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Absence of both Sos-1 and Sos-2 in peripheral CD4+ T cells leads to PI3K pathway activation and defects in migration

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Abstract

Sos-1 and Sos-2 are ubiquitously expressed Ras-Guanine Exchange Factors involved in Erk-MAP kinase pathway activation. Using mice lacking genes encoding Sos-1 and Sos-2, we evaluated the role of these proteins in peripheral T-cell signaling and function. Our results confirmed that TCR-mediated Erk activation in peripheral $CD4^+$ T cells does not depend on Sos-1 and Sos-2, although IL-2-mediated Erk activation does. Unexpectedly, however, we show an increase in AKT phosphorylation in Sos-1/2dKO $CD4^+$ T cells upon TCR and IL-2 stimulation. Activation of AKT was likely a consequence of increased recruitment of PI3K to Grb2 upon TCR and/or IL-2 stimulation in Sos-1/2dKO $CD4^+$ T cells. The increased activity of the PI3K/AKT pathway led to downregulation of the surface receptor CD62L in Sos-1/2dKO T cells and a subsequent impairment in T-cell migration.

Keywords

T cell; Sos; PI3K/AKT; Grb2; CD62L; Erk; signaling

INTRODUCTION

The Sos family consists of two highly homologous Ras-Guanine Exchange Factors (GEFs) involved in Ras-MAP kinase pathway activation [1, 2]. Sos-1 and Sos-2 (Sos-1/2) are ubiquitously expressed and function downstream of many signaling pathways and in particular, activated protein tyrosine kinases (PTKs) [3]. Stimulation of the T-cell antigen receptor (TCR) on T cells induces the activation of PTKs that phosphorylate multiple substrates including the adaptor molecule LAT. Phosphorylated LAT binds the SH2 domain of the adaptor Grb2. Consequently proteins bound to Grb2 SH3 domains such as Sos-1 and Sos-2 are recruited to LAT-based signaling complexes. Sos-1 and Sos-2 may also function

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CONFLICT OF INTEREST

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as scaffold proteins facilitating LAT oligomerization and clustering [4–6]. Sos-1 and Sos-2 may thus coordinate multiple downstream TCR signaling pathways.

In order to fully understand the role of Sos-1 and Sos-2 during T-cell activation we generated T-cell-specific Sos1 knockout mice (Sos1(T)$^{-/-}$) [7]. Our study of these mice showed that Sos-1 was critical for intrathymic Erk-1/Erk-2 activation and normal T-cell development [6–8]. The role of Sos-1 and Sos-2 is less clear in peripheral T-cell signaling and function. Previous studies that focused on Sos-1 and/or Sos-2 in peripheral T cells used RNA interference in either Jurkat T cells or peripheral human T cells [9–11]. Surprisingly, the use of Sos-1/2 siRNA in CD4^{+} human peripheral T cells revealed that TCR-mediated Erk activation did not depend on Sos-1/2 when the TCR was activated transiently, but led to a reduction in Erk phosphorylation upon sustained TCR activation [9, 11]. In Jurkat T cells, reduction of Sos-1 expression using siRNA was sufficient to decrease Erk phosphorylation upon TCR stimulation [10]. In order to better understand the contribution of Sos-1 and Sos-2 during the activation of peripheral T cells, we developed Sos-1(T) $^{-/-}$ mice that expressed Cre under the control of the *CD4* promoter. We crossed these Sos-1(T) $^{-/-}$ mice to pre-existing Sos2$^{-/-}$ mice.

With these mice we demonstrate and confirm that TCR-mediated Erk activation in peripheral CD4^{+} T cells does not depend on Sos-1 and Sos-2, however IL-2-mediated Erk activation is diminished in the absence of Sos1/2. Unexpectedly, activated Sos-1 and Sos-2 KO T cells showed an increase in AKT phosphorylation compared to WT CD4^{+} T cells. We show that enhanced recruitment of PI3K to Grb2 upon TCR and IL-2 stimulation in Sos-1/2dKO CD4^{+} T cells accounts for the increase in the active pool of AKT. As a consequence the expression of the surface receptor CD62L is downregulated in Sos-1/2dKO T cells. Finally we show that the absence of Sos-1 and Sos-2 led to impaired CD4^{+} T-cell migration.

RESULTS AND DISCUSSION

Increased recruitment of PI3K to Grb2 upon TCR activation in Sos-1/2dKO T cells

We first examined T-cell homeostasis and function in WT, Sos-1 KO, Sos-2 KO or Sos-1/2dKO mice. In lymph nodes (LNs) there was no difference in the numbers and percentages of CD4^{+}, CD8^{+}, regulatory T cells or T-cell memory subpopulations observed between WT and the various knockout mice (Supporting Information Fig. 1 A–C, Fig.2 A). Analysis of CD69 and CD25 (IL-2Rα) expression or cytokine production (IL-2 and IFN-γ) showed no significant difference between activated Sos-1/2dKO CD4^{+} or CD8^{+} T cells (Supporting Information Fig. 1 D–G, Fig.2 B).

To study peripheral T-cell proliferation, we stimulated CFSE-labeled T cells for 72 h with plate-bound anti-CD3 (Fig.1A). We observed a decrease in proliferation in T cells from single Sos-1 or Sos-2 KO relative to WT T cells. However no difference was observed when comparing WT and Sos-1/2dKO T-cell proliferation. Altogether our initial observations showed only mild differences between WT and Sos-deficient T cells.

In order to understand the role of Sos-1 and Sos-2 during T-cell activation, we performed a phosphotyrosine blot on lysates from $CD4^+$ T cells blasts isolated from WT and Sos deficient mice stimulated with anti-CD3 for the indicated times (Fig.1B). Deletion of either Sos-1 or Sos-2 or both proteins did not lead to major differences in the phosphotyrosine profile of T-cell activation. Quantification of Erk phosphorylation, especially when comparing Sos-1/2dKO T cells to WT controls, showed a modest increase (Fig.1C). One explanation for this result may be that RasGRP, the other major RasGEF in T cells, plays an important role in Sos-1/2dKO T cells. However, it was recently reported that depletion of RasGRP1 using siRNA showed decreases in TCR-mediated Erk activation in primary human $CD4^+$ T cells, and knockdown of both RasGRP1 and Sos-1 did not cause any decrease in Erk phosphorylation [11]. Because of the relatively minor roles reported for the RasGEFs RasGRP1 and Sos1/Sos2 in TCR-stimulated Erk activation in peripheral T cells, alternative Ras-independent pathways to Erk must be considered. Recently, it was shown that Pak1, in a molecular complex with Bam32 and PLC-γ1, could contribute to Erk phosphorylation in the absence of Sos-1/2 and RasGRP1 phosphorylation [12].

To evaluate other signaling pathways such as the PI3K/AKT pathway, we blotted for AKT phosphorylation upon early T-cell activation. Interestingly, after 2 min of TCR activation we observed an increase in the phosphorylation of AKT at Ser473 and Thr308 in cells lacking Sos1/2 (Fig.1B–D). Both Sos-1 and Sos-2 are involved in this process, as single Sos-1 or Sos-2 KO T cells showed an increase in AKT phosphorylation, but this effect was most pronounced in Sos-1/2dKO T cells (Fig.1C). In primary $CD4^+$ T cells, the phosphorylation of AKT at Thr308, which is regulated by PI3K activity, shows an increase at 2 and 5min in Sos-1/2dKO T cells compared to WT (Fig.1E). We were not able to detect any difference in the phosphorylation of AKT at Ser473 or Erk. The difference observed in the phosphorylation of AKT is more pronounced in T-cell blasts than in primary T cells, suggesting that activation and proliferation of naïve T cells exacerbated the difference between WT and Sos-1/2dKO T cells.

The activation of AKT is a well-known process. Phosphoinositide 3-kinase (PI3K) is activated upon TCR triggering and generates phosphoinositide-triphosphate $PI(3,4,5)P_3$ from $PI(4,5)P_2$ at the plasma membrane leading to the recruitment and phosphorylation of AKT [13]. Because AKT phosphorylation is increased in Sos-1/2dKO T cells, we hypothesized that PI3K might be recruited more efficiently to the plasma membrane via binding to Grb2 in the absence of Sos-1 and Sos-2. To test this hypothesis, the interaction between the p85-PI3K subunit and Grb2 was evaluated by immunoprecipitation (IP) in anti-CD3 stimulated $CD4^+$ T cells (Fig.1F). Immunoprecipitation of p85 followed by immunoblotting for Grb2 revealed a 3–4 fold increase in p85-Grb2 interaction upon T-cell stimulation in Sos-1/2dKO T cells compared to WT controls (Fig.1G). Reciprocal IP of Grb2 and immunoblotting for the p85-PI3K subunit confirmed that Grb2-p85 binding was increased upon stimulation in Sos-1/2dKO T cells (Fig.1H).

Sos-1 and Sos-2 are known to bind constitutively to Grb2 SH3 domains [14]. There is competition for the binding of Grb2 to several different proteins and Sos-1/2. Indeed, Grb2 has been shown in a mass spectrometry assay of activated HEK293T cells to bind to more than 90 different proteins[15]. In the absence of Sos-1/2 there would be less competition for

Grb2 SH3 domains so that these domains could bind more of the cellular pool of the p85 regulatory PI3K subunit or other proteins. As the binding of Grb2 SH3 domains to the proline-rich region of p85 [16] activates PI3K [17], enhanced recruitment and activation could result in more PI-3kinase-mediated activity.

It is possible that some effects of the absence of Sos-1/2 can be partially compensated for the increased activation of the PI3K pathway. It has been reported that in stimulated T cells isolated from mice expressing PI3K kinase-dead p110δ^{D910}, in addition to decreased AKT phosphorylation, Erk phosphorylation was greatly impaired compared to WT cells [18]. Moreover a study using PI3K inhibitors has shown that PI3K is required for plasma membrane targeting of RasGRP1 in B cells upon BCR stimulation [19]. Thus the increased activity of PI3K, leading to generation of PI(3,4,5)P_3 at the plasma membrane, in Sos-1/2dKO T cells $CD4^+$ T cells, could potentially lead to more recruitment of RasGRP1 to the plasma membrane, via binding of the basic/hydrophobic cluster of RasGRP1 to PI(3,4,5)P_3 [19]. This pathway may account for the increase in Erk phosphorylation observed in our studies (Fig.1B–D).

Other pathways could also be activated or inhibited in the absence of Sos-1/2 in this fashion. This sort of pathway rewiring of T cells, that is, the recruitment of additional components and engagement of normally inactive pathways, may explain why we only observed mild differences when studying T-cell phenotype and function.

PI3K is recruited to Grb2 upon IL-2 stimulation in Sos-1/2dKO T cells

We also decided to test the role of Sos-1 and Sos-2 in interleukin-2 receptor (IL-2R) signaling. A previous study, using siRNA in human peripheral T cells showed that knockdown of Sos-1/Sos-2, albeit incomplete, significantly reduced IL-2R-mediated Erk phosphorylation [11]. Consistent with these previous results, removal of both Sos-1 and Sos-2 almost entirely suppressed IL-2-dependent Erk phosphorylation (Fig.2A). Additionally, we tested other known mediators of IL-2R signaling. Stat-3/Stat-5 phosphorylation were not affected, however, there was an increase in AKT phosphorylation following IL-2 stimulation in Sos-1/2dKO T cells compared to WT (Fig.2A).

Because we had already shown an increased recruitment of PI3K to Grb2 upon TCR stimulation, we decided to test if this was also observed following IL-2 stimulation. We performed a Grb2 IP after IL-2 stimulation of $CD4^+$ T cells and blotted for the p85-PI3K subunit (Fig.2B). Similar to the findings observed after TCR activation, we found an increased recruitment of p85 to Grb2 upon IL-2 stimulation especially in the absence of Sos-1/2 after 2 and 5 minutes of stimulation. Thus, increased PI3K recruitment and activation accounts for the increase in AKT phosphorylation observed following IL-2 stimulation.

In contrast to the lack of a role for Sos-1/2 in Erk activation upon TCR stimulation, these enzymes are required for Erk phosphorylation following IL-2 addition, as shown previously [11]. This study also showed that RasGRP1 is probably not required for IL-2-mediated Erk phosphorylation. Perhaps the fact that RasGRP1 is dispensable in the IL-2 receptor signaling

pathway may explain why increased PI3K activity cannot affect Erk phosphorylation downstream of the IL-2R.

Sos-1/2dKO T cells have a defect in CD4$^+$ T-cell migration

CD62L (L-selectin) is a surface receptor involved in lymphocyte adhesion [20]. Downregulation of CD62L at the surface of T lymphocytes is a dynamic process that is highly dependent on PI3K activity upon TCR or IL-2 stimulation [21]. As we observed that the PI3K pathway was more active in Sos-1/2dKO T cells, we decided to test the impact of increased PI3K activity on CD62L expression upon stimulation through the TCR. We activated CD4$^+$ or CD8$^+$ T cells from WT or Sos-1/2dKO LNs for 3h using different concentrations of anti-CD3 antibodies, and examined CD62L status (Fig.3A, B and Supporting Information Fig.3A, B). Interestingly, Sos-1/2dKO T cells showed a more pronounced downregulation of CD62L upon TCR activation than WT controls. This downregulation is dependent on PI3K. This is confirmed with the use of a PI3K inhibitor, which is sufficient to inhibit most of the CD62L downregulation in either WT or Sos-1/2dKO T cells. (Supporting Information Fig.4). This result is consistent with our previous results showing that the PI3K pathway is more active in Sos-1/2dKO T cells (Fig.1, 2).

We next decided to evaluate the phenotype of T cells that had been stimulated for longer time frames. Sos-1/2dKO T cells blasts compared to WT had more effector memory T cells (CD44^{hi} CD62L^{lo}) and fewer central memory T cells (CD44^{hi} CD62L^{hi}) (Fig.3C). Evaluation of CD62L surface expression revealed that Sos-1/2dKO T cells expressed less CD62L at the surface than WT T cells, whereas CD44 expression was unaltered (Fig.3C, D).

A previous study showed that lymphocytes from mice deficient in CD62L did not bind to peripheral LNs HEVs and there was a severe reduction in the number of lymphocytes localized to peripheral LNs [22].We hypothesized that the decrease in CD62L expression on Sos-1/2dKO T cells could lead to a lymphocyte migration defect. To test this hypothesis in vivo, we used adoptive transfer experiments to compare the ability of Sos-1/2dKO and WT T cells blasts to home to secondary lymphoid organs (SLO). Blast T cells from both Sos-1/2dKO or WT mice were labeled with two different cytosolic dyes (Cell Trace Violet or Cell Trace Far Red), mixed at a ratio of 1:1 and then transferred into C57BL/6 host mice by tail vein injection. After 1h, we analyzed host mouse blood, LN and spleen for the presence of the transferred cells. We observed a significant defect in the migration properties of CD4$^+$ and CD8$^+$ Sos-1/2dKO T cells blasts into SLO compared to WT T cells (Fig.3E and Supporting Information Fig.3C).

We believe that the impairment in migration of Sos-1/2dKO CD4$^+$ T cells blasts is due to the fact that CD62L is expressed at lower levels at the surface of Sos-1/2dKO T cells. Recirculation of lymphocytes is important for immune surveillance and the generation of rapid and efficient adaptive immune responses. Thus Sos-1/2dKO mice may respond less effectively in models of infectious disease. Moreover, CD62L ligands can also be induced on the endothelium of inflamed tissues. Specifically, vascular CD62L ligands are expressed at cutaneous sites of chronic inflammation, acute dermatitis, rheumatoid arthritis, diabetes,

and asthma [23]. It would be interesting to test for the incidence or susceptibility of Sos-1/2dKO mice in these different diseases.

CONCLUDING REMARKS

Altogether our results using a genetic deletion identified PI3K/AKT as a signaling pathway activated in the absence of Sos-1 and Sos-2 in peripheral T cells upon TCR or IL-2R stimulation. We also definitively demonstrated a lack of Sos dependence for TCR-mediated Erk activation in mature T cells. We also showed that the PI3K/AKT pathway re-wiring led to a defect in Sos-1 and Sos-2 deficient peripheral T cell migration. Future studies will address the ability of Sos-1/2 KO mice to respond in models of inflammation or infectious diseases and will investigate other possible signal pathway re-wiring in Sos-1/2dKO $CD4^+$ T cells.

METHODS

Mice

Generation of Sos-1$(T)^{-/-}$ [7] and $Sos2^{-/-}$[24] mice was previously described . Sos-1/2dKO mice were obtained by crossing Sos-1$(T)^{-/-}$ with $Sos2^{-/-}$.

Cell Culture

Naïve $CD4^+$ T cells were purified from LNs by negative selection (90–98%) (Stem cell Technology #19852A) according to the manufacturer's specifications. T cells blasts were obtained from plate-bound anti-CD3ε (2 μg/ml) (BD Biosciences #553060), soluble anti-CD28 (1 μg/ml) (BD Biosciences #553294) and IL-2 (100U/ml) (NCI Biological Resource Branch) stimulation for 3 days and then expanded with IL-2 (100 U/ml) during 2 more days. T cells blasts were then IL-2 starved for 24h (resting phase).

Proliferation

For assessment of cellular proliferation by CFSE (carboxyfluorescein diacetate succinimidyl diester; Life Technologies #C34554), cells isolated from LN were labeled with 1 μM CFSE and stimulated with plate-bound anti-CD3ε (2μg/ml) for 72 h and processed for flow cytometric analysis.

Cell, stimulation, lysis, Immunoprecipitation and Western blotting analysis

For TCR stimulation $CD4^+$ T cells were incubated for 15 min at 4°C with biotinylated anti-CD3 (10μg/mL, BD Biosciences #553060). Cells were washed and stimulated for the indicated time by adding streptavidin (20 μg/ml final concentration). For IL-2 stimulation, rested T-cells blasts were stimulated with 1000 U/ml of IL-2.

For immunoprecipitation, antibodies were incubated with Protein A/G-agarose beads. Cells were lysed with 1% NP40. Precleared lysates were incubated with the beads coated with antibodies. Immunoprecipitates were analyzed by SDS-PAGE and western blot. (Detailed information is available in supplemental information)

Migration assays

Blasted $CD4^+$ T cells isolated from WT or Sos-1/2dKO, were loaded with Cell Trace Violet Stain (Life Technologies #C34557) or Cell Trace Far Red DDAO (Life Technologies #C34553). Cells were mixed at a ratio of 1:1 and $10x10^6$ cells were injected into the tail veins of C57BL/6 mice. Then, 1 h later, recipient mice were euthanized, and blood, spleen and LNs were removed for quantification of Cell Trace Violet-labeled and Cell Trace Far Red-labeled T cells by flow cytometry.

Flow cytometry

Single cell suspensions from lymph nodes were stained with the fluorochrome-conjugated monoclonal antibodies described in the text. Flow cytometry was performed using a FACSCalibur and CellQuestPro software (BD Biosciences) or on a FACSCanto II flow cytometer with the HTS module (BD Biosciences). Data were analyzed with FlowJo software (Tree Star, Inc.). All fluorochrome-conjugated antibodies were purchased from BD Biosciences (CD62L, CD44, CD4, CD8, CD69, CD25, IL-2, and IFN-γ Our work is MIATA compliant.

Statistical analysis

In all figures, data are presented as means ± SEM. Statistical significance was determined by two-tailed Student's *t* test with GraphPad Prism software: $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Jun JE, Rubio I, Roose JP. Regulation of Ras Exchange Factors and Cellular Localization of Ras Activation by Lipid Messengers in T Cells. Front Immunol. 2013; 4:239. [PubMed: 24027568]
2. Kortum RL, Rouquette-Jazdanian AK, Samelson LE. Ras and extracellular signal-regulated kinase signaling in thymocytes and T cells. Trends Immunol. 2013; 34:259–268. [PubMed: 23506953]
3. Rojas JM, Oliva JL, Santos E. Mammalian son of sevenless Guanine nucleotide exchange factors: old concepts and new perspectives. Genes Cancer. 2011; 2:298–305. [PubMed: 21779500]
4. Balagopalan L, Coussens NP, Sherman E, Samelson LE, Sommers CL. The LAT story: a tale of cooperativity, coordination, and choreography. Cold Spring Harb Perspect Biol. 2010; 2:a005512. [PubMed: 20610546]
5. Houtman JC, Yamaguchi H, Barda-Saad M, Braiman A, Bowden B, Appella E, Schuck P, Samelson LE. Oligomerization of signaling complexes by the multipoint binding of GRB2 to both LAT and SOS1. Nat Struct Mol Biol. 2006; 13:798–805. [PubMed: 16906159]
6. Kortum RL, Balagopalan L, Alexander CP, Garcia J, Pinski JM, Merrill RK, Nguyen PH, et al. The ability of Sos1 to oligomerize the adaptor protein LAT is separable from its guanine nucleotide exchange activity in vivo. Sci Signal. 2013; 6:ra99. [PubMed: 24222714]

7. Kortum RL, Sommers CL, Alexander CP, Pinski JM, Li W, Grinberg A, Lee J, et al. Targeted Sos1 deletion reveals its critical role in early T-cell development. Proc Natl Acad Sci U S A. 2011; 108:12407–12412. [PubMed: 21746917]
8. Kortum RL, Sommers CL, Pinski JM, Alexander CP, Merrill RK, Li W, Love PE, Samelson LE. Deconstructing Ras signaling in the thymus. Mol Cell Biol. 2012; 32:2748–2759. [PubMed: 22586275]
9. Poltorak M, Meinert I, Stone JC, Schraven B, Simeoni L. Sos1 regulates sustained TCR-mediated Erk activation. Eur J Immunol. 2014; 44:1535–1540. [PubMed: 24497027]
10. Roose JP, Mollenauer M, Ho M, Kurosaki T, Weiss A. Unusual interplay of two types of Ras activators, RasGRP and SOS, establishes sensitive and robust Ras activation in lymphocytes. Mol Cell Biol. 2007; 27:2732–2745. [PubMed: 17283063]
11. Warnecke N, Poltorak M, Kowtharapu BS, Arndt B, Stone JC, Schraven B, Simeoni L. TCR-mediated Erk activation does not depend on Sos and Grb2 in peripheral human T cells. EMBO Rep. 2012; 13:386–391. [PubMed: 22344067]
12. Rouquette-Jazdanian AK, Sommers CL, Kortum RL, Morrison DK, Samelson LE. LAT-independent Erk activation via Bam32-PLC-gamma1-Pak1 complexes: GTPase-independent Pak1 activation. Mol Cell. 2012; 48:298–312. [PubMed: 22981863]
13. Fruman DA, Bismuth G. Fine tuning the immune response with PI3K. Immunol Rev. 2009; 228:253–272. [PubMed: 19290933]
14. Buday L, Downward J. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. Cell. 1993; 73:611–620. [PubMed: 8490966]
15. Bisson N, James DA, Ivosev G, Tate SA, Bonner R, Taylor L, Pawson T. Selected reaction monitoring mass spectrometry reveals the dynamics of signaling through the GRB2 adaptor. Nat Biotechnol. 2011; 29:653–658. [PubMed: 21706016]
16. Kapeller R, Prasad KV, Janssen O, Hou W, Schaffhausen BS, Rudd CE, Cantley LC. Identification of two SH3-binding motifs in the regulatory subunit of phosphatidylinositol 3-kinase. J Biol Chem. 1994; 269:1927–1933. [PubMed: 8294442]
17. Pleiman CM, Hertz WM, Cambier JC. Activation of phosphatidylinositol-3' kinase by Src-family kinase SH3 binding to the p85 subunit. Science. 1994; 263:1609–1612. [PubMed: 8128248]
18. Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, et al. Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. Science. 2002; 297:1031–1034. [PubMed: 12130661]
19. Zahedi B, Goo HJ, Beaulieu N, Tazmini G, Kay RJ, Cornell RB. Phosphoinositide 3-kinase regulates plasma membrane targeting of the Ras-specific exchange factor RasGRP1. J Biol Chem. 2011; 286:12712–12723. [PubMed: 21285350]
20. Cyster JG. Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu Rev Immunol. 2005; 23:127–159. [PubMed: 15771568]
21. Sinclair LV, Finlay D, Feijoo C, Cornish GH, Gray A, Ager A, Okkenhaug, et al. Phosphatidylinositol-3-OH kinase and nutrient-sensing mTOR pathways control T lymphocyte trafficking. Nat Immunol. 2008; 9:513–521. [PubMed: 18391955]
22. Arbones ML, Ord DC, Ley K, Ratech H, Maynard-Curry C, Otten G, Capon DJ, Tedder TF. Lymphocyte homing and leukocyte rolling and migration are impaired in L-selectin-deficient mice. Immunity. 1994; 1:247–260. [PubMed: 7534203]
23. Grailer JJ, Kodera M, Steeber DA. L-selectin: role in regulating homeostasis and cutaneous inflammation. J Dermatol Sci. 2009; 56:141–147. [PubMed: 19889515]
24. Esteban LM, Fernandez-Medarde A, Lopez E, Yienger K, Guerrero C, Ward JM, Tessarollo L, Santos E. Ras-guanine nucleotide exchange factor sos2 is dispensable for mouse growth and development. Mol Cell Biol. 2000; 20:6410–6413. [PubMed: 10938118]

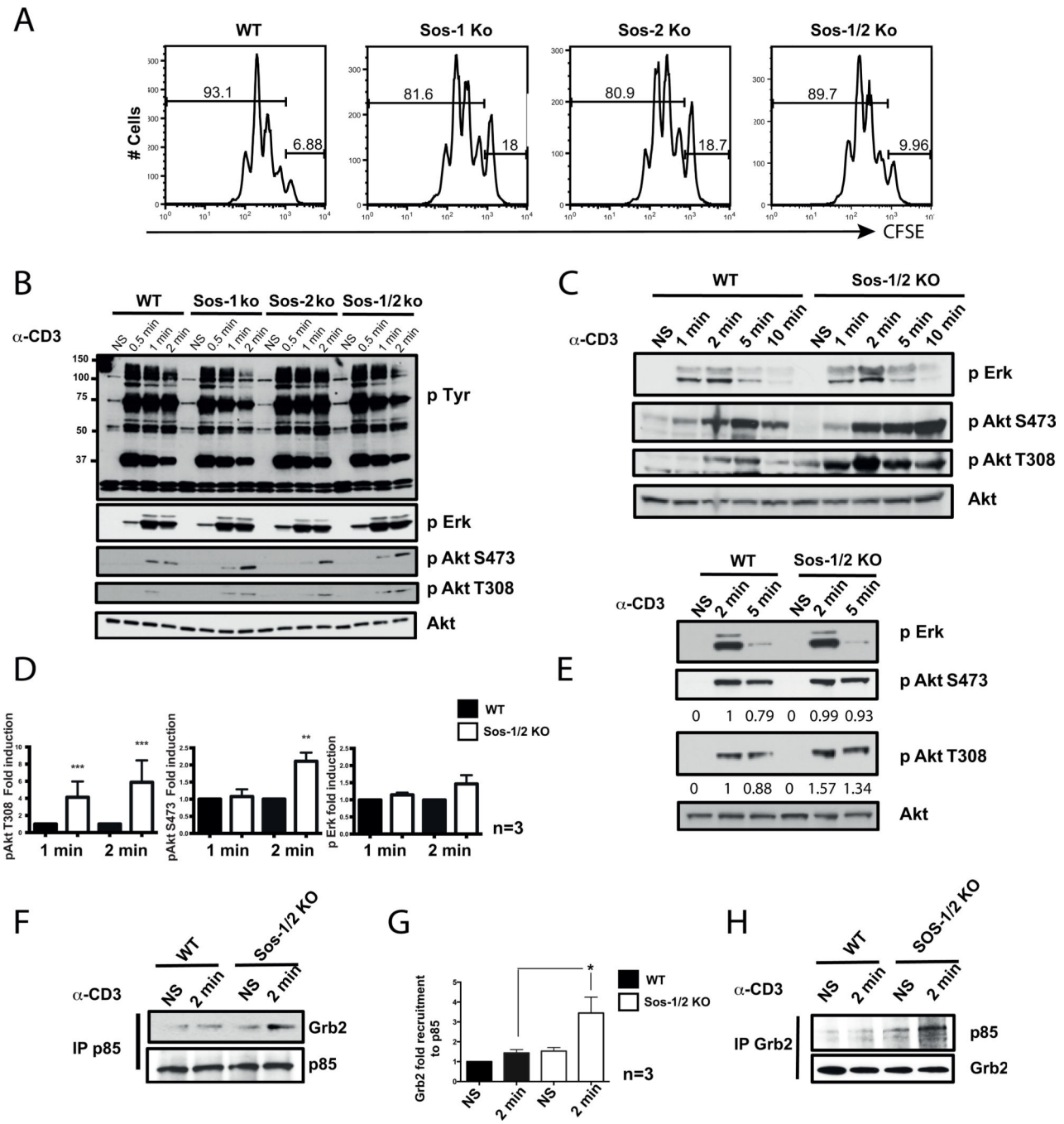

Figure 1. Increased recruitment of PI3K to Grb2 upon TCR activation in Sos-1/2dKO T cells leads to increased AKT phosphorylation

(A) Cells were isolated from LNs of wild-type (WT), Sos-1, Sos-2 and Sos-1/2 ko mice and labeled with CFSE prior to stimulation with CD3 and IL-2 for 3 days. Cellular proliferation was measured by flow cytometry. These results are representative of at least 3 independent experiments.

(B, C) Blast T cells from the indicated mice were stimulated for short kinetics (B) or extended time-point kinetics (C), with biotinylated anti-CD3 and cross-linked with

Streptavidin. Cell lysates were analyzed by immunoblotting with (B,C) pTyr, pErk, pAktS473 and pAktT308,; anti-Akt Ab was used as a loading control. One representative blot out of at least 2 independent experiments is shown.

(D) Quantitation of intensity of bands from (**B**) using Image J software. Evaluation of AKT and Erk phosphorylation is normalized to the corresponding loading signal and presented as fold induction compared to the level of WT phosphorylation. Bars show mean + SEM of at least 3 independent experiments. Different mice for the generation of blast T cells were used for each experiment.

(E) Primary $CD4^+$ T cells purified from LNs were stimulated the same way as in (**B**) and (**C**). Numbers below the band indicate fold-induction of phosphorylation compared with WT 2min stimulation. One representative blot out of at least 2 independent experiments is shown.

(F) Anti-CD3 stimulated T-cell lysates were immunoprecipitated using anti-p85 Abs and blotted for anti-Grb2. One representative blot out of at least 3 independent experiments is shown.

(G) Intensity of bands from three similar experiments as in (**F**) was quantified using Image J software. Results are represented as fold induction over non-stimulated WT. Bars show mean + SEM of 3 independent experiments. Different mice for the generation of blast T cells were used for each experiment. Statistical significance was determined by two-tailed Student's *t* test.*$P \leq 0.05$.

(H) Anti-CD3 stimulated T-cell lysates were immunoprecipitated using anti-Grb2 Abs and blotted for anti-p85. One representative blot out of at least 3 independent experiments is shown.

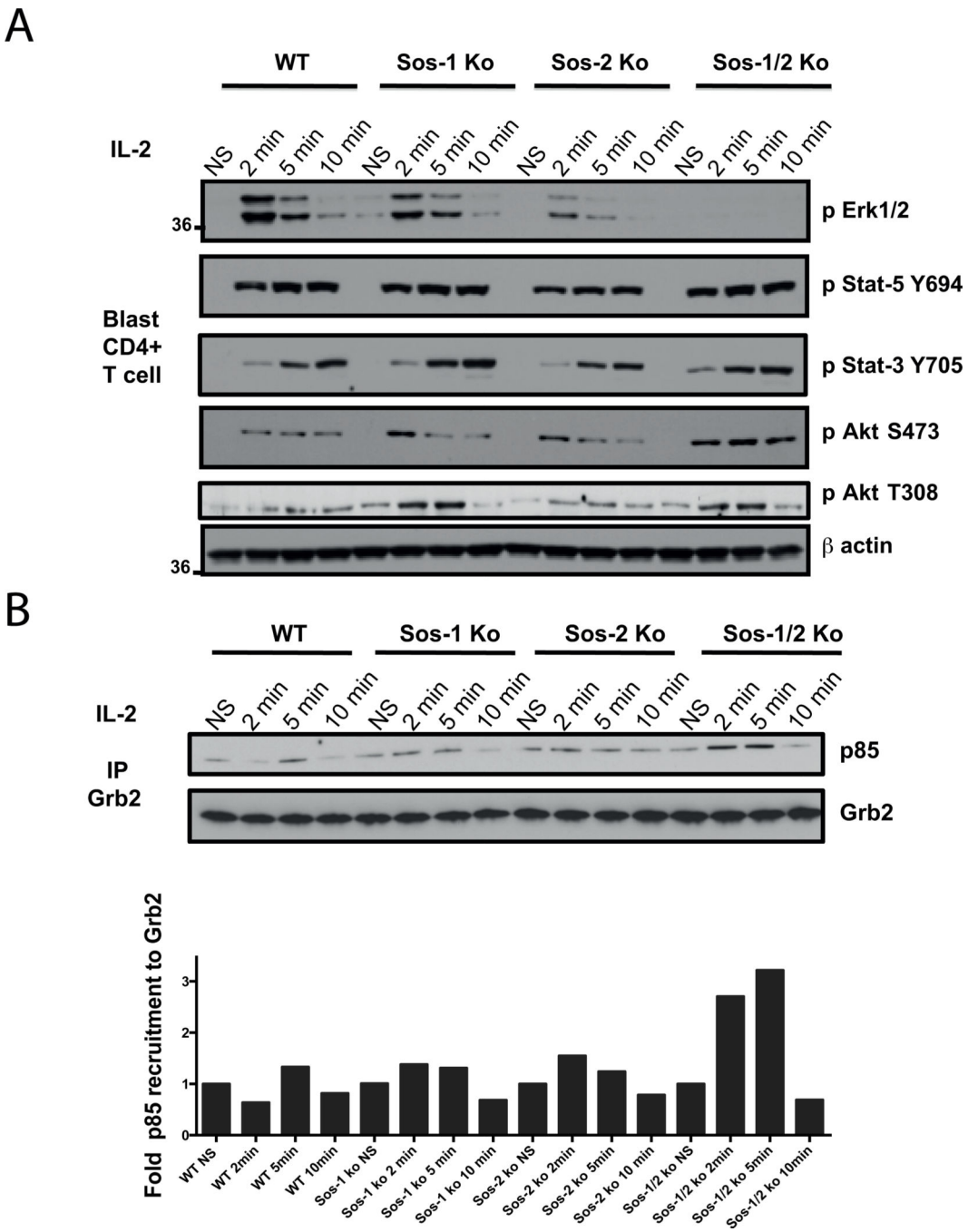

Figure 2. Increased recruitment of PI3K to Grb2 upon IL-2 stimulation in Sos-1/2dKO T cells leads to increased AKT phosphorylation

(A) Blast T cells from (WT), Sos-1, Sos-2 and Sos-1/2 ko mice were stimulated for the indicated times with IL-2. Cell lysates were analyzed by immunoblotting using the indicated Abs. β-actin was used as a loading control. One representative blot out of at least 3 independent experiments is shown.

(B) IL-2-stimulated cell lysates were immunoprecipitated using anti-Grb2 Ab and were blotted for anti-p85. (top) One representative blot out of at least 3 independent experiments

is shown. (bottom). The graph represents quantification of p85 binding to Grb2 normalized to Grb2 loading charge for this experiment.

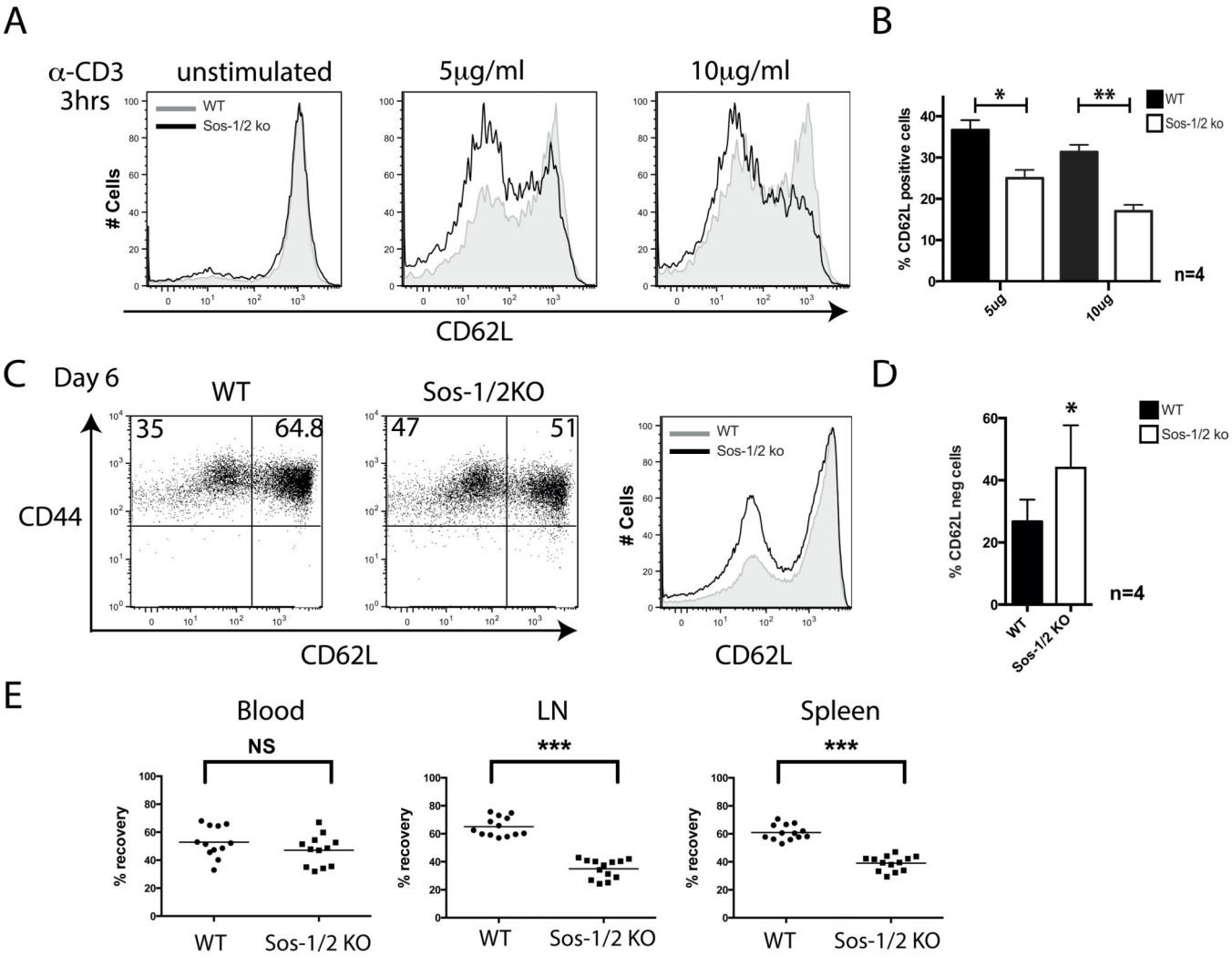

Figure 3. Sos-1/2dKO T cells have a defect in CD4$^+$ T-cell migration

(A) Cells isolated from LNs of WT and Sos-1/2dKO mice were stimulated for 3 h with anti-CD3. Flow cytometry evaluation of CD62L was performed after CD4$^+$ gating. These results are representative of at least 3 independent experiments.

(B) Quantification of CD62L-positive cells from the experiments in (A). Bars show mean + SEM out of 4 independent experiments. Cells from different mice were used for each experiment. Statistical significance was determined by two-tailed Student's *t* test. **P* ≤0.05, ***P* ≤0.01.

(C) Flow cytometry evaluation of CD44 and CD62L on blast T cells. Representative Dot plots out of 4 independent experiments and a histogram plot showing CD62L status comparison between WT and Sos-1/2dKO are shown.

(D) Quantification of CD62L-negative cells in (C). Bars show mean + SEM out of 4 independent experiments. Different mice for the generation of blast T cells were used for each experiment. Statistical significance was determined by two-tailed Student's *t* test. **P* ≤0.05.

(E) Recovery of co-transferred labeled WT or Sos-1/2dKO cells, presented as a percentage of total transferred cells recovered from the blood, LNs or spleen at 1 h after transfer. Each dot indicates an individual mouse, data are representative of three pooled experiments; horizontal bars indicate the mean. Statistical significance was determined by two-tailed Student's *t* test. ***$P \leq 0.001$.NS, not significant.