Supplementary materials

Supplementary Fig. 1. American ginseng-mediated cytotoxicity in H9C2 cells. H9C2 cells at 80-90% confluence were cultured in serum-free DMEM for 24 hours, and then treated with or without American ginseng extract (Am. g.) in serum-free DMEM as indicated for additional 24 hours. LDH release and cell survival were assessed by the Cytotoxicity Detection Kit ^{PLUS}. Cell viability = (LDH activity of cell lyate)/(LDH activity of cell lyate + LDH activity of culture media) $\times$ 100%. *$p < 0.05$ vs Am. g. (0), n=4. (A) American ginseng-induced LDH release into supernatant of H9C2 cells. (B) American ginseng-induced cell death of H9C2 cells. Serum starvation induced 30% of cell death in H9C2 cells.

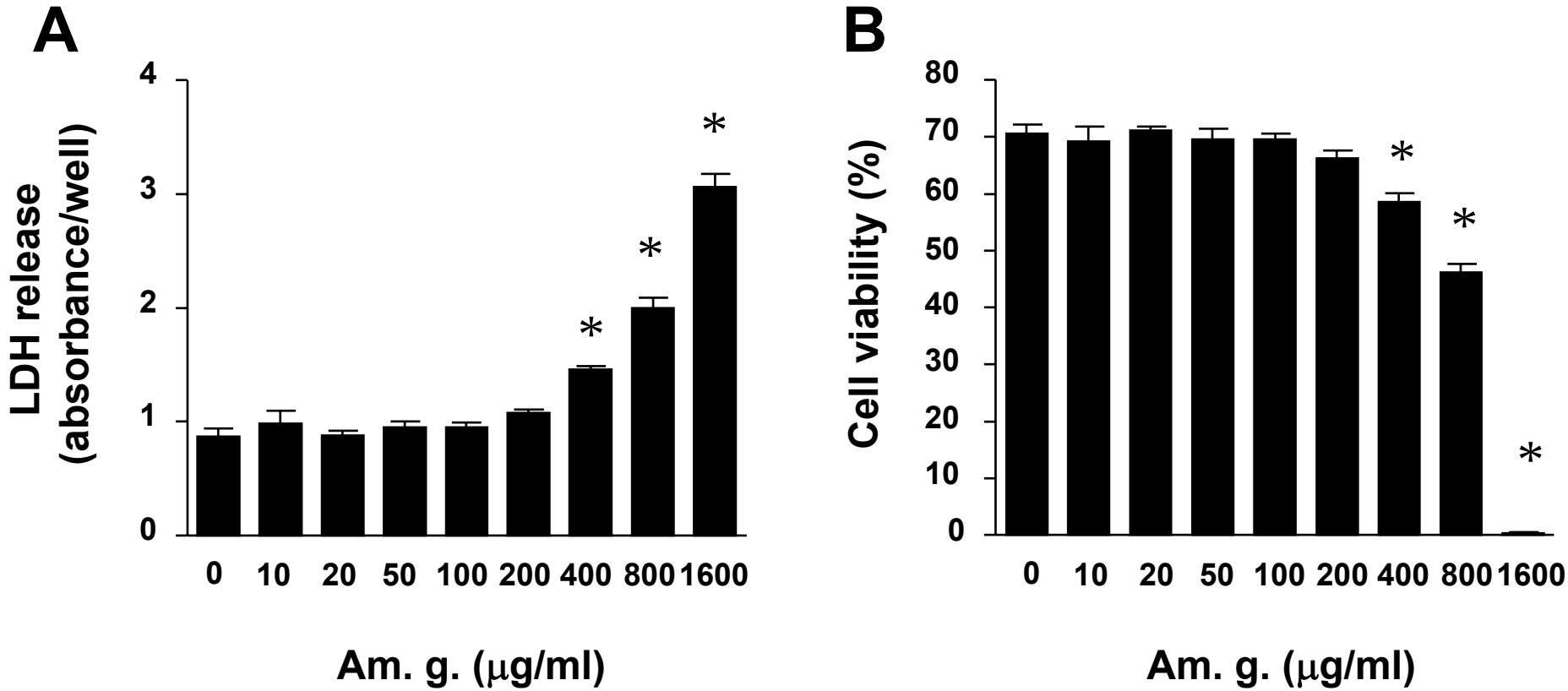
Supplementary Fig. 2. American ginseng-induced Nrf2 nuclear translocation in H9C2 cells. H9C2 cells at 80-90% confluence were cultured in serum-free DMEM for 24 hours, and the treated with or without American ginseng extract (Am. g.) in serum-free DMEM as indicated. Nuclear fractions were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce) following the manufacturer's instructions. Nuclear factions were confirmed by the immunoblotting of histone using anti-Histone H3 (FL-136, sc-10809, Santa Cruz). Unclear Nrf2 was assessed by the immunoblotting of Nrf2 using an antibody of Nrf2 (H-300, sc-13032, Santa Cruz). Results are representatives of duplicated experiments.

Supplementary Fig. 3. Effect of Nrf2 knockdown on American ginseng-mediated suppression of ROS and RNS formation in H9C2 cells. Cells infected with adenovirus of control scramble shRNA or Nrf2 shRNA for 48 hours, and then treated with TNFα (100 ng/ml) and American ginseng extract (Am. g., 200 μg/ml) for 1 hour. Free radical formation was determined as described in "*Methods*" *p < 0.05 vs Ad-scramble (-); #p < 0.05 vs Ang II (+) of Ad-scramble; § p < 0.05 vs Ang (+) of Ad-Nrf2 shRNA; †p < 0.05 vs Ad-Nrf2 shRNA (-); n=6.

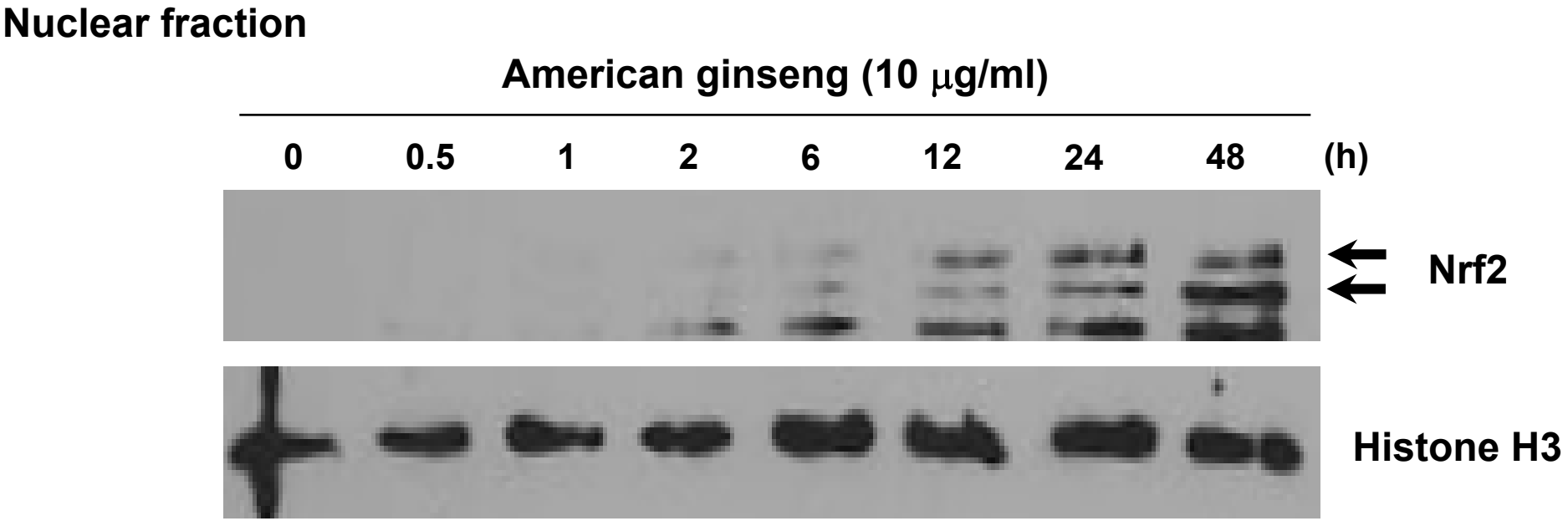
Supplementary Table 1. Primers for Q-PCR.

Supplementary Table 2. Primers for ChIP assay.

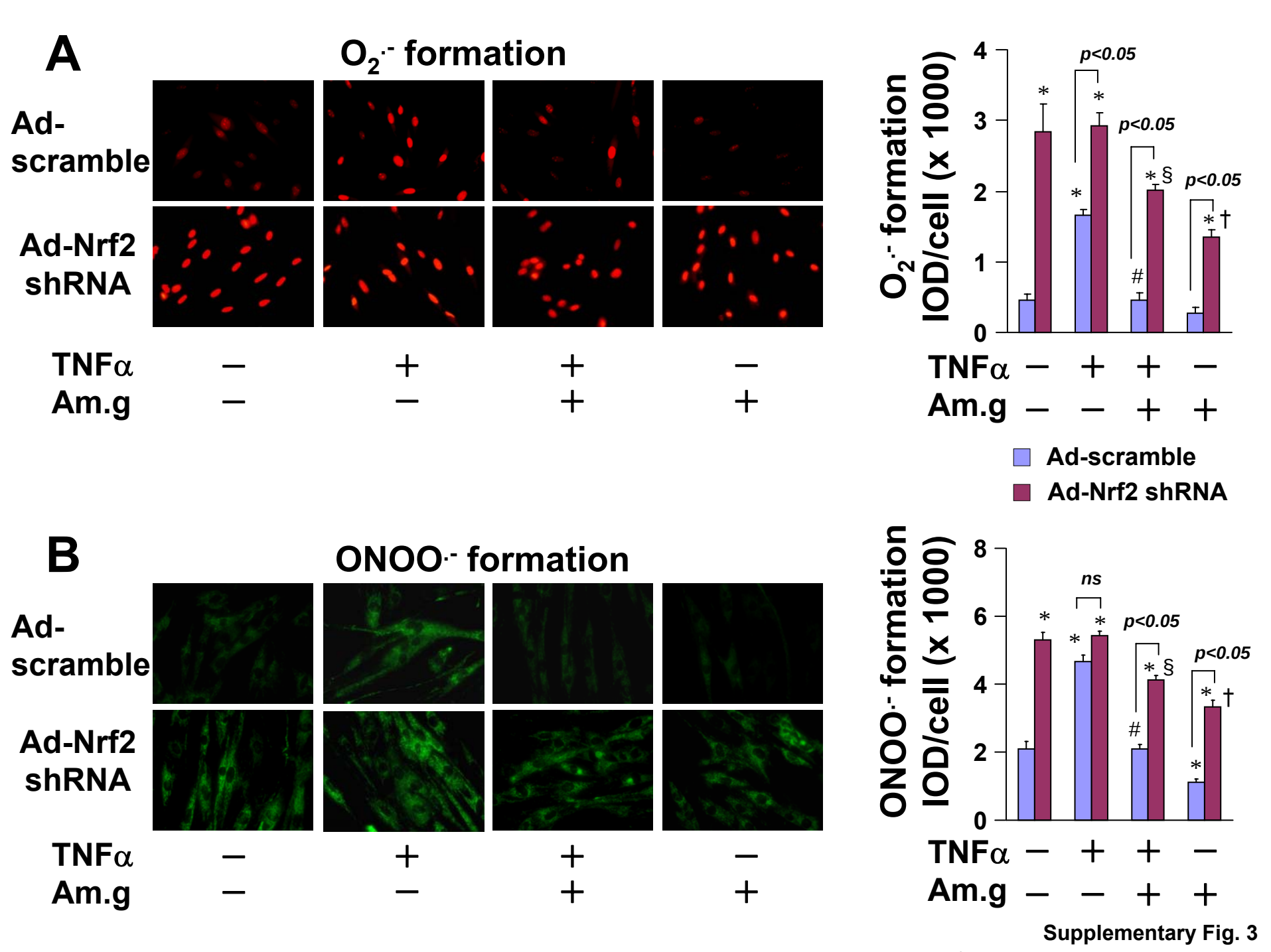
Supplementary Table 3. Cells were stimulated with or without American ginseng extract (Am. g., 50 μg/ml) for 12 hours and subjected to ChIP assay as described in "*Methods*". *A, p* < 0.05 vs vehicle, n=4. *ns*, non-significant.



Supplementary Fig. 1



Supplementary Fig. 2



Supplementary Fig. 3

Supplementary Table 1. Primers for Q-PCR

Gene	Species	Accession No.	5’ primer	3’ primer	PCR product size
NQO-1	Rat	NM_017000.3	ACTACGATCCGCCCCCAACTTCTG	CTTCGGCTCCCCTGTGATGTCGT	212
HO-1	Rat	NM_012580.2	TCCTGCGATGGGTCCTCACACTC	CAGCCGCCTCTACCGACCACAG	340
GCLc	Rat	NM_012815.2	TTGTTACTGAATGGCGGCGATGTT	GCGGGGGTGCTTGTTTATGG	357
SOD-2	Rat	NM_017051.2	GGACGCCGCAGAGCAGAC	GCCCCCGCCATTGAACTT	360
Txn-1	Rat	NM_053800.3	CTGATCGAGAGCAAGGAAGC	CAGAGCATGATTAGGCAAACT	319
Txnrd-1	Rat	NM_031614.2	AGTGGTTGGCGCGTCCTATGTC	TTCACGCCCACGGTCTCTAAGC	342
HO-1	Mouse	NM_010442.2	AGGAGATAGAGCGCAACAAGCAGA	CCAGTGAGGCCCATACCAGAAG	116
NQO-1	Mouse	NM_008706.5	CGGTATTACGATCCTCCCTCAACA	AGCCTCTACAGCAGCCTCCTTCAT	120
Trxnd-1	Mouse	NM_001042523.1	GTGTTGCTGGCGGTAGGAAGAGAT	GTCACCGATGGCGTAGATGTAAGG	144

Supplementary Table 2. Primers for ChIP assay

Gene	ARE location	5' primer	3' primer	PCR product size
NQO-1	-468 ~ -459	GATCTTGGACAGGGAGCAGTTGAA	ATTGGCAGGGAGAAGCAGTTTAGG	143
	-4683 ~ -4674	CCTGGCCTGGGAACACTGA	TCTTGGCCTATAAAACACATCCTG	181
	-5449 ~ -5440	CAAACACTGCCAACCTG	AGCTAAATCCCCAACCCCTGTGT	182
SOD-2	-886 ~ -877	GCTGCGGCTATCAAATGTT	GCAAAGGGGGTGGGAAAAA	158
	-3723 ~ -3714	CTGAGGGCAAGAGAAAAGAGAT	TACCCCTAAGTGAGTCCATTGAT	164
	-11188 ~ -11179	AGGATGTCTGCGATGGCTATTCT	GTCTCCCCAGTTCTAACGATTCAA	124
	-19099 ~ -19090	CTGGGAACTCCTGCGTCTCT	ATGGGCTTCGTTGTATCTTTCTG	173
Txn-1	-3769 ~ -3760	GACAAGGCAAATCACAGAC	ACACATACACATACATACATCC	165
	-11405 ~ -11396	GGCCCTCTGCTGGAAAAG	AGCACGGTCACTACTACG	151
	-11423 ~ -11414	GGCCCTCTGCTGGAAAAG	AGCACGGTCACTACTACG	151
	-11584 ~ -11575	GGCCCTCTGCTGGAAAAG	AGCACGGTCACTACTACG	151
	-19433 ~ -19424	TGGCACAGTTCTAAGGATTCATTC	CGGTGGTTATACGGTTTTGTTCTA	96
	-22521 ~ -22512	CCGCCTTCCTGTTCCTGCTCTC	TAAATGGGTGGGTATGGAACAAG	87

Supplementary Table 3. ChIP analysis of direct binding of Nrf2 to ARE on its target gene promoters

Gene	ARE location	direction	Chip assay		*p*
			Vehicle	Am. g.	
NQO-1	-468 ~ -459	5'→3'	1.00 ± 0.045	1.76 ± 0.141	*A*
	-4683 ~ -4674	3'→5'	0.99 ± 0.340	36.6 ± 12.42	*A*
	-5449 ~ -5440	3'→5'	0.99 ± 0.361	9.00 ± 1.803	*A*
SOD-2	-886 ~ -877	3'→5'	1.07 ± 0.110	1.72 ± 0.251	*A*
	-3723 ~ -3714	5'→3'	0.90 ± 0.075	2.23 ± 0.237	*A*
	-11188 ~ -11179	5'→3'	0.87 ± 0.145	0.82 ± 0.122	ns
	-19099 ~ -19090	5'→3'	0.91 ± 0.102	1.22 ± 0.176	ns
Txn-1	-3769 ~ -3760	3'→5'	1.04 ± 0.631	12.4 ± 3.893	*A*
	-11405 ~ -11396	5'→3'	1.22 ± 0.214	4.01 ± 0.265	*A*
	-11423 ~ -11414	5'→3'	1.22 ± 0.214	4.01 ± 0.265	*A*
	-11584 ~ -11575	3'→5'	1.22 ± 0.214	4.01 ± 0.265	*A*
	-19433 ~ -19424	3'→5'	1.07 ± 0.110	1.72 ± 0.251	*A*
	-22521 ~ -22512	3'→5'	0.93 ± 0.579	2.10 ± 0.995	ns