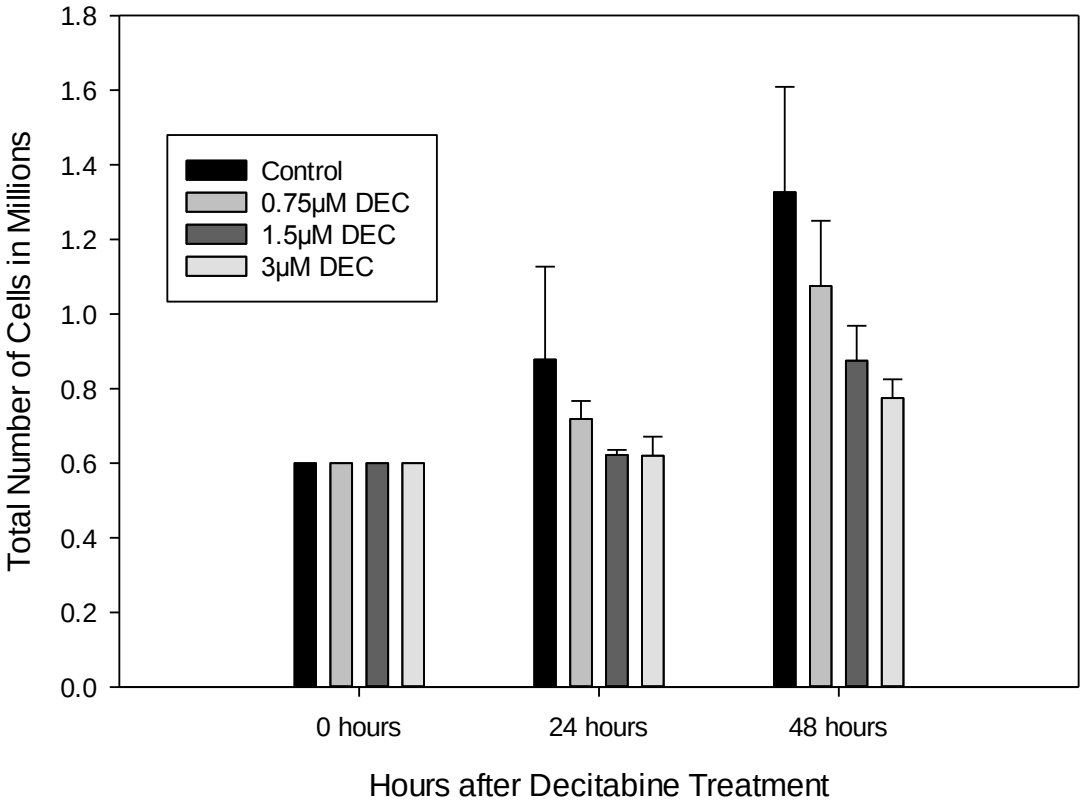
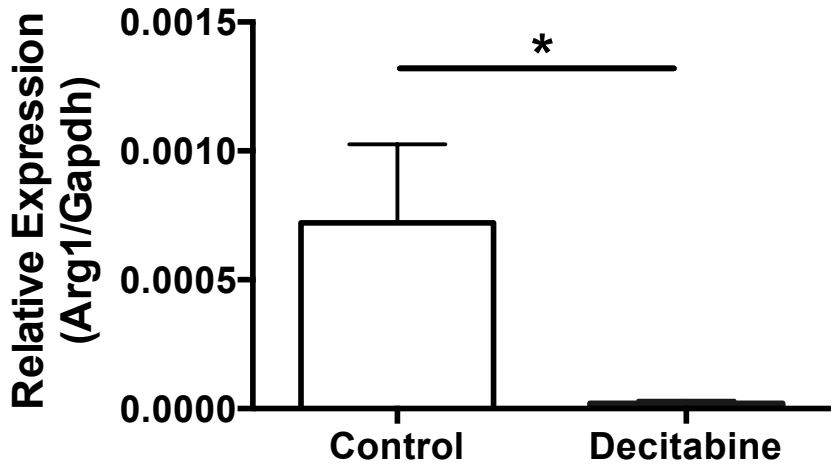
Group	Total Splenocytes ($X10^6$) (SEM)	% MDSC (SEM)	Total MDSC/Spleen ($X10^6$) (SEM)
No Treatment	**290 (53)**	**14.2 (1.3)**	**40.1 (5.6)**
Dec qd X 4	**70 (5.7)**	**0.9 (.26)**	**0.61 (.14)**
Aza qd X 4	**110 (41.6)**	**4.1 (1.2)**	**4.25 (1.38)**
Dec qod X 4	**73 (15.8)**	**0.9 (.05)**	**0.66 (.17)**
Aza qod X 4	**120 (37.8)**	**3.4 (2.3)**	**5.6 (4.7)**

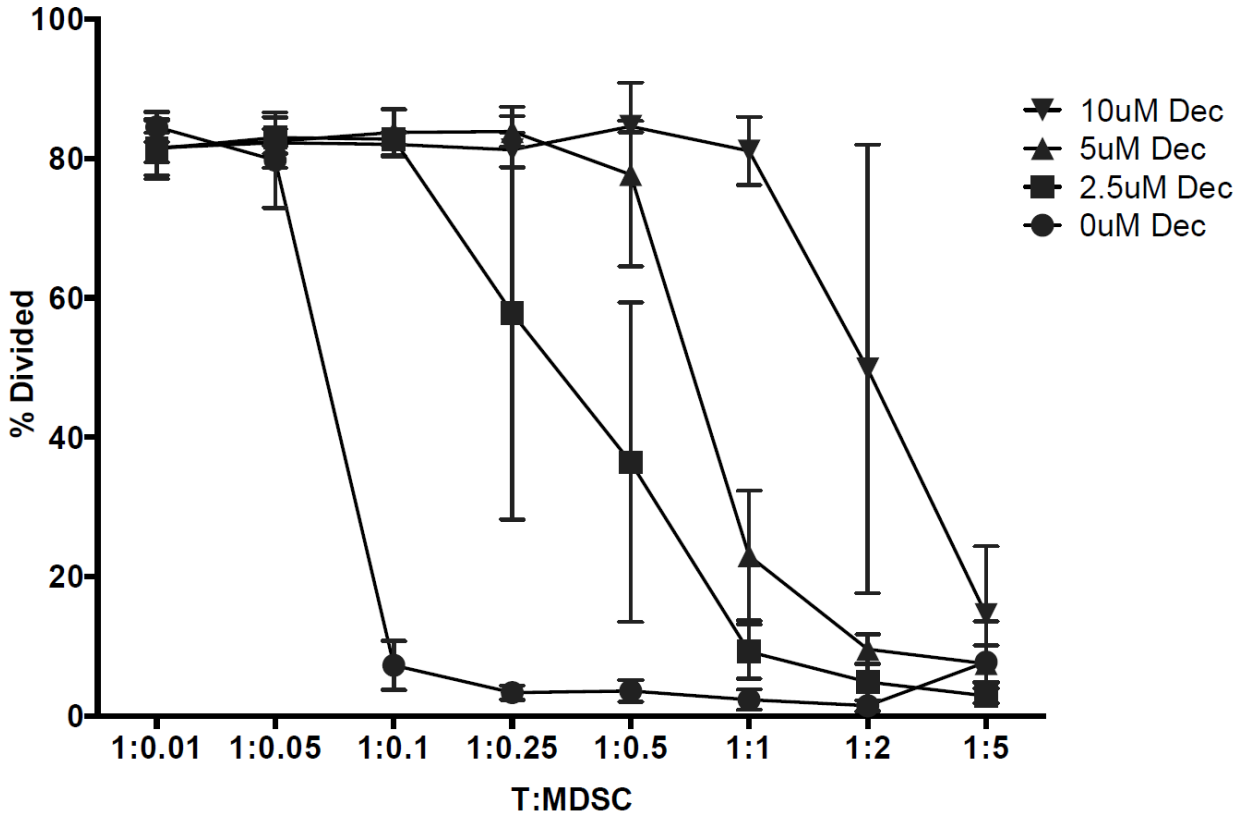
Supplemental Table S1: Effect of Dec or Aza on total spleen cell numbers, percent of spleen cells that were MDSC by flow cytometry and the total number of MDSC/spleen with Standard errors of Means (SEM) for each. 4T1 tumor-bearing mice were either untreated, or treated with Dec or Aza at 15 micrograms/mouse i.p. for 4 doses, either daily (qd) starting on Day 10 or every other day (qod), starting on Day 8. Spleens were harvested on Day 15. There were 3 mice in each group and these results are representative of 2 separate experiments.



Supplemental Figure S1: 4T1 cells were cultured in medium without or with varying concentrations of Dec, as shown. Cells were harvested and viable cells were counted (using Trypan blue exclusion) at 24 or 48 hours.

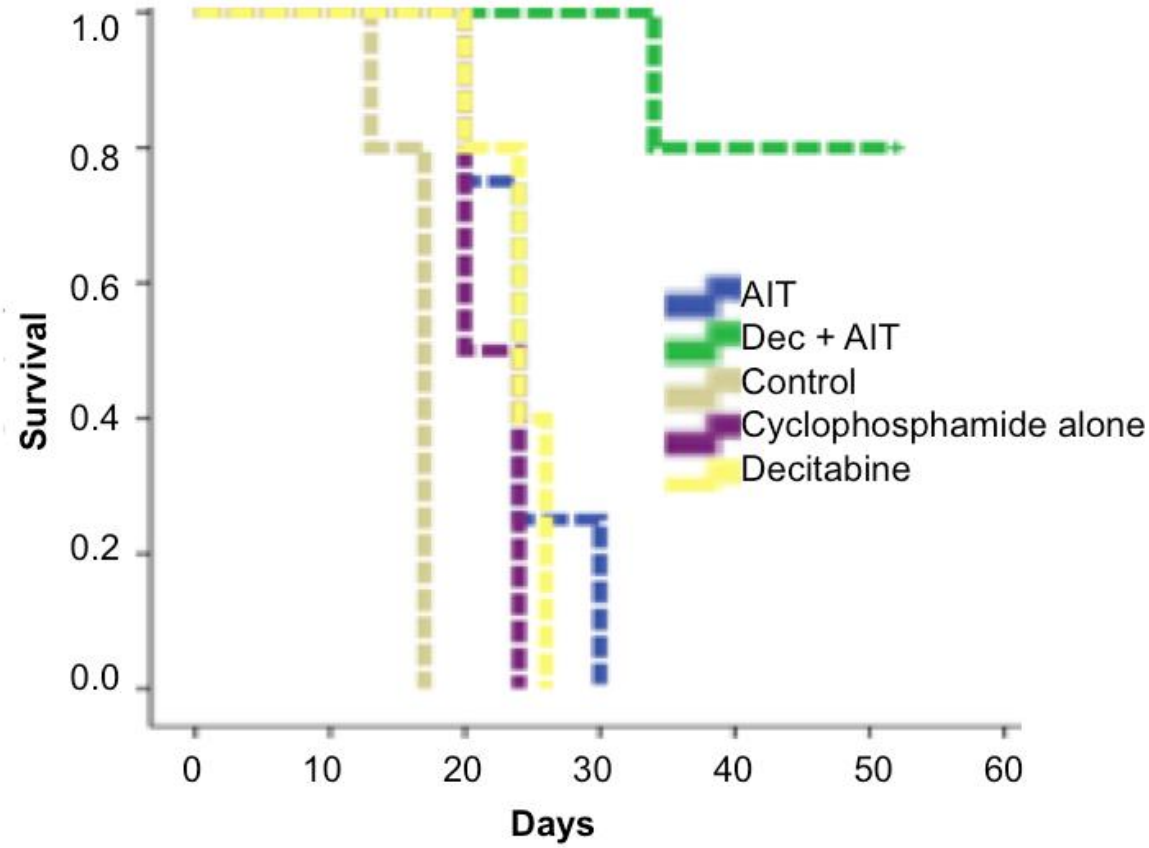


Supplemental Figure S2: Arginase 1 (Arg1) mRNA relative expression in MDSCs from 4T1-tumor bearing mice (Day 15) treated with decitabine or untreated (control). Gr1+ cells from the spleens of Day 15 4T1-tumor bearing mice, either untreated or treated with decitabine (15 µg i.p. daily for 4 doses on days 9 - 12) were isolated using magnetic bead isolation (EasySep Mouse PE Positive Selection Kit, STEMCELL Technologies, Vancouver, BC, Canada) using PE anti-Gr1 (Clone RB6-8C5 Biolegend). Harvested cells were confirmed to be greater than 70% CD11b+ Gr1+ by flow cytometry after selection. RNA was isolated from MDSCs using TRIzol reagent (Life Technologies), and 1000ng of RNA was reverse transcribed to cDNA using SuperScript IV (Life Technologies). Arginase 1 expression relative to GAPDH was measured using Taqman probes (Arg1 Mm00475988_m1 and GAPDH Mm99999915_g1) and run using the QuantStudio3 real-time PCR system. * = $p<0.05$, unpaired student's t test.

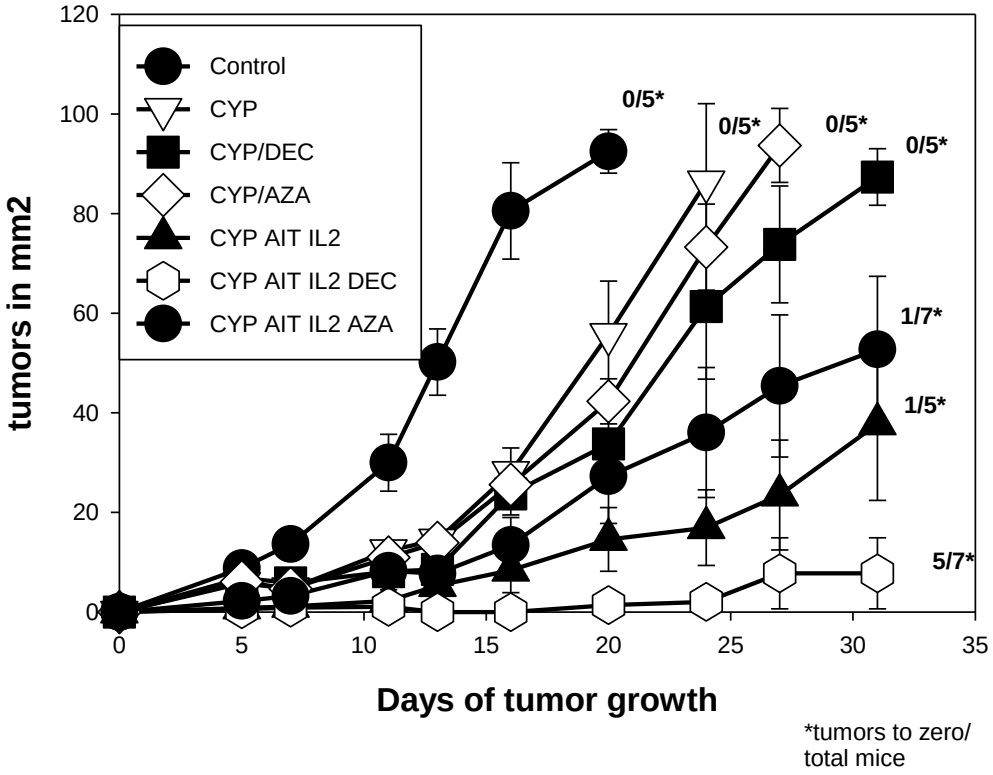


Supplemental Figure S3: In vitro Decitabine treatment of Gr1+CD11b+ cells from ADAM10 transgenic mice abrogrates their ability to suppress T cell proliferation.

ADAM10Tg MDSC isolated by magnetic bead depletion (>90% CD11b+Gr1+) were cultured for 1 week with 0, 2.5, 5, or 10uM decitabine and GM-CSF. For the T cell suppression assay, T cells were isolated by anti-CD90.2 magnetic bead selection (Miltenyi Biotec) from wild type C57Bl/6 spleens and labeled with Track-It Violet proliferation dye (Biolegend). MDSCs were enumerated using trypan blue exclusion and live cells were incubated with T cells at indicated ratios in cRPMI with anti-CD28 (2µg/ml, clone 37.5, Biolegend) on plates coated with anti-CD3ε (1µg/ml, clone 145-2C11, Biolegend). After 96hrs, cells were Fc blocked with unlabeled anti-CD16/32 (clone 2.4G2) and then stained with PE anti-CD3ε (Biolegend). Cells were analyzed on a BD LSRFortessa-X20 and percent divided was assessed by dilution of proliferation dye using FlowJo software (version 7.6, TreeStar, Ashland, OR).



Supplemental Figure S4: Adoptive immunotherapy in combination with decitabine resulted in a higher cure rate and greater efficacy than either therapy alone. Survival to humane endpoint for an AIT experiment;mean survival 48 days for AIT + Dec vs 30 days for AIT without Dec, $p<0.05$).



Supplemental Figure S5: Mice were inoculated with 50,000 4T1 cells, and AIT consisted of 2 X 10^7 lymphocytes expanded in IL-2 for 7 days, with or without Dec or Aza at 15 micrograms per mouse i.p. on days 3-6. At day 27, mean tumor sizes were 23.5 +/- 11.0 mm^2 for CYP/AIT, 7.8 +/- 7.1 for CYP/AIT + DEC and 45.4 +/- 14.3 for CYP/AIT + AZA. At Day 31, sizes were 37.8 +/- 15.4, 7.8 +/- 7.1 and 52.8 +/- 14.7, respectively.