Supplementary Figures

Supplemental Figure 1: Ligand independent growth of KITD814V bearing cells in the absence of Vav2 or Vav3. Proliferation as assessed by thymidine incorporation in KITD814V expressing WT, Vav2, and Vav3 deficient primary HSC/Ps in the absence of growth factors. Bars represent the mean [^{3}H] thymidine incorporation (in counts per minute) in primary BM cells expressing the indicated receptors. Data are pooled from experiments utilizing BM cells from two independent mice of each genotype.

Supplemental Figure 2: Inhibition of Rac rescues the tissue damage associated with mice transplanted with cells bearing KITD814V. (A) Left panel shows the percentage of EGFP+ leukemic cells in the peripheral blood, bone marrow, and spleen of mice transplanted with cells bearing KITD814V alone or in combination with RacN17 at the time of moribund. n=5, *p<0.001, KITD814V vs. KITD814V+RacN17. Right panel shows representative flow cytometric dot blot profiles from mice described in left panel at time of moribund. **(B)** Weight and size of spleens from various genotypes was assessed at time of moribund. Quantitative differences in the weight of spleens from the indicated genotypes are shown in the left panel and representative pictures of spleens are shown in the right panel. n=5, *p<0.001, KITD814V vs. KITD814V+RacN17. **(C)** Representative pictures of liver from mice transplanted with cells bearing KITD814V alone or in combination with RacN17.

Supplemental Figure 3: In vivo reduction of active Pak due to Rac inhibition in KITD814V bearing mice. Cell lysates derived from spleens of C3H/HeJ mice transplanted with cells bearing KITD814V with or without RacN17 were immunoblotted with antibodies that recognize phospho-Pak and total Pak.

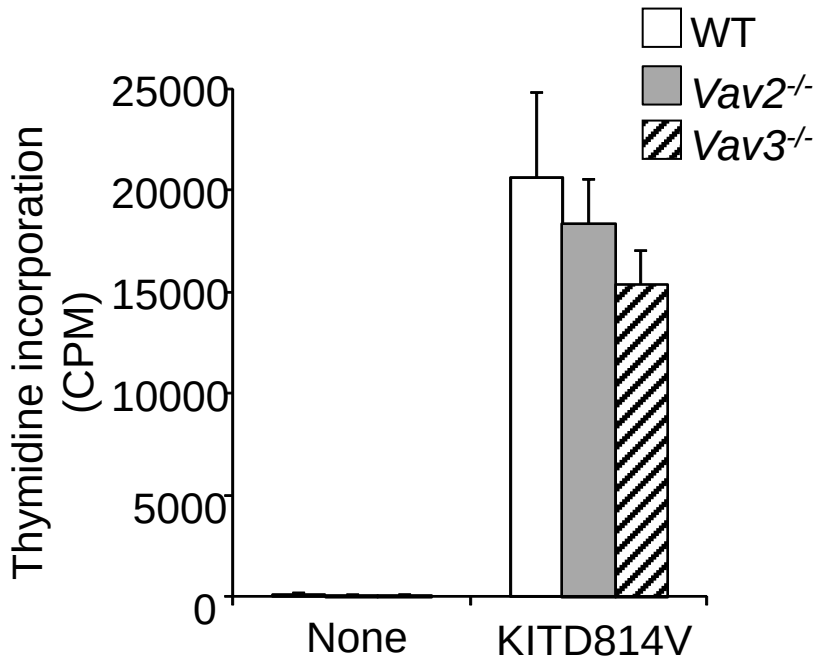
Supplemental Figure 4: Inhibition of Rac GTPases affects KITD814V growth due to increased apoptosis. (A) 32D cells bearing WTKIT grown in the presence of 5ng/mL IL-3 or KITD814V bearing cells grown in the absence of growth factors and indicated Rac inhibitor (NSC23766) were subjected to a [^{3}H] thymidine incorporation assay. **(B)** Murine mastocytoma cells, P815, and **(C)** human AML patient derived Kasumi-1 cells were cultured in the presence of NSC23766 and assessed for proliferation by measuring [^{3}H] thymidine incorporation. Bars represent mean ± SD from 2 to 4 independent experiments performed in replicates of four. *p<0.05. **(D)** 32D cells expressing KITD814V or WTKIT cells grown in the presence of a novel Rac inhibitor, EHop-016, were subjected to a [^{3}H] thymidine incorporation assay as described above. *p<0.05 **(E)** KITD814V bearing 32D cells were examined for survival in the presence of 5μM or 10μM EHop-016 for 0, 24, or 48 hours prior to subjecting the cells to Annexin V and 7-AAD staining. Survival was assessed by quantitating the percentage of Annexin V and 7-AAD negative (viable) cells. Bars denote the mean ± SD percentage of viable cells from one of two independent experiments performed in quadruplicate. *p<0.05, vehicle vs EHop-016 treated. **(F)** Cells described in (E) were treated with EHop-016 and cell lysates were subjected to immunoblotting with phospho-MLC, phospho-BAD, and β-actin.

Supplemental Figure 5: Expression of active RacV12 restores KITD814V induced hyperproliferation in Rac1/Rac2 deficient HSC/Ps. (A) Primary HSC/Ps were collected from WT and *MxCreRac1$^{flox/flox}$Rac2$^{-/-}$* mice following three consecutive intraperitoneal injections of polyI:C given at 48 hour intervals. These cells were transduced with KITD814V in the presence of empty vector or RacV12, sorted on EGFP and selected in puromycin and assessed for proliferation by [^{3}H] thymidine incorporation in the absence of growth factors. Bars represent the mean [^{3}H] thymidine incorporation (in counts per minute) in primary HSC/Ps expressing the indicated receptors. *p<0.05, empty vector vs RacV12. **(B)** KITD814V bearing WT or *Rac1-/-:Rac2-/-* HSC/Ps were lysed, and equal amount of lysates were subjected to western blotting using total Rac1, Rac2, pan-Rac, and ß actin antibody.

Supplemental Figure 6: Transduction efficiency of primary 5-FU treated bone marrow (BM) cells. 5-FU treated BM cells derived from WT, *Vav1*$^{-/-}$, and *Rac1*$^{flox/flox}$*Rac2*$^{-/-}$ mice were infected with retrovirus encoding the KITD814V receptor. EGFP expression on the x-axis is indicative of the transduction efficiency for each genotype. Transduced cells were sorted to homogeneity prior to being transplanted into recipients for MPN development and survival studies described in Figure 7.

Supplemental Figure 7: Genetic disruption of Rac2 enhances survival of KITD814V bearing mice. Cumulative survival using Kaplan-Meier log-rank test of recipient cohorts bearing KITD814V expressing wildtype (WT; n=11) (10) or *Rac2*$^{-/-}$ (n=10) BM. *p<0.05. Data showing the survival of KITD814V bearing mice has been previously shown (10).

Supplemental Figure 8: (A) 32D cells were transduced with KITD814V with or without dominant negative PakK299R. Flow cytometric dot plot profiles from a representative experiment are shown. **(B)** Primary HSC/Ps from WT and *MxCreRac1*$^{flox/flox}$*Rac2*$^{-/-}$ mice were harvested following three consecutive intraperitoneal injections of polyI:C given at 48 hour intervals. These cells were transduced with KITD814V in the presence of empty vector or PakT423 and assessed for growth by thymidine incorporation in the absence of growth factors. Bars represent the mean thymidine incorporation (in counts per minute) in primary HSC/Ps expressing the indicated receptors. *p<0.05, empty vector vs PakT423.



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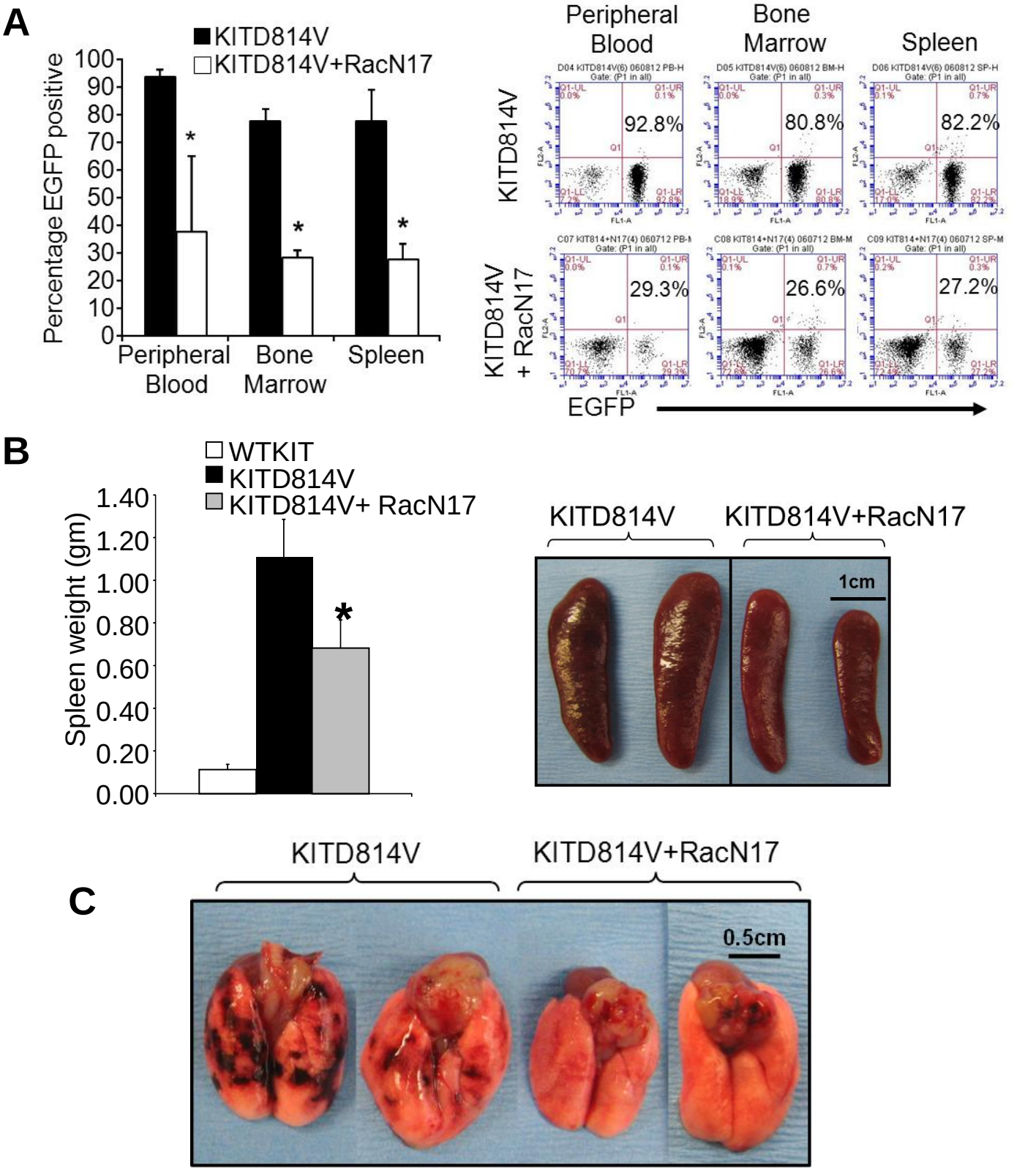

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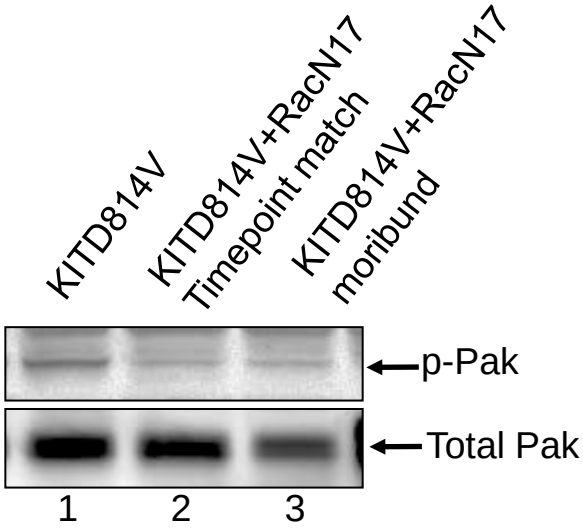

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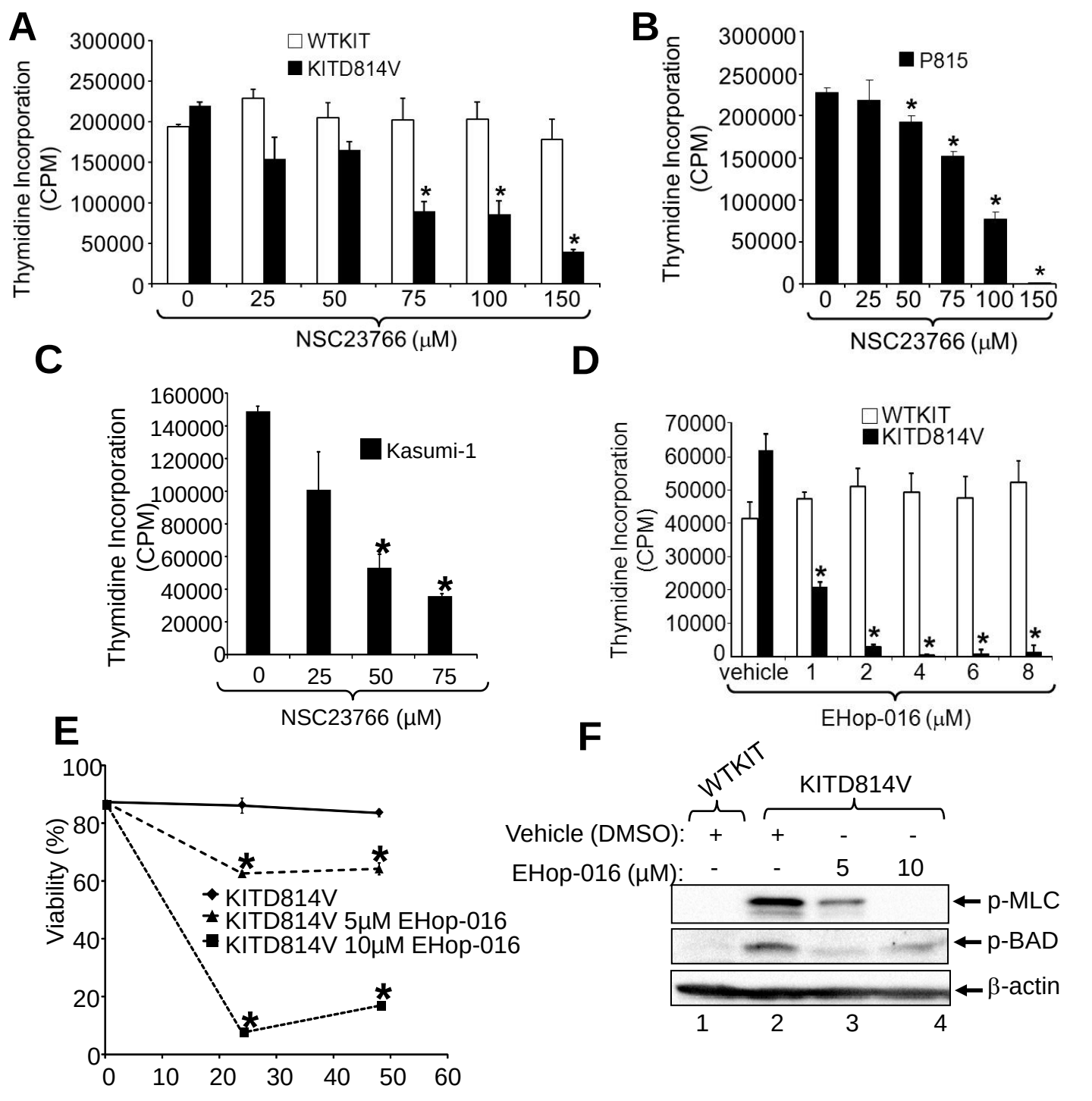

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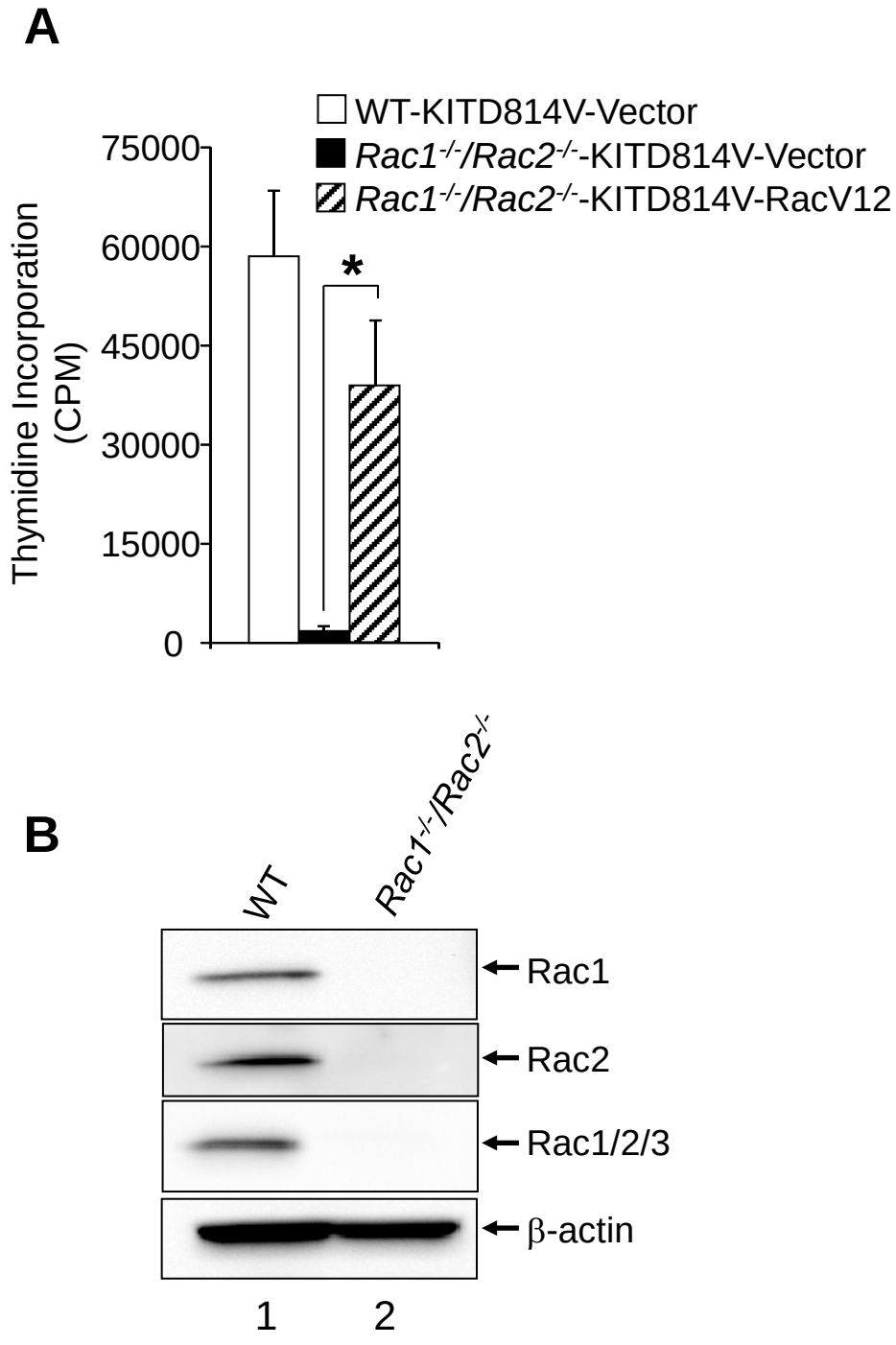

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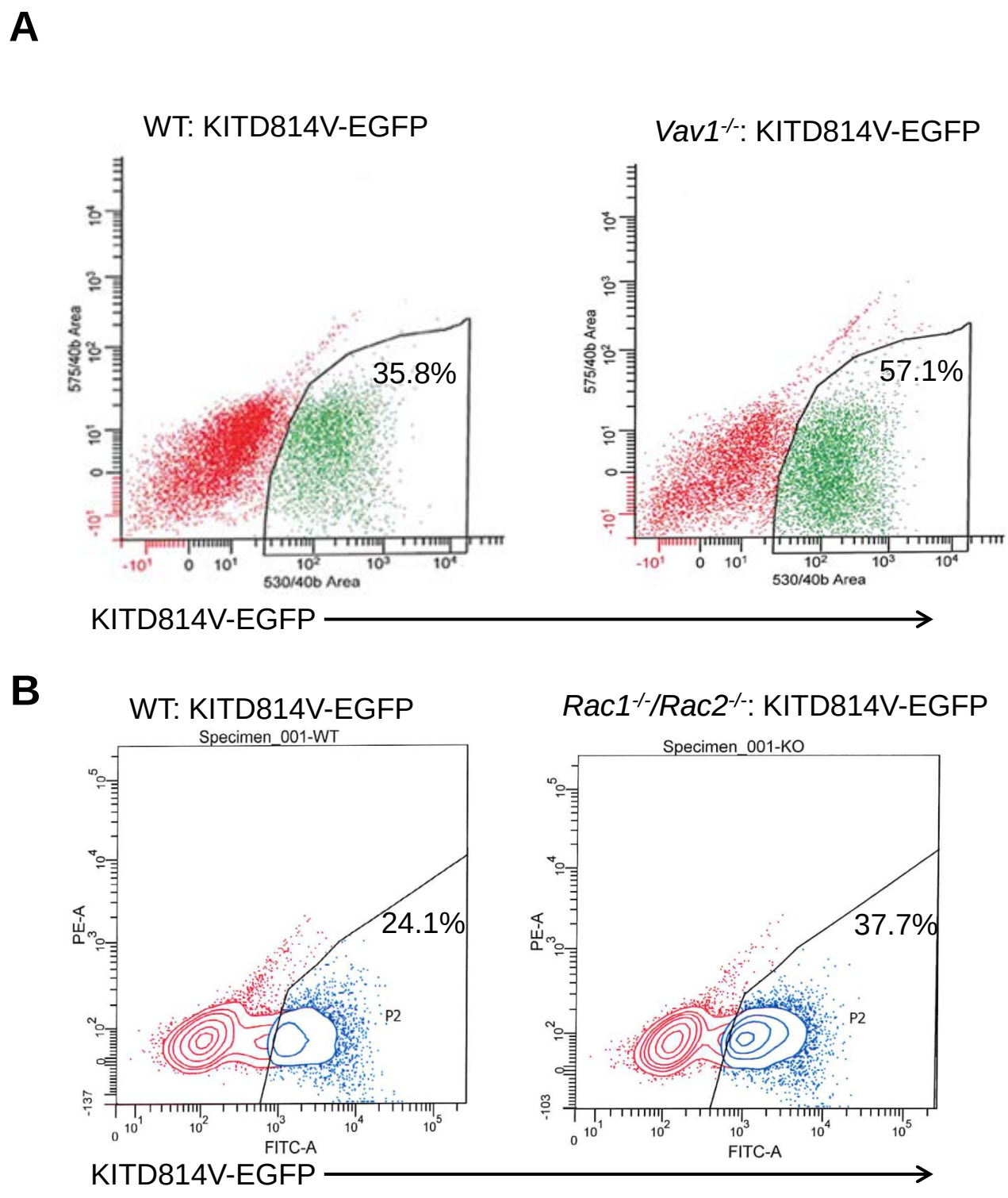

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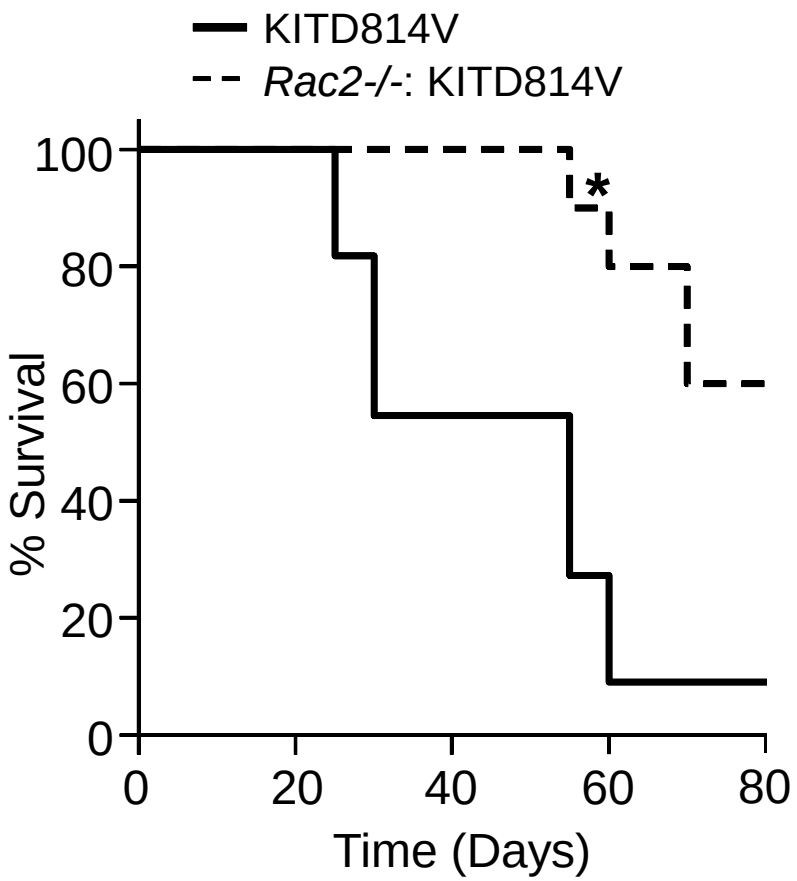

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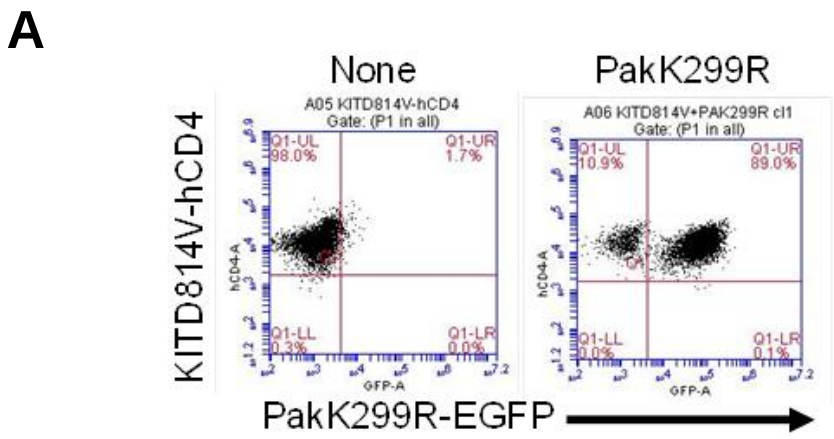

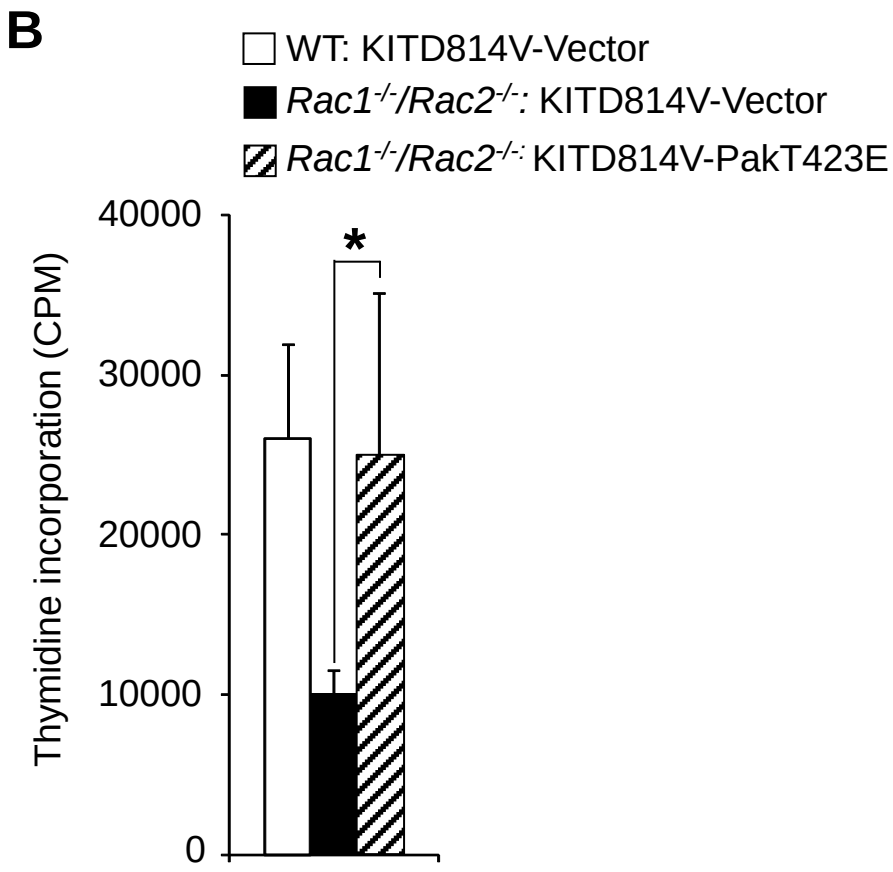

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