

Supplementary Figure 1.

a, Efficiency of VEGF-deletion in isolated peritoneal and tumor-associated macrophages as determined by real time PCR of genomic macrophage DNA. **b**, Transgenic mice (C57Bl/6J) expressing the polyoma middle T (PyMT) oncoprotein under the promoter of the mouse mammary tumor virus (MMTV)-long terminal repeat were bred to mice (C57Bl/6J), with both alleles of exon 3 of VEGF-A flanked by loxP sites (VEGF^{f/f}). Myeloid cell-specific knock out of VEGF was achieved by breeding male mice homozygous for the floxed VEGF allele and heterozygous for the PyMT oncogene (MMTV-PyMT/VEGF^{f/f}) with female mice (C57Bl/6J) homozygous for the floxed VEGF allele expressing Cre recombinase driven by the lysozyme M promoter (LysMCre+/VEGF^{f/f}). For our studies, we used female mice heterozygous for the PyMT oncogene carrying two floxed VEGF alleles and positive for Cre expression (MMTV-PyMT/LysMCre+/VEGF^{f/f}) designated as mutants (Mut) whereas female littermates negative for Cre expression (MMTV-PyMT/LysMCre-/VEGF^{f/f}) served as wildtype controls (WT). **c**, Whole-mount staining of mammary glands from 12 week old virgin mice. **d**, Determination of tumor onset by weekly mammary gland palpation of virgin PyMT-mice (n>5 for each group). **e**, Total tumor mass of WT mice and Mut mice at the age of 16 weeks (n>5 for each group). **f**, Representative image from double immunofluorescence for PyMT-antigen and PCNA on PyMT tumors. **g**, Visualization of blood vessels with immunostaining for CD 31 in wild type and mutant mammary tissue from different stages of tumor progression, **h**, Determination of tumor-cell proliferation by quantitative analysis of PCNA-positive cells. **i**, **j**, Gene-expression analysis on lysates of mammary tumors from mice at the age of 20

weeks for PGK-1, and VEGF (n=5). **k**, Representative Western Blot for VEGF in PyMT-tumors. **l**, Representative immunoblot analysis of anti-VEGFR2 immunoprecipitated lysates from PymT-tumors for VEGFR2 and phosphotyrosine (p-Tyr). Scale bars, 100 μ m; error bars, s.e.m.

Supplementary Figure 2.

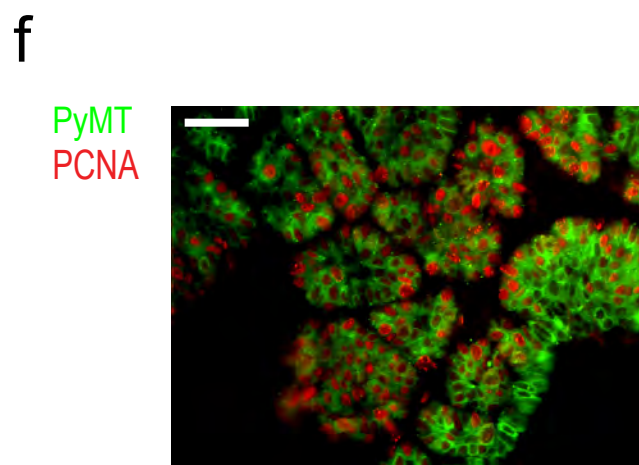
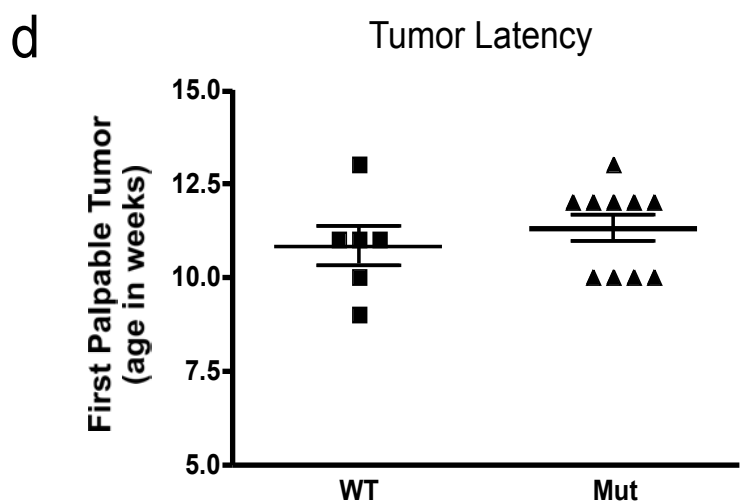
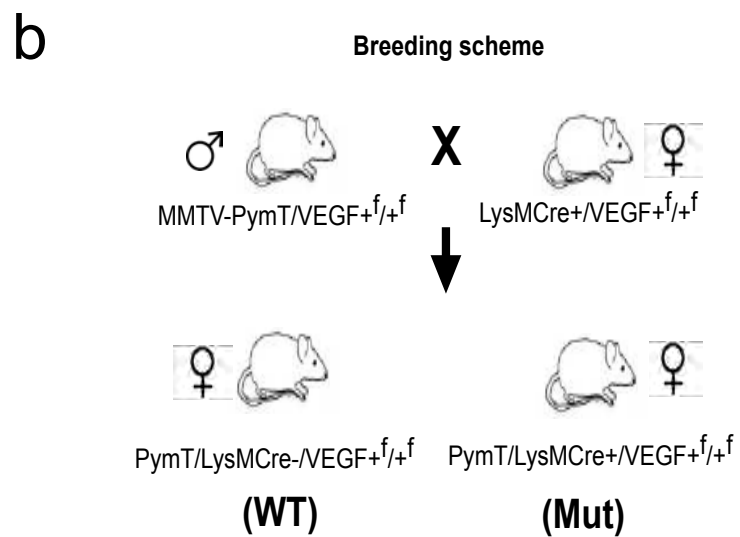
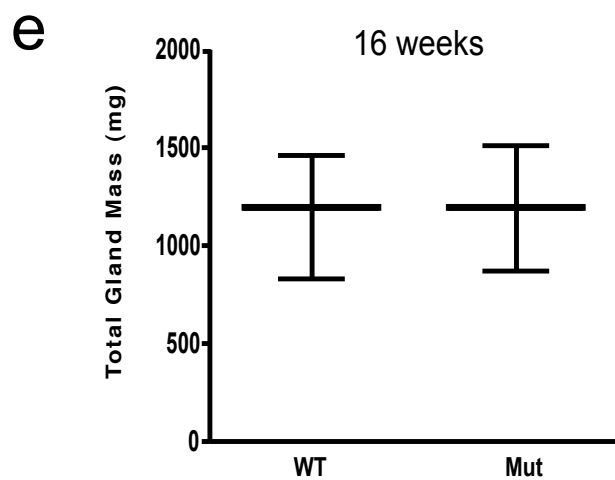
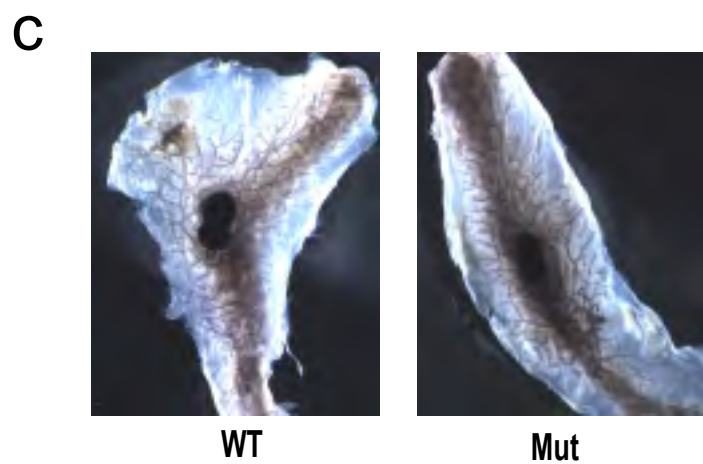
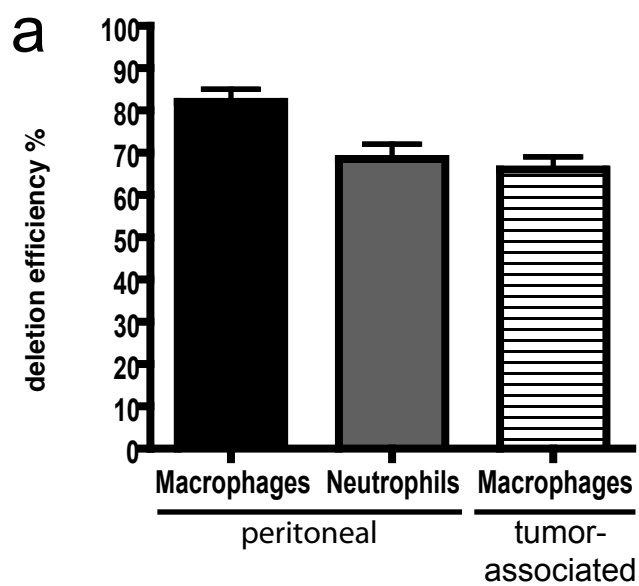
Flow cytometry analysis of single cell suspensions from subcutaneous day 6-8 LLC tumors from myeloid WT and Mut mice. **a**, C57Bl/6 bone marrow-derived cell marker CD 45.2 histogram on wide scatter gate. **b**, Gating on CD 11b+ cells and analysis of myeloid/granulocyte markers Gr-1 and macrophage marker F4/80 allows distinction of neutrophils and tumor-associated macrophages.

Supplementary Figure 3.

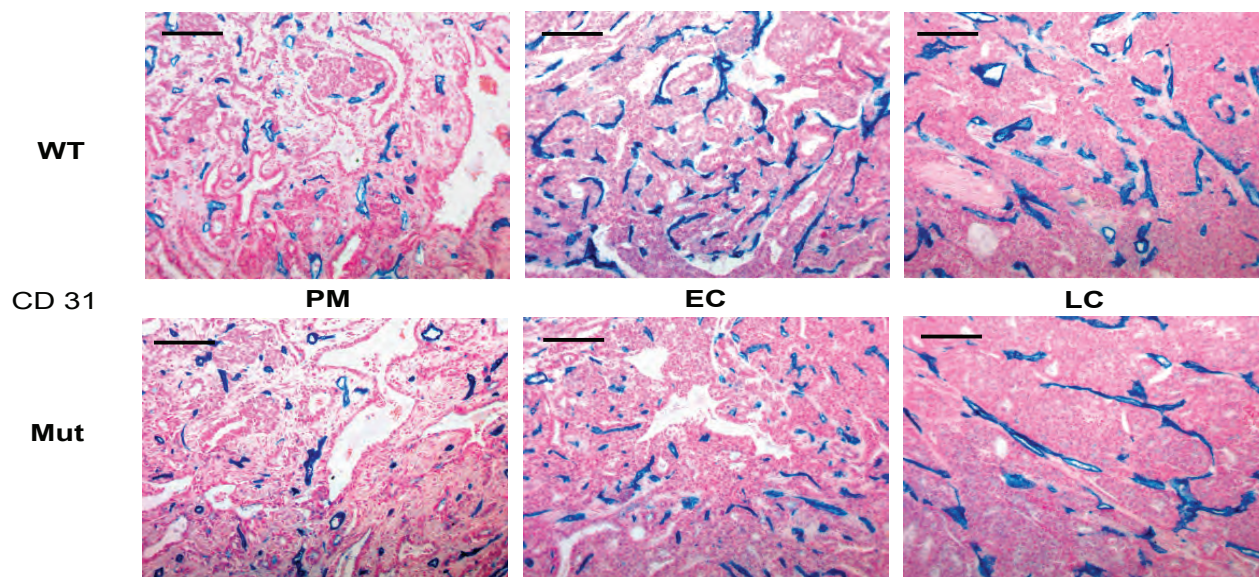
a, Left: Representative Western Blot for VEGF from LLC tumor lysates. Right: Quantitative analysis of VEGF signal intensities (WT n=5, Mut n=4). **b**, Gene-expression analysis for VEGF on total RNA extracts from LLC tumors (WT n=7, Mut n=6). **c**, (left) Immunohistochemical detection of tumor hypoxia with Pimonidazole on LLC isografts, (right) double staining for Pimonidazole and CD 31 on LLC tumors. **d**, Immunofluorescent double-staining for VEGF and F4/80 on LLC tumor sections. **e**, representative TUNEL-staining on CYCP-treated LLC tumors. **f**, Growth curve analysis of LLC isografts from WT (n=6) and Mut animals (n=8) treated with Cis-platinum (c-ddp) (5mg/kg) at days 6, 8 and 10 after tumor implantation. **g**, Response of tumors from WT and Mut mice to c-ddp treatment expressed as percentage of treated tumor volume to untreated tumor volume at certain time points. Scale bar, 100 μ m; error bars, s.e.m.

Supplementary Figure 4.

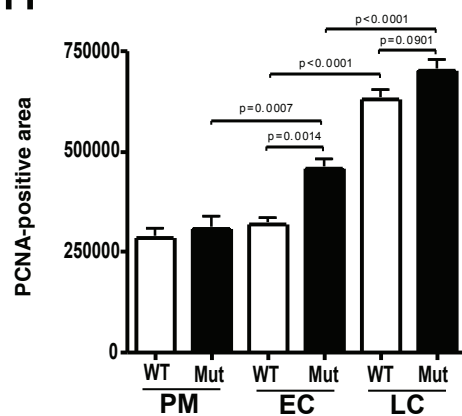
a, Representative Western Blot for VEGF from wildtype (WT) and VEGF nullizygous (null) fibrosarcoma isografts (genotype labeled in black) implanted into WT-mice or Mut-mice (null) with a myeloid cell-specific deletion of VEGF (genotype labeled in blue). **b**, Quantitative analysis of VEGF signal intensities in fibrosarcoma tumors (n=4 for each group). **c**, Gene-expression analysis for VEGF on total RNA extracts from fibrosarcomas (n=3 for each group). Error bars, s.e.m.



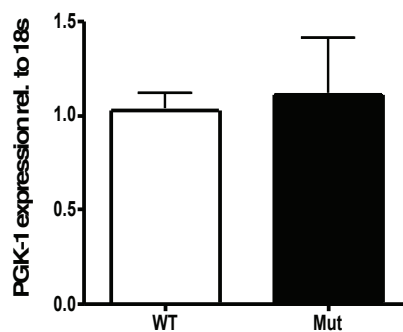
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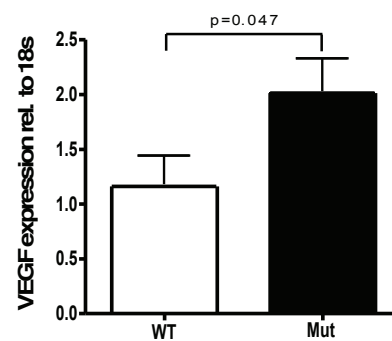
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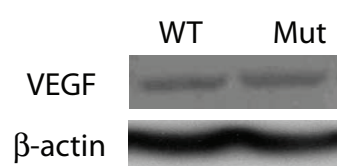
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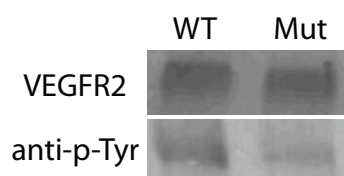
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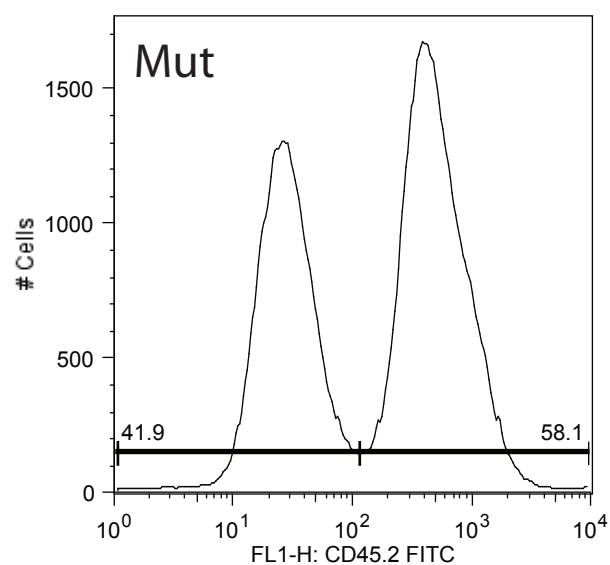
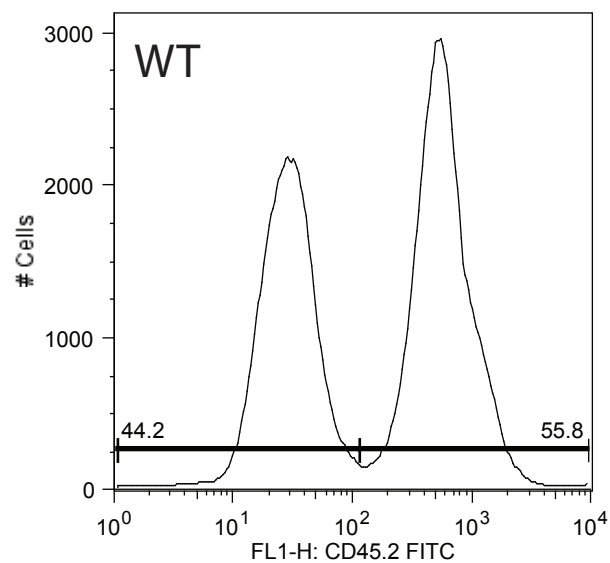
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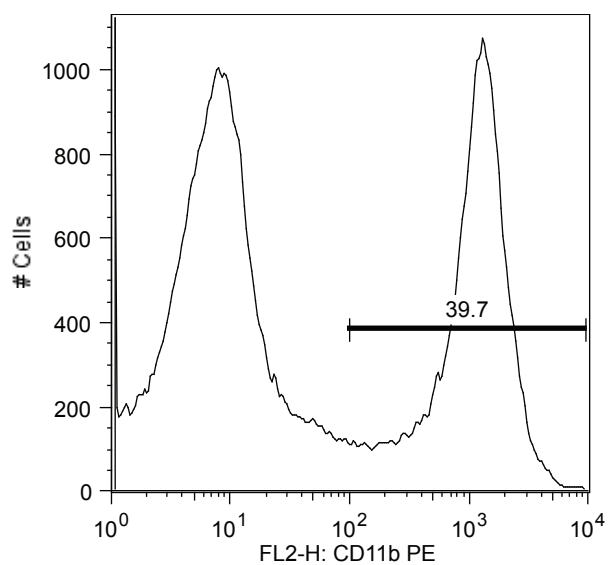
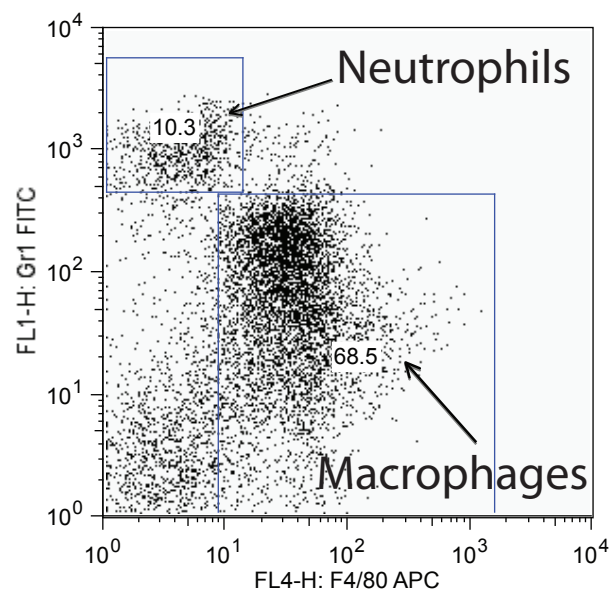


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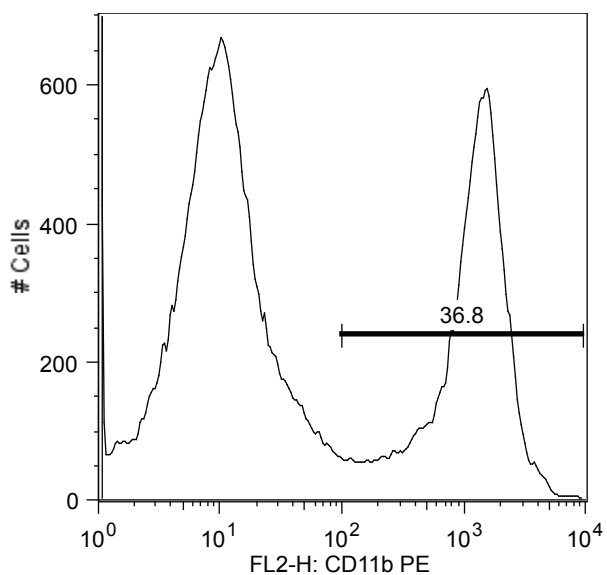
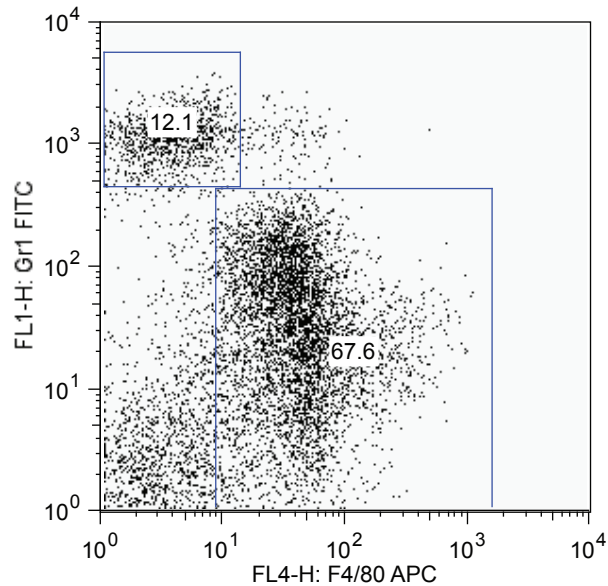


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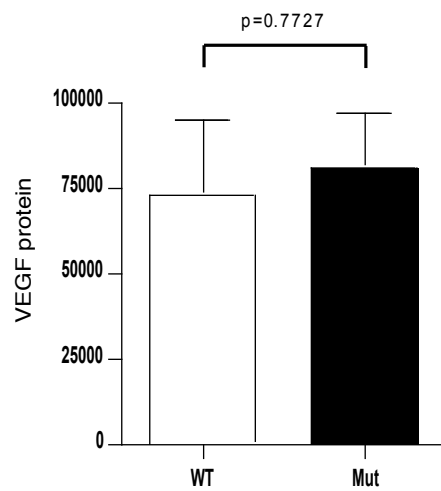
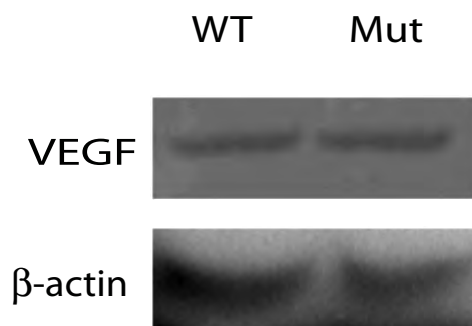
WT

CD11b⁺

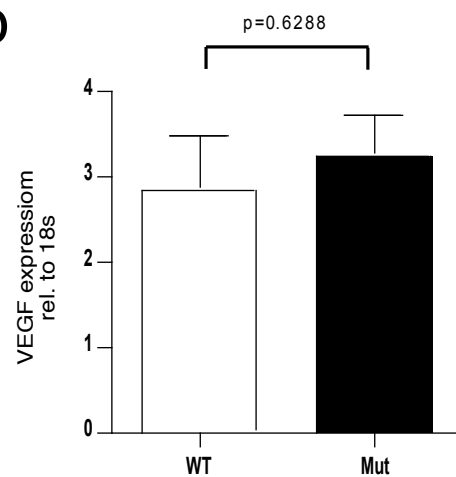
Mut

CD11b⁺

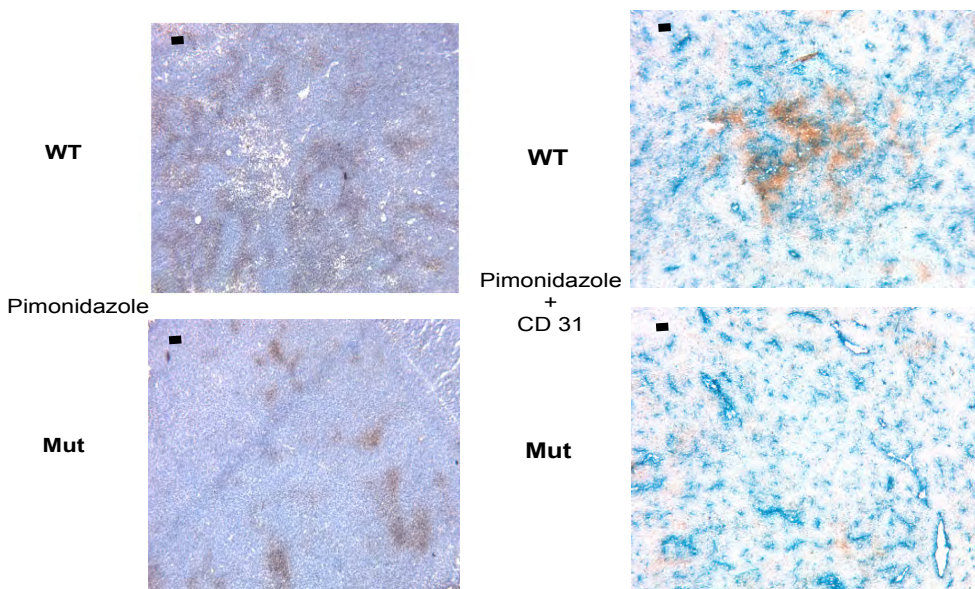
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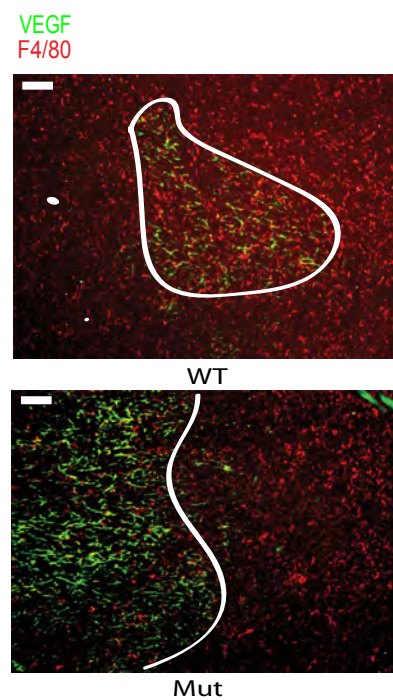
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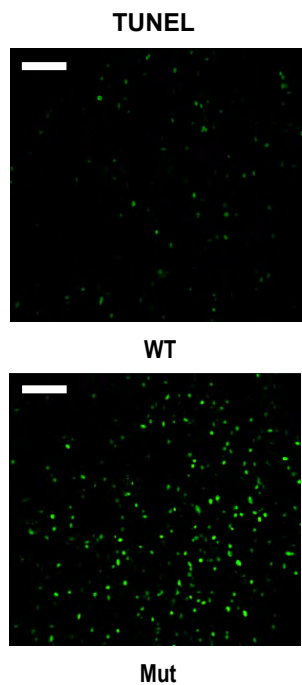
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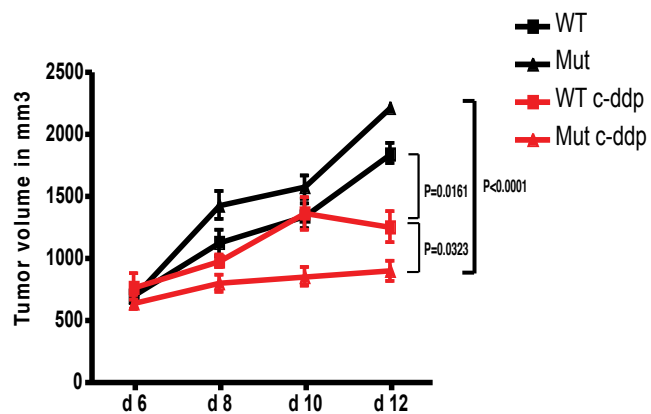
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