Supporting information

CD38-selective immuno-nano-DM1 conjugates for depleting multiple myeloma

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Materials and characterizations

Daratumumab (Dar, 21.7 mg/mL, M_w: 148 kDa, Shanghai TheraMabs Biotechnology Co., Ltd.), NHS-OEG$_4$-DBCO (97%, BroadPharm), mertansine (DM1, 99.4%, Suzhou BrightGene Biopharmaceutical Co., Ltd.), Dithiothreitol (DTT, >99%, Beyotime Biotechnology), 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT, Beijing Solarbio Science & Technology Co., Ltd.), 4,6-diamidino-2-phenylindole (DAPI, Beyotime Biotechnology), cell counting kit (CCK-8, Suzhou Fcmacs Biotech Co., Ltd.), ultrafiltration tubes with different molecular

weight cut-off (MWCO, Millipore) were purchased and used directly. Organic solvents such as dimethyl sulfoxide, anhydrous ethanol and acetonitrile were purchased from Sinopharm Chemical Reagent Co., Ltd for direct use.

Size and size distribution of polymersomes were measured by dynamic light scattering (DLS, Zetasizer Nano ZS90, Malvern Instruments, UK) equipped with a 633 nm He-Ne laser using back-scattering detection. In vitro cytotoxicity of polymersome-DM1 conjugates and polymersomes in different cell lines was determined by CCK-8 and MTT assays, via measuring the absorbance at 450 nm and 570 nm, respectively, using a microplate reader (Thermo Multiskan FC). Cellular uptake of polymersomes was studied by confocal laser scanning microscopy (CLSM, Leica, Germany) and flow cytometry (FACS Calibur, BD Biosciences, USA). DBCO functionality of Dar-DBCO was determined using mustard acid (SA) matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS, Bruker). The concentration of DM1 was determined by high-performance liquid chromatography (HPLC, Waters 1525) at an absorbance of 252 nm with a mobile phase of acetonitrile and water (v/v = 60/40). The amount of Dar was determined by HPLC equipped with a SEC column at an absorbance of 214 nm (mobile phase: PB (150 mM, pH 7.0): acetonitrile = 90: 10; column temperature: 30 ºC). In vivo bioluminescence imaging was performed using an infrared imaging system (Caliper IVIS Lumina II, USA) and analyzed using Living Image software. H&E images were captured by an inverted microscope (Eclipse Ci-L, Nikon). The femur and tibia of mice were scanned by micro-CT (Siemens, Germany) at a scanning rate of 6°/min and analyzed using Inveon Research Workplace software (Siemens, Munich, Germany).

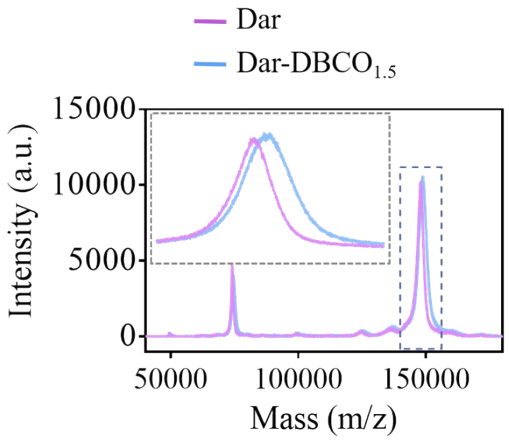

Fig. S1 MALDI-TOF-MS spectra of Dar-DBCO and Dar (Dar concentration: 1.0 mg/mL)

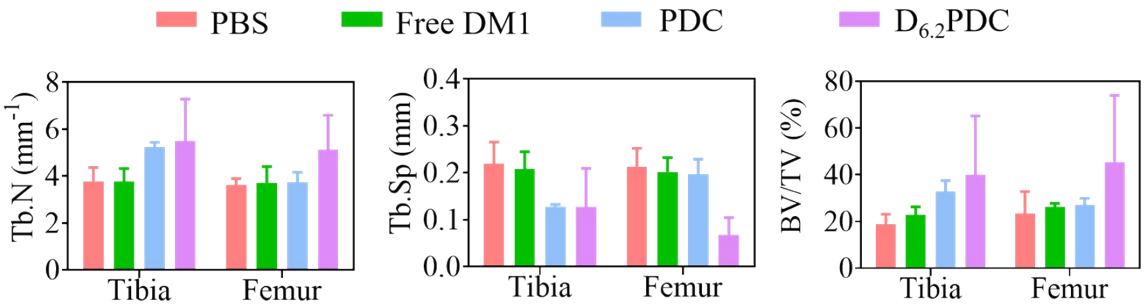

Fig. S2 Quantitative analysis of trabecular number (Tb.N), trabecular separation (Tb.Sp), bone volume fraction (BV/TV) for mice in different treatment groups (n = 3).

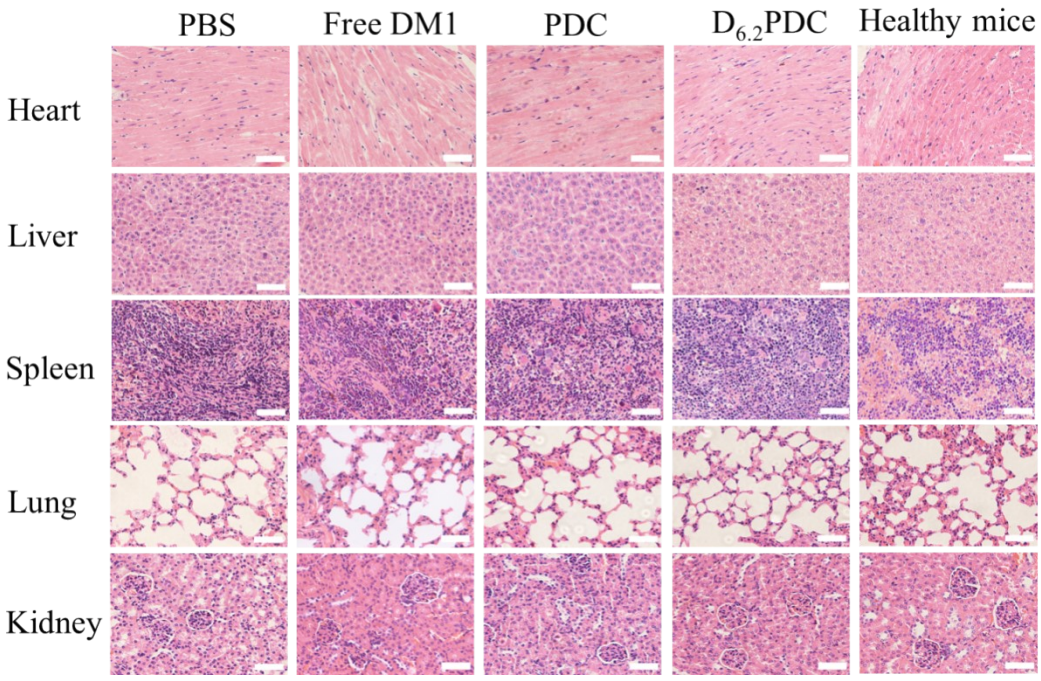

Fig. S3 H&E stained slices of major organs from different treatment groups. Scale bars: 50 μm.