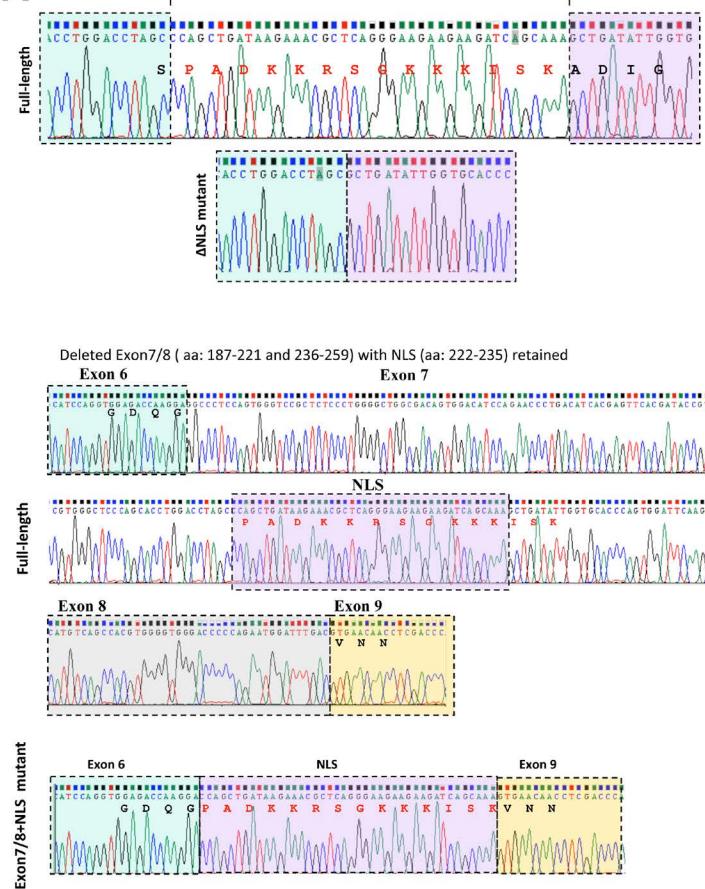
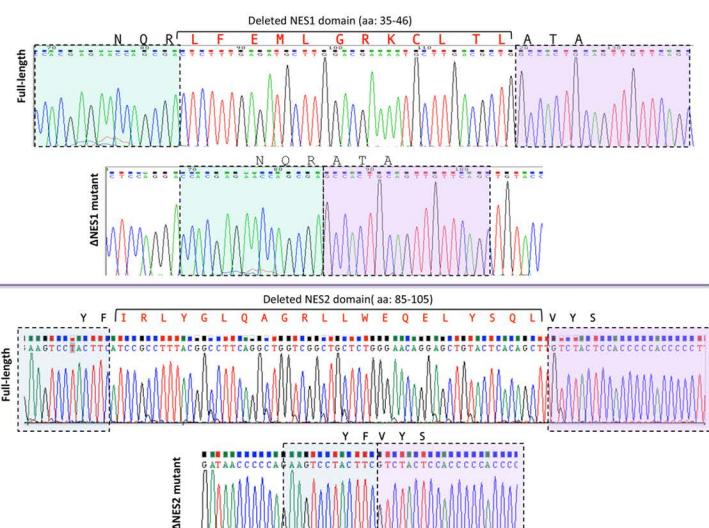


Supplementary Fig. S1

A



B



C

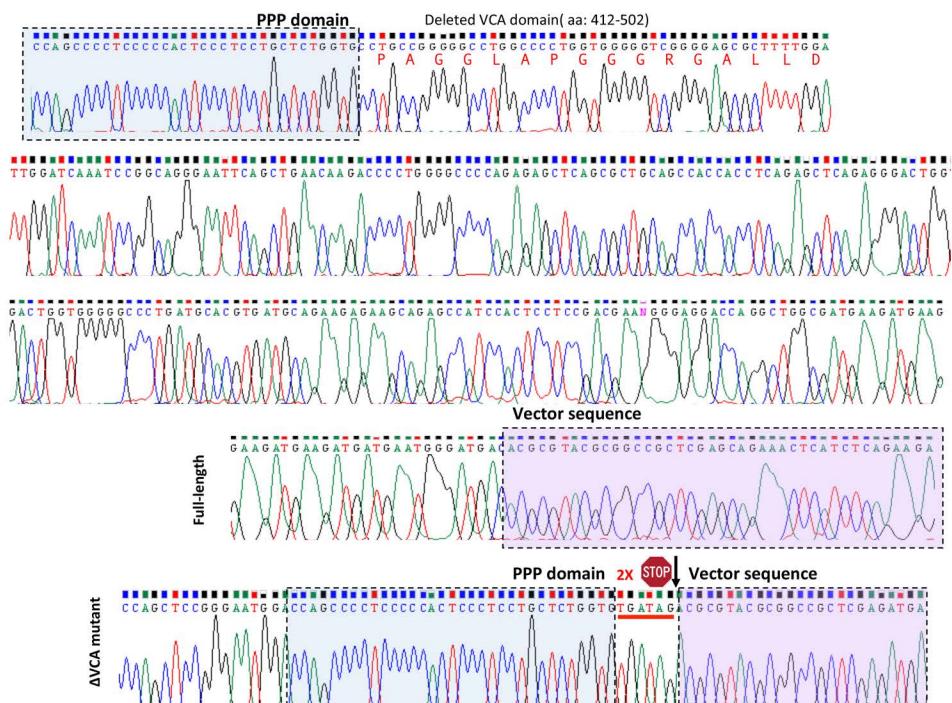
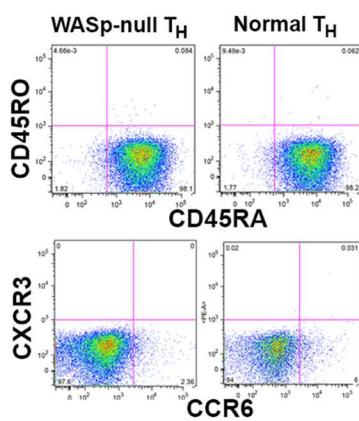
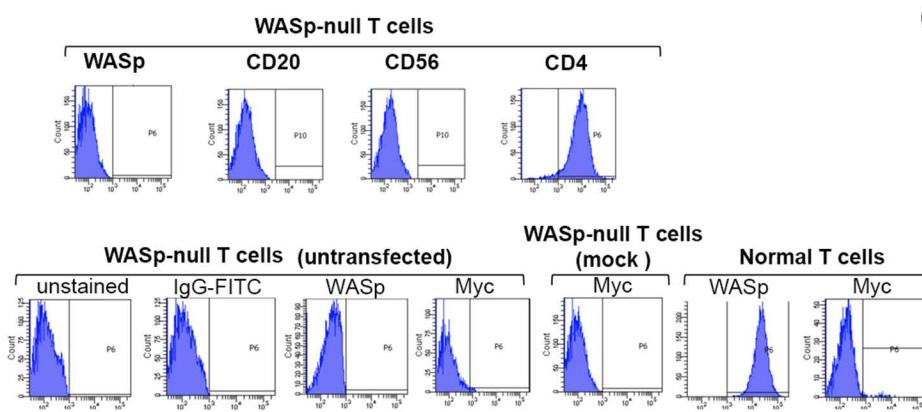
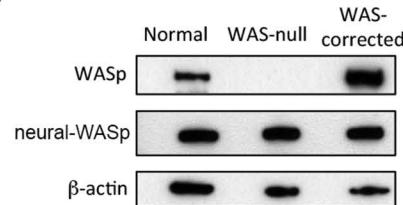
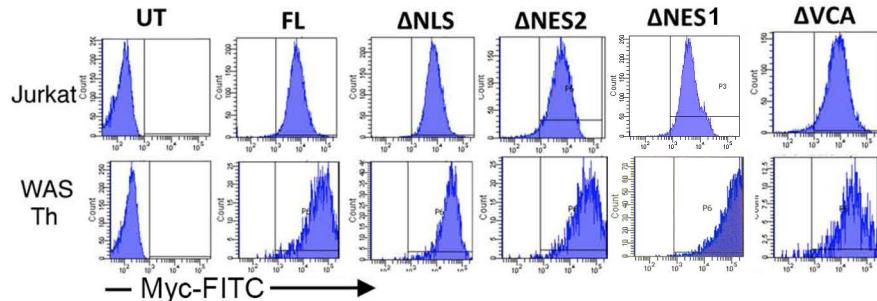
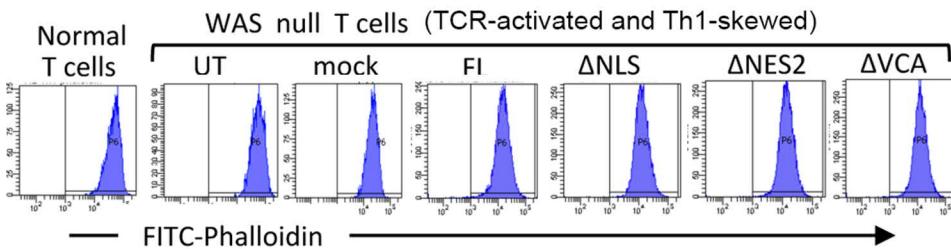


Figure S1. Chromogram of domain-deleted WASp mutants.

DNA sequence profile of WASp mutant lacking the NLS domain or exon7/8 domains (panel A), lacking the NES1 or NES2 domains (panel B) or lacking the VCA domain (panel C) are shown along with the corresponding sequences of full length WASp.

A**B****C****D**

WAS null T cells (TCR-activated and Th1-skewed)

**E****F**

MNase-digested chromatin

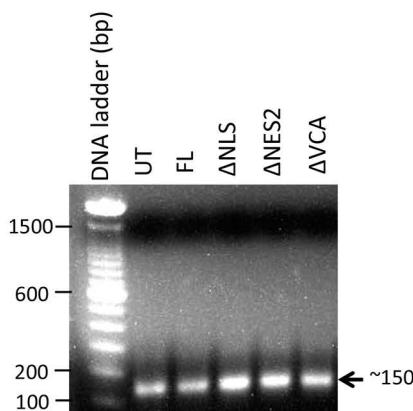


Figure S2. **(A and B)** Flow cytometry depicting differentiation status, phenotypic characteristics, and expression of WASp in the T_H cell line isolated from a WAS patient (WAS^{null}) and normal T_H control cells. **(C)** Western blot of the three indicated T_H lines depicting expression of WASp and Neural-WASP (N-WASP) in the same blot. **(D)** Flow cytometry showing stable expression of full-length (FL) WASp or its indicated mutants in Jurkat (top) and WAS^{null} T_H cells (bottom) performed on day 10 of transfection (day 7 of T_H1-skewing plus CD3/28 activation). Comparable mutant expression efficiency was obtained in T_H2 skewed cells (data not shown) **(E)** Flow cytometry with Phalloidin-FITC probe showing the total cellular F-actin content in the indicated cells. **(F)** The efficiency of micrococcal nuclease (MNase) digestion of chromatin is shown for the indicated WAS- mutant expressing T cells. The data is shown for T_H1 skewed cells.

Table S1: PCR Primers and Probes**A. Site Directed mutation primers**

Primer	Sequence
ΔNES1Forward	5'- GGACCACGAGAACCGAGCGAGCCACTGCAG -3'
ΔNES1 Reverse	5'- CTGCAGTGGCTCGCTGGTCTCGTGGTCC -3'
ΔNES2 Forward	5'-CCAGAAGTCCTACTTCGTCTACTCCACCCCCA-3'
ΔNES2 Reverse	5'-TGGGGTGGAGTAGACGAAGTAGGACTCTGG-3'
ΔNES2 Forward	5'-TCCGCCTTACGGCGTCACTCCACCCC-3'
ΔNES2Reverse	5'-GGGGTGGAGTAGACGCCGTAAAGGCCGA-3'
ΔNLS Forward	5'-AGCACCTGGACCTAGCGCTAGTATTGGTGCAC-3'
ΔNLS Reverse	5'-GTGCACCAATATCAGCGCTAGGTCCAGGTGCT-3'
ΔVCA Forward	5'-GAGGCGATGCCATGAGTGGGGCCCAATGGG-3'
ΔVCA Reverse	5'-GCGACCGCGTCTATCACACCAGAGCAGGAGGGAGTGGG-3'
ΔExon7(+NLS) Exon8 Forward	5'-CATCCAGGTGGAGACCAAGGACCAAGCTGATAAGAAACGCTCAGGAAAGA AGAAGATCAGCAAAGTGAACAACCTCGACCCAGAT-3'
ΔExon7(+NLS) Exon8 Reverse	5'-ATCTGGGTCGAGGTTGTTCACTTGCTGATCTTCTTCTTCCC TGAGCGTTCTTATCAGCTGGCTTGGTCTCCACCTGGATG-3'
ΔExon7- Exon8 Forward	5'-AGGTGGAGACCAAGGAGTGAAACAACCTCGACC-3'
ΔExon7- Exon8 Reverse	5'-GGTCGAGGTTGTTCACTCCTGGTCTCCACCT-3'

B. RT-PCR Primers and Probes of the genes studied

Gene (Assay ID)	Exon Boundary	Chromosome location
IFNG (Hs00989290_g1)	Ex2-3	Chr.12: 68548550 - 68553521
TBX21 (Hs00894392_m1)	Ex3-4	Chr.17: 45810610 - 45823485
IL4 (Hs00929861_g1)	Ex1-2	Chr.5: 132009373 - 132018368
GATA3 (Hs00922328_m1)	Ex4-5	Chr.10: 8096667 - 8117164
GAPDH (Hs00266705_g1)	Ex2-3	Chr.12: 6643657 - 6647536
CSF2 (Hs00929873_m1)	Ex2-3	Chr.5: 131409485 - 131411859

C. 5' end qPCR Primers and Probes of different gene locus based on NCBI36/hg18 (Mar. 2006)

Primer/ Probes	Sequence
TBX21_GAS Forward	5'-GATGAGGCACGTGAGGTTGA -3'
TBX21_GAS Reverse	5'-GGTATATGTGACCCAGGCAAGTC -3'
TBX21_GAS Probe	5'-6FAM-TTTCAGGCAAGGAAAA-3'

Intergenic Forward	5'-GATCTGCACTTGGCTTGCA-3'
Intergenic Reverse	5'-GTCTCACGGTGCCTACTAGGT-3'
Intergenic Probe	5'-6FAM-TGCCCTCCTCGGG-3'
IFNG5 Forward	5'- TCGCCCTGGTAAAATGTTGAC-3'
IFNG5 Reverse	5'- CCAACCACAAGCAAATGATCA-3'
IFNG5 Probe	5'-6FAM- CTTCATTCAACAAAGCAC-3'
GMCSF Forward	5'-GTGGGCTGTCGGTTCTT-3'
GMCSF Reverse	5'-GGAATCTCCTGGCCCTTATC-3'
GMCSF Probe	5'/56-FAM/AAAGGCTCA/ZEN/CCGTTCCCATGTGT/3IABkFQ/-3'
IL4 Forward	5'-CACGGACACAAGTGCATA-3'
IL4 Reverse	5'-ATGTCTGTTACGGTCAACTCG-3'
IL4 Probe	5'/56-FAM/TTAACACAGC/ZEN/CTCACAGAGCAGAAGAC/3IABkFQ/-3'
GATA3 Forward	5'-GCCTCAGCCACTCCTACA-3'
GATA3 Reverse	5'-CCTGACCGAGTTCCGTAGTA-3'
GATA3 Probe	5//56-FAM/TAACATCGA/ZEN/CGGTCAAGGCAACCA/3IABkFQ/-3'

D. 3' end qPCR Primers and Probes of different gene locus based on NCBI36/hg18 (Mar. 2006)

Primer/ Probes	Sequence
IFNG Forward	5'- ACTCATCCAAGTGATGGCTGAA-3'
IFNG Reverse	5'- TCCTTTTCGCTTCCCTGTTT-3'
IFNG Probe	5'-6FAM- TGTCGCCAGCAGCT-3'
TBX21 Forward	5'- CTCCAGTCCCTCCATAAGTACCA-3'
TBX21 Reverse	5'- CTCTCCGTCGTTCACCTCAAC-3'
TBX21 Probe	5'-6FAM- CCCGGCTGCATAT-3'
GAPDH Forward	5'- CAAGGCTGTGGGCAAGGT-3'
GAPDH Reverse	5'- GGCCATGCCAGTGAGCTT-3'
GAPDH Probe	5'-6FAM- ATCCCTGAGCTGAACG -3'
GMCSF Forward	5'-CCTCCTCCTCTCCTTACTTG-3'
GMCSF Reverse	5'-GGCACATCCACTCCTCATA-3'

GMCSF Probe	5'-/56-FAM/ACCTGTTCC/ZEN/CTCATCTCCCTCCA/3IABkFQ/-3'
IL4 Forward	5'-AACCGGCTCGACAGGAAC-3'
IL4 Reverse	5'-TCGTCTTACGCCTTCCAAGAA-3'
IL4 Probe	5'-/56-FAM/CCTGTGAAG/ZEN/GAAGCCAACCAGAGT/3IABkFQ/-3'
GATA3 Forward	5'-CACCGCCATGGGTTAGAG-3'
GATA3 Reverse	5'-TCATGATACTGCTCCTGAAA-3'
GATA3 Probe	5'-/56-FAM/CAAGTCGAA/ZEN/AGGGACTGCAGGGAC/3IABkFQ/-3'

Table S2: Reagent list

Reagents	Product number	Company name	Use
AF488 anti-mouse IgG	A21202	Invitrogen	IF, FACS
AF488 anti-rabbit IgG	A21206	Invitrogen	IF
FITC Anti-Human IFN γ	554551	BD Biosciences	FACS
PE Anti-Human IL4	12-7049	eBioscience	FACS
Anti-Human T-bet	50-5825	eBioscience	FACS
PE Anti-Human GATA3	12-9966	eBioscience	FACS
PE Anti-Human CD45RO	555493	BD Biosciences	FACS
APC Anti-Human CD45RA	304111	Biolegend	FACS
PE Anti-Human CXCR3	353705	Biolegend	FACS
APC Anti-Human CCR6	353415	Biolegend	FACS
Anti-Human GMCSF	50-7356-41	eBioscience	FACS
LIVE/DEAD® Fixable Violet Dead Cell Stain Kit	L34955	Life Technologies	FACS
AnkyrinG	sc-12719	Santa Cruz	WB
Anti-CD28	302902	BioLegend	Cell culture
Anti-CD3	300402	BioLegend	Cell culture
Anti-IL-4	500815	BioLegend	Cell culture/ Neutralization
Anti-IFNg	MAB285	R&D Systems	Cell culture/ Neutralization
Anti-IL12	MAB219	R&D Systems	Cell culture/ Neutralization
Arp2/3	ab77084	Abcam	WB
B23	sc-32256	Santa Cruz	WB
β -actin	NB600-503	Novus Biologicals	WB, ChIP
CRM1	sc-5595	Santa Cruz	WB
Flag M2	F1804	Sigma	uChIP, IP, WB
Histone H3	ab1791	Abcam	WB
Histone H3 (Trimethyl K27)	ab6002	Abcam	ChIP, WB
Histone H3 (Trimethyl K4)	ab8580	Abcam	WB, ChIP
Importin alpha	I1784	Sigma	WB
LaminB1	ab16048	Abcam	WB
LAMP1	H4A3	DSHB	WB
Myc	TA100010	Origene	WB
NF-kB (p65)	sc-109X	Santa Cruz	WB
NTF97 Importin beta	ab2811	Abcam	WB
NUP98	sc-32256	Santa Cruz	IP, WB
N-WASp	sc-20770	Santa Cruz	WB
pCD3 Zeta (Tyr142)	ab59175	Abcam	WB, FACS
p-STAT1 (Ser727)	9177S	Cell Signaling	WB
pZAP70 (Tyr319)	sc-12946-R	Santa Cruz	WB, FACS
RanBP2	ab64276	Abcam	IP, WB
RanBP3	NB100-74647	Novus Biologicals	WB
RbBP5	NB600-252	Novus Biologicals	IP, ChIP, WB
RNA Polymerase II (Ser2)	ab5095	Abcam	ChIP, WB
RNA Polymerase II (Ser5)	ab5408	Abcam	ChIP, WB
RyR1	34C	DSHB	WB
SPT5	sc-28678X	Santa Cruz	ChIP
STAT1	ab3987	Abcam	ChIP
STAT6	5397	Cell Signaling	ChIP
Tbet	sc-21749X	Santa Cruz	ChIP
WASp	sc-5300	Santa Cruz	WB, IF, ChIP
WASp	sc-8353	Santa Cruz	IP, IF
ZAP-70	sc-574	Santa Cruz	WB
AF488 Phalloidin	A12379	Invitrogen	FACS
Amaca Cell line nucleofactor kitV	NC9041615	Fisher Scientific	Cell transfection
Human GM-CSF Quantikine ELISA Kit	DGM00	R&D systems	ELISA
Human IFN-gamma Quantikine ELISA Kit	DIF50	R&D systems	ELISA
Human IL-4 Quantikine ELISA Kit	D4050	R&D systems	ELISA
Leptomycin B solution	L2913	Sigma	Cell culture
Mem-PER Membrane Extraction Kit	89826	Pierce	enrich membranes
Myc-DDK-tagged ORF clone of human WASp	RC203457	Origene	mammalian expression vector

QuickChange II Site-Directed Mutagenesis kit	200519	Stratagene	Site-Directed Mutation
Rh Histone H3.3	M2507S	NEB	HMTase assay
rhIL-12	I2276	Sigma	Cell culture
rhIL-4	I4269	Sigma	Cell culture
rhIL2	136	AIDS Reagent Program, NIH	Cell Culture
SAM (S-adenosylmethionine)	A7007	Sigma	HMTase assay

WB: western blot

IF: Immunofluorescence

ChIP: Chromatin Immunoprecipitation

IP: Immunoprecipitation

FACS: Fluorescence-activated cell sorting

RT-PCR: Real Time Polymerase Chain Reaction

HMTase assay: Histone Methylase assay