Supplementary data for

Polysaccharide from *Pleurotus nebrodensis* induces apoptosis via mitochondrial pathway in HepG2 cells

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Isolation and purification of PNA-2

Polysaccharides were isolated from fresh *P. nebrodensis* (1 kg) using alkaline extraction 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. Three fractions (noted as PNA-1, PNA-2 and PNA-3, **Fig.S1**) were collected, freeze-dried, and then used for activity evaluation. PNA-2 showed higher inhibition rates than others (**Fig.S2**).

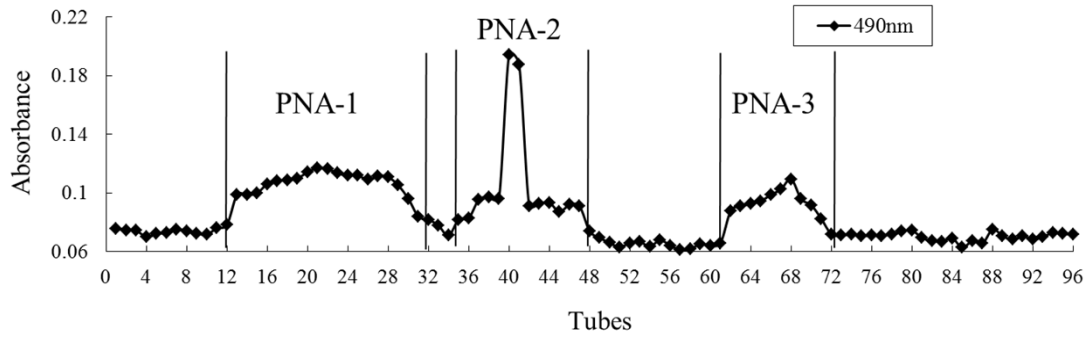

Fig.S1 Elution pattern of the purified polysaccharide from a Sepharose 2B gel-permeation column.

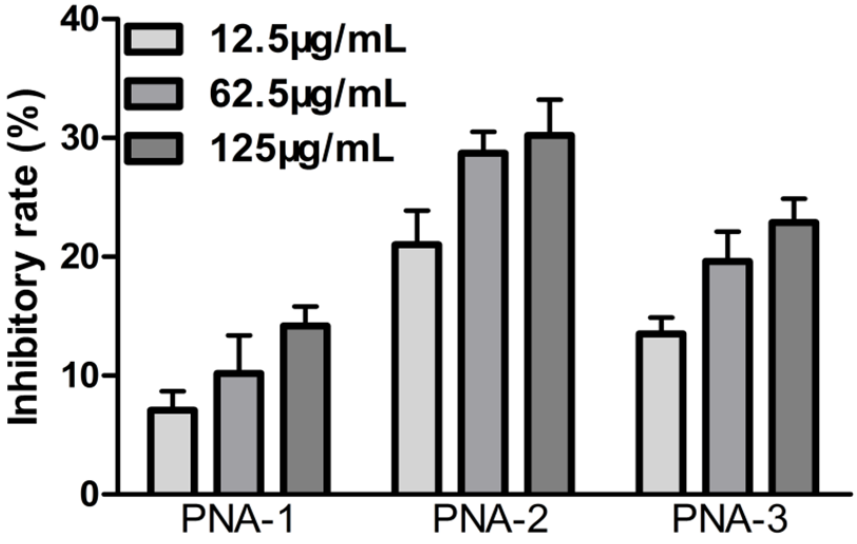

Fig.S2 Effects of PNA on the viability of HepG2 cells at different concentrations (n = 6).

Structural features of PNA-2

The molecular mass of PNA-2 was determined to be 37 kDa by HPLC and the percentage of total

sugar was determined to be 84.18% by the phenol–sulfuric acid method. The optical rotation was [α] -17.86 at RT (25 °C). The structural features of PNA-2 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 PNA-2 as follows (the article referenced has been submitted):

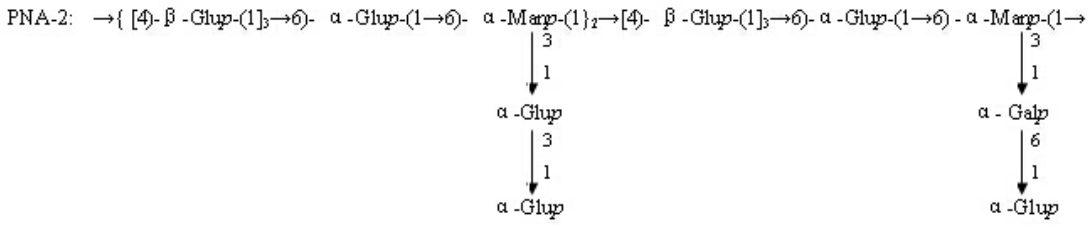

Fig.S3 The structure of the repeating unit of PNA-2.