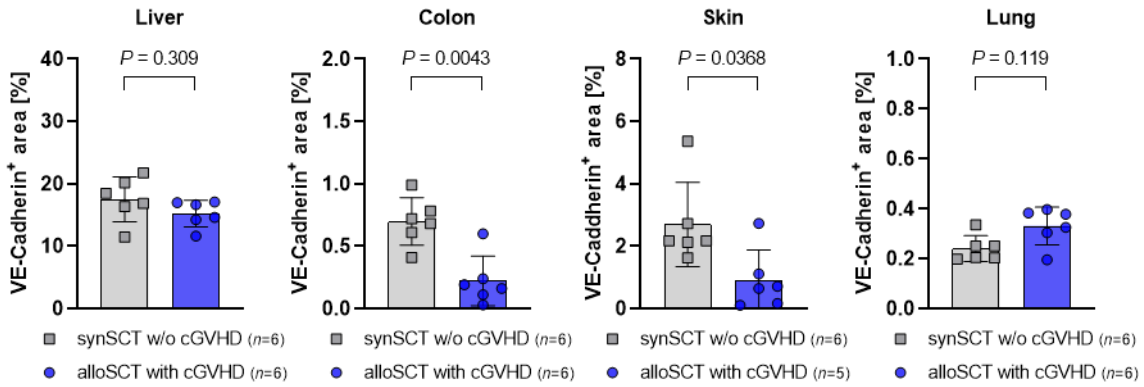
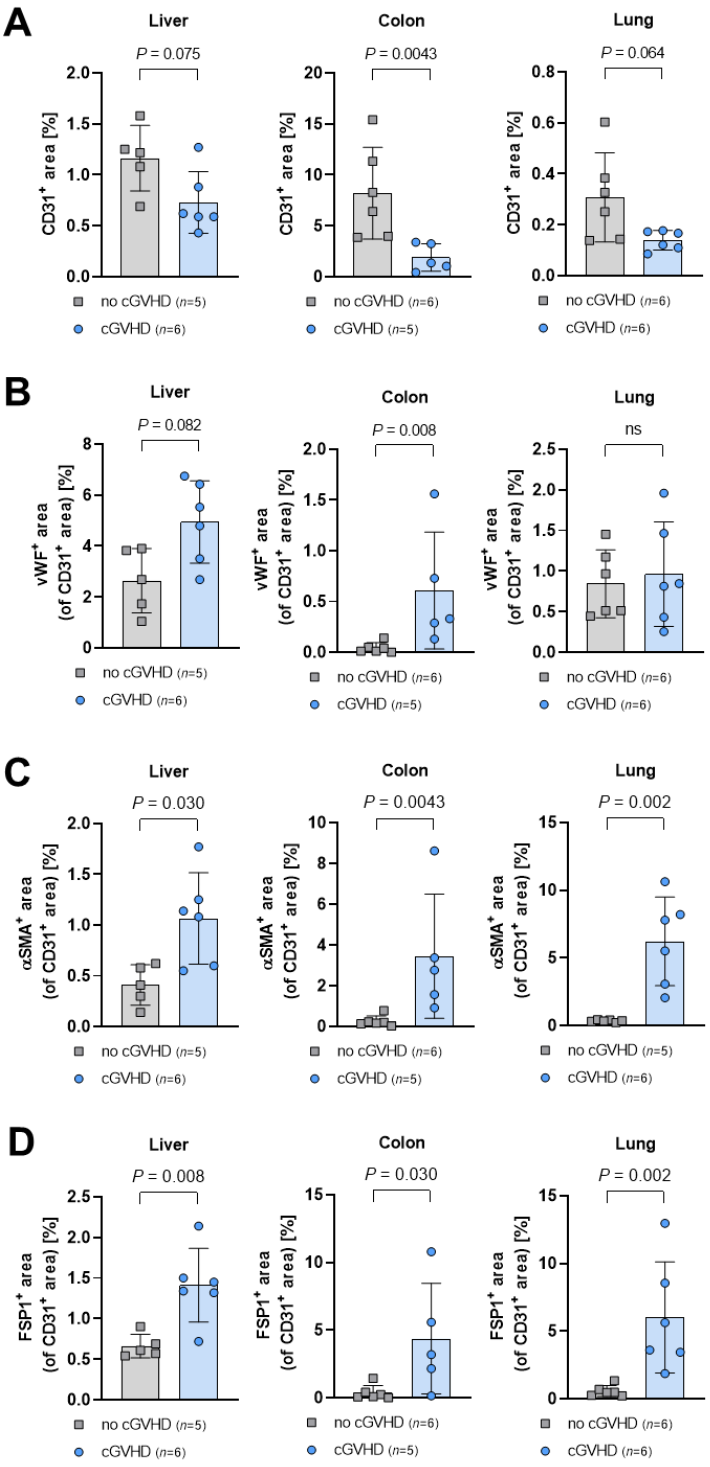
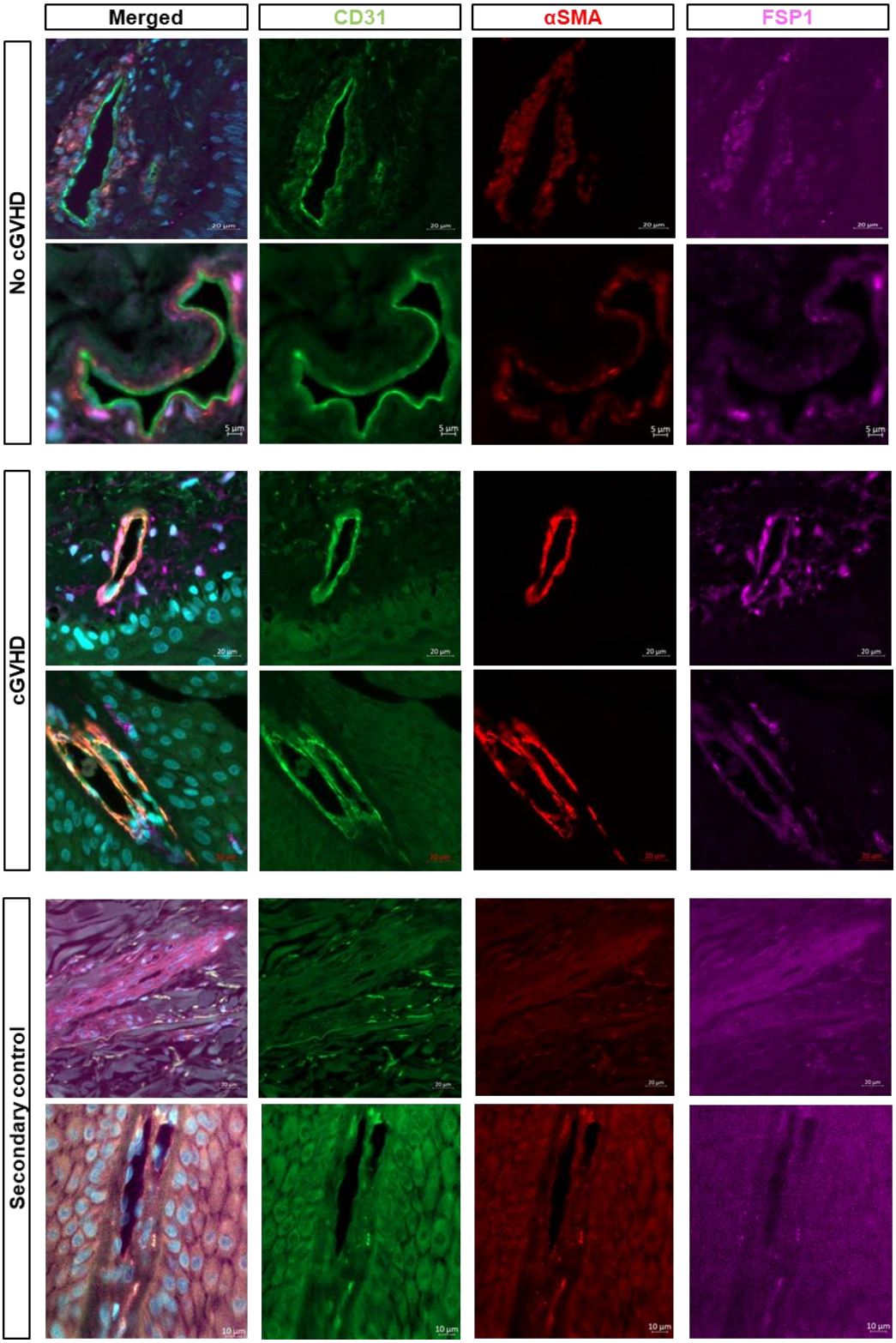
Supplementary Data and Tables



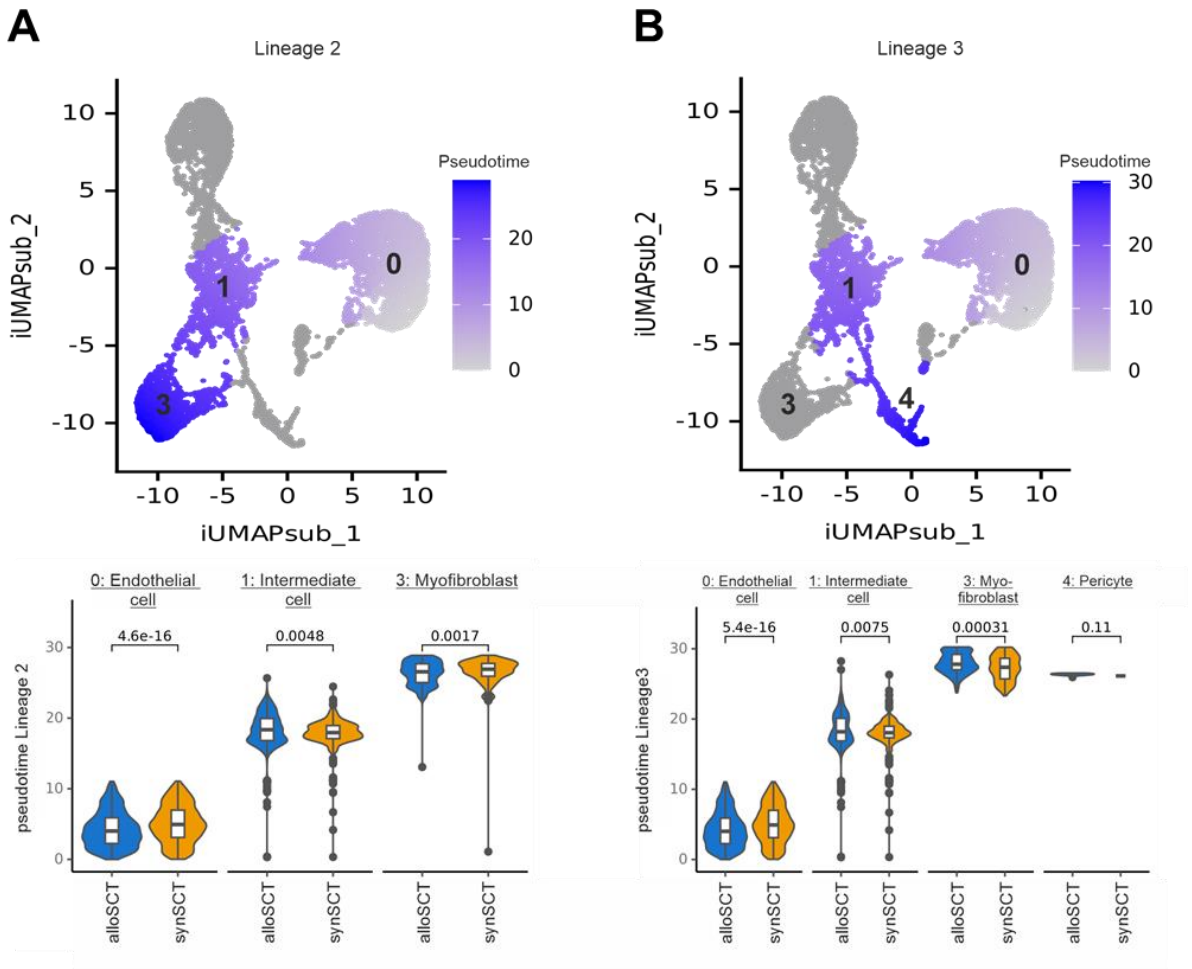
Supplementary Fig. 1. Confirmation of vessel loss during cGVHD with further endothelial marker.
Data shown from murine alloSCT cGVHD model C57BL/6 into B6D2F1 (dark blue bars) at day +125 after SCT, *n* =6 mice per group. Representative data shown from one out of two independent experiments. Quantification of VE-Cadherin expression in liver, colon, skin and lung by Immunofluorescence analysis, VE-Cadherin: marker for vessel density. Statistical analyses were performed by Mann-Whitney U test. Error bars indicate mean +SD.



Supplementary Fig. 2. Confirmation of vessel loss and EndMT events in further cGVHD model.
Data shown from xenograft cGVHD model PBMCs into NSG (light blue bars) at day +125 after SCT, *n* =5 or 6 mice per group. (**A**) Quantification of CD31 expression in liver, colon and lung by Immunofluorescence analysis, CD31: marker for vessel density. (**B**) Quantification of co-localized vWF/CD31 expression in liver, colon and lung by Immunofluorescence analysis, vWF: marker for endothelial damage. Quantification of EndMT events by co-localized αSMA/CD31 (**C**) and FSP1/CD31 (**D**) expression in blood vessels. Statistical analyses were performed by Mann-Whitney U test. Error bars indicate mean +SD. αSMA (alpha smooth muscle actin), cGVHD (chronic GVHD), EndMT (endothelial-to-mesenchymal transition), FSP1 (fibroblast-specific protein 1), SCT (stem cell therapy), vWF (von-Willebrand-Factor).

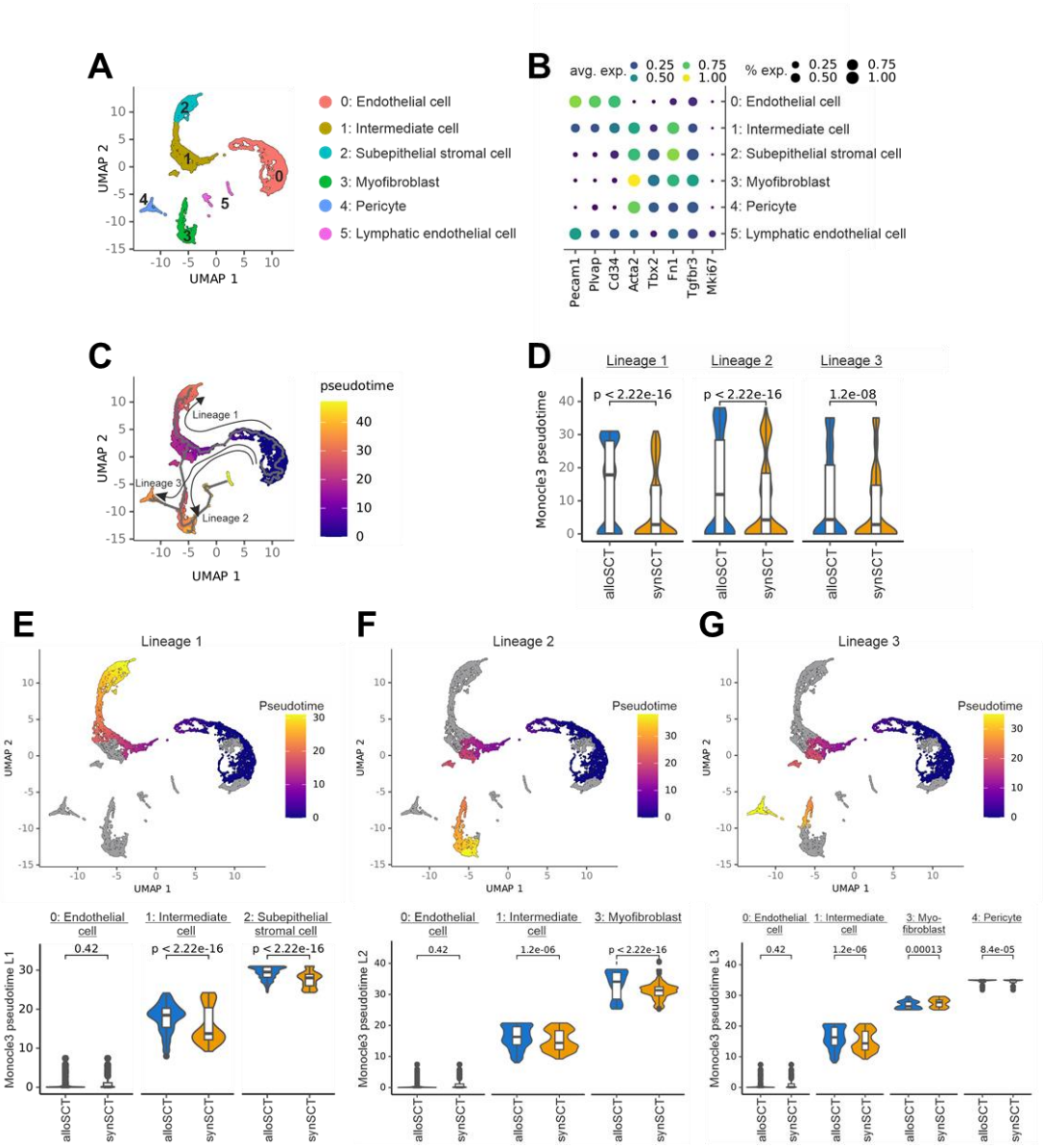


Supplementary Fig. 3. Immunofluorescence analysis of EndMT in human skin biopsies.
Representative images of Immunofluorescence analysis of EndMT events in skin biopsies from patients without clinical signs of cGvHD (n =2) and NIH moderate/severe cGVHD (n =2). Merged and single-color channel (CD31-green, αSMA-red, FSP1 violet) pictures used for Figure 1H. Skin samples from patients without cGVHD show αSMA and FSP1 staining in pericyte layer around the CD31 positive endothelial cells whereas in skin samples from cGVHD patients a co-localization with CD31 arises indicating EndMT events. αSMA (alpha smooth muscle actin), cGVHD (chronic GVHD), EndMT (endothelial-to-mesenchymal transition), FSP1 (fibroblast-specific protein 1).



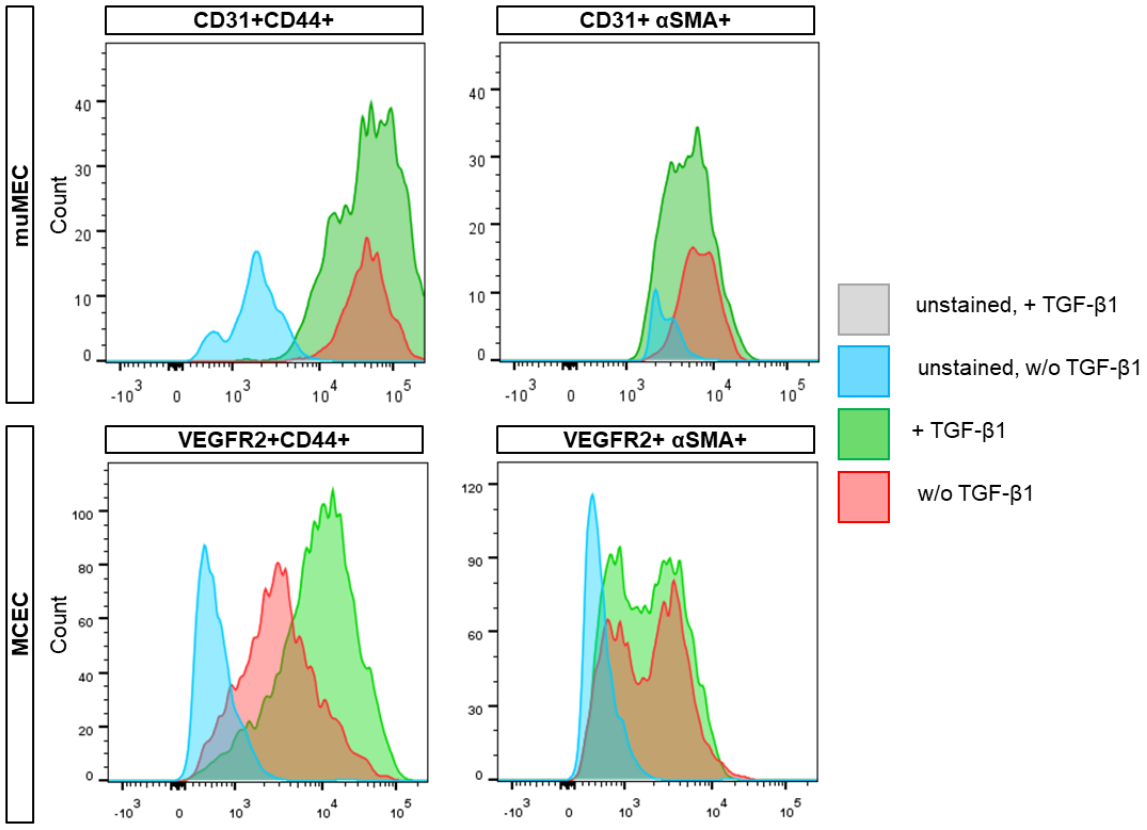
Supplementary Fig. 4. Pseudotime analysis of Lineage 2 and 3.

In murine alloSCT cGVHD model C57BL/6 into B6D2F1 at day +69 after SCT, colon cells from 3 individual mice per group (alloSCT and synSCT) were pooled prior scRNA-seq. (**A**) **Upper Panel**: UMAP embedding of *Acta2*- and *Pecam1*-positive cell clustering showing Slingshot-derived cell-wise pseudotime values for lineage 2. Dark grey points represent cells with no pseudotime values in this lineage. **Lower Panel**: Combined violin and box plots of lineage 2 pseudotime values for different cell types in alloSCT (blue) and synSCT (orange) recipients. (**B**) **Upper Panel**: UMAP embedding of *Acta2*- and *Pecam1*-positive cell clustering showing Slingshot-derived cell-wise pseudotime values for lineage 3. Dark grey points represent cells with no pseudotime values in this lineage. **Lower Panel**: Combined violin and box plots of lineage 3 pseudotime values for different cell types in alloSCT (blue) and synSCT (orange) recipients. Statistical analysis by Wilcoxon rank-sum tests. Lower and upper boxplot hinges represent the 25th and 75th percentiles, and center lines indicate medians. Acta2 (Actin Alpha 2, Smooth Muscle), Pecam1 (Platelet and Endothelial Cell Adhesion Molecule 1), SCT (stem cell therapy).

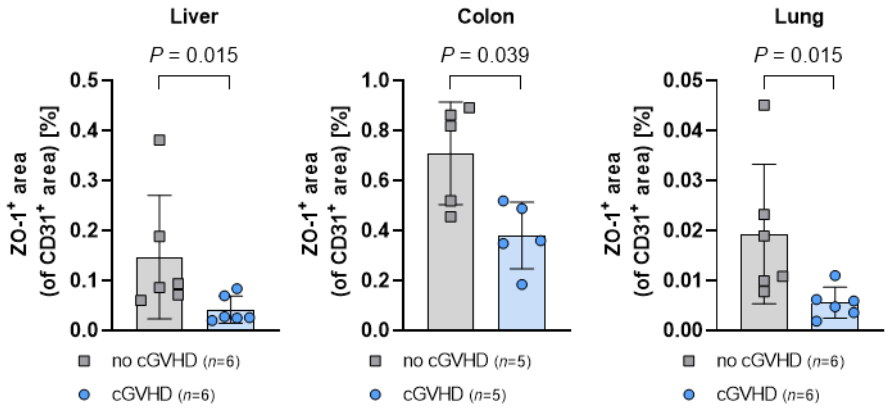


Supplementary Fig. 5. Pseudotime analysis with Monocle3 confirms progression from endothelial to mesenchymal cells during cGVHD.

In murine alloSCT cGVHD model C57BL/6 into B6D2F1 at day +69 after SCT, colon cells from 3 individual mice per group (alloSCT and synSCT) were pooled prior scRNA-seq. (**A**) UMAP embedding of *Acta2* and *Pecam1* positive colon cells, showing Monocle3 clusters and assigned cell type. A total of 8207 cells are shown. (**B**) Dot plot representation of scaled average expression using selected markers. Dot size reflects the percentage of cells. (**C**) UMAP embedding and Monocle3-derived cell-wise pseudotime values. Lineages 1, 2 and 3 are marked with arrows. (**D**) Combined violin and box plots of Monocle3 pseudotime values for the 3 different lineages in alloSCT (blue) and synSCT (orange) recipients. (**E-G**) **Upper Panel**: UMAP embedding and Monocle3-derived cell-wise pseudotime values for lineage 1 (**E**), 2 (**F**) and 3 (**G**). Dark grey points represent cells with no pseudotime values in this lineage. **Lower Panel**: Combined violin and box plots of lineage 1 (**E**), 2 (**F**) and 3 (**G**) pseudotime values for different cell types in alloSCT (blue) and synSCT (orange) recipients. Statistical analysis by Wilcoxon rank-sum tests. Lower and upper boxplot hinges represent the 25th and 75th percentiles, and center lines indicate medians. Acta2 (Actin Alpha 2, Smooth Muscle), cGVHD (chronic GVHD), Fn1 (Fibronectin 1), Mki67 (Marker of Proliferation Ki-67), Pecam1 (Platelet and Endothelial Cell Adhesion Molecule 1), Plvap (Plasmalemma Vesicle Associated Protein), SCT (stem cell therapy), Tbx2 (T-box transcription factor), Tgfbr3 (Transforming Growth Factor Beta Receptor 3).



Supplementary Fig. 6. Flow cytometry analysis of endothelial and mesenchymal markers in TGF-β stimulated muMEC and MCEC.
Representative histograms from two independent experiments. Co-expression of CD31 or VEGFR2 with CD44 (endothelial + adhesion/migration marker). Co-expression of CD31 or VEGFR2 with αSMA (endothelial + mesenchymal marker). αSMA (alpha smooth muscle actin), MCEC (immortalized mouse cardiac endothelial cells), muMEC (immortalized murine microvascular endothelial cells), TGF-β1 (Transforming Growth factor 1), VEGFR2 (vascular endothelial growth factor receptor 2).



Supplementary Fig. 7. Confirmation of loss of tight junction protein ZO-1 during cGVHD in further cGVHD model.
Loss of tight junction protein ZO1 in vessels in xenograft cGVHD model PBMCs into NSG (light blue bars) at day +125 after SCT. Quantification of co-localized ZO1/CD31 expression in liver, colon and lung by Immunofluorescence analysis, *n* =5 or 6 mice per group. Statistical analysis by Mann-Whitney U test. Error bars indicate mean +SD. cGVHD (chronic GVHD), SCT (stem cell therapy), ZO-1 (Zonula Occludens).

Supplementary Table 1. Patient and disease characteristics of Serum Samples. A) alloSCT recpients with cGVHD

Patient Nr.	1	2	3	4	5	6	7	8	9
Age at alloSCT	71	58	63	44	49	40	19	55	60
Gender	female	male	male	female	male	male	female	female	male
Donor	MRD	MRD	MUD	MUD	MUD	MUD	MRD	MRD	MUD
AlloSCT indication	DLBCL	AML	MDS	AML	AML	ALL	ALL	MDS	AML
Stem cell source	PB	PB	PB	PB	PB	PB	PB	PB	PB
Conditioning	RIC	RIC	RIC	MAC	RIC	MAC	MAC	RIC	RIC
aGVHD prior to cGVHD	yes	no	yes	no	no	yes	yes	no	yes
Onset of cGVHD day after alloSCT	235	371	356	228	166	321	355	187	489
Maximum grade cGHVD	severe	severe	moderate	moderate	severe	moderate	moderate	severe	moderate
GVHD prophylaxis	CSA, MMF	CSA, MMF	CSA, MMF	CSA, MTX	CSA, MMF	CSA, MTX	CSA, MTX	CSA, MMF	CSA, MMF

Abbreviations: MUD – matched unrelated donor, MRD = matched related donor, AML – acute myeloid leukemia, ALL = acute lymphoblastic leukemia, DLBCL = diffuse large B-cell lymphoma, MDS = myelodysplastic syndrome, PB = peripheral blood, MAC = myeloablative conditioning, RIC = reduced intensity conditioning, CSA = cyclosporine A, MTX = methotrexate, MMF = mycophenolate mofetil

Supplementary Table 1. Patient and disease characteristics of Serum Samples. B) Control alloSCT recipients without cGVHD

Patient Nr.	10	11	12	13	14	15	16	17	18
Age at alloSCT	68	61	67	53	54	37	28	54	68
Gender	female	male	female	male	male	male	male	female	male
Donor	MUD	MRD	MUD	MUD	MUD	MUD	MRD	MUD	MUD
AlloSCT indication	MDS	AML	AML	AML	MDS	ALL	ALL	AML	MDS
Stem cell source	PB	PB	PB	PB	PB	PB	PB	PB	PB
Conditioning	RIC	RIC	RIC	RIC	RIC	MAC	MAC	RIC	RIC
aGVHD present	no	no	yes	yes	no	no	yes	no	no
Onset of cGVHD day after alloSCT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Maximum grade cGHVD	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
GVHD prophylaxis	CSA, MMF	CSA, MMF	CSA, MMF	CSA, MMF	CSA, MMF	CSA, MTX	CSA, MTX	CSA, MMF	CSA, MMF

Abbreviations: MUD – matched unrelated donor, MRD = matched related donor, AML – acute myeloid leukemia, ALL = acute lymphoblastic leukemia, DLBCL = diffuse large B-cell lymphoma, MDS = myelodysplastic syndrome, PB = peripheral blood, MAC = myeloablative conditioning, RIC = reduced intensity conditioning, CSA = cyclosporine A, MTX = methotrexate, MMF = mycophenolate mofetil

Supplementary Table 2. Control patient without cGVHD characteristics of skin biopsies

Patient Nr.	1	2	3	4	5	6	7	8
Biopsy location	Right arm	Right arm	Left leg	Right ear	abdomen	Left leg	abdominal	abdominal
Sex	female	female	female	male	male	male	male	male
Age	46	21	29	78	41	34	47	56

Supplementary Table 3. Antibodies used for immunofluorescence staining of murine samples

Primary Antibodies					
Epitope	**Host**	**Clone**	**Dilution**	**Catalogue number**	**Company**
CD31	hamster	2H8	1:300	MA3105	Thermo Fisher Scientific, USA
vWF	rabbit	polyclonal	1:700	NB600-586	Novus Biologicals, USA
αSMA (conjugated with Cy3)	goat	1A4	1:300	C6198	Sigma-Aldrich, USA
FSP1	rabbit	polyclonal	1:300	07-2274	Merck, Germany
ZO-1	rabbit	polyclonal	1:200	61-7300	Thermo Fisher Scientific, USA
VE-Cad	goat	polyclonal	1:100	AF1002	R&D Systems, USA
Secondary Antibodies					
Host	**Reactivity**	**Fluorophore**	**Dilution**	**Catalogue number**	**Company**
goat	hamster	AF488	1:1000	A21110	Thermo Fisher Scientific, USA
donkey	rabbit	AF488	1:1000	A21206	Thermo Fisher Scientific, USA
donkey	rabbit	AF555	1:1000	A31572	Thermo Fisher Scientific, USA
donkey	rabbit	AF647	1:1000	A32795	Thermo Fisher Scientific, USA

Supplementary Table 4. Antibodies used for immunofluorescence staining of human samples

Primary Antibodies					
Epitope	**Host**	**Clone**	**Dilution**	**Catalogue number**	**Company**
CD31	goat	polyclonal	1:100	AF3628	R&D Systems, USA
αSMA (conjugated with Cy3)	goat	1A4	1:300	C6198	Sigma-Aldrich, USA
FSP1	rabbit	polyclonal	1:300	07-2274	Merck, Germany
Secondary Antibodies					
Host	**Reactivity**	**Fluorophore**	**Dilution**	**Catalogue number**	**Company**
donkey	goat	AF488	1:1000	A11055	Thermo Fisher Scientific, USA
donkey	rabbit	AF647	1:1000	A32795	Thermo Fisher Scientific, USA

Supplementary Table 5. Antibodies used for flow cytometry

Epitope	Fluorophore	Clone	Dilution	Catalogue number	Company
CD31	PE	MEC13.3	1:100	553373	BD Biosciences, USA
CD31	APC-Cy7	MEC13.3	1:200	102439	Biolegend, USA
CD146	FITC	P1H12	1:50	562229	BD Biosciences, USA
CD146	AF647	ME-9F1	1:200	562230	BD Biosciences, USA
αSMA	Cy3	1A4	1:300	C6198	Sigma-Aldrich, USA
CD44	PerCP-Cy5.5	IM7	1:200	103035	Biolegend, USA
CD133	PE-Cy7	315-2C11	1:200	141209	Biolegend, USA
VEGFR-2 (Flk1)	FITC	Avas 12alpha1	1:50	560680	BD Biosciences, USA

Supplementary Table 6. TaqMan primers used for qPCR. Obtained from Thermo Fisher Scientific.

Gene	TaqMan Assay ID	Amplicon Length
α-SMA (Acta2)	Mm00725412_s1	95
β-Actin	Mm00607939_S1	115
CD31 (Pecam1)	Mm01242584_m1	71
FSP-1 (S100a4)	Mm00803372_g1	87
GAPDH	Mm9999991515_g1	107
SMAD2	Mm00487530_m1	72
SMAD3	Mm01170760_m1	59
Snail1	Mm00441533_g1	79
TGF-β1	Mm01178820_m1	59
Tie-2 (Tek)	Mm00443254_m1	93
Twist	Mm00442036_m1	113
VE-Cadherin (Cdh5)	Mm03053719_s1	154
Vimentin	Mm01333430_m1	62
vWF	Mm00550376_m1	63