Supplemental Material

Material and methods

ELISA assay

Quantification of sIL-15/IL-15Rα complex in the plasma of HD and metastatic melanoma patients was performed by ELISA assay Human IL-15/IL-15R alpha Complex DuoSet ELISA DY6924 (R&D Systems) according to manufacturer's instructions.

Purification of NK cells

Freshly isolated NK cells were purified from peripheral blood mononuclear cells (PBMC) obtained from buffy coats using RosetteSep Human NK cell Enrichment Cocktail (Stemcell Thecnologies, Vancouver, Canada) according to manufacturer's instructions.

Purified NK cells were grown in RPMI-1640 medium (Euroclone) supplemented with 10% foetal bovine serum (FBS; Thermo-Fisher Scientific, Waltham, U.S.A.) and 600U/mL recombinant human IL-2 (rhIL-2 Proleukin; Novartis-Farma, Origgio, Italy).

Cytotoxicity assays

NK cytotoxicity activity was evaluated using as target cells K-562 (Human erythroleukemia cell line, ATCC, Manassas, VA, USA). K-562 cells were stained with 5μM Cell Tracker Green (CMFDA; Thermo-Fisher Scientific) and incubated with NK cells at 37°C at different Effector:Target (E:T) ratio. After 4 hours, propidium iodide (PI, Sigma-Aldrich) was added in a 1:200 ratio and cell viability was assessed by flow-cytometry (Cytoflex S Beckman-Coulter).
Dead target cells were identified as CMFDA+ PI+ and the percentage (%) of cell lysis was calculated as reported before [97].