SUPPLEMENTAL MATERIAL

Barrott et al., https://dx.doi.org/10.1084/jem.20160817

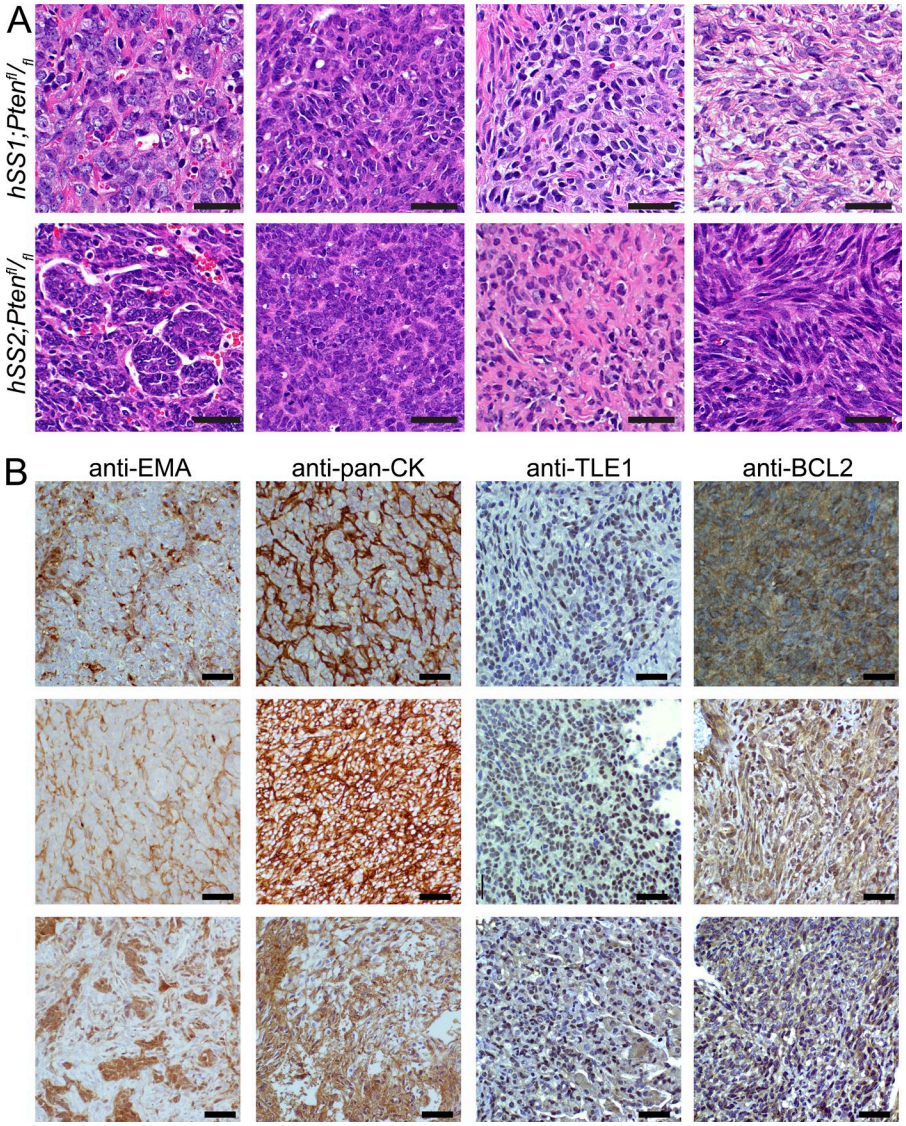

Figure S1. ***Pten* disruption enhances synovial sarcomagenesis.** (A) Photomicrographs of H&E histology demonstrating the range of mesenchymal and epithelial characteristics in *SS18-SSX*–induced synovial sarcomas from each *Pten* genotype in TATCre-injected mice. (B) Photomicrographs after immunohistochemistry on *hSS;Pten*$^{fl/fl}$ tumor tissue sections with noted primary antibodies, demonstrating characteristic SS staining patterns. Bars, 25 µm.

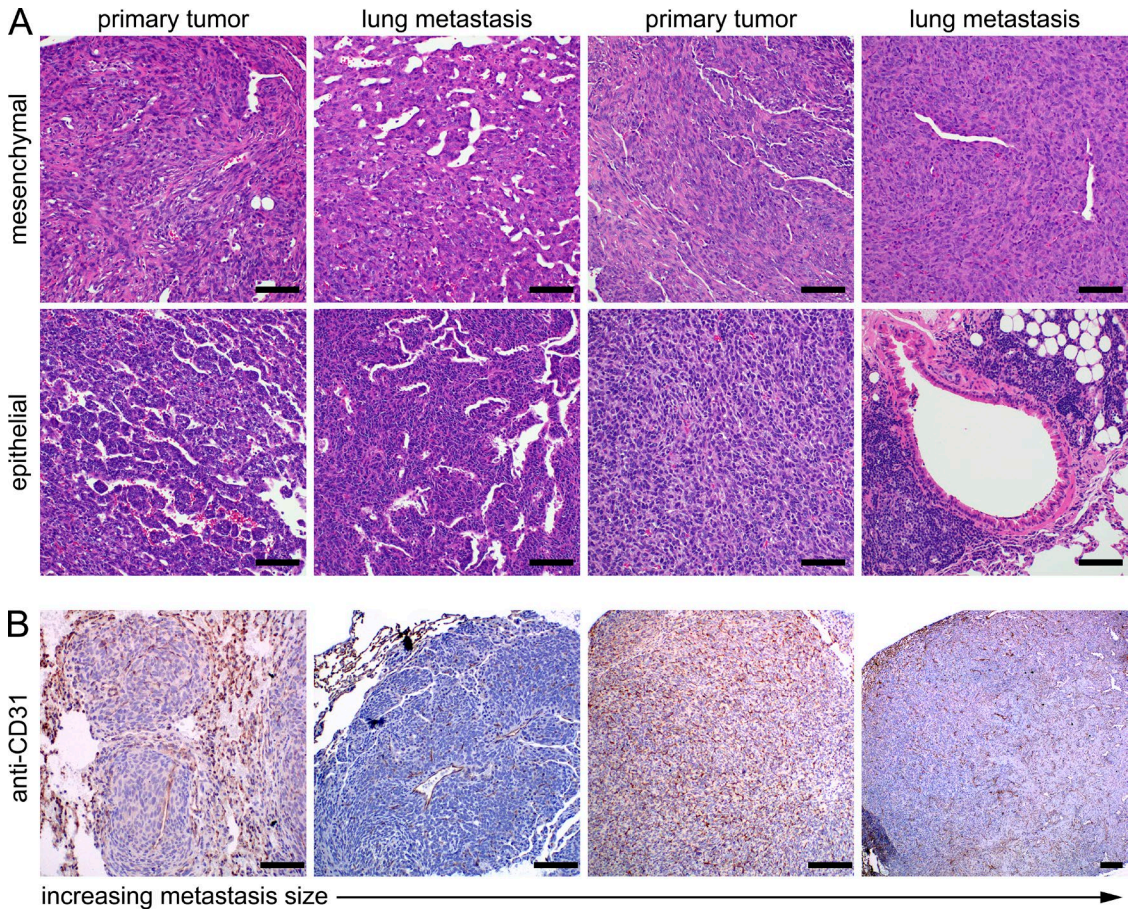

Figure S2. **Lung metastases retain primary tumor histology and E-cadherin expression and increases in vascularity with size.** (A) Photomicrographs of tumors with retained histological appearance between primary tumor and metastasis. (B) Examples of anti-CD31 immunohistochemistry photomicrographs demonstrating vascular density in *hSS;Pten* lung metastases. Bars, 100 µm.

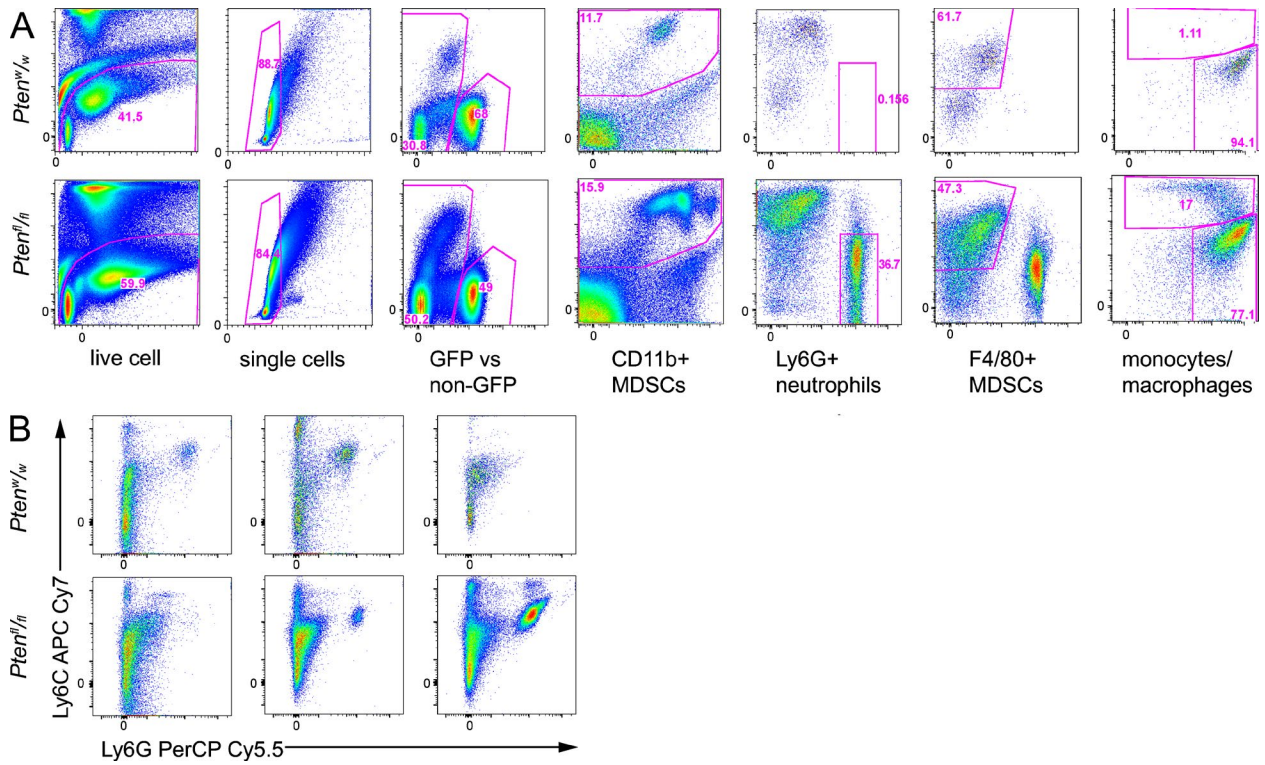

Figure S3. ***Pten* disruption associates with increased infiltration of myeloid-derived cells.** (A) Example flow cytometry comparing an *hSS;Pten*$^{w/w}$ tumor (top) to an *hSS;Pten*$^{fl/fl}$ tumor (bottom) for showing the hierarchal gates used for sorting F4/80^{+}/GFP^{-} monocytes/macrophages (sixth plot), their MHCII^{+}/Ly6C^{high} newly recruited monocyte (seventh) and MHCII^{+}/Ly6C^{mid} tissue macrophage (seventh plot) subpopulations, and Ly6C^{+}/Ly6G^{+} neutrophils (fifth plot). (B) Examples of CD11b^{+} flow cytometry comparing *hSS;Pten*$^{w/w}$ tumors (top) to *hSS;Pten*$^{fl/fl}$ tumors (bottom). Current display demonstrates Ly6C^{+}/Ly6G^{+} neutrophils, Ly6C^{high}/Ly6G^{-} monocytes, and Ly6C^{mid}/Ly6G^{-} macrophages.

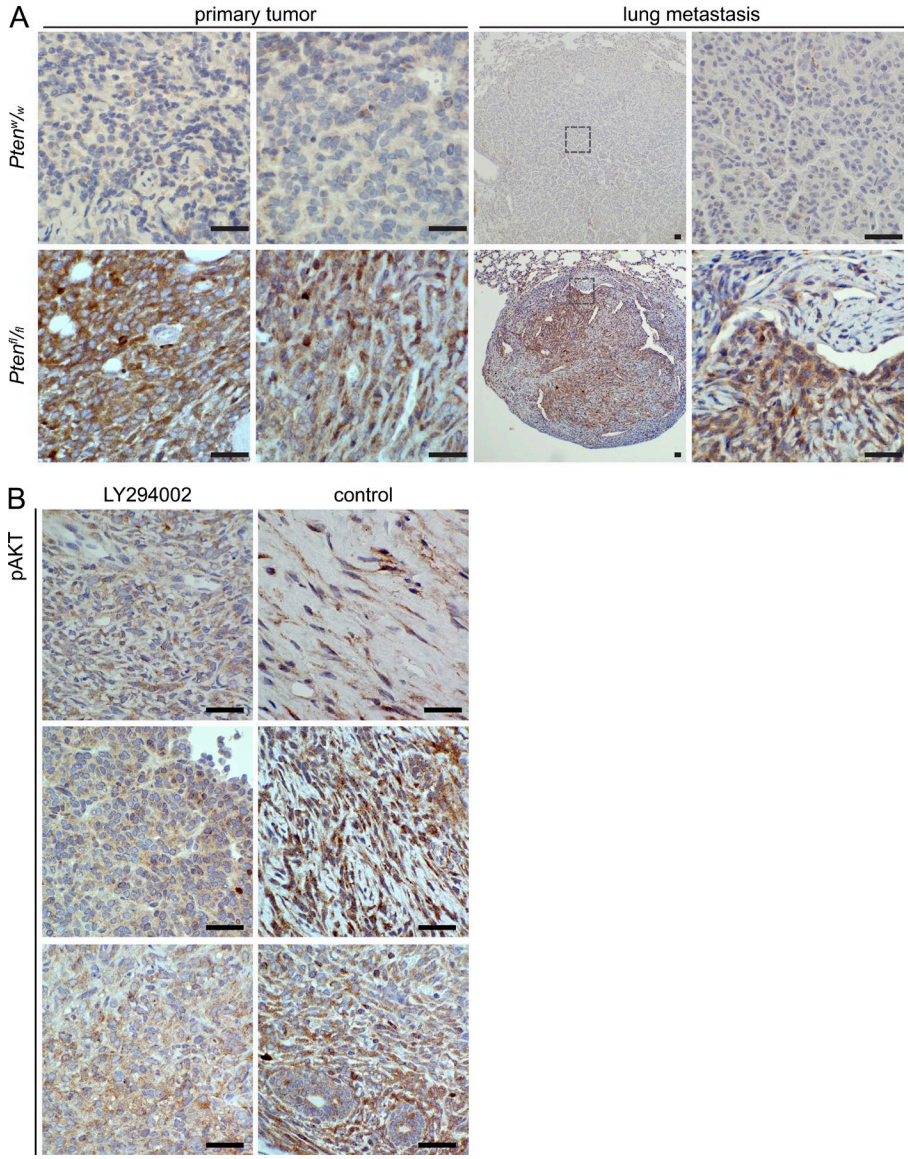

Figure S4. **PI3′-lipid signaling drives phosphorylation of AKT and downstream expression of CSF1.** (A) Representative photomicrographs of pAKT immunohistochemistry in *hSS;Pten*$^{w/w}$ and *hSS;Pten*$^{fl/fl}$ primary tumors (left) and *hSS;Pten*$^{fl/fl}$ lung metastasis (right). (B) Representative photomicrographs of pAKT immunohistochemistry in *hSS;Pten*$^{fl/fl}$ primary tumors after 1-wk treatment of LY294002 or control. Bars, 20 µm.

Tables S1–S5 are available as Excel files. Table S1 lists differential gene expression between *Pten*$^{w/w}$ and *Pten*$^{fl/fl}$ synovial sarcomas. Table S2 lists ingenuity pathway analysis of potential upstream regulators between *Pten*$^{w/w}$ and *Pten*$^{fl/fl}$. Table S3 lists differential gene expression between primary and lung metastases *Pten*$^{fl/fl}$ synovial sarcomas. Table S4 lists Ingenuity pathway analysis for potential upstream regulators between primary and lung metastases *Pten*$^{fl/fl}$ synovial sarcomas. Table S5 Nanostring gene expression on sorted cell poplulations with normalized values based on prevelance in flow cytometry.

Table S6. **Antibody list**

Protein	Company (cat. no.)	Application and dilution
phopho-Akt	Cell Signaling Technology (4060)	WB (1:1,000)
pan-Akt	Cell Signaling Technology (4391)	WB (1:1,000)
phospho-PTEN	Cell Signaling Technology (9551)	WB (1:1,000)
pospho-CSF1R	Thermo Fisher Scientific (MA5-15151)	WB (1:1,000) and IHC (1:50)
phospho-Akt	Santa Cruz Biotechnology, Inc. (sc-135650)	WB (1:200) and IHC (1:50)
GAPDH	EMD Millipore (MAB374)	WB (1:500)
Ly-6C	BD (560596)	Flow cytometry (1:1,000)
CD11b	BD (552850)	Flow cytometry (1:1,000)
MHC II (I-A/I-E)	BD (562367)	Flow cytometry (1:1,000)
F4/80	BD (565410)	Flow cytometry (1:1,000)
Ly-6G	BD (560602)	Flow cytometry (1:1,000)
E-cadherin	R&D Systems (FAB7481A)	Flow cytometry (1:100)
CD68	Abcam (ab125212)	IHC (1:500)
CD3	Abcam (ab16669)	IHC (1:200)
CD31	Abcam (ab28364)	IHC (1:1,000)
Ki67	Abcam (ab15580)	IHC (1:1,000)
GFP	Santa Cruz Biotechnologies, Inc. (sc-8334)	IHC (1:100)
BCL-2	Dako (M0887)	IHC (1:100)
Pan-CK	EMD Millipore (MAB3412)	IHC (1:1,000)
TLE1	Santa Cruz Biotechnology, Inc. (sc-9121)	IHC (1:100)
EMA	Dako (M0613)	IHC (1:100)

Table S7. **Primer list**

Gene	Forward sequence (5′–3′)	Reverse sequence (5′–3′)
Rosa26-LSL	GGATCGACGGTATCGTAGAG	CGCGAAAGCCACAGACATGTT
CSF1 (mouse)	ACAACACCCCCAATGCTAAC	GCTGTTGTTGCAGTTCTTGG
CSF1 (human)	GGAGTCTGTCTTCCACCTGC	GAATCCGCTCTCTGAGGCTC
GAPDH (mouse)	TGTCAGCAATGCATCCTGCA	CCGTTCAGCTCTGGGATGAC
GAPDH (human)	GTCAAGGCTGAGAACGGGAA	GCCTTCTCCATGGTGGTGAA
Rosa wt (genotyping)	GTTATCAGTAAGGGAGCTGCAGTGG	GGCGGATCACAAGCAATAATAACC
Rosa lox (genotyping)	AAGACCGCGAAGAGTTTGTCCTC	GGCGGATCACAAGCAATAATAACC
Pten (genotyping)	CAAGCACTCTGCGAACTGAG	AAGTTTTTGAAGGCAAGATGC