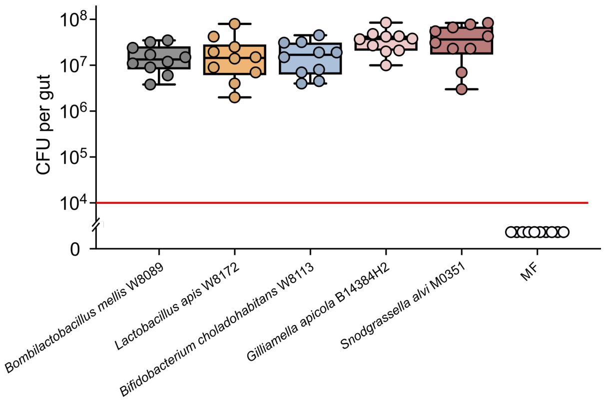
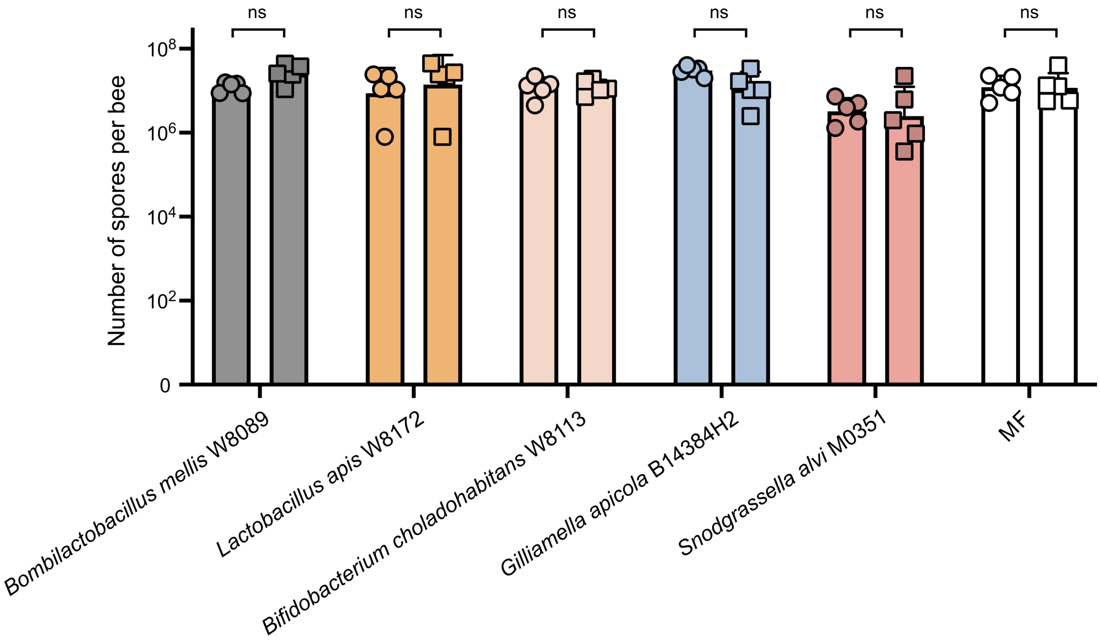
Supplementary Information

Engineered symbiotic bacteria interfering *Nosema* redox system inhibit microsporidia parasitism in honeybees

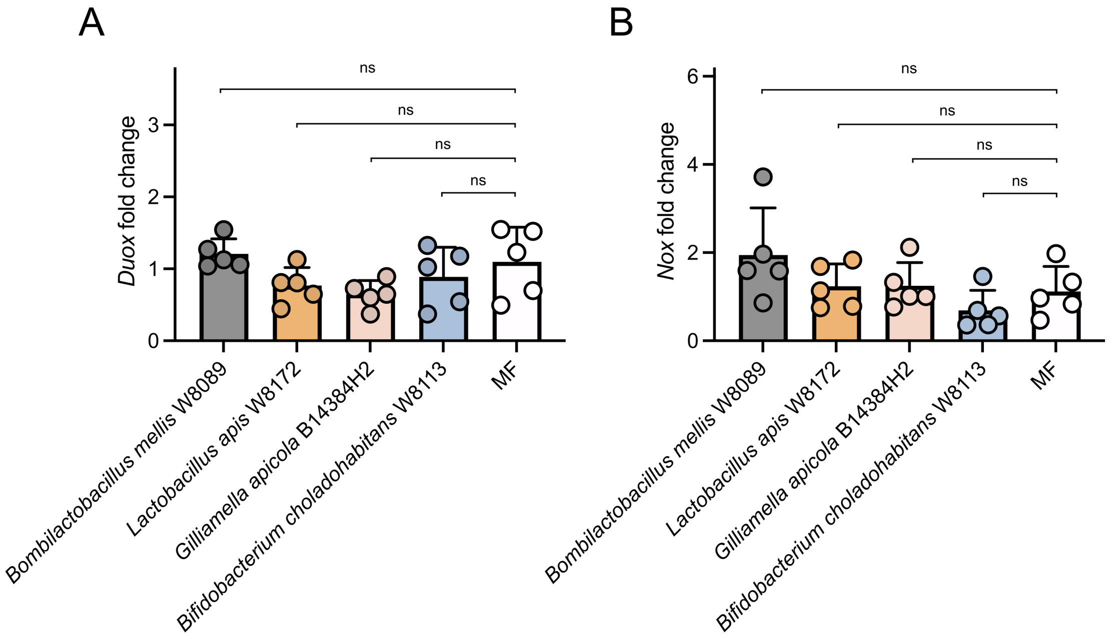
Lang H et al.



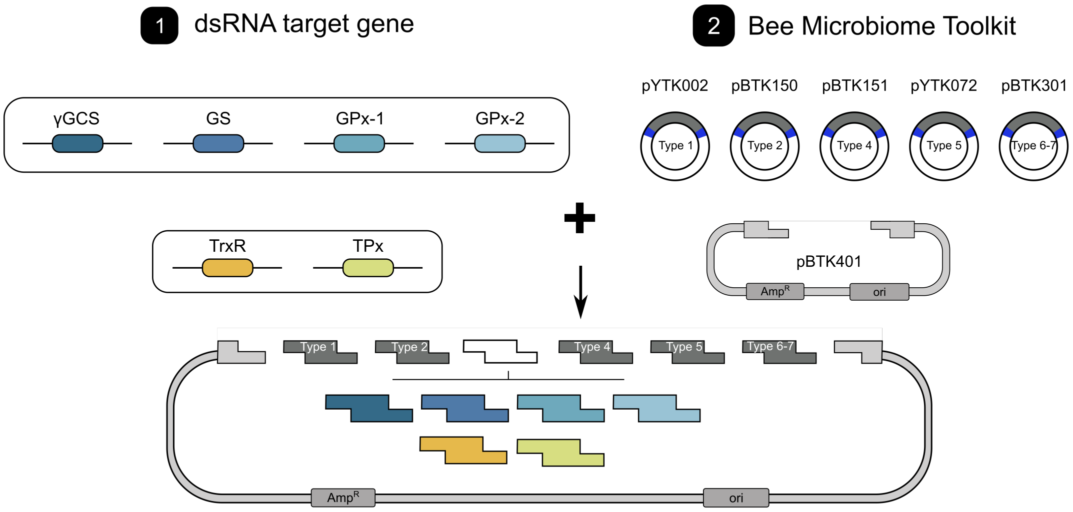
Supplementary Fig. 1. The graph showing the total CFU per gut estimated by bacteria culture for MF honeybees and honeybees mono-colonized with *B. choladohabitans* W8113, *B. mellis* W8089, *L. apis* W8172, and *G. apicola* B14384H2. Box plots indicate the median (middle line), 25th, 75th percentile (box), and 5th and 95th percentile (whiskers). n=10 biologically independent replicates.



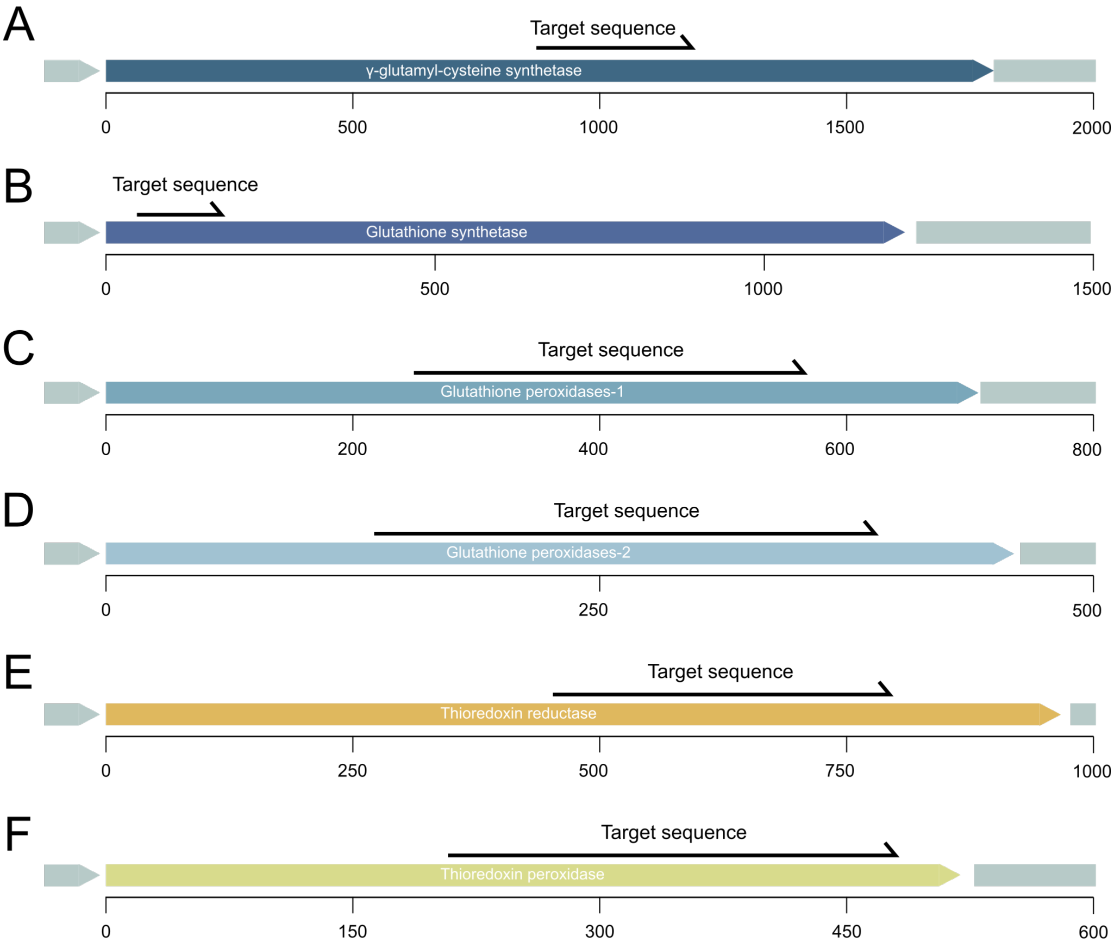
Supplementary Fig. 2. Absolute abundance of *N. ceranae* spores in the midgut 10 days post-infection for each colonization group. The shapes in the bar chart correspond to two independent source cup cages. Statistical analysis was performed by the two-sided Mann-Whitney U test, n = 5 bees for all groups. ns, not significant. Error bars represent mean SD.



Supplementary Fig. 3. The expression level of the *Duox* (A) and *Nox* (B) genes in the midgut following *B. choladohabitans* W8113, *B. mellis* W8089, *L. apis* W8172 and *G. apicola* B14384H2 colonization. No statistical significance between each colonization group and the MF group tested by the two-sided Mann-Whitney U test (n = 5 bees for all groups). ns, not significant. Error bars represent mean SD.



Supplementary Fig. 4. Schematic illustration of the Golden Gate assembly strategy for constructing plasmids expressing targeted dsRNA.



Supplementary Fig. 5. Design of *N. ceranae* targets. This diagram shows the overall gene organization for *N. ceranae* γ-glutamyl-cysteine synthetase (A), glutathione synthetase (B), Glutathione peroxidases-1 (C), Glutathione peroxidases-2 (D), thioredoxin reductase (E), and thioredoxin peroxidase (F). The targeted region in each gene is denoted, and the designed target sequences are listed in Supplementary Table 4.

Supplementary Table 1. List of bacterial strains.

Species and strain	**Source**	**Accession No.**
Escherichia coli MFD*pir*	1	/
Escherichia coli DH5α	Vazyme	/
Snodgrassella alvi M0351	2	JACFSG000000000
Bifidobacterium choladohabitans W8113	2	JACFQZ000000000
Bombilactobacillus mellis W8089	2	JACFRJ000000000
Lactobacillus apis W8172	2	JACFPG000000000
Gilliamella apicola B14384H2	2	JACFQC000000000

Supplementary Table 2. Plasmid list.

Name	**Use**	**Source**
pBTK501	Constitutive GFP	1
pYTK002	Type 2 YTK/BTK connector sequence part plasmid	1
pYTK072	Type 5 YTK/BTK connector sequence part plasmid	1
pBTK301	Type 6-7 BTK bridge connector sequence part plasmid	1
pBTK401	Type 8 origin of replication and origin of transfer plasmid	1
pBTK150	Type 2 BTK part: terminator, CP25 promoter, no RBS	3
pBTK151	Type 4 BTK part: reverse CP25 promoter, terminator, no RBS	3
pYTK001_T1T2	Insulated part vector with flanking terminators	3
pDS-GFP	Control dsRNA GFP	3
pDS-γGCS	dsRNA target γGCS	This Study
pDS-GS	dsRNA target GS	This Study
pDS-GPx-1	dsRNA target GPx-1	This Study
pDS-GPx-2	dsRNA target GPx-2	This Study
pDS-TrxR	dsRNA target TrxR	This Study
pDS-TPx	dsRNA target TPx	This Study

Supplementary Table 3. Primer list.

Purpose	Gene	Gene Symbol in NCBI	Forward	Reverse	Source
qPCR	β-tubulin (*N. ceranae*)	AAJ76_300077627	AGAACCAGGAACGATGGAGA	TCCTTGCAAACAATCTGCAC	4
qPCR	Duox (*A.mellifera*, Amel_HAv3.1)	LOC551970	CCCTAATAGCCCTCGTGAAC	GAGCATTCTCATAACGTGTG	5
qPCR	Nox (*A.mellifera,* Amel_HAv3.1)	LOC408451	ACCTGCTCTGTTTGGTCTCG	ACATCCACCACGTCGAACAA	This study
qPCR	γ-glutamyl-cysteine synthetase (*N. ceranae,* ASM18298v1)	AAJ76_1100057370	AACAGGCGAGGGAAGAAACC	CAACCCATACCTTGGCCCAT	This study
qPCR	Glutathione synthetase (*N. ceranae*)	AAJ76_600034116	TGGGAGTTTAATCGTTCTTGATGG	TCTAAACCGCCATTGCCAGA	This study
qPCR	Glutathione peroxidase (*N. ceranae*)	AAJ76_3500027152	GGCAAATCCACATGTAGAAGC	TCTGCTATAGACTACAAAGACAAGA	This study
qPCR	Glutathione peroxidase (*N. ceranae*)	AAJ76_3500027978	TGCAGTTGATTCAAAACAGTCAC	AGGCCTTTGTCATAAAATTCGTCT	This study
qPCR	Thioredoxin reductase (*N. ceranae*)	AAJ76_5800012528	GTGGTCCTGCAGCCTATTCT	TACTTCCTACCATCCCGCCT	This study
qPCR	Thioredoxin peroxidase (*N. ceranae*)	AAJ76_280004776	TGATTTTACTTTTGTGTGTCCAACT	CACCGTCCTGTCTTGGGAG	This study
qPCR	SSU rRNA (*N. ceranae*)	AAJ76_1700061205	GTCGCTATGATCGCTTGCC	TATTGTAGAGAGGTGGGAGATT	6
RNAI_Type3	γ-glutamyl-cysteine synthetase (*N. ceranae*)	AAJ76_1100057370	CGGTCTCATATGCCAAAAAGCAGGTGTTCTCCA	TGGTCTCAGGATGGGAGTTGCTTGCATTTCCAT	This study
RNAI_Type3	Glutathione synthetase (*N. ceranae*)	AAJ76_600034116	CGGTCTCATATGGCTAGAGCGGTGGGTTTAGT	TGGTCTCAGGATACCAAATGATACAAAACACTGTGAA	This study
RNAI_Type3	Glutathione peroxidase (*N. ceranae*)	AAJ76_3500027152	CGGTCTCATATGGCGCACTTCTAGACGATCTCC	TGGTCTCAGGATACATGTGGATTTGCCAAGAGT	This study
RNAI_Type3	Glutathione peroxidase (*N. ceranae*)	AAJ76_3500027978	CGGTCTCATATGTTCCTTGTTCGCAATTTTTAAACCA	TGGTCTCAGGATATAACCTCTGTTGGTCCATATCGC	This study
RNAI_Type3	Thioredoxin reductase (*N. ceranae*)	AAJ76_5800012528	CGGTCTCATATGAGGAGGAGACTCTGCTATGGA	TGGTCTCAGGATCGCAGTCGTACAATCCTCGT	This study
RNAI_Type3	Thioredoxin peroxidase (*N. ceranae*)	AAJ76_280004776	CGGTCTCATATGACGTTGTCAGTGATAGGAAGGA	TGGTCTCAGGATACACATGATCCATTGCGTCCA	This study
ds_RNAi	γ-glutamyl-cysteine synthetase (*N. ceranae*)	AAJ76_1100057370	TAATACGACTCACTATAGGGTTGGATTTCTGGGCAACGGT	TAATACGACTCACTATAGGGCGAACCTCGTCAGCAAAAGG	This study
ds_RNAi	Thioredoxin reductase (*N. ceranae*)	AAJ76_5800012528	TAATACGACTCACTATAGGGAGGAGGAGACTCTGCTATGGA	TAATACGACTCACTATAGGGCGCAGTCGTACAATCCTCGT	This study
Nosema apis	SSU rRNA (*N. apis*)	U97150	CCATTGCCGGATAAGAGAGT	TATATTTATTGTATTGCGCGTGCT	7
Nosema ceranae	SSU rRNA (*N. ceranae*)	DQ486027	AGAAACTACAACAGCATCACTGGGA	AGTGAATATTCCAATTCCCAACGACTT	7

Supplementary Table 4. Target sequences of dsRNA.

<table>
<tr><th>ID</th><th>Use</th><th>Length (bp)</th><th>GC %</th><th>Sequence Source</th></tr>
<tr><td>pDS-γGCS</td><td>γGCS knockdown</td><td>339</td><td>35</td><td>This Study</td></tr>
<tr><td colspan="5">CCAAAAAGCAGGTGTTCTCCATTTGATTTATTTATTTCTAATGACGCTAGAAATTTAGACAAGTACAACGATACAAATCCACCGATCCACGTGCCGATTTTTAATAAATTAATAAAGAAAGGTGTTGATGAAAAATTGTCGCGACATGTAGCATCTTTATTTATTCGCGATCCTATAGTCTCATATGACGAAACAGATGAAAGCACATTTGACGATTTTGAAAACATACAAAGTTCAAATTGGAGATCTGTAAGGTTTAAAGTACCAACAGAAAGTTCTGATAAAGATTTAAGAGGATGGAAAGTTGAAGTTCGTCCTATGGAAATGCAAGCAACTCCC</td></tr>
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<tr><td>pDS-GPx-1</td><td>GPx-1 knockdown</td><td>348</td><td>28</td><td>This Study</td></tr>
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<tr><td>pDS-GPx-2</td><td>GPx-2 knockdown</td><td>250</td><td>30</td><td>This Study</td></tr>
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References

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5. Azzouz-Olden F, Hunt A, DeGrandi-Hoffman G. Transcriptional response of honey bee (Apis mellifera) to differential nutritional status and Nosema infection. *BMC Genomics* **19**, 628 (2018).

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