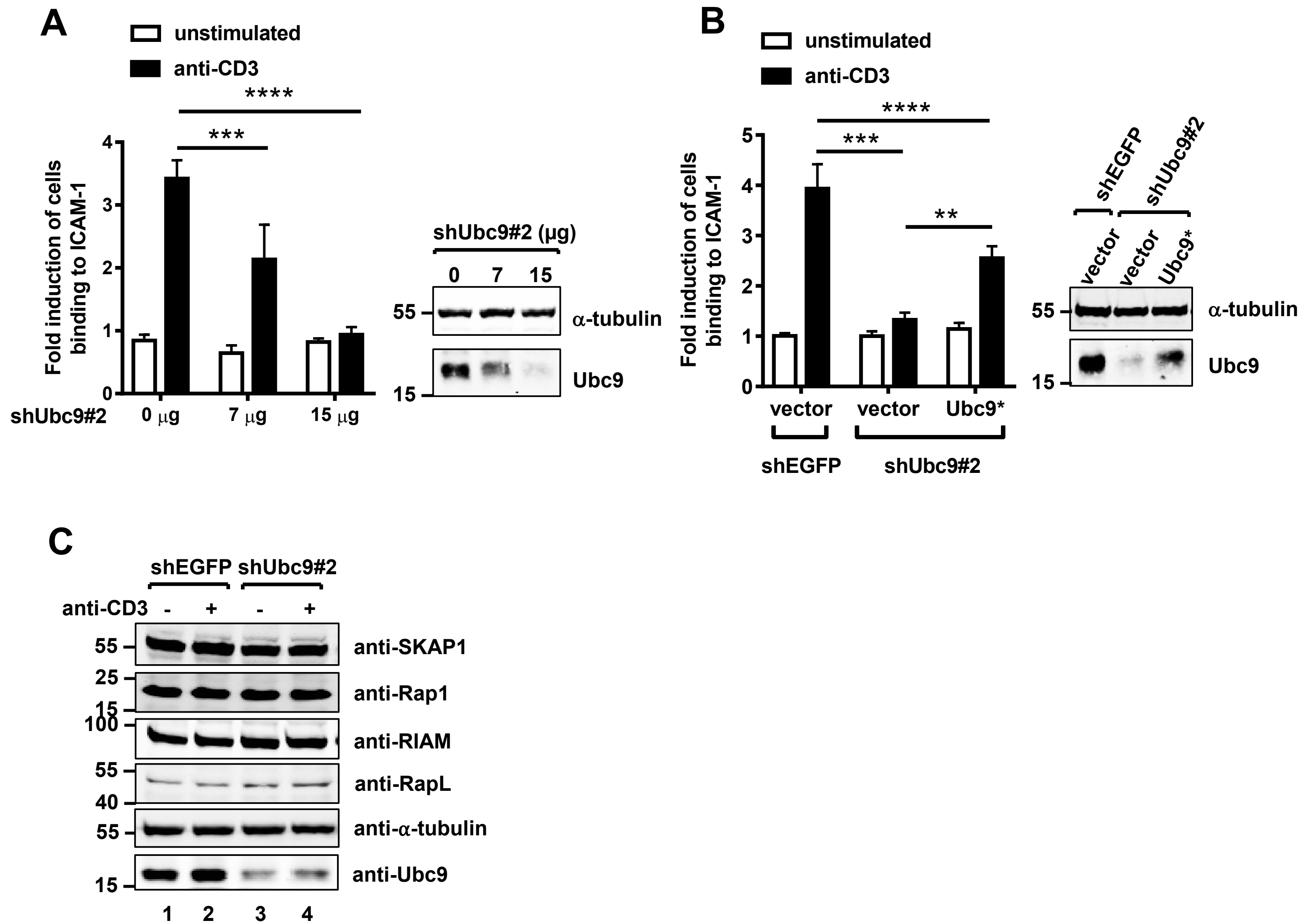
Supplemental Figure 1



Supplemental Figure 1. Knockdown of Ubc9 impaired TCR-mediated T-cell adhesion, but not affect the expression levels of RIAM, RapL, Rap1, and SKAP1. (A) Jurkat T cells were transfected with increased amount of shUbc9#2 construct. Cells were either left unstimulated or stimulated with anti-CD3 for 30 min before subjected to ICAM-1 binding assay. Error bars indicate the s.e. from three individual experiments. The knockdown effect of shUbc9#2 in the cells used in the ICAM-1 binding assay were assessed by Western blotting with anti-Ubc9 (right panel) (B) Stable Ubc9-knockdown Jurkat T cells (shUbc9#2) and the mock control cells (shEGFP) transfected with either empty vector or a Ubc9 expression construct (Ubc9*) containing 9 synonymous point mutations were left unstimulated or stimulated with anti-CD3 for 30 min, followed by *in vitro* ICAM-1 binding assay. Error bars indicate the s.e. from three individual experiment. The knockdown effect of shUbc9#2 and the re-expressed Ubc9 in the cells used in the ICAM-1 binding assay were assessed by Western blotting with anti-Ubc9 (right panel). (C) Stable Ubc9-knockdown Jurkat T cells (shUbc9) or the mock-knockdown control T cells (shEGFP) were either left unstimulated or were stimulated with anti-CD3 (OKT3). Whole cell lysate was prepared and subjected to Western blot analysis using antibodies against SKAP1, RIAM, Rap1 and RapL as where indicated. The anti-tubulin blotting was used as an internal control.

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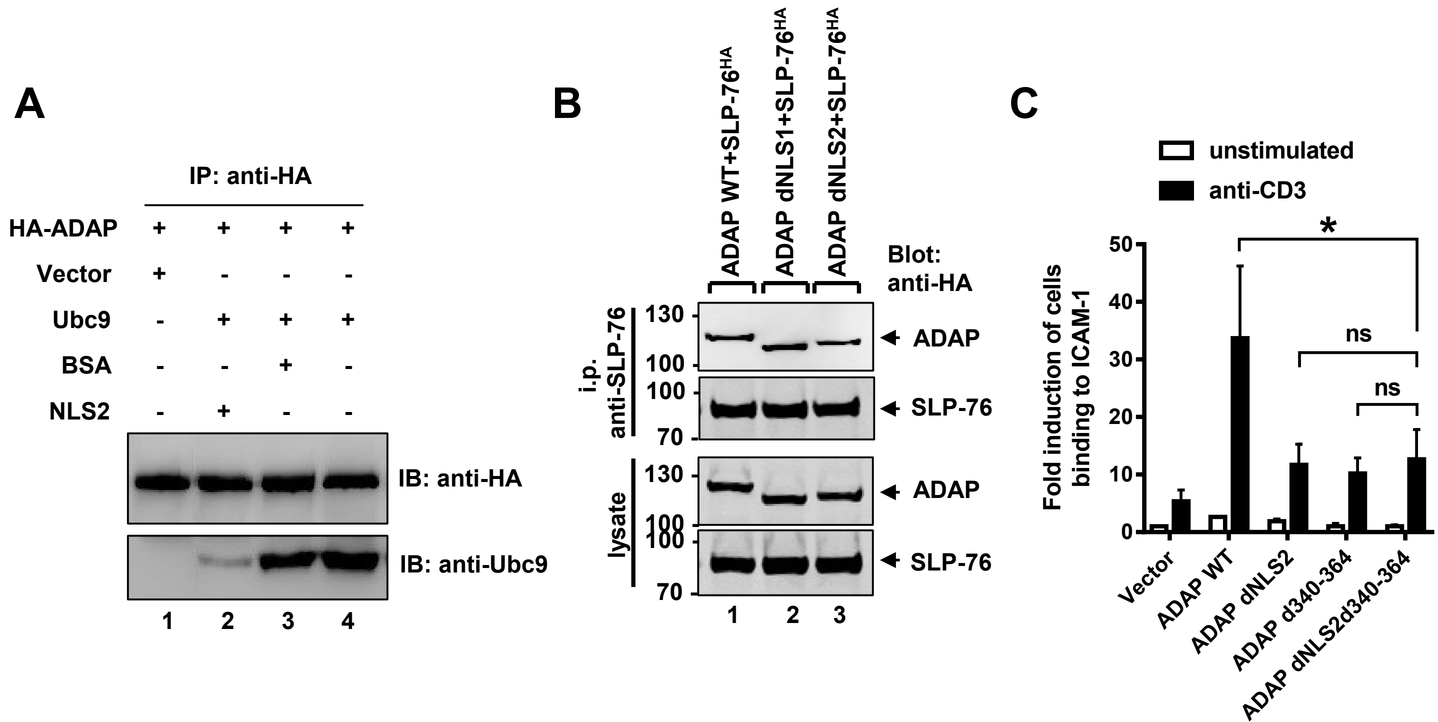
Supplemental Figure 2



Supplemental Figure 2. ADAP-Ubc9 binding is not required for ADAP-SLP-76 interaction but cooperates with the ADAP-SKAP complex for T-cell adhesion. (A) The effect of isolated NLS2 domain of ADAP on the binding of ADAP-Ubc9. The cell lysates of Jurkat T cells co-transfected with HA-ADAP and Ubc9 or with HA-ADAP and vector were incubated with either 10 µg of the synthesized NLS2 peptide or BSA as a control at 4ºC for 30 min. Then the ADAP in the lysates was pulled-down using anti-HA antibody, followed by Western blot analysis with anti-Ubc9 (lower panel) and anti-HA (upper panel) (B) Jurkat T cells were co-transfected with HA-tagged SLP-76 and together with either HA-tagged ADAP WT or mutants as indicated, followed by immunoprecipitation with anti-SLP-76 antibody and blotting with anti-HA. (C) Deletion of SKAP1-binding site on ADAP affect the TCR-mediated T-cell adhesion. An ADAP truncated mutant (ADAP d340-364) with deletion on SKAP1-binding site in proline-rich domain and a mutant with deletions on both NLS2 and SKAP1-binding site (dNLS2d340-364) were constructed with Quickchange. Cells transfected with either vector, ADAP WT, ADAP truncated mutants dNLS2, d340-364 or dNLS2d340-364 were either left unstimulated or stimulated with anti-CD3 followed by the *in vitro* T-cell adhesion assay as described in Materials and Methods. Error bars indicate the s.e. from three individual experiments.

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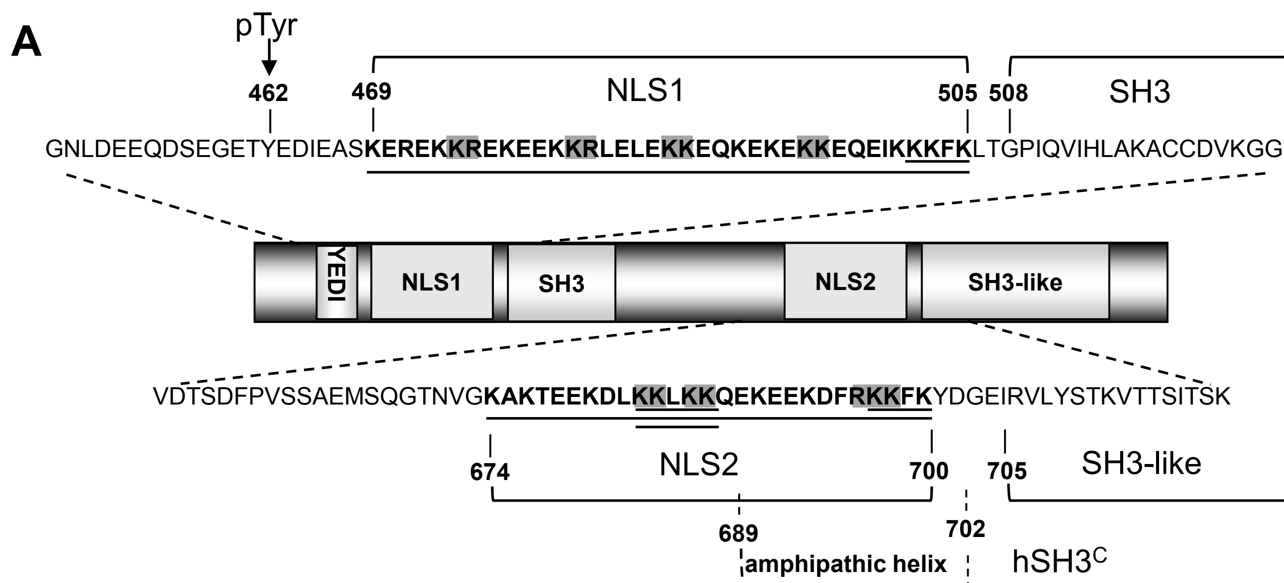
Supplemental Figure 3



B

NLS2	KAKTEEKDL**KKLKK**QEKEEKDFRKKFK
VSX-1	Q**K**R**KK**RR
AR	RKCYEAGMTLGAR**KLKK**
SV40	P**KK**KR**K**V
Nucleoplasmin	AVKRPAAT**KK**AGQAK**K**KKL**D**
NF-ATC1 (aa 682-684)	**R**R**K**
NF-ATC1 (aa 265-267)	**K**RR
NF-κB/p65	K**RK**R

Supplemental Figure 3. Sequence features of the ADAP-NLSs. (A) Schematic drawing of the NLSs of ADAP relative to the flanking sequences of the potential phosphorylation sites and SH3 domains. There is a potential phosphorylation sites at Y701 (in the YDGI motif) that overlaps with the C-terminal of the NLS2. However, Y701 was proven not phosphorylated (1). A phosphorylation sites at Y462 (in the YEDI motif) is locates at the N-terminal of NLS1. Further, two SH3 domains span residues 508-602 and residues 705-768 (2), which are each located at the C-terminal side of NLS1 and NLS2, respectively. The NLS2 does not overlap with the flanking SH3 domain, but overlaps with a pitch of 12 amino acids forming an amphipathic helix at the N-terminus of each SH3 domain (3). The combination of this amphipathic helix together with the SH3 domain was referred as helical SH3 (hSH3) domain. While the hSH3 domain mediates the interaction with the phospholipids, the amphipathic helix is not required for the phospholipid binding but it stabilizes SH3 domain. The NLS2 and its flanking hSH3 domain have non-overlapping roles in regulated TCR-mediated integrin activation and T-cell adhesion. (B) The ADAP NLS sequences were compared to those of the other known NLS-containing proteins by the EMBOSS Water Algorithm. The 5 amino acid motif (KKLKK) shared by ADAP-NLS2 and the other Ubc9-interacting NLSs from VSX-1 and AR is highlighted in bold and underlined. Sequence is aligned and identical residues compared with the NLS2 of ADAP are indicated in bold.

Reference:

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3. Heuer, K., M. Sylvester, S. Kliche, R. Pusch, K. Thiemke, B. Schraven, and C. Freund. 2006. Lipid-binding hSH3 domains in immune cell adapter proteins. J. Mol. Biol. 361:94-104.

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