## SUPPLEMENTAL MATERIAL

## Green et al., https://doi.org/10.1084/jem.20170356

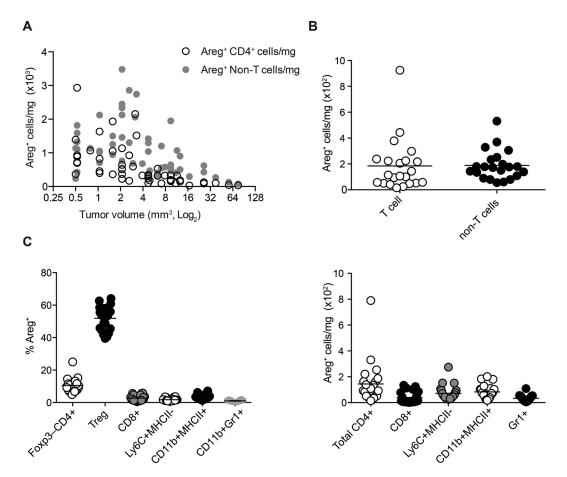


Figure S1. **Production of Areg by T cells and non–T cells in LLC lung tumors.** 150,000 LLC cells were injected intravenously into WT mice, and tumors that formed were analyzed from day 12 to 22. Individual tumor nodules as well as lungs from untreated mice were measured and weighed before being dissociated enzymatically and stimulated ex vivo with PMA and ionomycin for flow cytometric analysis of Areg production by the indicated cell populations. (A) Quantification of Areg-producing CD4<sup>+</sup> T cells and CD3<sup>-</sup> non–T cells in tumors of indicated volumes. Data points represent individual tumors from 20 mice. (B) Total numbers of Areg-producing T cells and non–T cells. (C) Percentages and numbers of indicated CD45<sup>+</sup> cell populations producing Areg upon ex vivo stimulation with PMA and ionomycin. In B and C, data points represent 22 individual tumors pooled from 10 mice. Horizontal lines indicate the mean.

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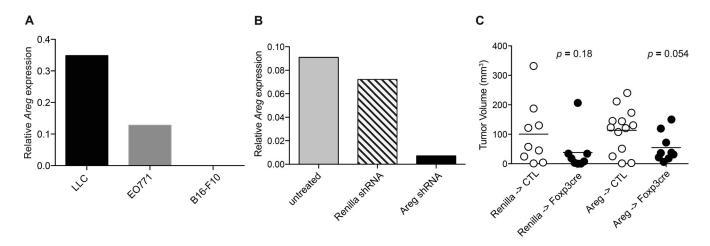
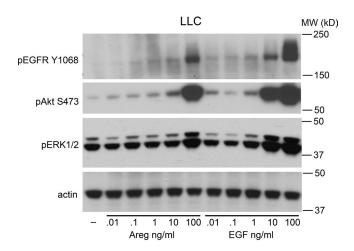


Figure S2. **Role of tumor-produced Areg in tumor progression.** (A) Plot shows quantitative real-time PCR data of Areg expression by indicated tumor cell lines. (B and C) LLC cells were transduced with shRNA expression plasmids targeting either control Renilla or Areg. (B) Relative expression of Areg in untreated LLC cells as well as cells expressing Renilla or Areg shRNA targeting plasmids. (C) 250,000 LLC cells expressing the indicated shRNA constructs were injected into  $Areg^{FL/FL}$  control (CTL) and  $Areg^{FL/FL}$  Foxp3<sup>YFP-cre</sup> mice (Foxp3cre), after which tumor-bearing lungs were harvested, and tumor volumes were measured at day 21–23. P-values as indicated; n = 8-13 per group, pooled from two independent experiments. Horizontal lines indicate the mean.



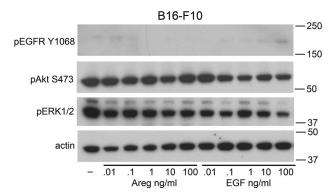


Figure S3. **EGFR signaling upon Areg stimulation in LLC and B16–F10 tumor cell lines.** LLC and B16–F10 cells were stimulated in vitro with indicated concentrations of rmAreg or recombinant mouse EGF for 10 min, followed by cell lysis and Western blotting for activation of phosphorylated signaling molecules in the EGFR pathway.

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