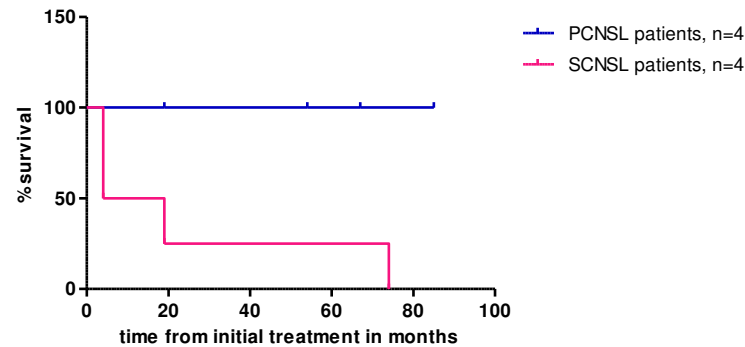


Figure S1

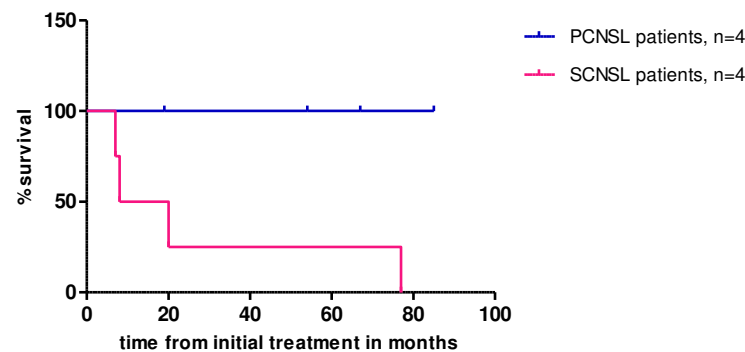
a:

Progression free survival of patients with established xenografts



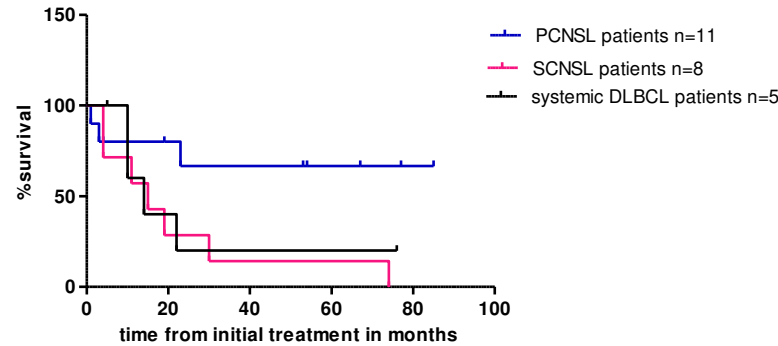
b:

Overall survival of patients with established xenografts



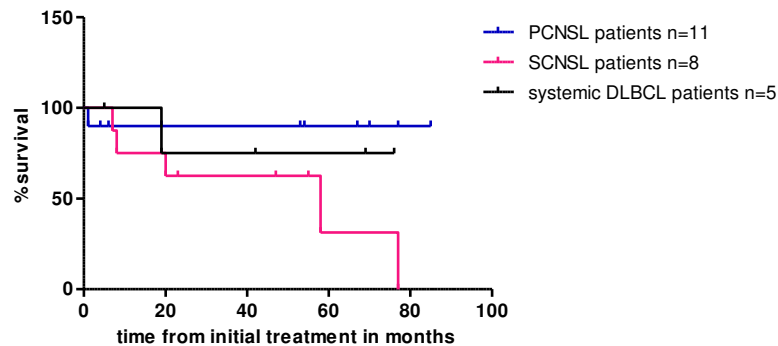
c:

Progression free survival of all patients



d:

Overall survival of all patients

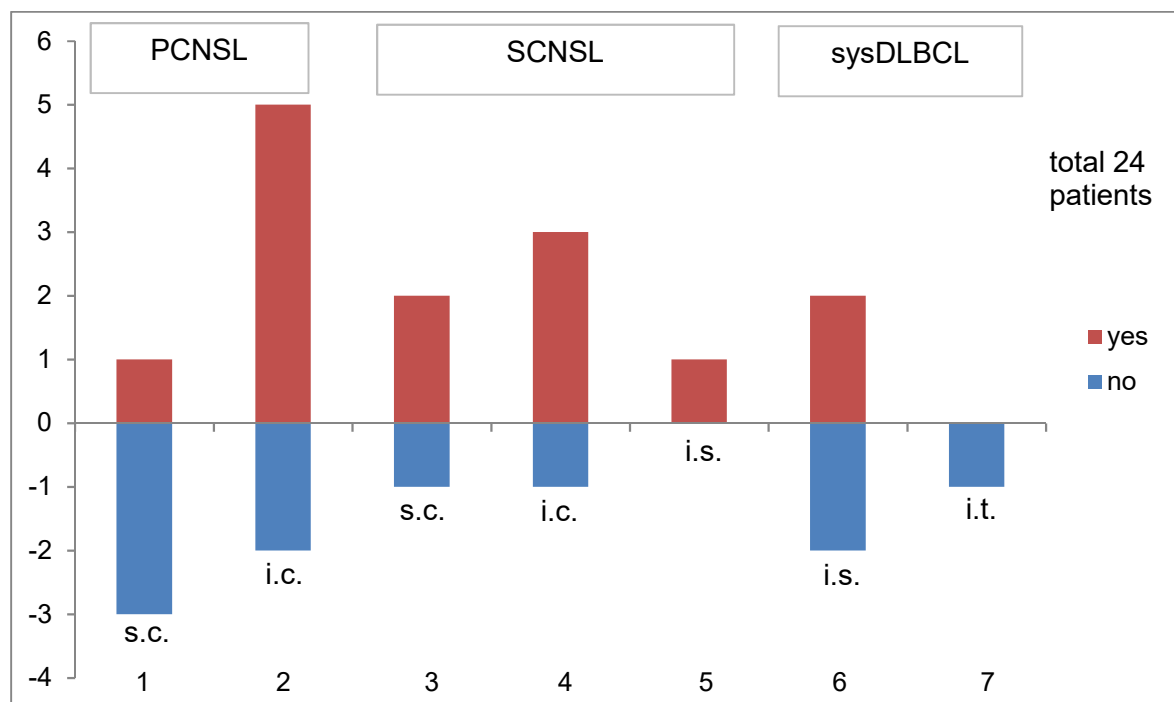


**Supplementary Fig. S1** Progression free survival (PFS) and overall survival (OS) of study patients.

a, b: PFS and OS of PCNSL and SCNSL patients with established xenografts (PCNSL n=4, SCNSL n=4). The 4 PCNSL patients are in ongoing remission, all 4 SCNSL patients died.

c, d: PFS and OS of all study patients (PCNSL n=11, SCNSL n=8, systemic DLBCL n=5). Two patients in the PCNSL patient cohort developed progressive disease after 3 and 23 months, respectively and 1 treatment related death occurred. Two patients were lost to follow up (FU). Five patients are in ongoing long-term remission without relapse, 1 patient is still in FU and in complete remission. Five of the PCNSL patients were treated with HCT-ASCT in first line, 1 in second line. Five of the SCNSL patients were treated with HCT-ASCT after first relapse, 1 patient is in ongoing remission after 55 months. Three of the 5 patients of the systemic DLBCL cohort developed progressive disease, 2 patients were successfully salvaged with HCT-ASCT or allogeneic stem cell transplantation, 1 patient died (for details see Supplementary Table S1).

Figure S2

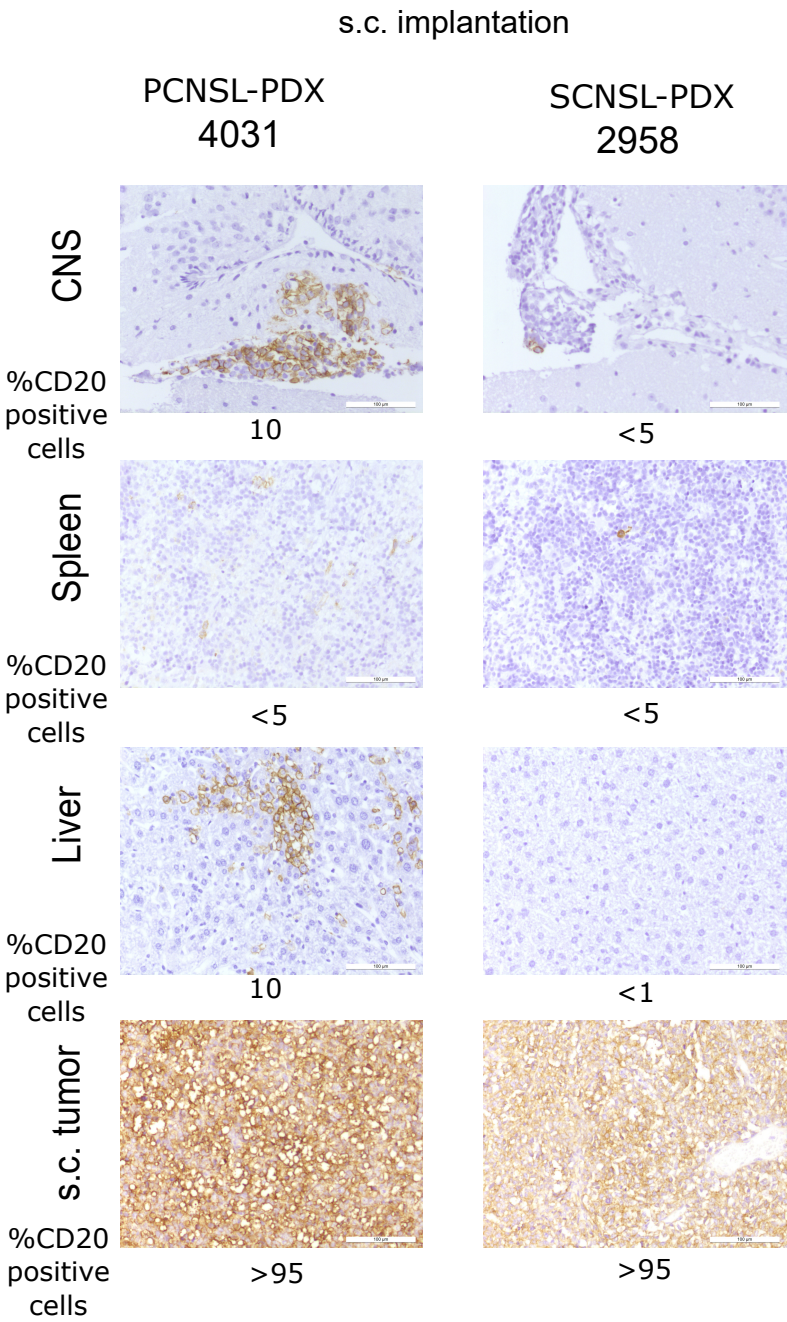


PCNSL=primary CNS lymphoma, SCNSL=secondary CNS lymphoma, sysDLBCL=systemic DLBCL, s.c.=subcutaneous, i.c.=intracerebral, i.s.=intrasplenic, i.t.=intratibial

**Supplementary Fig. S2** Lymphoma engraftment rates of different implantation routes.

Column chart shows engraftment rates of PCNSL patient samples after s.c. and i.c. implantation (columns 1 and 2), SCNSL patient samples after s.c., i.c. and i.s. implantation (columns 3-5) and systemic DLBCL patient samples (columns 6 and 7). "Yes" means successful engraftment of sample, "no" means engraftment of sample was not successful. After s.c. implantation 1 out of 4 PCNSL and 2 out of 3 SCNSL patient samples engrafted. After i.c. implantation 5 out of 7 (71%) PCNSL, 3 out of 4 SCNSL patient samples (75%) engrafted. One SCNSL patient sample was implanted primarily intrasplenically with successful engraftment. After i.s. implantation 2 out of 4 systemic DLBCL patient samples engrafted. One systemic DLBCL patient sample from a bone marrow biopsy was implanted intratibial (i.t.) with no engraftment.

Figure S3

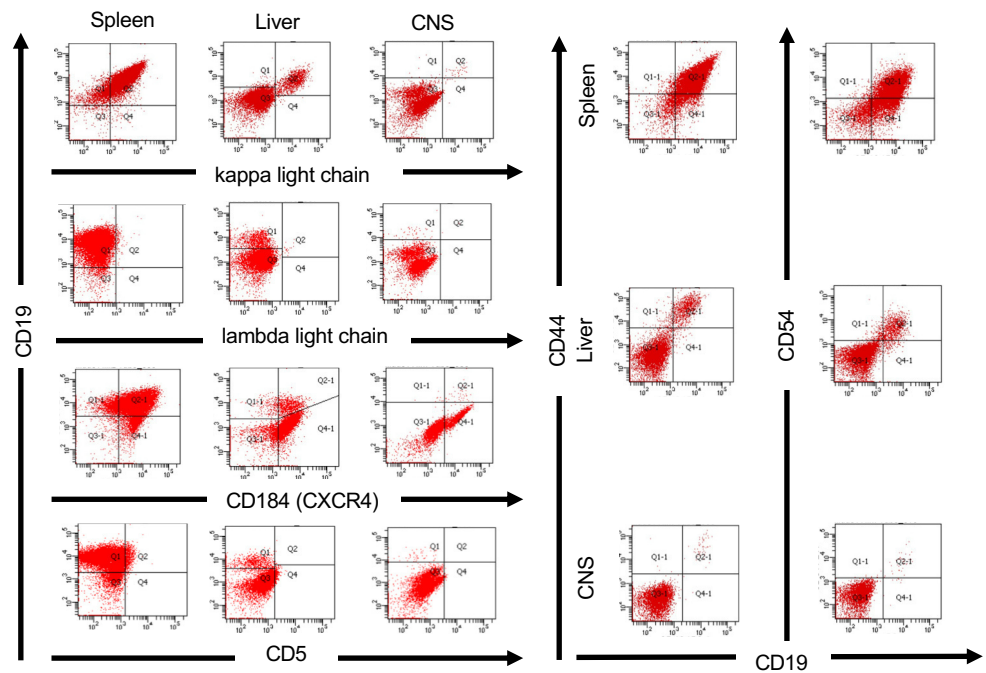


**Supplementary Fig. S3** CD20 staining of different organs after secondary s.c. implantation of PDX 4031 and PDX 2958 lymphoma cells into NSG recipient mice (N=3).

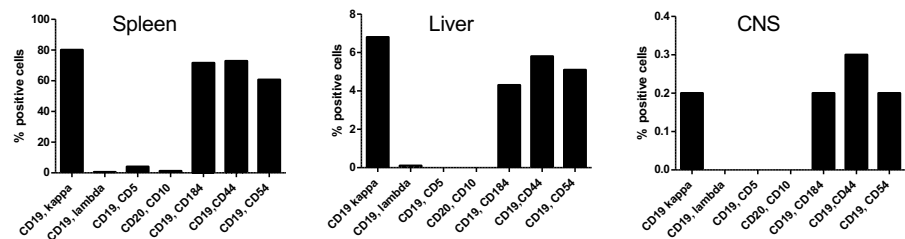
After s.c. implantation of 4031 CNS infiltration and infiltration of liver of approximately 10% was detected, whereas after implantation of 2958 only single cell infiltrates in CNS, spleen and liver were seen. Bar scale 100µm. Visual estimation of the percentage of infiltration in the respective organs was performed by two pathologists (S.D., H.E.S). For each PDX at least 3 slides were analysed, representative slide sections were selected and infiltration percentages were documented in 5% increments. Below 1 means no positive cells were detected in the 3 analysed slides.

Figure S4

a



b

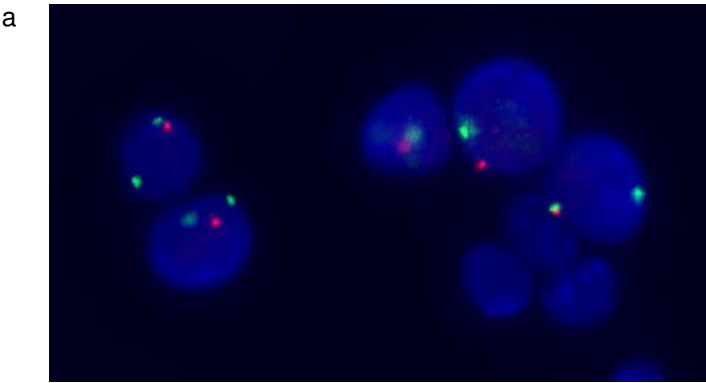


**Supplementary Fig. S4** Flow cytometric analysis of different organs after i.s. implantation of PDX 4113.

a: Flow cytometric analysis of PCNSL PDX 4113 showed monoclonal human CD19<sup>+</sup> cells with kappa light chain restriction as well as expression of adhesion molecules ICAM1 (CD54) and HCAM (CD44) and the chemokine receptor CXCR4 (CD184) in spleen, liver and CNS after secondary i.s. implantation. b: Percentage of antibody stained cells for PDX 4113 in the different organs.



Figure S5



b

PDX	DNA probes											
	XX/XY	% human	17p13/ TP53	% human	14q32/ IGH	% human	9p21/ CDKN2A	% human	18q21/ BCL2	3q27/ BCL6	cMYC	11q22/ ATM
4031 (PCNSL)	X0	320/400 (80%)	Deletion TP53	80/100 (80%)	Deletion IGH	80/100 (80%)	Deletion CDKN2A	80/100 (80%)	Normal pattern	Normal pattern	Normal pattern	Normal pattern
4113 (PCNSL)	XY	162/200 (81%)	Normal pattern	NA	Normal pattern	NA	Biallelic deletion CDKN2A	81/100 (81%)	Normal pattern	Normal pattern	Normal pattern	Normal pattern

100 interphase nuclei were analyzed. % human (cells with FISH signal/all analyzed cells). NA = not applicable

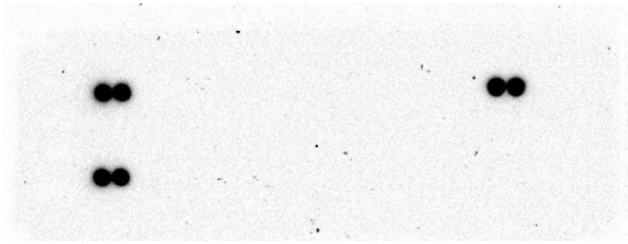
**Supplementary Fig. S5** Fluorescence in situ hybridization of PCNSL PDX 4031 and 4113.

a: Digital image of human cells with deletion 9p21/ CDKN2A (red) and two centromeres 9 (green). Mouse cells did not hybridize with human DNA probes.

b: Spleen xenograft lymphoma cells of PCNSL PDX 4031 and 4113 after i.s. implantation were subjected to FISH, using 8 DNA probes. PDX 4031 showed loss of X chromosome, TP53 deletion, deletion of 14q32/IGH and deletion of 9p21/CDKN2A. PDX 4113 showed biallelic deletion of 9p21/CDKN2A. No aberrations were observed in BCL2, BCL6, cMYC or 11q22/ATM.

Figure S6

control 1: medium (RPMI + 2% FSC)



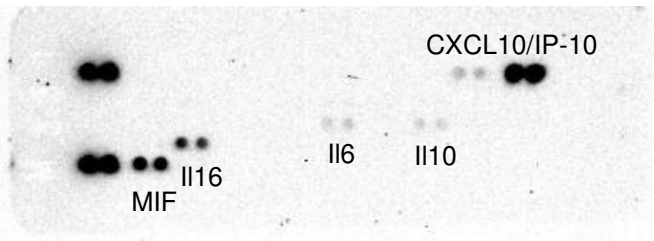
control 2: supernate of healthy mouse spleen cells



PDX 4031 (PCNSL)



PDX 2958 (SCNSL)

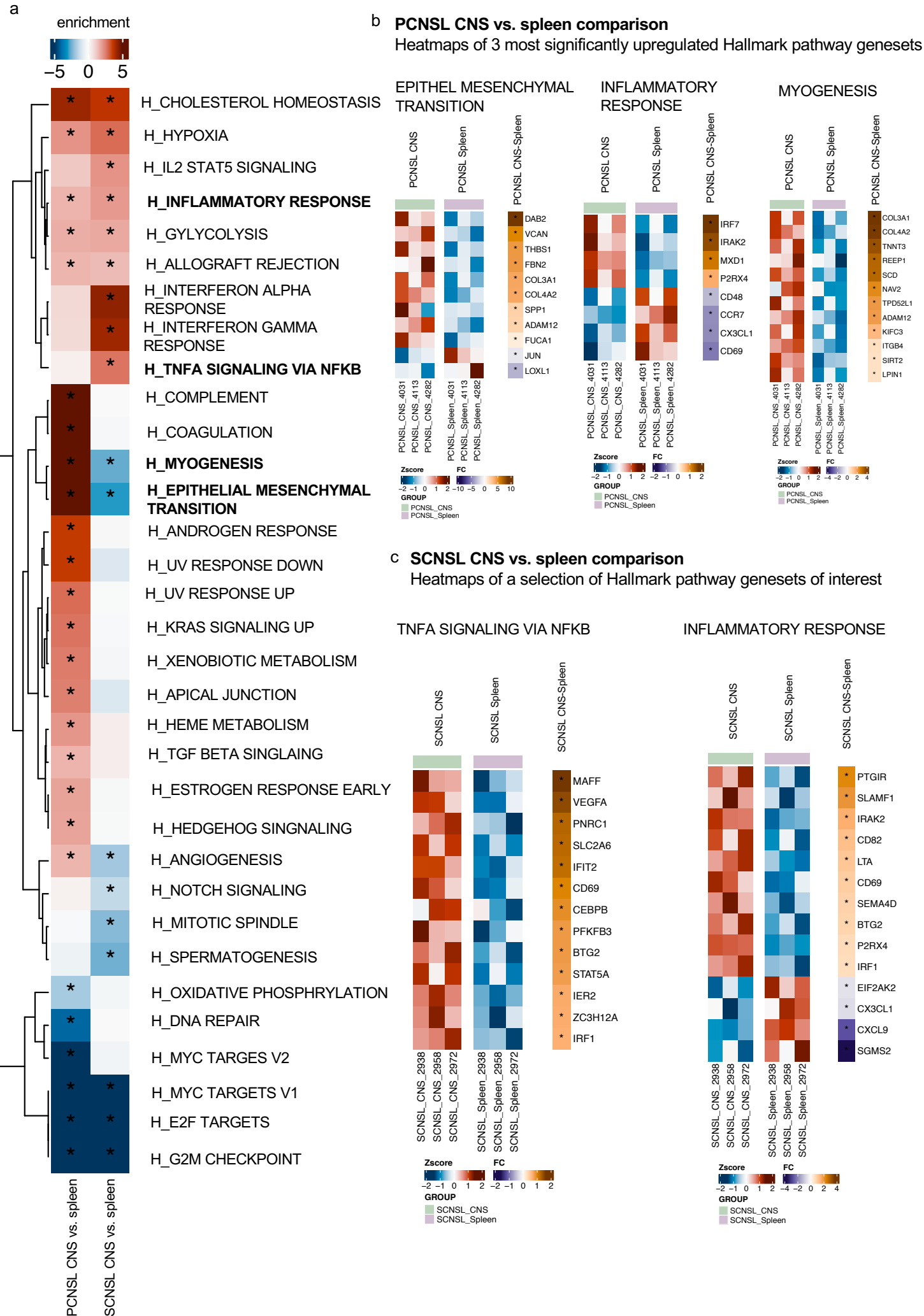


**Supplementary Fig. S6** Cytokine secretion profiles of spleen infiltrating lymphoma cells.

The supernatants of spleen xenograft lymphoma cells of PCNSL PDX 4031 and SCNSL PDX 2958 after i.s. implantation were analyzed with a cytokine array. Cells were cultured in RPMI + 2% FSC for 24h. Culture medium and supernatant of healthy mouse spleen cells were used as controls.

PCNSL PDX 4031 lymphoma cells secreted migration inhibitory factor MIF and macrophage inflammatory protein (MIPalpha and beta). SCNSL PDX 2958 lymphoma cells secreted MIF, IL16, IL6, IL10 and CXCL10/IP-10.

Figure S7



**Supplementary Fig. S7** Hallmark pathways that were significantly up- or down regulated in CNS versus spleen comparison in PCNSL and SCNSL.

a: Heatmap of significantly up- and downregulated hallmark pathways for PCNSL CNS versus spleen comparison (left side) and for SCNSL CNS versus spleen comparison (right side). Three significantly upregulated pathways for PCNSL CNS versus spleen comparison are marked in bold and shown as heatmaps in b. Two significantly upregulated pathways for SCNSL CNS versus spleen comparison are marked in bold and shown as heatmaps in c. The significance ( $p$ -value  $< 0.05$ ) is marked with asterisk. The enrichment score (color coded) represents either the positive or negative  $\log_{10}$   $p$ -values in case of depleted or enriched gene-sets respectively. Gene-sets were re-ordered according to the hierarchical clustering based on Euclidean distance.

b: Heatmaps of gene sets of the 3 significantly upregulated hallmark pathways (epithel mesenchymal transition, inflammatory response and myogenesis) showing only significantly regulated genes (marked with asterisk,  $p$ -value  $< 0.05$ ) for PCNSL CNS versus spleen comparison.

c: Heatmaps of gene sets of 2 significantly upregulated hallmark pathways (TNFA signaling via NFkB and inflammatory response) showing only significantly regulated genes (marked with asterisk,  $p$ -value  $< 0.05$ ) for SCNSL CNS versus spleen comparison.