Supplementary data for

Pleurotus nebrodensis polysaccharide(PN50G) evokes A549 cell apoptosis by ROS/AMPK/PI3K/AKT/mTOR pathway to suppress tumor growth

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Isolation and purification of PN50G

Polysaccharides were isolated from fresh *P. nebrodensis* (1 kg) using boiling bath method, and then proteins were discarded by Sevage assay. Furthermore, polysaccharides were purified using a gel permeation chromatography column (60 × 2.6 cm) packed with Sepharose 2B gel. Six fractions (noted as PN50E, PN50F, PN50G, PN50H, PN50I and PN50J **Fig.S1**) were collected, freeze-dried, and then used for activity evaluation. PN50G showed higher inhibition rates than others (**Fig.S2**).

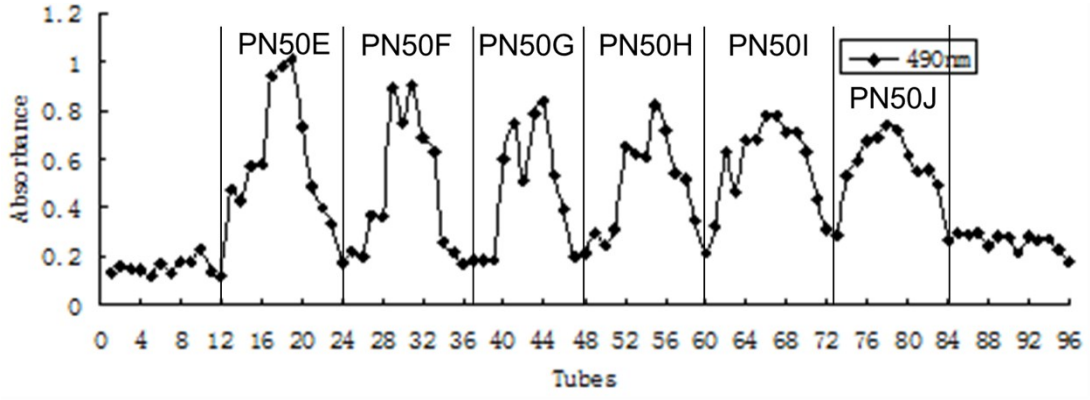

Fig.S1 Elution pattern of the purified polysaccharide from a Sepharose 2B gel-permeation column.

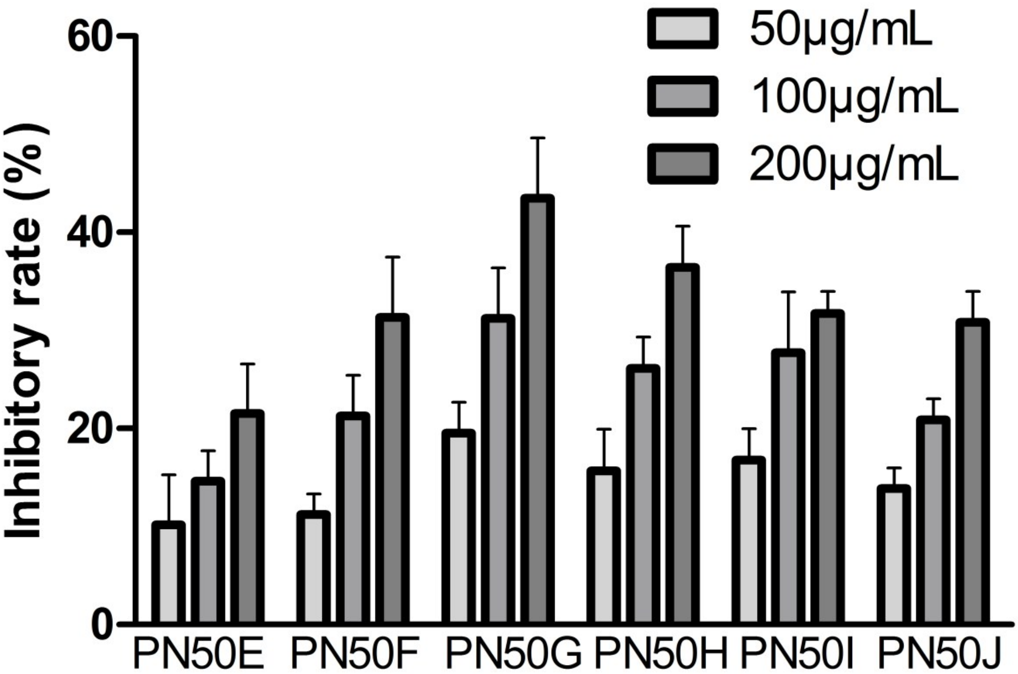

Fig.S2 Effects of PN50 on the viability of A549 cells at different concentrations (n = 6).

Structural features of PN50G

The molecular mass of PN50G was determined to be 261 kDa by HPLC and the percentage of total sugar was determined to be 92.40% by the phenol–sulfuric acid method. The structural features of PN50G were further investigated by chemical and instrumental analyses, including fourier-transform infrared spectroscopy, high-performance liquid chromatography, gas chromatography, periodate oxidation, smith degradation, methylation analysis, and 13C and 1H nuclear magnetic resonance spectroscopy. Results revealed the structure of the repeating units of PN50G as follows (the article referenced has been submitted):

PN50G: →[3)-α-Glu*p*-(1]$_4$→6)- α-Man*p*-(1→4)- β-Glu*p* -(1→6) - α-Man*p*-(1→ [3)- α-Glu*p* -(1]$_4$→

(Branch at the first α-Man*p*, 3←1: α-Gal*p*, 6←1: α-Glu*p*; branch at the second α-Man*p*, 3←1: α-Glu*p*, 6←1: α-Glu*p*)

Fig.S3 The structure of the repeating unit of PN50G.