A high-content cytokine screen identifies myostatin propeptide as a positive regulator of primitive chronic myeloid leukemia cells

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Supplementary information for:

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Summary:

Includes Supplementary Methods, Supplementary Figures and Supplementary Tables.

Supplementary Methods

Patient samples and CD34 enrichment

Mononuclear cells (MSCs) were isolated from primary samples using Lymphoprep (GE Healthcare Bio-Sciences AB, Sweden) and $CD34^+$ cells were obtained using magnetic separation (Miltenyi Biotec, Germany), both according to the manufacturers' instructions. The same procedure was used for healthy bone marrow (BM) control cells.

Cytokine screening

Stock solutions of 313 unique cytokines (Supplementary Table S2) were prepared by reconstitution of lyophilized cytokine according to the manufacturer's instructions (Peprotech, USA). Cytokines were further diluted in serum free StemSpan™ SFEM medium (Stem Cell Technologies, Canada) to a final concentration of 100 ng/ml and arrayed in Nunc 384-well clear polystyrene plates (Thermo Fisher Scientific, USA).

CML peripheral blood (PB) samples cells were stained with antibodies against CD34 (BioLegend, USA) and CD38 (BD Biosciences, USA) in PBS with 2% fetal bovine serum (FBS), and Draq7 (BioStatus, UK) was used as viability dye. Five hundred $CD34^+$ $CD38^{low}$ CML cells were sorted into the pre-made cytokine plates using a FACS Aria II (BD Biosciences). The $CD38^{low}$ gate was set to include the 10% of cells with the lowest CD38 expression. Plates were incubated at 37°C 5% CO_2 for 7 days prior to analysis of viable cell number using Draq5 (BioStatus) staining and the Cellomics Live Imaging system (Thermo Fisher Scientific). Wells with no cytokine were used as a baseline, and IL-1β (100ng/ml) was used as a positive control. Cytokines that caused at least a 2-fold increase in cell number were considered as positive hits.

The cytokines scoring as positive regulators in the original screen on PB CML samples were validated in a 96-well format using 2 000 $CD34^{+}CD38^{low}$ cells from CML BM and normal BM. The $CD38^{low}$ gate in the validation experiments was set to the lowest 5% of CD38-expressing cells. Plates were incubated in 37°C 5% CO_2 for 7 days prior to analysis of viable cell number using CountBright Absolute Counting Beads (Thermo Fisher Scientific) and Draq7 (BioStatus) on a Canto or LSR Fortessa (BD Biosciences).

Cell cultures of transgenic BCR-ABL mouse cells

The hips, femurs and tibias were harvested and crushed with pestle and mortar in PBS with 2% heat-inactivated FBS to isolate BM cells. Cells were filtered through a 70 uM filter mesh prior to enrichment of immature cells using MACS CD117 microbeads and an auto-MACS machine (both by Miltenyi) according to the manufacturer's instructions. Before sorting, the CD117 positive cells were stained with antibodies against CD117, Sca-1 and lineage markers (ter119, IgGM, CD3e, Nk1.1, CD4, CD19, Gr-1, Mac-1, B220, CD8a and CD8b) (ThermoFisher Scientific). DAPI was used for viability stain. Five thousand $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$ (LSK) BM cells were sorted into individual wells containing 500 ng/ml MSTNpp (catalog# 12012, lot# 0603297, Peprotech) or no cytokine in serum free StemSpan™ SFEM media (Stem Cell Technologies) supplemented with Penicillin Streptomycin (Invitrogen, USA), Antibiotic Antimycotic Solution (HyClone, USA). Plates were incubated in 37°C 5% CO_2 for 7 days prior to analysis of viable cell number using CountBright Absolute Counting Beads (Thermo Fisher Scientific) and DAPI on LSRFortessa (BD Biosciences).

Colony-forming assays

After 2 weeks, the number of colonies were counted. For replating experiments, colony dishes were resuspended in IMDM (Sigma-Aldrich, USA) with 2% FBS and replated in new MethoCult at a 1:4 ratio.

Co-culture experiments with primitive CML cells and mesenchymal stromal cells

Mesenchymal stromal cell (MSC) cultures were established from BM MNCs of three CML patients and three normal donors as described by Reinisch et al.[1] At passage 3, the MSCs were plated in 96-well tissue culture plates (Corning, USA) at 9 600 cells/cm^2, and left to incubate in 37°C 5% CO_2. After 24h, the media was removed and wells were carefully washed twice with PBS, prior to addition of serum free StemSpan™ SFEM media (Stem Cell Technologies) supplemented with Penicillin Streptomycin (Invitrogen). CML cells were sorted as described above, and 2 000 $CD34^+CD38^{low}$ were plated on top of the MSC monolayer, in media with or without 100 ng/ml of MSTNpp (Peprotech). After 3 days, the media with suspension cells was removed and the wells were carefully washed twice with PBS to recover residual leukemic cells. Viable cells were enumerated using CountBright Absolute Counting Beads (Thermo Fisher Scientific) and DAPI, on LSRFortessa (BD Biosciences).

MSTN RT-qPCR

Relative *MSTN* expression in CD34+ cells, MNCs and cultured MSCs from BM of three primary CML patients was assessed using reverse transcriptase quantitative PCR (RT-qPCR). In brief, 10 - 200 ng of RNA was converted to cDNA using M-MLV reverse transcriptase (Thermo Fisher Scientific). Quantitative PCR was then performed using Taqman universal PCR master mix (Thermo Fisher Scientific) with commercial Taqman assays (Thermo Fisher Scientific) targeting *MSTN* (Hs00976237_m1) and the control gene *HPRT1* (Hs01003267_m1). The reactions were performed according to the manufacturer's protocol, but with a total of 50 PCR cycles to enable detection of low-level expression. Relative expression values were calculated using the $2^{-\Delta\Delta Ct}$ method[2] with HPRT1 as internal control and pooled human skeletal muscle (Thermo Fisher Scientific) as calibrator sample.

RNA sequencing and GSEA

Cells were incubated with or without MSTNpp (Peprotech) at 37°C 5% CO_2 for 3h or 24h, prior to cell harvesting, centrifugation and extraction of RNA using PicoPure RNA Isolation Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. cDNA was generated using the SMARTer Ultra

Low Input RNA Kit for Sequencing v4 (Takara Bio, formerly Clontech Laboratories, USA). cDNA libraries were prepared using Nextera Library DNA Preparation Kit (Illumina, USA), with cDNA input adjusted according to the SMARTer protocol. RNA sequencing was performed on a NextSeq 500 (Illumina). The reads were aligned to the human reference genome hg19 using TopHat 2.0.7.[3] Aligned reads were summarized using FeatureCounts 1.5.1 from the Subread package.[4] Genes differentially expressed between samples treated with and without MSTNpp were identified using EdgeR.[5] To adjust for individual differences in gene expression between different patient samples before MSTNpp treatment, the data were analyzed as a paired design experiment using an additive generalized linear model with patient identity as the blocking factor. For the gene set enrichment analysis (GSEA), all genes were ranked according to the metric $-\log_{10}$(P-value for differential expression) modified with the sign of the fold change of the differential expression. The GSEA analysis was run with GSEA v2.2.4 (Broad Institute, USA) using the ranked gene list and gene sets from the Molecular Signatures Databases (MsigDB) curated gene sets collection (c2all.c6.0.symbols.gtm).

Phospho-flow cytometry

After fixation and permeabilization, cells were stained with phospho-SMAD2 (Ser465/467)/SMAD3 (Ser423/425) antibody (catalog# 11979, Cell Signaling Technologies, USA), anti-STAT5 (pY694) (catalog# 612599, BD Biosciences), anti-NF-κB p68 (pS529) (catalog# 560335, BD Biosciences), anti-p38 MAPK (pT180/pY182) (catalog# 560241, BD Biosciences), anti-JNK (pT183/pY185) (catalog# 562481, BD Biosciences), anti-Akt (pS473) (catalog# 562465, BD Biosciences), anti-Phosphotyrosine (catalog# 558008, BD Biosciences) and anti-ERK1/2 (pT202/pY204) (catalog# 562981, BD Biosciences). For experiments including CD38 and CD34 analysis, cells were also stained with CD38 antibody (BD Biosciences) during stimulation, and CD34 antibody (Biolegend) following fixation and permeabilization. Samples were analyzed on a LSRFortessa instrument (BD Biosciences).

Supplementary References

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2. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-408.
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5. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;26(1):139-140.

Supplementary Figures and Supplementary Tables

Figure S1

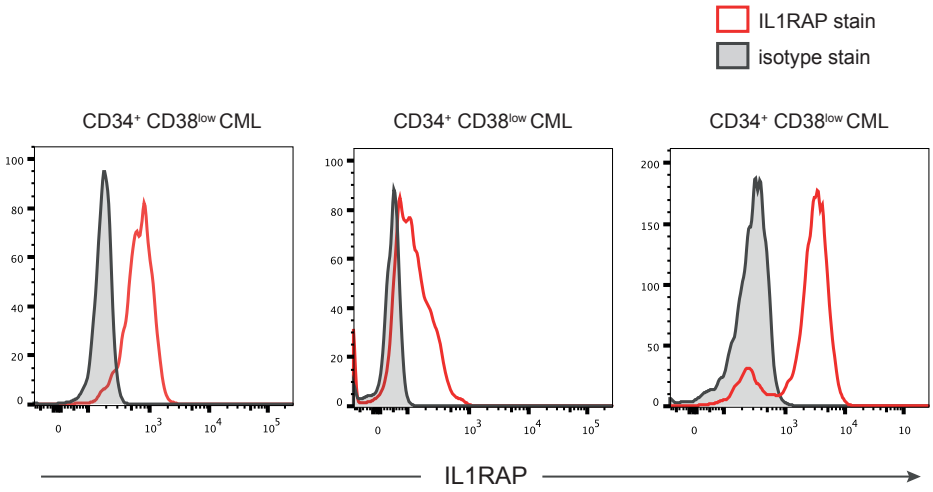

Figure S1. IL1RAP expression of $CD34^+$ $CD38^{low}$ CML cells. Histograms show the IL1RAP expression of the lowest 5% of the CD38-expressing $CD34^+$ cells from the 3 chronic phase CML patient samples used in the screen. Isotype control cells in gray and IL1RAP stained cells in red.

Figure S2

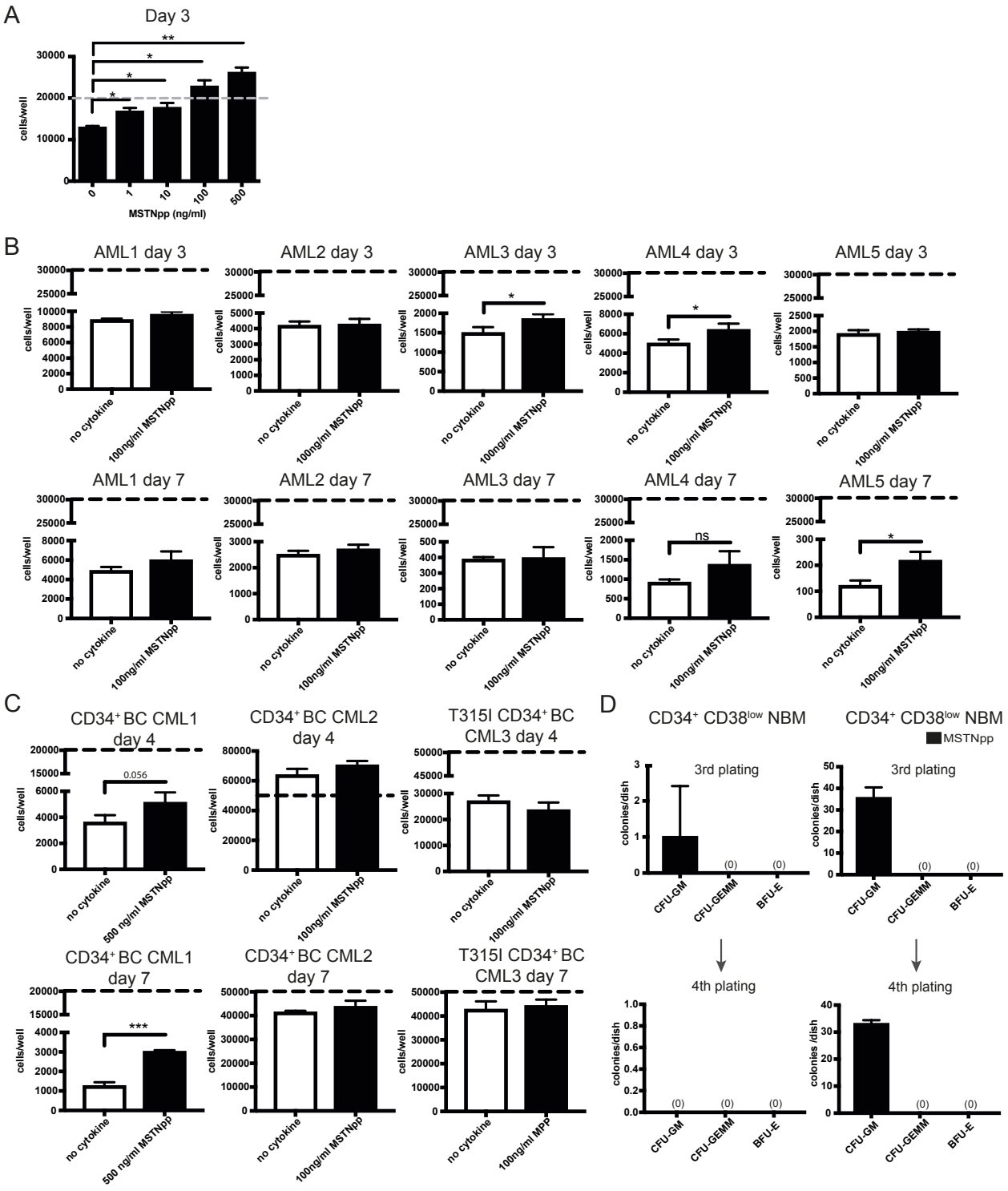

Figure S2. (A) Bar graph showing total cell numbers of CD34⁺ chronic phase CML cells from a single patient after 3 days in culture with increasing concentrations of MSTNpp. The dotted line indicates the number of seeded cells at day 0 (n=3). (B) Bar graphs showing absolute cell numbers of AML cells from 5 diagnostic patient samples, cultured with or without 100 ng/ml of MSTNpp in triplicates. Cell numbers were assessed at day 3 and day 7. The dotted line indicates the number of seeded cells. (C)

Bar graphs showing absolute cell numbers of $CD34^+$ BC CML cells from 3 patients, cultured with or without 100 ng/ml of MSTNpp in triplicates. Cell numbers were assessed at day 4 and day 7. One sample has a T315I-mutation and has been passaged in mouse. The dotted line indicates the number of seeded cells. (D) Colony type and number of $CD34^+CD38^{low}$ normal BM cells from Figure 2G after the 3rd and 4th replating of MSTNpp pre-cultured cells. Only MSTNpp treated cells were replated as there were no colonies left after the second plating for no cytokine control cells. AML, acute myeloid leukemia; BC, blast crisis; BM, bone marrow.

Figure S3

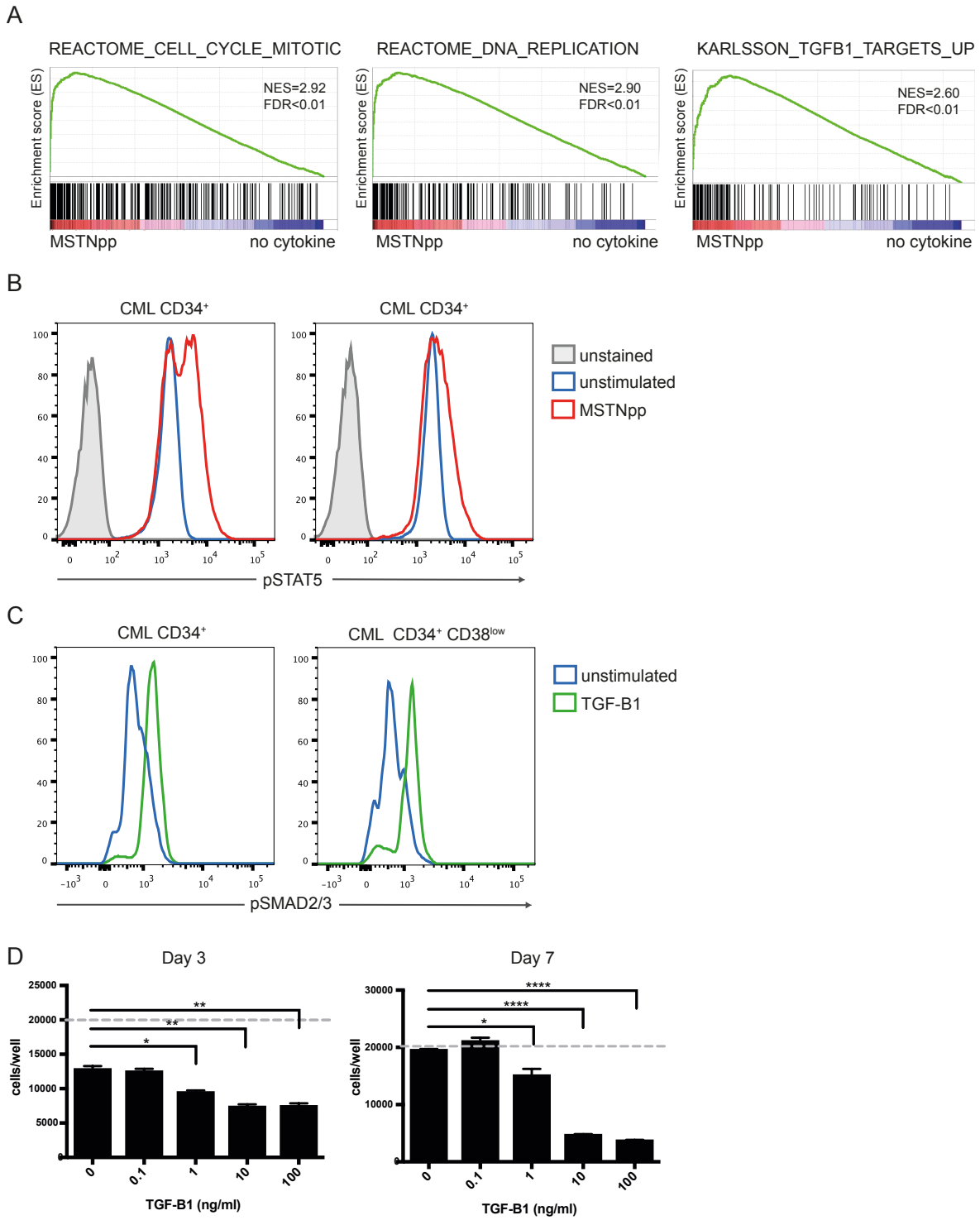

Figure S3. (A) GSEA showing upregulation of cell cycle genes, genes involved in DNA replication and TGF-B1 target genes after 24h of MSTNpp stimulation. Five individual chronic phase CML patient samples were used. (B) Histograms showing activation of STAT5 after 15-minute stimulation with MSTNpp in CD34^{+}chronic phase CML cells from two individual patients (also shown in Fig 4D), compared to unstimulated control. Unstained cells in gray, unstimulated cells in blue, and MSTNpp stimulated cells in red. (C) Histograms showing activation of SMAD2/3 after 15-minute stimulation

with TGF-β1 in $CD34^+$ and $CD34^+$ $CD38^{low}$ chronic phase CML cells from a single patient, compared to unstimulated control. Unstimulated cells in blue and TGF-β1 stimulated cells in green. (D) Bar graph showing absolute cell numbers of $CD34^+$ chronic phase CML cells from a single patient after 3 and 7 days in culture with increasing concentrations of TGF-β1. The dotted line indicates the number of seeded cells at day 0 (n=3).*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ and ****$p \leq 0.0001$

Figure S4

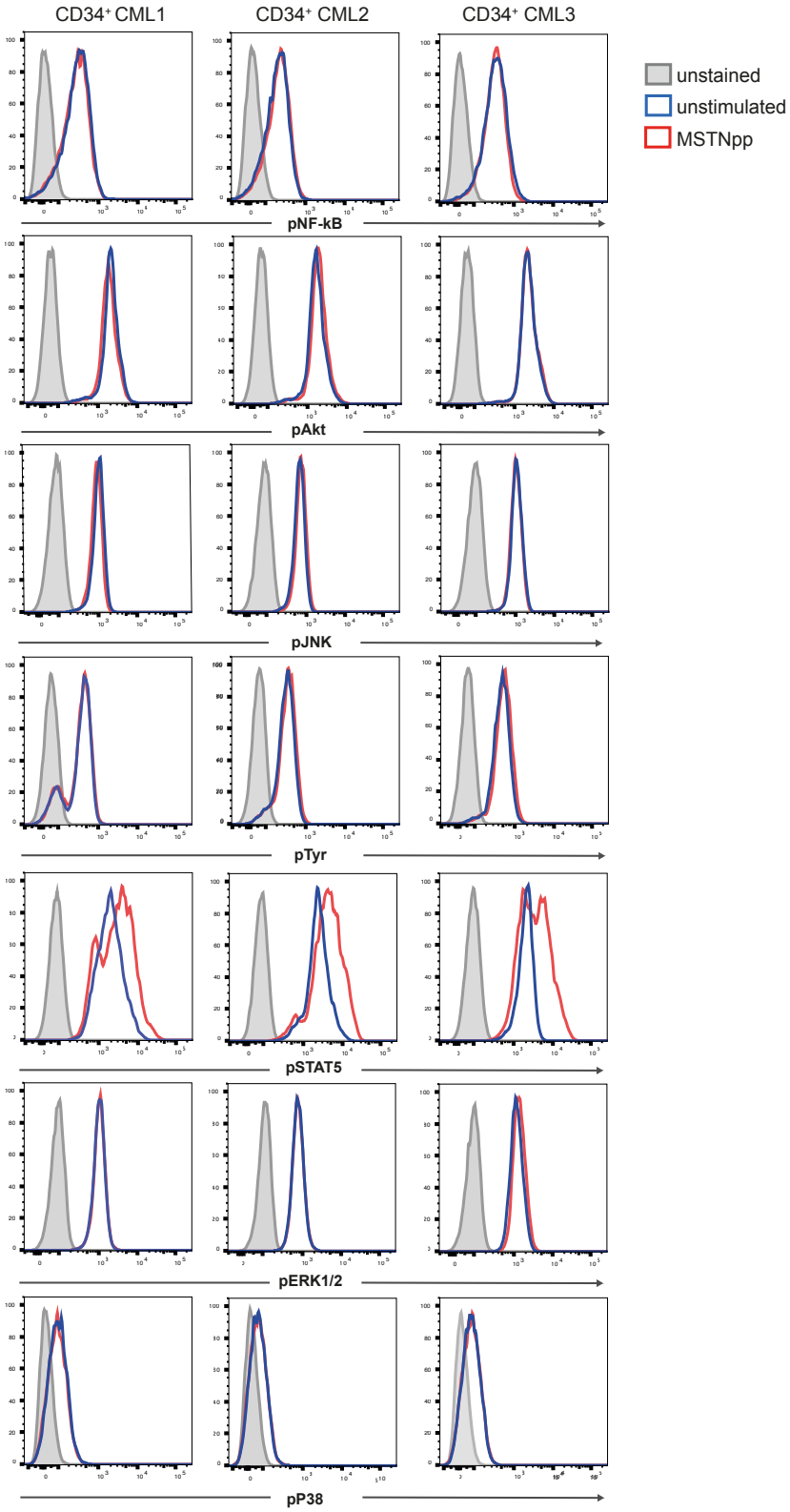

Figure S4. Histograms showing levels of pNF-κB, pAkt, pJNK, pTyrosine, pSTAT5, pERK1/2 and pP38 in CD34$^+$ cells from 3 individual chronic phase CML patients after 15-minute stimulation with 500ng/ml of MSTNpp, compared to unstimulated control cell. Unstained cells are shown in gray, unstimulated cells in blue and stimulated cells in red.

Supplementary Table S1: Clinical data of CML chronic phase patient samples

Patient #	Diagnosis	PB/BM	Gender	Age	Karyotype	Analysis
1	CML CP	BM	M	59	46,XY,t(2;9;22)(p22;q34;q11)	CFC-assay, RNA-seq, phospho-flow, co-culture
2	CML CP	PB	M	19	46,XY,t(9;22)(q34;q11)	RNA-seq, phospho-flow
3	CML CP	PB	M	73	46XY,t(9;22)(q34;q11)	384-well screen
4	CML CP	PB	M	71	45X,-Y, t(9;22)(q34;q11)	RNA-seq
5	CML CP	PB	F	32	46,XX,t(9;22;9;17)(q34;q11;q3?4;p12)	384-well screen
6	CML CP	PB	M	58	46,XY,t(9;22;16)(q34;q11;q13)	384-well screen, RNA-seq
7	CML CP	BM	F	65	46,XX,t(9;22)(q34;q11)	96-well screen, CFC-assay, phospho-flow, co-culture
8	CML CP	BM	M	78	46,XY,t(7;9;22)(q11;q34;q11)	96-well screen
9	CML CP	BM	M	44	46,XY,t(9;22)(q34;q11)	96-well screen
10	CML CP	PB	F	17	46,XX,t(9;22)(q34;q11)	MSTNpp culture
11	CML CP	PB	M	61	46,XY,t(9;22)(q34;q11)	phospho-flow
12	CML CP	PB	F	20	46,XX,t(9;22)(q34;q11)	CFC-assay, RNA-seq, MSTNpp culture, TGF-β1 culture, phospho-flow
13	CML CP	BM	F	59	46,XX,t(9;22)(q34;q11)/47,idem,+mar	phospho-flow
14	CML CP	BM	F	36	46,XX,t(9;22)(q34;q11)	MSC culture, RT-qPCR
15	CML CP	BM	M	73	46,XY,t(9;22)(q34;q11)	MSC culture, RT-qPCR
16	CML CP	BM	M	69	46,XY,t(9;22)(q34;q11)	MSC culture, RT-qPCR
17	CML CP	PB	F	30	46,XX,t(9;22)(q34;q11)	MSTNpp culture

Abbreviations: CML CP, chronic myeloid leukemia chronic phase; PB, peripheral blood; BM, bone marrow; M, male; F, female; CFC-assay, colony forming cell assay; RNA-seq, RNA-sequencing; phospho-flow, phospho-flow cytometry; MSTNpp, myostatin propeptide; RT-qPCR, reverse transcriptase quantitative PCR.

Supplementary Table S2: Cytokines included in the screen

PEPROTECH CATALOGUE NUMBER	RECOMBINANT PROTEINS
100-00AB	Human PDGF-AB
100-00CC	Human PDGF-CC
100-01	Human CD22
100-03	Human Heregulin beta-1
100-04	Human Epiregulin
100-05	Human IGF-BP5
100-06	Human PlGF-1
100-07	Human Prolactin
100-08	Human IGF-BP3
100-09	Human PTHrP
100-11	Human IGF-I
100-12	Human IGF-II
100-13A	Human PDGF-AA
100-14B	Human PDGF-BB
100-16A	Human TGF-alpha
100-17A	Human FGF-acidic
100-18B	Human FGF-basic
100-18C	Human FGF Basic (146AA)
100-19	Human KGF/FGF-7
100-20	Human VEGF 165
100-20A	Human VEGF 121
100-20B	Human VEGF-B
100-20C	Human VEGF-C
100-20D	Human VEGF-D
100-21	Human TGF-beta 1 (HEK293 cells)
100-21C	Human TGF-beta 1 (CHO-cell)
100-22A	Human SCGF-alpha

100-22B	Human SCGF-beta
100-23	Human FGF-9
100-25	Human FGF-8
100-26	Human FGF-10
100-27	Human FGF-17
100-28	Human FGF-18
100-29	Human FGF-16
100-30	Human FGF-6
100-31	Human FGF-4
100-32	Human FGF-19
100-34	Human FGF-5
100-35	Human TGF-beta 2 (Insect)
100-35B	Human TGF-beta 2 (Mammalian)
100-36E	Human TGF-beta 3 (E.coli)
100-39	Human HGF
100-41	Human FGF-20
100-42	Human FGF-21
100-44	Human EG-VEGF
100-45	Human Sonic Hedgehog (E.coli)
100-46	Human Prokineticin-2
100-47	Human HB-EGF
100-50	Human Betacellulin
100-51	Human Epigen
100-52	Human FGF-23
100-53	Human Klotho
100-55B	Human Amphiregulin (98 a.a)
100-56	Human PlGF-2
100-57	Human PlGF-3
100-58	Human Thrombomodulin
100-61	Human EGF-L7
110-01	Human sCD14
110-02	Human p16-INK4a
110-03	Human Sox2
110-10	Human Vimentin

120-00	Human Myostatin (GDF-8)
120-01	Human GDF-5/CDMP-1 (BMP-14)
120-02	Human BMP-2
120-03	Human BMP-7/OP-1
120-04	Human BMP-13/CDMP-2
120-05	Human BMP-4 (HeLa cells)
120-05ET	Human BMP-4 (E.coli cells)
120-06	Human BMP-6
120-07	Human GDF-2 (BMP-9)
120-09	Human TSG
120-10C	Human NOGGIN (Mammalian)
120-11	Human GDF-11(BMP-11)
120-12	Human Myostatin Propeptide
120-13	Human Follistatin
120-14	Human Activin A (Insect)
120-14E	Human Activin A (E.coli)
120-15	Human Activin B
120-16	Human CTGFL/WISP-2
120-17	Human WNT-1
120-18	Human WISP-1
120-19	Human CTGF (98 a.a)
120-20	Human WISP-3
120-21	Human Nanog
120-22	Human GDF-3
120-24B	Human BMP-3 (E.coli)
120-25	Human CYR61
120-26	Human NOV
120-28	Human GDF-15/MIC-1 (not recombinant)
120-29	Human sFRP-1
120-30	Human DKK-1
120-31	Human WNT-7a
120-35	Human Osteopontin
120-36	Human SPARC/Osteonectin

120-37	Human GDF-7
120-38	Human R-Spondin-1
120-39	Human BMP-5
120-40	Human BMP-10
120-41	Human GASP-1
120-42	Human Gremlin-1
120-43	Human R-Spondin-2
120-44	Human R-Spondin-3
120-46	Human DKK-3
130-01	Human MIA
130-02	Human MIA-2
130-03	Human OTOR
130-05	Human INSL5/INSL7 Hybrid
130-06	Human ANG-1
130-07	Human ANG-2
130-08	Human GLP-1 (7-36 a.a)
130-09	Human Visfatin
130-10	Human Relaxin-3
130-11	Human Vaspin
130-12	Human Maspin
130-13	Human PEDF
130-14	Human Neuroserpin
130-15	Human Relaxin-2
130-18	Human ANGPTL-3
130-20	Human C1 Inhibitor
140-04	Human PAI-1
140-06	Human PAI-2
140-07	Human sDLL-4
140-08	Human sDLL-1
140-09	Human Vitronectin
140-10	Human PAF-AH
140-13	Human Fetuin A/AHSG
140-14	Human Thymosin Beta 4

150-01	Human Endostatin
150-04	Human VCAM-1
150-05	Human ICAM-1
150-06	Human PECAM-1
150-11	Human Slit2-N
150-15	Human E-Selectin
150-16	Human VAP-1
150-17	Human Semaphorin 3A
150-18	Human Uteroglobin
160-01	Human TLR-3
160-02	Human FGFR1a (IIIc) Fc
160-03	Human FGFR2a (IIIc) Fc
200-01A	Human IL-1 alpha
200-01B	Human IL-1 beta
200-01RA	Human IL-1RA
200-02	Human IL-2 (1 x 100ug not avilable)
200-03	Human IL-3
200-04	Human IL-4
200-05	Human IL-5
200-06	Human IL-6
200-07	Human IL-7
200-08	Human IL-8 (77a.a) CXCL8
200-08M	Human IL-8 (72a.a) CXCL8
200-09	Human IL-9
200-10	Human IL-10
200-11	Human IL-11
200-12	Human IL-12
200-12P40	Human IL-12p40
200-12P80H	Human IL-12p80 (Insect cell)
200-13	Human IL-13
200-13A	Human IL-13 Variant
200-15	Human IL-15
200-16	Human IL-16 (130 a.a)
200-16A	Human IL-16 (121 a.a)

200-17	Human IL-17A (IL-17)
200-19	Human IL-19
200-20	Human IL-20
200-21	Human IL-21
200-22	Human IL-22
200-23	Human IL-23
200-24	Human IL-17E (IL-25)
200-25	Human IL-17F
200-27	Human IL-17D (IL-27)
200-28	Human IL-17B (IL-24)
200-31	Human IL-31
200-33	Human IL-33
200-34	Human IL-34
200-35	Human IL-24
200-36B	Human IL-36 beta (IL-1F8)
200-36G	Human IL-36 Gamma (IL-1F9)
200-36RA	Human IL-36RA
300-01A	Human TNF-alpha
300-01B	Human TNF-beta (E.coli)
300-02	Human IFN-gamma
300-02BC	Human IFN-beta (CHO)
300-02J	Human IFN-omega
300-02K	Human IFN-lambda2 (IL-28A)
300-02L	Human IFN-lambda1 (IL-29)
300-03	Human GM-CSF
300-04	Human MCP-1/MCAF (CCL2)
300-05	Human LIF
300-06	Human RANTES (CCL5)
300-07	Human SCF
300-08	Human MIP-1 alpha (CCL3)
300-09	Human MIP-1 beta (CCL4)
300-10	Human Oncostatin M (227 a.a)
300-10T	Human Oncostatin M (209 a.a.)
300-11	Human GRO/MGSA (CXCL1)

300-12	Human IP-10 (CXCL10)
300-13	Human Apo-SAA (1 x 100ug not available)
300-14	Human NAP-2 (CXCL7)
300-15	Human MCP-2 (CCL8)
300-16	Human PF-4 (CXCL4)
300-17	Human MCP-3 (CCL7)
300-18	Human TPO
300-19	Human flt3-Ligand
300-20	Human Lymphotactin (XCL1)
300-21	Human Eotaxin (CCL11)
300-22	Human ENA-78/CXCL5 (5-78 a.a)
300-22B	Human ENA-78/CXCL5 (8-78 a.a)
300-23	Human G-CSF
300-24	Human MCP-4 (CCL13)
300-25	Human M-CSF
300-26	Human MIG (CXCL9)
300-27	Human Leptin
300-28A	Human SDF-1 alpha (CXCL12)
300-28B	Human SDF-1 beta (CXCL12)
300-29	Human MIP-3 (CCL23)
300-29A	Human MIP-3 alpha (CCL20)
300-29B	Human MIP-3 beta (CCL19)
300-30	Human TARC (CCL17)
300-31	Human Fractalkine (CX3CL1)
300-32	Human Cardiotrophin-1
300-33	Human Eotaxin-2
300-34	Human MIP-4/PARC (CCL18)
300-35	Human Exodus-2 (CCL21)
300-36	Human MDC (67 a.a.) (CCL22)
300-36A	Human MDC (69 a.a.) (CCL22)
300-37	Human I-309 (CCL1)
300-38	Human HCC-1/CCL14 (72 a.a)
300-38B	Human HCC-1/CCL14 (66 a.a)

300-39	Human GRO-beta (CXCL2)
300-40	Human GRO-gamma (CXCL3)
300-41	Human GCP-2 (CXCL6)
300-42	Human NP-1
300-43	Human MIP-5 (CCL15)
300-44	Human LEC/NCC-4 (CCL16)
300-45	Human TECK (CCL25)
300-46	Human I-TAC (CXCL11)
300-47	Human BCA-1 (CXCL13)
300-48	Human Eotaxin-3 (CCL26)
300-49	Human BD-2
300-50	Human BRAK (CXCL14)
300-51	Human BD-1 (36 a.a)
300-51A	Human BD-1 (47 a.a)
300-52	Human BD-3
300-53	Human Apo-SAA1
300-54	Human CTACK (CCL27)
300-55	Human CXCL16
300-56	Human LD78-beta (CCL3L1)
300-57	Human MEC (CCL28)
300-58	Human LAG-1 (CCL4L1)
300-59	Human TFF2
300-60	Human TFF1
300-61	Human TFF3
300-62	Human TSLP
300-63	Human TAFA-2
300-65	Human BD-4
300-66	Human Chemerin
300-67	Human Nesfatin-1
300-69	Human MIF
310-01	Human sRANKL
310-02	Human sCD40Ligand/TRAP
310-03H	Human Fas Ligand (CHO cell)
310-04	Human sTRAIL/Apo2L

310-06	Human TWEAK
310-09B	Human LIGHT
310-10C	Human APRIL
310-11	Human 4-1BBL
310-13	Human BAFF
310-16	Human BCMA
310-17	Human TACI
310-22	Human AITRL
310-23	Human TL-1A
310-26	Human sCD23
310-27	Human HVEM-Fc
310-28	Human OX40 Ligand
310-29	Human sCD100
310-30	Human sCD27L
310-31	Human sCD34
350-02	Human ApoE3
350-04	Human ApoE4
350-05B	Human IGF-BP4
350-06B	Human IGF-BP2
350-07B	Human IGF-BP6
350-09	Human IGF-BP7
350-10	Human IGF-BP1
350-11	Human ApoA-I
350-12	Human ApoE2
410-01	Human TIMP-1
410-02	Human TIMP-2
450-01	Human beta-NGF
450-02	Human BDNF
450-03	Human NT-3
450-04	Human NT-4
450-05	Human CDNF
450-06	Human MANF
450-10	Human GDNF
450-11	Human Neurturin

450-12	Human Persephin
450-13	Human CNTF
450-14	Human Osteoprotegerin (OPG)
450-15	Human Pleiotrophin (PTN)
450-16	Human MIDKINE
450-17	Human Artemin
450-18	Human NNT-1/BCSF-3
450-19	Human Resistin
450-20	Human gAcrp30/Adipolean Variant
450-21	Human gAcrp30/Adipolean
450-22	Human RELM beta
450-24	Human Adiponectin
450-36D	Human Neuritin
450-37	Human GMF-beta
450-38	Human Galectin-3
450-39	Human Galectin-1
450-42	Human sCD30 Ligand

Supplementary Table S3: Upregulated and downregulated genes in primary $CD34^{+}$ $CD38^{low}$ chronic phase CML samples following 3 and 24 hours of MSTNpp stimulation

Supplementary Table S3 is uploaded as a separate file.