Supplementary Figures

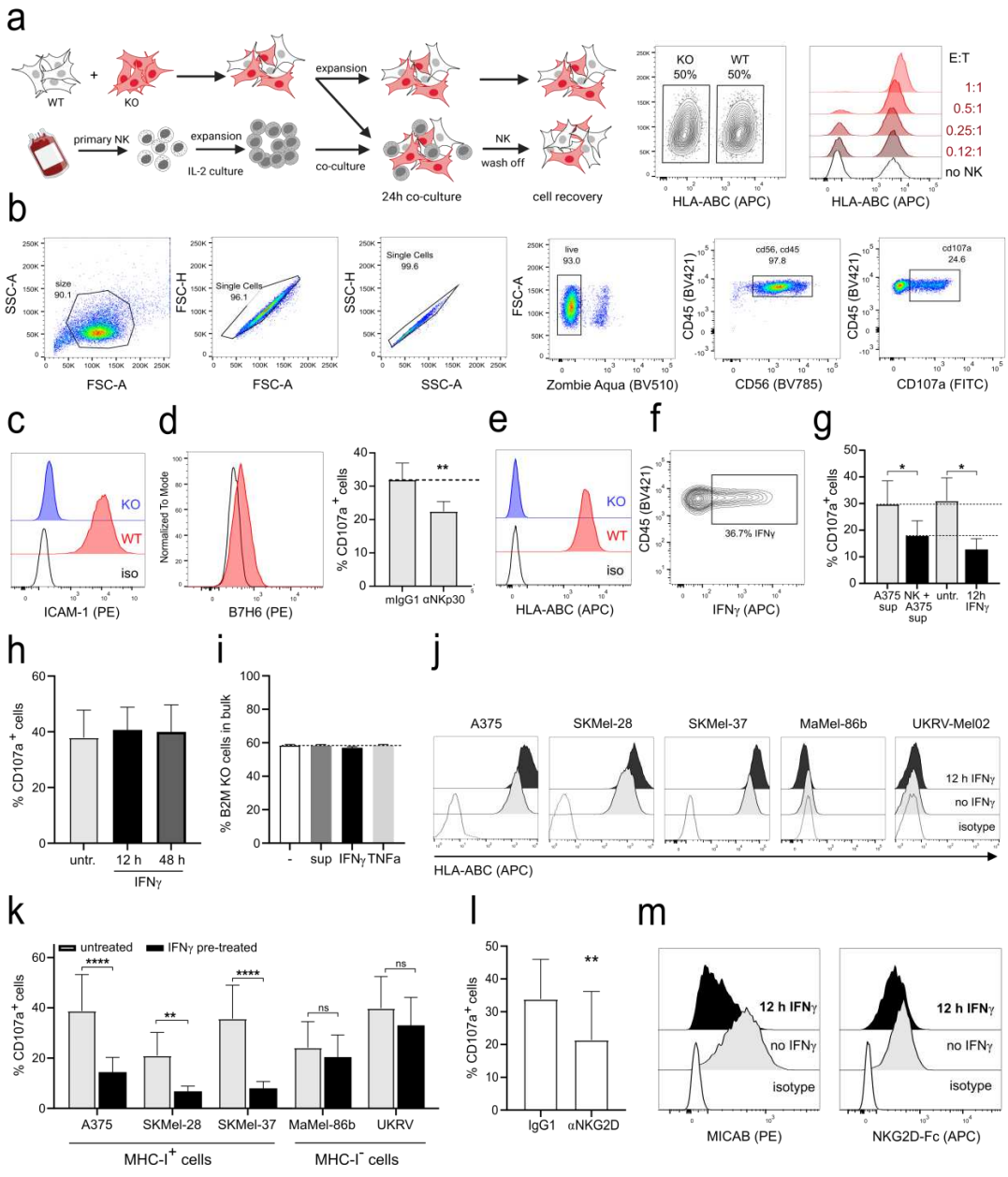

Figure S1

a) Experimental set-up mimicking the screen conditions. The ratio between WT vs KO melanoma cells was determined after 24-h co-culture with NK cells. Created with BioRender.com **b)** Representative gating strategy for detecting degranulation (CD107a) of NK cells. **c)** Expression of ICAM-1 on WT (red) and sorted ICAM-1 KO A375 cells (blue). **d)** Histogram showing the expression of B7H6 on A375 cells (left) and the degranulation of NK cells (right) after 4-h co-culture with A375 in the presence of NKp30 blocking mAb (n=5). **e)** Expression of HLA-ABC on WT (red) and sorted B2M KO A375 cells (blue). **f)** Intracellular staining of IFNγ in NK cells after 4-h co-culture with A375 cells. **g)** Effect of o/n pre-treatment of A375 cells with recombinant IFNγ or supernatant from 24-h NK/A375 cell co-culture on NK cells degranulation after 4-h co-culture (n=3). **h)** Degranulation of A375/NK cell supernatant-treated NK cells after 4-h co-culture with A375 cells. A375 cells were pre-treated or not with IFNγ for 24 and 48 h, washed and incubated for 4 h in a fresh media. Supernatant was then collected and used for NK cell treatment during the co-culture with untreated A375 cells (n=3). **i)** Changes in ratios between WT vs B2M KO A375 cells after 24-h treatment with supernatant (sup) from 24-h NK/A375 co-culture, recombinant IFNγ or TNFα in the absence of NK cells (n=3). **j)** Histograms showing the expression of MHC-I on different melanoma cell lines pre-treated or not for 12 h with IFNγ. **k)** Degranulation of NK cells after 4-h co-culture with MHC-I sufficient (left) and MHC-I deficient (right) melanoma cell lines treated or not with IFNγ o/n before the co-culture (n=3). **l)** Degranulation of NK cells after 4-h co-culture with A375 in the presence of NKG2D blocking mAb (n=6). **m)** Expression of MICA/B (left) or NKG2D ligands using NKG2D-Fc (right) on A375 cells

pre-treated or not with IFNγ 12 h. Statistical analysis was performed by two-tailed Student's *t* test (d, g, h, i, j, k, l), p-value *<0.05, **<0.01, ***<0.001, ****<0.0001.

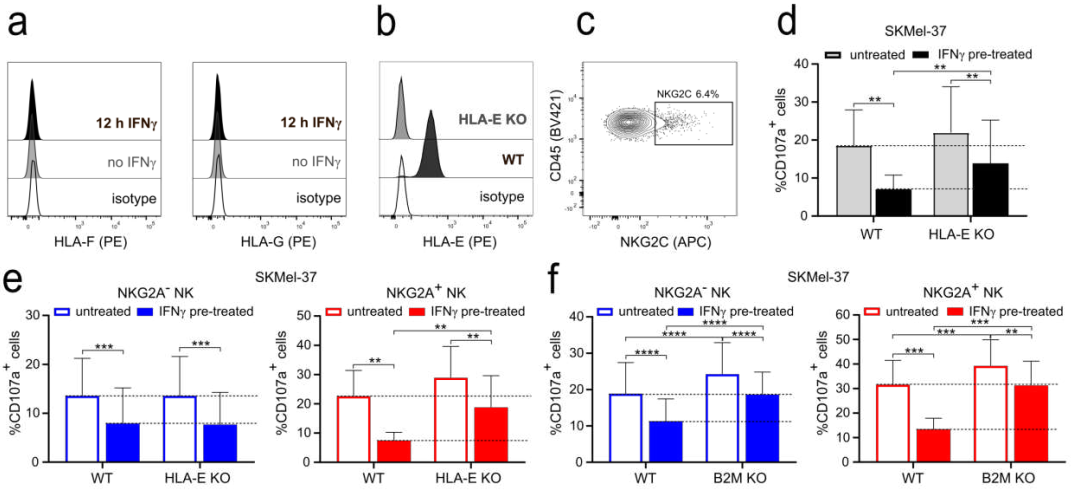

Figure S2

a) Expression of HLA-F and HLA-G on A375 cells pre-treated or not for 12 h with IFNγ. **b)** Expression of HLA-E on WT and HLA-E KO A375 cells after 12 h of IFNγ treatment. **c)** Representative dot plot showing the expression of NKG2C on NK cells. **d)** Degranulation of NK cells after 4-h co-culture with WT or HLA-E KO SKMel-37 cells pre-treated or not with IFNγ for 12 h before the co-culture (n=6). **e-f)** Degranulation of NKG2A$^-$ and NKG2A$^+$ NK cells after 4-h co-culture with WT, HLA-E KO or B2M KO SKMel-37 cells pre-treated or not with IFNγ for 12 h before the co-culture (n=6). Statistical analysis was performed by one-way ANOVA (multiple comparisons) test (d, e, f), p-value *<0.05, **<0.01, ***<0.001, ****<0.0001.

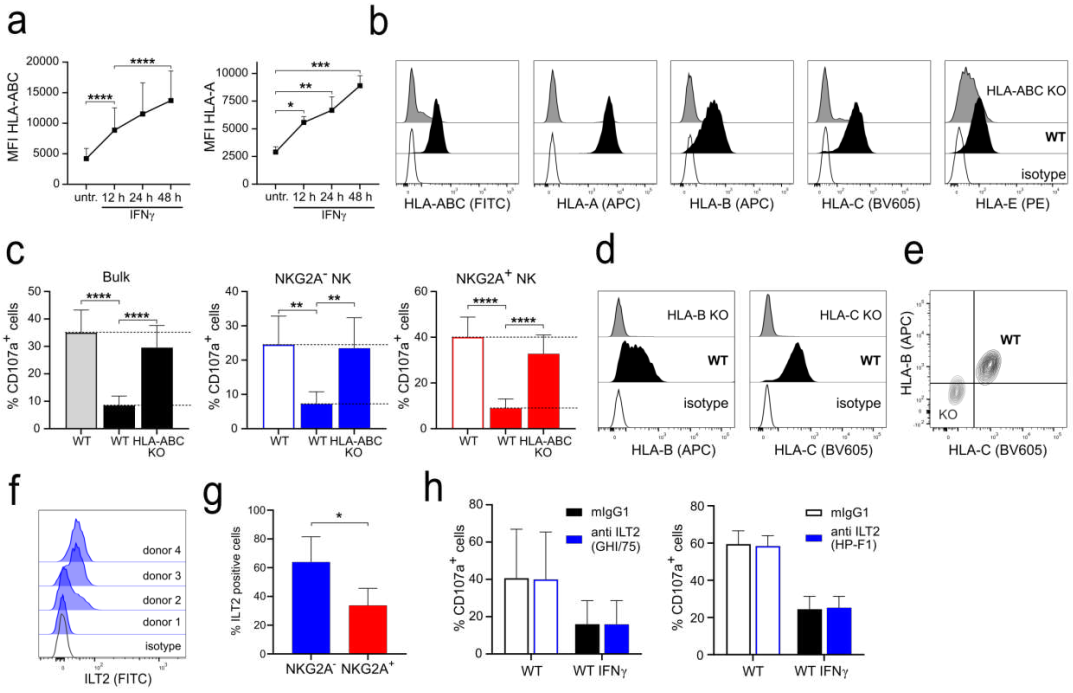

Figure S3

a) Expression of HLA-ABC and HLA-A on A375 cells pre-treated or not for 12, 24 and 48 h (n=3-5). **b)** Expression of individual HLA molecules on HLA-ABC KO A375 cells pre-treated with IFNγ for 12 h. **c)** Degranulation of bulk and NKG2A$^-$ and NKG2A$^+$ NK cells after 4-h co-culture with WT or HLA-ABC KO A375 cells pre-treated or not with IFNγ for 12 h before the co-culture (n=6). **d)** Histogram showing the expression of HLA-B and HLA-C on WT and HLA-B KO or HLA-C KO A375 cells treated or not for 12 h with IFNγ. **e)** Dot plot showing the expression of HLA-B and HLA-C on WT and HLA-BC dKO A375 cells treated for 12 h with IFNγ. **f)** Representative histograms showing the expression of ILT2 on NK cells from 4 different donors. **g)** Expression of ILT2 on NKG2A NK cell subsets (n=5). **h)**

Degranulation of NKG2A$^-$ NK cells after 4-h co-culture with A375 cells pre-treated or not with IFNγ 12 h before the co-culture in the presence of 2 different ILT2 blocking mAb (n=3-6). Statistical analysis was performed by one-way ANOVA (multiple comparisons) test (a, c, h) and two-tailed Student's *t* test (g), p-value *<0.05, **<0.01, ***<0.001, ****<0.0001.

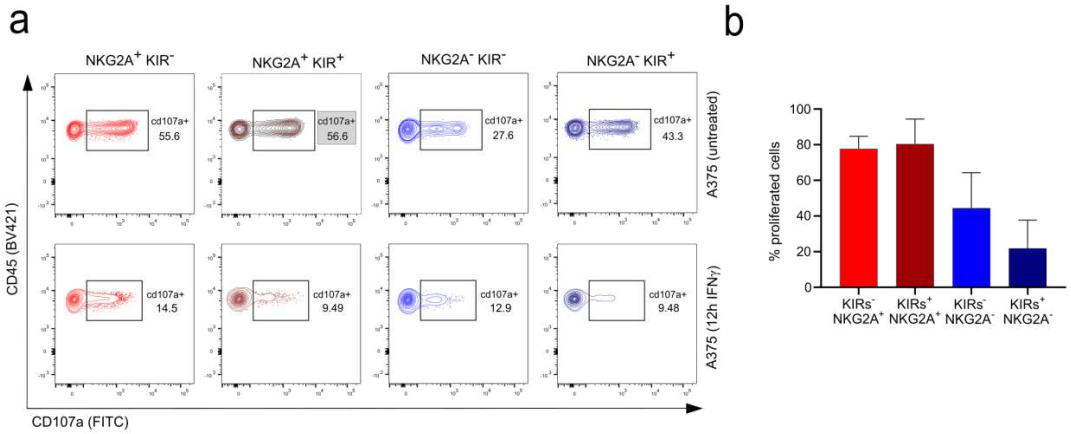

Figure S4

a) Representative dot plots showing the gating of CD107a$^+$ cells on different NK cell subsets after 4-h co-culture with A375 pre-treated or not with IFNγ 12 h before co-culture. **b)** Proliferation of individual NK cell subsets from isolated NKG2A$^-$ NK cells after 6 d of culture in IL-2-containing media. NKG2A$^-$ NK cells were isolated by depleting of NKG2A$^+$ NK cells using NKG2A magnetic beads, stained with CTV and analysed at d 6 (n=3).

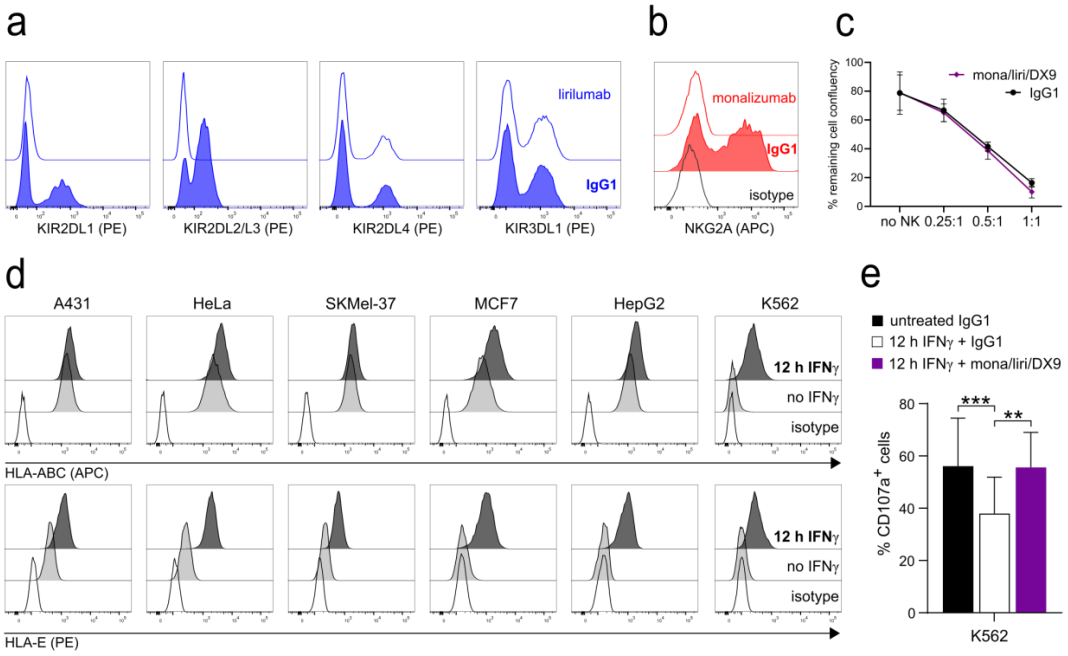

Figure S5.

a) Representative histograms showing the blocking effect of lirilumab mAb on individual KIRs expressed by most NK cell donors used in our assays. NK cells were incubated with 5 ug/ml of IgG1 or lirilumab for 15 min and stained with fluorochrome-conjugated mAbs against individual KIRs. **b)** Representative histograms showing the blocking effect of monalizumab on NKG2A detection on expanded NK cells. NK cells were incubated with 5 ug/ml of IgG1 or monalizumab for 15 min and stained with fluorochrome-conjugated NKG2A mAbs. **c)** Overall killing of B2M KO/WT cell mix (1:1) after 24-h co-culture with NKG2A$^+$ KIRs$^+$ NK cells in the presence of 5 ug/ml IgG1 or monalizumab + lirilumab + DX9 (n=3). **d)** Histograms showing the expression of HLA-ABC and HLA-E before and after 12-h IFNγ treatment of different tumor cell lines. **e)** Degranulation of 9d IL-2-cultured NK cells after 4-h co-culture with K562 cell line pre-treated or not with IFNγ for 12 h before co-culture in the presence of 5 ug/ml IgG1 or monalizumab + lirilumab + DX9 (n=6). Statistical analysis

was performed by one-way ANOVA (multiple comparisons) test (e), p-value **<0.01, ***<0.001.

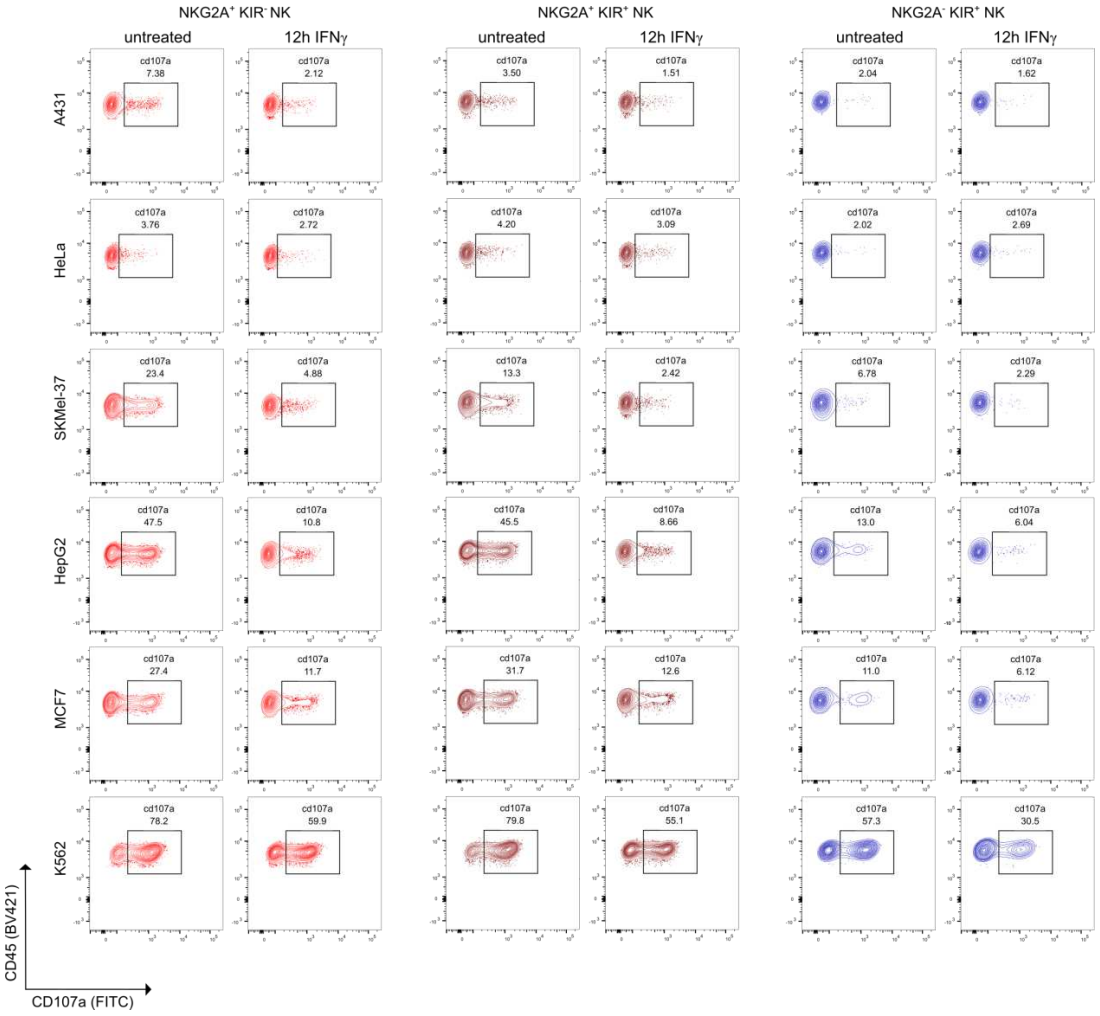

Figure S6.

Representative dot plots showing the degranulation of 9d IL-2-cultured NKG2A/KIRs NK cell subsets against untreated of 12-h IFNγ-pre-treated tumor cell lines.