Supplementary Information

Nalmefene non-enantioselectively targets myeloid differentiation protein 2 and inhibits Toll-like receptor 4 signaling: wet-lab techniques and *in silico* simulations

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Table S1 MM/PBSA derived binding free energies (kcal/mol) for nalmefene binding to murine MD-2

	ΔE_{vdW}	ΔE_{ele}	$\Delta G_{sol\text{-}polar}$	$\Delta G_{sol\text{-}nonpolar}$	$\Delta G_{binding}$
(+)-nalmefene	-47.3±0.3	-1.2±0.1	5.2±0.1	24.8±0.2	-18.4±0.4
(-)-nalmefene	-43.2±0.4	-1.1±0.1	4.7±0.1	21.9±0.2	-17.8±0.4
(+)-naltrexone[a]	-44.5±0.1	-1.6±0.1	5.5±0.1	23.7±0.1	-16.9±0.2[a]
(-)-naltrexone[a]	-47.8±0.3	-1.4±0.1	6.6±0.1	24.6±0.2	-18.0±0.3[a]

Numbers after ± present standard errors;

[a]SeeRef.16

Table S2. The decomposition of per-residue binding free energies (kcal/mol) of nalmefene and naltrexone binding to murine MD-2

	Compound			
Residue	**(+)-nalmefene**	**(-)-nalmefene**	**(+)-naltrexone[a]**	**(-)-naltrexone[a]**
Trp23		-2.3±0.4		
Ser48		-1.5±0.9	-1.5±.9	
Ile52		-1.6±0.3	-1.4±0.3	-2.3±0.5
Val61	-1.1±0.2	-1.2±0.3	-1.6±0.4	-1.2±0.3
Phe76	-2.3±0.7		-2.1±0.4	-1.7±0.4
Leu78	-2.0±0.3	-1.3±0.3		-1.4±0.3
Ile80				
Arg90	-1.1±0.2			
Glu92	-1.3±0.3		-1.5±0.4	-1.5±0.7
Phe119	-2.6±0.4	-1.8±0.3	-2.2±0.4	-1.5±0.4
Phe121	-1.3±0.4	-1.6±0.4	-1.5±0.5	-1.1±0.3
Ala135	-1.4±0.4			
Cys133	-1.0±0.3	-1.0±0.3		
Leu149	-1.2±0.3			
Phe151	-3.1±0.4	-3.1±0.4	-2.7±0.5	-2.2±0.5

Numbers after ± present standard error

[a]SeeRef.16

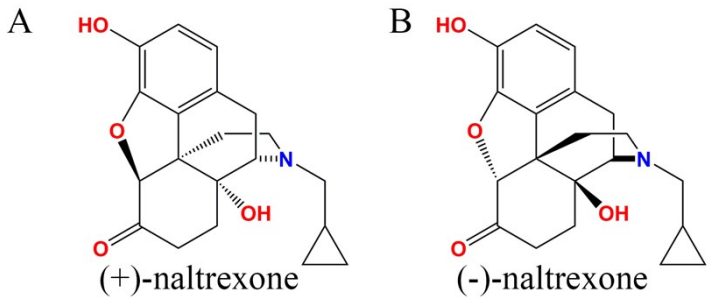

Figure S1. Structures of (+)-naltrexone and (-)-naltrexone.

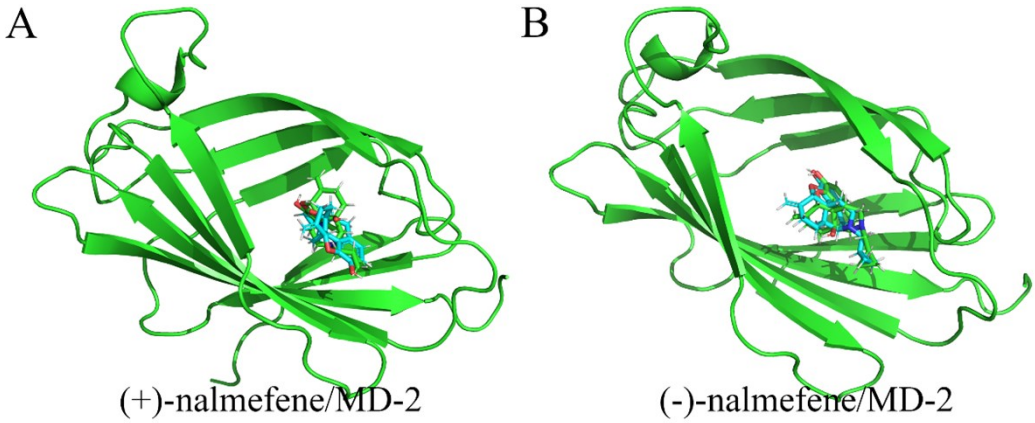

Figure S2. Alignment of docking poses from Autodock Vina (green stick) and Glide XP (cyan stick). Human MD-2 is shown as green cartoon.

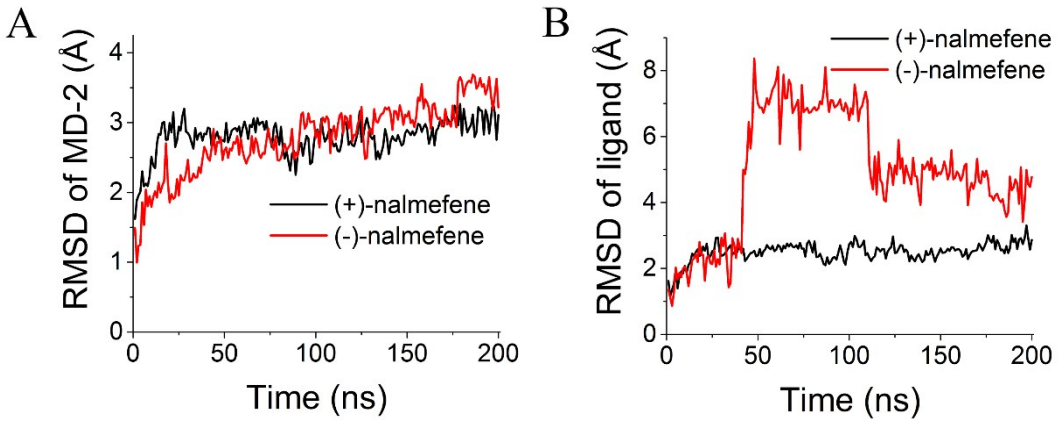

Figure S3. Time evolution of Cα RMSDs of human MD-2 (A) and ligand (B) during MD simulation Black and red colors indicate (+)-nalmefene and (-)-nalmefene, respectively.

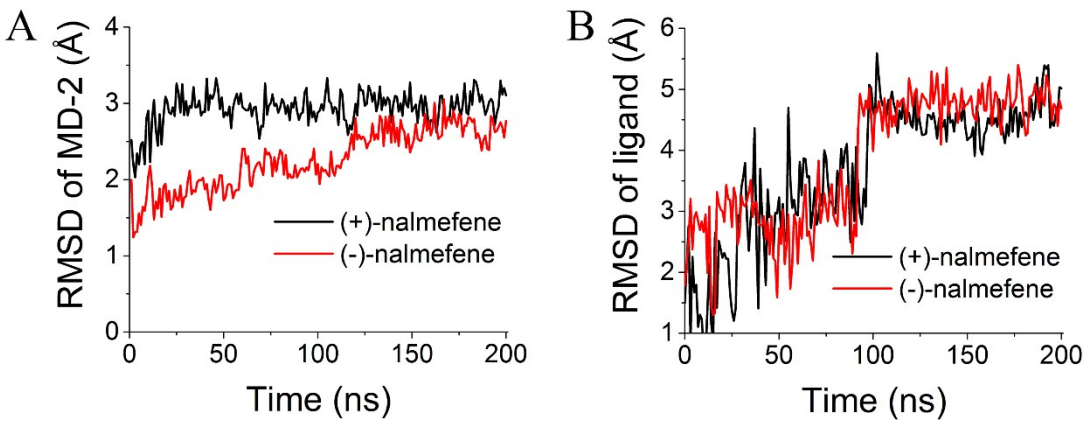

Figure S4. Time evolution of Cα RMSD of murine MD-2 (A) and ligand (B) during MD simulation. Black and red colors indicate (+)-nalmefene and (-)-nalmefene, respectively.

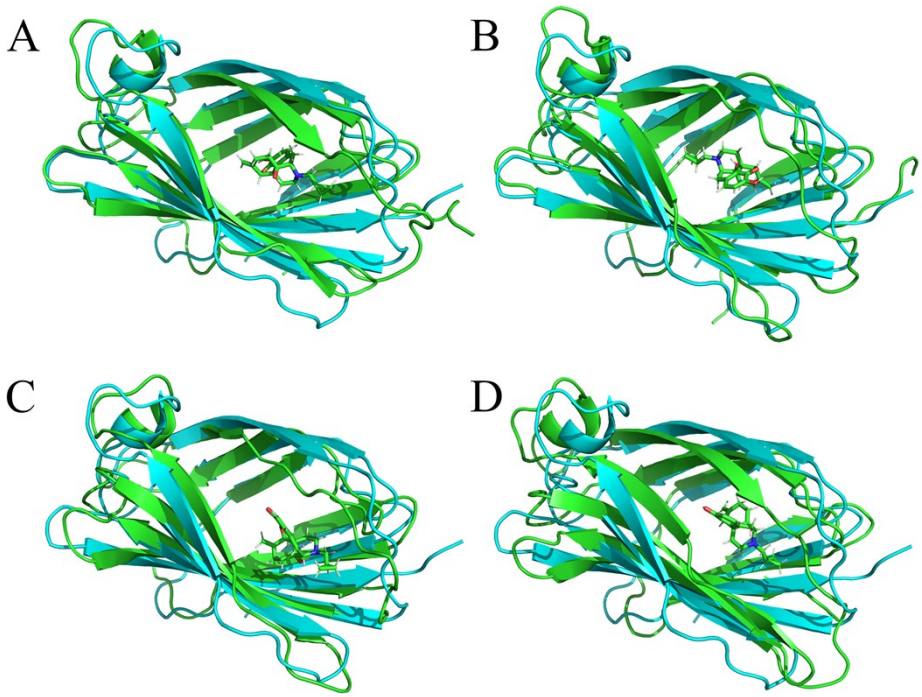

Figure S5. Overlap of the conformation of MD-2 ((A-B), human MD-2; (C-D), murine MD-2) bound to LPS ((A-B), PDB ID: 2E59; (C-D), PDB ID: 2Z64); both in cyan) with the representative structures of MD-2 interacted with nalmefene ((A, C), (+)-nalmefene; (B, D), (-)-nalmefene) with the lowest energy (in green).

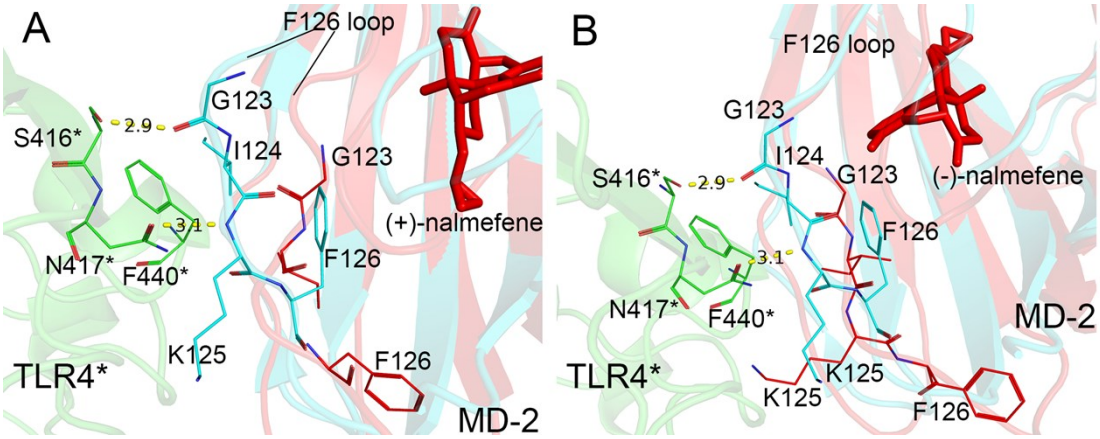

Figure S6. The main dimerization interface between the the F126 loop of MD-2 and TLR4* that is from the adjacent copy of TLR4-MD-2. Overlap of the conformation of active MD-2 (cyan) with the representative structures of MD-2 interacted with (+)-nalmefene (red) (A) and (-)-nalmefene (red) (B), with the lowest energy. TLR4* was shown as green cartoon, the key residues in dimerization interface were shown as stick. Binding of nalmefene to MD-2 abolishes most of the key interactions between MD-2 F126 loop and TLR4*.

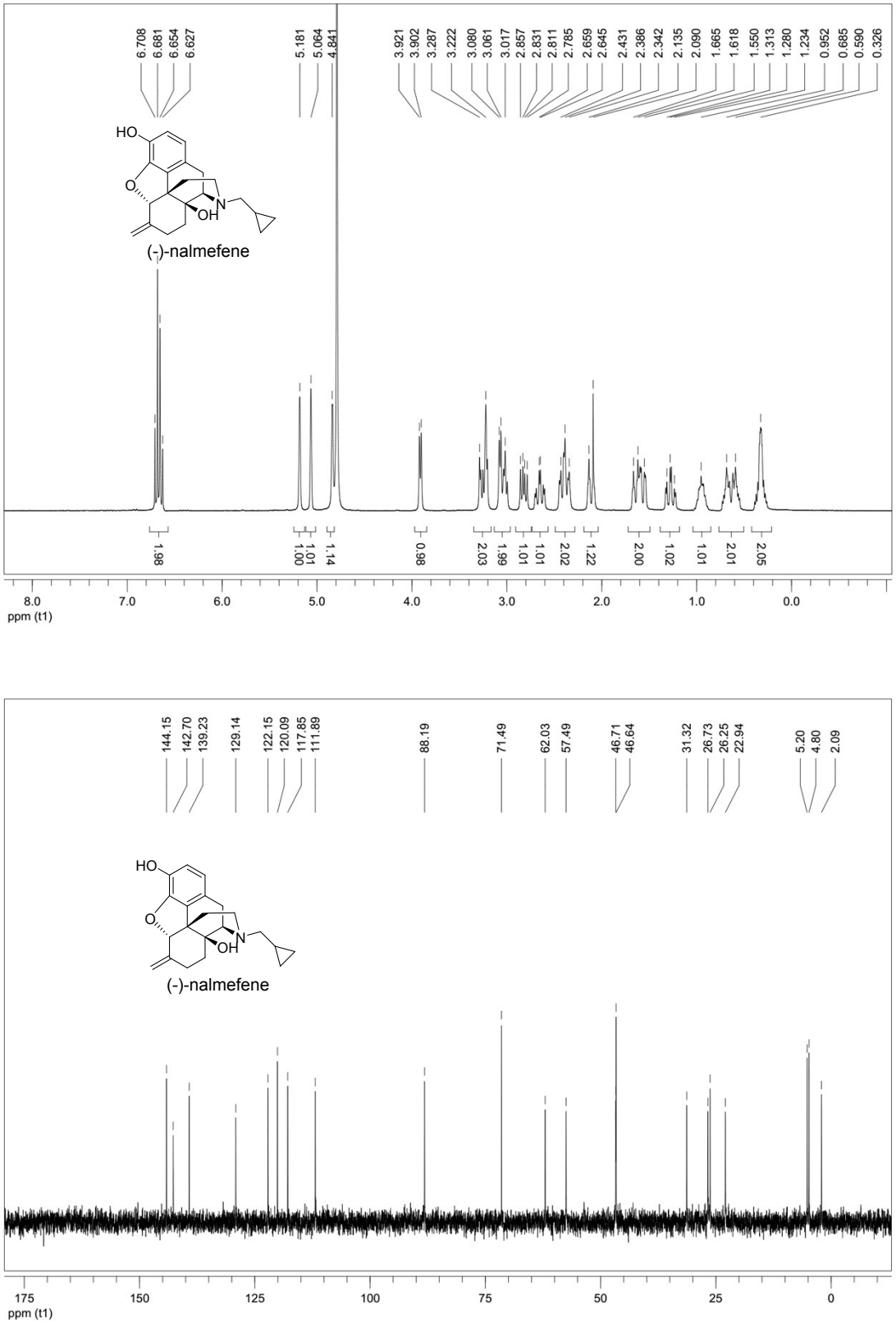

Figure S7. ^{1}H NMR and ^{13}C NMR spectra of (-)-nalmefene

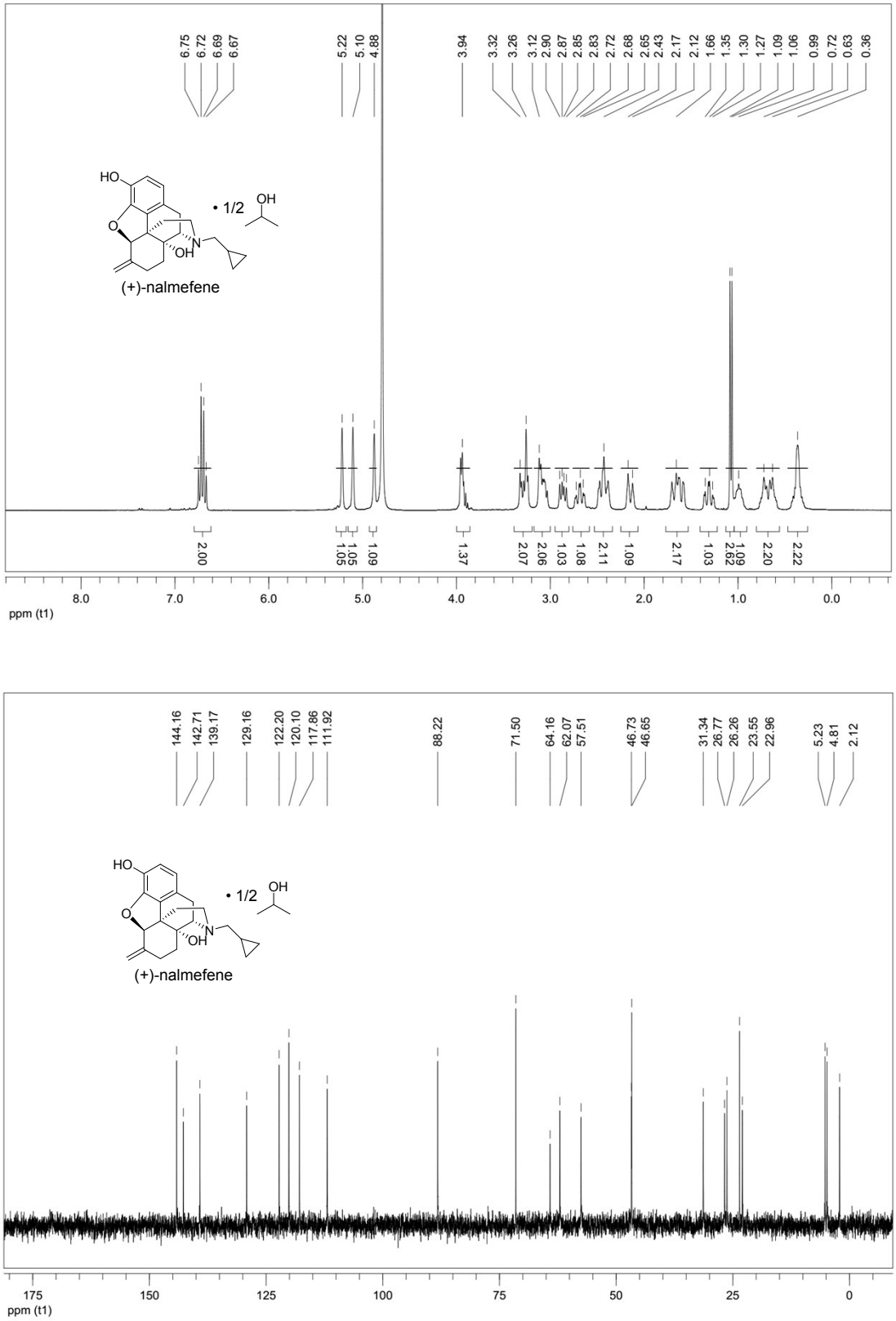

Figure S8. ^{1}H NMR and ^{13}C NMR spectra of (+)-nalmefene