Supplementary Materials

Downregulation of Heat Shock Protein 70 Impairs Osteogenic and Chondrogenic Differentiation in Human Mesenchymal Stem Cells

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Figure S1. HSP27 and HSP90 expression measured by Real-time RT-PCR in hMSCs during the growth and differentiation at Day 12 (A and C) and Day 26 (B and D).



Figure S2. Western Blot membrane of Figure 1A.



Figure S3. Full DNA gel images of Figure 2A.



Figure S4. Full Western Blot membrane of Figure 2B.

	Scrambled	HSP70 Knockdown	Reduction (%)
Day 6			
Growth Condition	□ 54.44±3.37	39.40±9.20	
Growth Condition+HS	• 58.26±0.36	43.92±8.42	
Differentiated	99.54±19.18		60.70
Differentiated+HS	• ••	45.30±0.87*	71.07
Day 12			
Growth Condition	-76.42±11.82	59.53±2.30	
Growth Condition+HS	\$5.46±12.80	70.65±4.55 •	
Differentiated	104.30±14.61	4 84.78±11.08	18.72
Differentiated+HS	* L _{149.09±14.96}	107.45±14.61	27.93

Table S1. Alkaline Phosphatase Activity (Unit[†])

[†]One enzyme unit of ALP is the amount of enzyme which releases 1 nmol p-nitrophenol per 15 minutes. Each sample had 10^4 seeded hMSCs. HS: heat shock;**P*<0.05; ***P*<0.01. N=4.

Table S2. Calcium Deposition ($\mu g/10^4$ seeded hMSCs)

ю.	Scrambled	HSP70 Knockdown	Reduction (%)
Day 19	•		1 <u>.</u>
Growth Condition	20.94±7.10	14.66±4.12	
Growth Condition+HS	* 16.56±5.37	16.24±7.23 *	
Differentiated	= 173.78±13.03*	*43.88±12.51	74.75
Differentiated+HS	* 202.26±6.87 **	* 83.11±10.96 — *	58.91
Day 26			
Growth Condition	61.73±3.60	51.14±6.95	
Growth Condition+HS	* 70.48±10.22	55.58±7.52 *	
Differentiated	= 275.57±5.65**	* 160.29±10.45 -	41.84
Differentiated+HS	* _ 306.43±11.10**	*186.37±12.17	39.18

P*<0.05; *P*<0.01. N=4.