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MYC Regulates the Anti-Tumor Immune Response through CD47 and PD-L1

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Abstract

The *MYC* oncogene codes for a transcription factor that is overexpressed in many human cancers. Here we show that *MYC* regulates the expression of two immune checkpoint proteins on the tumor cell surface, the innate immune regulator, CD47 (**C**luster of **D**ifferentiation 47) and the adaptive immune checkpoint, PD-L1 (programmed death-ligand 1). Suppression of MYC in mouse tumors and human tumor cells caused a reduction in the levels of CD47 and PD-L1 mRNA and protein. MYC was found to bind directly to the promoters of the CD47 and PD-L1 genes. MYC inactivation in mouse tumors downregulated CD47 and PD-L1 expression and enhanced the anti-tumor immune response. In contrast, when MYC was inactivated in tumors with enforced expression of CD47 or PD-L1, the immune response was suppressed and tumors continued to grow. Thus MYC appears to initiate and maintain tumorigenesis in part through the modulation of immune regulatory molecules.

MYC is a transcription factor that regulates the expression of a multitude of gene products involved in cell proliferation, growth, differentiation, and apoptosis (1–4). The *MYC* gene is genetically activated and overexpressed in many human cancers (1–4) and this overexpression has been causally linked to tumorigenesis (5, 6). Work with inducible transgenic mouse models has shown that growth of *MYC*-induced tumors is dependent on continuous expression of MYC (1–4, 7–10). For example, in the tetracycline-off mouse model (where *MYC* expression can be turned off by the addition of tetracycline or doxycycline), tumors grow only when *MYC* is “on.” When *MYC* is turned “off,” tumors regress.

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Supplementary Materials

Materials and Methods

Figs. S1-S17, Table S1

Supplemental References 46–54

MYC inactivation in mouse models results in tumor regression through the induction of proliferative arrest and apoptosis (1–3, 7, 8, 10–12). We have demonstrated that complete tumor clearance following the inactivation of oncogenes, including *MYC*, requires the recruitment of $CD4^+$ T cells and the secretion of Thrombospondin-1 (13, 14). Hence, a host-dependent immune response is required for sustained tumor regression. However, the mechanism by which oncogene inactivation elicits this immune response is unknown.

The host immune system generally serves as a barrier against tumor formation (15). Activation of the immune response can contribute to tumor regression (13, 16, 17) through both adaptive and innate immune effectors (18–20). Programmed death-ligand 1 (or PD-L1, also known as CD274 and B7-H1) is a critical “don’t find me” signal to the adaptive immune system (21–23), whereas CD47 is a critical “don’t eat me” signal to the innate immune system as well as a regulator of the adaptive immune response (24, 25) (Fig. S1A). These and similar molecules are often overexpressed on human tumors (22, 25). Therapeutic suppression of PD-L1 and other immune checkpoint molecules elicit an immune response against tumors and recently this strategy has been translated to the clinic, with very encouraging results (26–29).

To explore whether and how MYC regulates the anti-tumor response, we examined its effect on the expression of CD47 and PD-L1 in the Tet-off transgenic mouse model of MYC-induced T cell acute lymphoblastic leukemia (MYC T-ALL). When MYC was “on,” both CD47 and PD-L1 were expressed. However, *in vitro* or *in vivo MYC* inactivation resulted in a rapid downregulation of CD47 and PD-L1, both at the mRNA level, as detected by quantitative real-time PCR (qPCR), and at the protein level, as detected by flow cytometry (Fig. 1A–B) and immunofluorescence (Fig S1B). Expression of other immune-related surface receptors was not affected by MYC inactivation (Fig. 1A). Consistent with these observations, suppression of MYC expression in the human T-ALL cell lines, CCRF-CEM and Jurkat, either by treatment with a *MYC*-targeting shRNA (Fig. S2A) or with the bromodomain and extra-terminal (BET) inhibitor, JQ1 (30) reduced the expression of CD47 and PD-L1 (Fig. 1C). Treatment of MYC T-ALL cells with the chemotherapeutic drugs prednisone, cytoxan, cisplatin, or vincristine, resulted in tumor cell death. However, CD47 and PD-L1 were either unaffected or showed increased expression (Fig. S3A) and there was no effect on CD3, CD8, CD25, and CD69 expression (Fig. S3B–E).

We next investigated the effect of *MYC* inactivation on CD47 and PD-L1 in mouse and human solid tumors. In a Tet-off transgenic mouse model of hepatocellular carcinoma (HCC) (3), inhibition of MYC expression resulted in decreased levels of CD47 and PD-L1 protein (Fig. S4A–B) and mRNA (Fig. S4B); expression of the two proteins was unaffected by cisplatin treatment (Fig. S4A). In the human HCC cell line HEPG2, shRNA knockdown of MYC caused a reduction in the levels of both CD47 and PD-L1 mRNA (Fig. S4C). We also investigated the relationship between MYC expression and CD47 and PD-L1 expression in the human melanoma cell line SKMEL28 (Fig. 2A) and the human non-small cell lung cancer (NSCLC) cell line H1299 (Fig. 2B), as these cells represent tumor types that are often treated with immune checkpoint inhibitors in the clinic (31). We found that *MYC* shRNA knockdown and MYC functional suppression by JQ1 reduced the expression of

CD47 and PD-L1 mRNA and protein as measured by qPCR and flow cytometry, respectively.

In additional experiments we found that *MYC* shRNA knockdown (Fig. S2B) or JQ1 treatment of four independent primary human T-ALL samples reduced both CD47 and PD-L1 cell surface expression (Fig. S5). Cisplatin treatment increased CD47 and PD-L1 expression while CD8 expression was unaffected by the treatments (Fig. S5). Lastly, we examined publicly available gene expression data derived from human primary tumors. Notably, in human HCC, renal cell carcinoma (RCC), and colorectal carcinoma (CRC), MYC expression significantly correlated with the expression of both CD47 and PD-L1 (Fig. S6). Thus, MYC regulates CD47 and PD-L1 expression in multiple human tumor types.

MYC can act as a general transcriptional amplifier (that is, it can generally increase expression of many genes rather than specific target genes), but dosage-dependent specific effects have been reported (32–36). We applied ChIP (Chromatin ImmunoPrecipitation)-Seq analysis to mouse MYC T-ALL cells (34) and the human B cell line P493-6 (37, 38) and found high levels of MYC bound to the promoter regions of the genes coding for CD47 and PD-L1 (Fig. 2C, Fig. S7–S8). In contrast, we observed that both MYC T-ALL (Fig. S7) and P493-6 (Fig. S8) cells with high MYC levels had lower, often non-significant binding to the promoters of other cell surface immune molecules such as CD8a and CD25. Oncogenic levels of MYC bound the CD47 and PD-L1 gene promoters in human osteosarcoma U2OS cells, whereas low levels of MYC did not (Fig. S9). In a nuclear run-on assay with P493-6 cells, MYC induced expression of the CD47 gene along with other well-known target genes such as PDK1, CHEK1, CDK2, LDHA, and ODC1 (Fig. S10A–B). PD-L1 expression was too low to measure changes in this experiment. Thus, we conclude that MYC binds to the promoters and directly regulates the expression of the CD47 and PD-L1 genes. An alternative but not mutually exclusive possibility is that MYC suppression acutely affects CD47 and PD-L1 surface protein expression by reducing the half-lives of the two proteins. However, we did not observe the increased turnover of CD47 or PD-L1 proteins compared to other immune surface proteins in mouse MYC T-ALL cells when we inhibited protein synthesis by cycloheximide treatment (Fig. S11).

We have shown previously that MYC inactivation in mouse tumor models results in recruitment of immune cells to the tumors (13). To investigate the role of CD47 and PD-L1 in this process, we engineered MYC T-ALL 4188 cells to constitutively express CD47 or PD-L1 (Fig. S12A). In this overexpression system, CD47 and PD-L1 mRNA levels were unaffected by MYC inactivation (Fig. S12B). The recruitment of luciferase-labeled $CD4^+$ T cells (Fig. 3A), $CD69^+$ activated T cells, and $F4/80^+$ macrophages (Fig. 3B and Fig. S13) following MYC inactivation was suppressed when CD47 and PD-L1 were constitutively expressed by the tumor cells. CD47 or PD-L1 expression prevented the sustained tumor regression that has been observed with MYC inactivation (Fig. 4A) without affecting MYC expression (Fig. 4B). Enforced expression of CD47 or PD-L1 increased minimal residual disease (tumor cells remaining) resulting in tumor recurrence (Fig. 4C–D). Conversely, shRNA knockdown of CD47 or PD-L1 prevented the growth of MYC T-ALL cells *in vivo* (Fig. S14).

MYC inactivation induces tumor regression through both cell autonomous mechanisms, including proliferative arrest and induction of apoptosis, as well as through host-dependent mechanisms such as inhibition of tumor angiogenesis and induction of tumor cell senescence. We investigated the effect of enforced expression of CD47 or PD-L1 on these mechanisms. We found that CD47 or PD-L1 expression prevented the shutdown of angiogenesis following MYC inactivation, as measured by the presence of $CD31^+$ microvessels (Fig. 4E, S15A) and expression of Ang2 and Tie2 (Fig. S15C). The induction of tumor cell senescence as measured by β-galactosidase (SA-β-gal) (Fig. 4F, S15B) and p15Ink4b and p19ARF levels (Fig. S15D) was also affected, but we did not observe an effect on apoptosis or proliferation as evaluated by Annexin-V and 7-AAD (Fig. S16A), cleaved caspase 3 (CC3) (Fig. S16B, D), and Phospho-histone H3 (PH3) (Fig. S16C, E). Therefore, the downregulation of CD47 and PD-L1 appears to be required for the induction of sustained tumor regression, the shutdown of angiogenesis, and senescence induction promoted by MYC inactivation.

We conclude that MYC regulation of CD47 and PD-L1 expression has a direct role in the initiation and maintenance of MYC-driven tumorigenesis (Fig. 4). MYC overexpression may be one general mechanism by which tumor cells upregulate the expression of immune checkpoint regulators, thereby evading immune surveillance. MYC inactivation has been proposed to restore the immune response against tumors (Fig. S17) (39–41).

MYC suppression rapidly resulted in decreased mRNA and protein expression of CD47 and PD-L1, suggesting a transcriptional regulatory mechanism. MYC is a general transcriptional amplifier that can regulate gene expression through a multitude of mechanisms (32–34). However, as noted above, MYC also exhibits gene dosage transcriptional effects (36, 42). The relatively high levels of MYC expression that are associated with rapid proliferation and tumorigenesis may induce CD47 and PD-L1 expression.

Because transcription of their genes is regulated by MYC, CD47 and PD-L1 may be expressed at higher levels at steady state than other membrane proteins in tumors. Notably, MYC activation of the CD47 and PD-L1 genes appears to require higher levels of MYC binding to the CD47 and PD-L1 promoters compared with genes involved in normal cell growth; they may, therefore, represent promoters that have been “invaded” by oncogenic MYC levels (33, 42). Thus, these genes may be particularly sensitive to MYC withdrawal.

MYC activation may influence cancer immunoediting through the suppression of immune surveillance against tumor cells. We propose that during tumor evolution, high MYC expression results in increased expression of CD47 and PD-L1, suppressing both the innate and the adaptive immune response and favoring tumor growth (Fig. S17). Upon MYC inactivation, loss of the “don’t find me” and “don’t eat me” signals allow for the destruction of residual tumor cells and consequently, sustained tumor regression.

Although the effects of MYC on the expression of CD47 and PD-L1 were modest, the consequences on tumor regression were dramatic, consistent with reports that small influences on immune regulators can have marked effects (25). CD47 and PD-L1 both may also contribute to the tumor microenvironment through influence on T cell activation and

angiogenesis (13, 14, 22, 43–45). CD47 is the receptor for Thrombospondin-1, which may regulate cellular programs including angiogenesis, self-renewal and senescence (13, 14, 45). We speculate that therapies suppressing MYC expression and activity may restore an immune response against human cancers. MYC-overexpressing human cancers may be especially vulnerable to an immune checkpoint blockade.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and notes

1. Felsher DW, Bishop JM. Reversible tumorigenesis by MYC in hematopoietic lineages. Molecular cell. 1999; 4:199–207. [PubMed: 10488335]
2. Jain M, et al. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. Science. 2002; 297:102–104. [PubMed: 12098700]
3. Shachaf CM, et al. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. Nature. 2004; 431:1112–1117. [PubMed: 15475948]
4. Wu CH, et al. Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:13028–13033. [PubMed: 17664422]
5. Sharma VM, et al. Notch1 contributes to mouse T-cell leukemia by directly inducing the expression of c-myc. Molecular and cellular biology. 2006; 26:8022–8031. [PubMed: 16954387]
6. Weng AP, et al. c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. Genes & development. 2006; 20:2096–2109. [PubMed: 16847353]
7. Marinkovic D, Marinkovic T, Mahr B, Hess J, Wirth T. Reversible lymphomagenesis in conditionally c-MYC expressing mice. International journal of cancer. Journal international du cancer. 2004; 110:336–342. [PubMed: 15095297]
8. Hennighausen L, Wall RJ, Tillmann U, Li M, Furth PA. Conditional gene expression in secretory tissues and skin of transgenic mice using the MMTV-LTR and the tetracycline responsive system. Journal of cellular biochemistry. 1995; 59:463–472. [PubMed: 8749716]
9. Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. Nature reviews. Cancer. 2002; 2:764–776. [PubMed: 12360279]
10. D'Cruz CM, et al. c-MYC induces mammary tumorigenesis by means of a preferred pathway involving spontaneous Kras2 mutations. Nature medicine. 2001; 7:235–239.
11. Fisher GH, et al. Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. Genes & development. 2001; 15:3249–3262. [PubMed: 11751631]
12. Huettner CS, Zhang P, Van Etten RA, Tenen DG. Reversibility of acute B-cell leukaemia induced by BCR-ABL1. Nature genetics. 2000; 24:57–60. [PubMed: 10615128]

13. Rakhra K, et al. CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. Cancer cell. 2010; 18:485–498. [PubMed: 21035406]
14. Giuriato S, et al. Sustained regression of tumors upon MYC inactivation requires p53 or thrombospondin-1 to reverse the angiogenic switch. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:16266–16271. [PubMed: 17056717]
15. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001; 357:539–545. [PubMed: 11229684]
16. Gattinoni L, Powell DJ Jr, Rosenberg SA, Restifo NP. Adoptive immunotherapy for cancer: building on success. Nature reviews. Immunology. 2006; 6:383–393.
17. Galon J, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. The Journal of pathology. 2014; 232:199–209. [PubMed: 24122236]
18. Shanker A, et al. CD8 T cell help for innate antitumor immunity. J Immunol. 2007; 179:6651–6662. [PubMed: 17982055]
19. Corthay A, et al. Primary antitumor immune response mediated by CD4+ T cells. Immunity. 2005; 22:371–383. [PubMed: 15780993]
20. Qin Z, Blankenstein T. CD4+ T cell--mediated tumor rejection involves inhibition of angiogenesis that is dependent on IFN gamma receptor expression by nonhematopoietic cells. Immunity. 2000; 12:677–686. [PubMed: 10894167]
21. Parsa AT, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nature medicine. 2007; 13:84–88.
22. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Current opinion in immunology. 2012; 24:207–212. [PubMed: 22236695]
23. Tsushima F, et al. Interaction between B7-H1 and PD-1 determines initiation and reversal of T-cell anergy. Blood. 2007; 110:180–185. [PubMed: 17289811]
24. Jaiswal S, et al. CD47 is upregulated on circulating hematopoietic stem cells and leukemia cells to avoid phagocytosis. Cell. 2009; 138:271–285. [PubMed: 19632178]
25. Majeti R, et al. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell. 2009; 138:286–299. [PubMed: 19632179]
26. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015; 161:205–214. [PubMed: 25860605]
27. Chao MP, et al. Therapeutic antibody targeting of CD47 eliminates human acute lymphoblastic leukemia. Cancer research. 2011; 71:1374–1384. [PubMed: 21177380]
28. Woo SR, Corrales L, Gajewski TF. Innate immune recognition of cancer. Annual review of immunology. 2015; 33:445–474.
29. Galluzzi L, et al. Classification of current anticancer immunotherapies. Oncotarget. 2014; 5:12472–12508. [PubMed: 25537519]
30. Filippakopoulos P, et al. Selective inhibition of BET bromodomains. Nature. 2010; 468:1067–1073. [PubMed: 20871596]
31. Homet Moreno B, Parisi G, Robert L, Ribas A. Anti-PD-1 therapy in melanoma. Seminars in oncology. 2015; 42:466–473. [PubMed: 25965365]
32. Nie Z, et al. c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. Cell. 2012; 151:68–79. [PubMed: 23021216]
33. Lin CY, et al. Transcriptional amplification in tumor cells with elevated c-Myc. Cell. 2012; 151:56–67. [PubMed: 23021215]
34. Walz S, et al. Activation and repression by oncogenic MYC shape tumour-specific gene expression profiles. Nature. 2014; 511:483–487. [PubMed: 25043018]
35. Wiese KE, et al. The role of MIZ-1 in MYC-dependent tumorigenesis. Cold Spring Harbor perspectives in medicine. 2013; 3:a014290. [PubMed: 24296348]
36. Li Y, Choi PS, Casey SC, Dill DL, Felsher DW. MYC through miR-17-92 suppresses specific target genes to maintain survival, autonomous proliferation, and a neoplastic state. Cancer cell. 2014; 26:262–272. [PubMed: 25117713]

37. Schuhmacher M, et al. Control of cell growth by c-Myc in the absence of cell division. Current biology : CB. 1999; 9:1255–1258. [PubMed: 10556095]

38. Sabo A, et al. Selective transcriptional regulation by Myc in cellular growth control and lymphomagenesis. Nature. 2014; 511:488–492. [PubMed: 25043028]

39. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011; 331:1565–1570. [PubMed: 21436444]

40. Dranoff G. Experimental mouse tumour models: what can be learnt about human cancer immunology? Nature reviews. Immunology. 2012; 12:61–66.

41. Mittal D, Gubin MM, Schreiber RD, Smyth MJ. New insights into cancer immunoediting and its three component phases—elimination, equilibrium and escape. Current opinion in immunology. 2014; 27:16–25. [PubMed: 24531241]

42. Wolf E, Lin CY, Eilers M, Levens DL. Taming of the beast: shaping Myc-dependent amplification. Trends in cell biology. 2015; 25:241–248. [PubMed: 25475704]

43. Waclavicek M, et al. T cell stimulation via CD47: agonistic and antagonistic effects of CD47 monoclonal antibody 1/1A4. J Immunol. 1997; 159:5345–5354. [PubMed: 9548474]

44. Avice MN, Rubio M, Sergerie M, Delespesse G, Sarfati M. Role of CD47 in the induction of human naive T cell anergy. J Immunol. 2001; 167:2459–2468. [PubMed: 11509584]

45. Kaur S, et al. Thrombospondin-1 signaling through CD47 inhibits self-renewal by regulating c-Myc and other stem cell transcription factors. Scientific reports. 2013; 3:1673. [PubMed: 23591719]

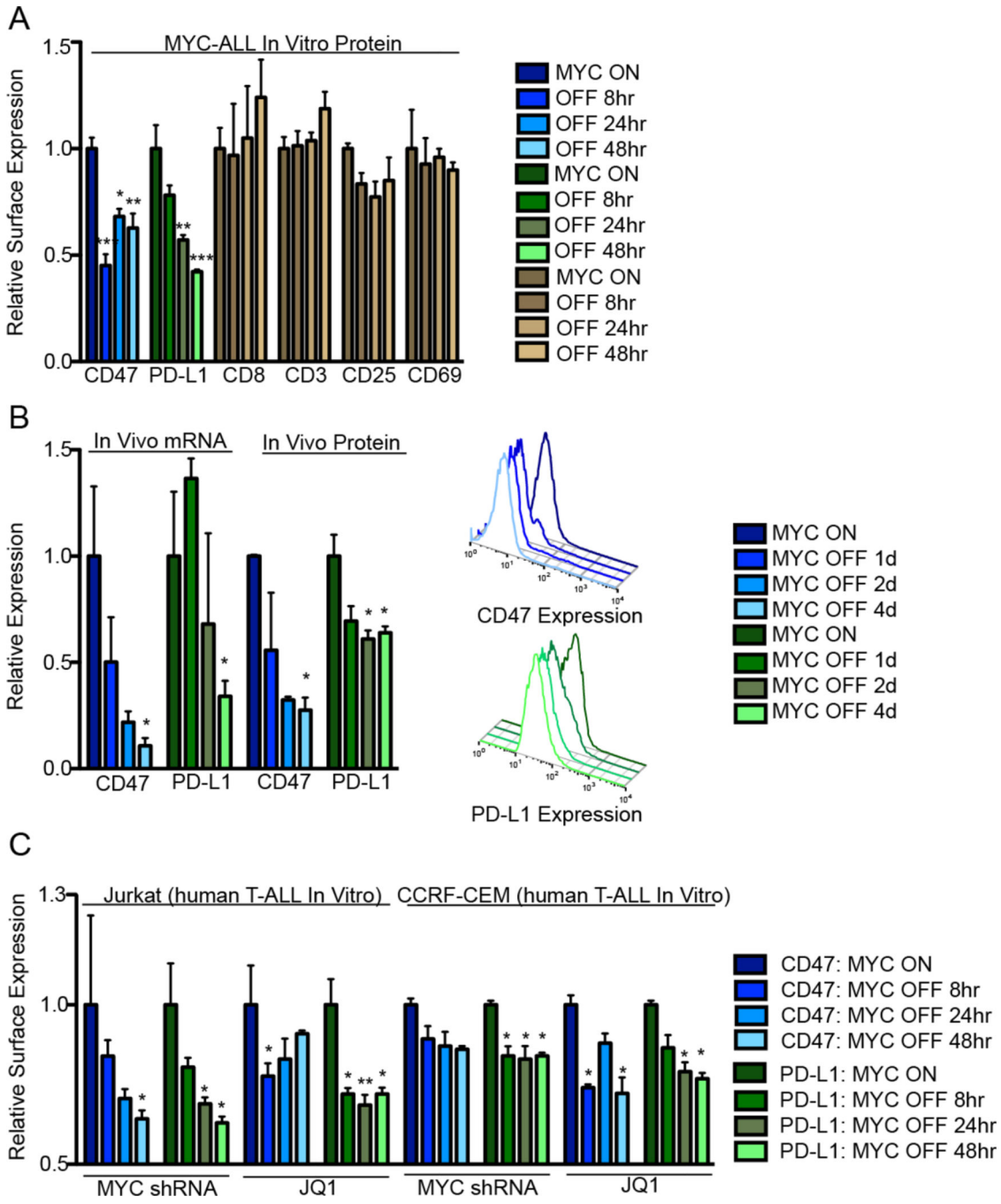

Fig. 1. MYC regulates the expression of CD47 and PD-L1 in murine and human leukemia and lymphomas

(A) Flow cytometry median fluorescence intensity (MFI) was used to determine the relative cell surface expression of CD47 (blue), PD-L1 (green), and other immune proteins following MYC inactivation in MYC T-ALL 4188 cells *in vitro* (n=3). **(B)** Tumors were harvested from primary MYC-driven lymphomas 0 or 4 days following MYC inactivation. mRNA and protein levels were quantified by qPCR and flow cytometry MFI (n=3 tumors per condition). Representative flow cytometry histograms are shown to the right. **(C)** CD47 (blue) and PD-

L1 (green) protein levels in Jurkat and CCRF-CEM cells were quantified by flow cytometry MFI following MYC inhibition by conditional shRNA knockdown or 10 μM JQ1 treatment (n=3 biological replicates).

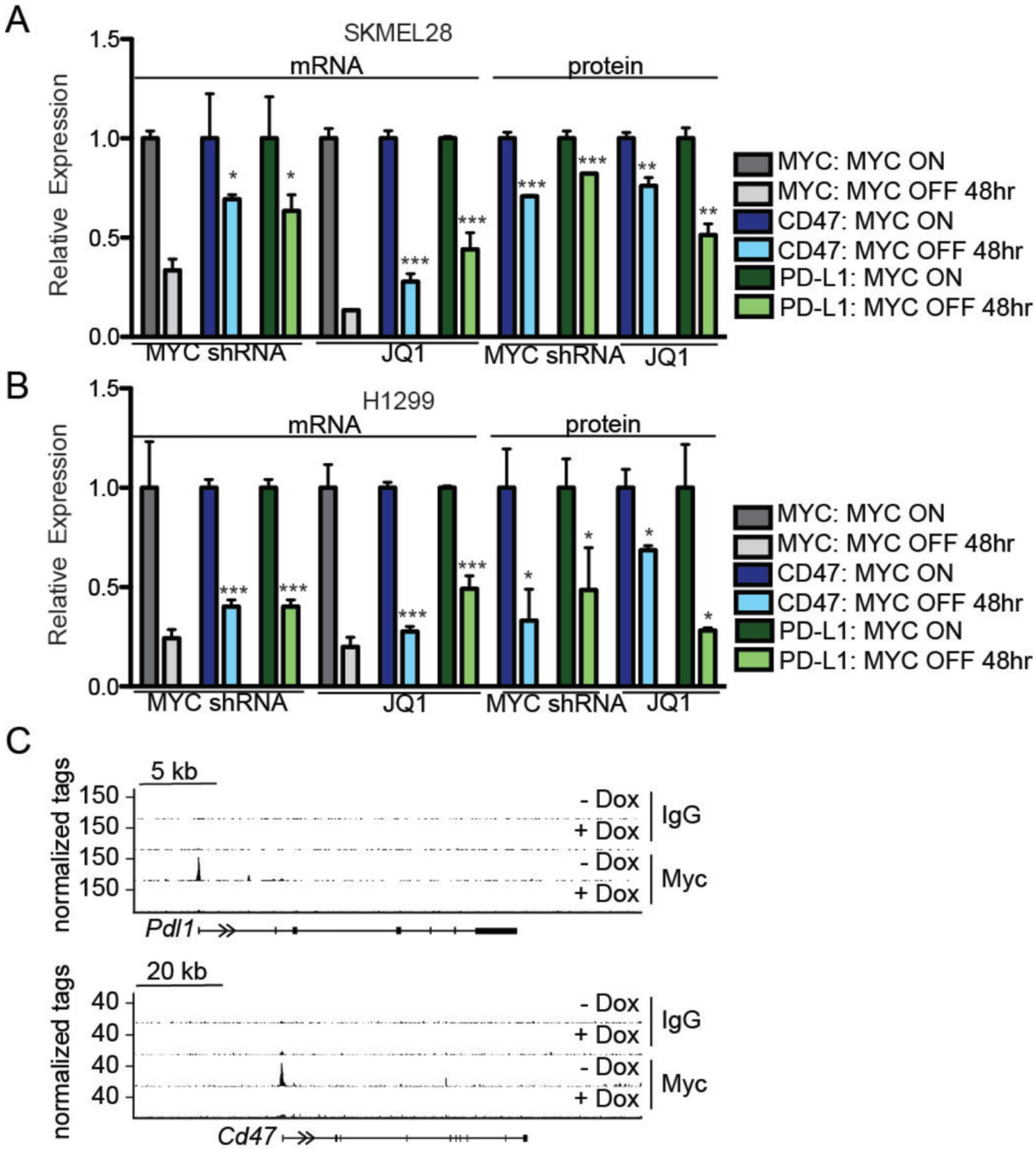

Fig. 2. MYC regulates CD47 and PD-L1 expression in human and mouse tumors and binds to the promoters of the corresponding genes

(A) and **(B)** The mRNA and protein levels of MYC (gray), CD47 (blue), and PD-L1 (green) in human melanoma SKMEL28 and human NSCLC H1299 cells were determined by qPCR and flow cytometry MFI, respectively, 48 hours after MYC inactivation *in vitro*. MYC was inactivated by 10 μM JQ1 treatment or MYC shRNA knockdown (n=3 biological and 3 technical replicates for qPCR and 3 biological replicates for flow cytometry). **(C)** ChIP-seq analysis of MYC binding to the promoter sequence of the genes encoding CD47 and PD-L1

in mouse MYC T-ALL cells. IgG was used as a negative control. ChIP-sequencing traces were generated from GSE44672 (34). Exons are represented as vertical bars, the untranslated region (UTR) is represented by a black line, and arrows indicate the direction of transcription.

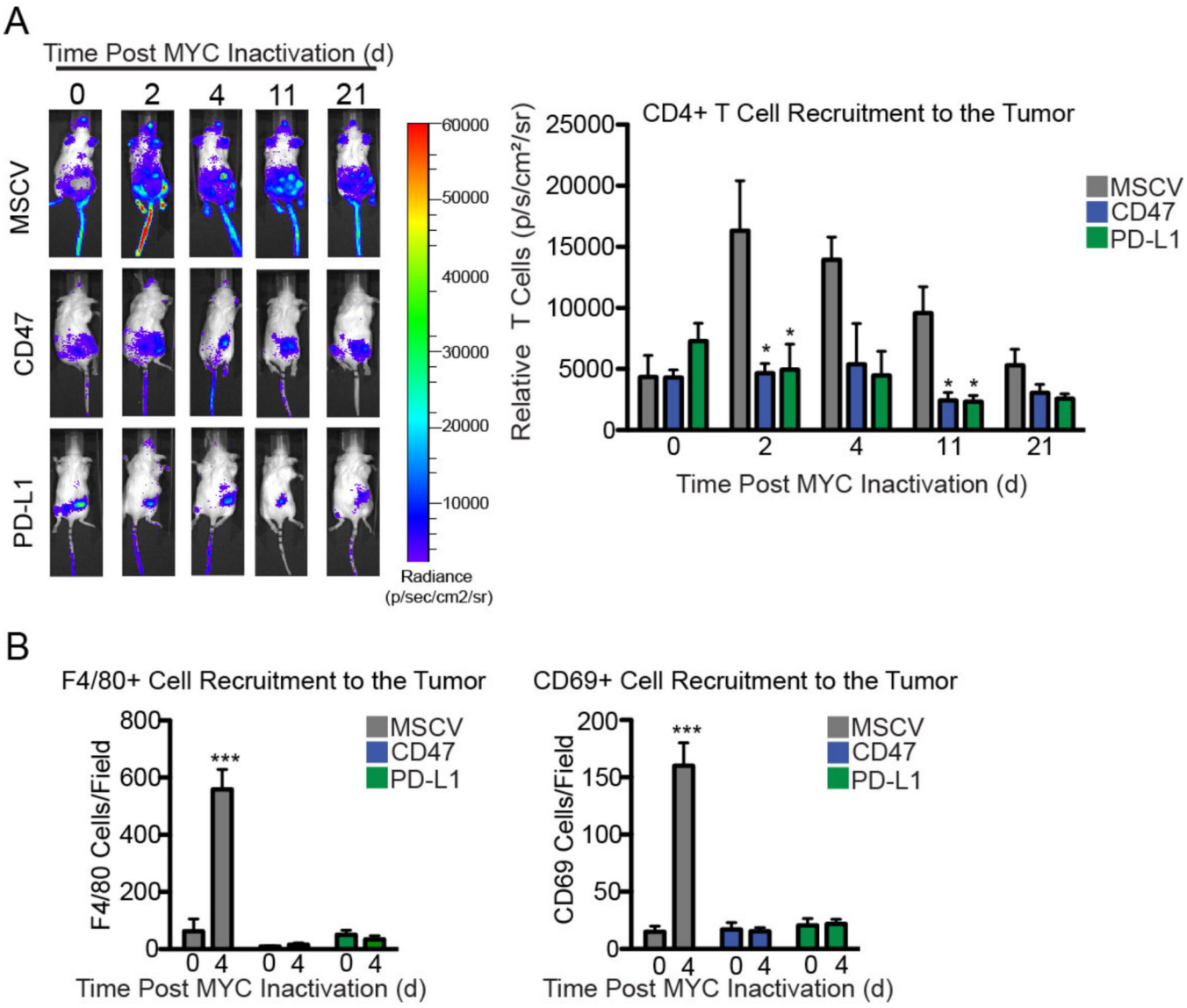

Fig. 3. Constitutive expression of CD47 and PD-L1 in mouse MYC T-ALL 4188 cells prevents recruitment of immune effectors after MYC inactivation

(A) Quantification of $CD4^+$ T cells in transplanted control (gray) or constitutive CD47 or PD-L1-expressing (colored) tumors before 2, 4, 11 or 21 days after MYC inactivation. Control, CD47-expressing, or PD-L1- expressing MYC T-ALL 4188 tumor cells were transplanted into FVB RAG1-/- mice one week after reconstitution with $fLuc^+$ $CD4^+$ T cells. Administration of Dox to inactivate MYC in established tumors is day 0. Left panel: representative bioluminescence images of tumor-bearing RAG1−/− animals. Right panel: average bioluminescence signal of the T cells is shown (n=5 tumors per group). **(B)** Quantification of $F4/80^+$ or $CD69^+$ cells in transplanted control (gray) or constitutive CD47 or PD-L1-expressing (colored) tumors before or 4 days after MYC inactivation by immunohistochemistry using markers for macrophages (F4/80) and activated T cells (CD69). Tumor cells were transplanted into WT FVB hosts. Administration of Dox to inactivate MYC in established tumors is day 0. The y axis denotes the number of positively

staining cells per field. For representative images, see Fig. S13. Data represent mean ± SEM derived from measurements of 3 independent tumors and 3 measurements per tumor.

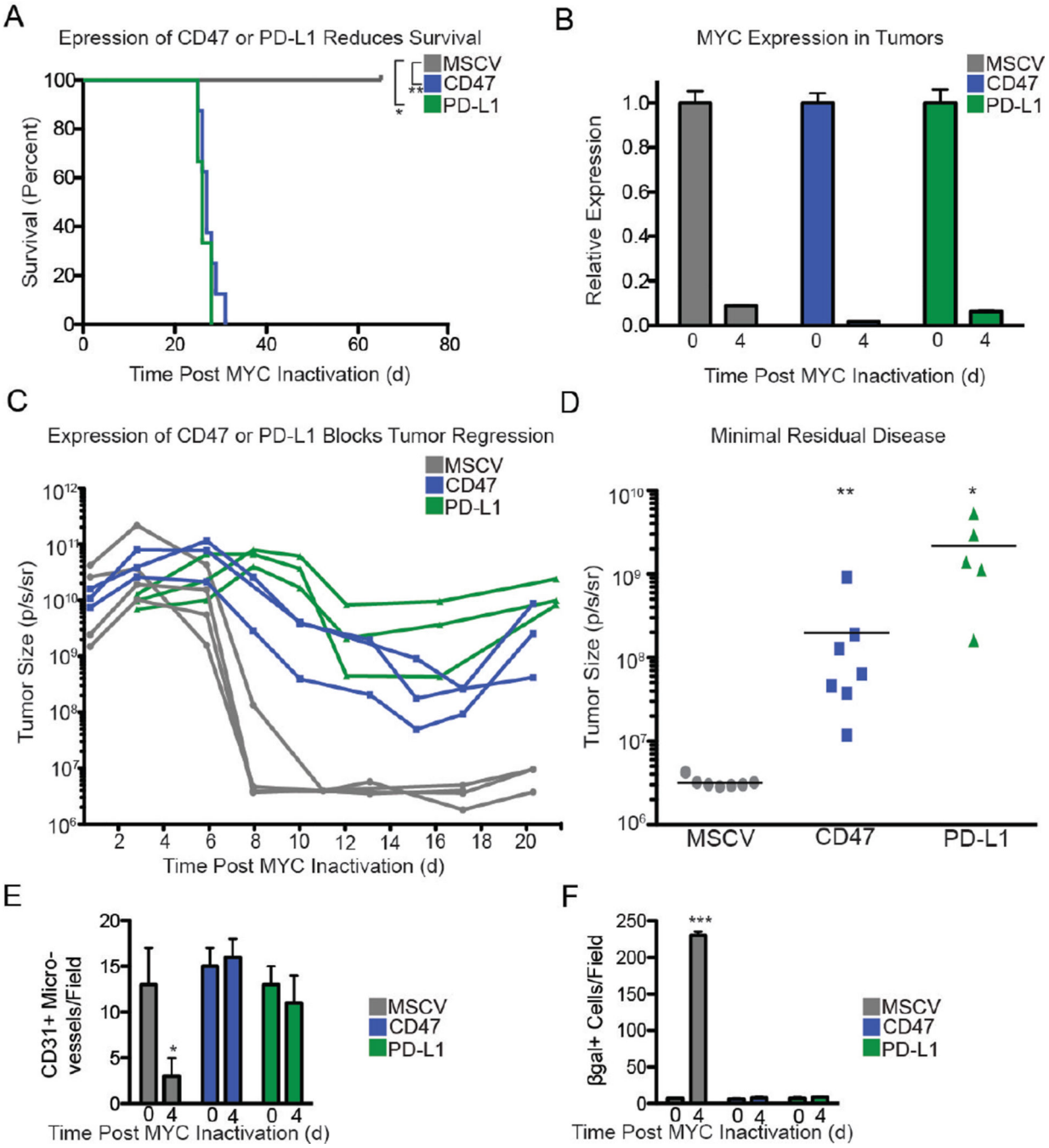

Fig. 4. Down-Regulation of CD47 or PD-L1 is Required for Tumor Regression, Shutdown of Angiogenesis, and Induction of Senescence upon MYC inactivation

(A) Survival after MYC inactivation of syngeneic FVB/N mice that had been transplanted with either MSCV control (gray), CD47-expressing (blue), or PD-L1-expressing (green) fLuc$^+$ MYC T-ALL cells. MYC was inactivated when tumors reached 1.5 cm^3 (d0). (n=5 for control, n=10 for CD47, and n=5 for PD-L1). **(B)** MYC expression before (d0) or after MYC inactivation (d4). **(C)** Bioluminescence imaging measurement of tumor burden before and after MYC inactivation in control (gray), CD47-expressing (blue), and PD-L1-

expressing (green) tumors. Three representative animals are shown per group. **(D)** Minimal residual disease (remaining tumor cells) after MYC inactivation was measured by bioluminescence imaging. (**E**) Angiogenesis was measured 0 and 4 days after MYC inactivation in control, CD47-expressing, and PD-L1- expressing tumors growing in WT FVB hosts by immunofluorescence for CD31. For representative images, see Fig. S15. **(F)** Control, CD47-expressing, and PD-L1-expressing tumors were analyzed by immunostaining for senescence associated β-galactosidease (SA-β- gal) in tumors described in (E). The y axis denotes the number of positively staining microvessels (E) or cells (F) per field. For representative images, see Fig. S15B. Data represent mean ± SEM derived from measurements of 3 independent tumors and 3 measurements per tumor.