Electronic Supplementary Material (ESI) for Nanoscale Advances.

Biomimetic yeast shell vaccine coated with layered double hydroxides induces robust humoral and cellular immune response against tumor

Dong-qun Liu[a,b,†], Shuai Lu[a,†], Lun Zhang[a,b], Ling-xiao Zhang[a,b], Mei Ji[a,b], Xiao-Ge Liu[a,b], Zhuo Yu[c,*], Rui-tian Li[a,*]

[a] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China

[b] School of Chemical Engineering, University of Chinese Academy of Science, Beijing 100049, China

[c] Beijing Tsinghua Changgung Hospital, Changping District, Beijing. 102218, China

[†] These authors equally contributed to this work

[*]Corresponding Author:

Rui-tian Liu

National Key Laboratory of Biochemical Engineering,

Institute of Process Engineering, Chinese Academy of Sciences,

Haidian District, Beijing 100190, China

Tel.: +86 10 82545017; Fax: +86 10 82545025

E-mail address: rtliu@ipe.ac.cn

Zhuo Yu

Beijing Tsinghua Changgung Hospital,

Changping District, Beijing. 102218, China

Tel: +86 10 56119544; Fax: +86 10 56118500

E-mail address: yza02214@btch.edu.cn

Table S1 List of the abbreviations and their full name

Abbreviation	full name
DCs	Dendritic cells
BMDCs	Mouse bone marrow-derived DCs
OVA	Ovalbumin
YS	Yeast shell
LDH	Layered double hydroxides
YSL	Yeast shell coated with LDH nanoparticle
CTL	Cytotoxic T cell
MHC I	Major histocompatibility complex class I

	6 h		24 h		4 D		7 D		
	PBS	**YSL**	**PBS**	**YSL**	**PBS**	**YSL**	**PBS**	**YSL**	**Normal range**
BUN	8 ±2.3	6.96 ±1.3	8.2 ±1.0	6.5 ±0.5	8.75 ±1.8	9.35 ±1.5	10.36 ±1.6	9.05 ±1.1	8~33
ALT	43.6 ±10.7	37.6 ±4.6	38 ±6	25 ±3.46	34.6 ±10.2	36.4 ±6.8	49.6 ±5.3	45.3 ±6.4	17~77
AST	77.2 ±11.7	87.2 ±14.1	79.6 ±13.1	61 ±12.7	63.3 ±7.5	74 ±7.3	96 ±17	112 ±29	54~298
LDH	727 ±135.2	729 ±178.7	844 ±110	980 ±191	831 ±60.4	867 ±89.9	914 ±87.8	940 ±98.5	215-1024
ALP	158 ±12.6	148 ±13.1	151.6 ±6.9	103.5 ±11.2	133 ±30.7	99 ±18.6	152 ±27	131 ±12	60~209

Table S2 Biocompatibility evaluation via serum biochemical parameters

Table S2 Serum biochemical analysis of heathy C57BL/6 mice subcutaneously injected with YSL particles. The blood of mice were collected at 6 h, 24 h, 4 day and 7 day post administration. Liver (ALT, AST and ALP), kidney (BUN) and Heart (LDH) function assays after treatment. Results were expressed as the mean ± S.D (n = 5). The normal ranges for all the biochemical parameters are shown in the right of the table.

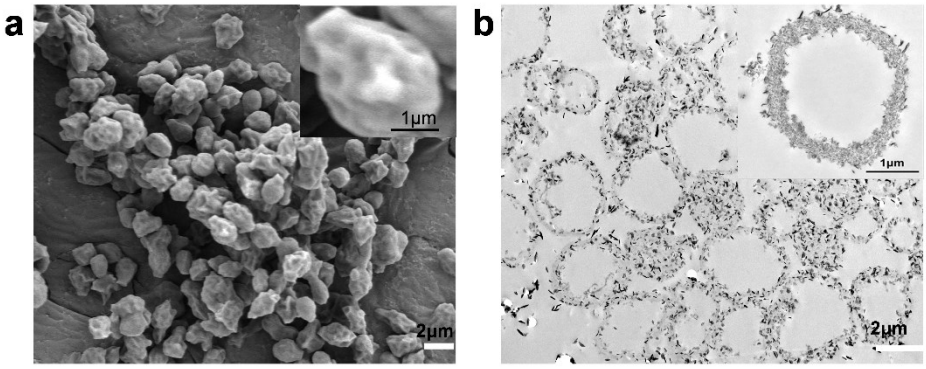

Fig. S1. Morphology of yeast shell particles. SEM images (a) and TEM micrograph (b) of yeast shell particles.

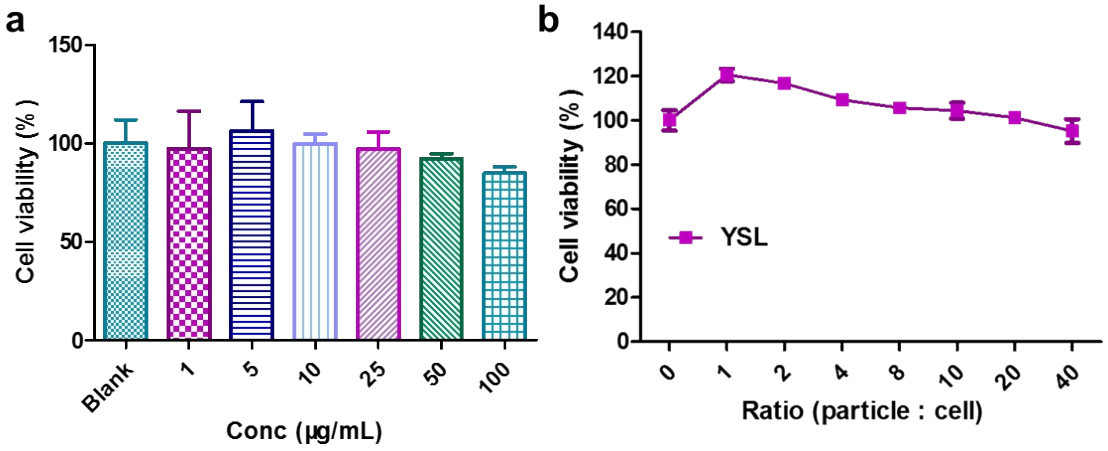

Fig. S2. Biocompatibility of LDH and YSL. The cytotoxicity of LDH (a) and YSL (b) on BMDCs was determined by MTT assay with untreated cells as a control. The mean and standard errors for triplicate experiments are shown.

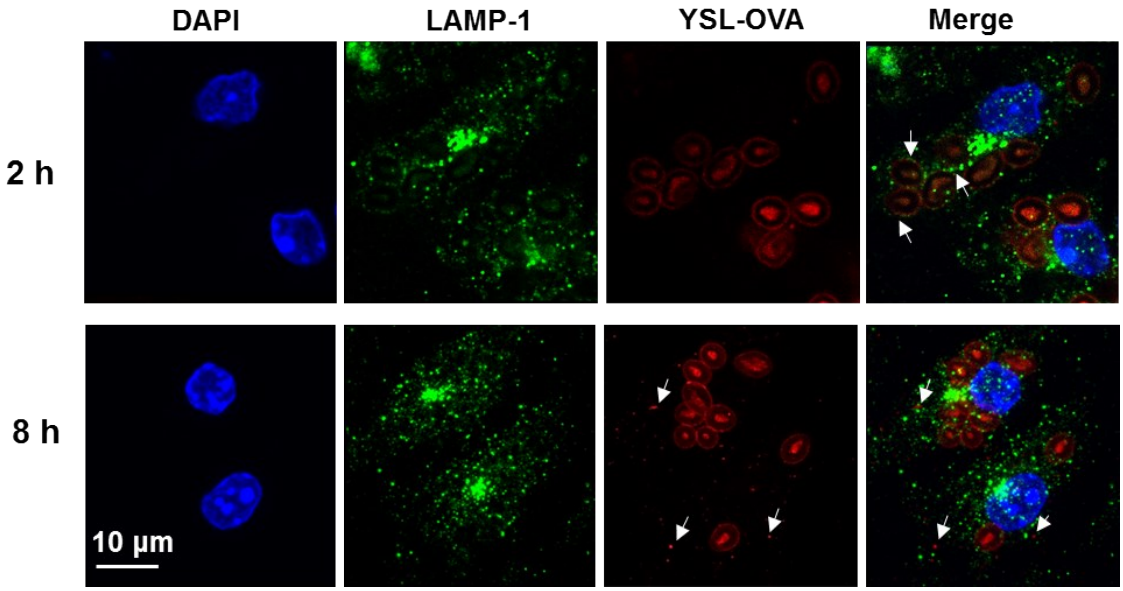

Fig. S3. Intracellular tracking of YSL-OVA in BMDCs. The BMDCs were incubated with YSL-OVA-Cy5 particles, and the images were obtained using confocal laser scanning microscopy after 2 and 8 h-incubation, followed by washing with PBS, fixation and staining with Lamp1 (green) which is a lysosome-associated membrane protein. The cell nuclei was stained with DAPI (blue).

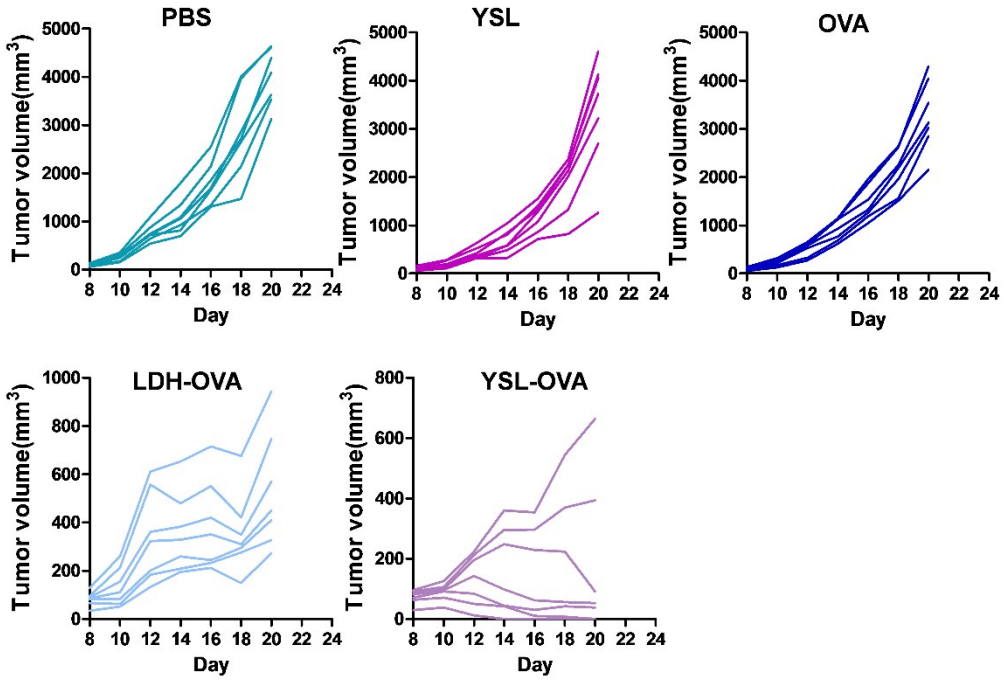

Fig. S4. Individual tumor growth kinetics. The mice were inoculated with EG.7 cells (1×10^6 cells/mouse) and were then immunized with various vaccines at day 4 and day 11 post incubation. Tumor volume was monitored every 2 days and calculated (n = 7).

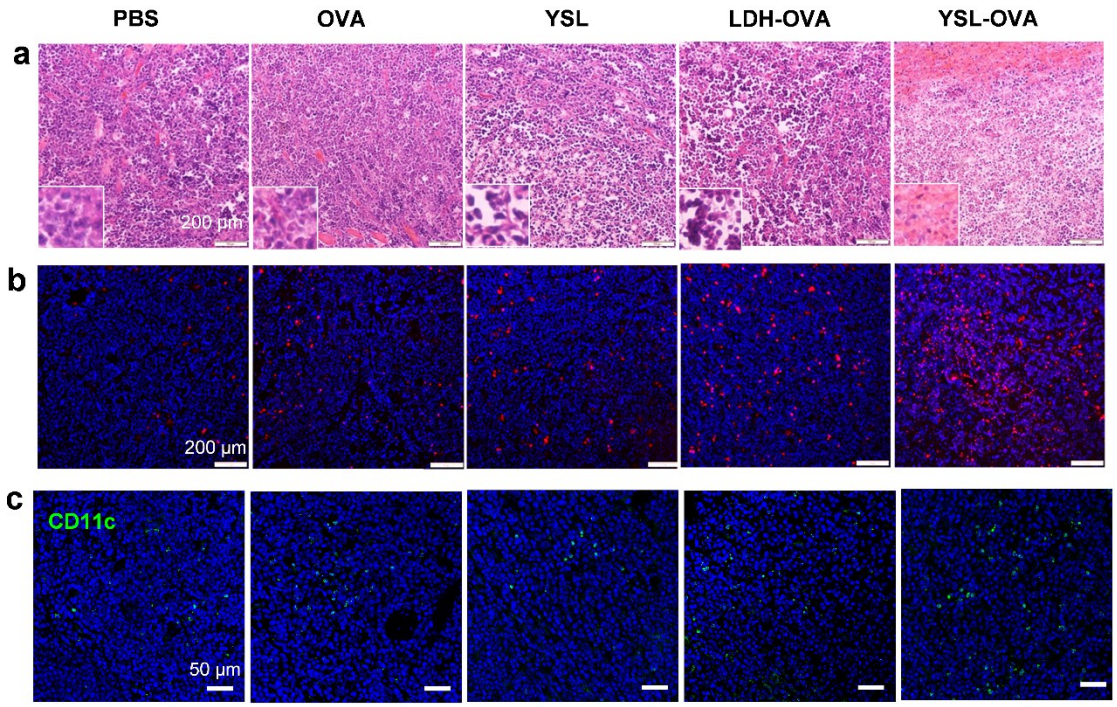

Fig. S5. Histological analysis and DCs infiltration in the tumor tissues. (a) The tumor tissues from the mice with various vaccinations were sectioned and stained with H&E. (b) TUNEL-positive cells in tumor sections excised from the mice seven days after the second treatment (c) Tumor tissues were assayed for DCs ($CD11c^{+}$) with immunofluorescence staining.

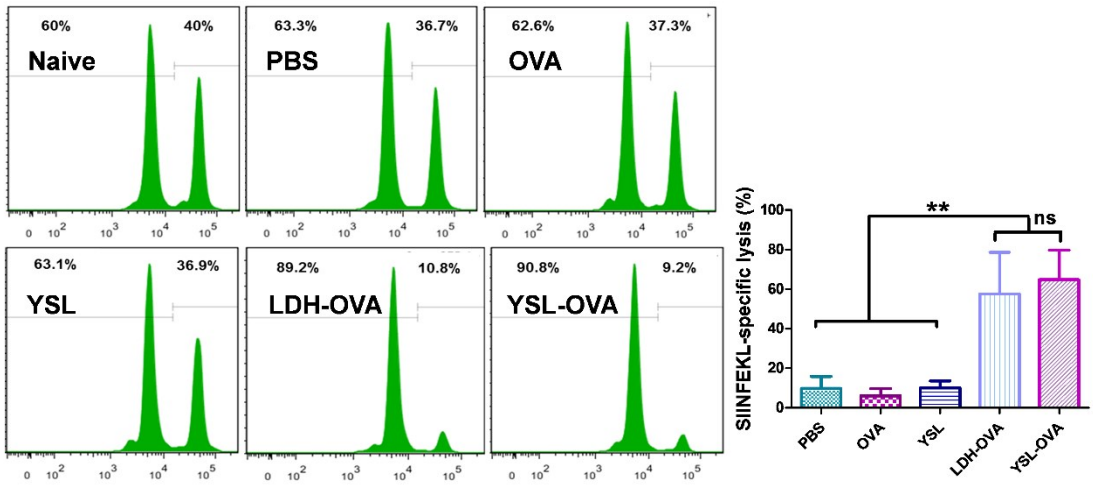

Fig. S6. *In vivo* CTL response induced by various vaccines. C57BL/6 mice were subcutaneously injected with vaccines on day 4 and boosted on day 11. Splenocytes from naive mice were pulsed with OVA peptide SIINFEKL or PBS and stained with high (SIINFEKL) or low (PBS) concentrations of CFSE, respectively. The cells were then mixed and injected i.v. into the vaccinated mice. After 18 h, splenocytes from the vaccinated mice were analyzed by flow cytometry and enumerated. A representative graph from each group is shown. *$p < 0.05$, **$p < 0.01$, $n = 4$.

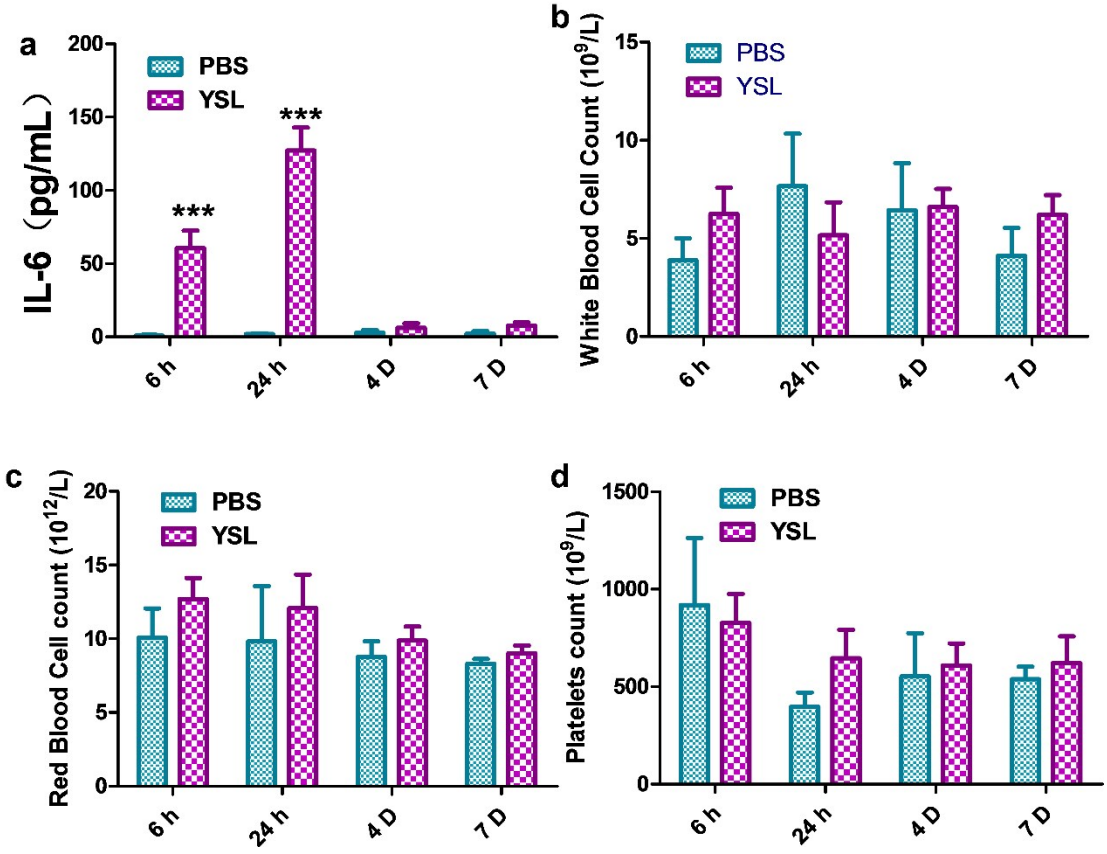

Fig. S7. Inflammation index of blood and blood routine analysis of heathy C57BL/6 mice subcutaneously injected with YSL particles. The blood of mice were collected at 6 h, 24 h, 4 day and 7 day post administration. (a) The level of IL-6 in serum were detected by ELISA kit. (b) White Blood Cell count, (c) Red Blood count and (d) Platelets count of mice immunized YSL particles were detected using Blood Routine. Data represent mean ±SD (n=5). Statistics were analyzed by TT-test. ***$p < 0.001$.