The quiescent fraction of chronic myeloid leukemic stem cells depends on BMPR1B, Stat3 and BMP4-niche signals to persist in patients in remission

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The quiescent fraction of chronic myeloid leukemic stem cells depends on BMPR1B, Stat3 and BMP4-niche signals to persist in patients in remission

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Supplemental figures legends

Supplemental Figure 1. (A) Beeswarm plots of *ID1*, *ID2*, *RUNX1* and *RUNX2* expression between group-A *BCR-ABL*$^+$ SCs from patients at remission (light blue; *n* = 122) and group-B *BCR-ABL*$^+$ SCs from patients at remission (purple; *n* = 123). Numbers of cells analyzed and numbers showing amplification for the selected genes are shown below the plot. The average gene expression level is indicated by red squares, and the boxes represent the median and quartiles of gene expression levels. *P*-values were calculated using the non-parametric Wilcoxon test. (B) Heat map shows hierarchical clustering of *ID1* and *RUNX1* genes in BMPR1B$^+$ cells (*n* = 8) from group-A *BCR-ABL*$^+$ SCs (left) or group-B *BCR-ABL*$^+$ SCs (right). (C) Heat map shows hierarchical clustering of IL2_Stat5 signaling pathway genes in BMPR1B$^+$ cells (*n* = 8) from group-A *BCR-ABL*$^+$ SCs (left) or group-B *BCR-ABL*$^+$ SCs (right).

Supplemental Figure 2. (A) *CD34, CD38, CD123, TPOR, BMPR1B, NANOG, c-Myc, FOXO1, FOXO3a, HIF2A, ALOX5, TWIST1, BCL2 and BCL-XL* mRNA levels from TF1-BAP cells. TF1-BA cells were used as comparators for each sample; N = 4. T-test significant *P*-values are indicated by red asterisks in the figure. (B) Dot plot showing BMP4 secretion, analyzed by ELISA, from TF1-BA and TF1-BAP supernatants.

Supplemental Figure 3. (A) Representative pictures of myeloid-derived (top), Erythroid-derived (middle) or mixed (bottom) CFC colonies from primary Normal Bone Marrow (NBM) CD34+, TF1 WT, TF1 BA or TF1-BAP cells. (B) Representative pictures derived LTC-IC colonies from TF1 BA or TF1-BAP cells.

Supplemental Figure 4. (A) Western blots showing BCR-ABL, CRKL and SMAD1/5/8 phosphorylation levels in TF1-BA and TF1-BAP cells following 2 h of TKI treatment; Scatter plot showing P-BCR-ABL/BCR-ABL ratio and P-CRKL (GAPDH used as internal control), n = 5-6. (B) Western blots showing Stat3 phosphorylation (Tyr705) levels (relative to Stat3) in TF1-BA and

TF1-BAP cells; Scatter plot showing P-Stat3/Stat3 ratio (GAPDH used as internal control) n = 3.

Supplemental Figure 5. (A) TF1-BAP cells treated with AG490 (25 μM) for 24 h and analyzed for their content of P-Smad1/5/8 (left) or P-Stat3 (right). (B-C) TF1-BAP cells treated with Imatinib (IM) (2 μM) or AG490 (25 μM) or E6201 (100 nM) for 24 h and analyzed for their P-CRKL (B), P-ERK (B) or cleaved caspase3 (C) contents. (D) TF1-BAP cells treated with AG490 (25 μM) + E6201 (100 nM) for 24 h and analyzed for their P-Smad1/5/8 (left) or P-Stat3 (right) contents.

Supplemental Figure 6. (A-B) ELISA quantification of BMP2 (A) and BMP4 (B) in BM plasma from healthy donors, CML patients at diagnosis and CCyR patients (at remission). Results from individual samples are expressed in picograms per milliliter, and horizontal lines represent mean values ± SEM of the indicated number of analyzed samples. (C) Mesenchymal stem cells from normal bone marrow donors were treated with Imatinib, AG490 or E6201 for 3 days. Comparative transcriptional expression of BMP4 gene, results from 2 individual samples analyzed are expressed in arbitrary units, and the horizontal line represents mean values ± SEM, determined compared to untreated controls. Unpaired t-test significant *P*-values are indicated by asterisks in the figures. (D) TF1-BA and TF1-BAP cells treated or not with BMP4 (20 ng/ml for 24 h) were analyzed for their content in P-Stat3.

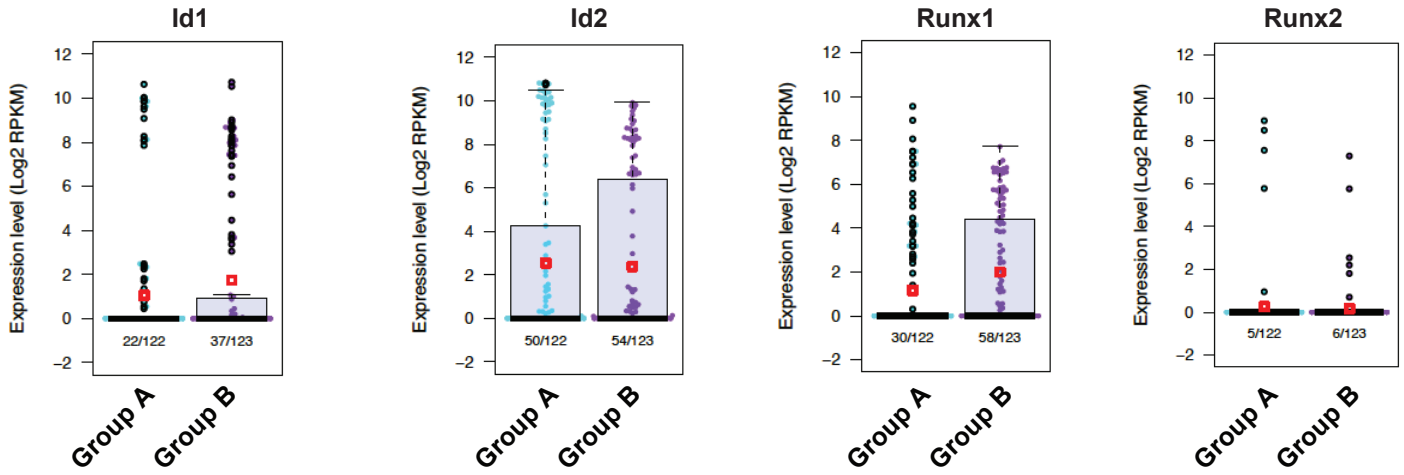
Supplemental Figure 7. (A) ELISA quantification of BMP4 in supernatants from HS5 and HS27A MSC cells. (B) TF1-BAP cells were seeded on top of a HS5 layer and then treated for 3 days with Imatinib (IM) (2 μM), with or without AG490 (25 μM) + E6201 (100 nM). Then TF1-BAP (GFP tagged) cells adherent fraction was harvested and analyzed for their content of Ki67-PE. Unpaired t-test significant *P*-values are indicated in the figure, $P<0.05$, *$P<0.01$, **

Supplemental Table1. Results from BMP signaling pathway GSEA between group-A BCR-ABL+ SCs from patients at remission and group-B BCR-ABL+ SCs from patients at remission.

Supplemental Table 2. Chemical structure of E6201 (Top) and IC50 on top target genes with 100nM E6201 (Bottom).

Supp Figure 1

A



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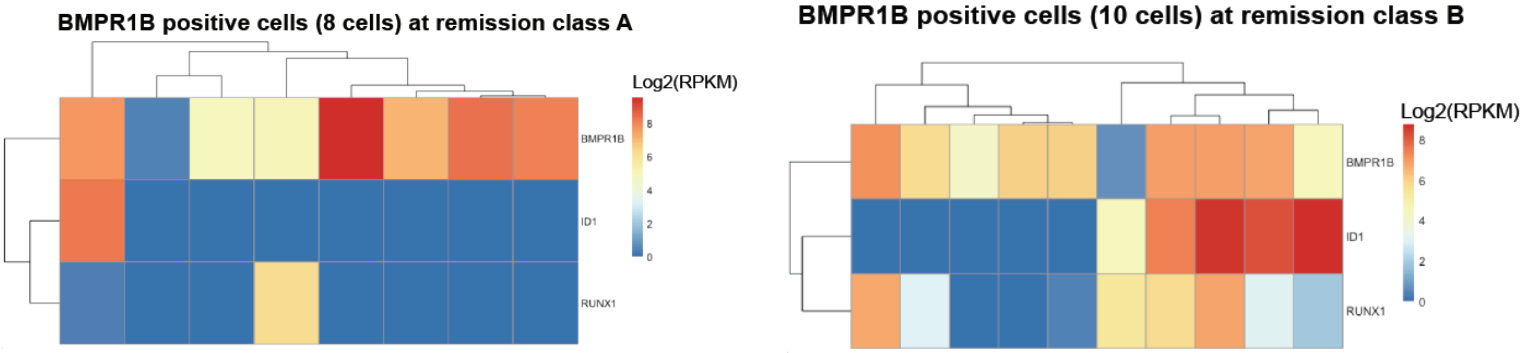

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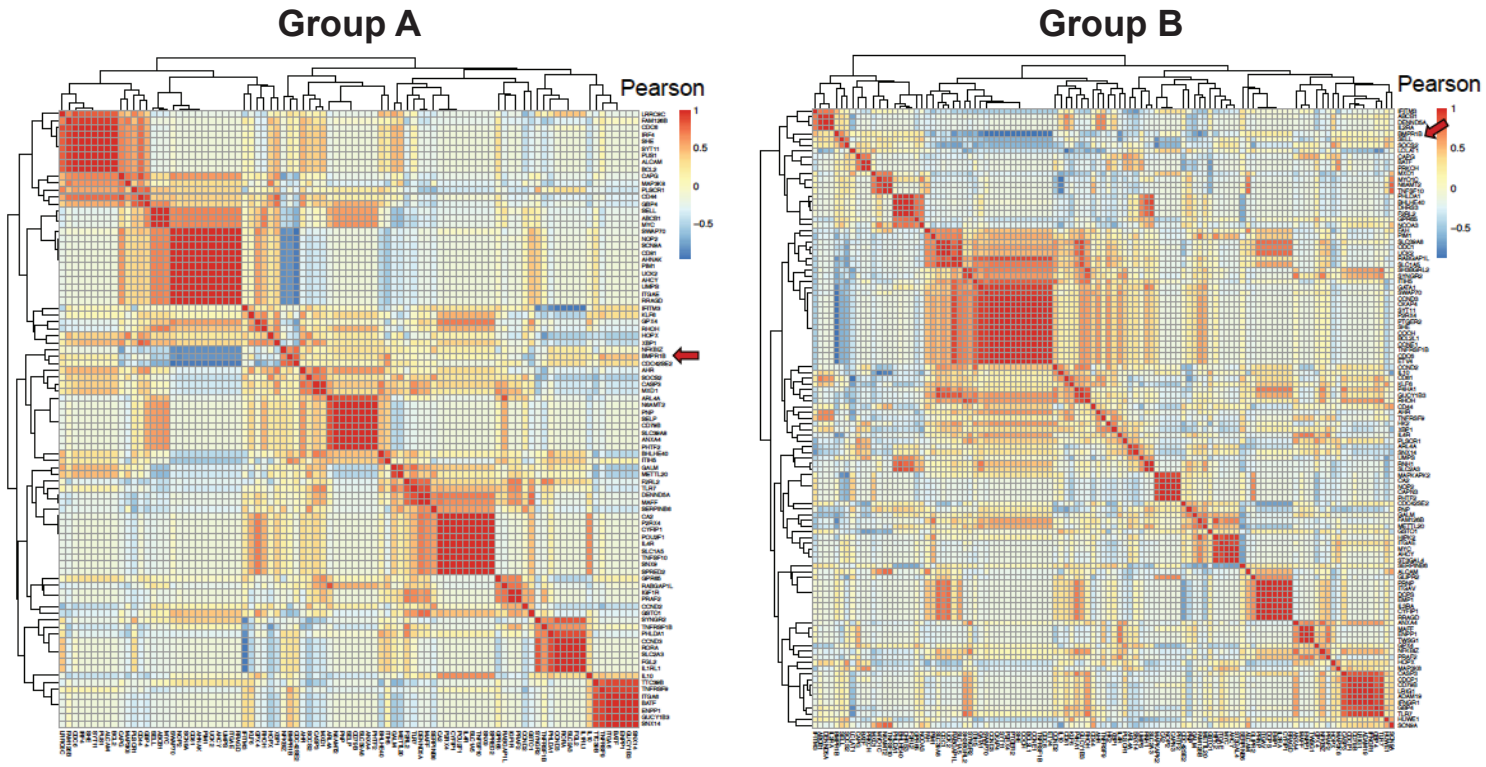

Supp Figure 2

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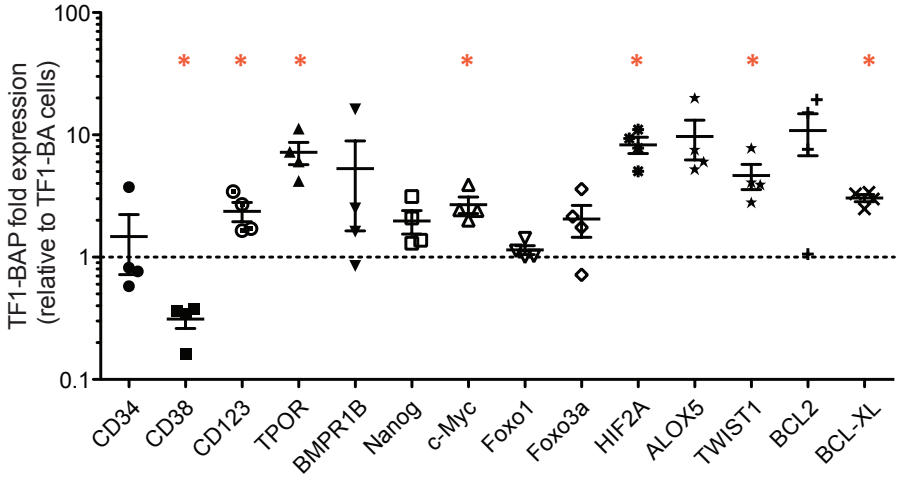

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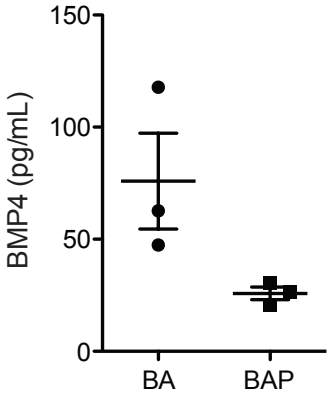

Supp Figure 3

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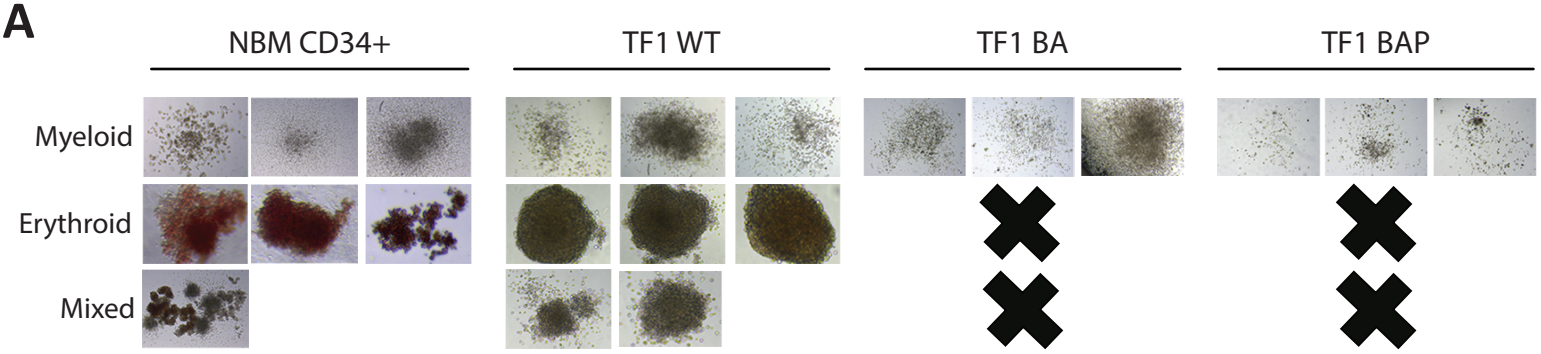

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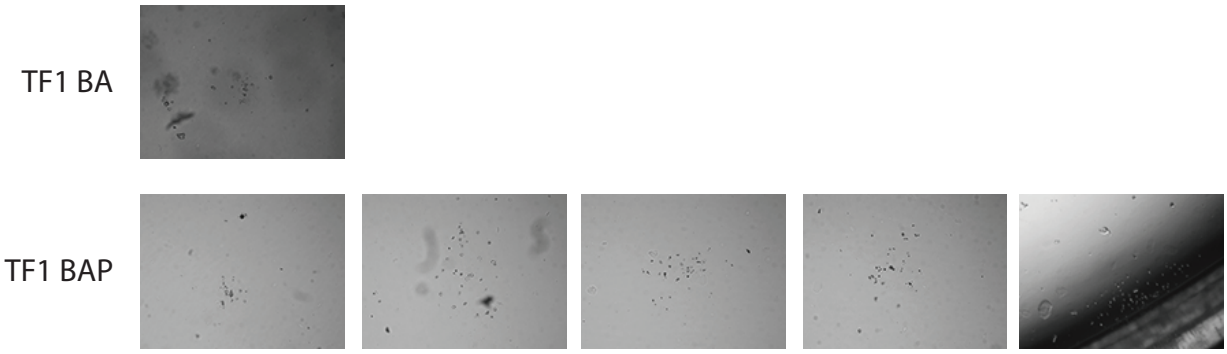
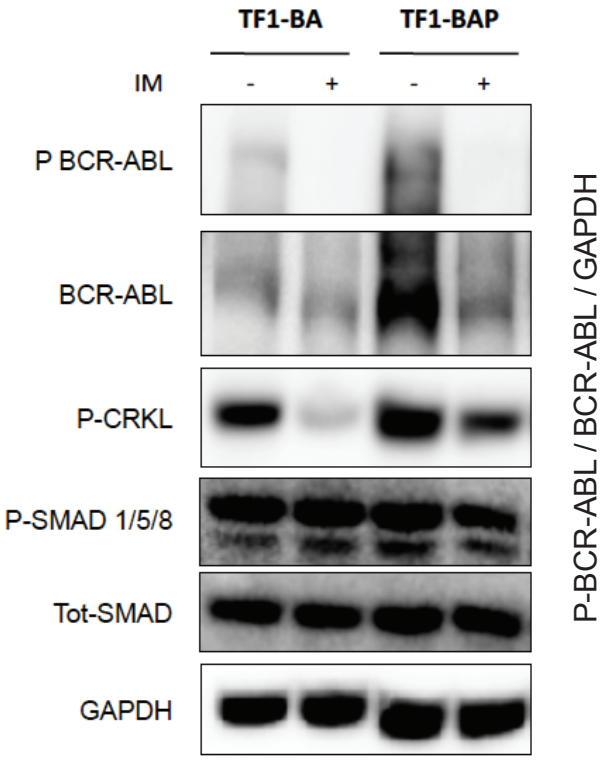
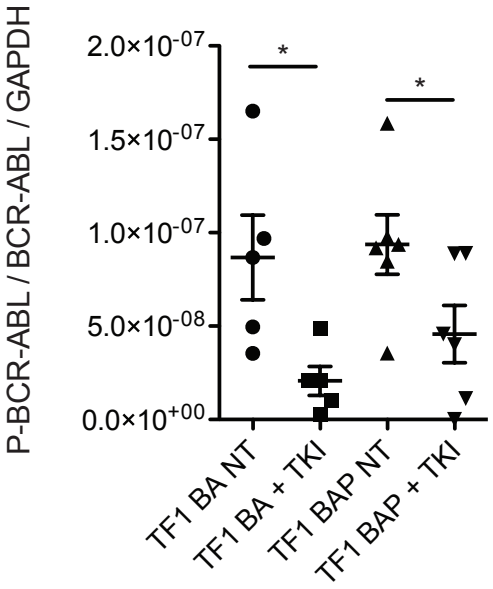
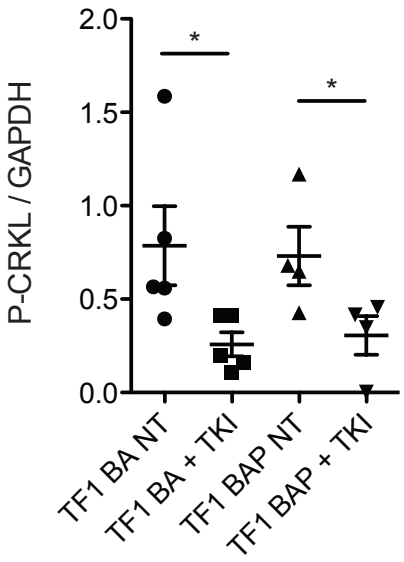


Supp Figure 4

A







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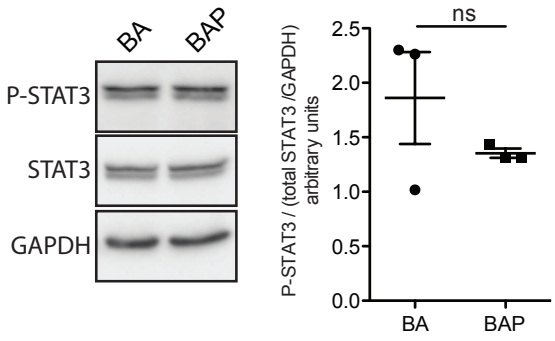
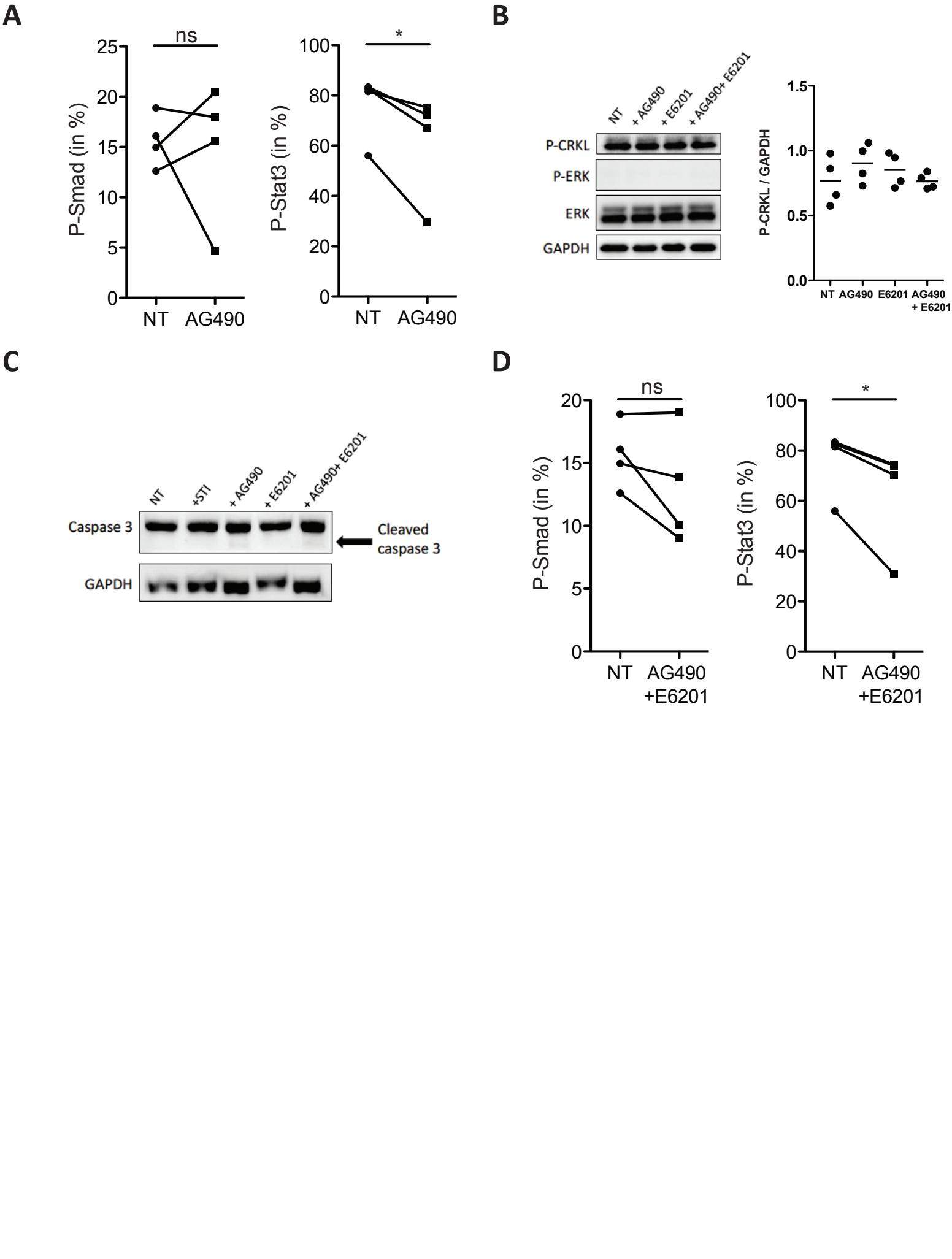
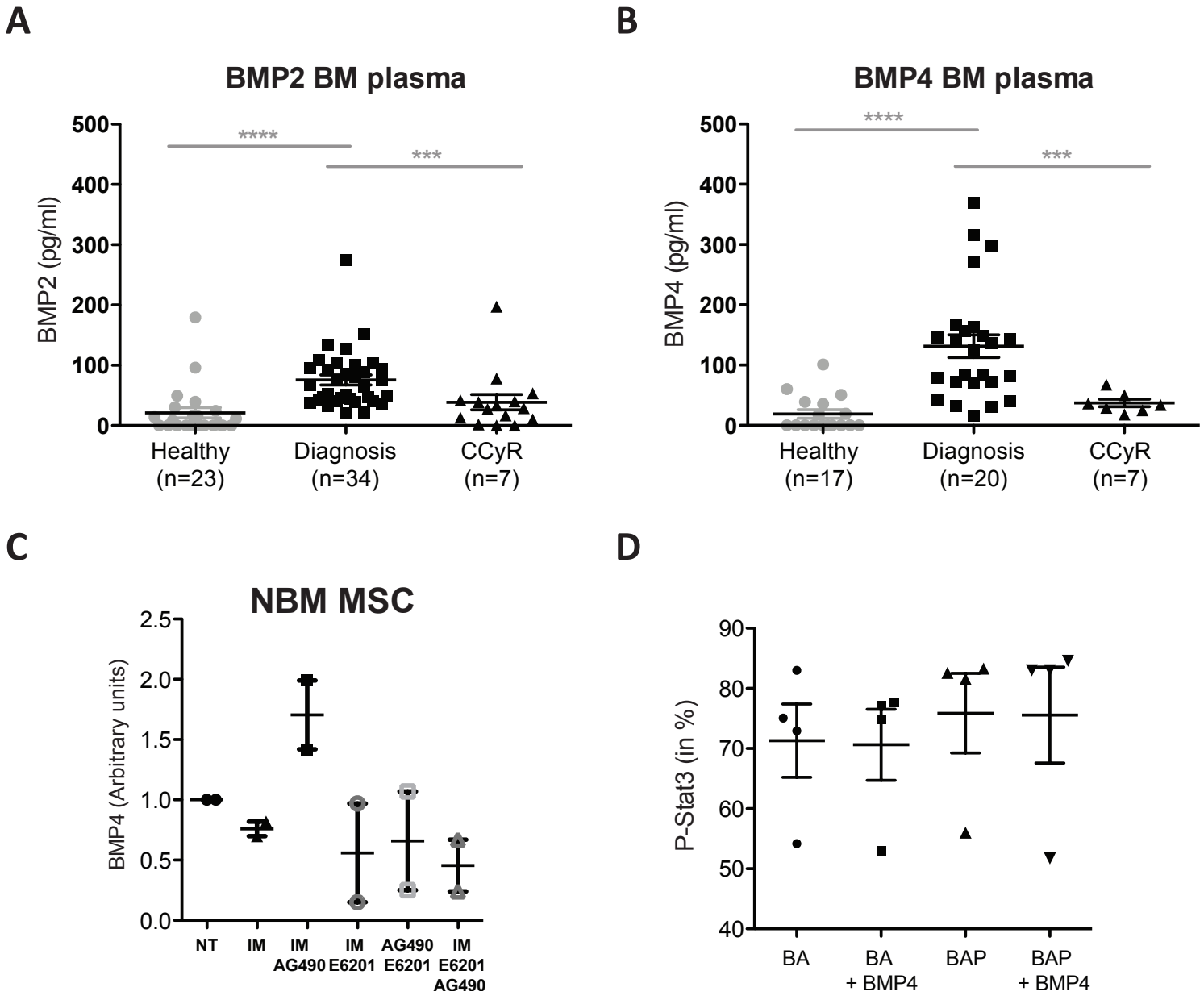


Supp Figure 5

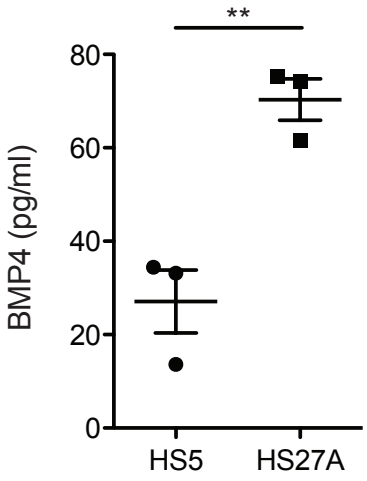


Supp Figure 6



Supp Figure 7

A



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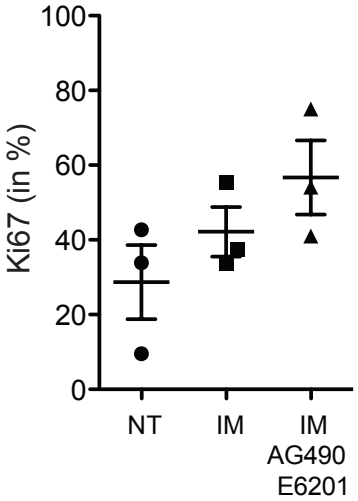
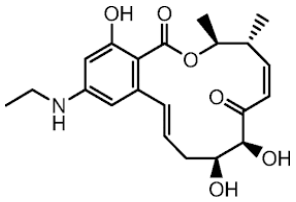


Supp Table S1. BMP Signaling pathway GSEA

PROBE	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
NOG	226	0,1885	0,0596	Yes
ID2	246	0,1850	0,1269	Yes
SMAD2	505	0,1511	0,1714	Yes
ACVR2B	618	0,1425	0,2190	Yes
BMP7	813	0,1303	0,2586	Yes
SMAD5	904	0,1269	0,3014	Yes
BMP8A	1382	0,1102	0,3215	Yes
ACVR2A	1395	0,1099	0,3614	Yes
RUNX3	1783	0,0991	0,3812	Yes
RUNX2	1789	0,0990	0,4174	Yes
ID3	1879	0,0962	0,4490	Yes
SMURF1	2069	0,0920	0,4747	Yes
ACVRL1	2663	0,0864	0,4811	Yes
BMPR1B	2892	0,0828	0,5017	Yes
SMAD7	3279	0,0756	0,5130	Yes
BMP2K	4437	0,0559	0,4839	No
TWSG1	5742	0,0344	0,4407	No
SMAD3	6223	0,0271	0,4301	No
RUNX1	6764	0,0195	0,4142	No
ACVR1B	7284	0,0132	0,3968	No
DAND5	7559	0,0102	0,3888	No
CHRDL1	7964	0,0065	0,3739	No
BMPR2	8283	0,0042	0,3618	No
BAMBI	8780	0,0000	0,3405	No
BMP15	8805	0,0000	0,3395	No
BMP2	8806	0,0000	0,3395	No
BMP5	8807	0,0000	0,3395	No
BMP6	8808	0,0000	0,3395	No
BMPER	8810	0,0000	0,3394	No
BMPR1A	8811	0,0000	0,3394	No
CER1	9171	0,0000	0,3241	No
CHRDL2	9198	0,0000	0,3230	No
GREM1	10032	0,0000	0,2873	No
SMAD6	13175	0,0000	0,1526	No
TNFRSF11B	13609	0,0000	0,1341	No
TNFSF11	13611	0,0000	0,1340	No
TWIST1	13699	0,0000	0,1303	No
ID1	14417	-0,0062	0,1018	No
SMAD9	16035	-0,0280	0,0429	No
SMAD1	16926	-0,0428	0,0204	No
SMURF2	17163	-0,0471	0,0277	No
BMP4	19096	-0,0836	-0,0244	No
FST	20088	-0,0944	-0,0321	No
SMAD4	20464	-0,1029	-0,0103	No
BMP1	22496	-0,1694	-0,0351	No
ENG	22856	-0,1986	0,0226	No

Supp Table S2

E6201



Gene	IC50@100nM
MEK1	0,1
MEK2	0,1
BMPR1B	0,45
BMPR1A	36
BMPR2	77
JAK1	89
JAk2	100