

Supplementary Materials for

Sterical Hindrance Promotes Selectivity of the Autophagy Cargo Receptor NDP52 for the Danger Receptor Galectin-8 in Antibacterial Autophagy

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Fig. S1. Stereo view of the hGal8_{C-CRD}-NDP52₃₇₁₋₃₈₁ complex.

Fig. S2. Electron density map for NDP52₃₇₁₋₃₈₁.

Fig. S3. Expression of wild-type or mutant Flag-tagged galectin-8 in transfected HeLa cells.

Table S1. Summary of thermodynamic parameters of ITC titrations.

Table S1. Thermodynamic parameters for the binding of galectin-8 to NDP52 as determined by ITC.

(galectin-8) ^a	(NDP52) ^b	ΔH (Kcal/mol)	ΔS (cal/mol/deg)	ΔG (Kcal/mol)	$K_d \pm s.d.$ (μM)	N	Chi ² /DoF
WT	WT	-7.46	-0.85	-7.20	5.2±0.2	1.02	2767
	L374A	-10.83	-19.9	-4.90	250.0±11.6	1.00	2236
	Y376A				NB		
	N378A				NB		
	Y380A				NB		
	Y380F	-3.66	5.88	-5.52	90.1±13.7	0.99	1598
V244A		-6.21	1.69	-6.71	12.0±0.4	0.88	1516
K246A		-5.93	3.43	-6.96	7.9±0.9	0.99	20340
K246R		-8.92	-4.85	-7.48	3.3±0.1	0.85	2000
K246S		-8.65	-5.56	-6.99	7.1±0.7	1.00	24270
E310A					(NB) ^c		
E310S					NB		
I312A	(WT) ^b				NB		
I312S					NB		
I312W					NB		
I312V		-5.12	3.38	-6.13	32.3±3.5	0.80	5409
I312L		-6.21	1.81	-6.75	11.3±0.9	1.62	8039
I312Q					NB		
Y314A		-6.88	-2.72	-6.07	35.7±2.0	0.99	2999
K321A		-6.50	-4.38	-5.20	140.2±19.4	1.00	10270
A323Y					NB		
A323F					NB		
A323V					NB		
A323M					NB		
A323S		-8.60	-11.2	-5.30	122.2±8.0	1.00	4426
N325A					NB		
H328A		-7.41	-4.76	-5.99	41.0±5.3	1.00	15850
W359A		-6.20	0.112	-6.23	27.2±0.9	0.96	1054
Y314L/W359L					NB		
Y314M/W359S		-6.44	-3.28	-5.43	88.4±6.5	1.00	4568
Y314L/H328G/W359 Δ					NB		
Y314M/K321Q/W359S					NB		
(Gal8 _{N-CRD}) ^d					NB		

^a Protein WT: human Galectin8(228-359)

^b Peptides WT: human NDP52(368-381) ARQNPGLAYGNPYS

Peptides L374A: ARQNPGAAYGNPYS

Peptides Y376A: ARQNPGLAAGNPYS

Peptides N378A: ARQNPGLAYGAPNYS

Peptides Y380A: ARQNPGLAYGNPAS

Peptides Y380F: ARQNPGLAYGNPFS

^c NB : No binding detectable

^d Gal8_{N-CRD}: human Galectin8(1-160)

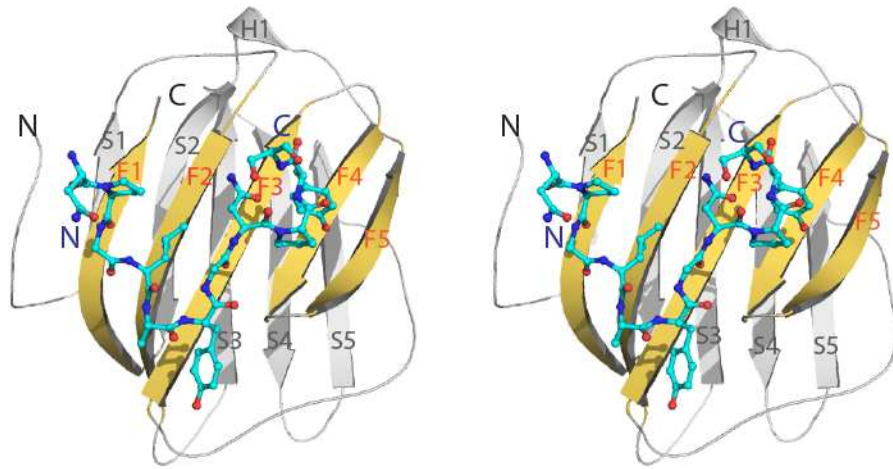


Figure S1. Stereo view of the hGal8_{C-CRD}-NDP52₃₇₁₋₃₈₁ complex. The β -sandwich structure of Galectin-8, depicted in yellow and grey, interacts with the hook-like structure of NDP52, depicted in cyan and purple.

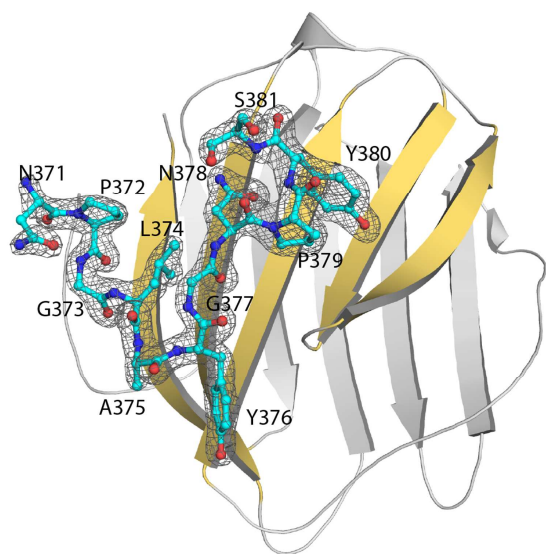


Figure S2. Electron density map for NDP52₃₇₁₋₃₈₁. The 2|Fo|-|Fc| electron density (grey mesh) for NDP52₃₇₁₋₃₈₁ contoured at 1.5 σ .

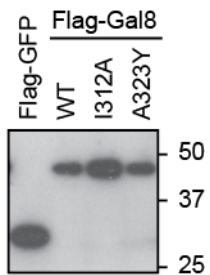


Figure S3. Expression of wild-type or mutated Flag-tagged galectin-8 in transfected HeLa cells. Western blot detecting the Flag tag in HeLa cells expressing Flag-tagged wild-type (WT) or mutant (I312A or A323Y) variants of galectin-8 (Gal8). To normalize for different expression levels, a ten-fold smaller volume of GFP-containing lysate was loaded.