

supplementary information

**Inhibition of A20 expression in tumor microenvironment exerts anti-tumor effect
through inducing myeloid-derived suppressor cells apoptosis**

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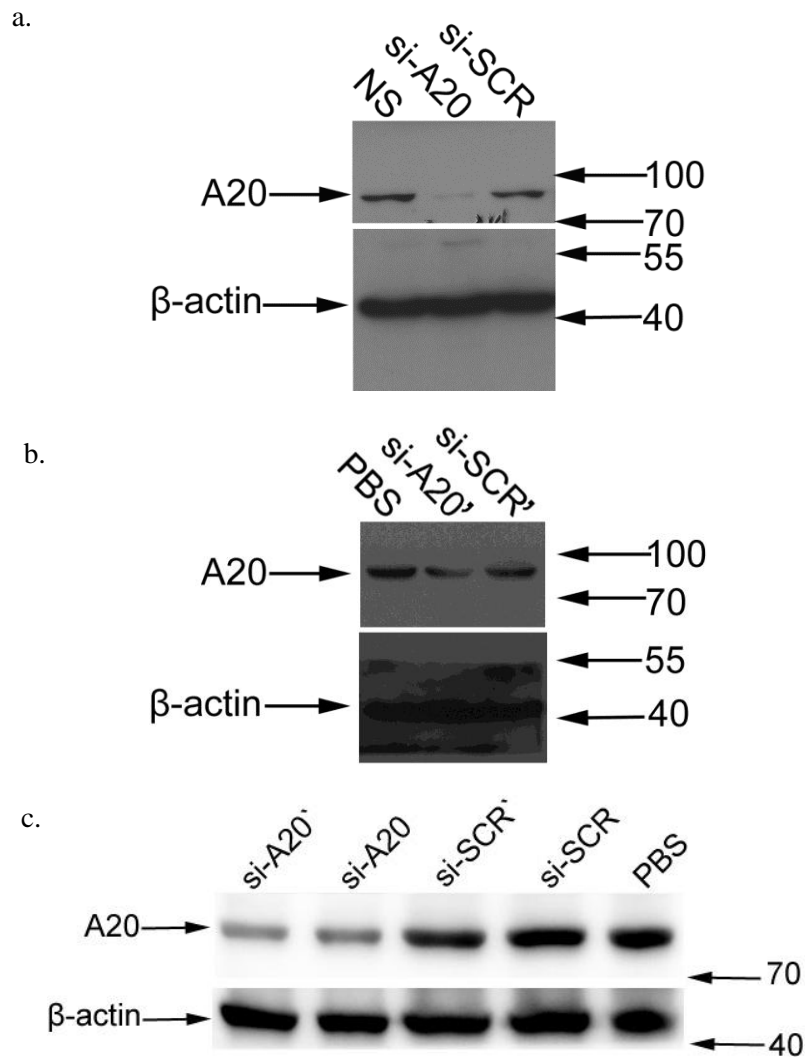
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Running title: A20 inhibition in tumor microenvironment

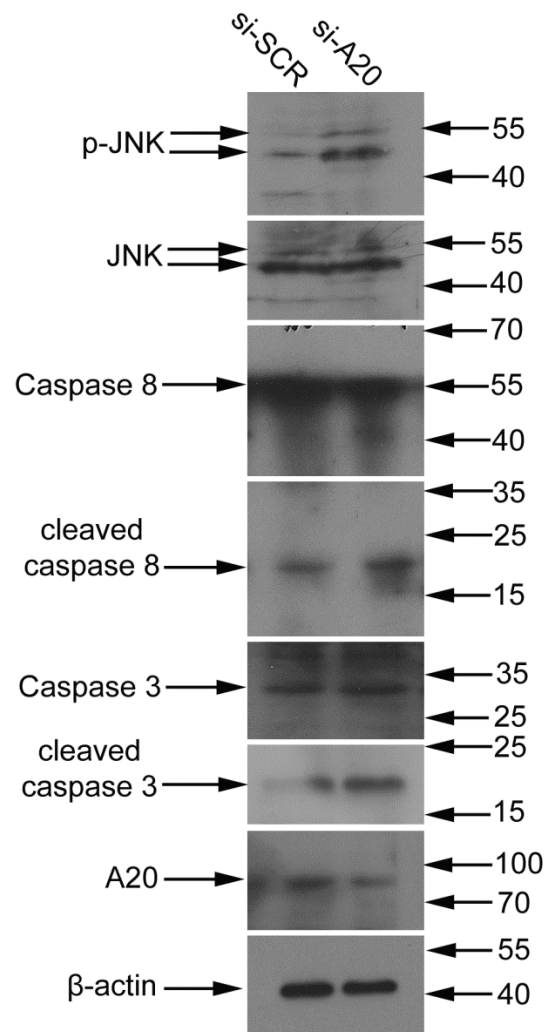
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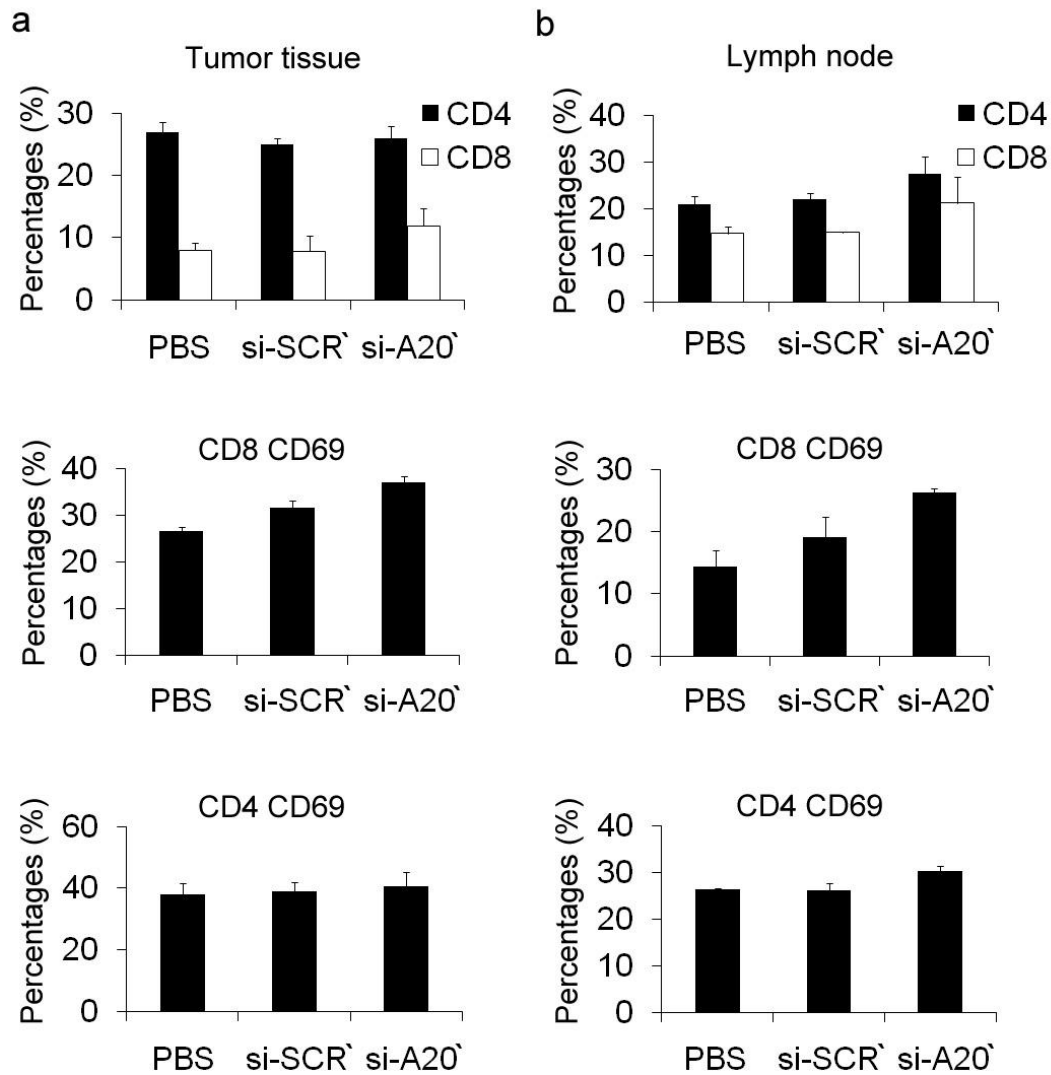
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Supplementary Figure S1. The interference efficiency of si-A20 was tested by western blotting in the L929 cells. (a,b) L929 cells were transfected for 48 hours and then stimulated with TNF- α (30ng/ml) for 1 hour. A20 protein was detected by western blot. β -actin was used as internal control. (c) Decrease of A20 expression in tumor tissue after si-A20 treatment. Tumor-bearing mice were treated with si-RNA intratumorally and CD11b⁺ cells are isolated from pooled tumor tissue suspension (3-5 mice per group). These cropped blots are used in the main figure (Figure 1) and these full-length blots are included in the supplementary figure.



Supplementary Figure S2. Si-A20 induces the apoptosis of MDSCs through JNK pathway. Gr-1⁺CD11b⁺ cells were isolated and transfected as mentioned above. p-JNK, activated caspase3 and activated caspase8 were analyzed by western blotting after treatment with si-SCR and si-A20. β-actin was used as internal control . These cropped blots are used in the main figure (Figure 7) and these full-length blots are included in the supplementary figure.



Supplementary Figure S3. Si-A20' treatment improves T cells activation in

tumor-bearing mice. E.G7 tumor model was established and mice were treated with

si-A20' as mentioned above. **(a)** Percentages of T cells in tumor tissue. Total number

of 100000 cells were analyzed. Cells were gated by CD3 lymphocyte region in tumors

and cell percentages were illustrated as percentages of the positive cells in gated

lymphocytes. For CD69 staining, cells were gated by CD4⁺ or CD8⁺ region. Numbers

illustrated indicate the percentage of the cells in gated cells. n=3 for each group. Two

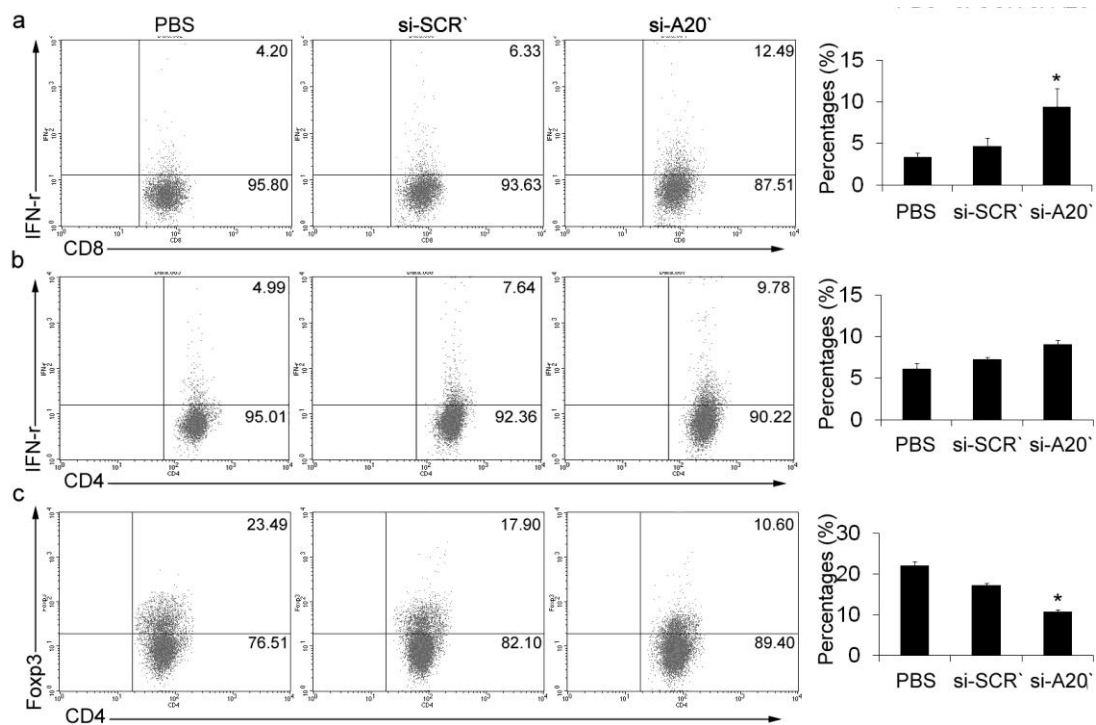
independent experiments were performed. **(b)** Percentages of T cells in Lymph node.

Total number of 30000 cells were subjected to flow cytometry assay. For CD69

staining, cells were gated by CD4⁺ or CD8⁺ region. The percentages of positive cells

in gated cells were illustrated. $n=3$. Two independent experiments were performed.

Data represent means \pm SD. (ANOVA test).



Supplementary Figure S4. Knockdown of A20 using si-A20' overcomes tumor-induced T-cell tolerance. (a, b) Analysis of antigen-specific T cell response. T cells isolated from lymph nodes were subjected to INF- γ intracellular staining after the re-stimulation with OT-I peptide (a) or OT-II peptide (b). Total number of 30000 cells were analyzed. Cells were gated by CD4⁺ or CD8⁺ region. Percentages of the positive cells are illustrated. Numbers illustrated indicate the percentage of the cells in total CD4 or CD8 cells. n=3 for each group. Independent experiments were repeated twice with similar results. Data represent means \pm SD. (c) Analysis of Treg cells in draining lymph nodes. Lymphocytes from immunized mice were subjected to Fc γ p3 intracellular staining. Total number of 30000 cells were analyzed. Cells were gated by CD4 positive region. Numbers illustrated indicate the percentage of the cells in total CD4 cells. n=3 for each group. Two independent experiments were performed. Data represent means \pm SD. * p < 0.05 (ANOVA test).