Supplementary Information

Multifunctional N-P-doped carbon dots for regulation of apoptosis and autophagy in B16F10 melanoma cancer cells and *in vitro* imaging applications

Vivek K. Bajpai[1], Imran Khan[2,3], Shruti Shukla[4], Sung-Min Kang[5], Faisal Aziz[3], Kumud Malika Tripathi[6], Deepika Saini[7], Hye-Jin Cho[8], Nam Su Heo[9], Sumit K. Sonkar[7,*], Lei Chen[10,*], Yun Suk Huh[2,*], Young Kyu Han[1,*]

[1] Department of Energy and Materials Engineering, Dongguk University-Seoul, 30 Pildong-ro 1-gil, Seoul 04620, Republic of Korea

[2] Department of Biological Engineering, Biohybrid Systems Research Center (BSRC), Inha University, 100 Inha-ro, Nam-gu, Incheon 22212, Republic of Korea

[3] The Hormel Institute, University of Minnesota, Austin, MN, 55912, USA

[4] Department of Food Science and Technology, National Institute of Food Technology Entrepreneurship and Management (NIFTEM), Sonipat, Haryana 131028, India

[5] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory School of Medicine, Atlanta, Georgia, 30332, USA

[6] Department of Chemistry, Indian Institute of Petroleum and Energy, Visakhapatnam 531035, Andhra Pradesh, India.

[7] Department of Chemistry, Malaviya National Institute of Technology, Jaipur, Jaipur 302017, India

[8] Reliability Assessment Center for Chemical Materials, Korea Research Institute of Chemical Technology (KRICT), 141 Gajeong-ro, Yuseong-gu, Daejeon 305-600,

Republic of Korea

[9] Research Center for Materials Analysis, Korea Basic Science Institute (KBSI), Daejeon 34133, Republic of Korea

[10] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China

Running head: Anticancer potential of N-P-doped carbon nanodots

Corresponding authors: Sumit K. Sonkar (E-mail: sksonkar.chy@mnit.ac.in); **Lei Chen** (E-mail: chenlei841114@hotmail.com); **Yun Suk Huh** (E-mail: yunsuk.huh@inha.ac.kr); **Young-Kyu Han (**E-mail: ykenergy@dongguk.edu)

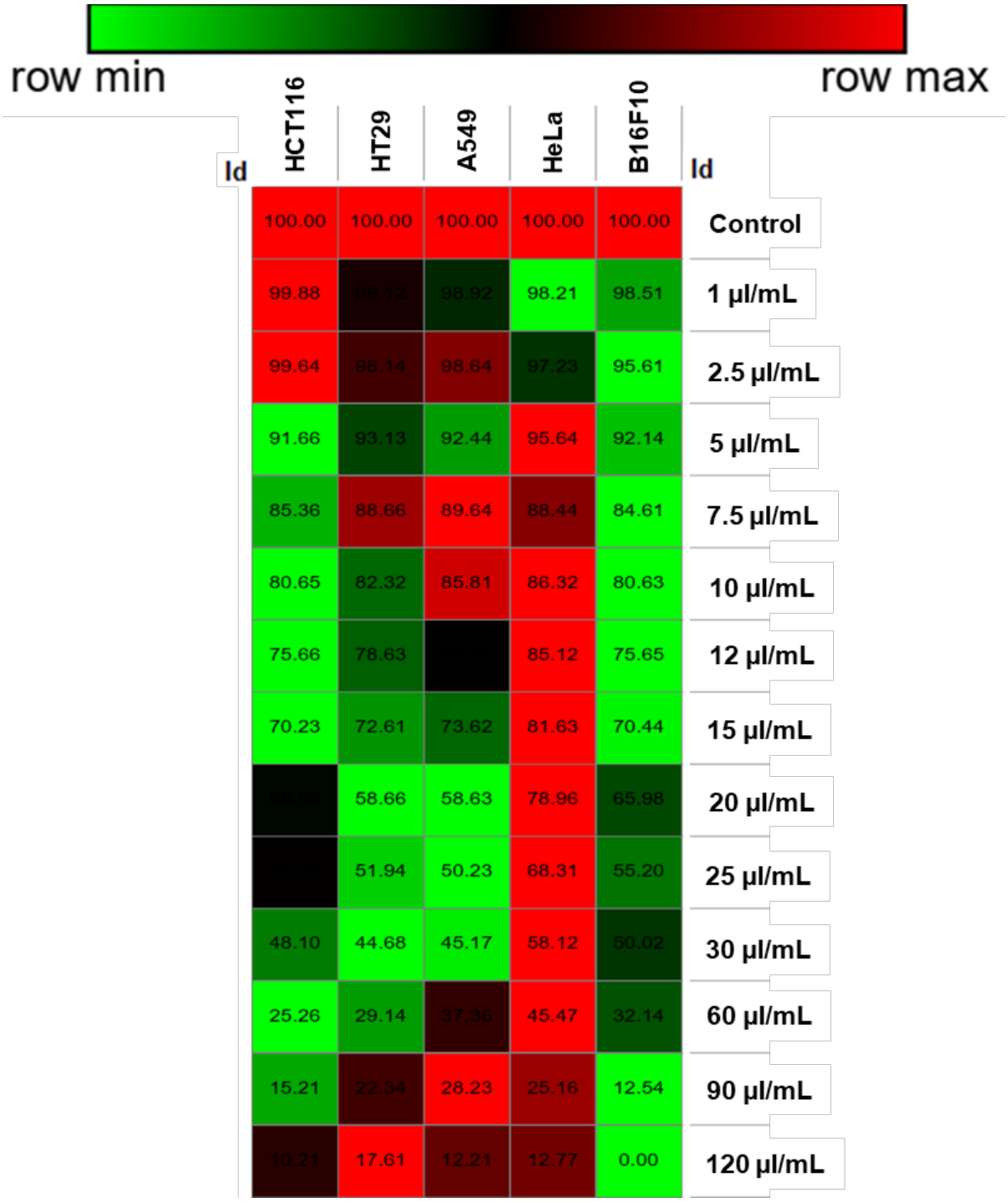

Figure S1 Heatmap showing the anticancer activities (% cell viability) of NPCDs (0-120 µL/mL) on different cancer cells HCT116, HT29, A549, Hela, and B16F10 cells.

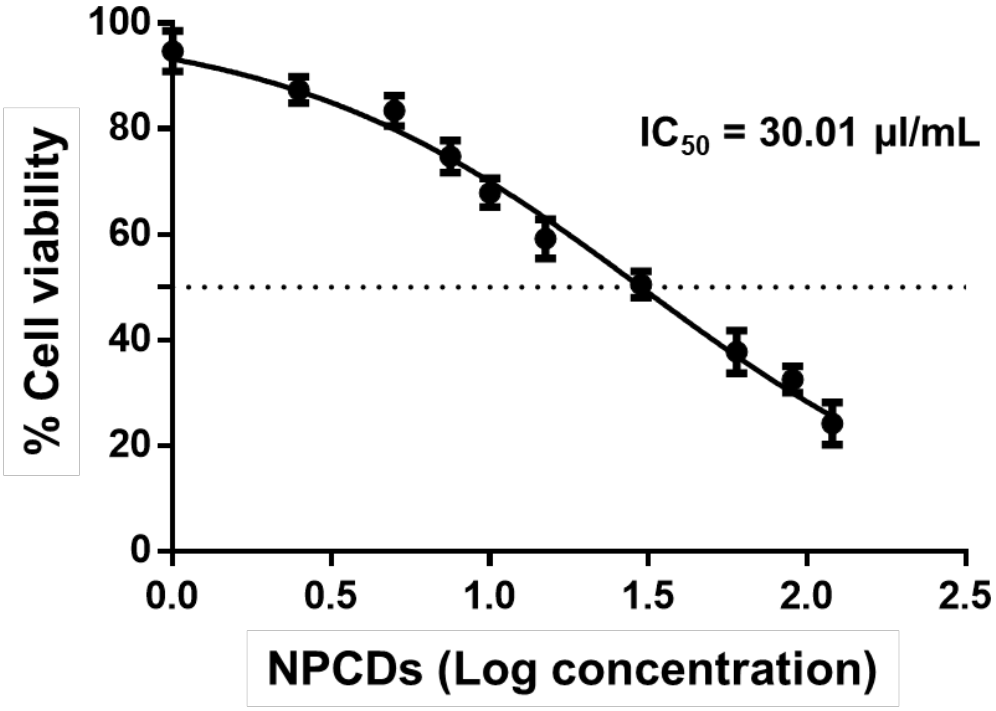

Figure S2 Anticancer activity of NPCDs evaluated by MTT assay, effect of NPCDs (0-120 µL/mL) on B16F10 melanoma cells.

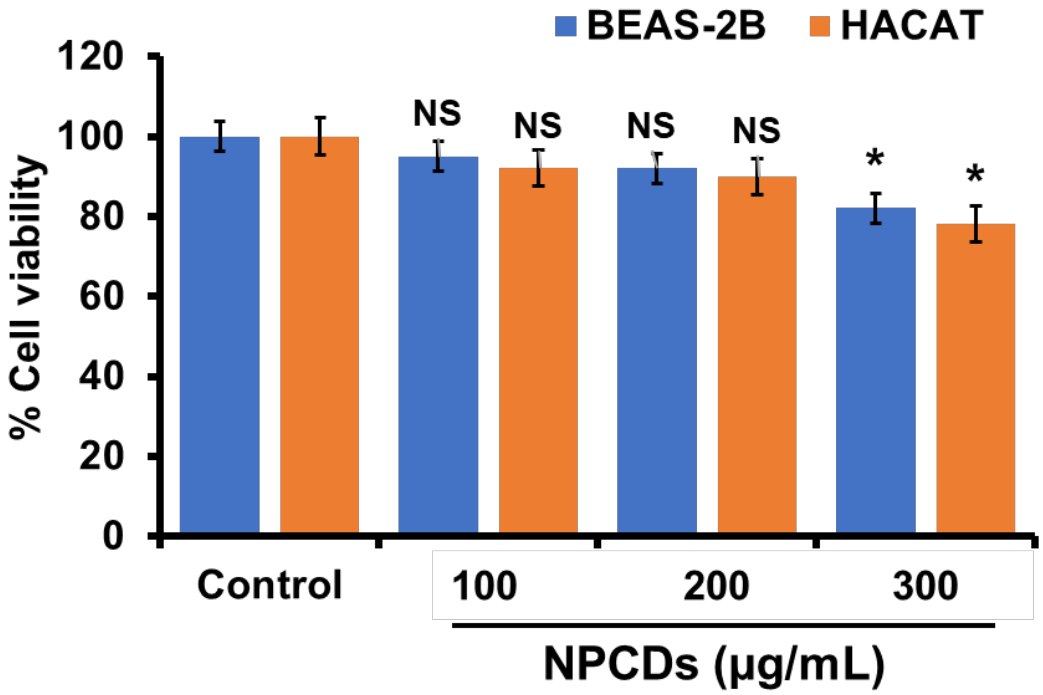

Figure S3 Cytotoxicity of NPCDs on BEAS-2B human lung epithelial cells and HACAT as human keratinocytes.

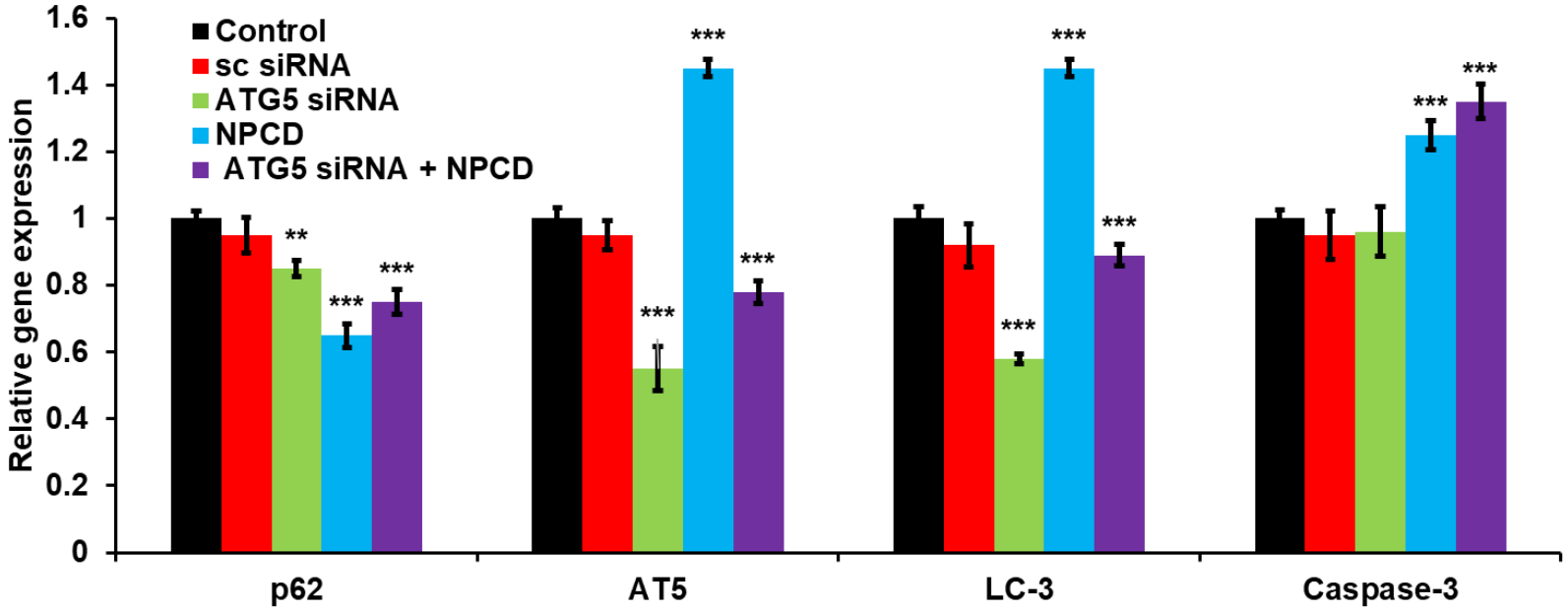

Figure S4. Autophagy attenuated by siRNA ATG5. The efficiency of siRNA mediated knockdown of ATG5 in B16F10 cells was examined by RT-PCR.

Table S1 Primary and secondary antibodies used in the study.

Sr. No.	Primary Antibody used	Dilution used	Secondary antibody used	Company
1	Bax (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
2	Bcl2 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
3	Caspase-3 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
4	β-actin (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
5	CDK-2 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
6	CDK-4 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
7	CDK-6 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
8	p21 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
9	LC-3 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
10	p62 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology
11	ATG-5 (Rabbit)	1:1000	Anti-rabbit IgG, HRP-linked	Cell signaling technology

Secondary antibody dilution used 1:10,000 (Cell Signaling Technology).

Restore™ PLUS Western Blot Stripping Buffer.

Table S2 Primers used in this study.

Primers	Sequence	Applications
atg5-f	AGGCAACCTGACCAGAAACA	Real time PCR
atg5-r	GAGGAAAGCAGAGGTGATGC	Real time PCR
p62-f	TGCCCAGACTACGACTTGTG	Real time PCR
p62-r	AGTGTCCGTGTTTCACCTTCC	Real time PCR
lc3-f	AGCAGCATCCAACCAAAATC	Real time PCR
lc3-r	CTGTGTCCGTTCACCAACAG	Real time PCR
caspase3-f	TGAGCCATGGTGAAGAAGGA	Real time PCR
caspase3-r	TCGGCCTCCACTGGTATTTT	Real time PCR
βactin-f	TCCCTGTATGCCTCTGGTCGT	Real time PCR
βactin-r	AAGCTGTAGCCTCTCTCGGTC	Real time PCR