

Spina bifida-predisposing heterozygous mutations in Planar Cell Polarity genes and Zic2 reduce bone mass in young mice

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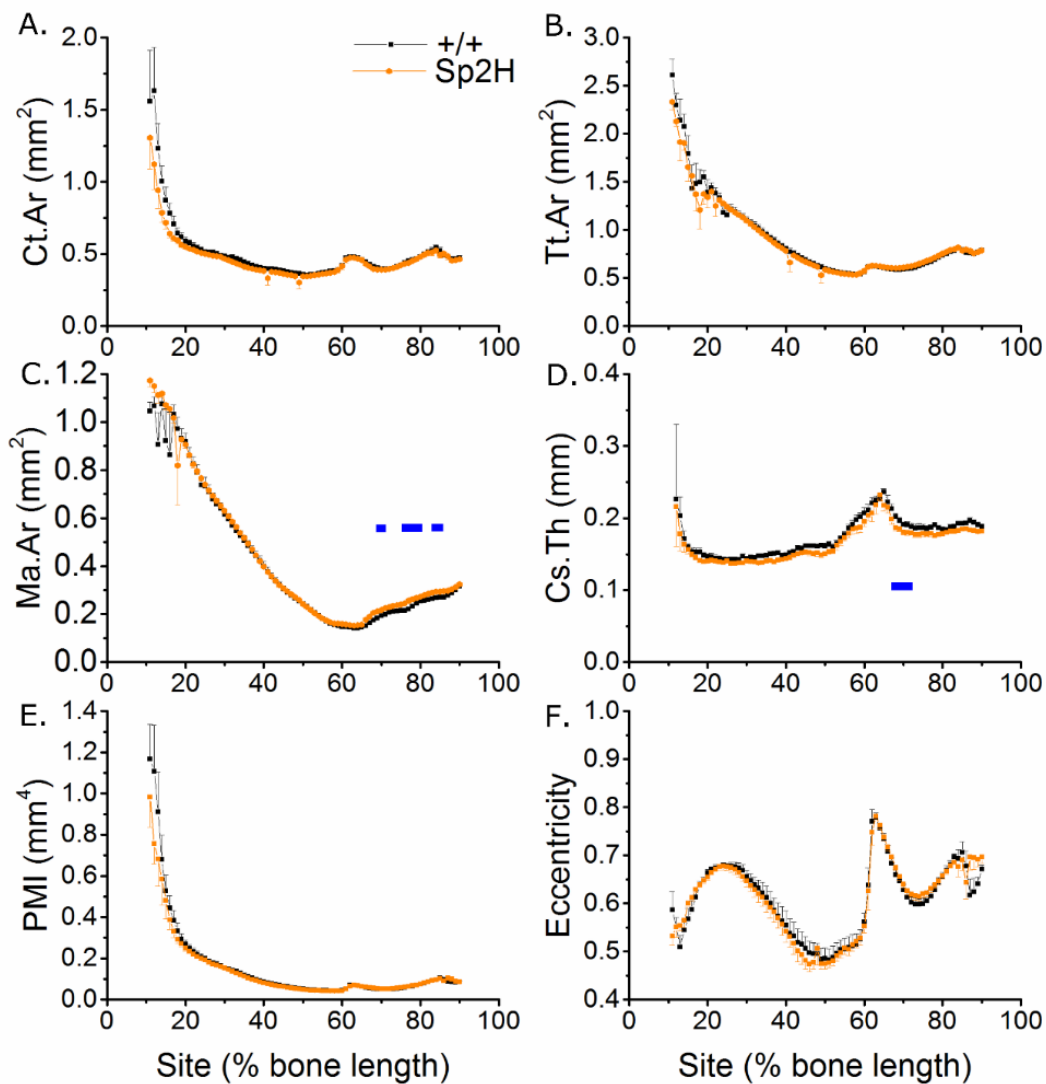
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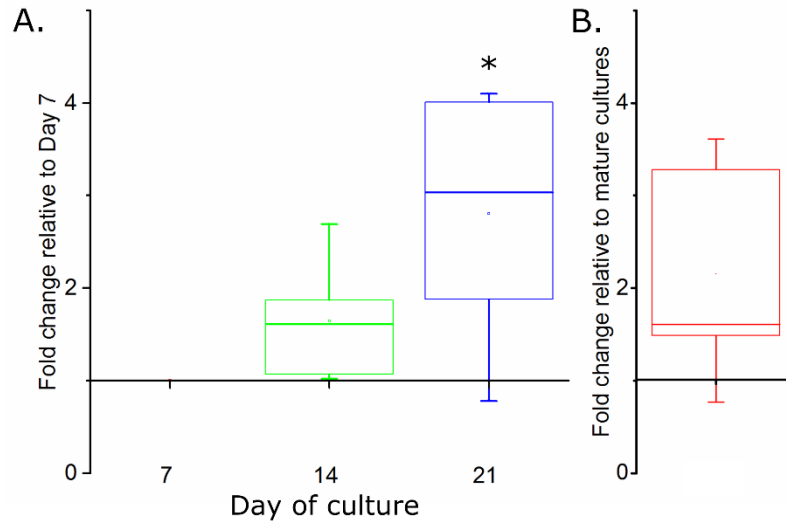
Keywords:

Bone, planar cell polarity, Vangl2, Celsr1, Zic2

Supplementary Figures and Legends:



Supplementary Figure S1: Heterozygous *Spotch* mutation of *Pax3* is associated with regionally smaller cortical bone mass in the tibia of young male mice. SSA μ CT analysis of cortical bone structure at each 1% site along the bone's length in 3-week-old male $Pax3^{+/+}$ and $Pax3^{Sp2H/+}$ littermates ($n = 8$ each). A) Ct.Ar, B) Tt.Ar, C) Ma.Ar, D) Cs.Th, E) PMI and F) eccentricity were quantified. Blue lines indicate regions of significant differences between genotypes by mixed model analysis with Bonferroni post-hoc.



Supplementary Figure S2: Zic2 expression increases in mineralising osteoblasts. A,B) qRT-PCR quantification of Zic2 mRNA expression was quantified in (A) osteoblasts cultured for 7, 14 or 21 days and their expression compared to 7 day cultures (set at 1 for each set of cultures) or (B) mature resorbing osteoclasts (day 9 after 2 days of medium acidification) versus mature osteoclasts (day 7). N = 5-6, * p < 0.05

Supplementary Table S1: Trabecular bone parameters in *Vangl2 Lp* heterozygous mice and WT littermates

	Body weight (g)	Tibial length (mm)	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (/mm)	Tb.Pf (/mm)	SMI
WT Mean	12.825	13.872	12.787	0.064	0.494	2.000	13.186	1.889
SEM	0.496	0.129	0.932	0.002	0.025	0.114	1.512	0.062
Lp/+ Mean	12.575	13.726	8.306	0.058	0.499	1.417	20.731	2.243
SEM	0.297	0.103	0.982	0.002	0.025	0.153	1.505	0.060
T-test p value	0.672	0.406	0.007	0.033	0.895	0.012	0.005	0.002

Supplementary Table S2: Trabecular bone parameters in *Celsr1 Crsh* heterozygous mice and WT littermates

	Body weight (g)	Tibial length (mm)	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (/mm)	Tb.Pf (/mm)	SMI
WT Mean	11.538	13.350	18.216	0.071	0.430	2.572	10.775	1.861
SEM	0.235	0.095	0.882	0.002	0.017	0.087	0.991	0.036
Crsh/+ Mean	10.775	13.240	12.914	0.061	0.398	2.083	19.396	2.229
SEM	0.313	0.089	1.651	0.002	0.027	0.208	1.575	0.074
T-test p value	0.082	0.396	0.047	0.019	0.320	0.128	0.002	0.002

Supplementary Table S3: Trabecular bone parameters in *Pax3 Sp2H* heterozygous mice and WT littermates

	Body weight (g)	Tibial length (mm)	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (/mm)	Tb.Pf (/mm)	SMI
WT Mean	9.913	13.002	10.570	0.056	0.426	1.878	21.797	2.255
SEM	0.280	0.088	0.868	0.001	0.028	0.144	1.223	0.046
Sp2H/+ Mean	9.713	13.031	10.400	0.056	0.445	1.867	20.228	2.151
SEM	0.237	0.053	0.498	0.001	0.017	0.075	1.260	0.054
T-test p value	0.594	0.781	0.868	0.814	0.571	0.948	0.386	0.270

Supplementary Table S4: Trabecular bone parameters in *Zic2* *Ku* heterozygous mice and WT littermates

	Body weight (g)	Tibial length (mm)	BV/TV (%)	Tb.Th (mm)	Tb.Sp (mm)	Tb.N (/mm)	Tb.Pf (/mm)	SMI
WT Mean	9.200	12.995	15.853	0.056	0.311	2.772	15.224	1.943
SEM	0.482	0.274	1.843	0.002	0.021	0.239	2.892	0.114
Ku/+ Mean	8.338	12.385	14.475	0.055	0.315	2.667	17.321	2.021
SEM	0.384	0.426	1.201	0.003	0.024	0.179	2.225	0.064
T-test p value	0.184	0.248	0.541	0.680	0.909	0.731	0.575	0.559

Supplementary Table S5: List of NTD-associated genes with "bone", "osteoblast" or "osteoblast" associated functional ontologies.

Msx2
 Dlx5
 Men1
 Ptch1
 Smad5
 Gli3
 Ski
 Rab23
 BMP7
 BMP2
 Nog
 Nf1
 Pitx2
 Rarg
 Rara
 Csk
 Fgfr1

Abi1
Abl1
Ptpn11
Lrp6
Twist1
Gja1
Cited2
Prkaca
Msx1
Tfap2a
Frem1
Tulp3
Hdac4
T
Ctnnbip1
Sfrp2
Wnt3a
Sfrp1
Cthrc1
Adar
Asf1a
Map3k7
Mapk8
Csnk1a1
Chuk
Trp53
Traf6
Rac1
Mapk9