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Supplementary Information

MgAl-Layered Double Hydroxide Nanoparticles Co-delivering siIDO and Trp2 Peptide Effectively Reduce IDO Expression and Induce Cytotoxic T-lymphocyte Responses Against Melanoma Tumor in Mice

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Table S1 List of the abbreviations and their full name

Abbreviation	Full name
LDH	Layered double hydroxides
NPs	Nanoparticles
DCs	Dendritic cells
BMDCs	Mouse bone marrow-derived DCs
Trp2	Tyrosinase-related protein 2
IDO	Indoleamine 2,3-dioxygenase
siIDO	Indoleamine 2,3-dioxygenase siRNA
TAA	Tumor-associated antigen
MHC I	Major histocompatibility complex class I
CTL	Cytotoxic T lymphocyte
LIs	LDH/siIDO
TL	Trp2/LDH
TLIs	Trp2/LDH/siIDO
TLSs	Trp2/LDH/Scrambled-siRNA
LNs	Lymph nodes
TA	Trp2/Alum
TAIs	Trp2/Alum/siIDO

Table S2 Physicochemical characterization of LDH, LIs and TLIs NPs

	Average particle size (nm)	Average zeta potential (mV)
LDH	164.2 ± 15.0	35.2 ± 0.7
LIs	396.1 ± 67.6	35.5 ± 0.8
TLIs	295.3 ± 23.3	28.5 ± 0.8

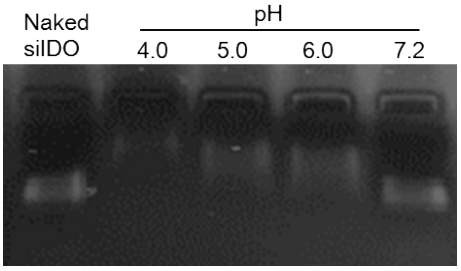

Figure S1. Stability of free siIDO in different pH solution. Free siIDO was incubated in pH4.0, 5.0, 6.0 or 7.2 for 15 min, the free siIDO in the samples was then detected by agarose electrophoresis using 2% agarose gel.

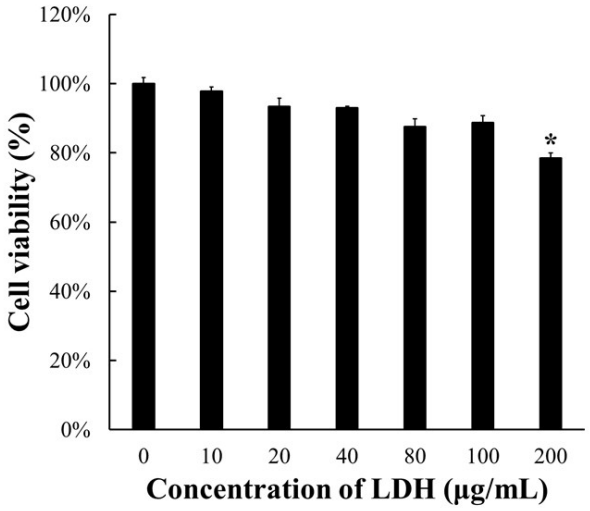

Figure S2. The effect of LDH nanoparticles on the cell viability of BMDC. LDH was added to BMDC culture to a final concentration ranging from 0 to 200 μg/mL. Cell viability of BMDC was detected using a CCK8 kit. The absorbance of dissolved crystals was measured at 450 nm and 600 nm. The data shown are expressed as the percentage of control values from three independent experiments with each experimental value being the average of six replicate wells (compared with control, $*p < 0.05$).

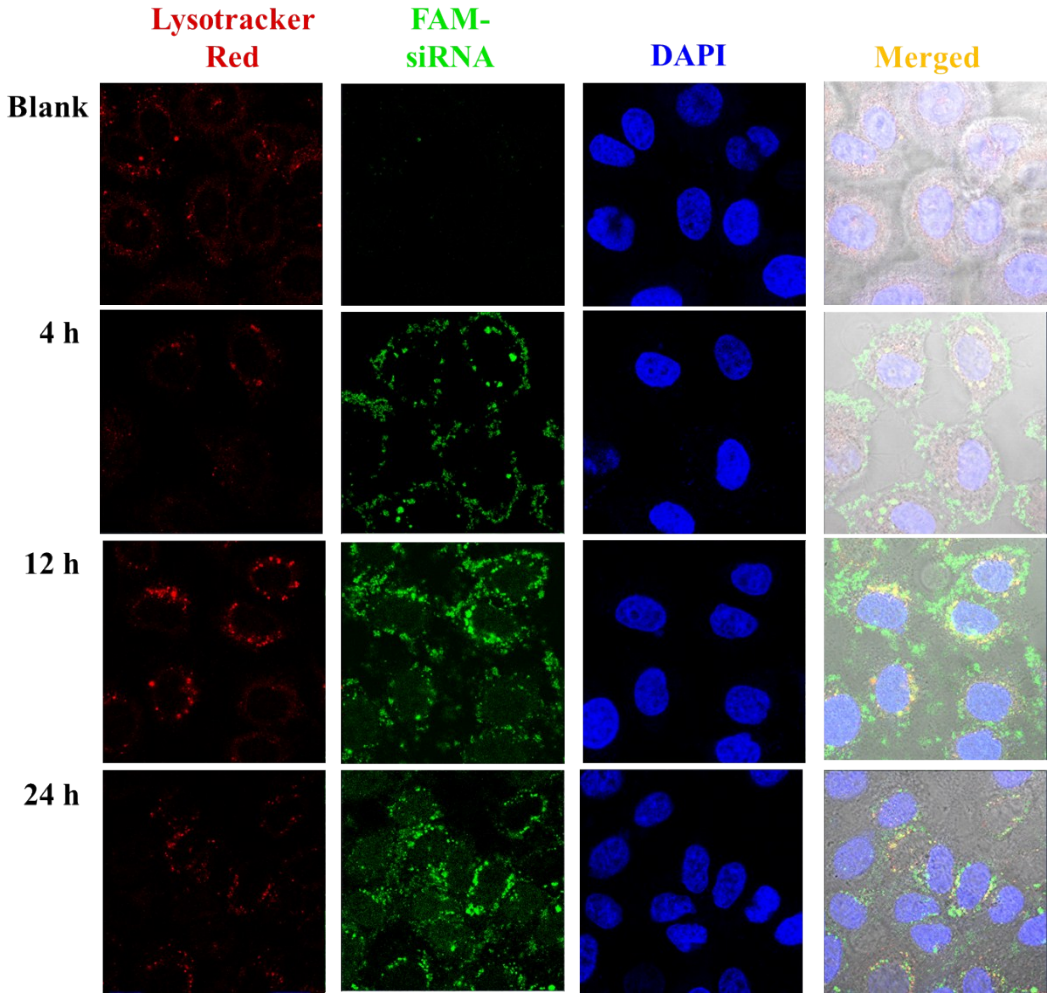

Figure S3. Internalization and distribution of TLIs in DC2.4 cells. The localization of TLIs in DC2.4 cells was visualized by CLSM at 4, 12 and 24 h after the nanoparticles were added to the cells. Nucleus, endosomes/lysosomes, TLIs, and co-localization of nanoparticles and endosomes were marked by blue, red, green and yellow, respectively.

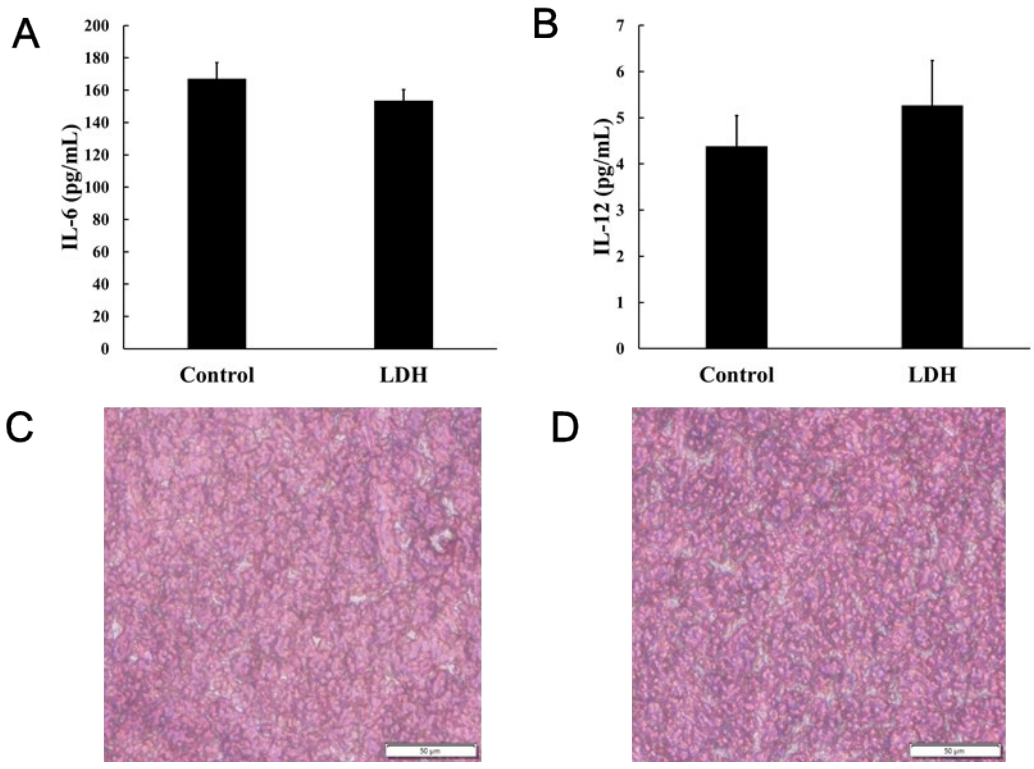

Figure S4. LDH did not show notable toxicity in mice. The levels of IL-6 (A) and IL-12 (B) in the sera of mice treated with 0.9% NaCl or LDH NPs (500 μg) were measured, and the LNs from NaCl- (C) and LDH-treated mice (D) were harvested, sectioned and stained with H&E. Compared with control mice, no significant difference in levels of IL-6 and IL-12, and apparent histopathological changes were observed in LDH-treated mice.