Supplementary Data

3,3′-Diindolylmethane stimulates exosomal Wnt11 autocrine signalling in human umbilical cord mesenchymal stem cells to enhance wound healing

Hui Shi[1, 3] , Xiao Xu[1, 3] , Bin Zhang[1, 3], Jiahao Xu[1], Zhaoji Pan[1], Aihua Gong[1], Xu Zhang[1], Rong Li[1], Yaoxiang Sun[1], Yongmin Yan[1], Fei Mao[1], Hui Qian[1], Wenrong Xu[1, 3]

[1] Key Laboratory of Laboratory Medicine of Jiangsu Province, School of Medicine, Jiangsu University, Zhenjiang, Jiangsu, P. R. China;
[2] The Affiliated Hospital, Jiangsu University, Zhenjiang, Jiangsu, P. R. China;
[3] These authors contributed equally to this work.

Contact: Wenrong Xu, Ph D. M D, Professor, School of Medical Science and Laboratory Medicine, Jiangsu University, 301 Xuefu Road, 212013, Tel: +86 511 85038215, Fax: +86 511 85038483, E-mail: icls@ujs.edu.cn; Hui Qian, Ph D. M D, Professor,School of Medical Science and Laboratory Medicine, Jiangsu University, 301 Xuefu Road, 212013, Tel: +86 511 85038334, Fax: +8651185038483, E-mail: lstmmmlst@163.com

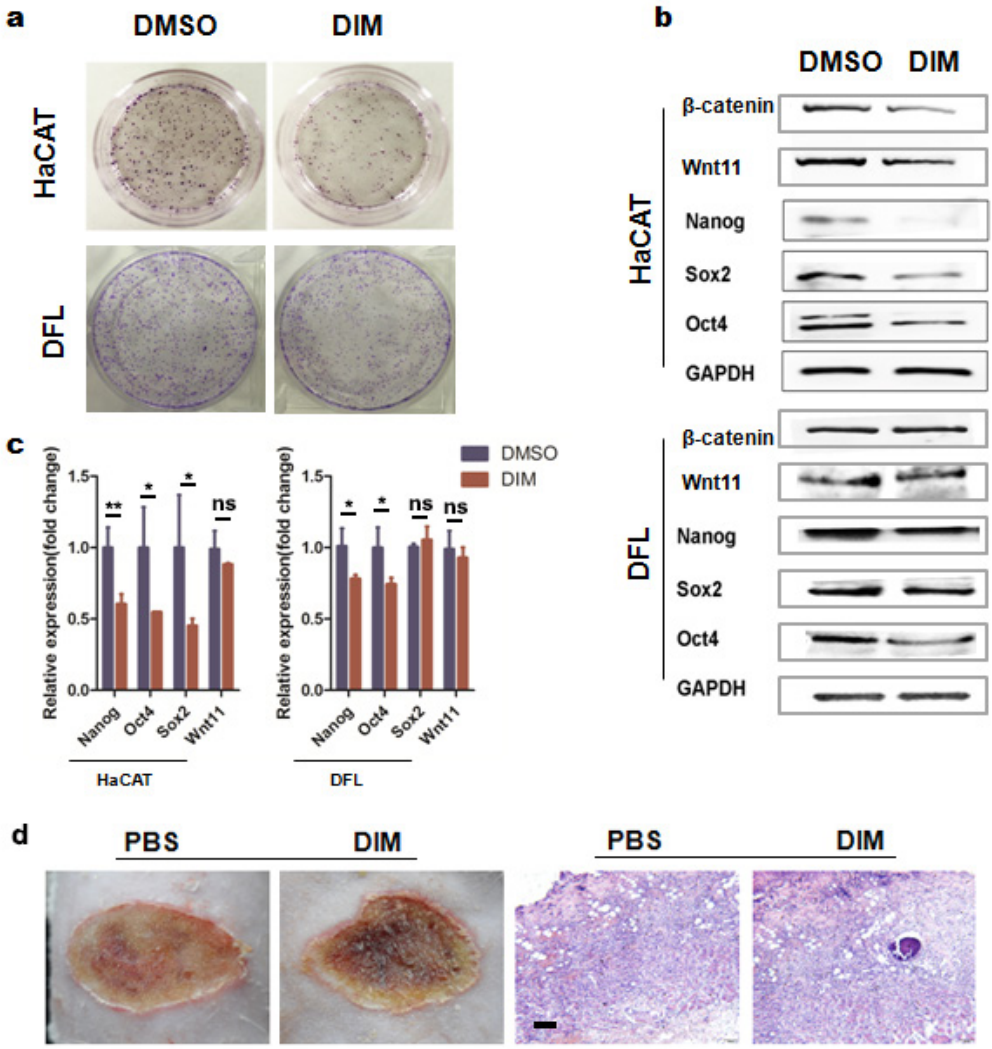
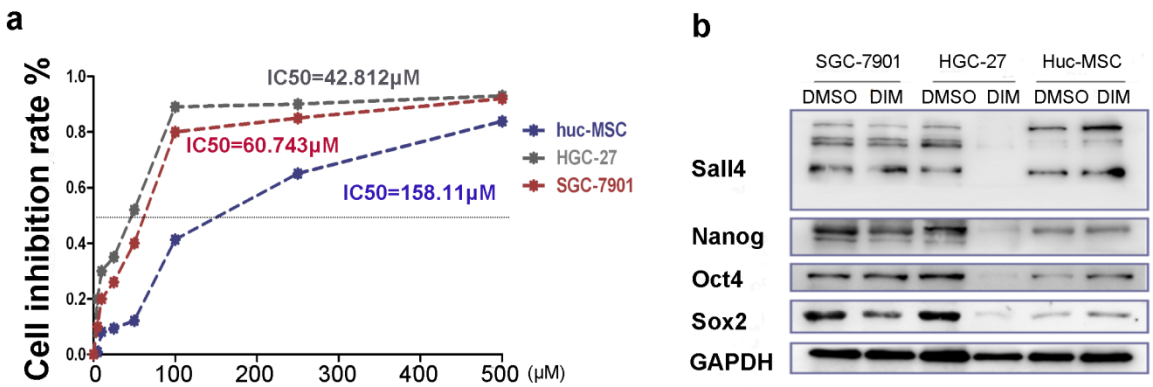


Figure S1. The effects of 50 μM DIM on HaCAT cells.
(a): HaCAT and DFL cells were treated with 50 μM DIM for 48 h and 0.1% DMSO as the control. The colony-forming assay was conducted to detect the proliferative capacity of skin cells. **(b):** Western blot for stemness markers in HaCAT and DFL cells after 50 μM DIM for 48 h. **(c):** Real-time PCR for the mRNA level of stemness markers and Wnt11 in HaCAT and DFL cells after 50 μM DIM for 48 h. **(d):**Representative images of the burned spot of rat skin and H&E staining.

Figure S2. The effects of 50 μM DIM on gastric cancer cells and hucMSCs.



(a): The MTT assay was conducted to show the effect of 50 μM DIM on the proliferation of gastric cancer cell lines SGC-7901, HGC-27, and hucMSCs for 48 h. The IC_{50} was calculated. **(b):** Western blot for stemness markers in SGC-7901, HGC-27, and hucMSCs after 50 μM DIM for 48 h.

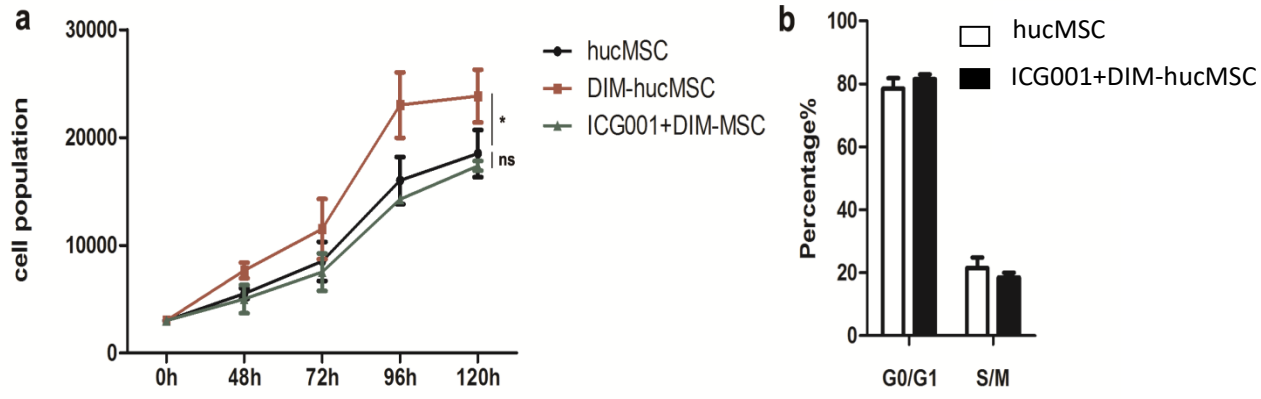


Figure S3. The effects of 20 μM/mL ICG001 on DIM-hucMSCs.
(a): The growth curve of hucMSCs,DIM-hucMSCs and ICG001 treated DIM-hucMSCs. **(b):** Cell cycle analysis of hucMSCs and ICG001 treated DIM-hucMSCs.

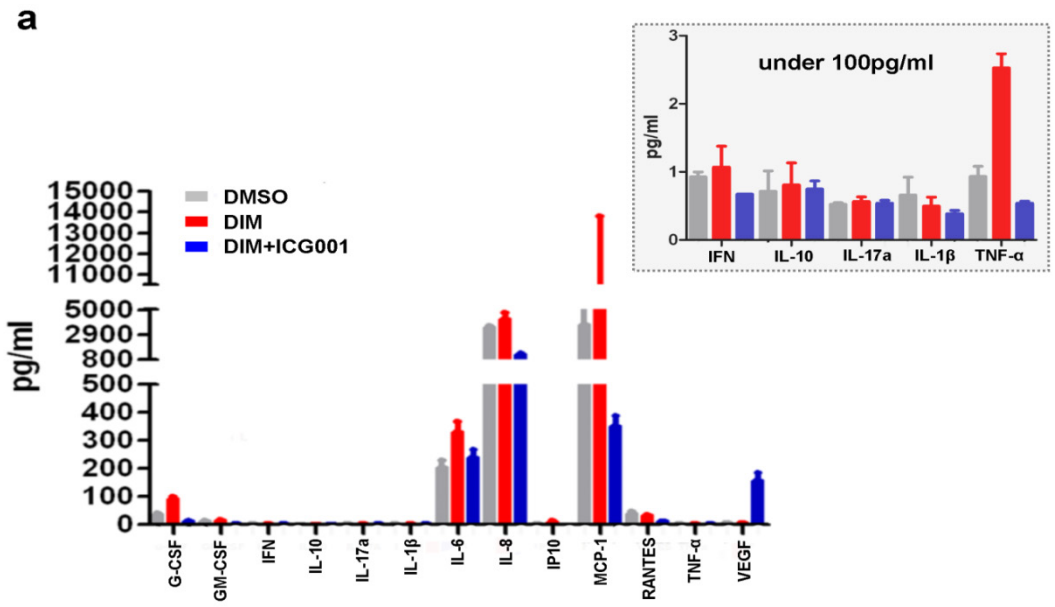


Figure S4. Luminex assay for secreted proteins and factors .
(a): HucMSCs were treated with 0.1% DMSO, 50 μM DIM, and ICG001(20 μM/mL), and the CdM was collected for the Luminex assay.

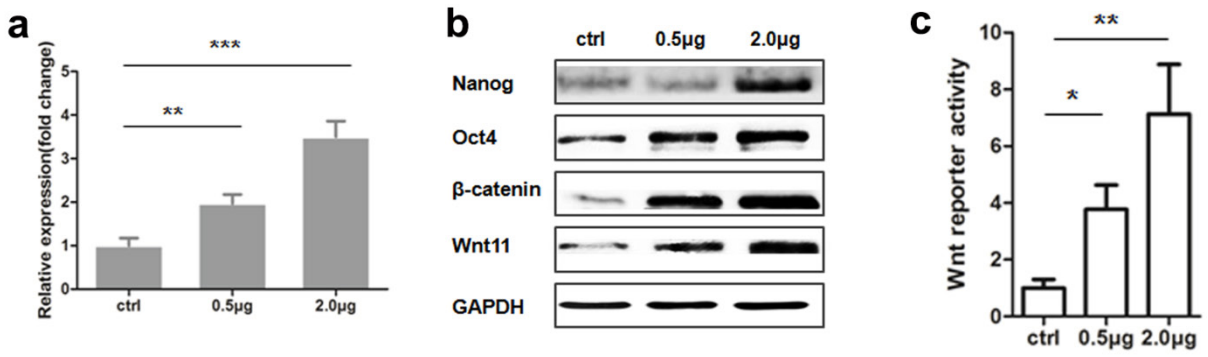


Figure S5. The effects of Wnt11 overexpression on Wnt/β-catenin activation.
(a): Real-time PCR to detect the mRNA level of Wnt11. **(b):** Western blot for the expression level stemness markers Oct4,Nanog and Wnt11, β-catenin.**(c):**Wnt reporter activity assay to detect the Wnt/β-catenin activation.

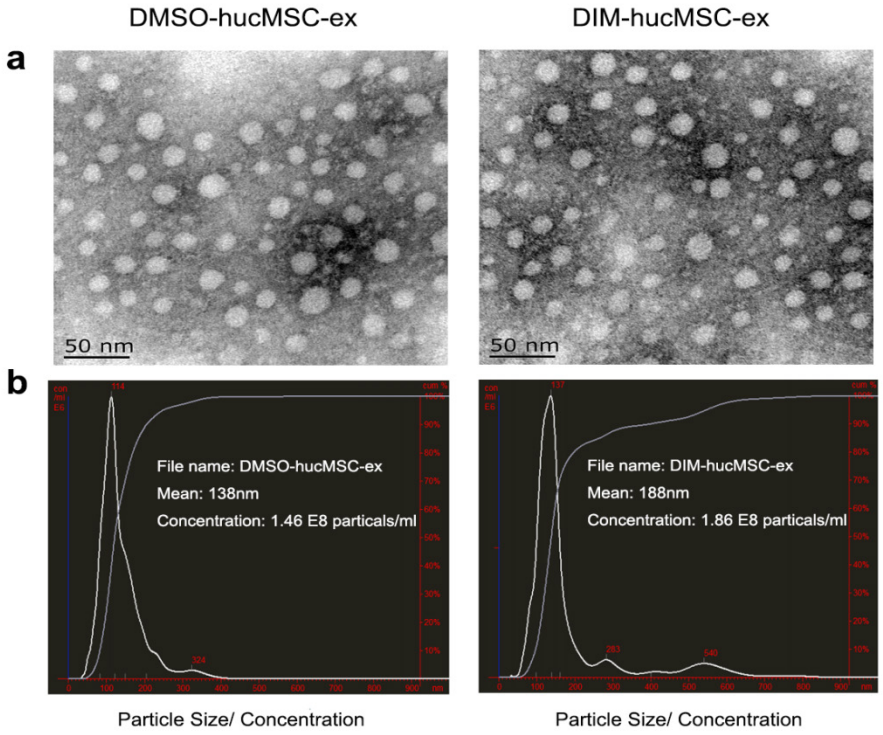


Figure S6. Characterization of exosomes from in vitro cell culture.
(a): Representative TEM image of exosomes extracted from hucMSCs treated with DIM and 0.1% DMSO as the control. Scale bar = 50 nm. **(b):** The exosome amount and size distribution were analysed using the NanoSight LM10 (Malvern) microscope. Exosome preparations (isolated from 50 mL CdM) were diluted 1:1000 with H_2O for tracking. Each

sample was analysed three times for 30 seconds.

Supplementary Table 1. Crieria for histological scores

Score	Epidermal and dermal regeneration	Cell infiltration	Granulation tissue
1–3	Minimal to moderate re-epithelialization with or without minimal developing glandular structure formation in the wound	Wound covered with thin to moderate cell layer	Granulation around wound edges only
4–7	Complete re-epithelialization with minimal developing glandular structure formation in the wound	Wound covered with thick cell layer	Granulation around wound edge and in 30%–50% of wound bed
8–10	Complete re-epithelialization with considerable developing glandular structure formation in the wound	Wound covered with very thick and densely populated cell layer	Thick granulation around wound edge and in >50% of wound bed

Supplementary Table 2. Sequences of real-time PCR primers

mRNA	Primer	Sequences (5′-3 ′)	Annealing temperature	Fragment size
Human-Sox2	Forward primer	ACACCAATCCCATCCACACT	60°C	224bp
	Reverse primer	GCAAACTTCCTGCAAAGCTC		
Human-Oct4	Forward primer	TTGAGGCTCTGCAGCTTAG	60°C	285bp
	Reverse primer	GCCGGTTACAGAACCACAC		
Human-Nanog	Forward primer	CCTGATTCTTCCACCAGTCC	60°C	292bp
	Reverse primer	TGCTATTCTTCGGCCAGTTG		
Human-Sall4	Forward primer	TCGATGGCCAACTTCCTTC	62°C	142bp
	Reverse primer	GAGCGGACTCACACTGGAGA		
Human-Wnt1	Forward primer	GATCGTCAACCGAGGCTGTC	64°C	115bp
	Reverse primer	CGTGCAGGATTCGATGGAAC		
Human-Wnt2	Forward primer	AGCTGGCAGGAAGGCTGTAA	63°C	91bp
	Reverse primer	CAGCCAGCATGTCCTGAGAG		
Human-Wnt3	Forward primer	GGCGCCTCTTCTAATGGA	60°C	188bp
	Reverse primer	AGAAGCGCAGTTGCTTGG		
Human-Wnt3a	Forward primer	GGCATGATCTCCACGTAGTT	63□	167bp
	Reverse primer	TACTCCTCTGCAGCCTGAAG		
Human-Wnt4	Forward primer	GCGAGCAACTGGCTGTACCT	64°C	119bp
	Reverse primer	AGGTTCCGCTTGCACATCTG		
Human-Wnt5a	Forward primer	CTCGCCATGAAGAAGTCCA	59□	157bp
	Reverse primer	TACCTAGCGACCACCAAGAA		
Human-Wnt6	Forward primer	GACGCATCCTGCAACAGGAC	65°C	106bp
	Reverse primer	AGCAGCTCGCCCATAGAACA		
Human-Wnt7b	Forward primer	CGAAGCGGAACTGGTACTGG	64°C	177bp
	Reverse primer	TGAAGCTCGGAGCACTGTCA		
Human-Wnt10b	Forward primer	GGCGCCAGGTGGTAACTGAA	66°C	178bp
	Reverse primer	GCTCCAGAATTGCGGTTGTG		
Human-Wnt11	Forward primer	ACAAGACAGGCAGTGCAACA	61°C	135bp
	Reverse primer	ACGTAGCAGCACCAGTGGTA		
Rat-collagen□α1	Forward primer	GCATGGCCAAGAAGACATCC	64°C	115bp

	Reverse primer	**CGTGCCATTGTGGCAGATAC**		
Rat-collagen α1	**Forward primer**	**TGGCACAGCAGTCCAATGTA**	**60°C**	**85bp**
	Reverse primer	**GGTTCTGGCTTCCAGACATC**		
Human/ Rat-β-actin	**Forward primer**	**GACCTGTACGCCAACACAGT**	**59°C**	**129bp**
	Reverse primer	**CTCAGGAGGAGCAATGATCT**		