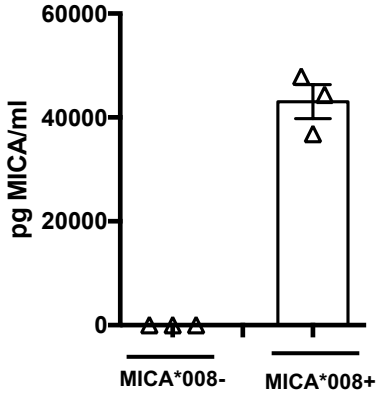
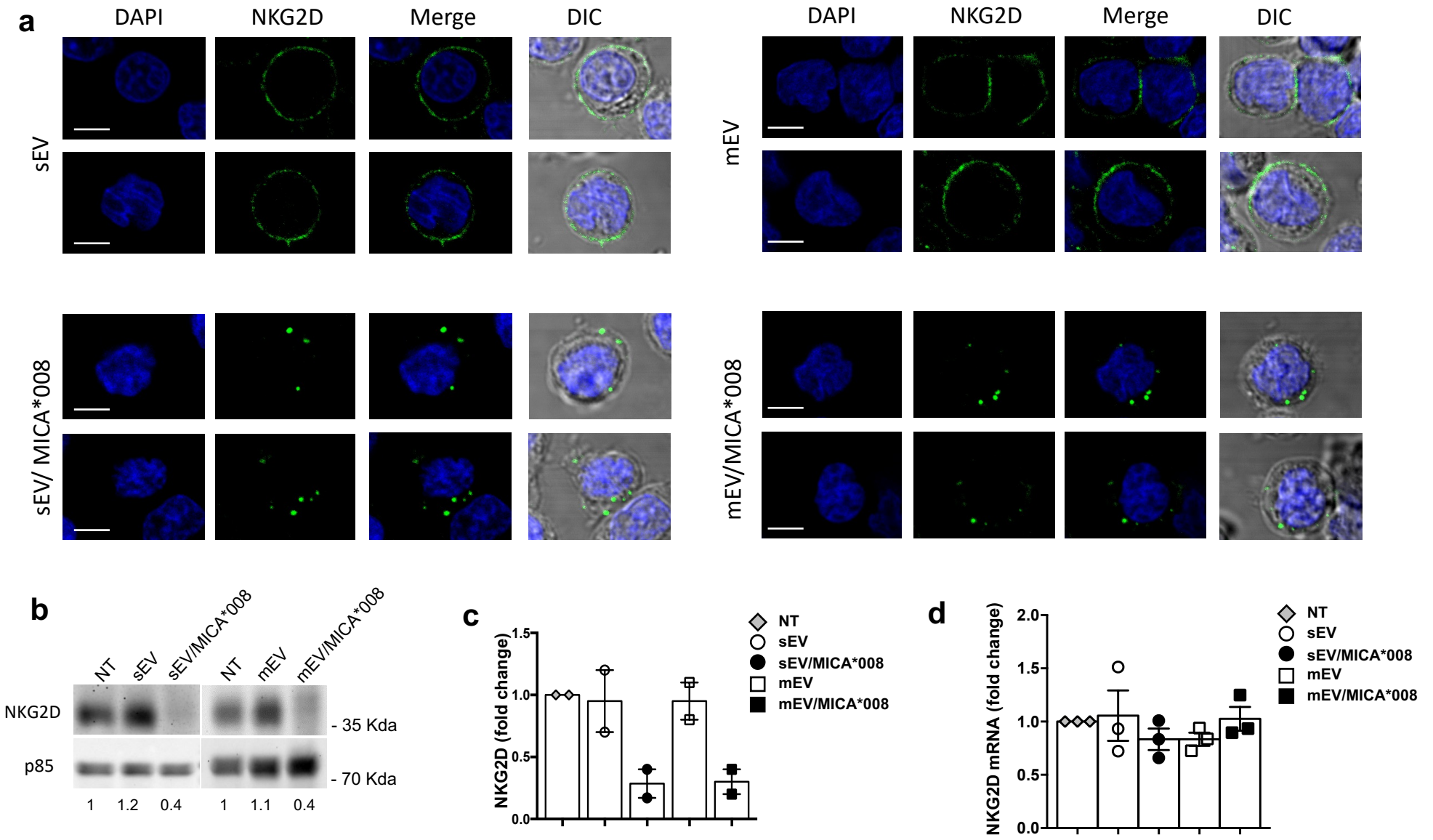
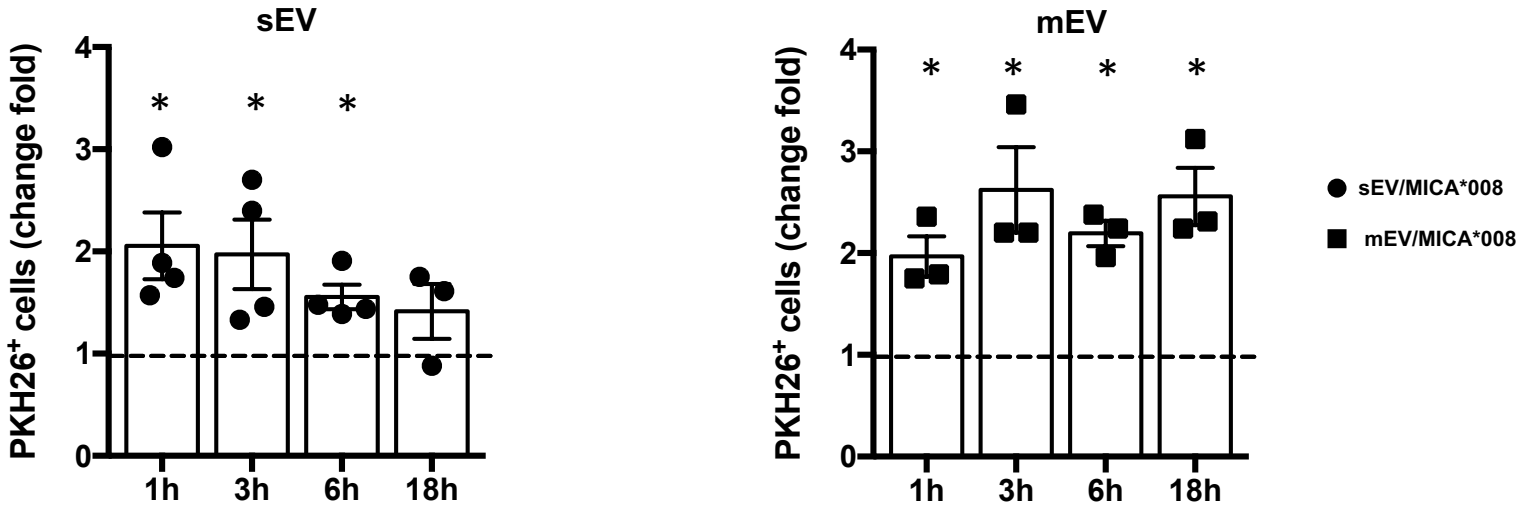


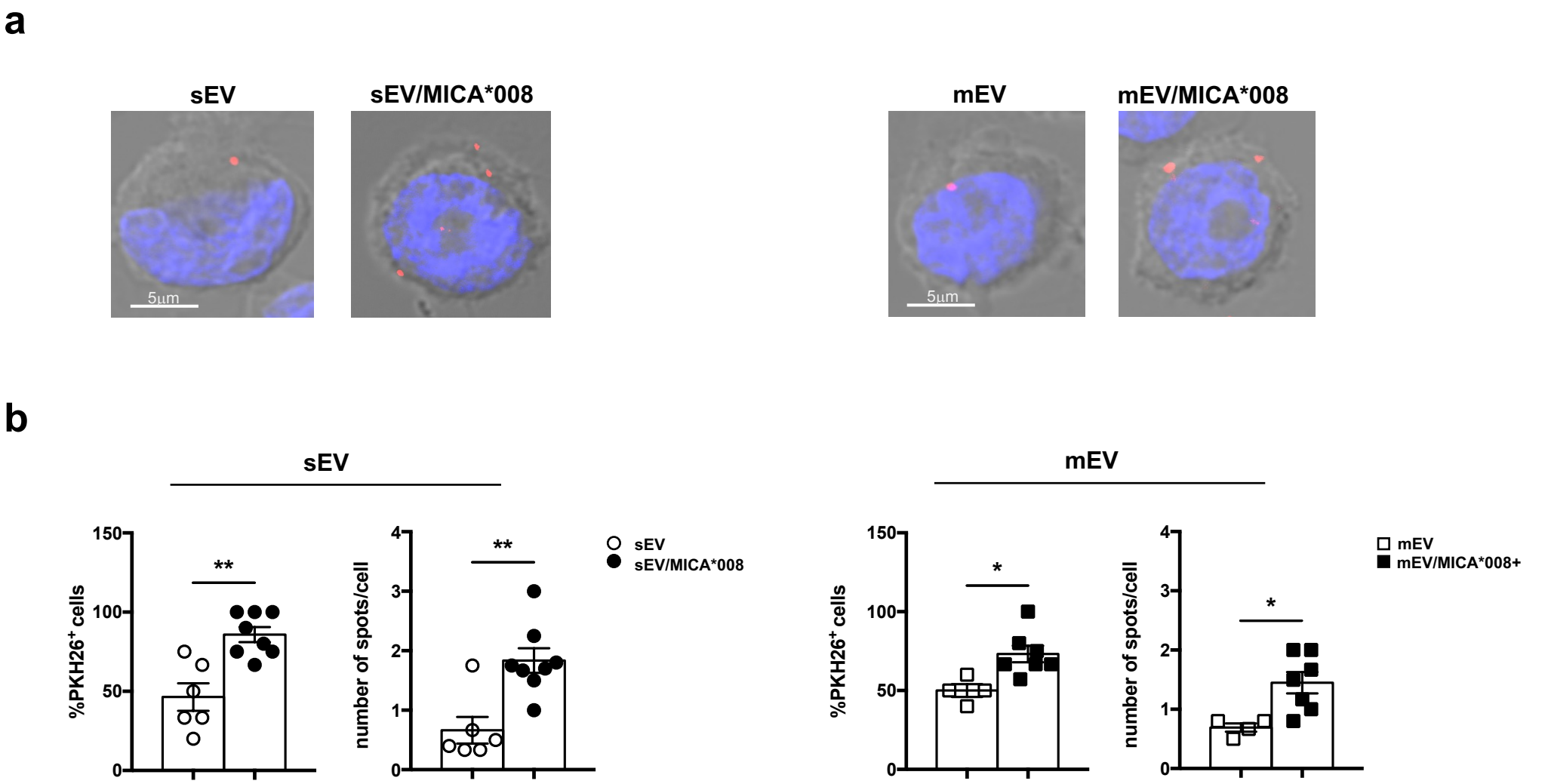
Supplementary Figure 1. Evaluation of soluble MICA in conditioned supernatants depleted of EVs. Conditioned supernatants derived from ARK MICA*008 transfectants or ARK transfected with an empty vector were depleted of EVs. The amount of soluble MICA was evaluated through a specific ELISA. The mean of three independent experiments is shown.



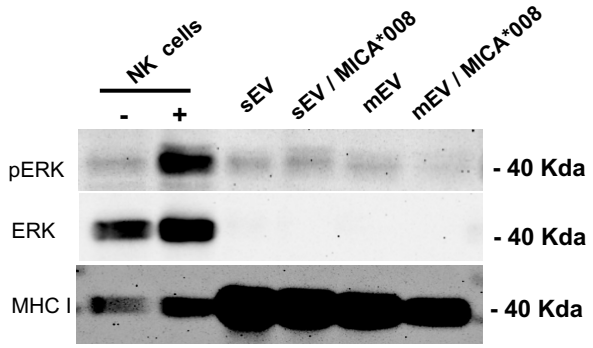
Supplementary Figure 2. MICA*008^{+}EVs induce NKG2D internalization and degradation. a) NKL cells were treated for 3 hrs with EVs (30 μg/ml of sEVs or 20 μg/ml of mEVs). After extensive washing cells were plated on poly-L-lysine coated slides, fixed and stained with anti-NKG2D mAb. DAPI was used to visualize the nuclei. Confocal images were acquired with a zoom 5 using 60x/1.35NA oil immersion objective. A single optical plane image is shown. DIC (differential interference contrast). Scale bar: 5μm. **b)** Western blot analysis of NKG2D in NKL cells treated for 24 hours with 30 μg/ml of sEVs or 20 μg/ml of mEVs. **c)** values relative to two independent experiments are shown. **d)** NKG2D mRNA was analyzed in NKL cells treated as described in b). The mean of three independent experiments is shown.



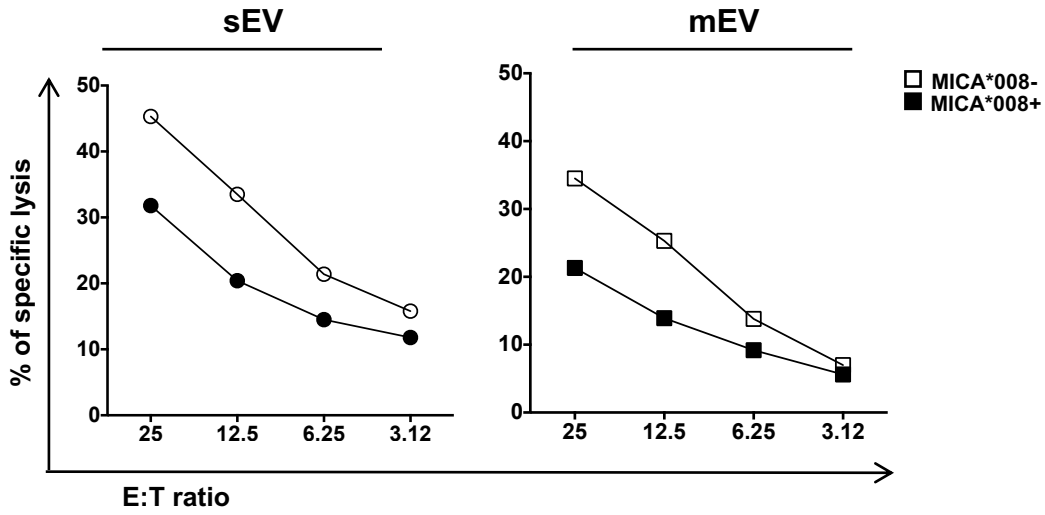
Supplementary Figure 3. Kinetics of uptake of PKH26 labelled EVs. NKL cells were treated for different times with PKH26-labeled sEVs (30 μg/ml) or mEVs (20 μg/ml) expressing or not MICA*008. Cells were harvested and the percentage of PKH26+ cells was evaluated by immunofluorescence and FACS analysis. Data are showed as relative expression of the percentage of PKH26+ cells referred to the cells incubated with EVs not expressing MICA*008 (=1) that is represented by the dashed line. The mean of at least three independent experiments is shown.



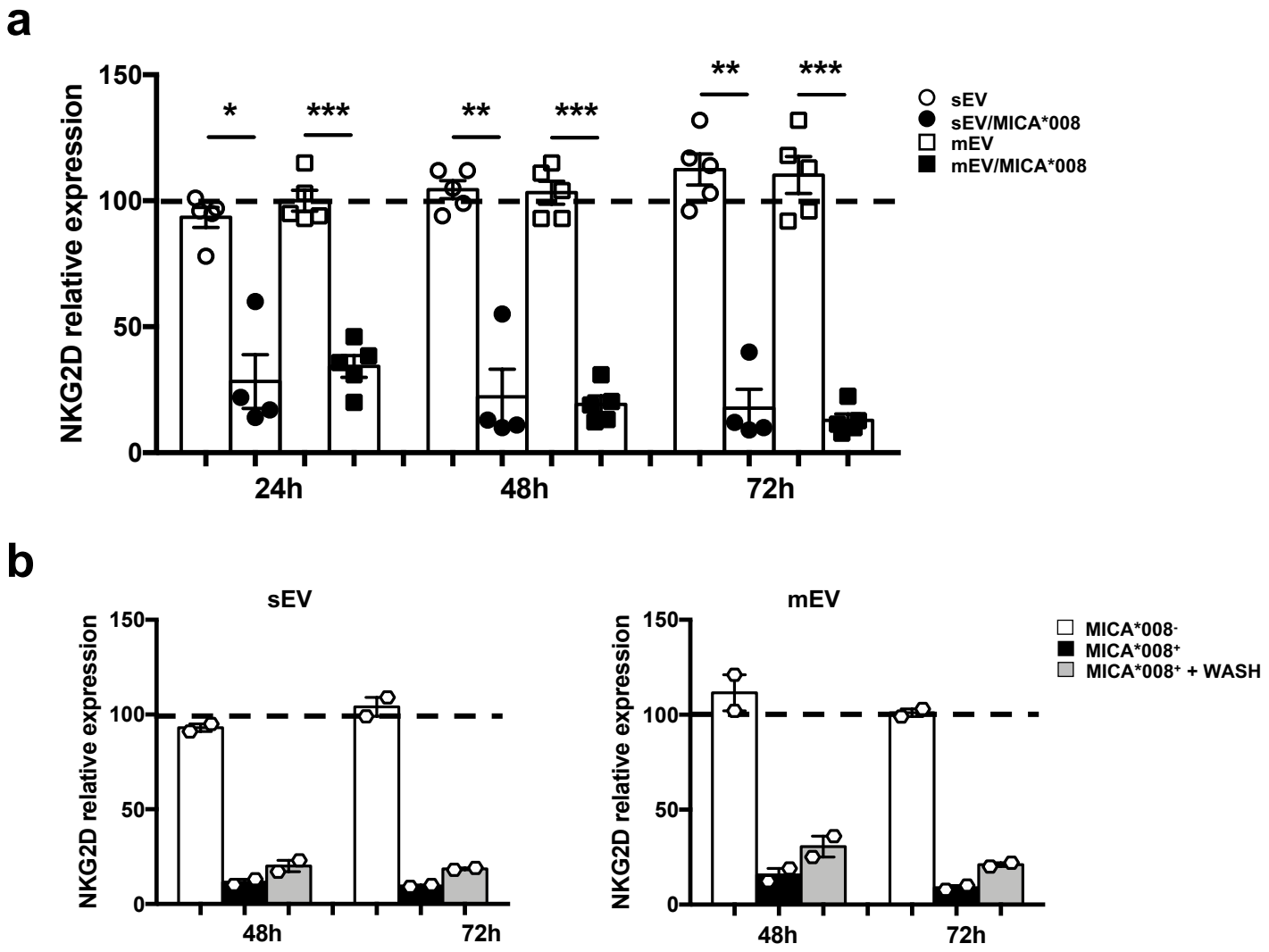
Supplementary Figure 4. MICA*008 EVs are internalized with higher efficiency by NK cells. NKL cells were treated for 3 hrs with PKH26-labeled EVs. After extensive washing cells were plated on poly-L-lysine coated slides, fixed and stained with DAPI to visualize the nuclei. Confocal images were acquired with a zoom 5 using 60x/1.35NA oil immersion objective. **a)** A single optical plane overlay the DIC (differential interference contrast) image is shown. Scale bar: 5µm. **b)** Data quantification was calculated on a total of 30 cells randomly acquired from one out of three independent experiments. Histograms represent the mean ± SD. * $p < 0.05$; ** $p < 0.01$, unpaired Student's *t* test.



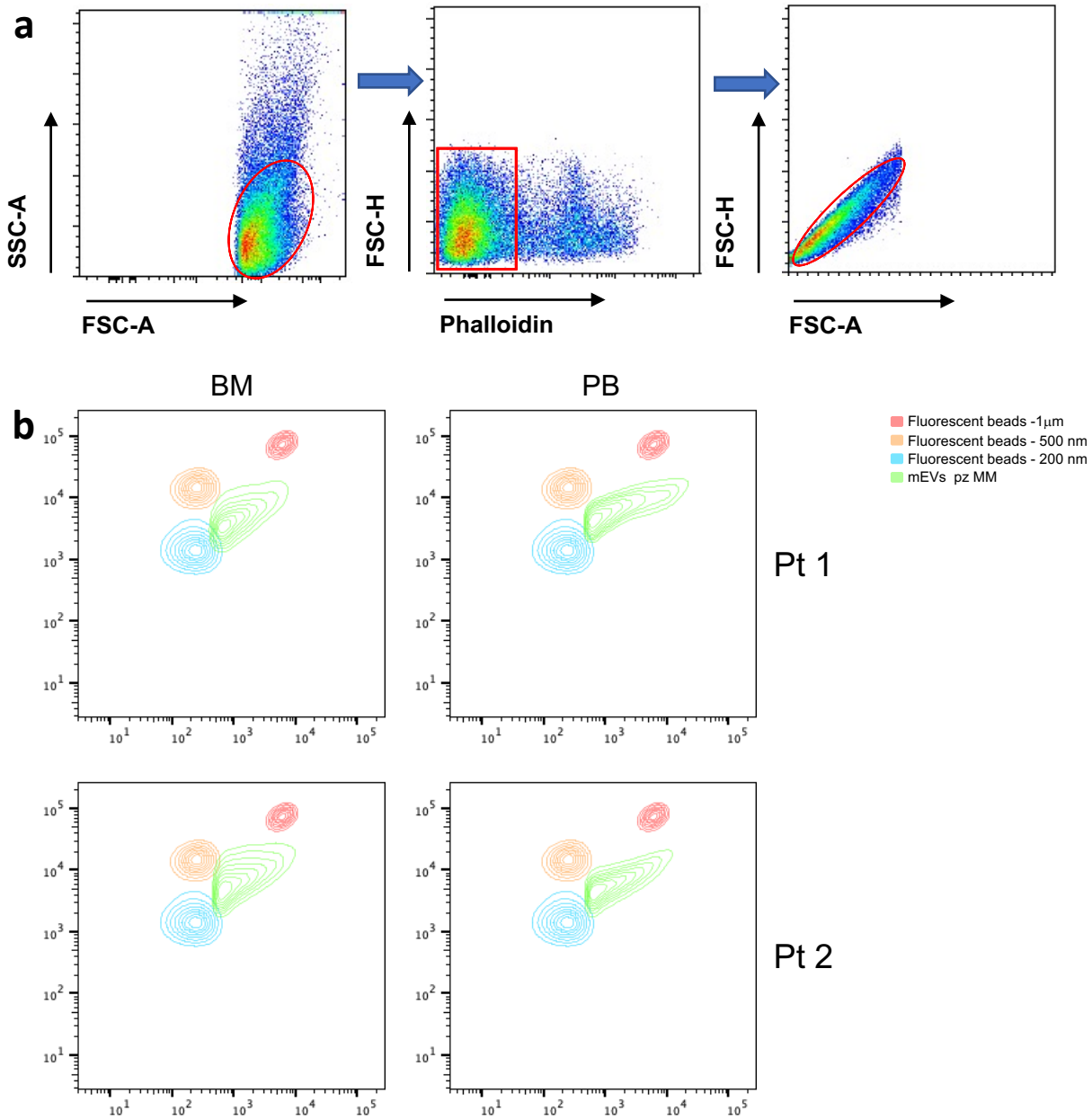
Supplementary Figure 5. Analysis of pERK expression in EVs expressing or not MICA*008.
Western blot analysis was performed on 20µg of lysate derived from EVs using anti-pERK, anti-ERK and anti-MHC I antibodies. As positive control of pERK induction, NKL cells were stimulated with anti-NKG2D mAb plus goat anti-mouse IgG for 15 min at 37°C before the lysis.



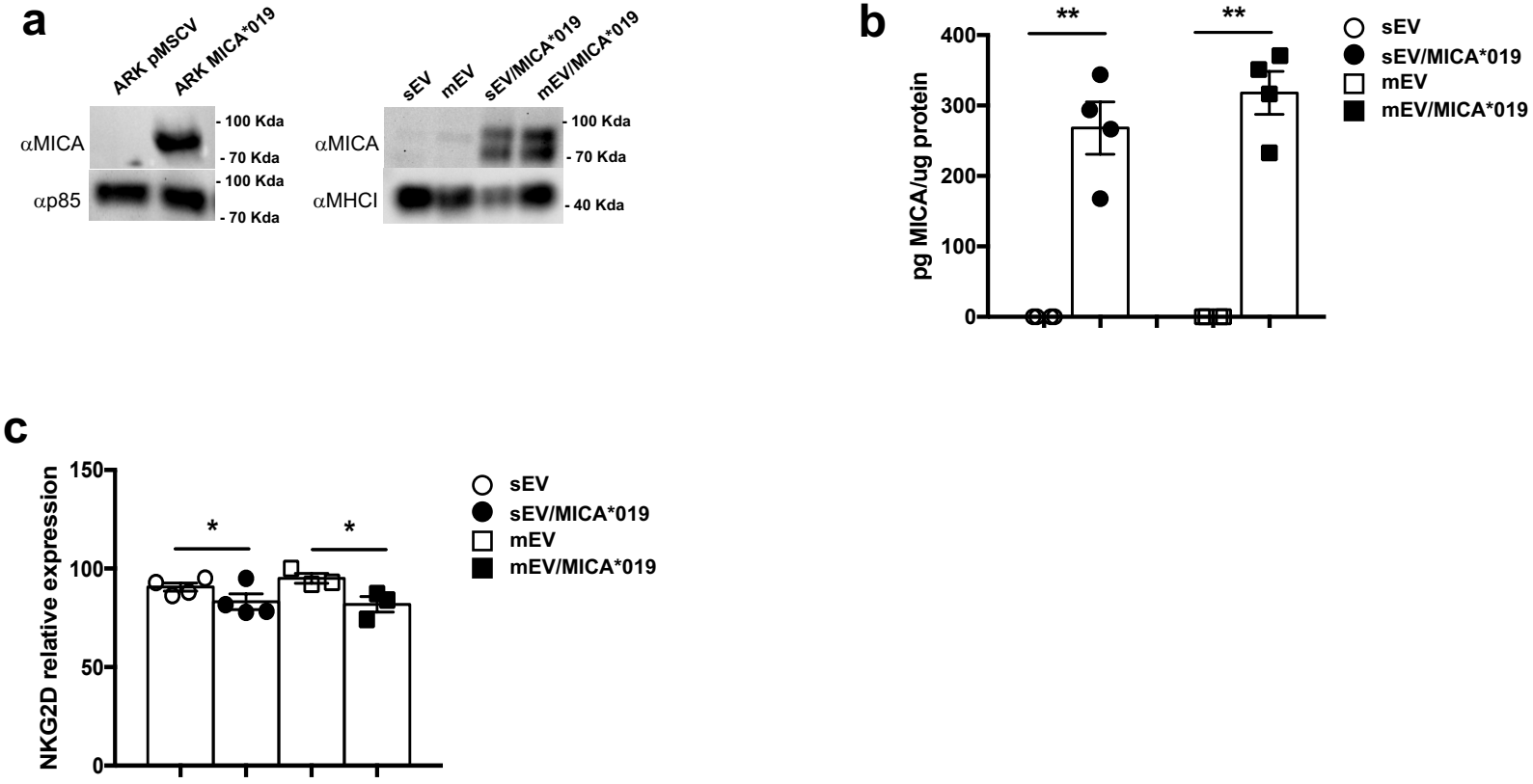
Supplementary Figure 6. MICA*008 positive EVs reduce NK cell killing vs K562 sensitive target cells. NKL cells were treated for 24 hours with 30 μg/ml of sEVs or 20 μg/ml mEVs expressing or not MICA*008. The cytotoxic activity of exosome- or microvesicle-treated NKL cells was evaluated against K562 target cells at different E:T ratio by performing the 7-AAD assay. A representative experiment is shown.



Supplementary Figure 7. Sustained NKG2D downmodulation induced by MICA*008$^+$ EVs. a) NKL cells were treated with 30 μg/ml of sEVs or 20 μg/ml mEVs expressing or not MICA*008. After 24, 48 or 72 hrs cells were harvested and NKG2D expression was evaluated by immunofluorescence and FACS analysis. Data are shown as relative expression of NKG2D, referred to the untreated group (=100%) that is represented by the dashed line. Values reported represent the mean of independent experiments ± SD. **b)** NKL cells were treated as described in a) for 24 hrs, EVs were removed from the culture through centrifugation and cells were resuspended in fresh medium for additional 24 and 48 hrs; after that NKG2D expression was evaluated. Data, showed as relative expression of NKG2D, referred to the untreated group (=100%) that is represented by the dashed line. Values reported represent the mean of two independent experiments ± SD.



Supplementary Figure 8 . Gating strategy of mEVs isolated from plasma derived from bone marrow (BM) or peripheral blood (PB) of MM patients.
5µg of mEVs were labelled with FITC/Phalloidin in combination with specific antibodies (i.e: anti-MICA, anti-MHCI or anti-CD138) for 60 min at room temperature. a) dot plots showing the gating strategy. The phalloidin negative population was gated and the doublets were removed by plotting FSC-H and FSC-A. A representative dot plot is shown. b) Fluorescent beads of defined size (i.e: 200 nm, 500 nm and 1µM) were used to visualize the size of the selected mEV population. Representative samples derived from two independent patients are shown.



Supplementary figure 9. MICA*019 long allele is associated to both sEVs and mEVs. a) Western blot analysis was performed on lysates derived from sEVs and mEVs fractions or from cell pellet of ARK/MICA*019 transfectants, using anti-MICA, anti-MHC I or anti-p85 antibodies. **b)** Lysates derived from sEVs and mEVs were assessed for the presence of MICA using a specific ELISA. Values are represented as the amount of MICA/μg of proteins and represent the mean of four independent experiments. **c)** The NKL cell line was incubated for 24 hours with 30 μg/ml of sEVs or 20 μg/ml of mEVs; cells were collected and NKG2D expression was evaluated by immunofluorescence and FACS analysis. Values reported represent the mean of at least three independent experiments. Relative expression of NKG2D was calculated considering the untreated group as 100%.