Supporting information

Characterization of galectin fusion proteins with glycoprotein affinity columns and binding assays

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Table S1. Results of ASF and fetuin coupling to CNBr-activated Sepharose. Glycoprotein amounts were calculated by Bradford assay.

Glycoprotein:	Amount [mg]	Unbound [mg]	Coupled [mg]	Coupled [%]	Binding capacity [mg/mL]
ASF (Batch I)	112.5	26.5	86.0	76.8	15.7
ASF (Batch II)	112.5	34.0	78.5	2.0	14.3
Fetuin (Batch I)	112.5	26.1	86.4	76.4	17.2
Fetuin (Batch II)	112.5	27.1	85.4	76.0	15.5

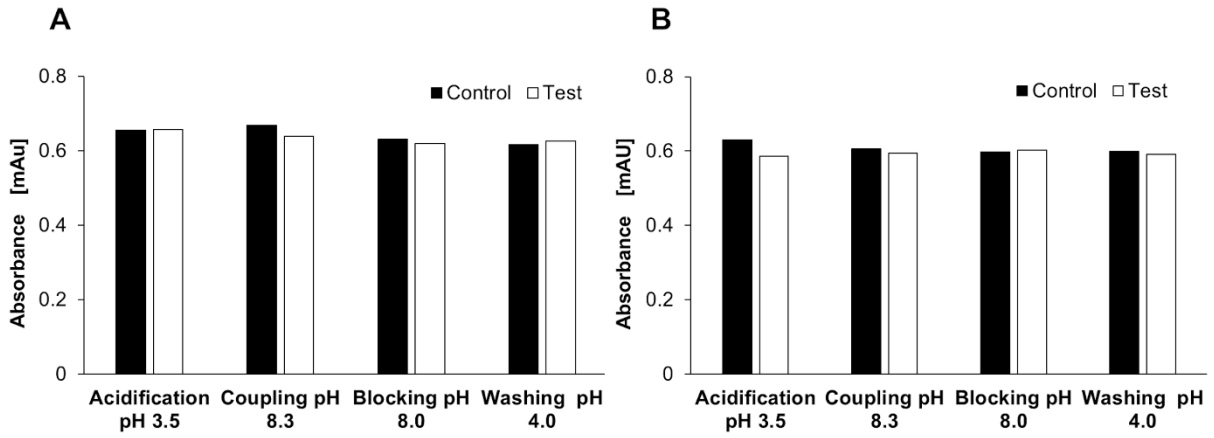

Figure S1. Enzyme-linked lectin assay (ELLA) of fetuin at different CNBr-activated sepharose coupling conditions.
A: Detection of the terminal of α2,3 sialic acids with *Maackia amurensis* lectin II (MAL II); **B:** Detection of terminal α2,6 sialic acids with *Sambucus nigra* elderberry bark lectin (EBL). Test: Treated fetuin sample with acidification solution pH 3.5, coupling buffer pH 8.0, blocking buffer pH 8.0, or washing buffer pH 4.0; Control: Untreated fetuin sample.

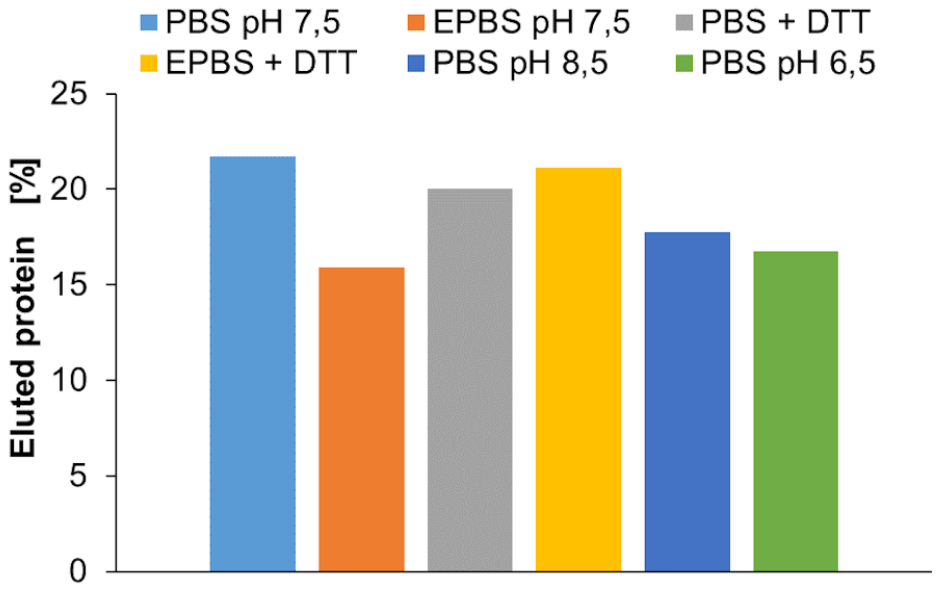

Figure S2. Comparison of buffers for binding HSYGal-3 on ASF-Sepharose.
3 mg of HSYGal-3 from one single expression batch was applied to the column in different buffers and eluted with lactose in a gradient mode (0-0.3 M lactose in PBS pH 7.5 within 15 min). The highest amount of eluted protein was observed for HSYGal-3 in PBS pH 7.5.

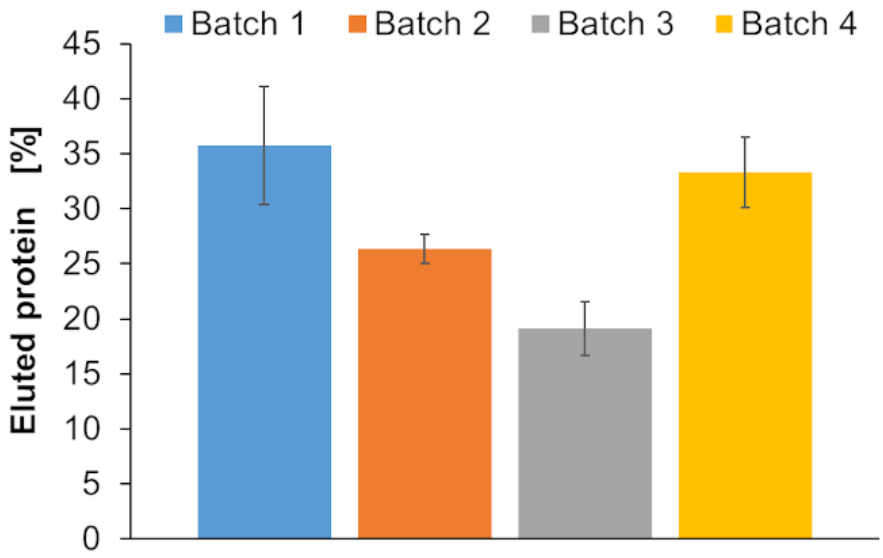

Figure S3. Dependency of expression batch on HSYGal-3 yield in ASF affinity chromatography.
4 mg of HSYGal-3 IMAC eluate of four different expression batches were applied to the ASF-Sepharose and eluted with lactose in a gradient mode (0-0.3 M lactose in PBS pH 7.5 within 15 min).

Table S2. Lactose concentrations for elution of galectins in glycoprotein affinity chromatography. Lactose concentrations were determined by gradient elution as the mean signal of two data points for ASF-Sepharose. For Fetuin-Sepharose lactose concentration was calculated once.

	Lactose concentration [mM]	
	ASF-Sepharose	**Fetuin-Sepharose**
HGal-1C2S	30 ± 5	20
HSDsRedMGal-1C2S	22 ± 2	27
HGal-3	36 ± 8	25
HSYGal-3	25 ± 4	22
HGal-8N	20 ± 0	32
HseGFPGal-8N	16 ± 1	30
HGal-8C	-	-
HSeGFPGal-8C	20 ± 0	23

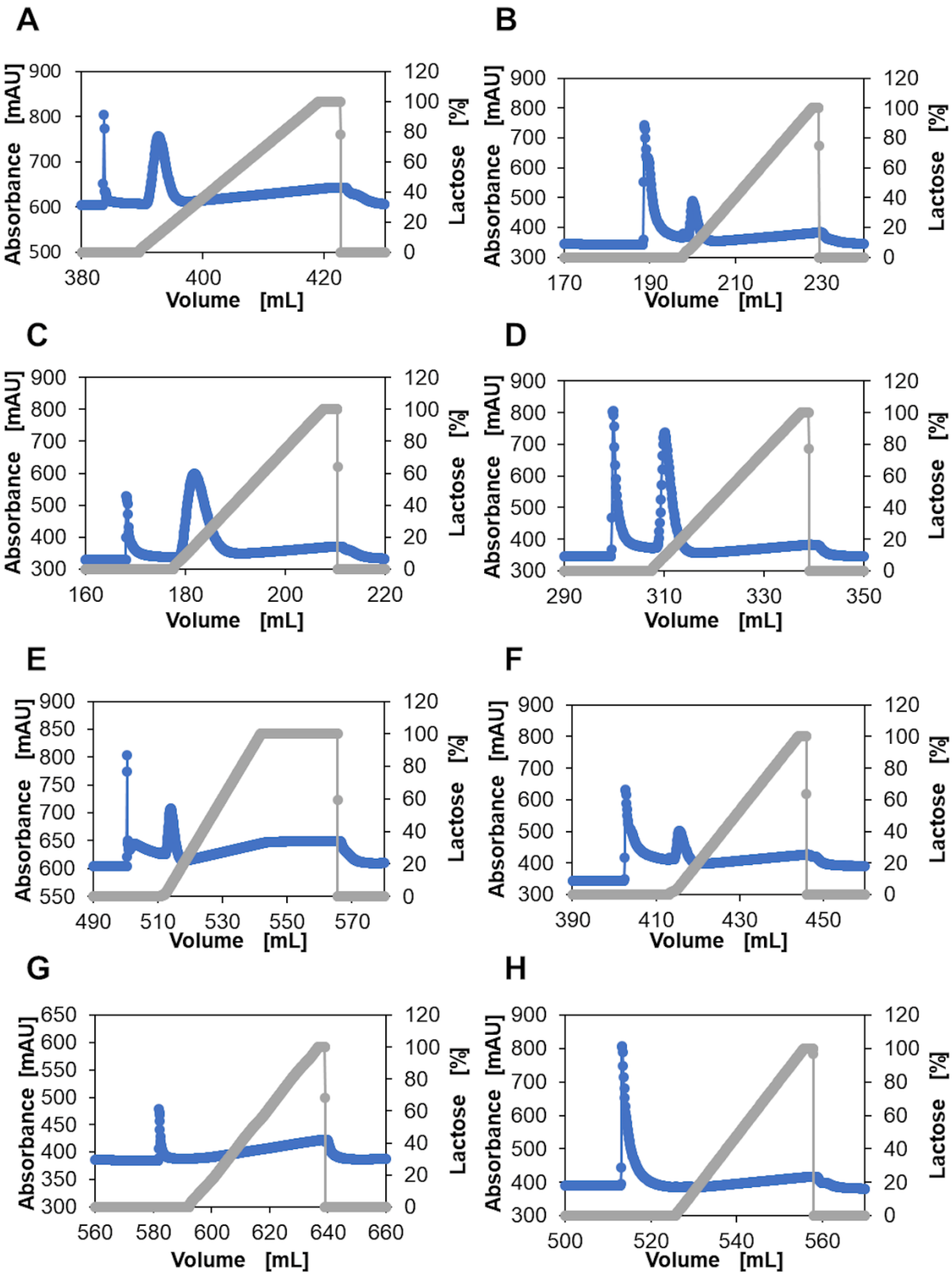

Figure S4. Elution profiles of galectin purification on ASF-Sepharose.
0.2 µmol galectin in PBS pH 7.5 was applied and eluted with lactose in a gradient mode (0-0.3 M lactose in PBS pH 7.5 within 15 min; 0%=0 mM lactose, 100%=0.3 M lactose). Blue line: Absorbance at 280 nm; Grey line: Concentration of lactose [%]. **A:** HGal-1C2S; **B:** HSDsRedMGal-1C2S; **C:** HGal-3; **D:** HSYGal-3; **E:** HGal-8N; **F:** HSeGFPGal-8N; **G:** HGal-8C; **H:** HSeGFPGal-8C.

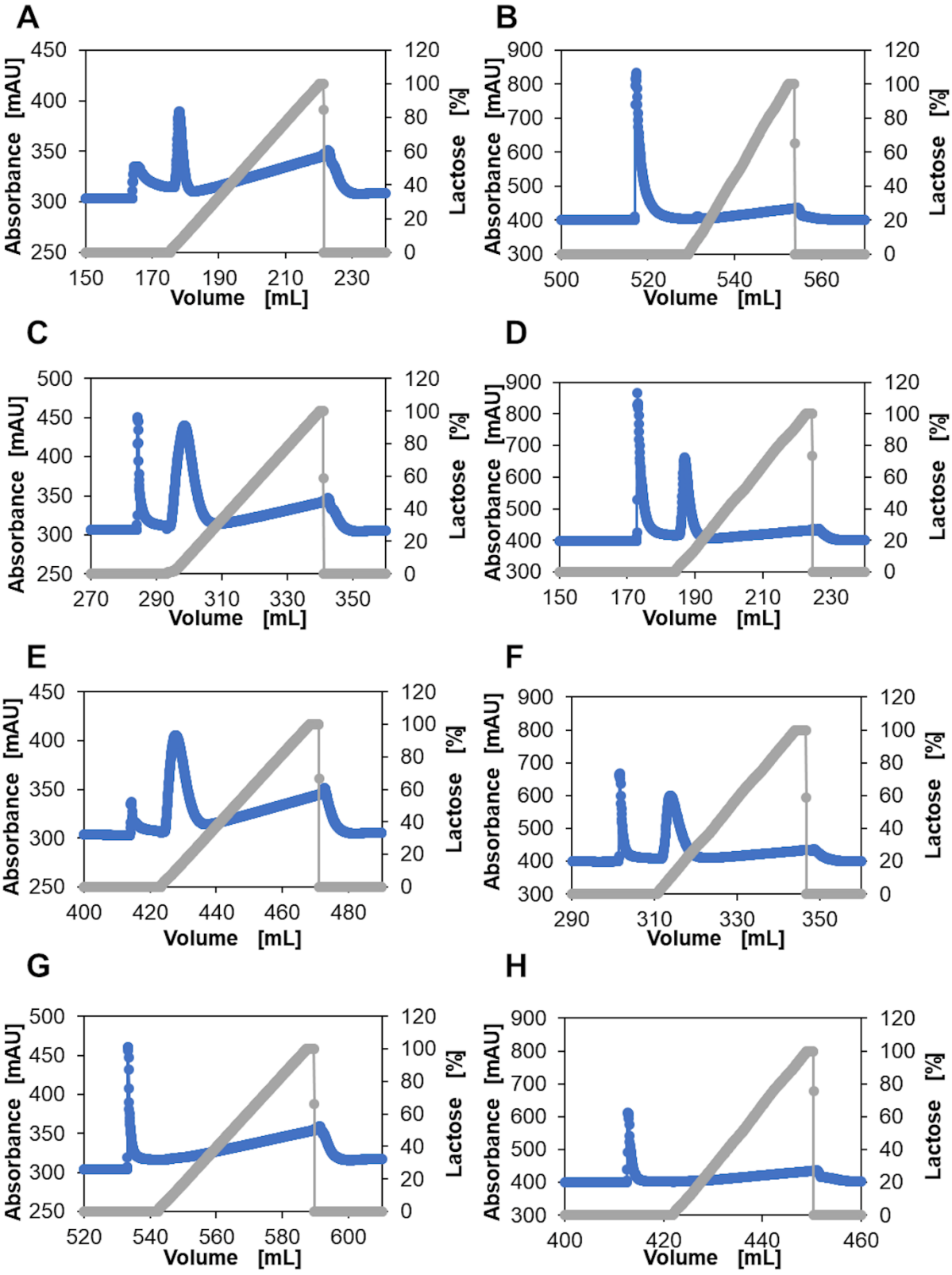

Figure S5. Elution profiles of galectin purification on Fetuin-Sepharose.
0.2 µmol galectin in PBS pH 7.5 was applied and eluted with lactose in a gradient mode (0-0.3 M lactose within 15 min; 0%=0 mM lactose, 100%=0.3 M lactose). Blue line: Absorbance at 280 nm; Grey line: Concentration of lactose [%]. **A:** HGal-1C2S; **B:** HSDsRedMGal-1C2S; **C:** HGal-3; **D:** HSYGal-3; **E:** HGal-8N; **F:** HSeGFPGal-8N; **G:** HGal-8C; **H:** HSeGFPGal-8C.

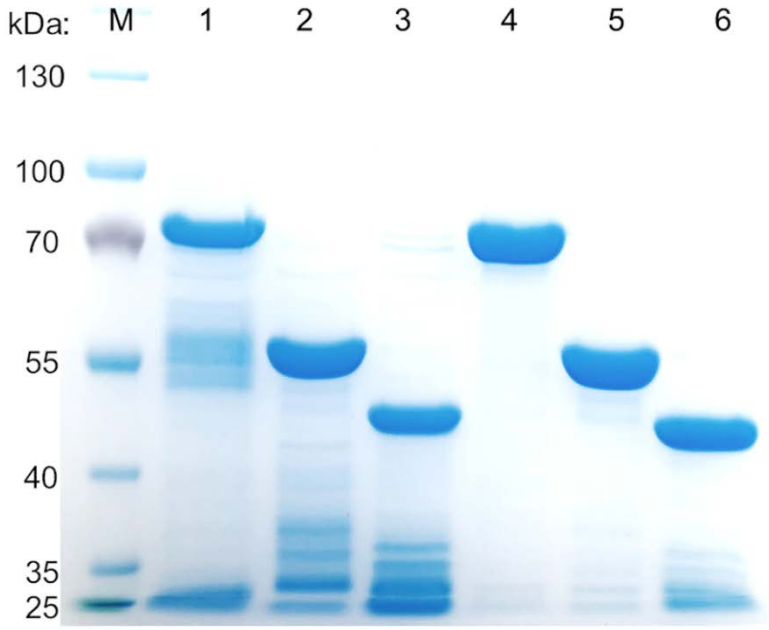

Figure S6. SDS-PAGE of Gal-3 fusion proteins after IMAC and ASF affinity chromatography.
M: Protein-Standard (10-180 kDa); **1:** HSYGal-3 IMAC eluate (74.2 kDa); **2:** HYGal-3 IMAC eluate (56 kDa); **3:** HSGal-3 IMAC eluate (46.9 kDa); **4:** HSYGal-3 ASF affinity resin eluate (74.2 kDa); **5:** HYGal-3 ASF affinity resin eluate (56 kDa); **6:** HSGal-3 ASF affinity resin eluate (46.9 kDa).

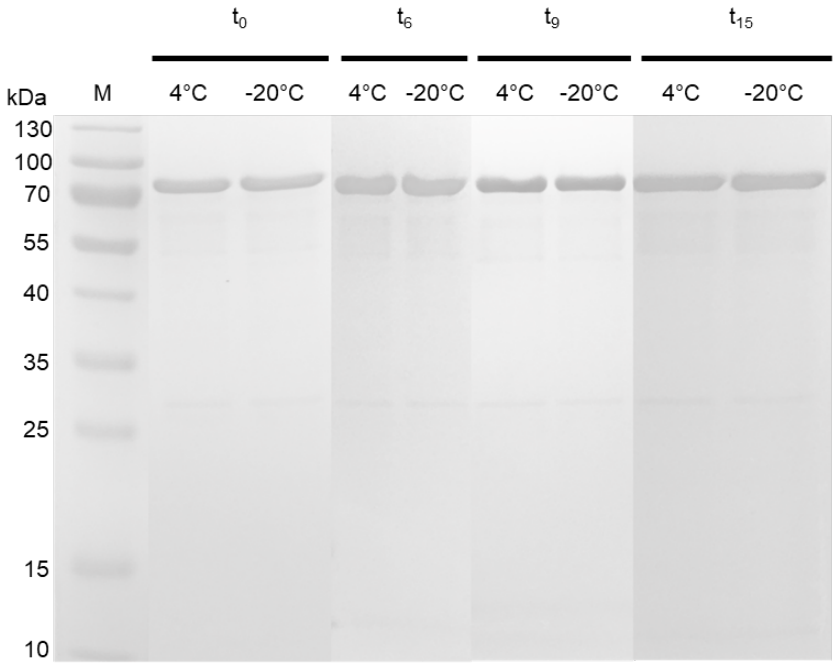

Figure S7. Analysis HSYGal-3 storage stability.
HSYGal-3 was purified with IMAC and ASF-Sepharose and stored at 4°C and -20°C in EPBS pH 7.5. Samples were taken on different days after purification (t_0, t_6, t_9, t_{15}) and in each case 5 µg protein was analyzed using discontinuous SDS-PAGE.

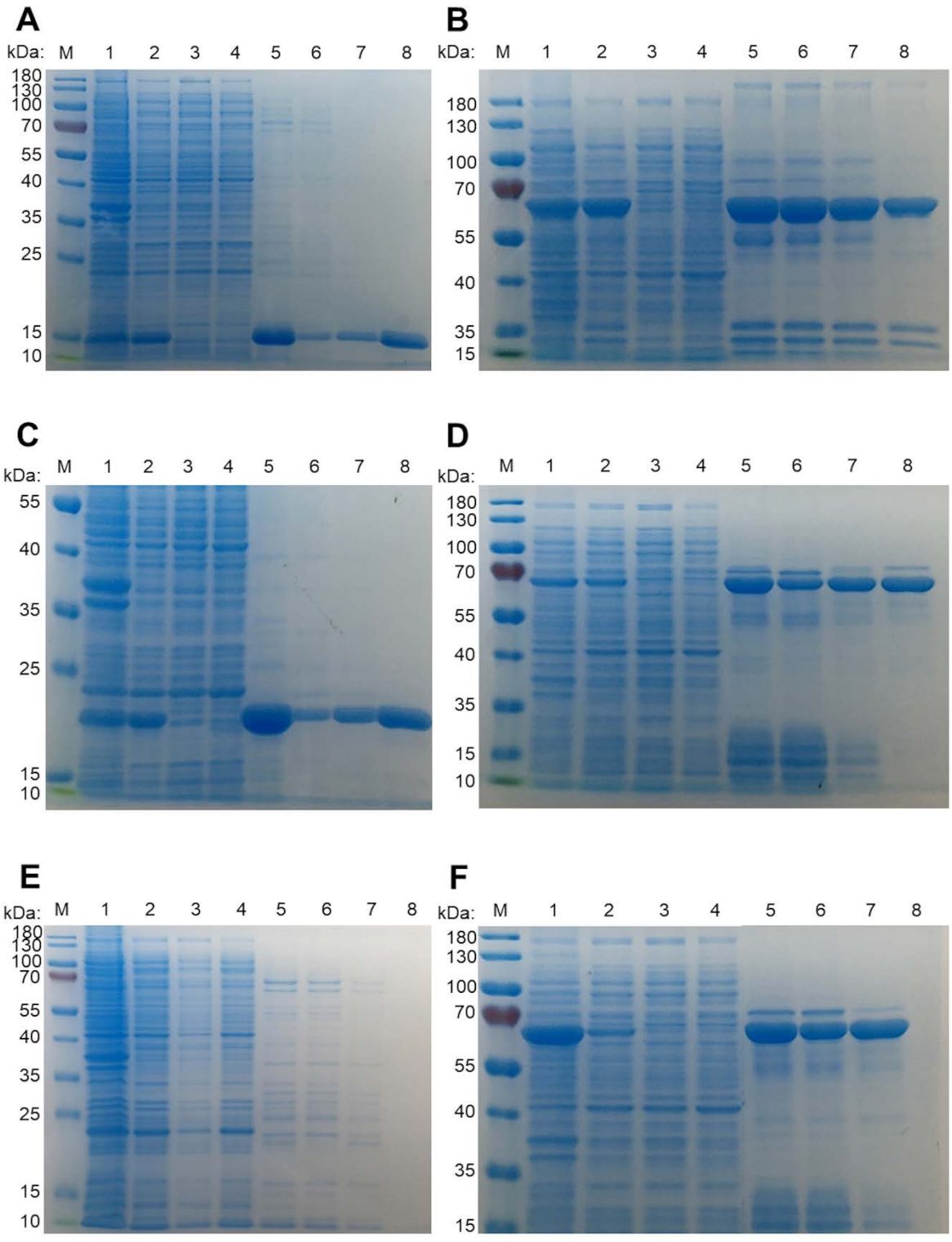

Figure S8. SDS-PAGE analysis of IMAC and ASF glycoprotein resin purified galectins**.**
A: HGal-1C2S (16 kDa); **B:** HSDsRedMGal-1C2S (64 kDa); **C:** HGal-8N (20.4 kDa); **D:** HSeGFPGal-8N (66.4 kDa), **E:** HGal-8C (17.8 kDa); **F:** HSeGFPGal-8C (63.9 kDa); M. Protein Standard (10-180 kDa); 1: Pellet; 2: Crude extract: 3: IMAC Flowthrough; 4: IMAC wash step; 5: IMAC eluate; 6: ASF affinity resin flowthrough; 7: ASF affinity resin wash; 8: ASF affinity resin eluate.

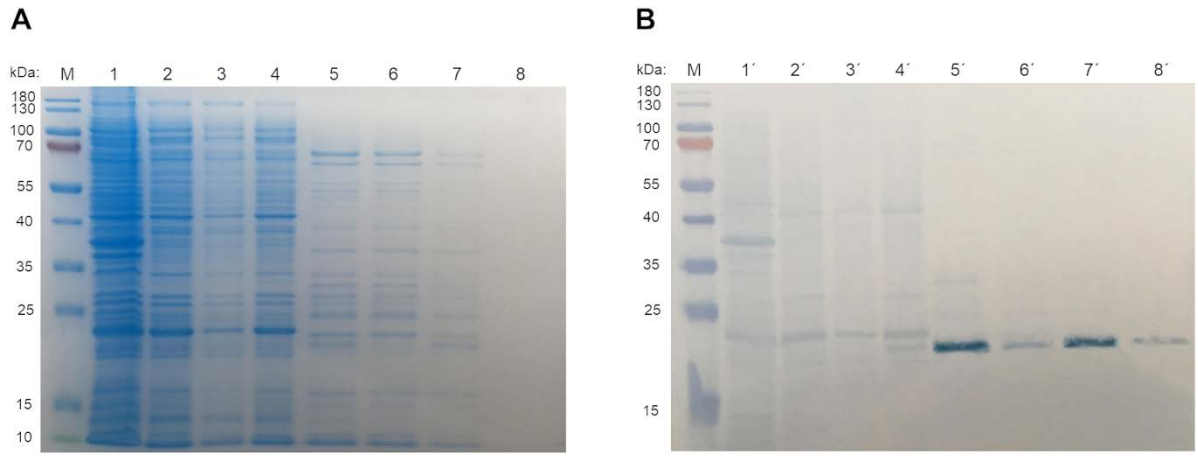

Figure S9. SDS-PAGE (**A**) and Western blot (**B**) of IMAC and ASF-affinity purification of HGal-8C. **A:** SDS-PAGE with Coomassie staining: M. Protein Standard (10-180 kDa); 1: Pellet; 2: Crude extract: 3: IMAC Flowthrough; 4: IMAC wash step; 5: IMAC eluate; 6: ASF affinity resin flowthrough; 7: ASF affinity resin wash; 8: ASF affinity resin eluate. **B:** Western blot analysis with anti-His_6-antibody: M. Protein Standard (10-180 kDa); 1´: Pellet; 2´: Crude extract: 3´: IMAC Flowthrough; 4´: IMAC wash step; 5´: IMAC eluate; 6´: ASF affinity resin flowthrough; 7´: ASF affinity resin wash; 8´: ASF affinity resin eluate; M_W (HGal-8C) =17.8 kDa.

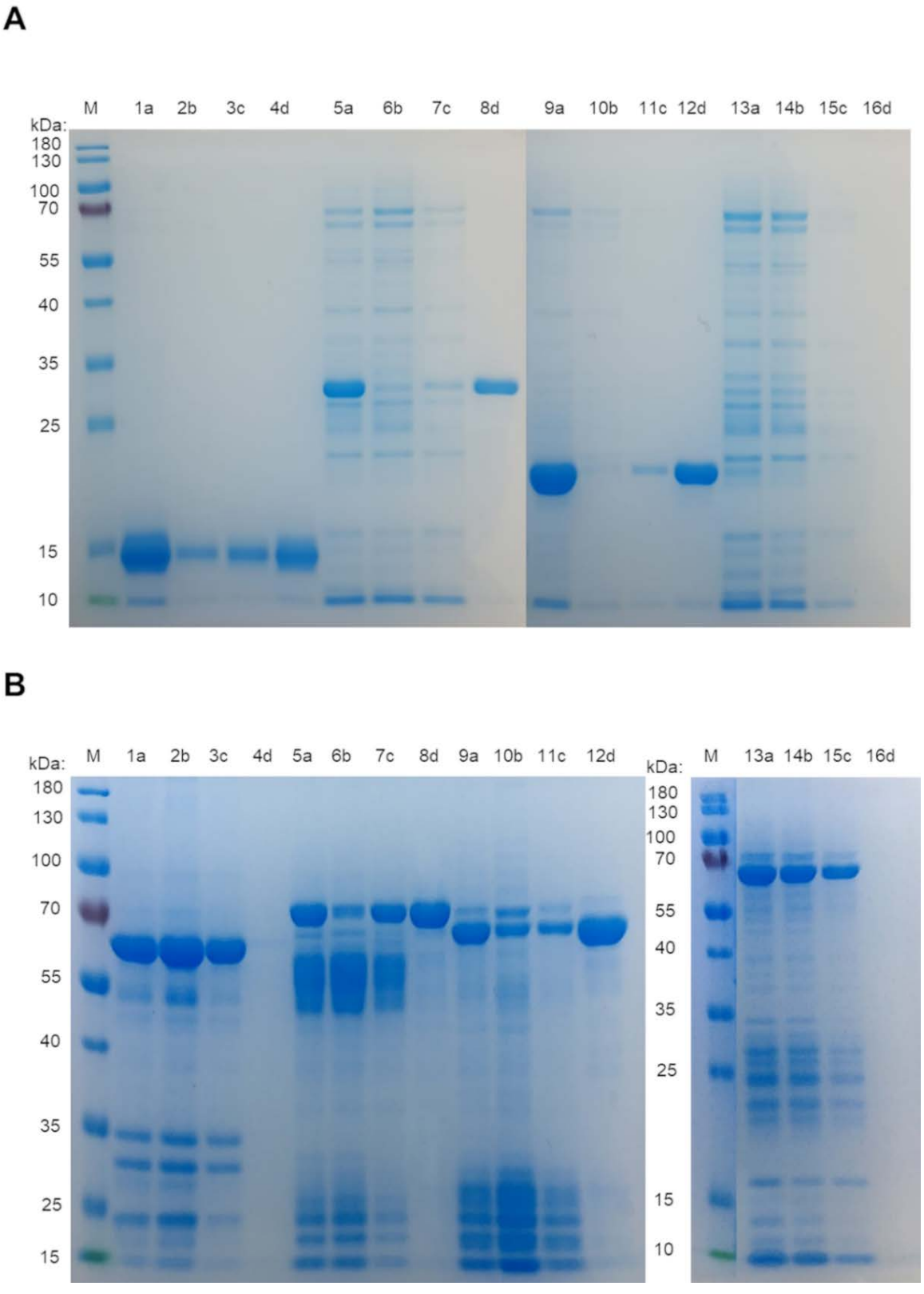

Figure S10. SDS-PAGE analysis of IMAC and fetuin affinity purified galectins.
A: His_6-tagged galectins (1-4:HGal-1C2S (16 kDa), 5-8 HGal-3 (28 kDa), 9-12: HGal-8N (20.4 kDa), 13-16 HGal-8C (17.8 kDa)); **B:** Galectin fusion proteins (1-4: HSDsRedMGal-1C2S (64 kDa), 5-8 HSYGal-3 (74 kDa), 9-12: HSeGFPGal-8N (66.4 kDa), 13-16 HSeGFPGal-8C (63.9 kDa)); for each galectin from left to right: a: IMAC eluate, b: fetuin affinity resin flowthrough, c: fetuin affinity resin wash, d: fetuin affinity resin eluate; M: Protein standard (10-180 kDa).

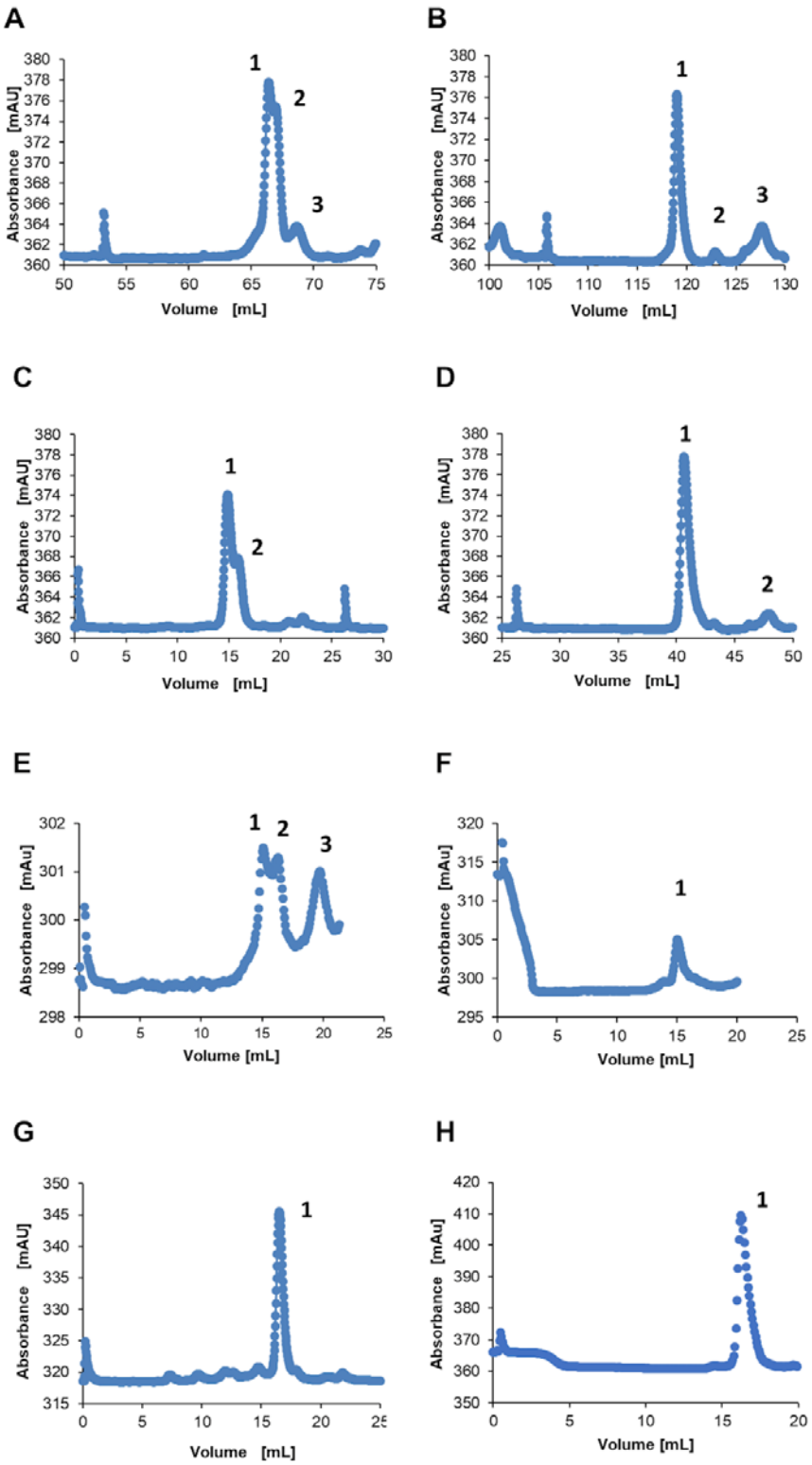

Figure S11. SEC profiles of Gal-3 proteins.
A: HSYGal-3 IMAC **B:** HSYGal-3 IMAC + ASF resin; **C:** HYGal-3 IMAC ; **D:** HYGal-3 IMAC + ASF resin resin; **E:** HSGal-3 IMAC; **F:** HSGal-3 IMAC + ASF resin resin; **G:** HGal-3 IMAC; **H:** HGal-3 IMAC + ASF resin resin. Column: Superdex 200 Increase 10/300 GL; Flow: 0.75 mL*min^{-1}; Buffer: PBS pH 7.5 Injection volume: 200 µL; Sample amount: 0.0024 µmol; M_W (theoretic for monomers): HSYGal-3: 74.2 kDa, HYGal-3: 56 kDa, HSGal-3: 46.9 kDa, HGal-3: 28 kDa.

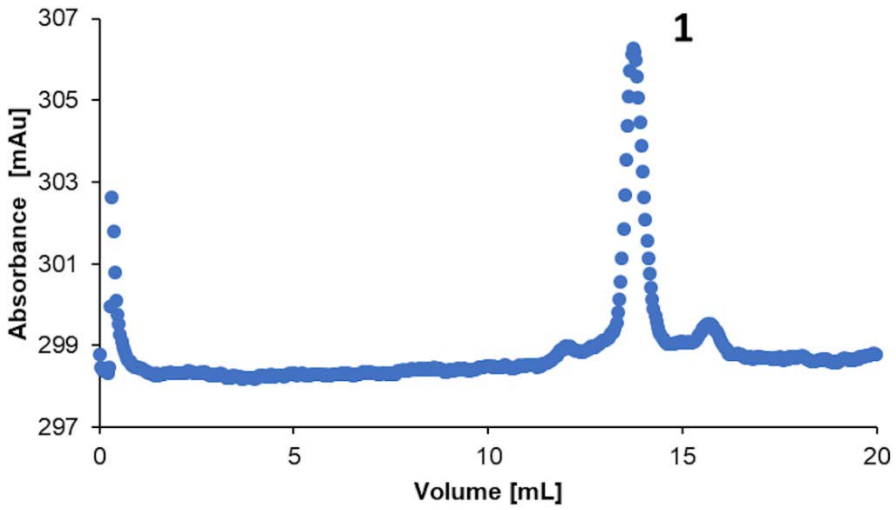

Figure S12. SEC profile of HSY eluate after IMAC.
Column: Superdex 200 Increase 10/300 GL; Flow: 0.75 mL*min^{-1}; Buffer: PBS pH 7.5 Injection volume: 200 µL; Sample amount: 0.0024 µmol; Peak 1: Elution volume: 13.73 mL, $MW_{(calc.)}$: 88.9 kDa; M_W (HSY-Monomer): 48.1 kDa.

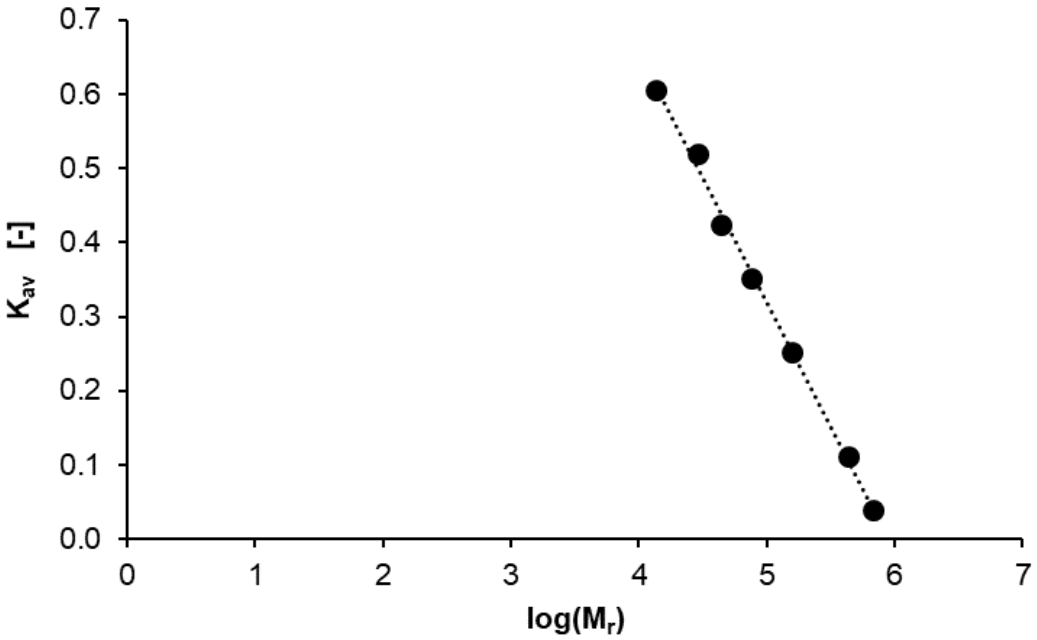

Figure S13. Linear regression of protein standards in SEC.
K_{av} is plotted over the logarithm of the molecular weight (M_r). Molecular weights of detected samples in SEC were calculated by equation y=-0.335x + 1.936 (R^2=0.997). Protein standards: Thyroglobulin (669 kDa), Ferritin (440 kDa), Aldolase (158 kDa), Conalbumin (75 kDa), Ovalbumin (44 kDa), Carbonic anhydrase (29 kDa), Ribonuclease A (13.7 kDa).

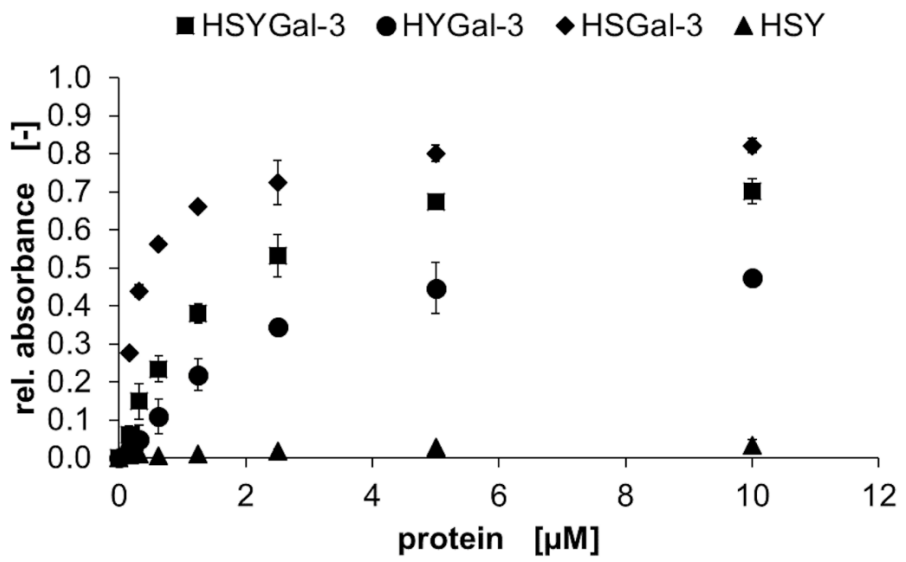

Figure S14. Binding of Gal-3 fusion proteins after IMAC.
The binding of IMAC-purified proteins HSYGal-3 (■), HSGal-3 (◆), HYGal-3 (●), and HSY (▲) (control) to ASF is shown. Bound galectins were detected using a peroxidase-conjugated anti-His_6-antibody and conversion of TMB as the mean signal of three data points. Errors indicate standard deviations.

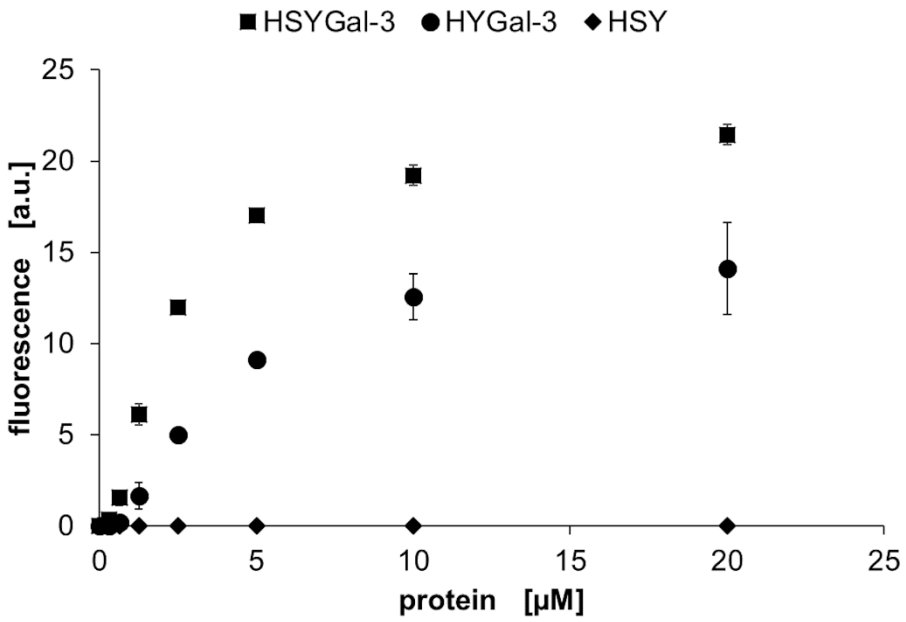

Figure S15. Binding of Gal-3 fusion proteins with fluorescence detection.
Binding of IMAC + ASF-Sepharose purified HSYGal-3 (■), HYGal-3 (●), and HSY (control, only IMAC) (◆) to ASF in a solid-phase binding assay. Fluorescence intensity was measured using excitation filter λ=485/20 and emission filter λ=528/20 as the mean signal of three data points. Error bars indicate standard deviations.

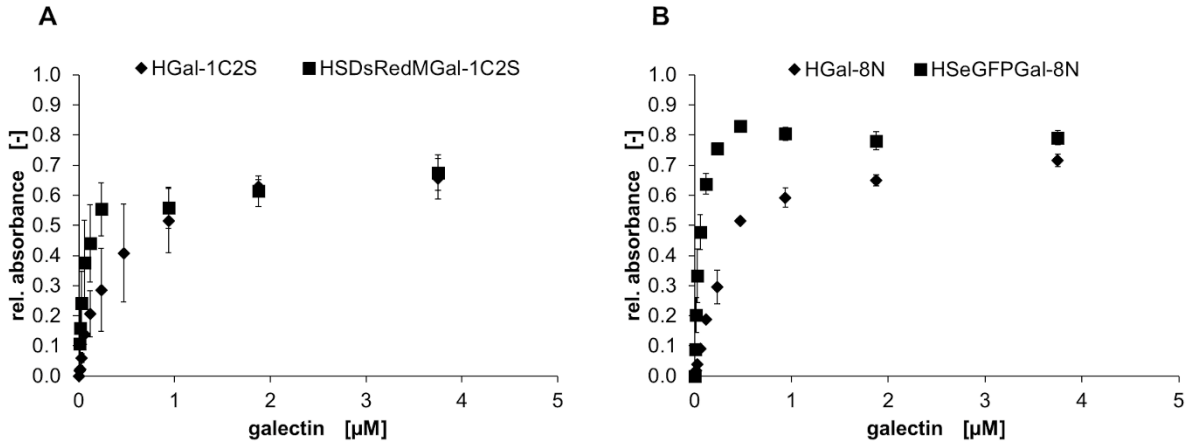

Figure S16. Binding of His_6-tagged and fusion proteins of Gal-1C2S and Gal-8N.
The binding of His_6-tagged proteins (◆) and fusion proteins (■) to ASF is shown for Gal-1C2S (**A**) and Gal-8N (**B**) in a solid-phase binding assay. Galectin binding was detected using a peroxidase-conjugated anti-His_6-antibody and conversion of TMB as the mean signal of three data points. Errors indicate standard deviations.

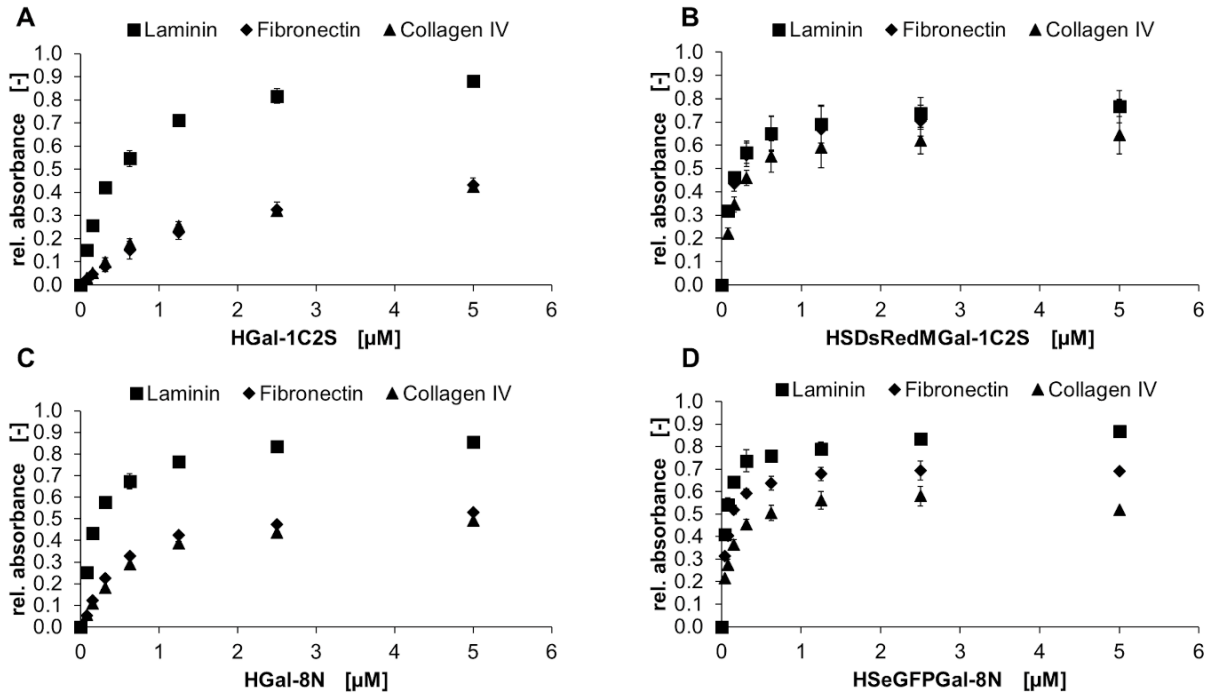

Figure S17. Binding of His_6-tagged and galectin fusion proteins on ECM glycoproteins.
The binding of galectins to laminin (■), fibronectin (◆), and collagen IV (▲) in a solid-phase assay is shown. **A:** HGal-1C2S; **B:** HSDsRedMGal-1C2S; **C:** HGal-8N; **D:** HSeGFPGal-8N. Galectin binding was detected using a peroxidase-conjugated anti-His_6-antibody and conversion of TMB as the mean signal of three data points. Errors indicate standard deviations.

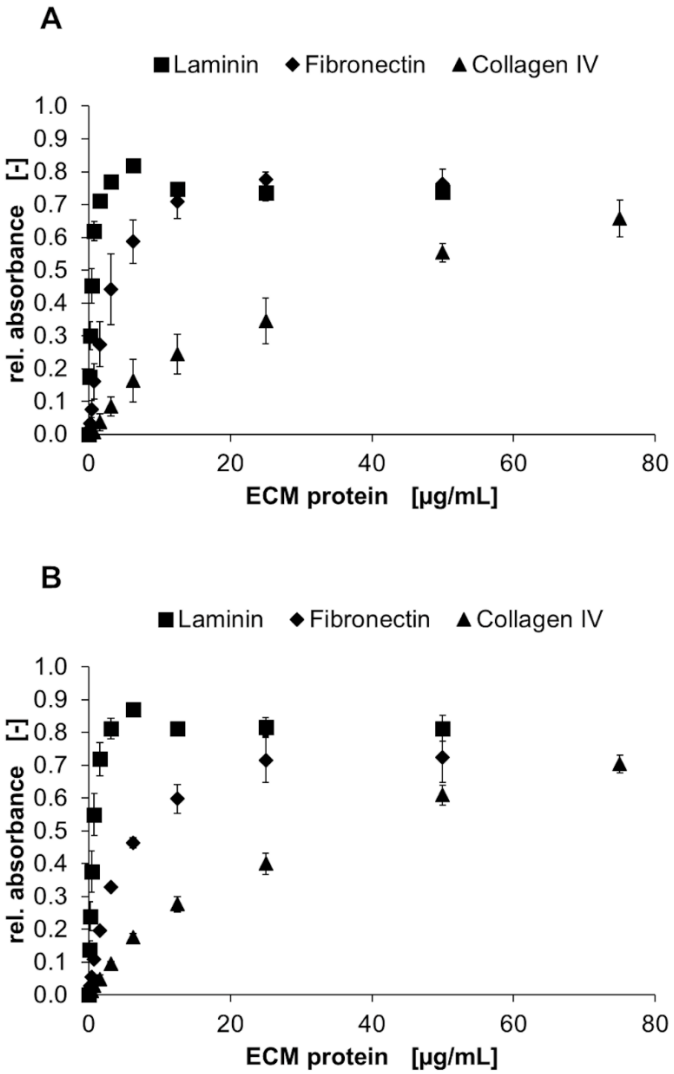

Figure S18. Binding of ECM glycoproteins to ASF-bound HGal-3 and HSYGal-3.
Binding curves of HGal-3 (**A**) and HSYGal-3 (**B**) for crosslinking laminin (■), fibronectin (◆), and collagen IV (▲) are shown. ECM glycoprotein binding was detected by primary anti-ECM protein-antibodies and corresponding secondary peroxidase-conjugated antibodies. Conversion of TMB was measured using three data points. Error bars indicate standard deviations.

Table S3. Calculated apparent K_D values of laminin, fibronectin, and collagen IV binding to ASF-bound HGal-3 and HSYGal-3 in a solid-phase binding assay. Values were calculated by non-linear regression assuming one-site saturation.

	Apparent K_D [µM]		
	Laminin	**Fibronectin**	**Collagen IV**
HGal-3	0.27 ± 0.03	5.1 ± 0.1	41.4 ± 8.5
HSYGal-3	0.45 ± 0.04	10.0 ± 1.1	34.7 ± 3.1

Table S4. *E.coli* strains used for recombinant expression of galectin constructs.

***E.coli* strain**	**Galectin construct**
BL21 (DE3)	pET28a::HYGal-3
Rosetta (DE3) pLysS	pET28a::HDsRedMGal-1C2S pETDuet-1:: HGal-1C2S pETDuet-1:: HGal-3 pETDuet-1:: HGal-8N pETDuet-1:: HGal-8C pET17b::HSDsRedMGal-1C2S pET17b::HSYGal-3 pET17b::HSeGFPGal-8N pET17b::HSeGFPGal-8C pET17b::HSGal-3 pET17b::HSY
Rosetta 2 (DE3) pLysS	pET28a::HeGFPGal-8N pET17b::HSGal-8N pET17b::HSGal-1C2S pET17b::HSDsRedM pET17b::HSeGFP