*Supporting Information*

# Tumor localization of oncolytic adenovirus assisted by pH-degradable microgels with JQ1-meidated boosting replication and PD-L1 suppression for enhanced cancer therapy

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## MATERIALS

Triethylamine ($Et_3N$, 99%), ethylene glycol vinyl ether (98%), *p*-toluenesulfonic acid monohydrate (PTSA, 98%), acryloyl chloride (98%), 2,2′-(ethylenedioxy) diethanethiol (95%), succinic anhydride (99%), were obtained from Energy Chemical Company (Shanghai, China) and used without any further purification. (+)-JQ1 (98%) was purchased from Beijing Zhongshuo Company (Beijing, China). Polyvinyl alcohol (PVA) were purchased from Acros (Belgium) with $M_n$ of 16000 and hydrolyzed degree of 87.0-89.0%. Vinyl ether acrylate (VEA), VEA-functionalized PVA (PVA-VEA) and thiolated PVA-VEA (PVA-VEA-SH) were prepared according to the method given in our previous reports [1, 2], and the functionalities of VEA and thiol groups were both determined as 2% by $^1$H NMR (Bruker ECX 300). Carboxyl-functionalized PVA-VEA (PVA-VEA-COOH) was synthesized by adding PVA-VEA and succinic anhydride into DMSO with stirring at room temperature overnight using $Et_3N$ as a catalyst, and the functionalities of carboxyl group were determined as 4% by $^1$H NMR. The chemical was calibrated against residual solvent peaks as the internal standard. For cell culture experiments, human lung adenocarcinoma A549 cells and human embryonic kidney cell line expressing the adenovirus E1 region (HEK293T) cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 1% nonessential amino acids, and 1 mM sodium pyruvate. All the medium and supplements were provided from Biological Industries. The cells were cultured at 37 °C in a humidified atmosphere with 5% $CO_2$. The adenovirus type 5 (OA) with an E1 deleted encoding an EGFP reporter gene was a kind gift from Dr. Ke Ren's lab at Chengdu Medical College in China. OA were propagated in HEK293T cells and purified by the CsCl gradient centrifugation and then stored at -80 ºC for use. The number of OA was quantified by optical density at 260 nm ($OD_{260}$), for which an absorbance values of 1 is equivalent to $1.1 \times 10^{12}$ viral particles (VPs) $mL^{-1}$. The experimental mice were purchased from the Model Animal Research Centre at Nanjing University (Nanjing, China). The animal experiments were performed in a good compliance with the Animal Management Rules of Ministry of Health in China and the guidance for Care and Use of Laboratory Animals of China Pharmaceutical University (Nanjing, China).

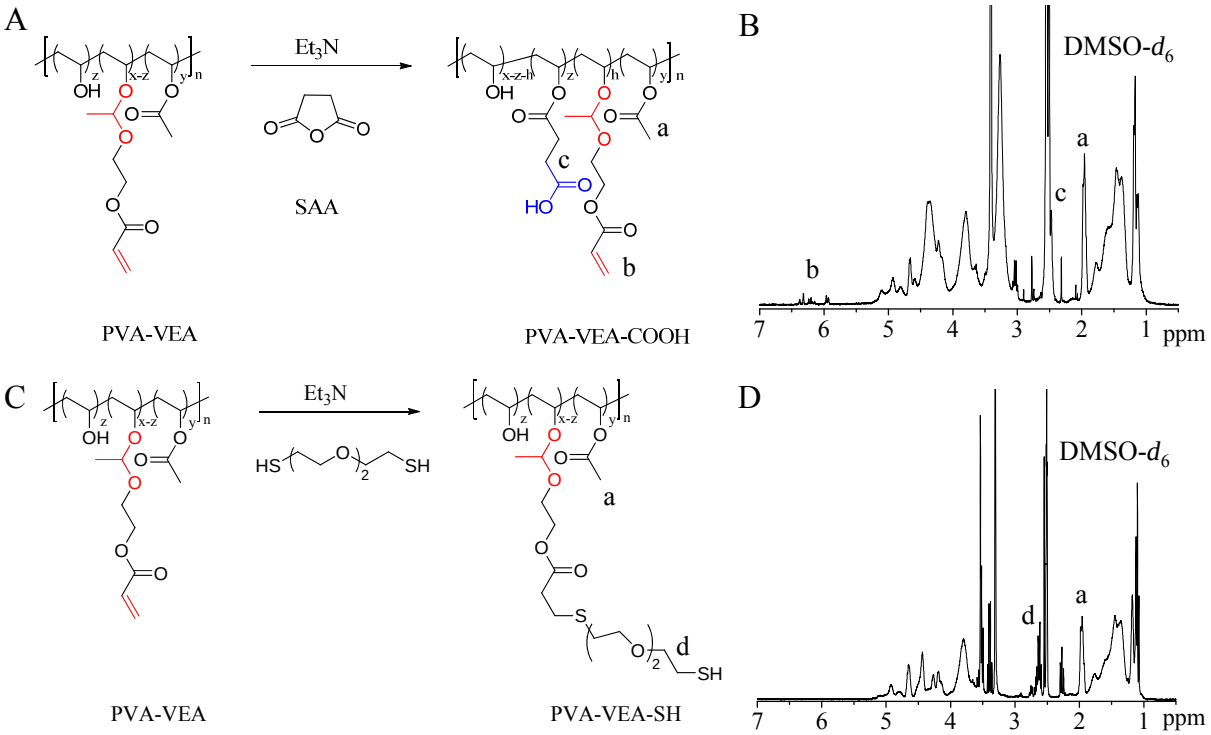


**Fig. S1** Synthetic route (A) and $^{1}$H NMR spectrum (300 MHz, DMSO-$d_6$, B) of VEA and carboxyl functionalized PVA (PVA-VEA-COOH) (conditions: succinic anhydride, $Et_3N$, room temperature, overnight); Synthetic route (C) and $^{1}$H NMR spectrum (300 MHz, DMSO-$d_6$, D) of thiolated PVA-VEA (PVA-VEA-SH) (conditions: 2,2'-(ethylenedioxy) diethanethiol, $Et_3N$, room temperature, overnight).

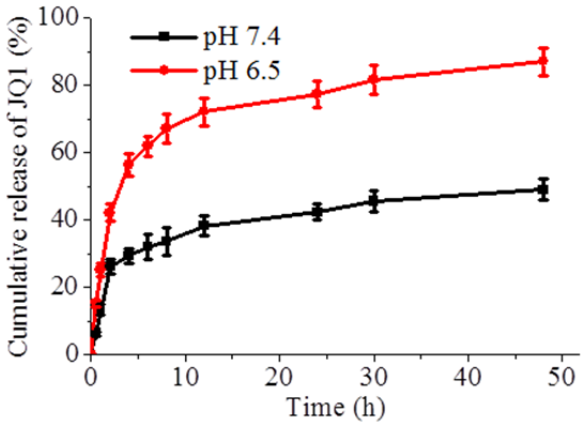


**Fig. S2** pH-triggered cumulative JQ1 release from AMGs at 37 °C determined by HPLC according to a standard curve with UV detection at 254 nm (Agilent1260, USA). A gradient elution at a flow rate of 1.0 mL/min was conducted for chromatographic separation using 0.1% formic acid in water and 0.1% formic acid in acetonitrile. Release experiments were conducted in triplicate, and the results are presented as the average ± SD.

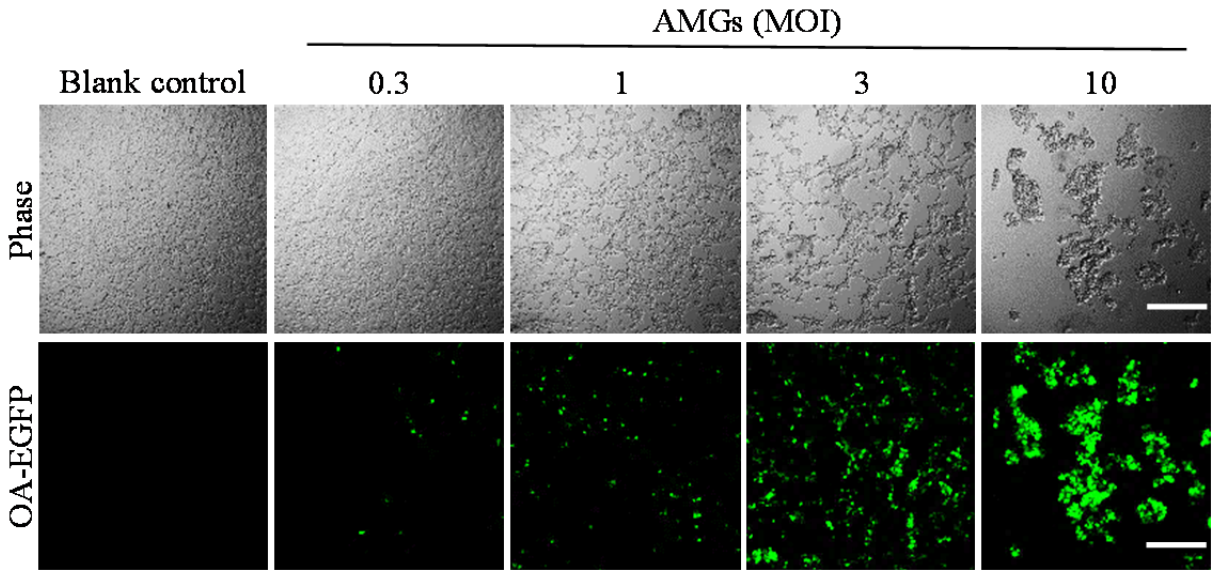


**Fig. S3** Fluorescence images of OA release from AMGs at different MOIs to infect HEK293T cells for 24 h incubation (scale bars: 200 μm).

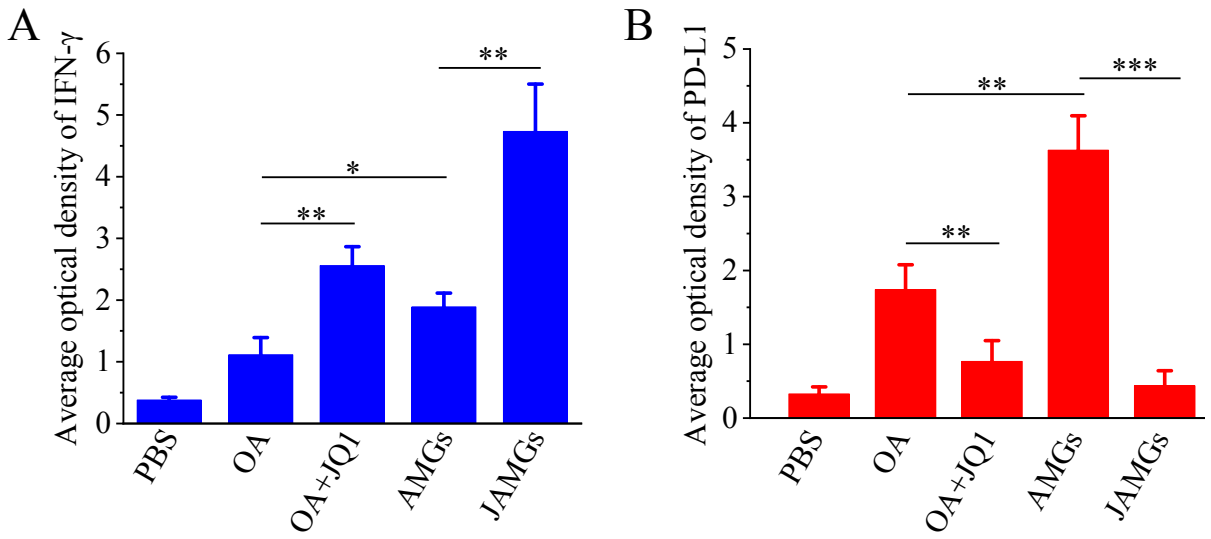


**Fig. S4** Statistical analysis of the expressions of IFN-γ (A) and PD-L1 (B) according to the IHC staining images of tumor tissues isolated from the nude mice after different treatment for 72 h using ImageJ software (n = 5). All the data were represented as the mean ± SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

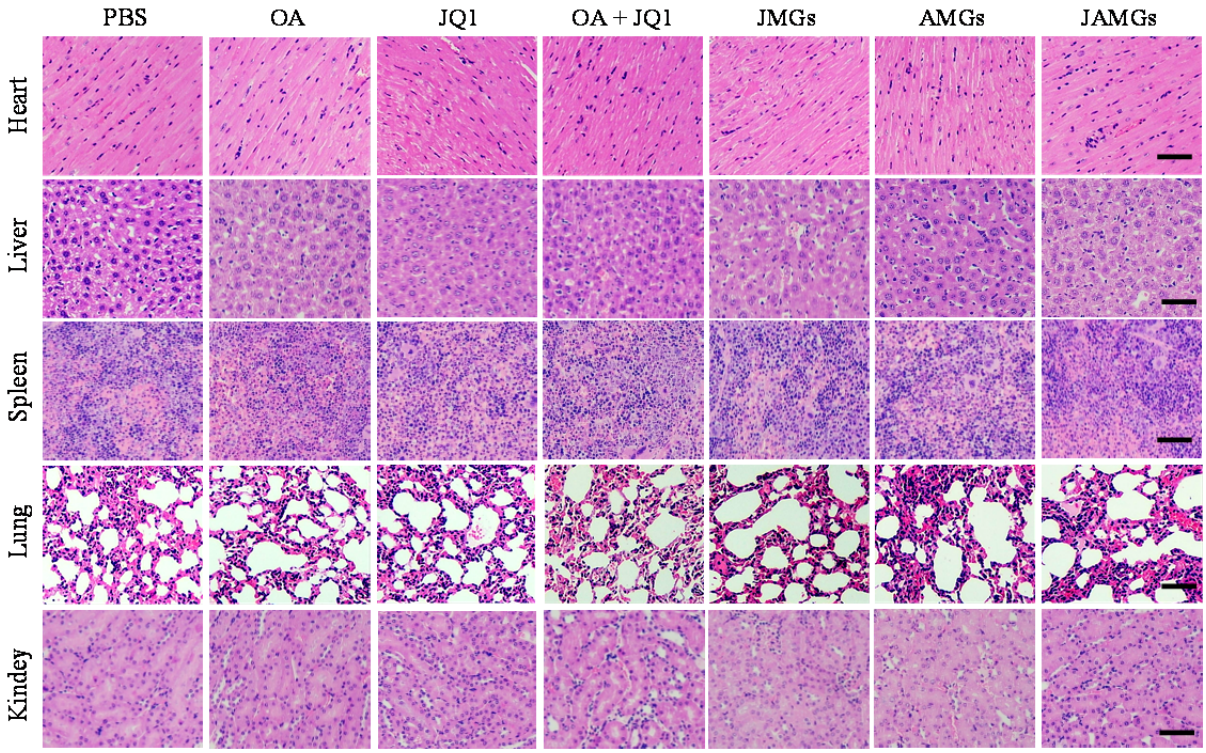


**Fig. S5** Representative sections of HE-stained normal organs including heart, liver, spleen, lung and kidney excised from A549-Luc lung tumor-bearing nude mice following with different treatments for 15 days (the images were observed by an Olympus BX41 microscope, scale bars: 100 μm ).

## REFERENCE

(1) W. Chen, Y. Hou, Z. Tu, L. Gao, R. Haag, *J Control Release,* 2017, **259**, 160-167.

(2) Y. Hou, W. Xie, K. Achazi, J.L. Cuellar-Camacho, M.F. Melzig, W. Chen, R. Haag, *Acta Biomater,* 2018, **77**, 28-37.