

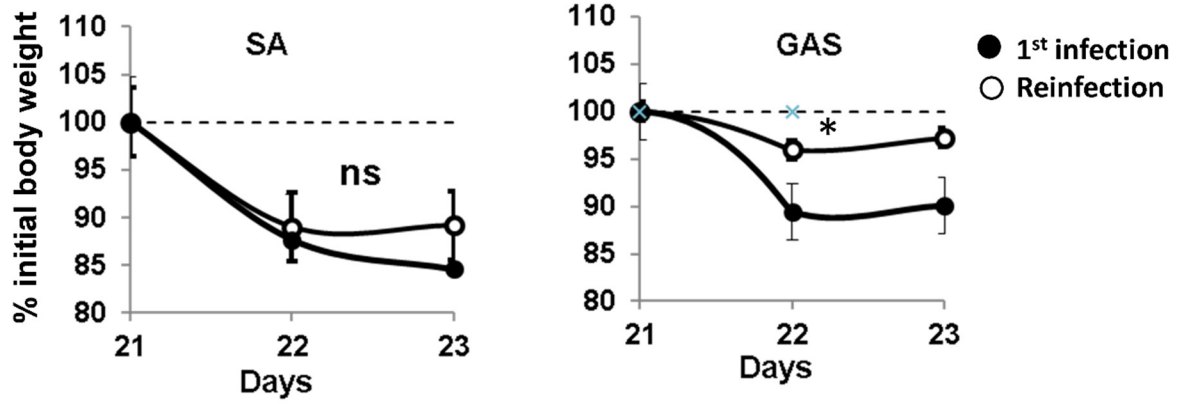
SUPPLEMENTAL INFORMATION

**O-acetylation of peptidoglycan limits
helper T cell priming and permits
Staphylococcus aureus reinfection**

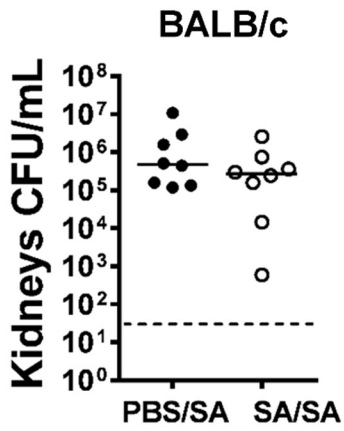
Marisel Sanchez, Stacey L. Kolar, Sabrina Müller, Christopher N. Reyes, Andrea J. Wolf, Chihiro Ogawa, Rajat Singhania, Daniel D. Carvalho, Moshe Arditi, David M. Underhill, Gislaine A. Martins, George Y. Liu

Supplemental Data

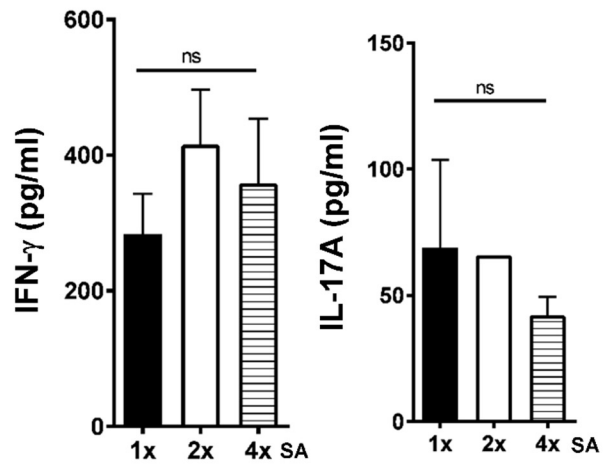
A



B



C



D

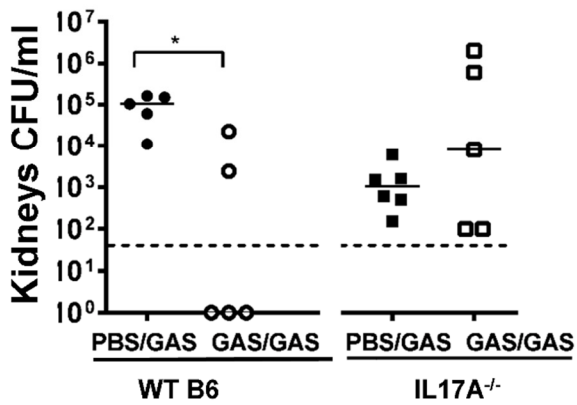


Figure S1, related to Figure 1. Prior *S. aureus* infection does not induce protective immunity against reinfection.

(A) Mouse body weight loss after reinfection with *S. aureus* or GAS. C57BL/6 mice (n=8-11) were injected i.p on d0 with 10^7 CFU of *S. aureus* (LAC USA300), GAS or PBS, and re-injected on d21 with 10^7 CFU of *S. aureus* or GAS. Shown is mouse body weights after infection relative to mouse body weights prior to infection.

(B) *S. aureus* does not induce protective immunity to reinfection in BALB/c mice. BALB/c mice (n = 8 per group) were injected i.p with 10^7 CFU of *S. aureus* (LAC USA300) or PBS on d0, and re-injected on d21 with 10^7 CFU of *S. aureus*. Bacteria from kidneys were recovered after 24hr.

(C) Spleen IL-17A and IFN- γ levels in mice infected multiple times with *S. aureus*. C57BL/6 mice (n =5) were infected i.p. once, twice (d0 and 21), or four times (d0, 21, 28 and 35) with *S. aureus* LAC (10^7). Splenocytes harvested 7 days after the last infection were stimulated with heat-killed *S. aureus* (MOI=10) for 48hr. Supernatants were analyzed by ELISA.

(D) Host resistance to GAS reinfection is IL-17A-dependent. WT C57BL/6 or IL-17A^{-/-} mice (n=8-11) were injected i.p. with 10^7 CFU of GAS or PBS on d0, and inoculated on d21 with 10^7 CFU of GAS. CFU were enumerated 24h after the last infection.

Data were analyzed by Student's t-test (A), Mann-Whitney U test (B and D) and ANOVA

(C). *p<0.05

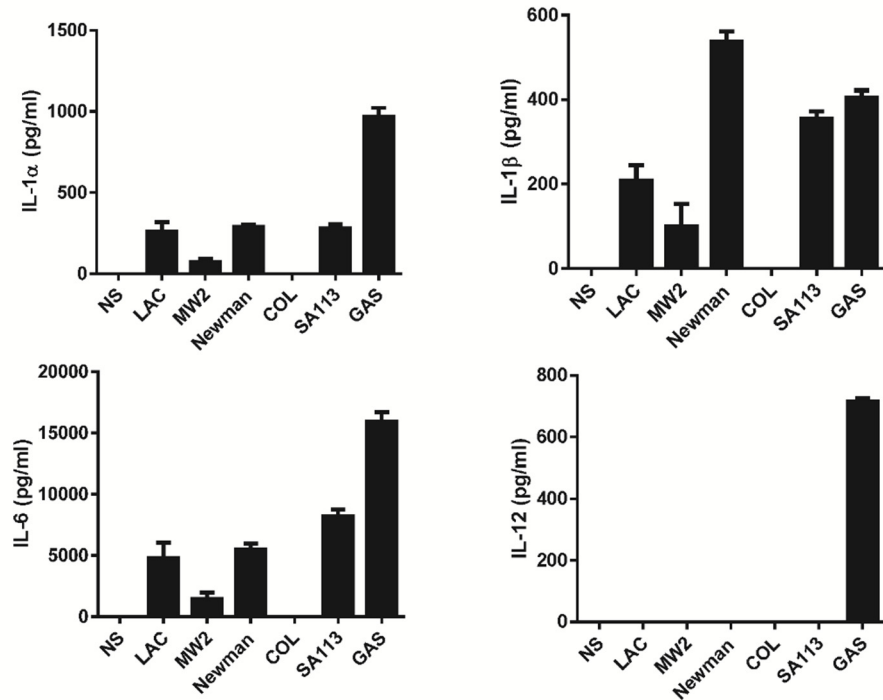


Figure S2, related to Figure 2. GAS induces higher pro-inflammatory cytokines

than *S. aureus*. Bone marrow-derived DC were left without any stimulation (NS) or incubated with strains of *S. aureus* (LAC, NW2, Newman, COL, SA113) or GAS (M49) at MOI=3. Shown are cytokine concentrations as measured by ELISA in the supernatant of DC treated for 24 hours. All data are representative of at least 2

experiments. Data are shown as mean \pm SEM and data analysis was performed using ANOVA. $p < 0.001$ for all comparisons between GAS and *S. aureus* strains in the IL-6, IL-

1 α and IL-12 assays. $p < 0.001$ for comparisons between GAS and MW2 or COL and

$p < 0.01$ for comparison between GAS and LAC in the IL-1 β assay.

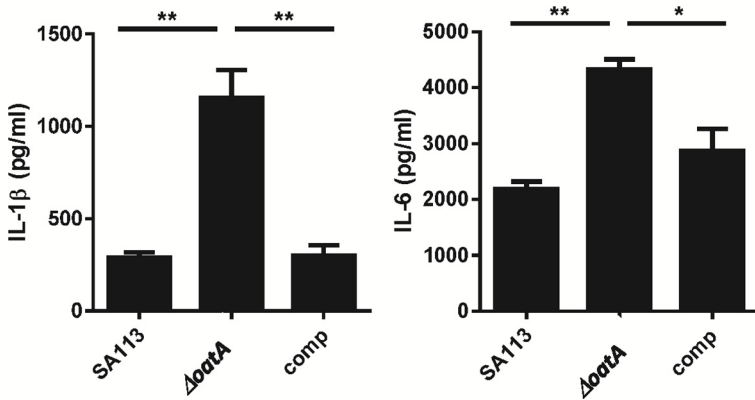


Figure S3, related to Figure 3. O-acetylation of *S. aureus* PGN limits the induction of pro-inflammatory cytokines critical for protective memory – complementation.

BMDC were stimulated with WT (*S. aureus* SA113), Δ oatA (isogenic SA113 mutant) or complemented Δ oatA at MOI of 10. Supernatants were harvested at 24h for cytokine determination. Data are representative of 2 experiments. Data analysis was performed using ANOVA. *p<0.05, **p<0.01.

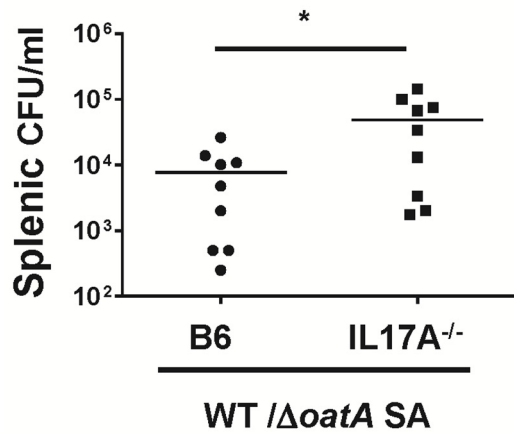


Figure S4, related to Figure 4. Adjuvancy with $\Delta oatA$ promotes development of protective immunity to *S. aureus* reinfection (replicate of Figure 4B).

WT or IL-17A^{-/-} mice were infected 3 times with *S. aureus* as in (3B). Seven days after the last infection, splenocytes were harvested and 5×10^7 purified total CD4⁺ T cells were transferred i.v. into naïve mice. The recipient mice were infected with WT *S. aureus* (2×10^7) the next day and spleen CFU were enumerated after 24h. Results are combined from two experiments (n=9). Data were analyzed by Mann-Whitney U test. *p<0.05.

Table S1, related to Figure 2. Top 100 differentially expressed (DE) genes in *S. aureus* – infected DC.

Up regulated			Down regulated		
Gene name	logFC	adj.P.Val	Gene name	logFC	adj.P.Val
Cxcl5	8.276260965	0.001757538	Cables1	-4.274064079	0.011157874
Ifit1b1	7.55967165	0.023792655	Eps8	-4.092140698	0.002280117
Cxcl3	7.53146553	0.002210827	Ppef1	-3.80390131	0.049940359
Ifit3	7.418134105	0.004698437	Scel	-3.669406733	0.007451206
Ifi205	7.308643237	0.024988002	Gm11802	-3.665304248	0.033066365
Oasl1	7.222203832	0.011705352	Hs3st3b1	-3.613421824	0.030672388
Cxcl10	7.118126794	0.011118908	Tmem53	-3.594471978	0.011161704
Apol9b	7.086312523	0.000945855	Gm17040	-3.428336331	0.039898647
Ifit2	7.069602878	0.002802861	Gm20479	-3.352359622	0.004698437
Acod1	7.049947282	8.59E-05	Cish	-3.321476495	0.006362765
Ifit3b	6.935922389	0.007264546	Klf4	-3.272038466	0.000652196
Cxcl2	6.911287497	0.004490447	Zfp985	-3.219724871	0.038431856
Rsad2	6.861075469	0.005366033	Gm29358	-3.180540771	0.042800377
Ifit1	6.749270341	0.00220908	Gm15539	-3.039777734	0.007421397
RP24-200K10.1	6.569804026	0.004490447	Dab2	-3.013540286	0.001450524
Lad1	6.545224447	0.031788907	Ipcef1	-2.99799254	0.044764698
Cxcl1	6.434693044	0.000924237	Lfng	-2.932189889	0.016497897
Cmpk2	6.336911589	0.000556966	Gm20667	-2.884708404	0.011835206
Cd40	6.328152237	0.008645666	Slc6a19	-2.776501982	0.007930912
Tgfp2	6.213556431	0.006663395	Irf4	-2.76055348	0.008452146
Zfp811	6.073426775	0.000556966	Tpbg1	-2.721861281	0.009373843
Isg15	5.938193453	0.000677871	Gm44993	-2.649622948	0.006290693
Ifi44	5.803170628	0.009972455	Socs2	-2.61799592	0.003395187
Il23r	5.789555857	0.001966358	Rab3il1	-2.489665856	0.029834473
Art3	5.72778396	0.017238026	Cbfa2i3	-2.435372806	0.018053754
Saa3	5.59241909	0.009306115	Diras2	-2.413912706	0.022936443
Ms4a4c	5.546987841	0.003860196	RP24-264F17.4	-2.39504215	5.97E-09
Gp6	5.496900346	0.00689812	Sfrp4	-2.39504215	5.97E-09
Irf7	5.480457378	0.000237172	Tmem154	-2.376231936	0.022187291
Mefv	5.283609598	0.033244253	RP23-424C4.7	-2.353208774	0.010131126
Igfp1	5.219355045	0.018053754	Gm37488	-2.352969242	2.66E-07
Mx2	5.190845548	0.011118908	Abcg1	-2.343813117	0.015491202
Usp18	5.147443514	0.001966358	Spef2	-2.33057104	0.043554021
Ifi208	5.002493492	0.008645666	Abcd2	-2.325538912	0.036068096
Tmem132e	4.991608985	0.001966358	Gm9200	-2.182227241	6.37E-05
Cd69	4.95724577	0.006084741	Pdgfc	-2.18186917	0.007904627
Gbp5	4.92062041	0.00689812	Gm6283	-2.172924457	0.016715782
Clec4e	4.859167184	0.017474019	Kcnn3	-2.170738125	0.027590488
Dnmt3c-ps	4.847013693	0.007595891	Gm16537	-2.163812692	0.047079738
Oas2	4.820270917	0.001085732	Gja5	-2.163563324	0.045263026
Ccl5	4.804367229	0.000109349	Vsn11	-2.135621062	0.011752484
Misp	4.726823543	0.004698437	Plekha6	-2.113198762	0.030898987
C530043A13Rik	4.716355389	0.012082776	Nov	-2.081544922	0.023792655
Tnfrsf10	4.639739514	0.006663395	F2r12	-2.046852258	0.028345738
Susd2	4.628342943	0.031308389	H2-DMb2	-1.953004347	0.018053754
1110032F04Rik	4.538302442	0.018015988	Tcf7l2	-1.889534511	0.03914933
Oasl2	4.503699454	0.00492614	Cnr2	-1.888498634	0.040391019
Gm19026	4.503432706	0.022804688	Gm7312	-1.851607683	0.00492614
Satr5	4.498522017	0.042600377	Prkea	-1.827775379	0.03914933
Gm37498	4.482543638	0.000945855	Cngb1	-1.783015711	0.031599779
Ifi213	4.44211772	0.00689812	Slitrik	-1.779755466	0.038017004
Ms4a6c	4.436039352	0.001966358	Coro2a	-1.767465807	0.011643208
Rtp4	4.415721903	0.00220908	2410002F23Rik	-1.735966307	0.023791926
Ms4a6b	4.415370332	0.020473715	Gm8883	-1.735875146	0.001966358
Nfkblz	4.402494948	0.006230693	Tspan9	-1.735875146	0.001966358
Gm12250	4.385415471	0.001966358	Pla2g15	-1.733080579	0.012297085
Akap12	4.358049911	0.011283635	Ehbp1	-1.689876823	0.012082776
Gpr31b	4.340435086	0.029393521	Ndst1	-1.65233401	0.04054382
Tnf	4.289375759	0.011416339	Elovl7	-1.646024237	0.03447392
Sifn1	4.281749805	0.007160073	Ptgs1	-1.635220447	0.010131126
Ifi206	4.263262307	0.020709818	Plekkg3	-1.6261926	0.022491721
Olfir56	4.243378809	0.024339174	Fam198b	-1.606588009	0.016855807
Il12a	4.233008208	0.004692253	Add3	-1.597231352	0.014430705
Il1f6	4.23285314	0.007958776	Mettl17	-1.561527522	0.033769634
Mx1	4.216272632	0.021621755	Tob1	-1.508131693	0.023070847
Phf11b	4.176442346	0.027063548	Pdia4	-1.498719199	0.030699893
Oas3	4.174599281	0.006663395	Smap2	-1.471669703	0.006495621
Hrc	4.121444384	0.045263026	Bank1	-1.45654223	0.039537511
Gm6904	4.11478936	0.011157874	Myc	-1.406037953	0.01099102
Hdc	4.068839149	0.03461938	Serp1	-1.349527614	0.01099102
Lrrc4	4.051572831	0.024931892	Lyl1	-1.335016366	0.039898987
Lox	4.02887478	0.038836655	Tmem64	-1.328256126	0.042543523
Isg20	3.996826572	0.003100707	Naaa	-1.310703435	0.010334628
Ppfia3	3.966903073	0.001757538	Pygl	-1.291318699	0.019000968
Rnf213	3.927840128	0.005644045	Ccne1	-1.281090088	0.032592984
4930512h18Rik	3.911354171	0.010131126	Rassf5	-1.270747699	0.023791926
Sifn8	3.905568141	0.008600906	Klh6	-1.265646223	0.040787976
Dlc1	3.902396816	0.029393521	Pcb2	-1.263736516	0.03777681
Gbp6	3.886047796	0.018015988	Pced1b	-1.257370657	0.04135682
Ifi47	3.861311919	0.021759571	Ttc21b	-1.255469465	0.003395187
Gadd45b	3.808123364	0.01057067	Ppp1r3b	-1.254801485	0.023848833
Gbp2b	3.775103872	0.034585315	Rell1	-1.215478673	0.019735392
Gbp3	3.756930864	0.002802861	Tube1b	-1.174101704	0.021810224
Cav1	3.750390681	0.013051927	Xbp1	-1.166752099	0.045263026
Ehf	3.745792616	0.023689257	Pik3cd	-1.164199636	0.037570479
Gm9230	3.705513543	5.14E-06	Arhgap18	-1.161609691	0.042407691
Marcks1	3.692575202	0.007878771	Rmnd5b	-1.141121182	0.015431932
Gbp4	3.68879321	0.01099102	Nek6	-1.09717858	0.006663395
Xaf1	3.653033716	0.001114989	Inpp4a	-1.090525979	0.030699893
Gbp2	3.649846645	0.022491721	Tarsl2	-1.079619707	0.047079738
Gpr84	3.644625351	0.038017004	Elovl5	-1.05190343	0.011644886
Fabp3	3.640528471	0.039898647	Lsp1	-1.045072534	0.007451206
Slco3a1	3.64041225	0.000652196	Lrp6	-1.042596784	0.04455417
Lcn2	3.62795797	0.034585315	Prkd3	-1.034595867	0.006615006
Adora2a	3.621892782	0.016616931	Svil	-1.034063117	0.010236601
Herc6	3.56194112	0.007171288	Net1	-1.032926295	0.006290693
Serpina3f	3.551811978	0.045297568	ldh3a	-1.024319532	0.039537511

Table S2, related to Figure 2. Top 100 differentially expressed (DE) genes in GAS – infected DC.

Up regulated			Down regulated		
Gene name	logFC	adj. P.Val	Gene name	logFC	adj. P.Val
Ii6	11.92565504	0.002977933	RP24-264F17.4	-2.395053	5.97E-09
Nos2	11.4362155	0.003588654	Sfrp4	-2.395053	5.97E-09
Ifi1b1	11.38253588	0.001502506	Gm37488	-2.35298	2.28E-07
Cxd10	11.28581534	0.000600022	Gm9200	-2.182238	4.00E-05
Ifnb1	11.08073149	0.004926138	Nek6	-2.53362	0.001253
Clic5	10.67493343	0.001721031	Pdha1	-1.100042	0.001552
Ifi205	10.58551432	0.001775033	Diaph1	-1.59417	0.002297
Tgtp1	10.51067886	0.0068754	Oxd1	-1.590951	0.002428
Oasl1	10.42458919	0.000856129	Acox3	-1.828995	0.002428
Art3	10.3536167	0.000600022	Svil	-2.316996	0.002428
Apol9b	10.21784429	9.19E-05	Gm26315	-4.438698	0.002642
Ptgs2	9.98562228	0.002453258	Ptgr2	-1.294722	0.002806
Cd40	9.954673734	0.000502477	Scd2	-4.735645	0.002959
Cxcl9	9.769725586	0.001601011	Dusp3	-2.401782	0.003085
Ilgp1	9.745396036	0.00058496	Plekhp3	-4.13716	0.003085
Ifi2	9.679735611	0.000295911	Mlec	-2.279296	0.003228
Rsad2	9.679333566	0.000426909	Cybb	-1.514946	0.00323
Ii12b	9.567986524	0.002411183	Sigmar1	-1.333353	0.00343
Ifi3b	9.5348465719	0.000591348	Ad3	-3.462191	0.00343
Tgtp2	9.534894421	0.000409134	Smap2	-2.336925	0.003744
Ifi3	9.442955671	0.000506816	Cnr2	-5.24615	0.003759
Cmpk2	9.38965751	4.74E-05	Aco1	-1.633093	0.00377
Ifi1	9.348647619	0.000242848	Elovl5	-2.053863	0.003813
Ms4a4c	9.262949638	0.000229743	Usp48	-1.029469	0.003832
Cd5	9.200596227	2.66E-06	Rmnd5b	-2.379615	0.003864
Ifi44	8.761873856	0.000600022	Gltp	-1.681018	0.00389
Cd69	8.706280766	0.000255677	Prkd3	-1.561261	0.004217
Isg15	8.655081653	7.13E-05	Vav2	-2.943265	0.004416
Spta1	8.540039212	0.001298765	Ctdsp2	-1.765209	0.004558
Oftr1028	8.52156186	0.000654661	ldh1	-2.876888	0.004558
Gm18445	8.450701309	0.007310744	Cbfa2l3	-5.004826	0.004558
Cxcl1	8.450401569	0.000846382	Tmem154	-5.16863	0.004558
Serpina3f	8.30659625	0.00063446	Nostrin	-2.678326	0.004561
Heatr9	8.254605825	0.000242848	Plcb2	-3.167049	0.004561
Ii12a	8.210930462	0.000155212	Kcnk3	-4.914203	0.004916
Angpt1	8.175011209	0.001012624	Rack1	-1.101944	0.005025
Asap3	8.165289278	0.002587011	903061700Rik	-1.776567	0.005025
Pou3f1	8.134511271	0.000409599	Rasf5	-2.743812	0.005025
Ii10	8.111730541	0.000527079	Eps8	-4.342723	0.005025
Acd11	8.097941162	4.00E-05	Elovl1	-1.193103	0.005068
Ifi208	8.064885725	0.000470031	Gm8883	-1.735886	0.005094
Ii23c	8.01806108	0.000232158	Tspan9	-1.735886	0.005094
Mx2	8.002808814	0.000650277	Dhcr7	-3.307879	0.005094
Serpina3g	7.994362375	0.001505419	Dnajc5	-1.082776	0.005106
Cxcl2	7.989586963	0.000600022	Lyl1	-3.257829	0.005106
Gbp10	7.967658044	0.003981694	Zdhc3	-1.200268	0.005224
Tnn	7.93541308	8.47E-05	Gnpda1	-2.073963	0.005224
Ii27	7.920100691	0.000430786	Gtf2i	-2.673164	0.005224
Apol9a	7.858038796	0.006970001	Mvb12b	-2.712294	0.005224
Gm4841	7.807324371	0.001275676	Fam1198b	-2.998844	0.005318
Akap12	7.796721521	0.000458335	Ap2m1	-1.042377	0.005546
Gm13285	7.795069916	0.000890959	Tuba1b	-2.320407	0.005546
Gbp4	7.749029064	0.000300992	Gpd1l	-2.250073	0.005599
Zfp811	7.713305383	9.14E-05	Cdk4	-1.167061	0.005845
Gm28347	7.627450479	0.000374399	Ttc21b	-1.348658	0.006
Lad1	7.595894415	0.004546622	Shtn1	-1.340223	0.006085
BC023105	7.591793222	0.001912952	Trpv2	-1.177904	0.006086
Usp18	7.578802032	0.000156405	Colgal1	-1.876987	0.006157
RP24-84E18.1	7.53065957	0.003765503	Gna12	-2.471543	0.006397
Gm15247	7.502087356	0.010512964	Lpl	-1.121299	0.006503
Rn225	7.470307682	0.013121972	Mapk14	-1.383555	0.006521
Gm6904	7.439457778	0.000441804	Hnmp1	-1.666525	0.006521
Gbp5	7.438084759	0.000447774	Dock2	-1.710902	0.006521
Ifi7	7.435367059	4.00E-05	Fads1	-2.803775	0.006521
Kir2	7.385612662	0.006946348	Tmem53	-5.568272	0.006521
Gm13889	7.360443364	0.01093355	Tex261	-1.088731	0.006547
Fam28f	7.349512485	0.000374399	Cdc42se2	-1.102097	0.006547
Tnfrsf10	7.234393175	0.000386448	Scel	-4.8574	0.006547
Misp	7.230440496	0.000308489	Ptgs1	-2.391829	0.006583
Edn1	7.23004541	0.000953592	Atic	-1.594352	0.006903
RP24-200K10.1	7.222394249	0.000675779	Tspan32	-3.109514	0.006758
Cxd11	7.196371752	0.00294373	Mdp1	-1.481649	0.006769
Dil1	7.173266395	0.007435966	Lrrc56	-1.35493	0.006857
Ifi1b2	7.15489473	0.003225458	Pfkfb3	-3.305515	0.00702
Kir1	7.146202294	0.031698567	Arhgap19	-3.625656	0.007275
Phf11a	7.087944996	0.013826494	Pira2	-1.403436	0.007309
Tnfrsf15	7.086482949	0.002484715	Lsp1	-1.330909	0.007434
Tnfr30c	7.074520472	0.002542704	Dhcr24	-2.5155	0.007434
Isg20	7.012746885	0.000155212	Cd300a	-1.666675	0.007511
Cxcl5	6.994240244	0.000652615	Kcnab2	-2.47607	0.007814
Ifi203-ps	6.983503962	0.010186846	Zfp94	-2.95249	0.008001
Hdc	6.950335284	0.01341865	241002F23Rik	-3.114725	0.008185
Smarca1-ps1	6.938088158	0.007792101	Fia2p15	-2.574789	0.008271
Ii1f6	6.926753788	0.000421733	D6Wsu183e	-1.741574	0.008273
Ifi213	6.921214509	0.000405624	Atg7	-1.826328	0.008413
Gm16685	6.899840499	0.008250788	Unc119	-2.501511	0.008496
Sifn4	6.874525408	0.001139934	kcnk6	-2.237797	0.008883
Gm7582	6.857646013	0.022803687	Lpin1	-4.555105	0.008883
Ilgb8	6.839211411	0.004226332	Mthc1	-1.288706	0.008886
Gm42519	6.801110086	0.042432945	1810011H11Rik	-4.193046	0.008886
Dnmt3c-ps	6.779928207	0.000584517	Esys1	-1.502352	0.008983
A630012P03Rik	6.777044852	0.000502477	Naaa	-1.750644	0.009353
Tnfr30b	6.761129789	0.000441804	Aspm	-4.095644	0.009353
Ifi206	6.759937527	0.001081373	Rab31l1	-4.510406	0.009451
Nts	6.716158061	0.000685743	Csf1r	-1.226853	0.009622
Olf56	6.709141182	0.001275676	Arhgap18	-2.315181	0.009622
Gm21742	6.693796159	0.040141442	Gm20479	-3.352371	0.009841
Tnfrsf11b	6.693468945	0.001145913	Wsb2	-1.138047	0.009888
Hsp1	6.671312799	0.000306284	Dip2b	-1.43747	0.010126
Tnf	6.664155478	0.000652128	Ipcef1	-5.967697	0.010139