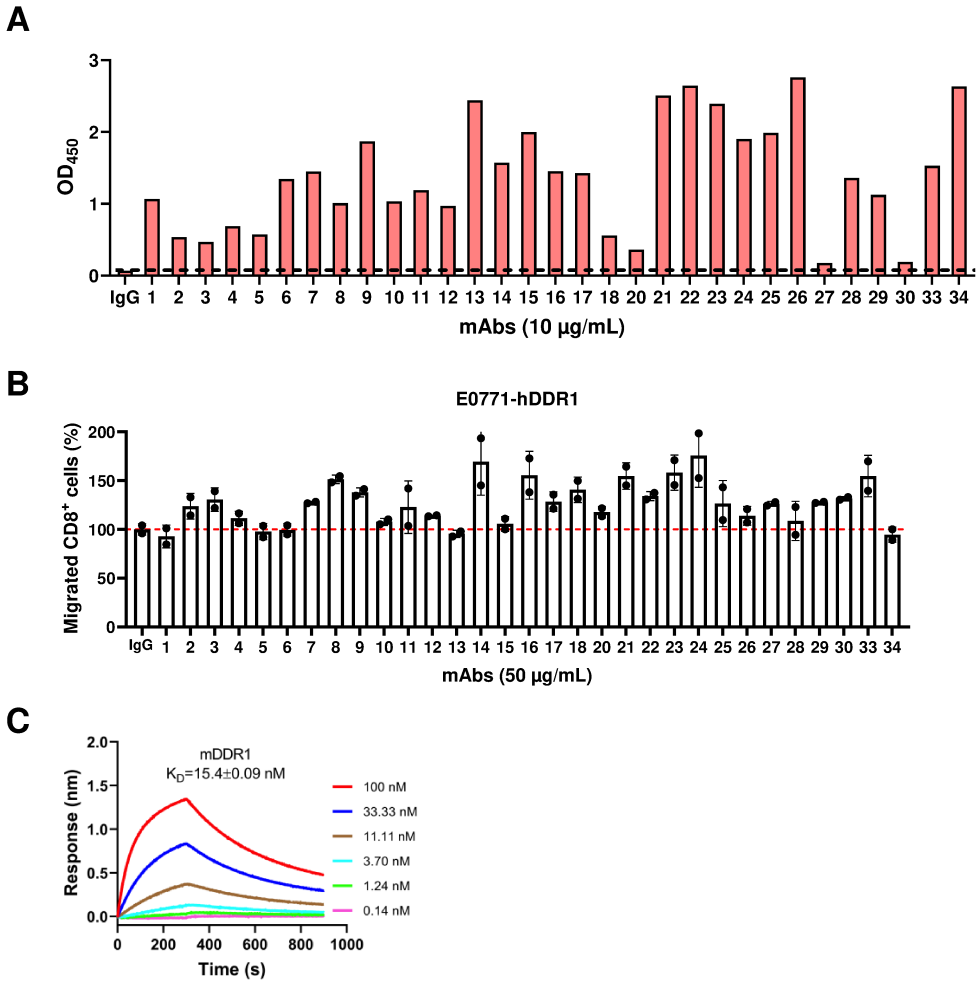

Figure S1 Screening of anti-hDDR1 ECD rabbit mAbs. (A) Binding of the 31 recombinantly expressed and purified rabbit mAbs to hDDR1 ECD as determined by ELISA. The dashed line is the OD_{450} of a control rabbit IgG1 as a cut-off for binders. (B) The 31 mAbs were assessed for their activity to enhance $CD8^+$ T cells migration mediated by E0771-hDDR1 conditioned media. The migrated $CD8^+$ cells were normalized with a rabbit isotype IgG1 produced in-house. The red dashed line indicates the migrated $CD8^+$ cells treated by rabbit isotype IgG1. (C) Binding affinity of rabbit mAb9 to mDDR1 ECD as determined by Octet RED96.

Table S1 Nine DDR1-DSL mutants which contain amino acids switch from hDDR1 to hDDR2.

Mutants	hDDR1	hDDR2	AA
mut1	TMYLSEAV	QFVLPGGSII	201-208
mut2	HTVGGLQYG	AVGYSMTE	218-226
mut3	RKSQELR	TQTHEYH	242-248
mut4	SNHSFSS	RNESATN	259-265
mut5	LRAFQAMQ	IRNFTTMK	277-284
mut6	HELGAR	FAKGVK	291-296
mut7	RGPAMA	SEASE	307-312
mut8	GEPMRHN	PNAISFP	315-321
mut9	GGNLGD	VLDDVN	323-328

Table S2 Summary of HDX-MS analysis for the PRTH-101-DDR1 epitope mapping experiment.

Data Set	**apo (control)**	**PRTH-101**
HDX reaction details	non-deuterated sample buffer (50 mM HEPES, 150 mM NaCl, pH 7.2); deuterated labeling buffer (50 mM HEPES, 150 mM NaCl, pHread 7.2); quench buffer (1 M glycine, 4 M GndHCl, 200 mM TCEP, pH 2.5); labeling temperature 20°C; HDX labeling dilution 1:9; protein amount 50 pmol	
HDX time course (sec)	15, 60, 600, 3600, 28800	15, 60, 600, 3600, 28800
HDX control samples	none	none
Back-exchange (mean / IQR over entire project)	unknown	unknown
# of peptides	98	98
Sequence coverage	75.42%	75.42%
Average peptide length / Redundancy	11.64 / 2.75	11.64 / 2.75
Replicates	3	3
Repeatability (avg. stddev of #D)	0.1375	0.1363
Significant differences in HDX (delta HDX > X D)	0.5771 D	

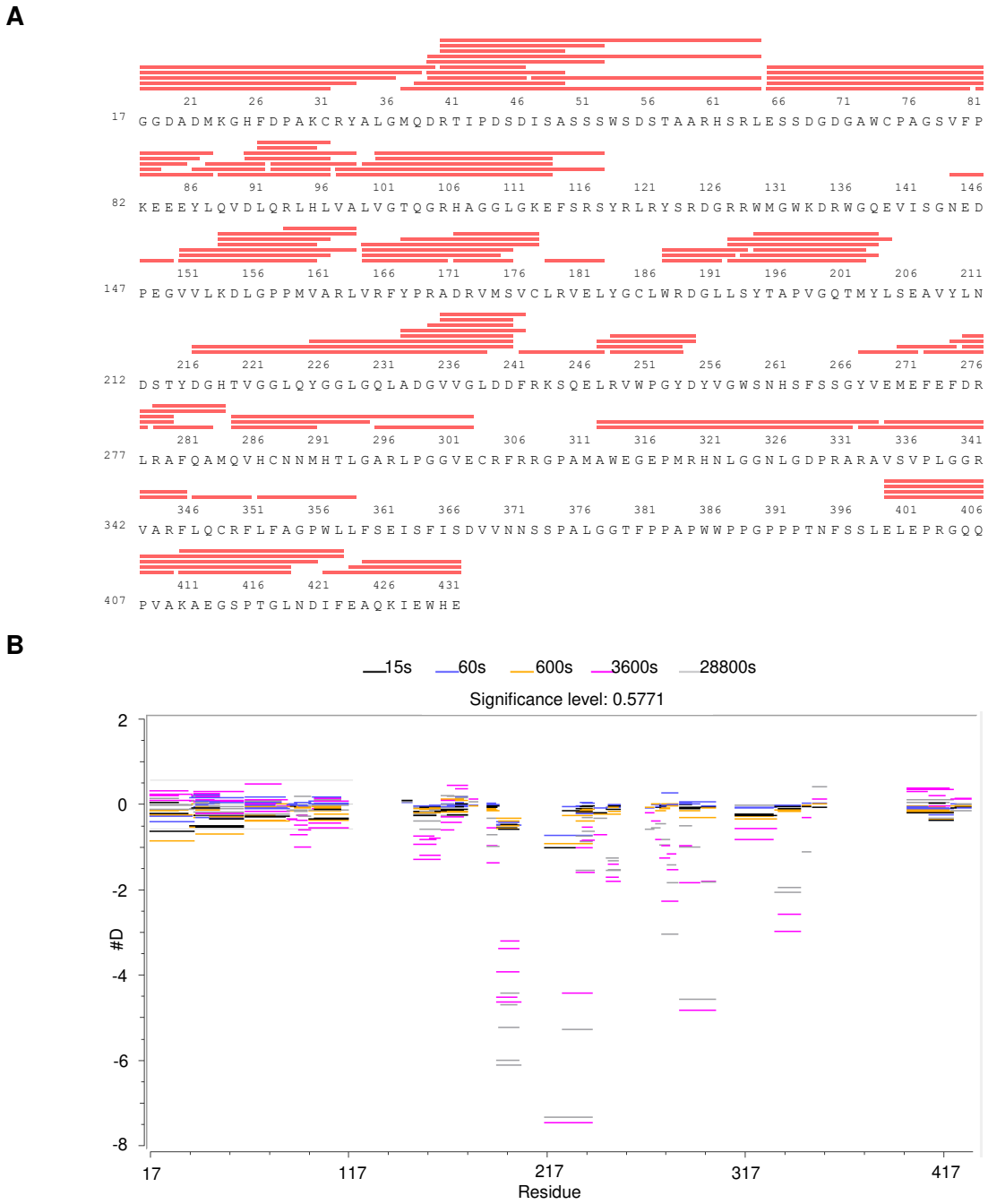

Figure S2 HDX-MS to identify the binding sites of PRTH-101. (A) DDR1 effective peptide coverage map for HDX-MS experiments with PRTH-101. Sequence coverage was 75.4 %. (B) Woods plot for PRTH-101-DDR1 epitope mapping. Depicted are deuteration differences from centroid analysis at different labeling time points in individual comparison experiments (PRTH-101–apo). In case of bimodal spectra the higher deuterated peak distribution was used for HDX difference calculation. The significance level was calculated as 0.5771 Da.

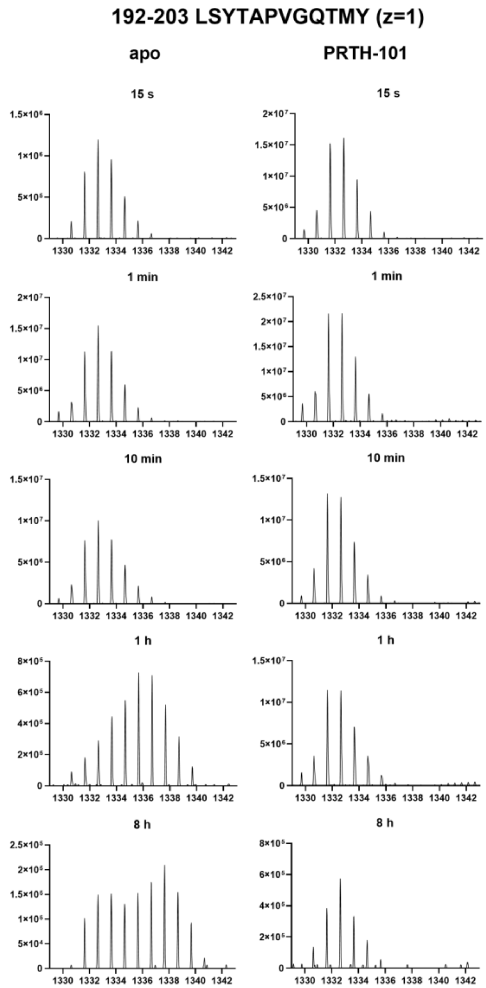

Figure S3 Spectra comparison of DDR1 peptide 192-203 in the apo state and with PRTH-101 bound. In the apo state bimodal peak distributions are visible at 1 h and 8 h labeling time points. These could be the result of mixed exchange kinetics caused by slow local dynamics of the protein which are absent in the PRTH-101 bound state.

Table S3 Data collection and refinement of the DDR1-DSL:PRTH-101 complex. Values in parenthesis correspond to the highest resolution shell.

Data collection	
Space group	$P2_12_12$
Cell dimensions	
a, b, c (Å)	117.1, 144.5, 52.3
α, β, γ (°)	90, 90, 90
Ellipsoidal resolution cut-offs, a, b, c (Å)	3.6, 3.1, 4.0
Wavelength (Å)	1.00001
Synchrotron, beamline	Swiss Light Source, PXI
Resolution (Å)	72.3 - 3.15 (3.56-3.15)
No. of unique observations	9760 (488)
Completeness (%)	86 (47)
Multiplicity	6.0 (6.7)
⟨I/σ(I)⟩	7.0 (1.8)
R_{merge} (%)	27.1 (99.3)
R_{pim} (%)	17.7 (59.2)
$CC_{1/2}$	0.99 (0.61)
Refinement	
Resolution	72.22 - 3.15
Number of reflections (test set)	9760 (496)
R_{work}/R_{free} (%)	27.8 / 30.9
No. of atoms:	
Protein	4588
Sugar	52
Metal ions	5
Average B-factor (Å^2):	
Protein	68.9
Sugar	78.7
Metal ions	82.2
RMSD bond lengths (Å)	0.007
RMSD bond angles (°)	1.23
Ramachandran plot (%)	
Favoured	93.3
Allowed	6.5
Outliers	0.2

RMSD = Root Mean Square Derivative

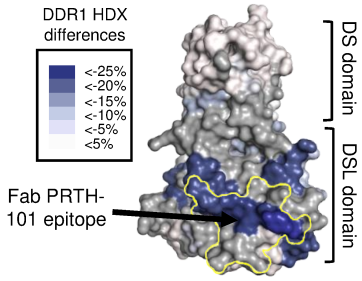

Figure S4 Comparison between protected peptides identified by HDX-MS and epitope from X-ray crystal structure. Overlap of the PRTH-101 epitope on the DDR1-DS and DSL domain surface with HDX data (based on PDB ID 4AG4). The epitope identified by the crystal structure is shown using a yellow outline. Observed deuteration differences are mapped onto the DDR1-DSL domain surface using a blue-to-red scale, as displayed by the inset.

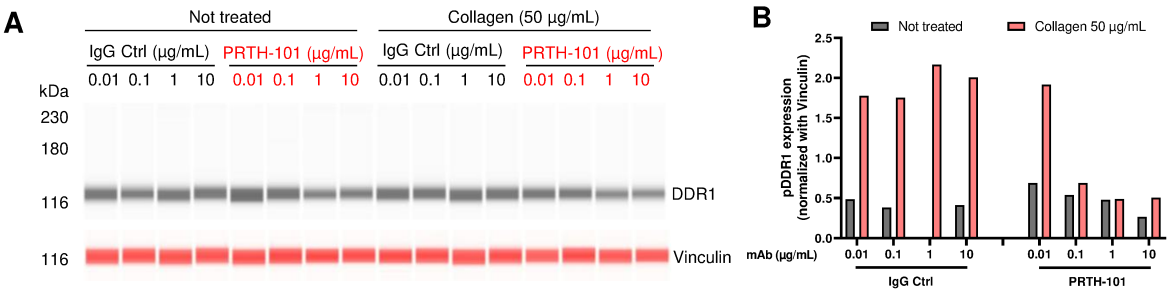

Figure S5 PRTH-101 inhibits DDR1 phosphorylation, but not alter total DDR1 expression. (A) Digital representation of data obtained for total DDR1 and Vinculin detection with different concentrations of IgG and PRTH-101 treatment. (B) pDDR1 was measured by JESS with different concentrations of IgG and PRTH-101 treatment.

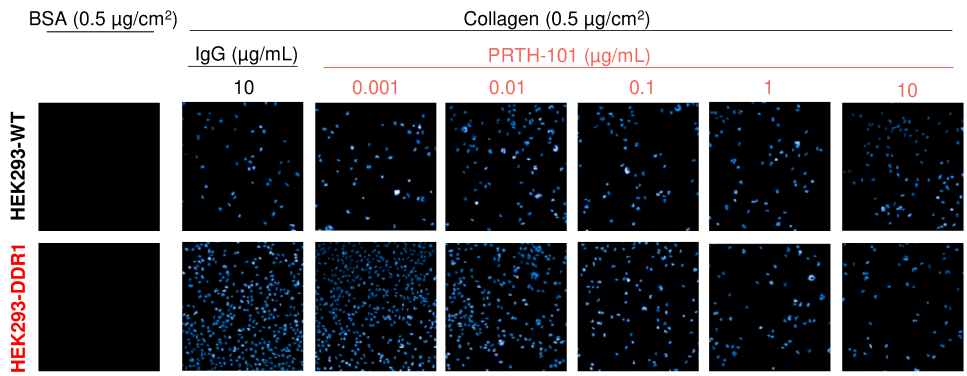

Figure S6 Functional characterization of PRTH-101 using collagen adhesion. Pictures of cell adhesion after 60 min using nuclei staining (Hoechst 33342). HEK293-WT or HEK293-DDR1 cells were detached and incubated for 2 h with/without IgG control or PRTH-101 for 2 h. Cells were seeded in plates coated with collagen I or BSA (0.5 µg/cm^2).

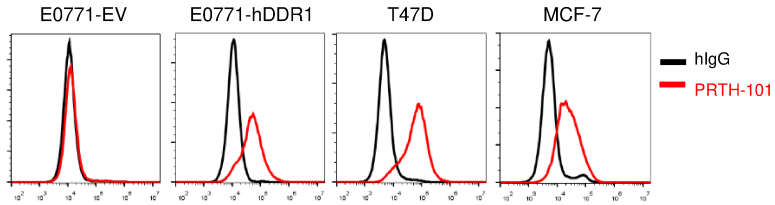

Figure S7 The binding of hIgG control and PRTH-101 to engineered E0771-EV, E0771-hDDR1, T47D and MCF-7 were determined with flow cytometry. Alexa Fluor 633 labelled goat anti-human IgG (H+L) was used as secondary antibody.

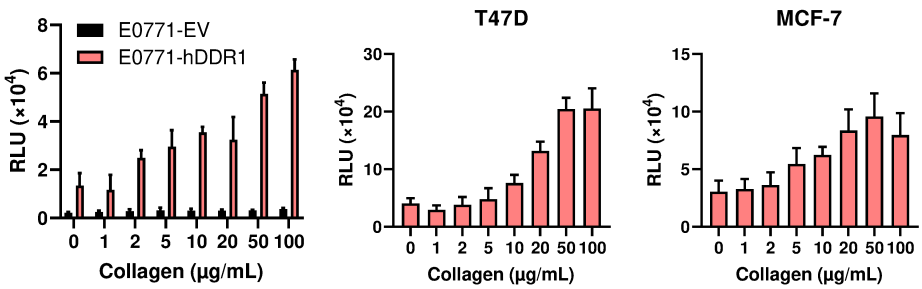

Figure S8 E0771-EV, E0771-hDDR1, T47D and MCF-7 were treated with different doses of collagen and shed DDR1 in the supernatant was detected by sandwich ELISA.

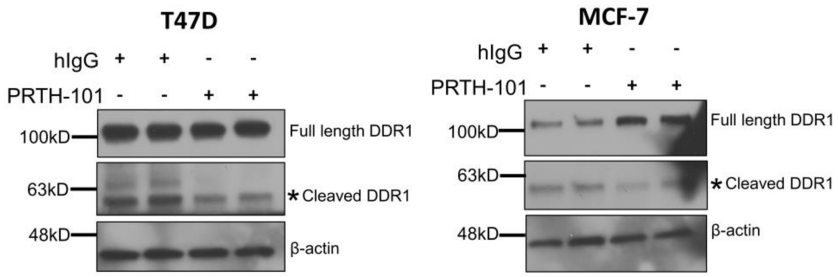

Figure S9 Assessment of full-length and cleaved DDR1 following PRTH-101 treatment in T47D and MCF-7 cell lysates using western blot detection. Cleaved DDR1 is for DDR1 after shedding DDR1 ECD. Antibody treated cells (24 hours) were washed with PBS twice and RIPA buffer was used to lysate cells. Cell lysates were used for WB detection. Full length and cleaved DDR1 on cell surface were detected by western blot using antibody D1G6 (Cell Signaling, #5583). Each treatment was duplicated.

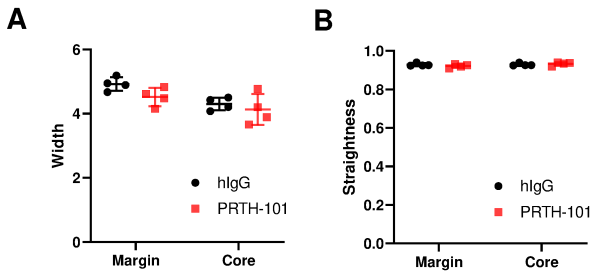

Figure S10 Comparison of collagen width (A) and straightness (B) in tumor margin and tumor core within hIgG or PRTH-101-treated tumors. The difference between two groups was calculated with two-tailed t-test.

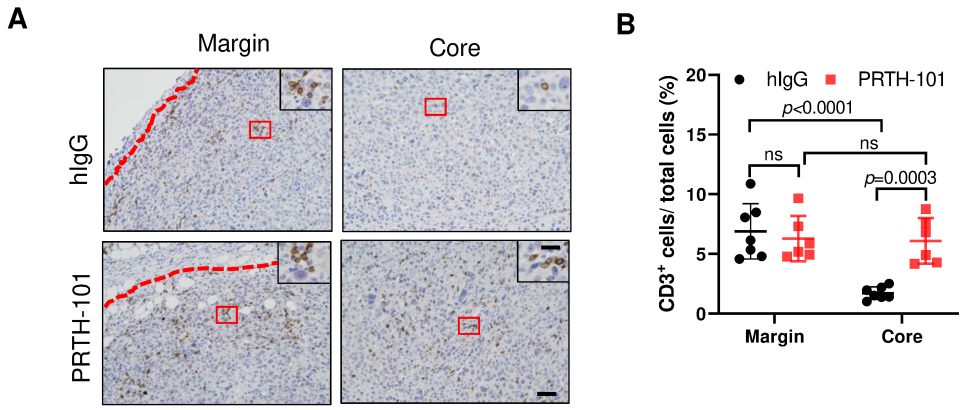

Figure S11 (A) Representative IHC images of CD3⁺ T cells in tumor margin and core for antibody-treated tumor. Scale bar: 50 µm, scale bar (inlet): 20 µm. An area on the tumor side with a depth 400-600 µm from the tumor-stroma border was defined as tumor margin which was separated by red dash line. (B) Quantification of CD3⁺ T cells in tumor margin and core for antibody-treated tumor. The difference between two groups was calculated with two-tailed t-test. n (hIgG) = 7, n (PRTH-101) = 6.

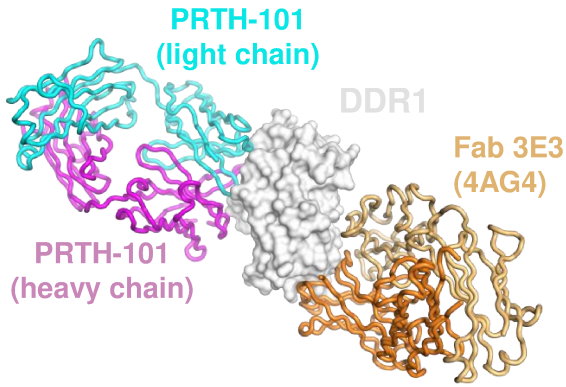

Figure S12 Comparison of the DDR1-DSL:PRTH-101 structure with published anti-DDR1 Fab 3E3 (PDB ID 4AG4, colored orange). Structures were superposed based on the DDR1-DSL domain. PRTH-101 and DDR1-DSL are colored as in figure 4A.

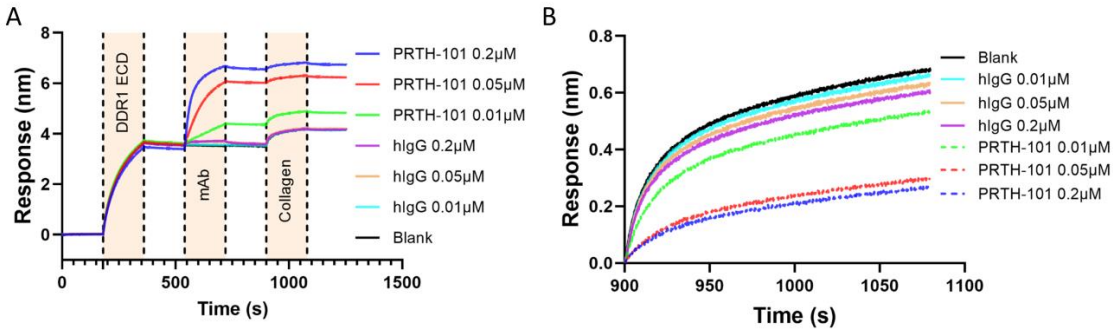

Figure S13 An *in vitro* Octet analysis of DDR1 ECD binding to collagen in the presence and absence of PRTH-101. (A) The binding response in Octet96Red system to test whether PRTH-101 impact the binding of DDR1 ECD to collagen. DDR1 ECD with His tag was captured by NTA-Ni sensor. After incubating with 30 μg/mL of isotype hIgG or PRTH-101, the sensor was dipped into 0.2 μM of collagen to test the binding response. Between each step the sensor was balanced in kinetic buffer for 180 s. (B) The response of collagen binding step after normalized with baseline control at 900 s. PRTH-101 treatments were marked as dash lines.