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}CD11b{sup}hiLy6G{sup}hiLy6C{sup}bw (LC3: ***p<0.0001, p62: ***p<0.0001) and G-MDSCs: CD11c{sup}CD11b{sup}hiLy6G{sup}Ly6C{sup}bw (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 μm. One representative experiment of 3 is shown. n = 5 mice per group. Results are expressed as mean ± SEM. Statistical significance was obtained by two-way ANOVA.
Supplemental Figure 2. Efficient deletion of Atg5 in MDSCs from Atg5ΔLysM 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 Atg5ΔLysM 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 Atg5ΔLysM 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 Atg5ΔLysM 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 LNs of naive Atg5ΔLysM and Atg5<sup>fl/fl</sup> control mice (n=4 mice per group). One representative experiment of three is depicted.
Supplemental Figure 3. Phenotypic analysis of Foxp3+ Tregs in tumors of Atg5ΔLysM 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 Atg5ΔLysM and Atg5fl/fl 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⁺CD40⁺ MDSCs and increased apoptosis in autophagy deficient M-MDSCs during melanoma induction. (A) Gating strategy and frequencies of CD115⁺CD40⁺ MDSCs in tumors of Atg5ΔLysM and Atg5fl/fl control mice (*p=0.0466), (n=4 mice per group). One representative experiment of three is shown. (B) Frequencies of VAD-FMK⁺ (*p=0.0137) and 7AAD⁺ (*p=0.0299) M-MDSCs or G-MDSCs in spleens of B16-F10 inoculated Atg5ΔLysM and Atg5fl/fl 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 μm. One representative experiment of 3 is shown. n = 4 mice per group. Results are expressed as mean ± SEM. Statistical significance was obtained by unpaired student’s t test.
Supplemental Figure 6. Enhanced IA^b expression in autophagy-deficient M-MDSCs isolated from peripheral blood. Representative histograms and MFI for IA^b 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 ± SEM. Statistical significance was obtained by unpaired student’s t test.