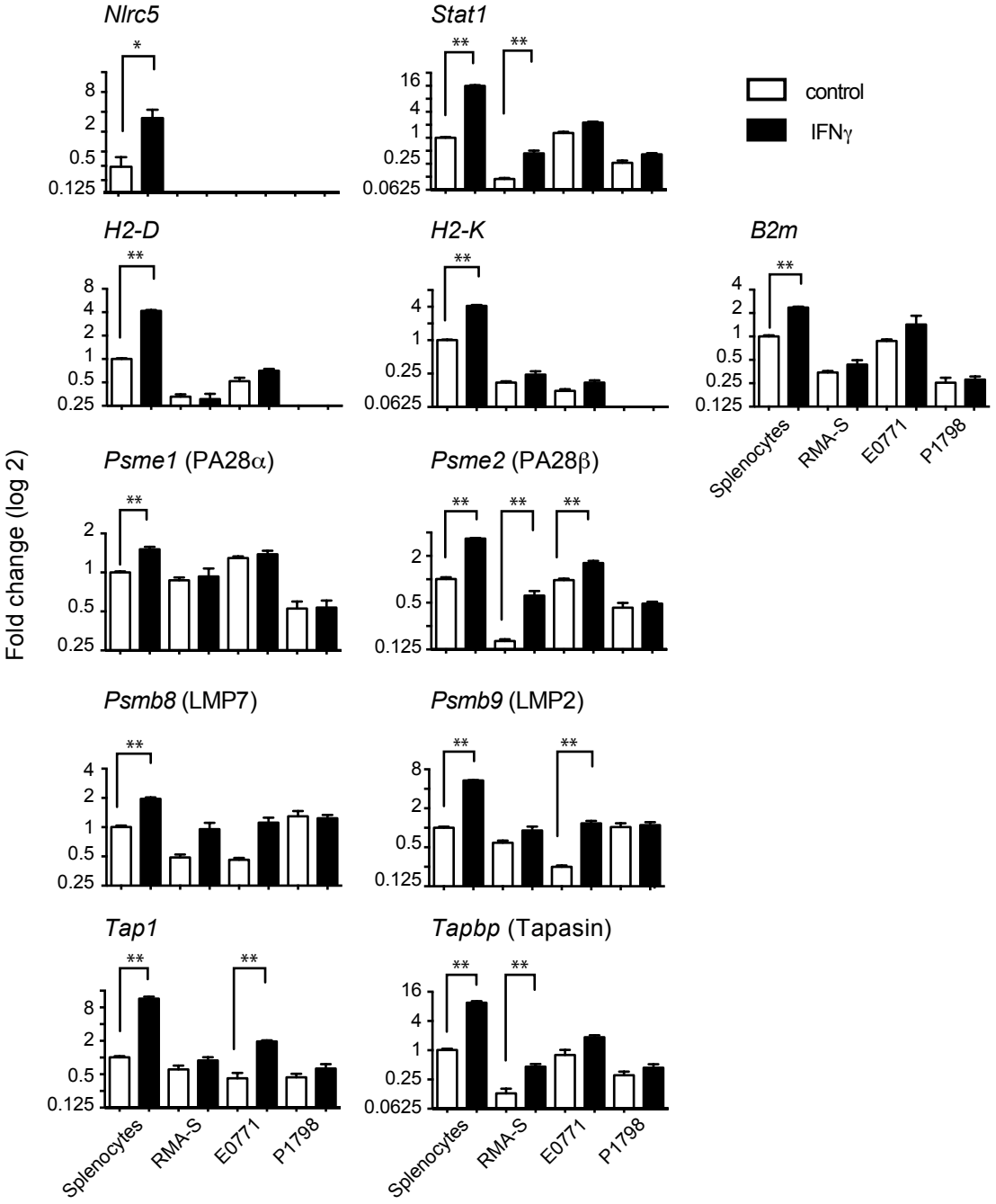

Supplementary Fig. S1. NLRC5 gene expresion in mouse tumor cell lines. Mouse T-lymphoma cell lines RMA-S [1] and P1798 [2] and the mammary carcinoma cell line E0771 [3] were evaluated by qPCR for the expression of *Nlrc5* and the indicated MHC-I pathway genes before and after stimulation with IFNγ (500 pg/ml) for 24h. C57BL/6 splenocytes stumulated with IFNγ served as control. Mann-Whittney U test: ** p <0.01.

1. Townsend A, Ohlen C, Bastin J, Ljunggren HG, Foster L, Karre K. Association of class I major histocompatibility heavy and light chains induced by viral peptides. Nature 1989; 340:443-8.
2. Thompson EA, Jr. Properties of a cell-culture line derived from lymphosarcoma P1798. Mol Cell Endocrinol 1980; 17:95-102.
3. Sirotnak FM, DeGraw JI, Schmid FA, Goutas LJ, Moccio DM. New folate analogs of the 10-deaza-aminopterin series. Further evidence for markedly increased antitumor efficacy compared with methot rexate in ascitic and solid murine tumor models. Cancer Chemother Pharmacol 1984; 12:26-30.

hsNLRC5: NP_115582.4 (1866 aa)
mmNLRC5 : NP_001028379.2 (1915 aa)

```
NP_115582      1     MDPVGLQLGNKNLWSCLVRLLTKDPEWLNAKMKFFLPNTDLDSRNETLDPEQRVILQLNKLHVQGSDTWQSFIHCVCMQL  80
NP_001028379   1     MDAESIRLNNENLWAWLVRLLSKNPEWLSAKLRSFLPTMDLDCSYEPSNPEV-IHRQLNRLFAQGMATWKSFINDLCFEL  79

NP_115582      81    EVPLDLEVLLLSTFGYDDGFTSQLGAEGKSQPESQLHHGLKRPHQSCGSSPRRKQCKKQQLELAKKYLQLLRTSAQQRYR  160
NP_001028379   80    DVPLDMEIPLVSIWGPRDEFSKQLGAGEESCPGPQLYHGAKRPFQSYGSSPRRKNSKKQQLELAKKYLKLLKTSAQQWHG  159

NP_115582      161   SQIPGSG-QPHAFHQVYVPPILR--RATASLDTPEGAIMGDVKVEDGADVSISDLFNTRVNKGPRVTVLLGKAGMGKTTL  237
NP_001028379   160   GVCPGAWLTPHS-PQTYIPPVLQWSRATAPLDAQEGATLGDPEAADNIDVSIQDLFSFKAHKGPRVTVLLGKAGMGKTTL  238

NP_115582      238   AHRLCQKWAEGHLNCFQALFLFEFRQLNLITRFLTPSELLFDLYLSPESDHDTVFQYLEKNADQVLLIFDGLDEALQP--  315
NP_001028379   239   AYRLRWRWAQGQLDRFQALFLFEFRQLNMITQLPTLPQLLFDLYLMPESEPDAVFQYLKENAQEVLLIFDGLDEALHADS  318

NP_115582      316   MGPDGPGPVLTLFSHLCNGTLLPGCRVMATSRPGKLPACLPAEAAMVHMLGFDGPRVEEYVNHFFSAQPSREGALVELQT  395
NP_001028379   319   VGTDNAGSALTLFSELCHGNLLPGCWVMTTSRPGKLPSCVPTEAATVHMWGFDGLRVEKYVTCFFSDLLSQELALKEMRT  398

NP_115582      396   NGRLRSLCAVPALCQVACLCLHHLLPDHAPGQSVALLPNMTQLYMQMVLALSPPGHLPTSSLLDLGEVALRGLETGKVIF  475
NP_001028379   399   NARLRGMCAIPALCTVTCFCLRRLLPGSSPGQSAALLPTITQLYLQMVETFSPSETLLDTSILGFGKVALRGLDTGKVVF  478

NP_115582      476   YAKDIAPPLIAFGATHSLLTSFCVCTGPGHQQTGYAFTHLSLQEFLAALHLMASPKVNKDTLTQYVTLHSRWVQRTKARL  555
NP_001028379   479   SVEDISPQLMSFGAVHSLLTSFCIHTRPGHEEIGYAFVHLSLQEFFAALYLMASHTVDKDTLVEYVTLNSHWVLRTKGRL  558

NP_115582      556   GLSDHLPTFLAGLASCTCRPFLSHLAQGNEDCVGAKQAAVVQVLKKLATRKLTGPKVVELCHCVDETQEPELASLTAQSL  635
NP_001028379   559   GLSDHLPAFLAGLASHTCHMFLCQLAQQDRAWVGSRQAAVIQVLRKLASRKLTGPKMIELYHCVAETQDLELARFTAQSL  638

NP_115582      636   PYQLPFHNFPLTCTDLATLTNILEHREAPIHLDFDGCPLEPHCPEALVGCGQIENLSFKSRKCGDAFAEALSRSLPTMGR  715
NP_001028379   639   PSRLSFHNFPLTHADLAALANILEHRDDPIHLDFDGCPLEPHCPEALVGCGQVENLSFKSRKCGDAFAEALCRSLPTMGS  718

NP_115582      716   LQMLGLAGSKITARGISHLVKALPLCPQLKEVSFRDNQLSDQVVLNIVEVLPHLPRLRKLDLSSNSICVSTLLCLARVAV  795
NP_001028379   719   LKTLGLTGSRITAQGISHLIQTLPLCSQLEEVSLHDNQLKDPEVLSLVELLPSLPKLQKLDLSRNSFSRSILLSLVKVAI  798

NP_115582      796   TCPTVRMLQAREADLIFLLSPPTETTAELQRAPDLQESDGQRKGAQSRSLTLRLQKCQLQVHDAEALIALLQEGPHLEEV  875
NP_001028379   799   TCPTVRKLQVRELDLIFYLSPVTETATQQSGASDVQGKDSLKEG-QSRSLQLRLQKCQLRIRDAEALVELFQKSPQLEEV  877

NP_115582      876   DLSGNQLEDEGCRLMAEAASQLHIARKLDLSNNGLSVAGVHCVLRAVSACWTLAELHISLQHKTVIFMFAQEPEEQKGPQ  955
NP_001028379   878   NLSGNHLEDDGCRLVAEAASQLHIAQKLDLSDNGLSQTGVTYVLKAMSTCGTLEDLHISLLNNTVVLTFAQEPREQEGSC  957

NP_115582      956   ERAAFLDSLMLQMPSELPLSSRRMRLTHCGLQEKHLEQLCKALGGSCHLGHL-HLDFSGNALGDEGAARLAQLLPGLGAL  1034
NP_001028379   958   KGRAPLISFVSPVTSELSQRSRRIRLTHCGFLAKHTETLCEALRASCQTHNLDHLDLSDNSLGGKGVILLTELLPGLGPL  1037

NP_115582      1035  QSLNLSENGLSLDAVLGLVRCFSTLQWLFRLDISFESQHILLRGDKTSRDMWATGSLPDFPAAAKFLGFRQRCIPRSLCL  1114
NP_001028379   1038  KSLNLSRNGLSMDAVFSLVQCLSSLQWVFHLDVSLESDCIFLRGAGTSRDALE----PKFQTGVQVLELSQRYTSRSFCL  1113

NP_115582      1115  SECPLEPPSLTRLCATLKDCPGPLELQLSCEFLSDQSLETLLDCLPQLPQLSLLQLSQTGLSPKSPFLLANTLSLCPRVK  1194
NP_001028379   1114  QECQLEPTSLTFLCATLEKSPGPLEVQLSCKSLSDDSLKILLQCLPQLPQLSLLQLRHTVLSSRSPFLLADIFNLCPRVR  1193

NP_115582      1195  KVDLRSLHHATLHFRSNEEEEGVCCGRFTGCSLSQEHVESLCWLLSKCKDLSQVDLSANLLGDSGLRCLLECLPQVPISG  1274
NP_001028379   1194  KVTLRSLCHAVLHFDSNEEQEGVCCG-FPGCSLSQEHMETLCCALSKCNALSQLDLTDNLLGDIGLRCLLECLPQLPISG  1272

NP_115582      1275  LLDLSHNSISQESALYLLETLPSCPRVREASVNLGSEQSFRIHFSREDQAGKTLRLSECSFRPEHVSRLATGLSKSLQLT  1354
NP_001028379   1273  WLDLSHNNISQEGILYLLETLPSYPNIQEVSVSLSSEQIFRMCFSKKEGAGTSLRLCECSFSPEQVSKLASSLSQAQQLT  1352

NP_115582      1355  ELTLTQCCLGQKQLAILLSLVGRPAGLFSLRVQEPWADRARVLSLLEVCAQASGSVTEISISETQQQLCVQLEFPRQEEN  1434
NP_001028379   1353  ELWLTKCHLDLPQLTMLLNLVNRPTGLLGLRLEEPWVDSVSLPALMEVCAQASGCLTELSISEIQRKLWLQLEFPHQEGN  1432

NP_115582      1435  PEAVALRLAHCDLGAHHSLLVGQLMETCARLQQLSLSQVNLCEDDDASSLLLQSLLLSLSELKTFRLTSSCVSTEGLAHL  1514
NP_001028379   1433  SDSMALRLAHCDLETEHSHLMIQLVETYARLQQLSLSQVSFNDNDGTSSKLLQNILLSSCELKSFRLTFSQVSTKSLTHL  1512

NP_115582      1515  ASGLGHCHHLEELDLSNNQFDEEGTKALMRALEGKWMLKRLDLSHLLLNSSTLALLTHRLSQMTCLQSLRLNRNSIGDVG  1594
NP_001028379   1513  AFGLGHCHHLEELDFSNNSLREEDTELLMGALQGTCRLKKLHLSFLPLGASSLALLIQGLSRMTLLQDLCLSHNQIGDVG  1592

NP_115582      1595  CCHLSEALRAATSLEELDLSHNQIGDAGVQHLATILPGLPELRKIDLSGNSISSAGGVQLAESLVLCRRLEELMLGCNAL  1674
NP_001028379   1593  TQCLAAILPKLPELRKFDLSHNQIGDVGTQCLAAILPKLPELRKFNLSHNQIGHVGTQCLAAILPKLPELRKFDLSRNQI  1672

NP_115582      1675  GDPTALGLAQELP--------------------------------QHL--------------------------RVLHLPFS  1698
NP_001028379   1673  GDVGTQCLAAILPKLPELRKFDLSGNRIGPAGGVQLVKSLTHFEHLEEIKLGNNALGEPTALELAQRLPPQLRVLCLPSS  1752

NP_115582      1699  HLGPGGALSLAQALDGSPHLEEISLAENNLAGGVLRFCMELPLLRQIDLVSCKIDNQTAKLLTSSFTSCPALEVILLSWN  1778
NP_001028379   1753  HLGPEGALGLAQALEQCPHIEEVSLAENNLAGGVPRFSKRLPLLRQIDLEFCKIEDQAARHLAANLTLFPALEKLLLSGN  1832

NP_115582      1779  LLGDEAAAELAQVLPQMGRLKRVDLEKNQITALGAWLLAEGLAQGSSIQVIRLWNNPIPCDMAQHLKSQEPRLDFAFFDN  1858
NP_001028379   1833  LLGDEVAAELAQVLPQMGQLKKVNLEWNRITARGAQLLAQGLVQGSCVPVIRLWNNPILNDVAQSLQSQEPRLDFSITDQ  1912
NP_115582      1859  QPQAPWGT  1866
NP_001028379   1913  QTL-----  1915
```

Supplementary Fig. S2. Amino acid sequence comparison between human and mouse NLCR5. Clustal-W analysis shows 62% sequence identity, and 80% similarity with 3% gaps.

NLRC5 elicits antitumor immunity by enhancing processing and presentation of tumor antigens to $CD8^+$ T lymphocytes

Galaxia M. Rodriguez et al.,

Supplementary methods

Gene expression analysis

RNA was extracted from B16 cells or its derivatives using RiboZol™ (AMRESCO, Solon, OH) and reverse transcribed using QuantiTect Kit (Qiagen). For qPCR reactions, 100ng of cDNA was used with SYBR Green mix (BioRad) and analyzed on an iQ5 Cycler (BioRad). All samples were amplified in triplicates and normalized for *36B4* housekeeping gene expression. Relative expression was calculated by ddCt method and corrected for primer efficiencies (1). The primers used for qPCR reactions are given in Table 1.

MTT assay for cell growth

Proliferation and viability of B16 cells was assessed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) reduction assay. Briefly, cells were plated at different densities in 96 well flat-bottom plates. 24h after, MTT solution (4mg/ml; 25µl) was added and cells were incubated for 16-20h at 37°C. The reaction was stopped by 10% SDS-HCl (100µl) and optical density was measured at 570nm in a microplate reader (Bio-Rad).

Flow cytometry for surface markers, intracellular cytokines and CD107 mobilization

Expression of cell surface and intracellular markers was evaluated by flow cytometry using FACS Canto flow cytometer (Becton Dickinson flow cytometry systems, Missisauga, ON,

Canada). For intracellular cytokine staining, the Cytofix/Cytoperm™ Plus kit (BD Biosciences) was used according to the manufacturer's instructions. Briefly, Pmel-1 cells were stimulated with antigenic peptides for 5h along with GolgiPlug™. The cells were washed, stained for cell surface markers, fixed, permeabilized and stained for TNFα as described previously (2). Cytotoxic granule exocytosis was assessed by flow cytometry for cell surface expression of CD107b (SEROTEC, Bio-Rad) (3). Flow cytometry data were analyzed using the FlowJo software (Tree Star Inc., Ashland, OR, USA). Antibodies used for flow cytometry are given in Table 2.

References

1. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29:e45.
2. Ramanathan S, Dubois S, Chen XL, Leblanc C, Ohashi PS, Ilangumaran S. Exposure to IL-15 and IL-21 enables autoreactive CD8 T cells to respond to weak antigens and cause disease in a mouse model of autoimmune diabetes. J Immunol. 2011;186:5131-41.
3. Betts MR, Brenchley JM, Price DA, De Rosa SC, Douek DC, Roederer M, et al. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. J Immunol Methods. 2003;281:65-78.

Table 1. The primer sequences used in gene expression analysis.

Gene ID	Gene name	Sense	Anti-sense	Amplicon size (bp)
Primer Sequences for mouse genes				
NM_010380.3	*H2d*	GGGAAACACAGAAAGCCAAG	AAGTCACAGCCAGACATCTG	124
NM_001001892.2	*H2k*	AGACACAGAAAGCCAAGGG	CACTTCACAGCCAGAGATCAC	122
NM_009735.3	*B2m*	TGGTCTTTCTGGTGCTTGTC	GGGTGGAACTGTGTTACGTAG	136
NM_009318.2	*Tapbp*	ATCGTAGCACCATGAAGCC	TGGCAGGTTTCTTAGACAGG	150
NM_013683.2	*Tap1*	TGGTTCTGGTTCTCTTGATTCTC	CTGGCTATGGTGAGAATGGAC	148
NM_013585.2	*Lmp2*	GAACATCTCCTACAAGTACCGTG	CTGTCGAATTAGCATCCCTCC	115
NM_010724.2	*Lmp7*	ATCGAGATTAACCCTTACCTGC	AGATGCGTTCCCCATTCC	121
NM_009283.4	*Stat1*	GCCGAGAACATACCAGAGAATC	GATGTATCCAGTTCGCTTAGGG	141
NM_011190.3	*Pa28b*	CCTTGCTCGCTTTGGTTAAG	ACCTTCTCCTGAATTGCCAC	133
NM_011189.1	*Pa28a*	GTGCTTTCGCTTTCCCTTC	TTGCTACACAGGTCTTCACG	147
NP_031501.1	*Rplp0*	TCTGGAGGGTGTCCGCAAC	CTTGACCTTTTCAGTAAGTGG	154
NM_001033207.3	*Nlrc5*	AGTGCAGCTGGTGAAGTCTC	TCCCGGACAGCAAGAGTTTC	380
Primer Sequences for human genes				
NM_032206.4	*NLRC5*	AGACCCAGAATGGCTGAACG	CTGGCTGGTGAACCCATCAT	241

Table 2. Antibodies used for flow cytometry.

Marker	mAb clone	Source	Cat. #
CD3ε	145-2C11	eBioscience	150-0031-82
CD8	a53-6.7	eBioscience	25-081-82
CD44	IM7	BD Pharmingen	553-133
CD62L	MEL14	eBioscience	17-0621-83
CD80	B7-1	eBioscience	17-0801-82
CD107b	M3/84	BioLegend	108506
H-2D^b	KH-95	BioLegend	115111
H-2K^b	AF6-88.5	BioLegend	116507
IL-2	JES6-5H4	BD Pharmingen	560538
PD-L1	B7-H1	BioLegend	124305
TNFα	MP6-XT22	eBioscience	1207321-82