Supporting Information

Discovery of MD-224 as a First-in-Class, Highly Potent, and Efficacious Proteolysis Targeting Chimera (PROTAC) Degrader of Murine Double Minute 2 (MDM2)

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Contents of Supporting Information:

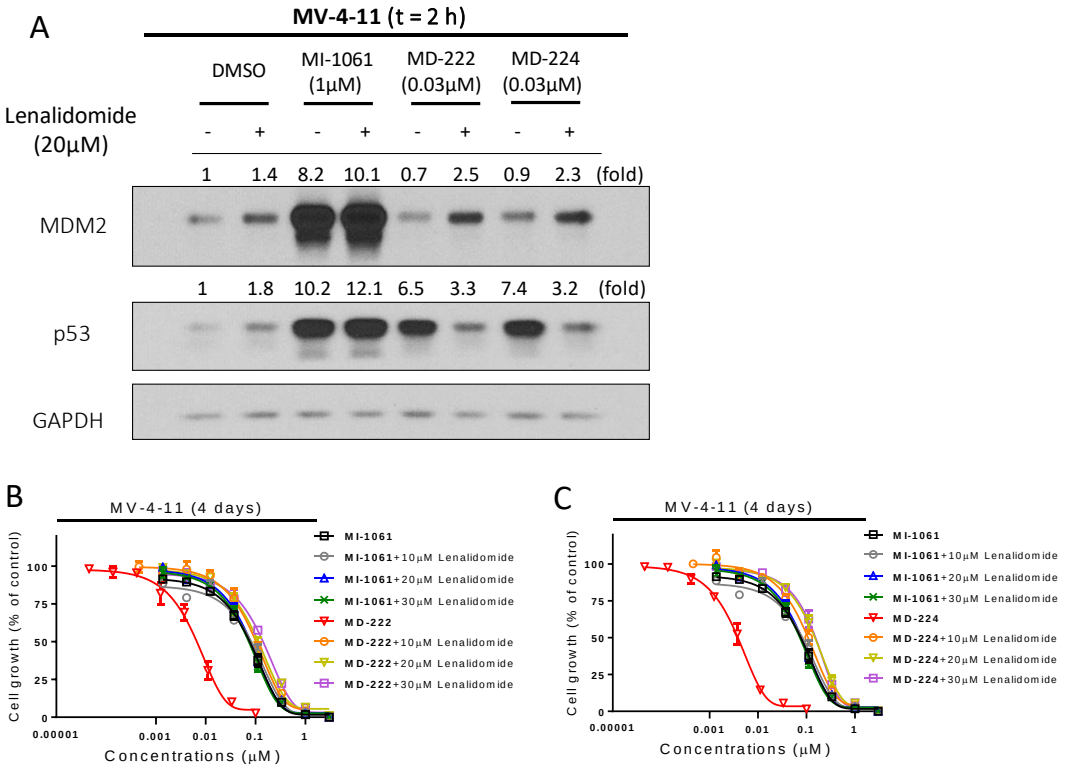


Figure S1. Activity of the MDM2 degraders is cereblon (CRBN)-binding dependent. MV-4-11 cells were treated with the MDM2 inhibitor **MI-1061**, and the degraders **MD-222** or **MD-224** for 2 h in the presence or absence of excess lenalidomide as a competitor: (A) Western blot data indicated that without lenalidomide competition, the MDM2 inhibitor **MI-1061** significantly accumulates both MDM2 and p53 proteins, while the MDM2 degraders degrade MDM2 and activate p53. Competition by 30 μM lenalidomide clearly rescued MDM2 protein from degradation and reduced p53 protein level; (B) and (C) Competition by excess lenalidomide significantly reduces cell growth inhibition activity of **MD-224** and **MD-222** in cell viability assay but fails to change the activity of the MDM2 inhibitor, **MI-1061**.

Figure S2. qRT-PCR analysis of mRNA levels of p53 target genes and *TP53* after treatment with the MDM2 inhibitor **MI-1061** and the MDM2 degraders **MD-222** and **MD-224** in MV4;11 cells. **MD-222** and **MD-224** more effectively activate the transcription of p53 target genes compared with much lower concentrations of the inhibitor **MI-1061**.

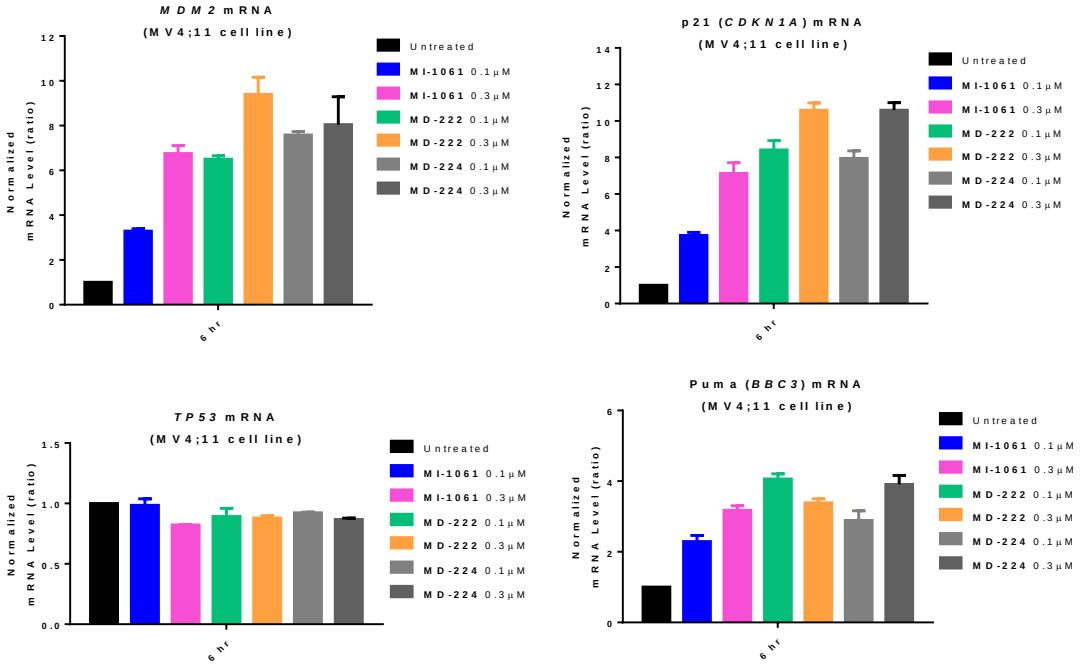


Figure S3. ^{1}H NMR spectrum for MDM2 degrader **MD-224**.

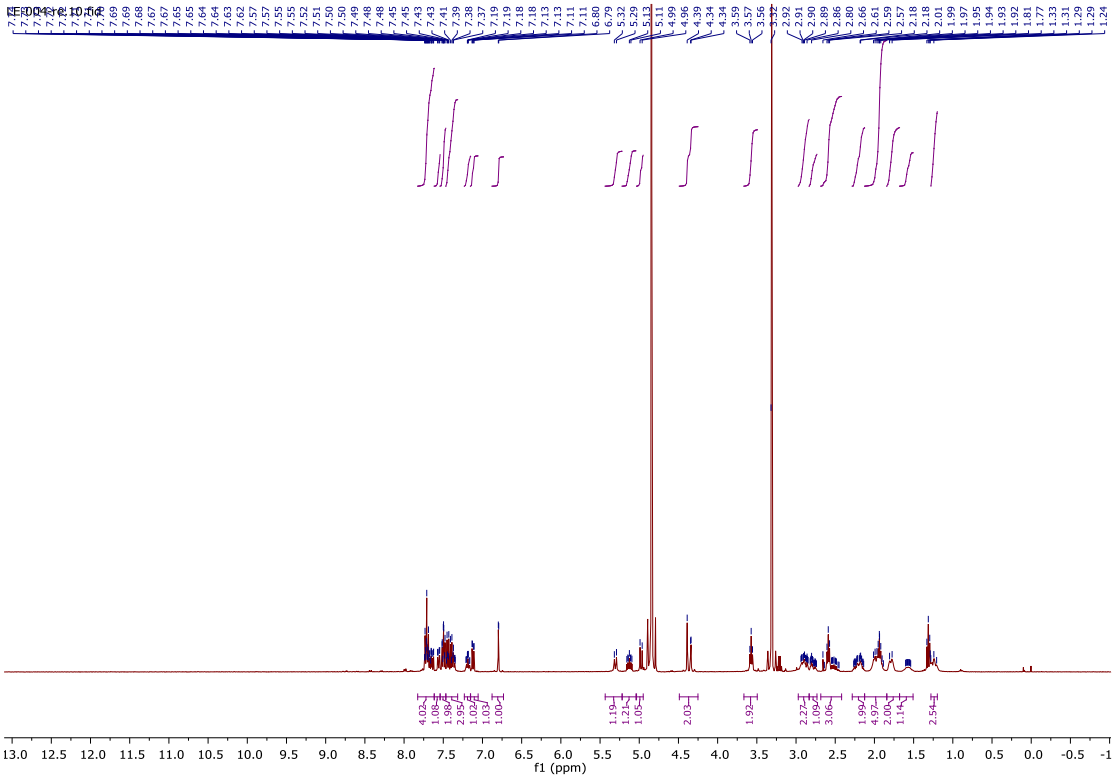
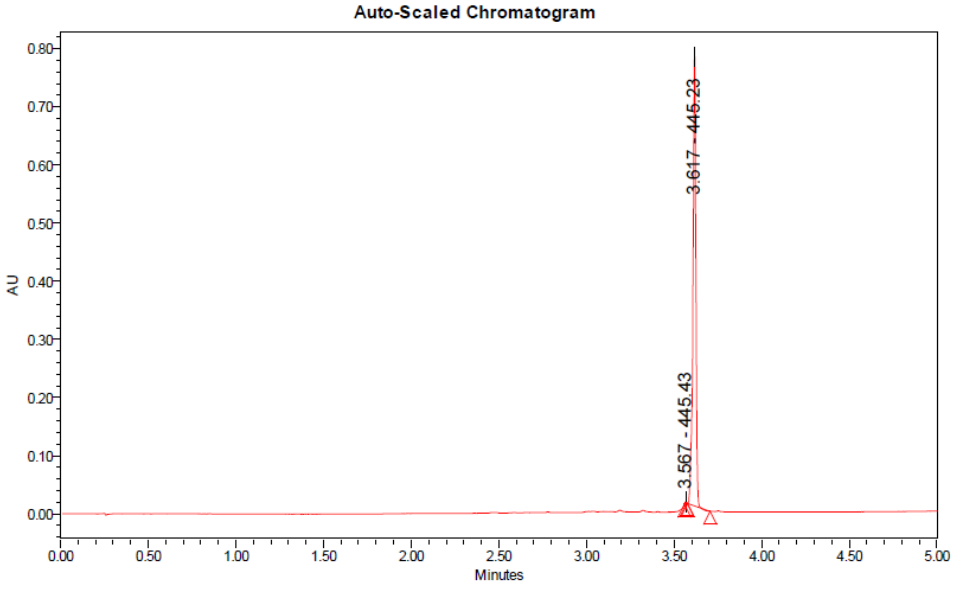


Figure S4. UPLC-MS results for MDM2 degrader **MD-224** (Original code name: LE-004).

SAMPLE INFORMATION

Sample Name:	LE-004-2	Acquired By:	System
Sample Type:	Unknown	Date Acquired:	4/22/2016 1:03:51 AM EDT
Vial:	2:A,4	Acq. Method Set:	10 to 100% B 5min Delay 5 min
Injection #:	1	Date Processed:	4/22/2016 8:56:01 AM EDT
Injection Volume:	3.00 ul	Processing Method:	test
Run Time:	5.0 Minutes	Channel Name:	254.0nm
Sample Set Name:	2	Proc. Chnl. Descr.:	PDA Spectrum (210-500)nm



Peak Results

	RT	Area	Height	% Area	Base Peak (m/z)
1	3.567	1676	3078	0.18	445.43
2	3.617	911394	775553	99.82	445.23