Supplementary Materials: Ex Vivo and In Vitro Studies Revealed Underlying Mechanisms of Immature Intestinal Inflammatory Responses Caused by Aflatoxin M1 Together with Ochratoxin A

Zi-Wei Wang, Ya-Nan Gao, Sheng-Nan Huang, Jia-Qi Wang and Nan Zheng

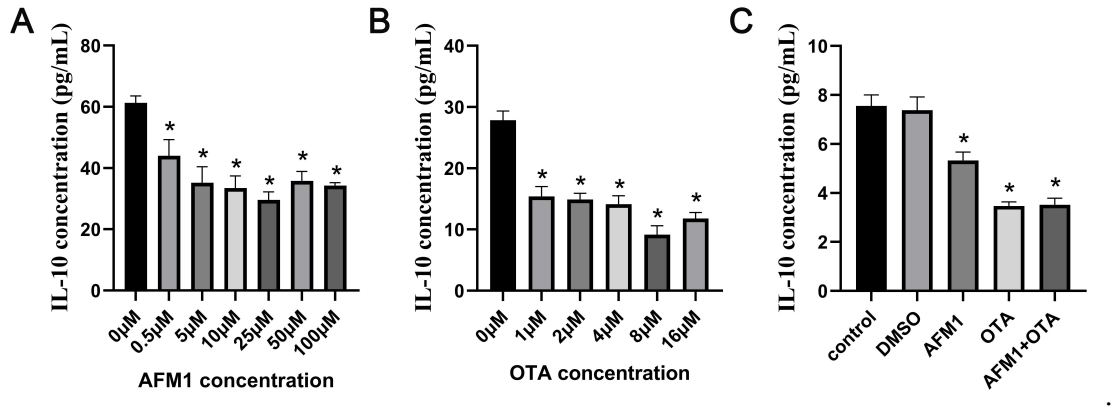

Figure S1. Effects of (A) various doses of AFM1, (B) various doses OTA and (C) AFM1+OTA treatment for 24h on the release of IL-10 from the isolated jejunal tissues. In panel C, the concentration of AFM1 and OTA was 50 μM and 4 μM, respectively. Results were shown as mean ± SEM (n ≥ 6). * $p < 0.05$ statistically significantly compared with control.

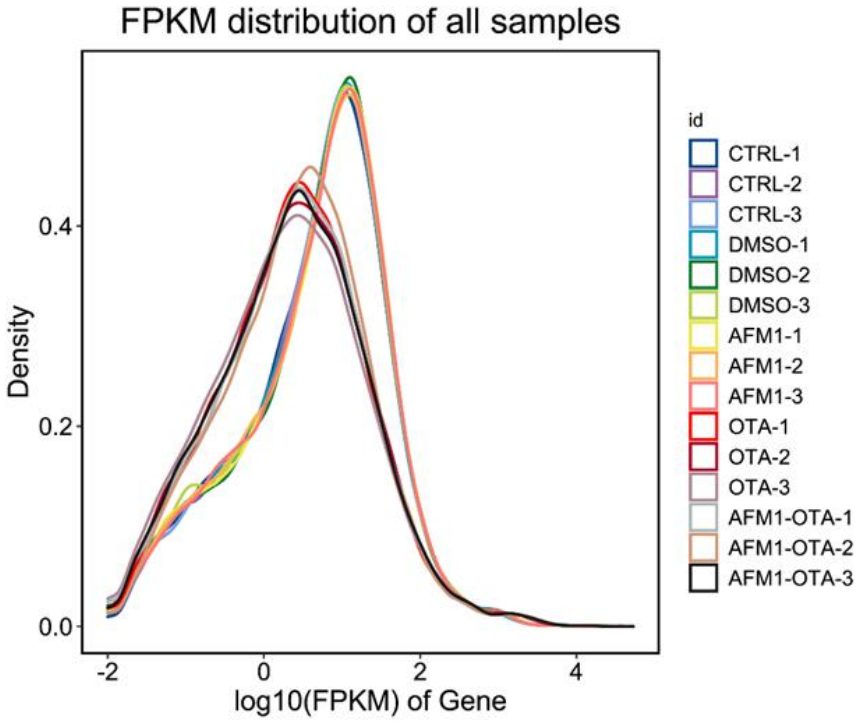

Figure S2. FPKM distribution of all samples.

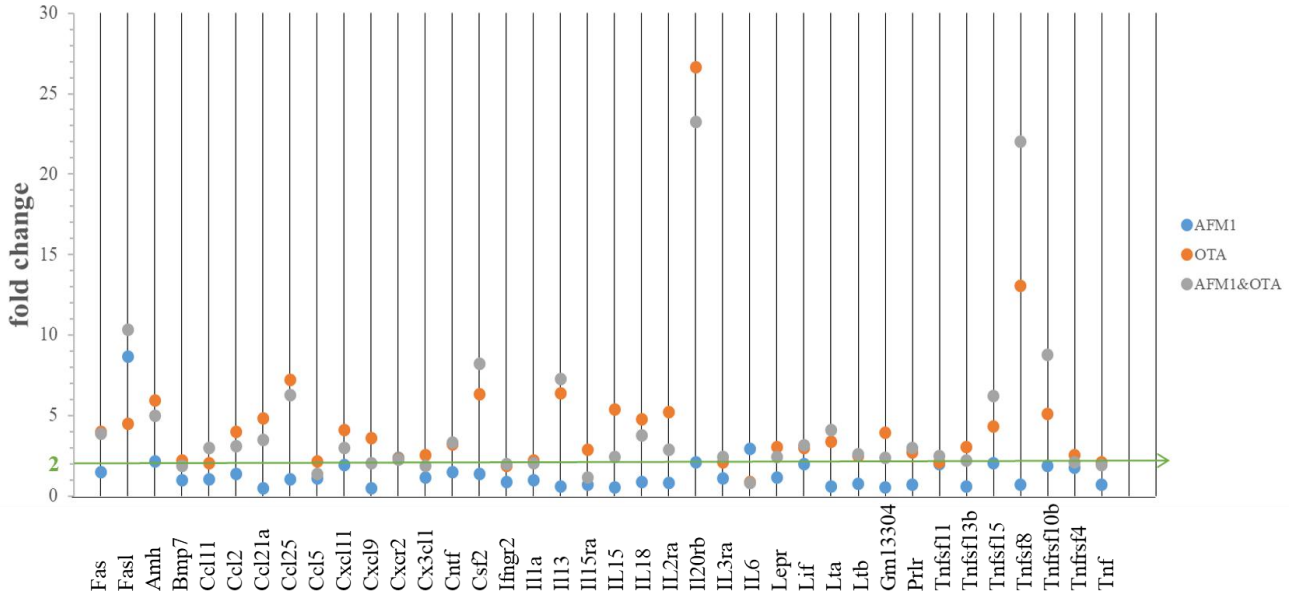

Figure S3. Relative expression of each gene (vs. DMSO group) involved in block 1 'Cytokine-Cytokine receptor interaction' pathway.

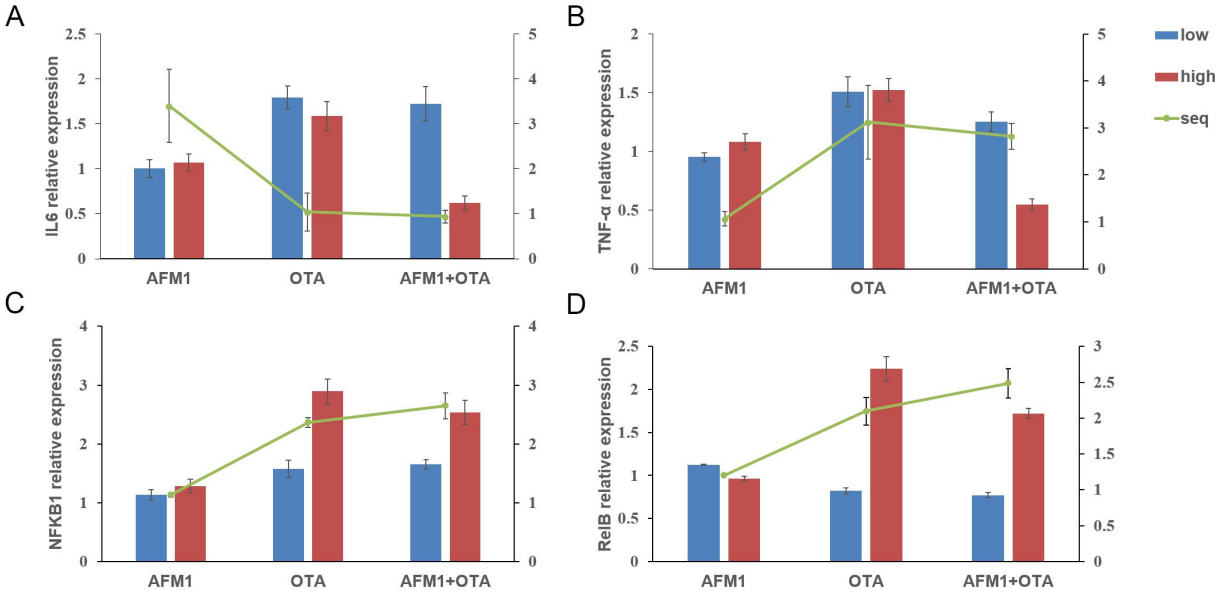

Figure S4. Relative gene expression (vs. DMSO group) of (A) *IL6* (B) *TNF-α* (C) *NFKB1* and (D) *RelB* using qPCR assay in FHs 74 Int cells and RNA-seq method in jejunal tissues of fetal mice. Blue bar and red bar represent the qPCR-data from toxin groups at low and high concentration, respectively (AFM1 low: 12.5 μM, AFM1 high: 50 μM; OTA low: 0.01 μM; OTA high: 2 μM). Green fold line represents the data from RNA-seq.

Table S1. List of primer sequence for Real-Time qPCR.

Gene		Sequence (5' to 3')
GAPDH (human)	F	GCAAGAGCACAAGAGGAAGAG
	R	TCTACATGGCAACTGTGAGGA
IL-6 (human)	F	CCTTCCAAAGATGGCTGAAA
	R	CAGGGGTGGTTATTGCATCT
TNF-α (human)	F	CTGAACTTCGGGGTGATCG
	R	GCTTGGTGGTTTGCTACGAC
NFKB1 (human)	F	AACAGAGAGGATTTCGTTTCCG
	R	TTTGACCTGAGGGTAAGACTTCT
RelB (human)	F	CATTGAGCGGAAGATTCAAC
	R	GCAGCTCTGATGTGTTTGTG

Table S2. List of several genes adjacent to NFKB1.

Gene name	Protein description	Fold change vs. DMSO group			
		DMSO	AFM1	OTA	AFM1+OTA
Nlrp3	NLR family, pyrin domain containing 3; the sensor component of the inflammasome, plays a crucial role in innate immunity and inflammation	1.00 ^{b}	3.16 ^{b}	4.98 ^{ab}	7.54 ^{a}
Pycard	PYD and CARD domain containing; Functions as key mediator in apoptosis and inflammation; Required for recruitment of caspase-1 to inflammasomes containing certain pattern recognition receptors, e.g., Nlrp3	1.00 ^{b}	0.83 ^{b}	3.28 ^{a}	3.86 ^{a}
Casp1	Besides apoptotic regulation, also plays a key role in cell immunity as an inflammatory response initiator: once activated through formation of an inflammasome complex, it initiates a proinflammatory response through the cleavage of the two inflammatory cytokines pro-IL1B and pro-IL18, releasing the mature cytokines	1.00	0.73	2.23	2.16
Il1b	interleukin 1 beta	1.00 ^{b}	8.27 ^{a}	0.68 ^{b}	0.18 ^{b}
Il1a	interleukin 1 alpha	1.00	0.98	2.22	2.07
Il18	interleukin 18	1.00 ^{b}	0.88 ^{b}	4.77 ^{a}	3.79 ^{a}
Tnf	tumor necrosis factor	1.00	0.73	2.13	1.93

Note: Different lowercase indicates statistical differences between groups ($p < 0.05$).