

SUPPLEMENTARY MATERIALS AND METHODS

R2 data analysis

The r2: Genomics Analysis and Visualization Platform (<http://r2.amc.nl>) was used for analyzing the correlation between *ADAR1* expression (*ADAR_103*) and the overall survival (OS) of patients with CC. The Tumor Cervical Squamous Cell Carcinoma dataset (CESC) (The Cancer Genome Atlas; n=292) was selected for analysis. Expression cutoff: 5759.8945 (min.grp=8). The curves were compared using the log-rank test. *ADAR1* expression on 10 normal squamous cervical epithelial samples, HSIL, and 21 invasive squamous cell carcinomas, each from different patients, was assayed on single HG_U133A arrays, GEO ID: GSE7803.

TCGA data analysis

Cervical cancer patient data were downloaded from FireBrowse (RNA data:CESC.rnaseqv2__illuminahisec_rnaseqv2__unc_edu__Level_3__RSEM_genes__data.data.tx, and clinical data: gdac.broadinstitute.org/CESC/Merge_Clinical/Level_1.2016012800.0.0). Genes without a gene name and duplicated names were removed. 306 tumors were present in the data. Log CPM were computed from the data and used for modified Z-score scaling as shown in (1). Normal, Primary Tumor and Metastatic sample types were assigned. The field patient.stage_event.clinical_stage was collapsed into 4 main stages. The *ISG* score is computed as the median expression among the Z-score scaled values for the genes part of the *ISG* list (Table S1).

CCLE analysis

Data for 20 cervical cell lines with positive *ADAR* RNA expression was downloaded from the DepMap Portal (OmicsExpressionProteinCodingGenesTPMLogp1.csv, Model.csv). The RNA data is Z-score scaled as described in (1).

27 **DepMap analysis**

28 The genetic dependency data from the CRISPR screen were extracted from the public data release
29 from the DepMap at the Broad Institute (<https://depmap.org/portal/>). This database facilitates the
30 construction of a model of cell population dynamics in CRISPR knockout screens, measured using
31 the Chronos score. A Chronos score of <0 indicates a significant role of the gene in the development
32 of the selected cell line. In this study, we downloaded the *ADARI* CRISPR (DepMap Public
33 23Q4+Score Chronos) dataset to evaluate the potential role of *ADARI* in the development of diverse
34 malignant tumors.

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36 **Cell lines and primary cultures of human fibroblasts**

37 SiHa (HTB35) cell line was grown in DMEM (Euroclone), whereas CaSki (CRM-CRL-1550) and
38 K562 cells (CCL-243) were cultured in RPMI (EuroClone). All cell lines were from ATCC, and were
39 supplemented with 10% fetal bovine serum (FBS) (Gibco), penicillin, streptomycin, and glutamine
40 (EuroClone). Primary cultures of human fibroblasts (HFs) derived from healthy skin were obtained
41 from patients attending the Dermatology Unit of the Sant'Andrea Hospital of Rome; all patients were
42 adequately and informed and the consent for the biopsy and the subsequent investigation was given
43 and collected in written form, in accordance with guidelines approved by the management of the
44 Sant'Andrea Hospital. HF isolation and culture were performed as previously described (2).

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46 **Real-Time (RT) PCR**

47 Reverse transcription was performed on 1 ug of purified RNA using random oligo(dT) and a reaction
48 mix of dNTPs, RNase inhibitor, reverse transcriptase and its buffer (all from Promega). cDNAs were
49 analyzed by RT-PCR, in a reaction mix of 20 µl, containing the Taqman probe, cDNA, and a Master
50 Mix containing dNTPs, buffer solution and Taq polymerase (Applied Biosystem). Gene expression
51 was quantified with the $2^{-\Delta\Delta C_t}$ method. Samples were normalized by comparison with the endogenous
52 constitutively expressed gene *GAPDH*.

53 **References**

- 54 1. Kung C-P, Cottrell KA, Ryu S, Bramel ER, Kladney RD, Bao EA, et al. Evaluating the
55 therapeutic potential of ADAR1 inhibition for triple-negative breast cancer. *Oncogene*.
56 2021;40:189–202.
- 57 2. Raffa S, Leone L, Scrofani C, Monini S, Torrisi MR, Barbara M. Cholesteatoma-associated
58 fibroblasts modulate epithelial growth and differentiation through KGF/FGF7 secretion.
59 *Histochem Cell Biol*. 2012;138:251–69.

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SUPPLEMENTARY TABLES

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Table S1. List of *ISG*

<i>CCL2</i>
<i>CCL5</i>
<i>CXCL10</i>
<i>CXCL11</i>
<i>CXCL9</i>
<i>IL10</i>
<i>IL12B</i>
<i>IL15</i>
<i>IL15RA</i>
<i>IL18</i>
<i>IFNAR1</i>
<i>IFNGR2</i>
<i>CXCL8</i>
<i>IL23A</i>
<i>IL12A</i>
<i>IL32</i>
<i>IFNB1</i>

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Table S2. Patients’ characteristics (IHC)

TOTAL PATIENTS	68
Age (years)	54 (42-66)
LSIL	17
HSIL/ <i>in situ</i> carcinomas (CIS)	10
Invasive carcinomas (IC)	41
<i>Histotype</i>	
Squamous carcinoma	34
Adenocarcinoma	7
<i>Grading</i>	
G1	3
G2	16
G3	22

Table S3. Patients’ characteristics (fresh biopsies)

TOTAL PATIENTS	82
Age (years)	51 (21-84)
LSIL	14
HSIL/ <i>in situ</i> carcinomas (CIS)	10
Invasive carcinomas (IC)	18
Normal mucosa	40
<i>Histotype</i>	
Squamous carcinoma	15
Adenocarcinoma	3
<i>Grading</i>	
G1	1
G2	9
G3	8
<i>NACT</i>	8
<i>Response to NACT</i>	
Progressive disease (PD)	0
Stable disease (SD)	2
Partial response (PR)	5
Complete response (CR)	1

68 **Table S4. Reagents used for FACS analysis of fresh biopsies.**

REAGENT	SOURCE
Fixable Viability Stain 780, APC-H7	BD Biosciences
Mouse anti-human CD45 antibody, clone HI30, BUV805	BD Biosciences
Mouse anti-human CD3 antibody, clone SK7, BV605	BD Biosciences
Mouse anti-human CD14 antibody, clone M5E2, BV605	BD Biosciences
Mouse anti-human CD19 antibody, clone SJ25C1, BV605	BD Biosciences
Mouse anti-human CD4 antibody, clone RPA-T4, BV605	BD Biosciences
Mouse anti-human CD5 antibody, clone UCHT2, BV605	BD Biosciences
Mouse anti-human CD16 antibody, clone 3G8, BUV737	BD Biosciences
Mouse anti-human CD94 antibody, clone HP-3D9, BV786	BD Biosciences
Mouse anti-human CD56 antibody, clone NCAM16.1, APC R-700	BD Biosciences
Mouse anti-human CD7 antibody, clone M-T701, BUV496	BD Biosciences
Mouse anti-human CD103 antibody, clone Ber-ACT8, BB700/BV480	BD Biosciences
Mouse anti-human CD127 antibody, clone A019D5, APC/Cyanine7	Biolegend
Mouse anti-human Tigit antibody, clone MBSA43, PeFluor610	Invitrogen
Mouse anti-human KIR (CD158a), clone HP-3E4, BV421	BD Biosciences
Mouse anti-human KIR (CD158b), clone CH-L, BV421	BD Biosciences
Mouse anti-human KIR (CD158e1), clone DX9, BV421	BD Biosciences
Mouse anti-human NKp30, clone p30-15, BV650	BD Biosciences
Mouse anti-human Eomes, clone WD1928, PE	Invitrogen
Mouse anti-human T-bet, clone 4B10, PeCy7	Invitrogen
Mouse anti-human Granzyme A, clone CB9, Alexa Fluor 488	Biolegend
Mouse anti-human Granzyme B, clone GB11, Alexa Fluor 647 / BV421	Biolegend
Mouse anti-human Granzyme K, clone G3H69, Alexa Fluor 647 / PerCP-eFluor 710	BD Biosciences
Mouse anti-human ROR γ T, clone AFKJS-9, Pe-eFluor610	Invitrogen
Mouse anti-human GATA3, clone TWAJ, eFluor660	Invitrogen
Mouse anti-human NKp44, clone P44-8, BUV395	BD Biosciences
Mouse anti-human IL-22, clone 22URTI, PE	Invitrogen
Mouse anti-human IFN γ , clone B27 (RUO), APC	BD Biosciences

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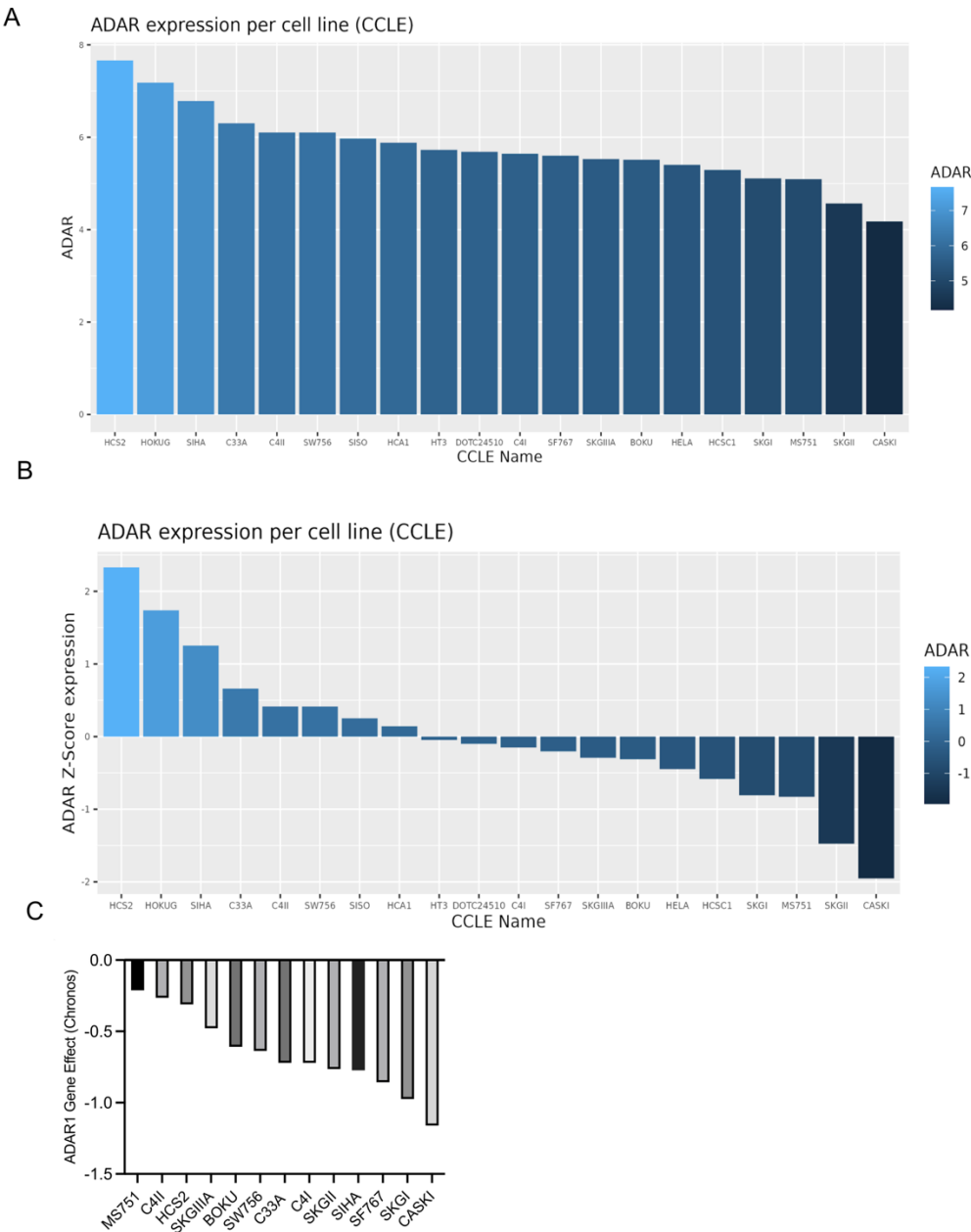
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SUPPLEMENTARY FIGURES

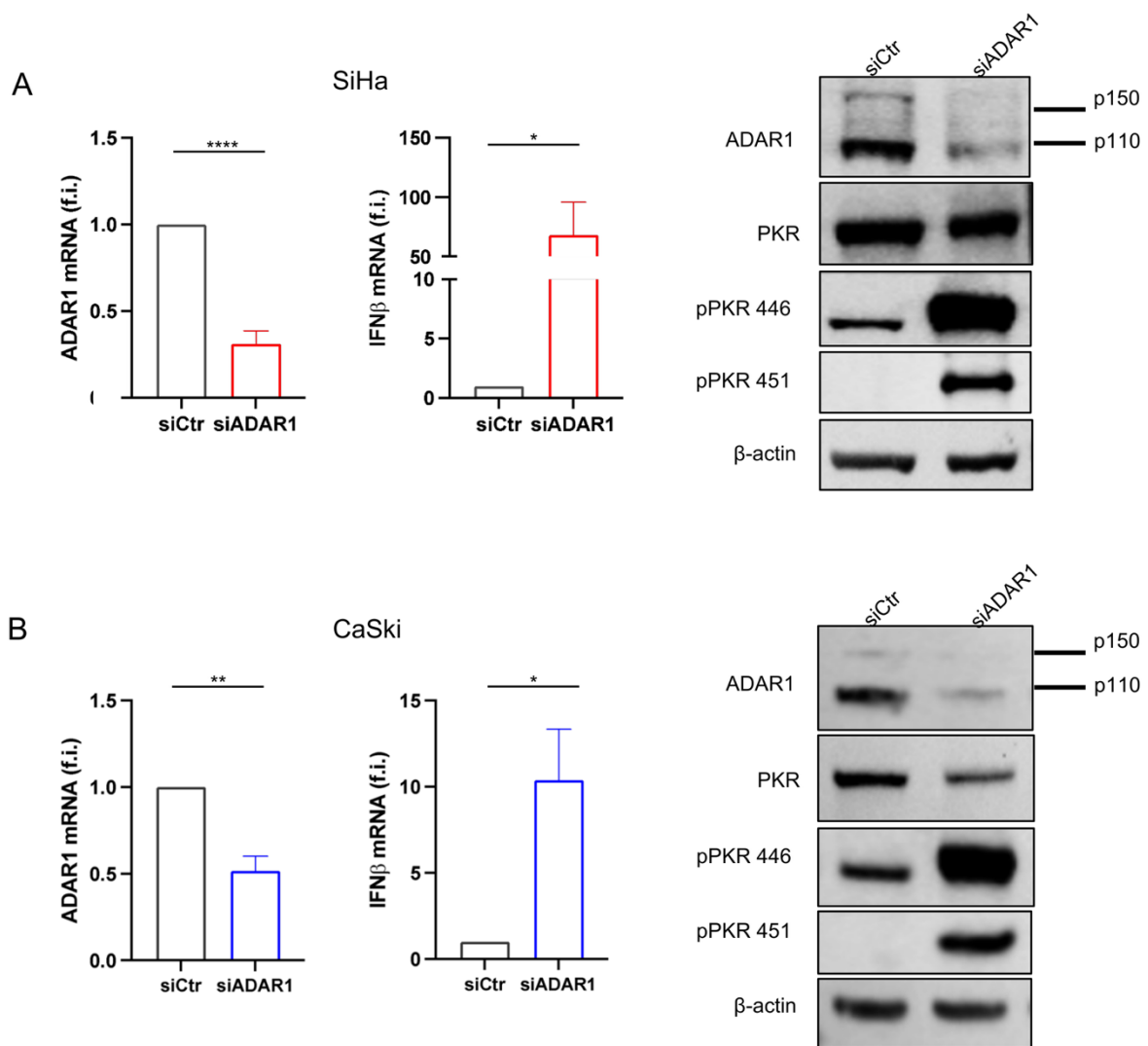
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75 **Supplementary Figure 1. *ADAR1* expression in CC-derived cell lines. A, B) Barplots sorted by**
76 ***ADAR* expression: A) raw counts and B) Z-score scaled per cell line in the CCLE data. C) *ADAR1*-**
77 **dependency scores in CRISPR knockout screens of different CC-derived cell lines. Lower Chronos**
78 **scores indicate stronger *ADAR1*-dependency.**

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81 **Supplementary Figure 2. ADAR1 silencing promotes *IFN-I* gene expression and activation of**

82 **the *IFN-I* pathway in SiHa and CaSki CC-derived cell lines. A) SiHa or B) CaSki cell lines were**

83 **transfected with an *ADAR1* siRNA (siADAR1) for 72 hrs. RT-PCR and immunoblotting were**

84 **performed to quantify *ADAR1* mRNA and protein expression, in parallel with *IFNbeta* and**

85 **phosphorylated PKR expression. *GAPDH* was used as control for RT-PCR. Results derive from 10**

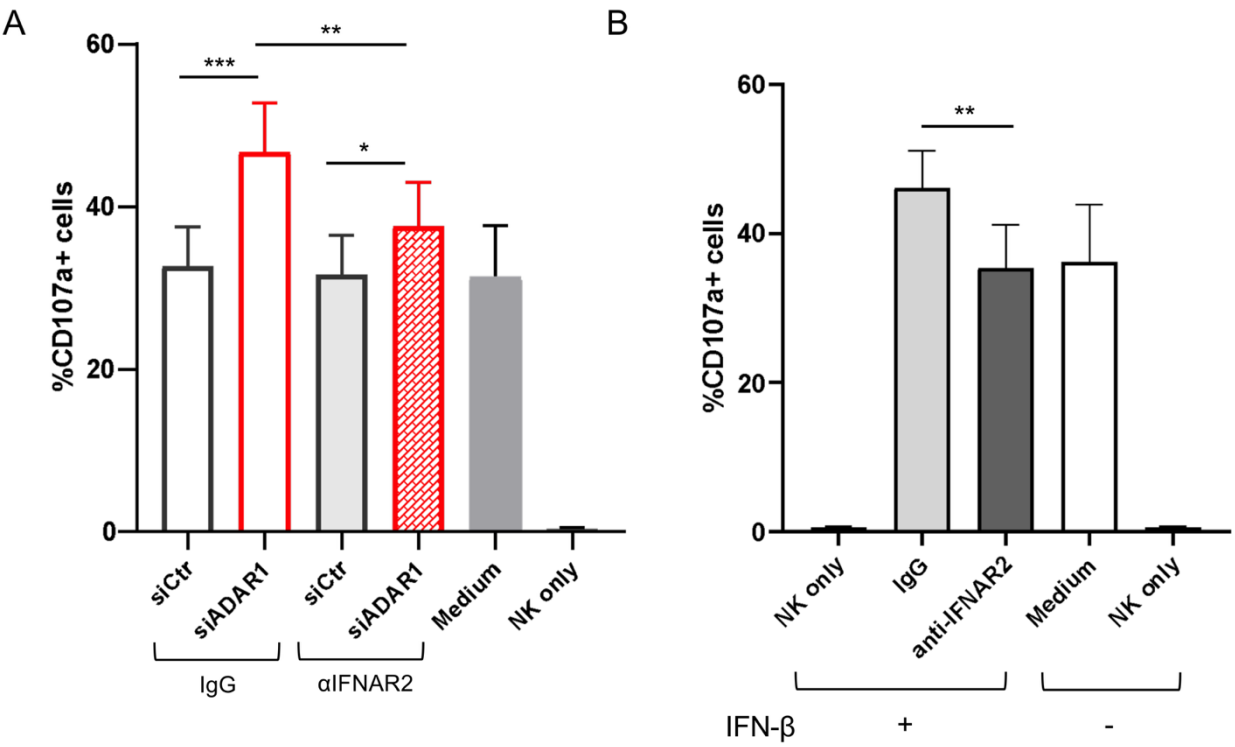
86 **(for SiHa) and 5 (for CaSki) independent experiments and from the average of triplicate**

87 **measurements $\pm$ SEM. A representative immunoblot is shown normalized to β -actin. Statistical**

88 **analysis was performed by paired *t*-test. * p < 0.05; ** p < 0.01; **** p < 0.0001.**

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91 **Supplementary Figure 3. ADAR1 silencing in CC-derived cell lines activates the expression of**
 92 ***ISGs* and of proinflammatory cytokines and chemokines and identifies ADAR1-regulated**
 93 **pathways.** SiHa (*left panels*) and CaSki (*right panels*) cell lines were transfected with an ADAR1
 94 siRNA for 72 hrs, then RNA was extracted to perform RNA-seq analysis. **A, B)** Volcano-plots. The
 95 *x*-axis shows effect size (logarithmized fold-change, FC), and the *y*-axis the negative log10 of *p*-
 96 values ($-\log_{10}(p\text{-value})$). The single horizontal dashed line marks the threshold for an unadjusted *p*-
 97 value of 0.05. The two vertical dashed lines demarcate the area outside of which there is at least one-
 98 fold difference in expression levels between siCtr and siADAR1 cells. The points highlighted in red
 99 indicate *ADAR1* and some *ISG*/pro-inflammatory genes. RNA-seq differential gene expression
 100 volcano plots display several hundreds of genes up- or down-regulated in both cell lines (see text for
 101 details) ($p < 0.05$ and $\log_{2}FC \geq |1|$). **C, D)** Heatmaps. Selected DEGs of samples were shown as Log
 102 counts per million (log cpm). Some up-regulated or down-regulated genes are indicated ($p < 0.05$ and
 103 $\log_{2}FC \geq |1|$). **E, F)** Pathway networks for statistically significant KEGG categories and relationships
 104 between genes.
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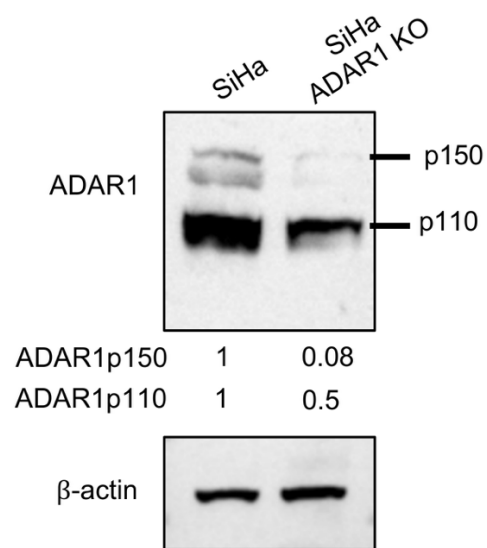
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108 **Supplementary Figure 4. Effects of IFN-I on NK cell degranulation upon *ADAR1* silencing. A)**
109 Conditioned media from siADAR1 or siCtr SiHa cells was collected at 72 hrs post-transfection and
110 used to stimulate for 18 hrs NK cells, in the presence of a blocking anti-IFNAR2 mAb or of an isotype
111 control IgG. Degranulation against K562 cells used as targets (E:T ratio of 1:2) was evaluated by
112 FACS analysis by CD107a expression on purified NK cells gated as CD56⁺. Pooled data ± SEM are
113 from two independent experiments with NK cells from six different donors. **B)** The same assay was
114 performed on NK cells cultured in the presence of IFNbeta (100 IU/ml, 18 hrs), used as a positive
115 control of the blocking anti-IFNAR2 mAb. Data ± SEM are from NK cells from four different donors.
116 Statistical analysis was performed by paired *t*-test. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

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121 **Supplementary Figure 5. Stable ADAR1 silencing in SiHa cell line.** CRISPR/Cas9 knock-out for
 122 *ADAR1* expression was performed on SiHa cell line (see materials and methods for details). A
 123 representative immunoblot to quantify ADAR1 protein expression is shown normalized to β -actin,
 124 14 days after transfection.

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