

Supplementary Material

Osteogenic Effect of Inducible Nitric Oxide Synthase (iNOS)-Loaded Mineralized Nanoparticles on Embryonic Stem Cells

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Fig. S1. Molecular functions of differentially expressed genes in the cells cultured with iNOS-MNPs. The molecular functions of regulated genes in the cells cultured with iNOS-MNPs were analyzed by ClueGO: functionally grouped network with terms as nodes linked, and functionally related groups partially overlap; the node size represents the term enrichment significance. Only significant term labels per group are shown.

Fig. S2. Functionally enriched KEGG pathways identified by ClueGO. (A) The size of the nodes reflects significance of the term. Network includes genes that are shared between different KEGG pathways (B) Chart represents significant KEGG pathways where bars show numbers of genes associated with each term. *P* values for terms is marked using an asterix (*) if the terms are significant ($0.001 < P \text{ value} < 0.05$).

Supplemental data 1. List of all differentially expressed genes and GO term data. A fold-change cut-off of >1.5 and a *P* value of <0.05 was applied to filter genes that were differentially regulated by iNOS-MNPs treatment compared to the control.

Supplemental data 2. Lists of differentially expressed genes used to identify the activation of bone-related molecular pathways induced by iNOS-MNP treatment ($P < 0.05$, with fold-change >1.5).

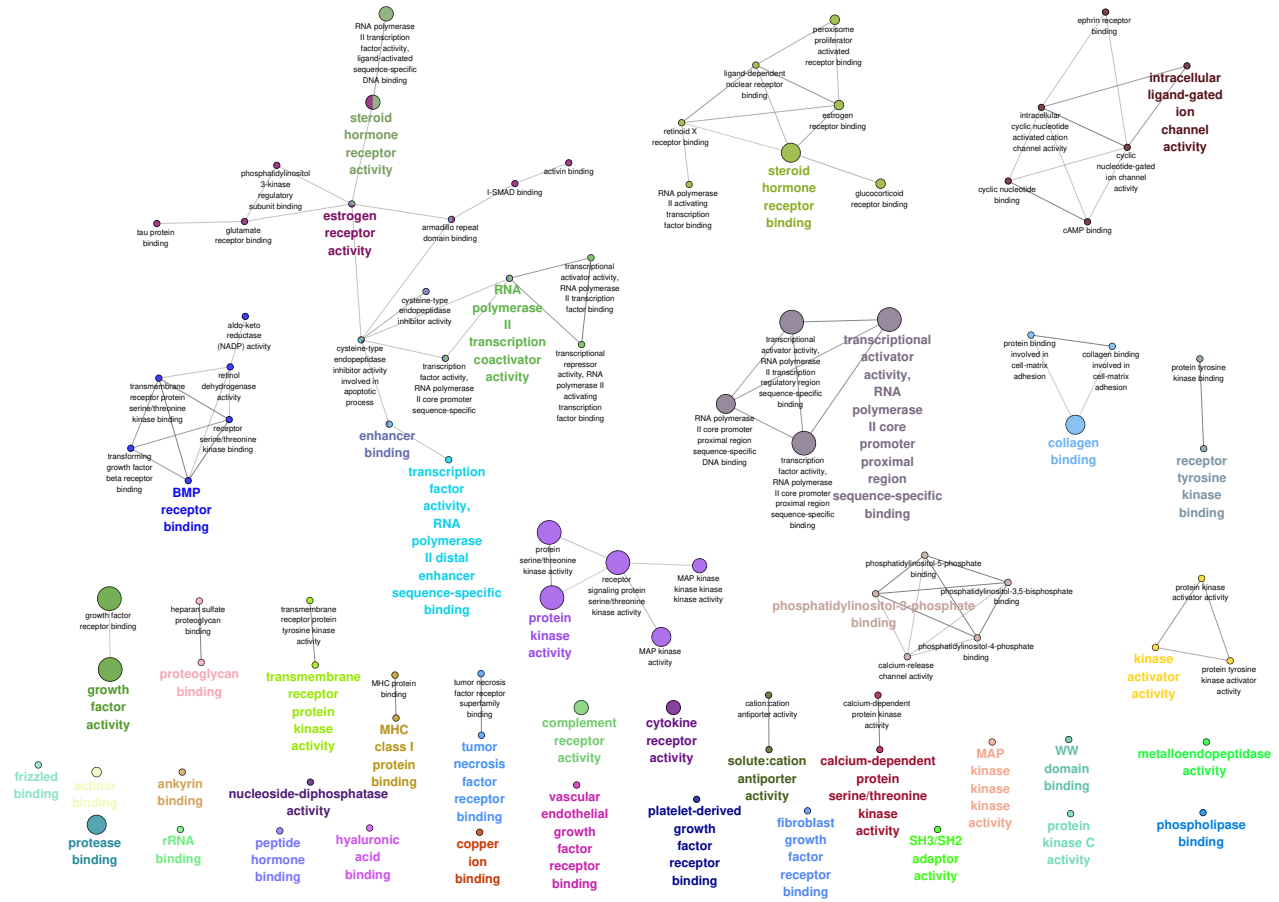
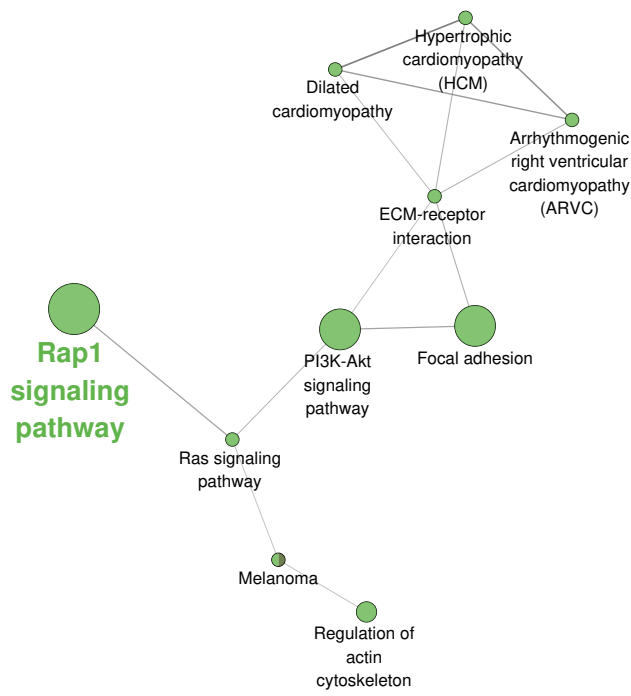


Figure S1

A



B

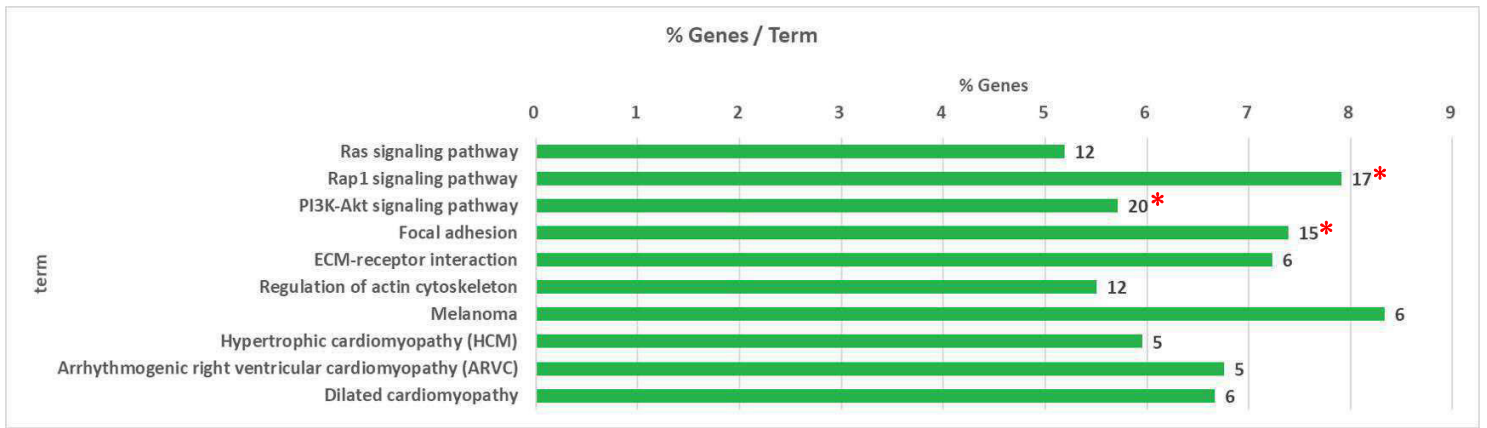


Figure S2





cell differentiation	cell proliferation	extracellular matrix	BMP signaling pathway	calcium ion binding	calcium mediated signaling	cartilage development	growth factor binding	MAPK cascade	notch signaling pathway	vasculature development	wnt signaling pathway	cell adhesion	cell association	growth factor activity	ossification	skeletal system development
Mfrp 4.749	Sox2 4.680	Albig 4.932	Bmp8a 4.764	Calr5 4.990	Jph2 4.926	Bmp8a 4.746	Scnf5a 4.664	Ngp1 4.408	Hes7 4.420	App1 4.522	Wnt10a 4.404	Nxk2-3 4.482	Nxk2-3 4.482	Fgf11 4.806	Atng 4.794	Bmp8a 4.746
Tiad2 3.916	Fam68a 3.699	Rarg2 3.586	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Pyg2 3.992	Jag2 3.992	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
H2-Dm1a 4.444	Fam68a 3.699	Rarg2 3.586	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Ect1 4.197	Trop2 4.444	Wnt9a 4.444	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Lnc55 4.749	Tgfb 4.582	Wnt9a 4.444	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Cxcl1 4.618	Mmp11 3.916	Emp2 3.916	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Wnt9a 4.618	Cb1 1.946	Lmn1 1.946	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Crysp1 4.088	Matt1 2.064	Tgfb1 2.064	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mrfg 4.131	Pdgfrb 1.708	Adam44 2.320	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Gm73 4.138	Gm73 4.138	Mmp11 3.916	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Sfrp1 4.148	Abcb1a 1.672	Wnt9a 4.444	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Gm1 4.093	Aim2 1.538	Mmp15 1.670	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mmp2 3.545	Imp2 1.533	Rarb1 1.641	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Klf2 3.374	Bok 1.517	Pdzr1 1.517	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Trid4 3.355	Npr3 0.620	Mmp1a 0.620	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Pgf 3.331	Cor1 0.620	Col2a2 0.620	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mov101 3.331	Cor1 0.620	Col2a2 0.620	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Osip1 3.036	Das1 0.490	Wnt9a 4.444	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Catpdr 2.881	Col2a2 0.620	Mmp8 0.620	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mmp1 2.858	Mmp1 2.858	Mmp1 2.858	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Fgg 2.833	Tack 0.447	Col4a4 0.447	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mmp1 2.833	Kam 0.436	Col4a4 0.436	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Tgfb 2.804	Term4 0.418	Wt 0.418	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Sox1 2.722	Mcom 0.406	Mmp13 0.406	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Selmp1 2.556	Es1 0.384	Wnt3 0.384	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Sox1 2.443	Lnc17 0.376	Mmp16 0.376	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Spry1 2.389	Lgt3 0.364	Evan 0.364	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Chrd 2.387	Trm7 0.356	Dmrt1 0.356	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Adam44 2.360	Wnt4 0.350	Emp1 0.350	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mmp12 2.338	P2ry7 0.307	Cannt1b 0.307	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mmp1 2.271	Acnt1c 0.305	Emp12 0.305	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Rarg1 2.191	Eph1 0.312	Cannt1 0.312	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
App4 2.126	Lef1 0.289	Adam23 0.289	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Fem3 2.105	Wnt3 0.286	Adam23 0.286	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Prca 1.969	Ros1 0.247	Adam23 0.247	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Prca 1.969	Ros1 0.247	Adam23 0.247	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Ad44131 1.858	Mmp15 0.230	Adam23 0.230	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Lmn1 1.853	C2 0.216	Adam23 0.216	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Matt1 1.848	Dmrt1 0.209	Adam23 0.209	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Gm2 1.839	Asn2 0.204	Adam23 0.204	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Fach 1.745	Dmrt2 0.195	Adam23 0.195	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Rgk4 1.726	Nkx1 0.191	Adam23 0.191	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Wnt9a 1.662	Sh3bp2a 0.183	Adam23 0.183	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.482	Bmp8a 4.746	Atng 4.794	Bmp8a 4.746
Mmp11 1.654	Kzmf1 0.174	Adam23 0.174	Bmp2 3.586	Calr6 3.697	Rarg2 3.586	Sox2 3.646	Scnf5a 4.664	Mpg2 3.699	Notch4 3.699	Exor 3.992	Wnt6 3.992	Nxk2-3 4.482	Nxk2-3 4.			