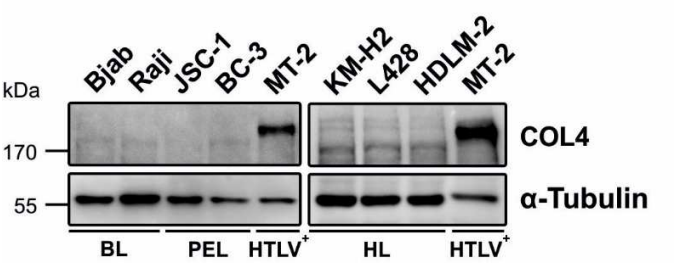
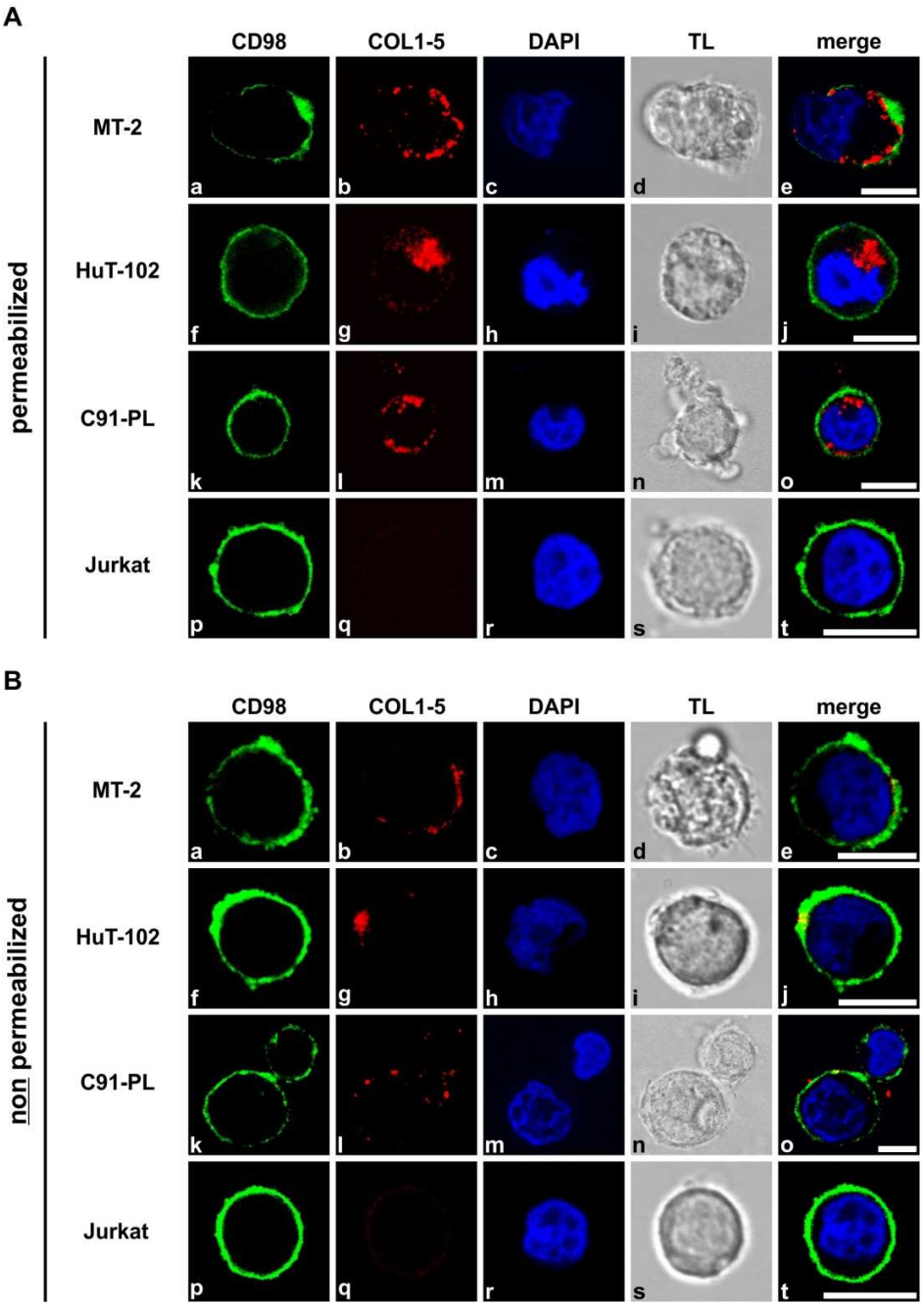
Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures

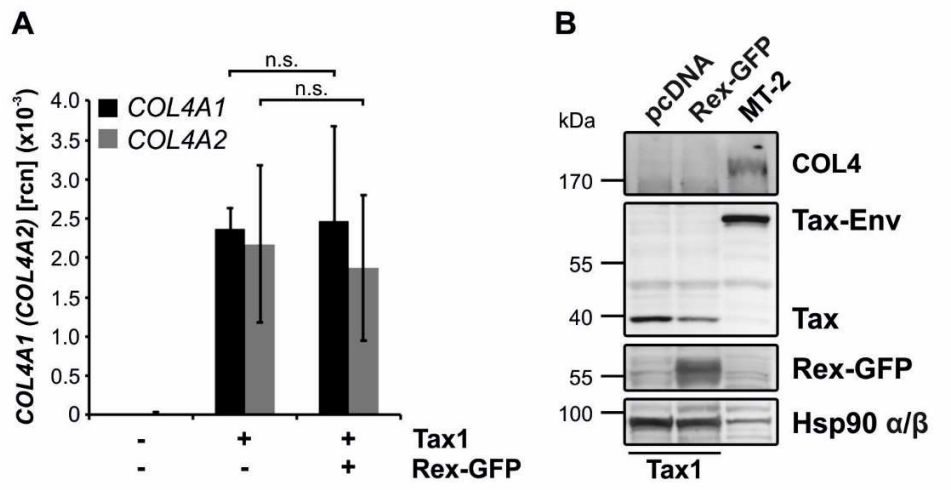


Supplementary Figure 1. COL4 protein is upregulated in HTLV-1 positive T-cells only. Immunoblotting shows COL4 protein expression in cell lines established from Burkitt lymphoma (BL; Bjab, Raji), Primary Effusion Lymphoma (PEL; JSC-1, BC-3) and Hodgkin Lymphoma (HL; KM-H2, L428, HDLM-2). Staining of COL4 protein in the HTLV-1 positive T-cell line MT-2 served as positive control, staining of α-Tubulin as loading control.

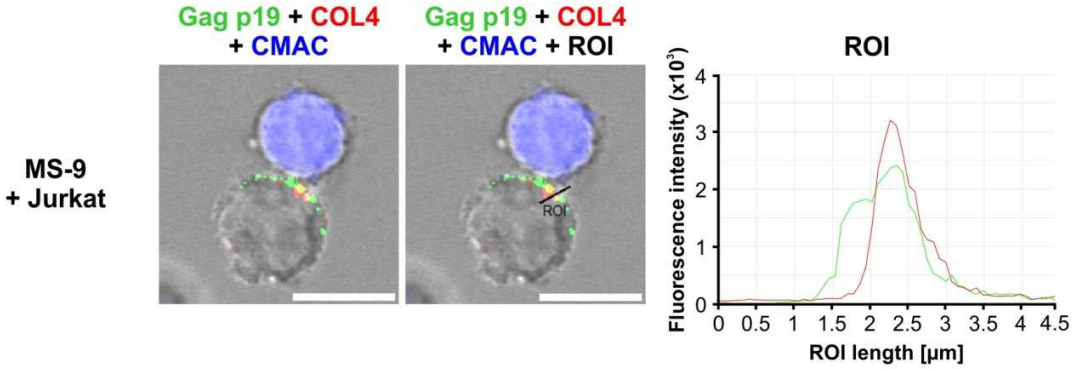


Supplementary Figure 2. COL1-5 protein localizes to extra- and intracellular compartments in HTLV-1 positive T-cells. (A, B) Confocal microscopy was performed in the HTLV-1 positive T-cell lines MT-2, HuT-102 and C91-PL and in the HTLV-1 negative T-cell line Jurkat. Cells were spotted on epoxy-resin coated coverslips and fixed, washed with PBS containing 0.1 % Tween®-20 and **(A)** were permeabilized by 0.2 % Triton™ X-100 for 20 min at 4 °C or **(B)** were left unpermeabilized by incubation with PBS for 20 min at 4 °C. Cells were washed twice and unspecific binding sites were blocked with PBS comprising 5 % FCS and 1 % BSA for 1 h at room temperature. Primary antibodies recognizing the plasma membrane marker CD98 (ab2528, abcam) or COL1-5 (2150-2206, BioRad) were applied for 45 min at 37 °C. Cells were washed three times and incubated with secondary

antibodies Alexa Fluor® 488 anti-mouse (CD98 in green) or Alexa Fluor® 647 anti-rabbit (COL1-5 in red) for 45 min at 37 °C one after the other with three washing steps in between. After three final washing steps, cells were fixed with *ProLong® Gold Antifade Mountant medium with DAPI* (Life Technologies) to stain the nuclei (blue). Images were acquired using a *Leica TCS SP5* confocal laser scanning microscope equipped with a 63x1.4 HCX PL APO CS oil immersion objective lens (Leica Microsystems). Images of transmitted light (TL) served as control. The scale bars represent 10µm.



Supplementary Figure 3. Rex does not cooperate with Tax in regulating COL4 expression. (A, B) $10*10^6$ Jurkat T-cells were co-transfected with a Tax expression vector (pEFneo-Tax1; 60 µg DNA) together with a mock (pcDNA) or Rex-GFP fusion protein expression vector (pCMV-Rex1-GFP; 40 µg). **(A)** *COL4A1* and *COL4A2* transcript levels were quantified by qPCR in untreated or Tax and Rex-GFP transfected Jurkat T-cells. The mean relative copy numbers (rcn), normalized on *ACTB*, of three independent experiments ± SD are depicted. Student's t-test was conducted for statistical analysis (n.s., not significant). **(B)** Western Blot analysis was carried out staining COL4, Tax, Rex-GFP protein (using GFP-specific antibodies), and Hsp90 α/β as loading control. Detection of COL4 and Tax protein in the HTLV-1 positive T-cell line MT-2 served as positive control.



Supplementary Figure 4. COL4 and Gag p19 partially co-localize and accumulate at the virological synapse. HTLV-1 negative Jurkat T-cells were prestained with the cell-permeable dye *CellTracker™ Blue 7-Amino-4-Chlormethylcumarin* (CMAC; Thermo Fisher Scientific; 45 min, 20 µM, 37 °C; blue). Pre-stained Jurkat and HTLV-1 positive MS-9 cells were co-cultured for 30 min at 37 °C at a ratio of 1:1 on poly-L-lysine coated coverslips. Cells were fixed in 2 % PFA for 1 h at room temperature and after five washing steps with PBS containing 0.1 % Tween®-20, cells were permeabilized by 0.2 % Triton™ X-100 for 20 min at 4 °C. Cells were washed twice and unspecific binding sites were blocked with PBS comprising 5 % FCS and 1 % BSA for 1 h at room temperature. Primary antibodies recognizing Gag p19 (mouse; TP-7, Zeptometrix) or COL4 (rabbit; ab6586, abcam) were applied for 45 min at 37 °C, followed by three washing steps. Cells were incubated with secondary antibodies Alexa Fluor® 488 anti-mouse (Gag p19 in green) and Alexa Fluor® 647 anti-rabbit (COL4 in red) for 45 min at 37 °C one after the other with three washing steps in between. After three final washing steps , cells were fixed with *ProLong® Gold Antifade Mountant medium without DAPI* (Life Technologies). Images were acquired using a *Leica TCS SP5* confocal laser scanning microscope equipped with a 63x1.4 HCX PL APO CS oil immersion objective lens (Leica Microsystems). A merge of CMAC (blue), Gag p19 (green) and COL4 (red) stain together with transmitted light is depicted. Images were analyzed using the LAS AF software (Leica Microsystems): A region of interest (ROI) was defined showing the corresponding signals of Gag p19 and COL4 fluorescence intensities. The scale bars represent 10 µm.

1.2 Supplementary Tables

Supplementary Table 1.

Transcriptional expression of components of the viral biofilm.

	average signals[a]				fold change[b]				
	Tax-positive		Tax-negative						
Gene	MT-2	Tesi	$CD4^+$	Tesi/Tet	MT-2 vs $CD4^+$	p value	Tesi vs Tesi/Tet	p value	probe set
AGRIN	68	150	65	122	1	0.79	1	0.13	212285_s_at
AGRIN	51	90	52	54	-1	0.97	2	0.41	217419_x_at
AGRIN	(5)	(6)	(6)	(9)	n.a.		n.a.		217410_at
AGRIN	(8)	(8)	(11)	23	-1	0.82	-3	0.04	212283_at
BST2	65	59	29	73	2	0.09	-1	0.02	1570198_x_at
BST2	39	49	(11)	32	4	0.12	2	0.09	1570197_at
BST2	468	730	516	650	-1	0.29	1	0.51	201641_at
COL1A1	263	161	(2)	65	272	0.06	2	0.17	1556499_s_at
COL1A1	108	53	(4)	28	38	0.04	2	0.27	202310_s_at
COL1A1	(12)	(4)	(5)	(17)	n.a.		n.a.		202311_s_at
COL1A1	(9)	(15)	(12)	(16)	n.a.		n.a.		202312_s_at
COL1A1	64	46	41	51	2	0.41	-1	0.89	217430_x_at
COL1A2	(2)	(2)	(2)	(2)	n.a.		n.a.		229218_at
COL1A2	(4)	(8)	(2)	(1)	n.a.		n.a.		202404_s_at
COL1A2	(3)	(13)	(18)	(17)	n.a.		n.a.		202403_s_at
COL2A1	(5)	(13)	(10)	(11)	n.a.		n.a.		213492_at
COL2A1	(4)	(5)	(1)	(2)	n.a.		n.a.		217404_s_at
COL3A1	(11)	1604	(5)	1614	n.a.		-1	0.99	215076_s_at
COL3A1	(6)	986	(3)	992	n.a.		-1	0.93	201852_x_at
COL3A1	(7)	492	(3)	449	n.a.		1	0.51	211161_s_at
COL3A1	(1)	57	(3)	70	n.a.		-1	0.14	232458_at
COL4A1	(7)	(13)	(2)	24	n.a.		-2	0.48	233652_at
COL4A1	1780	360	(16)	92	109	<0.01	4	<0.01	211980_at
COL4A1	855	128	(4)	40	267	0.02	3	<0.01	211981_at
COL4A2	1325	624	(4)	233	331	<0.01	3	0.06	211964_at
COL4A2	604	205	(8)	65	87	0.02	3	0.03	211966_at
COL4A2	33	(15)	(4)	(5)	10	0.03	n.a.		237624_at

Supplementary Table 1. continued

	average signals[a]				fold change[b]				
	Tax-positive		Tax-negative						
Gene	MT-2	Tesi	$CD4^+$	Tesi/Tet	MT-2 vs $CD4^+$	p value	Tesi vs Tesi/Tet	p value	probe set
COL4A3	(5)	(14)	(14)	(11)	n.a.		n.a.		216898_s_at
COL4A3	(1)	(7)	(1)	(7)	n.a.		n.a.		216896_at
COL4A3	(9)	(12)	(9)	(8)	n.a.		n.a.		216368_s_at
COL4A3	(3)	(7)	(2)	(11)	n.a.		n.a.		216367_at
COL4A4	(1)	(1)	(1)	(1)	n.a.		n.a.		214602_at
COL4A4	(10)	(3)	(1)	(4)	n.a.		n.a.		229779_at
COL4A4	(4)	(9)	(9)	(15)	n.a.		n.a.		241565_at
COL4A5	(2)	13	(1)	(14)	n.a.		-1	0.72	234387_at
COL4A5	(7)	(1)	(6)	(6)	n.a.		n.a.		213110_s_at
COL4A5	(8)	(9)	(6)	(8)	n.a.		n.a.		1563536_at
COL4A6	(14)	(16)	(2)	21	n.a.		-1	0.26	1564654_at
COL4A6	(7)	(3)	(7)	(6)	n.a.		n.a.		210945_at
COL4A6	(7)	(10)	(6)	(7)	n.a.		n.a.		213992_at
COL4A6	(6)	(5)	(3)	(2)	n.a.		n.a.		211473_s_at
COL5A1	(4)	(2)	(4)	(8)	n.a.		n.a.		212489_at
COL5A1	(5)	(18)	(11)	24	n.a.		n.a.		1556138_a_at
COL5A1	(2)	(2)	(4)	(2)	n.a.		n.a.		212488_at
COL5A1	(5)	(4)	(3)	(5)	n.a.		n.a.		203325_s_at
COL5A2	(8)	(4)	(18)	(8)	n.a.		n.a.		221730_at
COL5A2	(11)	(6)	(15)	(8)	n.a.		n.a.		221729_at
COL5A3	(12)	(4)	(15)	(4)	n.a.		n.a.		52255_s_at
COL5A3	(2)	(3)	(4)	(2)	n.a.		n.a.		218975_at
FUT4	(5)	(12)	(15)	(12)	n.a.		n.a.		244889_at
FUT4	(4)	(9)	(7)	(5)	n.a.		n.a.		1560995_s_at
FUT4	148	90	71	31	2	<0.01	3	0.03	209892_at
FUT4	98	38	61	31	2	0.32	1	0.37	209893_s_at
FUT9	(2)	(1)	(1)	(1)	n.a.		n.a.		207696_at
FUT9	(4)	(1)	(2)	(2)	n.a.		n.a.		216185_at
LGALS3	(4)	27	(6)	27	1	0.91	1	0.95	1557197_a_at
LGALS3	2043	4345	1014	4490	2	<0.01	-1	0.54	208949_s_at

[a] average signals were rounded to integers; values in brackets were deemed absent calls.

[b] values depicting the fold change of transcript expression were rounded to integers; p values were rounded on two decimals; vs, versus; n.a., not applicable.