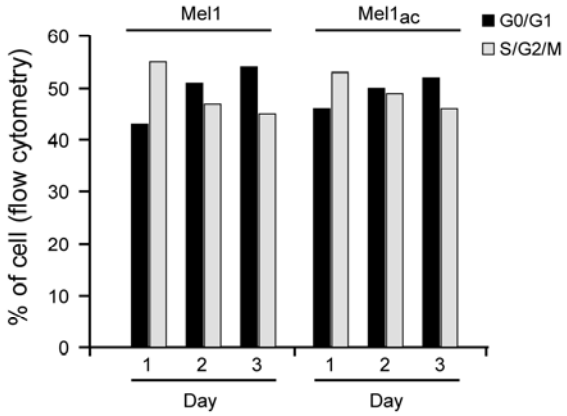
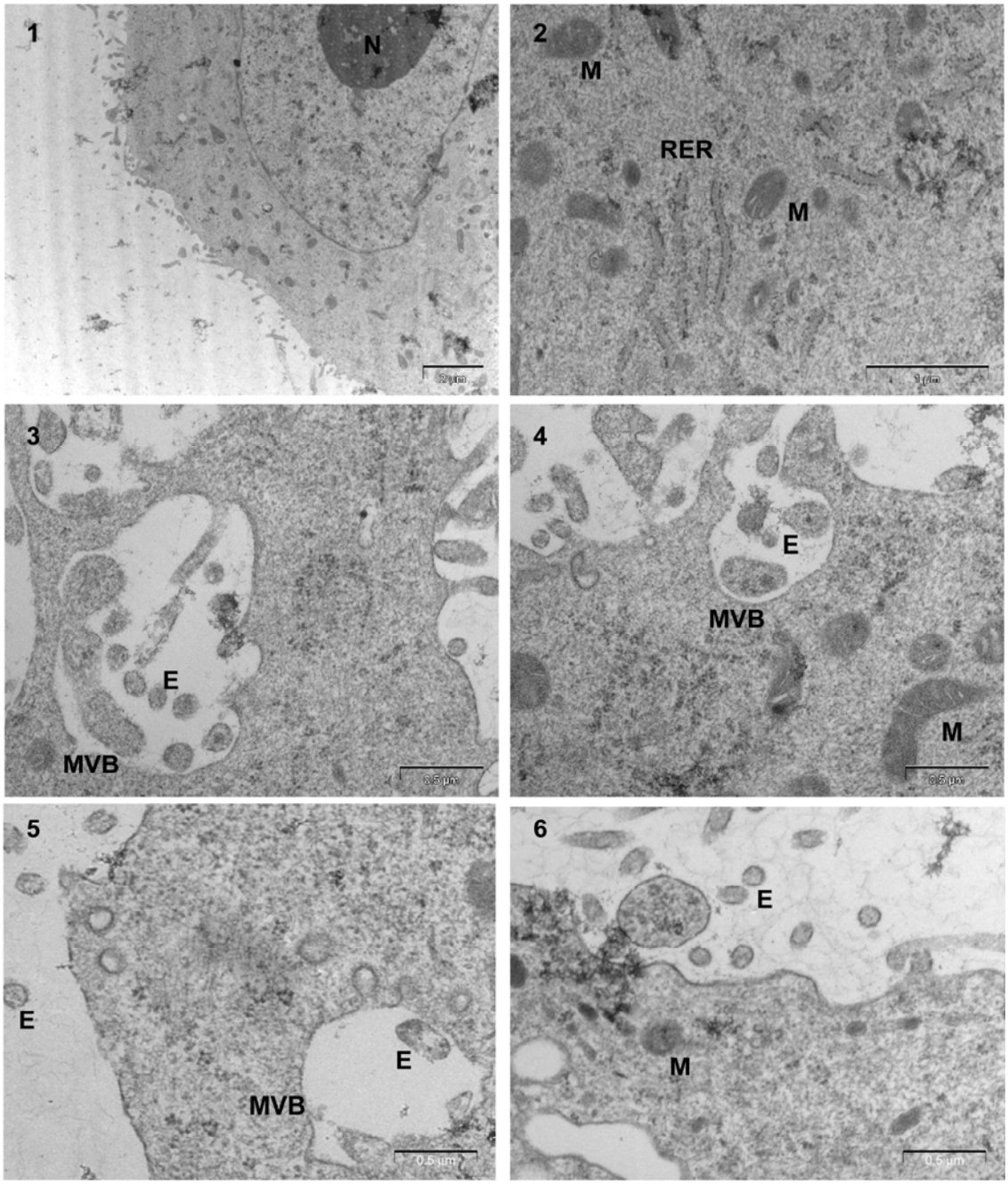
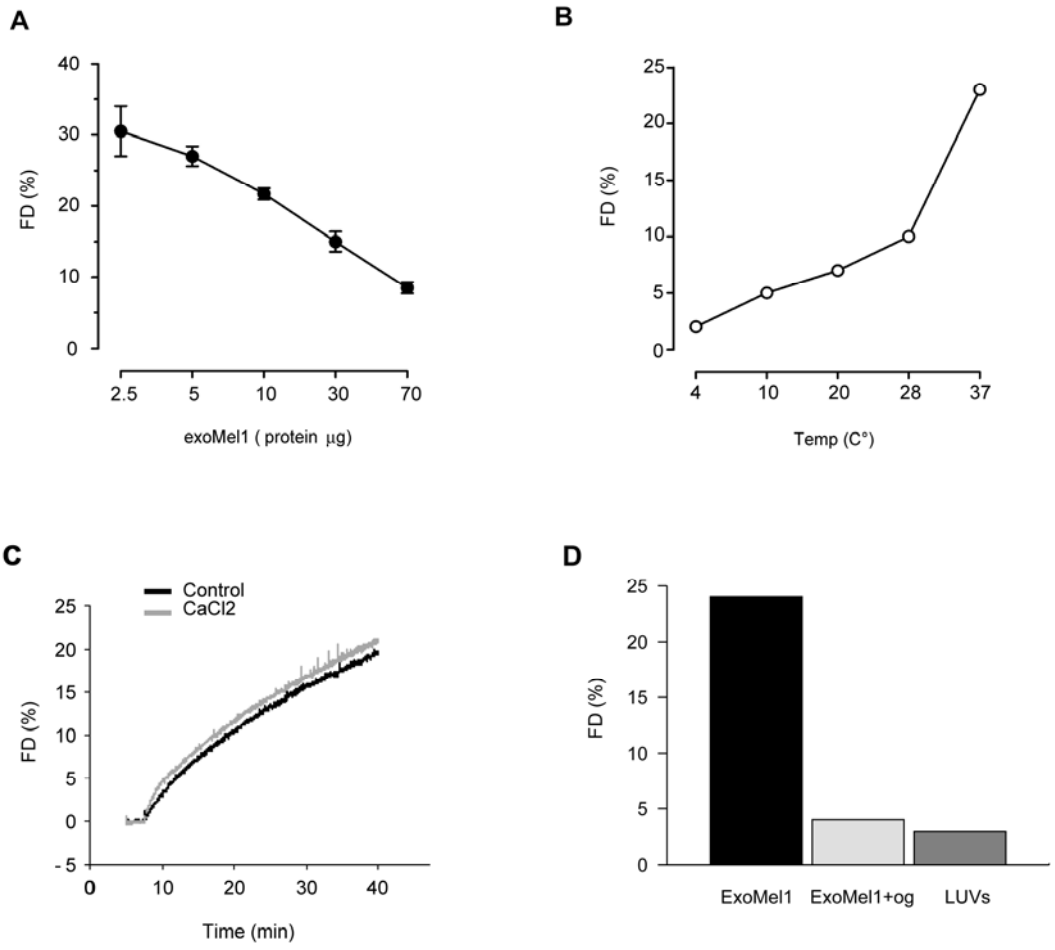
A


Mel1
Mel1ac
G0/G1
S/G2/M
% of cell (flow cytometry)
60
50
40
30
20
10
0
1
2
3
1
2
3
Day
Day

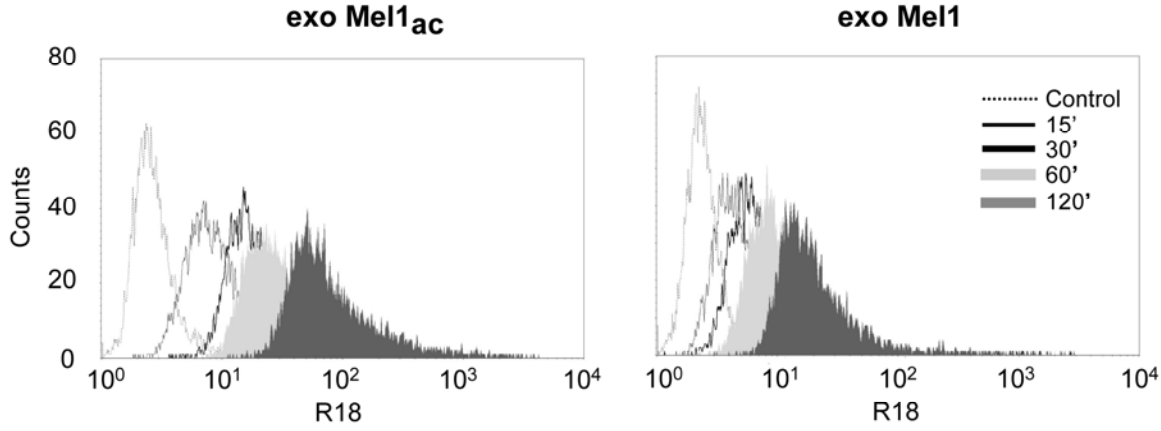
B


1
N
2
M
RER
M
3
E
MVB
4
E
MVB
M
5
E
E
MVB
6
E
M

Supp. Fig. 1



Supp Fig. 2



Supp. Fig. 3

Supplemental Fig.1. Cell vitality**.** *A.* Cell cycle distribution of Mel1 and $Mel1_{ac.}$ cells. Cells were incubated with propidium iodide and analyzed by flow cytometry to estimate the amount of G0/G1 (non-proliferative state) and S/G2/M (proliferative state) phases. Results are expressed as percentage of cells. *B.* Electron microscopy. Melanoma cells cultured in acidic (1-4) or buffered (5 and 6) conditions were analyzed by EM to detect morphological changes at ultrastructural level. No appreciable differences are detectable between Mel1 and $Mel1_{ac}$ cells. N=nucleus, M=Mithocondria, RER=Rough Endoplasmic Reticulum, E=Exosome, MVB=Multi-Vesicular Body. Bars: *B1,* 2µm, *B2,* 1µm, *B3-B6,* 0.5µm.

Supplemental Fig. 2. Fusion Test. *A.* R18-exoMel1 were incubated in different amounts with $1x10^6$ Mel1 cells and fusion activity tested. *B.* Membrane fusion between R18-exoMel1 and Mel1 cells ($1x10^6$) was evaluated at different temperature of incubation for 30 min**.** *C.* R18-exoMel1 were incubated with Mel1 cells in MES buffer or MES buffer containing 2mM $CaCl_2$ and fusion activity tested. *D*. Exosomes (10mg) were left untreated (exoMel1) or solubilized with octylglucoside (exoMel1+og), then reconstructed by dialysis, as compared to large unilamellar vesicles (LUVs) at the same concentration. All samples were R18-labeled and fusion efficiency tested on $1x10^6$ Mel1 cells, for 30 min at 37°C.

Supplemental Fig. 3. Flow cytometry analysis of exosome uptake at different pHs in melanoma cells. R18-exo$Mel1_{ac}$, or exoMel1 were incubated with $3x10^4$ cells at the corresponding pH for 15 min up 120 min. After cell washing, R18-exosome fluorescence was analyzed in parental cells. Note that exo$Mel1_{ac}$ uptake in acidic condition is more rapid than in buffered condition. The values of each peak are geometric means.