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Reagents

Cell culture media, penicillin and streptomycin (pen/strep), Lipofectamine2000 reagent, DTT, 1 Kb plus DNA Ladder, and all DNA primers and oligonucleotides (oligos) were from Invitrogen. FCS was from Atlanta Biologicals (S12450). Ampicillin, lysozyme, ascorbic acid (A4403), polybrene (107689), Flag-M2 Ab (F1804) and β-actin Ab (A5441) were from Sigma-Aldrich. Myc-Tag (9B11) and Histone 3 (H3) (9715) Abs were from Cell Signaling. β-glycerophosphate (sc-203323) and Rabbit polyclonal Abs to *c*-Myc (9E10, sc-40) and RNA Pol II (sc-899) were from Santa Cruz Biotechnology, Inc. ChIP Abs for Ser2P CTD (61083), H3K9ac (61251), H3K36me3 (61101), H3K4me3 (39915), H3K27me3 (39155), HDAC1 (40967), EZH2 **(**39901) were from Active Motif. GFI1 (ab21061) and LSD1 (ab17721) Abs were from Abcam. Secondary HRP-conjugated Abs anti-rabbit IgG (W4011) and anti-mouse IgG (W402B) were from Promega, as well as GoTaq Flexi DNA polymerase. TRIzol reagent (10296028) was from Life Technologies. All restriction enzymes were from New England Biolabs. Rapid DNA Ligation Kit was from Roche Applied Science. EndoFree Plasmid Maxi Kit and QIAquick Gel Extraction Kit were from Qiagen. Protein Marker (#161-0375) and protein concentration kit was from Bio-Rad. Mouse recombinant TNFα (410-MT) was from R&D Systems. Rabbit polyclonal Abs to *c*-Myc *(*9E10, sc-40), TFIID (TBP, sc-4000), MIZ-1 (B-10, sc-136985), FOXO1 (H-130, sc-67140), p53 (DO-1, sc-126), and OCT-1 (C-21, sc-232) were from Santa Cruz Biotechnology, Inc.

Cell lines and Primary murine BMSC

HEK293 cells were maintained in DMEM. BM cells were isolated from C57BL/6 mice femurs and tibia. Animal studies were approved by the IACUC at the VA Pittsburgh Healthcare System. After overnight incubation, the non-adherent cells were removed and the remaining stromal cell population was washed with PBS and maintained in ascorbic acid-free αMEM-10% FCS, 1% pen/strep proliferation media. BMSC were expanded for 2.5 weeks to reach optimal confluence. Co-cultures with MM cells or cytokine treatments and RNA preparation analyses were conducted as described for MC4 cells. Relative mRNA levels were calculated using the ΔΔCt method using *18S rRNA* for normalization. The *q*PCR primers are listed in Table S1.

DNAs

The -992/+111 m*Runx2-P1*-pGL2 was provided by Dr. Patricia F. Ducy (Columbia University), mGFI1-wt-pCDNA3.1 was provided by Dr. H. Leighton Grimes (Cincinnati Children's Hospital Medical Center), and the pGL4.10[luc2] reporter and pRL-TK plasmids were from Promega. Empty pGL4.10[luc2] basal activity and pRL-TK were not responsive to GFI1 co-transfection. To construct the various m*Runx2 P1* promoter-Luc reporter plasmids, the different DNA fragments of the m*Runx2* promoter were amplified from -992/+111 m*Runx2*-pGL2 using the DNA primers listed in Table S6, digested with XhoI/BglII and subcloned into Xho1/BglII cut pGL4.10[luc2] vectors. To generate the deletion or mutation constructs of the -974/+111 m*Runx2* promoter-pGL4.10[luc2], the PCR-based QuikChange II XL site-directed mutagenesis kit (Agilent Technologies) with the oligos listed in Table S7 was used to create the deletions Δ-108/-44 and Δ-37/-7 and the site-directed mutations in GFI1 site AAGCTT (L mutant **GG**GCTT, R mutant AAGC**CC**, and LR mutant **GG**GC**CC)**. Restriction enzymes were used to digest mGFI1 cDNA fragments and the p3xFlag-CMV10 (Sigma-Aldrich) or pCS2+MT (6x

Myc tag) vectors as noted in Table S8 before ligation to generate: mGFI1 cDNA encoding 1-423 aa in both vectors as well as 1-380, 239-423, 291-423, 341-423 aa in pCS2-MT. All constructs were verified by DNA sequencing.

Transfection of *Runx2 P1* promoter-Luc reporters and GFI1 constructs

The m*Runx2 P1* promoter-reporters (250 ng) were transfected into MC4 cells in 6-well plates with Lipofectamine2000, along with empty (EV) or wt mGFI1 expression vectors (250 ng) and pRL-TK (10 ng; Promega) as indicated in Figure legends. In some cases, the transfected cells were treated with TNFα. Harvested cells (48 h) were lysed in 1X Cell Lysis Buffer (Promega), and 20 μl supernatant was used to measure the Luc and renilla activities using the Dual-Luciferase® Reporter Assay System (Promega) in a Turner Biosystem Modulus Luminometer (Model 9300-010) or a Promega GloMax® 96 Microplate Luminometer w/Dual Injectors. The normalized (to renilla) relative Luc activities for each reporter construct were calculated as a percent of the activity of the -974/+111 m*Runx2*-pGL4.10[luc2]-wt co-transfected with EV. Transfections of Myc-mGFI1-wt and Myc-mGFI1-deletions for endogenous *Runx2* mRNA and ChIP analyses were carried out in 35-mm dishes using FugeneHD (E2311, Promega) according to the manufacturer instructions.

Protein lysates and Western blotting

SCR or ShGfi1 transduced MC4 cell cultures were treated with 1X lysis buffer (Cell Signaling) to make whole cell lysates, which were examined by western blotting with anti-GFI1 and anti-β-actin primary antibodies and analyzed as in the main text.

Whole cell extracts (WCE) for DNA binding studies

HEK293 cells transfected (48 h) with Flag-tagged or myc-tagged mGFI1-WT vectors were lysed in HKMG buffer [10 mM Hepes (pH 7.9), 100 mM KCl, 5 mM $MgCl_2$, 10% glycerol, 0.5 mM $ZnSO_4$, 1 mM DTT, 1% Nonidet P-40] to generate WCE.

Biotinylated oligo (B-oligo) pull-down assay

Non-biotinylated oligos or B-oligos from various m*Runx2* promoter regions as described in Table S9 were annealed to form double-stranded (ds) DNA. HEK293 WCE (50-300 μg), in vitro transcription/translation protein (10 μl reaction) or GST-fusion protein (10 μg) were incubated in 1 ml HKMG buffer at 4°C with 0.5 μg ds B-oligos plus 20-fold excess poly(dI-dC) (Sigma-Aldrich) for one h. In competition assays, non-biotinylated ds oligos were also added as indicated. Proteins were incubated for another hour with high capacity streptavidin agarose resin (Thermo Scientific) which was pre-blocked with HKMG buffer containing 100 μg/ml salmon sperm DNA, 100 μg/ml BSA and excess poly(dI-dC) overnight. Beads with proteins bound to B-oligo DNA were washed 6 times with HKMG buffer and the DNA-bound proteins were separated on 10% PAGE for IB with Abs against GFI1, Flag or myc.

Preparation of nuclear extracts from MC4 cells

MC4 cells untreated or TNFα-treated were collected, rinsed once with PBS, resuspended in 200 μl of cold buffer A [10 mM Hepes (pH 7.9); 10 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA; 1 mM DTT; 0.5 mM PMSF] and incubated on ice for 10 min before addition of 11 μl of 10% Nonidet P-40 (final concentration 0.5%) and vortexing (15 s vigorously 2 times with one min interval). After high-speed centrifugation at 4°C, the pellets were resuspended in 100 μl of

ice-cold buffer C [20 mM Hepes (pH 7.9); 400 mM KCl; 1 mM EDTA; 1 mM EGTA; 1 mM DTT; 1 mM PMSF]. Samples were placed on ice and subjected to vortexing for 15 s every 10 min over a period of 40 min. Finally, samples were centrifuged 10 min at 14,000 rpm, and the NE supernatant was collected and stored at -80°C.

EMSA

EMSA reactions were performed for 30 min at room temperature in binding buffer [10 mM Tris-HCl (pH 7.5), 75 mM NaCl, 0.5 mM ZnSO4, 1 mM EDTA, 0.5 mM DTT, 1 μg poly(dI-dC), 10% glycerol], 20 μg nuclear protein and 10 fmol *mRunx2* promoter ds B-oligos. As indicated, non-biotinylated oligos were added for competition or Abs were added to super-shift. The samples were run on 4% non-denaturing acrylamide gels in 0.5x TBE. DNA-protein complexes were transferred onto nylon membranes (Thermo Scientific) at 5 volts overnight and B-oligo DNA-protein complexes were probed with HRP-conjugated streptavidin (#20148 Thermo Scientific).

Table S1. *q*PCR primers for mRNA analysis

Gene	Forward primer (5′->3′)	Reverse primer (5′->3′)
m***Runx2***	CCTCTGACTTCTGCCTCTGG	ATGAAATGCTTGGGAACTGC
m***Gfi1***	CACACCTTCATCCACACAGG	CACACAGGTCACAGCCAAAG
m***Il6***	CAAAGCCAGAGTCCTTCAGA	GCCACTCCTTCTGTGACTCC
m***18srRNA***	GAGCGACCAAAGGAACCATA	CGCTTCCTTACCTGGTTGAT
m***Ocn***	TAGTGAACAGACTCCGGCGCTA	TGTAGGCGGTCTTCAAGCCAT
m***Alpl***	CACGGCCATCCTATATGGTAA	CTGGGCCTGGTAGTTGTTGT
m***Bsp***	AAGAAGAGGAAGAGGAAGAAAATGA	GCTTCTTCTCCGTTGTCTCC

Table S2. Murine and human *Runx2* ChIP primers

ChIP Amplicons*	Forward (5′->3′)	Reverse (5′->3′)
1 (-670)	AAGGCAAACAGAAGGAAGCA	TGCTGCTTTGCAGTAATTCG
2 (-366)	GGCTCCTTCAGCATTTGTGT	TCCCTTTCTCCCTCTGACAA
3 (-36)	TGAGGTCACAAACCACATGA	TGAAGCATTCACACAATCCAA
4 (+51)	CTTCATTCGCCTCACAAACA	TAAACGCCAGAGCCTTCTTG
5 (+150)	CGTTTTGTTTTGTTTCCTTGC	CCCAGTCCCTGTTTTAGTTG
6 (+363)	CAGGGACTGGGTATGGTTTG	ACGCCATAGTCCCTCCTTTT
7 (+33130)	AGGTAGCCCAGCAAAAACCT	CCCCTCTGTGAGCCAAAATA
8A (+78843)	ACTCTGTCCGGTCTCCAGTC	GAAAGAGGATGGAGGTGACG
8B (+79031)	TCGCTAACTTGTGGCTGTTG	GCTCACGTCGCTCATCTTG
8C (+79373)	CGACAGTCCCAACTTCCTGT	TGGGGGTGACCAGTCTCTTA
9 (+89651)	CAGATGCTTCCCTTCTGCTC	GAAGGAACAGTGGCACATCA
10 (+122904)	TCAGGAGTGTTGGCTGTGAG	TATGTCAGGGTGAGGGCTTC
11 (+141565)	GGCCCACCTATGTGTCTGTT	CAAGTGCACATGGAACCAAC
12 (+155686)	CCCAGAGCACTCAGTCTTCC	GGTCCCAAGAGTGACCTGAA
13 (+208775)	ATACCCCCTCGCTCTCTGTT	AGGTTGGAGGCACACATAGG
Human -97	GAAAGAGCAAGGGGGAAAAG	TGGAGAGGCAGAATCATGTG
Human +185	CACCGAGACCAACAGAGTCA	TGGTAACATGTGAAAAGCAAAGA
Human +66065	AAGGCCCCACCTCTAACACT	AGACAACAGGCGAGGCTAAA

*Numbers in parenthesis represent midpoints of amplicons relative to the *Runx2* transcription start site.

Table S3. MM Patient Characteristics used for H3K9ac ChIP analysis

ID	Age	Gender	Race	Newly Diagnosed	ISS Stage	Skeletal Disease
1	57	M	Unknown	?	Unknown	Y
2	58	F	White	N	Stage II	Y
3	63	M	White	N	Stage III	Y
4	68	F	White	Y	Stage III	Y
5	58	M	Native	N	Stage II	Y
6	73	F	White	N	Stage I	Y
7	50	F	Black	N	Stage III	N
8	64	M	White	N	Stage III	Y
9	83	M	White	N	Unknown	Y
10	70	M	White	N	Stage I	Y
11	65	M	White	Y	Stage III	Y
12	55	M	Unknown	Y	Stage II	N

Table S4. MM Patient Characteristics used for H3K27me3 ChIP analysis

ID	Age	Gender	Race	Newly Diagnosed	ISS Stage	Skeletal Disease
1	71	M	White	N	Unknown	N
2	67	M	White	N	Stage I	Y
3	50	M	White	N	Stage III	Y
4	57	F	White	Y	Stage I	Unknown
5	52	F	White	N	Stage II	Y
6	81	M	White	Y	Stage I	Unknown
7	66	F	White	N	Stage I	N
8	66	M	White	Y	Unknown	Y
9	66	M	White	Y	Stage II	Y
10	78	M	White	N	Stage I	Y
11	58	F	White	N	Stage II	Y
12	73	F	White	N	Stage I	Y

Table S5. MM Patient Characteristics used for Alizarin Red analyses

ID	Age	Gender	Race	Newly Diagnosed	ISS Stage	Skeletal Disease
1	60	M	White	Y	Stage I	N
2	76	M	White	Y	Stage I	Y
3	44	F	White	N	Stage I	Unknown
4	76	M	White	N	Unknown	Y
5	80	M	White	N	Stage I	Unknown

Note: patients 1 & 2 were used in Fig 6C and D, respectively.

Table S6: Primers used to clone fragments of *mRunx2* into XhoI/BglII cut pGL4.10 [Luc2] or pGL4.23 [Luc2]

5′ deletion	Primer (5′->3′)
-974 Forward	ACCG**CTCGAG**GGATGATGGCAAAAATAATGTAAACGATAC
-850 Forward	ACCG**CTCGAG**CCCCAGGCTAACACTTTTGTGAC
-700 Forward	ACCG**CTCGAG**AAGCAGCCACCCTGGGAAATCC
-476 Forward	ACCG**CTCGAG**CCTTAGCTACAGAGTTCTGCTC
-369 Forward	ACCG**CTCGAG**CTATAACCTTCTGAATGCCAGG
-250 Forward	ACCG**CTCGAG**CAGAGGAACACCCATAAGTAAAGAG
-128 Forward	ACCG**CTCGAG**GAAGCCACAGTGGTAGGCAG
-108 Forward	ACCG**CTCGAG**AGTCCCACTTTACTTTGAGTACTGTGAG
+111 Reverse	GGA**AGATCT**GCAGAGATTAACCATTTAAACGCCAGAGCC
+1 Reverse	GGA**AGATCT**CAATCCAAAAAAGCAAAAGCTTTAAAACTCC
-108 Reverse	GGA**AGATCT**GCCTACCACTGTGGCTTCCC

Table S7. Oligos to generate internal deletions or mutations in -974/+111 *mRunx2*-pGL4.10[Luc2]

change	Forward primer (5′->3′)	Reverse primer (5′->3′)
Δ-37/-7 (NdeI)	TCTCTCCAGTAATAGTGCTTGCA**TA**T GGATTGTGTGAATGCTTCATTCG	CGAATGAAGCATTCACACAATCCA**TA**TGCAA GCACTATTACTGGAGAGA
Δ-108/-44 (EcoRI)	GGGAAGCCACAGTGGTAGGCAG**AATT** CTTGCAAAAAATAGGAGTTTTAAAGC	GCTTTAAAACTCCTATTTTTTGCAAG**AATT**C TGCCTACCACTGTGGCTTCCC
Mutant left core	GTAATAGTGCTTGCAAAAAATAGGAG TTTTA**GG**GCTTTTGCTTTTTTGGATT GTGTGAATG	CATTCACACAATCCAAAAAAGCAAAAGC**CC**T AAAACTCCTATTTTTTGCAAGCACTATTAC
Mutant right core	GTAATAGTGCTTGCAAAAAATAGGAG TTTTAAAGC**CC**TTGCTTTTTTGGATT GTGTGAATG	CATTCACACAATCCAAAAAAGCAA**GG**GCAAT AAAACTCCTATTTTTTGCAAGCACTATTAC
Mutant both cores	GTAATAGTGCTTGCAAAAAATAGGAG TTTTA**GGGCCC**TTGCTTTTTTGGATT GTGTGAATG	CATTCACACAATCCAAAAAAGCAA**GGGCCC**T AAAACTCCTATTTTTTGCAAGCACTATTAC

Note: Restriction sites used for cloning are bolded; mutations or deletions (which form new restriction sites as indicated) are bolded and underlined

Table S8. Primers used to generate fragments of *mGfi1* cDNA

<table>
<tr><th>Vector</th><th>Forward (aa) primer (5′->3′)</th><th>Reverse (aa) primer (5′->3′)</th></tr>
<tr><td>p3xFlag CMV10 Not1/BamH1</td><td>(1)
ATAAGAATGCGGCCGCGATGCCGCGCTCA TTCCTGGTC</td><td>(423)
CGGGATCCTCATTTGAGTCCATGCTGA GTC</td></tr>
<tr><td rowspan="4">pCS2+MT EcoRI/XhoI</td><td>(1)
CCGGAATTCAATGCCGCGCTCATTCCTGG TCAAG</td><td>(380)
ACCGCTCGAGCTGACTGAAGGCTTTGC CGCACAC</td></tr>
<tr><td colspan="2">(239)
CCGGAATTCAAAGGTGGAGTCGGAGCTGCTTTG</td><td rowspan="3">These F primers were all used with (423) R-XhoI primer; (1) F-EcoRI primer was also was used with (423) R-XhoI primer.</td></tr>
<tr><td colspan="2">(291)
CCGGAATTCAGGCAAGACCTTCGGGCACGCGGTG</td></tr>
<tr><td colspan="2">(341)
CCGGAATTCATATCCCTGTCAGTACTGTGGCAAAAG</td></tr>
</table>

Note: Restriction sites used for cloning are bolded.

Table S9. Biotinylated *Runx2* promoter Oligos

Oligos*	**Forward (5′->3′)**	**Reverse (5′->3′)**
-108/-69	GTCCCACTTTACTTTGAGTACTGTGA GGTCACAAACCACA	TGTGGTTTGTGACCTCACAGTACTCA AAGTAAAGTGGGAC
-80/-41	TCACAAACCACATGATTCTGTCTCTC CAGTAATAGTGCTT	AAGCACTATTACTGGAGAGACAGAAT CATGTGGTTTGTGA
-62/-23	TGTCTCTCCAGTAATAGTGCTTGCAA AAAATAGGAGTTTT	AAAACTCCTATTTTTTGCAAGCACTA TTACTGGAGAGACA
Wild-type -40/-1	GCAAAAAATAGGAGTTTTAAAGCTTT TGCTTTTTTGGATT	AATCCAAAAAAGCAAAAGCTTTAAAA CTCCTATTTTTTGC
Left core mutant -40/-1	GCAAAAAATAGGAGTTTTA**GG**GCTTT TGCTTTTTTGGATT	AATCCAAAAAAGCAAAAGC**CC**TAAAA CTCCTATTTTTTGC
Right core mutant -40/-1	GCAAAAAATAGGAGTTTTAAAGC**CC**T TGCTTTTTTGGATT	AATCCAAAAAAGCAA**GG**GCTTTAAAA CTCCTATTTTTTGC
Both cores mutant-40/-1	GCAAAAAATAGGAGTTTTA**GG**GC**CC**T TGCTTTTTTGGATT	AATCCAAAAAAGCAA**GG**GC**CC**TAAAA CTCCTATTTTTTGC
-40/-19	GCAAAAAATAGGAGTTTTAAAG	CTTTAAAACTCCTATTTTTTGC
-31/-10	AGGAGTTTTAAAGCTTTTGCTT	AAGCAAAAGCTTTAAAACTCCT
-22/-1	AAAGCTTTTGCTTTTTTGGATT	AATCCAAAAAAGCAAAAGCTTT
+1/+40	GTGTGAATGCTTCATTCGCCTCACAA ACAACCACAGAACC	GGTTCTGTGGTTGTTTGTGAGGCGAA TGAAGCATTCACAC

*Bolded names were also made with the biotin label.

Table S10. Mouse *Il6* ChIP primers

Gene	Forward primer (5′->3′)	Reverse primer (5′->3′)
1 (-820)	CTGGAAATGTTTTGGGGTGT	TTCTCTGCATTCTCCCCAGT
2 (+61)	GAGCCCACCAAGAACGATAG	GTCTCAATAGCTCCGCCAGA
3 (+881)	GGTCAGCCCAGATAAGATGC	ACCATGCCCAGCCTAATCTA
4 (+3030)	TGTGAGCATCCACTTTTTGC	ATCCCATAATCAGCCACCAA
5 (+5057)	TGAGTGCTTCCCCATCTCTC	TCTGAGCAGCTGTCTGGAAA
6 (+6247)	CAGAACACGCCACAAGAAAA	GCCACTCCTTCTGTGACTCC

*Numbers in parenthesis represent midpoints of amplicons relative to the IL6 transcription start site.

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Figure S1. Kinetic assessment of *Runx2* mRNA down-regulation by MM cells, TNFα and IL7. (A) Analysis of *Runx2* mRNA steady-state levels in MC4 cells after 5TGM1 MM co-culture for 1.5, 3, 6, and 9 h in proliferation media. (**B**) Measurement of *Runx2* mRNA half-life in IL7 or TNFα treated MC4 pre-OB. Actinomycin D (2 μg/ml; Calbiochem #114666) was added at time 0 to cultures together with 5 ng of IL7 or TNFα. Samples were harvested at the indicated time points for *Runx2* mRNA analysis by *q*PCR. TNFα, but not IL7, decreased the $t_{1/2}$ of *Runx2* mRNA. The error bars represent SEM for 3 biological samples.

Figure S2. MM exposure induces the *mIl6* gene to adopt a more transcriptionally active state and increases *mIl6* mRNA levels in MC4 cells. MC4 cells cultured with or without 5TGM1 MM cells for 48 h in proliferation media were analyzed by ChIP-*q*PCR profiles for (**A**) Pol II occupancy and active chromatin marks (**B**) H3K9ac, (**C**) H3K4me3 and (**D**) H3K36me3. (**E**) Schematic representation of murine *Il6* gene with annotated amplicons used for ChIP analyses. Numbers in (**E**) represent midpoint of amplicons relative to *mIl6* TSS. Primer sequences are listed in Table S10. **(F)** *q*PCR analyses of *mIl6* mRNA levels in proliferating MC4 cells plus/minus 5TGM1 MM co-culture.

Figure S3. Identification of the functional GFI1 binding site in the *Runx2-P1* promoter. (**A**) Co-transfection of mGFI1-WT-pCDNA3.1 or EV control plasmids with m*Runx2-P1*-promoter-pGL4.10[luc2] reporter 5'-deletions as diagrammed (plus pRL-TK) into MC4 cells. The reference sample was 974/+111 *mRunx2-P1*-pGL4.10[luc2]+EV. (**B**) Analysis of GFI1-responsiveness of diagrammed internal and 3' deletions of the -974/+111 m*Runx2* promoter

reporter as in A. (**C**) The -108/-1 *mRunx2-P1* sequence (putative GFI1 recognition cores in yellow boxes) along with bars representing the B-oligos utilized below except oligo *e* (+1/+40) (see Table S9). (**D**) B-oligo pulldown screen for Flag-mGFI1-WT binding across the -108/+40 *mRunx2 P1* region as indicated using Flag-mGFI1-WT transfected HEK293 WCE. GFI1 protein was detected by Western blot with anti-Flag Ab. (**E**) Competition assays of -40/-1 B-oligo pulldown of Flag-GFI1 with the addition 0-32x unlabeled (no biotin) self [*d*; -40/-1] and non-self [*e*; +1/+40] competitor oligos, as indicated. (**F**) Competition assays done as in **E** using 40x unlabeled competitors (*d, f, g, h)* depicted in **C** as indicated, revealed that -31/-10 was sufficient for GFI1 binding. (**G**) Pulldown assays using -40/-1 B-oligos that were either wt or with mutations of the L core (AA->gg), R core (TT->cc) or both cores (LR) and Flag-mGFI1-WT transfected HEK293 WCEs. (**F**) EMSA of -40/-1 wt and LR mutant B-oligos incubated in the absence (-) or presence of NE from MC4 cells treated with TNFα (ng/ml as indicated; 48 h). In some cases, varying folds excess competitive non-biotinylated wt (lanes 13-17) or LR (lanes 20-24) oligos or Abs (lanes 27-32) for supershift were added as indicated. The B-oligo DNA-GFI1 protein complexes are indicated by an arrow and the supershifted DNA-GFI1 protein complex by an arrowhead. Experiments were all repeated at least three times and representative results are displayed here. (I) MC4 cells were transfected with wt -974/+111 m*Runx2-P1*-promoter-pGL4.10[luc2] (250 ng) plus pRL-TK (10 ng). After 6 h transfection, TNFα was added at increasing concentrations as indicated and luciferase in each sample was normalized by the renilla activity. The assay was repeated at least three times.

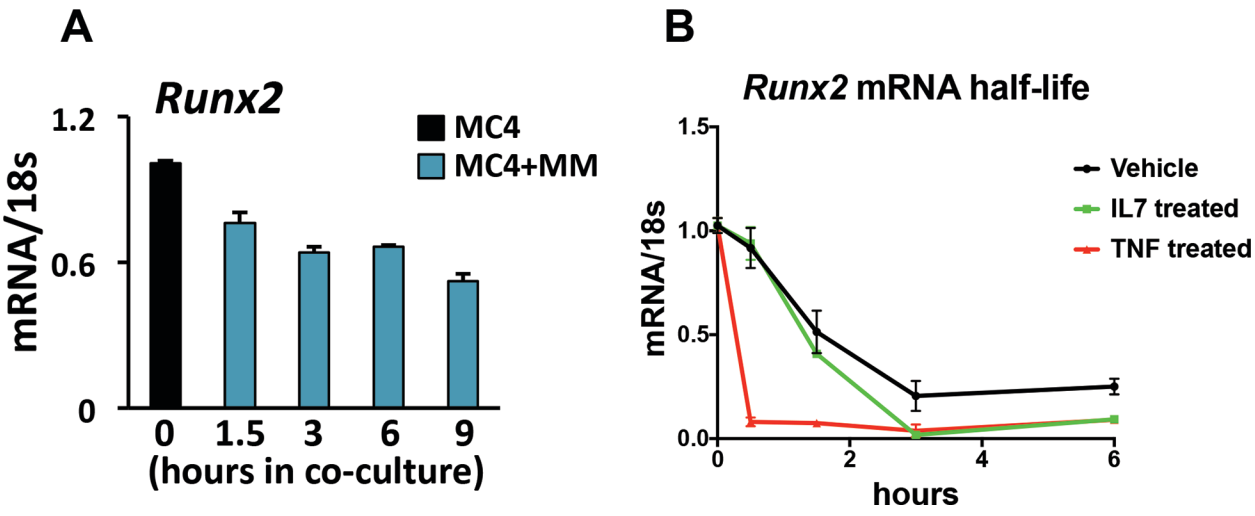
Figure S4. **GFI1 deletion protein expression and DNA binding in vitro, ectopic Myc-GFI1 effects on expression of several genes, binding along the *Runx2* gene, and GFI1 protein**

knockdown in stably transduced sh*Gfi1*-MC4 cells. (**A**) Several Myc-tagged mGFI1 constructs with deletions as depicted (top) were transfected into HEK293 cells and the resulting WCE were used to determine if the GFI1 deletions could bind biotinylated -40/-1 m*Runx2* ds-oligo (B-oligo). The proteins in the streptavidin-B-oligo inputs and pulldowns were analyzed by Western blot for GFI1 using antibody against Myc (9E10). (**B**) RNA samples from the experiment shown in Fig 3A were in which varying amounts of mGFI1-WT and EV plasmids were transfected as indicated into MC4 cells were also analyzed for *Sp7* (*Osx*), *Sp1*, *Ocn*, *Bsp, and Il6* mRNAs by *q*PCR as indicated. (**C**) Assessment of ectopic GFI1 binding along the Runx2 gene by Chip-*q*PCR using the amplicons depicted in Fig 1 and listed in Table S2. Empty vector (pCDNA3.1) or myc-mGFI1-WT was transfected into MC4 cells and ChIP using anti-myc antibody was performed to assess binding of ectopically expressed Myc-GFI1 at the *Runx2 P1* and *P2* promoters and within the structural gene. (**D**) Western blot analysis of GFI1 protein expression in control MC4 pre-OB cells, and MC4 cells stably transduced with Sh-scrambled (SCR) control and Sh*Gfi1* lentiviruses after 48 h culture with or without 5TGM1 MM exposure. β-actin was used as loading control.

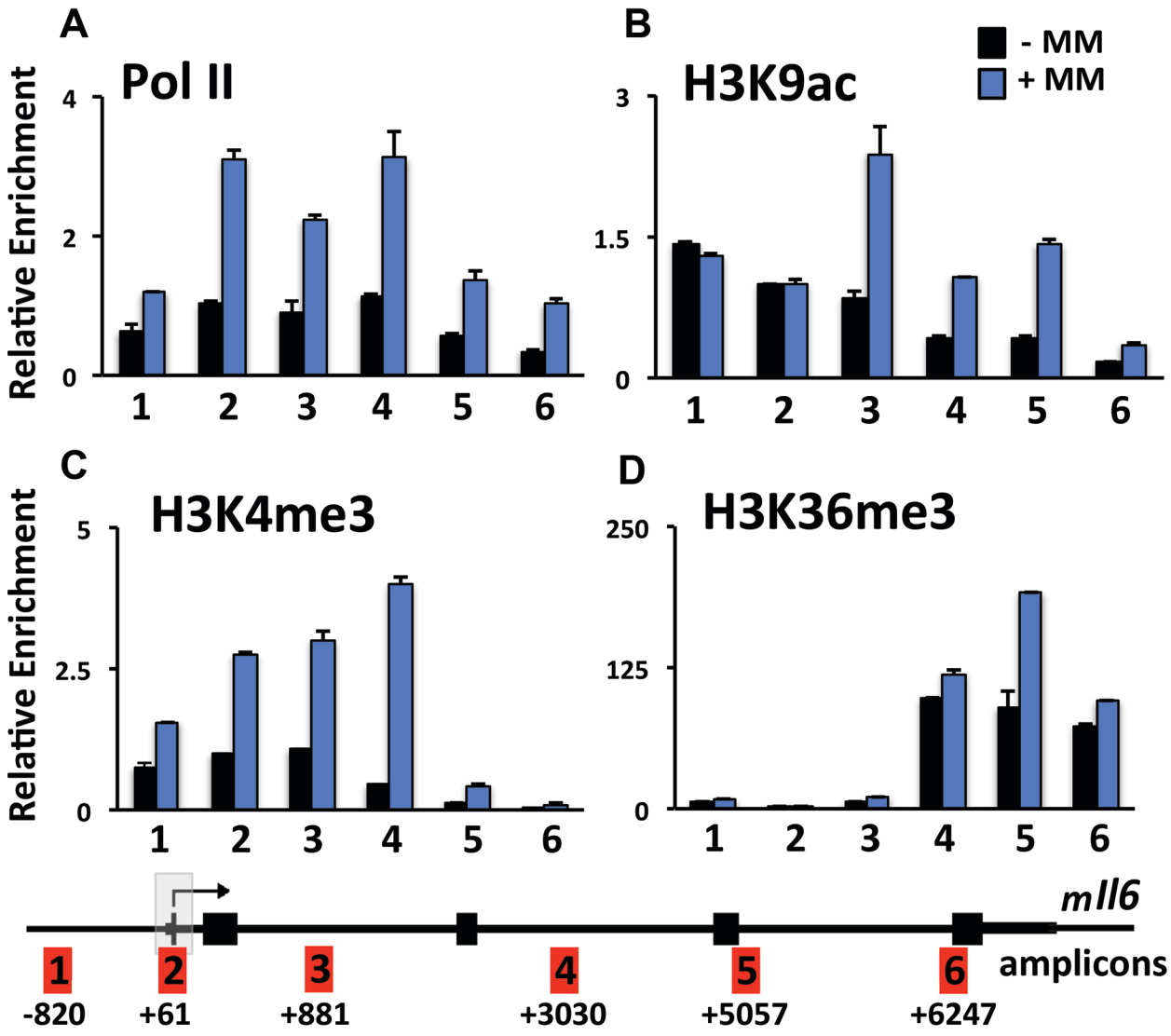
Figure S5. **Treatment with either HDAC1 inhibitor MC1294 or EZH2 inhibitor GSK126 rescued MM-BMSC OB differentiation – additional patient samples**. (A-C) MM-BMSC from patients #3-5 (Table S5) were cultured 21 days in osteogenic media supplemented with vehicle, MC1294 (10 μM) or GSK126 (2.5 μM) as indicated; the inhibitors were absent d14-21. Mineralization was assessed using Alizarin Red staining. Three independent wells from each treatment group are shown (the entire well next to a 5X magnification). Below each set is the density quantitation for the average of 6 wells/condition with SEM and significance indicated.

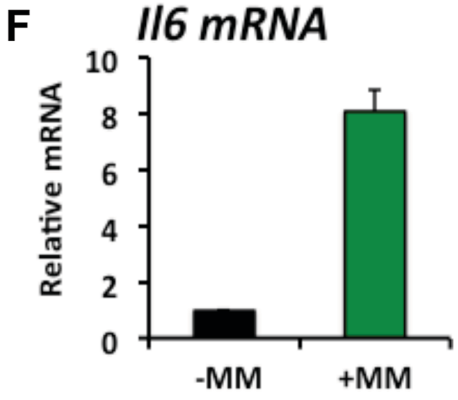
(**D**) Undifferentiated MM-BMSC (negative control) and (**E**) HD-BMSC treated with either DMSO or GSK126 and analyzed as in **A-C**.

Supplementary Figure S1

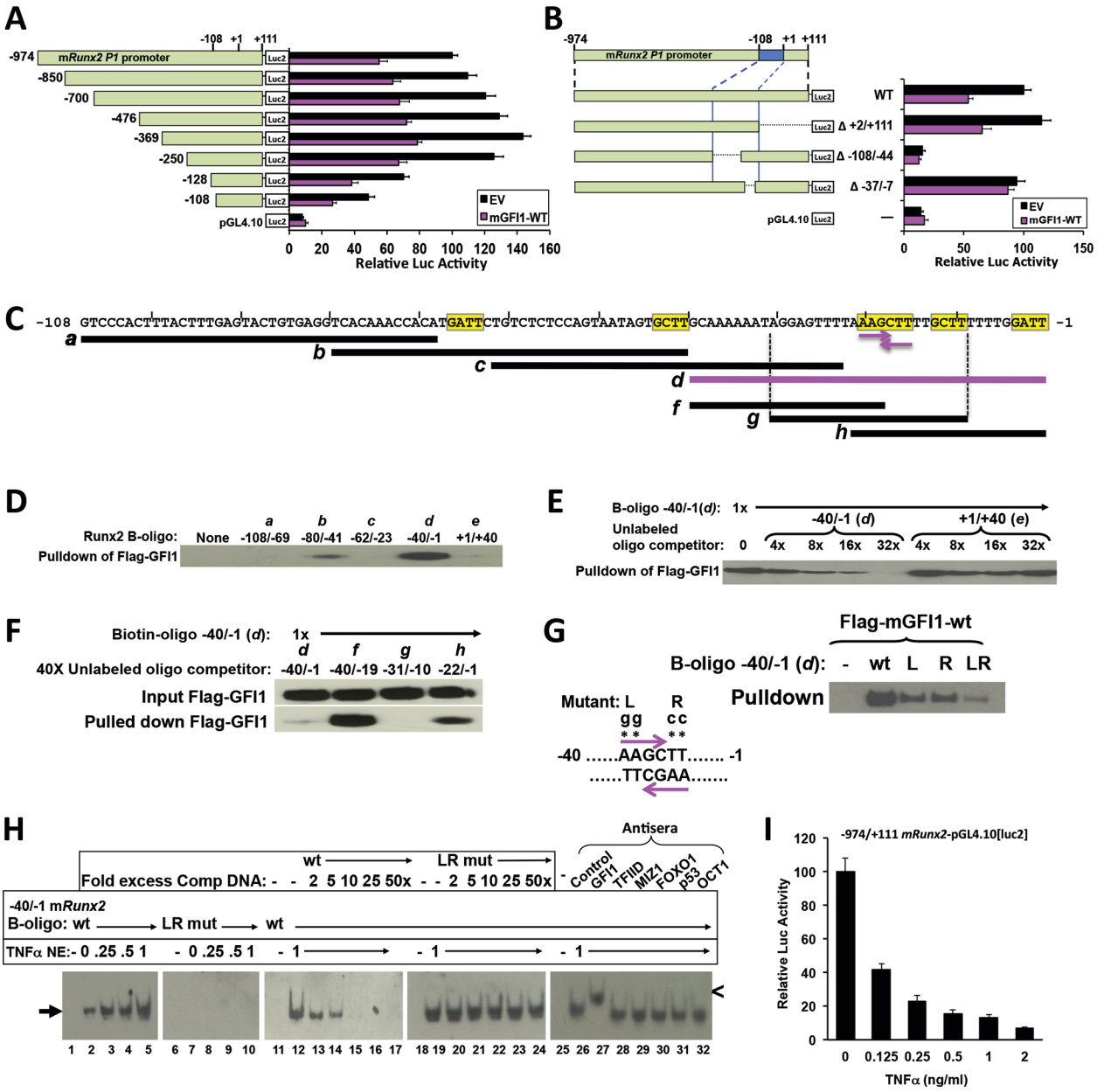


Supplementary Figure S2

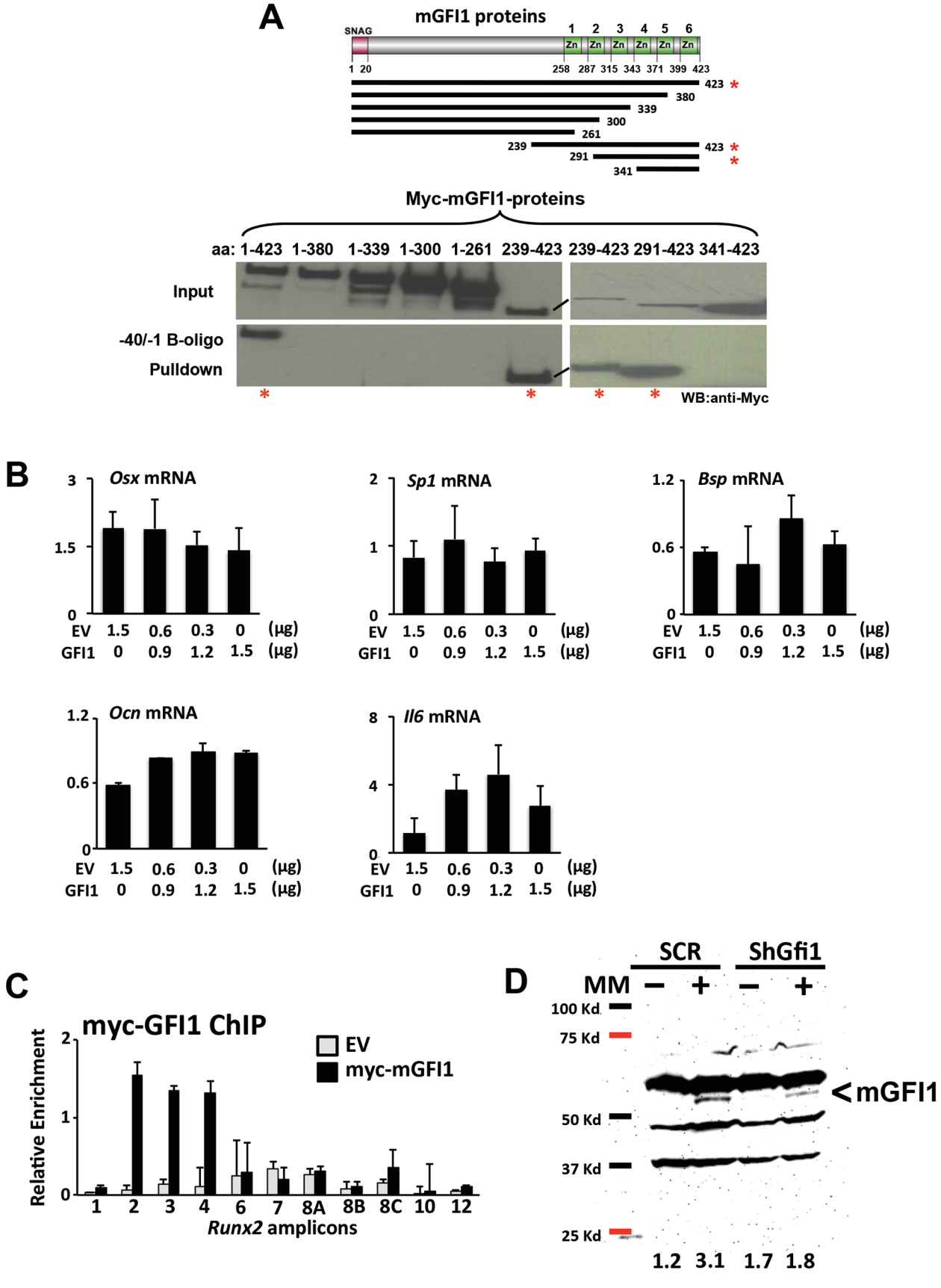




Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5