SUPPLEMENTAL DATA

Supplemental material.

Lysyl oxidase enzyme activity assay: LOX enzyme activity was measured as described in (1) by determining the production of hydrogen peroxidase by the oxidation of Amplex Red (Invitrogen, cat. A12222). Samples (media supernatant from cell cultures) were prepared in a final volume of 2.0 ml containing 1.2 M urea, 50 mM sodium borate (pH 8.2), 1 unit/ml of horseradish peroxidase, 10 μM Amplex Red and 10 mM 1,5-diaminopentane and were incubated at 37°C for 30 min. Samples were placed on ice and fluorescence was measured using a fluorescence spectrophotometer with excitation and emission wavelengths 567 and 584 nm respectively. Parallel assays were prepared with 500 mM BAPN to completely inhibit the activity of LOX and the difference in emission intensity was recorded. The amount of hydrogen peroxide produced by the action of LOX was determined by comparing fluorescence changes to a standard plot, relating fluorescence change to nmoles of hydrogen peroxide added to assays lacking LOX. The value corresponding to the amount of hydrogen peroxide produced was then normalized to total protein.

Iodination of PDGF-BB and binding of ^{125}I-PDGF-BB to cell surfaces: PDGF-BB was iodinated using the Bolton-Hunter reagent as previously described in (2). Binding of ^{125}I-PDGF-BB was measured in bone marrow cultures (isolated from wild type FVB mice) stimulated with 25ng/ml TPO in IMDM media with 10% FBS for a duration of three days. Next, cells were washed with cold binding buffer (25 mM Hepes, pH 7.4, 125 mM NaCl, 5 mM $MgSO_4$, 5 mM KCl and 1 mM $CaCl_2$, containing mg/ml BSA) and incubated in binding buffer at 4 ^{o}C for 30 min. The binding buffer was aspirated and fresh binding buffer was added at the different concentrations of ^{125}I-PDGF-BB for additional 4 h. After washing, cells were solubilized with 1% Triton X-100, 10% glycerol, 25 mM Hepes, pH 7.5, 1mg/ml BSA. Radioactivity was measured on a gamma counter. Non specific labeling was determined in the presence of unlabeled PDGF-BB. This experiment was performed in quadruplicates.

Immunohistochemistry: Paraffin-embedded spleen sections were blocked with avidin/biotin solutions (Vector Laboratories, cat. SP-2001), followed by blocking with 10% normal goat serum for 1 hour. Next, sections were stained with 5 μg/ml of rabbit polyclonal anti-CD41 (diluted 1000 fold) (Santa Cruz Biotechnology, cat. sc-15328) or rabbit IgG (Santa Cruz Biotechnology, cat. sc-2027) at 4 ^{o}C overnight. Labeling with rabbit secondary antibody and alkaline phosphatase method was performed according to the manufacturer's instructions (Vectastain, cat. Ak-5001). Finally, sections were develop using the alkaline phosphatase substrate kit (Sk-5100) followed by counterstaining with hematoxylin. Images were obtained at 200x original magnification using a Nikon eclipse 50i microscope and Spot imaging software (Insight Firewire).

Computational analysis of the LOX promoter region for predicted transcription factor binding sites. Regulatory Sequence Analysis Tools (RSAT, http://rsat.ulb.ac.be/) was used to predict binding sites for selected candidate transcription factors. A list of potential factors was selected based on (Yu, M. et al. 2009). Analysis was performed on 5 kb upstream of the transcription start site (TSS) for mouse LOX (GeneBank accession number: NM_010728) and human LOX locus (GeneBank accession number: NM_001178102). Predicted binding sites for GATA-1, RUNX-1 and Msx-1 (GATA-1 co-repressor) are shown in detail in a table format (Supplemental figure 8).

Supplemental Table 1. Comparison of wild type vs GATA-1^{low} MK properties. % MK was determined via FACS analysis of CD41-FITC stained BM cells, total MK number was calculated based on total BM cell count and %MKs and MK size was determined by the mean FSC-H value of CD41-FITC stained cells via FACS. Standard deviation bars represent the mean of n=6 mice per group (Wild type/BAPN group n=4 mice), *$p<0.05$.

Supplemental Table 2. Hematological parameters following *in vivo* administration of BAPN into control and GATA-1^{low} mice. Blood was collected via heart puncture and blood cell number was assessed on a Hemavet blood analyzer. Statistical analysis was performed to compare control and BAPN-treated mice for each genotype (wild type, GATA-1^{low}). Hct= hematocrit, RBC=red blood cells, WBC=white blood cells. Standard deviation bars represent the mean of n=6 mice per group (Wild type/BAPN group n=4 mice), *$p<0.05$ only for white blood cells in the GATA-1^{low} mice, but still within a normal range in mice.

Supplemental Figure 1: Immunofluorescence analysis of flow cytometry sorted MKs based on ploidy level. MKs were sorted as described in Methods. The identity of the sorted fractions (2N-4N and ≥8N MKs) was verified by immunofluorescence analysis. The 2N-4N fraction contains CD41-labeled cells (green) with a significantly smaller nucleus (stained with DAPI) as compared to the polyploid (≥8N) MK fraction.

Supplemental Figure 2: Ploidy analysis of LOX-inhibited and PDGF-treated MKs. A. Representative ploidy profiles are shown in histogram plots per treatment group as described in methods.. **B.** Summary of MK ploidy status per treatment group. Standard deviation bars represent the mean of ten independent experiments. No statistical difference was found when student t-test was applied (n=10, $p>0.05$).

Supplemental Figure 3: LOX enzymatic activity in purified MKs. Murine BM cells were isolated and cultured in IMDM without phenol red, with 10% BCS and 25 ng/ml TPO. 2 days post plating, MKs were claimed by MACS column purification and were cultured for an additional 2 days in 2 ml IMDM without phenol red, with 10% BCS and 25 ng/ml TPO. This procedure allowed us to obtain enough MKs for purification and enzyme assay. LOX is typically released out of cells, where it is active. Supernatant from MKs was then claimed and processed for LOX activity assay in the presence or absence (-/+) of 500 μM BAPN (LOX inhibitor) to assess for LOX specific activity, as described in methods. As shown, about half of the total of hydrogen peroxide detected is attributed to LOX activity. Data are averages, ± S.D of 3 independent experiments. Statistical analysis was applied using the Student's t-test, *$p<0.05$.

Supplementary figure 4: Effect of LOX inhibition on MK proliferation. Bone marrow was harvested and cultured in serum free media with 25 ng/ml PDGF-BB and in the presence or absence of 100 μM BAPN. Bone marrow was collected and MKs were isolated after 12 and 24 hours of treatment. Western blot analysis was performed on protein extracts using PCNA (Oncogene, cat.NA03) as a proliferation marker. Probing with anti-β-actin is used as loading control.

Supplemental Figure 5: Effect of LOX inhibition on PDGF-BB binding to MKs. Binding of PDGF-BB to a MK enriched fraction was evaluated as described under methods. Bars are the standard deviation of the mean value from 4 determinations. The 3000 pM PDGF-BB corresponds to 0.09 ng/ml of PDGF-BB, which is well in the physiological range.

Supplemental Figure 6. **Effect of LOX inhibition on GATA-1^{low} mice *in vivo*.** A. Growth curve analysis of BAPN-treated mice. Mice treated with BAPN or vehicle in the drinking water (0.2%/vol) were weighted every 7 days for the duration of 10 weeks. B. Effect of LOX inhibition on GATA-1^{low} on MK ploidy *in vivo*. Bone marrow was isolated and ploidy profile was determined by flow cytometry analysis as described in methods. Standard deviation bars represent the mean of n=6 mice per group (Wild type/BAPN group n=4 mice). No statistical difference was found when the student t -test was applied comparing treatments within the same genotype (Wild type, GATA-1^{low}).

Supplemental Figure 7. Immunohistochemistry analysis of CD41 stained cells. Spleen sections were stained as described in methods. IgG isotype was used as negative control. MKs are readily recognized by the large nucleus and morphology (hematoxylin stained). This is further verified by the CD41 immunostaining (an MK marker), represented by the red signal (see arrows).

Supplemental Figure 8. Predicted transcription factor binding sites in LOX promoter genes. Regulatory Sequence Analysis Tools (RSAT) was used to predict binding sites for selected candidate transcription factors. **A**. Promoter analysis of the 5 kb upstream of transcriptional start site (TSS) of the mouse LOX gene locus. **B.** Promoter analysis of the 5 kb upstream of transcriptional start site (TSS) of the human LOX gene locus.

Supplemental references:

1. Palamakumbura, A. H., and Trackman, P. C. (2002) *Anal Biochem* **300**, 245-251
2. Lucero, H. A., Ravid, K., Grimsby, J. L., Rich, C. B., DiCamillo, S. J., Maki, J. M., Myllyharju, J., and Kagan, H. M. (2008) *J Biol Chem* **283**, 24103-24117

Supplemental table 1.

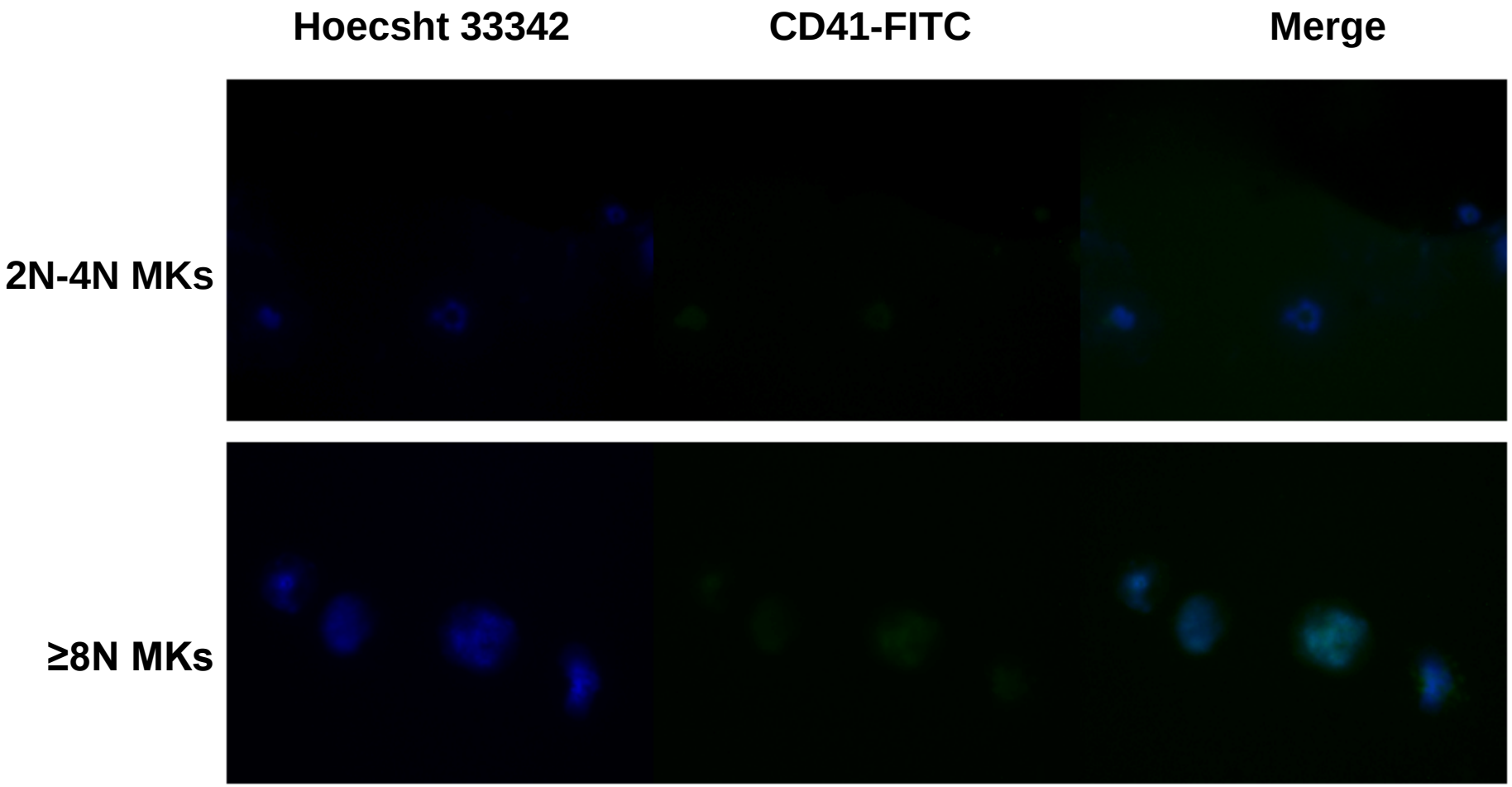
Comparison of Gata-1^{low} and wild type MK parameters

	%MKs	**total BM cells (x10^6)**	**total MK (x10^4)**	**MK size (mean FSC-H)**
Wild type	0.312	54.7	17.118	300.36
Gata-1^{low}	0.982	45.3	44.211	250.266
p value	<0.0001	0.068	<0.0001	0.007

Supplemental table 2.

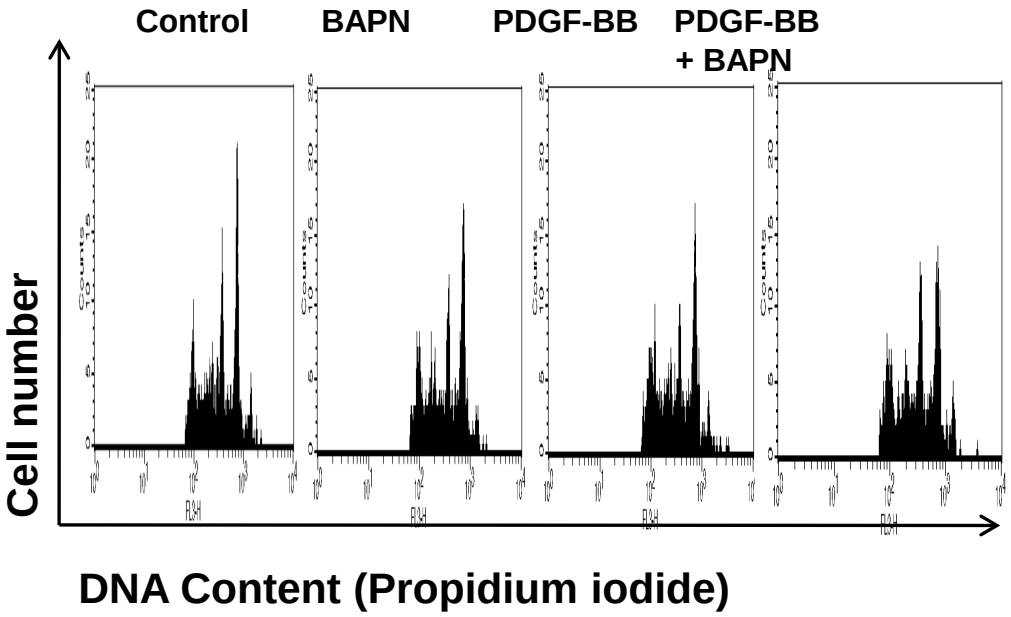
	Wild type				GATA-1^{low}			
	Control	STDEV (±)	BAPN	STDEV (±)	Control	STDEV (±)	BAPN	STDEV (±)
Hct %	42.10	3.32	44.23	1.45	33.43	12.80	37.88	6.19
RBC 10^{12}/L	8.93	0.58	9.35	0.27	6.30	2.28	6.80	1.15
WBC 10^{9}/L	4.27	1.47	7.53	2.70 *	3.31	1.39	4.17	0.90
Differential counts, %								
Neutrophils	23.96	3.63	23.95	3.61	20.27	7.32	22.09	2.93
Lymphocytes	68.62	3.64	68.34	9.48	73.23	8.15	70.36	2.91
Monocytes	5.79	1.64	3.87	1.77	5.77	1.56	6.14	1.75
Eosinophils	1.28	0.81	2.94	3.32	0.65	0.64	0.97	1.07
Basophils	0.35	0.30	0.91	1.08	0.08	0.09	0.44	0.48
Platelets 10^{9}/L	701.17	93.43	717.00	144.10	110.00	37.64	124.67	53.61
MPV 10^{-15}L	4.33	0.15	4.53	0.36	6.27	0.57	6.47	0.32

Supplemental figure 1.



Supplemental figure 2.

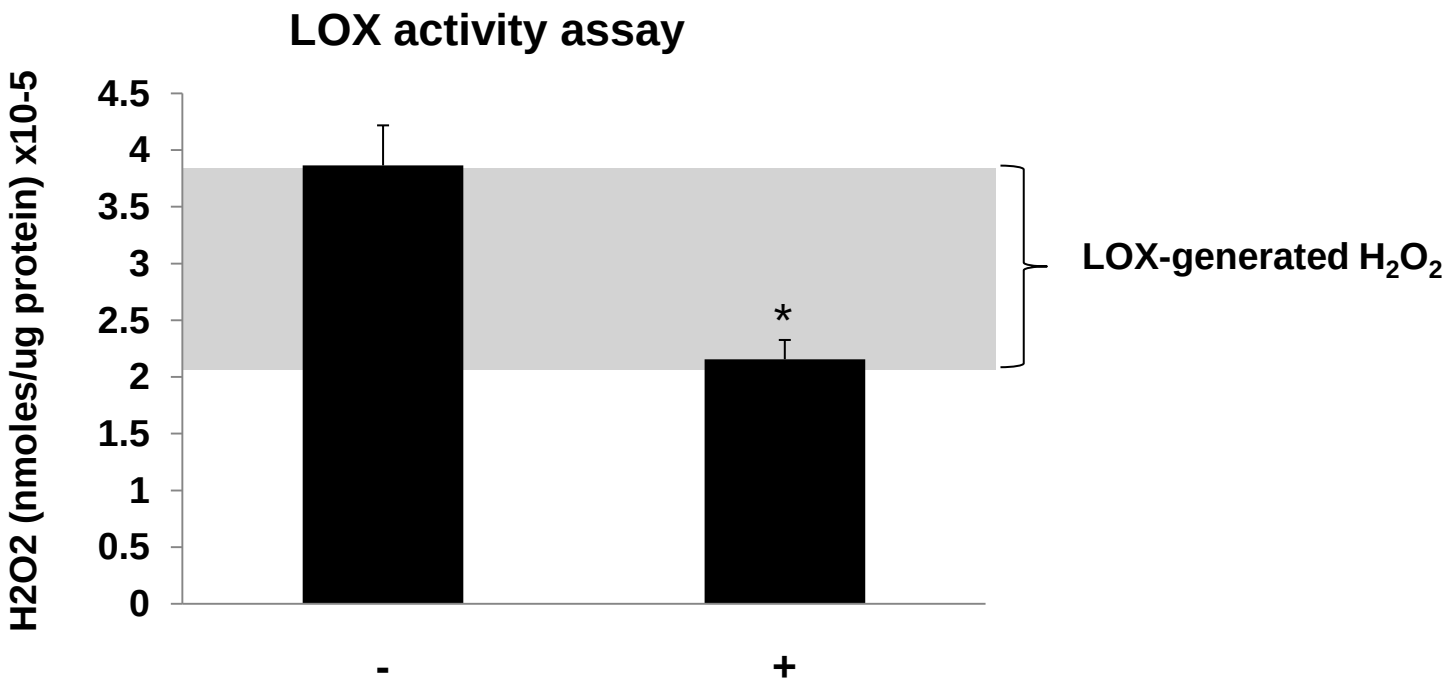
A.



B.

Treatment \ Ploidy	% 2N-4N	STDEV (±)	% 8N-64N	STDEV (±)
Control	26.70	3.52	58.12	4.09
BAPN	26.81	4.09	57.62	3.73
PDGF-BB	27.87	4.24	56.99	4.46
PDGF-BB + BAPN	27.70	4.10	56.47	4.32

Supplemental figure 3.



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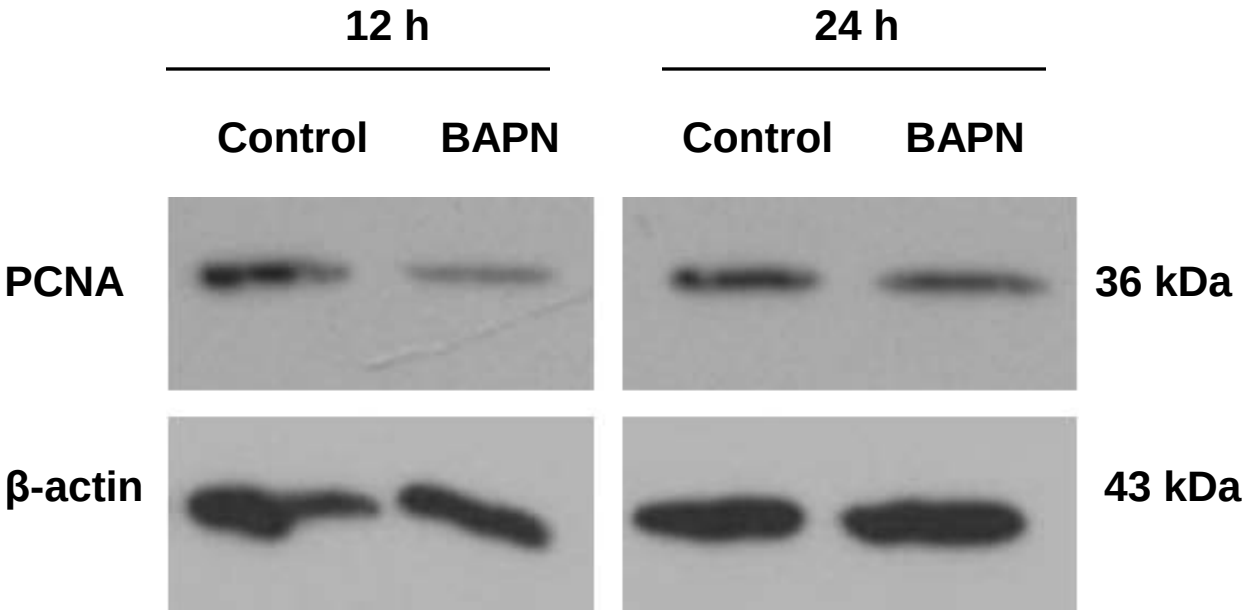

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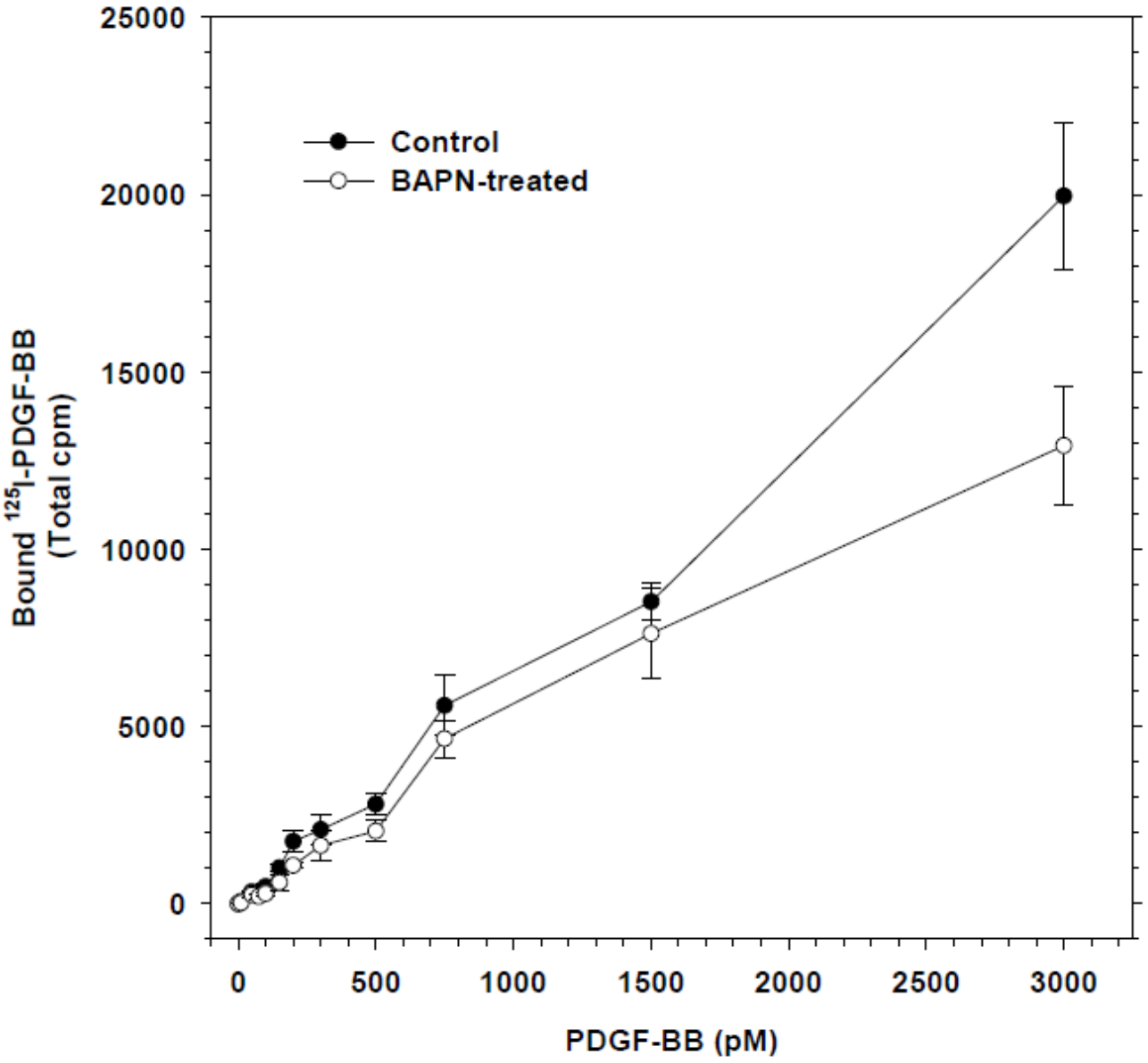
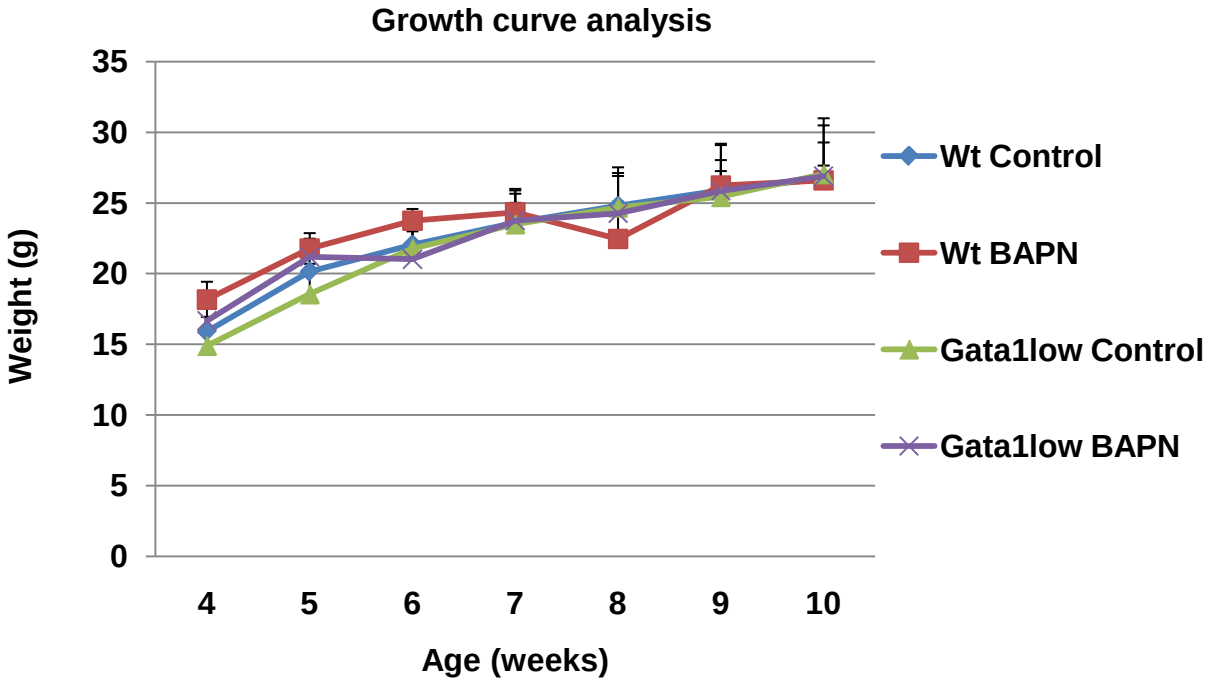


Supplemental figure 6.

A.



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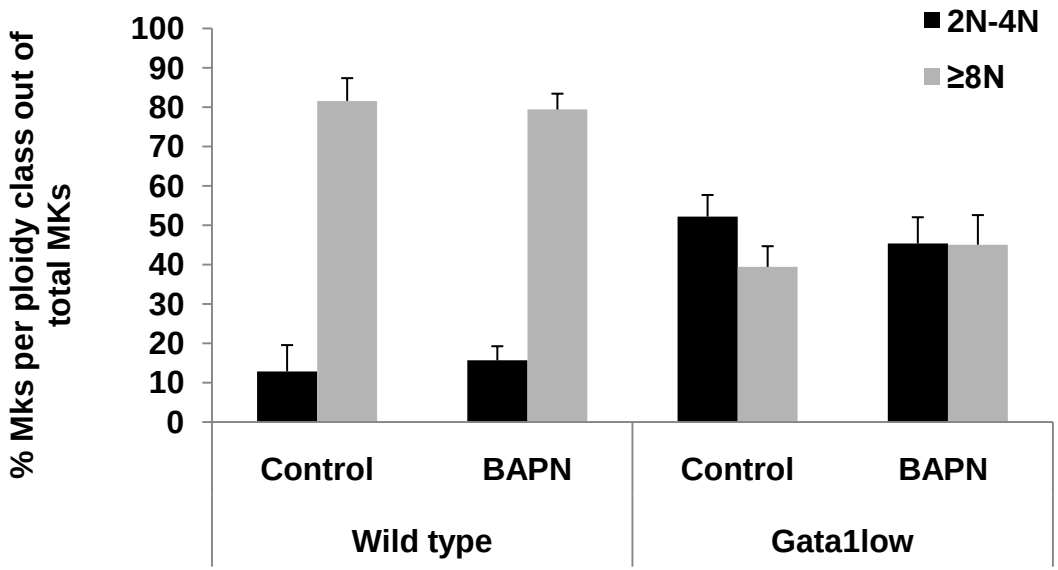

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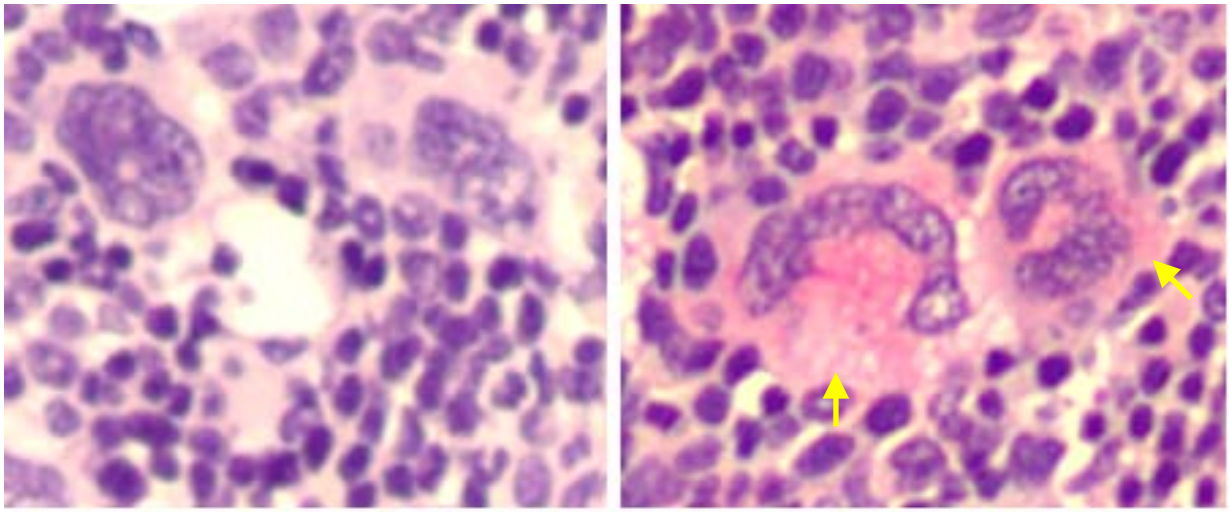

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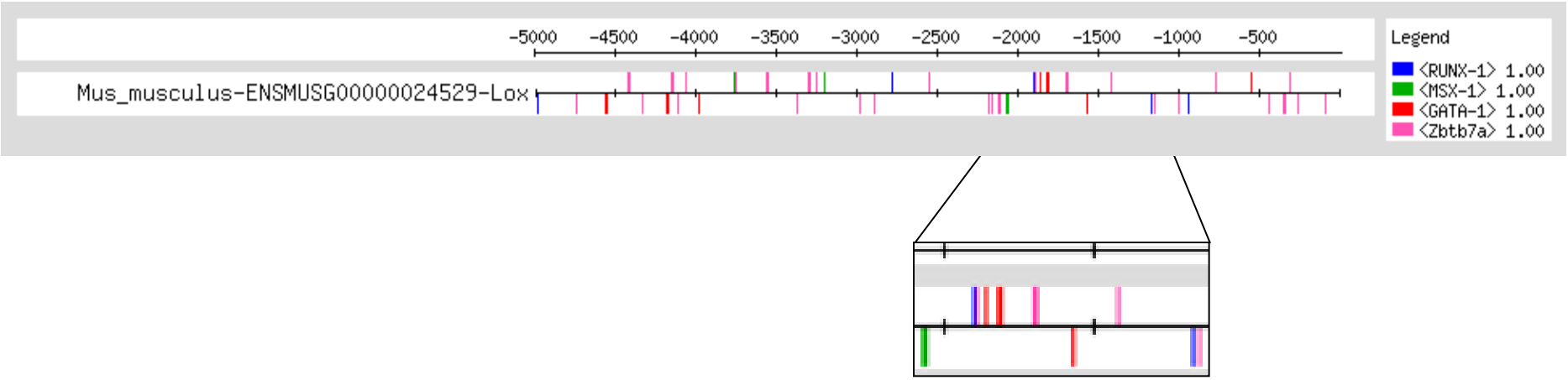
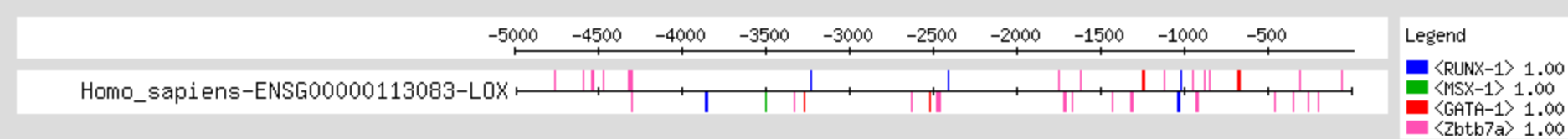
Supplemental figure 8.

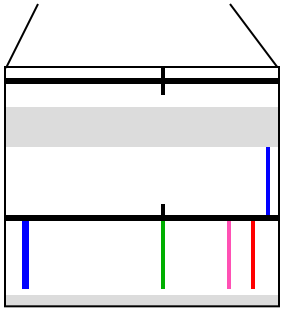
A.



Occurrence	Pattern	Start	End	matching_seq
Msx-1	cngtawnTg	-2070	-2062	ttctCTGTATGTGtatt
GATA-1	GATAAG	-1864 -1820	-1859 -1815	tggaAGATAAactg tgagAGATAAttat
Runx-1	TGTGGT	-1904 -1172	-1899 -1167	aaccTGTGGTcacg gtagTGTGGTtaga

Supplemental figure 8.
B.





occurrence	Pattern	Start	End	matching_seq
Msx-1	cngtawnTg	-3502	-3494	tctgCAGTATGTGccat
GATA-1	GATAAG	-3272	-3267	tcccAGATAAaatg
Runx-1	TGTGGT	-3857	-3852	tccaTGTGGTttga