Fig. S1. ACY-738 and Tubastatin are HDAC6 inhibitors. This experiment compares HDAC6 specific augmentation of suppressive Treg function by both HDAC6 inhibitors. Treg suppression assays with (top panels) wild type (WT) and (lower panels) HDAC6$^{-/-}$ Tregs were performed with the indicated concentrations of inhibitors. All assays were performed with APCs and effector T cells (TE) from HDAC6$^{-/-}$ mice to exclude confounding effects of HDAC6 inhibitor treatment by these HDAC6 specific inhibitors. Data representative of two independent experiments.

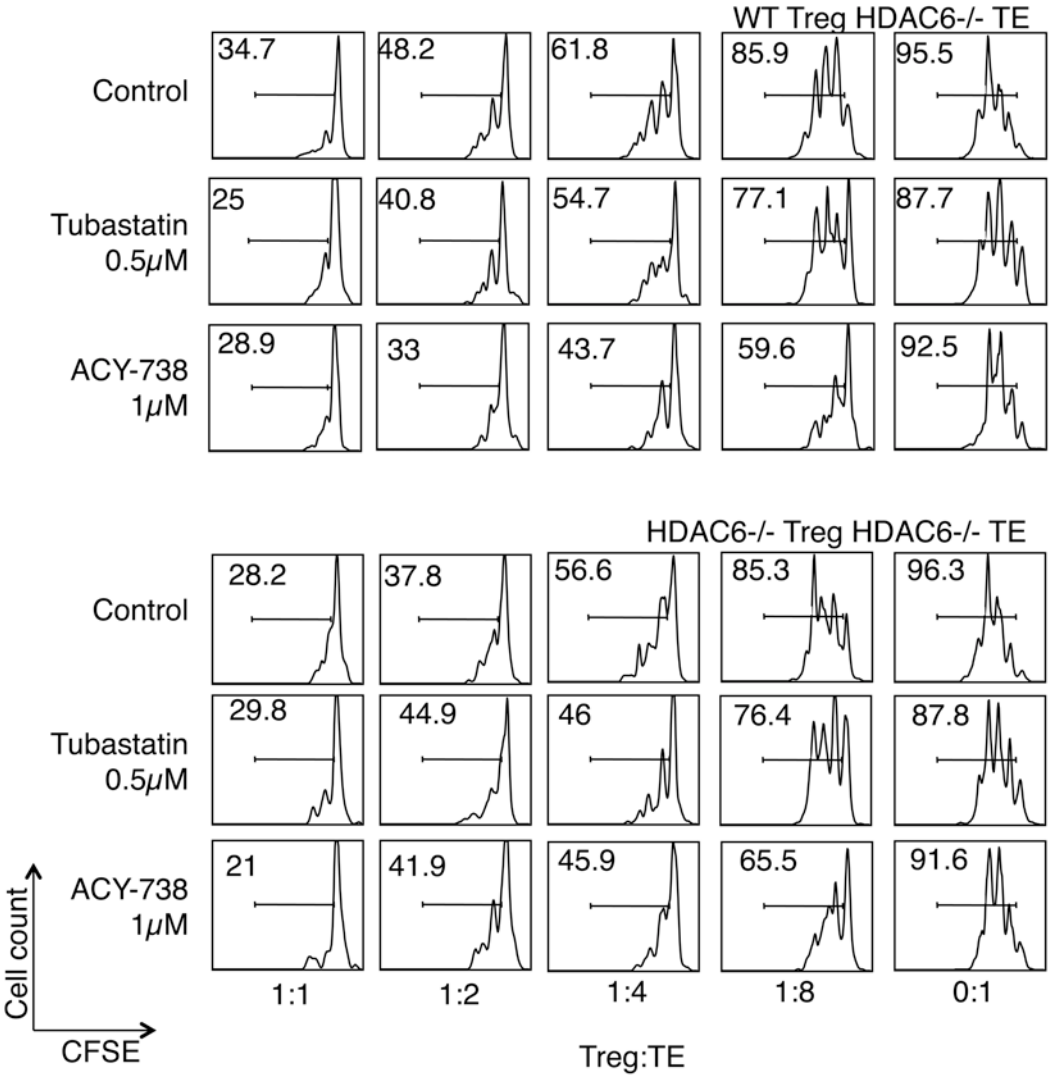

Fig. S2. Immunofluorescence of Tregs to assess the cytosolic and nuclear localization of transcription factors controlling Foxp3 expression. Scale bar: 10 µm.

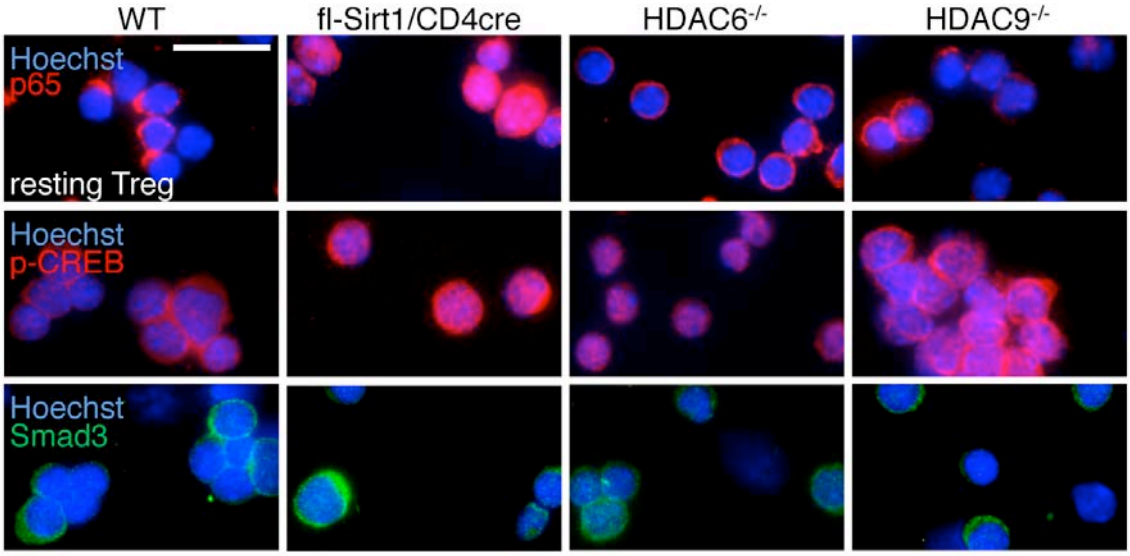

Fig. S3. Western blotting analysis of Treg lysates to compare Foxp3 transcription factors. Smad, SMA and Mothers against decapentaplegic homolog-3; CREB, cAMP response element-binding. Data representative of three independent experiments.

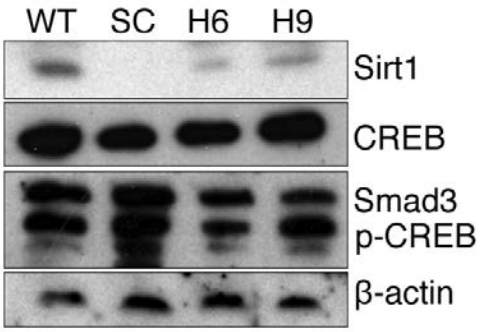

Fig. S4. Loss of Sirt1 does not affect conversion to iTregs. (**A**) Cumulative results from flow cytometric analysis of the induction of Foxp3 protein in wild-type (WT) and fl-Sirt1/CD4cre effector T cells treated for 4 to 5 days with TGF-β (2 to 3 ng/ml) and IL-2 (25 U/ml). Data pooled from 15 independent experiments. (**B**) $CD8^+$ effector T cells from fl-Sirt1/CD4cre mice were converted to iTregs at the same rate as were WT $CD8^+$ T cells. Experiment done in triplicate. (**C**) Baseline extent of $Foxp3^+$ staining in cell from mesenteric lymph nodes is equivalent between WT and Sirt1-deficient mice. Data pooled from three independent experiments.

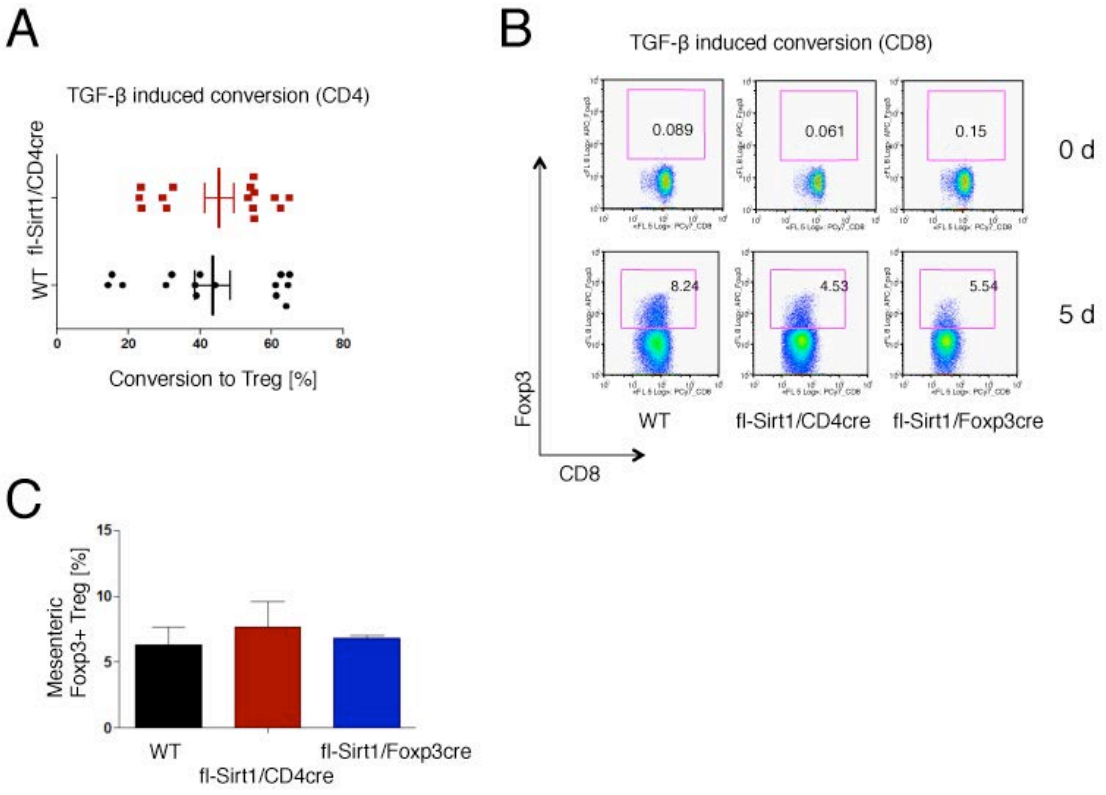

Fig. S5. Loss of HDAC9 does not alter TSDR methylation. Pyrosequencing of six CpG methylation sites from the TSDR of effector T cells from the indicated mice. Data are from two independent experiments.

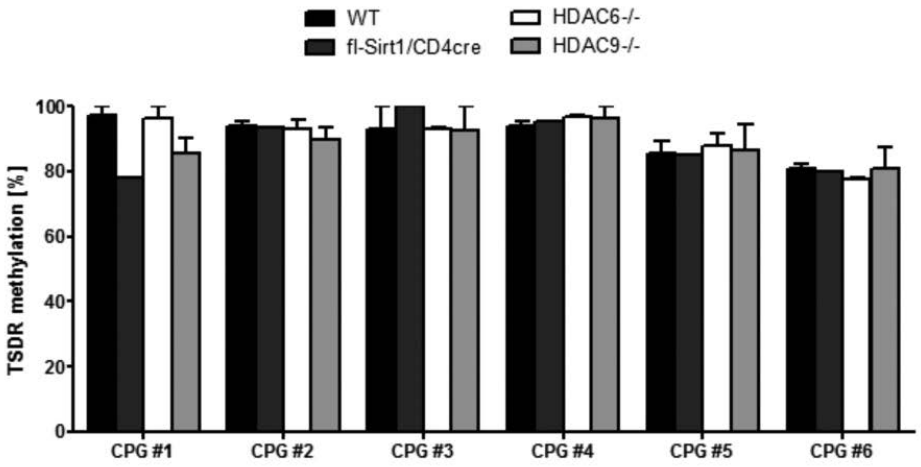

Fig. S6. Purity control of the effector T cells used for the pyrosequencing methylation assay (fig. S5). Almost all of the cells are negative for CD25 and Foxp3, indicating adequate purity of effector T cells for methylation analysis.

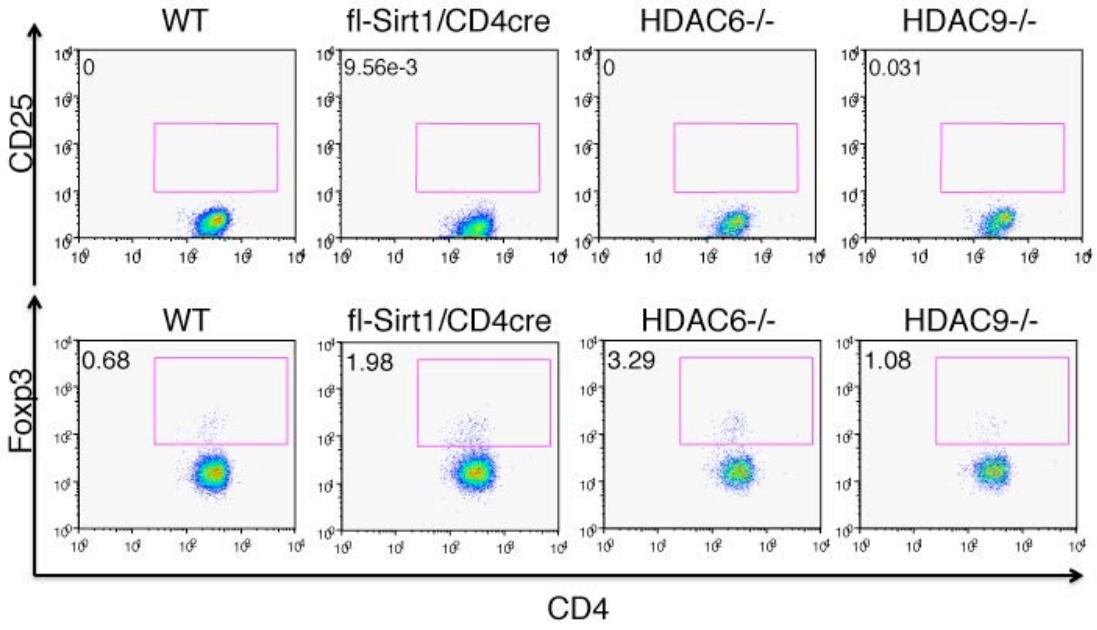

Fig. S7. Combined deletion of Sirt1 and HDAC9 produces minor improvements in Treg function. Tregs lacking HDAC9 or Sirt1 (colored panel, bottom row) are more potent than WT Tregs (bottom panel) at suppressing the proliferation of effector T cells. Inhibition of HDAC6 with ACY-738 (1 μM) improved Treg function in cells of either genotype. However, inhibition of Sirt1 with EX-527 (5 μM) had only a minimal additive benefit for HDAC9$^{-/-}$ Tregs (upper panel). Data representative of three independent experiments.

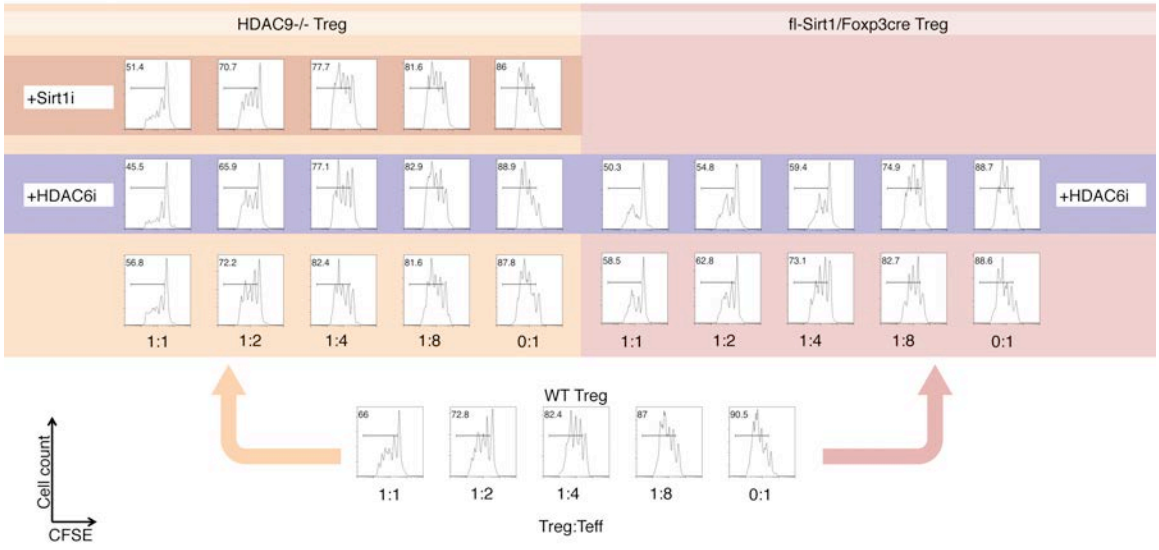

Fig. S8. Targeting of three HDACs does not improve Treg function any more than does use of dual HDACi. HDAC9$^{-/-}$ Tregs treated with ACY-738 (1 μM) and EX-527 (5 μM) did not exhibit any improvement in function than did WT Tregs treated with both HDAC inhibitors. Data representative of two independent experiments.

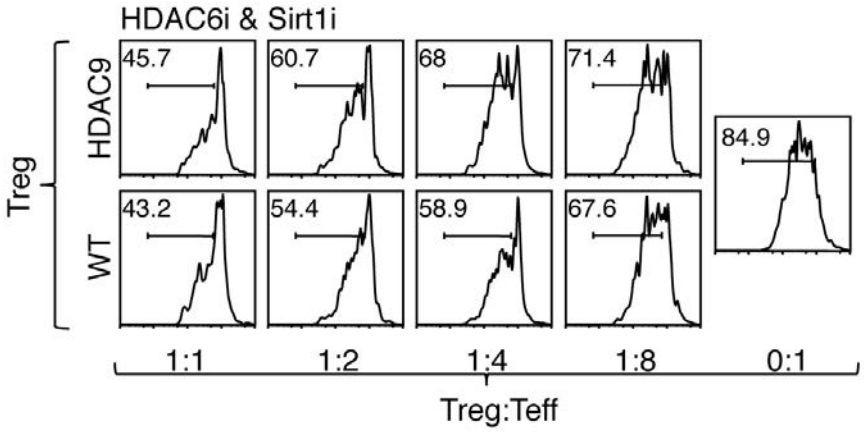

Fig. S9. Proliferation of effector T cells is not affected by HDAC inhibitors. Effector T cells (TE) injected into the B6/Rag1$^{-/-}$ mice from the homeostatic proliferation experiment in Fig. 5 showed no differences in Ki67 when treated with HDAC inhibitors or control (DMSO).

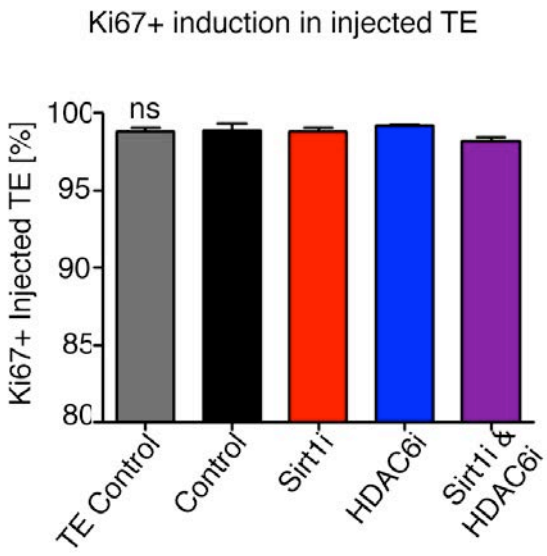

Fig. S10. Examples of image processing. (**A**) Raw immunofluorescence images obtained at 100× were processed with auto-contrast, merged, and then cropped. (**B**) Western blotting films were scanned or photographed, depending on the degree of background staining, and then were processed with auto-contrast and appropriate cropping.

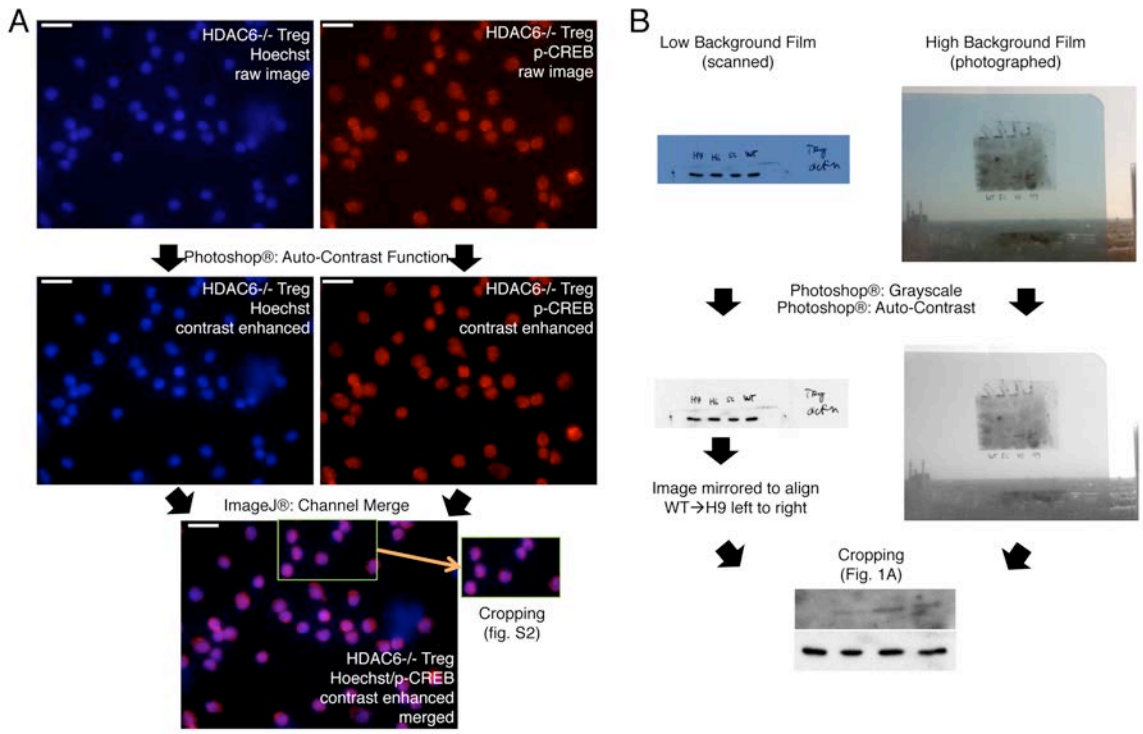

Table S1. Statistical analysis for Fig. 1C. Results of one-way ANOVA with Tukey's multiple comparison test. *P < 0.05, **P < 0.01, *** P <0.001. Diff, difference. q, q-value; Sig., statistical significance; CI, confidence interval; ns, not significantly different.

Tukey's Multiple Comparison Test	**Mean Diff.**	**q**	**Sig.**	**95% CI of diff**
WT_Control vs WT_ACY738	-0.6534	10.68	***	-0.9530 to -0.3537
WT_Control vs HS_WT_Control	0.2947	4.816	ns	-0.004942 to 0.5943
WT_Control vs HS_WT_ACY738	-0.3871	6.326	**	-0.6867 to -0.08745
WT_Control vs HSP70_Control	0.2776	4.538	ns	-0.02198 to 0.5773
WT_Control vs HSP70_ACY738	-0.07723	1.262	ns	-0.3769 to 0.2224
WT_Control vs HS_HSP70_Control	0.3778	6.175	**	0.07818 to 0.6774
WT_Control vs HS_HSP70_ACY738	0.1216	1.987	ns	-0.1781 to 0.4212
WT_ACY738 vs HS_WT_Control	0.9480	15.49	***	0.6484 to 1.248
WT_ACY738 vs HS_WT_ACY738	0.2663	4.352	ns	-0.03334 to 0.5659
WT_ACY738 vs HSP70_Control	0.9310	15.22	***	0.6314 to 1.231
WT_ACY738 vs HSP70_ACY738	0.5761	9.416	***	0.2765 to 0.8757
WT_ACY738 vs HS_HSP70_Control	1.031	16.85	***	0.7315 to 1.331
WT_ACY738 vs HS_HSP70_ACY738	0.7749	12.67	***	0.4753 to 1.075
HS_WT_Control vs HS_WT_ACY738	-0.6817	11.14	***	-0.9814 to -0.3821
HS_WT_Control vs HSP70_Control	-0.01704	0.2785	ns	-0.3167 to 0.2826
HS_WT_Control vs HSP70_ACY738	-0.3719	6.079	*	-0.6715 to -0.07229
HS_WT_Control vs HS_HSP70_Control	0.08312	1.359	ns	-0.2165 to 0.3827
HS_WT_Control vs HS_HSP70_ACY738	-0.1731	2.830	ns	-0.4727 to 0.1265
HS_WT_ACY738 vs HSP70_Control	0.6647	10.86	***	0.3651 to 0.9643
HS_WT_ACY738 vs HSP70_ACY738	0.3098	5.064	*	0.01021 to 0.6095
HS_WT_ACY738 vs HS_HSP70_Control	0.7649	12.50	***	0.4653 to 1.064
HS_WT_ACY738 vs HS_HSP70_ACY738	0.5086	8.313	***	0.2090 to 0.8082
HSP70_Control vs	-0.3549	5.800	*	-0.6545 to -

HSP70_ACY738					0.05525
HSP70_Control	vs	0.1002	1.637	ns	-0.1995 to 0.3998
HS_HSP70_Control					
HSP70_Control	vs	-0.1561	2.551	ns	-0.4557 to 0.1435
HS_HSP70_ACY738					
HSP70_ACY738	vs	0.4550	7.437	**	0.1554 to 0.7547
HS_HSP70_Control					
HSP70_ACY738	vs	0.1988	3.249	ns	-0.1008 to 0.4984
HS_HSP70_ACY738					
HS_HSP70_Control	vs	-0.2563	4.188	ns	-0.5559 to 0.04337
HS_HSP70_ACY738					

Table S2. Statistical analysis for Fig. 1G. Results of one-way ANOVA with Tukey's multiple comparison test. *P < 0.05, **P < 0.01, *** P <0.001. Diff, difference. q, q-value; Sig., statistical significance; CI, confidence interval; ns, not significantly different.

Tukey's Multiple Comparison Test	**Mean Diff.**	**q**	**Sig.**	**95% CI of diff**
WT_Control vs WT_EX527	-0.2114	4.732	*	-0.4207 to -0.002137
WT_Control vs HS_WT_Control	0.1130	2.529	ns	-0.09628 to 0.3222
WT_Control vs HS_WT_EX527	-0.1492	3.339	ns	-0.3584 to 0.06009
WT_Control vs HSP70_Control	0.2518	5.637	*	0.04259 to 0.4611
WT_Control vs HSP70_EX527	0.09279	2.077	ns	-0.1165 to 0.3020
WT_Control vs HS_HSP70_Control	0.3603	8.065	***	0.1511 to 0.5696
WT_Control vs HS_HSP70_EX527	0.07240	1.621	ns	-0.1369 to 0.2817
WT_EX527 vs HS_WT_Control	0.3244	7.261	***	0.1151 to 0.5336
WT_EX527 vs HS_WT_EX527	0.06223	1.393	ns	-0.1470 to 0.2715
WT_EX527 vs HSP70_Control	0.4632	10.37	***	0.2540 to 0.6725
WT_EX527 vs HSP70_EX527	0.3042	6.809	**	0.09492 to 0.5134
WT_EX527 vs HS_HSP70_Control	0.5717	12.80	***	0.3625 to 0.7810
WT_EX527 vs HS_HSP70_EX527	0.2838	6.352	**	0.07454 to 0.4931
HS_WT_Control vs HS_WT_EX527	-0.2621	5.868	**	-0.4714 to -0.05288
HS_WT_Control vs HSP70_Control	0.1389	3.108	ns	-0.07039 to 0.3481
HS_WT_Control vs HSP70_EX527	-0.02019	0.4520	ns	-0.2295 to 0.1891
HS_WT_Control vs HS_HSP70_Control	0.2473	5.537	*	0.03809 to 0.4566
HS_WT_Control vs HS_HSP70_EX527	-0.04058	0.9083	ns	-0.2498 to 0.1687
HS_WT_EX527 vs HSP70_Control	0.4010	8.976	***	0.1918 to 0.6103
HS_WT_EX527 vs HSP70_EX527	0.2419	5.416	*	0.03269 to 0.4512
HS_WT_EX527 vs HS_HSP70_Control	0.5095	11.40	***	0.3002 to 0.7187
HS_WT_EX527 vs HS_HSP70_EX527	0.2216	4.959	*	0.01231 to 0.4308
HSP70_Control vs HSP70_EX527	-0.1591	3.560	ns	-0.3683 to 0.05019
HSP70_Control vs HS_HSP70_Control	0.1085	2.428	ns	-0.1008 to 0.3177
HSP70_Control vs HS_HSP70_EX527	-0.1794	4.017	ns	-0.3887 to 0.02981
HSP70_EX527 vs HS_HSP70_Control	0.2675	5.989	**	0.05828 to 0.4768
HSP70_EX527 vs	-0.02039	0.4563	ns	-0.2296 to 0.1889

HS_HSP70_EX527				
HS_HSP70_Control vs HS_HSP70_EX527	-0.2879	6.445	**	-0.4972 to -0.07866