

### **Supplemental Figure 1**

Osteocytes positive for the CY3-labeled mouse chromosome probe. Photomicrographs of hematoxylin and eosin (H&E)-stained sections and fluorescence in situ hybridization (FISH) analysis with mouse Y chromosome paint probe (labeled with red CY3). Specimens processed for FISH analysis were obtained 15–30  $\mu\text{m}$  away from the H&E-stained section. The photomicrographs from the FISH analysis were taken with fluorescein isothiocyanate filters to show the tissue structure and were merged with photomicrographs of the same areas taken with a CY3 filter to show the red CY3-labeled mouse chromosome and with 4',6-diamidino-2-phenylindole to show the blue cell nuclei. The bright red dots, seen only in the osteocytes and osteoblasts, are CY3-labeled mouse chromosome probes. Original magnification, 630x. The arrow indicates an osteocytes positive for the CY3-labeled mouse chromosome probe (red dot) that is included in the area of detail. M, bone-like matrix; T, prostate cancer cells.

### **Supplemental Figure 2**

MDA PCa 118b-induced subcutaneous bone stained with alizarin red and alcian blue. MDA PCa 118b subcutaneous tumors harvested 1 to 4 weeks after cell injection stained positive for alizarin red, a bone-specific dye, but not for alcian blue, a marker for sulfated proteoglycans. Original magnification, 200x.

### **Supplemental Figure 3**

von Kossa-stained cross sections of bone (calvarial) organ culture left untreated or treated with 10 ng/ml of fibroblast growth factor 9 (FGF9). Each treatment condition included calvaria from three different mice. Original magnification, 1000x. Calvarial thickness area on the von Kossa-stained sections, as quantified by the OsteoMeasure system. \* $P < 0.01$  for untreated vs. FGF9-treated calvaria.

**Supplemental Table 1.** Genes expressed at different levels by cancer cells in the MDA PCa 118 variants vs the MDA PCa 2b and PC3 xenografts. Positive T scores indicate genes overexpressed by cancer cells in MDA PCa 118 variants

Probe Set	Gene Symbol	UniGene ID	T score	p Value
203423_at	RBP1	Hs.101850	50.67	0.000000908
210221_at	CHRNA3	Hs.89605	39.62	0.000002425
206502_s_at	INSM1	Hs.89584	37.8	0.000002927
<b>209540_at</b>	<b>IGF1</b>	<b>Hs.308053</b>	<b>33.27</b>	<b>0.000004868</b>
209498_at	CEACAM1	Hs.512682	28.77	0.000008689
219756_s_at	POF1B	Hs.267038	27.27	0.000010755
204712_at	WIF1	Hs.284122	25.45	0.000014148
<b>209541_at</b>	<b>IGF1</b>	<b>Hs.308053</b>	<b>25.26</b>	<b>0.000014578</b>
204684_at	NPTX1	Hs.84154	24.15	0.000017434
218963_s_at	KRT23	Hs.9029	21.96	0.000025434
206606_at	LIPC	Hs.9994	21.43	0.000028038
203305_at	F13A1	Hs.80424	20.83	0.00003141
210121_at	B3GALT2	Hs.181353	20.62	0.000032696
219966_x_at	BANP	Hs.448828	20.42	0.000033974
206128_at	ADRA2C	Hs.123022	20.39	0.000034148
218687_s_at	MUC13	Hs.5940	20.38	0.000034229
220213_at	C20orf17	Hs.150825	19	0.000045161
212848_s_at	C9orf3	Hs.412286	18.95	0.000045653
220979_s_at	SIAT7E	Hs.226440	18.93	0.000045841
209390_at	TSC1	Hs.69429	18.87	0.000046415
220002_at	FLJ10157	Hs.125020	18.84	0.000046736
207035_at	SLC30A3	Hs.111967	18.78	0.000047308
201477_s_at	RRM1	Hs.383396	18.6	0.000049228
212023_s_at	MKI67	Hs.80976	17.24	0.000066381
208121_s_at	PTPRO	Hs.160871	17.19	0.000067162
203304_at	BAMBI	Hs.348802	16.71	0.000075164
207540_s_at	SYK	Hs.192182	16.55	0.000078124
210169_at	KIAA0420	Hs.512856	16.53	0.000078516
213172_at	TTC9	Hs.79170	16.47	0.000079508
218219_s_at	LANCL2	Hs.134342	16.37	0.000081455
218435_at	DNAJD1	Hs.438830	16.3	0.000082918
212021_s_at	MKI67	Hs.80976	16.11	0.000086852
204791_at	NR2C1	Hs.108301	16.05	0.000088138
209122_at	ADFP	Hs.3416	15.8	0.00009376
<b>206404_at</b>	<b>FGF9</b>	<b>Hs.111</b>	<b>15.36</b>	<b>0.000104883</b>
214043_at	PTPRD	Hs.323079	15.21	0.000108992
219714_s_at	CACNA2D3	Hs.435112	14.95	0.000116734
205051_s_at	KIT	Hs.81665	14.95	0.00011675
212385_at	TCF4	Hs.359289	14.64	0.000126526
201887_at	IL13RA1	Hs.285115	14.27	0.000139957
202294_at	STAG1	Hs.138263	14.24	0.000141323
221100_at	C6orf15	Hs.272214	14.23	0.000141819
213348_at	CDKN1C	Hs.106070	14.17	0.000143859
222258_s_at	SH3BP4	Hs.17667	14	0.000151088
221841_s_at	KLF4	Hs.376206	14	0.000150824

201425_at	ALDH2	Hs.331141	13.84	0.000157918
217946_s_at	SAE1	Hs.32748	13.73	0.000163097
40020_at	CELSR3	Hs.55173	13.72	0.000163492
221840_at	PTPRE	Hs.437980	13.66	0.000166505
219961_s_at	C20orf19	Hs.436632	13.64	0.000167185
203961_at	NEBL	Hs.5025	13.61	0.000168765
202054_s_at	ALDH3A2	Hs.440662	13.56	0.000171084
204576_s_at	KIAA0643	Hs.155995	13.47	0.000175542
213596_at	CASP4	Hs.74122	13.35	0.000181788
206985_at	HSD17B3	Hs.477	13.24	0.000187922
201948_at	HUMAUANTIG	Hs.75528	13.19	0.000190728
206114_at	EPHA4	Hs.244624	13.04	0.000199806
214284_s_at	FGF18	Hs.87191	12.99	0.000202732
219142_at	RASL11B	Hs.8035	12.89	0.000209099
206191_at	ENTPD3	Hs.47042	12.88	0.00020922
217744_s_at	PERP	Hs.149620	12.87	0.000210277
215164_at	---	Hs.406753	12.81	0.0002139
204777_s_at	MAL	Hs.80395	12.7	0.000221702
203999_at	SYT1	Hs.154679	12.61	0.000227642
201677_at	DC12	Hs.458320	12.6	0.00022843
207413_s_at	SCN5A	Hs.169331	12.39	0.000243637
219305_x_at	FBXO2	Hs.132753	12.25	0.000254706
205055_at	ITGAE	Hs.389133	12.17	0.000261835
219513_s_at	SH2D3A	Hs.439645	12.09	0.000268535
201841_s_at	HSPB1	Hs.76067	12.05	0.00027169
208216_at	DLX4	Hs.172648	12.05	0.000271899
214811_at	KIAA0318	Hs.225014	11.91	0.000284999
212295_s_at	SLC7A1	Hs.14846	11.84	0.000291216
219229_at	SLCO3A1	Hs.113657	11.68	0.000307209
213082_s_at	SLC35D2	Hs.386278	11.66	0.000309708
201756_at	RPA2	Hs.79411	11.63	0.000312623
213174_at	TTC9	Hs.79170	11.61	0.000314627
218319_at	PELI1	Hs.7886	11.47	0.000330309
213721_at	SOX2	Hs.816	11.46	0.000330844
205165_at	CELSR3	Hs.55173	11.41	0.000336193
203797_at	VSNL1	Hs.2288	11.39	0.000338851
219918_s_at	ASPM	Hs.121028	11.2	0.000362102
204814_at	CADPS	Hs.441050	11.17	0.000365069
204105_s_at	NRCAM	Hs.7912	11.09	0.000376112
218044_x_at	PTMS	Hs.446525	11	0.000387652
221950_at	EMX2	Hs.202095	10.98	0.000391587
212290_at	SLC7A1	Hs.14846	10.85	0.00040998
218639_s_at	MGC11349	Hs.288697	10.85	0.000409153
212943_at	KIAA0528	Hs.30656	10.82	0.000414111
212224_at	ALDH1A1	Hs.76392	10.8	0.000416773
213241_at	PLXNC1	Hs.286229	10.78	0.000419603
212538_at	DOCK9	Hs.362177	10.75	0.000424994
204141_at	TUBB	Hs.512712	10.72	0.000428505
203690_at	TUBGCP3	Hs.9884	10.71	0.000431001

213317_at	---	Hs.21103	10.7	0.000432027
217452_s_at	B3GALT2	Hs.181353	10.7	0.000431788
207979_s_at	CD8B1	Hs.405667	10.69	0.000433154
211518_s_at	BMP4	Hs.68879	10.68	0.000435512
200608_s_at	RAD21	Hs.81848	10.68	0.000436
212399_s_at	KIAA0121	Hs.155584	10.63	0.000443841
200934_at	DEK	Hs.110713	10.61	0.000447146
204591_at	CHL1	Hs.388344	10.56	0.000455155
204975_at	EMP2	Hs.531561	10.52	0.000461274
209513_s_at	C9orf99	Hs.388160	10.5	0.000465603
218370_s_at	FLJ12903	Hs.369253	10.5	0.000465973
213826_s_at	---	Hs.477089	10.46	0.000472653
206158_s_at	ZNF9	Hs.2110	10.46	0.000472644
213253_at	SMC2L1	Hs.119023	10.39	0.000484232
212505_s_at	KIAA0892	Hs.112751	10.35	0.000491755
217143_s_at	TRD@	Hs.2014	10.28	0.000505783
202080_s_at	OIP106	Hs.457063	10.27	0.000506644
221677_s_at	DONSON	Hs.17834	10.27	0.000507025
216204_at	ARVCF	Hs.326730	10.25	0.000510819
217885_at	IPO9	Hs.445587	10.21	0.000518571
214924_s_at	OIP106	Hs.457063	10.17	0.000526946
212802_s_at	DKFZP434C212	Hs.287266	10.14	0.000532351
213417_at	TBX2	Hs.168357	10.12	0.000537167
201301_s_at	ANXA4	Hs.422986	10.03	0.000555692
208147_s_at	CYP2C8	Hs.282871	9.97	0.000569125
219225_at	PGBD5	Hs.12247	9.95	0.000573739
202413_s_at	USP1	Hs.35086	9.95	0.000571911
213547_at	TIP120B	Hs.154740	9.95	0.000573929
213054_at	KIAA0841	Hs.7426	9.93	0.000578563
204260_at	CHGB	Hs.2281	9.86	0.000593571
202869_at	OAS1	Hs.442936	9.84	0.000598289
208959_s_at	TXNDC4	Hs.154023	9.83	0.000600281
206460_at	SHREW1	Hs.25924	9.81	0.000604757
219619_at	DIRAS2	Hs.165636	9.78	0.000612033
218287_s_at	EIF2C1	Hs.309452	9.73	0.000623999
202510_s_at	TNFAIP2	Hs.101382	9.73	0.000625412
221522_at	ANKRD27	Hs.59236	9.73	0.000625066
221536_s_at	FLJ11301	Hs.436471	9.71	0.000629582
210892_s_at	GTF2I	Hs.408507	9.7	0.000631683
215335_at	---	Hs.406781	9.69	0.000635739
219882_at	FLJ23033	Hs.445826	9.66	0.000642237
209656_s_at	TM4SF10	Hs.8769	9.66	0.000641289
204742_s_at	APRIN	Hs.168625	9.66	0.000642285
201650_at	KRT19	Hs.309517	9.65	0.000645881
203086_at	KIF2	Hs.113319	9.63	0.000651361
208808_s_at	HMGB2	Hs.434953	9.59	0.000661849
203145_at	SPAG5	Hs.16244	9.53	0.000676108
204798_at	MYB	Hs.407830	9.47	0.000694845
205539_at	AVIL	Hs.333257	9.44	0.000701107

218876_at	CGI-38	Hs.412685	9.42	0.000707951
212621_at	KIAA0286	Hs.14912	9.38	0.000718272
213183_s_at	CDKN1C	Hs.106070	9.35	0.000729
220408_x_at	P38IP	Hs.435815	9.21	0.000771285
219404_at	EPS8L3	Hs.5366	9.18	0.000782791
218203_at	ALG5	Hs.339428	9.15	0.000790307
208854_s_at	STK24	Hs.168913	9.11	0.00080671
206450_at	DBH	Hs.223858	9.1	0.000807111
212826_s_at	SLC25A6	Hs.350927	-9.1	0.000809501
218404_at	SNX10	Hs.418132	-9.15	0.000792104
217099_s_at	GEMIN4	Hs.302421	-9.16	0.000788902
210976_s_at	PFKM	Hs.75160	-9.17	0.000786462
218665_at	FZD4	Hs.19545	-9.17	0.000786478
218807_at	VAV3	Hs.267659	-9.2	0.000774452
212333_at	DKFZP564F0522	Hs.23060	-9.21	0.00077336
204040_at	RNF144	Hs.78894	-9.29	0.000747368
212875_s_at	C21orf25	Hs.16007	-9.31	0.000739929
203622_s_at	LOC56902	Hs.262858	-9.31	0.00073971
201830_s_at	NET1	Hs.25155	-9.31	0.000740843
213713_s_at	LOC89944	Hs.436178	-9.32	0.000737439
208658_at	ERP70	Hs.93659	-9.36	0.000724543
218771_at	PANK4	Hs.26156	-9.37	0.000722259
218889_at	C10orf117	Hs.74899	-9.37	0.00072272
202106_at	GOLGA3	Hs.4953	-9.38	0.000719817
205074_at	SLC22A5	Hs.15813	-9.39	0.000716823
213060_s_at	CHI3L2	Hs.154138	-9.39	0.000716087
204319_s_at	RGS10	Hs.82280	-9.44	0.000702032
210250_x_at	ADSL	Hs.75527	-9.49	0.00068885
216309_x_at	JRK	Hs.142296	-9.5	0.000684082
201444_s_at	ATP6AP2	Hs.183434	-9.66	0.000642095
201125_s_at	ITGB5	Hs.149846	-9.68	0.000636343
221687_s_at	C9orf28	Hs.438972	-9.78	0.000612171
210275_s_at	ZNF216	Hs.406096	-9.89	0.000585491
217843_s_at	VDRIP	Hs.181112	-9.91	0.000581757
218909_at	RPS6KC1	Hs.30352	-9.95	0.000572452
203910_at	PARG1	Hs.430919	-10.08	0.000543913
213793_s_at	HOMER1	Hs.129051	-10.14	0.000531967
203815_at	GSTT1	Hs.268573	-10.15	0.000530392
206068_s_at	ACADL	Hs.430108	-10.2	0.000521088
219194_at	SEMA4G	Hs.444359	-10.28	0.000505294
219201_s_at	TWSG1	Hs.247302	-10.36	0.000490658
210740_s_at	ITPK1	Hs.408429	-10.43	0.00047652
208814_at	HSPA4	Hs.90093	-10.45	0.000474347
217236_x_at	IGHG1	Hs.413826	-10.45	0.000474116
219595_at	ZNF26	Hs.489608	-10.51	0.000463663
219319_at	HIF3A	Hs.420830	-10.51	0.000463124
217550_at	ATF6	Hs.433046	-10.66	0.000438692
218589_at	P2RY5	Hs.123464	-10.79	0.00041794
218526_s_at	RANGNRF	Hs.408233	-10.81	0.000414823

201517_at	NCBP2	Hs.240770	-10.88	0.000404457
212912_at	RPS6KA2	Hs.301664	-10.96	0.000393022
219347_at	NUDT15	Hs.144407	-11.03	0.000384301
215096_s_at	ESD	Hs.432491	-11.12	0.00037153
218856_at	TNFRSF21	Hs.159651	-11.14	0.000368995
218686_s_at	RHBDF1	Hs.57988	-11.14	0.00036973
220000_at	SIGLEC5	Hs.117005	-11.19	0.000363181
215317_at	---	Hs.185701	-11.2	0.000361932
204432_at	SOX12	Hs.43627	-11.24	0.000357285
205541_s_at	GSPT2	Hs.59523	-11.36	0.000342975
204129_at	BCL9	Hs.415209	-11.47	0.000329525
200827_at	PLOD	Hs.75093	-11.49	0.000327048
209061_at	NCOA3	Hs.382168	-11.57	0.000318693
213129_s_at	GCSH	Hs.513762	-11.58	0.000317463
212507_at	RW1	Hs.318783	-11.59	0.000316998
221359_at	GDNF	Hs.248114	-11.61	0.000314461
203187_at	DOCK1	Hs.437620	-11.74	0.000300884
203790_s_at	UK114	Hs.18426	-11.81	0.000293748
212658_at	LHFPL2	Hs.79299	-11.85	0.00029034
218352_at	RCBTB1	Hs.58452	-12.01	0.000275653
205677_s_at	DLEU1	Hs.344524	-12.07	0.000269976
202388_at	RGS2	Hs.78944	-12.4	0.000243158
208782_at	FSTL1	Hs.433622	-12.4	0.000243067
202956_at	BIG1	Hs.94631	-12.48	0.000237306
212345_s_at	CREB3L2	Hs.59943	-12.57	0.00023085
212929_s_at	FLJ10824	Hs.449662	-12.95	0.000205392
204497_at	ADCY9	Hs.20196	-13.13	0.000194135
207760_s_at	NCOR2	Hs.287994	-13.23	0.000188548
208886_at	H1FO	Hs.226117	-13.36	0.000181289
221530_s_at	BHLHB3	Hs.437282	-13.47	0.000175561
209009_at	ESD	Hs.432491	-13.49	0.000174818
201501_s_at	GRSF1	Hs.309763	-13.86	0.000156979
212737_at	GM2A	Hs.387156	-13.93	0.000154196
218764_at	PRKCH	Hs.315366	-13.99	0.000151244
209567_at	RRS1	Hs.71827	-14.18	0.00014341
219335_at	FLJ12969	Hs.119699	-14.34	0.000137372
202836_s_at	TXNL4	Hs.433683	-14.64	0.00012681
205751_at	SH3GL2	Hs.75149	-15.05	0.000113573
204316_at	RGS10	Hs.82280	-15.6	0.000098527
203421_at	TP53I11	Hs.433813	-15.91	0.00009127
210879_s_at	GAF1	Hs.24557	-16.7	0.000075336
213122_at	TSPYL5	Hs.173094	-16.72	0.000074889
203132_at	RB1	Hs.408528	-17.97	0.00005637
201709_s_at	NIPSNAP1	Hs.173878	-18.38	0.000051537
201848_s_at	BNIP3	Hs.79428	-18.65	0.000048667
203789_s_at	SEMA3C	Hs.171921	-19.53	0.000040498
221816_s_at	PHF11	Hs.308220	-19.72	0.000039
201432_at	CAT	Hs.395771	-20.02	0.00003674
202069_s_at	IDH3A	Hs.250616	-21.05	0.000030111

212539_at	CHD1L	Hs.429920	-23.07	0.000020927
204686_at	IRS1	Hs.390242	-24.17	0.000017394
212298_at	NRP1	Hs.173548	-24.72	0.000015902
203188_at	B3GNT6	Hs.8526	-26.31	0.000012394
218505_at	FLJ12270	Hs.413396	-28.48	0.000009048
216470_x_at	PRSS3	Hs.435699	-35.4	0.0000038
208905_at	CYCS	Hs.437060	-67.74	0.000000285

**Supplemental Table 2.** Sequences of primers used for polymerase chain reaction amplification of reverse-transcribed RNA (cDNA)

<b>Gene (Product Size)</b>	<b>Primer Sequence</b>
BMP2 (197 bp)	Sense: 5'-tcaagccaaacacaaacagc -3' Anti-sense: 5'-acgtctgaacaatggcatga-3'
BMP4 (297 bp)	Sense: 5'-cagcactggcttgagtatcctga -3' Anti-sense: 5'-cgtgtccagtagtcgtgtgatga-3'
BMP6 (244 bp)	Sense: 5'-ggaacgcctctgttcagttc-3' Anti-sense: 5'-ccctgtagcctcacctcag-3'
BMP7 (212 bp)	Sense: 5'-cggatcagcgtttatcaggt-3' Anti-sense: 5'-aactggggtgatgctctg-3'
Wnt-1 (210 bp)	Sense: 5'-cgacctcgtctacttcgagaaatcg-3' Anti-sense: 5'-cagctgacgtggcagcaccagtg-3'
Wnt-3 (209 bp)	Sense: 5'-ctgggccagcagtacacatctctg-3' Anti-sense: 5'-gatggccaggctgtcatctatg-3'
Wnt-3a (220 bp)	Sense: 5'-gcagctaccgatctggtggtc-3' Anti-sense: 5'-gacggtggtgcagttcca-3'
Wnt-4 (257 bp)	Sense: 5'-gaggagacgtgagagaaactc-3' Anti-sense: 5'-gtcactgcaaaggccacacctg-3'
Wnt-5a (367 bp)	Sense: 5'-ccgagcgggagcgcacccacgc-3' Anti-sense: 5'-ggtgctctcattgagcagcagtagtcaggg-3'
Wnt-7b (341 bp)	Sense: 5'-cgtgttctctgctttggcg-3' Anti-sense: 5'-tggctgcaggcagcggtgac-3'
Wnt-8a (81 bp)	Primers were purchased from SuperArray Bioscience Corp (Catalog No. PH02444A); sequences were not provided.
Wnt-10b (402 bp)	Sense: 5'-gataccacaaccgcaattct-3' Anti-sense: 5'-gcatgcagcttttctcta-3'
DKK-1 (510 bp)	Sense: 5'-caacgctatcaagaacctgc-3' Anti-sense: 5'-gatcttgaccagaagtgc-3'
PTHrP (242 bp)	Sense: 5'- gttggagtagccggttgcta -3' Anti-sense: 5'- tgcatcagatggtgaagga -3'
RANKL (158 bp)	Sense: 5'- cagtgggagatgtagactcatg -3' Anti-sense: 5'- gaaggggcacatgaccagggaaccaac -3'



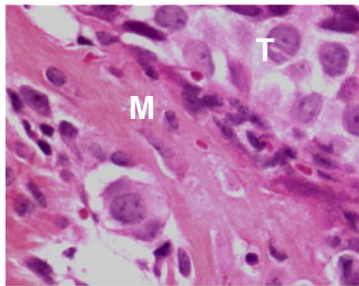
Osteoprotegerin (219 bp)	Sense:	5'-aaccccagagcgaaatac-3'
	Anti-sense:	5'-aagaatgcctcctcacac-3'
Endothelin-1 (245 bp)	Sense:	5'-gagcacattggtgacagacct -3'
	Anti-sense:	5'-cccagatgaaagaagagacc-3'
Fibroblast growth factor 9 (213 bp)	Sense:	5'-cagtgccacgtgcttatatag-3'
	Anti-sense:	5'-cacagccgattggcattctg-3'
Insulin-like growth factor 1 (361 bp)	Sense:	5'-tgccacggctggaccggagacg-3'
	Anti-sense:	5'-gcatgtcactctcactcctc-3'

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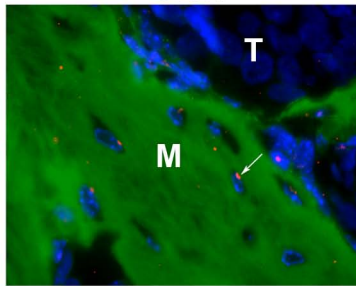
# Li et al. Supplemental Fig 1

## Mouse Probe

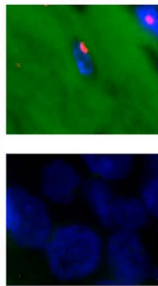
Hematoxylin and Eosin



FISH

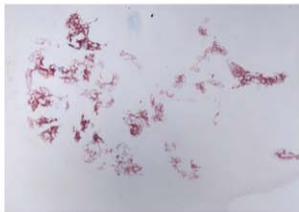


Detail



# Li et al. Supplemental Fig 2

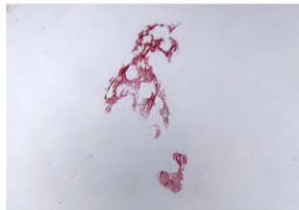
1 week



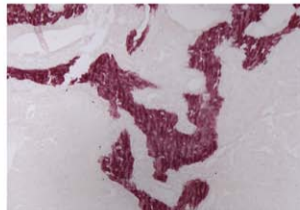
2 weeks



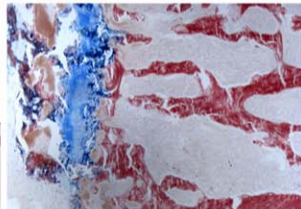
3 weeks



4 weeks



Positive Control  
(L5 growth plate)



# Li et al. Supplemental Fig 3

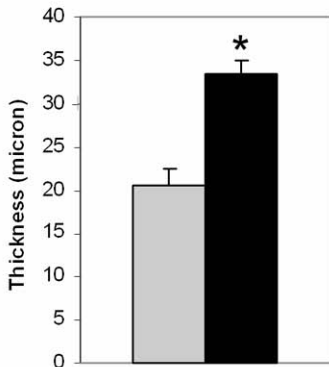


Untreated



FGF9 10ng/ml

Calvaria Thickness



■ Untreated    ■ FGF9 10ng/ml