SUPPLEMENTARY INFORMATION

Guiding the osteogenic fate of mouse and human mesenchymal stem cells through feedback system control

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Supplementary Information contains:
Supplementary Table S1
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Supplementary Table S1: Concentrations of extrinsic factors reported in osteogenic media.

Extrinsic Factors	Reported concentrations in the literature
AA2P, AA (μM)	10, 25, 50, 119.19, 200, 238.38, 250, 300, 476.76, >500
VD3 (nM)	0.1, 1, 10, 100, 1000
BMP-2 (ng/mL)	1, 3, 5, 6.25, 10, 12.5, 25, 30, 40, 50, 100, 150, 200, 250, 300, 400, 500, 800, 1000
Hep (μg/mL)	0.1, 0.2, 0.3, 0.5, 0.625, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 10, 15, 20, 30, 50, 100, 200, 2000
RA (nM)	< 1, 1, 2.5, 5, 10, 20, 100, 1000, 2500
Dex (nM)	< 1, 1, 10, 100, 1000
beta-GP (mM)	< 1, 5, 8, 10, 50

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Growth Factors:

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Figure S1| Double-objective FSC iterations using ALP expression as a biological fitness assay. (a) The representative iteration course change of the extrinsic factor concentrations. RA concentration increased in each iteration, whereas other factors showed a tendency of gradual decrease. Hep showed a sudden increase after the 7th iteration. (b) Contribution of RA for ALP activity. Removal of RA from an original cocktail completely abolished early ALP expression of D1 cells and L929 cells. D1 cells and L929 cells were seeded at 3125/cm^2. ALP activity was measured at day 3. Data show mean ± s.d. (n=3 per group). **: p<0.01 and *: p<0.05 compared to the original cocktail (ANOVA with a Dunnett's test).

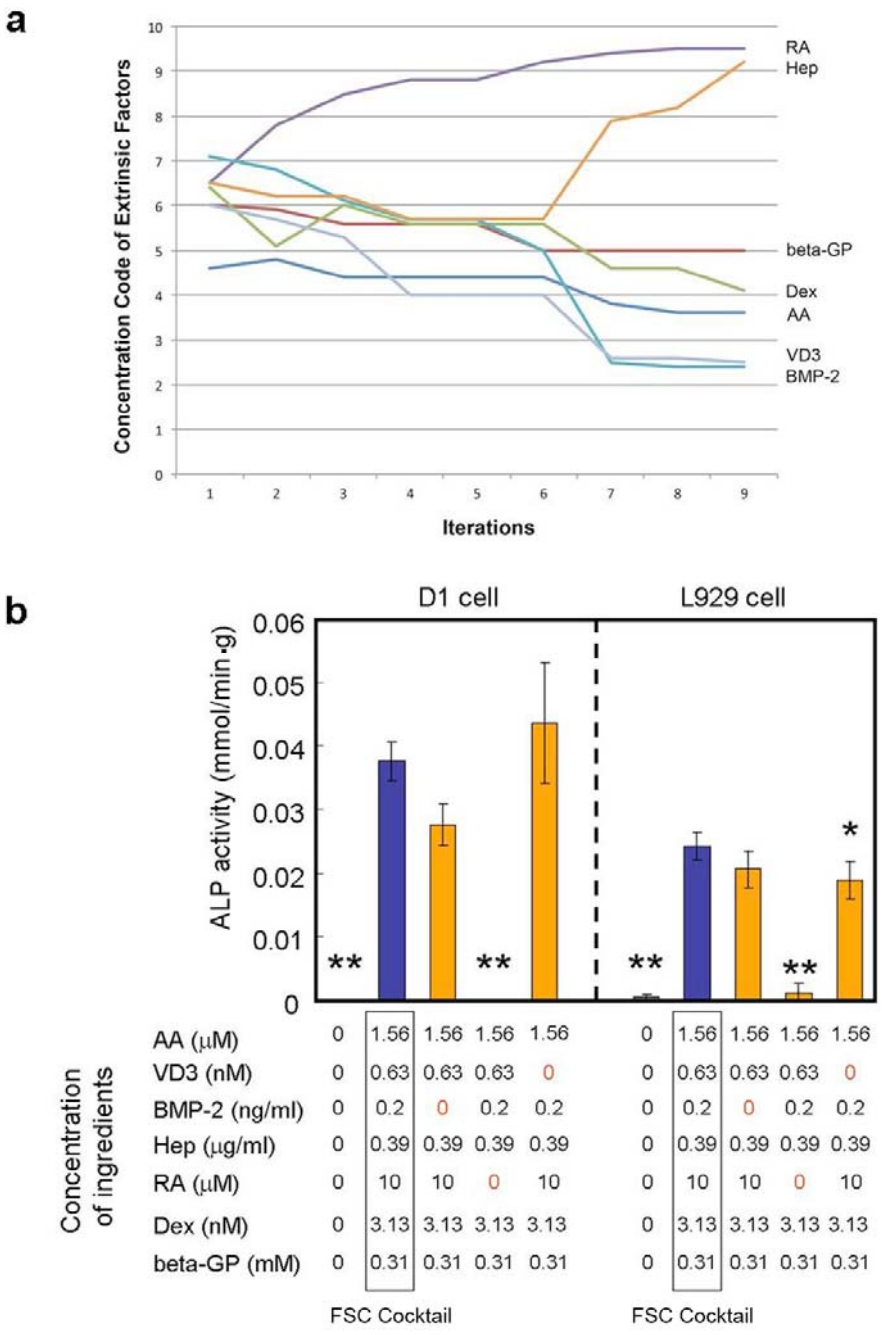


Figure S2| Classification of drug cocktails. (a) Classification of drug cocktails used in FSC-DE with mineralization assay in accordance with those mineralization indexes. Italic numbers: mineralization index. After 9 iterations, 14 cocktails (boxed) were found to induce the equivalent of or at least one half of Ca^{2+} deposition as compared to TB containing high dose of BMP-2 (100 ng/ml). (b) Classification of the above 14 drug cocktails (boxed) in accordance with BMP-2 concentration. Italic numbers: BMP-2 concentration. Eight drug cocktails containing less than 25 ng/ml BMP-2 were used in the further experiment.

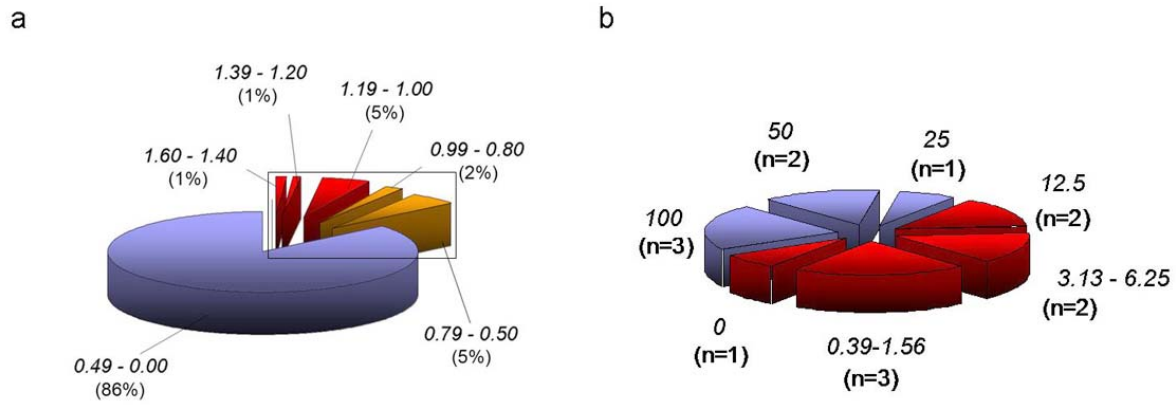
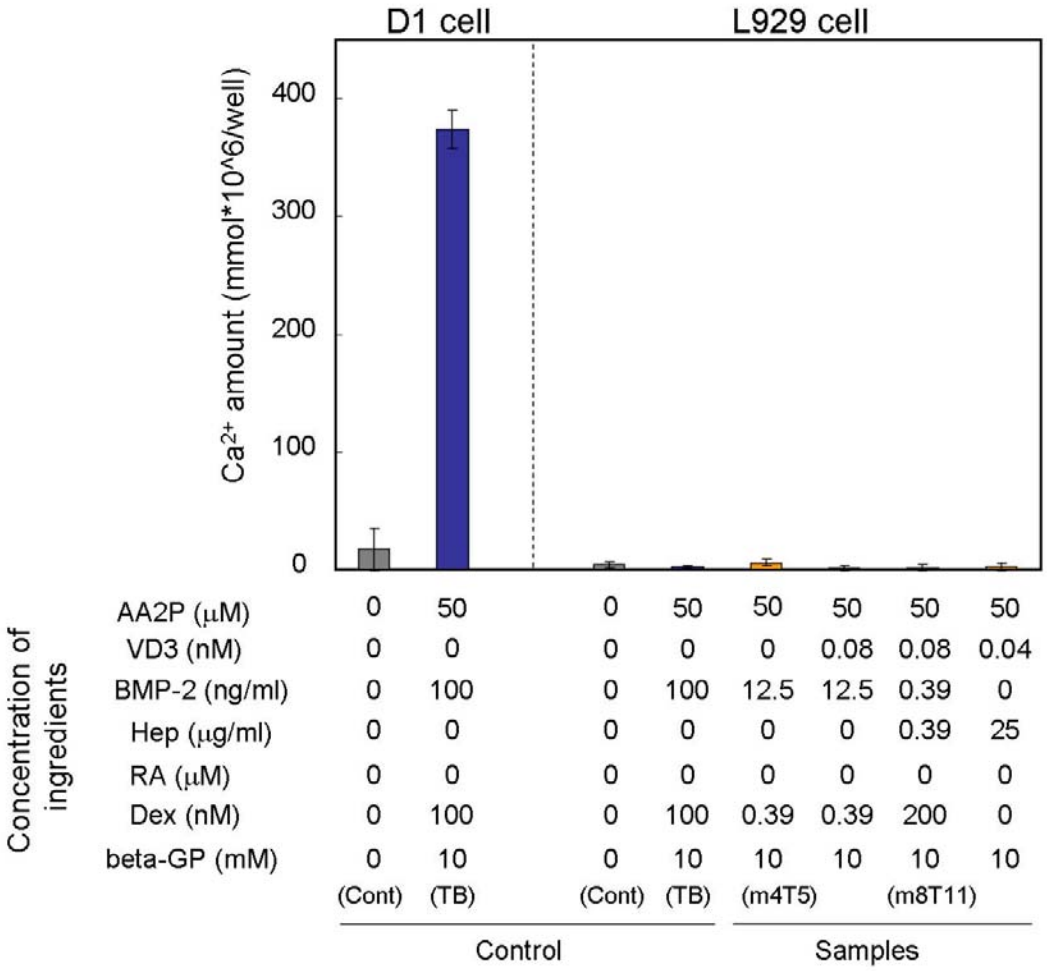


Figure S3| Effect of candidate cocktails on *in vitro* mineralization of L929 fibroblasts. None of the tested cocktails induced *in vitro* mineralization. Cells were seeded at 45,000/cm^2; once the cells became confluent, the media were changed to the basal media and then to the media with each drug cocktail. Ca^{2+} content in the well was measured at day 7. Data represent mean ± s.d. (n = 3 per group).



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