

Novel Humanized Monoclonal Antibodies Against ROR1 for Cancer Therapy

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Supplementary Information

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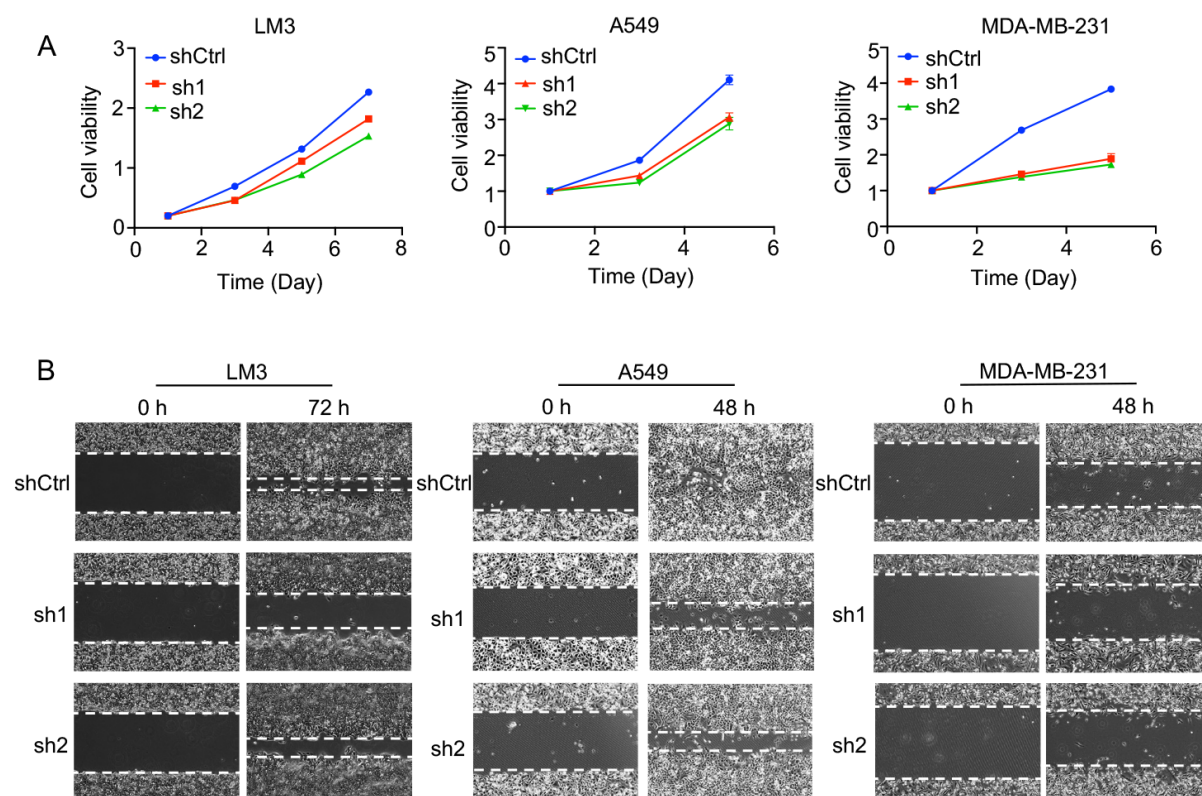


Figure S1. ROR1 knockdown inhibited cancer cell proliferation and migration.

(A) MTT assays show the effects of ROR1 knockdown on cell proliferation in A549, MDA-MB-231, and LM3 cells. (B) Wound healing assays show the effects of ROR1 knockdown on cell migration.

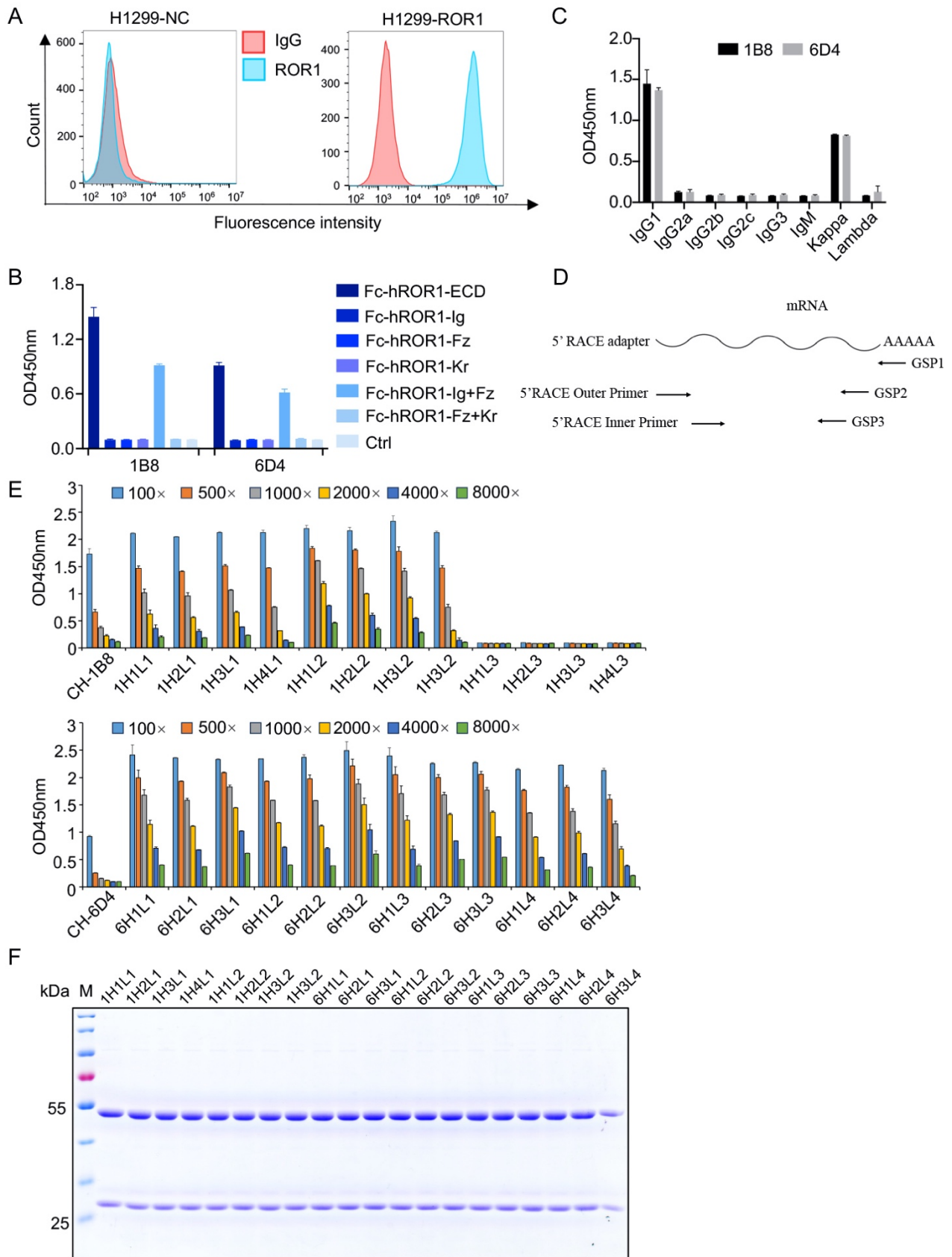


Figure S2. Development of humanized anti-ROR1 antibodies.

(A) Flow cytometric analysis of ROR1 overexpression (H1299-ROR1) and control (H1299-NC) cells. (B) ELISA assays show the binding epitopes of 1B8 and 6D4 antibodies with Fc-hROR1-ECD and other five Fc fusion proteins, each containing one or two extracellular domains of human ROR1. (C) Identification of murine anti-ROR1 mAb subtype by ELISA assays. (D) Schematic illustration of the primers used for 5'RACE assays. (E) ELISA assays were performed to evaluate the binding affinity between humanized anti-ROR1 antibody variants and recombinant extracellular domain of ROR1 protein. (F) The purity of purified humanized anti-ROR1 antibodies was determined by SDS-PAGE.

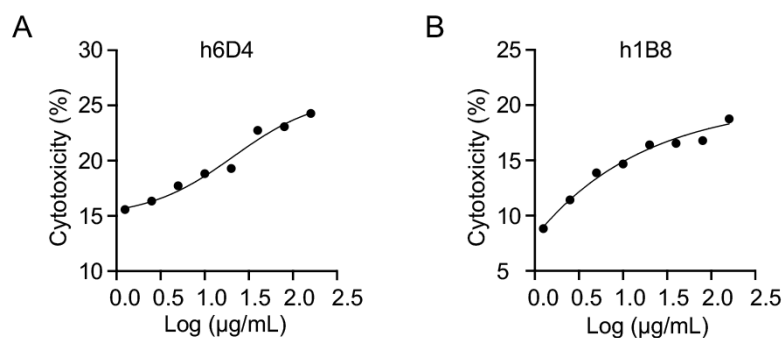


Figure S3. Humanized h1B8 and h6D4 antibodies induce ADCC killing of tumor cells.

The ADCC activities of h1B8 and h6D4 antibodies were measured by the cytotoxicity assay kit (Promega). Human PBMCs from healthy donors were incubated with MDA-MB-231 cells at an effector-to-target (E/T) ratio of 20:1 in the presence of various concentrations of h1B8 and h6D4.

Table S1. Sequences of primers used in this study.

Primers for 5' RACE	Sequence (5'-3')
GSP1-H*	GTAGAGGTCAGACTGCAGGAC
GSP2-H	CTCAGGGAAATAGCCCTTGAC
GSP3-H	AGATCCAGGGGCCAGTGGATAGAC
GSP1-L*	TTGCTGTCCTGATCAGTCCAAC
GSP2-L	TGTCGTTCACTGCCATCAATCTT
GSP3-L	TTGTTCAAGAAGCACACGACTGA
5'RACE Outer Primer	GCTGATGGCGATGAATGAACACTG
5'RACE Inner Primer	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG
Primers for mouse genotype	Sequence (5'-3')
HCC-1	TGTCCATTCAAGCAGACGAG
HCC-2	CCTTGCTCTAGGCAATCCTG
HCC-3	GGAGGAGGACAACTGGTCA
BRCA-1	GCCTTTATTGGCCCAACCTTG
BRCA-2	ATCCGCATGTAGCCTTCTTACCTG

*GSP: Gene specific primer; H, heavy chain; L, Light chain.