Supplementary Figures

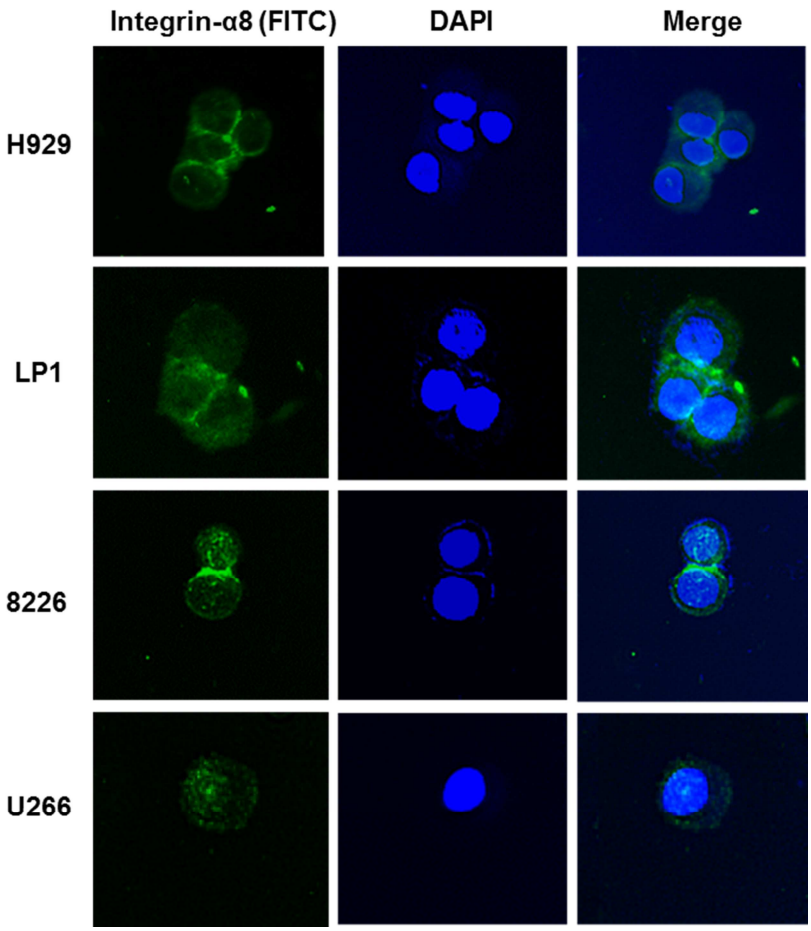

Figure S1. Immunofluorescence analysis of integrin-α8 in MM cells. Immunofluorescence staining of cytospin preparations of MM cells (H929, LP1, 8226, and U266) using primary antibody against integrin-α8 (green). Nuclei localization was observed by DAPI (blue) staining. Images are shown at x100.

Table S1. Genes up-regulated (≥2 fold) in PFS <12months group

Gene symbol	Fold change
HS.524873	23.87
LOC649923	9.98
MKX	8.94
FAM149A	8.68
LOC100132893	8.45
TMEM22	7.89
IGF1	7.76
ITGA8	7.69
GPRC5D	7.63
ISL2	6.18
FAM55D	6.17
LOC643382	5.54
C8ORF86	5.22
LOC651280	5.17
NAV2	5.15
LOC649201	5.06
C1ORF106	5.06
NBEA	5.04
MIR1236	4.88
BTLA	4.86
CYP2J2	4.49
FZD2	4.26
PTPRO	3.82
PAEP	3.69
LOC100133312	3.51
CCL3	3.50
PPARGC1A	3.48
SLC30A6	3.42
DUSP26	3.41
LOC100130100	3.39
TIGD7	3.36
FAM131C	3.34
LOC728557	3.32
NDRG4	3.23
LOC644331	3.13
KDELR3	3.00
ALG1	3.00

HS.567469	2.96
ALDOB	2.92
HIST1H2AC	2.90
LOC96610	2.87
LUZP2	2.80
FAM174A	2.75
FPGS	2.72
CD38	2.71
LDLRAD3	2.69
LOC651287	2.64
MEI1	2.63
LHB	2.60
DNAJC1	2.58
CXCL10	2.53
ABCC6	2.48
CD200	2.45
LOC644144	2.38
ZNF358	2.36
LOC100127899	2.36
HS.484967	2.35
ITGA7	2.34
DLG1	2.33
LOC642859	2.32
HS.499716	2.32
TSKU	2.31
HS.528873	2.28
TXNDC5	2.25
C14ORF124	2.25
HS.555255	2.21
MFAP5	2.20
CBWD5	2.18
ZNF460	2.18
ITM2A	2.17
LOC730051	2.12
SNORA71C	2.12
SLC35A5	2.10
SLC35B3	2.09
HS.26306	2.06
ECOP	2.06
LOC641849	2.06
CYP4V2	2.05

ARHGDIG	2.05
LOC349114	2.03
LOC647588	2.03
LOC727951	2.03
PDGFB	2.02
MAGEB1	2.00
PSPH	2.00

The table showed that the up-regulated genes in <12 months PFS group. Gene expression value was divided to the >12 months PFS group compared to control (Fold change ≥ 2).

Table S2. Biologic pathways associated in PFS > 12months group

Pathway name	Pathway Gene No.	No. of input genes in pathway	No. of pathway genes on chip	Genes	Corrected *P*-value	Impact factor	Corrected gamma *P*-value
Focal adhesion	203	4	199	PDGFB, IGF1, ITGA8, ITGA7	0.002	6.018	0.017
Glioma	65	2	65	PDGFB, IGF1	0.015	4.23	0.076
Melanoma	71	2	71	PDGFB, IGF1	0.017	4.063	0.087
Regulation of actin cytoskeleton	217	3	207	PDGFB, ITGA8, ITGA7	0.021	3.873	0.101
ECM-receptor interaction	84	2	82	ITGA8, ITGA7	0.023	3.793	0.108
Prostate cancer	90	2	89	PDGFB, IGF1	0.026	3.641	0.122
Toll-like receptor signaling pathway	102	2	102	CXCL10, CCL3	0.034	3.39	0.148
Cytokine-cytokine receptor interaction	263	3	259	PDGFB, CXCL10, CCL3	0.037	3.3	0.159

Pathways listed are the result of over two fold up-regulated genes (85genes) input, determined by Pathway-Express corrected *P*-value or corrected gamma *P*-value ($P < 0.05$). Corrected *P*-value was obtained using the classical statistics, and corrected gamma *P*-value was calculated using the impact analysis.

Table S3. Genes (≥2 fold) in early group with extreme PFS

Gene symbol	Fold change
LOC649923	30.16
MKX	18.72
LOC728253	16.67
LOC652869	16.62
ISL2	15.71
NBEA	10.46
LOC100131471	10.37
ST7L	9.68
CTNNA2	9.68
SIRT3	9.52
APOLD1	9.31
ALDOB	9.12
FZD2	8.99
ITGA8	8.18
TMEM174	7.55
LOC100128781	7.37
IFT80	6.61
FAM27A	6.05
NOC2L	5.98
LOC653119	5.85
CAMK2D	5.68
CAMSAP1L1	5.67
LOC646246	5.26
QPCTL	5.11
APBA2BP	5.03
SNRP70	5.02
DERL3	4.87
LOC653717	4.69
LOC650037	4.55
SLAMF7	4.55
PDGFB	4.54
EPPB9	4.50
RUFY2	4.49
LOC642859	4.41
ARHGEF12	4.32
LOC100134444	4.00
GPR89A	3.94

PSPH	3.83
BPNT1	3.80
ZNF611	3.73
LOC646746	3.66
MEI1	3.65
CCDC151	3.63
LOC642732	3.63
LOC728417	3.58
B9D1	3.56
WFS1	3.55
COL4A2	3.53
SLC39A10	3.51
OSGEPL1	3.44
LOC730153	3.43
HS.26306	3.43
TIGD7	3.35
PTPRO	3.35
HS.499716	3.30
HS.88156	3.30
CCDC110	3.28
LRFN3	3.25
ZKSCAN1	3.23
UNC13B	3.21
RPH3A	3.21
C11ORF24	3.18
TBC1D3C	3.18
SLC35A5	3.16
NAT8B	3.09
TBC1D9	3.06
SNX25	3.05
LOC100132948	3.04
LOC647436	3.02
ASNS	3.02
PCK2	3.02
GALK2	3.01
SLC1A5	2.99
ZNF804A	2.97
FAM164A	2.95
ITGA7	2.95
FKBP11	2.94
KIFAP3	2.91

ANKRD37	2.89
FAM13A	2.88
MFSD3	2.87
HM13	2.85
LOC729926	2.85
WNT10A	2.76
SLC6A9	2.75
NETO2	2.74
EXD2	2.73
CHRNB4	2.72
PRRT1	2.66
POMC	2.65
OR7E156P	2.64
SERPINA7	2.62
MAN2A1	2.62
C11ORF1	2.62
ALG5	2.59
ALDH1B1	2.57
PKDCC	2.57
SLC35B3	2.57
STARD5	2.55
LOC729349	2.55
USO1	2.55
SH2B2	2.55
ELMOD2	2.54
ZBTB42	2.53
FLRT2	2.51
RPLP0	2.50
SAR1B	2.50
CCDC51	2.45
LOC642897	2.45
ZNF358	2.45
ICAM2	2.40
MCEE	2.40
LCAT	2.40
MGAT2	2.39
PCMTD1	2.39
ICAM4	2.38
ZNF566	2.37
MC1R	2.36
C9ORF23	2.36

HS.336643	2.32
PREB	2.32
LOC728139	2.32
TMEM192	2.31
C2ORF42	2.28
SEC62	2.27
PHLPP2	2.27
DLG1	2.27
NFXL1	2.26
HS.128709	2.25
RSPRY1	2.24
SLC25A4	2.24
ZBP1	2.23
KIAA0114	2.22
METTL7A	2.21
LOC646463	2.19
DPM2	2.19
MARS	2.19
DNAJC19	2.18
C6ORF192	2.18
DNAJC24	2.17
PAAF1	2.16
CCS	2.16
MFSD6	2.16
GOT1	2.16
SLC38A9	2.14
SIGMAR1	2.14
SHMT2	2.13
SLC25A23	2.12
FLJ21986	2.11
LARP1B	2.09
MRPS21	2.08
SPHAR	2.08
METTL3	2.06
LOC390364	2.05
LOC641710	2.05
MRPS31	2.03
PIGF	2.03
PFKM	2.02
CLDN15	2.02
LOC100128337	2.02

PTGES2	2.01
FGD2	2.00
CYP4V2	2.00

The table showed that the up-regulated genes in early relapsed group. Gene expression value was divided to the late group compared to the control (Fold change ≥ 2).

Table S4. Biologic pathways associated with early relapsed group

Pathway name	Pathway Gene No.	No. of input genes in pathway	No. of pathway genes on chip	Genes	Corrected *P*-value	Impact factor	Corrected gamma *P*-value
Melanogenesis	102	5	102	CAMK2D, WNT10A, FZD2, MC1R, POMC	0.002	6.018	0.017
Insulin signaling pathway	138	4	137	PCK2, SH2B2, RHOQ, PFKM	0.015	4.23	0.076
ECM-receptor interaction	84	3	82	ITGA8, COL4A2, ITGA7	0.017	4.063	0.087
Focal adhesion	203	4	199	PDGFB, ITGA8, COL4A2, ITGA7	0.021	3.873	0.101
Regulation of actin cytoskeleton	217	4	207	PDGFB, ITGA8, ITGA7, ARHGEF12	0.023	3.793	0.108
Basal cell carcinoma	55	2	55	WNT10A, FZD2	0.026	3.641	0.122

Pathways listed are the result of over two fold up-regulated genes (163 genes) input, determined by Pathway-Express corrected *P*-value or corrected gamma *P*-value ($P < 0.05$). Corrected *P*-value was obtained using the classical statistics, and corrected gamma *P*-value was calculated using the impact analysis.

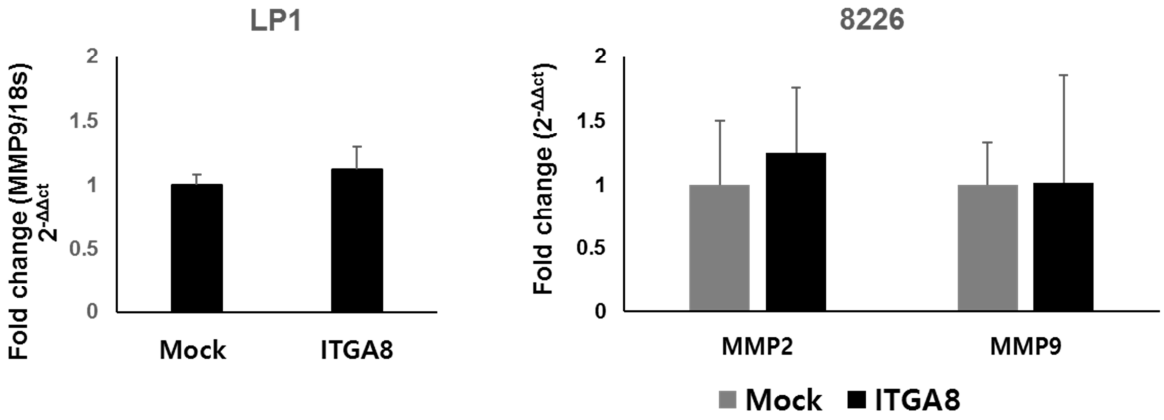

Figure S2. **The integrin-α8 effect on MMP2 and MMP9 mRNA expression.** MMP2 and MMP9 mRNA levels were measured in integrin-α8 overexpressed LP1 and 8226 cells by qRT-PCR. The relative levels of mRNA were normalized to 18s transcript and the data shown are the mean relative fold changes ±SD of three independent experiments compared to the control. Gene expression values in the samples were divided by those in the controls (i.e. each gene in the control is '1'). The sequences of the primers were MMP2: F 5'-aagtatggcttctgccctga-3', R '5-atttgttgcccaggaaagtg-3'; MMP9: F 5'-catcgtcatccagtttggtg-3', R 5'-agggaccacaactcgtcatc-3'.

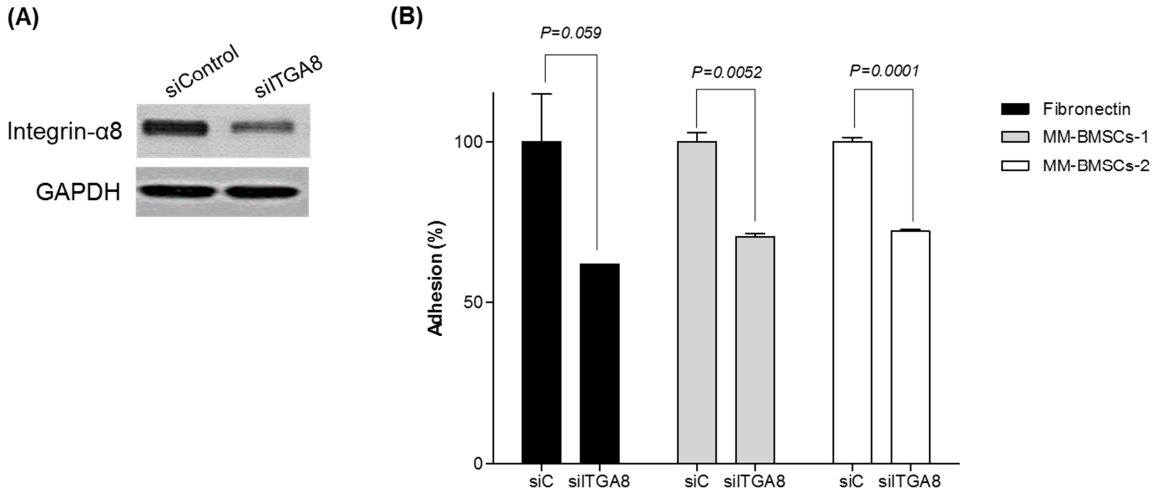

Figure S3. Effect of integrin-α8 silencing on MM cells adhesion. (A) Integrin-α8 silencing using siITGA8 or nontargeting siRNA was transfected in LP1 cells and confirmed with western blot analyses. GAPDH was used as a loading control. (B) Adhesion of integrin-α8 silenced LP1 and RPMI 8266 cells to FN or MM patient isolated BMSCs coated 96-well microplates. Unattached cells were washed out, after which adherent cells were determined based on the O.D. value. Data are presented as percentage of respective controls (mean ± SD of triplicates from 3 independent experiments).

Supplementary Materials and Methods

Immunostaining

H929, LP1, U266, and 8226 cell suspension ($4x10^3$cells/cytospin) were prepared for cytospins and centrifuged at 800 rpm for 4 minutes. MM cells on the slides were fixed for 30 minutes in 2% formaldehyde-PBS and blocked with the blocking buffer (4% BSA in PBS) for 30 minutes. Primary mouse antibody against integrin-α8 (Santa Cruz Biotechnology, Dallas, TX, USA) was incubated for 30 minutes and cells were washed for 3 times with washing buffer (0.1% tween20 in PBS) followed by goat anti-mouse IgG alexa 488 (Invitrogen, Carlsbad, CA, USA). The images were visualized by IN Cell Analyzer 2000 (GE Healthcare, Pittsburgh, PA, USA).

Small-interfering RNA (siRNA) transfection

Transfection with Oligonucleotide siRNA for ITGA8 or nontargeting control (Genolution, Seoul, Korea) was performed using Lipofectamine RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. siITGA8 sequence was S 5' CACCUUAUGCUCAUAAUAAUU 3' and AS 5' UUAUUAUGAGCAUAAGGUGUU 3'. The nontargeting siControl sequence was S 5' CCUCGUGCCGUUCCAUCAGGUAGUU3'and AS 5' CUACCUGAUGGAACGGCACGAGGUU 3'.

Cell adhesion assay

ITGA8 silenced LP1 (5×10^6/mL) or control were loaded into human fibronectin (Millipore, Germany) or MM patient derived BMSCs coated 96-well plates and incubated for 2–4 hours at 37℃. Non-adherent cells were washed out with se rum free IMDM 1640 media three times, after which 10 μl of CCK8 (Dojindo, Japan) was added into each well and incubated for 4 hours. The adhered cells were detected at 450 nm using a microplate reader (Becton Dickinson Labware, France).