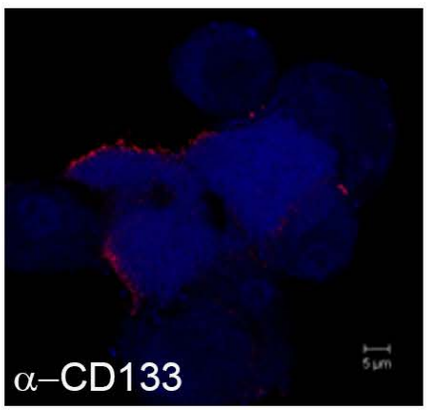
SUPPLEMENTARY FIGURE 1

a)



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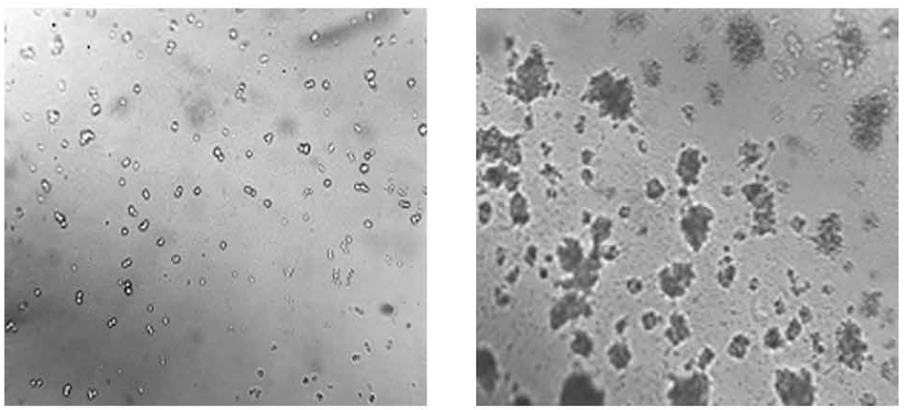
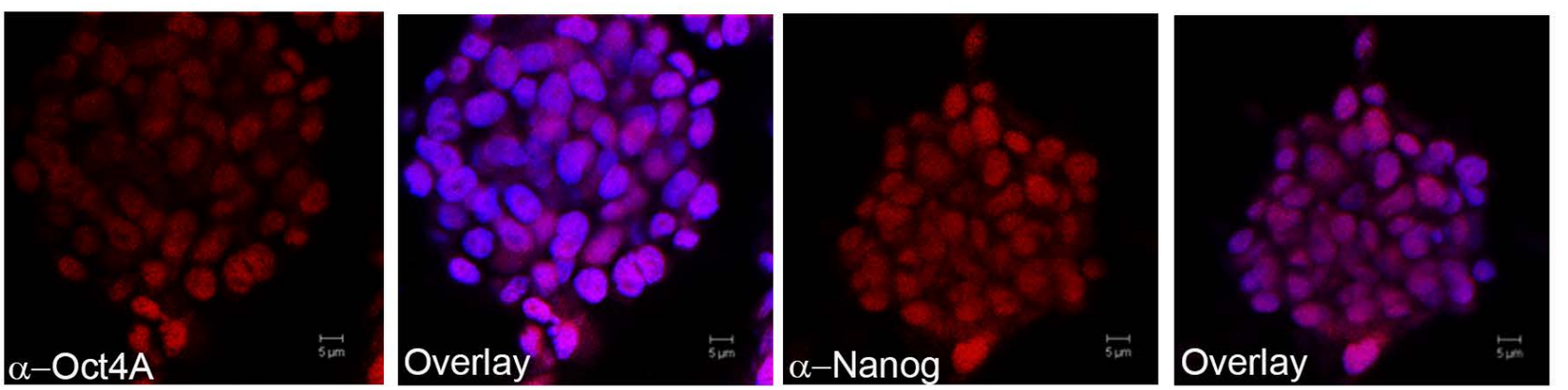
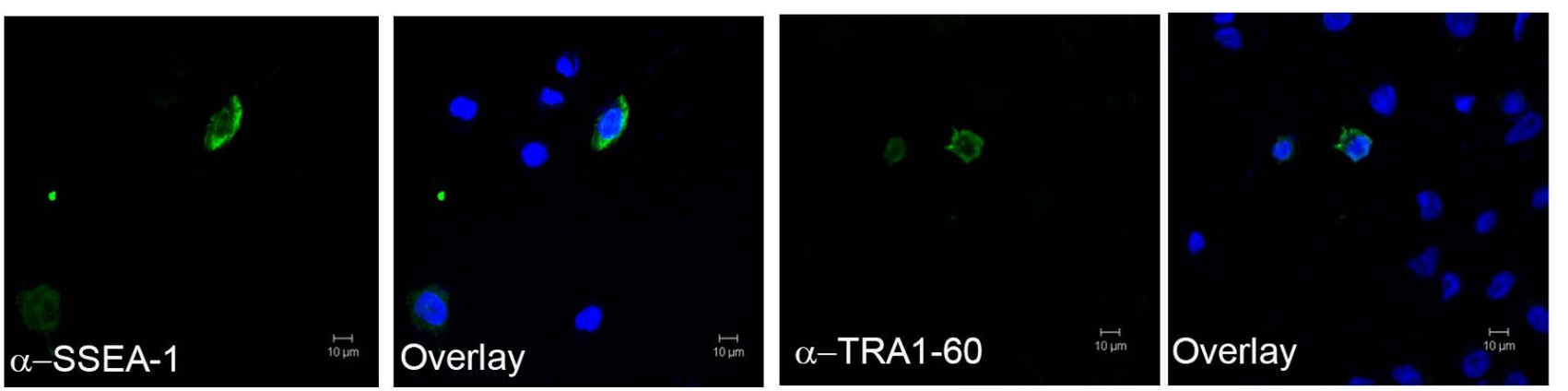


c)

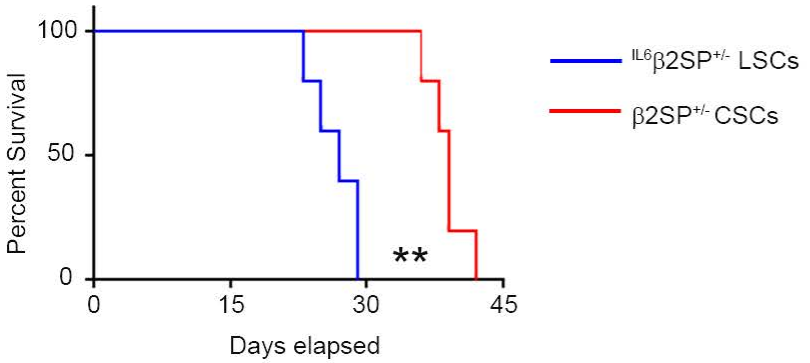


d)



SUPPLEMENTARY FIGURE 2

a)



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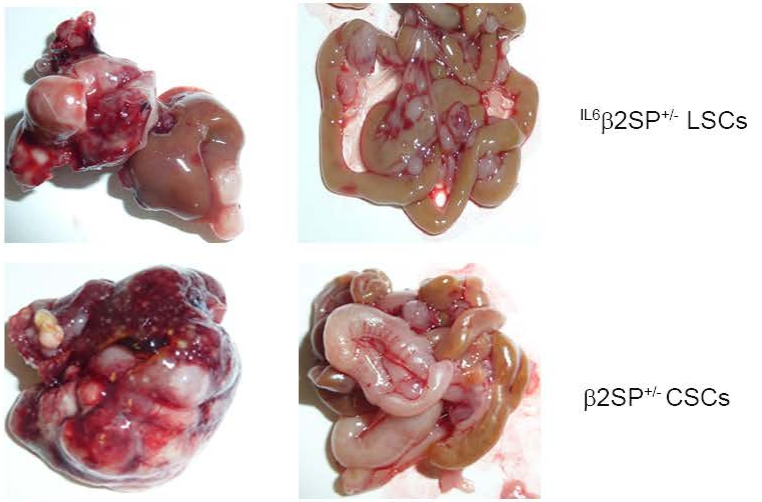
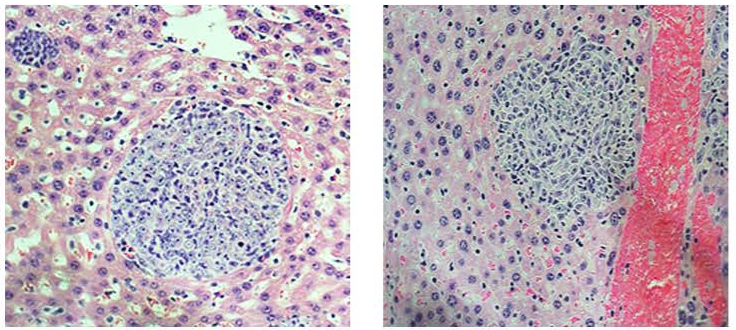


c)

IL6β2SP+/- LSCs

β2SP+/- CSCs

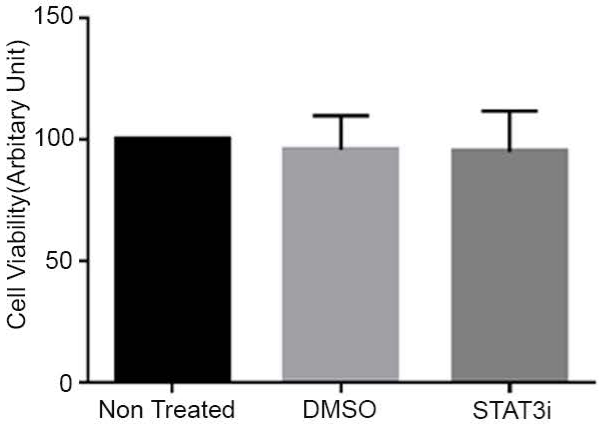


d)

Organ	IL6β2SP+/- LSCs	β2SP+/- CSCs
Liver	Primary	Primary
Lung	-	-
Kidney	-	-
Intestinal Lymph node	Metastasis	-
Bone	-	-
Heart	-	-

SUPPLEMENTARY FIGURE 3

a)



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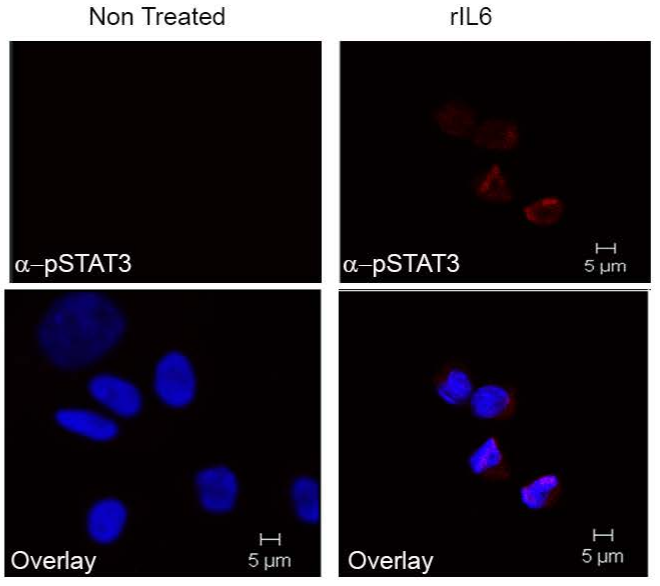

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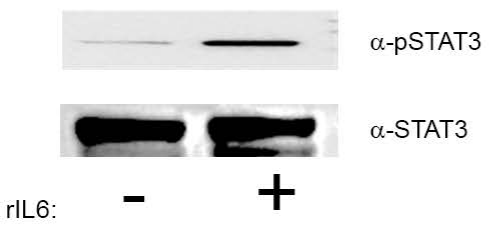

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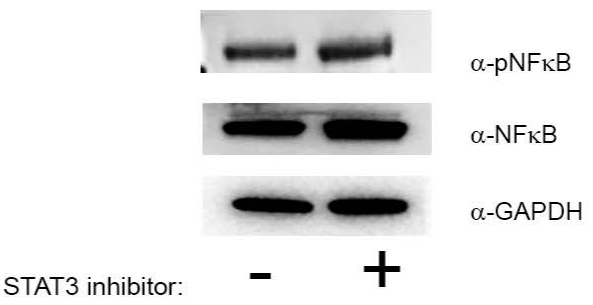
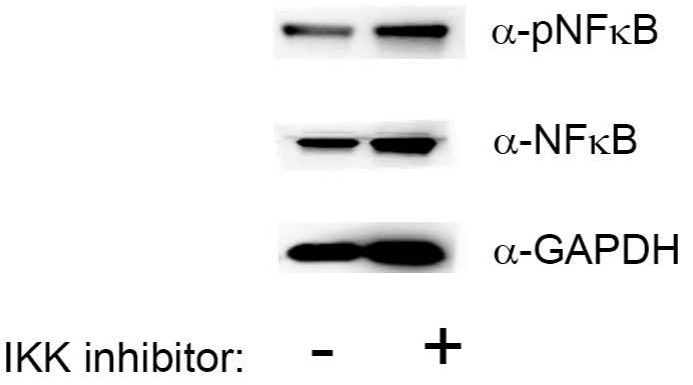
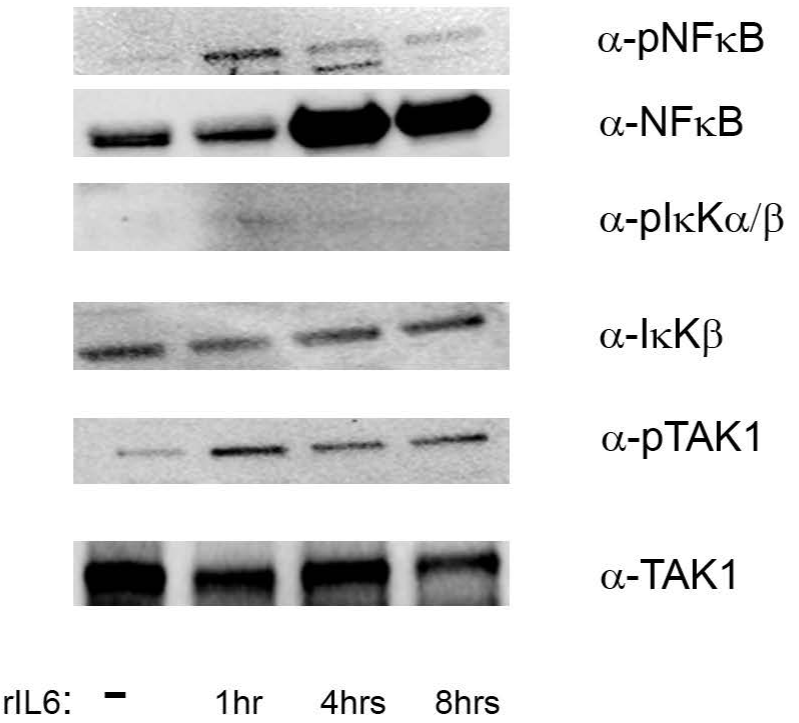


Supplementary Figure 4



Supplementary Figure 5



Supplementary Materials:

Supplementary Fig. 1. $CD133^+$ liver cells from $^{IL6}\beta2SP^{+/-}$ mice have stem-like properties. (a) Confocal microscopy images of α-CD133 immunohistochemical staining (red) and nuclear staining by Draq5 (blue). Immunofluorescent images were captured using PASCAL software on a Zeiss LSM 510 Meta confocal microscope. (b) Images of spheres formed in Matrigel by cells from $^{IL6}\beta2SP^{+/-}$ mice on days 1 and 6. (c) The stem cell–associated transcription factors Oct4A and Nanog were detected in Matrigel culture using fluorescence microscopy. (d) Confocal microscopy images of staining for the pluripotency markers α-SSEA-1 (green) and TRA-1-60 (green) and of nuclear Draq5 staining (blue).

Supplementary Fig. 2. Orthotopic inoculation of $CD133^+$ $^{IL6}\beta2SP^{+/-}$ LSCs showed poor survivability of mice and metastasis in intestinal lymph nodes. (a) $^{IL6}\beta2SP^{+/-}$ LSCs or $\beta2SP^{+/-}$ CSCs (1×10^5) were orthotopically injected into NSG mice. Kaplan-Meier survival curve is for overall survival of mice with orthotopic inoculation of $^{IL6}\beta2SP^{+/-}$ LSCs or $\beta2SP^{+/-}$ CSCs. The survival curve indicates that a statistically significant reduction of mice survivability upon orthotopic inoculation of $^{IL6}\beta2SP^{+/-}$ LSCs. (b) Representative images of liver tumor arising from $^{IL6}\beta2SP^{+/-}$ LSCs (left) and $\beta2SP^{+/-}$ CSCs (right). Intestinal lymph node metastasis were shown in $^{IL6}\beta2SP^{+/-}$ LSCs inoculated NSG mice. (c) Hematoxylin and eosin–stained sections of primary tumor tissues (original magnification x200). (d) Table represents the organs of primary tumor and metastasis upon orthotopic inoculation of $^{IL6}\beta2SP^{+/-}$ LSCs or $\beta2SP^{+/-}$ CSCs in NSG mice.

Supplementary Fig. 3. $^{IL6}\beta2SP^{+/-}$ LSCs do not have constitutive STAT3 phosphorylation. (a) Viability of $\beta2SP^{+/-}$ pIL6 derived mCSCs upon treated with S3I-201 (10 μM) was measured by

CyQUANT NF Cell Proliferation Assay reagent at 24 h. (b) Confocal microscopy images of immunohistochemical staining for α-pSTAT3 (red) and nuclear Draq5 staining (blue) of ^{IL6}β2SP$^{+/-}$ LSCs treated with or without recombinant IL6 (rIL6) for 1 h. Immunofluorescent images were captured using PASCAL software on a Zeiss LSM 510 Meta confocal microscope. (c) Western blotting for pSTAT3 and total STAT3 in untreated cells and cells treated with rIL6 (50 ng/ml) for 1 h. (d) Western blotting for pNFκB and total NFκB in untreated cells and cells treated with the STAT3 inhibitor S3I-201 (5 μM) 24 h.

Supplementary Fig. 4. IκK inhibition does not affect constitutive NFκB phosphorylation in ^{IL6}β2SP$^{+/-}$ LSCs. Western blotting for pNFκB, total NFκB, and GAPDH in untreated cells and cells treated with the IKK inhibitor amlexanox (2.5 μM) for 24 h.

Supplementary Fig. 5. Recombinant IL6 (rIL6) treatment induces the phosphorylation of TAK1 and NFκB in β2SP$^{+/-}$ CSCs in a time dependent manner. Western blotting for pNFκB, pTAK1, pIκK α/β, total NFκB, IκKβ and TAK1 in untreated cells and cells treated with rIL6 (50 ng/ml) for 1 h, 4h and 8h.

Invasion and migration assays

The invasion assay was performed using transwell inserts (Greiner Bio-One) in 24-well plates. The inserts were coated with 30 μl of phenol red–free Matrigel (BD Biosciences) for 1 h at 37°C. Approximately 1 x 10^5 cells in 100 μl of serum-free media were placed on the inserts, and 500 μl of media containing 10% fetal bovine serum was placed in each well of the 24-well plates. The

inserts were then placed in the media-containing wells, and the insert-containing plates were stored at 37°C overnight. The next day, the media was removed from the lower chambers, and the inserts were incubated with 500 µl of media containing 8 µM calcein-AM for 1 h at 37°C. Then, the inserts were transferred to new wells containing 500 µl of pre-warmed 1 mM EDTA to detach the cells from the outer surface of the insert. One hundred microliters of cell suspension was transferred to each well (triplicate) of 96-well plates, and fluorescence was measured using a SpectraMax microplate reader. The migration assay was performed in a similar fashion; however, the inserts were not coated with Matrigel, and cells inside the inserts were removed with cotton-tipped swabs after overnight incubation.

Subcutaneous injection of CD133^{+} LSCs into NSG mice

Freshly sorted CD133^{+} ^{IL6}β2SP$^{+/-}$ LSCs or CD133^{+} β2SP$^{+/-}$ LSCs (1 x 10^{5}) were subcutaneously injected into 8- to 10-week-old female NSG mice. The mice were humanely sacrificed in accordance with guidelines approved by MD Anderson's Institutional Animal Care and Use Committee.

Luciferase assays

^{IL6}β2SP$^{+/-}$ LSCs (1 x 10^{6}) were nucleofected with an empty plasmid, a plasmid encoding the NFκB response element, or a plasmid encoding luciferase under the cyotmegalovirus promoter using a Nucleofector kit (program Q25). Pharmacological inhibitors were added after 6 h of nucleofection.

Gross metastasis assay

$^{IL6}\beta2SP^{+/-}$ LSCs or shNFκB $^{IL6}\beta2SP^{+/-}$ LSCs (5×10^3) were injected intraosseously into the right legs of NSG mice. The right legs were amputated 2 weeks after injection, and the mice were humanely killed 2 weeks after amputation. Their livers were resected, and the gross metastases in each liver were counted. A digital camera was used to photograph the resected livers.

TAK1 mRNA expression analysis

The overall survival rates of HCC patients were stratified by TAK1 (MAP3K7) gene expression levels. HCC patients' overall survival data and TAK1 mRNA expression data were retrieved from The Cancer Genome Atlas (Liver Hepatocellular Carcinoma [TCGA, Provisional]; (http://www.cbioportal.org/, accessed Nov. 12, 2015). HCC patients were ranked by TAK1 gene expression level; the top 50% of patients were placed in the $TAK1^{High}$ group, and the bottom 50% of patients were placed in the $TAK1^{Low}$ group.

Statistical analyses.

Animals were randomly selected for control and treatment groups. All data were calculated as the mean+/- the standard error of the mean using GraphPad Prism v5.00. One-way/ Two-way ANOVA with Tukey's post-*hoc* test was used to analyze the data. Mann-Whitney's rank sum test was used to calculate the statistical significance of the results. To reduce the bias caused by influential points, we applied linear regression using Huber's M estimator (Huber 1981) for robust estimation when comparing the correlation between IL6 with B2Sp by cancer stage. F-test

was used to assess the overall heterogeneity by stage and t-test was used to compare the pairwise difference.