

Supplemental Table 1. Feed formulation and proximate analysis of experimental diets.

	ZnSO ₄	Zn-Nano
Casein	350	350
White fish meal	100	100
Gelatin	20	20
Fish oil	20	20
Soybean oil	40	40
Starch	200	200
Ascorbyl-2-polyphosphate	10	10
NaCl	10	10
CaH ₂ PO ₄ · 2H ₂ O	10	10
Vitamin mix	5	5
Mineral mix (Zn free)	5	5
Zn source	0.035	0.010
Betaine	10	10
Cellulose	219.965	219.99
Moisture	4.05	3.81
Crude ash	3.9	3.77
Crude protein	38.82	38.17
Crude lipid	7.13	7.53
Zn (mg kg ⁻¹)	23.46	23.01

Supplemental Table 2. Primers used for real-time quantitative PCR analysis

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Accession no.
<i>bpgd</i>	GCTCTGATGTGGCGAGG TGG	CGTAGAAGGACAGTG CAGTGG	JX992745
<i>g6pd</i>	CAGGAATGAACGCTGGG ATG	TCTGCTACGGTAGGTC AGGTCC	JX992744
<i>fas</i>	AACTAAAGGCTGCTGGT TGCTA	CACCTTCCCGTCACAA ACCTC	JN579124
<i>acca</i>	GGGGTTTTACGCTGCT TC	GGTTCTGATTGGGTCG TCCTG	JX992746
<i>srebpl-1</i>	CTGGGTCATCGCTTCTTT GTG	TCCTTCGTTGGAGCTT TTGTCT	JX992742
<i>ppary</i>	ACGCCCCGTTTCGTTATCC	TGAGCAGAGTCACCTG GTCATTG	JX992741
<i>dgat 1</i>	GCACCATCCACTGCTGTAT CA	CGCTCCAACCTTTGTCCG TC	MH663997
<i>fatp4</i>	TGCCCTCACATAGTTGCT G	CACTTCCTCGAACATCC CTCAT	MG637279
<i>i-fabp</i>	GACGGCACTGTGCTTAC	AAATCCTCTTAGCGTTG	MG637280

	TGG	ACACCT	
<i>znt1</i>	CACAAATGCGGATAGTGG GA	GGTCACTTGGAGCAACT GAAAC	KY652749
<i>znt5</i>	AAGAAAGGACAGAAGGGG ACG	ACCAAAGCGGAGCAGTC AAA	KY652750
<i>znt7</i>	GAACTCCACCTGCTCTTGA CC	CCGCCACATCTATCTGA ACG	KY652751
<i>zip4</i>	CATTCATAACTTCGCAGAC GG	CCAGAAAGCAACCCCAG ATT	KY652752
<i>mt</i>	ATCCTTGCGAGTGCTCCA	GCAGGAATCGCCCTTAC AC	EU124661
<i>mtf1</i>	CGAGTTGATGTTGCAGAGC C	GAGGTATGGAGGAAAG AAGGGA	KY652754
<i>β-actin</i>	GGACTCTGGTGATGG TGTGA	CTGTAGCCTCTCTC GGTCAG	EU161066
<i>rpl7</i>	GGCAAATGTACAGG AGCGAG	GCCTTGTTGAGCTT GACGAA	KP938522
<i>hprt</i>	ATGCTTCTGACCTGG AACGT	TTGCGGTTTCAGTGC TTTGAT	KP938523
<i>tuba</i>	TCAAAGCTGGAGTTC TCGGT	AATGGCCTCGTTAT CCACCA	KP938526
<i>b2m</i>	GCTGATCTGCCATGT GAGTG	TGTCTGACACTGCA GCTGTA	KP938520
<i>ubce</i>	TCAAGAAGAGCCAG TGGAGG	TAGGGGTAGTCGA TGGGGAA	KP938524
<i>gapdh</i>	TTTCAGCGAGAGAG ACCCAG	ATGACTCTCTTGGC ACCTCC	KP938521
<i>18srrna</i>	AGCTCGTAGTTGGAT CTCGG	CGGGTATTCAGGC GAGTTTG	KP938527
<i>elfa</i>	GTCTGGAGATGCTGC CATTG	AGCCTTCTTCTCAA CGCTCT	KU886307

Supplemental Table 3. Effect of dietary different Zn sources on growth performance and morphometrical parameters of juvenile *P. fulvidraco*.

	ZnSO ₄	Nano-Zn
IBM	4.08 ± 0.02	4.09 ± 0.02
FBM	22.59 ± 0.49	25.14 ± 0.27
WG	453.7 ± 11.5 a	515.1 ± 2.9 b
SGR	2.44 ± 0.03 a	2.60 ± 0.01 b
FI	20.11 ± 0.05	20.24 ± 0.38
FCR	1.09 ± 0.03 b	0.96 ± 0.02 a
VSI	6.13 ± 0.28 a	7.02 ± 0.28 b

ISI	1.30±0.03	1.37±0.05
CF	1.43±0.03 a	1.69±0.03 b
Survival	97.78±1.11	92.22±4.44

Values are means ± SEM (n=3 replicate tanks. For WG, SGR, FI and FCR, 26-30 fish each tank; for VSI, ISI and CF, six fish each tank). Values with different letters within the same row are significantly different at $P < 0.05$; CF, condition factor; FCR, feed conversion rate; IBW (g fish⁻¹), initial mean body weight; FBW (g fish⁻¹), final mean body weight; ISI, intestinal somatic index; SGR, specific growth rate; VSI, viscerosomatic index; WG, weight gain.

WG (%) = $100 \times (\text{final mean body weight} - \text{initial mean body weight}) / \text{initial mean body weight}$.

SGR (% d⁻¹) = $100 \times (\ln(\text{final mean body weight}) - \ln(\text{initial mean body weight})) / \text{day}$.

VSI (%) = $100 \times (\text{viscera weight}) / (\text{body weight})$.

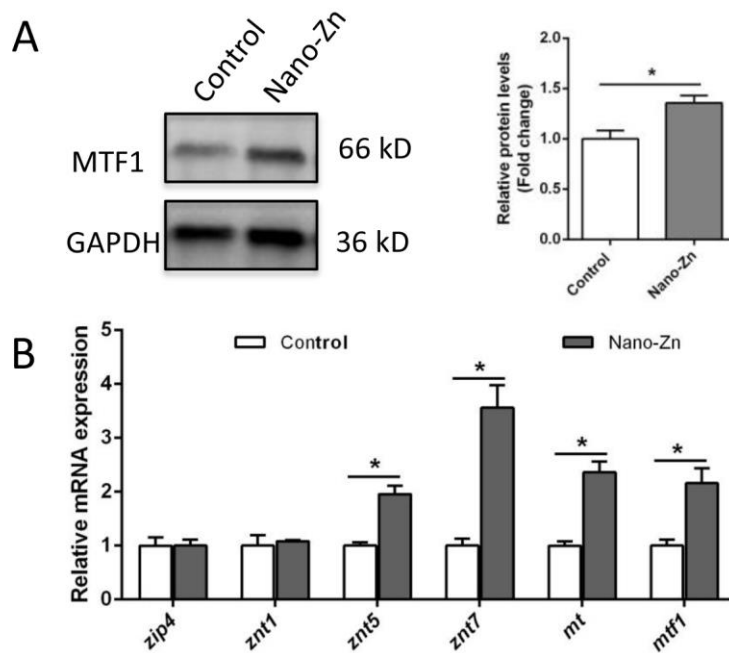
ISI (%) = $100 \times (\text{intestinal weight}) / (\text{body weight})$.

CF = $100 \times (\text{live weight, g}) / (\text{body length, cm})^3$.

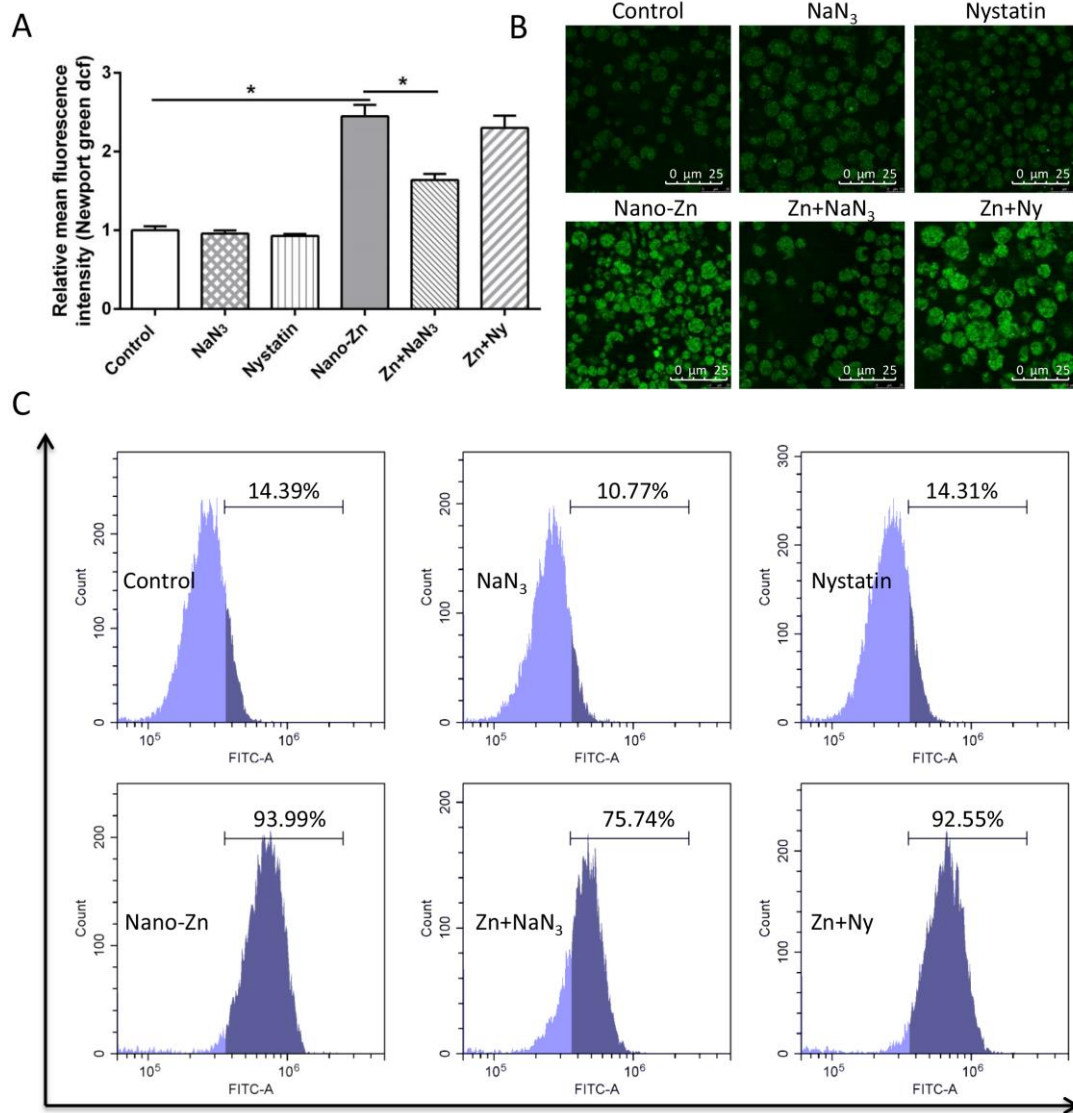
FI (g fish⁻¹): feed intake.

FCR = dry food fed (g) / wet weight gain (g).

Survival = $100 \times (\text{final fish number}) / (\text{initial fish number})$.



Supplementary Fig. 1. Intestinal epithelial cells didn't absorb Nano-Zn via caveolae-dependent and macropinocytosis pathways. (A) Protein levels of MTF1 of intestinal epithelial cells after 40 μ M Nano-Zn incubation for 12 h. (B) The mRNA levels of Zn transport protein after 40 μ M Nano-Zn incubation for 12 h. Values are means \pm SEMs, $n = 3-6$. Asterisks (*) indicate significant differences between control and Nano-Zn group ($p < 0.05$, $n = 3$).



Supplementary Fig. 2. Nano-Zn absorption is energy-consuming in the intestinal epithelial cells. (A) Free Zn²⁺ was quantified by calculating FL1 (green) mean fluorescence intensity of intestinal epithelial cells incubated for 12 h in 40 μ M Nano-Zn with 2-h 10 mM NaN₃ or 5 μ g/ml nystatin pretreatment. (B) Representative confocal microscopy stained with Zn²⁺ fluorescent probe (Newport green dcf). The primary intestinal epithelial cells from *P. fulvidraco* were incubated for 12 h in control or 40 μ M Nano-Zn containing medium with or without 2 h 10 mM NaN₃ or 5 μ g/ml nystatin pretreatment. (C) The presence of DCF-stained Zn²⁺ was demonstrated by flow cytometric analysis of green (FL1) fluorescence intensity. The primary intestinal epithelial cells from *P. fulvidraco* were incubated for 12 h in control or 40 μ M Nano-Zn containing medium with or without 2 h 10 mM NaN₃ or 5 μ g/ml nystatin pretreatment. Values are means \pm SEMs, n = 3-6. Asterisks (*) indicate significant differences between two groups ($p < 0.05$, n = 3). NaN₃, Sodium azide; Ny, Nystatin.

