Cells culture and BMSCs

Cells were maintained in RPMI 1640 media with 10% FBS (Sigma-Aldrich, Oakville, ON), 100 U/ml penicillin, and 100 mg/ml streptomycin (Sigma-Aldrich). For INA6 cells the culture media was supplemented with human rIL-6 (2.5 ng/ml). Primary $CD138^+$ MM cells were obtained from the BM aspirates of MM patients at the time of diagnostic procedure, after informed consent, in accordance with the Declaration of Helsinki, using positive selection with CD138 microbeads (Miltenyi Biotech, Auburn, CA). Residual $CD138^-$ bone marrow-derived mononuclear cells were cultured for 3 to 6 weeks to generate bone marrow stromal cells (BMSCs).

Quantitative reverse transcription polymerase chain reaction (RT-PCR)

The mRNA expression of integrin b7 was detected by RT-PCR in indicated MM cell lines and in sorted $CD138^+$ cells from MM patients with the forward primer GAATCAACCAGACGGTGACTTTCT and the reverse primer GCCCGGAGCCTCAGGA. The expression of GAPDH was detected with the following forward AACAGCGACACCCATCCTC and reverse CATACCAGGAAATGAGCTTGACAA primers. RT-PCR amplification was carried out with 250 ng of cDNA in a 25-µl reaction mixture containing 12.5 µl SYBR green mix (Bio-Rad, Mississauga, ON) and 1 ml RT^2 qPCR primers (SA Bioscences, Frederick, MD). Data quantification was carried out by the $2^{-\Delta\Delta Ct}$ method.

Multiple myeloma tissue microarray staining and scoring methodology

Myeloma tissue microarrays (TMA) were generated from formalin-fixed paraffin sections of pre-treatment diagnostic BM biopsies (n=79) of MM patients and used to evaluate the expression of integrin b7 as specific prognostic marker in MM by immunoperoxidase staining method under standardized protocol.

TMA sections were deparaffinized using xylene and graded alcohol and then subjected to heat-induced antigen retrieval technique (EDTA buffer at pH 8.0, CC1 Ventana, Tucson, AZ). Endogenous peroxidase activity was blocked by 3% hydrogen peroxide and sections were incubated with protein blocking solution for 5 minutes. TMAs were later incubated with anti-Integrin β7 antibody (1:200, US Biologicals, Swampscott, MA) and anti-Cyclin D2 (M20, 1:1000, Sana-Cruz, Santa-Cruz CA), utilizing Ventana Benchmarks automatic immunostainer (Ventana, Tucson, AZ). The staining conditions were established utilizing FFPE cell blocks of various myeloma and hematopoetic cell lines (MM1S and HL60 as positive and negative controls respectively) and normal control tissues (tonsil; BM). The staining pattern was reviewed and scored by a pathologist (A.M.) who was blinded to the clinical outcome of these patients. Positive cut-off was defined as >30% tumor cells staining (minimum count of 500 plasma cells), irrespective of intensity of staining, as established by Hans *et al*. [1]

NF-κB activity

NF-kB activity was investigated using the TransAM NF-kB p65 kit, a DNA-binding ELISA–based assay (Active Motif, Carlsbad, CA). NF-kB-p65 binding to a related consensus sequence on the plate-bound oligonucleotide was studied from nuclear extracts of $ITGB7^{silenced}$ and $ITGB7^{positive}$ MM cells as well as Jurkat cells (positive control), following the manufacturer's procedure.

The effect of *ITGB7* silencing on nuclear translocation of p65-NFkB was also examined

in *ITGB7*^{silenced} and control *ITGB7*^{positive} MM1S cells adherent to FN coated plates by immunofluorescence staining. Antibodies used for immunostaining included anti–p65-NFkB (Cell Signaling Technology, Danvers, MA) and corresponding Cy-3 labeled secondary antibody (Invitrogen). The slides were mounted with Vectashield anti-fade mounting medium containing 4',6-diamino-2-phenylindole, dihydrochloride (DAPI, Vector Laboratories). Immunocytochemical analysis was performed using an epifluorescence microscope (Olympus BX51) mounted with a multispectral color camera (Nuance FX, CRi, Woburn, MA) for image acquisition. Automated scoring of fluorescence was performed with the inForm^{TM} analysis software (CRi, Woburn, MA).

ITGB7 Blocking antibody and rescue experiments

To exclude the possibility of a non-specific and off-target effect of the ITGB7 shRNA constructs, blocking antibody and rescue experiments were also performed.

Myeloma cells at 5 x 10^5/mL were incubated with blocking anti-ITGB7 antibody (FIB504 clone[2]) or isotype control antibody at 20 µg/mL for 30 minutes at 4°C. Cells were then washed in PBS and carried out in Hepes-buffered saline with 0.1% BSA and 1 mM each $MnCl_2$, $MgCl_2$ and $CaCl_2$. Adhesion and migration assays were performed as described previously.

For the rescue experiments a silent mutant ITGB7 (ITGB7^{mut}) was prepared using PCR-based mutagenesis (MetaMorph Mutagenesis Kit, System Biosciences) with mutagenic PCR primers designed based on the sequences of ITGB7 shRNA #2 and the sequence of human wild type ITGB7 cDNA (GenBank: EU176336.1) cloned into the pLOC-GFP lentiviral vector (Clone Id PLOHS_100006445, Open Biosysytems). The PCR primers sequences were as follow:

Reaction 1:	forward GACACCGACTCTACTAGATTAAT
	reverse GGAATCAACTTACTCAGCTCCTGGTAGAC
Reaction 2:	forward CTGAGTAAGTTGATTCCTAAGTCTGCAGTTGG
	reverse CGCCCGGTTCATTAGCTAG

The mutagenic PCR fragments were then fused back to the pLOC empty (cut) lentiviral vector and the sequence of final construct (pLOC-ITGB7^{mut}) was confirmed by DNA sequencing. The introduced mutations to ITGB7 cDNA (from: GCTGAGTAA**AC**TGATTCCTAA to GCTGAGTAA**GT**TGATTCCTAA) are silent and do not cause any amino acid changes in the ITGB7 protein [Codon 361: AA**A** (Lysine; K) to AA**G** (Lysine, K) and codon 362: **C**UG (Leucine; L) to **U**UG (Leucine =L)] but they induce resistance to shRNA#2 because of changes in the corresponding ITGB7 mRNA seed sequence. Wild type ITGB7 (pLOC-ITGB7^{WT}) or silent mutant ITGB7 (pLOC-ITGB7^{mut}) lentiviral vectors were then packaged in HEK293T cells to generate lentiviral transductions particles that were used to infect MM1S and H929 ITGB7$^{silenced\ shRNA2}$ *cells*. Infection efficiency (GFP expression) and ITGB7 expression were analyzed by FACScan analysis. Blasticidin (for ITGB7 WT and mut expression) and Puromycin (for shRNA#2 expression) resistant cells from these lentiviral infections were selected and used in the adhesion and migration assays as described.

REFERENCES

1. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103(1):275-282.
2. Tidswell M, Pachynski R, Wu SW, et al. Structure-function analysis of the integrin beta 7 subunit: identification of domains involved in adhesion to MAdCAM-1. *J Immunol*. 1997;159(3):1497-1505. I.

Figure S1A. *ITGB7* (205718_at Affymetrix probe) and C-MAF (209348_s_at and 206363_at Affymetrix probes) expression in a cohort of myeloma patients treated with Total Therapy 2 and 3 based on NIH Gene Expression Ominibus dataset deposited by Shaughnessy JD Jr and colleagues under accession number GSE4581. *ITGB7* mRNA was identified in 124/130 (95.4%) even in the absence of detectable calls with the *C-MAF* probes.

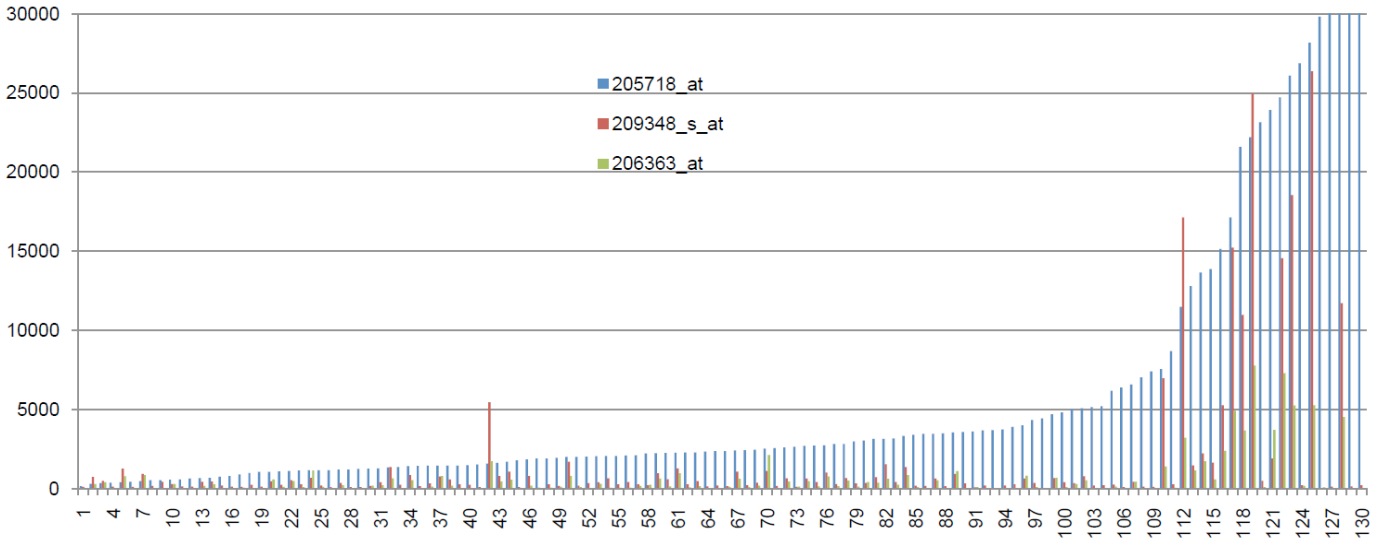


Figure S1B. *ITGB7* (205718_at Affymetrix probe) and C-MAF (209348_s_at and 206363_at Affymetrix probes) expression in the Mayo Clinic MM cell lines dataset deposited by J. Keats and colleagues in the MMRC Multiple Myeloma Genomics Portal MMPG (http://www.broadinstitute.org/mmgp/home). Red bar below indicates the cell lines studied in this manuscript.

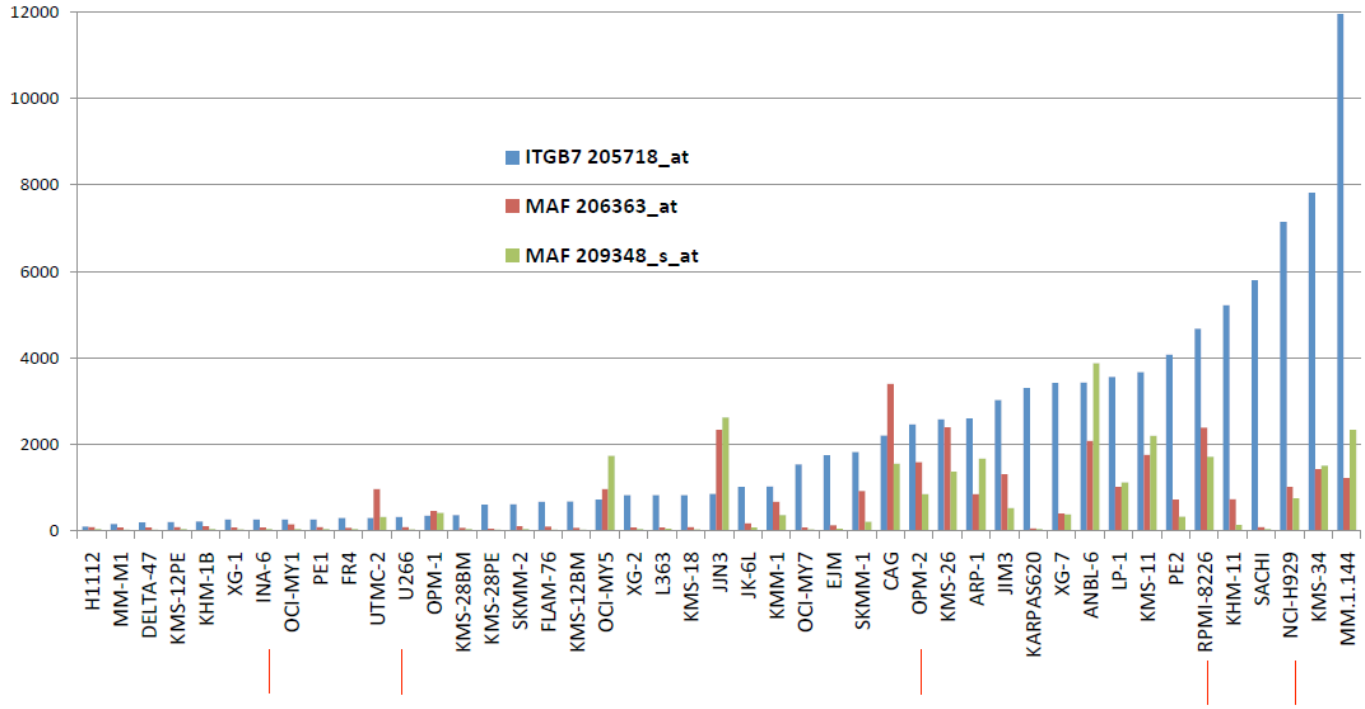
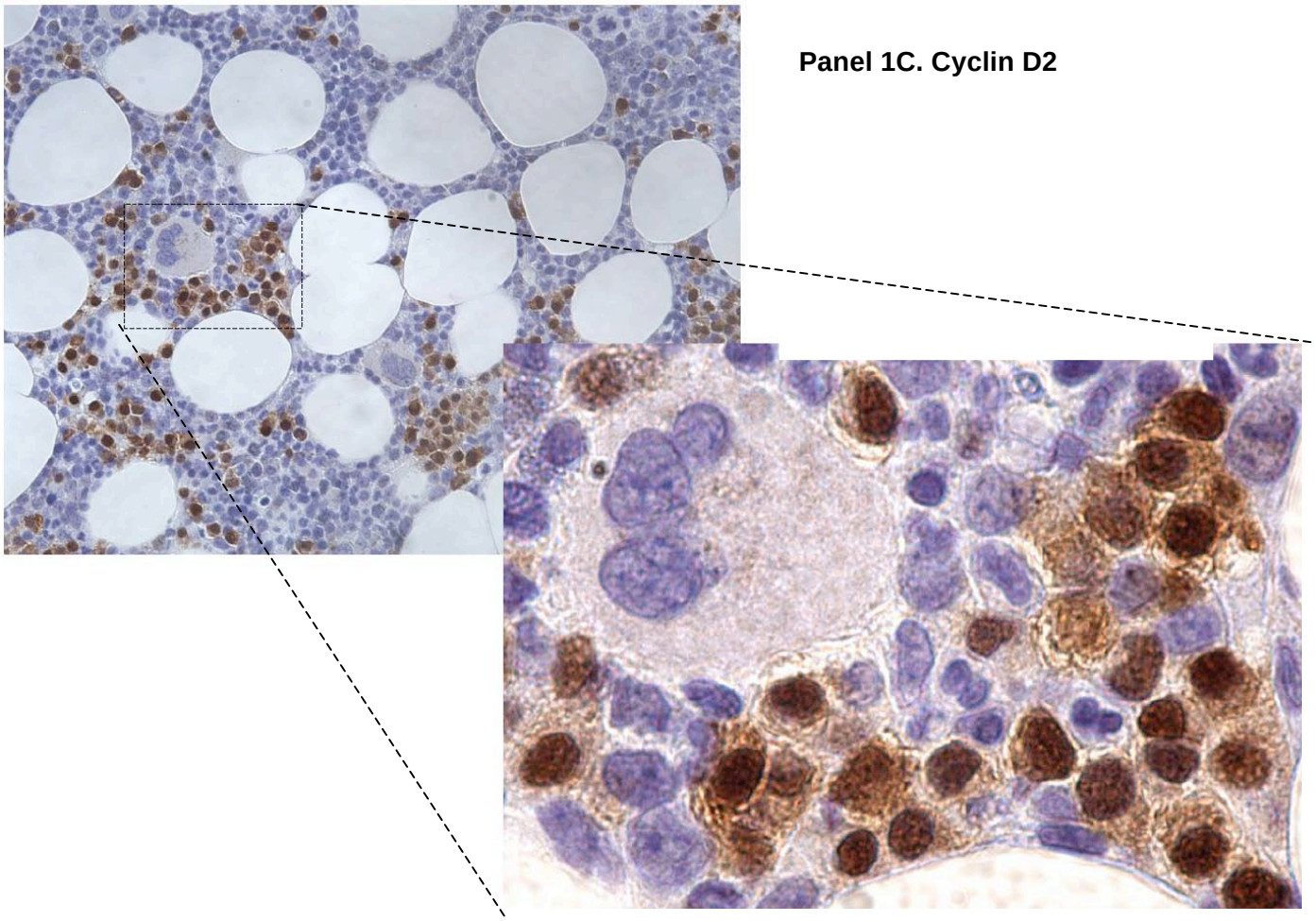
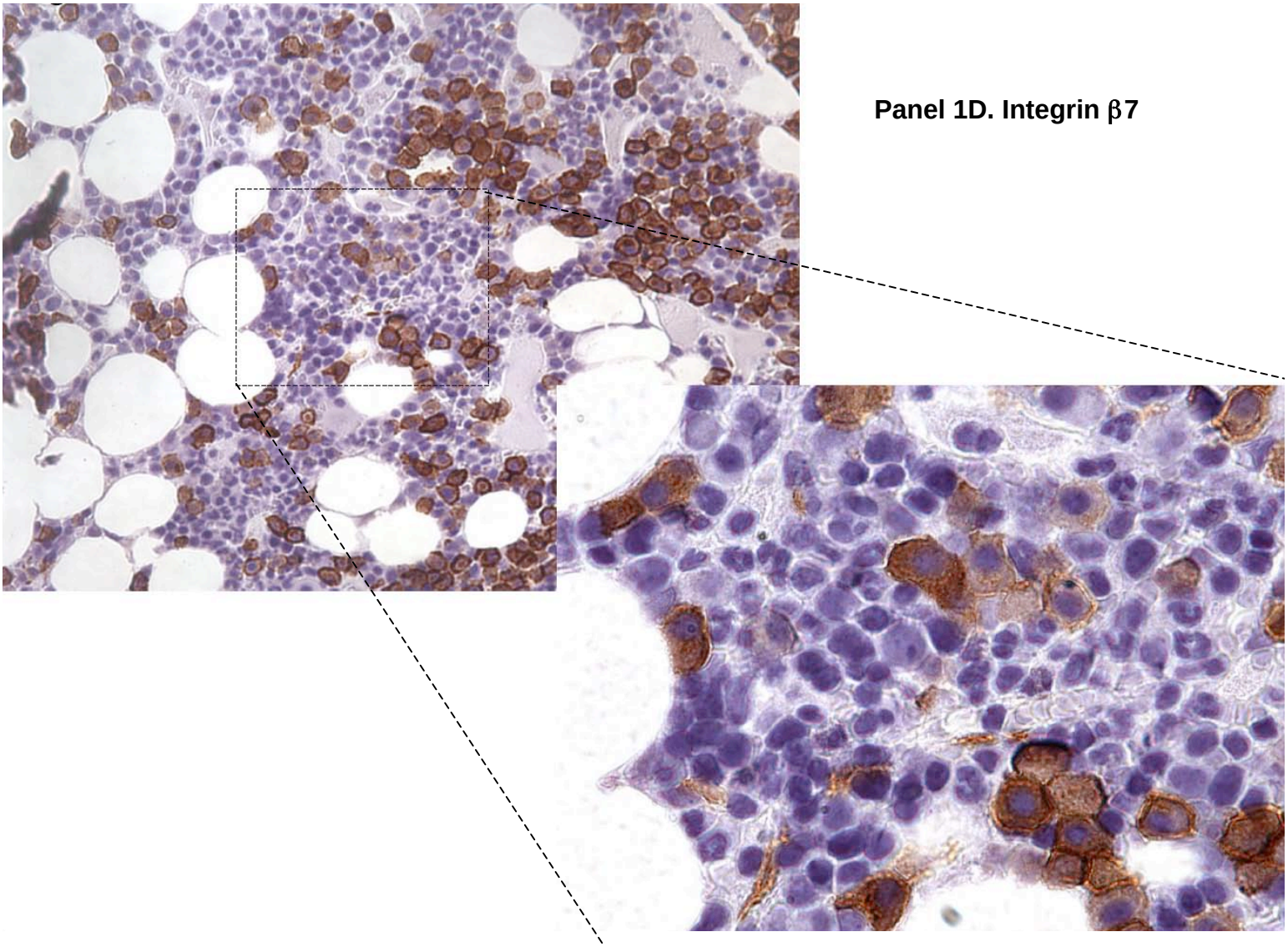


Figure S1C-D. Cyclin D2 (panel C) and Integrin β7 (panel D) immunohistochemical staining: shown are representative sections from multiple myeloma patients bone marrow biopsies (left 10x ; ---- doted square depicts a larger 60x oil immersion view of a section of the field)



Panel 1C. Cyclin D2



Panel 1D. Integrin β7

Figure S2A-B. *ITGB7* expression (205718_at Affymetrix probe) and its correlation with overall survival post salvage Bortezomib based on NIH Gene Expression Ominibus dataset deposited by Mulligan and colleagues under accession number GSE9782. Shown are the Kaplan-Meier survival curves of Bortezomib treated patients according to the quartiles distribution of *ITGB7* expression. **(A)** The median overall survival of patients in quartile 1 (Q1: low *ITGB7*) was 674 ±142 days versus 345 ± 67 for patients in quartile 4 (Q4: high *ITGB7*) (p=0.006). Shown in **(B)** are the Kaplan-Meier survival curves for patients in quartiles 1 and 2 (low ITGB7) versus quartiles 3 and 4 (high ITGB7).

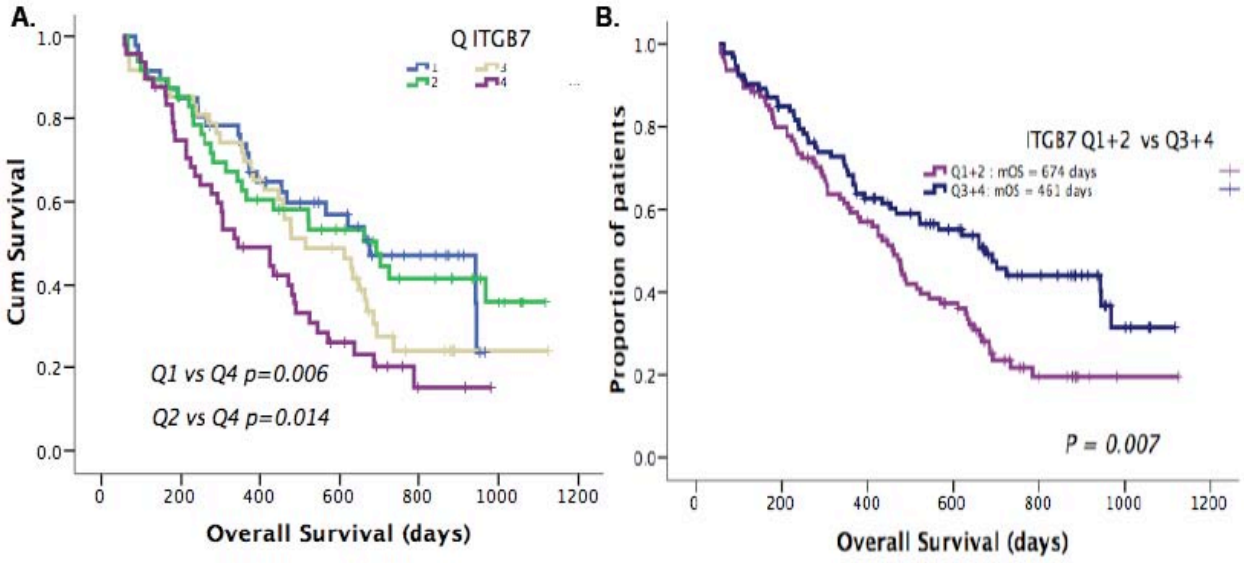
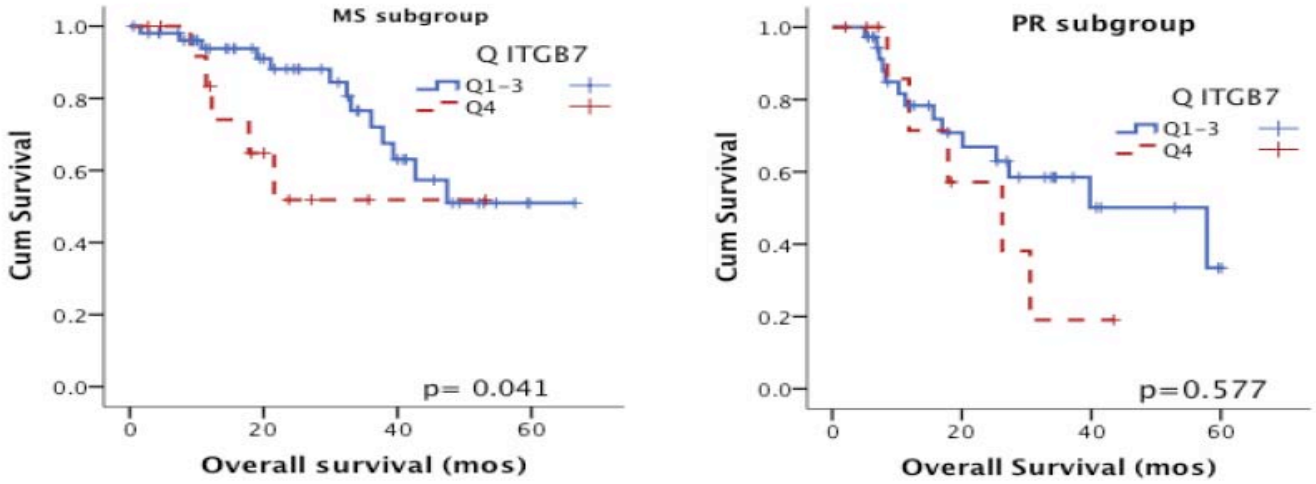


Figure S2C. *ITGB7* expression (205718_at Affymetrix probe) and its correlation with overall survival in the MS and PR myeloma molecular subroups. Expression Ominibus dataset deposited Shaughnessy JD Jr. and colleagues under accession number GSE4581. Shown are the Kaplan-Meier survival curves of MS and PR patients according to the quartiles distribution of *ITGB7* expression.



Molecular subgroup	ITGB7 Q 1-3 (Affymertix probe 205718_at)	ITGB7 Q4 (Affymertix probe 205718_at)
MS	n=54 2-yrs OS = 88.1%	n=14 2-yrs OS = 51.9%
PR	n=37 mOS = 57.8 mos (CI 20.7-95.0)	n=10 mOS = 26.8 mos (CI 9.1-43.0)

Figure S3. *ITGB7* silencing with lentivirial mediated delivery of *ITGB7* specific shRNAs (siRNA 1,4 and 5) in MM1S, H929 and INA-6 cells. Shown is integrin β7 expression in puromycin resistant cells transfected with *ITGB7* specific shRNAs (solid histogram: *ITGB7*^{silenced}) or scrambled oligonucleotides sequences (open histogram---- dashed line: *ITGB7*^{positive}) relative to control IgG1 istoype (open histogram – solid line) as determined by flow cytometry.

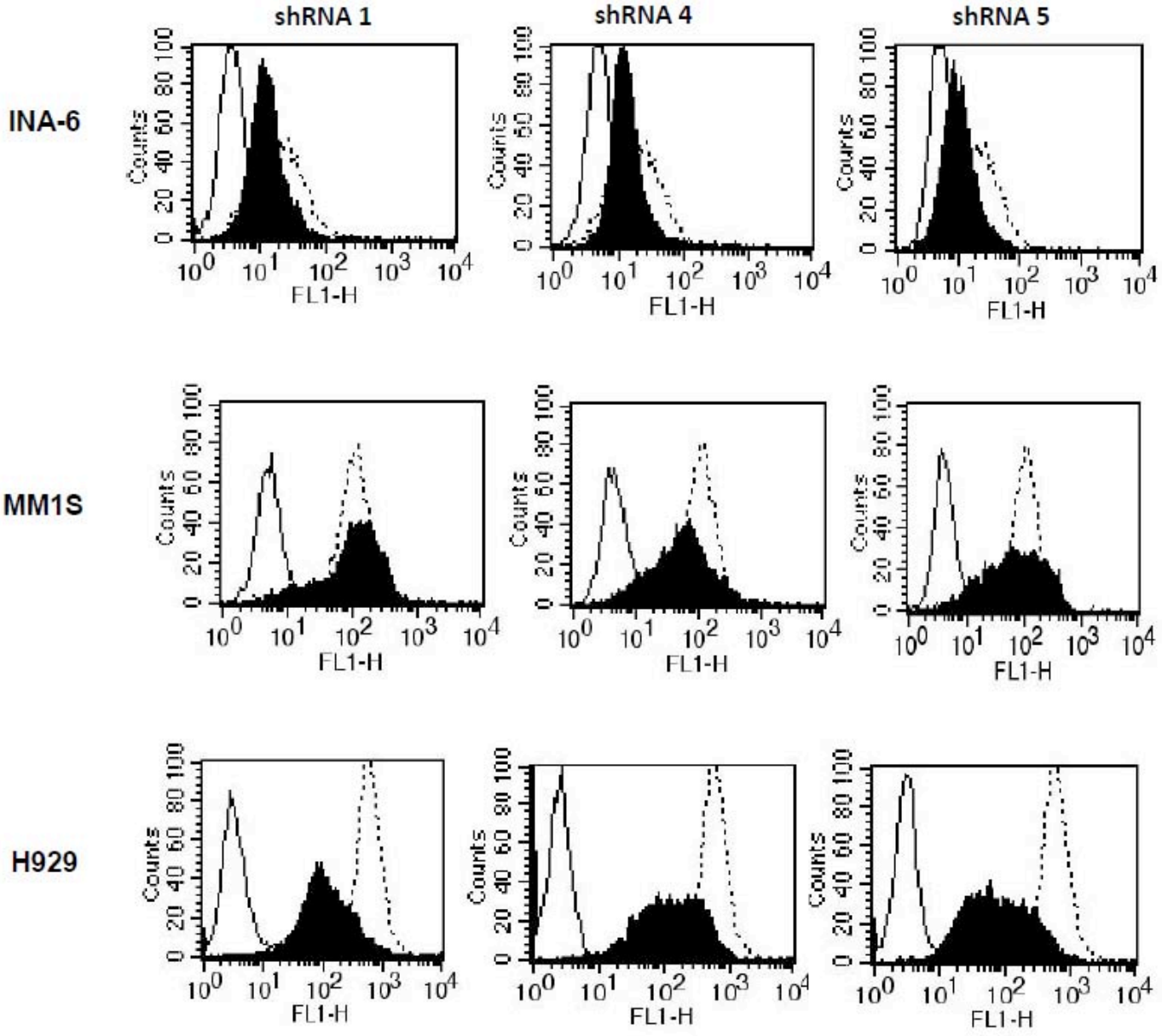


Figure S4A-C. Effects of *ITGB7* silencing on adhesion, migration and NF-κB activity in INA6 cells. (A) Adhesion of Calcein-AM labeled *ITGB7*^{silenced} (ITGB7 shRNA2 and ITGB7 shRNA3) versus *ITGB7*^{positive} (scrambled shRNA) and parental (non-transfected) INA-6 cells to BMSCs, FN and E-CDH coated 96-well microplates. Unattached cells were washed and adherent cells were measured in a fluorescence plate reader. Data are presented as percentage of respective controls (mean ± SD of triplicates from 3 independent experiments). **(B)** Transwell migration (8µm pores, Costar) of Calcein-AM labeled *ITGB7*^{silenced} (ITGB7 shRNA2 and ITGB7 shRNA3) versus *ITGB7*^{positive} (scrambled shRNA) and parental (non-transfected) INA6 cells to RPMI serum free media (cnt) or RPMI supplemented with SDF-1α (10 and 20nM). The fluorescence values were quantitated in a fluorescence multi-well plate reader using 494/517 nM filter set and % migrating cells relative to control are shown. Data is presented as the mean ± SD of triplicates from three independent experiments. **(C)** NF-κB-p65 transcription factor-binding to its consensus sequence on a plate-bound oligonucleotide was measured by ELISA in nuclear extracts from Jurkat, *ITGB7*^{silenced} (ITGB7 shRNA2 and ITGB7 shRNA3) and *ITGB7*^{positive} (scrambled shRNA) INA6 cells cultured on FN coated plates. Shown results represent means (± SD) of triplicate experiments (* p<0.05).

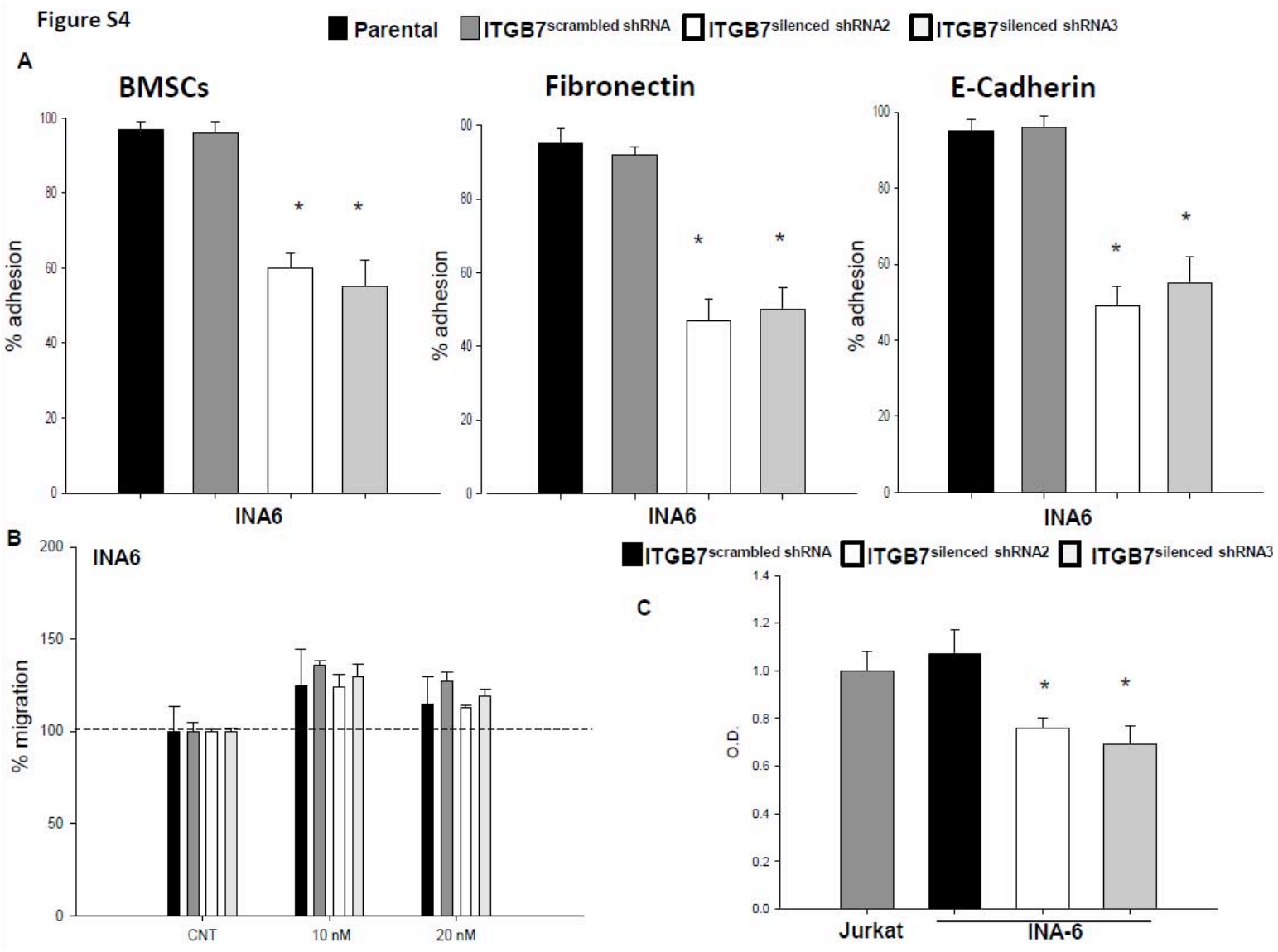
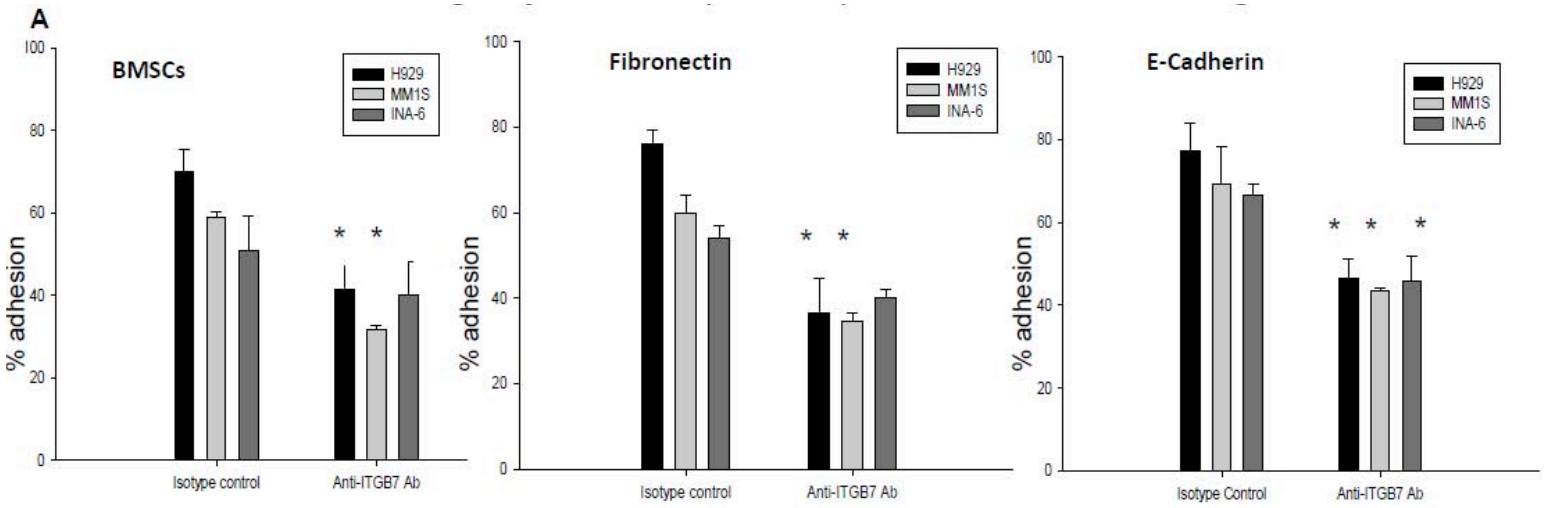


Figure S5A-B. Effects of *ITGB7* blocking antibody (FIB504) on cells adhesion and migration. (A) Adhesion of Calcein-AM labeled parental H929, MM1S and INA-6 cells to BMSCs, FN and E-CDH coated 96-well microplates. Unattached cells were washed and adherent cells were measured in a fluorescence plate reader. Data are presented as percentage of respective controls (mean ± SD of triplicates from 3 independent experiments). **(B)** Transwell migration (8μm pores, Costar) of Calcein-AM labeled parental H929, MM1S and INA-6 cells to RPMI serum free media (cnt) or RPMI supplemented with SDF-1α (10 nM). The fluorescence values, quantitated in a fluorescence multi-well plate reader using 494/517 nM filter set, are shown. Data is presented as the mean ± SD of triplicates from three independent experiments. Shown results represent means (± SD) of triplicate experiments (* p<0.05).



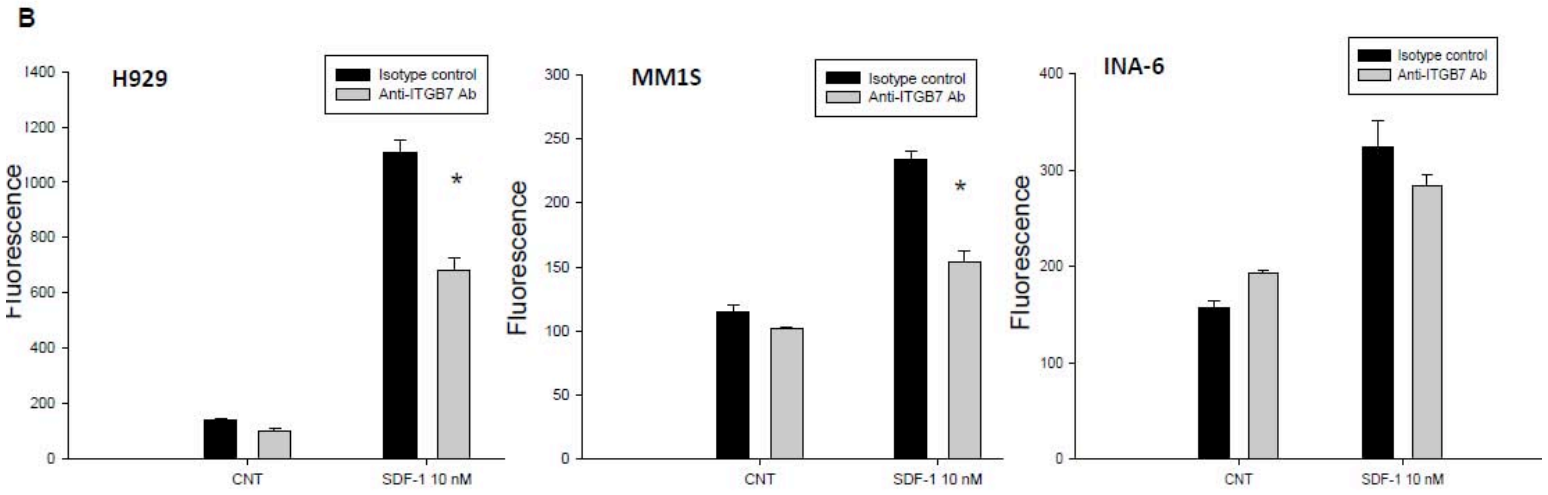


Figure S6. ***ITGB7* rescue with *ITGB7* silent mutant in ITGB7 silenced cells.** *ITGB7* silencing with lentivirial mediated delivery of *ITGB7* specific shRNA2 followed by rescue with lentiviral vectors expressing a silent mutant ITGB7 resistant to shRNA2 (pLOC-ITGB7mut) or wild type ITGB7 (pLOC-ITGB7 WT). In the top panels is shown the integrin β7 expression (solid black histogram) relative to control IgG1 istoype (open histogram), as determined by flow cytometry, in puromycin resistant MM1S and H929 cells transduced with lentiviral particules expressing scrambled oligonucleotides sequences (scrambled shRNA) or *ITGB7* specific shRNA2 (*ITGB7*^{shRNA2}). In the bottom panels is shown the integrin β7 expression in puromycin and blasticidin selected ITGB7silenced (*ITGB7*^{shRNA2}) MM1S and H929 cells infected with lentiviral particles expressing a silent mutant ITGB7 resistant to shRNA2 (pLOC-ITGB7mut) or wild type ITGB7 (pLOC-ITGB7 WT). Shown in the inset is the percent of ITGB7 positive cells.

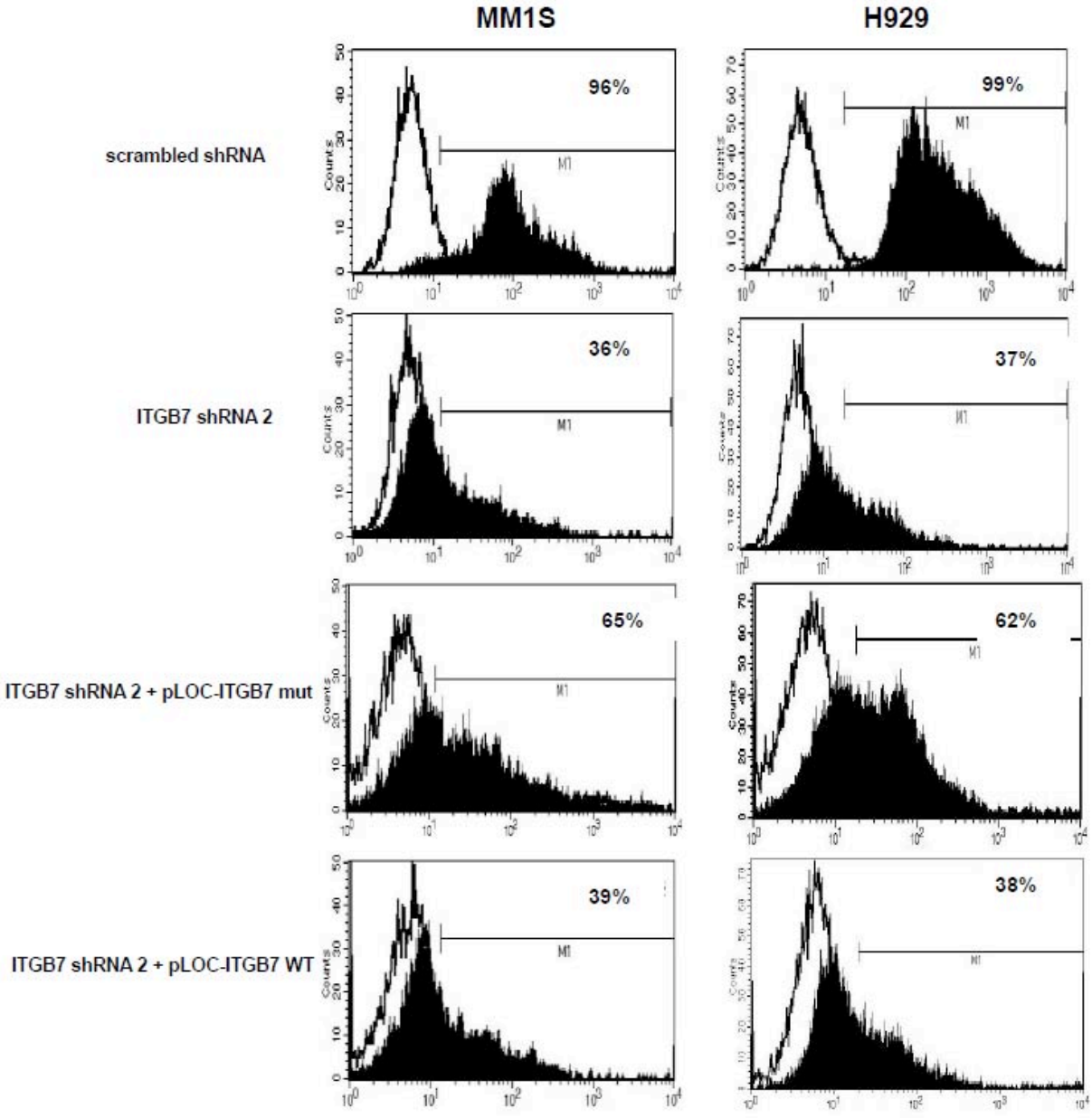
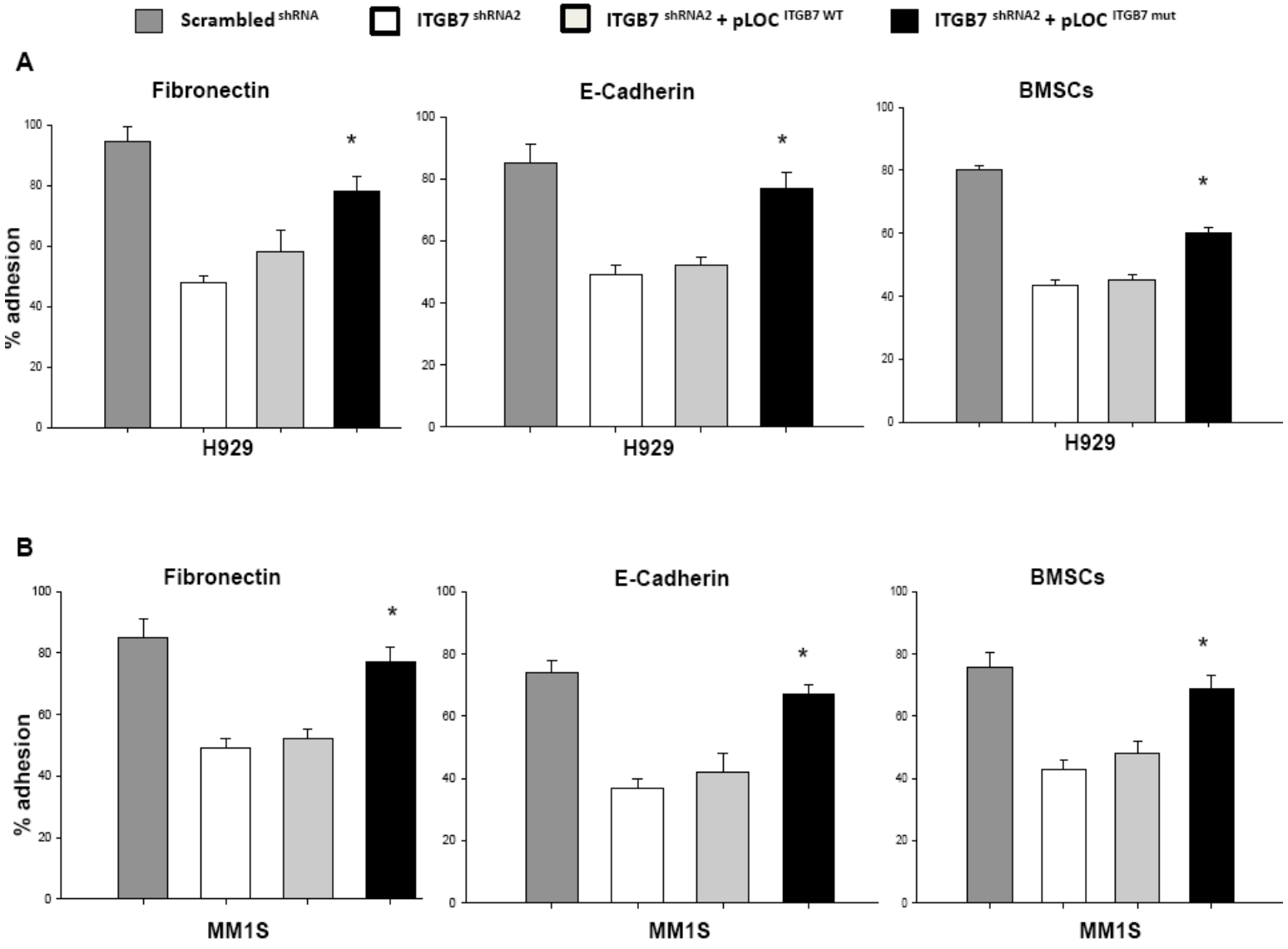
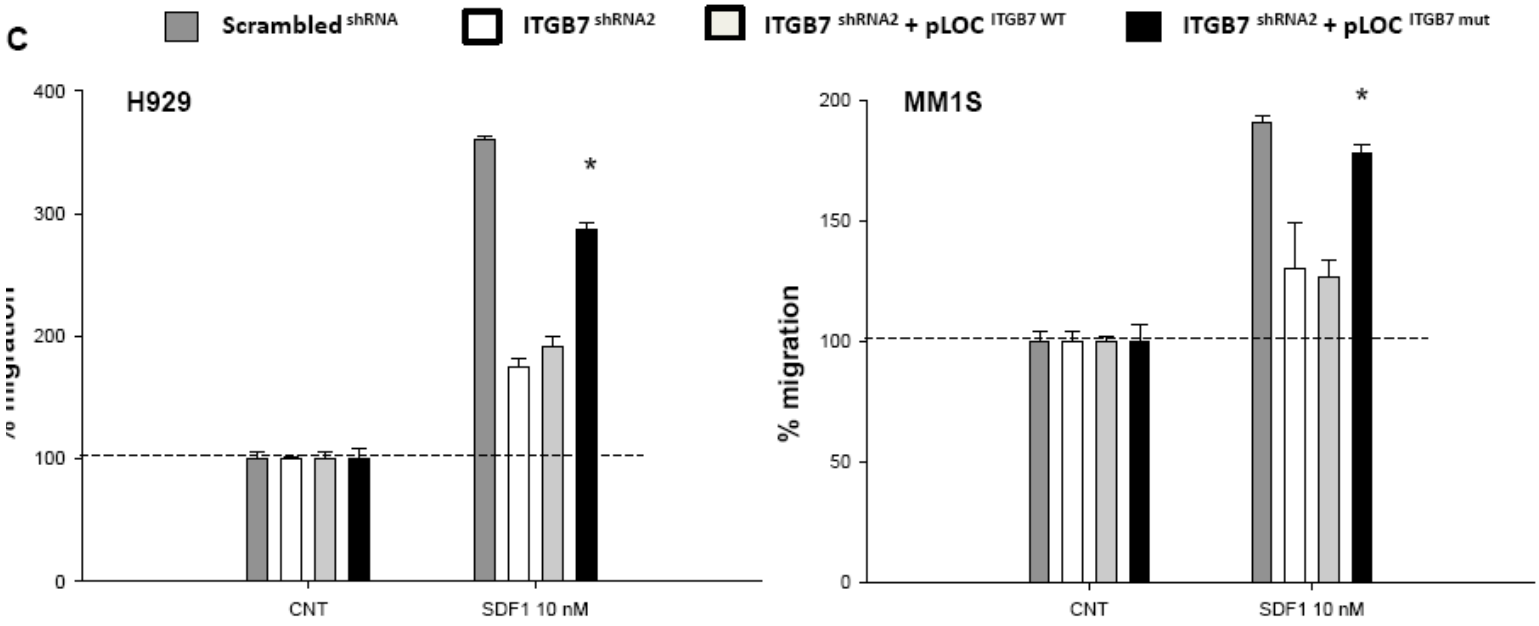


Figure S7A-D. Effects of *ITGB7* rescue in *ITGB7* silenced cells. *ITGB7*^{silenced} (ITGB7^{shRNA2}) cells were transduced with lentiviral particles delivering a lentiviral plasmid (pLOC) expressing WT *ITGB7* (ITGB7^{shRNA2} + pLOC$^{ITGB7\ WT}$) or silent mutant shRNA2 resistant *ITGB7* (ITGB7^{shRNA2} + pLOC^{ITGB7mut}). **(A-B)** Adhesion of Calcein-AM labeled *ITGB7*^{positive} (scrambled^{shRNA}) versus *ITGB7*^{silenced} (ITGB7^{shRNA2}) versus ITGB7^{shRNA2} + pLOC$^{ITGB7\ WT}$ versus ITGB7^{shRNA2} + pLOC^{ITGB7mut} H929 and MM1S cells to BMSCs, FN and E-CDH coated 96-well microplates. **(C)** Transwell migration (8μm pores, Costar) of Calcein-AM *ITGB7*^{positive} (scrambled^{shRNA}) versus *ITGB7*^{silenced} (ITGB7^{shRNA2}) versus ITGB7^{shRNA2} + pLOC$^{ITGB7\ WT}$ versus ITGB7^{shRNA2} + pLOC^{ITGB7mut} H929 and MM1S cells to RPMI serum free media (cnt) or RPMI with SDF-1α (10 nM). The fluorescence values, quantitated in a fluorescence multi-well plate reader using 494/517 nM filter set, % migrating cells to SDF-1α versus RPMI serum free media (cnt) are shown. **(D)** *ITGB7*^{positive} (scrambled^{shRNA}) or *ITGB7*^{silenced} (ITGB7^{shRNA2}) or ITGB7^{shRNA2} + pLOC$^{ITGB7\ WT}$ versus ITGB7^{shRNA2} + pLOC^{ITGB7mut} H929 and MM1S cells were added into 96 well-plates coated with BMSCs or uncoated plates and incubated at 37°C for 48 hours. Supernatant was collected and assayed for VEGF by ELISA based assay. Shown results in panels A, B, C and D represent means (± SD) of triplicate experiments (*indicates $p<0.05$ in ITGB7^{shRNA2} + pLOC^{ITGB7mut} versus ITGB7^{shRNA2} + pLOC$^{ITGB7\ WT}$ cells).

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ITGB7 shRNA2 + pLOC ITGB7 WT
ITGB7 shRNA2 + pLOC ITGB7 mut
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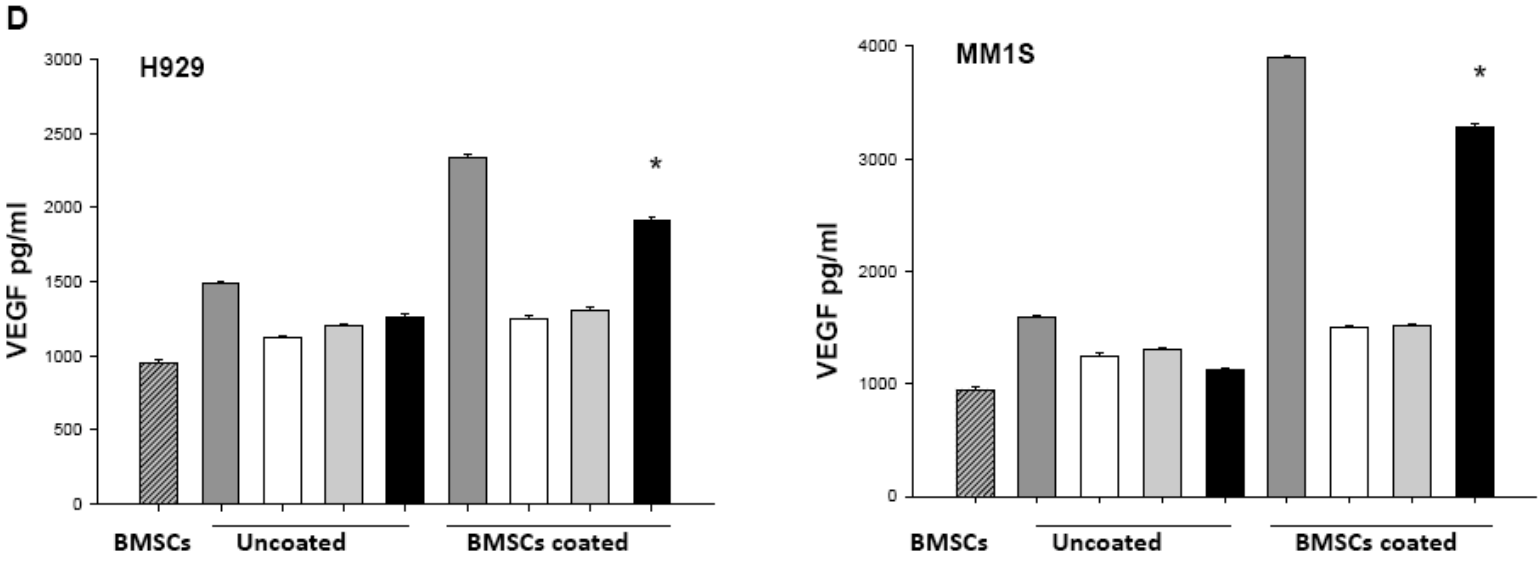
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MM1S
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2000
1000
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BMSCs
Uncoated
BMSCs coated
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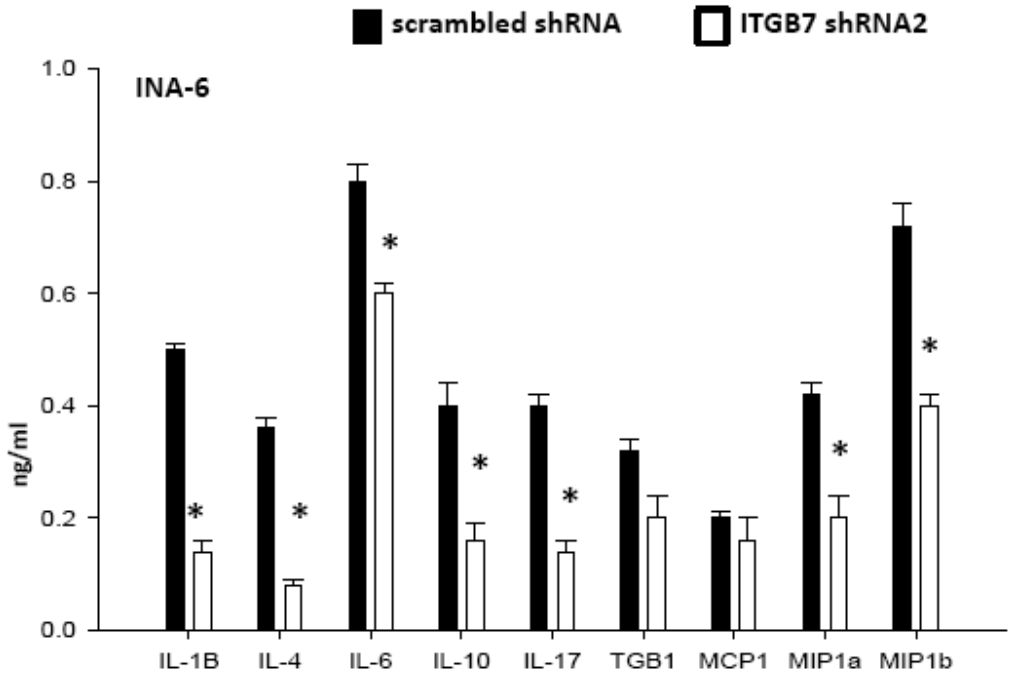
Figure S8A-C. Effects of *ITGB7* silencing on cytokines production in MM-BMSCs. *ITGB7*^{silenced} (ITGB7 shRNA2) versus *ITGB7*^{positive} (scrambled shRNA) in INA-6, H929 and MM1S cells were added into 96 well-plates coated with BMSCs or uncoated plates and incubated at 37°C for 48 hours. Supernatant was collected and assayed for multi-cytokines secretion by ELISA based assay. Data is presented as the mean ± SD of triplicates from two independent experiments. (*p<0.05)

A.



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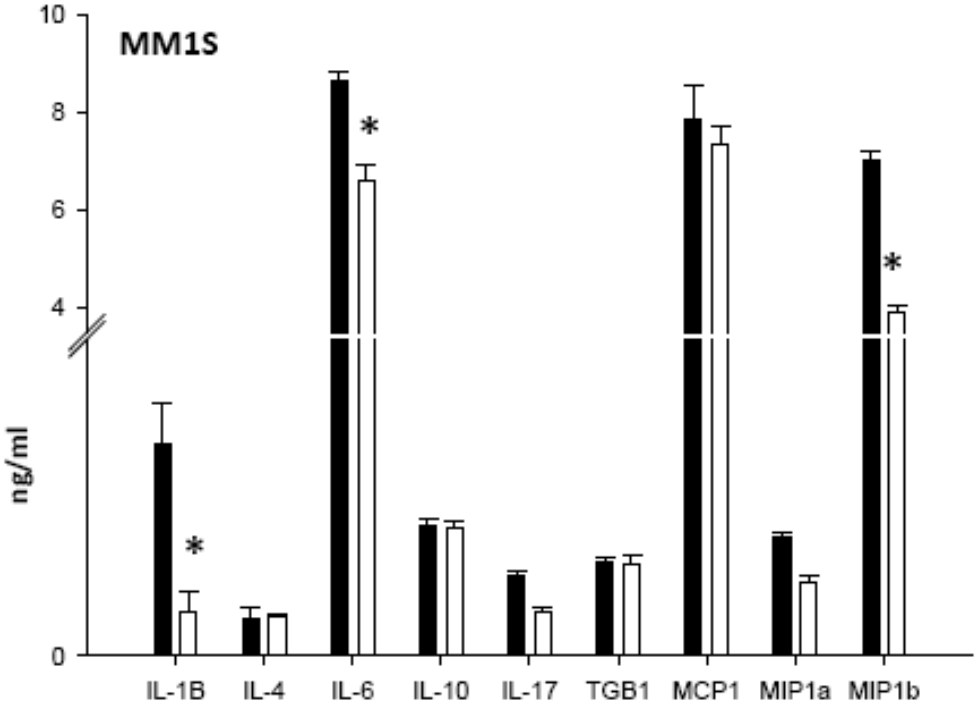

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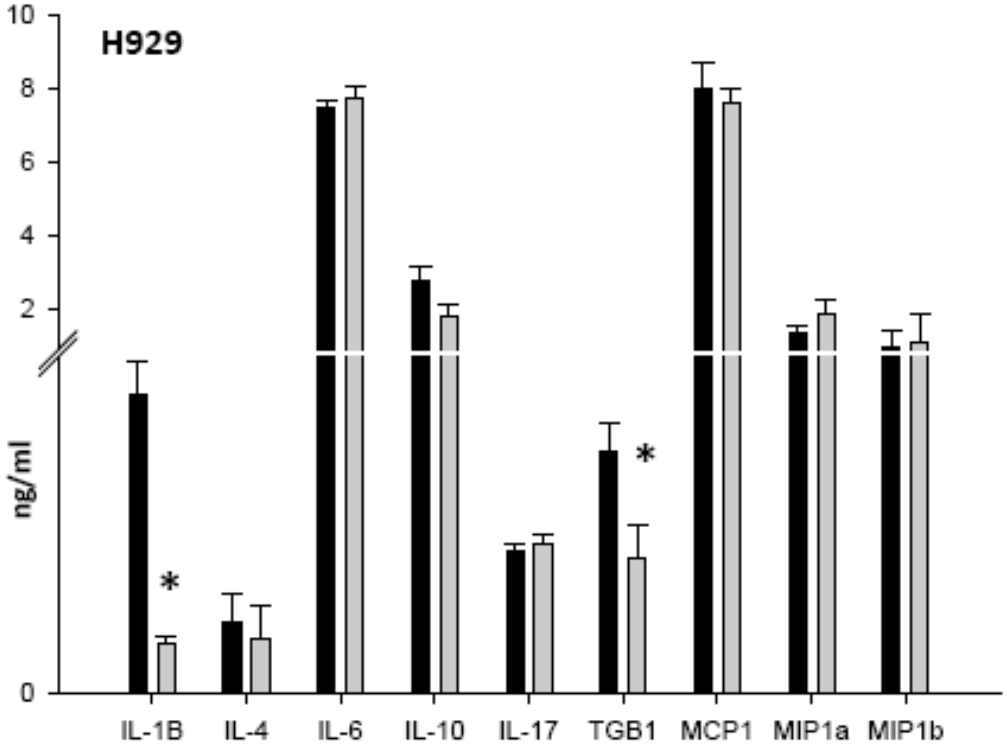
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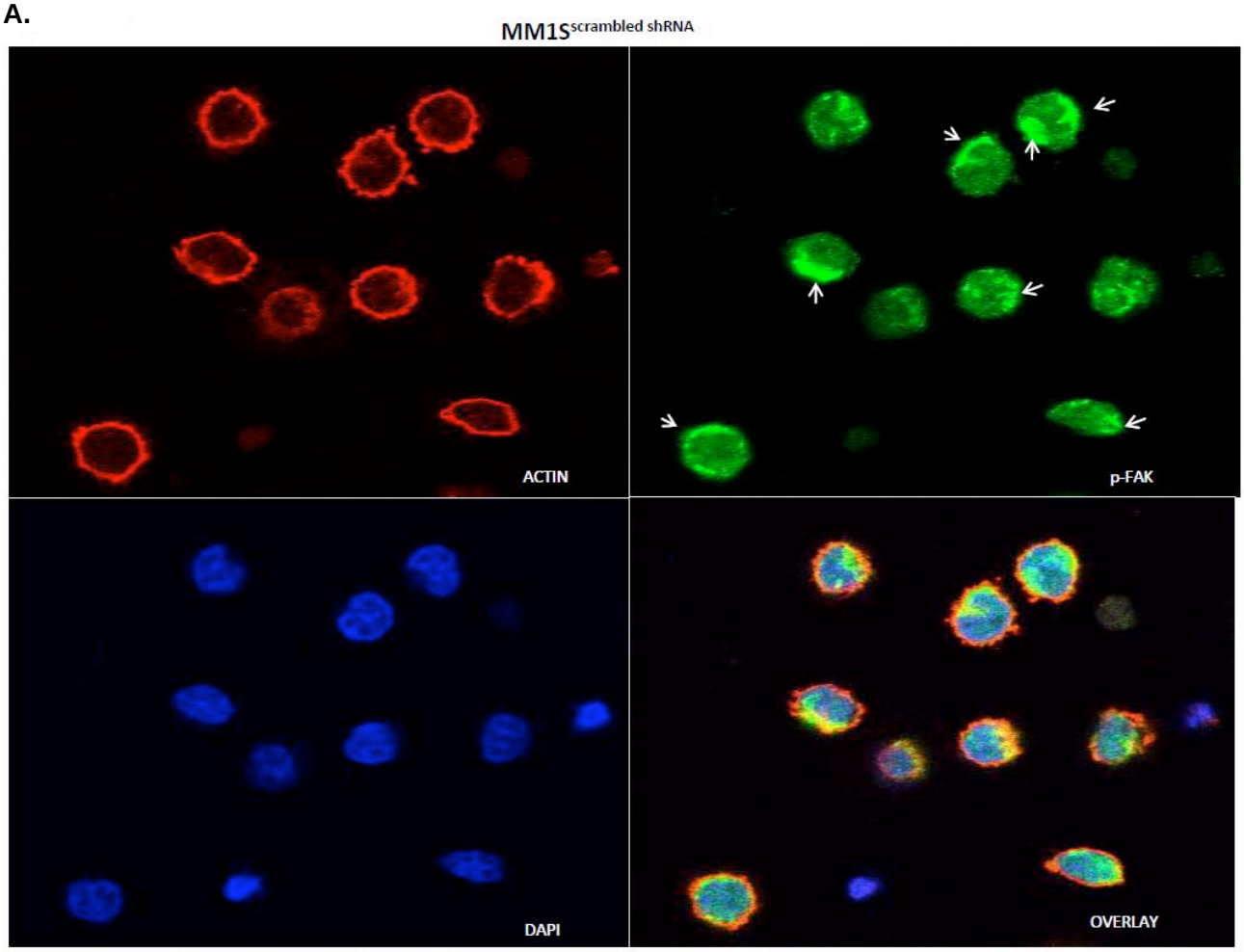
IL-4
IL-6
IL-10
IL-17
TGB1
MCP1
MIP1a
MIP1b

Figure S9A-C. FAK activation in MM1S cells. p-FAK localization and distribution in *ITGB7*^{positive} (A) and *ITGB7*^{silenced} (B) MM1S cells cultures on FN coated plates (A-B) or poly-Lysine coated plates (C) and measured by laser scan confocal microscopy. Shown are the images of immunostaining with anti-Actin (Red: Alexa-Fluoro-555 phalloidin), anti-phospho-FAK (Green: Alexa-Fluoro-488) and 4',6-diamino-2-phenylindole-dihydrochloride (DAPI) with overlay images.

A.



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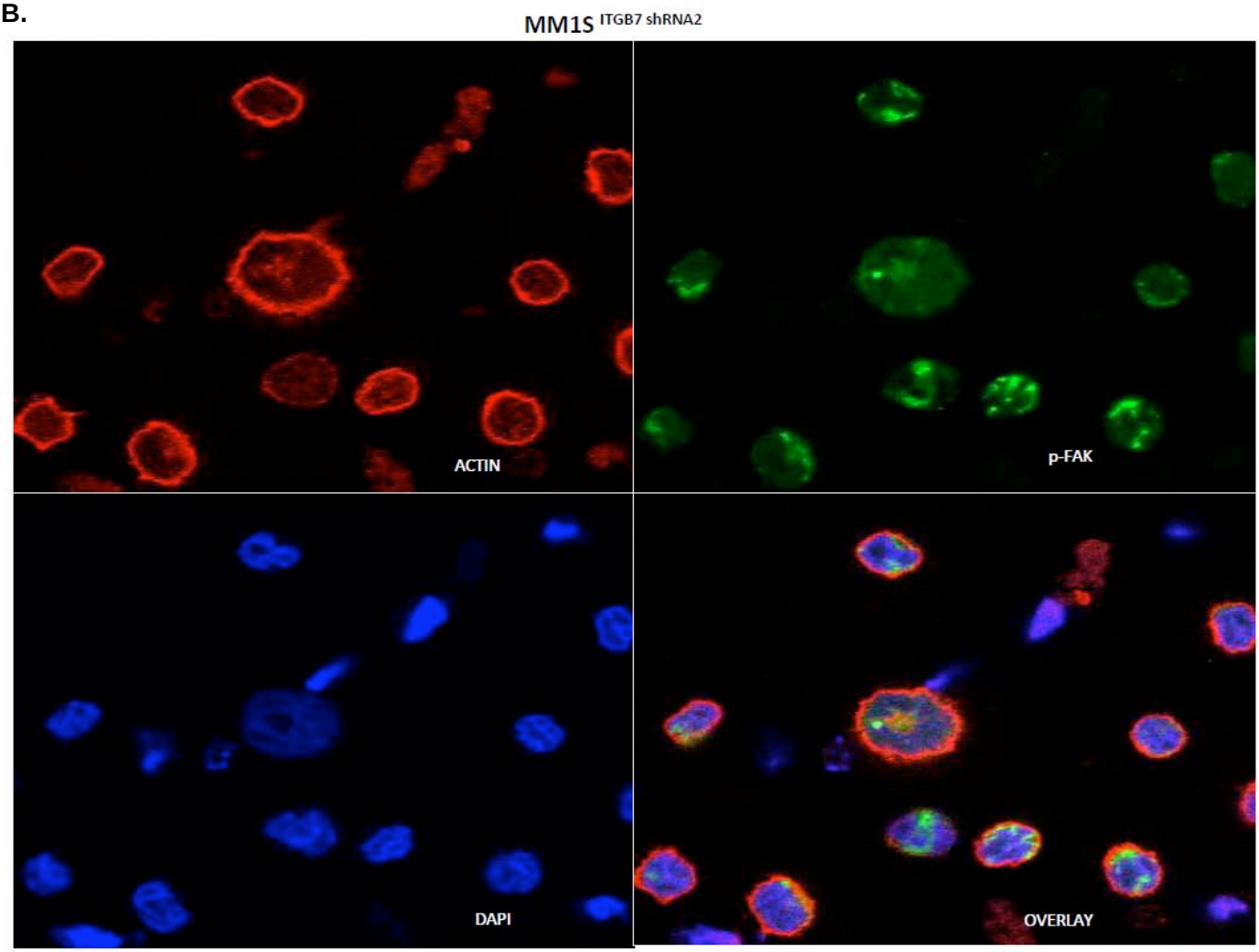

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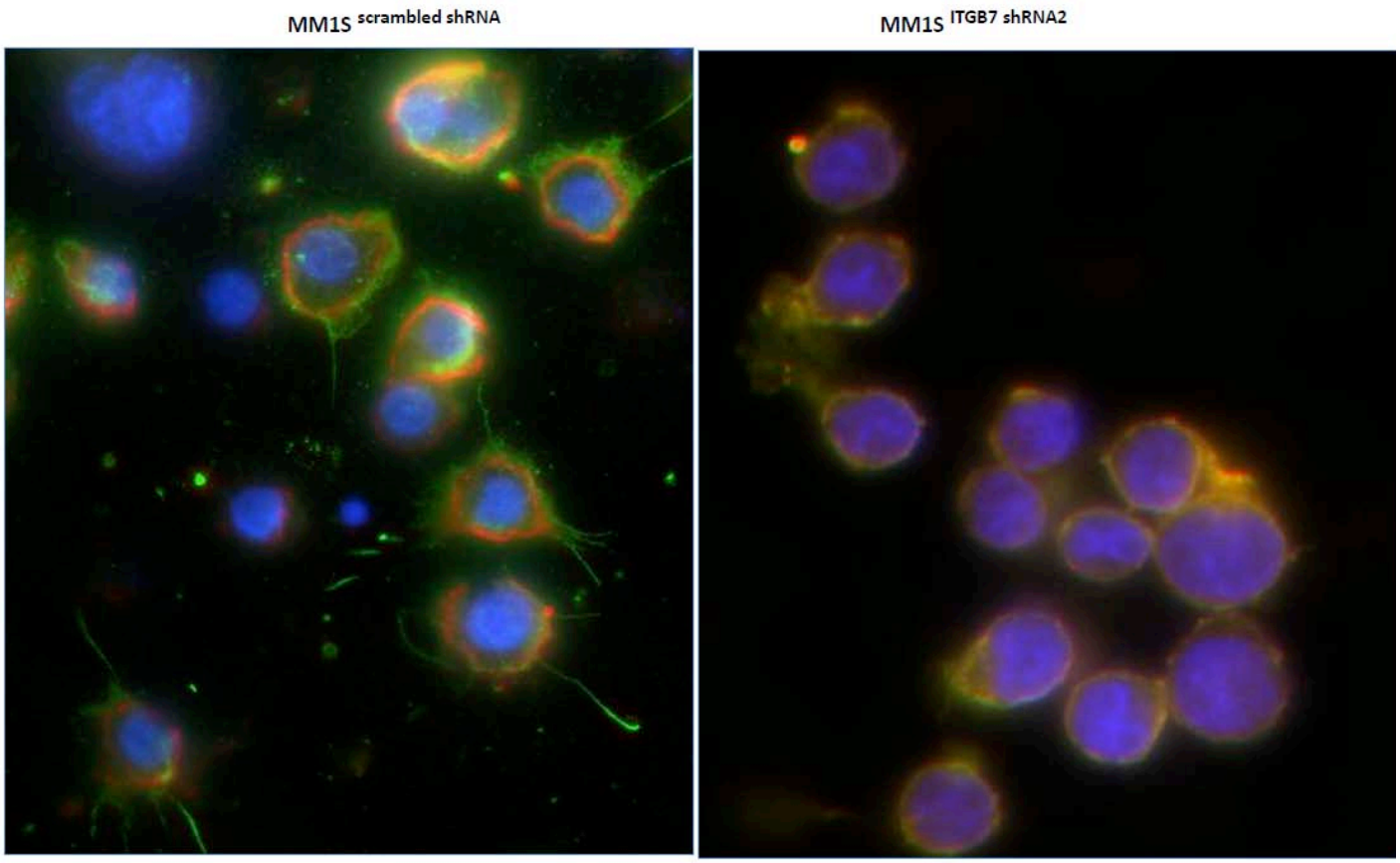

Figure S10. Rac1 activation and SUMOylation in MM1S cells. Pulldowns of activated Rac1 (GTP-Rac1 bound to GST-PAK1 beads) from MM1S *ITGB7*^{silenced} and *ITGB7*^{positive} cells cultured on FN were blotted with anti-SUMO1 antibody (middle blot) and reprobed for Rac1 (right). Also shown a western blot for total Rac1 in 10% of the imput lysate used for IP reaction (left) with α-tubulin loading control. GTP-Rac1-nS1 indicates the up-shifted band of Rac1 corresponding to SUMOylated Rac1.