

Figure S1. *Pbx1* and *Hox11^{aadd}* do not regulate patterning of distal or proximal superstructures, respectively. (A, A') Sagittal sections through the humeri of *Hox11^{aadd}* mutant and control embryos at E13.5 that were stained against SOX9 and COL2A1. (B, B') Sagittal sections through the elbows of *Pbx1* mutant and control embryos at E13.5 that were crossed to *ScxGFP* transgenic mice and stained against SOX9. Whereas *HoxA11* and *HoxD11* regulated the spatial organization of distal olecranon precursors, organization of the proximal DT precursors was unaffected in *Hox11^{aadd}* compound mutants (A, A'). Conversely, whereas *Pbx1* regulated the spatial organization of proximal DT precursors, organization of the distal olecranon precursors was unaffected in *Pbx1^{null}* mutants (B, B'). Scale bars: 100 μ m.

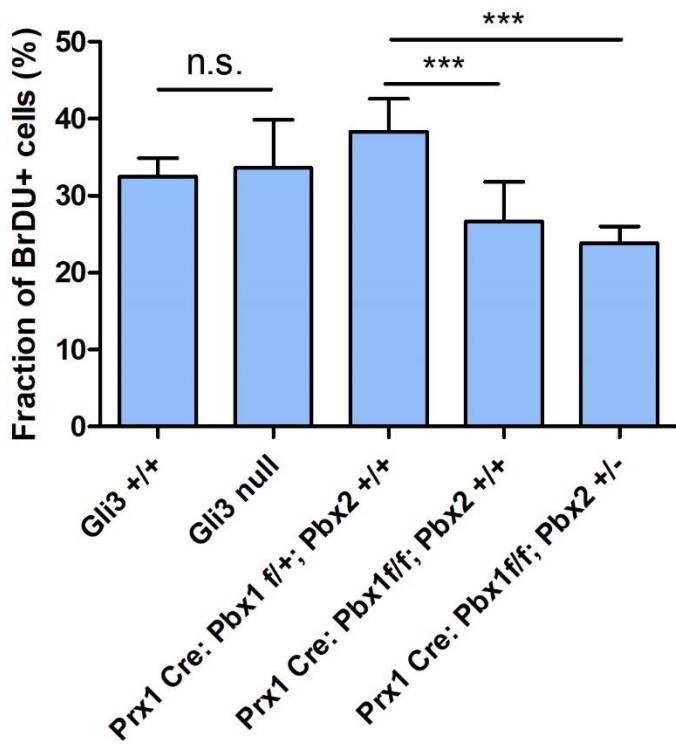


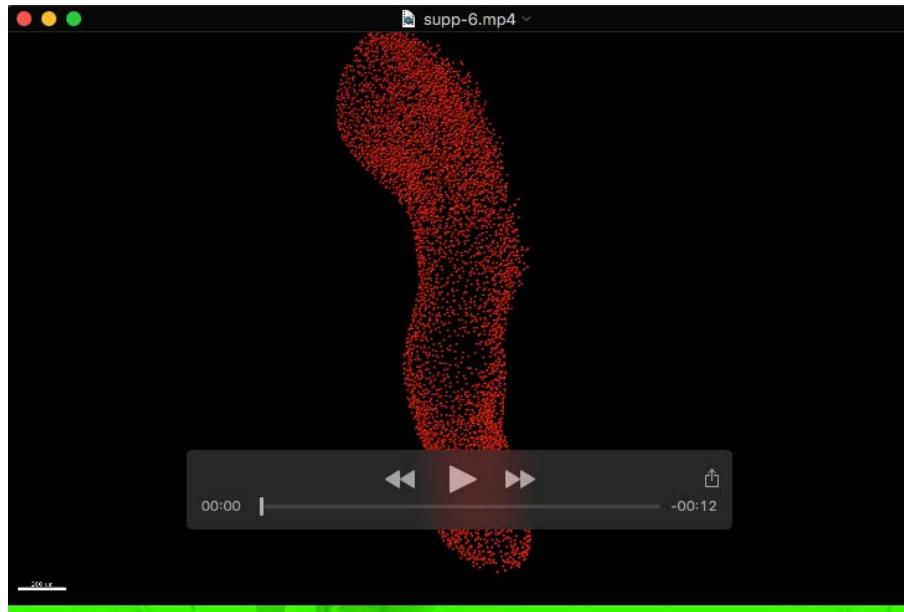
Figure S2. Quantification and comparison of proliferating cells among proximal humeral superstructure precursors in *Gli3* and *Prx1-Cre:Pbx1;Pbx2* control and mutant embryos. Paraffin sections taken from E13.5 *Gli3* and *Prx1-Cre:Pbx1;Pbx2* control and mutant forelimbs were immunostained for BrdU. The number of total *Sox9*⁺ and *Sox9*⁺/BrdU⁺ cells were counted in the region of the greater and deltoid tuberosity and the fractions of proliferating cells were calculated for both control and mutant embryos ($n \geq 6$). Whereas proliferation in both *Prx1-Cre;Pbx1*^{flaxed} and *Prx1-Cre;Pbx1*^{flaxed}; *Pbx2*^{het} mutant embryos was significantly decreased, *Gli3*^{null} embryos did not display significant changes in proliferation compared to control embryos. Statistical significance was determined as $p \leq 0.05$.

Table S1. Quantification of *Gli3^{null}* morphological variations within the deltoid tuberosity, patella and tibia.

Age (n)	Deltoid tuberosity		Patella			Tibia
	Severe/detached	Mild/dysplastic	Absent	Smaller	Deformed	Absent
E15.5 (4)	3/4	1/4	4/4	0/4	0/4	4/4
E17.5 (8)	3/8	5/8	0/4	5/8	3/8	8/8

Deltoid tuberosity morphologies were divided into two categories, namely severe or mild dysplasia. Detached deltoid tuberosity was categorized as severe phenotype. Patella morphologies were divided into three categories, namely absent, smaller or deformed. The tibia was absent in all *Gli3* mutant embryos.

gene	Ole_vs_D	Ole_vs_D	Ole_vs_D	Ole_vs_D	Ole_vs_D	Ole_vs_D							
	dChange (reduced)	Tlog2FC (reduced)	dChange (reduced)	Tlog2FC (reduced)	dChange (reduced)	Tlog2FC (reduced)	p-value (FDR)						
Nav	-4.14865	5.045-14 Hoxa2	-1.1512	0.000141 Pieno1	-0.8432	0.001444 Hd6t1	-0.56647	0.01526 Fgrf1	0.675382	0.001835 Mia	0.65361	1.088-06	
Hoxc5	-3.7619	5.045-14 Col1a1	-1.0778	0.000289 Gamm	-0.83972	9.016-05 Lamb1	-0.56408	0.001119 Cercam	0.677161	0.004345 Sctv	1.071125	5.046-14	
Pitx2	-3.40387	5.045-14 Ephb1	-1.0584	0.000111 Star8	-0.83715	0.00272 Cftr	-0.56227	0.041522 Ldha	0.685172	7.826-05 Cadre2	1.076673	0.000412	
Actr1	-3.20499	5.045-14 Pid1	-1.0563	5.156-05 Cxcl2	-0.83612	1.886-09 Glpr2	-0.56217	0.001409 Cpr	0.682582	0.015563 Fam181i	1.080127	3.096-05	
Hoxc6	-3.1742	5.045-14 Ank1	-1.04977	0.000049 Coro2b	-0.8359	0.00618 Mmp2	-0.55566	0.000881 ler5	0.68316	0.001059 Sct4a2	1.087374	0.000221	
Hoxa5	-3.02939	5.045-14 Tubb2b	-1.0942	3.306-13 grf1b	-0.83508	0.000452 Elavl1	-0.55458	0.036416 Impa2	0.696762	0.029668 Chaet	1.090667	1.476-05	
Hoxc6	-2.94419	5.045-14 Shx2	-1.0952	1.456-08 Fyn	-0.83302	0.000187 Bircl	-0.55326	0.025149 Gmnds	0.697403	0.002805 Tmem158	1.090901	5.216-07	
Hoxb4	-2.83826	5.045-14 Dnpo3	-1.09081	0.000522 Nrp2	-0.83106	4.516-05 Tagln2	-0.55223	0.013264 Phax2	0.699812	0.005201 Soc52	1.100538	2.356-07	
Hoxb5	-2.77666	5.045-14 Map1b	-1.08749	1.466-09 Tpm1	-0.83093	3.126-10 Efnb1	-0.54829	0.003108 Dhrs3	0.702053	0.010107 Acan	1.104008	0.00037	
Adh1	-2.73869	5.045-14 Vstm4	-1.08716	0.000168 Dmpk	-0.82753	0.015288 Grm	-0.54821	0.002524 Nrf2l2	0.704658	0.014864 Unsc	1.113077	5.506-07	
Ctgntf3	-2.71199	5.045-14 Trp53bl1	-1.08379	7.586-05 Ntm	-0.82514	0.017984 Esy1	-0.54255	0.024136 Gja3	0.705013	0.018824 Map2	1.121435	0.000281	
Hoxa2	-2.46659	5.045-14 Adams2	-1.08343	0.000233 I13	-0.82234	0.025267 Dik3	-0.54054	0.025656 Pthhl2	0.705822	0.022822 Pthhl2	1.126021	0.000237	
Hoxb5Sos	-2.45986	5.045-14 Nes	-1.08146	2.416-05 Pkd2	-0.82192	5.666-08 Pbx1	-0.53703	0.005073 Tmem131	0.707782	0.004259 Rarg	1.130266	3.566-09	
Crh	-2.45993	5.045-14 Rgct1	-1.07999	0.000498 Slc12	-0.82173	0.022101 Cd36	-0.53485	0.033155 Srv18	0.714725	0.007901 Gpr4	1.137559	1.376-05	
Pax1	-2.37227	5.045-14 Crsb2	-1.07883	0.000031 Bdh2	-0.82003	0.003544 Adams15	-0.53097	0.033155 Trappcd3a	0.715238	0.008449 Stmn2	1.138868	5.206-05	
Hoxa4	-2.33988	5.045-14 Dnpo3	-1.07578	0.000659 Peg3	-0.81911	0.000701 Zfp503	-0.52912	0.020501 Pax9	0.717446	0.027249 Homer2	1.146975	5.446-06	
Acta2	-2.33381	5.045-14 Peg10	-1.06925	4.026-06 Myo	-0.81095	0.011443 Tmem164	-0.52657	0.034722 Atoh8	0.727935	0.024875 Snai1	1.149585	1.596-07	
Pgm5	-2.24275	5.045-14 Crnp1	-0.98689	0.000701 Dok5	-0.80895	0.028285 Tmem4	-0.52489	0.03091 Gabpb1	0.727994	0.026988 Mfsd2a	1.161992	0.000149	
Meis1	-2.23013	5.045-14 Svil	-1.06677	1.356-05 Ston	-0.80716	0.008163 Tmsb4d	-0.52099	0.009033 Fam53b	0.72981	0.026509 Fmod	1.16736	1.988-07	
Vgl1	-2.20856	5.045-14 Fgf1	-1.0659	0.000993 Ebf2	-0.80343	1.506-05 Ppp1rlab	-0.51972	0.005254 Dusp1	0.740969	0.036382 Bnpip3	1.186573	8.456-09	
Hspb1	-2.09953	5.045-14 Rgct2	-1.05882	5.666-08 Pam1	-0.79795	0.032995 Smarc2	-0.47594	0.049871 Tbxb7	0.741412	0.004147 Foxp2	1.187366	5.696-06	
Myod1	-2.09621	5.045-14 Tgfb1	-1.05872	2.316-07 Tl006	-0.79775	0.033384 Tril	-0.47498	0.019169 Efem1	0.753222	0.014064 Mab2111	1.189824	8.826-07	
Msc	-2.05679	5.045-14 My4	-1.05777	0.000743 Mchr1	-0.79479	0.00287 Uchl1	-0.47111	0.014064 Fif1	0.7702	0.016903 Kera	1.19097	8.816-05	
Hoxb6	-2.02519	5.045-14 Hoxa2	-1.05704	0.000912 Khk	-0.79352	0.028076 Nflh	-0.46959	0.00362 Id1	0.775467	2.816-09 Plag16	1.231173	7.176-05	
Tnn1	-1.98928	5.045-14 Itih5	-1.05626	0.000883 Hoxa7	-0.79188	0.015957 Igf1	-0.46322	0.001008 Csnnp3	0.7755	0.042075 Foxp1	1.234903	7.746-11	
Akap4	-1.95538	2.876-12 Vrt1	-1.04861	0.000242 Cox5b	-0.78949	0.000412 Cox5b	-0.44225	0.002421 Tsc2d1	0.77745	1.196-08 Hir2	1.248122	2.416-06	
Meis2	-1.95349	5.045-14 Svil	-1.04677	1.356-05 Ston	-0.78746	0.005942 Sipr1	-0.43961	0.022499 Nrm1	0.780914	0.024243 Wwp2	1.250526	5.086-14	
Hoxb5	-1.94544	5.045-14 Tpox1	-1.04634	0.000188 Axin2	-0.78639	0.015210 Tpox1	-0.43520	0.027856 Tpox1	0.785053	0.027856 Tpox1	1.250526	5.046-14	
Stom24	-1.90487	2.711-12 Prrb2	-1.04541	0.000491 Mel3	-0.78562	0.020310 Hck5	-0.42279	0.005341 Dim3os	0.786617	0.027956 Wnt4	1.279519	2.356-07	
Hoxb2	-1.87411	5.045-14 Fgf13	-1.04587	0.001118 Par3	-0.78125	0.028844 Cond3	-0.4169	0.017178 Afet1	0.789193	1.386-05 C1c10a3	1.286553	8.676-13	
Cyp26a1	-1.81853	4.141-11 Nod1	-1.04509	5.616-05 Cyp26a1	-0.77737	0.017178 Cyp26a1	-0.41593	0.017322 Glz2	0.790571	0.015753 Foxc2	1.287162	1.566-07	
Ighbp3	-1.77634	5.045-14 Gata3	-1.04267	0.000241 Syt1	-0.77412	0.019265 Mest	-0.37191	0.019286 Colq3	0.799895	0.005-08 Edta1a	1.288975	3.206-12	
Myog	-1.75109	2.525-10 Gm26	-1.01183	0.000289 Tgpn9	-0.77049	0.000152 Gata10	-0.36682	0.005628 Vill	0.791205	0.008543 Erg	1.300608	3.606-06	
Gm1	-1.74188	5.045-14 Cym	-1.00868	0.001918 Tagin	-0.76802	0.02592 Rpl37	-0.36694	0.027873 Wnt5b	0.792157	0.003791 Ifitm1	1.308785	9.576-06	
Hoxa3	-1.73739	1.474-10 Runx4	-1.00692	0.000247 Famb5b	-0.76546	0.025656 Tuba1	-0.36388	0.045411 Ccrp2	0.792453	1.766-08 Rdlim5	1.310951	7.856-06	
Clu	-1.70541	5.045-14 Cdhd4	-1.00332	1.996-05 Zic4	-0.76581	0.002025 H3ta	-0.36388	0.026118 Fip1	0.793085	0.004492 LOC10263	1.326985	3.136-07	
Wnt2	-1.69163	1.302-09 Foxd1	-1.00001	0.01693 Kazal1	-0.76569	0.005675 H2afy	-0.36279	0.038401 Smad7	0.801303	0.002366 Nog	1.330008	5.856-07	
Gpx3	-1.66573	5.045-14 Ntn1	-0.99882	0.000931 Fam107a	-0.76511	0.003840 Blm8	-0.36284	0.038884 Epp6	0.802485	0.016846 Gloc1	1.36999	7.739-06	
Gfr2a	-1.62987	5.045-14 Cx3cr1	-0.99462	1.111-09 Chrn1	-0.76178	0.015753 Fcrl2	-0.36289	0.038893 Cx3cr1	0.803344	0.016696 Mecom	1.393335	9.446-10	
Fln	-1.61634	5.045-14 Tmem8c	-0.99446	0.000213 Tont2	-0.75672	0.015235 Wbp5	-0.36123	0.047223 Pkg1	0.804112	0.002362 Olecr1a	1.396226	9.896-07	
Hoxc8	-1.61596	5.045-14 Tubb2a	-0.99279	3.956-09 Tripob	-0.75779	0.003321 Ost	-0.36144	0.021436 Sem3d	0.806984	0.009668 Sox5	1.409572	5.196-07	
Zic1	-1.61358	8.745-14 Nrgn	-0.98913	0.002644 Camk2d	-0.75724	0.013015 Pgam1	-0.35973	0.009237 Hif1	0.813502	0.006032 Rrb2	1.411773	1.838-07	
Gm7325	-1.61041	1.002-08 Act1	-0.98642	0.002432 Pea15	-0.75676	1.306-06 Tbx5	-0.35824	0.041085 Barx2	0.814199	0.01067 Hd11b2	1.421657	2.526-10	
Irx3	-1.60624	1.265-08 Atp6v0e2	-0.98245	0.002865 Ptx3	-0.75624	0.015225 Flrt2	-0.346039	0.033388 Daam1	0.819055	0.004492 LOC10263	1.426985	3.136-07	
Hotai1	-1.58553	1.485-13 Dltd1	-0.98247	0.001444 Fgf17	-0.75566	0.006666 H1tf	-0.34572	0.027392 Thbd	0.819383	0.019401 Crf1	1.436459	5.046-14	
Plxn2a	-1.58123	1.495-10 Tmem132	-0.98141	0.004199 Pldx2	-0.75482	0.015095 H1tf	-0.34510	0.020388 Fndc1	0.819949	0.012133 Cdo1	1.43882	5.046-14	
Cdh19	-1.57471	1.595-07 Tnn1	-0.98144	0.004719 Cxcr1	-0.75457	0.015118 Chst13	-0.34490	0.020392 Hmef1	0.820472	0.011622 Hmef1	1.440701	2.916-07	
Gap43	-1.54357	5.045-14 Tbx2a	-0.98111	0.005991 Tmem132	-0.75345	0.015945 Srv18	-0.34458	0.028642 Fntf8	0.820506	0.000445 Sal1	1.457371	2.778-08	
Rspo1	-1.53032	7.811-07 Anxa2	-0.98099	0.000284 Spkr1	-0.75394	0.001386 Sps2	-0.34470	0.009033 Kank1	0.820491	0.004112 Hd9v9	1.462926	5.046-14	
Dlk1	-1.52904	5.045-14 Meox1	-0.98883	1.486-05 Mtrn1	-0.75612	0.010586 Pgse	-0.34426	0.021525 Scl2a1	0.821159	0.002225 Tbx5	1.461946	2.566-11	
Ajap1	-1.52481	2.355-08 Serpin1	-0.8751	0.000746 Sep1	-0.75538	0.034662 Gdf10	-0.57498	0.047986 Scl2a1	0.822531	3.199-06			
Egfl6	-1.51945	2.626-02 Lyve2	-0.87322	1.596-07 Col1a1	-0.75518	0.047482 Tpx2	-0.68183	0.045781 Bexl2	0.823030	0.002339 Ndr2	0.977639	0.000124	
Rpl39	-1.51924	8.805-06 Igap22	-0.87232	0.001037 Tnc	-0.66676	0.000554 Igf3	-0.578231	0.003210 H3tb2	0.932301	2.706-06			
Ackr4	-1.51892	2.416-05 Grin3a	-0.87244	0.000994 Aebp2	-0.66669	4.666-05 Lmo4	-0.58901	0.003073 Ibd1	0.935525	0.001084			
AW55198	-1.51861	2.595-07 Ramp2	-0.87248	0.000932 Mvk2	-0.66608	0.021043 Acr3	-0.62746	0.033155 Papss2	0.984647	0.001004			
Ifi27	-1.51827	3.165-06 Fznh2	-0.86788	5.506-07 Add3	-0.66195	0.00507 Pdh10	-0.62381	0.047327 Trbl1	1.002989	1.436-08			
Itgb6	-1.51808	0.995-06 Hmx1	-0.86761	0.001983 Vim	-0.66132	1.166-06 Jun	-0.62371	5.076-05 Nrn403	1.005516	0.000698			
Prokr1	-1.51795	6.965-07 Phnl1	-0.86711	1.176-06 Celf2	-0.66109	0.045206 Asc1	-0.624092	0.024444 Son1	1.005667	5.046-14			
Tpm2	-1.51633	0.038-10 Cld5	-0.86754	0.010586 Pgse	-0.66145	0.036462 Ctpb2	-0.624124	0.02508 Smox	0.969756				



Movie 1. *Sox9⁺/Scx⁺* progenitors contribute extensively to humeral morphology. This movie shows a three-dimensional (3D) reconstruction of a humerus and various protruding superstructures. Reconstruction was made from stacked images of E14.5 forelimbs from *Sox9-Cre^{ER}-tdTomato* transgenic embryos using Arivis Vision4D (Arivis) and Imaris (Bitplane) software. Forelimb was additionally stained against COL2A1 to label the outer surface of the long bone. Whereas first-wave *Sox9⁺* progenitors were labeled by tdTomato, the secondary wave of *Sox9⁺/Scx⁺* progenitors remained tdTomato-negative and contributed exclusively to superstructure formation, including bone eminences such as greater, lesser and deltoid tuberosities and various condyles, such as distal, medial and lateral humeral epicondyles.



Movie 2. *Sox9⁺/Scx⁺* progenitors contribute extensively to elbow morphology. This movie shows a 3D reconstruction of the elbow and various protruding superstructures. Reconstruction was made from stacked images of E15.5 forelimbs from *Col2a1-Cre^{ER}-tdTomato* transgenic embryos using Arivis Vision4D (Arivis) and Imaris (Bitplane) software. Forelimb was additionally stained against COL2A1 to label the outer surface of the long bone. Whereas first-wave *Sox9⁺* progenitors were labeled by tdTomato, the secondary wave of *Sox9⁺/Scx⁺* progenitors remained tdTomato-negative and contributed exclusively to superstructure formation, including protrusions such as the olecranon and various condyles, such as distal, medial and lateral humeral epicondyles.



Movie 3. *Sox9⁺/Scx⁺* progenitors contribute extensively to femoral morphology. This movie shows a 3D reconstruction of the proximal femur and various protruding superstructures. Reconstruction was made from stacked images of E15.5 hindlimbs from *Col2a1-Cre^{ER}-tdTomato* transgenic embryos using Arivis Vision4D (Arivis) and Imaris (Bitplane) software. Hindlimb was additionally stained against COL2A1 to label the outer surface of the long bone. Whereas first-wave *Sox9⁺* progenitors were labeled by tdTomato, the secondary wave of *Sox9⁺/Scx⁺* progenitors remained tdTomato-negative and contributed exclusively to superstructure formation, including bone eminences such as the greater, lesser and third trochanters.