Supplementary Information

SILVER NANOPARTICLES INTERFER WITH ESSENTIAL METAL HOMEOSTASIS IN A MULTI-CELLULAR FISH *IN VITRO* SYSTEM

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Content:

Table S1: EC50 values (in μM) calculated for RTgutGC cells exposed to cit-AgNP or $AgNO_3$

		Metabolic activity	**Lysosome membrane integrity**
L-15/FBS	$AgNO_3$	-	-
	cit-AgNP	-	925.4±666.5
L-15/ex	$AgNO_3$	5.2±1.1[a]	12.7±5.1[a]
	cit-AgNP	122.6±3.7[a]	53.4±4.6[b]

Values are mean and standard deviation from three independent experiments (n=3). EC50 values bearing different lettering are statistically different (t-test, $p<0.05$).

Table S2. Primers used for qPCR

Gene name	**Forward primer 5'→3'**	**Reverse primer 5'→3'**	**Repository ID***
MTb	GCTCTAAAACTGGCTCTTGC	GTCTAGGCTCAAGATGGTAC	M18104[a]
ATP7A	ACTGCCGCTCCTGGTGTGGTAAAC	ACGGTGGCGGTGTGGTGGATGTAG	XM_021561788
ZnT1	TGGATCCGGGCTGAGGTGATGG	CAAGCACAGCCCGAGGAGGTTG	NM_001124528
DMT1	AACAGTGTGGCGCTCTACGTGC	TTGCCCAGACAGGACACGTCCA	NM_001172513
GR	TACACCGCGCCTCATATCCTCATT	TGCCAGCCATTTCCACAGC	HF969248
ATP1a1	TGTGGCCGTCTTTCTGGGCATG	AGCAAATGGTGGAGGTGGAGCC	NM_001124459
EF1a	ATATCCGTCGTGGCAACGTGGC	TGAGCTCGCTGAACTTGCAGGC	NM_001124339
Ubiquitin	GCTGCGTCTTCGTGGAGGCATT	TTGGGGCGCAGGTTGTTTGTGT	NM_001124194

*GenBank (http://www.ncbi.nim.nih.gov/).

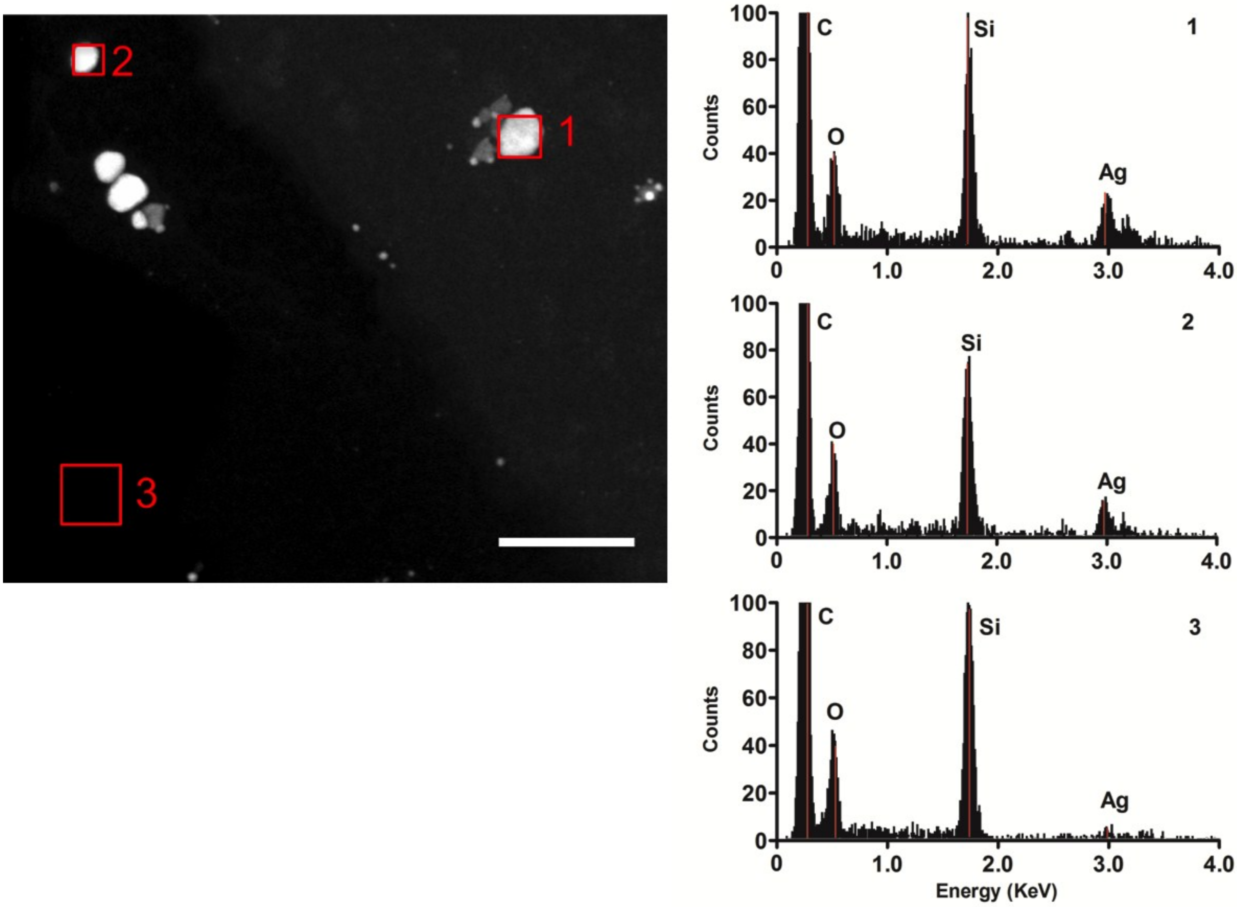

Figure S1: (A) STEM image of cit-AgNP showing a broad size distribution ranging from 20 to 30 nm. (B) EDX of area 1 and 2 shows the elemental silver composition, 3 shows the background. Scale bar = 100 nm

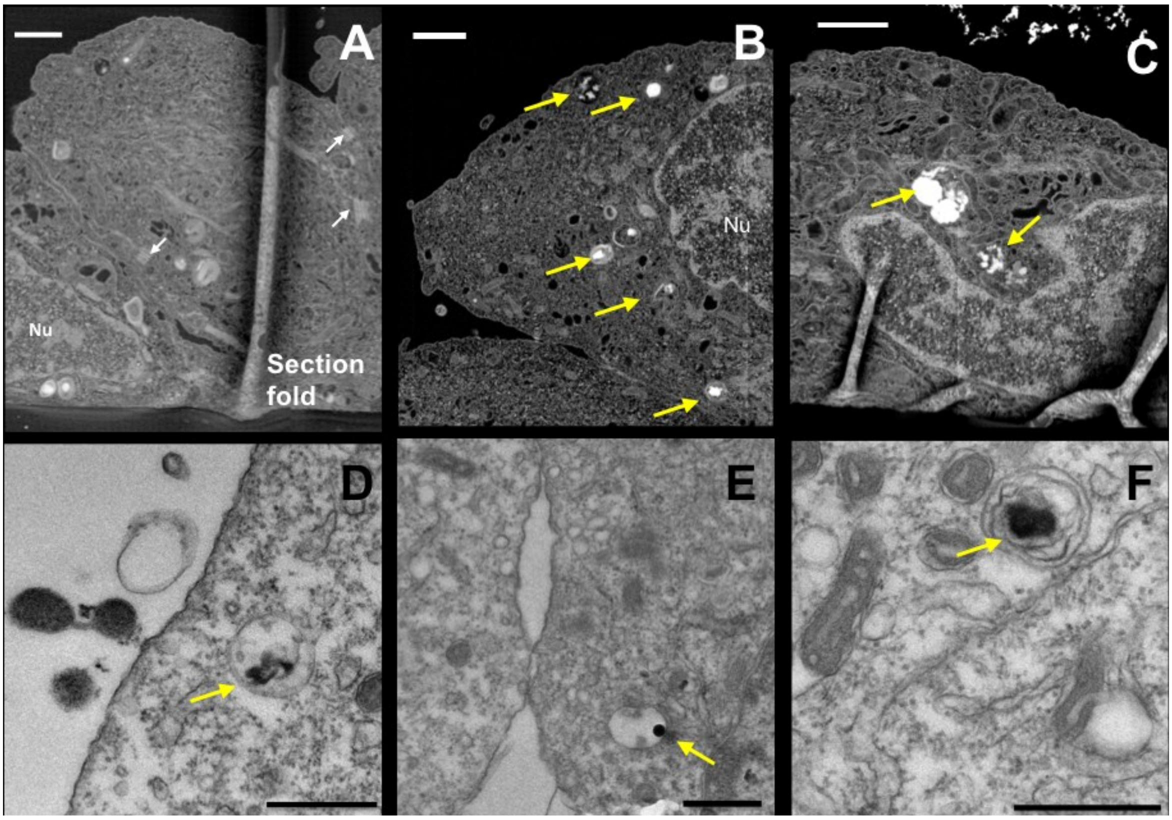

Figure S2: RTgutGC grown for 21 days on transwell inserts. Image A, B and C obtained by Scanning Electron Microscopy, shows a section of RTgutGC monolayer. Image D, E, F obtained by Transmission Electron Microscopy. A, shows control unexposed cells and white arrows indicates desmosomes, B, D, E, F cells exposed to 50 µM cit-AgNP for 3 hrs in L-15/ex and C cells exposed to 10 µM for 24 hrs in L-15/ex. Yellow arrows indicate electron dense material in a lysosome or autophagosome morphology. In A, B, C bar = 1 µm in D, E, F bar = 0.5 µm.

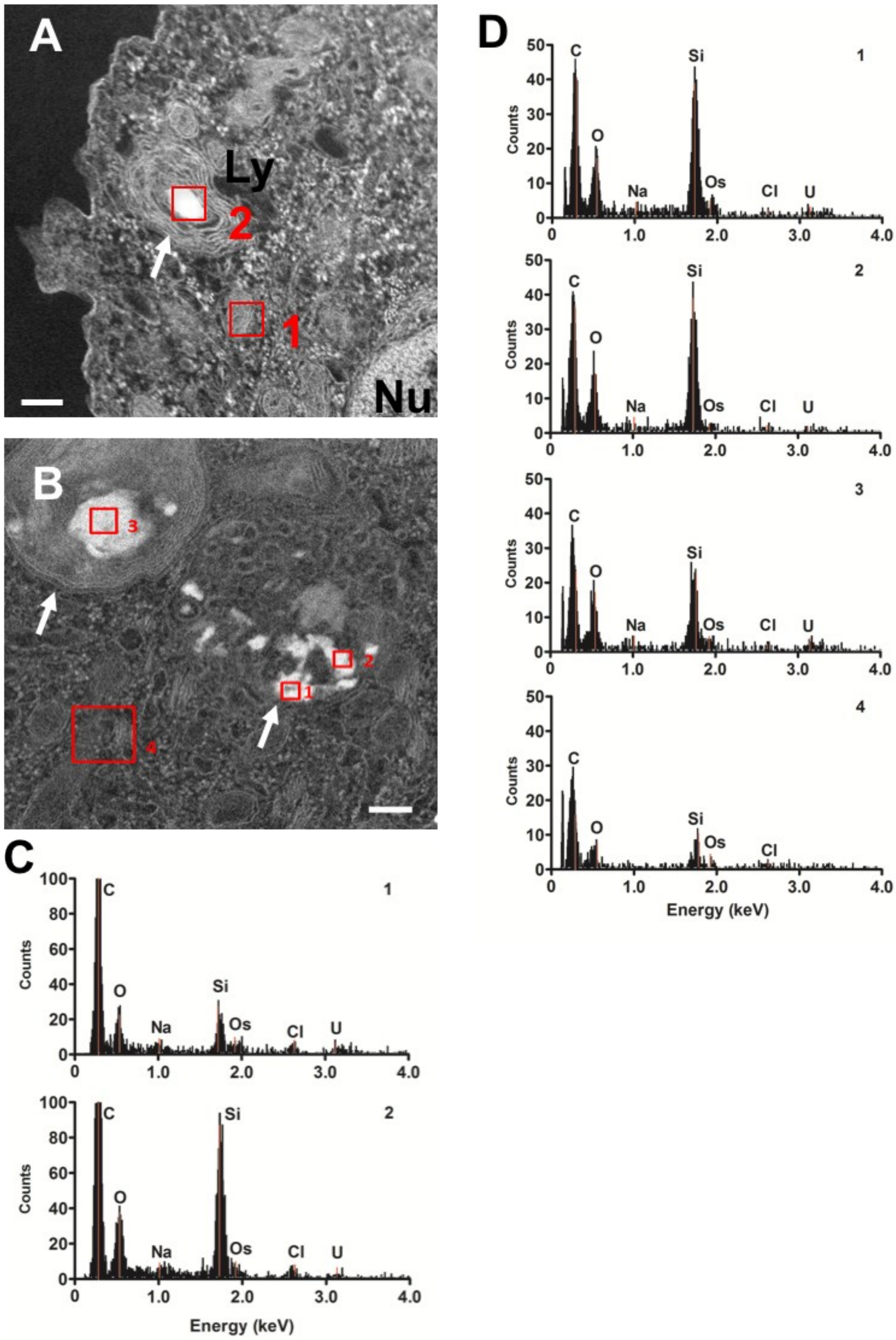

Figure S3: A and B shows STEM images of RTgutGC cells exposed for 24 hrs to 10 µM cit-AgNP in L-15/FBS and L-15/ex respectively. The corresponding EDX spectra are shown in panel C and D. Elemental Ag was not detectable. White arrows indicate possible lysosomes or autophagosomes. Scale bar is 200 nm in both images.

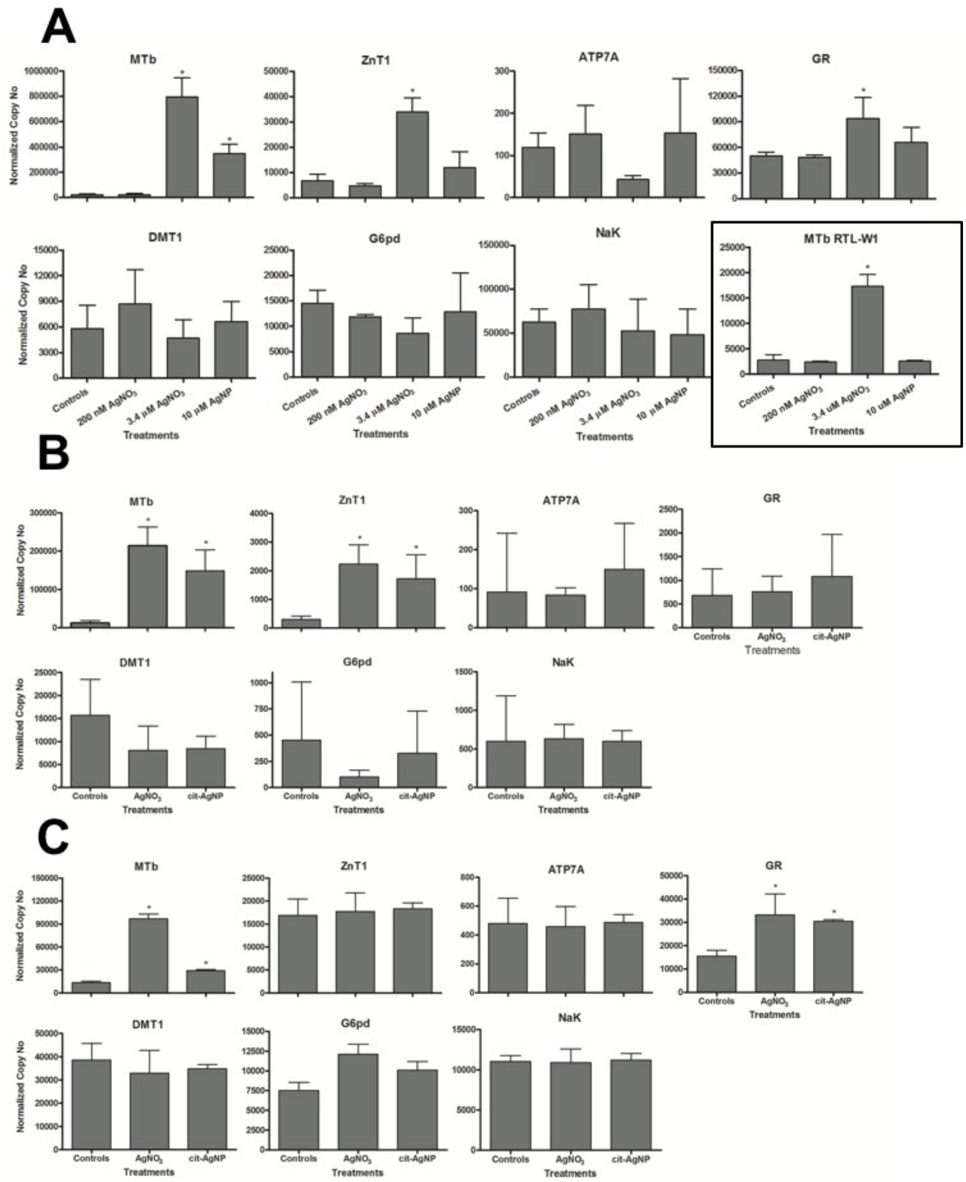

Figure S4: Normalized mRNA expression measured in RTgutGC (and RTL-W1 panel A only) cells. (A) toxic doses (~25% reduction in viability) of $AgNO_3$ and cit-AgNP and Ag concretion that were estimated to dissolve from cit-AgNP (200 nM = 2% of 10 µM) in L-15/ex (Yue et al., 2014); (B) non-toxic doses of $AgNO_3$ and cit-AgNP in L-15/ex; (C) not toxic doses of $AgNO_3$ and cit-AgNP in L-15/FBS. Values are means ± Sd (n=3). Statistical difference from controls for is indicated by an asterisk (*) (ANOVA, Dunnet test $p< 0.05$). Note that scales are different due to the wide variation in gene expression levels.

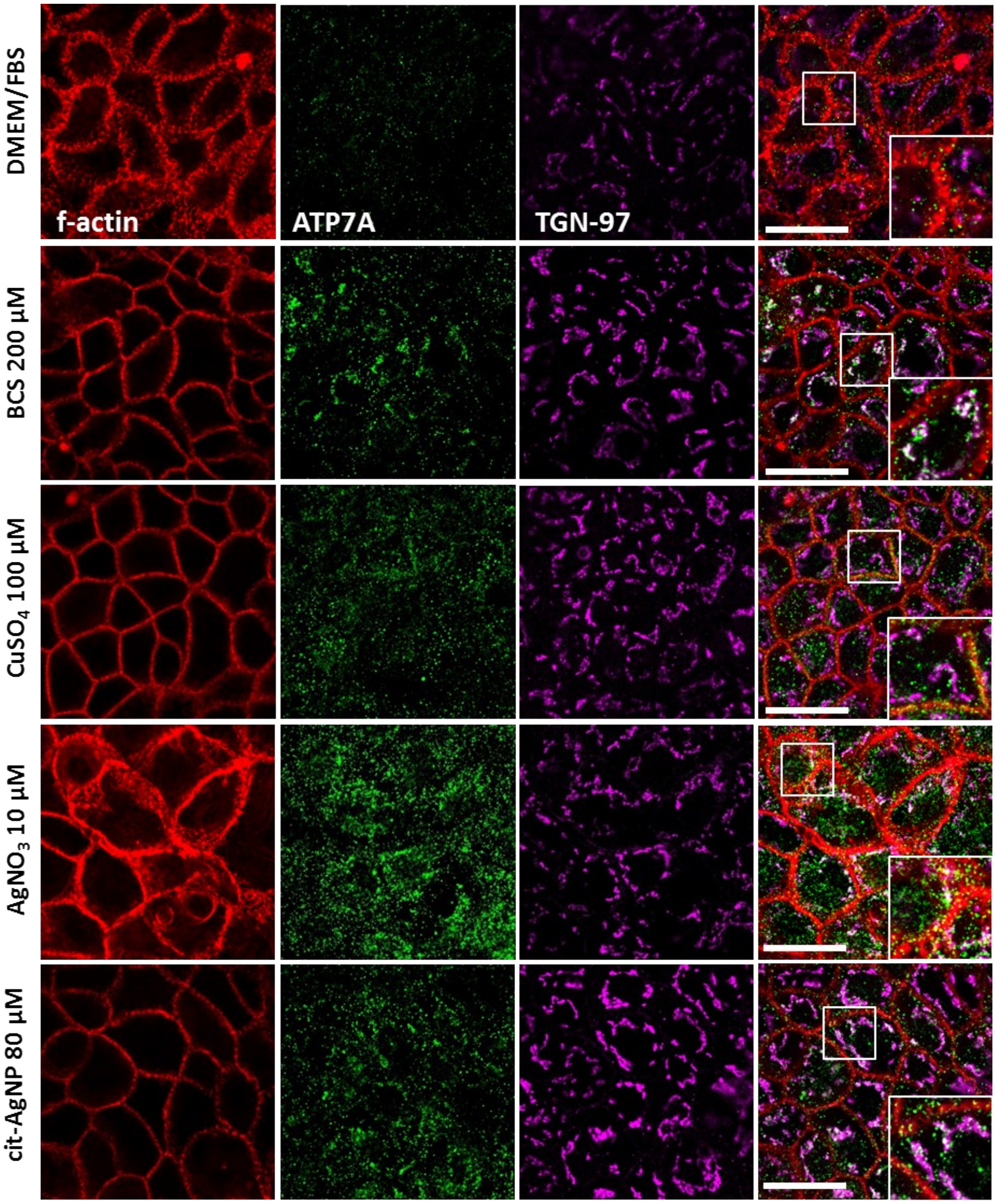

Figure S5. ATP7A protein trafficking. Caco2 cells were grown in the presence of 200 µM BCS for 18 hrs followed by the indicated treatment for 3 hrs and processed for immunostaining. Co-localization of ATP7A (in green) with cellular markers of cell periphery (in red, f-actin) and trans golgi network (TGN, in purple, Golgin97) is shown in insert panels. Insets are enlarged X2. Scale bars = 25 µm. Images were acquired using a laser scanning confocal microscope Leica SP5 and software 9LAS AF 2v. Insets are enlarged X2. Scale bars = 25 µm.