Supplementary Figure Legends

Supplementary Figure S1: (A) Experimental design; twenty mice of each ATF4$^{+/+}$/C57BL/6j (WT) and ATF4$^{+/-}$C57BL/6j heterozygous genotyped were divided into two groups (10mice/group). Group-1 received saline whereas group-2 received ATO (50µg/mouse in 200µl PBS, intra-peritoneal; daily for 10 days). Each of these two groups was sub-divided into two subgroups, whereas one subgroup received intratracheally (i.t.) saline. The other subgroup received i.t. fluorescent *E. coli* (2×10^7 *E. coli* in 30µl PBS). (B) Immunofluorescence staining of CHOP (Red) showing migration and localization from cytosol to nucleus in ATO–treated (2µM for 14h) peritoneal macrophages (F4/80, green) isolated from WT mice (Bar, 25µm).

Supplementary Figure S2: (A) Time-dependent response of bacterial clearance in saline vs. ATO-treated (2µM for 14h) Raw 264.7 cells. Note that saline-treated control cells effectively cleared fluorescence tagged *E. coli* within the period of 24h whereas ATO-treated Raw 264.7 cells were not able to clear this bacterial load and continued to show the presence of fluorescent tagged opsonized bioparticles (marked by yellow arrows). We also observed dissolution of cell membranes in these infected cells (marked by red arrow) (Bar, 25µm). (B) Effects of ATF4 on ATO-mediated impairment of engulfed *E.coli* clearance; Infection load of *E. coli* bioparticles were observed in Raw 264.7 cells transfected with ATF4 or scrambled siRNA. (B-I) Note here that control cells effectively cleared the fluorescence tagged *E. coli* within 24h. (B-II) ATO-treated (2µM, 14h) Raw 264.7 cells were not able to clear this bacterial load. (B-III and B-IV) Silencing ATF4 expression of Raw 264.7 cells restored the ATO-impaired clearance of engulfed bacteria as evidence by the diminished bacterial load in comparison to ATO-treated cells (Bar, 200µm). (C) Bacterial load of fluorescently labeled *E. coli* bioparticles were recorded through flow cytometry analysis in Raw 264.7 cells treated with ATO (2µM, 14h) either alone or in ATF4 silenced cells. Histogram representing the mean fluorescence intensity of bacterial load in Raw

264.7 cells. ***P<0.001, compared to control and ###P<0.001, compared to ATO-treatment group. NS- non significant compared to control.

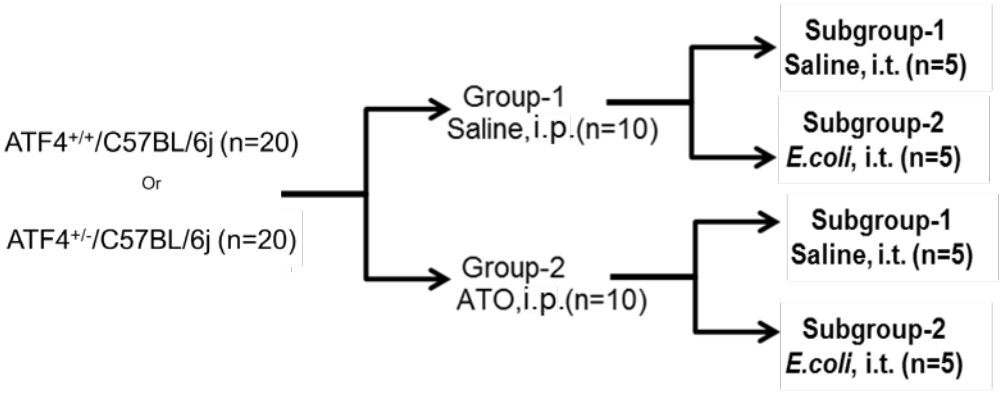
Supplementary Figure S3: (A) Western blot and mRNA expression analysis of ATF4 in scrambled and ATF4 siRNA transfected Raw 264.7 cells. (B) Western blot and mRNA analysis of ATF4, CHOP and GRP78 in scrambled and ATF4 siRNA transfected Raw 264.7 cells in the presence and absence of ATO (2µM, 14h). *P<0.05, **P<0.01 compared to scrambled siRNA and #P<0.05, ##P<0.01, compared to ATO-treatment group. NS- non significant compared to control.

Supplementary Figure S4: Interaction plot analysis between WT and $ATF4^{+/-}$ heterozygous mice for each treatment groups. ATO treated WT mice had significantly lower CD11b MFI as compared to the $ATF4^{+/-}$ mice. Significance was obtained using a two-way ANOVA with Bonferroni post-test comparison.

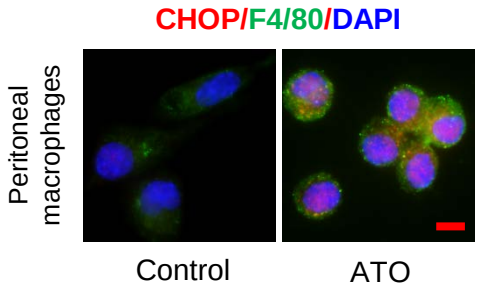
Supplementary Figure S5: (A) Alteration in mitochondrial membrane potential (MMP) was assessed using a fluorescent cationic dye, JC-1 (10µM, 15min) in the presence and absence of ATO (2µM for 24h). (B) Immunofluorescence staining of ATO-induced release of cytochrome c (Cyt c) (Green) in cytoplasm from mitochondria (Red). Mitochondria were stained with mitotracker, a red dye (250nM for 20min) (Bar, 50µm).

Supplementary Figure- S1

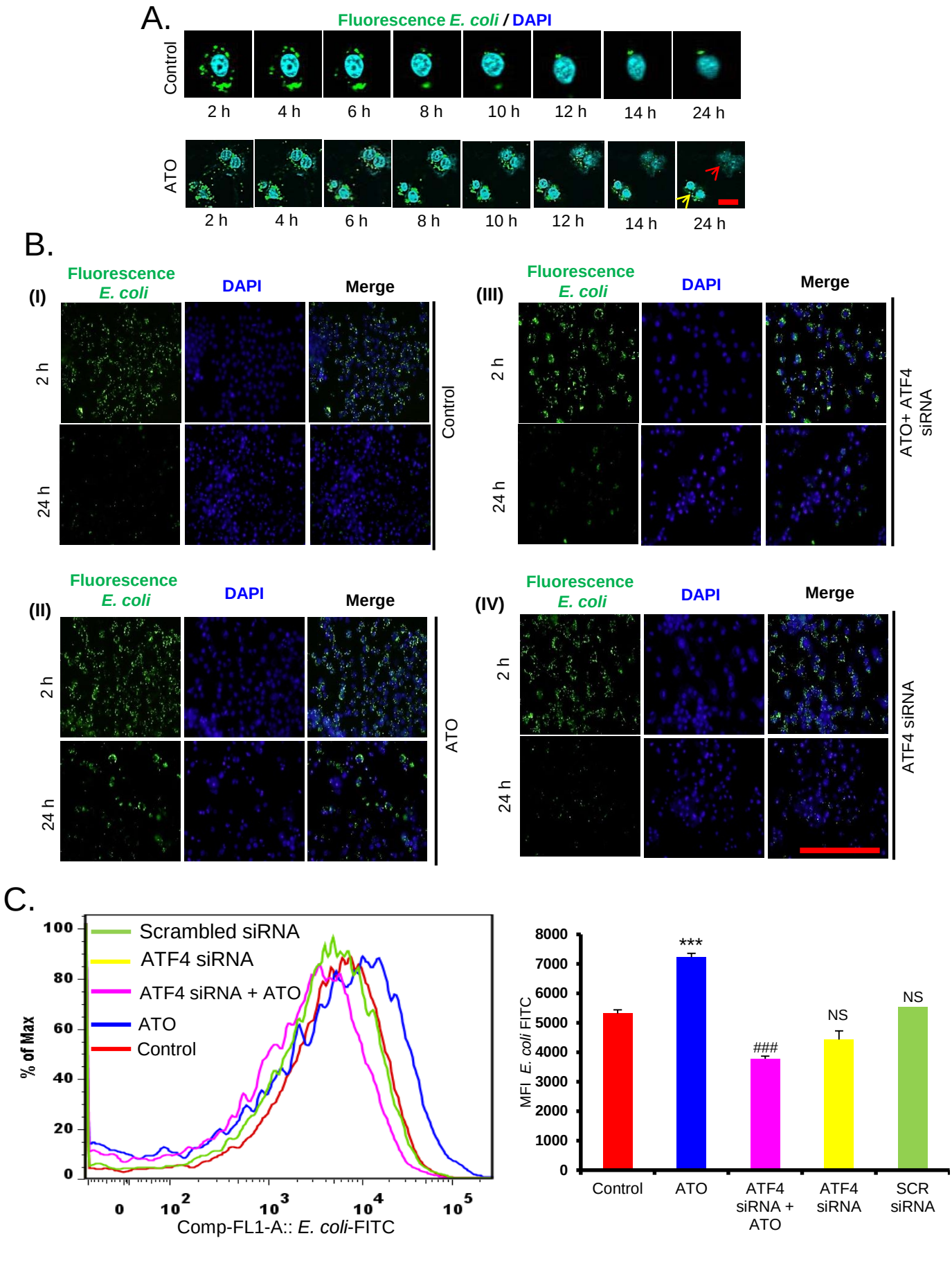
A.



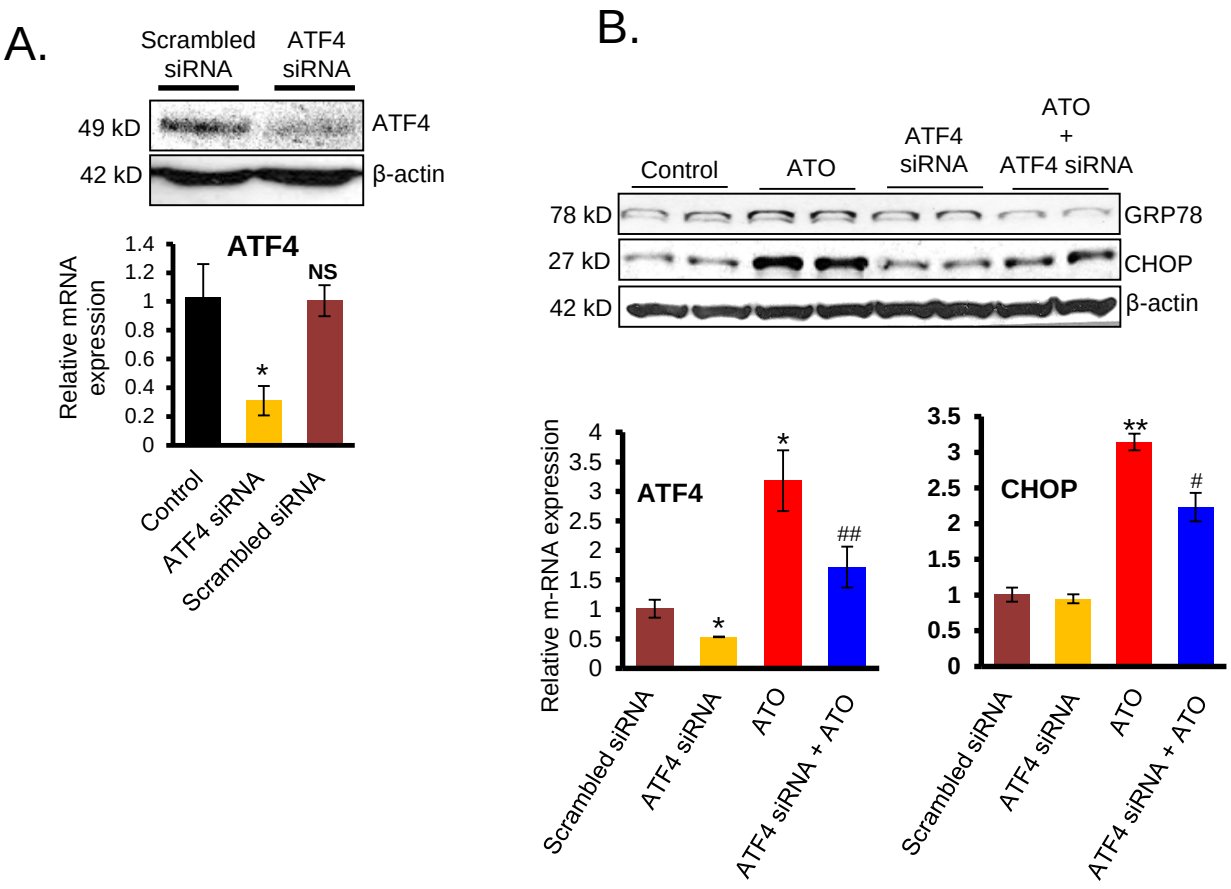
B.



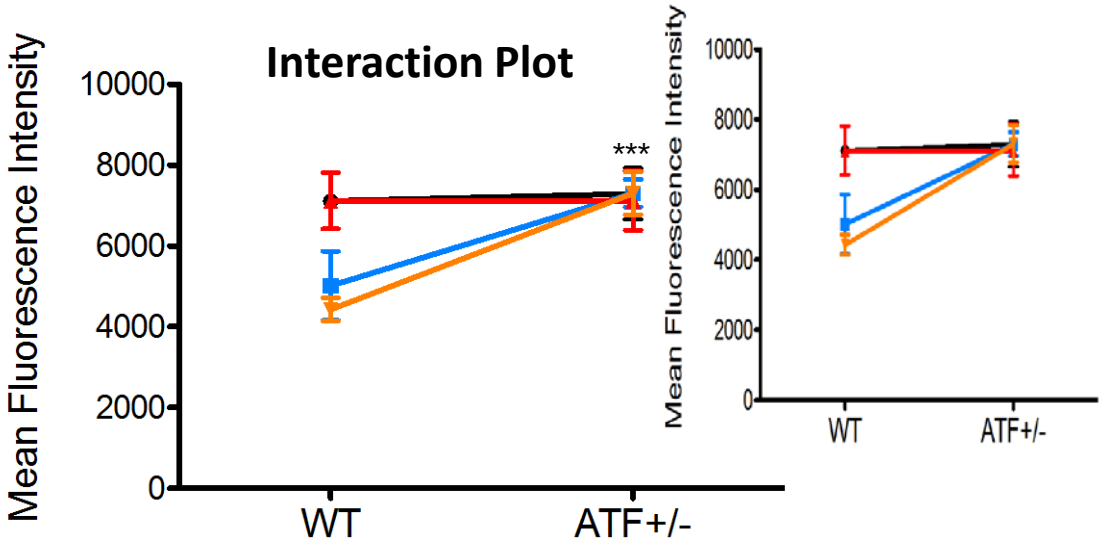
Supplementary Figure- S2



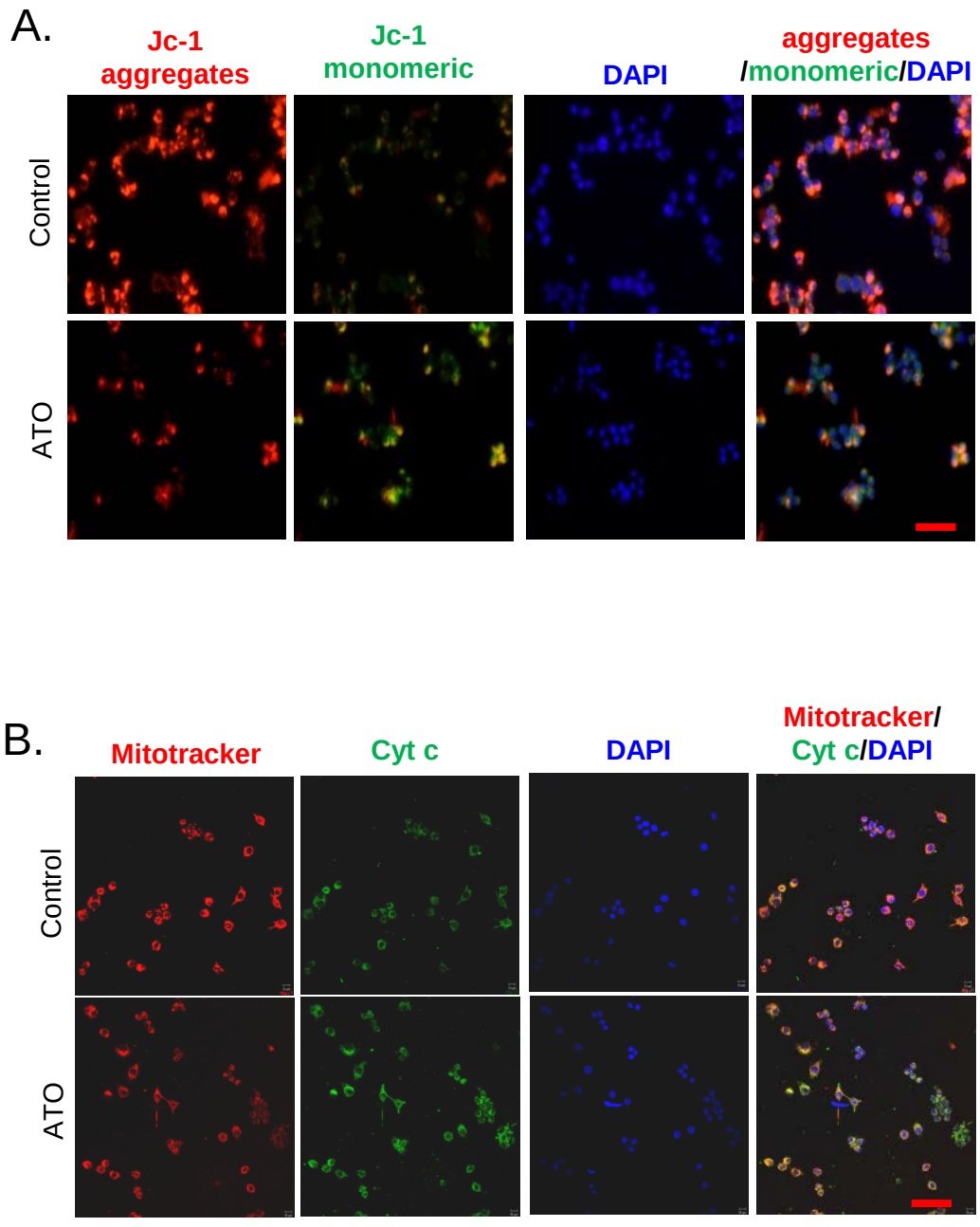
Supplementary Figure- S3



Supplementary Figure- S4



Supplementary Figure- S5



Supplementary Tables:

Table-SI List of Primers used in the study

Primers	Sequences
IL-1β	F- 5`-AAAGCCTCGTGCTGTCGGACC-3`
	R- 5`-CAGGGTGGGTGTGCCGTCTT-3`
TNF-α	F- 5`-AGCCCACGTCGTAGCAAACCAC-3`
	R- 5`-TCGGGGCAGCCTTGTCCCTT-3`
IL-10	F- 5-`GGCGCTGTCATCGATTTCTCCCC-3`
	R- 5-`GGCCTTGTAGACACCTTGGTCTTGG-3`
TGF-β	F- 5`-CGGCTGCTGACCCCCACTGA-3`
	R- 5`-ACGTTTGGGGCTGATCCCGTT-3`
ATF4	F- 5`-ATGGCCGGCTATGGATGAT-3`
	F- 5`CGAAGTCAAACTCTTTCAGATCCATT-3`
CHOP	F- 5`CTGCCTTTCACCTTGGAGAC-3`
	F- 5`CGTTTCCTGGGGATGAGATA-3`
ATF4 siRNA	MISSION® esiRNA esiRNA targeting mouse Atf4 From Sigma Aldrich.
Scrambled siRNA	From Ambion® | Life Technologies Cat No. 4390846
GAPDH	F-5`CAATGTGTCCGTCGTGGATCT-3`
	R-5`GTCCTCAGTGTAGCCCAAGATG-3`

Table-SII List of primary antibodies and horseradish peroxidase (HRP) conjugated secondary antibodies used in this study

Primary Antibodies	Company name	Applications	Dilutions
GRP78	Santa Cruz	Western Blot	400
CHOP	Cell signaling	Western Blot	800
ATF4	Cell signaling/Abcam	Western Blot, IF	800/ 100
Calpain-1	Cell signaling	Western Blot	800
Cleaved caspase-12	Cell signaling	Western Blot	800
Cleaved caspase-3	Cell signaling	Western Blot	1000
Bax	Cell signaling	Western Blot	1000
Cytochrome c	Santa Cruz	Western Blot, IF	500/100
p-IP3R (Ser 1756)	Cell signaling	Western Blot	800
VDAC	Cell signaling	Western Blot	1000
α-β tubulin	Cell signaling	Western Blot	1000
β-actin	Sigma	Western Blot	3000
Secondary Antibodies	**Company**	**Applications**	**Dilutions**
goat anti-rabbit	Pierce	IF	2000
goat anti-mouse	Pierce	IF	3000
mouse anti-goat	Pierce	IF	3000