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Supplementary Materials for

The $\alpha_v\beta_1$ integrin plays a critical in vivo role in tissue fibrosis

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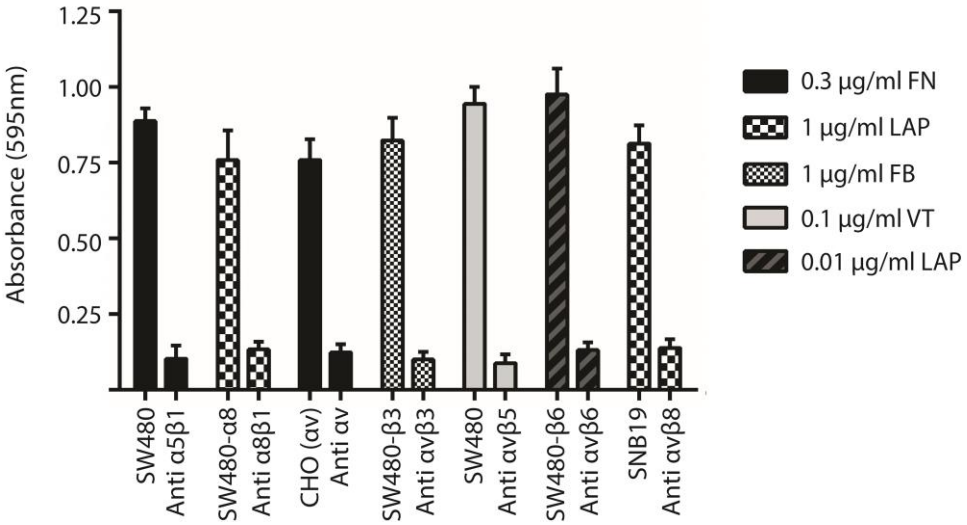


Fig. S1. Validation of cell adhesion conditions used to determine potency and specificity of $\alpha_v\beta_1$ inhibitors. Cell lines and adhesion assays were optimized to isolate the effects of individual integrin. Under the conditions used for α5β1 (SW480 cells adhering to wells coated with 0.3 µg/ml fibronectin (FN)), α8β1 (SW480 cells transfected with human α8 adhering to wells coated with 1 µg/ml TGFβ1 LAP), αvβ3 (SW480 cells transfected with human β3 adhering to wells coated with 1 µg/ml fibrinogen (FB), αvβ5 (SW480 cells adhering to 0.1 µg/ml vitronectin (VT), αvβ6 (SW480 cells transfected with human β6 adhering to wells coated with 0.01 µg/ml TGFβ1 LAP, or αvβ8 (SNB19 cells adhering to wells coated with 1 µg/ml TGFβ1 LAP) adhesion could be inhibited by prior incubation of cells with monoclonal antibodies blocking the relevant integrin. Since there are no specific blocking monoclonal antibodies to αvβ1, αvβ1 was evaluated by adhesion of α5 integrin subunit deficient CHO cells (engineered to express the αvβ1 integrin) adhering to 0.3 µg/ml fibronectin. Since there are no complex specific antibodies to αvβ1 and no antibodies that block hamster β1 integrins, specificity of adhesion was demonstrated by inhibition by monoclonal antibody against αv. Data represent mean ± s.e.m., n=3.

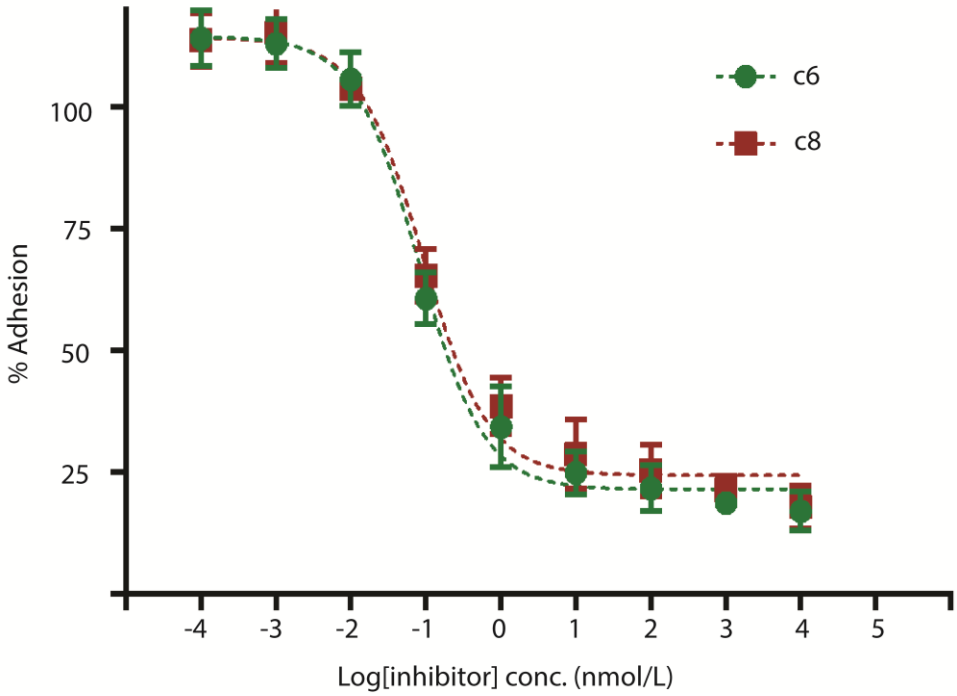


Fig. S2. C8 and C6 adhesion assays. Adhesion of α5 integrin subunit deficient CHO cells (engineered to express the αvβ1 integrin) to 0.3μg/ml fibronectin was performed in the presence of a range of concentrations of c8 and c6. Data represent mean ± s.e.m., n=3.

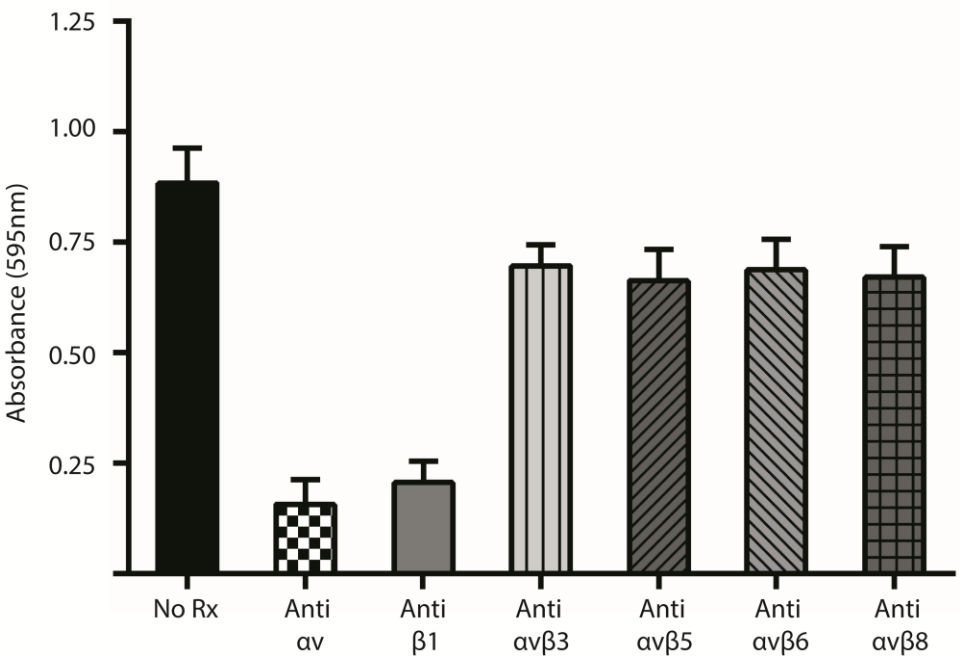


Fig. S3. Adhesion of WI38 cells in low concentration of TGFβ1 LAP. Adhesion of WI38 cells to wells coated with 0.3 μg/ml TGFβ1 LAP was performed after no pretreatment (No Rx) or pre-incubation with blocking monoclonal antibodies to αv or β1 or to αvβ3, αvβ5, αvβ6 or αvβ8 integrin. Data represent mean ± s.e.m., n=3.

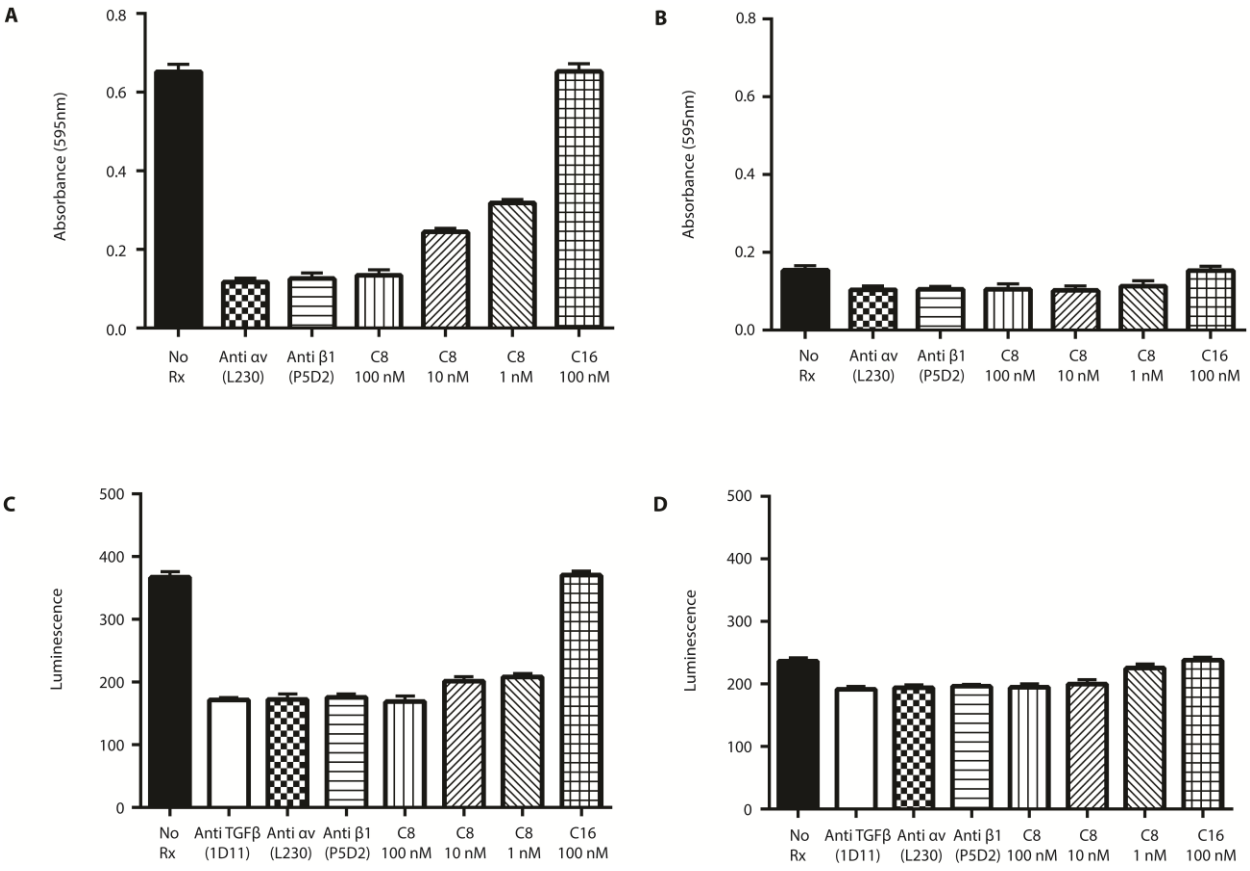
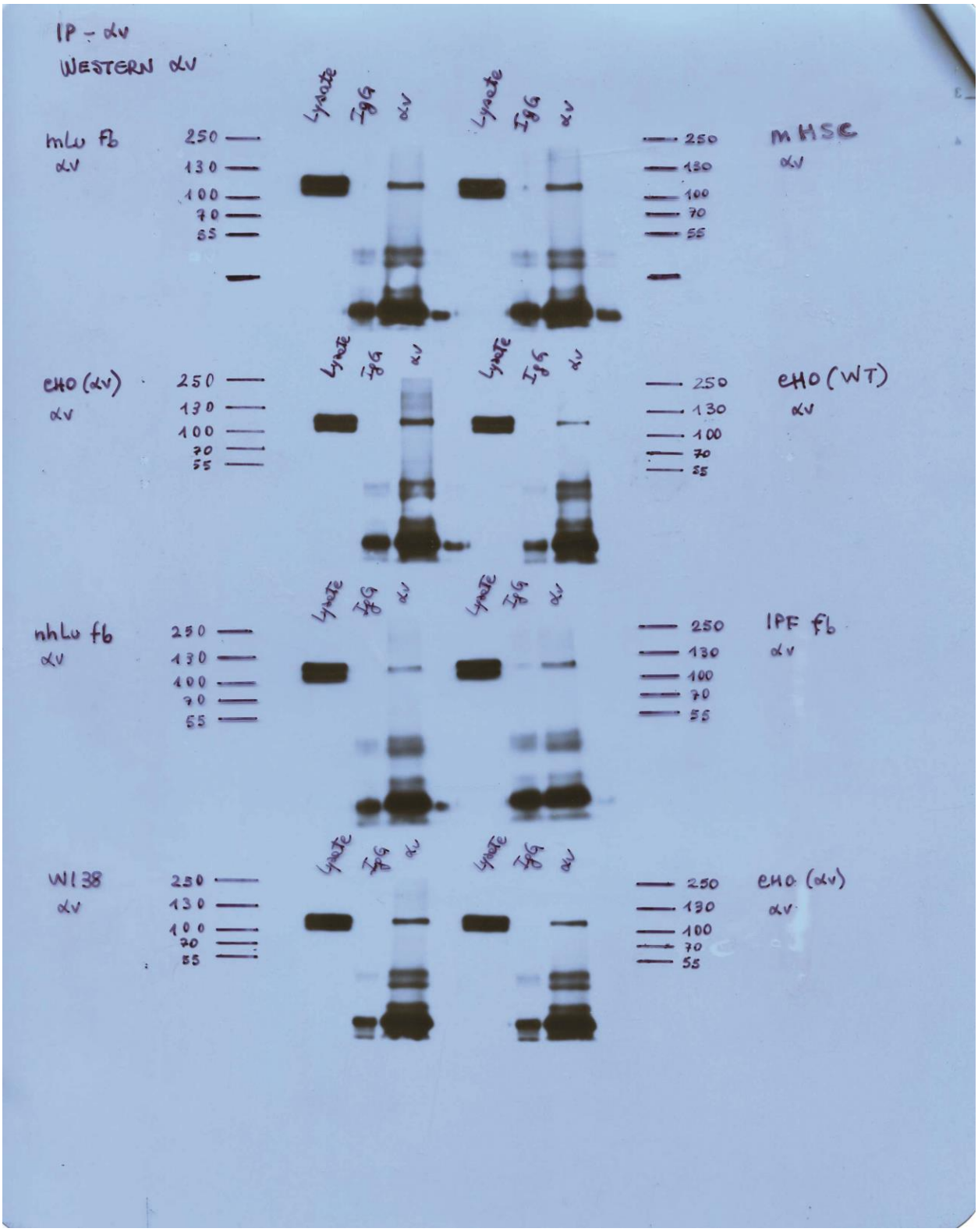


Fig. S4. Adhesion and TGFβ activation assays in A549 and 293 cells. Cell adhesion to 0.3 μg/ml TGFβ1LAP of A549 cells (A) or 293 cells (B) in the presence or absence of a range of concentrations of c8 or control small molecule c16 or blocking monoclonal antibodies to αv, or β1. TGFβ activity, assessed by co-culture bioassay with mink lung epithelial cells expressing luciferase under the control of a portion of the PAI1 promoter, for A549 cells (C) or 293 cells (D) in the presence or absence of a range of concentrations of c8 or c16 or blocking monoclonal antibodies to TGFβ, αv, or β1. Data represent mean ± s.e.m., n=3.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WESTERN αv
mLu fb
αv
Lysate
IgG
αv
250
130
100
70
55
mHSC
αv
CHO (αv)
αv
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αv
nhLu fb
αv
IPF fb
αv
WI38
αv
CHO (αv)
αv

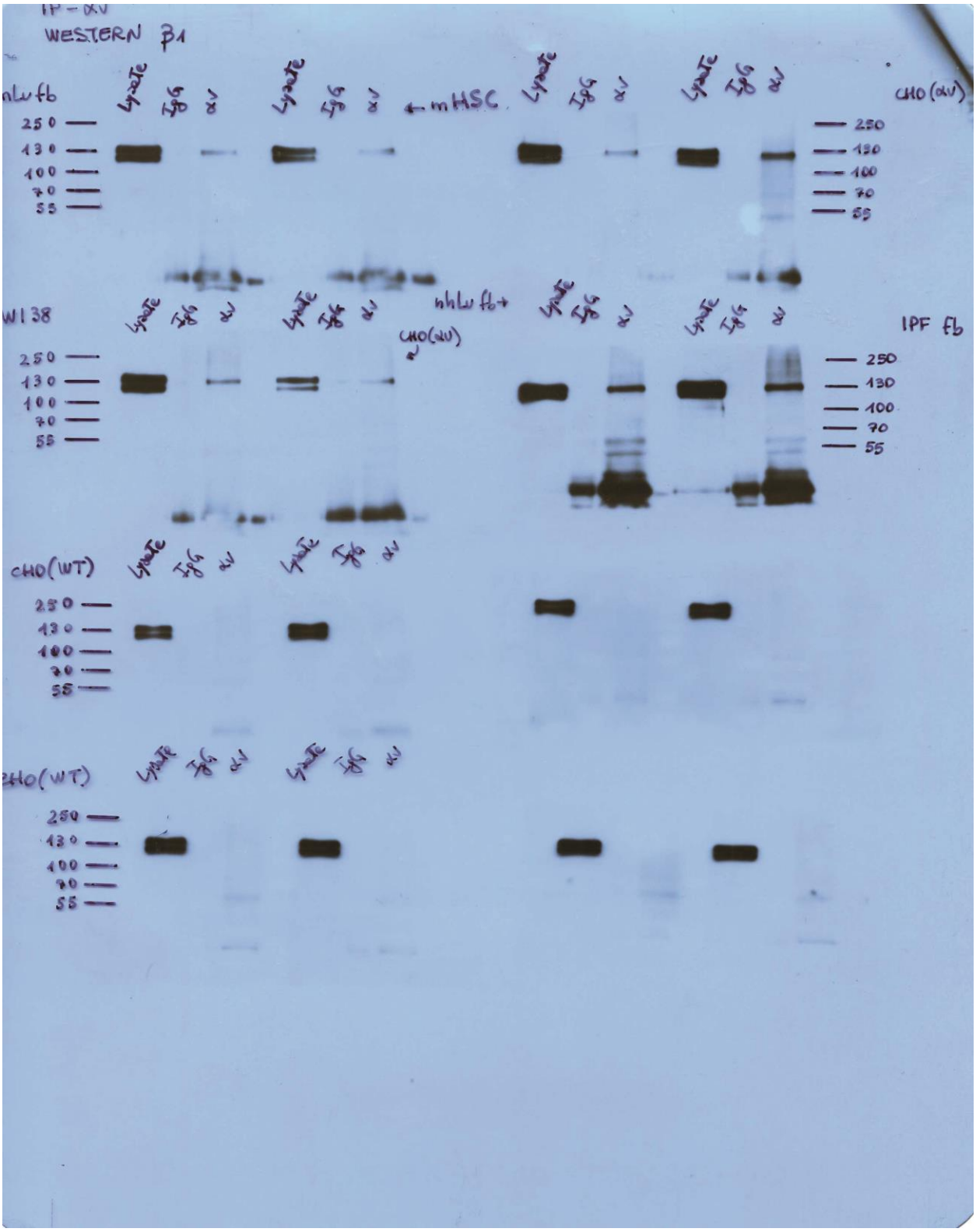
IP – αV
WESTERN β1
Lysate
IgG
αV
← mHSC
CHO(αV)
250
130
100
70
55
W138
IPF fb
CHO(WT)

Fig. S5. Original Western blot images of Fig. 2A.

α5β1 log [c8] (nM)	Absorbance (595nm)			BSA subtract			% Adhesion		
4	1.28	0.98	0.99	1.06	0.76	0.77	119	85	87
3	1.12	1.18	0.99	0.90	0.96	0.77	102	108	86
2	1.13	1.08	1.14	0.91	0.86	0.92	103	97	104
1	1.30	1.12	0.98	1.08	0.89	0.76	122	101	86
0	1.11	1.05	1.20	0.89	0.83	0.98	100	93	110
-1	1.17	1.12	1.11	0.94	0.90	0.89	106	101	100
-2	1.16	0.99	1.14	0.94	0.77	0.92	106	87	103
-3	1.11	1.09	1.10	0.89	0.87	0.88	100	98	100
-4	1.20	1.01	1.10	0.98	0.79	0.88	111	89	99
No Antibody	1.03	1.16	1.14						
Anti α5β1	0.39	0.33	0.25						
BSA	0.21	0.19	0.26						
No Ab (avg.)-BSA(avg.)			0.89						

α8β1 log [c8] (nM)	Absorbance (595nm)			BSA subtract			% Adhesion		
4	1.08	0.80	0.99	0.86	0.58	0.77	113	76	102
3	1.05	1.02	0.88	0.83	0.80	0.65	109	105	86
2	1.08	0.92	0.92	0.86	0.70	0.69	113	92	91
1	1.05	1.07	0.84	0.83	0.84	0.62	110	111	82
0	1.02	0.92	0.94	0.80	0.70	0.72	105	93	95
-1	0.98	1.07	0.91	0.76	0.85	0.69	101	113	91
-2	1.01	0.99	0.92	0.79	0.77	0.70	104	102	92
-3	1.13	1.11	0.99	0.91	0.89	0.77	120	117	102
-4	1.20	1.01	1.00	0.98	0.79	0.78	129	104	102
No antibody	1.17	0.89	0.87						
Anti α8β1	0.31	0.39	0.36						
BSA	0.25	0.20	0.21						
No Ab (avg.)-BSA(avg.)			0.76						

αvβ1 log [c8] (nM)	Absorbance (595nm)			BSA subtract			% Adhesion		
4	0.35	0.42	0.45	0.07	0.14	0.17	10	20	25
3	0.41	0.47	0.41	0.13	0.19	0.13	18	27	19
2	0.39	0.51	0.48	0.10	0.22	0.20	15	32	29
1	0.39	0.56	0.49	0.11	0.28	0.21	16	41	30
0	0.50	0.51	0.63	0.22	0.23	0.35	32	33	50
-1	0.79	0.75	0.66	0.51	0.47	0.38	73	68	55
-2	0.99	1.03	1.00	0.71	0.75	0.72	101	107	103
-3	1.14	1.12	1.00	0.85	0.84	0.72	123	120	103
-4	1.10	1.00	1.12	0.82	0.72	0.84	118	103	121
No antibody	0.92	1.06	0.96						
Anti αv	0.59	0.58	0.60						
BSA	0.25	0.32	0.27						
No Ab (avg.)-BSA(avg.)			0.70						

αvβ3 log [c8] (nM)	Absorbance (595nm)			BSA subtract			% Adhesion		
4	0.97	0.89	0.99	0.76	0.68	0.78	94	83	96
3	0.98	0.90	1.08	0.77	0.69	0.87	95	84	106
2	0.96	0.99	1.05	0.75	0.78	0.84	92	96	103
1	0.96	1.13	0.99	0.75	0.92	0.79	92	112	96
0	0.96	0.98	1.11	0.76	0.78	0.90	93	95	110
-1	0.99	0.97	1.14	0.79	0.76	0.94	96	93	115
-2	0.98	1.11	0.99	0.77	0.90	0.78	95	110	96
-3	1.12	1.01	1.19	0.91	0.80	0.98	112	99	121
-4	1.02	1.19	1.19	0.82	0.99	0.98	100	121	120
No antibody	0.91	1.00	1.16						
Anti αvβ3	0.44	0.42	0.47						
BSA	0.26	0.16	0.21						
No Ab (avg.)-BSA(avg.)			0.82						

αvβ5 log [c8] (nM)	Absorbance (595nm)			BSA subtract			% Adhesion		
4	1.10	0.97	1.00	0.90	0.78	0.80	96	82	85
3	1.19	1.19	1.12	0.99	0.99	0.93	105	105	98
2	1.18	1.29	1.10	0.99	1.09	0.90	105	116	95
1	1.26	1.14	1.20	1.06	0.95	1.00	112	100	106
0	1.16	1.03	1.32	0.96	0.83	1.12	102	88	118
-1	1.01	1.21	1.15	0.81	1.02	0.96	86	108	101
-2	1.18	1.10	1.15	0.98	0.91	0.95	104	96	101
-3	1.20	1.30	1.02	1.00	1.10	0.82	106	117	87
-4	1.13	1.19	1.13	0.93	1.00	0.93	98	105	99
No antibody	1.21	1.03	1.18						
Anti αvβ5	0.28	0.34	0.24						
BSA	0.20	0.20	0.19						
No Ab (avg.)-BSA(avg.)			0.95						

αvβ6 log [c8] (nM)	Absorbance (595nm)			BSA subtract			% Adhesion		
4	1.26	1.41	1.20	1.05	1.21	1.00	108	124	103
3	1.23	1.42	1.10	1.02	1.22	0.90	105	125	92
2	1.26	1.42	1.17	1.05	1.22	0.97	108	125	100
1	1.18	1.36	1.22	0.98	1.15	1.02	100	119	105
0	1.29	1.36	1.03	1.08	1.16	0.83	111	119	85
-1	1.36	1.02	1.31	1.15	0.82	1.11	119	84	114
-2	1.02	1.37	1.27	0.81	1.16	1.06	83	120	109
-3	1.11	1.20	1.10	0.91	1.00	0.90	93	103	92
-4	1.17	1.20	1.20	0.97	1.00	0.99	100	103	102
No antibody	1.21	1.31	1.02						
Anti αvβ6	0.80	0.74	0.85						

BSA	0.22	0.19	0.20
No Ab (avg.)-BSA(avg.)			0.97

αvβ8	**Absorbance**			**BSA subtract**			**% Adhesion**		
log [c8] (nM)	**(595nm)**								
4	1.01	0.88	0.99	0.79	0.66	0.77	97	81	95
3	0.98	1.04	0.99	0.76	0.82	0.77	93	100	94
2	1.07	0.88	0.97	0.84	0.66	0.75	104	82	92
1	0.98	1.07	0.96	0.76	0.85	0.74	94	104	91
0	0.94	1.06	0.95	0.72	0.83	0.73	88	103	90
-1	1.15	1.13	0.87	0.93	0.91	0.65	115	112	79
-2	1.21	0.92	0.94	0.99	0.70	0.72	122	86	89
-3	1.20	1.01	1.11	0.98	0.79	0.89	120	97	109
-4	1.19	1.19	1.18	0.96	0.97	0.96	119	120	118

No antibody	1.09	0.91	1.10
Anti αvβ8	0.48	0.50	0.41
BSA	0.25	0.23	0.18
No Ab (avg.)-BSA(avg.)			0.81

Table S1. Source data for Fig. 1 (D and E).

LAP conc. (μg/mL)	CHO WT Absorbance 595 nm			CHO (αv) Absorbance 595 nm			WI-38 Absorbance 595 nm		
0.01	0.24	0.21	0.22	0.35	0.36	0.33	0.41	0.40	0.41
0.03	0.23	0.19	0.27	0.49	0.53	0.43	0.42	0.44	0.43
0.1	0.20	0.26	0.21	0.66	0.63	0.69	0.57	0.47	0.55
0.3	0.24	0.21	0.23	0.87	0.72	0.80	0.76	0.57	0.67
1	0.21	0.30	0.21	0.99	0.90	0.84	0.79	0.77	0.82
3	0.31	0.25	0.35	1.08	0.99	1.00	0.96	0.88	0.93
10	0.39	0.40	0.36	0.99	1.17	1.00	0.99	0.98	0.99

Table S2. Source data for Fig. 2B.

C8 conc. log [c8] (nM)	Absorbance 595nm				BSA subtract			% Adhesion		
4	0.35	0.42	0.35	0.37	0.08	0.16	0.09	10	18	10
3	0.41	0.39	0.41	0.40	0.14	0.13	0.15	17	15	17
2	0.51	0.47	0.41	0.46	0.25	0.20	0.15	28	23	17
1	0.59	0.51	0.68	0.59	0.33	0.25	0.41	38	29	48
0	0.75	0.71	0.63	0.70	0.49	0.45	0.37	56	52	42
-1	1.09	0.97	0.99	1.02	0.83	0.71	0.73	95	82	84
-2	1.19	1.03	1.20	1.14	0.92	0.77	0.94	106	88	107
-3	1.14	1.12	1.20	1.15	0.87	0.86	0.94	100	98	107

No Antibody	1.14	1.16	1.11
anti β1	0.46	0.53	0.46
anti αv	0.46	0.50	0.43
BSA	0.29	0.26	0.25
No Ab (avg) - BSA (avg)			0.87

Table S3. Source data for Fig. 2C.

WI38 log [c8] (nM)	Luminescence			avg.	% TGF beta activation			avg.
4	220	270	250		-19	-10	-13	
3	410	370	305		15	8	-4	
2	345	420	425		3	16	17	
1	405	445	410		14	21	15	
0	480	515	480		27	33	27	
-1	875	790	755		96	82	75	
-2	960	885	841		111	98	90	
No Antibody	950	885	850	895	110	98	92	100
Anti TGFβ (1D11)	350	305	325	327	4	-4	0	0

CHO av log [c8] (nM)	Luminescence			avg.	% TGF beta activation			avg.
4	363	253	273		-1	-20	-16	
3	383	343	283		2	-5	-15	
2	313	383	333		-10	2	-6	
1	373	363	423		1	-1	9	
0	423	533	633		9	28	45	
-1	783	973	903		70	102	90	
-2	993	893	990		106	89	105	
No Antibody	1013	953	913	960	109	99	92	100
Anti TGFβ (1D11)	383	363	363	370	2	-1	-1	0

mHSC log [c8] (nM)	Luminescence			avg.	% TGF beta activation			avg.
4	225	263	245		-17	-11	-14	
3	401	362	310		14	7	-2	
2	342	415	421		3	16	17	
1	401	431	415		14	19	16	
0	475	510	473		27	33	26	
-1	865	802	765		96	84	78	
-2	955	875	835		111	97	90	
No Antibody	955	895	820	890	111	101	88	100
Anti TGFβ (1D11)	310	336	325	324	-2	2	0	0

mLufb log [c8] (nM)	Luminescence			avg.	% TGF beta activation			avg.
4	312	336	290		-2	2	-5	
3	345	354	306		4	5	-2	
2	375	409	369		8	14	8	
1	457	382	487		22	10	26	
0	467	504	429		23	29	17	
-1	871	869	925		87	87	96	

-2	993	898	952		107	92	100	
No Antibody	988	870	991	950	106	87	107	100
Anti TGFβ (1D11)	305	340	320	322	-3	3	0	0

nhLufb	**Luminescence**			**avg.**	**% TGF beta activation**			**avg.**
log [c8] (nM)								
4	275	315	305		-9	-2	-4	
3	335	305	355		1	-4	5	
2	305	365	415		-4	6	15	
1	425	405	485		17	13	27	
0	455	465	515		22	24	32	
-1	805	913	885		82	101	96	
-2	865	875	975		93	94	111	
No Antibody	965	925	835	908	110	103	87	100
Anti TGFβ (1D11)	395	335	255	328	11	1	-13	0

IPFfb	**Luminescence**			**avg.**	**% TGF beta activation**			**avg.**
log [c8] (nM)								
4	218	298	258		-15	-2	-9	
3	408	358	308		15	7	-1	
2	338	408	426		4	15	18	
1	408	421	428		15	18	19	
0	481	528	468		27	35	25	
-1	808	758	951		80	72	102	
-2	958	905	965		104	95	105	
No Antibody	945	878	984	936	101	91	108	100
Anti TGFβ (1D11)	298	328	308	311	-2	3	-1	0

Table S4. Source data for Fig. 2D.

Sirius red	C16 Oil	C8 Oil	C16 CCl_4	C8 CCl_4
% Area	2	2	6	3
	1	1	5	5
	2	2	5	2
	1	1	9	3
	1	2	6	5
	1	1	6	4
	1	2	7	2
	1	2	6	3

Hydroxyproline	C16 Oil	C8 Oil	C16 CCl_4	C8 CCl_4
(µg/mL)	54	71	135	86
	64	59	147	66
	61	51	108	66
	36	59	165	85
	38	35	113	95
	49	41	122	79
	59	87	169	91
	58	44	130	104
	40	34	159	99
	45	32	109	115

Table S5. Source Data for Fig. 3C.

Sirius red	C16 Oil	C8 Oil	C16 CCl_4	C8 CCl_4
% Area	2	2	6	3
	1	1	5	5
	2	2	5	2
	1	1	9	3
	1	2	6	5
	1	1	6	4
	1	2	7	2
	1	2	6	3

Hydroxyproline	C16 Oil	C8 Oil	C16 CCl_4	C8 CCl_4
(µg/mL)	54	71	135	86
	64	59	147	66
	61	51	108	66
	36	59	165	85
	38	35	113	95
	49	41	122	79
	59	87	169	91
	58	44	130	104
	40	34	159	99
	45	32	109	115

Table S6. Source Data for Fig. 3F.