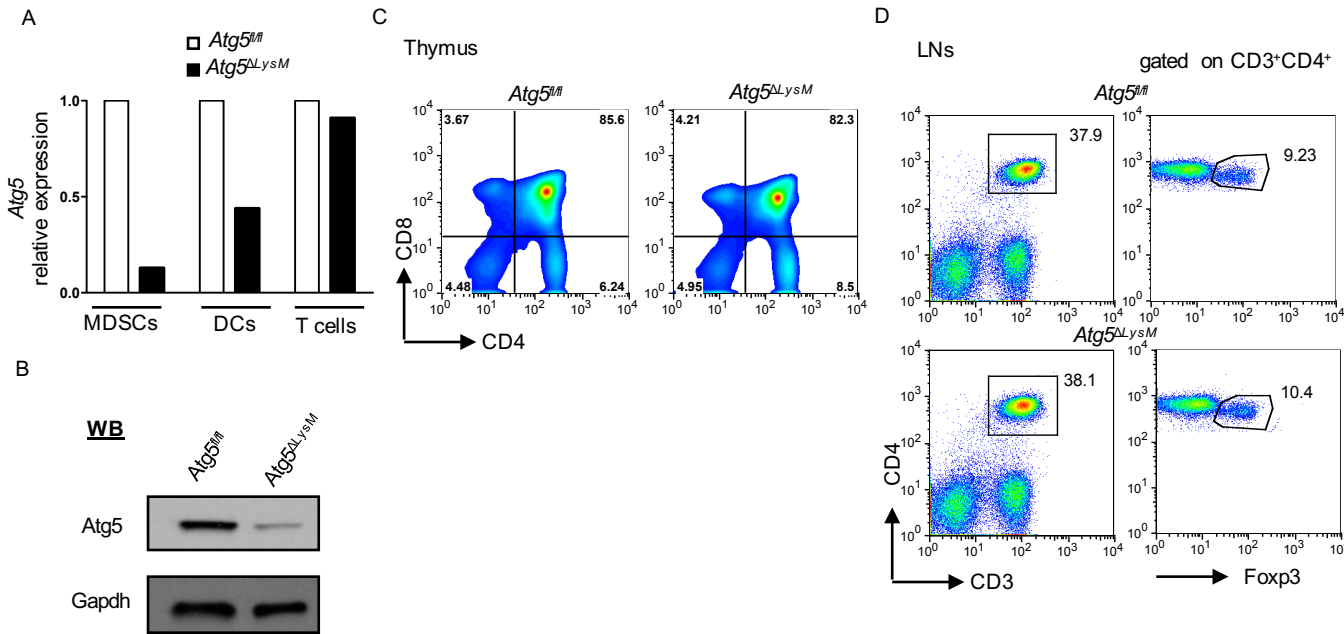
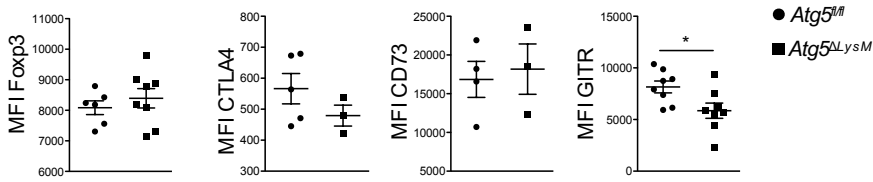


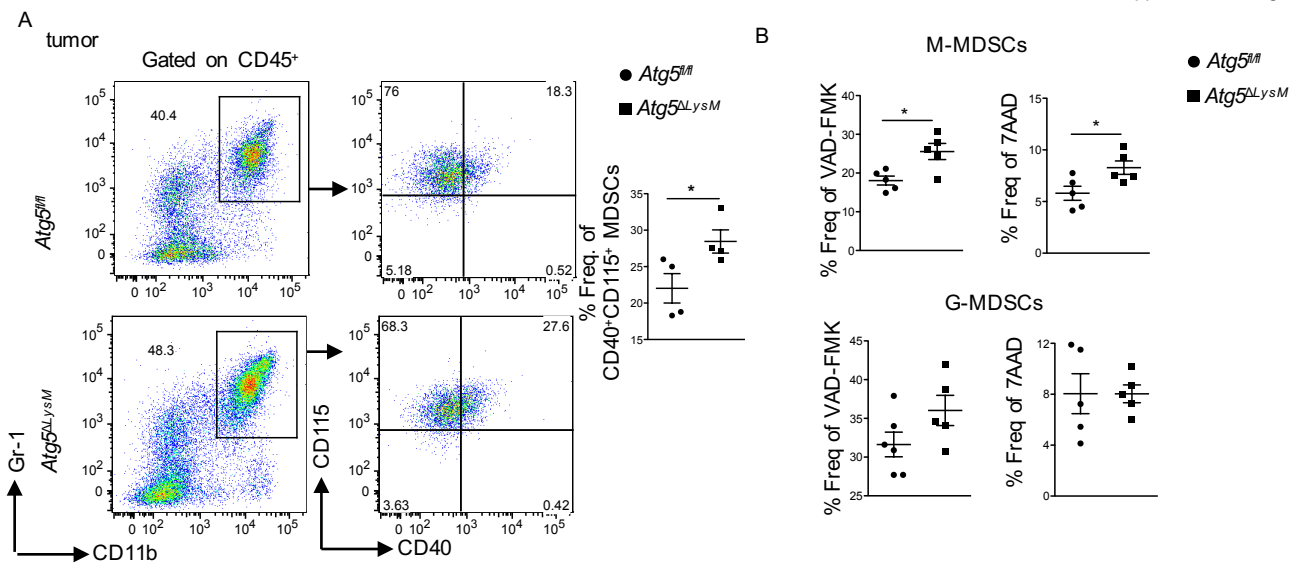
**Supplemental Figure 1. Up-regulation of autophagy pathway in MDSC subsets from melanoma-bearing mice.** Immunofluorescence confocal microscopy for LC3 (red), LAMP-1 (green), p62 (silver white), and DAPI (blue) and LC3 puncta/cell and p62 puncta/cell in sorted M-MDSCs: CD11c<sup>+</sup>CD11b<sup>hi</sup>Ly6G<sup>+</sup>Ly6C<sup>low</sup> (LC3: \*\*\* $p < 0.0001$ , p62: \*\*\* $p < 0.0001$ ) and G-MDSCs: CD11c<sup>+</sup>CD11b<sup>hi</sup>Ly6G<sup>+</sup>Ly6C<sup>low</sup> (LC3: \*\*\* $p < 0.0001$ , \* $p = 0.046$ , p62: \*\*\* $p < 0.0001$ ) from spleens and tumors of naïve and B16-F10-inoculated mice. Scale bars: 10  $\mu\text{m}$ . One representative experiment of 3 is shown.  $n = 5$  mice per group. Results are expressed as mean  $\pm$  SEM. Statistical significance was obtained by two-way ANOVA.



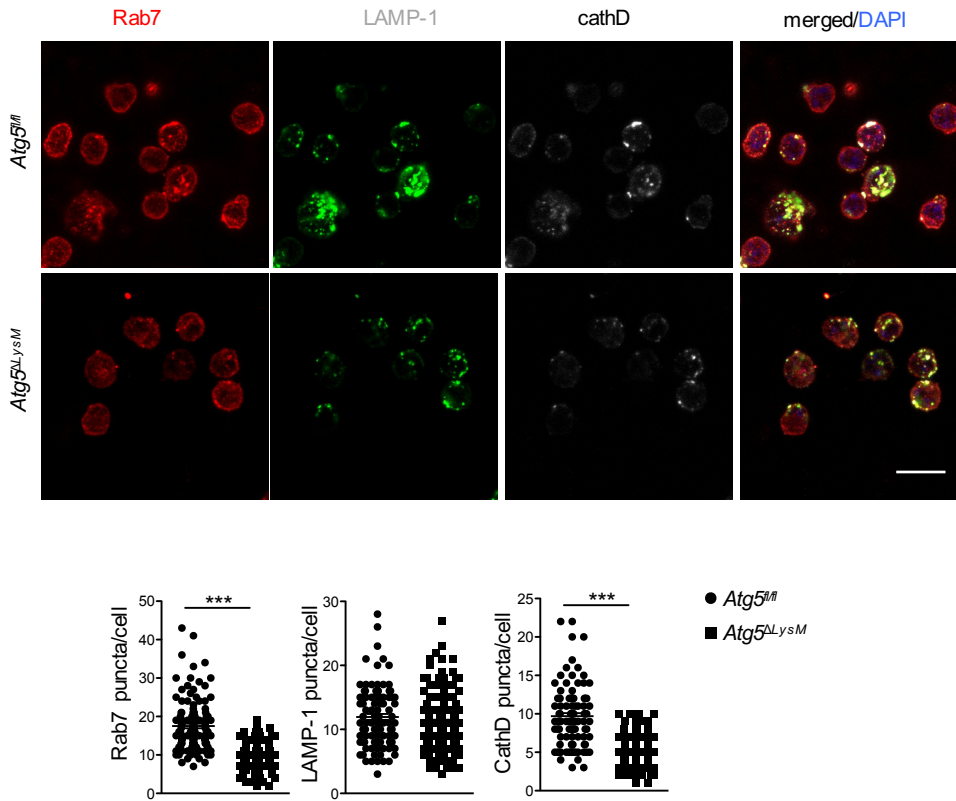
**Supplemental Figure 2. Efficient deletion of *Atg5* in MDSCs from *Atg5<sup>ΔLysM</sup>* mice.** (A) *Atg5* relative expression in sorted MDSCs (CD11c<sup>+</sup>CD11b<sup>+</sup>Gr-1<sup>+</sup>), DCs (CD11c<sup>+</sup>) and T cells (CD3<sup>+</sup>) from spleens of naive *Atg5<sup>ΔLysM</sup>* and *Atg5<sup>fl/fl</sup>* control mice (n=3 mice/group). (B) Representative images for protein expression of *Atg5* gene in M-MDSCs isolated from spleens of *Atg5<sup>fl/fl</sup>* compared to *Atg5<sup>ΔLysM</sup>* B16-F10 inoculated mice, normalized with GAPDH protein levels via western blot (n=3 mice/group). (C) Representative flow cytometric analysis of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the thymus of naive *Atg5<sup>ΔLysM</sup>* and *Atg5<sup>fl/fl</sup>* control mice (n=4 mice per group). (D) Representative flow cytometric analysis of CD3<sup>+</sup>CD4<sup>+</sup> and CD3<sup>+</sup>CD4<sup>+</sup>Foxp3<sup>+</sup> T cells in the LN's of naive *Atg5<sup>ΔLysM</sup>* and *Atg5<sup>fl/fl</sup>* control mice (n=4 mice per group). One representative experiment of three is depicted.

Gated on CD45<sup>+</sup>CD4<sup>+</sup>Foxp3<sup>+</sup>

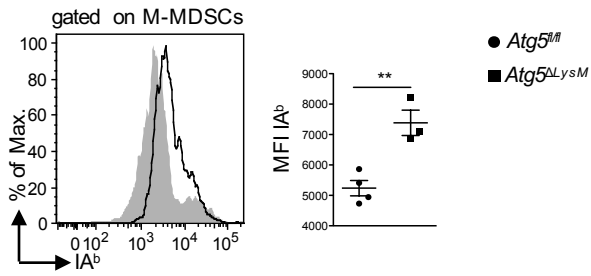
**Supplemental Figure 3. Phenotypic analysis of Foxp3<sup>+</sup> Tregs in tumors of *Atg5<sup>ΔLysM</sup>* mice.** Mean fluorescence intensity of Foxp3 (n=6-8 mice), CTLA4 (n=3-5), CD73 (n=3-4) and GITR (\*p=0.0274, n=8-9) expression in Tregs from tumors of *Atg5<sup>ΔLysM</sup>* and *Atg5<sup>fl/fl</sup>* control mice. Results are expressed as mean ± SEM. Statistical significance was obtained by unpaired student's t test.



**Supplemental Figure 4. Enhanced frequencies of CD115<sup>+</sup>CD40<sup>+</sup> MDSCs and increased apoptosis in autophagy deficient M-MDSCs during melanoma induction.** (A) Gating strategy and frequencies of CD115<sup>+</sup>CD40<sup>+</sup> MDSCs in tumors of *Atg5<sup>ΔLysM</sup>* and *Atg5<sup>fl/fl</sup>* control mice (\**p*=0.0466), (*n*=4 mice per group). One representative experiment of three is shown. (B) Frequencies of VAD-FMK<sup>+</sup> (\**p*=0.0137) and 7AAD<sup>+</sup> (\**p*=0.0299) M-MDSCs or G-MDSCs in spleens of B16-F10 inoculated *Atg5<sup>ΔLysM</sup>* and *Atg5<sup>fl/fl</sup>* control mice, *n*=5. Results are expressed as mean ± SEM. Statistical significance was obtained by unpaired student's *t* test.



**Supplemental Figure 5. Reduced lysosomal biogenesis and enzymatic activity of autophagy deficient M-MDSCs.** Representative confocal microscopy images for Rab7 (red)/LAMP-1 (green)/cathepsin D (silver white)/DAPI (blue). Rab7, Lamp-1 and CathD puncta/cell in sorted M-MDSCs from spleens of B16-F10-inoculated mice (Rab7: \*\*\* $p < 0.0001$ , CathD: \*\*\* $p < 0.0001$ ). Scale bars: 10  $\mu$ m. One representative experiment of 3 is shown.  $n = 4$  mice per group. Results are expressed as mean  $\pm$  SEM. Statistical significance was obtained by unpaired student's t test.



**Supplemental Figure 6. Enhanced IA<sup>b</sup> expression in autophagy-deficient M-MDSCs isolated from peripheral blood.** Representative histograms and MFI for IA<sup>b</sup> expression (\*\* $p=0.0055$ ) in M-MDSCs from peripheral blood of B16-F10 inoculated mice ( $n=4$  mice per group). One representative experiment of three is shown. Results are expressed as mean  $\pm$  SEM. Statistical significance was obtained by unpaired student's t test.