). Between incubations, the preparations were washed with PBS. Flow cytometry was performed with a FacSort (Beckman Coulter) and data were analyzed using the BD CellQuest Pro software (BD Biosciences).

Statistical analysis. Statistical analysis and comparisons were made with GraphPad Prism6. The numerical data are presented as mean ± SD or SEM. Differences between means were tested by Student's t test for two groups comparison. Where 3 or more groups were analysed one-way ANOVA was used. P-values are indicated by asterisks ((∗) $P < 0.05$; (∗∗) $P < 0.01$; (∗∗∗) $P < 0.001$).

Supplementary References

1. Méndez J, Stillman B (2000) Chromatin association of human origin recognition complex, Cdc6, and minichromosome maintenance proteins during the cell cycle: assembly of prereplication complexes in late mitosis. *Mol Cell Biol* 20:8602–8612.

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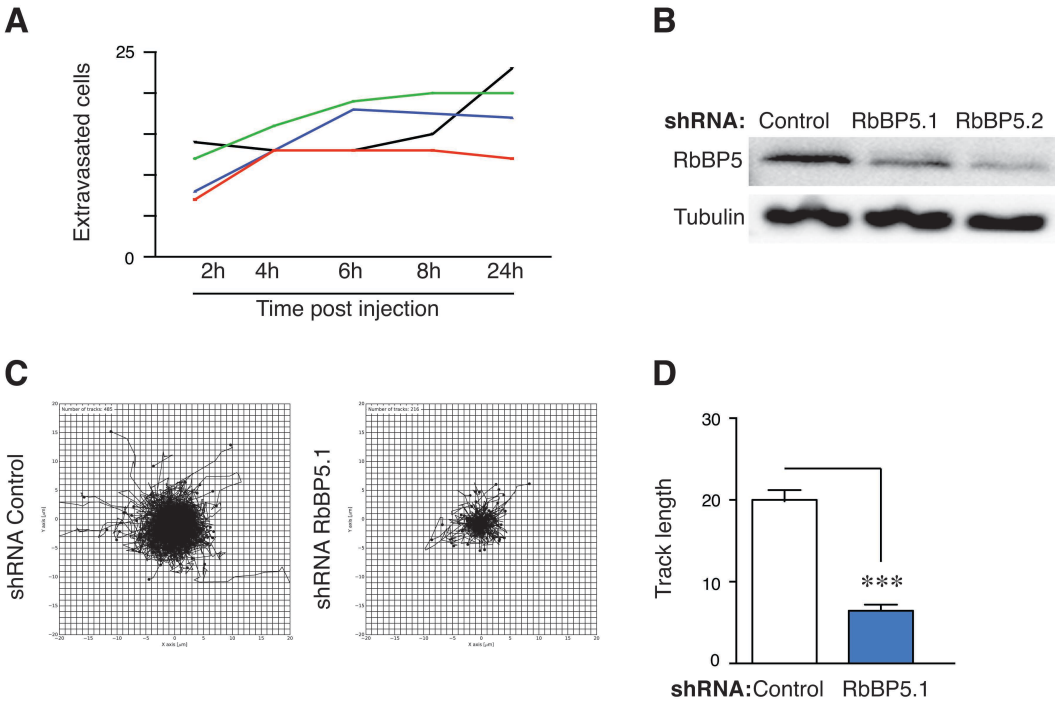

Fig. S1. RbBP5 function during cell migration in 3D. (A) Graph shows the number of cells that had extravasated at the indicated times. Each line represents an embryo. n=4 embryos. (B) Jurkat cells were infected with specific shRNAs for RbBP5. Cells were cultured for 2 weeks and, after sorting, knockdown expression confirmed by WB. (C) Tracks of Control or RbBP5-depleted cells in a 3D collagen matrix. (D) Quantification of track length of cells in (C).

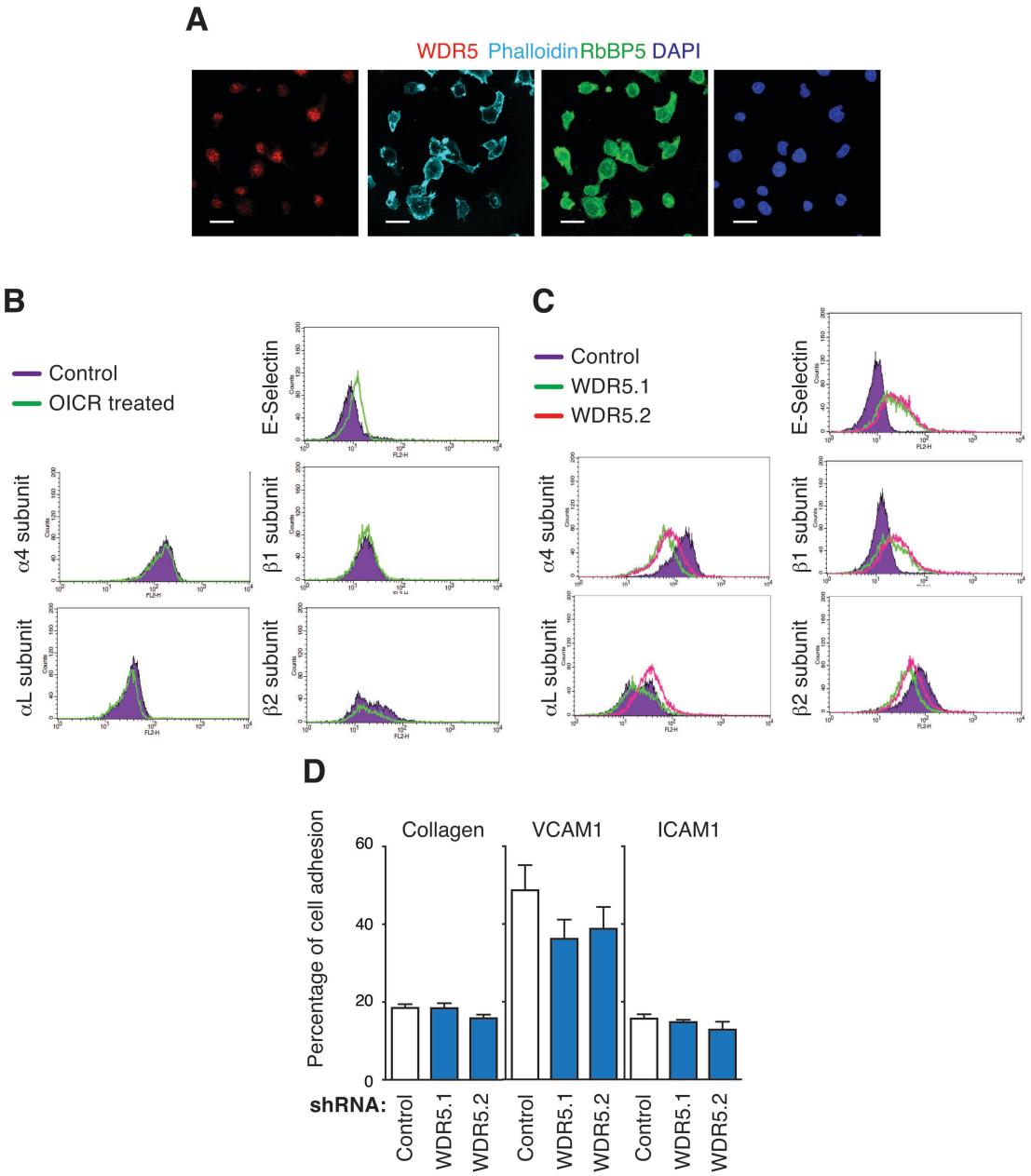

Fig. S2. WDR5 function in cell receptors expression. (A) Jurkat cells were cultured on poly- Lysine and then fixed and stained with Hoechst (blue), Phalloidin (cyan), anti-RbBP5 (green) and anti-WDR5 (red). Bar 10 µm. (B) Jurkat cells were incubated with OICR9429 for 1h and then cell receptors expression was analyzed by flow cytometry. (C) Expression analysis of cell receptors in control and WDR5 depleted Jurkat cells. (D) Control or WDR5 depleted Jurkat cells were cultured on collagen type I (10 µg/ml), ICAM1 and VCAM1 (both at 5 µg/ml). After 30 min cells were fixed and cell adhesion analyzed by crystal violet staining.

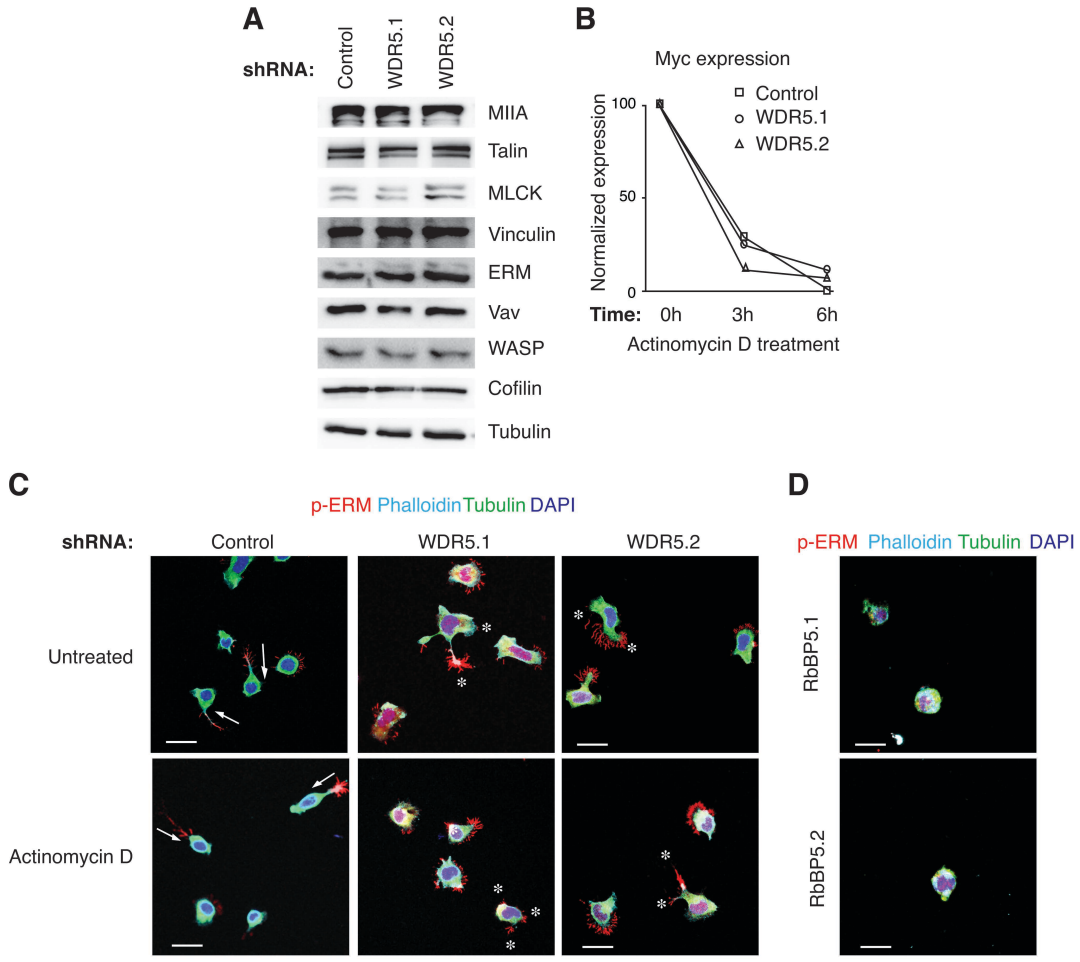

Fig. S3. WDR5 function during cell adhesion. (A) The expression of multiple cytoskeletal components of control or WDR5 depleted Jurkat cells was confirmed by WB. (B) Control or WDR5 depleted Jurkat cells were incubated with actinomycin D, then mRNA was collected at indicated times to determine by qPCR the transcriptional inhibition of c-Myc. (C) Control or WDR5 depleted Jurkat cells were preincubated with actinomycin D and then cultured on VCAM1 (2 μg/ml) for 20 min. Cells were fixed and stained for tubulin (green), phalloidin (cyan), phospho-ERM (red) and DAPI. White arrows indicate the presence of long trailing edges in control cells while asterisks show multiple cytoplasmic protrusions induced in WDR5 depleted cells. Bar 10 μm. (D) RbBP5 depleted cells were cultured on VCAM1 (2 μg/ml) for 20 min. Cells were fixed and stained for tubulin (green), phalloidin (cyan), phospho-ERM (red) and DAPI.

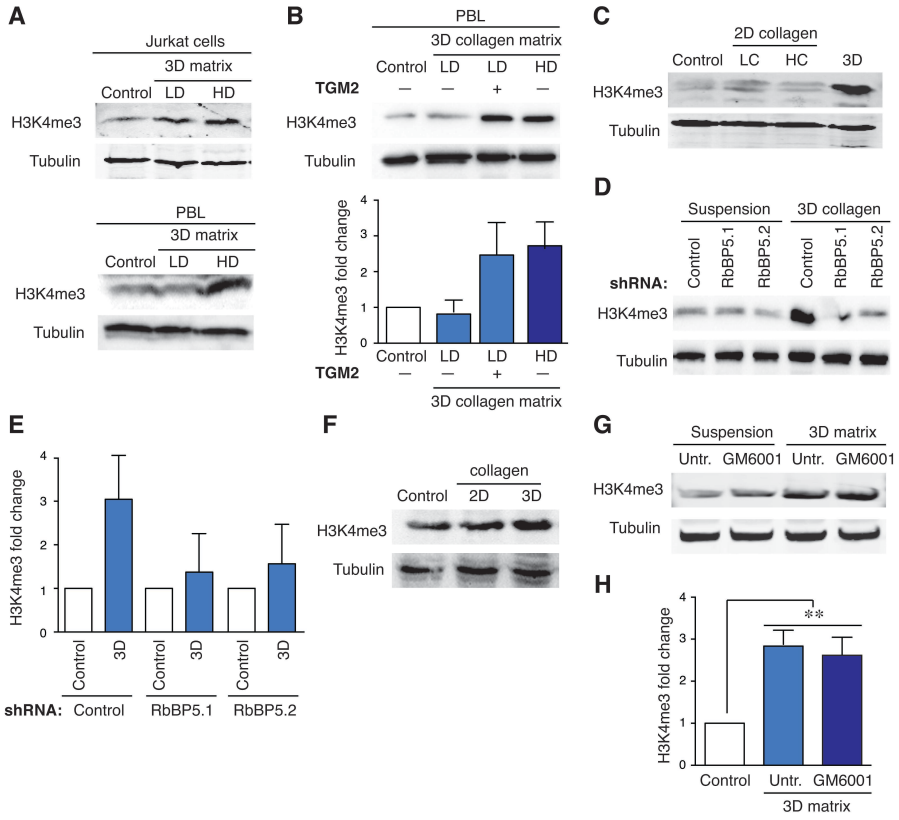

Fig. S4. H3K4 methylation induced by 3D conditions. (A) Jurkat or PBL cells as in Fig. 3A were lysed and sonicated to extract histones. Representative blots confirmed the results from colorimetric assays and showed increased H3K4me3 levels when cells were in 3D. (B) Levels of H3K4me3 in nuclear fractions isolated from PBL in suspension (Control), or cultured in a 0.4 mg/ml low density (LD) with and without the addition of TGM2 (10X, 1μg) crosslinking enzyme. Graph shows mean n=2 ± SD. (C) Samples from Jurkat cells as in Fig. 3C were analyzed to verify the result obtained by colorimetric assays. (D) Control or stable RbBP5 depleted cells were cultured in suspension or embedded in a 3D matrix, and H3K4me3 levels resolved by WB. (E) The graph shows the levels of H3K4me3 from (D) Mean n=2 ± SD. (F) Levels of H3K4me3 in nuclear fractions from MDA-MB-231 cells in adhesion to plastic (control), collagen (2D) and embedded in 3D conditions. (G and H) Jurkat cells were preincubated with GM6001 (40 μM) and then cultured inside a collagen matrix. Levels of H3K4me3 were tested by WB (G) and quantified (H). Mean n=3 ± SD.

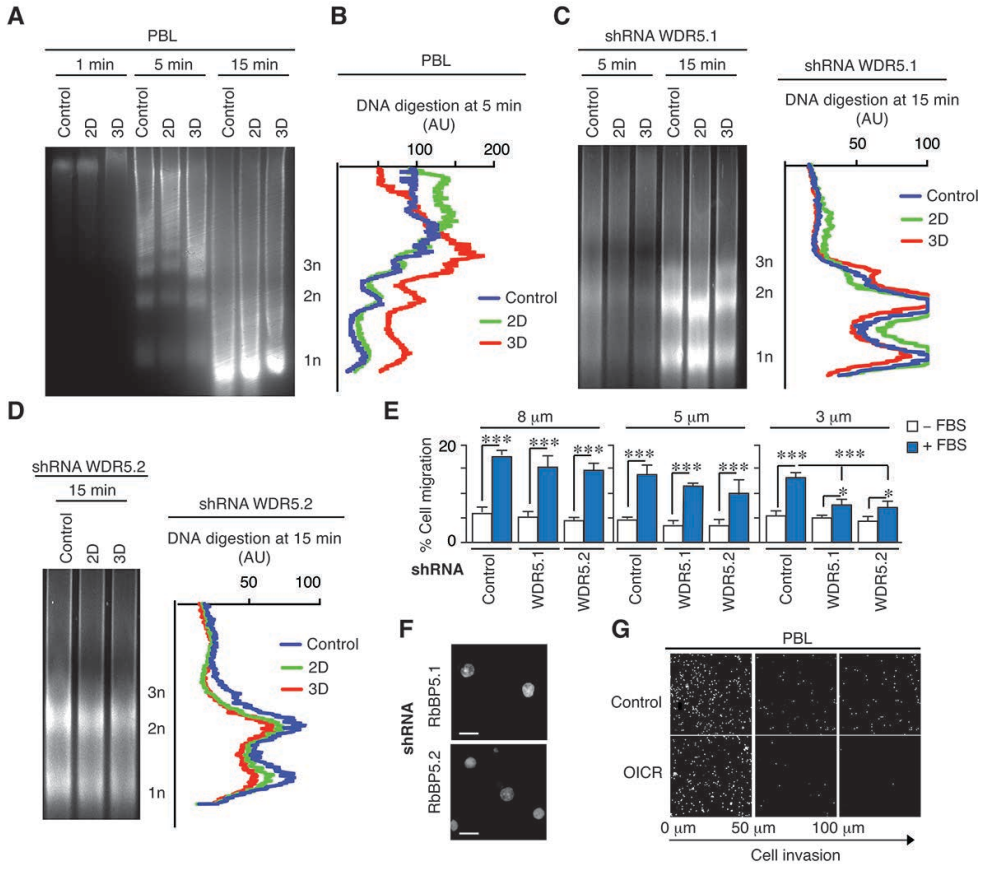

Fig. S5. 3D-mediated nuclear changes control chromatin conformation. (A) PBL cells were cultured in suspension, on 2D and 3D conditions. Then, cells were isolated and digested with micrococcal nuclease (MNase) at indicated times. DNA fragments were purified and resolved in agarose gel. (B) Nucleosomal releasing from cells in (A) was analysed after micrococcal digestion (5 min) and the signal profile of the mononucleosomes (1n), dinucleosomes (2n) and trinucleosomes (3n) determined. (C and D) WDR5 depleted cells were cultured in suspension, on 2D and 3D conditions. Then, nucleosomal sensitivity to DNA digestion analyzed as in (A) and (B). (E) Control or WDR5 depleted cells were seeded on 0.8 mg/ml of collagen gel in the upper chamber of transwells with different pore sizes and allowed to invade in response to 10% FBS for 24h. Cells were collected from the bottom chamber, stained and quantified. Mean n=3 ± SD. (F) RbBP5 depleted cells were embedded in 3D collagen fixed and stained with DAPI. (G) PBL cells were preincubated or not with OICR-9429, seeded on collagen gel in the upper chamber of transwells and allowed to invade

through collagen gel in response to 10% FBS for 6h. Then, cells were fixed, stained with DAPI and serial confocal sections were captured.

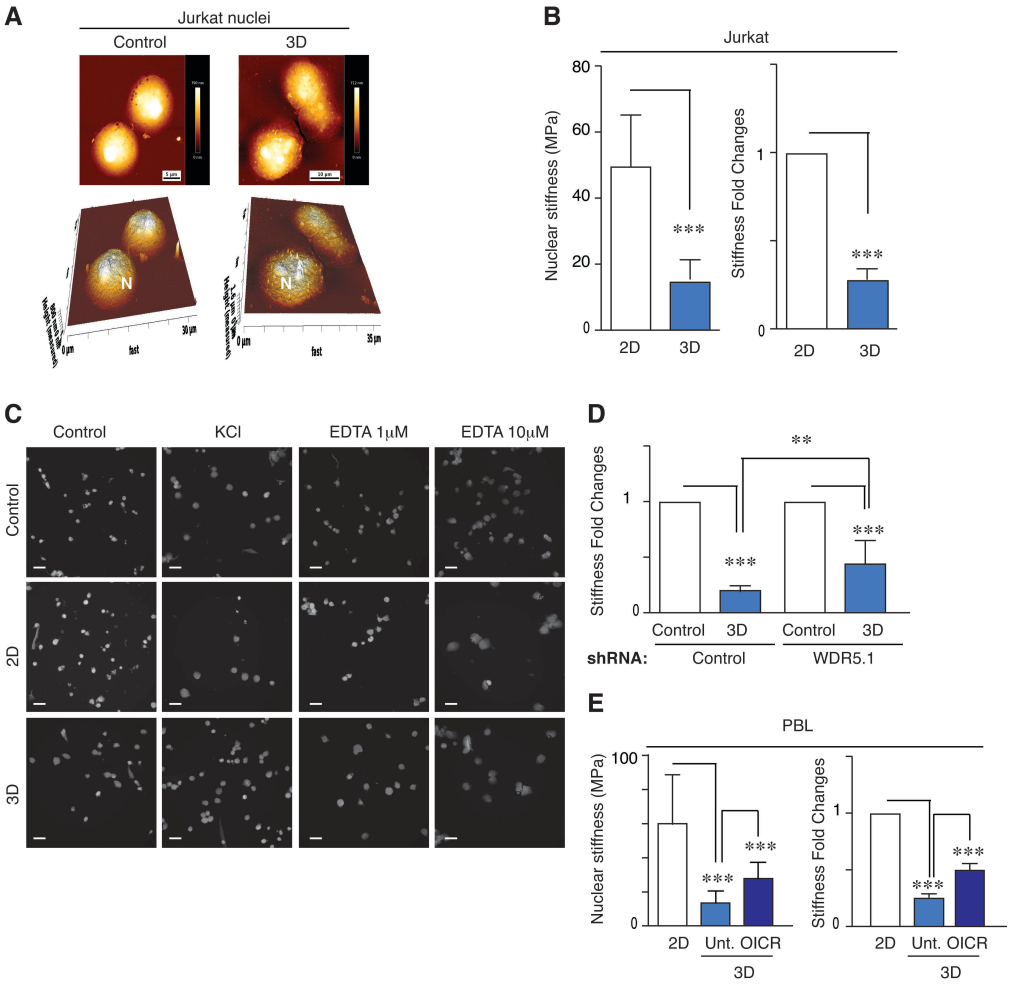

Fig. S6. 3D-mediated nuclear changes control nuclear stiffness. (A) Representative topography images captured from isolated nuclei of cells in suspension or in 3D, showing the nuclear deformation associated with changes in chromatin structure. "N" indicates the white areas corresponding to the nucleoli. (B) Nuclei from cells in suspension or cultured in 2D or 3D conditions were isolated and left to sediment onto a coverslip. Nuclear stiffness was analyzed by AFM. Mean n=30 nuclei ± SEM. (C) Isolated nuclei from Jurkat cells cultured in suspension, 2D or 3D conditions were sedimented on poly-Lysine coated coverslips incubated with KCl (1 μM) or different EDTA concentrations (1-10 μM), fixed and their nuclei stained with Hoechst and analyzed by confocal microscopy. (D) Isolated nuclei from WDR5 depleted cells cultured in suspension or in a 3D collagen gel were analyzed as in (B). Mean n=12-18 nuclei ± SEM. (E) Isolated nuclei from PBL cells cultured in suspension, or in 3D conditions treated or not with OICR-9429 (1 μM) were s analyzed by AFM.

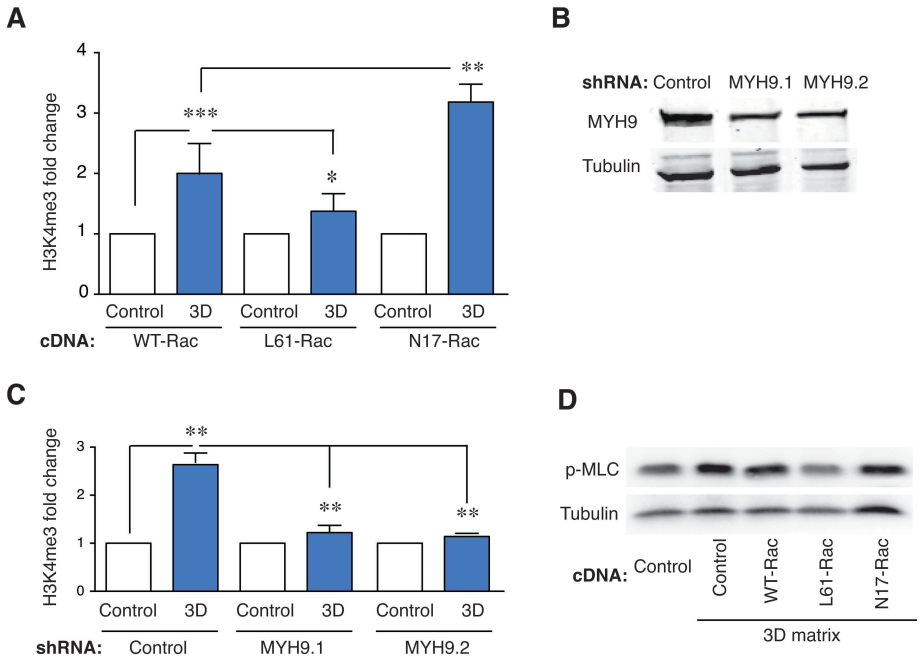

Fig. S7. Myosin activity controls the methyltransferase activity associated to WDR5. (A) Stable cells lines expressing empty, or wild type (WT-Rac), constitutively active (L61-Rac), or dominant negative (N17-Rac) forms of Rac were cultured in 3D matrix and H3K4 methylation quantified from nuclear fractions. Mean n=3± SD. (B) Jurkat cells were infected with specific shRNAs for non-muscle myosin heavy chain MYH9 and knockdown expression confirmed by WB. (C) Levels of H3K4me3 from nuclear fractions of Control or stable MYH9 depleted cells in suspension or embedded in collagen matrix. Mean n=3 ± SD. (D) Stable cells lines expressing empty vector, WT-Rac, L61-Rac or N17-Rac forms were cultured in 3D matrix and phospho-MLC levels analyzed by WB.

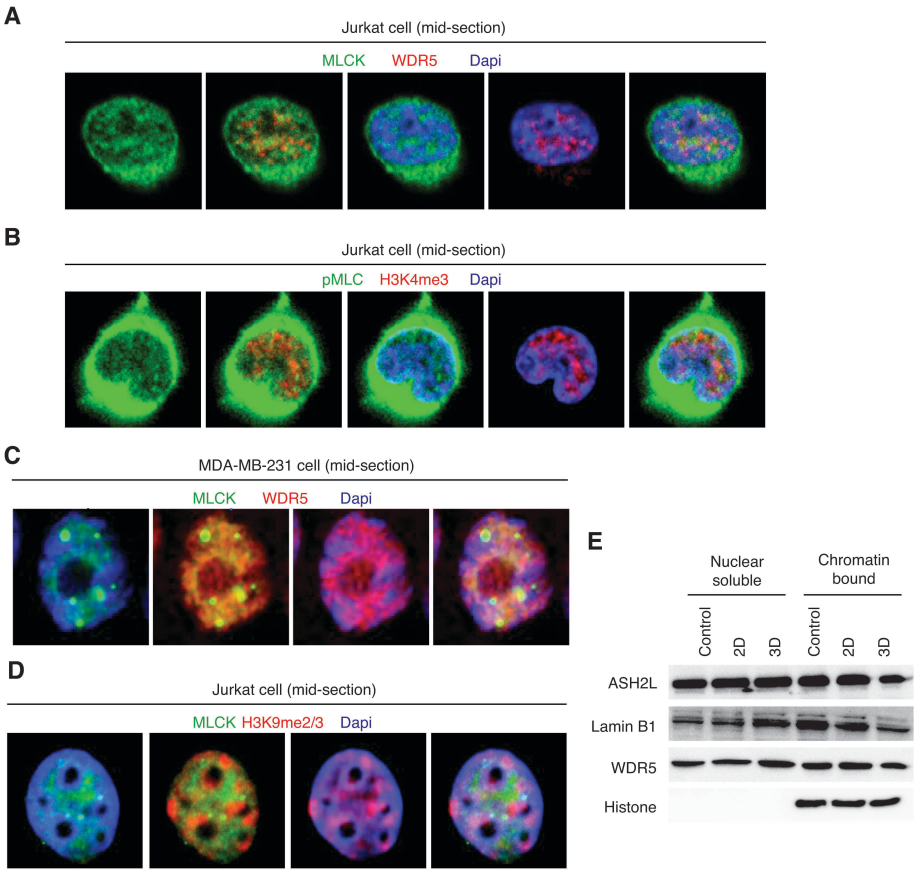

Fig. S8. MLCK and phospho-MLC localize both in the cytoplasm and nucleus of cells

(A) (A) Jurkat cells were cultured on poly-Lysine coated coverslips for 20 min, then fixed and stained to visualize MLCK, WDR5 and the chromatin. Mid-section shows a representative image of the protein localization inside the nucleus (B) Mid-section of representative Jurkat cell stained for phospho-MLC, H3K4me3 and the chromatin. (C) Mid-section shows a representative image of the protein localization inside the nucleus of MDA-MB-231 cells. (D) Mid-section of a representative nucleus from isolated nuclei sedimented on poly-Lysine coated coverslips and stained to visualize MLCK, the heterochromatin (H3K9me2/3) and the chromatin. (E) Jurkat cells were cultured in suspension, 2D and 3D conditions. Then, nuclear soluble and chromatin-bound fractions were purified and analyzed by WB. Lamin B1 and Histone were used as internal control of nuclear/chromatin and chromatin fractions.

Movie S1 and S2. Migration of WDR5 depleted cells in 3D collagen matrix. Representative movie of Control Jurkat (labeled with Red Cell-Tracer and Hoechst 33342) or WDR5.1 depleted (GFP^+ and labeled with CFSE and Hoechst 33342) cells migrating in a 3D collagen gel (1.7 mg/ml). Cell movement was tracked and the movie shows the migration of cells in brightfield (S1) and fluorescence channels (S2) through the collagen matrix during 3 hours.

Movie S3 and S4. Migration of RbBP5 depleted cells in 3D collagen matrix. Representative movie of the migration of Control Jurkat (labeled with Red Cell-Tracer) or RbBP5.1 depleted (GFP^+ and labeled with CFSE) cells migrating in a 3D collagen gel (1.7 mg/ml) in brightfield (S3) and fluorescence channels (S4).

Movie S5. Control cells migrating on 2D VCAM1 plate. Jurkat cells were plated on coated wells with VCAM1 (2 μg/ml). The movie shows the cell movement and trailing edge (long tail) formation.

Movies S6 and S7. WDR5 depleted cells migrating on 2D conditions. Stable cell lines depleted for WDR5 (S4 for shRNA WDR5.1 and S5 for shRNA WDR5.2) were plated on coated wells with VCAM1 (2 μg/ml). The movie shows how WDR5 depletion affects cell movement, impairs trailing edge formation and promotes multiple cell protrusions.

Movies S8 and S9. PBL migration on 2D conditions upon WDR5 inhibition. PBL were plated on coated wells with VCAM1 (2 μg/ml) in the absence (S6) or presence (S7) of the OCIR-9429 inhibitor (1 μM).

Movies S10-15. 3D reconstruction of the nuclear shape of representative isolated nuclei under swelling conditions. Isolated nuclei from Jurkat cells cultured in suspension (S8 and S9), 2D (S10 and S11) and 3D (S12 and S13) conditions were sedimented on poly-Lysine

coated coverslips and their nuclear elasticity analyzed by adding EDTA (movies S9, S11 and S13). 3D reconstructions show that EDTA addition increased the nuclear volume in isolated nuclei from cells in suspension and 2D conditions.