Supporting Information

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SI Materials and Methods

Immunoblot Analysis. Lysates of COS-7 transiently transfected with FuGene 6 (Roche) were fractionated by 15% SDS/PAGE and transferred to nitrocellulose membranes. Membranes were probed with anti-hexahistidine mAb (Bethyl) or anti-myc mAb (clone 9E10) followed by horseradish peroxidase–conjugated secondary reagents.

Flow Cytometry. Cells were incubated with the indicated mAb (10 μg/mL), Fc-fusion proteins (10 μg/mL; R&D Systems) or fluorochrome-conjugated tetramers (10 μg/mL) for 30 min on ice. After washing, cell-bound antibodies or Fc-fusion proteins were stained with fluorochrome-conjugated goat anti-mouse Ig antibodies or PE-conjugated anti-human IgG1 mAb (1:100; Southern Biotech), respectively. Fluorescence stainings were analyzed on a FACScalibur unit using CellQuest software.

Quantitative PCR. mRNA levels were determined by real-time PCR of human normal tissue purchased from Clontech (bone marrow), Stratagene (lymph node, skin), Applied Biosystems (all other tissues) and of cell lines or PBMCs followed by DNase I digestion and reverse transcription using SuperScriptII (Invitrogen). cDNA was amplified with primer pairs specific for NKp65 (5′-aggcacatttactggtgattcaa-3′/5′-tgttcatctatccacatccata-3′), KACL (5′-ataccagaaattggacagcc-3′/5′-gaatgtggtgccatttgtcc-3′), and 18S rRNA (5′-cggctaccacatccaaggaa-3′/5′-gctggaattaccgcggct-3′) using SYBR-Green chemistry on the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Data analysis was performed by ΔC_T method for relative quantification.

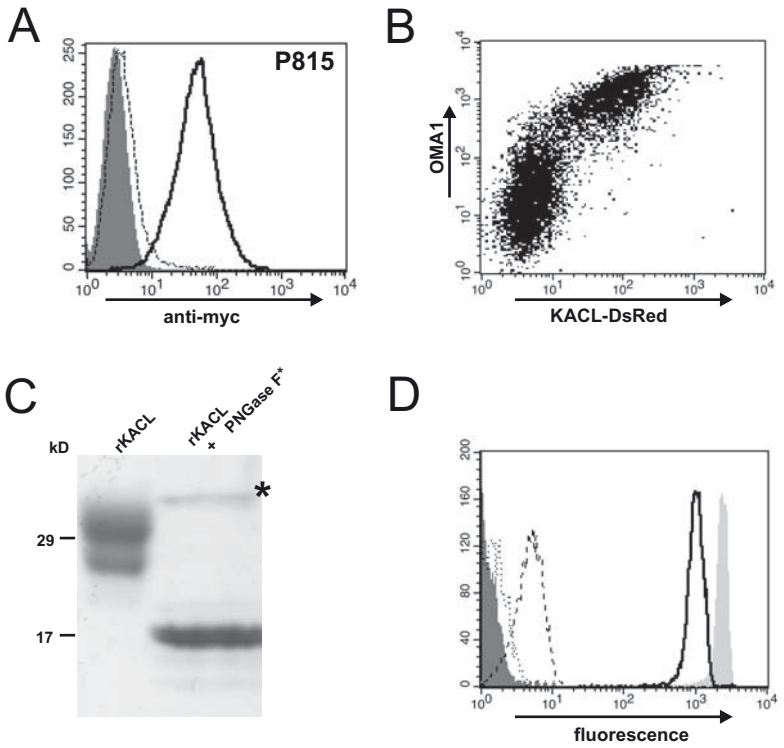

Fig. S1. MAb OMA1 is specific for KACL. (*A*) Anti-KACL mAbs were generated by immunizing mice with P815-KACL. Staining of mock-transfected (P815-neo) (dotted) or c-myc–tagged KACL-transfected P815 (P815-KACL) (solid) with anti–c-myc-tag mAb 9E10. Isotype control staining of P815-KACL is shaded. (*B*) MAb OMA1 specifically recognizes KACL on COS-7 transiently transfected with a KACL-pIRES-DsRed construct. (*C*) Soluble recombinant rKACL was produced in 293T, purified via affinity chromatography, and analyzed by SDS/PAGE. (*D*) OMA1 specifically binds imKACL, but not other members of the CLEC2 family. rKACL (solid black), rAICL (dotted), and rLLT1 (stippled) were immobilized on streptavidin-coated microspheres and stained with OMA1. OMA1 binding to uncoated microspheres (filled dark gray) and 9E10 to imKACL (filled light gray).

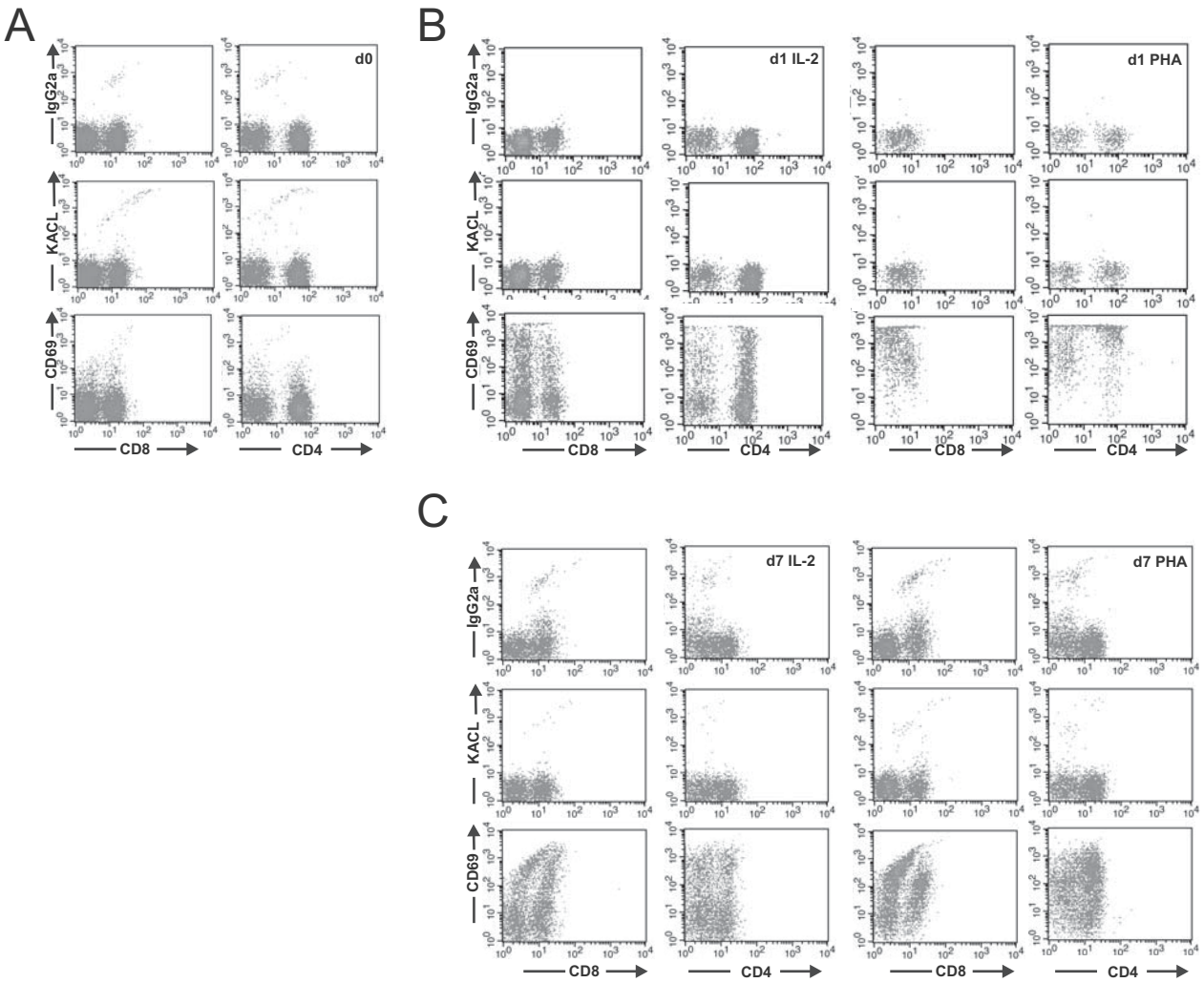

Fig. S2. KACL is not detectable on freshly isolated or activated T cells. (*A*–*C*) T cells were analyzed for KACL expression after isolation (*A*) or after 1 d (*B*) or 7 d (*C*) of treatment with 100 U/mL IL-2 (*Left*) or 1 μg/mL phythemagglutinine (*Right*), respectively. PBMCs were stained with anti-CD3, anti-CD4, anti-CD8, anti-CD69, or mAb OMA1. Dot plots depict staining of CD3-gated cells.

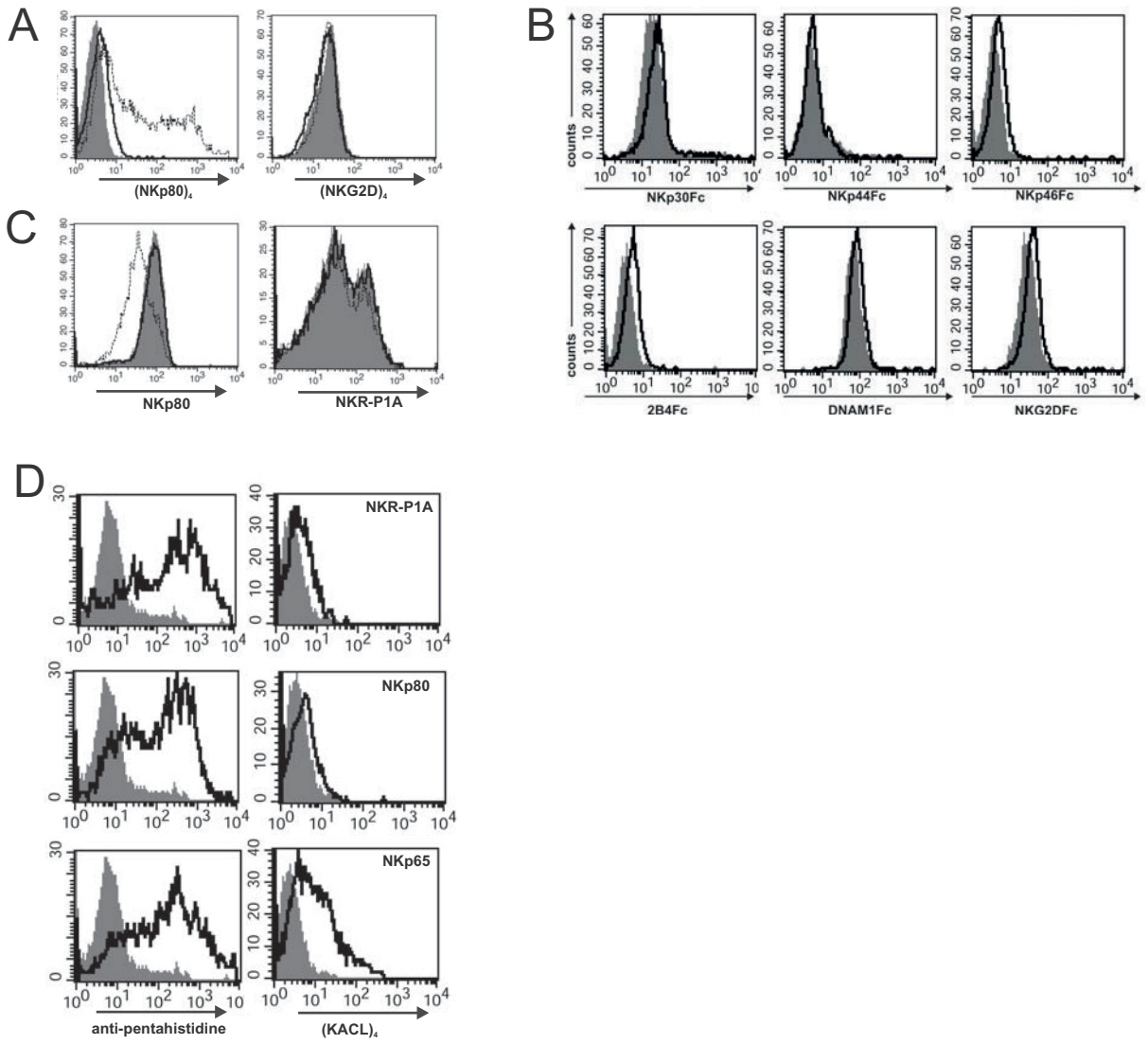

Fig. S3. No detectable interaction of KACL with known activating NK cell receptors. (*A*) KACL is not a ligand for NKp80 or NKG2D. Neither NKp80 tetramers nor NKG2D tetramers bind mock- (shaded) or KACL-transfected (solid) COS-7, whereas NKp80 tetramers bind AICL-transfectants (dotted). (*B*) Mock-transfected 293T (shaded) and KACL-transfected 293T (solid) were stained with ectodomains of known activating NK receptors. (*C*) Freshly isolated NK cells were cocultured with mock- (shaded), KACL- (solid), or AICL-transfected 293T (dotted) and stained for NKp80 (*Left*) or NKR-P1A (*Right*) surface expression. Neither NKp80 nor NKR-P1A is down-regulated after coculture with KACL-expressing 293T. (*D*) KACL is a ligand for NKp65. COS-7 cells were transiently transfected with histidine-tagged cDNA of NKR-P1A, NKp80, and NKp65, respectively, and surface expression monitored with anti–histidine-tag mAb (left) or KACL tetramers (*Right*). Shaded are stainings of mock-transfectants.

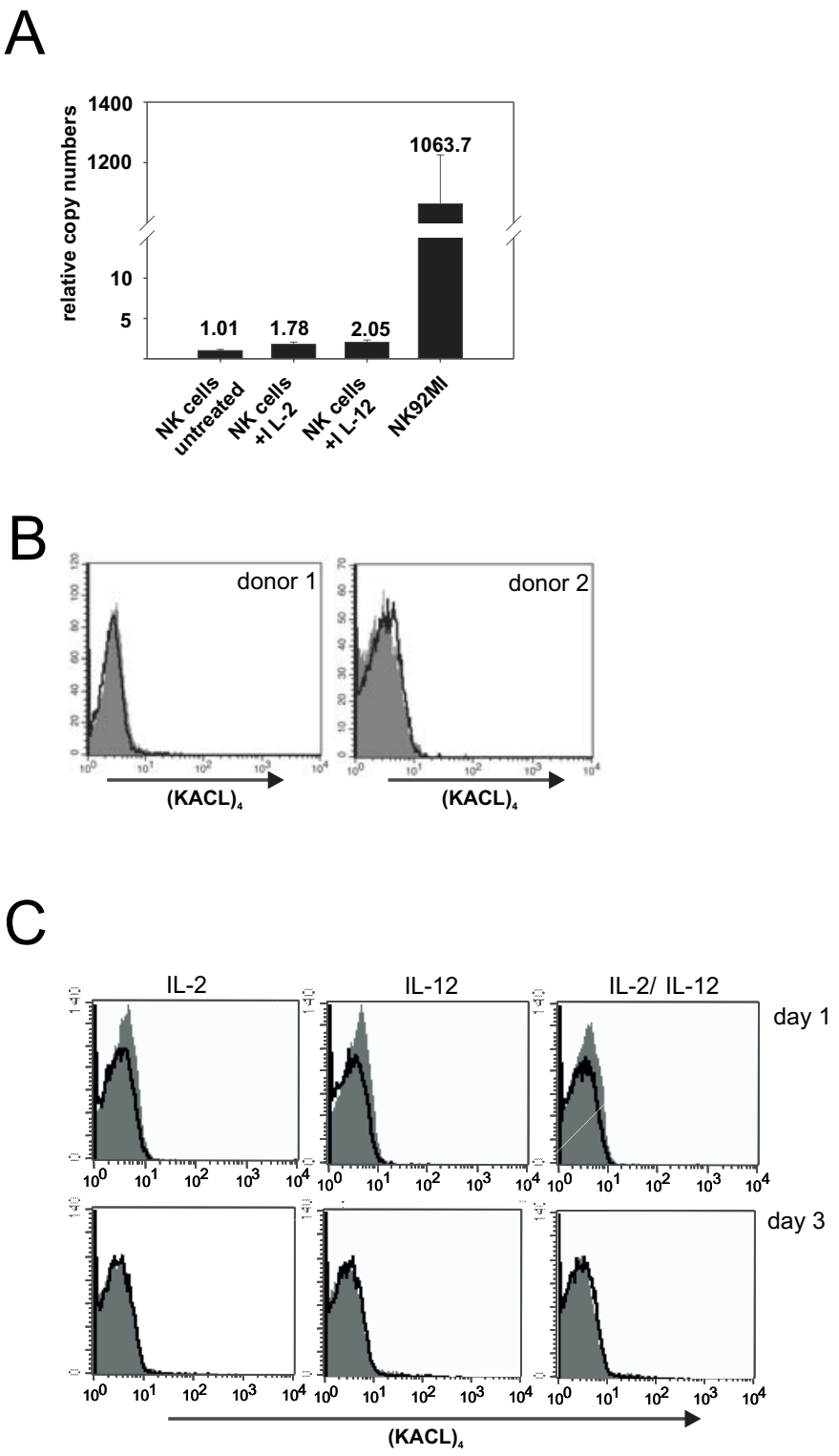

Fig. S4. Low-level NKp65 expression by human peripheral blood NK cells. (*A*) Relative copy numbers of NKp65 transcripts in NK92MI cells and in freshly isolated, human peripheral blood NK cells after 24 h of culture without cytokines (untreated), IL-2 (10 U/mL), or IL-12 (1 ng/mL) as determined by quantitative PCR. (*B*) Freshly isolated human peripheral blood NK cells of two unrelated healthy donors were stained with KACL tetramers (solid line) for NKp65 expression. (*C*) Purified peripheral blood NK cells of a healthy donor were stained with KACL tetramers (solid line) following 1 or 3 d of in vitro culture with IL-2 (100 U/mL) and/or IL-12 (5 ng/mL) for NKp65 expression. In *B* and *C*, filled histograms indicate control stainings with PE-conjugated streptavidin used for tetramerization of KACL monomers.