Oncogenic *IL7R* gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia

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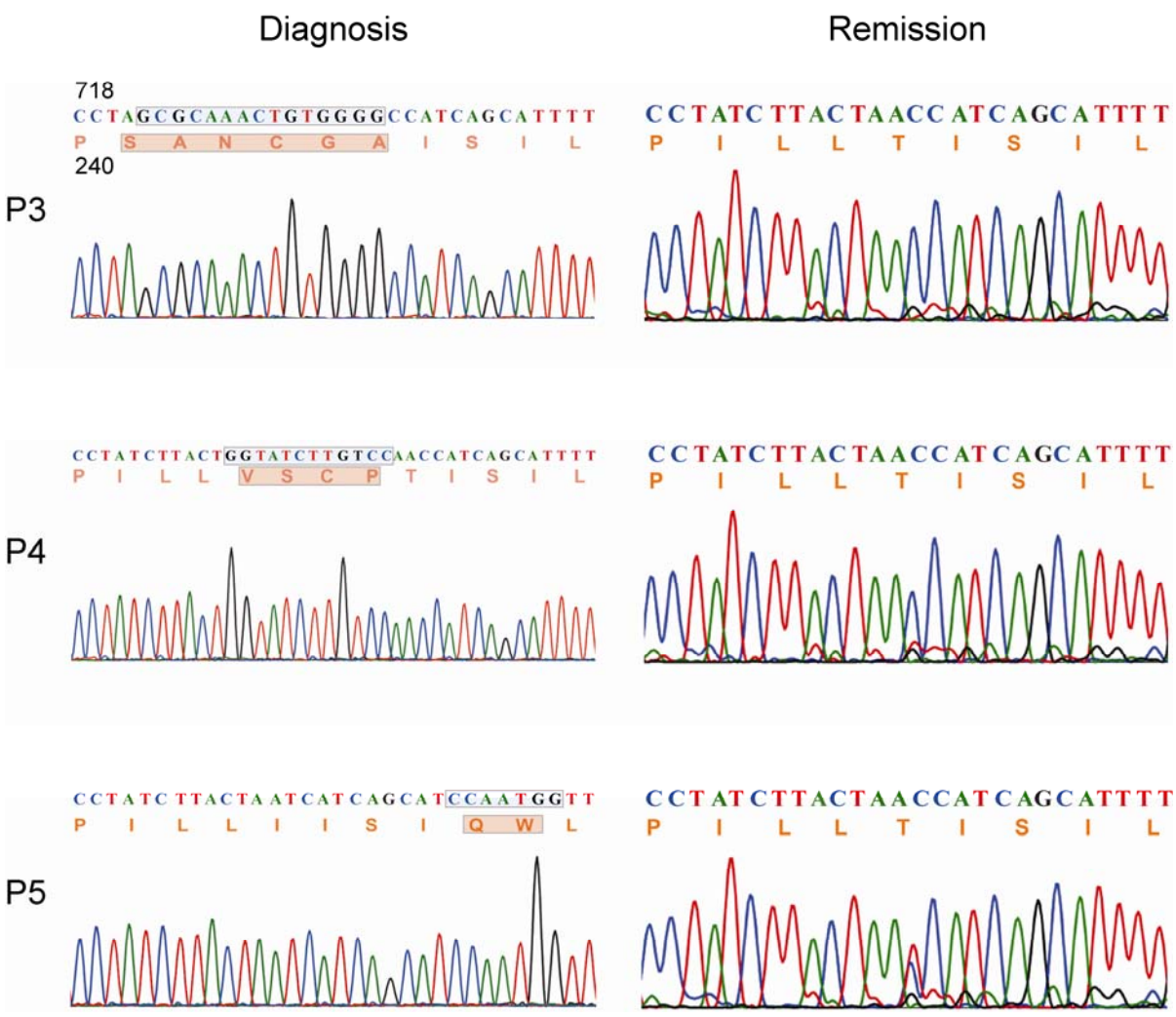
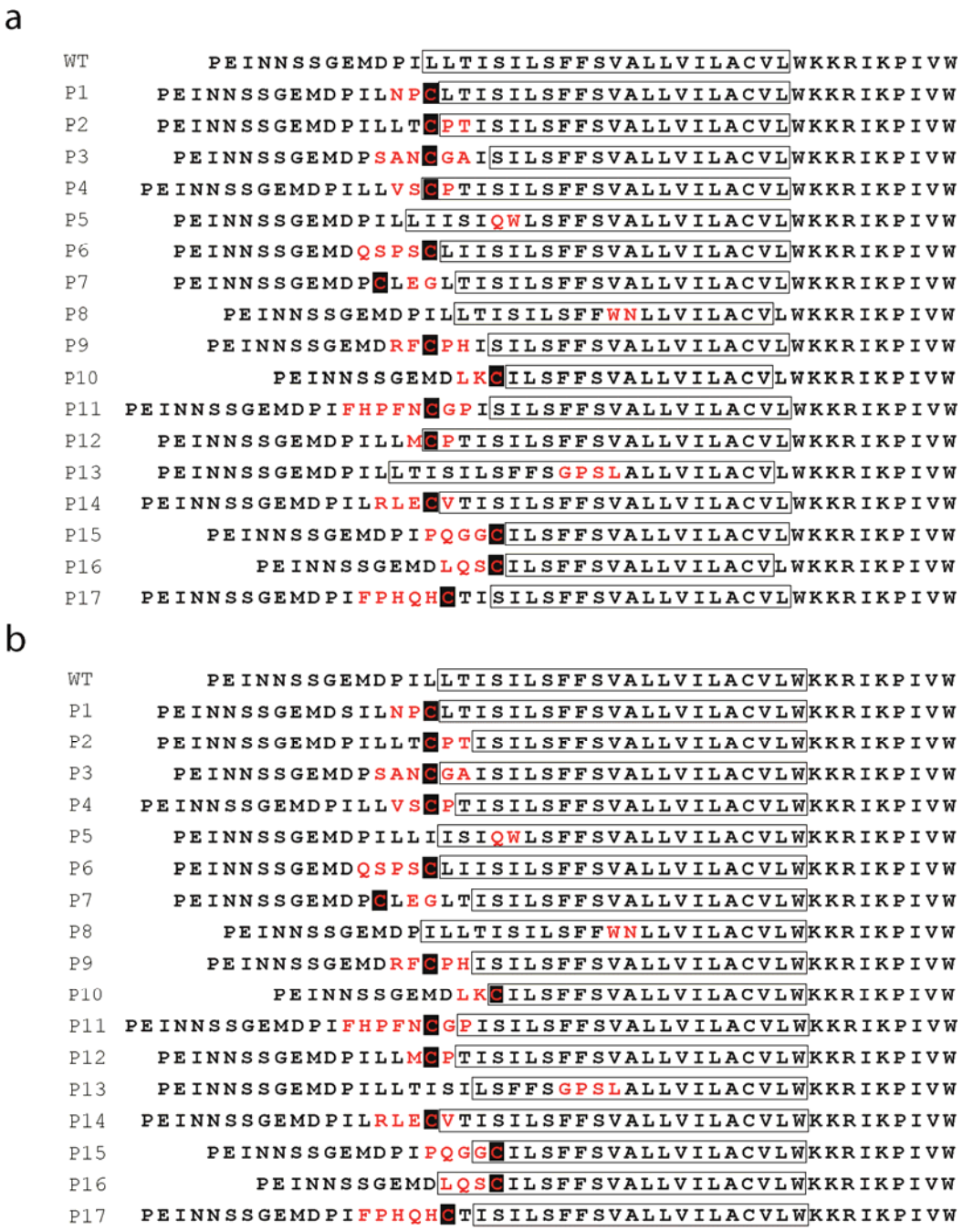
Supplementary Figures 1-19, Tables 1-2 and References

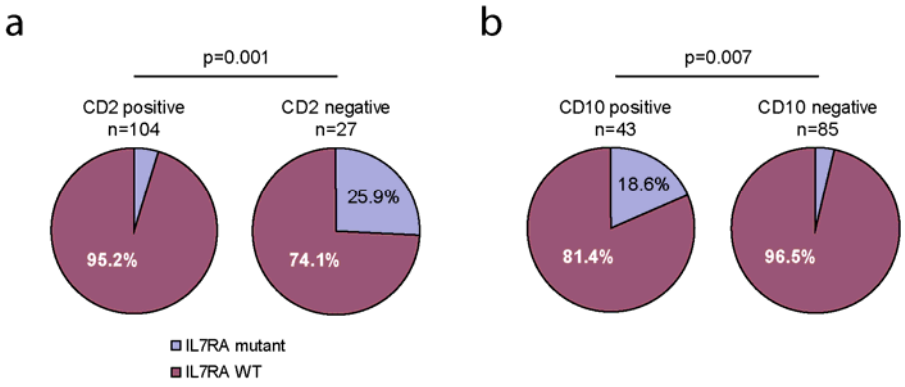
Supplementary Figures



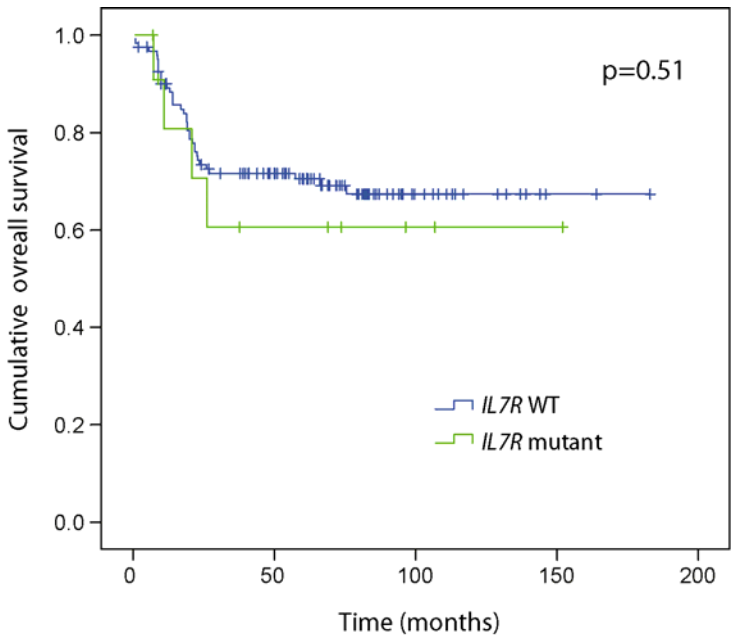
Supplementary Figure 1. *IL7R* exon 6 somatic mutations in pediatric T-ALL. DNA chromatograms of 3 patients from the Boldrini cohort showing the mutated sequences at diagnosis and lack of mutation at remission. The corresponding chromatograms of the remaining 2 patients from the same cohort are shown in Figure 1. Highlighted are the DNA and respective amino acid sequence alterations in each case. Patient P5 has a SNP (c.731C>T; p.T244I) in addition to the indicated QW mutation.



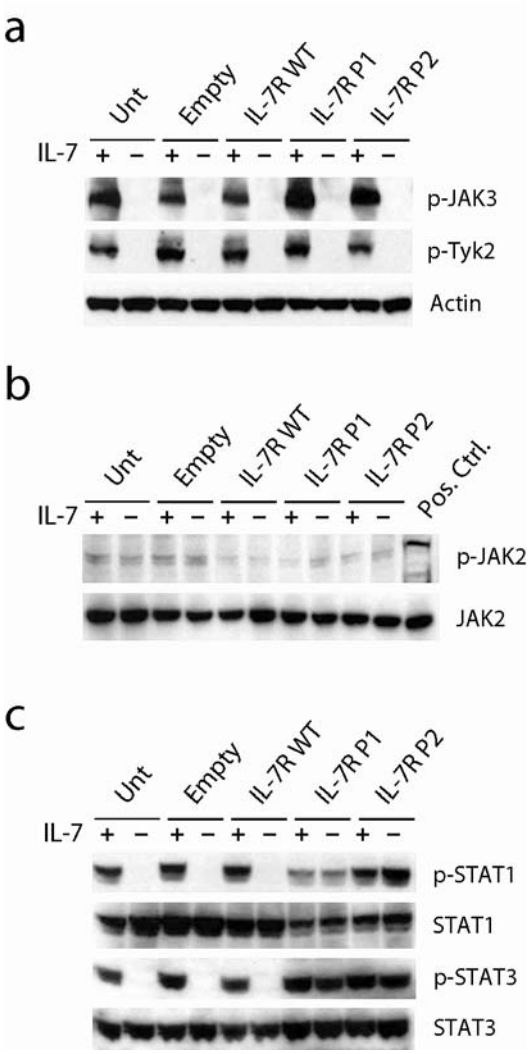
Supplementary Figure 2. The majority of unpaired cysteines created by *IL7R* mutations are predicted to localize extracellularly at the juxtamembrane-transmembrane domain interface. Prediction of core transmembrane helices (TMH, boxed) and localization of mutated amino acids (red), using two distinct bioinformatics tools. **(a)** According to DAS [1] using TM-Library size of 32, the cysteine (cyan) is located extracellularly in 12 patients (6 at the TMH border) and at the extracellular border within the TMH in 2 patients. **(b)** According to TMPRED [2] the cysteine is located extracellularly in 11 patients (4 at the TMD border) and within the TMD in 3 patients (with 1 at the extracellular border).



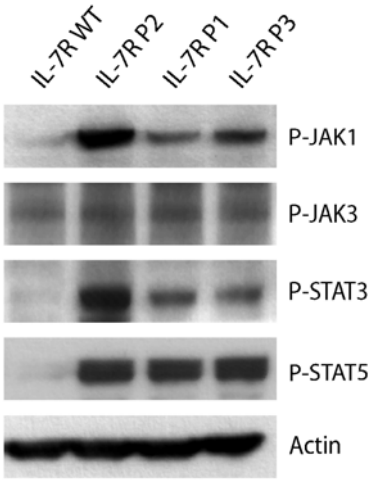
Supplementary Figure 3. Association of *IL7R* mutations with CD2 and CD10. Distribution of *IL7R* mutations among **(a)** CD2 and **(b)** CD10 positive *versus* negative patient samples. P values were calculated using Fisher's exact test.



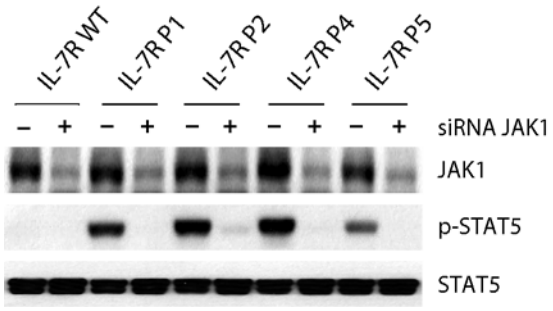
Supplementary Figure 4. Overall survival in pediatric T-ALL cases with and without *IL7R* mutation. Kaplan-Meier survival curve in pediatric T-ALL cases with (*IL7R* mutant, n=12) and without (*IL7R* WT, n=123) mutations in *IL7R* treated on DCOG ALL-7/-8 and -9 (n=66) and COALL-97 protocols (n=69). P value was calculated using Log-Rank test.



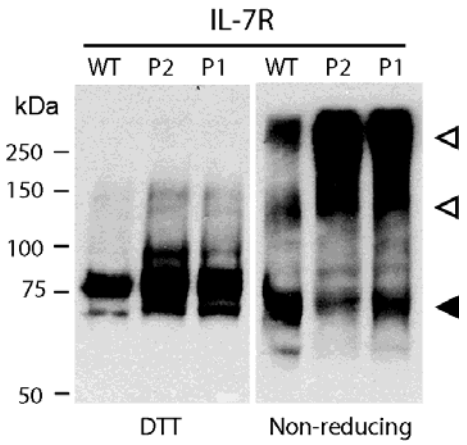
Supplementary Figure 5. Further characterization of JAK/STAT pathway activation in IL-7- versus mutant IL7R-dependent signaling in D1 cells. D1 cells expressing human WT or mutated (P1 and P2) IL-7Rα were cultured without IL-7 for 4 hr, stimulated or not with IL-7 for 20 min and evaluated by immunoblot for phosphorylation of **(a)** JAK3 and TYK2, **(b)** JAK2, and **(c)** STAT1 and STAT3. Whereas *IL7R* mutations induce constitutive phosphorylation of STAT1 and STAT3, they do not drive TYK2, JAK2 or JAK3 phosphorylation.



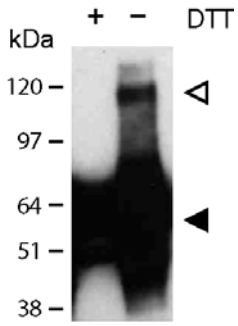
Supplementary Figure 6. *IL7R* mutations induce JAK1, STAT3 and STAT5, but not JAK3, phosphorylation in Ba/F3 cells. Ba/F3 cells expressing human WT or mutated (P1 and P2) IL-7Rα were cultured without IL-3 for 4 hr and evaluated by immunoblot for phosphorylation of the indicated proteins.



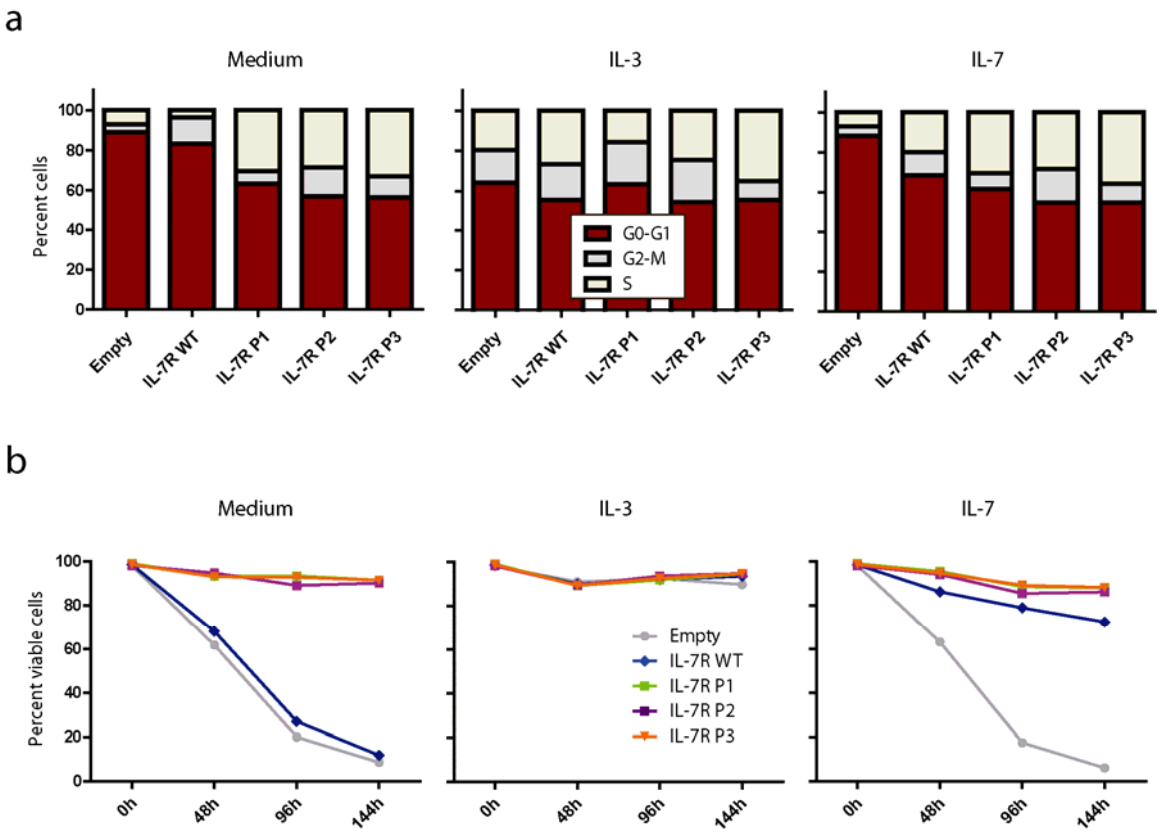
Supplementary Figure 7. JAK1 knockdown abrogates mutant *IL7R*-mediated signaling. 293T cells were transfected with WT or the indicated mutant IL-7Rα together with siRNA against JAK1 (+) or control non-targeting siRNA (-) and evaluated after 36 hr for JAK1 expression and STAT5 phosphorylation. This blot further illustrates that P5, which displays a mutation not introducing a *de novo* cysteine (T244I, I247_L248insQW; Table 1) is able to promote some degree of constitutive STAT5 phosphorylation, which is also sensitive to JAK1 knockdown.



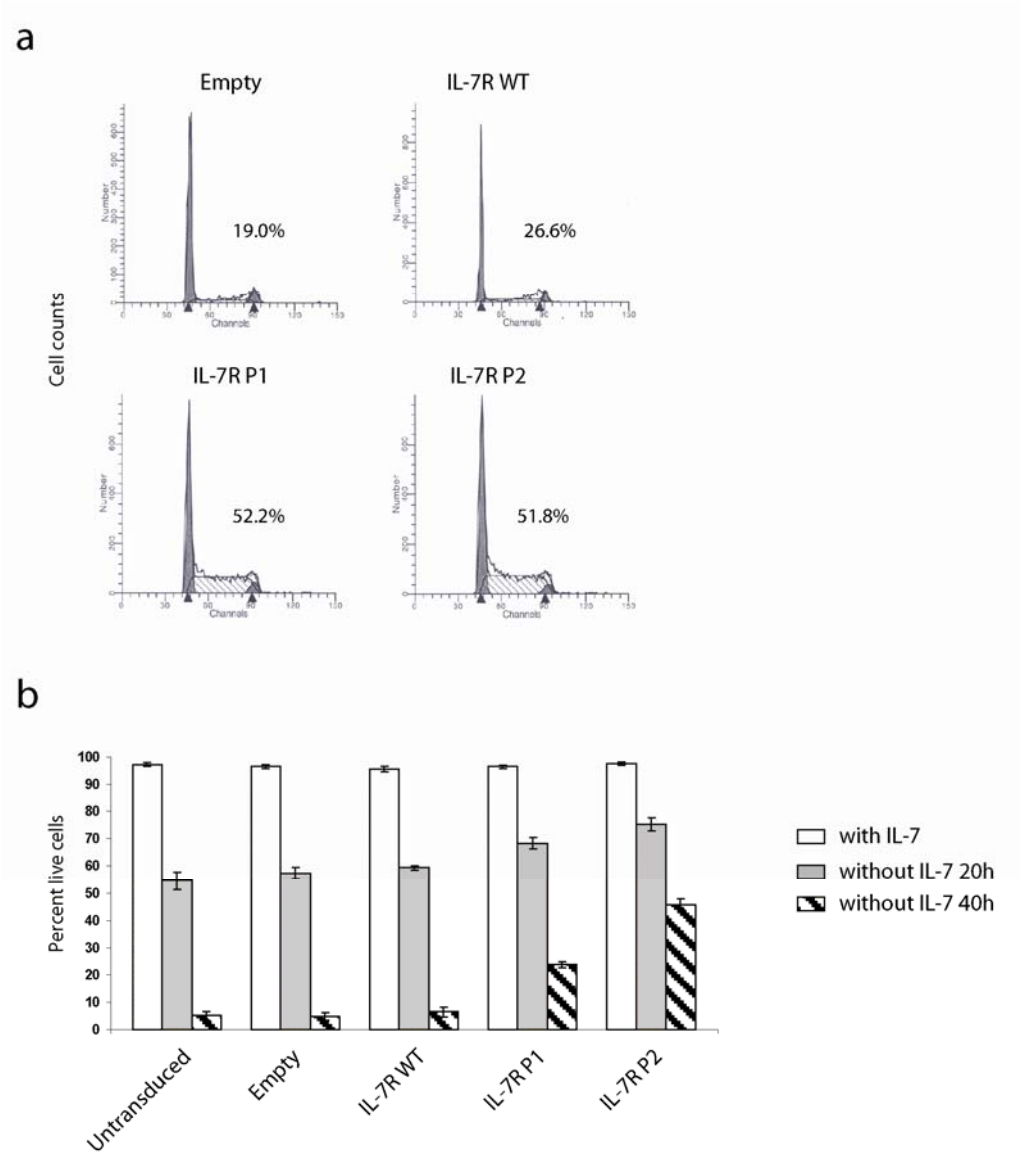
Supplementary Figure 8. IL-7Rα mutant, but not wild type, proteins constitutively form redox-sensitive dimers/oligomers in 293T cells. Lysates from 293T cells expressing wild type (WT) or mutant (P1 and P2) IL-7Rα were treated or not with the reducing agent DTT and analyzed for IL-7Rα expression by immunoblot. The monomeric and dimeric/oligomeric forms of the receptor are denoted by black and white arrows, respectively.



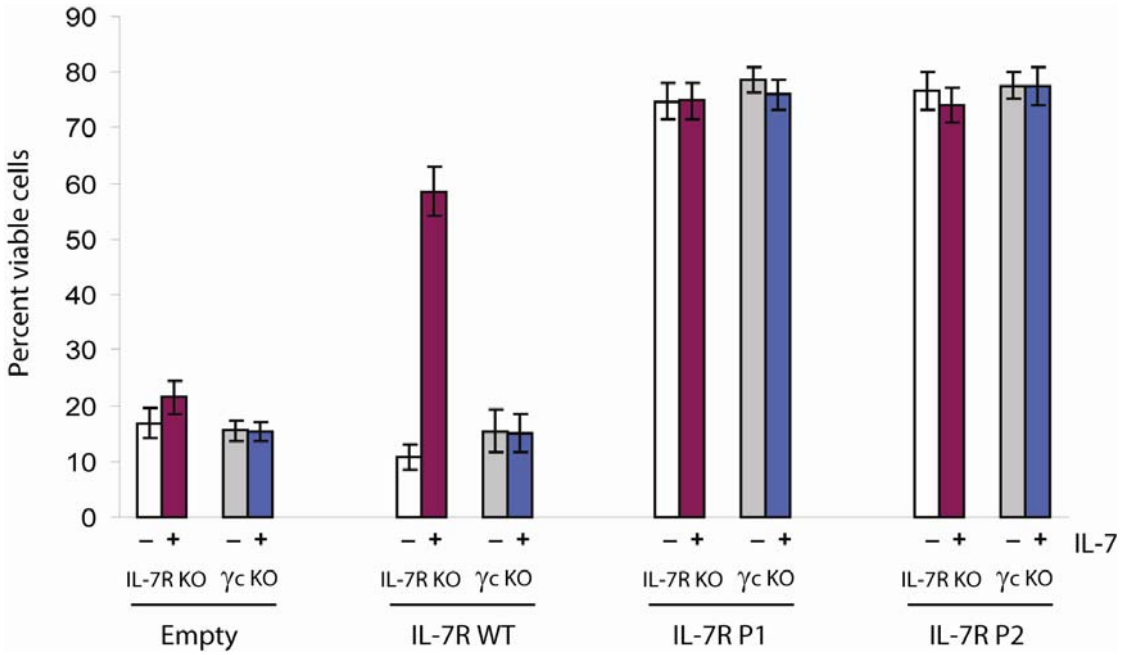
Supplementary Figure 9. IL-7Rα mutant proteins constitutively form redox-sensitive dimers/oligomers in *Il7r* -/- BM cells. Lysates from BM cells from *Il7r* -/- mice transduced with P1 mutant *IL7R* were treated or not with the reducing agent DTT and analyzed for IL-7Rα expression by immunoblot. The monomeric and dimeric forms of the receptor are denoted by black and white arrows, respectively.



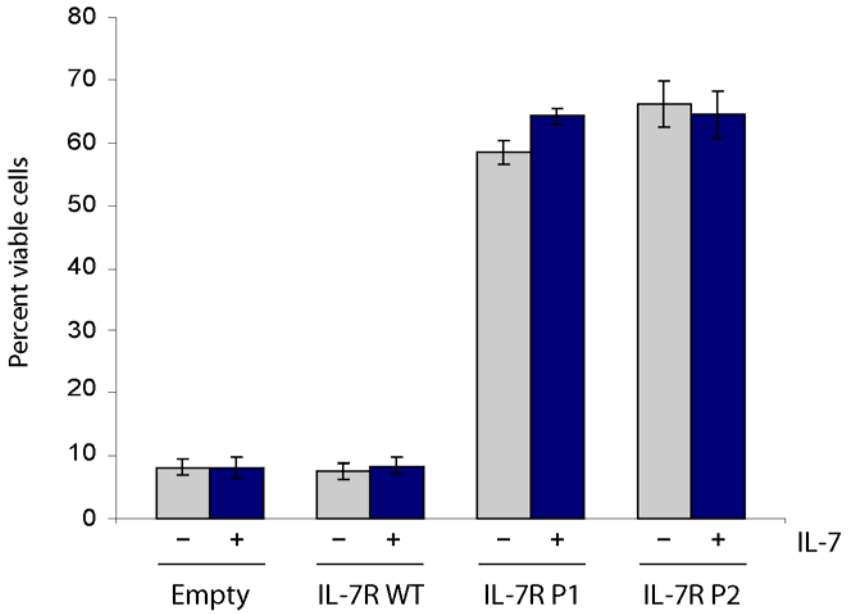
Supplementary Figure 10. *IL7R* mutations induce cell cycle progression and viability in Ba/F3 cells independently of IL-3 or IL-7. Ba/F3 cells stably expressing WT or mutated IL-7Rα were cultured in the absence of growth factors or with IL-3 or IL-7. **(a)** Cell cycle was determined at 96h. **(b)** Viability was evaluated by Annexin V/ 7-AAD staining at the indicated time points. Data represent average of triplicates ± sem.



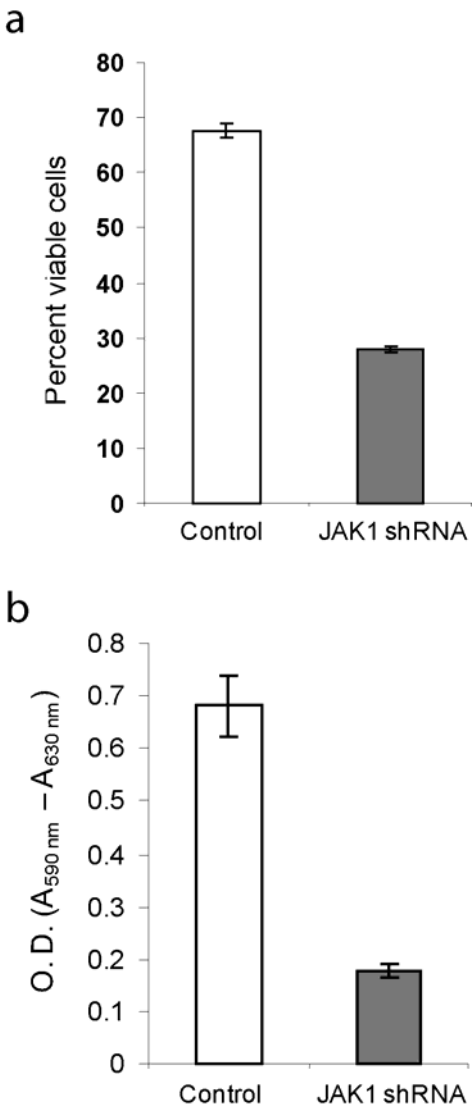
Supplementary Figure 11. *IL7R* mutations induce cell cycle progression and viability in D1 cells independently of IL-7. (a) D1 cells transduced with empty pMIG vector (Empty), IL-7Rα wild type (WT), IL-7Rα P1 or IL-7Rα P2 were analyzed for cell cycle distribution after 24h of IL-7 deprivation. Percentage of cells in cycle (S+G2/M) is indicated for each condition. **(b)** Viability of D1 cells transduced with empty pMIG vector (Empty), IL-7Rα WT, IL-7Rα P1 or IL-7Rα P2 was assessed after 20h and 40h of IL-7 deprivation. D1 cells cultured in the presence of IL-7 are shown as positive controls. Data represent average of triplicates ± sem.



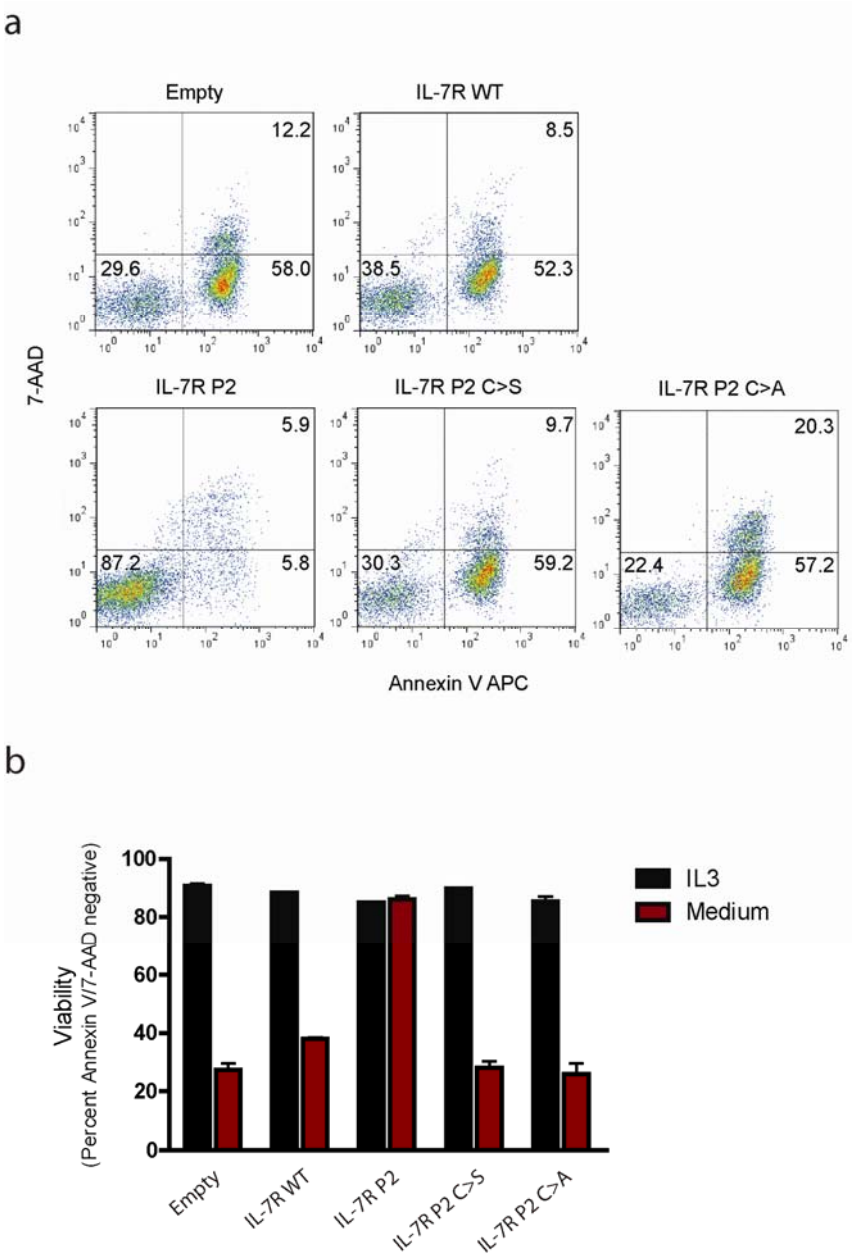
Supplementary Figure 12. *IL7R* mutations induce cell viability independently of γc expression. Bone marrow cells from *Il7r* -/- (IL-7R KO) or *Il2rg* -/- (γc KO) mice were transduced with the empty pMIG vector (Empty), *IL7R* (IL-7R) WT, *IL7R* P1 or *IL7R* P2 and cultured for 96h with or without IL-7. Viability was evaluated by Annexin V/ 7-AAD staining. Data represent average of triplicates ± sem.



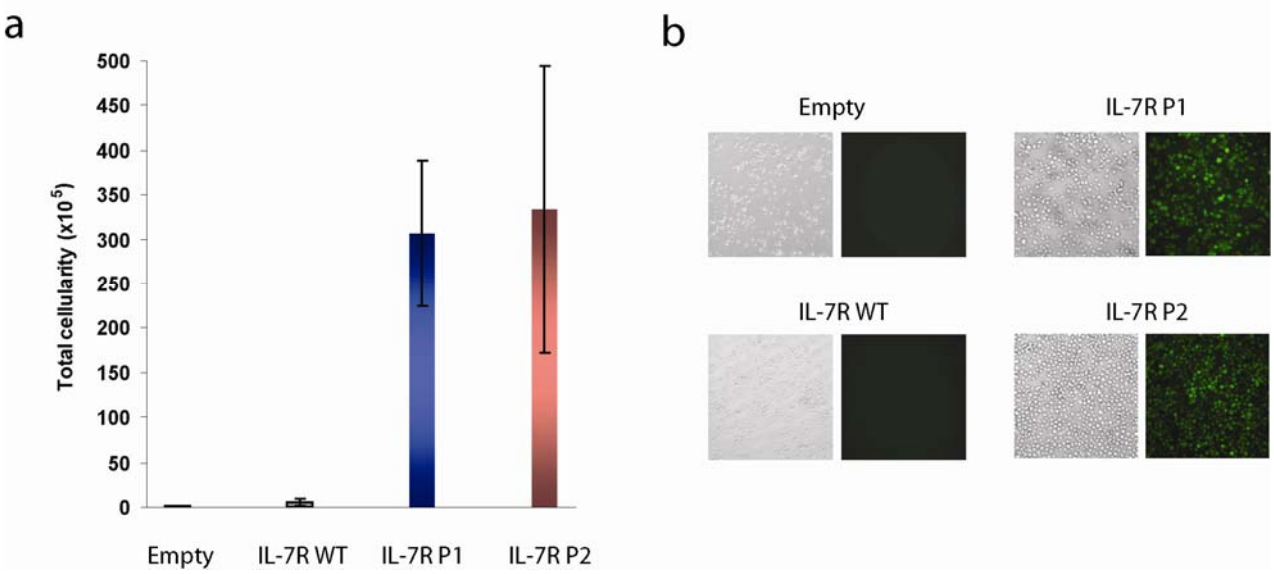
Supplementary Figure 13. *IL7R* mutations induce cell viability independently of JAK3 expression. Bone marrow cells from *Jak3* -/- mice were transduced with the empty pMIG vector (Empty), *IL7R* (IL-7R) WT, *IL7R* P1 or *IL7R* P2 and cultured for 96h with or without IL-7. Viability was evaluated by Annexin V/ 7-AAD staining. Mean ± sem is indicated for triplicates of each condition.



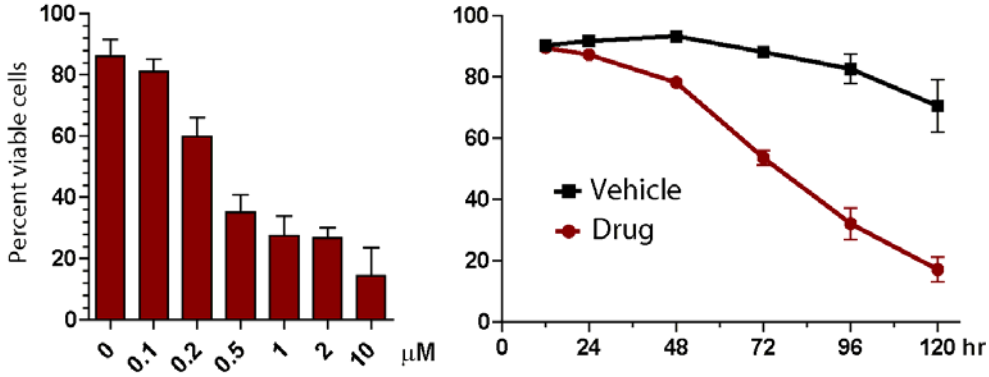
Supplementary Figure 14. *IL7R* mutations induce cell viability and proliferation in a JAK1-dependent manner. D1 cells expressing P1 mutant IL-7Rα were transduced with *Jak1* or control shRNAs. After 24 hr of infection, cells were cultured with IL-7 (50 ng/ml) and puromycin (5 μg/ml) for 48 hr, and then cultured in medium alone for 48 hr. Viability/Proliferation was analyzed by Annexin V/7-AAD staining **(a)** and by an MTT assay **(b)**. Mean ± sem is indicated for triplicates of each condition.



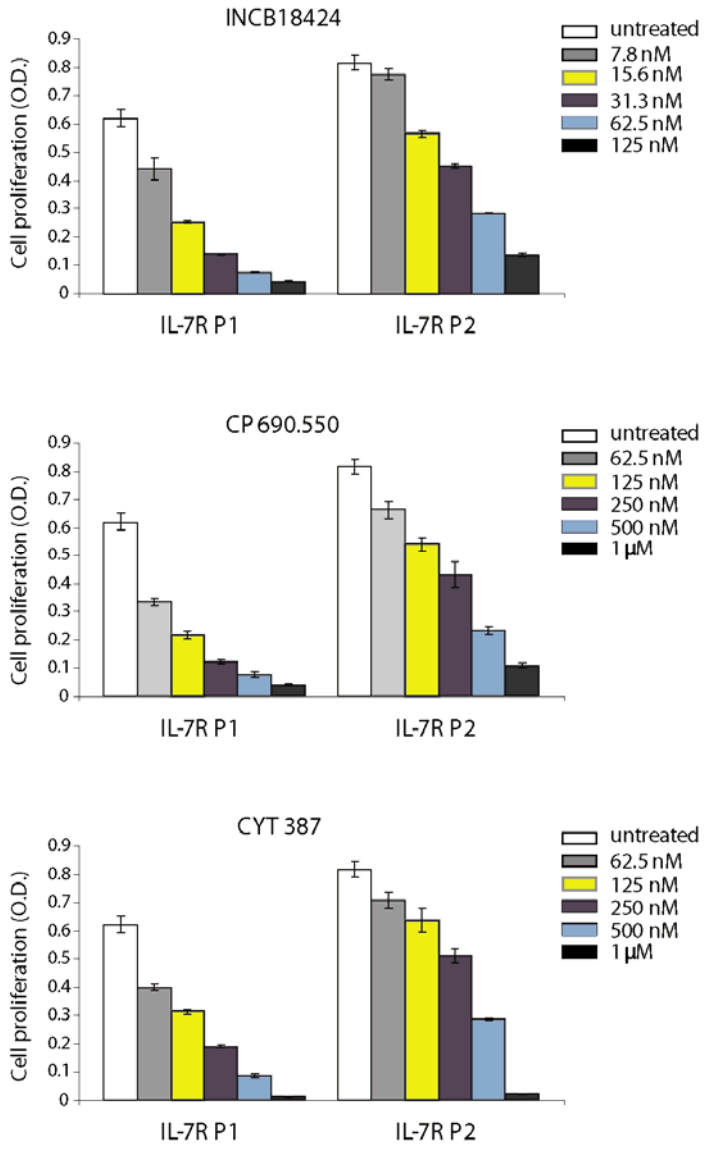
Supplementary Figure 15. Cell viability promoted by *IL7R* mutations depends on the presence of the cysteine introduced *de novo*. Ba/F3 cells stably transduced with pMIG vector (Empty), IL-7Rα WT, or IL-7Rα P2 or the indicated P2 cysteine mutants were cultured in cytokine-deprived medium or in the presence of IL-3, and analyzed for cell viability at 48h. Viability was evaluated by Annexin V/ 7-AAD staining. **(a)** Representative dot plots of cells cultured in the absence of cytokines. **(b)** Mean ± sem of duplicates of each condition.



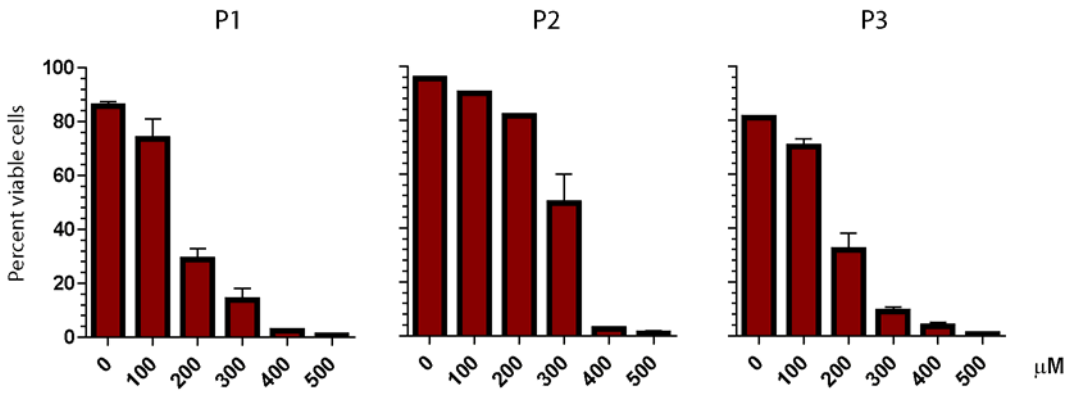
Supplementary Figure 16. Lymph node and liver infiltration in *Rag1* -/- mice subcutaneously injected with D1 cells. (a) Lymph node cellularity and **(b)** representative phase contrast and fluorescence images of lymph nodes from *Rag1* -/- mice 20 days post subcutaneous transplantation of D1 cells. Mean ± sem is indicated for triplicates of each condition.



Supplementary Figure 17. JAK inhibition reduces cell viability of P2 mutant IL-7Rα-expressing Ba/F3 cells. Ba/F3 cells stably expressing mutant IL-7Rα P2 were cultured in medium alone with or without JAK inhibitor I at the indicated doses for 72 hr (left) or for the indicated culture periods at 1μM (right) and analyzed for cell viability by flow cytometry. Mean ± sem is indicated for triplicates of each condition.



Supplementary Figure 18. JAK pharmacological inhibitors in clinical use reduce cell viability/proliferation of mutant IL-7Rα-expressing D1 cells. D1 cells stably expressing mutant IL-7Rα were cultured in medium deprived of IL-7 with or without the indicated clinically-relevant JAK inhibitors and viability/proliferation (shown as O.D.) was determined at 48 hr using an MTT assay. Mean ± sem is indicated for triplicates of each condition.



Supplementary Figure 19. Dose-dependent cell death induced by STAT5 inhibition in P1, P2 and P3 mutant IL-7Rα-expressing Ba/F3 cells. Ba/F3 cells stably expressing the indicated mutant IL-7Rα were cultured in medium alone with or without STAT5 inhibitor at the indicated concentrations for 72 hr and analyzed for cell viability by flow cytometry. Mean ± sem is indicated for triplicates of each condition.

Supplementary Table 1. Differentially expressed genes in IL7R mutant versus IL7R wild type pediatric T-ALLs (LIMMA analysis; cut-off FDR p-value=0.05).

Gene Title	Gene Symbol	ID	P.Value	adj.P.Val	logFC
similar to hypothetical protein MGC42630 /// hypothetical protein MGC42630 /// hypothetical LOC504188	LOC158318 /// MGC42630 /// LOC504188	1553590_at	3,77E-09	0,000103	1,284223
proprotein convertase subtilisin/kexin type 6	PCSK6	207414_s_at	3,54E-09	0,000103	1,435284
---	---	220701_at	2,07E-08	0,000378	-0,86895
carboxypeptidase A6	CPA6	1552511_a_at	1,04E-07	0,001417	1,247438
protein kinase (cAMP-dependent, catalytic) inhibitor alpha	PKIA	204612_at	1,62E-07	0,001478	-1,70916
interleukin 17D	IL17D	227401_at	1,38E-07	0,001478	-1,51526
dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	DPP4	203716_s_at	2,35E-07	0,001839	2,149853
BAI1-associated protein 2	BAIAP2	1556145_a_at	3,57E-07	0,002443	0,85672
Aryl hydrocarbon receptor	AHR	1559035_a_at	4,21E-07	0,002557	1,263743
hypothetical protein LOC253982	LOC253982	214993_at	6,09E-07	0,003328	0,657823
chromosome 2 open reading frame 23	C2orf23	204365_s_at	7,23E-07	0,003592	-0,77833
toll-interleukin 1 receptor (TIR) domain containing adaptor protein	TIRAP	1554091_a_at	1,34E-06	0,005045	-1,07611
tight junction protein 3 (zona occludens 3)	TJP3	213412_at	1,24E-06	0,005045	1,056165
hypothetical protein FLJ13841	FLJ13841	219995_s_at	1,38E-06	0,005045	1,495932
olfactory receptor, family 51, subfamily B, member 5	OR51B5	234775_at	1,14E-06	0,005045	-0,86369
suppressor of cytokine signaling 2	SOCS2	203372_s_at	1,96E-06	0,006691	2,636933
CDNA clone IMAGE:5265747	---	1555994_at	3,03E-06	0,009742	0,923981
F-box and leucine-rich repeat protein 16	FBXL16	227641_at	3,73E-06	0,011327	-1,11153
chromosome 1 open reading frame 142	C1orf142	230810_at	4,01E-06	0,011552	-0,71443
dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	DPP4	211478_s_at	4,52E-06	0,012143	2,123167
dehydrogenase/reductase (SDR family) member 9	DHRS9	219799_s_at	4,66E-06	0,012143	-0,69388
suppressor of cytokine signaling 2	SOCS2	203373_at	5,33E-06	0,01317	2,454919
EPH receptor B2	EPHB2	234158_at	5,54E-06	0,01317	0,806845
spondin 1, extracellular matrix protein	SPON1	209436_at	8,37E-06	0,018298	1,19454
Acyl-Coenzyme A oxidase 3, pristanoyl	ACOX3	243817_at	8,07E-06	0,018298	-0,77482
spondin 1, extracellular matrix protein	SPON1	213994_s_at	9,16E-06	0,019257	1,870146
Xg blood group (pseudoautosomal boundary-divided on the X chromosome)	XG	1563420_at	1,23E-05	0,021793	1,451362
spondin 1, extracellular matrix protein	SPON1	209437_s_at	1,15E-05	0,021793	1,246723
Protein kinase C, epsilon	PRKCE	216753_at	1,24E-05	0,021793	-0,81188
hypothetical protein MGC42630	MGC42630	227563_at	1,23E-05	0,021793	0,952581
zinc finger protein 335	ZNF335	78330_at	1,18E-05	0,021793	-0,95327
hypothetical gene supported by AK091454	LOC285382	242447_at	1,37E-05	0,023389	-0,8158
Ryanodine receptor 3	RYR3	241901_at	1,52E-05	0,025124	-0,944
v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	ETS2	201329_s_at	1,57E-05	0,025297	1,003391
LIM homeobox 6	LHX6	224556_s_at	1,62E-05	0,025355	0,707453
growth arrest-specific 2	GAS2	205848_at	1,7E-05	0,02579	2,442036
SH3-domain GRB2-like 3	---	230959_at	1,84E-05	0,027152	1,09072
Death-associated protein kinase 1	DAPK1	237409_at	2,1E-05	0,030229	1,255728
family with sequence similarity 49, member A /// family with sequence similarity 49, member A	FAM49A	208092_s_at	2,22E-05	0,03109	-0,89989
hypothetical gene supported by BC028053	LOC440569	1569386_at	2,34E-05	0,03183	0,901727
cysteinyl leukotriene receptor 2	CYSLTR2	220813_at	2,5E-05	0,03183	1,224922
protocadherin beta 13	PCDHB13	221450_x_at	2,41E-05	0,03183	0,676771
Adaptor-related protein complex 4, epsilon 1 subunit	AP4E1	241174_at	2,46E-05	0,03183	-0,90015
syndecan binding protein (syntenin) 2	SDCBP2	233565_s_at	2,62E-05	0,032525	-0,83116
src family associated phosphoprotein 1	SCAP1	205790_at	2,74E-05	0,032776	-1,33791
homeo box A9	HOXA9	209905_at	2,82E-05	0,032776	2,043246
Rho GTPase activating protein 10	ARHGAP10	239567_at	2,78E-05	0,032776	-1,09894
Rho GTPase-activating protein	RICS	203431_s_at	2,9E-05	0,033081	-1,34478
enhancer of zeste homolog 2 (Drosophila)	EZH2	203358_s_at	3,19E-05	0,034046	-0,65741
defensin, alpha 6, Paneth cell-specific	DEFA6	207814_at	3,3E-05	0,034046	1,288429
chromosome 1 open reading frame 105	C1orf105	214357_at	3,17E-05	0,034046	-1,08944
brain and acute leukemia, cytoplasmic	BAALC	218899_s_at	3,24E-05	0,034046	1,652154
chromosome 1 open reading frame 116	C1orf116	219856_at	3,17E-05	0,034046	0,519867
Potassium voltage-gated channel, KQT-like subfamily, member 1	KCNQ1OT1	237249_at	3,36E-05	0,034049	0,77784
olfactory receptor, family 5, subfamily U, member 1	OR5U1	234545_at	3,57E-05	0,035492	-0,88816
hypothetical gene LOC133874	LOC133874	1554115_at	3,76E-05	0,036728	-0,59776
spondin 1, extracellular matrix protein	SPON1	213993_at	3,88E-05	0,036764	1,5679
chromosome 1 open reading frame 165	C1orf165	219670_at	3,97E-05	0,036764	-0,95235
Hypothetical protein LOC441168	LOC441168	228362_s_at	4,02E-05	0,036764	0,930994
Solute carrier family 35, member F3	SLC35F3	231520_at	4,03E-05	0,036764	0,83601
Protein kinase (cAMP-dependent, catalytic) inhibitor alpha	PKIA	1563217_at	4,17E-05	0,037264	-1,61645
myosin, heavy polypeptide 14	MYH14	217660_at	4,23E-05	0,037264	0,906789
CDNA clone IMAGE:4828909	---	1563283_at	4,32E-05	0,037511	-1,00588
down-regulated in gastric cancer GDDR	GDDR	238222_at	4,48E-05	0,038288	-0,75619
v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	SRC	1558211_s_at	4,58E-05	0,038522	-0,7583
EPH receptor B2	EPHB2	210651_s_at	4,67E-05	0,038692	-0,68255
CDNA clone IMAGE:4694535	---	1564760_at	4,79E-05	0,039111	-0,8968
Chromosome 2 open reading frame 27	C2orf27	230336_at	4,9E-05	0,039372	1,039966
Vacuolar protein sorting 13A (yeast)	VPS13A	1570295_at	4,99E-05	0,039466	-0,87781
ethanolamine kinase 2	ETNK2	219268_at	5,05E-05	0,039466	-0,76813
uronyl-2-sulfotransferase	UST	205138_s_at	5,16E-05	0,039748	0,855043
hypothetical protein LOC144481	LOC144481	1559315_s_at	5,42E-05	0,040594	1,169696
thioesterase domain containing 1	THEDC1	222945_x_at	5,36E-05	0,040594	-1,00074
UTP15, U3 small nucleolar ribonucleoprotein, homolog (yeast)	FLJ12787	221038_at	5,56E-05	0,041075	-0,9038
Similar to ZNF43 protein	---	1565748_at	5,74E-05	0,041867	-0,69271
SLAM family member 6	SLAMF6	1552497_a_at	6,07E-05	0,043684	-0,6851
Four and a half LIM domains 2	FHL2	1557274_at	6,62E-05	0,046982	-0,70083
multimerin 2	MMRN2	219091_s_at	6,8E-05	0,047662	-0,67255
hypothetical gene supported by AK091527	FLJ34208	1566761_a_at	7,11E-05	0,048614	-0,64129
Amyloid beta (A4) precursor-like protein 2	APLP2	208701_at	7,05E-05	0,048614	-0,91316

Supplementary Table 2. Primers used for *IL7R*, *JAK1* (JH2), and *JAK3* (JH2) RT-PCR, sequencing and cloning.

Primer	Sequence (5' →3')	5'-3' position (size)	Fragment size
IL7R_exF	CCCTCCCTTCCTCTTACTCTCA	13 – 34 (22) [a]	589 bp
IL7R_exR	TGGCGGTAAGCTACATCGTG	601 – 582 (20) [a]	
IL7R_trF	AGCCAATGACTTTGTGGTGAC	515 – 535 (21) [a]	606 bp
IL7R_trR	ACATCCCCTCCAAGCCTCT	1120 – 1102 (19) [a]	
IL7R_inF	CAGAGGCTTGGAGGGGATGT	1101 – 1120 (20) [a]	416 bp
IL7R_inR	AATCATCTTTGTCGCTCACGGT	1516 – 1495 (22) [a]	
IL7R_hapF	CACTCACTGACCTGTGCTTTT	246 – 266 (21) [a]	673bp
IL7R_hapR	GGAGACTGGGCCATACGATA	918 – 899 (20) [a]	
IL7R_ex8F	TCCTATCTTACTAACCATCAGCATTT	806 – 831 (26) [a]	788bp
IL7R_ex8R	GACTGTGTAGTGGGGTTTTGCT	1593 – 1572 (22) [a]	
Jak1JH2_aF	AGGAGTGGCAGCCCGTCTA	1934 – 1952 (19) [a]	485 bp
Jak1JH2_aR	GGCCAGGAGGAGGTTTTTAGT	2418 – 2398 (21) [a]	
Jak1JH2_bF	AATTCAAAGTTGCCAAACAGCT	2321 – 2342 (22) [a]	527 bp
Jak1JH2_bR	GTCCACTTCAGTTGCTGGTTTT	2847 – 2826 (22) [a]	
Jak3JH2_aF	CGTAGATGGGGTGGCAGTG	1489 – 1507 (19) [a]	526 bp
Jak3JH2_aR	CAGATAGTTGAGGGCGTAGGC	2014 – 1994 (21) [a]	
Jak3JH2_bF	GCCTACGCCCTCAACTATCTG	1994 – 2014 (21) [a]	523 bp
Jak3JH2_bR	CACCATTCCACAGCCCATC	2516 – 2498 (19) [a]	
IL7R_exon6F	CAAAGCACCCTGAGACCCTAC	17400 – 17420 (21) [b]	278bp
IL7R_exon6R	TTCGTGAAATGCCTTAATCCC	17667 – 17657 (21) [b]	
IL7R 3U32	GTGGTACCCTCCCTCCCTTCCTCTTACTCTCA	(32) [c]	1470bp
IL7R 1434L39	GGGCCCGGGGTTTTGGTAGAAGCTGGACATGGTGACATA	(39) [c]	
hIL7R5'BglII	CGTACAGATCTCCCTTCCTCTTACTCTCA	(29) [c]	1476bp
hIL7R3'EcoRI	TACGGAATTCTAGCCGGGGTTTTGGTA	(27) [c]	
hIL7R_cP1s	AGATGGATCCTATCTTAAACCCAaGCCTAACCATCAGCAT	799 – 829 (40) [c]	151bp
hIL7R_cP2s	AGATGGATCCTATCTTACTAACTTcTCCCACCATCAGCATTTT	799 - 832 (43) [c]	
hIL7R_cP2a	AGATGGATCCTATCTTACTAACTgcTCCCACCATCAGCATTT	799 – 831 (42)	
hIL7R_BbsI	CAAAGATGTTCCAGAGTCTTCTTATGATCGGGGAGACTG	949 – 911 (39)	

[a] Positions according to NCBI reference of coding sequences NM_002185.2 (*IL7R*), NM_002227.2 (*JAK1*), NM_000215.3 (*JAK3*).
[b] Positions refer to flanking intronic regions of exon 6 according to NCBI reference of genomic sequence NT_006576.16
[c] The cloning restriction sites are underlined. Nucleotide changes for site-directed mutagenesis are noted in small caps. Positions refer to hybridization in the coding sequences of NM_002185.2 (*IL7R*).

Supplementary References

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2. Hofmann, K. & Stoffel, W. TMbase: A database of membrane spanning proteins segments. *Biol Chem Hoppe-Seyler* **374**, 166 (1993).