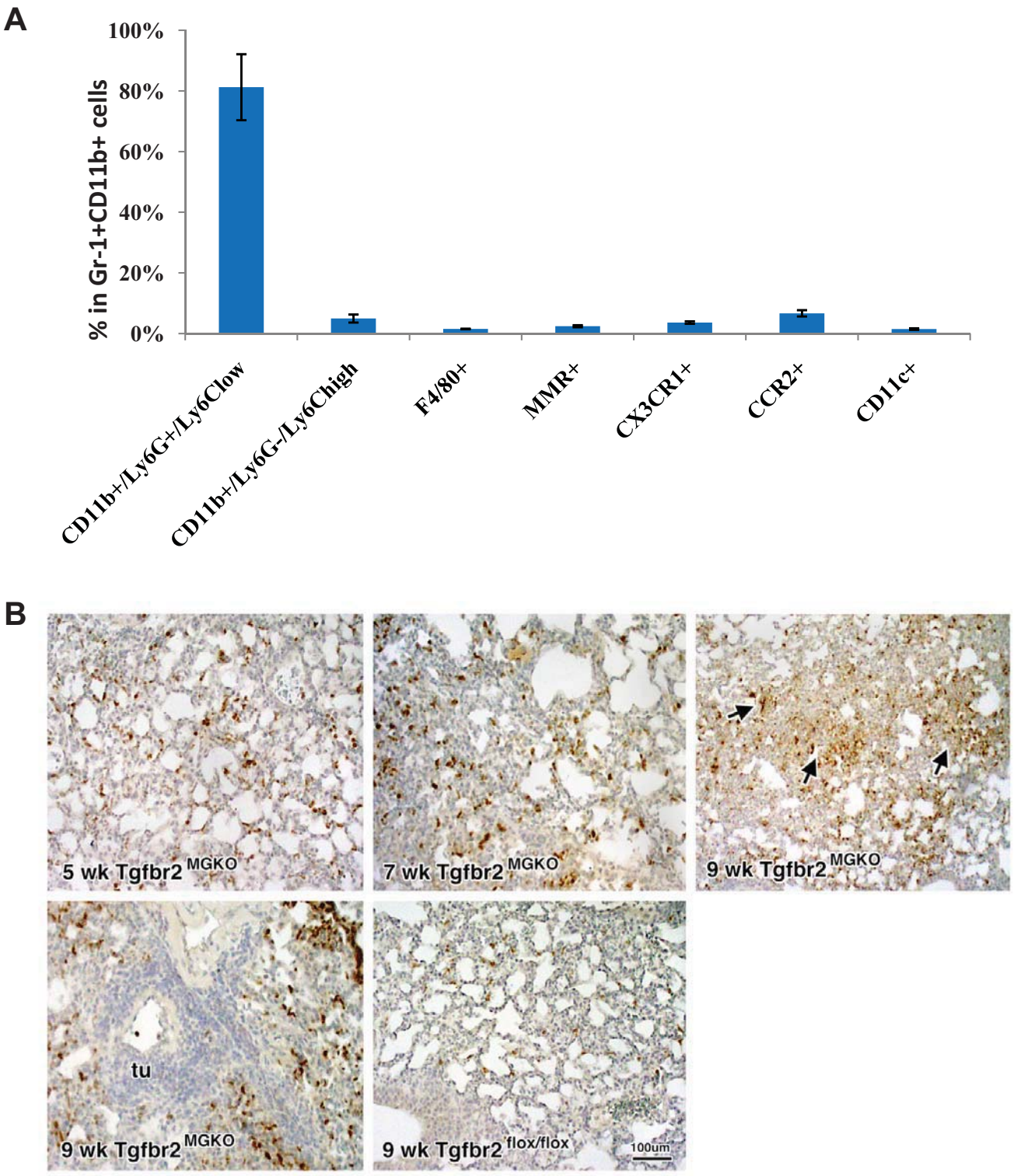
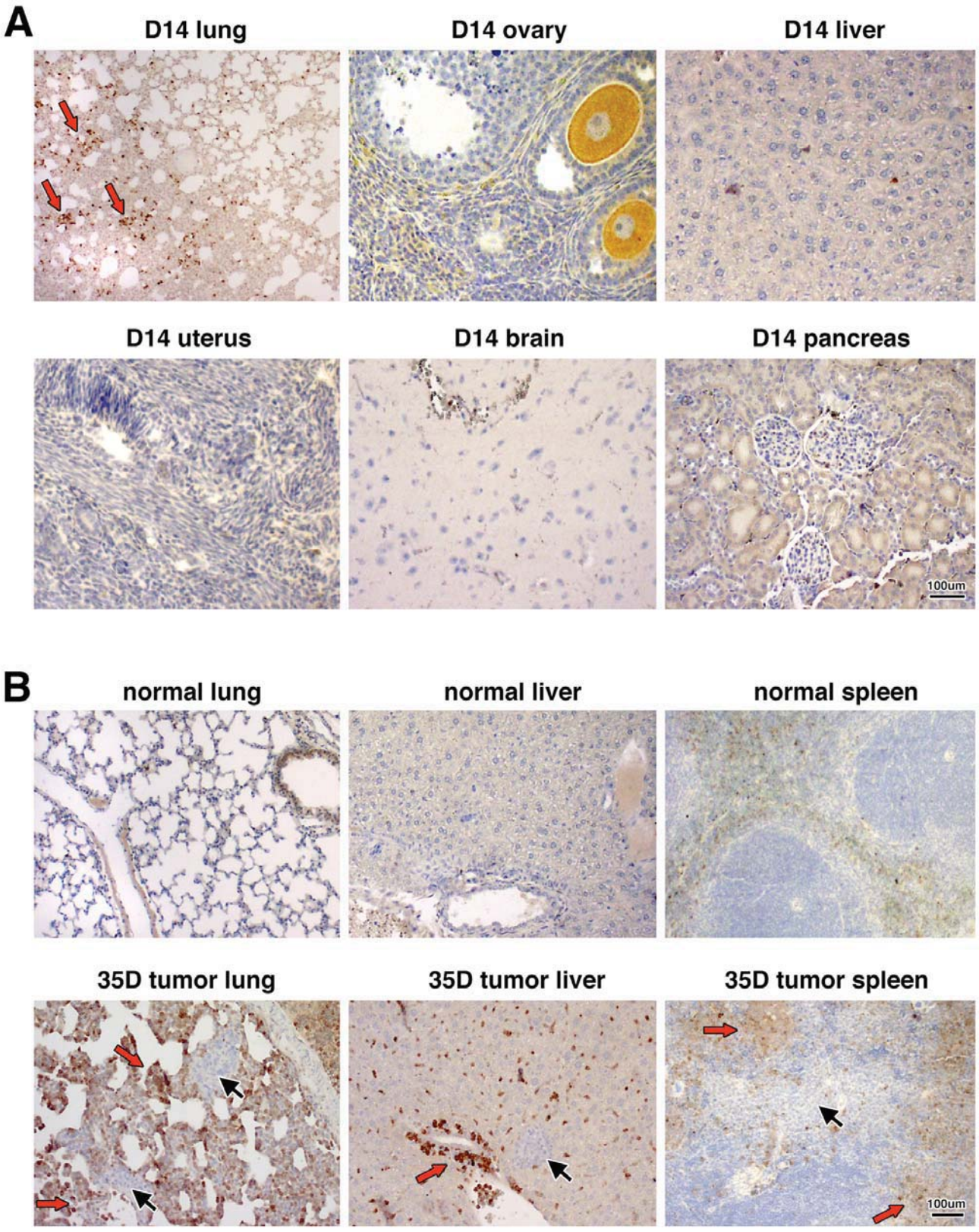
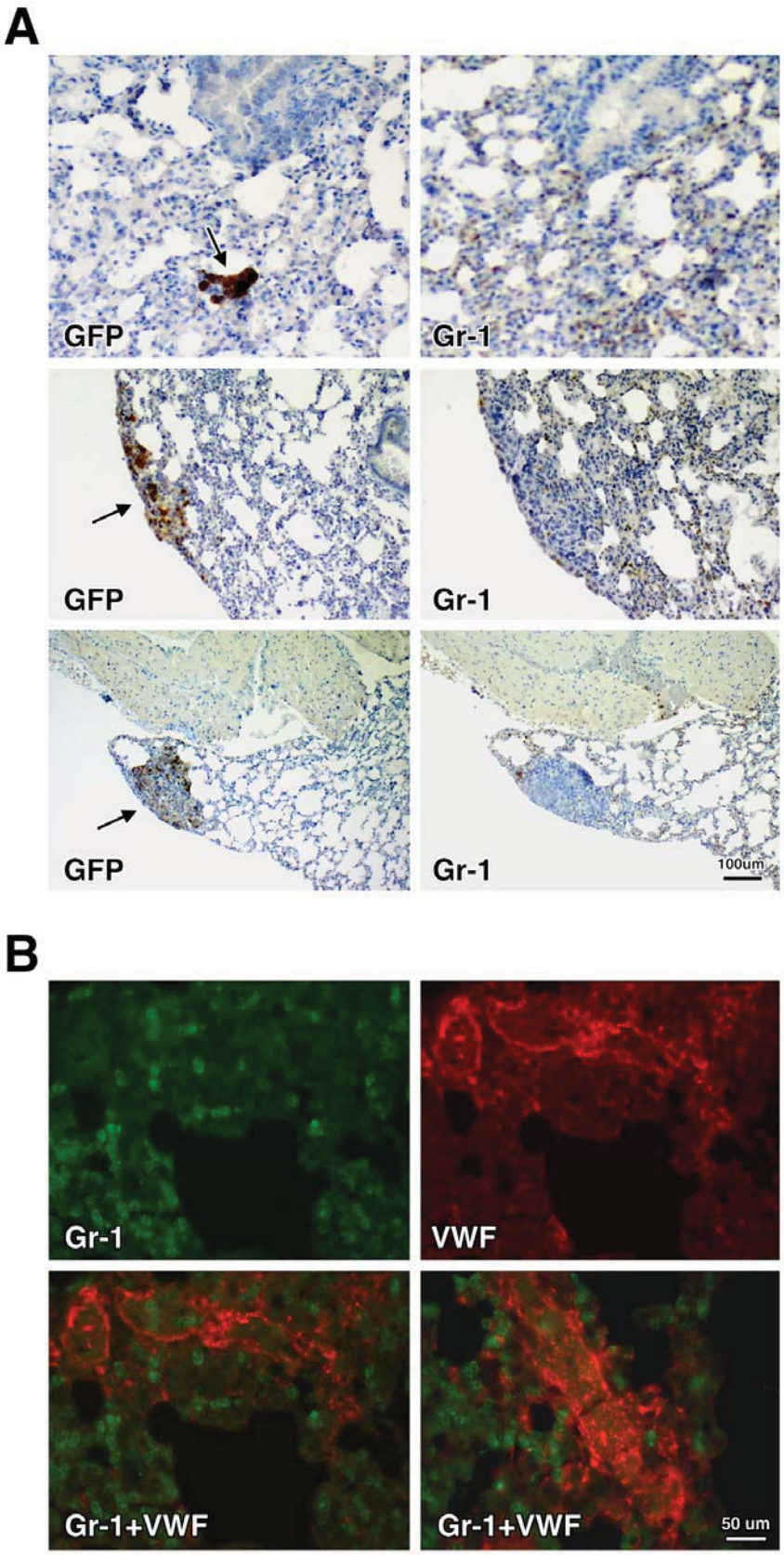


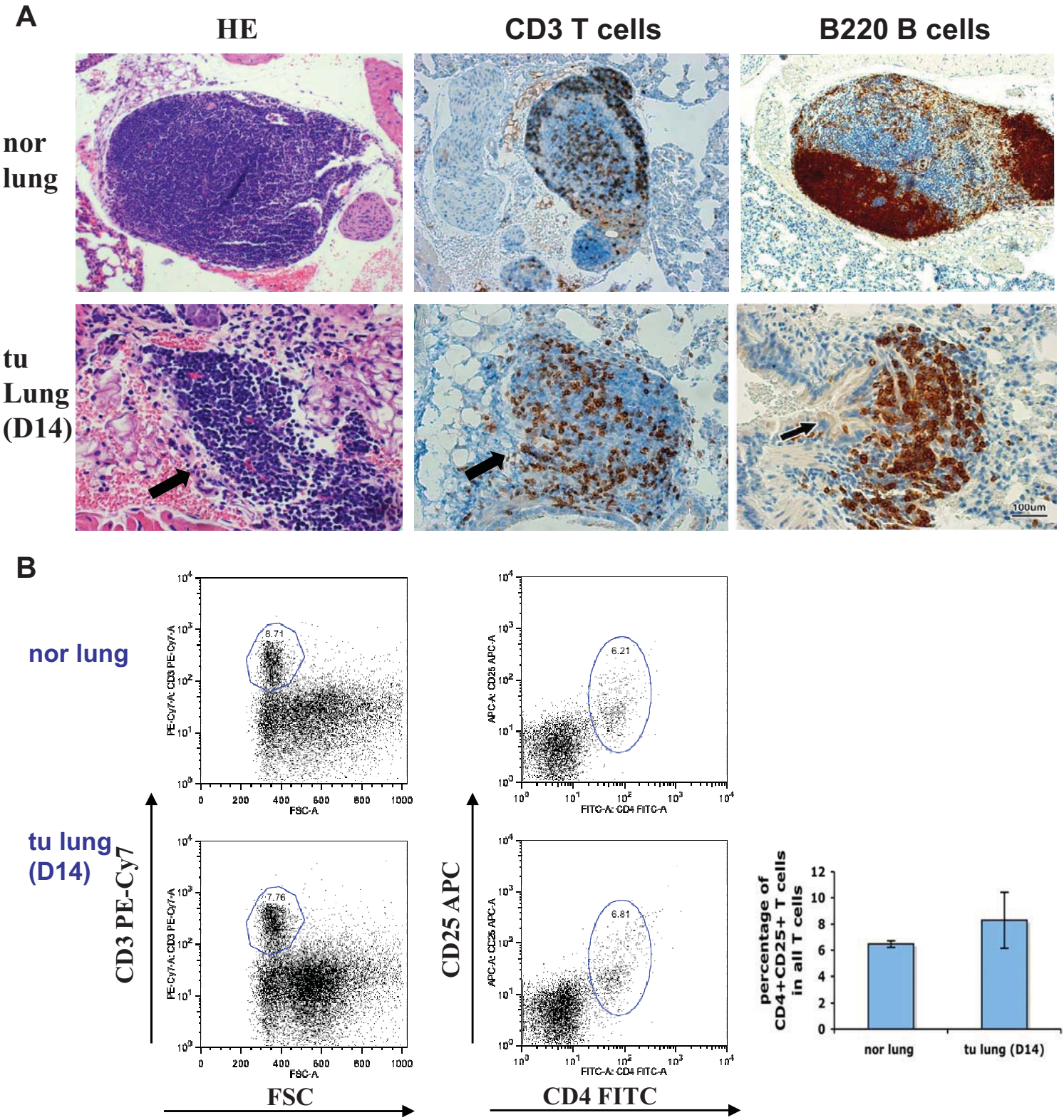
Supplementary figure 1: A, Gr-1+CD11b+ cells in the premetastatic lung are granulocytic but not macrophage lineage. Flow cytometry showed 81.2% of the cells are Ly6G+Ly6C low, 5% are Ly6G-Ly6C high, only 1.6%, 2.5%, 3.7%, and 6.7% of these Gr1+CD11b+ cells are F4/80+, MMR+, CX3CR1+, and CCR2+ respectively. B, Gr-1+CD11b+ cells infiltrate into the premetastatic lung in a genetic tumor model. IHC of Gr-1+CD11b+ cells in lungs of MMTV PyMT transgenic mice with deletion of TGFbeta receptor 2 ($Tgfbr2^{MGKO}$) from 5 to 9 weeks after birth as indicated in the figure. Arrows indicate Gr-1+CD11b+ cell clusters. “tu” indicates tumor metastasis nodules. Littermate $Tgfbr2^{flox/flox}$ mice were used as control.



Supplementary figure 2: A. Gr-1+CD11b+ cell infiltration exhibited certain organ specificity. IHC of Gr-1+CD11b+ cells in various organs of mice bearing 4T1 tumors on day 14 after tumor inoculation (s.c.), including ovary, uterus, brain, and pancreas. Red arrows indicate Gr-1+CD11b+ cell clusters. B: IHC of Gr-1+CD11b+ cells in lung, liver and spleen of mice bearing 4T1 tumors on day 35 after tumor inoculation (s.c.). Lung, liver and spleen from normal mice were used as controls. Red arrows indicate Gr-1+CD11b+ cell clusters. Black arrows indicate metastasis tumor nodules.

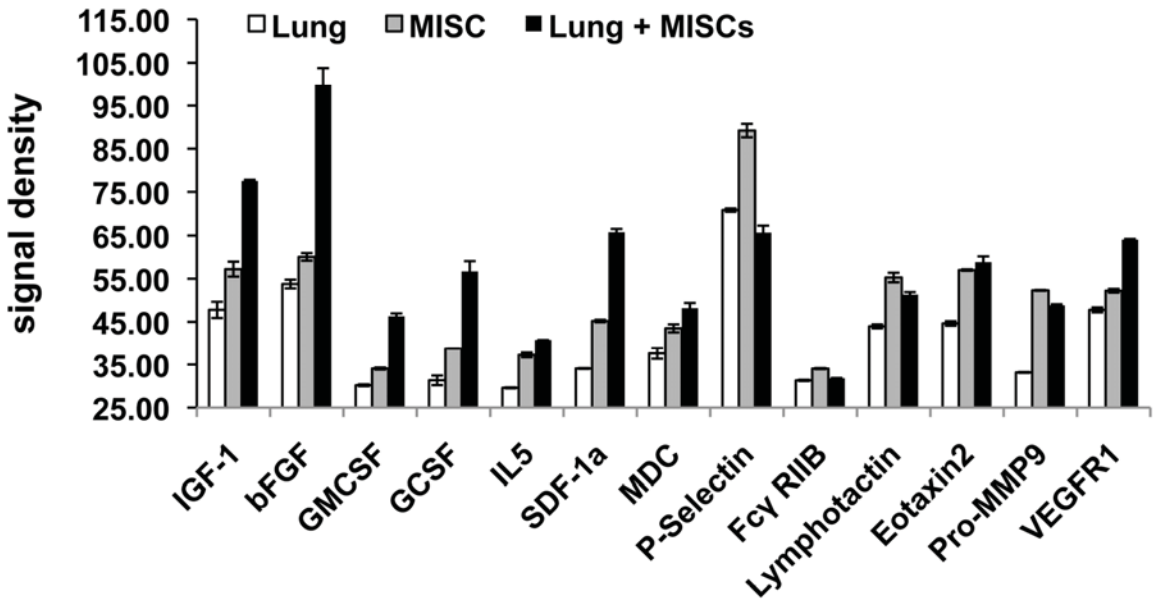


Supplementary figure 3: Gr-1+CD11b+ cells neither form a premetastatic niche nor incorporate into vasculature. A: IHC of Gr-1+CD11b+ cells and GFP in sequential lung sections from mice bearing 4T1 tumors. Arrows indicate 4T1-GFP cell clusters at early stage of metastasis tumor growth. No overlap between 4T1-GFP tumor cells and Gr-1+CD11b+ cell clusters was observed. B: Co-immunofluorescence staining of VWF8 (red) and Gr-1 (green) (upper panels as labeled) in lungs of 4T1 tumor bearing mice demonstrate that no Gr-1+CD11b+ cells were present in vasculature endothelium. Lower panels are the overlay images.

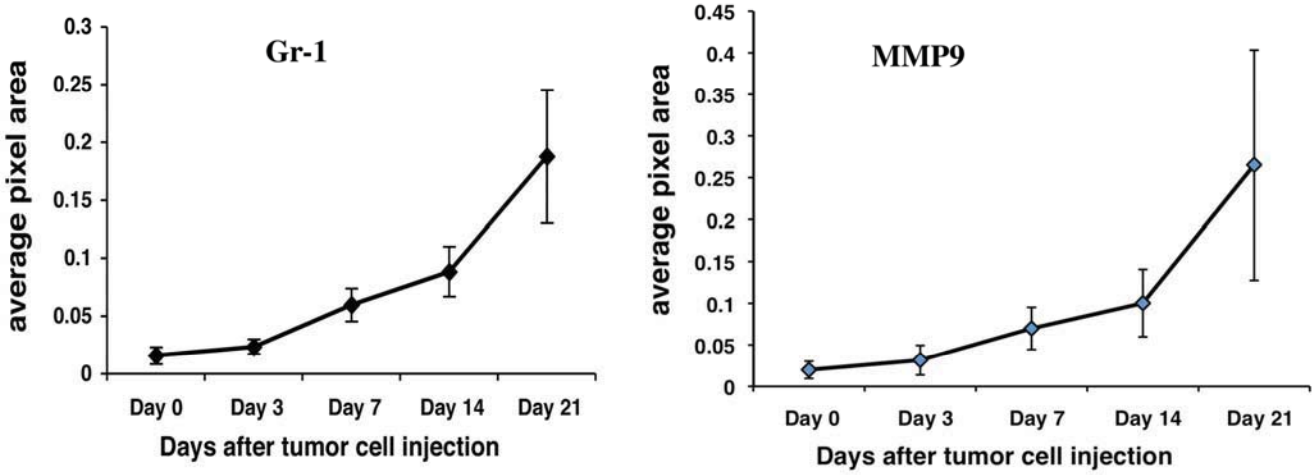


Supplementary figure 4: A: IHC of CD3+ T cells and B220+ B cells demonstrates the presence of bronchus-associated lymphoid tissue (BALT) in premetastatic lungs of 4T1 tumor bearing mice (day 7) as indicated by arrows (bottom three panels), as compared with lymph nodes (top three panels). B: Flow cytometry analysis of CD3+CD4+CD25+ T cells from lungs of normal mice and mice bearing 4T1 tumors 14 days after tumor inoculation (s.c.). The gates were set on 7AAD- and CD3+, CD4+CD24+ cell subset was analyzed. Three to four mice were used for each group. Quantitative data is shown on the right.

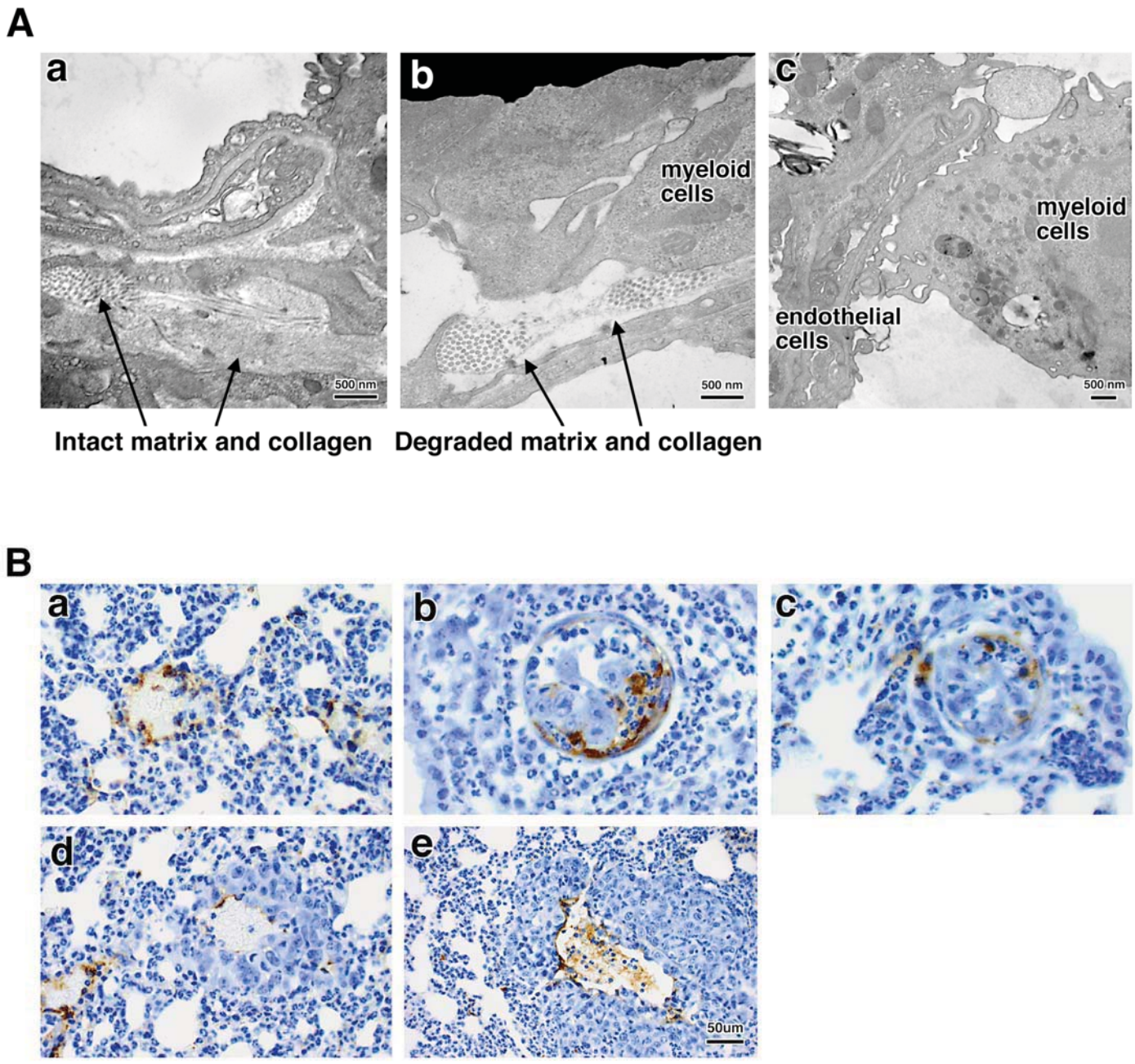
A.



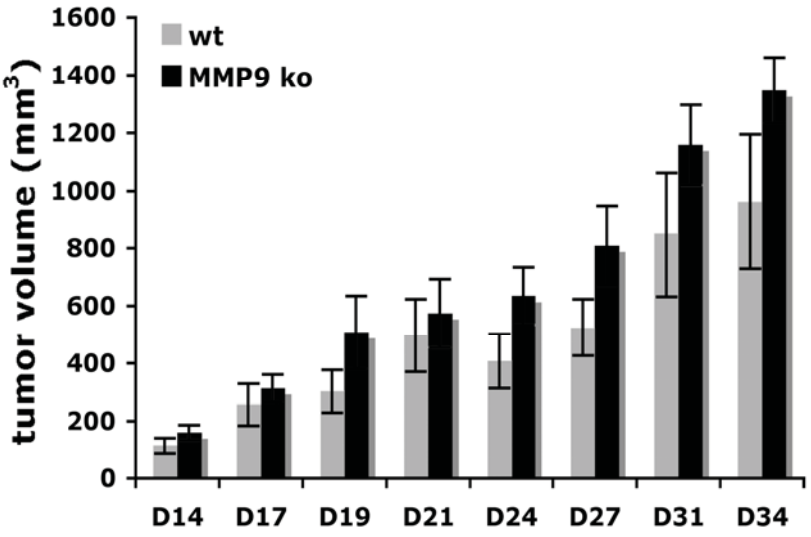
B.



Supplementary figure 5: A: Cytokine/chemokine protein profiling of in vitro co-culture of single cell suspension of lung cells and sorted Gr-1+CD11b+ cells. Shown is relative signal intensity in one experiment from two performed. B: Semi-quantitative data of co-immunofluorescence of Gr-1+CD11b+ cells (Gr-1+) and MMP9 in lungs of normal mice and mice bearing 4T1 tumors in premetastatic stage and also 21 days after tumor inoculation (figure 4A) by measuring pixel density using MetaMorph.



Supplementary figure 6: A: Electron microscopy of lung blood vessel in premetastatic phase. Aa: Intact matrix and collagen (arrows) in basal membrane of blood vessel in normal lung. Ab: A degraded matrix and collagen in premetastatic lung. Ac: Myeloid cells in close contact with endothelial cells of blood vessel. B: IHC of VWF staining in Lungs of mice bearing 4T1 tumor on day 35. Pictures were taken to demonstrate abnormal vasculature (a), tumor cells breaching lung capillaries (b, c & d), tumor cells grow into a metastasis nodules at the vicinity of lung capillaries (e).



Supplementary figure 7: Primary tumor growth of 4T1 tumors in wt and MMP9 knockout mice. 7-10 mice were used for each group.