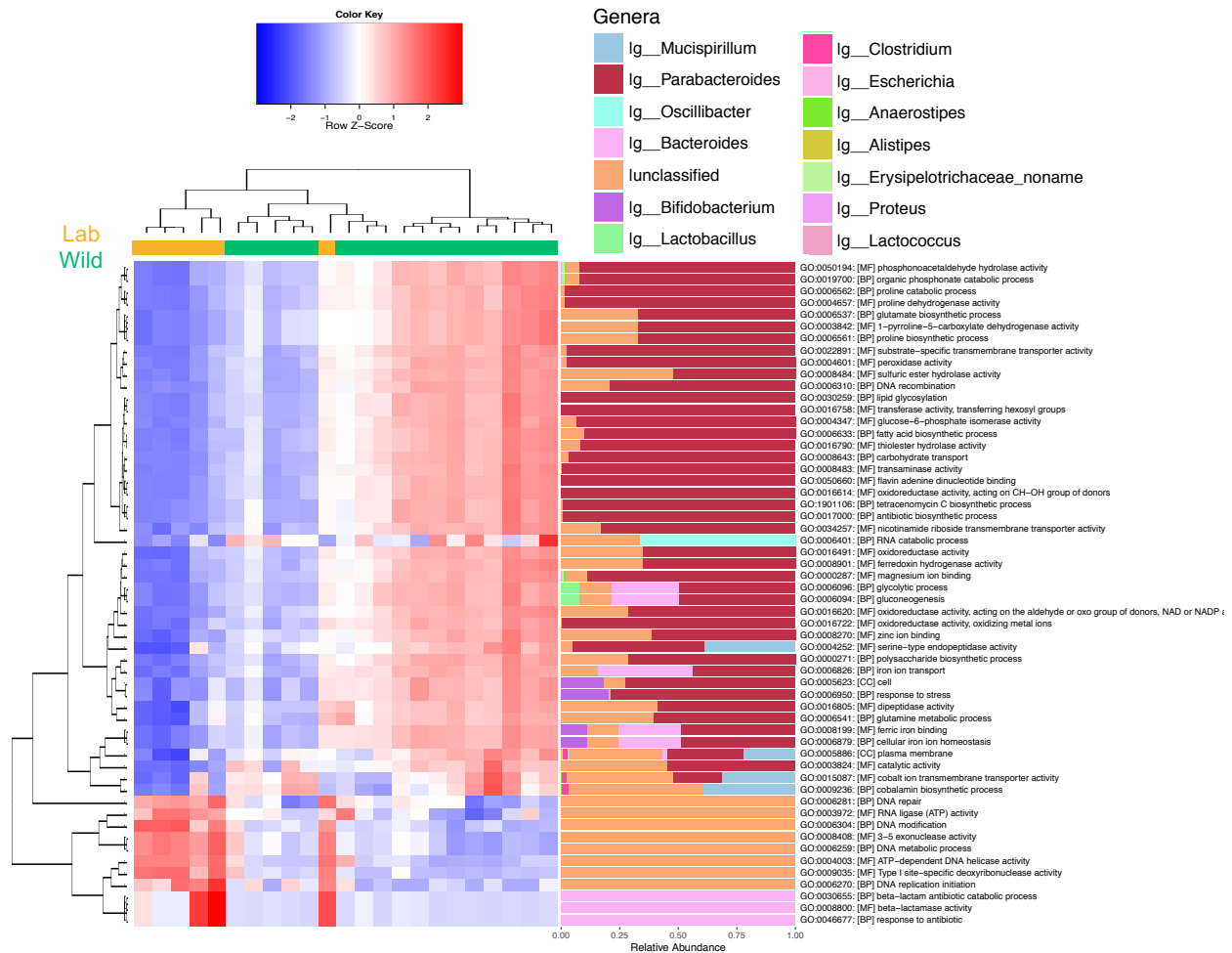


**Figure S1. Rewilding Increases the Proportion of Differentiated CD4<sup>+</sup> T Cell Populations, Related to Figure 1.**

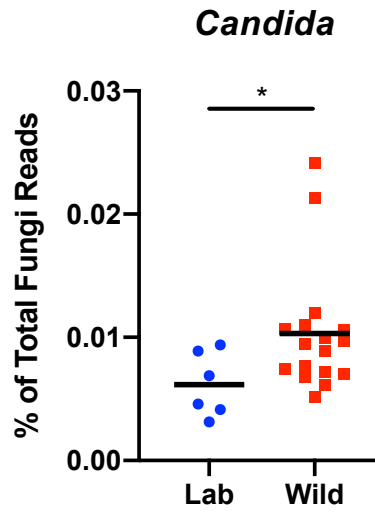
Additional quantification of T cell subsets from rewilded (Wild) mice and control mice maintained in the laboratory condition (Lab) described in Figure 1. **(A-B)** Quantification of CD62L<sup>hi</sup>CD44<sup>lo</sup>, CD62L<sup>hi</sup>CD44<sup>hi</sup>, and CD62L<sup>lo</sup>CD44<sup>hi</sup> CD4<sup>+</sup> T cells in the (A) peripheral blood and (B) MLNs. **(C)** Representative flow cytometry plots and quantification of mesenteric lymph node (MLN) cells expressing CD4<sup>+</sup>CD25<sup>+</sup>. N = 79 lab and 101 wild mice (Blood); N = 77 lab and 101 rewilded mice (MLN). \*\*\*\*  $P < 0.0001$  by two-tailed Student's *t*-test between groups, (A) to (C).





**Figure S3. Rewilded Mice Have Unique Bacterial Gene Family Signatures, Related to Figure 4.**

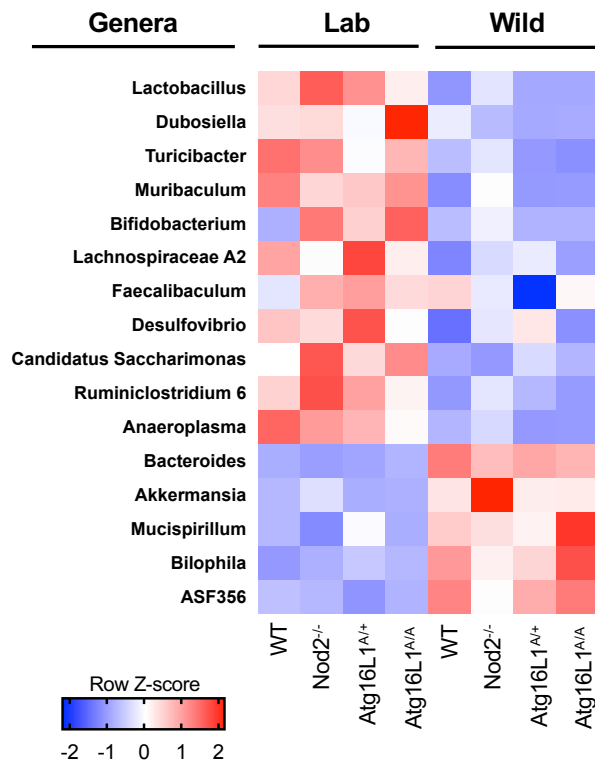
Differential gene family abundance differences and changes in microbial genera taxa of lab versus rewilded mice. Differentially abundant gene families were evaluated by a fold change difference of 1.8 in either direction and an adjusted p-value of 0.05 from Student's *t*-test between groups. N = 6 lab and 17 rewilded mice.



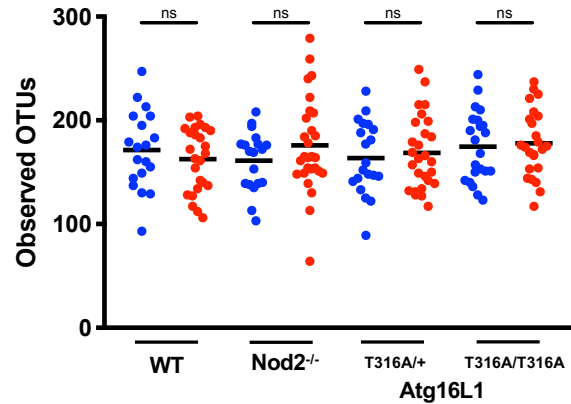
**Figure S4. Rewilded Mice are associated with an Increase in *Candida* Colonization, Related to Figure 4.**

*Candida* genus specific reads identified in the shotgun sequencing. N = 6 lab and 17 rewilded mice. \*  $P < 0.05$  by two-tailed Student's *t*-test between groups.

**A**

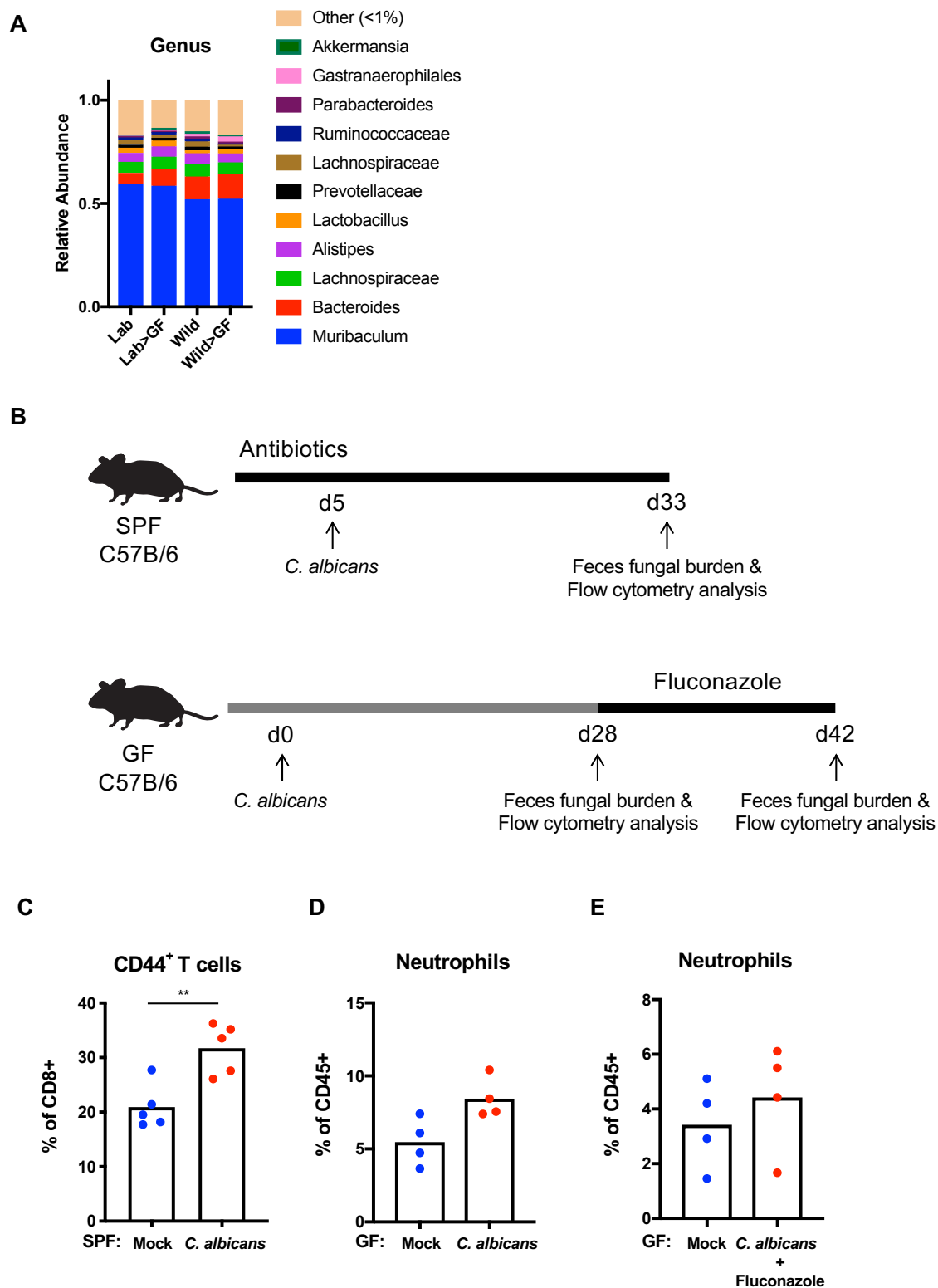


**B**



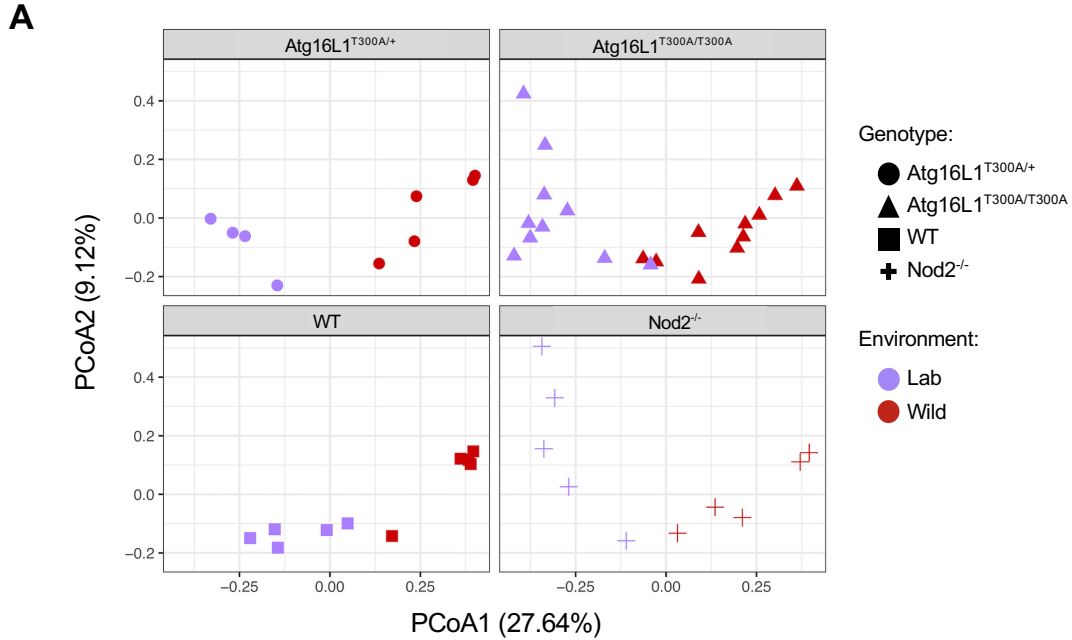
**Figure S5. Bacterial Community Structure of Wildtype, *Atg16l1*, and *Nod2* Mutant Mice in Lab and Wild Conditions, Related to Figure 5.**

**(A)** Heatmap of genera bacterial taxa significantly enriched in wildtype, *Atg16l1*, and *Nod2* mutant mice in lab and wild conditions as determined LefSe analysis (Fig. 4D) **(B)** Alpha diversity quantification through number of OTUs in the above mice. *ns*, not statistically significant

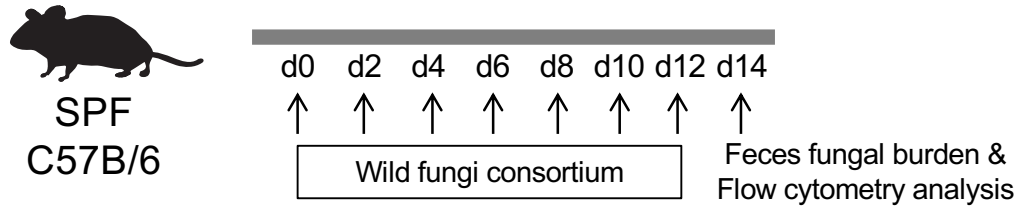


**Figure S6. Bacterial Community Structure of Germ-Free Mice Reconstituted with Microbiota from Lab and Rewilded Mice, Related to Figure 6. Legend continued on next page.**

**(A)** Stacked bar plots displaying the mean relative abundances of genus level taxa within the fecal microbial communities in lab (Lab), rewilded (Wild) mice, or germ-free (GF) mice reconstituted with cecal contents from either lab or rewilded mice. N>5 recipient mice per condition. **(B)** Experimental model of *C. albicans* colonization in SPF and germ-free mice. **(C)** Quantification of CD44<sup>+</sup>CD8<sup>+</sup> T cells in the peripheral blood of *C. albicans* colonized SPF mice. N = 5 mice per group. **(D)** Quantification of CD11b<sup>+</sup>Ly6G<sup>+</sup> neutrophils in the peripheral blood of *C. albicans* mono-associated and control mice. N = 4 mice per group. **(E)** Quantification of neutrophils in the peripheral blood of *C. albicans* mono-associated mice after treatment with antifungal drug (Fluconazole) for 4 weeks. N = 4 mice per condition. \*\*  $P < 0.01$  by two-tailed Student's *t*-test between groups.



**B**



**Figure S7. *Candida albicans* Mono-Colonization Increases Neutrophils in the Peripheral Blood, Related to Figure 7.**

**(A)** PCA of mycobiota ITS sequences in wildtype, *Atg16l1*, and *Nod2* mutant mice in Lab and Wild conditions. **(B)** Experimental model of wild fungi colonization in SPF mice.



<b>Serology</b>	<b>Lab</b>	<b>Wild</b>
MFIA SEND	0/5 (0%)	0/5 (0%)
MFIA PVM	0/5 (0%)	0/5 (0%)
MFIA MHV	0/5 (0%)	0/5 (0%)
MFIA MVM	0/5 (0%)	0/5 (0%)
MFIA MPV1	0/5 (0%)	0/5 (0%)
MFIA MPV2	0/5 (0%)	0/5 (0%)
MFIA NS1	0/5 (0%)	0/5 (0%)
MFIA MNV	0/5 (0%)	0/5 (0%)
MFIA GDVII	0/5 (0%)	0/5 (0%)
MFIA REO	0/5 (0%)	0/5 (0%)
MFIA EDIM (ROTA)	0/5 (0%)	0/5 (0%)
MFIA LCMV	0/5 (0%)	0/5 (0%)
MFIA ECTRO	0/5 (0%)	0/5 (0%)
MFIA MAV 1 & 2	0/5 (0%)	0/5 (0%)
MFIA MCMV	0/5 (0%)	0/5 (0%)
MFIA K	0/5 (0%)	0/5 (0%)
MFIA MTLV	0/5 (0%)	0/5 (0%)
MFIA POLY	0/5 (0%)	0/5 (0%)
MFIA HANT	0/5 (0%)	0/5 (0%)
MFIA MPUL	0/5 (0%)	0/5 (0%)
MFIA ECUN	0/5 (0%)	0/5 (0%)
MFIA CARB	0/5 (0%)	0/5 (0%)
MFIA PHV	0/5 (0%)	0/5 (0%)
MFIA LDV	0/5 (0%)	0/5 (0%)
MFIA Anti-Ig (Positive Ctrl)	5/5 (100%)	5/5 (100%)

**Table S1. Rewilded Mice Are Seronegative for Common Mouse Pathogens, Related to STAR Methods.**

Multiplexed Fluorometric ImmunoAssay results from serum of lab (Lab) and rewilded (Wild) mice (Animal Health Diagnostic Services, MFIA Mouse Assessment Plus Profile, Charles River). N = 5 lab and 5 rewilded mice.

<b>Infectious Disease</b>	<b>Lab</b>	<b>Wild</b>
LCMV	-	0/10 (0%)
MAV 1 & 2	-	0/10 (0%)
MHV	0/5 (0%)	0/20 (0%)
MNV	-	0/10 (0%)
Mousepox (Ectromelia)	-	0/10 (0%)
Mouse Parvovirus (MPV/MVM) P	0/5 (0%)	0/20 (0%)
MRV (EDIM)	0/5 (0%)	0/20 (0%)
PVM	0/5 (0%)	0/20 (0%)
REO	-	0/10 (0%)
SEND	0/5 (0%)	0/20 (0%)
TMEV/GDVII	0/5 (0%)	0/20 (0%)
Beta Strep Grp A	0/5 (0%)	0/20 (0%)
Beta Strep Grp B	0/5 (0%)	0/20 (0%)
Beta Strep Grp C	0/5 (0%)	0/20 (0%)
Beta Strep Grp G	0/5 (0%)	0/20 (0%)
B. bronchiseptica	0/5 (0%)	0/20 (0%)
B. hinzii	-	0/10 (0%)
Campylobacter Genus	-	0/10 (0%)
C. bovis	-	0/10 (0%)
CAR Bacillus	-	0/10 (0%)
C. kutscheri	0/5 (0%)	0/20 (0%)
C. piliforme	0/5 (0%)	0/20 (0%)
C. rodentium	0/5 (0%)	0/20 (0%)
K. oxytoca	0/5 (0%)	4/20 (20%)
K. pneumoniae	0/5 (0%)	0/20 (0%)
Helicobacter genus	-	0/10 (0%)
M. pulmonis	0/5 (0%)	0/20 (0%)
P. pneumotropicaHeyl	0/5 (0%)	0/20 (0%)
P. pneumotropicaJawetz	0/5 (0%)	0/20 (0%)
Ps. aeruginosa	0/5 (0%)	0/20 (0%)
Salmonella Genus	0/5 (0%)	0/20 (0%)
S. aureus	1/5 (20%)	19/20 (95%)
S. moniliformis	0/5 (0%)	0/20 (0%)
S. pneumoniae	-	0/10 (0%)
Cryptosporidium	-	0/10 (0%)
Demodex	-	0/10 (0%)
Entamoeba	1/5 (20%)	3/20 (15%)
Giardia	0/5 (0%)	0/20 (0%)
Mite	0/5 (0%)	0/20 (0%)
Pinworm	0/5 (0%)	0/20 (0%)
Pneumocystis	0/5 (0%)	0/20 (0%)
P. mirabilis	-	4/10 (40%)
Spirochete muris	0/5 (0%)	0/20 (0%)
Tritrichomonas genus	-	0/10 (0%)

**Table S2. Rewilded Mice Are Negative for Most Agents Detected by PCR Screening, Related to STAR Methods.**

TaqMan PCR testing results from direct animal sampling of lab (Lab) and rewilded (Wild) mice (Animal Health Diagnostic Services, Surveillance Plus PRIA Panel, Charles River). N = 5 lab and 20 rewilded mice.

Mouse/#	Repeats	Spa type	PVL	alpha	beta	delta/PSMs	Pigment
Wild WT Colony #1	Z1-D1-M1-D1-M1-N1-K1-B1	17/t216	-		+++	+++	normal
Wild WT Colony #2	Z1-D1-M1-D1-M1-N1-K1-B1	17/t216	-		+++	+++	normal
Wild WT Colony #3	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+++	normal
Wild WT Colony #4	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Nod2 Colony #1	Z1-D1-M1-D1-M1-N1-K1-B1	17/t216	-		+++	+++	normal
Wild Nod2 Colony #2	Z1-D1-M1-D1-M1-N1-K1-B1	17/t216	-		+++	+++	normal
Wild Nod2 Colony #3	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Nod2 Colony #4	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #1	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #2	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #3	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #4	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #5	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #6	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #7	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Wild Atg16L1 A/A Colony #8	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Lab Atg16L1 A/A Colony #1	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Lab Atg16L1 A/A Colony #2	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Lab Atg16L1 A/A Colony #3	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal
Lab Atg16L1 A/A Colony #4	U1-G1-F1-M1-E1-B1-B1-P1-B1	876/t786	+		+++	+	normal

**Table S3. Staphylococcus Aureus Isolates Identified from Stool of Rewilded Mice, Related to STAR Methods.**

Genotyping of *S. aureus* isolates from representative lab and rewilded mice by DNA sequence analysis of the protein A gene variable repeat region (spa typing) and a variety of additional DNA polymorphisms including the presence of pvl genes.