

Supplementary Materials for
Viral capsid structural assembly governs the reovirus binding
interface to NgR1

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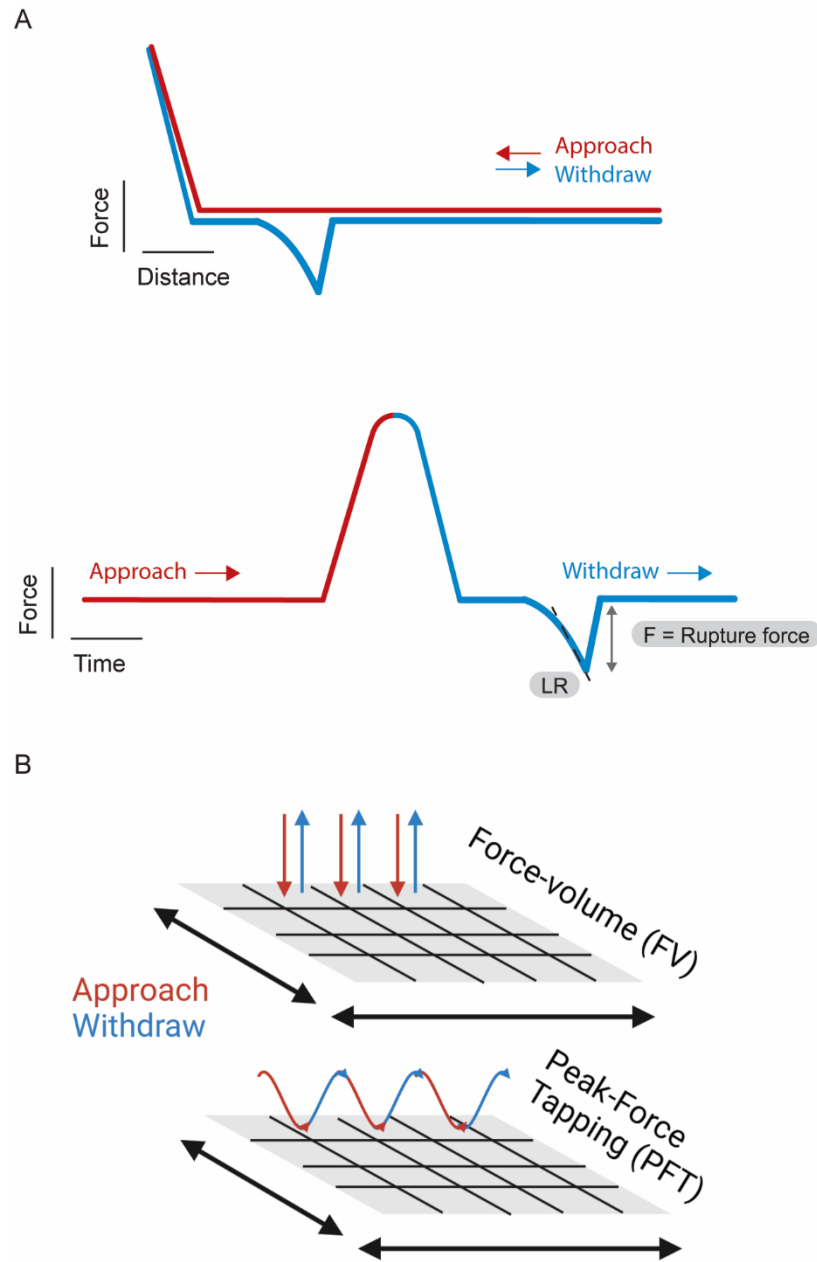
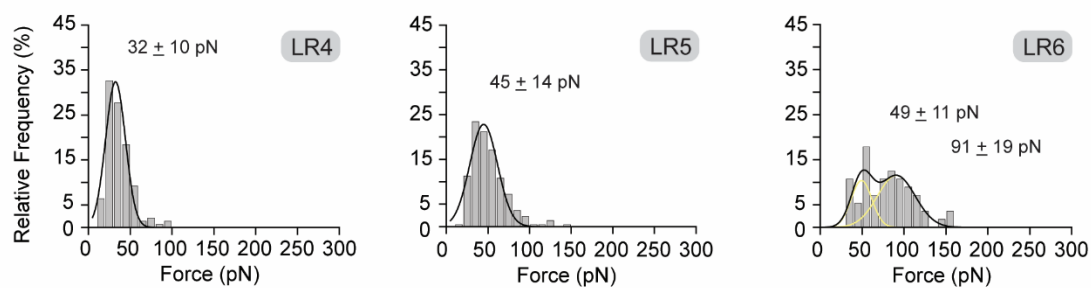


Fig. S1. Schematics of force curves and force mapping. (A) Each pixel, Force-distance (FD) curves (top curve) and Force-time curves (bottom curve) are recorded. The curves represent a specific adhesion event. Rupture forces are collected from the peak of an unbinding event. Loading rate (LR) corresponds to the slope of the curve just before rupture of the bond. (B) In force mapping, an array of force curves is collected. The AFM can be operated in FV mode, in which the tip follows a linear motion, or in PFT mode, with a sinusoidal movement of the tip.

hNgR1 - assembled hexamers

FFV



PFT

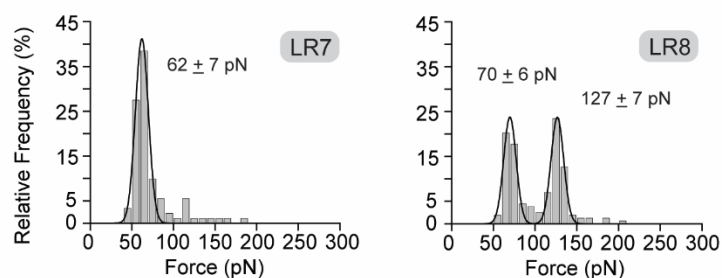


Fig. S2. Extraction of rupture forces of the interaction between hNgR1 and self-assembled $\mu_1\sigma_3\bar{3}_3$ heterohexamers. Rupture forces were collected from probing the assembled hexamers in different modes: Fast Force-volume (FFV; top three histograms) and Peak-Force Tapping (PFT; bottom two histograms). LRs were divided into different ranges (LR4-LR8). The distribution of rupture forces for each LR is plotted as histograms and fitted by multipeak Gaussian Fits. The maximum values of all force peaks are indicated.

hNgR1 - single hexamer

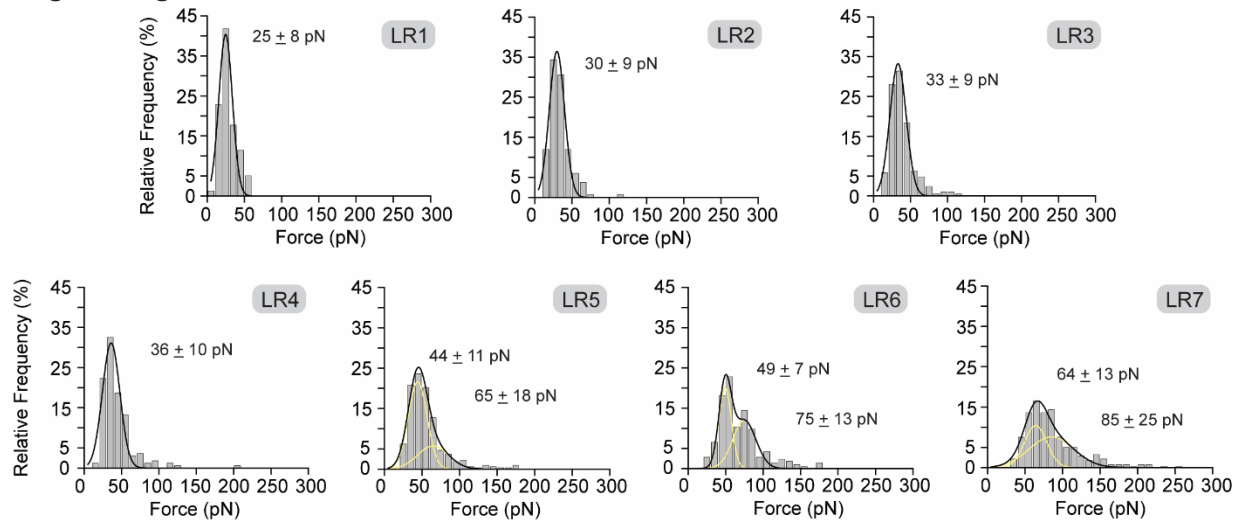



Fig. S3. Extraction of rupture forces of hNgR1 – single hexamer. LRs were divided into different ranges (LR1-LR7). The distribution of rupture forces for each LR is plotted as histograms and fitted by multi-peak Gaussian Fits. The maximum values of all force peaks are indicated.




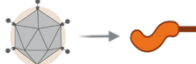

	$k_{\text{off}} [\text{s}^{-1}]$	$x_b [\text{nm}]$	$k_{\text{on}} [\mu\text{M}^{-1} \text{s}^{-1}]$	$K_D [\text{nM}]$
 $\sigma 3\mu 1 - \text{hNgR1}$	0.32 ± 0.21	0.86 ± 0.08	2.17 ± 0.20	147.25 ± 110.52
 $\text{mNgR1} - \text{T1L}$	0.65 ± 0.35	0.73 ± 0.07	6.94 ± 0.23	93.51 ± 53.63
 $\sigma 3\mu 1 - \text{mNgR1}$	0.23 ± 0.12	0.76 ± 0.05	2.99 ± 0.46	76.80 ± 51.86

Fig. S4. Kinetic parameters of the interactions between reovirus and hNgR1/mNgR1. Overview of the estimated kinetic parameters and their associated errors for the interactions between (represented by schematics on the left): i) reovirus T1L particle binding to hNgR1, ii) hNgR1 binding to single heterohexamers, iii) reovirus T1L particle binding to mNgR1, and iv) hNgR1 binding to single heterohexamers. The BE fit (for simple ligand-receptor bond) provides average k_{off} and x_u values, whereas the least-squares fit of a monoexponential decay provides average values for the k_{on} . The K_D is calculated using $k_{\text{off}}/k_{\text{on}}$.

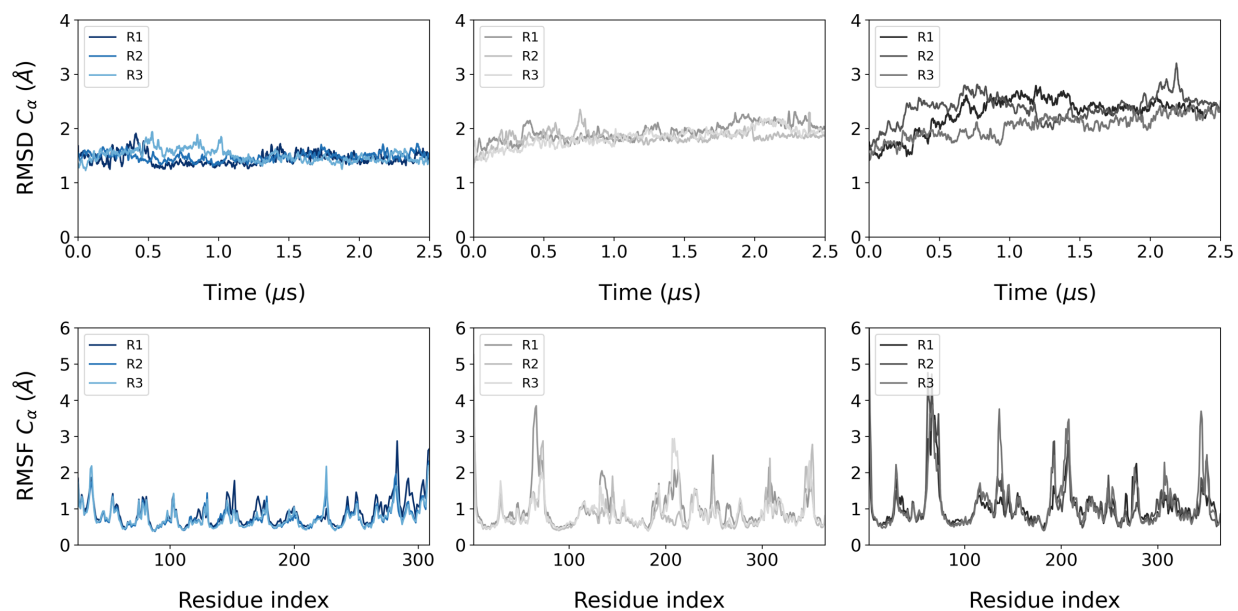
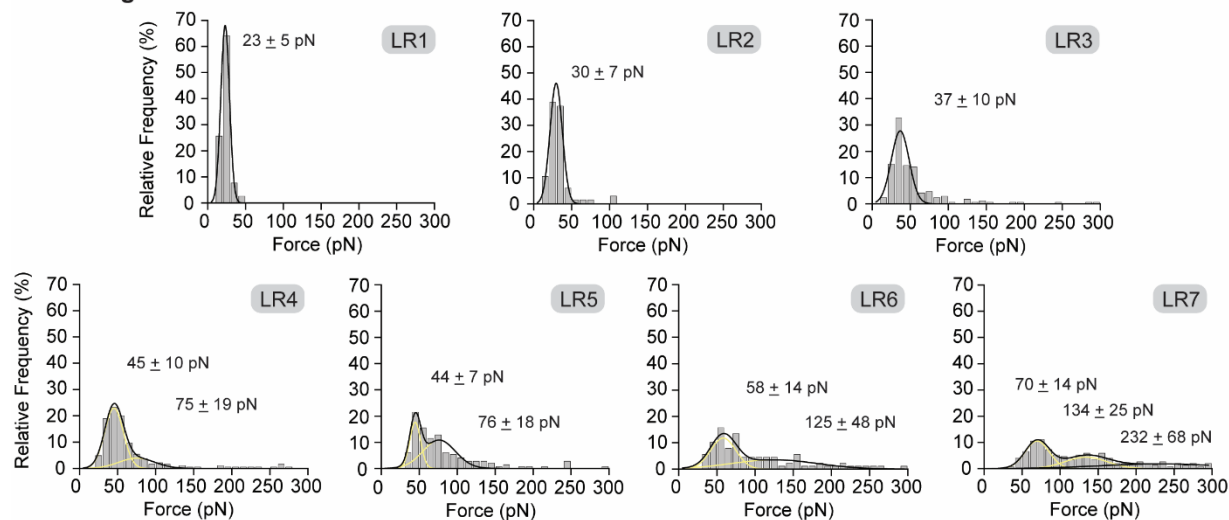


Fig. S5. Root Mean Square Deviation (RMSD, top panels) and Root Mean Square Fluctuations (RMSF, bottom panels) for hNgR1 and $\sigma 3$. RMSD and RMSF of the backbone C_α of hNgR1 (blue lines), $\sigma 3_A$ (light grey lines) and $\sigma 3_B$ (dark grey lines) during the MD simulations. R1, R2, and R3 denote MD replicas.

T1L - mNgR1



mNgR1 - single hexamer

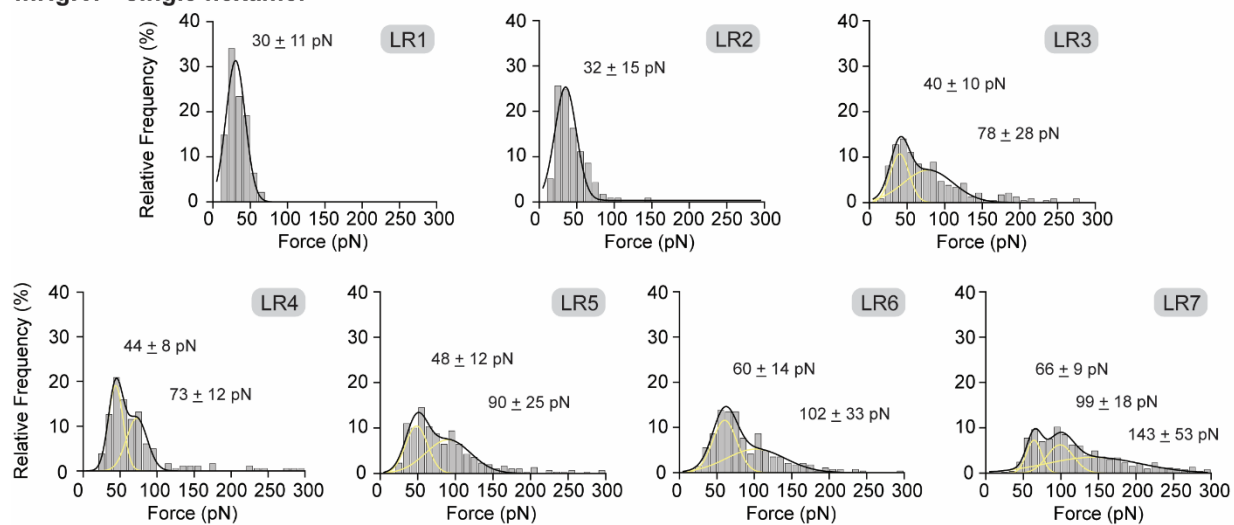


Fig. S6. Extraction of rupture forces of T1L – mNgR1 and mNgR1 – single hexamer. LRs were divided into different ranges (LR1-LR7). The distribution of rupture forces for each LR is plotted as histograms and fitted by multipeak Gaussian Fits. The maximum values of all force peaks are indicated.

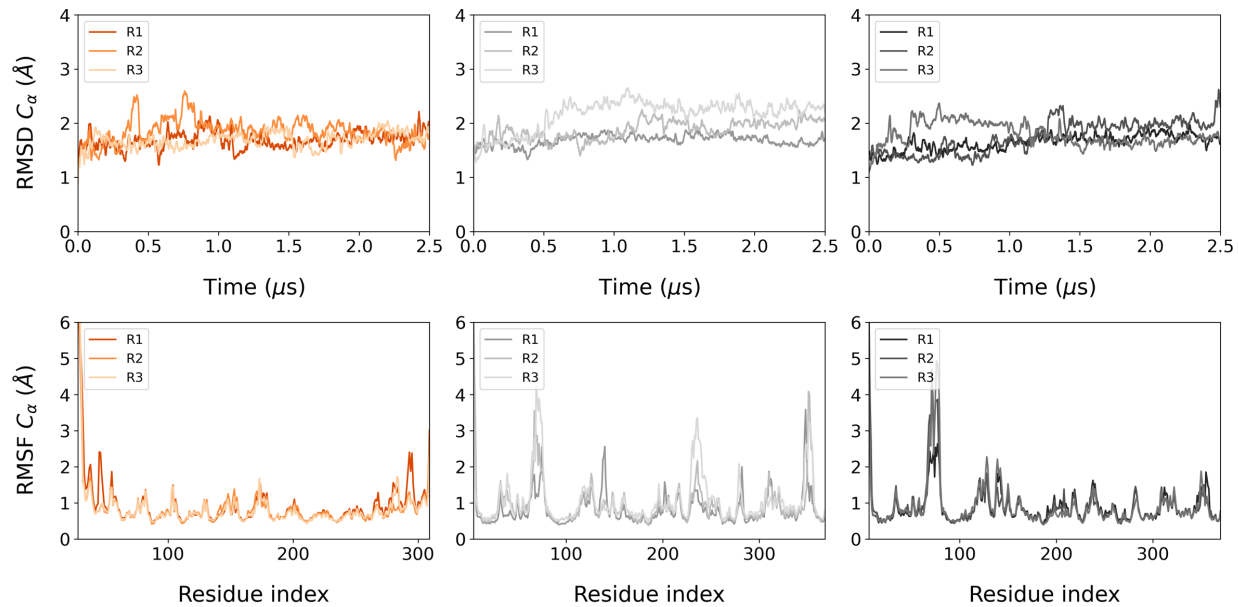


Fig. S7. Root Mean Square Deviation (RMSD, top panels) and Root Mean Square Fluctuations (RMSF, bottom panels) for mNgR1 and $\sigma 3$. RMSD and RMSF of the backbone C α of mNgR1 (orange lines), $\sigma 3_A$ (light grey lines) and $\sigma 3_B$ (dark grey lines) during the MD simulations. R1, R2, and R3 denote MD replicas.

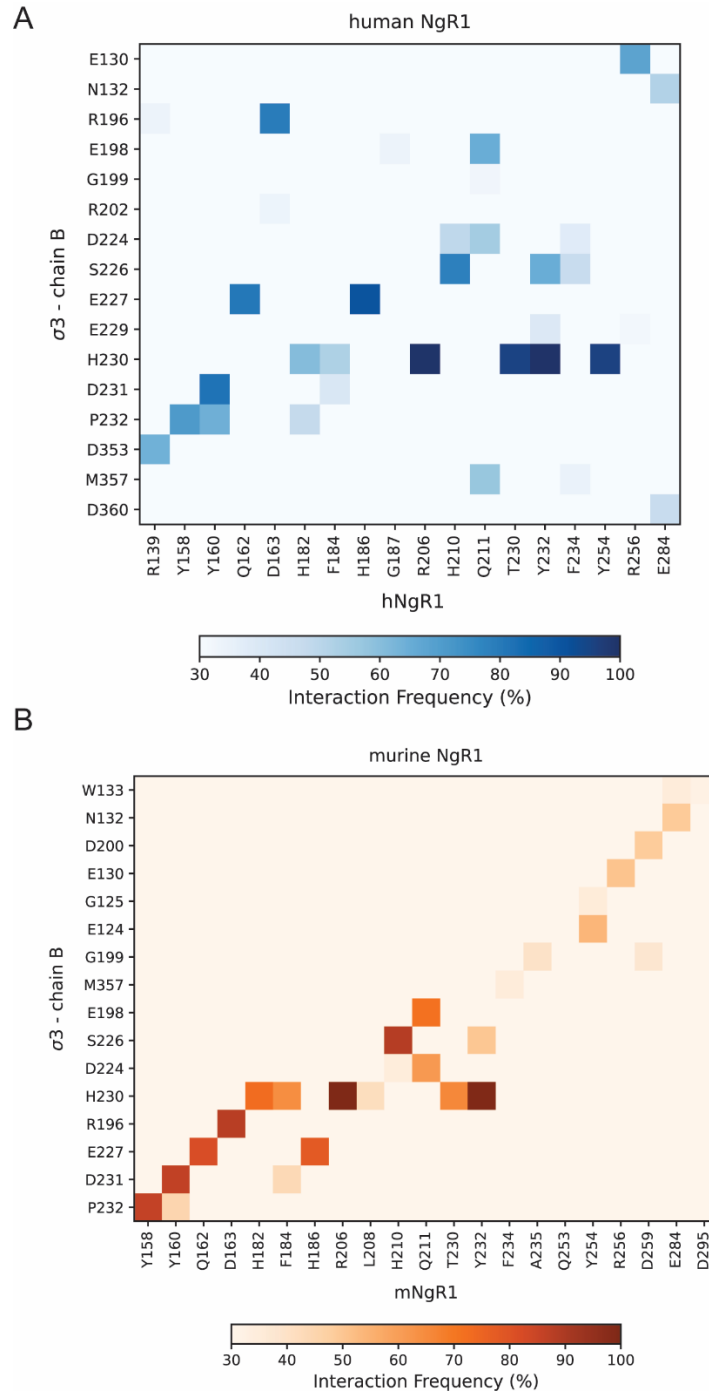


Fig. S8. Interactions occurring in the NgR1 complex with $\sigma 3_B$. Interactions are shown for the hNgR1 complex (panel A) and mNgR1 complex (B). Cells of the heat maps are coloured according to the increased number of interactions, from white to dark blue (A) and dark orange (B).

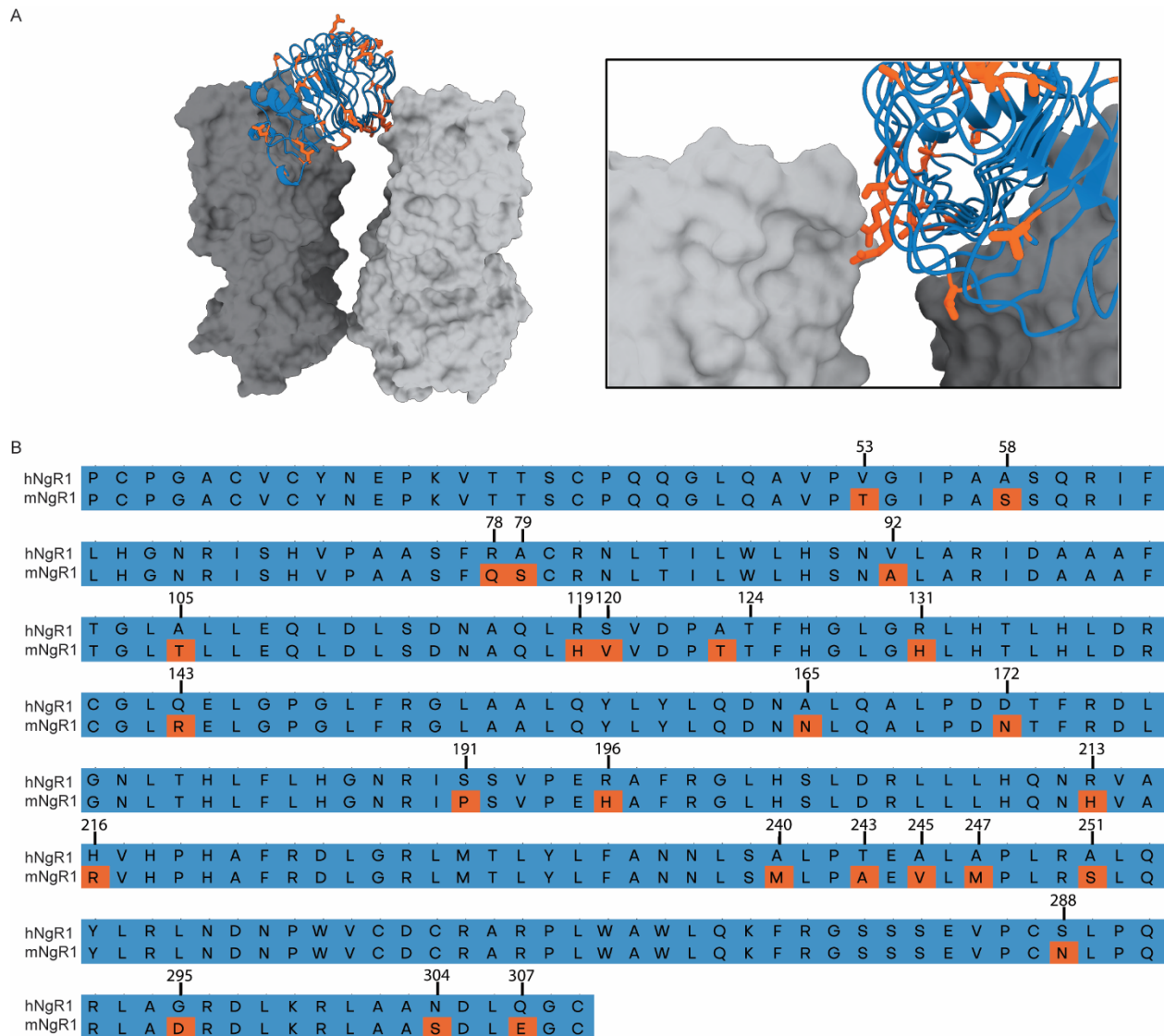


Fig. S9. Overview of diverse residues involved in the NgR1- $\sigma 3$ interaction. (A) Structural representation of the complex of hNgR1 with two $\sigma 3$ monomers. NgR1 is represented as blue cartoons, with regions involving diverse residues between human and murine NgR1 in orange, while $\sigma 3_A$ and $\sigma 3_B$ are shown as light and dark grey surfaces. A zoom-in on the interacting convex interface is reported on the right side of the figure. Residues that differ between hNgR1 and mNgR1 are shown as orange sticks. (B) Sequence alignment between human and murine NgR1.

Table S1. List of high frequent contacts conserved during the simulations between the human and murine NgR1 and $\sigma 3_B$.

$\sigma 3_B$	hNgR1	mNgR1	Frequency (%)	Interaction Type ^a	present initial structure
E227		Q162	80.5	hbss, vdw	yes
E227	Q162		82.0	hbss, vdw	yes
D231		Y160	86.4	hbss, vdw	yes
D231	Y160		81.9	hbss, vdw	yes
H230		R206	99.7	pc, vdw, hbss	yes
H230		Y232	99.4	ts, vdw	yes
H230	H182		60.6	vdw	yes
H230	R206		99.7	pc, vdw, hbss	yes
H230	T230		95.1	vdw, hbss	yes
H230	Y232		100.0	ts, vdw	yes

^aHydrogen Bonds (hb) backbone to backbone (bb) or side-chain to side-chain (ss); Salt bridges (sb); Pi-cation (pc), Pi-stacking (ps), T-stacking (ts) and van der Waals Interactions (vdw).

Table S2. List of contacts with a frequency higher than 40% between human and murine NgR1 and $\sigma 3_A$ during the simulations.

$\sigma 3_A$	hNgR1	mNgR1	Frequency (%)	Interaction Type ^a	present initial structure
D112		R189	47,2	sb, hbss	no
D112	R189		43,3	sb, hbss	no
S114		R143	63,5	vdw	no
E116	R119		61,8	sb, hbss	no
D117		R143	46,9	sb, hbss, vdw	no
D119	R119		42,6	sb, hbssB	no
R120		E144	69,9	sb, hbss	no
E130		R216	41,5	sb, hbss	no
E130		R189	41,2	sb, hbss	no
N132		N237	74,7	hbss, vdw	no
L134		P261	57,8	vdw	no
Q135		N237	63,9	hbss, vdw	no
R143		Q291	56,4	hbsb, vdw	no
I145		Q291	55,3	hbsb, vdw	no
D200		R267	46,9	sb, hbss	no

^aHydrogen Bonds (hb) backbone to backbone (bb) or side-chain to side-chain (ss); Salt bridges (sb); Pi-cation (pc), Pi-stacking (ps), T-stacking (ts) and van der Waals interactions (vdw).

Table S3. Protonation states of histidine residues (HID is protonated in δ , HIE in ϵ and HSP is double protonated), protonated aspartate (ASH) and protonated glutamate (GLH) in the simulated structures.

Protein	HID	HIE	HSP	ASH	GLH
hNgR1		65, 71, 89, 127, 133, 136, 182, 186, 202, 210, 216, 218, 220			
mNgR1	89, 119, 131, 213	65, 71, 127, 182, 196, 202, 210, 218, 220	133, 136, 186	163	284
$\sigma 3_A$		9, 53, 67, 70, 71, 79, 94, 107, 146, 190, 230, 251, 256, 304			
$\sigma 3_B$		9, 53, 67, 70, 71, 79, 94, 107, 146, 190, 230, 251, 256, 304			