Supplemental Information

to

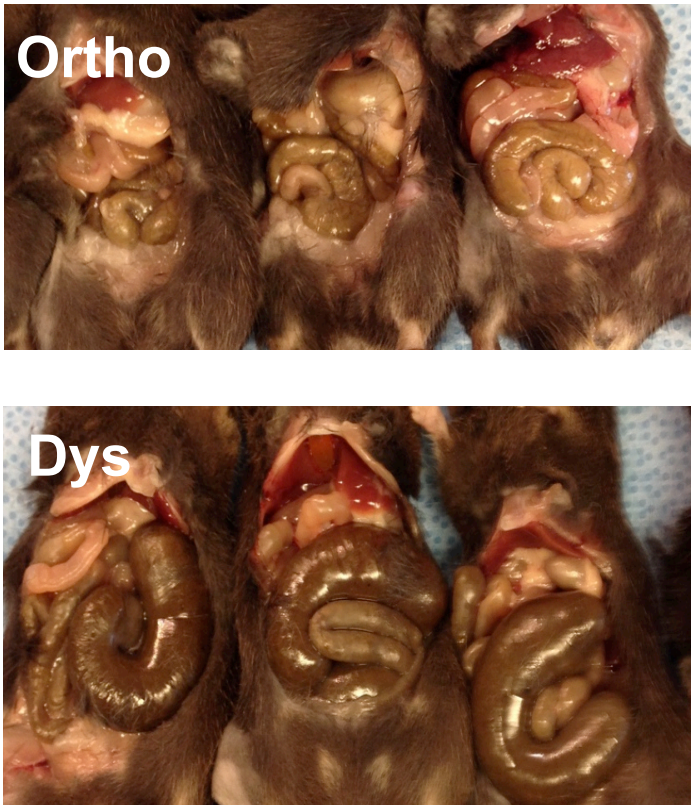
Gastrointestinal tract dysbiosis enhances distal tumor progression through suppression of leukocyte trafficking

Samir V. Jenkins,[1] Michael S. Robeson II,[2] Robert J. Griffin,[1] Charles M. Quick,[3] Eric R. Siegel,[4] Martin J. Cannon,[5] Kieng B. Vang,[6] and Ruud P.M. Dings[1*]

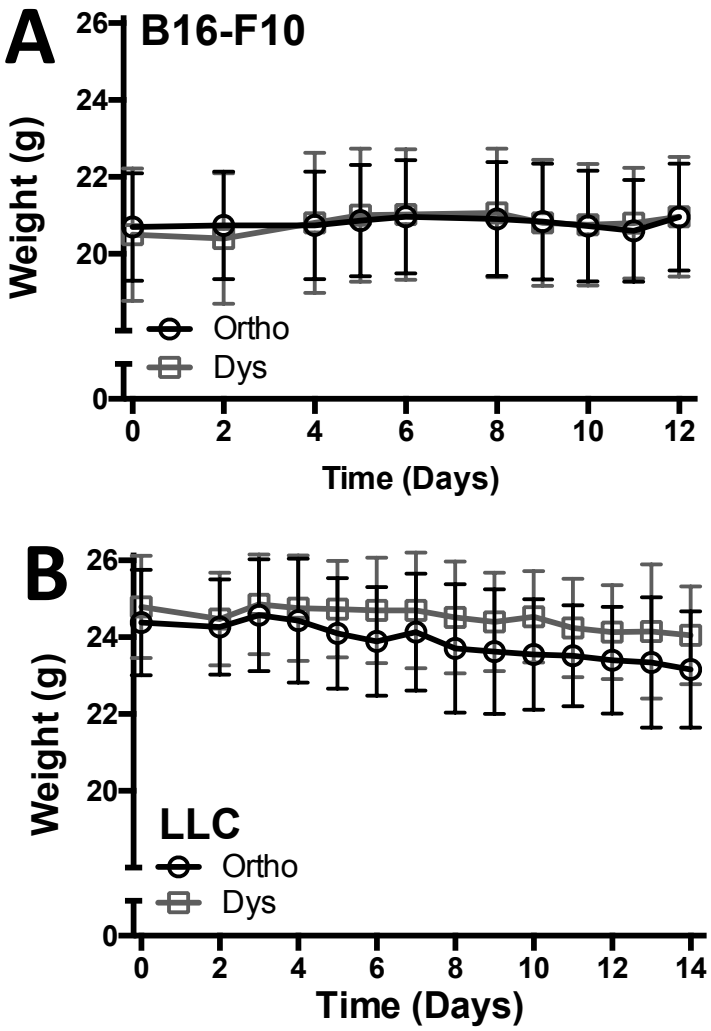
Departments of [1]Radiation Oncology, [2]Biomedical Informatics, [3]Pathology, [4]Biostatistics, and [5] Immunology and Microbiology, University of Arkansas for Medical Sciences, Little Rock, AR USA

[6]Center for Integrative Nanotechnology Sciences, University of Arkansas at Little Rock, Little Rock, AR USA

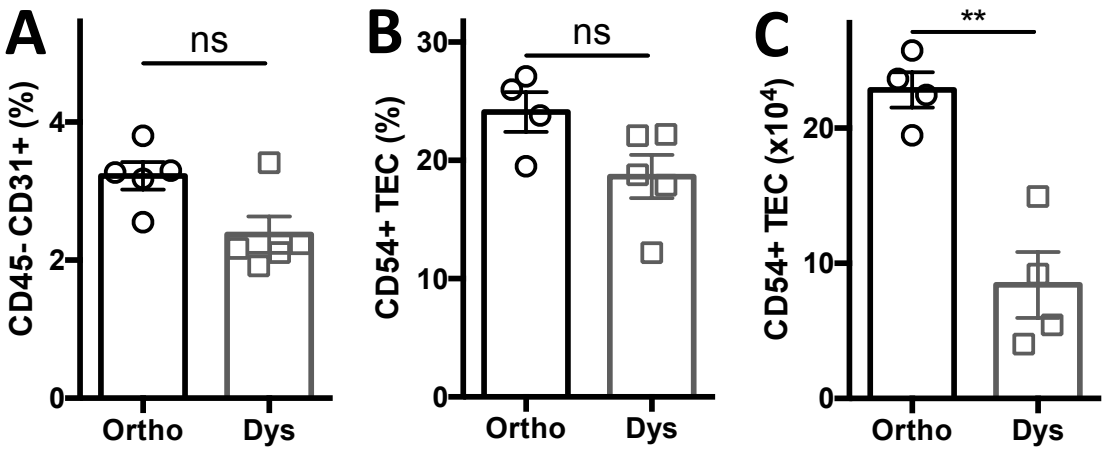
[*]**Corresponding Author:** Ruud P.M. Dings, 4301 W. Markham Street, Mail Slot #771, Little Rock, AR 72205. Email: rpmdings@uams.edu



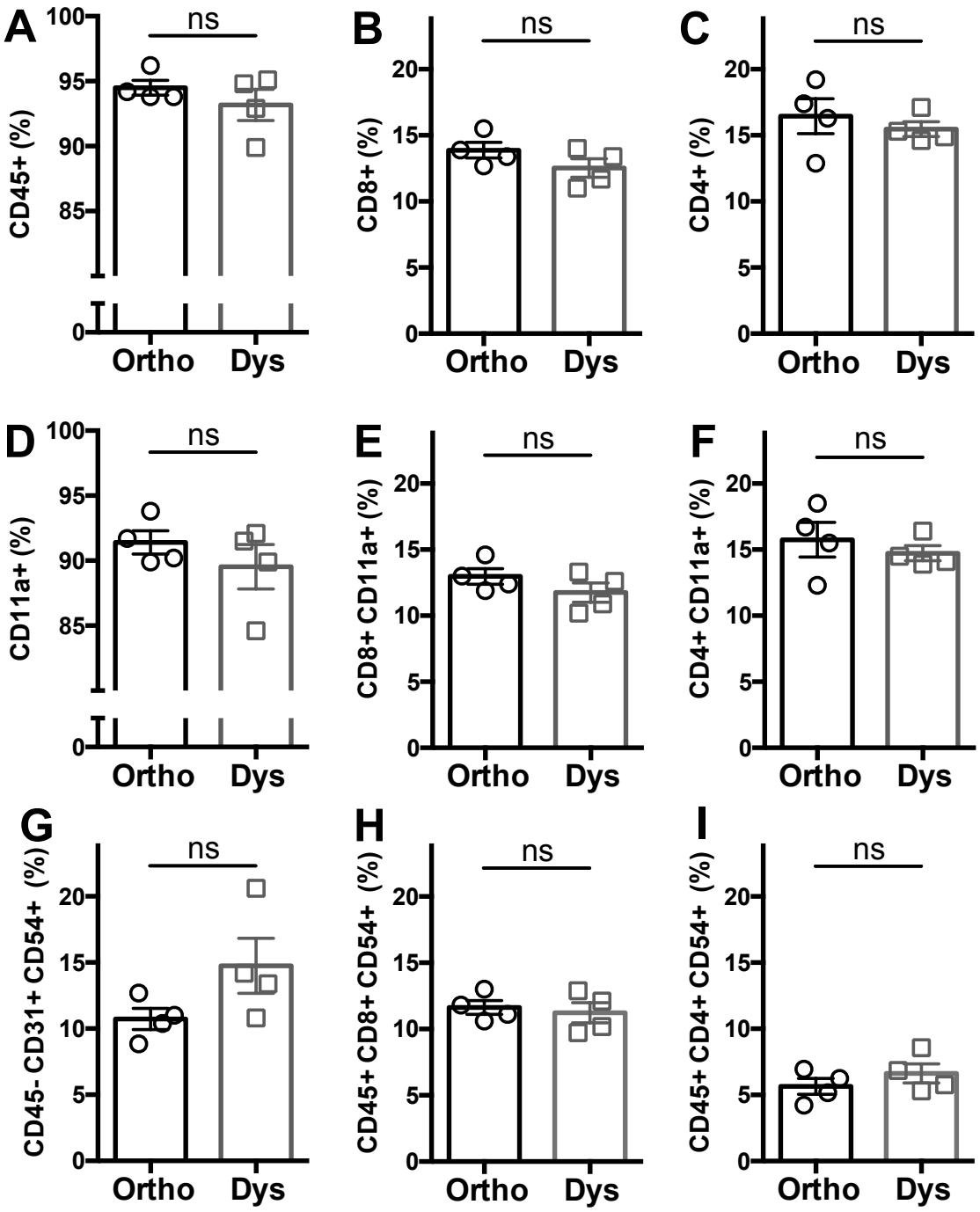
Supplemental Figure 1. Ceca are enlarged by antibiotic-induced dysbiosis in mice. Dysbiosis was induced by exposing mice to a cocktail of antibiotics in their drinking water 2 weeks before and during tumor development. Representative images of ceca in C57/BL6 mice bearing B16-F10 melanoma. Ortho = orthobiosis; Dys = dysbiosis



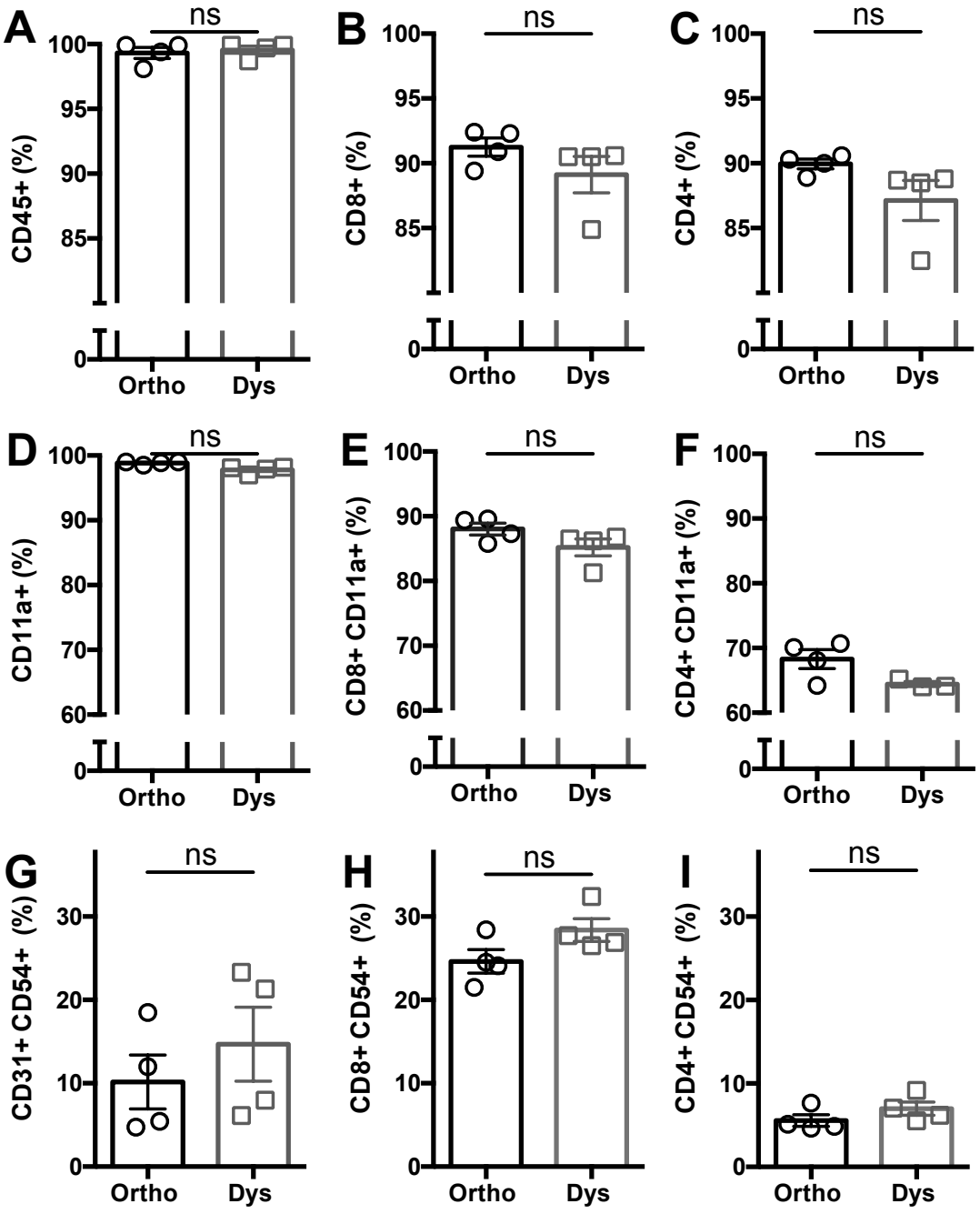
Supplemental Figure 2. Antibiotic-induced dysbiosis does not affect body weights. The body weights of (**A**) B16-F10 melanoma and (**B**) LLC-bearing mice. Data are shown as mean body weight in g ± SD (*n* = 15-17 animals per group pooled from 3 individual experiments.) -○- orthobiotic (Ortho) and -□- dysbiotic (Dys) mice.



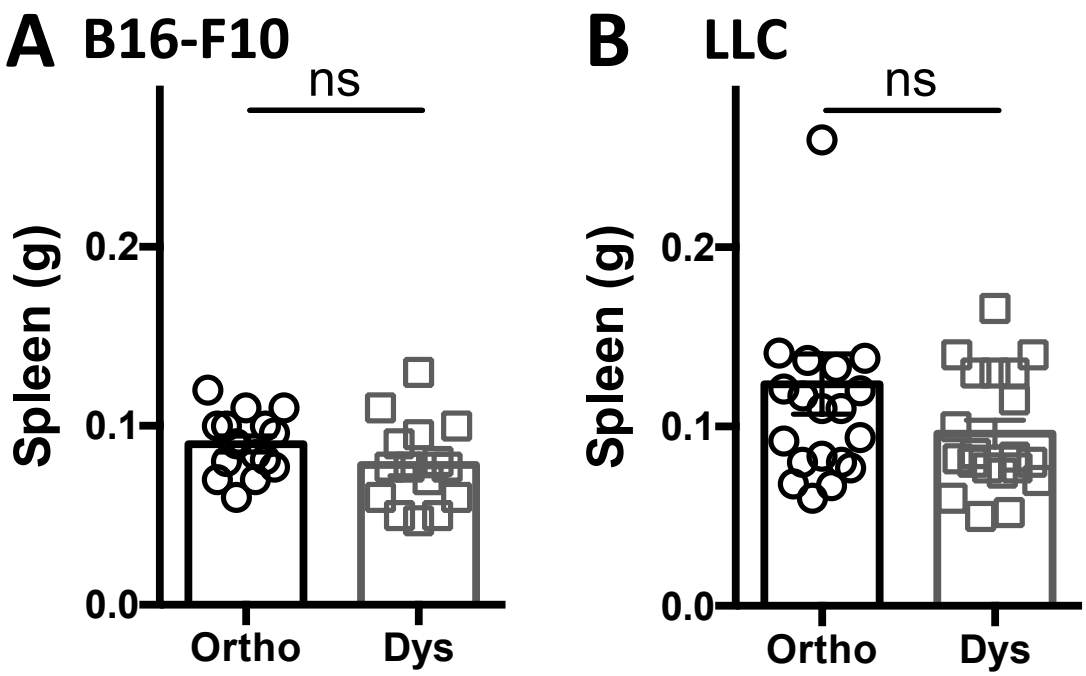
Supplemental Figure 3. ICAM-1 expression is suppressed under dysbiotic conditions in the LLC model. (**A**) Tumor endothelial cells (TEC; CD45- CD31+) are not reduced by antibiotic-induced dysbiosis. Quantification in (**B**) percentage and (**C**) absolute number of ICAM-1 (CD54+) TEC. Data are the mean ± SEM (*n* = 4-5 LLC-bearing mice per group). ***P* < 0.01, two-sided t-test. ns = not significant. -○- orthobiotic (ortho) and -□- dysbiotic (dys) mice



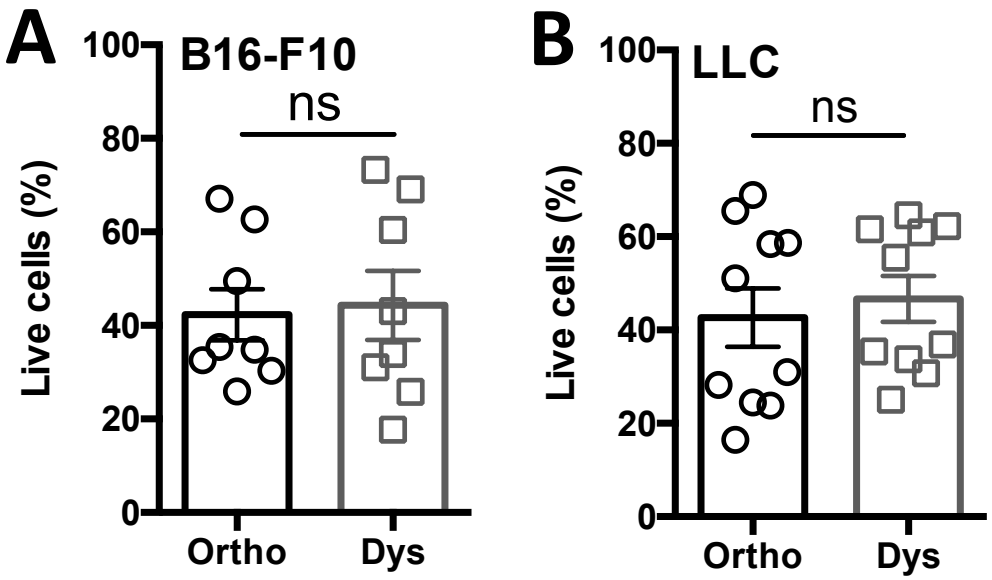
Supplemental Figure 4. Dysbiosis does not affect cell populations and adhesion molecules in the spleen. Dysbiosis does not change the amount of (**A**) CD45+ leukocytes, (**B**) CD8+ T cells or (**C**) CD4+ T cells in the spleen. ICAM-1 ligand, CD11a, expression is not affected by dysbiosis: (**D**) overall, (**E**) on CD8+ T cells, or (**F**) CD4+ T cells in the spleen. ICAM-1 (CD54) expression is not affected (**G**) on vasculature (CD45- CD31+), (**H**) CD8+ T cells (**I**) or CD4+ T cells in the spleen. Data are the mean ± SEM (*n* = 4). ns = not significant. -○- orthobiotic (ortho) and -□- dysbiotic (dys) mice.



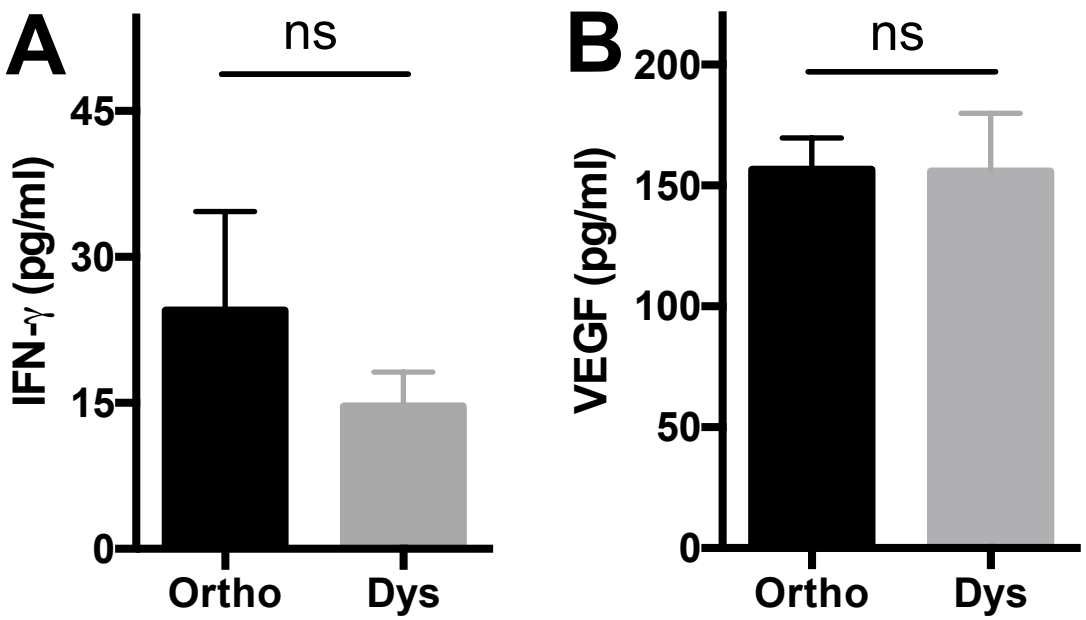
Supplemental Figure 5. Dysbiosis does not affect cell populations and adhesion molecules in the thymus. Dysbiosis does not change the amount of (**A**) CD45+ leukocytes, (**B**) CD8+ T cells or (**C**) CD4+ T cells in the thymus. ICAM-1 ligand, CD11a, expression is not affected by dysbiosis: (**D**) overall, (**E**) on CD8+ T cells, or (**F**) CD4+ T cells in the thymus. ICAM-1 (CD54) expression is not affected (**G**) on vasculature (CD45- CD31+), (**H**) CD8+ T cells (**I**) or CD4+ T cells in the spleen. Data are the mean ± SEM (*n* = 4). ns = not significant. -○- orthobiotic (Ortho) and -□- dysbiotic (Dys) mice.



Supplemental Figure 6. Antibiotic-induced dysbiosis does not affect spleens. The weights of (**A**) spleens in B16-F10 melanoma and (**B**) LLC bearing mice. Data are shown as mean spleen weight in grams ± SEM (*n* = 15-20 animals per group pooled from 3 individual experiments.). ns = not significant. -○- orthobiotic (Ortho) and -□- dysbiotic (Dys) mice.



Supplemental Figure 7. Dysbiosis does not change percentage of live cells in tumors. Percentage of live cells quantified by fixable viability dye in tumors with or without antibiotic-induced dysbiosis in mice bearing (**A**) B16-F10 melanoma or (**B**) LLC. Data are the mean ± SEM (*n* = 8–10 tumors per group, pooled from 2 individual FACS experiments). ns = not significant. -○- orthobiotic (Ortho) and -□- dysbiotic (Dys) mice



Supplemental Figure 8. Antibiotic-induced dysbiosis does not suppress IFN-γ or VEGF serum levels. Serum levels of (**A**) IFN-γ and (**B**) VEGF under orthobiotic (Ortho) and dysbiotic (Dys) conditions. ns = not significant.

Supplemental Table 1, Dysbiosis does not significantly affect adhesion molecules or cells populations in the spleen or thymus. Data are the mean (%) ± SEM (*n* = 4).

	Spleen		Thymus	
	Ortho	**Dys**	**Ortho**	**Dys**
CD45- CD31+ CD54+ (%)	10.7 ± 1.6	14.7 ± 4.2	10.2 ± 6.5	14.7 ± 8.9
CD45+ (%)	94.5 ± 1.1	93.2 ± 2.4	99.3 ± 0.9	99.6 ± 0.6
CD8+ (%)	13.9 ± 1.2	12.5 ± 1.4	91.3 ± 1.4	89.1 ± 2.8
CD4+ (%)	16.5 ± 2.7	15.5 ± 1.1	90.0 ± 0.7	87.1 ± 3.1
CD8+ CD54+ (%)	11.6 ± 1.0	11.2 ± 0.8	24.6 ± 2.8	28.4 ± 2.7
CD4+ CD54+ (%)	5.7 ± 1.2	6.6 ± 1.4	5.6 ± 1.4	7.0 ± 1.6
CD8+ CD11a+ (%)	13.0 ± 1.2	12.5 ± 1.4	88.0 ± 1.8	85.2 ± 2.6
CD4+ CD11a+ (%)	15.8 ± 2.6	14.7 ± 1.1	68.3 ± 2.9	64.4 ± 0.7