Supplementary Information Titles

Please list each supplementary item and its title or caption, in the order shown below.

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Journal: Nature Medicine

Article Title:	**Inflammasome-independent role of AIM2 in suppressing colon tumorigenesis by interfering with DNA-PK-dependent Akt activation**
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Supplementary Item & Number **(add rows as necessary)**	Title or Caption
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Supplementary Figure 1. Generation and assessment of inflammasome function in *Aim2*$^{-/-}$ mice. (**a**, top panel) *Aim2*$^{-/-}$ mice were generated on the C57BL/6 mouse background by targeted replacement of the *Aim2* coding region with a neomycin resistance gene. (**a**, lower panel) PCR analysis of wild type and/or neomycin-containing (*Aim2* knockout) alleles from *Aim2*$^{+/+}$, *Aim2*$^{+/-}$ and *Aim2*$^{-/-}$ mouse genomic DNA. (**b**) Bone marrow-derived macrophages from wild type and *Aim2*$^{-/-}$ mice primed with LPS (500 ug/ml for 3 hrs) and transfected with poly(dA:dT) (1 μg/ml) for IL-1β assessment by ELISA. Error bars represent standard error of the mean with triplicate samples/group.

Supplementary Figure 2. *Aim2*$^{-/-}$ mice have no defects in immune cell populations. (**a**) Flow cytometric analysis of total cellularity and the indicated immune cell populations were assessed from (**b**) spleen, (**c**) inguinal lymph node (ILN) and (**d**) thymus from naïve *Aim2*$^{+/+}$, *Aim2*$^{+/-}$ and *Aim2*$^{-/-}$ mice. Each data point represents cells isolated from a single mouse.

Supplementary Figure 3. *Aim2*$^{-/-}$ and wild type mice display similar clinical symptoms of disease during CAC. (**a**) Wild type, *Aim2*$^{-/-}$ and *Asc*$^{-/-}$ mice were subjected to the AOM/DSS model of CAC. Clinical symptoms of disease including rectal bleeding and diarrhea were assessed upon completion of each round of DSS administration. Error bars represent standard error of the mean with n≥12 mice/group. (**b**) Serum levels of FITC-dextran in AOM/DSS-treated wild type and *Aim2*$^{-/-}$ mice fasted and fed FITC-dextran. Error bars represent standard error of the mean with n≥4 mice/group. Ns, not

significant. *p<0.05, **p<0.01,***p<0.001. a,b, One-way ANOVA (Tukey's multiple comparisons test).

Supplementary Figure 4. Densitometric Analysis of immunoblots.

Densitometric analysis of (**a**) Caspase-1 p10, (**b**) pAkt (S473), (**c**) PDK1, (**d**) PI3K p85, (**e**) p-mTOR, (**f**) PTEN western blots on colon protein extracts from AOM/DSS-treated wild type and *Aim2*$^{-/-}$ mice, and densitometric analysis of (**g**) pAkt (S473) from AOM/DSS-treated wild type and *Asc*$^{-/-}$ mice (n=3 mice/group). Densitometric analysis of colon (**h**) pAkt (S473) levels in *APC*$^{Min/+}$ and *Aim2*$^{-/-}$/*APC*$^{Min/+}$ mice (n=3 mice/group). Densitometric analysis of colon (**i**) pAkt (S473), (**j**) pAkt (T308) and (**k**) PTEN levels in IGF-1-treated *Aim2*$^{+/+}$ vs. *Aim2*$^{-/-}$ mouse embryonic fibroblasts (MEFs) (Representative 1 of 3 independent experiments with quadruplicate samples/group) and (**l**) pAkt (S473) in *Asc*$^{+/+}$ vs. *Asc*$^{-/-}$ MEFs (Representative 1 of 3 independent experiments with triplicate samples/group). Densitometric analysis of (**m**) pAkt (S473) in IGF-1-treated HCT-116 cells expressing empty vector (EV) or AIM2 (Representative of 1 of 3 independent experiments with quadruplicate samples/group). Densitometric analysis of (**n**) pAkt (S473) in IGF-1-treated wild type and *Aim2*$^{-/-}$ colon organoid cultures (Representative of 2 of 3 independent experiments). Densitometric analysis of colon (**o**) active caspase-7 in AOM/DSS-treated wild type and *Aim2*$^{-/-}$ mice (n=3 mice/group). Densitometric analysis of (**p**) active caspase-3 levels in stuarosporin (staur)-treated wild type and *Aim2*$^{-/-}$ MEFs (Representative of 3 of 3 independent experiments). Densitometric analysis of (**q**) pDNA-PK (S2056) levels in bleocin (Bleo)-treated HCT-116 cells expressing empty vector (EV) or AIM2 (Representative of 1 of 3 independent experiments with triplicate

samples/group). Densitometric analysis of (**r**) pAkt (S473) in wild type and *Aim2*$^{-/-}$ MEFs treated with IGF-1 and DNA-PK inhibitors, NU7026 and NU7441 (Representative of 2 of 3 experiments with duplicate samples/group). Densitometric analysis of (**q**) active caspase-3 levels in wild type and *Aim2*$^{-/-}$ MEFs treated with staurosporin (staur) and the DNA-PK inhibitor NU7026 (Representative of 2 of 2 independent experiments with duplicate samples/group). Protein levels were normalized to the indicated loading control. Error bars denote standard error of the mean. Ns, not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, unpaired t test.

Supplementary Figure 5. AIM2 does not protect against experimental colitis.

(**a**) DSS model of acute experimental colitis. (**b**) Weight loss and (**c**) colon length reduction in response to experimental acute colitis. (**d**) Extended model of DSS-induced colitis. (**e**) Weight loss, (**f**) mortality, (**g**) colon length reduction in response to DSS treatment. Error bars represent standard deviation of the mean with n≥4 mice/group. No statistical significance was detected in weight loss, mortality or colon length between wild type and *Aim2*$^{-/-}$ mice. (**h**) IL-1β and IL-18 production in colon explants from chronic DSS-treated wild type, *Aim2*$^{-/-}$ and *Asc*$^{-/-}$ mice. Error bars represent standard error of the mean with n≥3 mice/group. (**i**) Weight loss (**j**) mortality and (**k**) colon weight to length ratio of *Rag1*$^{-/-}$ mice i.p. injected with naïve CD4^{+} T cells isolated from wild type or *Aim2*$^{-/-}$ mice. Error bars represent standard error of the mean with n≥11 mice/group. No statistical significance was detected in weight loss, mortality or colon weight to length ratio in *Rag1*$^{-/-}$ mice injected with wild type or *Aim2*$^{-/-}$ naive CD4^{+} T cells. d, days; w,

weeks. Ns, not significant. b,c,e,g,i,k, unpaired t test; f,j, log-rank test; h, ANOVA (Tukey's multiple comparisons test).

Supplementary Figure 6. AIM2 suppresses colon hyperplasia and dysplasia during CAC.

Representative images of hematoxylin and eosin-stained colons from day 55 AOM/DSS-treated wild type and *Aim2*$^{-/-}$ mice showing colon crypt hyperplasia and dysplasia (semiquantitative histopathology scoring shown in **Fig. 2d,e**). The images were taken at 200x magnification with scale bars = 100 μm.

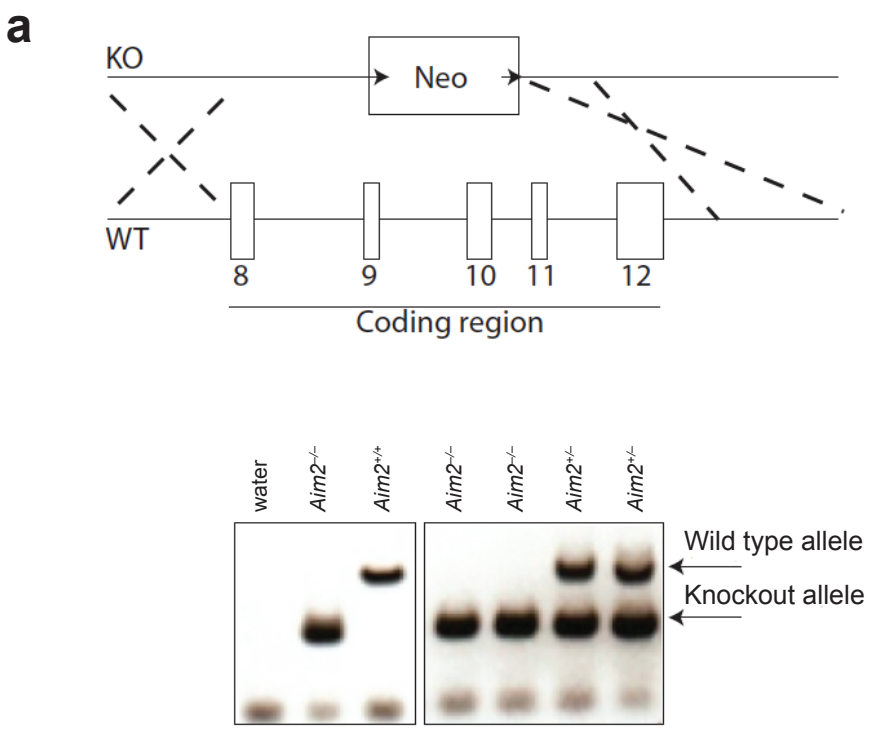
Supplementary Figure 7. AIM2 does not reduce MHC class II, MAPK, NF-κB and STAT3 activation during CAC.

(**a**) Quantitative PCR analysis of MHC class II (*I-Ab*) expression in the colon polyps and adjacent colon tissue of AOM/DSS-treated wild type and *Aim2*$^{-/-}$ mice (AU, arbitrary units). n≥5 mice/group. (**b**) Western blot analysis of MAPK, (**c**) NF-κB and (**d**) p-STAT3 expression in the colons of AOM/DSS-treated wild type and *Aim2*$^{-/-}$ mice with representative densitometry. Error bars represent the standard error of the mean. Ns, not significant. a, ANOVA (Tukey's multiple comparisons test), b,c,d, unpaired t test.

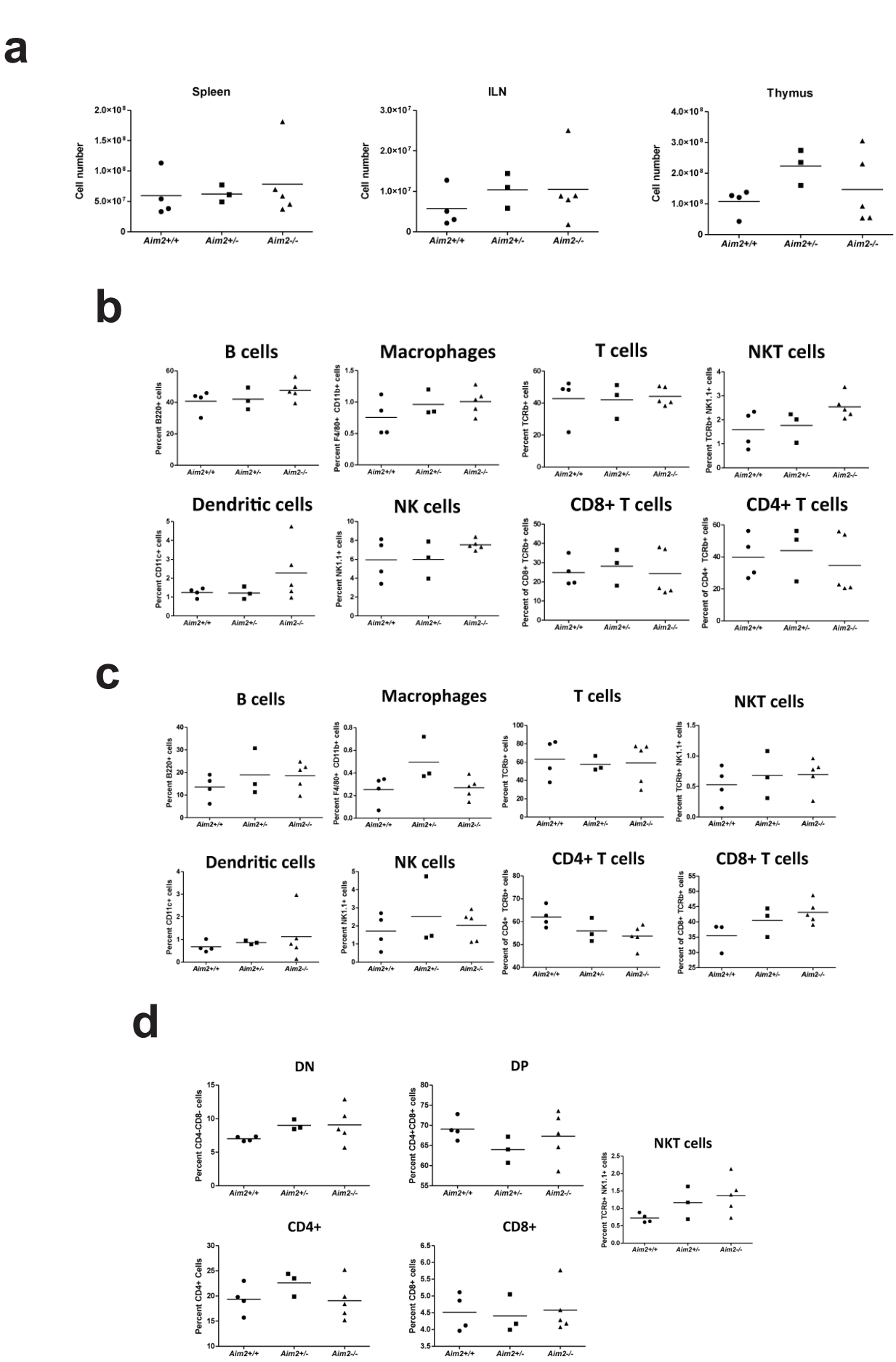
Supplementary Figure 8. AIM2 is expressed by human immune cells and colonic epithelial cells. (**a**) Levels of *AIM2* gene expression in immune organs and cells and (**b**) intestinal-specific organs and cells using publically available gene array data (NextBio Body Atlas: http://www.nextbio.com/b/search/ba.nb).

Supplementary Figure 9. Generation and differentiation of wild type and *Aim2*$^{-/-}$ in vitro organoid cultures. Colon epithelial cells were isolated from wild type and *Aim2*$^{-/-}$ mice on day 0 and differentiated into 3-dimensional epithelial spheroids (organoids) by culturing in matrigel in the presence of EGF, R-spondin, noggin and Wnt-3A for 4-5 days as described in the Materials and Methods and as previously described[41-43]. The images were taken at 10x magnification with scale bars = 100 μm.

Supplementary Figure 10. AIM2 expression in macrophages does not contribute to Aim2-mediated Akt suppression. (**a**) Western blot analysis of p-Akt from wild type and *Aim2*$^{-/-}$ bone marrow-derived macrophages stimulated with LPS or (**b**) IGF-1 with representative densitometry. The results are representative of 1 of 3 experiments with triplicate samples/group. ns, not significant. unpaired t test.



Supplementary Fig. 1



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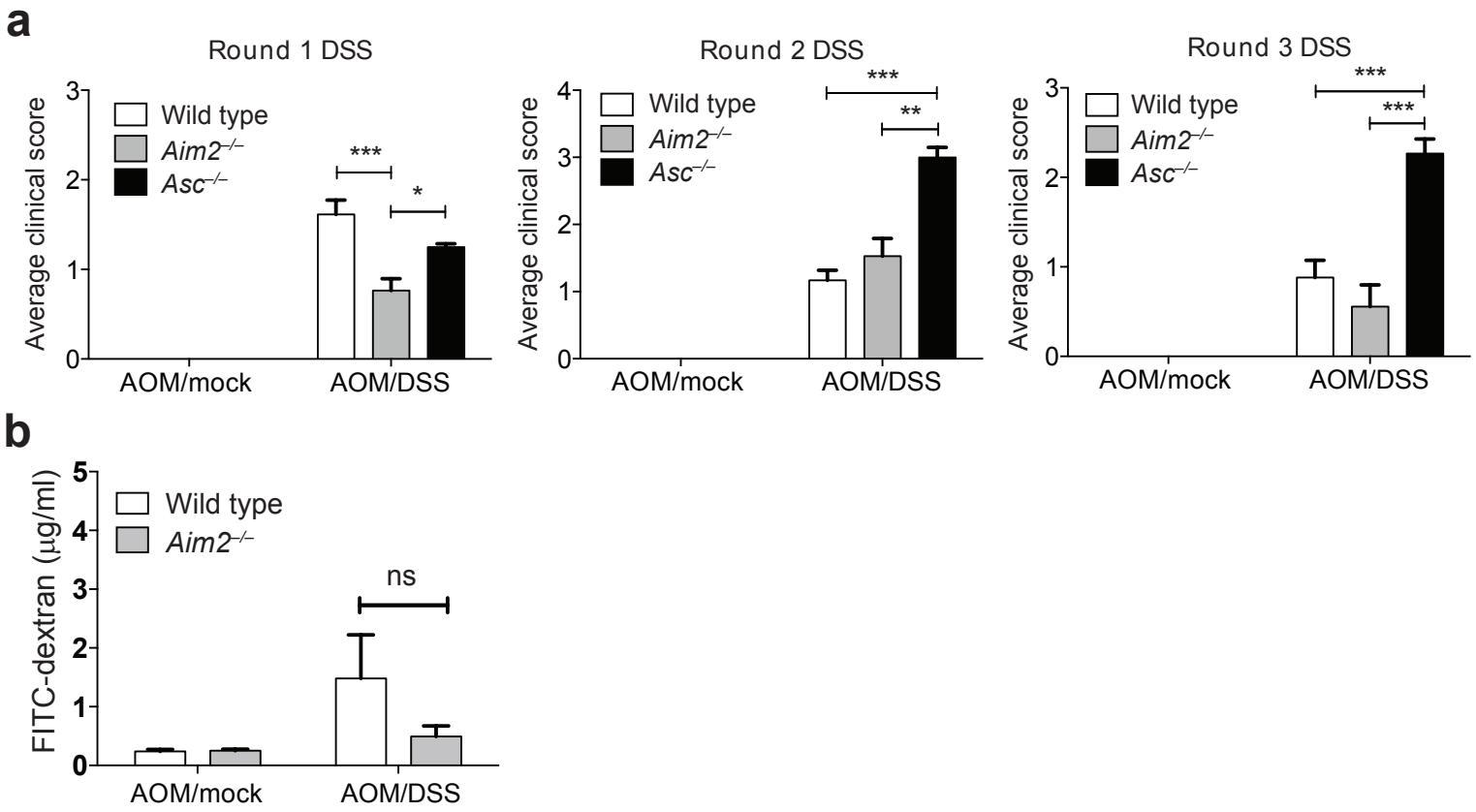

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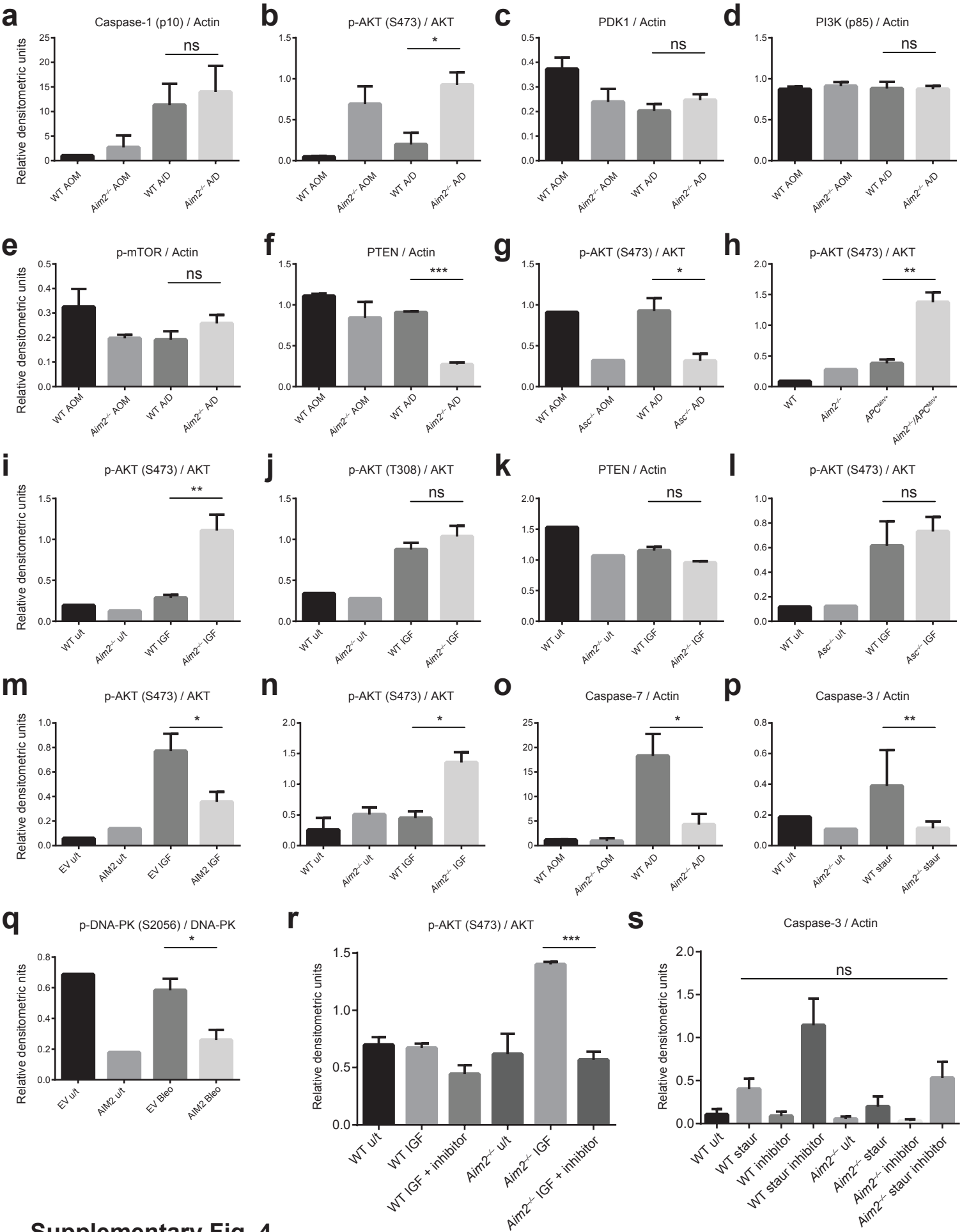

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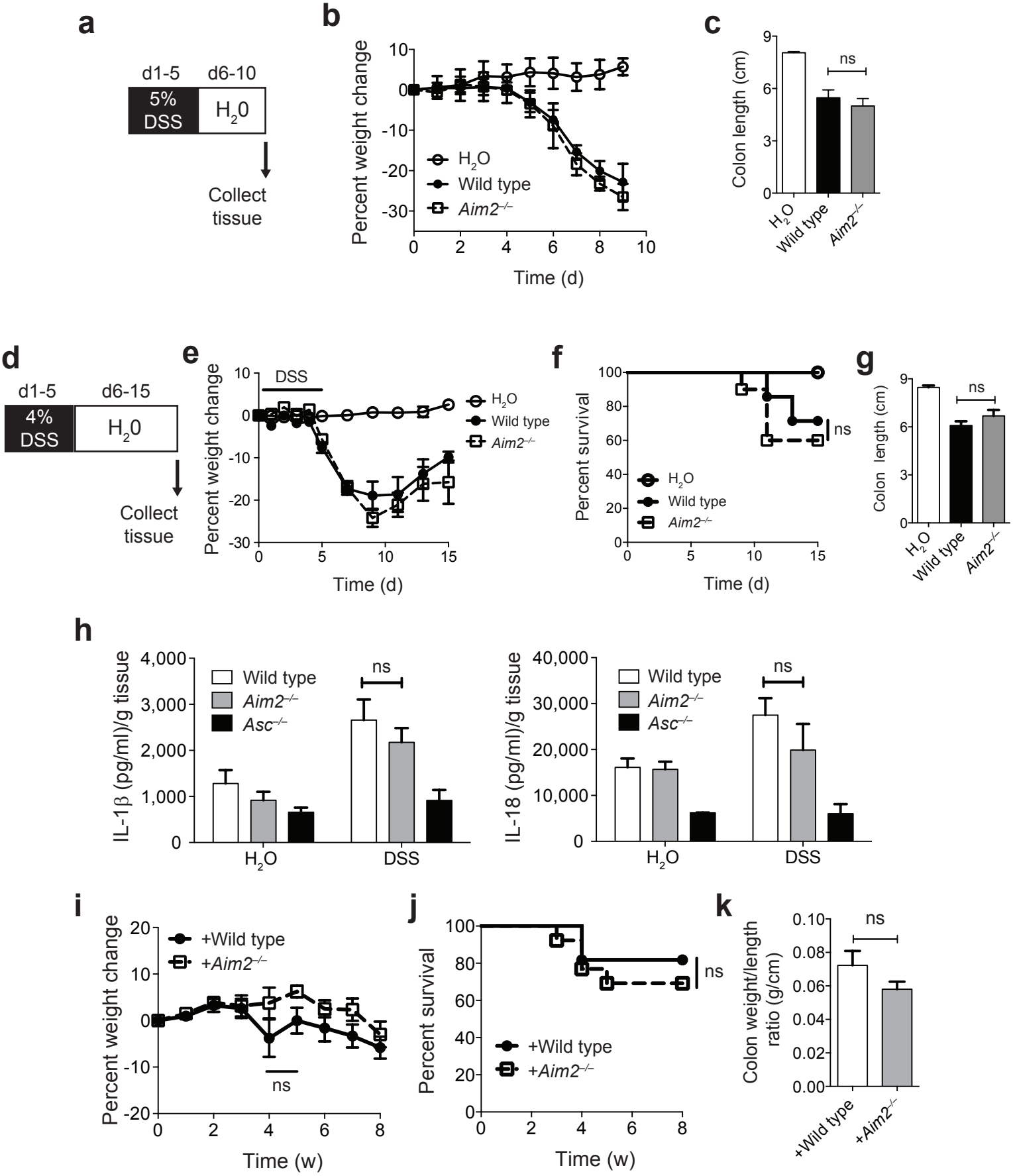

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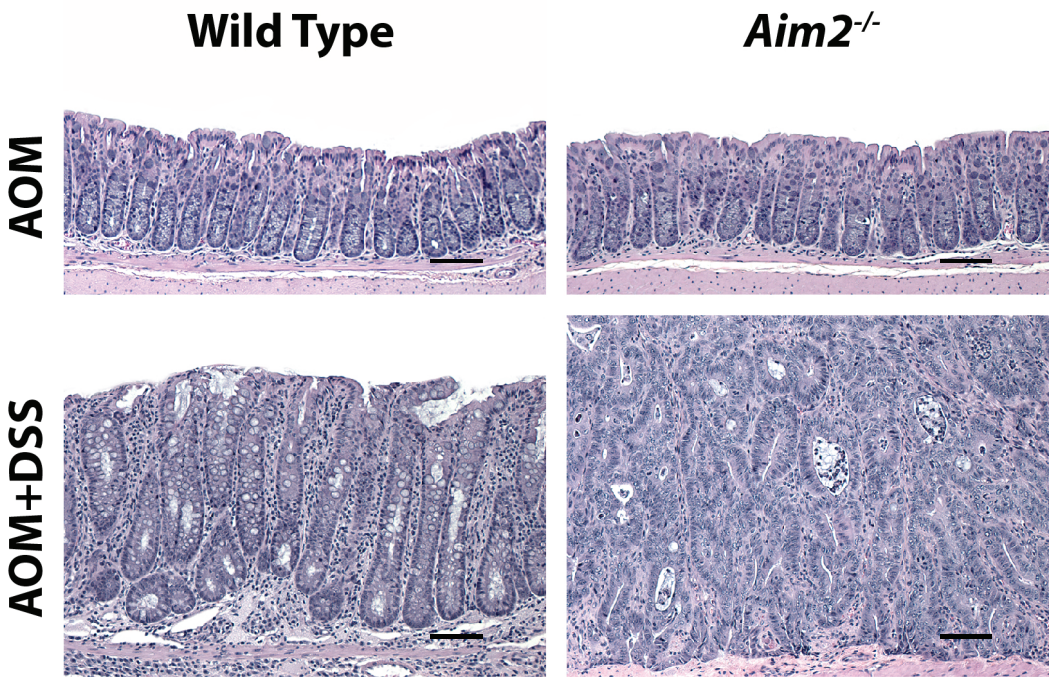

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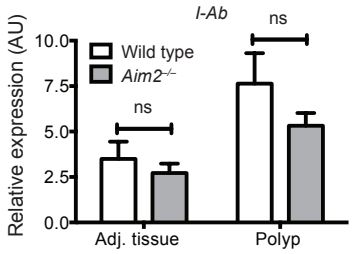

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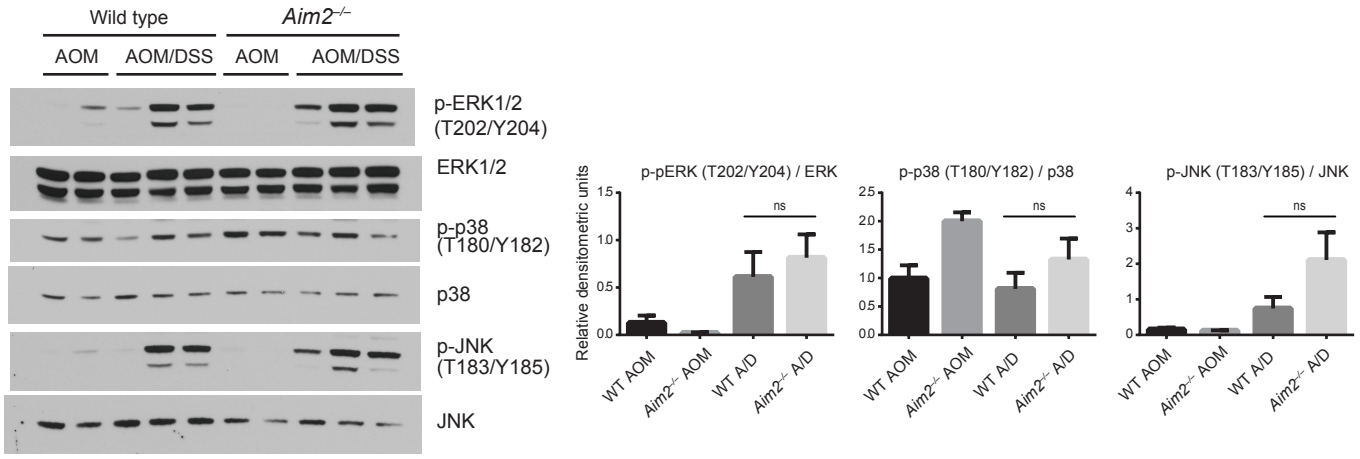

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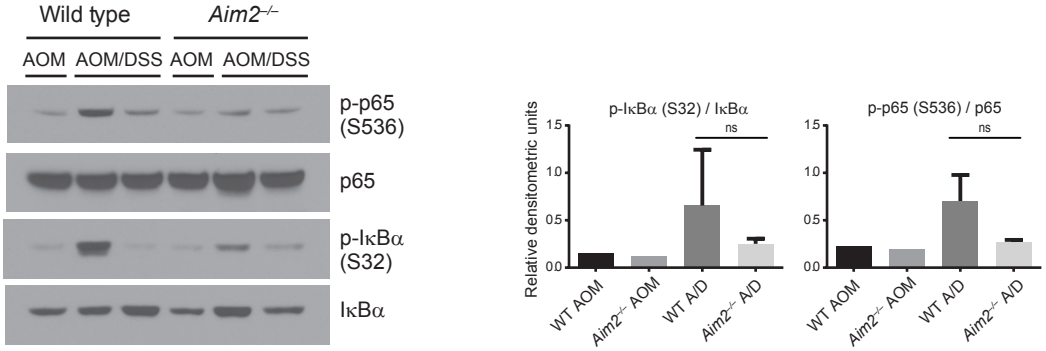

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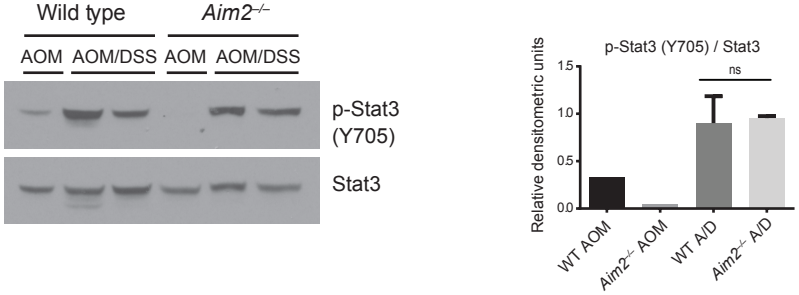

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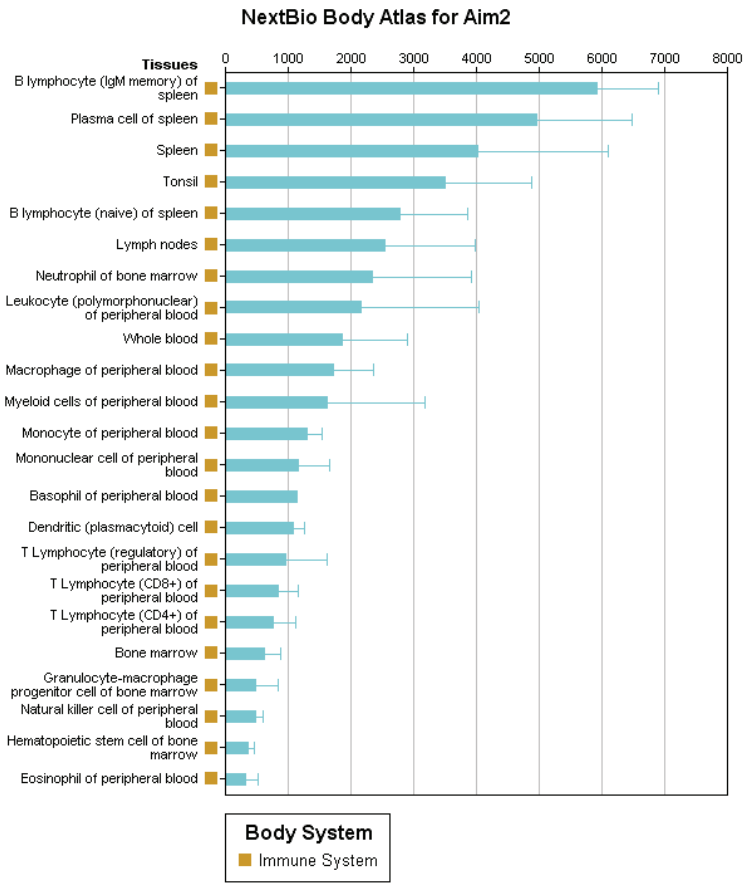

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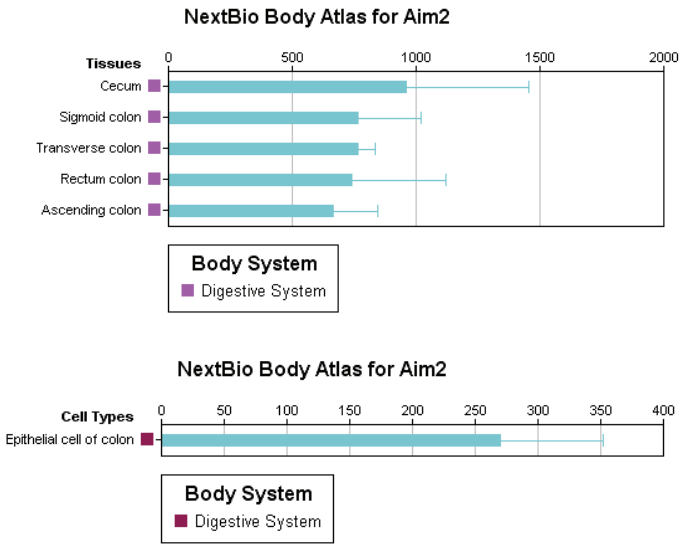

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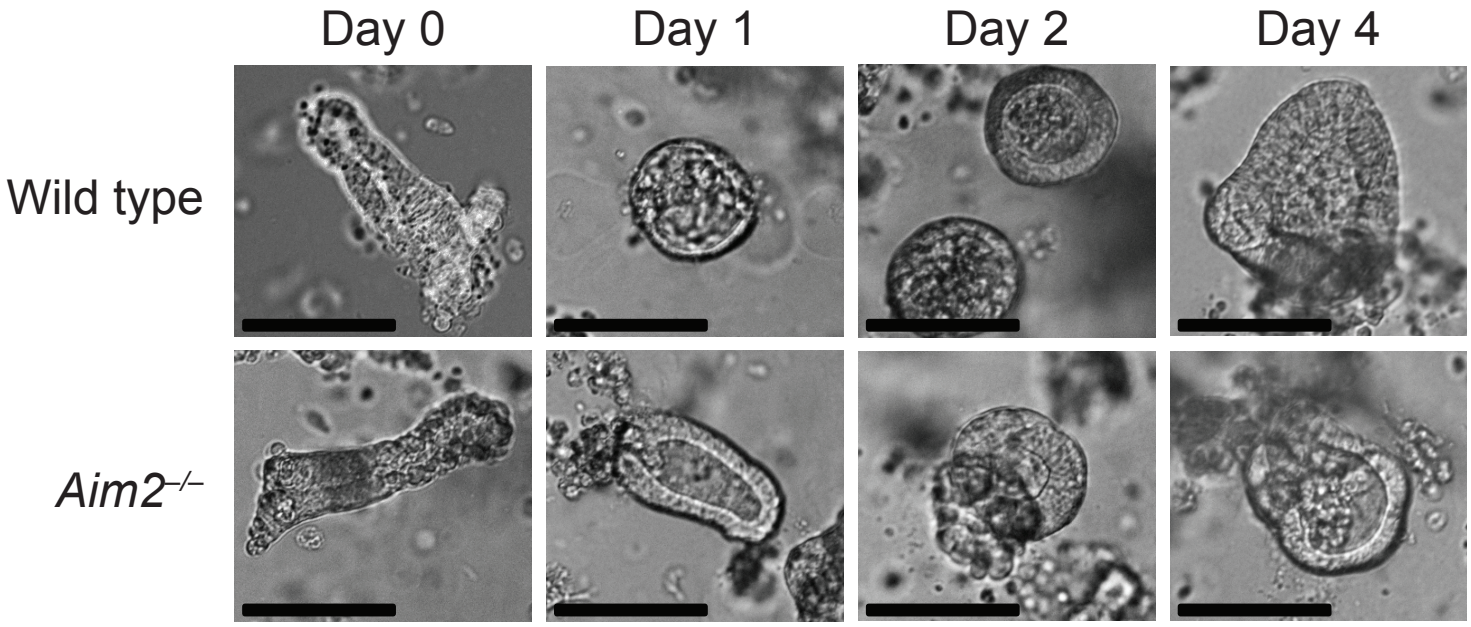

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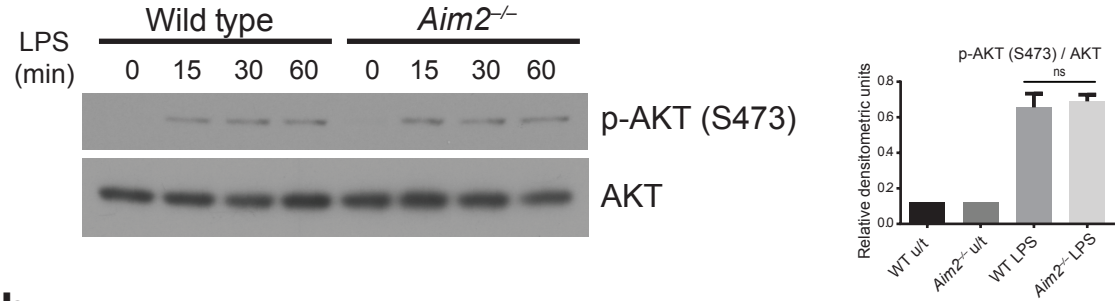

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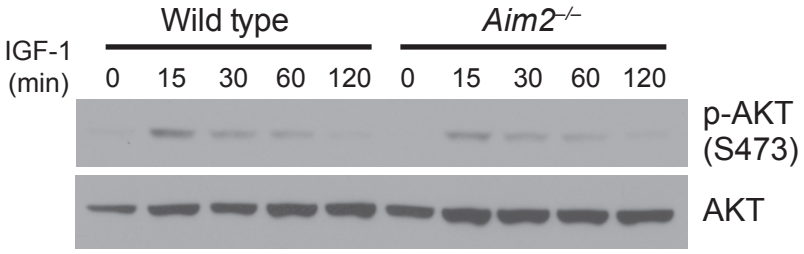

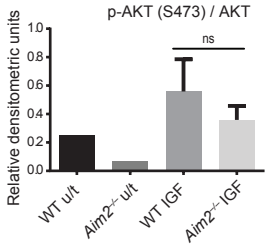

Supplementary Fig. 10