Supplemental Materials, Figures and Tables

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HGAL INHIBITS DIFFUSE LARGE B-CELL LYMPHOMA DISSEMINATION BY INTERACTING WITH MULTIPLE CYTOSKELETON PROTEINS
Supplemental Materials

Cell culture and medium

Human MDA-MB-231 derived breast cancer cell subline 4175 was cultured at 37°C and 5% CO₂ in Dulbecco’s Modification of Eagle’s Medium (DMEM) (Invitrogen-GIBCO, Grand Island, NY), supplemented with 10% fetal bovine serum (FBS) (Hyclone Logan, UT), 2 mM glutamine and 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen-GIBCO, Grand Island, NY). Human diffuse large B-cell lymphoma (DLBCL) cell lines BJAB, U2932 and TMD8 as well as Burkitt cell line Raji were cultured at 37°C and 5% CO₂ in RPMI 1640 medium (Invitrogen-GIBCO, Grand Island, NY), supplemented with 10% fetal bovine serum (FBS) (Hyclone Logan, UT), 2mM glutamine and 100 units/ml penicillin and 100 μg/ml streptomycin (Invitrogen-GIBCO, Grand Island, NY).

Antibodies, western blotting and immunoprecipitation

Monoclonal anti-HGAL antibody was generated in our laboratory as previously described1. Monoclonal antibodies for actin (C-2), α-tubulin (7-RY28) and β-tubulin (F-1) were purchased from Santa Cruz Biotechnology (Dallas, TX). RhoA antibody (ab187027) and de-tyrosinated α-tubulin (ab48389) antibody were purchased from Abcam (Cambridge, MA). Tyrosinated α-tubulin (YL1/2) antibody was purchased from Millipore-Sigma (Burlington, MA). Alexa-647 anti-Rabbit secondary antibody and Alexa-555 anti-Rat secondary antibody were purchased from Thermo Fisher Scientific (Waltham, MA).

A total of 5×10⁶ cells were washed once with ice-cold phosphate-buffered saline (PBS) and homogenized in RIPA buffer (1 x phosphate-buffered saline [PBS], 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 10 mM phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin, and 100 mM sodium orthovanadate) on ice for 30 min. Cell lysate was centrifuged at 14,000 g for 10 min at 4°C to remove insoluble material. Protein concentration of lysates was determined using Coomassie protein assay reagent (Thermo
Scientific, Rockford, IL) and samples were adjusted to equal protein concentration. For Western blotting, 20 μg of whole-cell lysates were separated on 12% SDS-PAGE, transferred to polyvinylidene difluoride membranes (BioRad Laboratories, Hercules, CA), blocked with 5% non-fat milk in PBS with 0.1% Tween 20 (PBST) for 1h, and immunoblotted with specified primary antibodies for 1h at room temperature or at 4°C overnight. The membranes were washed three times in PBST for 5 min and then immunoblotted with appropriate secondary antibodies conjugated with horseradish peroxidase and visualized by SuperSignal Western pico chemiluminescent substrate from Pierce Biotech (Rockford, IL).

For immunoprecipitation (IP), 400 μg of protein was precipitated for 1 to 2h with the indicated antibodies at 4 °C with rotation. Protein G-agarose (Invitrogen, Carlsbad, CA) was added and the mixture was rotated for an additional 1h. Precipitated complexes were washed four times in NP-40 buffer, boiled in protein loading buffer (2 x concentrate: 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, 0.004% bromophenol blue, 0.125 M Tris HCl (pH 6.8)), separated on 12% SDS-PAGE gel and immunoblotted with the indicated antibodies.

**Plasmids, cell transfection and stable cell lines**

Luciferase expression plasmid pLV430G oFL T2A mCherry was previously reported². pcDNA3.1-BirA* was purchased from OriGene (Rockville, MD) and was used as a template for subcloning. HGAL cDNA fused with GFP or BirA* at its C-terminal were subcloned into pCDH lentivirus vector to generate pCDH-HGAL-GFP or pCDH-HGAL-BirA* plasmids using standard techniques. These plasmids were used to generate 4175, BJAB, Raji, U2932 and TMD8 cell lines stably expressing HGAL protein. CRISPR/Cas9 HGAL lentivirus plasmid was purchased from Santa Cruz Biotechnology (Dallas, TX) to knockout HGAL protein in BJAB and Raji cells. Lentivirus plasmid encoding RhoA shRNA was purchased from Santa Cruz Biotechnology (Dallas, TX). pcDNA3-RhoA63L-V5 plasmid was generated using the QuickChange Site-
Directed Mutagenesis Kit (Stratagene, Bellingham, WA) according to the manufacturer’s instructions.

Lentivirus plasmids (pLV430G oFL T2A mCherry, pCDH-HGAL-GFP, CRISPR/Cas9 HGAL and RhoA shRNA) were packaged into virus particles and used to infect 4175, U2932 and TMD8, Raji and BJAB cells. Briefly, Amphi pack 293T cells were cultured on p100 plates in DMEM (10% FBS with antibiotics) to 70-80% confluence. Lentivirus plasmid, VPR and VSVG plasmids at ratio of 4:3:1 (e.g. 2 µg:1.5 µg:0.5 µg) were added and mixed into 500 µl of Opti-MEM I Reduced Serum Media (Gibco) to which 24 µl of Plus Reagent (Invitrogen) was added. This mixture (Mixture I) was incubated at room temperature for 10-15 min and then 12 µl of Lipofectamine 2000 (Invitrogen) in 500 µl of Opti-MEM (Mixture II) was added, mixed and left at room temperature for 20 min (Mixture III). Amphi pack 293T cells were washed once with 3 ml of serum free DMEM and then 3ml of Opti-MEM and prepared Mixture III was added to plates and gently shaken to evenly distribute the mixture. The plates were incubated for 4-5h at 37°C with 5% CO2. Then the suspension mixture fluid was discarded and 10 ml of full DMEM media (10% FBS with antibiotics) was added. The plates were left in the incubator for additional 48-72h and supernatant containing viral particles was collected into 15ml tubes, centrifuged at 8000g for 10 min and used for cell infection. To this end, 1x10^6 cells were spun down, re-suspended and incubated with 1ml of virus particles to which polybrene at final concentration of 5 µg/ml was added.

After plasmids transfection and virus infection, cells were incubated for 48h in humidified 37°C/5% CO2 incubator before utilization in subsequent experiments. GFP or mCherry positive cells were sorted using the Aria II instrument (BD Biosciences, San Jose, CA).

**RhoA-GTP pull down assay**

RhoA activation was measured by pull down assay (Cytoskeleton Inc., Denver, CO) according to the manufactures' instructions. 1000 µg of fresh cell lysate was used per assay.
Rho-binding domain (RBD) of the RhoA effector rhotekin was used to affinity-precipitate endogenous GTP-bound RhoA. The amount of precipitated RhoA was then quantified by western blotting using specific antibodies. The binding of glutathione S-transferase (GST)-RBD to GTP-RhoA inhibits its conversion to GDP, so the assay provides a reliable measure of the total amount of active RhoA present in the cell at any given time.

**CellTiter-AQueous MTS assay**

Proliferation of cells expressing or not expressing HGAL was measured by MTS assay according to the manufacturer’s instructions (Promega, Madison, WI) for 15 consecutive days. The initial cell concentration was 2000 cells/mL. Briefly, 20 µl of MTS reagent was added directly to the wells containing 100 µl of cells and cell plates were incubated at 37°C for 4h. Absorbance was measured at 490 nm on a SpectraMax Plus384 reader (Molecular Devices; Sunnyvale, CA). Background absorbance was subtracted using a set of wells containing medium only. All the experiments were performed in hexaplicates.

**Wound assay**

4175 cells stably transfected with HGAL or control vectors were grown to confluence in 35 mm dishes and wounded with a P200 pipette tip. Wounded monolayers were washed three times with complete growth medium and returned to the incubator for 1h to recover from wounding before experiments were begun. At times 0 and 24h, time-lapse images of the monolayers were photographed using 10× magnification (Carl Zeiss microscope, Thornwood, NY) and the wound width at the predetermined grid-marked points was measured using the AxioVision 4.5 measurement tool (Carl Zeiss, Thornwood, NY). All the experiments were performed in triplicates and were repeated at least three times.

**Chemotaxis assay**

Chemotaxis assays were performed in 24-well plates (Nalge Nunc International, Rochester, NY) containing 5.0 µm porous polycarbonate membranes inserts (Corning Incorporated Life
Sciences, Acton, MA) as was previously reported\textsuperscript{3}. Briefly, the inserts were coated with 0.3 ml of 10 μg/mL fibronectin (BD Biosciences, San Jose, CA) for 1h, washed once with 0.3 ml of PBS and equilibrated with RPMI 1640 medium. Cells were washed twice with no-supplemented RPMI 1640 and re-suspended at 5×10\textsuperscript{5} cells/ml in serum-free RPMI 1640 medium. IL-6 (R&D systems, Minneapolis, MN) at 10 ng/mL or SDF-1 (EMD Millipore Corporation, Temecula, CA) at 200 ng/ml in a total volume of 600 µL of non-supplemented RPMI 1640 medium or medium alone were added to the bottom of the wells and 100 µL of cells were loaded on the inserts. The optimal concentrations of inhibitors and stimulants were determined in titration assays, as shown for SDF1 (Supplementary Figure 17). BCR stimulations was performed with F(ab')-Goat anti-human IgM (Grand Island, NY) at 0.8 µg/ml. The cells were allowed to migrate for 4 h at 37°C and 5% CO\textsubscript{2}. The number of cells that migrated to the bottom portion of the well was assayed by flow cytometry. All the assays were performed in triplicates and repeated 3 times. In chemotaxis inhibition assays we used 1×10\textsuperscript{6} cells/ml and the following reagents: Latrunculin (Sigma, St. Louis, MO) at 0.1 µg/mL for 2h, R406 (ThermoFisher, Pittsburgh, PA) at 5 µM for 72h, ibrutinib (Abbvie, Chicago, IL) at 5 µM for 72h and colchicine or vinblastine (Sigma, St. Louis, MO) at different concentration (0, 2 nM, 10 nM and 50 nM) for 30 minutes.

\textbf{3D spheroid cell invasion assay}

96-well 3D spheroid cell invasion assay (3500-096-K, Cultrex, Trevigen, Gaithersburg, MD) was used for analyzing cell invasion \textit{in vitro} that was performed according to the manufacturer's instructions. Briefly, cells were re-suspended in 1×Spheroid Formation ECM. A total of 50 μl of cell suspension/per well was added to the 3D Culture Qualified 96 Well Spheroid Formation Plate and centrifuged at 200×g for 3 min at room temperature in a swinging bucket rotor. The cells were incubated at 37°C in a tissue culture incubator for 72h to promote spheroid formation. The 3D Culture Qualified 96 Well Spheroid Formation Plate was placed on ice in refrigerator for 15 min and 50 μl of Invasion Matrix was added to each well. The plates were centrifuged at 300×g at 4°C for 5 min in a swinging bucket rotor to eliminate bubbles and
position spheroids within the Invasion Matrix towards the middle of the well and then transferred to a tissue culture incubator set at 37°C for 3 to 6 days. To evaluate 3D culture cell invasion, photographs of the spheroid in each well were taken every 24h using the 4× objective and images were analyzed by ImageJ Fuji4. The assays were performed in triplicates and repeated 3 times.

**In vivo cell migration assay**

These *in vivo* studies were performed in NSG mice using the intraocular transplant approach as previously described in detail5,6 with modifications. In brief, the head of fully anesthetized NSG mice was fixed in a stereotaxic head holder with the eye exposed under a stereoscope. A total of 100,000 lymphoma cells with and 100,000 lymphoma cells without HGAL expression, respectively labeled with either green (GFP or CellTracker Green CMFDA Invitrogen) or red (CellTracker Orange CMRA Invitrogen) fluorescence, were injected (mixed in 2–4 µL) into anterior chamber of the eye using a glass micropipette connected to a precision Hamilton glass syringe. We have extensive experience using these dyes and have had observed no effect of either dye and of GFP expression on the motility *in vitro* or homing and migration of lymphocytes *in vivo*. The micropipette tip (diameter 20–40 µm) was inserted into the cornea near the limbus and the cells were deposited in a monolayer throughout the iris surface. The eye was washed on the outside with 1x PBS following the injection. The anesthetized mouse was then placed onto the stage of an upright Leica SP5 confocal imaging system and z-stack images spanning the full height of the anterior chamber were acquired noninvasively using a 10x objective in four quadrants covering the whole anterior chamber. Images of the four quadrants in each mouse were acquired within 10 min of injection (Day0) and on day 1 (24h) and day 3 (72h) post-injection. Image analysis was performed in Volocity software (Quorum technologies; [https://quorumtechnologies.com/](https://quorumtechnologies.com/)) to quantify the density of each cell type (HGAL+/−) in the anterior chamber based on the volume of objects (cells) independently
detected in the green and red channels relative to the total volume of the iris within the image\textsuperscript{6,7}.

Automatic detection of cells was done by the software in raw 3D images (unprocessed unenhanced z-stacks) using built-in algorithms with a detection threshold of 2–6 standard deviations from the noise signal in each channel\textsuperscript{8}. Individuals acquiring the images and performing the analysis were blinded to the conditions.

The relative density of each cell type on day 1 and day 3 was calculated in comparison to that of the same cell type on day 0 of the same mouse (i.e., normalized to baseline and expressed as %) as follows:

\[
\text{Relative Cell Density (day}_n\text{)} = \left( \frac{\text{Average Cell Density (day}_n\text{)}}{\text{Average Cell Density (day}_0\text{)}} \right)
\]

Where

\[
\text{Cell Density (day}_n\text{)} = \left( \frac{\text{Cell Volume (day}_n\text{)}}{\text{Iris Volume (day}_n\text{)}} \right) \quad \text{and} \quad \text{Cell Density (day}_0\text{)} = \left( \frac{\text{Cell Volume (day}_0\text{)}}{\text{Iris Volume (day}_0\text{)}} \right)
\]

Where the **Cell Volume** and **Iris Volume** are measured in the 3D confocal micrographs using the Volocity software.

The “Egress Index 24h and 72h” was used as a measure of the change in the relative density of each cell type at 24h and 72h after injection in the anterior chamber and was calculated as the inverse of the mean density ratio within 24h or 72h in each mouse:

\[
\text{Egress Index (day}_n\text{)} = \left( \frac{\text{Average Relative Density (day}_n\text{)}}{\text{Average Relative Density (day}_0\text{)}} \right)^{-1}
\]

Each experiment was performed in 3 animals and repeated twice.

**MMP activity assay**

MMP activity assay kit (Fluorometric-green (ab112146), Abcam, Cambridge, MA) was used for analyzing the matrix metalloproteinases activity of cell culture supernatant and was performed according to the manufacturer’s instructions. Briefly, 2× MMP green substrate working solution was prepared according to the protocol. Control (culture medium) or test
samples (the cell culture supernatant) were mixed with the same volume of 2× working solution to start the reaction. A total of 50 μL of the reaction samples was aliquoted to 96-wells of the assay plate in triplicates and incubated at 37°C for 60 min. Fluorescence intensity was measured by using a microplate reader with a filter set of Ex/Em = 490/525 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Green Substrate but no MMPs. The assays were performed in triplicates and repeated 3 times.

**Microtubule Binding Protein Spin-down Assay**

Microtubule binding protein spin-down assay kit (Cat. # BK029, Cytoskeleton, Denver, CO) was used and the experiments were performed according to the manufacturer’s protocol. Briefly, tubulin protein was incubated in general tubulin buffer at 35°C for 20 minutes, Taxol stock solution was added and mixed well. Aliquot was mixed with recombinant HGAL protein at room temperature for 30 minutes and then Taxol supplemented Cushion Buffer was added to each tube and centrifuged at 100,000 x g, at 4°C for 40 minutes. Supernatant and pellet portions were collected and suspended in 50 µl of 1x Laemmli sample buffer for western blotting.

**Confocal microscopy**

To investigate the colocalization of HGAL and α-tubulin following B cell activation, Raji cells were cultured on an anti-IgM F(ab’)2 functionalized planar membrane formed within a paralleled flow chamber. After 45 min incubation period, cells were fixed with 4% paraformaldehyde (Thermo Scientific, Waltham, MA) to terminate B cell activation. Membrane-permeabilized cells were labeled with HGAL rabbit plyclonal antibody (ProteinBiotech, Rosemont, IL) and mouse β-tubulin monoclonal antibody (Santa Cruz, Dallas, TX), followed by Alexa 547-anti-mouse and a Alexa-647 anti-rabbit secondary antibodies (ThermoFisher Scientific, Waltham, MA). Confocal images of the cells coupled to the planar bilayer were acquired on a Nikon A1R scanning laser confocal microscope with a 60X oil immersion.
objective (N.A. 1.4) at an acquisition rate of 2 seconds per frame. Colocalization of HGAL and α-tubulin in Hela cells, cultured in ibidi chamber slides, was similarly assessed.

Cellular distribution of tubulin was determined based on ImageJ analysis of acquired confocal images. Areas of interest containing individual cells were identified manually. Distributions of tyrosinated and detyrosinated tubulin were determined by measuring the fluorescence of cells labeled with the antibodies against tyrosinated tubulin (EMD Millipore, Temecula, CA) and detyrosinated tubulin (Abcam, Cambridge, MA), followed by fluorinated secondary antibodies goat anti-rat Alexa 555 (Thermo Scientific, Waltham, MA) and goat anti-rabbit Alexa 647 (Thermo Scientific, Waltham, MA), respectively.

**Apoptosis and cell death analyses.**

Apoptosis and cell death of Raji and BJAB cells treated with 20 nM colchicine were measured by PE Annexin V Apoptosis Detection Kit I (BD Biosciences, San Jose, CA) according to the manufacture’s protocol. Briefly, 1x10⁵ cells were washed with cold PBS twice, resuspended with 100 µl of 1X binding buffer, to which 5 µl of PE-Annexin V and 5 µl of 7AAD were added and incubated at room temperature for 15 min. The samples were analyzed by LSR-II Analyzer (BD Biosciences, San Jose, CA).

**Mouse xenografts model**

The institutional animal care and use committee (IACUC) at the University of Miami approved all animal procedures. Eight weeks old female BALB/c nude mice (The Jackson Laboratory, Bar Harbor, ME) were used for breast cancer cell line 4175. A total of 3x10⁶ cells were injected through tail vein in to BALB/c nude mice. Eight weeks old NOD/SCID mice (The Jackson Laboratory, Bar Harbor, ME) were used for BJAB, U2932 and TMD8 lymphoma cell lines. A total of 5x10⁶ cells were injected through tail vain into NOD/SCID mice. After injection, mice were monitored for survival daily. Body weight was measured and IVIS used for tracking tumor cell location and luciferase intensity weekly. At the end point, mice were euthanized, and tumor tissues were removed for photographing, tumor cell number counting, tumor size
measurement and immunohistochemistry analysis. The number of tumor sites was established by IVIS and corroborated by autopsy findings.

**Statistical analysis**

All statistical analyses were performed using Graph Pad Prism 6.0. Statistical significance was determined by Student t test or two-way ANOVA. p value < 0.05 was considered significant. Survival was calculated with the Kaplan-Meier survival curve method and differences in survival were calculated by long-rank test. For intraocular cell motility, data comparisons were made by ANOVA (multiple comparisons) or Student t-test (pairwise comparisons). Data presented as mean ± SD or SEM where specified or as Box and whiskers plots with min and max shown. Asterisks denote significance with * p<0.05, ** p<0.01, *** p<0.001, and **** p<0.0001.
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Supplementary Figure 1. Effect of HGAL on motility of 4175 breast cancer cells. A) Western blot of HGAL in 4175 HGAL and 4175 Mock cells; B) representative images of wound assay in 4175 Mock and 4175 HGAL cells; experiments were performed in triplicates; C) 24 hours migration distance of 4175 Mock and 4175 HGAL cells in wound assay (p=0.0051); D) MTS proliferation assay of 4175 Mock and 4175 HGAL cells.
Supplementary Figure 2. HGAL expression in the indicated lymphoma cell lines. Actin was used as a loading control.
Supplementary Figure 3

**Supplementary Figure 3.** HGAL expression inhibits TMD8 lymphoma cell growth and metastasis and prolongs mouse survival. Mice were injected intravenously with $5 \times 10^6$ luciferase-transduced human lymphoma cell line TMD8. D-luciferin was injected intraperitoneally (150 mg/kg body weight) 15 minutes before acquiring images. Bioluminescence images were taken weekly. A) representative bioluminescent images of mice with TMD8 Mock control and TMD8 HGAL lymphoma; B) tumor growth curve based on photon flux intensity (Mock group: 8 mice, HGAL group: 11 mice, not injected mice (Background) group: -5 mice) with significant difference between the TMD8 Mock and TMD8 HGAL lymphoma mice cohorts ($p=0.0038$). Normalized photon flux represents tumor photon flux (luminescence intensity) at the indicated time relative to photon flux measured post cell injection on Day 0 that was set to value
of 100. C) number of individual sites with lymphoma involvement in TMD8 Mock and TMD8 HGAL mice (p=0.0051); D) Kaplan-Meier plots of mice survival demonstrating significant difference between the TMD8 Mock and TMD8 HGAL mice (p=0.0051).
**Supplementary Figure 4. MTS proliferation assay of lymphoma cell lines.** The cell lines are indicated in the figure.
**Supplementary Figure 5. In vitro 3D spheroid cell invasion assay.**

A) Representative images of in vitro 3D spheroid cell invasion in U2932 Mock and U2932 HGAL cells, scale bar: 200 µm; B) quantitative analysis of surface area of U2932 Mock and U2932 HGAL spheroids over a four days period (p=0.001). C) Representative images of in vitro 3D spheroid cell invasion in TMD8 Mock and TMD8 HGAL cells, scale bar: 200 µm; D) quantitative analysis of surface area of TMD8 Mock and TMD8 HGAL spheroids over a four days period (p=0.001). Experiments were performed in triplicates and repeated twice.
Supplementary Figure 6. MMPs activity assay. The experiments were performed 3 times in triplicates.
**Supplementary Figure 7.** Longitudinal images of representative same mouse eye anterior chamber acquired at different time-points after injecting U2932 lymphoma cells (green GFP) in preliminary experiments to determine days at which experiments will be performed. The shown images were stitched horizontally from 4 consecutive images spanning the entire anterior chamber of the eye. Images were acquired with a digital camera installed in place of the eyepiece using a 5X air objective.
**Supplementary Figure 8. RhoA activation and expression.** (A and B) RhoA activation measured by rhotekin RGoA-GTP pull down assay in BJAB (A) and TMD8 (B) cells; (C and D) RhoA knockdown in BJAB (C) and Raji (D) cells.
Supplementary Figure 9. RhoA activation and expression upon BCR stimulation, inhibition and coactivation with SDF-1. A) RhoA activation measured by rhotekin RGoA-GTP pull down assay in TMD8 and BJAB cells upon BCR activation via anti-IgM F(ab)’2 without and with BTK inhibitor (ibrutinib) or SYK inhibitor (R406); B) RhoA activation measured by rhotekin RGoA-GTP pull down assay in TMD8 and BJAB cells upon BCR activation via anti-IgM F(ab)’2 without and with SDF-1 stimulation.
Supplementary Figure 10. Confocal microscopy images of HGAL and tubulin colocalization. A) HGAL reciprocally co-immunoprecipitated with β-tubulin in Raji cells. B) HeLa cells expressing exogenous HGAL. HGAL: green, tyrosinated tubulin: orange, de-tyrosinated tubulin: red; scale bar: 10 µm. C) Raji cell with endogenous HGAL protein expression following stimulation with soluble anti-IgM F(ab')2. HGAL: green, β-tubulin: red. Arrow indicated MTOC/ Scale bar: 5µm.
**Supplementary Figure 11. HGAL has no effect on tubulin polymerization.** Standard polymerization reactions without (general tubulin buffer 01 and general tubulin buffer 02 repeat) and with 10 µM paclitaxel (duplicate) or recombinant HGAL protein 2µg. (tetraplicate).
Supplementary Figure 12. Effect of HGAL on tubulin intracellular distribution. α-Tubulin was fluorescently labeled, and fluorescence intensity was measured at indicated distances from the cell surface (height) in the indicated cell lines (expressing and not expressing HGAL) in 50 cells for each experimental condition using confocal microscopy. α-Tubulin redistributed toward cell membrane only in the U2932 cells expressing HGAL (p<0.001) but not in other indicated cell lines. Raji HGAL and BJAB HGAL represent Raji HGAL KO and BJAB HGAL KO in which HGAL was re-expressed.
Supplementary Figure 13. MTS proliferation assays of the indicated lymphoma cell lines treated with colchicine at the indicated concentrations. Experiments were performed in triplicates and repeated 3 times.
Supplementary Figure 14. Apoptosis assess by staining with 7-AAD and PE-annexin in Raji and BJAB cells treated with colchicine 20nM for 5 hours. A representative result is shown from 3 repeated experiments.
Supplementary Figure 15. MTS proliferation assays of the indicated lymphoma cell lines treated with vinblastine at the indicated concentrations. Experiments were performed in triplicates and repeated 3 times.
Supplementary Figure 16. HGAL-mediated inhibition of lymphoma motility is mediated by tubulin. Shown are numbers of cells migrating in transwell experiments in the presence of the indicted stimulants and vinblastine 2nM. Means and standard deviations of 3 independent experiments performed in triplicates are demonstrated: **** p<0.0001
Supplementary Figure 17. Transwell chemotaxis dose response to SDF1 stimulation in the indicated cell lines.
### Supplementary Table 2. Cytoskeletal proteins interacting with HGAL

| Protein Symbol | Protein Name |
|----------------|--------------|
| ACTG1          | Actin, cytoplasmic 2 |
| ADD3           | Gamma-adducin |
| AFDN           | Afadin |
| ALDH18A1       | Delta-1-pyrroline-5-carboxylate synthase |
| ARHGAP25       | Rho GTPase-activating protein 25 |
| CCT2           | T-complex protein 1 subunit beta |
| CCT7           | T-complex protein 1 subunit eta |
| CLINT1         | Clathrin interactor |
| CRKL           | Crk-like protein |
| EHD1           | EH domain-containing protein 1 |
| EPB41L5        | Band 4.1-like protein 5 |
| EPS15          | Epidermal growth factor receptor substrate 15 |
| ERBIN          | Erbin |
| EZR            | Ezrin |
| GCSAM (HGAL)   | Germinal center-associated signaling and motility protein |
| MARK2          | Serine/threonine-protein kinase MARK2 |
| MSN            | Moesin |
| PICALM         | Phosphatidylinositol-binding clathrin assembly protein |
| PLEKHA5        | Pleckstrin homology domain-containing family A member 5 |
| PLS3           | Plastin-3 |
| PPFIBP1        | Liprin-beta-1 |
| SLC3A2         | 4F2 cell-surface antigen heavy chain |
| SNX1           | Sorting nexin-1 |
| SWAP70         | Switch associated protein 70 |
| TNS3           | Tensin-3 |
| TUBA1C         | Tubulin alpha-1C chain |
| TUBA4A         | Tubulin alpha-4A chain |
| TUBB           | Tubulin beta chain |
| TUBB2B         | Tubulin beta-2B chain |
| TUBB4B         | Tubulin beta-4B chain |
| WASF2          | Wiskott-Aldrich syndrome protein family member 2 |