



Supporting Information

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**Structurally Tunable Reduced Graphene Oxide Substrate
Maintains Mouse Embryonic Stem Cell Pluripotency**

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Supporting Information

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J. Zhao, Dr. D. Ye, Dr. X. Guo, Dr. J. Xi, Y. Xia, J. Qiao, Prof. J. Kang

Clinical and Translational Research Center of Shanghai First Maternity and Infant Health Hospital, School of Life Science and Technology, Tongji University, Shanghai 200092, China

E-mail: jhkang@tongji.edu.cn

J. Zhao, J. Cao, Y. Zhou, Prof. X. Yang

Institute for Regenerative Medicine, Shanghai East Hospital, School of Materials Science and Engineering, Tongji University, Shanghai 200092, China

E-mail: yangxw@tongji.edu.cn

Prof. M. Tang, Prof. R. Chai

Key Laboratory for Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Jiangsu Province High-Tech Key Laboratory for Bio-Medical Research, Southeast University, Nanjing 210096, China.

Co-Innovation Center of Neuroregeneration, Nantong University, Nantong 226001, China.

Institute for Stem Cell and Regeneration, Chinese Academy of Science, Beijing, China.

Email: renjie@seu.edu.cn

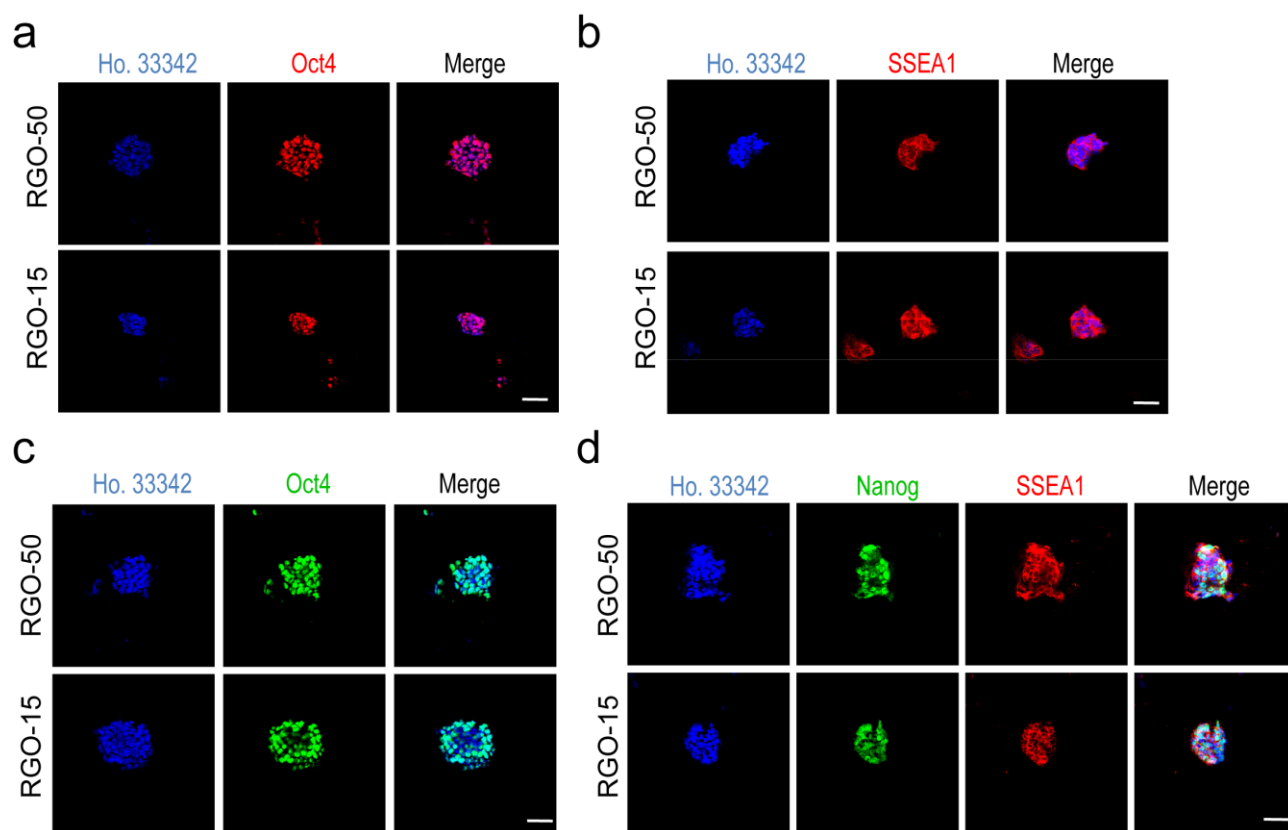


Figure S1. The protein levels of Oct4 (a) and SSEA1 (b) in NR3 cultured on RGO-50 and RGO-15 substrates. Scale bar: 50 μm in (a, b). The immunofluorescent staining of Oct4 (c), Nanog (d) and SSEA1 (d) in 46C cultured on RGO-50 and RGO-15 substrates. Scale bar: 50 μm in (c, d).

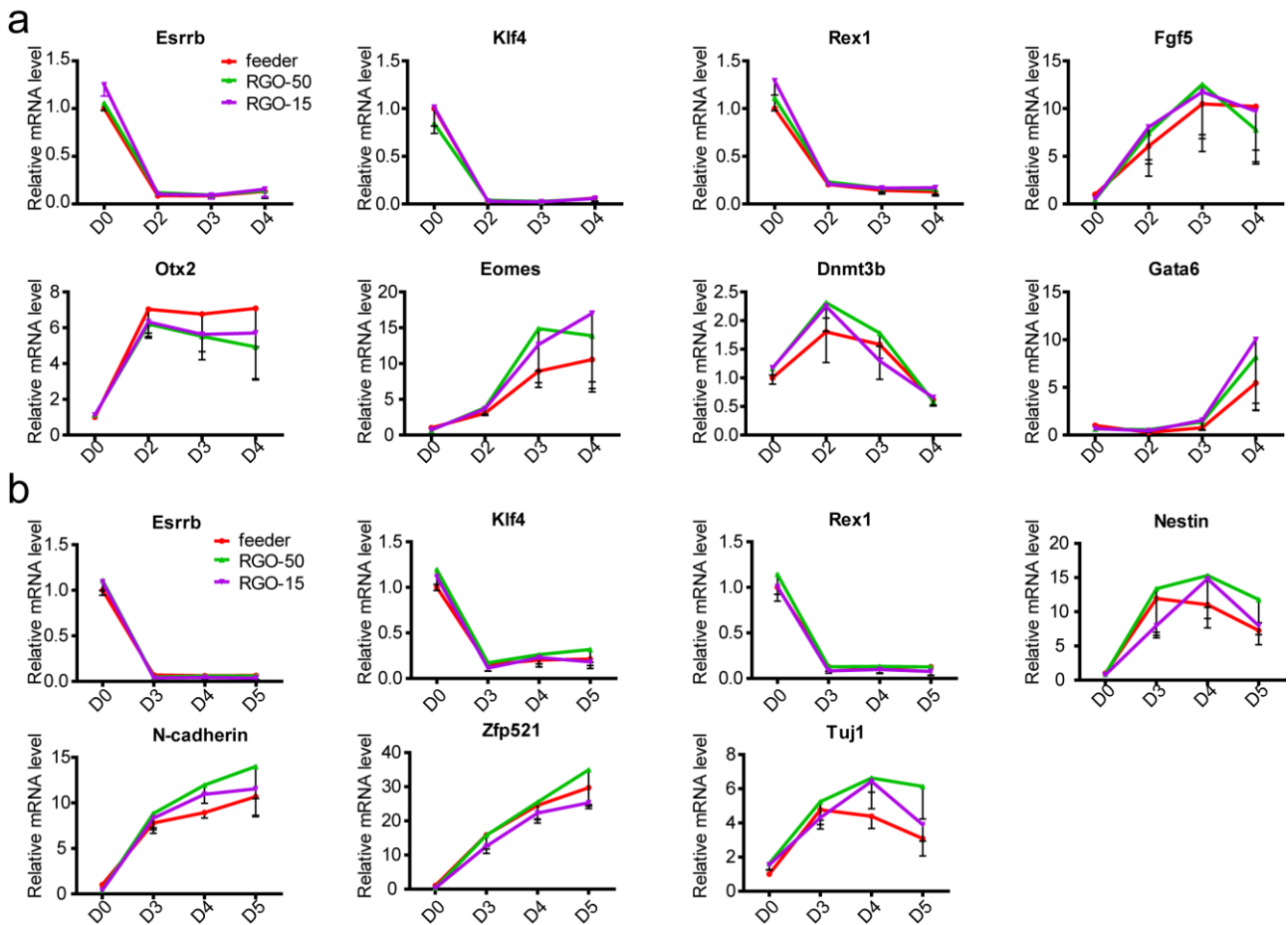


Figure S2. a) qRT-PCR was performed to analyse the relative levels of pluripotency gene and mesendoderm gene during early differentiation in 46C on RGO-50 and RGO-15 substrates. b) The relative expression levels of Esrrb, Klf4, Rex1, Nestin, N-cad, Zfp521 and Tuj1 in 46C cultured on RGO-50 and RGO-15 substrates.

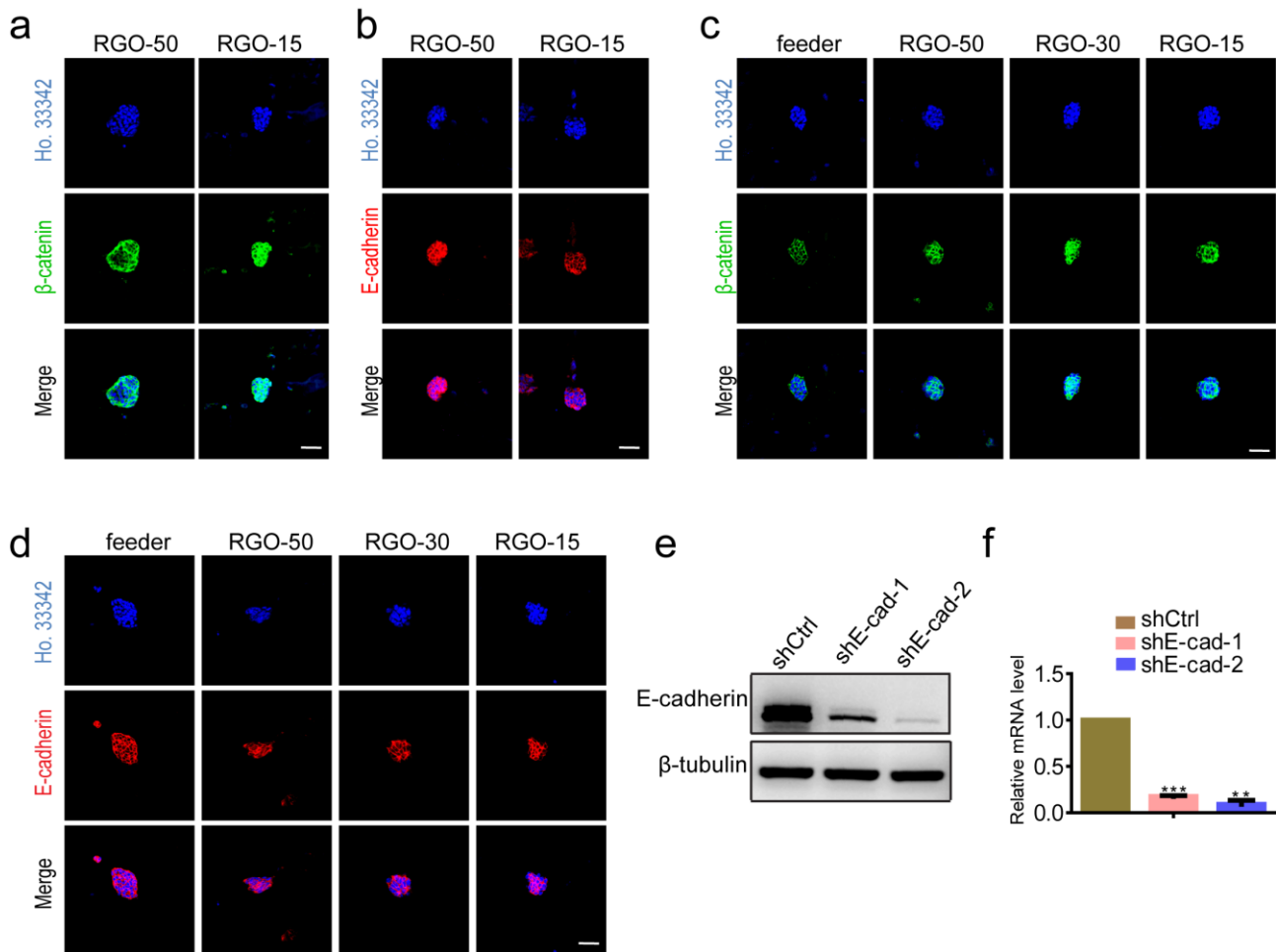


Figure S3. The fluorescence microscope images of β -catenin (a) and E-cadherin (b) in 46C on RGO-50 and RGO-15 substrates. Scale bar: 50 μ m. The protein expression levels of β -catenin (c) and E-cadherin (d) of NR3 cultured on feeder, RGO-50, RGO-30 and RGO-15 substrates. Scale bar: 50 μ m in (c, d). e) The western blotting of E-cadherin in shCtrl, shE-cad-1 and shE-cad-2 cultured on RGO-30 substrate. f) The expression level of E-cadherin in shE-cad-1 and shE-cad-2 cultured on RGO-30 substrate was confirmed by qRT-PCR. ** p < 0.01, *** p < 0.001 versus shCtrl group.

Table S1. The list of primer sequences

Application	Gene	Forward primer	Reverse primer
shRNA	shE-cad-1	CCGGGCTGGAATCTTTGTCCATGTACTC GAGTACATGGACAAAGATTCCAGCTTTTT	AATTCAAAAAGCTGGAATCTTTGTCCATGTACTC GAGTACATGGACAAAGATTCCAGC
		G	
shRNA	shE-cad-2	CCGGCCGAGAGAGTTACCCTACATACTC GAGTATGTAGGGTAACTCTCTCGGTTTTT	AATTCAAAAACCGAGAGAGTTACCCTACATACTC GAGTATGTAGGGTAACTCTCTCGG
		G	
qRT-PCR	Nanog	CAGGTGTTTGAGGGTAGCTC	CGGTCATCATGGTACAGTC
qRT-PCR	Oct4	TCTTTCCACCAGGCCCGGCTC	TGCGGGCGGACATGGGGAGATCC
qRT-PCR	Sox2	GATCAGCATGTACTCCCC	CCCTCCCAATTCCTTGTATC
qRT-PCR	Klf4	GTGCAGCTTGAGCAGTAAC	AGCGAGTTGAAAGGATAAAGTC
qRT-PCR	Esrrb	TGGCAGGCAAGGATGACAGA	TTTACATGAGGGCCGTGGGA
qRT-PCR	Rex1	GGAAGAAATGCTGAAGGTGGAGAC	AGTCCCATCCCCTTCAATAGC
qRT-PCR	Fgf5	AAAGTCAATGGCTCCCACGAA	GGCACTTGCATGGAGTTTTCC
qRT-PCR	Otx2	CCATGACCTATACTCAGGCTTCAGG	GAAGCTCCATATCCCTGGGTGGAAAG
qRT-PCR	Eomes	CCTGGTGGTGTTTTGTTGTG	TTTAATAGCACCGGGCACTC
qRT-PCR	Dnmt3b	AGCGGGTATGAGGAGTGCAT	GGGAGCATCCTTCGTGTCTG
qRT-PCR	Gata6	GACGGCACCGGTCATTACC	ACAGTTGGCACAGGACAGTCC
qRT-PCR	Nestin	CTGCAGGCCACTGAAAAGTT	GACCCTGCTTCTCCTGCTC
qRT-PCR	Zfp521	GAGCGAAGAGGAGTTTTTGG	AGTTCCAAGGTGGAGGTCAC
qRT-PCR	N-cadherin	TCCTGATATATGCCCAAGACAA	TGACCCAGTCTCTCTTCTG
qRT-PCR	Tuj1	TAGACCCCAGCGGCAACTAT	GTTCCAGGTTCCAAGTCCACC
qRT-PCR	E-cadherin	CAGCCTTCTTTTCGGAAGACT	GGTAGACAGCTCCCTATGACTG
qRT-PCR	GAPDH	GTGTTCTACCCCAATGTGT	ATTGTCATACCAGGAAATGAGCTT