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Supplemental information

**Intergenic risk variant *rs56258221* skews
the fate of naive CD4⁺ T cells via miR4464-BACH2
interplay in primary sclerosing cholangitis**

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SUPPLEMENTARY FIGURES AND FIGURE LEGENDS

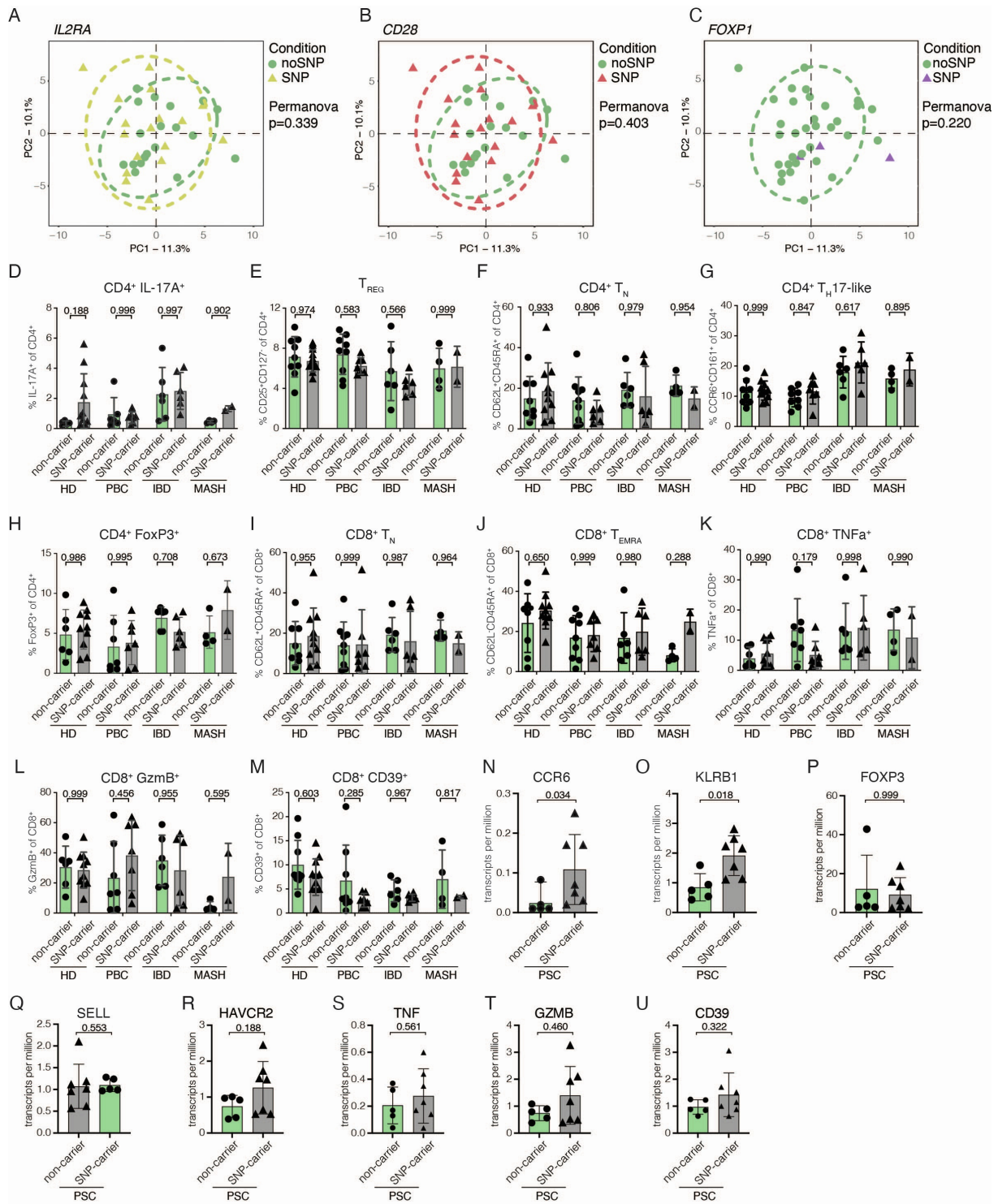


Fig. S1: People with PSC carrying *rs56258221* show distinct immunophenotypes. Related to Figure 1.

(A-C) Principal component analyses of the analyzed immunophenotyping data from peripheral blood of people with PSC, separated by the genotype for polymorphism *rs4147359* (*IL2RA*) (A), *rs7426056* (*CD28*) (B) and *rs80060485* (*FOXP1*) (C). (D-H) Frequencies of different CD4⁺ T cell subsets. (D) T_N, identified by CD62L/CD45RA. (E) T_H17 cells identified by IL-17A expression upon stimulation with PMA/Ionomycin. (F)

T_H17 cells identified by CCR6/CD161. **(G)** T_{REG} identified by CD127/CD25. **(H)** T_{REG} identified by FoxP3 expression upon stimulation with PMA/Ionomycin. **(I-M)** Frequencies of different CD8⁺ T cell subsets. **(I)** T_N, identified by CD62L/CD45RA. **(J)** T_{EMRA}, identified by CD62L/CD45RA. **(K)** CD39 expression on CD8⁺ T cells. **(L)** TNF α expression upon stimulation with PMA/Ionomycin. **(M)** GzmB expression upon stimulation with PMA/Ionomycin. Statistics: Normality distribution was tested by Kolmogorov-Smirnov test; normal distribution: Welch's t-Test or 2-way ANOVA; no normal distribution: Mann-Whitney U Test. p<0.05 was considered statistically significant. Data is presented as mean \pm SD and deriving from n \geq 2 repeats per experiment.

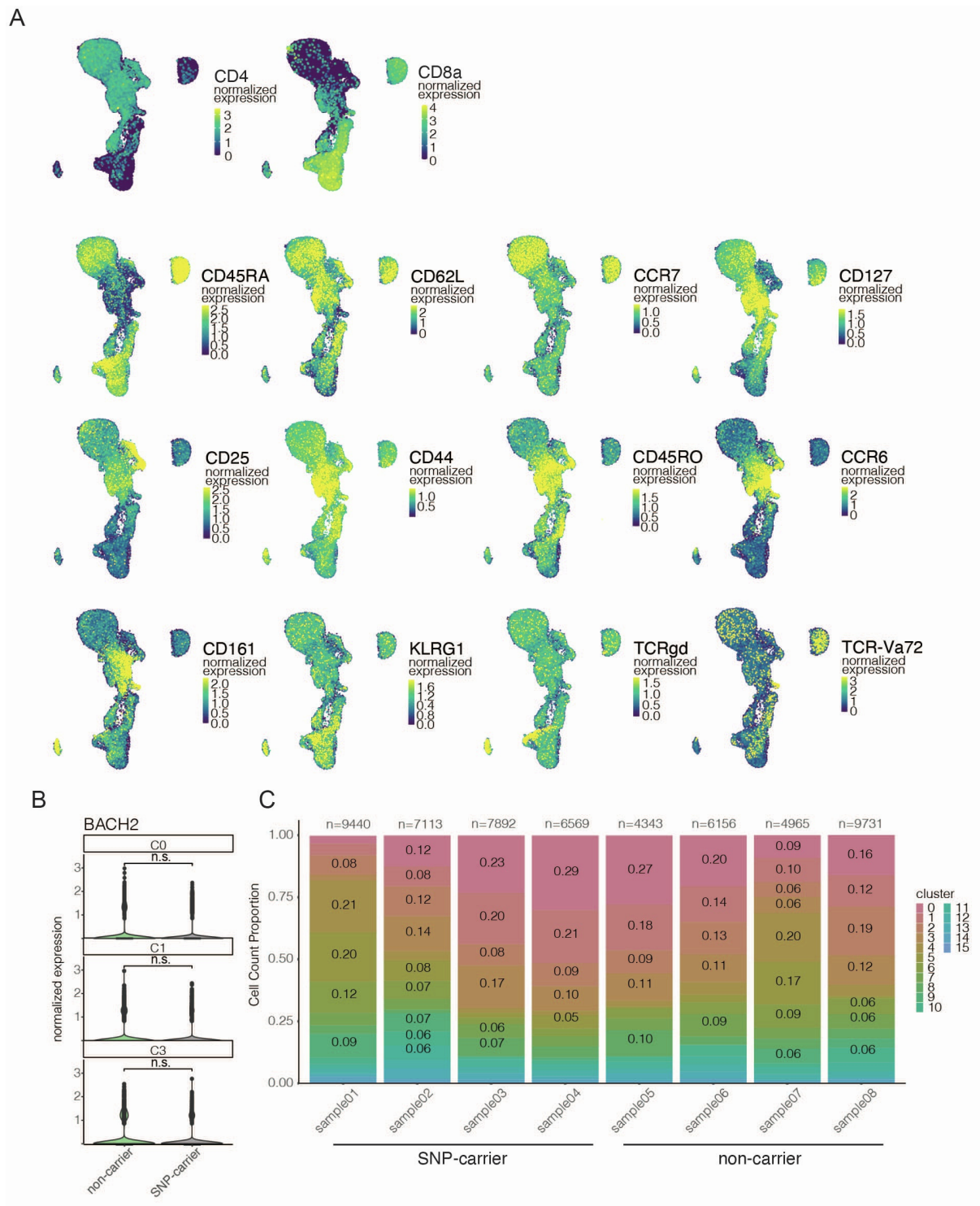


Fig. S2: Characterization of peripheral blood T cells from homozygous carriers of *rs56258221*. Related to Figure 2.

(A) Data of antibody-derived tags, resembling protein expression, mapped onto the UMAP from Fig. 2B.

Differentiation markers were used to assist cluster assignment. (B) BACH2 mRNA expression levels in CD4⁺

(C0, C1) and CD8⁺ T_N (C3) between SNP-carriers and non-carriers. (C) Distribution of cells per sample across the different clusters of T cells between SNP-carriers and non-carriers.

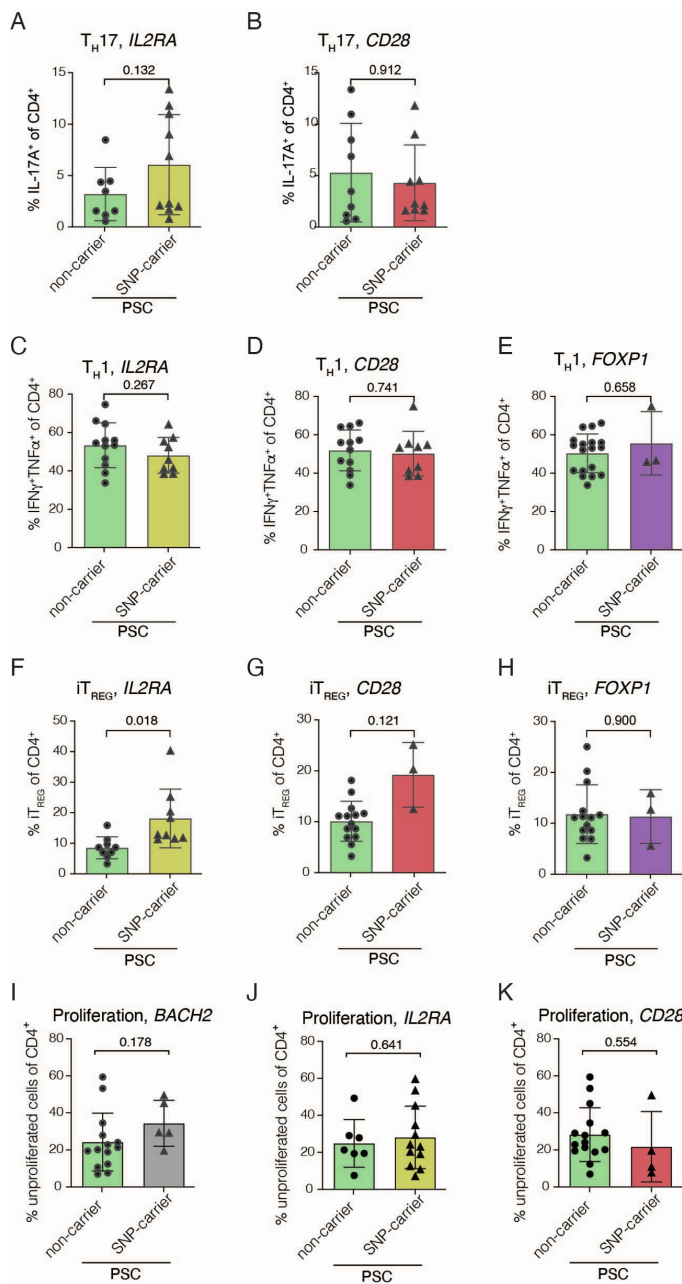


Fig. S3: Skewed differentiation of naive CD4⁺ T cells from people with PSC is associated with *rs56258221*.

Related to Figure 3.

(A-B) After 12 d of polarizing culture of CD4⁺ T_N from patients with PSC (n=18) the frequency of CD4⁺IL-

17A⁺ cells was determined and separated by *rs4147359* (*IL2RA*) (A), *rs7426056* (*CD28*) (B). *rs80060485*

(*FOXP1*) is not shown as no datapoints for SNP-carriers were recorded. (C-E) After 7 d of polarizing culture of

CD4⁺ T_N from patients with PSC (n=21), the frequency of T CD4⁺CXCR3⁺TBET⁺IFN γ ⁺TNF α ⁺ cells was

determined and data was separated into carriers and non-carriers of *rs4147359* (*IL2RA*) (C), *rs7426056* (*CD28*)

(D) and *rs80060485* (*FOXP1*) (E). (F-H) After 7 days of polarizing culture of CD4⁺ T_N from patients with PSC

(n=18), the frequencies of CD4⁺CD25⁺CD152⁺FOXP3⁺ cells were determined and data was separated into

carriers and non-carriers of *rs4147359 (IL2RA)* (F), *rs7426056 (CD28)* (G) and *rs80060485 (FOXP1)* (H). **(I-K)**

After 6 d of non-polarizing culture of *CellTrace Violet*-marked CD4⁺ T_N from patients with PSC, the frequency of divided and undivided cells was determined by flow cytometry. Data was separated into carriers and non-carriers of *rs56258221 (BACH2)* *rs4147359 (IL2RA)* (J), *rs7426056 (CD28)* (K). Data is presented as mean ± SD and deriving from $n \geq 2$ repeats per experiment.

SUPPLEMENTARY TABLES

Table S1: Clinical parameters of people with PSC, related to Figure 1A-M.

patient #	SNP-status	gender	year of birth	IBD status	other autoimmune disease	immunosuppr. therapy	transient elastography [kPa]	MELD-score	ANAs (Ref.: <1:80)	SMA (Ref.: <1:80)	SLA/LP (Ref.: <20relU/ml)
patient #1	non-carrier	cis	1967	PSC-assoc. colitis	/	/	10	6,0	negative	negative	negative
patient #2	non-carrier	cis	1968	PSC-assoc. colitis	Hashimoto's disease	/	8,8	7,1	1:80	negative	negative
patient #3	non-carrier	cis	1983	/	/	/	4,5	6,8	negative	negative	negative
patient #4	non-carrier	cis	1957	PSC-assoc. colitis	/	/	3,9	6,0	1:80	negative	negative
patient #5	non-carrier	cis	1968	/	/	/	5,3	6,0	negative	n.a.	n.a.
patient #6	non-carrier	cis	1982	PSC-assoc. colitis	/	/	60,4	33,4	1:640	negative	negative
patient #7	non-carrier	cis	1968	PSC-assoc. colitis	/	/	4,7	6,0	1:80	negative	negative
patient #8	non-carrier	cis	1965	/	/	/	16,5	15,8	1:160	negative	negative
patient #9	non-carrier	cis	1955	/	/	/	6,3	6,0	negative	negative	negative
patient #10	non-carrier	cis	1959	/	/	/	5,7	6,0	1:80	negative	negative
patient #11	non-carrier	cis	1965	PSC-assoc. colitis	/	Azathioprine	32	6,9	1:640	1:160	negative
patient #12	non-carrier	cis	1967	PSC-assoc. colitis	/	/	4,9	6,7	1:320	negative	negative
patient #13	non-carrier	cis	1978	PSC-assoc. colitis	Graves' disease	/	4,7	6,0	1:160	1:80	negative
patient #14	non-carrier	cis	1977	PSC-assoc. colitis	/	Azathioprine	4	6,2	negative	negative	negative
patient #15	non-carrier	cis	1974	PSC-assoc. colitis	/	/	3,7	6,0	1:320	negative	negative
patient #16	non-carrier	cis	1982	PSC-assoc. colitis	/	/	4,3	6,0	1:320	negative	negative
patient #17	non-carrier	cis	1941	/	/	/	4,1	6,0	negative	negative	negative
patient #18	non-carrier	cis	1966	/	/	/	7,8	6,0	1:160	negative	negative
patient #19	heterozygous	cis	1950	PSC-assoc. colitis	/	/	7,6	6,0	1:1280	negative	negative
patient #20	heterozygous	cis	1979	PSC-assoc. colitis	/	/	34,4	13,0	1:5120	1:180	negative
patient #21	heterozygous	cis	1979	PSC-assoc. colitis	/	/	11,7	6,0	1:320	negative	negative
patient #22	heterozygous	cis	1953	PSC-assoc. colitis	/	/	3,5	6,0	1:320	negative	negative
patient #23	heterozygous	cis	1986	PSC-assoc. colitis	/	/	7,5	7,2	1:80	negative	negative
patient #24	heterozygous	cis	1978	PSC-assoc. colitis	/	/	4,4	6,0	1:320	1:320	negative
patient #25	heterozygous	cis	1958	/	/	/	n.a.	18,9	1:160	1:80	negative
patient #26	heterozygous	cis	1963	/	/	/	70	14,5	1:320	1:80	negative
patient #27	heterozygous	cis	1957	PSC-assoc. colitis	Hashimoto's disease	/	7,2	6,0	1:80	1:80	negative
patient #28	heterozygous	cis	1976	/	/	/	11,3	7,8	1:160	negative	negative
patient #29	heterozygous	cis	1969	PSC-assoc. colitis	DM type 1	/	6,6	7,7	1:320	1:160	negative
patient #30	heterozygous	cis	1952	PSC-assoc. colitis	/	/	n.a.	9,6	1:160	negative	negative
patient #31	heterozygous	cis	1977	PSC-assoc. colitis	/	/	4,8	6,0	1:180	negative	negative
patient #32	heterozygous	cis	1969	/	/	/	3,6	6,0	1:160	negative	negative
patient #33	heterozygous	cis	1956	Crohn's disease	Graves' disease	/	n.a.	21,1	1:640	negative	n.a.
patient #34	homozygous	cis	1957	PSC-assoc. colitis	/	/	7,1	7,5	1:640	negative	negative
patient #35	homozygous	cis	1969	PSC-assoc. colitis	/	/	6	6,8	1:1280	negative	negative
patient #36	homozygous	cis	1963	PSC-assoc. colitis	/	/	37,4	6,0	1:640	negative	negative

Table S2: Clinical parameters of people with PSC, related to Figure 2A-J.

patient #	SNP-status	gender	year of birth	IBD status	other autoimmune disease	immunosuppr. therapy	transient elastography [kPa]	MELD-Score	ANAs (Ref.: <1:80)	SMA (Ref.: <1:80)	SLA/LP (Ref.: <20relU/ml)
patient #1	homozygous	cis	1969	PSC-assoc. colitis	/	/	6	6,8	1:1280	negative	negative
patient #2	homozygous	cis	1987	/	/	/	20	11,8	1:320	negative	negative
patient #3	homozygous	cis	1980	PSC-assoc. colitis	/	Vedolizumab	5,6	6,0	negative	negative	negative
patient #4	homozygous	cis	1975	PSC-assoc. colitis	/	/	5,3	6,0	negative	1:80	negative
patient #5	non-carrier	cis	1966	/	/	/	7,8	6,0	1:160	negative	negative
patient #6	non-carrier	cis	1979	/	/	/	4,7	6,0	1:160	negative	negative
patient #7	non-carrier	cis	1976	/	/	/	6,3	6,0	1:160	negative	negative
patient #8	non-carrier	cis	1972	Crohn's disease	/	/	6,7	6,0	1:160	negative	negative

Table S3: Clinical parameters of people with PSC, related to Figures 2K-L and 4D.

patient #	SNP-status	gender	year of birth	IBD status	other autoimmune disease	immunosuppr. therapy	transient elastography [kPa]	MELD-Score	ANAs (Ref.: <1:80)	SMA (Ref.: <1:80)	SLA/LP (Ref.: <20relU/ml)
patient #1	non-carrier	cis	1966	/	/	/	7,8	6,0	1:160	negative	negative
patient #2	non-carrier	cis	1979	/	/	/	4,7	6,0	1:160	negative	negative
patient #3	non-carrier	cis	1976	/	/	/	6,3	6,0	1:160	negative	negative
patient #4	non-carrier	cis	1972	Crohn's disease	/	/	6,7	6,0	1:160	negative	negative
patient #5	non-carrier	cis	1980	PSC-assoc. colitis	/	/	7,8	6,0	1:160	negative	negative
patient #6	non-carrier	cis	1994	PSC-assoc. colitis	/	Azathioprin	4,9	6,0	1:320	1:80	negative
patient #7	non-carrier	cis	1975	PSC-assoc. colitis	Psoriasis vulgaris	Ustekinumab	6,3	6,0	1:160	negative	negative
patient #8	homozygous	cis	1944	PSC-assoc. colitis	/	/	14,3	14,1	1:160	1:80	negative
patient #9	homozygous	cis	1957	PSC-assoc. colitis	/	/	7,1	7,5	1:640	negative	negative
patient #10	homozygous	cis	1986	PSC-assoc. colitis	/	/	5,2	6,0	1:640	1:80	negative
patient #11	homozygous	cis	1972	PSC-assoc. colitis	/	/	43,8	14,5	1:320	negative	negative
patient #12	homozygous	cis	1963	PSC-assoc. colitis	/	/	37,4	6,0	1:640	negative	negative
patient #13	homozygous	cis	1969	PSC-assoc. colitis	/	/	6	6,8	1:1280	negative	negative
patient #14	homozygous	cis	1987	/	/	/	20	11,8	1:320	negative	negative
patient #15	homozygous	cis	1980	PSC-assoc. colitis	/	Vedolizumab	5,6	6,0	negative	negative	negative
patient #16	homozygous	cis	1975	PSC-assoc. colitis	/	/	5,3	6,0	negative	1:80	negative

Table S4: Clinical parameters of people with PSC, related to Figure 3H+K.

patient #	SNP-Status	gender	year of birth	IBD status	other autoimmune disease	immunosuppr. therapy	transient elastography [kPa]	MELD-score	ANAs (Ref.: <1:80)	SMA (Ref.: <1:80)	SLA/LP (Ref.: <20relU/ml)
patient #1	non-carrier	cis	1982	PSC-assoc. colitis	/	/	4,3	6,0	1:320	negative	negative
patient #2	non-carrier	cis	1977	PSC-assoc. colitis	/	/	6	6,0	1:80	1:80	negative
patient #3	non-carrier	cis	1967	PSC-assoc. colitis	/	/	4,9	6,7	1:320	negative	negative
patient #4	non-carrier	cis	1967	PSC-assoc. colitis	/	/	6,1	6,0	1:160	negative	negative
patient #5	non-carrier	cis	1980	PSC-assoc. colitis	/	/	2,1	9,9	1:640	negative	negative
patient #6	non-carrier	cis	1977	PSC-assoc. colitis	/	/	7,6	6,0	1:160	negative	negative
patient #7	non-carrier	cis	1972	PSC-assoc. colitis	/	/	23	6,0	negative	negative	negative
patient #8	non-carrier	cis	1987	/	/	/	75	6,0	negative	negative	negative
patient #9	non-carrier	cis	1964	/	/	/	4,7	6,0	1:160	negative	negative
patient #10	non-carrier	cis	1975	PSC-assoc. colitis	/	Azathioprin	14	7,1	1:160	negative	23.0
patient #11	non-carrier	cis	1976	PSC-assoc. colitis	Hashimoto's disease	/	7,4	6,0	1:80	negative	negative
patient #12	heterozygous	cis	1964	PSC-assoc. colitis	/	/	4,8	6,9	1:320	negative	negative
patient #13	heterozygous	cis	1950	PSC-assoc. colitis	/	/	7,6	6,0	1:1280	negative	negative
patient #14	heterozygous	cis	1961	PSC-assoc. colitis	Rheumatoid arthritis	Baricitinib	12,9	6,0	1:160	negative	negative
patient #15	heterozygous	cis	1980	PSC-assoc. colitis	/	/	6,1	6,0	1:80	negative	negative
patient #16	heterozygous	cis	1953	PSC-assoc. colitis	/	/	3,5	6,0	1:320	negative	negative
patient #17	homozygous	cis	1963	PSC-assoc. colitis	/	/	37,4	6,0	1:640	negative	negative
patient #18	homozygous	cis	1994	PSC-assoc. colitis	/	/	71	6,6	1:640	1:160	negative
patient #19	homozygous	cis	1980	PSC-assoc. colitis	/	/	5,6	6,0	negative	negative	negative

Table S5: Clinical parameters of people with PSC, related to Figure 3G+J.

patient #	SNP-status	gender	year of birth	IBD status	other autoimmune disease	immunosuppr. therapy	transient elastography [kPa]	MELD-score	ANAs (Ref.: <1:80)	SMA (Ref.: <1:80)	SLA/LP (Ref.: <20relU/ml)
patient #1	non-carrier	cis	1997	Crohn's disease	/	/	7	8,3	1:160	negative	negative
patient #2	non-carrier	cis	1956	/	/	/	4,5	6,0	1:160	negative	negative
patient #3	non-carrier	cis	1962	PSC-assoc. colitis	/	/	5,6	6,0	1:80	1:160	negative
patient #4	non-carrier	cis	1980	PSC-assoc. colitis	/	/	2,1	9,9	1:640	negative	negative
patient #5	non-carrier	cis	1951	PSC-assoc. colitis	/	Vedolizumab	4,3	6,0	1:160	negative	negative
patient #6	non-carrier	cis	1974	PSC-assoc. colitis	/	/	3,7	6,0	1:320	negative	negative
patient #7	non-carrier	cis	1950	/	/	/	4,6	6,0	1:5120	negative	negative
patient #8	non-carrier	cis	1980	PSC-assoc. colitis	/	/	7,8	6,0	1:160	negative	negative
patient #9	non-carrier	cis	1987	PSC-assoc. colitis	/	/	5,8	6,0	1:640	1:160	negative
patient #10	non-carrier	cis	1987	PSC-assoc. colitis	/	/	6,1	6,0	1:160	negative	negative
patient #11	non-carrier	cis	1962	PSC-assoc. colitis	/	/	3,3	6,0	1:80	negative	negative
patient #12	heterozygous	cis	1999	PSC-assoc. colitis	/	Fligotinib	4,7	6,0	1:80	negative	negative
patient #13	heterozygous	cis	1977	PSC-assoc. colitis	/	/	4,8	6,0	1:180	negative	negative
patient #14	heterozygous	cis	1966	/	Hashimoto's disease	/	4,2	6,0	1:160	negative	negative
patient #15	heterozygous	cis	1988	PSC-assoc. colitis	/	/	7,8	6,0	1:160	negative	negative
patient #16	heterozygous	cis	1968	PSC-assoc. colitis	/	/	42	16,0	1:5120	negative	n.a.
patient #17	heterozygous	cis	1957	/	/	/	6,6	6,0	1:160	negative	negative
patient #18	heterozygous	cis	1976	PSC-assoc. colitis	/	/	4,9	6,0	1:80	negative	negative
patient #19	heterozygous	cis	2000	Crohn's disease	/	Ustekinumab	3,7	6,0	1:1280	negative	negative
patient #20	heterozygous	cis	1969	/	/	/	3,6	6,0	1:160	negative	negative
patient #21	heterozygous	cis	1977	/	/	/	4,9	7,5	1:160	negative	negative

Table S6: Clinical parameters of people with PSC, related to Figure 3F+I.

patient #	SNP-status	gender	year of birth	IBD status	other autoimmune disease	immunosuppr. therapy	transient elastography [kPa]	MELD-score	ANAs (Ref.: <1:80)	SMA (Ref.: <1:80)	SLA/LP (Ref.: <20relU/ml)
patient #1	non-carrier	cis	1988	PSC-assoc. colitis	/	/	28	6,0	1:80	1:160	negative
patient #2	non-carrier	cis	1961	Crohn's disease	Hashimoto's disease	/	4,7	6,0	1:320	negative	negative
patient #3	non-carrier	cis	1977	PSC-assoc. colitis	Hashimoto's disease	/	10,4	7,4	1:640	negative	negative
patient #4	non-carrier	cis	1963	/	/	/	6,2	6,0	1:80	1:80	negative
patient #5	non-carrier	cis	1979	/	/	/	4,7	6,0	1:160	negative	negative
patient #6	non-carrier	cis	1988	Crohn's disease	/	Vedolizumab	8,8	12,8	1:640	negative	negative
patient #7	heterozygous	cis	1967	/	/	/	9,3	6,0	1:160	negative	negative
patient #8	heterozygous	cis	1975	PSC-assoc. colitis	/	/	7,1	6,0	1:160	negative	negative
patient #9	heterozygous	cis	1976	/	/	/	7,4	6,0	1:320	negative	negative
patient #10	heterozygous	cis	1976	PSC-assoc. colitis	/	/	4,9	6,0	1:80	negative	negative
patient #11	heterozygous	cis	1950	/	/	/	9,3	6,0	1:160	negative	negative
patient #12	heterozygous	cis	1950	PSC-assoc. colitis	/	/	12,7	7,2	1:320	negative	negative
patient #13	heterozygous	cis	1965	/	/	/	6,7	6,0	1:160	negative	negative
patient #14	heterozygous	cis	1965	/	/	/	5	6,0	1:320	negative	negative
patient #15	heterozygous	cis	1959	PSC-assoc. colitis	/	/	12,3	6,0	1:5120	negative	negative
patient #16	homozygous	cis	1963	PSC-assoc. colitis	/	/	37,4	6,0	1:640	negative	negative
patient #17	homozygous	cis	1944	PSC-assoc. colitis	/	/	14,3	14,1	1:160	1:80	negative

*Abbreviations:

n.a.: information not available

/: patient without IBD; no immunosuppressive therapy; absence of tested autoantibodies

Table S7: Primers for generation of BACH2 plasmid, related to Figure 5F-H.

Primer	Sequence	Gene
Pr1	CACACGAGCTCGATACTGTACAGTAATGG	Forward primer (3'UTR BACH2-mRNA)
Pr2	CACACGTCGACAAAGCAAAGAATATACAG	Reverse primer (3'UTR BACH2-mRNA)
Fr1	(SacI)GAGCTCGATACTGTACANCCACTGGTCAACTTTGCCACTTGCA CTGAGTTTTGGGTCAAACCTATTTTCTTAAATGAAGTTGTAACCTTCG GTATAACTCAAGTATACTGTATATTCTTTGCTTTGTCGAC(SalI)	BACH2 3'UTR fragment (miR4464 site in bold letters)
Pr4	GAAAATACTCGAGACCCAAAACCTCAGTGC	Reverse primer (to mutate the miR4464 site)
Fr2	(SacI)GAGCTCGATACTGTACANCCACTGGTCAACTTTGCCACTTGCA CTGAGTTTTGGGTCTCGAGTATTTTCTTAAATGAAGTTGTAACCTTCG GTATAACTCAAGTATACTGTATATTCTTTGCTTTGTCGAC(SalI)	Mutated miR-4464 site in BACH2 3'-UTR (mutated part in bold letters)