Supplemental Information

CD200 ectodomain shedding into the tumor microenvironment leads to NK cell dysfunction and apoptosis

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Supplemental Figure 4

- Full-length
- Cleaved

PARP

- Full-length
- Cleaved

Caspase 8

- Full-length
- Cleaved

Caspase 9

- Cleaved

GAPDH
**Supplementary Table 1**

| #  | Gene  | Fold Change | Gene   | Fold Change |
|----|-------|-------------|--------|-------------|
| 1  | VCAN  | 3.14        | DCD    | -5.70       |
| 2  | APCDD1L | 2.97       | PIP    | -4.08       |
| 3  | MYCN  | 2.93        | SCGB2A2| -3.93       |
| 4  | SLC39A14 | 2.82       | ALOX15B| -3.39       |
| 5  | FBN3  | 2.80        | MUC1   | -3.37       |
| 6  | TNC   | 2.79        | ACTA1  | -3.19       |
| 7  | COL1A1| 2.76        | TMEM91 | -2.91       |
| 8  | FLJ22536 | 2.61       | KIA18881| -2.87      |
| 9  | MMP11 | 2.61        | SCGB1D2| -2.82       |
| 10 | COL5A1| 2.55        | MUCL1  | -2.80       |
| 11 | COL1A2| 2.55        | CFD    | -2.74       |
| 12 | CHCHD7| 2.54        | GPT    | -2.59       |
| 13 | COL5A2| 2.51        | HMGCS2 | -2.59       |
| 14 | GJB6  | 2.46        | UBIAD1 | -2.54       |
| 15 | PDGFA | 2.45        | FCGBP  | -2.52       |
| 16 | LUM   | 2.44        | PSAPL1 | -2.45       |
| 17 | CSPG4 | 2.42        | BR13BP | -2.34       |
| 18 | IGF2BP2| 2.37       | AZGP1  | -2.19       |
| 19 | SPON2 | 2.33        | CHCHD10| -2.14       |
| 20 | CREB5 | 2.19        | PROL1  | -2.08       |
| 21 | CALD1 | 2.17        | FCGBP  | -2.01       |
| 22 | COL6A3| 2.15        |        |             |
| 23 | COL1A2| 2.15        |        |             |
| 24 | COL3A1| 2.13        |        |             |
## Supplementary Table 2

| Tumor Type                                      | CD200 Z-Scores |
|------------------------------------------------|----------------|
| Adrenocortical_cancer                          | -1.523         |
| Bladder_cancer                                 | 0.71           |
| Brain_cancer_Astrocytoma                       | -1.131         |
| Brain_cancer_Glioblastoma                      | -0.192         |
| Brain_cancer_Glioma                            | -4.949         |
| Brain_cancer_Medulloblastoma                   | -0.774         |
| Brain_cancer_Meningioma                        | -1.803         |
| Brain_cancer_Neuroblastoma                     | -7.223         |
| Breast_cancer                                  | -1.924         |
| Colon_cancer                                   | 0.041          |
| Gastric_cancer                                 | -1.247         |
| Germ_cell_tumors                               | -1.113         |
| Head_and_neck_cancer                           | -0.674         |
| Head_and_neck_cancer_Hypopharyngeal_cancer     | 0.646          |
| Head_and_neck_cancer_Oesophageal_cancer        | 0.9            |
| Head_and_neck_cancer_Oral_SCC                  | 0.455          |
| Hematopoietic_cancer_AML                       | 1.529          |
| Hematopoietic_cancer_B_ALL                     | 2.899          |
| Hematopoietic_cancer_Burkitt_lymphoma          | -0.827         |
| Hematopoietic_cancer_CLL                       | -0.533         |
| Hematopoietic_cancer_DLBCL                     | 1.207          |
| Hematopoietic_cancer_FL                        | -0.24          |
| Hematopoietic_cancer_Mantle_cell_lymphoma      | -1.779         |
| Hematopoietic_cancer_Multiple_myeloma          | -1.122         |
| Kidney_cancer                                  | -1.247         |
| Liver_cancer                                   | 1.022          |
| Liver_cancer_Primary                           | 0.028          |
| Lung_cancer_ADENO                              | -0.492         |
| Lung_cancer_LCC                                | -1.718         |
| Lung_cancer_SCC                                | -1.587         |
| Lung_cancer_SCLC                               | 0.117          |
| Melanoma                                       | -1.399         |
| Melanoma_Metastasis                            | -0.392         |
| Mesothelioma                                   | -1.601         |
| Ovarian_cancer                                 | -2.347         |
| Pancreatic_cancer                              | 0.544          |
| Prostate_cancer                                | -0.585         |
| Sarcoma_Ewing_sarcoma                          | -1.003         |
| Sarcoma_Osteosarcoma                           | -0.366         |
## Supplementary Table 3

| Cancer Type                                      | NK Cells | B Cells | T Cells | D Cells | Gamma Delta | Eosinophils | Neutrophils |
|-------------------------------------------------|----------|---------|---------|---------|-------------|-------------|-------------|
| Head and Neck Cancer                            | 0.046    | 0.145   | 1.103   | 0.810   | 0.24        | 0.25        | 0.56        |
| Lung Adenocarcinoma                             | 0.492    | 2.129   | 1.07    | 0.767   | 1.203       | 0.89        | 0.76        |
| Lung SCL                                         | 1.548    | 0.478   | 1.007   | 1.387   | 1.04        | 0.63        | 0.85        |
| Lung Large Cell Carcinoma                       | 1.718    | 0.64    | 0.52    | 1.434   | 0.75        | 1.62        | 0.82        |
| Melanoma Primary                                | 1.219    | 1.715   | 0.77    | 1.162   | 1.37        | 1.15        | 0.48        |
| Melanoma Metastasis                             | 0.367    | 0.81    | 0.77    | 0.50    | 0.52        | 0.75        | 0.64        |
| Ovarian Cancer                                  | 2.347    | 0.472   | 1.023   | 1.128   | 0.55        | 0.74        | 0.80        |

"Z-Score" column indicates the statistical significance of the observed changes in cell types between different cancer types.
Supplementary Table 4 - Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies (Clone)  |        |            |
| anti-human CD207 (9H) | Beckman Coulter | Cat# IM3449; RRID: AB_131719 |
| anti-human CD123 (9F5) | BD Biosciences | Cat# 555642; RRID: AB395999 |
| anti-human CD8 (C8/144B) | Dako | Cat# M7103; RRID: AB_2075537 |
| anti-human CD4 (SK3) | BD Biosciences | Cat# 346320; RRID: AB_400250 |
| anti-human CD14 (MφP9) | BD Biosciences | Cat# 347490; RRID: AB_400310 |
| anti-human CD56 (NCAM16.2) | BD Biosciences | Cat# 559043; RRID: AB_397180 |
| anti-human HLA-DR (L243) | BD Biosciences | Cat# 347360; RRID: AB_400289 |
| anti-human Cytokeratin 14 (LL002) | Abcam | Cat#ab7800; RRID: AB_306091 |
| anti-human CD56-FITC (REA196) | Miltenyi Biotec | Cat#130-100-683; RRID: AB_2658729 |
| anti-human CD3-APC (HIT3a) | BioLegend | Cat#300312; RRID: AB_314048 |
| anti-human CD45-PE (H130) | BD Biosciences | Cat#560975; RRID: AB_2033960 |
| 7AAD | BD Biosciences | Cat#559925 |
| anti-human CD200 (OX-104) | BioLegend | Cat#329207; RRID: AB_2074064 |
| anti-human CD107a (H4A3) | BD Biosciences | Cat#562622; RRID: AB_2737684 |
| Antibody Description                              | Supplier               | Catalog Number | RRID         |
|--------------------------------------------------|------------------------|----------------|--------------|
| anti-human CD200R (OX-108)                       | BioLegend              | Cat#329306; RRID: AB_2074200 |
| anti-mouse NK1.1 (PK136)                         | ThermoFisher Scientific | Cat#MA1-70100; RRID: AB_2296673 |
| DAPI                                             | Sigma-Aldrich          | Cat#10236276001 |
| AF647 IgG1 isotype                               | BioLegend              | Cat#MCA1209A647; RRID: AB_322324 |
| PE Mouse IgG1, k Isotype Ctrl Antibody (MOPC-21)| BioLegend              | Cat#400112; RRID: AB_2847829 |
| APC Mouse IgG2a, k Isotype Ctrl Antibody (MOPC-173) | BioLegend              | Cat#400220; RRID: AB_326468 |
| FITC Human IgG1 Isotype Ctrl Recombinant Antibody | BioLegend              | Cat#403508; RRID: AB_2847831 |
| Purified Mouse IgG1, k Isotype Control (MOPC-21) | BD Biosciences         | Cat#555746; RRID: AB_396088 |
| CD200                                            | R&D Systems            | Cat#AF2724; RRID: AB_416669 |
| Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)     | Cell Signalling        | Cat#9101; RRID: AB_331646 |
| PARP (46D11)                                     | Cell Signalling        | Cat#9532; RRID: AB_659884 |
| Caspase 8 (1C12)                                 | Cell Signalling        | Cat#9746; RRID: AB_2275120 |
| Caspase 9                                        | Cell Signalling        | Cat#9502; RRID: AB_2068621 |
| Fas (4C3)                                        | Cell Signalling        | Cat#8023; RRID: AB_10860778 |
| FasL                                             | Cell Signalling        | Cat#4273; RRID: AB_2100652 |
| FADD                                             | Cell Signalling        | Cat#2782; RRID: AB_2100484 |
| Antigen                  | Supplier              | Cat. No.                  |
|-------------------------|-----------------------|---------------------------|
| GAPDH                   | Merck Millipore       | Cat#MAB374; RRID: AB_2107445 |
| Cleaved caspase-3       | Cell Signalling       | Cat#9661                  |
| CD200R                  | Abcam                 | Cat#ab198010              |
| CD200                   | R&D Systems           | Cat#AF2724                |
| NK1.1                   | ThermoFisher Scientific | Cat#MA1-70100             |
| Horse Anti-Goat IgG     | Vector Laboratories   | BP-9500-50                |
| Antibody (H+L), Biotinylated, R.T.U. |              |                           |
| Goat Anti-Rabbit IgG    | Vector Laboratories   | BP-9100-50                |
| Antibody (H+L), Biotinylated, R.T.U. |              |                           |
| Goat Anti-Mouse IgG     | Vector Laboratories   | BP-9200-50                |
| Antibody (H+L), Biotinylated, R.T.U. |              |                           |
| Donkey anti-Goat IgG    | Invitrogen            | Cat#A-11055; RRID: AB_2534102 |
| (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 |              |                           |
| Donkey anti-Rabbit IgG  | Invitrogen            | Cat#A-10042; RRID: AB_2534017 |
| (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 568 |              |                           |
| Donkey anti-Mouse IgG   | Invitrogen            | Cat#A-31571; RRID: AB_162542 |
| (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 647 |              |                           |
| Antibody/Molecule Type | Supplier | Catalog/RRID Information |
|------------------------|----------|--------------------------|
| Goat Anti-Rabbit IgG H&L (HRP) | Abcam | Cat#ab97051; RRID: AB_10679369 |
| Goat Anti-Mouse IgG1 (HRP) | Abcam | Cat#ab98693; RRID: AB_10674928 |
| Goat Anti-Mouse IgG2a heavy chain (HRP) | Abcam | Cat#ab97245; RRID: AB_10680049 |
| Rabbit Anti-Goat IgG H&L (HRP) | Abcam | Cat#ab97100; RRID: AB_10687752 |

**Chemicals, Peptides and Recombinant Proteins**

| Name | Supplier | Catalog/RRID Information |
|------|----------|--------------------------|
| Recombinant Human CD200 Peptide | R&D Systems | Cat#2724-CD-050 |
| Recombinant Mouse CD200 Peptide | R&D Systems | Cat#3355-CD-050 |
| Purified Mouse Anti-Human CD200 | BD Biosciences | Cat#552023 |
| Recombinant Human MMP3 protein | R&D Systems | Cat#513-MP-010 |
| Recombinant Human MMP11 (Catalytic domain) | Enzo Life Sciences | Cat#BML-SE282-0010 |
| Recombinant Human TIMP-3 Protein | R&D Systems | Cat#973-TM |
| Bovine Pituitary Extract | ThermoFisher Scientific | Cat#13028014 |
| Recombinant Human Fibroblast Growth Factor | PeproTech Ltd | Cat#AF-100-18B |
| Recombinant Human Epidermal Growth Factor | PeproTech Ltd | Cat#AF-100-15 |
| **Phorbol 12-myristate 13-acetate (PMA)** | Abcam | Cat#ab120297 |
| **Ionomycin Ca²⁺ Salt, Ca²⁺ ionophore** | Abcam | Cat#ab120116 |
| **Z-VAD-FMK (Pan-caspase inhibitor)** | Selleckchem | Cat#S8102 |
| **Z-IETD-FMK (Caspase 8 inhibitor)** | Selleckchem | Cat#S7314 |
| **Z-LEHD-FMK (Caspase 9 inhibitor)** | Selleckchem | Cat#S7313 |
| **ZB4 clone anti-Fas mAb** | Merck Millipore | Cat#05-338 |
| **GW9662 (PPARγ antagonist)** | Selleckchem | Cat#S2915 |
| **Dispase** | Worthington | Cat#LS02104 |
| **Collagenase III** | Worthington | Cat#LS004182 |
| **Hyaluronidase** | Worthington | Cat#LS005475 |
| **DNase I** | Stem Cell Technologies | Cat#07900 |
| **Donkey Serum** | Sigma-Aldrich | Cat#D9663 |
| **VECTASHIELD® Antifade Mounting Medium** | Vector Laboratories | Cat#H-1000-10 |
| **Protease Inhibitor Cocktail (100x)** | Cell Signalling | Cat#5871S |
| **QuantiTect Reverse Transcription Kit** | Qiagen | Cat#205313 |
| **TaqMan® Universal Master Mix II** | ThermoFisher Scientific | Cat#4440040 |
| **Bovine Serum Albumin Powder** | Fisher Bioreagents | Cat#BP9702-100 |
| **IncuCyte® Caspase-3/7 Reagent for Apoptosis** | Essen Bioscience | Cat#4704 |
| **TWEEN®20** | Sigma-Aldrich | Cat#P9416 |
| **IGEPAL®CA-630** | Sigma-Aldrich | Cat#I8896 |
| Product Name                                      | Brand              | Catalog Number |
|--------------------------------------------------|--------------------|----------------|
| Immobilon Forte Western HRP substrate            | Merck Millipore    | Cat#WBLUF0100  |
| Illumina™ TotalPrep™ RNA Amplification Kit        | ThermoFisher Scientific | Cat#AMIL1791  |
| Illumina HumanHT-12 v4 Expression BeadChip        | Illumina           | Cat#BD-901-1001 |
| RPMI 1640 Medium                                  | ThermoFisher Scientific | Cat#11875093 |
| DMEM Medium                                      | ThermoFisher Scientific | Cat#11960085 |
| Keratinocyte Serum Free Media                    | ThermoFisher Scientific | Cat#17005059 |
| Fetal Bovine Serum                               | ThermoFisher Scientific | Cat#10270106 |
| Horse Serum, Heat Inactivated                    | ThermoFisher Scientific | Cat#26050088 |
| Ficoll® Paque Plus                               | GE Healthcare      | Cat#GE17-1440-02 |
| Trans-Blot Turbo RTA Mini 0.2 µm PVDF Transfer Kit, for 40 blots | Bio-Rad            | Cat#1704272  |
| TGX™ FastCast™ Acrylamide Kit, 7.5%               | Bio-Rad            | Cat#1610171  |
| 96 well plates, flat bottomed, white walled      | Greiner Bio-One    | Cat#655098   |
| Pierce™ Protein Concentrator PES, 3K MWCO        | ThermoFisher Scientific | Cat#88526   |
| Pierce™ BCA Protein Assay Kit                    | ThermoFisher Scientific | Cat#23225   |
| RetroNectin® Recombinant Human Fibronectin Fragment | TaKaRa Bio        | Cat#T100A  |
| **Complementary DNA**                            |                    |               |
| CD200                                            | Gift of Alex Tonks, Department of | IMAGE consortium; Clone ID 5299899 |
| Retroviral Expression Vector | Haematology, Cardiff University, UK |
|-----------------------------|-------------------------------------|
| PINCO                       | Gift of Alex Tonks, Department of Haematology, Cardiff University, UK |

| Critical Commercial Assays | |
|---------------------------|------------------|
| RNeasy Plus Mini Kit      | Qiagen, Cat#74134 |
| CellTiter-Glo® Luminescent Cell Viability Assay | Promega, Cat#G7572 |
| CD200 Matched ELISA Antibody Pair Set, Human | Sino Biological, Cat#SEK10886 |
| Human CCL4/MIP-1 beta DuoSet ELISA kit | R&D Systems, Cat#DY271 |
| Human Fas Ligand/TNFSF6 ELISA Kit | R&D Systems, Cat#DY126 |
| Human IFN-gamma ELISpot kit | R&D Systems, Cat#EL285 |
| VECTASTAIN® Elite ABC-HRP Kit, Peroxidase | VECTOR Laboratories, Cat#PK-6100 |

| Biological Samples | |
|--------------------|--------------------------------|
| Fresh human tumour and PBMC samples | Hywel Dda and Cardiff and Vale University Health Boards |
| Paraffin embedded human tumour samples | Hywel Dda and Cardiff and Vale University Health Boards |
| Experimental Models: Cell Lines |   |   |
|---------------------------------|---|---|
| HeLa                           | ATCC® | CCL-2™ |
| NK92-MI                        | ATCC® | CRL-2408™ |
| NKL                            | Gifted to Edward Wang from Michael Robertson |   |
| NIH/3T3                        | ATCC® | CRL-1658™ |
| Primary CD19+ B Cells          | ATCC® | PCS-800-018™ |
| UWBCCC-1                       | Gifted from Vladimir Spiegelman |   |

| Experimental Models: Organisms/Strains |   |
|----------------------------------------|---|
| NU(NCr)-Foxn1™ mice                    | Charles River, UK |

| Oligonucleotides (TaqMan) |   |
|---------------------------|---|
| CD200 (FAM-MGB)           | ThermoFisher Scientific | Cat#Hs01033303_m1 |
| FAS (FAM-MGB)             | ThermoFisher Scientific | Cat#Hs00236330_m1 |
| FASLG (FAM-MGB)           | ThermoFisher Scientific | Cat#Hs00181225_m1 |
| FADD (FAM-MGB)            | ThermoFisher Scientific | Cat#Hs00538709_m1 |
| ADAM17 (FAM-MGB)          | ThermoFisher Scientific | Cat#Hs01041915_m1 |
| ADAM28 (FAM-MGB)          | ThermoFisher Scientific | Cat#Hs00248020_m1 |
| MMP3 (FAM-MGB)            | ThermoFisher Scientific | Cat#Hs00968305_m1 |
| MMP11 (FAM-MGB)           | ThermoFisher Scientific | Cat#Hs00968295_m1 |
| ACTB (FAM-MGB)            | ThermoFisher Scientific | Cat#Hs00357333_g1 |
| GAPDH (FAM-MGB)           | ThermoFisher Scientific | Cat#Hs02758991_g1 |

| Software and Algorithms |   |
|-------------------------|---|
| CIBERSORT                | Newman et al., 2015 | https://cibersort.stanford.edu |
| FlowJo v10.6.0          |   | https://www.flowjo.com |
| Software | Authors | URL |
|----------|---------|-----|
| GraphPad Prism v8 | | [https://www.graphpad.com](https://www.graphpad.com) |
| Image J | | [https://imagej.nih.gov/ij/](https://imagej.nih.gov/ij/) |
| Qupath | Bankhead *et al*., 2017 | [https://qupath.github.io](https://qupath.github.io) |
| ImageLab | | [https://www.bio-rad.com](https://www.bio-rad.com) |
| PRECOG | Gentles *et al*., 2015 | [https://precog.stanford.edu](https://precog.stanford.edu) |
| GSEA | Subramanian *et al*., 2005; Mootha *et al*., 2003 | [https://www.gsea-msigdb.org/gsea/index.jsp](https://www.gsea-msigdb.org/gsea/index.jsp) |
| R v3.4 | | [https://cran.r-project.org](https://cran.r-project.org) |
| **Other** | | |
| BD LSRFortessa | BD Biosciences | |
| Axio Scan.Z1 Slide Scanner | Zeiss | |
| QuantStudio™ 7 Flex Real-Time PCR System | ThermoFisher Scientific | |
| iScan Microarray Scanner | Illumina | |
| GelCount™ Colony Counter | Oxford Optronix | |
| CLARIOstar plate reader | BMG LABTECH | |

*Primary antibodies used in flow cytometry*
**Supplementary Figure 1. CD200R activation blocks MAPK signaling and NK cell killing activity**

Flow cytometric analysis of NK cell lines (A) and mouse neuronal cells for CD200R expression (B). Immunoblot of lysates from mouse neuronal cells incubated for 10 minutes with CD200 peptide and probed for phosphorylated ERK1/2, total ERK and GAPDH (C). HaCaT cell transduced with CD200 and control vector, CD200\(^{\text{POS}}\) and CD200\(^{\text{NEG}}\) respectively, viability after incubation with The NK\(^{\text{POS}}\) cell line (D). CD200\(^{\text{POS}}\) HaCaT cells were co-cultured with NK\(^{\text{POS}}\) cell line treated with CD200R ShRNA or vector (E). Time-lapse quantification of viable GFP-HeLa (target cell) cells in co-culture with NK\(^{\text{POS}}\) (effector) cell at an E:T ratio of 0:1, 5:1 and 10:1 (n=3) over 20 hours (F). Error bars represent mean ± SEM. Significance values, *<0.05, ***,<0.001.

**Supplementary Figure 2. Immune cell activation is impaired by CD200.**

Clariostar\(^{\text{TM}}\) quantification of CD200\(^{\text{POS}}\) HaCaT cells alone, co-cultured with NKPOS cell line and treated with CD200 blocking antibody (A). The NK\(^{\text{POS}}\) cell line was stimulated with PMA and Ionomycin for 4 hr (B). Unstimulated NK\(^{\text{POS}}\) cells were used as a baseline. The expression of CD107a was analyzed in live, CD56+ NK\(^{\text{POS}}\) cells by flow cytometry (n=3). Average CD107a positivity was calculated. Students t test was used to determine difference between CD107 population positivity, **** represents p<0.0001. Error bars represent mean ± SEM. Flow cytometric histogram analysis of the lymphocyte population forward scatter comparing day 0 (blue histogram) and 5 (red histogram) blood samples from HeLa\(^{\text{NEG}}\) and HeLa\(^{\text{POS}}\) grafted mice (C). Immunofluorescence labelling for phospho-ERK1/2 and DAPI to determine MAPK pathway activation in NK\(^{\text{POS}}\) cells: untreated, treated with epidermal
growth factor (EGF) for 4 hours, and after 4 hour co-cultured with HeLa<sup>NEG</sup> and HeLa<sup>POS</sup> (D). Error bars represent mean ± SEM. Significance values, *<0.05, ****<0.0001.

**Supplementary Figure 3. CD200 blocks NK cell killing**

Paraffin embedded sections labelled with anti-NK1.1 antibody by immunohistochemistry to determine NK cell infiltrate, used spleen sections as positive control (A). Paraffin embedded sections were also labelled with anti-cleaved caspase 3 and -CD200R antibodies by immunohistochemistry to determine the fraction of NK cells undergoing apoptosis and the proportion of remaining NK cells that expressed the CD200 receptor, respectively (B). Week 5 tumors from nude mice were grafted with 3x10<sup>6</sup> UWBCC1<sup>POS</sup> or UWBCC1<sup>NEG</sup> (n=5 each), histological analysis of tumor cellularity, necrosis and inflammatory cell infiltration, and data from paraffin embedded sections labelled with anti-NK1.1, -cleaved caspase 3 and -CD200R antibodies by immunohistochemistry (C). *=necrotic tissue area. Error bars represent mean ± SEM. Significance values, *<0.05.

**Supplementary Figure 4. NK<sup>NEG</sup> cells incubated with CD200 peptide**

Immunoblot of CD200 peptide (4 μg/1x10<sup>6</sup> cells for 8-hours) incubated NK<sup>NEG</sup> cells lysates for PARP, caspase 8, caspase 9 and GAPDH.

**Supplementary Figure 5. Over-expression of the Fas death receptor extrinsic apoptotic pathway in NK cells treated with CD200 peptide.**

Hierarchical clustering of differentially expressed genes from NK<sup>POS</sup> cells incubated with CD200 peptide for 2- and 4-hours compared to untreated cells (A). Gene set enriched
analysis using Gene Ontology and WikiPathway terms (B). The Gene Ontology term “regulation of extrinsic apoptosis signaling pathway via death domain receptors” (GO:1902041) was identified as enriched, with 39 of the 58 genes within the gene set found to be concordantly regulated after 2 hours (left hand side) and 4 hours (right hand side) (C). GSEA of the Biocarta “Fas Pathway” from differentially expressed genes comparing untreated and 2-hour (left panel) or 4-hour (right panel) CD200 peptide (4 μg/1x10^6 cells) treated NK^POS^ cells (D). ELISA determination of soluble FasL in culture supernatant of CD200 peptide treated NK^POS^ cells (E). GSEA of the Kegg “PPAR signaling Pathway” from differentially expressed genes comparing untreated and 2-hour (left panel) or 4-hour (right panel) CD200 peptide (4 μg/1x10^6 cells) treated NK^POS^ cells (F). Relative expression of PPAR^γ^ regulated genes comparing untreated and 2-hour (above panel) or 4-hour (below panel) CD200 peptide (4 μg/1x10^6 cells) treated NK^POS^ cells (G). Green bars are concordant genes, discordant regulated genes are red bars. Error bars are represented as mean ± SEM.

**Supplementary Figure 6. CD200 induced NK cell apoptosis.**

PBMCs were extracted from whole blood (n=4) using Ficoll-Paque separation then plated for overnight to remove adherent cells. The non-adherent population was treated with CD200 peptide for 4-hours before FACs analysis (A). RNA extracted from CD200 peptide treated CD45^+^CD3^-^ CD56^+^ CD200R^+^ and - flow sorted cells was analysed for FAS, FASL and FADD gene expression (B). Annexin V labelling was used to determine the frequency of apoptotic cells within the CD45^+^ CD3^-^ CD56^+^ CD200R^+^ and CD45^+^ CD3^-^ CD56^+^ CD200R^-^ fractions (C). Analysis for FAS, FASL and FADD gene expression relative to untreated cells after treatment with CD200 peptide, as well as pharmacological inhibition of ERK and PPAR-γ in NK^POS^ cells (D). Significance values, *<0.05. Error bars represent mean ± SEM.
**Supplementary Figure 7. CD200 ectodomain shedding**

Primary human BCC (n=4) and SCC (n=3) tissue lysates were probed for CD200 and GAPDH and quantitated using ImageJ (A). qPCR relative expression of mRNA extracted from primary human BCC (n=3) and SCC (n=3) tissues, with GAPDH normalization (B). qPCR relative expression of mRNA extracted from primary human BCC (n=3) and matched normal skin (n=3) for CD200, with GAPDH normalization (C). Transcriptomic analysis of BCC (n=4), SCC (n=3) and normal skin (n=3) unsupervised hierarchical clustering revealed 235 differential expressed genes (D), with gene set enrichment for metallocarboxypeptidase activity and metalloendopeptidase activity (E). The relative gene expression for BCC versus SCC datasets for known sheddases were analyzed (F). The expression of the sheddase substrates, ADAMs, MMP3 and MMP11 were also analyzed for BCC versus normal skin (G). Similarly, we analyzed the relative expression of ADAMs (H) and validated the findings by qPCR of primary human BCC (n=4) for ADAM 17 and 28, with GAPDH normalization (I). Error bars represent mean ± SEM.

**Supplementary Figure 8. Linear regression analysis of other immune cell phenotypes with CD200 prognostic z-scores.**

The PRECOG and iPRECOG databases were used to generate data matrices for the associations, in the form of z-scores, between CD200 expression and overall cancer survival outcomes, and immune cell type and overall cancer survival outcomes, respectively. Briefly, PRECOG and iPRECOG databases were downloaded and filtered for CD200 expression and NK cell status and rows with NA values were discarded. The resulting data matrix was then imported into GraphPad and a Normal QQ plot and residual plot was generated to test for normality and variance in the data, respectively. Normality of the data was confirmed, and


Supplementary Figure 9. Basal cell carcinoma NK cell immune evasion.

Expression of CD200 within primary BCC (n=3) and SCC (n=3) tissue was established before grafting into mice (A). Paraffin embedded sections from primary BCC (n=3) and SCC (n=3) xenografts were labelled with anti-CD200 via IHC (B). Differentially expressed genes from a publicly available BCC dataset (GSE58377) were extracted to determine expression of NK cell receptor genes for CD200\textsuperscript{High} vs CD200\textsuperscript{Low} BCC (C) and resistant vs sensitive BCC samples (D). Significance values, *<0.05. Error bars represent mean ± SEM.

Supplementary Table 1. Top differentially expressed genes when comparing BCC to normal skin; significance p<0.01

Supplementary Table 2. Meta z-scores generated from 39 cancers to indicate prognosis from CD200 expression (+ and – values indicate poor and good prognosis, respectively)

Supplementary Table 3. Meta z-scores generated from 23 cancers to indicate prognosis between CD200 expression and immune cells (iPRECOG)

Supplementary Table 4. Resources Table
Supplemental Methods

Tissue dissociation and culture

Tissue for cell culture was processed into single cells as described in our previously published protocols (1,2).

Peripheral Blood Mononuclear Cell (PBMC) extraction for NK cell analysis

PBMC cells were extracted using Ficoll-Paque PLUS (3mL) solution as per manufacturers guidelines.

NK cell degranulation assay

NK92MI cells were co-cultured with HeLa cells as described above at an effector:target ratio of 5:1. As a positive control for degranulation, NK cells were treated with a combination of PMA (100ng/mL) and ionomycin (2μg/mL) (3). Untreated NK cells were used for baseline expression. The CD107a antibody was added at the beginning of the co-culture. Isotype control for CD107a was also added to the co-culture. After 30 mins of co-culture, 1x monensin was added to the well to prevent the reinternalization of the protein. The suspension NK92MI cells were removed from the co-culture and pelleted by centrifugation at 120xg for 5 mins. Cells were washed with FACS buffer (0.05% sodium azide and 0.5% BSA in PBS) before staining with CD56 antibody. Cells were washed and processed as described above.

NK92MI cell shRNA

200,000 NK-92MI cells (10^5 cells/ml) were transduced with lentiviral particles carrying shRNA against CD200 receptor (sc-42956-SH) or control shRNA (sc-42956-SH) in the presence of 8 μg/ml. Cells were then grown for 40 hours in fresh NK-92MI medium before puromycin (sc-108071A, Santa Cruz) was added to a final concentration of 5 mg/ml for selection of transduced cells.
Cell conditioned media of cell lines and primary human BCC samples

HeLa cells were allowed to grow to 60-70% confluency, after which, media was replaced with 15mL of fresh media and cells allowed to grow for a further 48 hr. Primary human BCC samples were first dissociated as previously described and single cells were cultured in media for 48 hr. After 48 hr the conditioned media/supernatant were collected and concentrated in Pierce™ concentrators with a molecular weight cut off of 3K MWCO (ThermoFisher Scientific).

ELISA (soluble CD200, CCL4 and soluble FASL)

ELISA’s (Supplementary Table 4) were performed following the manufacturer’s guidelines, using the cell culture supernatant described earlier. The optical density was measured immediately, using the CLARIOstar plate reader set to 450nm. Wells containing concentrated media of each respective cell types served as the blank.

Human IFN-gamma ELISpot Assay (Interferon-gamma ELISA)

ELISpot assay was used for detecting the secretion of IFN-gamma from NK92MI cells following incubation with HeLa\textsuperscript{POS} and HeLa\textsuperscript{NEG} cell lines. In 6-well tissue culture plates NK92MI cells were incubated with either HeLa\textsuperscript{POS} or HeLa\textsuperscript{NEG} cell lines at a ratio of 1:10, for 4hr. Following incubation, NK92MI cells were removed from the supernatant and added at a concentration of 50,000 cells (100µL/well) per well of a 96-well plate that had been pre-coated with a monoclonal antibody specific for human IFN-gamma. ELISpot assay was then carried out as performed in manufacturers guidelines. The developed microplate was analyzed by
counting the number of spots in each well using the GelCount™ colony counter machine (Oxford Optronix).

**Cell viability assay – CellTiter® Glo**

Cells were plated (20,000 cells in 100 µL) in a white-walled 96-well plate and left overnight to adhere. NK cells were added to the wells at various effector-target ratios. Co-incubations were carried out for 4 hr after which NK cells and media were removed and the adherent tumor cells in wells were washed thoroughly with PBS. Fresh media was added to the wells and an equal amount of CellTiter-Glo® reagent was added to each well (100 µL) and subsequently incubated in the dark at room temperature for 20 mins. Luminescence was recorded using a CLARIOstar plate reader with wells containing only media serving as a blank.

**IncuCyte caspase-3/7 NK apoptosis and HeLa killing assays**

GFP transduced HeLa cells with and without CD200 expression were seeded into a flat-bottomed white walled 96-well plate at a density of 1,000 cells/well and left to adhere overnight. The next day the caspase-3/7 red reagent was diluted by 1:1000 in complete media to a final concentration of 5 µM. Media was aspirated from wells and 100 µL of diluted caspase-3/7 reagent was added to each well along with 5,000 NK92 cells. The plate was then placed into the IncuCyte Live-Cell Analysis System and pictures were taken at 10x magnification every 30 mins for 24 hr. The ability of either HeLaPOS or HeLaNEG cells to induce NK cell death could be determined as NK cells undergoing apoptosis turned red with the dye, which could subsequently be measured using the integrated IncuCyte software.
The ability of NK\textsuperscript{POS} cells to induce HeLa\textsuperscript{POS} or HeLa\textsuperscript{NEG} cell death in the presence or absence of exogenous CD200 peptide (4µg/10\textsuperscript{6} HeLa cells) was performed by incubating NK and HeLa cells at a ratio of 1:1 in a 96-well white walled plate in the presence or absence of exogenous CD200 peptide. As for the IncuCyte assay, the plate was placed into the IncuCyte Live-Cell Analysis System and pictures were taken at 10x magnification every 30 mins for 24 hr. Since the HeLa cells express GFP, which is maintained after cell division, the IncuCyte software was programmed to measure the levels of HeLa cell death using an image segmentation algorithm to identify individual GFP expressing HeLa cells.

**Western Blotting**

Samples were diluted in RIPA buffer to equal concentrations. Laemmli buffer (4x) was added to the samples, which were then heated at 95°C for 5 mins. TGXTM FastCastTM premixed acrylamide solutions (BioRad) were used to cast gels. 10ug of protein was loaded into the wells in addition to a molecular weight marker (PageRuler Plus, ThermoFisher), and run at 300V until the desired marker separation was achieved. Trans-Blot® Turbo™ Transfer System (BioRad) was used to transfer protein to the PDVF membrane. Membranes were incubated in blocking buffer of 10% milk or BSA (depending on antibody) in TBST for 1 hour at room temperature. Following incubation, membranes were incubated in the desired primary antibody (Supplementary Table 4) diluted in 5% BSA or milk in TBST and incubated overnight at 4°C on a roller. Following incubation, the membrane was washed in TBST before incubating in horseradish peroxidase (HRP)-conjugated secondary antibody (Abcam) diluted 1:5000 in TBST for 1 hour at room temperature. Membranes are washed again for 4 x 5 mins in TBST before antibody binding was detected by incubating Illumina Forte chemiluminescence reagent (Millipore) on the membrane and imaged using the ChemiDoc MP Imaging System (BioRad).
**RNA extraction and cDNA synthesis**

RNA was isolated using the Qiagen RNeasy Plus Mini Kit (Qiagen, UK) per manufacturer’s instructions. The quality of the extracted RNA was assessed using the Agilent RNA 6000 Nano kit. cDNA synthesis was performed using the Quantitect Reverse Transcription Kit (Qiagen, UK) in 0.2 mL PCR tubes as per manufacturer’s guidelines.

**Quantitative real-time PCR (qPCR)**

For qPCR gene expression studies, all reactions were performed using the TaqMan gene expression assay. Pre-designed TaqMan primer/probes were obtained from Applied Biosystems (Supplementary Table 4). Reactions were run using the TaqMan Universal Master Mix II, with UNG (Applied Biosystems) according to the manufacturer’s guidelines. Housekeeping genes (GAPDH and beta actin) were used as reference genes. All reactions were run in three technical replicates. All reactions were run on the QuantStudio 7 Flex Real-Time PCR system (Applied Biosystems) supplemented with the QuantStudio software. Gene expression analysis of qPCR data was analysed using the ΔΔCt method to calculate fold change ($2^{\Delta\Delta Ct}$) relative to control.

**Bioinformatic analysis**

Microarray data were analyzed using the Bioconductor packages in the R statistical program for data processing for all the subsequent steps. For analyzing Illumina microarray data, the ‘Lumi’ package was used for assessing quality control of the samples. Outliers were removed, before transforming and normalizing the data. Finally, genes were annotated and mapped at the probe level using ‘lumiHumanAll.db’ and ‘luminHumanIDMapping’ programs respectively. Genes/probes whose expression was absent between the samples were then filtered out. Next,
we performed comparisons between the treated groups and the untreated group by using the Limma program, again in the Bioconductor package to calculate the level of gene differential expression. A list of differentially expressed genes (DEGs) was obtained at a p value <0.05. Following this we used the expression values of DEGs to perform pathway analysis using Gene Set Enrichment Analysis (GSEA; Broad Institute) (4,5). Fold changes in gene expression were calculated from LogFC values, where fold changes of +1 or −1 indicate no change in gene expression relative to the control.

**Analyzing PRECOG (Prediction of Clinical Outcomes from Genomic Profiles) and CIBERSORT (Cell type Identification By Estimating Relative Subsets Of known RNA Transcripts) Datasets**

The pan-cancer resource from ~18,000 human tumors and associated overall survival outcomes across 39 malignancies, termed Prediction of Clinical Outcomes from Genomic Profiles (PRECOG; [http://precog.stanford.edu](http://precog.stanford.edu)) (6). Newman et al., (2015) applied an algorithm, termed Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT; [http://cibersort.stanford.edu](http://cibersort.stanford.edu)) (7), to the PRECOG resource in order to determine leukocyte representation in bulk tumor transcriptomes, and subsequently identified associations between 22 distinct leukocyte subsets and cancer survival. The significant differentially expressed genes used to identify activated and resting NK cells from 22 immune cell profiles after filtering were: Activated NK cells (*APOBEC3G, APOL6, CCL4, CCL5, CCND2, CD244, CD247, CD69, CD7, CD96, CDK6, CSF2, CST7, CTSW, DPP4, FASLG, GNLY, GPR171, GPR18, GRAP2, GZMA, GZMB, GZMH, GZMM, IFNG, IL12RB2, IL18R1, IL18RAP, IL2RB, KIR2DL1, KIR2DL4, KIR2DS4, KIR3DL2, KLRB1, KLRC3, KLRD1, KLRF1, KLRK1, LCK, LTA, LTB, NAALADL1, NCR3, NKG7, OSM, PRF1, PRR5L, PTGDR, PTGER2, TPTRCAP, PVRIG, SIPR5, SH2D1A, SOCS1, TBX21, TNFSF14,
TRDC, TXK, ZAP70) and Resting NK cells (AZU1, BPI, CAMP, CCL5, CD160, CD2, CD244, CD247, CD7, CD96, CDHR1, CEACAM8, CST7, CTSW, DEFA4, ELANE, GFI1, GNLY, GZMA, GZMB, GZMH, GZMK, GZMM, IL12RB2, IL18R1, IL18RAP, IL2RB, KIR2DL1, KIR3DL2, KLRB1, KLRC3, KLRC4, KLRD1, KLRF1, KLRK1, LCK, MGAM, MS4A3, NAALADL1, NKG7, NME8, PLEKHF1, PRF1, PRR5L, PTGDR, PTPRCAP, PVRIG, S1PR5, SH2D1A, TBX21, TEP1, TRBC1, TRDC, TTC38, TXK, ZAP70, ZNF135). Both PRECOG and immune-PRECOG databases were used to generate data matrices for the associations (in the form of z-scores), between CD200 expression and overall cancer survival outcomes, and leukocyte subsets and overall cancer survival outcomes, respectively. Briefly, PRECOG and immune-PRECOG databases were downloaded and filtered for CD200 expression and NK cell status and rows with NA values were discarded. Normal QQ plot and residual plots were generated to test for normality and variance in the data, respectively. Normality of the data was confirmed, and assumptions of equal variance were met. Finally, simple linear regressions were performed in GraphPad Prism to determine the correlation between immune cell phenotypes and CD200 prognostic z scores in a number of different cancer types. For the z-scores, negative values indicate good prognosis and positive values indicate poor prognosis.

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