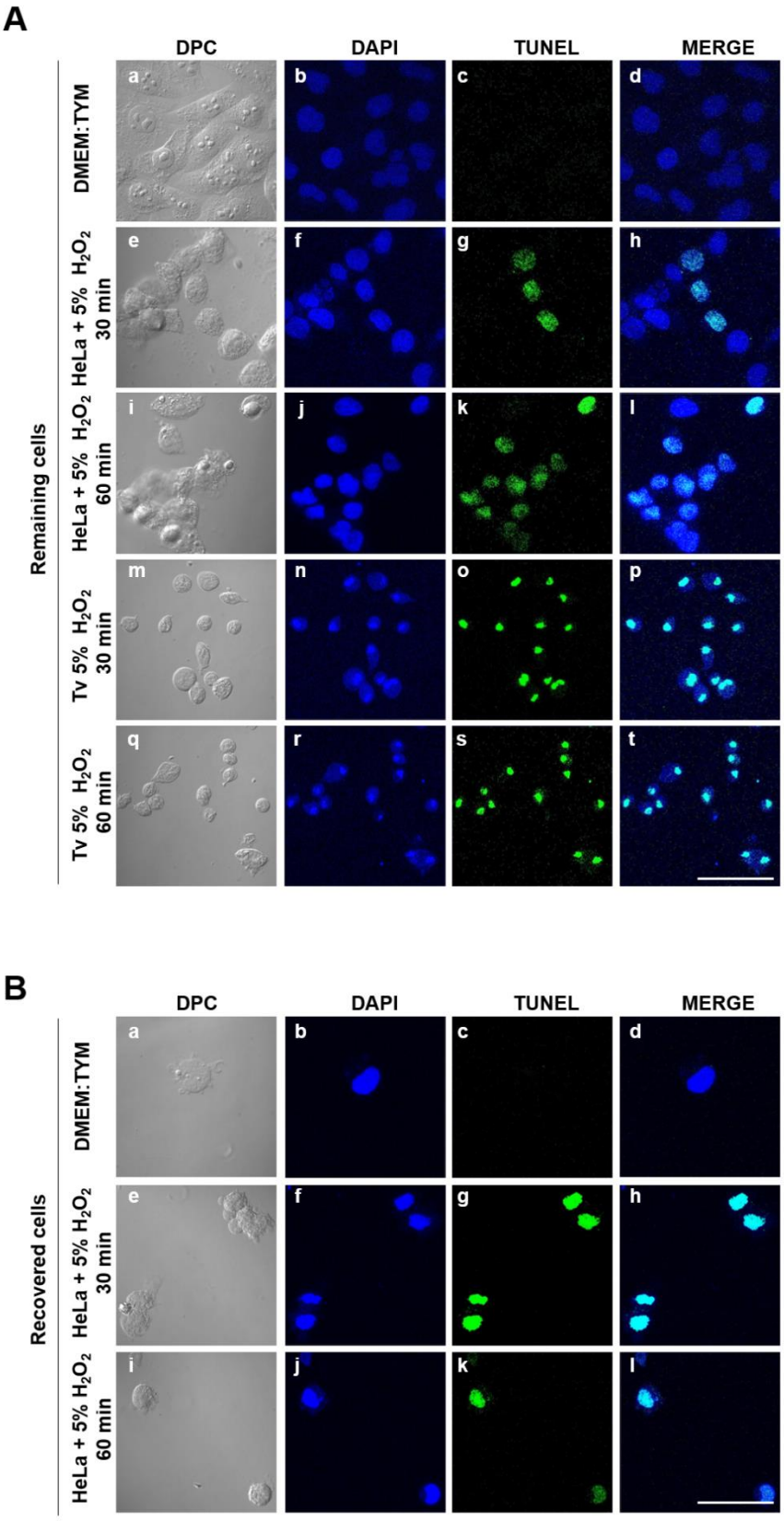
A
DPC
DAPI
TUNEL
MERGE
Remaining cells
DMEM:TYM
HeLa + 5% H2O2 30 min
HeLa + 5% H2O2 60 min
Tv 5% H2O2 30 min
Tv 5% H2O2 60 min
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q
r
s
t
B
DPC
DAPI
TUNEL
MERGE
Recovered cells
DMEM:TYM
HeLa + 5% H2O2 30 min
HeLa + 5% H2O2 60 min
a
b
c
d
e
f
g
h
i
j
k
l

Supplementary Fig. S4. Controls of induction of DNA fragmentation to HeLa cells and Tv. (A) TUNEL assay was performed after the incubation of HeLa cells and trichomonads with 5% H_2O_2 at 30 and 60 min at 37°C, as apoptosis inductor and positive control. After the incubation, the remaining cells attached to the coverslips (A) were incubated with TdT transferase and fluorescein-labelled nucleotide mix, as recommended by the manufacturer and observed by confocal microscopy (Zeiss, Germany). Differential Phase Contrast (DPC; panels a, e, i, m and q), DAPI (in blue, panels b, f, j, n and r) fluorescent TUNEL label (in green, panels c, g, k, o and s) and merge (panels d, h, l, p and t). HeLa cell monolayer incubated with the interaction medium was used as a negative control. (B) TUNEL assay was performed after the incubation of HeLa cells with 5% H_2O_2 at 30 and 60 min at 37°C, as apoptosis inductor and positive control. After the incubation, the cells released into the culture medium were incubated with TdT transferase and fluorescein-labelled nucleotide mix, as recommended by the manufacturer and observed by confocal microscopy (Zeiss, Germany). Differential Phase Contrast (DPC; panels a, e and i), DAPI (in blue, panels b, f and j), fluorescent TUNEL label (in green, panels c, g and k) and merge (panels d, h and l). HeLa cell monolayer incubated with the interaction medium was used as a negative control. These experiments were performed at least two independent times with similar results. The slides were observed at 63x magnification by confocal microscopy (Zeiss) 3D maximum projection. Scale bar = 20 µm.