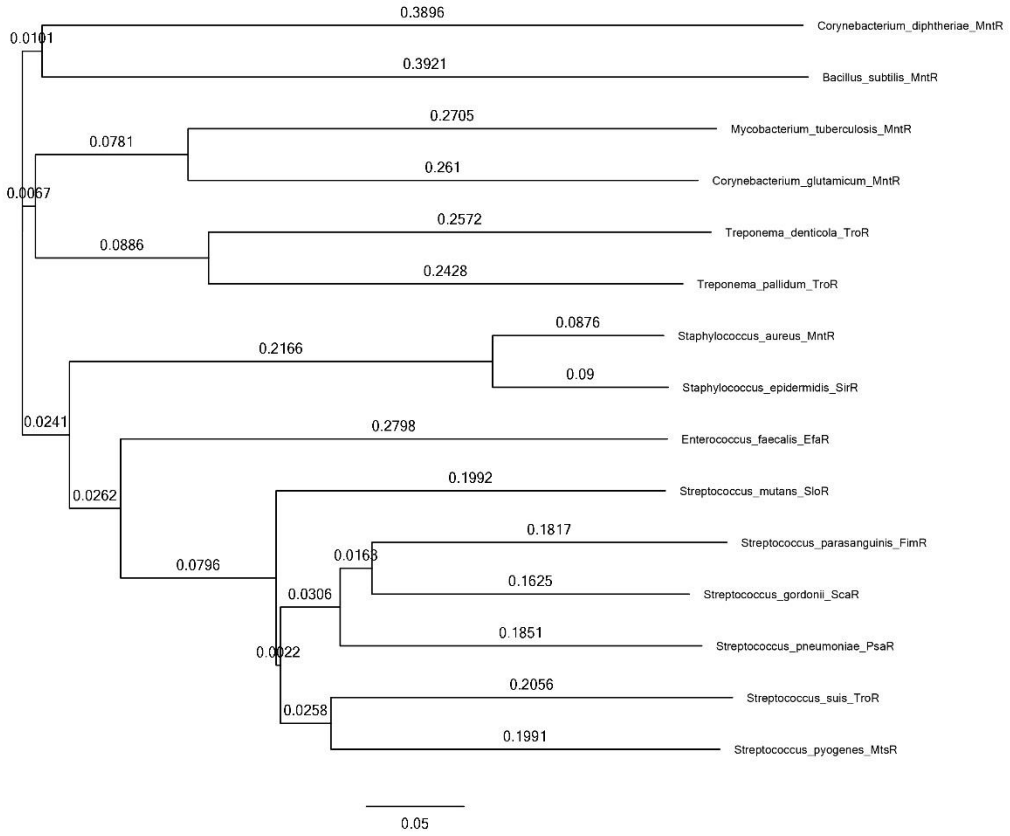

FIG S1 Phylogenetic analysis of the DtxR family metalloregulators. Protein sequences were aligned using Clustal Omega, and the generated phylogenetic tree was edited using FigTree v1.4.4. The GenBank accession numbers for the proteins are as follows: *Corynebacterium diphtheriae* MntR, WP_010935052.1; *Bacillus subtilis* MntR, NP_390332.2; *Mycobacterium tuberculosis* MntR, CCP45587.1; *Corynebacterium glutamicum* MntR, CAF19348.1; *Treponema denticola* TroR, WP_002678714.1; *Treponema pallidum* TroR, WP_010881614.1; *Staphylococcus aureus* MntR, WP_000954654.1; *Staphylococcus epidermidis* SirR, WP_001832139.1; *Enterococcus faecalis* EfaR, AAO80811.1; *Streptococcus mutans* SloR, WP_002262032.1; *Streptococcus parasanguinis* FimR, WP_014712822.1; *Streptococcus gordonii* ScaR, WP_012130850.1; *Streptococcus pneumoniae* PsaR, WP_000188498.1; *Streptococcus suis* TroR, WP_012027964.1; *Streptococcus pyogenes* MtsR, WP_011285412.1.

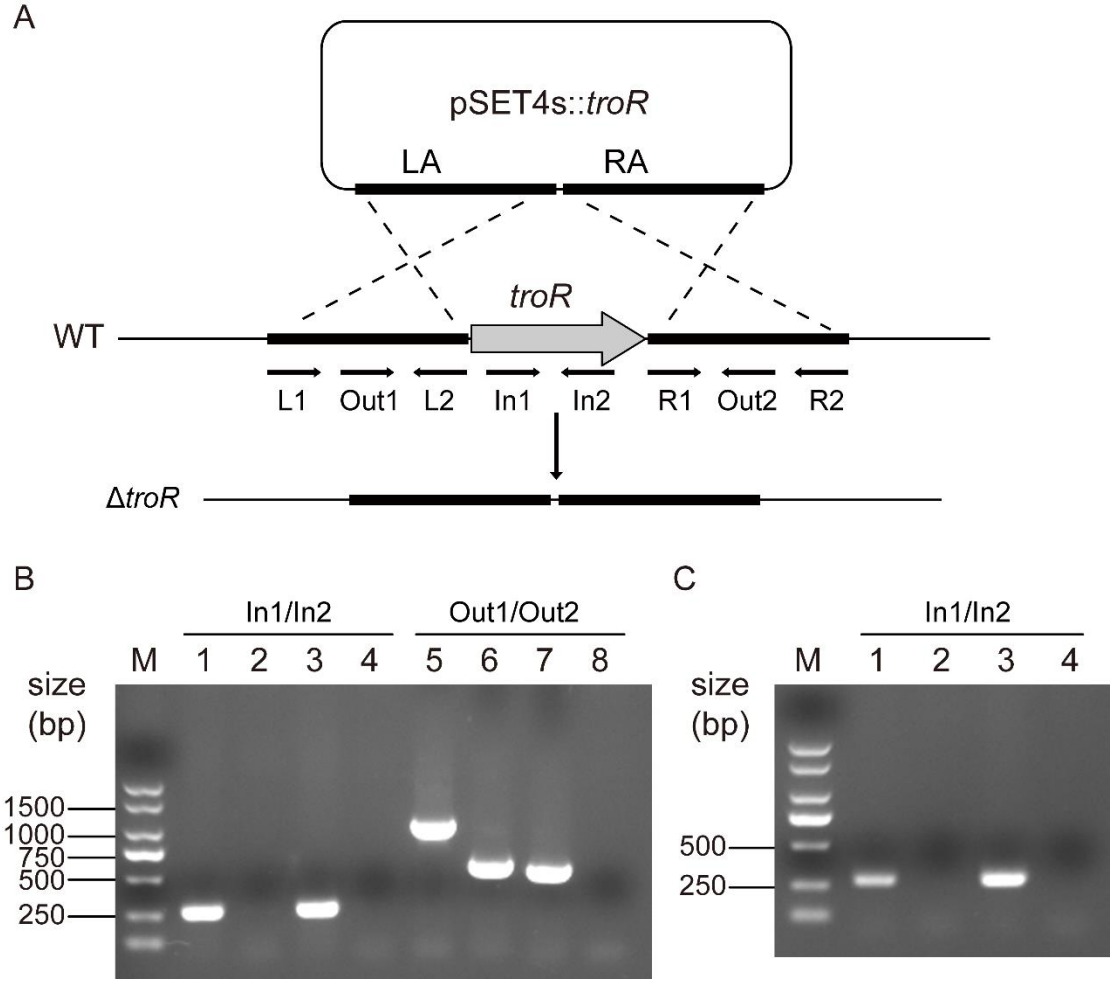

FIG S2 Construction and identification of the mutant and complementation strains. (A) Schematic representation of *troR* deletion in *S. suis* by homologous recombination. LA and RA indicate the left and right arms of *troR*. The primers used for construction and identification of the strains are indicated under the balck arrows. (B) Identification of the Δ*troR* and CΔ*troR* strains by PCR analysis. Genomic DNAs from the WT strain (lanes 1 and 5), Δ*troR* (lanes 2 and 6), CΔ*troR* (lanes 3 and 7) were used as the templates. PCR amplification with water as the templates served as the negative control (lanes 4 and 8). The primer pairs are indicated above the lanes. (C) Identification of the Δ*troR* and CΔ*troR* strains by reverse transcription PCR analysis. Total RNAs were isolated from the WT strain (lane 1), Δ*troR* (lanes 2), CΔ*troR* (lanes 3). cDNAs generated from these RNAs and water (lane 4) were used as the templates for PCR amplification. The primer pair is indicated above the lanes.

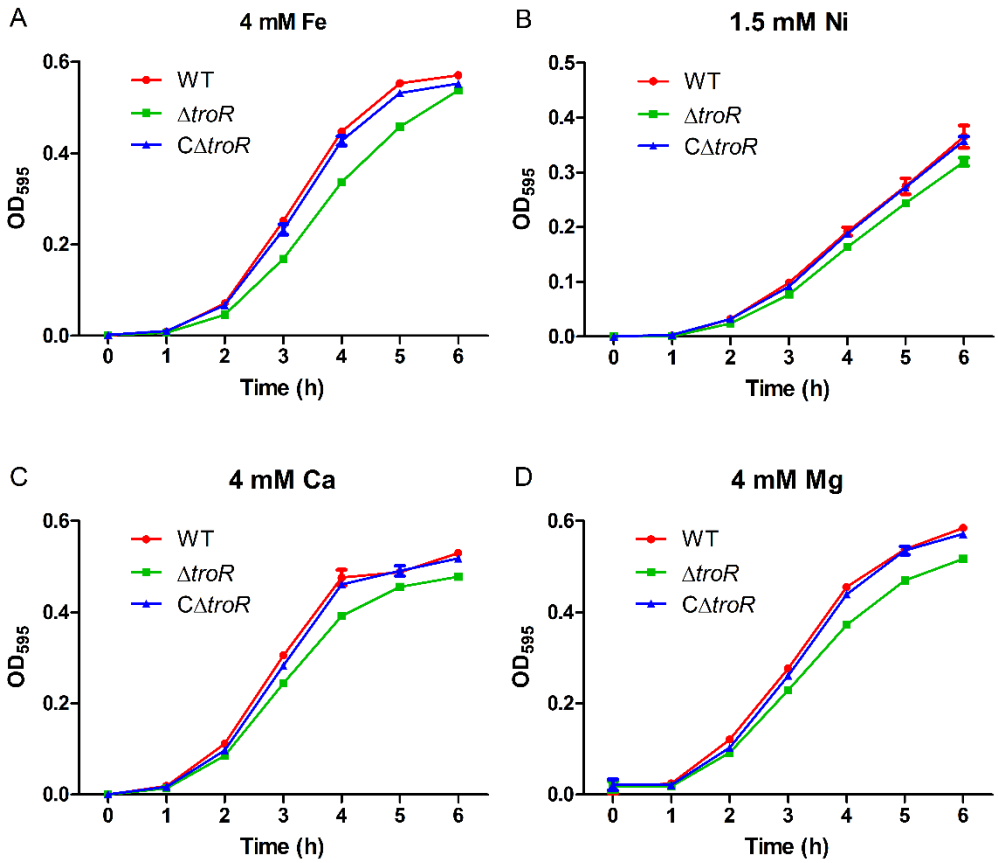

FIG S3 Growth curves of the *S. suis* strains in the presence of various metals. The WT, Δ*troR*, and CΔ*troR* strains were grown in TSBS medium supplemented with 4 mM Fe(II) (A), 1.5 mM Ni (B), 4 mM Ca (C), or 4 mM Ca (D). The strains were grown in 96-well plates (200 µl per well) at 37°C with linear shaking, and the OD_{595} values were measured hourly. At least three independent experiments were performed; the data shown are the means ± SDs from three wells in a representative experiment.

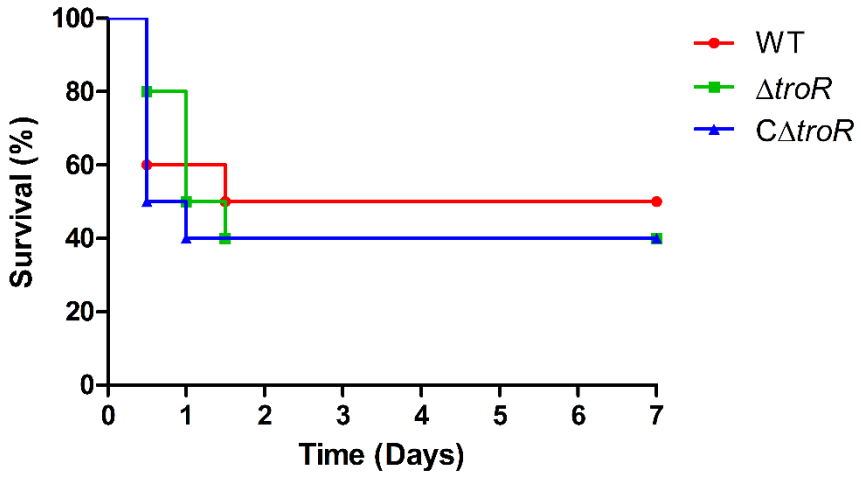

FIG S4 TroR plays no significant role in *S. suis* virulence in a mouse model of intraperitoneal infection. Four groups of BALB/c mice (10 mice per group) were inoculated by intraperitoneal injection of 3×10^8 CFU of the WT, Δ*troR*, and CΔ*troR* strains or PBS. There was no significant difference in the survival rates between the Δ*troR* and WT groups, and between the Δ*troR* and CΔ*troR* groups.

TABLE S1 Summary of the differentially expressed genes in the presence of Mn

Gene_id	readcount_Mn	readcount_Nor	log2FoldChange	padj	description
SSUSC84_RS03545	22.91230729	7.567272577	1.5983	0.019904	ECF transporter S component
SSUSC84_RS05785	54.5831151	19.08210212	1.5162	0.0008366	hypothetical protein
SSUSC84_RS03540	21.88103251	7.831805213	1.4823	0.038228	uridine phosphorylase
SSUSC84_RS08590	33.49920665	12.26981481	1.449	0.0087933	hypothetical protein
SSUSC84_RS03550	30.71983556	11.74074954	1.3876	0.014494	EcfT
SSUSC84_RS00965	63.38655466	25.14150482	1.3341	0.00035661	sugar ABC transporter permease
SSUSC84_RS03175	35.74213899	14.22428697	1.3293	0.011557	ComEC/Rec2
SSUSC84_RS10505	608.5943275	257.9754549	1.2382	6.67E-16	dihydrofolate reductase
SSUSC84_RS06145	245.4937476	109.1929148	1.1688	0.0058626	Nif3-like dinuclear metal center hexameric protein
SSUSC84_RS05780	89.17298939	39.6949737	1.1677	0.030825	sugar kinase
SSUSC84_RS02230	82.11512868	37.25648899	1.1402	0.0030723	PTS mannose-transporter subunit IIB
SSUSC84_RS04795	188.5688366	85.56864291	1.1399	0.00092969	septum formation inhibitor Maf
SSUSC84_RS06975	52.35333565	24.24778205	1.1104	0.010782	lipoprotein
SSUSC84_RS05500	31.79111109	14.95973792	1.0875	0.035848	2-dehydro-3-deoxy-phosphogluconate aldolase
SSUSC84_RS07855	2845.964211	1358.301965	1.0671	9.23E-07	pyruvate formate lyase-activating protein
SSUSC84_RS00740	67.45636828	32.84235314	1.0384	0.008147	copper chaperone
SSUSC84_RS09795	1381.401219	3959.403523	-1.5192	4.59E-36	metal-dependent transcriptional regulator
SSUSC84_RS00190	9.405353762	31.40564853	-1.7395	0.0016464	rod shape-determining protein MreD

SSUSC84_RS09305	135.4763964	646.74505	-2.2552	0.0040645	serine protease
SSUSC84_RS09770	634.8004027	3251.532107	-2.3567	5.62E-13	metal ABC transporter permease
SSUSC84_RS09775	598.5300396	4389.008315	-2.8744	5.21E-19	metal ABC transporter permease
SSUSC84_RS09780	872.6690645	7759.371472	-3.1524	2.77E-34	metal ABC transporter ATP-binding protein
SSUSC84_RS10665	4.740852848	56.09441512	-3.5646	1.81E-13	hypothetical protein
SSUSC84_RS09785	184.6791045	2696.747449	-3.8681	2.82E-49	hypothetical protein
SSUSC84_RS09790	450.1269953	13077.50139	-4.8606	3.71E-209	metal transporter

TABLE S2 Primers used in this study

Primer	Sequence (5'-3')[a]	Size (bp)	Target gene
L1	CG**GGATCC**ACAAGGGAATTGAAAACCAG	621	The left arm of *troR*
L2	TGTACTGGATGCTCTGCGATGAGTTTATTGGT		
R1	ATCGCAGAGCATCCAGTACAAGGACAAAGAG	636	The right arm of *troR*
R2	CG**GAATTC**GTCACTTTATCACAGGGGCT		
In1	TGCCAAGGCAGGTTATCTC	269	An internal region of *troR*
In2	GCCGTGTCTGGTAGCGTT		
Out1	AGCCTTCTGTGGTTTGCG	1089/601	A fragment containing *troR*
Out2	GATGACCTGGGGTATGCG		
C1	CG**GGATCC**AACCCTATTAGGGACAATAGCAG	835	*troR* and its promoter
C2	CG**GAATTC**TTAGGCTGGGCGATTGC		
QtroA1	TGGCTACATACAAATGAGGGTCTT	150	An internal region of *troA*
QtroA2	GTCCATCGCAAACCACAGAAG		
Q16S1	TAGTCCACGCCGTAAACGATG	159	An internal region of 16S rRNA
Q16S2	TAAACCACATGCTCCACCGC		

[a] The bold sequences are restriction sites.