<u>Manuscript title:</u> Distinct gene expression pattern define human osteoblasts' response towards BMP2-treatment -Is the therapeutic success all a matter of timing?

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		basal expression			BMP2 treated			
			G 1 (no response)	G 2 (viability个)	G 3 (function个)	G 1 (no response)	G 2 (viability个)	G 3 (function个)
BMP2 Signaling	receptors	Alk3	1,579 ± 0,200	1,012 ± 0,136	0,664 ± 0,083	1,254 ± 0,240	1,027 ± 0,208	0,865 ± 0,237
		BMPRII	2,055 ± 0,513	0,876 ± 0,133	0,507 ± 0,062	2,199 ± 0,422	1,277 ± 0,266	0,935 ± 0,148
		RGMb	0,196 ± 0,033	2,823 ± 0,661	2,074 ± 0,275	0,440 ± 0,101	4,139 ± 0,549	4,169 ± 0,387
		Alk6	2,241 ± 0,818	1,144 ± 0,145	0,501 ± 0,251	0,970 ± 0,115	1,990 ± 0,777	5,642 ± 2,871
		ActRIIA	1,189 ± 0,184	0,888 ± 0,156	0,857 ± 0,129	1,177 ± 0,190	0,879 ± 0,159	0,732 ± 0,174
		ActRIIB	0,948 ± 0,139	1,319 ± 0,146	0,845 ± 0,216	2,166 ± 0,825	1,353 ± 0,652	1,224 ± 1,004
	transcription factors	Smad1	1,732 ± 0,405	0,854 ± 0,130	0,642 ± 0,097	2,132 ± 0,558	1,246 ± 0,271	0,707 ± 0,199
		Smad4	0,711 ± 0,226	1,174 ± 0,271	1,881 ± 0,884	1,767 ± 0,658	1,725 ± 0,648	1,557 ± 0,645
		Smad5	1,955 ± 0,480	0,739 ± 0,159	0,813 ± 0,110	2,416 ± 0,734	1,468 ± 0,421	0,820 ± 0,115
		Smad8	0,876 ± 0,130	0,904 ± 0,109	1,263 ± 0,174	1,046 ± 0,122	0,893 ± 0,125	0,847 ± 0,130
	inhibitors	BAMBI	10,390 ± 5,057	0,803 ± 0,327	0,293 ± 0,158	12,510 ± 5,989	2,353 ± 0,987	0,405 ± 0,148
		Noggin	0,560 ± 0,109	1,166 ± 0,208	1,538 ± 0,188	1,415 ± 0,213	0,712 ± 0,164	3,595 ± 0,680
		Smad6	0,697 ± 0,152	1,129 ± 0,135	1,345 ± 0,261	1,510 ± 0,207	1,161 ± 0,148	1,534 ± 0,199
		Smad7	1,307 ± 0,188	0,969 ± 0,125	0,764 ± 0,139	0,917 ± 0,119	0,864 ± 0,131	1,125 ± 0,146
Wnt Signaling	receptors	LRP5	1,588 ± 0,277	0,941 ± 0,107	0,624 ± 0,076	1,385 ± 0,177	1,107 ± 0,155	0,814 ± 0,098
		LRP6	1,788 ± 0,155	1,112 ± 0,250	0,458 ± 0,146	1,610 ± 0,269	1,423 ± 0,329	0,713 ± 0,173
	inhibitors	DKK1	1,223 ± 0,197	0,963 ± 0,109	0,823 ± 0,154	1,145 ± 0,244	0,889 ± 0,109	0,697 ± 0,068
		DKK2	1,254 ± 0,231	1,014 ± 0,108	0,776 ± 0,102	1,147 ± 0,296	1,249 ± 0,394	1,235 ± 0,315
		SOST	1,145 ± 0,176	2,184 ± 0,733	0,384 ± 0,112	0,883 ± 0,150	0,878 ± 0,093	3,358 ± 0,337
Osteoclasto-		OPG	0,983 ± 0,133	0,602 ± 0,097	1,552 ± 0,366	1,084 ± 0,124	0,837 ± 0,122	1,794 ± 0,326
genesis		RANKL	0,522 ± 0,167	3,688 ± 2,012	0,619 ± 0,125	1,558 ± 0,971	1,572 ± 0,828	10,140 ± 5,713

## **Supplemental Figure 3**

≤ 0.25 fold

Supplemental Figure 3: Expression levels of genes involved in BMP2 and Wnt signaling. Osteoblasts were differentiated in the presence or absence of 50 ng/ml rhBMP2. After 48 h RNA was collected and RT-PCRs were performed. Densitometric analysis was performed with the individual samples (N = 7; n = 2 per group). For analysis the average signal intensity of the unstimulated cells was set as reference. Heat map shows the average gene expression  $\pm$  standard deviation. Response groups are displayed as group 1 (no response), group 2 (viability  $\uparrow$ ) and group 3 (function  $\uparrow$ ).

average

≥4 fold