JEM

Supplemental material

Fernandez et al., **https://doi.org/10.1084/jem.20182015**

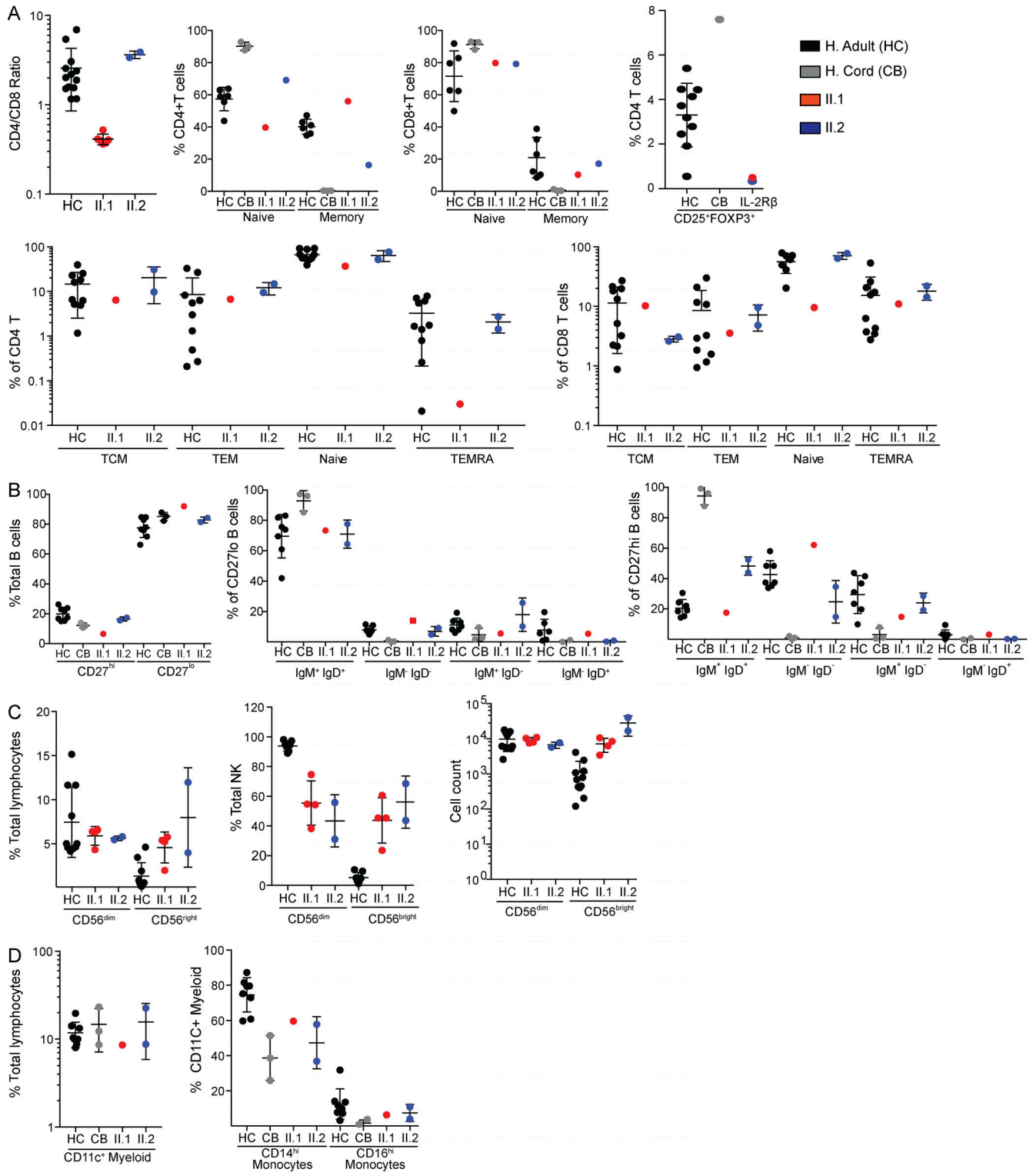

Figure S1. **Immunophenotyping of siblings with *IL2RB* homozygous mutation.** Immunophenotyping was performed by flow and mass cytometry to delineate major immune cell subsets of healthy controls and parents (HC, black circles), healthy cord blood (CB, gray circles), and both patients (II.1 red and II.2 blue circles). Frequencies of multiple immune cell populations are shown. **(A)** T cell subsets, including central memory (TCM), effector memory (TEM), and naive and effector memory with RA positivity (TEMRA) CD4+ and CD8+ T cells. **(B)** B cell subsets. **(C)** NK cell subsets. **(D)** Myeloid lineage cell subsets. Three experiments shown. Mean ± SD is shown.

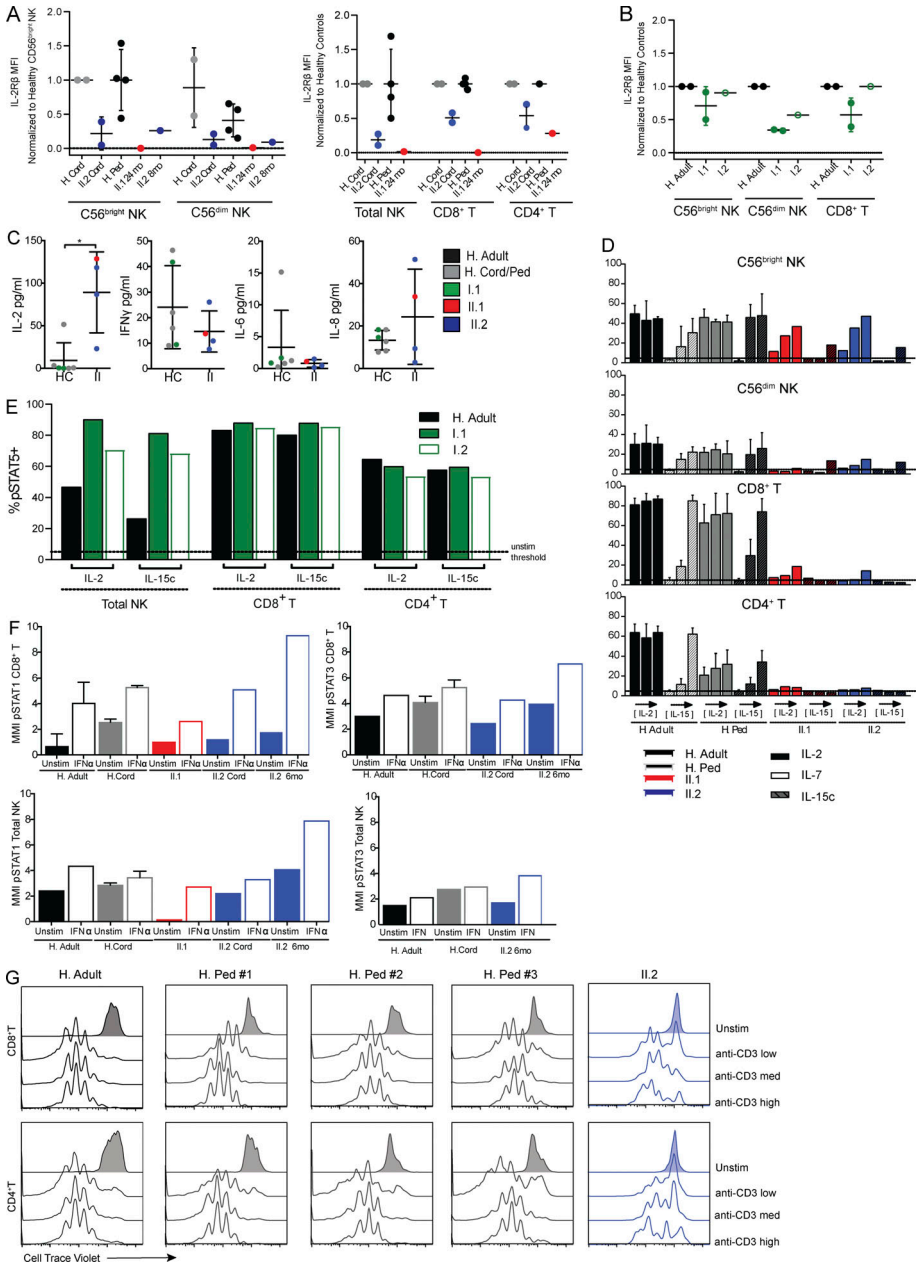

Figure S2. **Characterization of *IL2RB* homozygous mutation. (A)** Normalized surface IL-2Rβ protein expression MFI in CD56^{bright} and CD56^{dim} NK cells (left). Normalized surface IL-2Rβ protein expression MFI in total NK, CD8$^+$, and CD4$^+$ T cells is also shown (right). MFI shown is calculated as IL-2Rβ MFI – IgG1 isotype MFI and normalized to the mean for each cell type. Healthy cord and pediatric controls (age 12–30 mo) were normalized independently. For cord comparisons, two experiments with different healthy cord donors are shown. For pediatric comparisons, one experiment with four healthy age-matched controls is shown. **(B)** Relative IL-2Rβ surface protein expression levels in CD56^{bright} NK, CD56^{dim} NK, and CD8$^+$ T cells in heterozygous parents and adult healthy controls. The MFI is calculated as IL-2Rβ MFI – isotype MFI for each cell type. Two experiments with different healthy donors are shown. Material for the mother (I.2) was available for only one experiment. **(C)** A multiplexed 10-cytokine panel was measured by electrochemiluminescence in plasma from patients, parent (I.2), and healthy cord blood samples (n = 4) at multiple time points. IL-2, IFNγ, IL-6, and IL-8 levels are shown. Two experiments are shown. **(D)** Percentage of CD56^{bright} and CD56^{dim} NK, CD8$^+$, and CD4$^+$ cells with pSTAT5 signal above unstimulated level in response to 15-min stimulation with IL-2 (0.1, 1.0, or 10 µg/ml, left to right) or IL-15 complex (0.1, 10, or 1,000 ng/ml, left to right). Unstimulated %pSTAT5$^+$ level is set with 95% of cells below threshold (shown as dashed line). One experiment with II.1 (age 24 mo) and II.2 (age 8 mo) compared with healthy adults (H. Adult, n = 3) and healthy pediatrics (H. Ped, age 12–30 mo, n = 3) is shown. **(E)** pSTAT5 response in heterozygous parents. Percentage of total NK, CD8$^+$, and CD4$^+$ T cells with pSTAT5 signal above unstimulated level in response to IL-2 and IL-15c (both at 1 µg/ml for 30 min). Unstimulated %pSTAT5$^+$ level set with 95% of cells below threshold; shown as dashed line. One experiment with one healthy adult control shown. **(F)** STAT1 and STAT3 phosphorylation in response to IFNα on CD8$^+$ T and NK cells from patients and healthy controls. PBMCs were stimulated with IFNα 40 KU/ml for 30 min. MMI, mean isotopic mass index. Two experiments are shown. For CD8$^+$ T cell data, n = 2 healthy adult, n = 3 healthy cord; for NK cell data, n = 1 healthy adult and healthy cord. **(G)** Representative histograms show cell trace violet dilution in CD8$^+$ and CD4$^+$ T cells from patient II.2 (blue), pediatric (gray), and adult (black) healthy controls. PBMCs were stimulated (unfilled) with anti-CD28 and anti-CD3 at different concentrations (low, 1 µg/ml; med, 5 µg/ml; high, 10 µg/ml) or left untreated (filled) for 110 h. n = 3 for healthy adults, and n = 3 for healthy pediatric controls (age 12–30 mo). One experiment is shown. Unstim, no stimulation. Mean ± SD is shown.

Table S1. **Whole-exome sequencing variants**

Gene	Variant	Genotype	Inheritance	Inheritance type	Effect	HGVS.c	dbSNP ID	gnomAD MAF
STAC	chr3:36545905 G/A	Heterozygous	Mother	Compound heterozygous	Missense	NM_003149.2: c.787G>A	rs370295976	4.89E-05
	chr3:36570225 G/C	Heterozygous	Father		Missense	NM_003149.2: c.958G>C	rs150887393	4.19E-06
SI	chr3:164750310 C/A	Homozygous	Mother and father	Homozygous recessive	Missense	NM_001041.3: c.2736G>T	None	
MECOM	chr3:168845842 C/T	Homozygous	Mother and father	Homozygous recessive	Missense	NM_001105077.3: c.248G>A	rs768694075	5.07E-05
HTR3E	chr3:183823729 G/T	Homozygous	Mother and father	Homozygous recessive	Missense	NM_182589.2: c.942G>T	rs187832026	6.06E-04
NRROS	chr3:196386902 G/A	Homozygous	Mother and father	Homozygous recessive	Missense	NM_198565.1: c.388G>A	None	
MBOAT1	chr6:20212368 T/C	Homozygous	Mother and father	Homozygous recessive	Missense	NM_001080480.2: c.98A>G	rs200213957	1.36E-04
HOXA4	chr7:27170198 T/G	Heterozygous	Not inherited	De novo	Missense	NM_002141.4: c.155A>C	rs559797480	1.48E-04
ARHGAP32	chr11:128842466 C/T	Homozygous	Mother and father	Homozygous recessive	Missense	NM_001142685.1: c.3893G>A	rs148138101	2.30E-04
PARP16	chr15:65555604 T/C	Heterozygous	Father	Compound heterozygous	Missense	NM_017851.4: c.574A>G	rs779012579	1.67E-04
	chr15:65558941 G/A	Heterozygous	Mother		Missense	NM_017851.4: c.478C>T	rs775857143	3.70E-04
ZFHX3	chr16:72992059 C/A	Homozygous	Mother and father	Homozygous recessive	Missense	NM_006885.3: c.1986G>T	None	
DNAH9	chr17:11520833 C/T	Heterozygous	Mother	Compound heterozygous	Missense	NM_001372.3: c.1010C>T	rs3744574	5.99E-04
	chr17:11684466 C/T	Heterozygous	Father		Missense	NM_001372.3: c.7693C>T	rs756091562	4.09E-06
SIGLEC10	chr19:51914585 C/G	Homozygous	Mother and father	Homozygous recessive	Missense	NM_033130.4: c.1862G>C	None	
SRC	chr20:36012651 T/C	Heterozygous	Not inherited	De novo	Missense	NM_005417.4: c.95T>C	rs754705008	5.02E-05
SMTN	chr22:31481257 C/T	Homozygous	Mother and father	Homozygous recessive	Missense	NM_001207018.1: c.199C>T	rs769850768	9.01E-04
TLR7	chrX:12906073 G/A	Hemizygous	Mother	X-linked	Missense	NM_016562.3: c.2446G>A	None	
GRPR	chrX:16168773 G/C	Hemizygous	Mother	X-linked	Missense	NM_005314.2: c.759G>C	rs141598090	6.81E-05
CYBB	chrX:37665739 G/A	Hemizygous	Mother	X-linked	Missense	NM_000397.3: c.1414G>A	rs13306300	7.73E-04
HDAC8	chrX:71571612 C/T	Hemizygous	Mother	X-linked	Missense	NM_018486.2: c.1082G>A	rs781862582	9.00E-05
H2BFM	chrX:103294904 G/A	Hemizygous	Mother	X-linked	Missense	NM_001164416.1: c.361G>A	rs782027240	4.33E-05

MAF, minor allele frequency.