

Figure S1

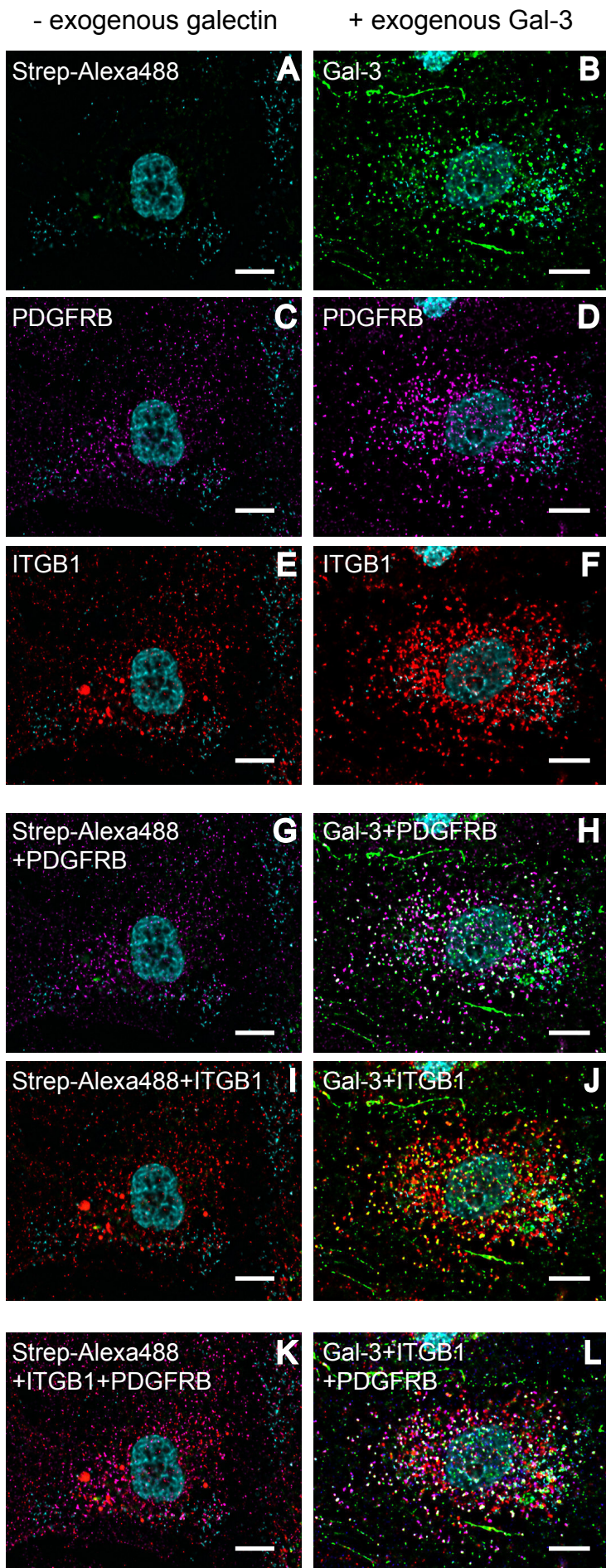


figure S2

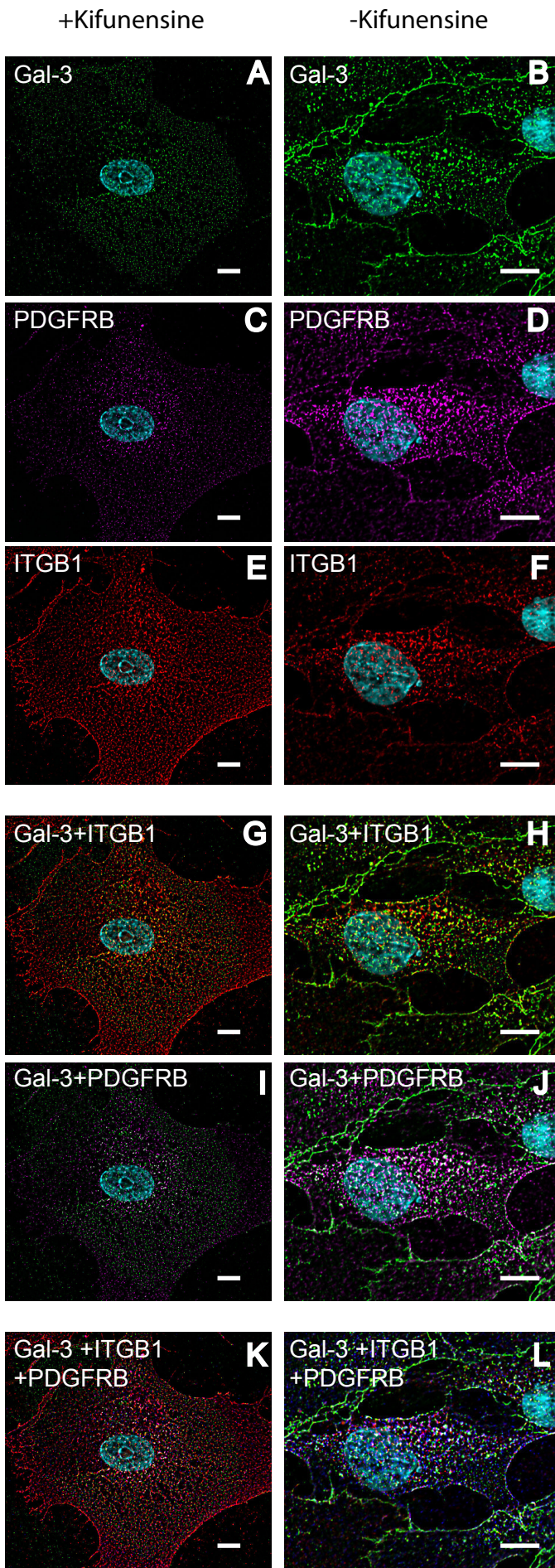


figure S3

Figure Legends

Supplemental figure S1: Gal-1 and Gal-3 are still active after coupling on Sepharose beads. Coomassie staining for asialofetuin detection after incubation of galectin beads with asialofetuin for 1h at RT in absence and presence of 0.1M Lactose and separation of the eluates by SDS-PAGE. Gal: galectin. PD: pull-down.

Supplemental figure S2: Exogenous Galectin-3 induces cross-linking of PDGFRB and ITGB1 on the cell surface of human mesenchymal RPE cells. Immunocytochemical staining of human RPE cells, pretreated before fixation with or without biotinylated Gal-3 for 30 min. Galectin-binding was visualized by Streptavidin-Alexa488 (green), PDGFRB by Alexa647 (magenta) and ITGB1 by Alexa568 (red). Gal-3 (B), PDGFRB (D) and ITGB1 (F) staining shows a clear punctuate staining pattern. Double staining of RPE cells with Gal-3 and PDGFRB as well as with Gal-3 and ITGB1 indicated a clear overlay of both staining patterns, visible by white (H) and yellow (J) spots. For visualization of the clustering of galectin, PDGFRB and ITGB1, PDGFRB staining was changed in silico to blue and the overlay is seen in white (L). Whereas exogenous galectin led to clear co-localization of PDGFRB and ITGB1 on human RPE cells, no crosslinking could be observed without addition of exogenous galectin (G, I and K). Representative images of one single cell from 4 independent experiments are shown. Scale bar: 10 μ m.

Supplemental figure S3: Complex-type N-glycosylation of Galectin-interactors is necessary for Gal-3 induced cross-linking of PDGFRB and ITGB1 on the cell surface of mesenchymal RPE cells. Immunocytochemical staining of human RPE cells, pretreated with or without 10 μ M Kifunensine. Before fixation cells were pretreated with biotinylatedGal-3 for 30 min. Galectin-binding was visualized with Streptavidin-Alexa488 (green) (A, B), PDGFRB by Alexa647 (magenta) (C, D) and ITGB1 by Alexa568 (red) (E, F). Overlay of PDGFRB and

galectin staining patterns is visible in white (J), overlay of ITGB1 and galectin staining patterns in yellow (H). For visualization of the clustering of galectin, PDGFRB and ITGB1, PDGFRB staining was changed in silico to blue and the overlay is seen in white (L). Whereas exogenous galectin led to clear co-localization of PDGFRB and ITGB1 on human RPE cells not treated with Kifunensine, no crosslinking could be seen on cell surface of human RPE cells treated with Kifunensine (G, I, K). Representative images from 2 independent experiments are shown. Scale bar: 10 μ m.

Supplemental table S1: Galectin interactors play a role in adhesion and binding processes and are mainly localized in membranes.

Gal-1 interacting proteins		Gal-3 interacting proteins		
	GO-Term	P-value	GO-Term	P-value
cellular components	integral component of plasma membrane	6.79E-05	intrinsic component of membrane	1.70E-18
	intrinsic component of plasma membrane	8.02E-05	integral component of membrane	9.26E-18
	integrin alpha3-beta1 complex	9.91E-05	intrinsic component of plasma membrane	7.08E-16
	receptor complex	2.51E-04	integral component of plasma membrane	6.09E-14
	integral component of membrane protein complex involved in cell adhesion	2.99E-04	plasma membrane part	8.46E-13
	integrin complex	3.01E-04	cell surface	3.11E-12
	external side of plasma membrane	3.01E-04	membrane part	4.27E-10
		3.17E-04	external side of plasma membrane protein complex involved in cell adhesion	6.21E-10
	intrinsic component of membrane invadopodium	3.60E-04	integrin complex	1.12E-08
		5.88E-04		1.12E-08
molecular functions	fibronectin binding	2.47E-06	receptor activity	5.21E-09
	protease binding	1.19E-04	molecular transducer activity	1.44E-07
	integrin binding	1.82E-04	cell adhesion molecule binding	2.99E-07
	extracellular matrix binding	5.80E-04	collagen binding	3.54E-07
	receptor binding	6.86E-04	integrin binding	1.01E-06
			transmembrane signaling receptor activity	1.81E-05
	collagen binding	9.54E-04	glycosaminoglycan binding	3.39E-05
	receptor activity	1.04E-03	receptor binding	4.83E-05
	cell adhesion molecule binding	1.35E-03	growth factor binding	5.95E-05
	macromolecular complex binding	2.10E-03	protein binding involved in cell-matrix adhesion	8.31E-05
lipoprotein particle receptor binding	2.77E-03			
signal transduction pathway associations	matrix metalloproteinase	1.75E-04	integrin	1.19E-11
			low density lipoprotein receptor related protein	2.74E-06
	integrin	1.94E-04	matrix metalloproteinase	1.38E-05
	lysosomal sphingomyelin phosphodiesterase 1, acid lysosomal	4.82E-04		
		2.20E-03	focal adhesion kinase 1	5.88E-05
	endocytic	9.83E-03	lysosomal interleukin 18 (interferon gamma inducing factor)	8.62E-05
			platelet derived growth factor	1.31E-03
			vascular endothelial growth factor receptor	1.42E-03
			1.65E-03	

		endocytic	3.04E-03
		lymphotoxin alpha (tnf superfamily)	3.77E-03
biological processes	integrin-mediated signaling pathway	6.31E-05	cell adhesion
	cellular defense response	1.04E-04	biological adhesion
	negative regulation of Rho protein signal transduction	1.04E-04	extracellular structure organization
	cell-substrate adhesion	1.91E-04	extracellular matrix organization
	negative regulation of Ras protein signal transduction	3.08E-04	cell-substrate adhesion
	negative regulation of small GTPase mediated signal transduction	3.08E-04	cell migration
	formation of primary germ layer	5.91E-04	cell motility
	regulation of body fluid levels	8.44E-04	localization of cell
	negative regulation of intracellular signal transduction	8.48E-04	cell-matrix adhesion
	cell adhesion	9.57E-04	locomotion

GeneRanker analysis of Gal-1 and Gal-3 interacting proteins based on the GO terms “cellular component”, “molecular function”, “signal transduction pathway associations” and “biological processes”. Top ten results with the according p-values are listed. GO: gene ontology.