SUPPLEMENTARY INFORMATION

Macrophage metabolic regulation by phosphoglucomutase 1 shapes the host immune response in pneumococcal meningitis

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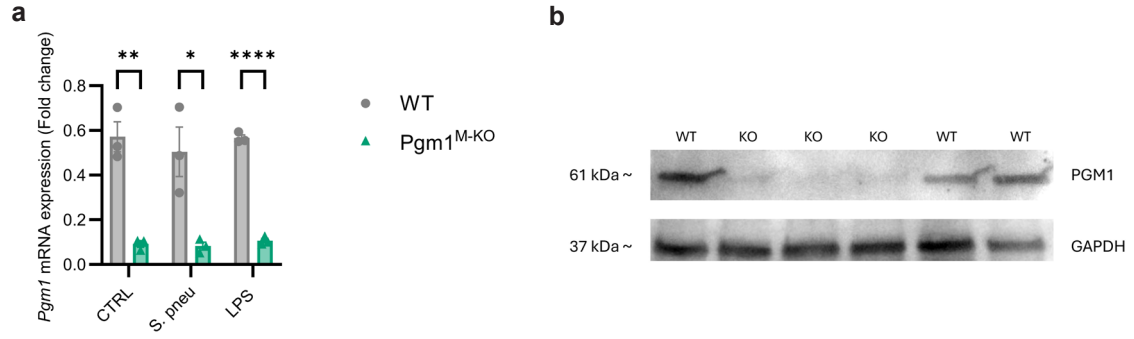
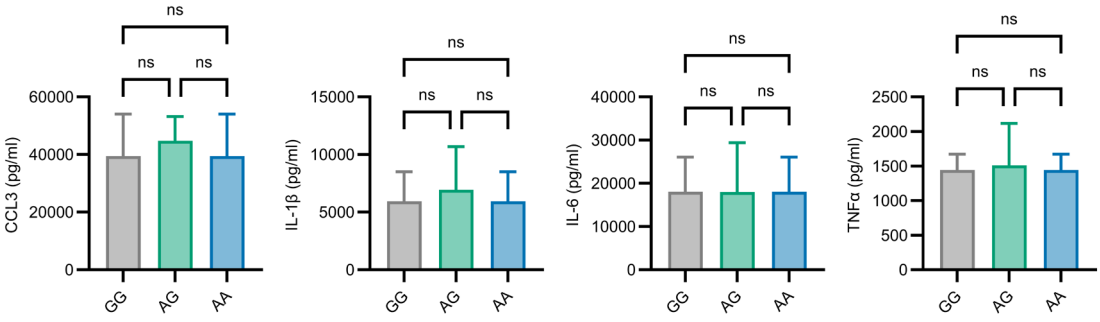
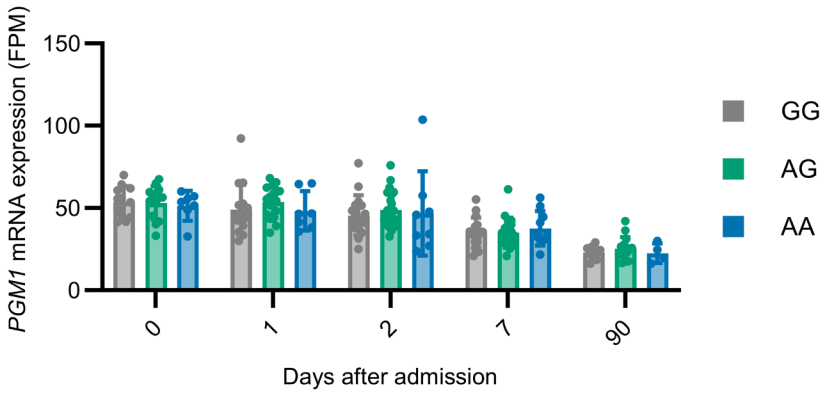
SUPPLEMENTARY FIGURES



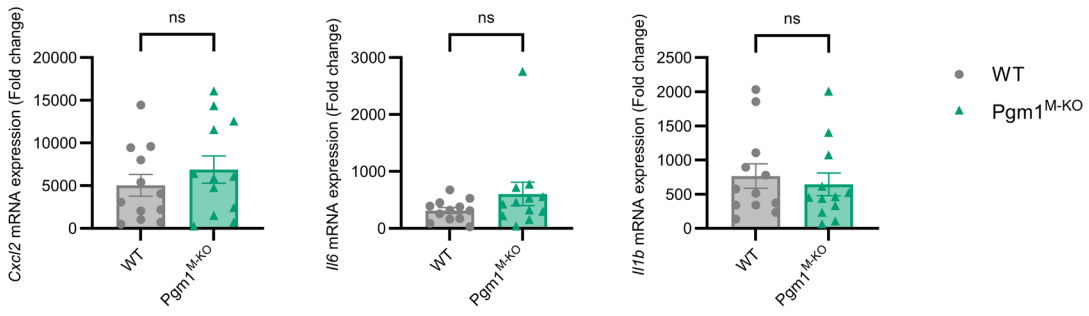
Supplementary Figure 1. Validation of *Pgm1* knockdown in bone marrow-derived macrophages. Knockdown of *Pgm1* was validated at the mRNA and protein levels in BMDMs derived from wild-type (WT) and Pgm1$^{M\text{-}KO}$ mice. (**a**) *Pgm1* mRNA expression in untreated BMDMs (CTRL), BMDMs infected with *S. pneumoniae* (MOI 1) and BMDMs stimulated with LPS (10 ng/mL) for 4 hours. (**b**) Western blot showing PGM1 protein expression in unstimulated wild type (WT) and Pgm1$^{M\text{-}KO}$ (KO) BMDMs with GAPDH used as a loading control. Data are shown as mean ± SEM. N = 3 mice per genotype. Group differences were assessed using a two-sided t test. *, p-value < 0.05; **, p-value < 0.01; ***, p-value <0.001; ****, p-value <0.0001. BMDMs, bone marrow-derived macrophages; MOI, multiplicity of infection; LPS, lipopolysaccharide; SEM, standard error of the mean.



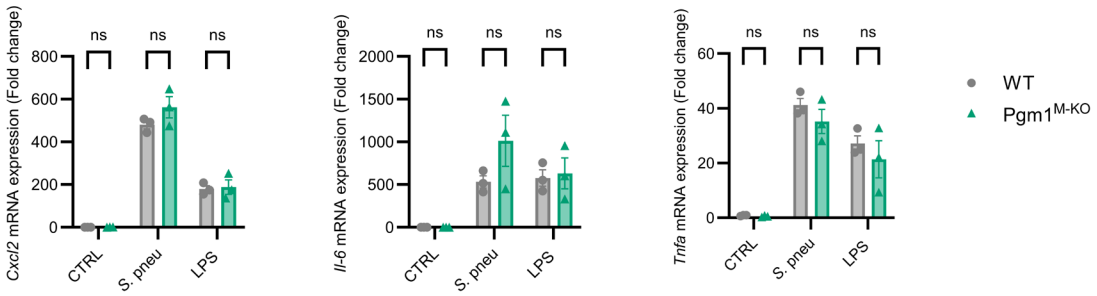
Supplementary Figure 2. PBMC cytokine production after LPS stimulation is not influenced by rs12081070 genotype. Cytokine concentrations in supernatants of PBMCs after 24 hours of stimulation with LPS (10 ng/mL). PBMCs were harvested from patients with the GG (N = 19), AG (N = 38) or AA (N = 14) genotype, one to five years after they were hospitalized for pneumococcal meningitis. LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell. Data are presented as median with interquartile range. Group differences are calculated with a Kruskal-Wallis test followed by a post-hoc two-sided Dunn's test. Ns, non-significant. PBMC, peripheral blood mononuclear cells; LPS, lipopolysaccharide.



Supplementary Figure 3. *PGM1* gene expression in whole blood of pneumococcal meningitis patients is not associated with rs12081070 genotype. *PGM1* fragments per million (FPM) in whole blood of patients with pneumococcal meningitis at days 0 (N = 7 AA, 13 AG, 12 GG), 1 (N = 7 AA, 18 AG, 16 GG), 2 (N = 8 AA, 24 AG, 17 GG), 7 (N = 10 AA, 19 AG, 16 GG), and 90 (n = 4 AA, 15 AG, 10 GG) after hospital admission, stratified by rs12081070 genotype. No significant differences in expression were observed between genotypes at any time point. Data are presented as median with interquartile range. Group differences were assessed using a Kruskal-Wal-lis test followed by a post-hoc two-sided Dunn's test.



Supplementary Figure 4. Cytokine gene expression in the brain is not influenced by Pgm1 during murine pneumococcal meningitis. Gene expression was determined in the brain wild-type (WT) and *Pgm1* knockdown ($Pgm1^{M\text{-}KO}$) mice, 20 hours after intracisternal infection with *S. pneumoniae*. Expression of *Cxcl2, Il6 and Il1β* were determined by qPCR in 12 mice per group. All data was normalized against the average data of uninfected wild type-mice. Values from individual mice are shown with the mean ± SEM. Group differences were assessed using a two-sided t-test. BMDMs, bone marrow-derived macrophages; MOI, multiplicity of infection; LPS, lipopolysaccharide; SEM, standard error of the mean; Ns, non-significant.



Supplementary Figure 5. Cytokine gene expression in bone marrow-derived macrophages is not in luenced by *Pgm1*. Gene expres-sion of *Cxcl2, Il6 and Tnfα* in untreated BMDMs, and BMDMs infected with *S. pneumoniae* (MOI 1) or stimulated with LPS (10 ng/mL) for 24 hours. Gene expression was determined by qPCR. All data was normalized against the average data of unstimulated wild type-mice. Data are shown as mean ± SEM. N = 3 mice per genotype. Group differences were assessed using a two-sided t test. BMDMs, bone mar-row-derived macrophages; MOI, multiplicity of infection; LPS, lipopolysaccharide; SEM, standard error of the mean; Ns, non-significant.

Supplementary Table 1. Clinical severity score for murine pneumococcal meningitis

Code	Item	Score
	Appearance	
	Weight loss	
1	< 5% weight loss	1
2	5-10% weight loss	2
3	11-15% weight loss	3
4	16-20% weight loss	4
5	≥20% weight loss	HEP
	Posture	
1	Slightly hunched back	1
2	Severe hunched back	2
	Coat	
1	Diminished/lack grooming	1
2	Piloerection	1
3	Combination 1+2	2
	Eyes	
1	Discharge from the eyes	1
2	Closed eyelids	1
3	Protruding eyes	1
4	Combination 1+2	2
5	Combination 1+3	2
	Behaviour	
	Activity	
1	Diminished activity	2
2	Inactive	3
3	Increased activity / aggressive behaviour	1
	Condition	
1	Within 5 sec to right when placed on back	2
2	Within 30 sec to right when placed on back	4
3	Inability to right after placing on back	HEP
4	Coma	HEP
	Body function	
	Respiration	
1	Laboured breathing	2
2	Irregular breathing,	2
3	Combination 1+2	4
	Procedure-specific indicators	
	Neurologic score	
1	Paresis	2
2	Coordination problem (including circling)	2
3	(Partial) seizure	2
4	Combination 1+2	4
5	Combination 1+3	4
6	Combination 2+3	4
7	Combination 1+2+3	6
8	Paralysis	HEP
9	Seizure > 5 minutes	HEP
10	≥ 2 seizures in 15 min	HEP

HEP, humane endpoint

Supplementary Table 2. Histopathological scoring method of brain tissue in bacterial meningitis mouse model.

Main category	Subcategory	Score			
		0	1	2	3
Meningeal infiltration		Absent	Focal mild infiltration	Multifocal mild or focal severe infiltration	Multifocal severe infiltration
Parenchymal infiltration		Absent	Focal mild infiltration	Multifocal mild or focal severe infiltration	Multifocal severe infiltration
Vascular inflammation	Large meningeal artery inflammation Small parenchymal vessel inflammation	Absent	Focal mild subendothelial infiltration /reactive changes	Multifocal mild subendothelial infiltration /reactive changes or focal severe vascular wall infiltration with obstruction and/or destruction of vessels	Multifocal severe vascular wall infiltration with obstruction and/or destruction of vessels
Ventriculitis		Absent	A few inflammatory cells in the ventricle	Groups of inflammatory cells in the ventricle with/without ependymal infiltration	Extension of inflammatory cells into the periventricular tissue
Hemorrhage		Absent	Focal small damage	Multifocal small or focal large damage	Multifocal large damages
Thrombosis		Absent	Focal mild with partial obstruction of vascular lumen	Multifocal mild with partial obstruction of vascular lumen or focal severe with complete obstruction of vascular lumen and destruction of vessel wall	Multifocal severe with complete obstruction of vascular lumen and destruction of vessel wall
Abscess		Absent	Focal small damage	Multifocal small or focal large damage	Multifocal large damages

Supplementary Table 3. List of primers

Gene	Organism	5' forward primer	3' reverse primer
Pgm1	*Mus musculus*	GAGCATCGTCTCTACCGTGG	TAACCAGGCGACCAATCCCG
B-actin	*Mus musculus*	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCA
Rpl13a	*Mus musculus*	GGGCAGGTTCTGGTATTGGAT	GGCTCGGAAATGGTAGGGG
Il6	*Mus musculus*	TCTATACCACTTCACAAGTCGGA	GAATTGCCATTGCACAACTCTTT
Tnfα	*Mus musculus*	CTGAACTTCGGGGTGATCGG	GGCTTGTCACTCGAATTTTGAGA
Cxcl2	*Mus musculus*	AAGTTTGCCTTGACCTGAAG	ATCAGGTACGATCCAGGCTTC
Il1β	*Mus musculus*	GCCACCTTTTGACAGTGATGA	AAGCTGGATGCTCTCATCAGG
Erdr1	*Mus musculus*	CGGTCAAGATGTTCACCCG	GCTTCTACGTGTGTGCTTTCG

SUPPLEMENTARY TABLES

Supplementary Table 4: Clinical characteristics of patients included in the Recall cohort compared to all pneumococcal meningitis patients

Characteristic	**Included** N = 71	**Not included** N = 1,129	**p-value**
Age – years[a]	61 (54-64)	61 (51-69)	0.44
Female sex	35/71 (49%)	578/1,129 (51%)	0.81
Immunocompromised state[b]	14/71 (20%)	299/1,129 (26%)	0.26
rs12081070 genotype			0.76
GG	19/71 (27%)	348/1,129 (31%)	
AG	38/71 (54%)	578/1,129 (51%)	
AA	14/71 (20%)	203/1,129 (18%)	
Clinical symptoms			
Temperature - °C[c]	39.2 (37.9-39.7)	39.0 (38.0-39.7)	0.69
GCS score[d]	12 (10-13)	11 (9-13)	0.054
Altered mental status (<14)	54/70 (77%)	869/1,128 (77%)	>0.99
Coma (<8)	8/70 (11%)	263/1,128 (23%)	0.019
Outcome			
Unfavourable outcome	17/71 (24%)	357/1,129 (32%)	0.19
Mortality	0/71 (0%)	87/1,129 (8%)	0.008
Hearing impairment	4/66 (6%)	52/977 (5%)	0.78
Cognitive impairment	12/57 (21%)	202/888 (23%)	0.92
Cranial nerve palsy	3/62 (5%)	52/935 (6%)	>0.99
Focal cerebral deficits[e]	5/64 (8%)	81/949 (9%)	>0.99

Data presented as n/N (%) or median (IQR). Group differences were tested with a Fisher's exact test for categorical variables and a Mann–Whitney U test for continuous variables. Abbreviations: GCS, Glasgow Coma Scale; GOS. [a]Age is known for all patients. [b]Immunocompromised state is defined as active cancer, diabetes, alcoholism, immunosuppressive treatment, splenectomy or HIV. [c]Temperature is known in 69 episodes included the in Recall study and in 110+ episodes not included. [d]GCS is known in 69 episodes included the in Recall study and in 1128 episodes not included. [e]Focal cerebral deficits are defined as aphasia or mono-or hemiparesis

Supplementary Table 5: Clinical characteristics of patients included in the SMS cohort compared to all pneumococcal meningitis patients

Characteristic	**Not included** N = 65	**Included** N = 1135	**p-value**
Age – years[a]	63 (54-70)	61 (51-68)	0.32
Female sex	28/65 (43%)	585/1,135 (52%)	0.20
Immunocompromised state[b]	18/65 (28%)	295/1,135 (26%)	0.77
Genotype rs12081070			0.62
GG	23/65 (35%)	344/1,135 (30%)	
AG	30/65 (46%)	586/1,135 (52%)	
AA	12/65 (18%)	205/1,135 (18%)	
Clinical symptoms			
Temperature - °C[c]	38.8 (37.8-39.4)	39.0 (38.0-39.7)	0.16
GCS score[d]	11 (8-13)	11 (9-13)	0.48
Altered mental status (<14)	50/65 (77%)	873/1,133 (77%)	>0.99
Coma (<8)	19/65 (29%)	252/1,133 (22%)	0.22
Outcome			
Unfavourable outcome	28/65 (43%)	346/1,135 (30%)	0.039
Mortality	14/65 (22%)	73/1,135 (6%)	<0.001
Hearing impairment	18/45 (40%)	373/992 (38%)	0.75
Cognitive impairment	8/38 (21%)	206/907 (23%)	0.019
Cranial nerve palsy	2/42 (5%)	53/955 (6%)	>0.99
Focal cerebral deficits[e]	5/41 (12%)	81/972 (8%)	0.39

Data presented as n/N (%) or median (IQR). Group differences were tested with a Fisher's exact test for categorical variables and a Mann–Whitney U test for continuous variables. Abbreviations: GCS, Glasgow Coma Scale; GOS. [a]Age is known for all patients.
[b]Immunocompromised state is defined as active cancer, diabetes, alcoholism, immunosuppressive treatment, splenectomy or HIV.
[c]Temperature is known in 63 episodes included the in SMS study and in 1112 episodes not included. [d]GCS is known in 65 episodes included the in SMS study and in 1133 episodes not included. [e]Focal cerebral deficits are defined as aphasia or mono-or hemiparesis