SUPPLEMENTARY DATA

Methods- Supplement

Genotyping

The following primers were used for genotyping by PCR. WT1 and WT2 were used for the wild type allele (197 bp product) and KO1 and KO2 were used for detection of the Sssbp2 null allele (457 bp product).

WT1,5′-GGAGGAGACTGCCGCTTAG – 3’

WT2, 5′ CACCTGTCAACCCATCACAG - 3’

KO1, 5′-GCCTGAAGAACGAGATCAGC - 3’

KO2, 5′-CAGAAGGTCGAGGGTTCAGA - 3’

Necropsy and histopathology

All organs were collected and fixed with 10% phosphate-buffered formalin and stored until they were processed for histology. A portion of each organ was embedded in paraffin, sectioned, and stained with hematoxylin and eosin were separately assessed by two different pathologists. Tissues were analyzed by light microscopy.

Protein Quantification

In order to verify protein was equally loaded, the filter was stained with Ponceau prior to immunoblotting. After immunoblotting, protein signal intensity was determined from densitometric scanning. The volume of protein density was measured by ImageQuant 5.2. Protein loading was normalized by Ponceau staining. This experiment was repeated for three

different mice from each group..

Real-time PCR

Total RNA was prepared by QIAGEN's RNeasy Micro (#74004) or Mini kits (#74106) according to the manufacturer's instructions. For first-strand cDNA synthesis, 1 μg RNA, 20 pmol oligo$(dT)_{12\text{-}18}$ and 200 units SuperScript II Reverse transcriptase (Invitrogen) were incubated a final volume of 20 μl. 1μl cDNA aliquots were added to 19 μl of PCR mixture containing 1x TaqMan Gene Expression Assay primers (Applied Biosystems) and 1x TaqMan Universal PCR Master Mix (4324018, Applied Biosystems). Each reaction was run in triplicate. Real-time PCR was performed in an ABI7900HT Sequence Detection System Machine (Applied Biosystems) using the following conditions: 40 cycles of 95^{o}C for 15 sec and 60^{o}C for 60 sec). The primers used were: Ssbp2: Mm00452502_m1; LDB1: Mm00440147_m1; and pTα: Mm00478363_m1. 18S rRNA (primer number 4319413E) served as an internal control. The specific position and length of the PCR products obtained were as described on the Applied Biosystems website.

Legends- Supplement

Table I. *Ssbp2* null mice are born alive

Crosses between *Ssbp2*$^{+/-}$ mice were monitored for live births. Tail snips from the offsprings were genotyped for the wild type and targeted alleles.

Table II. *Ssbp2*$^{-/-}$ mice are predisposed to lymphomas and carcinomas (A). Moribund mice (n=13) were euthanized, and all the organs examined for gross and histological abnormalities. Significant histological findings are reported.

(B) 12 "non- moribund" *Ssbp2*$^{-/-}$ mice between 60-80 weeks of age (median age= 68 weeks) were euthanized, organs collected and examined.

Table III. *Ssbp2*$^{-/-}$ mice are lymphopenic

Tail vein bleeds from *Ssbp2*$^{-/-}$ and age matched wild type were analyzed in an autoanalyzer for differential peripheral blood counts. Numbers in parantheses denote the number of mice showing anomalies. Mice aged 15-20 months.

Table IV. Immunophenotypes of *Ssbp2*$^{-/-}$*Trp53*$^{-/-}$ and *Ssbp2*$^{+/+}$*Trp53*$^{-/-}$ tumors

Tumors were stained with antibodies to CD3, CD4 and CD8 and analyzed by WinMDI 2.8 software. CD3 expression is using mean flurosence index (MFI) to show the expression level. Controls are 4 three months old wild type (*Ssbp2* $^{+/+}$,*Trp53*$^{+/+}$) mice. The table shows that while tumors from the *Ssbp2* $^{+/+}$, *Trp53*$^{-/-}$ mice exhibit different patterns of CD4/CD8 expression, most of the *Ssbp2* $^{-/-}$, *Trp53*$^{-/-}$ mice show an aberrant expression of $CD8^{+}CD4^{low}$ phenotype with a comparatively low expression of CD3.

Figures

Fig. 1 LMO2 expression in *Trp53*$^{-/-}$ and *Ssbp2* $^{-/-}$*Trp53*$^{-/-}$ thymic lymphomas. High LMO2 expression is only in *Ssbp2* $^{-/-}$ *Trp53*$^{-/-}$ but not in *Trp53*$^{-/-}$ thymic lymphomas. Nuclear extracts from thymic lymphomas from mice 1320, 1332, 1521 and 1386 (refer to Supplementary Table IV for immuno phenotype of these tumors) were analyzed by immunoblotting.

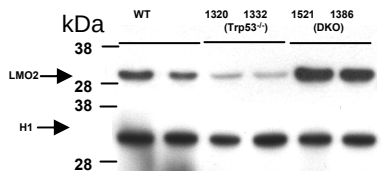
Fig.2 Marked increase in *Ssbp2* expression in DP thymocytes

Thymocytes from four wild type mice at four weeks of age were sorted for surface markers. Real-time PCRs were performed on purified cell populations using primers specific for *Ssbp2*. Results are average of two separate cDNA pools each with triplicate PCRs.

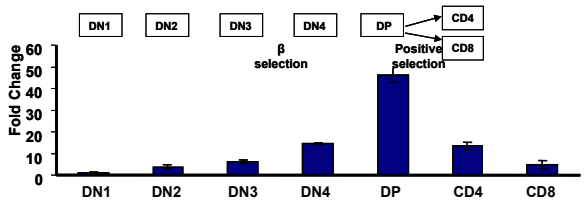
Fig.3 E2A half life is unaffected in *Ssbp2*$^{-/-}$ thymocytes

Nuclear lysates from short term cultured thymocytes were examined for E2A half life under conditions identical to Fig.6C. A non specific band detected by the antibody serves as internal control.

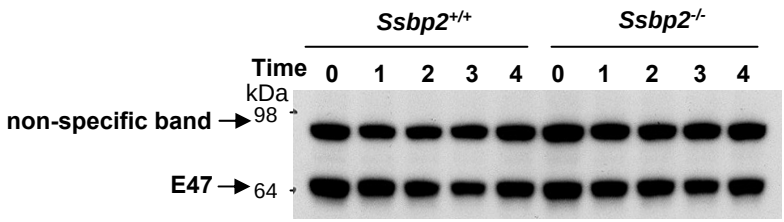
Wang_ et al., Supplementary Fig.1



Wang_ et al., Supplementary Fig.2



Wang_Supplementary Fig.3



E47 protein half life

Table I: Genotype of live births from *Ssbp2*$^{+/-}$ x *Ssbp2*$^{+/-}$ Crosses (n=172)

Genotype	No. of Pups	Expected(%)	Born(%)
Ssbp2$^{+/+}$	59	25	34
Ssbp2$^{+/-}$	79	50	46
Ssbp2$^{-/-}$	34	25	20

Table II A Histopathological Findings on Moribund Mice

Number (Gender)	Age (weeks)	Significant Pathological Findings
310 (M)	58	Chronic glomerulonephropathy, Anemia
373 (M)	82	Papillary Adenoma-lung, increased apoptosis in mesentric lymph nodes, glomeralunephropathy, Polyarteritis
435 (F)	64	Lymphocytic leukemia/lymphoma in spleen, lung, liver, pancreas, spinal cord
486 (F)	71	Systemic lymphoma, histiocytic sarcoma-multiple lymph nodes and spleen
559 (M)	68	Carcinoma-hardian gland, degenerative joint disease, generalized with compression of spinal cord
536 (F)	66	Lymphoma- lung, mediastinal lymph node, thymus, salivary gland; Histiocytic sarcoma- spleen
571 (F)	72	Lymphoplasmic infiltration in the lung, Uveitis, anemia
572 (F)	70	Lymphoma- jejunum, mesenteric lymph nodes, ovaries, uterus Adenoma-lung
576 (F)	65	Systemic lymphoma liver, kidney, lung, pancreas, urinary bladder
625 (F)	73	Lymphoma- pancreatic and spleen, thymus, lung mandibular lymph nodes
637 (M)	70	Pulmonary bronchio-alveolar carcinoma
692 (M)	66	Abnormal salivary gland with lymphoplasmocytic infiltration, mediastinal lymph node hyperplasia
693 (F)	60	Sialoadenitis, follicular hyperplasia in mandibular lymph node, generalized apoptosis of lymphocytes

Table II B Histopathological characterization of "non- moribund" mice

Number (Gender)	Age (weeks)	Significant Pathological Findings
294 (F)	80	Chronic glomerulonephropathy, Anemia
414 (M)	72	Lymphoma- lung, salivary gland, Dilatative cardiomyopathy
498 (M)	72	Lymphoma- spleen, Histocytic sarcoma- mandibular lymph node Sarcoma, unclassified- abdominal cavity
510 (F)	60	None
515 (M)	65	Cholecystitis
537 (M)	72	Chronic glomerulonephropathy
538 (M)	66	Lymphoma- mesenteric lymph node
545 (M)	66	Systemic polyarteritis, Cardiomyopathy
575 (F)	60	Adenocarcinoma- jejunum, Adenoma- lung, Systemic polyarteritis
616 (M)	73	Squamous cell carcinoma, leg
617 (M)	72	Lymphoma- lung, salivary gland
622 (M)	60	Chronic glomerulonephropathy

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Table III

Lymphopenia in 16-20 month old Ssbp2$^{-/-}$ mice

Mice	Number of mice analysed	WBC count, 109/liter (n)	Hb, g/dl(n)	Plt, 109/liter (n)	Granulocytes %	Lymphocytes %
+/+	9	7.8±1.8	14±2	1213±546	15±5	69±26
-/-	11	1.9±0.5(7)	6.0±2.5(6)	600±237(3)	73±8(7)	18±7(7)

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Table III. Immunophenotypes of *Ssbp2* $^{-/-}$ *Trp53*$^{-/-}$ and *Trp53*$^{-/-}$ tumors

Animal number	Genotype	CD4(%)	CD8(%)	CD4, CD8(%)	CD3 (MFI)
1685	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	0.7	51.2	47.8	25
1593	Ssbp2 $^{-/-}$, Trp53$^{-/--}$	0.2	76.9	19.7	44
1418	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	0.6	82.4	13.3	25
1477	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	0.5	86.6	11.6	38
1172	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	1.7	33.2	33.2	40
1521	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	3.3	21.4	42	15
111	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	0.2	51	46.3	18
60	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	1	82.5	12.7	24
44	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	0.3	28.1	70	18
42	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	1	77.6	16.1	17
1389	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	3.4	16.4	3.2	37
1386	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	0.5	59.4	6.4	18
1370	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	3	51.6	18.1	51
1330	Ssbp2 $^{-/-}$, Trp53$^{-/-}$	0.7	66.6	29.2	20
1445	Ssbp2 $^{+/+}$, Trp53$^{-/-}$	29	0.3	69.8	247
52	Ssbp2 $^{+/+}$, Trp53$^{-/-}$	56.7	2.6	21.7	121
71	Ssbp2 $^{+/+}$, Trp53$^{-/-}$	1.5	7.9	88.2	58
1845	Ssbp2 $^{+/+}$, Trp53$^{-/-}$	0.3	5.5	91.8	41
1320	Ssbp2 $^{+/+}$, Trp53$^{-/-}$	1.6	0.5	95.6	18
1332	Ssbp2 $^{+/+}$, Trp53$^{-/-}$	1.3	72.5	23.6	26
165	Ssbp2 $^{+/+}$, Trp53$^{-/-}$	15.9	4	5.7	10
control(4)	Ssbp2 $^{+/+}$, Trp53$^{+/+}$	8.97±2.13	3.8±0.83	84.3±2.59	58±11