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1	Binding of WIP to Actin is Essential for T Cell Actin Cytoskeleton Integrity and
2	Tissue Homing
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9	Supplemental figure legends
10	FIG S1 Generation of WIPAABD mice. (A) Genomic structure of murine Wipf1
11	gene, targeting construct, and predicted structure of the targeted allele after
12	homologous recombination. Exons are represented by black boxes. neo,
13	neomycin-resistance gene; DTA, diphtheria toxin A gene. The position of the PCR
14	primers used for genotyping, and the sizes of EcoRI and SacI restriction digests of
15	gDNA is shown. Probes A and B were used to detect the specific bands following
16	EcoRI and SacI digestion of genomic DNA, respectively. (B) PCR genotyping of
17	positive ES cell clones with primers a/b showing the expected ~4.0 Kb wild-type
18	(WT) and 5.7 Kb knock-in (KI) bands indicated with arrows. 1 Kb ⁺ , DNA molecular
19	weight marker. (C) Southern blot analysis of ES cell gDNA digested with EcoRI or
20	Sacl and detected with probes A or B, respectively. Probe A is a 629 bp fragment
21	derived from a region upstream of the 5' arm, while probe B is a 894 bp fragment
22	derived from a region downstream of the 3' arm. The sizes of the expected bands
23	is shown in A and indicated with arrows in C. (D) PCR genotyping of tail genomic

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DNA from WT, heterozygote and WIP∆ABD littermates with primers c/d, showing
a 176 bp WT and 140 bp KI band due to 36 bp deletion from exon 2 upon
integration of the targeting construct.

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FIG S2 Cellularity of thymus and bone marrow from WIP Δ ABD mice. (A) Thymus cellularity, representative FACS analysis of CD4⁺ and CD8⁺ cells, and percent CD4⁺ and CD8⁺ cells in the thymi of WIP Δ ABD mice and WT controls. N=6 each group. (B) Bone marrow cellularity, representative FACS analysis of B220⁺ and CD43⁺ or B220⁺ and IgM⁺ cells, and percent pro-B, pre-B, immature, and mature B cells in the bone marrow of WIP Δ ABD mice and WT controls. N=3 each group. Columns and bars represent mean±SEM. **p<0.01. n.s., not significant.

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36 **FIG S3** Defective *in vivo* homing of WIPAABD T cells to LNs. (A) Representative 37 FACS analysis of a mixture of equal numbers of Alexa fluor555-labeled WT T 38 cells (designated by red lettering) and Alexa fluor488-labeled WT or WIP∆ABD T cells (designated by green lettering) used for injection into genetically matched 39 40 WT recipients. (B) Representative FACS analysis of cells from the blood and LNs 41 of WT recipients obtained 1 h after *i.v.* administration of a 1:1 mixtures of equal 42 numbers of Alexa fluor555-labeled WT T cells (designated by red lettering) and 43 Alexa fluor488-labeled WT or WIP Δ ABD T cells (designated by green lettering). (C) Quantitative analysis of the homing index of WIPABD T cells relative to the 44 45 mean homing index of WT T cells set at 1.0. N=5 each group. *p<0.05.

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47 **FIG S4** Defective CHS response to oxazolone in WIPAABD mice. (A) CHS in WIPAAD mice and WT controls measured as the difference in thickness 48 49 between oxazolone- and vehicle-challenged ears. N=8 each group. (B) 50 Representative ear skin histology stained with hematoxylin and eosin (H&E) 24 h 51 post-challenge with oxazolone or vehicle, then visualized by light microscopy. 52 Bar=50 μ m. (C) *Ifn* γ and *II4* mRNA expression in ears of WIP Δ ABD mice and WT 53 controls 24 h post-challenge with oxazolone or vehicle. Data is represented as 54 fold increase in mRNA levels relative to vehicle-challenged ears of WT mice set at 55 1. N=8 each group. Columns or squares and bars represent mean±SEM. *p<0.05. 56

57 **FIG S5** Normal DC migration from the skin to the DLNs in WIP Δ ABD mice. (A) Representative FACS analysis, and percentages and numbers of CD11c⁺FITC⁺ 58 59 DCs in the DLNs of WIPAABD mice and WT controls 24 h following shaving and 60 painting the abdominal area with FITC or vehicle control. N=3 each group. n.d., 61 not detected. (B) Proliferation and secretion of IL-2, IFN- γ , and IL-13 by naïve WT 62 DO11.10 T cells stimulated with DCs isolated from WIP∆ABD or WT mice 24 h 63 following EC-sensitization with PBS or OVA, without further addition of OVA in 64 vitro. N=4 each group. Columns and bars represent mean±SEM. n.s., not 65 significant.

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FIG S6 Normal homing of adoptively transferred WT T cells into sites of cutaneous Ag challenge in WIP Δ ABD recipient mice. (A) Representative FACS analysis of CD4⁺KJ1-26⁺ cells in OVA- and PBS-challenged ears of WIP Δ ABD or

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75	Movies S1 and S2 Defective motility of T cells from WIPAABD mice. Live imaging
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73	Columns and bars represent mean±SEM. n.s., not significant.
72	mice recipients of OVA-stimulated WT DO11.10 $CD4^+$ cells. N=5 each group.
71	of CD4 ⁺ KJ1-26 ⁺ T cells in OVA- and PBS-challenged ears from WIP Δ ABD or WT
70	WT mice recipients of OVA-stimulated WT DO11.10 CD4 ⁺ cells. (B) Percentages

- of splenic T cells from WT (Movie S1) and WIP Δ ABD (Movie S2) mice stimulated
- with immobilized anti-CD3 and observed over a period of 20 min.















