

## **SUPPLEMENTARY MATERIALS**

**The p38 $\alpha$  MAPK positively regulates osteoblast function and postnatal bone acquisition**

Journal of Cellular and Molecular Life Sciences

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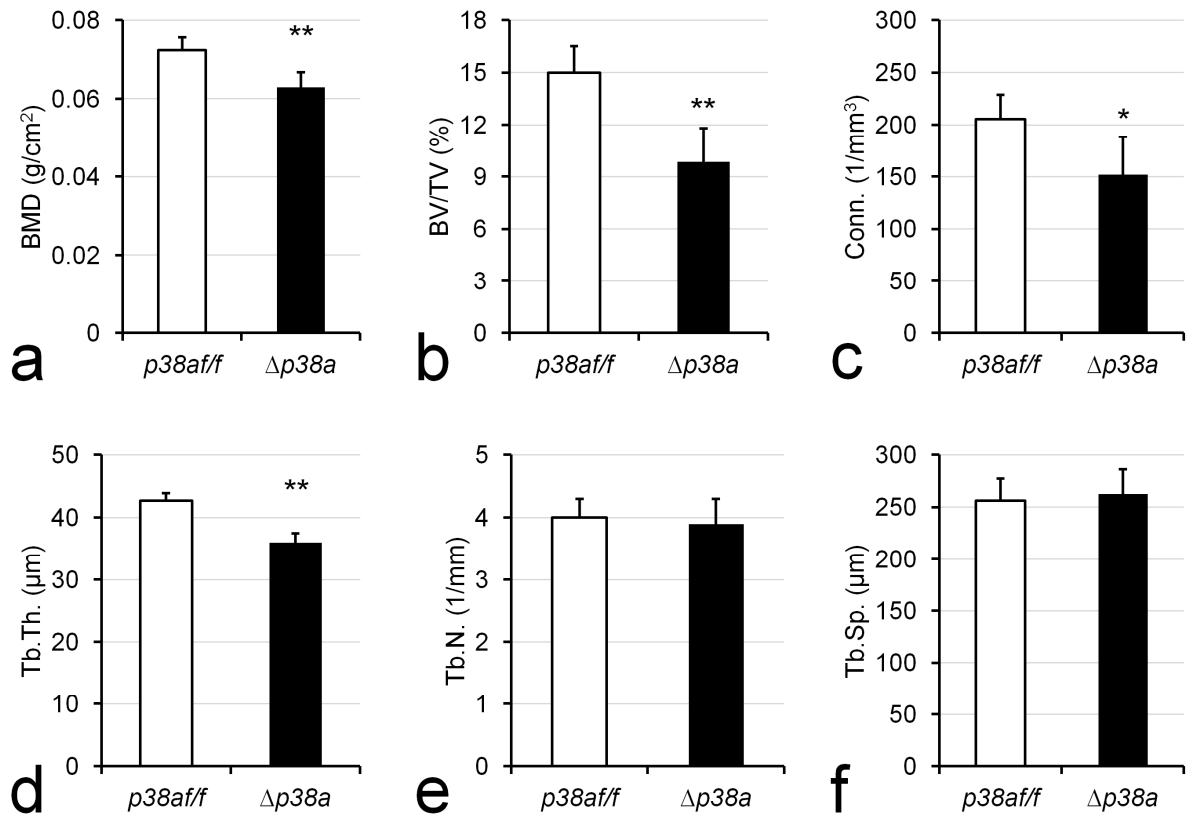
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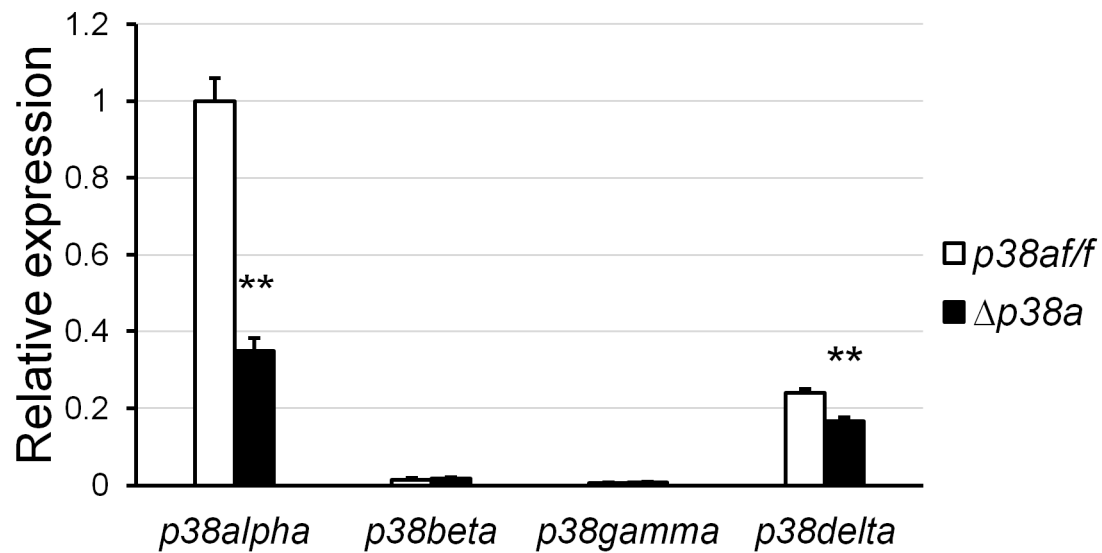
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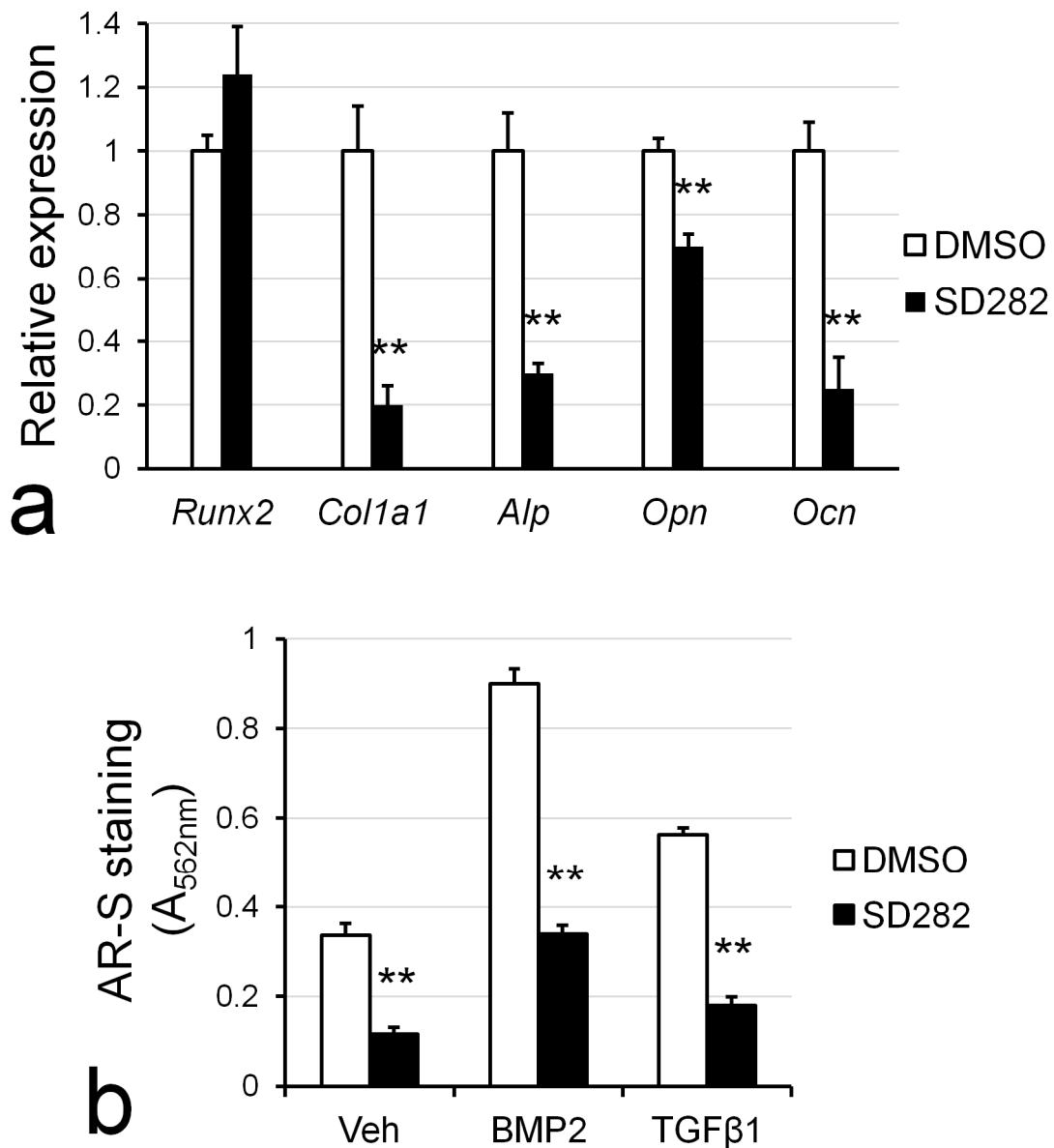
**Supplementary materials:** 3 figures + 2 tables



**Fig. S1** Decreased bone mineral density and altered microarchitecture of the 5<sup>th</sup> lumbar vertebra of  $\Delta p38a$  mice. (a) Lumbar BMD of *p38a<sup>ff</sup>* and  $\Delta p38a$  female mice at 12 weeks of age. (b-f) Trabecular bone microarchitecture was measured at the 5<sup>th</sup> lumbar vertebra of female *p38a<sup>ff</sup>* and  $\Delta p38a$  mice at 12 weeks of age.  $\mu$ CT parameters include (b) BV/TV: bone volume/total volume, (c) Conn: connectivity, (d) Tb.Th.: trabecular thickness, (e) Tb.N.: trabecular number, (f) Tb.Sp.: trabecular separation.. \* p<0.05, \*\* p≤0.01.



**Fig. S2** No compensation of *p38alpha* deletion in osteoblasts by *p38beta*, *p38gamma* and *p38delta* expressions. Primary osteoblasts were isolated from long bones of *p38af/f* and  $\Delta p38a$  mice and cultured for 10 days in osteogenic medium. Expressions of the different p38 members were assessed by real-time PCR. \*\*  $p \leq 0.01$ .



**Fig. S3** A selective p38 $\alpha$  inhibitor inhibits osteoblast function *in vitro*. (a,b) Primary osteoblasts were isolated from wild type mice and cultured in the absence (vehicle: DMSO) or presence of 10  $\mu$ M SD282, a selective p38 $\alpha$  inhibitor. (a) Osteoblast marker gene expression was assessed by real-time PCR after 7 days of culture in osteogenic medium. (b) Quantification of matrix mineralization was evaluated by AR-S staining after 14 days of culture in osteogenic medium supplemented with vehicle, TGF $\beta$ 1 (5 ng/mL) or BMP2 (50 ng/mL). \*\*  $p \leq 0.01$ .

**Table S1** SYBR Green primers for real-time PCR.

<b>Gene</b>		<b>Sequence</b>	<b>Location</b>	<b>Length</b>	<b>%GC</b>	<b>Tm</b>	<b>Product length</b>
<i>B2m</i>	F	CACTGACCGGCCTGTATGCT	41	20	60	60	87
	R	GTATGTTTCGGCTTCCCATTCTC	127	22	50	59	
<i>p38a</i>	F	CGCAAGGTCACTGGAGGAAT	276	20	55	59	83
	R	GGCACTTCACGATGTTGTTTCAG	358	22	50	59	
<i>p38b</i>	F	GTCCTGAGGTTCTGGCAAAGAT	728	22	50	58	70
	R	GGCATGGGAGGCAGAGACT	797	19	63	59	
<i>p38g</i>	F	ACGAGAACGTCATTGGGCTACT	248	22	50	59	81
	R	GCATCACCAGGTAGAAGTCTGTGA	328	24	50	59	
<i>p38d</i>	F	AGTCGACATCTGGTCTGTTGGTT	609	23	48	59	89
	R	TCAGCTGGTCCAGGTAGTCCTT	697	22	55	59	
<i>Runx2</i>	F	CGGACGAGGCAAGAGTTTCA	798	20	55	60	100
	R	GGGACCGTCCACTGTCACTT	897	20	60	58	
<i>Colla1</i>	F	CTGGCCTTGGAGGAAACTTT	428	20	50	57	72
	R	GCACGGAAACTCCAGCTGAT	499	20	55	59	
<i>Alp</i>	F	AGATGGCCTGGATCTCATCAGT	741	22	50	59	83
	R	G TTCAGTGC GGTTC CAGACATA	823	22	50	59	
<i>Opn</i>	F	CGAGGTGATAGCTTGGCTTATG	430	22	50	58	119
	R	GACTCACCGCTCTTCATGTGA	548	21	52	57	
<i>Ocn</i>	F	GGAGGGCAATAAGGTAGTGAACAG	114	24	50	59	110
	R	CACAAGCAGGGTTAAGCTCACA	223	22	50	59	
<i>Rankl</i>	F	GCACACCTCACCATCAATGC	493	20	55	59	82
	R	AGCCTCGATCGTGGTACCAA	574	20	55	59	
<i>Opg</i>	F	GACAACGTGTGTTCCGGAAA	544	20	50	58	96
	R	GGTAGGAACAGCAAACCTGAAGA	639	23	48	58	

**Table S2** Femoral bone microarchitecture of 12-week-old  $p38\alpha^{ff}$  and  $\Delta p38a$  female and male mice.

Parameters		$p38\alpha^{ff}$	$\Delta p38a$	p value	$\Delta$ (%)
<b>Female</b>		n=6	n=6		
Total	BMD (g/cm <sup>2</sup> )	0.079 ± 0.004	0.069 ± 0.004	0.001	-13.30%
Cortical	TV (mm <sup>3</sup> )	1.12 ± 0.03	1.16 ± 0.09	0.36	-
	BV (mm <sup>3</sup> )	0.46 ± 0.01	0.36 ± 0.02	0.001	-20.00%
	BMaV (mm <sup>3</sup> )	0.66 ± 0.02	0.78 ± 0.08	0.03	18.00%
	Ct.Th. (μm)	171.2 ± 3.7	136.6 ± 6.4	0.0001	-20.00%
Trabecular	BV/TV (%)	9.0 ± 1.5	5.7 ± 0.6	0.002	-37.90%
	Tb.Th. (μm)	49.4 ± 2.0	39.1 ± 2.4	0.0001	-20.70%
	Tb.N. (1/mm)	4.1 ± 0.3	3.9 ± 0.3	0.11	-
	Tb.Sp. (μm)	248.4 ± 20.0	260.9 ± 20.6	0.21	-
<b>Male</b>		n=4	n=4		
Total	BMD (g/cm <sup>2</sup> )	0.092 ± 0.008	0.082 ± 0.003	0.007	-11.30%
Cortical	TV (mm <sup>3</sup> )	1.25 ± 0.10	1.26 ± 0.03	0.72	-
	BV (mm <sup>3</sup> )	0.52 ± 0.05	0.44 ± 0.03	0.02	-16.20%
	BMaV (mm <sup>3</sup> )	0.74 ± 0.06	0.83 ± 0.03	0.02	13.70%
	Ct.Th. (μm)	173.2 ± 5.9	141.0 ± 9.5	0.002	-18.60%
Trabecular	BV/TV (%)	15.9 ± 3.0	11.1 ± 2.1	0.04	-30.20%
	Tb.Th. (μm)	49.2 ± 4.2	41.8 ± 3.6	0.04	-15.00%
	Tb.N. (1/mm)	5.1 ± 0.1	4.9 ± 0.3	0.27	-
	Tb.Sp. (μm)	189.4 ± 6.0	203.1 ± 15.2	0.17	-

μCT analyses were performed at femoral midshaft (cortical) and distal femur (trabecular) of control ( $p38\alpha^{ff}$ ) and mutant ( $\Delta p38a$ ) mice.

BMD: bone mineral density; TV: total volume; BV: bone volume; BMaV: bone marrow volume; Tb.Th.: trabecular thickness; Tb.N.: trabecular number; Tb.Sp.: trabecular separation.

Data are expressed as mean ± SD. p values were determined using unpaired *t* tests. Significant differences are expressed as percentage.