

SUPPLEMENTARY MATERIAL

Figure S1. GDNs are resistant to gastric and intestinal enzymatic digestion. GDNs were incubated first in pepsin solution at 37°C for 30 min, then with pancreatin and bile extract solution for another 30 min. The change of particle size **(a)** and surface charge **(b)** were measured using a Zetasizer (n=5).

Figure S2. The oral administration of GDNs does not induce systemic toxicity and change intestinal morphology. Mice were gavaged with PBS or GDNs for 7 days. The serum levels of **(a)** IFN- γ , **(b)** Alanine aminotransferase (ALT) and **(c)** Aspartate aminotransferase (AST) were measured after the last gavage (n=6). **(d)** Hematoxylin and eosin or Alcian blue and nuclear fast red stained slides of jejunum and colon were examined for morphology (n=6).

Figure S3. Oral administration of GDNs does not alter the intestinal microenvironment during steady-state. Mice were gavaged with GDNs for 7 days, **(a)** the 16S rDNA gene copies of different types of bacteria were quantified by real-time PCR from stool pellets, **(b)** the frequency of $\gamma\delta$ T cells in both intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) of small intestine, **(c)** the percentage of CD11b⁺Ly6C⁺ monocytes, **(d)** CD11b⁺Ly6G⁺ neutrophils, and **(e)** Foxp3⁺ regulatory T cells in both small intestine (SI) and large intestine (LI) and **(f)** the frequency of TNF- α secreting T cells in MLN were analyzed by FACS (n=10).

Figure S4. Cytotoxicity effect of GDNs on RAW264.7 mouse macrophages after 24 h incubation. RAW264.7 cells were incubated with indicated concentrations of GDNs for 24 h and

then stained with Annexin-V/PI to detect the mode of cell death. Annexin V⁺ alone indicates early apoptosis and PI⁺ alone represents necrosis. Cells at final stage of apoptosis take up both stains. Represent result of five independent experiments.

Figure S5. PGE₂ production in colonic mucosa during both steady-state (a) and on day 7 of DSS induced colitis (b). Data shows mean ± SEM (n=6).

Figure S6. Tissue biodistribution of orally administrated GDNs. Mice fasted overnight were fed with DiR-labeled GDNs 2 h or 4 h before organs harvested. The biodistribution of DiR-labeled GDNs was imaged with Kodak Image Station 4000MM Pro system. The images are representative of five independent experiments.

Figure S7. Intestinal macrophages are the major targets of orally administrated GDNs. Mice fasted overnight were fed with PKH26-labeled GDNs 2 h and 4 h before harvesting the intestine. Intestinal epithelial cells were isolated and stained for EpCAM (a). Lamina propria myeloid-derived cells were gated on CD11b and then further analyzed on F4/80 (b) or CD11c (c) (n=5).

Figure S8. The uptake of liposome in small and large intestines. Mice fasted overnight were orally administered two times commercially available liposome labeled with an equivalent amount of PKH26. Sections of jejunum, ileum and colon were stained with F4/80 (green) to identify macrophages and nuclei were labeled with DAPI. (n=6). Original magnification was 40×

(left panels) with enlargement of the indicated area shown in the right panels. Dotted line indicates basement membrane.

Figure S9. In vitro release curve of free MTX and GMTX in PBS at 37°C. Data shows mean \pm SEM (n=5).

Video S1. The internalization of GDNs by intestinal macrophages. 3D reconstruction of Z-series confocal images of the jejunum section revealed the internalization of PKH-26 (red) labeled GDNs by F4/80⁺ (green) macrophages (n=6).

Table S1. Composition of phosphocholine (PC) and phosphoethanolamine (PE) found in GDN

Formula	Name	Percentage
PC		
C40H80O8PN	PC(32:0)	1.53
C42H76O8PN	PC(34:4)	0.86
C42H78O8PN	PC(34:3)	10.67
C42H80O8PN	PC(34:2)	28.63
C42H82O8PN	PC(34:1)	13.88
C44H76O8PN	PC(36:6)	0.48
C44H78O8PN	PC(36:5)	4.07
C44H80O8PN	PC(36:4)	11.34
C44H82O8PN	PC(36:3)	18.36
C44H84O8PN	PC(36:2)	8.49
C44H86O8PN	PC(36:1)	0.72
C46H80O8PN	PC(38:6)	0.05
C46H82O8PN	PC(38:5)	0.02
C46H84O8PN	PC(38:4)	0.07
C46H86O8PN	PC(38:3)	0.32
C46H88O8PN	PC(38:2)	0.33
C48H86O8PN	PC(40:5)	0.00

C48H88O8PN	PC(40:4)	0.01
C48H90O8PN	PC(40:3)	0.08
C48H92O8PN	PC(40:2)	0.09
PE		
C37H68O8PN	PE(32:3)	0.02
C37H70O8PN	PE(32:2)	0.39
C37H72O8PN	PE(32:1)	2.28
C37H74O8PN	PE(32:0)	0.17
C39H70O8PN	PE(34:4)	0.37
C39H72O8PN	PE(34:3)	7.78
C39H74O8PN	PE(34:2)	37.01
C39H76O8PN	PE(34:1)	7.62
C41H70O8PN	PE(36:6)	0.30
C41H72O8PN	PE(36:5)	3.04
C41H74O8PN	PE(36:4)	14.98
C41H76O8PN	PE(36:3)	18.10
C41H78O8PN	PE(36:2)	5.12
C41H80O8PN	PE(36:1)	0.34
C43H74O8PN	PE(38:6)	0.04
C43H76O8PN	PE(38:5)	0.00
C43H78O8PN	PE(38:4)	0.12
C43H80O8PN	PE(38:3)	0.37
C45H84O8PN	PE(40:3)	0.29
C45H86O8PN	PE(40:2)	0.51
C47H86O8PN	PE(42:4)	0.01
C47H88O8PN	PE(42:3)	0.14
C47H90O8PN	PE(42:2)	1.01

Table S2. Identities of proteins found in GDNs

Protein name	Accession number	Molecular weight
(E)-beta-farnesene synthase	G8Z362 (+1)	64 kDa
(E)-beta-ocimene synthase	Q5CD81	71 kDa
1,2 rhamnosyltransferase	D0UZK1 (+2)	51 kDa
1,6-rhamnosyltransferase	A7ISD3	53 kDa
280 kDa protein	Q80H98	280 kDa
286 kDa polyprotein	Q15GA4 (+2)	286 kDa
2-phospho-D-glycerate hydrolase	D7NHW9	48 kDa

349 kDa polyprotein	D0EAL9	348 kDa
349-kDa polyprotein	Q9DTG5	349 kDa
Acidic cellulase	O22297	55 kDa
Acidic class I chitinase	Q8H986	34 kDa
Aconitate hydratase 1	D3GQL0	98 kDa
Actin	K7N8A0	42 kDa
Alcohol acyl transferase	A8W8Y0	51 kDa
Allene oxide synthase	Q84V85	59 kDa
Aminopeptidase	F8WL79	102 kDa
Apocytochrome f	Q09MG5	35 kDa
Ascorbate peroxidase	J7EIR8	28 kDa
Ascorbate peroxidase	B9VRH6	28 kDa
Auxin-response factor	G9I820	94 kDa
Beta-amylase	J7ICW8	65 kDa
Beta-galactosidase	Q8L5Q9	82 kDa
Beta-pinene synthase	A7BG60	69 kDa
Beta-tubulin	C0KLD1	50 kDa
Capsid protein	Q91QZ1	41 kDa
Capsid protein	Q3SAK9	31 kDa
Cation chloride cotransporter	D2U833	108 kDa
Chalcone synthase	C3VPJ0 (+3)	43 kDa
Chloride channel protein	D5LM39	86 kDa
Cinnamate 4-hydroxylase CYP73	Q9M4U0	61 kDa
Citrin	Q39627	55 kDa
Coat protein	G2XKD3	25 kDa
Coat protein	Q3L2I6	25 kDa
CRT/DRE binding factor	D5FV16	24 kDa
CTV.2	Q8H6S5	124 kDa
CTV.20	Q8H6Q8	364 kDa
CTV.22	Q8H6Q7	155 kDa
Cytochrome P450	Q1I1D7	54 kDa
Dehydrin	Q7Y045	27 kDa
DNA excision repair protein	F8WLD2	74 kDa
DNA-directed RNA polymerase subunit beta"	Q09MI8	159 kDa
Ethylene response 1	D2WKC9	83 kDa
Ethylene response sensor 1	D2WKD2	71 kDa
Ethylene-insensitive 3-like 1 protein	D7PVG7	70 kDa
Eukaryotic translation initiation factor 3 subunit E	G3CHK8	52 kDa
Fatty acid hydroperoxide lyase	A9NJG4 (+3)	56 kDa
F-box family protein	B8Y9B5	53 kDa
Fe(III)-chelate reductase	Q000W4	80 kDa
Fructokinase	Q6Q3H4	38 kDa
Gag-pol polyprotein	F8WL95	177 kDa

Gamma-terpinene synthase, chloroplastic	Q8L5K4	70 kDa
Glucose-1-phosphate adenylyltransferase	Q9SP43	57 kDa
Glutathione S-transferase	Q3HM93	24 kDa
GRAS family transcription factor	D0VEW6	57 kDa
Heat shock protein	F8WL87	34 kDa
Hsp90	H9NHK0	80 kDa
Jp18	Q8H6R4	23 kDa
Leucine-rich repeat family protein	G3CHK6	51 kDa
Limonoid UDP-glucosyltransferase	B2YGX9 (+1)	57 kDa
MADS-box protein	Q05KK0	30 kDa
Mechanosensitive ion channel domain-containing protein	F8WLB4	79 kDa
Monoterpene synthase	Q5CD82	70 kDa
MYB transcription factor	F8WLC4	23 kDa
NAC domain protein	A5YWA9	35 kDa
NAD(P)H-quinone oxidoreductase subunit 5, chloroplastic	Q09MC9	84 kDa
NBS-LRR type disease resistance protein	Q8H6R9	102 kDa
NBS-LRR type disease resistance protein	Q8H6S0	101 kDa
NBS-LRR type disease resistance protein	Q8H6R6	101 kDa
p1a	J9WR93	348 kDa
P23	Q1X8V8	24 kDa
P23	E7DSS0 (+4)	24 kDa
p27	G0Z9I6	27 kDa
p33	I3XHN0	34 kDa
p33 protein	B8YDL3	34 kDa
p33 protein	B9VB22	34 kDa
P346	P87587	347 kDa
p349 protein	B9VB56	349 kDa
p349 protein	I3RWW7	347 kDa
p349 protein	B9VB20	348 kDa
p349 protein	Q9WID7	347 kDa
P353	Q2XP16	353 kDa
Pectinesterase 1	O04886 (+1)	64 kDa
Peptidyl-prolyl cis-trans isomerase	F8WL74	58 kDa
Peroxidase	Q0ZA67	37 kDa
Phosphoenolpyruvate carboxylase	F1CT41	100 kDa
Phytoene synthase	B1PBV7 (+2)	50 kDa
Plastid-lipid-associated protein, chloroplastic	Q9ZWQ8	35 kDa
Pol polyprotein	Q94FM1	33 kDa
Pol polyprotein	Q94FM0	80 kDa
Poly C-binding protein	G9I825	62 kDa
Polygalacturonase inhibitor	O64460 (+7)	36 kDa
Polyprotein	I3XHM8	349 kDa
Polyprotein	C0STR9	175 kDa

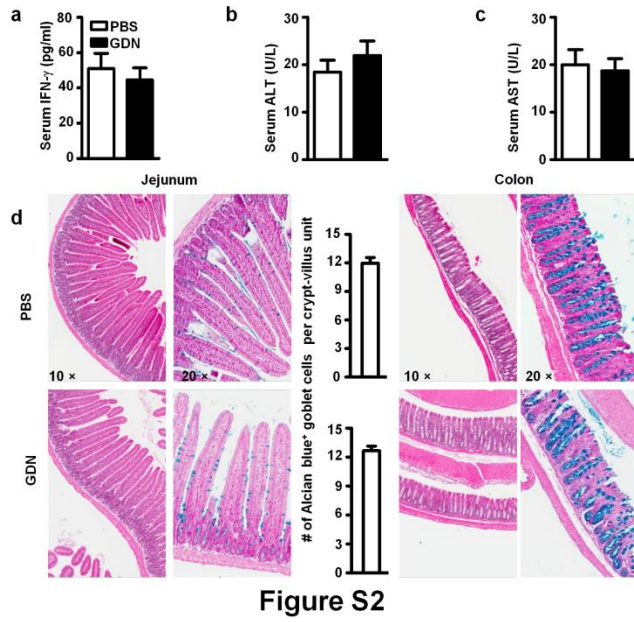
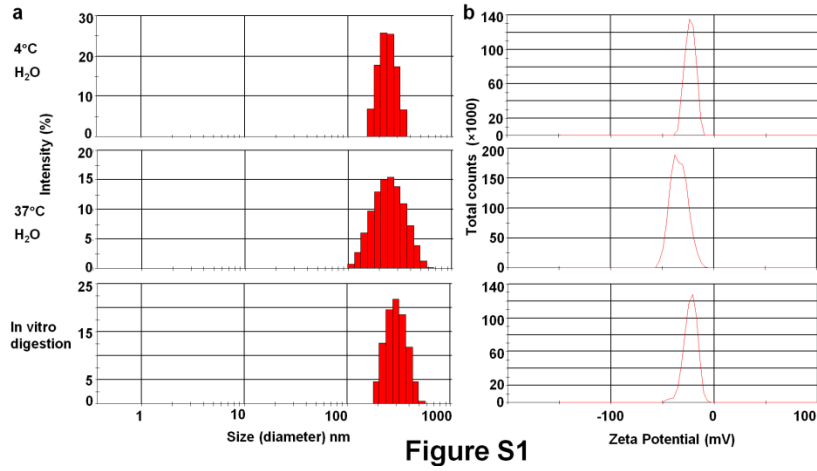
Polyprotein	H6U1F0	148 kDa
Polyprotein	B8QHP8	242 kDa
Polyprotein	I3V6C0	241 kDa
Polyprotein	C0STS0	175 kDa
Polyprotein	K0FGH5	241 kDa
Polyprotein	Q3HWZ1	241 kDa
PPR containing protein	F8WLA5	64 kDa
Probable phospholipid hydroperoxide glutathione peroxidase	Q06652 (+1)	19 kDa
Profilin	P84177	14 kDa
Protein ycf2	Q09MB4	268 kDa
PSI reaction center subunit II	A8C183	23 kDa
Putative 2b protein	A5JVP6	22 kDa
Putative eukaryotic translation initiation factor 1	D0EFM2	32 kDa
Putative gag-pol polyprotein	Q18L98	152 kDa
Putative movement protein	B5AMI9	40 kDa
Putative multiple stress-responsive zinc-finger protein	A1ECK5	18 kDa
Putative replicase polyprotein	B5AMJ0	227 kDa
Putative RNA-dependent RNA polymerase	I7CYN5	186 kDa
Putative terpene synthase	Q8RVR2	64 kDa
Putative uncharacterized protein	B5TE89	169 kDa
Putative uncharacterized protein	Q8JVF3	54 kDa
Putative uncharacterized protein ORF43	F8WLB0	52 kDa
Putative viral replicase	A5JVP4	121 kDa
Replicase	M1JAW3	285 kDa
Replicase polyprotein	H6VXK8	230 kDa
Replicase protein 1a	J9UF50 (+1)	118 kDa
Replicase protein 2a	J9RV45	95 kDa
Replicase-associated polyprotein	Q5EGG5	240 kDa
RNA recognition motif protein 1	G9I823	86 kDa
RNA-dependent RNA polymerase	J7EPC0	187 kDa
RNA-directed RNA polymerase L	Q6DN67	280 kDa
SEPALLATA1 homolog	A9CQM4	28 kDa
Sucrose synthase	Q9SLS2	92 kDa
Sucrose synthase	Q9SLV8 (+1)	93 kDa
Temperature-induced lipocalin	Q38JC1	22 kDa
Tetratricopeptide domain-containing thioredoxin	D0ELH6	37 kDa
Thaumatococcus-like protein	D2KU75	27 kDa
Translation elongation factor	C3VIC2	48 kDa
Ubiquitin/ribosomal fusion protein	D5LY07	18 kDa
UDP-glucosyltransferase family 1 protein	C6KI43	56 kDa
Vacuolar citrate/H ⁺ symporter	A0FKR1	57 kDa
Vacuolar invertase	Q944C8	65 kDa

V-type proton ATPase subunit E	Q9MB46	26 kDa
WD-40 repeat family protein	F8WL82	58 kDa

Table S3. Primer sets used in PCR analysis

Gene	Forward Primer	Reverse Primer
IL-1 β	GTTCCCATTAGACAACCTGC	GATTCTTTCCTTTGAGGC
IL-6	GATACCACTCCCAACAGACC	GCAATGGCAATTCTGATTGT
TNF- α	TCTATGGCCCAGACCCTCAC	GACGGCAGAGAGGAGGTTGA
IFN- γ	ACTGGCAAAAGGATGGTGAC	TGAGCTCATTGAATGCTTGG
Cox-2	TGAGTACCGCAAACGCTTCT	CTCCCCAAAGATAGCATCTGG
GPDH	AGGTCATCCCAGAGCTGAACG	ACCCTGTTGCTGTAGCCGTAT
β -actin	CTGTCCCTGTATGCCTCTG	ATGTCACGCACGATTTCC

SUPPLEMENTARY DATA



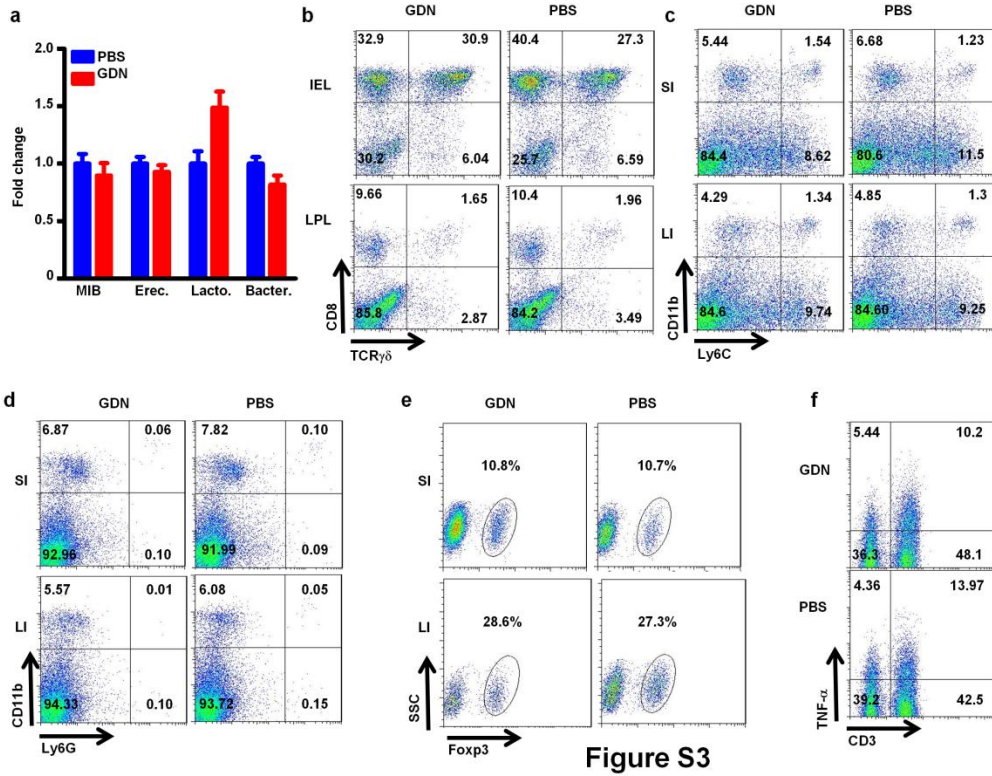


Figure S3

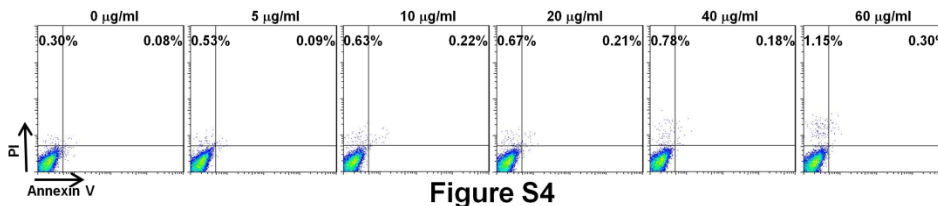


Figure S4

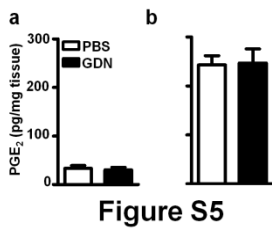


Figure S5

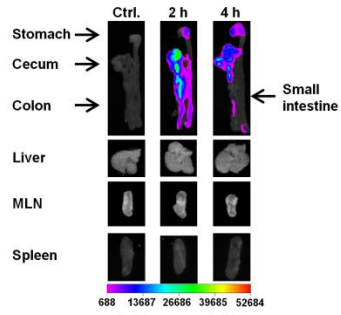


Figure S6

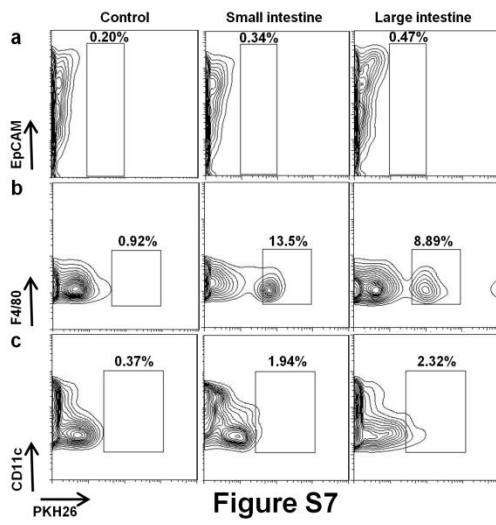


Figure S7

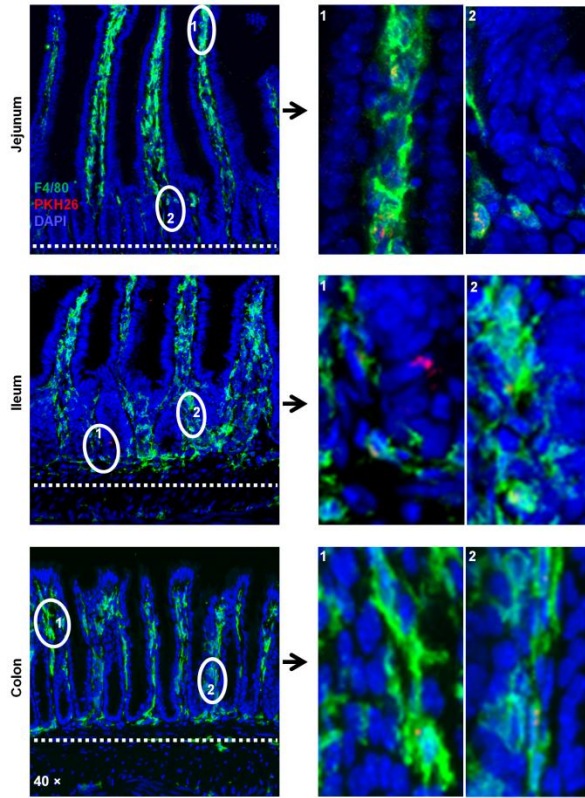


Figure S8

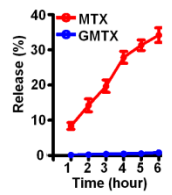


Figure S9