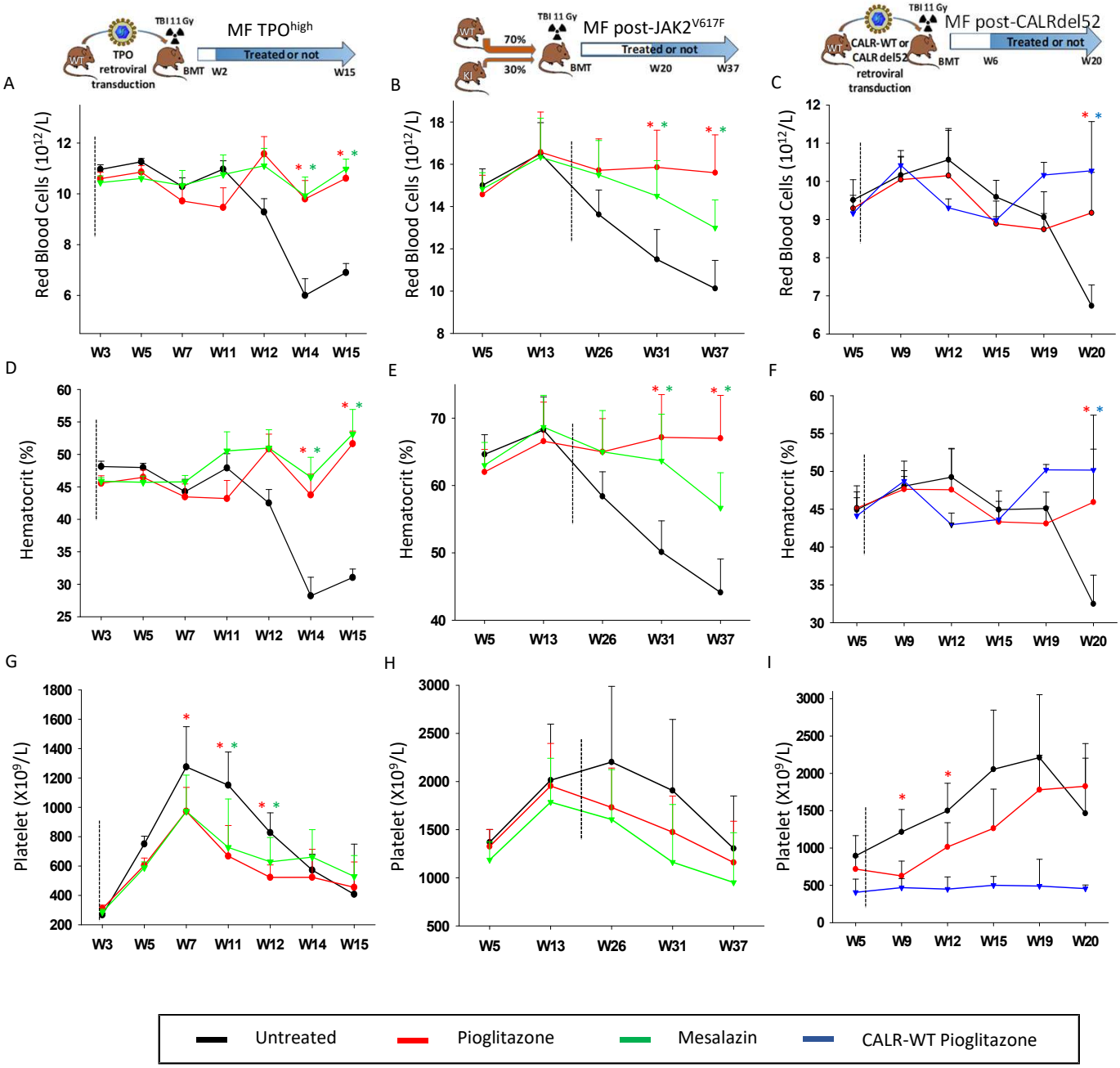
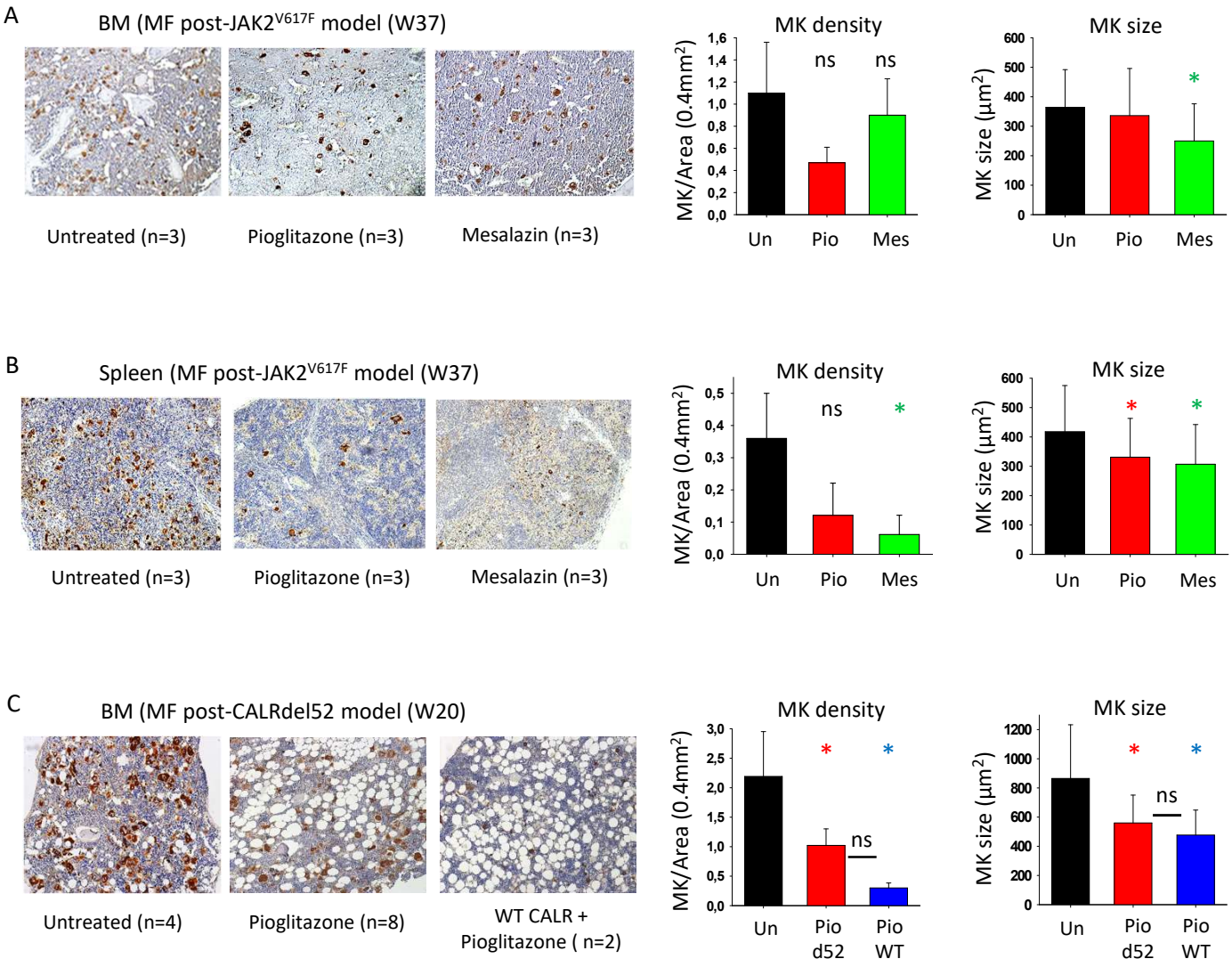
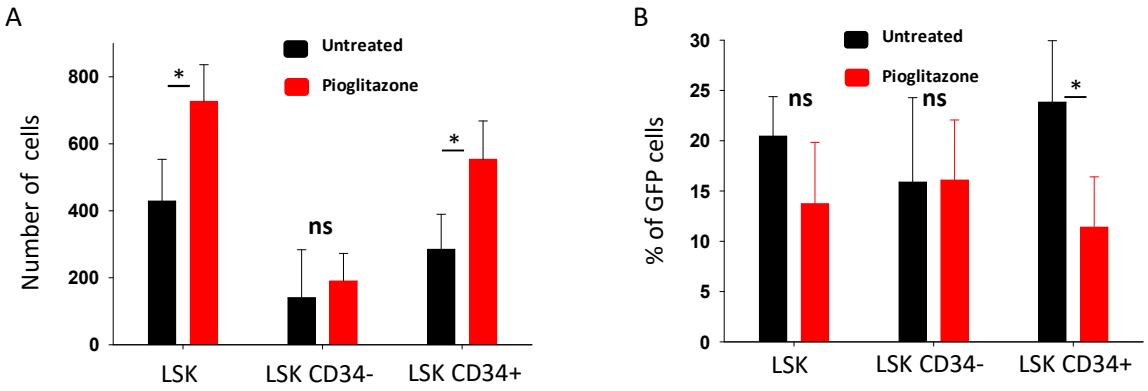


Supplemental Figure 1. Effect of PPAR-γ agonists on red blood cell count, hematocrit, and platelet count in the three mouse models of Myelofibrosis. PPAR-γ agonists counteract the decrease in red blood cell counts and hematocrit related to the development of BM fibrosis in the MF TPO^{high} model (A and D) , (B and E) MF post-JAK2V617F model, and (C and F) MF post-CALRdel52 model. PPAR-γ agonists help to decrease the high platelet count in the G) MF TPO^{high} model, H) MF post-JAK2V617F model, and I) MF post-CALRdel52 model. The black vertical dotted line indicates the initiation of treatment. The star denotes a statistically significant difference ($p < 0.05$). (n=10 mice/condition, excepted CALR-WT Pioglitazone n=5).

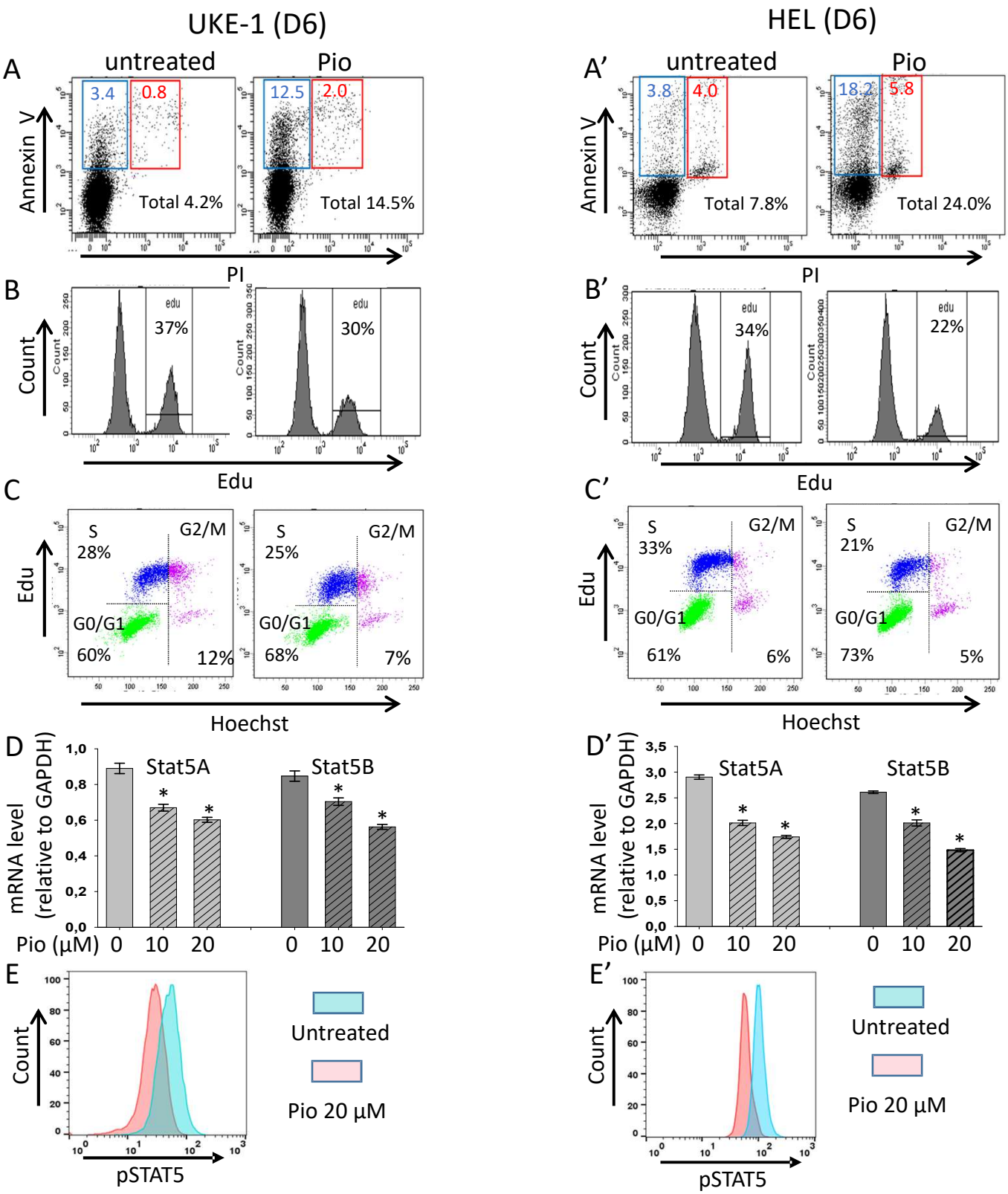
TBI: total body irradiation, Gy: Gray, BMT: bone marrow transplantation, W: week, WT: wildtype



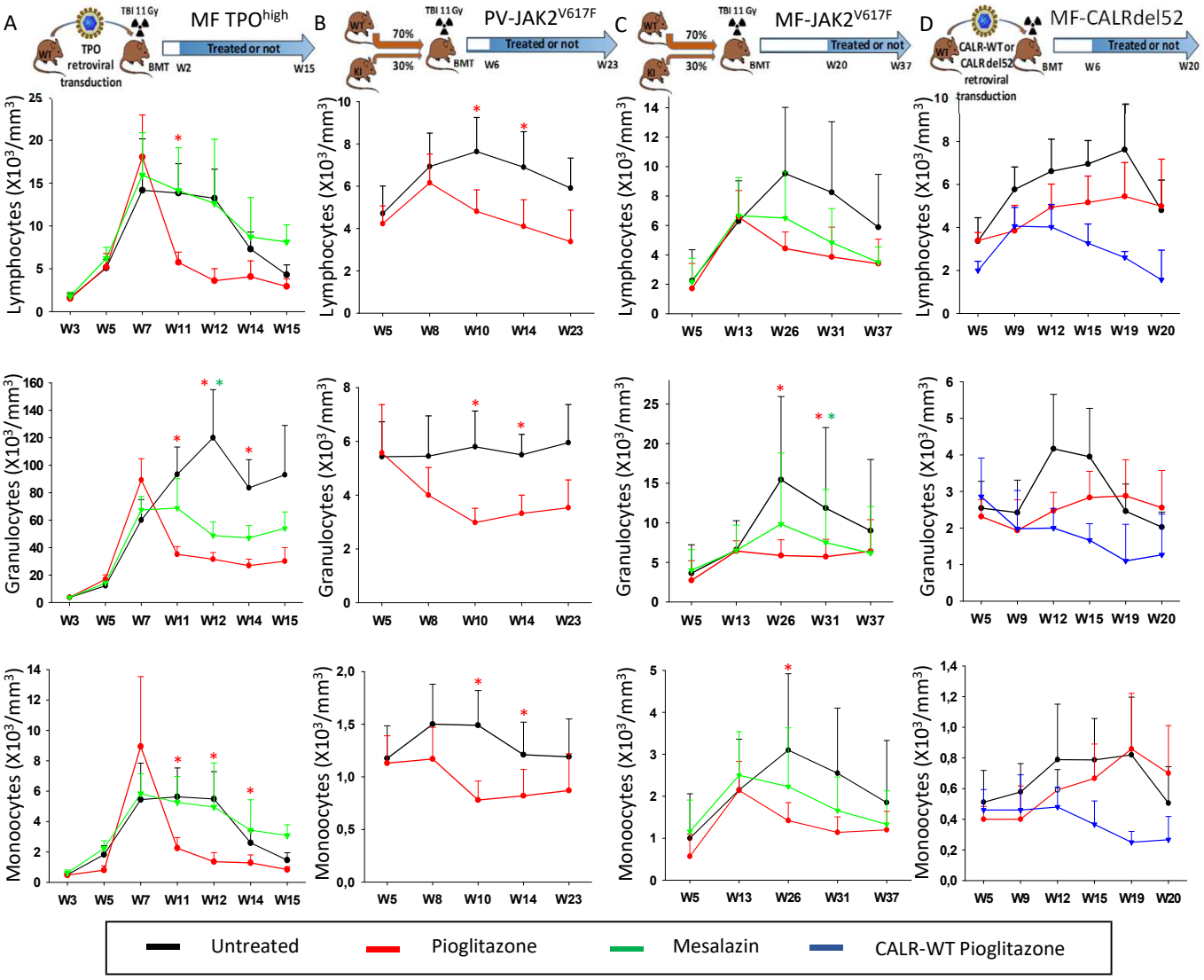
Supplemental Figure 2. Representative FvW staining and megakaryocytes (MK) analysis (density and size) in A) Bone marrow (BM) from MF post-JAK2V617F mouse model (W37), B) Spleen from MF post-JAK2V617F model (W37), C) BM from CALRdel52 mouse model (W20). (MK density/area (0,04mm2), number of area read/condition > 200, mean +/- SD ; MK size measurement is performed using image J software over more than 50 MK/condition, mean +/- SD ; The star denotes a statistically significant difference ($p < 0.05$); ns = not significant).

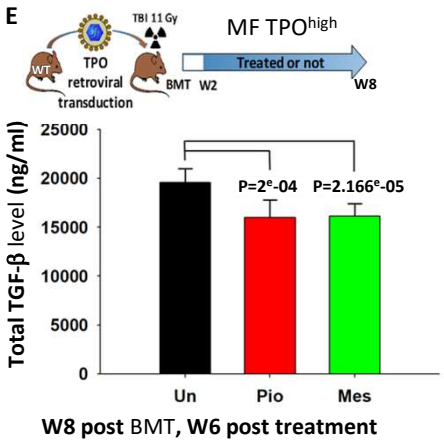


Supplemental Figure 3. Effect of pioglitazone on the LSK population in JAK2^{V617F} mice (W23). A) The LSK (Lin^{-}, Sca^{+}, Kit^{+}) hematopoietic population is greater in the BM of treated mice, especially the short-term progenitors (LSK CD34^{+}). B) The frequency of JAK2^{V617F}-GFP cells is lower in the LSK population from the BM of treated mice, especially in the short-term progenitors (LSK CD34^{+}). The star denotes a statistically significant difference ($p < 0.05$); ns= not significant.



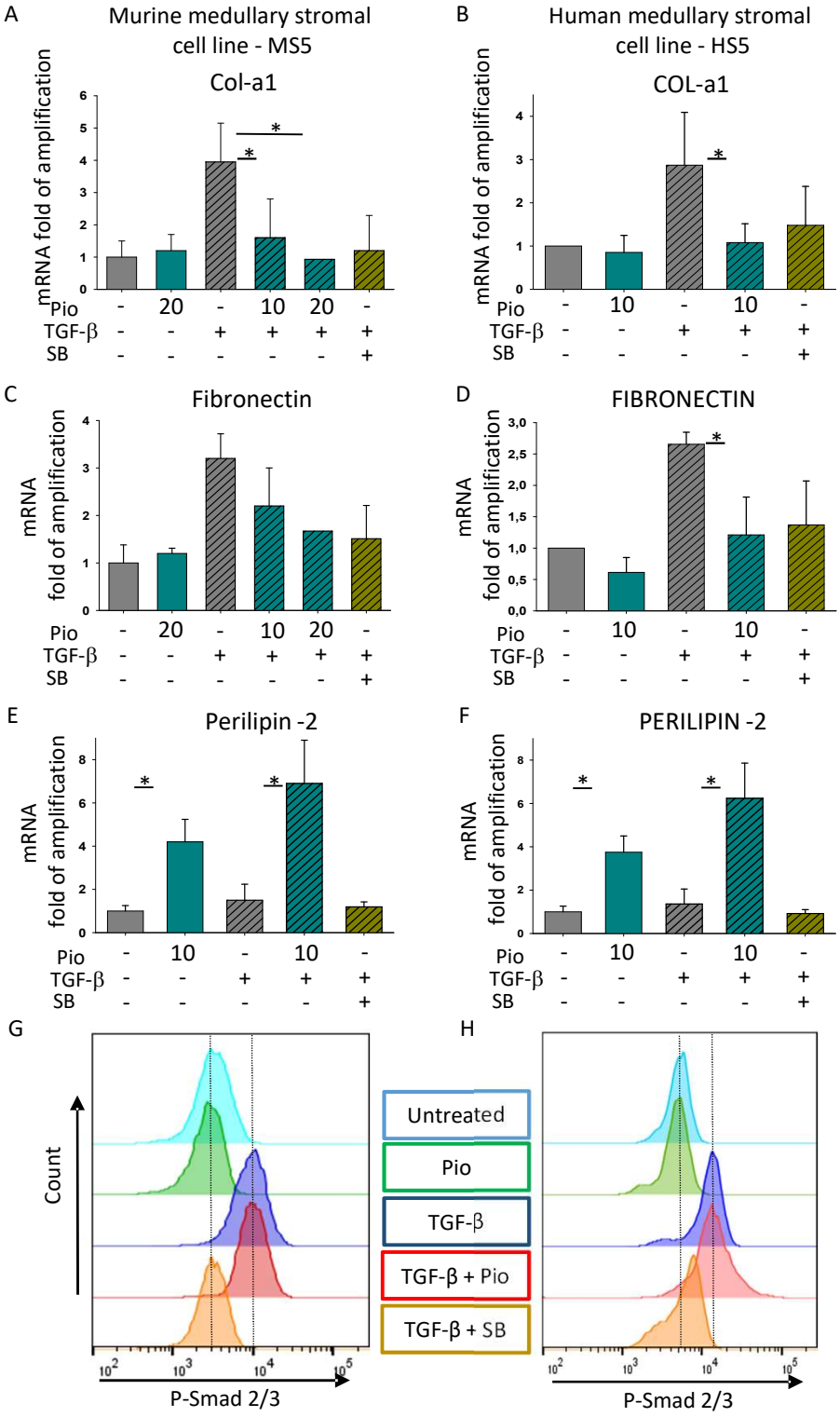
Supplemental Figure 4. Pioglitazone promotes apoptosis and impairs the cell cycle and Stat5 activity of JAK2^{V617F} cells. Pioglitazone (20µM) increases the proportion of apoptotic cells of A) UKE-1 cells and A') HEL cells (blue frame = early apotosis (Annexin V positive, propidium iodide (PI) negative) ; red frame = late apoptosis (Annexin V positive, PI positive). Pioglitazone decreases the proliferation of B) UKE-1 and B') HEL cells, with an increased proportion of C) UKE-1 and C') HEL cells in the G0/G1 phase of the cell cycle. Pioglitazone decreases Stat5A and B mRNA levels in D) UKE-1, D') HEL cells and reduce STAT5 activity (pSTAT5) in E) UKE-1, E') HEL cells (Representative of five independent experiments). The star denotes a statistically significant difference ($p < 0.05$).



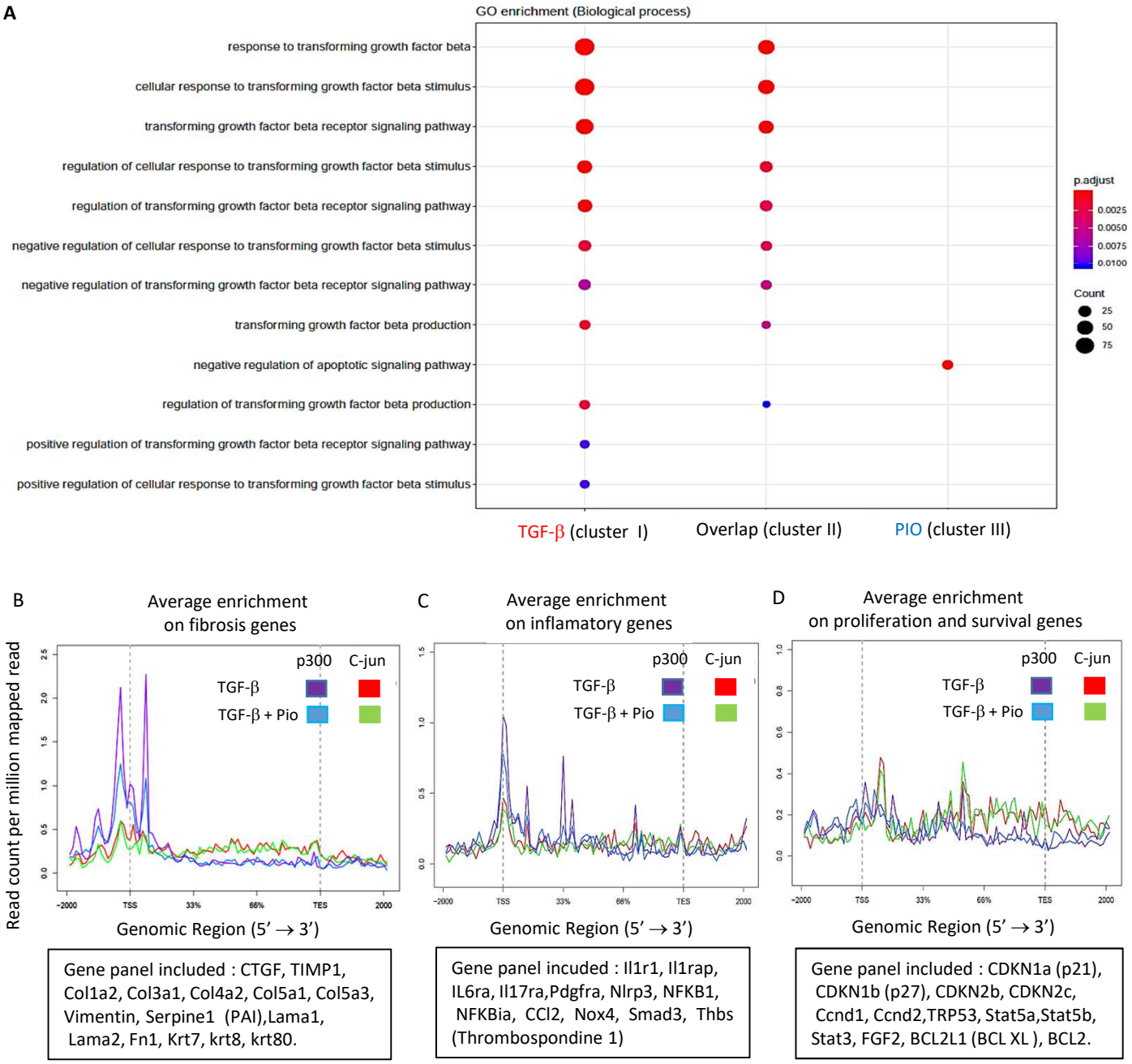


Supplemental Figure 5: PPAR-γ agonists decrease leukocytosis related to MF in all leukocyte subpopulations, and reduce Total TGF-β protein level in MF TPOhigh model.

Reduction in lymphocytes, granulocytes and monocytes counts in **A**) MF TPOhigh model, **B**) PVJAK2V617F model, **C**) MF post-JAK2V617F and **D**) MF post-CALRdel52 model. PV: Polycythemia Vera. MF: Myelofibrosis. TBI: total body irradiation. Gy: gray. BMT: bone marrow transplantation. WT: wild type. W: week. The star denotes a statistically significant difference ($p<0.05$). **E**) Quantification of TGF-β level in mice plasma from MF TPOhigh model treated or not with PPAR-γ agonists (Un = untreated ; Pio = pioglitazone ; Mes = mesalazin ; n=10/group)

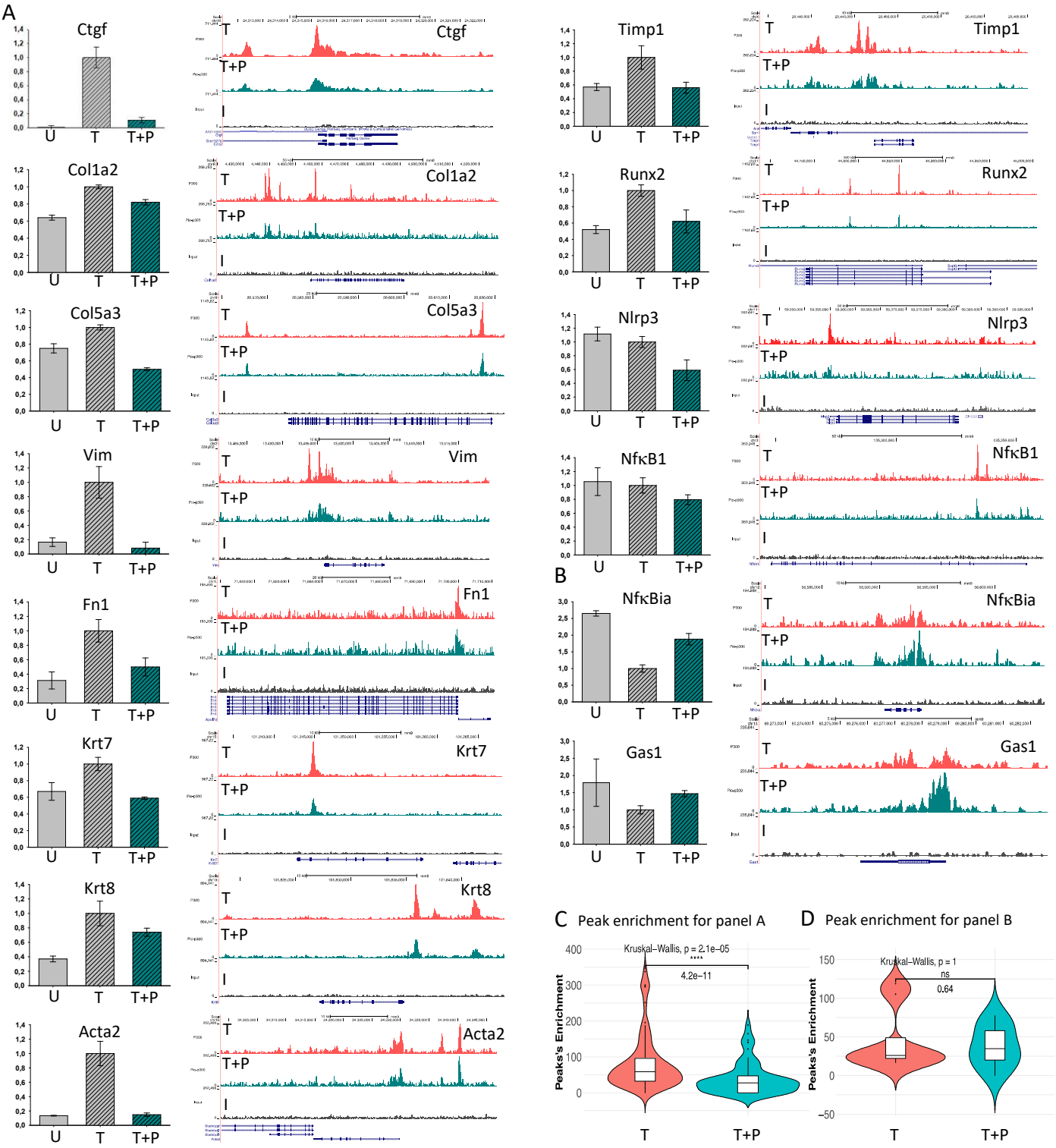


Supplemental Figure 6. Repression of TGF-β1 pro-fibrotic target gene expression by pioglitazone is not dependent on Smad phosphorylation. Pioglitazone counteracts the induction of transcription of collagen a1 and fibronectin genes by TGF-β1 in MS5 BM stromal cells (A and C), and HS5 BM stromal cells (B and D). Transcriptional induction of the PPAR-γ target gene, perilipin 2, with pioglitazone is not counteracted by TGF-β1 in MS5 BM stromal cells E) or HS5 BM stromal cells F). The antagonist effect of pioglitazone on the induction of fibrotic-genes by TGF-β1 is not dependent on Smad2/3 phosphorylation in MS5 BM stromal cells G) or HS5 BM stromal cells H). The star denotes a statistically significant difference ($p < 0.05$). COL-a1: collagen1 a1, Pio: pioglitazone, SB: SB431542 (inhibitor of TGF-β1 receptor)



Supplemental Figure 7. Additional results of ChIP-seq performed on MS5 BM stromal cells stimulated by TGF-β1, with or without pioglitazone. A) GO enrichment analysis of the three clusters of genes with the anti-p300 Ab. The size of the circle represents the number of peaks and the color the adjusted p-value. Pioglitazone reduces TGF-β production and TGF-β signaling pathways. Analysis of the average enrichment after ChIP with anti-p300 or anti c-Jun Abs on a panel of genes involved in B) fibrosis, C) inflammation, or D) proliferation and survival. ChIP with anti-p300 Ab shows that the addition of pioglitazone (in blue) decreases the average enrichment in fibrosis-related genes and, to a lesser extent, inflammation-related genes relative to TGF-β 1 alone (in purple). Pioglitazone has no effect on proliferation- and survival-related genes. ChIP with anti-c-jun Ab shows no difference between TGF-β1 alone (in red) and TGF-β1 + pioglitazone (in green) stimulation on fibrosis-, inflammation-, or proliferation/survival-related genes..

GO: gene ontology, Pio: pioglitazone, Ab: antibody, TSS: transcription start site, TES: transcription end site



Supplemental Figure 8: Comparison of mRNA gene expression and ChIP-seq analysis in MS5 BM stromal cells stimulated by TGF-β1 with or without pioglitazone. We have targeted a panel of 14 genes related to myelofibrosis (*Ctgf, Col1a2, Col5a3, Vim, Fn1, Krt7, Krt8, Acta2, Timp1, Runx2, Nlrp3, NfκB1, NfκBia, Gas1*) and whose mRNA level is modulated after induction by TGF-β alone (T) versus TGF- β + Pioglitazone (T + P). For each indicated gene, the mRNA level quantified by RT-qPCR (on the left, mean +/- SD, relative to GAPDH and normalized on TGF-β condition, n=3) was compared to the size and the localization of it's P300 ChIP-seq peaks (on the right). A) and B) present the panel of genes repressed or unrepressed by pioglitazone treatment respectively. C) In panel A, the decrease of mRNA level in presence of pioglitazone was associated to a significant decrease in p300 ChIP-seq peaks enrichment. D) No significant modulation of p300 ChIP-seq peaks enrichment was observed in panel B. (U=untreated, T=TGF-β, T+P=TGF-β+pioglitazone, I=input).

Sup.Material 1	sex	age	JAK2^{V617F}	allele burden (%)	EPO mU/ml	CFC
Patient 1	F	78	Positif	85	<4	performed
Patient 2	F	61	Positif	38	<4	performed
Patient 3	M	62	Positif	72	<4	performed
Patient 4	F	58	Positif	34	<4	performed
Patient 5	F	81	Positif	32	<4	not performed
Patient 6	M	68	Positif	79	<4	performed

Supplemental Material 1. Information about PV-patients (Figure 3D and H).

Sup.Material 2	sex	age	driver mutation	allele burden (%)	Others mutations	Fibrosis	CFC
Patient 1	M	80	JAK2^{V617F}	45.31		Grade MF-3	performed
Patient 2	F	79	CALRdel52	not performed		Grade MF-3	performed
Patient 3	F	68	CALRdel52	46		Grade MF-2	performed
Patient 4	M	58	JAK2^{V617F}	88	ASXL1 and NFE2	Grade MF-2	performed
Patient 5	F	45	JAK2^{V617F}	67	NFE2	Grade MF-2	performed
Patient 6	M	62	JAK2^{V617F}	not performed		Grade MF-2	performed
Patient 7	F	65	JAK2^{V617F}	48		Grade MF-2	performed
Patient 8	M	85	MPL 515L	100	TET2, CUX1 and ZRSR2	Grade MF-2	performed

Supplemental Material 2. Information about MF-patients (Figure 3E and I).

Human Primers			
Gene	Protein	Forward primer 5'-3'	Reverse primer 5'-3'
CCN2	Connective tissue growth factor	CGA GGA GTG GGT GTG TGA C	CAG GCA GTT GGC TCT AAT CAT AG
COL1A1	Collagen type 1 α1	GGC AAA GAA GGC GGC AAA G	GCA CCA GCA GGA CCA TCA G
FN1	Fibronectin 1	AGA CCA GCA GAG GCA TAA GG	ACT CAT CTC CAA CGG CAT AAT G
ADIPOQ	Adiponectin	GCC TAC CAC ATC ACA GTC TAT ATG	AGT CCA TTA CGC TCT CCT TCC
PLIN2	Adipophilin	TGC TGT GAC GAC TAC TGT GAC TG	GAC TGT GTT AAT GCT GCC ACT GAC
LAMA1	Laminin α1	AAG GAG TCG GAA GCG GAA G	CTG AAG CGG AGA CAC TGA ATC
PAI 1	Plasminogen Activator Inhibitor 1	GGC TGG TGC TGG TGA ATG	GGC GTG GTG AAC TCA GTA TAG
EP300	Histone Acetyltransferase P300	GAC ACC TAC ACC ACC AAC AAC	AGA GCG TGC TGT GCT CTG
TGFB1	Transforming Growth Factor β1	GGA CAT CAA CGG GTT CAC TAC	GCA CGC AGC AGT TCT TCT C
THBS1	Thrombospondin 1	TGC TCC AAT GCC ACA GTT C	ATC GGT TGT TGA GGC TAT CG
TIMP1	Tissue Inhibitor Of Metalloproteinases 1	TGT TGT TGC TGT GGC TGA TAG	ACG CTG GTA TAA GGT GGT CTG
FOS	Fos Proto-Oncogene	GGC AAG GTG GAA CAG TTA TCT C	CTT CAG CAG GTT GGC AAT CTC
JUN	Jun Proto-Oncogene	CGA GAG CGG ACC TTA TGG	CGT TGC TGG ACT GGA TTA TC
STAT5A	Signal transducer and activator of transcription 5A	AGA GGC TGG TCC GAG AAG	TGT CTG GTT GAT CTG AAG GTG
STAT5B	Signal transducer and activator of transcription 5B	ACA GAG GTT GGT CCG AGA AC	CTG GTT GAT CTG GAG GTG TTT C
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	GTA TCG TGG AAG GAC TCA TGA CC	GTT CAG CTC AGG GAT GAC CTT
Murine Primers			
Gene	Protein	Forward primer 5'-3'	Reverse primer 5'-3'
Ccn2	Connective tissue growth factor	AGG ACC GCA CAG CAG TTG	GCA GTT GGC TCG CAT CAT AG
Col1α1	Collagen type 1 α1	CAG TGG CGG TTA TGA CTT CAG	GGC TGC GGA TGT TCT CAA TC
Col1α2	Collagen type 1 α2	ACG ATG TTG AAC TTG TTG CTG AG	GCA CCA CCA ATG TCC AGA GG
Col5α3	Collagen type 5 α3	CCA GCC AAT CAG TCT GTC CTT C	TGC CAC CTG CCA TCC ATA ATG
Fn1	Fibronectin 1	AGT CAG TGT CTC CAG TGT CTA C	CAG AAT GCT CGG CGT GAT G
Vim	Vimentin	ACT AGC CGC AGC CTC TAT TCC	GAG AAG TCC ACC GAG TCT TGA AG
Acta2	Actin alpha 2	TCA GGG AGT AAT GGT TGG AAT GG	GTT GGT GAT GAT GCC GTG TTC
Krt7	Keratin 7	GTT GCT GAA GAA GGA TGT GGA TG	TCT GCT AAC TCT GTC TCG TGA AG
Krt8	Keratin 8	ACC ACC AGC GGC TAC TCA G	CAT CAG AAG ACT CGG ACA CCA G
Adipoq	Adiponectin	GCT CTC CTG TTC CTC TTA ATC	TGC CAT CTC TGC CAT CAC
Plin2	Perilipin 2	GTG CCA GAG GTG CCG	AAC TGT ATT GAT GCT GCC ATT GAC
Lama1	Laminin α1	CGT GGA TGG CGT CAA GTT C	TTC GTT GTC TGC TCT GTA AGT G
Pai1	Plasminogen Activator Inhibitor 1	CTC CTC ATC CTG CCT AAG TTC	GTC CCG CTC TCG TTT ACC
Ep300	Histone Acetyltransferase P300	AAC CAC CAC CAG CAA CAG	CAG AAG GAG CAG CAG GAA G
Tgfb1	Transforming Growth Factor β1	AGC AAC AAT TCC TGG CGT TAC	GTA TTC CGT CTC CTT GGT TCA G
Thbs1	Thrombospondin 1	CAT CTT CCT GGC TTC CTT GAG	TCC TCC ACT GAC ACC ACT TG
Timp1	Tissue Inhibitor Of Metalloproteinases 1	ATC TCT GGC ATC TGG CAT CC	ACG CTG GTA TAA GGT GGT CTC
Runx2	Runt-related transcription factor 2	ACA ACA GCA ACA GCA ACA ACA G	CAG CAC GGA GCA CAG GAA G
Nlrp3	NOD-like receptor family, pyrin domain containing 3	AGC CTT GAA GAA GAG TGG ATG G	TGC GTG TAG CGA CTG TTG AG
Gas1	Growth arrest-specific gene 1	GCC TTG CTG TGC CTG ATG G	TCG GCG TAC TGG CTG TAG G
Fos	FBJ osteosarcoma proto-oncogene	CCG ATG ACC TTG GCT TCC	CGT TGC TGA TGC TCT TGA C
Jun	Jun proto-oncogene	GCC AAG AAC TCG GAC CTT C	GTC GGT GTA GTG GTG ATG TG
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	GCA TTG TGG AAG GGC TCA TGA CC	GTT CAG CTC TGG GAT GAC CTT G
Il15	Interleukin 15	TTT GGG CTG TGT CAG TGT AGG	ATT CCA GGA GAA AGC AGT TCA TTG
Tnf	Tumor necrosis factor	CTC CAG GCG GTG CCT ATG T	GA GAG CGT GGT GGC CC
Ifng	Interferon γ	CTC AAG TGG CAT AGA TGT GGA AG	AGG TGT GAT TCA ATG ACG CTT ATG
Il1b	Interleukin 1β	GCA GCA GCA CAT CAA CAA GAG	CAC CAG CAG CAG GTT ATC ATC ATC ATC
Il5	Interleukin 5	CGC TCA CCG AGC TCT GTT G	CCA ATG CAT AGC TGG TGA TTT TT
Nfkb1	Nuclear factor κ-light-chain-enhancer of activated B cell	ACG ACA GAA TCC TCA GCA TCC	CCA CCA GCA GCA GCA GAC
Nfkbia	NF-κB inhibitor α	TGA CCT GGT TTC GCT CTT GTT G	GCT CTC CTC ATC CTC GCT CTC
Il1ra	Interleukin 1 receptor antagonist	TGT CTT GTG CCA AGT CTG GAG ATG	GAC GGT CAG CCT CTA GTG TTG TG
Il12b	Interleukin 12b	CTC AGG ATC GCT ATT ACA ATT CCT C	TTC CAA CGT TGC ATC CTA GGA TC
Il6	Interleukin 6	AGA GGA GAC TTC ACA GAG GAT ACC	CAT TTC CAC GAT TTC CCA GAG AAC

Supplemental material 3: Human and murine qPCR primers.

Sample	Ab Target	Ab Vendor	Ab Cat No.	Ab Lot No.	Ab Volume	Chromatin amount
TGF-b1	c-Jun	Santa Cruz	sc-1694	A2413	20 µL	30 µg
TGF-b1 + Pioglitazone	c-Jun	Santa Cruz	sc-1694	A2413	20 µL	30 µg
TGF-b1	p300	Santa Cruz	sc-585	B0211	20 µL	30 µg
TGF-b1 + Pioglitazone	p300	Santa Cruz	sc-585	B0211	20 µL	30 µg

Supplemental material 4: ChIP, reaction data and technical details.